بسم الله الرحمن الرحيم

EVALUATION OF RE-PACKED WHOLE MILK POWDER SOLD IN KHARTOUM STATE

By

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DEDICATION

To my dear family
Father, mother and sisters
To my husband
To my dear friends and colleagues

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My thanks and praise to Allah who gave patience and will to accomplish this work.

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ABSTRACT

This study was carried on 27 samples of re-packed whole milk powder. The samples were collected from Khartoum State (Khartoum, Khartoum North and Omdurman). From each town the samples were collected from groceries of three different locations according to income level (high, middle and low income) and hygiene measures.

The analysis of the samples was carried out to determine the chemical composition and bacteriological content of repacked whole milk powder sold in Khartoum State.

According to the effect of city on the chemical composition of whole milk powder, the results showed that fat, protein and total solids content were not significantly affected by city ($P > 0.05$). Although the highest fat content was found in Khartoum (26.62%), while the highest protein content was found in Omdurman (26.40%) and highest total solids content was found in Khartoum (95.04%).

Titratable acidity and ash content were significantly affected by city ($P < 0.05$). The highest acidity content was found in Omdurman (0.15%) and the highest ash content was found in Khartoum (6.18%).

The effect of area did not show any significant variation ($P > 0.05$) in fat, protein, acidity, total solids and ash content of whole milk powder.

The microbiological results showed that the total bacterial count, coliform count and proteolytic bacterial count were significantly differ according to city ($P < 0.01$), the highest count were recorded in Khartoum North, with values of Log_{10} 4.12, 2.35 and 2.34 cfu_{10}/gm, respectively.

The lypolytic bacterial count and yeasts and moulds count were not significantly affected by city ($P > 0.05$), the high lipolytic count was found in Omdurman ($Log_{10}2.43$ cfu_{10}/g) and high yeasts and moulds count was found in Khartoum North ($Log_{10}2.38$ cfu_{10}/g).

The effect of area showed significant variation ($P < 0.01$) in total bacterial count, coliform count and yeasts and moulds count, but did not show any significant variation ($P > 0.05$) in proteolytic and lypoytic bacterial count of whole milk powder.

خلاصة الأطروحة

أجريت هذه الدراسة على 27 عينة من اللبن المجفف الكامل الدسم المعاد تعبئته، جمعت العينات من مدينة الخرطوم (الخرطوم، الخرطوم بحري وامدرمان). ومن كلية مدينة من ثلاثة مواقع مختلفة على حسب مستوي دخل الفرد (مستوي عالي، متوسط بسيط) ومستوى الصحة. تم تحليل هذه العينات لمعرفة مدى مطابقة الشروط الكيميائية والميكروبيولوجية التي يجب أن تتوفر في اللبن المجفف كامل الدسم المعاد تعبئته. على حسب تأثير المدينة على التكوين الكيميائي للبن المجفف فقد أوضحت النتائج أن الدهن والبروتين والمواد الصلبة الكلية لم تتأثر معنوياً باختلاف المدينة، وقد كانت أعلي نسبة للدهن في الخرطوم (26.62%) وللبروتين في امدرمان (26.40%) وللمواد الصلبة الكلية في الخرطوم (95.04%). كما اثبتت النتائج أن محتوي الحموضة والرماد في اللبن المجفف قد تأثرت معنوياً بالمدينة وسجلت أعلي نسبة للحموضة في امدرمان (0.15%) كما سجلت أعلي نسبة للرماد في الخرطوم (6.18%).

قد أوضحت النتائج أنه لم يكن للموقع أي تأثير معنوي واضح في محتوى (الدهن، البروتين الحموضة، محتوي المواد الصلبة الكلية والرماد) للبن المجفف.

أوضحت النتائج أن العدد الكلي للبكتريا وبكتريا القولون والبكتريا المحللة للبروتين قد تأثر معنوياً بالمدينة، أعلى عدد منها وجد في الخرطوم بحري بقيم (Log_{10} 4.12, 2.35, 2.34 cfu/gm) على التوالي. البكتريا المحللة للدهون والخمائر والفطريات لم تتأثر معنوياً بالمدينة ورصدت أعلي نسبة للبكتريا المحللة للدهون في امدرمان (Log_{10} 2.43 cfu/gm) وأعلي نسبة للخمائر والفطريات سجلت بالخرطوم بحري (Log_{10} 2.38 cfu/gm).

وقد كان لتأثير المنطقة اختلافات معنوية في العدد الكلي للبكتريا وبكتريا القولون والخمائر والفطريات ولم يكن لها أي تأثير معنوي للبكتريا المحللة للبروتين والبكتريا المحللة للدهون للبن المجفف الكامل الدسم.

CHAPTER ONE
INTRODUCTION

The seasonal fluctuation in milk production or in market demand as well as the need to supply milk to locations where there is shortage of fresh milk, have created a demand for milk which can be kept for extended periods of time at low storage costs.

Drying the milk is an efficient method of preservation, drying also greatly reduces the volume of the milk, which is an advantage for long distance shipping and extended storage (Rosenthal, 1991).

Moisture content and water activity of foods are critical parameters that influence shelf life. In milk powder, excessive moisture causes caking and promotes spoilage and loss of quality. Water activity (A_W) of milk powder influences enzymatic and oxidative changes that occur during storage and growth of microorganisms. Thus, a manufacturer must not only determine the maximum moisture content required to maximize yields and profitability but, also the maximum or minimum water activity to give maximum stability during storage (Farkye *et al*., 1998)

Milk powder is produced today in large scale in modern plants. The powder produced can be stored for long periods of time without significant deterioration of the taste or nutritive value (Alfa Laval, Dairy Hand book).

Nijdam and Longrish (2005) stated that milk is spray dried for easier storage, handling, and transport. The functional properties of the resultant milk powder, such as particle size distribution, bulk density, flowability and solubility, determine its storage, handling and transport capabilities. And he noted that milk powder is generally considered a

product of good microbiological quality; however, several factors may contribute to change in its physical and chemical properties which reduce shelf –life and thus its commercial value.

- Whole milk powder is usually obtained by removing water from pasteurized, homogenized whole milk. It may also be obtained by blending fluid, condensed or skim milk powder with liquid or dry cream or with fluid, condensed or dry milk, provided the composition of the whole milk powders of good standards (USDEC, 2006).
- Whole milk powder must contain between 26 and 40% (by weight) on an ``as is'' basis and not more than 5.0% moisture (by weight) on a milk-solids-not-fat (MSNF) basis. By removing moisture to the greatest extent possible, microbial growth is prevented (Alfa Laval, Dairy Handbook).

Different researchers agree that the hygienic conditions under which raw milk is produced are the main factorsaffecting powder quality. Raw milk used by powder manufacture is required to treatment, for which no have been established on microbiological parameters such as total bacterial count and pathogenic bacteria. Therefore, some dairy industries have set their own standards on the quality of raw milk purchased for processing (Mastieri *et al*., 2000).

Whole milk powder will have approximately half the useful life of non fat milk powder under the same condition of packaging and storage.

The properties required for milk powder packaging include, protection against contamination from dirt and microorganisms, protection from light, insects and odour (Tokley, 1982)

The type of packaging used for milk powder influences chemical and physical changes that occur during storage. Also, product loss during

shipping, handling and storage is influenced by package construction and design (Farkye *et al*., 1997).

Due to shortage of raw milk production to meet the market demand of fresh milk, necessitated the importation of whole milk powder to use as recombined or reconstituted milk to supply the market. In addition, milk powders packaged in small containers are imported for consumption, therefore, the importation of milk powders is increased where it is imported in large containers and re-packed in small retail containers for sale in the local market. The process of repacking and the conditions of storage and distribution might affect the quality of milk powder.

The objectives of this study are:

1) To study the chemical characteristics of whole milk powder.
2) To study the possibility of re-contamination by bacteria that influences the shelf life of repacked whole milk powder.
3) To determine whether re-packaged whole milk powder in the market comply with Sudanese standards.

CHAPTER TWO
LITERATURE REVIEW

2.1 Definition of milk powder

Milk powder is a product of lower water activity and better keeping qualities and it is produced in large scale in modern plants. The powder produced can be stored for long periods of time without significant deterioration of taste or nutritive value (Rosenthal. 1991). And he stated that skim milk can be dried into skim milk powder to obtain a shelf life of about two years, while whole milk powder can be stored for only six months because of fat oxidation.

2.1.1 Whole milk powder

Whole milk powder is a soluble powder made by spray drying fresh whole milk. And no other dairy ingredient comes as close to the composition of fresh milk as whole milk powder (Thompson, 1996).

Whole milk powder is usually obtained by removing water from pasteurized, homogenized whole milk (USDEC, 2006)

Quality standards for whole milk powder are as follows: fat content 24.0%, moisture content 4.0%, solubility index 1.0, and total bacterial count 30000 - 50000 cfu/gm (Alfa-Laval Dairy Handbook).

