RELATIONSHIP AMONG MILK DENSITY, COMPOSITION, AND TEMPERATURE

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ABSTRACT

RELATIONSHIP AMONG MILK DENSITY, COMPOSITION, AND TEMPERATURE

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Equations for estimating milk density at 4°C from fat, protein, and lactose and other solids (LOS) contents were developed based on data collected over a whole year from Ontario and Alberta milk producers. Density was measured by an Anton Par Model DMA 45 density meter. Although milk density was mainly explained by composition, including seasonal factors in equations increased accuracy. "Weight over weight" to "weight over volume" conversions of milk components employing the developed equations were equivalent to the calculation with empirical density. The average difference between estimated and actual w/v values was 0.000 with the standard deviation of 0.002. Density of producer milk at 4, 16, 28, and 40°C was determined, and an equation for density estimation at any temperatures between 4 and 40°C was constructed. A formula with a cubic term of the temperature exhibited the best density prediction with the standard deviation of residuals of 2.31e-4.

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1. Introduction

Milk payment systems for farm milk vary from country to country. Some countries determine the price of milk in per unit volume, for example \$/L, and others in per unit weight, such as \$/kg (Bulletin of the IDF 305, 1995). In a survey conducted by the International Dairy Federation (IDF) during the period of 1992/1993, 14 countries out of 22 indicated that their calculation of a compositional price for producer milk is based on a standard milk price, while 12 countries, including Canada, assess the price according to the composition criteria. The composition criteria also differ among countries. Many payment schemes started by paying on fat only, mainly because this was the only parameter which could be easily tested for a large number of samples. However, as testing methods have improved, schemes have become sophisticated to encourage farmers to provide milk for different market needs (Harding, 1995). Milk fat is, therefore, the most widely used component, determining milk price in 21 countries. Eighteen countries adopt crude (total) protein, the amount of protein including non-protein-nitrogen (NPN), as a parameter, whereas France and Australia use true protein, which is protein without NPN, as the value of protein. Other components used for milk assessment are solids-non-fat, lactose, and milk solids (Bulletin of the IDF 305, 1995).

At the present time, Canadian milk producers are paid according to the amount of milk components they produce, namely, fat, protein, and lactose and other solids (LOS) (LOS is defined as total solids – fat – protein). Milk composition is determined by infrared automated milk analyzers which are calibrated using reference milk samples. Composition of reference milks is determined by wet chemistry on a weight over weight (w/w) basis at 20°C, while bulk milk is measured at farms in units of volume (hL) at 4°C.

Therefore, composition results must be converted from weight over weight (w/w) unit at 20°C to weight over volume (w/v) unit at 4°C. The simplest procedure to achieve this conversion is to multiply the w/w results by the density of the milk at 4°C. Accurate estimation of milk density at 4°C (ρ_4) is, therefore, necessary in this payment system. Most jurisdictions use a constant density factor to effect weight/volume conversions. The difficulty with this practice is that milk density varies with composition (USDA, 1965; Walstra, 1984).

A preliminary survey conducted by Paul Sauvé, Canadian Laboratory Services, identified five different procedures, used by Canadian provinces, to convert w/w values to w/v values (Kouaouci *et al.,* 1997). In British Columbia, Alberta, Saskatchewan, and New Brunswick, the w/w values are multiplied by 1.02969, while Manitoba uses a factor of 1.032. These procedures assume that milk density is constant regardless of the variation in composition. The province of Quebec is unique among the provinces, because it includes empirically measured density values in the reference results used to calibrate the infrared milk analyzers. The provinces of Ontario, Prince Edward Island, and Nova Scotia use calibration standards provided by the Laboratory Services Division University of Guelph (LSD). In this system reference values for fat, protein and total solids are determined on a w/w basis. In fat conversion, the milk density at 20°C varies according to the fat content (Table 1.1). Calculated w/v values at 20°C are then converted to w/v at 4°C using the expansivity factor of fat derived from a fat content-expansion table (Table 1.2). The expansivity derived from fat is also applied to protein, LOS and or total solids at their respective levels.

Table 1.1: Whole milk density at 20°C used to convert w/w fat content to w/v at 20°C for the purpose of milk analyzer calibration in Ontario.

Fat w/w (%) of reference samples	**Density at 20°C used for w/v conversation of fat content (g/cm^3)**
0-0.99	1.032
1.00-1.99	1.031
2.00-2.99	1.030
3.00-3.99	1.029
4.00-4.99	1.028
5.00 and more	1.027

Table 1.2: Fat content–expansion factor for conversion of w/v at 20°C results to w/v at 4°C for the purpose of Ontario milk pricing.

The expansivity factor is added to value of w/v at 20°C according to its fat content.

Fat w/w (%) of reference samples	**Expansivity factor to be added**
0.00-1.56%	0.00
1.57-3.79%	0.01
3.80-5.50%	0.02
5.51-6.95%	0.03
6.96% and more	0.04

Previous workers, Kouaouci *et al.* (1997), evaluated the effects of different conversion procedures using a set of real milk composition values ranging from 2.8 to 6% fat. They observed a wide range of difference in fat and protein estimates among provinces. This study recommended conducting an investigation to define the relationship between milk composition and milk density at 4°C.

Kouaouci *et al.* (1997) reported the interim project progress based on 276 Ontario observations and 117 Quebec observations in July 1997. The study showed that the average milk density in Ontario and Quebec is 1.0336, which is higher than conversion factors currently in use by provinces other than Quebec. This report also recommended a model to predict milk density at 4°C from its components for interim use during the 1997/98 dairy year:

$$\text{Density} = -.0002878\ \text{Fat} + .003664\ \text{Protein} + .0008347\ \text{LOS} + 1.01783 \qquad [1]$$

where density is in units of g/cm^3 and milk components are given in units of g/g percent.

However, Kouauci *et al.* stressed that the model above was an interim recommendation only because their results to that point indicated the need of revision to experimental protocol and more observations. For example, analysis of variance (ANOVA) of combined data of Ontario and Quebec milk showed a significant effect of sample source. Equation [1] did not take seasonal effect into account since the data did not cover a whole year. Therefore, an additional experiment is needed to collect data throughout a year, to investigate seasonal effect, and to construct a model for calculating milk density at 4°C, at which temperature the volume of milk is measured at farm.

Further, the effects of temperature on milk density should be investigated in order to develop a model equation for predicting milk density at various temperatures. This will provide adequate information on expansivity of milk as a function of temperature.

Objectives of this research were to:

1. assess seasonal variation in the relationship between milk density and components
2. develop a model to predict the density of Ontario producer milk at 4°C from proximate analysis values
3. test the model using milk from other provinces
4. use the models to simulate the w/w to w/v conversion of composition
5. develop a model to predict the density of Ontario producer milk as a function of w/w

analysis and temperature in the range of 4-40°C

This project is the first large-scale investigation on the density of Canadian milk. Moreover, it should be noted that the provinces from which the data on milk density were collected, namely Ontario, Quebec, and Alberta, produce about 83% of the national milk production (Dairy Farmers of Ontario, 1997) (Table 1.3). Therefore, the outcome of this thesis can be a good depiction of Canadian milk density. The results of this study provide an enhancement to the current milk pricing system in Canada.

Table 1.3: Production of butterfat by province during dairy year of 1996/1997.

Province	**Production M kg Butterfat**	**Percentage**
Prince Edward Island	2.287	1.37
Nova Scotia	4.088	2.44
New Brunswick	3.149	1.88
Quebec	69.041	41.29
Ontario	60.412	36.13
Manitoba	5.586	3.34
Saskatchewan	3.736	2.23
Alberta	10.260	6.14
British Colombia	8.640	5.17
Canada	**167.199**	99.99

Source: Dairy Farmers of Ontario (1997)

2. Literature Review

2.1. Definitions of Density and Specific Gravity

Density of a substance at a specific temperature is defined as the mass divided by the volume at that temperature. It is expressed in kg/m^3 in the SI units. According to the c.g.s. units, density measurements are expressed in grams of mass per cubic centimetre (g/cm^3). However, this absolute density is seldom used. More commonly, density is expressed in grams per millilitre (g/mL) (Lewin, 1972), which is called relative density. In setting up the standards of mass and volume, it was intended that 1 mL should equal 1 cm^3 exactly, but subsequent measurements showed that 1 cm^3 = 0.999973 mL (Lewin, 1972). The difference between grams per cubic centimetre and grams per millilitre is negligible for most purposes. The symbol of density is ρ, and since density closely depends on temperature, it is usually denoted with temperature of samples at which the density was measured. For example the density at 20°C is expressed as ρ_{20}.

Another quantity is the specific gravity (sp.gr.) or specific weight, which is the density of a substance relative to the density of some other substance chosen as a standard, usually water. It is obtained by weighing a given volume of a sample and then dividing the weight by the same volume of water at a specific temperature. Specific gravity can be expressed as $\rho_{product}/\rho_{water}$, and is a dimensionless quantity. When stating the sp.gr., it is desirable to state both the sample and water temperatures. Frequently, they are the same. The sp.gr. of a sample at 20°C relative to water at 4°C can be written as $S.g._4^{20}$. Specific gravity is equivalent to density if the water temperature is 3.98°C, where its density is 1.000 g/mL (999.972 kg/m^3).

2.2. Methods of Measuring Density

The method for density determination can be chosen from several options depending on the needs for speed and accuracy and availability of equipment. The classic methods are hydrometers or lactometers. Hydrometers are hollow glass bodies with a broad bottom and a narrow stem. They rely on the principle that the same body displaces equal weights for all liquids in which it floats. When placed in a uniform glass cylinder filled with the liquid to be tested, the hydrometer sinks. The deeper the hydrometer sinks, the lower is the density of the solution (Giese, 1995). The lactometer, a form of hydrometer specially designed for milk, provides the most rapid method of determining the specific gravity of milk (Vanstone and Dougall, 1960). The lactometer, which was most common for milk, is a combination of thermometer and hydrometer known as the "Quevenne" lactometer. The value recorded is known as the lactometer reading (L.R.). Although it is designed to give a correct reading when used in milk at a temperature of 60°F (15.6°C), specific gravity of milk can be determined in the temperature range from 50 to 70°F (10.0 to 21.1°C) by adjusting the readings. The adjustment is carried out by adding 0.1 to the L.R. for each degree that the temperature exceeds 60°F, and by subtracting 0.1 from the L.R. for each degree that the temperature is less than 60°F. Specific gravity is then calculated using the following formula (Canada Department of Agriculture, 1915):

$$(\text{L.R.} + 1000)/100 = \text{specific gravity} \qquad [2]$$

The density hydrometer is similar to the lactometer but the scale is calibrated to read density at 20°C (68°F) in milk from which the Recknagel's effect (see section 2.5.1) has been eliminated by warming the milk to 40°C and cooling to 20°C (Vanstone and

Dougall, 1960). Hydrometers are suitable for rough determinations. Even with a large amount of sample (25-50 mL), the accuracy is not higher than ±0.001 (Lewin, 1972).

A more accurate, but less rapid method than lactometers, is the Westphal Balance method (Vanstone and Dougall, 1960). The Westphal Balance consists of a beam, one arm of which is graduated and the other is equipped with a counterpoise. A plummet with a volume of 5 mL is hung from the graduated arm and immersed in a cylinder of liquid to be measured (Vanstone and Dougall, 1960). A weight that is equal to the weight of 5 mL of the liquid should be placed on the other end of the beam to keep balance (McKennell, 1960). The specific gravity is determined by the weight needed to counterpoise the beam.

The pycnometer has been one of the most common methods of density determination. It ascertains density by measuring the weight of a known volume of liquid in a vessel, the volume of which has been calibrated in terms of the weight of pure water that the vessel holds (Giese, 1995). Various types of devices have been used for measuring a volume so that the filling and weighing of the vessel are reproducible and convenient. The specific gravity bottle is a type of pycnometer. The bottle has a capacity of 50 mL and is provided with a perforated well-fitting stopper so that exactly the same volumes of different liquids can be weighed (Vanstone and Dougall, 1960).

The Babcock bottle method, having similar mechanisms to that of pycnometers, was used to examine the temperature effect on density of milk and fluid milk products for a large project in the United States (USDA, 1965; Sherbon, 1988). The study used 8% Babcock test bottles, each with a capacity of 50 mL, and experimenters read the changes in volume of weighed samples at various temperatures in the calibrated part of the neck of the bottles. This method was used because lactometers were not available with a range sufficient to test cream, milk, and skim milk.

The density of fluid can also be determined by measuring the distance that a drop of product falls in a density gradient column (Stull *et al.*, 1965; Sherbon, 1988). The drop of liquid rises or falls to a position of floating equilibrium when immersed in a vertical medium of immiscible fluid with a continuous graduation of density as a function of column height. This method has the advantage that it requires as little as 0.1 mm^3 sample and attains a precision of about 5×10^{-6} g/mL (Lewin, 1972).

Measuring density by the density meter has recently become common. In the present study milk density is to be measured by the Anton Par Model DMA 45 digital density meter, which has an accuracy of ±0.0001 g/cm^3 when it is used with a circulating thermostat having an accuracy of ±0.05°C. The measuring principle of the instrument is based on the change of the natural frequency of a hydrogen filled hollow oscillator, when filled with different liquids. The mass, and thus the density of the liquid, changes this natural frequency due to a gross mass change of oscillator caused by the introduction of the liquid. The electronic measurement of the time period is used to automatically calculate the density by a built-in arithmetic processor. The hollow oscillator is also surrounded by another tube through which water is pumped to maintain the sample in the oscillator at the desired temperature. This instrument is calibrated on a daily basis by measuring the density of pure water at 4.0°C and ensuring that the value is within .0001 g/cm^3 of the theoretical value of 1.0000 g/cm^3.

2.3. Density of Liquid

The density of water peaks at the temperature of 3.98°C (Weast, 1984) and decreases by approximately 0.03% per °C rise in temperature (Lewin, 1972). Density of water in grams per cubic centimetre (g/cm^3) and grams per millilitre (g/mL) are shown in Table 2.1.

Table 2.1: Density in grams per cubic centimetre and grams per millilitre of air-free water at 1 atm.

Temperature (°C)	**Density of water (g/cm^3)**	**Density of water (g/mL)**
0	0.999841	0.999868
3.98	0.999973	1.000000
10	0.999710	0.999728
15	0.999102	0.999129
20	0.998207	0.998234
25	0.997048	0.997075
30	0.995651	0.995678

Adopted from *Physical Methods of Chemistry, Part 4*, Lewin (1972) p.62

In the book of *Physical Methods of Chemistry, Part 4*, Lewin (1972) summarized some rules for explaining the influence of molecular composition on liquid density: density increases with increasing molecular weight, polar molecules have greater density than non-polar molecules of similar molecular weight, the branching of a carbon chain usually produces relatively small changes in density.

The molar volume of a compound, defined as molecular weight divided by density, also gives insight into the effect of substances on the density. It indicates the nature of molecular shapes, packing, and forces. For example, ring formation generally leads to a concentration of molar volume, whereas double-bond formation leads to an expansion. The transformation of a double bond into a single bond greatly reduces the molar volume (Lewin, 1972).

In mixed systems, density changes with concentration of the components. For two-component liquid systems, a plot of density versus concentration generally gives a somewhat curved line. When the concentration is expressed in volume units (vol.%, molarity, or g/litre), the plot is more likely to be linear than with weight units. If mixing occurs at all concentrations without a change in volume, the relation between density and concentration is perfectly linear over the whole range. In binary liquid mixtures, the volume change due to mixing depends upon the mixed substances, which have different intermolecular forces. Moreover, the molecular packing structure of the mixture is different from that of the pure components (Lewin, 1972).

2.4. Composition, Structure, and Variability of Milk

2.4.1. Composition

Walstra (1984) discusses in his widely used textbook *Dairy Chemistry and Physics* the diverse composition of milk. Milk is a complex fluid containing many compounds in several states of dispersion. The components include water, fat, protein, lactose, mineral substances, organic acids, and miscellaneous other compounds. In milk fat, triacylglycerols are the major lipid class accounting for 97-98% of the total lipid. In addition, small amounts of several substances such as di- and mono-acylglycerols, free cholesterol and cholesterol esters, unesterified fatty acids, and phospholipids are present (Christie, 1983). Milk proteins include several kinds of proteins, namely caseins, which are insoluble at pH 4.6, and whey or serum proteins. Caseins represent about 76% of the total milk proteins. Milk also contains a number of minor proteins such as enzymes. The protein content is often calculated by multiplying total nitrogen by a factor of 6.38. However, it should be noted that 4 to 8% of the nitrogen of milk is present in the form of small molecules and is called non-protein-nitrogen (NPN) (Walstra, 1984; Bulletin of IDF, 1995; Goff and Hill, 1993; Jenness, 1988). Lactose is a reducing disaccharide composed of glucose and galactose, giving a slightly sweet taste. The minerals in milk are inorganic salts, partly ionized and partly present as complex salts. Finally, milk has many miscellaneous components, such as vitamins.

2.4.2. Structure

Physical structure is as important as composition in determining properties of milk. Main structural elements are fat globules, casein micelles, and serum. Milk fat exists in the form of globules surrounded by a membrane called milk fat globule membrane (MFGM), which maintains the integrity of the globules and separates them from the aqueous environment. MFGM, occupying about 2% of the mass of the total fat globules, consists mainly of polar lipids and proteins, and many enzymes (Christie, 1983). It prevents flocculation and coalescence of fat globules and protect the fat against enzyme action. Milk fat and milk fat globule are not identical because about half of MFGM is not lipid materials and about 0.4% of the fat of milk is found outside the globules (Walstra, 1984). Milk minus fat globules is called milk plasma. Casein micelles consist of water, casein, salts, and some minor components such as lipase and proteinase. A slight amount of casein is found in solution, not in the micelles.

2.4.3. Variability

Although all cows' milk contains the basic elements of fat, protein, lactose, and other solids, there is considerable variation such as the ratio of constituents or size and stability of structural elements. This may be caused by natural variation or changes occurring after the handling or processing of milk (Walstra, 1984).

Natural variation may be caused by the differences in genetic characteristics between breeds or between individuals and in physiological conditions such as stage of lactation or age of cows and environment, including feeding, climate, season, and stress (Walstra, 1984).

Breed variation

In the short term, the main factor available to the farmer to alter milk composition is selection of breed. Table 2.2 lists the composition of milk of various breeds.

Table 2.2: Typical composition (w/w %) of milk of various breeds.

Breed	Fat	Protein	Total solids
Holstein	3.54	3.29	12.16
Ayrshire	3.95	3.48	12.77
Guernsey	4.72	3.75	14.04
Jersey	5.13	3.98	14.42
Brown Swiss	3.99	3.64	13.08

(Goff and Hill, 1993)

Age of cows

As cows advance in age, milk fat percent tends to decline.

Lactation variation

Milk fat contents are high soon after calving, decline to the lowest point from the third to seventh month of lactation, and generally increase toward the end of lactation.

Season

In Ontario, Canada, maximum annual fat contents occur during the winter months, usually peaking in November or December. Minimum fat contents occur in August. Seasonal trends in protein contents follow a similar trend with some differences. The seasonal variation is not as great. The minimum occurs in July, and the maximum occurs in October (Goff and Hill, 1993).

Climate

The principal effects of climatic and regional factors are due to variation in feed and stage of lactation (Goff and Hill, 1993).

Individual cow variation

Individual cow's milk varies according to the factors above and other factors such as inheritance and condition of the cow. In 1966, low and high milk fat tests for different cows for all months in the University of Nebraska dairy herd were as follows: Brown Swiss 3.0 to 7.6, Guernsey 3.7 to 7.4, Holstein 2.4 to 9.0, and Jersey 3.1 to 9.1 (University of Nebraska, 1967).

Generally, the individual cow's milk varies more than herd milk, and the bulk milk involving several herds has less variation than does single herd milk (Boden, 1942). Therefore, handling milk in large quantities reduces the variations.

2.5. Factors Affecting Milk Density

The density of milk is the summary result of the densities of its various components. It is dependent on the amount of dissolved or suspended matter, changes in chemical composition of the constituents, and variations in physical states of components. Thus, milk density is influenced by various factors such as temperature history of samples, biological differences of milk, and processing of milk. Among the various constituents, the variation in fat content is known to be the main cause in milk density variation (Davies, 1936; Walstra, 1984). However, according to Sherbon (1988), variations in the composition of fat and in the proportions of lactose, proteins, and salts may influence the milk density much less than variations due to the physical state of fat.

2.5.1. Recknagel's phenomenon

Increased density of cold stored milk has been known for a long time. Recknagel (1883) was the first researcher to determine some of the conditions under which the increase in specific gravity occurred (Sharp and Hart, 1936). Thus, this increase in density during storage was named Recknagel's phenomenon. He showed that the escape of air bubbles was not the factor but attributed the increase to an increase in the hydration of the casein at the low temperatures. However, he did not study skim milk. Toyonaga (1898) related the increase to the solidification of fat and found that the process could be repeated by warming and cooling the sample. No increase in specific gravity was observed in almond oil emulsified in gum solution, but 4% emulsion of milk fat in gum solution showed the same increase on standing at 15°C as did whole milk. Richmond (1920), from observation in the variation of the specific heat of milk on standing, stated that most of the rise in density was caused by the solidification of the fat. Sharp and Hart (1936) confirmed that the fluctuation of the value of specific gravity was due to the variation in physical states of fat. The liquid-solid fat ratio played an important role because solid fat has a higher specific gravity than liquid fat at the same temperature (Herrington, 1964). Sharp *et al.* reported significant differences in specific gravity between the samples held 24 hours at 2°C and then warmed to desired temperatures (15 and 30°C), and the same milk that was held at 45°C for 30 seconds and then cooled to those temperatures. They concluded that the fat caused the variations because fat-free milk showed no such variation and the variation in whole milk was linearly related to the fat content. This study suggested that the specific gravity used in calculating the composition of milk should be determined when the fat is in a definitely known and reproducible physical state, which was the liquid state.

Other possible factors causing Recknagel's phenomenon are the degree of hydration of the proteins (Davies, 1936; Sherbon, 1988) and change in the casein (Vanstone and Dougall, 1960).

2.5.2. Temperature history

To avoid Recknagel's phenomenon, milk should be either heated to liquefy the fat or held sufficiently long to allow attainment of maximum density (Boden, 1942). The former method, first suggested by Sharp and Hart (1936), is carried out by warming milk samples to 40-45°C for one-half to five minutes to ensure that all fat is in liquid state, followed by cooling to the temperature of density measurement (Vanstone and Dougall, 1960). Later, the British Standards Institution in the specification No. 734 (1955) recommended to warm the milk to 40°C for five minutes and measure density at 20°C in order to ensure that the milk fat is in a reproducible state. Some researchers favoured the latter procedure. Yet, they had to determine the sufficient length of holding time needed for accomplishment of stable density. Hilker (1961) investigated the change in the specific gravity of milk fat with various holding times and found that the samples that had been held at a desired temperature (32–50°F or 0-10°C) more than 4 hours showed the maximum values. Whitnah (1957), in his study of maximum density, measured density after pasteurization and 10 hour suspension of the samples at 3°C. The United States Department of Agriculture (USDA) (1965) found that cream samples (18% fat) required at least 15 hours to come to equilibrium at 40°F (4.4°C). Homogenized cream contracted more slowly than non-homogenized samples. Cream was examined because the effect of slow crystallization of fat on milk density was most easily observed in high fat products where the total contraction is larger than that of whole milk. The research also reported that pre-chilling in ice water did not shorten the time needed for equilibration at 40°F.

As seen in the previous works, controlled temperature history is necessary to secure high precision and accuracy with density determination.

2.5.3. Variance in density of milk fat

Even accounting for Recknagel's phenomenon and the importance of controlling temperature history of milk samples, variance in density of milk fat has still been observed. Old literature, especially, presented a wide range of milk fat density or specific gravity. For example, *The Chemistry of Milk* written by Davies, W.L. in 1936 claimed that milk fat was not of consistent density and introduced the following ranges of specific gravity at the stated temperatures found by various researchers (Table 2.3).

Table 2.3: Ranges in the specific gravity of milk fat measured at the stated temperature.

Researcher	Specific gravity of milk fat	Temperature
Fryer and Watson	0.936-0.942	15.5/15.5°C
Koestler	0.9355-0.9448	15.5/15.5°C
Bell and Richmond	0.9094-0.9135	100/100°F (37.8°C)
U.S. Standard butter-fat	not less than 0.905	40/40°C
Rahn	0.8973-0.8986	50/50°C
Allen and Richmond	0.8655-0.8685	100/15.5°C[1]

[1] 100/15.5°C = samples were at 100°C and the water was at 15.5°C
(Davies, 1936)

In the 1940s, the specific gravity of milk fat was already known to vary slightly due to variations in the composition of fat. In the United States Jenness *et al.* (1942) found a range of 0.8867 to 0.8910 at 60°C. McDowell (1954) reported a range of 0.8885 to 0.8916 at 60°C for New Zealand milk fat.

Riel (1956) observed the specific gravity of milk fat obtained from 29 factories across eight provinces of Canada during twelve months of 1953/1954. The range of specific gravity at 40°C/25°C was 0.9056 to 0.9090 with the average of 0.9072. This study found significant differences between months and between provinces. Higher specific gravity was typical of summer fat compared to winter fat, and the variance for months was greater than that for provinces. In addition, low but significant correlation was reported between specific gravity and each of the properties of Reichert-Meissl value and refractive index, which indicated that the fluctuation in specific gravity of milk fat was associated with changes in the fat composition. Bailey's (1945) statement that specific gravity of oils generally increase with lower molecular weights of their fatty acids, supported this claim.

2.5.4. Effect of processing on milk density

In 1914 Wiegner noted that reduction in fat globule size had no detectable influence upon specific gravity of milk. Trout (1950) also claimed that homogenization did not change the density of milk. However, Trout, Halloran and Gould (1935) found a slight decrease in the specific gravity of homogenized samples. Walker (1945) examined the density of homogenized and sterilized milk, and found that the density increased during a period of two to three days after processing. The density of his processed milk finally reached that of original raw milk. Walker attributed this gradual increase of density to a slow solidification of fat in the processed milk samples. The inconsistency in the results of various researchers might be due to various temperatures used and different types of homogenising valves employed.

Rutz *et al.* (1955) found no significant effect on milk density of one-stage homogenization at pressures ranging from 500 to 3500 psi, but a highly significant increase in density occurred at pressures between 15 and 300 psi. He used mixed breed milk, having fat 4% and TS 13%, that was pasteurized at 62°C for 30 minutes and homogenized at 59°C. Processed milk was immediately cooled to 20°C, held 18 hours at 2°C, and then analytical balance and a bulb determined its density.

