

APPENDICES

APPENDIX A

JOURNAL OF ENGINEERING AND APPLIED SCIENCE, VOL. 67, NO. 8, DEC. 2020, PP. 2265-2278
FACULTY OF ENGINEERING, CAIRO UNIVERSITY

INVESTIGATING LIGHT PROPAGATION IN FULL AND SKIMMED MILK BASED ON SPECTROSCOPY AND MONTE CARLO ANALYSIS

N. A. I. MUHAMAD KAMIL¹, I. H. ZAKARIA², W. Z. WAN ISMAIL^{1*},
I. ISMAIL¹, J. JAMALUDIN¹, S. R. BALAKRISHNAN¹, M. SAHRIM¹

ABSTRACT

Milk quality can be determined through its fat composition. It is important to know the fat composition in milk to ensure consumption of the right product for health reasons. Spectroscopy can be used to study the fat composition in milk. In this paper, light propagation in milk based on visible and near infrared (NIR) spectra is investigated. Samples comprise skimmed and full milk. Full milk shows higher absorbance at visible (VIS) spectra compared to skimmed milk. The analysis on NIR spectra also shows that full milk has higher absorbance peak than skimmed milk due to higher amount of fat globule. Fourier-transform infrared spectroscopy (FTIR) analysis is done to study the chemical compounds such as C=C and O-H in milk samples. Through FTIR, the unsaturated fatty acid and water element in the samples were analyzed. Both milk samples show higher water element than carbon. Numerical modeling based on Monte Carlo method is also done to support experimental results. The modeling results show that full milk has a larger photon count compared to skimmed milk. This is attributed to the large fat globule in full milk that has higher absorbance over skimmed milk. Thus, characterization of milk fat based on spectroscopy techniques can monitor milk adulteration issues, which indirectly gives us guidance on healthy dairy intakes.

KEYWORDS: Absorbance, Transmittance, Milk fat, Monte Carlo, Spectroscopy, Photon count and photon loss.

1. INTRODUCTION

Light propagation consists of three main light phenomena: absorption, scattering and transmission [1]. Based on [2], light absorption refers to the disappearance of photon after hitting a particle, whereas light scattering involves changes in photon direction when light hits particle or molecule, as shown in Fig. 1. Transmission intensity, I

¹ Member, Advanced Devices and Systems, Department of Electrical and Electronics Engineering, Faculty of Engineering and Built Environment, Universiti Sains Islam, Malaysia.

² Engineer, Berryhak Sdn. Bhd., George Town, Penang, Malaysia.

* Corresponding Author: ikusuzakiah@usim.edu.my

decreases for both absorbed and scattered light. The attenuation due to absorption can be found using Eq. (1). Then, the attenuation due to scattering can be measured using Eq. (2) where I_0 , α , and τ are the incident intensity before attenuation, absorption coefficient and turbidity respectively [2]. The light absorbance can be calculated using $\log I_0/I$ or $\log(1/T)$. T refers to light transmittance.

$$I = I_0 \cdot 10^{-\alpha x} \tag{1}$$

$$I = I_0 \cdot e^{-\tau x} \tag{2}$$



Fig. 1. Transmitted light disturbed by either (a) absorbance (b) scattering [2].

The exposure of a turbid medium to the light source causes the medium to change to hazy and cloudy states due to the presence of the insoluble and suspended medium. The high opaqueness of a medium causes the medium to become more turbid with high degradable of light intensity. Turbidity is an important parameter to measure the quality of liquid, providing a wide range of applications for mankind. Recent water quality monitoring system applies Internet of Things (IOT) [3].

Milk, the most consumed dairy product in the world, is an example of turbid media. Fat, carbohydrates, proteins and mineral contents in milk are important to develop a growth-mechanism effect and well-nourishes diet for mankind. Nevertheless, the role of milk as a nutritious food is questioned as the composition of milk has been altered results in health problems. Full milk composition is being altered by adding water to increase milk volume, indirectly affecting the milk's quality [4-6].



Spectroscopy is widely applied to monitor food quality. The authentication of raw milk from reconstituted milk using FTIR spectroscopy [5] is done. The effectiveness of mid-infrared spectrometer is analyzed and results show mineral compositions of bulk milk [6]. Many spectroscopic analyses are done on milk samples to characterize milk fat including Laser spectroscopy [7], Fiber-optic spectroscopy [8], MEMS-based Fourier Transform Infra-Red (FTIR) spectrometer sensing [9] and Raman spectroscopy [10]. There are many applications based on spectroscopy analysis such as the use of Fourier Transform Infra-Red (FTIR) to identify the mineral drug substance (purified bentonite) in a drug product [11] and to analyze the chemical properties of "*Nicotiana Plumbginifolia*" as an important element in ethnomedicine [12].

Light propagation in milk samples can be modelled based on Monte Carlo technique. Monte Carlo method applies the stochastic model that analyzes the random probability distribution or pattern which is not precisely predicted [13]. Monte Carlo (MC) algorithm offers a randomized statistical sampling to solve the complex structure in milk composition and are widely used to study biological materials such as human tissues [1].

Previous spectroscopy studies of milk samples focus on experimental or theoretical technique and only one type of spectrometer is utilized. Hence, this research aims to investigate light propagation in milk samples based on experimental and theoretical studies. Three spectroscopy techniques are applied and modeling is done based on Monte Carlo simulation. Two types of milks are compared and analyzed based on their optical properties. Full milk shows higher light absorption than skimmed milk because of larger fat content in it.

2. MATERIALS AND METHODS

2.1 Materials and Experimental Set Up

Three different types of spectrometers are used such as Ocean Optic Flame Near-Infrared (NIR) Spectrometer, Perkin Elmer Lambda 750 UV/VIS/NIR Spectrometer and Varian 3100 Excalibur Series Fourier Transform Infra-Red (FTIR) Spectrometer. These

spectrometers are used as they can offer large size sampling, cost-effective, fast and reliable measurement.

2.2 Sample Preparations

Ultra-High Temperature (UHT) milk samples consist of skimmed and full milk from brand 'Dutch Lady'. Firstly, both samples are diluted with a ratio of 1:100 for water and milk respectively. Distilled water is used as a reference and comparison with milk samples. A prepared sample is placed in the 10 mm plastic cuvette and lightly shaken and stirred to mix the solution before being placed in Ocean Optic Flame Near-Infrared (NIR) Spectrometer and Perkin Elmer Lambda 750 UV/VIS/NIR Spectrometer at spectrum ranges of 950-1650 nm and 350-860 nm respectively. A droplet of undiluted solution from both milk samples are tested in a Varian 3100 Excalibur Series for FTIR spectroscopy. The FTIR spectroscopy is used to identify and differentiate any chemical compounds in both samples. The wavelength ranges for FTIR Spectroscopy are from 550 to 3950 cm^{-1} with the analysis is done in Varian Resolution Software. The experiments are repeated 5 times to ensure consistency and accuracy of the measurement. The output is averaged out with the standard deviation $\sim 0.01-0.03$.

2.3 Theoretical Analysis

Many modeling techniques can be applied to analyze the light propagation in random media such as Monte Carlo [14-19], Finite Element Modeling (FEM) [20] and Finite Different Time Domain (FDTD) [21]. Vaskova *et al.* [10] state that mathematical evaluation can be used to measure the accurate milk fat content for the quality control of the milk product. Here, Monte Carlo modeling technique is used to simulate the photon count and photon loss in milk. The selected technique can be used to observe the amount of photon transmitted and loss in skimmed and full milk where the scattering factors such as internal coefficient, anisotropic parameters and forward power are analyzed. Besides that, Monte Carlo can track the movement of photons inside the samples which experience absorption, scattering and power loss. Fundamentally, the



photon propagation is initiated by photon launching followed by photon absorption, scattering and is terminated by photon detection as depicted in Fig. 2.

