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Use of color sensor device with resazurin test for rapid screening of raw milk with low microbiological quality

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ABSTRACT

The microbiological quality of raw milk is important for the dairy industry because it can directly affect the quality and shelf life of dairy products. To evaluate the microbiologdical quality of raw milk, the resazurin test, a rapid dye reduction assay, was commonly used. However, resazurin test results are usually read by the eye, which can cause errors in judging the milk quality. In this study, a color sensor device based on the RGB system for measuring the color resulting from the resazurin test was developed. The device was embedded with VEML6040, an RGB-based color sensor, and operated using an Arduino microcontroller. The results showed that there is a reverse correlation between the percentages of the blue color and the number of microorganisms, both with the resazurin test performed in test tubes and in reduced-scale microtubes. A linear regression line with an R^2 of approximately 0.85 was obtained from the plot between the percentages of blue values from the microtube resazurin test and the corresponding numbers of microorganisms. This suggests that using the blue values to classify milk according to its microbiological quality is possible. With the present dataset, the blue value of 25% could be used as a cut-off value to separate raw milk with microbial loads of 7 log cfu/ml or higher from milk with lower microbial loads.

Keywords: milk quality, dye reduction assay, rapid method, colorimetric device

INTRODUCTION

The microbiological quality of raw milk is important for the dairy industry because it can directly affect the quality and shelf life of dairy products. Contamination of microorganisms in raw milk can occur during and after the milking process (Elmoslemany et al., 2009). The level of microbial contaminants is one of the crucial factors that affect milk quality because it directly affects the sensory qualities of raw milk and processed products and their shelf life (Murphy et al., 2021, Barbano et al., 2006). According to the Thai Agricultural Standard, raw milk can be divided into 3 quality categories based on the plate count values. The categories include premium quality milk, good quality milk, and standard quality milk, which have limits for microbial loads of less than 200,000 cfu/ml, between 200,000 and 400,000 cfu/ml, and between 400,000 and 600,000 cfu/ml, respectively (Thai Agricultural Commodity and Food Standard, 2005). Raw milk having microorganisms higher than 600,000 cfu/ml is considered to have a lower microbiological quality than the standard requirement or poor microbiological quality.

Analysis of standard plate count (sometimes referred to as total plate count, total bacterial count, or bacterial count), which reflects general microbial quality, is routinely performed in the dairy production chain (Mendonca et al., 2014). Nevertheless, the time taken to obtain the results for the standard plate count method is usually long, up to 48 hours (Rosmini et al., 2004, Duncan et al., 2016). Therefore, dye-reduction assays have been used in the dairy industry to indirectly evaluate milk microbiological quality (Braide et al., 2015). One of the most common methods based on the dye-reduction principle is the resazurin test. In this test, resazurin, a dye indicator for oxidation-reduction potential, was used. The color that changes after testing a milk sample, which correlates with the number of microorganisms in raw milk, is used to classify milk quality.

The principle of a dye reduction assay is the reduction of dye by substances in a sample, resulting in a change in the color of the dye, which is related to the metabolic rates of living cells in the sample. For the resazurin test, resazurin is reduced to resorufin and then to hydroresorufin, which turns the color from blue to colorless (Ibanez et al., 2019). A milk sample that still appears blue after the resazurin test is regarded as being of excellent quality. Milk samples that have turned mauve, pink, pale pink, or white are regarded as good, fair, poor, and bad quality, respectively (Al-shamary and Abdalali, 2011). For quality control purposes, it is important for the dairy industry or milk collection center to rapidly screen out milk that has poor microbiological quality before it enters the bulk tank or the processing lines.

Although the resazurin test is a quick (one-hour) test with great potential for screening milk quality, it can have errors due to individuals' color judgment, which can appear in a variety of shades. In addition, when there are many samples to analyze, it takes time to manually read and record the color results. Color sensors have been used in previous research for evaluating the quality of foods. Some examples include the use of sensors to screen the quality of tomatoes (Adamu and Shehu, 2018) and measure the color of flavored milk using an RGB camera-based image technique (Minz and Saini, 2021).

In this study, therefore, we aimed to develop a colorimetric system with an RGB-based color sensor for measuring the milk colors resulting from the resazurin test and for screening out milk with poor microbiological quality.

MATERIALS AND METHODS

Designing of colorimetric device

A box with a light-tight lid was designed using the program "360 Fusion" and printed using a 3D printer. The box was built to accommodate a six-well microplate, which was used to contain the sample for color measurement. An RGB color sensor VEML6040 was assembled into the lid of the box, with a white-light LED lamp installed next to the sensor as a light source. The sensor that is attached to the lid could be moved to the position above each well in the six-well microplate when the color measurement was taken.

