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

BY

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This is to certify that the thesis prepared by **Wassu Assen Abegaze**, entitled **"Determination of fat and protein content of milk using He-Ne laser light scattering"** and submitted in partial fulfillment of the requirements for the degree of **Master of Science in physics**. Compiles with the regulations of the University and meets the accepted standards with respect to originality.

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Declaration

I, the undersigned, declare that this research is my original work, has not been presented for a degree in any of other University and that all sources of materials used for this research have been dully acknowledged.

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This research has been submitted for examination with my approval as University adviser:

Name: Prof. A.V. Gholap

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Dedication

I dedicate this thesis manuscript

To

My Family and My Wife

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Abstract

This study was initiated with the objectives to enhance the accuracy of determining the intended components inside the selected milk samples like the amount of fat and protein contents and to develop optical method for the determination of the amount of fat and protein content in the milk samples. I implemented the ratio of the scattered to transmitted light intensity as the principal analysis parameter to minimize the power fluctuation of the light source during doing the experiment. The amount of fat contents and protein contents of five (Family, Etete, Harme, Sholla and Mama) homogenized milk samples were determined. In this work we used laser light scattering method. Finally, the data analysis was carried out using curve and surface fitting method. My result shows that, the amount of fat contents in each (Family, Etete, Harme, Shola and Mama) homogenized milk samples are 2.700%, 2.500%, 2.800%, 2.900% and 2.800% respectively. But the amount of fat content written in the packets to each (Family, Etete, Harme, Shola, Mama) milk samples are 2.700%, 2.800%, 2.800%, 2.700% and 2.700% respectively which are almost consistent to experimental results. The amount of protein contents that is found in each (Family, Etete, Harme, Shola and Mama) homogenized milk samples become 3.600%, 3.900%, 3.800%, 3.500% and 3.500% respectively. The amount of protein contents written in the packets to each (Family, Etete, Harme, Shola, Mama) milk samples are also 3.500%, 3.500%, 3.500%, 3.500% and 3.500% respectively which are also almost consistent to experimental results. It may be concluded that, the laser light scattering method provides a simple and direct method to determine fat and protein contents in investigated milk samples.

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Introduction

This work discusses how we can determine the amount of fat and protein in the milk samples that are sold in Ethiopian market by laser light scattering technique.

LASER is an acronym for Light Amplification by the Stimulated Emission of Radiation. The concept consists of an excited state atom encountering a photon of the same energy that corresponds to the difference between the excited and lower states of the atom. When such a photon is encountered, it causes the emission of another photon of the same energy. Laser radiation is highly monochromatic (almost of single wave length), highly directional and intense. It is coherent in time and space and also polarized. These properties are characteristics of all lasers regardless of a specific laser type and its technical data [1]. The properties of laser beam are exploited in many fascinating applications in industries, agriculture, medicine and scientific disciplines. In helium-neon laser [He-Ne] Ali Javan, William Bennet Jr. and Donald Heriot used a mixture of 10 part of He to 1 part of Ne $(10:1)$ as the active medium [2]. Its usual operation wave length is 632.8nm in the red region.

Most diary industries widely used the chemical analysis methods for measuring the milk components. The chemical methods for measuring fat content of milk sample are the Geber method, the Rose-Gottelieb method, the Babcock method etc. Among these method, Geber method and the Rose-Gottelieb method are the most widely used methods. Some of the chemical methods for measuring protein content of milk sample are the Kjiedhal determination of N-methods, the Udy dye binding method and the formal titration method. Kjiedhal determination of N-methods is internationally accepted and widely used in dairy industries [3, 4]. These chemical methods for measuring the amount of fat and protein content in milk samples are expensive, time consuming, labor consuming and low in frequency range. They have been partially not totally replaced by rapid and relevant methods like spectroscopy analysis methods which is including infrared, middle infrared, near infrared, ultraviolet, etc and ultrasonic method [5, 6]. Nowadays, spectroscopic analysis methods are most preferable and more precise but they are instrumentally too complex and they are not cheap.

Based on laser light scattering theory, laser light scattering methods have been used to gather information about the size, structure, shape, composition and concentration of matters [7, 8].

In this study, determination of fat and protein content in the milk samples is performed by dual-angle laser light scattering method in which experimentally, scattered light intensity (at a scattering angle, $90^0 \pm 0.1^0$) and the transmitted light (at transmitted angle, $0^0 \pm 0.1^0$) intensities were measured and the ratio of the scattered light to the transmitted light intensity which is called scattered-transmitted- ratio (STR) was adopted as an optical parameter to determine the amount of fat and protein content. The objective of using this scattered-transmitted- ratio (STR) is to minimize the effect due to fluctuation of power of light source and this improved the accuracy and precision of the experiment that we done.

Objectives of the Study

General objective

The main (general) objective of this research is to determine the amount of fat and protein content in the milk samples by using He-Ne laser light- scattering experimental technique.

Specific objectives

- To introduce a model of direct, easy and precise method for testing milk quality.
- To determine the optical properties of the sample such as amount of light scattering and transmission for different concentrations of milk samples under the study.
- To measure the intensity of the transmitted and scattered light through the milk samples.
- To compare amount of fat and protein contents of this experimental result with amounts written on the packets.

Organization of the Thesis

This thesis is organized into five chapters.

In the first chapter, we describe milk sample and its components, lasers light interaction with matter and He-Ne laser beam transmission through milk solution. In second chapter, Introduction of Beer-Lambert law, its mathematical derivation, application to determine protein and fat content in milk sample and scattering of laser light are described. In third chapter, Materials and methods used in this work are presented. This chapter has two sections. The first section deals with the description of different chemicals, samples of milk types and instruments used to carry

out this research. The second section deals with methods of sample preparation, determination of protein and fat in the selected homogenized milk samples, descriptions of the apparatus and data collections are presented. In Chapter four, analysis of experimental data by curve and surface fitting method for fat and protein determination are presented. Here, scattered-transmitted-ratio (STR) or $'Y_1'$, concentration of fat in the solution(x_1), scattered-transmitted-ratio (STR) or $'Y_2'$ of the milk solution in which both the fat and the protein exist, concentration of protein in the milk solution (x_2),results and discussion are described and Conclusion and Recommendation (Future prospects) are discussed in Chapter five . Finally, References /Bibliography and appendices are listed.

Chapter-1

1. Milk and Its Components

Milk is used throughout the world as a human food in at least one form or more. Because of its high nutritive value, milk is considered as the most important diet items of many people [9]. Nutritionally, milk has been defined the most nearly perfect food. The demand of consumers for safe and high quality milk has placed a significant responsibility on dairy producers, retailers and manufacture to produce and market safe with virtually no quality control at all levels [10]. It is also an emulsion of butter fat globules with a water-based fluid. Each fat globe is surrounded by a membrane consisting of phospholipids and protein. These emulsions keep the individual globules from joining together into noticeable grains of butter fat [11]. In general, milk can be described as a solution of lactose, soluble protein, fat minerals, vitamins and other components. Milk's value is determined primarily by its amount of protein and fat contents [12]. Among different physical properties of milk, milk is a white liquid extracted from the mammary gland of mammals. This white color of milk is due so many fat globules and colloidal protein molecules in milk that enough light at all wavelengths gets scattered giving the appearance of white light which cause to the white color of milk [13].

1.1 Light Matter Interaction

When the beams of light is incident on material medium and propagate through it, various light matter interaction phenomenon takes place. Within any conditions of material medium, when light tries to propagate through the given medium, reflection on the surface, absorption inside the medium by internal suspended components, transmission, and scattering properties of light can occur [14].

1.2 He-Ne Laser Beam Transmission through Milk Solution

As a beam of light is incident on the milk solution, various light-milk 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, scattering, transmission and absorption inside the milk by suspended internal components [15]. In general, when a collimated He-Ne laser beam travels through the milk sample, only a fraction of the light energy is transmitted through the sample and the rest is lost by reflection, absorption

and scattering. Figure (1.2.1) illustrates the interaction between the laser light and the milk sample.

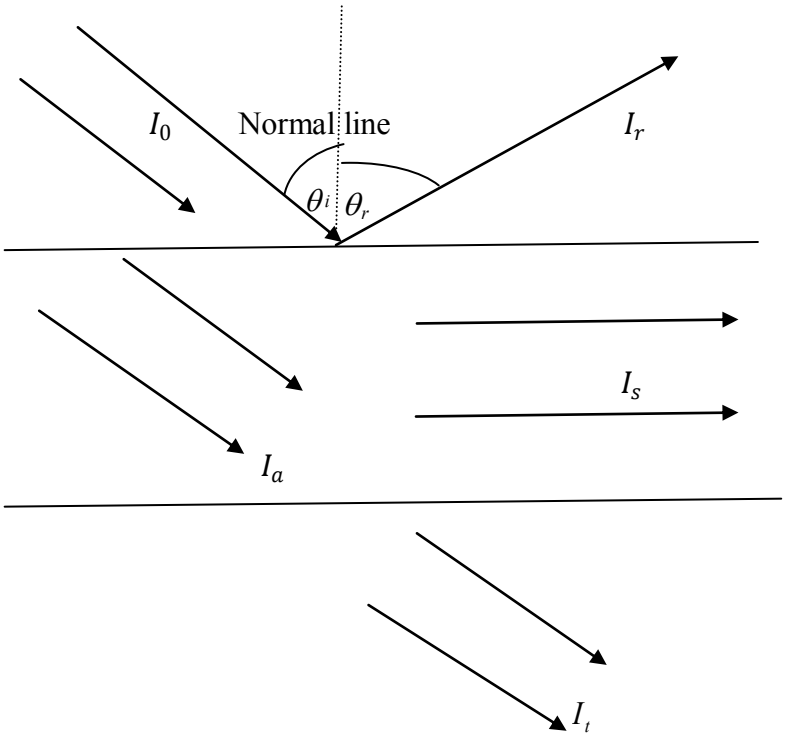

Figure 1.2.1: Reflection, absorption, scattering and transmission of light in a sample

Assume that we have the total incident light intensity(I_o), the reflected intensity (I_r), absorbed intensity (I_a), transmitted intensity (I_t), and the scattered intensity (I_s). According to the conservation law of energy the normalized total incident intensity of light fulfills the following condition.

$$I_o = I_r + I_a + I_t + I_s . \tag{1.2.1}$$

If we divide both sides of equation (1.2.1) by $'I_o'$, we have equation (1.2.2) bellow.

$$R + A + T = 1. \tag{1.2.2}$$

Where, R- Reflectance (the ratio of reflected to incident intensities)

T- Transmittance (the ratio of transmitted to incident intensities)

A- Absorbance in which the sum totals of absorbed and scattered fraction of light intensities.

When light moves from a medium with refractive index "n_1" into a second medium of refractive index "n_2", both reflection and refraction (transmission) of the light may occur, as shown in figure (1.2.2). Light intensity will be reflected because of a refractive index mismatch between the air and the sample boundary [16]. Intensity of reflected light does not only depend on the difference in refractive index mismatch but also depend on polarization, angle of incident light, and surface shape.

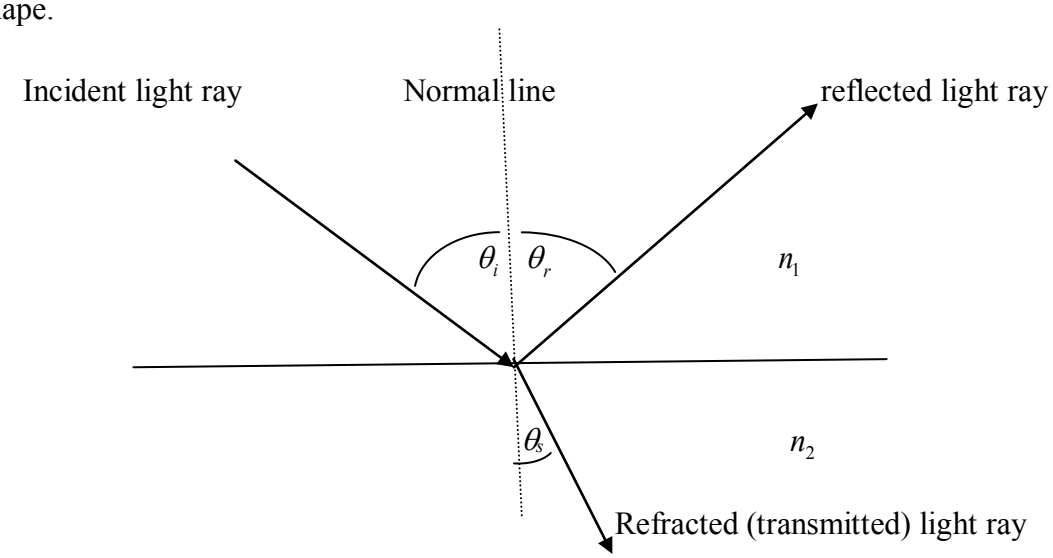

Figure 1.2.2: Reflection and refraction (transmission) of light ray by the medium

The relationship between the angles that the incident, reflected and refracted rays make to the normal of the interface given as θ_i, θ_r *and* θ_t in figure (1.2.3.) is given by the law of reflection and Snell's law [17].

