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DETERMINATION OF FAT AND PROTEIN CONTENT OF MILK USING He-Ne LASER LIGHT SCATTERING

By

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Ermias Abebe

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To my sister, Menbere

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Abstract

The main aim of of this thesis is to build up simple, an accurate and fast method for the determination of fat and protein content in milk. Based on the laser scattering theory, the ratio of the scattered light intensity to the transmitted light intensity, which is called scattered-transmitted method,is taken on as the optical parameter characterizing the fat and protein content. In this way, the influence of the fluctuation of the power of the light source is reduced and the accuracy of determination is enhanced consequently. The method we used can be implemented in diary industries due to its simple methodology and its economic importance because all milk products are sold on basis of fat and protein content.Finally, the data analysis is carried out using curve and surface fitting method. The fat contents of mama and shola homogenized milk types are determined to be 2.9% and 2.7%, respectively. The protein content of mama milk is 3.6% and for shola milk is 3.4%.

Introduction

Laser (Light Amplification by stimulated emission of radiation) has some particular and unique characteristics. These include its monochromatic (the single wave length color of a laser beam), its ability to be collimated (the parallel nature of the laser beam), time and space coherence (synchronized phase of the light waves), its brightness, high power intensity and its focusing to a very small spot. All these properties have helped to the application of laser for many industrial and scientific uses. A helium neon (He-Ne) laser is a type of small gas laser. Its usual operation wave length is 633nm, in the red portion of the visible spectrum. It was first invented by Ali Javan, William Bennet Jr. and Donald Heriot at bell Labs [1].

In this paper, we used He-Ne laser for the determination of fat and protein content in the milk. It has been so difficult for breading organization, the diary industries as well as for control laboratories to determine fat and protein content rapidly, precisely and with less labor cost. Most diary industries widely used the chemical analysis methods for measuring the milk components. The most prevalent chemical methods for measuring fat content are the Geber method, the Rose-Gottelieb method, the Babcock method and the Tesa method. The chemical methods for measuring protein content mainly include the Kjiedhal determination of N-methods, the Udy dye binding method, the formal titration method, etc. The Kjiedhal determination of N-methods

is internationally accepted and widely used in dairy industries as well as in control laboratories [2, 3]. However, these chemical methods for measuring fat and protein content in milk are expensive, time, labor consuming and low in frequency. These methods have been partially replaced by rapid methods such as spectroscopy analysis methods (including the infrared, middle infrared, near infrared and the ultraviolet, etc) and ultrasonic method [4, 5]. Even though spectroscopic analysis method are most preferable and more precise nowadays, they are too complex instrumentally and very expensive.

For many years measurement techniques based on laser light scattering theory have been applied to obtain information about the size, structure, shape, composition and concentration of substances [6, 7]. In this thesis, the determination fat and protein content in the milk is carried out by dual-angle Laser scattering method. That is, scatter light intensity (at a scattering angle, $90^o \pm 0.1^o$) and the transmitted light (at transmitted angle, $0^o \pm 0.1^o$) intensities were measured experimentally and the ratio of the scattered light to the transmitted light intensity was calculated as an optical parameter to determine the fat and protein content. We assigned this ratio as scattered-transmitted ratio (STR). The basic aim of using the STR is to reduce the effect of fluctuation of the power light source. This in turn improves the accuracy and precision of the experiment.

Chapter 1

Interaction of He-Ne laser beam with milk components

Milk is an emulsion of butter fat globules with a water-based fluid. Each fat globe is surrounded by a membrane consisting of phospholipid and protein. These emulsions keep the individual globules from joining together into noticeable grains of butter fat[8]. In general, milk can be described as a solution of lactose, soluble protein, fat minerals, vitamins and other components.

when a beam of light is incident on the milk solution, various light-milk interactions happen. These interactions can occur at the surface or inside the milk components and the rest of light penetrates the milk solution. Due to this interaction of light with the milk components there might be reflection on the surface and attenuation due to scattering and absorption inside the milk by internal components [9].

The absorption properties are strongly dependent on the wave length of the incident light while reflection and scattering have negligible effect on the wave length of the incident light. All these processes give a concrete information about the size, concentration, motion and shape of the constituents of milk. The information that

we obtain has a paramount importance in calculating the concentration of the constituents for this specific work.

1.1 Transmission of He-Ne laser beam through milk solution

When a collimated He-Ne laser beam impinges on a sample ,only a fraction of the light energy is transmitted through the sample. The rest is lost by three processes. These processes are reflection,absorption and scattering. Figure (1.1) illustrates the interaction between the Laser light and the sample.

Assume that the total incident light intensity is I_o, the reflected intensity is I_r, absorbed intensity I_a, transmitted intensity I_t and the scattered intensity I_s. According to the conservation principle we relate these quantities as

$$I_o = I_r + I_s + I_a + I_s \tag{1.1.1}$$

1.2 Reflection

Light intensity will be reflected because of a refractive index mismatch between the air and the sample boundary [10]. The intensity of the reflected light doesn't only depend on the difference in refractive index mismatch but also depend on polarization, Angle of incident light, structure and shape of the surface.

If irradiance of the incident beam is I_o, there will be a reflected beam of irradiance, I_r back into air. The ratio $\frac{I_r}{I_o}$ is called reflectance (R).

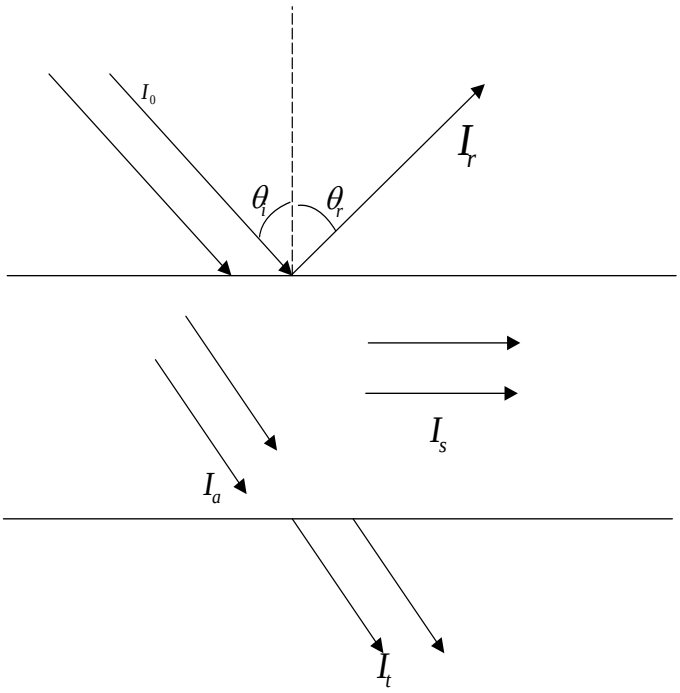

Figure 1.1: reflection, absorption, scattering and transmission of light in a sample

The reflectance, R can be expressed using Fresnel law

$$R = \frac{1}{2}\left[\left(\frac{n_1\cos(\theta_i) - n_2\cos(\theta_t)}{n_1\cos(\theta_i) + n_2\cos(\theta_t)}\right)^2 + \left(\frac{n_2\cos(\theta_t) - n_1\cos(\theta_t)}{n_2\cos(\theta_i) + n_2\cos(\theta_t)}\right)^2\right] \tag{1.2.1}$$

Where n_1 and n_2 are the reflective indices for the external medium (air) and the material respectively, θ_i is the incident angle, and θ_t is the transmitted angle θ_t is given by Snell's law as

$$n_1 \sin\theta_i = n_2 \sin\theta_t \tag{1.2.2}$$

or

$$\theta_t = \sin^{-1}\left(\frac{n_1}{n_2}\sin(\theta_t)\right) \tag{1.2.3}$$

For a beam perpendicular to the sample and the external medium is air, $n = 1$ and the index refraction of the sample, $n_2 = n$, the reflectance can be given by

$$R = \left(\frac{n-1}{n+1}\right)^2 \tag{1.2.4}$$

Since index of refraction of milk is 1.462 [11], the reflectance has been evaluated to be about 3.5%. This shows that about 3.5% of a He-Ne laser incident intensity is reflected back to air.

1.3 Absorption

The beam that is propagating through the material is very often absorbed by the material. The light interacts with the material in variety of ways, including excitation of electronic transition and molecular vibration. This leads the energy of the electromagnetic radiation to convert into different forms of energy in the milk solution.

