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NAME: Mr. Jigme Wangdi

THIS THESIS HAS BEEN ACCEPTED BY

(Associate Professor Pravee Vijchulata, Ph.D.) **THESIS ADVISOR**

(Associate Professor Pornsri Chairatanayuth, Ph.D.) **COMMITTEE MEMBER**

(Associate Professor Suwapong Swasdiphanich, Ph.D.) **COMMITTEE MEMBER**

(Associate Professor Somnuk Wongtong, Ph.D.) **PROGRAM CHAIRMAN**

APPROVED BY THE GRADUATE SCHOOL ON _____

(Associate Professor Vinai Artkongharn, M.A.) **DEAN**

THESIS

VARIATION OF RAW MILK FAT GLOBULES AND THEIR GRAVITY SEPARATION CHARACTERISTICS

JIGME WANGDI

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the Requirements for the Degree of

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Experiment 1 The study was conducted to investigate gravity separation characteristics of raw milk fat globules (MFGs) during 0 to 8 hrs of holding milk in the glass columns at 4 °C. Means fat content, fat globule size, number of fat globules and total surface area of the raw milk samples were 4.15 %, 3.41 µm, 4.46 x 10^{10} globules/g and 7,612 cm^2 /g respectively. A significant increase ($p<0.01$) in milk fat content was observed at the top fraction was observed throughout the time intervals. On the other hand, a significant decrease in milk fat content was observed at middle and bottom fractions. At 2 h time an increasing rate of 15.84 % h^{-1} at the top fraction and a decreasing rate of 4.03% h^{-1} and 6.09% h^{-1} in fat content was found at the middle and bottom fractions. A significant interaction effect ($p<001$) between time and milk fraction was also observed. There was a significant ($p<0.01$) increase in raw milk fat globules size, i.e. from an averaged 3.38 to 3.56 µm at the top fraction of glass column at 2 h time interval. A significant decrease ($p<0.01$) in milk fat globules size was found until 6 h and 4 h of time interval at the middle and bottom fractions respectively. **Experiment 2** The best mixing indicators among the milk composition and the efficiency of Kasetsart University (KU) versus International Standard Organization (ISO) plungers in mixing raw bulk milk were investigated. Fat content was found to be the best indicator for mixing milk prior to sampling. Fat content stabilized after 10, 5 and 10 times of stirring raw milk truck's chamber I, II and III of 4,700, 3,900 and 7,400 kg capacities respectively. A significant difference ($p<0.05$) was observed in the efficiency of two types of plunger in mixing raw bulk milk. The KU designed plunger was found to be more efficient in mixing small chambers and it was found equally efficient as ISO plunger in mixing larger chambers. Adopting maximum variation of plus or minus 0.1% in fat content between the samples collected at different mixing intervals at least 15 times of stirring for chamber I and II, and 20 times of stirring for chamber III would be required for KU plunger to obtain representative samples. Whereas 20 times of stirring would be necessary using ISO plunger to obtain representative samples for all the chambers with capacity of less than 7,400 kg. **Experiment 3** The effects of homogenization on raw milk fat globules were investigated. A significant difference ($p<0.01$) in milk fat globules size was observed between raw and homogenized milk. The mean fat globules size for the raw milk was 3.37 µm; and for milk homogenized at different times were 0.98 µm (1h) and 0.96 µm (3h). A significant increase ($p<0.01$) in a total surface area was also observed after homogenization of milk. Means total fat surface area of raw milk increased from 7063 cm^2 / g to an average about 24,810 cm^2 /g (1 h) and 25246 cm^2 / g after homogenization.

Student's Signature | Thesis Advisor's Signature | 9/5/06 Date

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Jigme Wangdi
May 2006

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INTRODUCTION

The challenges faced by the dairy industry today is to assure high standards of the starting milk quality, as it provides greater flexibility to the processor in terms of holding milk prior to processing and in development of quality products. A highly perishable nature of the milk further aggravated by a long time lag between milking, collection, and transportation to the dairy plant increase the risk of milk deteoriation; thereby makes difficult to maintain the quality of starting milk. Therefore, to ascertain the suitability of milk for heat treatment and payment purpose, the milk delivered to the dairy plant is subjected to quick quality control tests. However, during holding and/or transportation of milk for a long duration gravity separation of fat globules due to lower density as compared to skim milk leads to formation of strata of milk in the tanks. This causes problem in securing an accurate butterfat sample from the farm tank, which ultimately results in variation of milk fat tests result, sub standard milk product compositions and might even renders milk unsuitable for the processing. Consequently, producer might suffer loss of payment due to abnormal milk fat content of sample mainly if samples are collected after improper or inadequate mixing. Gravity separation, on the other hand is adopted as one of the critical processing steps in separating cream from the rest of the milk constitutes and in development of dairy products of different milk fat contents. It is also used in removing high concentration of microorganisms. In order to address the problems associated with gravity separation proper agitation of milk prior to sampling is imperative mainly because reliable results for quality and compositional tests depend upon the collection of representative sample and its subsequent proper handling. The importance of proper mixing of milk is well understood and most dairy plants mixed the milk in bulk tanks prior to sampling and processing. However, the length of mixing time varies mainly due to variety of the bulk tank designs (size and shape), variation of agitator types (manual plunger, motor driven agitator to air agitation) and their speeds. For instance, in Thailand different designs of plunger are adopted by different dairy plants in mixing milk in the bulk tanks; although, International Standard Organization (ISO) specified

standard plunger is available. This could be due to lack of comparative information on the efficiency of ISO standard to the locally designed plunger.

Milk fat content and globules size play a very significant role in products development. Thus, it is important to avoid agitation and mechanical damages to milk fat globules because this could affect the quality of products made with the milk. Besides milk fat globules damage resulted from excessive agitation or pumping would also reduced the efficiency of skimming of raw milk and also reduce churning efficiency. Therefore, the milk in the tanks should be agitated in such a way that there is minimum or no impact on the stability of milk fat globules. Hence the mixing of milk should be very smooth and the length of time for proper agitation should be adequate.

Objectives

1. To study the variation of raw milk fat globules
2. To investigates the gravity separation characteristics of natural raw milk fat globules
3. To evaluate the proper agitation techniques for raw bulk milk
4. To determine the effect of homogenization on milk fat globules

LITERATURE REVIEWS

World dairy industry and milk consumption trends

Breeding cows are able to produce more milk than their offspring requires mainly due to development in advance technologies, improvement in management practices especially in the field of feedings and nutrition. The excess milk produced is harvested by man to make a significant contribution to his diet and income. The total world milk production of different species was estimated at 557 million tonnes in 1998, dominated by cow's milk accounting about 90% and buffalo, sheep and goats milk accounts about 6%, 1.7% and 1.5% respectively (Kelly, 2001). Tracy *et al*. (1958) advocated that the world dairy industry would continue to grow with increasing per capita consumption of various dairy products. Today, the tangible growth in dairy industry could be substantiated by the ever increasing milk and dairy product consumption as advocated by Tracy *et al.* (1958). Statistics showed that about 94 % of the world milk supply is utilized as processed milk (Harding, 1995). Jost (2000) reported that 60% of cows' total milk produced was processed into dairy products (i.e. yogurt, butter, cheese and milk deserts) with the rest consumed as loose milk. The per capita milk consumption (as fluid milk and processed products) averaged 107 kg world-wide, but varies from 380 kg in Europe and 280 kg in North America and Oceania, to 50 kg in Southern Asia and 20 kg in Eastern Asia (Goff, 1999). The decreasing trend of raw milk consumption from 49 % in 1953 to 33 % in 1995; with increasing trend in milk products consumption especially cheese from 13 % in 1955 to 33 % in 1995 was reported (Harding, 1995). There was increased consumption of fresh dairy products (i.e. yogurt, fresh cheese and milk deserts) with stagnating liquid milk consumption in industrialized countries. The highest growth rates in milk products consumption was about 4-5 % per annum in East and South Asia (Jost, 2000). Australian Trade Commission (ATC) (2005) reported that the growth rate of milk consumption increased by 14 per cent between 1989 and 1993 and current growth rate of approximately 20 per cent per annum; with annual per capita milk consumption expected to reach 35 kilograms by 2007 in Thailand.

On the other hand, the per capita butter consumption decreased in most countries over the baseline, with the exceptions being Poland, Brazil, and Mexico (Agriculture Outlook, 2003).

Nutritional values of milk

Milk is considered as the most nutritious food ever. Whole milk about 24 g would provide about 90 to 150 kcal depending upon the fat and milk solid not fat (MSNF) content (Posati and Orr, 1976). Besides, milk is associated with many of the vitamins (A, D and E), pigments, and other compounds, which enhance the nutritive as well as the commercial value (Olson, 1950). A quartz of milk provides all of the calcium and phosphorous needed by an individual for one day, a liberal amount of vitamin A and riboflavin, one third or more of protein, one eighth or more of iron, one fourth of the energy and some of the vitamins B, C and D. Furthermore, milk is reported as a highly digestible food with digestibility of 99% for fat, 97% for protein and 98 % for carbohydrates. In addition, milk and dairy products in diet would help in digestion process through coagulation in the stomach; and as well as increase the life span and promote virility and fertility (Adam, 1947).

The relation of milk consumption to the human health especially association of milk fat to arthrosclerosis is of concerned. This could be mainly attributed to the fact that the milk fat contains about 70% saturated fatty acids, 25% monounsaturated acids, and 5% polyunsaturated fatty acids which highly exceeds the ideal milk fat recommended. Various government and health professional organization in America recommended that healthy person above 2 years to consume a diet that provide an average of no more than 30% of total calories as fat, less than 10% of total calories as SFAs, and less than 300 mg of cholesterol a day (Miller *et al.,* 2000). However, the authors in their reports on the effects of individual fatty acid on blood lipid levels reported that the major fatty acids, i.e. long chain saturated fatty acids (SFAs) mainly stearic acid; short chain SFAs; monounsaturated fatty acids (MUFAS); polyunsaturated fatty acids (PUFAs), and possibly conjugated linoleic acid (CLA) and sphingolipids found in milk fat does not have hypercholesterolemic effect. CLA has a powerful anti-cancer potential and prevent the arteries from getting clogged easily. Milk is known to have a high concentration of CLA, and

experimental animal studies indicated that CLA may also reduce the risk of chronic heart disease (CHD) (Miller *et al*., 2000).

The advantages of milk as articles of diet greatly outweighed the disadvantages. Thus, even today, milk could be still considered as "Natures most nearly perfect food" especially in the developing countries and this could be justified by the ever increasing growth rates in milk and milk products consumption.

Milk composition and its variations

Milk is the normal product of mammary gland secretion, with a very complex composition. The constituents, such as milk fat, milk sugar and casein are not found else where, either in the body or in nature, and practically it is only food stuff which contains all of the different substances known to be essential for human nutrition. It is estimated to contain more than 100,000 molecular species. However, the main compositions of milk are water, milk solids (total solids), i.e. fat, protein, lactose and other solids, i.e. minerals and vitamins. The average gross composition of cows' milk consists of about 87% water and 13% dry substances, i.e. 4.1% fat, 3.6% protein (79.5 % total casein protein, 19.3 % total whey protein and 1.2 % fat globule membrane protein), 4.9% lactose and 0.7% ash (de Wit, 1981; Tetra Pak, 1995). Non-water constitutes are present in different physical forms; dissolved (lactose), colloidally dispersed (protein) and emulsified in water (fat) due to its relatively small particles (Table 1). These physical characteristics are used to facilitate the commercial analytical separation of the major constitutes of milk.

Table 1 Relative size of different particles in milk.

Types of particles	Size (mm)
Fat globules	10^{-2} to 10^{-3}
Casein-calcium phosphates	10^{-4} to 10^{-5}
Whey proteins	10^{-5} to 10^{-6}
Lactose, salts and other substances in true solutions	10^{-6} to 10^{-7}

Source: Tetra Pak (1995)

A number of factors are reported to affect the quantities of the various main constituents of milk. The factors are the breed and individuality of the cow, time of milking, season of the year and the cow's feed and living conditions (Lampert, 1947; Tetra Pak, 1995). Among these factors the breed of the cow has a great influence upon the composition of milk and the greatest difference was found in the fat content and other composition shows less variation (Lampert, 1947). The average milk compositions of different breeds are presented in Table 2.

Table 2 Average milk compositions of different breeds.

Breed	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Ayrshire	3.85	3.35	4.95	0.69
Guernsey	4.90	3.85	4.95	0.75
Holstein	3.40-4.00	3.25	4.60	0.73
Jersey	5.14	3.80	5.00	0.75
Milking Shorthorn	3.65	3.30	4.80	0.69

Source: Robinson and Wilbey (1998).

The fat concentration is most sensitive to dietary changes, followed by protein concentrations; whereas the concentration of lactose, vitamins, minerals and other solid constitutes of milk does not respond much to the dietary alterations (Walder *et al*., 2004). This finding could be substantiated by statistical parameters illustrated in Table 3.

Table 3 Variations in the composition of raw milk.

Milk composition		Trocher (1925) individual milk	Overman *et al.* (1939) 3-day composites	Herrington *et al.* (1972) bulk tank
N		676	2426	868
Fat (%)	Mean	3.95	4.37	3.53
	SD	0.78	0.82	0.28
	CV	0.20	0.19	0.08
CP (%)	Mean	3.24	3.74	3.13
	SD	0.40	0.52	0.14
	CV	0.12	0.14	0.05
Lactose	Mean	4.64	4.89	4.82
(%)	SD	0.37	0.38	0.16
	CV	0.08	0.08	0.03
Ash (%)	Mean	0.70	0.72	0.72
	SD	0.05	0.05	0.01
	CV	0.07	0.07	0.02
TS (%)	Mean	-	13.73	12.02
	SD	-	1.23	0.63
	CV	-	0.09	0.05

Sources: Compiled by Jenness (1988).

The concentration of milk fat is influenced by many factors including diets, breeds, stage of lactations, nutrition, frequency of feeding, genetics, management and environment (Anonymous, 2005a). The trend of milk fat production is a seasonal operation depending on the availability of feed, climatological conditions and on traditional management practices. A strong influence of season on fat component as compared to other components of the milk, with relatively high fat percentage during the autumn months and during summer months was reported (Berg, 1988). Yadav *et al.* (1994) reported that the milk fat exhibits pronounce seasonal trend being higher in winter than in summer.

Diseases in general caused an increase in fat and salt content, and diminishing trend of lactose content (Lampert, 1947). Today, mastitis is the predominant disease intensively studied, and it is reported to reduce fat and casein content and increase whey content of the milk (Table 4).

Table 4 Change in milk constituents associated with elevated somatic cell counts.

Constituent	Normal Milk %	High SCC Milk %	Normal %
Milk nonfat solids	8.9	8.8	99
Fat	3.5	3.2	91
Lactose	4.9	4.4	90
Total protein	3.61	3.56	99
Total casein	2.8	2.3	82
Whey protein	0.8	1.3	162
Sodium	0.057	0.105	184
Chloride	0.091	0.147	161
Potassium	0.173	0.157	91
Calcium	0.12	0.04	33

Source: Harmon (1994).

The differences in sampling procedures, especially accuracy in sampling and the number of sample size could have also resulted in variation of milk constituents. For instance, the variation of the milk constituents from the farm was much lower than that of the individual farmer (Table 5). This difference could have resulted from different sample size, besides other factors reported.

Table 5 Comparison of raw milk composition between individual and farm in Thailand.

Milk composition	N	Mean	SD	95% CI	p-value
Fat					
Farm	31,705	3.63	0.63	0.169 - 0.232	0.000
Individual	2,293	3.82	1.65		
Protein					
Farm	31,705	3.07	0.26	0.122 – 0.146	0.000
Individual	2,301	3.21	0.47		
Lactose					
Farm	31,674	4.48	0.34	0.141 – 0.171	0.000
Individual	2,289	4.33	0.51		
SNF					
Farm	31,705	8.26	0.49	0.006 – 0.049	0.012
Individual	2,301	8.23	0.66		
Total solids (TS)					
Farm	31,472	11.90	0.87	0.021 – 0.102	0.000
Individual	2,301	12.05	1.79		

Source: Srongsumod and Kiarsunthorn (2005).

Feed is the principal cause of variation in the composition of fat, although several other factors are believed to influence (Herrington, 1948). Likewise, Nickerson (1995) reported a change in nutrition, i.e. type of diets, nutrient content of the diet, sizes of the forage and ratio of roughage to concentration would affect the fat composition. With manipulation of diet, variation in fat content by over a 3.0 per cent unit could be achieved (Harding, 1995). However, the moderate reduction in the percentage of milk fat caused by changes in feeding management could be compensated by an increase in milk yield (Harding, 1995; Walder *et al*., 2004). Hence, variation in fat content has been studied far more intensively than those in the other constituents, mainly because of its greater economic value.

Milk fat

Fat is a principal component of milk, exists in microscopic globules, dispersed within the aqueous phase of milk (McBean and Speckmann, 1988; Burgess, 2001). It makes up from 2.5 – 6 % of milk and varies between breed of cattle and with feeding practices (Tetra Pak, 1995). Milk fat serves as a major source of energy and essential structural components for the cell membrane of the neonates in all mammalian species. The energy value contributed by milk fat to the body is about 38 kJg^{-1} compared with about 17 kJg^{-1} for protein and 16 kJg^{-1} for carbohydrates (Gurr, 1998). Likewise, Burgess (2001) reported fat as a rich source of energy yielding approximately 9 kcal per gram; as a carrier of the fat soluble vitamins A, D, E and K; and it contains significant amount of essential fatty acids (linoleic and arachidonic) required by human beings.

Milk fat has always had an important bearing on the economics of milk and milk products mainly because it is considered as the indicator of the quality milk; besides milk is normally sold on the basis of its fat content (Hasanuzzaman *et al.*, 2002). Milk fat is the oldest and most widespread compositional payment criteria adopted. However, today crude protein level is increasingly being used as a payment basis due to negative impact of dietary fat on health, and its parallel importance in the products development. These payment criteria were reported to vary between countries and individual processors (Harding, 1995; Hurley, 2004). Although, in the recent years considerable changes on milk payment system was evident; it was reported that the milk fat would still play a very significant role in determining the price of milk both with regards to base price and differential for milk fat content exceeding the base price arrangement. Besides, the cost of dairy and food products that contain milk solids would depend to a considerable extent on the amount of fat which they contains (Harding, 1995).

