COMPOSITION AND HYGIENIC QUALITY OF SUDANESE WHITE SOFT CHEESE IN KHARTOUM NORTH MARKETS

By

LEMYA MOHAMED WARSAMA

B.Sc. (Hon.) in Animal Production

University of Khartoum

1999

A dissertation submitted in partial fulfilment for requirement of the degree of M.Sc. in Dairy Production and Technology

Supervisor: Dr. Ibtisam Elyas Mohamed Elzubeir

Department of Dairy Production

Faculty of Animal Production

University of Khartoum

Dec. 2003

DEDICATION

TO MY FAMILY

Π

قال تعالى [وإن لكم في الأنعام لعبرة نسقيكم مما في بطونه من بين فرثٍ ودمٍ
لبناً خالصاً سائغاً للشاربين]

صدق الله العظيم

(سورة النحل الآية 66)

ACHNOWLEDGEMENTS

My faithful thanks and praise to Allah.

No words of mine can express my great thanks and gratitude to my supervisor Dr. Ibtisam Elyas Mohamed for her proper guidance, leadership, friendly attitude and supervision throughout this work. I would like to appreciate the help offered by Dr. Abdel Moneim Abu Nikhaila, Dept. of Dairy Production.

I am very much indebted to all the technical staff of the Department of Dairy Production, Faculty of Animal Production for their cooperation and help.

Last but not least my appreciation is due to my husband Hytham Ahmed for his patience, financial support and encouragement during the course of this work. Thanks are due to Mrs Elmagam Ali Hassan who typed this thesis.

ABSTRACT

The study based on surveying thirty sex samples of Sudanese white soft cheese from Bahrry (Elmahata Elwasta) and Elsafia (Khartoum North).

The comparison of the chemical composition (total solids, total protein, fat, ash and acidity) and some of the microbial hazards (*E. Coli, Salmonella* spp. and *Staphylococcus aureus*) associated with cheese were estimated. The mean of total solids, ash, fat, protein and acidity for the samples were (47.8, 6.2, 14.0, 15.9 and 0.4) respectively. The results indicated that there was nonsignificant differences between total solids, ash, fat, acidity and protein in all batches in different groups ($P > 0.05$).

When comparing Bahrry (Elmahata Elwasta) and Elsafia a nears different results were obtained for bacteriological results. High total bacteria and coliforms counts were observed in all cheese samples. Positive isolates for 2 *E. coli*, 4 *Salmonella* spp. and 4 *S. aureus*, were respectively found in all restaurants in Bahrry and Elsafia. Similarly 4, 2, 2 of *Salmonella, S. aureus* and *E. coli* were respectively found in supermarkets and groceries.

Π

خلاصة الأطروحـة

إهتمت هذه الدراسة بالمسح الميداني لبعض العينات من الجبنة السودانية البيضاء المباعة في منطقة الخرطوم بحري بغرض تحديد جود ا ،

تمّ تجميع **36 عينة من المطاعم ، البقالات والسوبرماركت** في **كل من بحري (المحطة الوسطى) والصافية.**

شملت **التحاليل الكيميائية للعينات تقدير نسبة الدهون ، البروتين ، الرماد ، المواد الصلبة الكلية ودرجة الحموضة الى جانب العد البكتيري والتصنيف لبعض أنواع البكتيريا**

بلغ المتوسط الحسابي للمواد الصلبة الكلية ، الرماد ، الدهن ، البروتين ونسبة الحموضة 47.8 ، 6.2 ، 14.0 ، 15.9 و 0.4 على

) . بينما $P > 0.05$ التوالي . أظهرت النتائج انه لا يوجد إختلافاً معنوياً في هذه المكونات الكيميائية عند مقارنة جميع العينات (

أظهرت نتائج التحليل الباكتيري وجود اختلافات في العينات من مصادر الجمع المختلفة.

رصدت معدلات عالية للعدد الكلي للبكتيريا ولأعداد بكتيريا القولون في **جميع عينات الجبن. أيضاً تمّ عزل عدد 4 *E. coli* 2, *Salmonella spp.* 4, *S. aureus* 2**

في **مطاعم منطقتي بحري (المحطة الوسطى) والصافية الى جانب السبوبرماركت والبقالات.** *E. coli* 2 *Salmonella spp.*4, *S. aureus* 2

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Cheese was originally developed as a mean of preserving raw milk in time of excess production and cheese is generally considered to be relatively ‘safe’ food. However, the spread of some diseases by cheese has been demonstrated and as a result, most cheese is now produced from milk that has been pasteurized (Professional Food Microbiological Group, 1998). They added that although cheese outbreaks don’t present a large proportion of all outbreaks of food borne illness, on some occasions the consequences for the victims have been particularly severe. Moreover, organisms such as *Listeria monocytogenes*, *Brucella melitensis* and *Escherichia coli* O157 have been involved in cheese associated outbreaks and they have caused severe infections.

Listeria monocytogenes can cause meningitis and septicemia with up to 30% mortality as well as abortion in pregnant women (Professional Food Microbiological Group, 1998). Some *Salmonella* spp. can cause septicemia and may result in long-term illness such as reactive arthritis, *Brucella melitensis* causes undulant fever, a severe condition that can be long lasting and incapacitating (Professional Food Microbiological Group, 1998). Verocytotoxin producing strains of *Escherichia coli* (including *E. coli* O157) may cause haemorrhagic colitis, haemolytic uraemic syndrome and renal failure, which may result in death, particularly in young children.

It is indisputable that some outbreaks of food borne illness have been clearly linked with the consumption of cheese, the majority of those reported being associated with cheese made from unpasteurized milk. Whilst pathogens can gain access to cheese after curd formation, it is clear that many food borne pathogens are fecal in origin. Moreover, pathogens may also be excreted into the milk directly from the udder (Rampling, 1996). Correctly controlled milk pasteurization kills such bacteria. Moreover, pasteurization was designed to destroy the vegetative pathogens that may be found in raw milk (Professional Food Microbiological Group, 1998). They also added that pasteurization of milk dramatically reducing the 19th century diseases such as milk borne tuberculosis, brucellosis and typhoid fever that were widely recognized.

One of the most frequent foods borne microbial disease is staphylococcal food poisoning, which is caused by *S. aureus* or it's metabolites (Holeckova *et al.*, 2002).

Hence, the present study was undertaken with following objectives:

1- To estimate the chemical composition of Sudanese white cheese from the markets in Khartoum North.

2- To estimate the hygienic quality and to determine some potentially food borne pathogens associated with Sudanese cheese.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cheese

Cheese is a fresh matured product obtained after coagulation of milk, cream, skim or partly skimed milk, butter milk or a combination of these products (FAO/WHO, 1973).

Cheese is important for human nutrition when other animal protein is not available (Kosikowiski, 1966). It is an excellent, tasty, 99% digestible energy food, which is suitable for all age groups (Kosikowiski, 1977). It contains high quality proteins, vital minerals and vitamins (Ibrahim, 1971).

Babiker (1987) demonstrated that the chemical composition of cheese was affected by the manufacturing method and type of milk used. Hofi *et al.* (1982) demonstrated that the moisture content of cheese made from raw and pasteurized milk was 60.9±2.17 and 62.18±0.46 respectively. However Khalid (1991) studied the effect of sodium chloride concentration on the yield and chemical composition of Sudanese white cheese and concluded that the yield of fresh cheese was 22.9 and 23.9% for milk salted with 6% and 8% sodium chloride respectively.

2.1.1 Sudanese white cheese

Soft cheese characterized by high moisture content (55 – 80), it is made from whole milk standard with cream or skim milk.

Moreover, it is made from the milk of cows, ewe's goats or any mixture of them (Kosikowiski, 1977).

In Sudan the nomadic people in eastern central and in eastern areas (latitudes 12^{o} to 16^{o} and longitudes 26^{o} to 36^{o}) have surplus of milk during the rainy season. Hence they made cheese as a method of milk preservation (Ibrahim, 1971).

The most famous making cheese centers in Sudan are Eldueem in White Nile State and Elobeid in Northern Kordofan State (Ibrahim, 1971).

2.1.1.1 Sudanese white cheese

This cheese is traditionally manufactured in Eldueem, Elobied and Nyala areas. The traditional method of manufacturing Sudanese white cheese was described by Babiker (1987). Milk is brought to the factory immediately after milking and warm during winter time. Salt is added at the rate of 8 – 10%. The salt is first dissolved in a small amount of milk and sieved with cheese cloth into the milk. Rennet tablets (two tablets/100 kg milk) were first crushed and mixed with a small amount of water and added to the milk and stirred for two minutes. After coagulation the curd is cut into small cubes and transferred into tins or cans covered with a cloth until next morning. Whey is added to cheese in tins and sealed with soldering.

2.1.1.2 Chemical composition of Sudanese white cheese

Abdalla (1992) determined the chemical composition of white cheese made from pasteurized milk and preserved in whey at 9^{o}C for 60 days. He found the fat content, protein, total solids and ash content decreased in curd during ripening while the acidity and salt content did not change.

Ahmed and Khalifa (1989) found that the yield of cheese from fresh cow's milk, recombined milk with 1% fat and 2% fat were 19.0, 35.0 and 41.5 kg/100 kg milk respectively. The moisture content was 56.06, 68.43 and 66.70 and the total proteins were 21.32, 14.01 and 11.02%. While the soluble nitrogen yielded 0.33, 0.50 and 0.41%, respectively. The acidity was 0.40, 0.90 and 1.20% while the ash content was 4.48, 4.82 and 4.13%, respectively.

Ibrahim (1971) studied some characteristics of white cheese purchased in Khartoum area. He found that the pH of cheese varied between 3.6 and 8.3 with a mean of 4.6.

Allah Gabo (1986) studied the composition and quality of white cheese and found that the pH of cheese was 3.9 while total solids content was 38 – 85%, the protein content was 22.6% and the fat content was 12.65%. Moreover Abdel Razig (1996) reported that the cheese yield of fresh cows milk with 2% sodium chloride was 19.0% and chemical composition of the cheese was 23.4%, 15.3%, 44.09% and 2.21% for fat, protein, total solids and ash respectively.

2.2 Type of common bacteria in cheese

2.2.1 Useful bacteria

There are some microorganisms that have many advantages, their amino acids, cellular protein and vitamins supplements improve nutritive value of foods (Bantwart, 1980). Similarly, Jay (1986) reported that there are numbers of microorganisms that ferment lactose and produce lactic acid and these bacteria include the genera of *Streptococcus, Lactobacillus* and *Leuconostoc*.

Foster *et al.* (1961) and Bantwart (1980) reported that the production of lactic acid by the hydrolysis of lactose is important in cheese making. They added that the main lactic acid formers are the homofermenting *Streptococci* (*S. lactis* and *S. cremoris*), these organisms are responsible for the development of cheese flavour. Moreover, for each type of cheese a specific bacterium takes change and developed the curd in a predestined manner. Variations can be expected due to differences in cultures, rate of acid production, moisture, salt concentration, prior treatment of milk and curd and storage temperature (Kosikowiski, 1977).

2.2.1.1 Starter culture

Starter cultures are used to convert lactose into lactic acid which reduces the pH of the system to bring about multitude of reactions occurring in cheese processing (Scott, 1986). He also

added that the most important activities of starter culture bacteria are glycolysis and proteolysis.

Franklin and Sharpe (1963) and Chapman and Sharpe (1981), reported that mixed starter cultures produce more acid than the individual bacteria.

Bantwart (1980) reported that *Leuconastoc* species (*L. cremoris, L. dextramium* and *L. mesenteroides*) are responsible for the production of aroma and flavour in cheese.

2.2.1.2 Bacteria of cheese spoliage

There are some species of bacteria that cause spoilage in food such as *Acetobacter, Micrococcus, Moraxella, Pseudomonas* and *Flavobaetorum* (Jay, 1986).

The defects that can occur in milk due to microbial growth are off-flavouring, lipolysis, gas production and souring (Bantwart, 1980).

Kosikowiski (1977) reported that coliforms bacteria grow well in cold or warm cheese causing slit eyes. He also reported that coliforms don't survive in pasteurized cheese milk but may be present as a result of post pasteurization contamination.

Ibrahim *et al.* (1981) reported that the complete inactivation of streptococci during the early stage of cheese manufacture. However, several pathogenic bacteria e.g. (*Salmonella* spp., *Brucella* spp., *Compylobacter* spp. and *Listeria* spp.) can survive in cheese made from raw milk and may not be destroyed by some heat process.

Coveney *et al.* (1994) studied the microbial status of Irish cheeses and emphasis on selected pathogenic and spoilage microorganisms. They found that the incidence of coliforms were higher in soft, semi-soft and semi-hard cheeses than in hard types. Also they found high level of *Staphylococcus aureus* as well as contamination with fecal *Streptococci*.

Massa *et al.* (1992) reported that high concentration of fecal coliforms was observed in 41 samples of Mozzarella cheese. They identified 3 isolates *Klebsiella pneumoniae,* 2 isolates *Klebsiella oxytoca* and 1 isolate for each *Enterobacter aerogenes* and *Escherichia coli*.

Brocklehurst and Lund (1988) studied the effect of pH on initiation of growth of Cottage cheese spoilage bacteria and found that strain of *Pseudomonas spp.* grow at pH 4.8 when incubated at 7°C and *E. agglomeraus* grow at pH 3.8 when incubated at 7°C.

2.2.3 Cheese and food-borne diseases

Foster *et al.* (1961) and Kosikowsiki (1977) reported that the incidence of coliforms on the surface of ripened cheese has assumed greater importance. Since it was discovered that the enteropathogenic *E. coli* contaminating such cheese had caused several food poisoning epidemics as they reported. Yang and Jones (1969) reported that the isolation *Escherichia coli* from pasteurized dairy products was due to post contamination of the product.

Kosikowiski (1977) reported that cheeses made from raw milk which drawn by hand or milk drawn from infected udders are usually contaminated by *Staphylococcus aureus*.

Foster *et al.* (1961) and Kosikowiski (1977) reported that some dairy products, which were prepared by pasteurized milk, had been found to contain *Staphylococcus aureus* as a result of post-pasteurization contamination. They also added that *Staphylococcus aureus* might also be present in cheese if the initial numbers of organisms in raw milk were very high.