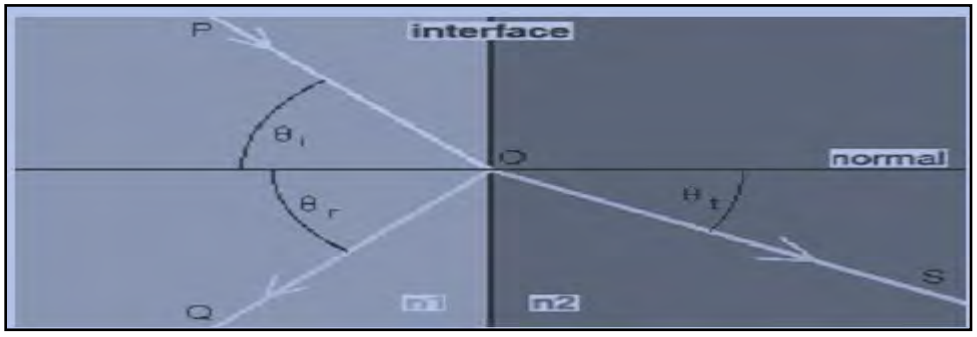

Figure 1.2.3 Reflection and refraction of light passing from one medium to another medium

Based on the assumption that the two materials are both non-magnetic, the Fresnel equations may be used to calculate the reflection coefficient "R". If the light is polarized with the electric

field of the light perpendicular to the plane of the diagram above (s-polarized), the reflection coefficient s-polarized is given by:-

$$R_S = \left[\frac{\sin(\theta_t - \theta_i)}{\sin(\theta_t + \theta_i)} \right]^2. \tag{1.2.3}$$

Since, $\sin(x \pm y) = \sin(x)\cos(y) \pm \cos(x)\sin(y)$ *and* $n_1 \sin(\theta_i) = n_2 \sin(\theta_t)$, we can write equation (1.2.3) as:-

$$R_S = \left[\frac{n_1 \cos(\theta_i) - n_2 \cos(\theta_t)}{n_1 \cos(\theta_i) + n_2 \cos(\theta_t)} \right]^2. \tag{1.2.4}$$

If the incident light is polarized in the plane of the diagram (p-polarized), the reflection coefficient p-polarized is given by:-

$$R_P = \left[\frac{\tan(\theta_t - \theta_i)}{\tan(\theta_t + \theta_i)} \right]^2 = \left[\left(\frac{n_2 \cos(\theta_i) - n_1 \cos(\theta_t)}{n_2 \cos(\theta_i) + n_1 \cos(\theta_t)} \right)^2 \right]. \tag{1.2.5}$$

If the incident light is unpolarized (containing an equal mix of s- and p-polarizations), the reflection coefficient is given by:-

$$R = \frac{1}{2}\left[R_S + R_P \right]. \tag{1.2.6}$$

Substituting the value of R_P and R_S , we can write equation (1.2.6) as follows.

$$R = \frac{1}{2}\left[\left(\frac{n_1 \cos(\theta_i) - n_2 \cos(\theta_t)}{n_1 \cos(\theta_i) + n_2 \cos(\theta_t)} \right)^2 + \left(\frac{n_2 \cos(\theta_i) - n_1 \cos(\theta_t)}{n_2 \cos(\theta_i) + n_1 \cos(\theta_t)} \right)^2 \right], \tag{1.2.7}$$

where n_1 and n_2 are the reflective indices for the external medium (air) and the material medium respectively, θ_i is the incident angle and θ_t is the transmitted angle.

When the light is incident in the direction normal to the medium surface, i.e. $\theta_i = \theta_t = 0^0$ and if the external medium is air (n_1 = 1), and n_2 = n, equation (1.2.7) can be written as follows.

$$R = \left(\frac{n-1}{n+1} \right)^2 . \tag{1.2.8}$$

Since index of refraction of milk is 1.462 [18], the reflectance has been evaluated by using equation (1.2.8) to which it be come about 0.03521or 3.521%. This indicates that about 3.521% of He-Ne laser incident intensity is reflected back to the air.

By applying Snell's law of equation (1.2.9) bellow, the transmitted angle (θ_t) is given by:-

$$n_1 \sin(\theta_i) = n_2 \sin(\theta_t) , \tag{1.2.9}$$

$$\Rightarrow \quad \theta_t = \sin^{-1}\left(\frac{n_1}{n_2} \sin(\theta_i) \right). \tag{1.2.10}$$

Chapter- 2

2. Theory

2.1 Introduction to Beer-Lambert's law

Beer-Lambert's law states that, for low concentrations the absorption coefficient is proportional to the concentration of the absorbing atomic system in the sample. This can be expressed in equation form as: - $\mu_a \approx c = ac$.

Where a -constant depending on material and the wave length of the light beam used.

μ_a -Absorption coefficient and "c" is the concentration of the absorbing sample.

In optics, this law is an empirical relationship that relates the absorption and scattering of light with the properties of the material through which light is traveling [21]. If a beam of light having initial intensity (I_0) is propagating through the three sates (solids, liquids or gaseous), there is the decreasing of intensity. This decreasing of intensity can be due to absorption. 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 enable us to obtain information about the size, concentration, and components of milk sample. Absorption is due to a partial conversion of light energy into heat, certain vibrations of molecules of the absorbing 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 solution [7, 8].

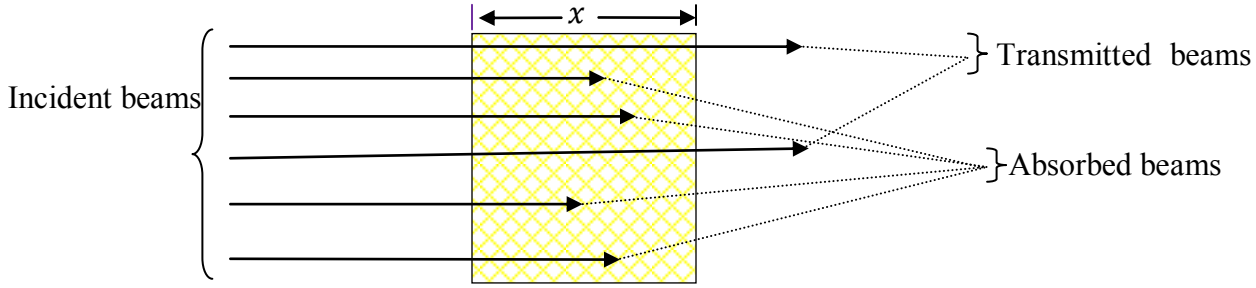

Figure 2.1.1: Absorbed and transmitting of light beam by matter

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 [20]. This implies that, the absorption of He-Ne laser light by the milk solution helps to investigate the physical property of milk. If all the wavelength of the He-Ne laser light would get absorbed and having high absorption coefficient, the milk would be black that is why the absorption coefficient of milk is smaller than the scattering coefficient.

2.2 Mathematical Derivation of Beer-Lambert's law

The relationship between the absorption of light in an absorbing medium of thickness $'x'$ can be calculated using Beer-Lambert's law [21]. For plane waves, the fractional reduction of the beam intensity $\frac{dI}{I}$ is directly proportional to an infinitesimal path length $'dx'$ in its direction through a homogeneous medium with absorption coefficient (μ_a).

$$\text{i.e, } \frac{dI}{I} = -\mu_a dx \tag{2.2.1}$$

Integrating and evaluating equation (2.2.1) as definite integral in traversing finitesimal thickness $'x'$ we can find that:-

$$\int_{Io}^{I} \frac{dI}{I} = -\mu_a \int_0^x dx \tag{2.2.2}$$

The reduced intensity (I) of equation (2.2.2) can be written as follows.

$$I = I_o \exp(-\mu_a x) \tag{2.2.3}$$

Applying the Beer- Lambert law and assuming $'z'$ as an axis parallel to the direction of motion of the photons, $'A'$ the area, $'dz'$ as finitesimal thickness and $'x'$ thickness (along the z- axis) 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, $\mu_t Axdz$, divided by the area of the slab, $\mu_t xdz$. 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:-

$$\frac{dI_z}{I_z} = -\mu_t xdz \tag{2.2.4}$$

The solution to equation (2.2.4) can be obtained by integrating it both sides as a function of $'z'$.

$$\ln(I_z) = -\mu_t xz + c \tag{2.2.5}$$

For the slab of real thickness $'d'$ the difference in incident light intensity (I_o) at $z = 0$ and transmitted intensity (I_t) at $z = d$ is given by equation (2.2.6) bellow.

$$I_t = I_o exp(-\mu_t xd) \tag{2.2.6}$$

where μ_t - represents the total extinction coefficient due to scattering and absorption [22].

2.3 Application of Beer- Lambert's law

2.3.1 Beer-Lambert's law Application for Determination of Fat Content

Introducing the Beer-Lambert law of equation (2.2.6), when a laser beam passes through the fat milk solution, we have that:-

$$I_t = I_o exp(-\mu_t x_1 d) \tag{2.3.1.1}$$

where I_t - Intensity of the transmitted light

I_o - Intensity of the incident light

x_1 - Concentration of fat in the milk solution

d - Thickness of the cuvette which is assumed to be the distance light travels in the sample

μ_t - Total extinction coefficient which can be defined as.

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

Where μ_{sf} -scattering coefficient of fat in the milk solution

μ_{af} - Absorption coefficient of fat in the milk solution

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 is 1.50 cm^{-1} of milk solution is much larger than its absorption coefficient ($\mu_{sf} >> \mu_{af}$) [23].So extinction coefficient is approximately equal to the scattering coefficient which gives as:-

$$I_t = I_o exp\left(-\mu_{sf} x_1 d\right) \tag{2.3.1.2}$$

By applying law of conservation of energy which states about that the energy balance of incident, scattered, transmitted and absorbed light energy, we will have:-

$$I_o S_o = \oint_s I_{si} d_{si} + I_t S_o \tag{2.3.1.3}$$

Where I_0 -incident intensity

S_o - cross-sectional area of the incident laser beam

I_{si} - Intensity of the scattered light of fat in i^{th} direction

d_{si} - Corresponding cross-sectional area of the scattered light

Assume that, $I_s s_R = \oint_s I_{si} d_{si}$ (2.3.1.4)

where I_s - intensity of the scattered light perpendicular to the incident light

S_R -is the corresponding spherical cross-sectional area

These enable us to write equation (2.3.1.4) as equation (2.3.1.5) bellow.

$$S_R = \oint_s \frac{I_{si} d_{si}}{I_s} \tag{2.3.1.5}$$

Up on employing equation (2.3.1.5) into equation (2.3.1.4), we will have:-

$$I_o = u_1 I_s + I_t, \tag{2.3.1.6}$$

where $u_1 = \frac{S_R}{S_O}$ is called uniform scattered coefficient for fat content of milk which is constant to thin milk solution. Taking the ratio of equation (2.3.1.6) and equation (2.3.1.2), we can obtain equation (2.3.1.7).

$$Y_1 = \frac{I_s}{I_t} = \frac{1}{u_1}\left[\exp(\mu_{sf} x_1 d) - 1\right]. \tag{2.3.1.7}$$

Introducing the new term $'\mu_1'$ to equation (2.3.1.7) it can be written as:-

$$Y_1 = \frac{1}{u_1}[exp(\mu_1 x_1) - 1], \tag{2.3.1.8}$$

where $\mu_{1=}\, \mu_{sf} d$

Y_1- Describes the scattered -transmitted -ratio (STR) of the laser beam.