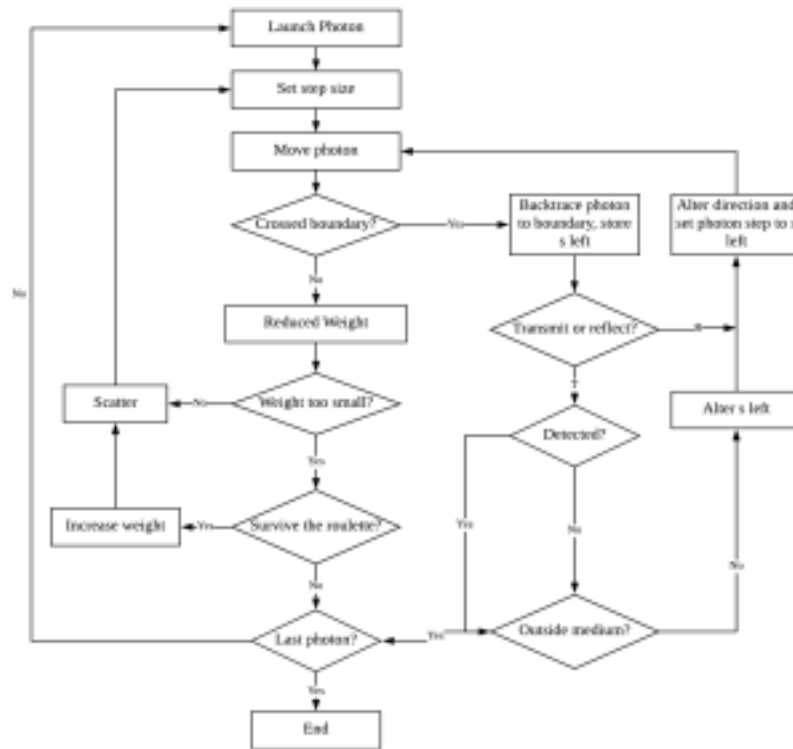


Fig. 2. Modeling of light propagation based on Monte Carlo technique [17].

In Monte Carlo modeling technique, four main steps are involved; photon launching, photon absorption, photon scattering and finally, photon detection with continuous propagation [14].

2.3.1 Photon initialization

At the first stage, the photon is launched, followed by the step length expressed by Eq. (3) [15].

$$\Delta s = \frac{-\ln(rnd)}{\mu_t} \quad (3)$$



While μ_t refers to internal coefficient, rnd is a random number that is launched between the interval of 0 and 1 and will be sampled at every executing loop, μ_a is the absorption coefficient and, μ_s is the scattering coefficient as shown in Eq. (4) [16].

$$\mu_t = \mu_s + \mu_a \quad (4)$$

At the same time, virtual weight is assigned to each photon before any movement. The first photon position exists at 3D space, (x_1, y_1, z_1) . The first polar position at 3D space is given by Eq. (5) [15].

$$\begin{aligned} x_1 &= x_0 + \mu_x \Delta s \\ y_1 &= y_0 + \mu_y \Delta s \\ z_1 &= z_0 + \mu_z \Delta s \end{aligned} \quad (5)$$

2.3.2 Photon absorption

The second stage involves photon absorption. After the absorption, photon reduces its weight (w) by setting the threshold for every iteration. The reduction of photon weight is given by Eq. (6) [16].

$$w_l = w_{l-1} \left(1 - \frac{\mu_a}{\mu_t}\right) \text{ where } l = 1, 2, 3, \dots \quad (6)$$

Russian roulette technique is used in the modeling because the sole calculation time to estimate the light propagation is not realistic. Thus, random value (rnd) is set based on Eq. (7) [16].

$$rnd = \frac{w_0}{10} \quad (7)$$

Where w_0 refers to the initial photon weight. If the random number exceeds the threshold value, the photon will be terminated, photon weight is increased by $1/p$.

2.3.3 Photon scattering and propagation

The third stage consists of photon scattering and propagation. When photon reduces its weight, but still alive in the roulette, they can be scattered. Hence, at this stage, the deflection angle can be measured based on the phase function. Deflection angle, θ is calculated based on Eqs. 8 and 9 [16].



$$\theta = \cos^{-1} \left[\frac{1}{z \cdot g} \cdot \left\{ 1 + g^2 - \left(\frac{r_{nd}}{H} + (1 + g^{-2\alpha}) \alpha^{-1} \right) \right\} \right] \quad (8)$$

$$\text{Where: } H = \frac{(1+g^2)^{2\alpha}}{(1+g)^{2\alpha} - (1-g)^{2\alpha}} \quad (9)$$

Where g is the anisotropy factor referring to scattering function, H is Henvey-Greenstein phase function and α is the weight factor. The new position (x_1, y_1, z_1) can be generated using the obtained phase function, deflection angle and the azimuthal angle, φ . φ is randomly chosen from the uniform distribution within the interval of $(0, 2\pi)$, expressed by Eqs. (10-12) [16].

$$x_{i+1} = x_i + \Delta s \cdot \left(\frac{\sin(\theta)}{\sqrt{1-u_z^2}} (u_x \cdot u_z \cdot \cos(\varphi) - u_y \cdot \sin(\varphi)) + u_x \cdot \cos(\theta) \right) \quad (10)$$

$$y_{i+1} = y_i + \Delta s \cdot \left(\frac{\sin(\theta)}{\sqrt{1-u_z^2}} (u_y \cdot u_z \cdot \cos(\varphi) + u_x \cdot \sin(\varphi)) + u_y \cdot \cos(\theta) \right) \quad (11)$$

$$z_{i+1} = z_i + \Delta s \cdot (-\sin(\theta) \cdot \cos(\varphi) \cdot \sqrt{1-u_z^2} + u_z \cdot \cos(\theta)) \quad (12)$$

where, (u_x, u_y, u_z) represents the values of photon propagation in every iteration, i .

2.3.4 Photon detection

The photon is detected at the final stage. Photon detection is done by considering its weight and the optical properties of the measured medium such as refractive index, number of emitted photon, position, absorption coefficient and scattering coefficient [17]. Table 1 shows the weight of milk fat contents, scattering coefficient and absorption coefficient. The parameters in the table are used to record backscattered count and photon loss during scattering process. The internal coefficient is determined from Eq. (4) whereas the scattering and absorption coefficients are based on Qin et al. [26].

Table 1. Scattering, absorption and internal coefficients for both milk samples.

Type of medium	Weight of Milk Fat Contents, gram (g)	Scattering coefficient, μ_s	Absorption Coefficient, μ_a	Internal Coefficient, μ_i
Skimmed Milk	0.0	1.65	0.55	2.20
	0.1	1.68	0.56	2.24
Full Milk	3.2	2.36	1.80	4.16
	3.8	2.36	1.9	4.26

Here, modeling is used to support experimental analysis in terms of light or photon transmitted in milk, not as a direct comparison with the experimental results.

3. RESULTS AND DISCUSSION

The experiments are done using various spectrometer techniques whereas the theoretical analysis is done based on Monte Carlo modeling.

3.1 Experimental Analysis

The experimental analysis is carried out based on Visible (VIS), Near Infra-Red (NIR) and Infra-Red (IR) spectra.

3.1.1 Visible (VIS) spectrum

Figure 3(a) shows the absorbance spectra of skimmed milk, full milk and water from 380 nm – 860 nm. The spectra are plotted using UV WinLab software in Perkin Elmer Lambda, 750 UV/VIS/NIR Spectroscopy. The samples in Visible (VIS) spectrum are analyzed based on spectrum continuity [18] instead of observing the milk fat spectrum at certain peaks, as analyzed in Near Infra-Red (NIR) and Infra-Red (IR) spectra [9, 10, 19, 22]. It is clearly shown that the absorbance of the skimmed milk is much lower than full milk. The almost linear line is observed for water sample due to weak light absorbance in the visible spectrum compared to Near Infra-Red (NIR) and Infra-Red (IR) spectra.

3.1.2 Near infra-red (NIR) spectrum

Figure 3(b) shows the absorbance spectra for skimmed milk, full milk and water from 960 -1660 nm using Optic NIR Spectrometer. From the fig., we observe that the absorbance of full milk shows higher absorbance over skimmed milk and water respectively. The three strong absorption peaks observed at 972, 1158 and 1392 nm in the NIR spectrum. In Fig. 3.2(b), the absorbance shows negative values from 1050 nm until 1100 nm which we attribute that to the background noise and spectrometer calibration.



INVESTIGATING LIGHT PROPAGATION IN FULL AND SKIMMED MILK...

