THE pH- AND FREEZING POINT VALUES OF MILK IN THE WESTERN AND SOUTHERN CAPE AND FACTORS AFFECTING THESE VALUES.

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November 2003

DECLARATION

I declare that the dissertation hereby submitted by me for the Master of Science in Food Science degree at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

Peter Vassen

November 2003

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CHAPTER 1

1.1 INTRODUCTION

The chemical composition of milk renders it as an extremely valuable commodity in the human diet (Robinson, 1981). The principal components of milk are water, fat, protein, lactose, and minerals. Milk also contains trace amounts of other substances such as pigments, enzymes, vitamins, phospholipids, and gases (Bylund, 1995). Milk is however, a highly perishable commodity since its chemical composition makes it an ideal medium for the growth of microorganisms, including pathogens, which may be present in raw milk due to mastitis or introduced accidentally during subsequent handling (Porter, 1980). The activity of some microorganisms on the other hand is clearly advantageous, a view confirmed by the numerous fermented milk products, including a vast variety of cheeses, which are available on the food market. It is due to the presence of spoilage and the pathogenic microorganisms that the hygienic quality of milk has been of paramount importance and why the health controls exercised on milk and milk products have exceeded those of any other food class ("*Hygienic milk production*", 1985).

It is not only the keeping quality of milk, but also the consumer that demands the highest standards in milk production. The consumer regards the quality image of milk as the most important attribute and the dairy industry should thus strive to maintain this position. The quality reputation of the dairy industry within the food industry is equally high, and we should constantly endeavour to foster the high quality features of dairy products and further enhance their unique attributes. This satisfactory position has not been easily achieved and has only been realized through sound practices during milk production and product manufacturing, by legislation and by tight marketing standards. Many of these steps have been viewed as restrictive, and the vigorous enforcement of new standards has not always been popular. When viewed in perspective, these steps have largely ensured the successful development of the South African dairy market ("*Hygienic milk production*", 1985).

The analytical methods employed in the determination of the quality or acceptability of milk, are those that are best suited to measure the actual microbial and chemical quality of milk routinely under practical conditions. Sampling methods and analytical procedures for any test used should be uniform in all jurisdictions. Practical and uniform quality standards are required in order to ensure continuous conformance to quality control requirements and to facilitate unprejudiced acceptance of milk. Furthermore, judicious and uniform interpretations of compliance with these standards should prevail explicitly ("*Standard methods*", 1960).

The analytical methods used for the testing of milk quality can be classified into two groups, namely microbiological and chemical methods. Both types of methods can be further classified according to their intended purpose, namely for the acceptability of raw milk at intake and for the payment of ex-farm milk. The focus of this paper is based on two properties of milk, namely the pH-value and the freezing point. These criteria are respectively used to determine the acceptability and authenticity of raw milk at intake.

The pH-value of milk indicates its hydrogen ion concentration and is influenced by acid development ("*South African Dairy Training Board*", 1990; Lück & Du Toit, 1968). According to Smith (1970), the pH of fresh milk produced from healthy udders, usually falls between 6.50 and 6.70. Johnson and Doan (1942) concluded that the pH of normal herd milk falls between 6.40 and 6.79. The pH specifications for raw milk at reception and according to the *South African Dairy Training Board* (1990) should fall between 6.60 and 6.75. According to this reference, values lower than 6.60 indicate bacterial deterioration and values higher than 6.75 indicates the possibility of mastitis milk, accidental or wilful adulteration with alkali such as detergents or bicarbonate of soda. Jenness and Patton (1959) stated that a low pH-value can also indicate the presence of colostrum. Milk with pH-values outside these specifications should be considered as unacceptable for distribution or processing.

The presence of extraneous water in milk is illegal in most countries (Harding, 1983) including South Africa ("*Agricultural Products Standards Act*", 1994). Added water significantly reduces the value of milk since it increases transportation- and manufacturing-costs, reduces product yields and may contribute to bacterial contamination. The measurement of the freezing point of milk provides the most reliable means of detecting and measuring the percentage of extraneous water in milk (Harding, 1983). Freezing point determinations are carried out by means of the thermistor cryoscope method and are expressed in degrees Celsius (°C). The legal upper limit of the South African freezing point specification is -0.512°C ("*Agricultural Products Standards Act*", 1994). This law does not state a lower limit. Lück (1984) and the *South African Dairy Training Board* (1990) however, specified lower limits of -0.541°C and -0.550°C, respectively.

It is normally accepted that raw milk, which freezes between -0.512°C and -0.550°C, contains no added water. A freezing point above -0.512°C indicates added water, whereas one below -0.550°C, points to either sour milk or adulteration by the addition of soluble substances such as milk powder or sugar (*"South African Dairy Training Board",* 1990).

The pH-value is termed a physical property of milk; examples of other physical properties of milk include viscosity, density, and refractive index. Each physical property of milk is a resultant determined by the contributions of its constituents, i.e. chemical identity and concentration of the constituents (Jenness et al., 1959). Freezing point is termed a colligative property of milk. Colligative properties of solutions are properties that depend on the concentration of solute molecules or ions in solution, and not on the chemical identity of the solute (Ebbing, 1993).

The factors that affect the composition of milk can be divided into two broad areas, namely physiological and environmental/managerial factors. The physiological influence is governed by heredity and non-heredity factors. Heredity factors include variation among breeds and individual variation within breeds. Non-hereditary factors includes amongst other, age, stage of lactation, and pregnancy of the cow. Environmental factors include season, temperature, and nutritional factors. In general, the dairy farmer has little control over the physiological factors but has some control over the environmental factors. A thorough understanding of the factors that affect the yield and the composition of milk enables the dairy farmer to partially manage the environment in an effort to produce the desired changes in milk yield and composition (Schmidt et. al., 1988).

Literature reveals that the most recent specification for the pH-value of South African milk is stated in the pH module of the *South African Dairy Training Board* (1990), and according to G. Venter (Training and Development Manager of Milk South Africa, personal communication, 10 March, 2003) and Dr. J. Floor (Manager Laboratory Services of Clover, personal communication, 11 August, 2003), the compilation of these pH specifications was conducted prior to this date. The most recent reference for the South African freezing point specification is cited in an article by Lück (1984). The South African standards for pH and freezing point of raw milk are therefore, at least 13 and 19 years old, respectively. During this period, changes may have taken place regarding the physiological and environmental factors that affect the composition of milk. If such changes had occurred, they may have affected the pH and freezing point.

The most notable change, increased milk production, that occurred over the years shows that the present lactating cow produces higher fat- and protein-masses compared to her 1980 predecessor. The higher milk production however, resulted in a decrease in both the fat and protein percentage of raw milk (Robertson, 2000). Annual average figures obtained from the South African National Dairy Animal Improvement Scheme (2002) for registered cows milked twice a day, showed the following: From 1975 to 2001, the average lactation yield of Holstein and Jersey breeds showed increases in milk production from 4 388 to 7 561 kg and from 3 393 to 5 185 kg, respectively. The higher milk production per lactation was accompanied by an increased fat production from 161 to 260 kg and from 165 to 230 kg for Holsteins and Jerseys, respectively. Despite this increase in fat produced, the fat percentages decreased from 3.68 to 3.45 and from 4.86 to 4.45 for the Holstein and Jersey breeds, respectively. The same trend is noticeable for protein, in which case, the mass in kilograms produced per lactation increased from 146 to 239 and from 135 to 188, while the protein percentages decreased from 3.33 to 3.16 and from 3.96 to 3.64 for the Holstein and Jersey breeds, respectively. These changes in fat and protein percentages may well be accompanied by other compositional changes, which may have a significant influence on the current South African specifications for pH and freezing point.

The high quality standards that the South African Dairy Industry is known for and lives up to are also being compromised. The consequence may well be the rejection of normal milk and vice versa.

In addition, R. Fourie (Quality Assurance Manager of Ladismith Cheese, personal communication, July 22, 2003) stated that at the dairy plant where she is employed, the average monthly pH-values of milk received for the months of February, June and July 2003 were 6.75, 6.76 and 6.77, respectively. According to the information supplied by Dr. J. G. Conradie (Manager Producers Milk Quality of Parmalat, personal communication, July 22, 2003), the average pH of producers milk as received per plant over the period 1999 to 2002 were 6.77, 6.76, 6.78, 6.80, 6.71, 6.77, and 6.74, respectively. The highest average monthly pH obtained at any factory was 6.88 and the lowest was 6.65. Dr. J. G. Conradie (Manager Producers Milk Quality of Parmalat, personal communication, July 22, 2003) also supplied information regarding freezing point values from the seven diary plants in question. The average freezing point over the period 1999 to 2002 was -0.523 °C. The highest and lowest monthly average freezing points noted were -0.515 °C and -0.533°C, respectively.

The objective of this study is to determine the current natural pH- and freezing point-values of raw bulk milk. Secondly, to determine how the milk composition, the physiological and the environmental/managerial factors relate to milk pH and freezing point. The aim is not to reinvent the wheel by investigating the factors that affect the pH and freezing point but to substantiate the findings of the thesis. For practical reasons, the scope of this study will be limited to the Western Cape and the Tsitsikama regions of South Africa. The outcome of this research is to determine if the current South African pH and freezing point values of raw milk are still applicable or whether this topic warrants further research to establish new standards.

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CHAPTER 2

LITERATURE REVIEW

2.1 THE pH-VALUE OF MILK

2.1.1 ACIDS AND BASES: OVERVIEW

It is essential to cover acid-base principles and the equilibria involved to understand the factors affecting the pH of milk. The terms acid and base apply to two groups of compounds with opposite sets of characteristics and were first recognized by simple properties (Slabaugh & Parsons, 1971). Acids have a sour taste, produce a prickling sensation on the skin, whereas bases are bitter, and have a slippery feeling (Petrucci, 1985). In addition, acids and bases change the colour of certain dyes called indicators, such as litmus and phenolphthalein. Acids change litmus from blue to red and basic phenolphthalein from red to colourless. Bases change litmus from red to blue and acidic phenolphthalein from colourless to red (Stine, 1981). Acids react with active metals, such as iron and zinc, to release hydrogen (Snyder, 1992). Acids and bases neutralize, or reverse, the action of one another. During neutralization, acids and bases react with each other to produce ionic substances called salts (Ebbing, 1993; Snyder, 1992).

Antoine Lavoisier was one of the first chemists who tried to explain what makes a substance acidic (Ebbing, 1993). In 1777, he proposed that all acids contain a common element-oxygen (Petrucci, 1985). In 1810, Humphry Davy proposed that hydrogen, not oxygen, is the common element in acids. He proved this by showing that hydrogen chloride dissolves in water to give hydrochloric acid, which only contains hydrogen and chlorine. Although some chemists argued that chlorine was a compound of oxygen, chlorine was eventually proven an element. Chemists then noted that hydrogen, not oxygen, must be the essential constituent of acids (Ebbing, 1993).

2.1.2 ARRHENIUS CONCEPT OF ACIDS AND BASES

When studying theories, new theories are devised when the former theories no longer explain all the known facts. The theories of acids and bases serve as an excellent example of this progression of knowledge (Canham, 1996). A simple theory of acids and bases was first devised by the Swedish chemist, Svante Arrhenius in 1884, who stated, “an acid is a hydrogen-containing substance that dissociates to produce hydrogen ions, and a base is a hydroxide-containing substance that dissociates to produce hydroxide ions in aqueous solutions”. Arrhenius postulated that hydrogen ions are produced by the dissociation of acids in water, and that hydroxide ions are produced by the dissociation of bases in water (Hein, Best, Pattison, & Arena, 2001).

According to the Arrhenius theory, a strong acid completely ionizes in aqueous solution to give H_3O^+ and an anion; a strong base completely ionizes in aqueous solution to give OH^- and a cation (Ebbing, 1993). According to the Arrhenius theory, weak acids and bases do not completely ionize in solution (Hill, 1986). They exist in reversible reaction with the corresponding ions (Ebbing, 1993).

There are however, two major flaws in the Arrhenius theory. Many acid-base reactions in aqueous solution occur in solvents other than water or without a solvent (Ebbing, 1993). The Arrhenius theory assumes that the solvent has no influence on acid-base properties. If hydrogen chloride is dissolved in water, the solution conducts electricity, but if it is dissolved in a solvent like benzene, the solution does not conduct electricity. This difference in the properties of hydrogen chloride when dissolved in these two different solvents means that the type of solvent does affect the behaviour of the solute (Canham, 1996).

The second flaw of the Arrhenius theory is that it considers salts to be neutral compounds, yet there are many salts that contradict this rule. For example, solutions containing phosphate ions and carbonate ions are basic, whereas those of ammonium ions are slightly acidic and those of aluminium ions are very acidic. A solution of sodium dihydrogen phosphate is acidic but that of disodium hydrogen phosphate is basic (Canham, 1996).

2.1.3 BRØNSTED-LOWRY CONCEPT OF ACIDS AND BASES

To provide a more realistic model of acid-base behaviour, the Danish chemist, Johannes N. Brønsted, and, independently, the British chemist, Thomas M. Lowry, during 1923 devised a theory that involved the solvent in the acid-base phenomenon. Even though there have been newer and more sophisticated theories of acid-base behaviour, Brønsted-Lowry theory still provides the most convenient framework for understanding acids and bases (Canham, 1996).

According to the Brønsted-Lowry theory, acid-base reactions can be seen as proton-transfer reactions and acids and bases can be defined in terms of this proton (H^+) transfer (Ebbing, 1993). According to the Brønsted-Lowry concept, an acid is the species donating a proton in a proton-transfer reaction. A base is the species accepting the proton in a proton-transfer reaction. Acids and bases can be ionic compounds (salts) as well as molecular substances and their reactions are not restricted to aqueous solutions (Ebbing, 1993).

To be precise, we should say *hydrogen nucleus* instead of proton, the term proton is conventional (Ebbing, 1993). In an acid-base theory, a proton refers to a particular H-atom that has lost an electron, i.e., H^+. Since the H^+ is just a lone proton and is the nucleus of the H atom, we speak of the transfer of a proton (Petrucci, 1985). The protons located in the nuclei of other atoms are not involved in acid-base equilibria (Dillard & Goldberg, 1978).

The hydrogen ion, H^+, is not a bare proton but a proton chemically bonded to water, called the hydronium ion, H_3O^+ (Ebbing, 1993; Ladd, 1998). For simplicity, H^+ is used instead of H_3O^+, with the explicit understanding that H^+ is always hydrated in solution (Hein et al., 2001). The hydronium ion is in turn hydrogen bonded to three neighbouring water molecules, thus, it is more correctly written as $H_9O_4^+$. (Figure 2.1). However, for simplicity, we usually ignore the three molecules of hydration (Canham, 1996).

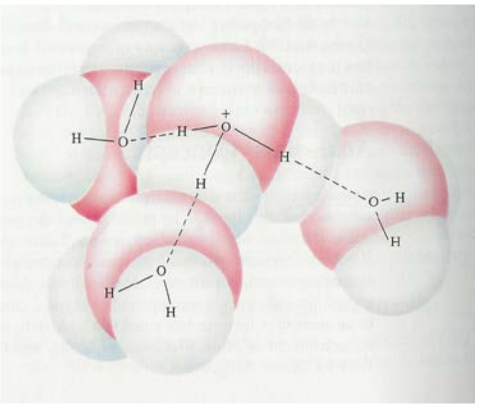

Figure 2.1 The Hydronium ion, H_3O^+ (Ebbing, 1993).

Petrucci (1985) makes the following summary concerning the comparison of the Arrhenius and Brønsted-Lowry theories. Any species that is an acid by the Arrhenius theory remains an acid in the Brønsted-Lowry theory. The same is true for bases. Certain species, because they do not contain a hydroxide group, are not classified as a base by the Arrhenius theory, but are classified as such by the Brønsted-Lowry. The Brønsted-Lowry theory accounts for a substance that can act as either an acid or a base (amphiprotic), while the Arrhenius theory does not clearly account for this behaviour (Kotz & Purcell, 1987).

2.1.4 SELF-IONIZATION OF WATER

The central feature of the Brønsted-Lowry theory is the importance of the solvent, which self-ionizes by its own acid-base reaction (Canham, 1996). Ionization is the formation of ions (Hein et al., 2001). Water thus undergoes a slight self-ionization, also called auto-ionization, to give the hydronium ion and the hydroxide ion:

$$\underset{\text{Base(1)}}{H_2O(\ell)} + \underset{\text{Acid(2)}}{H_2O(\ell)} \leftrightarrows \underset{\text{Acid(1)}}{H_3O^+(aq)} + \underset{\text{Base(2)}}{OH^-(aq)}$$

During self-ionization, the water molecule that donates the hydrogen ion is the acid and the water molecule that accepts the hydrogen ion is a base. When we consider the reverse process, the hydronium ion acts as a hydrogen ion donor (an acid) and the hydroxide ion is the hydrogen ion acceptor (a base). Two species that differ in formula by a hydrogen ion are called a conjugate acid-base pair. In this case, water (1) is the conjugate base of the hydronium ion (1) and water (2) is the conjugate acid of the hydroxide ion (2). The ability of a substance to act as either an acid or a base is called amphiprotic behaviour (Canham, 1996).

The equilibrium constant, K_c, for the self-ionization of water is noted as follows:

$$K_c = \frac{[H_3O^+]\,[OH^-]}{[H_2O]^2}$$

The equation can be rearranged as follows:

$$[H_2O]^2.\ K_c = [H_3O^+]\,[OH^-]$$

The activity of water is constant and essentially 1, so it is explicitly excluded from the equilibrium expression. The equilibrium value of the ion product $[H_3O^+]\,[OH^-]$ is called the ion-product constant for water, which is written K_w (Ebbing, 1993; Snyder, 1992). Like all equilibria, the self-ionization of water is temperature-dependent (Canham, 1996). At 25°C, the value of K_w is 1.0×10^{-14}. Thus, the ion-product for water at 25°C can be written as:

K_w	=	$[H_3O^+]\,[OH^-]$	=	1.0×10^{-14} at 25°C
The ion product constant of water	is	the product of the hydronium ion concentration times the hydroxide ion concentration	and	at 25°C, equals 0.00000000000001, which is a constant at this temperature.

Using the formula of K_w, the concentrations of H+ and OH- ions can be calculated in pure water. These ions are produced in equal numbers in pure water, so their concentrations are equal. Thus at 25 °C, the concentrations of both H^+ and OH^- are 1.0 x 10^{-7} M in pure water (Ebbing, 1993). The concentration of one ion increases proportionally as the concentration of the other ion decreases. Thus, if enough acid were added to raise the H^+ concentration to 10^{-6} M, the OH^- concentration would decrease to 10^{-8}M. In the extreme, the highest possible concentration of H^+ is 10^0 M and the lowest possible concentration of OH^- is 10^{-14} M. In the other extreme, the highest concentration of OH^- is 10^0 M, when the H^+ concentration is at 10^{-14} M (Moore, Clark, & Vodopich, 1998).

The terms dissociation and ionization are often used interchangeably to describe chemical processes taking place in water. Strictly speaking, the two however differ. During the dissociation of a salt, the salt already exists as ions. When it dissolves in water, the ions separate, or dissociate, and increase in mobility. During the ionization process, ions are produced by the reaction of a compound with water (Hein et al., 2001).

2.1.5 RELATIVE STRENGTHS OF ACIDS AND BASES

According to the Brønsted-Lowry theory, acids are proton donors and bases are proton acceptors. This statement is somewhat misleading, for the acid-base theory is more accurately a competition for the proton between the acid and the base, with the base winning (Canham, 1996). The competition for the proton in the acid-base reaction can be ranked according to the relative strengths of acid and base. The stronger acids are those that lose their protons more easily than others do. The stronger bases are those that hold on to protons more strongly than others do. The strongest acids have the weakest conjugate bases, and the strongest bases have the weakest conjugate acids. The direction for an acid-base reaction always favours the weaker acid and weaker base (Ebbing, 1993).

In binary acids (monoprotic acids), e.g. H-X, the strength of an acid depends on how easily the proton, H^+, is lost or removed from an H-X bond in the acid species. There are two factors important in determining relative acid strengths of binary acids. One is the polarity of the bond to which the hydrogen atom is attached. The hydrogen atom should have a partial positive charge. The more polarized the bond is, the easier the proton is removed and the greater the acid strength will be. The second factor is the strength of the bond, i.e. how tightly the proton is held. The strength of the bond is in turn depended on the size of the atom X. The larger the X atom, the weaker the bond and the greater the acid strength (Ebbing, 1993).

In going down a column of the periodic table, the size of atom X increases, the H-X bond strength decreases, and the strength of the binary acid increases. Going across the row of the periodic table, the atomic radius of the elements decreases slowly, thus the relative strengths of the binary acids of these elements are less dependent on the sizes of atoms X. The polarity of the H-X bond thus becomes the dominant factor in determining acid strength. Going across a row of elements of the periodic table, the electronegativity of the elements increases, thereby increasing polarity of the H-X bonds, which results in a stronger acid (Ebbing, 1993).

Another type of acid is the oxoacid. An oxoacid has the structure:

H-O-Y (Ebbing, 1993)

For all the common inorganic acids, the ionisable hydrogen atoms are covalently bonded to oxygen atoms. For example, nitric acid (HNO_3) is more appropriately written as $HONO_2$ (Canham, 1996). The formulas of these acids may be written as either HYO or HOY, depending on the convention used. Formulas of oxoacids are generally written with the acidic H first, followed by the characteristic element (Y), then O atoms (Ebbing, 1993).

For a series of oxoacids with the same structure, differing only in the atom Y, the acid strength increases with the electronegativity of Y. For example,

$HIO < HBrO < HClO$

For a series of oxoacids, $(HO)_mYO_n$, the acid strength increases with n, which is the number of O atoms bonded to Y (excluding O atoms in OH groups). For example,

$HClO < HClO_2 < HClO_3 < HClO_4$ (Ebbing, 1993)

Another type of acid is a polyprotic acid. A polyprotic acid is a substance that can donate more than one proton (Shriver & Atkins, 1999). An example of a polyprotic acid is H_2SO_4, which ionizes by losing a proton to give HSO_4^-; HSO_4^- in turn ionizes to give SO_4^{2-}. HSO_4^- can lose another proton, so it is also acidic. However, because of the negative charge of the HSO_4^- ion, which tends to attract protons, its acid strength is reduced from that of the uncharged species. The acid strength is therefore in the order

$HSO_4^- < H_2SO_4$

The acid strength of a polyprotic acid and its anions decrease with increasing negative charge. Each equilibrium has an associated acid-ionization constant (Ebbing, 1993).