Software Development

A software program to command the sensor was developed using an Arduino IDE program based on the RGB system. The original raw data obtained from the readings of red, green, and blue by the VEML6040 sensor was converted by the coding to percentages of red, green, and blue (%RGB), which, when combined, made up 100%. The reading was taken every second, 10 times for each sample.

Preparation of raw milk samples with different levels of microorganisms

Raw milk samples were collected from milk collection centers of different dairy cooperatives in Chiang Mai, Thailand. The samples were collected aseptically in a 1000-ml bottle and kept in an ice box while being transported to the laboratory for analysis. The original raw milk sample from each source was divided into subsamples with different levels of microorganisms. To prepare a set of subsamples for each original milk sample, a 30-ml portion of each sample was transferred to a 50-ml conical tube for 8–10 tubes in total. Each tube was then incubated at 37 °C in the water bath. The first tube was collected immediately (0 h, or identical to an original milk sample). The rest of the tubes were collected at the end of each hour. Plate counting was performed on each sample using the dilution-spread plate technique on Plate Count Agar (PCA).

Resazurin test

The resazurin solution was prepared in sterile distilled water at a concentration of 0.005% (w/v). The solution was kept from light and oxygen, stored at 4 °C, and used within 2–3 days. Each milk sample and the subsamples were subjected to the resazurin test in the traditional test tube and microtube configurations.

The traditional resazurin test was performed in glass test tubes according to the protocol described by Harrigan (1998) with a ratio of resazurin solution to the milk of 1:10. First, a 10-ml portion of a raw milk sample was transferred to a test tube. After that, one milliliter of the resazurin solution was added to the milk, and the tube was tightly closed and inverted twice. The tube was incubated for 1 h in a water bath at 37 °C. The modified resazurin test in the 1.5-ml microtube configuration was performed using the same ratio of resazurin solution to milk, but with a reduced volume of 1 ml. The milk-resazurin content was mixed by inverting the microtube 2–3 times, and the microtube was incubated for 1 h in the water bath set at 37 °C. For each replicate of the milk sample, the resazurin test was carried out in five microtubes. The resazurin test for each sample or subsample was carried out in triplicates.

Measuring color values from resazurin test using color sensor device

The results of the resazurin tests performed in two different configurations were read using the color sensor device installed with sensor VEML6040. After 1 h incubation of the milk samples subjected to the resazurin test, the contents of the test tubes and the microtubes were transferred into the wells of the six-well microplate. For the test performed in the test tubes, 5 ml of each replicate was transferred to each well. For the test performed in microtubes, the contents of 5 tubes (1 ml per tube) from each replicate were transferred to a well. The microplate was placed in the box of the color sensor device for color measurement. The digital data of the colors red, green, and blue were converted to percentages of red, green, and blue (%RGB), as described above.

Enumeration of microorganisms in raw milk samples.

To enumerate microorganisms in raw milk samples, a plate count was performed using the drop plating method on Plate Count Agar (PCA). Ten-fold serial dilution was carried out by diluting the raw milk samples in 0.1% (w/v) peptone water (mostly, dilutions 10^{-2} to 10^{-7} were used). Then, in triplicate, a 10-µl portion of each dilution was dropped on PCA. The plates were incubated for 48 h at 32 °C (De Silva et al., 2016)., and the average numbers of colonies from an appropriate dilution (dilution giving approximately 5–50 colonies) were calculated into colony-forming units per milliliter of milk.

Data analysis

The data from the sensors' digital colors were presented as mean values. The average numbers of microorganisms were converted to log colony-forming units per milliliter of milk. The linear regression model and the coefficient of determination (R^2) were generated using Microsoft Excel.

RESULTS AND DISCUSSION

Resazurin test color as observed by eye and color sensor

In this study, a color sensor device embedded with the VEML6040 color sensor was made. The device was used to measure the color resulting from the resazurin test of raw milk samples and subsamples from three different sources, which contained different levels of microorganisms.

After transferring the content in the test tubes and microtubes to the six-well microplate in order to read the RGB values, it was observed that each color that appeared to the eye corresponded to a wide range of digital color values of red, green, and blue, as well as a wide range of numbers of microorganisms (Table 1). This confirmed that the color sensor had a better capacity than the human eye to differentiate the colors. It also pointed out the limitations of manual color reading to accurately judge the microbiological quality of milk.

