ABSTRACT

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Grease accumulation in sewers is the leading cause of sewer blockages resulting in Sanitary Sewer Overflows. Nationwide Fats, Oils, and Grease (FOG) control programs exist to address this problem, but the guidelines with respect to wastewater from dairy-based food service establishments (FSEs) are unclear, with no existing scientific investigation on potential separation of dairy products. The separation tendencies of wastewater originating from dairy-based FSEs were investigated, and significant separation of dairy constituents (Kjeldahl Nitrogen and fats) was observed under acidic conditions (pH 4-5.4), with maximum separation at the isoelectric point of casein proteins (pH = 4.6). Physical treatment at field-scale grease interceptors for dairy-based FSEs showed accumulation of dairy constituents, particularly fats, at pH 4.4-5 caused by dairy products souring. Separation induced by souring in neutralized pH persisted but to a lesser extent (10% vs. 2%). Based on research conducted, physical treatment of wastewaters originating from dairy-based FSEs is recommended.

THE PRODUCTION AND FATE OF FATS, OILS, AND GREASE FROM SMALL DAIRY-BASED FOOD SERVICE ESTABLISHMENTS.

By

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Preface

In recent years there has been growing concern for sewer collection system capacity, management, operation and maintenance (CMOM), and the prevention of sewer system overflows (SSOs). Grease accumulation in sewers has been cited as the leading cause of sewer blockages resulting in SSOs. Such blockages can be attributed to fats, oils, and grease (FOG) discharged from food preparation and manufacturing facilities, concentrated residential areas, and even single family homes. Utilities have responded to this problem by implementing FOG management programs directed to food service establishments (FSEs) with obvious FOG production (frying, cooking using oil, meat processing, etc). While current FOG regulations have successfully reduced FOG blockages and subsequent SSO events, they have not been eliminated. At issue here is whether oils and fats from coffee and dairy products contribute to pipe deposits and thus SSOs. In this study the potential accumulation of FOG originating from dairy sources, such as ice creams, frozen dairy drinks, and hot dairy-based beverages, was investigated.

While nationwide FOG control programs are being updated, many state municipalities, such as Connecticut, do not cite dairy FOG as a contributor to pipeline blockages while others such as Florida, the towns of Louisburg and Salisbury in North Carolina among others regard dairy products as a source of FOG and enforce installation of grease abatement devices (GADs) in all their FSEs including coffee and ice cream shops. The number of states and municipalities regulating FOG from coffee and ice cream shops is limited, and so is the scientific-based knowledge to promote sound regulations. Thus an important consideration is to evaluate the nature of dairy products served in such FSEs (coffee shops, ice cream shops).

The fundamentals of dairy physics and chemistry were reviewed in order to understand the behavior of dairy products under different conditions and assess their potential to form a separate phase when discharged into sewer systems. These findings suggested that a dairy solid phase, composed of different proteins and fat

globules has a high potential to separate. The time frame required for the separation process to take place and thus whether currently used interceptors and grease abatement devices could be efficient were of great interest.

Temperature and pH were noted as the most significant environmental factors that are expected to cause separation of dairy products in wastewater. Research studies were conducted to examine the effects of temperature and pH on separation employing synthesized dairy wastewater in batch settings. In the case of warm tap water, the higher kinetic energy of the system caused more rapid separation while in room temperature systems, the separation occurred at a slower rate. Nevertheless, in both cases ultimately comparable results were obtained. The effects of detergents and other surface-active materials used for cleaning were also studied. The addition of detergents slightly enhanced the separation of dairy constituents except for vanilla latte, in which slightly lower separation levels were observed. As a general trend, separation for mixtures with higher fat content resulted in a complete floating layer, as observed for half and half, or a mixture of floating and sinking phases (ice cream), signaling the entanglement of fats and proteins in separated phases. Further separation of the phases was also witnessed in coffee-based synthetic wastewater. The separation of samples was found to be relatively fast, with samples found to reach steady fractionation after 3 hours, which could become more compact via centrifuging.

In an attempt to quantify the partitioning tendencies, Standard Method 5520B for FOG analysis was used. However, this test was determined to be incompatible for dairy fat determination as upon addition of hexane to the mixture, a viscous gelatin layer formed because of the potential low solubility of dairy fats and proteins in non-polar hexane. An industry acceptable standard method for testing dairy products was employed, and the separated phases (curd layers) were found to hold almost all the fat residing in the sample, with little fat residing in the solution for samples of higher fat (whole milk and ice cream:<4.%). Fresh wastewater sample representing practices in a local coffee shop were collected and found to hold a small mass fraction of fat (average of 0.02 ± 0.004% for first shift, and 0.03 ± 0.003% for second shift).

Samples were also collected from grease interceptors of a specialty coffee shop and an ice creamery for the 3-4 week duration. Based on analysis of these GADs, with an average retention time of 2 days obtained for the ice cream grease interceptor, separation of dairy constituents from wastewater was observed. In the case of the interceptor treating the specialty coffee shop wastewater, the floating and the settling fractions could be witnessed, with the degree of partitioning reducing from the inlet to the outlet chambers. Similar to the dairy dilutions tested, the solution segment of the samples had a marginal fat content, and fats partitioned into the sediment phase and were found to be the major constituent of the floating phase. Accumulation of separated layers, as well as increase in fat content of each phase, was observed during the 3 weeks of GAD sampling and laboratory study. In the interceptor samples collected from the ice cream facility, on the other hand, separation could be witnessed prior to the most recent cleaning of the interceptor, and the separated phases were found to be most commonly floating, slowly sinking only when disturbed in the sampling column. Furthermore, partitioning in this GAD seemed to be slow, with the greatest fractionation occurring in the outlet compartment.

Because souring of dairy product is accompanied by the production of lactic acid, the pH reduces to values close to the zero point of charge (ZPC) pH, at which point separation and curdling of dairy constituents takes place. To investigate whether separation as a result of souring is merely pH-induced, a 7-day study was conducted where souring of 1:10 milk dilutions took place in neutralized pH conditions, using NaOH solution. Although reduced for advanced stages of souring (i.e., for day 6 and 7 of the experiment), separation persisted despite the neutral pH ranges. Adjusting the ionic strength of an identical system by addition of equal portions of 1 M NaCl in place of 1 M NaOH used for neutralizing pH, slightly enhanced separation, and the extent of separation was comparable to the degree of separation in an identical sample prepared without the addition of NaCl or NaOH.

Further studies of GAD interceptors, as well as emphasis on protein and fat suspension and emulsion will help better determine the fate of dairy constituents. Sampling for this study occurred during the colder months of December and January,

when the sales of frozen dairy beverages and desserts are minimal. Continuing this study in the warmer months would be beneficial to account for variations in sales, potentially leading to different retention times. Seasonal studies should also investigate the effects of temperature on GAD separations, and the affinity of dairy constituents with one another. Characterization of fresh wastewater and sewer samples would be beneficial in mapping the fate of dairy products in wastewater. Based on laboratory and field observations, it is expected that partitioning tendencies of wastewaters rich in milks and ice cream-like products is substantial enough to justify installation of grease traps.

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Table of Contents

List of Tables

List of Figures

List of Diagrams

Glossary

Terminology for Food Service Establishments:

1. **Ice Creamery:** Retail store supplying soft or frozen ice cream, custard, shakes, sundaes, and/or yogurt in one or more serving options (cones, cups, etc.)
2. **Regular Coffee Shops:** Coffee shops that serve only pre-made coffee drinks, without serving any specialty drinks prepared by personnel per customer's request.
3. **Specialty Coffee Shops:** Coffee shops that in addition to serving only coffee, prepare coffee drinks that may use dairy product, which are prepared on site per customer's request.

Terminology for Dairy Systems:

1. **Creaming:** deposition of aggregates of dairy fats to the surface in milks and/or other dairy products.
2. **Curd:** separated settled or floated phase of dairy products comprised of proteins and fats, which form as a result of dissolution of calcium phosphate at ZPC pH (see definition 4).
3. **Inherent Fat:** the commonly used estimation of fat content for dairy product.
4. **Serum**: the suspended or clear solution of dairy products, which contains aqueous dairy constituents. At ZPC pH of caseins, curdling of milk occurs, which results in separation of the casein proteins and fats from the serum. At this pH, the serum appears as a transparent solution distinguishable from the separated curd layer. Serum and suspended phase are interchangeably used throughout this thesis.
5. **Zero Point of Charge pH (ZPC pH):** also known as isoelectric point, describes the acidity level at which the electric charge density of the surface is zero, which should promote agglomeration and separation of materials from a bulk fluid phase.
6. **Curdling:** Separation of dairy constituents, such as proteins and fats, from the serum into another phase.
7. **Curd:** The separated fats, proteins, and other dairy constituents

1. Introduction

The U.S. EPA has found that Sanitary Sewer Overflows (SSOs) caused by poor sewer collection system management pose a substantial health and environmental challenge as they spill raw sewage into basements or out of manholes and onto city streets, playgrounds and into streams, before it can reach a treatment facility (National Pollution Discharge Elimination System). Such overflows represent health risks to communities (as they carry bacteria, viruses, and parasites) as well as having the potential to damage properties, our water resources budget and recreational water supplies. While there are different causes that contribute to SSOs, such as pipe breaks, increased inflow due to infiltration, power failures and an insufficient system capacity, most studies attribute over 47% of the SSOs to pipe blockages (National Pretreatment Program). Many of these blockages are a result of retention of fats, oils and grease (FOG) in the sewer lines. Food service establishments (FSE) have the potential to contribute heavily to sewer outflows. If discharged without installation of a grease abatement device (GAD), their wastewater can contain large quantities of fats, vegetable oils, lards, shortenings, margarine, butter, grease, and other FOG-based products.

Although an average of 47% of the nationwide SSOs is attributed to FOG clogs, the impact of FOG can exceed this percentage on a state by state basis. Moreover, the Wall Street Journal reported that 75% of the sewer systems in the U.S. worked only at half capacity because of FOG clogs (Russell, J.M., 2002). The problem becomes more severe as rainfall adds to the sewer load and water levels exceed the already reduced capacity of the pipelines.

Once discharged, FOG solidifies, resulting in build-up and blockages in sewage pipelines, and reduces the capacity of the sewage collection systems. Consequently,

preventing the discharge of FOG from FSEs by implementing best management practices (BMPs) or installation of GADs will help diminish the number of SSOs attributed to retention of FOG. EPA and other state environmental agencies are seeking solutions and have implemented the practice of regulating FOG discharges. FSEs serving and preparing hot food have been identified as the major contributors to FOG buildups. As generation of FOG imposes a threat to our water supplies, almost all states have come up with FOG control programs, and depending on the state, different food groups and classes of FSEs are defined as sources for FOG. While almost all FOG ordinances clearly define FOG to be a byproduct of cooking, and baking practices, using oils, and grease, the regulations imposed on FSEs serving only dairy products, such as ice creams, smoothies, and specialty coffees are unclear.

2. Objectives

Wastewater originating from dairy-based FSEs is rich in dairy constituents, including dairy fats and proteins. While vegetable oils and animal fats have minimal solubility in water and tend to separate into another phase, making physical treatment of such wastewaters a feasible solution for the removal of FOG, dairy systems are more complex. Wastewater originating from dairy-based FSEs, such as specialty coffee shops and ice creameries, are in essence diluted dairy systems, and are hence diluted protein suspensions and fat emulsions. Although more stable than vegetable oils, dairy suspensions can separate into different phases under certain conditions. Therefore, wastewater discharged from dairy-based FSEs is comprised of dairy FOG, and if separated, may contribute to FOG build-up and blockages in the sewer lines.

Most of the current focus on FOG in wastewater collection systems has been oriented to FSEs preparing and serving food to public with obvious FOG production (frying, cooking using oil, meat processing, etc). However, other wastewater discharging facilities, such as specialty coffee and dairy shops are less obvious. Because of dairy FOG characteristics, the industry claims that physical treatment may be ineffective with their wastewater (Starbucks, 2009). Nevertheless, some evidence exists to the contrary and GADs have been observed to accumulate FOG from dairy wastewater (WSSC, 2010). There is a need to scientifically investigate whether further regulations to control FOG discharges from coffee and dairy shops could limit the amount of dairy fat or other suspended solids entering sanitary sewage systems and thus preventing FOG deposits.

No systematic studies on whether wastewaters rich in dairy products will separate, and if the currently used interceptors or traps are efficient in removing dairy FOG are

currently available. In order to assists policy makers and regulators, there is a need to better understand whether partitioning occurs in dairy wastewater, and quantify its tendency to form separate phases that would either float or precipitate. To date, there is no research available to assess whether current FOG removal devices (GADs) can remove dairy fats and residues, and whether such devices are effective only under certain conditions.

The objectives of this research are (1) to study the separation tendencies of dairy containing wastewater on a scientific basis, and (2) to investigate whether physical treatment of such wastewaters employing GADs will effectively reduce or eliminate the discharge of dairy FOG to collection systems. These objectives were achieved by a combination of laboratory and field observations. In the laboratory, the effects of different environmental conditions, such as pH and temperature, and temporal changes caused by souring on the stability of synthetic and real-time dairy-containing wastewater were investigated. In the field, the feasibility of physical treatment of dairy-containing wastewater was examined by monitoring and sampling GADs treating wastewater from different types of dairy-based FSEs.

3. Literature Review

3.1 Dairy Chemistry and Physics

While it is argued that restaurants and other FSEs that use animal fats are heavily responsible for generation of FOGs in sewer pipelines, there has been little research on the potential FOG from FSEs serving dairy products, such as ice cream shops or coffee shops serving hot and cold beverages. In the following section, the properties of different milk constituents prone to separation, their chemistry, and the processes of partitioning are discussed.

Milk Constituents and Properties

Milk is a complex fluid containing as many as 100,000 different molecular species in different dispersed states. Milk composition depends on many environmental, physiological (age and stage of lactation), and genetic factors. Nevertheless, regardless of its origins, water clearly predominates, giving an average of 89.3% of its composition. Table 1 gives the average composition of milk in lowland breeds. Besides water, milk contents include lactose, fats, proteins, minerals, organic acids, and other miscellaneous compounds (Webb et al., 1975). Table 1 shows that the nonfat solid portion of milk averages 8.8%, and the weight percentage of fat in dry matter has a mean value of 31%. It can thus be clearly seen that fat is one of the principle compounds found in dairy products, averaging 3.9 weight percent.

Fats, consisting of numerous different lipids, also have nutritional value and provide consumers with energy. They include fatty acids, and contain fat soluble vitamins, including vitamins A, D, E, and K. The two most abundant lipid classes in milk include neutral (bearing an overall net charge of zero) glycerides (tri-, di-, and monoglycerides), and

saturated and unsaturated forms of free fatty acids (C_4-C_{10}), each with a distinct water solubility. Although composed of many different lipids, nearly all fats exist in separate small globules, with a size distribution of 0.1 - 15.0 μm. Each globule is surrounded by a thin protective membrane, giving a total area of approximately 80 m^2/L and preventing the globules from coalescence and flocculation (Walstra et al., 1984).

Table 1. Data for Netherlands milk, but typical of lowland breeds. Table reproduced from Dairy Chemistry and Physics (Webb et al., 1975)

Component	Average Content % [1](w/w)	Range % (w/w)	Average % of Dry Matter
Water	87.3	85.5-88.7	
Solids-non-fat	8.8	7.9-10.0	69
Fat in dry matter	31	21-38	
Lactose	4.6	3.8-5.3	36
Fat	3.9	2.4-5.5	31
Protein	3.25	2.3-4.4	26
Casein	2.6	1.7-3.5	20
Mineral substances	0.65	0.53-0.8	5.1
Organic acids	0.18	0.13-0.22	1.4
Miscellaneous	0.14		1.1

Another important class of milk compounds is protein. Milk proteins consist of amino acid residues joined "head to tail" by peptide linkages and can be further grouped into two subdivisions. The protein content of milk averages to 32 g/kg, out of which 80% is categorized as caseins. Caseins are a group of phosphate-containing milk-specific proteins that precipitate upon acidification. They tend to self-associate and form micelles of different sizes. Their stability is dependent on the system's pH and temperature. An interesting characteristic of caseins is their different Ca^{2+} bonding capacities, with a sedimentation

[1] Sum of the principle constituents (i.e., water, lactose, fat, protein, minerals, organic acids, and other miscellaneous compounds) gives a value of 100%.

constant of 7.5 Svedberg[2] units. The remaining proteins that are soluble under acidic conditions are known as whey or milk serum proteins. Thus, it is expected that proteins, and specifically casein micelles, can separate to some extent when heated and under acidic conditions. One should expect that as milk is heated to introduce acidic compounds such as coffee, caseinate might eventually precipitate out. In addition, proteins, particularly caseins are surfactive molecules, they tend to form a layer at the air-water (AW) interface to reduce surface tension, leading to separation of protein species (Walstra, 1984). Other compounds mixed with beverages, such as chocolate and vanilla may also alter the situation. Therefore, their effects must also be considered. Another important aspect is the interaction of phosphate and Ca^{2+}, which forms bridges among the casein sub-micelles and acts as a cementing agent in the micelle structure, tends to be favored at lower pH values (Walstra, 1984).

Considering its high fat content, milk can more or less be described as an oil-in-water emulsion. Like any other emulsion, milk is not entirely stable, and any fat and plasma interactions occur separately in each globule. The structural components of milk are small, so diffusion into and out of milk occurs within a few seconds, resulting in a very rapid partition equilibrium between fat globules and plasma or between casein micelles and serum. Other substances include nonfat solids, fat in dry matter, lactose, and proteins. Another characteristic of milk is its tendency to uptake apolar substances quite easily because of their relatively high solubility in the fat globules (Walstra, 1984).

In short, milk is a very complex system consisting of diverse components, which behave differently under different conditions. When mixed with other surfactive material,

[2] Svedberg unit is a measure of time and is defined as 10^{-13} sec. It is a non-SI unit used in determining the rate of sedimentation of a macromolecule, notably in centrifugation.

such as detergents, grains such as coffee, or chocolate, and other flavors used in ice creams, their chemistry and physical properties change.

Effect of pH and Temperature on Dairy Wastewater Properties

When it comes to analyzing the tendency of a dairy wastewater to separate into different phases, pH is an important factor. A typical wastewater from a coffee shop may contain milk products, as well as coffee and detergents. While at typical milk pH of 6.6-6.8, most proteins are fully homogenized with the water; most caseins are insoluble at pH of 4.6 (Walstra, 1984). This pH might be approached since coffee is acidic. As pH decreases, calcium phosphate present in the casein micelles dissolves, and enlargement of these micelles occurs due to a decrease in their surface potential. Such enlargement results in precipitation of the micelles. The zero point of charge of the caseins is at pH = 4.6; at this pH it loses its net charge and forms internal salt bridges (Walstra, 1984). The high hydrophobicity of the casein then makes it insoluble. Although as already mentioned, milk pH tends to fall anywhere between 6.6 to 6.8, it is important to understand the influence of acidic conditions in FOG formation in coffee shop drains since coffee is relatively acidic and lowers the acidity level of the system to values below the ZPC pH, which may result in formation of long insoluble protein complexes.

Temperature is another variable that affects milk in many different ways. As already discussed, milk is a very complex system, and changing the temperature results in a wide range of both reversible and irreversible changes. Heating milk is an important preparatory step in making hot beverages, and here a description of some of the few changes accomplished by heating that are relevant to the purpose of this study are presented. Some of

the reversible processes include cold agglutination of fat globules, state of crystallization of fat globules and state of association of caseins.

One prominent irreversible reaction is the transfer of calcium and phosphate from solution to the colloidal state. This process has a very long relaxation time and affects casein micelles properties. Another change caused by heating is an increase in the titratable acidity of milk and reduction of pH. As indicated above, reduction of pH causes further entanglement of casein micelles and reduces their solubility. For temperatures above 60^0C, the solubility of whey proteins decreases due to heat denaturation, and they become largely associated with casein micelles. Moreover, immunoglobulin, a species present in milk which causes gelation and cold agglutination of fat globules becomes denatured and inactive during heating, thus reducing the possibility of formation of clusters of fat. Therefore, although this process can cause intensive separation of milk fat from plasma, it is not considered in this study as milk used in industry is homogenized. Deactivation of immunoglobulin is not the only change occurring in homogenizing, and denaturation of fat globule membranes takes place as well (Walstra, 1984).

Variations in temperature also affect density. Density is an important property that helps determine the separation tendencies of milk constituents. If given enough time, substances of higher and lower densities separate as sediments or flocs. The density of liquid milk fat at 20^0C is about 915 kg/m^3. For proteins, this value is 1400 and for lactose it is 1780 kg/m^3. Therefore, the density of milk is averaged to be about 1030 kg/m^3. The diverse densities of composing substances in the mixture can cause floatation of fat content and sedimentation of proteins given sufficient retention time. The density of a suspension of several compounds is a function of many parameters such as temperature, water, and fat content, and the density of skim milk, milk, and cream can be obtained using equation 1. For

density of dairy products concentrated by removing water, Equation 2 can be used (Walstra, 1984).

$$\rho^{20} = \frac{1000}{0.123\, m_F + 0.9665} \quad (1)$$

$$\frac{1}{\rho_c} = \frac{R}{\rho_o} + \frac{1-R}{\rho_w} \quad (2)$$

In Equations 1 and 2, ρ is the density in kg/m^3, m_F is the fat content, and subscripts o, c, and w denote initial milk, concentrated milk, and water respectively. R is the concentration factor relating the ratio of dry matter content in the concentrated milk to that in the dilute milk (Walstra, 1984). In the Fundamentals of Dairy Chemistry, density of milk is defined as a function of temperature for two temperature ranges of 0 to 10^{0}C and 10 to 40^{0}C. For the first range:

$$Density = 1.003073 - 0.000179\, T - 0.000368\, F + 0.003744\, N \quad (3)$$

,where density is in kg/m^3, T = temperature in ^{0}C, F and N refer to percent fat and percent nonfat solids respectively (Webb, 1975). A linear relationship for the second temperature range is determined using equation 4. The coefficients appear in Table 2.

$$D - 1 = a - bT + cT^2 - dT^3 \quad (4)$$

Table 2. Coefficients for determining density of different temperatures. Table reproduced from Webb et al., 1975 (Table 8.3).

	Whole Milk	Skim Milk	Average difference/ 1% fat
a	350	366	4.8
b	3.59	1.46	0.39
c	0.09	0.023	0.0061
d	0.11	0.0016	0.00002

Using the densities of pure and tap water, a graphical representation of milk density is produced for better illustration and appears as Figures 1 - 3. A sharp spike appears in Figure

1 at 10^0C. This is due to the fact that two different correlations are obtained for 1-10^0C and 10-40^0C. A very gradual decrease in the lower range is seen, followed by a more dramatic drop in the densities of dairy products. Skim and 1% milk show very similar results, while whole milk shows lower values, which follows from considering its higher fat content. It is clearly seen that an increase in temperature reduces density. Considering the temperatures of the dairy products used in hot beverages or in cold deserts, the immediate response of the components of the system can be seen. Moreover, for the entire temperature profile, the density of milk is greater than that of water and therefore milk should settle in the solution. This is not the case, however, since the proteins with highest densities settle and the lighter fat globules float if the two systems were to mix.

