EVALUATION OF HYGIENE AND CHEMICAL COMPOSITION OF MILK FROM SALE POINTS IN KHARTOUM STATE

BY:

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Dedication

To my family……
With love
Rihab

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ABSTRACT

This study was carried out in Khartoum State to evaluate the hygiene and quality of milk supplied to consumers which come from various areas in and near by Khartoum State. Milk was collected from sales points in Khartoum, Khartoum North and Omdurman. The collection of samples was during winter and summer season. Total of 143 samples raw milk samples were collected, 33 samples during summer season and 110 samples during winter season. Fifty nine samples were collected from Khartoum, 29 samples from Khartoum North and 55 samples from Omdurman. From each area samples were collected in the early morning.

Milk samples were examined to determine the composition (fat, protein, ash, total solids, acidity and freezing point) and total bacterial count as affected by season of the year and area of study. The presence of brucella, clot on boiling and alcohol test were also carried out.

The results indicated that there were significant ($P< 0.05$) differences in fat percent between the different seasons (4.33± 0.545% in winter and 4.11± 0.450% in summer). Similarly there was significant ($P< 0.001$) increase in protein, ash and acidity between the different seasons, 3.29± 0.319%, 0.62± 0.111% and 0.21± 0.030%, respectively in winter and 2.92± 0.400%,0.53± 0.106% and 0.23± 0.036%, respectively in summer season. Total solids and freezing point were 13.18±

0.827% and -0.53± 0.031° C, respectively in winter and 12.70± 0.942% and -0.50± 0.095° C, respectively in summer season. Those values were significantly different (P< 0.01). Similarly highly significant differences (P< 0.001) were obtained for total bacterial count between the two seasons (8.3×10^{7}± 1.071 cfu/ml in winter and 2.75×10^{16}± 1.702 cfu/ml in summer).

High percent of protein (3.29± 0.319%), fat (4.33± 0.545%), ash (0.62± 00.111%) and total solids (13.1± 0.827%) were found in winter season.However, the percentage of acidity (0.21± 0.030%) and total bacterial count (2.75×10^{16}± 1.702 cfu/ml) were high in summer. Milk samples tested for alcohol test in summer season were positives (81.8%), while only 58.8% of the milk samples tested in winter season were positive. During summer season 36.4% of milk samples tested for clot on boiling test were positive, while only 15% of the milk samples were positive in winter season.

When comparing the results of the three areas studied, significant differences were obtained for protein (P< 0.001), which was high in milk samples collected from Khartoum North (3.41± 0.252%) followed by samples collected from Omdurman (3.32± 0.346%) and Khartoum (2.99± 0.341%). Significant differences were also obtained for milk acidity and fat

(P< 0.01). Low acidity were found in Khartoum North (0.19± 0.163%) followed by Khartoum (0.22± 0.039%) and Omdurman (0.22± 0.034%) milk. High fat were reported in Khartoum (4.40± 0.341%) followed by Omdurman (4.28± 0.494%) and Khartoum North (4.01± 0.390%) milk. However, non-significant differences were found for total solids, ash and freezing point (P> 0.05). The highest (P< 0.01) total bacterial count of the collected milk was found in Khartoum ($5.13\times 10^{10}\pm 5.146$ cfu/ml) followed by Omdurman ($1.05\times 10^{10} \pm 2.591$ cfu/mml) than Khartoum North ($9.3\times 10^{7}\pm 0.668$ cfu/ml). Milk samples when tested for alcohol test in Omdurman presented 72.7% positive, followed by Khartoum (69%) and Khartoum North (44.8). However, milk samples tested for clot– on– boiling test in Omdurman showed 29.1% positive, followed by Khartoum (24.1%) and Khartoum North (3.4%).

In Khartoum area non- significant differences were found (P> 0.05) for total solids and fat percent, while significant differences were observed for acidity (P< 0.05) ash and total bacterial count (P< 0.01). In Khartoum North area there were no significant differences in total solids, protein, fat and ash, while the freezing point and total bacterial count (P< 0.001) and acidity (P< 0.01) were highly differed. In Omdurman there

were no significant differences in total solids, protein, fat and freezing point, but there were significant differences in ash ($P< 0.05$) acidity and total bacterial count ($P< 0.001$).

The comparison between sales points showed that there were no significant differences in total solids, while there were highly significant differences in proteins, acidity and total bacterial count ($P< 0.001$) ash and fat ($P< 0.01$) and freezing point ($P< 0.05$).

The results of Brucella in Khartoum State for bulk milk were 77.3% from 110 samples tested. High incidence was found in Khartoum North (82.8%), followed by Khartoum (76.9%) where low incidence was reported in Omdurman (73.8%).

بسم الله الرحمن الرحيم

ملخص الأطروحة

أجريت هذه الدراسة بغرض اختبار مدي جودة وصحة اللبن الطازج المعروض للمستهلك في المدن الرئيسية الثلاث بولاية الخرطوم (الخرطوم ، أم درمان والخرطوم بحري) خلال فصلي الصيف والشتاء. وقد استخدمت طريقة التحليل الكيميائي والميكروبي للعينات التي تم جمعها – عينة من الألبان. تصنيفها الفصلي كالآتي: 110 عينة في فصل الشتاء (نوفمبر 143وعددها مارس)، و 33 عينة في فصل الصيف (أبريل- يونيو).

وقد تم جمع العينات من نقاط تجمع بائعي الألبان عند مداخل المدن الثلاث خلال فترات الصباح الباكر من كل يوم عمل ومن ثم التوجه بها مباشرة إلي معمل قسم الألبان بشمبات لاختبارها وتحليلها لمعرفة مكونات اللبن والعد البكتيري ومدي وجود بكتيريا البروسيلا .

شملت الدراسة فحص المكونات التالية في اللبن: نسبة الدهون، نسبة البروتين، نسبة الرماد، نسبة المواد الصلبة الكلية، نسبة الحموضة، درجة التجمد، نسبة التخثر عند الغليان واختبار الكحول) كذلك لوحظ ازدياد معنوي $P< 0.05$(. وتوصلت الدراسة للآتي: اختلفت نسبة الدهن اختلافاً معنويا) وأيضا درجة التجمد والمواد الصلبة الكلية $P< 0.001$في نسب البروتين والرماد ونسبة الحموضة ( ) بين الفصلين P). بينما اختلف العدد الكلي للبكتريا اختلافا معنويا ($0.001 > P$ < 0.01) فكانت نسبة الدهن 0.545% ±4.33 والبروتين % 0.319 ±3.29 والرماد% 0.111 ±0.62 والجوامد الكلية 0.827% ±13.18 وهي اعلي في الشتاء عنها في الصيف، أما نسبة الحموضة (% 0.030 ±0.21) ودرجة التجميد ($0.53 \pm 0.031° C$) والعدد الكلي للبكتريا ($8.3 \times 10^7 \pm 1.071$ cfu/ml.) فقد كانت اعلي في فصل الصيف

وعند المقارنة بين المدن الثلاث لوحظ عدم وجود فرق معنوي في نسب المواد الصلبة) ونسبة P) بينما اختلفت نسبة البروتين ($0.001 > P$ الكلية والرماد ودرجة التجمد ($0.05 <$) اختلفت اختلافا معنويا بين P) ، والعدد الكلي للبكتريا ($0.01 > P$ الحموضة والدهن ($0.01 <$

المدن الثلاث. فكان اعلي عدد كلي للبكتريا في منطقة الخرطوم (cfu/ml
1.05×10^{10} 2.591 ± 5.13cfu/ml) تليها ام درمان (5.146×10^{10} ± cfu/ml) ثم الخرطوم بحري ($9.3 \times 10^{8} \pm 0.668$.

اما في منطقة الخرطوم فلا يوجد فرق معنوي في نسب المواد الصلبة الكلية والدهن ، بينما اختلفت نسبة البروتين ودرجة التجميد ونسبة الحموضة ($P < 0.05$)، وكذلك الرماد والعدد الكلي ($P < 0.01$). وفي منطقة الخرطوم بحري لم يتم رصد أي فرق معنوي في نسبة المواد للبكتريا ($P < 0.001$)، والعد الصلبة الكلية ونسب البروتين والدهن والرماد، بينما اختلفت درجة التجمد ($P < 0.001$) اختلافا معنويا بين نقطتين من نقاط ، ونسبة الحموضة ($P < 0.01$) والعد الكلي للبكتريا ($P < 0.001$) تجمع الألبان التي أخضعت للدراسة. وكذلك في منطقة ام درمان لم ترصد فروقات معنوية في المواد الصلبة الكلية والبروتين والدهون ودرجة التجميد بينما وجدت فروقات معنوية في نسبة الرماد ($P < 0.05$) ونسبة الحموضة والعد الكلي للبكتريا ($P < 0.001$).

وعند المقارنة بين نقاط التجمع لم تلاحظ فروق معنوية في المواد الصلبة الكلية، بينما اختلفت نسبة البروتين ونسبة الحموضة والعد الكلى للبكتريا اختلافا معنويا كبيرا ($P<0.001$)، وكذلك كانت نسب الدهن والرماد ($P< 0.01$) ودرجة التجمد ($P< 0.05$).

وعند إجراء اختبار الكحول وجد ان اعلي نسبة موجبة كانت في فصل الصيف (81.8%) عنها في فصل الشتاء (58.8%) ولكنها تختلف من مدينة إلي أخرى فكانت أعلاها في ام درمان (72.7%) تليها الخرطوم (69%) ثم الخرطوم بحري (44.8%).

وعند إجراء اختبار التخثر عند درجة الغليان ظهرت النتائج كالآتي: نسبة التخثير في فصل الصيف كانت 36.4% وفي فصل الشتاء 15%، أما القراءات لعينات المدن الثلاثة كانت كالأتي: أعلاها في منطقة ام درمان (29.15%) تليها منطقة الخرطوم (24.1%) واخيرا منطقة الخرطوم بحري (3.4%) .

واخيرا تم رصد ميكروب البروسيلا في 85 عينة من مجموع العينات التي بلغت 110 أي

بنسبة 77.3% تقريبا بالنسبة للولاية ، وكانت بنسبة اكبر في منطقة الخرطوم بحري حيث بلغت 82.8% تليها منطقة الخرطوم (76.9%) ثم منطقة أم درمان بنسبة 73.8%.

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CHAPTER ONE

INTRODUCTION

Milk is highly nutritious food, which is suitable for both children and adults as an excellent source of energy, protein, vitamins and minerals. However, due to its rich nutritional composition, it is also ideal for microbial growth. Fresh raw milk is easily deteriorated to become unsuitable for processing and human consumption (FAO, 2001).

The use of milk and milk products as human food has got a very long history. The milk; as it is meant to be first and sole food of offspring of mammals; is more or less a complete food as it contains in a balanced form all the necessary elements for building up and maintaining of human and animal body. Milk is also a perishable product. It is an ideal media for microorganisms and as a liquid it is very easily contaminated and invaded by bacteria. Thus, milk can transmit zoontic diseases of microbial origin to people from sick animals and/or people carrying certain diseases can contaminate the milk with pathogenic bacteria during it is handling (Siirtola, 2000).

Milk hygiene measuring are absent in Sudan. The raw distributed milk for the consumer never finds the real quality control measures needed to be a precious food. Previous studies in Khartoum State showed that the raw milk was highly contaminated and some producers practice milk ration by antibiotics and other additives, bad milking practice and bad handling of milk may produce milk of high bacterial count. Milk dealers are less conscious about the problems of unhealthy milk they handle. In addition, the transfer of milk from milking places to consumers takes long time (2–4 hours) without cooling and distributed to consumers through many means (sales men, venders, groceries …etc). This investigation is an attempt to evaluate milk supplied to consumers in Khartoum State by sale points with the following objectives:

- Assessment of chemical composition (fat %, protein %, ash % and total solids %) acidity % and freezing point degree C of milk collected from sale points in Khartoum State in winter and summer seasons.
- Determination of the level of microbial contamination of milk distributed in Khartoum State.
- Investigation of the prevalence of Brucella in milk distributed in Khartoum State.