Since equation (2.3.1.8) is an exponential expression, we can approximate the expression by using the appropriate polynomial function. This can be approximated by using Taylor series expansion. If a function $'f'$ and its derivative are defined throughout a closed interval of two points $[a,b]$ containing the term $'k'$ and if $'x'$ is defined in that closed interval, then the function $'f'$ can be expanding as:-

$$f(x) = f(k) + f'(k)(x-k) + \frac{f''(k)(x-k)^2}{2!} + \ldots + \frac{f^n(k)(x-k)^n}{n!} \tag{2.3.1.9}$$

This leads us to expand the expression in equation (2.3.1.8), i.e. $\exp(\mu_1 x_1)$ as follows.

$$\exp(\mu_1 x_1) \simeq \exp(\mu_1 k) + \mu_1 \exp(\mu_1 k)(x_1 - k) + \frac{\mu_1^2 \exp(\mu_1 k)(x-k)^2}{2!} + \ldots \tag{2.3.1.10}$$

Since the concentration of fat (x_1) is small, so that the series is only approximated to the second term and the term $'k'$ is taken to be zero.

Employing equation (2.3.1.10) into equation (2.3.1.8), we can obtain equation (2.3.1.11) bellow.

$$Y_1 = \frac{1}{u_1}\left[\frac{\mu_1^2 x_1^2}{2} + \mu_1 x_1 - 1\right] \tag{2.3.1.11}$$

We can deduce that equation (2.3.1.11) is an approximate expression relation between scattered-transmitted- ratio (Y_1) and the concentration of fat (x_1) which implies that, if we obtain $'Y_1'$ from the experiment, the concentration of fat in the milk solution (x_1) can be determined using this equation.

2.3.2 Beer-Lambert's law Application for Determination of Protein Content

Applying similar mathematical approach as we did in deriving the relation between $'Y_1'$ and $'x_1'$ above, we can deduce the expression describing the relation between $STR(Y_2)$ of the thin milk solution in which both fat and protein exist, the concentration of the fat (x_1) and the concentration of protein (x_2) in the milk solution. As we derive for $'Y_1'$ above, the expression of the thin milk solution $'Y_2'$ can be expressed by equation (2.3.2.1) bellow as follows.

$$Y_2 = \frac{1}{u_2}\left[\exp\left(\mu_{sf} x_1 d + \mu_{sp} x_2 d\right) - 1\right], \tag{2.3.2.1}$$

where Y_2- STR of the milk solution in which both the fat and the protein exist
x_2- Concentration of protein in the milk solution
x_1 -concentration of the fat in the milk concentration
u_2- Uniform scattered coefficient of milk protein content
μ_{sf} - Scattered coefficient of the fat in the milk sample
μ_{sp}- Scattered coefficient of the protein in the milk sample
d - distance that light travel in milk sample.

Applying the same approach what we did to calculate $'Y_1'$ equation (2.3.2.1) can be expanded by using Taylor series expansion to the quadratic term as equation (2.3.2.2) bellow.

$$Y_2 = \frac{1}{u_2}\left[\frac{1}{2}{\mu_1}^2{x_1}^2 + \frac{1}{2}{\mu_2}^2{x_2}^2 + \mu_1 x_1 + \mu_2 x_2 + \mu_1\mu_2 x_1 x_2 - 1\right]. \tag{2.3.2.2}$$

where $\mu_1 = \mu_{sf} d$ and $\mu_2 = \mu_{sp} d$.
The last expression of equation (2.3.2.2) implies that, the protein concentration (x_2) is determined by substituting the value of $'x_1'$ which can be calculated by using equation (2.3.1.11) and using the experimental calculating value of $'Y_2'$ into equation (2.3.2.2).

2.4 Scattering

When light hits a small object (a particle or a molecule) and thereby changes its direction, the phenomena that happens is called light scattering. It is the physical process by which suspended particle in the medium of different index of refraction diffuses incident radiation in all direction. In scattering, there is no transformation of energy. There is only spatial redirection of energy. The scattering of light may be thought of as the redirection of light that takes place when an incident light ray encounters an obstacle, in our case, the scattering particle. Generally we have, Raleigh scattering, Raman scattering and Mie scattering. Among these scattering techniques, the researcher uses Mie scattering that occurs when the dimensions of the scattered particles is much

larger than the wavelength of the incident electromagnetic radiation [19]. 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 distribution of the incident beam when it interacts with particles in material medium of thickness x as shown in the figure (2.4.1).

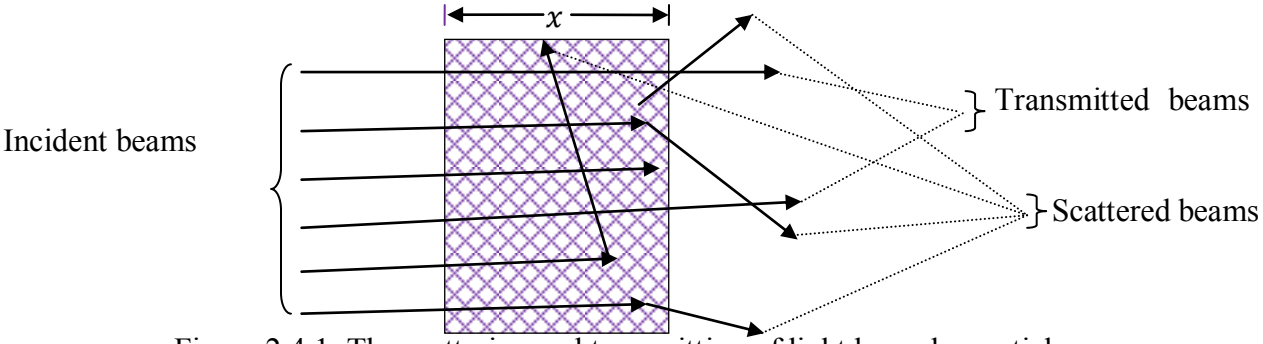

Figure 2.4.1: The scattering and transmitting of light beam by particles

The scattering properties of an ensemble of particles are expressed by the scattering coefficient, $\mu_s(mm^{-1})$ bellow in equation (2.4.1) as follows.

$$\mu_s = \frac{I_s}{I_o d} = \frac{Intensity\ of\ total\ power\ scattered\ by\ particles}{dis\tan ce\ from\ sample to\det ector \times Incident\ \text{int}\ ensity} \qquad (2.4.1)$$

The inverse of the scattering coefficient, $\frac{1}{\mu_s}$ (mm) is the mean free path between two successive scattering events. The scattering coefficient is therefore a useful parameter in determining the concentration of fat and protein in the milk as it enables us to calculate scattered-transmitted-ratio (STR). This parameter (STR) also enables us to determine the content of fat and protein in the milk.

Chapter- 3

3. Materials and Methods

In this Chapter, the materials and methods used for this study are explained. The first Section of this Chapter describes the samples, chemicals and instruments used to perform this research. While, the Second section of this Chapter explains the different methods that applied to determine the amount of fat and protein content in five selected milk samples. The experimental procedures used to analyze determination of fat and protein content in milk samples is also described.

3 .1 Materials, chemicals and Laboratory Apparatus

Materials used to this experiment for standard sample preparation are:-beakers, volumetric flasks, measuring cylinders, Balance, magnetic stirrer with hot plate, syringe, milk sample and rectangular glass cuvette (1cm, 1cm, 4cm).

Chemicals used to prepare standard sample are distilled water and ethylene- diamine -tetra - acetic acid (*EDTA*)

The laboratory apparatus used for this work are also:- 5mw He-Ne laser source emitting at 632.8 nm wavelength, chopper, chopper controller (Standard research system INC, model SR 540) with optimum chopper frequency 550 Hz, photodiode detector, lock-in amplifier (Standard research systems model SR 830 DSP) and spectrometer with instrument number 16156.

3.1.1 Description of Some Laboratory Apparatus

3.1.1.1 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 this 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). It 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 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.

3.1.1.2 Optical Chopper

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

3.1.1.3 Photo Detector

The output current is proportional to 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 had a maximum sensitivity in the red portion of the visible electromagnetic radiation.

3.2 Milk Samples

Five homogenized milk samples have been selected for this experimental work. These milk samples are commercially produced Family, Etete, Harme, 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 almost the same size.

Figure 3.2.1: Preparation of milk sample for experimental work

The average diameter of the fat in the milk sample after it is homogenized is $2000nm$ and that of the protein is $120nm$ [24]. 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. Therefore, the scattered light of the laser has only a profound effect on the protein and fat particles in such a way that STR can absolutely determine the content of fat and protein in the milk solution.

3.2.1 Experimental Work to Determine Fat Content

The first experiment was to measure the scattered and transmitted light intensity to calculate scattered- transmitted-ratio (STR) by diluting the milk with different proportion of the reagent ethylene- diamine- tetra- acetic acid ($EDTA$) solution to carry out the fat content in the milk. The primary advantage of using this $EDTA$ solution is to dissolve the protein in the milk without dissolving the fat in the milk [25]. This solution makes the milk to contain only fat molecules. Therefore, we can easily determine the concentration of fat by calculating scattered-transmitted-

ratio (STR) with scattered and transmitted intensity from the experimental reading and using curve fitting analysis method.

3.2.1.1 Procedures for Determination of Fat Content

Determination of fat content was carried out using the following experimental procedures:-

- We used $100\,ml$ of ethylene- diamine- tetra- acetic acid ($EDTA$) solution.
- We initially added $10\,ml$ of milk to the $EDTA$ solution and read the transmitted and scattered light intensities.
- By increasing the concentration of the milk in steps of $1\,ml$, we read the corresponding transmitted and scattered light intensities for each increment of the milk concentration.
- We analyzed the relation between scattered-transmitted-ratios (STR) with milk concentration.

3.2.2 Experimental Work to Determine Protein Content

The second experiment was also performed and read transmitted and scattered intensity to calculate STR to investigate the protein content in the milk by diluting the milk sample with different proportion of 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.

3.2.2.1 Procedures for Determination of Protein Content

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

- We used $100\,ml$ of distilled water.
- We initially added $10\,ml$ of milk to the distilled water. After that, read transmitted and scattered light intensities.
- By increasing the concentration of the milk in step of $1\,ml$, we read the corresponding transmitted and scattered light intensities for each increment of the milk concentration.
- We analyzed the relation between STR with the concentration of milk.

3.3 Experimental Methods and Procedures

A 5 mw He-Ne laser source emitting at 632.8 nm (Melles Griot) is operating in continuous wave was first modulated using chopper controller (Stanford research systems SR 540) and directed (transmitted) horizontally to the sample box (cuvette) of volume, 1cm× 1cm ×4 cm, which contained (filled) by the milk sample solution. The reference signal was obtained from the chopper controller and feed into the Lock-in amplifier (Stanford research system SR 830 DSP) and we put the photodiodes.

3.3.1 Methods to Measure the Scattering Intensity

The photodiode was 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). Which means that we aligned the photodiodes to detect the scattering intensity at an angle of $90^0 \pm 0.1^0$ with respect to the initial light intensity. After connecting the photodiodes to the lock-in amplifier, the corresponding scattered light intensity was read as shown in the figure (3.3.1.1) as follows.