2.1.2 Skim milk powder

This is a skim milk reduced to a powdered concentrate. It can be found in two forms, regular and instant, but both are made from milk in a spray drying process, both types have the same nutrient composition. The regular variety is more compact and requires less storage space than the instantized variety, but it is more difficult to reconstitute (Thompson, 1996).

Quality standards for skim milk powder are as follows: fat content 1.5% moisture content 5.0%, solubility index 2.0 and total bacterial count not more the 50.000 cfu/gm. In the production of skim milk powder, the milk is clarified in conjunction with fat separation, and this is also the

case if the fat content is standardized in a direct standardization system (Alfa-Laval Dairy Hand book).

2.1.3 Flavored skim milk powder

This may be packaged in a variety of forms from a low caloric diet drink (artificially sweetened) to the other end of the scale, as cocoa mix or malted milk (Thompson, 1996).

2.1.4 Partially skimmed milk powder

Is a powder product of milk originally obtained by means of skimming, concentration and drying of milk (Thompson, 1996).

2.1.5 Whole milk powder with condiment

Is a powder product of milk or milk powder origin with condiments, less than 70% solids content (Thompson, 1996).

Branson *et al*. (2003) noted that the raw materials should comply with the corresponding hygienic standards. They also showed that organoleptic requirements of milk powder should be as follows: homogenous cream, white or light yellow, with pure milk odor, and dry and homogenous powder without foreign substances. They recommended that the product should be stored in a dry and ventilated area, and the product should not be stored with any poisonous or harmful materials that could influence the product quality, and the product should be protected from exposure to the sun and rain during transportation. And they stated that Milk powder is used for a wide variety of applications such as bakery industry, chocolate industry and various types of ready cooked meals in the foodstuff industry.

If the powder is to be mixed with water into recombined milk for direct consumption, it must have the correct fat content, good solubility, correct taste and the correct nutritive value (Alfa-Laval Dairy Hand book).

2.2 Manufacture of milk powder

Milk used for making milk powder (whether whole or skim milk), is not pasteurized before use. The milk is pre- heated in tubular heat exchangers before being dried. The preheating temperature depends on the season (which affects the stability of the protein in milk) and the characteristics desired for the final powder product. The pre-heated milk is fed to an evaporator to increase the concentration of total solids. The solids concentration that can be reached depends on the efficiency of the equipment and the amount of heat that can be applied without degrading the milk protein (Rosenthal, 1991).

The milk concentrate is then pumped to the atomizer of drying chamber; the milk is dispersed as a fine fog-like mist into rapidly moving hot air steam, which causes the individual mist droplets to instantly evaporate. Milk powder falls to the bottom of the chamber, from where it is removed (Rosenthal, 1991). Finer milk powder particles are carried out of the chamber a long with the hot air stream and collected in cyclone separators (Rosenthal, 1991).

Roller- drying and spray drying are the principal processes used to produce milk powder because of their low processing costs (Alfa- Laval Dairy Hand Book).

2.2.1 Roll-drying powder

In the production of roll-dried powder, the pre-treated milk is distributed onto rotating steam. Heated rolls coming into contact with the hot roll surface (Alfa-Laval Dairy Hand book).

The water in the milk will be evaporated and removed by a flow of air. Since the high temperature of the heating surface causes the protein

to be converted to a form in which it is not easily soluble and causes brown discolorations of the product (Alfa-Laval Dairy Hand book).

Roll drying is not used for producing powder satisfying strict demands on solubility, appearance, taste and aroma.

Rosenthal (1991) stated that because of the uneven and more intense heat treatment, roller dried powder is less soluble and slightly discolored and this powder is preferred in the confection industry.

2.2.2 Spray-drying powder

The spray- drying is carried out in two stages. In the first stage the pre treated milk is concentrated by evaporation to dry solids content 45-55%. In the second stage the concentrate is fed into a drying tower for final drying and this process takes place in three stages, firstly dispersion of the concentrate into very fine droplets, secondly mixing of the finely dispersed concentrate into a stream of hot air which quickly evaporates the water and thirdly separation of the dry milk particles from the drying air (Rosenthal, 1991).

He noted that in pray drying the temperature of air entering the drying chamber is approximately 180-200^{o}C and its outlet temperature is 80 - 90^{o}C. As milk douplets lose water, the latent heat of evaporation continuously loose their surface, so the temperature of the milk never exceeds 75^{o}C.

Evaporation is a necessary production stage for high quality powder. If the milk is spray dried without prior concentration the powder particles will be very small and will have a high air content, poor wettability and short shelf life (Alf-Laval Dairy Hand Book). In addition, the process will then be uneconomical.

Ozmen and Languish (2003) stated that spray dryers have to be cleaned frequently due to the deposition of the powders on the walls. They also added that the build up of the powder deposits in a spray dryer

is undesirable because they undergo oxidation and browning or scorching and will degrade the quality of the final product if they fall off and mix with it.

2.3 Packaging of milk powder

Rosenthal (1991) reported that there are many types of packaging materials for milk products, the container must not only protect the food from contamination and spoilage, but it must also be convenient attractive and informative as well. The packaging is the last link in the processing chain, and poor packaging undoes all the previous, careful steps.

Thomas *et al.* (2004) stated that packaging plays an important role in protecting and preserving the quality of food manufacturing and distribution process. He also added that the shelf-life of any food is limited due to the occurrence of many deteriorative processed and reactions within the food materials. These include physical, chemical and interactions between food and the ambient environment across the packaging material.

Rosenthal (1991) reported that the general packaging factors to be considered for dairy products are: non toxicity and compatibility with the product, resistant to impact, ability to provide sanitary odor- and light protection, to be temperature resistant or evident, to be easy to use and dispose of, to comply with size, shape and weight limitation and to provide the defined appearance and cost.

Tokley (1982) stated that initially, all milk powder wills is packed into some form of bulk container of which there are two distinct types: 25 kg multi- wall paper bag with polythene liner and 500-1000kg bulk bin.

He also stated that the multi- wall bag is the most versatile of all the packs of milk powder and it is due to this versatility that is likely to

remain the major bulk container for milk powder. Its key features are that it is robust, relatively low in cost and is capable of being packed at high protection rates 9 - 10 ton/hr being achievable with modern filling/ sealing equipment.

It is a convenient pack for major end users such as recombining plants and repackaging station, where adequate protection must also be coupled with ease of access to clean uncontaminated powder.

Rehman *et al*. (2001) noted that plastic bags have been introduced recently as an alternative to paper bags in milk powder packaging as it is believed that plastic bags are more durable and would preserve the quality of milk powder during storage better than paper bags.

Farkye and Obispa (1997) showed that the type of packaging used for milk powder influences chemical and physical changes that occur during storage. The chemical analysis during storage showed an increase in moisture content and water activity of milk powder packaged in paper bags, polyethylene liners and stored at 38^{o}C and 98% relative humidity. Also they showed that caking and increased browning occurred in some samples packaged in polyethylene lined paper bags.

Thomas *et al*. (2004) showed that milk powder is prone to such reactions and interactions with package resulting in degradation of its edible quality due to change in smell, colour and texture, which ultimately renders it nutritionally and economically unacceptable.

They also stated that the filled milk powder is manufactured and packed in bulk in Europe, imported to Africa via sea freight containers to be re-packed into small packages and distributed in many African countries. Because of the tropical conditions the packed product is exposed to rather high levels of relative humidity and temperature during transportation and storage which influences the stability of the powder. The most common sizes of milk powder bags are 25 kg and 15 kg,

although other sizes are also employed, since it is very easy to vary the weight of powder in the bags to meet specific requirements of customers (Alfa-Laval Dairy Hand book).

Tokley (1982) stated that considerable interest in the bulk handling of milk powder has been generated by the upsurge in increased costs of handling and transportation. For major users of milk powder such as the large recombining factories which have developed systems around the 25 kg bag being a recipe unit as well as a powder container, conversion to the bulk pack concept could be delayed by the need for plant re-design and re-equipment.

2.4 Defects of Milk powder

Milk powder is now considered as a food ingredient mainly because of the functional properties of milk protein. During storage of milk powder, mainly physicochemical damages, mainly dependent on lactose glass transition occur (Thomas *et al*., 2004).

The main physiochemical and biochemical damages occurring upon storage are lactose crystallization, sticking and caking problems, and biochemical reactions especially Maillard reaction (Thomas *et al*., 2004).

2.4.1 Lactose crystallization

Thomas *et al*. (2004) stated that lactose is the most abundant component in fresh whole milk (4.9%) and in freshly spray dried milk powder, lactose is amorphous. During storage, an increase in temperature or relative humidity enhances lactose crystallization which is one of the main phenomena responsible for the modification of the surface chemical composition of milk powder particles. During the storage of milk powder, lactose crystallization enhances the migration of internal fat onto the

particle surface, and generates a network of capillary in the whole particle.