- Evaluation of milk distributed in Khartoum State by using clot on boiling and alcohol test as quality control tests.

CHAPTER TWO

LITERATURE REVIEW

2.1. Milk:

Milk is defined by Bath *et al*. (1985) and Foley *et al.* (1974) as the physiological secretion from the mammary gland of mammals. Harding (1999) described milk as a natural food of the young to be as close to being nature's perfect food.

2. 2 . Milk composition:

Milk is a complex mixture of fats, proteins, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water (Hurley 2002). Non – water constituents are present in different physical forms; dissolved (lactose), colloidaly dispersed (protein) and emulsified in water (fats) as reported by Harding (1999).

ElFaki (1988) mentioned that the available data in Sudan revealed the chemical composition of milk of the local breeds (Kenana and Butana) as follows: water (86.8%), fat (4.39%), protein (3.2%) and total solids (13.19%). Hassan (1985) worked on bulk milk supplied to Gazira Cooperatives, found that the fat content ranges from 4.63% to 5.26%, protein content ranged from 3.03 to 3.28 %, solids–non –fat ranged from 8.44% to 9.0% and totals solids ranged from 13.08% to 14.34%. Hussain (1985) reported that the milk composition of Zebu crossed with Friesian is as follows: fat 4.7 % and total solids 12.5 %. Also Salih (2001) found that the chemical composition of venders milk samples collected from Khartoum State were fat 5.56 %, protein 2.80 %, total solids 16.15 % and ash 0.98 %. Abdalla (2002) found that the mean value of total solids, protein, fat, ash and lactose for whole milk samples collected from Khartoum State were 12.02 %, 3.11 %, 3.82 %, 0.72 % and 4.57 %, respectively.

Eddleman (1999) worked on the commercial milk, found that the average composition as

follows: protein 3.3%, lactose 4.8%, fat 3.8%, Ash 0.71% and total solids 12.8% with the fact that the commercial milk is a blend of many animals of many breeds. Also the same author found that the Holstein milk has about 3% fat but Jersey milk has 5% fat. That fat content for individual cows' ranges from 1% to 9%.

Webb *et al.* (1980) and Clarence *et al.* (1982) found that in temperate breeds an average milk composition as follows: water 87.3%, fat 3.7%, protein 3.5%, total solids 12.8%, lactose 4.8%, solids non fat 9.15% and ash 0.65%. However, Harding (1999) reported that cow's milk contains about 87.4% water and about 12.6% milk solids (total solids) the latter comprising about 3.9% fat, 3.2% protein, 4.6% lactose and 0.9% other solids', i.e. minerals, vitamins, etc.

2.3. Factors affecting milk composition:

Eddleman (1999) stated that the composition of cow's milk varies from cow to cow within breeds and from breed to breed, it also varies during lactation, seasonally, and regionally and there are many factors which cause these variation. Also Johnson (1980) stated that milk from individual cows may show a day–to–day variation, which is influenced by mental and physical condition of the animal, in addition to variation due to environmental factors. Also Stallings (1998) and Agabriel *et al*. (2001) reported that environment, breed, and nutrition are three broad main factors that affecting milk composition.

Every summer we see a reduction in milk, fat and protein concentration as a result of hot weather. Usually the average fat content cow on DHI in Virginia drops from approximately 3.7% in March to 3.4% in August and protein drops from 3.2% to 3.1%. Generally, milk protein will not fluctuate as much as milk fat. Also Breed will affect milk composition. Total solids of milk produced by Holstein cows in a recent experiment averaged 12.4% versus 14.6% for Jerseys. Holsteins produced less milk fat (3.7% VS 5.1%), solids–not –fat (8.7% VS 9.5%) and protein (3.1% VS 3.7%) (Stallings, 1998).

Factors affecting the solids content of milk are reviewed by Campabadal (1999) and Eddleman (1999). These factors may include genetic and environmental factors, including environmental temperature, age of cow, health of the cow, management of milking and feed management.

Buchberger *et al*. (2002) in German found highly significant differences for the factors year, farm

and season in fat, protein, casein, lactose, freezing point and pH. Also Reinoso and Simon (1999) assessed the influences of the seasonal variation upon milk constituent. They found that the season of the year significantly affected the content of solids–non–fat and total solids. However, Sharma *et al*. (2002) collected 50 samples from herd of Jersey cross breeds during summer (April–June) and winter (November–February) season, the seasonal influences on cross bred cow milk composition was evaluated. He found that solids–non–fat and total solids content varied among seasons; and the values were higher during winter (8.983% and 13.639%, respectively) and lower during summer season (8.835 and 13.403 %, respectively).

Auldits *et al*. (2000) studied in detail the seasonal variation of milk composition and concluded that improved nutrition may partly obviate seasonal variation in milk yield and composition but that nutrition alone is not the primary cause. Although, Ireland and Stallings (1998) found that with diets containing 17% acid detergent fiber (ADF) (65% Starch) and 25% fiber (53% starch) dry matter intake and milk production were reduced with higher fiber lower starch diets. Milk fat content increased from 3.36% to 3.69% by increasing fiber from 17% to 25% ADF. Milk protein concentration did not change; however there was a trend of less percent protein with the higher fiber ration. Also Guthrie (1994) found that the cows on the high roughage ration produced 3.21% versus 3.01% milk fat percent for cows fed low roughage ration.

Harding (1999) reported that the seasonal effect on milk yield and composition are largely attributed to extremes in environmental temperature. Cow's responses to environmental changes are influenced by age, body condition, plane of nutrition, production and genotype. The consumption of roughage is reduced during environmental heat stress, resulting in decreased milk production as well as percentage fat. Similarly milk protein and lactose percentages are lower during the warm season.

Percentages of fat, total solids and solids–non– fat are greatest during the winter months. Most of seasonal variation in solids–non–fat is due to variation in the milk protein content, however, percentages of fat and protein are lowest during the summer season and highest during cooler months, in part due to seasonal change in forage quality and availability.

Latitude is another environmental factor that affects milk production and composition because of its relation ship with the angle of solar radiation and day length. About 50% of the worlds cows are located at latitudes between 30ºN and 30ºS but heat stress also occurs at these latitudes (Harding, 1999).

Smit *et al*. (2000) studied the differences in nutrient content of whole milk in five localities in South Africa (Pretoria, Bloem Fontion, Durban, Cape Town and Port Elizabeth) between winter and summer. They reported that the variation was attributed to the different composition of the feed used and the feeding practices. Guthrie (1994) stated that the season of the year cause some variation in milk fat test. This variation is probably caused by changes in types of feed available at the various seasons and the interaction of these and temperature effects. Also he noted that, highest milk fat test occur in fall and winter and the lowest in spring and summer. These variations are likely to be the result of changes in the eating habits of cows during hot and cold weather as he reported.

Also Pinto *et al*. (2002) studied the fatty acid composition of milk fat and it is variation due to geographical and seasonal effect. They showed that an effect of season was observed only in the case of short chain fatty acids, with significantly ($P< 0.05$) average value, which was lower in summer than in spring. Moreover, analysis with geographic area revealed significant differences ($P< 0.05$) for all fatty acids.

However, El Faki (1988) working on the herd of the university of Khartoum Farm, found that composition of milk was significantly affected by the season of the year with lowest value being in summer season; 3.1%, 12.1% and 6.8% for protein, total solids and fat respectively, as compared to 3.4

%, 12.6% and 8.1% protein, total solids and fat respectively, for the winter season.

Ballou *et al*. (1995) collected 2272 bulk milk samples for 12 months to evaluate milk composition, he found that yearly means were 3.73% fat, 3.16% protein and 4.64% lactose, and they showed significant ($P< 0.01$) effect of the month factor for all parameters.

Guo *et al*. (2001) in U.S.A. conducted a study on chemical composition of the goat milk over 12 months beginning in April 1996. They found that chemical composition of milk varied widely during the year. Moreover, the contents of fat and total solids decreased over the first 20 weeks from 3.6% and 12.7% to 3.0% and 11.3% respectively, then increased to peak values of 13.4% and 4.4% in January. The crude protein and casein content also decreased over the first 20 weeks from 3.5 % and 2.7% to 3.2% and 2.3% respectively, before increasing gradually to 3.8% and 2.9 % in February. The ash content declined during the first 20 weeks from 0.82 % to 0.78% and then increased sharply to 0.9% by week 36. Also he reported that goat's milk had a similar composition to Holstein– Friesian cows (protein 2.98%, fat 3.7 %, ash 0.71% and lactose 4.46%).

ALAmin (1994) compared the local breed (Butana) and cross breed (Holstein–Friesian). He found that constituent of Butana milk was higher than the Holstein– Friesian and there were significant differences ($P< 0.01$) between constituent except for the protein. Also he found that milk constituent of Holstein– Friesian cow averaged 10.67%, 7.49%, 3.18%, 3.44%, 3.43% and 0.62% for total solids (T.S), solids– non– fat (SNF), fat, protein, lactose and ash, respectively. Whereas constituent of Butana milk averaged 14.14%, 8.86%, 5.29%, 3.5%, 4.69% and 0.67% for T.S, SNF, fat, protein, lactose and ash, respectively. He conducted that milk composition was adversely affected by summer season in both breeds.

Also Guthrie (1994) found that the breed of cow influences greatly the milk fat content, since Jerseys and Guernseys produce milk with the highest fat content, while Ayrshires and Brown Swiss being intermediate. Milking Shorthorns and Holsteins produce milk with the lowest amount of fat and the milk fat percent within a breed is also highly variable and is influence by genetics as he reported. The same author stated that the health, the age of the cow, milking variations, excitement of the cow prior to or during milking, improper milking techniques, and improper operation or cooling equipment, are some

factor that influence milk fat percent.

Mason (2001) reported that approximately 49%– 60% of the variation in milk protein can be attributed to genetics. Although milk protein and fat generally increase or decrease together and there is evidence that protein can be increased without increasing milk fat.

The same author mentioned that mammary infection (mastitis) and high SCC (> 200.000) are known to depress milk protein concentration. Cow that have high SCC have a high level of plasmin and plasminogen, therefore, casein percentage and amount are lower for cows with high SCC. Also he stated that the growth hormone reduce the plasmin content of milk, which would reduce protein degradation and increase milk protein.

Mohamed (1996) compared between mastitic cow's milk and normal cow's milk and found highly significant ($P < 0.001$) differences for total solids, casein, ash, chloride, lactose and casein number. She also reported significant decrease in fat ($P < 0.01$) and total protein content ($P < 0.05$).

2.4. Freezing point:

Milk is a variable biological fluid, in which the fat, protein and; even the natural water content all vary; from cow to cow and from herd to herd. In spite of the wide variations which occur, compositional quality has been used as an indication of adulteration (Harding, 1999).

There are a few mill degrees difference in the freezing point of milk from different breeds of cows but there is little significant lactational effect. The greatest impact on freezing point of genuine milk is abnormal water intake by the cow. The freezing point can be abnormally elevated (–0.500º H and above) if herd is deprived of water for some time then given ad lib access prior to milking. Very bad feeding can also give elevated freezing points, but developed acidity will depress the freezing point, and

masking the confirmation of adulteration (IDF, 1983).

Physiological, geographical and seasonal factors had only slight and irregular effects on the freezing point. The presence of rising water and pasteurization respectively, led to an increase in freezing point of about 1.5 and 2 mill degrees (Asperger, 1975).