Ibrahim *et al.* (1981) reported that the complete inactivation of *Streptococci* during early stage of cheddar cheese production results in the massive growth of *S. aureus* and production of enterotoxins. However, Speek (1972) and Price and Lee (1970) reported that pathogenic bacteria could be controlled by the use of starter culture, such as *Lactobacilli*, which produce antibiotics and can suppress the growth of *Pseudomonas*.

Araujo *et al* (2002) investigated the presence of some pathogenic bacteria in soft cheese and found that *Staphylococcus aureus* was in about 20% of the samples, while, *Aeromonas hydrophila* and *Aeromonas caviae* were detected in 17.7% of the samples and *Escherichia coli* was isolated from 21.1% of the samples.

Pourshahe *et al.* (1998) reported an outbreak of food borne botulism, which was associated with contaminated locally made cheese in Iran.

De Buyser *et al.* (2001) estimated the proportion of diseases due to milk and milk products in France and found that *Salmonella spp.* were responsible for 29 outbreaks, *Listeria monocytogenes* for 10 outbreaks, *E. coli* for 11 outbreaks and *Staphylococcus aureus* for 10 outbreaks.

Geiss *et al.* (1993) reported that there were 600 cases of salmonella outbreaks in South West Germany due to contaminated cheese. Moreover, Miller and Paige (1998) found that brucellosis transmission via the food chain especially through contaminated raw milk and cheese, while tuberculosis has limited transmission via raw milk. De Reu *et al.* (2002) found the incidences of coliforms, B-glucuronidase positive *E. coli* and *S. aureus* were higher in soft than in blue veined, semi hard, hard and fresh cheeses.

2.2.3.1 Salmonellaceae

It belongs to the family Enterobacteriaceae, only five species are recognized, they are Gram negative, short rods that are aerobic and don't produce pigment on culture media (Barrow and Felthman, 1993). Most of species ferment glucose and other sugars with the production of acid and gas, they don't ferment lactose (Barrow and Felthman, 1993).

Carson and Dewitt (2002) reported that Salmonella food poisoning is a bacterial food poisoning caused by Salmonella bacterium and responsible for about 15% of all cases of food poisoning. It can occur when someone drinks unpasteurized milk or

eat any food contaminated during preparations, poor hygiene can also allow such carrier to spread the infection to others.

Vlaemynck (1994) mentioned that numbers of cells that caused various diseases varies from one cell of *Salmonella typhi* to several millions of *Salmonella anatum*. He also added that contamination of raw milk usually takes place by Salmonella from external sources (feces, the farmer, water pollution and dust).

Salmonellae continue to be a major concern for the dairy industry, since these bacteria have caused recent outbreaks of illness and have been isolated from various dairy products in the market places (El-Gazzar and Marth, 1992). Non-typhoid *Salmonella* spp. continue to figure prominently in many epidemiological registries as the leading cause of food borne disease. Moreover, the disease can spread and cause a chronic form, such as reactive arthritis, ostemyelitis, cardiac inflammation or neural disorders D'Aoust (1994). Vlaemynck (1994) mentioned that the reduction in Salmonella number due to freezing temperatures is limited. Hence Salmonella spp. can survive a prolong time in frozen foods. Also he mentioned that all Salmonella are readily destroyed at milk pasteurization temperatures and are unable to ferment sucrose.

Kasrazadeh and Genigeorgis (1994) reported that the minimum temperature allowed growth of Salmonella in Hispanic type soft cheese was 8^{o}C. They also mentioned that the addition of 0.3% sodium benzoate (pH 6.6) and addition of 0.3% potassium sorbate to

cheese pH (6.0) had a significant delaying or preventing growth of Salmonella.

L'Ecuyer et al. *(1996) detected 22 cases of Salmonella infections in persons consuming food from hospital kitchen. Moreover, they mentioned that this outbreak of Salmonellosis was due to contamination of the kitchen with cross contamination via equipment.*

2.2.3.2 *Staphylococcus aureus*

This species belongs to the family Micrococcaceae that consist of twenty-three species and four sub-species (Barrow and Felthman, 1993). They are Gram positive cocci and they are catalase positive (Asperger, 1994). Moreover, he also reported that staphylococci are non motile, spores are not produced, colonies are smooth and colonial pigment is variable from gray or gray-white with yellowish tint.

The natural habitat of *S. aureus* is warm-blooded animals including humans. Moreover, 10 to 40% of people are asymptomatic carriers of *S. aureus* mostly in the mucosal membrane (Noble, 1981). Moreover, Adesiyun *et al.* (1997) reported that enterotoxin producing staphylococcal species (*S. aureus* in particular) are the leading cause of food-borne disease.

In Slovakia, sheep cheese and Bryndza cheese are considered to be traditional products, which are mostly made from unpasteurized milk and therefore can contribute to the source of Staphylococcus enterotoxicosis (Simko, and Brartko, 1996).

2.2.3.3 Escherichia coli

***Escherichia coli* is a member of the family Enterobacteriaceae, the bacterium is Gram negative, non-spore forming straight rods. It can also grow in media with glucose as the sole organic constituent. It ferments lactose producing acid and gas (Barrow and Felthmen, 1993).**

Maher *et al.* (2001) observed that during manufacture and ripening of smear ripened cheese there was growth of *E. coli* O157: H7 to a level that permitted survival during an extended storage of the cheese. However, Ansay and Kaspar (1997) suggested that serotype O157: H7 was not prevalent in dairy product ingredients and processing environments. Similarly, Spano *et al.* (2003) showed that curd at 8^{o} C, for 5 minutes resulted in the loss of culture ability of *E. coli* O157: H7 during the production of mozzarella cheese.

Papageorgiou *et al.* (1998) indicated that the addition of 4% mesophilic starter culture to pasteurized mixture of ewes and goats' milk resulted in high quality of pichtogalo chanion cheese that was free from *E. coli*. Similarly, Saad *et al.* (2001) reported that the addition of type O lactic culture might be an additional safeguard to well established good manufacturing practices and hazard analysis control points programs in the control of growth of *E. coli* O157: H7 in cheese. Similarly, Caridi (2002) suggested the use of *Lactobacilus paracasei* subsp paracasei strains to increase the safety of cheese made from raw goats milk because these cultures strongly inhibited *E. coli* without adverse sensory changes.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Source of cheese sample

Thirty six samples of Sudanese white cheese were collected from Bahrry (Elmahata Elawasta) and Elsafia. The survey involved a total of three restaurants three supermarkets and three groceries from each location. The collection of the samples was done in duplicate.

3.2 Analysis of cheese samples

The samples were collected in clean sterile bottles and transported to the laboratory of the Department of Dairy Production, Faculty of Animal Production, University of Khartoum, for chemical and microbiological examinations.

3.2.1 Chemical analysis

3.2.1.1 Protein content

The protein content was determined by Kjeldahl method (AOAC, 1990). In a Kjeldahl flask, 3 grams of cheese were added. Two Kjeldahl tablets (1 mg $NaSO_4$ and equivalent of 0.1 gm Hg) were added. Twenty five milliliters of concentrated sulfuric acid (density of 1.86 g/ml 20^{o}C) were added to the flask. The mixture was then digested on a heater until a clean solution was obtained (2.5 hours), and the flasks were removed and left to cool. The

digested sample was poured into a volumetric flask (100 ml) and diluted to 100 ml with distilled water. The distillate was received in a conical flask containing 25 ml of 4% boric acid plus three drops of indicator (bromcerol green plus methyl red). The dilution was continued until the volume in the flask was 75 ml. The flask was then removed from the distillator.

The distilate was then titrated against 0.1 N HCl until the end point was obtained (red colour). Protein content was calculated as follows:

$$\text{Nitrogen (\%)} = \frac{\text{T x 0.1 x 0.014}}{\text{Weight of sample}} \text{ x } 100$$

$$\text{Protein (\%)} = \text{Nitrogen (\%) x 6.38}$$

Where:

T: Titration figure

0.1: Normality of HCl

0.014: Atomic weight of Nitrogen

3.2.1.2 Fat content:

The fat content was determined by Gerber's method according to AOAC (1990) as follows:

In a clean dry Gerber tube, 10 ml of sulfuric acid (density 1.815 gm/ml at 20^{o}C) were poured, then 3grams of minced cheese sample were added. Amyl alcohol (1-2 ml) was added to the mixture followed by addition of distilled water. The contents were

thoroughly mixed till no white particles could be seen. The Gerber tube was centrifuged at 1100 revolution per minutes (rpm) for 4-5 minutes, and the tubes were then transferred to a water bath adjusted at 65^{o}C for three minutes. The fat percent was then read out directly from the fat column.

3.2.1.3 Total solids content

Total solids was determined according to the modified method of AOAC (1990). Two grams of cheese samples were weighed and placed in a clean dried porcelain dish and heated on a steam bath for 10-15 minutes. The dish was then placed in an oven at 100^{o}C for three hours, then cooled in weighed quickly. Weighings were repeated until the difference between two readings was < 0.1 milligram. The total solids contents were calculated from the following equation:

$$T.S\% = W_1/W \times 100$$

Where:

W_1 = Weight of sample after drying

W = Weight of sample before drying

3.2.1.4 Ash content

Ash content was determined according to the method described in AOAC (1990). Two grams of cheese samples were weighed in a suitable crucible and evaporated to dryness on a steam bath. The sample was placed in a muffle furance (550^{o}C)

for 1.5 – 2 hours, then cooled in a desicator and weighed. The ash content was calculated using the following equation:

$$\text{Ash (\%)} = \frac{W_1}{W_2} \times 100$$

Where:
W_1 = Weight of ash
W_2 = Weight of sample

3.2.1.5 Titratable acidity

Titratable acidity was determined according to AOAC (1990). Ten grams of cheese were weighed and placed in a conical flask and distilled water at 40^{o}C was added until the volume in the flask was 105 ml. The sample was then vigorously agitated and filtered through filter paper (Whatman No. 41). Twenty five milliliters of the filtrate were placed in a porcelain dish and five drops of phenophtalein indicator were added. The sample was titrated against 0.1 N NaOH until a faint pink colour appeared. The acidity was calculated as follows:-

$$\frac{T \times 4}{W}$$

Where:

T: Titration figure

W: Weight of sample

3.2.2 Microbiological examination

The samples were enumerated for counting standard plate count, coliforms bacteria, *E. coli, Staphylococcus aureus,* and

***Salmonella* spp. The samples were collected in a clean sterile bottles and kept immediately at 4^{o}C.**

3.2.2.1 Preparation of the media

All media were obtained in dehydrated forms and prepared according to the manufactures instructions.

3.2.2.2 Types of culture media used for bacteriological examination of cheese

3.2.2.2.1 Solid media

3.2.2.2.1.1 Plate count agar (Merck, 74065)

The medium consisted of 5 grams casein enzymic hydrolystate, 2.5 grams of yeast extract, 1.0 gram of dextrose and 12 grams agar. The medium was prepared by suspending 23.5 grams of powder in one litter of distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121^{o}C for 15 minutes.

3.2.2.2.1.2 Salmonella and shigella agar (SS agar) (Hi media, M108)

This a selective differential medium. It consisted of 5 grams beef extract, 10 grams lactose, 8 grams bile salts, 10 grams sodium titrate, 8.5 grams sodium thiosulphate, 1.0 gram ferric citrate, 15 grams agar, 0.00033 brilliant green and 0.025 grams nuteral red. The medium was prepared by susbending 63.1 grams of powder in one liter of distilled water then boiled until dissolved completely and steriled by water bath at 100^{o}C for 30 minutes.

3.2.2.2.1.3 Mannitol salt agar (Hi media, M118)

The medium consisted of 1.0 gram meat extract, 5 grams casein peptone, 5 grams meat peptone, 75 grams sodium chloride, 10 grams D (-) Mannitol, 0.025 gram phenol red, and 15 grams agar. The medium was prepared by suspending 111 grams of powder in one litre of distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121^{o}C for 15 minutes.

3.2.2.2.1.4 Metachromgelb Agar (Sifin, 1108097)

The medium consisted of 4.4 grams peptone (meat), 4.4 grams peptone (casein), 2.7 grams yeast extract, 10.0 grams lactose, 10.0 grams sucrose, 5.0 grams sodium chloride, 0.725 grams metachrom yellow, 0.4 gram blue water and 8.0 grams agar. The medium was prepared by suspending 45.6 grams in one liter of distilled water then boiled until dissolved completely and sterilized by autoclaving at 121^{o}C for 15 minutes.

3.2.2.2.1.5 Nutrient agar (S.d. Fine. Chem Ltd 74056)

This medium, consisted of 10 grams meat extract, 10 grams peptone, 5 grams sodium chloride, 20 grams agar. The medium was prepared by suspending 20 grams of powder in one liter of distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121^{o}C for 15 minutes.

3.2.2.2.1.6 Urea agar media (Hi media M112)

The medium consisted of 10 grams peptone, 1 gram dextrose, 1.2 grams Na_2HPO_4, 0.8 gram K $(HPO_4)_2$, 5 grams sodium chloride, 15 grams agar and 0.012 gram phenol red. The

medium was prepared by suspending 2.4 grams of powder in 95 ml of distilled water and boiled to dissolved completely. The pH was adjusted to 6.8 and then sterilized by autoclaving at 121^{o}C for 15 minutes. Then cooled to 50^{o}C and aseptically 5 ml of sterile 4% urea solution were added and mixed well. The medium was then distributed (10 ml amounts) into sterile test tubes and allowed to set in a slope position.

3.2.2.2.2 Semi-solid media

3.2.2.2.2.1 Hugh and Leifson (OF) medium

This medium contained 2.0 grams peptone, 5.0 grams of sodium chloride, 0.3 gram of dipotassium hydrogen phosphate, 3 grams of agar and 0.2% of bromothymol blue. It was prepared according to Barrow and Felthman (1993) by dissolving the solid in one liter of distilled water. The medium was filtered followed by the addition of indicator and sterilized.