The absorption property of an ensemble of particles is represented by the absorption coefficient $\mu_a(mm^{-1})$, defined as

$$\mu_a = \frac{The\ total\ power\ absorbed\ by\ particles\ in\ unit\ volume}{The\ intensity\ of\ incident\ power} \tag{1.3.1}$$

$$\mu_a = \frac{I_a}{I_o} \tag{1.3.2}$$

The inverse of the absorption coefficient $\frac{1}{\mu_a}$ (mm) is the absorption length, which is the mean distance traveled by photons before it gets absorbed. Thus, the absorption of He-Ne laser light by the milk solution helps to investigate the property of the components of milk.

1.4 Scattering

Scattering of light in milk solution is caused by the refractive index mismatch at microscopic boundaries such as fat globules, protein, minerals, vitamins and many others. Scattering changes the angular distribution of the incident beam when it interacts with a particle as shown in the figure (1.2). The scattering properties of an

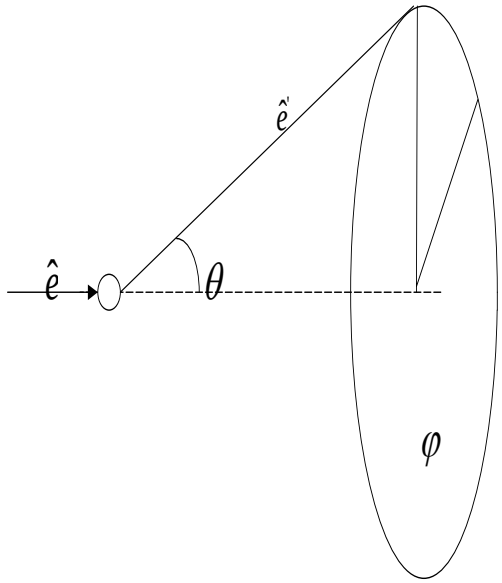

Figure 1.2: The scattering of light by a single particle from incident direction ê

ensemble of particles are expressed by by the scattering coefficient, μ_s (mm^{-1})

$$\mu_s = \frac{I_s}{I_o} = \frac{The\ total\ power\ scattered\ by\ particles}{The\ intensity\ of\ incident\ power} \tag{1.4.1}$$

The inverse of the scattering coefficient, $\frac{1}{\mu_s}$, is the mean free path between two successive scattering events (mm). The scattering coefficient is therefore a useful parameter in determining the concentration of fat and protein in the milk.

Chapter 2

The Beer-lambert law

In optics, the Beer Lambert law is an empirical relationship that relates the absorption and scattering of light to the properties of the material through which light is traveling [12].

When a beam of light is passed through matter in solids, liquids or gaseous states, its propagation is affected by two important ways,

- The intensity will always to a great or lesser extent will decrease as the light penetrates further into the medium.
- The velocity of light will be less in the medium than in free space.

The loss of intensity is chiefly due to absorption and scattering. If monochromatic light is passed through a certain thickness of solid or of liquid enclosed in a transparent cell, the intensity of the transmitted light may be much smaller than that of the incident beam of light, owing to absorption. However, the decrease in intensity of the light beam is not only due to absorption . Some of light passing through the material is scattered, i.e. a fraction of a beam does not continue in its original straight path but is deflected to other direction, due to the fact that the velocity of light in matter differs from that in vacuum.

2.1 Derivation of Beer-Lambert law

The parameter used to describe the interaction of electromagnetic radiation with matter is the complex index of refraction, Which is the combination of a real part and an imaginary part

$$\tilde{n} = n - ik \tag{2.1.1}$$

Here, n is also called the index of refraction and k is the damping of an electromagnetic (EM) wave inside the material due to absorption and scattering.

An EM wave travels in the material with velocity, v and angular frequency, ω, the time varying electric field of the wave is described by

$$E(z,t) = E_0 \exp i\omega\left(t - \frac{z}{v}\right) \tag{2.1.2}$$

Where only the real part of E has physical significance. For simplicity, the radiation is assumed to be a plane wave, and its direction of propagation is defined by z. The index of refraction $\tilde{n}$ is defined to be the ratio of the speed of light in a vacuum, c to the speed of the EM wave in the medium, v

$$\tilde{n} = \frac{c}{v} \tag{2.1.3}$$

substituting equation (2.1.3) into equation (2.1.1)gives

$$\frac{1}{v} = \frac{n}{c} - i\frac{k}{c} \tag{2.1.4}$$

Again, substituting equation (2.1.4) into equation (2.1.2) yields

$$E(z,t) = E_0 \exp i\omega\left(t - z(\frac{n}{c})\right) \exp -\left(\frac{k\omega}{c}\right)z \tag{2.1.5}$$

The expression in equation (2.1.5) describes a propagating electromagnetic wave with an exponentially damped amplitude due to k-term. This term causes the EM to reduce as it travels further in the material. The intensity of the wave, which corresponds to the energy it carries with it, is simply the square of the magnitude of the waves electric field. The intensity of the wave is therefore

$$I(z) = I_0 \exp -\left(\frac{2\omega k}{c}\right) z \tag{2.1.6}$$

According to the Beer Lambert law, if we consider z as an axis parallel to the direction of motion of the photons, and A, dz and x as the area, the thickness (along the z-axis) and the concentration of particles in a three dimensional slab through which light is traveling, then the fraction of photons absorbed in the slab is equal to the total opaque area of the particle in the slab, $\mu_t A x dz$, divided by the area of the slab, or $\mu_t x dz$.

Expressing the number of photon absorbed by the slab as dI_z, and the total number of photons on the slab as I_z, the fraction of photons absorbed by the slab is given by

$$\frac{dI_t}{I_t} = -\mu_t x dz \tag{2.1.7}$$

The solution to equation (2.1.7) can be obtained by integrating both sides as a function of z.

$$\ln(I_z) = -\mu_t x z + c \tag{2.1.8}$$

For the slab of real thickness, d, the difference in light intensity I_0 at $z = 0$, and I_t at $z = d$, is given by

$$I_t = I_0 \exp(-\mu_t x d) \tag{2.1.9}$$

Where $\mu_t = \frac{2\omega k}{c}$, which represents the extinction coefficient of an EM wave due to scattering and absorption.

2.2 Application of the Beer lambert law for testing the milk fat content

Applying the Beer-Lambert law of equation (2.1.9), when a laser beam passes through the fat milk solution, we have

$$I_t = I_o \exp(-\mu_t x_1 d) \tag{2.2.1}$$

Where, I_t represents the intensity of the transmitted light, I_0 is the intensity of the incident light, x_1 represents the concentration of fat in the solution, d representees the thickness of the cuvette which is assumed to be the distance that light travels in the milk solution. μ_t is the total extinction coefficient which defined as

$$\mu_t = \mu_{sf} + \mu_{af} \tag{2.2.2}$$

In equation (2.2.2), μ_{sf} and μ_{af} describe scattered and absorption coefficient of fat in the solution, respectively.

However, the absorption coefficient of the fat in thin milk solution is very weak ($0.003cm^{-1}$) at a wave length of 633nm. In contrast the scattering coefficient ($1.50cm^{-1}$) of a milk solution is much larger than its absorption coefficient ($\mu_{sf} \gg \mu_{af}$)[13]. so the extinction coefficient is approximately equal to the scattering coefficient.