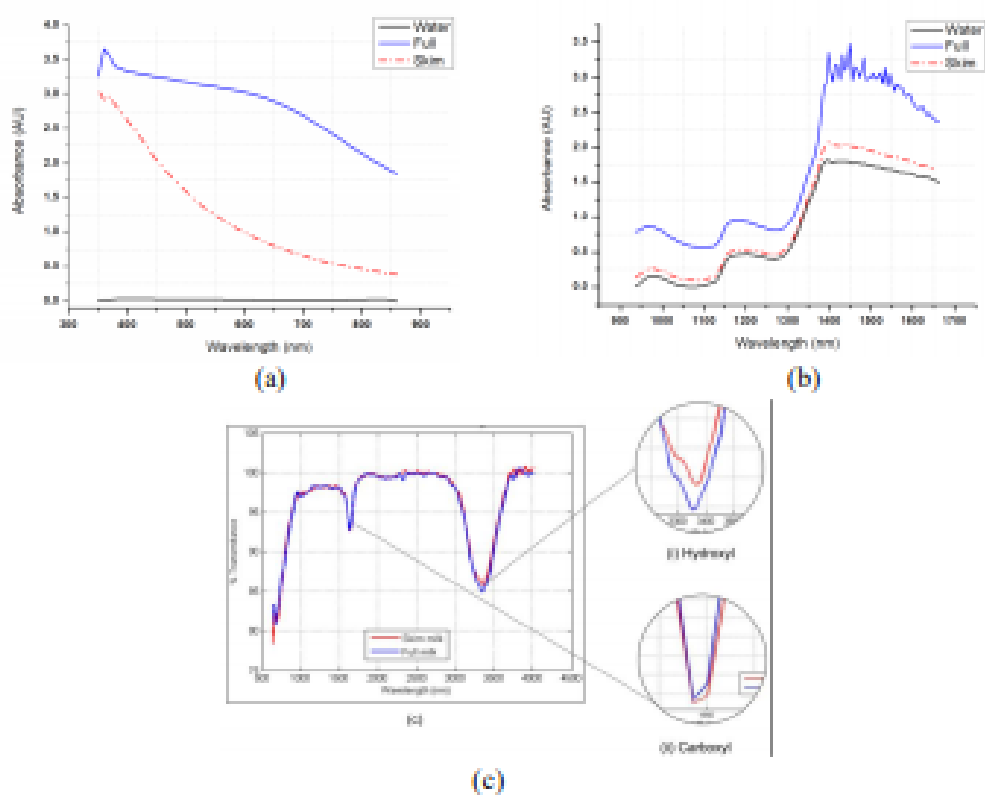


Fig. 3. Spectrum analysis of different milk samples (a) Visible (VIS) spectrometer versus wavelength (b) Near infra-red (NIR) absorbance versus wavelength (c) FTIR transmittance versus wavelength.

3.1.3 Fourier transform infra-red (FTIR) spectrum

Investigating light propagation in milk is continued using Fourier Transform Infra-Red (FTIR) spectroscopy. Transmission spectra for both milk samples are shown in Fig. 3(c). The fig. shows the transmission of two main functional groups in milk samples, hydroxyl and carboxyl group. A small valley from 1500-1680 nm represents alkene (chemical bond, C=C) and a broad valley from 3300 - 3500 nm represents hydroxyl (chemical bond, O-H) groups. The C, O and H refer to carbon, oxygen and hydrogen respectively. A broad hydroxyl spectrum (O-H) is observed from the graph due to high water composition in the samples. The dominated water bands in the spectra can affect the absorption of other milk fat components such as triglycerides and saturated



fatty acid [22]. We observe slight changes at alkene (C=C) stretch that is attributed to the unsaturated fatty acid which characterizes skimmed and full milk [9, 10, 22].

In addition, strong water (O-H) absorption was observed at 960, 1440 and 1950 nm using MEMs spectrometer [9] whereas Raman spectroscopy was used to characterize milk fat consist of triglycerides groups, saturated and unsaturated fatty acid. The characterization is based on chemical bonds C=O, CH₂ and C=C stretching. The functional groups based on absorbance wavelength are summarized in Table 2.

Table 2. Chemical compounds at varied wavelength ranges in FTIR analysis.

Wavelength Peak/ Ranges (cm ⁻¹)	Functional Group	Chemical Bonds	Types of Chemical Compounds
3300 – 3500	Hydroxyl	O-H	Water
1500-1680	Alkene	C=C	Unsaturated fatty acid

3.2 Theoretical Analysis

The Monte Carlo technique is used to measure the forward photon count for different anisotropy values, g in both skimmed and full milk. Henvey-Greenstein suggests three main scattering types based on anisotropic value, g where purely forward scattering exists for $g=1$, while purely backscattering and isotropic scattering exist for $g=-1$ and $g=0$ respectively [23].

Henvey-Greenstein phase function is commonly used to analyze the light scattering in biomedical fields such as tissues and cell [14, 24] where most of biological tissues have anisotropic value, g greater than 0.7 ($g > 0.7$) [25]. Hence, forward scattering in the range of $0 < g < 1$ is applied to analyze the milk fat due to the disordered and random media [26].

Numerical modeling based on Monte Carlo technique is shown in Fig. 4. Different internal coefficients are measured to observe their effects on various anisotropic parameters. The forward photon count in skimmed milk are measured and compared using internal coefficient of 2.24 and 2.20 respectively (Fig. 4(a) and 4(b)). Meanwhile, Fig. 4(c) and 4(d) show photon count and photon loss for both samples based on various anisotropy parameters. High forward power is analyzed when high internal coefficient is applied to the skimmed milk. Besides that, there is a high incline

INVESTIGATING LIGHT PROPAGATION IN FULL AND SKIMMED MILK...

when g parameters approach 1 for both internal coefficients (Fig. 4(a)). The photon loss for skimmed milk can be analyzed by varying the internal coefficient. Based on the graph in Fig. 4(b), when g increases until 0.8, the photon loss increases gradually and starts to decrease when g is larger than 0.8. The measured photon loss for anisotropy parameter, g approaches 1 is lower than the g approaches 0. Fig. 4(c) and (d) distinguish skimmed milk and full milk in terms of photon count and photon loss.

Based on both graphs, forward photon count in full milk is higher than skimmed milk whereas the photon loss in skimmed milk is lower than in full milk. This is due to the fact that skimmed milk has less fat globules than full milk. The large fat molecules in full milk absorb and scatter more light over skimmed milk.

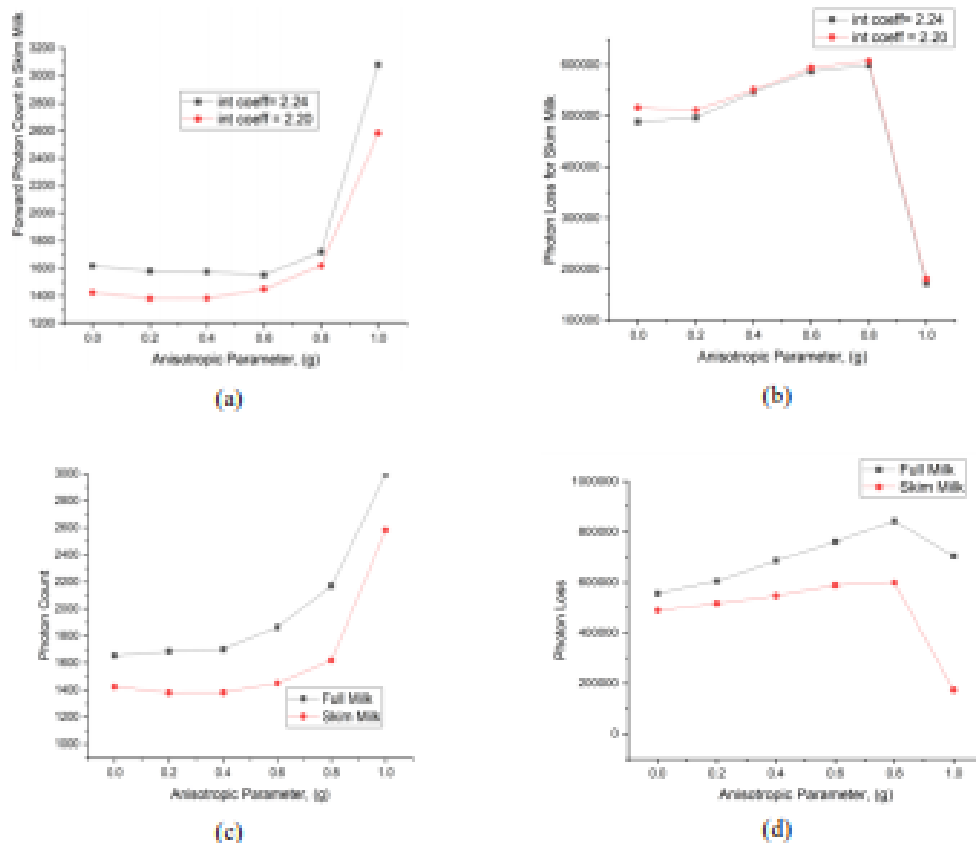


Fig. 4. (a) Forward photon count of skimmed milk (b) Photon loss with various internal coefficients measured in skimmed milk (c) Photon count for skimmed milk and full milk (d) Photon loss for skimmed milk and full milk.



