PRODUCTION OF EXOPOLYSACCHARIDES IN A KENYAN FERMENTED MILK, *MURSIK*

SALOME CHELAGAT MUIGEI

A thesis submitted to the Graduate School in partial fulfillment for the requirements of the Master of Science Degree in Food Science and Technology of Egerton University

EGERTON UNIVERSITY

APRIL, 2014

DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not, wholly or in parts, been presented for an award of a degree, diploma or certificate in this or any other University.

Salome Chelagat Muigei

Reg. No: KM16/1986/07

Signature…….. Date……

RECOMMENDATION

This thesis is the candidate's original work and has been prepared with our guidance and assistance; it has been submitted with our approval as the official University supervisors.

Signature…… Date……

Prof. Anakalo Shitandi, PhD.

Professor of food science, and Registrar of research and Extension,

Kisii University.

Signature…… Date……

Dr. P.S. Muliro, PhD.

Senior Lecturer,

Dept. Dairy and Food Science &Technology, Egerton University.

COPYRIGHT

DEDICATION

I dedicate this work to my son Allan, and daughters Ruth and Rebecca for their support and prayers.

ACKNOWLEDGEMENT

I wish to thank the Almighty God for his love, care, strength and guidance during my entire study**.** My sincere thanks are extended to Egerton University, led by the Vice Chancellor, Prof. James. K. Tuitoek for their support towards my study through provision of a tuition waiver. I also thank the Board of Post Graduate Studies, Egerton University, lead by Prof. Michael A. Okiror for providing me with a grant which enabled me to accomplish my research work. I wish to acknowledge the support from the entire staff of the Department of Dairy and Food Science and Technology, led by Dr. A. K. Faraj for their sincere support. Special thanks go to my Supervisors, Prof. A. A. Shitandi and Dr. P. S. Muliro for their tireless support and effort in guidance during the entire period of my study and research. My appreciation is also extended to my fellow colleagues and friends for their support through prayers, encouragement and assistance during the entire period of my study. Special thanks go to Mr. J. Kioko Mutungi for his support during the entire period of study, especially during data collection.

My sincere gratitude goes to the National Council for Science and Technology, lead by Prof. Shaukat Abdul Razak, for their support through provision of a research grant which greatly supported my research. I also extend my gratitude to Kenya Agricultural Research Institute (KARI), Njoro station for allowing me to carry out part of my research work in their Biotechnology Laboratory. I wish to thank my family for the immense support. Special thanks go to my children, Allan, Ruth and Rebecca for their prayers and understanding when I wasn't available for them during my study. I also thank my parents, bothers and my sisters for their support, prayers and encouragement. Special thanks to Joshua Katam and Julia Kietany for supporting my family during my study. To all that I have mentioned, I will always appreciate your support and contribution to my success in this work. 'May God Bless You All'.

ABSTRACT

The aim of this research was to establish whether *Mursik*, a Kenyan traditional fermented milk product, contained Exopolysaccharides (EPS) and EPS producing Lactic acid Bacteria (LAB). It also sought to establish the optimum conditions for their production in *Mursik*. Samples of *Mursik* were collected from 39 randomly selected homesteads in Njoro District, Nakuru County, Kenya, and transported to Egerton University for analysis. Their EPS levels were determined by isolating them and quantifying by phenol-sulphuric acid method. EPS producing LAB were screened using sucrose agar and identified by phenotypic and biochemical methods. Optimum incubation period for EPS production in traditional *Mursik* was determined by fermenting *Mursik* in gourds and determining their EPS levels after every 24 hours for 5 days. The effect of incubation period and growth medium on EPS yield by *Mursik* culture were determined by propagating it in four different growth media, Reconstituted skim milk (RSM), RSM with 5% sucrose (RSM-S), Whey (WH) and WH with 5% sucrose (WH-S) at 20 ± 1ºC and determining their EPS levels after every 6 hrs for 30 hours. The data obtained was used to compute for analysis of variance (ANOVA) and means separated by least significance difference (LSD). The study established that the EPS levels in traditional *Mursik* differed with households at 5% level of significance ($P< 0.05$), and ranged between 17.86 - 59.77 mg/L glucose equivalent (Glu.equiv), the mean being 44.83 mg/L Glu.equiv. A total of 122 EPS producing LAB were isolated and characterized into five genera namely, *Lactococcus* (76%), *Lactobacillus* (11%), *Enterococcus* (7%), *Leuconostoc* (5%) and *Weissella* (1%). Their EPS yields ranged between 44.3 and 449.9 mg/L Glu.equiv. EPS production during traditional fermentation of *Mursik* was not growth associated and its optimum incubation period was 4 days (mean yield being 73 mg/L Gluc.Equiv). The EPS yields by the *Mursik* culture in the four growth media and at the five incubation periods were significantly different ($P< 0.05$). Based on the mean EPS level, the growth media and incubation periods ranked from the highest to the lowest as; RSM-S>WH-S>WH>RSM and 24hrs>18hrs>30hrs >12hrs >6hrs respectively. However, 18 and 24 hours were not significantly different. The Growth medium/incubation period interaction which yielded maximum EPS level (141.5 mg/L Glu.equiv) from *Mursik* culture was RSM-S/ 24 hours. From these results, it was therefore concluded that *Mursik* contains EPS and EPS producing LAB. Although their levels were low, they can be improved by supplementing the milk with sucrose and incubating for 24 hrs. Therefore, it is recommended that *Mursik* should be developed as a functional food product by enhancing production EPS during its fermentation. This study has a significant impact on development of *Mursik* as a functional food product and its commercialization.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMS AND ABREVIATIONS

ANOVA	Analysis of Variance.
CFU (cfu)	Colony forming units
CRD	Completely randomized design.
EPS	Exopolysaccharides
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drugs Act.
GDI	Guilford Dairy Institute.
GRAS	Generally Regarded as Safe
KCC	Kenya Cooperative Creameries.
LAB	Lactic acid bacteria
Log	Logarithm base 10 (Log_{10})
LSD	Least significance difference.
ml	Milliliters
RSM	Reconstituted Skim milk.
RSM-S	Reconstituted Skim milk with 5% sucrose.
SAS	Statistical analysis for Science
T.A	Titratable acidity
TCA	Trichloroacetic acid
WH	Whey
WH-S	Whey with 5% sucrose.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Exopolysaccharides (EPS) are polysaccharides that are secreted by bacteria onto their cell walls or into the growth medium (Ruas-Madiedo and Reyes-Gavilan, 2005; Kumar *et al.,* 2007). They are categorized as capsular EPS and ropy EPS based on whether they are attached or unattached on the bacterial cell wall respectively (Hassan *et al.,* 2003; Ruas-Madiedo and Reyes-Gavilan, 2005; Hassan 2008). They are also categorized based on their monomer composition as homopolysaccharides and heteropolysaccharides. Homopolysaccharides are the EPS that have only one type of monomer in their polymer such as glucan (with only glucose) and fructan (with only fructose), whereas heteropolysaccharides have several different types of monomers such as glucose, fructose and rhamnose on one polymer (Vuyst and Degeest, 1999; Vaningelgem *et al.,* 2004a; Ruas-Madiedo and Reyes-Gavilan, 2005). Production of EPS by Lactic acid bacteria (LAB) is important in fermented milk products since they improve their rheological properties (Awad *et al.,* 2005; Ayala-Hernandez *et al.,* 2008; Hassan, 2008) and some have the ability to enhance growth of health beneficial microbiota in the gut of the consumers (Korakli *et al.,* 2002; Korakli and Vogel, 2006; Bleau *et al.,* 2010; Lebeer *et al.,* 2010). Several traditional fermented food and milk products have been established to have EPS and/or EPS producing LAB and these include *Viili*, a Finish traditional fermented milk (Ruas-Madiedo *et al.,* 2006), Burkina Faso traditional fermented milk (Savadogo *et al.,* 2004), *Ogi*, Nigerian traditional fermented Maize, sorghum or millet products, and *Fufu*, Nigerian fermented cassava (Sanni *et al.,* 2002).

Production of EPS by LAB has recently attracted a lot of interest, due to their unique properties and safety nature of LAB (Jolly *et al.,* 2002; Korakli, *et al.,* 2003). Due to their ability to improve rheological properties of fermented food as biothickeners, stabilizers and gelling agents, they are good alternatives for the plant and algae-based polysaccharides especially those that are chemically modified (Korakli, *et al.,* 2003; Tieking *et al.,* 2003; Awad *et al.,* 2005; Doleyres *et al.,* 2005; Saija *et al.,* 2010). Some LAB EPS have prebiotic properties and these include fructooligosacchride kestoses and nystoses (Korakli, *et al.,* 2003; Tieking *et al.,* 2003), glucooligosaccharides (Vasileva *et al.,* 2010), and β-glucan (Russo *et al.,* 2012). They selectively stimulate growth or activity of *Bifidobacteria* and/or *Lactobacillus* species in the intestines, hence improve their gut colonization which is antagonistic to the effects of pathogenic bacteria

(Korakli *et al.,* 2003; Tieking *et al.,* 2003; Macfarlane *et al.,* 2006; Salazar *et al.,* 2008). Other health benefits due to increased *Bifidobacteria* and *Lactobacillus species* in the gut include inhibition of growth and adherence of pathogenic microorganisms in the gut (Macfarlane *et al.,* 2006; Chichlowiski *et al.,* 2007; Callaway *et al.,* 2008), reduction of blood cholesterol levels, anti-cancer activity (Ruas-Madiedo *et al.,* 2006) anti-tumor activity, and immuno-modulation (O'Connor *et al.,* 2005; Kim *et al.,* 2010; Bleau *et al.,* 2010). Studies have shown that EPS interact with milk proteins and as a result improve their rheological properties. According to Hassan *et al.,* (2003, 2008) and Ayala-Hernandez *et al.,* (2008), EPS molecules in the fermented milks interact with the milk proteins (whey proteins and casein) forming a network which results in improved viscosity, inhibition of syneresis and improved rheological properties. EPS producing LAB are now being used industrially to improve rheological (viscosity and texture) of fermented milk products such as yoghurt and cheese (Skiver and Karychev, 2007; Vasilean and Segal, 2011). However, the limitation for use of these LAB EPS as a bioingredient has been their low production capacities. According to Badel *et al.,* (2011), with a few exceptions such as *Lactobacillus rhamnosus*, most LAB produce below 1g/Liter EPS, unlike the non-food grade species such as *Xanthomonas campestris* (source of xanthan biothickener) which produces between 30 and 50 g/liter. These levels are far below the economical levels (10 -15g/L) required for production of biothickeners (Welman and Maddox, 2003; Badel *et al.,* 2011). EPS production by LAB can be manipulated through biotechnological techniques such as genetic and metabolic engineering to improve their EPS yields, EPS quality as well as induce production of tailor made EPS products with specific functional properties for particular applications (Kleerebezem *et al.,* 2002; Badel *et al.,* 2011).

In Kenya, traditional fermented milk products are mainly produced by pastoral communities such as the Maasai, Borana, Kalenjin and Gusii and Somali. These products are spontaneously fermented in traditional containers such as gourds which may or may not be smoked, with application of one or more additives such addition of cow's or goat's blood. Some of these fermented milks include *Kule naoto* produced by the Maasai (Mathara *et al.,* 2008), *Mursik* produced by the Kalenjin (Mathara *et al.,* 1995), *Suusac* produced by the Somali (Lore *et al.,* 2005) and *Ambere amaruranu* produced by the Gusii (Mokua, 2004). Some of these traditional fermented milk products such as *Mursik* (Mathara *et al.,* (1995) and *Kule naoto* (Mathara *et al.,* 2008) have been claimed to have health beneficial effects on consumers' health.

Mursik is traditionally, produced by spontaneous fermentation of cow milk in a traditionally prepared gourd (FAO, 1990; Mathara *et al.,* 1995), and referred to as '*Sotet'* in Kalenjin (Figure 1). It is mainly produced by the Kalenjin communities in Kenya, who consume it as a major part of their diet due to its delicious taste and belief that it improves health (Mathara *et al.,* 1995). It is also highly valued by the Kalenjin as a special drink that is shared in special occasions to symbolize success of certain activities such as during successful marriage negotiations, victory in athletics and other events. According to the studies by Mathara *et al.,* (1995) and Nakamura *et al.,* (1999), *Mursik* culture consist of LAB which include *Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides* subsp. *dextranicum, Lb.curvatus, Leuc.paramesenteroides* and *Lb. planturum*. The claimed health beneficial effects of *Mursik* are attributed to presence of the LAB. Mathara *et al.,* (1995) established that some LAB species in *Mursik* have antimicrobial properties against certain pathogens such as *Staphylococcus aureus* and *salmonella typhimurium*. A study by Mathara *et al.,* 2008, established that *Kule naoto* traditional fermented milk produced by the Maasai and is similar to *Mursik*) contain *Lactobacillus plantarum* species which have probiotic properties. However, there are no reports on presence of EPS and/or EPS producing LAB in *Mursik*. Therefore, the objective of this study was to establish production of EPS in *Mursik.* This has a significant impact on the functional properties of *Mursik*.

1.2 Statement of the problem

Studies have shown that EPS produced by LAB have the ability to enhance certain health beneficial effects on consumers. This includes the ability to improve of growth of the health beneficial microbiota in the gut (as prebiotics), ability to enhance attachment and colonization of the gut by some probiotics, immunomodulation and antitumor activities. They also improve the rheological properties such as texture, water holding properties and melting properties in fermented milk products. Since they are regarded as natural food additives, consumers prefer them over the commercial plant, algal based additives, especially those that are chemically modified, and those that are obtained from non-food grade microorganisms. *Mursik,* a Kenyan traditional fermented milk product is mainly consumed due to its delicious taste and belief that it enhances immunity against common diseases (Mathara *et al.,* 1995). This is mainly attributed to the effects of the LAB species that are responsible for fermentation of *Mursik*. However, it has not been established whether it contains EPS and/or EPS producing LAB. Therefore, the aim of

this study was to establish whether *Mursik* contains EPS and/or EPS producing LAB. Their presence will significantly have an impact on the functional properties of *Mursik* and its development as a commercial product. It will also provide a new source of EPS producing LAB that can be used for production of other functional food products.

1.3 General objective

To establish production of EPS in a Kenyan traditional fermented milk, *Mursik.*

1.4 Specific objectives

1. To determine production of EPS in *Mursik.*
2. To determine the incubation period at which EPS yield is maximum during traditional fermentation of *Mursik.*
3. To determine the effects of incubation period and growth medium composition on EPS production by the *Mursik* culture.

1.5 Hypotheses

1. *Mursik* fermenting bacteria do not produce EPS during fermentation.
2. Incubation period does not have any significant effects on EPS yield in *Mursik.*
3. Incubation period and composition of the growth medium have no effect on EPS yield by *Mursik* culture.

1.6 Justification

LAB produce various metabolites in milk during fermentation which are responsible for the sensory, nutritive and health beneficial properties in the products. Various studies have shown that EPS produced by some LAB play a significant role in imparting these properties in the fermented milks. Health beneficial properties that have been established for LAB EPS include the ability to improve growth of health beneficial microbiota such as *Bifidobacteria species* in the gut as prebiotics (Korakli *et al.,* 2002), ability to enhance attachment and colonization of the gut by probiotics which antagonizes the effects of pathogenic microorganisms (Welman and Maddox, 2003; Lebeer *et al.,* 2010; Russo *et al.,* 2012). Some LAB EPS enable probiotic LAB to adapt to the gut environment by protecting them against the non-selective gut antimicrobial system, innate immunity (Lebeer *et al.,* 2010) and by enhancing attachment of probiotics to the gut (Welman and Maddox, 2003). LAB EPS have also been shown to induce

immunity against diseases by acting as immunomodulators. Bacterial polysaccharides induce immunological response systems in the gastrointestinal mucosa (O'Connor *et al.,* 2005) and immuno-suppression activities such as increased production of cytokine IL-10 by the macrophages (Bleau *et al.,* 2010). A study by Kim *et al.,* 2010 also established that the cell-bound EPS by *Lactobacillus acidophilus* 606 have ant-tumour activity against colon cancer cells. EPS and/or EPS producing LAB improve the rheological properties of food products and since they are regarded as natural food additives, consumers prefer them over the commercially available plant and algal based additives, especially the chemically modified biothickeners and the EPS obtained from non-food grade microorganisms such as xanthan gum. They improve the texture and other rheological properties such as water holding, gelling and melting properties in the fermented milk products (Awad *et al.,* 2005; Hassan *et al.,* 2008; Badel *et al.,* 2011). *Mursik* is consumed due to its delicious taste and believe that it enhance immunity against common diseases such as diarrhea (Mathara *et al.,* 1995). There no reports on production of EPS or presence of EPS producing LAB in *Mursik*. Therefore, it is not known whether the claimed health benefits are due to presence of EPS or EPS producing LAB. The aim of the study therefore, was to establish whether *Mursik* contains EPS and/or EPS producing LAB in *Mursik*. This study has a significant effect on development and commercialization of Mursik as a functional food product and as source of EPS or EPS producing starter cultures for application in other food products.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Functional fermented milk products

Functional foods are foods that are consumed as part of normal diet but impart health beneficial effects such as improved health and/or ability to resist occurrence of certain diseases, in addition to provision of the intended nourishment (Shih, 2003; Toma and Pokrotnieks, 2006). The belief that fermented milk products are functional foods dates back to olden days including the days of Abraham in the Bible. The concept of fermented milk products being associated with health enhancement to the consumers was first reported in early 1900 by the Eli Metchinkoff, and as a result, he was awarded a Nobel Prize of Medicine in 1908 (Parves *et al.,* 2006; Anukam and Reid, 2007). Metchinkoff hypothesized that the long healthy lives of the Bulgarian peasants was due to regular consumption of fermented milk such as yoghurt which contained bacteria that provided immunity against disease causing microorganisms in the gut (Parves *et al.,* 2006; Anukam and Reid, 2007). The health beneficial bacteria were later discovered to be the Lactic acid bacteria (LAB) species and were later referred to as 'Probiotics' (Parves *et al.,* 2006; Toma and Pokrotnieks, 2006; Anukam and Reid, 2007). 'Probiotics are "Live microorganisms, which when administered in adequate amounts, confer health benefits on the host" (FAO/WHO 2001). *Lactobacillus* and *Bifidobacteria* species are the most commonly used bacteria as probiotics, but other candidates established include some species of yeast such as *Saccharomyces boulardii*, Fungi, and non-LAB bacteria such as *Bacillus species* (Parvez, *et al.,* 2006; Toma and Pokrotnieks, 2006). Another way for health enhancement through modification of the gut microbiota involves the use of prebiotics. These are 'non-digestible food ingredients that stimulate the growth of bifidogenic and LAB in the gastrointestinal tract' (Patel and Goyal, 2012). These include the carbohydrates that escape metabolism and absorption in the upper gut hence end up in the lower gut (colon) where they are utilized by the health beneficial gut microbiota, and as result influence composition and nature of the predominant microbiota in the colon (Kaplan and Hutkins, 2000; Cummings and Macfarlane, 2002; Korakli and Vogel, 2006). According to Korakli and Vogel, (2006) and Macfarlane *et al.,* (2006), non-digestible oligosaccharides such as oligofructose, galactooligosaccharides and Lactulose are the most commonly used prebiotics in functional foods in order to increase the population of the health promoting gut microbiota, mainly *Bifidobacteria* and *Lactobacillus* species. They are mainly

found naturally in plants such as chicory and Jerusalem artichokes (Korakli and Vogel, 2006; Macfarlane *et al.,* 2006) and in human milk (Branca and Rossi, 2002). Similar oligosaccharides, mainly oligofructans are synthesized by some LAB as exopolysaccharides (EPS) (Korakli *et al.,* 2003; Tieking *et al.,* 2003). A study by Korakli *et al.,* (2002) established that *Lactobacillus sanfranciscensis* produced a fructan type of EPS which exhibited prebiotic properties. Presence of EPS and EPS producing LAB in fermented milks such as yoghurt and cheese, especially the low-fat types, are also important since they improve their texture and mouth feel properties (Awad *et al.,* 2005; Hassan, 2008; Badel *et al.,* 2011). Since EPS are naturally synthesized products, consumers prefer them in food over other food additives, mainly plant based thickeners and biothickeners obtained from non-food grade microorganisms such as xanthan gum (Khurana and Kanawijia, 2007; Badel *et al.,* 2011).

2.2 Kenyan Fermented Milk, *Mursik*

In Kenya, traditional fermented milks have been consumed for centuries due to the inherent belief that they promote health and wellbeing, especially among the pastoral communities such as the Maasai, Turkana, Borana, and the Highland Kalenjin (Mathara *et al.,* 1995; Mokua, 2004; Mathara *et al.,* 2008). These include *Kule naoto* (Maasai), *Amabere amururanu* (Gusii), *Iria ri matii* (Meru), *iria imata* (Kikuyu) and *Mursik* (Kalenjin), all of which are a variety of acidified milks that are very similar, (FAO, 1990; Mureithi *et al.,* 2000; Mokua, 2004), but produced by different communities. During their production, no starter cultures are used, but their production technology mostly involve pouring raw or boiled milk into a fermentation container, covering, and incubating under ambient temperatures (22°C - 35°C) for several days (FAO, 1990; Mureithi *et al.,* 2000). Fermentation is achieved by natural cultures in the milk or on the inner surface of the fermentation container. In some communities, the cultured milk is colored and/or flavored with wood charcoal from particular tree species (FAO, 1990; Mathara *et al.,* 1995; Mureithi *et al.,* 2000; Mokua, 2004).

