Optical Characteristic Properties of Probiotic Drink Using Spectrophotometer UV/Vis and FTIR

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Abstract. This research aims to qualitively observe the light absorbtion response from probiotic drink to several spectra of electromagnetic waves. This absorption response will later be related to physical properties of probiotic drink. The Observations in the infrared region used Bruker ATR Alpha FTIR Spectrometer while the observations in the UV and visible light region used Thermo Scientific Evolution 220 UV-Visible Spectrophotometer. The measuring time was done until there is a change in probiotic drink absorption. The measurement results obtained were in the form of the transmittance spectrum of the wavelength. Measurements in the infrared region showed some probiotic drink content such as protein at ~6123 nm, lactose at ~9532 nm, and glucose at wave number ~1100-800 cm⁻¹. In the UV region, it is related to the reduction amount of protein while visible light region was associated with changes in the constituent particles of probiotic drink along with changes in pH. All observations refer to the literature. The results of this observation certainly do not represent all the contents of probiotic drink but this method can also be utilized for freshness detection of other similar drinks that contain milk.

1. Introduction
Lately, the tendency of people to maintain a healthy diet continues to increase. A Healthy diet is believed to be able to prolong human life and affect the beauty and fitness of the human body itself. Milk is one of the foods that are more people likely to consume due to the tendency of a healthy diet. The increasing number of interests in dairy product, and the necessary on milk can be seen from the fact in the last three decades, that world milk production has increased by more than 50%, from 482 million tons in 1982 to 831 million tons in 2017 [1-2]. The increasing demand for milk, the biggest challenge that must be faced by the dairy industry is milk processing because basically fresh or good milk for consumption has a relatively short shelf life [3].

Milk that is popular and widely consumed is cow's milk because the population of dairy cows is relatively high and each cow can produce milk from 7-20 l/day [4]. Fresh cow's milk (raw) generally consists of water (85-88,7%), protein (2,3-4,4%), fat (2,4-5,5%), minerals (0,53-0,8%), carbohydrates (lactose) (3,8-5,3%) and organic acids (0,13-0,22%) [3,5-6]. The high nutrient content in milk provides microorganism media such as pathogenic bacteria to grow. Therefore milk becomes easily...
damaged and causes various diseases for consumption [7]. Efforts to reduce the presence of microbial contamination and to extend the time to store milk are to preserve the milk [8].

The preservation process in milk was done by adding other microbes to the regular fermentation cultures provide therapeutic benefits such as modification of the immune system, reduction in cholesterol, alleviation from lactose intolerance, faster relief from diarrhea, and restoration of a healthy vaginal microbiota [9-11]. One of the fermented milk products consumed by the consumer in Indonesia is the probiotic drink from Japan.

Probiotic drink must be stored at room temperature 0C-10C because in these conditions probiotic drink bacteria are not active so that the quality of probiotic drink can be maintained. Storage above 10C will result in a decrease in the quality of probiotic drink because the probiotic drink bacteria are active and produce lactic acid which causes probiotic drink to become acidic and the number of live bacteria will decrease [12]. The degradation in the quality of probiotic drink should have an impact on its physical properties. One of the physical properties that will be observed in this research is the light absorption properties of probiotic drink.

At present, there are several studies developed on milk monitoring records using optical properties of milk to determine the level of freshness and quality of milk [13-15]. Most of these studies do observations that tend to be microscopic and specific from one of the ingredients found in milk, for example, only observe protein, fat or lactose. In this research, probiotic drink is characterized using Spectrophotometer UV/Vis and Fourier Transform Infrared (FTIR). The aim of this study is to see the light response to the optical properties and observe the degradation of probiotic drink. This research will be limited to macroscopic observations and the sample used is only probiotic drink, it means that the analysis of the sample does not tend to one of the probiotic drink contents.

2. Material and Method

2.1. Probiotic Drink Sample

Before the experiment, probiotic drinks of the same brand and the same expiration period were collected from market. In SpectoVis Plus Vernier measurement, probiotic drinks are first diluted with a volume ratio of 1:1. Then a probiotic drink is put into a 3.5ml open cuvette with dimensions of 12.5mm x 10mm x 45mm. The aim of the sample treatment is for light to penetrate the sample. The measurements are performed every 10 hours for 20 hours. In the UV/Vis Spectrometer measurement, probiotic drinks are not diluted, because the cuvette used is very thin (with a volume of 0.35ml) and the measurement is performed at a certain interval of time for 8 days. Finally, the Fourier Transform Infrared Spectroscopy (FTIR) analysis of probiotic drink was performed on a drop of the sample (volume less than 1ml) and the measurement interval was the same as the UV/Vis Spectrometer.