3.2.2.2.2.2 Kligler sugar iron agar (Hi media M078)

This medium contained (grams/liter): peptic digest of animal tissue (15.00), beef extract (3.00), yeast extract (3.00), peptone (5.00), lactose (10.00), dextrose (1.00), ferrous sulphate, 20, sodium chloride (5.00), sodium thiosuphate (0.30), phenol red (0.05), and agar (15.00). It was prepared by suspending 57.5 grams in 1000 ml distilled water. Then the medium was distributed into sterile test tubes, it was sterilized by autoclaving at 121^{o}C for 15 minutes. After sterilization the tubes were put in a slope position to solidify.

3.2.2.2.2.3 Motility medium

The medium consisted of 1.0 gram gelatin, 2.5 grams peptone, 1.0 gram beef extract, 5.0 grams sodium chloride and 20.0 grams agar. It was prepared by soaking the gelatin in water for 30 minutes and the other ingredients were then added and heated to dissolve completely. After sterilization the medium was distributed into 10 ml volumes into sterile tubes.

3.2.2.2.3 Liquid media

3.2.2.2.3.1 Nutrient broth (Hi media M 002)

This medium consisted of 10 grams lab-lemco beef extract, 5 grams peptone and 13 grams sodium chloride. It was prepared by dissolving 28 grams of the powder into one liter of distilled water, mixed well and distributed into test tubes then sterilized.

3.2.2.2.3.2 Peptone water (Oxoid L37)

This medium contained 10.0 grams peptone and 5.00 grams sodium chloride, it was prepared by dissolving 15 grams in one liter of distilled water, mixed well and then distributed into test tubes and sterilized.

3.2.2.2.3.3 MR-VP medium

This medium consisted of 5 grams peptone, 5 grams dipotassium phosphate and 5 grams glucose. It was prepared by dissolving 15 grams of the solid in one liter of distilled water and distributed into test tubes and sterilized

3.2.2.2.3.4 Koser citrate medium (Oxoid CM65)

The medium contained 1.5 grams sodium amonium phosphate, 1.0 gram potassium dihydrogen phosphate, 0.2 gram magnesium sulphate, 3.0 gram sodium citrate and bromothlymol blue. It was prepared by dissolving 5.7 grams in one liter of distilled water, distributed into Mac-cartney bottles and sterilized.

3.2.2.2.3.5 Peptone water sugars

These were prepared according to Barrow and Felthman (1993). Sterile peptone water (900 ml) was prepared and sterilized by steaming for 20 minutes. Ten grams of appropriate sugar in 90 ml of distilled water were added to sterile peptone water and 1% bromoresol blue indicator was also added. The medium was then distributed in 5 ml volumes in sterile test tubes and steamed to 5 minutes at 121^{o}C.

3.2.2.3 Sterilization

3.2.2.3.1 Sterilization of culture media

Plate count agar, Mannitol salt agar, nutrient broth, peptone water, MR – VP medium, motility medium and Koser citrate medium were sterilized by autoclaving at 15 pound pressure for 15 minutes at 121^{o}C. While Hugh and Leifson medium and sugars were sterilized by autoclaving for 5 minutes at 121^{o}C. Salmonella Shigella agar were heated only.

3.2.2.3.2 Sterilization of equipment

Glassware such as petri-dishes, test tubes, pipetes, flasks and bottles were sterilized in a hot oven at 160^{o}C for one hour, whereas mixer, distilled water and tips were sterilized by autoclaving for 15 minutes at 121^{o}C.

3.2.2.4 Culturing of the specimens

3.2.2.4.1 Dilution methods

Five grams of the cheese were added to warm (45^{o}C) 15 ml of 2% sodium citrate and blinded for 2 minutes. Then one ml from the mixture (cheese and sodium citrate) was transferred with sterile 1 ml graduated pipette to (9 ml) sterile normal saline in a screw capped bottle and mixed thoroughly. Using another sterile pipette 1 ml of the prepared dilution was transferred to a second dilution bottle. This process was repeated to make ten fold dilutions from 10^{-1} to 10^{-7} (Richardson, 1985). Then 1 ml from each selected dilutions was cultured on duplicate plates contain, the selected media and incubated at 32^{o}C for 48 hr. the plates containing in 30-300 cfu were enumerated.

3.2.2.5 Examination of cultures

Growth on solid media was examined visually with naked eye for colonies appearance and changes in media. Growth on liquid media was examined for colour, turbidity and sediment formation.

3.2.2.6 Purification of organisms

Purification was done by subculturing of a well isolated typical colonies on nutrient agar medium. After the growth of the

plates were checked by Gram's stain for purity, it was transferred to a plate containing a fresh solidified corresponding medium (Barrow and Felthman, 1993).

3.2.2.7 Identification of organisms

The purified isolates were identified according to the criteria outlined by Barrow and Felthman (1993) as follows:-

3.2.2.7.1 Primary tests

Morphological appearance:

Shape of the cell

Oxidation and fermentation

Motility test

Aerobic growth

Glucose test

Catalase test

Oxidase test

3.2.2.7.1.1 Cultural appearance

Colonies of *Escherichia coli* were slightly blue with dark centers in metachromgelb media. *Staphylococcus aureus* is typically circular, smooth, convex and yellow in colour in mannitol salt agar media. Colonies of *Salmonella* spp. were yellow with black centres in SS agar.

3.2.2.7.1.2 Shape of the cell

It was done by Grams stain as described by Harrigan and McCance (1976) as follows:-

Crystal violet was added to smears on slides for one minute, followed by washing with distilled water. Lugol's iodine was added for one minute then removed by washing with distilled water. The slides were decolorized by alcohol for ten seconds and the residue was removed by distilled water. The slides were counter-stained with bacteriological Gram saffranin for 30 – 60 seconds and washed with distilled water. The slides were then dried with a filter paper and a drop of immersion oil was added followed by examining under the microscope. Gram positive organisms appeared purple, while Gram-negative ones appeared pink.

3.2.2.7.1.3 Catalase test

The organisms to be tested were put on sterile slides. A drop of 3% hydrogen peroxide (H_2O_2) was added to the colony and emulsified. Evaluation of gas immediately or after 5 minutes indicated a positive result.

3.2.2.7.1.4 Oxidase test

Two or three drops of teteramethyl-P-phenylene diamine dihydrochloride were placed on a filter paper. Before the drops dried, the tested organism was removed with a platinium wire or glass rod and smeared across the surface of the impregnated paper. A positive reaction was shown by the development of dark purple colour within 10 seconds.

3.2.2.7.1.5 Oxidation of Fermentation test (OF)

Duplicate tubes of Hugh and Lifson's medium were inoculated by stabbing with a sterile straight wire. The medium in one of the tubes was covered with a layer of soft sterile paraffin oil to a depth of about one centimeter. The tubes were then incubated at 37^{o}C and examined daily for 14 days. Colour changes to yellow in both opened and covered tubes indicated fermentative organisms while change in uncovered tube only indicated oxidative organisms. However, the negative OF test showed no changes of colour in both tubes.

3.2.2.7.1.6 Glucose fermentation test

Glucose media were inoculated with a 24 hours growth in broth sugar medium, and the cultures were examined daily for up to 7 days. The change of colour to yellow indicated a positive reaction. Gas was accumulated in the Durham's tubes, when produced.

3.2.2.7.1.7 Motility test

The test of motility was read out from the mortality medium, the test organism was inoculated by inserting the loop in stright line (Barrow and Felthman, 1993).

3.2.2.7.2 Secondary biochemical tests

3.2.2.7.2.1 Coagulase test

The coagulase test was performed using kits (Staphytect, Oxoid). The tested organism was emulsified in both test and control kits. Coagulatation or clot formation was observed for positive test.

3.2.2.7.2.2 Hydrogen sulphide test

Kliger sugar iron agar medium was used to test the ability of the organisms to produce hydrogen sulphide (H_2S). Heavy inoculum was cultured on the surface of Kliger iron slope. It was incubated at 37^oC and examined daily for 7 days. A positive reaction was indicated by black colour while yellow or pink colour was considered as a negative result for H_2S. Similarly lactose and glucose fermentation were recorded from this medium. They changes to yellow when positive. Similarly, gas production was also recorded.

3.2.2.7.2.3 Voges-Proskaur (VP) test

3.2.2.7.2.4 Methyl red (MR) test

Glucose phosphate medium was inoculated and incubated at 37^oC for 2 days. About 2 drops of methyl red solution were added to it, well shaken and examined. The red colour indicated a positive result and yellow colour indicated negative result.

The same culture was then used for VP test after MR test result was taken. It was done by addition of 0.5 ml of 5% α-naphthol solution. It was then well shaken and the tubes were

sloped. They were examined after one hour. A positive reaction was indicated by production of pink colour.

3.2.2.7.2.5 Indole test

To a 48 hours culture (peptone water), 0.5 ml of Kovacs' reagent was added. It was shaken well and examined after one minute. Production of a red colour was indicative of a positive result.

3.2.2.7.2.6 Urease test

Urease activity is shown by alkali production (ammonia) from urea splitting by the test organism. Inoculum of the tested organisms was cultured on the surface of urea agar slope and incubated at 37^{o}C and examined daily for 7 days. A positive test indicated by pink or red colour.

3.2.2.7.2.7 Citrate utilization

Using a sterile straight wire (to avoid carry over of the medium) a small inoculum of the test organism were taken from suspention and incubated at 37^{o}C on Koser citrate medium, that was in chemically clean tubes and was examine daily for 7 days for either turbidity or production of blue colour which was an indication of positive reaction.

3.2.2.7.2.8 Fermentation of sugars

A twenty four hours culture was inoculated into peptone broth with sugars (Mannitol, lactose, arbinose, rahamnose, xylose, maltose, glucose) and incubated at 37^{o}C for up to seven days. Change in colour to yellow indicated a positive reaction. Gas was accumulated in the Durham tubes when produced.

3.2.3 Statistical analysis

The data of the present study were analyzed statistically using complete randomized design. ANOVA test and the least significant difference were used to determine the difference between means. The analysis was carried out using SAS program (1989).

CHAPTER FOUR

RESULTS AND DISSCUSION

4.1 Chemical composition

The fat content revealed values of 21.3±5.5%, 17.5±3.3% and 18.6±6.0% for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurants, supermarkets and groceries, respectively. While values of 12.0% to 27%, 15.5% to 20%, and 15% to 20% were reported for minimum and maximum values, respectively (Table 1).

The cheese samples collected from Elsafia's showed values of 18.5±1.8%, 18.0±1.8% and 19.6±3.0% for mean±standard deviation for restaurants, supermarkets and groceries, respectively (Table 1 and Fig. 1).

Values of 17.0% to 20%, 10.2% to 20%, and 16.5% to 22.5% were found for minimum and maximum values, respectively (Table 1 and Fig. 1). There was no significant differences between these values (Table 2 and Table 3).

The average of fat content ranged between 17.5 – 21.33% in the present survey, the low values of fat might be due to storage as reported by Nuser (2001) she indicated that the fat content was high in the beginning of storage period, then gradually decreased towards the end of storage period. Moreover some fat must have leaked from curd into the brine solution, which partially might

explain the decrease in fat content in curd during the storage period (Abdalla, 1992).

Table 1. Comparison between chemical composition of white soft cheese in Khartoum North.

Place	*Source*	*Fat %*			*Protein %*			*Acidity %*			*Ash %*			*Total solid %*		
		Mean±sd	**Min**	**Max**	**Mean±sd**	**Min**	**Max**	*Mean±sd*	**Min**	**Max**	**Mean±sd**	**Min**	**Max**	**Mean±sd**	**Min**	**Max**
Bahrry	Restrant	21.3±5.5	21.0	27.0	15.0±2.2	12.5	17.0	1.9±1.0	1.0	3.1	5.4±2.8	4.2	8.7	46.3±3.3	43.8	50.1
	Supermarket	17.5±3.3	15.0	20.0	14.5±1.6	12.7	15.4	1.8±0.7	1.3	2.7	5.0±1.3	4.0	6.6	42.8±4.7	42.8	45.3
	Grocery	18.6±6.0	15.0	20.0	14.3±2.5	11.7	16.3	1.8±0.7	1.2	2.6	5.9±0.4	5.5	6.3	46.5±6.1	42.8	5
Elsafia	Restrant	18.5±1.8	17.0	20.0	12.3±1.3	11.5	13.9	1.3±0.2	1.1	1.4	10.7±8.4	5.2	5.5	41.8±17.4	22.4	56.1
	Supermarket	18.0±1.8	10.2	20.0	16.3±5.2	12.3	22.3	1.2±0.0	1.1	1.2	8.9±4.7	5.4	9.1	45.1±8.5	39.2	54.8
	Grocery	19.6±3.0	16.5	22.5	12.4±1.0	11.7	13.6	1.2±0.7	1.1	1.3	10.5±7.6	5.2	9.3	48.4±10.1	41.6	61.0

