Quality Evaluation of Traditionally Fermented Milk (*Roub*)

Produced and Sold In Khartoum State, Sudan

By

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DEDICATION

To my mother and father

To my dear husband

To the soul of my grandfather who wished to see this day

Asked Allah to forgive him and let the paradise his place
ACKNOWLEDGMENT

All grateful to Allah for the assistance, health and give me the ability to accomplish this work.

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TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGMENT</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF CONTENTS</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>V</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>Vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>VII</td>
</tr>
<tr>
<td>المستخلص</td>
<td>VIII</td>
</tr>
<tr>
<td>CHAPTER ONE: INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER TWO: LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>2.1 Fermentation and fermented dairy products</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1 Definition of fermentation</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2 Fermentation pathways</td>
<td>4</td>
</tr>
<tr>
<td>2.1.3 History of fermentation</td>
<td>5</td>
</tr>
<tr>
<td>2.1.4 Functional properties of fermented dairy products</td>
<td>6</td>
</tr>
<tr>
<td>2.1.5 Antimicrobial activity of Probiotics</td>
<td>8</td>
</tr>
<tr>
<td>2.1.6 Probiotics as protective culture</td>
<td>9</td>
</tr>
<tr>
<td>2.2 Fermented dairy products in the world</td>
<td>9</td>
</tr>
<tr>
<td>2.2.1 Dahi</td>
<td>9</td>
</tr>
<tr>
<td>2.2.2 Suusac</td>
<td>10</td>
</tr>
<tr>
<td>2.2.3 Lben</td>
<td>10</td>
</tr>
<tr>
<td>2.2.4 Kajmak</td>
<td>11</td>
</tr>
<tr>
<td>2.3 Traditional dairy products in Sudan</td>
<td>11</td>
</tr>
<tr>
<td>2.3.1 Gariss</td>
<td>11</td>
</tr>
<tr>
<td>2.3.2 Roub</td>
<td>12</td>
</tr>
<tr>
<td>2.3.2.1 Preparation method</td>
<td>13</td>
</tr>
<tr>
<td>2.3.2.2 Uses of roub</td>
<td>14</td>
</tr>
<tr>
<td>CHAPTER THREE: MATERIALS AND METHODS</td>
<td>15</td>
</tr>
<tr>
<td>3.1 Sample collection</td>
<td>15</td>
</tr>
<tr>
<td>3.2 Sample analysis</td>
<td>15</td>
</tr>
<tr>
<td>3.2.1 Fat content</td>
<td>15</td>
</tr>
<tr>
<td>3.2.2 Protein content</td>
<td>16</td>
</tr>
<tr>
<td>3.2.3 Total solids content</td>
<td>17</td>
</tr>
<tr>
<td>3.2.4 Ash content</td>
<td>17</td>
</tr>
<tr>
<td>3.2.5 Titratable acidity</td>
<td>17</td>
</tr>
<tr>
<td>3.2.6 Bold of Solids-non-fat</td>
<td>18</td>
</tr>
<tr>
<td>3.3 Microbiological examination</td>
<td>18</td>
</tr>
<tr>
<td>3.3.1 Sterilization of equipment</td>
<td>18</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (Cont'd)

3.3.2 Preparation of sample dilutions 18
3.3.3 Preparation of media 18
3.3.3.1 Plate count agar medium (PCA) 18
3.3.3.2 MRS agar medium 19
3.3.3.3 MacConkey agar medium 19
3.3.3.4 Yeast extract agar medium 19
3.4 Counting 20
3.4.1 Total viable bacterial count 20
3.4.2 Lactic acid bacteria count 20
3.4.3 Coliforms count 20
3.4.4 Yeasts and moulds count 20
3.5 Statistical analysis 20

CHAPTER FOUR: RESULTS AND DISCUSSION 21
4.1 Chemical composition of *roub* collected from areas under study 21
4.2 Effect of storage period on the chemical composition of *roub* 23
4.3 Microbiological characteristics of *roub* collected from areas under study 29
4.4 Effect of storage period on microbiological population 31

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS 37

REFERENCES 38
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chemical composition of <em>roub</em> samples collected from Khartoum, Khartoum North and Omdurman (Mean ±SD)</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Effect of storage period on chemical composition of <em>roub</em> samples collected from areas under study (Mean ±SD)</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>Effect of storage period on chemical composition of <em>roub</em> samples collected from Khartoum (Mean ±SD)</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>Effect of storage period on chemical composition of <em>roub</em> samples collected from Khartoum North (Mean ±SD)</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>Effect of storage period on chemical composition of <em>roub</em> samples collected from Omdurman (Mean ±SD)</td>
<td>37</td>
</tr>
<tr>
<td>6</td>
<td>Microbiological characteristics of <em>roub</em> samples collected from Khartoum, Khartoum North and Omdurman (Mean ±SD)</td>
<td>39</td>
</tr>
<tr>
<td>7</td>
<td>Effect of storage period on microbiological characteristics of <em>roub</em> samples collected from areas under study (Mean ±SD)</td>
<td>43</td>
</tr>
<tr>
<td>8</td>
<td>Effect of storage period on microbiological characteristics of <em>roub</em> samples collected from Khartoum (Mean ±SD)</td>
<td>44</td>
</tr>
<tr>
<td>9</td>
<td>Effect of storage period on microbiological characteristics of <em>roub</em> samples collected from Khartoum North (Mean ±SD)</td>
<td>45</td>
</tr>
<tr>
<td>10</td>
<td>Effect of storage period on microbiological characteristics of <em>roub</em> samples collected from Omdurman (Mean ±SD)</td>
<td>46</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pathway of fermentation</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Protein coagulation</td>
<td>5</td>
</tr>
</tbody>
</table>
CHEMICAL AND MICROBIOLOGICAL CHARACTERISTIC OF TRADITIONALLY FERMENTED MILK (ROUB) PRODUCED AND SOLD IN KHARTOUM STATE, SUDAN

Safinaz Adil Sir El-khatem Osman

Degree of M.Sc. (Dairy Production and Technology)

ABSTRACT

This study was conducted to evaluate the quality of roub during shelf life. Thirty roub samples were collected from Khartoum, Khartoum North and Omdurman areas for physico-chemical (fat, protein, TS, SNF, ash, acidity) and microbiological (TVBC, coliform bacteria, lactic acid bacteria, yeasts and moulds) characteristics were determined at 1, 7, 14, 20-day intervals.

Results showed that area from which samples were collected had a significant (P<0.001) effect on all physico-chemical characteristics, while the storage period had a significant (P<0.001) effect on protein, ash and acidity.