Milk fat have greater function than any other milk constitutes in product development mainly because the technological ability, rheological and sensory properties of many dairy products depend on the fat globules size distribution and on the composition of their membrane that affect their interaction with the protein (van Vliet, 1988; Cho *et al.,* 1999; Lopez and Defour, 2001). The most distinctive roles the milk fat plays in dairy products concerns flavor, and this

rich pleasing flavor of milk fat is not adequately duplicated by another type of fats. The typical milk flavor is derived from over 120 compounds, with most significant contribution from that of free fatty acids, i.e. methyl ketones and dimethyl sulphide (Burgess, 2001). Thus, milk fat could have been considered as the most complex among the common fats exhibiting unique physical, chemical and biological properties not easily duplicated by other fats (Lampert, 1947). Its complexity among the common fats could be substantiated by a wide melting range, i.e. in between – 40 to + 40 $^{\circ}$C (Walstra *et al*., 1995) and the unique fatty acid profile of milk fat, comprising approximately 62% saturated fatty acids (SFAs), nearly 30% monounsaturated fatty acids (MUFAs), 4% polyunsaturated fatty acids (PUFAs), and remaining 4% comprised of other types of lipids (Miller *et al*., 2000).

Gravity separation

The formation of a cream layer on the surface of milk is an everyday phenomenon, and this natural creaming process or gravity separation is adopted as one of the critical processing steps in removing or concentrating milk fat prior to the invention of cream separator (Ma and Barbano, 2000). It is also used to remove a high percentage of microorganisms present in raw milk (Dellaglio *et al.,* 1969). On the other hand, variation in milk composition and bacteria counts of farm bulk-milk samples was mainly caused by gravity creaming due to the association of bacteria with fat globules, which cause both fat and bacteria to concentrate on the surface (Belknap *et al.*, 1978; Jackson, 1981; Goodridge *et al.*, 2004). Servello *et al.* (2004) reported that bacterial cell counts were directly correlated with fat content, but somatic cell counts were independent of fat content.

Milk fats are suspended in the water of milk (skim milk) as fat droplets due to lower specific gravity of milk fat (between 0.91 and 0.93 g/cm^3) as compared to non fat fraction of milk (between 1.0295 and 1.035 g/cm^3). The fat globule buoyed up by a force greater than its own weight, thus tends to rise or float (Warner, 1976). The fat globules rise very slowly in accordance to Stokes' equation; $V_s = a(\rho_s-\rho_f)\, d^2/18\eta$, where a = acceleration due to gravity, ρ = density, η = viscosity, and subscripts *s* and *f* refer to skim phase and fat globules, respectively (Herrington,

1948; Mulder and Walstra, 1974; Fox, 1995; Walstra, 1995). The rate of rising of different fat globules size is illustrated in the Figure 1. It indicates that the larger fat globules would move much faster than the smaller fat globules. A simple calculation adopting the Stokes's equation shows that if each of the globules rise independently, the average globule with a diameter of about 3 μm would rise at the rate of 2.54 cm in about 6 hrs and the smallest globules would rise about 1/10 of the rate (Prentice, 1992).

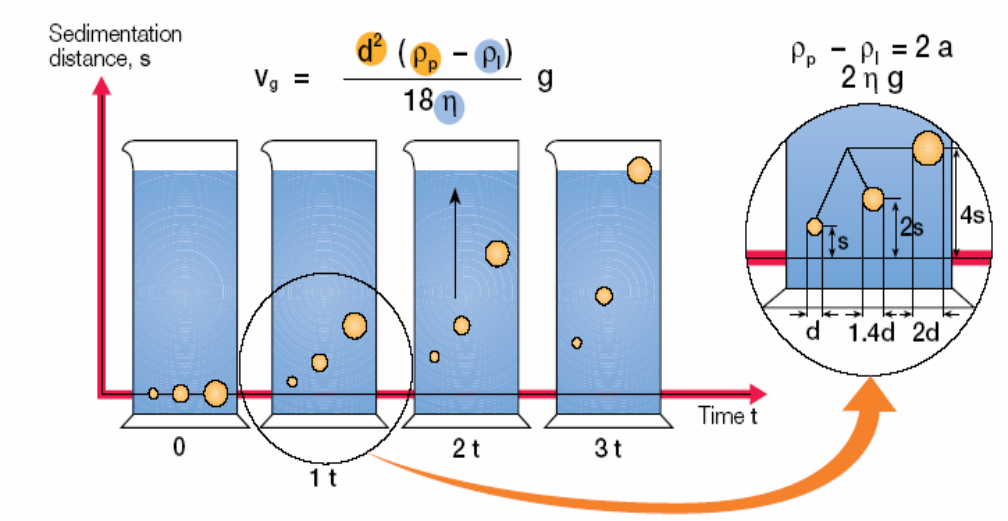

Figure 1 Floatation velocities of different fat globules size.

Source: Tetra Pak (1995).

However, in practice the milk fat globules (MFGs) were found to rise much faster than predicted by Stokes law. This could be substantiated by the formation of cream layer within 20 to 30 min of holding cold milk (Anonymous, 2005b). The onset of creaming was observed after approximately 30 min (Jackson, 1981) and 40 to 50 min (Servello *et al., 2004*). The fat content for the final cream on the surface due to gravity separation was reported to vary in between 10 to 28 % during the shelf life of milk (Lampert, 1947). The thickness of the cream layer could reach maximum after 4 hours of quiescent holding (Walstra *et al.*, 1999; Servello *et al.*, 2004). However, the creaming rate was reported to vary (Servello *et al.*, 2004). The gravity separation of raw milk is affected by several factors, such as the concentration of agglutinin, size of fat

globules, fat content, agitation, warming or heat treatment, homogenization, and temperature (Walstra, 1995). The action of cold agglutination increased the sizes of the fat globule mass; hence, increase in the diameter. This results in increase in both the surface area and the volume of the globule, but at the different rates. Volume increased more rapidly than surface area, therefore the buoyancy increases more rapidly than resistance. Hence, a larger fat globule would move better against the friction because the surface area for each unit of volume is less for the larger globule, thus its resistance is less than the smaller globule (Warner, 1976). The greater the pressure used, the smaller would be the size of the fat globules (Figure 2). Hence, no appreciable cream layer would be formed on homogenized milk due to the small size of the fat globules and their inability to coalesce greatly decreases their ability to rise to the surface (Lampert, 1947). The creaming and depth of the cream layer formed would depend on milk temperature (Ma and Barbano, 2000; Servello *et al*., 2004). In the recent studies conducted by Ma and Barbano, (2000), the rate of gravity separation of fat globules was observed much greater in the milk stored at 15 °C than at 4 °C.

The possibility of deriving different milk products of range of fat contents through gravity separation by holding raw milk at 4 °C for 48 h without agitation was reported. The possible products are skim milk (0.2% fat), low fat milks (1-2% fat) and cream (containing about 25 to 27% fat) (Ma and Barbano, 2000). These products are found very similar to the products derived by centrifugation in a commercial separator, i.e. the fat reduced-milks (skim milk up to 0.3%, semi-skimmed milk 1.5-1.8%) and high fat cream (Harding, 1995).

Ma and Barbano (2000) advocated that, this simplicity of gravity separation could be adopted by small scale dairy product manufacturers to produce milks with range of fat contents without investment on cream separator. However, the question arises on the feasibility to store the milk for such a longer period, considering the availability of resources with the small scale dairy product manufacturers.

Cream separators have replaced the gravity separation process in large dairy industry and the separation pace is accelerated by many folds. However, even today in many areas with low

milk production, poor processing facilities and inadequate marketing supports the gravity separation still plays a very important role in the process of products development. For instance, gravity separation is used as a critical processing steps in the production of Italian hard cheese Parmigiano Reggiano and Grana Padano (Nizzalo, 1969).

Fat globule size distribution

The fat globules have a distinct effect on some physical properties of the products, the more so at the high fat contents. Fat globule scatter light (Mulder and Walstra, 1974), and this optical properties is used in estimating fat globule size or fat content in milk (Walstra, 1965). Total fat content and fat globule size distribution affects the viscosity of milk and its importance in the processing and manufacturing of milk products (Mulder and Walstra, 1974; Attaie and Richter, 2000). For instance, Camembert cheese produced with native small MFGs contained more moisture, was less firm and had a more elastic texture than cheese made of milk containing large MFGs, which is explained by the larger surface area of small MFGs having a higher water binding capacity and that the thinner casein stands are formed in cheese with small MFGs (Michalski *et al.*, 2003). On the other hand, the low-fat Cheddar cheese made with large fat globules resulted in the best texture, flavor and color compared with cheeses produced with small or conventional MFGs. Emmental cheese produced from ripened small fat globules (~3 μm) was found less flexible and less firm than large fat globule cheese (~6 μm). The small fat globule cheese contained more moisture and underwent greater proteolysis than the large fat globules after 52 days of manufacturing. However, lypolysis was found to be three-fold lower in small fat globule cheese. The use of native milk fat globules with different sizes could lead to a range of new dairy products with different physio-chemical and functional properties (Michelski *et al.,* 2004). Thus, several studies were conducted to understand the nature of milk fat globule size distributions. Fat in milk is predominantly present in spherical globules of different sizes. Fat globules size is most commonly reported as the volume-surface average diameter (d_{vs}) value defined as $\sum N_i d_i^3 / \sum N_i d_i^2$, where N_i is the number of fat globules of diameter d_i (Thieband *et al*., 2003). The d_{vs} of most milk was reported to range in between 2.5-5 μm (Walstra, 1969; Mulder and Walstra, 1974). For instance, Warner (1976) reported that the fat globules size in cows' milk

ranges between 3.3 and 4.0μm; in buffalo milk between 3.9 and 5.0 μm; and in goat milk between 2.2 and 3.5 μm. The individual fat globule size of goat milk ranges from 0.73 to 8.58μm in diameter with d_{vs} of 2.76 μm. This was observed much smaller than the mean d_{vs} of 3.51 μm for bovine milk with fat globules size ranging from 0.92 to 15.75 μm in diameter (Attaie and Richter, 2000). The range of fat globules size reported varies (Table 6).

Table 6 The range of fat globules size in bovine milk.

References	Range (μm)	Average (μm)
Lampert (1947)	0.1 – 20	3
Fleischmann's figure quoted by Jenness and Patton (1959)	0.1 – 22	-
Warner (1976)	0.1 – 10	3.3 – 4.0
Bath *et al.* (1978)	0.5 – 20	3
Wong *et al.* (1988)	<0.2 – 20	-
Walstra *et al.* (1999)	<0.2 – 15	-
Mulder and Walstra (1974); Michalski *et al.* (2001)	0.1 – 10	4
L.L.Van Slyke's figure quoted by Winston and Winston (2002)	<1 – 20	2.5
Attaie and Richter (2000)	0.92 – 15.75	3.51

The number estimated per mL of milk was in between 10^{10} to 10^{11} fat globules and it develops a surface area of 5 to 11 m^2 per 100 gram (g) of milk (Walstra *et al.,* 1995). Attaie and Richter (2000) found larger specific surface area (SSA) of the fat particle in caprine milk (21,778 cm^2 / ml) than the bovine milk (17,117 cm^2 / ml). This could be attributed to high distribution of smaller fat globules size in caprine milk.

The frequency distribution of fat globules size in bovine milk was composed of three sub-distribution; namely 'small particles' with diameters less than 1 μm contains less than 10% of

the total volume fat; 'main' between 1 to 8 μm in diameters comprise about 90% of the total volume of fat; and 'large globule' greater than 8 μm in diameters are lower in number, and it account for 1-3% of the total volume of milk fat. Fat globules with diameters below 1 μm account for 80% or more of the total number of globules (Keenan and Dylewski, 1995). Attaie and Richter (2000) found 90% of the total particles in goat and bovine milk were less than 5.21 μm and 6.42 μm in diameter respectively, based on the volume frequency distribution. The frequency of size distribution of fat globule would greatly depend on the measurement method employed (Walstra, 1969). The methods employed in determining the fat globules size are scanning electron microscopy, spectroturbidimetry, fluorescence microscopy, the coulter counter, electroacoustics or Laser Light Scattering (Michalski *et al*., 2001). The results of the fat globule size distribution would also depend on whether a casein-dissolving agent is used, and if so, which agent, and if not used an apparent increase in the globule diameter may be resulted due to fat globules aggregate formed through casein interactions (Evers, 2004b).

A fair correlation between average globule size and the fat content was observed (Walstra, 1995). The shapes of the size distribution of fat globules were found to be constant with variations on d_{vs} and total fat content (Walstra 1969; Hood, 1981). However, the shape of size distribution of fat globules could be altered considerably by various treatments, particularly homogenization. It would also depend upon the type of machine and conditions during homogenization and primarily upon homogenizing pressure (Walstra, 1995). Figure 2 illustrates size distribution of milk fat globules of unhomogenized and homogenized raw milk. It indicates that the greater the homogenizing pressure used, the smaller would be the size of the fat globules (Tetra Pak, 1995).

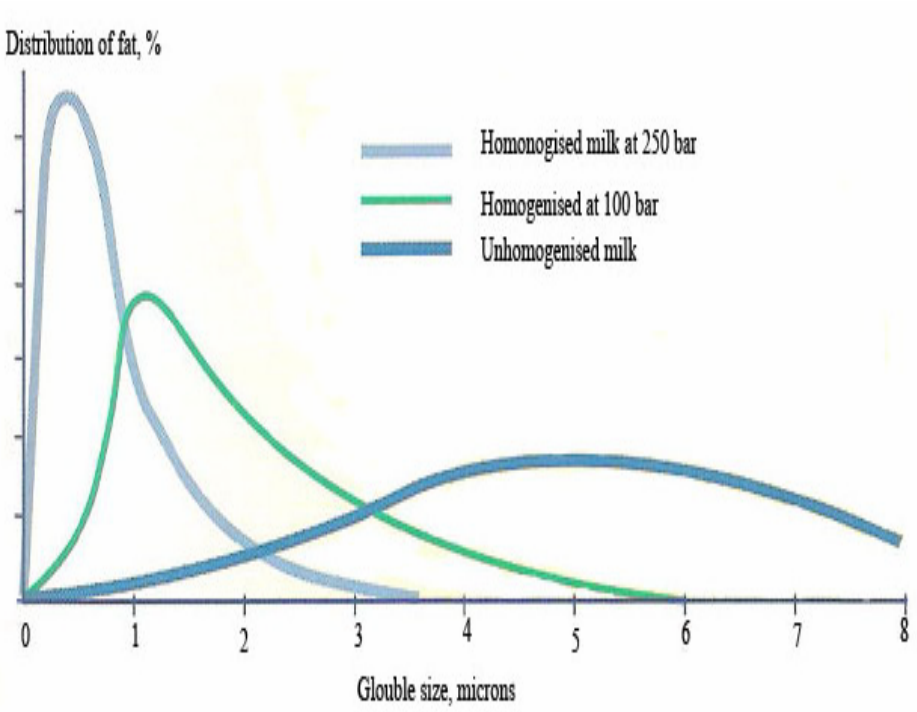

Figure 2 Size distribution of MFG of unhomogenized and homogenized raw milk.

Source: Tetra Pak (1995).

Factors affecting the variation of fat globules size are stage of lactation, where the average globule size decreased with advancing lactation (d_{vs} = 4.4 to 2.9 μm); breeds (Jersey = 4.5 μm, Holstein Friesian = 3.8 μm) producing higher fat gives milk with larger fat globules (Mulder and Walstra, 1974). Similarly, Warner (1976) reported that fat globules size in milk vary with species of animal, breed, stage of lactation, frequency of milking and other factors. The diets that cause an increase in total fat content increased the fat globule size rather than the numbers of globule (Hood, 1981).

Agitation of milk

Sample collection is a fundamental step in the process of evaluating the milk producer's product to determine its compliance with legal bacteriological and chemical standards. Therefore, sample collected should represent the volume and reach the laboratory in the same condition it left the bulk tank, with no change in its bacteriological, chemical, or physical condition (Belknap,

1976). Thus, obtaining representative sample and subsequent proper handling are keys to reliable results for quality and compositional tests. Hence, the importance of proper procedure used in collecting, handling and transporting samples from farm to laboratory cannot be overlooked. However, there is no established recommendation on a standard method for obtaining representative sample for a fat test from the farm tank. This is mainly constrained by usage of wide variety of style and design of tanks, as well as variations in methods of agitation, type of agitator, number of agitator on the tank, force of the agitator and volume of the product held (Walstra, 1969; Likas and Calbert, 1976; Grace *et al*., 1992). The bulk tanks variable such as size, shape, percent fill and temperature at the time of milk pick-up, as well as the shape and rotation speed of agitator would likely affect mixing behavior (Servello *et al.*, 2004). Similarly, Goodridge *et al.* (2004) reported possible variables, i.e. percent fill, agitator speed, number of agitators, and herd specific variables, such as fat content, would also play a role in mixing time behavior. Servello *et al*. (2004) in their survey of tank characteristics identified several potential variables that would likely affect the mixing time. Impeller varied greatly with respect to shape, position in the tank (off-centered or not) and rotation speed. Rotation speeds for the 34 tanks of 6313 bulk tanks surveyed ranged from 23 to 40 rpm, with vast majority were close to the average of 35 rpm. The distance of the agitator from the tank outlet ranged from 36 – 175 cm, with an average of 108 cm. In addition different impellers (flat turbine versus angled-blade marine) produced different flow patterns during mixing.

Representative sample can only be obtained after adequate agitation of the milk. Agitation is done by inverting, stirring, by pouring to and from one product container to another of the same volume for small container. For the large container agitation is done using mechanical agitator or manual stirrer of sufficient surface to produce adequate disturbance of the products by up and down circular motion of a disc-shaped stirrer, initially placed on the bottom of the tank, pulling up on the near side of the tank, and then pushing down on the far side (Goodridge *et al,*. 2004). The extent of agitation would depend on the period of time over the milk has been at rest. For instances, at least 5 min of plunging or stirring with an agitator was recommended if samples are collected within 30 min after filing the container and at least 15 min of agitation was recommended for a longer period of holding time (ISO, 1997).