Short (1956) investigated the effect of pasteurisation, homogenization at 3000 lb/in^2 at 145°F (62.8°C), and sterilization on milk samples with fat contents of 0.05, 3, and 6%. Density was measured by the process of displacement immediately after warming to 40°C for five minutes and cooling to 20°C. He found that influences of pasteurization, carried out by two methods of warming to 145°F for 30 minutes and heating to 161°F (71.7°C) for 15 seconds, were very small. This study concluded that homogenization slightly increased the density of whole milk but not of skim milk, and sterilization, 95°C for an hour, decreased the density of both milks. He attributed this decrease in density to denaturation of the soluble proteins above 70°C. Short also showed that dispersion of the fat by homogenization increased the time lag for the physical state of the fat to attain equilibrium after a temperature change.

2.6. Previous Works on Relationship between Milk Density and Components

The relationship between milk density or specific gravity and composition of milk, especially fat and solids-non-fat (SNF) content, has been investigated for more than a century. The idea that a relation exists between specific gravity, fat and solids in milk occurred first to Behrend and Morgen (Behrend and Morgen, 1879; Overman *et al.*, 1925). Equations were proposed to determine the fat content of milk from the specific gravity and total solids content because at that time no simple method for determination of fat in milk was available (Sharp and Hart, 1936). The development of the Babcock and Gerber methods for the determination of fat shifted the use of the equation to the determination of total solids or SNF from the fat content and the specific gravity (Sharp and Hart, 1936). Richmond's formula for calculation of total solids was widely used in England. The formula was expressed as follows:

$$\text{Total solids (\%)} = L/4 + 1.2\,F + 0.14 \qquad [3]$$

where L = Quevenne lactometer reading (L.R.) at 60°F (15.6°C)

F = fat (%) in milk.

This equation was based on milk in which the Recknagel contraction had proceeded to completion and which had been selected throughout a twelve month period during 1892/1893 (Boden, 1942).

In the United States, the formula of Babcock was commonly used (Babcock, 1892):

$$\text{Total solids (\%)} = (L + 0.7\,F)/3.8 + F \qquad [4]$$

Babcock later stated this relation as total solids (%) = $L/4 + 1.2\,F + 0.14$, the same as Richmond's formula.

In Canada either of the following formula was employed to calculate SNF in milk (Canada Department of Agriculture, 1915):

$$\text{SNF (\%)} = (\text{fat (\%)} + \text{L.R. at } 60°\text{F})/4 \qquad [5]$$

$$\text{SNF (\%)} = 0.2 \times \text{fat (\%)} + \text{L.R. at } 60°\text{F}\,/4 \qquad [6]$$

A number of equations for describing the relation had to be published because the formulae worked well only when applied to the data from which they were derived. None of the formulae could be the universal equation. Overman *et al.* (1925) examined the accuracy of Babcock's formula with 1158 milk samples from individual cows and 134 random samples from mixed milk to find a considerable difference between calculated and measured total solids values. They claimed that the accuracy of the formula increased as the number of cows contributing to the milk was increased. This study adjusted Babcock's formula to its milk samples by changing the intercept. The lack of agreement could be due to the difference in the method employed to determine the value of each factor.

After progress in the measuring methods of fat and solids, the need for a simple accurate method of calculating the weight per given volume of milk and other dairy products was recognised by the industry (Hilker, 1961). Bearce (1914) investigated the specific volume and expansion values of cream. Sommer (1932) and Fendsen (1950) published a formula for calculating the weight of a gallon of ice cream mix. Pien and Maurice (1938) showed formulae for calculating weights of a litre of milk, skim milk, cream, and condensed milk from their components. Hilker (1961) proposed a formula that could be applied to all milk products. It was expressed by the following equation:

$$W = (A + B + C)/100 \qquad [7]$$

where W = sp.gr. of mixture

A = fat (%) × sp.gr. of fat at given temperature

B = SNF (%) × factor for SNF

C = water (%) × sp.gr. of water at a given temperature

This study determined a factor to adjust specific gravity of solids because simple addition of density of water × percentage of water and density of solids × percentage of solids did not yield the density of the mixture. However, Hilker assumed that the specific gravity of solids was not affected by temperature. Walstra (1984) also suggested that the density of milk could be derived by summation over all of its components according to:

$$1/\rho = \Sigma\,(m_x/\rho_x) \qquad [8]$$

where m_x is the mass fraction of component x

ρ_x is apparent density of component x in the mixture

Usually, ρ_x is not the density of the pure substance because a change in volume occurs when two components are mixed. They reported ρ_{20} values of 918 for milk fat, 1400 for protein, 1780 for lactose, and 1850 for the residual components of milk, when using 998.2 kg/m^3 for the ρ_{20} of water.

With the development in methods of density measurement, many studies have measured milk density at various temperatures with various methods. Oguntunde and Akintoye (1991) tested the density of cow's milk and soymilk by a 50-mL pycnometer at 25°C. Bakshi and Smith (1984) determined the milk density in the temperature range of 0 to 30°C with vibrational density meter (Mettler DMA 35). Watson and Tittsler (1961) measured the density of 101 milk samples in the temperature range of 1 to 10°C by pycnometers with a capacity of 46 mL. This study reported that the density was more closely correlated with the percentage of SNF than the percentage of fat in milk.

In Ontario, Canada, Biggs (1978) constructed equations for density prediction of Ontario producer milk based on research conducted during April to July 1978. Density was determined with 1-litre volumetric flasks. Because this work was not published and a detailed procedure was not given in the final report to Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), the exact number of samples used in this study is unknown. However, some statistical tables indicated that the study measured at least 800 samples from 23 different farms. Developed formulae were as follows:

$$D = 1.02641 + 0.00152\ F \qquad [9]$$

$$D = 1.017008 + 0.0011898\ TS \qquad [10]$$

$$D = 1.00689 + 0.002827\ SNF \qquad [11]$$

$$D = 1.007664 + 0.0001653\ F + 0.0026703\ SNF \qquad [12]$$

$$D = 1.007125 + 0.0001227\ F + 0.00327\ P + 0.00281\ L \qquad [13]$$

where D = milk density at 4°C, F = fat (%), TS = total solids (%)

SNF = solids-non-fat (%), P = protein (%), L = lactose (%)

A limitation of this research is the small number of farms and the short time period.

2.6.1. Volume-weight conversion factors project in the United States

The latest large-scale investigation on determining volume-weight conversion factors for milk was carried out in the United States in 1965. Previous conversion factors, in which the effects of SNF content and temperatures were ignored, were inadequate (USDA, 1965). This research included 8000 samples from 13 regions and lasted for a full year to take geographic location and seasonal effect into account. The specific gravity of raw and processed whole milk, skim milk, and cream were measured at four temperatures (40, 50, 68, and 102°F or 4.4, 10, 20, 38.9°C), using the Babcock bottle methods (see section 2.2). Fat, protein, and total solids contents were determined by the Babcock test, Mojonnier procedure, and air-drying oven at 100°C, respectively.

Geographical effect

This investigation first calculated regional regression equation for each product and temperature tested. The equation predicted the pound per gallon value of a fluid milk product based on its w/w contents of fat and SNF. The research, secondly, constructed regression equations for each product, using the data from all participating regions at each temperature. Then, the magnitude of the effects of sample source was evaluated, comparing the differences in predicted pound per gallon values between the regional and the all region equations using identical composition data. For example, in the case of raw producer milk at 40°F, milk samples were collected from New York (18), North Texas (74), Oklahoma (48), Puget Sound (407), and Washington D.C. (62), where the numbers in brackets show the number of samples obtained. The regression coefficients were calculated for each region; therefore, five equations were defined, and the predicted w/v values with average milk composition were compared to that of the equation derived from data of all five regions. Predicted weight per gallon value with 4.00% fat and 8.95% SNF varied from 8.621 to 8.627 among regional equations, but the average agreed with the predicted value, 8.625, calculated from the all region equation. The study attributed small differences among regions to differences in personnel, laboratory equipment, or laboratory conditions, as well as in the milk itself. The research concluded that there appeared to be little or no regional effect on weight per gallon of fluid milk products.

Seasonal effect

Raw producer milk from three regions was collected monthly throughout a year, so that the differences in weight due to season could be analyzed. The number of samples obtained from the regions of Puget Sound, Washington, D.C., and North Texas, was 407, 62, and 74, respectively. The greatest difference for any month from the testing period average was .008 pound per gallon and the variation between the month of the highest actual weight and the month of lowest actual weight was 0.014 pound per gallon. The difference in computed weight per gallon value by the universal equation and actual weight per gallon value determined by the bottle test varied from -0.008 to 0.005. The researchers, however, concluded that the effect of variations in product composition could explain practically all the monthly weight differences.

Supporting this conclusion, Boden (1942) commented in his research on the estimation of solids in milk, "the risk of confusion would more than counterbalance any increase in accuracy". He also strongly recommended to investigate milk samples throughout a year when developing an equation because the seasonal divergences were sometimes positive and sometimes negative.

Universal equation

Because the use of a number of different equations was impractical in computing weights of fluid milk products, single equations at 40, 50, 68, and 102 °F (4.4, 10, 20, and 39.8°C) were established as universal equations. The basic formula was as follows:

Specific gravity = 100/ specific volume factor

Specific volume factor = A/sp.gr. of butterfat + B/sp.gr. of SNF + C/sp.gr. of water

or [14]

Specific gravity = 100/(100 + A × butterfat factor - B × SNF factor) [15]

where A = butterfat (w/w %), B = SNF (w/w %), C = water (w/w %)

Butterfat factor = 1/sp.gr. of butterfat –1

SNF factor = 1/sp.gr. of SNF –1

Specific gravity of butterfat was computed from the density values determined by Sharp (1928), and that of SNF was calculated from a number of skim milk samples in several regions at each of four temperatures. Specific gravity and factors for butterfat and SNF determined by this study are shown in Table 2.4. Although changes in SNF composition such as protein and lactose was known to result in slight changes in specific gravity of SNF, the committee agreed that the small effect resulting from this change would have no appreciable effect on computed weight per gallon of fluid milk products. In the universal equation, specific gravity of fluid milk products at 40°F was calculated by the following equation:

$$\text{Specific Gravity} = 100/(100 + 0.03928\,F - 0.39221\,\text{SNF}) \quad [16]$$

where F is butterfat content (%), SNF is SNF content (%)

To show the reliability of all region and universal equations, the weight per gallon of each fluid milk product was computed using samples selected at random from each participating region for each group of products. Then, the calculated values were compared with the actual weight per gallon value determined by the bottle method. As a result, it was concluded that weights computed by the universal equation differed from actual weights slightly more than those computed by the all region equations. However, these differences were minute enough to permit the use of a single universal equation in the computation of unit weight of fluid milk products.

This research indicated that the specific gravity of a fluid milk product depends primarily upon the proportionate amounts of fat and SNF and temperature at which the density is measured. Differences in specific gravity associated with geographic location, breed of cow, and seasons of the year are relatively unimportant.

Table 2.4: Values of specific gravity for butterfat and SNF, factors for butterfat and SNF, and weights per gallon of water at different temperatures.

Factors were used in equations for specific gravity prediction of fluid milk[1].

Temperature	**Sp.gr. Butterfat**	**Butterfat factor**	**Apparent sp.gr. SNF**	**SNF factor**	**Pounds per gallon H_2O**
40°/40°F	.9622	.03928	1.6453	.39221	8.3364
50°/50°F	.9541	.04811	1.6275	.38556	8.3341
68°/68°F	.9330	.07181	1.6167	.38146	8.3217
102°/102°F	.9133	.09493	1.5952	.37312	8.2752

[1] Specific gravity of fluid milk at a temperature
= 100/(100 + A × butterfat factor - B × SNF factor)
where A = % by weight of butterfat in the mixture and B = % by weight of SNF in the mixture (USDA, 1965)

2.7. Effects of Temperature on Density

Many studies have been conducted to reveal the effect of temperature on milk density and to find a general rule that explains the expansion of milk. The thermal behaviour of milk is complex because the components of milk have different expansion characteristics. The major component is water, which has maximum density at 3.98°C (Lewin, 1972). However, the changes of volume of milk with temperature is greater than that of water (Fleischmann, 1893). Another heat sensitive component is milk fat. Richmond (1953) said high fat milk had a greater coefficient of thermal expansion than low fat milk (Short, 1955). Density of milk fat decreases as the temperature increases, and it does not have a maximum density at 4°C (Hilker, 1961), unlike water. Hillker (1961) measured the specific gravity of milk fat over the temperature range of 36 to 165°F (2.2 to 73.9°C) to find that milk fat showed the maximum density at the lowest temperature, 36°F, and the minimum at the highest, 165°F. The density of milk decreases as the temperature is raised to about 40°C (Short, 1955, 1956; Wegener, 1953). Davies (1936) claimed that the temperature of maximum density of milk was –0.3°C, but the temperature would naturally vary with the content of soluble and fat constituents. Whitnah (1957) reported that commercially pasteurised and homogenised milk had its maximum density around –5.2°C and the temperature of maximum density of the water-milk mixture approached that of water, 4.0°C, in a linear manner as milk was diluted with water.

2.7.1. Expansivity of milk

Until the middle 20th century, a table based on the work of Bearce (1914), the National Bureau of Standards, was used by milk producers to calculate the price of milk and estimate weight yields of dairy products from the volume in gallons and the percentage of fat by weight (Watson & Tittsler, 1961). However, the accuracy of the table was doubtful due to the separation of fat and the fact that the density at temperatures lower than 20°C were obtained by extrapolation from the determinations made in the range of 20 to 50°C (Watson & Tittsler, 1961).

Several studies used specific milk composition to investigate milk density over a wide range of temperatures. Whittaker *et al.* (1927) measured the density of skimmed milk with 8.96% solids, including 0.7% fat over a temperature range of 5-80°C. Wegener (1953) reported density of pasteurized milk in the range of 10 to 75°C; however, he did not take the effect of milk composition into account. Thomsen (1953) developed a table for calculating the weight of milk per gallon over the range of 36 to 160°F (2.2 to 71.1°C). Rutz *et al.* (1955) investigated milk density at temperatures between 4 and 49°C and found that the coefficient of the change in density between 18-49°C was 0.00038, which was different from that between 4-18°C, 0.00018. Rutz's findings suggested that the relationship between milk density and temperature was not linear. Short (1955) measured the density of raw milk between 10°C and 45°C and reported that the temperature coefficient of milk varied with composition. Data obtained from whole milk of 3% fat and 8.7% SNF and skim milk of 0.02% fat and 8.9% SNF were fitted to empirical equations expressed as:

$$D\text{-}1 = a + bt + ct^2 + dt^3 \qquad [17]$$

where D = density (g/mL) and t = temperature (°C).

The coefficients determined are presented in Table 2.5. According to this study, the relative importance of the linear term is less and that of the cubic term was greater for skim milk than whole milk. This downward deviation from linearity as SNF increased might indicate a relationship between extent of hydration and concentration of solids (Sherbon, 1988).

Table 2.5: Estimate of density from temperature for whole milk and skim milk.

Coefficients a, b, c, and d in the equation $D - 1 = a + bt + ct^2 + dt^3$ where D = density (g/mL) and t = temperature (°C).

	a	b	c	d
Whole milk	3.50×10^{-2}	-3.58×10^{-4}	4.9×10^{-6}	-1.0×10^{-7}
Skim milk	3.66×10^{-2}	-1.46×10^{-4}	2.3×10^{-6}	-1.6×10^{-7}

(Short, 1955; Sherbon, 1988)

Watson and Tittsler (1961) determined the density of raw milk in the range of 1 to 10°C with 101 samples to cover the temperature range in which milk was frequently handled. The study constructed the best-fit equations for predicting density from the fat and SNF content and the temperature. The equation containing all three parameters showed the best accuracy, while SNF was more important to determine density than fat content. The equation is as follows:

$$D = 1.003073 - .000179\,T - .000368\,F + .003744\,SNF \qquad [18]$$

where D = density (g/cm^3), T = temperature (°C), F = fat (%), SNF = SNF (%)

Densities of fluid milk products at 40, 50, 68, and 102°F (4.4, 10, 20, and 39.8°C) were examined to obtain sufficient data to permit the calculation of weight per gallon value from their w/w composition at each temperature (USDA, 1965). However, equations to describe the relationship between expansivity of milk products and temperatures were not developed (see section 2.6.1).

Phipps (1969) presented viscosity data for milk products with fat contents of 0 to 50% and temperatures of 40 to 80°C. This study developed an equation that relates viscosity and density to fat percentage and temperature. Bakshi and Smith (1984) expanded the work of Phipps', relating fat, temperature, viscosity, and density in the temperature range of 0 to 30°C, at which milk is handled often. Sample density was measured with a vibrational densitometer (Mettler DMA 35), having an accuracy of ± .001 g/cm^3. This study reported that the density of fluid milk increased as temperature decreased, and that density decreased as fat content increased.

In Ontario, Biggs (1978) constructed an equation to estimate the expansivity of producer milk between 4 and 20°C based on 26 milk samples with fat content varying from 0.04 to 4.6% and SNF content varying from 8.3 to 10.2%. The density measurement was done using 1-litre volumetric flasks. This study concluded that the expansivity of milk between these temperatures was mainly a function of fat content explained by the following equation:

$$\text{Expansivity } 20°\text{C} / 4°\text{C} = 1.00266 + (0.00034136\ F) \qquad [19]$$

where F = fat (%) determined by Mojonnier method

The number of samples used in this study seems to be small but the province of Ontario currently uses this equation in its w/w to w/v conversion of milk components.

2.8. Thermal Characteristics of Milk Fat

Classically, specific gravity of milk fat had been determined at 15°C (60°F) by a number of authors. Different researchers and even the same author declared different values of specific gravity of milk fat, ranging from 0.93 to 0.946 (Hilker, 1961). One of the reasons for disagreement of specific gravity value of milk fat is the difference in the physical state caused by a lag in melting and solidification of fat with changes in temperature (Sharp and Hart, 1936)(See section 2.5). Sharp and Hart (1936) reported that the temperature history of the milk influenced its specific gravity if it was measured immediately on reaching a desired temperature. Since then care has been paid to ensure equilibrium in the state of fat by tempering milk samples at the desired temperature (Whitnah, 1957; Watson & Tittsler, 1961; Hilker, 1961). The length of holding time varied depending on the researchers (see section 2.5.2).

Milk fat is liquid above 40°C and usually completely solidified below -40°C (Walstla, 1984). At intermediate temperatures it is a mixture of crystals and oil. Solid fat content at a temperature ranges greatly, depending on the temperature history of the sample. At 0°C, solid fat may differ from about 45% to 90%. And the temperature at which 50% of the fat is solid may be between –5°C and 20°C (Walstra, 1984).

Solidification and melting of milk fat is more intricate than of most other fats because of its complicated composition. Anhydrous milk fat (AMF), from which the membrane material has been almost completely removed, is a complex mixture of triacylglycerols, composed of more than 60 different fatty acids, with unique chemical and thermal properties. Besides the fatty acid composition, the position of residues on the triglyceride molecule also affects the melting point (Walstra, 1984). Multiple component fat has various melting points according to the distribution of fatty acid among the triglyceride molecules. A two-stage dry fractionation process can fractionate the AMF into three fractions: high melting (HMF), middle melting (MMF), and low melting (LMF). The HMF (m.p. 42°C) shows a wide melting range similar to a plastic fat. The MMF (m.p. 33°C) resembles the original AMF, and the LMF (m.p. 16°C) is liquid at room temperature. Solid fat content curves, obtained by differential scanning calorimetry (DSC), shows that HMF possesses no solids > 42°C, and that MMF has no solids > 37°C (Dimick *et al.*, 1996).

Crystallization of fat is initiated by the presence of suitable nuclei, and crystal growth follows it. Crystal growth is relatively slow in natural fats, much slower than in pure triglycerides. Generally the growth rate of crystals depends on the degree of supersaturation, on the rate of diffusion of molecules to the crystal surface, and on the time needed for a molecule to attain a perfect fit into a vacant site on the crystal lattice (Murder and Walstra, 1974). A triglyceride molecule will almost fit the crystal lattice for a time before diffusing away again to make way for another molecule, until at last one exactly fits the vacant site. Because of the existence of the very great number of different though similar triglycerides, the growth is much delayed through this competition phenomenon. Cooling rate affects not only the time needed to reach the equilibrium but also the amount of solids. Very slow cooling gives less solid fat than rapid cooling to the desired temperature. Cooling to a low temperature and then warming to the final temperature gives more solid fat than direct cooling to the final temperature (Walstra, 1984).

Crystallization of milk fat in globules behaves differently from that of fat in bulk. A smaller proportion of the fat solidifies if it is present as smaller globules, and it takes longer to reach equilibrium (Herrington, 1964; Murder and Walstra, 1974). Even in bulk the slowness in reaching maximum crystallization has been observed by many authors (Herrington, 1964; Mulder, 1947; Phipps, 1957). When a small quantity of milk fat is quickly cooled to 25°C, it takes about one hour to complete 50% of the eventual crystallization. For every 5°C lower temperature, crystallization rate is roughly doubled (Murder and Walstra, 1974). When the fat is in globules, crystallization is even slower. In this project the density of milk is measured 24 hours after heat treatment to ensure that crystallization is complete and each sample has the same density as before treatment.

2.9. Factors Influencing Fat Globule Size

Nearly all the milk fat in milk is in separate small globules. The state of fat dispersion may influence crystallization rate and milk density. A slow crystallization rate was obtained in more finely dispersed fat (Walstra & Beresteyn, 1975).

General information on fat globule dispersion is best provided by a frequency distribution, in which the number of globules, N, is plotted against globule diameter d (μm). In whole milk, small globules (< 2 μm) comprise about 80% of the total number of globules but only a few percent of the fat. Main globules (2-8 μm) include about 94% of the fat. Large globules (> 8 μm) are few in number and represent about 2-3% of the fat (Murder and Walstra, 1974).

Globule size shows considerable variation. The main factors influencing globule size of fresh milk are breed (Brunner, 1974), individual cow, and stage of lactation (Walstra, 1969). For example, Jersey and Guernsey give milk with larger fat globules than cows of Friesian type.

The globules can become larger through creaming, flocculation and coalescence and smaller through disruption. Agitation, vibration, pumping milk through the pipelines, and heating milk at high temperatures can cause coalescence or disruption. The incorporation of air severely impairs stability. Properties of the milk also influence the stability of fat globules. Small globules are more stable than large ones in almost every respect. With increasing size, collision energy and deformability increase, and clumping and disruption can occur. Clumping or coalescence increases considerably with fat content.

Temperature is an important variable. Since solid fat globules cannot be disrupted nor can they coalesce, cold milk cannot be homogenized. With liquid globules, disruption and coalescence are possible. In addition, storage at 4°C may weaken the membrane, and subsequent increase in temperature may cause local volume changes and pressure differences within globules.

Homogenization causes smaller fat globules with a large surface area. A product is homogenized by forcing it, at a high temperature, through a narrow slit called a homogenizing valve. Efficiency of homogenization in terms of globule size is influenced by pressure, type of valve, repeated homogenization, fat content, and temperature during homogenization. With increasing fat content, homogenization efficiency decreases, particularly at high pressure and low temperature (Goulden & Phipps, 1964). High temperature increases the homogenization efficiency.

3. Materials and Methods

3.1. Relationship between Milk Density and Its Components

3.1.1. Sample collection

Ontario

University of Guelph Laboratory Services Division (LSD) collects milk samples from all 7500 Ontario milk producers for payment testing (AFLSC, 1997). Ontario samples used in this study consisted of reference samples and samples used for infrared instrument calibration. Reference samples were selected from all of the producer milk samples as follows. A schedule for chemical analysis with infrared milk analyzers was organized to ensure that a sample from every producer was tested once a week. After proximate analysis by the milk analyzer, LSD selected five samples from each of the three infrared instruments out of the 2000 samples processed each day, and technicians analyzed their chemical components manually. The results, fat (w/w %), protein (w/w %), and total solids (w/w %), were converted to the w/v values and used to calibrate milk analyzers on a daily basis. These five samples were chosen with some selectivity to obtain sufficient range of fat content. These samples were kept at 4°C for at least twenty-four hours and tested for density. Density was measured at 4.0°C using an Anton Par Model DMA 45 density meter, which is accurate to ± 0.0001 g/cm^3.

Milk for calibration samples was collected from the same eight herds in Ontario every other week. These samples were tested for their chemical composition manually by LSD and for density. Herd #1 consisted of about 30 Jersey cows with a few cows of other breeds, herd #2 about 30 of single breed of Holsteins, herd #3 about 30 Jersey, herd #4 about 40 Holsteins plus other breeds, herd #5 about 12 Guernsey, herd #6, #7, and #8 are single breed of Holsteins with 37, 35, and 35 cows, respectively. The number of cows was estimated from the amount of milk that the herd usually produced.

From May 1997 to March 1999, the University of Guelph tested 866 reference samples and 313 calibration samples. The data set containing the result of reference milk is defined as "Ontario random data set" and the data regarding calibration milk is referred as "Ontario non-random data set".

Quebec

Programme d'Analyse des Troupeaux Laitiers Du Quebec (PATLQ) transferred its results on the milk composition and density tests to the University of Guelph. The PATLQ prepared the calibration milk samples in order to calibrate milk analyzers following the procedure below. Nine milk samples were collected twice a month. Generally, the same producers were used each month. The samples were divided into two aliquots, and dichromate preservative was added to one of the sub-samples. Density and total solids were determined on the fresh sub-sample. Fat and protein were determined on the preserved sub-sample. Density was measured at 4.0°C using an Anton Par Model DMA 55 density meter, which is accurate to ± 0.0001 g/cm^3. This data set was named "Quebec non-random". In addition, the data of 30 random samples, which were collected in a similar way to Ontario reference milk, were transferred to the University of Guelph and called "Quebec random" data set.

Alberta

Between March 1998 and February 1999, eight Alberta samples were collected once or twice a month. The 102 results were named "Alberta random" data set. These samples were randomly selected from all Alberta milk producers by computer random number selection prior to testing, except one or two samples in each set which the lab specifically picked because of their high fat content. Fat, protein, and LOS contents were determined at the Central Milk Testing Laboratory in Alberta, and then the samples were sent to the University of Guelph for density determination. These samples were packed in a box with an ice pack and transferred by overnight courier service. The samples were taken out of the box and kept at 4°C until required for density measurement. Alberta random data were analyzed together with Ontario random data and the combined data set was called "Ontario-Alberta random" data set.