The storage period significantly (P<0.001) affected on the protein and ash contents of roub from Khartoum, while all physico-chemical characteristics of roub from Khartoum North were significantly (P<0.001) affected by the storage period except SNF, and only TS and SNF were significantly affected by the storage period for samples collected from Omdurman.

All microorganisms tested were significantly (P<0.001) by the area from which samples were collected and coliform bacteria was not detected in samples collected from Omdurman.

The storage period significantly (P<0.001) affected all microorganisms tested. TVBC and yeasts and moulds count were not significantly (P>0.05) affected by the storage period for samples collected from Khartoum, while only coliform bacteria was significantly (P<0.001) affected by the storage period for samples collected from Khartoum North, and all microorganisms were significantly (P<0.001) affected by the storage period for samples collected from Omdurman. Coliform bacteria were not detected in samples collected from Omdurman.
تقييم الخصائص الفيزيوكيميائية والميكروبية للألبان المخبأة تقليديا (الروب) المنتج والمبايع في ولاية الخرطوم، السودان

صافيناز عادل سر الختن عثمان

ماجستير العلوم في إنتاج وتغذية الألبان

المستخلص

أجريت هذه الدراسة لتقييم الخصائص الكيميائية والميكروبية للروب أثناء فترة التخزين فترة الحفظ. جمعت ثلاثين عينة روب من كل من الخرطوم، الخرطوم شمالي وأم درمان. تم تحديد الخصائص الفيزيوكيميائية (الدهون، البروتين، الجواهر الكلية، الرماد، الحموضة والميكروبية (العدد الكلي للبكتريا الحية، بكتريا حامض اللاكتيك، بكتريا القولون، الخمار والعنق) في الأيام 10، 17، 20 يوم.

أوضح النتائج أن المنطقة التي جمعت منها العينات كان لها تأثير معنوي على كل الخصائص الفيزيوكيميائية، وكان لفترة التخزين تأثير معنوي على البروتين، الرماد والحموضة فقط. أثرت فترة التخزين معنوي على البروتين والرماد والروب المجمع من منطقة الخرطوم، بينما تأثر كل الخصائص الفيزيوكيميائية معنوي للروب المجمع من منطقة الخرطوم شمال فترة التخزين، وتأثرت فقط الجواهر الكلية للروب المجمع من منطقة أم درمان معنوي بفترة التخزين.

كل الميكروبات التي تم اختبارها تأثيرة معنوية بالمنطقة التي جمعت منها العينات ولم بكتريا القولون لم توجد في العينات التي جمعت من منطقة أم درمان.

أثرت فترة التخزين معنوي على كل الميكروبات التي تم اختبارها. العدد الكلي للبكتريا الحية والخمار والعفن لم تتأثر معنوي بفترة التخزين للعينات التي جمعت من منطقة الخرطوم، في حين أن بكتريا القولون تتأثر معنوي بفترة التخزين للعينات التي جمعت من الخرطوم شمال. وكل الأحياء الميكروبات تتأثر معنوي بفترة التخزين للعينات التي جمعت من منطقة أم درمان.
CHAPTER ONE
INTRODUCTION
CHAPTER ONE
INTRODUCTION

Some of the major fermentation processes are based on the use of lactic acid bacteria which produce organic acids. The presence of fermentative lactic acid bacteria is crucial to the intrinsic properties of fermented food products (Ehrmann et al., 2002; Soomro et al., 2002).

However, the role of fermented milk in human nutrition is well documented and the virtues of these products were known to man even during the ancient days of civilization. These products have long been an important component of nutritional diet, and the medicinal and nutritional properties of various fermented foods have been experienced by several generations. The scientific community gave impetus to these beliefs in 1910, when Eli Metchnikoff suggested that man should consume milk fermented with lactobacilli to prolong his life. He postulated the desirable bacteria in the Bulgarian milk that could help in suppressing the undesirable and disease causing bacteria in the intestine of human beings (Panesar, 2011). The observation proved the way for exploring the potentials of lactic cultures and cultured products in the alleviation of human and animal disorders. Recently, importance has been given to produce fermented milk with improved health attributes particularly the therapeutic properties of these products (Panesar, 2011).
The objectives of this study are:

1) To determine the chemical composition and bacterial load of traditionally made  *roub* collected from different areas in Khartoum State.

2) To determine the quality of  *roub* during shelf life of 20 days
CHAPTER TWO
LITERATURE REVIEW
CHAPTER TWO
LITERATURE REVIEW

2.1 Fermentation and fermented dairy products

2.1.1 Definition of fermentation:

The word "fermentation" is derived from the Latin meaning "to boil," since the bubbling and foaming of early fermenting beverages seemed closely a kind to boiling (Shurtleff and Aoyagi, 2007).

Fermentation is the chemical transformation of organic substances into simpler compounds by the action of enzymes (complex organic catalysts) which are produced by microorganisms such as molds, yeasts or bacteria. Enzymes act by hydrolysis, a process of breaking down or predigesting complex organic molecules to form smaller (and in the case of foods, more easily digestible) compounds and nutrients. For example, the enzyme protease breaks down protein molecules first into polypeptides and peptides, then into numerous amino acids, which are readily assimilated by the body. The enzyme amylase works on carbohydrates, reducing starches and complex sugars to simple sugars, and the enzyme lipase hydrolyzes complex fat molecules into simpler free fatty acids. In some fermentation, important by-products such as alcohol or various gases are also produced (Shurtleff and Aoyagi, 2007).

Another definition by the International Dairy Federation (IDF) published general standards of identity for fermented milks that could be briefly defined as follows: fermented milks are prepared from milk and/or milk products ((e.g. any one or combinations of whole, partially or fully skimmed, concentrated or powdered milk, buttermilk powder, concentrated or powdered whey, milk protein (such as whey proteins, whey protein
concentrates, soluble milk proteins, edible casein and caseinates), cream, butter or milk fat all of which have been manufactured from raw materials that have been at least pasteurized)) by the action of specific microorganisms, which results in a reduction of the pH and coagulation (Khurana and Kanawjia, 2007).

Figure 1: Pathway of fermentation

2.1.2 Fermentation pathways:

The general pathway for fermentation of milk involves the production of lactic acid from lactose. This lowers the pH and results in a variety of products. In figure 1, glucose can be replaced by many different sugars, including lactose to produce the same product.

The protein responsible for curdling of milk is casein, and with the drop of pH, casein molecules coagulate in a structure similar to that shown below.
2.1.3 History of fermentation:

Human beings are known to have made fermented foods since Neolithic times. The earliest types were beer, wine and leavened bread (made primarily by yeasts) and cheeses (made by bacteria and molds). These were soon followed by East Asian fermented foods, yogurt and other fermented milk products, pickles, sauerkraut, vinegar (soured wine), butter, and a host of traditional alcoholic beverages. More recently, molds have been used in industrial fermentation to make vitamins B-2 (riboflavin) and B-12, textured protein products (from Fusarium and Rhizopus in Europe) antibiotics (such as penicillin), citric acid, and gluconic acid. Bacteria are now used to make the amino acids lysine and glutamic acid. Single-celled protein foods such as nutritional yeast and microalgae (spirulina, chlorella) are also made in modern industrial fermentations.