2.1.6 SOLUTIONS OF A STRONG ACID AND BASE

Strong acids and bases are completely dissociated into ions in an aqueous solution (Hill, 1986). In a strong acidic or basic solution, the self-ionization of water, as a source of H^+, is ignored when applying Le Chatelier's principle (Ebbing, 1993). If 0.10 mol of a strong acid, such as HCl, is diluted with water up to 1.0 ℓ of aqueous solution, giving 0.10 M HCl, the concentration of H^+ from HCl is 0.10 M. The reason is that a strong acid ionizes completely and the $[H^+]$ from the self-ionization of water is minute in comparison. Although the $[H^+]$ is ignored in the self-ionization of water, the self-ionization equilibrium still exists and is responsible for the presence of a small amount of OH^-. The ion-product constant for water is used to calculate this concentration. At 25°C, the OH^- ion concentration is calculated as follows:

$$K_w = [H_3O^+][OH^-]$$

$$1.0 \times 10^{-14} = 0.10 \times [OH^-]$$

Thus $[OH^-] = 1.0 \times 10^{-13}$ (Ebbing, 1993)

The concentrations of H^+ and OH^- ions are altered in water when substances are dissolved in it. In a neutral solution, like pure water, the H^+ and OH^- are equal. In an acidic solution, the concentration of H^+ is greater than that of OH^- and in a basic solution; the concentration of OH^- is greater than that of H^+.

To summarize:
In an acidic solution, $[H^+] > 1.0 \times 10^{-7}$ M.
In a neutral solution, $[H^+] = 1.0 \times 10^{-7}$ M.
In a basic solution, $[H^+] < 1.0 \times 10^{-7}$ M (Ebbing, 1993).

2.1.7 SOLUTIONS OF A WEAK ACID AND A BASE

Weak acids and bases partially dissociate into ions in aqueous solution (Scott, 2001; Selinger, 1998; Hill, 1986). For a weak acid, the concentrations of ions in solution are determined from the acid-ionization constant, K_a, which is the equilibrium constant, K_c, for the ionization of a weak acid. The acid-ionization equilibrium of a weak monoprotic acid (HA) in an aqueous solution is given the general formula:

$$HA(aq) + H_2O(\ell) \leftrightarrows H_3O^+(aq) + A^-(aq)$$

The corresponding equilibrium constant is

$$K_c = \frac{[H_3O^+]\,[A^-]}{[HA][H_2O]}$$

Rearranging this equation gives

$$K_a = [H_2O]\,K_c = \frac{[H_3O^+]\,[A^-]}{[HA]}$$

The equation indicates that the acid-ionization constant, K_a, is equal to the constant $[H_2O]\,K_c$. Due to the fact, that water is a constant, it is assigned the value of 1 and can therefore be explicitly excluded from the equilibrium constant equation, in which $[H^+]$ then can be substituted for $[H_3O^+]$.

$$K_a = \frac{[H^+]\,[A^-]}{[HA]}$$ (Ebbing, 1993)

Because the values of acid ionization constants can involve very large or very small exponents, the most useful quantitative measure of acid strength is the pK_a, where

$$pK_a = -(\log_{10} Ka)$$

The stronger the acid, the more negative the pK_a (Canham, 1996).

The relevant constant for bases is identified as the base ionization constant, K_b. For a base, A^-, the general equation for the equilibrium can be written as

$$A^-(aq) + H_2O(\ell) \leftrightarrows HA(aq) + OH^-(aq)$$

The corresponding base ionization expression would be

$$K_b = \frac{[HA][OH^-]}{[A^-]}$$

Similarly as $pK_a = -(\log_{10} Ka)$ for acids, pK_b for bases is defined as

$$pK_b = -(\log_{10} K_b)$$

There is a mathematical relationship between the acid ionization constant K_a of an acid and the base ionization constant K_b of its conjugate base. The product of the two terms equals the ion product constant K_w, thus:

$$K_w = [K_a] \times [K_b]$$ (Canham, 1996)

This can be expressed more conveniently in logarithmic form as

$$pK_w = pK_a + pK_b \qquad \text{where } pK_w = 14.00 \text{ at } 25\ °C$$

Thus the stronger the base, the weaker the conjugate acid. Conversely, a strong acid will have a weak conjugate base (Canham, 1996).

2.1.8 ACID-BASE PROPERTIES OF SALT SOLUTIONS; HYDROLYSIS

A salt may be regarded as an ionic compound obtained by a neutralization reaction in an aqueous solution. A salt can be neutral, acidic, or basic in character. According to the Brønsted-Lowry concept of acids and bases some ions can act as acids or bases, thus the acidity or alkalinity of a salt solution is determined by individual ions in the solution. This acid-base property of a salt is due to the hydrolysis of the individual ions. Hydrolysis of an ion is the reaction of an ion with water, to produce the conjugate acid and hydroxide ion or the conjugate base and the hydrogen ion. For normal salts, those in which the anion has no acidic hydrogen atoms, the following set of rules can be applied, to decide whether a salt solution will be neutral, acidic or basic:

- A salt of a strong base and a strong acid. The salt has no hydrolysable ions and gives a neutral aqueous solution. An example is NaCl.
- A salt of a strong base and a weak acid. The anion of the salt is the conjugate of the weak acid. It hydrolyzes to give a basic solution. An example is NaCN.
- A salt of a weak base and a strong acid. The cation of the salt is the conjugate of the weak base. It hydrolyzes to give an acidic solution. An example is NH_4Cl.
- A salt of a weak base and a weak acid. Both ions hydrolyse and whether the solution is acidic or basic depends on the relative acid-base strengths of the two ions. If the K_a is larger than the K_b, the solution is acidic. If the K_b is larger than the K_a, the solution is basic (Ebbing, 1993).

2.1.9 THE pH OF A SOLUTION

The H^+ concentration (or $[H_30^+]$) in an aqueous solution varies over a very wide range. For this reason, it is practical and convenient to use the logarithmic quantity called pH to measure the concentration of H^+ (Horton, Ochs, Rawn, & Scrimgeour, 1996). The concentration of hydronium ions determines the acidity of a solution. Mathematically, pH is defined as the negative logarithm to the base 10 of the hydronium ion concentration expressed in molarity, i.e. $pH = - \log [H_3O^+]$ (Bylund, 1995; Sherman, A, Sherman, S, & Russikof, 1984). The Danish biochemist S.P.L. Sørensen devised the pH-scale while working on the brewing of beer (Ebbing, 1993). The pH-scale ranges from 0 to 14. A neutral solution, with a hydronium ion concentration of 1.0 × 10^{-7} M at 25°C, therefore has a pH of 7.00. For an acidic solution, with a hydronium ion concentration of greater than 1.0 × 10^{-7} M, the pH is less than 7.00. Comparatively, for a basic solution, where the hydroxide-ion concentration is greater than the hydronium ion concentration, the pH is greater than 7.00 (Ebbing, 1993; Masterton & Slowinski, 1973). Table 2.1 indicates the relationship between pH and the concentrations of H^+ and OH^-.

Table 2.1 Relation of H^+ and OH^- to pH (Horton et al, 1996)

pH	OH^- (M)	H^+ (M)
0	1	10^{-14}
1	10^{-1}	10^{-13}
2	10^{-2}	10^{-12}
3	10^{-3}	10^{-11}
4	10^{-4}	10^{-10}
5	10^{-5}	10^{-9}
6	10^{-6}	10^{-8}
7	10^{-7}	10^{-7}
8	10^{-8}	10^{-6}
9	10^{-9}	10^{-5}
10	10^{-10}	10^{-4}
11	10^{-11}	10^{-3}
12	10^{-12}	10^{-2}
13	10^{-13}	10^{-1}
14	10^{-14}	1

The pH of a solution is accurately measured with a pH meter, which basically consists of an electrode and a calibrated potentiometer. The glass electrode consists of a bulb of glass, partly made of a special composition sensitive to hydrogen ions. Within the bulb is a chloride buffer to maintain a high, constant, hydrogen ion activity. A silver-silver chloride electrode dips into this buffer solution. When the glass bulb is immersed in a solution containing hydrogen ions, an electrical potential is set up across the glass membrane, owing to the difference in hydrogen ion concentration on the two sides. This potential is measured against a standard saturated-calomel-electrode with a vacuum tube voltmeter. Most instruments are calibrated to read directly in terms of pH. (Wilson & Walker, 2000; Jenness & Patton, 1959).

Acid-base indicators also measure pH. They are not as accurate as the pH meter, because normally they only indicate a value above or below a certain level.

The colour change of an indicator involves establishment of equilibrium between an acid form and a base form, which have different colours (Ebbing, 1993).

Paper strips, impregnated with several indicators, can also measure pH values. They give a definite colour for different pH ranges and can give the pH to the nearest integer value or better (Ebbing, 1993).

2.1.10 BUFFERS

A buffer is a solution, characterized by the ability to resist changes in pH when limited amounts of acid or base are added to it. Buffers contain either a weak acid with its conjugate base or a weak base with its conjugate acid. In a buffer, the acid and base species are at equilibrium. The buffering system operates on the principle, whereby a buffer solution resists pH change through its ability to combine with both H^+ and OH^- ions (Ebbing, 1993).

Two important characteristics of a buffer are its pH and its buffer capacity. The latter refers to the amount of acid or base with which the buffer can react before a significant pH-change occurs. The buffering capacity is dependent on the amount of acid and conjugate base in the solution (Ebbing, 1993).

The pH of a buffer, for different concentrations of conjugate acid and base, can be calculated according to the Henderson-Hasselbalch equation:

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log \frac{[\mathrm{base}]}{[\mathrm{acid}]}$$

By substituting the value of $\mathrm{p}K_\mathrm{a}$ for the conjugate acid, and the ratio [base]/[acid], the pH of the buffer can be obtained.

The components, affecting the buffering capacity of milk, are carbon dioxide, proteins, phosphate, citrate, and a number of minor constituents (Jenness et al., 1959).

2.1.11 FACTORS AFFECTING THE pH OF MILK

Lück and Smith (1975) investigated the influence of the concentration of sodium, potassium, chloride, calcium, magnesium, lactose, total nitrogen, non-casein nitrogen, and casein on the pH-value of fresh milk. He found that a proportional relationship exists between pH and sodium, chloride and non-casein nitrogen, and an inverse relationship existed between pH and potassium, lactose, calcium and magnesium. He also found that when the influences of different constituents were combined, a high sodium concentration increases the pH-value, while high calcium content decreases the pH-value.

The general effect when diluting milk is an increase in the pH and lowering of the titratable acidity. Concentrating the milk lowers the pH and increases the titratable acidity. Diluting and concentrating milk, results in a shift in the distribution of calcium and phosphate between the dissolved and colloidal states. Upon concentration, dissolved calcium and phosphate are shifted to the colloidal state with the subsequent release of hydrogen ions. Dilution of the milk shifts the calcium and phosphate from the colloidal state to the dissolved state, with acceptance of hydrogen ions (Jenness et al., 1959).

Lück and Du Toit (1968) statistically investigated the possibility of estimating the total bacterial count in raw farm milk, meant for manufacturing in warm countries, by means of a pH test, consisting of approximately 400 samples, using regression equations. The relationship was not consistent enough (with $r = 0.32$, $sd = 1.19$) to determine the bacterial count by means of a pH test on the receiving platform. Milk with a pH of 6.60 may contain more than 200 000 000 bacteria per mℓ.

2.1.12 SUMMARY OF FACTORS AFFECTING pH

The pH-value of milk is the resultant determined by each ionisable component of milk. Each ionisable component is characterized by an ionization constant; this together with its concentration, determine the concentration of dissociated hydrogen ions present in milk. The sum of hydrogen ions, as determined by the concentration and ionization constants of the ionisable components of milk, determines the pH of milk; thus $pH = -\log [H^+]$. The concentration of the buffering components of milk influences the $[H^+]$ and therefore, has a marked influence on the measured pH of milk. The task of evaluating the individual effect of each ionisable component on the pH of milk is impractical, due to the presence of the vast number of milk constituents (Table 2.2). A more practical approach would be to evaluate the effect of the main constituents that affect the pH of milk.

Table 2.2 Approximate concentration of constituents in cow milk (adopted from Jenness et al., 1959)

Constituent or group of constituents	Mass/ℓ
1. Water	860-880 g
2. Lipids in emulsion phase	
a. Milk fat (a mixture of mixed acyltriglycerides)	30-50 g
b. Phospholipids (lecithins, cephalins, sphingomyelins, etc.)	0.30 g
c. Cerebrosides	*
d. Sterols	0.10 g
e. Carotenoids	0.10-0.60 mg
f. Vitamin A	0.10-0.50 mg
g. Vitamin D	0.4 ug
h. Vitamin E	1.0 mg
i. Vitamin K	trace
3. Proteins in colloidal dispersion	
a. Casein (alpha, beta, gamma fractions)	25 g
b. B-lactoglobulin(s)	3 g
c. Alpha-lactalbumin	0.7 g
d. Albumin probably identical to blood serum albumin	0.3 g
e. Euglobulin	0.3 g
f. Pseudoglobulin	0.3 g
g. Other albumins and globulins	1.3 g
h. Mucins	*
i. Fat globule protein (*)	0.2 g
j. Enzymes	*
Catalase, Peroxidase, Xanthine oxidase,	N L
Phosphatases (acid and alkaline), Aldolase,	N L
Amylases (alpha and beta), Lipase and other esterases	N L
Proteases, Carbonic anhydrase & Salolase (*)	N L

Table 2.2 Approximate concentration of constituents in cow milk (adopted from Jenness et al., 1959) (Continued)

Constituent or group of constituents	Mass/ℓ
4. Dissolved materials	
a. Carbohydrates	
1. Lactose (alpha and beta)	45-50 g
2. Glucose	50 mg
3. Other sugars	traces
b. Inorganic and organic ions and salts	
1. Calcium+	1.25 g
2. Magnesium+	0.10 g
3. Sodium	0.50 g
4. Potassium	1.50 g
5. Phosphates+ (as PO_4^{3-})	2.10 g
6. Citrates+ (as citric acid)	2.00 g
7. Chloride	1.00 g
8. Bicarbonate	0.20 g
9. Sulfate	0.10 g
10. Lactate (*)	0.02 g
c. Water soluble vitamins	
1. Thiamine	0.4 g
2. Riboflavin	1.5 mg
3. Niacin	0.2-1.2 mg
4. Pyridoxine	0.7 mg
5. Pantothenic acid	3.0 mg
6. Biotin	50 ug
7. Folic acid	1.0 ug
8. Choline (total)	150 mg
9. Vitamin B_{12}	7.0 ug
10. Inositol	180 mg
11. Ascorbic acid	20 mg

Table 2.2 Approximate concentration of constituents in cow milk (adopted from Jenness et al., 1959) (Continued)

Constituent or group of constituents	Mass/ℓ
d. Nitrogenous materials not proteins or vitamins (as N)	250 mg
1. Ammonia (as N)	2-12 mg
2. Amino acids (as N)	3.5 mg
3. Urea (as N)	100 mg
4. Creatine and creatinine (as N)	15 mg
5. Methyl guanidine (*)	*
6. Uric acid	7 mg
7. Adenine	*
8. Guanine	*
9. Hypoxanthine (*)	*
10. Xanthine (*)	*
11. Uracil-4-carboxylic acid (orotic acid)	50-100 mg
12. Hippuric acid	30-60 mg
13. Indican	N L
14. Thiocyanate	N L
e. Gases (milk exposed to air)	
1. Carbon dioxide	100 mg
2. Oxygen	7.5 mg
3. Nitrogen	15.0 mg
f. Miscellaneous	
1. Esters of phosphoric acid not yet identified (as phosphorus)	0.10 g
5. Trace elements (form of occurrence not elucidated)	N L
Usually present	
Rb, Li, Ba, Sr, Mn, Al, Zn, B, Cu, Fe, Co, I	N L
Occasionally present or questionable	
Pb, Mo, Cr, Ag, Sn, Ti, V, F, Si	N L

(*) = Presence, identity, or concentration uncertain,
(+) = Partly in colloidal dispersion,
(N L) = Not listed

2.2 FREEZING POINT OF MILK

2.2.1 BASIC CONCEPTS

It is essential to cover the principles governing freezing point depression, to explain the factors affecting the freezing point of milk. Freezing point depression is a colligative property. Colligative properties of solutions are properties that depend on the concentration of solute molecules or ions in solution, but not on the chemical identity of the solute (Kenkel, Kelter, Hage, 2001; Kroschwitz & Winokur, 1987). These properties apply to solutions containing non-volatile solutes. A non-volatile solute is one that exerts negligible vapour pressure (Freemantle, 1987).

Particles in a liquid are in a state of constant motion, known as Brownian motion (Freemantle, 1987). When left open to the atmosphere, some particles of a liquid escape into the gas phase. This is called evaporation (Williams, Embree, Debey, 1981). The rate of evaporation increases with increasing surface area, increasing temperature, and decreasing external pressure. The pressure exerted by these escaping particles is called the vapour pressure of the liquid (Freemantle, 1987).

Boiling occurs when the vapour pressure of the liquid equals the external pressure (Fernandez & Whitaker, 1975). The temperature at which this occurs is called the boiling point of the liquid. When a liquid is heated, its particles absorb more energy and therefore move faster. They bump into each other more often and bounce further apart. This makes the liquid expand. At the boiling point, the particles absorb enough energy to exert a force, large enough, to overcome the forces holding them together and they break away from the liquid to form a gas (Gallagher & Ingram, 1989).

In a liquid, the motion and thus the kinetic energies of particles are sufficiently high to prevent the attractive forces, which tend to hold the particles together in a crystal lattice. However, as the liquid cools, the attractive forces overcome the motions of those particles with low kinetic energies. As a result, these particles are held together in fixed positions thus forming a crystal lattice. The temperature, at which the kinetic energy and the attractive forces of the particles are equal, is the freezing point of the substance. At this temperature, the solid- and the liquid phases are in equilibrium (Freemantle, 1987; Lewis & Waller, 1982; Compton, 1979;).

2.2.2 VAPOUR PRESSURE OF A SOLUTION

In a pure solvent, particles can escape or evaporate from the surface of the liquid (See Figure 2.2). If the solvent however, contains dissolved solute, the evaporation of the solvent particles is reduced. If the solute has a lower vapour pressure than the solvent, the vapour pressure of the solution will be reduced. The extreme case occurs with a solution containing a non-volatile solute. In this case the vapour pressure of the solution is almost entirely due to the vapour pressure of solvent particles (Freemantle, 1987). Vapour pressure-lowering of a solvent is a colligative property, equal to the vapour pressure of the pure solvent minus the vapour pressure of the solution (Ebbing, 1993).

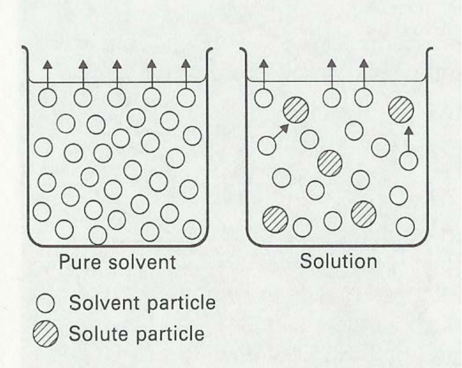

Figure 2.2 Non-volatile solute particles inhibit solvent particles from escaping from the surface and thus lowering vapour pressure (Freemantle, 1987).

In 1886, the French chemist, Francois Marie Raoult, observed that the partial vapour pressure of a solvent above a solution (P_A), containing either a non- or volatile solute, is equal to the product of the vapour pressure of the pure solvent ($P°_A$) and the mole fraction of the pure solvent in solution (X_A) (Ebbing, 1993; Petrucci, 1985; Lippincott, Garrett, Verhoek, 1977, Lee, 1970).

$$P_A = P°_A X_A$$

If the solute is however non-volatile, P_A constitutes the total vapour pressure of the solution. The reason for this is, that in such a solution, the mole fraction of the solvent is always less than 1, and therefore the vapour pressure of the solution (P_A) is less than that of the pure solvent ($P°_A$). A non-volatile solute is thus responsible for the lowering of the vapour pressure.

In general, Raoult's law is observed to hold for dilute solutions, i.e. solutions in which X_A is close to 1. If the solvent and solute are chemically similar, Raoult's law may hold for all mole fractions (Ebbing, 1993; Fine & Beall, 1990).

Raoult's law is displayed graphically in Fig 2.3, where vapour pressures of two solutions have been plotted against the mole fraction of a solvent. In the case of the "ideal solution", i.e. a solution in which both solvent and solute follow Raoult's law for all the values of the mole fraction, vapour pressure is found to be proportional to the mole fractions of the solvent. In this case the vapour pressure follows Raoult's law for all concentrations of a solute. For the "non-ideal solution", Raoult's law is followed for low solute concentrations (mole fraction of solvent near 1), but the vapour pressure deviates at other concentrations.

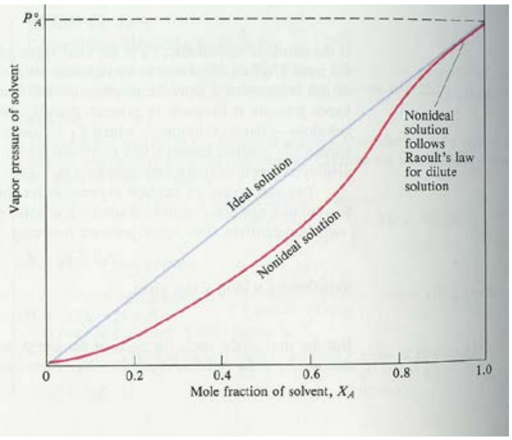

Figure 2.3 A plot of vapour pressures of solutions showing Raoult's law (Ebbing, 1993)

You can obtain an explicit expression for the vapour-pressure lowering of a solvent in a solution, assuming Raoult's law holds and that the solute is a non-volatile non-electrolyte. The vapour pressure lowering, $\triangle P$, is

$$\triangle P = P^\circ_A - P_A$$

Substituting Raoult's law gives

$$\triangle P = P^\circ_A - P^\circ_A X_A$$

$$P^\circ_A (1-X_A)$$

As the sum of the mole fractions of the components of a solution must equal 1, i.e. $X_A + X_B = 1$. So $X_B = 1 – X_A$. Therefore,

$$\triangle P = P^\circ_A X_B$$

From this equation, it is evident that the vapour pressure lowering is a colligative property, i.e. a property that depends on the concentration, but not on the nature, of the solute (Ebbing, 1993).