30

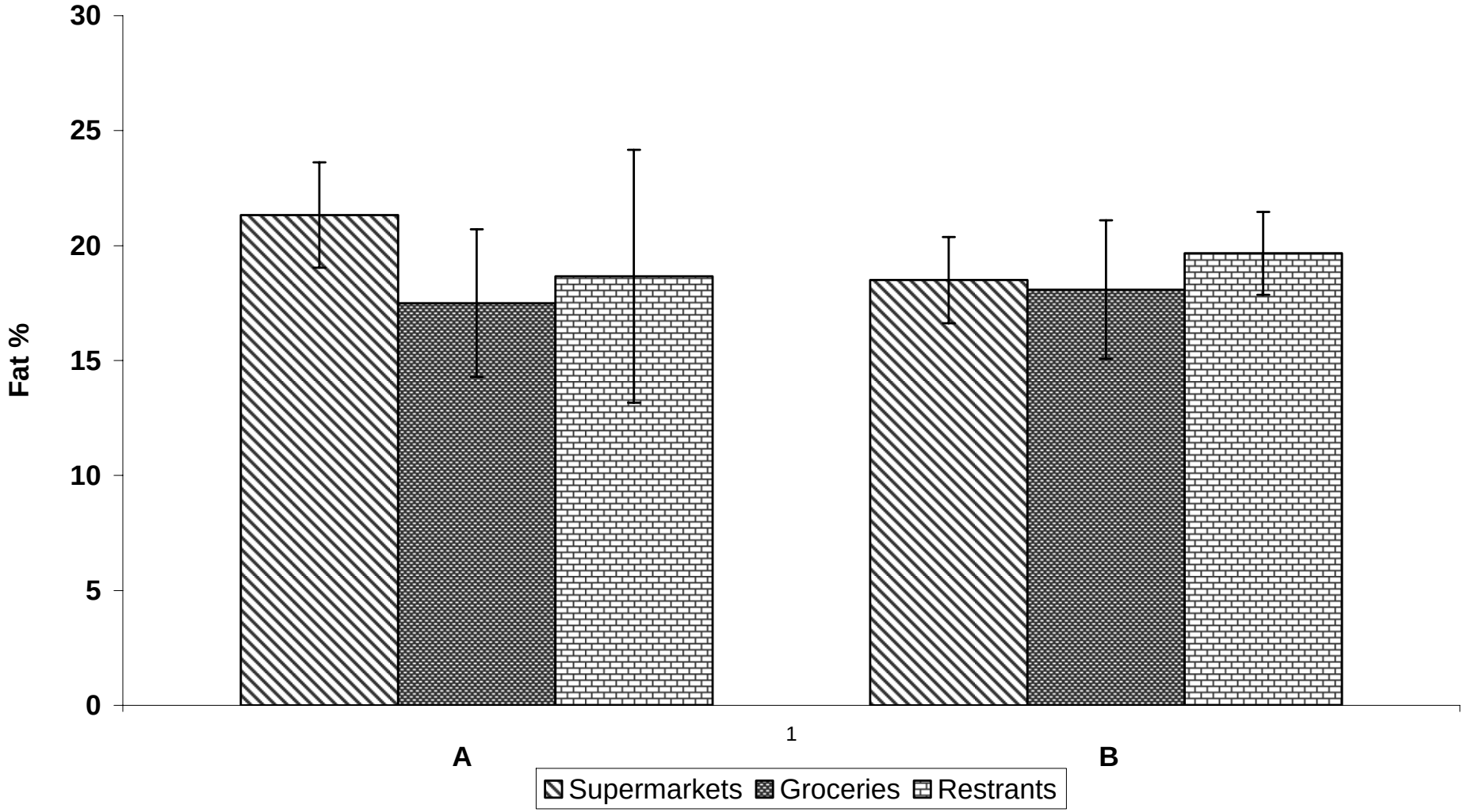

Figure 1: Comparison of the Fat % of white sot cheese marketed in Khartoum North

A: Bahri **B: Elsafia**

Table 2. Comparison of chemical composition of cheese obtained from Khartoum north markets using Duncan's Multiple Rang test.

Constituent	Restaurants	Groceries	Supermarkets
TS	**44.07[a]**	**47.48[a]**	**43.95[a]**
Fat	**19.86[a]**	**19.17[a]**	**17.71[a]**
Protein	**13.40[a]**	**13.39[a]**	**15.41[a]**
Acidity	**1.62[a]**	**1.53[a]**	**1.50[a]**
Ash	**8.08[a]**	**8.27[a]**	**6.93[a]**

Means within the same row bearing the same superscripts are not significantly different (P> 0.05)

Table 3. Comparison of chemical composition of cheese from Khartoum North markets using ANOVA test.

Constituent	Mean	F. value	Probability
TS	**47.8**	**0.52**	**0.5^{NS}**
Ash	**6.2**	**0.25**	**0.7^{NS}**
Fat	**14.0**	**1.61**	**0.2^{NS}**
Protein	**15.9**	**1.30**	**0.2^{NS}**
Acidity	**0.4**	**0.10**	**0.9^{NS}**

NS = Non significant (P > 0.05).

The protein content recorded values of 15.0±2.2%, 14.5±1.6% and 14.3±2.5% for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurants, supermarkets and groceries respectively (mean±standard deviation). While values of 12.5 to 17.0%, 12.7 to 15.4%, and 11.7% to 16.3%) were found for minimum and maximum values respectively, (Table 1 and Fig. 2).

Cheese samples collected from Elsafia's restaurants, supermarkets and groceries showed values of 12.3±1.3%, 16.3±5.2% and 12.4±1.0% for mean±standard deviation respectively. Values of 11.5% to 13.9%, 12.3% to 22.3% and 11.7% to 13.6% for minimum and maximum values, were found respectively, (Table 1 and Fig. 2).

Protein content in Elsafia's supermarkets and groceries (Table 1 and Fig. 2) showed lower values. This result agreed with that of (Khalid, 1991 and Abdel Razig, 1996). They reported that protein decreased considerably due to degradation of protein and loss in whey. Although all these values of protein, showed non significant differences between them (Table 2 and 3).

The acidity revealed values of 1.9±1.0%, 1.8±0.7% and 1.8±0.7% for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurants, supermarkets and groceries respectively, for mean±standard deviation. Values of 1.0% to 3.1%, 1.3% to 2.7% and 1.2% to 2.6% were found for minimum and maximum values

respectively, (Table 1 and Fig. 3). While cheese samples collected from Elsafia's showed values of 1.3±0.2%, 1.2±0.0% and 1.2±0.7%

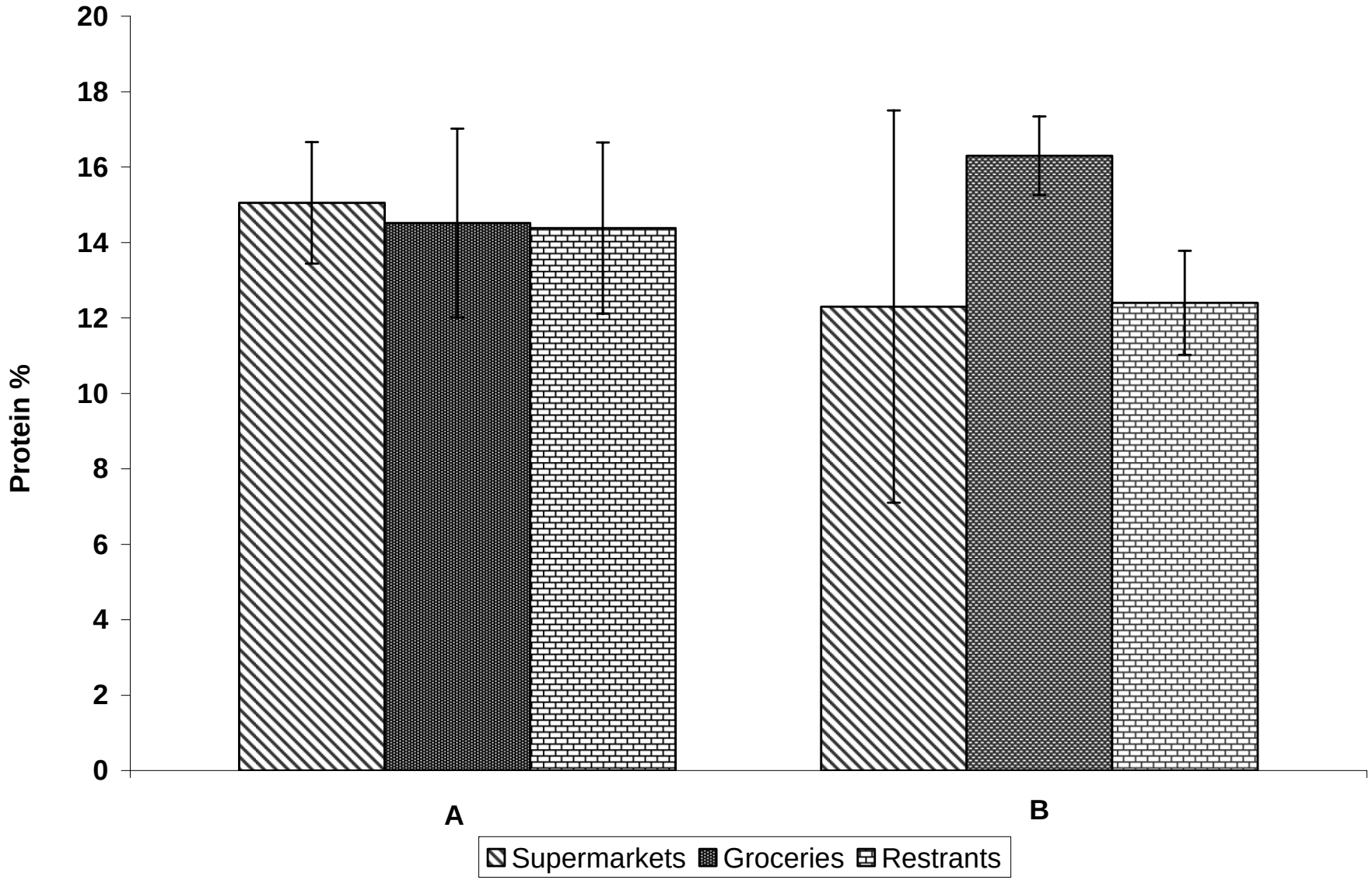

Figure 2. Comparison of protein % of white soft cheese in Khartoum North

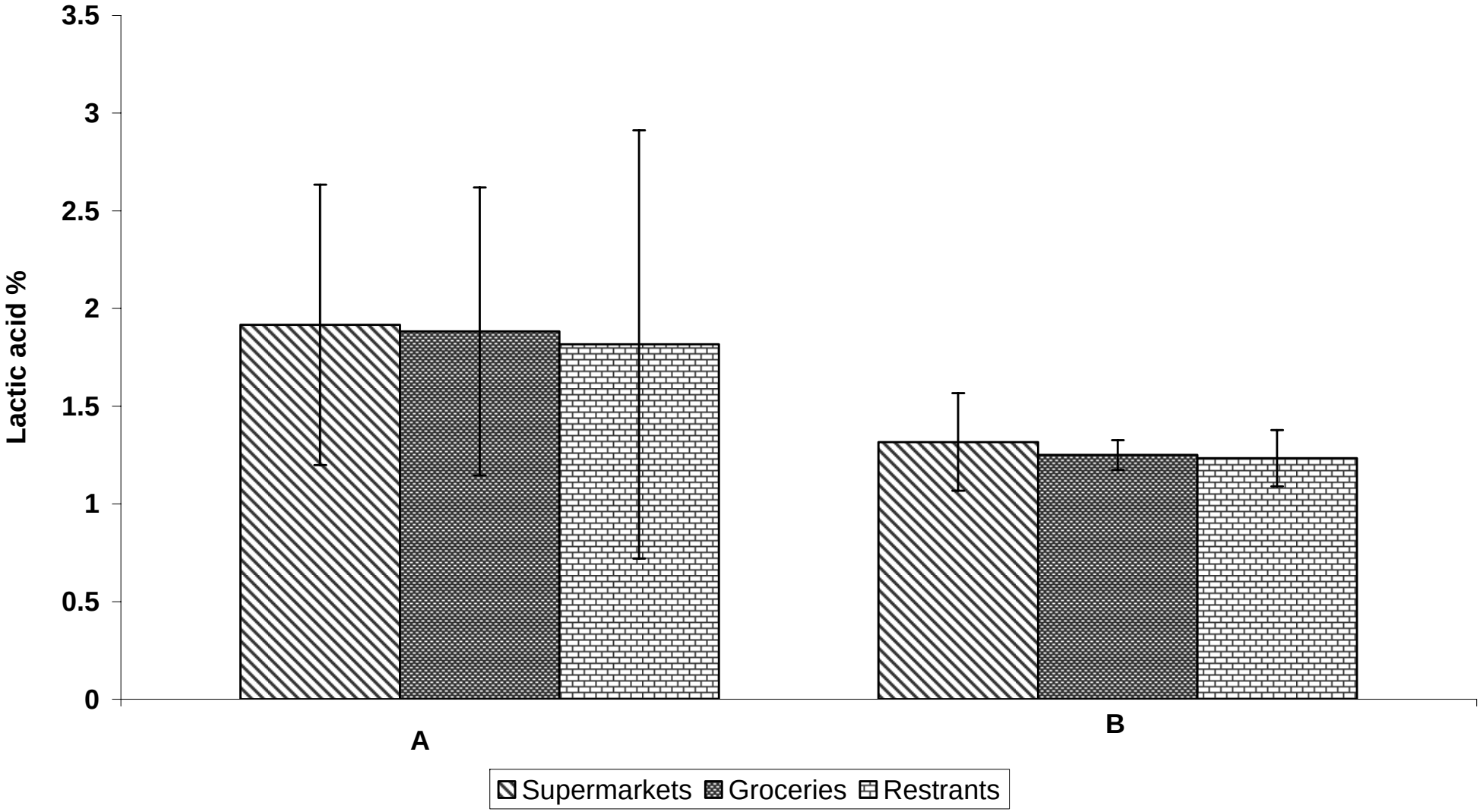

Figure 3. Comparison of Acidity between white soft cheese marketed in Khartoum North

For mean±standard deviation, respectively. While values and 1.1% to 1.2% and 1.1% to 1.3% were found for minimum and maximum levels, respectively (Table 1 and Fig. 3).

The acidity content of cheese samples varies between 1.2% in Bahrry restaurant samples to 1.3% in Elsafia's groceries. There was no significant differences between all values of the acidity. This was explained by Hamed (1998) who reported that the high acidity of raw milk cheese could be due to the fact that the storage temperature activated the natural microflora of raw milk to developed acidity as the result of lactose fermentation since the cheese was stored at room temperature. Moreover Nofal *et al.* (1981) reported that whether at room temperature or in the refrigerator about 45 – 80 % of increase in the acidity was mainly due to lactic acid formed by the predominating lactic acid bacteria.

The ash content values were 5.4±2.8%, 5.0±1.3% and 5.9±0.4% for mean±standard deviation for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurants, supermarkets and groceries respectively (Table 1 and Fig. 4). Values of 4.2 % to 8.7%, 4.0% to 6.6% and 5.5% to 6.3% were reported for minimum and maximum values respectively. While cheese samples collected from Elsafia showed values of 10.7±8.4%, 8.9±4.6% and 10.5±7.6% for mean±standard deviation, respectively (Table 1 and Fig. 4). Values of 5.2% to 5.5%, 5.4 % to 9.1%, and 5.2% to 9.3% were reported for minimum and maximum values respectively (Table 1and Fig. 4).