Stocker *et al.* [27] have done simulation using scattering coefficient, μ_s and reduced scattering coefficient, μ_r at different wavelength for untreated and treated raw milk with Ethylenediaminetetraacetic acid, EDTA based on integrating sphere measurements. The scattering properties and refractive index depend on the size of milk fat droplet, where, a high fat globule yields a high refractive index as well as scattering properties [27]. Thus, numerical modeling based on Monte Carlo can support the experimental results where the light propagation in milk is analyzed theoretically in terms of photon count and photon loss.

4. CONCLUSIONS

The milk fat analysis in skimmed and full milk is done based on light propagation in milk. All experimental measurements are done using NIR, VIS and FTIR spectrometers. Full milk sample shows the highest absorbance due to high fat globule in full milk compared to others. A large particle of fat globule inside the medium can provide high absorbance at certain wavelength which specifies chemical properties of water and unsaturated fatty acid. The numerical modeling based on Monte Carlo shows that full milk has higher photon loss and forward photon count than skimmed milk. The higher internal coefficient in full milk results in higher photon count and photon loss. Full milk sample has higher photon count and photon loss due to higher absorption and scattering in the sample. This study can be used to assist the authority to monitor milk adulteration issue to ensure good human well-being.

CONFLICT OF INTERESTS

The authors have declared no conflict of Interests.

REFERENCES

1. Meretska, M. L., Uppu, R., Vissenberg, G., Lagendijk, A., Ijzerman, W. L., and Vos, W. L., "Analytical Modeling of Light Transport in Scattering Materials with Strong Absorption", *Optics Express*, Vol. 25, No. 20, pp. A906-A921, 2017.
2. https://www.nbi.dk/~ogendal/personal/lbo/L_S_brief_intro.pdf. (Accessed 10/10/2020).
3. Sathish, P., and Gandla, S., "Smart Water Quality Monitoring System with Cost-Effective using IoT", *Heliyon*, 6, 2020.

4. Abegaõ, L. M. G., Alessandra, A. C. P., Sergio, C. Z., Marcio, A. R. C. A. and Jose, J. R. J., "Measuring Milk Fat Content by Random Laser Emission", *Scientific Reports*, Vol. 6, pp. 4-7, 2016.
5. Du, L., Lu, W., Gao, B., Wang, J., and Yu, L., "Authenticating Raw from Reconstituted Milk Using Fourier Transform Infrared Spectroscopy and Chemometrics", *Journal of Food Quality*, Vol. 2019, 2019.
6. Malacarne, M., Andrea, S., Mauro, P., Giorgio, Z., Giulio, V., Martino, C., Giuseppe, B., and Massimo, D. M., "Investigation on the Effectiveness of Mid-Infrared Spectroscopy to Predict Detailed Mineral Composition of Bulk Milk", *Journal of Dairy Research*, Vol. 85, No. 1, pp. 83-86, 2018.
7. Montemurro, M., Schwaighofer, A., Schmidt, A., Culzoni, M. J., Mayer, H. K., and Lendl, B., "High-Throughput Quantitation of Bovine Milk Proteins and Discrimination of Commercial Milk Types by External Cavity-Quantum Cascade Laser Spectroscopy and Chemometrics", *Analyst*, Vol. 144, No. 18, pp. 5571-5579, 2019.
8. Katsumata, T., Aizawa, H., Komuro, S., Ito, S., and Matsumoto, T., "Quantitative Analysis of Fat and Protein Concentrations of Milk Based on Fibre-Optic Evaluation of Back Scattering Intensity", *International Dairy Journal*, Vol. 109, p. 104743, 2020.
9. Amr, M., Sabry, Y. M., Khalil, D., "Near-Infrared Optical MEMS Spectrometer-Based Quantification of Fat Concentration in Milk." *National Radio Science Conference, NRSC, Proceedings*, Vol. 3, No. 3, pp. 409-416, 2018.
10. Vasková, H., Bucková, M., Zálesáková, L., "Spectroscopic Analysis of Milk Fat and Its Mathematical Evaluation", *International Journal of Biology and Biomedical Engineering*, Vol. 10, pp.168-175, 2016.
11. Ouhaddouch, H., Cheikh, A., Idrissi, M. O. B., Draoui, M., and Bouatia, M., "FT-IR Spectroscopy Applied for Identification of a Mineral Drug Substance in Drug Products: Application to Bentonite", *Journal of Spectroscopy*, Vol. 2019, ID 2960845, 2019.
12. Chandra, S., "Fourier Transform Infrared (Ft-Ir) Spectroscopic Analysis of *Nicotiana Plumbaginifolia* (Solanaceae)", *Journal of Medical Plant Study*, Vol. 7, No.1, pp. 82-85, 2019.
13. Prah, S. A., and Keijzer, M., "A Monte Carlo Model of Light Propagation in Tissue", *Dosimetry of Laser Radiation in Medicine and Biology I*, pp. 102-111, 1989.
14. Vinckenbosch, L., Lacaux, C., Tindel, S., Thomassin, M., and Obara, T., "Monte Carlo Methods for Light Propagation in Biological Tissues", *Mathematical Biosciences*, Vol. 269, pp. 48-60, 2015.
15. Song, S., Kobayashi, Y., and Masakatsu, G. F., "Monte-Carlo Simulation of Light Propagation Considering Characteristic of Near-Infrared LED and Evaluation on Tissue Phantom", *Procedia CIRP* 5, pp. 25-30, 2013.
16. Bocklin, C., "Modeling of Light Propagation in Tissue", *ETH Zurich Research Collection*, Vol. 9, No. 2, 2014.



17. Romanov, O. G., Tolstik, A., Velez, F. F., Quijano, N. O., Garcia, I. S., and Diego, J. L. A., "Modeling of Light Propagation in Turbid Media: Application to Biological Tissues", *Nonlinear Phenomena in Complex Systems*, Vol. 15, No. 4, pp. 395-402, 2012.
18. Jain, P., and Sarma, S. E., "Light Scattering and Transmission Measurement Using Digital Imaging for Online Analysis of Constituents in Milk", *Proceedings, Optical Measurement Systems for Industrial Inspection IX, SPIE Optical Metrology, Germany*, 2015.
19. Forcato, D. O., Carmine, M. P., Echeverria, G. E., Pecora, R. P., and Kivatinitz, S. C., "Milk Fat Content Measurement by a Simple UV Spectrophotometric Method: An Alternative Screening Method", *Journal of Dairy Science*, Vol. 88, No. 2, pp. 478-481, 2010.
20. Mahmoud, F. F., El-Shafei, A. G., Abdelrahman, A. A., and Attia, M. A., "Finite Element Modeling of Large Deformation Viscoelastic Frictional Contact Systems", *Journal of Engineering and Applied Science*, Vol. 59, No. 1, pp. 65-85, 2012.
21. Botros, A. Z., and Nasr, K. M. A., "Finite Difference Time Domain in One Dimension Remote Sensing", *Journal of Engineering and Applied Science*, Vol. 47, No. 3, pp. 587-605, 2000.
22. Tsenkova, R., Atanassova S., Itoh, K., Ozaki, Y., and Toyoda, K., "Near Infrared Spectroscopy for Biomonitoring: Cow Milk Composition Measurement in a Spectral Region from 1,100 to 2,400 Nanometers", *Journal of Animal Science*, Vol. 78, No. 3, pp. 515-522, 2000.
23. Prahl, S. A., Van Gemert, M. J., Welch, A. J., "Determining the Optical Properties of Turbid Media by Using the Adding-Doubling Method", *Applied Optics*, Vol. 32, No. 4, pp.559-68, 1993.
24. Golshan, A. M., Tarei, M. G., Ansari, M. A., and Amjadi, A., "The Propagation of Laser Light in Skin by Monte Carlo- Diffusion Method: A Fast and Accurate Method to Simulate Photon Migration in Biological Tissues", *Journal of Laser in Medical Science*, Vol. 2, No. 3, pp. 109-114, 2011.
25. Ding, H., Nguyen, F., Boppart, S. A., and Popescu, G., "Optical Properties of Tissues Quantified by Fourier-Transform Light Scattering", *Optics Letters*, Vol. 34, No. 9, pp. 1372-1374, 2009.
26. Qin, J., and Lu, R., "Measurement of the Absorption and Scattering Properties of Turbid Liquid Foods Using Hyperspectral Imaging", *Applied Spectroscopy*, Vol. 61, No. 4, pp. 388-396, 2007.
27. Stocker, S., Foschum, F., Krauter, P., Bergmann, F., Hohmann, A., Happ, C. S., and Kienle, A., "Broadband Optical Properties of Milk", *Applied Spectroscopy*, Vol. 71, No. 5, pp. 951-962, 2017.