A general rule of at least five (5) minutes of constant agitation for tanks less than 1,500 gallons; and at least 10 minutes of constant agitation for tanks of 1,500 gallons capacity or more was recommended (Belknap, 1976; Blush, 2002). There are no prescribed international standards on mixing length, besides the general consensus that the adequate agitation times of five minutes for tanks less than 1,000 gallons and 10 min for tanks of 1,000 gallons and more would be necessary. Likewise, mechanical mixing of milk for at least 5 min or until attainment of sufficient homogeneity is recommended (Goodridge *et al.*, 2004). However, mixing time required to mix the milk homogeneously would differ with availability of facilities and regulation pertaining to jurisdictions. For instance, bulk milk trucks in developed countries were installed with mechanical agitator. Hence, milk during transportation is periodically agitated, thus it would require less time of agitation prior to sampling. In such tank, with a periodical, time programmed agitation system, sampling could be carried out after only a short duration of agitation (1 min to 2 min) (Goodridge *et al.*, 2004). In New Zealand, continuous mixing of all tanks is necessary and this has shown to maintain homogeneity even with tanks up to 30,000 liters. However, it specifies following agitation conditions for storage silos and vats that, "Silos and vats should be provided with continuous agitation sufficient to thoroughly mix the milk to give a variation in fat content of less than 0.1% in milk volume down to 10% of the rated capacity of the vat or silo, prevent thermal layering (the variation in temperature in the vat or silo should not exceed 1 $^{\circ}$C), and ensure that foaming or churning of milk does not occur". However, the impacts of continuous mixing on the quality of milk is not studied (Goodridge *et al.*, 2004). Servello *et al.* (2004) recommended that hourly agitation of bulk tanks as currently prescribed in many jurisdictions to be maintained; but the duration of intermittent agitation should be reduced from 5 to 2 min to reduce the impact of agitation on fat globule stability.

The time required for agitation of milk varies from 3-10 minutes and it was recommended that the milk should be agitated minimum of 5 minutes for all tanks (Flake *et al.,* 1970). They also emphasized a need to determine adequate agitation time for individual bulk tank, although it would not be practical. The Ontario Milk Act requires at least 5 min of agitation of bulk tank milk prior to sampling. Most other Canadian provinces also use the 5 min standard, although it is not officially legislated by all Canadian provinces (Goodridge *et al.*, 2004). Dairy

Industry committee (1952) states that "agitation should provide sufficient degree to assure homogeneity within five minutes of operation, and the fat content taken at different levels in the tank at extreme distances from the source of agitation should not vary more than plus minus 0.1% to the fat content throughout the capacity volume". Likewise, regulations for the state of Washington (DOA, 1952) states that on farm holding tanks the uniformity of fat content throughout the capacity volume should be restored at 3 minutes with deviation not over or under 0.1%.

Adequate agitation is determined using a series of milk fat samples taken from a full tank at specified time intervals (e.g. 3 minutes, 4 minutes, 5 minutes) while mixing is in progress until at least five milk fat tests stabilized at a definite value for a particular tank (Grace *et al.*, 1992). Adhering to the determined adequate agitation time for particular tank, a significant uniformity in the components and milk quality through out the volume of milk was obtained (Manning, 2004). The agitation time required to mix the milk homogeneously increased with increasing volume of milk in the tank (Likas and Calbert, 1976).

Inadequate agitation results to erroneous butterfat and drug residue tests. Such result, referred to as "Spike" could result to distrust; leads to credibility gap between producer, handler, laboratory, field man, sanitarian, and truck driver; the payment basis would be destroyed; the milk producers would be unjustly penalized; besides much time and efforts would be wasted (Barnum 1972; Manning, 2004). Whereas vigorous agitation would induce lipolysis in raw milk through disruption of milk fat globule membrane rendering milk triglycerides more accessible to the milk lipase (Deeth and Fritz-Gerald, 1995). The amount of lipolysis would depend on the mode of agitation, the severity and duration of agitation, the amount of lipase present, the fat content, hardness of the fat and the vulnerability of the milk fat globule membrane (Claypool, 1965). The temperature of the milk during agitation would have a major influence on the activation of lipase, with greatest at 37-40 $^{\circ}$C and least at cold storage temperatures below <5 $^{\circ}$C. However, the relationship between temperature and activation of lipase was reported complex mainly due to its dependence on the conditions of mechanical treatment, the characteristics of the milk and its age and previous temperature history (Deeth and Fitz-Gerald, 1995).

A change in the composition and structure of the milk fat globule membrane would occur within the mammary gland (Evers, 2004a). Milk harvesting, subsequent milk handling and treatment effect could also change the milk fat globule membrane (MFGM) and the fat globule as a whole (Evers, 2004b). Hence, proper handling and adequate agitation of milk is therefore necessary to obtain representative samples, which ultimately reflect the true components and quality of milk being produced by the dairy herd. However, agitation of milk should be smooth and adequate so that there is less or no disruption of the fat globule membranes.

Standard specification of plunger

In view of the different shapes and sizes of containers used, no specific design of manual plunger was recommended for all purposes; however it was reported that the plungers should be of sufficient area to produce adequate disturbance of the products without developing rancid flavor; besides during agitation the scratching of the inner surface of the product containers should not occur (ISO, 1997; First Commission Directive 87/524/EEC, 1987). ISO (1997) recommended different forms of plunger for different sizes of the container as follows:

(a) A form of plunger (Figure 3) suitable for the mixing of liquids in buckets or in cans should have the following dimensions: a disc 150 millimeters (mm) in diameter, perforated with six holes each of 12.5mm in diameter on a circle 100mm in diameter, the disc being fixed centrally to a metallic rod, the other end of which forms a loop handle. The length of the rod, including the handle, should be approximately 1 meter (m).

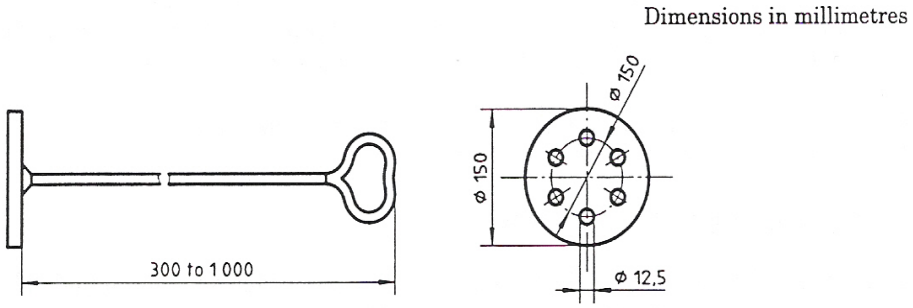

Figure 3 Recommended agitator (plunger) for cans and buckets.

Source: ISO (1997)

(b) A suitable plunger (Figure 4) recommended for bulk tanks should have the following approximate dimensions a rod not less than 2 m in length, fitted with a disc 300 mm in diameter perforated with 12 holes each 30 mm in diameter on a circle 230 mm in diameter.

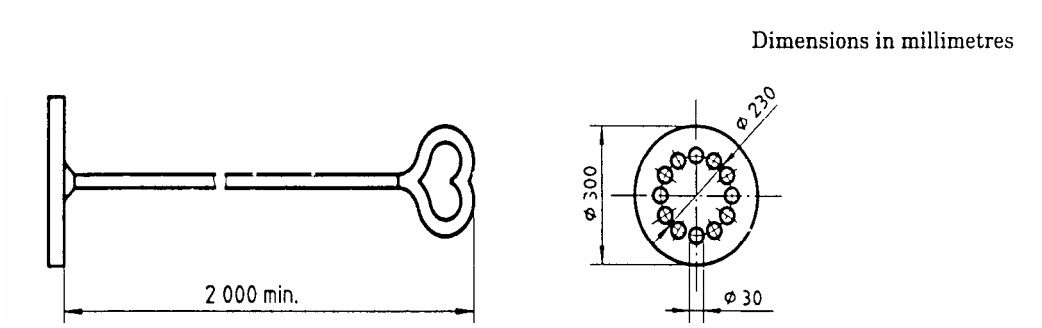

Figure 4 Recommended suitable agitator (plunger) for road, rail and farm tanks.

Source: ISO (1997)

(c) For mixing the contents of large vessels, mechanical agitation or agitation by clean compressed air with minimal air pressure and volume is recommended to prevent rancid flavor development.

Various types of mechanical agitators, i.e. built-in, and removable agitator are used, but the technical characteristics and construction of built-in agitators would depend upon the product to be mixed in the vessels or tanks. Removable agitators are usually provided with a propeller and it is introduced into transport, road and rail tanks through the manhole. However, no attempt has been made to describe any of them in the international standard (ISO, 1997).

Homogenization of milk

Homogenized milk is defined as "milk which has been treated in such manner as to insure break-up of the fat globules to such an extent that after 48 hrs of quiescent storage no visible cream separation should occur on the milk (Warner, 1976). However, it should be noted that homogenization is not a process that improves the sanitary quality of milk but a process which, when improperly done, may easily damage both the flavor and appearance of the milk (Trout, 1950). Properly processed homogenized milk should not form a cream layer, and the consumer could no longer use the depth of the cream layer as a criterion, to judge the richness of the milk. Hence presence of a certain % of fat should be assured by processor and distributor of homogenized milk, as there are possibilities, that some unscrupulous processor may take advantage of the lack of cream line to reduce the fat content of the milk to be homogenized to the minimum state standard. Some states have minimum fat standards for homogenized milk; a few requires the minimum % of fat to be printed on the containers closure; other have no regulations whatsoever specific to the products (Trout, 1950). The author also reported that the best fat content for the homogenized milk should be about 4 %.

Many variables are associated with the processing of homogenized milk that no specific routine could be prescribed for carrying out the process (Trout, 1950). However, the first step in the production of homogenized milk should start with milk of high quality milk, not only of low bacterial count but also of good flavor. Effective homogenization could be achieved by adhering to the two fundamental rules by the milk processor in homogenizing milk, i.e. (a) the fat must be in a liquid state at the time of homogenization and (b) all homogenized milk should be pasteurized, either prior to processing or immediately afterwards (Trout, 1950).

Effects of homogenization on raw milk fat globules

Warner (1976) reported that fat globule would rise to the surface to form cream layer because the oil in water emulsion is not stable enough to resist separation of the two immiscible phases. Homogenization is a standard industrial process, universally practiced as a means of stabilizing the fat emulsion against gravity separation by reducing the fat globules to approximately 1 micron in diameter, accompanied by an increase in the fat/plasma interfacial surface area by a four to six-fold (Tetra Pak, 1995). In classical homogenization process, milk heated at 60 to 70 $^{\circ}$C is forced under moderate pressure (20 – 50 MPa), and high velocity through a narrow opening (opening valve) results into fat globule disruption. Likewise, Early (1998) reported that milk at 55-80 $^{\circ}$C (high enough to liquefy) fed to the first stage valve at 100 – 400 ms^{-1} through valve set with a gap about 0.1mm would results in reduction of fat globules size to an average of 1 to 2 μm by a combination of high velocity turbulence. Turbulence, shear and cavitations are the main physical causes that reduced the fat globule size (Tetra Pak, 1995; Thieband *et al.*, 2003). Figure 5 illustrates raw and homogenized milk fat globules. It shows that the fat globules after homogenization are reduced and uniformly distributed throughout the milk. The homogenization reduced the size of fat globules in milk and cream (from 1- 8 μm in raw milk to 0.3 μm in homogenized milk) and prevent creaming and coalescence during long shelf-storage (Thieband *et al*., 2003). Anonymous (2005b) reported that homogenization of milk prevents creaming by decreasing the diameter and size distribution of the fat globules, causing the speed of rise to be similar for the majority of globules by formation of a recombined membrane which is much similar in density to the continuous phase. Homogenization greatly increases the number of fat globules and the total globule surface area; and reduces the average volume of fat per globule (Warner, 1976). The diameter of the globules in homogenized milk was reported to average about 1 or 2 μm depending upon pressure and other factors (Warner, 1976). Trout (1950) referred to Babcock that all fat globules examined should be 2 μm or less in diameter for properly homogenized milk. Similarly, Trout (1950) referred to the Creamery Package Manufacturing Company (1944) that the efficiency of homogenizer would be considered good, when 90 % of the fat globules observed under microscope were less than 2 μm in diameter and if excessive clustering or clumping was not observed.

a) **b)**

Figure 5 Fat globules in raw milk (a) versus homogenized milk(b).

Source: Tetra Pak (1995)

Several methods such as consumer reaction, appearance, microscopic examination, fat rising during quiescent storage for 48 hours, centrifugal separation and refraction of light were used to determine the efficiency of homogenizer. However, today the microscope examination of fat globule size and USPH index (Tetra Pak, 1995) are the most common method adopted in measuring the efficiency of homogenizer. USPH index is defined as "the fat percentage of the milk in the top 100 ml (1/9 of volume) of milk in a quartz bottle, or of proportionate volumes in containers of other sizes, does not differ by more than 10 percent of itself from the fat percentage of the remaining milk (9/10 of volume) as determined after through mixing" (Trout, 1950). Trout (1950) referred to Burr and Weise (1914) that the microscopic examination and counting of fat globules would require a great skill and experience. Other methods such as Farrell index and NIZO value are also used to determine the efficiency of homogenizers.

Homogenization efficiency would depend on several factors, (a) the velocity at which the liquid passes through the valve, (b) the force with which it strikes the wall of the chamber surrounding the valve, (c) the angle of impact, (d) the degree of mechanical perfection of operation of the machine and (e) temperature used in processing. Proper operation of the

homogenizer could only be obtained by keeping the machine and homogenizing valve in good operating condition, free of grooves and worn place (Trout, 1950). Therefore, timely assessment and monitoring of the homogenizer would be necessary to assure proper functioning.

MATERIALS AND METHODS

Experiment 1 Gravity separation of raw milk fat globules

Experiment 1.1 Raw milk in glass columns

On arrival of the bulk milk truck, the milk in different chambers (chamber I, II and III) was thoroughly mixed using manual plunger. Raw milk sample about 1500 ml each was collected in plastic bottles from three chambers of the bulk milk truck. Then samples collected from each chamber were transferred to five different glass columns of 250 ml capacity with 3.5 cm in diameter. These glass columns were set up in a cool room at 4 $^{\circ}$C for different time intervals at 0, 2, 4, 6 and 8 hour.

1.1.1 Sample collection and analysis

The graduated glass columns were divided into five equal parts. At every stipulated time interval, glass column each representing different chambers were removed from the cool room. Then, samples (50 ml) were collected from the top opening of glass column using 10 ml pipette. The samples collected from three fractions, i.e. top (250-200 ml), middle (150-100) and bottom (50-0 ml) of the graduated glass column after every time interval were analyzed for the fat content and other milk composition using calibrated ultrasonic milk analyzer (Eon Trading, 2001). Two fractions in between top – middle (150-200 ml) and middle – bottom (50-100 ml) were discarded.

1.1.2 Raw milk fat globules size determination

After analysis of the milk composition, the milk samples of same fraction from different chambers for the given time interval were commingled. The samples for determination of fat globules size distributions were prepared. A dilution of 1 part of milk to 25 parts of cold distilled water was prepared and a drop of dilution was transferred to the center of the glass slide

(75 x 24mm) using rubber dropper. A small drop of Rhodamine dye was transferred over the dilution and the solution was thoroughly mixed by tilting the slides. Then a glass cover slide (22 x 22mm) was gently placed over the solution and the fat globule size was determined by light scattering microscope (Microscope model LM series 2000, MEIJI, Japan) equipped with a camera at 40 X magnification power. Five films per slide were observed and average diameter of fat globules was determined by the computer software.

1.1.3 Statistical analysis

The statistical models used were:

$$Y_{ijkl} = \mu + C_i + F_j + \gamma_{ij} + T_k + FT_{jk} + \varepsilon_{ijkl}$$

$$Y_{jkl} = \mu + F_j + T_k + FT_{jk} + \varepsilon_{jkl}$$

Where; Y_{ijk} = the dependent variables;

μ = overall mean;

C_i = effect of containers as blocked (i = 1, 2 and 3);

F_j = main effect of the fractions (j=1, 2 and 3);

γ_{ij} = sampling error of main plot (i=1, 2 and 3; j = 1, 2 and 3);

T_k = sub plot (Main effect of storage time, (k = 0, 2, 4, 6, and 8 h);

FT_{jk} = interaction between storage time and fractions;

ε_{ijkl} = the sub plot error $\sim$ NID (0, σ_e^2)

Statistical differences among means were compared using Duncan's multiple range test (DMRT) according to Cody and Smith (1997).

Experiment 1.2 Raw milk in bulk storage tanks

Two bulk storage tanks namely ST2 and ST3, with approximate capacity of 8,000 kg each used to hold incoming milk prior to processing at the Dairy Center, Kasetsart University

(KU) were used for the study. The milk in the storage tanks was kept overnight at quiescent state for about 9 hours at 5 $^{\circ}$C.

1.2.1 Sample collection and analysis

Samples (60ml) were collected at the quiescent state after 9 hrs of holding time from the top surface and bottom of the tanks. Samples from the bottom were collected after draining out about five liters of milk. All samples collected were analyzed for the fat content using calibrated ultrasonic milk analyzer.

1.2.2 Statistical analysis

The simple Randomized Complete Block Designed (RCBD) model was used:

$$Y_{ijk} = \mu + S_i + T_j + \varepsilon_{ijk}$$

Where, Y_{ijk} = the dependent variables;

μ = overall mean;

S_i = storage tank as blocked (i = 1 and 2);

T_j = treatment effect, where j=1 (top) and 2 (bottom);

ε_{ijk} = error $\sim$ NID (0, σ_e^2)

Mean differences were compared using DMTR.