For early societies, the transformation of basic food materials into fermented foods was a mystery and a miracle, for they had no idea what caused the usually sudden, dramatic, and welcomed transformation.
Some societies attributed this to divine intervention; the Egyptians praised Osiris for the brewing of beer and the Greeks established Bacchus as the God of wine. Likewise, at many early Japanese miso and shoyu breweries, a small shrine occupied a central place and was bowed to daily. In ancient times fermentation joined smoking, drying and freezing as basic and widely practiced food preservation techniques. Hasseltine and Wang (1980) reported that "probably the first fermentation was discovered accidentally when salt was incorporated with the food material, and the salt selected certain harmless microorganisms that fermented the product to give a nutritious and acceptable food." The process was taken a step further by the early Chinese who first inoculated the basic foods with molds, which created enzymes in salt-fermented soy foods such as miso, soy sauce, soy nuggets, and fermented tofu (Shurtleff and Aoyagi, 2007).

2.1.4 Functional properties of fermented dairy products:

According to the Canadian Dairy Commission fermented milk products have numerous functional properties:

1- **Preservation:** bacteria are inhibited from growing through pH reduction when lactic acid is formed, and shelf life is increased.

2- **Flavour enhancement:** the sour characteristic of fermented milk products comes from fermentation products (lactic acid, diactyl, carbon dioxide, ethanol); these products act as excellent flavour carriers for herbs, spices and other flavourings.

3- **Texture enhancement:** some fermented milk products (sour cream or crème fraîche) can add body and thickness to sauces, dips or vinagrettes.

4- **Reducing caloric content:** many fermented milk products come in low fat or fat free varieties and can be used to substitute for higher fat ingredients.
5- **Emulsification:** milk proteins help stabilize fat emulsions in salad dressings, soups and cakes.

6- **Foaming and whipping:** crème fraîche is capable of being whipped like whip cream.

7- **Nutritional benefits:** fermented milk products may contain probiotic which are live microorganisms used as food supplements, that provide health benefits, when consumed, by improving the intestinal microbial balance of the host (Fuller, 1989). Microorganisms commonly used as probiotic include bifidobacteria, lactic acid bacteria and certain yeasts.

Since ancient times, food has been considered essential and indispensable to human life. Numerous studies clearly show that an individual’s quality of life is linked to daily diet and lifestyle (Moura, 2005).

Interest in the role of probiotic for human health began as early as 1908 when Metchnikoff associated the intake of fermented milk with prolonged life (Hattingh and Viljoen, 2011). However, the relationship between intestinal microbiota and good health and nutrition has only recently been investigated. Therefore, it was not until the 1960’s that health benefit claims began appearing on foods labels.

In recent years, there has been an increasing interest in probiotic foods, which has stimulated innovation and fueled the development of new products around the world. Probiotic bacteria have increasingly been incorporated into foods in order to improve gut health by maintaining the microbial gastrointestinal balance. The most popular probiotic foods are produced in the dairy industry because fermented dairy products have been shown to be the most efficient delivery vehicle for live probiotic to date (Rigobelo, 2012).
2.1.5 Antimicrobial activity of probiotic

Foodborne pathogens are the major concern for food safety, and various methods of food processing and preservation have been developed in order to improve food and reduce the incidence of food infection and intoxication. Several studies have been carried out to investigate the types of antimicrobials produced by probiotic and the range of pathogens susceptible to them. Common antimicrobials produced include bacteriocins (antimicrobial proteinaceous substances, e.g. nisin), hydrogen peroxide and organic acids such as lactic and acetic acids. In vitro and in vivo studies by Wang et al. (2004) showed suppression of the pathogen *Helicobacter pylori* by *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* BB12 contained in yoghurt.

Milk fermented by *Lb. acidophilus* and *Lb. casei* was found to possess substances inhibitory to *Staphylococcus aureus*, *Listeria monocytogenes*, *Listeria innocua*, *Escherichia coli* O157:H7, *Enterococcus faecium* and *Enterococcus faecalis* (Millette et al., 2007). Yazid et al. (1999) showed varying inhibitory effects of certain probiotic bacteria against pathogens including *E. coli*, *Salmonella enteritidis*, *Listeria* spp. and *Bacillus cereus*, with *Bifidobacterium breve* C11 being the most antagonistic and *Bifidobacterium infantis* being the least antagonistic. It was also concluded that the presence of a combination of probiotic provided a better inhibitory activity than the presence of a single probiotic.

Probiotic could be used to prevent and treat illnesses caused by these pathogens, as a result of their ability to inhibit growth of pathogens, and thus provide an alternative in the face of increasing antibiotic resistance among pathogens (Toure et al., 2003).
2.1.6 Probiotic as protective cultures

Protective cultures are microorganisms (usually lactic acid bacteria) which play an inhibitory role against pathogens within a food product during storage (Rodgers, 2008). The use of protective cultures helps to provide further safety to food, and reduces the use of chemical/synthetic antimicrobials, and also minimizes processing (Soomro et al., 2002; Kesenkas et al., 2006).

Some probiotic have been found to possess protective qualities. Pidcock et al. (2002) demonstrated that *Lb. acidophilus* and bifidobacteria, coupled with starter culture, proved to be inhibitory against *L. monocytogenes* and *E. coli* in Hungarian salami. Similarly, *Lactobacillus reuteri Protectis*, which is used as a probiotic in dairy products and fermented sausages, is inhibitory against *Helicobacter pylori* (Muthukumarasamy and Holley 2006).

Some strains of *Enterococcus faecium*, which are used in meat products as probiotic, are anti-listerial (Franz et al., 2003). However, a range of probiotic used in dairy were ineffective against certain strains of *Clostridium botulinum*, thus indicating that the use of probiotic exclusively may not guarantee safety of some foods (Rodgers et al., 2003).

2.2 Fermented dairy products in the world:

2.2.1 Dahi is the traditional fermented milk in Bangladesh and more or less similar to yogurt-like products. It is produced from cow and buffalo milk or from a mixture of them by a traditional method using an indigenous non-descriptive starter culture (previously made dahi) containing lactic acid bacteria (LAB) and other fermentative organisms. It is served as a dessert after typical Bangladeshi polao dishes that are highly nutritious and rich. It is believed that dahi assists in digestion and cures intestinal disorders such as
constipation, diarrhea, and dysentery. This suggests that it might have some antipathogenic properties that offer protection from these disorders. LAB have the ability to rapidly produce a number of antimicrobial substances, such as organic acids, free fatty acids, ammonia, diacetyl, \( \text{H}_2\text{O}_2 \) and bacteriocin, which have the capacity to inhibit growth of a variety of foodborne spoilage and pathogenic organisms (Haarun-ur-Rashid et al., 2007).