Oil droplets are stressed inside the particles and forced to spread onto the particle surface. Lactose crystallization is correlated with a decrease of milk powder's flowability, and involves modification of protein structure and damages the solubility of milk powders (Thomas *et al*., 2004).

2.4.2 Sticking and caking

Foster *et al*. (2005) stated that high fat powders, such as cream powders and cheese powders have been known to experience sticking and caking problems during processing and storage. During processing, high fat powders cause smearing, where the powder builds up on the inside of the dryers, cyclones and fluidized beds. During storage, lumps of powder, are formed which are difficult to break up form. Milk fat has been stated as the cause of caking in a number of studies. Viscous liquid bridges may cause flow difficulties in fat-containing powders. If the temperature is increased during storage, some fat may melt forming liquid bridges of fatty composition.

Thomas *et al*. (2004) stated that a powder collapse occurs when it is not rigid enough to support its own weight. Collapse is the shrinkage of powder particles, and it induces important structural changes in powders. Particle volume decreases since particles are crushed, thus collapse is linked to a decrease in porosity and an increase in density. Also they noted that particle agglomeration is a problem, since caked milk powders are seen as low quality products by consumers and impair flowability. They stated that collapse, caking and sticking are inter-linked and occur simultaneously during the storage of milk powder and it is difficult to study each phenomenon separately, that the initial stage agglomeration

corresponds to the collapse, particle shrink and the number of inter-particle contact increase and the Particles then start to adhere (sticking), before being strongly stuck (caking).

Both fat and lactose have a strong influence on the stickiness of milk particles. The stickiness of these particles is particularly sensitive to small changes in the fat content between 0 - 5%, which is due to a rapid change in surface fat coverage from 0 - 35% (Nijdam and Longrish 2005).

2.4.3 Maillard reaction

Thomas *et al*. (2004) stated that Maillard reaction is a biochemical reaction between proteins and reducing sugars in food. In milk products, it begins by lactose condensation on some amino acid residues (lactosylation) and involves a wide range of chemical reactions.

This reaction is mainly induced by heat treatment and generally damages milk powder quality. Nutritional quality is also worsened, because essential amino acid residues of milk proteins are less available when linked to lactose and because the digestibility of milk proteins lessened and this reaction implies food safety problems (Thomas *et al*. 2004).

2.4.4 Oxidation

Thomas *et al*. (2004) stated that oxidation is the saturation of unsaturated fatty acids with molecular oxygen. In the first stage of the reaction, free radicals are formed and yield to hydroperoxides, which further react. In the second stage of oxidation the degradation products bring oxidative off-flavors and polymerize with proteins. Oxidation increases with storage time and, thus, damages the quality of milk powders. And they added that oxidation occurs even at very low water

activities, since the migration of hydrophobic compounds (fats) does not depend on water mobility.

In the primary stage of milk powder, oxidation was dominant during the first six months of storage period and that in the secondary stage, oxidation dominated during the subsequent six months of storage period(Farkye *et al*., 2001).

Oxidation limits the product's shelf life and adversely affects quality and consumer acceptability (Farkye *et al*., 2001).

Milk powders packed with inert gases or under vacuum are less oxidized than powders packed in. When particles disintegrate, oxidation is increased because the amount of lipid exposed to air is increased.

Functionality and flavour changes in milk and milk powder are normally the result of enzyme reactions.

2.4.5 Lipolysis and proteolysis

Chen *et al*., (2003) studied lipase activity and the free fatty acid content in whole milk powders. Lipase activity was found to be stable throughout eight months of storage. Free fatty acids were constantly liberated from triglycerides by lipases and accumulated during powder storage. Although lipases were active even at 3^{o}C, larger values of free fatty acids were obtained at higher storage temperatures of 25^{o}C. They also reported that this is detrimental to milk powder quality, since free fatty acids readily oxidize and bring off-flavours.

Chen *et al*., (2003) stated that proteolysis in milk powder has been measured by monitoring changes in nitrogen levels such as the decrease in casein nitrogen or increase in non- protein nitrogen (NPN). These changes have been linked to changes in functionality, such as

microstructure changes (e.g. casein flocculation) and increase in viscosity in ultra high temperature (UHT) milk. Thomas *et al*. (2004) stated that the storage of whole milk powders did not significantly affect the proteolytic activity.

2.5 Storage of milk powder

The critical factor in the storage of dairy products is temperature. The recommended temperature varies according to the nature of the product, but any rise, and for some products any drop in temperature, could be detrimental. A reliable temperature control and monitoring system will prevent any temperature problems (Alfa-Laval Dairy Hand book).

Beside temperature, the length of time for which the product is stored from the date of production to the date of consumption must also be monitored, and every dairy product has a definite shelf life at recommended storage conditions (Alfa-Laval Dairy Hand book).

Farkye and Obispa (1998) stated that moisture content and water activity of foods are the critical parameters that influence shelf life in milk powder. Excessive moisture cause caking and promotes spoilage and loss of quality, water activity of milk powder influences enzymatic and oxidative changes that occur during storage and growth of micro-organisms.

Farky *et al*. (2001) noted that milk powders are hygroscopic, and they tend to attract water readily from humid atmospheres. When moisture levels are excessive milk powder may become sticky, caked or lumpy, and exhibit reduced flowability and solubility. They reported that these changes affect the ease of use of the product, requiring grinding for example, and may affect the flavour, but do not represent a healthy or safety problem. Also they stated that skim milk powder has low moisture

and fat contents, and, when stored in dry cool conditions, has a shelf life in excess of two years. Moreover specifically, when stored at 15^{o}C and relative humidity of 75%, skim milk powder has a minimum shelf life of two years. When dried skim milk product was stored in optimal conditions no change in colour was observed, even during two years of storage at 35^{o}C.

Extended storage of dried milk products may result in increased solubility of proteins, the insolubility is generally attributed to the Maillard reaction, which involves reducing sugars and proteins (Farky *et al*. 2001).

Farkye *et al*. (2001) demonstrated that, the most apparently visible change in milk powder after storage is a light brown colour. This colour can develop in milk powder stored at room temperature, as well as in milk powder refrigerated for a period of three years.

The contents of methionine and tryptophan did not change significantly during storage of milk powders at a temperature ranging from -40 to 40^{o}C.

The available lysine decreased at high water activity and highest temperature. Loss of lysine after six months of storage at 20^{o}C and any water activity level was less than 8%. However, pronounced losses of lysine were observed during storage at a temperature above 40^{o}C and six months storage period accompanied with high water activity.

The amount of thiamine (B_1), riboflavin (B_2), niacin, calcium, pantothenate, biotin and pyridoxine, present in dried milk are quite comparable to those of market milk and are not affected by storage for six months at 35^{o}C. Vitamin C content is slightly reduced during storage for six months. Vitamins A and D are photosensitive and break down rapidly if exposed to light.

The area where the dry milk is stored should be kept as cool as possible. If the storage container is transparent or translucent then it should be put into an opaque container or stored in a dark room. Generally milk powder made from good quality milk and containing low microbial counts is microbiologically safe during storage, provided the moisture content is kept low during storage (Thompson, 1996).

The storage stability of the filled milk powder along barrier characteristics of the packaging was assessed in order to evaluate the theoretical shelf life of the powder in its current packaging system (Thompson, 1996).

2.6 Milk powder microbiology

The relationship between the manufacturing process and raw milk quality has been examined in Germany. Raw milk for use in infant feeding formulations should not contain in excess of 10,000 cfu/ml as an initial count. Further, that raw milk counts in excess of 100,000 cfu/ml can result in counts of over 10,000 cfu/gm in the dried product (Lovell, 1990).

The earliest large-scale examination of the microbiology of the spray-dried milk was the experiment the principles established, carried out in 1942 are still valid. The work was basically directed to extending the shelf-life of whole milk powder, but it also identified the basic microbiological factors a valuable contribution to the significance of count has been made by Kwee *et al*. (1986). The work focused on total bacteria, thermodurics, thermophiles, spore-formers, psychrotrophs, coliforms, yeasts and moulds. These were measured in low, medium, and high-heat powders, and at various intermediate stages including before and after pre-heating, and after concentration. They established that pre-heating had the most significant effect, and that with the exception of

thermoduric, thermophiles, and sporeforming organisms, counts were reduced to negligible proportions in the stage of processing.

This work is important in that it enables manufactures to evaluate the quality of the process from the spectrum of micro-organisms found in the final product (Flint, 2006).

2.6.1 Coliforms

Coliforms may be defined as gram-negative, oxidase-negative, non-sporing rods which can grow aerobically or facultative unaerobically in the presence of bile salts or other surface-active agents with similar growth inhibiting properties, and which are able to ferment lactose with production of acid and gas within 48 hr at 37^{o}C (Lovell, 1990).

The coliforms count in raw milk should be less than 100 cfu/ml and count typically is much less when things are done properly. Coliform counts in the hundreds /ml may indicate a problem with dirty cows being milked. When it rises into the thousands it often means there are dirty zones in the milking system where bacteria are growing and shedding large numbers into the raw milk (Winston, 2003).