Harding (1999) stated that the osmotic pressure or salt balance of a cow's milk has to be in balance with that of her blood. Since the osmotic pressure of a cow's blood can only vary within narrow limits, it follows that the salt balance of her milk–and hence the freezing point, dedicated by salt balance– can only vary within narrow limits.

Bradley *et al.* (1993) reported that the lactose and chloride account for about 75% of the freezing point depression of milk and changes in the concentration of one tends to compensate for changes in the concentration of the other. Most of the variation among normal milk samples has been attributed to changes in the non– chloride ash fraction of the milk as he reported.

Luck and Dresner (1975) in South Africa, found that the evening milk appeared always to be more concentrated (freezing point –0.539 ± 0.0093º C) than morning milk (freezing point– 0.532 ± 0.0079º C). A smaller freezing point –0.530ºC was observed during winter months (November–February) than that during summer months (May–August) –0.543º C. They also stated that the official recommended freezing point standard of South Africa was –0.530º C. Velden *et al*. (1984) mentioned that the time of milking had significant influence on the freezing point. Mean freezing point of post meridiam (p.m.) milking was 0.007º C lower than the freezing point of ante meridiam (a.m) Milking.

However Zee *et al*. (1982) found that average freezing point of un adulterated milk (from p. m. and a. m. milking) in tanks on 70 farm evenly distributed over the Netherlands was –0.533± 0.004º C. This suggested that the requirement under Netherlands legislations that the freezing point of farm milk should be below – 0.53º C. However overnight storage at 4º C slightly increase the freezing point.

Barest and Wickes (1980) found that cows fed 100% of metabolizable energy requirement produce milk of significantly ($P< 0.05$) lower freezing point than from cows fed 80% (M.E), they also gave significantly ($P< 0.05$) more milk with higher solids non fat and protein content and lose less live

weight. The significant correlation between freezing point and the milk components was that between freezing point and solids non fat.

Bradley *et al*. (1993) mentioned that the freezing point value of –0.517º C has been established in some areas as normal for milk and milk that freezes at or below that value is presumed to be free of added water. Also Harding (1999) stated that the average freezing point of normal milk is about– 0.5º C below the freezing point of water. However, Aagaard *et al*. (1998) in Denmark, found that grade one milk has a freezing point of less than –0.518º C.

Mohammadi (1988) found that about 90.3% of the samples collected from Khartoum North had freezing point in range from –0.540 to –0.574º C with an average of –0.5583º C, while the freezing point of the University of Khartoum farm ranged from –0.541 to –0.569º C with an average of –0.5525º C. The average freezing point of the samples collected from El Sagana, Burri and Omdurman were –0.5565, –0.5560 and –0.5580º C respectively. However Ibrahim (1989) found that the average freezing point in Dairy Land and University farm were –0.539 and –0.548º C, respectively. This indicates that there was no addition of water in both farms.

Boor *et al.* (1998) evaluated the quality of raw milk collected from selected farms in New York State and found that the mean values for milk Freezing point range form –0.539 to –0.545º C with an average –0.543º C. Dayyani *et al*. (2000) found that raw milk form Iran milk industry had freezing point ranged farm –0.535 to –0.540º C with an average –0.537º C. Factors as season of the yea; age; health and breed of the cow; access to water; feed; weather; ambient temperature; time of milking (morning or evening milk); and possibly other factors, many of which are apparently interrelated (Bradley *et al.*, 1993).

2.5. Titratable acidity:

Lactic acid bacteria are a number of organisms, which are concerned in the production of lactic acid from lactose in milk. Since they produce relatively large amounts of lactic acid, while other form smaller amounts. The principal acid producing bacteria in milk include the genera Streptococcus, Lactobacillus and Leuconostoc (Jay, 1986).

According to Harding (1999) the natural acidity of normal milk is less than 0.16 % lactic acid or

equivalent.

Lin and Lin (1987) reported that low– acidity grade B milk has a normal acidity of 0.11– 0.18% but coagulates in the presence of 70% alcohol. Also they stated that the samples of milk from 598 cows in Taiwan were examined during the period from winter 1981 to summer 1982, and found that low acidity of milk was given by 25 (4.2%) of cows. Causative factors of low acidity of milk were related to the physiological condition of the cow, only 6 cows gave true low acidity grade B milk; the other 19 cows were affected by mastitis– related disorders.

Mohammadi (1998) found that the acidity of 82.9% of raw milk samples collected from Khartoum North at even intervals was 0.18%– 0.21%. The samples collected from ALSagana, Burri and Omdurman were 0.19 %, 0.20 % and 0.21% respectively. Also Abdalla (2002) working in Khartoum State, found that the titratable acidity of whole milk in the range of 0.18% to 0.22% lactic acid with the mean of 0.193± 0.015%. However, Ibrahim (1989) found that the average lactic acid percentage in Dairy Land and University farm were found to be 0.177± 0.022 and 0.172± 0.023; respectively. ELTayeb (1973) reported that the mean titratable acidity was 0.18 % for venders in the Sudan and 0.20% for dairy farms.

Butkus *et al*. (1974) reported that mastitis decreases titratable acidity and increase pH

significantly (P< 0.05) as compared to healthy cow's, the effects were found to increase with the severity of infection. However, Molina (2001) collected 96 samples of raw cow's milk from 11 small farms from the milk collection centers. They found that the average of the results among farms were statistically significant (P< 0.05) for acidity and pH and there were significant correlation between pH and acidity (r = –0.4656).

Have *et al*. (1980) reported that the differences in build up of titratable acidity between milk from spring calving and autumn calving cows was due to differences in casein and phosphate contributions. Sebela and Klicnik (1977) found that milk acidity was positively correlated with contents of Ca, P and citric acid and negatively correlated with chloride content. Dayyani *et al*. (2000) found that the acidity of raw milk in Iran milk industry factory ranged from 14.15 to 14.52% with an average 14.34%.

Belyaev (1974) found the cows with daily milk yield of 12.12 kg, whose ration consisted of 40% concentrate produce milk with titratable acidity of 19 –21 T (0.19– 0.21%), while those with daily milk yield of 7– 8 kg whose ration consisted of 10.9 % concentrated produce milk titratable acidity of 20–23 T (0.20– 0.23 %) .

2.6. Sources of contamination in the milk:

Microbial contamination of bulk milk originates from three main sources: within the udder, the teats and udder exterior, and milk handing and storage equipment. Bacterial contamination can originate from multiple sources such as mastitis cows, dirty udders and poorly cleaned milking equipment (Bramley and Mckinnon, 1990 and Harding, 1999). Milk, when it leaves the healthy udder, is relatively free from bacteria. While some contamination with bacteria from the milking environment and equipment is inevitable, the total bacterial count of cooled milk, produced under good hygienic condition, should be lower than 10,000 bacterial/ml. Mastitic cows can produce milk with very high bacterial counts, milk from individual cows may contain millions of organisms per milliliter of milk. Dirty teats may contribute up to 100,000 bacteria/ml and contaminated milking equipment is often the major source of bacteria in milk, visually clean surfaces should not contribute more than 1000 bacteria/ml. However, surfaces in effectively cleaned and sterilized or plant containing old milk residues will elevate the bulk milk count by at least 10,000/ml. Bacteria are present in the air, dust and water–especially any water containing traces of milk

residues which may have been left in the milking plant overnight, as such residues provide a very good source of food for bacteria enabling the bacterial count to increase rapidly (Harding, 1999).

Godefay and Molla (2000) found that milk samples collected from the udder contained mainly staphylococci and micrococci as udder specific bacteria, while samples taken at later stage were additionally contaminated with bacteria of environment origin (especially Enterobacteriaceae). Also they stated that the lack of knowledge about clean milk production, use of unclean milking equipment and lack of potable water for cleaning purposes were some of the factors which contributed to the poor hygienic quality of raw milk in the studied farms.

2.7. Type of bacteria in milk:

There are many different microorganisms (mainly bacteria) which can find access to milk and there are three broad temperature ranges classifying their optimum growth rates. Organisms with an optimum growth rate at low temperature (0– 15º C) are the psychrophiles, at medium temperature (20– 40º C) are called the mesophiles and at high temperature (45– 55º C) the thermophiles (Harding, 1999).

2.8. Sanitary control of milk:

Nickerson and Sinskey (1974) reported that good sanitary procedures on the farm are prompt cooling to suitably low temperature and quick delivery to processing plants that lead to the prevention of spoilage of raw milk. Regarding sanitation, the air of the stable or barn should be kept as free of dust as possible. To facilitate this, milking should be done before feeding, the coat of milking cows should be clean and the udder should be clean before the cow is milked.

Clothes and hands and milkers should be clean, and all the equipment that can come in contact with milk including milking machines pails and milk cans or bulk cooling tanks should be thoroughly cleaned and treated with chlorine solution or other suitable sanitizing agents (Abdalla, 1999).

Peckham (1966) stated that the clean healthy cows, proper sterilization of utensils, prompt cooling of milk and minimum storage time, are the principal factors that help to assure the production of low bacterial count.

Jurczak and Zdziarski (1998) conducted a study on hygienic quality of raw milk samples collected from milk collection center. Their result indicated no seasonal variation in microbial quality; this

was attributed to new polish standards requiring cooling raw milk.

Cifrian *et al.* (1998) in Spain, found that the hygienic quality of milk delivered to industry has improved significantly over the last 10 year. In 1995, 41% of farm supplied milk with a total bacterial count less than 100,000 cfu/ml a 12% improvement compared with 1994; as a result of low prevalence of mastitis, tubercolosis and brucellosis (Kiss, 1997).

2.9. Bacteriological quality of milk:

Milk bacteriology can be used to investigate milk quality (total bacterial count) and intramammary infection (mastitis)(Biggs, 2003). Bacteriological quality was based on the total bacterial count and somatic cell count (Litwinczuk *et al.*, 2002).

According to Aagaard *et al*. (1998) grade one milk in Denmark, has total bacterial count of less than 30,000 bacterial/ml. But Reena *et al.* (2003) stated that the total bacterial count (T.B.C) in the normal milk was below 500 bacterial/ml. Also Murphy and Boor (2000) stated that the total of bacterial from a normal animal ranged from few hundreds to few thousands.

Hussain (2001) conducted a study to evaluate the quality of milk supplied to consumers from various sources during summer and winter seasons in Khartoum State. She found that the total bacterial count was significantly ($P< 0.001$) affected by season of the year, with the highest total bacterial count being in summer season (Log_{10} 6.895± 0.678 cfu/ml), while the lowest count was in winter season (Log_{10} 5.563± 0.572 cfu/ml). Also she stated that the highest total bacterial count in Khartoum (Log_{10} 6.245± 0.921 cfu ml), followed by Omdurman (Log_{10} 6.140± 0.872 Cfu/ml) and Khartoum North (Log_{10} 5.911± 0.878 cfu/ml) .

Heeschen *et al*. (1987) compared bacterial count in milk samples collected from market and farm during summer and winter season. They reported that 450 market milk samples and 785 farms samples during summer and 250 and 68 samples, respectively in winter season, showed decrease in bacterial count from 500,000 to 100,000/ml for farm milk samples and from 3,000,000 to near 500,000/ml for market milk samples in summer and winter season, respectively.

Boycheva *et al.* (2002) showed that total number of bacterial in the milk samples collected during autumn was higher than in the spring season, the average was $3.92 \times 10^6/cm^3$ compared to $2.03 \times 10^6 /cm^3$,

respectively.

Desmasures and Gueguen (1997) found that there were seasonal variations in the concentration of lactobacilli and yeasts over the 2 years monitored.

Ballou *et al.* (1995) reported that the yearly mean for 1116 samples analyzed for psychrotrophic bacteria count (PBC) was 720 cfu/ml. The PBC was significantly ($P< 0.01$) affected by month, and PBC elevated in winter. This was attributed to lower temperatures which are optimal for proliferation or because inefficient sanitation of milking equipment in the colder season contributes to increased growth or psychrotrophic bacteria.