Ontario-Alberta random data set was used to develop an equation for density prediction of producer milk. Seasonal effects were assessed with Ontario and Quebec non-random samples. Table 3.1 is a summary of samples collected by data set and month.

It should be noted that all milk samples collected in this project were herd milk, which is a mixture of individual cow's milk.

Table 3.1: Period of data collection and the number of samples tested for random and non-random data.

(a) Ontario random data (Reference) and non-random data (Calibration)

Month		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Refer -ence	'97					49	104	51		10	44	27	
	'98			64	45	65	52	47	34	47	45	40	44
	'99	49	49										
Total 866		49	49	64	45	114	156	98	34	57	89	67	44
Calibr -ation	'97					24	25	24		8	40	8	
	'98			8	16	16	16	16	8	16	16	16	16
	'99	16	16	8									
Total 313		16	16	16	16	40	41	40	8	24	56	24	16

(b) Alberta random data

Month	Mar'98	Apr'98	May'98	Jun'98	Jul'98	Aug'98	Sep'98	Oct'98	Nov'98	Dec'98	Jan'99	Feb'99
N = 102	8	8	8	8	14	8	8	8	8	0	8	16

(c) Quebec non-random data (Calibration)

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
'97	18	18	18	18	18	18	18	18	18	18	18	18
'98	17	17	17	18	18	18	18	18	18	18	18	9
TOTAL 420	35	35	35	36	36	36	36	36	36	36	36	27

3.1.2. Density measurement

Density measurements were made using an Anton Par Model DMA 45 digital density meter. For density measurement at 4.0°C, milk samples were cooled and held at 4°C for at least twenty-four hours to obtain maximum density. These samples were then transferred to a 4.0°C circulating water bath, accurate to ±0.1°C. The samples remained in the water bath for the duration of testing. This circulating water bath was also used as the source of water to pump through the density meter to ensure that the sample being tested was maintained at 4.0°C during measurement. Although the density meter requires the use of a circulating water bath with an accuracy of ±0.05°C to yield the accuracy of ±0.0001 g/cm^3, daily reproducibility test with distilled water showed that the instrument is accurate and precise to ±0.0001 g/cm^3 (section 2.2). Five-mL syringes were used to inject the samples into the oscillating tube of the density meter, preventing air from entering the sample. Density measurement was done in duplicate. Calibration samples and Alberta samples followed the same procedure stated above. To investigate the temperature effect, milk density was determined at the temperature of 4.0, 16.0, 28.0, and 40.0°C by the same density meter. The density meter was calibrated for the measurement at each temperature.

The Quebec laboratory (PATLQ) determined the density of all Quebec samples, using the same instrument and procedure. Agreement of density measurement between the Ontario and Quebec laboratories was confirmed by testing samples from the same source. During the period of May to July 1997, the previous workers investigated 96 milk samples. In March 1999, eight additional Ontario calibration samples, packed in a box with cooling agents, were shipped to the PATLQ by courier service and the two laboratories determined their density on the same date.

Reproducibility of density determinations between the Quebec and Ontario laboratories was excellent. Table 3.2 shows the statistical summary.

Table 3.2: Inter-laboratory comparisons of milk density determination (g/cm^3) of PATLQ (Quebec) and University of Guelph (Ontario).

	PATLQ T=4°C	U. of G. T=4°C	Mean	Difference From Averaged Density[1] PATLQ	Difference From Averaged Density[1] U of G
Mean	1.0334	1.0334	1.0334	0.0000	0.0000
Minimum	1.0313	1.0314	1.0314	-0.0002	-0.0002
Maximum	1.0359	1.0360	1.0360	0.0002	0.0002
Range	0.0046	0.0046	0.0046	0.0004	0.0004
STD	0.0008	0.0008	0.0008	0.0001	0.0001
STD of Differences as % of range				1.3953	
Observations				104	

[1] Difference between a density value determined from a laboratory and the average density of the two laboratories.

3.1.3. Controlling the temperature history of the samples

The effect of temperature history on milk density was examined to determine the timing of measuring density after heating because all Ontario reference samples are heated to 40°C during the process of chemical composition analysis.

The change in density after heat treatment at 40°C and storage at temperatures of 4, 16, 28, and 40°C as a function of time was examined by the following procedure. Five samples were selected from the raw producer milk samples. These samples were not heat treated because their chemical compositions were not determined. (Although all bulk tank samples are delivered to the laboratory, not all samples are required to be tested.)

After being held at a desired temperature for 20 minutes, the first sample density was determined at that temperature and then placed in a 40°C (±1°C) circulating water bath. This first density measurement was done only for 4°C. After 20 minutes, the sample was transferred to the original water bath for 20 minutes to ensure that it had been cooled to the temperature of measurement. The sample's density was then measured at the temperature, and the sample was maintained at the temperature. Density of the second sample was tested ten minutes later than the first sample. This pattern continued for the third, fourth, and fifth samples. After one hour had elapsed since the measurement of the first sample, density measurements were taken again. Sample one was measured first, followed by sample two ten minutes later and sample three after an additional ten minutes, and so on. Density of samples was measured 0, 1, 2, 3, 4, and 6 hours after the heat treatments. After measuring the 6-hour sample, all samples were held in the water bath at the desired temperature, where they remained for 18 hours. Density measurements were then taken for the final time starting with sample number one and then sample two, three, four, and five following in ten-minute intervals. The whole experiment was repeated three times.

3.1.4. Analysis of composition

All Ontario reference samples were heated and kept at 40°C for five minutes before being tested for composition to liquefy the milk fat and unify the structure. The samples were then immediately cooled to 20°C and used for measuring fat, protein, and total solids content.

The fat content was measured by the Mojonnier method. In this method the fat content of a pre-weighed 10-mL milk sample was dissolved in ethyl ether with a repeated extraction procedure aided by the presence of ammonium hydroxide and ethyl alcohol. Following the evaporation of organic solvents, the total fat was gravimetrically related to the original sample weight and the result was reported as percent fat on a w/w basis.

The percentage of protein was determined by the IDF-macro Kjeldahl method using the Gerhard system, with a 1.0g sample. This method was a means of determining the total nitrogen content from both organic and inorganic substances. A weighed amount of the sample was digested with concentrated sulphuric acid and potassium sulphate in the presence of copper (II) sulphate as a catalyst to convert the bound nitrogen in the organic matter of the sample into ammonium sulphate. Ammonium sulphate was further treated with a sodium hydroxide solution to liberate ammonia which is distilled and absorbed in an ample amount of boric acid solution which in turn was titrated with standardized acid. Percent nitrogen was then obtained by titration of this solution with an acid solution, and by calculation. The nitrogen content value was multiplied by the conversion factor of 6.38 to obtain the protein content in raw milk. This is based on an assumption of 15.67% average N in milk proteins (Jenness, 1988).

Determination of total solids followed the oven method. A milk sample was placed in an aluminium dish then weighed. This sample was then transferred into a steam bath and heated for 15 minutes. The sample was then placed into an oven at 100-102°C and kept there for three hours. After heating, the sample was cooled in a desiccator and its weight was measured. LOS content was calculated by the following formula:

$$\text{LOS (\%)} = \text{total solids (\%)} - \text{fat (\%)} - \text{protein (\%)} \qquad [20]$$

Detailed procedures can be found in *Central Milk Testing Laboratory Operations Manual*

(1992) and *Analytical Procedures for Milk Analysis* (1991).

Composition analyses were performed at one of Ontario, Alberta, or Quebec laboratories. Reproducibility between these laboratories is confirmed by regular inter-lab agreement tests conducted by Canadian laboratory services (CLS) Ottawa (CLS, 1998), within the framework of the Canadian Laboratory Accreditation Program.

3.1.5. Statistical analysis

Collected data were analysed as an analysis of covariance and as a combined model of multiple regression and one way classification using SAS software version 6.12 (SAS Institute, Cary, NC, 27513, USA). The data set contained date, density (g/cm^3), and protein, fat, and LOS contents (%).

3.2. The effect of Temperature on Milk Density

3.2.1. Experimental design

The experiment was designed as a complete random design of four treatments, 4.0, 16.0, 28.0, and 40.0°C, with nine replications. Twenty-two samples were tested in each replication. A replication of a treatment was conducted within a day.

3.2.2. Sample preparation and composition determination

Samples were selected from Ontario producer milk samples. Samples for this experiment were first subjected to the regular analysis for payment purpose by the infrared milk analyzer, the System 4000 MilkoScan (Foss Electric, Denmark). System 4000 results were not suitable for our purpose because the instrument is calibrated in w/v units. The residual portion of the sample, not used by the Systems 4000 instrument, was analyzed by the MilkoScan FT 120 (Fourier Transform Infrared)(Foss Electric, Denmark) to obtain results on a w/w basis. Twenty-two samples were collected with some selection to obtain a wide fat range. Chemical compositions of fat, protein, total solids, and LOS were measured immediately, while the samples were still at 40°C.

Milk sample analytical procedures followed the steps below. A sample under a vibration pipette was pumped to a heat exchanger and heated to 40°C. After passing the heat exchanger, the sample was homogenized through the built-in two step homogenizer with a pressure of 200 bar. Then, liquid sample passed through the inline filter and into the cuvette, where the interferometer measurements took place, and the Fourier transformed end results obtained.

This composition determination relies on the specific absorbing chemical group existing in fat, protein, and lactose. Determination of fat uses absorption of carbonyl groups ($C = O$) in triglycerides at 5.7 μm (Fat A) or that of carbon-hydrogen (CH) at 3.4 to 3.5 μm (Fat B). Absorption of amide groups (CONH) at 6.5 μm is suitable for protein determination. Lactose content can be determined by absorption of hydroxyl groups (OH) at 9.6 μm. Total solids content is calculated as the sum of the fat, protein, and lactose plus a mineral content or determined through the absorption of hydroxyl groups of water molecules at 4.3 μm.

Fourier transform infrared (FTIR) is a recent technique which leads to a means of measuring fat, protein, and lactose in milk without the need for local calibration. If this technique develops successfully, infrared instruments throughout the world may be calibrated with the same basic milk samples and improved agreement in test results between different countries of the world may be achieved (Harding, 1995).

Several tests ensured the accuracy of composition determination on a daily basis. A pilot test checked agreement between the measurement of samples and a control. At first a control sample, the first sample of a batch of 2.0% UHT homogenized milk, was defined and the data stored. Everyday a pilot test measured one sample from the remaining 2.0% milk batch and checked the agreement of its results to that of the control. When a new batch of 2.0% UHT control milk was introduced, a new control sample was defined. A repeatability check was also conducted ahead of daily measurement. A reference sample, which had been poured from a carton of 2.0% homogenized milk in good condition, was measured for its composition 11 times to evaluate repeatability. The first measurement did not count, so in total 10 measurements were computed for the standard deviations. The purging efficiency check examined the residual volume of the previous sample in the total volume of the cell after a single pumping sequence of sample through the cell. Two hundred mL of whole milk and 200 mL of water with 0.1% S-6060 Zero Liquid Concentrate solution added were taken and poured into 11 sample beakers each. The 22 consecutive analyses of water and milk proceeded using the sequence water, water, milk, milk, water, water, and so on. MilkoScan FT 120 calculated the purging efficiency using the following formula. The purging efficiency should be greater than 99% for ordinary milk (Foss Electric, 1996).

$$\text{water to product } (\text{sum } M1 - \text{sum } W2)*100/(\text{sum } M2 - \text{sum } W2) \quad [21]$$

$$\text{product to water } (\text{sum } W1 - \text{sum } M2)*100/(\text{sum } W2 - \text{sum } M2) \quad [22]$$

where $M1$ = first milk reading, $M2$ = second milk reading,

$W1$ = first water reading, and $W2$ = second water reading

Agreement between compositions determined by FT 120 and by chemical methods was also confirmed by measuring three sets of Ontario non-random samples with the two methods.

FT 120 had excellent repeatability and accuracy. The pilot test was in high agreement. The repeatability test also depicted good precision of this instrument (Table 3.3). Table 3.4 gives the satisfactory results on the purging efficiency. Composition determination with FT 120 sufficiently matched the results derived from wet chemistry (Table 3.5).

Table 3.3: Repeatability of composition determination by FT 120.

	Fat	**Protein**	**Total solids**
Mean of daily means[1]	2.013	3.224	10.900
Mean of daily STD[2]	6.667e-3	1.243e-2	1.962e-2
STD of daily STD[3]	1.372e-3	6.206e-3	5.489e-3

[1] Calculated by averaging the means of daily determination of 10 reference milk.
[2] Computed by averaging the standard deviations of daily determination of reference milk.
[3] Standard deviation of the standard deviations of daily determination of reference milk.

Table 3.4: Purging efficiency test of FT 120.

	Fat	**Protein**	**Total solids**
Water to Product	99.92	99.83	99.68
	99.88	99.38	99.69
Average	**99.00**	**99.61**	**99.68**
	Fat	**Protein**	**Total solids**
Product to Water	99.74	99.48	99.56
	99.59	98.63	99.08
Average	**99.67**	**99.06**	**99.32**

Table 3.5: Difference in determined milk composition between FT 120 and wet chemistry.

The value determined by FT 120 minus the value from wet chemistry

	Fat	Protein	Total solids
Average	0.0425	0.0196	0.1638
STD	0.0180	0.0241	0.0359
Min	0.01	-0.03	-0.21
Max	0.07	0.06	0.085

3.3. Model Development

Since the prices of milk are based on milk fat, protein, and LOS, these were the primary factors used in the models. Interactions among fat, protein, and LOS variables and temporal and geographical factors were also included in the model. To calculate the temporal factor, each month was assigned a number, and each of those numbers constituted a level: that is, twelve levels were defined in total. The geographical factors represented the difference between provinces. This full model is expressed as follows:

$$\text{Density}_{ijk} = \text{Intercept} + \beta_1 F_{ijk} + \beta_2 P_{ijk} + \beta_3 L_{ijk} + \beta_4 F_{ijk}*F_{ijk} + \beta_5 F_{ijk}*P_{ijk} + \beta_6 F_{ijk}*L_{ijk} + \beta_7 P_{ijk}*P_{ijk} + \beta_8 P_{ijk}*L_{ijk} + \beta_9 L_{ijk}*L_{ijk} + \beta_{10} F_{ijk}*P_{ijk}*L_{ijk} + T_i + S_j + E_{ijk} \quad [23]$$

for $i = 1, 2$ $j = 1,\ldots,12$ $k = 1,\ldots., n$

where density is in units of g/cm^3 at 4°C

F is the fat (w/w %), P is the protein (w/w %), L is the LOS (w/w %)

T is the provincial factor, S is the seasonal factor

The symbol * represents an interaction among factors

E is the error

The model included the following assumptions:

1. Regression variables did not interact.
2. Values were measured without error.
3. Errors were uncorrelated and followed $N(0,\sigma^2)$.

All density values were measured with duplicates to minimize errors.

Because temporal and geographical factors were inconvenient for the practical use of a formula, a simple multiple regression model, not including temporal and geographical factors, was constructed and evaluated. A simple regression model with fixed intercept of 1.0000 was also examined, hypothesizing that, when milk including zero percent of fat, protein, and LOS, the intercept of a formula for density estimation corresponded to the density of water (at 4.0°C). Simplified models were developed from full models above by removing non-significant ($p > 0.05$) regression variables.

An outlier in the regression setting is a point that lies far from the fitted line and produces a large residual. The outliers can heavily influence the position of the least-square line. In order to find a best-fit equation that describes the main body of the observations, outliers should be defined and eliminated. After fitting models to data, observations with residuals larger than three STDR (STD of residuals) were defined as outliers and eliminated. Regression results with and without outliers were compared in terms of Analysis of variance (ANOVA) statistics and the averages and the standard deviations of residuals. Then, the regression equation for each model was constructed without outliers.

3.3.1. Parameters used in the evaluation of models

The most important parameters indicating adequacy of a model are the Mean square of error (MSE) and the standard deviation of residuals (STDR). The MSE is the estimate of the variation of response (σ^2). The estimated σ is shown as 'root-MSE', which is literally the rooted value of MSE. The method of least squares, which SAS employs in deciding the estimates of coefficients, was originally designed to construct a regression line that has the minimum MSE. Therefore, the value of root-MSE, is a good indicator to compare which model fits better to the data. The standard deviation of residuals is closely related to root-MSE. Squared standard deviation of residuals is the sum of residuals divided by total degrees of freedom, which is calculated by subtracting one from the number of total observation. MSE is the sum of residuals divided by degrees of freedom of error. The standard deviation of residuals can also describe how well the model fits the data, yet root-MSE is a better indicator since the degrees of freedom of the errors is employed in order to make it an unbiased estimator of σ.

By looking at the distribution of residuals, especially its skewness and kurtosis, researchers can asses if the model confirms the assumption that errors are uncorrelated and follow a normal distribution of $N(0,\sigma^2)$. The residuals are often plotted in the vertical direction against the corresponding values of the predicted response in the horizontal direction to assess the assumption of homogeneity of error variance. If it goes according to plan, the pattern of this plot will be an unstructured horizontal band centred at zero. A fan pattern indicates that the error variance increases with the predicted response. The residuals are also often plotted against independent variables. If the model is adequate, horizontal scattered bands will result. A curved pattern shows that the relation between y and x has a quadratic (x^2) or cubic (x^3) structure.

4. Results and Discussion

4.1. Deciding the Timing for Density Measurement

Change in density as a function of tempering time after heating to 40°C and cooling to 4°C was determined as the difference from the initial density. The difference was greatest when the density was measured immediately after heating and cooling (Figure 4.1). Table 4.1 gives the statistic summary of the difference in density. The mean and the standard deviation of discrepancy in density became smaller during the holding at 4.0°C. After the samples had been held 24 hours, there was very little or no difference in density compared to the density before heating. Therefore, milk samples were stored at 4°C for 24 hours prior to density measurement, to ensure maximum density during the whole experiment.

At temperatures of 16, 28, and 40°C, the rate of density change was calculated as a function of holding time at the specified temperature. For example, if the density value of sample A at a holding time of one hour was 1.0333, and that at two hours holding was 1.0335, the difference between these values, 0.0002, was reported as a change in density at 2-hours holding point. The change was greatest between the holding time of the first hour and hour zero (Table 4.1). The differences in density became small after the samples were held at that temperature for at least an hour. Milk samples spoiled after the temperature was maintained for 5 hours at 40°C, and 6 hours at temperatures of 28°C and 16°C. Therefore, density measurements at 16, 28, and 40°C were made after the temperature had remained constant for an hour, and before 5 hours had passed.

Table 4.1: Statistical summary of difference in density after heating to 40°C and cooling to the desired temperature as a function of time.

(a) 4°C

	0 hour	1 hour	2 hours	3 hours	4 hours	6 hours	24 hours
Mean	-6.1e-4	-2.8e-4	-1.8e-4	-1.4e-4	-1.1e-4	-9.3e-5	-4.7e-5
Maximum	-4.5e-4	-2.0e-4	-5.0e-5	0.0000	5.0e-5	5.0e-5	1.5e-4
Minimum	-8.0e-4	-4.0e-4	-3.0e-4	-3.0e-4	-2.0e-4	-2.0e-4	-1.5e-4
Range	3.5e-4	2.0e-4	2.5e-4	3.0e-4	2.5e-4	2.5e-4	3.0e-4
STD	9.4e-5	5.2e-5	8.4e-5	8.5e-5	7.0e-5	7.0e-5	7.4e-5

(b) 16°C

Time	1 hour	2 hours	3 hours	4 hours	6 hours
Mean	1.3e-4	8.3e-5	1e-5	3.3e-5	2.0e-5
Maximum	4.0e-4	2.0e-4	1.0e-4	2.0e-4	1.0e-4
Minimum	-5.0e-5	0.0000	-1.0e-4	-5.0e-5	-5.0e-5
Range	4.5e-4	2.0e-4	2.0e-4	2.5e-4	1.5e-4
STD	1.1e-4	4.9e-5	6.2e-5	6.5e-5	4.0e-5

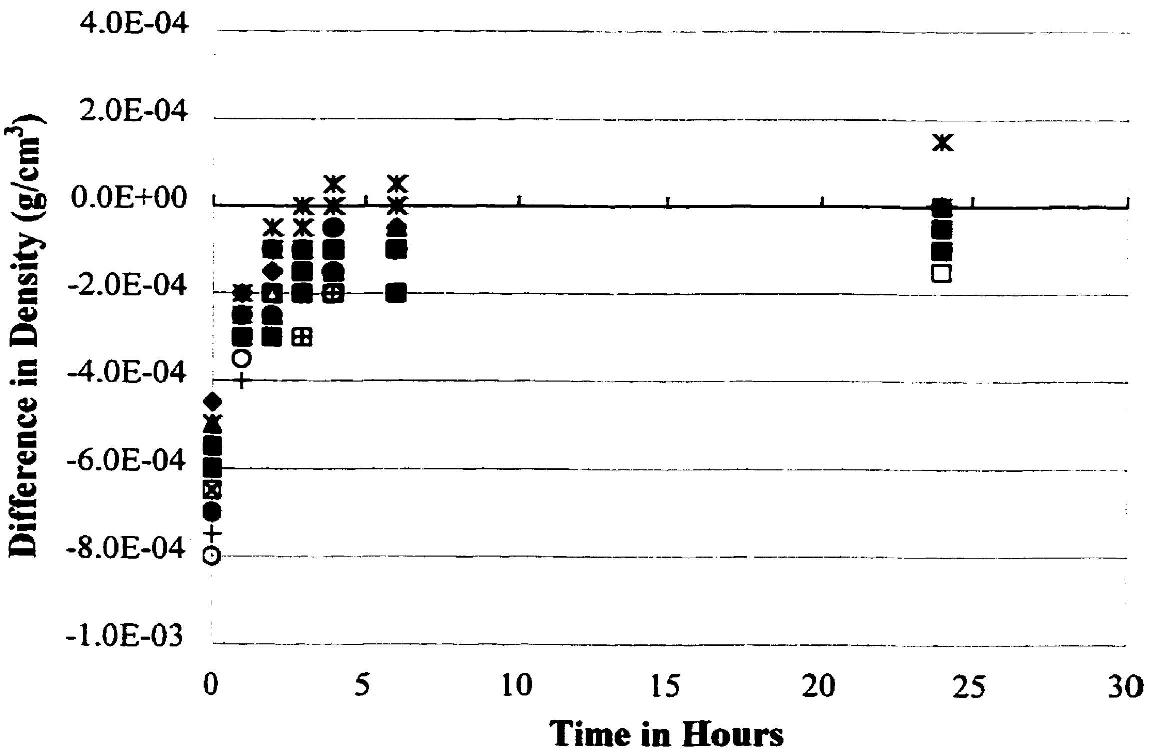
(c) 28°C

Time	1 hour	2 hours	3 hours	4 hours	6 hours
Mean	1.6e-4	6.7e-5	4.0e-5	3.7e-5	2.7e-5
Maximum	4.0e-4	2.5e-4	2.0e-4	2.0e-4	2.0e-4
Minimum	-2.0e-4	-1.0e-4	-1.0e-4	0.0000	-1.0e-4
Range	6.0e-4	3.5e-4	3.0e-4	2.0e-4	3.0e-4
STD	1.7e-4	9.8e-5	8.9e-5	5.5e-5	7.5e-5

(d) 40°C

Time	1 hour	2 hours	3 hours	4 hours	6 hours
Mean	3.1e-4	1.8e-4	8.8e-5	6.7e-5	8.7e-5
Maximum	9.5e-4	5.0e-4	3.0e-4	3.0e-4	2.5e-4
Minimum	-1.0e-4	0.0000	-5.0e-5	-1.0e-4	-1.0e-4
Range	1.05e-3	5.0e-4	3.5e-4	4.0e-4	3.5e-4
STD	3.1e-4	1.5e-4	9.0e-5	9.6e-5	9.9e-5

Figure 4.1: Difference in density after heating to 40°C and cooling to 4°C as a function of time.



Each point represents one observation (N = 15).

4.2. Relationship between Density and Milk Composition at 4°C

4.2.1. Statistical summaries of data sets

Statistical characteristics of Ontario-Alberta random, Ontario random, Alberta random, Ontario non-random, and Quebec non-random data sets are summarised in Table 4.2. Random and non-random data sets showed similar variability, except Quebec non-random data, which had relatively low minimum density (1.0280) relative to other data sets.

Plots of relationship among density, fat, protein, and LOS for Ontario-Alberta random are presented in Figure 4.2 through Figure 4.4. Density was most clearly correlated with protein. The relationship between fat and density was less clear, but there appeared to be some positive correlation. Density seemed to be independent of LOS content. It should be noted that high fat milk tended to be high in protein. Although fat has a lower density than water, multiple regression may assign a positive coefficient to the fat variable because of this interaction.

Table 4.2: Statistical summary of average, standard deviation, minimum, and maximum of density and composition for each data set.

Ontario-Alberta random N = 968

Variable	AVE	STD	Minimum	Maximum
Density	1.0337	8.730e-4	1.0312	1.0364
Fat	3.884	5.644e-1	2.095	5.720
Protein	3.318	2.622e-1	2.084	4.253
LOS	5.573	2.416e-1	3.537	7.482

Alberta random N = 102

Variable	Mean	STD	Minimum	Maximum
Density	1.0337	8.703e-4	1.0319	1.0364
Fat	3.721	5.523e-1	2.155	5.039
Protein	3.309	2.585e-1	2.908	4.079
LOS	5.486	1.549e-1	5.026	6.487

Ontario random N = 866

Variable	Mean	STD	Minimum	Maximum
Density	1.0337	8.734e-4	1.0312	1.0364
Fat	3.904	5.629e-1	2.095	5.720
Protein	3.319	2.627e-1	2.084	4.253
LOS	5.583	2.473e-1	3.537	7.482

Ontario Non-random N = 313

Variable	Mean	STD	Minimum	Maximum
Density	1.0340	9.667e-4	1.0319	1.0367
Fat	4.158	5.985e-1	2.795	5.990
Protein	3.403	3.321e-1	2.930	4.330
LOS	5.502	7.682e-2	5.217	5.660

Quebec Non-random N = 420

Variable	Mean	STD	Minimum	Maximum
Density	1.0339	1.347e-3	1.0280	1.0368
Fat	4.084	5.499e-1	1.872	5.815
Protein	3.373	2.927e-1	2.497	4.071
LOS	5.517	1.910e-1	3.752	5.775

Figure 4.2: Plots of relationship (a) density versus fat (b) density versus protein for Ontario-Alberta random data.