Hence, equation(2.2.1)can be rewritten as

$$I_t = I_o \exp(-\mu_{sf} x_1 d) \tag{2.2.3}$$

According to the energy conservation principle: (i.e energy balance of incident, scattered, transmitted and absorbed light energy), we have

$$I_o S_o = \oint_s I_{si} ds_i + I_t S_o \tag{2.2.4}$$

Where S_o is the cross-sectional area of the incident beam, I_{si} represents the intensity of the scattered light of fat in i direction and ds_i is the cross-sectional area of the scattered light. Suppose

$$I_s S_R = \oint_s I_{si} ds_i \tag{2.2.5}$$

Where I_s is the intensity of the scattered light perpendicular to the incident light and S_R is the corresponding spherical cross-sectional area. Then equation (2.2.5) can be rewritten as

$$S_R = \oint_s \frac{I_{si} ds_i}{I_s} \tag{2.2.6}$$

Substituting equation (2.2.6)into equation (2.2.5), we obtain

$$I_o = C_1 I_s + I_t \tag{2.2.7}$$

Where $C_1 = \frac{S_R}{S_o}$. C_1 is called uniform scattered coefficient. For thin milk solution, C_1 is a constant. Taking the ratio of equation (2.2.7) and equation (2.2.3), we get

$$Y_1 = \frac{I_S}{I_t} = \frac{1}{C_1}\left[\exp(\mu_{sf} x_1 d) - 1\right] \tag{2.2.8}$$

Or equation (2.2.8) can be written as

$$Y_1 = \frac{1}{C_1}(\exp(\mu_1 x_1) - 1) \tag{2.2.9}$$

Where $\mu_1 = \mu_{sf} d$ and Y_1 describes the scattered -transmitted ratio (STR) of the laser beam. Since equation (2.2.9) is an exponential expression, we can approximate

the expression by using the appropriate polynomial function. Therefore, equation (2.2.9) can be approximated by using Taylor series expansion. Note that, if f and its derivative are defined throughout a closed interval [a,b] containing k, and if x is in that interval, then

$$f(x) = f(k) + f^{'}(k)(x-k) + \frac{f^{''}k(x-k)^2}{2!} + ... + \frac{f^n k(x-k)^n}{n!} \tag{2.2.10}$$

So, the expression, $\exp \mu_1 x_1$ in equation (2.2.9) can be expanded as

$$\exp \mu_1 x_{1\simeq} \exp \mu_1 k + \mu_1 \exp \mu_1 (x_1 - k) + \frac{\mu_1^2 \exp \mu_1 k (x_1 - k)^2}{2} + ... \tag{2.2.11}$$

Since the concentration of fat, x_1, is small so that the series is only approximated to the second term and k is taken to be zero. Substituting equation (2.2.11) into equation (2.2.9), we obtain

$$Y_1 = \frac{1}{C_1}\left[\frac{\mu_1^2 x_1^2}{2} + \mu_1 x_1 - 1\right] \tag{2.2.12}$$

Equation (2.2.12) is an approximate expression relation between scattered-transmitted ratio, Y_1 and and the concentration of fat, x_1. If we can get Y_1 from the experiment, the concentration of fat in the milk solution can be calculated using equation (2.2.12).

2.3 Application of the Beer lambert law for testing the milk protein content

Following similar mathematical approach as we used in deriving the relation between Y_1 and x_1, we can deduce the expression describing the relation between the three quantities: the STR (Y_2) of the thin milk solution in which both the fat and the protein exist, and the concentration of the fat (x_1) and the concentration of protein

(x_2)in the milk solution. The expression can be written as

$$Y_2 = \frac{1}{C_2}\left[(\exp \mu_{sf} x_1 d + \mu_{sp} x_2 d) - 1\right] \tag{2.3.1}$$

Where Y_2 represents the STR of the milk solution in which both the fat and the protein exist, x_2 represents the concentration of protein in the milk solution, x_1 represents the concentration of the fat in the milk concentration, C_2 represents the uniform scattered coefficient, μ_{sf} and μ_{sp} represents the scattered coefficient of the fat and the protein in the milk respectively, and d represents the distance that light travels in milk.

In the same way, equation (2.2.12) can be expanded into Taylor series to the quadratic term as

$$Y_2 = \frac{1}{C_2}\left[\frac{1}{2}\mu_1^2 x_1^2 + \frac{1}{2}\mu_2^2 x_2^2 + \mu_1 x_1 + \mu_2 x_2 + \mu_1 \mu_2 x_1 x_2 - 1\right] \tag{2.3.2}$$

Here, μ_1=$\mu_{sf} d$ and $\mu_2 = \mu_{sp} d$. Y_1 is measured experimentally and x_1 can be calculated by applying equation (2.2.12). The protein concentration, x_2 is determined by substituting the value x_1 and using the experimental value Y_2 into equation (2.3.2).

Chapter 3

Experimental set up and methodology

3.1 Experimental method

The experimental work was done mainly in two parts. These parts were focused on measuring the transmitted and scattered laser light intensity with respect to the incident beam direction.

First the scattered and transmitted light intensity were measured by diluting the milk with different proportion of ethylene diamine tetra acetic acid (EDTA) solution to carry out the fat content in the milk. The second experiment was performed to investigate the protein content by diluting the milk with different proportion of distilled water. The corresponding scattered and transmitted laser light intensity were also measured experimentally.

3.2 Measurement of scattered -transmitted ratio (STR) to determine the fat content

Before the concentration was examined by the system we designed, chemical reagent was needed to dilute the milk samples. The reagent we used is (EDTA) solution. The

primary advantage of using this EDTA solution is to dissolve the protein in the milk with out dissolving the fat in the milk [14]. This solution makes the milk to contain only fat molecules. Therefore, we can easily determine the concentration of the milk by measuring the (STR) from the Experiment and using curve fitting analysis.

3.3 Measurement of Scattered-transmitted ratio (STR) to determine the protein content

The second Experiment was done to determine the protein content in the milk samples. In order to find out the protein concentration, we first diluted the milk by distilled water. When the milk is diluted with distilled water, it contains both fat and protein. Since the fat concentration has already been determined in the first experiment so that the protein concentration would be easily calculated using surface curve fitting method.

3.4 Milk samples

Two samples of milk have been selected for the experiment. These milk samples are commercially produced Shola and Mama homogenized milk types. The aim of using homogenized milk type for the experiment is that homogenization makes the components of the milk to have uniform size. The fact that the scattered constants are correlated with the size of the particles tasted homogenization will result in forming the fat and protein to become the same size.

The average diameter of the fat in the milk after it is homogenized is 2000nm, and that of the protein is 120nm [18]. The other particles (the lactose, the inorganic salt, etc) have much smaller diameter compared to the fat and the protein. Thus, only the

big molecules of fat and protein can cause obvious light scattering according to Mie scattering.On account of this light scattering of the other particles in the milk solution can be ignored. Therefore, the scattered light of the laser has only a profound effect on the protein and fat particles in such away that the STR can absolutely determine the content of fat and protein in the milk solution.

3.5 Experimental methods and procedures

A 5mw He-Ne laser source emitting at 632.8nm (Melles Griot), operating in continuous wave, was first modulated using chopper controller (Stanford research systems SR 548) and directed horizontally to the sample box of volume, 1cm× 1cm ×3cm, which contained the milk solution as shown in the figure (3.1). The reference signal was obtained from the chopper controller and fed into the Lock-in amplifier (Stanford research system SR 830 DSP). Then after we put three photodiodes (New port 818 SL) around the sample box. The two photodiodes were placed perpendicular to the sample box so as to detect the scattered light intensity, I_s, with respect to the initial beam intensity, I_o. We aligned the photodiodes to detect the scattering intensity at an angle, $90^o \pm 0.1^o$ with respect to the initial light intensity. After connecting the photodiodes to the lock -in amplifier, the corresponding scattered light intensity were read from the digital oscilloscope.

The third photodiode was placed parallel to the initial beam direction at an angle of $0^o \pm 0.1^o$ so that the collimated transmitted light intensity, I_t passing through the sample was measured by connecting the photodiode to the lock-in Amplifier. The photodiode we used had a sensitivity in the visible range of wavelength (390nm-780nm). The diaphragm, aperture 1mm, was mounted on each photodiode to screen

off optical noise.