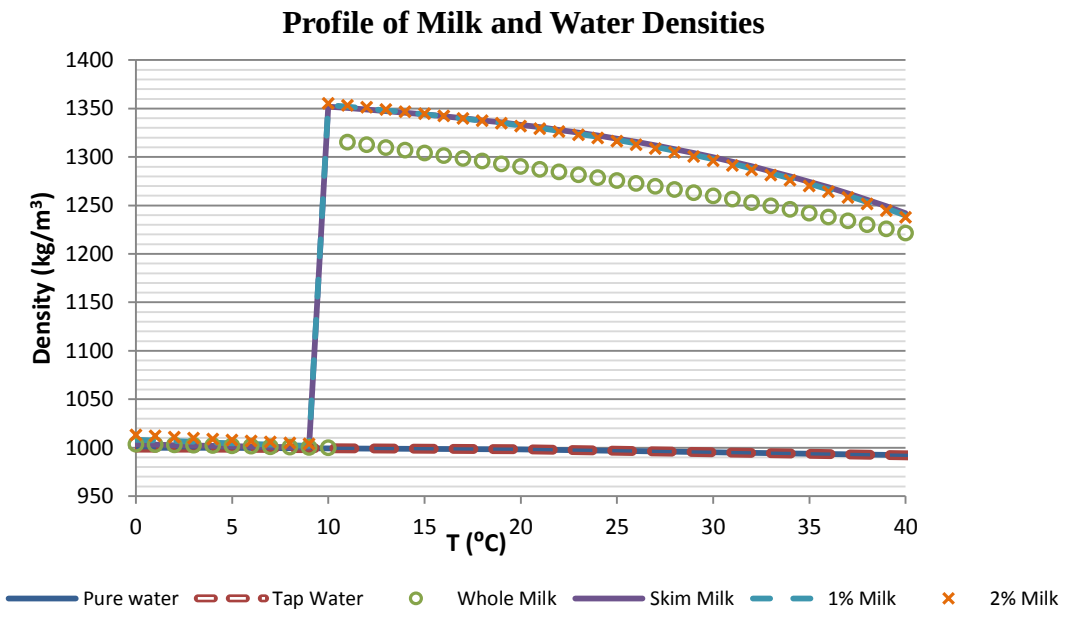


Figure 1. Densities of whole milk, skim milk, 1 and 2% milk compared to water. As can be seen, there is a very small difference between the densities of 1 and 2% and Skim milk, which tend to intersect at most points, calculated from equations 3 and 4, and Table 2 (Webb et al., 1974)

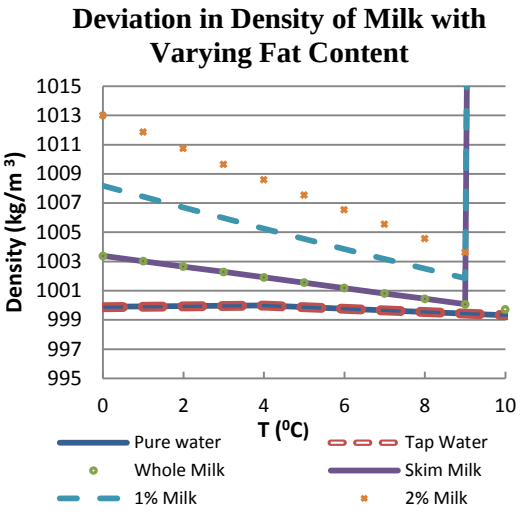


Figure 2. Densities of whole, skim, 1 and 2% milk compared to water at lower Temperature Range (0-10^{0}C).

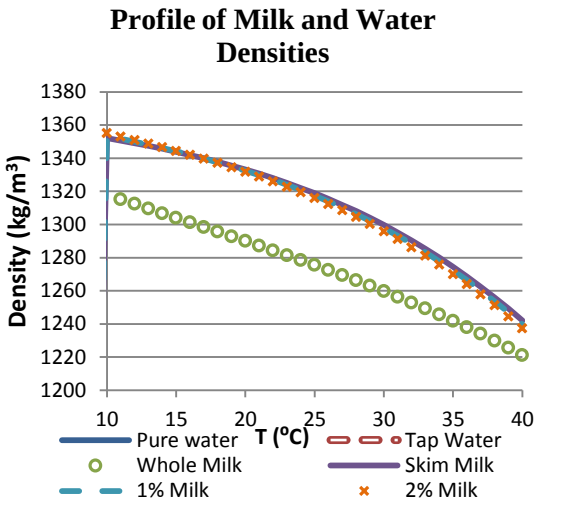


Figure 3. Densities of Whole, skim, 1 and 2% milk compared at higher temperature range (10-40)^{0}C.

For clarity, a focused graphical representation of each temperature range appears in Figures 2 and 3. It can be seen that as the fat content of milk increases, its density reduces. Whole milk has the lowest density, while based on calculations reduced fat milk is the more dense form. As can be seen from Figure 2, the density of 2% milk is highest and the same trend continues for most of the higher temperature range (10-40^{0}C). Whole milk has the overall lowest density. Deviations are higher for lower temperatures and results are counterintuitive as it is expected that skim milk should have the higher density. Therefore, it can be seen that increasing fat content reduces the density of milks. . It is also interesting to see that deviations in densities of skim, 1 and 2% milk are minimal for higher temperature ranges

Dairy Partitioning

Because of the diversity found in its building blocks, milk can undergo physical changes quite frequently. One example is creaming of fat globules and crystallization that occur upon cooling. Creaming is a rapid phenomenon in which fat globules aggregate to

loose, large flocs at low temperatures and form a new structural element. It occurs as a result of the difference in density between fat globules (920 kg/m^3 at room temperature) and milk plasma (1030 kg/m^3). To consider the creaming of a single globule, Stoke's velocity can be calculated, which appears in Equation 5, where α is the acceleration defining the field (gravity or centrifugation), subscripts *p* and *f* denote density of milk plasma and fat globules, (1030 kg/m^3 and 920 kg/m^3 respectively), *d* is the particle diameter, and finally η_p is the viscosity of milk plasma, which is not necessarily that of milk (Walstra, 1984).

$$\upsilon_s = \frac{-a(\rho_p - \rho_f)d^2}{18\eta_p} \tag{5}$$

For Equation 5 to hold, several conditions must be fulfilled. For one, the concentration of fat globules must be very low (Volume fraction of fats to total volume of plasma < 10^{-3}), which is rarely the case in milk, but for a diluted milk solution as found in dairy wastewaters, the concentrations may be lowered to these amounts. Another aspect is the size of the fat globules. For colloidal particles with d <1 μm, Brownian motion disturbs the creaming process. Finally, the globules must be smooth spheres, which is not the case for most homogenized fat globules (Walstra, 1984). Having stated these conditions, the Stoke's equation can be used as a useful reference, but since the globules exhibit some variability in size, Equation 6 can be used to find the proportion q of the fat reaching the cream layer per unit time where N is the number of particles/unit area, d is the diameter of fat globules and ν is their individual globule velocity, and D is the depth of the layer of milk (Walstra, 1984).

$$q = \frac{\sum N_i d_i^3 v_i}{D\sum N_i d_i^3} \tag{6}$$

It is also important to review the colloidal stability of milk in some detail prior to covering other dairy products. By now, it is clear that milk consists of both hydrophobic and hydrophilic colloidal particles and the following characteristics apply to them. First, they are subject to Oswald ripening, where large particles tend to grow while smaller particles start to disappear. Dairy colloidal particles tend to settle or cream unless Brownian motion prevails (i.e., d <1 μm). Moreover, if they are fluid, as are fat globules at moderate temperatures, they coalesce with each other if the thin film of continuous phase between the closely approaching globules is ruptured somehow. Homogenization of milk enhances this phenomenon as during the process the membrane covering fat globules is damaged (Walstra, 1984).

It is also possible for substances dissolved in one or both phases to accumulate or adsorb onto the interface. If both phases are fluids, the interface has no specific adsorption site and adsorption to the interface continues until it is packed with a monomolecular layer. In general, solute is adsorbed if it lowers the interfacial tension. This is expressed in Gibb's equation, Equation 7, where γ is the surface tension (N/m), Γ is the surface excess or adsorbed amount in (mol/unit area), and c is the concentration[3] of the solute in the bulk fluid, and finally R and T correspond to the ideal gas constant and temperature. Equation 7 states that for a solute that lowers the surface tension, the surface excess increases, resulting in a decrease in surface tension. If this principle is applied to milk, milk constituents are likely to absorb at air-water (AW) and oil-water (OW) interfaces in order to reduce surface tension. Therefore, proteins, consisting of hydrophobic and hydrophilic parts, can readily be adsorbed onto OW and AW interfaces.

$$d\gamma = -RT\Gamma d\ln c \tag{7}$$

[3] For simplicity, concentration is used in equation 7 in place of the activity of the solute in solution.

Another important parameter is the contact angle θ, which depends on the adsorption of surfactants as dictated by interfacial tensions (Equation 8):

$$\cos\theta = \frac{\gamma_{AS} - \gamma_{WS}}{\gamma_{AW}} \quad (8)$$

, where γ_{AS} (N/m) is the interfacial tension between surfactant molecules and air, γ_{WS} is the water-surfactant interfacial tension, and γ_{AW} is the air-water interfacial tension. This principle also determines whether spreading occurs in a sample. For example, triglycerides do not spread on water, but natural fats spread because small quantities of surfactants in milk (proteins) lower γ_{OW} (oil-water interfacial tension). Milk fat does not spread on plasma (skim milk) surfaces because the adsorbing proteins lower γ_{AW} to such a value that $(\gamma_{AO} + \gamma_{OW}) > \gamma_{AW}$. Reaching of surface and forming a film by dairy fat is retarded since proteins lower the surface tension to a greater extent and take precedence over fats as a general rule. The protein layer on top would then repel the fat globules, keeping them suspended in the liquid phase.

Additionally, the introduction of air bubbles to a dairy system also changes milk chemical and physical characteristics. This process takes place frequently in many specialty coffee and dairy FSEs while preparing hot or frozen drinks, such as cappuccinos, smoothies, ice creams and whipped cream. If air is beat into a liquid containing surfactant, such as milk, a foam layer forms. Despite their difference in interfacial tension, this foam has much in common with an oil-in-water emulsion as they are both dispersions of apolar fluid phases in a polar liquid. To explain foaming of milk products, it is noted again that milk proteins, such as caseinates, contain both hydrophobic and hydrophilic parts. Therefore, they adsorb strongly to the air-water interfaces and stabilize the foam. As air start dissolving, bubbles shrink. During this time, the micellelacious part stays at the interface and other adsorbed substances presumably desorb. Further shrinkage of air bubbles causes the micelles to touch

each other and form a layer. Finally with the full dissolution of air, an empty folded sac consisting of a membrane of casein micelles remains. This phenomenon is particularly common in skim milk that has been foaming at temperatures above 20^0C, which is the case in most hot beverages containing foamed milk (Walstra, 1984).

Association of fat globules with air bubbles is somewhat different. It is argued that fat globules only occasionally make contact with the air water interface and only when they are caught in a foam lamella. The membrane material spreads over the bubble, which ruptures the film. Liquid fat de-stabilizes the foam to a smaller extent than solid fat globules. This occurs because the globule and the adsorption layer of the AW interface repel each other. When the lamella becomes thinner than the globule diameter, deformation of globules takes place, which is much more easily done in liquid fat as opposed to solid fats because solids show a higher resistance to flattening (Walstra, 1984). For association of air bubbles in high-fat dairy products such as cream, it is believed that as soon as bubbles are formed and before proteins can adsorb on the surface, fat globules become attached to the surface and are held there by surface forces. The foam layer on top of the cream is enriched in fat. In practical situations beating often goes on in such a way that foam is not formed. In whipped cream, the fat globules need to be fully attached to air bubbles in order to have stable foam. The entrapped air makes the fluid very light with a much lower density (Walstra, 1984).

Finally another milk product that is used to make hot beverages is powdered milk. Although powdered milk can be made via several processes, it is most commonly produced by spraying small droplets of milk (or concentrated milk) into hot air (Walstra, 1984). Dry powder particles are then made within a second. The water content is found to be 3% or 4% for skim milk. The spray drying produces roughly spherical particles with a size distribution of 5-100 μm and air bubbles become embedded in the particles, which tend to expand during

drying. Particle aggregates are comprised of several individual spheres. Milk powder particles consist of continuous masses of amorphous lactose, fat globules, casein micelles, and whey proteins. The fat globules in milk powder are changed by the evaporation and spraying process and considerable splitting of fat globules occurs. As far as proteins are concerned, drying is so rapid that denaturing of proteins is negligible, whey proteins remain soluble, and most enzymes active. A small fraction of powder may remain insoluble as small proteinaceous lumps may remain after dissolving the powder in water. Moreover, creaming is also possible in the form of flecks of fat globules held together by gelled proteins. Another important consideration is the age of milk powder. If dried milk is held for several months in relatively moist conditions (mole fraction of water = 0.5) or at high temperatures, a substantial portion of the protein becomes insoluble. Since proteins help stabilize the dairy emulsion, their insolubility in water can result in higher partitioning tendencies. Therefore proper storage is vital (Walstra, 1984).

Ice cream is another dairy product that should be considered. Ice cream typically contains 10% milk fat, 11% milk solids-not-fat, and 14% sugar. It is made by beating air into cream while rapidly cooling the mixture to -4 to -6^{0}C. The structural elements here are ice crystals, air cells, fat globules, fat granules, and often lactose crystals. Although ice creams and creams contain high concentrations of fat globules, their susceptibility to coalescence are low because of homogenization, and only partial coalescence of fat globules are observed in ice cream. Additionally, emulsifiers are added to ice cream to destabilize the fat globules so that they can form granules during beating (Marshall et al., 2003).

Temporal Changes in Milks

The changes in milk characteristics are not exclusive to the influence of environmental conditions, such as pH and temperature. Dairy systems hosts a wide range of native biological and enzymatic activity, and because of their high nutritional values, they are a good substrate for almost all types of microorganisms. Many of these changes are inevitable with passing of time and can cause instability in the emulsion, and ultimately cause separation in milk systems. In this section, a brief overview of some of these changes is presented.

Similar to other foods rich in sugar, milks can also undergo fermentation processes, in which lactose is metabolized by different microorganisms to compounds of smaller molecular weight. In dairy systems, the most important fermentation process results in production of lactic acid, although propionic and butyric fermentations can also take place. Depending on the nature of the bacteria, fermentation can take different pathways, leading to a range of products from lactic acid, or a combination of lactic acid, acetic acid, alcohols, CO_2 as well as other intermediates. The homofermentative bacteria phosphorylate[4] the lactose, and hydrolyze the lactose-P, and conversion of these species to glucose and other smaller molecular weight sugars takes place. The glucose is ultimately metabolized to pyruvate. The bacterial group *N streptococci* all have lactic dehydrogenase, and produce four molecules of lactic acid from a molecule of lactose (Walstra et al., 1984).

Other homofermentative bacteria, such as *S. thermophiles* and *Lactobacillus Bulgaricus* do not cause phosphorylation. Here the hydrolysis of lactose occurs, producing 2 molecules of lactic acid from a molecule of lactose. Bacteria of genus *Propionibacterium,* ferment lactic acid to propionic acid, acetic acid, CO_2, and water (Reaction 1).

[4] The addition or take up of a phosphate group to proteins or other organic compounds (Merriam-Webster.com)

$$3CH_3\text{-}CHOH\text{-}CO_2 \rightarrow 2CH_3\text{-}CH_2\text{-}CO_2H + CH_3\text{-}CO_2H + CO_2 + H_2O \quad \text{(Reaction 1)}$$

Other fermentations, such as formation of butyric acid from lactic acid (Reaction 2), caused by *Clostridium* bacteria, or the production of diacetyl (Reaction 3) from pyruvic acid by *Streptococcus thermophiles* and *Lactobacillus Bulgaricus* is also possible. In fresh milk, the insoluble calcium forms complexes with phosphoric acid ($CaHPO_4$), and with casein (Ca_4Caseinate) (Slyke et al., 1916). With the formation of lactic acid, $CaHPO_4$ converts into a mono-calcium phosphate ($Ca(H_2PO_4)_2$), and the calcium available in the calcium caseinate complexes at first reduces and finally turns into uncombined caseins (Walstra et al., 1984).

$$2\ CH_3\text{-}CHOH\text{-}CO_2H \rightarrow CH_3\text{-}CH_2\text{-}CH_2\text{-}CO_2H + 2\ CO_2 + 2\ H_2 \quad \text{(Reaction 2)}$$

$$2\ CH_3\text{-}CO\text{-}CO_2H + O \rightarrow CH_3\text{-}CO\text{-}CO\text{-}CH_3 + 2\ CO_2 + 2\ H_2O \quad \text{(Reaction 3)}$$

With the reduction of pH with formation of lactic and butyric acids, the dissolution of calcium phosphate occurs, which in turn results in the decrease in association of cations with proteins since at this time the negative charge of the proteins diminishes. The ionic strength, and the Ca^{2+} activity increases, and several lactic acid bacteria can break down citrate, which may also increase the Ca^{2+} activity. Therefore, the changes caused by lactic fermentation are not quite similar to those caused by addition of other acids, such as HCl (Walstra et al., 1984). It is speculated that due to the diverse changes caused during enzymatic and fermentation reactions, the stability of dairy emulsions will differ significantly from acidified fresh milk.

Another phenomenon taking place in dairy system is lipolysis. The presence of lipoprotein lipase enzymes in milks serves this purpose, which includes degrading triglycerides in milk into free fatty acids in a process known as lipolysis, which gives milk a rancid, soapy flavor. Most milks contain approximately 1-2 mg of this enzyme per liter, and at a pH of 8.5, temperature of 37^oC, the presence of divalent cations, and agitation can

stimulate lipase action. Although when active at optimal conditions, the enzyme can produce more than 3000 molecules of fatty acid per second, its activity is inhibited partly by the pH range of milk (i.e., 6.6-6.8), and partly because the enzyme is bound to casein micelles, which reduces the concentration of the free enzyme. Another inhibitor of this process is the fat globule membrane, which protects the triglycerides against the enzyme, and because of the low interfacial tension of the globules, the enzyme cannot penetrate the membrane. Lipase is known to be large deactivated by heating; however, heat-resistant microbial or somatic cell associated lipase in pasteurized milk can also cause lipolysis (Santos et al., 2003)

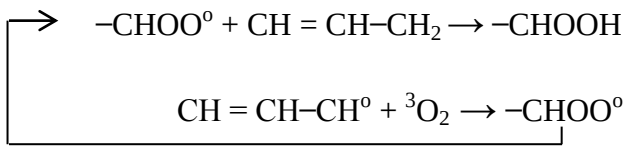
Similar to fats, proteins also undergo proteolysis, or the degradation of proteins, which can take place in milk at temperatures as low as 4^{o}C (Santos et al., 2003), and is often stimulated by the presence of different protease groups. The principle proteolytic enzyme is found to be the alkaline proteinase plasmin, which can exist in both active and inactive forms (plasminogen). The active plasmin enzymes hydrolyze casein micelles, while their inhibited counterpart can also become fully functional by a class of enzymes known as plasminogen activators (Enright et al., 1999).

Pasteurization or heat treatment of milks results in higher proteolytic activity, most likely attributed to inactivation of inhibitors of plasminogen activators (Enright et al., 1999). Both as a membrane, sheltering the fat globules, or as casein micelles, proteins play an important role in the stability of dairy emulsions, and their deterioration can destabilize dairy systems and induce separation (Kaylegian et al., 2007). Therefore, it is important to link the degree of proteolysis of milk with separation or instability in milk systems.

Finally, oxidation of unsaturated fatty acids can also take place in milk, which can be summarized into three steps. First is the direct oxidation of CH groups by O_2 via the following reaction:

$$O_2 + -CH = CH - CH_2 \rightarrow -CHOOH - CH = CH - \qquad \text{Reaction 4}$$

Once the oxidation is initiated, a chain reaction sets in, involving ROO◦, demonstrated in Reaction 5.



Reaction 5

Lipids in many dairy products are subject to autoxidation, in which Cu acts as a catalyst. During this process the triglycerides are also attacked, and in high concentrations of Cu, oxidation of triglycerides takes place even in the absence of phospholipids (Walstra et al., 1984).

While Cu is naturally found within milk's structure, external Cu entering the system plays a more important role in facilitating this process and is active as a catalyst for concentrations between 20 and 500 μg/kg. In aqueous systems, Cu is active as an oxidative catalyst against the triglycerides if proteins and phospholipids are present. Other metal catalysts include Fe, although inactive in the presence of proteins. Another parameter that can induce oxidation of fats is light, particularly in the short wavelength range. The susceptibility of milks to autoxidation, particularly by light, is significantly reduced by homogenization.

3.2 FOG and Dairy-Based Food Service Establishments

Wastewater Characterization and Grease Abatement Devices

The most important parameters that should control the fate of FOG in sewer systems are wastewater composition, temperature and pH. Chemical properties of FOG components and the livelihood of microorganisms found in wastewater are both strongly influenced by pH. The pH of typical wastewater tends to fall around 6.5 (Gross, 2004). Temperature can also influence biochemical reactions and many physical treatment processes. Wastewater discharged from FSEs typically contains high concentrations of FOG. FOG is removed from these drains via plumbing the kitchen drains through GADs. These devices hold the wastewater long enough to allow the fats to float. Congealed FOG tends to be lighter than water, and the grease needs to cool enough to congeal and separate from the water carrying the grease away from the kitchen. Therefore, the temperature of water entering the grease trap as well as the temperature of the actual device is of importance. A long retention time may be required to allow for sufficient separation of grease. Since most FSEs tend to use very hot temperatures to clean their dishes, the wastewater entering the GAD tends to be too hot, resulting in insufficient retention times for partitioning to occur (Scherfius, 2010). Overall, the temperature, time and emulsion properties of the FOG in the wastewater affect the efficiency of the interceptor.

Dish detergents are also important constituents that need to be considered as they have surfactive properties and can keep the FOG suspended or emulsified in the waste stream, and can ultimately allow the grease to escape and cause further problems. Typical outflow of a restaurant ranges between 2-4 gallons/unit area/day; thus it is essential to

understand the characteristics and load of wastewater prior to beginning of study (Gross, 2004).