Mursik is Kenyan traditional fermented milk produced by the Kalenjin community, mainly the Kipsigis, Nandi, Tugen, Pokot and Maasai (Mureithi *et al.,* 2000). It is a soft cheese-like product that is made by spontaneous fermentation of raw or boiled (mainly) milk (Mathara *et al.,* 1995; FAO, 1990) in a traditional gourd. According to Mathara *et al.,* (1995), *Mursik* forms a major part of the Kalenjin diet due to its excellent flavor, delicious taste and belief that it improves health. *Mursik* is also used as a special diet for the newly delivered mothers, initiates and invalids because it is believed to make them strong and immune against common diseases

(Mathara *et al.*, 1995). A study by Nakamura *et al.*, (1999), established that the *Mursik* culture is generally composed of *Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides* subsp. *dextranicum, Lactobacillus curvatus, Leuconostoc Paramesenteroides* and *Lb. planturum*, some of which produce EPS (dextran). According to Mathara *et al.*, (1995) some of the LAB strains in *Mursik*, such as *Lactococcus lactis* (formally *Streptococcus Lactis)* have antimicrobial activities against pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Another study by Mathara *et al.*, (2008) established that *Lactobacillus planturum* isolated from *Kule naoto* (similar product made by the Maasai community) had good probiotic properties.

Mursik is produced through spontaneous fermentation of raw or boiled milk in traditionally prepared gourds. The production process involves preparation of the fermentation gourd, by washing with warm water using a special palm stick known as '*sosiot'*, which is made from palm tree branches. It is then dried in the shade for a few hours and then its inner surface is smoked by crushing and rubbing hot charcoal from a burnt wood splint, called '*itet'*. The wood splints are mainly made from special trees/shrubs such as *Senna didymobotrya* ('Senetwet' in Kalenjin), *Dombeya torrida* (Silibwet), *Lantana kitu* (Mwokyot) and *Olea europaea ssp.africana* (Emitiot) (Mureithi *et al.*, 2000; Heidi, 2009). Raw or boiled milk is then poured into the prepared gourd, tightly corked and incubated in a cool and undisturbed place at room temperature for 7-10 days. During fermentation, the milk is occasionally checked for whey formation, which if present, is drained off and the gourd refilled with fresh milk.

Figure 1: Materials used for production of traditional *Mursik*: A; *Senna didymobotrya* plant that is used for smoking *Mursik* fermentation gourds, B; Kalenjin traditional fermentation containers, gourd (referred to as '*Sotet*') that is used for *Mursik* fermentation.

The main aim of applying the charcoal in the gourd is to improve the flavour of *Mursik*, pasteurize the gourd, colour the milk, impart flavour to the milk, absorb the natural milk flavour which is undesirable, preserve the milk and impart the medicinal value to the milk (only when medicinal species are used (Mureithi *et al.,* 2000). Currently, *Mursik* is one of the most widely consumed traditional fermented milk products in Kenya by both Kalenjin and non-Kalenjin communities. It is marketed informally through small food kiosks and in some hotels as part of traditional delicacies.

2.3 Modulation of the Gut Microbiota by prebiotics

The type and population of the microbiota available in the gut greatly influence the health of an individual (Woodmansey, 2007). According to Kolida and Gibson, (2007), there are two main types of fermentations that are carried out in the gut; saccharolytic and proteolytic fermentation. The authors further state that saccharolytic fermentation results in production of short-chain fatty acids, acetate, propionate and butyrate which are utilized by the host for ATP generation, immunity stimulation, and other uses, whereas proteolytic fermentation results in production of phenolic compounds, amines and ammonia, some of which are toxic and carcinogenic to the host's body. The type of fermentation (saccharolytic or proteolytic) which predominates in the gut mainly depends on the type of the substrate available in the gut and the intestinal microbiota involved (Woodmansey, 2007; Van den Broek *et al.,* 2008). This processes can be manipulated by either use of health promoting microorganisms (probiotics) to manipulate the composition of gut microbiota or by use of prebiotic (a specific limiting nutrient) stimulate growth of the existing health promoting microbial species in the gastrointestinal tract (Toma and Pokrotnieks, 2006). The Probiotics antagonize growth and establishment of the detrimental microorganisms in the gut through several mechanisms which include; direct and indirect competition for nutrients, competition for physical attachment sites, production of antimicrobial compounds such as bacteriocins, organic acids and short chain fatty acids, enhancement of host immune system activity or by synergistic interaction of two or more of the above mechanisms (Miles, 2007; Chichlowski, *et al.,*2007; Callaway, *et al.,* 2008). In addition, some oligosaccharides (prebiotics) have been shown to directly hinder pathogenic microorganisms from attaching on to the gut epithelium (Macfarlane, *et al*., 2008).

2.4 Exopolysaccharides

2.4.1 Background

Exopolysaccharides (EPS) are polysaccharides which are synthesized and excreted by certain species of microorganisms on to the surface of their cell wall as a cohesive layer (capsular EPS) or into the growth medium as a loosely attached/un-attached slime (ropy) EPS (Ruas-Madiedo and Reyes-Gavilan, 2005; Kumar *et al.,* 2007). EPS biosynthesis has been reported by a wide variety of microorganisms including moulds, yeasts, bacteria and algae (Korakli and Vogel, 2006; Parves *et al.,* 2006; Kumar *et al.,* 2007). The physiological role of EPS to the excreting microorganisms is not clearly established, but the capsular form is thought to provide protection to the cell against unfavorable environmental conditions such as desiccation, phagocytosis, phage attacks, antibiotic activity and other antibacterial effects (Jolly *et al.,* 2002; Kumar *et al.,* 2007). Depending on when and where they are produced, EPS produced may be desirable or undesirable. Some of the undesirable effects of EPS include formation of adherent biofilms on inert and biological surfaces leading to fouling of pipelines, onset of dental caries (Kumar *et al.,* 2007) and spoilage of products such as soft drinks (Korakli and Vogel, 2006). However, according to Kumar *et al.,* (2007), these biopolymers have extensive applications in several industries including food and pharmaceutical industries. In the food industry, EPS application has been focused as agents for improving rheological properties of fermented food product, mainly as biothickeners, texturizers, stabilizer, or gelling agents, as alternatives for plant and algal polysaccharides (Awad *et al.,* 2005; Doleyres *et al.,* 2005; Hassan, 2008). EPS producing LAB have been shown to improve rheological properties of certain fermented milk products such as yoghurt and cheese (Awad *et al.,* 2005; Hassan, 2008). EPS from LAB are more preferred by consumers unlike other food additives such as the chemically modified plant based thickeners and biothickeners obtained from non-food grade microorganisms such as xanthan gum (Vuyst and Degeest, 1999; Badel *et al.,* 2011). This is mainly because LAB are classified as GRAS (generally regarded as safe) food additives and their EPS are naturally synthesized (Korakli *et al.,* 2003; Badel *et al.,* 2011). Currently, EPS application has expanded into the possibility of developing multifunctional additives to improve both rheological properties of food as well as provide health beneficial properties. Some strains of EPS producing LAB can synthesize EPS which have prebiotic properties such as fructooligosaccharides, (Korakli *et al.,* 2003; Tieking *et al.,* 2003). Certain EPS producing LAB species can produce Fructans, and glucans, types of oligosaccharides such as

fructooligosacchride kestoses and nystoses which have the ability to stimulate growth of *Bifidobacteria* (bifidogenic effects) and other health promoting microorganisms in the gut (Korakli *et al.,* 2003; Tieking *et al.,* 2003; Salazar *et al.,* 2008). Other health beneficial effects associated with LAB EPS include reduction of blood cholesterol levels, ant-carcinogenicity (Ruas-Madiedo *et al.,* 2006), ant-temporal activity and immunomodulation (O'Connor *et al.,* 2005; Ruas-Madiedo and Reyes-Gavilan, 2005; Lebeer *et al.,* 2010; Kim *et al.,* 2010). Apart from being sources of health beneficial EPS, LAB are also regarded as a potential sources of a large variety of highly specific enzymes, *glycosyltransferases*, which can be used to construct specifically tailored prebiotics and compounds for specific applications (Kleerebezem *et al.,* 2002; Korakli and Vogel, 2006; Badel *et al.,* 2011). Through biotechnological techniques such as genetic engineering and metabolic engineering, synthesis of targeted polysaccharides such as those with optimized rheological properties or with certain biological characteristic can be directed (Kleerebezem *et al.,* 2002; Badel *et al.,* 2011). However, the major limitations for exploitation of the EPS from LAB is that very few EPS or EPS producing LAB have been established (Korakli and Vogel, (2006) and their yields are generally low compared to those from non-food grade microorganisms such as *Xanthomonas campestris* (Badel *et al.,* 2011).

2.4.2 Biosynthesis of Exopolysaccharides.

Biosynthesis of EPS closely resembles the process of synthesis of bacterial cell wall polymer such as peptidoglycan, teichoic acid and lipopolysaccharide (Cerning *et al.,* 1992; Cerning, 1995; Ruas-Madiedo and Reyes-Gavilan, 2005; Kumar *et al.,* 2007). Several enzymes are involved in EPS biosynthesis (Degeest and Vuyst, 2000; Welman and Maddox, 2003; Kumar *et al.,* 2007). These enzymes can be classified into four groups as; intracellular enzymes which are involved in phosphorylation of glucose to glucose- 6-phosphate and phosphoglucomutase, and then conversion of glucose-6-phosphate to glucose-1-phosphate; intracellular enzymes such as uridine diphosphate- glucose pyrophosphorylase (UDP-glucose pyrophosphorylase) which catalyze conversion of glucose-1-phosphate to uridine diphosphate glucose (UDP-Gucose), components of the sugar nucleotide which are key molecules for EPS synthesis; *glycosyltransferases* which are involved in transfer of the sugar nucleotides (UDP-Glucose or UDP-Galactose and others) to a repeating unit attached to the glycosyl lipid carrier (isoprenoid alcohol) and the enzymes situated outside the cell membrane and the cell wall which are involved in the polymerization of the EPS (Degeest and Vuyst, 2000; Welman and Maddox, 2003; Kumar *et al.,* 2007; Badel *et al.,* 2011). According to Korakli and Vogel, (2002), synthesis

of homopolysaccharides is mainly catalyzed by specific *glycosyltransferases*, mainly *glucosyltransferases* which catalyze glucans formation and *fructosyltransferases* which catalyze fructan formation. The process of biosyntheses of heteropolysaccharides is complex, and it involves presence of various acceptor molecules and enzymes (Vuyst and Degeest, 1999; Welman and Maddox, 2003; Korakli and Vogel, 2006). EPS yield and quality are greatly affected by the nutritional and environmental conditions in which the microorganism is exposed, and hence increase in yield can be achieved by manipulating these conditions (Zisu and Shah, 2003; Vaningelgem *et al.,* 2004b; Kumar *et al.,* 2007).

2.4.3 Physico-Chemical Properties of the Exopolysaccharides from Lactic Acid Bacteria

EPS from LAB can be classified based on monomer composition as homopolysaccharides and heteropolysaccharides, where the former are composed of a single type of monosaccharide, and the later composed of two or more different types of monosaccharide (Vaningelgem *et al.,* 2004a; Ruas-Madiedo and Reyes-Gavilan, 2005; Korakli and Vogel, 2006). Homopolysaccharides are further classified based on their monomer composition as; α-D-glucans which are mainly composed of α-1, 6 and / or α-1, 3 linked glucose residues (such as *dextans* and *mutans*), β-D-fructans which are mainly composed of β-2, 6-linked fructose molecules (such as *levans*), β-D- glucans and Polygalactans (Vaningelgem *et al.,* 2004a; Ruas-Madiedo and Reyes-Gavilan, 2005). They may have a similar backbone structure but vary in their degrees of branching and linkage sites depending on the producing bacterial strains (Ruas-Madiedo and Reyes-Gavilan, 2005). Some may be oligosaccharides (short chained polysaccharides of 2 – 10 monomers) such as fructooligosaccharides (FOS) and inulin (Tieking *et al.,* 2003; Korakli and Vogel, 2006). Heteropolysaccharides are composed of repeating units, made of between two to eight monomers (Vuyst and Degeest, 1999; Ruas-Madiedo and Reyes-Gavilan, 2005). Most often, heteropolysaccharides are composed of a combination of D-glucose, D-galactose and L-rhamnose (the classical monosaccharide) in different ratios (Ruas-Madiedo and Reyes-Gavilan, 2005; Korakli and Vogel, 2006). Other sugar monomers that may form the heteropolysaccharide structure include L-fructose, N-acetyglucosamine, N-acetylgalactosamine, glucuronic acid, D-ribose, xylose, and non-carbohydrate components such as glycerol, phosphates, pyruvyl and acetyl groups (Vaningelgem *et al.,* 2004a; Ruas-Madiedo and Reyes-Gavilan, 2005). According to Ruas-Madiedo and Reyes-Gavilan, (2005), the physic-chemical characteristics of EPS are mainly determined by their monomer composition, chain length, structure of subunits, molar mass and radius of gyration, and this greatly influence their

functionality in the food systems. The molecular mass of LAB EPS greatly varies between 10 kDa to 200 kDa (Vaningelgem *et al.,* 2004a; Mozzi *et al.,* 2006), whereby heteropolysaccharides are generally smaller (range between 40 to 60 kDa) and homopolysaccharides are larger (Vuyst and Degeest, 1999; Ruas-Madiedo and Reyes-Gavilan, 2005). High molecular mass EPS are mainly excreted into the growth medium (as ropy EPS) and hence are the ones which mainly affect the physical properties such as texture and viscosity of the fermented milks, whereas Low molecular mass EPS are mainly excreted onto the cell wall of the microorganism where they remain attached capsules (Zisu and Shah, 2003; Ruas-Madiedo and Reyes-Gavilan, 2005). Growth medium composition, mainly the nature of nitrogen and carbohydrates sources, and the bacteria strain greatly affect the molecular mass of EPS (Zisu and Shah, 2003; Vaningelgem *et al.,* 2004b).

2.5 Factors which affect Exopolysaccharide yields by Lactic acid bacteria.

2.5.1 Bacteria strain

Production of EPS varies between different LAB species and strains (Zisu and Shah, 2003, Vaningelgem *et al.,* 2004a; Mozzi *et al.,* 2006). Studies by Vaningelgem *et al.,* (2004a) and Mozzi *et al* (2006) established that different LAB species and strains produce different EPS levels as well as EPS with different monomer composition and molecular mass, and this was attributed to the diversity in their genetic ability to synthesize EPS. EPS production is related to the activities of EPS synthesizing enzymes in a given bacteria (Degeets and Vuyst, 2000; Mozzi *et al.,* 2001). EPS biosynthesis involves the activities of several enzymes, EPS specific (*glycosyltranferases*) and non-EPs specific (Housekeeping) enzymes (Vuyst and Degeest, 1999; Welman and Maddox, 2003, Mozzi *et al.,* 2006; Badel *et al.,* 2011). Housekeeping enzymes catalyze production of sugar nucleotides which are used for construction of EPS and other biopolymers required by the cell, such as peptidoglycan, lipoteichoic acid, tecichoic acid and others (Vuyst and Degeest, 1999; Welman and Maddox, 2003). The EPS phenotype is controlled by *eps*-genes which are either located in the plasmid DNA (For most mesophillic LAB) or in chromosomal DNA (in most thermophillic LAB) (Vuyst and Degeest, 1999; Welman and Maddox, 2003; Dapour and Lapointe, 2005). These genes encode for the type of the *glycosyltranferase*s which produce the specific types of EPS in a given microorganism (Welman and Maddox, 2003). Different *glycosyltranferases* catalyze production of different EPS, mainly based on the monomer composition, molecular mass and structure (Welman and Maddox, 2003;

Badel *et al.,* 2011). Therefore, the type of EPS produced depends on the type of EPS enzymes that an organism produces and their concentration (Welman and Maddox, 2003).

2.5.2 Growth medium composition

The yield and composition of EPS produced by LAB are influenced by the composition of the growth medium (Vuyst *et al.,* 1998; Zisu and Shah, 2003; Ruas-Madiedo and Reyes-Gavilan, 2005; Mozzi *et al.,* 2006). The nature and concentration of carbon (sugar) and nitrogen source, as well as other nutrients such as mineral salts, vitamins also affect EPS production by a given LAB (Mozzi *et al.,* 2001; Ricciardi *et al.,* 2002; Zisu and Shah, 2003; Vaningelgem *et al.,* 2004a). A study by Bergmaier *et al* ., (2003) established that whey permeate supplemented with yeast extract, vitamins and salt increased EPS yield by *Lactobacillus rhamnosus* 95995M by 5 to 8%. According to Vuyst *et al.,* (1998), enough nitrogen is required for synthesis of essential elements by the cell during active growth. Complex nitrogen sources such as yeast extract, peptone and whey protein hydrolysates contain several peptides and amino acids which are important for cell growth and EPS production (Zisu and Shah, 2003; Vaningelgem *et al.,* 2004a; Vaningelgem *et al.,* 2004b). According to Vaningelgem *et al.,* (2004b), whey hydrolysates contain peptides and free amino acids which are readily available for utilization by the bacterial cells. Studies have shown that whey permeate, a by-product from the dairy industry can improve EPS yield by certain LAB such as *Lb.rhamnosus* 95995M (Bergmaier *et al.,* 2003 and *Streptococcus thermophilus* (Zisu and Shah, 2003; Vaningelgem *et al.,* 2004b). In addition, these whey protein products were able to improve EPS yields without affecting the rate of cell growth (Zisu and Shah, 2003; Vaningelgem *et al.,* 2004b), implying they contained nutrients that were mainly used for EPS biosynthesis rather than for cell growth (Zisu and Shah, 2003). Addition of other milk proteins in growth media of some LAB has been shown to stimulate capsule formation (Ricciardi *et al.,* 2002; Vaningelgem *et al.,* 2004b). According to Georgalaki *et al.,* (2000), LAB species contain limited abilities to synthesize amino acids which are essential for their growth, whereas milk contains insufficient amounts of these amino acids and low molecular mass peptides. However, LAB species have complex proteolytic systems that aid them to hydrolyze milk proteins to amino acids and low molecular mass peptides (Georgalaki *et al.,* 2000; Zisu and Shah, 2003). Therefore, increased milk protein in the growth medium improves EPS production (Zisu and Shah, 2003; Vuyst *et al.,* 1998).

The EPS yield by a particular LAB strain is also greatly affected by the nature of carbon source (sugar) in the growth medium (Looijesteijn *et al.,* 1999; Ruas-Madiedo and Reyes-

Gavilan, 2005; Vaningelgem *et al.,* 2004a). Looijesteijn, *et al.,* (1999) established that EPS yield by *Lactobacillus delbrueckii bulgaricus*, increased up to three times when it was grown on glucose compared to fructose. *Lb.casei, Lb. rhamnosus,* and *Streptococcus thermophilus* have been shown to produce different EPS yield when grown in different sugar sources (Degeest and Vuyst, 2000; Mozzi *et al.,* 2001). According to Degeest and Vuyst (2000), some LAB strains are not able to utilize certain carbon sources for EPS biosynthesis due to their genetic potentials. However, the best carbon source for optimum EPS production mainly depends on the strain involved (Looijesteijn *et al.,* 1999; Ruas-Madiedo and Reyes-Gavilan, 2005). This is because, the strain determines the type of carbon to utilize based on the available EPS synthesizing genes it contains (Degeest and Vuyst, 2000; Welman and Maddox, 2003; Mozzi *et al.,* 2006). Sugars are very important for both bacteria growth as well as for synthesis of sugar nucleotides, which are important precursors for EPS production (Welman and Maddox, 2003). Therefore, according to Vuyst *et al.,* (1998), it is important to maintain a balanced carbon/nitrogen ration in a medium in order to optimize EPS yield by a given strain.