2.2.2 Suusac: in Kenya is traditionally prepared by spontaneous fermentation of unheated milk in smoke-treated gourds. The fermentation is carried out at ambient temperature (26–29°C) for 1–2 days. The product is a white, low-viscosity product with a distinct smoky flavour and astringent taste. Spontaneous fermentation of unheated milk takes advantage of the action of naturally occurring mixed microflora inherent in the milk (Lore et al., 2005).

2.2.3 Lben: is a refreshing cultured product obtained by spontaneous fermentation of cow’s milk. Occasionally, goat’s milk alone or in combination with cow’s milk is used; however, the same product is made in different arab countries and it is known as iben or leben (in North African countries) and laban (in the Middle East). The traditional stages of manufacture of lben or other related products are as follows: raw milk is left to sour spontaneously at ambient temperature until coagulation occurs which may take up to 24–72 h depending on the temperature during the summer and winter seasons, respectively. On gelation, the product is called rayeb, and may be consumed as such; however, by churning the fermentate, the product is separated into lben and raw butter called zebda beldia literally meaning ‘butter of the country’ (in other Middle Eastern countries the same product is known as zibdeh baladieh, samna and in some instances, the
mutton fat dripping is also samen or samneh). In the rest of the world butter-related product is known as butter oil, ghee or anhydrous milk fat (Benkerroum and Tamime, 2004).

2.2.4 Kajmak: is a dairy product with a soft and creamy texture made by fermentation of milk fat. It is produced on the Balkans Peninsula (Serbia, Montenegro and Bosnia), as well as in Turkey, Afghanistan and Iran. Clotted cream from England and Indian Malay are very similar to kajmak in the first step of the production, but kajmak differs in a longer period of fermentation needed to obtain the final product (Jokovic et al., 2008).

There are other different dairy products well known around the world such as: Kefir, Koumiss, Filmjölk, Viili, Acidophilus milk, Cultured Buttermilk, Sour Cream, Crème fraîche (Pavlaka, 2007).

2.3 Traditional fermented dairy products in Sudan:

2.3.1 Gariss is a fermented camel’s milk in the Sudan, which is not always available for the family as camels are often driven far away in search of pastures (Dirar, 1993; Abdelgadir et al., 1998).

The method of gariss preparation was described by Dirar (1993). Gariss is fermented in large skin bag (locally named “Si’in”) which contains large quantity of previously sour product, while in the absence of starter from previous lot, fermentation is initiated by adding to the container a few seeds of black cumin (Nigella sativa) and one onion bulb. Two large skin bags with fermented milk are hung onto the saddle of special camel called the gariss camel. Moreover, fresh camel milk is added to the Si’in whenever part of the fermented product has been consumed.
Fermentation of gariss takes place while the camels are on the move and due to the inherent jerk in the camel’s walk; the milk in the bags is gently shaken during fermentation (Mirghani, 1994).

2.3.2 *Roub* is the major fermented milk product in the country. It is mainly produced from surplus milk of the rainy season by nomadic tribes. The housewife makes it a point during this season to turn as much milk into *roub* each evening. About 80% of the rainy season milk is turned into *roub* by the household in this season. A report by the Arab Organization for Agricultural Development (AOAD, 1983) estimated the milk turned into dairy products in Sudan to be 65% of the annual total; Dirar (1993) gave an estimate of 50–60%. *Roub* makes about 90% of all fermented milk products, as an offhand estimate. It must be stated that the aim of souring milk into *roub* is not to obtain a fermented milk for consumption. Milk souring here is only an expedient to facilitate the extraction of butter from it, the remaining sour milk is *roub*. It could therefore be said that *roub* is the by-product of butter production not the other way round as it is commonly held in urban thought. Pastoralists commonly waste *roub* away by spillage on the ground or in natural water bodies or give it to the dogs or young animals and wild birds.

Every few days the accumulated butter is boiled to give ghee or butter oil, called *samin*. The unboiled butter or *furssah* finds no other use except that part of it is fed to babies. All other fermented dairy products of the Sudan are fermented to be consumed whole without removing the butter in advance.

2.3.2.1 Preparation method

*Roub* preparation methods differ only slightly from one area to another. Most of *roub* is made from cow’s milk but milk from sheep and goats is likewise processed. All milk is obtained by hand milking and grossly
contaminated (Rufa’I, 1990) as it is true for other Third World countries (FAO, 1990; Abdelgadir et al., 1998).

The containers used for fermentation and churning are the Si’ in and the Bukhssa. The former is a leather bag made from whole goat or sheep skin while Bukhssa is a large gourd with a lidded narrow mouth.

The Si’ in, as a rule, is not washed and the traces of roub from the previous batch serve as a starter when fresh milk is added in the course of making a new batch of roub. The Bukhssa, on the other hand, is often washed with water, dried and smoked using wood of select trees. When roub is to be prepared in such a container, a starter, in the form of a small quantity of roub from a previous batch has to be added with the new milk.

When Si’ in is used for churning, the container is distended by blowing through the operator’s mouth into it and then tightly tying the Si’ in’s mouth with a rope or a thong. Obviously this state of affairs is incongruent with good manufacturing practice. Nothing is known by way of scientific investigation about the health implications of this act. It is certainly safer to use the Bukhssa, the washed and smoked gourd which is not blown into through the mouth. One more point worth mentioning with respect to the process of churning is the fact that, during churning, cold water is added to the milk. This is claimed to facilitate the separation and conglomeration of the butter in one lump. However, the water in a nomad’s tent or a villager’s hut is not expected to be totally uncontaminated and it is logical to assume that pathogens can get access to roub through this route.

It is clear that for a hazard analysis of critical control point (HACCP) approach, roub production provides a number of critical control points which are easily identifiable along its processing course.
2.3.2.2 Uses of roub:

Freshly prepared roub which is available early in the morning is a pleasantly sour product with an unmistakable characteristic buttery flavour, very well liked by those who consume it habitually.