Experiment 2 Comparative efficiency of mixing raw milk using milk fat as an indicator

Experiment 2.1 Mixing efficiency of manual plungers

The experiment was conducted on the chambers of bulk milk truck delivering raw milk to Dairy Center, Kasetsart University (KU) after approximately 3.5-4 hrs of transportation from milk collection center (MCC). The capacity of the chambers was 4,700 kg, 3,900 kg, and 7,400 kg for chamber I, II and III respectively. Two different plungers (Figure 6) with following specification were used; a) the ISO standard made of stainless steel rod 2 m in length, fitted with a disc 300 mm in diameter perforated with 12 holes each 30 mm in diameter on a circle 230 mm in diameter (ISO, 1997) and b) the KU design made of stainless steel rod of 1.80 m in length, fitted with a stainless disc of 230 mm in diameter perforated with 4 holes each 62 mm in diameter on a circle of 205 mm in diameter from the centre.

Figure 6 Different manual plungers; a) ISO standard and b) KU design.

2.1.1 Raw milk samples

Prior to transferring milk from the truck to storage tank, milk in each chamber was thoroughly mixed using manual plunger. In this study, samples were collected at uniform interval

of 0, 5, 10, 15, 20, 25 and 30 times of stirring. During each sampling time 60 ml of raw milk were collected in plastic bottles of 80 ml capacity with the help of plastic jug from the top opening of each chamber. Samples were collected from all three chambers adopting the similar method and were analyzed for fat content and other milk compositions at the laboratory using calibrated ultrasonic milk analyzer, Ekomilk-M (Eon Trading, 2001).

2.1.2 Statistical analysis

Data were analyzed under split plot in complete randomized design (CRD) using PROC General linear model (GLM) in Statistical Analysis System (SAS). The statistical model used was:

$$Y_{ijkl} = \mu + C_i + \gamma_{k(i)} + S_j + T(C)_{il} + ST(C)_{ij} + \varepsilon_{ijkl}$$

Where Y_{ijkl} = the dependent variable;

μ = the overall mean;

C_i = the effect of chambers as main plots (i = 1, 2 and 3),

$\gamma_{k(i)}$ = the main plot error;

S_j = the effect of stirrers (j = 1 and 2);

$T(C)_{il}$ = stirring times nested with containers;

$ST(C)_{ij}$ = the interaction effect between the plunger and container;

ε_{ijkl} = the subplot error at NID (0, σe^2).

Means differences were compared using DMTR.

Experiment 2.2 Mixing efficiency of motor driven agitator

Two bulk storage tanks (ST2 and ST3) about 8000 kg capacity preinstalled with motor driven agitator of different mixing efficiency were used for the study. Initially, the adequate time required to mix the milk homogeneously prior to sampling was reassessed for both storage tanks.

Thereafter, agitator blade in storage tanks requiring more time to attain homogeneity was replaced (Figure 8). Then the adequate agitation time required to mix the milk homogeneously was again determined for both tanks.

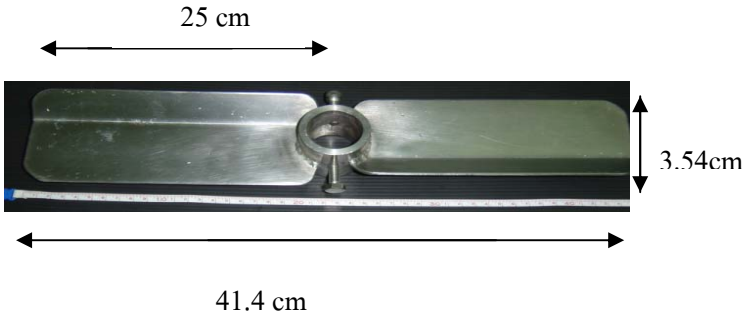

Figure 7 Specification of old agitator blade.

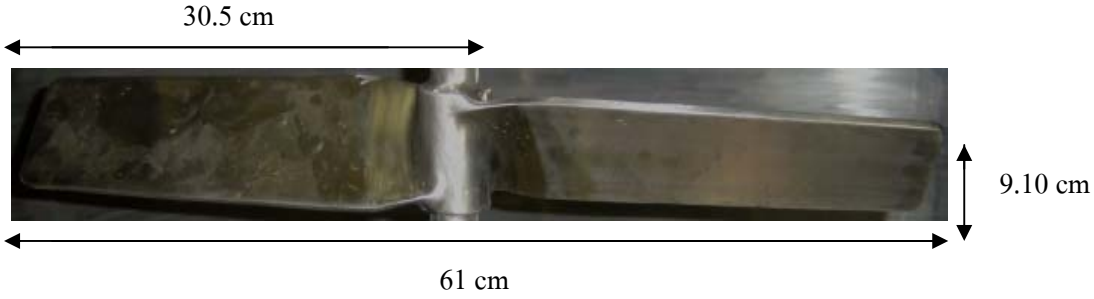

Figure 8 Specification of new agitator blade.

2.2.1 Sample collection and analysis

Initially, samples were collected after mixing interval of 0, 10, 20, 30, 40, 50 and 60 min from the top and 0, 30 and 60 min from the bottom for ST2; and 0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 min from the top and 0, 30, 60 and 90 min from the bottom for ST3. Likewise, after replacement with new agitator blade in ST3, samples were collected from both tanks again from the top and bottom at the mixing time interval of 0, 5, 10, 15, 20, 25 and 30 min. The samples collected were determined for milk fat content using calibrated ultra sonic milk analyzer.

2.2.2 Total cost of mixing raw milk prior to processing

Actual labor and power energy required to mix the milk prior to processing was used to determine the total cost of mixing raw milk in the bulk tank. Total power consumed in mixing raw milk was recorded for 1 week for both storage tanks before and after determining adequate time of agitation; and replacement of old agitator blade with the new one in ST3. The operation times of mixing milk in the storage tanks was determined based on the incoming milk to the dairy center. In average storage tanks were used 208 times each per annum in mixing raw milk prior to processing. Daily minimum wages (Baht 175/day) for Bangkok (Hewitt, 2005) and actual cost per unit (2.68/Kwh) of energy consumed was derived from the expenditure incurred at KU Dairy center.

2.2.2 Statistical analysis

Data gathered were statistically analyzed using factorial RCBD design as follow:

$$Y_{ijkl} = \mu + B_i + T_j + L_k + TL_{jk} + \varepsilon_{ijkl}$$

Where, Y_{ijkl} = the dependent variable;

μ = overall mean;

B_i = Blocked by storage tanks (i= 1and 2);

T_j = treatment or time of mixing (j= 0, 5, 10……, 25 and 30)

L_k = sampling effect (j=1(top) and 2(bottom);

ε_{ijkl} = main error$\sim$NID (0, σ_e^2)

Statistical differences among different means were compared using DMRT.

Experiment 3 Effects of homogenization on the characteristics of raw milk fat globules

Effect of homogenization on characteristics of raw milk fat globules was studied. Milk preheated at 60-70 °C was homogenized using commercially imported (T) and locally fabricated (P) homogenizers at 2500 psi using standard homogenizing head.

3.1 Sample collection and analysis

Milk samples about 500 ml were collected at 0 h (prior to homogenization), 1 h and 3 h after homogenization during the daily processing period. The samples collected were analyzed for the fat content using calibrated ultrasonic milk analyzer.

3.1.1 USPH Index

Milk samples (200 ml) raw and homogenized were transferred in plastic bottles of 200 ml and were kept in a refrigerator for 48 hrs at 5 °C (Harding, 1995). Then, the fat content of samples from the upper part, i.e. 1 / 10 (a) and samples from the bottom, i.e. 9 / 10 (b) of the plastic bottle were determined using ultrasonic milk analyzer. The following equation (Ertugay *et al.,* 2004) was adopted to calculate the homogenization index /USPH index of the sample:

$$\text{USPH Index} = (a - b) / a \times 100$$

3.1.2 Fat globule size determination

The samples were prepared and fat globule size was determined for the raw milk and milk homogenized at different time intervals following similar procedure as in section 1.1.2.

3.2 Statistical analysis

The data collected was analyzed using simple model as hereunder:

$$Y_{ijk} = \mu + H_i + T_j + \varepsilon_{ijk}$$

Where, Y_{ijk} = the dependent variables;

μ = overall mean;

H_i = blocked by homogenizer, where i = 1 and 2;

T_j = Time of homogenization (j = 0, 1 and 3 h);

ε_{ijk} = main error$\sim$NID (0, σ_e^2)

Mean differences were compared by DMTR.

RESULTS AND DISCUSSIONS

Experiment 1 The gravity separation of fat globules

Experiment 1.1 Raw milk in glass columns

1.1.1 Milk constituents

The components of tested raw bulk milk samples are described in Table 7. Average mean of bulk milk composition, i.e. protein, SNF and TS was found lower than the average standard milk composition reported for dairy farm in Thailand (Table 5). It could be explained in part by the standard deviation associated with bulk tank sampling error of 0.01 % for milk protein and 0.093 % for the milk fat (Dickinson and Stainsby, 1998). Besides, many factors, i.e. breed, feed, season, region and herd health are reported to affect the gross composition of milk (Jenness and Patton, 1959).

Table 7 Means and standard deviations of bulk milk constituents determined using ultrasonic milk analyzer.

Parameters	N	Bulk milk (KU)
Fat (%)	99	3.85 ± 0.34
Protein (%)	99	3.01 ± 0.07
SNF (%)	99	8.11 ± 0.22
Total solids (%)	99	11.96 ± 0.30
Specific gravity	99	1.028 ± 0.52
Freezing point (°C)	99	-0.540 ± 0.82

1.1.2 Gravity separation

1.1.2.1 Fat content

Table 8 illustrates milk fat content (%) of different milk fractions at different time intervals. There was a significant influence ($p<0.01$) of time on milk fat content. A significant difference ($p<0.01$) in milk fat content was observed among different fractions. Besides, there was significant interaction effect ($p<0.01$) between time and fraction. A significant increase ($p<0.01$) in fat content was observed throughout the time intervals at the top fraction. A rapid increase in fat content was observed during 2 h time interval from 3.85 to 5.07%, an increase in fat content by about 31.69%. Fat content as high as 7.07% was obtained at the top fraction after 8 h of time interval; an increase in fat content by about 83.63% from the initial fat content of 3.85%. A rapid increase in fat content at the top fraction could be explained by the rising of larger fat globules to the top fraction, as these larger fat globules are reported to comprise about 95 % of the total fat volume (Walstra, 1995). Warner (1976) reported that fat globule would rise to the surface to form cream layer because the oil in water emulsion is not stable enough to resist separation of the two immiscible phases.

Table 8 Means and standard deviations of fat content (%) of different milk fractions in glass columns at different time intervals.

Time (h)	Fractions		
	Top	Middle	Bottom
0	3.85 ± 0.06^{e}	3.85 ± 0.06^{a}	3.85 ± 0.06^{a}
2	5.07 ± 0.01^{dx}	3.54 ± 0.03^{by}	3.35 ± 0.03^{by}
4	5.92 ± 0.01^{cx}	3.27 ± 0.04^{cy}	3.02 ± 0.07^{cy}
6	6.41 ± 0.02^{bx}	3.13 ± 0.06^{dy}	2.80 ± 0.05^{dz}
8	7.07 ± 0.09^{ax}	3.05 ± 0.07^{dy}	2.74 ± 0.04^{dz}
Average	5.67 ± 1.16^{x}	3.37 ± 0.31^{y}	3.15 ± 0.04^{z}

N = 33 for each fraction at the given time interval.

^{abcde} means within the same column are significantly different ($p<0.01$).

^{xyz} means within the same row are significantly different ($p<0.01$).

On the other hand, a gradual decrease in fat content was observed at the middle and bottom fractions with increasing time interval. There was a significant decrease ($p<0.01$) in milk fat content until 6 h of time intervals; thereafter, a gradual non significant decrease ($p>0.05$) in milk fat content was observed at the middle and bottom milk fractions. The decrease in fat content observed was highest at 2 h time interval, with decreasing rate of 8.05% and 12.99% for the middle and bottom fractions respectively. The decrease in milk fat was more pronounced at the bottom fraction (Figure 9). After 8 h of time interval a fat content as low as 3.05% and 2.74% was obtained, a decrease in fat content by about 20.8% and 28.80% at the middle and bottom fractions respectively. Average fat depletion observed after three hours and 1.5 hours of creaming at the bottom of tanks was about 30% (Servello *et al.*, 2004) and 60% (Jackson,1981), respectively. This difference in depletion rate of fat content at the bottom could be due to different methods employed in sample collection. Besides, the creaming rate was reported to vary (Servello *et al*., 2004).

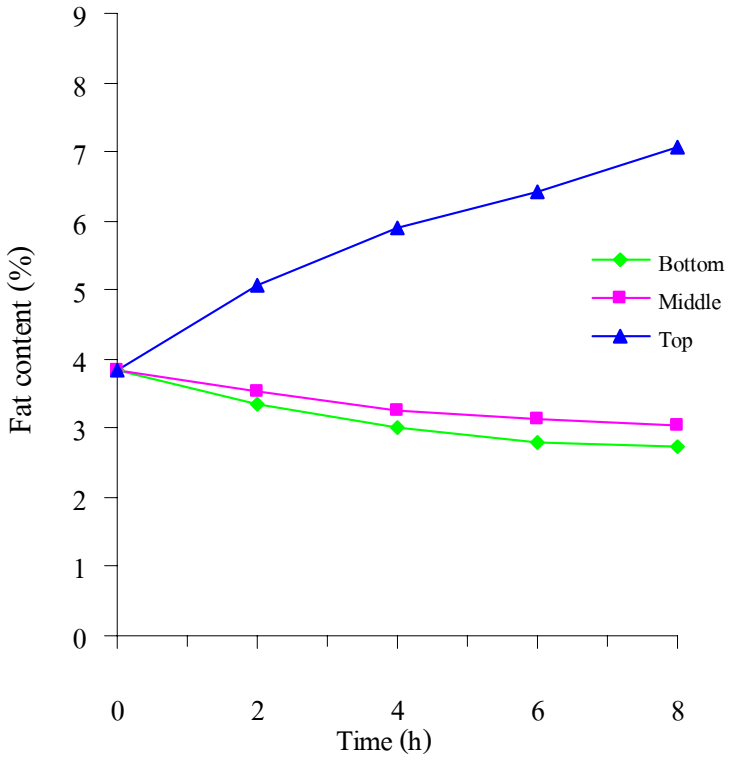

Figure 9 Changing pattern of fat content of different milk fractions at different time intervals.

A number of investigations have confirmed that the fat globules exist largely as separate entities and it rise in close accordance with Stokes' equation. Gravity separation of fat globules is caused mainly due to lower density of fat globules as compared to the continuous phase. In accordance to the Stokes' equation the larger MFGs would have risen faster to the surface. This would have lead to depletion of the larger MFGs at the bottom fraction, which comprised major volume of fat. Ma and Barbano (2000) reported that the fat globules size and fat content would have already redistributed in the column after 2 h of quiescent holding of milk at 4 $^{\circ}$C and 15 $^{\circ}$C with larger fat globules and greater fat concentrating in the top fraction.

In accordance to Stokes' equation, it was estimated that an average fat globules size of approximately 4 μm in diameter would take several hours to rise 2.54 cm (Winton and Winton 2002). Prentice (1992) estimated that the average globule size with diameter of about 3 μm would rise independently at the rate of 2.54 cm in about 6 hrs and the smallest globules would rise about

1/10 of the rate. However, in this study, a thin cream layer was visible at the surface after 2 h of time interval. This indicates that the creaming might have started much earlier; for instance, it could have started as early as 50 min of storage (Walstra *et al.*, 1999). This early creaming phenomenon could be explained by clustering of the individual fat globules at low temperature by the action of cold agglutinin, where a larger globule comes in contact with a smaller globule, and the two joined globules rise together, and on rising clustering continues until substantial size is formed, thereby rise more rapidly than any of the individual globules because of their greater effective radius. The rate of rise is directly proportional to the square of the radius of fat globules in accordance to Stokes' equation. Hence the larger fat globules would rise faster than the smaller fat globules. This early natural creaming also indicates that there are other factors affecting the gravity separation besides the fat globules size. The factors affecting the gravity separation of the raw milk are concentration of agglutinin, fat content, agitation, warming, heat treatment, temperature and homogenization (Walstra, 1995).

With gravity separation milk of different fat contents and fat globules size could be obtained at different time intervals. Although, during the course of experimental time intervals milk products similar to cream separator could not be obtained. However, it is felt that this gravity separation could be adopted by small dairy products manufacturers as an alternative to cream separator in milk fat standardization, and in development of milk products of different natural milk fat globules size as desired. In particular, the cheese processing industry could adopt this gravity separation method in deriving small natural milk fat globules as many cheese makers prefer to use milk with small fat globules. Besides, the natural small milk fat globules cannot be replaced by the homogenized one for manufacturing cheese. On the other hand, gravity separation could results in variation of milk fat tests mainly if samples are collected without or after inadequate agitation of milk in the bulk tanks. Consequently, this could leads to loss of payment for the milk producers. This necessitates proper mixing of milk prior to sampling or processing.

1.1.2.2 Changing rate of fat content

Table 9 shows the changing rate (% h^{-1}) of fat content at different fractions of glass column at different time intervals. The highest increasing rate (15.84% /h) of fat content was observed at the top fraction at 2 h time interval. On the other hand, the decreasing rate of fat content, i.e. 4.03% h^{-1} and 6.49%h^{-1} was observed at 2 h of time interval at the middle and bottom fractions respectively. The changes in fat content continued but the rate of change decreased with increasing time intervals (Figure 9). This could be explained by increasing in volume and surface area of the fat globules with increasing fat content (Table 14), whereby the buoyance increases more rapidly than resistance. Therefore, a larger fat globule move better against the friction because the surface area for each unit of volume is less for the larger globule, thus its resistance is less than that of the smaller globule (Warner, 1976). Hence, early depletion of larger fat globules at the lower portion is evident. The small fat globules are reported to rise very slowly as it has a less tendency to form clumps (Prentice, 1992). Besides, the adsorption of large quantity of protein particles on it surface reduced the density difference between the milk fat and plasma. Thus, the rising speed of fat particles is reduced.