2.5.3 Growth condition

Growth temperature, Oxygen potential and pH requirements vary with the EPS producing LAB (Vuyst *et al.,* 1998; Vaningelgem *et al.,* 2004b). Every bacteria species and strain has its minimum, optimum and maximum temperature and pH for growth. According to Degeest *et al.,* (2001), LAB may produce EPS as a primary or secondary metabolite. As a primary metabolite, EPS production is growth associated whereby it starts simultaneously with the bacteria growth, and maximum levels occurs mainly at/or towards the end exponential phase (Degeest *et al.,* 2001; Vuyst *et al.,* 1998). In growth associated EPS production, optimum EPS yields are obtained when the LAB are grown at their optimum growth conditions such as temperature, pH, oxygen potential and in optimum growth media (Vuyst *et al.,* 1998; Vuyst and Degeest, 1999; Degeest *et al.,* 2001; Zisu and Shah, 2003). Growth associated EPS production has been reported mainly in thermophillic LAB such as *Streptococcus thermophilus* (Vuyst *et al.,* 1998; Zisu and Shah, 2003), and as a secondary metabolite, EPS production is not growth associated, hence it occurs mainly when the rate of bacteria growth is minimum (Vuyst and Degeest, 1999; Cerning, 19995; Degeest *et al.,* 2001). Non-growth associated EPS production has been reported mainly in mesophillic LAB (Vuyst and Degeest, 1999; Cerning, 19995). This mainly because in these bacteria EPS biosynthesis competes with other cellular metabolic activities for growth factors such as sugar metabolites and enzymes. Therefore, optimum EPS yields occurs when they are

propagated at conditions which do not support bacteria growth such as sub-optimal growth conditions such temperature, pH and growth media that don't favour their growth (Cerning, 1995; Vuyst and Degeest, 1999; Degeest *et al.,* 2001). EPS biosynthesis in mesophillic LAB is similar to biosynthesis of cell wall polymers such as peptidoglycan and lipoteichoic acid (Cerning, 1995; Vuyst and Degeest, 1999; Badel *et al.,* 2011). They both require sugar nucleotides, similar enzymes, sugar nucleotides and isoprenoid lipid carrier (Cerning, 19995, Vuyst and Degeest, 1999; Badel *et al.,* 2011). During active cell growth, there is a high demand for cell wall polymers, hence the sugar nucleotides, enzymes and isoprenoid lipid carrier are mainly diverted towards biosynthesis of cell wall polymer than towards EPS biosynthesis and vice versa (Vuyst and Degeets, 1999; Degeest *et al.,* 2001; Welman and Maddox, 2003; Badel *et al.,* 2011). Based on this, mesophillic LAB produce higher EPS yields in conditions which do not favour bacterial growth such as sub-optimal growth temperatures and pH since at those conditions, the demand for cell wall polymers is low hence most of the sugar nucleotides and enzymes are available for EPS biosynthesis (Cerning, 1995; Vuyst and Degeest, 1999; Degeest *et al.,* 2001; Badel *et al.,* 2011). According to Vuyst and Degeest (1999) and Degeest *et al.,* (2001) optimum EPS yields by most LAB are obtained at close to pH 6. In addition, it has been shown that EPS yields are generally higher in pH-controlled systems than in un-controlled systems (Zisu and Shah, 2003; Vaningelgem *et al.,* 2004b). According to Degeest *et al.,* (2001), varying conditions in the culture during fermentation results in variation in EPS yields and composition, this is mainly because the changes result in different sugar activation and inter-conversion enzymes, which results in switching on or off certain enzymatic activities (Degeest *at al.,* 2001).

2.5.4 Presence of adjunct culture

Some non-EPS producing LAB can improve EPS yields by some EPS producing species/strains. A study by Zisu and Shah (2003) established that *Streptococcus thermophilus* 1275 produced higher EPS yields when it was grown with a non-EPS producing variant of *Streptococcus thermophilus* than when it was grown alone. This was attributed the results to the mutual relationship that may have existed between the strains, in which the non-EPS producing variants may have been a source of stimulatory metabolites such as amino acids and peptides to the EPS producing strain, which improved its growth and EPS production (Zisu and Shah, 2003).

CHAPTER 3
3.0 MATERIALS AND METHODS

3.1 Study site

The study was carried out in Njoro District, Kenya. Njoro District is found within Nakuru County in the Rift valley region, bordering the complex Mau Forest on the Eastern edge, between Longitudes 35° 45′ and 35° 46′ (East) and Latitudes 0° 16′ and 1° 10′ (South). Samples of *Mursik* were collected from randomly selected homesteads in three locations, namely Mauche, Njoro and Mukungugu Divisions, where the Kalenjin communities are mainly settled. The analyses were carried out at the Department of Dairy and Food science and Technology (Dairy chemistry and Dairy microbiology Laboratories), Egerton University, Njoro, and at Kenya Agricultural Research Institute (KARI), Njoro Station (Biotechnology Laboratory).

3.2 Study Survey

Prior to sampling the *Mursik*, a study survey was carried to establish the traditional techniques used for *Mursik* production in Njoro District. Thirty (30) randomly selected Kalenjin homesteads in Mauche, Njoro and Mukungugu Divisions, were contacted during the survey. The survey was conducted using questionnaires in which the interviewees responded by filling, or being assisted to fill by an assistant. During the study survey, households from where *Mursik* samples were to be collected were also identified.

3.3 Sampling.

Mursik samples were collected from 39 randomly selected households. Prior to sampling, the household owners were requested to prepare the *Mursik* traditionally so as to be ready after three days and sampling was done on the fourth day. One liter *Mursik* samples were collected from every household using sterile screw capped bottles (Figure 2), and 40 ml sub-sample was drawn from each into 100 ml sterile screw caped bottles for microbiological analysis. The samples were then transported in a cool box to Egerton University, Department of Dairy and Food Science and Technology, in within 2 hours. They were analyzed immediately or stored at 4 - 5 °C for not more than 24 hours before analysis.

Figure 2: Collection of *Mursik* samples at Lawina Village (Njoro Division); (a) Homogenization of the *Mursik* prior to sampling (b) Sample collection (c) One liter samples of *Mursik* collected from different households

3.4 Experimental design

The experiment was carried out in a Completely Randomized Design (CRD). Samples of *Mursik* were randomly collected from thirty nine households in Njoro District. The samples were analyzed for Exopolysaccharides (EPS) yields and LAB counts. EPS producing LAB were also screened from the *Mursik* samples. The effect of the growth media composition and Incubation period on EPS production by *Mursik* culture was studied by inoculating the culture on four different growth media (Reconstituted Skim milk (RSM), RSM with 5% sucrose, Whey, Whey with 5% sucrose) and incubating at 20± 1ºC for 30 hours. EPS yields were determined in each growth medium after every 6 hours (after 6, 12, 18, 24 and 30 hours). Mesophillic milk culture was used as a control and was subjected to similar treatments as the *Mursik* Culture. The data obtained was used to compute for Analysis of Variance (ANOVA) using SAS statistical package (SAS system for windows, v.6.12, USA) and least significant difference (LSD) test was used to determine whether there were significant differences between the treatments at 5 % level of significance.

3.5 Experiment 1: Production of Exopolysaccharides in *Mursik*.

3.5.1 Enumeration of Lactic acid bacteria in *Mursik.*

Lactic acid bacteria (LAB) in *Mursik* were enumerated using M17 agar (Himedia) for *Lactococcus* and MRS agar (Himedia) for *Lactobacillus* and *Leuconostoc*. Briefly, each *Mursik* sample was shaken vigorously for 1 minute to homogenize, and then 10 ml was drawn and transferred into 90 ml 2% Sterile Bacteriological peptone (Himedia) solution to make 10^{-1} dilution. From the10^{-1} dilution, Serial dilutions were further made by transferring 1 ml sample into 9 ml 2% sterile bacteriological peptone solution subsequently up to 10^{-8} dilution level. The last three dilutions (10^{-6}, 10^{-7} and 10^{-8}) were propagated to enumerate LAB using pour plate method. In brief, I ml sample was transferred from each dilution into sterile Petri-dishes in duplicate, then the appropriate selective media was added at 45 ºC (molten), allowed to solidify for 5 to 10 minutes and then incubated appropriately. Both *Lactobacillus* and *Lactococcus* species were enumerated in each sample. M17 agar plates were incubated aerobically at 30 °C for 48 -72 hours whereas MRS agar plates were incubated in 3.5 Liter anaerobic jars each with a 3.5 Liter anaerobic gas production kit (AnaeroGas Pack, Himedia) at 35 °C for 48 – 96 hours. The LAB counts were determined by counting the colony forming units (cfu) in the plates with between 30 and 300 cfu. Average counts of the duplicate plates were computed and each multiplied by the reciprocal of its dilution factor to obtain the LAB counts in colony forming units per milliliter (cfu/ml). The LAB counts (cfu/ml) were then converted into Logarithm_{10} values (Log cfu/ml) for convenience during data analysis.

3.5.2 Isolation of Exopolysaccharides from *Mursik*.

Exopolysaccharides (EPS) were isolated from the *Mursik* samples as described by Adebayo-tayo *et al.,* (2011) with a few modifications. Each *Mursik* sample was first homogenized in the high speed homogenizer (Janke and Kunkel, Ultra-Turrax) for 1 minute and then 50 ml samples were transferred into clean centrifuge tubes in duplicate. To each 50 ml sample, 17% TCA (80% m/v) (Lobachemie, 99% purity) was added, mixed and allowed to stand for 20 minutes to precipitate the protein. They were then centrifuged at 4930 x g (mLw T23D centrifuge, radius = 9cm, 7000 revolutions per minute) for 20 minutes. Clear supernatant was extracted from the centrifuged samples by decantation into clean beakers and then EPS isolated by two-step alcohol precipitation method as follows; to the clear supernatant, twice the volume (100 ml) of chilled absolute ethanol (Scharlau, Ethanol analytical grade, Spain) at -10 °C or

below was added and then kept in the fridge at 4 ºC for overnight (12 - 16 hours). The mixture was then centrifuged at 4930 x g for 20 minutes. The EPS extract (precipitate) was obtained by carefully decanting off the supernatant. The EPS extract was re-dissolved in 5 ml distilled water and then the EPS extraction process repeated as described in step one above (using 17 % TCA, chilled absolute ethanol and centrifugation at 4930 x g for 20 minutes). Finally, the EPS extract was dried at 45 ± 2 °C for 48 hours and quantified by phenol sulphuric acid method as described in section 3.5.3.

3.5.3 Quantification of the Exopolysaccharide Extracts.

The EPS extracts from the *Mursik* samples (in part 3.5.2) were analyzed for total carbohydrate content using Phenol-sulphuric acid method as follows; 1 ml of 5% phenol (Panreac, analytical grade, Spain) solution was added to each EPS extract, followed by addition of 5 ml fast running concentrated sulphuric acid (using a pipette with a cut tip to ensure immediate mixing). The solution was allowed to stand at room temperature for 10 minutes, then shaken and incubated at 30 °C for 20 minutes. The carbohydrate content was determined by immediately measuring the absorbance of the samples at 490 nm in a UV-Visible Spectrophotometer (Thermo-Scientific, *Evolution 300^{TM} UV-Vis)* with a Vision pro software (Thermo-Scientific, Vision *pro*^{TM} software) and interpreting using a glucose standard calibration curve with a linearity of 0.99. The amount of EPS in every sample was expressed as milligrams per liter glucose equivalent (EPS mg/L Gluc Equiv).

3.5.4 Screening for Exopolysaccharides Producing Lactic acid bacteria from *Mursik*.

The LAB from *Mursik,* that had been cultured in the selective LAB agar plates (M17, and MRS) in section 3.5.1 were screened for EPS production as follows; For every sample, 5 - 6 LAB colonies were randomly selected from the selective agar plates, each purified by streaking on its original selective agar (M17 and MRS agar) and incubating as earlier done. The pure isolates were first confirmed to be LAB by catalase, cytochrome-*e*-oxidase and gram stain tests as follows; catalase activity test was done by picking a small amount of purified bacteria from a pure plate culture using a sterile wire loop, smearing on a clean glass slide, and flooding with 4% hydrogen peroxide. The flooded culture was observed for production of gas bubbles (Barrow and Felthman, 1993). The bacteria which produced gas bubbles were catalase positive and those which did not produce gas were catalase negative. Presence of Cytochrome-*e*–oxidase was tested using Tetra methyl-*p*-phenylenediamine dihydrochloride using the method described by Barrow

and Felthman, (1993). Oxidase positive bacteria produced a blue black colour when smeared on a filter paper soaked with methyl-*p*-phenylenediamine dihydrochloride reagent, whereas the oxidase negative bacteria did not change colour. Gram stain test was done by staining each isolate with ammonium Oxalate-Crystal Violet stain (Primary) and Safranin (counter stain) as described by Barrow and Felthman, (1993). The stained bacteria were observed in a microscope, using the immersion oil objective (x 100). Gram positive isolates appeared purple/deep blue coloured (primary stain colour), whereas gram negative isolates appeared red (counter stain colour). Therefore, Catalase negative, Cytochrome-*e*-oxidase negative, and gram positive test confirmed that the bacteria isolates were LAB. Also by gram stain, the LAB was characterized based on cell morphology (cell shape) and cell arrangement. The LAB which appeared ovoid (spherical) were referred to as cocci and those that appeared rectangular shaped were rods. The cocci and the rods were characterized as single, paired or chained cells based on their cell arrangements. The confirmed LAB were then screened for EPS production by streaking on Sucrose Hiveg agar (Himedia) and incubating at their optimum growth temperatures as earlier done. EPS positive LAB developed large or small mucoid (Figure 3) and/ or ropy colonies on the sucrose agar (Barrow and Felthman, 1993, Ruas-Madiedo *et al.,* 2005). The EPS positive LAB were streaked on the appropriate agar (M17 or MRS agar) and further characterized by phenotypic and biochemical tests as shown in section 3.5.5.

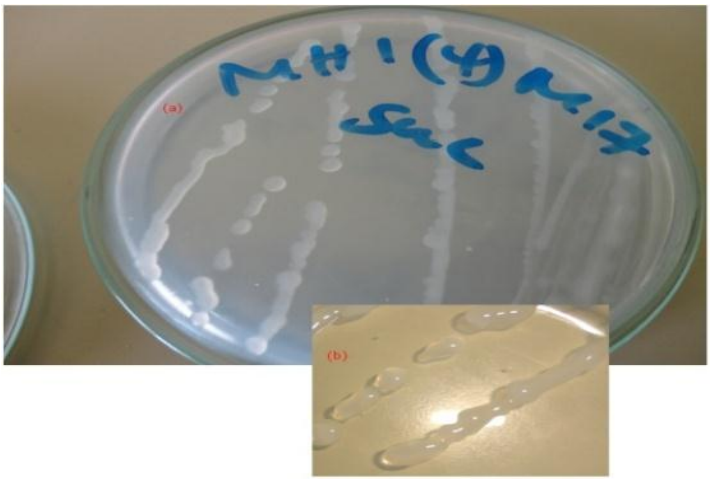


Figure 3: Mucoid colonies for an EPS positive LAB (MH1 (4) M17) Isolated from *Mursik* when grown on Sucrose agar

3.5.5 Characterization of the Exopolysaccharide Producing Lactic acid Bacteria Isolates from *Mursik*.

The EPS producing LAB obtained from *Mursik* were characterized by phenotypic and biochemical tests to the genera and species level where possible as follows;

3.5.5.1 Production of gas in MRS broth.

Each isolate was transferred into MRS broth with inverted Durham tubes as described by Beukes *et al.,* (2001) and Coeuret *et al.,* (2003), and incubated at their optimum growth temperatures. Gas positive LAB (Heterofermentative) produced gas which partially or fully displaced the broth from the Durham tubes whereas gas negative (Homofermentative) LAB did not displace the broth in the Durham tubes.

3.5.5.2 Tolerance to Sodium chloride (Salt).

Salt tolerance was tested by streaking the bacteria on M17 or MRS agar (*Lactococcus* or *Lactobacillus* respectively) with 4 and 6.5 % sodium chloride, NaCl (Rankem, AR) using the method described by Thamaraj and Shah (2003). The LAB grew (colonies) on the agar with the salt concentration that it tolerated.

3.5.5.3 Temperature Tolerance.

This was done using the method described by Beukes *et al.,* (2001) and Coeuret *et al.,* (2003. *Lactococcus* species (M17 isolates) were tested by growing in M17 broth at 10, 30, 40 and 45 ºC for 48 hours (aerobically), whereas *Lactobacillus* species were tested by growing in MRS broth at 35 and 45 ºC for 48 hours (anaerobically). The LAB grew at the temperatures they tolerated and that was indicated by cloudiness of the broth after incubation. Negative tolerance was indicated by clear appearance of the broth.

3.5.5.4 Arginine hydrolysis test.

Arginine hydrolysis test was done by growing the LAB in Arginine Dihydrolase Hiveg™ Broth (Himedia) followed by confirmation by Nessler's test using the method described by Barrow and Felthman, (1993). Arginine positive LAB grew and changed the colour of the Arginine Dihydrolase Broth from clear red to purple whereas the negative bacteria did not change the colour (Figure 4(a)). In nessler's test, arginine positive isolates were indicated by development of a brown colour upon addition of nessler's reagent to the test organism solution (Figure 4(b)).

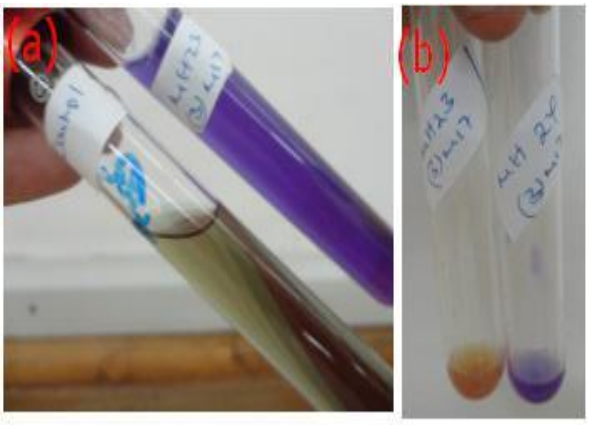


Figure 4: Arginine dihydrolase test reactions by Lactic acid bacteria isolated from *Mursik* (a) Arginine dihydrolase negative (lower brown-red tube) and positive test (upper purple test tube) when grown on arginine dihydrolase broth. (b) Nessler's Positive (Brown) and negative (purple) tests**.**

3.5.5.5 Vancomycin resistance test

This was done by streaking each isolate on M17 or MRS agar (for *Lactococcus* and *Lactobacillus/Leuconostoc* isolates respectively) and then placing a 30 micro-gram (mcg) Vancomycin disc (SD045-5CT, Himedia) on the agar where the culture was heavily streaked. The streaked plates were incubated as follows; M17 isolates at 30 ºC aerobically and MRS isolates at 35 ºC anaerobically for 24 - 48 hours. The cultures were observed for presence of inhibition zones around the 30 mcg vancomycin discs. Vancomycin resistant isolates did not have any inhibition zone around the discs whereas susceptible isolates had inhibition zones (Figure 5).

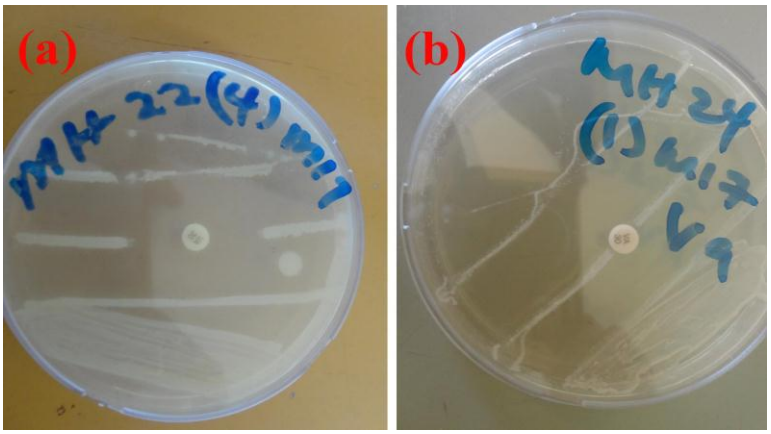


Figure 5: A Vancomycin disc **(**30 microgram, mcg) placed in the agar plate (white circular disc) that is streaked with the EPS producing LAB from *Mursik*; (a) A Vancomycin Susceptible LAB; (b) A Vancomycin resistant LAB.

3.5.5.6 Litmus milk Test.

This was done by growing the test organisms in litmus milk using the method described by Barrow and Felthman, (1993). The Litmus milk was prepared by dissolving 100 g of skim milk powder (New KCC, Kenya) and 750 mg of Litmus powder (Medispan) in 1000 ml distilled water. The litmus milk was then distributed into test tubes, 10 ml each, sterilized at 121° C for 15 minutes, and cooled to ambient temperature. Active test organisms (M17 and MRS Isolates) from 24 hour culture broths were each inoculated into Litmus milk tubes in duplicate using a sterile wire loop and incubated as follows; M17 Isolates were incubated aerobically at 30 °C and MRS isolates incubated anaerobically at 35°C for up to 14 days. The cultures were examined on daily basis to establish the various reactions (changes) in the Litmus milk tubes (Figure 6). The changes (test reactions) were interpreted as follows; Pink colour indicated Acid production (A); Blue colour indicated Alkali production (Alk); White colour indicated reduction of the litmus indicator (R); Presences of clot (C) indicated coagulum formation; and No Change (NC) of the litmus milk indicated negative litmus test (original blue colour retained).

Figure 6: Different Litmus test reactions by the EPS producing Lactic acid bacteria isolated from *Mursik* **(**a) Acid, Reduction and Coagulum formation (ARC) (b) ARC reaction and a negative control (No Change) (c) Various Litmus test reactions by different EPS producing LAB Isolates from *Mursik*.

3.5.6 Isolation and Quantification of Exopolysaccharides from the Exopolysaccharide Producing Lactic acid bacteria obtained from *Mursik*.