Titini *et al*. (1991), reported that even raw milk of good quality might have psychrotrophic bacterial count in the range of 1×10^3 to 1×10^4 cfu/ml, although psychrotrophic bacteria have been shown to cause keeping quality problems in fluid milk at levels of more than 1×10^6 cfu/ml. Bacterial counts during spring, summer, fall and winter were 4.8×10^4, 6.0×10^4, 7.7×10^4 and 6.0×10^5 cfu/ml respectively, meaning that bacterial count during winter season was the highest.

Mohammadi (1988) examined 290 samples of vendors' milk in Khartoum State for total bacterial count and found that 45.4% of venders had total bacterial count ranging between 5.0×10^5 and 5.0×10^6 cfu/ml.

Barakat (1995) collected 80 samples of milk from dairy farms in Khartoum State and found that total bacterial count ranged between 4.5×10^5 to 9.5×10^6 cfu/ml. He also stated that 53.7 % of tested milk samples were satisfactory according to tropical grading of raw milk, but were not so when compared with milk grading in some developed countries.

Ali (1988) collected five and eight milk samples from Kuku and Gazira dairy plant, respectively. Milk samples were examined for total bacterial count before and after pasteurization. The mean bacterial counts were 3.4×10^6 cfu/ml and 4.3×10^6 cfu/ml for raw milk, and 4.4×10^5 cfu/ml and 1.99×10^4 cfu/ml for pasteurized milk in Kuku and Gazira dairy plant, respectively.

Lukac (1990) conducted a study on bacteriological quality of raw milk collected daily from dairy farms and bulk milk tanks in Croatia, the results indicated great variability of bacteriological quality of milk samples which was attributed to ecological conditions and milk handling as well as husbandry

practices and feeding condition.

Hayes *et al*. (2001) collected milk samples from two bulk milk tanks for one farm, they found that the total bacterial count of raw milk ranged from 8.9×10^3 to 180.0×10^3cfu/ml in tank one and 70×10^3 to 150.0×10^3 cfu/ml in tank two.

Godefay and Molla (2000) conducted study on bacteriological quality of raw cow's milk taken at different sampling points from four dairy farms and a milk collection center in and around Addis Ababa. They observed that the high increase in the mean total aerobic plate count in milk samples taken from the bucket (1.1×10^5 cfu/ml), storage container before cooling (4×10^6 cfu/ml) and upon arrival at the processing plant (1.9×10^8 cfu/ml). They reported that the hygienic quality of raw milk collected from collection center was poor with mean total bacterial count of 1.3×10^7 cfu/ml, which was attributed to lack of knowledge about clean milk production.

Boor *et al.* (1998) examined 855 samples of raw milk from bulk tanks across the New York State and found that 10% of the samples had SPC ≤ 2500 cfu/ml, 50% of the samples had SPC ≤ 10,000 cfu/ml and 10% had SPC ≥ 60,000 cfu/ml. The SPC ranged from 6400 to 22,000 cfu/ml with an average of 11.4000 cfu/ml for all sample.

Dayyani *et al.* (2000) examined 48 samples of raw milk from Iran milk industry factory, for total bacterial count and found that mean of total bacterial count ranged between 1.02×10^6 to 2.49×10^6 cfu/ml with an average 1.76×10^6 cfu/ml. They concluded that control of hygienic condition on milk production is essential.

Cempirkova (2001) found that the farms with loose housing system and cross type milking platform had always–lower levels of microbial contamination and the average total bacterial count ranged from 17000 to 29000 cfu/ml. While farms with stanchion housing and milking machines in the cattle shed had an average bacterial count of 46,000 to 100,000 cfu/ml. Also Boni *et al.* (1998) found that the total bacterial count and somatic cell count were lower in traditional farms .

Litwinczuk *et al*. (2002) stated that season, number of cows and milking method were significantly influenced hygienic quality of raw milk.

2.10. Importance of hygienic quality of milk:

The hygienic quality of milk at the point of production is of importance from both public health and consumer perception point of view,

making it important for milk to be produced with a low bacterial count and the count, by adequate temperature control, is to be kept low until the point of processing (Harding, 1999). The microbial count of raw milk affect quality, shelf – life and safety of milk and other dairy products.

2.11. Brucella:

Brucella species exhibit pathogenicity toward a wide variety of

animals, including dairy cattle and goats. Of the many species within the genus, *Brucella abortus* is the only significant species with respect to animal and human health. Brucella is the causative agent of undulant fever (Flowers *et al*., 1993). Brucellosis is a chronic zoonosis that plays an importance role in public health, it is presente in raw milk and non pasteurized dairy product (Langoni *et al*., 2000).

The disease causes serious economic losses in terms of abortions, diminish milk production, culling of infected animal, rejection of consig– nments containing infected animal, cost on research control and eradication programs, failure in financial investments, expenses on hospitalization and treatment of people and reduction of their working hours (Chukwu , 1985).

Brucellosis in cattle was reported in all part of the Sudan and the prevalence rate was found higher in cattle compared to other animal species. The first incidence of bovine brucellosis in Sudan was reported from a dairy herd in Khartoum where *B. abortus* was isolated from aborted cow (Bennett, 1943).

Musa (1995) found that about 69.7% of cattle herds tested in Darfur State had brucellosis. Also he reported that the prevalence of the disease was higher in commercial cattle (21.5%) followed by sedentary cattle (20.2%), nomadic cattle (15.2%) and seminomadic cattle (8.5%).

Musa *et al*. (1990) reported that the prevalence of the disease in different animal species including cattle, and concluded that the highest prevalence rates were encountered in intensive farming system and under nomadic conditions. Moreover, cattle were found most affected (13.9%) followed by camels (7.76%).

Habiballa (1977) reported that in some parts of Sudan, the incidence of brucellosis ranged from

6.7% to 28.5% in dairy farms, while incidence up to 36.0% was reported in livestock in Gazira.

Abdalla (1966) found that 3% of cattle tested in Wadi Halfa was positive

Langoni *et al*. (2000) reported that in 49 analyzed samples, 15 (30.61%) contained *Brucella abortus*. Srinand *et al*. (2000) found that the prevalence of *Brucella abortus* in milk was 11.4% in 70 Argentine cattle. Cifrian *et al*., (1998) in span reported that low prevalence of brucellosis and tuberculosis; since 99.4% and 98.6% of the cows were free from the two diseases, respectively.

2.12. Alcohol stability test:

The alcohol test is used by the milk industry as a reception test for milk in the plant to measure the heat stability of milk (Molina *et al*., 2001). The alcohol test gives an index of casein stability which is reduced with increase in acidity (H^+). Either high acidity or unbalanced mineral salts will cause milk to coagulate at sterilizing temperature of 115–121º C. A high albumin content also favors coagulation (Foley *et al*., 1974 and Harding, 1999).

Shekarforoush *et al*. (2003) collected 140 samples of bulk milk from milk collection centers and examined them for alcohol test with single and double volumes of 68% ethanol, to evaluate the efficacy of alcohol coagulation test for differentiation between acceptable and unacceptable bulk milk. Sensivity of single and double volume alcohol test for differentiation between high acid milk and normal milk were 45.7% and 55.5%, respectively. They concluded that the alcohol test is not accurate test for the evaluation of the quality of bulk cow's milk, and they suggested that this test be removed from routine testing carried out in milk collection centers and industrial dairy plant.

Dayyani *et al*. (2000) in Iran collected 48 samples of raw milk and tested them for alcohol 62% test and alcohol 72% test. They found that the positive results of alcohol 62% were 4.2% and alcohol 72% test was 29.2 of the samples tested.

Molina *et al*. (2001) collected 96 samples of raw cow's milk from milk collection center. The alcohol test was carried out at four– ethanol concentration (70, 75, 80, and 85% V/V). They reported that 75% (V/V) concentration of ethanol is recommended for the alcohol test at the milk collection centers.

2.13. Clot on boiling test:

Colt on boiling test is used during milk collection for detection of instability of milk proteins for heating because either lactic acid or rennin enzymes produced by bacterial (Siirtola, 2000). In summer, milk coagulate on boiling when it reaches a titratable acidity of about 0.24%. In winter and during late lactation milk coagulates on boiling at about 0.20% acidity (Foley *et al*., 1974).

Girgis *et al*. (2001) studied the preservative effect of lactoperoxidase system (LPS) on cow's milk at 30 and 8º C. They found that activation of LPS caused a considerable slowing down in the rate of increase in titratable acidity during storage; it also caused a delay in clot on boiling test (C.O.B).

CHAPTER THREE

MATERIAL AND METHODS

3.1. Sources and number of samples:

This study was based on collecting raw milk samples from Khartoum State. One hundred and ten samples in winter season, (November, December, January and March) thirty three samples in Summer season, (April, May and June) from sales point in Khartoum, Omdurman and Khartoum North, in order to determine the milk quality, (contamination, adulteration by addition of water, milk composition and to test for the presence Brucella organisms in milk).

3.2. Collection and transportation of milk samples:

Milk sample were collected randomly in the evening from sale points, from Khartoum (Al Sagana, the60street and Child city), Bahry (Kuku and Shambat), Omdurman, (Al Fetaihab, Libya market, Al Ahamda and El Azhary street).

The samples were collected under aseptic conditions in labeled sterilized bottles and placed into ice box (under refrigerated conditions), then transported to laboratory for analysis.

3.3: Milk composition:

3.3.1: Determination of fat content:

Fat content was determined using Gerber method (Bradley *et al.,* 1993). Ten ml of sulphuric acid (specific gravity 1.820–1.825, 15.5º C) was measured into a Gerber butyrometer. From a well mixed sample at 24° C, a sample of milk was withdrawn using 10.94 ml pipette.

Milk was allowed to drain into the butyrometer slowly at first to prevent a violent reaction with the acid then the pipette was permitted to drain normally. One ml amyle alcohol (sp.g 0.814– 0.816, 15.5º C) and distilled water at 20º C were added and the lock stopper was inserted securely. With the stopper end up, the butyrometers were grasped at the graduated column and shaken until the curd was completely digested. Holding the butyrometer at the stopper and neck, the butyrometers were inverted at least four times to mix the acid remaining in the bulb with the rest of the contents. The butyrometers were then centrifuged at 1100 revolution per minutes (rpm) for four minutes.

The butyrometer was placed in a water– bath at 65º C, leaving only the bulb exposed, for five minutes. The straight line at the bottom of the fat column was pushed gently upwards until it coincided with the nearest whole percent graduation mark. The scale at the bottom of the meniscus at the top of the fat column was read promptly to the nearest 0.05% graduation, the lower reading was subtracted from the upper reading and the difference was recorded as the fat content.

3.3.2: Determination of protein content:

Total nitrogen was determined using Kjeldahl method (Bradley *et al.,* 1993). Ten ml of milk samples were weighed into Kjeldahl digestion flasks. Twenty five ml of sulfuric acid (sp.g, 1.84, nitrogen free) were added to the flasks. Two catalyst Kjeldahl tablets (each tablet contains 1gram of Na_2So_4 and the equivalent of 0.1gm Hg). The flasks were heated on a Kjeldahl digestion heater for three hours or until the solution became clear. The flasks were then cooled to room temperature and the solutions were diluted to 100 ml, by graduated pipette using distilled water. Five ml of the samples were transferred to distillator and then ten ml of 40% NaOH were added.

