CHAPTER 4

INVESTIGATING QUALITY OF MILK USING SPECTROMETRY AND SCATTERING THEORY

This chapter analyses the study of milk quality using spectrometry experiments and scattering theory. The whole study was carried in two parts, theoretical and experimental approaches. The theoretical part involved a computation modeling of Mie efficiency and its angular scattering using MATLAB software. On the other hand, the experimental approach was conducted using Ocean Optic Visible-Near-Infrared (VIS-NIR) and Near-Infrared (NIR) spectrometer to analyse the spectrum of reflectance, transmission, and absorbance of the samples tested.

This chapter is used to achieve the second objective; to analyse milk quality after fermentation using spectrometry technique.

Results in Chapter 4 have been published as:

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4.1 Introduction

Milk is a dairy product that contains dissolved proteins, carbohydrates, fat, and many minerals. However, milk can turn bad after being kept at room temperature for several days. The endurance of milk could depend on its fat and protein composition. In this chapter, the comparison for the quality of milk after being kept at room temperature for several days had been investigated using spectrometry technique. Modeling based on scattering theory is also provided to compare the light propagation in milk, water, and air. A VIS-NIR spectrometer was used to observe the light absorption, transmission, and reflectance whereas a modeling approach was applied to study the scattering, absorption, and extinction efficiencies. The milk samples consist of full cream milk kept at room temperature for 8 days, 11 days, 14 days, and 17 days. The results show that milk without fermentation has higher light absorbance and lower transmission compared to milk with fermentation, due to changes in milk composition after the fermentation process. Milk scatters more light compared to water and air due to its fat globule and protein ingredients. The output of this study can be used as a reference for studies involving bacteria or microorganisms in milk. It also can be used to compare the quality of milk with and without air exposure.

4.2 Theoretical Study

For the computation of Mie efficiencies, there are two input parameters which are the complex refractive index, m and the parameter size, x as shown in Equation (4.1) and (4.22) (Bakar et al., 2016).

$$m = m' + im'' \tag{4.1}$$

$$x = ka = \frac{2\pi}{\lambda} a \tag{4.2}$$

where:

m' = Real refractive index

im'' = Imaginary refractive index

k = Wave number in ambient medium

a = Sphere radius.

The key parameters of Mie theory are to compute the amplitudes of scattered field. The coefficients of $a_n$and b_n are required to obtain the Mie efficiency using Spherical Bessel function *n* (*n= 1, 2,..*) for higher order and work well in the wider range of size parameters, as shown in Equation (4.3) to (4.5) (Mätzler, 2002).

$$h_n^{(1)}(z) = j_n(z) = iy_n(z) \tag{4.3}$$

$$a_n = \frac{m^2 j_n(mx)[xj_n(x)]' - j_n(x)[mxj_n(mx)]'}{m^2 j_n(mx)\left[xh_n^{(1)}(x)\right]' - h_n^{(1)}(x)[mxj_n(mx)]'} \tag{4.4}$$

$$b_n = \frac{j_n(mx)[xj_n(x)]' - j_n(x)[mxj_n(mx)]'}{j_n(mx)\left[xh_n^{(1)}(x)\right]' - h_n^{(1)}(x)[mxj_n(mx)]'} \tag{4.5}$$

The efficiency of extinction Q_{ext} and scattering, Q_{sca} can be identified in forward-scattering theorem and in the integration of the power scattered in all directions. The absorption efficiency, Q_{abs} can be identified with the equation of energy conservation (van der Molen, 2007) whereas the backscattering efficiency, Q_b is applicable to monostatic radar (Mätzler, 2002).

$$Q_{ext} = \frac{2}{x^2}\sum_{n=1}^{\infty}(2n+1)Re({a_n}^2 + {b_n}^2) \tag{4.6}$$

$$Q_{sca} = \frac{2}{x^2}\sum_{n=1}^{\infty}(2n+1)(|a_n|^2 + |b_n|^2) \tag{4.7}$$

$$Q_{ext} = Q_{sca} + Q_{abs} \tag{4.8}$$

$$Q_b = \frac{1}{x^2}\left|\sum_{n-1}^{\infty}(2n+1)(-1)^n(a_n - b_n)\right|^2 \tag{4.9}$$

where

x=parameter size

n= Spherical Bessel function order *n* (*n*= *1, 2,…*)

The efficiency of radiation pressure can be proven by the Two-Stream Models and correlates with the asymmetry parameter (Meador & Weaver, 1980).

$$Q_{pr} = Q_{ext} + Q_{sca}(cos\theta) \tag{4.10}$$

where $cos\theta$ = scattering angle.

Amplitude function S_1 and S_2 indicate the scattering properties or the scattering of an electromagnetic wave from spherical particle. The scattering function are required for the scatterer far field (van der Molen, 2007).

$$S_1(cos\theta) = \sum_{n=1}^{\infty}\frac{2n+1}{n(n+1)}(a_n\pi_n + b_n\tau_n) \tag{4.11}$$

$$S_2(cos\theta) = \sum_{n=1}^{\infty} \frac{2n+1}{n(n+1)} (a_n\tau_n + b_n\pi_n) \tag{4.12}$$

Where $\pi_n = \frac{2n-1}{n-1} cos\theta \, . \, \pi_{n-1} - \frac{n}{n-1}\pi_{n-2}$ (4.13)

$$\tau_n = ncos\theta . \pi_n - (n+1)\, \pi_{n-1} \tag{4.14}$$

4.3 Research Methodology

The study was done using experimental and theoretical method. The analysis of light absorption and scattering in milk were done based on Mie scattering theory. The scattering, absorption, extinction, and backscattering efficiencies are analysed in a homogeneous dielectric sphere and its angular scattering using MATLAB. The analysis was also repeated for water and air.

4.3.1 Theoretical Approach

The theoretical part was used to determine the characteristics of light in disordered medium with a Monte Carlo model using MATLAB software. The light propagation efficiency with the justification of Mie coefficient matrix was computed. Additionally, the angular functions were also computed to produce the Mie angular efficiency**.** Figure 4.1 shows the flow chart of the theoretical approach based on theory in section 4.2.

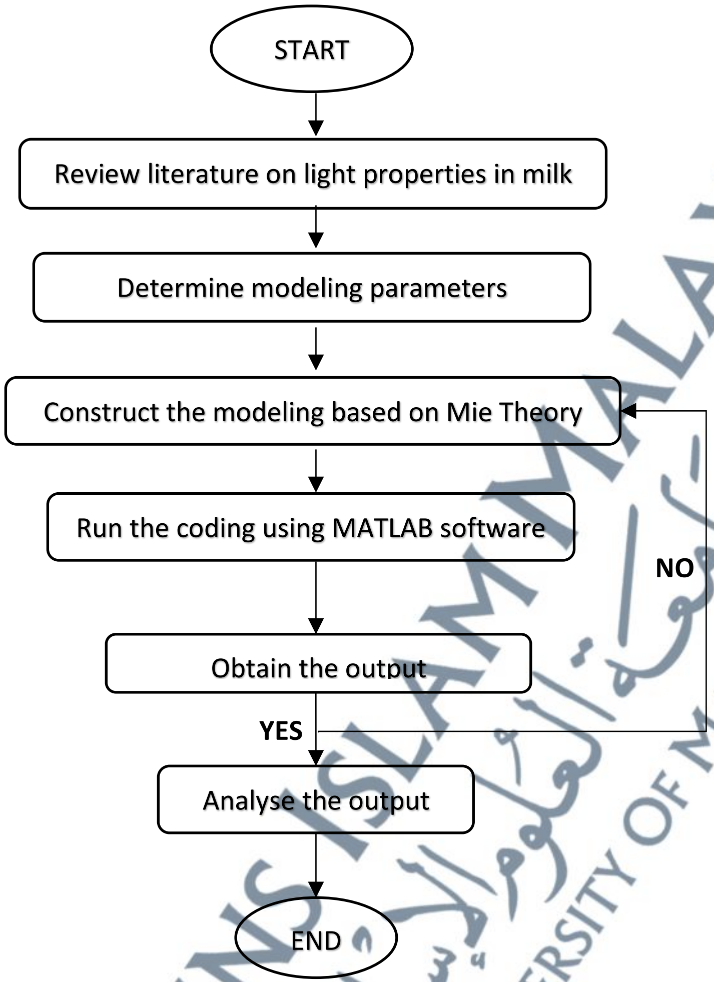


Figure 4.1 : Flow Chart Of The Theoretical Approach.