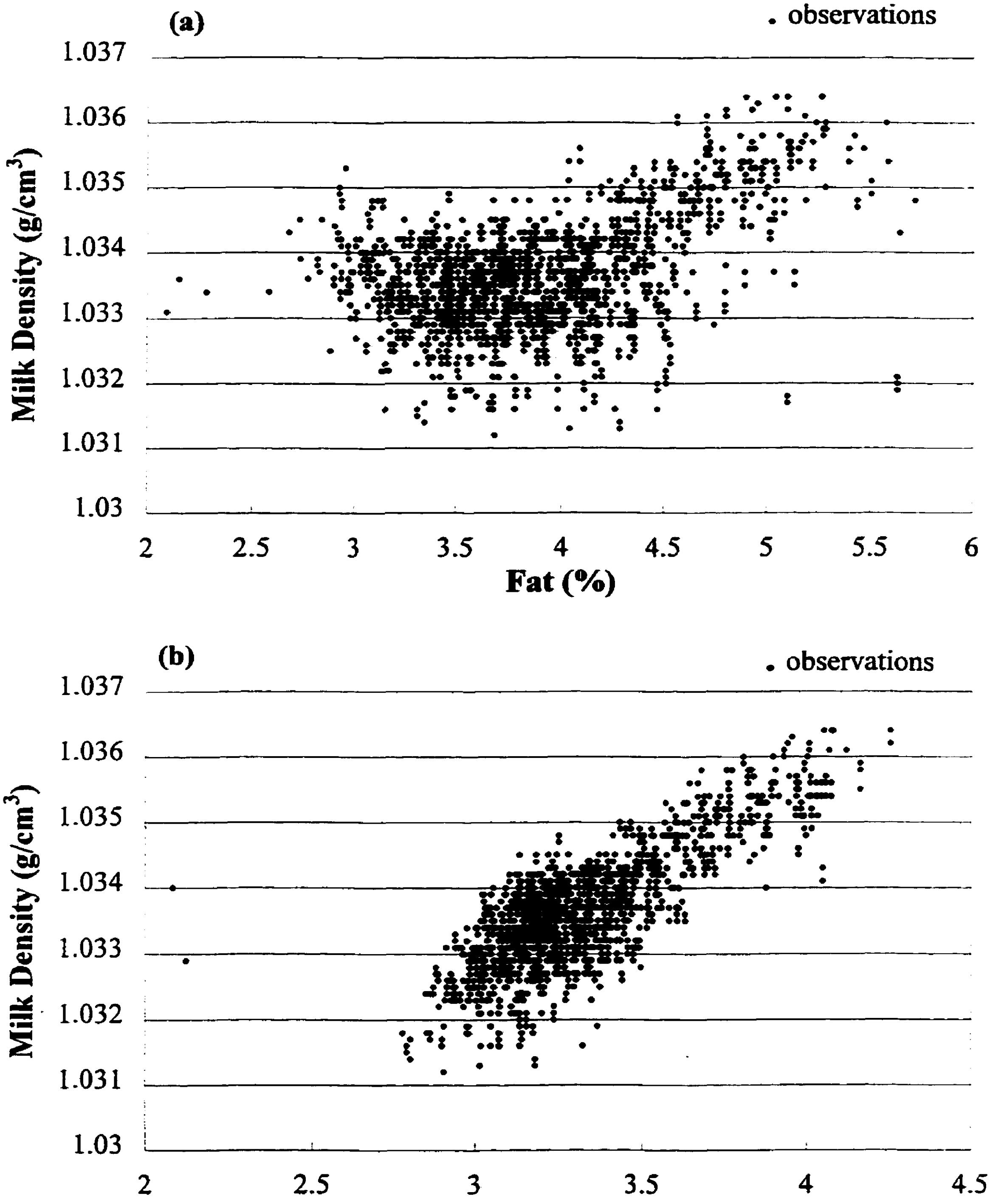


Figure 4.3: Plots of relationship (a) density versus LOS (b) fat versus protein for Ontario-Alberta random data.

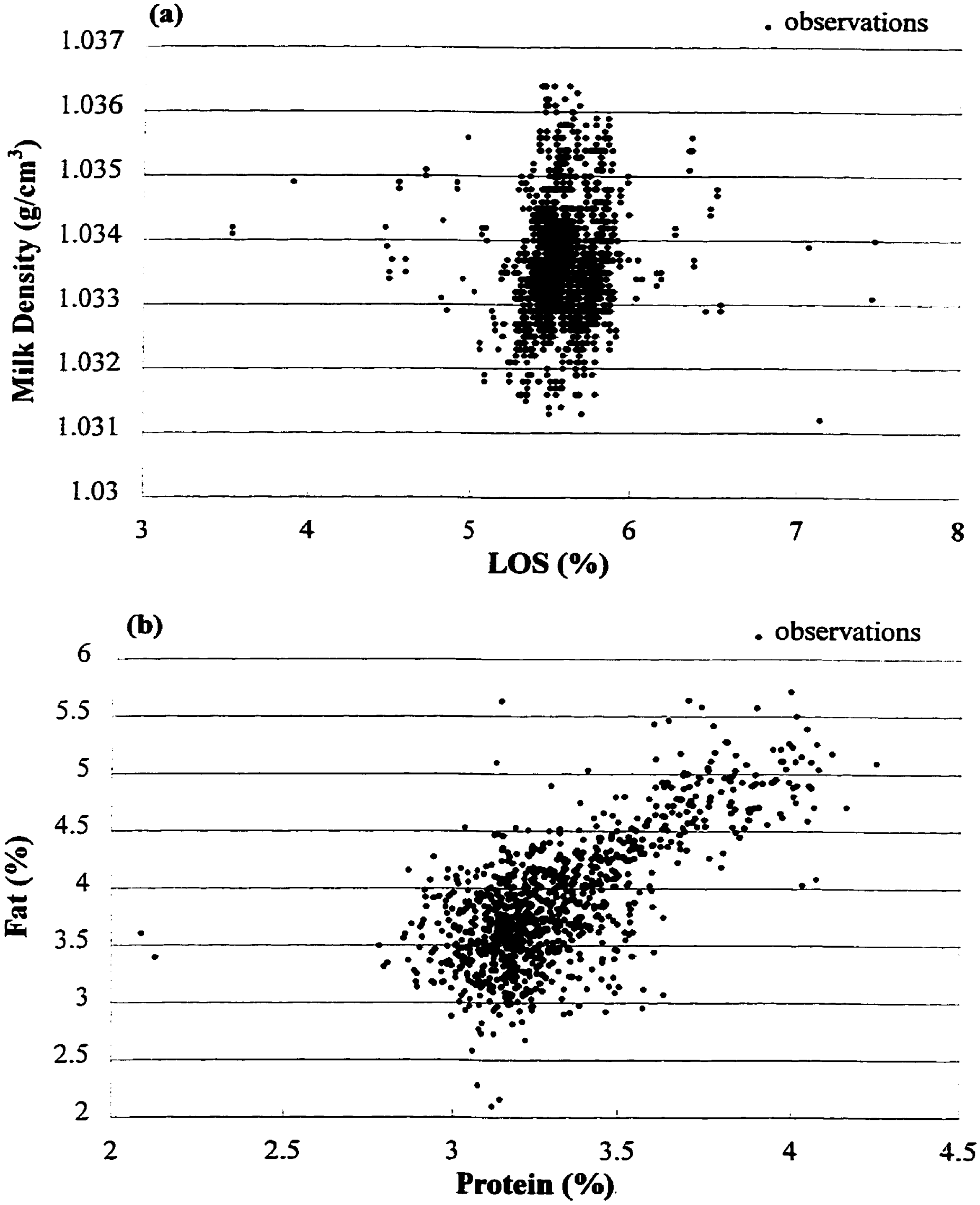
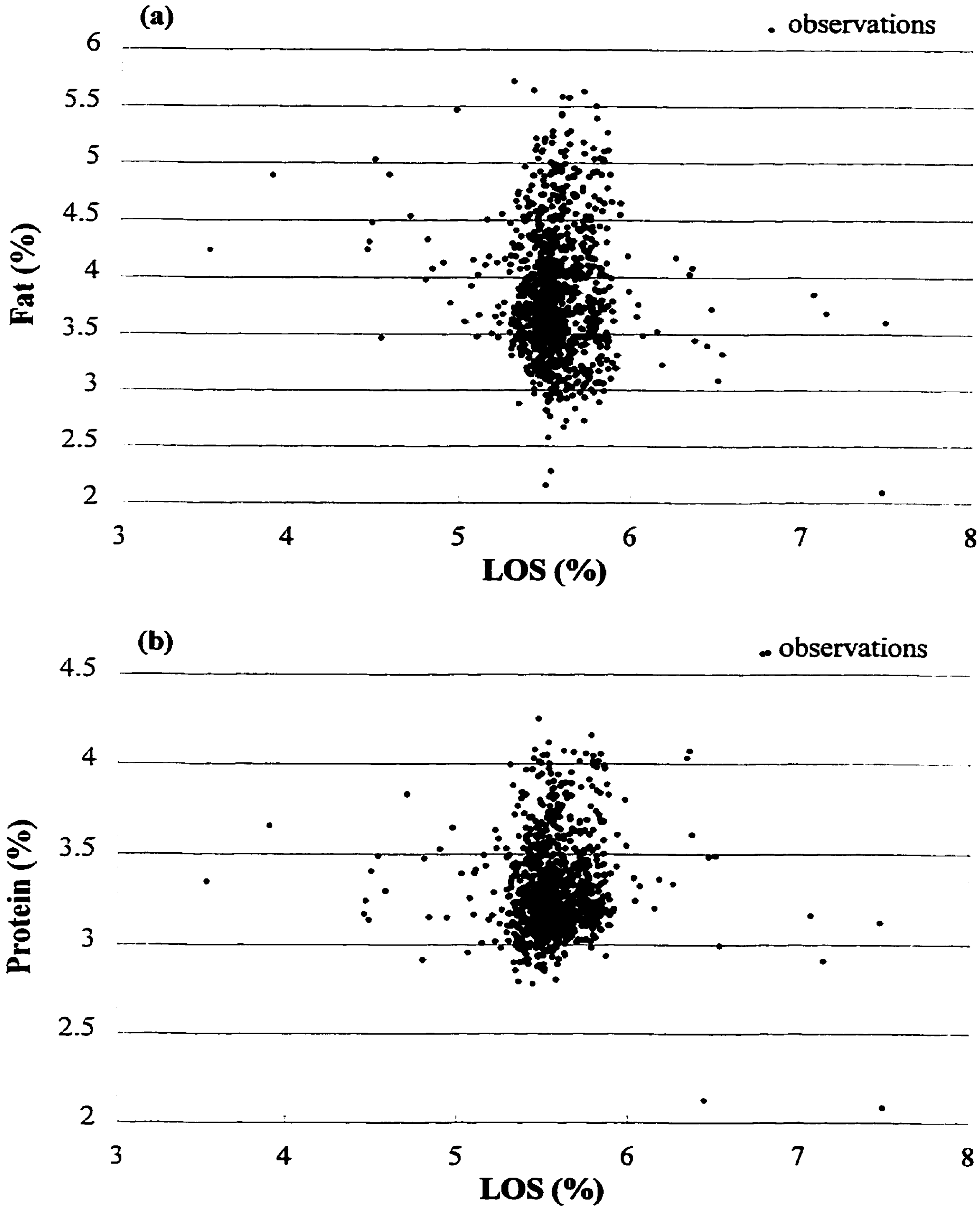


Figure 4.4: Plots of relationship (a) fat versus LOS (b) protein versus LOS for Ontario-Alberta random data



4.2.2. Developing a formula to predict milk density from composition

Models listed in Table 4.3 were evaluated to obtain the best equation explaining the relationship between milk density and composition.

Table 4.3: A list of the models examined in the model selection.

Data set	Code	Model
ON & AB random	R1	Predicted density = all composition variables & interactions + season + region
ON & AB random	R2	Predicted density = all composition variables & interactions + season
ON & AB random	R3	Predicted density = all composition variables & interactions (open intercept)
ON & AB random	R4	Predicted density = 1 + all composition variables & interactions (fixed intercept)
ON & AB random	R5	Predicted density = reduced composition variables & interactions + season
ON & AB random	R6	Predicted density = reduced composition variables & interactions (open intercept)
PQ non-random	Q1	Predicted density = all composition variables & interactions + season
PQ non-random	Q2	Predicted density = all composition variables & interactions
PQ non-random	Q3	Predicted density = reduced composition variables & interactions + season
PQ non-random	Q4	Predicted density = reduced composition variables & interactions
ON non-random	O1	Predicted density = all composition variables & interactions + season
ON non-random	O2	Predicted density = all composition variables & interactions
ON non-random	O3	Predicted density = reduced composition variables & interactions + season
ON non-random	O4	Predicted density = reduced composition variables & interactions
USDA (1965)	US	Predicted density = 100/(100 + .03928Fat - .39221(Protein + LOS))
Biggs (1978)	Biggs	Predicted density = 1.007664 + 0.0001653Fat + 0.0026703 SNF

where ON = Ontario, AB = Alberta, PQ = Quebec, US = an equation for estimation of milk density at 4.4°C constructed by USDA in 1965, and Biggs = an equation based on Ontario milk developed by D.A. Biggs in 1978.

Regression results of full models with and without outliers can be compared in Table 4.4 and 4.7. Since deleting outliers decreased the estimation of error variance, root-MSE and STDR, and made the distributions of residuals closer to the Normal distribution, data without outliers were used in further investigation. ANOVA statistics and estimated coefficients for models fitted to the data without outliers are summarized in Table 4.5, 4.6, 4.8, and 4.9. In Ontario-Alberta random data set, estimated error variance became larger when a model was forced to take a pre-determined intercept. Therefore, only models with an open intercept were used for data sets of Quebec non-random and Ontario non-random.

The provincial factor was not significant ($p > 0.05$) in Ontario-Alberta random data set. The results of F-test (Table 4.10) also indicate that model R1 and R2 are identical, which means that the provincial factors can be ignored without losing precision.

The seasonal factors were significant ($p < 0.05$) in all data sets (Table 4.10, 4.11, and 4.12). However, the difference in estimated error variance between the simple regression and the model with seasonal factors was small.

The results of F-tests in Table 4.10 through Table 4.12 indicate that differences between full and reduced models were not significant ($p > 0.05$), except between O1 and O3. However, the difference in root-MSE and STDR between O1 and O3 was very small. Therefore, the reduced equation (O3) was employed in further investigation. Reduced models with seasonal factors showed slightly lower root-MSE than simple regressions. However, the simplicity obtained by omitting seasonal factors may justify slightly reduced precision. At least four significant digits are used in the coefficients to maintain the same accuracy in terms of root-MSE and STDR as the parameter estimates that SAS provided. Equations constructed from models R5 (3.85e-4), R6 (4.65e-4), Q3 (1.51e-4), Q4 (2.15e-4), O3 (2.10e-4), and O4 (2.54e-4) were used in further analysis as best-fit equations for the corresponding data set. A number in the bracket is the standard deviation of residuals of the equation.

Coefficients of variances seem to be related to the theoretical contribution of the constituents to density. The coefficients of simple effects of protein fat, and LOS have the largest value followed by an interaction between protein and fat. The simple effect of protein seems to have a larger impact on density than fat. The density of each component appears to explain the difference in the magnitude of influence to milk density. Protein is more influential to density than fat because difference in density between water and fat is much smaller than that between water and protein (section 2.6). Opposed to the fact that protein has the smallest density among SNF (section 2.6), protein has larger coefficients than LOS, which is probably due to the small variation in the LOS content. Plots of density and components confirm these tendencies (Figure 4.2, 4.3, and 4.4). Protein positively correlated with density most clearly among components. Fat content also had a positive correlation with density, although the relation was not as clear as that between fat and density. Density varied regardless of LOS contents, and the variation in LOS contents was small.

Table 4.4: Comparison of ANOVA statistics and the characteristics of residuals before and after eliminating outliers in model R1, R3, and R4.

R1 includes seasonal and regional factors and regression variables. R3 and R4 are simple multiple regressions with open intercept (R3) and fixed intercept (R4).

		Full observations	Without outliers
Model R1	# of outliers (%)	0	24 (1.14%)
	SSE	3.826e-4	3.100e-4
	MSE	1.8e-7	1.5e-7
	Error DF	2090	2066
	Root MSE	4.278e-4	3.874e-4
	R-square	0.7623	0.7996
	Average of residuals	-3.005e-15	-1.208e-15
	STDR	4.256e-4	3.853e-4
	Max/Min of residuals	1.25e-3 / -2.22e-3	1.08e-3 / -1.14e-3
	Skewness	-0.5371	-0.2144
	Kurtosis	1.5246	0.2118
Model R3	# of outliers (%)	0	18 (0.85%)
	SSE	5.150e-4	4.535e-4
	MSE	2.5e-7	2.2e-7
	Error DF	2102	2084
	Root MSE	4.950e-4	4.665e-4
	R-square	0.6800	0.7077
	Average of residuals	-2.797e-15	-1.363e-15
	STDR	4.938e-4	4.654e-4
	Max/Min of residuals	1.27e-3 / -1.96e-3	1.18e-3 / -1.39e-3
	Skewness	-0.4819	-0.2888
	Kurtosis	0.4926	-0.2485
Model R4	# of outliers (%)	0	14 (0.66%)
	SSE	5.184e-4	4.717e-4
	MSE	2.5e-7	2.3e-7
	Error DF	2103	2089
	Root MSE	4.965e-4	4.752e-4
	R-square	0.6779	0.6967
	Average of residuals	-2.060e-8	-1.508e-8
	STDR	4.954e-4	4.742e-4
	Max/Min of residuals	1.34e-3 / -2.15e-3	1.31e-3 / -1.42e-3
	Skewness	-0.4753	-0.2563
	Kurtosis	0.5087	-0.2324

Table 4.5: Summary of ANOVA statistics and parameter estimates of full models for the combined random data sets of Ontario and Alberta.

R1 includes seasonal and regional factors and regression variables.
R2 has seasonal factors and regression variables.
R3and R4 are simple regressions with open and fixed intercepts, respectively.

	Model R1	Model R2	Model R3	Model R4
SSE	3.100e-4	3.100e-4	4.535e-4	4.717e-4
MSE	1.5e-7	1.5e-7	2.2e-7	2.3e-7
Error DF	2066	2067	2084	2089
Root MSE	3.874e-4	3.873e-4	4.665e-4	4.752e-4
R-Square	0.7996	0.7996	0.7077	0.6967
STDR	3.85e-4	3.85e-4	4.65e-4	4.74e-4
Intercept	0.83133	0.83123	0.84959	1.0000
FAT	4.3649e-2	4.3674e-2	3.7636e-2	-2.8590e-5
PROT	6.1365e-2	6.1389e-2	5.5120e-2	1.3648e-2
LOS	3.4736e-2	3.4753e-2	3.2170e-2	5.0730e-3
F*F	7.1900e-6	7.1566e-6	2.8037e-6	2.6155e-6
F*P	-1.2353e-2	-1.2359e-2	-1.0660e-2	-4.2573e-4
F*L	-8.4643e-3	-8.4688e-3	-7.3327e-3	-4.8191e-4
P*P	-8.1457e-4	-8.1489e-4	-6.3949e-4	-2.5068e-4
P*L	-1.0200e-2	-1.0204e-2	-9.2123e-3	-2.1325e-3
L*L	1.3854e-4	1.3847e-4	1.5810e-5	1.1136e-4
F*P*L	2.3879e-3	2.3890e-3	2.0699e-3	2.0617e-4
Type AB	-1.7565e-6			
ONR	0.0000			
Season 1	-4.3588e-5	-4.3813e-5		
2	3.4436e-5	3.4006e-5		
3	7.7783e-5	7.7570e-5		
4	4.5012e-5	4.4741e-5		
5	-3.5845e-4	-3.5858e-4		
6	-6.4578e-4	-6.4589e-4		
7	-6.7994e-4	-6.8016e-4		
8	-3.2881e-4	-3.2906e-4		
9	-4.5091e-4	-4.5109e-4		
10	-3.0554e-4	-3.0565e-4		
11	-2.0851e-4	-2.0870e-4		
12	0.0000	0.0000		

Table 4.6: Summary of ANOVA statistics and parameter estimates of reduced models R5 and R6.

R5 contains seasonal factors and regression variables.
R6 involves only regression valuables.

	Model R5	Model R6
SSE	3.101e-4	4.535e-4
MSE	1.5e-7	2.2e-7
Error DF	2068	2086
Root MSE	3.872e-4	4.663e-4
R-Square	0.7996	0.7077
STDR	3.85e-4	4.65e-4
Plots location		Figure 4.12
Intercept	0.83109	0.84692
FAT	4.3711e-2	3.8196e-2
PROT	6.1422e-2	5.5653e-2
LOS	3.4779e-2	3.2744e-2
F*F		
F*P	-1.2349e-2	-1.0795e-2
F*L	-8.4761e-3	-7.4308e-3
P*P	-8.2511e-4	-6.3576e-4
P*L	-1.0210e-2	-9.3142e-3
L*L	1.3895e-4	
F*P*L	2.3904e-3	2.0946e-3
Season 1	-4.3875e-5	
2	3.3962e-5	
3	7.7284e-5	
4	4.5144e-5	
5	-3.5806e-4	
6	-6.4589e-4	
7	-6.8021e-4	
8	-3.2909e-4	
9	-4.5107e-4	
10	-3.0592e-4	
11	-2.0851e-4	
12	0.0000	

Table 4.7: Comparison of ANOVA statistics and the characteristics of residuals before and after eliminating outliers from Quebec non-random data and from Ontario non-random data.

Q1 includes seasonal factors and regression variables.
Q2 is a simple multiple regression.
O1 includes seasonal factors and regression variables.
O2 is a simple multiple regression.

		Full observations	Without outliers		Full observations	Without outliers
# of outliers (%)	**Model Q1**	0	12 (2.86%)	**Model O1**	0	10 (1.46%)
SSE		3.208e-5	9.22e-6		3.976e-5	2.952e-5
MSE		8e-8	2e-8		6e-8	5e-8
Error DF		398	386		665	655
Root MSE		2.8e-4	1.5e-4		2.4e-4	2.1e-4
R-square		0.9578	0.9868		0.9380	0.9528
Average of resid		1.348e-15	4.463e-16		-8.96e-16	-5.31e-16
STDR		2.77e-4	1.50e-4		2.41e-4	2.09e-4
Max residual		1.28e-3	3.71e-4		7.21e-4	5.96e-4
Min residual		-1.43e-3	-4.04e-4		-1.64e-3	-5.58e-4
Skewness		-0.3783	-0.1977		-0.753	0.061
Kurtosis		4.0594	-0.3294		5.848	0.087
# of outliers (%)	**Model Q2**	0	9 (2.14%)	**Model O2**	0	7 (1.01%)
SSE		4.300e-5	1.894e-5		5.290e-5	4.370e-5
MSE		1.1e-7	5e-8		8e-8	7e-8
Error DF		409	400		676	669
Root MSE		3.2e-4	2.2e-4		2.8e-4	2.6e-4
R-square		0.9434	0.9733		0.9175	0.9303
Average of resid		1.200e-15	2.707e-16		-1.77e-16	-8.07e-16
STDR		3.20e-4	2.15e-4		2.78e-4	2.54e-4
Max residual		1.51e-3	6.41e-4		8.06e-4	7.33e-4
Min residual		-1.57e-3	-6.18e-4		-1.64e-3	-7.41e-4
Skewness		-0.2990	-0.3012		-0.574	0.0268
Kurtosis		3.7752	-0.2375		3.150	-0.163

Table 4.8: Summary of ANOVA statistics and parameter estimates of full and reduced models for Quebec non-random data.

Q1 includes seasonal factors and all regression variables.
Q2 is a simple regression.
Q3 is a simplified model developed from Q1.
Q4 is a reduced regression model.

	Model Q1	Model Q2	Model Q3	Model Q4
SSE	9.22e-6	1.894e-5	9.27e-6	1.896e-5
MSE	2e-8	5e-8	2-8	5e-8
DF error	386	400	387	402
Root MSE	1.5e-4	2.2e-4	1.5e-4	2.2e-4
R-Square	0.9868	0.9733	0.9867	0.9733
STDR	1.50e-4	2.15e-4	1.51e-4	2.15e-4
Plots				Figure 4.5
Intercept	0.91363	0.94510	0.95505	0.96838
FAT	1.8791e-3	-1.1677e-3	-8.5207e-3	-6.8242e-3
PROT	2.3604e-2	1.6211e-2	1.1618e-2	9.6244e-3
LOS	3.0830e-2	2.2781e-2	2.3058e-2	1.8293e-2
F*F	2.7825e-4	2.2233e-4	2.8223e-4	2.2089e-4
F*P	-4.1219e-3	-2.2592e-3	-1.0135e-3	-5.2707e-4
F*L	-1.8242e-4	1.8125e-4	1.6926e-3	1.1882e-3
P*P	9.0048e-4	6.3702e-5	8.7083e-4	
P*L	-3.9555e-3	-1.9213e-3	-1.7510e-3	-6.7306e-4
L*L	-1.9729e-3	-1.7014e-3	-1.9334e-3	-1.6641e-3
F*P*L	5.6123e-4	3.0741e-4		
Season			Season	
1	1.2304e-4		1.3025e-4	
2	-2.2672e-5		-1.8962e-5	
3	-4.3729e-5		-4.2427e-5	
4	2.2969e-5		2.9807e-5	
5	-1.4293e-5		-1.1635e-5	
6	-2.6645e-4		-2.6232e-4	
7	-3.5832e-4		-3.5154e-4	
8	-3.1635e-4		-3.1178e-4	
9	-3.1577e-4		-3.1275e-4	
10	-1.4312e-4		-1.4335e-4	
11	2.8891e-5		2.8022e-5	
12	0.0000		0.0000	

Table 4.9: Summary of ANOVA statistics and parameter estimates of full and reduced models for Ontario non-random data.

O1 includes seasonal factors and all regression variables.
O2 is a simple regression with open intercept.
O3 is a simplified model developed from O1.
O4 is a reduced regression model.