Table 9 Changing rate (% h^{-1}) of fat content of different milk fractions at different time intervals.

Time (h)	Fractions		
	Top	Middle	Bottom
0	0	0	0
2	15.84	-4.03	-6.49
4	13.44	-3.77	-5.39
6	11.08	-3.12	-4.55
8	10.46	-2.60	-3.60

1.1.2.3 Protein and Solid not fat (SNF) content

Table 10 and 11 illustrate the milk protein (%) and SNF content (%) of milk samples of different fractions in glass column at different time intervals. A significant difference ($p<0.01$) in protein and SNF content (%) was observed between 0 h and rest of the time intervals (2, 4, 6 and 8 h) at the top fraction. A gradual non significant decrease ($p>0.05$) in protein and SNF content was observed after 2 h of time intervals at the top fraction. Whereas in the middle and bottom fraction, a non significant increase ($p>0.05$) in protein and SNF content was evident with increasing time intervals.

Table 10 Means and standard deviations of milk protein (%) of different fractions in glass columns at different time intervals.

Time (h)	Fractions		
	Top	Middle	Bottom
0	3.01 ± 0.01^{a}	3.01 ± 0.01	3.01 ± 0.02
2	2.89 ± 0.06^{by}	2.98 ± 0.05^{x}	3.01 ± 0.03^{x}
4	2.93 ± 0.02^{by}	3.02 ± 0.01^{x}	3.02 ± 0.03^{x}
6	2.88 ± 0.05^{by}	2.99 ± 0.04^{x}	3.02 ± 0.02^{x}
8	2.89 ± 0.04^{by}	3.02 ± 0.01^{x}	3.02 ± 0.01^{x}

N = 33 for each fraction at the given time interval.

$^{a\,b}$ means within the same column are significantly different ($p<0.01$).

$^{x\,y}$ means within the same row are significantly different ($p<0.01$).

Table 11 Means and standard deviations of SNF (%) of different milk fractions in glass columns at different time intervals.

Time (h)	Fractions		
	Top	Middle	Bottom
0	8.09 ± 0.02^{a}	8.11 ± 0.03^{b}	8.13 ± 0.02^{a}
2	7.87 ± 0.06^{by}	8.13 ± 0.01^{bx}	8.18 ± 0.02^{ax}
4	7.84 ± 0.10^{by}	8.18 ± 0.04^{ax}	8.14 ± 0.19^{ax}
6	7.82 ± 0.02^{by}	8.19 ± 0.01^{ax}	8.21 ± 0.02^{ax}
8	7.77 ± 0.06^{by}	8.21 ± 0.02^{ax}	8.21 ± 0.02^{ax}

N = 33 for each faction at the given time interval.

$^{a\,b}$ means within the same column are significantly different ($p<0.01$).

^{xy} means within the same row are significantly different ($p<0.01$).

Milk fat and protein are the major variable constitutes in milk. Fat and protein vary considerably seasonally between breeds and herds; on the average a one point (0.1%) change in milk fat is associated with 0.4 point (0.04%) change in solid not-fat, and in protein, since protein is the major variable constituent within the solid not-fat portion (Bath *et al.*, 1978).

The difference in the protein and SNF content at the top fraction at 0 hr with the rest of the time intervals could be explained by the coalescence (and also partial coalescence) of fat globules which leads to decrease in the surface area causing a proportional desorption of membrane substances, e.g. phospholipids, into aqueous phase (Walstra, 1995). Phospholipids account about 1 % of the milk fat and are concentrated in the fat globule membrane (Warner, 1976). A very slight increase in protein and SNF content with increasing time intervals could be in part attributed to a high protein load at the surface of small particles of fat globules that reduces the densities differences between two phases. This caused a gradual precipitation of those small MFGs engulfed with protein to the lower surface. It could also be explained by the Recknangel phenomenon, which states that during cooling of milk the separate fatty acids solidify and it caused a small rise in the specific gravity of the milk. The affect on specific gravity was as much

as 0.001 over a period of a few hours or a day or two depending upon the temperature (Warner, 1976).

1.1.2.4 Correlation of milk composition on time

Correlation **a**mong individual milk composition in relation to time intervals at different fractions is illustrated in Table 12. The highest correlation was found in fat content followed by SNF and then protein. A correlation of fat content was highest at the top (0.87) followed by bottom (0.56) and then the middle fractions (0.39). This indicates that the time has significant effect on the fat content. A high correlation for fat content as compared to other compositions indicates that the time has strong significant effect on milk fat content than the SNF and protein content. Following regression equations (Figure 10) was derived for the fat content, i.e. $y = 0.3923x + 4.1007$, $y = -0.0999x + 3.7641$ and $y = -0.1332x + 3.7135$ at the top, middle and bottom fractions respectively. With this equation we could predict the rate of change in fat content at different fractions and at the same time it could also be used in fat standardization. For instance, with the regression equation derived above milk with 2 % fat content could be obtained after 13 hrs and 18 hrs of gravity separation at the bottom and middle fractions respectively. Whereas cream with 18 % fat content could be obtained at the top (1/5 of the milk volume) fraction after 35.6 hrs of quiescent holding of milk at 4 $^{\circ}$C. Since, milk could be stored for several days at low temperature prior to processing. It is felt that, the gravity separation could be adopted especially in developing countries by small dairy products manufacturers as an alternative to cream separator in milk fat standardization; and in development of milk products of different fat contents and of different natural milk fat globules size as desired. In particular, the cheese processing industry could adopt this gravity separation method in deriving small natural milk fat globules as many cheese makers prefer to use milk with small fat globules.

Table 12 Regression equation for bulk milk constitutes on time at different fractions in glass columns.

Fractions	Y	A	+	b1x	p-value	R^2
Top	Protein	2.97055	+	-0.0127 x	0.0014	0.061
(N =165)	SNF	8.01164	+	-0.0336x	<0.0001	0.11
	Fat	4.10170	+	0.3922x	<0.0001	0.87
Middle	Protein	2.99588	+	0.0025x	0.3804	-0.0014
(N = 165)	SNF	8.11297	+	0.0129x	0.0441	0.02
	Fat	3.76545	+	-0.0999x	<0.0001	0.39
Bottom	Protein	3.00794	+	0.0021x	0.3423	0.06
(N=165)	SNF	8.13018	+	0.0145x	0.1103	0.01
	Fat	3.71442	+	-0.1332x	<0.0001	0.56

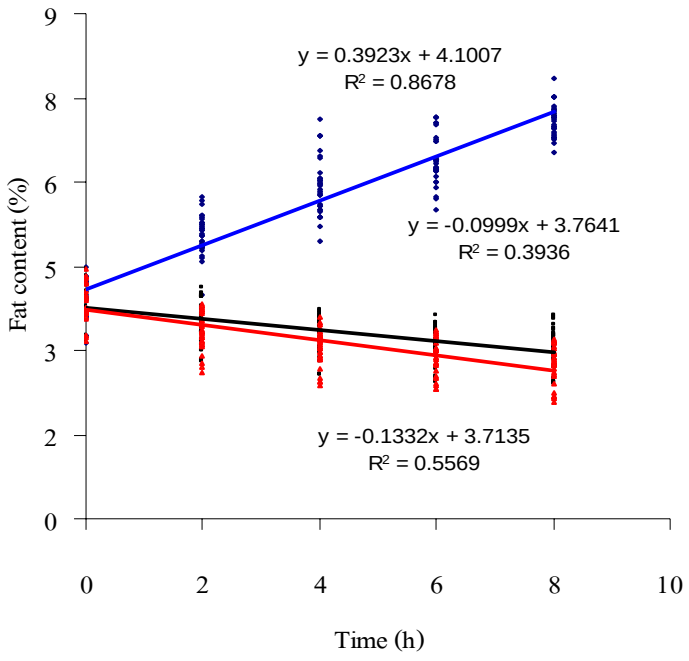

Figure 10 Correlation of raw milk fat at different fractions in relation to different time intervals.

1.1.3 Milk fat globules size distribution

Table 13 illustrates raw MFGs size of different fractions at different time intervals. There was a significant influence ($p<0.01$) of time on the fat globule size. A significant difference ($p<0.01$) was also observed in the fat globule size among the milk fractions. Significant increase ($p<0.01$) in the size of MFGs at the top fraction was observed after 2 h of time interval. The MFGs size at the top fraction increased significantly ($p<0.01$) from a diameter of 3.38 to 3.56 μm after 2 h of holding time intervals; thereafter, there was not much changes in the size of fat globules. In the middle fraction, a gradual decrease in MFGs size was observed with increasing time intervals. However, a significant decrease ($p<0.01$) in the MFGs size was observed after 6 h time intervals (Figure 11). Similarly, a gradual decrease in MFGs size was observed at the bottom fraction with increasing time interval, but a significant decrease ($p>0.01$) in size was observed only after 4 h time interval. Milk fat globules size as low as 2.80 μm and 2.70 μm was obtained after 8 h time intervals at the middle and bottom fractions respectively. As the holding time increases, the distributions of fat globules size become more stable.

Table 13 Means and standard deviations of fat content (%) and MFGs size in diameter (μm) of milk fractions at different time intervals.

Time (h)	Fractions					
	Top		Middle		Bottom	
	Fat (%)	Size (μm)	Fat (%)	Size (μm)	Fat (%)	Size (μm)
0	4.15 ± 0.01^{e}	3.38 ± 0.01^{b}	4.15 ± 0.01^{a}	3.41 ± 0.05^{a}	4.15 ± 0.01^{a}	3.43 ± 0.05^{a}
2	5.39 ± 0.18^{d}	3.56 ± 0.12^{ax}	3.82 ± 0.08^{b}	3.26 ± 0.05^{ay}	3.60 ± 0.05^{b}	3.13 ± 0.27^{aby}
4	6.48 ± 0.05^{c}	3.55 ± 0.06^{ax}	3.47 ± 0.05^{c}	3.12 ± 0.05^{aby}	3.08 ± 0.28^{c}	2.95 ± 0.16^{bcy}
6	6.88 ± 0.02^{b}	3.55 ± 0.07^{ax}	3.31 ± 0.03^{d}	3.11 ± 0.06^{aby}	3.01 ± 0.54^{c}	2.76 ± 0.32^{bcdy}
8	7.18 ± 0.09^{a}	3.55 ± 0.08^{ax}	3.24 ± 0.15^{d}	2.84 ± 0.16^{by}	2.78 ± 0.34^{c}	2.70 ± 0.18^{dy}

N = 15

$^{a\,b\,c}$ means within the same column are significantly different ($p<0.01$).

$^{x\,y}$ means within the same row in respect to the same parameter are significantly different ($p<0.01$)

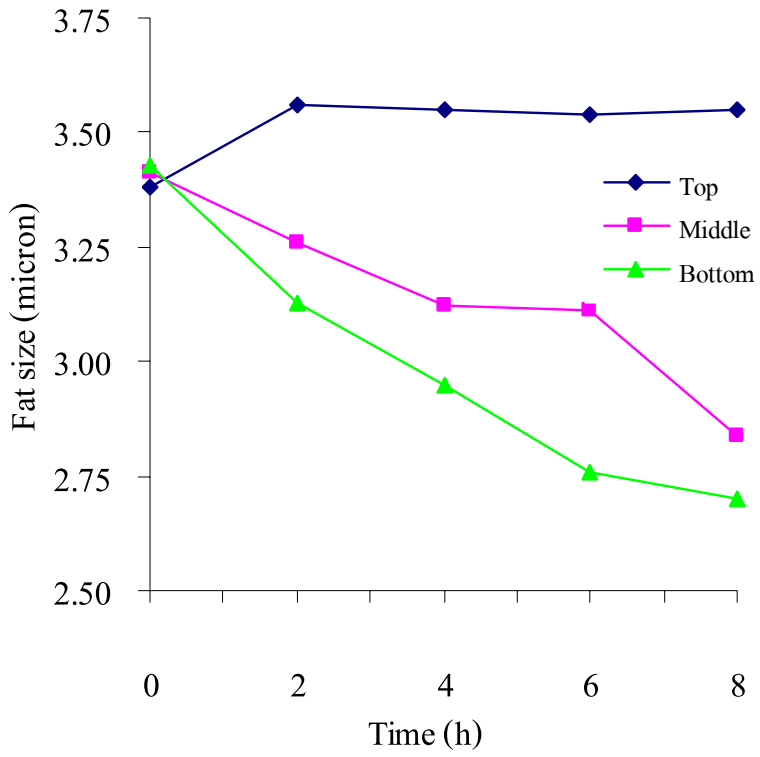

Figure 11 Changing patterns of fat globule sizes of different milk fractions at different time intervals.

Milk fat globules size has crucial influence on the stability and technological properties of milk. Thus, the particle size of the fat droplets present in dairy and other food emulsions is important in defining properties such as flavor release, mouth feel and the emulsion stability. Larger emulsion droplets can lead to poor flavor release, greasy mouth feel, and poor stability due to creaming. Emulsification to a smaller droplets size tends to reduce creaming and improve the taste of a product. Hence, the nature of the fat globules was studied intensively; more so with the MFGs size distribution. The raw MFGs size in this study was observed using light scattering microscopic and the fat globules size were determined using inbuilt computer software. The fat globules size ranging from 0.1 to 12.12 μm with an average diameter of 3.41 μm was observed for the bulk raw milk. This finding agrees with most of the previous findings (Table 6). A slight difference or variation in the fat globule size reported could be attributed to the different methods

of measurement employed (Walstra, 1969). For instance, the results of the fat globule size distribution would also depend on whether a casein-dissolving agent is used, and if so, which agent, and if not used an apparent increase in the globule diameter may be resulted due to fat globules aggregate formation through casein interactions (Evers, 2004b).

A reducing trend of fat globules size was observed at the middle and bottom fraction as the time interval increased. This changing pattern of fat globules size of different fractions over period could be explained by the continuous rising of the larger fat globules to the top resulting to depletion of larger fat globules at the middle and bottom fractions. Insignificant changes ($p>0.05$) in the fat size at the top fraction indicate that most of the lager fat globules would have risen to the surface at the early hours of the time intervals. Ma and Barbano (2000) reported that after 2 h of quiescent holding of milk at 4 $^{\circ}$C and 15 $^{\circ}$C the fat globules size and fat content were already redistributed in the column with larger fat globules and greater fat concentrating in the top fraction. King (1957) found non-significant increase in the fat size of the fat globules with corresponding increase in the fat percentage in milk obtained from Ayrshires and Friesian, which were between day 45 and 165 of lactation period.

A fair correlation between the average diameter of milk fat globules with the fat content was reported (Walstra, 1995). Wiking *et al.* (2004) observed a correlation ($r = 0.54$) between average diameter of the MFG and the diurnal fat yield of cows. Similarly, in this study a significant correlation ($r = 0.59$, $p<0.01$) between the fat content and average fat globules size was observed for the raw bulk milk. In contrast, Walstra (1969) did not observed positive correlation between the fat content and average globule size within a lactation period and between different cows within a breed (Friesian and Jersey).

1.1.4 Particle size distributions

The fat globules have redistributed within 2 h of time interval among different milk fractions of the glass columns. The changes in the shape of the raw milk fat globules distribution illustrated in Figure 12-14 could substantiate the findings. The change in the shape was rapid in

the bottom fraction (Figure 12) followed by the middle fraction (Figure 13) in the early time intervals. The change in the particles distribution at the top fraction (Figure 14) was very gradual. At middle and bottom fractions, the curve of the particles distribution shifts toward the abscissa until 4 h and with increasing time intervals it remained almost constant. Whereas, the curve at the top fraction (Figure 14) shift away from the abscissa very gradually with increasing time intervals, but then it was a very slow process. This could be explained by the changes occurred in the milk fat content and the size of fat globules resulted due to rising of the larger fat globules to the surface. The shape of the size distribution was found almost constant (Figure 12-14). This agrees with the findings of Walstra (1969) and Hood (1981), who reported that the shape of the size distributions remained almost constant despite variation in fat content and average diameter of the fat globules.

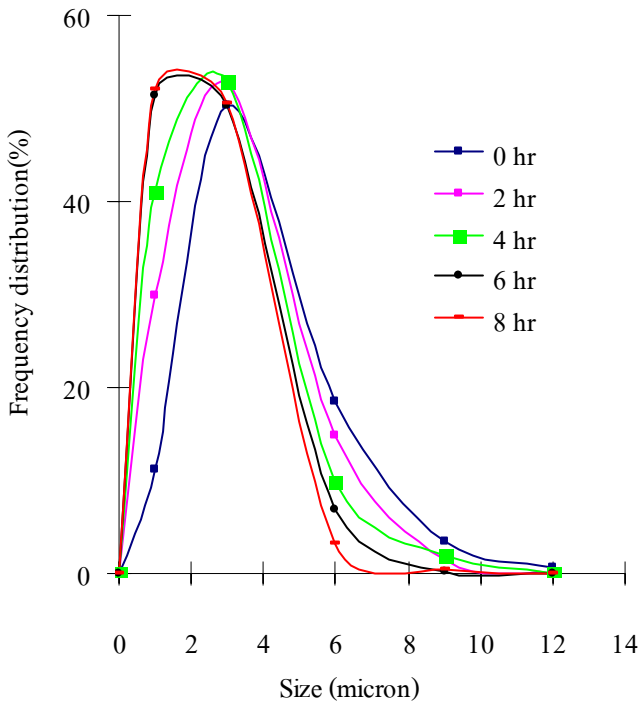

Figure 12 Particles size distribution of fat globules at bottom milk fraction in glass columns at different time intervals.