4.3.2 Experimental Approach

The Ocean Optic Flame NIR spectrometer and VIS-NIR spectrometer were used to observe the characteristics of light propagation in milk. Every experiment was repeated 10 times to ensure the accuracy of the output. Five samples of milk with different days of exposure were used where the sample turn to yogurt after 14 days of air exposure. Figure 4.2 shows the flow chart of the experimental method.

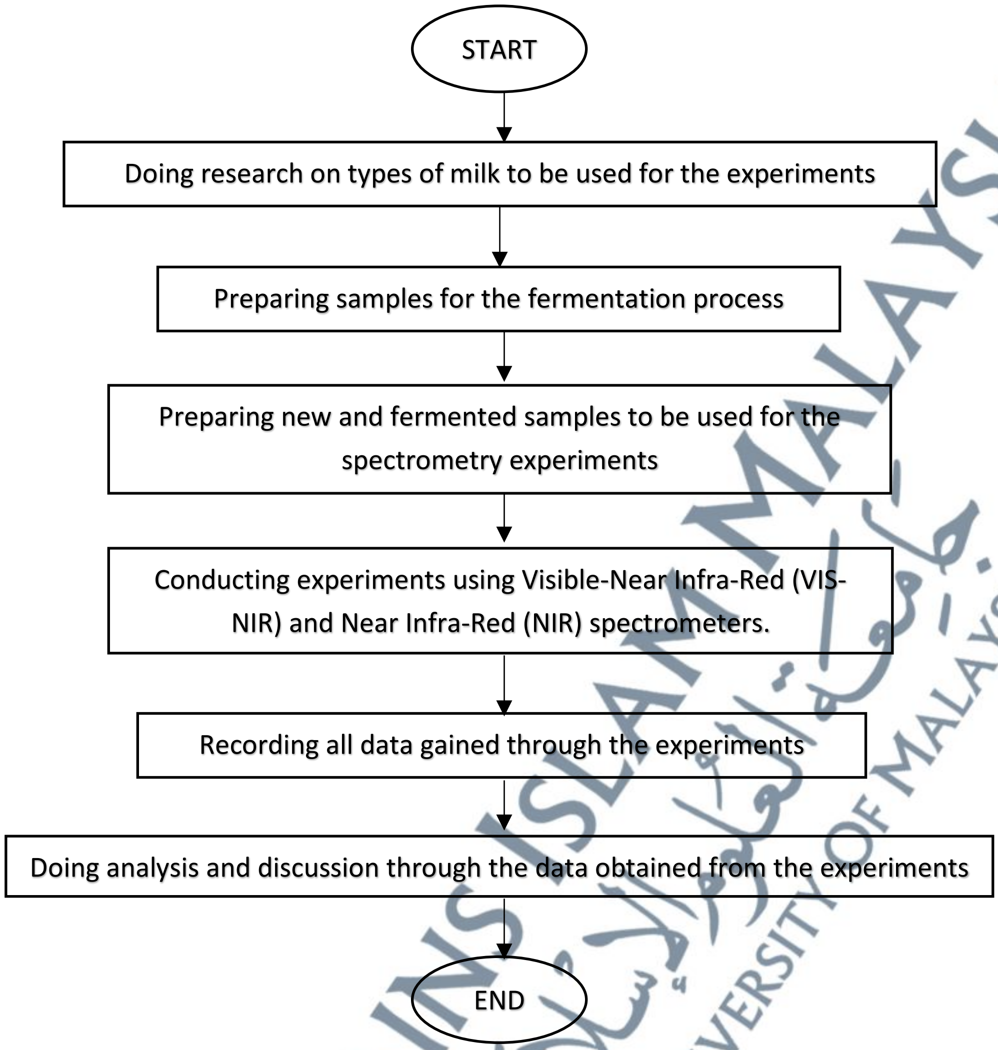


Figure 4.2 : Flow Chart Of The Experiment Approach.

4.3.2.1 Experimental Set-Up

Two spectrometers with different wavelength ranges were used to observe the light propagation in milk: Ocean Optic Flame Near-Infrared (NIR) spectrometer and VIS-NIR spectrometer. The spectrometers are small and do not have any detachable parts which contribute to easy handling, portable and compact. The VIS-NIR spectrometer can capture the wavelength of light from 350nm to 1000nm whereas the NIR spectrometer can capture the wavelength from 950nm to 1650nm. The experiments were repeated 10 to 15 times for each sample in order to observe the stability and reliability of the results. Table 4.1 shows the apparatus needed for spectrometry experiments.

Table 4.1: The Apparatus Used for Spectrometry Experiments.

NO	Apparatus	Application
1.	Tungsten Halogen Light Source HL-2000-HP-FHSA	Halogen light source with wavelength range from 360nm until 2400nm.
2.	Fiber Optic Cables QP450-2-XSR	Two fibre optics cables were used to connect the tungsten light source to the cuvette holder and the cuvette holder to the spectrometer.
3.	Cuvette (Quartz and Plastic)	Plastic cuvette and quartz cuvette were used to place sample to measure the light absorbance and fluorescence respectively.
4.	CUV 1cm Cuvette Holder and Cuvette Cover	The samples were placed inside the cuvette holder by using a cuvette (diameter~1 cm)
5.	Flame-T-VIS-NIR & Flame-NIR+ Spectrometer	Two spectrometers which were used in the experiments; VIS-NIR spectrometer (wavelength from 350nm-1000nm) and NIR spectrometer (from 950nm-1650nm).
6.	Ocean View 2.0 Software	Software used for analysing absorbance and fluorescence of light intensity spectrum.

Figure 4.3 shows the VIS-NIR and NIR spectrometers used in the spectrometry experiments whereas figure 4.4 shows the apparatus used in the spectrometry experiments.

Figure 4.3: The VIS-NIR And NIR Spectrometers.

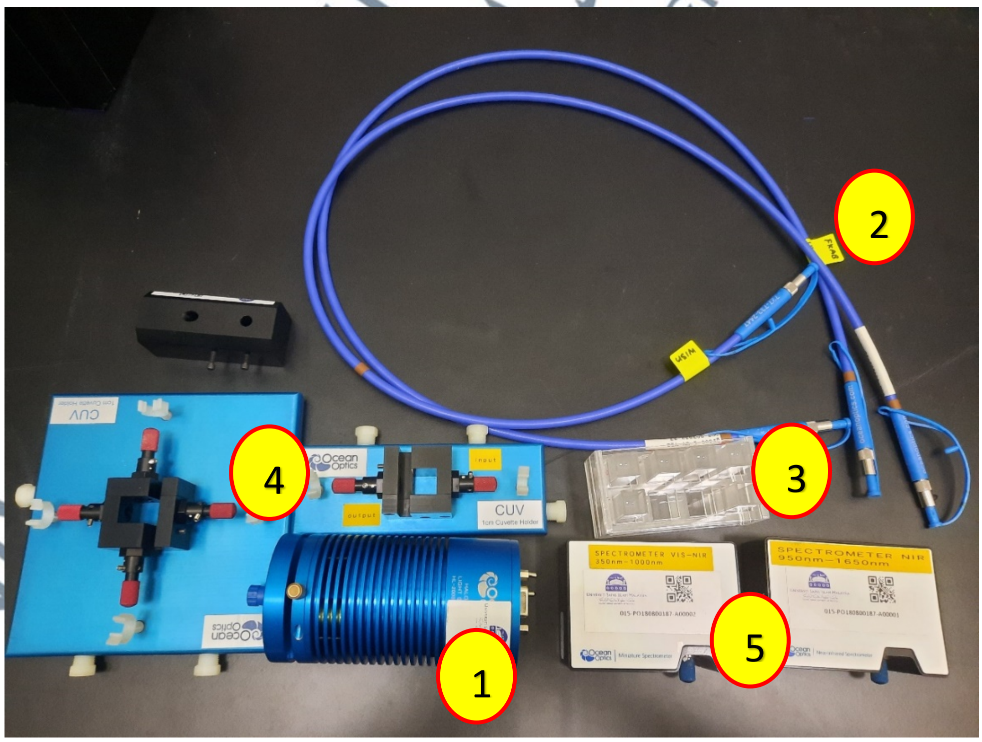


Figure 4.4: The Apparatus Used for Spectrometry Experiments.