State of Regulations on FOG Discharge

In the guidelines instituted to reduce FOG discharge, several states list dairy products as a source for FOG, one of which is the Florida Department of Environmental Protection. Promoting policies to drastically reduce FOG discharge to the sewers, Florida is specific in identifying FOG sources and lists them as cooking meats, mayonnaise and salad dressings, butter, ice cream and other dairy products, creams and sauces. All FSEs, including ice cream shops, and coffee shops, discharging FOG from the listed sources are required to abide with applicable state, local or federal rules and regulations, which includes installation of properly sized and operational GADs (FOG BMP, FL). Another state that includes dairy-based FSEs is North Carolina (Fats, Oils, Grease and Wax Control Program). The city of Salisbury, NC enforces the ordinance and installation of interceptors in all FSEs as well, so any facility preparing food or serving prepared food, including ice cream shops and other dairy shops, are obligated to use GADs (FOG Regulations, Salisbury). Similarly, the town of Louisburg, NC lists dairy products as a contributor to FOG, and requires all commercial kitchens to install grease traps. This city also prescribes residential practices to diminish the discharge of FOG in the sewer (FOG Regulations, Louisburg, NC). The Hampton Roads Planning District Commission, VA also implements a "fat free drain program" in which dairy products are listed as FOG contributors, and requires all restaurants and other FSEs to install grease traps (FatFreeDrains.com). Another city where dairy products are recognized as a FOG source is Indianapolis where installation of grease interceptors by all restaurants and other FSEs are called for. In Columbus, Ohio all new or remodeled FSEs seeking a plumbing permit have

been required to install outside interceptors since 2005. The City of Los Angeles followed somewhat confusing guidelines as it does not define a specific definition for FOG discharge. They have successfully minimized their SSOs and require the ordinance to be enforced in all FSEs that have the potential to generate waste FOG unless a conditional waiver is granted. While there was no mention of coffee shops or dairy shops as being included in their FOG regulations in June 2010, in their most recent document, dairy products are included as a source for FOG, and all FSEs as well as big apartment buildings to install grease traps (Protecting your sewer systems from FOG, lacounty.gov).

Many other environmental departments and municipalities, however, do not list dairy products as a source for FOG; one of which is Connecticut. In the FOG ordinance instituted by the Department of Environmental Protection in Connecticut, establishments producing hot/cold dairy products have negligible influence on the sewerage intake of FOGs and they do not enforce regulations on potential FOG originating from dairy sources (FOG guidance, CT). The city of San Francisco also arrived at a similar conclusion, and does not require The city of San Francisco is another site in the state of California which does not identify coffee shops or any other FSE serving pre-packaged food as a threat to sewerage and imposes no regulations on them for grease abatement actions Their FOG Control Ordinance includes limiting total oil and grease discharge. FSEs are defined as facilities engaged in preparation of food for consumption by public such as restaurants, commercial kitchens, caterers, schools, hotels, etc. Although this city does not list coffee shops and ice cream shops as contributors to FOG discharge, they define FOG as organic polar compounds derived from vegetable, plant, or animal sources composed of long chain triglycerides, which is the major form of dairy fat and perhaps leaving dairy shop FOG discharge open to negotiation. It seems surprising that with their FOG definition, they should leave dairy shops out of FOG

pretreatment program (San Francisco FOG Ordinance). Orange County also follows similar guidelines, and physical treatment of wastewater originating from small FSEs, which do not engage in cooking/basking are not required.

Finally other states follow somewhat confusing guidelines. For example, the New York City Department of Environmental Protection requires that grease interceptors be installed in all waste lines receiving grease, including those originating from pot wash sinks, food scrap sinks, scullery sinks, meat, poultry, and fish preparation sinks, floor drains, automatic dishwashers, and other plumbing fixtures in all restaurants, cafeterias, clubs, butcher shops, slaughterhouses, fish markets, supermarket food processing areas, delicatessens, and other non-residential establishments where grease may be introduced into the drainage system. The state provides guidelines to FSEs regarding proper installation and adequate sizing, and maintenance of interceptors as their best management practices (BMPs). There is no mention of coffee shops, ice cream shops, or other institutes introducing FOGs to the pipelines via dairy fats, but as stated above, the FSEs include all non-residential facilities that introduce grease into the drains. Therefore, this code may include dairy shops as part of FSE, yet no records, or documents directly citing coffee or dairy shops were found (Preventing Grease Discharge). The state of Texas Commission on Environmental Quality states that fats, oils, and grease come from meat fats in food scraps, cooking oil, shortening, lard, butter and margarine, gravy, and food products such as mayonnaise, salad dressings, and sour cream" (keeping Fats, Oils, and Grease out of Our Sewers, State of Texas). Since the state mentions sour cream as a source for FOG generation, other dairy products such as creams and ice creams with a typical fat content of approximately 10% may be considered as a source for them. This state does not impose ordinance on FSEs but prescribes best management practices for both commercial and household kitchens.

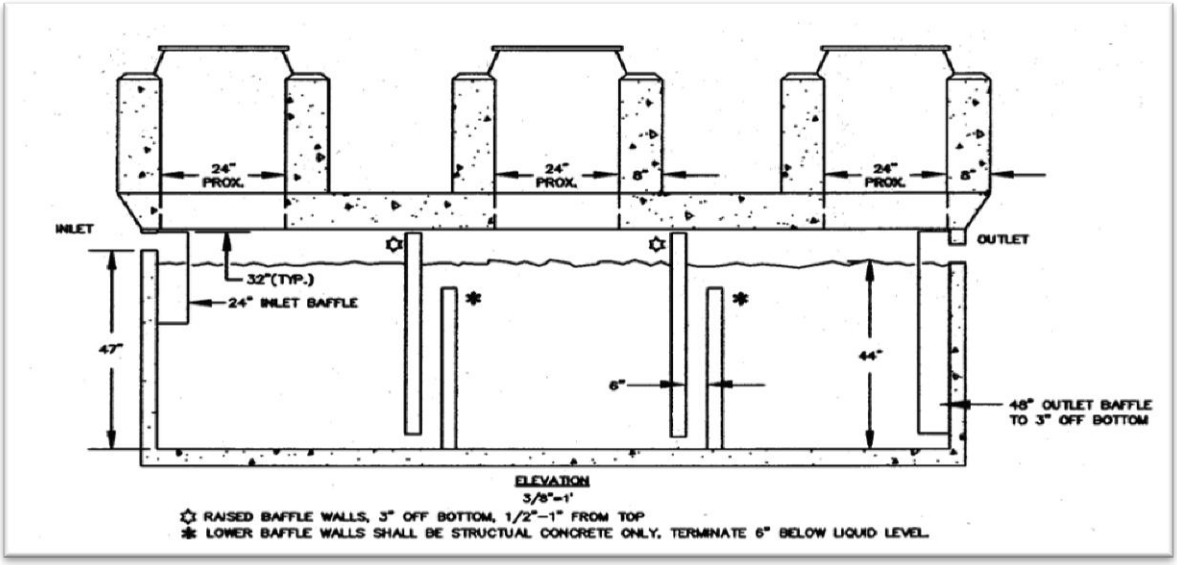
In summary, it is seen that while a number of states do regulate FSEs, their definition of FOG may be inconsistent with one another, and coffee and ice cream shops are not always included in their FOG control programs. For example, the states of New York and Connecticut, the city of San Francisco, and Orange County do not categorize coffee shops and ice cream shops as obligated facilities in their FOG control program. On the other hand, Florida is very rigid and counts such establishments as sources of FOG and enforces regulations, and L.A. County, a few cities in North Carolina, and Colorado include dairy products as a source of FOG.

Grease Interceptors

FSEs can install GADs to accumulate FOG from their wastewater and prevent it from reaching the collection systems. Physical treatment of FOG-containing wastewater includes retarding the flow in order to allow FOG and other suspended material to separate. This is most commonly done through installation of grease interceptors (installed outside the facility), or grease traps (installed inside the FSE). The grease interceptors/traps are commonly passive and are comprised of 2-3 compartments. The GADs retard the flow of wastewater enough to allow partitioning of suspended solids, and FOG from the wastewater. Suspended material will then separate (settle or float) based on their density. Because of the lower density of fats and grease, FOG tends to rise in the system and float on the surface of wastewater in each GAD compartment. The design of the grease interceptors used in this study appears in Figure 4. In Figure 4a, the overall design of the interceptors are shown while in 4b the location of the opening to each chamber is displayed. As can be seen, the baffles are placed such that the fluid portion of the wastewater would pass to the consecutive

compartments. GADs are flow-based systems, and consequently the retention time of an interceptor (t_R=V/Q) varies with the flow.

(a)



(b)

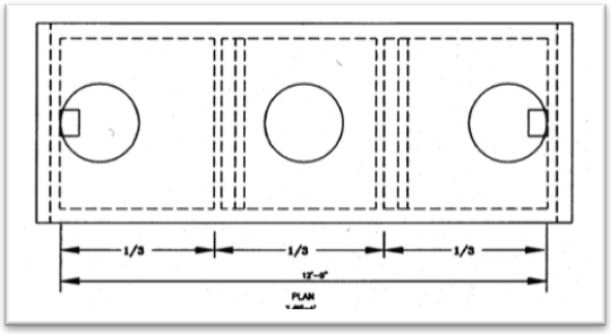


Figure 4. (a) General Design of a 1600 gal Grease Interceptor, WSSC Grease and Sand/Oil Interceptor Detail 1998, (b) Design of Manholes for Grease and Oil/Sand Interceptor, Inlet (left) to Outlet (right), WSSC Grease Interceptor Design, '98

Proper upkeep, design and maintenance of GADs can prevent FOG from reaching the sewage. This factor is particularly important as failure to clean the interceptor will result in excessive accumulation of the separated materials, leading to their discharge into the collection system.

4. Methods and Materials

4.1 Initial Field Studies

A visit to the University of Maryland Stamp Student Union Coffee Bar was done on Wednesday, Aug 4th, 2010. This visit was mainly aimed to understand the practices of a typical specialty coffee shop and to estimate the composition of coffee shop wastewater for future reconstruction of synthetic samples. Most of the mixing for preparation of hot dairy beverages such as cappuccino, and café mocha were done inside the consuming cup. The Frappuccino drinks on the other hand were made inside a blender consisting of a requested number of espresso shots, a shot of the requested flavor, milk with specific fat content, and ice. This drink with or without ice is the most popular drink during the academic year and accounts for approximately half of their entire sales during regular semester hours. The coffee shop used whole and skim milk although 2% milks are also offered, which were made by mixing equal portions of whole and skim. Table 3 reports the average consumption of whole and skim milk, on a per day basis, obtained after monitoring the daily consumption of milk in the establishment, and talking to the employees. As can be seen, whole milk has a higher demand than skim. Approximately 80% of the entire milk was heated for use while only 20% is consumed chilled. Whipped cream sprays were used in the drinks. Thus it seems that none of the whipped cream would enter the sewerage system.

Table 3. Average Daily Consumption of Milk in Student Union Coffee Bar

Milk	Summer (L/day)	Semester (L/day)
Skim	28	57
Whole	38	76

Due to health regulations, coffee pots holding regular and decaffeinated coffee were

cleaned and replaced with freshly brewed coffee every two hours. The samovars are cylindrical in shape, with a diameter of 8.5" (22 cm) and a height of 17" (43 cm). The volume of regular coffee made each day was about twice that of decaffeinated coffee, which is filled only to half capacity. Therefore approximately 0.95 and 0.47 L of regular and decaffeinated coffee enter the drain every 2 hours. If the Coffee Bar only provided coffee in this manner, it would be classified as a regular coffee shop. However, because of the dairy additives and options available, the Coffee Bar is a "specialty" coffee shop. Ice cream sale in this location seems to be minimal.

This specialty coffee shop used four sinks, each with dimensions of 44.5cm x 26.cm x 23cm (17.5"x10.5"x9"). Three of the sinks are used for cleaning practices, as prescribed by the health department, which means two of the sinks hold water for first dipping and sterilizing. The rinsing sink, on the other hand is used only as needed. There is an additional rinsing sink; its use was omitted during sampling to assist in obtaining samples for this facility.

During regular semester hours, the sinks are drained an average of four times per day while during the summer this number reduces to twice a day. It seems that the only source of milk entering the drain in this specialty coffee shop is from cleaning the milk pots and blenders. From this visit, the portions used to prepare synthetic wastewaters were selected. Furthermore, after observing daily practices of the Coffee Bar, it was important to conduct tests to determine the effects of sterilizers and detergents.

4.2 Laboratory Studies

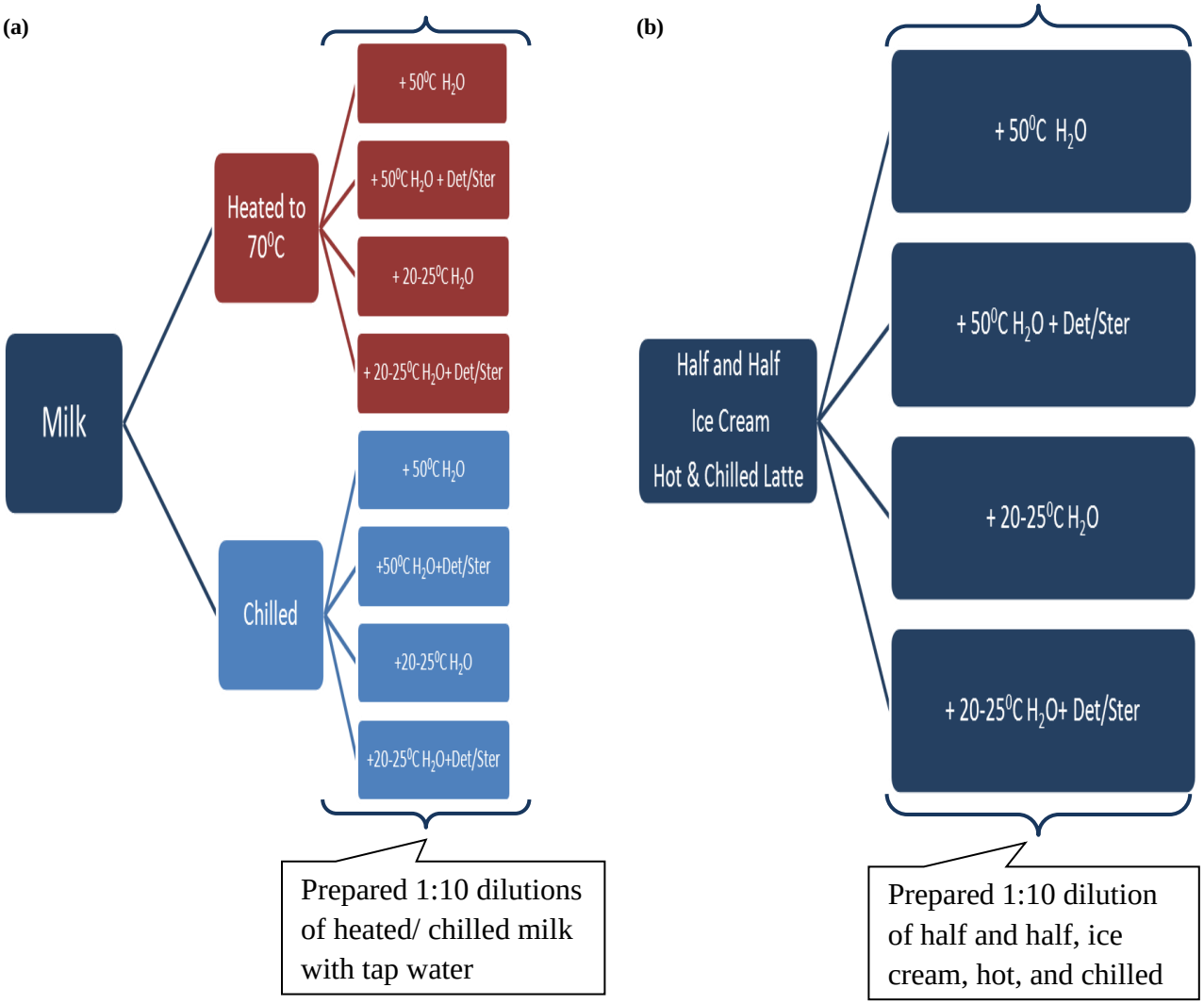
Partitioning Behavior of Dairy

To study the behavior of dairy-containing wastewater, 1:10 dilutions of wastewater were prepared, and for each batch pH effects were studied. As can be seen in Diagram 1a, both chilled and heated Cloverland Farm's milk samples were used for this analysis while Diagram 1b illustrates

the procedure used for half and half, ice cream, and hot and chilled lattes, obtained from the University of Maryland Coffee Bar. All the milk samples and dairy-containing drinks used in this study were prepared with Cloverland Farm milk, and the ice creams were obtained from the university of Maryland Dairy shop. The separation tendencies of milks at 0, 25, and 80^{o}C were examined using Cloverland Farm's milk. Additionally, 1:10 dilutions of the milk, or other dairy products/drinks and tap water were prepared, the samples were exposed to different water temperatures (25, and 52^{o}C) , which were found to be commonly used during cleaning practices, and the partitioning tendencies of the samples were studied at different pH conditions [7 – 2.9].

To investigate the effects of detergents and sterilizers, an aliquot of a solution containing equal portions of EcoLab manual dish detergent and sterilizer obtained from a dairy-based FSE (the Coffee Bar) was mixed with tap water for preparation of the synthetic wastewater samples. Wastewater samples from rinse and sterilizing sinks were obtained from University of Maryland Dining Services. Equal volumes of water, detergent + water dilution from sink 1 and sterilizer solution from sink 3 were mixed. Similar 1:10 dilutions of dairy: detergent: water were made, incorporating sink samples. For these tests, the tap water was replaced by a 1:4 sink-mix: tap water, containing 50 mL of the detergent and sterilizer cocktail in a total sample volume of 200 mL. Samples were prepared in 200-250 mL Erlenmeyer Flasks and tested in 50 mL centrifuge tubes. These samples were allowed to sit for 3 hours and were monitored frequently during this time. A 9 M HCl solution was used to study the effects of pH on stability of the synthetic wastewater samples at different pH levels.

Diagram 1. Experimentation to Investigate Partitioning Tendencies of Synthesized Dairy Wastewater



Methodology for Quantification of Fractioned Fat

To quantify the fate of fat upon separations and examine the fraction of fat that remains in the suspended phase, 1:10 dilutions of ice cream, whole, 2%, and skim milk were prepared in 250 mL Erlenmeyer flasks, and 100 mL of each batch was drawn using a 100 mL pipette, and weighed in a 200 mL Erlenmeyer flask. The pH of the samples were reduced to 4.8 using concentrated HCl and allowed to sit until aggregates had separated into distinguishable phases (~

3 hours). At this point, no additional dissolution of caseinate should occur. The samples were then transferred to pre-weighed tubes and centrifuged at3600 rpm for 5 minutes. This step was added to obtain distinct phases, and avoid interference of fractions of serum retained within the curd with measurements for the mass fraction of separated phases as well as their fat content. In testing of milk dilutions, the serum was first separated and weighed using a pipette. The mass of the tube plus the settled curd layer was then obtained and subtracted from the empty dry centrifuge tube to obtain an accurate measurement of the settled curd. The curd was then transferred to a clean and pre-weighed 80 mL beaker using a spatula. Portions of the curd tended to adhere to the tube/spatula; to separate them for analysis, known volumes of distilled water, (approximately 13 to 16 mL) were added to wash the tube of all the curd flocs. After a final mass measurement, the curd was thoroughly dispersed in the water using a magnetic stirring bar, and the beaker sealed. After mixing for 10-15 minutes until all the curd was completely suspended in the water, the seal was removed and 11 mL of sample was drawn for testing using a milk pipette. The Gerber Fat Method was then used for determination of the fat content of the curd (separated phase). Testing of fat fraction of serum is important in determining the efficiency of removal of dairy-based FOG via physical treatment of dairy-containing wastewater.

Effects of Souring of Milk on Stability of Dairy Emulsion

To investigate the temporal changes of dairy wastewater systems, 1:10 (m/m) dilutions of skim and whole milks with DI water were prepared. Two sets of each milk type was used, each consisting of six- 200 mL 1:10 Milk: DI water dilution; 1 M NaOH solution was added to set 2 to neutralize the acidity of the samples, while set 1 underwent souring without any chemical addition. Furthermore, since the addition of NaOH changes the ionic strength of the samples, a

separate set of six replicas of 200 mL 1:10 dilutions were prepared, to which 1 M NaCl was added (set 3). 1 M NaCl was added to set 3 in volumes identical to the volume of 1 M NaOH required for neutralizing the pH for each of the six samples of set 2, so that the number of moles of NaOH added to the sample 2 of set 2 (s2d2) was equal to the number of moles of NaCl in sample 2 of set 3 (s3d2). The six replicas for each set were labeled s_id1-d6 where s_i corresponds to the three sets examined. The pH, turbidity, and the extent of separation of the dilutions were monitored on a daily basis for 7 consecutive days. The Gerber Fat Method 15.085 (Wehr, et al., 2004), and modified total Kjeldahl nitrogen methods for Protein of bulk milk (15.131), protein-Nitrogen, or the direct true protein method (15.133), and Non-Protein Nitrogen (approximately 5% of all milk nitrogen) (15.135) were employed for determination of Nitrogen content of milk (Wehr et al., 2004) were used to determine the fractionation of the fat and nitrogen during the souring of dairy-containing wastewater.

4.3 Field Methods

Wastewater Collection Procedure

Wastewater samples for FOG analysis were collected from the Coffee Bar, located at University of Maryland, CP Adele H. Stamp Student Union. Figure 5 shows the set up for sample collection, which was carried out for the first and second shift during Fall 2010 and Spring 2011 semesters. To maintain consistent conditions, the sampling took place from 11AM – 3PM for the first shift, and 4-9

Figure 5. Set up for Sample Collection, The Coffee Bar, Student Stamp Union, 2/7/11

PM during the second shift. Samples were collected by placing a 1000 mL beaker underneath the sink drain, shown in Figure 5. Once the beaker is full (~15 min), the pH and temperature of the wastewater was measured. After collecting enough drainage, the liquid was thoroughly mixed with a spatula to make the mixture homogeneous and approximately 100-200 mL was collected in a 1-gallon container. These steps were repeated every time the sinks are used. After all the wastewater was collected, the temperature of the wastewater was measured, and the sample was transferred to the University of Maryland Environmental Engineering Laboratory. Once the sample was inside the lab, it was mixed with a spatula, separated into two batches, mixed well, and equal aliquots of each batch for testing was mixed for further testing (pH and fat content). The pH was also measured using calibrated pH probes.

Procedure for Testing Wastewater Samples

Wastewater samples collected in this study appeared to be much diluted, and fat measurements of the bulk sample fell very close to the detection limit of the Gerber Fat Determination method, with a high degree of uncertainty. However, based on theory, upon curdling of milk (pH induced separation at the isoelectric point), essentially the entire fat content of the sample will be concentrated in the separated curd layer. Therefore, the measurement of low fat solutions were carried out by concentrating the fat in the settling phase via acidification with concentrated HCl, centrifuging the sample at 3600 rpm for 5 minutes, followed by Gerber analysis of the settled curd layer.

Field Testing of Grease Abatement Devices

GADs treating wastewater of a specialty coffee shop and an ice creamery were chosen for testing. The details of each visit are presented in Table 4.