The distillate was received in a conical flask containing 25 ml of 2% boric acid and 3 drops of indicator (bromocresol green + methyl red). The distillation was continued until the volume in the flasks was reached 75 ml. The flask was then removed from the distillator, and the distillate was titrated against N/10 HCl until the end point was obtained (red colour). The protein content was calculated as follows:

$$\text{Protein (\%)} = \frac{T \times N \times 0.014 \times 20 \times 6.38 \times 100}{\text{weight of sample}}$$

where:

T= Titrant volume

N= Normality of the HCl (0.1)

0.014= Atomic weight of nitrogen/1000

* for milk: one part nitrogen equals 6.38 parts protein

3.3.3: Determination of ash content:

Ash was determined by gravimeteric method (Bradley *et al.,* 1993). The principle of the method is to burn away all organic matter at a temperature of 540– 550° C .Five grams of milk were weighed

accurately into a dry clean pre– weighed crucibles and evaporated to dryness on a steam bath. The crucibles were placed in a muffle furnace at 550º C for 1.5 hours. The crucibles were removed and placed in a desicator to cool and weighed. Heating, cooling and weighing were repeated several times until the difference between weighings was less than 0.1 gram. The ash content was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{weight of residue} \times 100}{\text{weight of sample}}$$

3.3.4: Determination of total solids content:

Total solids content of milk was determined by using forced draft oven method (Bradely *et al.,* 1993)

Five grams of milk were weighed accurately into clean dried flat bottomed pre–weighed aluminum dishes. The dishes were placed on a steam bath for 30 minutes, then transferred to an oven and held for 2 hours. at 102 + 1º C. The dishes were removed from the oven and cooled in a desicator, the dishes were weighed on a sensitive balance. Heating, cooling and weighing were repeated until the difference between weighings was less than 0.1 mg. The total solids contents were calculated as follows:

$$\text{Total solids (\%)} = \frac{W_2 - W_1}{S} \times 100$$

where:

w_1 = weight of empty crucible

w_2 = weight of crucible and sample after drying

s = weight of sample and crucible before drying

3.3.5: Freezing point test:

The freezing point was determined by using a FISKE MS Cryoscope manufactured by Fiske Med. Sci. Inc. U.S.A the theory and technique of analysis used were described by the manual supplied with the machine.

Accuracy and calibration was done by using standards supplied with the machine. After 45 minutes of setting on the machine, 2.0 ml milk samples were taken in a sample tube and emmersed in the bath then the machine was run. The result is immediately obtained in a digital screen.

3.3.6 Titratable acidity test:

The acidity of milk samples was determined according to AOAC (1990). Ten milliliters of milk sample were measured into a white clean and dry procelain dishes and five drops of phenolphtalein indicator were then added.

The mixtures were then titrated against N/9 NaOH until a faint pink colour lasting for not less than 30 seconds was obtained. The titration figure was divided by 10 to give the acidity of the sample expressed as percentage of lactic acid.

3.3.7. Clot on boiling test (C.O.B):

The clot on boiling of milk samples were determined according to Foley *et al*. (1974).

Two milliliters of milk samples were taken in test tubes and placed in boiling water for five minutes until boiling point was reached and then examined for precipitation.

Positive results were obtained if milk samples were cloted.

3.3.8. Alcohol stability test:

The alcohol test of milk samples was determined according to Foley *et al.* (1974). Two milliliters of milk samples were taken in test tubes; an equal volume of 75% ethyl alcohol was added. The mixture was shaked and observed to see if any curd flakes appear. Positive result was obtained if flocculation occur.

3.4. Microbiology of milk:

3.4.1. Standard plate count (total viable bacterial count):

Plate count agar medium was used to determine total viable bacterial count (Richardson, 1985)

3.4.1.1. Preparation of the medium:

The medium was obtained in a dehydrated form (Oxoid limited England).The medium was prepared according to manufacturer's instruction by suspending 23.5 gram in a liter of distilled water and dissolved by heating followed by sterilization.

3.4.1.2 Preparation of the diluent:

Ringer solution was prepared by dissolving one tablet (Oxoid) in 500 ml distilled water. The solution was distributed into 9 ml amount into clean test tubes and sterilized.

3.4.1.3. Sterilization:

Plate count agar (medium) and ringer solution were sterilized in the autoclave at 15 pounds pressure at a temperature of 121º C for 15 minutes. Petri dishes and glass ware were sterilized in hot air oven at 160º C for 1.5 hours.

3.4.1.4. Dilution:

Sterile dilutions of milk samples were prepared by using the following technique:

From the bottles containing milk samples, one ml of milk was transferred with sterile pipette into 9 ml ringer solution in a test tube and mixed thoroughly, using another sterile pipette one ml of prepared dilution was transferred into the second tube. The process was repeated to make ten fold dilutions from 10^{-1} to 10^{n} (n number of dilution), primary test showed that 10^{-5} to 10^{-7}(in winter), 10^{-12} to 10^{-16}(in summer) dilution were the most suitable dilutions for plating.

3.4.1.5. Plating technique:

New sterile one ml pipette was used to transfer one ml amount of selected dilutions into two sterile Petri dishes, then 10–12 ml of standard plate count agar at 45–46º C, were poured under sterile condition into each plate. The Petri dishes were rotated four times clock wise and four times anti clock wise to ensure thorough mixing. The mixture was allowed to solidify, them plates were inverted and incubated at 37º C for 48hours.

3.4.1.6. Counting:

Developed colonies on plates counting 25–250 colonies were counted using colony counter and the total viable bacterial count was calculated by multiplying the number of colony forming units by the reciprocal of the dilution used.

3.4.2 Milk Ring test:

The milk ring test (MRT or ART) is an agglutination reaction used to diagnose brucella as antigen, the test determined according to Harrigan and McCance (1976).

One drop (0.03 ml) of stained *brucella abortus* antigen was added to one ml of a well –mixed milk sample in a small narrow agglutination test tube. The sample was mixed thoroughly by shaking but avoids frothing. The mixture was incubated at 37º C for 30– 60 minutes and then the test was examined for ring formation.

If the cream layer was deeply coloured and the milk beneath the cream layer was white or nearly so, the test was regarded as positive, indicating the presence of brucella in milk. If the cream layer was white and milk beneath deeply coloured or the cream layer was the same colour as milk layer, the test was recorded as negative.

3.5. Statistical analysis:

Statistical analysis was performed using Statistical Analysis System (SAS, 1988). All the data of this experiment were analyzed statistically by using complete randomized design (C.R.D). Similarly the least significant difference test (L.S.D) was used to detect difference between means.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1. Effect of season of the year on milk quality and chemical composition:

Table 4.1 present the effect of season on milk quality and chemical composition. Mean total solids (T. S) content was significantly (P< 0.01) affected by season of the year with the highest content being in winter season (13.18± 0.827%). While the lowest total solids was in summer season (12 .70± 0.942%).

The results in this investigation are consistent with those obtained by Harding (1999), Reinoso and Simon (1999), Agabriel *et al.* (2001) and Sharma *et al.* (2002) who reported that the season of the year significantly affected the (T. S) content , with high (T.S) during winter and low (T.S) in summer season. The average (T.S) content found in winter season was agreed with that found in Sudan (13.19%) for local breeds (Kenana and Butana) as reported by ElFaki (1988).Moreover The results of T. S content in summer season were in agreement with those reported by Webb *et al.* (1980) and Clarence *et al.* (1982) for temperate breeds (12.8 %).

The average total solids content was lower than that reported by Salih (2001) who reported 16.15% for venders milk in Khartoum State, also the results equal or less than that reported by Hassan (1985) who reported the T.S ranged between 13.08–

14.34% for bulk milk . But the results of T. S content were higher than that reported by Hussein (1985) who reported 12.5% for zebu cattle crossed with Friesian.

The variation in T.S content (SD= 0.827and 0.942) in this investigation is probably due to variation in percentages of fat. High T. S probably due to high fat content of milk obtained form local breeds which were characterized by producing high fat milk as reported by ElFaki(1988). It might also be due to stage of lactation or due to seasonal changes in forage quality and availability and low T.S probably due to low fat content resulted form adulteration or due to low forage consumption during the summer season as reported by Harding (1999).

Table 4.1 shows that the mean protein content of milk was high in winter (3.29± 0.319%) and low in summer season (2.92± 0.400%), protein significantly (P< 0 . 001) affected by the season of the year.

These results agreed with those of Stallings (1998) who found that average protein percent of cows on DHI in Virginia, drops from 3.2% in March to 3.1 in August. Also Harding (1999) stated that percentage of protein is lowest during summer season and highest during cooler months . However, Buchberger *et al.*

(2002) reported highly significant difference for the factors year, farm and season for protein content.

The average protein content in summer was corresponding with Salih (2001) who reported 2.8% for venders milk in Khartoum State, mean protein content in winter season agreed with that reported by Eddleman (1999) who reported (3.3%) for commercial milk in India. Similarly Hassan (1985) in Gazira (Sudan) reported (3.28%), ElFaki (1988) reported 3.2% for local breeds and Dayyani *et al.* (2000) reported 3.12% for raw milk in lran. The results were less than that reported by Webb *et al.* (1980) and Clarence *et al.* (1982) who reported 3.5% for temperate breeds.

The variation in protein content was probably due to the species, the breed, the stage of lactation, the presence of an intramammary infection and type of feed (Hurley, 2002).

Table 4.1 also shows the average fat content of milk was significantly ($P< 0.05$) affected by season of the year, thus, the highest fat in winter season (4.33± 0.545). This variation is probably caused by changes in types of feed available and changes in the eating habits during hot and cold weather (Guthrie, 1994).

The results of milk fat in this investigation indicate relatively little variation (SD= 0.45 and 0.545) because milk was usually

taken from different farms where the product was usually mixed. It might also be due to the fact that samples were collected from farm had similar dairy breed, similar type of feed, and similar stage of lactation. High fat content was probably due to milk obtained from local breeds as reported by ElFaki (1988). While the low fat content is probably due to adulteration of milk.

The results in the study were consistent with those obtained by ElFaki (1988) who reported 4.39% for local breeds (Kenana and Butana). But higher than that reported by Eddleman (1999) for Holstein milk fat (3%) and commercial milks (3.8%) in India. Also Dayyani *et al.* (2000) reported 3.58% for raw milk in Iran, and Webb *et al* (1980) and Clarence *et al.* (1982) reported 3.7% for temperate breeds. However, our results were lower than those reported by Salih (2001) for venders milk (5.56%), Hassan (1985) for Gazira cooperative milk (5.06%) and Eddleman (1999) and Stallings (1998) for Jersey (5.1%).

Table 4.1 shows that the average ash content of milk was significantly ($P< 0.001$) affected by season of the year with the highest mean being in winter season (0.62± 0.111%), while the lowest mean was in summer season (0.53± 0.106%). Ash content had the lowest standard deviation (0.111 and 0.106) among milk constituents in this study. The small variation among samples may

be due to the fact that ash and lactose content are only components of milk showing little variation.

The ash results were in agreement with the values reported by Webb *et al.* (1980) and Clarence *et al.* (1982) reported 0.65% for temperate breeds. While the results were disagreed with that reported by Eddleman (1999) who reported 0.71% and Salih (2001) who reported 0.98% for venders milk in Khartoum State.

Table 4.1 also shows that the average freezing point of milk in winter and summer season were –0.53± 0.031 and –0.50± 0.095º C, respectively. Moreover, the freezing point significantly ($P< 0.01$) affected by season of the year. The mean freezing point in winter season (–0.53) indicated that there was no adulteration or addition of water , while mean freezing point in summer (–0.50) revealed addition of water or might be due to abnormal water intake by the cow or might be due to bad feeding habit due to high environmental temperature in the summer season (Harding, 1999).

The results of this study were in disagreement with the reports of Luck and Dresner (1975) who reported smaller freezing point in winter months (–0.532º C) than that during summer month (–0543º C).