Whey was used as the growth medium for testing EPS production by the EPS positive LAB isolates obtained from *Mursik*. This was done using the method described by Adebayo-Tayo *et al.,* (2011) with a few modifications as follows;

3.5.6.1 Preparation of the Whey Medium.

Sweet cheese whey was prepared at Guildford Dairy institute (GDI), Department of Dairy and Food Science and Technology, Egerton University, by following the GDI procedures for Gouda cheese manufacture. Good quality raw milk, meeting GDI minimum acceptable quality specifications was used. This was milk that was free from objectionable odours (Organoleptic test), alcohol negative (72% ethanol) and with specific density of 1.028 to 1.032, based on lactometer test, was selected. The milk was tested for presence of inhibitory substances by starter culture activity test as follows; 100 ml milk was boiled in a beaker, cooled to 45 °C, inoculated with 2% thermophillic starter culture for yoghurt (YF-811, Chr Hansen) and incubated at 45 ºC for three hours. Titratable acidity (T.A) was monitored from 0 to 3 hours to establish whether there was inhibition of growth of starter culture bacteria. Increase in titratable acidity from 0.18 % lactic acid (L.A) to 0.4 % L.A (and above) in three hours indicated absence of inhibitory substances. The milk was then pasteurized at 63 °C /30 minutes and cooled to 30 - 32 °C. Active Mesophillic starter culture (CH-22, Chr Hansen) was added at the rate of 2% and ripened for 30 minutes. Rennet (CHY-MAX® Powder Extra NB, Chr Hansen) was added to the milk as per the manufactures instructions (3 g/Liter milk) and allowed to set for 45 minutes. The curd was cut into 5 mm cubes, allowed to heal for 10 minutes and then the whey/curd mixture (Figure 7$_{(a)}$) were gently stirred while raising the temperature gradually to 35 - 38 °C in within 30 – 60 minutes. The curds were separated from whey and filtered using muslin clothe followed by centrifugation at 4930 x g for 15 minutes to obtain clear whey (Figure 7$_{(b)}$). The whey was distributed into falcon tubes, each 90 ml (Figure 7$_{(c)}$) and sterilized in the autoclave at 121 °C for 15 minutes. They were then cooled to room temperature after which they inoculated with the EPS producing LAB cultures.

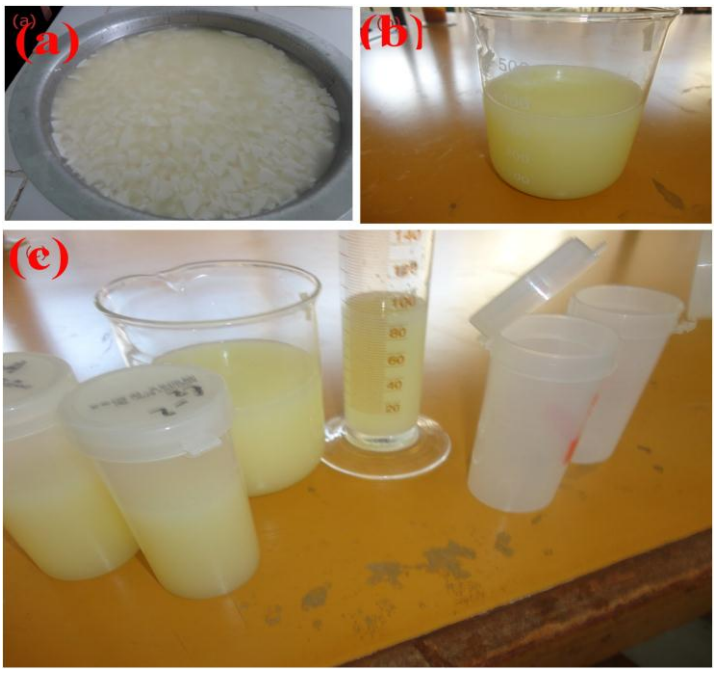


Figure 7: Whey medium used for testing Exopolysaccharide production by EPS producing LAB isolated from *Mursik*; (a)Whey-curd mixture after curd formation and cutting; (b) Clear whey obtained after separation of curd; (c) Distribution of whey into falcon tubes (each 90ml) before sterilization.

3.5.6.2 Preparation of the Exopolysaccharide producing Lactic acid bacteria Cultures.

Prior to inoculation of the EPS producing LAB cultures into the whey media, they were prepared according to the method described by Adebayo-Tayo and Onilude (2008) with a few modifications (Figure 8). The method was slightly modified by using MRS broth instead of Modified Exopolysaccharide Selective Medium as the growth medium for the inoculums was used. The isolates were first reactivated by streaking on M17 and MRS agar (for M17 and MRS isolates respectively) and incubated aerobically at 30 °C for 48 hours (M17 isolates) and anaerobically (in the 3.5 liter anaerobic jar each with a 3.5 Liter anaerobic gas production kit) at 35 °C for 48 hours (MRS isolates). From the M17 and MRS agar plates, each culture was transferred to 10 ml MRS broth using a sterile wire loop and incubated at 30 °C (aerobically) and at 35 °C (anaerobically) respectively for 24 hours. From the 24 hour MRS broth culture (both M17 and MRS Isolates), one loop-full of each culture was transferred into another 10 ml MRS broth (2^{nd} MRS broth) and incubated as above, after which they were ready for use as inoculums in the whey media.

3.5.6.3 Propagation of the Exopolysaccharide producing Lactic acid bacteria Isolates in Whey.

Each 10 ml MRS broth cultures (2nd MRS broth) prepared in section 3.5.6.2 above were wholly transferred into 90 ml whey medium (Figure 8) and incubated aerobically at 30 °C and 35 °C (M17 and MRS isolates respectively) for 30 hours. Immediately after incubation, the cultures were put in a water bath with boiling water for 10 minutes to inactivate potential polymer degrading enzymes, and then cooled to room temperature before EPS isolation.

3.5.6.4 Isolation and Quantification of the Exopolysaccharides from the Whey cultures.

EPS were isolated from each whey culture (Figure 8) as described by Adebayo-Tayo *et al.,* (2011). Briefly, 10 ml samples from each culture were transferred from a well mixed whey culture into 100 ml falcon tubes in duplicate. Proteins and cells were precipitated by adding 17 % TCA (80 % w/v), allowed to stand for 20 minutes, and then centrifuged at 4930 x g for 20 minutes. A clear supernatant was obtained by decanting the clear liquid from the centrifuged sample. EPS was precipitated from the supernatant by addition of two volumes (20 ml) of chilled ethanol (Scharlua, AR Spain) at below -10 °C and allowed to precipitate in the fridge at 4 °C for overnight. The EPS extract was harvested by centrifuging the ethanol-EPS extract mixture at 13000 x g (Eppendorf Centrifuge, 5804) for 25 minutes. The extract was obtained by carefully decanting off the clear liquid (supernatant) and then drying the EPS extract at 45 °C in a hot air oven for 48 hours. The EPS was quantified by Phenol-Sulphuric acid method as earlier described in section 3.5.3.

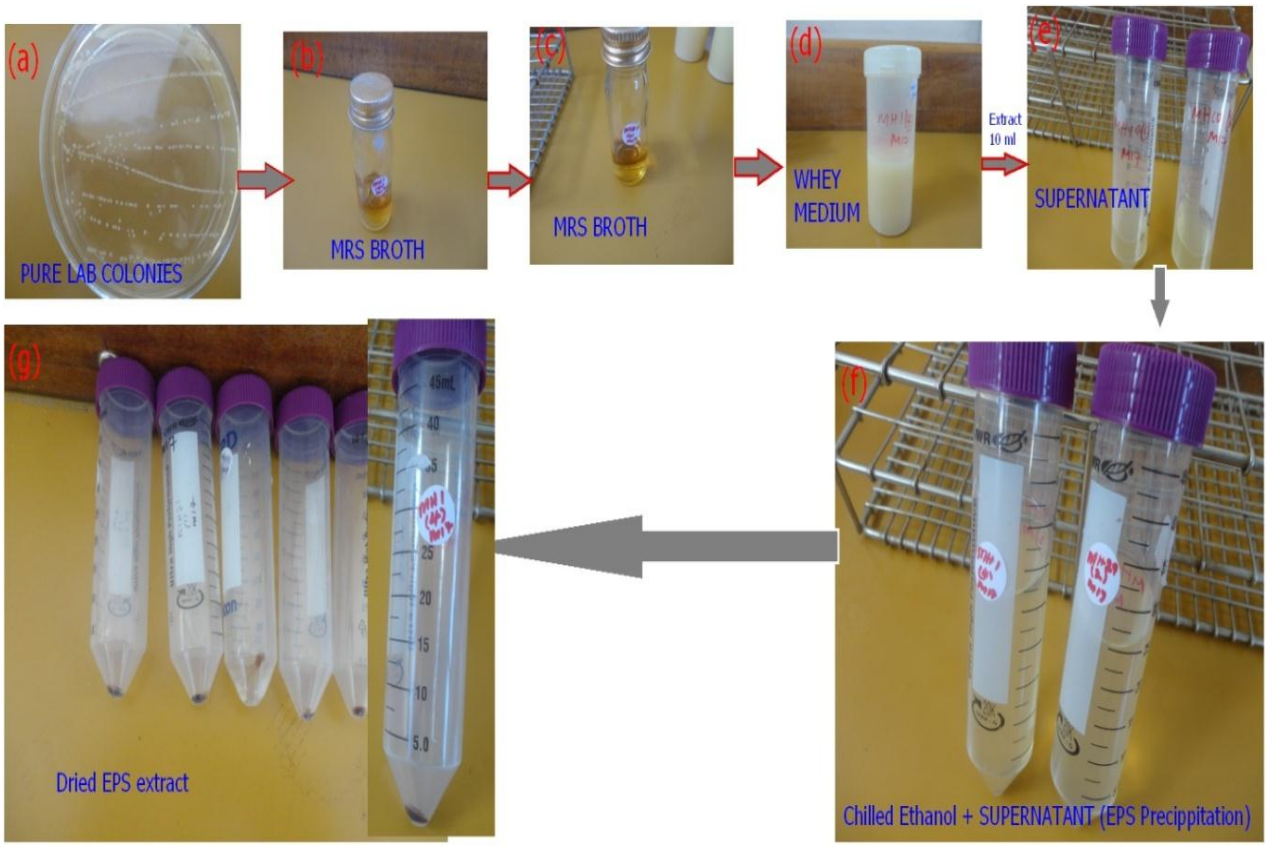


Figure 8: Flowchart for Isolation of Exopolysaccharides from the EPS Producing LAB obtained from *Mursik*: (a) EPS positive LAB streaked on M17/MRS agar; (b) Transfer of the EPS positive LAB into MRS broth; (c) Transfer of a loop-full culture from the MRS broth into 10 ml MRS broth; (d) Transfer all of the 10 ml MRS broth culture into 90 ml Whey (e) Supernatant obtained from 10 ml whey culture after protein precipitating and centrifugation (f) EPS precipitation from the cell/protein free supernatant; (g) EPS extract obtained after alcohol precipitation, centrifugation and drying.

3.5.7 Preservation and Storage of Exopolysaccharide Producing Lactic acid bacteria.

The EPS producing LAB from *Mursik* were preserved by freezing in MRS broth with 20% glycerol at - 18 to - 20 ºC. To avoid loss of the isolates, they were reactivated and preserved after every 6 months. Short term preservation was done using Litmus milk at 2 - 4 °C with monthly reactivation and repeated preservation.

3.6 Experiment 2: Effect of Incubation Period on Exopolysaccharides Production in *Mursik* during Traditional Fermentation Process.

Three gourds were obtained from three households in Lawina Village, Njoro District. The gourds selected were those that had been ripened for *Mursik* preparation and were being used regularly for serving fresh milk and for fermentation of *Mursik* in the homesteads. The milk for fermentation was obtained from GDI. Good quality raw milk, meeting GDI minimum acceptable quality specifications as earlier described in section 3.5.6.1 was selected and tested for presence of inhibitory substances by starter culture activity test (section 3.5.6.1). The milk was used for preparation of traditional *Mursik* as described in section 3.6.1.

3.6.5 Traditional *Mursik* fermentation.

Fermentation gourds were first cleaned and dried a day before *Mursik* preparation and then applied burning charcoal fines from burned dry stems of *Senna didymobotrya* species (*senetwet*) using a bow shaped palm branch (*sosiot*). It was then allowed to cool for 4 hours before filling the milk. The milk was heated (to the boiling point), cooled to 20 ± 1 °C and then filled into the gourds. The gourds were properly corked, labeled (G1, G2 and G3) and incubated at room temperature, in a box placed in cool and undisturbed place in GDI dairy pilot plant. During fermentation, the milk was sampled daily (every 24 hours) for 5 days and examined for Titratable acidity, pH, Temperature, Lactic acid bacteria (*Lactobacillus* and *Lactococcus*) counts and EPS isolation and quantification.

3.6.5.1 Determination of Titratable Acidity, Temperature and pH of *Mursik* during incubation.

(i) Titratable acidity determination.

Titratable acidity (T.A) was determined by titrating 9 ml of a well mixed *Mursik* sample with 0.1 Normal Sodium Hydroxide (0.1N NaOH) using 3 - 4 drops of 1% phenolphthalein indicator solution to a permanent faint pink colour. The amount of titer that was utilized to get the permanent faint pink colour was determined. T.A for the sample was computed as follows; Amount of titer x 0.1 = T.A (% Lactic acid).

(ii) Temperature and pH determination.

This was examined using a milk analyzer (Lactoscan MCC30, Bulgaria) as per the manufacture's procedure.

3.6.5.2 Enumeration of Lactic acid bacteria in *Mursik* during Incubation.

The LAB (*Lactococcus* and *Lactobacillus*) were enumerated using LAB selective media, MRS Agar for *Lactobacillus and Leuconostoc species* and M17 agar for *Lactococcus species* by pour plate method as earlier described in section 3.5.1.

3.6.5.3 Exopolysaccharide isolation and Quantification in *Mursik* during Incubation.

Production of EPS was monitored on daily basis by isolation and quantification as follows; a sample of 25 ml was drawn from each gourd (treatment) after every 24 hours and immediately put in a water bath with boiling water for 10 minutes. They were cooled to room temperature and then 10 ml sample transferred from each sample into 100 ml falcon tubes in duplicate. EPS was isolated and quantified as earlier described in section 3.5.6.4 and 3.5.3 respectively.

3.7 Experiment 3: Effect of Growth Medium and Incubation time on EPS Production by *Mursik* culture.

The Kenyan fermented milk (*Mursik*) culture was propagated in four different growth media namely, whey, whey with 5% sucrose, Reconstituted skim milk and Reconstituted skim milk with 5% sucrose (Table 1). Mesophillic fermented milk culture was used as a control and hence was propagated in the same media as the *Mursik* culture. After propagation, EPS yield from the different growth media were determined at 5 different incubation periods, after every 6 hours of incubation (6, 12, 18, 24 and 30 hours) at 20 ± 1 ºC. The growth media were prepared and inoculated as shown in section 3.7.1.

Table 1: The composition the growth media that were used to test EPS production by *Mursik* culture.

Growth Medium (Treatment)	Code	Composition
1. Whey	WH	Whey only
2. Whey-Sucrose	WH-S	Whey + Sucrose (50g sucrose per liter of whey)
3. Reconstituted Skim milk	RSM	12.5% Skim milk powder dissolved in distilled water
4. Reconstituted skim milk –Sucrose	RSM-S	12.5% Skim milk powder dissolved in distilled water + Sucrose (50g sucrose per liter of RSM)

3.7.1 Growth Media Preparation

Whey and Reconstituted Skim milk media were prepared as follows;

3.7.1.1 Whey and Whey-sucrose media

Whey for the media preparation was a by-product from Gouda cheese processing at GDI dairy pilot plant prepared as described earlier in section 3.5. 6.1. The whey was divided into two portions. The first portion was added 5% sucrose (50g / Liter) to make Whey-sucrose (WH-S) medium and the second portion remained as plain whey (WH) medium. For each medium, 200 ml were distributed into 500 ml screw capped bottles and sterilized at 121 °C for 15 minutes. They sterilized media were allowed to cool to room temperature before being inoculated with the cultures.

3.7.1.2 Reconstituted skim milk and Reconstituted skim milk-Sucrose media.

Reconstituted skim milk (RSM) was prepared by dissolving 12.5% skim milk powder (New K.C.C, Kenya) in warm distilled water at 45 °C. It was divided into two portions. One portion was added 5 % Sucrose (50g/Liter) to make Reconstituted skim milk with sucrose (RSM-S) whereas the second portion remained as plain RSM. Each medium was distributed into 500 ml screw capped bottles, each 200 ml, sterilized at 121 °C for 15 minutes and cooled to room temperature, after which they were ready for inoculation.

3.7.2 Inoculation of the culture into the growth media.

Three days old *Mursik* culture was obtained from a house hold in Belbur Village, Njoro District. It was kept in the fridge at 4 °C up to the next day when it was used for the analysis. The culture *Mursik* culture had 8.72 Log cfu/ml *Lactococcus* and 8.39 Log cfu/ml *Lactobacillus* counts as determined using M17 and MRS agar respectively. Mesophillic fermented milk culture was used as a control medium and was composed of the freeze dried Mesophillic milk culture (CHN-22, DVS) manufactured by Chr Hansen (Denmark), which only constitutes *Lactococcus lactis* species. Before inoculation into the different growth media, the mesophillic milk culture was first propagated in sterile RSM and incubated at room temperature (20 ± 1°C) for 16 hours. The culture was then kept in the fridge at 4 °C until the next day when it was used. The *Lactococcus* count in the Mesophillic milk culture was 8.43 Log cfu/ml when enumerated using M17 agar. Each growth medium (WH, WH-S, RSM, RSM-S) was inoculated with 5 %

Mursik culture and incubated at 20 ±1 °C in the water bath for 30 hours. The control culture, 5% Mesophillic milk culture, was inoculated into each growth medium (as control samples) and incubated at similar conditions as the *Mursik* samples. All the treatments and control samples were monitored during incubation for LAB growth, EPS production and change pH after every 6 hours as shown below (section 3.7.3).

3.7.3 Monitoring of the EPS production, LAB growth and pH in the cultured media during incubation.

The treatments, *Mursik* cultures and control (mesophillic milk) samples were each monitored for EPS production, LAB growth and changes in pH after every 6 hours for 30 hours as follows;

3.7.3.1 Lactic acid bacteria count.

Lactococcus and *Lactobacillus* species were enumerated using the selective media; M17 and MRS agar respectively as earlier described in section 3.5.1.

3.7.3.2 Change of pH.

The pH was d**e**termined using a milk analyzer as described in section 3.6.3.

3.7.3.3 EPS isolation and Quantification:

EPS was isolated and Quantified as earlier described in section 3.5.6.4 and 3.5.3 respectively.

3.7.4 Data Analysis

The data obtained was subjected to Analysis of Variance (ANOVA) test using SAS statistical package (SAS system for window v.6.12, USA) to establish whether there were significant differences between the four growth media (RSM-S, WH, WH-S and RSM), the five incubation periods (6, 12, 18, 24 and 30 hours) and the two culture (*Mursik* and Mesophillic milk culture) in EPS production. Least significance Difference (LSD) test was used to establish the means which were significantly different at 5 % level of significance. From the data the best growth medium, incubation periods and culture, and interactions based on EPS yields were established.

CHAPTER 4

4.1 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Traditional Technology for *Mursik* Production in Njoro District.

The traditional production technology for Mursik in Njoro district is shown in Table 2. According to the results, *Mursik* is mainly done by the Kalenjin communities. It is mainly produced from boiled milk by spontaneous fermentation in traditional gourds (Figure 1 B), plastic containers or metallic cans. Although most homesteads admitted that gourds produce good quality *Mursik* in terms of taste, aroma and consistency, plastic and metallic containers are used especially for commercial production. During production, fresh raw milk is first boiled and allowed to cool to room temperature before filling into the fermentation container. When fermentation is in the gourd, it should be prepared by washing with warm water using a bow shaped palm branch called *sosiot* and drying in the shed for at least one day. It is then applied charcoal fines on the inner surface by crushing while rubbing charcoal from burnt (hot) wood splints from specific trees or shrubs using another *sosiot*. It is allowed to cool to room temperature before filling the milk. When a plastic or metallic can is used, the charcoal fines may be introduced by filling the milk into a charcoal applied gourd and then transferring into the fermentation container, or the charcoal may be crushed separately and added into the milk before or after filling the can. After filling, the milk is incubated mainly in a cool undisturbed place designated for incubation (called *Kapsoton or Lengu*) where it takes 3 -5 days to ferment and become ready for consumption as *Mursik.* The most commonly used plant species for charcoal application in gourds is a shrub called *Senna didymobotrya* (Figure 1 A). Other trees/shrubs include *Dombeya torrida* (Silibwet), *Lippia kituensis* (Mwokyot) and *Olea europaea ssp.africana* (Emitiot). They improve the flavor and consistency of *Mursik*, eliminate the bitter flavor from the gourd, impart the grey colour to *Mursik,* and some have medicinal effects. The incubation period for *Mursik* can be accelerated by fermenting the milk in a previously used gourd (without cleaning) or previously prepared *Mursik* as starter culture (back slopping), and by incubating in a warm place. However, the *Mursik* produced by the modified methods were reported to be of low quality in terms of flavour and consistency, mainly characterized by flat taste or lack of sourness, bitter taste and with a watery / light consistency. The shelf-life of *Mursik* (traditional) is two to three days after fermentation at ambient

temperatures. *Mursik* is mainly consumed by all age groups, as a food, refreshment and a health beneficial food. It is believed to improve appetite, speed healing and provide strength to invalids, delivered mothers and initiated boys. It is also believed to prevent certain diseases especially stomach problems such as diarrhea.

Table 2: The technology for production of traditional *Mursik* that is used in Njoro District obtained during Study Survey.