The significance of coliforms, primarily as indicator, is un-diminished, and their presence is a clear evidence of contaminations.

The presence of *Escherichia coli* after the heat treatment stage in the drying process can be regarded as adventitious contamination.

E. coli under suitable condition can spoil milk and many dairy products usually with the production of gas. It has been responsible for the early blowing of various types of milk products (Winston, 2003).

2.6.2 *Staphylococcus aureus*

In spray drying process small proportion of *S. aureus* could survive the drying conditions.

It was observed that whilst *S. aureus* occurred in raw milk supplies, there was no connection with the types isolated from milk powder, and that the most likely source was the plant contamination.

The presence of *S. aureus* is a clear evidence of contamination during manufacture, only 2% of *S. aureus* survived spray drying (Lovell, 1990).

2.6.3 Yeasts

Debaryomyces hansenii has been isolated from cheese and other foods. Cells appear spherical to short oval and are arranged singly, in paris or in short chains and propagate by multipolar budding. On slide cultures pseudomycelium is absent or, if present, is very primitive. It is unable to ferment lactose (Lovell, 1990).

Yeasts can break down protein and fat and therefore cause quality defects in cheese and butter.

Yeasts grow best in an acid environment and are therefore usually found in acid products as cultured milk. Yeasts develop most vigorously at about 30^{o}C in the presence of air.

Yeasts are generally undesirable organisms from the dairy point of view because they can cause fermentation in milk and dairy products, adversely effecting both flavour and smell (Alfa-Laval Dairy Hand book).

2.6.4 Moulds

Moulds are commonly found in dairy products. On malt extract agar, colonies are white in colour and appear yeast-like and butyrous, particularly when older.

Other research into the microbiology of dried milk has been concerned with yeasts and moulds. Aboul-Khier *et al*. (1985) have isolated *Aspergillus, penicillium, Mucor spp*. from arange of dried products including whole milk, skim milk and ice cream mixes.

The commonest species of moulds in milk and dairy products, do not in practice survive high temperature short time pasteurization (HTST). The presence of moulds in pasteurized products is therefore asign of re contamination.

Many different families of moulds exist, those which are of the greatest importance in the dairy industry are *Penicillium* (Alfa-Laval Dairy Hand book).

2.6.5 Proteolytic bacteria

Proteolytic bacteria produce protein splitting-enzymes, and this category comprises a very large number of species which grow both aerobically and unaerobically. Proteolysis is favored by an almost neutral pH, that is by the destruction of acid former by heat, or after neutralization of acids by products of other organisms and by storage at a low temperature.

Actively proteolytic bacteria are found among the species of *Pseudomonas, Micrococcus, Proteus, Acromobacter, Flavobacterium, Serratia, Alcaligenes*, all of which are genera of non spore forming bacteria as well as of the *Bacillus* and *Clostridium* genera of spore formers (Rosenthal 1990).

Bacteria of the genus *Pseudomonas* have been associated with the spoilage of raw milk and dairy products due to the production of thermos table Proteolytic enzymes (Martin *et al*., 2005).

Chen *et al*. (2004) stated that *Bacillus spp* are widely distributed in the environment and can be introduced into milk and milk powder during production, handling and processing. An additional problem with *Bacillus spp* is that they sporulate during milk powder processing, and the spores are extremely resistant to heat and chemical reagents.

2.6.6 Lipolytic bacteria

Milk fat may be decomposed by several of lipase excreting bacteria which are found in many bacterial genera such as: *Pseudomonas, Proteus, Acromobacter, Clostridium* and others (Rosenthal, 1990).

Milk is a good medium for lipase production which can be stimulated by lipids, such as glycogen, can also enhance the production of extracellular lipases. The lipolytic activities of *Pseudomonas spp* are relatively heat-stable. For instance, the crude lipase produced by *psychotropic Pseudomonas* spp are isolated from raw milk retained 55-100% activity after heat treatment at 63^{o}C for 30 min in raw milk, and 75-100% activity after heat treatment at 63^{o}C for 30 min in skim milk. Therefore, lipolytic enzymes produced by both *pseudomonas* and *Bacillus* species are likely to survive thermal processing during milk powder manufacture (Chen *et al*., 2003).

Lipolysis is observed even in a restricted water environment such as in milk powders. Lipase activity was found to be stable after eight months of storage (Mastieri *et al*., 2000).

Many proteinases and lipases produced by psychrotrophs (mainly *Pseudomonas)* of dairy origin have been reported to survive pasteurization and UHT treatments and these enzymes cause functionality and flavour defects during storage (Chen *et al*., 2004).

CHAPTER THREE
MATERIAL AND METHODS

3.1 Collection and analysis of whole milk powder samples

3.1.1 Collection of samples

Twenty seven samples of whole milk powder were collected from Khartoum State. Nine samples were collected from Khartoum, nine samples form Khartoum north and nine samples from Omdurman cities.

From each City the samples were collected from groceries of three different locations according to income level (high, middle and low income) and hygiene measures.

Three foil repacked milk powder bags (400 g) were taken from each location.

The whole milk powder samples were analyzed in the laboratory of the Department of Dairy Production, Faculty of Animal Production, University of Khartoum.

3.1.2 Sample analysis

The samples were analyzed for protein, fat, total solids, ash, titrable acidity, total bacterial count, coliform count, lipolytic count, yeasts and moulds count and proteolytic count.

3.2. Chemical analysis

3.2.1. Fat content

To an ordinary milk butyrometer, 10 ml of Gerber sulphuric acid and cold water were added so that a layer is formed on top of the acid about six mm deep. And 1.69 gm of dried milk was added by means of the dry stem less funnel, followed by addition of 1 ml amyl alcohol (Gravity). Sufficient hot water was added (about 70^{o}C) to bring the level of the liquid up to about 5 mm below the shoulder, this allowed all air entrapped in the neck to escape stopper the tube, and then mixed and placed in a water bath at 65^{o}C for 3-10 minutes and centrifuged at 1100 rpm for 5 minutes (AOAC, 2003).

3.2.2 Protein content

It was determined by kjeldahal method according to AOAC (2003). In a kjeldahal flask, 3 gm of milk powder were added, and two kjeldahal tablets (1 mg NaSo4 and equivalent of 0.1 gm hg) were added, followed by addition of 25 ml concentrated sulfuric acid (density of 1.86g/ml at 20^{o}C).

The mixture was digested on a heater until clean solution was obtained (3 hours). The flasks were removed and left to cool. The digested samples were 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 +3 drops of indicator.

The distillate was then titrated against 0.1 N HCl until the end point was obtained (red color).

The protein content was calculated as follows:

Nitrogen (%) = T × 0.1 × 0.014 / weight of sample × 100

Protein (%) = Nitrogen (%) × 6.38

Where:

T : Titration

0.1 : Normality of HCl

0.014: Atomic weight of nitrogen/1000

3.2.3 Total solids content

Total solids content was determined according to modified methods of AOAC (2003).

Two grams of milk powder were weighed and placed in a clean dried porcelain dish. The weight of the sample and dish were recorded. The dishes were heated on a steam bath for 10-15 minuets, and then the dishes were transferred to an air oven for 12 hours at 70^{o}C. The dishes were placed into a desiccator to cool and then weighed. Heating, cooling and weighing were repeated until the difference between two successive weighings was < 0.1 mg.

Total solids content was calculated as follows:

Total solids (%): $(W_1 / W_{0)} \times 100$

Where:

W_1 : Weight of sample after drying

W_0 : Weight of sample before drying

3.2.4 Ash content

Ash content was determined according to modified method of AOAC (2003).

Two grams of milk powder samples were weighed in a suitable clean and dry crucible and evaporated to dryness on a steam bath. The crucibles were placed in a muffle furnace at 550^{o}C for two hours, cooled in a desiccator and weighed.

The ash content was calculated as follows:

Ash (%) = (W1 / W2) × 100

Where:

W_1: Weight of ash

W_2: Weight of sample

3.2.5 Titratable acidity

Thirteen grams of milk powder were weighed and placed in a conical flask then 100 ml of distilled water at 40^{o}C were added and mixed for not more than 1min to avoid formation of excessive foam (Case and Williams. 1992).

Eighteen ml were placed in a porcelain dish and 5 drops of phenolphthalein indicator were added. The samples were titrated against 0.1 NaOH until a faint pink colour appeared.

The acidity was calculated as follows:

T × 9 / W

Where:

T: Titration figure Weight of sample (18 ml)

3.3 Microbiological examinations

3.3.1 Preparation of media

3.3.1.1 Solid media

3.3.1.1.1 Plate count agar medium (Merek.74065)

This medium consists of 0.5 gm casein enzymic hycholysate, 2.5 gm yeast extract powder, 1 gm dextrose and 15 gm agar.

An amount of 23.5 gm were added to 1 liter of distilled water in a flask, mixed well then boiled until dissolved completely and sterilized by autoclaving at 121 ^{o}C for 15 minuets.