The average freezing point of milk in summer season (–0.5º C) was in agreement with that reported by Harding (1999) who reported –0.5º C below the freezing point of water for normal milk. While the recommended freezing point standard officials of South Africa was –0.530º C (Luck and Dresner, 1975), was in agreement with the finding of the winter season in this study.

The average freezing point in this study was higher than that reported by Ibrahim (1989) who reported –0.539 and –0.548º C for Dairy Land and University farm respectively, and Boor *et al.* (1998) who reported –0.539º C to –0.545º C for raw milk in New York State .

Titratable acidity means of milk were 0.21± 0.030% in winter and 0.23± 0.036% lactic acid in summer season. Acidity significantly ($P< 0.001$) affected by the season of the year (Table 4.1). The deviation of acidity is expected under tropical conditions, due to the fact that high temperature enhance the growth and multiplication of lactic acid bacteria, sported to Harding (1999).

The findings in summer season were in agreement with those reported by Mohammadi (1988) who reported 0.21% in Omdurman and Khartoum North. The results in winter season were higher than those reported by Ibrahim (1989) who found

0.172 and 0.177% for Dairy Land and University farm respectively, ElTayeb (1973) who reported 0.18% for venders milk and 0.20% for dairy farms in Sudan, and approaching the levels reported for whole milk (0.22% lactic acid) by Abdalla (2002).

Table 4.1 shows that total bacterial count of milk was significantly ($P< 0.001$) affected by the season of the year with the lowest count being in winter season ($8.3\times10^{7}\pm$ 1.071 cfu/ml), while the highest count being in summer season ($2.75\times10^{16}\pm$ 1.702 cfu/ml).

These results agreed with findings of Hussain (2001) and Heeschen *et al.* (1987) who reported that highest bacterial counts were found during summer season. However, these results disagree with those reported by Titinie *et al.* (1991) who found that total bacterial count was highest during winter season compared to summer season.

The results indicated very poor quality of raw milk in Khartoum State, and high S.D (1.071and 1.702) indicated great variability of bacteriological quality of milk samples. This might be attributed to environmental conditions and milk handing or might be due to husbandry practices and feeding conditions (Lukac, 1990). The high total bacterial counts might be due to the

lack of knowledge about the production of clean milk, lack of potable water for cleaning purposes, the long distance from production farms to marketing centers and absence of cooling system during handling and transportation sported to FAO (2001) and Godefay and Molla (2000).

4.2. Effect of area of collection on milk quality and chemical composition:

Table 4.1 shows that the mean total solids content of milk from Khartoum was 13.03± 0.958%, Omdurman was 13.19± 0.787% and Khartoum North was 12.94± 0.863%. The mean total solids of milk from these cities were not significantly ($P< 0.05$) different. The standard deviation of 0.958, 0.787 and 0.863 is due to the variation between minimum and maximum values.

Mean total solids content was higher than those reported by Hussain (1985) for cross bred cows (12.5%) and Abdalla (2002) for whole milk in Khartoum State (12.02%). Results of this study agreed with ElFaki (1988) for local breed (13.19%) while T.S content equal or less than those reported by Hassan (1985) in Gazira cooperative (13.08– 14 .34%).

Table 4.1 also shows that the mean milk protein (%) in this study was significantly ($P< 0.001$) different when compared with the mean in Khartoum 2.99± 0.341%, Omdurman 3.32± 0.346%, and Khartoum North 3.41± 0.252%.

The above values for milk protein from Omdurman and Khartoum North exceed those reported by Abdalla (2002) for whole milk in Khartoum State (3.11%), and those reported by Salih (2001) for venders milk in Khartoum State (2.8%).

The results also showed that the mean fat content of milk from Khartoum was 4.40± 0.582%, Omdurman 4.28± 0.494%, and Khartoum North 4.01± 0.390%. Fat % significantly ($P< 0.01$) differed in these localities.

The values in this investigation is in agreement with those found by ElFaki (1988) for local breed; Kenana and Butana; (4.39%) and lower than those reported by Hassan (1985) for Gazira cooperatives (4.63– 5.26%) and Salih (2001) for venders milk in Khartoum State (5.56%), but higher than those reported by Abdalla (2002) for whole milk in Khartoum State (3.82%).

It can be seen from table 4.1 that the mean ash contents of milk from Khartoum, Omdurman and Khartoum North were 0.61± 0.138, 0.61± 0.110 and 0.57± 0.079%, respectively. There were no significant ($P< 0.05$) differences between these results for ash content.

The ash results in this study is lower than those reported by Salih (2001) for venders milk in Khartoum State (0.98%) and Abdalla (2002) who reported 0.72% for whole milk. This might be

due to weather and type of feed(Harding, 1999 and Stallings, 1998). The differences between the results of milk collected from Khartoum, Omdurman and Khartoum North were inconsistent with those reported by Smit *et al.* (2000) who found that there were differences in milk composition between five localities. He also reported that the variations were attributed to the different composition of feed used and the feeding practices.

The average freezing point of milk in Khartoum, Omdurman and Khartoum North were –0.52± 0358, –0.53± 0.077 and –0.52± 0.021º C, respectively. There were non significant differences (P> 0.05) between the freezing point obtained for milk from Khartoum, Omdurman and Khartoum North (table 4.1).

The freezing point values in this study are in agreement with the official recommended freezing point –0.53º C for raw milk, standard of South Africa (Luck and Dresner, 1975), while the results were higher than those reported by Ibrahim (1989) for University farm milk (–0.548º C).

The mean titratable acidity of milk collected from Khartoum North (0.19 ± 0.163%) lower than those from Khartoum(0.22± 0.039%) and Omdurman (0.22± 0.034%).Moreover, the comparison of titratable acidity between the three cities revealed highly significant variation (P< 0.01) as shown in Table 4.1

It was observed that the lower acidity of milk being in Khartoum North, this might be due to the fact that milk samples collected immediately after milking and transported to the laboratory, because farms are located near sale points, hence were subjected to less unfavorable conditions.

The above acidity results were in agreement with Mohammadi (1988) who reported acidity of 0.195% for milk from Khartoum North and 0.21% for Omdurman milk, and Abdalla (2002) for whole milk in Khartoum State who reported 0.18% – 0.22%. The results were higher than the finding of Ibrahim (1989) who reported 0.177% and 0.172% for Dairy Land and University farm, respectively.

Table 4.1 shows that the milk samples collected from Khartoum revealed high significant ($P< 0.01$) total bacterial count ($5.13\times10^{10}\pm5.146$ cfu/ml) followed by Omdurman ($1.05\times10^{10}\pm$ 2.591 cuf/ml). While the lowest total bacterial count was found in milk collected from Khartoum North ($9.3\times10^{7}\pm$ 0.668 cfu/ml). The lowest bacterial count being in milk from Khartoum North, which might be due to the fact that most of the milk in this area was cooled in the milk factory before distribution. It might also be due to the fact that farms and milk centers in Khartoum North are located near sale points. However, in Khartoum and Omdurman,

farms are located far from collection points and consumption areas, and it need to be transported from farms to consumer by means that make milk not subjected to contamination. Also long distance for transportation, high temperature and absence of cooling methods, all those factors and other factors, which might promote the bacterial growth (FAO, 2001).

The above results are in agreement with Hussain (2001) who reported that total bacterial count of milk was high in Khartoum area followed by Omdurman and the lowest bacterial count being in Khartoum North.

The results higher than that reported by Dayyani *et al.* (2000) for Iran raw milk (1.76×10^6 cuf/ml) and Godefay and Molla (2000) who reported 1.3×10^7cuf/ml for milk collected from collection centers in and around Addis Ababa.

Table 4.1: Effect of season and area of collection on milk quality and chemical composition.

Item	Total Solids (%)	Protein (%)	Fat (%)	Ash (%)	Freezing Point (° C)	Acidity (%)	TBC (cfu/ml)
Season	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Winter	13.18 ± 0.8527^{a}	3.29 ± 0.319^{a}	4.33 ± 0.545^{a}	0.62 ± 0.111^{a}	$- 0.53 \pm 0.031^{b}$	0.21 ± 0.030^{b}	$8.3 \times 10^{7} \pm 1.071^{b}$
Summer	12.70 ± 0.942^{b}	2.92 ± 0.400^{b}	4.11 ± 0.450^{b}	0.53 ± 0.106^{b}	-0.50 ± 0.095^{a}	0.23 ± 0.036^{a}	$2.75 \times 10^{16} \pm 1.702^{a}$
S . L	* *	* * *	*	* * *	* *	* * *	* * *
Area of colection							
Khartoum	13.03 ± 0.958^{a}	2.99 ± 0.341^{b}	4.40 ± 0.582^{a}	0.61 ± 0.138^{a}	-0.52 ± 0.358^{a}	0.22 ± 0.039^{a}	$5.13 \times 10^{10} \pm 5.146^{a}$
Omdurman	13.19 ± 0.787^{a}	3.32 ± 0.346^{a}	4.28 ± 0.494^{a}	0.61 ± 0.110^{a}	-0.53 ± 0.077^{a}	0.22 ± 0.034^{a}	$1.05 \times 10^{10} \pm 2.591^{a}$
Khartoum North	12.94 ± 0.863^{a}	3.41 ± 0.252^{a}	4.01 ± 0.390^{b}	0.57 ± 0.079^{a}	-0.52 ± 0.021^{a}	0.19 ± 0.163^{b}	$9.3 \times 10^{7} \pm 0.668^{b}$
S . L	NS	* * *	* *	NS	NS	* *	* *

In this table and the following tables: means within each column bearing the same superscripts are not significantly different (P> 0.05).

S.L = significance level.

* * * = P < 0.001

* * = P < 0.01

* = P < 0.05

NS = Not Significant.

TBC = Total Bacterial Count.

4.3 Quality and chemical composition of milk from sale points in Khartoum, Khartoum North and Omdurman:

Table 4.2 shows that mean T.S content did no show any significant difference (P> 0.05) between the three sale points studied in Khartoum.

The highest T.S content was in Child city collecting point (13.1± 0.998%), while the lowest T.S was found in Al Sagana (12.93± 1.059%). The high SD of 1.059 and 0.998 is due to variation between minimum and maximum values. This variation is probably due to variation in fat %.

The total solids results for milk from Khartoum North did not show any significant difference (P> 0.05). Yet, the highest T.S content was found in Kuku center (13.15± 0.919%), while the lowest total solid was in Shambat (12.71± 0.767%).

Table 4.2 shows that the mean T.S content did not show any significant difference (P> 0.05) between the four collection points in Omdurman. The highest T.S was in ALAhamda collecting point (13.63± 0.519%), while the lowest T.S was in AL Fetaihab sale points (13.04± 0.674%).

The above results agreed with those reported by EL Faki (1988) for local breed (13.19%) and equal or less than that

reported by Hassan (1985) for bulk milk supplied by Gazira cooperative which varied between 13.08 to 14.34%.

The results were higher than those reported by Abdalla (2002) for whole milk in Khartoum State (12.02%) and lower than that reported by Salih (2001) for venders milk in Khartoum State (16.15%).

Table 4.2 also showed that the average protein % of milk was significantly ($P< 0.05$) affected in Khartoum. Higher protein values were found in Al Sagana (3.12± 0.254%). While the lowest protein content was found in the 60 street (2.85± 0.361%).

In Khartoum North milk collected from Kuku had the highest protein content (3.43± 0.212%), while the samples from Shambat had the lowest protein (3.38± 0.275%), and did not show any significant difference between the two points studied ($P> 0.05$). Also protein content in Omdurman did not show any significant difference ($P> 0.05$), with the highest protein in AL Ahamda (3.44± 0.257%), and lowest protein values were in Al Azhary street (3.24± 0.364%) .