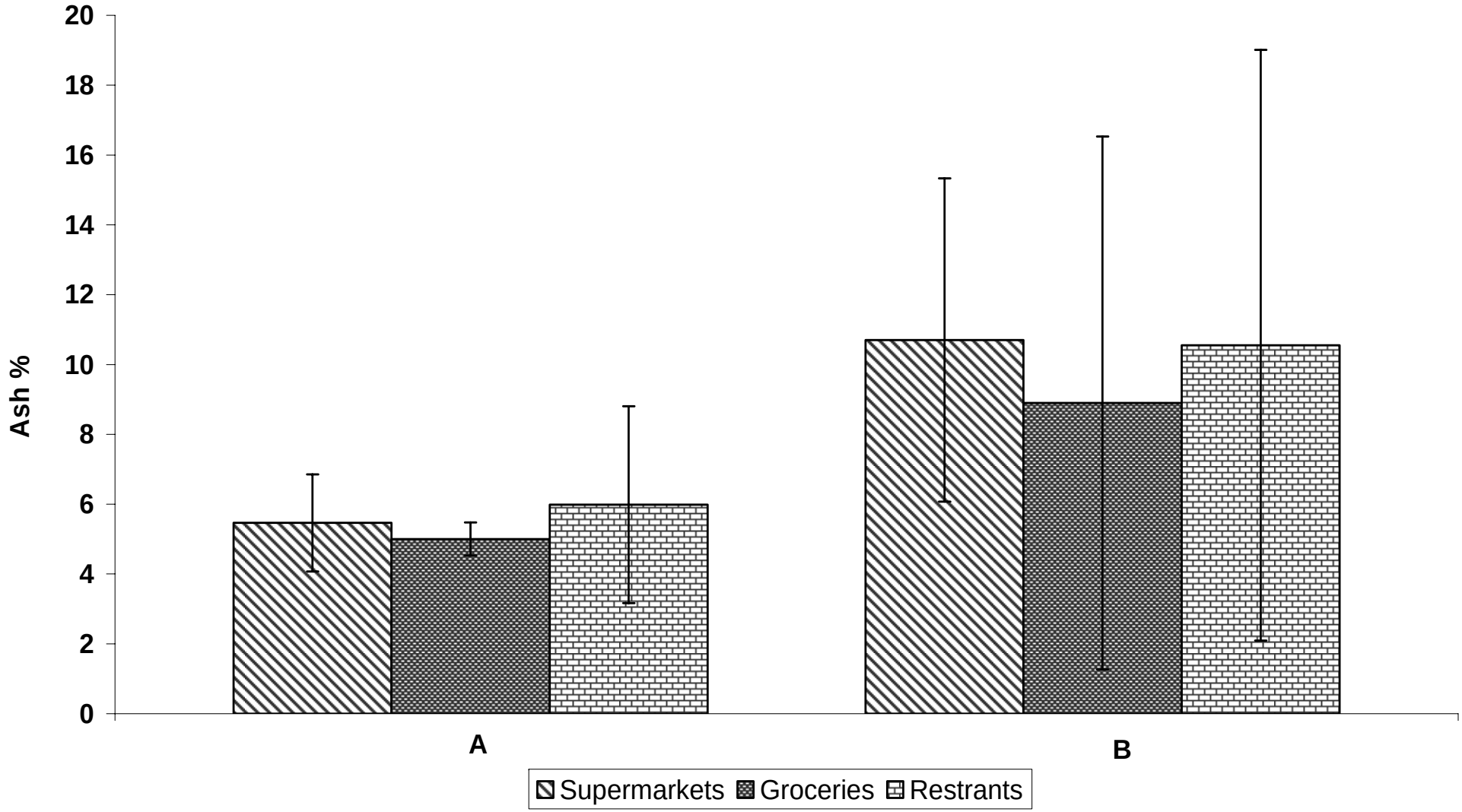

Figure 4. Comparison of the ash % of the white soft cheese marketed in Khartoum North

Ash content was higher in Elsafia's samples when compared to Bahrry's (Elmahata Elwsta) samples. There was no significant differences between values of ash content (Table 2 and 3). Abdel Razig (1996) reported that increase in ash content during picking might be due to decrease in moisture content or absorption of salt by curd.

Total solids content revealed values of 46.3±3.3%, 42.8±4.7%, 46.5±6.1% for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurant, supermarkets and groceries, for mean + standard deviation (Table 1 and Fig. 5). While values of 43.8% to 50.1 %, 42.8% to 45.3% and 42.8% to 53.6% were reported for minimum and maximum values, respectively. Similarly total solids content revealed values of 41.8±17.4%, 45.1±8.5% and 48.4±10.9% for cheese samples collected from Elsafia's restaurants, supermarkets and groceries (Table 1 Fig. 5). While values of 22.4% to 56.1%, 39.2% to 54.8%, and 41.6% to 61.0% for minimum and maximum, respectively (Table 1 and Fig. 5).

The average of total solids content ranges between 48.4 % and 41.8%, in the present survey. This values agreed with Hamed (1998) and Nuser (2001).

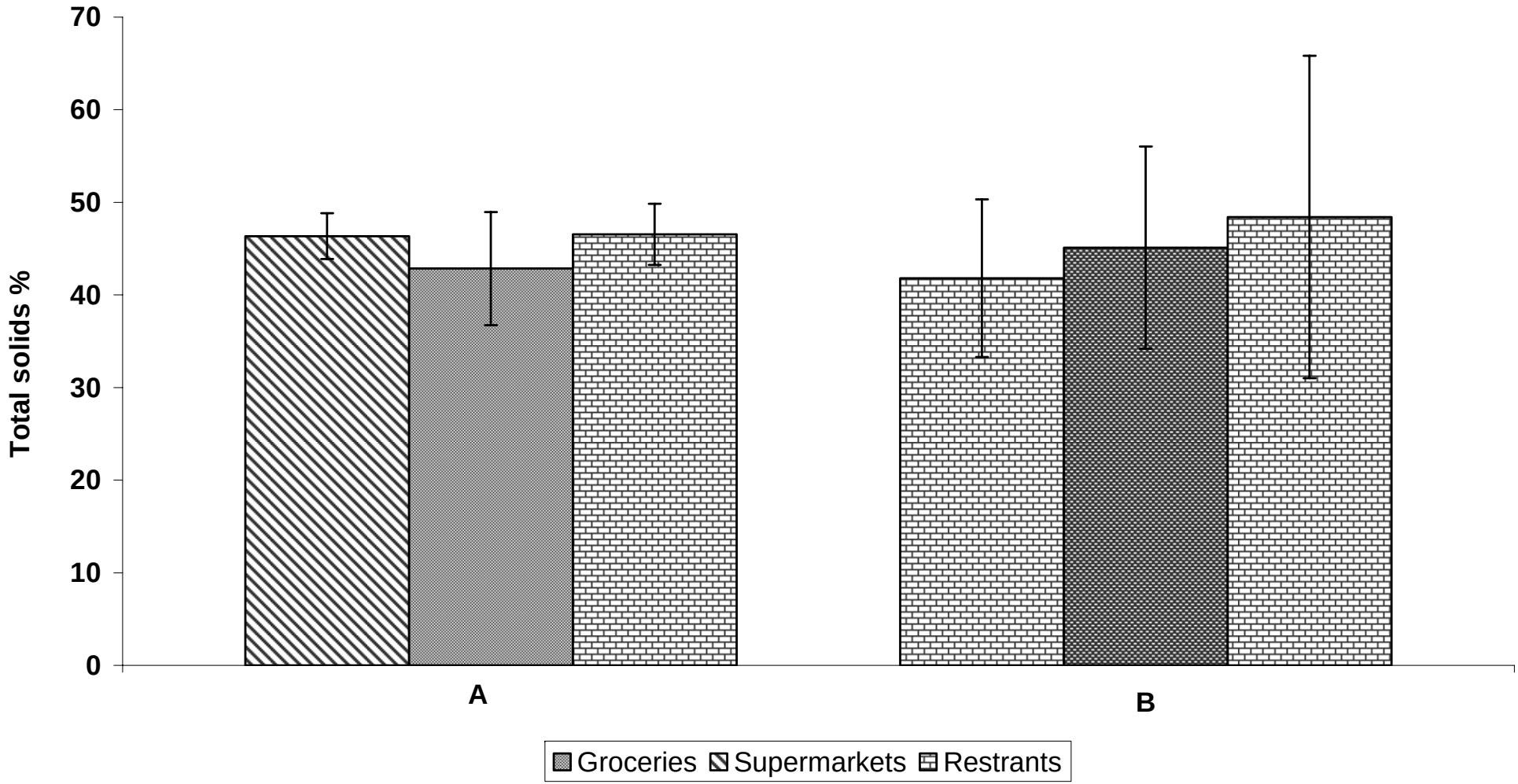

Figure 5. Comparison of the total solids % of white soft cheese marketed in Khartoum North

4.2 Bacteriology of Sudanese white cheese in Khartoum North markets

In the present survey three different types of the causative agent food borne diseases (*E. coli, Salmonella* spp., and *S. aureus*) were examined in Sudanese white soft cheese. Colonies of *E. coli* appeared blue with dark centers in Metachromgelb agar, colonies of *Salmonella* spp. appeared yellow with black centers in Salmonella and Shigella agar and *Staphylococcus aureus* appeared circular, smooth and the are yellow in colour in Mannitol salt agar.

Purification was done by subculturing different colonies in Nutrient agar medium to identify the pathogenic bacteria isolated from the cheese samples using the primary test and secondary biochemical test (Table 4).

4.2.1 Food borne organisms isolated from Sudanese white soft cheese

A total of 38 food borne pathogens were found in the samples of white soft cheese. Of these 10 (26.32 %) isolates were identified for each of *S. aureus*, *Salmonella typhi* and *Salmonella paratyphi*, and 8 (21.05 %) isolates of *E. coli* (Table 5). In Bahrry restaurants and groceries, 2 isolates (5.26 %) for each of *S. aureus, E. coli*, *Salmonella typhi,* and *Salmonella paratyphi* and *E. coli*, *Salmonella typhi,* and *Salmonella paratyphi,* respectively. While the supermarket surveyed in Bahrry revealed the absence of these food borne pathogens **(Table 5). In Elsafia restaurants, 6 (15.79 %) *S. aureus,* 2 (5.26 %) *E. coli,* 2 (5.26 %) *Salmonella typhi* and 2 (5.26 %) *Salmonella paratyphi* were found. Also in Elsafia groceries, 2 (5.26 %) isolates for each of *E. coli, Salmonella typhi* and *Salmonella paratyphi* could be detected. In Elsafia supermarkets 2 (5.26 %) isolates of *S. aureus, S. paratyphi* and *Salmonella typhi* were found (Table 5).**

Table 4. Primary and secondary biochemical tests used for the identification of the pathogenic bacteria isolated from cheese samples.

Test	***Organisms***			
	E. coli	***S. aureus***	**S. typhi**	**S. pratyphi**
Gram reaction	-	+	-	-
Shape	***Bacilli***	Cocci	***Bacilli***	***Bacilli***
Oxidase test	-	-	-	-
Catalase test	+	+	+	+
OF test	F	F	F	F
Motility	+	-	+	+
Indole	+	ND	-	-
Mexhyle red (MR)	+	ND	+	+
Voges. Proskaur (VP)	-	+	-	-
Coagulase	ND	+	ND	ND
H_2S	+	ND	+	+
Urease	+	ND	+	-
Sugars:				
Gas from glucose	+	+	–	+
Lactose	+	+	–	–
Maltose	+	+	+	+
Mannitol	+	+	+	+
Arbinose	+	ND	+	+
Rahamnose	+	ND	–	+
Xylose	+	–	+	-

+ = positive reaction
– = negative reaction
H_2S = Hydrogen suphide
F= Fermentative
OF: Oxidation fermentation reaction
ND: Not done.

Table 5. The incidence of some pathogens in white soft cheese collected from Khartoum North.

		S. aureus	***E. coli***	**S. typhi**	**S. pratyphi**
Bahrry	Restaurants	2	2	2	2
	Groceries	0	2	2	2
	Supermarkets	0	0	0	0
Elsafia	Restaurants	6	2	2	2
	Groceries	0	2	2	2
	Supermarkets	2	0	2	2
	Total	10	8	10	10

4.2.2 The counts of food borne pathogens isolated from Sudanese white cheese

4.2.2.1 *Salmonella spp.*

The log of *Salmonella* spp. counts was found to be 4.0±0.0, 3.9 and 4.0, for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurants; for mean±standard deviation, minimum and maximum level, respectively (Table 6). While the cheese samples collected from Elsafia's restaurants showed counts for *Salmonella* spp. of 4.1±0.1, 3.9 and 4.5 for mean±standard deviation, minimum and maximum levels, respectively (Table 6 and Fig. 6).

***Salmonella* spp. in cheese samples collected from Bahrry's (Elmahata Elwsta) supermarkets were negative in the present survey. While the cheese samples collected from Elsafia's supermarkets showed the log counts of 4.1±0.0, 3.9 and 4.0 for mean + standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 6).**

The log of salmonella counts was found to be 5.8±0.0, 5.8 and 5.8 for cheese samples collected from Bahrry's (Elmahata Elwsta) groceries for mean±standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 6). While the cheese samples collected from Elsafia's groceries revealed log counts of 4.1±0.1, 3.9 and 4.5 for mean±standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 6).

Table 6. Comparison of the log counts of isolated, E. coli, *S. aureus* and *Salmonella* spp. coliform and total count from white cheese in Khartoum North.