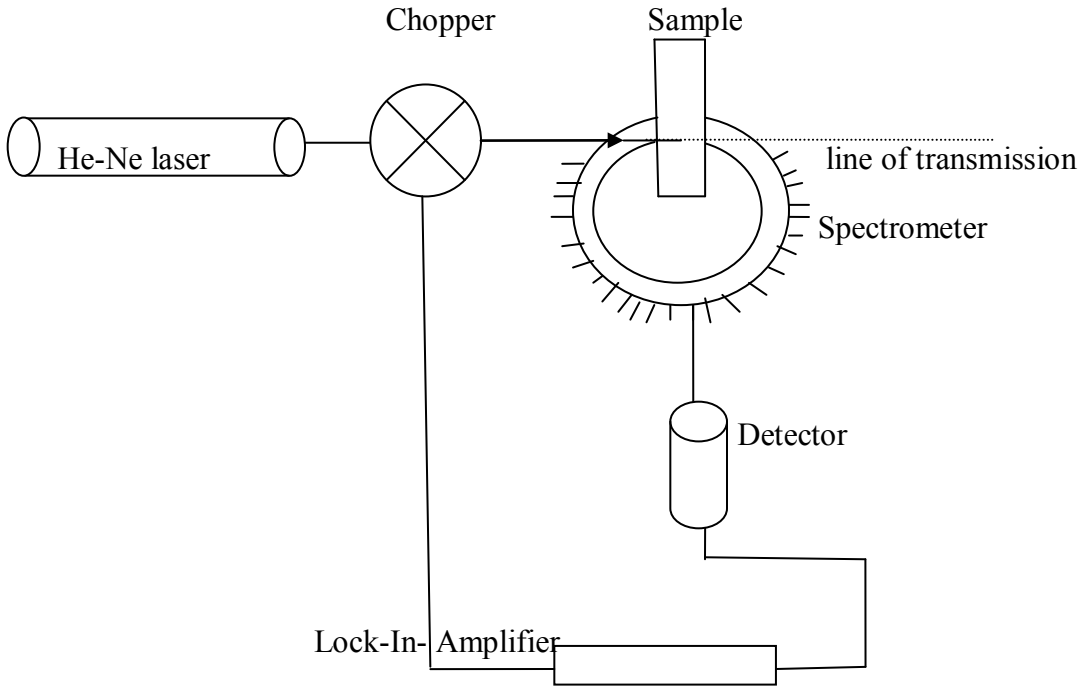

Figure 3.3.1.1: Schematic diagram of the experimental setup for scattering intensity measurement

Figure 3.3.1.2: Photograph of experimental set up used for scattering intensity measurement

3.3.2 Methods to Measure Transmitted Intensity

In this case, the photodiode was placed parallel to the initial beam direction at an angle of $0^0 \pm 0.1^0$ so as to that the collimated transmitted light intensity (I_t) passing through the sample was measured by connecting the photodiode to the lock-in Amplifier as shown in the figure (3.3.2.1) bellow as follows.

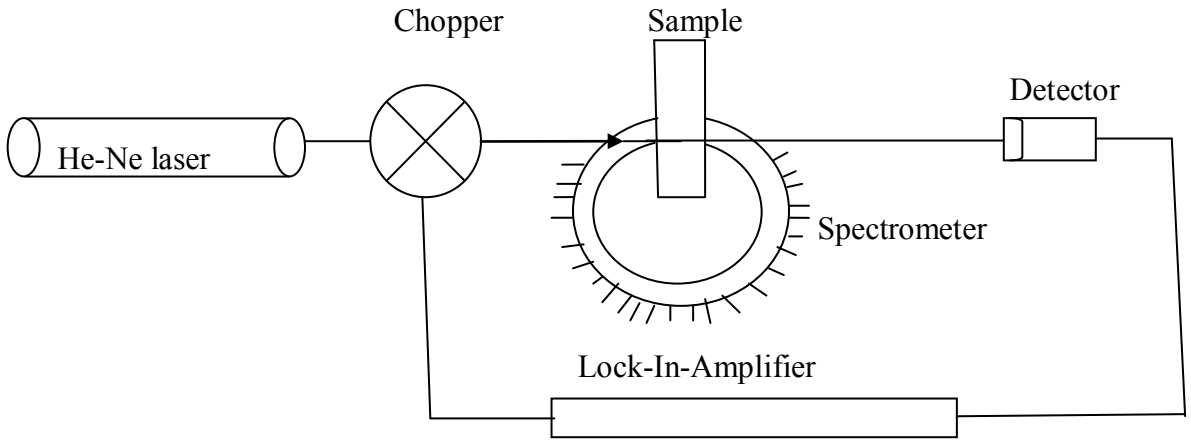

Figure 3.3.2.1: Schematic diagram of experimental setup for transmission intensity measurement

Figure 3.3.2.2: Photograph of experimental set up used for transmission intensity measurement

The transmitted light through the milk sample was detected with photo diode detector connected to the lock-in-amplifier. The lock-in amplifier again connected to the chopper frequency controller (550 Hz) which is fixed for all phase measurements to purify the signal from other interfering frequency noise signals.

3.4 Data collection

Data for this research are collected experimentally and listed in appendix for each five selected milk samples.

Chapter 4

4. Results and Discussion

4.1 Data Analysis by Curve and Surface Fitting Methods

Curve fitting is the process of constructing a curve or mathematical function that has the best fit to a series of data points. Curve fitting can involve either interpolation where an exact fit to the data is required or smoothing in which a smooth function is constructed that approximately fits the data. The idea of curve fitting is to find a mathematical model that fits our data. The curve fit finds the specific coefficients (parameters) which make that function match our data as closely as possible. In curve fitting, we have raw data and a function with unknown coefficients. We want to find values for the coefficients such that the function matches the raw data as well as possible. Fitted curves can be used as an aid for data visualization to infer values of a function where no data are available and to summarize the relationships among two or more variables.

Surface fitting is the generation of a physical surface to pass through or close to a set of existing data points. It is the techniques that use three-dimensional point data to create a raster containing a surface [26, 27]. We want to find values for the coefficients such that the function matches the raw data. Fitted curves can be used as an aid for data visualization to infer values of a function where no data are available and to summarize the relationships among two or more variables. Then we have analyzed our experimental data 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 anywhere along the curve [28]. Fitting is performed for the following two purposes. The first purpose is to determine a physical quantity and a measure of its uncertainty from experimental data when there is a well-established relationship between variables. The second purpose is the goal might be to establish mathematical relationship between dependent variable and independent variables.
In this study, we use a curve fitting method for the first purpose. That is, we have a dependent variable, scattered-transmitted-ratio (Y_1) and an independent variable, which is the concentration of milk (X_1), and a given set of data points with the form, X_r, Y_r, for r =1, 2... m . The purpose is to construct a curve which interpolates or approximates these points.

Also we use surface fitting method for the second purpose. It is a method of fitting a function of two variables to a given set of points [29]. We have a single dependent variable which is the scattered-transmitted-ratio in which both fat and protein exist (Y_2), and two independent variables which are concentration of fat (x_1) and protein concentration (x_2).

The data points are denoted by the form, 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.

4.2 Milk Fat Testing Method

In order to determine the fat content of the milk solution, first we diluted the milk with ethylene-diamine tetra-acetic acid (*EDTA*) solution. The *EDTA* solution dissolves the protein in the milk without 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 samples. We analyzed the relation between scattered-transmitted-ratio (STR) and milk concentration by using curve fitting method. Calculated results obtained for each homogenized milk sample (Family, Etete, Harme, Shola, Mama) are analyzed as follows.

Let us start the amount of fat content data analyzed by Family homogenized milk sample and to each other homogenized milk sample as we have mentioned above.

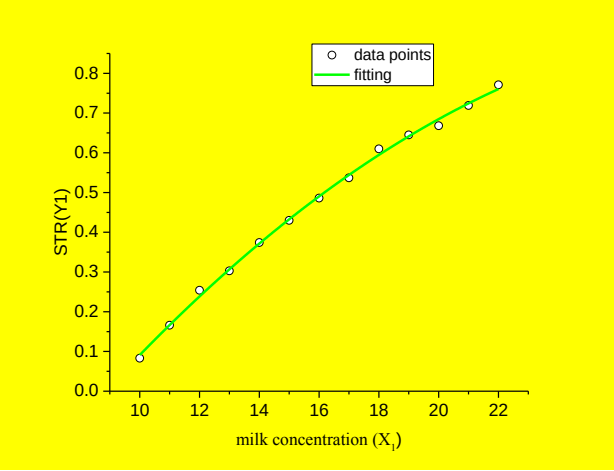

Figure 4.2.1: The relation between STR (Y_1) and milk concentration (X_1) for Family homogenized milk.

The curve fitting shows that the relation between them is quadratic. This indicates that our theoretical derivation based on Beer-Lambert's law for thin fat solution would be absolutely verified by our experimental result. According to the curve fitting method, the relation between scattered-transmitted-ratio (Y_1) and the milk concentration (X_1) of this milk sample is given by the equation (4.2.1) bellow.

$$Y_1 = -0.002X_1^2 + 0.113X_1 - 0.861 \qquad (4.2.1)$$

The fat concentrations (x_1) corresponding to the scattered-transmitted-ratio (Y_1) can be figured out as we see bellow.

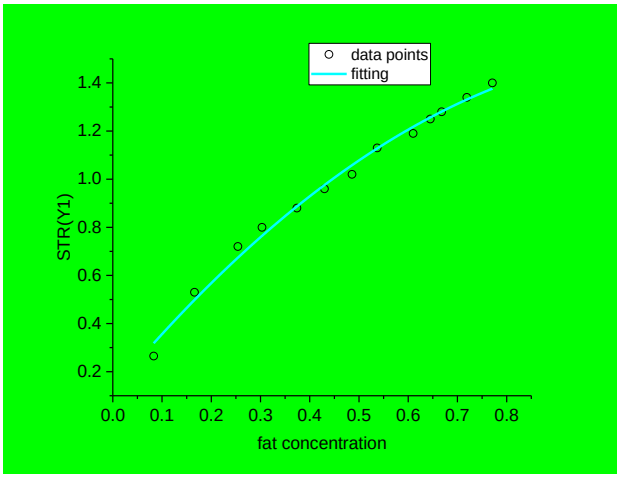

Figure 4.2.2: Illustrates the relation between $STR(Y_1)$ and the fat concentration (x_1)for Family homogenized milk.

From equation (4.2.1) according to our theoretical assumption, the relation between fat concentrations $(x_1)$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 (x_1) and $STR(Y_1)$ can be written as:-

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

where A, B and C are constants to be determined.

Comparing equation (4.2.1), (4.2.2) and substituting the value of each calculated term from appendix we can determine each constant as follows:-

$$Ax_{1f}^2 = -0.002X_{1m}^2, \tag{4.2.3}$$

$$A = -\frac{0.002X_{1m}^2}{x_{1f}^2} = -2.857, \tag{4.2.4}$$

and $$Bx_{1f} = 0.113X_{1m}, \tag{4.2.5}$$

$$B = \frac{0.113X_{1m}}{x_{1f}} = 4.264. \tag{4.2.6}$$

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:-

$$Y_1 = -2.857x_1^2 + 4.26431x_1 - 0.861 \tag{4.2.7}$$

From equation (4.2.1) and equation (4.2.7), we can easily determine the fat content of Family milk sample.

The fat content of the milk at which $'Y_1'$ is equal for the two values is given by equation (4.2.8) bellow as follows.

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

where m_f - fat content of the milk.

For instance, when the STR (Y_1) is 0.083, the milk concentration is 10% and that of the fat is 0.265%. Hence, substituting the values into equation (4.2.8) the fat content becomes 2.650%. This reveals that the amount of fat content that is found in Family homogenized milk is nearly equal to 2.700%.

For Etete homogenized milk sample, we used the same testing method as before to calculate constants and the fat content for Family homogenized milk sample above we get the following.

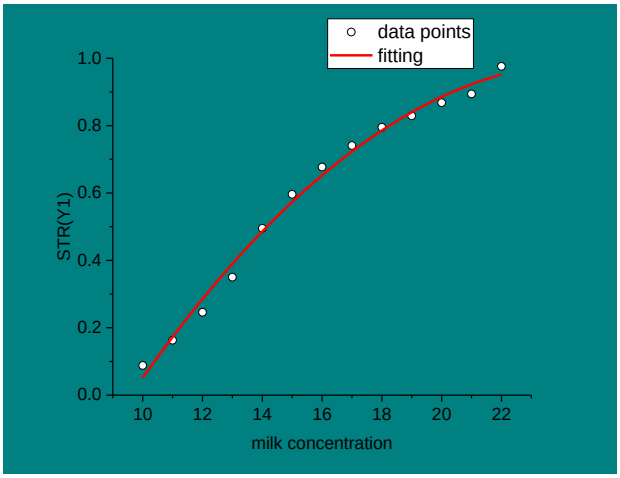

Figure 4.2.3 Describes the relation between $STR\,(Y_1)$ and milk concentration (X_1) for Etete milk type.