	Model O1	Model O2	Model O3	Model O4
SSE	2.952e-5	4.370e-5	2.997e-5	4.371e-5
MSE	5e-8	7e-8	5e-8	7e-8
DF error	655	669	659	670
Root MSE	2.1e-4	2.6e-4	2.1e-4	2.6e-4
R-Square	0.9528	0.9303	0.9521	0.9303
STDR	2.09e-4	2.54e-4	2.10e-4	2.54e-4
Intercept	0.86546	0.48708	0.89996	0.48892
FAT	-6.4777e-4	8.4458e-2	-3.0695e-3	8.4456e-2
PROT	1.4846e-2	1.2320e-1	3.4153e-3	1.2329e-1
LOS	5.0408e-2	1.1696e-1	4.3538e-2	1.1629e-1
F*F	9.6276e-5	2.0608e-5		
F*P	-1.8604e-3	-2.5413e-2	-1.3747e-4	-2.5366e-2
F*L	3.2093e-5	-1.5019e-2	6.1122e-4	-1.5029e-2
P*P	5.3565-4	5.0736e-4		4.5283e-4
P*L	-2.3907e-3	-2.1991e-2		-2.1991e-2
L*L	-3.8997e-3	-3.5951e-3	-3.8319e-3	-3.5346e-3
F*P*L	2.2736e-4	4.5026e-3		4.5063e-3
Season			Season	
1	-2.0494e-4		-2.0689e-4	
2	-2.2529e-4		-2.2585e-4	
3	-1.4211e-4		-1.2866e-4	
4	-1.1463e-4		-1.0512e-4	
5	-4.1878e-4		-4.1396e-4	
6	-4.0082e-4		-3.8815e-4	
7	-6.0888e-4		-6.1070e-4	
8	-2.9981e-4		-3.0290e-4	
9	-4.8173e-4		-4.6919e-4	
10	-3.4271e-4		-3.3847e-4	
11	-3.0166e-4		-2.9810e-4	
12	0.000		0.000	

Table 4.10: Summary of the F-test. Comparing models from Ontario-Alberta random.

R1 includes seasonal and regional factors. R2 has seasonal factors.
R3 and R4 are simple regressions with open and fixed intercepts, respectively.
R5 is a reduced model developed from R2.
R6 is a reduced simple regression derived from R3.

Comparisons	Conditions	F-values	Pr>F
R3 vs. R4	Full observations	13.88	2.00e-4
R3 vs. R4	Without outliers	16.73	3.11e-16
R1 vs. R2	Without outliers	0	1
R3 vs. R1 and R2	Without outliers	53.13	7.30e-156
R5 vs. R2	Without outliers	0.67	0.41
R6 vs. R3	Without outliers	0	1

Table 4.11: Summary of the F-test. Comparing full and reduced models based on Quebec non-random.

Q1 includes seasonal factors. Q2 is a full model of simple regression.
Q3 is a reduced model of Q1. Q4 is a reduced simple regression.

Comparisons	Conditions	F-values	Pr>F
Q1 vs. Q2	Without outliers	29.07	6.86e-52
Q3 vs. Q4	Without outliers	26.97	4.29e-51
Q1 vs. Q3	Without outliers	2.09	0.15
Q2 vs. Q4	Without outliers	0.21	0.81

Table 4.12: Summary of the F-test. Comparing full and reduced models based on Ontario non-random.

O1 includes seasonal factors. O2 is a full model of simple regression.
O3 is a reduced model developed from O1. O4 is a reduced simple regression.

Comparisons	Conditions	F-values	Pr>F
O1 vs. O2	Without outliers	22.47	3.56e-47
O3 vs. O4	Without outliers	27.47	2.38e-47
O1 vs. O3	Without outliers	2.50	0.042
O2 vs. O4	Without outliers	0.15	0.696

4.2.3. Comparing Quebec equations and Ontario-Alberta equations

To calibrate milk analyzers as a part of milk pricing system, the province of Quebec converts the w/w composition of calibration samples to w/v values by multiplying corresponding empirical density (see section 1). The current system is represented by the equations derived from Quebec non-random data (section 4.2.2). To assess the impact of applying the Ontario-Alberta equation to Quebec milk, models Q4 and R6 were applied to Quebec non-random data set. Quebec random data were also used to evaluate how accurate the density estimation was with Q4 and R6. In Quebec non-random, ten outliers, which were detected when the model Q1 was applied to the data set in section 4.2.2, were deleted from the data and the remaining part was used throughout the calculation.

Table 4.13 gives the statistical summary of predicted density and residuals for Quebec non-random and Quebec random. Plots of residuals versus predicted density and predicted density versus observed density are shown in Figure 4.5 and 4.6. Q4 had the best fit to Quebec non-random data, showing small error variation and no bias. However, when Q4 was fitted to Quebec random, it depicted some bias. The majority of the residuals of Q4 were positive, centring at 2.0e-4, and Q4 tended to overestimate the density of Quebec random data. This disagreement may imply inadequacy of using only a few herds to establish the relationship between density and composition.

R6 did not fit well to Quebec non-random since residuals formed a slanted belt, instead of a horizontal band. Density was overestimated at low density (1.030-1.033) and underestimated at high density (1.034-1.036). The distributions of residuals of R6 had high skewness and kurtosis due to an extreme outlier that was not eliminated by the first screening. When the extreme was omitted, the skewness and kurtosis of residual distribution appeared to be close to the Normal distributions.

Representing the Ontario and Alberta producer milk, R6 may have low bias and high variability. On the other hand, Quebec non-random data are more likely to have high bias and low variability since they were collected from the same herd repeatedly. It can be considered that R6 explains less at low and high density because the population group of Quebec non-random is slightly different from that of Ontario-Alberta random.

In Quebec random, unlike Q4, the residual plots of R6 gave a horizontal band that centred at zero. The predicted versus observed density plot indicated that R6 was unbiased, showing balanced plots around the line $y = x$, although the regression line made it difficult to find this tendency. R6 had larger variance than Q4. This unbiased prediction of R6 was supported by the fact that the average of predicted density of R6 had the closest value to the average of observed density.

Using reference milk collected from a few herds repeatedly may be risky since non-random data are likely to be biased. When an equation developed from non-random data is applied to milk collected randomly, the equation may overestimate or underestimate the density because non-random samples may not be adequate representatives of the population of milk. The non-random data may have the mean far from the mean of the real population, or an equation developed from non-random data may have little flexibility due to its small variability.

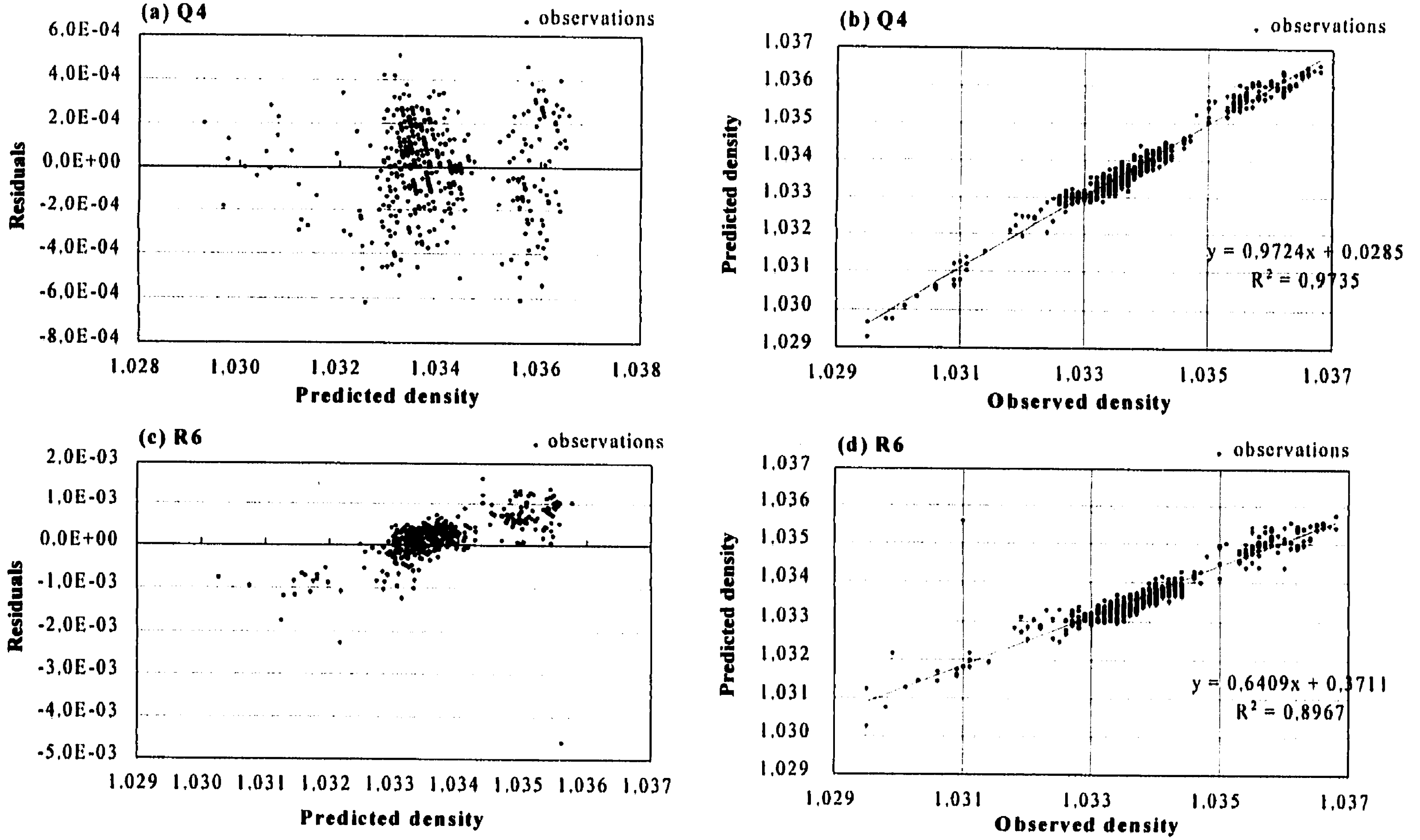
Table 4.13: Statistical summary of predicted density and residuals computed from the Quebec non-random and Quebec random data sets by use of equations Q4 and R6.

Q4 is a simple regression developed from Quebec non-random.
R6 is a simple regression based on ON-AB random.

	PQ non-random		PQ random	
Equations	Q4	R6	Q4	R6
Predicted density average[1]	1.0339	1.0337	1.0329	1.0330
Predicted density STD	1.29e-3	8.86e-4	8.838e-4	6.169e-4
Average of residuals	-2.703e-6	1.986e-4	1.813e-4	5.988e-5
STDR	2.130e-4	5.495e-4	2.474e-4	4.866e-4
Skewness	-0.334	-2.029	-0.210	-0.241
Kurtosis	0.321	14.971	1.390	-0.792
Skewness(omit extreme)		-0.592		
Kurtosis(omit extreme)		2.253		

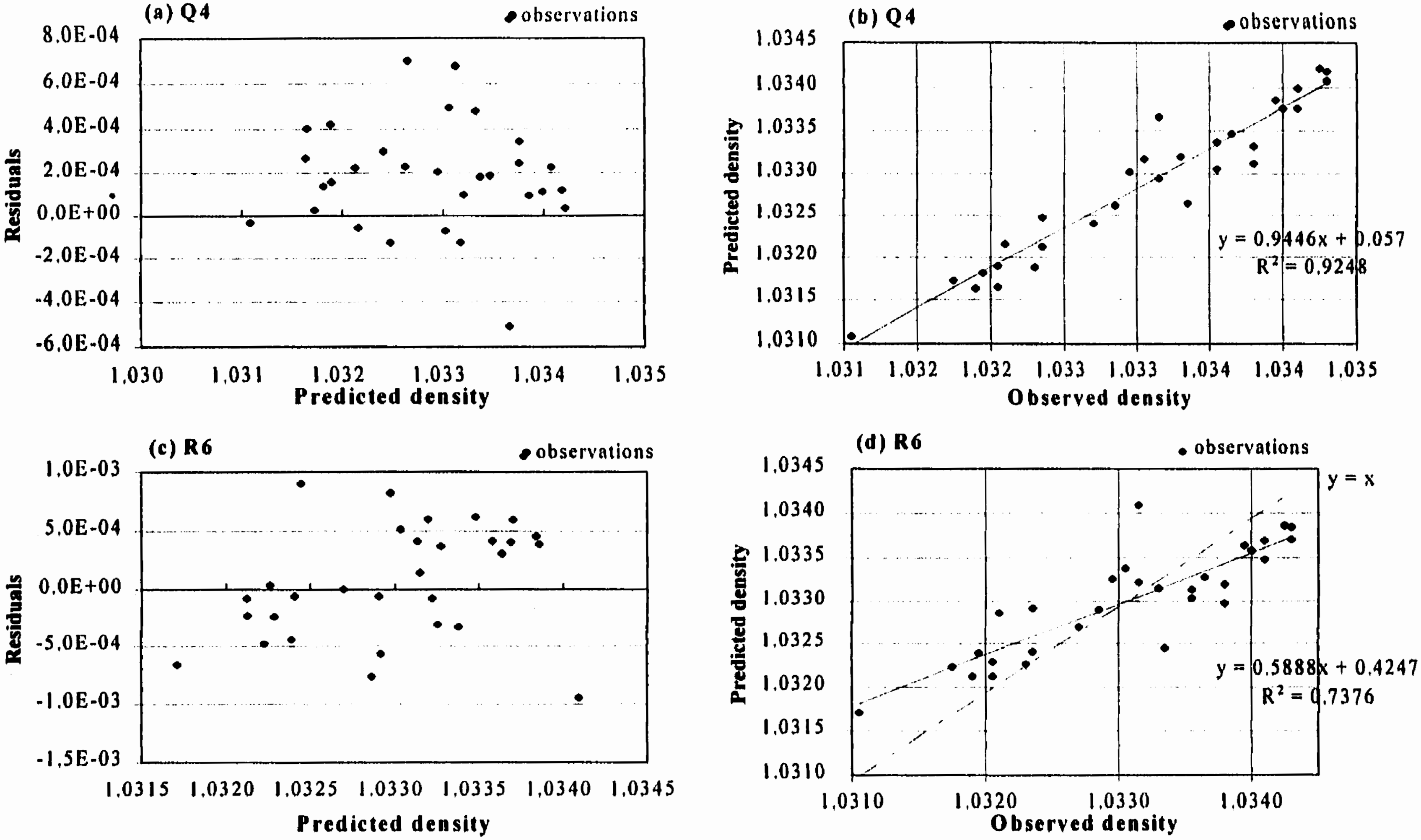
[1] Average density of Quebec non-random sample is 1.0339 with the standard deviation of 1.315e-3, and Quebec random has an average density of 1.0331 with the standard deviations 8.998e-4.

Figure 4.5: Q4 and R6 fitted to Quebec non-random data set. Plots of (a) Q4 residuals versus predicted density (b) Q4 predicted density versus observed density (c) R6 residuals versus predicted density (d) R6 predicted density versus observed density.



Q4 is a simple regression developed from Quebec non-random. R6 is a simple regression derived from Ontario-Alberta random.

Figure 4.6: Q4 and R6 fitted to Quebec random. Plots of (a) Q4 residuals versus predicted density (b) Q4 predicted density versus observed density (c) R6 residuals versus predicted density (d) R6 predicted density versus observed density.



Q4 is a simple regression developed from Quebec non-random. R6 is a simple regression derived from Ontario-Alberta random.

4.2.4. Fitting Ontario-Alberta equations to Ontario non-random data set

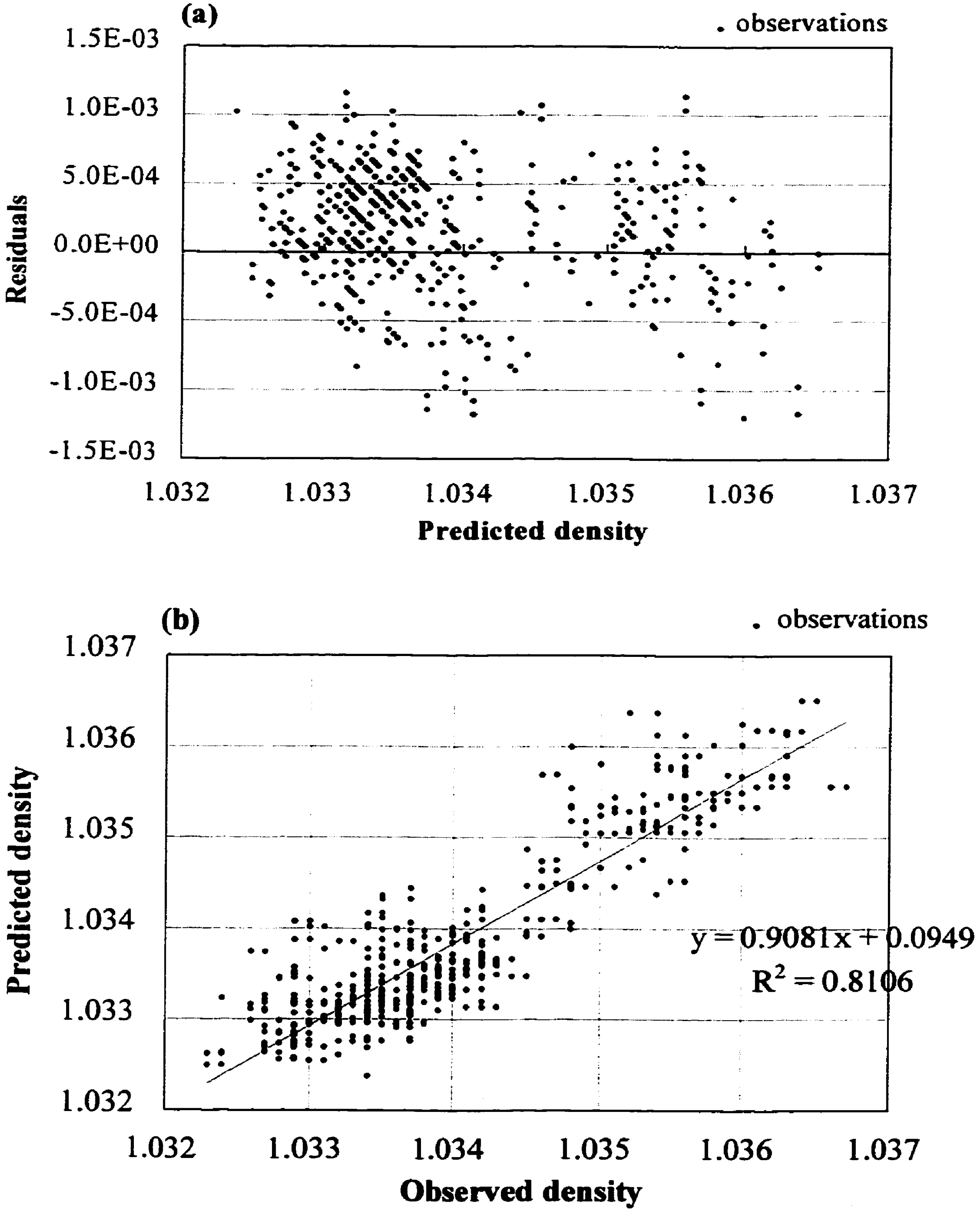
In order to evaluate flexibility of Ontario-Alberta equation, formulae R5 and R6 (see section 4.2.2) were fitted to Ontario non-random data set. Ten outliers that were defined in the analysis of model O4 were omitted from Ontario non-random data set. Table 4.14 gives the average of predicted density and STDR. Equations R5 and R6 were adequate in explaining the relation between density and components of Ontario non-random samples. Predicted density of R5 and R6 matched to original density with small STDR. Residual versus predicted density plot and predicted density versus observed density plot for R6 (Figure 4.7) depicted a good fit of the formulae by presenting well-scattered horizontal band.

Table 4.14: The average of predicted density and the standard deviation of residuals (STDR) for Ontario non-random data set analyzed by equations of R5 and R6.

	R5	R6
Averaged predicted density	1.0338	1.0338
STDR	4.120e-4	4.316e-4

Figure 4.7: Plots of (a) residuals versus predicted density (b) predicted density versus observed density of equation R6 fitted to Ontario non-random data set.

R6 is a simple regression derived from Ontario-Alberta random data set.



4.2.5. Seasonality of milk

In each data set of Ontario and Quebec non-random, monthly averages of observed density and compositions were computed. Figure 4.8 and 4.10 depict the average of observed density as a function of time. In both Quebec and Ontario, the change in density followed a certain pattern. Summer milk (from May to August) tended to have lower density, while winter milk (from November to March) possessed higher values. The variation between the month of the highest actual density and the month of the lowest actual density was 1.09e-3 in Quebec and 1.08e-3 in Ontario.

Monthly change in milk composition followed a similar trend to that of density. Figure 4.9 (Ontario) and Figure 4.11 (Quebec) give the average of each component, fat, protein, and LOS, as a function of time of year. It can be clearly seen in Ontario milk that change in fat content resembled a mountain shape that had its sides in summer and peaks in the fall. The protein content had the same pattern as that of fat, whereas LOS showed no specific structure throughout the year. Fat, protein, and LOS contents of Quebec milk exhibited a similar tendency to Ontario. It can be concluded that seasonal change in milk density is strongly related to changes in the amount of components. Fall milk, which is high in fat and protein, is likely to show high density and summer milk with low percentages of fat and protein tends to have low density.

Secondly, equations developed in section 4.2.2 were investigated to see how well they explain the seasonal fluctuation of density and components. Predicted density was computed with O3 and O4 for Ontario and with Q3 and Q4 for Quebec, using the monthly average compositions. The values of predicted density are plotted in Figure 4.8 and Figure 4.10 together with the monthly average of empirical density. Formulae with and without seasonal factors were compared to evaluate how much seasonal factors contributed to improve the accuracy of density prediction.

Q3 predicted the density of Quebec milk accurately throughout two years. Figure 4.10 indicates that predicted density of Q3 follows almost the same curve as that of observed density. Simple regression Q4 had a tendency to overestimate in summer and underestimate in winter, yet the difference between the average of observed density and predicted density was small with a maximum and minimum of 2.39e-4 and –3.33e-4, respectively.

In Ontario non-random, O3 gave larger residuals than Q3 with the Quebec data. The difference between monthly density and predicted density in O3 and O4 had a maximum and minimum of 8.57e-5 and –3.34e-4, 3.06e-4 and –4.09e-4, respectively. O4 tended to overestimate density of summer milk and underestimate that of fall and winter milk. However, the differences were small. Figure 4.8 indicates that O4 explained the seasonal fluctuation of density to some extent as the plots of predicted density of O4 followed a similar curved pattern to that of actual density.

Equations with seasonal factors explained the seasonal fluctuation in density slightly better than did those without seasonal factors. This significance of seasonal effect may imply the presence of some factors that cannot be explained by the amount of milk constituents. The factor may be differences in chemical composition of fat triglycerides and casein in the protein fraction.

Selection of the optimum equation must balance the value of precision lost by simplification and the need for accuracy. An example of a practical conclusion regarding seasonal differences of raw milk can be seen in a study by the USDA (1965). The researchers investigated the seasonality of mixed breed milk in three regions using similar calculations and comparisons to those carried out in the previous paragraphs. The study reported that the variation between the month of the highest actual weight and the month of the lowest actual weight in any one of the three regions was 0.014 pounds per gallon, which agrees with 0.0017 g/cm^3. This investigation also found that the computed weight per gallon minus actual had the highest value of +0.005 pounds per gallon and the lowest of –0.008 pounds per gallon, which correspond to 6.0e-4 g/cm^3 and –9.6e-4 g/cm^3, respectively. Although there were some monthly differences in density prediction, the USDA concluded that the influence of season was much less important than variations in composition and that the seasonal factors were negligible.

As Boden (1942) pointed out (section 2.6.1), the seasonal divergences were sometimes positive and sometimes negative. The sum of these differences should be near zero over a year. Taking the fact that simple regressions explained the monthly density differences adequately, it may be concluded that monthly compositional change in milk is the most important factor in density prediction. In addition, for the purpose of density estimation in payment system, overestimation in winter and underestimation in summer may cancel out the effect of season on milk price.

Figure 4.8: Monthly average of observed density and predicted density calculated from the average composition of the month in Ontario non-random milk analyzed by equations O3 and O4.