Place	*Source*	**E. coli *cfu/ml***			***S. aureus* cfu/ml**			***Salmonella* cfu/ml**			Coliform			*Total bacteria count*		
		Mean±sd	**Min.**	**Max.**	**Mean±sd**	**Min.**	**Max.**	**Mean±sd**	**Min.**	**Max.**	**Mean±sd**	**Min.**	**Max.**	**Mean±sd**	**Min**	Max
Bahrry	Restaurant	5.6±0.1	5.5	5.7	6.8±0.1	6.7	6.9	4.0±0.0	3.9	4.0	6.9±1.4	5.8	8.8	9.0±0.4	8.4	9.4
	Supermarket	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7±0.4	6.0	7.1	9.0±0.5	8.2	45
	Grocery	5.8±0.0	5.8	5.9	0.0	0.0	0.0	5.8±0.0	-	-	6.6±0.5	5.8	7.0	8.6±0.5	8.2	9.3
Elsafia	Restaurant	4.8±0.1	4.8	4.9	8.1±1.3	6.9	9.8	4.1±0.1	3.9	4.5	6.1±0.2	5.8	6.4	9.0±0.7	9.3	10.8
	Supermarket	0.0	0.0	0.0	9.8±0.0	9.8	9.8	4.0±0.7	3.9	4.0	6.3±0.5	5.8	7.1	9.0±0.5	8.2	9.4
	Grocery	4.7±0.0	4.7	4.8	0.0	0.0	0.0	4.1±0.1	4.0	4.3	6.3±1.2	5.1	7.8	9.2±0.2	8.6	9.4

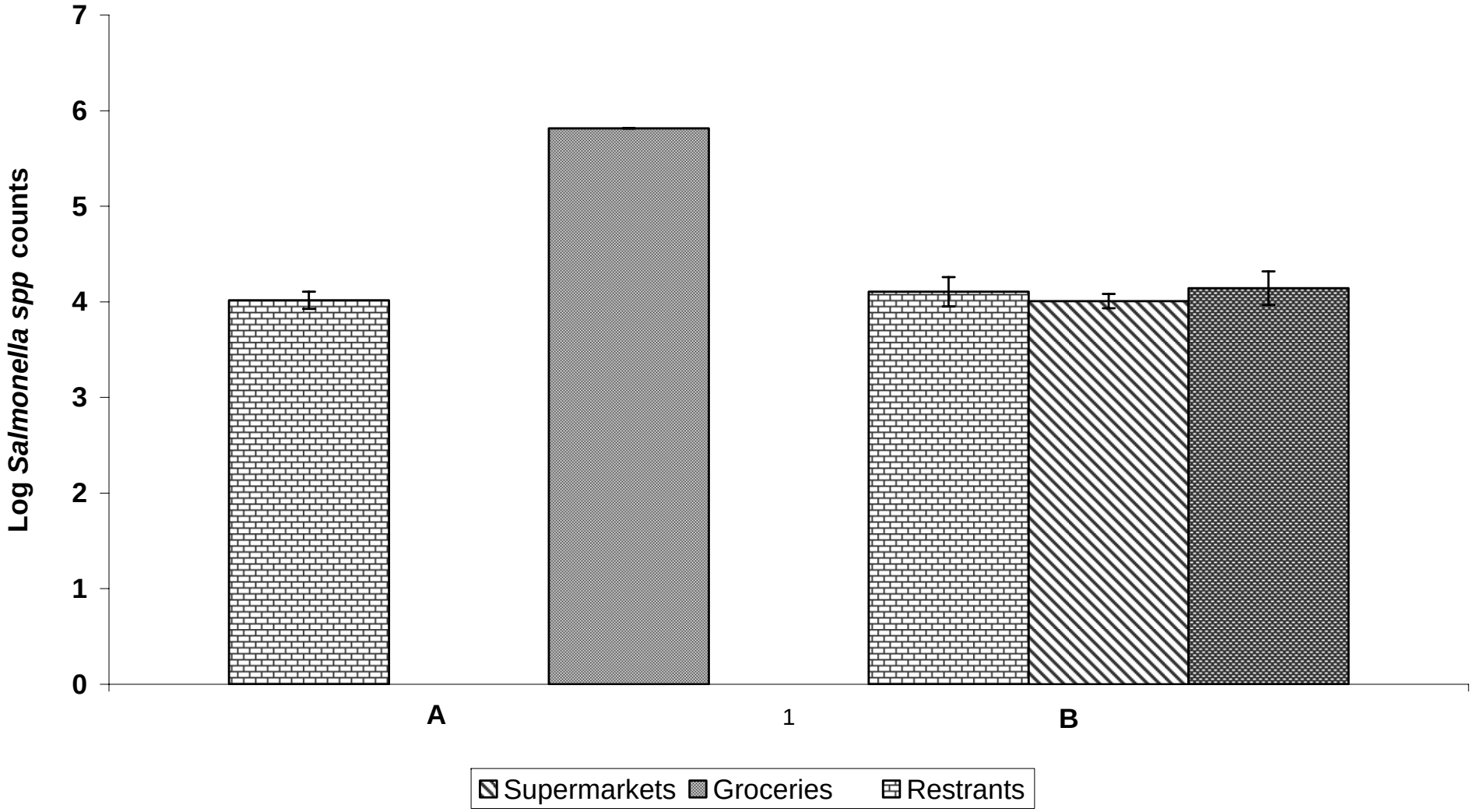

Figure 6. Comparison of *Salmonella spp.* counts of white soft cheese marketed in Khartoum North

The log counts of *Salmonella* spp. was found to be higher in both Elsafia's restaurant and groceries (Table 6 and Fig. 6). However it revealed a negative result in Bahrry's (Elmahata Elwsta) supermarkets compared to Elsafia's supermarkets (Table 5 and Table 6).

Positive isolation of *Salmonella* spp. specially in the restaurants and groceries suggested that large numbers of people were at high risk for subjecting to those food borne diseases. This might be due to mishandling or improper hygiene. According to Asperger *et al.* (1994) raw milk contamination usually takes place by salmonella from external sources (feces, the farmer, water pollution and dust). Similarly, Carson and Dewitt (2002) reported that Salmonella food poisoning can occur when someone drinks unpasteurized milk or eat any food contaminated during preparations, poor hygiene can also allow such carrier to spread the infection to others. However, the present result disagreed with Ahmed (1997) who found that *Salmonella* spp. was absent in the Sudanese white cheese. Hence the present study supported El-Gazzar and Marth, (1992) who reported that salmonellae continue to be a major concern for the dairy industry. Since these bacteria have caused recent outbreaks of illness and have been isolated from various dairy products in the market places (El-Gazzar and Marth, 1992; Geiss *et al.*, 1993; L'Ecuyer *et al.* 1996 and De Buyser *et al.*, 2001).

4.2.2.2 *Staphylococcus aureus*

The log count of *Staphylococcus aureus* was found to be 6.8±0.1, 6.7 and 6.9 for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurant, for mean±standard deviation, minimum and maximum values respectively, (Table 6 and Fig. 7). While the cheese samples collected from Elsafia's restaurant were found to have 8.1±1.3, 6.9 and 9.8 log counts for mean±standard deviation, minimum and maximum values, respectively. However *Staphylococcus aureus* during the present survey revealed a negative result for cheese samples collected from Bahrry's (Elmahata Elwsta) supermarkets (Table 6 and Fig. 7). While the cheese samples collected from Elsafia's supermarkets revealed log counts of 9.8±0.0, 9.8 and 9.8 for mean±standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 7). The present survey revealed a negative isolation for *Staphylococcus aureus* in cheese samples collected from groceries in both Bahrry and Elsafia. However, the log count of *Staphylococcus aureus* is very high in both Bahrry and Elsafia restaurants (Table 6 and Fig. 7).

This result is higher in compared with Ahmed (1997) who found that the maximum log counts of *Staphylococcus aureus* in Sudanese white cheese was 3.5. Also this high counts of Staphylococcus agreed with the result of Johnson *et al.* (1990).

They also reported that cfu of *S. aureus* numbering 10^6 + 10^8/g or ml of food must be present inorder to produce sufficient amounts of toxins

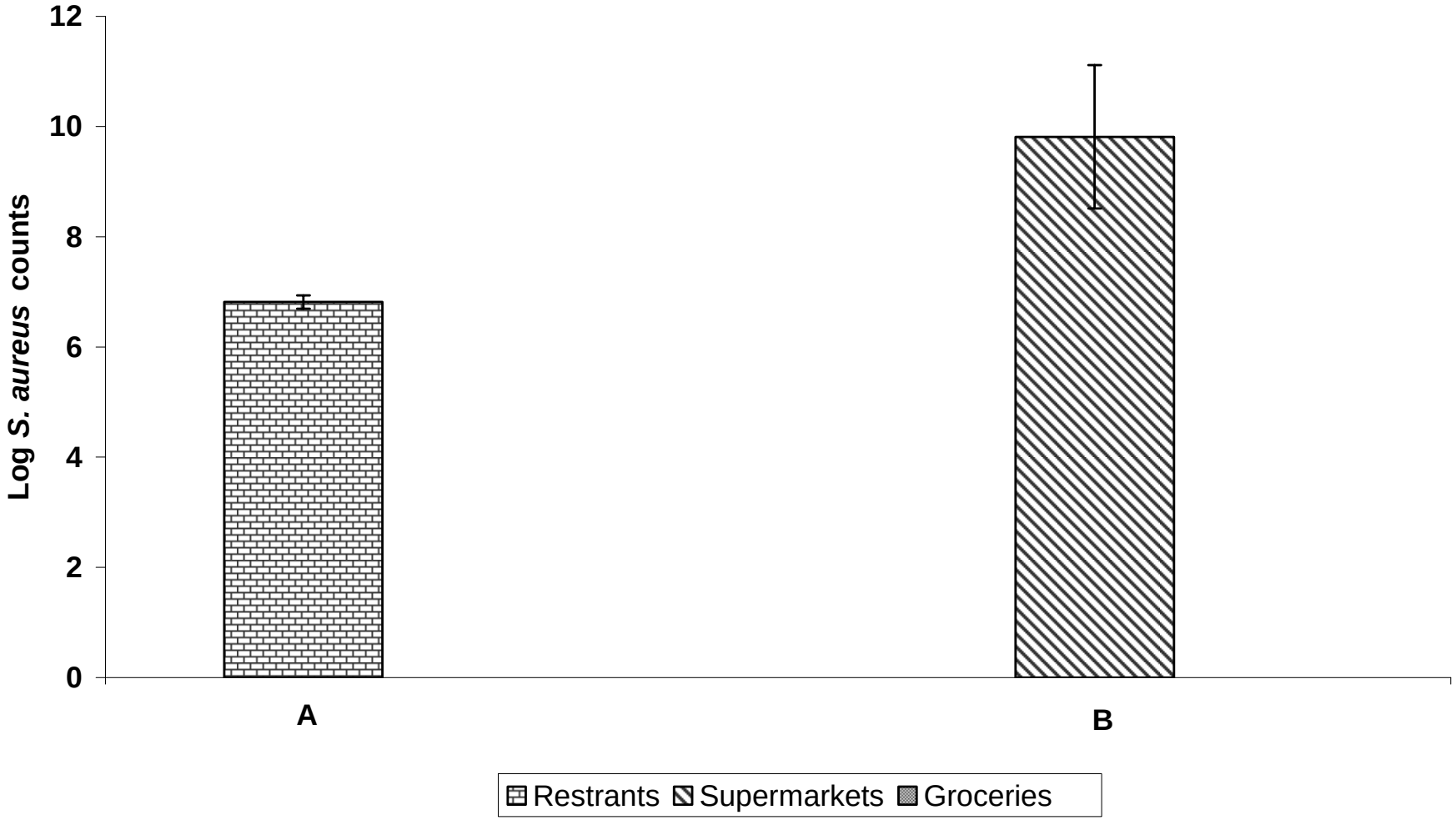

Figure 7. Comparison of *S. aureus* counts of white soft cheese marketed in Khartoum North

for poisoning. Moreover, Simko and Brevtko *et al.* (1996) reported that pasteurized milk was a major source of *Staphylococcus aureus* enterotoxicosis. Moreover, *Staphylococcus aureus* have been found in various types of cheese and involved in outbreaks of food poisoning (Harrigan and McCance, 1976; Khalid, 1981; De Buyser *et al.*, 2001; and De Reu *et al*, 2002). Moreover, Noble (1981) reported that 10 – 40% of people are asymptotic carriers of *S. aureus,* mostly at the mucosal membrane.

4.2.2.3 *Eschriechia coli*

The log of *Eschriechia coli* counts was found to be 5.6±0.1, 5.5 and 5.7 for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurants for mean±standard deviation minimum and maximum values respectively (Table 6 and Fig. 8). While the cheese samples collected from Elsafia's restaurants revealed log values of 4.8+0.0, 4.8 and 4.9 respectively (Table 5 and Fig. 8).

***Escherichia coli* during the present survey revealed a negative isolation for cheese samples collected from supermarkets in both Bahrry and Elsafia (Table 6 and Fig. 8). The log of *E. coli* was found to be 5.8±0.0, 5.8 and 5.9 for cheese samples collected from Bahrry's (Elmahata Elwsta) groceries for mean + standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 8). While the cheese samples collected from Elsafia's groceries showed lower values (4.7±0.0, 4.7 and 4.8) for**

mean±standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 8).