Table 4. Sampling Dates of GADs

Date and Time		T_{air} (^{0}C)	Visited Interceptors
Monday	12/20/2010 2-3 PM	-3	Ice Creamery
Thursday	1/6/2011 10-12 PM	4	Ice Creamery and Specialty Coffee Shop
Friday	1/14/2011 10-12 PM	0	Ice Creamery and Specialty Coffee Shop
Thursday	1/20/2011 10-12 PM	6	Ice Creamery and Specialty Coffee Shop

The two initial visits to the ice cream shop interceptor took place 6 months after the last cleaning date of June 2010. Nonetheless, this interceptor was purged again on Monday 1/9/11, and the consecutive visits represent conditions found in a newly cleansed GAD. Three visits were conducted to a specialty coffee shop interceptor. This interceptor appeared to be well maintained. The 2 interceptors were equivalent in volume (1600 gal) and the general design of GADs of this volume (Figure 4).

Sampling of Grease Interceptors

To measure the extent of separation in each interceptor, a long sampling column (d=5.5 cm, L> 2m) (Figure 6) was used, and using a ruler/meter the height of different phases found within the column was then measured. Once the column was inserted in the GAD compartment, a cable connecting the bottom to its cover was pulled and then secured. This covers the bottom of the tube, and the device was then removed from the chamber for

(a)

(b)

Figure 6. Sampling with Column of 2nd(a) and 3rd(b) Chambers of ice creamery GAD – 1/6/11

measurements of floating phases. Since flow affects the distribution of sediment and/or floating phase/s, height measurements of each fraction were made using a ruler, which was repeated for 3 points within each chamber to represent the spreading of the separated material. The average measurements were reported as the extent of separation for each phase.

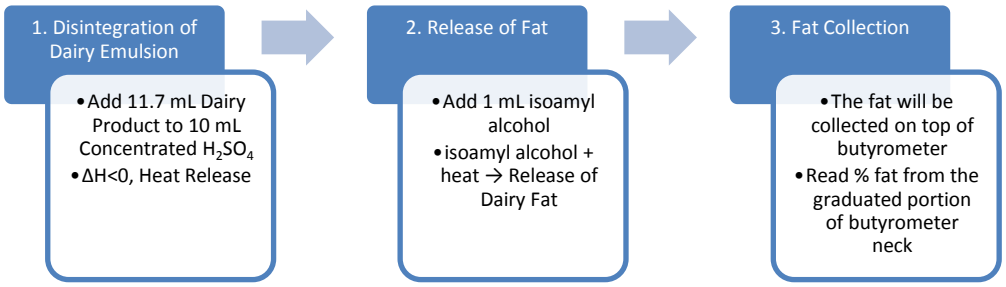
For further testing, each draw was discharged by releasing the cable and collecting the sample into another container. Representative samples were brought to the laboratory. To maximize consistency in analysis, the sampling positions of all compartments closely matched. The total height of wastewater in the column rose from 100 – 110 cm. The samples were brought to the laboratory for further testing, including pH determination, final height measurement and weighing of bulk and segregated phases to determine the degree of separation in the collected samples. Finally, the Gerber Fat analysis was carried out to determine the dairy fat content of each segment.

4.4 Analytical Methods

Determination of Fat Content

Because of the low solubility of dairy fat in hexane, the Gerber Fat Method (15.085) was adapted from the Standard Methods for the Examination of Dairy Products (Wehr et al, 2004, Rossel et al., 1999), to determine the mass percentage of fat trapped in different separated layers. The outline of this method appears in Diagram 2.

Diagram 2. Standard Methods for Examination of Dairy Product, Gerber Fat Method (15.085)



Since the butyrometer used in this method (Figure 7) is not compatible with the available centrifuge, it was replaced with Pyrex centrifuge tubes in the initial steps. Ten mL of concentrated H_2SO_4 was poured in the centrifuge tubes as prescribed for digestion of the emulsion and 11 mL of the sample and 1 mL of isoamylalcohol was added to the centrifuge tube. The samples were mixed by inverting the tube rapidly for at least 30 seconds; immediately after the digestion of the emulsion by the acid, they were centrifuged. A pipette was used to ensure complete transfer of samples to and from these tubes to the butyrometer. The fat fraction of all the milks and ice creams were also determined, using the described modified version of the Gerber method.

Figure 7. Standard Gerber Butyrometer Used for Milk Fat Determination (www.coleparmer.com)

To map the fractionation of fat upon pH induced separation, 200 mL 1:10 (m/m) dairy: water samples were prepared in 250 mL Erlenmeyer flasks, and using concentrated HCl, the pH was reduced to 4.8 to cause curdling. The Gerber Fat Method was then adapted, and the fat content of the separated phases were measured. The *Standard Methods for the Examination of*

Water and Wastewater (5520B) was employed for determination of FOG in synthetic dairy samples.

To quantify the fractioned fat in each layer, a mass balance equation was written (equation 9), and knowing the total mass of fat present in the bulk solution, the fraction of total fat residing in each layer were found using equations 10 and 11.

$$m_{BulkSoln}.x_{Fat,BulkSoln} = m_{curd}.x_{Fat,curd} + m_{Serum}.x_{Faat,Serum} \quad (9)$$

$$y_{Fat,Curd} = \frac{m_{curd}.x_{Fat,curd}}{m_{BulkSoln}.x_{Fat,BulkSoln}} \quad (10)$$

$$y_{Fat,Serum} = \frac{m_{Serum}.x_{Serum}}{m_{BulkSoln}.x_{Fat,BulkSoln}} \quad (11)$$

where x_i corresponds to mass percent of fat measured using the Gerber Fat Method and m_i is the mass of each layer/solution.

Determination of Total Kjeldahl Nitrogen

The mass of nitrogen in the bulk/separated phases was followed, by first obtaining the total mass of the sample used for analysis, obtaining the mass of the total Kjeldahl nitrogen sample portion as described by the Standard Methods for the Examination of Dairy Products Methods 15.131 and 15.133 (Wehr et al., 2004). Equation 12 was used to calculate the mass percentage of nitrogen available in the tested sample.

$$\% N = \frac{14007(V_s - V_b)M}{W} \quad (12)$$

where V_s and V_b are the volumes in mL (expressed to at least the nearest 0.05 mL) of the standard volumetric solution of acid used in the determination for the milk sample and blanks, respectively. M is the molarity (to 4 decimal places) of the standard volumetric solution of acid, and W is the mass, in grams to the nearest 0.1 mg of the test portion. Once the % N (m/m) of

nitrogen was determined, this number was multiplied by the mass of the total sample/separated phase (i.e., floating, serum, settling, or bulk solution) to obtain the total mass of nitrogen available and used for mass balance analysis.

Turbidity

The Nephelometric Method (2130 B) was used for determination of the turbidity in this study using a Hach 2100 N turbidimeter (Eaton, et al., 2005).

Measurement of pH

The pH of samples was measured at room temperature (20-25^{o}C) electronically using a pH meter following preparation steps described in the Standard Methods for Examination of Dairy Products 2.0413 (Wehr et al., 2004).

5. Results and Discussion

5.1 Dairy Suspension and pH

Figure 8 displays the effect of pH when various dairy products were mixed with water at room temperature and pH values and centrifuged after 3 hours.As shown in Figure 8, acidification of dairy systems, as expected, promoted separation of the dairy constituents, which was maximum for pH ranges approaching the ZPC of casein proteins at pH of 4.6 (Walstra, et al., 1984). Results also illustrated that based on the fat content of the mixtures, the dissociated aggregate could either rise, sink or remain in suspension. Thus to assess the effect of pH, each dilution was categorized into three groups of sinking, suspended, and floating phases. The volume of each phase was then measured and results reported as percentage of total volume. Based on the visible clarity of the solution/suspended phase, the number and colors of the lines crossing the columns were altered to reflect observations.

Floating is associated with fat content. This was readily observed in dilutions prepared with higher fat-content dairy, and as illustrated in Figure 8, the tendency of formation of a floating phase increased with the inherent fat characteristics of the dairy material (half and half, ice cream). Additionally the samples showed the greatest extent of separation at pH range close to the isoelectric point of milk proteins (4.66). As Figure 8 illustrates, a larger fraction of sinking occurred for the milk samples, with fat ≤4% as opposed to ice cream and half and half mixtures Curdling (separation of dairy constituents) was observed in the pH range of 5.4-4.

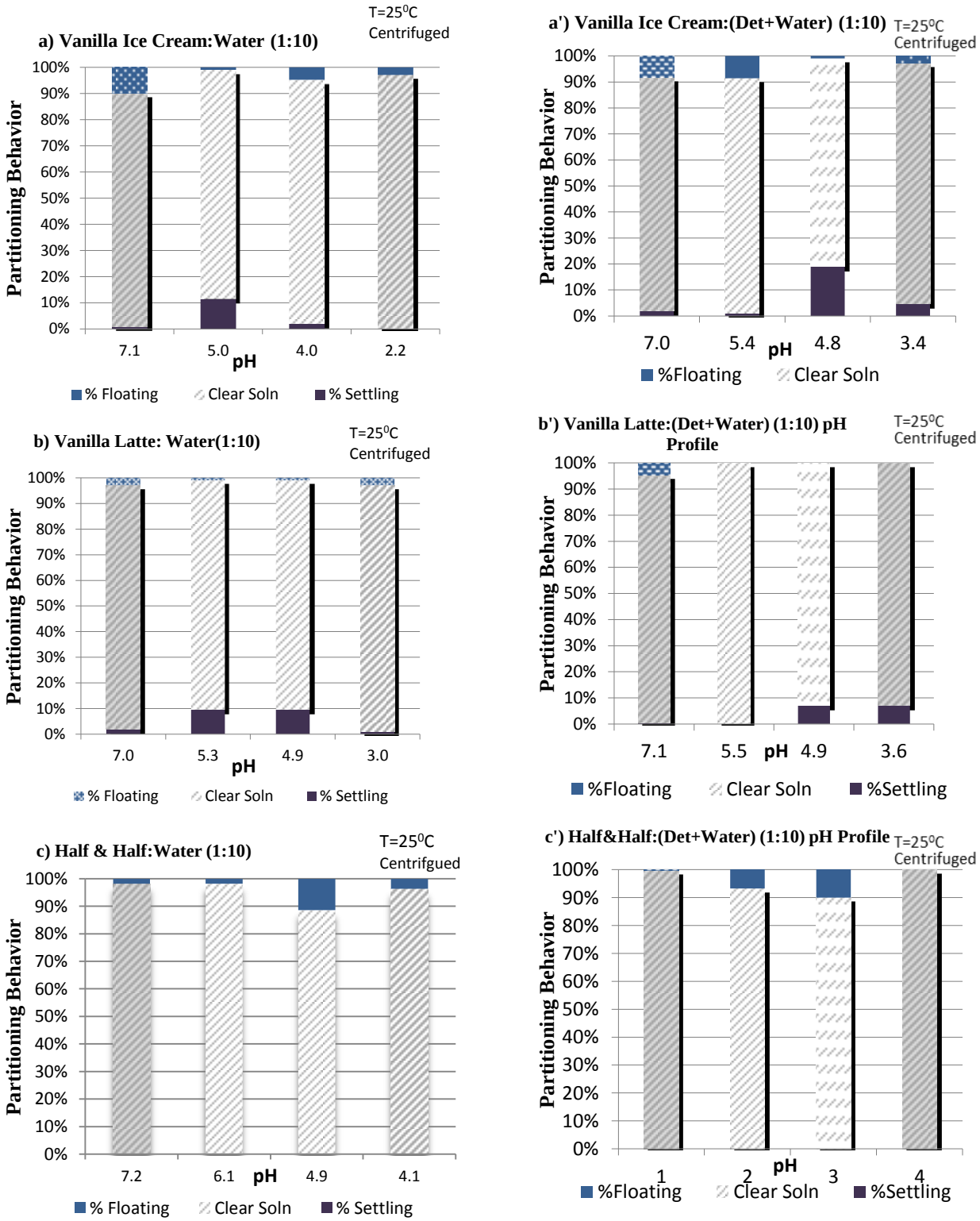


Figure 8. Separation of vanilla ice cream, vanilla latte and half and half: water and vanilla ice cream, vanilla latte, and half and half: (detergent + water) mixtures at different pH values. Samples centrifuged after 3 hours- (testing in duplicates).

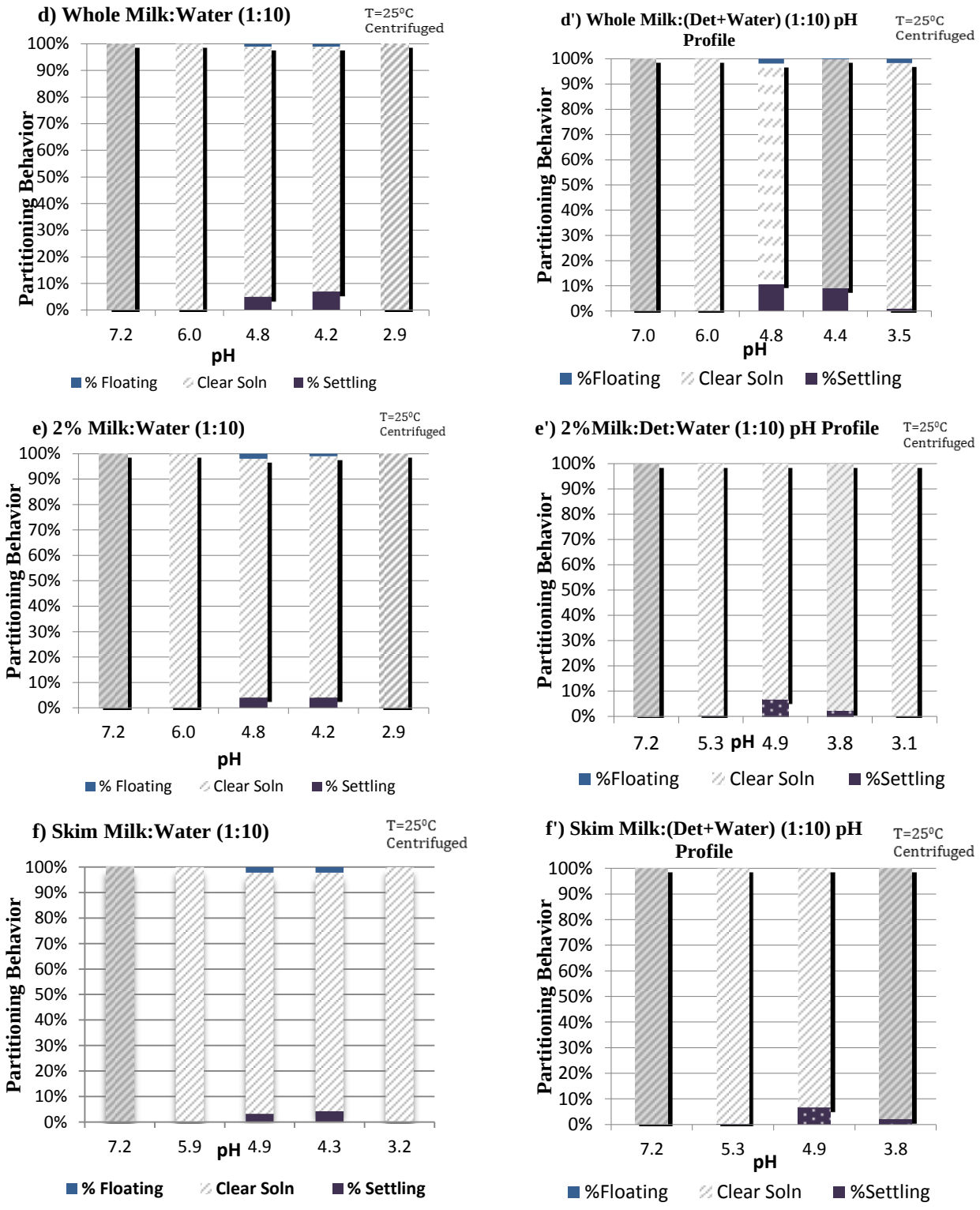


Figure 8-continued. Separation of whole, 2%, and skim milk: water and whole, 2%, and skim milk: (detergent + water) mixtures at different pH values. Samples centrifuged after 3 hours (testing in duplicates)..

As a general note, it can be said that introduction of detergents to the system enhances the separation tendencies of the samples. In almost all dairy products, similar or better partitioning occurred with added detergent (Figure 8). This separation may be the result of number of modifications, such as salting out, co-solvent effects, and/or surface tension. The only exception here is vanilla latte, in which some degree of partitioning occurred for all pH values with slightly lower separation observed upon addition of EcoLab detergents-sterilizer cocktail (1 part detergent and sterilizer with 4 parts of tap water) used in the synthetic wastewater. Moreover, it seems that the addition of the detergent mixture also slightly improves the buffer capacity, and the samples were more resistant to pH change.

Figure 9 clearly demonstrates the degree of separation with increasing inherent fat in synthetic samples with and without addition of detergent/sterilizers. The fractionation of dairy constituents into separate phases reduces with the fat content of the sample. Although no fat measurements were completed in this exercise, the inherent fat content of each sample can be estimated based on the data available in literature (ice cream ~14%, half and half ~8%, whole milk ~3-4%, skim milk ~0-0.5%, Walstra et al., 1984). It can also be seen that except for the the latte mixture, the degree of separation appears to have increased with the addition of the detergent/sterilizer cocktail. This is quite evident for the case of ice cream, in which the separation increased from 11.4% to 19.0%. This observation is speculated to have been caused by the potential replacement of casein micelles by the surfactants available in detergents. Since these surfactants tend to be smaller than the casein micelles, they make the system more prone to subsequent separation. Another explanation could be salting out of dairy constituents caused by the

addition of detergents/sterilizers (Goff, H.D., 1997)

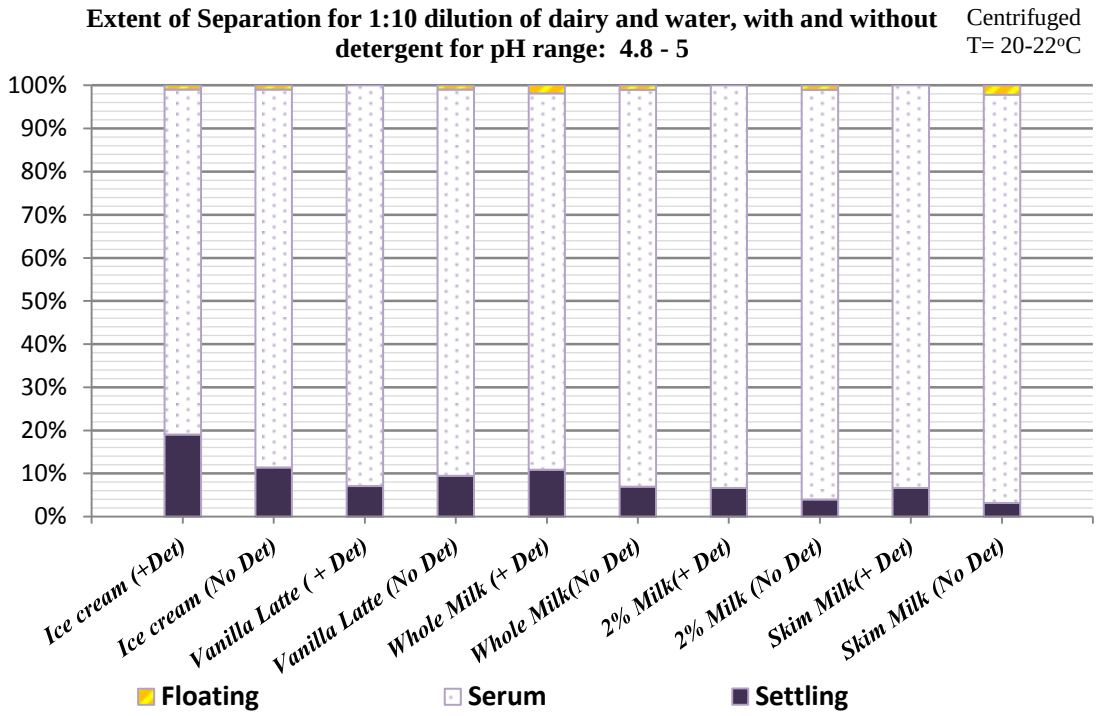


Figure 9. Extent of Separation for 1:10 dilutions of dairy and tap water, with and without addition of detergent and sterilizers (45 mL in 200 mL synthetic sample for pH range of 4.8-5.

5.2 Dairy Suspension and Temperature

Temperature was found to have significant effects on separation of phases in dairy systems (Figure 10). Separation increased significantly with an increase in temperature, and was found to be related to the inherent fat content of milk samples. Partitioning of fat in a floating phase occurred at 80^{o}C, with the half and half demonstrating the maximum separation (12.5% v/v), whole milk (3.33% v/v), 2% fat milk (1.67% v/v), and skim milk (0.33% v/v). The separation was observed to occur in heated samples within 5 minutes of reaching of the maximum temperature except for half and half, which took 10 minutes for the floating phase to reach its maximum volume. The samples were studied for an additional 3 hours, after which no

additional change could be seen. The only separation observed at room temperature was for half and half (7.5% v/v).

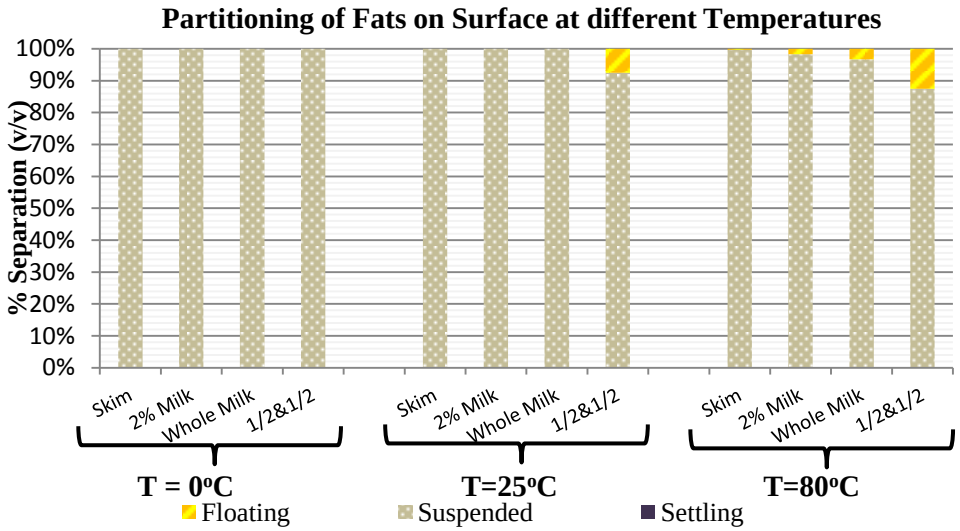


Figure 10. Temperature profile of milk and half and half samples. The extent of creaming/separation reported on a volume basis, allowed to rest for 3 hours after first observation of partitioning

These observations can be explained by denaturing of membrane proteins of fat globules in milk, which destabilizes the emulsion and causes coalescence of fat in the sample (Wehr et al, 1984). It is also interesting to see that separation in the samples very closely matches the inherent fat content of milk samples. Although separation could be seen with heating of the samples, mixing of the samples during heating, as observed in practices employed in the Coffee Bar, greatly retards separation. The results of heating of samples to 70^0C, the average measured temperature of coffee purchased from the Coffee Bar, with simultaneous agitation by inverting the sample, appear in Table 5. As summarized in the table, diminishing of the separated phases was observed for all milk samples (1.9% v/v and 0.95% v/v for whole milk and 2% milk, respectively).