Investigating the Quality of Milk using Spectrometry Technique and Scattering Theory

Nur Ain Insyirah Muhamad Kamil
Advanced Devices and System (ADS)
Faculty of Engineering and Built Environment
Universiti Sains Islam Malaysia
Negeri Sembilan, Malaysia

Zatunnur Syakirah Nor'aini
Advanced Devices and System (ADS)
Faculty of Engineering and Built Environment
Universiti Sains Islam Malaysia
Negeri Sembilan, Malaysia

Wan Zakiah Wan Ismail
Advanced Devices and System (ADS)
Faculty of Engineering and Built Environment
Universiti Sains Islam Malaysia
Negeri Sembilan, Malaysia

Sharma Rao Balakrishnan
Advanced Devices and System (ADS)
Faculty of Engineering and Built Environment
Universiti Sains Islam Malaysia
Negeri Sembilan, Malaysia

Juliza Jamaludin
Advanced Devices and System (ADS)
Faculty of Engineering and Built Environment
Universiti Sains Islam Malaysia
Negeri Sembilan, Malaysia

Imeza Ismail
Advanced Devices and System (ADS)
Faculty of Engineering and Built Environment
Universiti Sains Islam Malaysia
Negeri Sembilan, Malaysia

Musab Sahrim
Advanced Devices and System (ADS)
Faculty of Engineering and Built Environment
Universiti Sains Islam Malaysia
Negeri Sembilan, Malaysia

Abstract—Milk is a dairy product that contains dissolved proteins, carbohydrates, fat, and many minerals. Milk enhances body growth and provides vital energy and fatty acids. 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. Our work aims to compare the quality of milk after being kept at room temperature for several days using spectroscopy methods. 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.

Keywords—light propagation; absorbance; transmittance; reflectance; scattering; milk; spectroscopy

I. INTRODUCTION

It is crucial to monitor the quality of milk in order to ensure we gain sufficient nutrients and minerals and prevent the occurrence of diseases. Cow milk consists of water (87%), fat (4%), proteins (3.4%), lactose (4.8%), and minerals (0.8%) [1]. Fat composition is not similar in full cream milk and skimmed milk. A layer of cream forms on the milk's surface if it is exposed for several days. This cream consists of spheres of various sizes floating in milk surrounded by a fat globule membrane. The membrane is responsible for fat protection against enzymes and prevents any globule coalescing into butter grains [1]. The spectroscopy technique can be used to observe the optical properties of milk based on light absorbance, transmission and scattering. Mie scattering theory is used to compute the absorption coefficient (μ_a), the scattering coefficient (μ_s), and the phase function $p(\theta)$, where θ is the scattering angle [2]. Mie theory is used to calculate the spectral dependence for the extinction cross section of nanoparticle suspensions [3]. The pump source energy passes

Corresponding author: Wan Zakiah Wan Ismail (drwanzakiah@uisim.edu.my)

www.etasr.com

Kamil et al.: Investigating the Quality of Milk with Spectroscopy and Scattering Theory

through the turbid media depending on optical properties such as the refractive index, scattering, anisotropic factor, and laser light absorption [4]. The optical properties of milk based on backscattering intensity can be used to study fat and protein concentrations [5]. The complex fluid of milk is made up of many components such as water, lipids, lactose and protein [6, 8]. Spectroscopy is widely used to measure the optical properties of samples based on light propagation and fluorescence. Color spectroscopy is used to obtain information about the atoms and molecules [8-9]. The absorbance spectroscopy is a technique used to measure the amount of absorbed light [10, 11] with the determination of solution concentration based on Beer's Law [12]. NIR spectrometer and VIS-NIR spectrometer with different wavelength ranges are used to determine the accuracy of the intensity spectrum in the spectroscopy analysis [13].

Many recent studies on light propagation in milk involve backscattering [5], external cavity-quantum cascade laser spectroscopy [6], and laser diffraction and centrifugation [7]. Authors in [8] introduced the simplified NIR spectroscopy in measuring the end of milk fermentation by transforming sugar to lactic acid. The key characteristic of the fermentation process is the pH end point value, in the range of 4.4-4.5 [8]. This technique is quite complicated and costly. To the best of our knowledge, no comparison has been conducted using milk after several days' exposure and water. The previous studies also do not provide a modeling approach on light scattering in milk. Our previous work [14] compared the optical properties of full cream and skimmed milk using different spectrometer types. We found that full cream milk has higher absorption due to its higher fat content. This research is continued in the current paper, which aims to study the light propagation in various milk samples for different exposure at room temperature durations based on spectroscopy techniques using Visible (VIS) and Near Infra-Red (NIR) spectrometers. The technique is simpler and cheaper than the ones used in previous studies as indicated in the experimental section. The output shows that the newly opened milk sample absorbs more light than the other samples. A modeling approach based on Mie theory was also provided to compare light scattering in milk, water, and air.

II. THEORETICAL FRAMEWORK

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 (1) and (2) [12].

$$m = m' + im'' \quad (1)$$

$$x = k\alpha = \frac{2\pi}{\lambda} \alpha \quad (2)$$

where m' is the real refractive index, im'' is the imaginary refractive index, k is wave number in the ambient medium, and α is the sphere radius.

The key parameters of Mie theory are the computed amplitudes of the scattered field. The coefficients a_n and b_n are required to obtain the Mie efficiency using Spherical Bessel function n ($n=1, 2, \dots$) of higher order and work well in the wider range of size parameters [15].

The efficiency of extinction Q_{ext} and scattering Q_{scat} can be identified in forward-scattering theorem and in the integration of the power scatters in all directions. The absorption efficiency Q_{abs} can be identified with the equation of energy conservation [16]. Meanwhile, the backscattering efficiency Q_b is applicable to monostatic radar [15]. Equations for absorption, scattering and backscattering efficiency are:

$$Q_{ext} = \frac{2}{x^2} \sum_{n=1}^{\infty} (2n+1) \text{Re}(a_n^2 + b_n^2) \quad (3)$$

$$Q_{scat} = \frac{2}{x^2} \sum_{n=1}^{\infty} (2n+1) (|a_n|^2 + |b_n|^2) \quad (4)$$

$$Q_{ext} = Q_{scat} + Q_{abs} \quad (5)$$

$$Q_b = \frac{1}{x^2} \left| \sum_{n=1}^{\infty} (2n+1) (-1)^n (a_n - b_n) \right|^2 \quad (6)$$

where x is the parameter size and n is the spherical Bessel function order n .

The efficiency of radiation pressure can be proven by the Two-Stream Model and correlates with the asymmetry parameter [17].

$$Q_{pr} = Q_{ext} + Q_{scat}(\cos\theta) \quad (7)$$

where θ is the scattering angle.

Amplitude functions S_1 and S_2 indicate the scattering properties or the scattering of an electromagnetic wave from a spherical particle. The scattering function is required for the far field scatterer [16]:

$$S_1(\cos\theta) = \sum_{n=1}^{\infty} \frac{2n+1}{n(n+1)} (a_n \pi_n + b_n \tau_n) \quad (8)$$

$$S_2(\cos\theta) = \sum_{n=1}^{\infty} \frac{2n+1}{n(n+1)} (a_n \tau_n + b_n \pi_n) \quad (9)$$

where

$$\pi_n = \frac{2n-1}{n-1} \cos\theta \cdot \pi_{n-1} - \frac{n}{n-1} \pi_{n-2}$$

$$\tau_n = n \cos\theta \cdot \pi_n - (n+1) \pi_{n-1}$$

III. METHODOLOGY

The research is conducted using experimental and theoretical methods. The light absorption and scattering analysis in milk are based on Mie scattering theory. The scattering, absorption, extinction, and backscattering efficiencies are analyzed in a homogeneous dielectric sphere and its angular scattering using MATLAB. The analysis is also repeated for water and air.

A. Modeling Approach

The modelling part is used to determine the characteristics of light in a disordered medium using MATLAB. The light propagation efficiency with the justification of Mie coefficient matrix is computed. The angular functions are also computed to produce the Mie angular efficiency. Figure 1 shows the flowchart of the constructed modeling approach.



UNIVERSITI SAINS ISLAM MALAYSIA
جامعة العلوم الإسلامية الماليزية
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

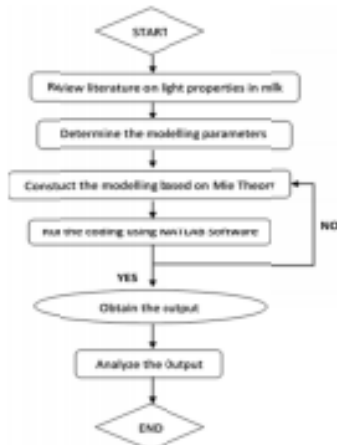


Fig. 1. The flowchart of the modeling approach.