The protein content of milk samples obtained from the 60 street (2.85± 0.361%) agreed with those reported by Salih (2001) for vender's milk (2.80%), while the other results were in agreement with those reported by Ballou *et al*. (1995), Dayyani *et*

al. (2000), and Abdalla (2002) they reported3.1% Hassan (1985) reported (3.03– 3.28%) and Eddleman (1999) reported 3.3% for commercial milk in Indiana.

Table 4.2 present the results of fat percentage for milk from the three cities. It is clear that mean fat content did not show any significant differences (P> 0.05) between collections points in any city studied. In Khartoum, milk collected from 60 street had the lowest fat content (4.24± 0.618%), while the highest fat was in Al Sagana (4.63± 0.552%). Yet, the highest mean fat content was for milk collected from Shambat (4.03± 0.395%) and lowest in Kuku (3.99± 0.397%) in Khartoum North city. However, in Omdurman, Al Azhary street had the lowest fat content (4.18± 0.488%), while the highest fat content was found in AL Ahamda (4.54± 0.680%).

The above results were consistent with those obtained by EL Faki (1988) who reported 4.39% for local breed. The results were higher than those reported by Abdalla (2002) for whole milk (3.82%) but lower than those reported by Hassan (1985) who claimed that the fat ranged from 4.63 to 5.26%.

The results showed that the ash content of milk was significantly (P< 0.01) affected in Khartoum area, also ash was significantly affected (P< 0.05) in Omdurman. While the milk

samples collected from Khartoum North did not show any significant difference (P> 0.05) as shown in table 4.2.

In Khartoum the lowest ash content was in Child city (0.56± 0.126%), while the highest ash content was found in AlSagana (0.68± 0.096%). In Khartoum North, the highest ash was in Kuku (0.58± 0.085), While the lowest ash in Shambat (0.56± 0.073%). However, in Omdurman, the highest ash content was found in AL Ahamda (0.72± 0.070%), while the lowest ash was found in the AL Fetaihab collecting point (0.58± 0.132%).

The results of Al Ahamda were in agreement with the results of Eddleman (1999) who reported 0.71% for commercial milk and Abdalla (2002) who reported 0.72% for whole milk in Khartoum State.

Also the results obtained from Al Saganan (0.68%) agreed with ash content of temperate breed milk (0.65%) as reported by Webb *at al.* (1980) and Clarence *et al.* (1982). Ash content of milk obtained during this study was lower than those reported by Salih (2001) for venders milk (0.98%).

Table 4.2 shows that average freezing point of milk in Khartoum was significantly (P< 0.05) affected by the different sale points studied, they ranged between –0.50± 0.034º C In Child city to –0.53± 0.035º C in 60 street. The average freezing point of the different collecting points in Khartoum North was highly significant (P< 0.001). The freezing point was high in kuku (– 0.51± 0.025º C) and low in Shambat (– 054± 0.009º C). However, in Omdurman the freezing point did not show any significant differences (P> 0.05), the highest freezing point being in Al Azhary (–0.46± 0.243º C), while the lowest freezing point was in Libya market and Al Ahamda (–0.56± 0.013º C).

The results obtained from Al Azhary (–0.46º C) revealed adulteration by addition of water, while the other results indicate that the milk freezing point is normal.

The freezing point values of milk were in agreement with those reported by Ibrahim (1989) for Dairy Land Farm (–0.539º C) and for University farm (–0.548º C) and Mohammadi (1988) also reported freezing point of–0.5583º C for milk samples collected from Khartoum North and –0.5525º C for University of Khartoum and –0.556º C for samples collected from Al Saganan, Burri and Omdurman.

Table 4.2 present the results of the acidity of milk. The average lactic acid percentage in Khartoum was significantly ($P< 0.05$) affected by the different collection points studied, with highest acidity values being in 60 street and Child city (0.23± 0.040%), while the lowest acidity was in Al Saganan (0.19± 0.016%). In Khartoum North, the acidity results were significantly affected ($P< 0.01$). The highest acidity (0.20± 0.017%) was in milk samples collected from Kuku, while the lowest acidity (0.19± 0.011%) was in milk samples collected from Shambat. However acidity was significantly ($P< 0.001$) differed in the milk samples collected from Omdurman, with the highest acidity being in Libya market (0.24± 0.032%), and lowest acidity was obtained in milk collected from Al Ahamda (0.18± 0.011%).

The acidity percentage revealed a minimum of (0.18± 0.011%) lactic acid for milk collected from AL Ahamda, this result in agreement with that reported by Ibrahim (1989) who reported 0.177% for Dairy Land farm. Low acidity in AL Ahamda was probably due to the fact that the farms and milk sale points in AL Ahamda located near consumption areas, also the samples were transported to the laboratory immediately after collection. But samples collected from Libya market revealed a maximum values (0.24± 0.032%) of lactic acid. The presents finding was

higher than the reported by Abdalla (2002) for whole milk in Khartoum State (0.22%). The highest acidity in Libya market (0.24%) might be due to the fact that the farms are located at longer distance from collection points and consumption areas, or might be due to the fact that this area suffers from lack of potable water for cleaning purposes.

Table 4.2 shows that in Khartoum the total bacterial count of milk was highly significantly ($P< 0.01$). Affected samples collected from Child city showed the highest bacterial count ($2.88\times10^{12}\pm 5.995$ cfu/ml) and lowest count was obtained in samples collected from Al Saganan ($2.57\times10^{7}\pm 1.163$ cfu/ml). In Khartoum North, the total bacterial count was significantly ($P< 0.001$) affected with highest count in Shambat ($2.45\times10^{8}\pm 0.572$ cfu/ml) and lowest in samples collected from Kuku ($3.72\times10^{7}\pm 0.486$ cfu/ml). Total bacterial counts of Milk samples from Omdurman were highly significantly different ($P< 0.001$), the highest count was obtained in samples collected from Al Azhary ($3.55\times10^{11}\pm 3.424$ cfu/ml), while the lowest count ($1.70\times10^{8}\pm 0.191$cfu/ml), was from Al Fetaihab.

The results of total bacterial count showed great variation in milk samples collected form 60 street, Child city and Al Azhary, respectively. This might be due to the fact that the milk coming to

Khartoum form different areas, which were far from sale points and subjected to various factors which can influence its quality. These various factors and various farms make grate variation between samples collected from sale points (Table 4.2). Moreover, the result obtained during the present study was found to influence the acidity of milk as shown in Table 4.1 and 4.2.

The results of total bacterial count were higher than that reported by Barakat (1995) who reported 4.5×10^5– 9.5×10^6 cfu/ml), Hussain (2001) and Dayyani *et el.* (2000). The results obtained from AlSaganan, Kuku, Shambat, Al Ahamda, and Al Fetaihab were slightly equals or exceeds that reported by Godefay and Molla (2000) who reported 1.3×10^7 cfu/ml for raw milk collected from sale points in and around Addis Ababa, and 1.9×10^8 cfu/ml for milk samples collected upon arrival at the processing plant. These highest bacterial count is expected under tropical condition, in addition to absence of sanitary control for production, handing and transportation (FAO, 2001).

Table 4.2: Effect of sale points in Khartoum, Khartoum north and Omdurman on milk quality and chemical composition .

Item	Total solid (%)	Protein (%)	Fat (%)	Ash (%)	Freezing Point (°C)	Acidity (%)	T B C (cfu/ml)
Khartoum	Mean ± SD	Mean± SD	mean± SD	mean± SD	mean± SD	mean ± SD	mean ± SD
60 Street	12.99 ± 0.843^{a}	2.85 ± 0.361^{b}	4.24 ± 0.618^{a}	0.57 ± 0.156^{b}	-0.53 ± 0.035^{b}	0.23 ± 0.040^{a}	$1.23 \times 10^{12} \pm 5.245^{a}$
Child city	13.17 ± 0.998^{a}	$3.01 \pm .355^{ab}$	4.35 ± 0.533^{a}	0.56 ± 0.126^{b}	-0.50 ± 0.034^{a}	0.23 ± 0.040^{a}	$2.88 \times 10^{12} \pm 5.995^{a}$
Al Sagana	12.93 ± 1.059^{a}	3.12 ± 0.254^{a}	4.63 ± 0.552^{a}	0.68 ± 0.096^{a}	-0.52 ± 0.035^{b}	0.19 ± 0.016^{b}	$2.57 \times 10^{7} \pm 1.163^{b}$
S.L	NS	*	NS	* *	*	*	* *
Khart. North							
Kuku	13.15 ± 0.919^{a}	3.43 ± 0.212^{a}	3.99 ± 0.397^{a}	0.58 ± 0.085^{a}	-0.51 ± 0.025^{a}	0.20 ± 0.017^{a}	$3.72 \times 10^{7} \pm 0.486^{b}$
Shambat	12.71 ± 0.767^{a}	3.38 ± 0.275^{a}	4.03 ± 0.395^{a}	0.56 ± 0.073^{a}	-0.54 ± 0.009^{b}	0.19 ± 0.011^{b}	$2.45 \times 10^{8} \pm 0.572^{a}$
S.L	NS	NS	NS	NS	* * *	* *	* * *
Omdurman							
Al Azhary Street	13.05 ± 0.910^{a}	3.24 ± 0.364^{a}	4.18 ± 0.488^{a}	0.59 ± 0.106^{b}	-0.46 ± 0.243^{a}	0.22 ± 0.025^{b}	$3.55 \times 10^{11} \pm 3.424^{a}$
Libya market	13.27 ± 0.719^{a}	3.40 ± 0.316^{a}	4.19 ± 0.442^{a}	0.60 ± 0.090^{b}	-0.56 ± 0.013^{a}	0.24 ± 0.032^{a}	$2.40 \times 10^{9} \pm 0.435^{b}$
Al Ahamda	13.63 ± 0.519^{a}	3.44 ± 0.257^{a}	4.54 ± 0.680^{a}	0.72 ± 0.070^{a}	-0.56 ± 0.013^{a}	0.18 ± 0.011^{b}	$3.89 \times 10^{8} \pm 0.344^{b}$
Al Fetaihab	13.04 ± 0.674^{a}	3.25 ± 0.411^{a}	4.51 ± 0.331^{a}	0.58 ± 0.132^{b}	-0.55 ± 0.007^{a}	0.19 ± 0.15^{b}	$1.70 \times 10^{8} \pm 0.191^{b}$
S.L	NS	NS	NS	*	NS	* * *	* * *

4.4 Effect of variation sale points in Khartoum State **on milk quality and chemical composition:**

Table 4.3 presents the results obtained from the different sales points in milk composition and microbial quality. It shows that T.S content did not reveal any significant difference (P> 0.05) between the centers studied, the highest T.S content was in ALAhamda (13.63± 0.519%), while the lowest T.S was found in Shambat (12.71± 0.767%).

Table 4.3 shows that the protein content results were highly significant (P< 0.001) affected, with highest protein in ALAhamda (3.44± 0.257%) and lowest protein was in the 60 street (2.85± 0.361%). The average fat content was significantly affected (P< 0.01). Yet highest fat was found in milk obtained from ALSagana (4.63± 0.552%) and lowest fat (3.99± 0.397%) was in samples collected from Kuku.

It can be seen from table 4.3 that the mean ash content of milk samples collected from ALAhamda (0.72± 0.070%) was high, while low ash (0.56± 0.073%), (o.56± 0.126%) was found in Shambat and Child city, respectively.

The findings in this study were in agreement with those of Smit *et al* (2000) who found that the nutrient content of whole milk differed between the five location studied in South Africa and that the variation was attributed to the different composition of the feed used and the feeding practices.