Little specks of tiny butter pieces are always a float its face roub is drunk as is or made into pudding and used to top aceda for breakfast. It is this product that any modernization or preservation attempt should aim at as the day wears on, the product loses its original pleasant flavour and turns more and more sour till at one point the whey (safwa) separates from the curd (kush-kush) which floats on top being fully impregnated with gas. Such roub is put to a number of uses. It is mixed with fresh milk to give ratiya, or milk is milked directly into the container with roub to give a product with a fluffy head of foam called umgufufu, a treat for children. In hot weather, roub is diluted with 2 or 3 volumes of water to give ghubasha, a thirst quencher (Dirar, 1997). Sometimes pastoralists make a salad of a mixture of roub.
CHAPTER THREE
MATERIALS AND METHODS
CHAPTER THREE
MATERIALS AND METHODS

In areas where the survey was carried out, the practice of “roub” making was as follows:-

Raw cow's milk is usually for roub making; however, there are still some people who prefer heating milk before processing. It is clear that it is just a matter of taste and liking; people who prefer boiling of milk say that the resultant "roub" is better in taste and they call it "roub barid" (moderately acidic). They usually (unless the container is new and is not used before) do not add any of the previous roub (as a starter culture) to the raw milk under the processing, while some producers prefer adding part of the previous "roub" as a starter culture. Milk for roub making is generally left for 12 hrs after which the fermentation is complete and some people leave it for 24 hrs, but the fermentation period can go up or down according to the ambient temperature. Coagulation of milk is usually noticed by experience. After being curdled, milk is shaken for 10 minutes after which a separation of butter is obtained and water is added to ease the collection of butter (Abdalla and Hussain, 2009).

The experiment was carried out at the Department of Dairy Production, Faculty of Animal Production - University of Khartoum during the period from July to March, 2013.

3.1 Sample collection:

A total of 30 samples((10 samples from each of Khartoum (soug 6 and soug Elbagari ), Khartoum North (soug Zaraeb hay Elnasr and soug 6 in Elhaj yousif) and Omdurman(Alsoug Alshabei, soug Alrahma, soug Khalifa, soug Alshingiti and soug Sabreen) )) were collected.
Ro*ub in these areas was traditionally manufactured and sold in the local market. Samples were collected in sterile polyethylene bags, preserved in sample containers in ice at ≤10 °C and transported to the laboratory. The analysis was carried out immediately on arrival, or the collected samples stored at refrigerate temperature for 20 days. Physicochemical and microbiological analysis were carried out for 1, 7, 14, 20 days intervals.

3.2 Sample analysis:

The chemical composition of *roub* was determined as follows:

3.2.1 Fat content

The fat content was determined by Gerber method according to Bradley *et al.* (1992) as follows: ten milliliters of sulfuric acid (specific gravity 1.815 gm/ml at 20°C) were poured into a clean dry Gerber tube, followed by the addition of 10.94 ml of well mixed *roub* sample, 1 ml of amyl alcohol (density 0.814-0.816 gm/ml at 20°C) and distilled water (at 20°C) were added. The content was then thoroughly mixed till no white particles could be seen. Gerber tubes were centrifuged at 1100 revolutions per minute (rpm) for 5 min and the tubes were then transferred into a water bath at 65°C for 3 min. The fat percent was then read out directly from the fat column.

3.2.2 Protein content

The protein content was determined by Kjeldahl method (AOAC, 2000). In a clean dry Kjeldahl flask, 9 ml of *roub* sample were placed, and then 25 ml of concentrated H₂SO₄ were added followed by addition of two Kjeldahl tablets (CuSO₄). The mixture was then digested on a heater until a clear solution was obtained after 3 hr. 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, then 5 ml were taken, neutralized using 10 ml of 40% sodium hydroxide (NaOH) and the neutralized solution
was then distilled. The distillate was received in a conical flask containing 25 ml of 2% boric acid plus three drops of indicator (bromocresol green plus methyl red). The distillation was continued until the volume in the flask was 75 ml. The flask was then removed from the distillator, and the distillate was titrated against 0.1N HCl until the end point was obtained (red color).

The protein content was calculated as follows:

\[ \text{Nitrogen (\%)} = \left( \frac{T \times 0.1 \times 20 \times 0.014}{\text{weight of sample}} \right) \times 100 \]

\[ \text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.38 \]

Where: \( T \) = Titration figure
\( 0.1 \) = Normality of HCl
\( 0.014 \) = Atomic weight of nitrogen/1000
\( 20 \) = Dilution factor
\( 6.38 \) = Conversion factor of milk nitrogen into protein

### 3.2.3 Total solids content

Total solids content was determined according to the modified method of AOAC (2000). Five millilitres of milk were placed in a clean dried flat-bottomed aluminum dish and heated on a steam bath for 10 min. The dishes were then dried in an air oven at 100°C for 3 hr, after which they were transferred to a millilitres to cool and then weighed. Heating, cooling and weighing were repeated several times until the difference between two successive weighing was less than 0.5 mg. The total solids content was calculated as follows:

\[ \text{Total solids (\%)} = \left( \frac{W_1}{W_2} \right) \times 100 \]

Where: \( W_1 \) = Weight of sample after drying
\( W_2 \) = Weight of the original sample
3.2.4 Ash content

The ash content was determined according to AOAC (2000). Five millilitres of roub were weighed into suitable clean dry crucibles which were placed in a muffle furnace at 550°C for 2 hr, cooled in a disiccator and weighed. The ash content was calculated as follows:

\[
\text{Ash (\%)} = \left( \frac{W_1}{W_2} \right) \times 100
\]

Where: \( W_1 \) ≡ Weight of ash
\( W_2 \)≡ Weight of the original sample

3.2.5 Titratable acidity

Titratable acidity was determined according to AOAC (2000). Ten millilitres of roub were placed in a white porcelain dish and 5 drops of phenolphthalein indicator were added. The sample was titrated against 0.1N NaOH till a faint pink color was obtained. The acidity was calculated as follows:

\[
\text{Titratable acidity (% lactic acid)} = \frac{T}{W}
\]

Where: \( T \) = Titration figure
\( W \) = Weight of the sample

3.3 Microbiological examination

3.3.1 Sterilization of equipment

Glassware such as flasks, test tubes, Petri dishes, pipettes and bottles were sterilized in a hot oven at 170°C for two hr, whereas distilled water was sterilized by autoclaving at 121°C for 15 min (Marshall, 1992).
3.3.2 Preparation of sample dilutions

Eleven millilitres from a homogeneous *roub* sample were added to 99 ml of sterile distilled water in a clean sterile flask, and then shaken to make $10^{-1}$ dilution. One ml from the previous dilution ($10^{-1}$ dilution) was aseptically transferred to 9 ml sterile distilled water. This procedure was repeated to make serial dilutions of $10^{-1}$ to $10^{-8}$ (Houghtby *et al*., 1992).

3.3.3 Preparation of media:

All media were obtained in a dehydrated form and stored in a hygroscopic environment in a cool dry place away from light and prepared according to the manufacturer’s instructions.