Table 1. Ranges of digital values of colors read by VEML6040 in relation to colors observed by eye resulted from the resazurin test performed using the traditional test tube and microtube formats

Config-uration	Value measured	Color observed by the eye				
		Blue	Mauve	Pink	Pale pink	White
Test tube	%R	33.25 – 36.36	34.27 – 39.73	37.40 – 38.54	36.31 -39.22	34.50 -38.61
	%G	36-76 – 39.84	35.37 – 39.41	36.88 – 37.57	35.96 – 39.26	36.52 – 42.70
	%B	24.21 – 27.98	24.90 – 26.95	24.57 – 25.04	23.50 – 26.19	21.85 – 24.87
Microtube	%R	33.17 – 36.69	34.67 – 39.00	36.51 – 38.60	36.09 – 39.07	34.50 – 39.06
	%G	37.75 – 39.71	36.08 – 39.16	36.70 – 37.85	36.18 – 39.15	36.01 – 42.27
	%B	25.32 – 27.53	24.92 – 26.80	24.53 – 25.63	23.49 – 26.20	21.89 – 24.93
MO (log cfu/ml)		5.08 – 6.60	5.03 - 7.20	6.28 – 7.60	7.19 – 8.61	7.11 – 9.90

MO: microorganisms

Colorimetric analysis of resazurin test

The digital values of the blue color (%B), measured by the color sensor device, showed a reverse correlation with the numbers of microorganisms, whereas those of the red and green colors (%R and %G) had inconsistent correlations with the numbers of microorganisms in raw milk. When the levels of microorganisms were approximately 7 to 8 log cfu/ml, a turning point in the red and green curves was observed (Figure 1) for every set of samples tested, regardless of the sources. The same pattern was observed for the resazurin test performed in both configurations (test tube and microtube), and the %R, %G, and %B for the two configurations also showed similar trends (Figure 1). This indicated that it is possible to perform the resazurin test in the microtube configuration.