Table 5. Separation induced by heating while agitating milk samples

	Floating Phase	Suspended Phase	Settling Phase
Whole Milk	1.90%	98.10%	< 0.1%
2% Milk Fat	0.95%	99.05%	< 0.1%
Skim Milk	< 0.1%	> 99.9%	< 0.1%

It was also seen that heated whole milk created cloudy samples at pH of 5.4, and took a longer time compared to chilled whole milk dilutions for separated phases to reach constant volumes (120 minutes as opposed to 50 minutes), which in both samples resulted in 20% (v/v), which after centrifuging at 3600 rpm separation was reduced to a more compact layer of 10% (Figure 11 a and b). The patterns of the settling phase in Figure 11 demonstrate the density of this phase. Overall this phase appeared to be loosely packed, and became more compact with passing of time, which is represented in the graph as the density of patterns in the settling phase. Studying the suspension of synthetic wastewater created by mixing heated whole milk and warm tap water (50^{o}C), it was seen that the increased temperature of the system reduced the rate of separation to only 90 minutes (Figure 11c). In all three scenarios, a 10% settling phase was observed. Similar observations were made in similar samples containing detergents. A summary of the

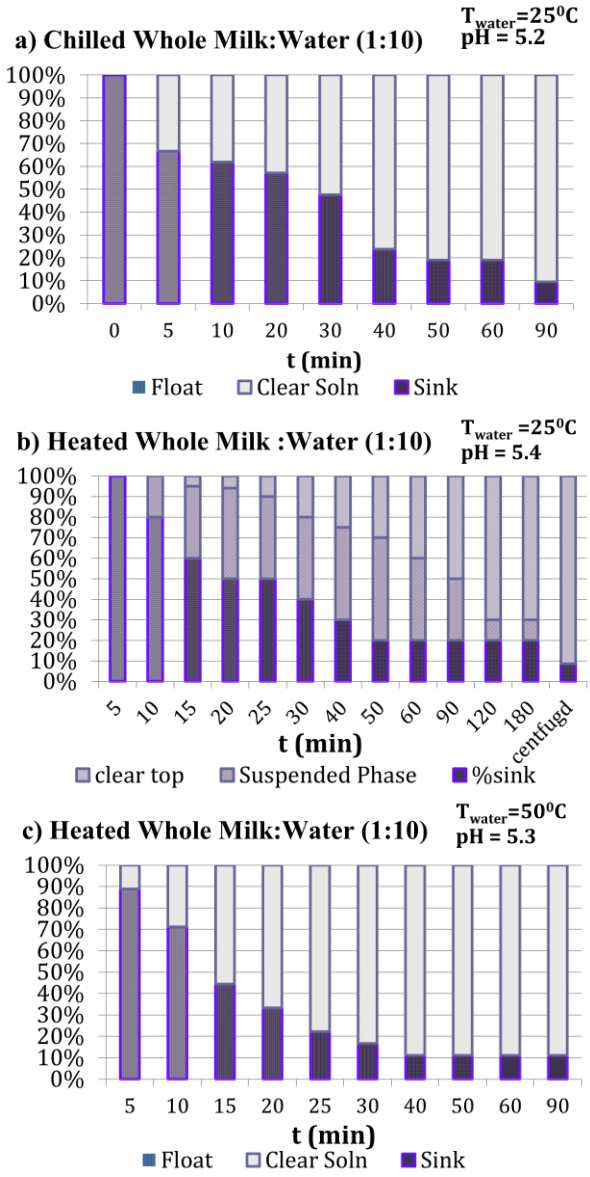


Figure 11. Partitioning tendencies of chilled and heated whole milk in room temperature and warm tap water mixtures to study the effects of heating of milk and use of warm tap water on separation tendencies. (a) Chilled whole milk: room temperature tap water (1:10) (25^{0}C), (b) heated whole milk: room temperature tap water (1:10) dilution at 25^{0}C, and (c) heated whole mill: warm tap water (50^{0}C) at 1:10 dilution rate.

observations made in dairy containing synthetic wastewater samples, using different dairy products with and without incorporation of detergents appears in Table 6.

Table 6. Summary of Results for Separation of Dairy: Water and Dairy: (Det + Water) dilutions

Sample (1:10)	T (^{0}C)	pH Effects	Det. Effects
Heated Milk: tap water Dilution	25	• Separation occurring at pH [5.2-4], with maximum partitioning as pH→4.8. • More resistance to curdle, Formation of colloids for pH ~5.3	slightly enhanced separation for pH [5.3-4]
	50	• Similar behavior as 25^{0}C; higher kinetic energy→faster settling • More compact precipitate phase	slightly enhanced separation for pH [5.3-4]
Chilled Milk: tap water Dilution	25	• Separation occurring at pH [5.2-4], with maximum partitioning as pH→4.8.	slightly enhanced separation for pH{5.3-4]
	50	• Similar behavior as 25^{0}C, higher kinetic energy→faster settling • More compact precipitate phase	slightly enhanced separation for pH [5.3-4]
Hot Vanilla Latte	25	• Separation occurring at pH [5.2-4], with maximum partitioning as pH→4.8. • Portion of precipitant rises after 30 min and density of settling layer ↓	slightly reduced separation tendencies at pH [5.3-4]
	50	• Similar behavior as 25^{0}C, higher kinetic energy→faster settling • More compact precipitate phase	slightly reduced separation tendencies at pH [5.3-4]
Chilled Vanilla Latte	25	• Similar behavior as hot vanilla latte; more curdling, less dense, • Centrifuging produces similar results for both hot and chilled	slightly reduced separation tendencies at pH [5.3-4]
	50	• Similar behavior as 25^{0}C, higher kinetic energy→faster settling • More compact precipitate phase	slightly reduced separation tendencies at pH [5.3-4]
Creams (half & half, and ice creams)	25	• Formation of both settling and floating layer, based on fat content. Half and half(floating), ice cream(floating of foam and fat, settling of curd + additives), maximum separation at pH [5.4-4.8]	slightly enhanced separation for pH [5.3-4]
	50	• Similar behavior as 25^{0}C, higher kinetic energy → faster settling • More compact precipitate phase	slightly enhanced separation for pH [5.3-4]

As outlined in Table 6, heated and chilled milk, and heated and chilled Vanilla Latte started curdling within the pH range of 4 - 5.4, with maximum separation taking place at pH between 4.5-4.9. In hot vanilla latte, a portion of the settled material slowly started to rise after 30 minutes, and the settled phase became less loosely packed. In ice cream samples, both settling and floating phases were formed, while only a floating phase could be seen in half and half-containing synthetic samples. In all samples prepared by mixing warm tap water (50^{o}C) and dairy products, faster separation (settling/floating) could be seen. The addition of detergents also appeared to slightly enhance separation for the pH range of 4 – 5.4, except for latte where a slightly smaller separation could be seen.

It thus appears that increasing the temperature of the water does not induce any changes, such as denaturing of fats or proteins as only faster rates of partitioning were observed. Once again, it was seen that the addition of detergents slightly increased the rate of separation in samples of milks and creams by approximately 1-2%.

One interesting observation during testing of ice cream synthetic samples was that ice creams containing acidic fruit flavors can have pH values close to ZPC pH, in the range of [4 – 5.3]. For example, the addition of water to raspberry ice cream resulted in instantaneous separation of the dairy mixture from the syrup, and the pH of the sample was measured to be 3.8. Figure 12 illustrates this finding. The 50^{o}C raspberry ice cream-water (1:10) dilution resulted in a separation of a relatively

Figure 12. Separation of dairy species in raspberry ice cream: water 1:10 dilution at sample temperatures of 50^{0}C (left) and 25^{0}C (right) in the first 5 minutes. The pH of the samples without any addition of acid was 3.8.

condensed 12.5% (v/v) floating layer while partitioning of dairy constituents in the 25°C sample resulted in a relatively dispersed 14% fat layer after 1 hour. Eventually both systems separated to approximately the same extent, but the warm sample did so at a faster rate.

5.3 Fractionation of Fat upon pH-induced Separation

While extensive separation, particularly in pH ranges of 4.6-5.4 could be observed, since FOG by definition is derived from fat, it is important to quantify the fate of fat upon separation. In an experiment designed to quantify the different fractions of fat retained within the separated curd (i.e., floating and/or settling) and serum phases, it was found that the available fat within the sample will be concentrated within the separated phases. Results obtained from this experiment appear in Tables 7 and 8.

Table 7 provides the mass of dairy product used in mixing of the samples and the dilution ratio of dairy to water. Fat fractions of the bulk samples were determined, and 100 mL of each sample, initially neutral, was acidified to 4.8. After distinguishable phases were obtained and the sample centrifuged at 3600 rpm for 5 minutes, mass fractions of the separated layers were determined and reported in Table 6. As can be seen, almost the entire fat content was trapped in the curd, and very little (≤0.06%) of the fat remained in the serum.

Table 7. Mass and Mass Fractions of Fat in Dairy Products and Curd, Centrifuged after 1.5 hours at 3600 rpm; pH =4.8, T = 20^{0}C

	Mass of Dairy Product (g)	Total Mass: (g) $m_{Milk}+m_{H2O}$	x_{Milk} [5]	Unseparated Fat (g)	Curd (g)	x_{curd} [6]
Vanilla Ice Cream	7.47	74.730	10	1.084	5.11	6.84%
Whole Milk	10.01	100.01	10	0.280	4.88	4.88%
2% Milk	10.01	100.04	10	0.170	2.05	2.05%
Skim Milk	10.02	100.01	10	0.020	1.89	1.89%

[5] Milk mass percent in the sample
[6] Mass percent of curd in the sample

Table 8. Mass Fractions and Mass Balance for Dairy: Water Dilutions

	Bulk Solution		Serum		Curd		**Total Fat = Curd Fat + Ser. Fat**	Recovery
	Gerber x^{7}_{Fat}	g Fat	Gerber x_{Fat}	g Fat	Gerber x_{Fat}	g Fat		
Vanilla Ice Cream	1.45%	1.084	0.06%	0.042	20.30%	1.04	1.08	99.6%
Whole Milk	0.28%	0.280	0.01%	0.010	5.30%	0.26	0.26	95.7%
2% Milk	0.17%	0.170	0.00%	0.000	8.30%	0.17	0.17	100.1%
Skim Milk	0.02%	0.020	0.00%	0.000	1.10%	0.02	0.02	103.9%

Figure 13 provides a visual presentation of the fractionation of fat into separated phases. The mass of fat recovered in the curd layer was greater than 92% of total fat for all 4 samples.

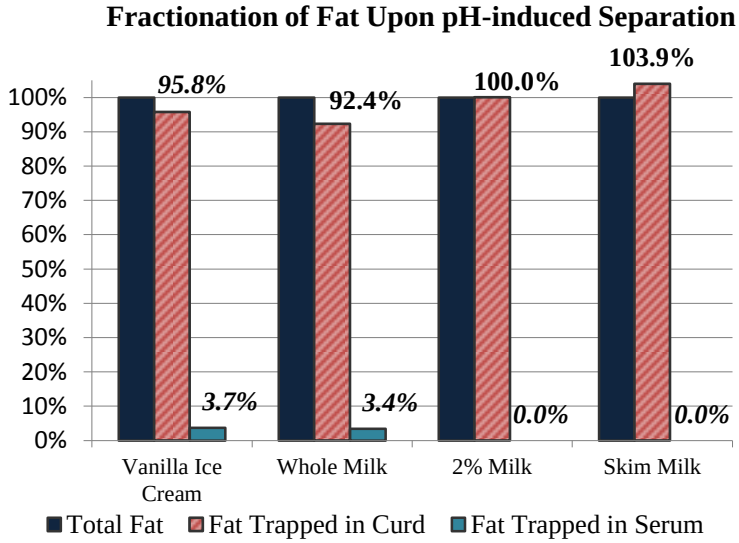


Figure 13. Fractionation of Fat upon pH-induced Separation for 1:10 dairy: water dilutions.

5.4. Wastewater Analysis

Fresh Wastewater- The Coffee Bar

In fresh wastewater samples obtained from the Coffee Bar at the University of Maryland, the fat content was determined using the Gerber Method. The fat content was determined by using bulk measurements on wastewater samples, and also by curdling the samples using concentrated HCl, the results of which appear in Table 9. The samples collected reflect the

[7] Mass percentage of fat in the phase measured

performance of different shift operations. The fat content was approximately 0.02%. The dilution rate of milk products in the collected wastewater sample were estimated based on a volumetric comparison of the curd layer of the wastewater samples with previously tested synthetic milk wastewaters of 1:10 and 1:100 dilution, and the rate was estimated to be approximately 2:25.

Table 9. Bulk Sample Fat Measurements vis-à-vis Determination of Fat from Curd

	Curd Fat Determination	Bulk Measurement
Shift 1 (11AM-3PM)	0.02% ± 0.004%	0.03% ± 0.01%
Shift 2 (4PM – 9PM)	0.03% ± 0.003%	0.02% ± 0.02%

Although the fat contents as determined in both bulk and curdled samples, are essentially equal, analysis of curdled samples improved the accuracy of the measurements (average uncertainty of ± 0.015 for Bulk measurements vs. 0.0035% for Curdled samples).

Grease Interceptors

To assess the efficiency of physical treatment of wastewater rich in dairy FOG, the grease interceptor of an ice creamery and a specialty coffee shop were visited. It should be noted that the performance of a grease interceptor greatly depends on its maintenance and upkeep. In order to relate the degree of separation and accumulation of separated material and assess the efficiency of a grease interceptor, it would have been ideal to start monitoring of a grease interceptor close to its most recent purge date. This could not be done since according to records of WSSC, the grease interceptor of the ice creamery visited, had not been purged for approximately 6 months due to renovations and frequent shut-down days. The variations in the retention time of the interceptor is also important as the retention time (t_R[8]) varies with flow, and is likely to be different for different seasons, and even times of day. The longer the retention time, the more efficient the grease interceptor, as the suspended material will have a longer time

[8] The retention time: t_R= volume of grease interceptor / volumetric flow rate

to separate. Another factor that should be considered is that the manhole is the only point of access to the different chambers of the interceptor, through which only a limited view of each interceptor chamber is possible. Sampling in order to identify the extent of separation in the visited grease interceptors, therefore, can be used under the assumption that the conditions in the interceptor chambers remain the same throughout the chamber. This assumption was used in this study although it is possible for the separated material to adhere to the walls of the interceptor.

Ice Creamery

The grease interceptor treating wastewater originating from an ice creamery was visited 4 times. The dye test employed clearly identified the inlet compartment of the grease interceptor after 40 minutes. This interceptor consists of 3 compartments and has a maximum volume capacity of 1600 gallons. During all visits, the interceptor appeared to be in almost static conditions, and it took approximately 40 minutes for the dye dispensed in the sink to appear in the inlet. Using the water consumption of this facility, provided from the local water utility, the average residence times were calculated assuming that the water usage for purposes other than cleaning/preparing of goods in the restaurant was negligible. The calculations appear in Table 10.

Table 10. Water Consumption of a Maryland Ice Creamery – Data from WSSC

Start Date	End Date	Δt [d]	$V_{\text{water consumption}}$[gal][9]	Q[10] [gal/d]	t_R=1600/Q [d]
12/9/2009	3/10/2010	91	71,000	780	2.05
3/10/2010	6/10/2010	92	78,000	847	1.89
6/10/2010	9/10/2010	92	78,000	847	1.89
9/10/2010	12/10/2010	91	40,000	439	3.64

[9] Volume of the water consumption of the FSE, passed through the grease interceptor
[10] Average volumetric flow rate of the grease interceptor

As can be seen, the average residence times for the first 3 intervals fall within close range of one another (approximately 1.9 days), while the residence time of last interval, from June to September is significantly longer. This is due to the fact that this FSE was under renovation and was shut down for long periods during this time. The most recent purge of this grease interceptor at the time of first visit dated back to June 2010, followed by another cleaning on 1/14/11. Figure 14 a-c illustrates the inlet, intermediate, and outlet compartments of the interceptor 6 months from the purge in June, while Figure 14 e-f correspond to the purge date of 1/14/11.

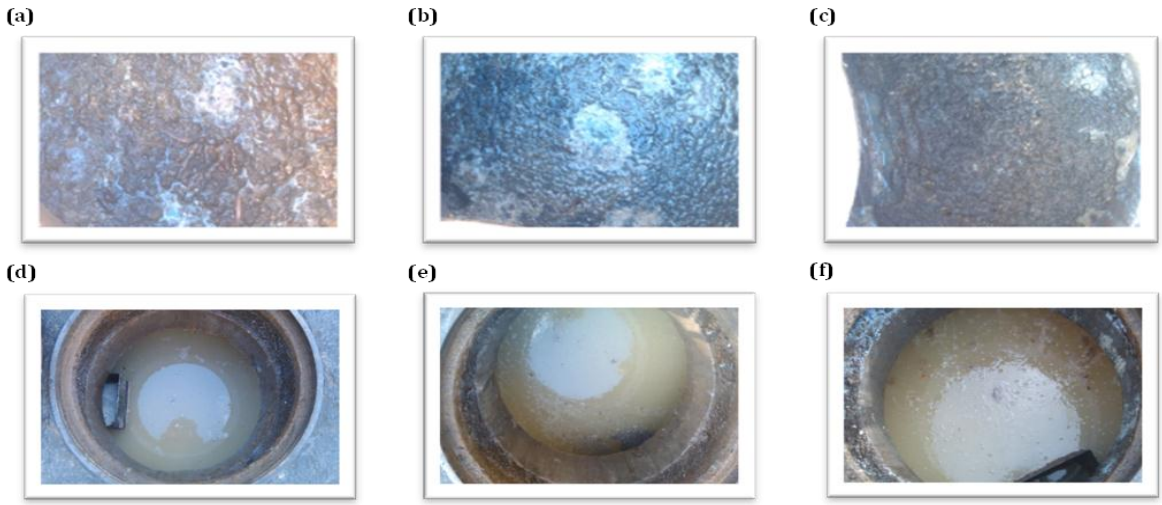


Figure 14. Inlet (a) and (d), Middle (b) and (e), and Outlet (c) and (f) Chamber of Ice Cream Shop's grease interceptor sampled on 12/20/10 [a-c air temperature of 3^{0}C], and 1/14/11 [d-f air temperature of 0^{0}C] respectively.

Figure 14 clearly indicates accumulation of separated material from June to December, 2010. No significant difference could be observed in the quality of wastewater among the three chambers although a settling layer, most likely consisting of food solids, could be seen in the inlet and intermediate chambers. The floating layer in each compartment consisted of large dark and cream colored clusters spread throughout the interface. The measured phases for the samples collected for this study appear in Figure 15. The pH values for each compartment is reported on the graph, and fell very close to the casein ZPC pH for all measurements, where curdling is expected to occur.

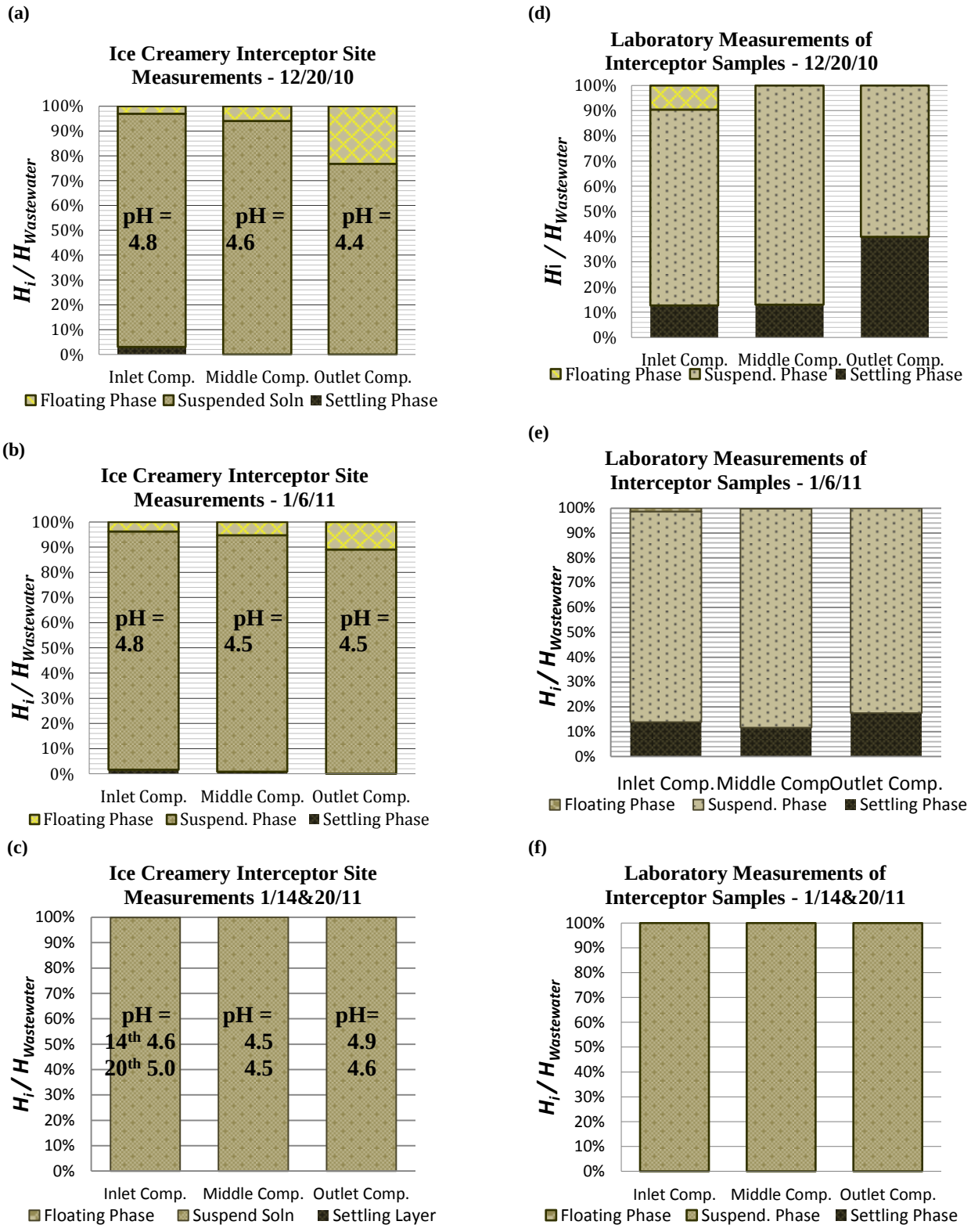


Figure 15. Degree of Separation in the ice cream shop's grease interceptor,–Purge Date 06/2010 (a-d), and 01/10/2011 (e-f)

As demonstrated in Figure 15, the settling phase tended to form in the inlet compartment, and most of the separated material contributed to the floating phase. Furthermore, ice creams are prepared by rapid cooling and incorporation of air bubbles in cream mixtures; therefore, they have a lower density than milk, and separation for this type of FSEs was expected to occur with formation of a floating phase (Walstra, et al., 1984). It was interesting to note that the extent of separation in the outlet chamber was much greater than the inlet and intermediate chambers. This was contrary to expected observations. It, therefore, appears that separation of dairy constituents available in the wastewater of an ice cream shop is much slower than anticipated. It is speculated that the addition of stabilizers used during manufacturing of ice creams resulted in retarding of separation. The outlet chamber, during the 2 visits up to 1/14/11 appeared to exceed the maximum capacity of separated material as measurements indicated the outflow chamber to hold a total of 13% (v/v) separated material. This signals the importance of maintenance of the grease interceptors. The visits of 1/14 and 1/20/2011 showed no measurable signs of separation, most likely due to the shortage of sale of dairy product during this time as stated by the store manager. Testing of the same samples in the laboratory after allowing them to sit for an additional 3 hours, demonstrated increase in the extent of separation. It was also seen that once the separated materials were interrupted by the sampling column, they tended to sink in the solution.