2.2. Characterization

2.2.1. Transmittance and Fluorescence Methods

Probiotic drink recording using the transmittance and fluorescence methods performed using SpectroVis Plus Vernier. The spectroVis setup system is shown in Fig.1. SpectroVis employs an LED and tungsten bulb to transmit light through a solution containing the sample. The transmitted light then passes through a high-quality diffraction grating and the diffracted light is sorted and collected by the linear CCD array detector [28]. SpectroVis is directly connected to LabQuest or a computer USB port. The data recording from SpektroVis plus is shown in software Logger Pro 3.14.1.
2.2.2. **Diffuse Transmittance Method**

The recording of fermented milk was performed using a single beam type spectrophotometer with diffuse transmittance method or diffusion transmittance. Measurements of transmittance are measured accurately by conventional methods by collecting and integrating scattered light using the integrating sphere ISA-220. The arrangement of accessories needed on the Thermo Scientific Evolution 220 UV/Visible Spectrophotometer is shown in Figure 2.

2.2.3. **Reflectance Method**

Reflection method is performed by using Bruker ATR Alpha FTIR Spectrometer. Fourier Transform Infrared (FTIR) spectrometer is a characterization device used to determine the functional groups and chemical bond structures in a material. The resulting spectrum shows absorption and molecular transmission that are unique for each type of material because each different functional group has a different vibrational energy. Generally, the infrared radiation area is divided into near IR regions (14290-4000 cm\(^{-1}\)), far IR (700-200 cm\(^{-1}\)), and middle-IR (4000-666 cm\(^{-1}\)). The areas that are most used for the purposes of limited investigation are in the middle-IR region.

3. **Results**

The spectrum results from the measurement of probiotic drinks are shown in the following figure. Each measurement spectrum shows different absorption at different wavelengths. PH measurements are performed to support the condition of probiotic drinks. In FTIR measurements, each probiotic drink at a certain time interval is labeled to facilitate analysis and identification of wave number as shown in Table 1.
Fig. 3: SpectroVis Vernier Bands; a) Transmittance vs Wavelength; b) Fluorescence vs Wavelength

Fig. 4: Spectroscopy bands; a) Visible light area; b) UV area

Figure 5: FTIR bands related to pH of probiotic drink

| Table 1. Identified bands FTIR spectra of probiotic drink |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | 0 Hours cm⁻¹     | 20 hours cm⁻¹   | 8 days cm⁻¹     | 9 days cm⁻¹     | Expired cm⁻¹   |
| 3262,81          | 3278,34          | 3279,55         | 3279,46         | 3277,57         |                 |
| 1639,16          | 1636,95          | 1638,46         | 1638,81         | 1637,29         |                 |
| 1421,71          |                 |                 |                 |                 |                 |
| 993,93           | 996,58           | 996,83          | 996,32          |                 |                 |
| 1050,28          | 1053,58          | 1053,58         | 1053,40         | 1054            |                 |
4. Discussion

4.1. Optical Characteristic Properties of Fermented Milk Using SpectroVis Plus Vernier

The measurement of probiotic drinks using SpectroVis Vernier is in the visible light region of 400nm-750nm. This measurement is an initial measurement performed to determine whether probiotic drinks have physical changes when left open at room temperature 27°C. The Graph in Figure 3a. shows that for a different time, the spectrum of probiotic drinks has a relatively similar absorption. It means that the highest transmitted light is at a wavelength of 600nm-750nm while the smallest transmitted light is at a wavelength of 400nm-500nm. According to Melenteva, in the Visible and Short-Wavelength NIR (Vis/SW-NIR) region (400-1100 nm) typically containing the significant absorption of the milk components [27]. If we saw closer to the spectrum of probiotic drinks, in 10 hours the graph is overlapped each other. After 20 hours there is a slight difference from light absorption when passing probiotic drinks. This condition is believed that probiotic drinks decrease in quality.

In addition, SpectroVis Plus Spectrophotometer can also be used to measure the fluorescence intensity of a material. SpectroVis measures fluorescence intensity using green lights with a wavelength of 500 nm and purple lights with a wavelength of 405 nm. According to McCarthy, in the area of visible light, riboflavin in milk absorbs strongly at a wavelength of 470 nm [5]. For this reason, fluorescence measurements at a wavelength of 405 nm are performed because strong absorption is at that range and changes in the physical properties of probiotic drinks can be related to previous measurements.

Figure 3b. Shows fluorescence from probiotic drinks for 20 hours. It can be seen that the fluorescence curve tends to shift to the positive x-axis which states that the longer the monitoring time the greater the wavelength. If it is connected with the fluorescence understanding by McCarthy, that fluorescence occurs when the molecule that is reconnected in its basic state and the energy $\Delta E'$ is released and converted to another form such as electricity or thermal therefore the wavelength is longer [5]. This statement is certainly compatible because the longer the light is passed by Yakult and the less light is dissipated. This shift certainly affects the physical nature of Yakult which will later be studied more deeply in the advanced experiment.