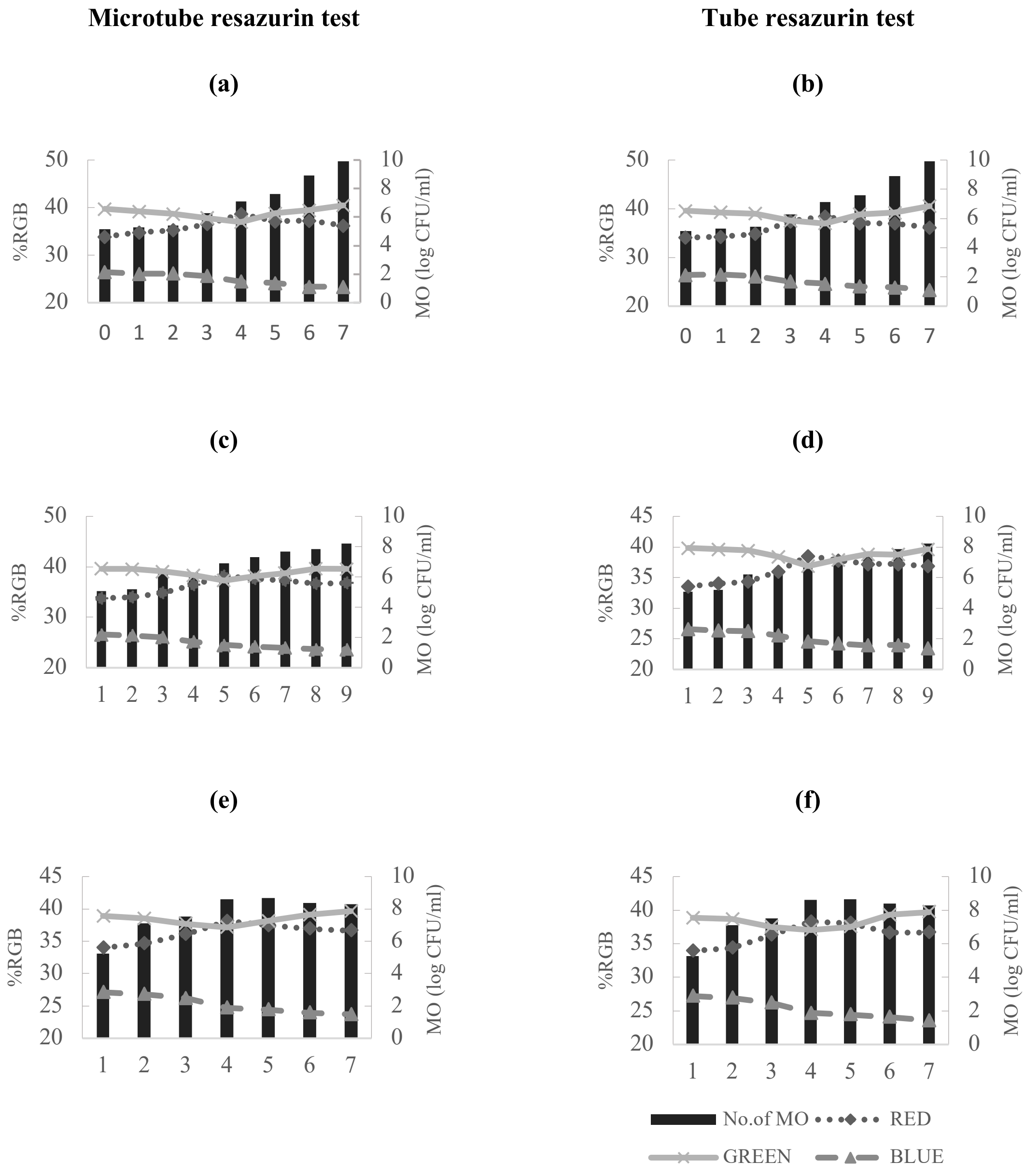

Figure 1. Results from the resazurin test carried out in the microtube and tube configurations. The digital color values of red, green, and blue (displayed in percentages; %RGB) measured using a color sensor device embedded with VEML6040 are presented together with the log number of microorganisms (MO) per milliliter. The results are presented by the source of raw milk: (a) and (b): Cooperative S, (c) and (d): cooperative W, (e) and (f): cooperative O.

Correlations between blue color values and numbers of microorganisms

As observed from the previous experiment, the blue color values obtained by the color sensor showed a reverse correlation to the number of microorganisms in raw milk. This correlation pattern was observed in milk sample sets from all three sources (three cooperatives). When plotting the digital blue values and the numbers of microorganisms using the combined data from all sources, a linear regression pattern can be seen for both microtubes and test tube configurations (R^2 of 0.8459 and 0.7313, respectively). As discussed above, it seemed to be possible to perform the resazurin test in the microtube configuration, which is a smaller scale, having 10 times fewer volumes of both the sample and the resazurin reagent. However, it is suggested that a correlation curve to be used for the prediction of microorganism levels or for the classification of milk quality based on resazurin reduction should be constructed from the database of the resazurin test results performed in the same configuration.

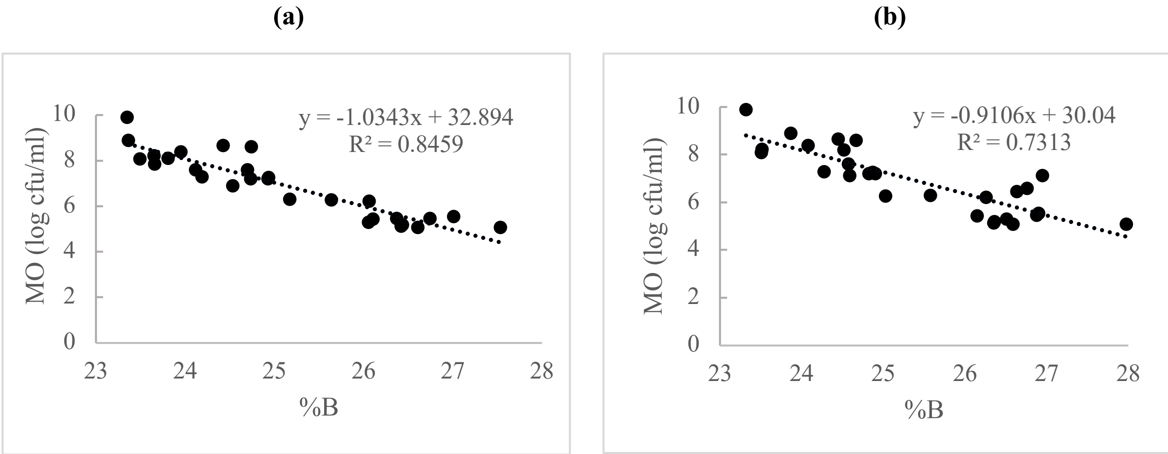

Figure 2. Correlation curves of %B values measured using the color sensor VEML6040 and the numbers of microorganisms (MO, displayed in log cfu/ml). The curves were constructed from the results obtained from the resazurin test performed in microtubes (a) and test tubes (b).