Meanwhile, the whole experimental set-up for investigating the absorbance and transmission spectrum is shown in figure 4.5.

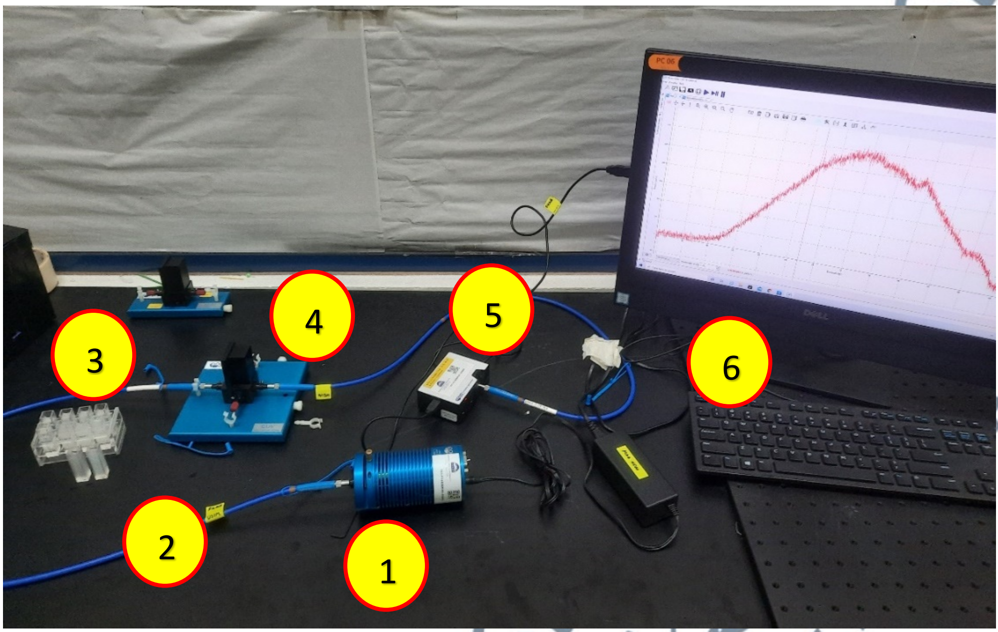


Figure 4.5: The Experimental Set-Up Used for Spectrometry Experiments.

On the other hand, the set-up for reflectance spectrum is different from the absorbance and transmission spectrum setup. NIR spectrometer was used to observe the reflectance intensity spectrum of the samples. The sample was placed on the stage RTL-T. The reflectance spectrum of the sample was observed by placing the fiber probe. The probe transmits the light from the halogen light and then the probe was placed on the stage RTL-T. The reflectance spectrum can be observed using NIR spectrometer when the light propagates inside the medium. Figure 4.6 shows the experimental set-up for the reflectrance spectrometry.

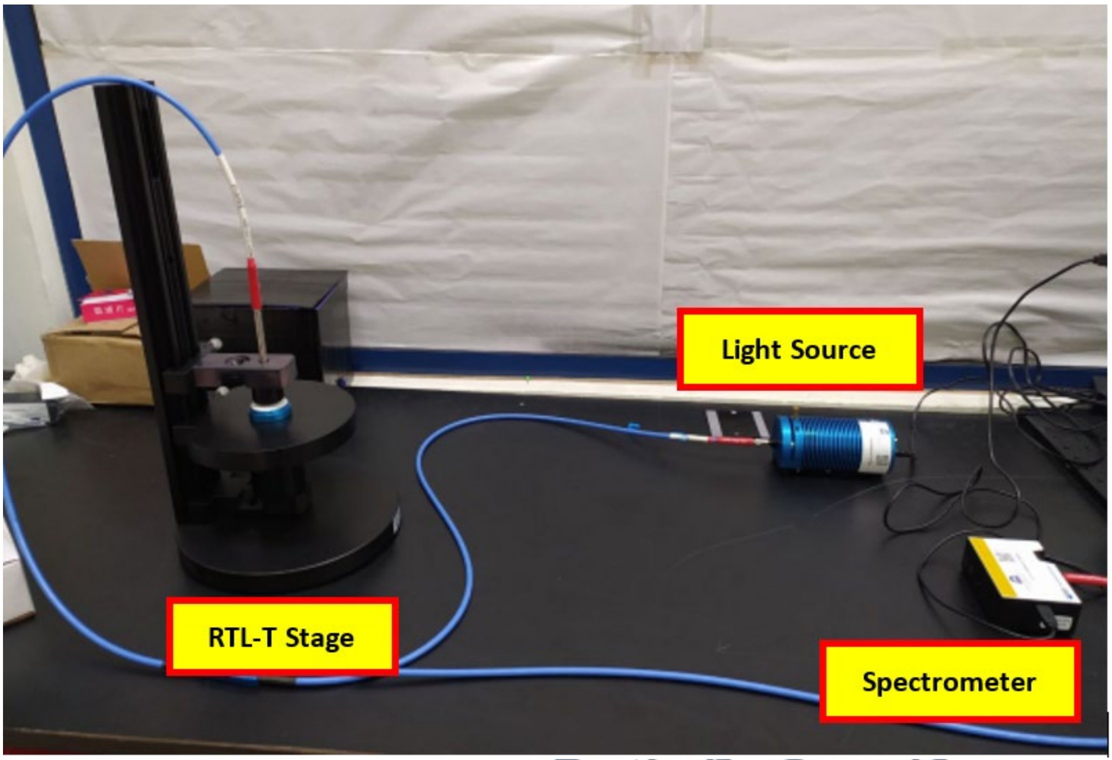


Figure 4.6: Experimental Set-Up For Reflectance Spectrometry.

4.3.2.2 Ocean Optic Software

Ocean View software is used to acquire and simulate the data captured from the spectrometers. The software allows the user to select the experimental analysis as shown in figure 4.7.

Figure 4.7: Application Wizard In Ocean View Software.

4.3.2.3 Sample Preparations

The milk samples were kept in room temperature for 8 days, 11 days, 14 days, and 17 days. The samples were diluted with a ratio of 1:100 for milk and water, respectively. The prepared samples were placed in a cuvette of 10 mm and shaken lightly. Each samples were placed into 10 mm plastic cuvette. The cuvette was placed into the cuvette holder and was illuminated by a halogen lamp as the light source. The light from the cuvette was collected by VIS-NIR spectrometer (as shown in Figure 4.5). The spectrum graph was displayed on the computer using OceanView software. Figure 4.8 shows the diluted milk samples used in the experiments while figure 4.9 shows the cuvette in a cuvette holder. Milk samples for 8 and 11 days are still cloudy but milk samples after 14 days become transparent with large milk particles are observed.

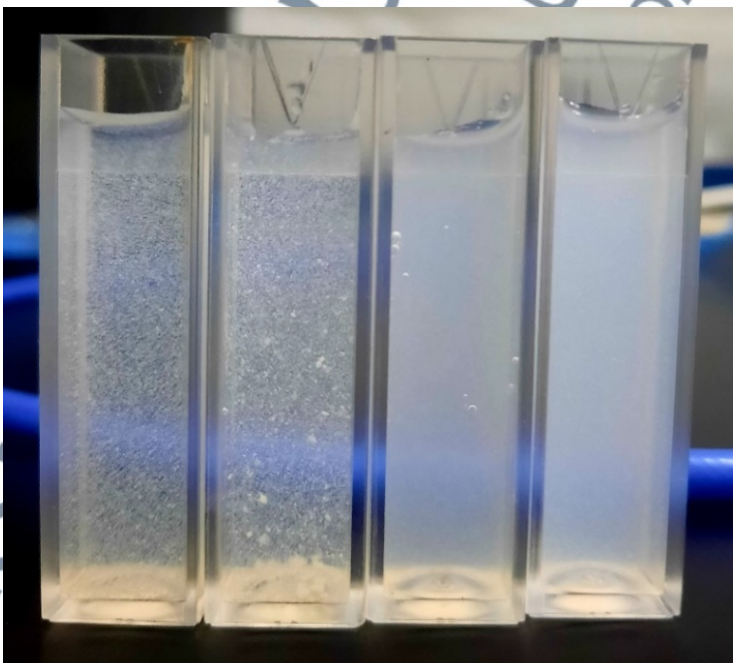


Figure 4.8: Fermented Full Cream Milk Samples After Dilution With Water For 8 Days, 11 Days, 14 Days, And 17 Days (From Right).