Traditional Technology for *Mursik* Production (Cont).			**Responses in Percentage (%)**
(i)	Type of Milk used	Raw cow milk	23.3
		Boiled cow milk	76.7
(ii)	Type of container used for fermentation	Gourds	56.7
		Plastic or Metallic containers	6.7
		Either gourd, plastic or metallic cans	36.7
(iii)	Incubation conditions	Cool conditions	90.0
		Warm conditions	10.0
(iv)	Incubation period (days)	3 - 5 days	93.3
		6 - 7 days	6.7
(v)	Method of accelerating *Mursik* fermentation.	None	46.7
		Addition of previously made *Mursik* (back slopping)	26.7
		Use of previously used fermentation gourd without washing	13.3
		Other methods	13.3
(vi)	Quality of the *Mursik* made using the accelerated fermentation method.	Same as traditional *Mursik*	13.3
		Higher than traditional *Mursik*	10.0
		Lower than traditional *Mursik*	20.0
		No response	56.7
(vii)	Belief that *Mursik* imparts health benefits to consumers	No	16.7
		Yes	83.3

4.1.2 Production of Exopolysaccharides in *Mursik.*

4.1.2.1 Lactic acid bacteria count in the *Mursik.*

The LAB counts in the *Mursik* from Njoro District are shown on Table 3. There was a significant difference between the *Lactococcus* and *Lactobacillus/Leuconostoc* counts at 5 % level of significance. The LAB of the genus *Lactococcus* were dominant over the genus *Lactobacillus/Leuconostoc*, the average *Lactococcus* count being 9.12 Log cfu/ml and the average *Lactobacillus/Leuconostoc* count being 7.77 Log cfu/ml. The mean counts for *Lactococcus* species were not significantly different in all the samples obtained from the three locations (Mauche, Njoro and Mukungugu), whereas those for *LactobacilluslLeuconostoc* were significantly different at 5% level of significance.

Table 3: Lactic Acid Bacteria counts in *Mursik* sampled from Njoro District.

1. **LAB counts per Genus**	Mean (Log cfu/ml)	SD
Lactococcus	9.12^{a}	± 0.442
Lactobacillus /Leuconostoc	7.77^{b}	± 0.917

2. **LAB counts per Division**	***Lactococcus*** **(Mean (Log cfu/ml) ± SD**	***Lactobacillus /Leuconostoc*** **Mean (Log cfu/ml) ± SD**
Mauche	9.121 ± 0.514^{a}	7.107 ± 0.7589^{c}
Njoro	9.124 ± 0.424^{a}	8.516 ± 0.5064^{a}
Mukungugu	9.099 ± 0.273^{a}	8.074 ± 0.3175^{b}

The Means in the same column followed by the same superscript are not significantly different at $P< 0.05$. n= 38 (Mauche=17, Njoro=13, Mukungugu=8).

4.1.2.2 Exopolysaccharide Levels in the *Mursik*.

The mean EPS levels in *Mursik* per household in Njoro District are presented in Table 4. There was a significantly difference between the mean EPS levels in the *Mursik* obtained from the different Households in Njoro District at 5% level of significance. The mean EPS level which significantly differed based on LSD test are shown in Table 4. They ranged between 17.86 and 59.77 mg/L glucose equivalents (Gluc.Equiv), the average being 44.83 mg/L Gluc.Equiv. The mean EPS levels in the *Mursik* also differed between Locations at 5% level of significance. The mean EPS level of the *Mursik* obtained from Njoro Division was not significantly different from

that from Mukungugu Division, but both were significantly different from that from Mauche Division (Table 5). The average EPS level for Mukungugu was the highest, with 52.56 mg/L Gluc.Equiv, followed by that of Njoro and Mauche, with 45.9 and 35.41 mg/L Gluc.Equiv respectively. The EPS levels per households and locations showed a great variation as indicated by the big standard deviation (SD) values. This may be due to variation in the traditional techniques for *Mursik* preparation in each household. During sampling, each household was requested to prepare *Mursik* traditionally as they do in their households. These traditional techniques may have varied in each household, especially the way they prepare the gourd before filling the milk, the plant species used for smoking the gourd and incubation condition.

Table 4: Exopolysaccharides levels in *Mursik* sampled from different households in Njoro District.

Sample (HouseHold)	Mean EPS (mg/L glucose Equivalents)	Sample (Household)	Mean EPS (mg/L glucose Equivalents)
1. MH39	59.77^{a}	14. MH19	50.38^{cde}
2. MH37	58.62^{ab}	15. MH25	48.41^{de}
3. MH14	58.54^{ab}	16. MH32	46.21^{ef}
4. MH36	58.06^{ab}	17. MH22	39.65^{fg}
5. MH29	58.01^{ab}	18. MH11	36.03^{gh}
6. MH27	56.64^{abc}	19. MH33	35.89^{gh}
7. MH20	56.33^{abc}	20. MH13	33.46^{gh}
8. MH38	55.92^{abc}	21. MH24	32.35^{ghi}
9. MH31	54.66^{abcd}	22. MH16	32.09^{hi}
10. MH28	54.38^{abcd}	23. MH21	25.49^{ij}
11. MH34	53.35^{abcde}	24. MH23	22.89^{jk}
12. MH12	51.79^{bcde}	25. MH15	18.24^{jk}
13. MH26	51.59^{bcde}	26. MH18	17.86^{k}

The Means with the same superscript are not significantly different at $P< 0.05$. n = 2.

Table 5: Exopolysaccharide levels in *Mursik* per Location in Njoro District.

Location	EPS mean mg/L glucose Equivalents)	SD
Mauche	35.41^{b}	± 15.37
Njoro	45.90^{ab}	± 12.60
Mukungugu	52.56^{a}	± 8.66
Overall, mean	44.83	

The Means with the same superscript are not significantly different at $P< 0.05$. n=26 (Mauche =7, Njoro=12, Mukungugu=7).

4.1.2.3 Exopolysaccharide Producing Lactic acid bacteria screened from *Mursik*.

(i) Composition of Exopolysaccharide producing Lactic acid Bacteria.

A total of 122 EPS producing LAB were isolated from *Mursik*. Based on phenotypic and biochemical characteristics they were classified into five genera as follows, *Lactococcus* (76%), *Lactobacillus* (11%), *Enterococcus* (7%), *Leuconostoc* (5) and *Weissella* (1%) (Figure 9). Upon further characterization, 88 *Lactococcus* isolates were identified as *Lactococcus lactis subsp.lactis* while 6 isolates were not identified to the species level. All the *Lactobacillus* isolates were homofermentative and were not characterized to the species level. The *Enterococcus* and *Weissella* isolates, were not identified to the species level. These results clearly show that the *Mursik* contain a variety of EPS producing LAB, but the predominant species are *Lactococcus lactis susp.lactis* (76%). This could be because the predominant LAB species in the *Mursik* were also *Lactococcus* (Section 4.1.2.1, Table 3).

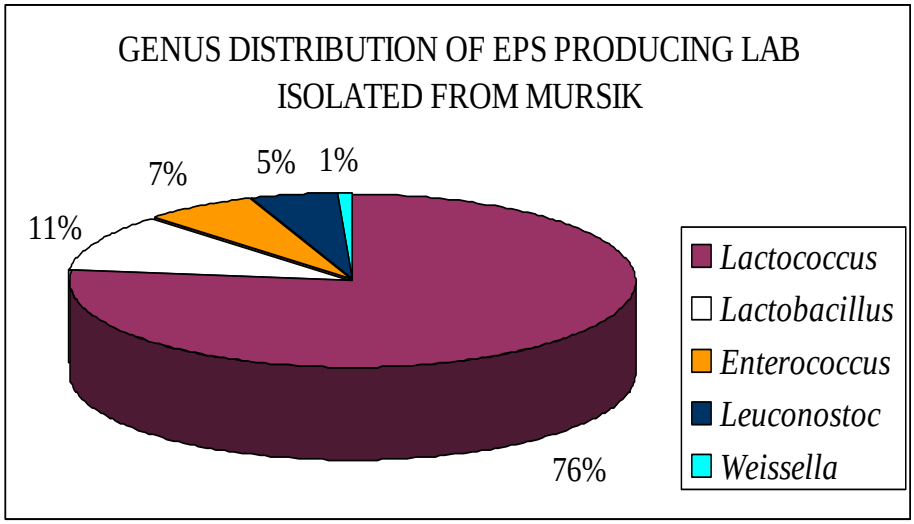


Figure 9: Genera Distribution for the EPS producing LAB isolated from *Mursik.*

Note: The classification was based on criteria described by Garvie *et al.,* 1986; Simpson *et al.,* 1988; Millere *et al.,* 1989; ; Mackey *et al.,* 1993; Facklam and Elliot; 1995; Thunnel, 1995; Facklam *et al.,* 1989; Coeuret *et al.,* 2003.

(ii) Exopolysaccharides Yields from the Exopolysaccharide Producing Lactic acid bacteria from *Mursik*.

The EPS levels produced by the EPS producing LAB isolates (in section 4.1. 3.1) varied between both genera and species. Seventy four (74) *Lactococcus lactis species* were tested and found to produce between 55.7 and 449.9 mg/L Gluc.Equiv. The majority (48%) of these isolates produced between 101 and 200 mg/L Guc.Equiv and very few (5%) produced over 400 mg/L Gluc.Equiv (Figure 10). Twelve (12) *Lactobacillus* isolates were tested and they produced EPS levels between 44.3 and 298.4 mg/L Gluc.Equiv (Figure 11). The majority (67%) produced between 201 and 300 mg/L Gluc.Equiv. Seven (7) *Enterococcus* isolates were tested and they produced EPS levels between 62.2 and 345.9 mg /L Gluc.Equiv (Figure 12). The majority (43%) produced between 101 and 200 mg/L Gluc.Equiv. EPS production by the *Leuconostoc mesenteries* and *Weissella species* were each tested on one isolate which produced193.55 mg/L and 244.5 mg/LGluc.Equiv.

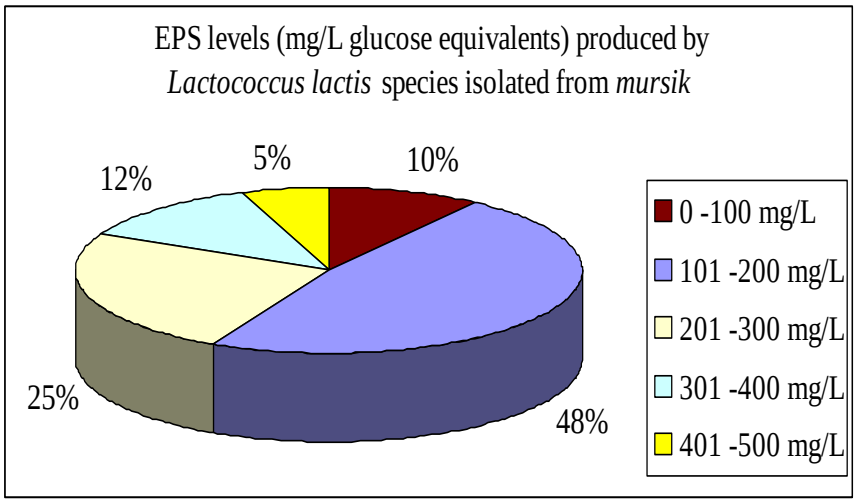


Figure 10: Production of EPS by *Lactococcus lactis species* isolated from *Mursik.*

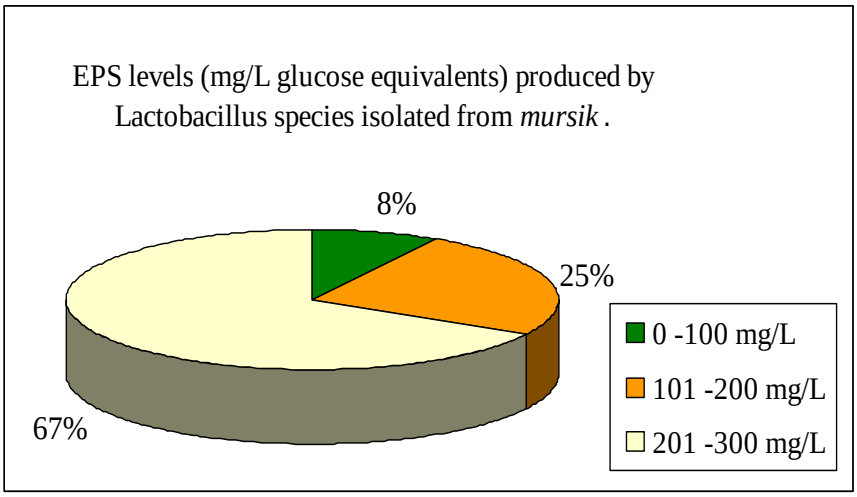


Figure 11: Production of EPS by *Lactobacillus species* isolated from *Mursik.*

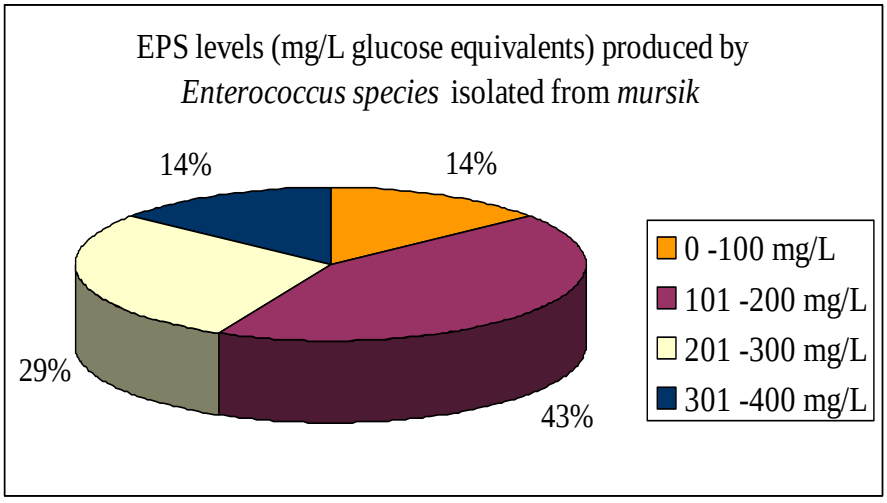
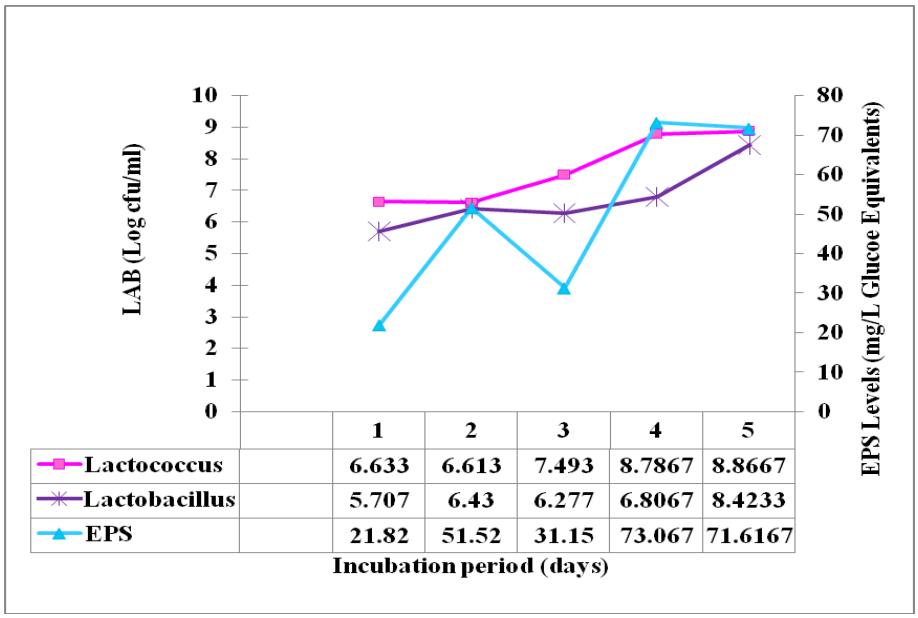


Figure 12: Production of EPS by *Enterococcus* species isolated from *Mursik.*

4.1.3 Effect of incubation period on exopolysaccharides yield during traditional fermentation of *Mursik.*

4.1.3.1 Exopolysaccharide production and lactic acid bacteria growth in *Mursik*.

Growth of both *Lactococcus* and *Lactobacillus* species during traditional fermentation of *Mursik* is shown in Figure 13. Initially the LAB growth rate was generally low, between day one and two. However, the growth rate for *Lactococcus* increased between day two and four and reached stationary phase between day four and five. On the other hand the *Lactobacillus* growth rate remained slow up to day four when it started increasing at a faster rate up to day five. The *Lactococcus* population attained its maximum level of 8.8 log cfu/ml on day four whereas the *Lactobacillus* increased to 8.4 log cfu/ml by day five. The *Mursik* culture produced between 21.82 and 73.07 mg/L Gluc Equiv EPS during the 5 days incubation period. The EPS production was not growth associated. The levels were higher when the rate of LAB growth was low and vice versa. Its rate of production increased between day 1 and 2 after which it slightly decreased on day 3 and increased again between day 3 and 4 to the maximum level of 73.1 mg/L Gluc.Equiv. The level went down slightly again on day 5 when the *Lactococcus* growth was at the stationary phase.



	1	2	3	4	5
Lactococcus	6.633	6.613	7.493	8.7867	8.8667
Lactobacillus	5.707	6.43	6.277	6.8067	8.4233
EPS	21.82	51.52	31.15	73.067	71.6167

Figure 13: LAB growth and EPS production in *Mursik* during traditional fermentation process.

4.1.3.2 Titratable acidity development and change in pH of *Mursik* during Incubation.

The Titratable acidity in the *Mursik* during traditional fermentation increased steadily from 0.21 % to 1.02% Lactic acid (L.A) during the 5 days incubation period, whereas the pH

decreased steadily from pH 6.73 to pH 4.78 (Figure 14). These changes were attributed to the breakdown of lactose (milk sugar) by the LAB resulting in production of lactic acid and other by-product such as carbon-dioxide and, acetic acid. This indicates active growth of the LAB in *Mursik* during incubation.

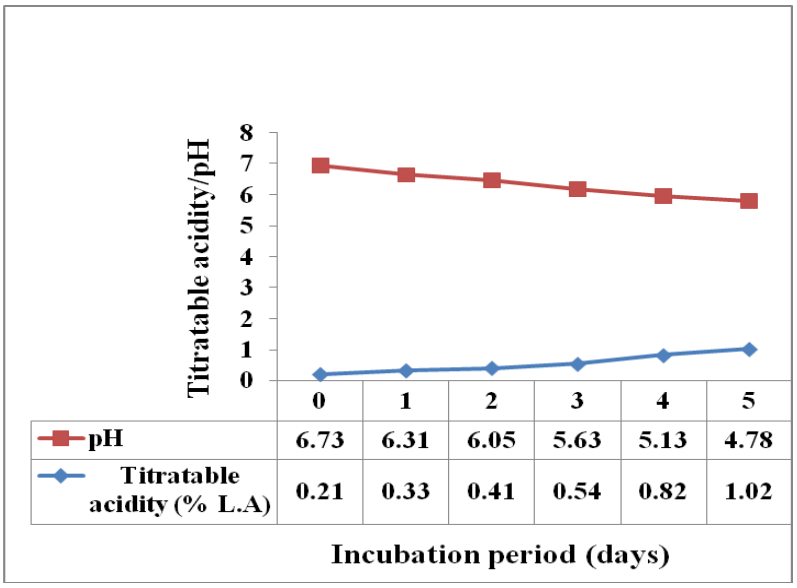


Figure 14: Titratable and pH of *Mursik* during Traditional fermentation process.

4.1.4 Effect of growth medium and incubation period on exopolysaccharide production by *Mursik* culture.

4.1.4.1 Lactic acid bacteria growth and exopolysaccharide production by *Mursik* and Mesophillic milk cultures in the different growth Media during incubation.

The *Mursik* LAB (*Lactococcus* and *Lactobacillus*) grew on all the four growth media, Reconstituted Skim milk with sucrose (RSM-S), Whey (WH), Whey with sucrose (WH-S) and Reconstituted skim milk (RSM). Their growth at 20 ± 1 °C for 30 hours followed the normal sigmoid growth curves (Figure 15. In all the media, LAB growth was initially slow at between 6 and 12 hours. After 12 hours, Logarithmic growth occurred, and the *Lactococcus* species increased faster to a level slightly above that of the *Lactobacillus* species by the 18th hour (the end of logarithmic growth phase). After the 18th hour, the LAB entered the stationary phase at between 18 and 30 hours. The *Lactobacillus* species dominated the culture at between 6 to 12 hours of incubation, after which a shift of the dominant species occurred where the *Lactococcus* species dominated the culture at 18 and 30 hours (Figure 15). The *Mursik* culture (inoculums) had 8.72 Log cfu/ml *Lactococcus* and 8.39 Log cfu/ml *Lactobacillus* counts at the time of inoculation into the growth media. This implies that although *Lactococcus species* were slightly

more than *Lactobacillus species* in the culture during inoculation, their initial growth was lower compared to that for *Lactobacillus species*.

Mursik culture produced different EPS yields on the different growth media during after the 30 hours incubation period. The EPS yields from RSM-S ranged between 35.95 and 141.5 mg/L Gluc Equiv, from WH-S was between 30.38 and 55.75 mg/L Gluc Equiv, from RSM was between 22.64 and 37 mg/L Gluc Equiv and from WH was between 14.2 and 78.13 mg/L Gluc Equiv. RSM-S was the most superior medium for EPS production (Figure 15) by *Mursik* culture, with an optimum yield of 141.5 mg/L Gluc Equiv. As earlier observed, the EPS yield in this medium increased at a higher when the rate of LAB growth was low and vice versa (figure 15). The EPS levels increased steadily from 35.95 to 126.6 mg/L Gluc.Equiv at between 6 and 12 hours incubation periods, when the LAB growth rate was low, and slightly decreased to 107 mg/L Gluc.Equiv at 12 and 18 hours when LAB growth were at the logarithmic phase. The levels increased again to 141.5 mg/L Gluc.Equiv (Maximum level) when LAB growth was at the stationary phase (18 to 24 hours), and again decreased slightly to 138.5 mg/L Gluc.Equiv at between 24 to 30 hours, when LAB growth was towards the end of stationary phase. WH-S also produced higher EPS yields than WH and RSM which did not favour much EPS.