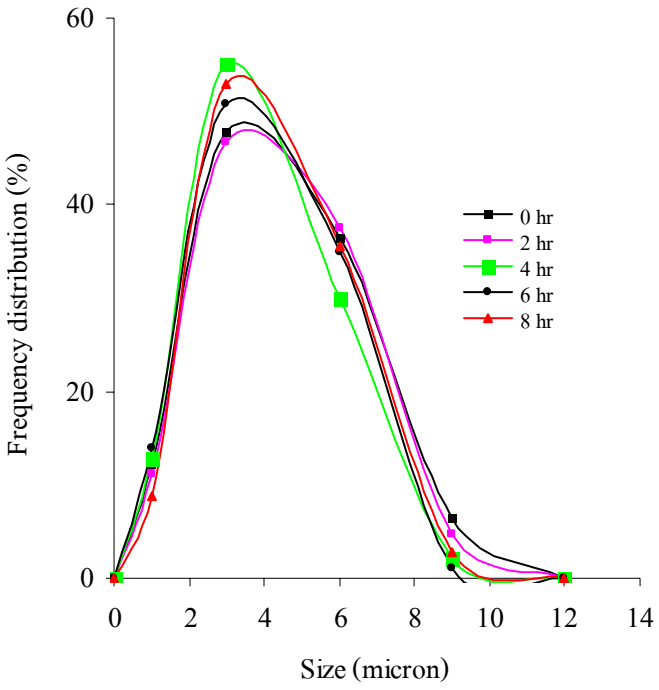

Figure 13 Particles size distribution of fat globules at middle milk fraction in glass columns at different time intervals.

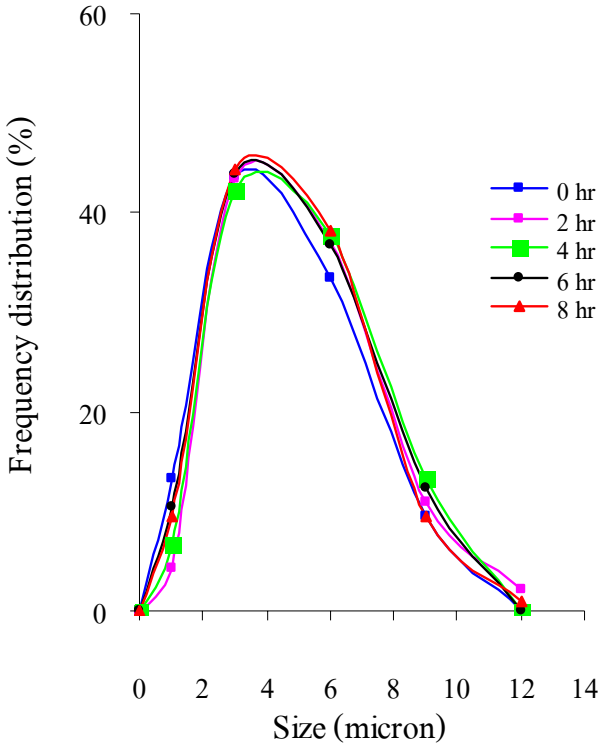

Figure 14 Particles size distribution of fat globules at top milk fraction in glass columns at different time intervals.

Milk fat globules are composed of three sub populations; one containing the small globules with diameter less than 1 μm, which comprised little of the fat content; the medium size population containing about 94% of the fat and the last sub population including the larger fat globules with a diameter larger than 8 μm contributing about 2-3% of the fat volume (Walstra, 1969). This was later confirmed by the Michalski *et al.* (2001) using laser light scattering microscope. Similarly, in this study three sub population groups were observed; namely small globules less than 1μm (not shown), accounts about 78% of the total number of globules; the medium size (1-8 μm) accounts about 20.51-21.54 % of the total number of fat globules; and the larger groups (>8μm) accounts about 0.49–1.49 % of the total fat globules. This finding was close to Keenan and Dylewski (1995), who reported that the fat globules below 1 μm in diameter

account 80% or more of the total globule number, but they comprised less than 10% of the total volume of milk fat, and fat globules between 1-8 μm in diameter contained 90 % or more of the total volume of milk fat. The larger fat globules comprised about 1-3 % of the total volume of fat.

Table 14 shows different parameters estimated for the raw milk at different fractions at different time intervals. An increasing trend of fat volume and surface area, with decreasing trend in total surface area was evident with increasing fat content. This could substaintiate that the larger fat globules comprised or contributes major volume of the % fat in the milk. In this study, an average total surface area of 7,612 cm^2 / g was estimated for the raw bulk milk, and this result was lower than the findings of Attaie and Richter (2002). Attaie and Richter (2002) estimated a total surface area of 17,117 cm^2 per ml for the bovine milk.

On the other, an increase in the total surface area with decreasing fat globules size was evident. This could be due to more numbers of smaller fat globules. The number of fat globules in the milk was reported to range in between 1.5 to 3 billion globules per cm^3 (Winton and Winton, 2002). In this study, total fat globules estimated (Table 14) for the raw bulk milk averaged 4.46 x 10^9 per gram. This was close to the range reported, i.e. in between 10^{10} to 10^{11} fat globules per ml (Walstra *et al.,* 1999). The number of fat globules increased with increasing fat content at the top fraction, and decreased at the bottom fraction. However, the number of fat globules reported should be cautioned as all of the globules found in milk would have included; besides, the globules of 0.1 to 1 μm in diameter are difficult to measure and seldom are accurately enumerated and measured (Attaie and Richter, 2000). Fat globules lower than 0.1 μm were not counted, besides difficulty it was the lowest limit used for the study.

Table 14 **Volume, surface area and total surface area of different fat globule sizes of different fractions at different time intervals.**

Fractions	Time (h)	Fat content (%)	Mean diameter (μm)	Volume(μm^3)	Surface Area (μm^2)	No. of globules/g	Total Surface area/g (cm^2)
Top	0	4.15 ± 0.01^{e}	3.38 ± 0.01^{bx}	20.22 ± 0.18	35.89 ± 0.21	2.14×10^9	7674.98
	2	5.39 ± 0.18^{d}	3.56 ± 0.12^{ax}	23.62 ± 0.34	39.82 ± 0.82	2.34×10^9	9467.76
	4	6.48 ± 0.05^{c}	3.55 ± 0.06^{ax}	23.44 ± 1.19	39.60 ± 1.35	2.88×10^9	11406.40
	6	6.88 ± 0.02^{b}	3.54 ± 0.07^{ax}	23.18 ± 1.30	39.31 ± 1.48	3.09×10^9	12156.60
	8	7.18 ± 0.09^{a}	3.55 ± 0.08^{ax}	23.44 ± 1.40	39.60 ± 1.67	3.19×10^9	12638.6
Middle	0	4.15 ± 0.01^{a}	3.41 ± 0.05^{ax}	20.83 ± 0.95	36.61 ± 1.10	2.07×10^9	7599.69
	2	3.82 ± 0.08^{b}	3.26 ± 0.05^{ay}	18.10 ± 0.80	33.33 ± 1.35	2.19×10^9	7329.21
	4	3.47 ± 0.05^{c}	3.12 ± 0.05^{aby}	15.91 ± 0.61	30.59 ± 1.03	2.27×10^9	6951.46
	6	3.31 ± 0.03^{d}	3.11 ± 0.06^{aby}	15.78 ± 0.33	30.39 ± 0.78	2.19×10^9	6641.85
	8	3.24 ± 0.15^{d}	2.84 ± 0.16^{by}	11.99 ± 0.85	25.34 ± 0.47	2.81×10^9	7134.60
Bottom	0	4.15 ± 0.01^{a}	3.43 ± 0.05^{ax}	21.14 ± 0.85	36.97 ± 0.99	2.04×10^9	7561.88
	2	3.60 ± 0.05^{b}	3.13 ± 0.27^{aby}	16.11 ± 0.36	30.84 ± 0.46	2.32×10^9	7180.57
	4	3.08 ± 0.28^{c}	2.95 ± 0.16^{bcy}	13.56 ± 2.62	27.35 ± 3.49	2.36×10^9	6472.70
	6	3.01 ± 0.54^{c}	2.76 ± 0.32^{bcdy}	11.06 ± 0.67	23.99 ± 0.99	2.83×10^9	6802.66
	8	2.78 ± 0.34^{c}	2.70 ± 0.18^{dy}	10.33 ± 1.12	22.92 ± 1.64	2.80×10^9	6426.82

^{abcde} means within the same fractions for the same column at different time intervals are significantly different ($p<0.01$).

^{xy} means among different fractions within the same column at same time interval are significantly different ($p<0.01$).

Experiment 1.2 Raw milk in storage tanks

Table 15 and 16 illustrate the fat content (%) and the changes in fat content of milk samples collected from different portions (top and bottom) of the storage tanks (ST2 and ST3) after 9 hrs of quiescent holding. There was no significant differences ($p>0.05$) among the storage tanks. A significant increase ($p<0.05$) in fat content for milk samples collected from top surface, and a significant decrease ($p<0.05$) in fat content at the bottom was observed for both storage tanks.

Table 15 Least square means and standard error of fat content (%) for milk samples collected after 9 hrs of holding time.

Samples	N	Tanks		Average
		ST2	ST3	
Original	16	3.69 ± 0.17^{bx}	3.55 ± 0.18^{bx}	3.62 ± 0.12^{b}
Top	16	9.46 ± 0.17^{ax}	9.48 ± 0.18^{ax}	9.47 ± 0.12^{a}
Bottom	16	2.54 ± 0.17^{cx}	2.53 ± 0.18^{cx}	2.53 ± 0.12^{c}

$^{a\,b\,c}$ means within the same column are significantly different ($p<0.05$).

^{x} means within the same row are not significantly different ($p>0.05$).

The fat content at the surface increased from 3.70 % and 3.55 % to 9.46% and 9.48% for ST2 and ST3 respectively, an increased by about 156.31% (ST2) and 167.2% (ST3). On the other hand, the fat content at the bottom decreased from 3.70 % and 3.55% to 2.53% and 2.54% for ST2 and ST3 respectively a decreased by about 31.07% (ST2) and 28.17% (ST3).

Table 16 Rate of change in fat content (%) of milk samples collected from the top and bottom of the storage tanks after 9 hrs of holding time.

Sample	N	Tanks		Average
		ST2	ST3	
Original	16	0	0	0
Top	16	156.32 ± 3.59^{ax}	167.12 ± 2.07^{ax}	161.72 ± 2.09^{a}
Bottom	16	-31.07 ± 3.59^{bx}	-28.17 ± 2.07^{bx}	-29.62 ± 2.09^{b}

$^{a\,b}$ means within the same column are significantly different ($p<0.05$).

^{x} means within the same column are significantly different ($p<0.05$).

The increase in fat content at the top fraction could be explained by the rising of fat globules resulting to concentration of the larger fat globules at the surface. The larger globules, as they begin to rise, tend to form adventitious clumps with others that they encounter, trapping other small globules and these adventitious clumps with many fat globules rise faster than a single globule (Prentice, 1992). Similarly, Warner (1976) reported that by action of cold agglutinin the fat globule mass increases in size, resulting to increase in both the surface area and volume of the globule. This large fat globule mass would move better against the friction because the surface area each unit of volume is less for the larger mass of fat globule, thus it resistance is less as compared to the smaller globule. The larger fat globules comprised major volume of fat content in the milk (Walstra, 1995). Whereas, a slow decreasing rate of fat content at the bottom fraction indicates that the larger fat globules still exist as an individual entity, and it rise accordance to the Stokes' law. The smaller fat globules rise slowly as the tendency to form clump is less (Prentice, 1992). The fat depletion at the bottom of storage tanks determined was 31.07% (ST2) and 28.17% (ST3) after 9 hrs of quiescent holding at 5 $^{\circ}$C. A similar result was obtained in this study conducted under laboratory condition, i.e. an average fat depletion of 28.80% was recorded at the bottom of glass column after 8 h of quiescent holding of milk. This suggests that the gravity separation of fat globules under same condition would be almost constant. Servello *et al.* (2004) and Jackson (1981) observed depletion rate of 60% and 30% after 1 1/2 hrs and 3 hrs of quiescent

holding respectively. This difference in the depletion rate could be attributed to the differences in the sampling method adopted.

Experiment 2 Efficiency of mixing raw milk in bulk tank

Experiment 2.1 Using manual plunger

2.1.1 Milk composition

The changing analysis values of milk composition sampling at various stirring times are illustrated in Table 17-19 for milk protein, solid-not fat (SNF) and fat respectively. The protein and SNF content in milk mixed with both plungers, stabilized after 5 times of stirring with no significant differences ($p>0.05$) with the rest of the stirring times (Table 17 and 18). Whereas statistically the milk fat content stabilized after 10, 5 and 10 times of stirring for chamber I, II and III respectively (Table 19).

The shortest time required to attain homogeneity in protein content could be explained by its nature of existence in dispersion form of a very tiny particles size ranging from 10^{-4} to 10^{-5} mm (Tetra Pak, 1995). These tiny particles could be easily disturbed and redistributed with mild agitation. Whereas, early stabilization of SNF could be explained by early stabilization of protein, which is one of the SNF components and the other water soluble components of SNF, i.e. lactose, minerals and certain vitamins which exists in true solution. The early stabilization of protein and SNF could also be in part explained by the nature of variation of milk components, where fat shows the widest variation, followed by protein, lactose and then ash (Table 3).

The difficulty in stabilization of fat content was caused by stratification of fat globules during storage or transportation from gravity creaming and also from the action of cold agglutinin. Fat globule tends to float due to lower density as compared to skim milk; depleting the fat content at the lower portion of the container forming cream layer at the surface. Cream starts to form after 40-50 minutes after cooling milk to 5 °C (Servello *et al.,* 2004).

Milk fat was found to be the best indicator among the three milk constituents, namely milk fat, protein and SNF in mixing raw milk prior to sampling. It is so because protein and SNF

stabilized earlier than the fat on mixing milk in the tanks. Thus considering milk protein and SNF alone in quality milk payment might jeopardize milk quality. This ultimately would leads to biased payment of milk to the producers. Today crude protein level and SNF is increasingly being used as a payment basis due to negative impact of dietary fat on health and also due to its parallel importance in the products development. These payment criteria for milk were reported to vary between countries and individual processors (Harding, 1995; Hurley, 2004). Thus, no matter which composition is used as the basis to test the quality of milk for payment, milk fat should not be overlooked. As reported by Harding (1995, besides dependence of the cost of dairy and food products that contain milk solids on the amount of fat which they contain, the milk fat would still continue to play a very significant role in determining the price of milk both with regards to base price and differential for milk fat content exceeding the base price arrangement.

Table 17 Least square means and standard errors of protein (%) as an indicator for adequate mixing of raw milk in different chambers.

Stirring number	Chamber I	Chamber II	Chamber III
0	2.78 ± 0.033^{b}	2.63 ± 0.033^{b}	2.67 ± 0.033^{b}
5	3.05 ± 0.033^{a}	3.07 ± 0.033^{a}	3.06 ± 0.033^{a}
10	3.06 ± 0.033^{a}	3.07 ± 0.033^{a}	3.07 ± 0.033^{a}
15	3.07 ± 0.033^{a}	3.07 ± 0.033^{a}	3.06 ± 0.033^{a}
20	3.08 ± 0.033^{a}	3.08 ± 0.033^{a}	3.07 ± 0.033^{a}
25	3.07 ± 0.033^{a}	3.08 ± 0.033^{a}	3.08 ± 0.033^{a}
30	3.08 ± 0.033^{a}	3.07 ± 0.033^{a}	3.07 ± 0.033^{a}

N of individual stirring number for each chamber = 20.

[ab]means within the same column are significantly different ($p<0.05$).

Table 18 Least square means and standard errors of SNF (%) as an indicator for adequate mixing of raw milk in different chambers.

Stirring number	Chamber I	Chamber II	Chamber III
0	7.19 ± 0.095^{b}	6.72 ± 0.095^{b}	6.86 ± 0.095^{b}
5	8.04 ± 0.095^{a}	8.10 ± 0.095^{a}	8.08 ± 0.095^{a}
10	8.07 ± 0.095^{a}	8.11 ± 0.095^{a}	8.11 ± 0.095^{a}
15	8.12 ± 0.095^{a}	8.11 ± 0.095^{a}	8.10 ± 0.095^{a}
20	8.14 ± 0.095^{a}	8.14 ± 0.095^{a}	8.12 ± 0.095^{a}
25	8.13 ± 0.095^{a}	8.14 ± 0.095^{a}	8.13 ± 0.095^{a}
30	8.14 ± 0.095^{a}	8.13 ± 0.095^{a}	8.13 ± 0.095^{a}

N of individual stirring number for each chamber = 20.

^{ab} Means within the same column are significantly different ($p<0.05$).

Table 19 Least square means and standard errors of fat content (%) as an indicator for adequate mixing of raw milk in different chambers.

Stirring number	N	Chamber I	N	Chamber II	N	Chamber III
0	15	9.55 ± 0.170^{a}	12	9.63 ± 0.188^{a}	20	9.58 ± 0.182^{a}
5	20	5.15 ± 0.157^{b}	20	4.47 ± 0.157^{b}	20	4.96 ± 0.157^{b}
10	20	4.81 ± 0.157^{bc}	20	4.33 ± 0.157^{b}	20	4.24 ± 0.157^{bc}
15	20	4.12 ± 0.157^{c}	20	4.20 ± 0.157^{b}	20	4.19 ± 0.157^{bc}
20	20	4.02 ± 0.157^{c}	20	4.02 ± 0.157^{b}	20	4.03 ± 0.157^{c}
25	20	3.95 ± 0.157^{c}	20	3.96 ± 0.157^{b}	20	4.03 ± 0.157^{c}
30	20	3.98 ± 0.157^{c}	20	3.98 ± 0.157^{b}	20	3.99 ± 0.157^{c}

^{abc} means within the same column are significantly different ($p<0.05$).

2.1.2 Comparative plunger efficiency

Comparative effectiveness of KU versus ISO standard designed plungers using protein, SNF and fat composition in raw milk as indicators are illustrated in Table 20-22. Using both types of plunger, protein and SNF stabilized after 5 times of agitation (Table 20 and 21). Statistically, milk fat content in all three chambers mixed with KU plunger stabilized after 5 times of stirring. Whereas, statistically milk mixed with ISO standard plunger attained homogeneity in milk after 15 times of stirring for chamber I and II, and 10 times of stirring for the chamber III (Table 22). A significant interaction effect ($p<0.05$) between stirrer and stirring times nested with container was observed. A significant difference ($p<0.05$) in the mixing efficiency was also observed between the stirrers (Table 23).