2.2.3 BOILING POINT ELEVATION AND FREEZING POINT DEPRESSION

The normal boiling point of a liquid, is the temperature at which its vapour pressure equals 1 atm. The addition of a non-volatile solute to a liquid reduces its vapour pressure, therefore temperature of the solution must be increased to a value greater than the normal boiling point to achieve a vapour pressure of 1 atm. Figure 2.4 illustrates the vapour pressure curve for the solution as influenced by temperature. The curve is below the vapour pressure curve of the pure liquid solvent. The boiling point elevation, $\triangle T_b$, is a colligative property of a solution equal to the boiling point of the solution minus the boiling point of the pure solvent. The boiling point elevation is found to be proportional to the molal concentration, c_m, of the solution (for dilute solutions).

$$\triangle T_b = K_b c_m$$

The constant of proportionality, K_b, (called the boiling point elevation constant), depends only on the solvent (Lide, 2003, Ebbing, 1993; Fine & Beall, 1990;).

Figure 2.4 also shows the effect of a dissolved solute on the freezing point of a solution. By decreasing the temperature of a solution, below its freezing point, the pure solvent usually freezes out of solution. Sea ice, for example, is almost pure water. During freezing, the vapour-pressure curve for the solid is unchanged and thus the freezing point of the solution shifts to a lower temperature. The freezing-point-depression, $\triangle T_f$, is a colligative property of a solution equal to the freezing point of the pure solvent minus the freezing point of the solution (Ladd, 1998; Ebbing, 1993; Ladd & Lee, 1986).

Freezing-point-depression, like boiling-point elevation, is proportional to the molal concentration, c_m, of the solution (for dilute solutions).

$$\triangle T_f = K_f c_m$$

In the above formula, K_f is the freezing-point-depression constant and is only depended on the solvent.

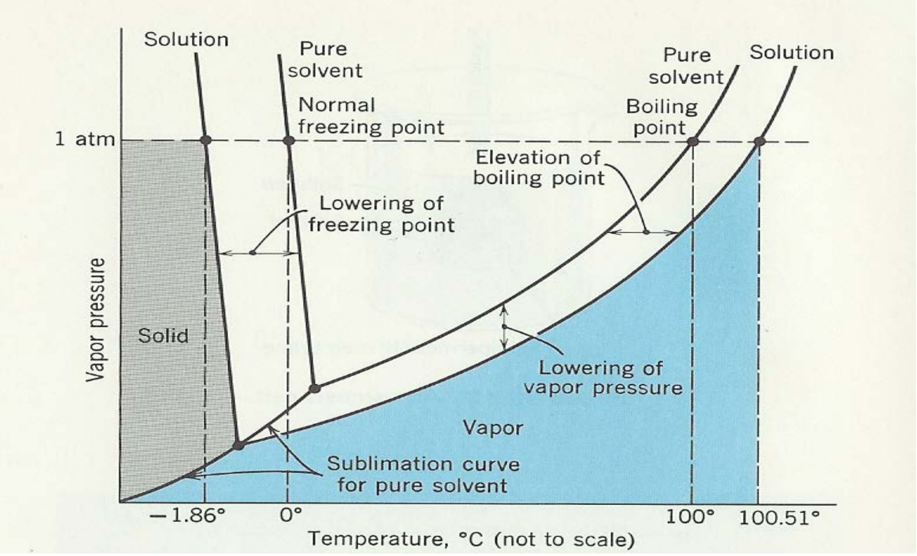

Figure 2.4 Displacement of freezing and boiling points of a one molal aqueous solution (Slabaugh & Parsons, 1971).

The freezing-point-depression constant for water is 1.858°C/*m* (= 0.1858°C/0.100 *m*). As pure water freezes at 0.000°C, the freezing point depression of a 0.100 *m* solution is calculated as follows: 0.000°C – 0.1858°C = -0.1858°C. Thus, a 0.100 *m* solution freezes at 0.1858°C below the freezing point of pure water.

2.2.4 THE RELATIONSHIP BETWEEN FREEZING POINT EXPRESSED IN DEGREE CELSIUS AND DEGREE HORVET

Freezing point determinations, in South Africa, are carried out by means of the thermistor cryoscope method, which has, with time, largely replaced the cumbersome Horvet procedure. Freezing-point-depressions, cited in literature, are often expressed in degrees Horvet (°H). Freezing point determinations, for this study, were conducted by means of a thermistor cryoscope and thus the freezing point is expressed in °C and not in nominal °C or °H (Lück, 1983).

When Horvet, the inventor of the Horvet cryoscope, did his original work, he used a thermometer calibrated by the United States Bureau of Standards, to determine the freezing point of sucrose solutions. He used 7 % w/v and 10 % w/v sucrose solutions with freezing points of -0.422 °C and -0.621 °C, respectively as standards for the calibration of other cryoscope thermometers. It is possible that Horvet assumed that these figures gave the true freezing points for these solutions. However, they actually represented values obtained when only the Horvet cryoscope and Horvet technique were used. The true freezing points of the above sucrose standards were -0.40746 °C and -0.59968 °C, respectively. Hence, the observed depressions of the sucrose solutions recorded by Horvet were too large and the interval between them was 0.199 °C and not -0.192 °C.

Results recorded in literature, which were obtained with the Horvet apparatus, are therefore not true freezing points. Some countries still use the Horvet method and record their results accordingly. Both methods of expressing results are acceptables providing the method of cryoscope calibration is given. It is possible also to correct results from one method to another using the following formulae, see Table 2.3 ("*International Dairy Federation*", 1991).

$$T_c = 0.9656\ T_H \text{ and } T_H = 1.0356\ T_c$$

Where,

T_c is the freezing point in degrees Celsius and T_H is the freezing point in degrees Horvet

Table 2.3 Relationship between °C and °H (“*International Dairy Federation*”, 1991)

Sodium Chloride (grams made up to 1 litre) 20 °C	Freezing Point °H (nominal °C as measured by Horvet apparatus)	Freezing Point °C
000	0.000	0.000
6.859	-0.422	-0.408
7.820	-0.480	-0.464
8.151	-0.500	-0.483
8.317	-0.510	-0.492
8.482	-0.520	-0.502
8.648	-0.530	-0.512
8.813	-0.540	-0.521
8.979	-0.550	-0.531
9.145	-0.560	-0.41
10.155	-0.621	-0.600

Sucrose solutions have with time been replaced by more stable sodium chloride solutions (“*International Dairy Federation*”, 1991)

2.2.5 MILK CONSTITUENTS AND FREEZING POINT DEPRESSION

The freezing point of an aqueous solution is directly related to the concentration of its water-soluble constituents. If one or more substances are dissolved in water, the freezing point will be lowered in direct proportion to the molality of the solution. Thus, the freezing point depression is dependent on the numbers of dissolved molecules and/or ions. The freezing point of milk will therefore be lower than that of water. The higher the freezing point (i.e. closer to 0 °C) the more likely it is that the sample contains extraneous water. The opposite nomenclature is used if freezing point depressions (FPD) are referred to, i.e. the lower the freezing point depression (closer to 0 °C) the more likely it is that the sample contains extraneous water (Harding, 1983).

The freezing point depression, $\triangle T$, is generally calculated by means of Raoult’s law and Clausius Clapeyron’s equation as follows:

$$\triangle T = \frac{R.T^2}{r} \cdot \frac{m}{1000 . M}$$

Where,

R = the molar gas constant; being equal to 8.314 J/(K . mol);
T = freezing point temperature of water in K
r = solidification resp. melting energy of water in kJ/kg
m = mass in g of dissolved matter in 1000 g of water
M = relative molecular mass of dissolved matter in g/mol

For aqueous solutions, the result is:

$$\triangle T = \frac{8.314 \cdot 273.15^2}{333.5 \cdot 1000} \cdot \frac{m}{M}$$

$$\triangle T = 1.86 \cdot \frac{m}{M}$$

If several substances are dissolved in water, then

$$\triangle T = 1.86 \frac{(m_1 + m_2 + \dots m_n)}{M_1 + M_2 + \quad M_n}$$

In the case of a salt solution, the decrease in the freezing point is determined by the presence of ions. The total relative molecular mass (M) of a salt is composed of its various elements (M1 + M2 + … Mn). The same is valid for the total mass (m) of the dissolved salt, the ion portions of which result as follows:

$$m = m \frac{M_1}{M} + m \frac{M_2}{M} + \dots m \frac{M_n}{M}$$

Thus, the freezing point decrease of the salt solution is calculated as being

$$\triangle T = 1.86 \left(\frac{m M_1}{M_1 M} + \frac{m M_2}{M_2 M} + \dots + \frac{m M_n}{M_n M}\right)$$

$$\triangle T = n \cdot 1.86 \frac{m}{M}$$

(Kessler, 1984)

Milk is a complex aqueous solution and Raoult's law can therefore only be used for a rough calculation of the relation between the concentration of the milk constituents' and the freezing point. Fat globules exist as an emulsion in milk and casein as a colloidal suspension; therefore, it is evident that these constituents will have practically no effect on the freezing point depression. The freezing point of milk is therefore largely dependent upon the concentration of the lactose and the salts. Lactose accounts for about 55 % and chloride 25 % of the freezing point depression. The remaining 20 % are made up of other water-soluble constituents such as calcium, potassium, magnesium, lactates, phosphates, citrates, etc (Harding, 1983).

See Table 2.4 for a summary of milk ingredients and their effects on freezing point depression.

Table 2.4 Milk ingredients and their effects on freezing point decrease (Adopted from Kessler, 1984).

Milk ingredients	%	Relative Molecular Mass kg/kmol	Milk ingredients g/1 000 g of water	Freezing point decrease in ° C
Water	87.5	N L	N L	N L
(Proteins	3.33)			
Casein	2.66	107 … 109	30.4	-0.000 001
Whey protein	0.67	15-70…103	7.66	-0.000 407
Fat	3.78	N L	43.2	-0.000 000
Lactose	4.65	342	53.14	-0.289
(Salts	0.80)	N L	9.14	N L
NaCl	0.085	58	0.97	-0.062
$NaHCO_3$	0.019	84	0.22	-0.0095
KCl	0.072	74	0.82	-0.041
K_2SO_4	0.013	174	0.15	-0.0045
KH_2PO_4	0.083	136	0.95	-0.026
K_2HPO_4	0.075	174	0.86	-0.0275
$CaHPO_4$	0.052	136	0.59	-0.016
$Ca_3(PO4)_2$	0.062	310	0.71	-0.021
$K_3C_6H_5O_7$	0.052	306	0.59	-0.014
$Mg_3(C_6H_5O_7)_2$	0.059	402	0.67	-0.0075
$Ca_3(C_6H_5O_7)_2$	0.137	498	1.57	-0.0145
Salts incl. Proteins	0.091	N L	1.04	-0.0000
Sum of salts				-0.2435
Lactose				-0.289
Total freezing point decrease				-0.533

N L Not listed

The freezing point of milk is its most constant physical property, although the concentration of the soluble constituents of milk can vary substantially. The total molality of the different soluble constituents must therefore be relatively constant. The secretory processes of the mammary gland are such that the osmotic pressure of milk is kept in equilibrium with that of blood. Any depression of the synthesis of lactose is compensated by increases in the concentration of sodium and chloride. A variation of 0.1 % chloride was found to be equivalent to 1.75 % lactose. It must not be thought, however, that the freezing point of milk is invariant. It varies somewhat, but within very narrow limits (Harding, 1983; Jenness et al., 1959).

There seems to be agreement on the fact, that the main factor, regulating the relatively constant freezing point of milk, is the complementary relationship between lactose and chloride (Harding, 1983). Chloride, sodium, non-casein nitrogen and potassium are the most important constituents in regulating the lactose content (Lück et al., 1974). It is, however, not clear, which changes in the milk composition are responsible for the observed variations in the freezing point. This is evidently a complex question without a simple answer. Some results imply that deviation from the complementary lactose/chloride relation and variation in potassium content are the most important factors accounting for the freezing point. Other results imply, that the normally observed variations in the freezing point of milk were caused, mainly by the variation in the chloride free ash fraction, probably soluble phosphates, (Harding, 1983).

2.2.6 ESTIMATION OF THE PERCENTAGE OF EXTRANEOUS WATER IN MILK

When the effect of added water on the freezing point of milk is determined by calculation, it should be borne in mind that the percentage of water added (W %) relates to the total quantity of milk (water and dry matter), whereas the freezing point depression is expressed by the quantity of dissolved matter per 1000 g of water (Kessler, 1984).

According to Harding (1983), using the actual or assumed freezing point standard, -0.512 °C, as reference, the percentage of extraneous water in the suspect sample can be calculated from the following equation:

$$W = \frac{(C - D)\,(100 - S)}{C}$$

Where,

W = % (m/m) of extraneous water in the suspect milk sample
C = actual or assumed reference freezing point of genuine milk
D = the freezing point of the suspect milk sample
S = the % (m/m) of total solids in the suspect milk sample

2.2.7 SUMMARY OF FACTORS AFFECTING FREEZING POINT

The freezing point of milk is largely dependent upon the concentration of lactose, and salts. Lactose accounts for about 55 %, chloride 25 % and the remaining 20 %; due to other water-soluble constituents such as calcium, potassium, magnesium, lactates, phosphates, citrates, etc (Harding, 1983). Analyses of the lactose and chloride content of milk are therefore imperative in the study of the freezing point of milk.

2.3 FACTORS AFFECTING THE COMPOSITION OF MILK

2.3.1 INTRODUCTION

Milk, being a liquid secreted by a biological being with many chemical processes in her body, will not have the same composition at all times, but will vary under the interaction of environmental and physiological factors (Robertson, 1997). The physiological factors are governed by heredity and nonhereditary factors. Heredity factors include variation among breeds and individual variation within breeds. Nonhereditary factors include age, stage of lactation and pregnancy of the cow. Environmental factors include season, temperature and nutrition of the cow (Schmidt et al, 1988). A brief discussion of the influence of the factors affecting milk composition will be dealt with below.

2.3.2 AGE OF THE COW

It is well known that cows produce more milk as they become older. Cows of most breeds are considered matured at the age of six. First-lactation cows, freshening at 24 months of age, produce approximately 75 % of the milk produced by mature cows. Three- (second lactation), 4-, and 5- year old cows produce approximately 85 %, 92 %, and 98 % of milk produced by mature cows, respectively. There is some variation among breeds; the Brown Swiss matures somewhat slower than other breeds (Schmidt et al, 1988). The increased production with age is the result of an increase in body size and udder capacity (Robertson, 1997). Depeters & Cant (1992) found that milk protein content peaked for cows at 3 years of age and gradually declines with advancing age.

As milk production increases with age, there is a slight decrease in solids-non-fat and milk-fat percentages up to the 5th lactation, beyond this there, is little change (Schmidt et al, 1988). According to Ng-kwai-hang et al. (1982) the proportion of casein in milk protein decreased as cows become older. When cows are 8 to 9 years of age, a slight reduction in the amount of milk produced occurs and this reduction continues until their death (Schmidt et al, 1988).

2.3.3 BODY WEIGHT OF THE COW

A general relationship exists between the body weight of cows and the amount of milk produced. Larger cows will produce more milk, because they have more udder secretory tissue and larger digestive systems (Schmidt et al, 1988).

2.3.4 BREED OF THE COW

The composition of milk of different breeds differs significantly (Table 2.5). For the more common dairy breeds, the Friesland/Holstein breed has the lowest fat, protein, lactose, total solids and solids-non-fat content; while the Jersey breed has the highest content for each of these components.

Table 2.5 Composition of milk of different breeds of cows in RSA. (Robertson, 1997)

Breed	Fat %	Protein %	Lactose %	Ash %	Total Solids %	Solids-non-fat %*
Frieslands	3.56	3.22	4.87	0.68	12.33	8.54
Ayrshire	3.66	3.35	4.81	0.68	12.50	8.62
Guernsey	4.37	3.54	4.96	0.74	13.61	9.13
Jersey	4.49	3.75	5.00	0.70	13.94	9.36

*Values obtained from (Norman, Kuck, Cassell & Dickinson, 1977)

2.3.5 COLOSTRUM

During the first 4 to 5 days after calving, the milk obtained is known as colostrum, which has a markedly different composition from that of normal milk. Colostrum contains high levels of most of the milk constituents except for lactose and potassium (Robertson, 1997). The most striking difference is the high percentage of protein in colostrum. Most of the increase in protein is attributable to the globulins, particularly immune globulins, which carry the immune bodies, or antibodies. Antibodies can be absorbed by the intestinal tract during the first 24 hours of the calf's life and gives the newborn calf passive immunity from its dam. The fat and ash percentages are also somewhat higher in colostrum than in normal milk. These differences cause the total solids content to be approximately double than that of normal milk (Schmidt et al, 1988). The progressive transition from colostrum to normal milk is normally rapid (Table 2.6).

Table 2.6 The progressive change of colostrums into normal milk (Robertson, 1997).

Time calving	Total Solids %	Protein %	Albumin + Globulin %	Lactose %	Chlorides%	Fat %
0 h	27.0	17.6	11.3	2.2	0.15	5.1
6 h	20.5	10.0	6.3	2.7	0.16	6.8
36 h	12.2	4.0	1.0	4.0	0.16	3.6
4 days	11.9	3.8	0.8	4.7	0.13	2.8
7 days	12.1	3.3	0.7	5.0	0.11	3.5

2.3.6 DAY-TO-DAY VARIATIONS

Both milk yield and composition of milk, as produced by a single cow or a herd, may vary considerably from day to day. In general, daily variation in milk yield is related to the completeness of milking. Factors such as temperature, feeding, esterus, excitement and shortage of drinking water, may also cause short term variation, but the influence of these together with factors such as disease, cows being under-fed, stage of lactation and age are usually of longer duration.

Depressions in milk yield are usually accompanied by a higher fat percentage, because there is a general inverse relationship between the milk yield and the fat content. First-drawn milk, or foremilk, may contain as little as 1 % milk fat, whereas the fat content of the last drawn milk can be as high 8 to 15 %. Incomplete milking will therefore result in milk with a low fat content. The milk obtained during the next complete milking will normally have a higher than normal fat content. (Schmidt et al, 1988).

With unequal intervals between milkings, milk yield following the shorter interval will be lower with a higher fat percentage and little, if any change in protein percentage on a fat-free basis (Robertson, 1997). (Table 2.7)

Table 2.7 Milk yield and composition as influenced by milking interval (Robertson, 1997).

	Preceding milking intervals (hours)		
	5	7	12
Milk Yield (kg)	15.00	17.00	29.00
Fat %	6.63	6.53	3.93
Fat kg	1.10	1.10	0.99
SNF %	9.43	9.48	9.59

If cows are milked twice a day at equal intervals, the milk yield is usually slightly higher with a lower fat percentage during the morning's milking (Table 2.8).

Table 2.8 Milk yield and composition as influenced by morning and evening milking (Robertson, 1997)

Time	Milk	Fat		SNF	
	Kg	%	kg	%	kg
Morning	8.36	3.66	0.31	8.60	0.72
Evening	7.42	3.76	0.28	8.59	0.64

2.3.7 EXERCISE OF THE COW

Excessive exercise has a marked decreasing influence on milk yield. The lower milk volume will cause the fat percentage to increase. The net result will however be a slight loss of fat (Robertson, 1997).

2.3.8 FEED OF THE COW

The influence of feeding on milk production and composition is complicated. Various nutrients are needed for a cow to produce at an optimum level within her genetic potential. An ideal ration should contain approximately 16 % crude protein, 10-11 megajoules (MJ) digestible energy/kg, 0.6 % calcium, 0.4 % phosphorus and at least 17 % fibre. Factors such as protein and fibre quality, mineral and vitamin status, physical structure of feed should be taken into account (Robertson, 1997).

Diets of cows, based largely on grazing or hay, are normally classified as low-energy diets, while those based on concentrates constitute high-energy diets. Production of milk, fat, solids-non-fat, protein, and casein is usually lower for cows fed low-energy diets compared with that of cows fed high-energy diets. The percentages of solids-non-fat, total protein, casein, ß-lactoglobulin and ά-lactalbumin in milk are generally higher, as the proportion of concentrate in the diet is increased, whereas the fat percentage decreases (Bartsch, Graham, McLean, 1979).

2.3.9 GESTATION

A marked drop in milk production occurs toward the end of pregnancy. The exact reason for this decrease is not known. An increase in the solids-non-fat content of the milk, which continues until the end of lactation, occurs from the 4th to the 5th month of gestation (Schmidt et al, 1988).

2.3.10 HORMONES

Several hormones have been used commercially and experimentally to increase milk production and change the composition of milk. The two most common, are thyroxine, and the growth hormone, bovine somatotropin (BST) (Schmidt et al, 1988).

Thyroxine, which is secreted by the thyroid gland, has, as one of its major functions the control of the metabolic rate. Cows receiving thyroxine, via the feeding of thyroprotein, an artificial precursor of thyroxine, have elevated body temperatures and increased heart- and respiration rates (Schmidt et al, 1988).

The short term feeding of thyroxine at 10 to 15 g per animal per day results in an increase in milk production, which may be as high as 15 to 20 %. A transitory increase in the milk-fat occurs, but the concentration soon returns to normal. To make thyroxine supplementation effective, additional feed must be fed for the synthesis of the extra milk and the maintenance of the animal (Schmidt et al, 1988).

Several problems arise from the feeding of thyroprotein to dairy cattle. Firstly, thyroprotein supplemention seems only to be effective for periods of 60 to 120 days. Secondly, when the supplementation is withdrawn, a marked drop in milk production occurs. During feeding of thyroprotein, the animal’s thyroid gland becomes inactive. When the thyroprotein is withdrawn, the thyroid gland requires time to return to an active state. During this time, a drop in milk production occurs. In many cases, the decrease in milk, produced after thyroprotein withdrawal, is equal to the additional amount produced during thyroprotein supplementation. Several research workers have withdrawn thyroprotein at a gradual rate instead of abruptly, but no consistent beneficial effects have been obtained (Schmidt et al, 1988).