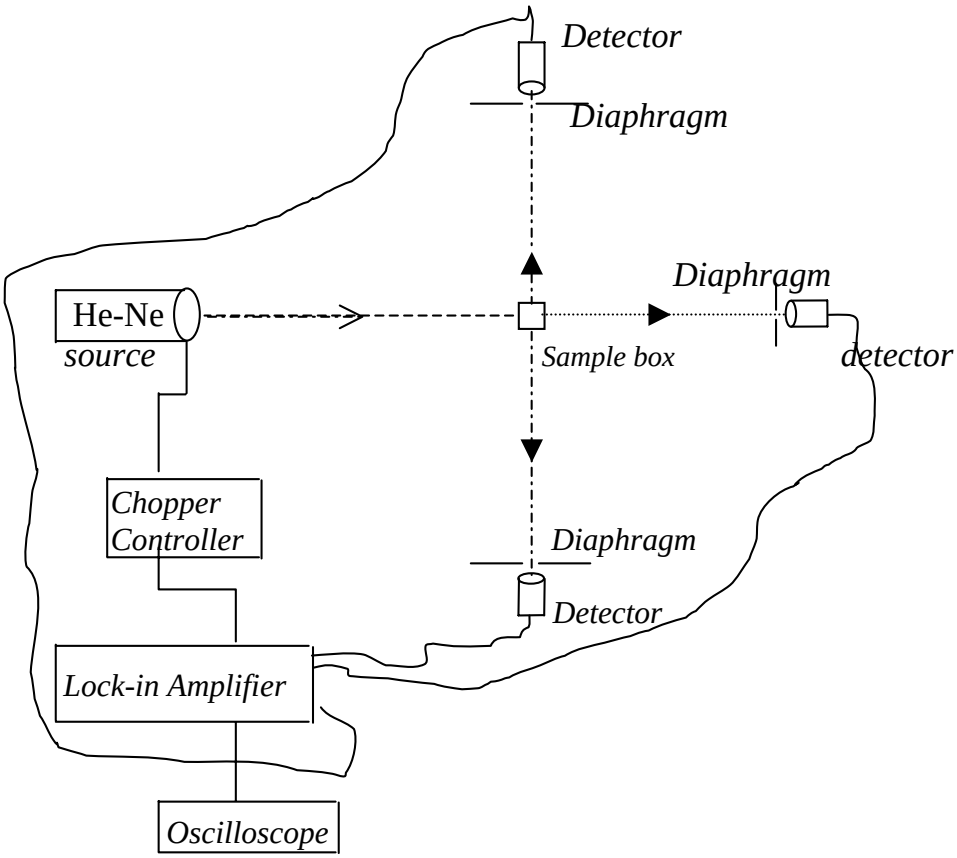

Figure 3.1: The schematic diagram of the experimental setup.

3.6 Experimental procedures for the determination of fat content

The determination of fat content were carried out using the following experimental procedures,

- We used 100ml of EDTA solution .

- we initially added 10ml of milk to the EDTA solution and read the transmitted and scattered light intensities.
- By increasing the concentration of the milk in steps of 2ml, we read the corresponding transmitted and scattered light intensities for each increment of the milk concentration.
- We analyzed the relation ship between scattered-transmitted ratio (STR) with the concentration milk.

3.7 Experimental procedures for the determination of protein content

The following experimental procedures were performed for the determination of protein content in the milk samples.

- We used 100ml of distilled water.
- We initially added 10ml of milk to the distilled water and the the transmitted and scattered light intensities.
- By increasing the concentration of the milk in step of 2ml, we read the corresponding transmitted and scattered light intensities for each each increment of the milk concentration.
- We analyzed the relation between scattered-transmitted ratio (STR) with the concentration of milk.

3.8 Description of the apparatus

3.8.1 The Lock-in Amplifier

A lock-in Amplifier is a device which is used to detect a very small signal all the way down to a few nano volts. Its function is to single out the components of the signal at specific frequency or phase. In the Experiment we used a Lock-in Amplifier in order to improve both accuracy and reliability of our measurement. It ensures good signal to Noise ratio (SNR). Noise signals at frequencies other than the reference frequency are rejected and do not affect the measurement.

A Lock-in Amplifier is used together with a low band filter to remove much of unwanted Noise while allowing through the signal of interest. To achieve the objective of to detecting weak signals, the REF IN channel supply the same frequency and phase to the Lock-in Amplifier to ensure that the instrument 'track' any changes to the frequency of the signal of interest since the original signal is locked in. The reference signal must be at the same frequency with the signal of interest. The REF out terminal from the chopper controller supplied the reference signal. The signal was observed on a Tektronix TDS 350 oscilloscope and the values of the received intensities in arbitrary units were read from the digital oscilloscope.

3.8.2 Optical chopper

The model SR 540 optical chopper is used to square wave modulate the continuous light signal of He-Ne laser light. The optimum chopper frequency must be set so that the Lock-in Amplifier measures only the required signal out of the ambient Noise signal.

3.8.3 The photo detector

The out put current is proportional to the the laser power that entered to the detector. The coefficient of proportionality depends on the wavelength of the laser source and can be varied by changing the supply voltage. Hence, the photo detector maintained a linear response with the incident radiation. The photodiode we used was a New Port Model 818 SL. It had a maximum sensitivity in the red portion of the visible electromagnetic radiation.

Chapter 4

Result and discussion

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4.1 Curve and surface fitting method

We have analyzed our experimental result using curve and surface fitting method. Curve fitting, also known as regression analysis used to find the ”best fit” line or curve for a series of data points. The curve fit will produce an equation that can be used to find points any where along the curve [16]. Fitting is usually performed for one of the two purposes. On the one hand, to determine a physical quantity and a measure of its uncertainty from experimental data when there is a well-established relationship between variables. on the other hand, the goal might be to establish a mathematical relation ship between a dependent variable and independent variables. In this work we use a curve fitting method for the first purpose. That is, we have a dependent variable, scattered-transmitted ratio or Y_1 and an independent variable, the concentration of milk or X_1, and a given set of data points (X_r, Y_r), for r=1,2,...,m. The aim is to construct a curve which interpolates or approximates these points.

Surface fitting is a method of fitting a function of two variables to a given set of

points [20]. We have a single dependent variable (the scattered-transmitted ratio in which both fat and protein exist or Y_2), and two independent variables (concentration of fat, x_1 and protein concentration, x_2). The data points are denoted (x_{1r}, x_{2r}, Y_{2r}), for r=1,2,...,m. The aim is to construct a surface which interpolates or approximates the given data points.

The following data are collected from the experiment and presented in the table shown below for the two milk samples. In the tables, the calculated values of fat and protein concentration as well experimental obtained values of milk concentration and STR values are included. Moreover, the distance between the set of data points from the smooth function is presented and the error will be analyzed.

concentration of milk $\chi_1\%$	**concentration of fat** x_1	**Scattered light**	**transmitted light**	**STR**	**error=** exp(−4)
10	0.290	15.423	25.431	0.6067	0.2537
12	0.348	34.983	21.624	1.6178	0.0331
14	0.409	42.733	16.453	2.5973	0.4028
16	0.464	48.103	13.569	3.5451	0.0541
18	0.530	57.881	12.974	4.4613	0.0793
20	0.583	63.440	11.867	5.3459	0.0026
22	0.638	64.667	10.432	6.19897	0.299
24	0.688	66.910	9.531	7.0202	0.1875
26	0.754	68.157	8.727	7.8099	0.4596
28	0.812	72.811	8.498	8.5680	0.4837
30	0.870	73.929	7.954	9.2945	0.3577
32	0.928	74.701	7.478	9.9894	0.0163
34	0.986	76.965	7.225	10.6526	0.3944
36	1.044	78.967	6.998	11.2843	0.4102
38	1.102	79.114	6.657	11.8843	0.4301
40	1.175	82.026	6.587	12.4526	0.3346

Table 4.1: experimental data of milk concentration ,fat concentration and STR for mama milk

concentration of milk $X_1\%$	**concentration of protein** $x_1\%$	**Scattered light**	**transmitted light**	**STR**	**error=** $\exp(-4)$
10	0.361	35.447	26.147	1.3557	0.3652
12	0.435	57.553	22.796	2.5248	0.3103
14	0.504	71.881	19.569	3.6732	0.0943
16	0.578	80.916	16.853	4.8013	0.0131
18	0.648	93.794	15.873	5.9090	0.0120
20	0.720	98.130	14.026	6.9963	0.0977
22	0.792	103.990	12.897	8.0632	0.3159
24	0.864	105.380	11.568	9.1096	0.3574
26	0.936	107.133	10.565	10.1357	0.0799
28	1.001	111.023	9.965	11.1413	0.3782
30	1.080	113.056	9.324	12.1266	0.2742
32	1.152	116.016	8.862	13.0914	0.0351
34	1.224	118.841	8.467	14.0358	0.0954
36	1.298	120.247	8.038	14.9598	0.1174
38	1.368	121.974	7.689	15.8634	0.0309
40	1.440	124.561	7.438	16.7466	0.1642

Table 4.2: experimental data of milk concentration,protein concentration and STR for mama milk

Using the collected data from the experiment, the fat and protein content of the milk samples are analyzed in the following sections using curve and surface fitting method.