Figure 15[11] also demonstrates that the extent of separation is greater in the outlet samples in both the laboratory and on-site measurements for the first two visits. Although testing was done to determine the fat content of the separated phases, the mass fraction of fat trapped within each phase could not be measured, as the fat content of the sample exceeded the detection limit of the butyrometer (> 25% for mass fraction of dairy fat). Although the grease interceptor was

[11] Results of this study also appear in the Appendix.

constrained in its performance, it appeared to have been successful in reducing the discharge of dairy constituents prone to separation. During the last two visits, more significant accumulation along the walls of the interceptor could be seen (Figure 14e). Therefore, although, no measurable separation could be observed for the last two visits, accumulation of separated material towards areas of no access could have been possible as the only access to the interceptor is through the limited area of the manholes.

Specialty Coffee Shop

The interceptor of a specialty coffee shop was chosen for the second field investigation of this study. The interceptor is identical to the previous GAD, and sampling of each chamber was carried out as previously described. A film of milky colored aggregates could be observed on the interface of the inlet and middle compartments, whereas the outlet chamber showed significantly less partitioning. Figure 16 shows the 3 compartments of this interceptor on 1/14/2011.

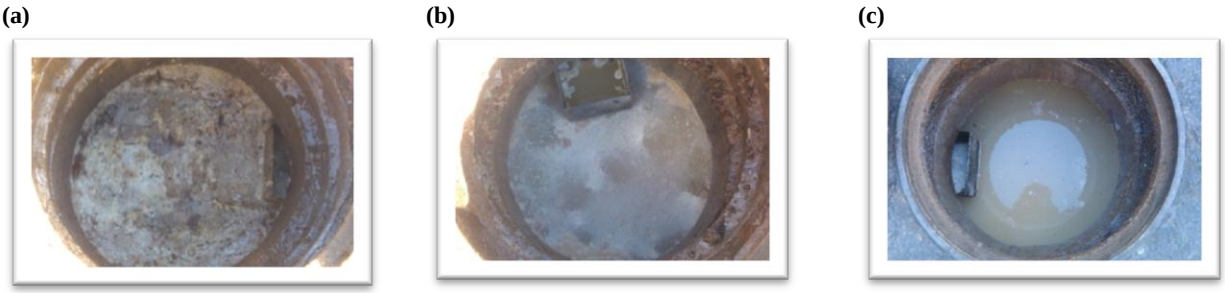


Figure 16. Inlet (a), Middle (b), and Outlet (c) Chambers of Specialty Coffee Shop grease interceptor, 1/14/2011

As seen in Figure 16, a milky-colored floating phase covered the inlet and intermediate compartments, and little separation was observed for the initial visits of 1/6 and 1/14/2011. This interceptor was last cleaned during the week of 12/20/2010, and in contrast to the ice cream shop interceptor, partitioning of dairy constituents appeared to diminish from the inlet to outlet chambers. Looking at Figure 16 a and b, it could be seen that the separated phases are denser towards the interceptor walls. The on-site measurements of this interceptor indicated a more

settling-dominated separation than the ice cream shop's interceptor. Figure 17 provides a graphical presentation of the degree of separation in the different compartments of the specialty coffee shop grease interceptor.

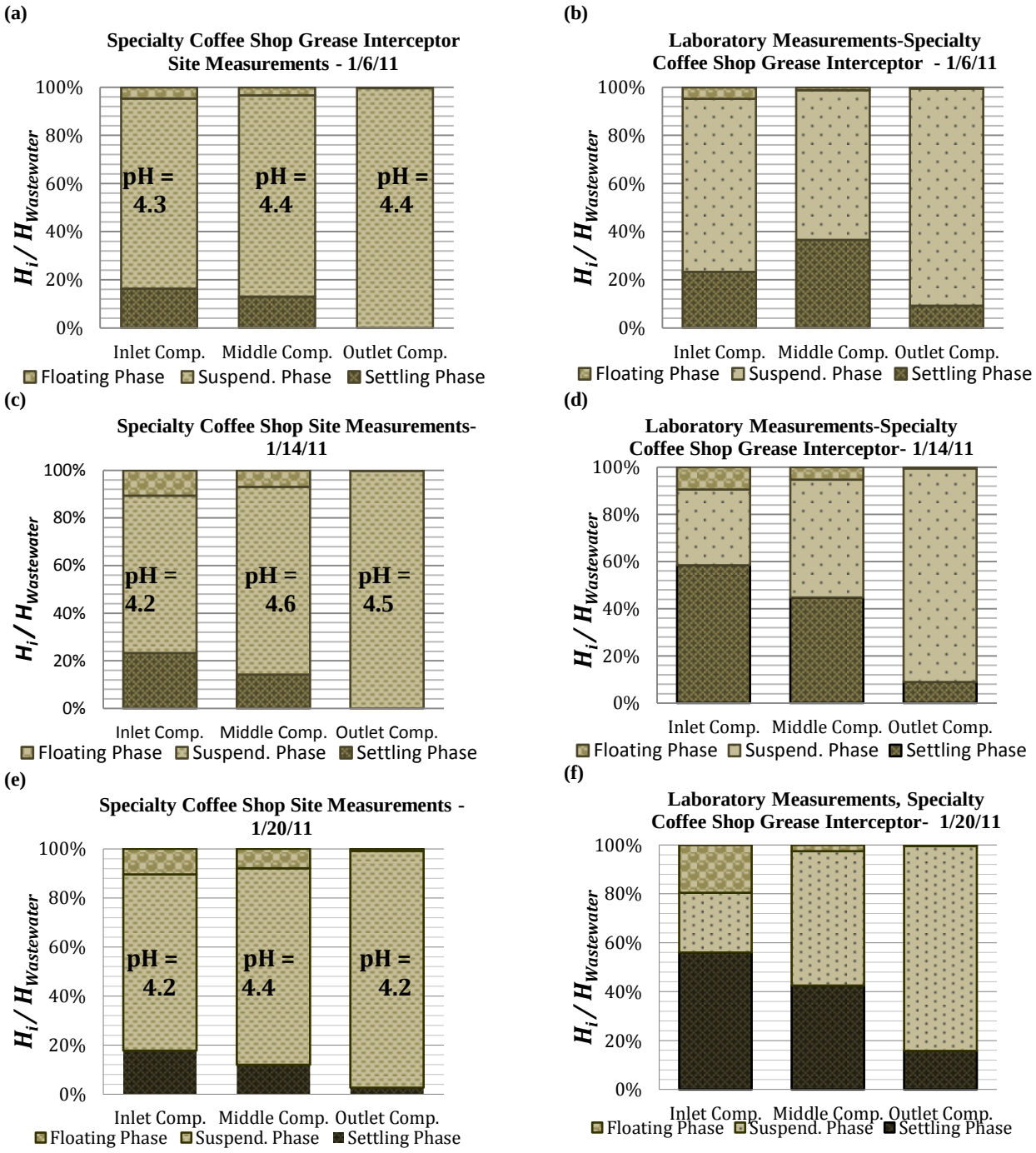


Figure 17. The Extent of Separation in a Specialty Coffee Shop Grease Interceptor, Sampled and Measured on site and in Laboratory- Measurements made the same day as collection at approximately 4:30 PM

Considering the increase in the extent of separation in the laboratory, increasing the retention time will enhance removal efficiency of dairy constituents prone to separation. The quality of wastewater based on measurements and visual inspection, as demonstrated in Figures 17 and 18, improved significantly moving from inlet to outlet compartments. The outlet compartment demonstrated the smallest separation tendencies, as shown in Figures 17 and 18, where the quality of water from the initial visit of 1/6 (a) to the 3rd visit of 1/20 (b) is seen.

(a)

(b)

Figure 18. Specialty Coffee Shop Interceptor Samples Collected from Inlet (right), Middle (Center), and Outlet Compartments (Left) on 1/6/11 (a), and on 1/20/11

From pH values shown on Figure 17, it is seen that the pH range fell close to the casein ZPC [4.2-4.6], but was nonetheless lower than the pH of the ice creamery wastewater [4.4 - 5.0]. Furthermore, the phases were in liquid form while wastewater obtained from the ice cream shop wastewater appeared to hold dense clusters. Considerable separation was observed in this grease interceptor. Allowing the samples to rest in the laboratory for 3-4 hours resulted in a greater extent of separation, which is demonstrated in Figure 17b, d, and f. The measurements indicated a larger settling phase,

which was expected based on laboratory studies, considering the lower fat content of dairy product used in specialty coffee shops as opposed to ice creameries.

Looking at Figures 17 and 18, the separated fractions increased with time of visit, and a higher extent of separation , particularly for the settling phases, were observed in laboratory during the subsequent laboratory measurements. As shown in Figure 17 a greater increase in the extent of separation (floating and settling phases) for the first two weeks of study was observed, which was more dramatic in samples taken from the inlet compartment. Although the extent of separation of floating phases did not differ substantially from 1/14 to 1/20, the sample appeared to become more dense and compact. This could be the result of temperature and also higher concentrations and passing of time on separated fats and proteins. Finally, the degree of separation in the GAD also depends on the retention time. The water consumption of this specialty coffee shop could not be obtained in time for this study, and hence no t_R could be calculated.

The fractionation of fat into the separated phases was obtained using the Gerber Fat Method. Tables 11-12 summarize the results. Dilutions of floating compounds with distilled water (1:11) were made for testing to determine the fat content, and measurements were higher than 9.1%, resulting in greater than 100% fat content, except for the floating phase of the intermediate chamber for 1/14/11 (9.0% mass percent fat). The mixing of other fatty and/or organic compounds with isoamyl alcohol during the analytical method could have resulted in higher than normal readings. For practical purposes this phase was regarded as pure fat and is demonstrated as such in Table**s** 11 and 12. The fat content of the serum/suspended phase was measured to be lowest (0.5-0.15%). The fat content of the sediment phase was found to reduce from the inlet to the outlet

compartments. Furthermore, the accumulation of fat within each phase was found to increase from 1/6/11 to 1/20/11. For example, the fat content of the settled phase increased from 4.3% for 1/6/11, to 5.49% in the second week of analysis (1/14/11), and finally to 8.25% on 1/20/2011.

Table 11. Percentage of Fat Trapped in Each Phase of the Specialty Coffee Shop Grease Interceptor

Sample Date	Chamber	Measured Floating Fat % (1:11 Dilution)	% X_{fat}[12], Floating Phase	Measured % $X_{Fat,\ Suspended\ Phase}$	Measured % $X_{Fat,\ Settled\ Phase}$
1/6/2011	Inlet	14.25%	100 %	0.100%	4.30%
	Intermediate	NA[13]	NA	0.050%	1.60%
	Outlet	NA	NA	0.10%	0.35%
1/14/2011	Inlet	16.70%	100%	0.10%	5.49%
	Intermediate	9.00%	99%	0.05%	3.00%
	Outlet	NA	NA	0.10%	0.49%
1/20/2011	Inlet	15%	100%	0.15%	8.25%
	Intermediate	NA	NA	0.05%	4.00%
	Outlet	NA	NA	0.10%	0.70%

Table 12. Determination of Fat Content of Partitioned Phases in the Specialty Coffee Shop GAD, Laurel, MD

Sample Date	Chamber	$M_{floating}$ (g)	$M_{fat\ in\ Floating\ Phase}$ (g)	$M_{suspended\ Phase}$ (g)	$M_{fat\ in\ Suspended\ Phase}$ (g)	$M_{Sediment}$ (g)	$M_{fat\ in\ Sediment}$ (g)
1/6/11	Inlet	34	34	596	3.0	296	12.7
	Intermediate	NA	NA	651	0.33	289	4.6
	Outlet	NA	NA	827	< 0.001%	74	0.3
1/14/11	Inlet	77	77	897	0.90	759	41.7
	Intermediate	10.65	10.54	1396	0.70	1139	34.2
	Outlet	NA	NA	1891	1.9	121	0.6
1/20/11	Inlet	94	94	548	0.82	257	21.2
	Intermediate	NA	NA	748	0.37	220	8.8
	Outlet	NA	NA	807	0.81	113	0.8

[12] % Mass fraction of fat of the analyzed phase

[13] Separated phase was not significant enough for analysis (i.e., mass of separated phase prior to dilution < 0.001 g)

This interceptor successfully showed retention of dairy material prone to separation during the course of study. An increase in the extent of separation, as well as accumulation of fat in the floating phase was found. The maintenance of this interceptor provided evidence to conclude that physical treatment of wastewater rich in dairy constituents can successfully remove dairy components prone to separation in wastewater originating from a specialty coffee shop. This separation, although significant was somewhat expected as the pH of the wastewater was found to close to the casein ZPC pH, which induces separation. Since acidification follows souring of milk, the pH of wastewater contained within the interceptor induces separation of dairy constituents.

4.5 Separation of Milk upon Souring

The reversibility of separation induced by souring with respect to pH was tested in 3 batches of samples (1:10 dilution of skim, 2% fat, and whole milk with DI water) for seven consecutive days to investigate the extent of separation of diluted milk without any chemical addition with neutralized samples via NaOH addition. Influence of ionic strength on separation was also tested by addition of equal volumes of 1 M NaCl solution as 1 M NaOH required for neutralizing samples of set 2. Figure 19 summarizes the changes in pH and turbidity for each day of the experiment, and Figure 20 illustrates the extent of separation for each set during the course of study. Testing was done using 6 replica of 1:10 milk: DI water and each curve on Figures 19 a-c' represents the changes in pH and turbidity observed for each day of the experiments. Figure 20 includes the mean separation behavior of these samples for each set. Substantial separation could be observed in samples allowed to spoil without any chemical addition. The pH reduced from neutral (6.8-7) to 3.5, and the turbidity increased during the first 3 days of study, but reduced eventually once the separation had occurred and a transparent serum remained. Samples

neutralized by addition of NaOH also experienced separation from day 2 to day 5, after which although the separated phase was still present, it reduced greatly (14.5 to 7.0% from day 5 to day 7). Furthermore the samples containing NaOH were more turbid (2682 NTU for day 7 of set 2 with NaOH addition, compared to 135 NTU and 110 NTU for day 7 of sets 1 without any chemical addition and 3 with NaCl addition). The samples of set 2, neutralized via NaOH addition gained a pale green color from day 5. Samples with salt addition showed the greatest extent of separation and the overall turbidity of the samples were measured to be lowest.

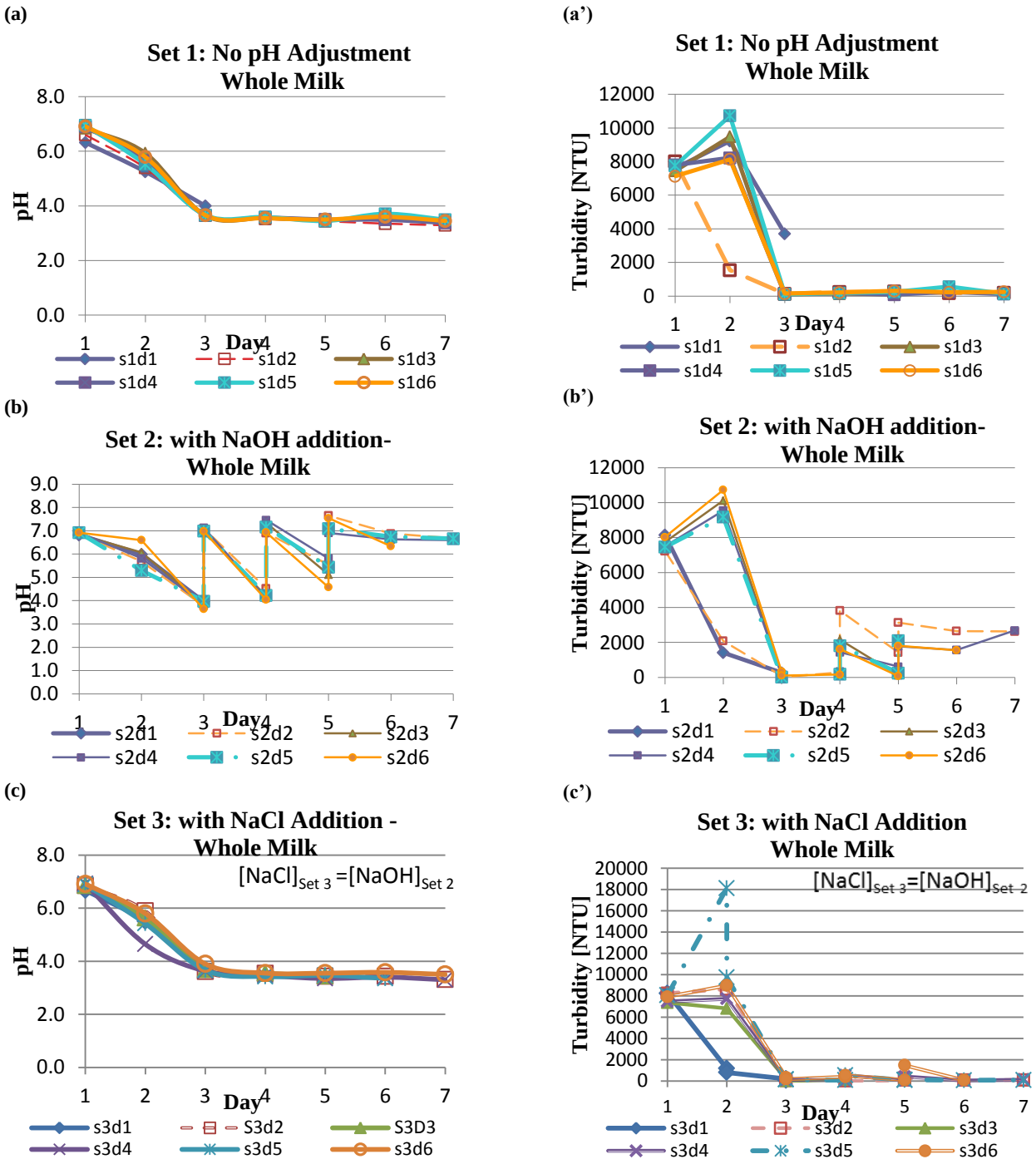


Figure 19. Changes observed in pH and Turbidity of 3 sets of samples, each containing 6 replica of 200 mL batches of 1:10 whole milk: DI water dilution. Each curve corresponds to the behavior of individual samples s_id1-s_id6 where s_i represents sets 1 – 3.

As can be seen from Figure 20, the pattern of the settled phase changes throughout the course of this study. These patterns were chosen to represent how densely the separated phase

were held together; the solid bars show compact and closely packed separated phase, while the patterns show loosely-packed material. A relatively large degree of deviation was found in the fraction of the separated phases, but an interesting observation was the how the separated materials were packed together. The separated phases in some of the samples appeared to be compact and dense, while others appeared to be loosely packed. Another contributing factor to deviations in the extent of separation is the rate at which samples began spoiling. Some of the samples started separation by the second day, while others remained suspended. The deviation is particularly high in the second day of the experiment.

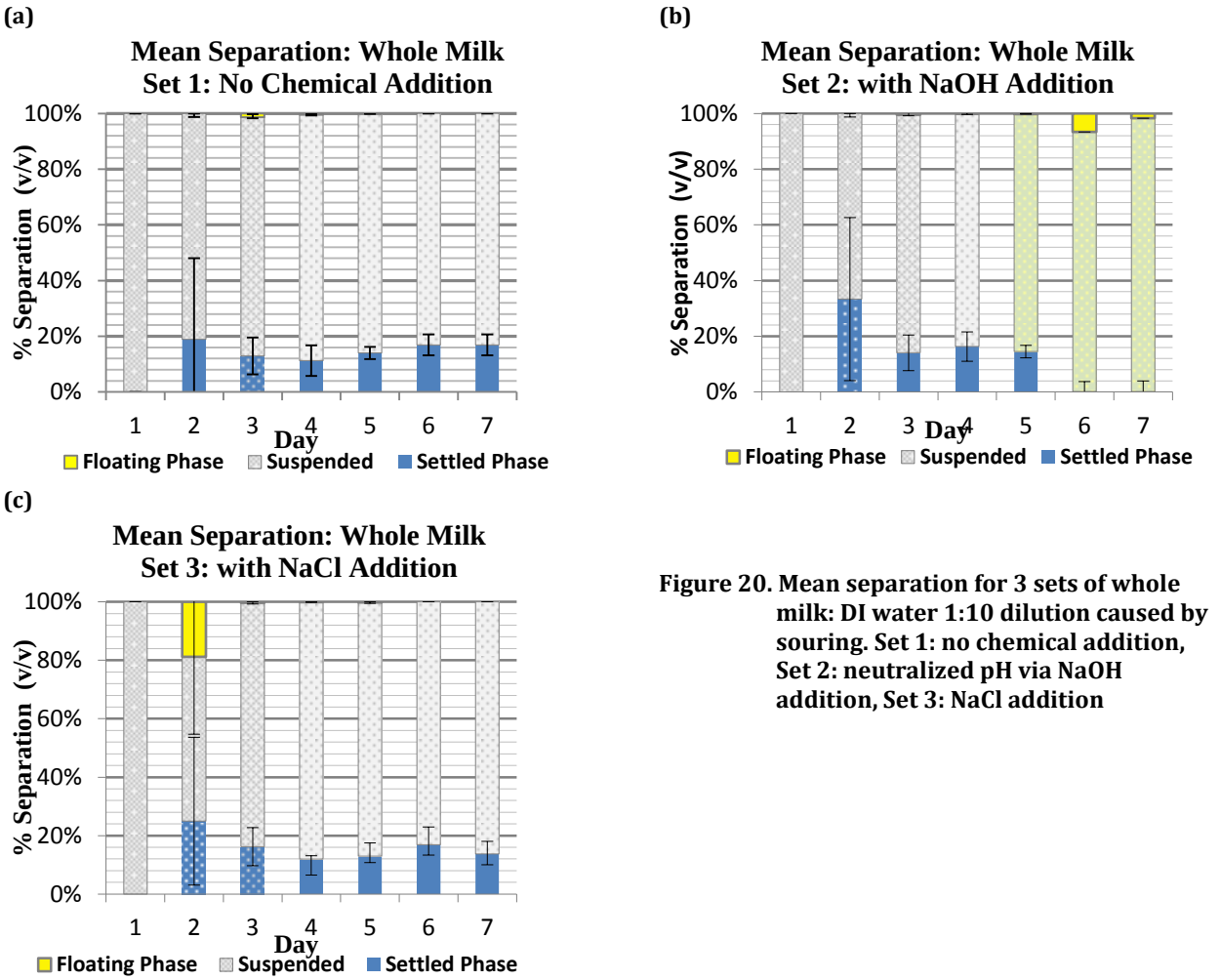


Figure 20. Mean separation for 3 sets of whole milk: DI water 1:10 dilution caused by souring. Set 1: no chemical addition, Set 2: neutralized pH via NaOH addition, Set 3: NaCl addition

To relate the separation with fractionation of fat and organic nitrogen assumed to be proteins[14] in the samples, total Kjeldahl nitrogen and the Gerber fat method were performed on the samples of days 3, 5, and 7, and their sum compared with the original organic nitrogen[15] content of the sample obtained on the first day of the experimentation. The results appear Figure 21**.** The initial mass of nitrogen and fat measured in the sample were found to be 0.94±0.02 g and 0.57 ± 0.01 g, respectively. The data labels provide the mass of nitrogen or fat (g) available in each phase, while the percentage of mass of nitrogen for each phase appears on the y axis.