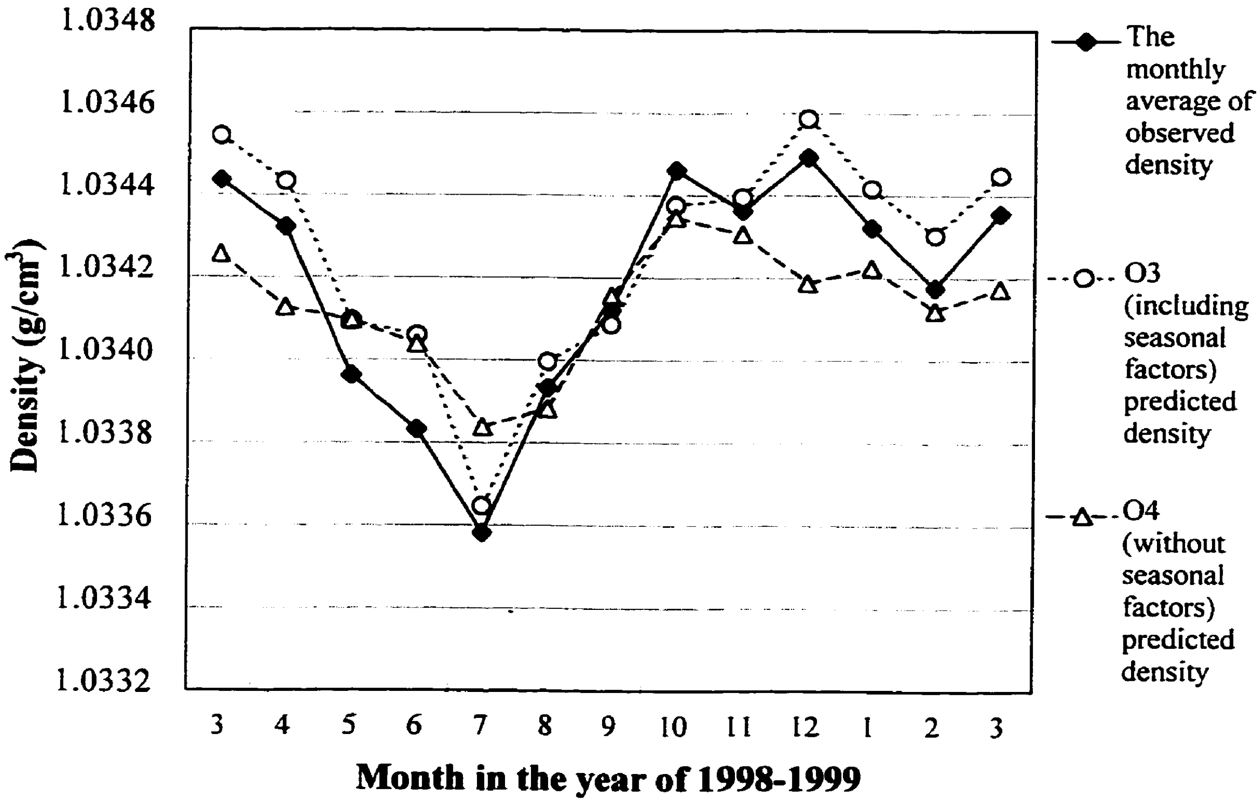
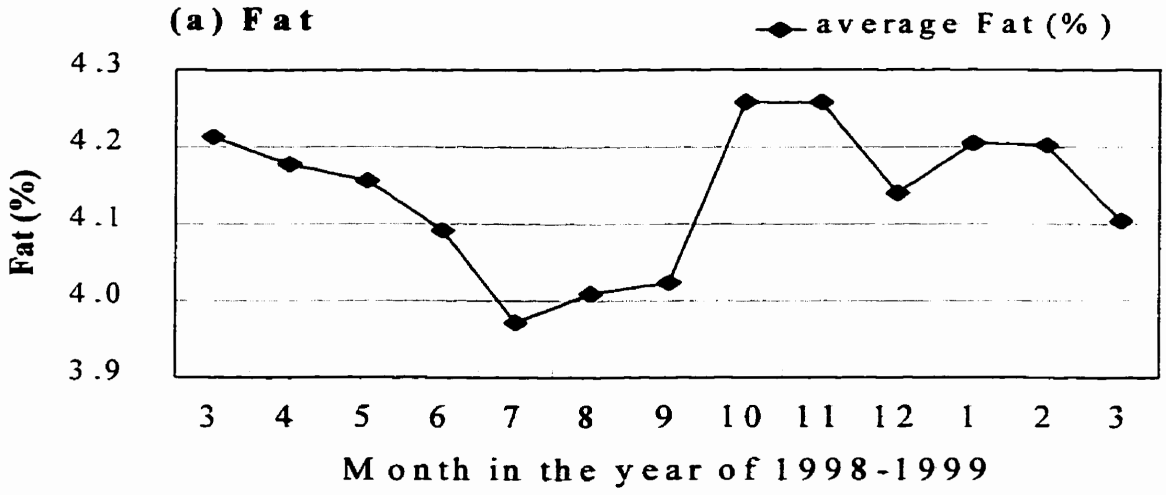
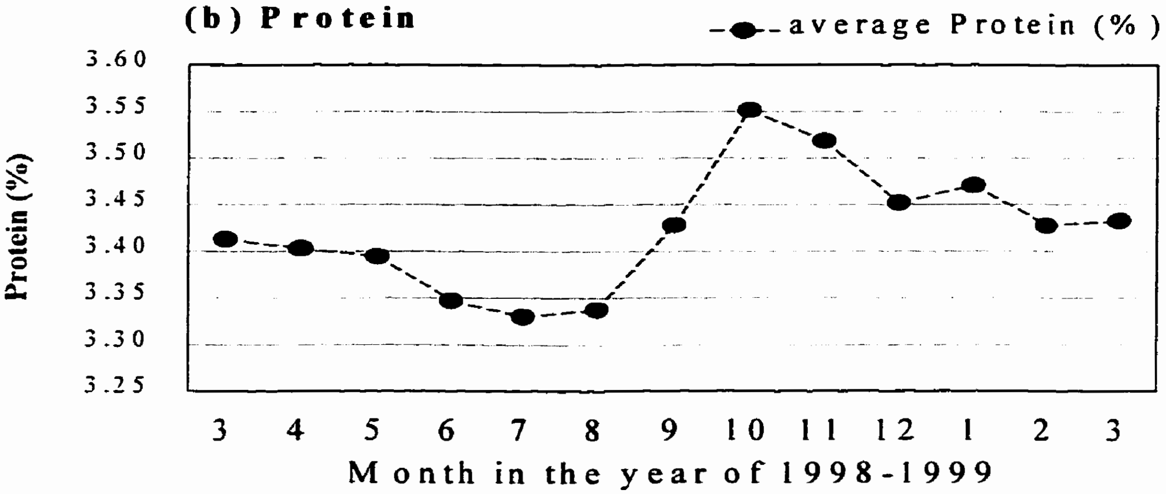


Figure 4.9: Monthly differences in average composition in Ontario non-random milk. (a) Fat (b) Protein (c) LOS


(a) Fat


(b) Protein

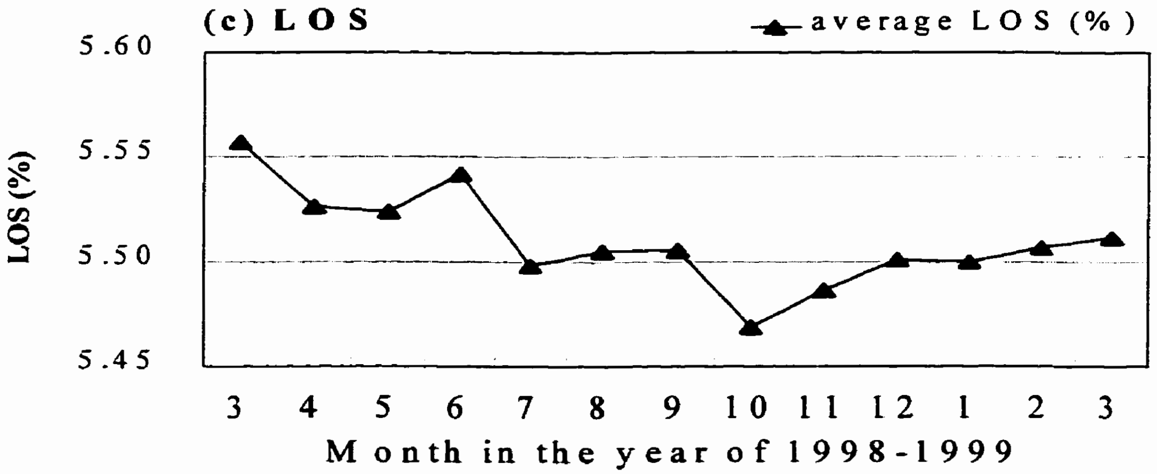

(c) LOS

Figure 4.10: Monthly average of observed density and predicted density calculated from the average composition of the month in Quebec non-random milk analyzed by equations Q3 and Q4.

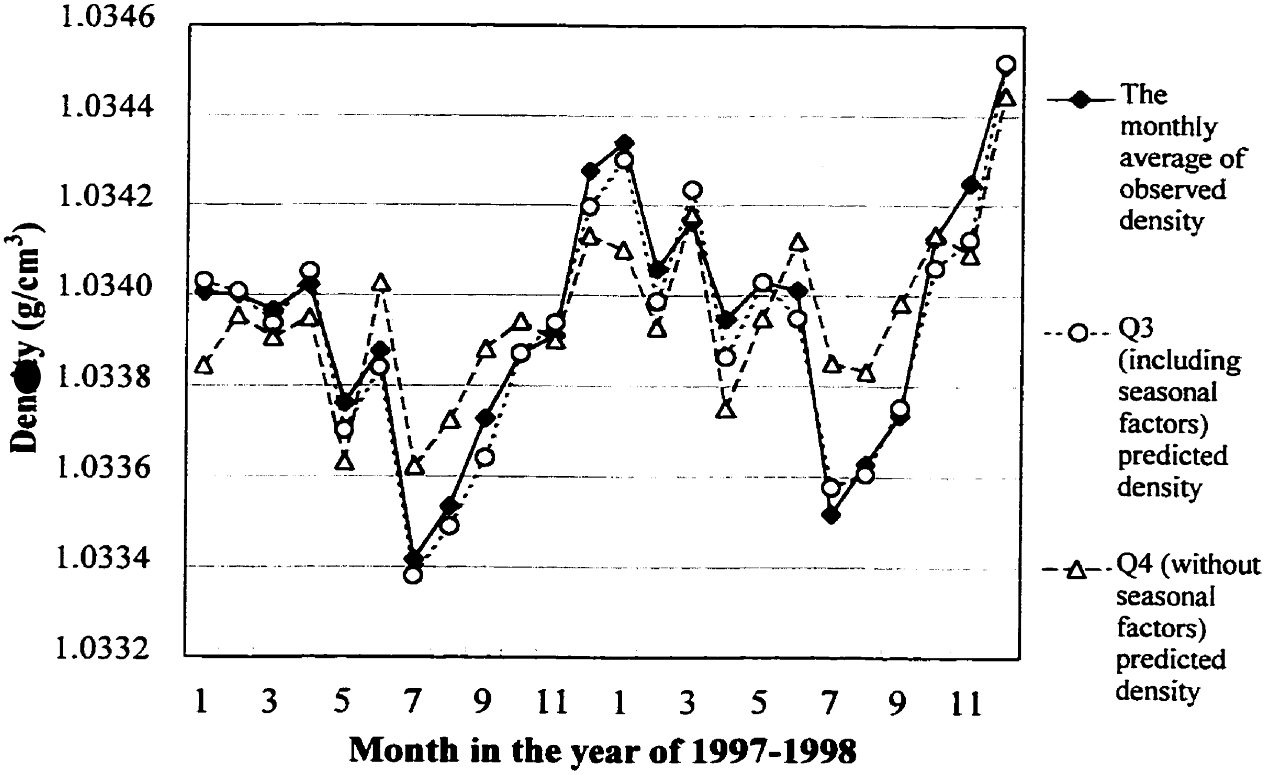
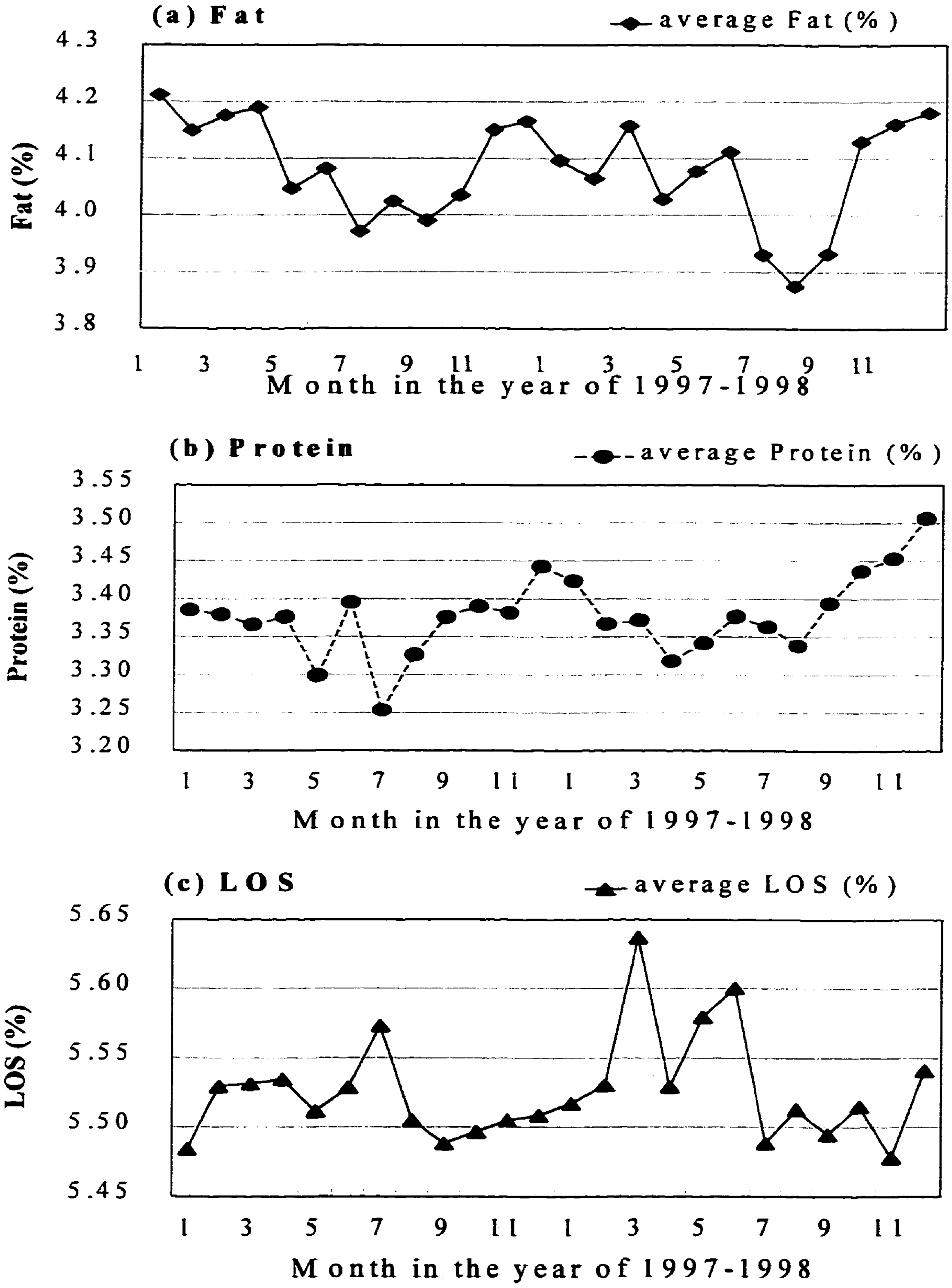


Figure 4.11: Monthly differences in average composition in Quebec non-random milk. (a) Fat (b) Protein (c) LOS



4.2.6. Fitting various models to combined random data set of Ontario and Alberta

Since the Ontario-Alberta data set was considered representative of producer milk in Ontario and Alberta, various equations were fitted to this data set for evaluating the reliability of the formulae. This data set was 'cleaned' by omitting 24 outliers that had been detected when model R1 had been fitted to the data set (section 4.2.2). Equations of R6, Q4, US, and Biggs were evaluated. The average and the standard deviation of predicted density and residuals are summarized in Table 4.15. The plots of residuals versus predicted density or observed density and predicted density versus observed density are depicted in Figure 4.12 and 4.13. None of the formulae except R6 seemed to be adequate in describing Ontario-Alberta data set. All of their residuals had very large standard deviations, and some residuals had extreme positive values.

The reason why equations derived from different milk sources did not fit well to Ontario-Alberta random may be attributed to the following point. The data sets from which the equations had been developed may have high bias such as a different mean values from that of Ontario-Alberta random data. As a consequence, those formulae may be valid only within the original data set and are inadequate with other sources of milk. Supporting the result in section 4.2.3, this result indicates the problem in using a formula based on a few herds or an equation developed in a different country to establish the relationship between density and composition.

Table 4.15: Statistical summary of predicted density and residuals for the combined data set of Ontario and Alberta random computed by the following equations.

R6 = a simple regression developed from Ontario-Alberta random.
Q4 = a simple regression driven from Quebec non-random.
US = an equation constructed by the study of USDA in 1965 (See section 2.6.1).
Biggs = a formula developed by D.A. Biggs in 1978 from Ontario producer milk.

Equations	R6	Q4	US	Biggs[2]
Predicted average[1]	1.0337	1.0338	1.0338	1.0320
Predicted STD	7.242e-4	1.193e-3	1.227e-3	9.954e-4
Residual average	4.826e-5	-9.806e-5	-6.292e-5	1.660e-3
STDR	4.637e-4	9.282e-4	9.556e-4	7.659e-4
Skewness	-0.301	4.637	0.717	0.472
Kurtosis	-0.197	47.994	9.658	5.717

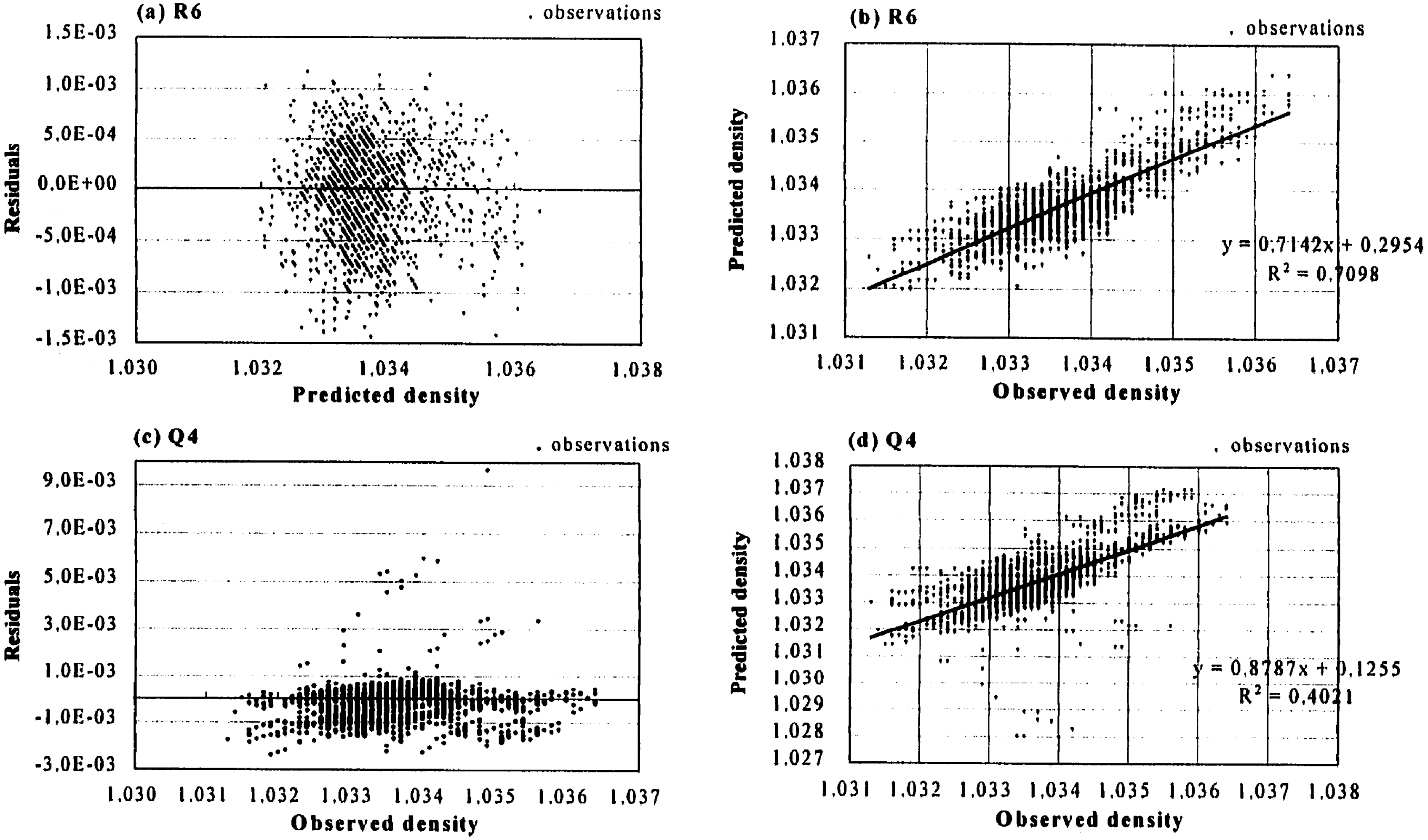
[1] Combined data set Ontario and Alberta random has the average density of 1.0337 with the standard deviation of 8.730e-4.

[2] Calculated by an equation containing fat and SNF.

$$\text{Density} = 1.007664 + 0.0001653\ \text{Fat}(\%) + 0.0026703\ \text{SNF}(\%)$$

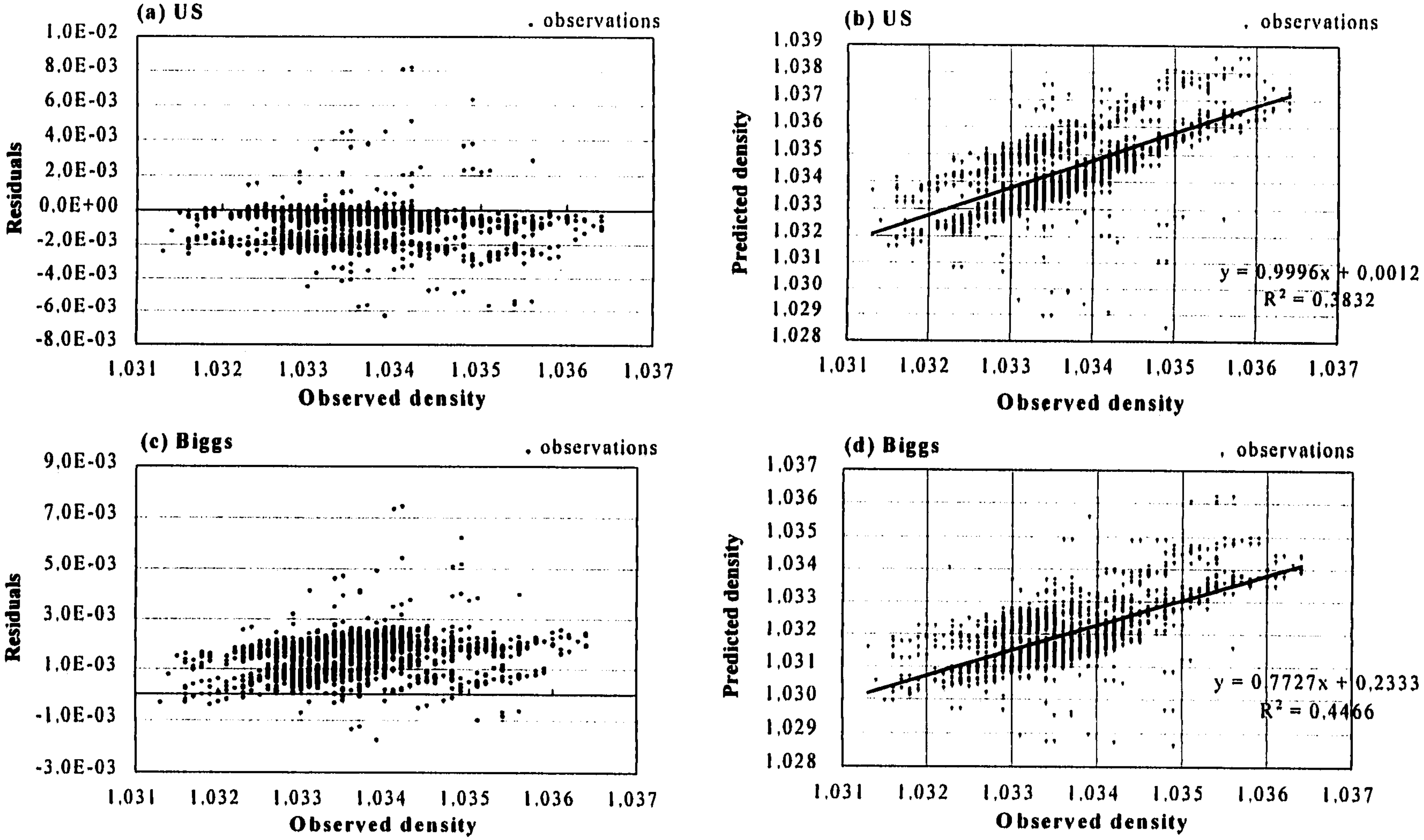
where $\text{SNF}(\%) = \text{protein}(\%) + \text{LOS}(\%)$

Figure 4.12: R6 and Q4 fitted to Ontario-Alberta random. Plots of (a) R6 residuals versus predicted density (b) R6 predicted versus observed density (c) Q4 residuals versus observed density (d) Q4 predicted versus observed density.



R6 is a simple regression developed form ON-AB random. Q4 is a simple regression derived from Quebec non-random.

Figure 4.13: US and Biggs fitted to Ontario-Alberta random. Plots of (a) US residuals versus observed density (b) US predicted versus observed density (c) Biggs residuals versus observed density (d) Biggs predicted versus observed density.



US is an equation constructed by USDA in 1965. Biggs is a formula developed from Ontario milk by D.A. Biggs in 1978.

4.2.7. Comparisons among equations and current systems

Effects of various weight/volume conversion procedures were evaluated using three sets of milk composition and corresponding density values. The fat, protein, and LOS values in the selected composition ranged from 2.2 to 5.0%, 3.0 to 4.0%, and 5.3 to 5.8%, respectively. These values are real data selected from reference milks examined at The Alberta Central Milk Testing Centre for their composition and at the University of Guelph for their density. Simulated conversions were conducted using equations of R6, Q4 (section 4.2.2), US (section 4.2.2 and 2.6.1), and a Biggs equation developed by D.A. Biggs (1978) using Ontario producer milk (section 2.6). Current conversion systems in the province of Ontario, Alberta, and Quebec were also simulated and compared with the result of the equations above. Equation Q4 represented the current Quebec conversion procedure. See section 1 for details about w/v computing policies in provinces. Multiplication of reference w/w values with the corresponding empirical density at 4.0°C gave actual w/v values. Chemical reference values (w/w), actual w/v values, and w/v estimates by equations are given in Table 4.16, 4.18, and 4.20, for fat, protein, and LOS, respectively. Table 4.17 and Figure 4.14 depict the differences of w/v estimates from actual w/v values. Table 4.19 and Figure 4.15 present the differences for protein estimates and Table 4.21 and Figure 4.16 give those for the LOS estimates.

For the conversion of fat and protein, R6, Q4, US demonstrated the most accurate estimations. The values of estimated w/v minus actual w/v remained very close to zero regardless of the fat and protein content. Among the three models, US estimates tended to be slightly higher than actual w/v values, showing maximum and minimum differences of 0.005 and –0.001, respectively. In contrast, R6 was more likely to have a negative discrepancy from the real w/v and had a maximum difference of 0.001 and a minimum of –0.004. Q4 estimates were well balanced on both positive and negative sides and the difference had the highest and lowest values of 0.002 and -0.002.

Biggs, current ON, and current AB estimates always produced lower results than real w/v values. Particularly, current AB, which simply multiplies w/w contents by a constant factor of 1.02969, showed the lowest estimates and the discrepancy from actual w/v increased as the fat and protein content increased. This loss of accuracy in high fat and protein contents samples of the current Alberta system reveals the limitation of using a constant factor. Although it is based on a combined factor of density at 20°C and expansivity which makes up the density difference between 20°C and 4°C, the current Ontario system was less accurate at high levels of fat and protein. Biggs w/v predictions were constantly lower than actual density by 0.005 - 0.01%. Even though it used fat and SNF content for predicting density, the Biggs formula was inadequate, possibly due to small sample size and short-term experiment. LOS estimation depicted the same tendency as that of fat and protein except that current ON and AB did not show a decrease in accuracy even at high LOS content. Variances between predicted and actual w/v in LOS were larger than those in fat and protein.

Since formulae of R6, Q4, and US showed good conversion of w/v milk components, a conversion procedure based on regression of empirical density on w/w milk composition is probably the most accurate. Reliability of w/v conversion of components can increase by switching the current systems in Ontario and Alberta to the one employing an equation that estimates milk density from components.