Table 4.3 shows that milk samples collected from Khartoum, Omdurman and Khartoum North revealed significant differences ($P< 0.05$) for mean freezing point. In Libya market and ALAhamda (–0.56± 0.013º C), while highest freezing point was in samples collected from ALAzhary street (–0.46± 0.243º C). Low freezing point in Libya market (–056± 0.013º C) probably attribute to high acidity, due to fact that the developed acidity will depress the freezing point (IDF, 1983)

Table 4.3 shows that the average lactic acid ($P< 0.001$) was (0.24± 0.032%) in Libya market followed by ALAhamda (0.18 ± 0.011%). Also it shows significant differences ($P< 0.001$) between the milk of the different sales points studied for total bacterial count, with the highest count being in Child city (2.88×10^{12}± 5.995 cfu/ml), and the lowest count was in ALSagana (2.57×10^{7}± 1.163 cfu/ml).

Table 4.3: comparison of the different sale points on milk quality and chemical composition :

Item	Total solid (%)	Protein (%)	Fat (%)	Ash (%)	Freezing point^{o} C	Acidity (%)	T B C (cfu/ml)
Center	mean ± SD	mean ± SD	mean ± SD	Mean ± SD	mean ± SD	mean ± SD	mean ± SD
60 Street	12.99 ± 0.843^{a}	2.85 ± 0.361^{c}	4.24 ± 0.618^{bc}	0.57 ± 0.156^{c}	-0.53 ± 0.035^{ab}	0.23 ± 0.040^{ab}	$1.23\times10^{12}\pm5.245^{a}$
AL Sagana	12.93 ± 1.059^{a}	3.12 ± 0.254^{b}	4.63 ± 0.552^{a}	0.68 ± 0.096^{ab}	-0.52 ± 0.035^{ab}	0.19 ± 0.016^{d}	$2.57\times10^{7}\pm1.163^{c}$
Child city	13.17 ± 0.998^{a}	3.01 ± 0.355^{bc}	4.35 ± 0.533^{abc}	0.56 ± 0.126^{c}	-0.50 ± 0.034^{ab}	0.23 ± 0.040^{ab}	$2.88\times10^{12}\pm5.995^{a}$
AL Azhary street	13.05 ± 0.910^{a}	3.24 ± 0.364^{ab}	4.18 ± 0.488^{bc}	0.59 ± 0.106^{c}	-0.46 ± 0.243^{a}	0.22 ± 0.25^{bc}	$3.55\times10^{11}\pm3.424^{ab}$
Libya market	13.27 ± 0.719^{a}	3.40 ± 0.316^{a}	4.19 ± 0.442^{bc}	0.60 ± 0.096^{bc}	-0.56 ± 0.013^{b}	0.24 ± 0.032^{a}	$2.40\times10^{9}\pm0.435^{bc}$
AL Ahamda	13.63 ± 0.519^{a}	3.44 ± 0.257^{a}	4.54 ± 0.680^{ab}	0.72 ± 0.070^{a}	-0.56 ± 0.013^{b}	0.18 ± 0.011^{d}	$3.89\times10^{8}\pm0.344^{c}$
AL Fetaihab	13.04 ± 0.674^{a}	3.25 ± 0.411^{ab}	4.51 ± 0.331^{ab}	0.58 ± 0.132^{c}	-0.56 ± 0.007^{b}	0.19 ± 0.015^{d}	$1.70\times10^{8}\pm0.191^{c}$
Kuku	13.15 ± 0.919^{a}	3.43 ± 0.212^{a}	3.99 ± 0.397^{c}	0.58 ± 0.085^{c}	-0.51 ± 0.025^{ab}	0.20 ± 0.017^{cd}	$3.72\times10^{7}\pm0.486^{c}$
Shambat	12.71 ± 0.767^{a}	3.38 ± 0.275^{a}	4.04 ± 0.395^{c}	0.56 ± 0.073^{c}	-0.54 ± 0.009^{b}	0.19 ± 0.011^{d}	$2.44\times10^{8}\pm0.572^{c}$
S . L	NS	* * *	* *	* *	*	* * *	* * *

4.5. Clot on boiling and alcohol test for milk samples collected from Khartoum State:

It can be seen in table 4.4 that the clot on boiling test showed that 24.1% 3.4% and 29.1% of the milk samples tested were positive in Khartoum, Khartoum North and Omdurman respectively, while alcohol stability test showed 69%, 44.8% and 72.7% of the milk sample tested were positive in Khartoum, Khartoum North and Omdurman, respectively.

Khartoum North revealed minimum percentage of clot on boiling and alcohol test. This was attributed to the fact that Khartoum North has a minimum bacterial count and acidity (dairy farms located near the sales points and cooling facilities is also available). About 55.2% of the milk samples collected from Khartoum North were acceptable.

4.6. Clot on boiling and alcohol test for milk samples collected during summer and winter season:

Table 4.5 shows that the colt on boiling tests were 15% and 36.4% of the milk samples tested were positive during winter and summer season, respectively. However, alcohol tests showed 58.8% and 81.8% were positive in winter and summer season, respectively.

High percentage of clot on boiling and alcohol test in summer season might be due to high acidity and total bacterial counts (Girgis *et al*., 2001 and Shekarforoush *et al*., 2003).

Libya market had the highest positive results (58.8%) for clot on boiling test in samples collected during both summer and winter season due to high acidity in milk samples, which attributed to poor hygienic control in milk production, handing and transportation.

4.7. Prevalence of brucella in milk samples collected from Khartoum State:

Table 4.6 presents the prevalence of brucella in milk collected Khartoum, Khartoum North and Omdurman. Samples collected from Khartoum North showed high prevalence (82.8%) of brucella, followed by Khartoum (76.9%), while the lowest prevalence was found in the milk samples collected from Omdurman (73.8%).

Table 4.4: Percentages of alcohol and colt on boiling test for milk sample collected from Khartoum State.

Area of study	No. of samples tested	Clot on boiling test (%)		Alcohol test (%)	
		+ve	-ve	+ve	-ve
Khartoum	29	7 (24.1)	22 (75.9)	20 (69)	9 (31)
Khartoum North	29	1 (3.4)	28 (96.6)	13 (44.8)	16 (55.2)
Omdurman	55	16 (29.1)	39 (70.9)	40 (72.7)	15 (27.3)

Table 4.5: Percent of alcohol and colt on boiling test for milk samples collected during summer and winter season:

points / Season	No. of samples tested	Colt on boiling test (%)		Alcohol test (%)	
		+ ve	- ve	+ ve	- ve
Winter					
Al Sagana	9	0 (0)	9 (100)	4 (44)	5 (56)
Al Azhary street	10	1 (10)	9 (90)	10 (100)	0 (0)
Libya market	17	10 (58.8)	7 (41.2)	16 (94.1)	1 (5.9)
Al Ahamda	7	0 (0)	7 (100)	1 (14.3)	6 (85.7)
Al Fetaihab	8	0 (0)	8 (100)	3 (37.5)	5 (62.5)
Kuku	15	1 (6.7)	14 (93.3)	9 (60)	6 (40)
Shambat	14	0 (0)	14 (100)	4 (28.6)	10 (71.4)
Total	80	12 (15)	68 (85)	47 (58.8)	33 (41.2)
Summer					
60 street	10	4 (40)	6 (60)	8 (80)	2 (20)
Child city	10	3 (30)	7 (70)	8 (80)	2 (20)
Al Azhary street	13	5 (38.5)	8 (61.5)	11 (84.6)	2 (15.4)
Total	33	12 (36.4)	21 (63.6)	27 (81.8)	6 (18.2)

AL Fetaihab revealed a maximum percentage of brucella (100%), while the minimum percentage was found in Libya market (58.8%).

The above results were in disagreement with Habiballa (1977) who reported 6.7– 28.5% of brucella in dairy farms in some parts of Sudan and 36.0% in livestock in Gazira. Similarly, Abdalla (1966) reported 3% prevalence of brucella in Wadi Halfa.

From this study it is clearly that there is high prevalence of brucella in Khartoum State. This high prevalence might be due to the type of test used; Ring Test (Bulk milk test), or due to absence of hygienic consciousness of the farmers about brucella hazard for animals and humans. Or might be due to lack of knowledge about hygienic practices (no certified animal against brucella, no brucella free animal for milk, the local custom of drinking raw milk before heat treatment, or due to the fact that the public herd have one or two sirs (male) for reproduction makes the transmission of disease is easy and by

uncontrolled movement of humans and stock across political borders).

Table 4.6: Prevalence of brucella in milk samples collected from Khartoum State:

Area of study	Sale points	Total no. of sample test	+ ve %	- ve %
Khartoum	60 street	10	7 (70)	3 (30)
	Al Sagana	19	16 (84.2)	3 (15.8)
	Child city	10	7 (70)	3 (30)
Sub total		**39**	**30 (76.9)**	**9 (23.1)**
Omdurman	Al Azhary street	10	9 (90)	1 (10)
	Libya market	17	10 (58.8)	7 (41.2)
	Al Ahamda	7	4 (57.1)	3 (42.9)
	Al Fetaihab	8	8 (100)	0 (0)
Sub total		**42**	**31 (73.8)**	**11 (26.2)**
Khartoum North	Kuku	15	11 (73.3)	4 (26.7)
	Shambat	14	13 (92.9)	1 (7.1)
Sub total		**29**	**24 (82.8)**	**5 (17.2)**
Total		**110**	**85 (77.3)**	**25 (22.7)**

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1: Conclusions:

From this study the following conclusion could be drawn:

1. Milk collected during winter season had better composition and quality than that collected during summer season. Milk collected during summer season was highly contaminated (total bacterial count). Samples tested during summer season were positive 36.4% and 81.8% for clot on boiling test and alcohol test respectively, while only 15% and 58.8% of samples tested during winter season were positive.
2. No significant variations or differences observed in the milk composition between the three areas studied except in protein and fat. Milk from Khartoum was highly contaminated followed by milk from Omdurman and the milk from Khartoum North was the least contaminated (total bacterial count). Samples collected from Khartoum North showed less positive 3.4% and 44.8% clot on boiling test and alcohol test, respectively. While 29.1% and 72.7% of the milk samples collected from Omdurman were

positive for colt on boiling and alcohol test, respectively and samples collected from Khartoum ware 24.1% and 69% positive for clot on boiling test and alcohol test, respectively.

3. In Khartoum, AL Sagana had the lowest total bacterial count. In Khartoum North, Kuku had the lowest count. While in Omdurman, Al Fetaihab had the lowest total bacterial count.
4. Milk from Khartoum North showed high prevalence of brucella (82.8%), followed by Khartoum (76.9%) and low prevalence was found in Omdurman (73.8%).

5.2. Recommendations:

According the findings of the present study, we suggest the following recommendations:

1. We strongly recommend the use of lactoperoxidase system of milk preservation, which has a bacteriostatic effect on the raw milk and effectively extends the shelf–life of the raw milk under tropical conditions for 7–8 hours. In addition to the preservation, it has no adversely effect no the nutritional characteristics of raw milk. The lactoperoxidase system was approved by the FAO/WHO Expert Committee on food Additives in 1989 and by the Codex Alimentarius Commission in 1991 (FAO, 2001). Because this system is cheap, easy to use and readily applicable in developing countries with minimum of training requirements

as reported by (FAO, 2001). However, cooling is rarely an applicable preservation technique for small scale producers in studied areas due to the absence of reliable electrical supply and/or economic constraints. Hence sale points with facilities should be stimulated.

2. The problem of high prevalence of brucella can be reduced by raising the awareness of farmers about the brucella free animals and where they can diagnose and vaccinate their animals and the importance of eradication of the brucella in their herds. They should also trained never to mix milk obtained from brucellosis animals with the clean milk.
3. Establishment of proper collection points and centers with facilities
4. Quality control and quality assurance programs for examination and grading of milk should be initiated.
5. Prevention of offering raw milk for sales and processing it into heated products.
6. Eradication of milk and milk borne diseases.

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