As demonstrated in Figure 21, the TKN available in the serum phase in sets 1 and 3 is decreasing from day 3 to day 5 (0.49 to 0.38 g, respectively), which corresponds to an increase in the nitrogen available in the separated phases. In contrast, the serum TKN is increasing from 0.61 to 0.81g from day 3 to day 5, which is consistent with the minimum extent of separation observed for set 2 on day 7. Considering that acidic conditions with pH $\leq$3.5 are toxic to lactobacters responsible for souring of milk, it is expected that their activities were prolonged for set 2 because of neutral pH conditions, corresponding to more changes observed in the overall structure of the dairy system.

The fat content of the serum, on the other hand, showed a clear transfer from the separated phases to the serum phase, increasing from 0.095 to 0.194 and 0.097 to 0.497 for set 1 and set 2. Once again the fat available in the serum phase of the second set for day 5 and day 7 was greater than that of set 1, signaling re-suspension of the fat in the serum. Finally the third set, showed almost identical fat content in the serum phase and settling phases, perhaps an indication of salting out of fats in this set.

[14] 95% of all milk nitrogen constitutes protein nitrogen (Walstra, et al., 1984)
[15] Refer to Appendix for comparison of initial organic nitrogen and organic nitrogen on day 3, 5, and 7 of the experiment.

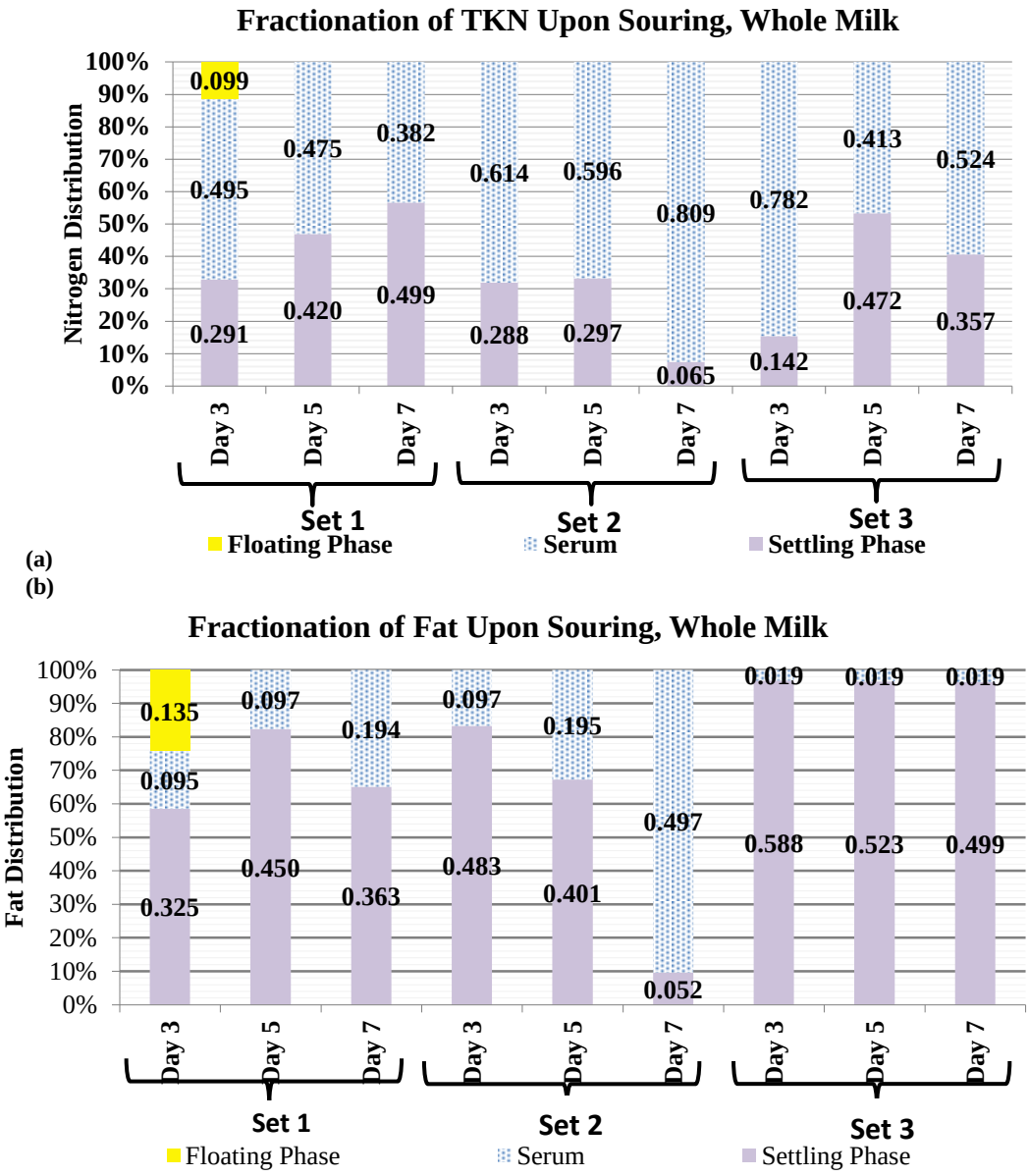


Figure 21 . Fractionation of total Kjeldahl nitrogen (a) and fat (b) into different phases during souring of milk, 1:10 Whole Milk and DI Water Solution. The data labels appearing in the Figures correspond to the mass of nitrogen and fat (in grams) for Set 1: no chemical addition, Set 2: with NaOH addition, and Set 3: with NaCl Addition

Similar experiments on 1:10 dilutions of skim milk: DI water produced somewhat different results. Similar trends in pH and turbidity were observed, with pH reducing from neutral pH range of 7-7.1 to 3.5-3.6 in set 1 and set 3. Figure 22 provides the pH and turbidity profiles of the sample for skim milk samples. Set 2, as in the case of whole milk, was neutralized with the addition of NaOH. The turbidity of the samples began increasing during

the initial phase of spoiling prior to separation. The turbidity of set 3, containing NaCl, was the lowest among the three samples while turbidity of set 2, containing NaOH, did not deviate dramatically from sets 1 and 3.

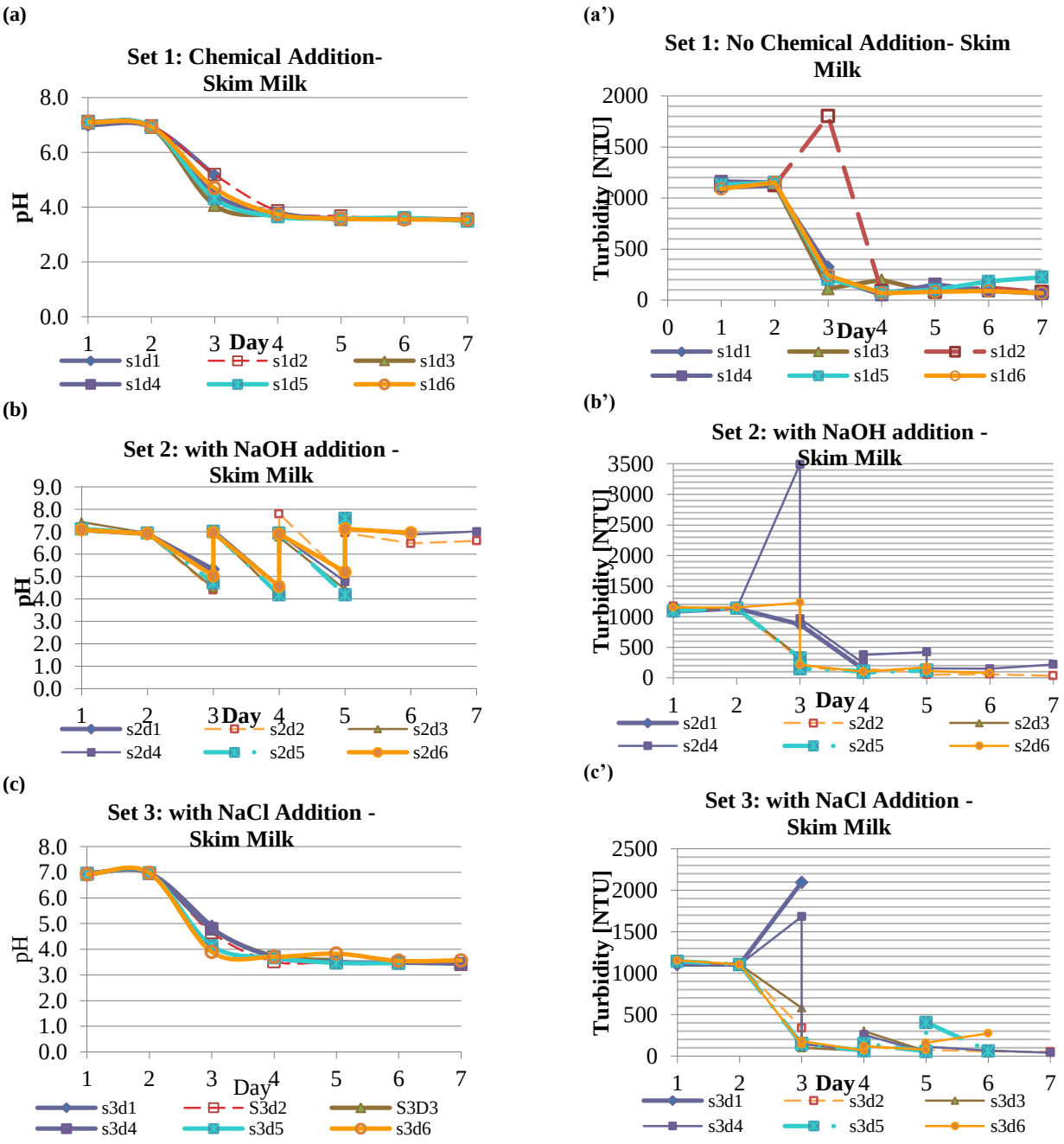


Figure 22. Changes observed in pH and Turbidity of 3 sets of samples, each containing 6 replica of 200 mL batches of 1:10 skim milk: DI water dilutions where the group of curves correspond to the behavior of individual samples s_id1-s_id6; s_i represents sets 1 – 3. sid1-d6

Separation in skim milk could be observed in all batches on the third day of experimentation, while signs of separation or very small formation of separate phases could be observed on the second day (Figure 23). As can be seen from Figure 23, once again the addition of NaOH to the sample reduced the extent of separation for the last two days of experiments. The extent of separation was found to be lower in skim milk samples than the whole milk dilutions, partly attributed to the minimal fat content of the sample. Sets 1 and 3 showed similar trends for separation, while separation in set 2 for days 6 and 7 reduced dramatically, and the samples became re-suspended.

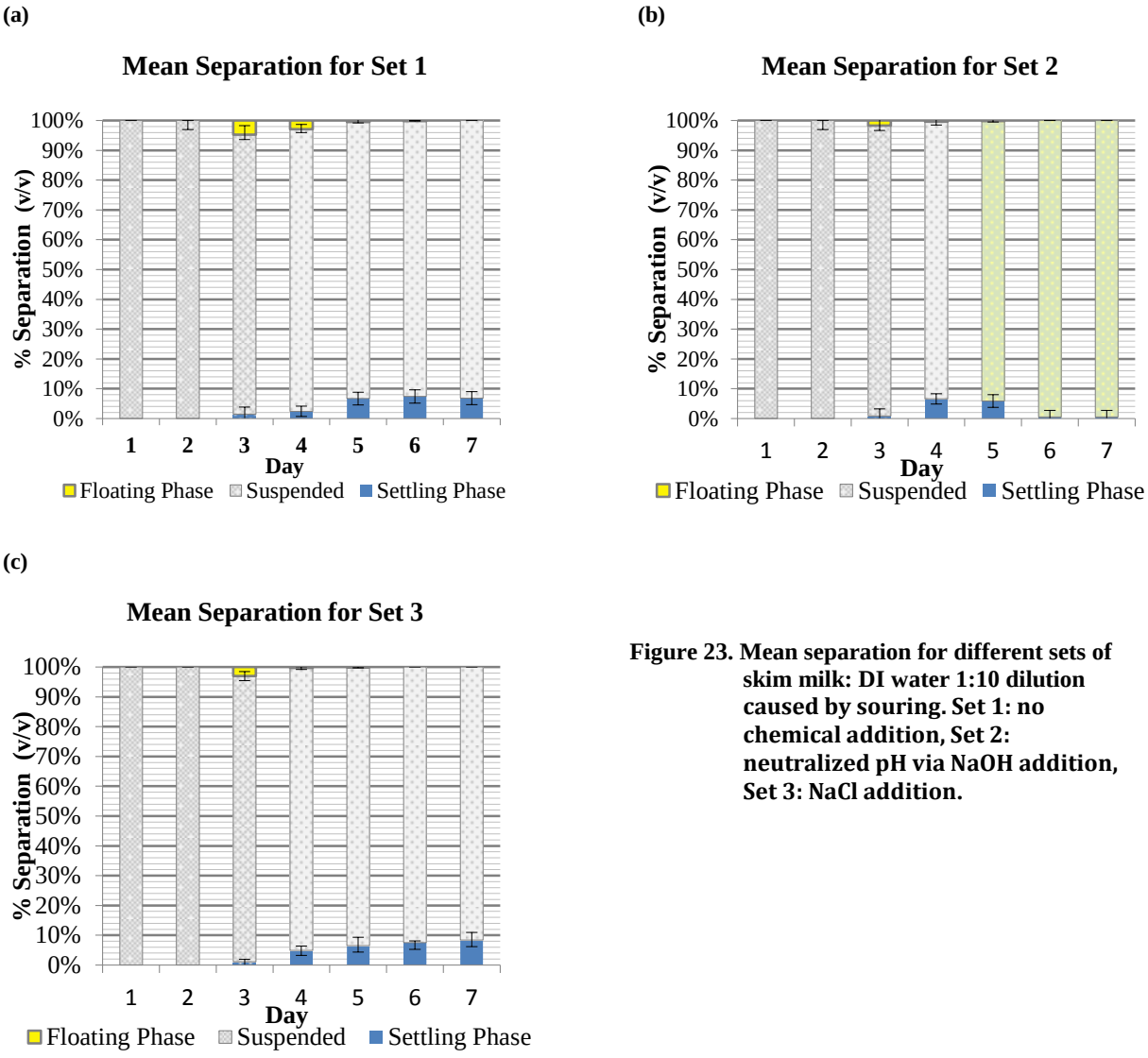


Figure 23. Mean separation for different sets of skim milk: DI water 1:10 dilution caused by souring. Set 1: no chemical addition, Set 2: neutralized pH via NaOH addition, Set 3: NaCl addition.

The TKN and Gerber Fat tests were performed to determine the fractionation of N and fat in the separated layer, and the results are demonstrated in Figure 24. The total nitrogen of diluted skim milk was measured to be 0.806 ± 0.0248 g. The mass percentage of fat of undiluted skim milk was measured to be 0.1%, and determined to be too low for accurate measurements of separation induced by souring of diluted skim milk. The fractionation of nitrogen into different layers appears in Figure 24. As shown aside from the measurements of day 3, the recovery falls within 5% of initial TKN.

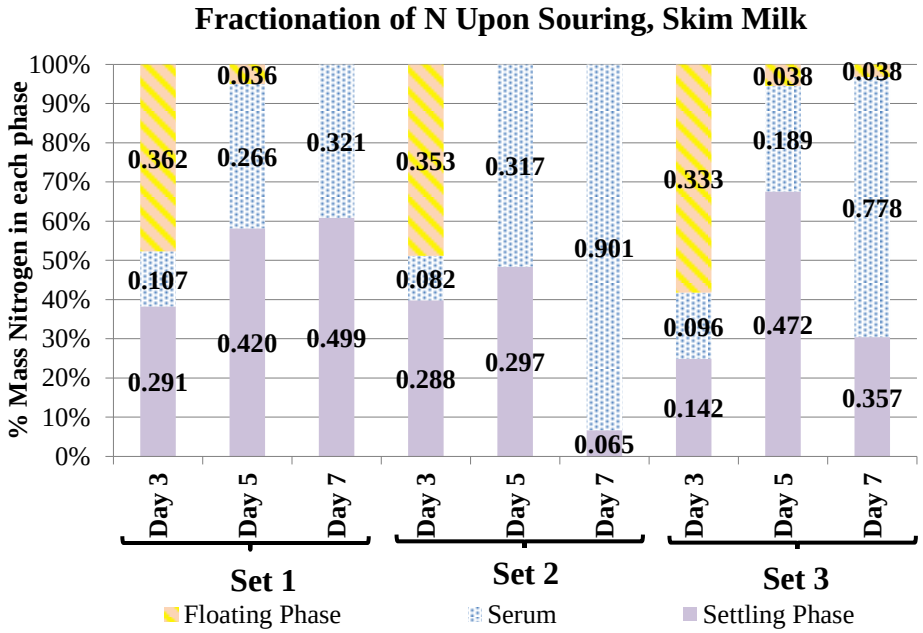


Figure 24. Fractionation of Nitrogen into the Separated Phases during Souring of Skim Milk: DI water 1:10 Dilution

The testing for casein nitrogen content as prescribed by Wehr et al was also conducted on all samples of whole milk and skim milk. It is interesting to note that Day 7 Set 2 for skim milk was the only sample for which a casein phase, with a mass of 0.4920 g, could be obtained. Since proteolysis or the degradation of proteins heavily influences the casein proteins, it can be concluded that for whole milk samples, the serum TKN content on day 7 of the experiment did

not correspond to increase in solubility of the casein micelles at neutralized pH conditions (Walstra et al., 1984).

The deviation between total nitrogen measured on the third day of experiment, and the TKN content of the sample measured on the first day are quite high, with the maximum occurring for Day 3, Set 1 sample (18.0%)[16]. The measurements made on the fifth and seventh day of the analysis all fall within the 5% deviation limit from the initial TKN content of the samples.

As illustrated in Figure 24, the mass of TKN retained in the serum increases from day 3 to day 7 for all sets. Similar to observations made in this experiment, it appears that addition of the base and neutralizing of the pH of the sample during later stages of souring greatly reduces the fractionation potential of dairy constituents, including nitrogen into a separate phase and causes the dairy constituents to enter the serum phase, and appearance of a pale green colored fluid (suspended phase) as oppose to a transparent serum.

Overall the addition of NaOH to the samples caused complete or partial re-suspnsion of the separated material in both skim and whole milk samples. TKN here is assumed as representative of the protein fraction of the system. It can be seen that for sets 1 and 3, the nitrogen content of the serum increases with passing of time despite the persistence of a settling phase. This could be attributed to the degradation of the proteins during souring of milk. The TKN content of the suspended phase for set 2, was greatest among the three sets for day 5 and 7. Although separation and reduction of pH from neutral values to 4.5-4.8 could be seen on day 3, addition of NaOH did not influence separation dramatically from day 3 to day 5 although a greater fraction of TKN was retained within the suspended phase on Day 5 of the experiment.

[16] For detailed report of analyses, refer to Appendix I: Mass Balance of Distributed Nitrogen in the Separated Phases for 1:10 dilution of Skim Milk

This observation shows that neutralizing dairy systems during advanced stages of souring does not retard separation, and although to a smaller extent, separation induced by souring persists in neutralized dairy samples.

As discussed above, souring of dairy constituents induces separation that is not merely pH-induced. Based on results, separation takes place after day 2. Considering the elevated biological activity of wastewater samples, which could potentially increase the rate of souring of dairy products and result in separation before the observed timeframe in laboratory studies. Furthermore, it was seen that during advanced levels of souring (day 6 and 7), the degree of separation reduced. These advanced stages could occur at faster rates in wastewater conditions. Another important factor is that once dairy product mix with the bulk wastewater, a highly diluted mixture of dairy and wastewater will be obtained, which could potentially cause deviation in the behavior of dairy products. As soon as the protein shelter layer is removed, dairy fats will not differ from other fatty material, and it is likely that they will have a high affinity with other FOG components in the wastewater. Furthermore, proteins will form a film on solid surfaces, which could encourage adsorption of organic compounds on the surface and enhance formation of build-up in the collection system. Therefore, physical treatment of wastewaters rich in dairy constituents using GADs is recommended. From this analyses, a longer retention time is recommended for GADs treating ice creamery wastewater, or the incorporation of another compartment to these grease interceptors could be beneficial. For the wastewater discharged from specialty coffee shops, the 3-compartment design with a volume of 1600 gallons was sufficient although frequent maintenance is required to ensure high removal efficiencies.

6. Concluding Research and Field Studies

While FOG blockages have been recognized as one of the major contributors to SSOs (epa.gov), it was seen that in their attempt to address this challenge, many states' definition of FOG may be inconsistent with one another, and coffee and ice cream shops are not always included in FOG control programs. Several cities and states, including Florida, the City of Milwaukee, Oregon, the town of Louisburg and the city of Salisbury in North Carolina, have included such facilities in their FOG ordinance programs, while others leave dairy-serving FSEs to operate under loose conditions. These facilities tend to serve prepared foods and hot/cold beverages, and do not involve preparing/making food. Most states tend not to require these places to install grease traps and consider their FOG input negligible, if at all. However, dairy products can contain high concentrations of fats and proteins, which may separate in the sewerage. Therefore, a study of composition, structure, and properties of milk and other dairy products and their possible contribution to FOG input is necessary. Furthermore, the efficiency of grease interceptors in treating wastewaters rich in dairy components needed to be addressed.