Secondly, the same w/v conversion was simulated with the Ontario-Alberta random data set to compare equations R6, Q4, and US. A statistical summary of differences between computed w/v values and real w/v for fat, protein, and LOS is given in Table 4.22. In the previous simulation with selected data sets, Q4 appeared to have the most accurate conversion. However, when the equations were applied to the whole data set of Ontario-Alberta random, R6 conversion was the most precise and accurate (Table 4.22), having the smallest standard deviation (0.002). Q4 and US also depicted a sufficient conversion, but they contained some extremes, as indicated by a large minimum and maximum (-0.052 and 0.013 in Q4 and –0.035 and 0.044 in US).

Table 4.16: Weight/weight (w/w) reference fat converted to weight/volume (w/v).

Comparing the conversion by use of constructed equations, observed density, and current system in ON and AB. Real w/v = empirical density × reference fat.

Reference Fat w/w	Real w/v	R6	Q4 (Current PQ)	USDA	Biggs	Current ON	Current AB
2.155	2.227	2.227	2.229	2.229	2.222	2.230	2.219
2.282	2.358	2.358	2.359	2.360	2.353	2.360	2.350
2.881	2.975	2.975	2.975	2.975	2.969	2.977	2.967
2.969	3.069	3.067	3.068	3.069	3.061	3.068	3.057
3.216	3.322	3.322	3.322	3.323	3.316	3.319	3.311
3.367	3.479	3.477	3.477	3.478	3.471	3.475	3.467
3.506	3.621	3.621	3.620	3.621	3.614	3.618	3.610
3.608	3.727	3.727	3.728	3.729	3.721	3.723	3.715
3.722	3.847	3.845	3.845	3.847	3.839	3.840	3.833
3.945	4.076	4.074	4.074	4.075	4.068	4.075	4.062
4.111	4.250	4.246	4.248	4.250	4.241	4.246	4.233
4.264	4.409	4.407	4.408	4.410	4.401	4.403	4.391
4.603	4.760	4.760	4.762	4.764	4.753	4.752	4.740
4.726	4.890	4.888	4.890	4.892	4.881	4.878	4.866
4.900	5.078	5.074	5.077	5.081	5.067	5.057	5.045
5.039	5.222	5.218	5.220	5.224	5.210	5.195	5.189
AVE	3.832	3.830	3.831	3.833	3.824	3.826	3.816
STD	0.914	0.913	0.914	0.915	0.913	0.907	0.907

Table 4.17: Fat differences: converted w/v values minus actual w/v values.

Reference Fat w/w	R6	Q4 (Current PQ)	USDA	Biggs	Current ON	Current AB
2.155	0.000	0.001	0.001	-0.005	0.002	-0.008
2.282	0.000	0.001	0.001	-0.005	0.002	-0.008
2.881	0.000	0.000	0.000	-0.006	0.003	-0.008
2.969	-0.001	-0.001	0.000	-0.008	-0.001	-0.012
3.216	0.000	0.000	0.000	-0.007	-0.003	-0.011
3.367	-0.002	-0.002	-0.001	-0.008	-0.005	-0.012
3.506	-0.001	-0.001	0.000	-0.007	-0.004	-0.011
3.608	0.000	0.001	0.002	-0.006	-0.004	-0.012
3.722	-0.003	-0.002	-0.001	-0.009	-0.007	-0.015
3.945	-0.003	-0.002	-0.001	-0.008	-0.001	-0.014
4.111	-0.003	-0.002	0.000	-0.008	-0.003	-0.016
4.264	-0.002	0.000	0.001	-0.008	-0.005	-0.018
4.603	0.001	0.002	0.004	-0.006	-0.008	-0.020
4.726	-0.002	0.000	0.002	-0.009	-0.012	-0.024
4.900	-0.004	-0.001	0.003	-0.012	-0.021	-0.033
5.039	-0.004	-0.002	0.001	-0.012	-0.027	-0.034
AVE	-0.002	-0.001	0.001	-0.008	-0.006	-0.016
STD	0.002	0.001	0.001	0.002	0.008	0.008
MIN	-0.004	-0.002	-0.001	-0.012	-0.027	-0.034
MAX	0.001	0.002	0.004	-0.005	0.003	-0.008

Table 4.18: Weight/weight (w/w) reference protein converted to weight/volume (w/v).

Comparing the conversion by use of constructed equations, observed density, and current system in ON and AB. Real w/v = empirical density × reference protein.

Reference Protein w/w	**Real density w/v**	**R6**	**Q4 (Current PQ)**	**USDA**	**Biggs**	**Current ON**	**Current AB**
2.995	3.092	3.093	3.093	3.093	3.086	3.089	3.084
3.056	3.157	3.156	3.156	3.156	3.150	3.152	3.147
3.096	3.199	3.197	3.197	3.198	3.192	3.203	3.188
3.162	3.269	3.266	3.267	3.268	3.261	3.261	3.256
3.204	3.313	3.310	3.311	3.312	3.304	3.304	3.299
3.271	3.382	3.380	3.381	3.383	3.375	3.383	3.368
3.337	3.451	3.448	3.450	3.451	3.444	3.450	3.436
3.42	3.536	3.535	3.535	3.536	3.529	3.536	3.522
3.482	3.599	3.600	3.600	3.601	3.594	3.599	3.585
3.519	3.638	3.638	3.639	3.640	3.633	3.638	3.623
3.59	3.712	3.712	3.714	3.715	3.707	3.711	3.697
3.663	3.790	3.789	3.790	3.792	3.783	3.786	3.772
3.703	3.832	3.831	3.833	3.835	3.825	3.827	3.813
3.807	3.941	3.939	3.942	3.945	3.934	3.934	3.920
3.93	4.072	4.068	4.070	4.073	4.062	4.060	4.047
4.079	4.227	4.224	4.226	4.229	4.217	4.213	4.200
AVE	3.576	3.574	3.575	3.577	3.569	3.571	3.560
STD	0.337	0.337	0.338	0.338	0.337	0.335	0.333

Table 4.19: Protein differences: converted w/v values minus actual w/v values.

Reference Protein w/w	**R6**	**Q4 (Current PQ)**	**USDA**	**Biggs**	**Current ON**	**Current AB**
2.995	0.000	0.000	0.000	-0.006	-0.003	-0.008
3.056	-0.001	-0.001	0.000	-0.006	-0.005	-0.010
3.096	-0.002	-0.002	-0.001	-0.007	0.004	-0.011
3.162	-0.002	-0.002	-0.001	-0.008	-0.008	-0.013
3.204	-0.003	-0.002	-0.001	-0.009	-0.009	-0.014
3.271	-0.002	-0.001	0.001	-0.007	0.001	-0.014
3.337	-0.002	-0.001	0.001	-0.007	0.000	-0.015
3.42	-0.001	-0.001	0.000	-0.007	0.000	-0.014
3.482	0.000	0.001	0.002	-0.005	0.000	-0.014
3.519	0.000	0.001	0.002	-0.005	0.000	-0.014
3.59	0.000	0.002	0.003	-0.005	-0.002	-0.015
3.663	-0.001	0.000	0.002	-0.007	-0.005	-0.018
3.703	-0.001	0.001	0.003	-0.006	-0.005	-0.019
3.807	-0.001	0.002	0.005	-0.007	-0.007	-0.021
3.93	-0.004	-0.002	0.001	-0.010	-0.012	-0.025
4.079	-0.003	-0.002	0.001	-0.010	-0.014	-0.027
AVE	-0.001	0.000	0.001	-0.007	-0.004	-0.016
STD	0.001	0.001	0.002	0.002	0.005	0.005
MIN	-0.004	-0.002	-0.001	-0.010	-0.014	-0.027
MAX	0.000	0.002	0.005	-0.005	0.004	-0.008

Table 4.20: Weight/weight (w/w) reference LOS converted to weight/volume (w/v).

Comparing the conversion by use of constructed equations, observed density, and current system in ON and AB. Real w/v = empirical density × reference LOS.

Reference LOS w/w	**Real w/v**	**R6**	**Q4 (Current PQ)**	**USDA**	**Biggs**	**Current ON**	**Current AB**
5.342	5.516	5.516	5.516	5.516	5.504	5.512	5.501
5.390	5.567	5.565	5.564	5.566	5.555	5.562	5.550
5.410	5.593	5.593	5.594	5.595	5.585	5.585	5.571
5.431	5.613	5.611	5.612	5.613	5.600	5.604	5.592
5.461	5.643	5.639	5.640	5.642	5.631	5.636	5.623
5.468	5.652	5.648	5.649	5.651	5.639	5.643	5.630
5.480	5.666	5.664	5.665	5.668	5.656	5.657	5.643
5.503	5.688	5.688	5.691	5.691	5.674	5.676	5.666
5.522	5.714	5.712	5.715	5.718	5.705	5.701	5.686
5.534	5.719	5.719	5.721	5.722	5.706	5.709	5.698
5.553	5.740	5.736	5.738	5.740	5.729	5.732	5.718
5.607	5.794	5.796	5.800	5.804	5.787	5.786	5.773
5.737	5.937	5.932	5.938	5.945	5.926	5.920	5.907
5.819	6.015	6.011	6.015	6.022	6.006	6.005	5.992
AVE	5.704	5.702	5.704	5.707	5.693	5.695	5.682
STD	0.132	0.131	0.133	0.135	0.133	0.130	0.130

Table 4.21: LOS differences: converted w/v values minus actual w/v values.

Reference LOS w/w	**R6**	**Q4 (Current PQ)**	**USDA**	**Biggs**	**Current ON**	**Current AB**
5.342	0.001	0.000	0.001	-0.011	-0.004	-0.015
5.390	-0.002	-0.003	-0.002	-0.012	-0.005	-0.017
5.410	0.000	0.001	0.003	-0.008	-0.007	-0.022
5.431	-0.003	-0.001	0.000	-0.014	-0.010	-0.021
5.461	-0.004	-0.003	-0.001	-0.012	-0.006	-0.020
5.468	-0.004	-0.003	-0.001	-0.013	-0.009	-0.022
5.480	-0.002	0.000	0.002	-0.010	-0.009	-0.023
5.503	0.000	0.003	0.003	-0.014	-0.012	-0.022
5.522	-0.002	0.001	0.004	-0.010	-0.013	-0.028
5.534	0.000	0.002	0.003	-0.013	-0.010	-0.021
5.553	-0.005	-0.002	0.000	-0.011	-0.008	-0.022
5.607	0.002	0.005	0.009	-0.007	-0.009	-0.021
5.737	-0.005	0.002	0.008	-0.011	-0.017	-0.029
5.819	-0.004	0.000	0.007	-0.009	-0.010	-0.023
AVE	-0.002	0.000	0.003	-0.011	-0.009	-0.022
STD	0.002	0.002	0.004	0.002	0.003	0.004
MIN	-0.005	-0.003	-0.002	-0.014	-0.017	-0.029
MAX	0.002	0.005	0.009	-0.007	-0.004	-0.015

Figure 4.14: Simulation of **Fat** weight/volume conversion.

Difference is calculated by subtracting real w/v from estimated w/v values. Comparing density computed by equations developed in this study compared to that calculated by other studies and current conversion system.

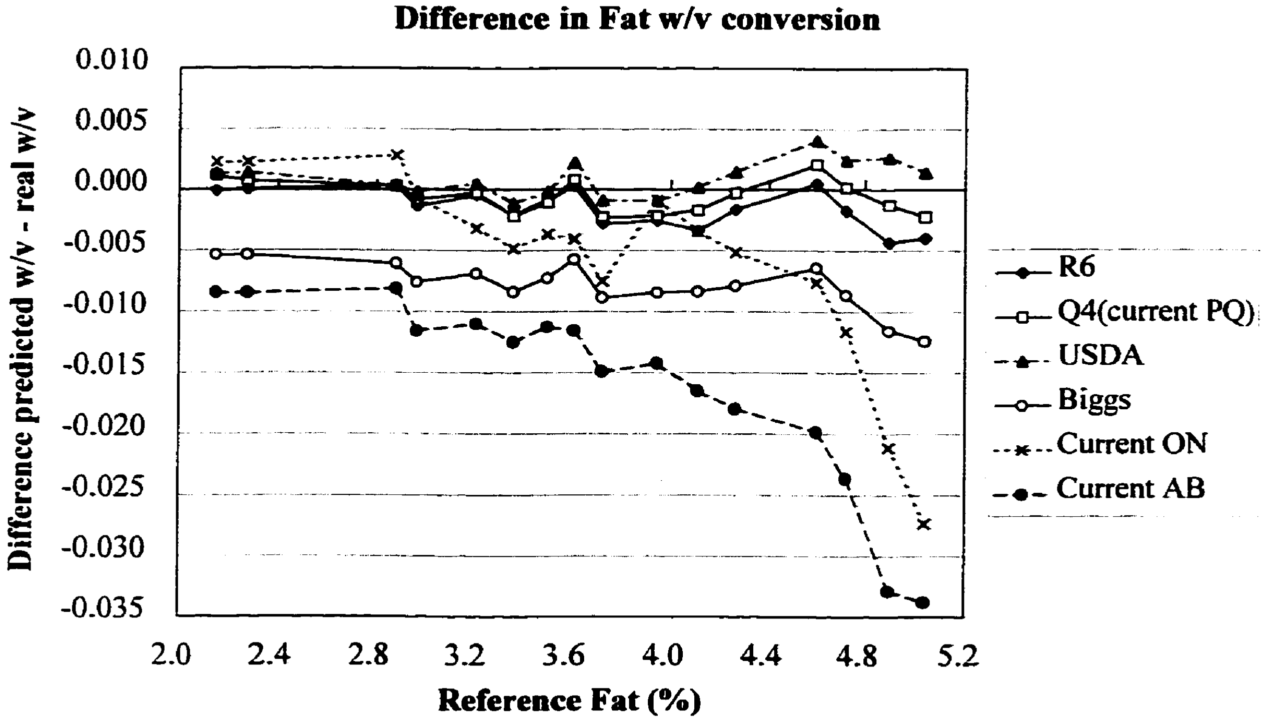


Figure 4.15: Simulation of **Protein** weight/volume conversion.

Difference is calculated by subtracting real w/v from estimated w/v values. Comparing density computed by equations developed in this study compared to that calculated by other studies and current conversion system.

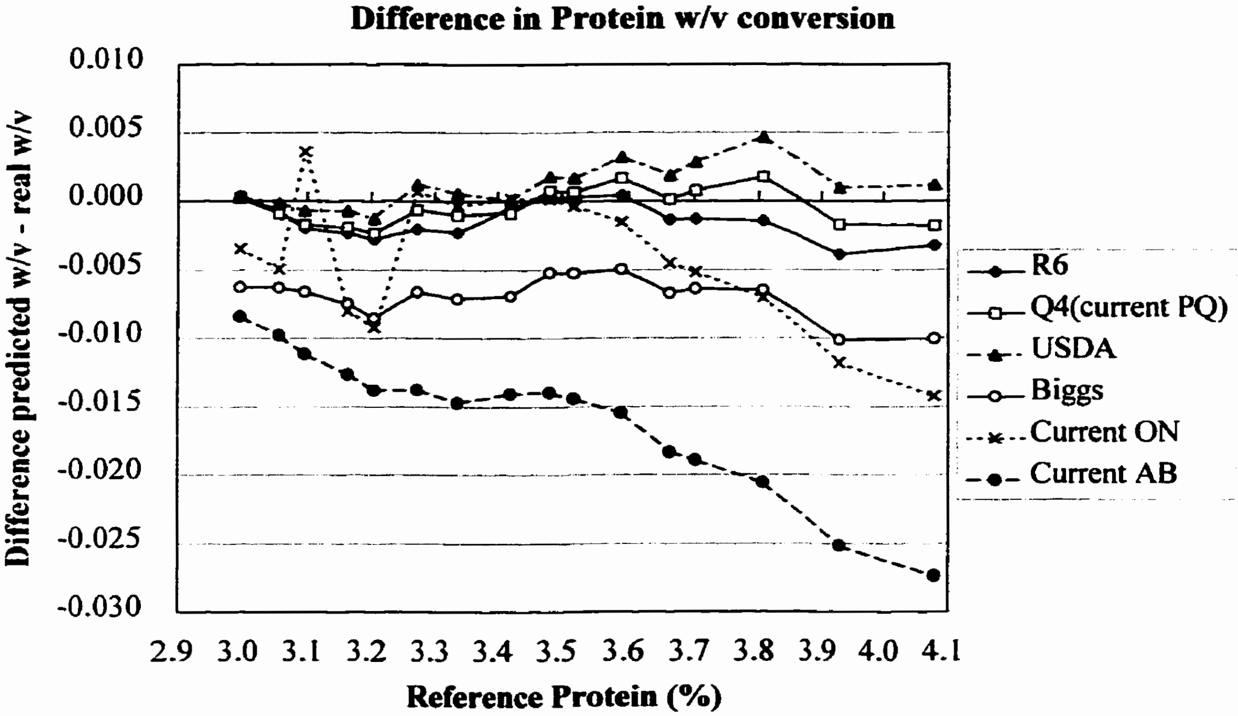


Figure 4.16: Simulation of **LOS** weight/volume conversion.

Difference is calculated by subtracting real w/v from estimated w/v values. Comparing density computed by equations developed in this study compared to that calculated by other studies and current conversion system.

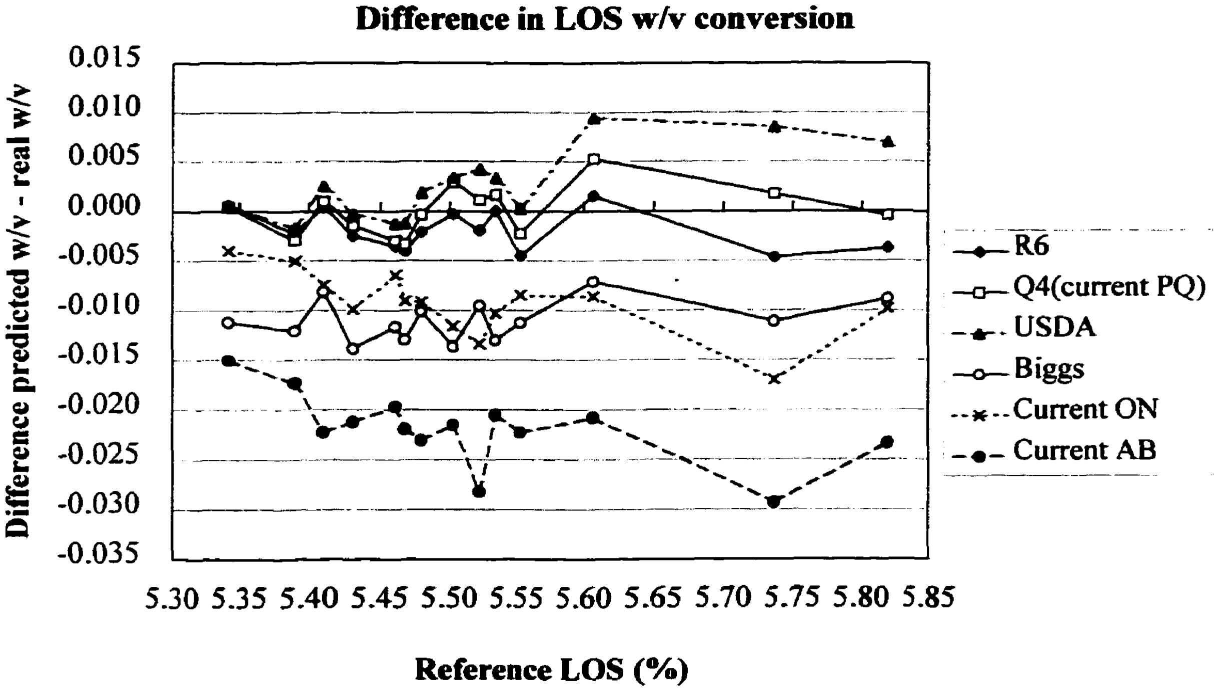


Table 4.22: Comparison of various equations in w/v conversion of Ontario-Alberta random data set.

Statistical summary of difference, which are defined as converted w/v minus real w/v values. Real w/v = empirical density × composition (%).

	Equation	**R6**	**Q4**	**US**	**Biggs**	**Current ON**	**Current AB**
Fat	AVE	0.000	0.000	0.003	-0.006	-0.005	-0.016
	STD	0.002	0.004	0.005	0.003	0.006	0.005
	MIN	-0.006	-0.052	-0.035	-0.032	-0.035	-0.035
	MAX	0.007	0.013	0.024	0.007	0.010	-0.006
Protein	AVE	0.000	0.000	0.003	-0.005	-0.004	-0.014
	STD	0.002	0.003	0.004	0.003	0.005	0.004
	MIN	-0.004	-0.041	-0.027	-0.025	-0.016	-0.029
	MAX	0.007	0.009	0.023	0.005	0.018	-0.005
LOS	AVE	0.000	0.001	0.005	-0.009	-0.009	-0.022
	STD	0.003	0.005	0.006	0.004	0.004	0.005
	MIN	-0.007	-0.045	-0.029	-0.026	-0.022	-0.038
	MAX	0.009	0.013	0.044	0.012	0.005	-0.009

4.3. Temperature Effect

4.3.1. Statistical summaries

Statistical summaries of milk density and the composition for temperatures of 4, 16, 28, and 40°C are shown in Table 4.23. Fat, protein, and LOS contents had a mean around 3.9-4.0%, 3.4%, and 5.53%, respectively. Figure 4.17 gives plots of density and temperature.

Table 4.23: Statistical summary of milk compositions and density determined at each temperature of 4, 16, 28, and 40°C.

Temperature = 4°C

Variable	N	Mean	STD	Minimum	Maximum
Density	396	1.0341	7.167e-4	1.0321	1.0364
Fat	396	4.02	4.96e-1	2.64	5.95
Protein	396	3.44	2.23e-1	3.03	4.21
LOS	396	5.54	9.97e-2	5.15	5.76

Temperature = 16°C

Variable	N	Mean	STD	Minimum	Maximum
Density	396	1.0304	6.525e-4	1.0280	1.0322
Fat	396	3.95	4.46e-1	2.68	5.41
Protein	396	3.40	1.87e-1	3.04	4.07
LOS	396	5.53	1.03e-1	5.10	5.71

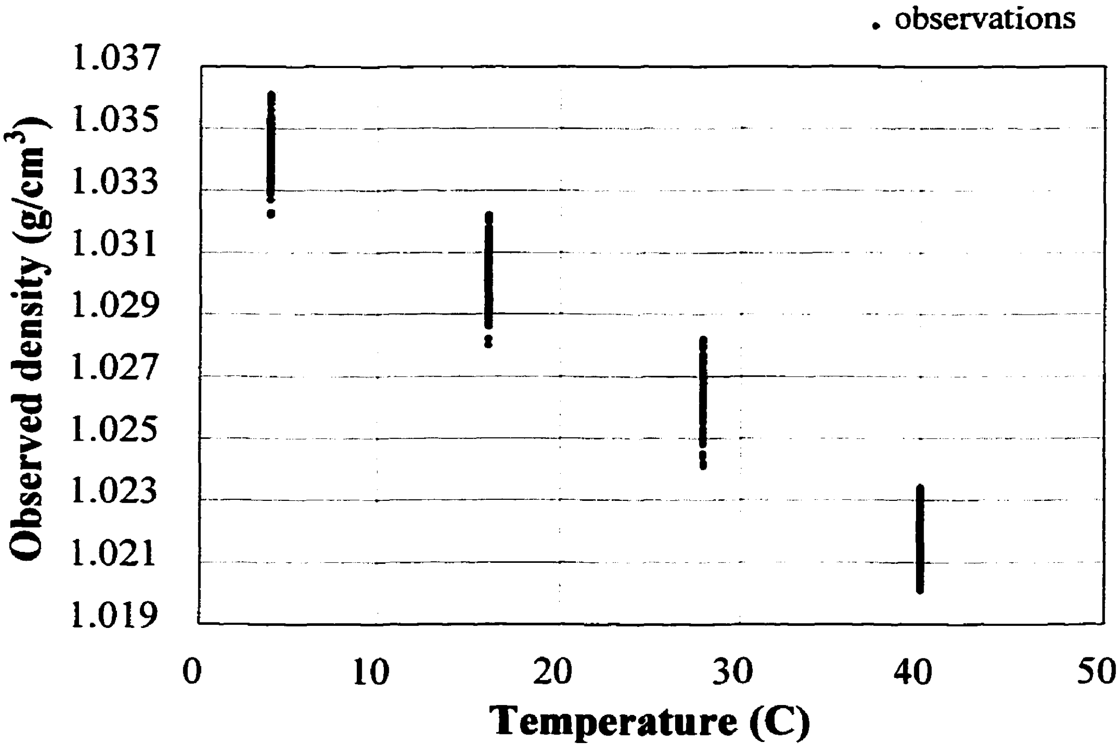
Temperature = 28°C

Variable	N	Mean	STD	Minimum	Maximum
Density	396	1.0265	6.730e-4	1.0241	1.0282
Fat	396	3.92	4.42e-1	2.75	5.58
Protein	396	3.39	2.18e-1	3.01	4.51
LOS	396	5.52	1.16e-1	5.09	5.77

Temperature = 40°C

Variable	N	Mean	STD	Minimum	Maximum
Density	396	1.0218	6.273e-4	1.0201	1.0234
Fat	396	4.00	4.73e-1	2.71	5.88
Protein	396	3.40	2.45e-1	2.96	4.33
LOS	396	5.53	8.77e-2	5.26	5.76

Figure 4.17: Plot of density versus temperature.



Density was determined at 4, 16, 28, and 40°C (N = 396 for each temperature).

4.3.2. Developing models

An equation that predicts milk density at any temperatures between 4 and 40°C was developed. The primary factors of compositional analysis, fat, protein, and LOS and their interactions were used in the full model, as well as the temperature at which the sample density had been measured. Because Short (1955) indicated the importance of cubic terms of temperature in skim milk, cubic, quadratic, and linear terms of temperature were evaluated. Table 4.24 gives the list of the models examined.

Table 4.24: The models used in examining temperature effect.