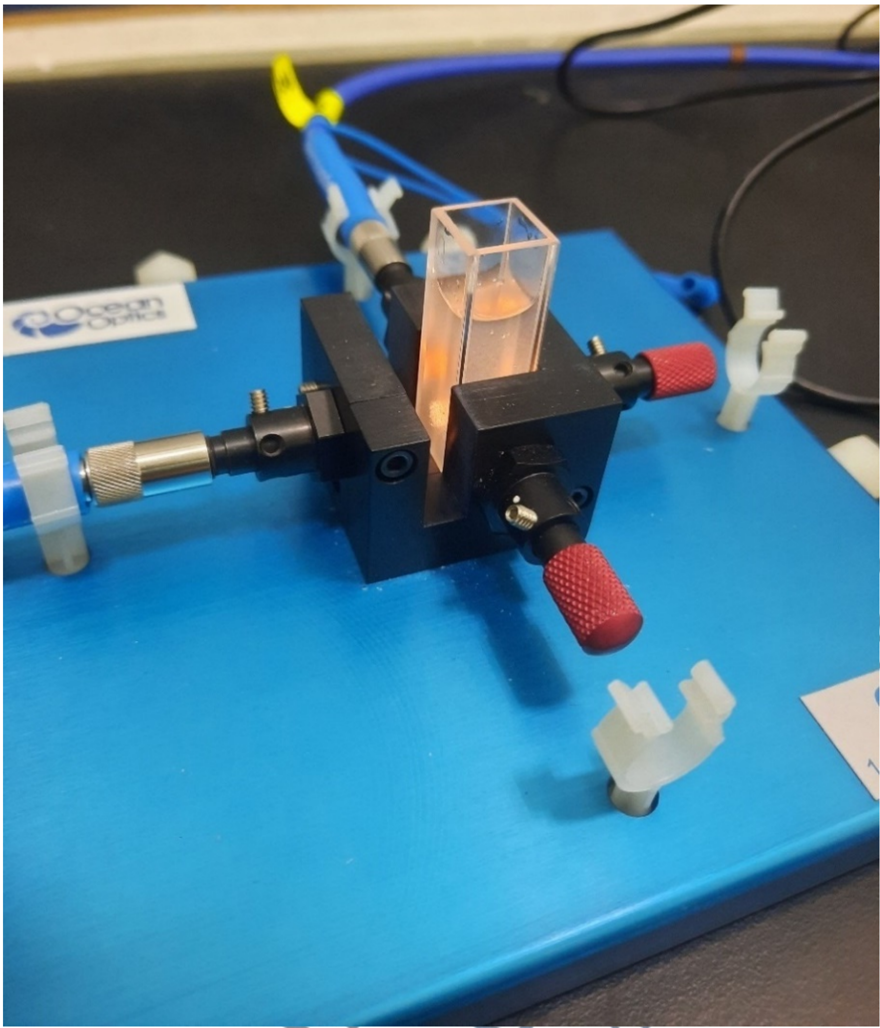


Figure 4.9: The Cuvette Was Placed In The Cuvette Holder For The Spectrometry Experiments.

4.4 Results and Discussion

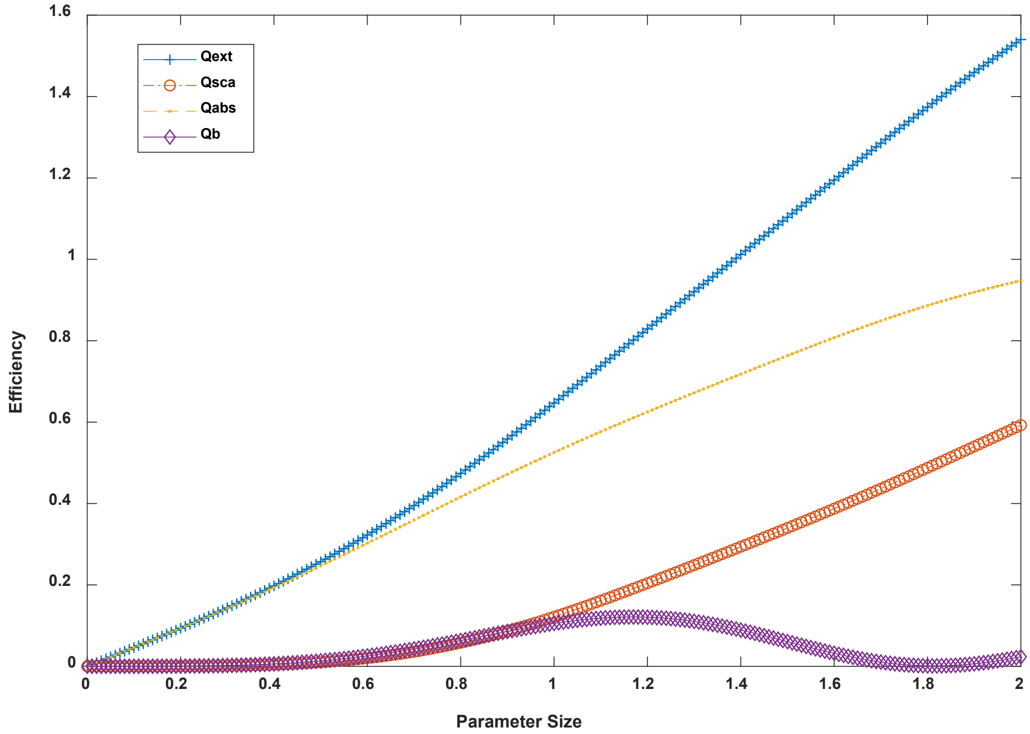
Previous works (Katsumata et al., 2020b) (Montemurro et al., 2019b) have analysed fat and protein concentrations of milk through backscattering and spectrometry. (Ransmark et al., 2019) studied the homogenization efficiency to control the development of cream layer on fresh milk and Aljaafreh et al. (Aljaafreh, 2015a) used NIR spectrometry to study yogurt fermentation automation. Meanwhile, we studied the quality of milk which were kept in the room temperature using VIS and NIR spectrometers. We did a simple modeling to compare the light propagation in milk, water and air. The modeling analysis uses Mie theory to compute the efficiency of scattering, absorption, extinction, backscattering, asymmetry parameter and radiation pressure whereas the experimental section shows the output in terms of absorbance, transmission and reflectance. The output from the theoretical and experimental studies were analysed and discussed thoroughly in this section.

4.4.1 Modeling based on Mie Scattering Theory

The measurement of scattering, extinction and absorption efficiency based on Mie theory were done using MATLAB Software. The important input parameters are complex refractive index and the parameter size, x (Ren et al., 2017). The modeling was done for milk, water, and air. Figures 4.10 and 4.11 summarize the modeling results, where Equation (4.3) to (4.7) were used to plot graph in Figure 4.10. Figure 4.11 is plotted based on Equation (4.8) to (4.9). The extinction, forward scattering, absorption and backscattering efficiencies are represented by Q_{ext}, Q_{sca}, Q_{abs} and Q_b respectively.

Figure 4.10 shows clearly that milk has better scattering efficiency compared to water and air. At parameter size 2, the scattering efficiency in milk (Figure 4.10(a)) reaches 0.6, while the scattering efficiency in water (Figure 4.10 (b)) and scattering efficiency in air (Figure 4.10(c)) are 0.7 and 5×10^{-7} respectively. It proves that the least light scattering occurs in the air. Meanwhile, milk and water consist of particles which can scatter the light. We presume that the light scattering and absorption are affected by the size and concentration of the particles, the incident light wavelength and sample size (Dahm, 2013). Milk depicts the highest efficiency of light absorption compared to water and air due to composition of fat globules and protein. Figure 4.10 also shows that the forward scattering is more efficient compared to the backscattering for all samples due to larger particles size of the samples. Figure 4.11 shows the scattering angle of milk, water and air respectively.