3.3.3.1 Plate count agar medium (PCA):

The medium consisted of 5 gm casein enzymic hydrolysate, 2.5 gm yeast extract, 1.0 gm dextrose and 15 gm agar. The medium was prepared by suspending 23.5 gm of the powder in one litre of distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121°C for 15 min.

3.3.3.2 MRS agar medium:

This medium consisted of protease peptone 10.00 gm, beef extract 10.00 gm, yeast extract 5.00 gm, dextrose 20.00 gm, polysorbate 801.00 gm, ammonium citrate 2.00 gm, sodium acetate 5.00 gm, magnesium sulphate 0.10 gm, manganese sulphate dipotassium 0.05 gm and phosphate 2.00 gm and the agar 15 gm and the final pH 6.5±0.2 at 25°C. The manufacturer’s instructions were followed by dissolving 55.15 gm from MRS medium in a litre of distilled water; the mixture was heated to boiling and sterilized in an autoclave at 121°C for 15 min. The medium was left to cool (45-46°C), poured into sterile Petri dishes (15-18 ml) and left to solidify (Harrigan and McCance, 1976).
3.3.3.3 MacConky agar medium:

The medium consisted of 20 gm peptic digest of animal tissue, 10 gm lactose, 5 gm bile salts, 0.075 gm neutral red and 12 gm agar. The medium was prepared by suspending 47 gm of the powder in one litre distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121°C for 15 min.

3.3.3.4 Yeast extract agar medium

The medium consisted of 5 gm tryptone, 3 gm yeast extract and 15 gm agar. The medium was prepared by suspending 23 gm of the powder in one liter distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121°C for 15 min.

3.4 Counting

Plates were counted using colony counter. The number of colony-forming units (cfu) in each dilution was obtained by multiplying the number of colonies in the petri dish by the reciprocal of each dilution (Harrigan and McCance, 1976).

3.4.1 Total viable bacterial count

Plate count agar was used for the enumeration of total bacteria count, 1ml quantities of each sample decimal dilutions was streaked in dried plate with plate count agar and incubated at 31 ±1°C for 48 ± 3hrs (Hougby et al., 1992).

3.4.2 Lactic acid bacteria count

MRS agar was used for the enumeration of Lactobacilli, 1ml quantities of each sample decimal dilutions $10^5,10^6,10^7$ was streaked in dried plate with MRS agar and incubated at 35°C for 48 ± 3hrs (DeMan et al.,1960).
3.4.3 Coliform bacteria count

MacConky agar was used for the enumeration of coliforms, 1ml quantities of each sample decimal dilutions $10^5, 10^6, 10^7$ was streaked in dried plate with MacConky agar and incubated at 35°C for 48 hr (Harrigan and McCance, 1976).

3.4.4 Yeasts and moulds count

This was determined by plating suitable dilution of sample in yeast extract agar plate which was acidified to pH 3.5 using 10% as indicated in Harrigan and McCance (1976). Plates were incubated at 35°C for 3-5 days (Harrigan and McCance, 1976).

3.5 Statistical analysis

The samples were analyzed for total viable bacterial count, lactic acid bacteria count, coliform bacteria count and yeasts and moulds count. Data were analyzed by Statistical Analysis System (SAS, ver. 9) using 3×4 factorial design. GLM procedures were used to determine the effect of area and storage period and their interaction on the physic-chemical and microbiological characteristics of roub. Mean separated was carried out by Duncan multiple range test at $p \leq 0.05$. 
CHAPTER FOUR

RESULTS AND DISCUSSION
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Chemical composition of roub collected from areas under study:

The present study showed significant variations between the collected samples in all chemical components (Table 1), and also showed that the fat, TS and ash contents were significantly (P< 0.001) higher in samples from Khartoum, while protein and acidity were higher (P< 0.001) in samples from Omdurman. The fat content was higher in Khartoum, and this might be due to difference in agitation conditions during roub production. These findings are in disagreement with Hassan et al. (2008) who reported that the fat content in collected gariss samples was 2.8%-3.6%.

The highest protein content was in Omdurman, and this might be due to absence of coliform bacteria which left more nutrient substance for the other population specially the lactic acid bacteria this might be express the highest acidity also in Omdurman. These results are in agreement with Haj et al. (2007) who reported that the protein content in stirred yoghurt in Khartoum state was 2.66%-3.97%.
Table (1). Chemical composition of *roub* samples collected from Khartoum, Khartoum north and Omdurman (mean ±SD)

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Area from which samples were collected</th>
<th>Khartoum</th>
<th>Khartoum North</th>
<th>Omdurman</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td></td>
<td>1.98±0.929&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.91±0.654&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.46±0.804&lt;sup&gt;a&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td>2.59±0.565&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.06±0.584&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.97±0.606&lt;sup&gt;a&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>Total solids</td>
<td></td>
<td>7.19±1.783&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.73±1.333&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.47±0.937&lt;sup&gt;c&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>Ash</td>
<td></td>
<td>0.579±0.194&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.567±0.162&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.529±0.131&lt;sup&gt;b&lt;/sup&gt;</td>
<td>***</td>
</tr>
<tr>
<td>Acidity</td>
<td></td>
<td>2.63±0.680&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.48±1.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.96±0.557&lt;sup&gt;a&lt;/sup&gt;</td>
<td>***</td>
</tr>
</tbody>
</table>

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

***≡ P< 0.001

SL ≡ significance level
SD ≡ standard deviation
4.2 Effect of storage period on the chemical composition of roub:

From results in Table (2) the fat, TS contents of samples collected from the three cities did not significantly (P>0.05) change during the storage period, while protein, ash and acidity were significantly (P<0.001) increased as the storage period progressed towards the end. Fat content showed two peaks at day 7 (2.05%±1.031%) and day 20 (1.98%±0.945%) in Khartoum, while in Khartoum North increased at day 14 (2.05%±0.689%) before decreasing at the end, and Omdurman the results showed a slight decrease with the progress of storage period (Tables 3, 4 and 5). Similar results reported by Ahmed and El Abid (2002) and Haj et al. (2007) who reported that the mean of fat was 2.17%-4.51% in stirred yoghurt. However, these findings are in disagreement with Hassan et al. (2008) who stated that the average fat levels of gariss was 4.85%±0.66% and 3.46%±1.18% in transhumance and nomadic herders respectively.