concentration of milk $X_1\%$	**concentration of protein** $x_1\%$	**Scattered light**	**transmitted light**	**STR**	**error=** $\exp(-3)$
10	0.341	35.447	26.147	0.8972	0.2618
12	0.476	57.553	22.796	1.9633	0.1829
14	0.554	71.881	19.569	3.01414	0.0522
16	0.612	80.916	16.853	4.0433	0.1651
18	0.680	93.794	15.873	5.0509	0.2123
20	0.680	98.130	14.026	6.0369	0.2308
22	0.748	103.990	12.897	7.0031	0.2205
24	0.816	105.380	11.568	7.9441	0.1815
26	0.884	107.133	10.565	8.8653	0.0342
28	0.952	111.023	9.965	9.7649	0.0341
30	1.022	113.056	9.342	10.6429	0.0641
32	1.088	116.016	8.862	11.4992	0.2910
34	1.156	118.8411	8.467	12.3341	0.5467
36	1.224	120.247	8.038	13.1473	0.7331
38	1.292	121.974	7.689	13.9389	0.0323
40	1.361	124.561	7.438	14.7098	0.9241

Table 4.3: experimental data of milk concentration ,protein concentration and STR for shola milk

concentration of milk $X_1\%$	**concentration of fat** $x_1\%$	**Scattered light**	**transmitted light**	**STR**	**error=** $\exp(-4)$
10.00	0.271	13.740	54.800	0.2507	0.2434
12	0.324	20.616	26.350	1.2078	0.1324
14	0.378	33.750	20.430	2.1334	0.0958
16	0.432	38.424	12.920	3.0338	0.0639
18	0.486	39.742	11.590	3.9062	0.0635
20	0.540	43.845	9.652	4.7514	0.0137
22	0.594	44.556	8.962	5.5688	0.0044
24	0.648	46.148	7.895	6.3594	0.0179
26	0.702	46.148	7.243	7.1228	0.0268
28	0.756	49.374	6.978	7.8591	0.0311
30	0.810	51.542	6.645	8.5682	0.0308
32	0.864	53.739	6.387	9.2498	0.0259
34	0.918	53.915	5.958	9.9044	0.0164
36	0.972	55.485	5.745	10.5318	0.0220
38	1.026	56.580	5.495	11.1322	0.0166
40	1.081	56.580	5.233	11.7053	0.0399

Table 4.4: Experimental data of milk concentration ,fat concentration and STR for shola milk

4.2 Milk fat testing method

In order to determine the fat content of the milk solution, we had first diluted the milk with EDTA solution. The EDTA solution dissolves the protein in the milk with out dissolving the fat. This was done to determine only the fat content due to the fact that the protein had been already dissolved in the milk. We measured the transmitted and scattered intensity of light from the experiment for different concentration of milk. We analyzed the relation between STR and milk concentration by using curve fitting method. Results obtained for mama and Shola homogenized milk are reported as follows.

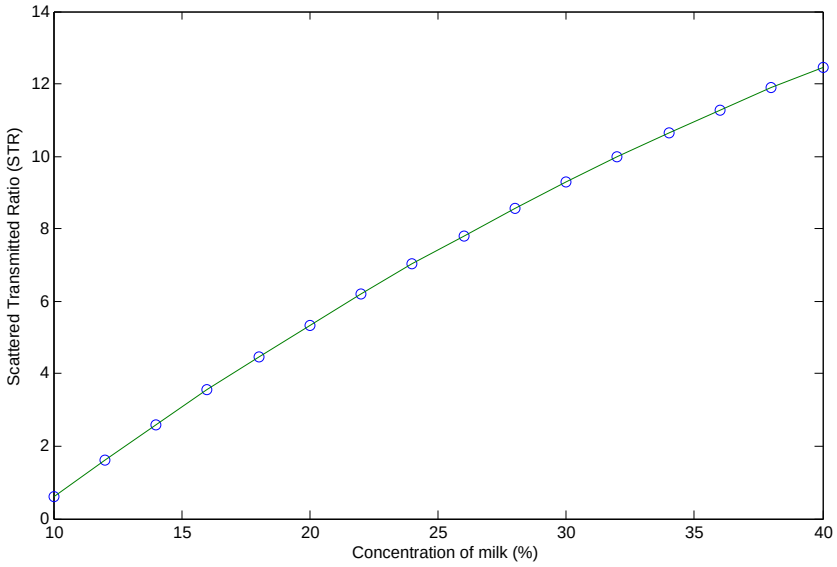

Figure 4.1: The relation between STR Y_1 and milk concentration for mama homogenized milk.

The curve fitting shows that the relation between them is quadratic. This asserts that our theoretical derivation based on Beer-lambert law for thin fat solution would

be absolutely verified by our experimental result. According to our curve fitting result for the relation between STR, Y_1 and the milk concentration, X_1 is given by

$$Y_1 = -0.004X_1^2 + 0.5925X_1 - 4.9230 \tag{4.2.1}$$

The fat concentrations corresponding to the milk concentration can be figured out

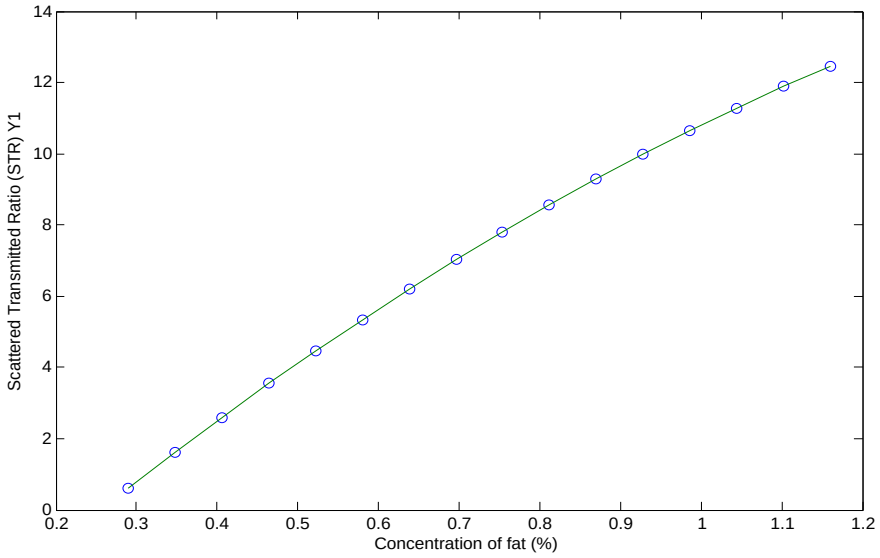

Figure 4.2: illustrates the relation between STR, Y_1 and the fat concentration, x_1 for mama homogenized milk.

from equation (4.2.1). According to our theoretical assumption,the relation between fat concentration, x_1 and STR, Y_1 is quadratic. Therefore, the fat concentration corresponding to each milk concentration can be obtained using the following relation.The quadratic relation between the fat concentration and STR can be written as

$$Y_1 = Ax_1^2 + Bx_1 + C \tag{4.2.2}$$

Where A, B and C are constants .

Comparing Equation (4.2.1) and (4.2.2), we have

$$Ax_{1f}^2 = -0.004X_{1m}^2 \tag{4.2.3}$$

and

$$Ax_{2f}^2 = -0.004X_{2m}^2 \tag{4.2.4}$$

Taking the ratio of equation (4.2.3) and (4.2.4), we have

$$\frac{x_{1f}}{x_{2f}} = 0.833 \tag{4.2.5}$$

The correspondingly difference between the two quantities can be given by

$$x_{2f} - x_{1f} = 0.0582 \tag{4.2.6}$$

From equation (4.2.5) and (4.2.6), the values of x_{1f} and x_{2f} are 0.290 and 0.348. These fat concentrations are corresponding to the milk concentration of 10% and 12%, respectively. Since the ratio and the difference between the consecutive value is constant so that we can calculate the fat concentration corresponding to each milk concentration and the calculated results are shown in the respective tables.

By using the calculated value of x_1 and the experimental value of Y_1, the relation between the two quantities can be deduced from the curve fitting as

$$Y_1 = -4.7x_1^2 + 20.4310x_1 - 4.9230 \tag{4.2.7}$$

From equation (4.2.1) and equation (4.2.7), we can easily determine the fat content of mama homogenized milk. Despite the fact that X_1 and x_1 have different values,

they have the same value of Y_1. The fat content of the milk at which Y_1 is equal for the two values is given by

$$m_f = \frac{x_1}{X_1} \times 100\% \tag{4.2.8}$$

Where m_f is the fat content of the milk.