(a)



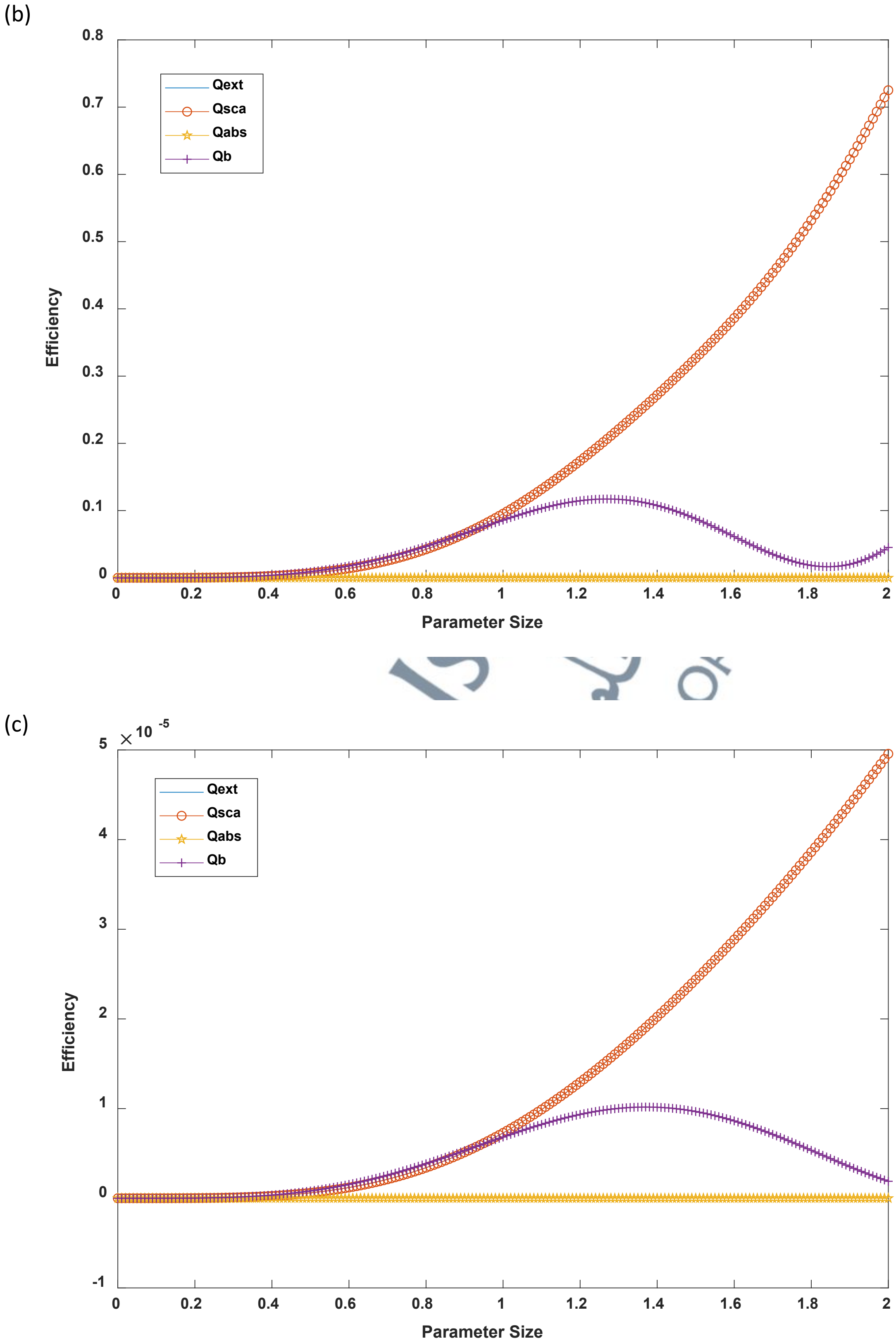
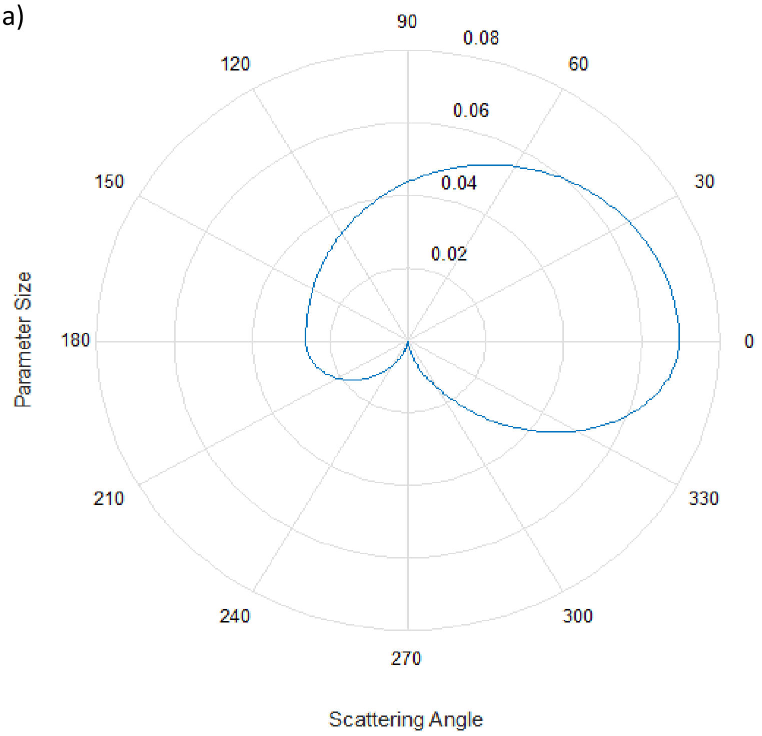
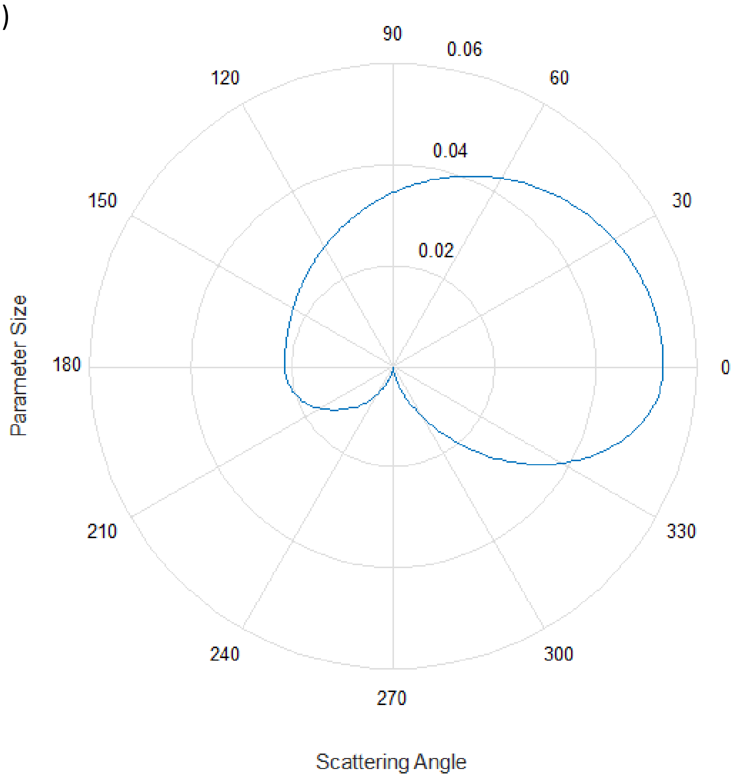


Figure 4.10 : The Efficiencies Based On Mie Theory For (a) Milk, (b) Water And (c) Air.