Potential application of correlations between blue values and numbers of microorganisms

The correlations between the percentages of B values from the color sensor and the numbers of microorganisms can theoretically be used to predict raw milk quality based on the level of microorganisms. With the available dataset, it is observed that when the levels of microorganisms were 7 log cfu/ml or above, the B values were almost always less than 25% (Figure 3). However, at the levels of microorganisms of 6 log cfu/ml or lower, the blue values appeared to cover a wider range.

Based on the observations above, we propose that a cut-off blue value (in this case, 25%) could be used to separate milk of low microbial quality (milk with extremely high microbial loads) from the milk of higher quality.

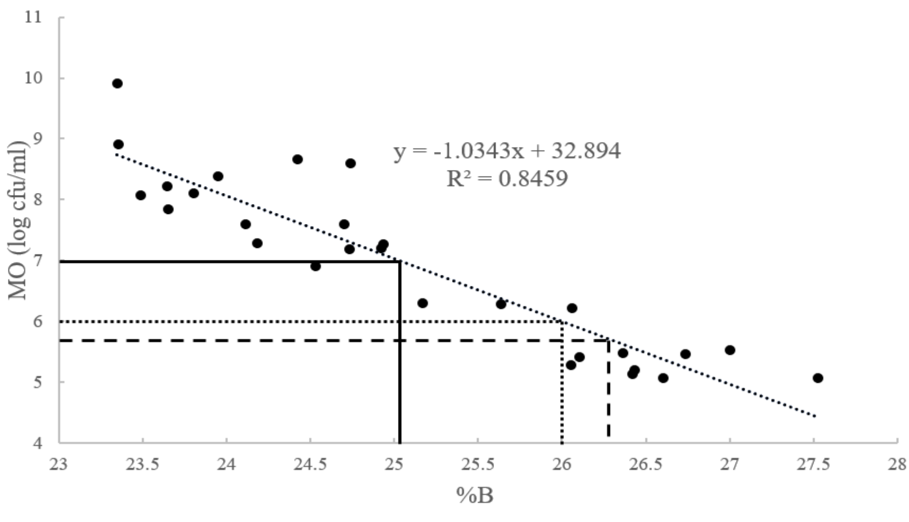

Figure 3. A demonstration of how the correlations between the blue values (%B) and the numbers of microorganisms in milk can be used. In this example, the blue value of 25% can be used as a cut-off value to separate raw milk with microbial loads of 10^7 cfu/ml (7 log cfu/ml) or higher from milk of higher quality (with lower microbial loads).

Color sensors are increasingly used to evaluate food quality. In this study, it is shown that an RGB color sensor could be used to measure the color resulting from the resazurin reduction test. This test is routinely used to evaluate raw milk quality entering the processing plants (O'Grady et al., 2020), and having a color sensor device as an alternative to the traditional manual reading would be of great benefit to the dairy industry. Researchers have long observed inconsistent correlations between the resazurin test colors and the numbers of microorganisms, which can be caused by the temperature of raw milk, the amount of cream, and the types of microorganisms in milk (Quigley et al., 2013). In this study, although with a limited amount of raw milk samples, we interestingly observed a reverse correlation between the blue values measured by the RGB-based VEML6040 color sensor and the numbers of microorganisms. Such correlation was consistent for the resazurin test results obtained in both a traditional glass test tube and a modified microtube. A linear regression model derived from this correlation would be particularly useful for the dairy industry and the milk collection units to improve the overall quality of bulk tank raw milk. It can also prevent milk with unacceptably high microbial loads from entering dairy processing plants. However, more samples are needed to construct an accurate predictive model. This point should be considered in future research work.

CONCLUSIONS

In this study, a color sensor device based on an RGB system for measuring the color resulting from the resazurin test was developed. The device was embedded with VEML6040, an RGB-based color sensor, and operated using an Arduino microcontroller. It was used to measure the color from the resazurin test of raw milk samples from three sources with different levels of microorganisms. The results showed that there is a reverse correlation between the blue values (expressed in percentages) and the numbers of microorganisms, both with the resazurin test performed in test tubes or in reduced-scale microtubes. The plot between the percentages of blue values from the resazurin test performed in microtubes and the numbers of microorganisms showed a linear regression pattern with an R^2 of approximately 0.85. This suggests that it is possible to use the blue values from the color sensor device to predict or screen milk quality. With the present limited dataset, the blue value of 25% could be used as a cut-off value to separate raw milk with microbial loads of 10^7 cfu/ml (7 log cfu/ml) or higher from the milk of better quality (milk with lower microbial loads). However, to increase the accuracy of prediction, more samples were needed to construct a linear regression model, and the color sensor may need to be further refined.

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