Growth of the LAB in Mesophillic milk culture (control) during the 30 hours incubation period also followed a normal sigmoid curve pattern (Figure 16) as the *Mursik* LAB culture. Similarly, the EPS yields differed in the four growth media, but the levels were generally low compared to that for *Mursik* culture. The EPS yield (in mg/L Gluc.Equiv) ranged between 7.23 and 47.25 mg/L in RSM-S, 16.18 and 65 mg/L in WH-S, 5.88 and 60.08 mg/L in RSM, and 10.23 and 47.5 mg/L in WH. The most superior medium for the EPS production by Mesophillic milk culture was WH-S, with a maximum yield of 65 mg/L Gluc.Equiv. Unlike the *Mursik* culture, EPS production in WH-S by Mesophillic milk culture occurred steadily regardless of the LAB growth rate, and the maximum level was attained at 24 hours. A similar trend was also observed in RSM-S, WH and RSM growth media. The EPS yields by both *Mursik* and mesophillic milk culture decreased in all the four media towards the end of stationary phase, at between 24 and 30 hours. Generally, *Mursik* culture produced higher EPS yields in all the four growth media compared to Mesophillic milk culture.

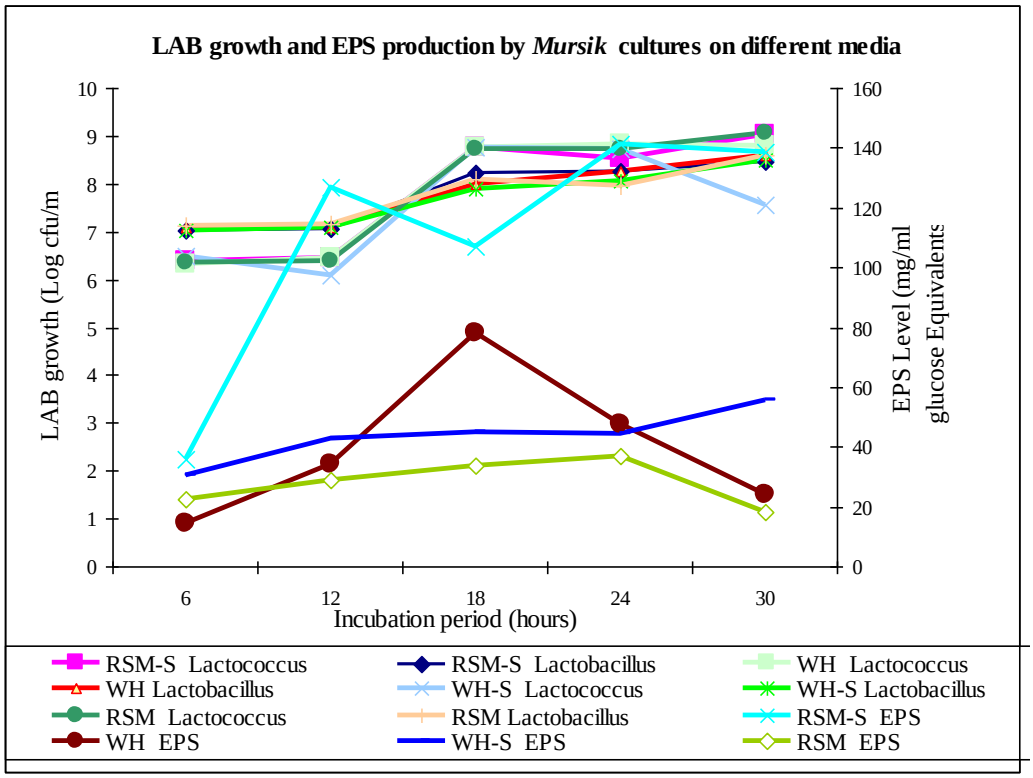


Figure 15: LAB growth and EPS production by *Mursik* culture in different growth media.

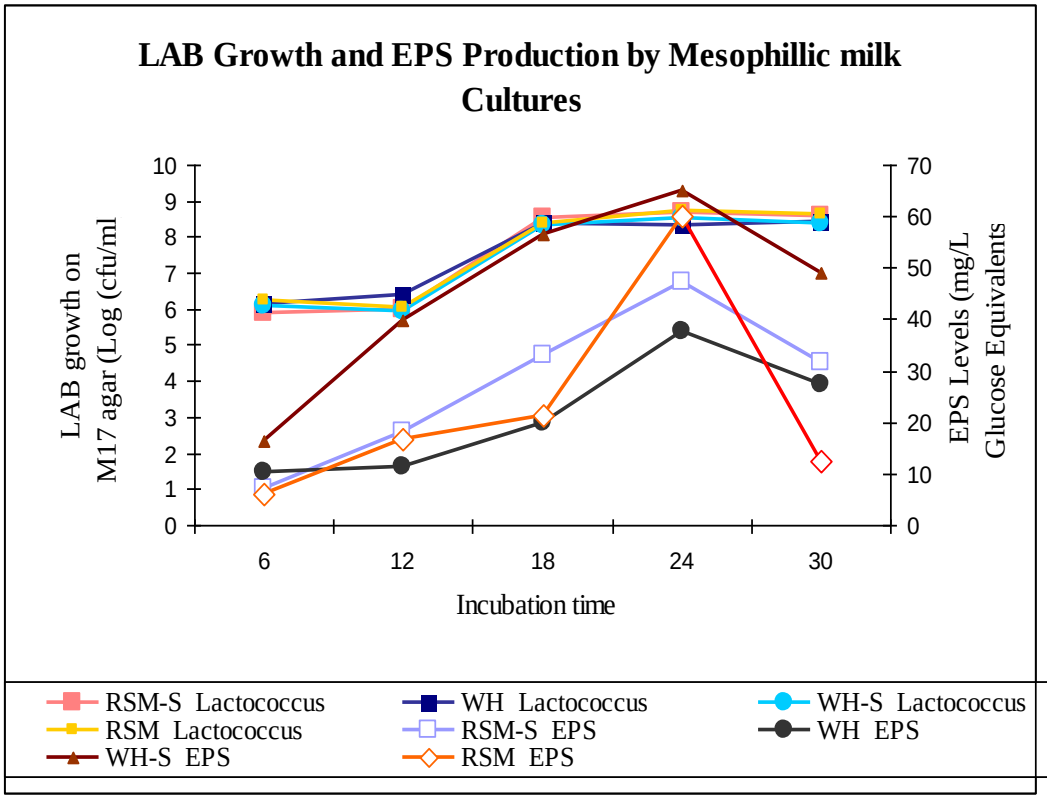


Figure 16: LAB growth and EPS production by Mesophillic milk cultures.

4.1.4.2 Change of pH by *Mursik* and Mesophillic milk culture on different growth media.

The changes in pH by *Mursik* and Mesophillic milk cultures during incubation (0 -30 hours) in all the four media (Figure 17) were uniform. The pH dropped from pH 6.10 to pH 4.20 by *Mursik* culture and from pH 5.95 to pH 4.15 by Mesophillic milk culture. The EPS production experiments were carried out in uncontrolled pH conditions, which is a common condition for many traditional food fermentation processes. The pH drop indicates that the LAB cultures were active in the four different growth media.

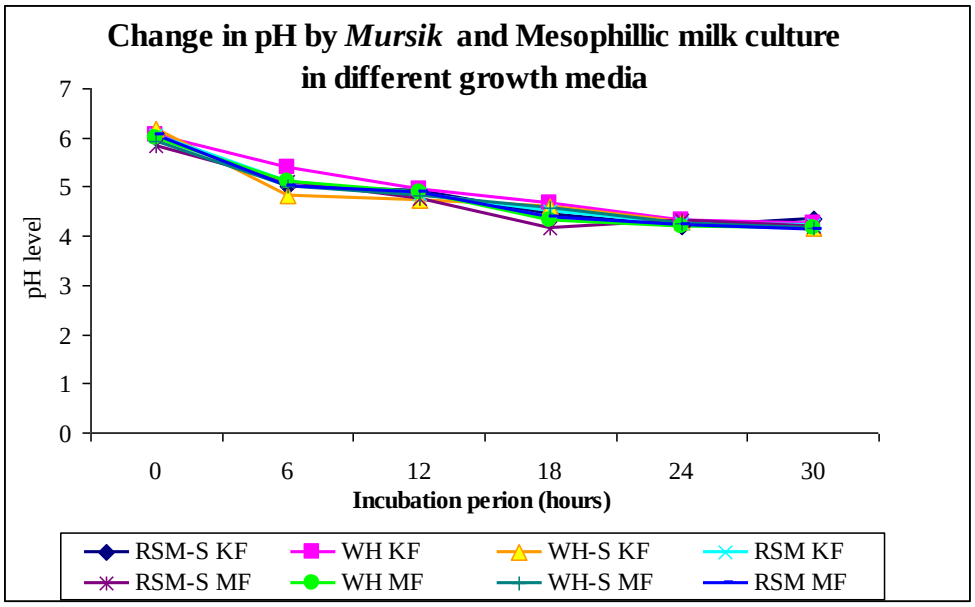


Figure 17: Change in pH in different growth media during growth of the *Mursik* and Mesophillic milk cultures.

4.1.4.3 Effect of growth media and incubation period on exopolysaccharide yield by *Mursik* Culture.

Results from analysis of variance indicated that the four growth media {Reconstituted Skim milk with 5% sucrose (RSM-S), Whey with 5% sucrose (WH-S), Whey (WH) and Reconstituted skim milk (RSM), the five incubation periods {6, 12, 18, 24 and 30 hours} and the two culture (*Mursik* and Mesophillic milk) were significantly different in EPS production at 5 % level of significance. The specific variations based on LSD ($P< 0.05$) are shown in the following results.

(i) Effect of Culture

There was a significant difference between the mean EPS yield from *Mursik* culture and that from Mesophillic milk culture at 5 % level of confidence (Table 6). The EPS yield from

Mursik culture was higher than that from Mesophillic milk culture, the mean levels being 53.98 mg/L and 30.73 mg/L Gluc.Equiv respectively. The EPS levels showed a great variation as indicated by big standard deviation (SD) values because the Mean EPS yields was computed from all EPS yields from the four different growth media each at the five different incubation periods. Some growth media (RSM and WH) and incubation periods (6 and 12 hours) produced very low EPS levels whereas others (RSM-S and WH-S, and 18, 24 and 30hours) produced relatively high EPS levels.

Table 6: Exopolysaccharides levels (in mg/L glucose Equivalents) produced by different culture.

Culture	Mean EPS	SD
Mursik culture	53.98 [a]	± 40.97
Mesophillic fermented milk culture	30.73 [b]	±18.87

Means with the same superscript are not significantly different at $P< 0.05$. LSD = 2.6917. n = 40.

(ii) Effect of growth media.

The average EPS yields from the four growth media were all significantly different at 5 % level of confidence (Table 7). Based on LSD test, all the growth media significantly differed from each other. RSM-S and WH-S, both of which were supplemented with 5 % sucrose produced higher EPS yield, the average being 68.7 and 44.6 mg/L Gluc.Equiv respectively, whereas RSM and WH which were not supplemented with sucrose produced low EPS yields (average yields of 30.5 and 25.7 mg/L Gluc.Equiv respectively). This clearly shows that sucrose stimulated EPS production by both the *Mursik* and Mesophillic fermented milk cultures. The four growth media ranked from the highest to the lowest based on mean EPS yields as follows; RSM-S> WH-S > WH > RSM. The EPS levels for each growth media showed a big variation as indicated by big SD values because the means were computed from all the EPS yields obtained from *Mursik* and Mesophillic milk cultures, each at different incubation periods. The EPS levels from *Mursik* greatly varied from that of Mesophillic milk culture at each incubation period, the former producing higher yields in each media than the later. The EPS yields in each growth media varied at each incubation period, where higher yields were observed at longer incubation periods (18 -30 hours) and lower yields at shorter incubation periods (6 – 12 hours).

Table 7: Exopolysaccharides levels (in mg/L glucose Equivalents) produced in different Growth media.

Growth medium	Mean EPS	SD
Reconstituted skim milk with 5 % sucrose (RSM-S)	68.7 [a]	± 52.18
Whey with 5 % Sucrose (WH-S)	44.6 [b]	± 14.15
Whey (WH)	30.5 [c]	± 20.28
Reconstituted skim milk (RSM)	25.7 [d]	± 15.28

Means with the same superscript are not significantly different at $P< 0.05$. LSD = 3.8067. n=20.

(iii) Effect of the incubation Period

The EPS yields from the five incubation periods were significantly at 5 % level of confidence (Table 8). The EPS levels showed a great variation at each incubation period as shown by big SD values because the means were computed from the EPS yields produced by both *Mursik* and Mesophillic milk culture in each of the four different growth media. Generally, EPS yields by *Mursik* culture were higher than those produced by Mesophillic milk culture; and RSM-S and WH-S produced higher yields than RSM and WH growth media. Based on LSD test all incubation periods except 18 and 24 hours were significantly different. Generally, the EPS levels increased with increase in incubation period from 6 hours to the optimum period at 24 hours after which it started decreasing as observed at 30 hours. It was clear that shorter incubation periods (6 and 12 hours) produced lower EPS levels than longer incubation periods (18 – 30 hours). However, the EPS yields produced at 18 and 24 hours were higher than the yields at 30 hours. This implies that prolonged incubation period result in decrease in the EPS yield. The incubation periods which produced optimum EPS yields were 18 and 24 hours, with the mean yields of 52.8 and 56.7 mg/L Gluc.Equiv respectively, where both were not significantly different. Based on mean EPS yield, the incubation periods can be ranked from the highest to the lowest level as follows; 24 hours > 18 hours >30 hours >12 hours > 6 hours.

.

Table 8: Exopolysaccharide levels (in mg/L glucose Equivalents) produced after different incubation periods.

Incubation Time (hours)	Mean EPS	SD
24	56.7 [a]	± 35.99
18	52.8 [a]	± 27.65
30	44.6 [b]	± 39.60
12	39.9 [c]	± 35.75
6	17.8 [d]	± 10.81

Means with the same superscript are not significantly different at $P< 0.05$. LSD = 4.256. n=16.

(iv) Interactions between Culture and Growth medium.

There was a significant difference between the different interactions between culture and growth media at 5% level of significance (Table 9)**.** The mean EPS yields for each interaction showed a wide range of variation because the means were computed from EPS yields produced by each culture in each growth medium at each of the five different incubation periods (6,12, 18, 24, and 30 hours). Generally, shorter incubation periods (6 and 12) produced lower EPs yields than longer incubation periods (18 -30 hours). From the results, it was evident that the interaction between the growth medium and culture influenced EPS production by both *Mursik* and Mesophillic milk cultures. Both cultures (*Mursik* and Mesophillic milk cultures) produced higher EPS yields in the media supplemented with sucrose (RSM-S and WH-S). However, Culture/Growth medium (interaction) which produced the best EPS yield was *Mursik*/RSM-S with the mean yield being 109.9 mg/ L Gluc.Equiv. This was followed by Mesophillic milk culture/WH-S with the mean yield of 45.3 mg/L Gluc.Equiv. While *Mursik* culture produced its optimum yield in RSM-S, Mesophillic milk culture (Control) produced its optimum yield in WH-S. This implies that, both cultures varied in their growth media requirements for EPS production. The EPS yield by *Mursik* culture in the four growth media can be ranked based on EPS yield from the highest to the lowest as follows; RSM-S > WH-S > WH > RSM, whereas for Mesophillic milk culture, can be ranked as follows; WH-S > RSM-S, WH and RSM, where RSM-S, WH and RSM were not significantly different. It was also observed that the interaction between *Mursik* culture and the other growth media produced higher EPS yields compared to the

interaction with Mesophillic milk culture. The best culture/growth interaction was *Mursik*/RSM-S, with an optimum yield of 109.9 mg/L Gluc.Equiv.

Table 9: Exopolysaccharide Levels (in mg/L Glucose Equivalents) due to the Interaction between Growth Medium and Culture.

Growth Medium	*Mursik* Culture		Mesophillic milk Culture	
	Mean EPS	SD	Mean EPS	SD
RSM-S	109.94^{a}	± 41.737	27.51^{b}	± 15.221
WH	34.13^{c}	± 24.439	26.83^{b}	± 15.546
WH-S	43.78^{b}	± 10.204	45.32^{a}	± 17.811
RSM	28.10^{d}	± 7.383	23.26^{b}	± 20.621

Note: Means in the same column with the same superscript are not significantly different at $P<$ 0.05. LSD= 5. 383. n = 4.

(v) Interaction between Culture and Incubation period

There was a significant difference between the interaction between incubation periods and cultures at 5 % level of significance (Table 10). The EPS levels showed a wide range of variation as indicated by big SD values because the means were computed from EPS yield produced by each culture, *Mursik* and Mesophillic milk culture, in each of the four growth media at each incubation periods. EPS yields were higher RSM-S and WH-S than in RSM and WH. For *Mursik* culture, the mean EPS yield at all incubation periods except 18 and 24 hours were significantly different. The optimum EPS yield from *Mursik* culture occurred at 18 -24 hours, the mean being 66.0 and 60.8 mg/L Gluc.Equiv respectively. For Mesophillic milk culture, the mean EPS yields at all incubation periods were significantly different, and the optimum yield (52.5 mg/L Gluc.Equiv) was at 24 hours. For *Mursik* culture, the incubation periods can be ranked from the highest to the lowest based on the mean EPS yields as follows; 18 hours > 24 hours >30 hours >12 hours > 6 hours, whereas for Mesophillic milk culture, they can be ranked as follows; 24 hours > 18 hours > 30 hours > 12 hours > 6 hours. In both cultures, 6 and 12 hours produced generally low yields and prolonged incubation (30 hours) resulted in decrease in yields. Generally, it was observed that the interaction between all incubation periods with *Mursik*

culture resulted in higher EPS yields than their interaction with Mesophillic milk culture. Optimum EPS yield occurred from the interaction between the *Mursik* cultures with 18 hours incubation period, the mean yield being 66 mg/L Gluc.Equiv. This was not significantly different with the yield due the interaction between *Mursik* culture and 24 hours.

Table 10: Exopolysaccharide levels (in mg/L glucose Equivalents) for the interaction between culture and Incubation period.

Incubation Time (Hours)	*Mursik* culture		Mesophillic milk culture	
	Mean EPS	**SD**	**Mean EPS**	**SD**
6	25.79^{c}	± 9.027	9.88^{e}	± 4.922
12	58.22^{b}	± 42.793	21.55^{d}	± 11.813
18	66.00^{a}	± 31.977	39.67^{b}	± 14.808
24	60.80^{ab}	± 50.787	52.51^{a}	± 12.549
30	59.12^{b}	± 51.587	30.03^{c}	± 14.685

Means in the same column with the same letter are not significantly different at $P< 0.05$. LSD = 6.019. n = 10.

(vi) Interaction between Incubation period and growth media.

There was a significant difference in the average EPS yields due to the interactions between incubation periods and growth media at 5 % level of significance (Table 11). The optimum EPS yield in the different growth media occurred at different incubation periods, mainly between 18 and 24 hour. The mean EPS levels during the 30 hours incubation period for the different growth media ranged between 21.59 and 94.38 mg/L in RSM-S (optimum being at 24 hours), between 23.28 and 54.84 mg/L in WH-S (optimum being at 18 – 24 hours), between 12.21 and 62.81 mg/L in WH (Optimum being at 18 hours), and between 14.25 and 48.54 mg/L in RSM (optimum being at 24 hours). RSM-S produced higher EPS levels compared to WH-S, WH and RSM at 12 to 30 hour incubation periods followed by WH-S. This indicates that, the media sucrose supplemented improved EPS yields. Based on EPS yields, the incubation periods for each growth medium can be ranked from the highest to the lowest as follows; RSM-S, 24 hours > 30 hours > 12 hours > 18 > 6 hours, where 24 and 30 hours, and 12 and 18 hours were not significantly different; WH, 18 hours > 24 hours > 30 hours > 12 hours > 6 hours, where 24 and 30 hours, and 6 and 12 hours were not significantly different; WH-S, 24 hours > 30 hours >

18 hours > 12 hours > 6 hour, where 18, 24 and 30 hours were not significantly different; and RSM, 24 hours > 18 hours > 12 hours >30 hours > 6 hours, where 6 and 30 hours were not significantly different. In all the growth media, 6 to 12 hours produced lower EPS levels whereas 30 hours resulted in reduction of the EPS yields. The growth medium/incubation period interaction which produced the highest EPS yield was RSM-S/24 hours, with the mean EPS yield of 94.4 EPS mg/L Gluc.Equiv. Prolonged incubation period (30 hours) resulted in reduction of the EPS level to 85.1 mg/L Gluc.Equiv but the level was not significantly different from the optimum level.

Table 11: Exopolysaccharides levels (mg/L glucose Equivalents) for the interaction between Growth medium and Incubation period.