(a)


90
0.08
120
60
0.06
150
0.04
30
0.02
Parameter Size
180
0
210
330
240
300
270
Scattering Angle

(b)


90
0.06
120
60
0.04
150
30
0.02
Parameter Size
180
0
210
330
240
300
270
Scattering Angle

(c)

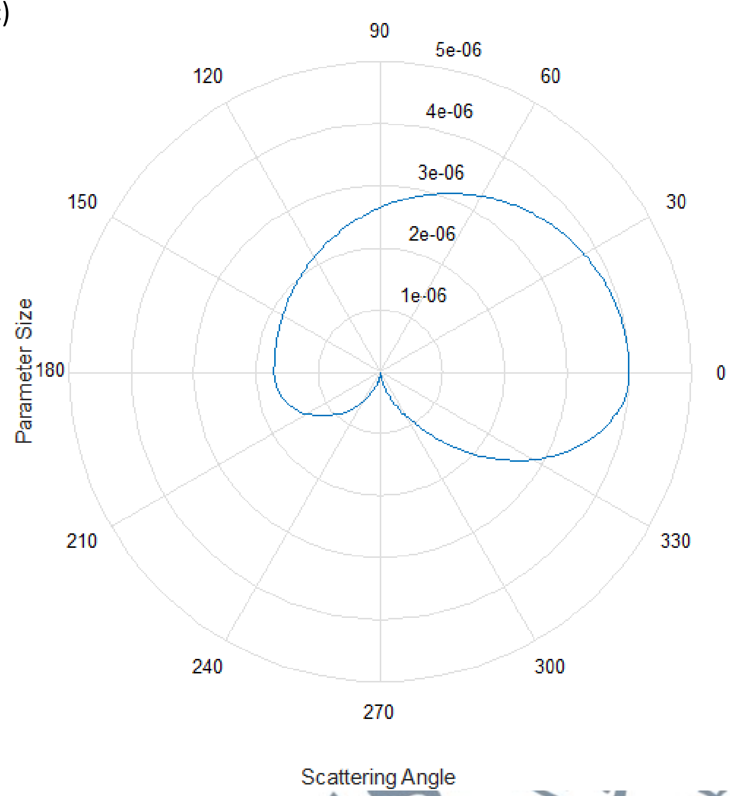


Figure 4.11: Angular Scattering Of (a) Milk, (b) Water And (c) Air.

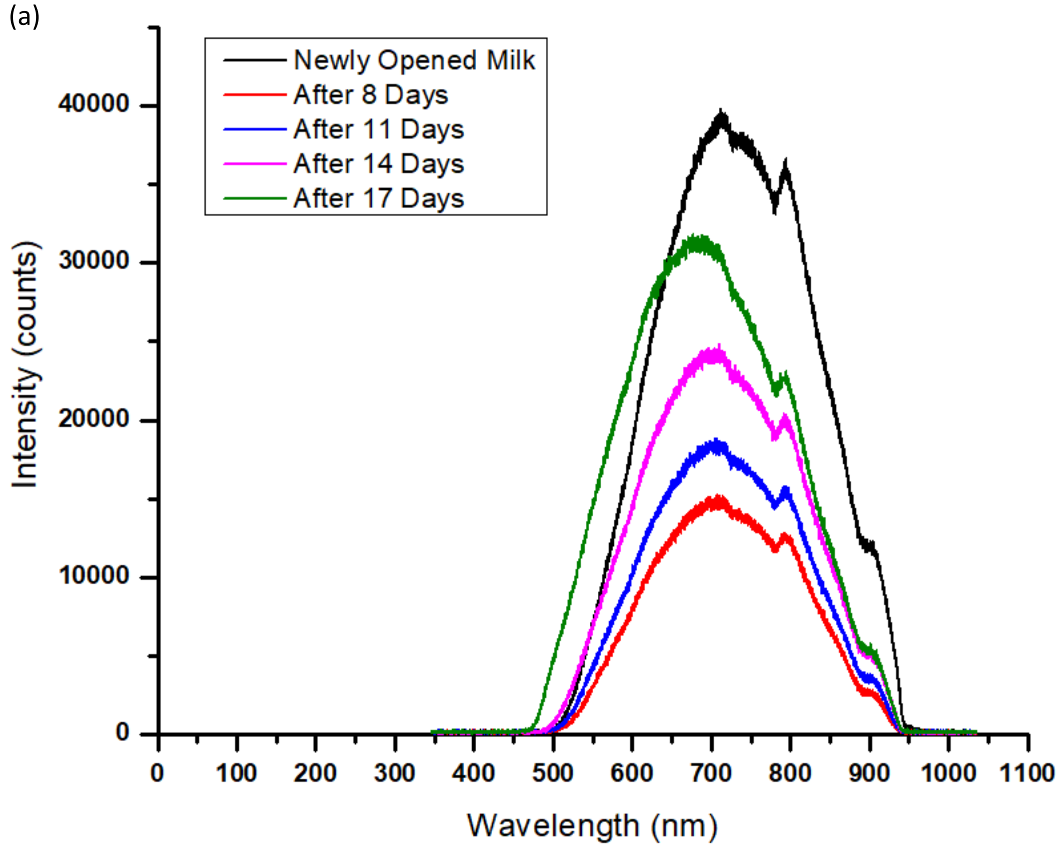
From Figure 4.11, we observe that milk has larger value of angular scattering compared to water and air. It is clearly shown that milk has higher scattering effect compared to water and air. We attribute that to the milk contents which mostly consist of fat and protein concentrations. Fat and protein in milk can act as the scatterers which scatter the light when it is exposed to the light source (Andrews, 2019).

4.4.2 Experimental Results

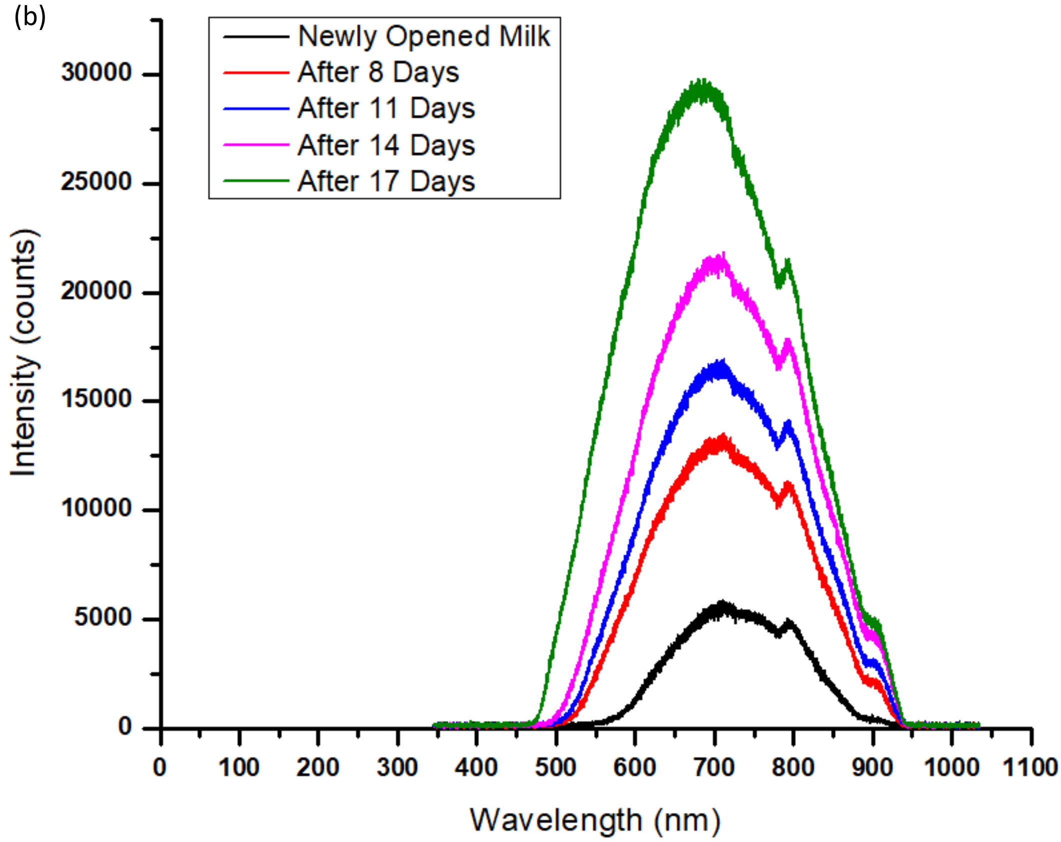
The spectra of reflectance, transmission and absorbance of the samples are observed from the spectrometer. The samples involve fresh full cream milk which are kept in room temperature for 8 days, 11 days, 14 days and 17 days. The experiment studied using NIR Spectrometer and VIS-NIR Spectrometer with wavelength ranges of 950 nm to 1650 nm and 350 nm to 1000 nm, respectively. Figure 4.12 depicts the absorbance, transmission and reflectance spectra of various milk samples.