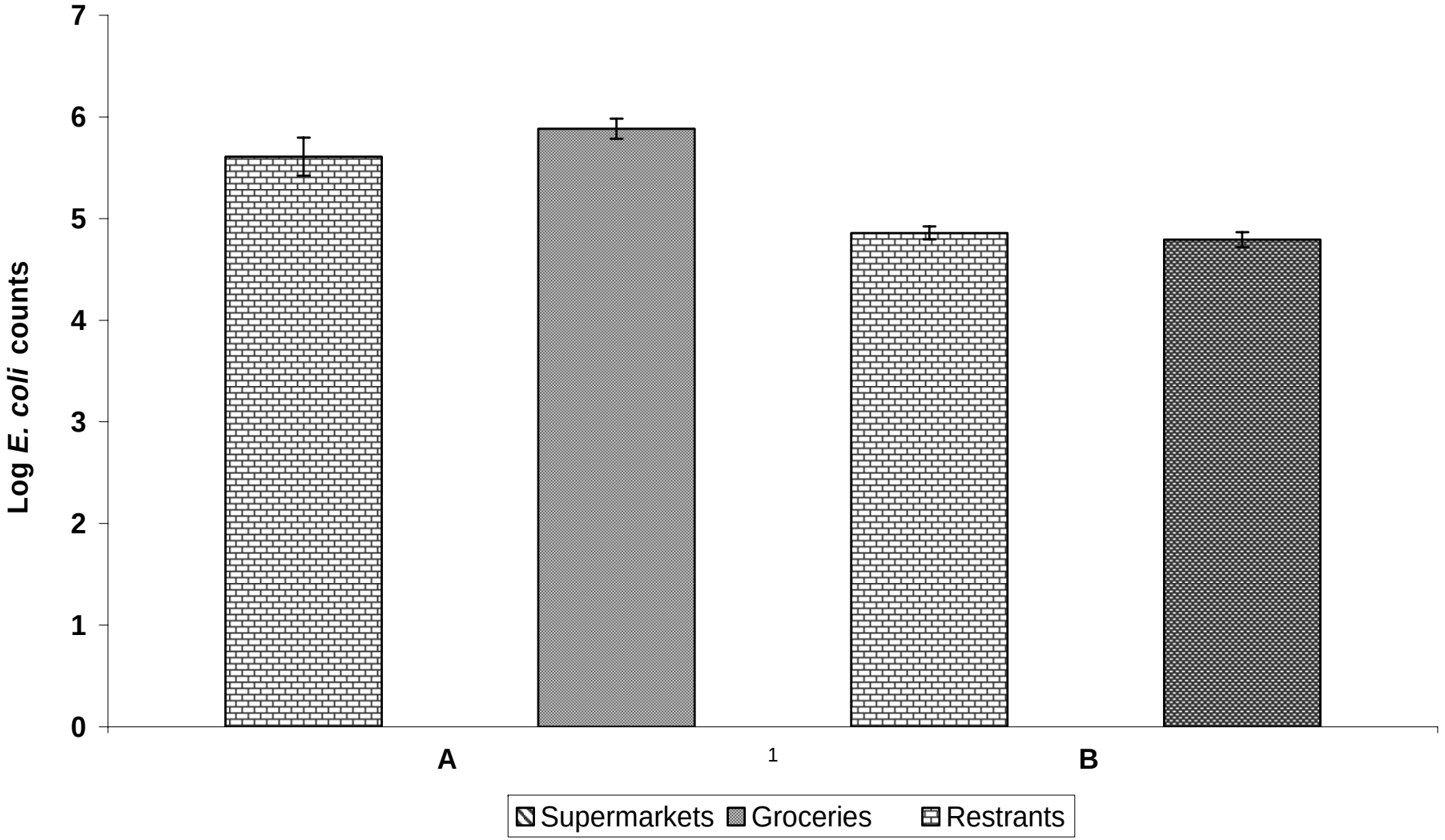

Figure 8. Comparison of *E. coli* counts of white soft cheese marketed in Khartoum North

The log of *E. coli* in restaurants in both Bahrry and Elsafia were very high and this might be due to post contamination of the product as stated by Yang and Jones (1961) and Kosikowiski (1977). Similarly this result (Table 4 and Fig. 3) could be attributed to the traditional method used for the distribution of the product which subjecting it to contamination. Moreover Adesiyun (1997) reported that unrefrigerated transportation of milk from dairy farms, through collection centers, to the major processing plants may have been responsible for the high counts of *E. coli* and *S. aureus* in the milk.

The log count of *E. coli* in both Bahrry's (Elmahata Elwsta) and Elsafia's groceries was high. This was similar to Maher *et al.* (2001) who resulted that the manufacturing procedure encouraged some growth of *E. coli* O157:H7. Moreover they also reported that it might be due to mishandling and poor hygiene. *Escheirchia coli* in cheese samples from supermarkets in both Bahrry and Elsafia (Table 6 and Fig. 8) was absent and had a negative result. This interesting result was compared to those samples collected from restaurants and groceries which might be due to good quality and good hygiene.

4.2.2.4 Coliforms counts

The log counts of coliforms bacteria was found to be 6.9±1.4, 5.8 and 8.8 for mean±standard deviation, minimum and maximum values respectively (Table 6 and Fig. 9), for cheese

samples collected from Bahrry's (Elmahata Elwsta) restaurants. While the cheese samples collected from Elsafia's restaurants showed log counts of

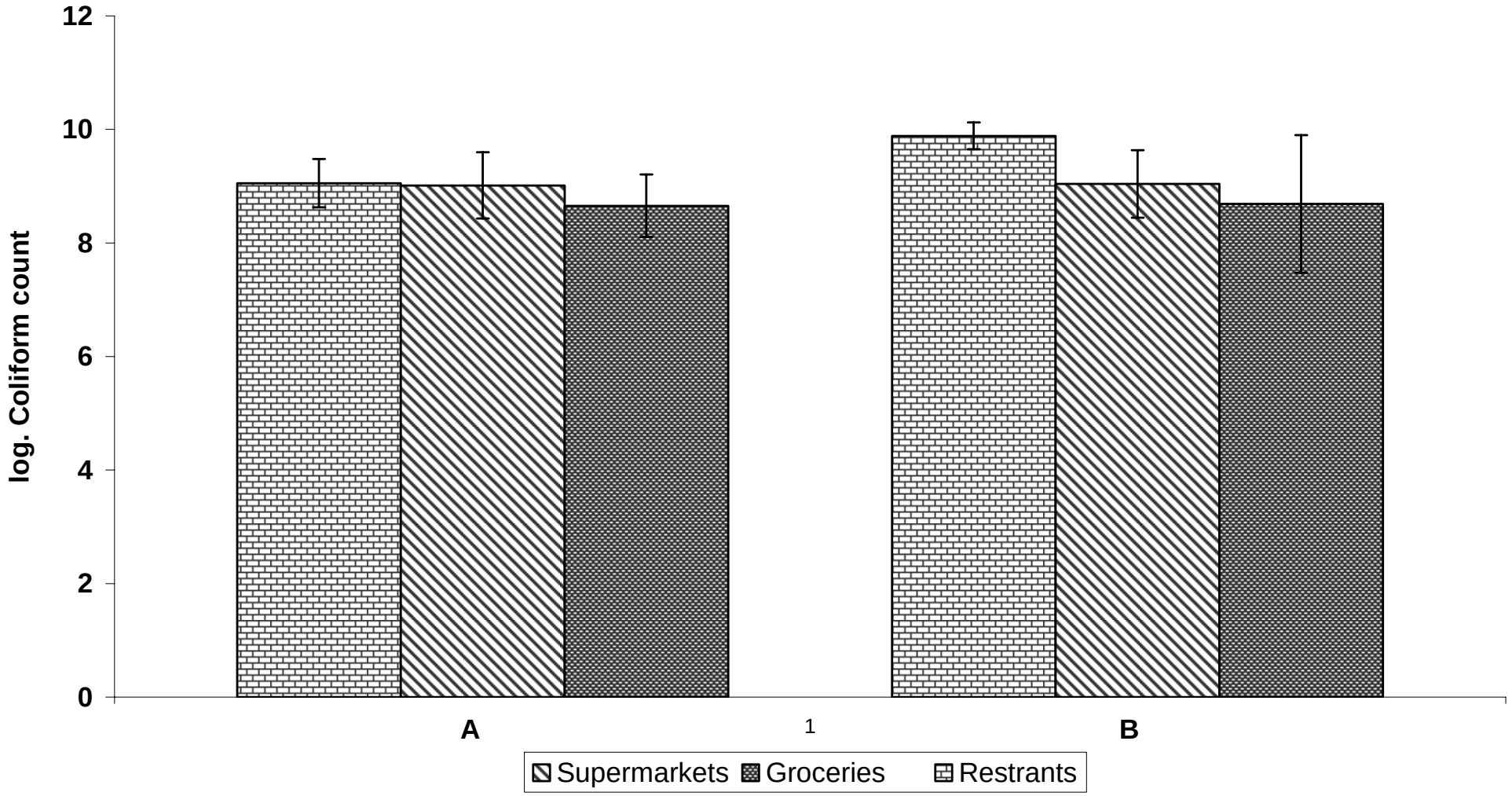

Figure 9. Compareson of Coliforms counts of white cheese marketed in Khartoum North

6.1±0.2, 5.8 and 6.4 for mean±standard deviation minimum and maximum values, respectively (Table 6 and Fig. 9). Similarly the log counts of coliforms bacteria was found to be 6.7±0.4, 6.0 and 7.1 respectively, for cheese samples collected from Bahrry's (Elmahata Elwsta) supermarkets (Table 6 and Fig. 9). While the cheese samples collected from Elsafia's supermarkets had a log counts of 6.3±0.5, 5.8 and 7.1 for mean±standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 9).

The log counts of coliform in cheese samples were very high specially in restaurants compared to supermarkets (Table 6 and Fig. 9). Similarly the log counts of coliforms bacteria was found to be 6.6±0.5, 5.8 and 7.0 for mean±standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 9), for cheese samples collected from Bahrry's (Elmahata Elwsta) groceries. While the cheese samples collected from Elsafia's groceries revealed log counts of 6.3±1.2, 5.1 and 7.8 respectively (Table 6 and Fig. 9).

This result agreed with Kosikowiski (1977), Massa *et al.* (1992) and Coveney *et al.* (1994). Moreover, Kosikowiski (1977) reported that coliforms bacteria grow well in cold or warm cheese causing slit eyes. He also reported that coliforms don't survive in pasteurized cheese milk but may be present as a result of post pasteurization contamination. Similarly, Massa *et al.* (1992) reported that high concentration of fecal coliforms was observed in Mozzarella cheese. Coveney *et al.* (1994) also found that the

incidence of coliforms were higher in soft, semi-soft and hard cheese.

4.2.2.5 Total bacteria counts

The log counts of total bacteria was found to be 9.0±0.4, 8.4 and 9.4 for mean±standard deviation, minimum and maximum values respectively, for cheese samples collected from Bahrry's (Elmahata Elwsta) restaurants (Table 6 and Fig. 10). Similarly, cheese samples collected from Elsafia's restaurants, showed log counts of 9.0±0.7, 9.3 and 10.8 for mean±standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 10).

Total bacteria counts was found to be 9.0±0.5, 8.2 and 9.4 for mean±standard deviation, minimum and maximum values respectively (Table 6 and Fig. 10) for cheese samples collected from Bahrry's (Elmahata Elwsta) supermarkets. The cheese samples collected from Elsafia's restaurants revealed values of 9.2±0.2, 8.6 and 9.4 for mean±standard deviation, minimum and maximum values respectively (Table 6 and Fig. 10).

The log counts of total bacteria was found to be 8.6±0.5, 8.2 and 9.3 for mean±standard deviation, minimum and maximum respectively (Table 4 and Fig. 5) for cheese samples collected from Bahrry's (Elmahata Elwsta) groceries while cheese samples collected from Elsafia groceries showed values of 9.2±0.2, 8.6 and 9.4 for mean±standard deviation, minimum and maximum values, respectively (Table 6 and Fig. 10).

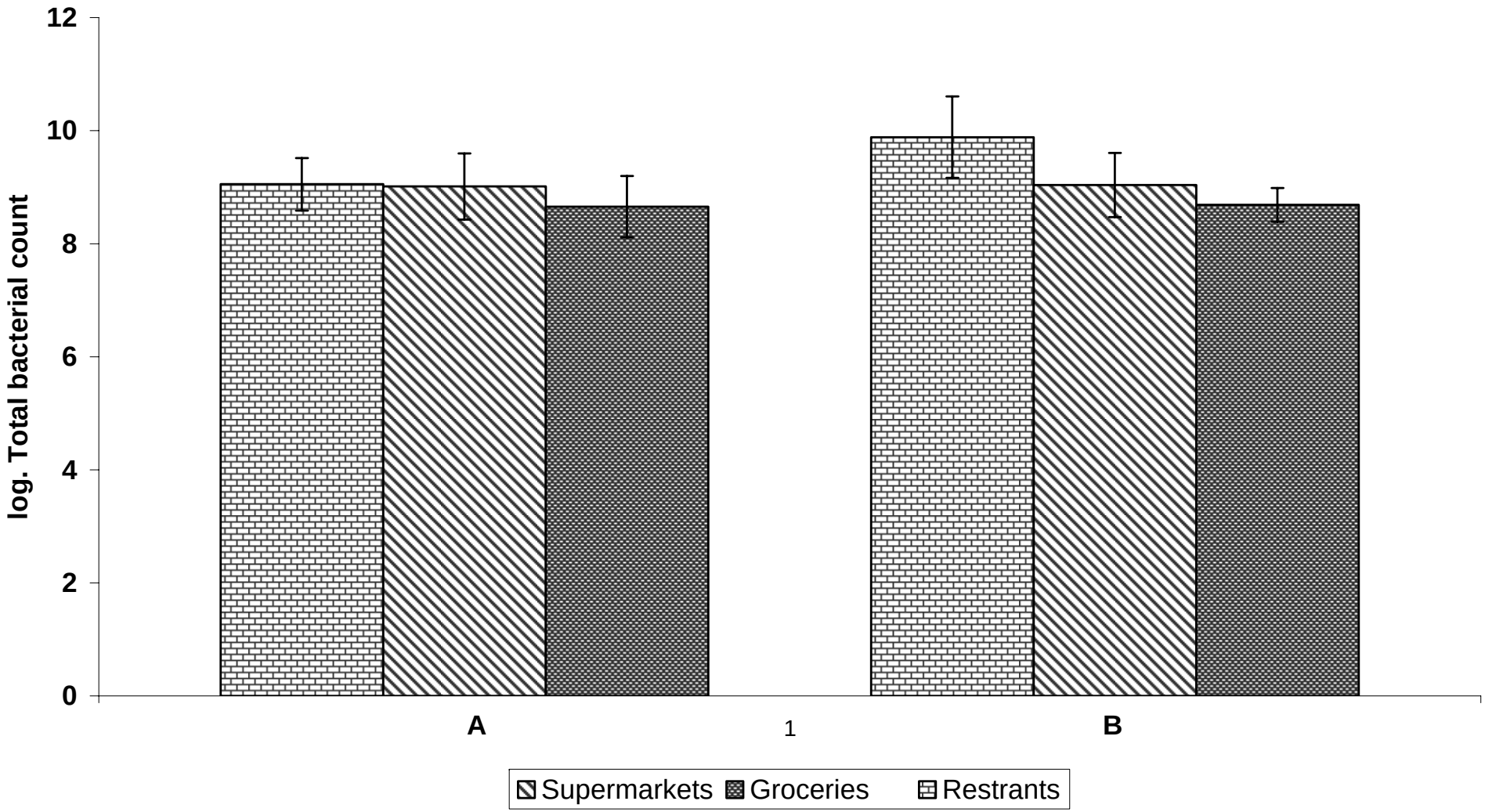

Figure 10. Comparison of total bacterial count in white soft cheese marketed in Khartoum North

The results indicated the presences of pathogenic bacteria (Salmonella and coliforms) as shown in Table 5 during the present survey. It is disagreed with Ahmed (1997) who demonstrated the absence of coliform and salmonella in Sudanese while soft cheese. Moreover, The high counts of pathogenes in cheese samples in the present survey (*Salmonella, E. coli, Staphylococcus* and coliforms) influence the counts of total bacteria (Table 6 and Fig. 10).