The equation that relates milk concentration (X_1) with STR (Y_1) for this homogenized milk sample is given by equation (4.2.9) bellow.

$$Y_1 = -0.00419X_1^2 + 0.20914X_1 - 1.62087 \tag{4.2.9}$$

The fat concentrations (x_1) corresponding to the scattered-transmitted-ratio (Y_1) can be figured out as follows:-

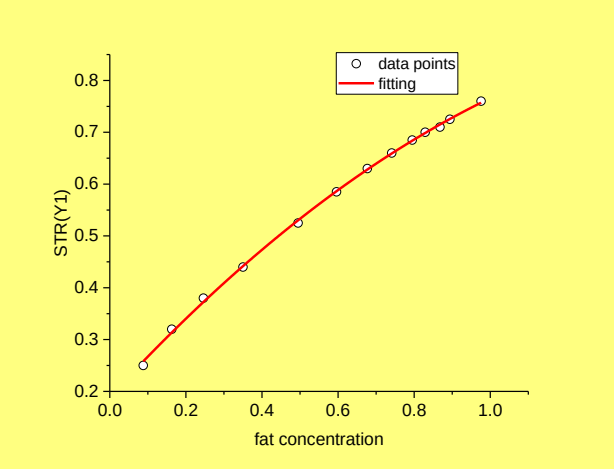

Figure 4.2.4: Illustrates the relation between $STR(Y_1)$ and the fat concentration (x_1)for Etete homogenized milk.

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

$$Y_1 = -6.704x_1^2 + 8.3656x_1 - 1.62087 \qquad (4.2.10)$$

The fat content of the milk at which $'Y_1'$ is equal for the two values is given by equation (4.2.11) bellow as follows.

$$m_f = \frac{x_1}{X_1} \times 100\% \qquad (4.2.11)$$

where m_f - fat content of the milk.

For instance, when the STR (Y_1) is 0.088, the milk concentration is 10% and that of the fat is 0.250%. Hence, substituting the values into equation (4.2.11) the fat content becomes 2.500%. This shows that, the amount of fat content which is found in Etete milk sample is 2.500%.

For Harme homogenized milk sample, we used the same testing method as before to calculate constants and the fat content above we have the following.

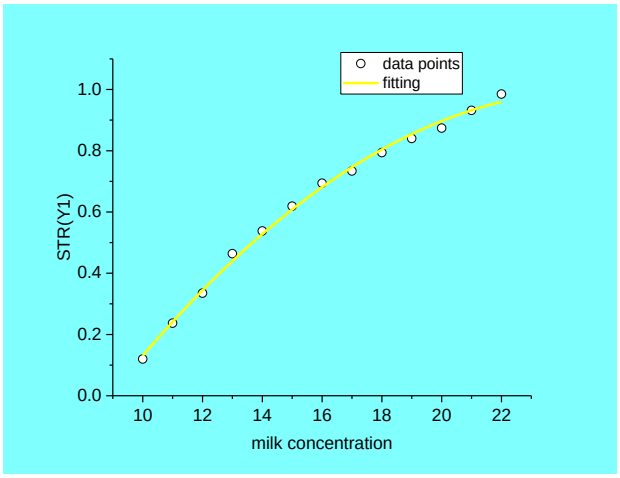

Figure 4.2.5: Describes the relation between $STR(Y_1)$ and milk concentration (X_1) for Harme milk type.

The equation that relates milk concentration (X_1) with $STR(Y_1)$ for this milk type is given by:-

$$Y_1 = -0.00378X_1^2 + 0.189914X_1 - 1.39103 \qquad (4.2.12)$$

The fat concentrations (x_1) corresponding to scattered-transmitted-ratio (Y_1) can be also figured out as follows:-

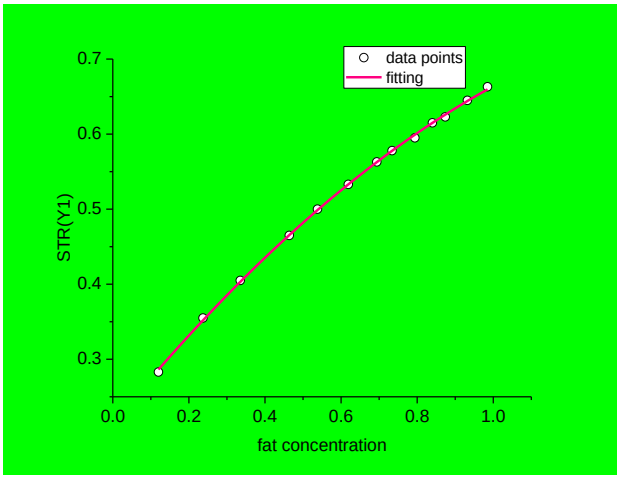

Figure4.2.6: Illustrates the relation between $STR(Y_1)$ and the fat concentration (x_1) for Harme homogenized milk.

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

$$Y_1 = -4.7197x_1^2 + 6.7107x_1 - 1.39103 \qquad (4.2.13)$$

The amount of fat content of the milk at which $'Y_1'$ is equal for the two values is given by:-

$$m_f = \frac{x_1}{X_1} \times 100\% \qquad (4.2.14)$$

where m_f - fat content of the milk.

For instance, when the STR (Y_1) is 0.120, the milk concentration is 10% and that of the fat is 0.283%. Hence, substituting the values into equation (4.2.14) the fat content becomes 2.830%. This indicates that the amount of fat content in Harme homogenized milk is nearly 2.800%.

For Shola homogenized milk sample, we used the same testing method as before to calculate constants and the fat content above and we get the following result for it.

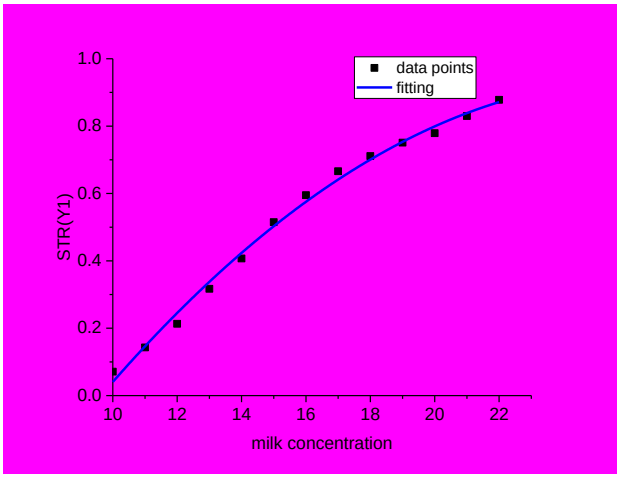

Figure 4.2.7: Describes the relation between $STR(Y_1)$ and milk concentration (X_1) for Shola milk type.

The equation that relates milk concentration (X_1) with $STR(Y_1)$ for this milk type is given by:-

$$Y_1 = -0.00347X_1^2 + 0.17967X_1 - 1.41136 \qquad (4.2.15)$$

The fat concentrations corresponding to the scattered-transmitted-ratio is figured out as follows.

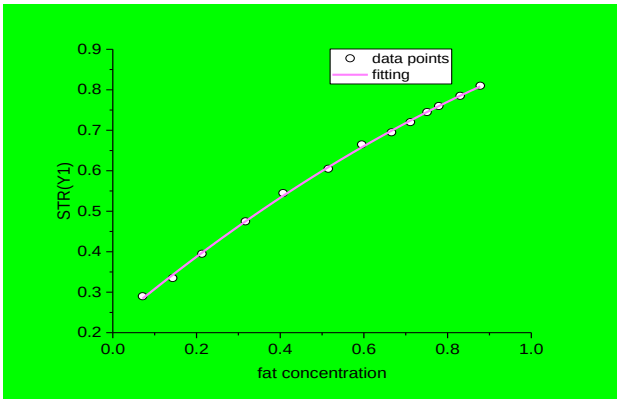

Figure 4.2.8: Illustrates the relation between $STR(Y_1)$ and the fat concentration (x_1) for Shola homogenized milk.

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

$$Y_1 = -4.12604x_1^2 + 6.19552x_1 - 1.41136 \qquad (4.2.16)$$

The fat content of the milk at which $'Y_1'$ is equal for the two values are also given by equation (4.2.17) bellow as follows.

$$m_f = \frac{x_1}{X_1} \times 100\% \qquad (4.2.17)$$

where m_f - fat content of the milk.

For instance, when the STR (Y_1) is 0.071, the milk concentration is 10% and that of the fat is 0.290%. Hence, substituting the values into equation (4.2.17) the fat content becomes 2.900. This shows for us that, the amount of fat content in Shola homogenized milk is equal to 2.900%.

For Mama homogenized milk sample, we used the same testing method as before to calculate constants and the fat content above and we have the following results to it.

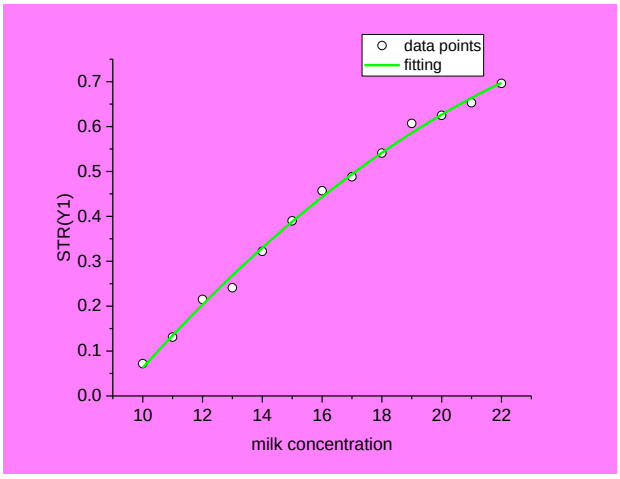

Figure 4.2.9: describes the relation between $STR(Y_1)$ and milk concentration (X_1) for Mama homogenized milk type.

The equation that relates milk concentration with STR for this milk sample is given by:-

$$Y_1 = -0.00174X_1^2 + 0.10856X_1 - 0.84901 \qquad (4.2.18)$$

The fat concentrations corresponding to the scattered-transmitted-ratio can be figured out in the figure (4.2.10) bellow as follows.

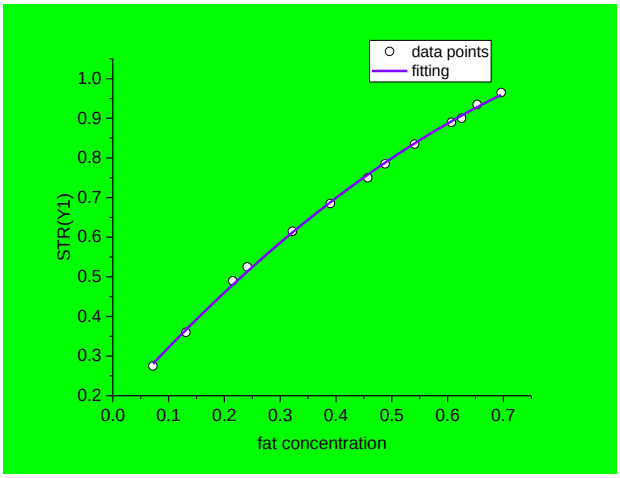

Figure 4.2.10: Illustrates the relation between $STR(Y_1)$ and the fat concentration (x_1) for Mama homogenized milk.

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

$$Y_1 = -2.30067x_1^2 + 3.94764x_1 - 0.84901. \qquad (4.2.19)$$

The fat content of the milk at which $'Y_1'$ is equal for the two values is given by equation (4.2.20).

$$m_f = \frac{x_1}{X_1} \times 100\%, \qquad (4.2.20)$$

where m_f -fat content of the milk.

For instance, when the STR (Y_1) is 0.072, the milk concentration is 10% and that of the fat is 0.275%. Hence, substituting the values into equation (4.2.20) the fat content becomes 2.750%. This indicates to us that, the amount of fat content which is found in Mama homogenized milk is nearly equal to 2.800%.

Equation (4.2.7), (4.2.10), (4.2.13), (4.2.16) and (4.2.19) 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. This implies that the higher the concentration of the fat in the milk sample the larger the value of STR.

4.3 Milk Protein Testing Method

In this study, each selected milk samples were diluted with distilled water of different proportion before testing. In this case, the milk samples contain both fat and protein because of that distilled water can dissolve neither the fat nor the protein in the selected milk sample. For this reason the protein test must be carried out after the fat concentration being tested. It would be better to test the protein concentration independently. However, we have not yet obtained a reagent which can rapidly dissolve the fat without dissolving the protein. By applying the same procedures in milk

fat testing method above and using appendix $'B'$ we can determine the protein content of each selected milk sample as follows. From the curve fitting method the relation between the change of scattered-transmitted-ratio (STR) and the milk concentration of each selected homogenized milk sample (X_2) can be figure out as follows as much as possible.

Let us start the protein content of data analyze by Family homogenized milk sample and to each other homogenized milk sample as we have mentioned each above.