Incubation Time (Hours)	RSM-S		WH		WH-S		RSM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
6	21.59^{c}	± 16.734	12.21^{c}	± 3.239	23.28^{c}	± 8.982	14.25^{c}	± 9.773
12	72.21^{b}	± 62.579	22.86^{c}	± 14.232	41.41^{b}	± 5.773	22.85^{c}	± 7.184
18	70.14^{b}	± 44.547	62.81^{a}	± 17.932	50.91^{ab}	± 8.081	27.48^{b}	± 7.513
24	94.38^{a}	± 54.630	28.89^{b}	± 10.641	54.84^{a}	± 11.801	48.54^{a}	± 14.931
30	85.10^{a}	± 62.137	25.60^{b}	± 2.557	52.31^{ab}	± 7.599	15.28^{c}	± 4.676

Means in the same column with the same letter are not significantly different at $P< 0.05$. LSD = 11.6. n =8.

(vii) Interaction between Culture, Growth medium and Incubation period.

There was a significant difference between the mean EPS levels due to the interaction of all the three factors, culture, growth medium and incubation period, at 5 % level of significance as shown in Table 12. The interaction between *Mursik* culture and RSM-S produced higher EPS yields at all incubation periods compared to its interaction with WH-S, WH and RSM. Both *Mursik* and Mesophillic milk culture produced higher EPS yields in WH-S compared to WH and RSM. This implies that sucrose supplementation stimulated EPS yields by both *Mursik* and mesophillic milk culture. However, *Mursik* culture produced higher EPS levels in all the

growth media compared to Mesophillic milk culture. Optimum yields in both *Mursik* and Mesophillic milk culture optimum yields were obtained at 24 and 18 hours incubation periods respectively, whereas at 30 hours, EPS reduction was observed. *Mursik* culture/ RSM-S/24 hours produced the highest EPS yield (mean yield being 141.5 mg/L Gluc.Equiv). These observations are in agreement with the earlier results, where the interaction between *Mursik* culture and 18 - 24 hour resulted in the highest EPS yield (Table 10), and where the interaction between RSM-S and 24 hours produced the best EPS yields (Table 11).

Table 12: Average levels of Exopolysaccharides (mg/L glucose equivalents) for the interaction between Growth medium, Incubation period and Culture

	***Mursik Culture* (mean ± SD)**				**Mesophillic milk Culture (mean ± SD)**			
Incubation Time (hrs)	**RSM-S**	**WH**	**WH-S**	**RSM**	**RSM-S**	**WH**	**WH-S**	**RSM**
6	35.95^{d} (±2.333)	14.20^{c} (±2.404)	30.38^{c} (±4.632)	22.64^{bc} (±0.707)	7.23^{c} (±3.076)	10.23^{c} (±3.147)	16.18^{d} (±4.349)	5.88^{c} (±2.369)
12	126.60^{b} (±0.990)	34.30^{b} (±8.132)	42.95^{b} (±8.980)	29.02^{abc} (±1.591)	18.23^{c} (±1.502)	11.43^{c} (±4.278)	39.88^{c} (±3.147)	16.68^{bc} (±0.177)
18	107.08^{c} (±21.248)	78.13^{a} (±3.571)	45.15^{ab} (±7.920)	33.65^{ab} (±0.071)	33.20^{b} (±6.647)	47.50^{a} (±3.748)	56.68^{ab} (±0.601)	21.30^{b} (±4.101)
24	141.50^{a} (±5.374)	20.05^{c} (±3.677)	44.65^{ab} (±1.018)	37.0^{a} (±3.246)	47.25^{a} (±6.435)	37.73^{ab} (±3.712)	65.00^{a} (±1.626)	60.08^{a} (±11.028)
30	138.55^{ab} (±16.435)	$23.95b^{c}$ (±2.828)	55.75^{a} (±10.889)	18.22^{c} (±0.007)	31.65^{b} (±10.677)	27.25^{b} (±0.849)	48.88^{bc} (±2.722)	12.35^{bc} (±5.586)

Means with the same letter in the same column are not significantly different at $P< 0.05$. LSD= 12.04. n = 2.

4.1 DISCUSSION.

4.2.1 Traditional Production Technology of *Mursik* in Njoro District.

Traditionally, *Mursik* is produced by spontaneous fermentation of raw or boiled cow milk in a traditionally prepared gourd. It is not only a refreshing drink, but it is believed to be health beneficial food product. According to Mathara *et al.,* (1995), Kalenjins consume *Mursik* mainly due to its delicious flavour and belief that it improves health mainly that it makes one strong, speeds recovery by the sick and enhances immunity against certain diseases. Due to its taste and health enhancing claims, other non-Kalenjin communities also consume *Mursik*. Therefore, *Mursik* is now being produced and sold informally at farm gates, in food Kiosks and in some Hotels. During the study, it was established that the traditional production technology for *Mursik* by the Kalenjin has been gradually changing in Njoro District. Some of the changes established include, use of boiled milk instead of raw milk for *Mursik* production instead, use of plastic and metallic cans instead of only gourds as *Mursik* fermentation containers and optional application of charcoal fines in the milk used for *Mursik* unlike in the traditional method where *Mursik* should only be prepared in smoked gourds. In addition, other non-traditional techniques have been used for *Mursik* production such as acceleration of *Mursik* fermentation by back-slopping or incubation in warm conditions, and addition of crushed cold charcoal into the milk prior to fermentation in plastic or metallic containers. These changes in traditional *Mursik* production technology is attributed to several factors which include change in life styles of the *Mursik* producing community (Kalenjin), commercialization of *Mursik* which require production of larger volumes, different consumer demands, health awareness by consumers and lack or loss of traditional skills for *Mursik* preparation. In the past, the Kalenjins were mainly pastoralists and women observed the traditional ways of life, which include preparation of traditional gourds for drinking fresh milk and production of *Mursik*. These skills were passed from old to the young generation. However, due to education and acquisition of new ways of life such as crop farming, running of businesses and employment in various sectors, the young women do not find time to acquire and practice these traditional techniques. This has therefore resulted in loss of the traditional skills and /or acquisition of alternative techniques that are quick and non-tedious for *Mursik* preparation such as use of plastic/metallic cans and accelerated methods of fermentation. Production of *Mursik* for commercial purposes and different consumer demands have mainly contributed to production of *Mursik* in plastic or metallic cans which enables production of larger quantities and/or production

of *Mursik* without charcoal fines. Some consumers prefer *Mursik* with charcoal fines whereas others prefer without. Therefore, commercial producers have adopted ways for application of the charcoal fines such as crushing hot charcoal from the traditionally used plant species, cooling and adding into the milk just before fermentation in the plastic or metallic cans. Alternatively, the charcoal may be introduced into the milk by first filling the milk in a smoked gourd and then transferring into the plastic/metallic can. Due to consumer health awareness and frequent occurrences of food borne of diseases, *Mursik* is now produced from boiled milk and rarely from raw milk. From the study survey, it was established that the change from the traditional *Mursik* production technology have a significant effect on the final Mursik quality. Fermentation of *Mursik* in plastic or metallic cans and use of back slopping techniques was reported to result in lower quality products that are mainly characterized by watery/light consistency and lack of the typical *Mursik* flavor. This quality difference is mainly attributed to the variation in the composition of the fermentation culture in the *Mursik* produced using the two techniques (traditional and the modified method). According to Rattanachaikunsopon and Phumkhachorn (2010), the quality of the finished product in spontaneous fermentation depends on the microbial load and microbial spectrum of the raw material. Studies have shown that the main fermentation microorganisms in traditional *Mursik* are Lactic acid bacteria, LAB (Mathara *et al.,* 1995, Nakamura *et al.,* 1999). The LAB mainly originates from the gourd, where they become selectively established on the inner rough surface with time during gourd ripening and successive *Mursik* fermentation. However, when plastic or metallic cans are used, the main source of fermentation culture is milk, which is basically a different microbial profile from that which is responsible for fermentation in the traditional *Mursik*. This therefore may be the cause of the difference in quality between the traditional (made in gourds) and non-traditionally (made in plastic/metal cans or cultured by back slopping) *Mursik*. Accelerating *Mursik* fermentation by incubating the milk in a warm place may also results in imbalance of the LAB responsible for fermentation since the conditions may favour growth of certain LAB species over other species resulting in a shift in composition of the fermentation culture. According to Rattanachaikunsopon and Phumkhachorn (2010), back-slopping is a better approach for accelerating fermentation process in products whose microbial ecology is not well defined since it optimizes the fermentation processes and reduces the risks of fermentation failure. However, successive propagation of the cultures may result in a shift of ecosystem (change of microbial profile), loss of some strains, and loss of some metabolic traits in some LAB strains, especially the plasmid DNA

encoded traits, and this may subsequently results in loss of the original properties of the product (Rattanachaikunsopon and Phumkhachorn, 2010). From the study survey, it was clear that *Mursik* production technology in Mursik is gradually changing and hence it may eventually result loss of the original *Mursik* product. Therefore, there is need for protection of the original *Mursik* LAB culture, and this can be achieved through development and patenting of the typical *Mursik* starter culture, and preservation using the current culture preservation techniques such as Freeze drying. This will also enable large scale (industrial) production and extensive commercialization of *Mursik* both in Kenya and in other countries.

4.2.2 Production of Exopolysaccharides in *Mursik*

Production of EPS by LAB is important in fermented milk products since they improve their rheological properties, mainly the texture, water holding properties, viscosity, gelling properties, and melting properties (Welman and Maddox, 2003; Awad *et al.,* 2005; Hassan, 2008; Badel *et al.*, 2011). They impart a smooth and creamy body to fermented milks such as Stirred yoghurt, low fat yoghurt and low milk solids yoghurt (Vuyst and Degeest, 1999). They also enhances taste of the fermented milk products and improve colonization of the gut by probiotics by increasing the residence time of the fermented milk in the mouth and in gut respectively (Welman and Maddox, 2003). Many traditional fermented milk and food products have been established to contain EPS producing LAB and these include the Nigerian fermented cereal products, *Ogi* and *fufu* (Sanni *et al.,* 2002), the Scandinavian ropy fermented milk products, *Viilli* and *Langfil* (Vuyst and Degeest, 1999; Ruas-Madiedo *et al.,* 2006) and *Kefir* (Vuyst and Degeest, 1999). In this study, it was established that the traditionally produced *Mursik* consist of EPS and EPS producing LAB. However, the EPS levels were low, ranging between 17.86 to 59.77 mg/L Gluc. Equiv, the mean being 44.83 mg/L Gluc.Equiv. These levels varied within households and Locations. The predominant LAB species in the *Mursik* were *Lactococcus* species, the mean counts being 9.12 Log cfu/ml, whereas the mean counts of *Lactobacillus* species were 7.79 Log cfu/ml. The screened EPS producing LAB from the *Mursik* were also predominated by the *Lactococcus* species (76%). The low EPS yields in *Mursik* is attributed to several factors which include the composition of EPS producing LAB in *Mursik*, growth medium composition and growth conditions. Although *Mursik* production technology is generally similar among the Kalenjins, different households have different household practices for *Mursik* fermentation. These include variations in the methods of gourd preparation, *Mursik* incubation conditions, the type of plant species used for application of charcoal fines in the gourds and the hygiene and cleaning practices for the gourds after and before

use. These variations results in differences in the LAB profile and/or composition of the EPS producing LAB in the *Mursik*. The method for preparation of the gourd (ripening) is very important since it determines the type of LAB which becomes established on the inner surface of the gourd, which eventually becomes the major fermentation culture in the gourd-fermented *Mursik*. These cultures therefore determine the quality of the final *Mursik* product that is produced using the gourd. Hygiene practices used for cleaning and maintenance of the gourd are also important since they affect the microbiological quality of the gourd, especially establishment of non-desirable microorganisms on the inner surface of the gourd. Poor cleaning and storage of the gourd may result in growth of undesirable microorganisms such as yeast and moulds, which eventually become contaminants in *Mursik*. The observed difference in LAB counts and EPS levels in *Mursik* obtained from different locations in Njoro District may have generally emanated from household differences.

Composition of LAB and /or EPS producing LAB species affects the EPS yield in fermented milks. According to Mozzi *et al.,* (2006), EPS production by different LAB varies with genera, species and strains, where thermophillic LAB generally produce more EPS than mesophillic LAB. A study by Vuyst *et al.,* (1998) as well as by Zisu and Shah (2003) established that EPS yields by *Streptococcus thermophilus* species are affected by the composition of EPS producing strains in the culture. LAB species vary in their ability to produce EPS due to variation in their genetic potential (Welman and Maddox, 2003; Mozzi *et al.,* 2006). EPS biosynthesis is a complex process which involve activities of different enzymes, mainly non-EPS specific (housekeeping) and EPS specific (*glysosyltranferases*) enzymes (Vuyst and Degeest, 1999; Welman and Maddox, 2003; Badel *et al.,* 2011). Housekeeping enzymes catalyze production of sugar nucleotides (EPS precursors) whereas *glycosyltranferases* are involved in the assembly of the sugar nucleotides into EPS polymers (Vuyst and Degeest, 1999; Welman and Maddox, 2003; Badel *et al.,* 2011). EPS production phenotype is encoded by specific genes, called *eps*-genes, that are either located in the plasmid DNA in most mesophillic LAB, or in the chromosomal DNA in most thermophilic LAB (Kleerebezem *et al.,* 2002; Wellman and Maddox, 2003; Badel *et al.,* 2011). Each specific *eps*-gene encodes for a specific *glycosyltransferases,* which catalyzes biosynthesis of a particular type of EPS (Kleerebezem *et al.,* 2002; Wellman and Maddox, 2003; Badel *et al.,* 2011). *Glycosyltranferases* determine the monomer composition and the structure (type of linkages and the side chains) of EPS to be produced (Vuyst and Degeest, 1999; Welman and Maddox, 2003), whereas their concentration determines the amount of EPS (yield) to be

synthesized (Welman and Maddox, 2003). Biosynthesis of each type of homopolysaccharide is catalyzed by one specific *glycosyltransferase*, whereas each heteropolysaccharide biosynthesis, is catalyzed by several types of *glycosyltransferases* depending on the monomer composition (Welman and Maddox, 2003; Badel *et al.* 2011). Therefore, the *eps*-genes and EPS producing enzymes that a given bacteria can produce determines the nature and the yield of the EPS to be produced by a given microorganism (Welman and Maddox, 2003). Most Mesophillic LAB produce heteropolysaccharides (Jolly *et al.,* 2002; Badel *et al.,* 2011). In this study, it was established that the predominant EPS producing LAB in the *Mursik* were *Lactococcus lactis* species. These isolates produced low EPS levels, ranging between 101 -200 mg/L Gluc.Equiv. According to Cerning *et al.,* (1992), *Lactococcus lactis subsp.cremoris* strains have been reported to produce between 25 and 600 mg/L under controlled conditions.

Production of EPS by LAB is affected by growth condition, mainly pH, temperature, incubation period and oxygen potential (Vuyst *et al.,* 1998; Zisu and Shah, 2003; Degeest *et al.,* 2001). Zisu and Shah (2003) found that EPS yields by *Streptococcus thermorphilus* were low when propagated under un-controlled pH system compared to the controlled pH system. Since *Mursik* fermentation is done under un-controlled pH conditions, this could have contributed to the observed low EPS yields. *Mursik* fermentation is mainly done at ambient temperatures (20 ±1 ºC), which may not be optimum for EPS production by some of EPS producing LAB. EPS production by Mesophillic LAB mainly occurs when the rate of LAB growth is low (Cerning *et al.,* 1992; Cerning, 1995; Vuyst and Degeest, 1999; Degeest *et al.,* 2001). This is because they synthesize EPS in almost a similar process as the cell wall polymers such as peptidoglycan, teichoic acid, and lipoteichoic acid (Cerning *et al.,* 1992; Degeest *et al.,* 2001). Both EPS and cell wall polymers require sugar nucleotides, housekeeping enzymes and isoprenoid lipid carriers for their assembly and production (Cerning *et al.,* 1992; Cerning, 1995; Vuyst and Degeest, 1999; Degeest *et al.,* 2001). Therefore, at sub-optimal conditions, the LAB growth rate is generally low hence most of the sugar nucleotides, housekeeping enzymes and isoprenoid lipid carriers are directed towards EPS biosynthesis than towards cell wall polymers formation (Cerning *et al.,* 1992; Cerning, 1995; Vuyst and Degeest, 1999; Degeest *et al.,* 2001).

Growth medium composition, mainly the carbon and nitrogen sources also determine the EPS production by LAB (Vuyst *et al.,* 1998; Zisu and Shah, 2003; Vaningelgem *et al.,* 2004b). LAB species vary in their abilities to utilize different carbon sources for EPS production (Looijesteijn *et al.,* 1999; Degeest and Vuyst, 2000). The major source of carbon (sugar) in the

milk is lactose. This may have not been a good carbon source for EPS production by some of the EPS producing LAB (genera, species or strains) in *Mursik*. Carbon source may also be a limiting factor for EPS biosynthesis during active cell growth, since it is utilized for energy generation in the glycolysis system as well as for production of sugar nucleotides, the precursors for biosynthesis of EPS and cell wall polymers (Vuyst and Degeest, 1999; Welman and Maddox, 2003; Badel *et al.,* 2011). Therefore, during active cell growth, most of the carbon is utilized for cell wall polymer formation than EPS production and vice versa.

Production of EPS degrading enzymes (*glycohydrolases*) by some LAB affect EPS yield by some LAB species. They are mainly produced during prolonged incubation, and they hydrolyze the already formed EPS polymers in the culture resulting in reduction of the EPS yields (Cerning, 1995; Ruas-Madiedo and Reyes-Gavilan, 2005). This was also observed in this study when the EPS levels in *Mursik* reduced upon incubation beyond four days during traditional *Mursik* fermentation process and beyond 24 hours (at 30 hours) during propagation of *Mursik* culture in the four different growth media in the laboratory.

The method for isolation of EPS from *Mursik* may have also contributed to the low EPS yield observed in *Mursik*. EPS may have been lost during the isolation process either with the retentate as co-precipitates with protein during precipitation with Trichloroacetic acid (TCA) or with the supernatant during EPS extract recovery from chilled ethanol by centrifugation and decantation. It has been reported that substantial amounts of EPS are lost during the isolation process due to the complexity of the EPS isolation and purification method (Cerning *et al.,* 1992; Cerning, 1995; Ruas-Madiedo and Reyes-Gavilan, 2005). According to Ruas-Madiedo and Reyes-Gavilan (2005), precipitation of protein with TCA from the culture during EPS isolation results in up to 50% loss of EPS as co-precipitates with the protein (Ruas-Madiedo and Reyes-Gavilan, 2005). An alternative method which involves the use of proteases such as pronase requires application of heat treatment to the culture to inactivate the enzymes, and this also interferes with EPS isolation (Ruas-Madiedo and Reyes-Gavilan, 2005). Other methods of EPS isolation include membrane filtration, ultra filtration and diafiltration. However, the method which involve protein precipitation with TCA and EPS precipitation with chilled ethanol is more preferred in research since it results in EPS with fewer impurities (Ruas-Madiedo *et al.,* 2005). Purification of the EPS extract is mainly done by dialysis using membranes of different cut-off (1000 – 12000 Da) sizes. In this study, protein precipitation in the *Mursik* samples was done using 17% TCA (with 80% m/v concentration) followed by EPS precipitation using chilled absolute ethanol and recovery of

the EPS extract by centrifugation. Since dialysis was not done, the EPS extracts obtained were generally crude extracts. In conclusion, the findings from this study indicated that the traditional *Mursik* produced in Njoro district contains EPS. The EPS levels were generally low, but this was consistent with the low levels reported for most LAB species, especially *Lactococcus* species which were predominant in the *Mursik*. However, it has been reported that LAB EPS have excellent physico-chemical properties even at low levels (Jolly *et al.,* 2002; Vuyst and Degeest, 1999), and the threshold levels required for the EPS to impart health beneficial effects on consumers have not been established.

4.2.3 EPS Producing LAB Isolated from *Mursik* and their EPS Production Capacities.

Production of EPS has been reported in many LAB including the *Lactobacillus* species such as *Lb. casei, Lb.rhamnosus, Lb.helviticus, Lb.plantarum Lb.acidophilus, Lb.curvatus, Lb.reuteri* (Homofermentative *Lactobacillus*), *Lb.brevis, Lb.fermentum* (heterofermentative *Lactobacillus*) (Badel *et al.,* 2011), *Leuconostoc mesenteries* (Vasileva *et al.,* 2010), *Weissella confusa* (Katina *et al.,* 2009) and *Lactococcus lactis subsp.cremoris* (Ramos *et al.,* 2001; Vuyst and Degeest, 1999). Homopolysaccharide production, mainly dextran (α-glucan polymer) has been reported in *Leuconostoc mesenteroides species (*Badel *et al.,* 2011*), Weissella confusa species* (Katina *et al.,* 2009), whereas heteropolysaccharide production has been reported in *Lactococcus lactis* subsp. *cremoris* (Ramos *et al.,* 2001; Vuyst and Degeest, 1999) and homofermentative *Lactobacillus* such as *Lb. casei* (Kojic *et al.,* 1992), *Lb.plantarum* and *Lb.rhamnosus* (Badel *et al.,* 2011). In this study, *Mursik* culture was established to consist of a variety of EPS producing LAB consisting of *Lactococcus* species (76%), homofermentative *Lactobacillus* species (11%), *Enterococcus* species (7%), *Leuconostoc* species (5%) and *Weissella* species (1%). Their EPS production capacities varied between genera and within the species, the yields ranging between 44.3 - 449.9 mg/L glucose equivalents. Majority (48%) of the *Lactococcus* lactis species (predominant species) produced between 101 and 200 mg/L glucose equivalents and very few (17%) produced above 300 mg/L glucose equivalents.