For instance, when the STR (Y_1) is 0.6072, the milk concentration is 10% and that of the fat is 0.290%. Hence, substituting the values into equation (4.2.8), the fat content becomes 2.9%. If we select other values X_1 and x_1 at which Y_1 is the same for both values, the fat content is very closer to 2.9%. This reveals that the fat content of mama homogenized milk is 2.9%.

For shola homogenized milk sample, we used the same testing method as before to calculate the fat content. Figure (4.3) describes the relation between STR and milk concentration, X_1 for shola milk type. The equation that relate milk concentration with STR for this milk type is given by

$$Y_1 = -0.0034X_1^2 + 0.5517X_1 - 4.9234 \tag{4.2.9}$$

Using similar procedures as we have used so far to calculate the fat concentration corresponding each milk concentration ,the relation between x_1 and Y_1 for shola homogenized milk is given by

$$Y_1 = -4.7x_1^2 + 20.4310x_1 - 4.9230 \tag{4.2.10}$$

Equation (4.2.7) and (4.2.10) have the same expression. This shows that for any homogenized milk type the concentration of fat and STR can be represented by a standard curve. The difference lies on the concentration of the fat in the milk types. For instance, the STR of mama and shola milk at milk concentration of 10% are 1.61779 and 0.25074 ,respectively. The value of the STR indicates that the higher the

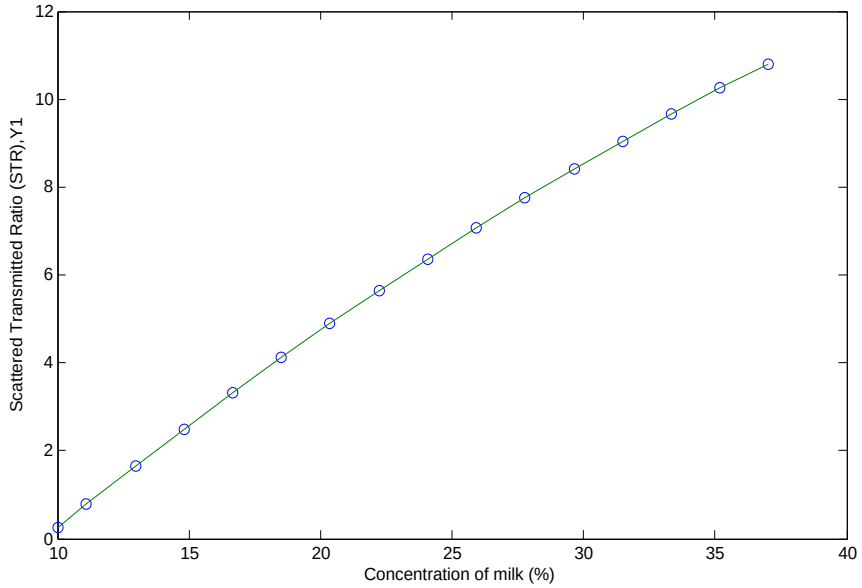

Figure 4.3: The relation between concentration of milk with STR Y_1 for shola homogenized milk.

concentration of the fat in the milk sample the larger the value of STR. Since the milk concentration and the fat concentration of shola milk at $Y_1 = 0.25074$ are 10% and 0.27% respectively. Applying equation (4.2.8), the fat content of shola homogenized milk to be 2.7 %. If we select any other point at which Y_1 equal for both X_1 and x_1, the fat content is nearly equal to 2.7%. From this result we can say that the fat content of shola homogenized milk is approximately equal to 2.7%.

4.3 Milk protein testing method

Each milk samples was diluted with distilled water of different proportion before the testing. In this case the milk samples contain both fat and protein because water can dissolve neither the fat nor the protein in the milk sample. For this reason the protein

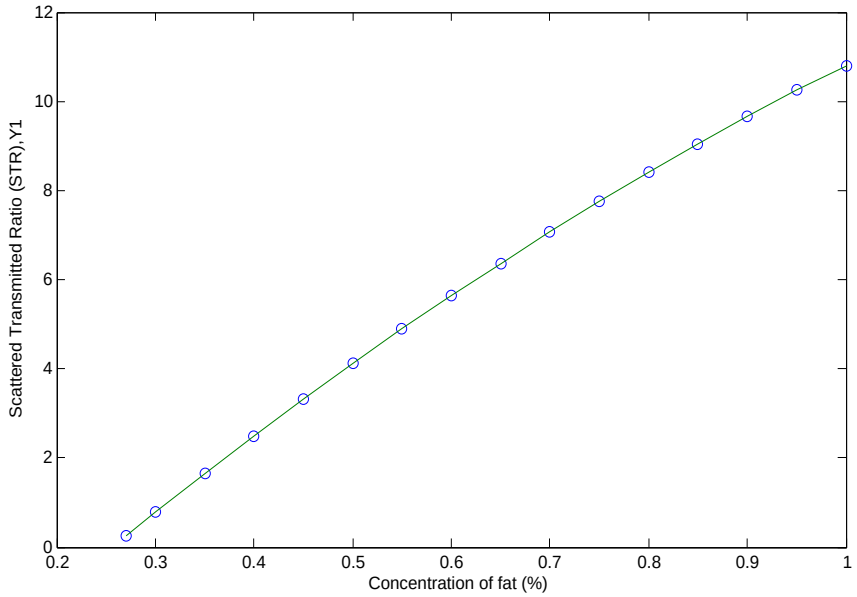

Figure 4.4: illustrates the relation between concentration of fat with STR (Y_1) for shola homogenized milk.

test must be carried out after the fat concentration being tested. It would be better to test the protein concentration independently, nevertheless we haven't yet obtained a solution which can rapidly dissolve the fat with out dissolving the protein. Figure (4.5) and figure (4.6) illustrate the change of STR with milk concentration for thin pure fat milk solution (Y_1) and the fat protein milk solution (Y_2) for Mama and shola milk samples, respectively. From the curve fitting method the relation between the STR, (Y_2) in which both fat and protein exist and the milk concentration of mama homogenized milk, X_2 can be given by the equation

$$Y_1 = -0.0026X_2^2 + 0.6406X_2 - 4.7951 \tag{4.3.1}$$

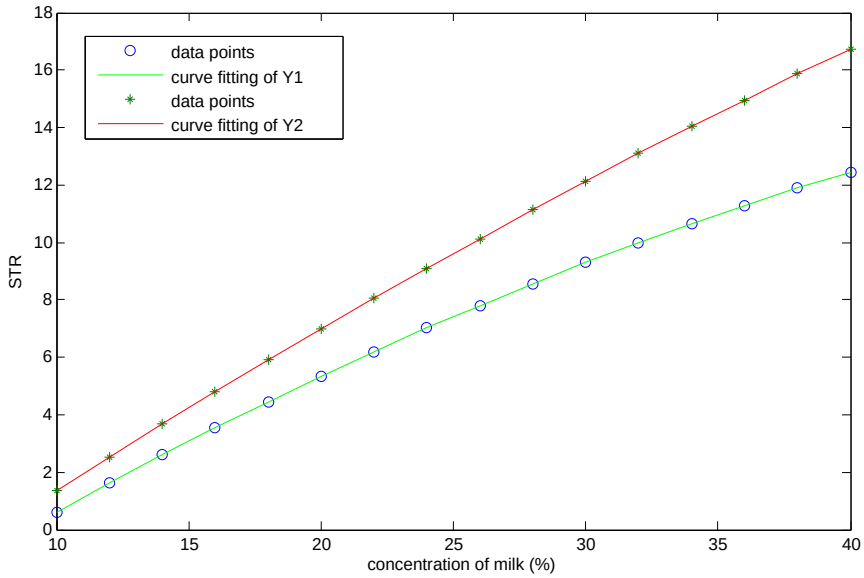

Figure 4.5: the change of scattered transmitted ratio (STR) with milk concentration for thin pure fat solution (Y_1 and the fat protein solution Y_2 for mama homogenized milk).