Table 20 Least square means and standard errors of protein (%) used as indicator for the efficiency of plungers.

Stirring number	Chamber I		Chamber II		Chamber III	
	KU	ISO	KU	ISO	KU	ISO
0	2.69 ± 0.05^{b}	2.81 ± 0.04^{b}	2.43 ± 0.05^{b}	2.57 ± 0.05^{b}	2.72 ± 0.04^{b}	2.45 ± 0.06^{b}
5	3.07 ± 0.05^{a}	3.03 ± 0.04^{a}	3.08 ± 0.05^{a}	3.08 ± 0.05^{a}	3.05 ± 0.04^{a}	3.09 ± 0.06^{a}
10	3.08 ± 0.05^{a}	3.05 ± 0.04^{a}	3.08 ± 0.05^{a}	3.08 ± 0.05^{a}	3.05 ± 0.04^{a}	3.09 ± 0.06^{a}
15	3.08 ± 0.05^{a}	3.05 ± 0.04^{a}	3.07 ± 0.05^{a}	3.08 ± 0.05^{a}	3.06 ± 0.04^{a}	3.08 ± 0.06^{a}
20	3.09 ± 0.05^{a}	3.05 ± 0.04^{a}	3.08 ± 0.05^{a}	3.09 ± 0.05^{a}	3.08 ± 0.04^{a}	3.08 ± 0.06^{a}
25	3.08 ± 0.05^{a}	3.06 ± 0.04^{a}	3.08 ± 0.05^{a}	3.09 ± 0.05^{a}	3.08 ± 0.04^{a}	3.09 ± 0.06^{a}
30	3.08 ± 0.05^{a}	3.08 ± 0.04^{a}	3.07 ± 0.05^{a}	3.08 ± 0.05^{a}	3.08 ± 0.04^{a}	3.09 ± 0.06^{a}
Average	3.02 ± 0.02	3.02 ± 0.02	2.98 ± 0.02	3.01 ± 0.02	3.02 ± 0.02	3.00 ± 0.02

N of individual stirring number for each chamber = 10

[ab] Means with different superscripts within the same column are significantly different ($p<0.05$).

Table 21 Least square means and standard errors of SNF (%) used as an indicator in for the efficiency of plungers.

Stirring number	Chamber I		Chamber II		Chamber III	
	KU	ISO	KU	ISO	KU	ISO
0	6.92 ± 0.13^{b}	7.26 ± 0.12^{b}	7.85 ± 0.06^{b}	6.13 ± 0.13^{b}	6.92 ± 0.12^{b}	6.13 ± 0.13^{b}
5	8.12 ± 0.13^{a}	7.96 ± 0.12^{a}	8.13 ± 0.06^{a}	8.10 ± 0.13^{a}	8.05 ± 0.12^{a}	8.10 ± 0.13^{a}
10	8.16 ± 0.13^{a}	8.04 ± 0.12^{a}	8.13 ± 0.06^{a}	8.11 ± 0.13^{a}	8.06 ± 0.12^{a}	8.11 ± 0.13^{a}
15	8.16 ± 0.13^{a}	8.04 ± 0.12^{a}	8.16 ± 0.06^{a}	8.11 ± 0.13^{a}	8.11 ± 0.12^{a}	8.11 ± 0.13^{a}
20	8.19 ± 0.13^{a}	8.05 ± 0.12^{a}	8.15 ± 0.06^{a}	8.13 ± 0.13^{a}	8.16 ± 0.12^{a}	8.13 ± 0.13^{a}
25	8.18 ± 0.13^{a}	8.06 ± 0.12^{a}	8.16 ± 0.06^{a}	8.13 ± 0.13^{a}	8.17 ± 0.12^{a}	8.13 ± 0.13^{a}
30	8.17 ± 0.13^{a}	8.07 ± 0.12^{a}	8.16 ± 0.06^{a}	8.12 ± 0.13^{a}	8.16 ± 0.12^{a}	8.12 ± 0.13^{a}
Average	7.98 ± 0.05	7.93 ± 0.04	8.10 ± 0.02	7.83 ± 0.05	7.95 ± 0.05	7.83 ± 0.05

N of individual stirring number for each chamber = 10

^{ab} Means with different superscripts within the same column are significantly different ($p<0.05$).

Table 22 Least square means and standard errors of fat content (%) as indicator for the efficiency of different plungers.

Stirring	Chamber I				Chamber II				Chamber III			
number	N	KU	N	ISO	N	KU	N	ISO	N	KU	N	ISO
0	7	9.71 ± 0.31^{a}	8	9.87 ± 0.20^{a}	4	9.43 ± 0.41^{a}	10	9.33 ± 0.20^{a}	10	9.40 ± 0.28^{a}	10	9.66 ± 0.24^{a}
5	10	4.72 ± 0.26^{bc}	10	5.51 ± 0.18^{b}	10	4.44 ± 0.26^{b}	10	4.91 ± 0.17^{b}	10	4.49 ± 0.26^{b}	10	4.98 ± 0.24^{b}
10	10	4.31 ± 0.26^{c}	10	4.58 ± 0.18^{c}	10	4.30 ± 0.26^{b}	10	4.60 ± 0.17^{b}	10	4.40 ± 0.26^{b}	10	4.65 ± 0.24^{bc}
15	10	3.97 ± 0.26^{c}	10	4.29 ± 0.18^{cd}	10	4.02 ± 0.26^{b}	10	4.24 ± 0.17^{cd}	10	4.18 ± 0.26^{b}	10	4.32 ± 0.24^{c}
20	10	3.86 ± 0.26^{c}	10	4.15 ± 0.18^{d}	10	3.99 ± 0.26^{b}	10	4.13 ± 0.17^{d}	10	3.95 ± 0.26^{b}	10	4.07 ± 0.24^{c}
25	10	3.83 ± 0.26^{c}	10	4.09 ± 0.18^{d}	10	3.97 ± 0.26^{b}	10	4.10 ± 0.17^{d}	10	3.85 ± 0.26^{b}	10	4.05 ± 0.24^{c}
30	10	3.89 ± 0.26^{c}	10	4.06 ± 0.18^{d}	10	3.98 ± 0.26^{b}	10	4.04 ± 0.17^{d}	10	3.88 ± 0.26^{b}	10	4.05 ± 0.24^{c}
Average		4.90 ± 0.09		5.22 ± 0.0		4.79 ± 0.11		5.05 ± 0.06		4.88 ± 0.09		5.11 ± 0.08

[abc] Means with different superscripts within the same column are significantly different ($p<0.05$).

The statistical inferences of the shortest stirring times for two plunger designs with different chamber capacities of milk truck tank are not practically acceptable in terms of both the manual sampling management and the relatively large variable of milk composition values between different mixing time intervals. The difference of analysis values are far beyond the acceptable range recommended by Grace *et al.* (1992) that " adequate agitation is that degree of agitation, which at full tank capacity, results in a variation in fat content of the milk in the tank of not more than ± 0.1 % level as determined by an official AOAC milk fat test". Adhering to the above recommendation by using the variable of fat composition as indicator, a trend requiring different mixing times for different chambers were evident in milk agitated with the two plungers (Table 22 and 23).

Milk in Chamber I and II agitated with KU plunger attained homogeneity after 15 times of stirring, whereas 20 times of stirring was required for chamber III (Table 22). More stirring times were required as the size of the chamber increased. Similar trend requiring more stirring times to attain milk homogeneity was observed with increase in the volume of milk in the tank (Likas and Calbert, 1954). This may be due to small surface area of the disc which in turn could not produce sufficient disturbances to disperse the clustered milk fat that has formed during transportation. The plunger should be long and the disc should be large enough to provide sufficient disturbances during mixing (ISO, 1997).

On the other hand, the milk agitated with ISO standard plunger attains homogeneity of milk after 20 times of stirring for all chambers. More stirring times required to attain homogeneity of milk in the small chamber could be attributed to the inconveniences encountered during mixing motion of the plunger with large disc diameter. The occasional contact of plunger to the side surface of the tanks while mixing resulted in non uniformity of mixing cycles. On the other hand, more stirring times required to attain homogeneity in milk mixed with KU plunger for larger chamber could be attributed to its short stirring rod. A more complete mixing motion can be attained with a slight improvement of its length. This emphasizes that the proportionality of plunger to the size of containers was imperative for effective mixing.

Table 23 Least square means and standard errors for milk composition (%) used for the efficiency of plungers in mixing raw milk.

Chamber	Plunger	N	Protein	N	SNF	N	Fat
I	KU	70	3.02 ± 0.02	70	7.98 ± 0.05	65	4.91 ± 0.08
	ISO	70	3.02 ± 0.02	70	7.93 ± 0.05	70	5.25 ± 0.09
	p-value		0.7273		0.3951		0.0063
II	KU	70	3.06 ± 0.017	70	8.10 ± 0.050	66	4.79 ± 0.09
	ISO	70	2.98 ± 0.017	70	7.83 ± 0.050	70	5.09 ± 0.08
	p-value		0.0016		0.00002		0.0852
III	KU	70	3.01 ± 0.018	70	7.95 ± 0.050	70	4.95 ± 0.09
	ISO	70	3.00 ± 0.021	70	7.87 ± 0.050	70	5.06 ± 0.08
	p-value		0.5397		0.2991		0.0784
Total Average	KU	70	3.03 ± 0.010	70	8.01 ± 0.03	67	4.88 ± 0.05
	ISO	70	3.00 ± 0.010	70	7.88 ± 0.03	70	5.13 ± 0.05
	p-value		0.0187		0.0012		0.00004

Experiment 2.2 Efficiency of motor driven agitator

Table 24 illustrates mean milk fat content of milk samples from the storage tanks (ST3 and ST2) used as an indicator in determining adequate mixing time prior to sampling. A significant difference ($p<0.01$) in mixing time was observed for both tanks. The samples collected from the surface of the tanks showed milk homogeneity after 60 min and 10 min of mixing for ST3 and ST2 respectively. Whereas samples collected from the bottom showed milk homogeneity only after 60 min (ST3) and 30 min (ST2) of mixing. Thus, milk was smoothly mixed for 60 min (ST3) and 30 min (ST2) prior to sampling.

Table 24 Least square means and standard error of fat content (%) as indicator for adequate milk mixing prior to sampling from the storage tanks.

Mixing	ST3				ST2			
Time (Min)	N	Top	N	Bottom	N	Top	N	Bottom
0	8	10.29 ± 0.31^{ax}	5	2.67 ± 0.09^{by}	8	8.90 ± 0.16^{ax}	5	2.76 ± 0.17^{by}
10	8	9.31 ± 0.31^{b}		-	8	3.81 ± 0.16^{b}		-
20	8	8.23 ± 0.31^{c}		-	8	3.81 ± 0.16^{b}		-
30	8	7.53 ± 0.31^{dx}	5	3.09 ± 0.09^{aby}	8	3.81 ± 0.16^{bx}	5	3.68 ± 0.17^{ax}
40	8	6.50 ± 0.31^{d}		-	8	3.80 ± 0.16^{b}		-
50	8	4.61 ± 0.31^{e}		-	8	3.80 ± 0.16^{b}		-
60	8	3.92 ± 0.31^{ex}	5	3.55 ± 0.09^{ax}	8	3.80 ± 0.16^{bx}	5	3.82 ± 0.17^{ax}
70	5	4.04 ± 0.40^{e}		-		-		-
80	5	4.02 ± 0.40^{e}		-		-		-
90	5	4.04 ± 0.24^{ex}	5	3.73 ± 0.09^{ax}		-		-

^{abcd} means within the columns are significantly different ($P<0.01$).

^{xy} means within the same row for the respective tank are significantly different ($p<0.01$)

Table 25 illustrates least square means of milk fat content (%) used as an indicator to determine the adequate mixing time prior to sampling for the storage tanks (ST3 and ST2) after

replacement of agitator in ST3. A significant difference ($p<0.01$) in mixing time and least square means of fat content (%) for samples collected from different portions, i.e. top and bottom was observed. The milk samples collected from the top surface showed homogeneity after 5 min and 10 min of mixing for ST3 and ST2 respectively. A significant interaction effects ($p<0.01$) between the mixing time and samples collected from different portions of tanks was observed. Statistically, samples collected from the bottom for both tanks showed homogeneity after 10 min of mixing. A non significant difference ($p>0.05$) in between samples collected from top and bottom was observed after 10 min (ST3) and 15 min (ST2) of mixing (Figure 15). Therefore, it would be necessary to mix the milk for at least 10 min (ST3) and 15 min (ST2) prior to sampling.

Table 25 Means and standard deviations of fat content (%) as an indicator for adequate mixing of raw milk in storage tanks after replacement of agitator blade in ST3.

Mixing Time	ST3		ST2	
(Min)	Top	Bottom	Top	Bottom
0	8.96 ± 0.43^{ax}	2.32 ± 0.20^{cy}	8.79 ± 0.47^{ax}	2.21 ± 0.04^{cy}
5	3.44 ± 0.27^{bx}	2.74 ± 0.31^{by}	4.16 ± 0.86^{bx}	2.72 ± 0.42^{by}
10	3.40 ± 0.30^{bx}	3.39 ± 0.31^{ax}	3.72 ± 0.23^{cx}	3.47 ± 0.49^{ay}
15	3.39 ± 0.30^{bx}	3.38 ± 0.33^{ax}	3.71 ± 0.22^{cx}	3.59 ± 0.35^{ax}
20	3.40 ± 0.31^{bx}	3.40 ± 0.31^{ax}	3.70 ± 0.23^{cx}	3.69 ± 0.26^{ax}
25	3.41 ± 0.32^{bx}	3.40 ± 0.31^{ax}	3.70 ± 0.24^{cx}	3.71 ± 0.25^{ax}
30	3.41 ± 0.29^{bx}	3.40 ± 0.30^{ax}	3.69 ± 0.25^{cx}	3.71 ± 0.28^{ax}

N=8

^{abc} Means within the same column are significantly different ($P<0.01$).

^{xy} Means within the same rows for the respective tank are significantly different ($p<0.01$).

Initially, longer duration of mixing time required to attain milk homogeneity in ST3 could be explained mainly due to small size of agitator blade, which in turn could not produce sufficient disturbances to displace the cream layer formed at the surface due to rising of fat globules. Servello *et al.* (2004) reported that mixing behavior in the bulk tanks is affected by

variables such as size, shape, percent fill and temperature at the time of pick up, as well a the shape and rotation speed of the agitation. It was observed that the milk fat content analyzed for samples collected from the top surface stabilized earlier than the bottom samples. Thus, establishing adequate agitation time based on the samples collected from one portion could jeopardize the quality of milk. Thus, if used for payment purpose could cause a loss of payment for milk producers. Therefore, possibly it would be necessary to consider sampling from top and bottom portions in determining adequate agitation time in mixing milk for the tanks.

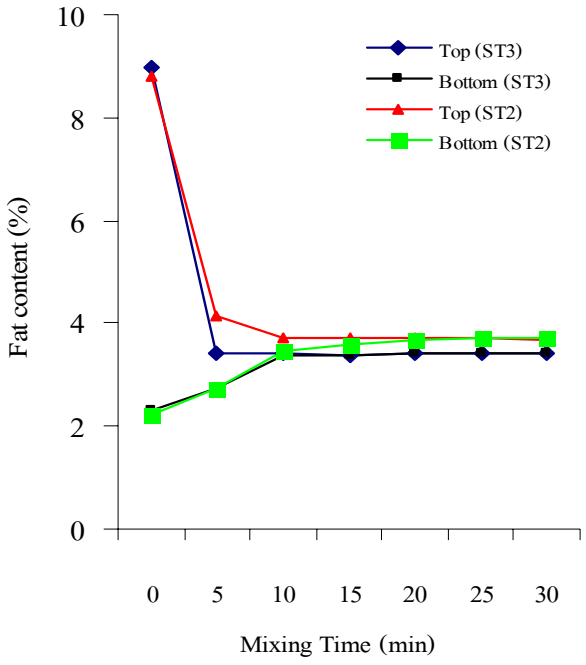

Figure 15 Mean mixing efficiency of power driven agitator after replacement of agitator blade in ST3.

2.2.1 Total power energy consumed

Table 26 shows total power consumed for mixing raw milk in the two bulk storage tanks (ST2 and ST3). The total power consumed was calculated based on the initial mixing time adopted, i.e. 30 min and 60 min for ST2 and ST3 respectively. Initially, the total power energy

consumed per annum was 90.688 Kwh (ST2) and 118.56 Kwh (ST3). The power energy consumed was drastically reduced after replacement of the agitator in ST3; and adhering to the determined adequate mixing time of 15 min (ST2) and 10 min (ST3). The estimated power energy consumed per year was 47.424 Kwh (ST2) and 25.029 Kwh (ST3). Adhering to the determined adequate mixing time for both storage tanks after replacement of the agitator in ST3, total power energy consumed decreased from 209.248 Kwh to 72.453 Kwh per annum. With this, the dairy plant in total could save about 136.795 Kwh of power energy per annum. Besides, the impact on the stability of the fat globules would have reduced drastically, although this was beyond the scope of this study.

Table 26 Total power energy consumed (Kwh) in operation of agitator in mixing raw milk in the storage tanks prior to processing.

Tanks	Initial*		Final**	
	Daily	Yearly	Daily	Yearly
ST2	0.436	90.688	0.228	47.424
ST3	0.570	118.56	0.120	25.029
Total	1.006	209.248	0.348	72.453

Note: * Based on 30 min (ST2) and 1hr (ST3) of mixing time.

** Based on 15 min (ST2) and10 min (ST3) of mixing time.

2.2.2 Total costs of operation

Table 27 shows total costs of mixing bulk raw milk in the storage tanks. It was estimated based on actual operation time used in mixing milk prior to processing, i.e. 104 h (ST2) and 208 h (ST3). Initially, the estimated cost of operating agitator to adequately mix the milk prior to sampling was ฿2518.04 and ฿4867.74 for ST2 and ST3 respectively. After replacement of agitator blade in ST3 and determining the actual adequate mixing time required for both tanks, i.e. 15 min (ST2) and 10 min (ST3) of mixing time prior to sampling was adequate. The operation

time required was drastically reduced from 104 and 208 to 52 h (ST2) and 34.67 h (ST3). In total, the dairy plant could approximately save around ฿ 5296.36 per annum. A drastic reduction in cost of operating agitator in mixing raw milk prior to sampling or processing was resulted mainly due to reduction of labor cost and power energy consumed.