B. 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 (the sample turns to yogurt after 14 days of air exposure). Figure 2 shows the flowchart of the experimental method.

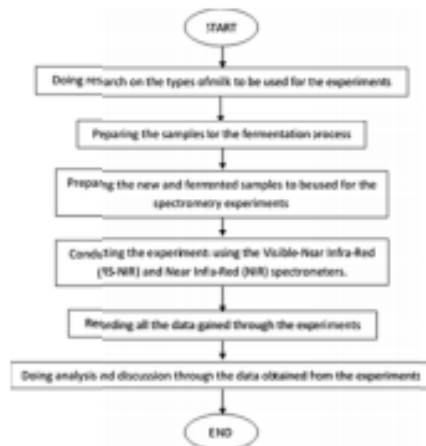


Fig. 2. The flowchart of the experiment.

The milk samples were kept at room temperature for 8, 11, 14, and 17 days. The samples were diluted with a ratio of 1:100 of milk and water respectively. The prepared samples were placed in a cuvette of 10mm and shaken lightly. The cuvette

was placed into a cuvette holder and was illuminated by a halogen lamp (the light source). The light from the cuvette was collected by the VIS-NIR spectrometer (Figure 3). The spectrum graph was displayed on a computer screen using the OceanView software. Figure 4 shows the milk samples used in the experiments. Milk samples of 8 and 11 days were still cloudy but milk samples after 14 days became transparent with large milk particles observed.

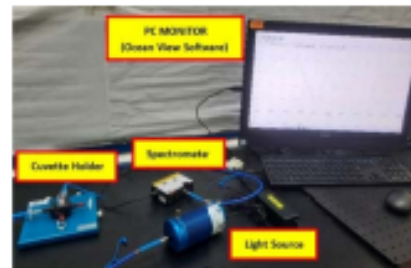


Fig. 3. The experimental set-up for the spectrometry technique.

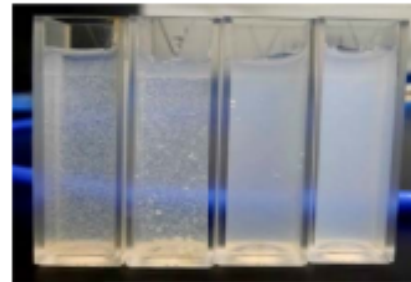


Fig. 4. Fermented full cream milk samples after dilution with water after 8, 11, 14, and 17 days (from right to left) of exposure.

The set-up for reflectance spectroscopy is different from the absorbance and transmission spectroscopy setup. It can be used in liquid samples and solids. NIR spectrometer was used to observe the reflectance intensity spectrum of the samples. The samples were placed on the stage RTL-T. The reflectance spectrum of a sample in the cuvette was observed by a fiber probe. The probe transmits the light from the halogen light and then it was placed on the stage RTL-T. The reflectance spectrum can be observed by using a NIR spectrometer when the light propagates inside the medium.

IV. RESULTS AND DISCUSSION

Previous works [5, 6] have analyzed fat and protein concentrations of milk through backscattering and spectroscopy. Authors in [7] studied the homogenization efficiency to control the development of the cream layer on fresh milk and authors in [8] used NIR spectroscopy to study yogurt fermentation automation. In this paper, we study the quality of milk which is kept at room temperature using VIS and NIR spectroscopy. We do 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 study are analyzed and discussed thoroughly in this section.

A. Modeling based on Mie Scattering Theory

The measurements of scattering, extinction and absorption efficiency based on Mie theory were conducted in MATLAB. The input parameters were the complex refractive index and the parameter size x [18]. Modeling was done for milk, water, and air. Figures 5 and 6 summarize the modeling results.

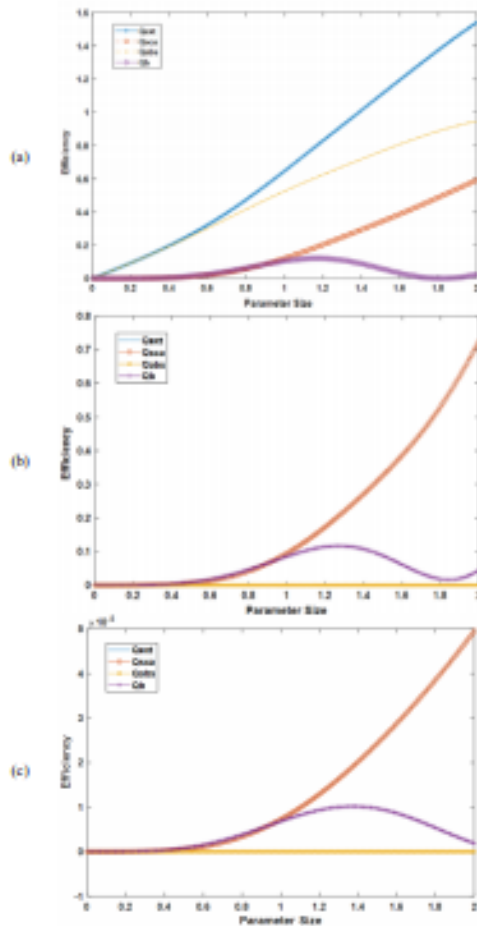


Fig. 5. Mie theory based efficiencies for (a) milk, (b)water, and (c) air.

Equations (3) to (7) were used in Figure 5. Figure 6 is plotted based on (8)-(9). The extinction, forward scattering, absorption, and backscattering efficiencies are represented by Q_{ext} , Q_{sca} , Q_{abs} and Q_b respectively. Figure 5 shows clearly that milk has better scattering efficiency than water and air. At parameter size 2, the scattering efficiency in milk (Figure 5(a)) reaches 0.6, while the scattering efficiency in water (Figure 5(b)) and air (Figure 5(c)) are 0.7 and 5×10^{-4} respectively, proving that the least light scattering occurs in the air, whereas 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 [19]. Milk depicts the highest efficiency of light absorption due to its composition of fat globules and proteins. Figure 5 also shows that the forward scattering is more efficient compared to the backscattering for all samples due to the larger particles size of the samples. Figure 6 shows the scattering angle of milk, water and air respectively.

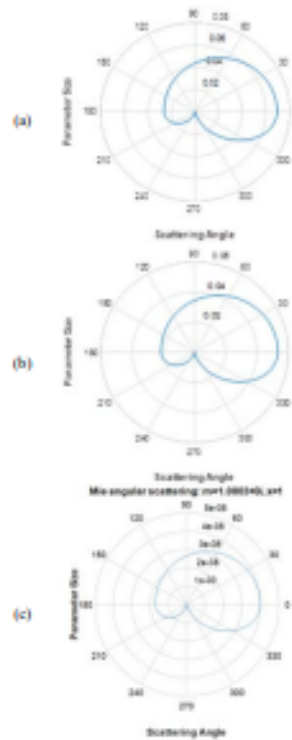


Fig. 6. Angular scattering for (a) milk, (b) water, and (c) air.

We observe that milk has larger value of angular scattering than water and air. It is clearly shown that milk has higher scattering effect. We attribute that to the milk contents which mostly consist of fat and proteins which can scatter light [20].



B. Experimental Results

The spectra of reflectance, transmission, and absorbance of the samples were monitored with the spectrometer. The samples of fresh full-cream milk were kept at room temperature for 8, 11, 14, and 17 days. The experiment utilized an NIR Spectrometer and a VIS-NIR Spectrometer with wavelength ranges of 950nm-1650nm and 350nm-1000nm respectively. Figure 7 depicts the absorbance, transmission and reflectance spectra of the milk samples.

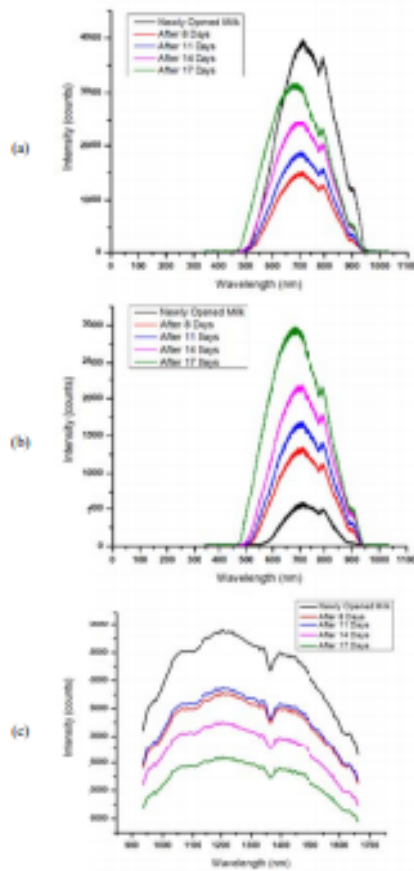


Fig. 7. (a) Absorbance, (b) transmission, and (c) reflectance of the milk samples for various days of fermentation.