4.2. Optical Characteristic of Fermented Milk Using Spectrophotometer UV/Vis

A subsection

The measurement results using UV/Vis Spectrophotometer were not significantly different from SpectroVis Plus except that the transmission percentage of probiotic drinks that were passed tended to be higher than in measurements using SpectroVis Vernier. This is due to the measurement of probiotic drinks in the UV / Vis spectrophotometer using cuvette with a thickness of 1 mm. While the previous measurements using cuvette with a thickness of 12.5 mm. Of course, this is in accordance with the Lambert-Beer Law, the longer the light passing through the material, the less light can be transmitted but the lighter absorbed.

Judging from the size of the constituent particles of milk. The biggest milk constituent particles are fat clumps or fat globule (1-10μm), micelle proteins or casein micelles (0.05-0.25 μm), lactose, salt and minerals (0.0001-0.001 μm). In probiotic drinks, the type of milk used is SMP which is dominated by micelle casein and lactose. Thus we can interpret that the light passed by Yakult is getting bigger because the size of casein micelle is getting smaller. According to Michael et al at normal pH, casein micelles are spread evenly but at a lower pH level, the protein can no longer survive in solution, the protein will thicken because of the acid produced from fermentation [14]. A similar statement is also supported by Klaenhammer that milk experiences decay if milk is at a lower pH level, at that time lactic acid bacteria can grow and produce lactic acid [17]. In line with what was reported by Sinaga that the size of casein micelle in milk depends on pH, the lower the pH in milk, the smaller the size of casein micelle [16]
Figure 4b shows measurements at the UV wavelength. Absorption obtained does not differ greatly from absorption at the wavelengths of visible light. It's just that there is a dominant peak at a wavelength of 287 nm. Some studies that have been carried out state that there is dominant milk absorption for 280 nm wavelength [5, 26]. It is associated with temperature increases. The higher the temperature, the smaller the amount of protein contained in milk or vice versa [26]. This means that milk is in a bad condition because the amount of protein contained in milk begins to decrease so that milk decreases in quality.

4.3. Optical Characteristic Properties of Fermented Using FTIR
Figure 5 shows the spectrum of FTIR measurements for probiotic drinks. The curve shows no wavelength that shifts during the measurement of probiotic drinks. However, the sharpness of the absorption peaks obtained varies and causes some peaks to be unreadable. When associated with the literature this observation is similar to that reported by [21-22 in 25] which states that the characteristic band for α-lactose appears at wave number 920 cm\(^{-1}\), for β-lactose at 950 cm\(^{-1}\) and 833 cm\(^{-1}\). Then the characteristic bands found in the spectrum are 800-1000 cm\(^{-1}\) for carbohydrates, and bands at 3200 cm\(^{-1}\) for stretching between molecules [24]. The presence of the C = O group was confirmed to be at 1700 cm\(^{-1}\). In addition, it was also observed the characteristics of stretching and bending of the glycosidic bond COC (the bond that connects the two groups of sugars) in the spectrum (~1100 and ~800 cm\(^{-1}\)) [24]. The content of the probiotic drink further has a strong absorption in the amide (II) group (CONH) of the protein at ~6465 nm, the hydroxyl (OH) group of lactose at ~9610 nm and the carbonyl ester group (CTO) from lipids at ~5723 nm.

From this guide, Figure 5 shows that the content of probiotic drinks observed is in the intended range of the literature, namely at wave numbers ~1632 cm\(^{-1}\) or at ~1632 nm, which means the protein in the probiotic drink also contains lactose in wave number 1049 cm\(^{-1}\) or at a wavelength of ~9532 nm. However, there is no dominant absorption at a wavelength of ~5723 nm. This means that there is no lipid in the content of probiotic drinks, or the amount is too small so that it does not show the dominant absorption. There is also glucose at 925 cm\(^{-1}\) and 996 cm\(^{-1}\). From here, the main constituents of probiotic drinks consist of lactose, protein, and glucose [12]. Furthermore, Table 1 explains that when the condition of probiotic drinks decreases in quality, there are some ingredients that are lost, especially glucose. Because one indicator of damage to probiotic drinks is the sour taste caused by lactic acid. So that it removes sweetness from the sugar.

5. Conclusions
Analysis has been performed against various lengths with a probiotic drink of electromagnetic waves. An analysis of probiotic drinks with several electromagnetic wavelengths has been performed. In the visible light region, there is no dominant absorption at a certain wavelength but the longer the measurement, the more light is transmitted. This change is due to a particle size such as casein micelle shrinking because the pH decreases. At the UV region, there is a dominant absorption at a wavelength of 287 nm. In the Infrared region, there is absorption at ~1632 cm\(^{-1}\) wave number related to protein, ~1049 cm\(^{-1}\) related to lactose or carbohydrate and ~1100-800 cm\(^{-1}\) is related to glucose. For longer measurements, this content is not all found in probiotic drinks.

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