Table 27 Total cost (Baht) of operation in mixing bulk raw milk prior to processing.

Tanks	Initial*		Final**	
	Daily	Yearly	Daily	Yearly
ST2	12.11	2518.04	6.08	1264.60
ST3	23.40	4867.74	3.97	824.82
Total	35.51	7385.78	9.78	2089.42

Note: 8 h of working per day; 1 person; ฿175 daily minimum wages/ day and ฿2.68 for unit (Kwh) of power consumed

* Based on 30 min (ST2) and 1hr (ST3) of mixing time.

**Based on 15 min (ST2) and 10 min (ST3) of mixing time.

2.2.3 Return on investment (RoI)

RoI for ST3 = (Final Value – Initial Value) / Initial Value

= (16050 + 824.82) – 4867.74 / 4867.74

= 2.47

Note: Expenditure incurred on agitator blade and the services = Baht 16,050.00.

With the RoI value obtained, we conclude that the investment was feasible and the invested cost could be recovered within 2.47 years of operation.

The margin of profit the milk plant operator could expect is strictly limited, and the main aim of every plant manager should be to increase production efficiency to generate higher profit. Hence, it is necessary to make the best use of the resources available keeping the operating cost to

the minimum (Hall *et al.,* 1953). “Plant operating efficiency is defined as the ability to produce the desired products with a minimum of effort, expense, and waste without sacrifice of the workers’ welfare, although perfection in efficiency may not be achievable, but the various operations must conform to recognized standards of efficiency and should all the times equal to or above these standards” (Tracy *et al.,* 1958). Hence, there is a need to timely monitor the efficiency of the production processes. For example, in Ontario reduction of the agitation time from 5 to 2 min had saved about $ 1.2 million per year mainly due to more efficient use of both trucks and truck drivers (Goodridge *et al.,* 2004). Likewise, if different dairy plants in the country reassess and adhere to the new agitation time, huge power energy could be saved; besides labor cost would also be reduced, thereby ultimately curb the overall production costs.

Experiment 3 Effects of homogenization on raw milk fat globules

3.1 USPH index

Table 28 and 29 illustrate the least square means of milk fat content (%) and USPH index of raw and homogenized milk. There was no significant difference ($p>0.05$) in fat content between the raw and homogenized milk. However, a slight increase in fat content was observed after homogenization of milk at 2,500 pounds pressure. In contrast, Trout (1950) reported that milk homogenized at 2,000 – 3,000 pounds pressure and tested for milk fat using Babcock test shows about 0.1 to 0.15 percent lower than the test of the milk before homogenization. The difference between the Babcock test of homogenized and nonhomogenized milk could be due to one or more of several factors reported such as a) the specific gravity of the acid used; b) the volume of acid; c) the maximum heats of reaction obtained; d) the length of time of shaking the acid-milk mixture and e) the completeness of digestion and the amount of foreign matter in or at the base of the fat column.

Table 28 Least square means and standard error of fat content (%) for raw and homogenized milk.

Homogenizer	Homogenization Time (h)					
	N	0	N	1	N	3
T	14	3.87 ± 0.07	14	3.89 ± 0.07	13	3.92 ± 0.08
P	10	3.68 ± 0.08	10	3.75 ± 0.08	10	3.75 ± 0.08
Average		3.80 ± 0.05		3.83 ± 0.05		3.84 ± 0.06

There was a significant difference ($P<0.01$) in USPH index between the raw and homogenized milk. Average least square means of USPH index for the raw milk was 79.37 %; and for the homogenized milk sample was 5.54 % (1 h) and 5.42 % (3 h). The means USPH index for homogenized milk sample decreased slightly with increasing time of operation. However, there was no significant difference ($p>0.05$) in the USPH index for milk homogenized

at different time intervals. The slow decreasing trend in the USPH index with increasing time of operation was difficult to explain, however a slight variation might have occurred while removing the cream layer from the top surface-using pipette. Trout (1950) reported that in drawing off of the top portions it was impossible to get all the cream, a variable portion always remain because the tip of the pipette would be held at varying distances below the surface of the liquid. As such, an error in sampling was expected resulting to inevitable small variation of result reported.

Table 29 Least square means and standard error of USPH index for raw and homogenized milk.

Homogenizer	Homogenization Time (h)					
	N	0	N	1	N	3
T	14	79.38 ± 0.58^{ax}	14	5.35 ± 0.58^{by}	13	5.33 ± 0.58^{by}
P	10	79.36 ± 0.68^{ax}	10	5.82 ± 0.68^{by}	10	5.53 ± 0.68^{by}
Average		79.37 ± 0.43^{a}		5.54 ± 0.43^{b}		5.42 ± 0.44^{b}

^{ab} means within the same row are significantly different ($p<0.01$).

^{xy} means within the same column are significantly different ($p<0.01$)

The vast differences in the USPH index between raw and homogenized milk could be explained by formation of numerous small fat globules by homogenization process; besides the natural agglutinin that aids in formation of larger clusters could have been destroyed due to homogenization. Hence, no appreciable cream layer would be formed on homogenized milk due to the small size of the fat globules and their inability to coalesce greatly decreases their ability to rise to the surface (Lampert, 1947). This differences could also be explained in part by an increase in the specific gravity of fat globules resulted by adsorption of casein particles in the newly formed fat globule surface. Trout (1950) reported that in non homogenized milk about 2% of the casein was observed on the surface of fat globule; whereas in homogenized milk 25% of casein was found adsorbed on the surface of fat globules. Gravity creaming or skimming of homogenized milk was reported questionable (Trout, 1950). In this experiment gravity separation of fat globules was evident even in the homogenized milk. It could be substantiated by increasing fat content at the top surface (1/10 of the milk volume) of the container after 48 h of quiescent

holding at 5 °C. Trout (1950) reported that the MFGs size in homogenized milk should be less than 2 μm to prevent creaming. In this study, substantial number of fat globules greater than 2 μm was observed after homogenization. Rising of these fat globules individually could have triggered the gravity separation of fat globules in homogenized milk. The separation of fat globules determined as the means USPH index falls within the acceptable range value in between 1 to 10 (Tetra Pak, 1995). A value of homogenization efficiency below 10 % indicates that homogenizer is very efficient (Ertugay *et al*., 2004). Thus the homogenizers used in this study could be considered equally efficient or the milk is properly homogenized.

3.2 Effects of homogenization on different parameters of milk fat globules

Table 30 illustrates effects of homogenization on different parameters of milk fat globules, i.e. fat globule size, volume, surface area, total surface area and number of fat globules. A significant difference ($p<0.01$) was observed between the raw and homogenized milk in all the parameters studied with exception to fat content. However, there was no significant difference ($p>0.05$) between the milk homogenized at the different time intervals. This could be due to the same pressure (2500psi) used in the homogenization of milk. The greater the homogenization pressure used, the smaller would be the size of the fat globules (Tetra Pak, 1995).

A particles size of fat globules ranging from 0.1 – 10.10 μm with an average diameter of 3.37 μm was observed in this study for the bulk raw milk. Whereas, the mean fat globule size after homogenization was found to be 0.98 μm (1 h) and 0.96 μm (3 h) with particle size ranging in between 0.1 to 2.37 μm. In average, the raw milk fat globules size was reduced below 1μm, a decrease by about 3.44 (1h) and 3.51 (3h) times after homogenization. The result on the reduction of size after homogenization agrees with the previous report. Tetra Pak (1995) reported that the fat globules should be reduced to approximately 1 μm in diameter to stabilize the fat emulsion against gravity separation. Warner (1976) reported that the fat globules size in milk homogenized would average about 1 to 2 μm depending upon the pressure used and other factors. However, the size reported might depend upon the measurement method employed (Walstra, 1995). The methods employed in determining the fat globules size are scanning electron microscopy,

spectroturbidimetry, fluorescence microscopy, the coulter counter, electroacoustics or Laser Light Scattering (Michalski *et al*., 2001). In addition, the results of the fat globule size distribution would also depend on whether a casein-dissolving agent is used, and if so, which agent, and if not used an apparent increase in the globule diameter may be resulted due to fat globules aggregate formation through casein interactions (Evers, 2004b).

Table 30 Least square means and standard error of different parameters for raw and homogenized milk fat globules.

Parameters	Time (h)	Homogenizers				Average
		N	T	N	P	
Fat content (%)	0	6	3.66 ± 0.03	5	4.02 ± 0.11	3.84 ± 0.05
	1	6	3.74 ± 0.03	5	4.04 ± 0.11	3.89 ± 0.05
	3	6	3.76 ± 0.03	5	4.04 ± 0.11	3.90 ± 0.05
Mean diameter (μm)	0	6	3.39 ± 0.04^{a}	5	3.35 ± 0.08^{a}	3.37 ± 0.04^{a}
	1	6	0.99 ± 0.04^{b}	5	0.96 ± 0.08^{b}	0.98 ± 0.04^{b}
	3	6	0.99 ± 0.04^{b}	5	0.93 ± 0.08^{b}	0.96 ± 0.04^{b}
Surface Area (μm^2)	0	6	36.22 ± 0.79^{a}	5	35.45 ±1.70^{a}	35.85 ± 0.85^{a}
	1	6	3.12 ± 0.79^{b}	5	2.92 ± 1.70^{b}	3.01 ± 0.85^{b}
	3	6	3.07 ± 0.79^{b}	5	2.72 ± 1.70^{b}	2.89 ± 0.85^{b}
Volume (μm^3)	0	6	20.55 ± 0.66^{a}	5	20.05 ±1.49^{a}	20.31 ± 0.74^{a}
	1	6	0.52 ± 0.66^{b}	5	0.47 ± 1.49^{b}	0.49 ± 0.74^{b}
	3	6	0.51 ± 0.66^{b}	5	0.43 ± 1.49^{b}	0.46 ± 0.74^{b}
No. of fat globules/g	0	6	1.86 x 10^{9}	5	2.09 x 10^{9}	1.96 x 10^{9b}
	1	6	7.49 x 10^{10}	5	8.96 x 10^{10}	8.27 x 10^{10a}
	3	6	7.68 x 10^{10}	5	9.79 x 10^{10}	8.83 x 10^{12a}
Total Surface area/g	0	6	6721 ± 807.34^{b}	5	7405 ± 1749^{b}	7062 ± 907.54^{b}
(cm^2)	1	6	23380 ± 807.34^{a}	5	26151 ± 1749^{a}	24897 ± 907.54^{a}
	3	6	23582 ± 807.34^{a}	5	626626 ± 1749^{a}	25529 ± 907.54^{a}

$^{a\,b}$ means within the same column against respective parameter are significantly different ($p<0.01$).

Note: Total no. of globules per g = fat content x 10^{12} μm^3 / density of fat x 100 x 1.032 * volume of fat (μm^3)

The decrease in raw milk fat globules size after homogenization was accompanied by a significant increase ($p<0.01$) in the number of fat globules. Warner (1976) reported an increase in number of fat globules by about three times. There was a significant increase ($p<0.01$) in the total surface area of fat globules after homogenization. However, a significant difference ($p>0.05$) was not observed in the total surface area among the milk homogenized at different time intervals. The mean total surface area of fat globules was about 7063 cm^2 per g of bulk raw milk. This finding was close to Attaie and Richter (2000), who estimated the total surface area of the bovine milk to be 17,117 cm^2/ml. It was low as compared to the findings of Warner (1976), who estimated a total surface area of bovine milk to be about 17,500 cm^2 per ml of milk. Whereas, total surface area for the milk homogenized at different times was estimated about 24,810 cm^2 (1 h) and 25,246 cm^2 (3 h) per g in this study, an average increased in the total surface area by about 4 times as compared to the raw milk (Table 30). This finding was close to Tetra Pak (1995); the fat/plasma interfacial surface area would increased by about four to six folds. The total surface area was reported to increase by 6 or 8 folds after homogenization (Trout, 1950). An increase in total surface area after homogenization could be explained by increased in number of fat globules. Average total number estimated was about 1.96×10^9 fat globules per gram for the raw bulk milk. Whereas a total number of 8.27×10^{10} (1h) and 8.83×10^{10} (3h) fat globules per g was estimated after homogenization. The number of fat globules was reported to increase by many folds after homogenization. On the average, each fat globule in normal milk was reported to divide into approximately 1,200 smaller globules after homogenization (Trout, 1950). In this study, after homogenization the number of fat globules estimated increased by about 47 times, which means each fat globule was divided into approximately 43 smaller fat globules after homogenization. However, the extent of the increase in numbers resulting from the homogenization is seldom accurately determined mainly because it would depend on many factors, i.e. the size of the globules existing in the original milk and by the extend of their division. The fat globules size in homogenized milk would depend primarily upon the homogenizing pressure used and on other factors, such as condition of the valves and temperature of the milk (Trout, 1950). Since, it was derived by applying the mathematical formula; it would depend upon the respective radii of the fat globules. Thus, the result reported might greatly differ. It should also be noted that counting of fat globules required great skill and experiences in microscopic examination (Trout, 1950).

3.3 Particle distributions

Figure 16 illustrates particles size distribution of raw and homogenized milk fat globules. In raw milk about 70 % of the fat globules observed were less than 3 μm in diameters. Whereas, in homogenized milk about 75 % of fat globules were found to be ≤ 1 μm with maximum fat globules size of about 2.37 μm in diameter. In this study, after homogenization of milk almost above 90 % of the fat globules size was observed below 2 μm in diameter. Trout (1950) reported that the efficiency of homogenizer is considered good, when 90 % of the fat globules observed under a microscope were less than 2 μm in diameter. Thus, the homogenizers used in this study could be considered equally efficient using milk fat globules size as an indicator. A significant decrease ($p<0.01$) in unit surface area and volume of fat globules was observed after homogenization.

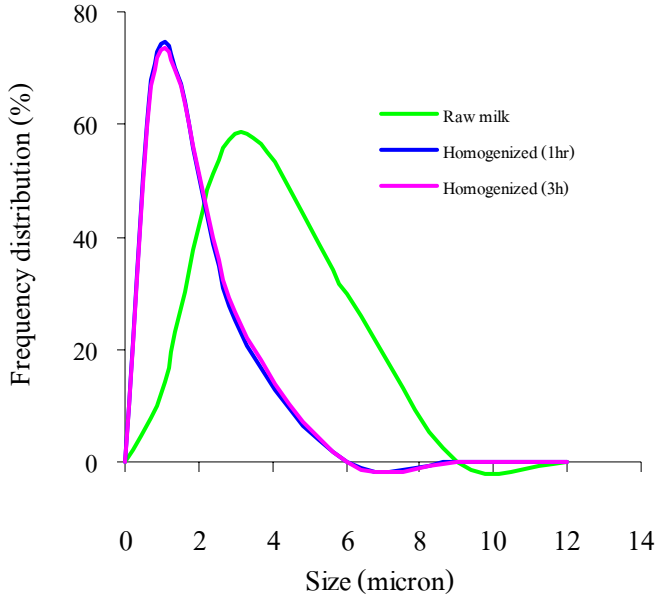

Figure16 Fat globules size distributions curve of raw and homogenized milk.

CONCLUSIONS

The fat globules being insoluble and lighter than the skim milk rise to the surface in the form of a cream line during storage or transportation. The rate of fat globules separation was found to be much faster than predicted by Stokes equation especially at the early hour of holding milk at low temperature. The fat content increased at the surface and decreased at the bottom of the glass column as the holding time increases. Like wise, the fat globules size at the top fraction increased; whereas, a decrease in fat globules size was evident at the bottom fraction with increasing holding time. The gravity separation process will continue at the decreasing rate until the fat globules at the bottom are completely depleted or the fat globules at the bottom attain same density as of the continuous phase. This leads to formation of strata of milk in the containers with different milk fat contents and fat globules sizes. Thus proper understanding of the gravity separation characteristic of raw milk fat globules will help small dairy products manufactures, especially in developing countries in deriving milk products of different milk fat contents and fat globules size. The gravity separation of natural raw milk fat globules depends on the fat content, fat globule size distribution and time.

Homogenization reduces the fat globules size by many folds and renders even distribution of smaller fat globules. It increases the number and total surface area of fat globules thereby the gravity separation is drastically reduced in homogenized milk. This gravity separation characteristic of modified fat globules with proper understanding could be adopted in assessing and controlling the quality of homogenized milk.

Milk fat was found to be the best indicator among the three milk constituents, namely milk fat, protein and SNF in mixing raw milk prior to sampling. Comparing KU versus ISO designed plungers for manually mixing milk in the milk truck chamber prior to sampling, the KU plunger performed relatively better for small size milk chamber. However, both plungers are equal in their effectiveness in mixing milk for larger chamber. With slight increase in the length of the KU plunger rod, at least 15 times of proper stirring motion are required for chamber of less than 7,500 kg capacity. As for the ISO standard plunger, at least 20 times of stirring are required

for similar sample homogeneity. By reducing the mixing time resources (time, labor and energy) for the dairy plant will be saved; besides it will also reduce the potential impact of agitation on fat globules stability. Therefore, determining adequate time of mixing raw milk empirically upon installation for each individual tank would be imperative. Besides, a timely monitoring and evaluation of the production efficiency must be in place at all the time.

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BIOGRAPHY

NAME	JIGME WANGDI
NATIONALITY	BHUTANESE
DATE OF BIRTH	1 JANUARY, 1976
PLACE OF BIRTH	THIMPHU, BHUTAN
EDUCATION	1985 completed primary education from Dechencholing Primary School, Thimphu, Bhutan.
	1993 completed high school from Sainik School Imphal, Manipur, India
	1998 completed Bachelor of Science in Agriculture from Central Luzon State University, Nueva Ecija, Philippines
WORKING EXPERIENCE	1999 Livestock Extension Officer, Ministry of Agriculture
	1999-2004 District Livestock Officer, Ministry of Agriculture