Figure 4.3.1: The change of scattered transmitted ratio (STR) with milk concentration (X_2) for Family homogenized milk.

The relation between scattered-transmitted-ratio (Y_2) and the concentration of milk (X_2) for Family homogenized milk is given by equation (4.3.1) bellow as follows.

$$Y_2 = -0.0012X_2^2 + 0.09846X_2 - 0.68805 \qquad (4.3.1)$$

The relation between protein concentration (x_2) and $STR(Y_2)$ for Family milk sample also figure out as follows.

Figure 4.3.2: The relation between concentration of protein (x_2) with $STR(Y_2)$ for Family homogenized milk

The relation between scattered-transmitted-ratio (Y_2) and the concentration of protein (x_2) for Family homogenized milk can also given by the equation (4.3.2) bellow.

$$Y_2 = -0.0041198x_2^2 + 1.28269x_2 - 0.00818, \quad (4.3.2)$$

The amount of protein content of this homogenized milk samples is determined by the formula that is given by equation (4.3.3) bellow as follows.

$$m_p = \frac{x_2}{X_2} \times 100\%, \quad (4.3.3)$$

where m_p - 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. From appendix $'B'$ we have $Y_2 = 0.162$ the value of milk concentration, $X_2 = 10\%$ and the protein concentration, $x_2 = 0.360\%$ for Family homogenized milk sample. Hence, substituting the corresponding values into equation (4.3.3) the protein content becomes 3.600%. This indicates that amount of protein content for this Family homogenized milk sample is equal to 3.600%.

By applying the same approach for Etete homogenized milk sample we will have the following.

Figure 4.3.3: The change of scattered transmitted ratio (STR) with milk concentration (X_2) for Etete homogenized milk.

The relation between scattered-transmitted-ratio (Y_2) and the concentration of milk (X_2) for Etete homogenized milk is given by the equation (4.3.4) bellow as follows.

$$Y_2 = -0.00426X_2^2 + 0.22231X_2 - 1.67284 \quad (4.3.4)$$

The relation between protein concentration (x_2) and $STR(Y_2)$ for Etete milk sample also expressed as follows.

Figure 4.3.4: The relation between concentration of protein (x_2) with $STR(Y_2)$ for Etete homogenized milk.

The relation between scattered-transmitted-ration (Y_2) and the concentration of protein (x_2) for Etete homogenized milk also given by the equation (4.3.5) bellow.

$$Y_2 = -0.0773x_2^2 + 0.43833x_2 - 0.33007 \tag{4.3.5}$$

The amount of protein content that found in this homogenized milk sample is determined by the formula given by equation (4.3.6) bellow as follows.

$$m_p = \frac{x_2}{X_2} \times 100\%, \tag{4.3.6}$$

where m_p - 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. From appendix $'B'$ we have $Y_2 = 0.144$ the value of milk concentration, $X_2 = 10\%$ and the protein concentration, $x_2 = 0.385\%$ for this Etete homogenized milk sample. Hence, substituting the corresponding values into equation (4.3.6), the protein content becomes 3.850%. This shows that the amount of protein content which is found in this homogenized milk sample is nearly equal to 3.900%.

By applying the same approach what we have done above, we will have the following for Harme homogenized milk sample.

Figure 4.3.5: The change of scattered transmitted ratio (STR) with milk concentration (X_2) for Harme homogenized milk.

The relation between scattered-transmitted-ratio (Y_2) and the concentration of milk (X_2) for Harme homogenized milk can also given by equation (4.3.7).

$$Y_2 = -0.00335X_2^2 + 0.17755X_2 - 1.18685 \qquad (4.3.7)$$

The relation between protein concentration (x_2) and $STR(Y_2)$ for Harme homogenized milk sample also as follows.

Figure 4.3.6: The relation between concentration of protein (x_2) with $STR(Y_2)$ for Harme homogenized milk.

The relation between scattered-transmitted-ratio (Y_2) and the concentration of protein (x_2) for Harme homogenized milk is also given by equation (4.3.8) bellow.

$$Y_2 = -0.13247x_2^2 + 0.60359x_2 - 0.24412 \qquad (4.3.8)$$

The amount of protein content that is found in this homogenized milk samples is determined by the following formula given by equation (4.3.9) bellow as follows.

$$m_p = \frac{x_2}{X_2} \times 100\%, \tag{4.3.9}$$

where m_p - 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. From appendix $'B'$ we have Y_2=0.232, the value of milk concentration, X_2=10% and the protein concentration, x_2=0.38% for this Harme homogenized milk sample. Hence, substituting the corresponding values into equation (4.3.9), the protein content becomes 3.800%. This shows amount of protein content that is found in this milk sample is equal to 3.800%.

By applying the same approach what we have done above, we will have the following for Shola homogenized milk sample.

Figure 4.3.7: The change of scattered transmitted ratio (STR) with milk concentration (X_2) for Shola homogenized milk.

The relation between scattered-transmitted-ratio (Y_2) and the concentration of milk (X_2) for Shola homogenized milk is given by the equation (4.3.10) bellow.

$$Y_2 = -0.00388X_2^2 + 0.20342X_2 - 1.57145 \tag{4.3.10}$$

The relation between protein concentration (x_2) and $STR(Y_2)$ for Shola homogenized milk sample also figure out by the figure (4.3.8) bellow as follows.

Figure 4.3.8: The relation between concentration of protein (x_2) with $STR(Y_2)$ for Shola homogenized milk.

The relation between scattered-transmitted-ratio (Y_2) and the concentration of protein (x_2), for Shola homogenized milk is also given by the equation (4.3.11) bellow.

$$Y_2 = -0.19269x_2^2 + 0.76805x_2 - 0.27495 \tag{4.3.11}$$

The protein content of this milk sample is determined by the formula given by equation (4.3.12).

$$m_p = \frac{x_2}{X_2} \times 100\%, \tag{4.3.12}$$

where m_p - 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. From appendix $'B'$ we have $Y_2 = 0.103$, the value of milk concentration, $X_2 = 10\%$ and the protein concentration, $x_2 = 0.350\%$ for this Shola homogenized milk sample. Hence, substituting the corresponding values what we get into equation (4.3.12), the protein content becomes 3.500%. This indicates the amount of protein content that is found in this homogenized milk sample is equal to 3.500%.

By applying the same approach what we have done above, we will have the following for Mama homogenized milk sample.

Figure 4.3.9: The change of scattered transmitted ratio (STR) with milk concentration (X_2) for Mama homogenized milk.

The relation between scattered-transmitted-ratio (Y_2) and the concentration of milk (X_2) for Mama homogenized milk is given by equation (4.3.13).

$$Y_2 = -0.00213X_2^2 + 0.12574X_2 - 0.92944 \qquad (4.3.13)$$

The relation between protein concentration (x_2) and $STR(Y_2)$ for Mama homogenized milk sample also expressed as follows.

Figure 4.3.10: The relation between concentration of protein (x_2) with STR (Y_2) for Mama homogenized milk.

The relation between scattered-transmitted-ratio (Y_2) and the concentration of protein (x_2) for Mama homogenized milk can also given by the equation (4.3.14) below as follows.

$$Y_2 = -0.39651x_2^2 + 1.20875x_2 - 0.23789 \qquad (4.3.14)$$

The protein content of this homogenized milk samples is then determined by the following formula which is given by equation (4.3.15) bellow.

$$m_p = \frac{x_2}{X_2} \times 100\%, \tag{4.3.15}$$

where m_p - protein content of the milk sample.

For the determination of protein content in this milk sample, we used similar method that we followed to determine the fat content. From appendix $'B'$, we have $Y_2 = 0.099$, the value of milk concentration, $X_2 = 10\%$ and the protein concentration, $x_2 = 0.350\%$ for this Mama homogenized milk sample. Hence, substituting the corresponding values into equation (4.3.15), the protein content becomes 3.500%. This shows the amount of protein content that is found in this homogenized milk sample is equal to 3.500%.

As we have observed above, equation (4.3.2), (4.3.5), (4.3.8), (4.3.11) and (4.3.14) have the same expression. This shows that for any homogenized milk type the concentration of protein and STR can be represented by a standard common curve. The dissimilarity of these graphs 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 other milk sample, the STR will be larger. Which means that, concentrated protein milk sample will result the larger value of STR.

As we have seen above the relation between scattered-transmitted-ratio (Y_1) and fat concentration (x_1) was somewhat easily calculated. However, it is challenging to overcome the relation between scattered- transmitted-ratio (Y_2) and protein concentration (x_2) which is caused by that of the scattered-transmitted-ratio (Y_2) represents both fat and protein concentration. So, determination of protein content can be performed by applying surface fitting method. In short, an exclusive curved surface which denotes the relation between scattered-transmitted-ratio (Y_2), fat concentrations (x_1) and protein concentration (x_2) in the milk solution was analyzed through curved surface fitting.

The three dimensional curved surfaces with their surface equations for each homogenized milk sample (Family, Etete, Harme, Shola, Mama) are shown in the figure (4.3.11), (4.3.12), (4.3.13), (4.3.14) and (4.3.15) bellow as follows, respectively.

The three dimensional curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Family homogenized milk sample is represented by figure (4.3.11) bellow.

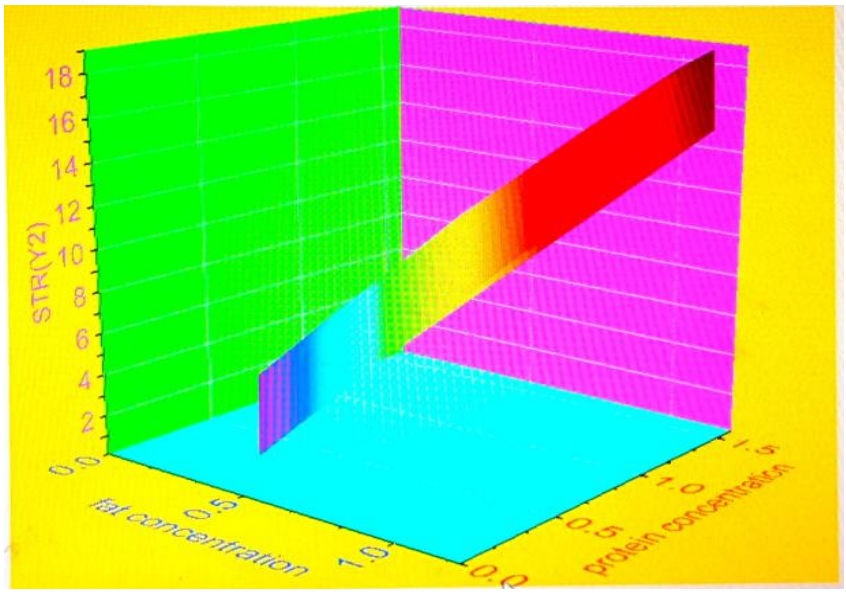

Figure 4.3.11: The curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Family homogenized milk.

The surface fitting equation for this Family homogenized milk sample is represented by the equation (4.3.16) bellow as follows.

$$Y_2 = -0.001x_1^2 - 0.004x_2^2 + 0.136x_1 + 0.058x_2 + 0.008x_1x_2 - 1.869 \qquad (4.3.16)$$

The curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for this Etete homogenized milk type also represented by figure (4.3.12) bellow as follows.

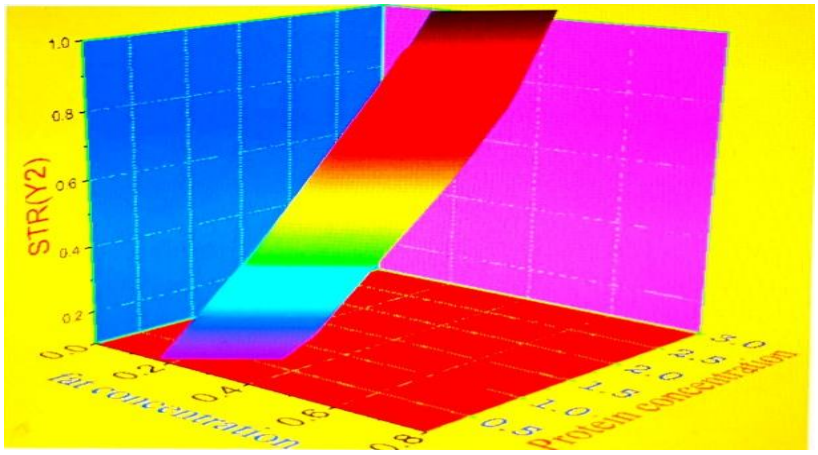

Figure 4.3.12: The curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Etete homogenized milk.