Figure 4.12 (a) shows the absorbance spectrum of milk samples with different numbers of days exposure. It is clearly shown that the absorbance spectra in freshly opened full cream milk is higher than the milk that has been kept in the room temperature for 17 days at room temperature. Absorbance of milk reaches the peak at 700 nm due to high attenuation coefficient which quickly absorbs the light around 700 nm. When milk samples are kept in the room temperature, the samples undergo physical changes where the particles in milk aggregate and create lumpy structures. The lumpy structures affect the light absorption (Hahn, Sramek, et al., 2012). The lumpy structures are clearly observed in Figure 4.8 which we believe that it is due to particle aggregation. Hence, the quality of milk from fresh milk to fermented milk is gradually decreasing as the protein concentration decreases due to milk coagulation (Thomsen et al., 1990b).

(a)


Newly Opened Milk
After 8 Days
After 11 Days
After 14 Days
After 17 Days
Intensity (counts)
Wavelength (nm)
0
10000
20000
30000
40000
0
100
200
300
400
500
600
700
800
900
1000
1100

(b)


Newly Opened Milk
After 8 Days
After 11 Days
After 14 Days
After 17 Days
Intensity (counts)
Wavelength (nm)
0
5000
10000
15000
20000
25000
30000
0
100
200
300
400
500
600
700
800
900
1000
1100

(c)

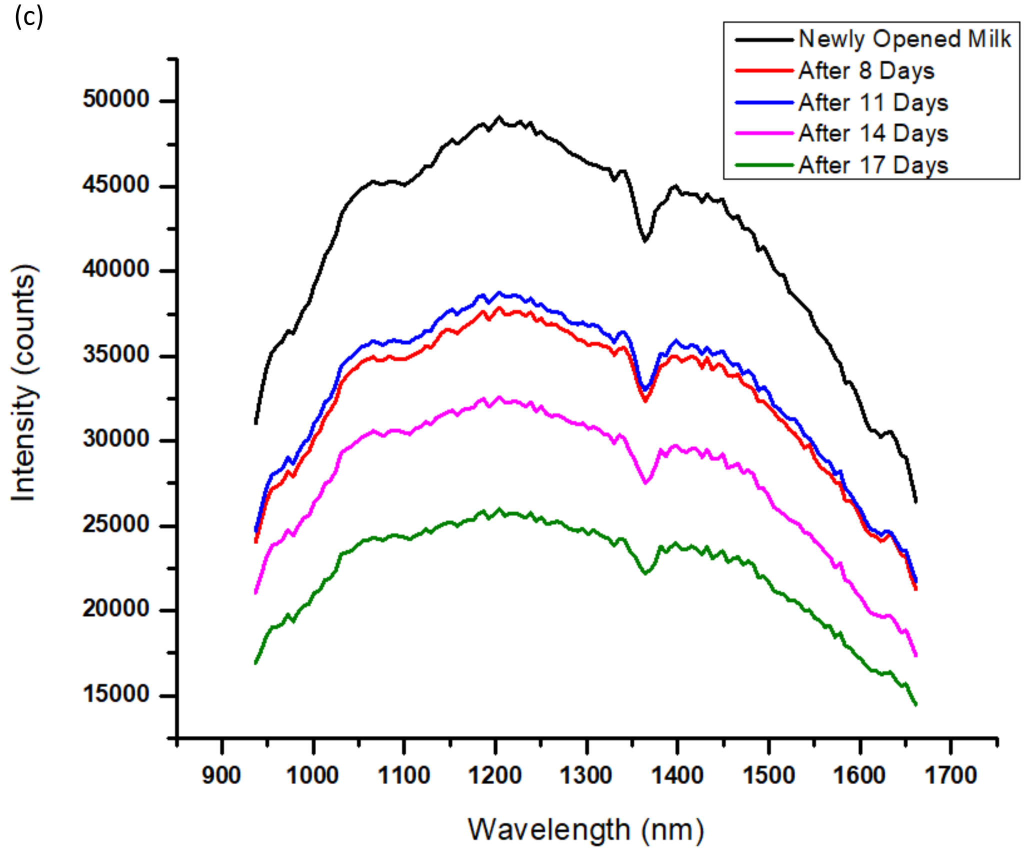


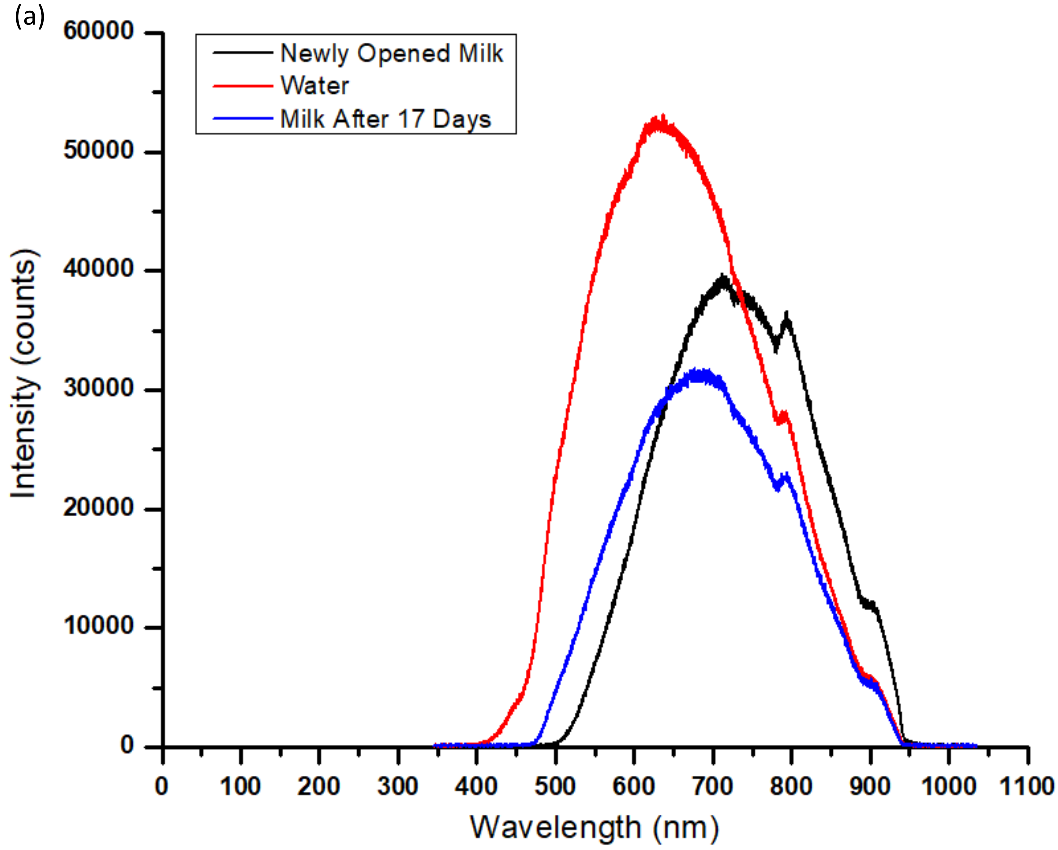
Figure 4.12: (a) Absorbance, (b) Transmission And (c) Reflectance Of Milk Samples For Several Days Of Fermentation.

Figure 4.12 (b) shows the transmission spectra of newly opened milk and fermented milk samples. The newly opened full cream milk sample has lower light transmission rather than the fermented milk sample. The transmission of light in fermented milk after 17 days is higher compared to the rest of samples as the particles in milk aggregate during the coagulation (Hahn, Sramek, et al., 2012)(Thomsen et al., 1990b). The protein thickens due to fermentation process which produces the lactic acid (Lu et al., 2013b). The fermented milk experiences decay and the pH level decreases as the lactic acid bacteria grows to produce the acid (Fernandes, 2009).