Figure 7(a) shows the absorbance spectrum of the milk samples for different days of exposure. It is clearly shown that the absorbance spectra in freshly opened full-cream milk is higher than the milk that has been kept at room temperature for 17 days. The absorbance of milk reaches its peak at 700nm due to high attenuation coefficient which quickly absorbs the light

around 700nm. When milk samples are kept at room temperature, they undergo physical changes where the particles aggregate and create lumpy which affect the light absorption [21] (see Figure 4). Hence, the quality from fresh to fermented milk is gradually decreasing as the protein concentration decreases due to milk coagulation [22].

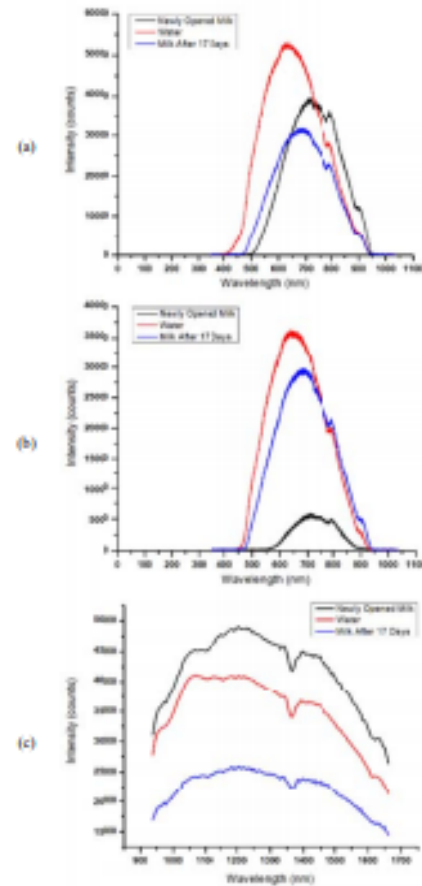


Fig. 8. (a) Absorbance, (b) transmission, and (c) reflectance of newly opened milk sample, water, and milk sample after 17 days.

Figure 7(b) shows the transmission spectra of freshly opened and fermented milks. The newly opened full-cream milk has lower light transmission than the fermented milk. The light transmission in fermented milk after 17 days is higher than in the rest of the samples as the particles in milk aggregate during the coagulation [21, 22]. The protein thickens due to the fermentation process which produces lactic acid [23]. The fermented milk experiences decay and the pH level decreases as the lactic acid bacteria grow to produce the acid [24].

UNIVERSITI SAINS ISLAM MALAYSIA
الجامعة الإسلامية العلوم
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

Figure 7(c) shows the reflectance spectra of the milk samples. The reflectance of newly opened milk is higher than the fermented milk's. The low reflectance values for fermented milk are recorded due to its high water absorption [25]. Hence, we suppose that the presence of fat globules and protein micelles in milk affect the light reflectance. The reflectance intensity decreases over the fermentation process due to the changes of protein and fat globules [26].

Figure 8 shows the spectra comparison of milk samples and water. Figure 8(a) shows that water absorbs most of the light at 600nm whereas the absorption peak of milk is at 700nm. Milk and water depict similar transmission peaks at ~ 650nm (Figure 8(b)). Water sample shows higher transmission spectrum as it is more transparent than milk. Newly opened milk samples have higher reflectance than water due to their fat and protein composition. The size and shape of particles, the composition, and the concentration of the tested samples can affect the absorption, transmission, and reflectance of the samples respectively [26]. Newly opened milk samples consist of various particle compositions whereas the fermented milk samples have experienced physical state changes.

V. CONCLUSION

In conclusion, this research investigates the quality of milk for samples freshly opened and after being kept at room temperature for several days using spectroscopy and scattering theory. The optical properties of milk samples were investigated using VIS and NIR spectrometers. Newly opened milk samples have higher light absorbance and lower light transmission compared to the fermented milk, due to the aggregation of the fat and protein particles in milk during the fermentation process. Besides that, modeling based on scattering theory was done to compare the 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 at room temperature for several days. This is proved by both naked eye observation and spectroscopy. The outcome of this study can be useful in supporting future analysis studies on dairy products.

ACKNOWLEDGEMENT

We acknowledge the support of the Ministry of Education of Malaysia under the FRGS grant (FRGS/1/2018/STG02/USIM/02/2) and Universiti Sains Islam Malaysia (USIM) for the funding and support.

REFERENCES

- [1] G. H. Schmidt, L. D. V. Vleck, and M. F. Hutjens, *Principles of Dairy Science*, Subsequent edition. Englewood Cliffs, NJ, USA: Prentice Hall, 1988.
- [2] A. Kienle, M. S. Patterson, L. Ott, and R. Steiner, "Determination of the scattering coefficient and the anisotropy factor from laser Doppler spectra of liquids including blood," *Applied Optics*, vol. 35, no. 19, pp. 3404-3412, Jul. 1996, <https://doi.org/10.1364/AO.35.003404>.
- [3] A. A. Ramazanka, A. A. Lizanova, A. K. Mazurenko, M. F. Kerechaniina, V. V. Ivanov, and S. V. Gaponenko, "Preparation and Optical Properties of Isopropanol Suspensions of Aluminum Nanoparticles," *Journal of Applied Spectroscopy*, vol. 87, no. 4, pp. 662-667, Sep. 2020, <https://doi.org/10.1007/s10812-020-01051-w>.
- [4] M. A. Ansari, M. Erfanzadeh, and E. Mohajerani, "Mechanisms of Laser-Tissue Interaction: II. Tissue Thermal Properties," *Journal of Lasers in Medical Sciences*, vol. 4, no. 3, pp. 99-106, 2013.
- [5] T. Katsumata, H. Aizawa, S. Komuro, S. Ito, and T. Matsumoto, "Quantitative analysis of fat and protein concentrations of milk based on fibre-optic evaluation of back scattering intensity," *International Dairy Journal*, vol. 109, Oct. 2020, Art. no. 104743, <https://doi.org/10.1016/j.idairy.2020.104743>.
- [6] M. Montenegro, A. Schwanighofer, A. Schleich, M. J. Colucci, H. E. Mayer, and B. Lendl, "High-throughput quantitation of bovine milk proteins and discrimination of commercial milk types by external cavity-quantum cascade laser spectroscopy and chemometrics," *Analyst*, vol. 144, no. 18, pp. 5571-5579, Sep. 2019, <https://doi.org/10.1039/C9AN00746F>.
- [7] E. Ranarumk, B. Svensson, I. Svodberg, A. G. Östman, and T. Skoglund, "Measurement of homogenisation efficiency of milk by laser diffraction and centrifugation," *International Dairy Journal*, vol. 96, pp. 95-97, Sep. 2019, <https://doi.org/10.1016/j.idairy.2019.04.011>.
- [8] A. Aljadhaf and H. Steiner, "Evaluation of Using NIR Simplified Spectroscopy in Yogurt Fermentation Automation," presented at the 8th International Conference on Researches in Engineering, Technology and Sciences, Istanbul, Turkey, Aug. 2015.
- [9] M. B. Alhamrani, E. H. Anzaz, and G. A. El-Hiti, "Spectroscopic Characterization, Hirschfeld Surface, DFT, and TD-DFT of tert-Butyl Phenethylcarbamate and 1,1-Dimethyl-3-Phenethylurea," *Journal of Applied Spectroscopy*, vol. 87, no. 4, pp. 736-744, Sep. 2020, <https://doi.org/10.1007/s10812-020-01063-6>.
- [10] F. Alorifi, S. M. A. Ghaly, M. Y. Shalaby, M. A. Ali, and M. O. Khan, "Analysis and Detection of a Target Gas System Based on TDLAS & LabVIEW," *Engineering, Technology & Applied Science Research*, vol. 9, no. 3, pp. 4196-4199, Jun. 2019, <https://doi.org/10.48084/etasr.2736>.
- [11] M. M. Nadarishvili, G. Marniasvili, D. Jishiasvili, G. Abreashvili, C. Ramana, and J. Ramden, "Investigation of the Visible Light-Sensitive ZnO Photocatalytic Thin Films," *Engineering, Technology & Applied Science Research*, vol. 10, no. 2, pp. 5524-5527, Apr. 2020, <https://doi.org/10.48084/etasr.3392>.
- [12] N. A. Bakar, Haize Cui, A. Abu-Siada, and Shengtao Li, "A review of spectroscopy technology applications in transformer condition monitoring," in *2016 International Conference on Condition Monitoring and Diagnosis (CMD)*, Xi'an, China, Sep. 2016, pp. 372-375, <https://doi.org/10.1109/CMD.2016.7757895>.
- [13] R. A. Viscarra Rossel, D. J. J. Walvoort, A. B. McBratney, L. J. Janik, and J. O. Skjenstad, "Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties," *Geoderma*, vol. 131, no. 1, pp. 59-75, Mar. 2006, <https://doi.org/10.1016/j.geoderma.2005.03.007>.
- [14] N. A. I. Mahamad Kamil *et al.*, "Investigating Light Propagation in Full and Skimmed Milk based on Spectroscopy and Monte Carlo Analysis," *Journal of Engineering and Applied Science*, vol. 67, no. 8, pp. 2265-2278, Dec. 2020.
- [15] C. Matzler, "MATLAB functions for Mie scattering and absorption," Institut für Angewandte Physik, Bern, Switzerland, Research Report 2002-08, Jun. 2002.
- [16] K. L. van der Molen, "Experiments on scattering lasers: from Mie to random," Ph.D. dissertation, University of Twente, Enschede, Netherlands, 2007.
- [17] W. E. Meador and W. R. Weaver, "Two-Stream Approximations to Radiative Transfer in Planetary Atmospheres: A Unified Description of Existing Methods and a New Improvement," *Journal of the Atmospheric Sciences*, vol. 37, pp. 630-643, Mar. 1980.
- [18] Y. Ren, H. Qi, X. Yu, and L. Ruan, "A forward-angle-scattering method for the determination of optical constants and particle size distribution by collimated laser irradiation," *Optics Communications*, vol. 389, pp. 258-264, Apr. 2017, <https://doi.org/10.1016/j.optcom.2016.12.060>.
- [19] D. J. Dahm, "Explaining Some Light Scattering Properties of Milk Using Representative Layer Theory," *Journal of Near Infrared Spectroscopy*, vol. 21, no. 5, pp. 323-339, Oct. 2013.