The effect of bovine somatotropin stimulation on milk production has been known for about 50 years. Its use was limited because of the high cost of the naturally produced compound that had to be extracted from the pituitary glands of slaughtered animals. Much interest has developed in its use after it became possible to produce rather large amounts with bacteria through synthetic production by using bioengineering methods of recombinant DNA. A number of short-term studies indicate that growth hormone injections could attain increases of 10 % to 40 % in milk production. After the withdrawal of the injections, milk production returned to pre-injection levels. This is in contrast to the precipitous drop seen after the withdrawal of thyroprotein. Recombinant-derived growth hormone increased fat-corrected milk in a dose-dependent manner from 23 to 41 % over controls, whereas the pituitary-derived version resulted in only a 16 % increase. Supplementing bovine somatotropin did not affect the milk composition as regards fat, protein, and lactose content (Schmidt et al, 1988).

2.3.11 LACTATION STAGE

The relationship of milk yield to the milk fat and protein percentages of a cow during a normal lactation period is shown in Figure 2.5.

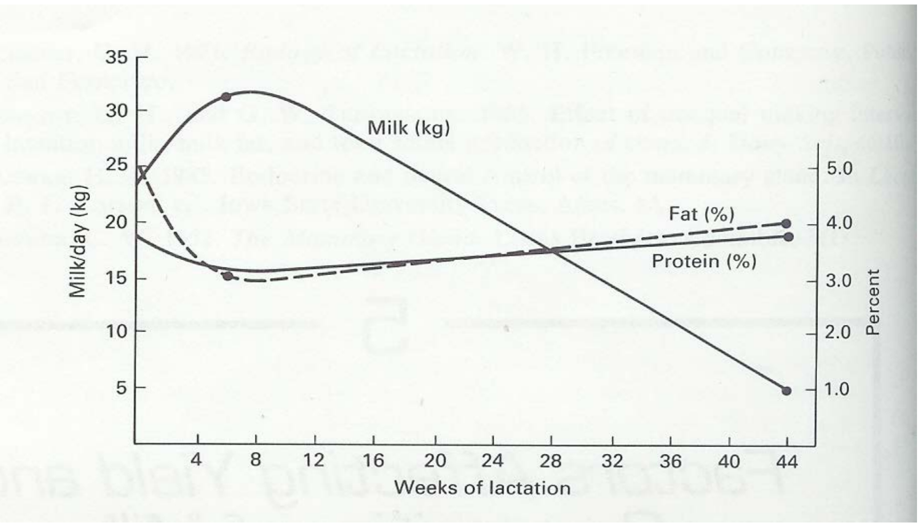

Figure 2.5 Milk yield, fat-, and protein percentages of Holstein cows with advancing lactation (Schmidt et al, 1988).

A general inverse relationship exists between milk yield and total solids content. As yield increases, the percentages of fat and protein decrease and vice versa. At the onset of normal lactation, the protein and milk-fat percentages decreases to a minimum, more or less at the stage where maximum milk production occurs and thereafter gradually increases towards the end of lactation (Depeters et al, 1992; Schmidt et al, 1988). Lactose content tends to move with the milk yield. It rises to a maximum at about the 7th week of lactation and then slowly declines until about mid-lactation after which the decline becomes more pronounced (Robertson, 1997). The ash content shows a very slight increase with advancing lactation (Schmidt et al, 1988). The calcium and phosphorus contents will decrease during the first month, then remain relatively constant and increase during the last 3 months. During the latter stage of lactation, the sodium chloride content of the milk may increase sharply (Robertson, 1997).

2.3.12 MASTITIS

Mastitis is an inflammatory change of the mammary gland, along with physical, chemical, and microbiological changes, which is characterized by an increase of somatic cells, especially leukocytes in the milk, and by pathological changes in the mammary tissue. The somatic cell count (SCC) of milk provides a useful and convenient indirect measure of the prevalence of udder infection in dairy herds (Conradie, 1985).

Mastitis has a marked and characteristic effect on milk yield and composition. Udder infection, like other metabolic disturbances, lowers milk yield. A decrease in milk yield is linear, with increasing somatic cell count (Jones et al., 1984; Raubertas & Shook, 1982; Meijering et al., 1978). An indication of the expected loss in yield, on herd level, as influenced by mastitis, is given in Table 2.9.

Table 2.9 Influence of somatic cell count (SCC) on milk yield (Robertson, 1997)

SCC X 1 000/ mℓ	Degree of Mastitis	Milk Loss (%)
< 200	Slight	2.7
200 to 500	Middling	4.1
500 to 1 000	Bad	6.4
> 1 000	Severe	8.8

Under the influence of mastitis most of the major milk constituents tend to decrease in concentration, (Table 2.10)

Table 2.10 Milk composition as influenced by mastitis (Robertson, 1997)

Milk constituent	Effect	Composition %	
		Normal	Mastitic
Fat	Decrease	3.8	3.6
Total Protein	Slight decrease	3.6	3.5
Casein	Decrease	2.8	2.3
Whey proteins	Increase	0.8	1.2
Lactose	Decrease	4.9	4.4
Na^+	Increase	0.05	0.08
Cl^-	Increase	0.10	0.18
Ca^{++}	Decrease	0.13	0.09

With mastitis, lactose secretion is reduced presumably due to damaged epithelium and reduced enzyme activity (Robertson, 1997). A percentage of less than 4.6 % in the milk of an individual cow is indicative of mastitis (Munro, Grieve, & Kitchen, 1984). An osmotic effect also may account for reduced lactose secretion (Robertson, 1997).

In the mastitic udder the permeability of the blood-milk barrier increases. Due to this increased permeability, more blood substance, including sodium and chloride, which occurs in markedly higher concentrations in blood than in milk, enters the milk. The resulting ionic changes alter the osmotic balance between the milk and the blood. To maintain osmotic equilibrium, the secretory tissue in the udder secretes less lactose (Robertson, 1997; Munro et al, 1984).

In mastitic milk, the concentration of casein decreases, whereas concentration of certain whey proteins, such as albumin and globulin, increases. Generally, milk fat concentration is slightly depressed in mastitic milk. The total protein content stays relatively constant, because the decrease in casein is countered by an increase in the albumin and globulin percentage. Total solids and solids-non-fat content in mastitic milk also decrease, mainly due to the decrease in fat and lactose (Robertson, 1997; Fox, Shook, & Schultz, 1984).

2.3.13 PHOTOPERIOD

The effects of various lighting regimes were investigated on milk production. In a field trial involving 13 dairy herds, cows were exposed to 16 - 16.25 and 9 - 12 hours of light per day, respectively. The longer exposure resulted in 2.2 kg higher milk yield per cow, per day, and a drop of 0.16 % milk-fat percentage (Schmidt et al, 1988).

2.3.14 MILK SAMPLING

In any analytical procedure, the value of the result is dependent upon the extent to which the sample tested is representative of the total volume of product to be examined. It is difficult to over-emphasize the importance of obtaining a representative sample for testing. Creaming will take place when milk is left undisturbed (Robertson, 1997). (Table 2.11)

Table 2.11 The influence of creaming on the fat content in the upper 25 % layer of undisturbed milk (Robertson, 1997)

Time (Hours)	Fat %
0	5.25
½	5.36
1	6.96
3	12.00
5	13.40

The rising fat globules serve as a filter; and will cause bacteria and somatic cells to rise with them. Thorough agitation of milk to ensure homogeneity, but without causing churning of the fat, is thus essential before a sample for any analytical purpose is taken (Robertson, 1997).

Factors such as lipolysis, churning and bacteriological activity will cause deterioration of the milk and may thus influence the accuracy of analysis. As high temperatures enhance these influences, it is advisable to store milk samples prior to analysis, at a temperature between 0 °C and 4 °C (Robertson, 1997).

To facilitate thorough mixing of a sampling before analysis, it is advisable that the sample bottles should be of a convenient size and should not be filled to more than 75 % capacity (Robertson, 1997).

2.3.15 SEASON

In South Africa, milk production is high during summer (September to February) and low during winter (March to August). Fat and protein percentages follow an inverse pattern to milk production (Robertson, 1997).

In the northern part of the United States, cows freshening in the autumn and early winter produce considerably more milk and higher milk-fat yields than do those freshening in late winter, spring, and summer. Previously, differences were as high as 680 kg; however, the differences have been reduced over the years because of better feeding and management of cows throughout the year (Schmidt et al, 1988).

There are several reasons for the higher production of cows freshening in autumn and early winter. Milk production is usually lower during summer, because of the higher environmental temperatures (Sharma et al., 1983; Thatcher, 1973) and the undernutrition that exists on some farms, where summer drought results in poor pastures, which serves as the major source of feed. Cows freshening in fall have their peak production during winter, when feeding and management are usually better. Most dairy farmers have more time to spend with the cows and more emphasis is placed on the feeding program at this time (Schmidt et al, 1988). When these cows are turned out on pasture, during spring and summer, a boost in milk production occurs. This boost occurs during the declining phase of lactation and has a significant effect on the milk yield per lactation. The cows are also dry during the latter part of the summer, when high environmental temperatures and poor feeding cause a decrease in milk production (Schmidt et al, 1988).

2.3.16 TEMPERATURE AND HUMIDITY

Climatic factors, such as environmental temperature, solar radiation, relative humidity, wind-speed, and their interactions, influence animal performance (Sharma et al., 1983). Environmental temperature is usually the most important climatic factor that affects milk production. The magnitude of this influence is dependent on factors such as air movement, humidity, period of exposure, breed, and individuality of the cow. High relative humidity, extensive periods of exposure, and excessive radiation accentuate the influence of high temperatures. Air velocity at higher temperatures helps to cool the animals and minimize the influence (Robertson, 1997; Schmidt et al, 1988).

Temperatures between 4 and 24 °C have little, if any effect, on the milk production of most dairy animals. In this temperature range, known as the comfort zone, no body processes are directly involved in maintaining body temperature (Schmidt et al, 1988). Rodriquez et al. (1985) states that milk yields decline rapidly at temperatures greater than 29°C, while chloride content increases at temperatures above 21°C. The fat and protein percentages will decrease as the temperature increases (Robertson, 1997; Rodriquez et al., 1985; Feagan, 1979).

When the temperature drops below 4 °C, there is no adverse effect on milk production, if sufficient feed is supplied and protection from the elements is provided. Temperatures of -15 °C and below may have a detrimental effect on milk yield. Larger breeds are more tolerant of low temperatures than the smaller breeds. Milk-fat content, as well as solids-non-fat and total solids percentages, increase with decreasing temperature (Schmidt et al, 1988).

Regarding the influence of low temperatures on the freezing point depression of milk, the following case study is noteworthy: During the night of 13/14th September 2001 an abnormal cold front swept the highveld of Gauteng, Mpumalanga and Kwazulu Natal. The front was accompanied by a piercing wind, causing the sensible temperature to drop to –13°C and below. A large volume of milk, collected the next morning from different producers in different road tankers, showed freezing points as low as –0.542 °C, which was markedly lower than the norm of about –0.535 °C for the same milk immediately before and after passing of the cold spell. According to Mr. Robertson, (Assistant Director:ARC-ANPI Elsenburg Dairy Laboratory, personal communication, 10 March, 2003) who was responsible for investigating the phenomenon, reasons for the abnormal low freezing points could have been the following:

- Hunger and thirst due to the inaccessibility of feed and free flowing water was maybe the main cause. The low temperature of the water may also have resulted in restricted intake. This viewpoint is confirmed by the research of Henningson (1963).
- Exposure to the severe cold weather may have cause blood to flow from the external organs, including the udder, to the internal organs in an effort to maintain essential life functions. Under these circumstances, blood circulation through the udder will diminish,

resulting in lower milk production. The effect hereof on the freezing point is unknown.

The milk yield and protein percentages may decrease slightly during cold weather. Conversely, with increasing temperatures, there is a slight decrease in milk yield, milk-fat, solids-non-fat, and total solids percentages (Schmidt et al, 1988). See Figure 2.6. The decrease in yield may cause an increase in the milk-fat percentage. High temperatures cause a decrease in feed consumption, and an increase in water intake, body temperature and respiration rate. The smaller breeds, particularly the Jersey, are more tolerant of high temperatures than are the larger breeds, particularly the Holstein. The smaller breeds have a larger surface area per unit of body weight and can dissipate heat somewhat more rapidly than do larger cows. The brown Swiss is an exception; it is much more tolerant to heat than is the Holstein (Schmidt et al, 1988).

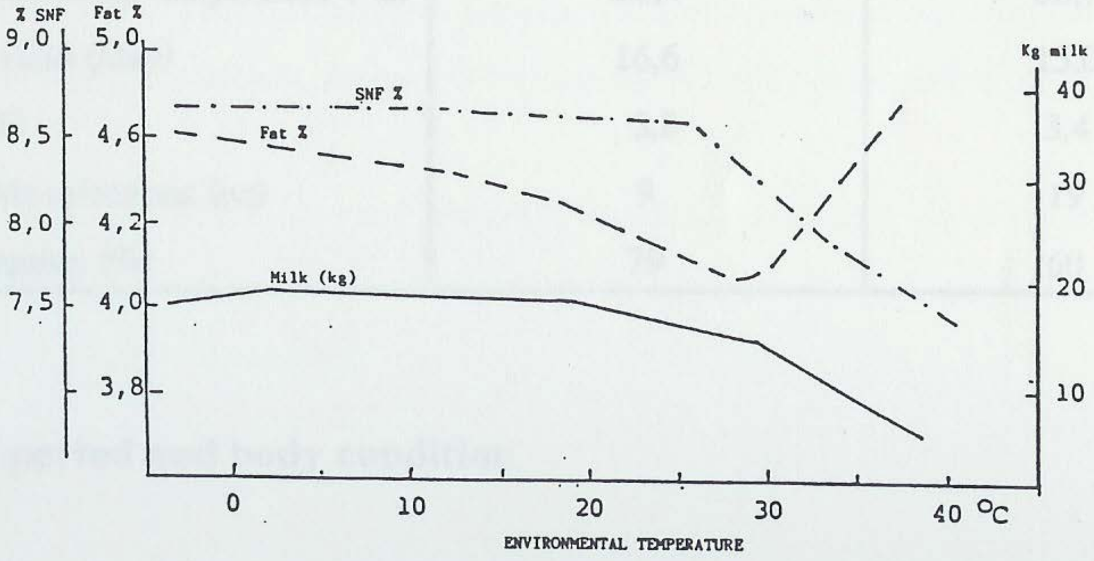

Figure 2.6 Influence of environmental temperature on milk production and composition (Robertson, 1997)

2.3.17 WATER INTAKE BY THE COW

Lactating dairy cows require 3 to 5 litres of water for each kg of milk they produce. Restrictions on the availability and quantity of water will result in a lower milk yield and fat percentage. Cows that have water constantly available will produce about 7 % more milk and 6 % more fat than those that have access to water 2 or 3 times a day (Robertson, 1997).

2.4 CONCLUSION AND DISCUSSION

It is clear that many factors have an influence on the composition of milk. It is a complicated task to determine what the combined effect of the interactions of these factors will be. The effect that it has on the milk composition is nonetheless critical, because it is the milk composition that determines the pH and freezing point (Figure 2.7)

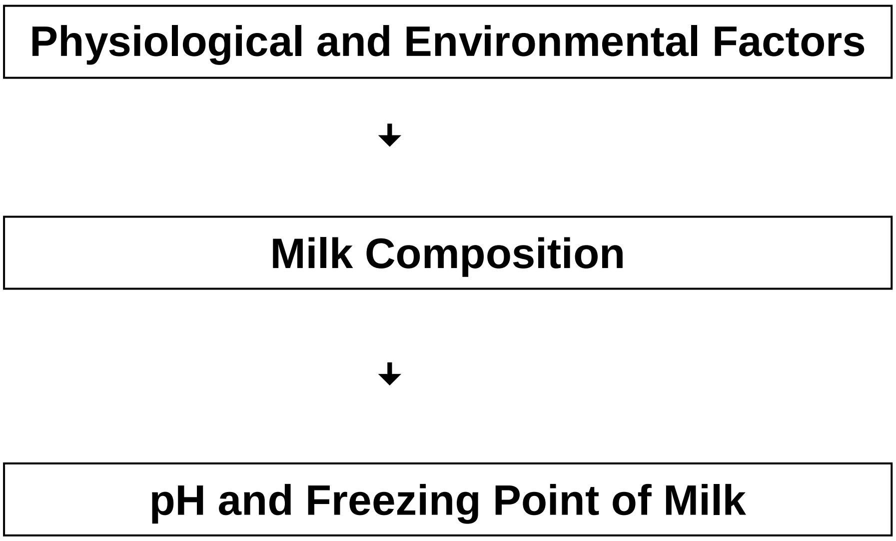

Figure 2.7 Summary of factors influencing the pH and freezing point of milk.

For practical reasons, the research will only concentrate on the influence that certain physiological and environmental factors has on the pH and freezing point of milk. The influence of the following factors was investigated, namely breed, season, feed, temperature, rainfall, humidity, air movement, radiation, sunlight, and evaporation.

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CHAPTER 3

pH- AND FREEZING POINT-VALUES OF MILK

ABSTRACT

Variables from 21 564 and 28 083 herd milk samples, originating in the Western Cape and the Tsitsikama regions of the eastern cape of South Africa, were correlated with the pH- and freezing point-values of raw cows' milk, respectively.

The pH-values obtained, showed the following statistically characteristics, namely: mean (6.72), median (6.72), mode (6.70), range (0.24), minimum (6.60) and maximum (6.84). Fourteen point two percent of the samples had pH-values above 6.75 and none below 6.60, which are the upper and lower limits of the current South African specifications, respectively.

The corresponding statistics of the freezing point values were: mean (-0.523°C), median (-0.522°C), mode (-0.521°C), range (0.043°C), minimum (-0.545°C) and maximum (-0.502°C). Freezing points higher than the current South African specification, being ≤ -0.512 °C, were found in 1.88 % of the samples, while the lower accepted freezing point, being ≥ -0.541, was exceeded by 1.28 % of the samples.

The influence of 17 numerical variables on the pH- and freezing point-values of milk were investigated. pH was significantly ($p < 0.05$) influenced by the content of chloride ($r = + 0.35$), magnesium ($r = + 0.21$), calcium ($r = - 0.20$), average humidity ($r = - 0.19$), somatic cell count ($r = + 0.16$), average temperature ($r = - 0.15$), lactose ($r = - 0.10$), evaporation ($r = - 0.09$), fat ($r = - 0.07$), average rainfall ($r = - 0.05$), wind speed ($r = + 0.04$) and total bacterial count ($r = -0.02$), whereas protein, total solids, solids-non-fat, hours of sunshine, and radiation showed no significant ($p < 0.05$) influence. Freezing point was significantly ($p < 0.05$) influenced by wind speed ($r = + 0.36$), lactose ($r = + 0.35$), solids-non-fat ($r = - 0.35$), protein ($r = - 0.28$), total solids ($r = - 0.23$), fat ($r = - 0.20$), somatic cell count ($r = - 0.19$), radiation ($r = + 0.17$), chloride ($r = + 0.15$), total bacterial count ($r = - 0.14$), average temperature ($r = + 0.12$), sunshine ($r = + 0.06$), evaporation ($r = - 0.05$) and average humidity ($r = - 0.05$), whereas calcium, magnesium, and rainfall showed no significant ($p < 0.05$) influence.

The influence of the non-numerical variables, viz. region, season, breed, and feed on the pH- and freezing point-values was investigated. All of these significantly ($p < 0.05$) influenced pH, whereas freezing point was only influenced by region, breed, and feed.

3.1 INTRODUCTION

The pH-value of raw milk is determined to calculate the amount of developed acid and is one of the intake tests to determine the acceptability of raw milk ("*South African Dairy Training Board (a)*", 1990; Lück, Kriel and Mostert, 1973; Lück and Du Toit, 1968;). The *South African Dairy Training Board (a)* (1990) states that pH specifications of raw milk should fall between 6.60 and 6.75. Milk with pH-values outside this range, should be considered as unacceptable for distribution or processing.

The adulteration of milk with added water is illegal in most countries (Harding, 1983), including South Africa ("*Agricultural Products Standards Act*", 1994). The measurement of the freezing point of milk provides the most reliable means of detecting and measuring the percentage of extraneous water in milk (Harding, 1983). The legal upper limit of the South African freezing point specification is -0.512°C ("*Agricultural Products Standards Act*", 1994). This law does not indicate a lower limit. Lück (1984) and the *South African Dairy Training Board (b)* (1990) however, suggest lower limits of -0.541°C and -0.550°C, respectively.

The pH-value of milk, like other physical properties such as viscosity, density, and refractive index, is the resultant, determined by the contributing chemical milk constituents and their concentrations (Jenness & Patton, 1959). Freezing point, a colligative property of milk, is dependent on the concentration of solute molecules or ions in solution, and not on the chemical identity of the solute (Ebbing, 1993).

The factors that affect the composition of milk can be divided into two broad categories, namely physiological and environmental/managerial factors. The physiological influence is governed by heredity and non-heredity factors. Heredity factors include variation among breeds and individual variation within breeds, whereas non-hereditary factors includes amongst other, age, stage of lactation and pregnancy of the cow. Environmental factors include season, temperature, and nutritional factors (Schmidt, Van Vleck, & Hutjens, 1988; Munro, Grieve & Kitchen, 1984; Sharma, Rodriguez, Mekonnen, Wilcox, Bachman & Collier, 1983; Ng-kwai-hang, Hayes, Moxley, & Monardes, 1982; Raubertas, & Shook, 1982; Feagan, 1979; Meijering, Jaartsveld, Verstegen, & Tielen, 1978; Norman, Kuck, Cassell, & Dickinson, 1977; Thatcher, 1973).

Literature reveals that the most recent specification for the pH-value of South African milk is stated in the pH module of the *South African Dairy Training Board (a)* (1990). According to G. Venter (Training and Development Manager of Milk South Africa, personal communication, 10 March, 2003) and Dr. J. Floor (Manager Laboratory Services of Clover, personal communication, 11 August, 2003), the compilation of these pH specifications was conducted before this date. The most recent reference for the South African freezing point specification is cited in an article by Lück (1984). In conclusion, the South African standard for pH and freezing point on raw milk is based on data that is at least 13 and 19 years old, respectively.

During this period, changes may have taken place regarding the physiological and environmental factors that affect the composition of milk. If such changes had occurred, they may have affected both the pH and freezing point.