3.3.1.1.2 Nutrient agar medium (S.S.D five. Chemlid 74056)

This medium consists of 5 gm peptic digest of animal tissue, 1.5 gm beef extract, 1.5 gm yeast extract, 5 gm sodium chloride and 15 gm agar.

An amount of 28 gm were added to 1 liter distilled water in a flask, mixed well and sterilized by autoclaving at 121^{o}C for 15 minuets.

3.3.1.1.3 MacConkey agar medium (Oxoid.M 77)

This medium consists of 20.00 gm peptic digest of animal tissue, 10 gm lactose, 1.5 gm bile salt, 5 gm sodium chloride, 0.001 gm crystal violet, 0.05 gm neutral red and 15 gm agar.

The medium was prepared by suspending 51.55 gm in 1 liter of distilled water in the flask boiled to dissolve and sterilized by autoclaving at 121˚C for 15 minutes.

3.3.1.1.4 Yeast extract agar

This medium was prepared by dissolving 23 gm in one liter of distilled water and sterilized by autoclaving at 120˚C for 15 minuets, cooled to 50^{o}C and stored until used

3.3.1.2 Semi solid media

3.3.1.2.1 Hugh and Leifson (OF (medium)

This medium contained 2.0 gm peptone, 5 gm sodium chloride, 0.3 gm dipotassium hydrogen phosphate, 3 gm agar and 0.2% bromothymol blue. It was prepared according to Barrow and Feltham (1993) by dissolving in one liter of distilled water. The medium was filtered followed by the addition of indicator and sterilized.

3.3.2. Sterilization

3.3.2.1 Sterilization of equipments

Glassware such as Petri dishes, test tubes, pipettes, flasks and bottles were sterilized in a hot oven at 160^{o}C for 1 hour, whereas mixer, distilled water and tips were sterilized by autoclaving for 15 minuets at 121^{o}C.

3.3.3 Preparation of sample dilution

Eleven grams of milk powder were dissolved in 99 ml of sterile distilled water to make 10^{-1} dilution, then one ml from the above mentioned was specially transferred to 9 ml sterile distilled water. This procedure was repeated to make serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{7} and 10^{-8}. From each dilution, 1 ml was transferred to Petri-dishes (in duplicate) to make serial dilutions. The culture medium was poured aseptically into each Petri-dish, mixed gently, left to solidity and incubated (in inverted position) (Houghtby *et al*., 1992).

3.3.4 Examination of cultures

Growth on solid media was examined usually with naked eye for colony appearance and changes in media.

3.3.5 Counting of bacteria

3.3.5.1 Total bacteria count

It was determined according to Houghtby *et al*. (1992) using standard plate count agar medium. After preparation of serial dilutions, 1 ml from each dilution (in duplicate) was transferred to Petri dishes, followed by addition of about 18 ml of liquid medium (at 45^{o}C). The plates were gently mixed and incubated (in inverted position) at 32^{o}C for 48 hrs.

3.3.5.2 Coliform bacteria count

It was determined according to Christen *et al*. (1992) using MacConkey agar medium. After preparation of serial dilutions, 1 ml from selected dilution (in duplicate) was transferred to Petri dishes, followed by addition of about 18 ml of liquid medium (at 45^{o}C).The plates were gently mixed and incubated (in inverted position) at 37^{o}C for 24 hrs.

3.3.5.3 Proteolytic bacteria count:

It was counted and determined according to Frank *et al*. (1992) by using composition of standard plate count gar medium plus 10% sterile skim milk. After preparation of serial dilutions, 1 ml from each dilution (in duplicate) was transferred to Petri dishes, followed by addition of about 18 ml of liquid medium (at 45^{o}C).The plates were gently mixed and incubated (in inverted position) at 37^{o}C for 72 hrs. Colonies were confirmed by using HCl.

3.3.5.4 Lipolytic bacteria count

It was determined according to Zaki (1988) using nutrient agar medium. After preparation of serial dilutions, 1 ml from each dilution (in duplicate) was transferred to Petri dishes, followed by addition of about 18 ml of liquid medium (at 45^{o}C). The plates were gently mixed and incubated (in inverted position) at 37^{o}C for 72 hrs. Colonies were confirmed by using copper sulfate.

3.3.5.5 Moulds and Yeasts count

It was determined according to Frank *et al*. (1992) using yeast extract agar medium.

After preparation of serial dilutions, 1 ml from each dilution (in duplicate) was transferred to Petri dishes, followed by addition of about 18 ml of liquid medium (at 45^{o}C). The plates were gently mixed and incubated (in inverted position) at 25^{o}C for 4 – 5 days.

3.3.6 Purification of organisms

Purification was done by sub-culturing of a well isolated typical colony on nutrient agar medium. After the growth, were checked by Gram stain for purity, it was transferred to a plate containing fresh solidified corresponding medium (Barrow and Feltham, 1993).

3.3.7 Identification of organisms

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

3.3.7.1 Primary tests

3.3.7.1.1 Shape of the cell:

It was done by grams stain as described by Barrow and Feltham (1993) as follows:

Crystal violet was added to smears on slides for one minute, followed by washing with distilled water. Logo'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 oil immersion was added followed by examining under the microscope. Gram positive organisms appeared purple, which Gram negative organisms appeared pink.

3.3.7.1.2 Oxidase test

The oxidase test was performed using oxidase test paper. The tested organism was removed with a platinum wire or glass rod and smeared across the surface of the oxidase test paper.

The positive reaction was shown by the development of dark purple color within 10 seconds.

3.3.7.1.3 Catalase test

The organisms to be tested were put on sterile slides. A drop of 3% hydrogen peroxide ($H_2 O_2$) was added to the colony and emulsified.

Evaporation of gas immediately or after 5 minutes indicated a positive result.

3.3.7.1.4 Oxidation Fermentation test:

Duplicate tubes of Hugh and Leifson's medium were inoculated by organism 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. However, the negative test showed no changes of colour in both tubes.

3.4 Statistical analyses

Statistical analyses were performed using the MSTATC (1990). General Linear Models (GLM) were used to determine the quality of whole milk powder.

The samples were analyzed for fat, protein, ash, total solids, titratable acidity, total bacterial count, coliform count, lipolytic count, yeasts and moulds count and proteolytic count. Means were separated using Duncan Multiple Range Test with $P \leq 0.05$ (Freed, 1990).

CHAPTER FOUR

RESULTS

4.1 Chemical composition of whole milk powder as affected by city

The results in Table (1) showed that the fat content and protein content of milk powder were not significant affected by city ($P > 0.05$), although the highest fat content was found in Khartoum North (26.62±0.208%), while in Omdurman and Khartoum it was (26. 52±0.208%) and (26.61±0.208%) respectively.

The highest protein content was found in Omdurman (26.40±0.196%) and lowest protein content was found in Khartoum North (25.08±0.196%).

The titratable acidity was significantly affected by city ($P < 0.05$), the highest titratable acidity being found in Omdurman (0.15±0.002%) and while in Khartoum and Khartoum North it was (0. 14±0.002%).

Total solids content was not significantly affected by city ($P > 0.05$), the highest total solids content was found in Khartoum (95.04±0.099%) and lowest content was found in Omdurman (94.03±0.099%).

The results showed that the ash content of milk powder was significantly affected by city ($P < 0.05$), it percentage was 6.18±0.079%, 6.01±0.079% and 5.67±0.079% in Khartoum, Khartoum North and Omdurman respectively.

Table (1): Chemical composition of whole milk powder as affected by city

Parameter	Area				
	Khartoum North	Omdurman	Khartoum	Grand mean	S.L.
Fat	26.62±0.208	26.52±0.208	26.61±0.208	26.583±4.209	N.S
Protein	25.08±0.196	26.40±0.196	26.20±0.196	25.893±4.154	N.S.
Total solids	94.96±0.99	94.03±0.99	95.04±0.99	94.676±7.944	N.S.
Acidity	0.14±0.002[a]	0.15±0.002[a]	0.14±0.002[b]	0.14 ± 0.31	*
Ash	6.01±0.079[b]	5.67±0.079[ab]	6.18±0.079 [a]	5.953±1.992	*

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

In this and the following tables:

*** = (P< 0.001)
** = (P< 0.01)
* = (P< 0.05)
N.S.= Not significant (P> 0.05)
S.L.= Significant level

4.2 Chemical composition of whole milk powder as affected by area

The results in Table (2) showed that the fat content of milk powder was not significantly affected by area (P> 0.05), although the fat content was high in high income area (26. 56±0.619%).

The protein content was not significantly affected by area (P> 0.05). However, the high protein content was found in middle and low income area (26.49±0.189%).

The titratable acidity and total solids content were not significantly affected by area (P> 0.05), the highest acidity content was found in high and low income area (0.15±0.002%), and highest total solids was found in high income area (95.03±0.076%)

The result showed that the ash content was significantly affected by area (P< 0.05). The highest ash content was found in middle income area (6.04±0.085%).