The protein content progressively increased towards the end of storage period in Khartoum North and Omdurman, while in Khartoum the content increased at day 7 (2.83%±0.447%) before decreasing to 2.44%±0.585% at the end. This might be due to addition of skimmilk during processing, However, in Khartoum North and Omdurman yeasts and moulds decreased (Tables 3, 4 and 5) this might be due to hygienic procedures.
Table (2). Effect of storage period on chemical composition of *roub* samples collected from areas under study (mean± SD)

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Storage period (days)</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Fat</td>
<td>1.77±0.836</td>
<td>1.79±0.904</td>
</tr>
<tr>
<td>Protein</td>
<td>2.36±0.682</td>
<td>2.57±0.699</td>
</tr>
<tr>
<td>Total solids</td>
<td>6.82±1.347</td>
<td>6.86±1.652</td>
</tr>
<tr>
<td>Ash</td>
<td>0.517±0.162</td>
<td>0.557±0.159</td>
</tr>
<tr>
<td>Acidity</td>
<td>2.75±0.822</td>
<td>2.69±0.719</td>
</tr>
</tbody>
</table>

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

***≡ P< 0.001

NS≡ Not significant

SL ≡ Significance level

SD ≡ Standard deviation
Table (3). Effect of storage period on chemical composition of *roub* samples collected from Khartoum (mean± SD)

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Storage period (days)</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Fat</td>
<td>1.95±0.941&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05±1.031&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>2.52±0.718&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.83±0.447&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total solids</td>
<td>7.29±2.156&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.40±2.197&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>0.592±0.195&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.599±0.186&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acidity</td>
<td>2.56±0.577&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.68±0.606&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

***≡ P< 0.001
NS≡ Not significant
SL ≡ Significance level
SD ≡ Standard deviation
Table (4). Effect of storage period on chemical composition of *roub* samples collected from Khartoum north (mean± SD)

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Storage period (days)</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Fat</td>
<td>1.76±0.664&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.84±0.756&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>1.79±0.439&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.92±0.536&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total solids</td>
<td>6.36±1.282&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.64±1.431&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>0.569±0.168&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.587±0.165&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acidity</td>
<td>2.16±1.162&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.59±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in the same row bearing similar superscripts are not significantly different (P>0.05)
***≡ P< 0.001
NS≡ Not significant
SL ≡ Significance level
SD ≡ Standard deviation
### Table (5). Effect of storage period chemical composition of *roub* samples collected from Omdurman (mean± SD)

<table>
<thead>
<tr>
<th>Chemical Composition</th>
<th>Storage period (days)</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Fat</td>
<td>1.62±0.804&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50±0.862&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>2.78±0.559&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.96±0.598&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total solids</td>
<td>6.53±1.019&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.55±1.063&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>0.392±0.104&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.485±0.095&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acidity</td>
<td>2.56±0.491&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.82±0.459&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

***≡ P< 0.001

NS≡ Not significant

SL ≡ Significance level

SD ≡ Standard deviation
These results are in accordance with Haj et al. (2007) who reported that the protein content of stirred yoghurt was 2.17%-4.51% and in agreement with Ahmed and El-Abid (2002), However these results are in disagreement with Uzeh et al. (2006) who reported that the protein content in Nono was 6.40%.

The maximum TS content was in day 7 (7.40%±2.197%) in Khartoum, in day 14 (7.00%±1.506%) in Khartoum North and in day 7 (6.55%±1.063%) in Omdurman (Tables 3, 4 and 5), These results are in agreement with Ahmed and El-Abid (2002) and Haj et al. (2007) who reported that the storage period had a significant (P<0.001) effect on the chemical composition except total solids.

Ash content increased to a maximum at day 7 (0.599%±0.186% and 0.587%±0.165% in Khartoum North and Omdurman respectively), and day 20 (0.580%±0.178% and 0.583%±0.172% in Khartoum and Khartoum North respectively), while in Omdurman the content increased progressively towards the end (Tables 3, 4 and 5). These findings are in line with Haj et al.( 2007) and Ahmed and El Abid (2002), however these results are not in line with Hassan et al. (2008) who reported that the ash contents in gariss were 1.30%±0.17% and 0.87%±0.13% in transhumance and nomadic herders respectively. The highest acidity was found in samples from Omdurman and this might be due to effect of agitation which played a major role in the fermentation process of the product by increasing the fermentability (Hassan et al., 2008), and also might be due to absence of coliform bacteria left more nutrient substance.

Acidity in Khartoum increased to at day 7 (2.68%±0.606%), before decreasing at day 14 (2.59%±0.803%) followed by an increase towards the end, while in Khartoum North the acidity increased to a maximum at day 14 (2.63%±1.030%) before slightly decreasing towards the end, and in Omdurman the acidity progressively increased towards the end (Tables 3, 4 and 5), These findings are in accordance with Ahmed and El Abid (2002) and Hassan et al.
(2008) who stated that the mean acidity expressed as lactic acid percent of gariss was 2.29%±1.25% and 2.24%±0.68% in samples collected from transhumance and nomadic herders respectively. These results are not in line with Haj et al. (2007), Uzeh et al. (2006) who stated that total titratable acidity was in Nono 1.37% and in Wara was 0.48%.

4.3 Microbiological characteristics of roub collected from areas under study:

The microbiological examination of roub samples from three cities under study revealed that there was a significant variation (P<0.001) in the microbiological population, with the highest TVBC, LAB and yeasts and moulds being in Khartoum North, while coliform bacteria was high in Khartoum and not detected in Omdurman (Table 6).

The findings of Khartoum and Khartoum North were in disagreement with Lore et al. (2005) who reported that high total viable bacterial counts were observed conversely, relatively lower numbers of fungal flora and coliform bacteria were encountered. This might be due to contamination during processing and handling.
Table (6). Microbiological characteristic of *roub* samples collected from Khartoum, Khartoum north and Omdurman (mean ±SD)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Area from which samples were collected</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Khartoum</td>
<td>Khartoum North</td>
</tr>
<tr>
<td>Total viable bacterial count</td>
<td>7.38±1.357&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.51±0.833&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coliforms</td>
<td>6.29±2.497&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.39±3.026&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>7.41±1.276&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.75±0.777&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yeasts and Moulds</td>
<td>7.17±1.316&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.490.685&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

***≡ P< 0.001

NS≡ Not significant

SL ≡ Significance level

SD ≡ Standard deviation

ND≡ not detected
Absence of coliform bacteria in Omdurman might be due to high acidity of *roub* samples in Omdurman. The production conditions of *roub* in Omdurman might have contributed to the decrease or absence of coliform bacteria in these samples. These findings are in line with Lore *et al.* (2005) who reported that the inhibition of *E. coli* and other coliform bacteria by high acidity caused by the production of organic acids (e.g. lactic acid) in fermented products.

### 4.4 Effect of storage period on microbiological population:

TVBC, coliform bacteria count and yeasts and moulds count decreased towards the end of storage period, while results of LAB revealed that fermentation is mainly done by LAB which increased to a maximum at day 14 (7.77%±1.148%) before decreasing towards the end.