The most notable change that occurred over the years, shows that the present lactating cow, due to increased milk production, produces higher fat- and protein masses, compared to her 1980 predecessor. The higher milk production however, resulted in a decrease in both the fat- and protein percentage of raw milk (Robertson, 2000). Annual average figures obtained from the "*South African National Dairy Animal Improvement Scheme*" (2002) for registered cows, milked twice a day, showed the following: From 1975 to 2001, the average lactation yield of Holstein and Jersey breeds showed increases in milk production from 4 388 to 7 561 kg and from 3 393 to 5 185 kg, respectively. The higher milk production per lactation was accompanied by increases in fat production from 161 kg to 260 kg and from 165 kg to 230 kg for Holsteins and Jerseys, respectively. Despite this increase in fat produced, the fat percentages decreased from 3.68 to 3.45 and from 4.86 to 4.45 for the Holstein and Jersey breeds, respectively. The same trend is noticeable for protein, in which case, the kilograms produced per lactation increased from 146 to 239 and from 135 to 188, while the protein percentages decreased from 3.33 to 3.16 and from 3.96 to 3.64 for the Holstein and Jersey breeds, respectively. These long term changes in milk yield as well as fat- and protein-percentages, as illustrated in Figure 3.1 A-D, may well be accompanied by other compositional changes, which may have a significant influence on the current South African specifications for both the pH and freezing point.

The high quality standards that the South African Dairy industry is known for and lives up to are also being compromised. The consequence may well be the rejection of normal milk and vice versa.

The aim of this study is to determine the current natural pH- and freezing point- values of raw bulk herd milk. Secondly, to determine how the milk composition, the physiological and the environmental/managerial factors relate to the observed milk pH- and freezing point values. The aim is not to reinvent the wheel by investigating the factors that affect the pH and freezing point but to substantiate the findings of the thesis. For practical reasons, the scope of this study will be limited to the Western Cape and the Tsitsikama region of the Eastern Cape. The outcome of this dissertation is to ascertain if the current South African pH- and freezing point values for raw milk are still applicable or whether this topic warrants further research to establish new specifications.

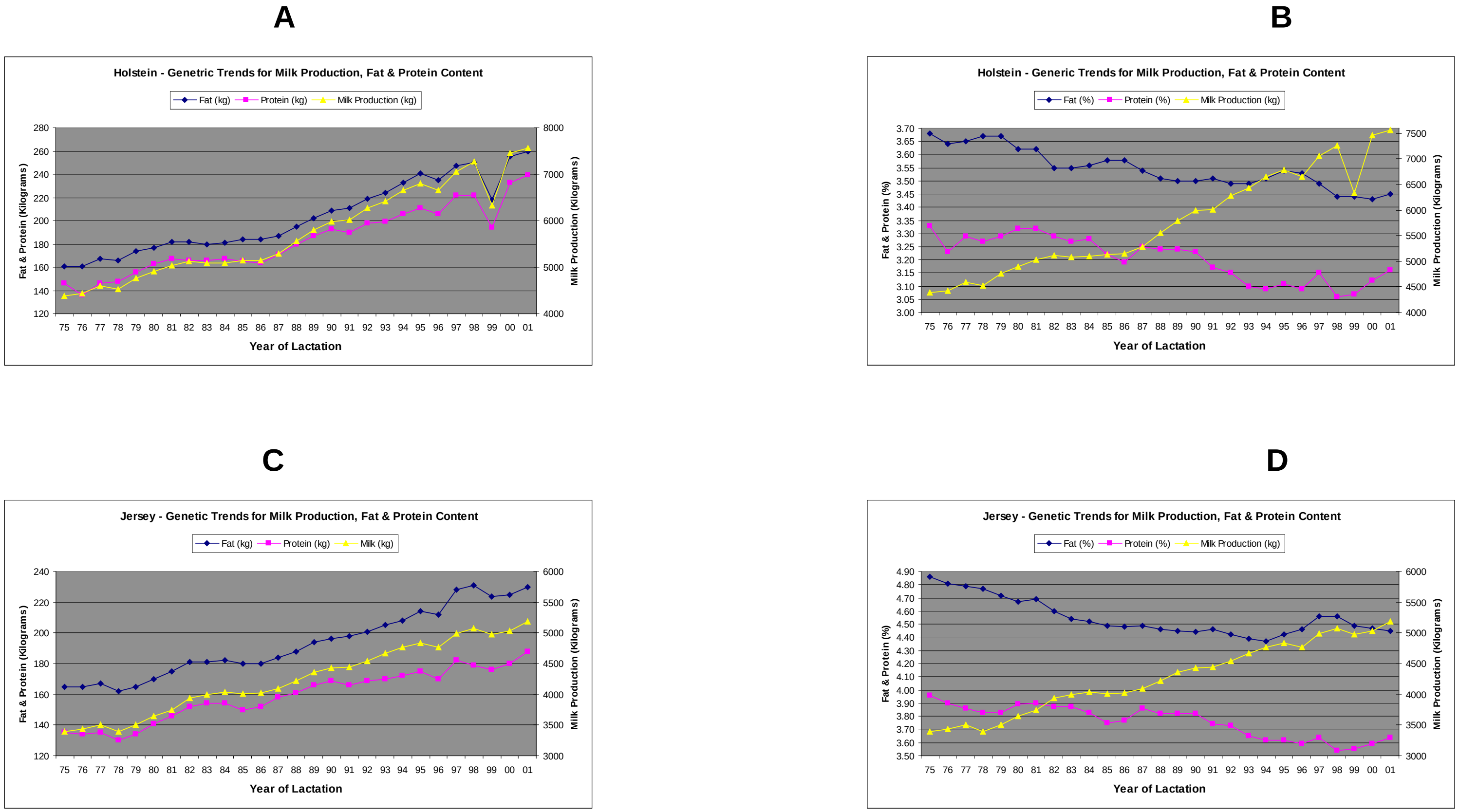

Figure 3.1 Yield and compositional changes ("*South African National Dairy Animal Improvement Scheme*", 2002)

A-Genetic trend of Holsteins for milk production, fat, and protein (kg),

B-Genetic trend of Holsteins for milk production (kg), fat, and protein (%),

C-Genetic trend of Jerseys for milk production, fat, and protein (kg),

D-Genetic trend of Jerseys for milk production (kg), fat, and protein (%).

3.2 MATERIALS AND METHODS

3.2.1 THE COLLECTION OF DATA

Five manufacturers of dairy products, operating in the Western Cape and Tsitsikama regions of South Africa, participated in this study. Data collection comprised both newly determined and historically documented analyses. Two laboratories, namely the ARC-ANPI Elsenburg Dairy Laboratory and the laboratory of a manufacturer in the Tsitsikama, participated in the analyses of the samples. All chloride, calcium, magnesium, total solids, solids-non-fat, lactose, and somatic cell count determinations were conducted at the ARC laboratory, where 9 521 samples, of which pH determinations were done on 939, were analysed for freezing point, fat, lactose and total bacterial counts. The Tsitsikama laboratory analysed 20 625 samples for pH, fat, protein and total bacterial counts. This laboratory also determined the freezing point of 18 652 of these samples. Data, at both laboratories, were collected over a five-year period, as from 1998 to 2003. Field officers of the respective manufacturers completed questionnaires, whereby information such as the breed, locality of milk farmers, feeding regime and date of milking was gathered.

3.2.2 THE EFFECT OF BREED ON pH AND FREEZING POINT

Four herd groupings according to breed, namely Jerseys, Holsteins, mixed Jersey and Holsteins and other crossbreeds, were included in the study.

3.2.3 THE EFFECT OF FEEDING REGIME ON pH AND FREEZING POINT

Three groups of feed regimes were investigated. The groupings were: mainly grazing (low energy), mainly concentrates (high energy), and a mixture of grazing and concentrates (grazing-concentrate).

3.2.4 THE EFFECT OF SEASON ON THE pH AND FREEZING POINT

The influence of the summer (September to February) and the winter (March to August) seasons were investigated.

3.2.5 THE SAMPLING OF MILK

Herd milk samples comprising of mixed morning and evening milk, were used in this study. Field officers of the respective milk manufacturers drew individual milk samples from the bulk tanks of the milk suppliers. The following method was used for sampling. The milk was stirred for at least 5 minutes before a sample was taken directly from the tank, or by means of a sterile dipper, into a sterile bottle. The sample bottles were filled approximately 80 % to capacity and were placed on ice water to maintain a temperature between 0°C and 5°C. Care was taken to prevent freezing of the samples or the floating of the bottles in the ice water. The samples were taken to the laboratory where they were tested within 36 hours of milking.

3.2.6 THE DETERMINATION OF THE pH-VALUE OF MILK SAMPLES

The pH-value of milk was determined, using a Technic T + C 1003 pH-meter and a Knick calimatic pH-meter, according to the method specified by the "*Milk Industry Foundation*" (1959).

3.2.7 THE DETERMINATION OF THE FREEZING POINT VALUE OF MILK SAMPLES

The freezing point (FP) of milk was determined, using either a Fiske MS or an Advanced Cryoscope Model 4D3, according to the method described in "*International Dairy Federation (a)*" 108B (1991).

3.2.8 THE DETERMINATION OF MILKFAT, PROTEIN, AND THE LACTOSE CONTENT IN MILK

The fat content of the milk was determined, using a N. Foss Electric, Denmark, Milkoscan 133B, according to the "*International Dairy Federation*" 141C (2000) method.

3.2.9 THE DETERMINATION OF THE TOTAL SOLIDS CONTENT IN MILK

The total solids content was calculated using the formula: TS = fat % + protein % + lactose % + ash %. A factor of 0.70 %, as suggested by Jenness and Patton (1959), was substituted for the ash content.

3.2.10 THE DETERMINATION OF THE SOLIDS-NON-FAT CONTENT IN MILK

The same formula as for calculating the total solids content, but omitting the fat percentage, was used for the calculation of the solids-non-fat content of the milk.

3.2.11 THE DETERMINATION OF THE SOMATIC CELL COUNT IN MILK

The somatic cell count of the milk was determined, using a Bently Somacount 300, according to the method described by "*Bently Instruments*" (1994).

3.2.12 THE DETERMINATION OF THE BACTERIAL COUNT IN MILK

The bacterial count on the milk was determined according to the "*International Dairy Federation (b)"* 100B (1991) method, using standard plate count agar (Oxoid CM 325).

3.2.13 THE DETERMINATION OF THE CHLORIDE CONTENT IN MILK

The chloride (Cl^-) content was determined according to the method described in "*International Dairy Federation*" 17A (1972).

3.2.14 THE DETERMINATION OF THE CALCIUM CONTENT IN MILK

The calcium (Ca^{++}) content was determined according to the method described by Robertson (1964).

3.2.15 THE DETERMINATION OF THE MAGNESIUM CONTENT IN MILK

The magnesium (Mg^{++}) content was determined according to the method described by Robertson (1964).

3.2.16 THE CLIMATIC PATTERNS IN THE REGIONS WHERE DATA WERE COLLECTED

The climatic patterns namely, average daily temperature, rainfall, humidity, radiation, wind speed, and evaporation for the study were obtained by the Agricultural Research Council Agromet, Infruitec, Stellenbosch.

3.2.17 THE STATISTICAL ANALYSES OF THE DATA

Statistical analyses, consisting of the calculation of correlation coefficients, the anova- and t-distribution tables, and regression diagrams and histograms, were done by means of the SAS program (1999), at ARC-Infruitec-Stellenbosch.

3.3 RESULTS AND DISCUSSION

3.3.1 MILK COMPOSITION

The statistical data comprising the number of observations, mean, minimum, and maximum values of the pH, freezing point, fat, protein, lactose, somatic cell count, total bacterial count, chloride, calcium and magnesium values, as found in the milk samples investigated, are listed in Table 3.1.

The average chloride value, 88.78 mg/100 mℓ, is markedly lower than the average values quoted in literature, e.g. Lück, Mostert and Smith (1974), 146.78 mg/ 100 mℓ; Webb, Johnson and Alford (1974), 119 mg/100 mℓ, and Jenness and Patton (1959), 100 mg/mℓ. It is pointed out that the relatively high value of Lück et al. (1974), is an average figure determined over the lactation periods for only 15 Holsteins, while the other values are for bulk milk. Data cited in Jenness et al. (1959) indicate that in comparison with the Brown Swiss, Holstein's milk contains a relatively high chloride content.

The average calcium level of 112.68 mg/100 mℓ is lower than the 137.50 mg/100 mℓ found by Lück et al (1974); the 132.1 mg/100mℓ by Webb et al. (1974), and the 132.1 mg/100 mℓ found by Jenness et al. (1959). Robertson (1964) analysed 351 herd milk samples, collected during winter and summer in the Western- and Southern-Cape, and found an average value of 125.9 mg/100 mℓ.

An average magnesium level of 7.71 mg/100 mℓ again is lower than the values quoted by Lück et al. (1974), 10.2 mg/100 mℓ, Webb et al. (1974), 10.8 mg/100 mℓ, Jenness et al. (1959), 10.0 mg/100 mℓ, and Robertson (1964), 13.4 mg/100 mℓ.

From the above it is clear that the chloride, calcium, and magnesium levels that were found in the samples analysed are lower than the historical values quoted in literature. A number of unknown factors may be responsible for this phenomenon.

One reason could be the increased milk production, which has already been pointed out (South African National Dairy Animal Improvement Scheme, 2002).

Table 3.1 The statistical data of the criteria investigated

	n	Median	Minimum	Maximum	SD
pH	21 564	6.72	6.60	6.84	0.04
Freezing point	28 083	-0.523	-0.545	-0.502	0.0067
Fat (%)	26 176	3.64	2.88	5.50	0.36
Protein (%)	26 865	3.18	2.52	4.33	0.19
Lactose (%)	9 456	4.78	4.40	5.10	0.11
Total Solids (%)	9 310	12.40	10.96	15.05	0.53
Solids-non-fat (%)	9 310	8.69	7.83	9.91	0.23
Somatic cell count/mℓ	14 480	371 423	3 270	1 X 10^6	190 228
Bacterial count/mℓ	17 273	41 008	1 000	30 000	67 475
Chloride (mg/100mℓ)	650	88.78	60.65	135.00	13.65
Calcium (mg/100mℓ)	481	112.68	97.92	131.20	5.08
Magnesium (mg/100mℓ)	392	7.71	3.10	14.75	2.56

3.3.2 THE pH OF THE MILK SAMPLES

Descriptive statistics for samples analysed as well as the current South African pH specification for raw milk, viz. ≥ 6.60 and ≤ 6.75, ("*South African Dairy Training Board (a)*", 1990) are listed in Table 3.2.

The number of observations (n), the mean, median, and mode were 21 654, 6.72, 6.72 and 6.70, respectively for the obtained pH-values. The lowest and highest pH-values observed were 6.60 and 6.84, respectively. The pH thus varied over a range of 0.24 pH units.

The observed pH variation of 0.24 units is wider than the range of the current pH specification, which is 0.15. The upper pH limit of 6.84 found is higher than the corresponding limit of 6.75 as stated in the specification. Although the pH difference of 0.09 units appears to be small, it must be borne in mind that pH is measured in a logarithmic scale and that the difference in actual hydrogen ion activity will be considerable.

The current pH specification, with a lower limit of 6.60, mean 6.675, and upper limit 6.75 and the observed pH-values with lower limit 6.60, mean 6.72, median 6.72, mode 6.70, and upper limit 6.84, are displayed as histograms in Figures 3.2 A & B. In Figure 3.2 C, the histograms are displayed in an overlapping manner.

Figure 3.2 C graphically illustrates that in comparison with the current specification, the range of the observed pH-values has widened and generally shifted to higher levels. The cumulative percentage distribution of pH, which is listed in Table 3.3, indicates that 14.2 % of the observed pH-values exceed the upper limit set by the current specification. Higher pH-values can result from alkaline detergent residues and mastitis. Good milking parlour management and the normal mean freezing points and somatic cell counts observed (Table 3.1), would however discourage this line of thought. It is therefore concluded that the pH-values obtained must be the result of changes in the yield and composition of milk.

The more alkaline pH, together with the higher mean (Table 3.2) found, is of such magnitude that the current specification can be regarded as suspect, and justifies the statement that it is outdated.

Table 3.2 Descriptive statistics for the obtained pH values and the South African (S.A.) specification

	Obtained values	S.A. specification
n	21 564	N L
Mean	6.72	6.675
Median	6.72	N L
Mode	6.70	N L
Range	0.24	0.15
Minimum	6.60	6.60
Maximum	6.84	6.75
Kurtosis	-0.24	N L
Skewness	-0.07	N L
Standard Deviation	0.04	N L

N L - Not Listed

Table 3.3 Cumulative percentage distribution for the obtained pH of milk

pH	Cumulative %
6.60	0.55
6.61	1.29
6.62	2.26
6.63	3.87
6.64	6.01
6.65	9.01
6.66	12.76
6.67	17.86
6.68	23.81
6.69	30.56
6.70	40.15
6.71	49.51
6.72	58.20
6.73	66.25
6.74	73.64
6.75	80.32
6.76	85.80
6.77	90.92
6.78	94.42
6.79	96.73
6.80	98.21
6.81	99.03
6.82	99.56
6.83	99.87
6.84	100.00

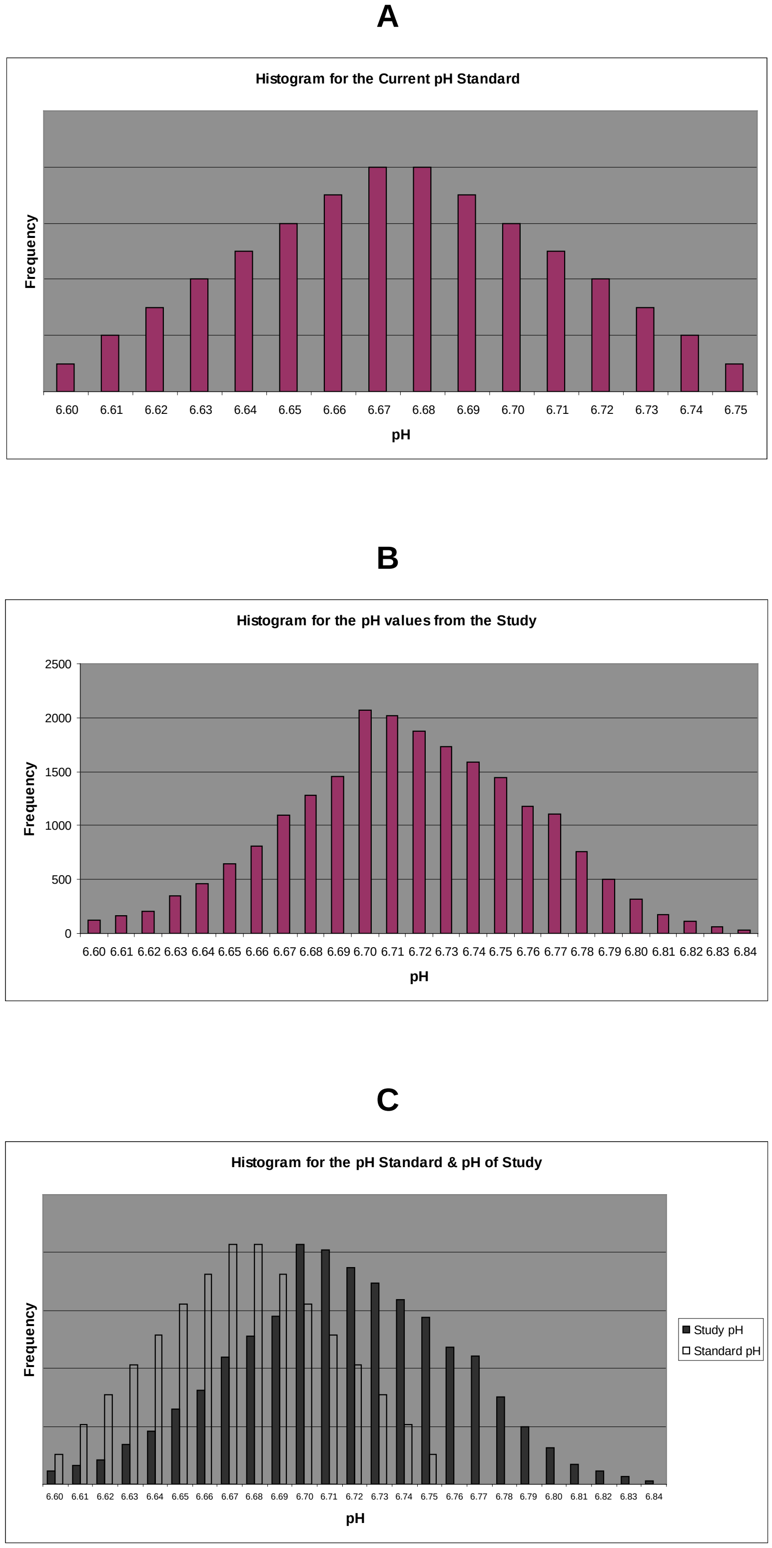

Figure 3.2 pH Histograms, A-pH histogram of current standard, B-pH histogram of the study, C-histogram of both current pH standard and pH from the study.

3.3.3 THE FREEZING POINT OF MILK

Descriptive statistics for samples analysed and the current South African freezing point specification, of ≥ -0.512°C, are listed in Table 3.4. Although no legal lower limit is specified, Lück (1984) suggested a value of -0.541°C.

For the observed freezing points, the number of observations (n), the mean, the median, and the mode were 28 803, -0.523°C, -0.522°C and -0.521°C, respectively. The lower- and upper-values observed were -0.545°C and -0.502°C, thus giving a range of 0.043°C. This range is wider than 0.029°C, obtained from the difference between the specified upper value and the lower value suggested by Lück (1984).

If it is accepted that the range of -0.512° to -0.541°C, with a mean of -0.527°C, is the current norm or specification, then Figure 3.3 A displays the histogram of the current norm. Similarly Figure 3.3 B displays the histogram for the observed freezing points, with range -0.502°C to -0.545°C, mean -0.523°C, median -0.522°C, and mode -0.521°C. In Figure 3.3 C, the data of the “accepted” current norm and that of the observed data are displayed in an overlapping manner.

From the accumulative percentage of the observed freezing points, listed in Table 3.5, it is evident that the freezing points of 1.88 % of the samples studied, are higher than the current specification, while only 1.28 % are lower than the suggested lower limit of -0.541°C. These observations are graphically illustrated in Figure 3.3 C. These small percentages of samples falling outside the “accepted” range, taking into account that the 1.88% of samples with freezing points above the legal limit may well have been adulterated with water, may not carry enough weight to support the argument that the current standard is no longer valid.