4.3 Chemical composition of whole milk powder as affected by replication

The results in Table (3) showed that fat, protein, total solids and ash content was not significantly affected by replication (P> 0.05).

The highest fat content was found in first replication (26.89±1.062%), while the highest protein content was found in second replication (26.29±1.062%). The highest total solids content was found in second replication (95.03±0.059%) and highest ash content was found in first replication (6.02±0.925%).

The titratable acidity was significantly affected by replication (P< 0.05). The results showed that the high acidity content was in first

Table (2): Chemical analysis of whole milk powder as affected by area

Parameter	Area				
	1	2	3	Grand mean	N.S
Fat	26.56±0.619	26.52±0.69	26.34±0.208	26.473±4.201	N.S
Protein	26.08±0.19	26.49±0.19	26.49±0.189	26.353±4.191	N.S
Total solids	95.03±0.06	94.96±0.06	94.83±0.076	94.94±7.95	N.S
Acidity	0.15±0.002	0.14±0.002	0.15±0.002	0.14 ± 0.31	N.S
Ash	5.78±0.085[a]	6.04±0.085[b]	6.03±0.085[ab]	5.95 ± 1.991	*

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

Table (3): Chemical analysis of whole milk powder as affected by replication

Parameter	Area				
	1	2	3	Grand mean	N.S
Fat %	26.89±1.062	26.64±1.062	26.34±1.062	26.62±4.21	N.S
Protein %	26.09±1.062	26.29±1.062	25.29±1.062	25.89±4.15	N.S.
Total solids %	94.86±0.059	95.03±0.059	94.93±0.059	94.94±7.95	N.S.
Acidity %	0.15±0.002 [a]	0.14±0.002[b]	0.14±0.002[b]	0.14±031	*
Fat %	26.89±1.062	26.64±1.062	26.34±1.062	6.01±1.99	N.S

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

replications (0.15±0.002%), followed by second and third replication (0.14±0.002%).

4.4 Chemical composition of whole milk powder as affected by area with in city:

The results in Table (4) showed that in Khartoum North the fat, protein, titratable acidity and total solids content were not significantly affected by area within city (P> 0.05). However, the fat content was high in middle income area (27.08±0.287%), and protein content was high in high income area (26.40±0.322%), and titratable acidity was high in middle income area (0.15±0.004%), and total solids content was high in high income area (95.04±0.922%). The ash content was higher in low income area (5.98±0.157%) (P< 0.05).

In Omdurman the ash content was significantly affected by area within city ($P< 0.05$), and the high ash content was found in low income area (6. 18±0.126%).

The fat, protein, acidity and total solids content were not significantly affected by area within city($P>0.05$), although fat content was higher in middle income area (26.68±0.612%), protein and acidity were high in high income area(26.22±0.190% and 0.15±0.004% respectively) and total solids content was high in middle income area (95.01±0.132%).

The results showed that in Khartoum the fat, protein, acidity, total solids and ash content were not significantly affected by area within city ($P> 0.05$). However, the highest fat content was in hig5 income area (26.88±0.277%), protein in high income area, acidity in middle income

Table (4): Chemical analysis of whole milk powder as affected by area within city

Parameter		Fat (%)	Protein (%)
Khartoum North	High income	26.62±0.287	26.40±0.322
	Middle income	27.08±0.287	26.32±0.322
	Low income	26.62±0.287	25.89±0.322
	Grand mean	26.773±4.22	26.20±4.18
	S.L	N.S.	N.S
Omdurman	High income	26.34±0.612	26.22±0.190
	Middle income	26.68±0.612	25.95±0.190
	Low income	26.67±0.612	25.81±0.190
	Grand mean	26.56±4.20	25.99±4.16
	S.L	N.S.	N.S.
Khartoum	High income	2.6.07±.0.27	26.85±0.217
	Middle income	26.94±0.27	25.97±0.217
	Low income	26.88±0.27	26.37±0.217
	Grand mean	26.53±4.20	26.39±4.19
	S.L	N.S.	N.S.

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

Total solids (%)	Titratable Acidity (%)	Ash (%)
95.04±0.922	0.14±0.004	5.67±0.157[a]
94.95±0.922	0.15±0.004	5.35±0.157[b]
94.83±0.922	0.14±0.004	5.98±0.157[a]
94.94±7.95	0.14±0.31	5.66±1.94
N.S.	N.S.	*
94.89±0.132	0.15±0.004	6.10±0.126[a]
95.01±0.132	0.13±0.004	5.75±0.126[b]
94.91±0.132	0.13±0.004	6.18±0.126[a]
94.93±7.95	0.130.36	6.01±3.47
N.S.	N.S.	*
94.60±0.217	0.14±0.004	6.33±0.172
95.10±0.217	0.15±0.004	6.25±0.172
95.00±0.217	0.14±0.004	5.95±0.172
94.93±7.95	0.14±0.31	6.17±2.03
N.S.	N.S.	N.S.

area, total solids in middle income area and ash content in high income area.

4.5 Chemical composition of whole milk powder as affected by replication within area

Table (5) presents the data concerning the effect of replication of sampling in each city.

In low income area the fat, protein, titratable acidity, total solids and ash content did not show any significant variation between replication of sampling (P> 0.05).

In middle in come area no significant variation was observed in fat, protein, acidity and total solids content (P> 0.05), the only variation observed was in ash content which was higher in third replication (6.18±0.126%) and lower in second replication (5.75±0.126%) (P< 0.05).

In high income area, except for ash which was high in third replication (5.98±0.126%) and total solids which was high in first

replication (95.06±0.922%) (P< 0.05), no significant effect was observed in fat, protein and acidity between replication of sampling (P> 0.05).

4.6 Microbial examination of whole milk powder as affected by city

The results in Table (6) showed that total bacterial count was significantly affected by city (P< 0.01), with the highest count being found in Khartoum North (Log_{10} 4.12±0.038 cfu/gm) followed by Omdurman (Log_{10} 3.52±0.038 cfu/gm) and Khartoum (Log_{10} 3.25±0.038 cfu/gm).

Table (5): Chemical analysis of whole milk powder as affected by replication within area

Parameter		Fat (%)	Protein (%)
High income area	1st	26.27±0.287	26.40±0.322
	2nd	26.94±0.287	26.22±0.322
	3rd	27.10±0.287	25.89±0.322
	Grand mean	26.77±4.22	26.17±4.17
	S.L	N.S.	N.S.
Middle income area	1st	26.42±0.612	26.22±0.190
	2nd	26.77±0.612	25.95±0.190
	3rd	26.50±0.612	25.81±0.190
	Grand mean	26.56±4.20	25.99±4.16
	S.L	26.32±0.276	26.39±0.287
Low income area	1st	26.32±0.276	26.64±0.287
	2nd	26.95±0.276	26.16±0.287
	3rd	26.32±0.276	26.39±0.287
	Grand mean	26.53±4.20	26.39±4.19
	S.L	N.S.	N.S.

Total solids (%)	Titratable Acidity (%)	Ash (%)
95.06±0.922[a]	0.14±0.004	5.67±0.126[ab]
94.75±0.922[b]	0.15±0.004	5.35±0.126[b]
95.01±0.922[a]	0.14±0.004	5.98±0.126[a]
94.94±7.95	0.14±0.31	5.66±1.64
*	N.S	N.S.
94.89±00.132	0.15±0.004	6.10±0.126[ab]
96.01±00.132	0.13±0.004	5.7[b]±0.126[b]
94.97±00.132	0.14±0.004	6.18±0.126[a]
95.29±7.97	0.14±0.30	5.99±1.99
N.S.	N.S	*
94.79±00.217	0.14±0.004	6.07±0.172
94.89±00.217	0.15±0.004	6.28±0.172
95.11±00.217	0.14±0.004	6.18±0.172
94.93±7.95	0.14±0.31	6.17±2.02
N.S.	N.S.	N.S.

Table (6): Microbial count of whole milk powder as affected by city (Log_{10} cfu/gm).

Microbial count	City				
	Khartoum North	Omdurman	Khartoum	Grand mean	S.L
Total bacteria	4.12±0.038[a]	3.52±0.038[b]	3.25±0.038[b]	3.63±1.58	**
Coliform bacteria	2.35±0.037[a]	2.03±0.037[b]	2.19±0.037[ab]	2.19±1.22	*
Proteolytic bacteria	2.34±0.007[a]	2.26±0.007[b]	2.32±0.007[a]	2.36±1.24	*

Lipolytic bacteria	2.28±0.022	2.43±0.022	2.39±0.022	2.37±1.26	N.S.
Yeasts and moulds	2.38±0.001[a]	3.52±0.001[b]	3.25±0.001[b]	2.33±1.25	N.S

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

The coliform bacterial count was significantly affected by city (P< 0.05) with the highest count being found in Khartoum North (Log_{10} 2.35±0.037 cfu/gm) while in Omdurman and Khartoum was Log_{10} 2.03±0.037 cfu/gm and Log_{10} 2.19±0.037 cfu/gm respectively.