The highest coliform bacteria count found in Khartoum (6.29%±2.497%) which indicate unhygienic practices during *roub* processing, while the highest yeasts and moulds count was found in Khartoum North which indicates unhygienic practices during processing and handling.

All microbial populations tested except TVBC decreased with the advancement of storage period in samples collected from all cities, while TVBC increased with storage period in Khartoum and decreased in Khartoum North and Omdurman (Tables 7, 8, 9 and 10).

In Omdurman TVBC was decreasing towards the end of storage period; however, coliform bacteria were not detected. This might be refer to hygienic practices during production of *roub* leading to low initial load of bacteria at the beginning of production.
Table (7). Effect of storage period on microbiological characteristic of *roub* samples collected from areas under study (mean± SD)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Storage period (days)</th>
<th></th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Total viable bacterial counts</td>
<td>7.22±0.995&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.33±0.898&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.05±1.281&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coliform bacteria count</td>
<td>4.18±3.559&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.13±3.452&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.87±3.735&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>7.59±0.904&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.42±0.880&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.77±1.148&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yeasts and Moulds</td>
<td>7.10±1.050&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.93±1.059&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.92±1.145&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in the same row bearing similar superscripts are not significantly different (P>0.05)
***≡ P< 0.001
NS≡ Not significant
SL ≡ Significance level
SD ≡ Standard deviation
Table (8). Effect of storage period on microbiological assessment of *roub* samples collected from Khartoum (Mean ±SD)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Storage period (days)</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Total viable bacterial count</td>
<td>7.19±1.258 a</td>
<td>7.34±1.195 a</td>
</tr>
<tr>
<td>Coliform bacteria count</td>
<td>6.11±2.442 b</td>
<td>6.12±2.304 b</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>7.55±1.027 a</td>
<td>7.03±0.849 b</td>
</tr>
<tr>
<td>Yeasts and moulds</td>
<td>7.20±1.203 a</td>
<td>7.22±1.179 a</td>
</tr>
</tbody>
</table>

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

***≡ P< 0.001

NS≡ Not significant

SL ≡ Significance level
SD ≡ Standard deviation
Table (9). Effect of storage period on microbiological assessment of *roub* samples collected from Khartoum north (Mean ±SD)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Storage period (days)</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Total viable bacterial count</td>
<td>7.48±0.957</td>
<td>7.67±0.656</td>
</tr>
<tr>
<td>Coliform bacteria count</td>
<td>6.44±2.389</td>
<td>6.26±2.201</td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>7.96±0.717</td>
<td>7.82±0.825</td>
</tr>
<tr>
<td>Yeasts and moulds</td>
<td>7.55±0.601</td>
<td>7.51±0.755</td>
</tr>
</tbody>
</table>

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

***≡ P< 0.001

NS≡ Not significant

SL ≡ Significance level

SD ≡ Standard deviation
Table (10). Effect of storage period on microbiological assessment of *roub* samples collected from Omdurman (Mean ±SD)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Storage period (days)</th>
<th>SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Total viable bacterial count</td>
<td>6.98±0.624&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.98±0.598&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coliform bacteria count</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lactic acid bacteria count</td>
<td>7.28±0.827&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.14±0.798&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yeasts and moulds</td>
<td>6.55±1.017&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.07±0.518&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

***≡ P< 0.001

NS≡ Not significant

SL ≡ Significance level

SD ≡ Standard deviation

ND≡ not detected
The findings of TVBC are in agreement with the findings of Mathara et al. (2004), Savadago et al. (2004), Al-Tahiri (2005), Hassan et al. (2008) and Abdalla and Hussain (2009) who reported that the mean total viable bacterial count was 8.14, 7.56 in El-Obied, Nyala respectively. However, these findings are in disagreement with Lore et al. (2005) and Awad et al. (2006) who reported a reduction of means log total bacterial counts of the samples. However, at the end of storage period the mean log increased.

Moreover Coliform bacteria findings are in agreement with Savadago et al. (2004), Al-Tahiri (2005) and Lore et al. (2005) who reported that the low coliforms numbers were encountered (<1log_{10} cfu/ml), Cetinkaya and Soyutemiz (2006) and Abdalla and Hussain (2009). However these results are not in line with Uzeh et al. (2006) who stated that the mean coliform count was 4.25×10^7 cfu/ml for Wara.

Results of LAB are in accord with Mathara et al. (2004), Savadago et al. (2004), El-Baradei et al. (2008), Jokovic et al. (2008) and Abdalla and Hussain (2009) who reported that the mean lactobacillus count was 7.80%, 7.90% and 7.50% in El-Obeid, Nyala and Abu Naama areas respectively. However these results are not in line with Abdalgadir et al. (2008) and Hassan et al. (2008) who reported that the mean log lactobacilli spp. counts were 6.83%± 0.33% and 6.55%± 0.32% in gariss from transhumance and nomadic herders respectively.

Results of yeasts and moulds are in line with Hassan et al. (2008) who reported that the log yeasts counts comprised means of 6.99% ±0.13% and 7.02% ±0.3% in samples collected from transhumance and nomadic herders respectively. However, these findings are in disagreement with Uzeh et al. (2006) who reported that the mean fungal count of Wara was 1.31× 10^7 cfu/g and Awad et al. (2006).
CHAPTER FIVE
CONCUSSION AND RECOMMENDATIONS
CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:

Physic-Chemical composition of roub had a significant difference (P<0.001) between all contents, and storage period affect significantly (P<0.001) in all physic-chemical composition except fat, TS also storage period did not affect significantly (P>0.05) in all contents of samples collected from Khartoum except protein and ash, samples from Khartoum North affected significantly (P<0.001) in all physic-chemical composition, while samples from Omdurman affected significantly (P<0.001) by storage period in all contents except TS.

Microbiological characteristics affected significantly by difference of areas from which samples were collected, and coliform bacteria was not detected in Omdurman.

Storage period affect significantly (P<0.001) in all microorganisms except TVBC and yeasts and moulds in samples collected from Khartoum, and samples from Khartoum North did not affect significantly (P>0.05) by storage period except coliform bacteria, while all microorganisms in Omdurman affect significantly (P<0.001) by storage period and coliform bacteria was not detected in Omdurman.

6.2 Recommendations

1. More researches should be conducted in processing methods of roub.
2. More research is needed to improve the quality of the product.
3. Isolation and identification of pathogenic bacteria in roub.
4. Learning and training the producers hygienic practices should conducted during roub processing.
5- Encourage the producers to improve roub quality by pay good price to hygienic roub.
REFERENCES


Canadian dairy commission 2011-10-17.