Table 3.4 Descriptive statistics for freezing point and the "accepted" South African (S. A.) specification

	Obtained Values	S. A. specification
N	28083	N L
Mean	-0.523	-0.527
Median	-0.522	N L
Mode	-0.521	N L
Range	0.043	0.029
Minimum	-0.545	-0.541
Maximum	-0.502	-0.512
Kurtosis	0.15	N L
Skewness	-0.26	N L
Standard Deviation	0.007	N L

N L – Not Listed

Table 3.5 Cumulative percentage distribution for freezing point (FP) of milk

FP (°C)	Cumulative %	FP (°C)	Cumulative %
-0.545	0.09	-0.522	55.54
-0.544	0.20	-0.521	62.53
-0.543	0.36	-0.520	69.42
-0.542	0.63	-0.519	74.95
-0.541	0.87	-0.518	79.76
-0.540	1.18	-0.517	84.10
-0.539	1.64	-0.516	87.45
-0.538	2.22	-0.515	90.34
-0.537	3.01	-0.514	93.00
-0.536	3.95	-0.513	95.07
-0.535	5.09	-0.512	97.62
-0.534	6.70	-0.511	98.12
-0.533	8.50	-0.510	98.60
-0.532	10.63	-0.509	98.92
-0.531	12.99	-0.508	99.19
-0.530	15.97	-0.507	99.40
-0.529	19.56	-0.506	99.56
-0.528	23.46	-0.505	99.74
-0.527	28.07	-0.504	99.83
-0.526	33.17	-0.503	99.90
-0.525	38.48	-0.502	100.00
-0.524	43.72	-	-

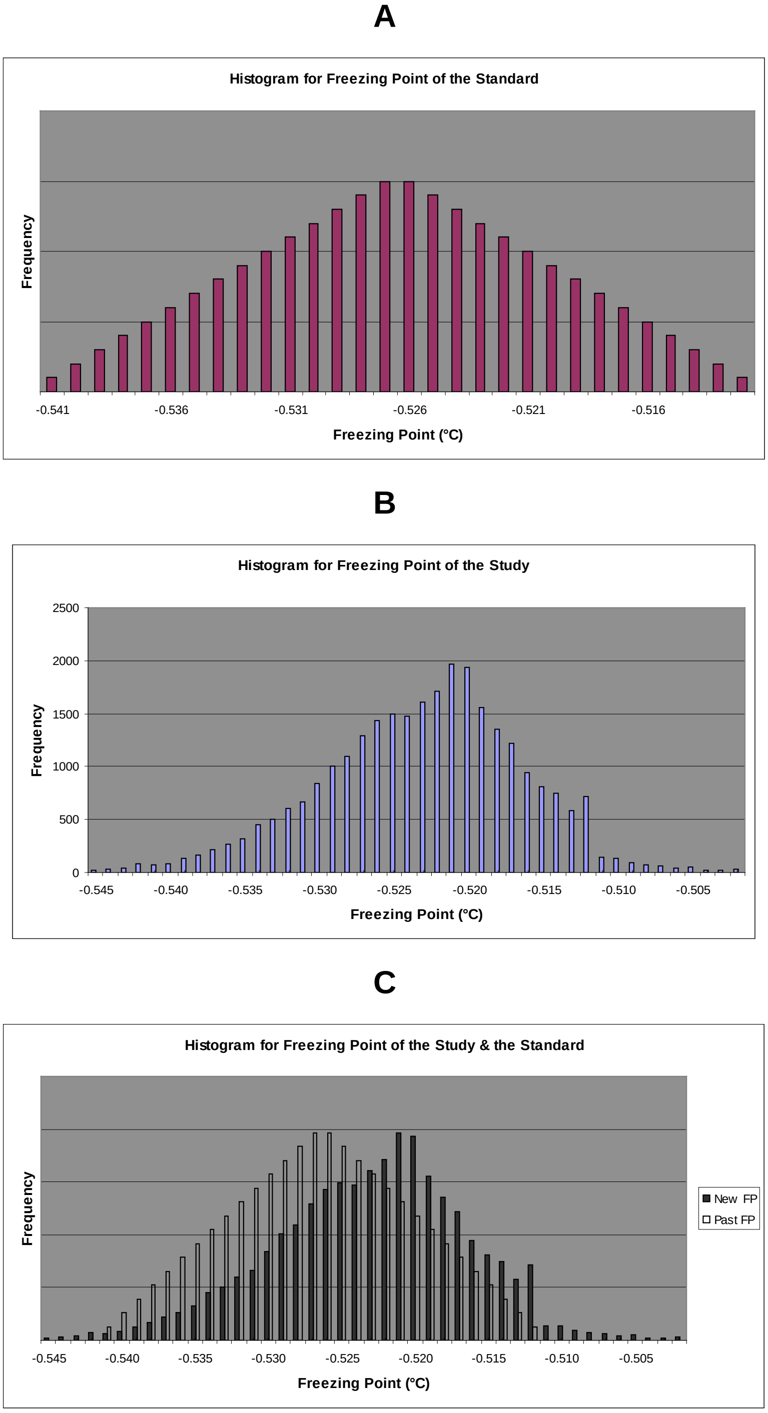

Figure 3.3 Freezing point (FP) Histograms, A-FP histogram of current standard, B-FP histogram of the study, C-histogram of both current FP standard and FP from the study.

3.3.4 STATISTICAL ANALYSES FOR THE INFLUENCE OF NUMERICAL VARIABLES ON pH

Statistical analysis, by means of scatter diagrams, regression curves, and Pearson correlation coefficients, for the influence of the individual numerical variables on the pH of milk provides more clarity regarding the observed shift in the pH of milk. The relationship between the numerical variables, fat, protein, lactose, total solids, solids-non-fat, somatic cell count, total bacterial count, chloride, calcium, magnesium, average temperature, rainfall, average humidity, wind speed, evaporation, sunshine, and radiation, and the pH of milk were investigated. Pearson correlation coefficients are listed in Table 3.6. Scatter diagrams and regression curves of fat, lactose, somatic cell count, total bacterial count, chloride, calcium, magnesium, average temperature, rainfall, average humidity, wind speed, and evaporation exercising a significant influence ($p < 0.05$) are illustrated in Figures 3.4 A-L. Protein, total solids, solids-non-fat, sunshine, and radiation did not have a significant influence.

None of the significant relationships are consistent enough to determine the pH of milk. The variables, chloride ($r = + 0.35$), magnesium ($r = + 0.21$), somatic cell count ($r = + 0.16$) and wind speed ($r = + 0.04$), correlated positively, while calcium ($r = - 0.20$), average humidity ($r = - 0.19$), average temperature ($r = - 0.15$), lactose ($r = - 0.10$), evaporation ($r = - 0.09$), fat ($r = - 0.07$), average rainfall ($r = -0.05$), and total bacterial count ($r = -0.02$) correlated negatively, with pH.

In accordance with the above, Lück and Smith (1975) analysed 215 samples, and found that chloride ($r = + 0.79$) correlated significantly ($p < 0.05$) positive whereas lactose ($r = - 0.76$) as well as calcium ($r = - 0.55$) correlated significantly negative with pH. Magnesium which, of these four solutes, in both studies showed the smallest significant correlation coefficient, correlated positively in the present study and negatively ($r = - 0.34$) in the study conducted by Lück et al., (1975).

Table 3.6 Correlation between individual variables and the pH of milk

Variables	pH		
	r	p	n
Fat (%)	-0.07	<0.0001	17 518
Protein (%)	+0.01	0.25	18 071
Lactose (%)	-0.10	0.002	891
Total Solids (%)	-0.01	0.70	872
Solids-non-fat (%)	-0.02	0.58	872
Somatic Cell Count/mℓ	+0.16	<0.0001	6 181
Bacterial Count/mℓ	-0.02	0.02	9 165
Chloride (mg/100mℓ)	+0.35	<0.0001	627
Calcium (mg/100mℓ)	-0.20	<0.0001	458
Magnesium (g/100mℓ)	+0.21	<0.0001	373
Average Temperature (°C)	-0.15	<0.0001	7 999
Rainfall (mm/day)	-0.05	<0.0001	8 313
Average Relative Humidity (%)	-0.19	<0.0001	7 517
Wind speed (m/s)	+0.04	0.0003	7 744
Evaporation mm/day	-0.09	<0.0001	7 141
Sunshine (hours/day)	+0.01	0.18	7 238
Radiation (MJ/m^2/day)	+0.07	0.13	451

r = Pearsons correlation coefficient

p = Probability

n = Number of observations

Probability < 0.05 = Significant influence on the pH and freezing point

3.3.5 STATISTICAL ANALYSES FOR THE INFLUENCE OF NUMERICAL VARIABLES ON FREEZING POINT

The influence of 17 numerical variables, namely fat, protein, lactose, total solids, solids-non-fat, somatic cell count, total bacterial count, chloride, calcium, magnesium, average temperature, rainfall, average humidity, wind speed, evaporation, sunshine, and radiation on the freezing point of milk, were statistically analysed by means of scatter diagrams, regression curves, and Pearson correlation coefficients. The Pearson correlation coefficients are listed in Table 3.7. The scatter diagrams and regression curves for significant ($p < 0.05$) relationships are illustrated in Figures 3.5A-L.

The variables, wind speed ($r = + 0.36$), radiation ($r = + 0.17$), chloride ($r = + 0.15$), average temperature ($r = + 0.12$), and sunshine ($r = + 0.06$) correlated positively, while lactose ($r = - 0.35$), solids-non-fat ($r = - 0.35$), protein ($r = - 0.28$), total solids ($r = - 0.23$), fat ($r = - 0.20$), somatic cell count ($r = - 0.19$), bacterial count ($r = - 0.14$), average humidity ($r = - 0.05$), and evaporation ($r = - 0.05$) correlated negatively, with freezing point. None of these relationships are, however, consistent enough to determine the freezing point of milk.

The data of Sato, Hankinson, Gould, and Armstrong (1956) confirmed the negative influence of the total solids and solids-non-fat on the freezing point and Pinkerton and Peters (1957) found a similar influence for lactose. In contrast with the present study, Mussenden, Hodges, and Hiley (1977) found a negative relationship between the freezing point and the chloride.

Numerous authors, Kessler (1984); Harding (1983); Dermott (1968) and Henningson (1963) stated that the freezing point of milk is almost solely dependent on the concentration of the lactose and the salts, with lactose accounting for about 55 % and chloride for about 25 % of the freezing point depression. The remaining 20 % is due to other water-soluble constituents such as calcium, potassium, magnesium, lactates, phosphates, citrates, etc. (Harding, 1983).

The positive correlation found between chloride and freezing point is in contrast with the expected negative correlation, but supports the argument by Harding (1983) and Shipe (1959), that a complementary relationship exists between lactose and chloride. It is suggested that this complementary relationship is the reason why the obtained freezing points remained within 96.84 % of the “accepted” range (-0.512°C to -0.545°C, Table 3.5), although yield and compositional changes did occur in the milk.

As indicated in Table 3.6, most of the variables investigated correlated significantly ($p < 0.05$) with the freezing point. As it is however, only the lactose and the salts, mainly chloride, which directly influences the freezing point of milk, explanations for the remaining significant correlation coefficients with freezing point is not clear. The highly significant ($p < 0.0001$) correlation of wind speed with freezing point may be explained on the hand of the correlation ($r = + 0.46$, $p < 0.0001$) found between wind speed and chloride. The reason for the relationship between wind speed and chloride is however, not clear. It is known that wind, either hot or cold, as well as the speed thereof, influences the physiology of the cow (Robertson, 1997). It is questionable whether this influence is such, that it is capable of influencing the chloride content of the milk, but justifies further investigation.

Table 3.7 Correlation between individual variables and the freezing point of milk.

Variables	Freezing point		
	r	p	n
Fat (%)	-0.20	<0.0001	24 279
Protein (%)	-0.28	<0.0001	24 884
Lactose (%)	-0.35	<0.0001	9 412
Total Solids (%)	-0.23	<0.0001	9 266
Solids-non-fat (%)	-0.35	<0.0001	9 266
Somatic Cell Count/mℓ	-0.19	<0.0001	13 960
Bacterial Count/mℓ	-0.14	<0.0001	16 375
Chloride (mg/100mℓ)	+0.15	<0.0001	620
Calcium (mg/100mℓ)	-0.05	0.26	451
Magnesium (g/100mℓ)	-0.06	0.22	367
Average Temperature (°C)	+0.12	<0.0001	13 139
Rainfall (mm/day)	+0.01	0.19	13 629
Average Relative Humidity (%)	-0.05	<0.0001	12 470
Wind speed (m/s)	+0.36	<0.0001	10 741
Evaporation mm/day	-0.05	<0.0001	12 026
Sunshine (hours/day)	+0.06	<0.0001	7 654
Radiation (MJ/m^2/day)	+0.17	<0.0001	3 109

r = Pearsons Correlation Coefficient
p = Probability
n = Number of observations
Probability < 0.05 = Significant influence on the pH and freezing point

3.3.6 STATISTICAL ANALYSES FOR THE INFLUENCE OF NON-NUMERICAL VARIABLES ON pH AND FREEZING POINT

The influence of the non-numerical variables, viz. region, season, breed, and feed, on the pH (Table 3.8) and freezing point of milk (Table 3.9) were investigated statistically by means of analyses of variance.

The statistical data indicate that the pH (Table 3.8) was significantly ($p < 0.05$) influenced by region, season, breed, and feed and the freezing point (Table 3.9) significantly by region, breed, and feed, respectively.

The influence of groupings within the individual non-numerical variables on the pH (Table 3.10) and freezing point (Table 3.11) were statistically investigated by means of t-grouping.

The pH (Table 3.10) was found to be significantly lower:

- in the Western Cape (pH = 6.71) than in the Tsitsikama (pH = 6.72),
- during summer (pH = 6.71) than in winter (pH = 6.72),
- in receding order for Jerseys (pH = 6.722), Holstein-Jerseys (pH = 6.715), Crossbreed (pH = 6.714), and Holstein (pH = 6.712),
- and for milk produced from concentrates (pH = 6.71) and mixed rations (pH = 6.71) as against grazing (pH = 6.72).

Significantly lower freezing point depressions (Table 3.11) were observed:

- in the Western Cape (-0.527°C) than in the Tsitsikama (-0.521°C),
- in increasing order for Holstein-Jerseys (-0.520°C), Crossbreed (-0.521°C), Holstein (-0.524°C), Jersey (-0.526°C), and Ayrshire (-0.533°C),
- and in milk produced from concentrates (-0.527°C) and mixed rations (-0.527°C) as against grazing (-0.521°C).

Table 3.8 Analyses of variance for the influence of Province, Season, Breed and Feed on the pH of milk.

Source of variability	df	SS	MS	F	P
Province	1	0.01607675	0.01607675	8.23	**0.0041***
Error	21562	42.10227549	0.00195261		
Corrected Total	21563	42.11835224			
Season	1	0.89667199	0.89667199	469.03	**<0.0001***
Error	21562	41.22168024	0.00191177		
Corrected Total	21563	42.11835224			
Breed	3	0.10114241	0.03371414	17.18	**<0.0001***
Error	15510	30.44026447	0.00196262		
Corrected Total	15513	30.54140688			
Feed	2	0.05447399	0.02723699	13.86	**<0.0001***
Error	15511	30.48693290	0.00196550		
Corrected Total	15513	30.54140688			

*** level of significant difference**

Df = degrees of freedom, SS = sum of squares, MS = mean square, F = variance ratio, P = significance level

Table 3.9 Analyses of variance for the influence of Province, season, breed and feed on the freezing point (°C) of milk.

Source of variability	**df**	**SS**	**MS**	**F**	**P**
Province	1	0.27918686	0.27918686	8091.33	**<0.0001***
Error	28081	0.96891925	0.00003450		
Corrected Total	28082	1.24810612			
Season	1	0.00007124	0.00007124	1.60	0.2055
Error	28081	1.24803488	0.00004444		
Corrected Total	28082	1.24810612			
Breed	4	0.09105659	0.02276415	539.29	**<0.0001***
Error	22582	0.95321606	0.00004221		
Corrected Total	22586	1.04427266			
Feed	2	0.23010830	0.11505415	3191.47	**<0.0001***
Error	22584	0.81416436	0.00003605		
Corrected Total	22586	1.04427266			

*** Level of significant difference**

Df = degrees of freedom, SS = sum of squares, MS = mean square, F = variance ratio, P = significance level

Table 3.10 t Grouping for the influence of Province, Season, Breed and Feed on the pH of milk

t grouping	Mean	n	Variable		
Province					
A	6.72	20625	**Tsitsikama**	Alpha	0.05
B	6.71	939	**Western Cape**	Error Degrees of Freedom	21562
				Error Mean Square	0.001953
				Critical Value of t	1.96007
				Least Significant Difference	0.0029
				Harmonic Mean of Cell Sizes	1796.223
Season					
A	6.72	12323	**Winter**	Alpha	0.05
B	6.71	9241	**Summer**	Error Degrees of Freedom	21562
				Error Mean Square	0.001912
				Critical Value of t	1.96007
				Least Significant Difference	0.0012
				Harmonic Mean of Cell Sizes	10561.76

Means to which the different letters were assigned differs significantly from each other and vice versa.

Table 3.10 t Grouping for the influence of Province, Season, Breed and Feed on the pH of milk (Continued)

t grouping	Mean	n	Variable		
Breed					
A	6.722	252	**Jersey**	Alpha	0.05
B	6.718	5701	**Holstein & Jersey**	Error Degrees of Freedom	15510
B C	6.714	861	**Crossbreed**	Error Mean Square	0.001963
C	6.712	8700	**Holstein**	Critical Value of t	1.96012
				Least Significant Difference	0.0045
				Harmonic Mean of Cell Sizes	738.0013
Feed					
A	6.72	14289	**Grazing**	Alpha	0.05
B	6.71	246	**Grazing & Concentrate**	Error Degrees of Freedom	15511
B	6.71	979	**Concentrate**	Error Mean Square	0.001966
				Critical Value of t	1.96012
				Least Significant Difference	0.0051
				Harmonic Mean of Cell Sizes	581.7928

Means to which the different letters were assigned differs significantly from each other and vice versa.

Table 3.11 t Grouping for the influence of Province, Season, Breed and Feed on the freezing point of milk

t grouping	**Mean**	**n**	**Variable**		
Province					
A	-0.521	18652	**Tsitsikama**	Alpha	0.05
B	-0.527	9521	**Western Cape**	Error Degrees of Freedom	28081
				Error Mean Square	0.000035
				Critical Value of t	1.96005
				Least Significant Difference	0.0001
				Harmonic Mean of Cell Sizes	12586.18
Season					
A	-0.523	11649	**Summer**	Alpha	0.05
A	-0.523	16434	**Winter**	Error Degrees of Freedom	28081
				Error Mean Square	0.000044
				Critical Value of t	1.96005
				Least Significant Difference	0.0002
				Harmonic Mean of Cell Sizes	13633.85

Means to which the different letters were assigned differs significantly from each other and vice versa.

Table 3.11 t Grouping for the influence of Province, Season, Breed and Feed on the freezing point of milk (Continued)

t grouping	**Mean**	**n**	**Variable**		
Breed					
A	-0.520	5256	**Holstein & Jersey**	Alpha	0.05
A	-0.521	758	**Crossbreed**	Error Degrees of Freedom	22582
B	-0.524	15717	**Holstein**	Error Mean Square	0.000042
C	-0.526	696	**Jersey**	Critical Value of t	1.96007
D	-0.533	160	**Ayrshire**	Least Significant Difference	0.0008
				Harmonic Mean of Cell Sizes	539.9611
Feed					
A	-0.521	13067	**Grazing**	Alpha	0.05
B	-0.527	618	**Grazing & Concentrate**	Error Degrees of Freedom	28584
B	-0.527	8902	**Concentrate**	Error Mean Square	0.000036
				Critical Value of t	1.96007
				Least Significant Difference	0.0004
				Harmonic Mean of Cell Sizes	1660.223

Means to which the different letters were assigned differs significantly from each other and vice versa.

3.4 CONCLUSIONS

The chloride-, calcium-, and magnesium-levels obtained, were lower than that of historical levels. It is concluded that this is due to the gradual increase of milk yield per cow, which has also resulted in a decrease in fat- and protein-percentages, over the past two decades.

Of the 17 numerical variables investigated, fat, lactose, somatic cell count, chloride, calcium, magnesium, average temperature, rainfall, average humidity, wind speed, and evaporation, correlated significantly with pH and fat, protein, lactose, total solids, solids-non-fat, somatic cell count, total bacterial count, chloride, average temperature, average humidity, wind speed, evaporation, sunshine and radiation with freezing point. None of these individual correlations were however, consistent enough to calculate the pH and freezing point, but it does indicate that a number of factors, either directly or indirectly, have a bearing on the pH and freezing point.

For the influence of the 4 non-numerical variables investigated, viz. region, season, breed, and feed on the pH and freezing point of milk, a significant influence was found for all variables on the pH, but only region, breed, and season influenced the freezing point.

Fourteen point two percent of the samples had pH-values above 6.75, and none below 6.60, which are the upper and lower limits of the South African specifications, respectively. This variation is of such magnitude that the current specification can be regarded as suspect, and thus justifies the statement that the current specification is outdated.

Since a small percentage, 1.88 %, of the samples studied gave freezing points higher than the current specification, being ≤ -0.512°C, ("*Agricultural Products Standards Act*", 1994), it would thus appear that the freezing point specification is still valid. As the samples were drawn from bulk tanks, unintentional contamination with water residues from, e.g. the milk machine, may have contributed to the higher freezing points of these samples. The freezing point of an even smaller percentage, 1.28 %, of the samples was found to be below the lower limit of ≥ –0.541°C suggested by Lück (1984).

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3.3.6 STATISTICAL ANALYSES FOR THE INFLUENCE OF NON-NUMERICAL VARIABLES ON pH AND FREEZING POINT

The influence of the non-numerical variables, viz. region, season, breed, and feed, on the pH (Table 3.8) and freezing point of milk (Table 3.9) were investigated statistically by means of analyses of variance. The statistical data indicates that the pH (Table 3.8) was significantly ($p < 0.05$) influenced by region, season, breed, and feed and the freezing point (Table 3.9) significantly by region, breed, and feed, respectively.