Code	Models
T1	Predicted density = a cubic term of temperature + all composition variables & interactions
T2	Predicted density = a quadratic term of temperature + all composition variables & interactions
T3	Predicted density = a linear term of temperature + all composition variables & interactions
T4	Predicted density = a cubic term of temperature + reduced composition variables & interactions
T5	Predicted density = a quadratic term of temperature + reduced composition variables & interactions

Observations with residuals larger than three STDR were defined as outliers and eliminated. Regression results with and without outliers were compared in terms of ANOVA statistics in Table 4.25. The F-test (Table 4.27) indicated that the T1, T2, and T3 were significantly ($p < 0.05$) different from each other. Looking at the the STDR and root-MSE, those values in T1 and T2 were closer to each other than to the result of T3. This tendency may imply that T2 loses accuracy by reducing the term of temperature from cubic to quadratic, but the loss is even larger when the model is simplified from quadratic to linear. In addition, plots of residual versus predicted density of T3 (Figure

4.18(c)), had a curved structure, which indicated that the model needed to include higher terms of temperature. The same curve was also detected in plots of residuals against temperature of T3 (Figure 4.18(d)). No evident pattern was observed in T1 and T2 (Figure 4.18). Therefore, reduced models (T4, T5) were developed only from T1 and T2. The results of the F-test indicated that the reduced models were equivalent to the full models in terms of precision of density prediction (Table 4.27). ANOVA statistics and estimated parameters for T1, T2, T4, and T5 are summarized in Table 4.26.

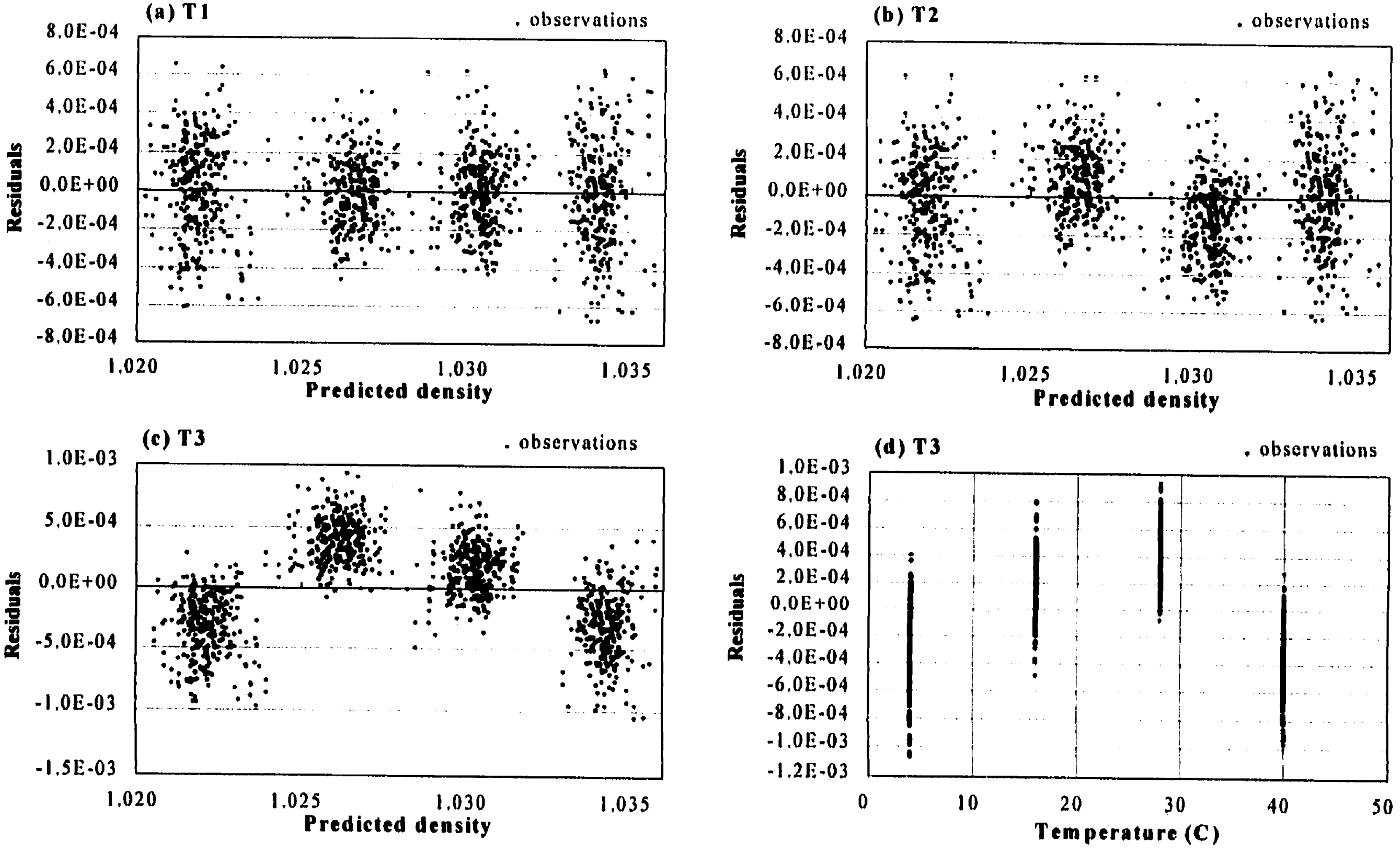
The difference in accuracy between T4 and T5 was significant (Table 4.27), but seemed to be small. In Table 4.28, which shows the STDR calculated for total observations (without outliers) and for each temperature, the STDR values of T5 were similar to those of T4. The difference in STDR between T4 and T5 was largest at 16°C, which indicated that the cubic model had some advantage in explaining the relationship between density and composition at this temperature. Four significant digits were used for developed models to ensure the same accuracy as the calculation in SAS results.

Table 4.25: Comparison of ANOVA statistics and the characteristics of residuals before and after eliminating outliers in model T1, T2, and T3.

T1 includes a cubic term of temperature and regression variables.
T2 has a quadratic term of temperature and regression variables.
T3 contains a linear term of temperature and regression variables.

		Full observations	Without outliers
Model T1	# of outliers (%)	0	36 (2.3%)
	SSE	1.163e-4	8.225e-5
	MSE	7.0e-8	5.0e-8
	Error DF	1570	1534
	Root MSE	2.722e-4	2.316e-4
	R-square	0.9966	0.9975
	Average of residuals	-9.412e-16	-3.557e-16
	STDR	2.711e-4	2.306e-4
	Max/Min of residuals	1.29e-3 / -1.61e-3	6.57e-4 / -6.75e-4
	Skewness	-0.2372	-0.0423
	Kurtosis	3.2106	-0.0749
Model T2	# of outliers (%)	0	30 (1.9%)
	SSE	1.279e-4	9.564e-5
	MSE	8.0e-8	6.0e-8
	Error DF	1571	1541
	Root MSE	2.853e-4	2.491e-4
	R-square	0.9962	0.9970
	Average of residuals	-3.409e-16	-5.200e-16
	STDR	2.840e-4	2.480e-4
	Max/Min of residuals	1.34e-3 / -1.67e-3	6.83e-4 / -6.83e-4
	Skewness	-0.1857	-0.0123
	Kurtosis	2.6535	-0.1388
Model T3	# of outliers (%)	0	5 (0.3%)
	SSE	2.614e-4	2.471e-4
	MSE	1.7e-7	1.6e-7
	Error DF	1572	1567
	Root MSE	4.078e-4	3.971e-4
	R-square	0.9923	0.9926
	Average of residuals	-6.877e-16	-8.789e-16
	STDR	4.060e-4	3.960e-4
	Max/Min of residuals	1.09e-3 / -2.00e-3	1.01e-3 / -1.14e-3
	Skewness	-0.3638	-0.2451
	Kurtosis	0.2211	-0.4104

Figure 4.18: Plots of residuals versus predicted density for model (a) T1 (b) T2 (c) T3. (d) T3 Plots of residuals versus temperature



T1 includes cubic terms of the temperature variable. T2 includes quadratic terms of the temperature variable.
T3 includes linear terms of temperature variable.

Table 4.26: Summary of ANOVA statistics and parameter estimates of full and reduced models of cubic and quadratic models.

T1 includes a cubic term of temperature and regression variables.
T2 has a quadratic term of temperature and regression variables.
T3 contains a linear term of temperature and regression variables.
Reduced model T4 is constructed from T1, and reduced model T5 is derived from T2.
Thirty-six outliers were eliminated from data in the investigation of T1 and T4 and 30 were omitted in T2 and T5.

	Model T1	Model T2	Model T4	Model T5
SSE	8.225e-5	9.564e-5	8.239e-5	9.625e-5
MSE	5.0e-8	6.0e-8	5.0e-8	6.0e-8
Error DF	1534	1541	1537	1546
Root MSE	2.316e-4	2.491e-4	2.315e-4	2.495e-4
R-Square	0.9975	0.9970	0.9974	0.9970
STDR	2.306e-4	2.480e-4	2.308e-4	2.490e-4
Intercept	0.98101	0.99859	0.97504	0.98225
FAT	-6.7821e-3	-9.7152e-3	-4.3046e-3	-2.9369e-3
PROT	5.5892e-3	3.2153e-3	5.5689e-3	2.8970e-3
LOS	1.5276e-2	1.0487e-2	1.6768e-2	1.4676e-2
F*F	5.5683e-5	5.8502e-5		
F*P	6.7728e-4	1.3667e-3	3.4442e-4	
F*L	8.9114e-4	1.4672e-3	4.4809e-4	4.1257e-4
P*P	-5.5558e-4	-4.1551e-4	-5.9487e-4	
P*L	2.9438e-5	3.4492e-4		
L*L	-1.3056e-3	-1.0058e-3	-1.3719e-3	-1.1676e-3
F*P*L	8.1657e-5	2.2102e-4		
T	-3.2773e-4	-2.4374e-4	-3.2763e-4	-2.4359e-4
T^2	2.6856e-6	-2.0736e-6	2.6823e-6	-2.0781e-6
T^3	-7.1800e-8		-7.1800e-8	

Table 4.27: Summary of the F-test. Comparing models with cubic, quadratic, and linear terms of temperature using data sets without outliers.

T1 includes a cubic term of temperature and regression variables.
T2 has a quadratic term of temperature and regression variables.
T3 contains a linear term of temperature and regression variables.
Reduced model T4 is constructed from T1, and reduced model T5 is derived from T2.

Comparisons	Conditions	F-values	Pr>F
T1 vs. T2	Without outliers	35.68	2.12e-46
T2 vs. T3	Without outliers	93.86	6.80e-295
T1 vs. T3	Without outliers	93.17	0.000
T1 vs. T4	Without outliers	0.870	0.456
T2 vs. T5	Without outliers	1.976	0.081
T4 vs. T5	Without outliers	28.73	1.62e-46

Table 4.28: Standard deviations of residuals (STDR) calculated by the reduced cubic equation (T4) and the reduced quadratic formula (T5) for total observations and for each temperature.

Data set used	Model T4	Model T5	Difference T5-T4
All temperatures	2.308e-4	2.490e-4	1.82e-5
4°C	2.814e-4	2.849e-4	3.50e-6
16°C	1.938e-4	2.075e-4	1.37e-5
28°C	1.812e-4	1.824e-4	1.20e-6
40°C	2.548e-4	2.582e-4	3.40e-6

36 data were omitted in T4 and 30 eliminated in T5.

4.3.3. Comparing equations from previous works

Formulae for estimating density of whole milk constructed in previous studies were tested for their universality employing empirical data collected in this study. Table 4.29 gives the list of equations examined by being fitted to the data set shown in the middle column. Data set was "cleaned" by removing 36 outliers that had been defined when model T4 was fitted to the full observations. Table 4.29 also presents the average of predicted density and the STDR.

USDA's equation at 40°F (4.4°C) and at 102°F (39.8°C) and Watson and Tittsler's formula calculated at 4°C appeared to fit to the corresponding data set to some extent. Both formulae had as small STDR as that of T4, which indicated that the error variance of the two equations were as small as that of T4. However, Figure 4.19 and 4.20 depict that USDA's and Watson's formulae overestimate most densities and that the residuals became larger as predicted density increased. This disagreement among equations may be due to the discrepancy in the technique employed for determining density or composition. As an illustration, Goff and Hill (1993) pointed out that for raw milk, the Babcock method produces slightly higher results (0.021% fat) than does the Mojonnier fat determination. In addition, USDA (1965) pointed out that specific gravities determined by the Watson lactometer were slightly lower than those determined by the Babcock bottle method. The difference in determined specific gravity of mixed breed producer milk between the two methods was 0.00056 at the maximum and 0.00035 in average. In order to evaluate the validity of equations developed in the past, further investigation will be needed to examine the agreement of various processes used in density determination and composition measurement.

Table 4.29: Summary of the average of predicted density and the standard deviation of residuals (STDR) calculated by fitting equations developed in the past to the data set collected in this study.

Source	Equations	Data set used	The average of predicted density[1]	STDR
USDA(1965)	[2]Specific gravity of milk (40°F /40°F) $= 100/(100 + 0.03928 \times \text{Fat}(\%) - 0.39221 \times \text{SNF}(\%))$	Density at 4°C	1.0347	3.00e-4
USDA(1965)	[3]Specific gravity of milk (102°F/102°F) $= 100/(100 + 0.09493 \times \text{Fat}(\%) - 0.37312 \times \text{SNF}(\%))$	Density at 40°C	1.0224	3.15e-4
Watson & Tittsler (1961)	Density $(\text{g/cm}^3) = 1.003073 - 0.000179 \times \text{Temp}(°\text{C}) - 0.000368 \times \text{Fat}(\%) + 0.003744 \times \text{SNF}(\%)$ temperatures between 1 and 10°C	Density at 4°C	1.0344	2.47e-4
Equation T4	[4]Density $= .9750 - .0003276T + .000002682T^2 - .00000007180T^3 - .004305F + .005569P + .001677L + .0003444F{*}P + .0004481F{*}L - .0005949P{*}P - .001372L{*}L$	Density at 4°C	1.0340	2.82e-4
		Density at 40°C	1.0218	2.55e-4

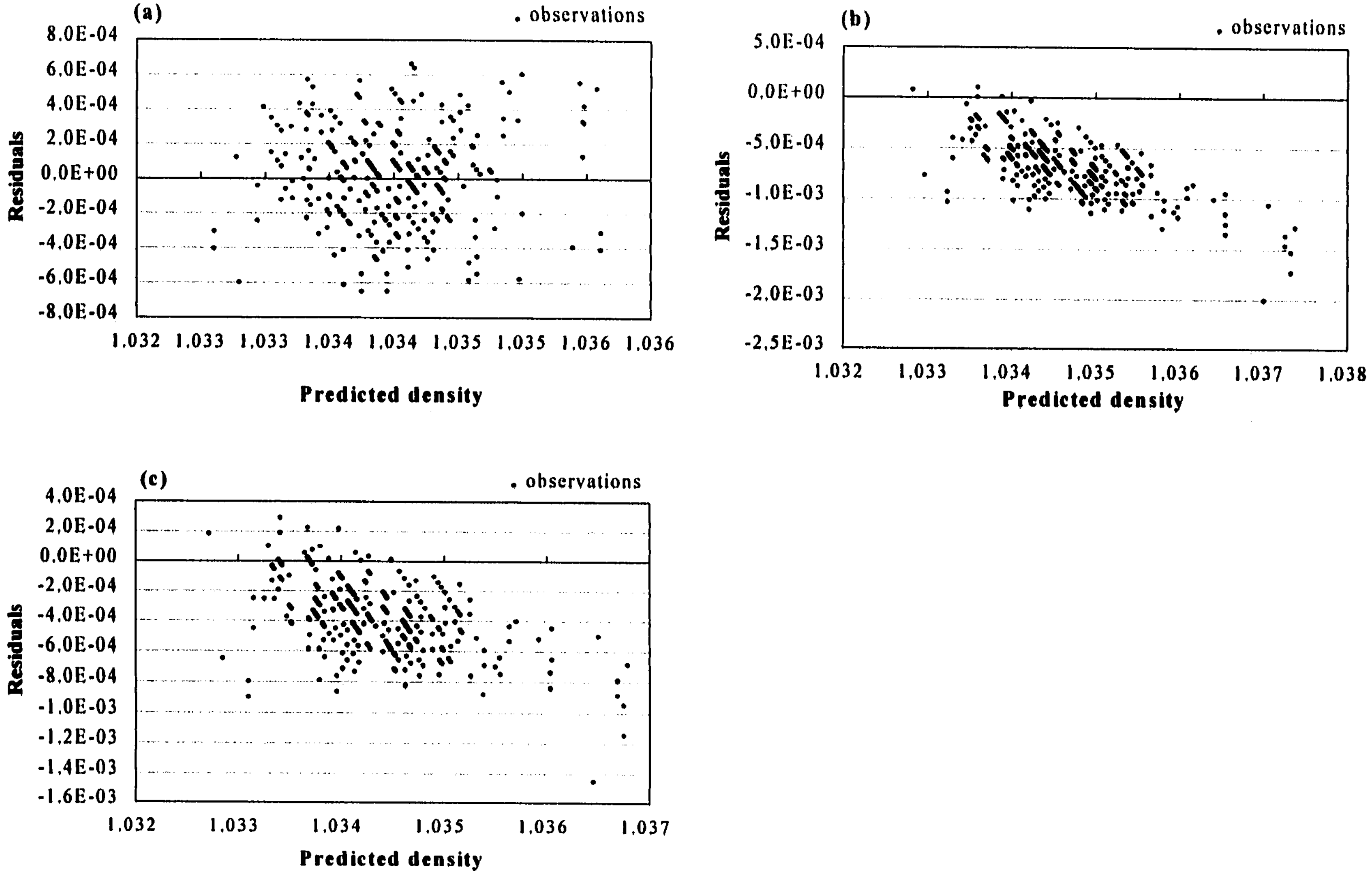
[1] The average of observed density at 4°C and 40°C is 1.0341 and 1.0218, respectively.

[2] Converted to density at 40°F (4.4°C) by multiplying the value by the density of water at 3.98°C = 1.0000 (Weast, 1983), assuming the difference between the density of water at 3.98°C and 4.4°C is small.

[3] Converted to density at 102°F (39.8°C) by multiplying the value by the density of water at 40°C = 0.99224 (Weast, 1983), assuming the difference between density of water at 39.8°C and 40°C is negligible.

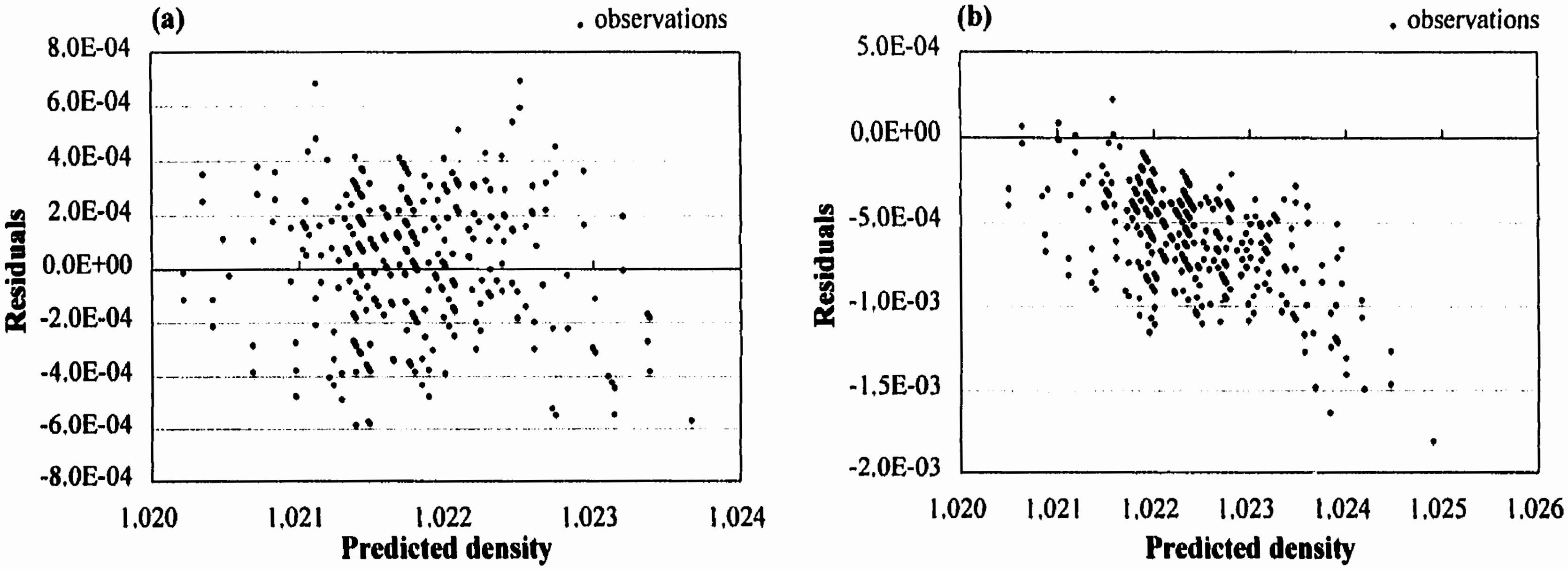
[4] Where T is temperature (°C), F is the fat (%), P is the protein (%), and L is the LOS (%).

Figure 4.19: Plots of residuals versus predicted density of equation (a) T4 (b) US (c) Watson & Tittsler fitted to the data at 4°C.



T4: simplified equation including a cubic term of temperature. US: Density at 4.4°C = 100/(100 + 0.03928 × Fat (%) – 0.39221 × SNF (%)). Watson & Tittsler: Density = 1.003073 – 0.000179 × Temp (°C) – 0.000368 × Fat (%) + 0.003744 × SNF (%).

Figure 4.20: Plots of residuals versus predicted density of equation (a) T4 and (b) US fitted to the data at 40°C.



T4 is a simplified equation including a cubic term of temperature.
US: Density at 40°C = 100/0.99224 × (100 + 0.09493 × Fat (%) – 0.37312 × SNF (%)).

5. Conclusions

Herd milk in Ontario and Alberta was examined for its density at 4.0°C and composition over a whole year. Quebec participated in this study by sending data of random samples and non-random samples that were used for calibrating infrared milk composition analyzers. Density measurement was done following 24 hour holding of samples at 4°C.

Best-fit equations for milk density estimation from fat, protein, and LOS contents were developed by the least squares method for Ontario-Alberta random, Quebec non-random, and Ontario non-random data. The Ontario-Alberta data showed no significant difference between provinces. Since seasonal factors were significant in all of the three data sets, two types of formulae were constructed for each data set. One adjusted its intercept according to the month of the year by using seasonal factors and the other ignored seasonal fluctuation by containing only regression variables.

Examination of seasonal fluctuation of milk density showed that changes in density are strongly related to changes in the amount of components. Fat, protein, and LOS compositions were the most important elements in density estimation. Particularly, for the purpose of milk pricing, an equation without seasonal factors can be used since overestimation of density in summer and underestimation in winter cancel out throughout a year.

Equations developed from Ontario-Alberta random and Quebec non-random were compared by fitting them to data sets of Quebec non-random and Quebec random. The Quebec non-random formula (Q4) was slightly biased in density prediction of Quebec random data. Its estimates had larger values than actual density but the variance was small. The Ontario-Alberta equation (R6) did not fit well to Quebec non-random data since they tended to over or underestimate density when the milk had high or low density. However, R6 fitted to the Quebec random data set with slightly large variance but low bias. The average of predicted density of R6 had closer value to the mean of observed density than did Q4. Biased prediction of Q4 may indicate the risk of using limited numbers of herds in density prediction.

None of the equations constructed from other sources of samples, Quebec non-random, USDA (1965), and Biggs (1978), explained the relation between density and composition of Ontario-Alberta random data. This limitation in the use of equations may attribute to different methods employed in measurement, short period of experiment, or small numbers of herds used.

Simulation in w/v conversions of fat, protein, and LOS demonstrated that experimentally determined equations produced very close estimations to actual w/v values. A conversion system with a constant conversion factor seemed to be unfair because its underestimation of w/v values increased as the milk had higher fat or protein content.

As a summary of the first part, the current Ontario and Alberta milk pricing system can be enhanced by employing equation R6 for density estimation. Among the developed equations, R6 is the most appropriate since it is a good representative of Ontario and Alberta herd milk. More than 950 randomly chosen samples contributed to the equation. Since the sample collection lasted more than a year, seasonal variation of milk density can be explained by this simple regression, avoiding complexity of seasonal factors. Most importantly, R6 demonstrated excellent performance in w/v conversion of w/w composition for Ontario-Alberta random data. The equation R6 is expressed as:

$$\text{Density} = 0.8549 + 0.03820\ F + 0.05565\ P + 0.03274\ L - 0.01080\ F*P - 0.007431\ F*L - 0.0006358\ P*P - 0.009314\ P*L + 0.002095\ F*P*L \qquad [24]$$

where F is the fat content (%), P is the protein (%), and L is the LOS (%)

To obtain a Canadian universal equation for density estimation, equation R6 should be tested for the data from other provinces although Ontario produces a considerable percentage of Canadian milk. Randomly chosen milk samples should be used in that investigation to avoid bias.

Although the equation R6 is adequate for density estimation, there is a significant effect of season on milk density. Seasonal factors determined in this study are not applicable to other countries particularly those in Southern Hemisphere. Since seasonal changes in milk density are strongly influenced by changes in feeding practice, climate of the region is a key element in the seasonal effect. In future work, seasonal factors determined according to the climatic zone may help construct a universal equation that can be applied to all milk in the world.

In the second section, formulae were constructed for density estimation of producer milk at any temperatures between 4.0 and 40.0°C. Density measurements at temperatures of 16.0, 28.0, and 40.0°C were carried out after one to four hours of tempering at the desired temperature. Density at 4.0°C was measured after holding the samples at 4.0°C for 24 hours. Milk density could be explained by regression variables of fat, protein, LOS, interactions of the three variables, and temperature. The term of temperature should be at least quadratic. An equation with a cubic term showed slightly better fit than that with a quadratic term. Equations regarding temperature-milk density relationship developed by other studies depicted small error variance when fitted to the data collected by this study, but they had some bias in their prediction. The developed equation (T4) is presented as follows:

$$\text{Density} = 0.9750 - 0.0003276\ T + 0.000002682\ T^2 - 0.00000007180\ T^3 - 0.004305\ F + 0.005569\ P + 0.001677\ L + 0.0003444\ F*P + 0.0004481\ F*L - 0.0005949\ P*P - 0.001372\ L*L \qquad [25]$$

where T is the temperature (°C), F is the fat content (%), P is the protein (%), and L is the LOS (%)

This equation enables researchers to estimate the density of raw milk with any composition at temperatures between 4.0 and 40.0°C. It is also useful for calculating the expansivity of a milk sample.

Since this study used only raw milk samples, further research to develop equations for density estimation of fluid milk products such as skim milk, low fat milks, processed whole milk, and creams would be useful to the dairy industry.

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