The surface fitting equation for this Etete homogenized milk sample is represented by equation (4.3.17) bellow.

$$Y_2 = -0.007x_1^2 - 0.002x_2^2 + 0.048x_1 + 0.031x_2 + 0.015x_1x_2 - 2.951 \qquad (4.3.17)$$

By applying the same approach that we have used above, the curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Harme homogenized milk sample is represented by the following surface bellow.

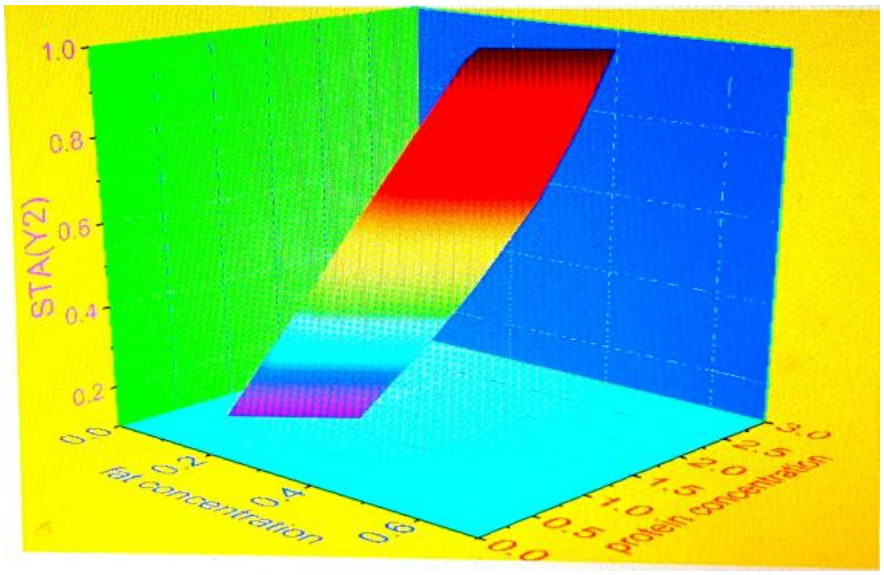

Figure 4.3.13: The curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Harme homogenized milk.

The surface fitting equation for that of Harme homogenized milk sample is represented by equation (4.3.18) as follows:-

$$Y_2 = -0.009x_1^2 - 0.001x_2^2 + 0.376x_1 + 0.04x_2 + 0.016x_1x_2 - 2.635 \qquad (4.3.18)$$

The curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Shola homogenized milk sample can be looks like the following.

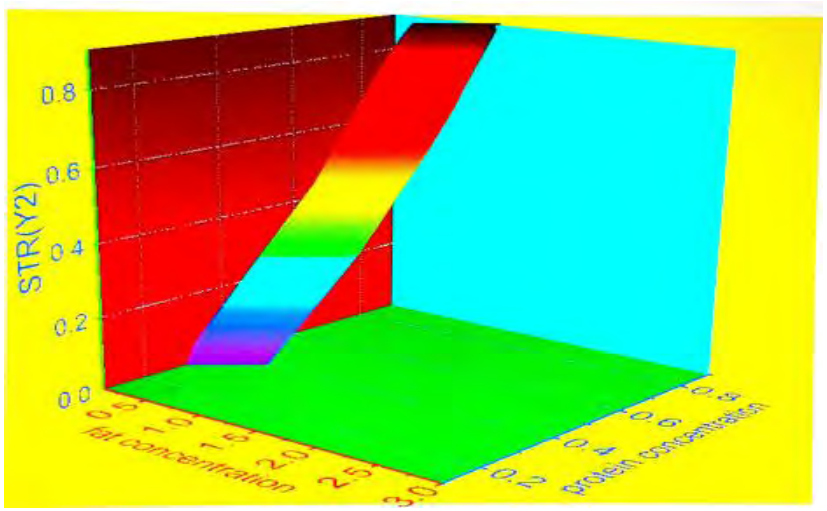

Figure 4.3.14: The curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Shola homogenized milk.

The surface fitting equation for this Shola homogenized milk sample is represented by equation (4.3.19) bellow as follows.

$$Y_2 = -0.004x_1^2 - 0.0002x_2^2 + 0.254x_1 + 0.415x_2 + 0.01x_1x_2 - 2.686 \qquad (4.3.19)$$

Applying the same procedure, the curved surface representation to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Mama homogenized milk sample is figure auto bellow as follows.

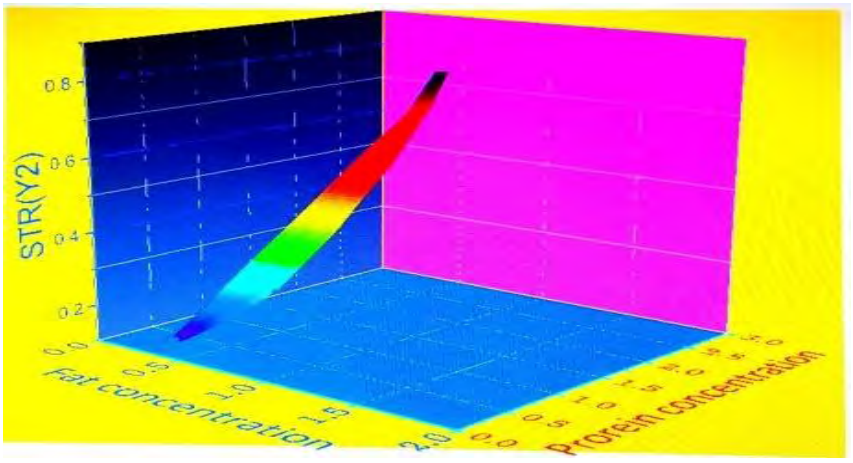

Figure 4.3.15: The curved surface to concentration of fat (x_1), concentration of protein (x_2) and STR (Y_2) for Mama homogenized milk.

The surface fitting equation of this Mama homogenized milk sample is represented by equation (4.3.20) bellow.

$$Y_2 = -0.002x_1^2 - 0.001x_2^2 + 0.118x_1 + 0.048x_2 + 0.005x_1x_2 - 2.087. \quad (4.3.20)$$

As we have observe above that what we have calculated, equations (4.3.16), (4.3.17), (4.3.18), (4.3.19) and (4.3.20) have the same expression without the constant coefficients. This shows that for any homogenized milk type the concentration of protein, fat and scattered-transmitted-ratio can be represented by a standard common surface curve. The dissimilarity of these surfaces 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 and fat concentration compared to the other milk sample, the STR will be larger. Which means that, concentrated protein milk sample will result the larger value of scattered-transmitted-ratio (STR).

The three coordinate axis are the fat concentration in the milk solution (x_1), the protein concentration in the milk solution (x_2) and STR (Y_2).

4.4 Error Analysis

The measure of the distance between the set of data points and the specified function $'f(x)'$ is needed and has been presented in the corresponding tables in the error row. The distance from the single data points (x_r, Y_r) to the specified function $'f(x)'$ can be simply taken as the norm as equation (4.4.1) [30, 31].

$$\varepsilon_r = |Y_r - f(x_r)|, \quad (4.4.1)$$

where $'\varepsilon_r'$ is called the residual of the points. With this definition, the residual is regarded as the function of the coefficients contained in the function $f(x)$; however, the term is also used to mean the particular value of $'\varepsilon_r'$ which corresponds to the fitted values of the coefficients. 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 the following formula given by equation (4.4.2) bellow [32,33].

$$E_r = \sqrt{\sum_{r=1}^{m} \varepsilon_r^2}. \quad (4.4.2)$$

Therefore, the error in fitting the fat concentration with scattered-transmitted-ratio (STR) can be calculated using equation (4.4.2).Putting $'r'$ from m =1,2...13 using error row of appendix $'A'$ Tables, (3.4.1), (3.4.2), (3.4.3),(3.4.4) and (3.4.5) the error in calculating the fat content of Family, Etete, Harme, Shola and Mama homogenized milk sample become $0.002\%, 0.003\%$, $0.003\%, 0.017\%$ and 0.002% respectively.

Similarly, using the error row of appendix $'B'$ Tables, (3.4.6), (3.4.7), (3.4.8), (3.4.9) and (3.4.10), the error for the protein content of Family, Etete, Harme, Shola and Mama

homogenized milk sample become 0.002%, 0.003% , 0.003%,0.015% and 0.016% respectively.

4.5 Result

According to our experimental data analysis, the amount of fat content of Family, Etete, Harme, Shola and Mama homogenized milk sample become $2.700\% \pm 0.002\%$, $2.500\% \pm 0.003\%$, $2.800\% \pm 0.003\%$, $2.900\% \pm 0.017\%$ and $2.800\% \pm 0.002\%$,respectively. This implies that, Harme and Mama homogenized milk samples have the same fat content.

The amount of protein content that are found in Family, Etete, Harme, Shola and Mama homogenized milk samples become $3.600\% \pm 0.002\%$, $3.900\% \pm 0.003\%$, $3.800\% \pm 0.003\%$,$3.500\% \pm 0.015\%$ and $3.500\% \pm 0.016\%$,respectively. Thus, the amount of fat and protein content of each (Family, Etete, Harme, Shola, Mama) homogenized milk sample can be generalized in the table (4.5.1) bellow as follows.

Milk samples used	Amount of fat and protein Contents	
	Fat	Protein
Family	2.700%	3.600%
Etete	2.500%	3.900%
Harme	2.800%	3.800%
Shola	2.900%	3.500%
Mama	2.800%	3.500%

Table 4.5.1 Generalized amount of fat and protein content of each homogenized milk samples

This implies that, Shola and Mama homogenized milk samples have the same protein content. The error in calculating the fat content of Family, Etete, Harme, Shola and Mama homogenized milk sample become $0.002\%, 0.003\%, 0.003\%, 0.017\%$ and 0.002% respectively. Similarly, the error calculating for the protein content of Family, Etete, Harme, Shola and Mama homogenized milk sample become 0.002%, 0.003%, 0.003%, 0.015% and 0.016%, respectively. From our data analysis we observe that, for any homogenized milk type the concentration of protein and STR can be represented by a standard common curve. The dissimilarity of these graphs 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 other milk sample, the STR will be larger. Concentrated protein milk sample will results the larger value of STR and also for any homogenized milk type the concentration of protein, fat and scattered-transmitted-ratio can be represented by a standard common surface curve. The dissimilarity of these surfaces is that the scattered-transmitted-ratio value for the same concentration of the milk samples is not the same. This implies that, if the milk sample has high protein and fat concentration compared to the other milk sample, the STR

will be larger. Which means that, concentrated protein milk sample will result the larger value of scattered-transmitted-ratio.

Comparison between amount of fat and protein content of experimental results of this study with amount of fat and protein content written on the packets to each selected milk samples is tabulated in the table (4.5.2) bellow.

Milk samples	Experimental results of fat and protein		Amount of fat and protein written on the packets	
	Fat	Protein	Fat	Protein
Family	2.700%	3.600%	2.700%	3.600%
Etete	2.500%	3.900%	2.800%	3.500%
Harme	2.800%	3.800%	2.800%	3.500%
Shola	2.900%	3.500%	2.700%	3.500%
Mama	2.800%	3.500%	2.700%	3.500%

Table 4.5.2 Amount of fat and protein content comparison between experimental results of this study and written on the packets of milk samples.

The amount of fat and protein content of the two homogenized milk samples (Shola, Mama) is done by Ermias Abebe. The comparison between experimental results of this study with his results is shown in the table (4.5.3) bellow.

Milk samples	Results of this study		Ermias's Results	
	Fat	Protein	Fat	Protein
Shola	2.900%	3.500%	2.700%	3.400%
Mama	2.800%	3.500%	2.900%	3.600%

Table 4.5.3 Amount of fat and protein content comparison between experimental results of this study with Ermias result.

If we compare these fat content and the protein content of the two homogenized milk sample (Shola , Mama) with fat and protein content of this study, the difference between fat content and protein content is 0.1% and 0.2% for fat content and 0.1% and 0.1% for protein contents.

Generally, our experimental result strongly suggests that, the scattered and transmitted light intensities of He-Ne laser can absolutely determine the fat and protein content accurately and precisely as we have expected.