The influence of groupings within the individual non-numerical variables on the pH (Table 3.10) and freezing point (Table 3.11) were statistically investigated by means of t-grouping.

The pH (Table 3.10) was found to be significantly lower:

- in the Western Cape (pH = 6.71) than in the Tsitsikama (pH = 6.72),
- during summer (pH = 6.71) than in winter (pH = 6.72),
- in receding order for Jerseys (pH = 6.722), Holstein-Jerseys (pH = 6.715), Crossbreed (pH = 6.714), and Holstein (pH = 6.712),
- and for milk produced from concentrates (pH = 6.71) and mixed rations (pH = 6.71) as against grazing (pH = 6.72).

Significantly lower freezing point depressions (Table 3.11) were observed:

- in the Western Cape (-0.527°C) than in the Tsitsikama (-0.521°C),
- in increasing order for Holstein-Jerseys (-0.520°C), Crossbreed (-0.521°C), Holstein (-0.524°C), Jersey (-0.526°C), and Ayrshire (-0.533°C),
- and in milk produced from concentrates (-0.527°C) and mixed rations (-0.527°C) as against grazing (-0.521°C).

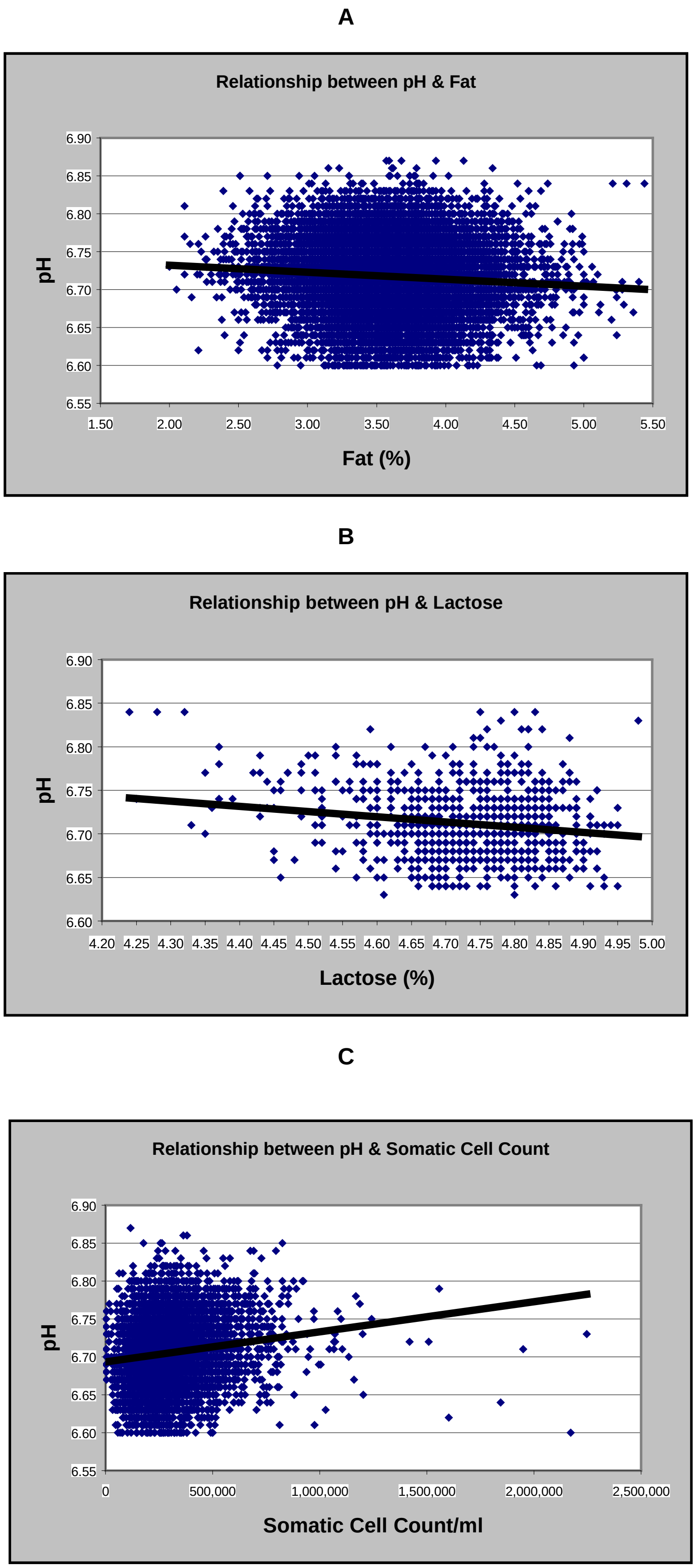


Figure 3.4 Scatter diagrams and regression curves for relationship between pH & individual numerical variables

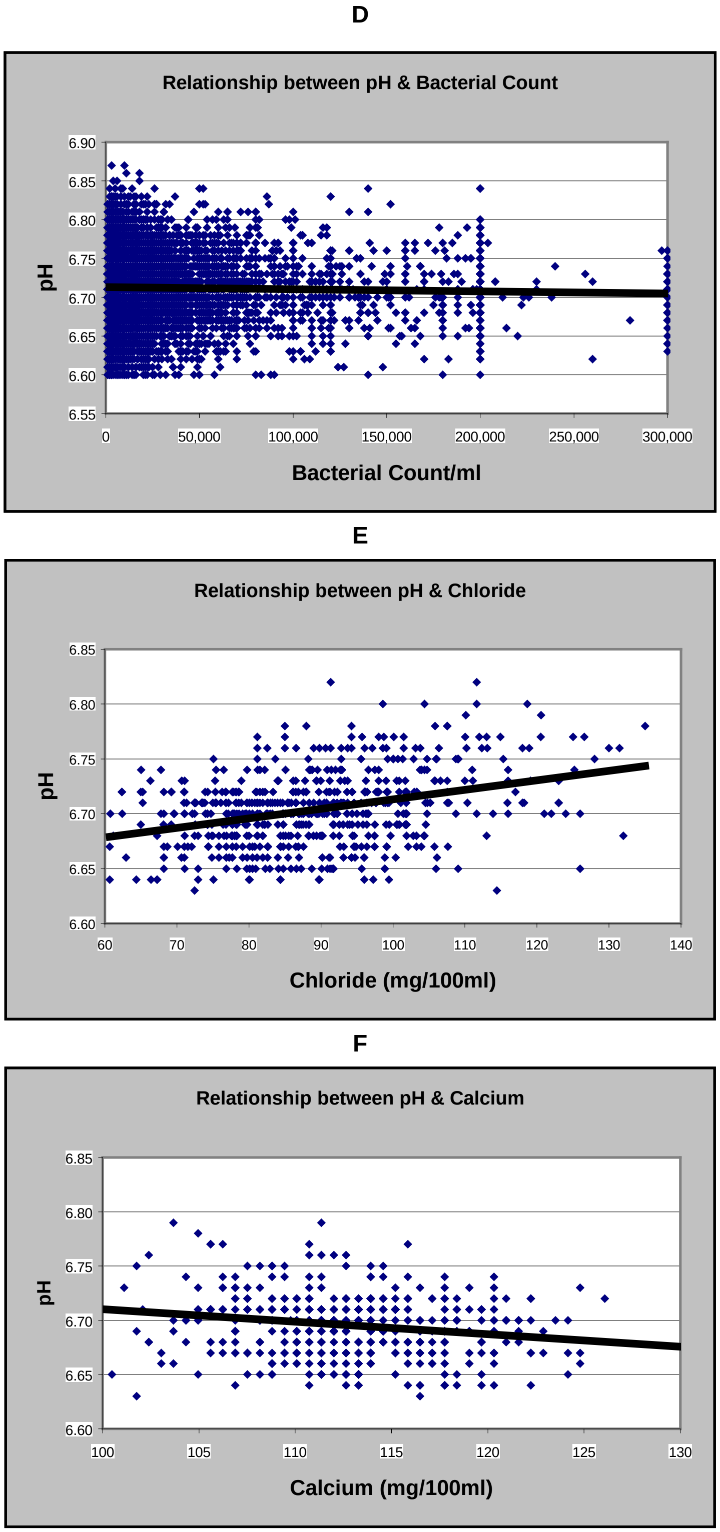


Figure 3.4 Scatter diagrams and regression curves for relationship between pH & individual numerical variables continues

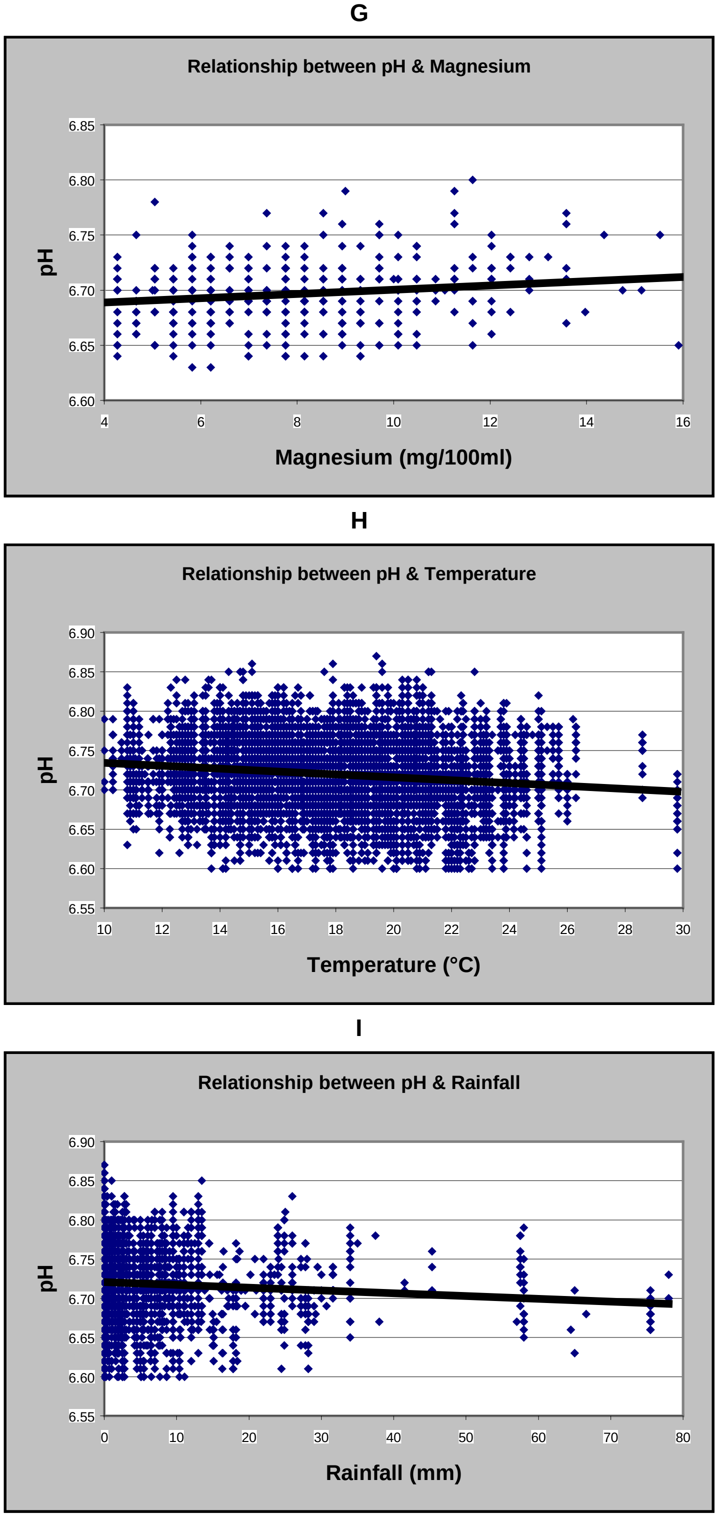
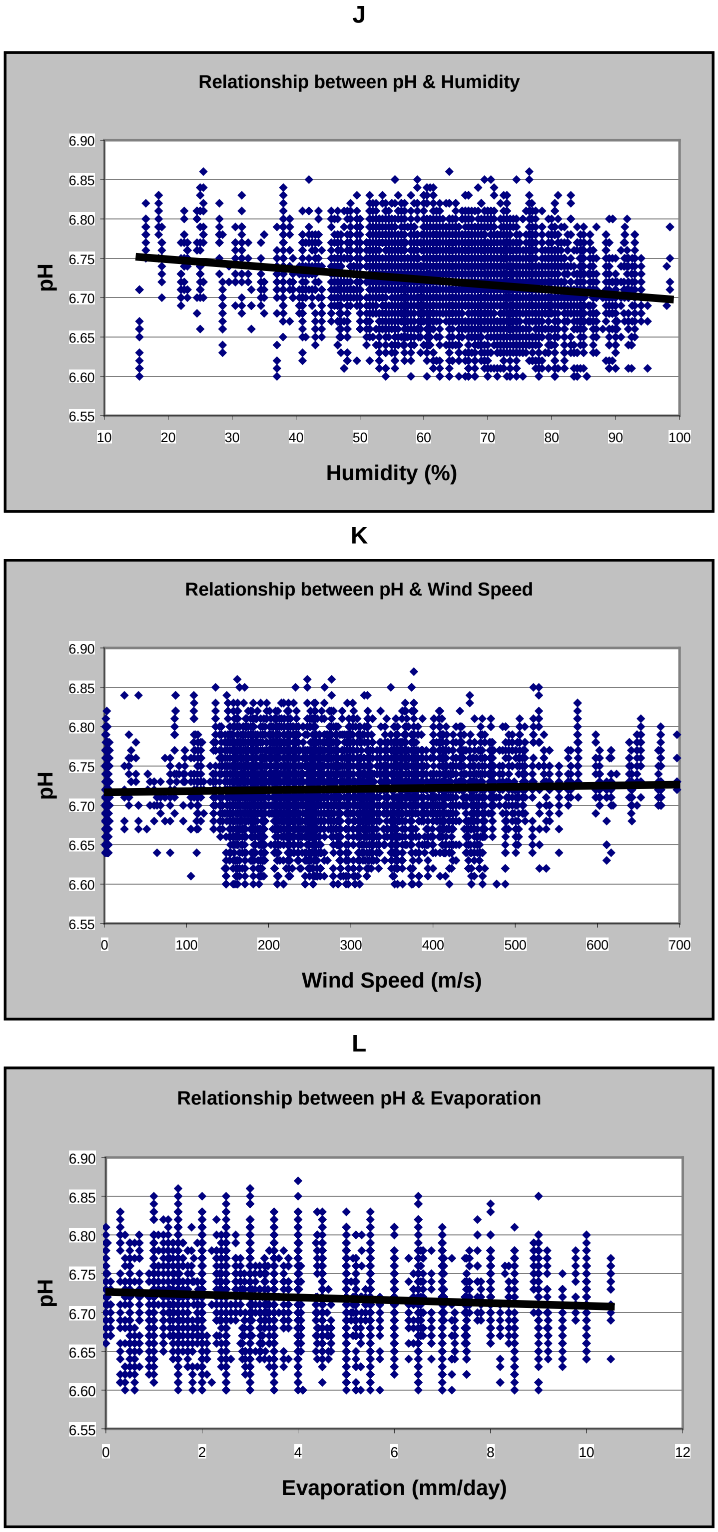


Figure 3.4 Scatter diagrams and regression curves for relationship between pH & individual numerical variables continues



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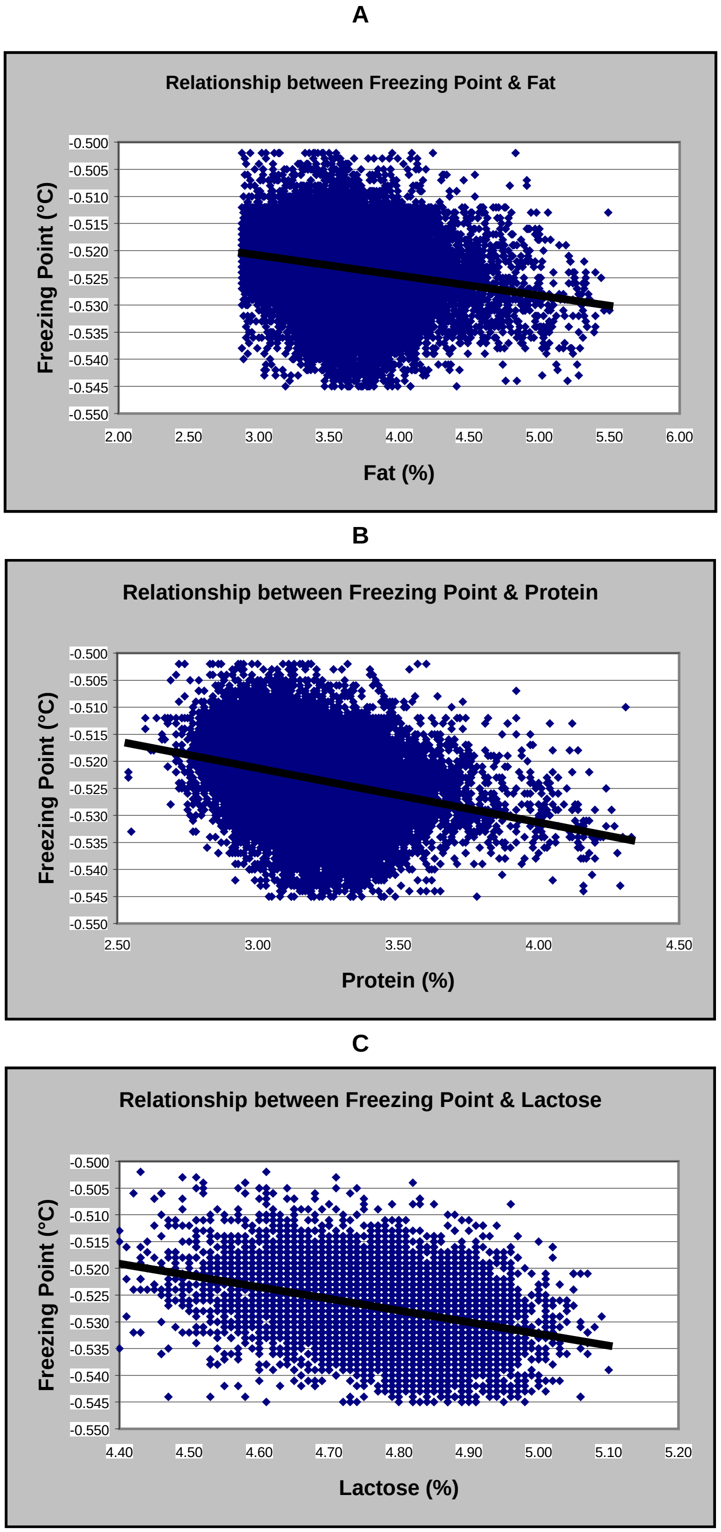


A

Relationship between Freezing Point & Fat

B

Relationship between Freezing Point & Protein

C

Relationship between Freezing Point & Lactose

Figure 3.5 Scatter diagrams & regression curves for the Relationship between freezing point & individual numerical variables

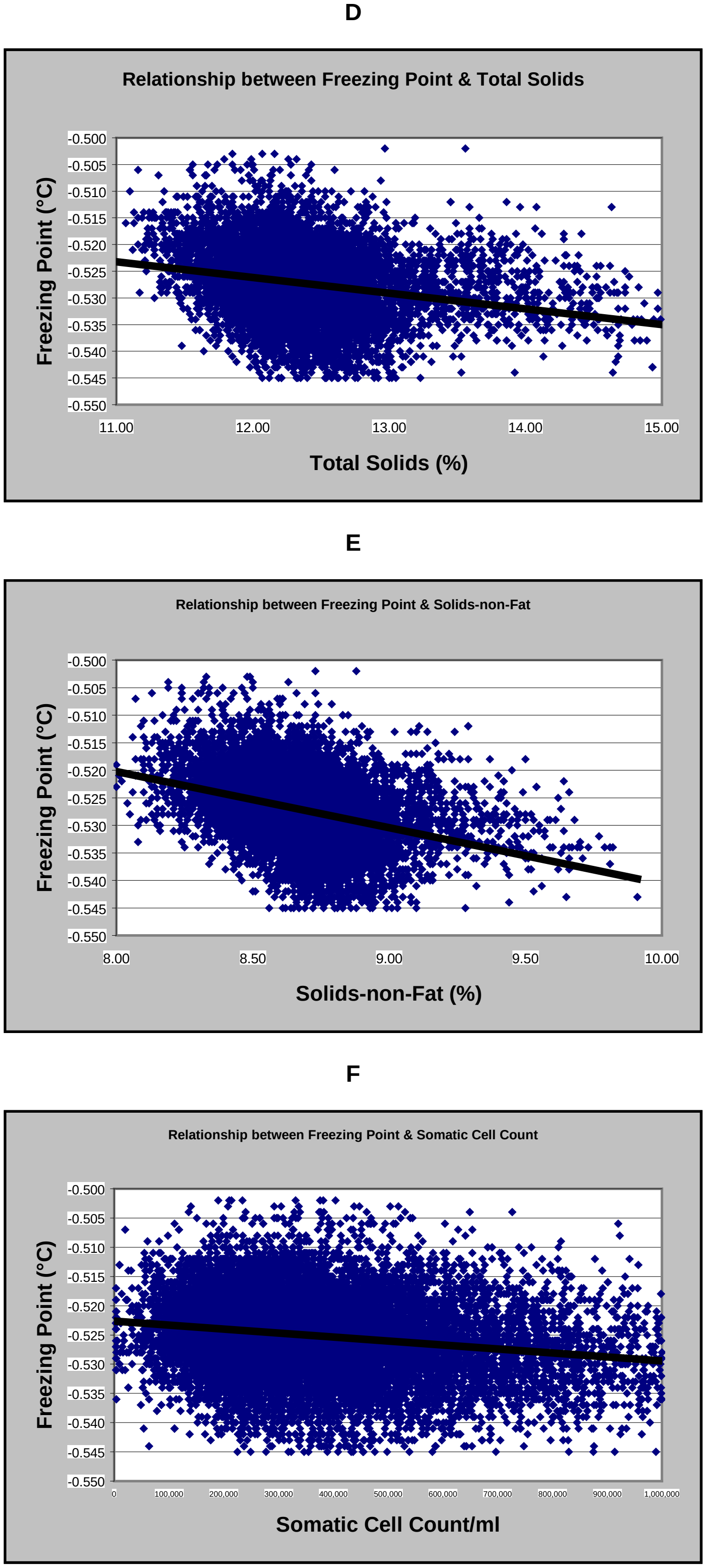
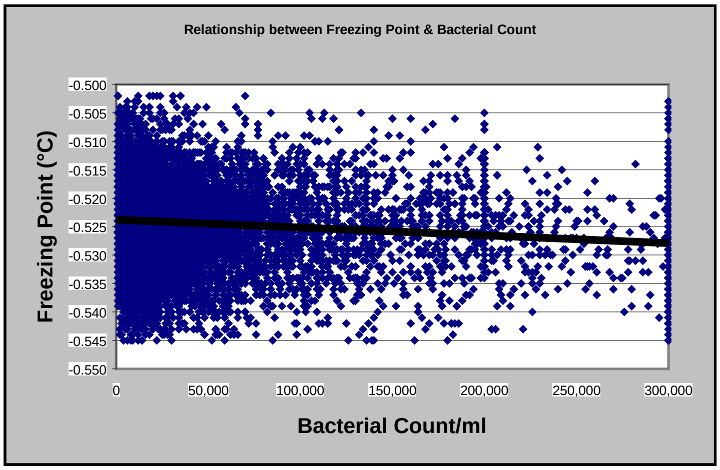
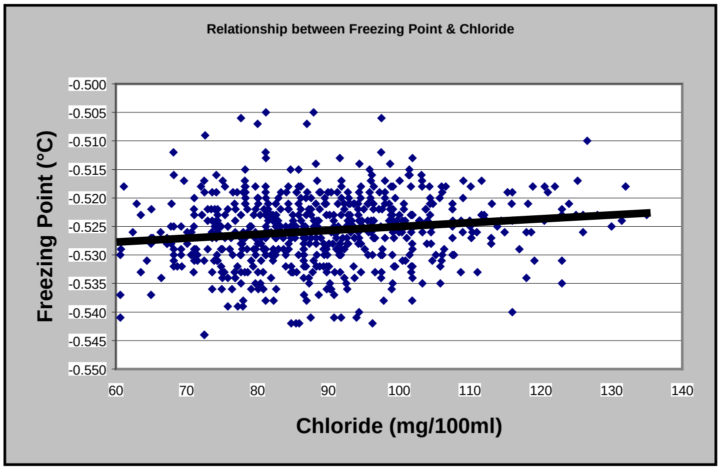