The relation between (Y_2) and the concentration shola homogenized milk, X_2 can also given by

$$Y_1 = -0.0027X_2^2 + 0.5956X_2 - 4.7951 \tag{4.3.2}$$

The relation between (Y_1) and fat concentration (x_1) was easily obtained. However, it is difficult to get the relation between (Y_2) and protein concentration (x_2) because Y_2 represents both fat and protein concentration. Furthermore, it can not be figured out from the difference between Y_1 and Y_2. So, the determination of protein content can be performed by surface fitting method. In detail, an exclusive curved surface, which depicts the relation between Y_2, x_1 (fat concentration in milk solution) and x_2 (protein concentration in the milk solution), was obtained through curved surface

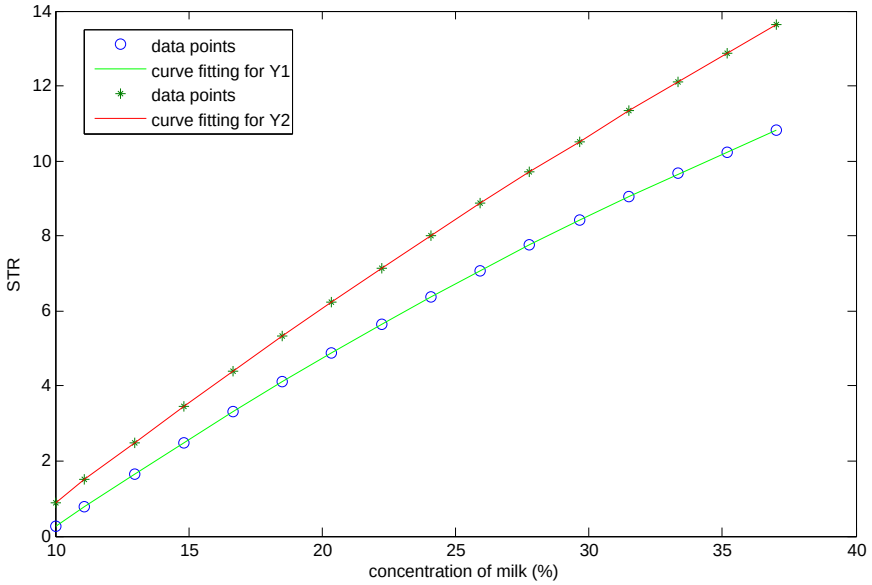

Figure 4.6: The change of scattered transmitted ratio, STR (Y_2) with milk concentration thin pure fat solution (X_1) and the fat protein solution (X_2) for shola homogenized milk.

fitting. The three dimensional curved surface for mama and shola milk samples are shown in (4.7) and (4.8), respectively. The surface equation for the two milk samples is represented by

$$Y_2 = -30.1754x_1^2 - 43.0672x_2^2 + 8.5567x_1 - 1.3934x_2 + 86.4035x_1x_2 - 4.7951 \quad (4.3.3)$$

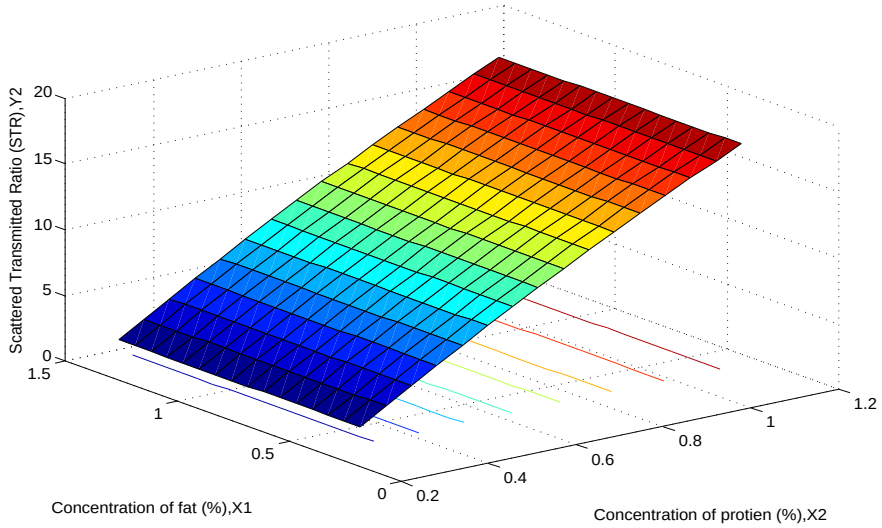

Figure 4.7: the curved surface of concentration of fat, concentration of protein an STR (Y_2) for mama homogenized milk.

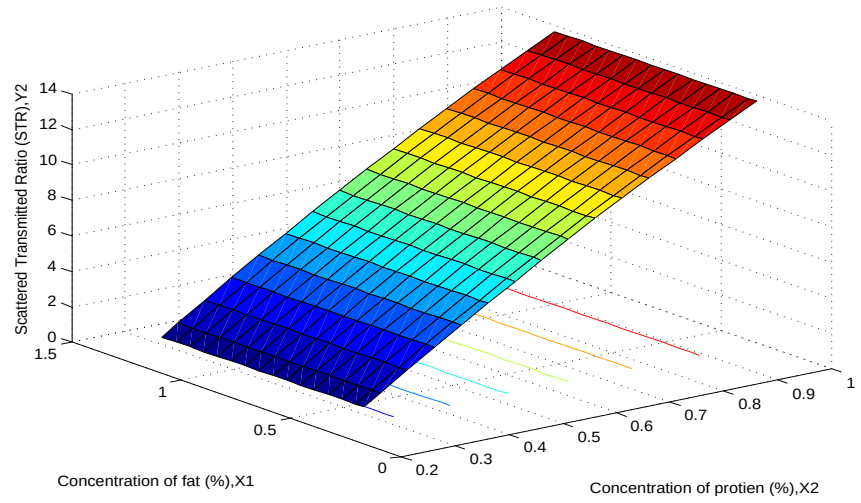

Figure 4.8: the curved surface of concentration of fat, concentration of protein an STR (Y_2) for shola homogenized milk.

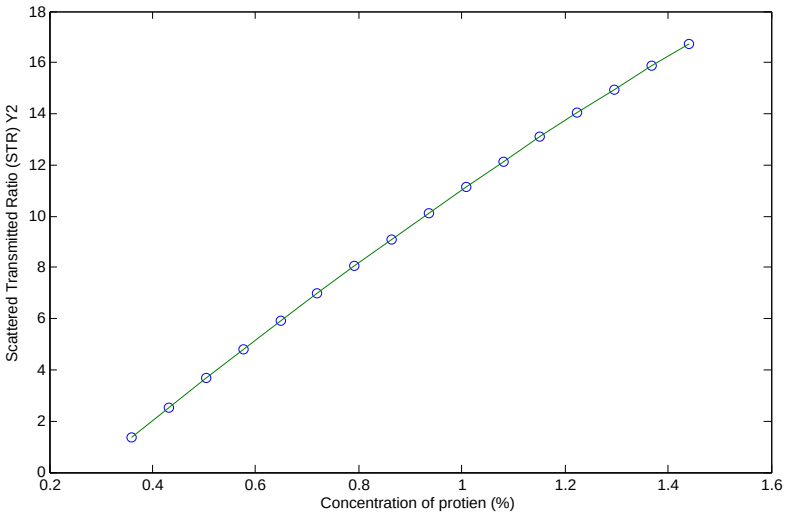

Figure 4.9: The relation between concentration of protein (x_2) with STR (Y_2) for mama homogenized milk

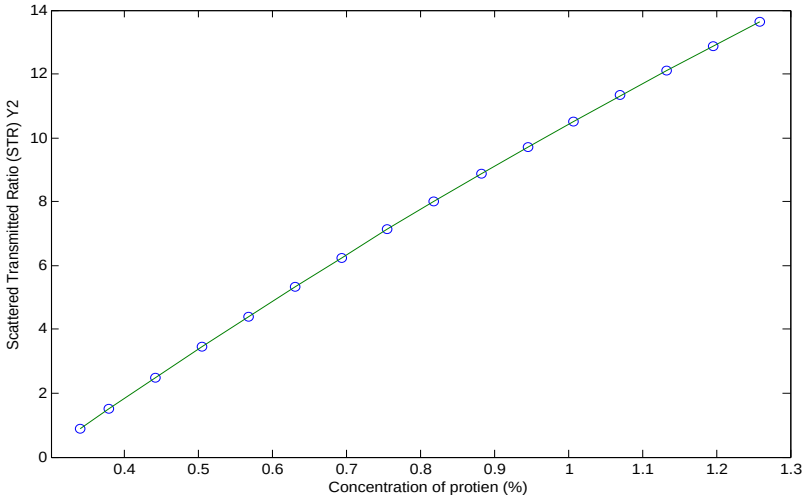

Figure 4.10: The relation between concentration of protein (x_2) with STR (Y_2) for shola homogenized milk

The three coordinate axis are the fat concentration in the milk concentration x_1, the protein concentration in the milk solution x_2 and the STR Y_2, respectively. By substituting the determined value of fat concentration, x_1 into equation (4.2.8), the milk protein concentration, x_2 can be calculated through the formula (4.3.3). After the determination of protein concentration corresponding to the concentration of the milk solution, we can easily analyze the relation between STR Y_2 and the protein concentration, x_2 independently. Figure (4.9) and figure (4.10) illustrate the relation between the two quantities for mama and shola homogenized milk, respectively.