In order to investigate the potential contribution of dairy components to FOG blockages, the chemistry and physics of dairy systems were studied. Milk consists of many colloidal particles, such as fats globules and proteins that can separate. Milk proteins become insoluble at lower pH values (i.e., 4.6). Although the pH of most wastewaters tends to be higher and fall in the neutral range, it should be taken into account that coffee and almost all of the fruits used for flavoring many dairy desserts/beverages acidify the water, and thus can make the conditions more favorable for casein micelles to become insoluble in water. Another important aspect is heating milk for the preparation of hot beverages. Heat coagulation of caseins occurs, as well as damage to the fat globule membrane, thus more rapid coalescence of fat globules may take place.

This can be readily observed during heating of milk. Furthermore, heating increases the acidity of milk, and coupled with pH reduction from coffee, it is likely that caseins will become insoluble. Heating also causes denaturation of whey proteins, and they will become entangled with the casein micelles. Moreover, whipping and cooling dairy products, as seen in whipped cream and ice creams, also cause crystallization of fat crystals and their stronger association with one another, making flocculation of fats more likely.

Having examined the physics and chemistry of dairy products and wastewater characteristics, it was hypothesized that partitioning and separation of different phases, namely fats and proteins occurs in wastewater originating from coffee shops and ice cream shops. It should also be mentioned that proteins may start to settle at higher temperatures, making the adsorption of fat globules at the air-water interface more likely. Thus a combination of both substances will be present in the floating phase. Another inevitable and noteworthy factor is spoiling of milks, which is accompanied by production of many acids, such as acetic acid and lactic acids. Finally, the effects of different surfactive material used for cleaning should also be considered as they will influence the surface tension and may delay the separation process.

The literature review was followed by several characterization studies including dairy: water and dairy: detergent: water dilutions under different pH and temperature conditions. Separation was clearly seen under different conditions. Separation took place in two forms of settling and floating phases. Under acidic conditions, curdling of the dairy systems, dissolution of caseins and separation of fats and proteins took place (Webb et al., 1975, Walstra, et al., 1984), which was observed in the laboratory. Whether the dissociated curd layer floats or settles depends on the fat content of the mixture. The effects of temperature on milks were also studied, and creaming (formation of a fat film at the air/water interface) was observed. Creaming,

however, was retarded by mixing, a practice commonly employed in coffee shops. Furthermore, it was observed that for heated whole milk samples, at pH 5.2-5.5, fine particles formed, and a higher resistance to curd separation was witnessed. Raising the temperature of the mixture to 50^0C resulted in a more rapid settling/floating. The maximum separation occurred as pH approached the zero point of charge (4.66) in all samples. Finally, introduction of detergents and sterilizers slightly enhanced separation of dairy constituents at lower pH values [4-5.3].

To quantify the FOG available in both real and synthetic wastewater samples, the Standard Method for FOG analysis (5520B, Eaton et al., 2006) was employed and found to be incompatible with dairy systems since mixing of non-polar hexane with dairy constituents, exhibiting different degrees of molecular polarity, created a viscous gelatin layer (Rossel et al., 1991). The Gerber Fat Method, adopted from the Standard Methods for the Examination of Dairy Products, was used for determination of fat content of samples (Wehr et al., 2004). The fat content of synthetic and fresh wastewater samples, collected from the University of Maryland's Coffee Bar, was successfully determined by this method although the fat content of the wastewater samples were very low (averaged mass percentage at 0.02±0.004% and 0.03 ±0.003% for 1^{st} and 2^{nd} shifts).

It was found that more than 92% of all the dairy fat will reside in the separated curd layer, with only a small fraction remaining in the suspended solution/serum for samples of higher fat content, such as ice cream and whole milk dilution (3.7% to 3.4% for ice cream and whole milk 1:10 dilutions). It was seen that in samples of low fat milk (i.e., 2% and skim), 100% of the fat resided in the curd

Sampling of grease interceptors treating wastewater originating from two dairy-using FSEs, (a specialty coffee shop, and an ice creamery) took place. Four visits to the interceptor of

the ice cream shop were made. During the initial 2 visits, the grease interceptor was excessively full. Substantial separation was witnessed for the two visits. The samples appeared to have fractioned predominantly in form of a floating phase, however, disrupting the samples resulted in sinking of a portion of the separated material, and separation as both floating and settling layers could be observed. The last two visits took place after the most recent cleaning, after which no significant and quantifiable separation could be observed in the samples, which may have been caused by the minimal sale of dairies expected during the cold season. The pH levels of all the collected samples were found to fall close to the ZPC pH [4.5-5]. The extent of separation in this interceptor grew from inlet to outlet compartments, and hence separation of ice cream products may be slow paced. Furthermore, during the first two weeks of study, prior to the purge of 1/10/11, the degree of separation seemed to have reduced, particularly in the outlet compartment, signaling the flow of material out of the interceptor and into the sewer lines.

The grease interceptor of a specialty coffee shop was also studied, and separation in form of both floating and settling phases was observed. The initial visit to this interceptor took place within 1-2 weeks from the last purge date, and retention of dairy constituents prone to separation, including fats could be observed. The mass fraction of fat in the three phases of floating, suspended, and settling phases increased during the first two weeks, and little change was observed from the second to third week of study. This fraction of separated material reduced moving from inlet to outlet compartment. Mass fractions of fat in all phases for the 3 samples were determined. The fat content of the floating phase was analytically measured to be greater than 100%. The floating phase did, however, resemble other fatty material. The settling phase followed the floating phase in highest fat content. The mass of fat available in the settling phase reduced from inlet to outlet chambers. Finally and similar to observations made in synthetic

samples, the suspended phase had the smallest fraction of fat. The pH of all the samples also fell close to ZPC [4.5-5], and as already studied at this pH range, separation of casein proteins and fats is expected.

The retention time of the ice cream shop interceptor was calculated to be approximately 2 days. It should be noted that once the samples were transferred to the lab for further testing and allowed to remain in a static condition, higher degrees of separation in almost all samples occurred. Overall, the specialty coffee shop interceptor appeared to successfully treat the wastewater and separate dairy constituents prone to separation from bulk wastewater. Concluding from the initial two visits to the ice cream shop interceptor separation of dairy material appeared to take place. Nevertheless, this interceptor was not well maintained for the beginning phase of sampling, and the visits following the latest purge date do not represent common conditions found in warmer seasons when the sale of dairy desserts/produce is higher.

While considerable separation could be witnessed in the grease interceptors, neutralizing the synthetic wastewater samples during a 7-day study appeared to significantly reduce the extent of separation in the samples for advanced souring stages (after day 5) while separation of constituents in the initial phase (day 1-5) persisted. In this 7-day experiment, transfer of TKN from the separated phases to the serum occurred in all samples. The samples with NaOH addition held the greatest fraction of fat in the suspended/serum phase. It was also seen in the samples with NaCl that the fat available in the serum remained constant in the serum phase (0.019 gr) during the 7 days, signaling salting out of the dairy fat with the addition of salts to the sample. Taking into account the salinity of wastewater samples, salting out of dairy fat could potentially be taking place in bulk wastewater.

Although the addition of NaOH to maintain near-neutral pH appeared to reduce the separation tendencies of dairy constituents in synthetic wastewater for advanced stages of souring (i.e., day 6 and 7), the affinity of fats and other dairy constituents with other organic compounds found in wastewater systems should also be considered. The association of dairy compounds with other FOG or organic material in the wastewater systems could potentially cause or contribute to the accumulation of FOG in the sewer lines. Salting out of the proteins and fats could also take place in wastewater samples, regardless of pH, and resulting in partitioning of dairy constituents en route to the treatment facilities. Overall, it is expected that dairy products in wastewaters from dairy shops and specialty coffee shops will separate, and installation of GADs can be considered as a treatment option.

In summary, it was seen that significant separation could be witnessed in the GADs at acidic pH values, and although neutralization of pH via NaOH addition was found to reduce the degree of fractionation of dairy constituents upon spoiling, some separation still persisted. Furthermore, it should be noted that while the dairy constituents did remain suspended to a greater extent in neutralized samples, once exposed to bulk wastewater, they could associate with other organic and FOG material in bulk wastewater and contribute to build up. Therefore based on observations made in laboratory settings, and inspection of GADs, physical treatment of wastewater originating from dairy-based food service establishments is recommended to avoid entanglement of dairy constituents and their potential contribution to FOG buildup within the sewer lines.

7.0 Future Work and Recommendations

As part of future work, since one of the key aspects of this study is to reduce FOG blockages, it is important to study the grease interceptors treating wastewaters rich in dairy components more thoroughly. The sampling for this study took place during colder months, when the sale of frozen desserts and dairy beverages high in fat and proteins are low if not minimal. Therefore, it would be constructive to continue this study in warmer months and study how the introduction of different dairy products in the wastewater influences the overall partitioning behavior of the wastewater and monitor the efficiency of interceptors in removing the separable material. Furthermore, as already discussed, temperature affects the stability of dairy suspensions. In cooler temperatures, crystallization/ partial crystallization of fats and proteins takes place, which makes them more partial to coagulation. The crystallized/partially crystallized fat globules will no longer consist of liquid fat, but rather needle-like crystals, which when agitated can pierce the membrane, and enhance coalescence of fats (Goff, et al. 2003). Higher temperatures can cause denaturation of membrane proteins and whey proteins, causing creaming of milk products (Walstra et al., 1984). It would be thus important to study the interceptor during longer periods of time and investigate the effects of composition and temperature on the behavior of dairy wastewater.

Another focus of future study should be the analysis of the effects of detergent-grade surfactants on dairy emulsion stability. During characterization studies, it was seen that addition of detergents and sterilizers slightly enhanced pH-induced separation in synthetic samples. As already explained and demonstrated in Figure 25, the proteinaceous membrane covering the fat globules and the casein micelles surrounding the globules lower the interfacial tension between fat and water, and in doing so stabilize the emulsion. Addition of surfactive material such as detergents, however, can cause displacement of casein proteins and other emulsifying agents found in dairy systems by surfactants. Although surfactants are reduce the free Gibbs energy of the emulsion more than proteins do, they provide a thinner shelter for the fat globule, and hence make the system more susceptible to subsequent separations (Goff, et al., 2003). Furthermore, some surfactants can potentially cause deformation of proteins in fat globule membranes, leaving a larger area of the fat exposed (Goff, et al. 2003).

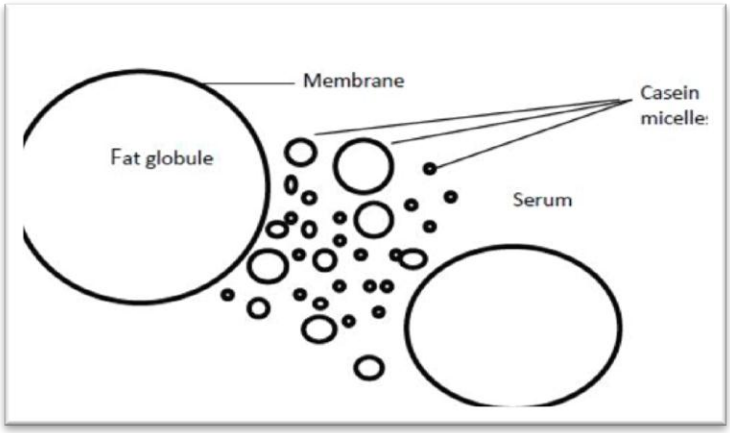


Figure 25. Dairy composition and Structure, picture reproduced from Walstra et al., Figure 1.1.

Appendix I

Extent of Separation in the Ice Cream Shop's Grease Interceptor

Table 13. Extent of Separation in the Ice Cream Shop's Grease Interceptor - On Site Measurements

Date	Compartment	$H_{Floating\ Layer}/H_{ww}$			$H_{Settling\ Layer}/H_{ww}$			$H_{Suspended\ Layer}/H_{ww}$		
	Inlet Comp.	3.0%	±	0.3%	3.0%	±	0.3%	94.0%	±	0.3%
12/20/2010	Middle Comp.	5.9%	±	0.5%	0.0%	±	0.0%	94.1%	±	0.5%
2:00 PM	Outlet Comp.	23.2%	±	28.1%	0.0%	±	0.0%	76.8%	±	28.1%
	Inlet Comp.	4.2%	±	2.6%	1.3%	±	2.3%	94.5%	±	1.8%
1/6/2011	Middle Comp.	6.2%	±	1.3%	0.0%	±	0.0%	93.8%	±	1.3%
1:00 PM	Outlet Comp.	11.0%	±	6.6%	0.0%	±	0.0%	89.0%	±	0.0%
	Inlet Comp.	0.0%	±	0.0%	0.0%	±	0.0%	100.0%	±	0.0%
1/14/2011	Mid Comp.	0.0%	±	0.0%	0.1%	±	0.1%	99.9%	±	0.1%
1:00 PM	Outlet Comp.	0.0%	±	0.2%	0.0%	±	0.0%	99.8%	±	0.0%
	Inlet Comp.	0.0%	±	0.0%	0.0%	±	0.0%	100.0%	±	0.0%
1/20/2011	Mid Comp.	0.0%	±	0.0%	0.0%	±	0.0%	100.0%	±	0.1%
1:00 PM	Outlet Comp.	0.0%	±	0.0%	0.0%	±	0.0%	100.0%	±	0.0%

Table 14. Extent of Separation in the Ice Cream Shop's GAD – Laboratory Measurements

Date	Compartment	pH	$H_{Floating\ Layer}/H_{ww}$		$H_{Settling\ Layer}/H_{ww}$		$H_{Suspended\ Layer}/H_{ww}$	
Monday	Inlet Comp.	5.0	9.6% ±	0.6%	12.8% ±	1.9%	77.6% ±	2.0%
12/20/2010	Middle Comp.	4.6	0.0% ±	0.0%	13.0% ±	0.5%	87.0% ±	0.5%
5:00 PM	Outlet Comp.	4.4	0.0% ±	0.0%	40.0% ±	0.0%	60.0% ±	0.0%
Thursday	Inlet Comp.	4.8	1.4% ±	0.0%	13.8% ±	0.0%	84.8% ±	0.0%
1/6/2011	Middle Comp.	4.5	0.1% ±	0.0%	11.6% ±	0.0%	88.3% ±	0.0%
4:00 PM	Outlet Comp.	4.5	0.1% ±	0.0%	17.4% ±	0.0%	82.5% ±	0.0%
Friday	Inlet	4.6	0.0% ±	0.0%	0.0% ±	0.0%	100.0% ±	0.0%
1/14/2011	Mid Comp.	4.5	0.0% ±	0.0%	0.1% ±	0.0%	100.0% ±	0.0%
4:00 PM	Outlet	4.9	0.0% ±	0.0%	0.2% ±	0.0%	100.0% ±	0.0%
Thursday	Inlet	5.0	0.0% ±	0.0%	0.0% ±	0.0%	100.0% ±	0.0%
1/20/2011	Mid Comp.	4.5	0.0% ±	0.0%	0.0% ±	0.0%	100.0% ±	0.0%
4:00 PM	Outlet	4.6	0.0% ±	0.0%	0.0% ±	0.0%	100.0% ±	0.0%

Extent of Separation in the Specialty Coffee Shop's Grease Interceptor

Table 15. Extent of Separation in the Specialty Coffee Shop GAD – On-Site Measurements

Date	Compartment	$H_{Floating\ Layer}/H_{ww}$	$H_{Settling\ Layer}/H_{ww}$	$H_{Suspended\ Layer}/H_{ww}$
Thursday	Inlet Comp.	4.7% ± 2.9%	16.3% ± 5.47%	79.0% ± 7.2%
1/6/2011	Middle Comp.	3.3% ± 1.5%	13.0% ± 11.3%	83.7% ± 11.7%
11:00 AM	Outlet Comp.	0.3% ± 0.0%	0.0% ± 0.0%	99.7% ± 0.0%
Friday	Inlet	10.6% ± 1.9%	23.3% ± 3.71%	99.9% ± 0.0%
1/14/2011	Mid Comp.	7.0% ± 1.4%	14.2% ± 3.70%	99.9% ± 0.1%
11:00 AM	Outlet	0.2% ± 0.0%	0.0% ± 0.0%	99.8% ± 0.0%
Thursday	Inlet	10.5% ± 5.5%	17.7% ± 1.20%	71.8% ± 6.4%
1/20/2011	Mid Comp.	7.9% ± 0.3%	11.9% ± 1.62%	80.2% ± 1.4%
11:00 AM	Outlet	0.9% ± 0.8%	2.6% ± 2.73%	96.5% ± 0.0%

Table 16. Extent of Separation in the Specialty Coffee Shop GAD – Laboratory Measurements

Date	Compartment	pH	$H_{Floating\ Layer}/H_{ww}$	$H_{Settling\ Layer}/H_{ww}$	$H_{Suspended\ Layer}/H_{ww}$
Thursday	Inlet Comp.	4.3	4.8% ± 0.0%	23.4% ± 0.0%	71.9% ± 0.0%
1/6/2011	Mid Comp.	4.4	1.2% ± 0.0%	36.6% ± 0.0%	62.2% ± 0.0%
4:30 PM	Outlet	4.4	0.6% ± 0.0%	9.3% ± 0.0%	90.1% ± 0.0%
Friday	Inlet	4.2	9.5% ± 0.0%	58.4% ± 0.0%	32.2% ± 0.0%
1/14/11	Mid Comp.	4.6	5.3% ± 0.0%	44.7% ± 0.0%	50.0% ± 0.0%
4:30 PM	Outlet	4.5	0.5% ± 0.0%	9.0% ± 0.0%	90.5% ± 0.0%
Thursday	Inlet	4.2	19.5% ± 0.0%	58.4% ± 0.0%	32.2% ± 0.0%
1/20/11	Mid Comp.	4.4	2.5% ± 0.0%	44.7% ± 0.0%	50.0% ± 0.0%
4:30 PM	Outlet	4.2	0.5% ± 0.0%	9.0% ± 0.0%	90.5% ± 0.0%

Mass Balance of Distribution of Nitrogen and Fat in 1:10 Whole Milk: DI Water Dilution

Table 17. Mass of Total Kjeldahl Nitrogen and Fat Recovered in the separated phases, 1:10 Whole Milk: DI Water Dilution

	Separated Phases	N (g)	Fat (g)
Day 3, Set 1	Floating Phase	0.0997	0.135
	Serum	0.495	0.095
	Settling Phase	0.291	0.325
	Σm_i [17] (g)	0.885	0.555
	% Deviation from initial measurement of fresh milk	5.34%	2.56%
Day 3, Set 2	Floating Phase	0.000	0.000
	Serum	0.614	0.097
	Settling Phase	0.288	0.483
	Σm_i (g)	0.902	0.580
	% Deviation from initial measurement of fresh milk	3.60%	-1.71%
Day 3, Set 3	Floating Phase	0.000	0.0000
	Serum	0.782	0.0194
	Settling Phase	0.142	0.588
	Σm_i (g)	0.924	0.607
	% Deviation	1.19%	-3.52%
Day 5, Set 1	Floating Phase	0.000	0.0000
	Serum	0.475	0.0967
	Settling Phase	0.420	0.450
	Σm_i (g)	0.895	0.547
	% Deviation from initial measurement of fresh milk	4.35%	4.08%
Day 5, Set 2	Floating Phase	0.000	0.0000
	Serum	0.596	0.195
	Settling Phase	0.297	0.401
	Σm_i (g)	0.893	0.597
	% Deviation from initial measurement of fresh milk	4.55%	-4.66%

[17] Mass of nitrogen/fat obtained for each phase

Table 17 Continued. Mass of N and Fat Recovered in the separated phases, 1:10 Whole Milk: DI Water Dilution

	Separated Phases	N [gr]	Fat [gr]
Day 5, Set 3	Floating Phase	0.000	0.000
	Serum	0.413	0.019
	Settling Phase	0.472	0.523
	Σm_i (g)	0.886	0.542
	% Deviation from initial measurement of fresh milk	5.32%	-0.45%
Day 7, Set 1	Floating Phase	0.000	0.000
	Serum	0.382	0.194
	Settling Phase	0.499	0.363
	Σm_i (g)	0.881	0.557
	% Deviation from initial measurement of fresh milk	5.79%	2.31%
Day 7, Set 2	Floating Phase	0.000	0.000
	Serum	0.809	0.497
	Settling Phase	0.0647	0.0523
	Σm_i (g)	0.874	0.549
	% Deviation from initial measurement of fresh milk	6.55%	3.61%
Day 7, Set 3	Floating Phase	0.000	0.000
	Serum	0.524	0.0192
	Settling Phase	0.357	0.499
	Σm_i (g)	0.881	0.518
	% Deviation from initial measurement of fresh milk	5.82%	2.71%

Mass Balance of Distributed Nitrogen in the Separated Phases for 1:10 dilution of Skim Milk

Table18. Fractionation of Nitrogen in the separated phases of (1:10) Skim Milk: DI Water Dilution

		Total Mass (g)	N (g)
Day 3, Set 1	Floating Phase	4.88	0.362
	Serum	194	0.107
	Settling Phase	0.402	0.187
	Σm_i[18](g)		0.656
	% Deviation		18.0%
Day 3, Set 2	Floating Phase	5.33	0.353
	Serum	214	0.082
	Settling Phase	0.698	0.253
	Σm_i (g)		0.687
	% Deviation		14.2%
Day 3, Set 3	Floating Phase	6.440	0.333
	Serum	213	0.096
	Settling Phase	0.429	0.253
	Σm_i (g)		0.68
	% Deviation		14.8%
Day 5, Set 1	Floating Phase	0.797	0.036
	Serum	198	0.265
	Settling Phase	0.674	0.623
	Σm_i (g)		0.888
	% Deviation		-4.32%
Day 5, Set 2	Floating Phase	0.000	0.000
	Serum	209	0.317
	Settling Phase	0.576	0.574
	Σm_i (g)		0.892
	% Deviation		-0.59%
Day 5, Set 3	Floating Phase	0.603	0.0383
	Serum	209	0.189
	Settling Phase	0.576	0.576
	Σm_i (g)		0.803
	% Deviation		5.17%

[18] Mass of nitrogen/fat obtained for each phase

Table18 Continued. Fractionation of Nitrogen in the separated phases of (1:10) Skim Milk: DI Water Dilution

		Total Mass (g)	N (g)
Day 7, Set 1	Floating Phase	0.000	0.000
	Serum	235	0.321
	Settling Phase	5.46	0.695
	Σm_i (g)		1.02
	% Deviation		-4.45%
Day 7, Set 2	Floating Phase	0.000	0.000
	Serum: Non Casein N	233	0.409
	Casein in Serum	5.19	0.492
	Settling Phase	2.12	0.0680
	Σm_i (g)		0.970
	% Deviation		0.25%
Day 7, Set 3	Floating Phase	0.409	0.0380
	Serum	237	0.778
	Settling Phase	0.576	0.139
	Σm_i (g)		0.955
	% Deviation		1.73%

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