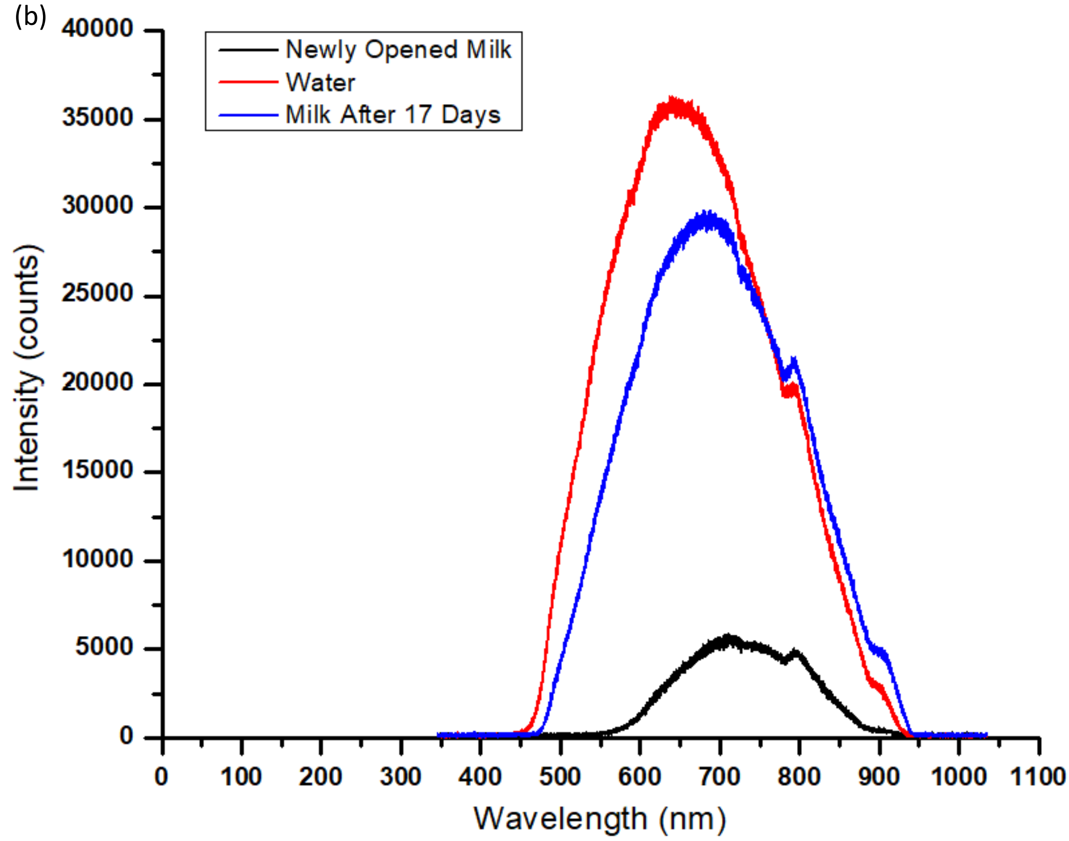
Figure 4.12 (c) indicates the reflectance spectra of milk. The reflectance of newly opened milk bottle is higher than the fermented milk. The low reflectance values for fermented milk are recorded due to high water absorption (Aernouts et al., 2011). Hence, it is possiblethat the presence of fat globules and protein micelles in milk affecting the reflectance of source light. The reflectance intensity decreases over the fermentation process due to the amount changes of protein and fat globules (Räty & Peiponen, 1999).

Figure 4.13 shows the spectra comparison of milk samples and water. Figure 4.13 (a) shows that water absorbs most of the source light at 600 nm whereas the absorption peak of milk is 700 nm. Milk and water depict similar transmission peaks, ~ 650 nm (Figure 4.13 (b)). Water sample shows higher transmission spectrum as it is more transparent than milk. Newly opened milk sample has higher reflectance than water due to fat and protein composition in milk. The size and shape of particles, composition and concentration of the tested samples can affect the absorption, transmission, and reflectance of the samples respectively (Viscarra Rossel et al., 2006). It is because the newly opened milk sample consists of various particle compositions whereas the fermented milk samples have experienced the physical state changes.

(a)


Newly Opened Milk
Water
Milk After 17 Days
Intensity (counts)
Wavelength (nm)
60000
50000
40000
30000
20000
10000
0
0
100
200
300
400
500
600
700
800
900
1000
1100

(b)


Newly Opened Milk
Water
Milk After 17 Days
Intensity (counts)
Wavelength (nm)
40000
35000
30000
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
600
700
800
900
1000
1100

(c)

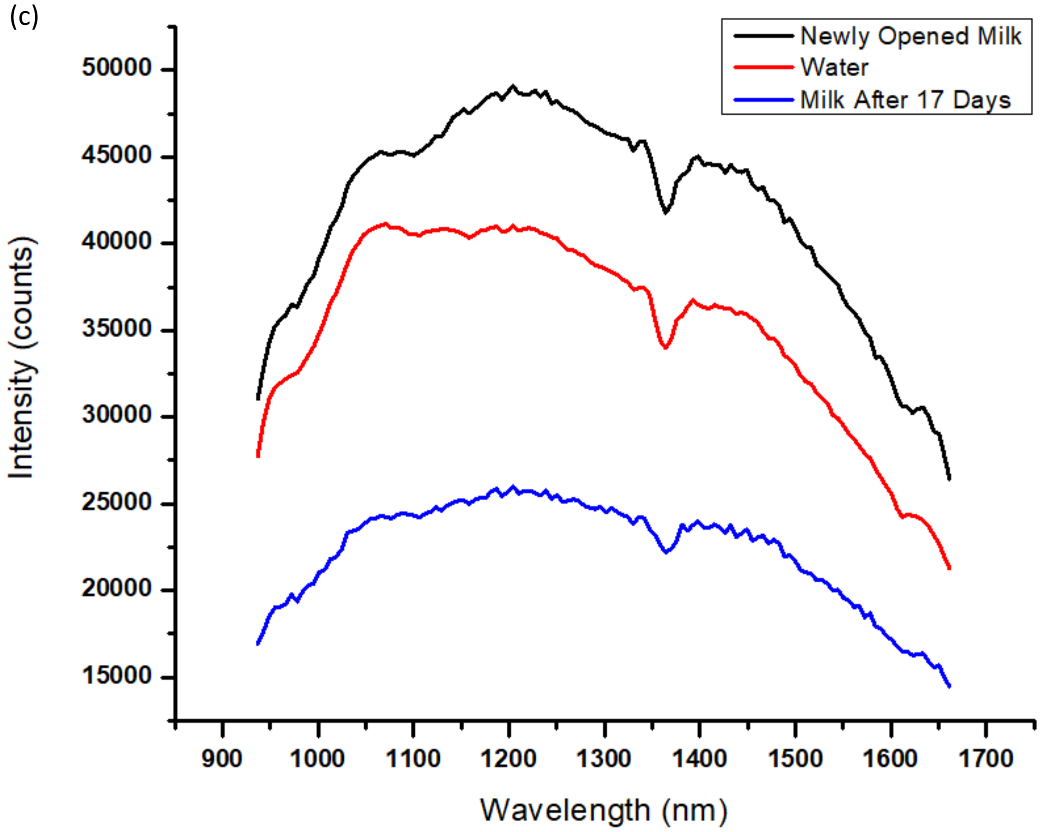


Figure 4.13: (a) Absorbance, (b) Transmission And (c) Reflectance Of Newly Opened Milk Sample, Water And Milk Sample After 17 Days.

4.5 Conclusion

In conclusion, this research investigates the quality of milk for newly opened sample and samples which are kept in room temperature for several days based on spectrometry technique and scattering theory. The optical properties of milk samples are investigated using VIS and NIR spectrometers in order to study the quality of milk. Newly opened milk sample has higher light absorbance and lower light transmission compared to the fermented milk. It is due to the aggregation of fat and protein particles in milk during fermentation process. Besides that, modeling based on scattering theory is done to compare light propagation in milk, water and air. The modeling shows that milk scatters more light compared to water and air due to the presence of fat globule, protein and minerals. The outcome of the study shows that the quality of milk is reduced when it is kept in the room temperature for several days. It is proved by the naked eye observation and the spectrometry technique. Thus, the outcome of the research is useful to support future chemical analysis study on dairy product.