The equation of the curve shown in figure (4.9) and figure (4.10) can be represented by

$$Y_2 = -2.12725x_2^2 + 17.6523x_2 - 4.79495 \tag{4.3.4}$$

Equation (4.3.4) stands for the two milk samples. This means that the two milk samples can be characterized by the same standard curve equation. The dissimilarity of the two graph is that the scattered transmitted ratio (STR) value for the same concentration of the milk samples is not the same. This implies that if the milk sample has high protein concentration compared to the second milk sample, the STR will be larger. In other words, concentrated protein milk sample will result in larger value of STR.

The protein content of the milk samples is then determined by the following formula

$$m_p = \frac{x_2}{X_2} \times 100\% \tag{4.3.5}$$

Where m_P is the protein content of the milk sample.

For the determination of protein content in the milk samples we used similar method that we followed to determine the fat content. For example, at Y_2=6.2381

the value of milk concentration, X_2=20.37 and the protein concentration, x_2=0.693 for shola milk. Hence, substituting the corresponding values into equation (4.2.10), the protein content becomes 3.4%. If we go through the milk concentration and protein concentration at which Y_2 equal for both quantities,the protein content is nearly equal to 3.4%. Following similar mathematical procedure, the protein content of mama homogenized milk is calculated to be 3.6%.

4.4 Data analysis

The distance between the set of data points from the specified function, $f(x)$ has been presented in the corresponding tables in the error row. The error in fitting the data points to the specified function, $f(x)$ and the distance from the single points (x_i, Y_i)can be simply taken as

$$\epsilon_r = (Y_i - f(x)) \tag{4.4.1}$$

Where ϵ_r is called the residual points. However, we need a measure of a distance for the set of data points as a whole and the error function E_r can be given by

$$E_r = \sqrt{\sum_{r=1}^{m} \epsilon_r^2} \tag{4.4.2}$$

Therefore, the error in fitting the fat concentration with STR can be calculated using equation (4.4.2). Putting ϵ_r from $m = 1, 2.....16$ using error row of table (4.1), the error in calculating the fat content of mama milk is 0.0228.

Similarly,substituting the errors in equation (4.4.2) from table (4.4), the error in calculating the fat content of shola milk is to be 0.021.Using the error row of table

(4.2) and table (4.3), the error for the protein content of mama and shola homogenized milk is determined to be 0.014 and 0.015, respectively.

4.5 Discussion

The primary goal of this experimental work is to investigate the fat and protein content of different milk samples. The scattered and transmitted light intensities of a He-Ne laser light are very essential physical quantities which give important information about the composition and concentration of the milk samples. By deriving a theoretical formula based on Beer-Lambert which related the concentration of fat and protein with scattered-transmitted ratio (STR), we could able to calculate the fat and protein content of two commercially produced milk samples. According to our experimental result, the fat content of mama homogenized milk is calculated to be $2.900\% \pm 0.0228\%$ and the protein content is $3.60\% \pm 0.014\%$. The fat and protein content of shola milk sample are $2.70\% \pm 0.021\%$ and $3.40\% \pm 0.015\%$, respectively. These small errors were mainly due to the imperfect reading of the scattered and transmitted light intensities because of the interference other light sources and the misalignments of the photo detector perpendicular or exactly at 90^o and parallel or exactly at 0^o with respect to the incident beam direction to measure scattered and transmitted light intensities, respectively. On the whole, our experimental result strongly suggest that the scattered and transmitted light intensities of He-Ne laser can absolutely determine the fat and protein content accurately and precisely.

4.6 Recommendations and suggestions

The experimental work with its high accuracy can be applied to quality control laboratories to control the fat and protein content of different food stuffs.Due to its simple methodology and high accuracy, it can be implemented in diary industries to determine the fat and protein content of different diary products. Further more, this experimental work also can be used in hospital for diagnostic purposes by investigating the fat and protein concentration of human blood so that it will help to treat diseases which caused by high fat and protein concentration.

Chapter 5

Conclusion

Through data analysis,the concrete methods for testing the fat and protein content were obtained.the fat should be determined through curve fitting and protein should be determined through surface fitting.

It can be concluded that the laser light scattering provides a simple and direct method to determine fat and protein content in liquid milk accurately and precisely with less labor cost.Result of this experimental work indicates the feasibility of using STR for milk fat and protein analysis.

compared to other methods of determination of fat and protein content,it has the following advantage:

1.We can test many milk samples with reduced labor cost.

2.The experiment was carried out by using only one kind of monochromatic light.This in general helps to reduce the complexity and cost of the instrument.

Bibliography

[1] F.A. Jenkins, *Fundamentals of optics*, 4^{th} ed., Mc Graw-Hill, Tokyo (1976)

[2] Sommer H., *control of health and nutritional status in diary cows*, *vetmed.*, rev (1985) :10:13.

[3] IDF standard , *Determination of fat content of dried milk ,dried whey ,dried butter milk and dried butter serum*, international diary federation,(1987)

[4] Sato t .Yoshino m,Furukewas,Someya ,Yano,Uozumij,Iwamato M, *Analysis of milk constituents by the near infrared spectrphotometric method* ,japan J Zootech ,Sci,(1964);58:689-706

[5] Barbano D.M,clark JL. *infrared milk-analysis chalenges for the future* ,J Diary sci (1989):72:1672-36

[6] Yuz,*a study of light scattering characterstic of human white blood cells.* ,Pc spie 266-77(1996).

[7] Hank J. *application of Mie theorey to large particles* , IEEE south east CoN'99 conference ,Lexigton kentucky

[8] WWW.national diary council.org, National diary council /nutrition /products/figure 0.2 Pdf,

[9] WWW.amazon .co.uk/ Laser beam interaction -materials principle .Application/dp/3540594019-60k

[10] WWW.Med phys.Ucl.ac.uk/ research /borl /home pages / florian /thesis/Pdf-files /page 45-58 pdf

[11] WWW.Wikipedia ,the free encyclopidia,milk/html

[12] WWW.Wikipedia ,the free encyclopidia,Beer-lambert law/html

[13] Zoumin Zhao and Risto myllyla , *(*the effects of optical scattering on pulsed Photo acoustic measurement in weakly absorbing liquids) , Infotech oulu ,university of ollu,Finland (1957).

[14] WWW.sci-toys.com/ ingredients / EDTA .html-7k

[15] Duck F.A,*(*Physical properties of tissue),a comprehensive reference book ,San Diego:Academic,(1990)

[16] Cox M.G and hayes JG , curve fitting :"A guide and suit of algorithm for the none special user NPL ",(1973)

[17] Renka R.J , "multivariate interpolation of large sets of scattered data ACM trans math .soft ware (1998) 14 139-148 R.M.A.Azzam and N.M. Bashara , *Ellipsometry and polarized light*, chap.3, p.257, 2^{nd} ed., Elselvier: Amsterdam (2003).

Patel C.K.N and tan A.c ,Rev .Mod .phys.517-50(1981) , Rev .Mod .phys.517-50.

[18] Van stravern H.J,moes C.J.M,VAN Marlie ,prahl S.A and Germent M.J *Light scatteringin interalipid -10% in the wave length of 400-1100nm*,Applied optics (1991)31,4507-4514

[19] R.M.A.Azzam and N.M. Bashara, *Ellipsometry and polarized light*, chap.4, p.286, 2^{nd} ed., Elselvier: Amsterdam (2003).

[20] Driver I,Feather J.W ,king PR and dawson JB, "The optical properties of aqueuos suspensions of intralipid,a fat emulsion,"phys.Med.BIOl,12,1927-1930

[21] Cox M.G and hayes J.G (1973), curve fitting :"A guide and suit of algorithm for the none special user NPL "

[22] Renka R.J , "multivariate interpolation of large sets of scattered data ACM trans math .soft ware (1998) 14 139-148