4.6 Discussion

The primary goal of this experimental work was to investigate (determine) the fat and protein content of five 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's law which relate the concentration of fat and protein with scattered-transmitted ratio (STR), we have able to calculate the fat and protein content of five commercially produced milk samples. According to our experimental result, the fat content of Family, Etete, Harme, Shola and Mama homogenized milk sample become $2.700\% \pm 0.002\%$, $2.500\% \pm 0.003\%$, $2.800\% \pm 0.003\%$, $2.900\% \pm 0.017\%$ and $2.800\% \pm 0.002\%$, respectively. This implies that, Harme and Mama homogenized milk samples have the same fat content.

The amount of protein content that are found in Family, Etete, Harme, Shola and Mama homogenized milk samples become $3.600\% \pm 0.002\%$, $3.900\% \pm 0.003\%$, $3.800\% \pm 0.003\%$, $3.500\% \pm 0.015\%$ and $3.500\% \pm 0.016\%$, respectively. This implies that, Shola and Mama homogenized milk samples have the same protein contents.

The amount of fat and protein content of the two (Shola, Mama) homogenized milk samples is $2.700 \pm 0.021\%$, $2.900 \pm 0.023\%$ and $3.400 \pm 0.015\%$, $3.600 \pm 0.014\%$, respectively which is done by Ermias Abebe. If we compare these fat and protein content of these homogenized milk sample with fat and protein content of this study, the difference between fat and protein content is 0.1%, 0.2% and $0.1\%, 0.1\%$, 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 90^0 and parallel or exactly at 0^0 with respect to the incident beam direction to measure scattered and transmitted light intensities respectively.

Chapter- 5

5. Conclusion and Recommendations (Future prospects)

5.1 Conclusion

The current study demonstrated that, laser light scattering method is helpful for the concerned governmental regulatory bodies to monitor quality of milk products in the market. The fat should be determined through curve fitting and protein can be determined through surface fitting.

We can conclude that, the laser light scattering provides a simple and direct method to determine fat and protein content in liquid milk samples accurately and precisely. It may also be applied to milk powder and other dairy products by applying specific calibration.

The result of this experimental work indicates the feasibility of using scattered-transmitted-ratio (*STR*) for milk fat and protein analysis.

Compared to other methods like spectroscopy determination of fat and protein content of the milk sample, laser light scattering technique has the following some 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 laser light.

3. It helps us to reduce the complexity, difficulty calibration and cost of the instrument used.

5.2 Recommendations (Future prospects)

It would a great interest if further investigations are to be carried out to examine other microbial quality and safety of milk products. The study will create awareness among community or consumers levels in the town. 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 applied in diary industries to determine the amount of fat and protein contents of different dairy products. Furthermore, this experimental work also can be used in hospital for diagnostic purposes by investigating the fat and protein concentration of human blood. So, it helps to treat diseases which caused by high fat and protein concentration. Further research may improve it, if necessary, for other milk samples in different place and time. Thus, it is recommended for food quality (milk) inspection centers to use light scattering techniques for their quality standard based on the amount of fat and protein contents which is found in the milk samples.

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Appendices

Appendix A: Raw Data for Fat Concentration Measurements

Concentration of milk (X_1%)	Concentration of fat (x_1%)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= Exp(-4)
10	0.265	51.550	4.300	0.083	0.082
11	0.530	39.780	6.580	0.166	0.165
12	0.720	35.960	9.130	0.254	0.253
13	0.800	33.380	10.120	0.303	0.302
14	0.880	31.360	11.720	0.374	0.373
15	0.960	29.790	12.800	0.430	0.429
16	1.020	28.060	13.630	0.486	0.485
17	1.130	27.040	14.520	0.537	0.536
18	1.190	25.670	15.670	0.610	0.609
19	1.250	25.020	16.150	0.645	0.644
20	1.280	24.240	16.200	0.668	0.667
21	1.340	23.390	16.820	0.719	0.718
22	1.400	22.730	17.520	0.771	0.770

Table3.4.1:- X_1, experimental (I_t, I_s) and calculated (x_1, STR) data for Family milk sample

Concentration of milk (X_1%)	Concentration of fat (x_1%)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= exp(-4)
10	0.250	75.620	6.680	0.088	0.080
11	0.320	59.780	9.760	0.163	0.155
12	0.380	46.070	11.310	0.246	0.238
13	0.440	36.070	12.630	0.350	0.342
14	0.525	29.260	14.480	0.495	0.487
15	0.585	25.340	15.110	0.596	0.588
16	0.630	23.500	15.910	0.677	0.669
17	0.660	21.870	16.200	0.741	0.733
18	0.685	20.860	16.580	0.795	0.787
19	0.700	20.440	16.940	0.829	0.821
20	0.710	19.640	17.050	0.868	0.860
21	0.725	19.070	17.050	0.894	0.886
22	0.760	18.670	18.230	0.976	0.968

Table3.4.2:- X_1, experimental (I_t, I_s) and calculated (x_1, STR) data for Etete milk sample

Concentration of milk (X_1 %)	Concentration of fat (x_1 %)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= Exp(-4)
10	0.283	62.740	7.500	0.120	0.117
11	0.355	42.760	10.150	0.237	0.234
12	0.405	35.050	11.730	0.335	0.332
13	0.465	29.910	13.890	0.464	0.461
14	0.500	26.530	14.270	0.538	0.535
15	0.533	24.750	15.310	0.619	0.616
16	0.563	23.1300	16.050	0.694	0.691
17	0.578	22.020	16.170	0.734	0.731
18	0.595	20.940	16.630	0.794	0.791
19	0.615	20.240	17.010	0.840	0.837
20	0.623	19.580	17.120	0.874	0.871
21	0.645	18.810	17.540	0.932	0.929
22	0.663	18.230	17.950	0.985	0.982

Table3.4.3:- X_1, experimental (I_t, I_s) and calculated (x_1, STR) data for Harme milk sample

Concentration of milk (X_1 %)	Concentration of fat (x_1 %)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= exp(-3)
10	0.290	77.190	5.510	0.071	0.067
11	0.335	60.540	8.630	0.143	0.139
12	0.395	49.030	10.450	0.213	0.209
13	0.475	38.470	12.210	0.317	0.313
14	0.545	32.480	13.210	0.407	0.403
15	0.605	27.710	14.260	0.515	0.511
16	0.665	25.670	15.280	0.595	0.591
17	0.695	23.850	15.890	0.666	0.662
18	0.720	22.710	16.140	0.711	0.707
19	0.745	21.850	16.400	0.751	0.747
20	0.760	21.130	16.470	0.779	0.775
21	0.785	20.570	17.070	0.830	0.826
22	0.810	20.130	17.680	0.878	0.874

Table3.4.4:- X_1, experimental (I_t, I_s) and calculated (x_1, STR) data for Shola milk sample

Concentration of milk (X_1%)	Concentration of fat (x_1%)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= exp(-4)
10	0.275	55.920	4.030	0.072	0.070
11	0.360	42.200	5.520	0.131	0.129
12	0.490	37.570	8.060	0.215	0.213
13	0.525	35.130	8.450	0.241	0.239
14	0.615	33.280	10.730	0.322	0.320
15	0.685	31.170	12.150	0.390	0.388
16	0.750	29.650	13.540	0.457	0.455
17	0.785	28.460	13.890	0.488	0.486
18	0.835	27.470	14.850	0.541	0.539
19	0.890	26.030	15.790	0.607	0.605
20	0.900	25.600	16.000	0.625	0.623
21	0.935	24.660	16.110	0.653	0.651
22	0.965	23.650	16.470	0.696	0.694

Table3.4.5:- X_1, experimental (I_t, I_s) and calculated (x_1, STR) data for Mama milk sample

Appendix B: Raw Data for Protein Concentration Measurements

Concentration of milk (X_2%)	Concentration of protein (x_2%)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= Exp(-4)
10	0.360	35.590	5.780	0.162	0.159
11	0.470	33.000	8.200	0.248	0.245
12	0.545	29.300	9.660	0.330	0.327
13	0.630	26.070	10.930	0.419	0.416
14	0.660	24.870	11.150	0.448	0.445
15	0.710	22.690	11.260	0.496	0.493
16	0.790	21.650	13.020	0.601	0.598
17	0.810	20.810	13.220	0.635	0.632
18	0.845	19.850	13.470	0.679	0.676
19	0.900	19.700	14.730	0.748	0.745
20	0.940	18.480	14.940	0.808	0.805
21	0.950	18.380	15.240	0.829	0.827
22	0.995	17.460	15.980	0.915	0.912

Table 3.4.6:- X_2, experimental (I_t, I_s) and calculated (x_2, STR) data for Family milk sample

Concentration of milk (X_2%)	Concentration of protein (x_2%)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= Exp(-4)
10	0.385	65.280	9.380	0.144	0.138
11	0.433	49.870	11.730	0.235	0.229
12	0.475	37.700	13.200	0.350	0.344
13	0.533	29.570	14.670	0.496	0.490
14	0.580	24.660	15.500	0.629	0.623
15	0.608	21.850	15.790	0.723	0.717
16	0.635	20.450	16.680	0.816	0.810
17	0.650	19.130	16.840	0.880	0.874
18	0.665	18.100	16.860	0.931	0.925
19	0.680	17.680	17.230	0.975	0.969
20	0.710	16.760	18.010	1.075	1.069
21	0.723	16.510	18.10	1.096	1.090
22	0.745	15.390	18.200	1.183	1.177

Table 3.4.7:- X_2, experimental (I_t, I_s) and calculated (x_2, STR) data for Etete milk sample

Concentration of milk (X_2%)	Concentration of protein (x_2%)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= Exp(-4)
10	0.380	38.440	8.910	0.232	0.225
11	0.450	31.740	11.630	0.366	0.361
12	0.475	28.090	12.700	0.452	0.447
13	0.553	25.340	14.560	0.575	0.570
14	0.595	23.220	15.650	0.674	0.669
15	0.623	21.810	16.210	0.743	0.738
16	0.638	20.450	16.210	0.793	0.788
17	0.663	19.440	16.620	0.855	0.850
18	0.680	18.390	16.640	0.905	0.900
19	0.698	17.940	17.010	0.948	0.943
20	0.713	17.210	17.170	0.998	0.993
21	0.745	16.590	18.110	1.092	1.087
22	0.753	16.380	18.260	1.115	1.110

Table 3.4.8:- X_2, experimental (I_t, I_s) and calculated (x_2, STR) data for Harme milk sample

Concentration of milk (X_2%)	Concentration of protein (x_2%)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= exp(-4)
10	0.350	69.670	7.180	0.103	0.098
11	0.415	52.250	9.900	0.189	0.184
12	0.475	41.670	11.570	0.288	0.283
13	0.550	33.580	12.890	0.384	0.379
14	0.645	26.120	14.500	0.555	0.550
15	0.660	24.540	14.520	0.592	0.587
16	0.705	22.680	15.490	0.683	0.678
17	0.760	21.270	16.560	0.779	0.774
18	0.780	20.660	17.340	0.839	0.834
19	0.805	19.660	17.580	0.894	0.889
20	0.830	18.710	17.970	0.960	0.955
21	0.855	18.180	18.150	0.998	0.993
22	0.855	18.160	18.260	1.006	1.001

Table 3.4.9:- X_2, experimental (I_t, I_s) and calculated (x_2, STR) data for Shola milk sample

Concentration of milk (X_2%)	Concentration of protein (x_2%)	Transmitted Intensity (I_t)	Scattered Intensity (I_s)	Scattered-Transmitted -Ratio(STR)	Error= exp(-3)
10	0.350	54.180	5.390	0.099	0.097
11	0.470	39.340	8.070	0.205	0.203
12	0.540	34.830	9.390	0.270	0.268
13	0.620	32.190	11.410	0.354	0.352
14	0.680	29.310	12.620	0.431	0.429
15	0.735	27.910	13.500	0.484	0.482
16	0.775	26.660	14.500	0.544	0.542
17	0.800	25.220	14.530	0.576	0.574
18	0.850	24.190	15.460	0.639	0.637
19	0.860	23.380	15.600	0.667	0.665
20	0.920	22.190	16.430	0.740	0.738
21	0.940	21.610	16.850	0.780	0.778
22	0.960	20.910	17.010	0.814	0.812

Table 3.4.10:- X_2, experimental (I_t, I_s) and calculated (x_2, STR) data for Mama milk sample