The proteolytic bacterial count was significantly affected by city (P< 0.05) with the highest count being in Khartoum North (Log_{10} 2.34±0.077 cfu/gm) and lowest count being found in Omdurman (Log_{10} 2.26±0.077cfu/gm). The lipolytic bacterial count and yeast and mould bacterial count were not significantly affected by city (P> 0.05), that the highest lipolytic count was found in Omdurman (Log_{10} 2.43±0.022 cfu/gm), while highest yeast and moulds count were found in Khartoum North (Log_{10} 2.38±0.011 cfu/gm), followed by Khartoum (Log_{10} 2.35±0.011 cfu/gm) and Omdurman (Log_{10} 2.25±0.011 cfu/gm).

4.7 Microbial examination of whole milk powder as affected by area

The result in Table (7) showed that the total bacterial count was significantly affected by area (P< 0.05). The total bacterial count was

highest in high income area (Log_{10} 4.78±0.038 cfu/gm), followed by low income area (Log_{10} 4.67±0.038 cfu/gm) and middle income area (Log_{10} 4.44±0.038 cfu/gm).

The coliform count was significantly affected by area with the highest count being in middle income area (Log_{10} 2.29±0.0240 cfu/gm), followed by high income area (Log_{10} 2.16±0.0240 cfu/gm) and middle income area (Log_{10}2.12±0.0240cfu/gm). Proteolytic and lipolytic bacterial counts were not significantly affected by area (P> 0.05). The

Table (7): Microbial count of whole milk powder as affected by area

Microbial count (Log_{10} cfu/gm)	Area				
	1	2	3	Grand mean	S.L.
Total bacteria	4.78±0.038	4.44±0.038[b]	4.67±0.038[b]	4.63±1.76	*
Coliform bacteria	2.12±0.240[b]	2.29±0.240[a]	2.16±0.240[ab]	2.19±1.21	**
Proteolytic bacteria	2.28±0.037	2.29±0.037	2.32±0.037[a]	2.29±1.24	N.S.
Lipolytic bacteria	2.39±0.022	2.35±0.022	2.32±0.022	2.35±1.25	N.S.
Yeasts and moulds	2.37±0.011[a]	2.33±0.011[a]	2.23±0.011[b]	2.31±1.24	**

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

Area 1:High income area

Area 2:Middle income area

Area 3:Low income area

proteolytic count was high in low income area (Log_{10}2.32±0.037 cfu/gm and low count in high income area (Log_{10} 2.28±0.037 cfu/gm), while the highest lipolytic count was found in low income area (Log_{10} 2.39±0.022 cfu/gm).

Yeasts and moulds count were significantly affected by area ($P< 0.01$). The highest counts were observed in high income area (Log_{10} 2.37±0.011 cfu/gm) and lowest counts were observed in low income area (Log_{10} 2.23±0.011 cfu/gm).

2.8 Microbial examination of whole milk powder as affected by replication

Table (8) showed the results of samples taken repeatedly in each area of sampling in each city.

The total bacterial count was significantly affected by replication ($P< 0.01$), the highest count being found in the first replication (Log_{10} 4.76±0.048 cfu/gm), while the lowest count was in the second replication (Log_{10} 4.45±0.048 cfu/gm).

The coliform, proteolytic and yeasts and moulds counts were not significantly affected by replication ($P> 0.05$). The highest coliform count was in the second replication (Log_{10} 2.31±0.130 cfu/gm), while the highest count in proteolytic bacteria was in the first replication (Log_{10}

2.33±0.032 cfu/gm). The lipolytic bacterial count was significantly affected by replication of sampling (P< 0.01) with highest count being in the second replication (Log_{10} 2.44±0.021 cfu/gm) and the lowest count in the third replication (Log_{10} 2.28 ±0.021cfu/gm).

Table (8): Microbial examination of whole milk powder as affected by replication.

Microbial count (Log_{10} cfu/gm)	Replication				
	1	2	3	Grand mean	S.L.
Total bacteria	4.76±0.048[a]	4.45±0.048[b]	4.59±0.048[b]	4.60±1.75	**.
Coliform bacteria	2.03±0.130	2.31±0.130	2.23±0.130	2.19±1.21	N.S
Proteolytic bacteria	2.33±0.032	2.24±0.032	2.24±0.032	2.27±1.23	N.S.
Lipolytic bacteria	2.36±0.021[a]	2.44±0.021[a]	2.28±0.021[b]	2.36±1.25	**
Yeasts and moulds	2.30±0.021	2.32±0.021	2.34±0.021	2.32±1.24	N.S

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

Yeasts and moulds count were not significantly affected by replication (P> 0.01). The highest counts were observed in third replication (Log_{10} 2.34±0.021 cfu/gm) and lowest counts were observed in first replication (Log_{10} 2.30±0.021 cfu/gm).

4.9 Microbial examination of whole milk powder as affected by area within city

The results in Table (9) showed that in Khartoum North the total bacterial count, lypolytic bacterial count and proteolytic bacteria count were not significantly affected by area (P>0.05). The highest total bacterial count was found in middle income area (Log_{10} 4.50±0.002 cfu/gm), and lowest count was in high income area (Log_{10} 4.22 ±0.002cfu/gm). The highest lypolytic count was found in low income area (Log_{10} 2.31±0.030 cfu/gm), and lowest count was in high income area (Log_{10} 2. 05±0.030 cfu/gm). The highest proteolytic count was found in low income area (Log_{10} 2.45±0.030 cfu/gm) and lowest count was found in middle income area (Log_{10} 2.21±0.030 cfu/gm).

The coliform count and yeast and mould count were significantly affected by area (P< 0.05). The highest coliform count was found in middle income area (Log_{10} 2.64±0.051 cfu/gm) followed by low income area (Log_{10} 2.37±0.051 cfu/gm) and high income area (Log_{10} 2.05±0.051 cfu/gm).The highest yeasts and moulds was found in high income area

(Log_{10} 2.46±0.0345 cfu/gm), followed by middle income area (Log_{10} 2.45±0.045 cfu/gm) and low income area (Log_{10} 2.21±0.045 cfu/gm).

In Omdurman, the total bacterial, lypolytic, proteolytic and yeasts and moulds counts were not significantly affected by area (P> 0.05). The

Table (9): Microbial count of whole milk powder as affected by area within city (Log_{10} cfu/gm)

measurement		Total bacteria count	Coliform	Lipolytic	Proteolytic
Khartoum North	High income	4.22±0.062	2.05±0.051^{b}	2.05±0.030	2.35±0.030
	Middle income	4.50±0.062	2.64±0.051^{a}	2.30±0.030	2.21±0.030
	Low income	4.40±0.062	2.37±0.051^{b}	2.31±0.030	2.45±0.030
	Grand mean	4.37±1.17	2.35±1.25	2.22±1.22	2.33±1.25
	S.L	N.S.	*	N.S.	N.S.
Omdurman	High income	4.37±0.070	2.41±0.053^{a}	2.43±0.032	2.19±0.248
	Middle income	4.15±0.070	2.26±0.053^{ab}	2.42±0.032	2.43±0.248
	Low income	4.24±0.070	2.09±0.053^{b}	2.31±0.032	2.32±0.248
	Grand mean	4.25±1.68	2.25±1.23	2.38±1.26	2.31±1.24
	S.L	N.S.	*	N.S.	N.S.
Khartoum	High income	4.74±0.029^{a}	2.11±0.042	2.55±0.040	2.29±0.027
	Middle income	3.35±0.029^{b}	1.98±0.042	2.15±0.040	2.24±0.027
	Low income	4.47±0.029^{b}	1.99±0.042	2.35±0.040	2.18±0.027
	Grand mean	4.18±1.64	2.026±1.16	2.35±1.25	2.36±1.145
	S.L	**	N.S.	N.S.	N.S.

Yeast and moulds
2.46±0.045[b]
2.45±0.045[a]
2.21±0.045[b]
2.37±1.26
*
2.28±0.020
2.38±0.020
2.37±0.020
2.34±1.25
N.S.
2.34±0.013[a]
2.16±0.013[c]
2.25±0.013[b]
2.25±1.2
**

highest total bacterial count was in high income area (Log_{10} 4.37±0.070 cfu/gm), followed by low income area (Log_{10} 4.24±0.070 cfu/gm) and middle income area (Log_{10} 4.15±0.070 cfu/gm), while the highest lipolytic

Count was high income area (Log_{10} 2.43±0.032 cfu/gm) and the lowest count was in low income area (Log_{10} 2.31±0.032 cfu/gm). The highest proteolytic count was found in the middle income area (Log_{10} 2.43±0.029 cfu/gm), while the lowest count was found in the high income area (Log_{10} 2.19±0.029 cfu/gm). The highest yeasts and moulds count was found in middle income area (Log_{10} 2.38±0.020 cfu/gm), followed by low income area (Log_{10} 2.37±0.020 cfu/gm) and high income area (Log_{10} 2.28±0.020 cfu/gm).The coliform count was significantly affected area ($P< 0.05$). The highest count was in high income area (Log_{10} 2.41±0.053 cfu/gm), followed by middle income area (Log_{10} 2.26±0.053 cfu/gm) and low income area (Log_{10} 2.09±0.053 cfu/gm).