-
- [20] L. C. Andrews, *Field Guide to Atmospheric Optics, Second Edition*, 2nd ed. Washington, DC, USA: SPIE--The International Society for Optical Engineering, 2019.
- [21] C. Hahn, M. Sramek, S. Nöbel, and J. Hinrichs, "Post-processing of concentrated fermented milk: influence of temperature and holding time on the formation of particle clusters," *Dairy Science & Technology*, vol. 92, no. 1, pp. 91–107, Jan. 2012, <https://doi.org/10.1007/s13594-011-0046-1>.
- [22] S. L. Thomsen, S. L. Jacques, and S. T. Flock, "Microscopic correlates of macroscopic optical property changes during thermal coagulation of myocardium," in *Laser-Tissue Interaction*, Jan. 1990, vol. 1202, pp. 2–11, <https://doi.org/10.1117/12.17605>.
- [23] M. Lu et al., "Milk Spoilage: Methods and Practices of Detecting Milk Quality," *Food and Nutrition Sciences*, vol. 4, no. 7, pp. 113–123, Jul. 2013, <https://doi.org/10.4236/ins.2013.47A014>.
- [24] R. Fernandes, Ed., *Microbiology Handbook: Dairy Products*, 3rd ed. Cambridge, UK: Royal Society of Chemistry, 2009.
- [25] B. Aernouts, E. Polshin, J. Laensmeryn, and W. Saey, "Visible and near-infrared spectroscopic analysis of raw milk for cow health monitoring: Reflectance or transmittance?," *Journal of Dairy Science*, vol. 94, no. 11, pp. 5315–5329, Nov. 2011, <https://doi.org/10.3168/jds.2011-4354>.
- [26] J. A. Raty and K.-E. Peiponen, "Reflectance Study of Milk in the UV-Visible Range," *Applied Spectroscopy*, vol. 53, no. 9, pp. 1123–1127, Sep. 1999.



APPENDIX C

%Scattering calculation by Monte Carlo amplified by gain for a simple
%3D scattering medium.

%(1)declaration of variables

```
lambdap=532E-9;% pump wavelength(532nm)
ss=100; %scatterers density(1/m^3)
sccros=6.793E-13; %scattering cross section
ls=1/(ss*sccros); %scattering mean free path
c = 0.4; % attenuation coefficient (1/m)
g=0.9; %scattering asymmetry factor
w0 = 0.8; % single-scattering albedo
N = 1e5; % number of photons to trace
ns = 100; % max number of scatters per photon
E = zeros(N,2); % initialize detector
Epump = 100E-3; %Pumping energy(2mJ)
planck = 6.63E-34; %planck constant
light = 3E8; %speed of light
Cpabs =3.7E-20; %absorption cross section at pumping wavelength( $3.7 \times 10^{-20} \text{m}^2$ )
Ndye=100; %Concentration of dye
```

%(2)Reading values for measured fluorescence spectrum

```
tic %Start counting time for simulation
[lambda, y] = textread('pol_gold_short.txt','%f%f'); % dye spectrum from text file
%wavelength distribution from dye spectrum (530-700nm)
cdf=cumsum(y/(sum(y))); %cumulative sum of intensity value
%plot(x,cdf); %plot the cumulative sum
```

%(3)Photons start doing loop

```
for i=1:N %start of photon loop
p=[0,0,0]; % initial position (x,y,z)
L=0;% summation of path length
%muz = sqrt(rand);% diffuse illumination
muz = 1; %Direction of laser through z direction
phi = 2*pi*rand; % azimuthal scattering angle
%mux = sqrt(1-muz^2)*cos(phi); % initial x-direction cosine
mux = 0; %Direction of laser
%my = sqrt(1-muz^2)*sin(phi); % initial y-direction cosine
muy = 0; %Direction of laser
r=0; % initial distance from source
w = (Epump * lambdap)/(planck * light * N); % initial photon weight
```

```
[~b]=min(abs(cdf-rand)); %the minimum of random number to cumsum
```

```
Plambda=lambdap(b); %wavelength of emitted photon.
```

```
E(i,2)=lambdap(b); %intensity detection for each lambda
```

%(4) Scattering loop

```

for j=1:ns
s = -ls*log(rand); % geometric path length
p = p + s*[mux,muy,muz]; % move photon
L=L+norm(s*[mux,muy,muz]); %updated total path length
    s = - log(rand)/c; % geometric path length
    %z = z + muz*s; % move photon

%Photon detection condition (Limit the size of sample)
if (p(3)<0 && p(3)<0.01) %Photon inside the sample(<1cm) is detected. z axis is
chosen,p(3).p(1)=x axis,p(2)=y axis
%if (p <0)
    E(i,1)=E(i,1)+w;
else
    E(i,1)=0;
    %break,
end % count photons leaving out top

%scattering events based on Henyey-Greenstein scattering
w0 = exp(Cpabs * Ndye * s); % scattering albedo using gaussian function
w = w*w0;%absorb fraction of photon packet
mu= 1 - 2*rand; % isotropic scattering
%mu= (1+g^2-((1-g^2)/(1+g-2*g*rand))^2/(2*g)); % Henyey-Greenstein scattering
phis = 2*pi*rand; % azimuthal scattering angle
[mux,muy,muz]=chgdir(mux,muy,muz,mu,phis); % new direction
muz=muz*mu-sqrt(1-muz^2)*sqrt(1-mu^2)*cos(phis); %new direction for z
mux=mux*mu-sqrt(1-mux^2)*sqrt(1-mu^2)*cos(phis);%new direction for x
muy=muy*mu-sqrt(1-muy^2)*sqrt(1-mu^2)*cos(phis);%new direction for y

end %end of scattering loop
L=L+norm(s*[mux,muy,muz]); %updated total path length

%(5)amplification by dye within photon loop(Assume all dyes excited
Ndye=10E-3; %Concentration of dye (10^-3Molar)
Cemi=E(i,2)*57E-9/pi; %stimulated emission cross section
gain=exp(Cemi*Ndye*L);% gain value assuming all dye molecules are excited
Ndyefact=exp(gain*L); %amplification factor for the dye molecules

end % end of photon loop

%(6)Intensity detection
E(:,1) = E(:,1)/N; % Intensity normalized to no of photon
Enorm=E(:,1).*Ndyefact; %Intensity normalized to no of photons after amplification

[histlambda,indx]=histc(E(:,2), lambda); %Build histogram graph for intensity
detection
plotout=accumarray(indx,E(:,1),[length(lambda) 1]); %accumulate array of intensity
detection
y=(plotout.*histlambda);%/max(plotout.*histlambda);

```

```
plot(lambda,y); %plot graph lambda vs hist of graph  
toc %Finish counting time for simulation
```