CONCLUSION AND RECOMMENDATIONS

Conclusion:

The present study concluded that there is non-significant variation in the chemical content of the Sudanese white soft cheese marketed in Khartoum North. However, high bacterial load are found to be associated with cheese samples collected from different sources and location. Moreover, the study was able to identify some potentially food-borne pathogens, such as *Staphylococcus aureus, E. coli, Salmonella typhi* and *Salmonella paratyphi,* especially in cheese samples collected from the restaurants followed by groceries compared to those collected from supermarkets. This might indicate that the level of hygiene and storage of the product and its handling played a major role in the contamination of the cheeses.

Recommendations

Hence the following recommendation are suggested:-

1. Processing of high quality milk that produced from healthy animals in a hygienic manner using hygeinic utensils.

2. Ensurance of the safety of milk products by proper handling, storage and marketing.

3. Preventions of post processing contamination of dairy products particularly the Sudanese white cheese which was used by large population.

4. **Periodic check out for all food distributing centers by the official authorities.**
5. **Health certificate for all people in contact with food to ensure that they are free from diseases, especially food borne infections.**
6. **Further studies are needed to demonstrate the vehicles and sources of contaminations with food borne diseases and poisonings. Similarly molecular characterizations of those pathogens and their toxins are needed to determine the levels of the health hazards that might a rise.**

REFERENCES

Abdalla, M.O. (1992). Effect of processing conditions on the microbiological quality of white pickled cheese. Ph.D. Dissertation, University of Tennessee, Knoxuille, U.S.A.

Abdel Razig, A.K. (1996). The production of white soft cheese from different milk sources. M.Sc. Thesis, University of Khartoum, Sudan.

Adesiyun, A.A.; Webb, L.A.; Romain, H. and Kaminjolo, J.S. (1997). Prevalence and characteristics of strain of *Escherichia coli* isolated from milk and feces of cows on dairy farms in Trinided. J. of Food Prot., 60(10): 1174-1181.

Ahmed, Awatif, M. (1997). The common microorganisms in the Sudanese white soft cheese. Sud. J. Vet. Sci. Anim. Husb, 36(2): 109-112.

Ahmed, T.K. and Khalifa, N.A. (1989). The manufacture of white soft cheese (Gibna Beyda) from recombined milk. Sudan J. Animal Prod., 2: 63-69.

Al-Ashmaway, A.M. (1990). Hand book of food hygiene: Fluid milk. Dairy Products, Fats and Oils, eggs, El-Fardoos Publishing Co. Cairo, Egypt.

Allah Gabo, I.H. (1986). Studies on the composition and quality of Gibna Beyda. M.Sc. Thesis, University of Khartoum, Sudan.

Ansay and Kaspar (1997). Survey of retail cheeses, dairy processing environments and raw milk for *Escherichia coli* O157:H7. Lett. Appl. Microbiol., 25(2): 131-134.

AOAC (1990). Official methods of analysis, 15th edition. Association of Official Analytical Chemist, Washington, D.C. USA.

Araujo, V.S.; Pagliares, V.A.; Gueiroz, M.L. and Freitas-Almedia, A.C. (2002). Occurrence of *Staphlylococcus* and enteropathogens in soft cheese commericalized in the city of Rio De Janeiro, Brazil. J. Appl. Microbiol., 92(6): 1172-1177.

Asperger, H (1994). *Staphylococcus aureus* In: The significance of pathogenic microorganisms in raw milk. International Dairy Federation .

Babiker, M.A. (1987). Technological studies on Sudanese white soft cheese. M.Sc. Thesis, University of Khartoum, Sudan.

Bantwart, G.J. (1980). Basic Food Microbiology. Second edition, New York, USA, pp. 112-135.

Barrow and Felthman (1993). Manual for the identification of medical bacteria, 3rd, Cambridge.

Brocklehurst, T.F. and Lund, B.M. (1988). The effect of pH on the initiation of growth of cottage cheese spoilage bacteria. Int. J. Food Microbial. 6(1): 43-49.

Caridi, A. (2002). Selection of *Escherichia coli* – inhibiting strains of *Lactobacillus casei subsp. paracasei*. J. Ind. Microbiol. Biotechnol., 29(6): 303-308.

Carson, Rosalyn and Dewitt, D.M. (2002). Gale Encyclopedia of Medicine. Cited in www.healthatoz.com/health a to z /AtoZ/ency/Salmonella_food_poisoning_html.

Chapman, H.B. and Sharpe, M.E. (1981). The microbiology, Vol. 2. Robinson, R.K. APFP.Sci. Publ., London.

Coveney, H.M.; Fitzgerald, G.F. and Daly, C. (1994). A study of the microbiological status of fresh farm house cheeses with emphasis on selected pathogenic and spoilage micro-organisms. J. Appl. Bacteriol., 77(6): 621-630.

D'Aoust, J.Y. (1994). Salmonella and international food trade. Int. J. Food Microbiol. 24(1-2): 11-31.

De Buyser, M.L.; Dufour, B.; Maire, M. and Lafarge V. (2001). Implication of milk and milk products in food borne disease in France and in different industerialised countries. Int. J. Food Microbiol., 20:67(1-2): 1-17.

De Reu, K.; Debeuckelaere, W.; Botteldoorn, N.; De Block, J. and Herman, L. (2002). Hygeinic parameters, toxins and pathogens occurance in raw milk cheeses. Journal of Food Safety, 22 . Avilable at http:// www. foodscipress.com/ JFSV22.3.htm.

El-Gazzar, F.E. and Marth, E.H. (1992). Salmonellae, salmonellosis, and dairy foods: a review. J. Dairy Sci. 75(9): 2327-2343.

FAO/WHO (1973). Definition and describtion in the tropics and subtropics. Rndoc Wageninen.

Foster, M.E.; Nelson, F.E.; Speek, L.M.; Poetch, N.R. and Olsen, C.J. (1961). Dairy Microbiology Prentio-Hall Inc. Englewood.

Franklin, J.C. and Sharpe, M.E. (1963). The incidence of bacteria in milk and Cheddar cheese and their association with flavor. J. of Dairy Res. 30: 87-99.

Geiss, H.K.; Ehrhad, I.; Rosen-Wolff, A.; Sonntag, H.G.; Pratsch, J.; Wirth, A.; Kruger, D.; Knossmann-Sehanbacher, I.; Kuhn, A. and Treiber-Klotzer, C. (1993). Food borne outbreak of a *Salmonella enteritidis* epidemic in a large pharmaceutical industry. Gesundheitswesen, 55(3): 130 135.

Gorden, J.F. and Shapton, N. (1977). Starter P. 147. Elesvier Applied Science Publishers. J. Soc. Dairy Tech, 30, 15. Cited in cheese making practice (1989).

Hamed, O.I. (1998). The effect of processing conditions on yield, chemical composition and sensory characteristics of white soft cheese. M.Sc. Thesis, University of Khartoum, Sudan.

Harrigan, W.F. and Margaret, E. McCance (1976). Laboratory Methods in Food and Dairy Microbiology. 25-29.

Hofi, A.A.; Hagrass, A.E.; Foyed, A.E. and Haggag, H.F. (1982). Detection of milk powdered in some dairy products. I. white cheese. Egypt. J. Dairy Sci., 10: 171-179.

Holeckova, B.; Holoda, E.; Fotta, M.; Kalinacoue, V.; Gondol, J. and Grolmus, J. (2002). Occurrence of enterotoxigenic *Staphylococcus aureus* in food. Ann. Agric. Environ. (9): 179-182.

Ibrahim, A.E. (1971). Studies of some characteristics of Sudanese white cheese. The Sudan Journal of Veterinary Science and Animal Husbandry; 12: 31-39.

Ibrahim, G.F.; Randford, D.R.; Baldock, A.K. and Ineland, L.B. (1981). Inhibition of growth of *Staphyococcus aureus* and enterotoxin. J. of Food Protection. 44(2): 189-193.

Jay, M.J. (1986). Modern Food Microbiology, 3rd ed., Van. Nostrand Reinhold Company, USA, New York. 18-21 and 242-243.

Johnson, M. E., Riesterer, B. A.; Chen, C.; Tricomi, B. and Olson, N. F. (1990). Effect of packaging and storage condition on the calcium lactate crystilization on the surface of cheddar cheese. Journal of Dairy Science, 73 (11): 3033-3041.

Kasrazadeh, M. and Genigerorgis, C. (1994). Potential growth and control of salmonella in Hispanic type soft cheese. Int. J. Food. Microbiol., 22(2-3): 127-140.

Khalid, A. S. (1981). Growth and enterotoxin production by Staphylocoocus aureus in Sudanese semi preserved food with special reference to white soft cheese. Ph. D. Thesis, Unversity of Khartoum, Sudan.

Khalid, E.A. (1991). The effect of salt concentration on the yield and chemical composition of Sudanese white cheese. M.Sc. Thesis, University of Khartoum, Sudan.

Kosikowiski, F.V. (1966). Cheese and fermented milk food, Edwards Brothers Inc., Ann. Arbor, Michigan, U. S. A.

Kosikowiski, F.V. (1977). Cheese and fermented milk food. Published by the author Department of Food Sci. Cornell Univ., Ithaca, New York.

L'Ecuyer, P.B.; Diego, J.; Murphy, D.; Trovillien, E.; Jones, M. and Sahm, D.F, Fr. (1996). Nosocomial outbreak of gastroenteritis due to *Salmonella senftenbe*. Clin. Infect Dis., 23(4): 734-742.

Maher, M.M.; Hordan, K.N.; Upton, M.E. and Coffey, A. (2001). Growth and survival of *E. coli* O157: H7 during the manufacture and smear-ripened cheese produce from raw milk. J. Appl. Microbiol., 90(2): 201-207.

Massa, S.; Cardini, F.; Sinigaglia, M. and Guerzoni, M.E. (1992). *Klebsiella pneumoniae* as a spoilage organism in Mozzarella cheese. J. Dairy Science, 75(6): 1411-1414.

Miller, A.M. and Paige, J.C. (1998). Food borne infections. Food Anim. Pract. 14(1): 71-89.

Noble, W. C. 1981. Microbiology of human skin, 2nd ed. Lloyd-Luke Ltd.,London, United Kingdom.

Nofal, A.; Elhami, M.; ElGazzar, H.; Abu-El-Kheir, A., El Gazzar, H.; Kheir, A. A. and Abu E. (1981). Studies on acceleration of manufacturing Domati cheeses. III. Effect of storage on yield and properties of cheese manufactured by the suggested methods. Agriculture Research Review. 59 (6): 301-312.

Nuser, S.M. (2001). The effect of cooking and vacuum packing on the quality of white soft cheese. M.Sc. Thesis, University of Khartoum, Sudan.

Papageorgiou, D.U.; Ibrahim, A.; Bori, M. and Doundounkis, S. (1998). Chemical and bacteriological characteristics of Pichtogalo chanion mesophilic starter cultures for its production. J. Food Prot., 61(6): 688-692.

Pourshahe, M.R.; Saific, M.; Shafiee, A.; Vahdani, P.; Aslani, M. and Salemian, J. (1998). An outbreak of food-borne botulism associated with contaminated locally made cheese in Iran. Scand J. Infect. Dis. 30(1): 92-94.

Price, R.J. and Lee, J.S. (1970). Inhibition of *Pseudomonas* species by hydrogen peroxide producing *Lactobacilli*, J. Milk Food Technol. 33: 13-18.

Professional Food Microbiological Group (1998). Food safety and cheese science and technology. Food Science and Technology Today, 12(2) 117-121.

Rampling, A. (1996). Raw milk cheeses and salmonella. British Medical Journal, 312: 67-68.

Richardson, H.G. (1985). Standard methods for the examination of dairy products, 6th ed, pp. 133-150. American Public Health Association, Washington, D.C.

Saad, S.M.; Vanzin, C.; Oliveira, M.N. and Franco B.D. (2001). Influence of lactic acid bacteria on survival of *Escherichia coli* inoculated Minas cheese during storage at 8.5 degrees C. J. Food Prot. 64(8): 115-150.

Scott, R. (1986). Cheese making practice. Second eddition, Elesvier Applied Science Publishers at Essex IG 11 JU, England.

Simko, S. and Brartko, P. (1996). Resistance to antibiotics in *Staphylococcus aureus* at ewe mastitis in sheep milk and its products. Vet Med. Czech, 8: 241-249.

Spano, G.; Goffnedo, E.; Beneduco, L.; Tavantio, D.; Dupuy, A. and Massa, S. (2003). Fate of *Eschenichia coli* 0157: H7 during manufacture of mozzarella cheese. Lett. Appl. Microbiol., 36(2): 73-76.

Speek, M.L. (1972). Control of food-borne pathogens by starter cultures. J. Dairy Sci. 55: 1019-1023.

Vlaemynck, G. (1994). Salmonella. In: The significance of pathogenic microorganisms in raw milk. International Dairy Federation.

Yang, H.Y. and Jones, G.A. (1969). Physiological characteristics of enter pathogenic and non pathogenic coliform bacteria isolated from Canadian pasteurized dairy products. J. Milk Food Technol., 32: 102-109.