In Khartoum, the total bacterial count and yeasts and moulds counts were significantly affected by area ($P< 0.01$). The highest total bacterial count was found in high income area (Log_{10} 4.74±0.029 cfu/gm) and lowest count in middle income area (Log_{10} 3.35±0.029 cfu/gm). The highest yeasts and moulds count was found in high income area (Log_{10} 2.34±0.013 cfu/gm) and lowest count in middle income area (Log_{10} 2.16±0.013 cfu/gm).

The coliform, lypolytic and proteolytic counts were not significantly affected by area (P> 0.05).The highest coliform count was found in high income area (Log_{10} 2.11±0.042 cfu/gm) and lowest count in middle income area (Log_{10} 1.98±0.042 cfu/gm).The highest lypolytic count was found in high income area (Log_{10} 2.55±0.042 cfu/gm) and lowest count in middle income area (Log_{10} 2.15±0.042 cfu/gm).The highest proteolytic count was found in high income area (Log_{10} 2.29±0.027 cfu/gm) and lowest count in low income area (Log_{10} 2.18±0.027 cfu/gm).

4.10 Microbial examination of whole milk powder as affected by replication within area of sampling

The results in Table (10) showed that in high income area the total bacterial count (P< 0.001) and proteolytic bacterial count were (P< 0.05) significantly affected by replication.

The highest total bacterial count was found in second replication (Log_{10} 4.74±0.062 cfu/gm) and lowest count in a third replication (Log_{10} 4.38±0.062 cfu/gm), while the highest proteolytic count was in third replication (Log_{10} 2.31±0.030 cfu/gm) and lowest count in second replication (Log_{10} 2.10±0.030 cfu/gm).

The coliform, lipolytic and yeasts and moulds count were not significantly affected by replication (P> 0.05). However, the highest coliform, lipolytic and yeasts and moulds count, (Log_{10} 2.07±0.021, Log_{10} 2.57±0.050 cfu/gm and Log_{10} 2.28±0.065 cfu/gm respectively).

In middle income area the total bacterial, Proteolytic bacterial and yeasts and moulds counts were not significantly affected by replication (P> 0.05). However the highest total bacterial count was in third replication (Log_{10} 4.33±0.075 cfu/gm) while the highest proteolytic count

Table (10): Microbial examination of whole milk powder as affected by replication within (Log_{10} cfu/gm)

measurement		Total bacteria count	Coliform	Lipolytic	Proteolytic	Yeast and moulds
High income area	Replication 1	4.43±0.080	2.03±0.021	2.31±0.050	2,30±0.040[a]	2.26±0.065
	Replication 2	4.74±0.080	2.07±0.021	2.57±0.050	2.10±0.040[b]	2.28±0.065
	Replication 3	4.38±0.080	1.98±0.021	2.17±0.050	2.31±0.040[a]	2.22±0.065
	Grand mean	4.51±1.77	2.02±1.24	2.41±1.24	2.24±1.22	2.25±1.28
	S.L	***	N.S.	N.S.	*	N.S.
Middle income area	Replication 1	4.30±0.075	2.34±0.029[b]	2.63±0.022[b]	2.37±0.045	2.29±0.063
	Replication2	4.20±0.075	2.33±0.029[a]	2.32±0.022[b]	2.30±0.045	2.31±0.063
	Replication 3	4.33±0.075	1.09±0.029[b]	2.47±0.022[a]	2.28±0.045	2.38±0.063
	Grand mean	4.27±1.68	2.02±1.44	2.41±124	2.23±1.24	2.25±1.25
	S.L	N.S.	***	*	N.S.	N.S.
Low income area	Replication 1	5.00±0.091	1.83±0.030	2.42±0.052[a]	2.32±0.044	2.30±0.067
	Replication 2	3.52±0.091	2.52±0.030	2.36±0.052[a]	2.33±0.044	2.38±0.067
	Replication 3	4.51±0.091	2.60±0.030	2.04±0.052[b]	2.36±0.044	2.44±0.067
	Grand mean	4.34±1.70	2.32±1.24	2.27±1.23	2.34±1.25	2.37±1.26
	S.L	N.S.	N.S.	*	N.S.	N.S.

was found in first replication (Log_{10} 2.37±0.045 cfu/gm), and the highest yeasts and moulds count was in third replication (Log_{10} 2.38±0.063 cfu/gm).

The coliform count was highly significantly effected (P< 0.001), with highest count being in first replication (Log_{10} 2.34±0.029 cfu/gm). The lipolytic count was high in first replication (Log_{10} 2.63±0.022 cfu/gm) (P<0.05).

In low income area, the total bacterial, coliform, proteolytic and yeasts and moulds count were not significantly affected by replication within area (P> 005).The lipolytic bacterial count was significantly affected by replication with area (P< 0.05), with the highest count being found in first replication (Log_{10} 2.42±0.052 cfu/gm).

CHAPTER FIVE

DISCUSSION

The investigation focused on whether the repacked whole milk powder sold in Khartoum State complies with Sudanese standards for milk powder or not, and how much the environment of repacking affects the quality of milk powder.

The practice of repacking whole milk powder in the Sudan started recently by importing the powder in sacs of 50 kg weight then the repacking takes place in small factories. From a survey carried out it was noticed that the environmental conditions of repacking lack the sanitation practices so that the possibility of recontamination is possible.

Although the packing takes place in aseptic containers, but the environment is of poor sanitary measurements. Therefore, the samples were collected from repacked milk powder by local plants.

The results showed that the average of total bacterial count was Log_{10} 4.78±0.038 cfu/gm while coliform count ranged between Log_{10} 1.09±0.029-2.64±0.051 cfu/gm, the proteolytic count was Log_{10} 2.43±0.029 cfu/gm, while lypolytic counts was Log_{10} 2.57±0.052 cfu/gm and yeasts and moulds count was Log_{10} 2.44±0.067 cfu/gm.

These results do not conform with the Sudanese standards, which stated that the total bacterial count should be less than Log_{10} 4.0 cfu/gm and proteolytic, lipolytic and yeasts and moulds count should be less than Log_{10}1.0 cfu/gm, and the milk powder must be free from coliform bacteria.

This high load of bacteria is an indicator of recontamination. Because the imported milk powder always complies with universal

standards, therefore, no bacterial growth will be observed during and after processing.

The recontamination could be attributed to different factors such as: The recontamination during repackaging, the unsanitized storage area, no aseptic conditions are observed during sack opening, the use of unsanitized equipment for repackaging, no sanitary standards are used for workers, there might not be routine health check up for workers, the temperature and humidity of storage and transportation might not comply with standards.

In addition, the contamination means may occur during handling and storage, because whole milk powder is a sensitive product, it easily absorb the foreign bodies and moisture from the surrounding and this lead to growth of bacteria.

According to results, the chemical composition of whole milk powder was found to be as follows: The fat content 26.32±0.276 – 26.89±0.062%, protein content 25.06±0.196–26.49±0.198%, total solids content was 94.03±0.99–96.1±0.132%, titratable acidity 0.14±0.004 – 0.15±0.004% and ash content 5.35±0.126 – 6.33±0.172%.

The results of chemical composition obtained during the present study comply with Sudanese standard which states that the fat content should be in the range of 26 – 42%, protein content 27%, titratable acidity 0.15%, total solids 95% and ash content 7.3%. This means that the imported milk powder was processed according to the universal standards for milk powder.

For example USA standards state that the fat content is 26.0%, protein content is 27%, total solids content is 95% and titratable acidity is less than 0.15%.

Whole fresh milk should be recommended due to the fact that the importation of whole milk powder will affect the local fresh milk production, beside the safety consideration other than bacterial contamination. In addition to nutritional aspects of milk powder compared to fresh milk. The investigation suggests that importation should be limited if not totally abandoned to encourage the local production of fresh healthy milk.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:

Since whole milk powder is re-packaged in small packages and distributed to difference areas, no significant variation was observed concerning the chemical composition.

The study revealed high bacterial load of re-packaged milk powder meaning recontamination during re-packaging.

Due to packaged retail containers status of grocery store had no effect on the quality of whole milk powder.

6.2 Recommendations:

1. Establishment of an efficient program for the assurance of high quality whole milk powder.
2. The responsible governmental bodies should issue laws and regulations that the re-packaging of whole milk powder should comply with Sudanese standards of milk powder.
3. Sanitary re-packaging retails are recommended.
4. A frequent examination of repacked milk to detect environmental hazards with storage recontamination.

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