Figure 3.5 Scatter diagrams & regression curves for the Relationship between freezing point & individual numerical variables continues

G



H



I

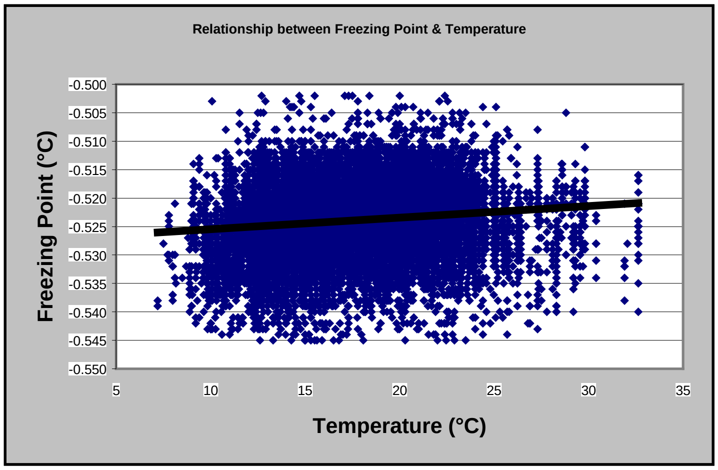
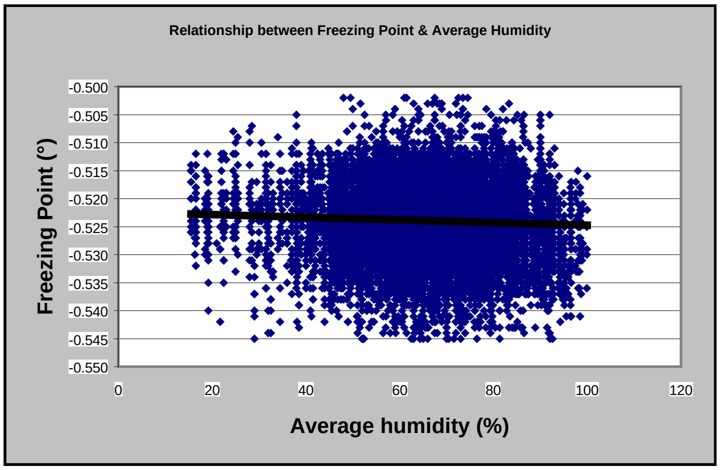


Figure 3.5 Scatter diagrams & regression curves for the Relationship between freezing point & individual numerical variables continues

J



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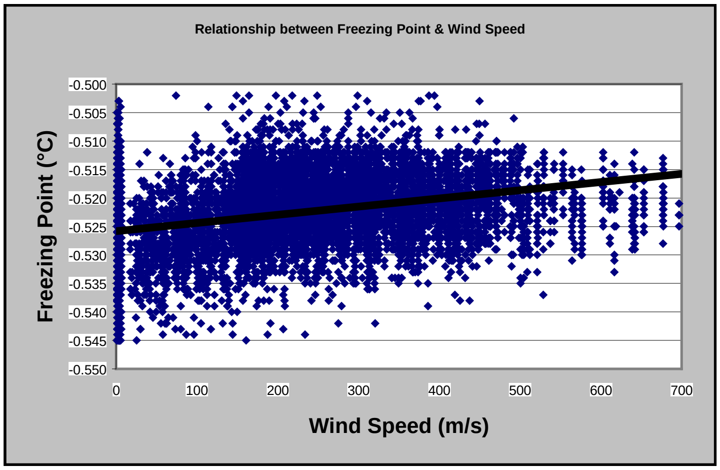

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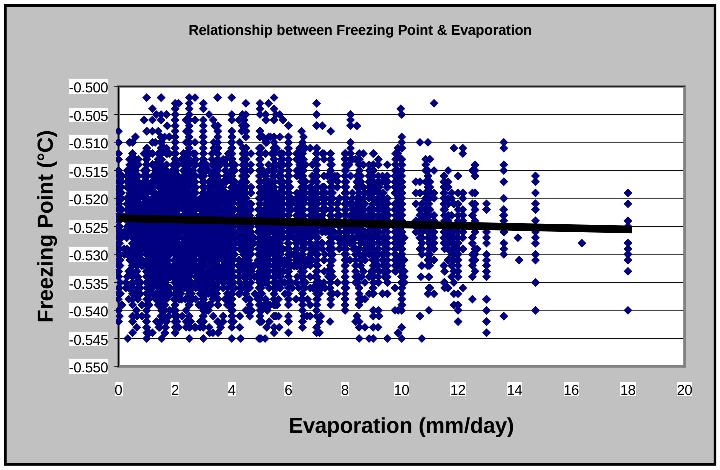
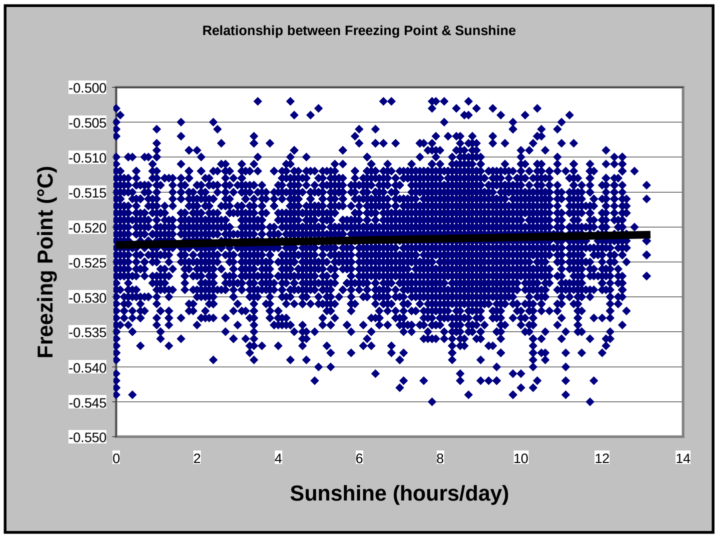


Figure 3.5 Scatter diagrams & regression curves for the Relationship between freezing point & individual numerical variables continues

M



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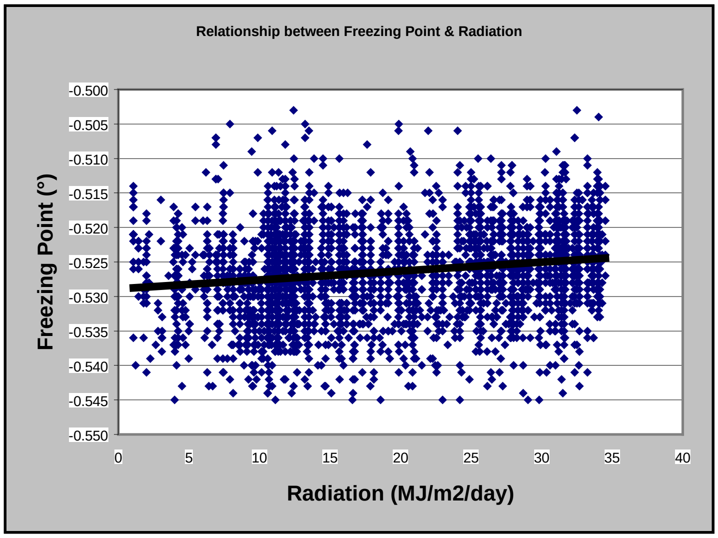

Figure 3.5 Scatter diagrams & regression curves for the Relationship between freezing point & individual numerical variables continues

CHAPTER 4

GENERAL CONCLUSIONS AND DISCUSSION

This study was conducted to determine if the current pH- and freezing-point specifications, in view of the changes that occurred in milk yield and composition (Robertson, 2000; "*South African National Dairy Animal Improvement Scheme*", 2002), are still applicable. As both pH and freezing point are influenced by various factors, the influence of 17 numerical- and 4 non-numerical-variables on these two properties of milk, were statistically investigated.

The mean pH of the samples was found to be 6.72, and range from 6.60 to 6.84. As the pH-values of 14.2 % of the samples were above the accepted upper limit of 6.75 ("*South African Dairy Training Board*," 1990), it would appear that the hypothesis, that the pH specification might be outdated, at least holds true, as regards the upper pH limit.

Although the variables, chloride ($r = + 0.35$), magnesium ($r = + 0.21$), somatic cell count ($r = + 0.16$) and wind speed ($r = + 0.04$), correlated significantly ($p < 0.05$) positively, while calcium ($r = - 0.20$), average humidity ($r = - 0.19$), average temperature ($r = - 0.15$), lactose ($r = - 0.10$), evaporation ($r = - 0.09$), fat ($r = - 0.07$), average rainfall ($r = -0.05$), and total bacterial count ($r = -0.02$) correlated negatively, with pH, none of these individual relationships are however consistent enough to determine the pH of milk. It does however indicate that a large number of numerical factors, either directly or indirectly, have a bearing on the pH of milk.

It was further found that the pH was significantly lower in the Western Cape (pH = 6.71) than in the Tsitsikama (pH = 6.72), during summer (pH = 6.71) than in winter (pH = 6.72), in receding order for Jerseys (pH = 6.722), Holstein-Jerseys (pH = 6.715), Crossbreed (pH = 6.714), and Holstein (pH = 6.712), and for milk produced from concentrates (pH = 6.71) and mixed rations (pH = 6.71) as against grazing (pH = 6.72). The influence of these on pH is certainly indirectly through their influence on the physiology of the cow.

Freezing points obtained had a mean, median, and mode of -0.523°C, -0.522°C, and -0.521°C, respectively, with a range from -0.502°C to -0.545°C. It appears that the freezing point specification is still however, valid because only a small percentage, 1.88 %, of the samples studied gave freezing points higher than the current specification, being ≤ -0.512°C, ("*Agricultural Products Standards Act*", 1994). As the samples were drawn from bulk tanks, unintentional contamination with water residues from, e.g. the milk machine, may have contributed to the higher freezing points of these samples. The freezing point of an even smaller percentage, 1.28 %, of samples was found to be below the lower limit suggested by Lück, (1984), being ≥ –0.541°C. Attention is been called to the fact, that this lower freezing point value is not specified by the "*Agricultural Products Standards Act*", (1994)

The variables, wind speed ($r = + 0.36$), radiation ($r = + 0.17$), chloride ($r = + 0.15$), average temperature ($r = + 0.12$), and sunshine ($r = + 0.06$) correlated significantly ($p < 0.05$) positively, while lactose ($r = - 0.35$), solids-non-fat ($r = - 0.35$), protein ($r = - 0.28$), total solids ($r = - 0.23$), fat ($r = - 0.20$), somatic cell count ($r = - 0.19$), total bacterial count ($r = - 0.14$), average humidity ($r = - 0.05$), and evaporation ($r = - 0.05$) correlated negatively, with freezing point. Although none of these correlations are consistent enough to determine the freezing point of milk, it indicates, as in the case of pH, a number of numerical variables, either directly or indirectly, also influence the freezing point of milk.

Significant lower freezing-point-depressions, were observed in the Western Cape (-0.527°C) than in the Tsitsikama (-0.521°C), in increasing order for Holstein-Jerseys (-0.520°C), Crossbreed (-0.521°C), Holstein (-0.524°C), Jersey (-0.526°C), and Ayrshire (-0.533°C), and in milk produced from concentrates (-0.527°C) and mixed rations (-0.527°C) as against grazing (-0.521°C). The influence of these variables on freezing point, as in the case of pH, is surely due to their indirect influence on the physiology of the cow

It is concluded that based on the data analysed, the pH of milk is less acidic, than is generally indicated in literature. To prevent rejection of normal milk, it is suggested that further investigation may be justified. If such a study is undertaken the following should be considered;

- Representative samples should be gathered proportionally as wide as possible from the South African milk shed.
- The inclusion of sodium, potassium, phosphorus, sulphur, carbonate and citrate determination, in the investigation.
- Inclusion of seasonal calving

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CHAPTER 5

SUMMARY

The milk yield per cow has gradually increased over the past two decades, and resulted in compositional changes of the milk that altered the physical properties of milk.

pH a physical property, which is used as a rejection test on the milk receiving platform, is a resultant of the milk constituents and their concentrations. The current South African specification states that the pH of raw milk should fall within the limits, 6.60 and 6.75.

Freezing point, a colligative property of milk, is depended on the concentration of solute ions in solution and not on the chemical identity of the solute. The measurement of the freezing point of milk provides the most reliable means of detecting and measuring extraneous water in milk. The current “accepted” South African specification states that the freezing point of raw milk should fall between -0.512 °C and -0.545°C.

The specifications for pH and freezing point are at least 13 and 19 years old, respectively, and may be outdated, resulting in the rejection of acceptable milk or vice versa. The purpose of this study was therefore to determine if these specifications are still applicable or whether this topic warrants further research to establish new specifications.

Seventeen variables as determined in 21 564 and 28 083 herd milk samples, originating in the Western Cape and the Tsitsikama regions of the Eastern cape of South Africa, were correlated with the pH- and freezing point values of raw cows' milk, respectively.

The pH-values obtained showed the following statistically characteristics, namely: mean (6.72), median (6.72), mode (6.70), and range (6.60 to 6.84).

The corresponding statistics of the freezing point values were: mean (-0.523°C), median (-0.522°C), mode (-0.521°C), and range (-0.545 to -0.502°C).

pH was significantly ($p < 0.05$) influenced by chloride ($r = + 0.35$), magnesium ($r = + 0.21$), calcium ($r = - 0.20$), average humidity ($r = - 0.19$), somatic cell count ($r = + 0.16$), average temperature ($r = - 0.15$), lactose ($r = - 0.10$), evaporation ($r = - 0.09$), fat ($r = - 0.07$), average rainfall ($r = - 0.05$), wind speed ($r = + 0.04$) and

total bacterial count ($r = -0.02$), whereas protein, total solids, solids-non-fat, sunshine, and radiation showed no significant ($p < 0.05$) influence.

Freezing point was significantly ($p = 0.05$) influenced by wind speed ($r = + 0.36$), lactose ($r = + 0.35$), solids-non-fat ($r = - 0.35$), protein ($r = - 0.28$), total solids ($r = - 0.23$), fat ($r = - 0.20$), somatic cell count ($r = - 0.19$), radiation ($r = + 0.17$), chloride ($r = + 0.15$), total bacterial count ($r = - 0.14$), average temperature ($r = + 0.12$), sunshine ($r = + 0.06$), evaporation ($r = - 0.05$) and average humidity ($r = - 0.05$), whereas calcium, magnesium, and rainfall showed no significant ($p < 0.05$) influence.

Statistical analyses indicate that the pH was significantly ($p < 0.05$) influenced by region, season, breed, and feed and the freezing point by region, breed, feed, and not by season.

The findings of this study justify the hypothesis that the pH specification, being ≥ 6.60 and ≤ 6.75, is outdated, because 14.2% of samples fell outside the upper limit. It would therefore appear that the freezing point standard is still valid because a small percentage, 1.88 %, of the samples studied, gave freezing points higher than the current specification, being ≤ -0.512°C. As samples drawn from bulk tanks might contain water residues, due to unintentional contamination, it may have contributed to the higher freezing points of these samples. The freezing point of an even smaller percentage, 1.28 %, of samples were found to be below the suggested lower limit.

KEY WORDS

pH, freezing point, physical property, colligative property, specification.

OPSOMMING

Melkopbrengs per koei, as gevolg van beter genetika, voeding en bestuur, neem voortdurend toe. Produksietoenames gedurende die afgelope twee dekades was sodanige dat moontlike gepaadgaande verandering in melksamestelling die fisiese eienskappe van die melk kon beinvloed.

pH is ‘n fisiese eienskap van melk wat deur die aanwesigheid en konsentrasie van verskeie melkbestandele daargestel word en word as ‘n aanvaardingstoets benut.

Die huidige Suid-Afrikaanse pH-spesifikasie vir roumelk stel ‘n minimum en maksimum waarde van 6.60 en 6.75 onderskeidelik.

Die vriespunt van melk word bepaal deur die gesamentlike konsentrasie van die molekule of ione en nie deur die chemise identiteit van die opgelosde stowwe nie. Die vriespuntsbepaling van melk verskaf is die mees betroubaarste metode vir die opsporing en meting van bygevoegde water in melk. Die huidige “aanvaarbare” Suid-Afrikaanse vriespuntspesifikasie vir roumelk is maksimum, -0.512°C, en minimum, -0.545°C.

Die spesifikasies vir pH en vriespunt is onderskeidelik ten minste 13 en 19 jaar oud en mag dus as gevolg van tussentydse veranderinge in melksamestelling verouderd wees. Indien wel, kan dit tot gevolg hê dat aanvaarbare melk afgekeur word en andersom.

Die doel van hierdie studie was om te bepaal of die pH- en vriespuntspesifikasies nog geldig is of aanpassing daarvan deur verdere studie ondersoek moet word.

Sewentien veranderlikes, soos bepaal van 21 564 en 28 083 kuddemelkmonsters, afkomstig vanaf die Wes-kaap- en die Tsitsikama-streke van Suid-Afrika, is afsonderlik met die pH en vriespunt gekorrelleer.

Die pH-waardes het die volgende statistiese eienskappe getoon, nl: gemiddelde (6.72), mediaan (6.72), modus (6.70), en verspreiding (6.60 tot 6.84).

Die ooreenstemmende statistiek vir die vriespunte was as volg: gemiddelde (-0.523°C), mediaan (-0.522°C), modus (-0.521°C), en verspreiding (-0.545°C tot -0.502°C).

pH was betekenisvol ($p < 0.05$) beïnvloed deur chloried (r = + 0.35), magnesium (r = + 0.21), kalsium (r = - 0.20), gemiddelde humiditeit (r = - 0.19), somatiese seltelling (r = + 0.16), gemiddelde temperatuur (r = - 0.15), laktose (r = - 0.10), verdamping ($r = -0.09$), vet ($r = -0.07$), reënval ($r = -0.05$), windspoed ($r = +0.04$) en bakteriese-telling ($r = -0.02$), terwyl proteien, totale vastestof, vetvrye vastestofinhoud, sonskyn, en bestraling geen betekenisvolle ($p < 0.05$) invloed getoon het nie.

Vriespunt was betekenisvol ($p < 0.05$) beïnvloed deur die windspoed ($r = +0.36$), laktose ($r = +0.35$), vetvrye vastestofinhoud ($r = -0.35$), proteïen ($r = -0.28$), totale vastestof ($r = -0.23$), vet ($r = -0.20$), somatiese seltelling ($r = -0.19$), bestraling ($r = +0.17$), chloried ($r = +0.15$), bakteriese-telling ($r = -0.14$), gemiddelde temperatuur ($r = +0.12$), sonskyn ($r = +0.06$), verdamping ($r = -0.05$) en gemiddelde humiditeit ($r = -0.05$), terwyl kalsium, magnesium, en reënval geen betekenisvolle ($p < 0.05$) invloed getoon het nie.

Statistiese ontledings toon dat die pH betekenisvol ($p < 0.05$) beïnvloed deur streek, seisoen, ras en voer en die vriespunt slegs deur die streek, ras en voer beïnvloed is.

In die studie is gevind dat die pH in die geval van 14.2 % van die monsters hoër as die gespesifiseerde maksimum van 6.75 was. Die hipotese, dat die pH spesifikasies, nl., ≥ 6.60 and ≤ 6.75, dus verouderd is, word hierdeur bevestig. In die geval van die vriespunt, het slegs 1.88 % van die waardes die maksimum gespesifiseerde vriespunt van –0.512°C oorskrei. Hierdie persentasie moet beoordel word in die lig daarvan dat 'n gedeelte van die monsters moontlik tydens produksie onopsetlik met water residu's besoedel is. Slegs in 1.28 % van die monster is vriespunte van laer as –0.545°C gevind. Dit word dus afgelei dat die huidige vriespuntspesifikasie, ≤ -0.512°C en ≥ -0.545°C, nog geldig is.

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pH, vriespunt, fisiese eienskap, saambindende eienskap, spesifikasie.