Generally, LAB have been reported to produce low EPS yields compared to the non-food grade microorganisms such as *Xanthomonas campestris* whose EPS product, xanthan gum, is widely used as a biothickener in many food products (Cerning, 1990; Cerning, 1995; Broadbent *et al.,* 2003, Badel *et al.,* 2011). This low yields have been a key limitation for their application as sources of biothickeners in foods as in the case of the EPS from non-food grade microorganisms (Vuyts and Degeest, 1999; Jolly *et al.,* 2002; Badel *et al.,* 2011). According to Badel *et al.,* (2011),

X. campestris produces between 35 to 50 g/L EPS, whereas LAB species hardly produce up to 1 g/L, the heteropolysaccharides being even much lower. However, some LAB species such as *Lactobacillus rhamnosus* have been reported to produce up to 2.7g/Liter (Badel *et al.,* 2011), but they are still far much below the recommended level for economical production of EPS bioingrdient, which should be 10 -15 g/L (Welman and Maddox, 2003). Several studies have shown that there is a wide variation in EPS production by different LAB. The EPS yield, EPS molecular mass and EPS monomer composition vary with different LAB species and strains (Vaningelgem *et al.,* 2004a; Mozzi *et al.,* 2006). In this study, variation in EPS yield by different genera and species of the EPS producing LAB from *Mursik* was observed. There is no much information about EPS production by *Lactococcus lactis* subsp.*lactis* compared to that for *L.lactis subsp.cremoris*. The later has been reported to produce between 25 to 600 mg/L (Cerning *et al.,* 1992; Vuyts and Degeest, 1999). Generally, low EPS yields have been reported in different LAB genera and species including *Lactobacillus, Enterococcus, Leuconostoc* and *Weissella,* although a few high yielder have also been reported (Mozzi *et al.,* 2006; Badel *et al.,* 2011). The diversity in production of EPS by the EPS producing isolates from *Mursik* is mainly attributed to their genetic diversity (species and strain genetic variation), their variation in growth medium requirements and growth condition (temperature, pH, oxygen potential) requirements. As earlier discussed, the genetic potential for a given LAB greatly affects its production of EPS. EPS yield, monomers composition and EPS structure by a given LAB depend on the type of eps-*genes* and the *glycosyltranferases* (EPS specific enzymes) that it contains (Kleerebezem *et al.,* 2002; Welman and Maddox, 2003). There is a correlation between activities of certain enzyme such as phosphoglucomutase, UDP-galactose -4-epimerase and UDP-glucose pyrophosphorylase and formation of sugar nucleotides (Degeest and Vuyst, 2000).

Growth medium composition, mainly the carbon and nitrogen sources, greatly affect EPS yields by different LAB. According to Looijesteijn *et al.,* (1999) and Degeest and Vuyst, (2000), different LAB species vary in their abilities to metabolize different carbon sources and synthesize different EPS from them. EPS producing LAB require sugars (carbon source) for generation of energy during their metabolic activities (Cerning, 1990; Broadbent *et al.,* 2003), synthesis and transportation of the sugar nucleotides and synthesis of the EPS polymers (Welman and Maddox, 2003). Some LAB have been shown to produce different EPS yields and EPS composition from different carbon sources and this is mainly due to their abilities to utilize the carbon sources (Vuyst and Degeest 1999; Degeest and Vuyst, 2000). The growth medium that was used for the analysis

of EPS production by the EPS producing LAB from *Mursik* was whey, whose major carbon source is lactose. Therefore, lactose may have been a limiting factor for production of EPS by some of the isolates. Growth condition (temperature, pH) may have also been a limiting factor for EPS production by some isolates. This is because different LAB strains have different optimum temperature and pH conditions at which they produce EPS (Zisu and Shah, 2003; Vaningelgem *et al.*, 2004b). Mesophillic LAB mainly produce higher EPS yields at sub-optimal growth conditions, when there is minimum demand for cell wall polymers, and hence most of the enzymes, sugar nucleotides and isoprenoid lipid carriers are directed towards EPS biosynthesis (Cerning *et al.*, 1992; Cerning, 1995; Vuyst and Degeest, 1999; Degeest *et al.*, 2001). Similarly, EPS production by LAB is affected by the pH of the medium. According to Vuyst and Degeest (1999), most LAB species produce optimum EPS yields at pH conditions close to pH 6. In addition, EPS levels are generally higher in cultures subjected to controlled pH conditions than those in un-controlled pH conditions. Zisu and Shah, (2003), in their study, established that low EPS yields were obtained when *Steptococcus thermophilus* species were incubated under un-controlled pH system compared to a when they were incubated under pH-controlled system. Propagation of the EPS producing isolates from *Mursik* in this study was done under un-controlled pH condition and this may have contributed to the observed low yields. Production of EPS degrading enzymes (*glycohydrolases)* by some LAB strains (Cerning *et al.*, 1992; Cerning, 1995; Zisu and Shah, 2003).

4.2.4 Production of EPS in *Mursik* during Traditional Fermentation Process.

Mursik is a product of spontaneous fermentation composed of mixed and undefined culture. These cultures naturally come from the fermentation container, gourd, and some are from the milk. During traditional fermentation process, the rate of growth for the LAB, *Lactococcus* and *Lactobacillus/Leuconostoc*, were both initially low (day one and two), but after day two, the *Lactococcus* species consistently increased faster than the *Lactobacillus/Leuconostoc species* and reached the maximum counts of 8.8 Log cfu/ml on day four, after which they entered the stationary phase (day four to five). On the other hand, the *Lactobacillus* growth rate remained slow between day two and four, and then they increased to the maximum counts of 8.4 mg/L Log cfu/ml on day five. This growth pattern is attributed to the influence of symbiotic and/or competitive relationship between *Lactococcus* and *Lactobacillus* species. Growth of the *Lactococcus* species was more favoured compared to the growth of *Lactobacillus* species between day two and day four. After day four, the *Lactococcus* species growth entered the stationary phase, while the growth rate for *Lactobacillus* species increased. The initial predominance of

Lactococcus species in the *Mursik* was either because the initial growth conditions in the culture were more favourable to them than *Lactobacillus* species or they competed for growth factors such as nutrients better than *Lactobacillus* species. In addition, the nutrients in milk may have not been readily available for assimilation by the *Lactobacillus* species at the initial stages of growth, either due to inability to hydrolyze certain biomolecules. Growth conditions may have improved later due to hydrolysis of the nutrients by the *Lactococcus* species resulting in the observed increase in the growth rate of the *Lactobacillus* species at between day four and five. In addition, growth of the *Lactococcus* species may have modified the conditions in medium hence making them favourable for growth of the *Lactobacillus* species. According to Phumkharchorn *et al.,* (2010), during fermentation in mixed cultures, certain metabolites such as organic acids (lactic acid, acetic acid, formic acid), carbon dioxide, hydrogen peroxide, bacteriocins, amino acids and peptides are produced and some may stimulate growth of some species in the culture, especially those which depend on others for metabolism of complex nutrients which they cannot hydrolyze or assimilate. According to Georgalaki *et al.,* 2000, LAB species generally have limited abilities to synthesize amino acids and peptides for their growth, but they obtain them from milk by hydrolyzing milk proteins. Variation in growth requirements by the different LAB in a mixed culture affects the microbial ecology of the culture, and may result in shift of ecology or culture succession at certain stages of growth.

Production of EPS in *Mursik* during traditional fermentation process was not growth associated. Higher EPS levels were produced when LAB growth rate was low and vice versa. The EPS level increased steadily between day one and day two, after which it rapidly decreased between day two and three. Another rapid increase was observed between day three and four after which it slightly decreased between days four and five. The increase was mainly observed when the rate of LAB growth was low, between day one and two, and day three and four, and decreased was observed when the rate of LAB growth was high, between day two to three (high growth rate for *Lactococcus species*). The EPS levels rapidly decreased between day two and three implying that, other than retarded EPS biosynthesis, EPS was also being broken down or lost. According to Vuyst and Degeest (1999) and Degeest *et al.,* (2001), EPS production in Mesophillic LAB occurs mainly when the rate of LAB growth is low since the enzymes and sugar nucleotides are mainly diverted towards EPS production and not towards cell wall polymer formation. Similarly, EPS levels have been observed to be high when mesophillic cultures are propagated at sub-optimal growth conditions such as low temperatures and pH, because at such conditions, bacterial cell

growth is also low (Cerning *et al.,* 1992; Cerning, 1995; Vuyst and Degeest, 1999; Degeest *et al.,* 2001). The decrease in EPS levels observed during Mursik incubation, between day 2 to 3 and day 4 to 5, is attributed EPS breakdown by EPS degrading enzymes (*glycohydrolase*s) which may have been produced by some LAB. These *glycohydrolases* hydrolyze EPS in the culture resulting in decrease of the EPS yields (Vuyst *et al.,* 1998; Zisu and Shah, 2003; Vuyst and Degeest, 1999). This occurs mostly at the stationary growth phase or during prolonged incubation periods (Zisu and Shah, 2003; Vuyst *et al.,* 1998; Vuyst and Degeest, 1999). EPS degradation is affected by certain growth conditions especially temperature and pH as well as bacteria strain (Zisu and Shah, 2003, Vuyst *et al.,* 1998).

4.2.5 Effect of Growth medium and Incubation period on Exopolysaccharides production by *Mursik* Culture.

Exopolysaccharide (EPS) yields by the EPS producing LAB vary depending on several factors which include the LAB strain, composition of the growth media and growth conditions. Species and strain variations are mainly due to their genetic differences. Studies by Mozzi *et al.,* (2006) established that EPS producing LAB strains produce different types of EPS mainly based on monomer composition, yield and molecular mass. EPS production phenotype in the EPS producing LAB is mainly controlled by the *eps* genes that they posses and they vary with the species and strains (Welman and Maddox, 2003; Mozzi *et al.,* 2006). The *eps* genes determine the type of *glycosyltransferases* to be synthesized by a given LAB strain, and hence the type of EPS to be produced (Welman and Maddox, 2003; Badel *et al.,* 2011). One particular homopolysaccharide is synthesized by one specific *glycosyltransferase* whereas biosynthesis of one heteropolysaccharide is a complex process which involves several steps and intervention of several *glycosyltranferases* and housekeeping enzyme (Welman and Maddox, 2003; Badel *et al.,* 2011). In this study, it was established that both *Mursik* and Mesophillic milk culture (control) produced different EPS yields. This difference was attributed to variation in their LAB (culture) composition and their variation in their genetic potentials for production of EPS. Basically, *Mursik* and Mesophillic milk cultures vary in their LAB composition, whereby *Mursik* contains an un-defined mixed culture that is composed of different species of *Lactococcus* and *Lactobacillus* (section 4.2.3) and Mesophillic milk contains a mixture of two strains of *Lactococcus lactis* species. The EPS producing isolates from *Mursik* produced different EPS yields (4.2.3) and this was attributed to variation in their genetic potentials for EPS production.

Growth medium composition, mainly carbon and nitrogen, influence EPS production by LAB. Studies have shown that the nature and composition of the growth medium (carbon, nitrogen, mineral salts and vitamins) affect EPS production by a given strain (Looijesteijn *et al.,* 1999; Ricciardi *et al.,* 2002; Zisu and Shah, 2003; Vaningelgem *et al.,* 2004b). Energy production is very essential during EPS biosynthesis since it is required for production of large numbers of sugar nucleotides, polymer formation, bacterial cell growth and other metabolic activities in the cell (Badel *et al.,* 2011; Broadbent *et al.,* 2003; Welman and Maddox, 2003; Jolly *et al.,* 2002). Carbon (sugar) is required for synthesis of sugar nucleotides such as UDP-glucose, UDP-galactose and dTDP-rhamnose which are the donors of the monomers during biosynthesis bacterial polysaccharides including EPS (Degeets and Vuyst, 2000; Degeest *et al.,* 2001; Welman and Maddox, 2003; Badel *et al.,* 2011). The nature and concentration of carbon source strongly influences EPS production by LAB (Degeets and Vuyst, 2000; Vaningelgem *et al.,* 2004a; Ruas-Madiedo and Reyes-Gavilan, 2005), but the most suitable for EPS production mainly depends on the ability of the bacteria strain to utilize it (Degeest and Vuyst, 2000; Ruas-Madiedo and Reyes-Gavilan, 2005). Some LAB can produce high amount of EPS on one type of carbon source than on another. This was established by Looijesteijn *et al.,* (1999) where *Lactobacillus delbrueckii bulgaricus* produced up to three times EPS yield on glucose than on fructose as a carbon source. In this study, it was observed that *Mursik* culture produced higher EPS yields in the media that were supplemented with sucrose (Reconstituted skim milk with 5 % sucrose, RSM-S, and Whey with 5 % sucrose, WH-S) than in the non-sucrose supplemented media (Reconstituted skim milk, RSM, and whey, WH). The main carbon source in non-supplemented media (RSM and WH) was lactose whereas in the supplemented media it was mainly sucrose. From the findings, it was clear that sucrose stimulated EPS production by *Mursik* culture better than in the Mesophillic milk culture. Studies have shown that addition of sucrose into the growth media for EPS production increase EPS yields by certain EPS producing LAB specie which include *Lactobacillus sanfranciscensis* LTH2590 (Korakli *et al.,* 2003), *Weissella* species (Tayuan *et al.,* 2011) and *Leuconostoc mesenteries* (Moosavi-Nasab *et al.,* 2010). Nitrogen sources (proteins) are also important nutrients for EPS production by LAB. Enough nitrogen is required for formation of the various cell components and enzymes required during EPS biosynthesis (De Vuyst *et al.,* 1998; Broadbent *et al.,* 2003; Korakli and Vogel, 2006). Complex proteins are good nitrogen sources since they contain several peptides and amino acids that are important for both cell growth and EPS production (Vaningelgem *et al.,* 2004(a); Vaningelgem *et al.,* 2004(b); Zisu and Shah, 2003).

By products such as whey hydrolysates from the dairy industry contain peptides and amino acids that are readily available for assimilation by the bacteria cells (Vaningelgem *et al.,* 2004(b)). Milk proteins such as whey concentrates, whey permeate and casein have been shown to improve EPS yields by certain LAB such as *Lactobacillus rhamnosus* (Bergmaire *et al.,* 2003), *Streptococcus thermophilus* (Zisu and Shah, 2003) and *Lactobacillus delbrueckii bulgaricus* (Ricciardi *et al.,* 2002). According to Georgalaki *et al.,* (2000), LAB species have limited abilities for synthesizing amino acids which are essential for their growth. However, they can hydrolyze milk proteins into the simple peptides and amino acids for their assimilation (Georgalaki *et al.,* 2000). Cheese whey is a by-product from cheese manufacture that consist of whey proteins, mainly lactoalbumin and Lactoglobulin (≈ 20%) (Walstra *et al.,* 2006). It also contains the water soluble kappa-casein / glycomacropeptide (0.8 -1 % protein) that is formed after cleavage of kappa-casein by chymosin enzyme during cheese curd formation and mineral salts (Walstra *et al.,* 2006). However, the influence of the nitrogen source on EPS production depends on the LAB strain and its EPS production Kinetics. EPS production by most thermophillic LAB is growth associated hence their EPS products are classified as primary metabolites whereas in mesophillic LAB it is non-growth associated hence are secondary metabolites(Degeest *et al.,* 2001). In growth associated EPS production, the rate of EPS production is highest at the exponential phase of LAB growth hence any approach that will increase LAB growth rate also increases the rate of EPS production (Degeest *et al.,* 2001). In non-growth associated EPS production, the rate of EPS production is high when the rate of LAB production is lowest, hence EPS yield is optimum towards the end of stationary phase (Degeest *et al.,* 2001). Therefore, in mesophillic LAB, any approach that will result in slow rate of bacterial growth will optimize EPS yield. Therefore, increased nitrogen may increase EPS yield in thermophillic LAB but reduce the yield in mesophillic LAB. Some LAB can produce different LAB at different stages of growth, one as a primary metabolite and another as a secondary metabolite (Degeeest *et al.,* 2001). In this case, nitrogen source will influence the composition of the primary metabolite EPS in the overall EPS yield by the given LAB.

Incubation period has been shown to affect EPS production by LAB. This was also observed in this study, both in *Mursik* and Mesophillic milk (control) cultures. It was established that shorter incubation periods (6 -12 hours) were insufficient for EPS production whereas prolonged incubation periods (30 hours) resulted in decrease in EPS levels. Optimum incubation period for EPS production by *Mursik* culture was 18 – 24 hours, the best being 24 hours. Production of EPS in mesophillic LAB is not growth associated (Cerning, 1995; Vuyst and

Degeest, 1999; Degeest *et al.*, 2001), whereas in thermophillic LAB, it is mainly growth associated (Ricciardi *et al.*, 2002; Bergmaire *et al.*, 2003; Vaningelgem *et al.*, 2004(b)). Studies have shown that EPS degradation occurs upon prolonged incubation of some LAB cultures (Vuyst *et al.*, 1998; Ricciardi *et al.*, 2002), and this is mainly due to production of *glycohydrolases* (Cerning *et al.*, 1992; Cerning, 1995; Vuyst *et al.*, 1998; Zisu and Shah, 2003). According to Ricciardi *et al.*, 2002 and Vuyst *et al.*, (1998), EPS degradation may be induced by certain environmental conditions such as pH and temperature surrounding the cell. However, some LAB species such as *Streptococcus thermophillus* ST 111 do not produce EPS degrading enzymes (Vaningelgem *et al.*, 2004(b)).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS.

5.1 Conclusions.

The findings of this study showed that the traditional *Mursik* produced in Njoro District contains Exopolysaccharides (EPS) and a variery of EPS producing Lactic acid bacteria (LAB). The EPS producing LAB are predominated by the *Lactococcus* species. The EPS levels in *Mursik* are generally low and this is attributed to the low potential for EPS production by the *Lactococcus* species and the other LAB species. The EPS producing LAB isolates produce generally low EPS yields which vary depending on the genus and species. The EPS yield in traditional *Mursik* is influenced by incubation period, the optimum level being obtained after four days. Incubation period and growth medium composition, mainly the carbon source also influences the EPS yield by the *Mursk* culture. The optimum EPS levels by the *Mursik* culture (as starter) are obtained after incubation for 18 to 24 hours at 20 ± 1 ºC and by supplementing milk with 5% sucrose. The optimum EPS yield in sucrose supplemented milk is obtained after incubation for 24 hours at 20 ± 1 ºC. Prolonged incubation of the *Mursik* (over 24 hours) results in reduction of EPS levels.

5.2 Recommendation.

Based on the findings of this study, *Mursik* should be developed as functional food product by optimizing its EPS yields during fermentation. EPS yields in traditional *Mursik* should be optimized by incubating the milk for 4 days. Also, a defined *Mursik* culture should be developed and preserved for production of *Mursik* using modern technologies. This wil guarantee production of good quality and safe product, and application of technologies for improved EPS yield. The EPS yields by *Mursik* culture should be improved by supplementing milk with sucrose and incubating for 24 hours at 20 ± 1 ºC. In future, more studies should be done to establish the effects of other sugars (other than sucrose) such as glucose, fructose, galactose, maltose among others, and other growth conditions (temperatures, pH levels and oxygen potential) on the EPS yield by the *Mursik* culture and the EPS producing LAB in *Mursik*. In addition, monomer composition and molecular mass of the EPS produced by the *Mursik* culture and EPS producing LAB from *Mursik* should be established in order to determine their application in food as sources of EPS and starter cultures for production of functional foods, improvement of food rheological properties, and sources of EPS specific enzymes (*glycosyltransferases*) for biotechnological modification of EPS production by other LAB species.

6.0 REFERENCES

Adebayo-Tayo, B. C., Onilude, A. A. 2008. Screening for Lactic acid Bacteria strains Isolated from some Nigerian fermented foods for Exopolysaccharide production. World Applied Science Journal, 4: 741 - 747.

Adebayo-Tayo, B. C., Onilude, A. A., Joe, A. U. 2011. Efficiency of Formulated Media on Production of Bio-Molecules by some selected EPS- Producing Lactic acid bacteria strains. Electronic Journal of Environmental, Agriculture and Food Chemistry, 10: 1837 - 1847.

Anukam, K. C., Reid, G. 2007. Probiotics: 100 years (1907-2007) after Elie Metchnikoff's Observation. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, A. Méndez-Vilas (Ed.): 466 - 474.

Awad, S., Hassan, A. N., Muthukumarappan. 2005. Application of Exopolysaccharides-producing cultures in Reduced fat Cheddar cheese: Texture and Melting properties. Journal of Dairy Science, 88: 4204 - 4213.

Ayala-Hernandez, I., Gogg, H. D., Corredig, M. 2008. Interactions between Milk Protein and Exopolysaccharides produced by *Lactococcus lactis* observed by scanning Electron Microscopy. Journal of Dairy science, 91: 2583 - 2590.

Badel, S., Bernardi, T., Michaud, P. 2011. New perspectives for Lactobacilli exopolysaccharides.

Barrow, G.I., Felthman, R. K. A. 1993. Barrow and Felthman, Manual for Identification of Medical bacteria, Third edition. Cambridge University Press: 188 - 238.

Bergmaier, D., Champagne, C. P., Lacroix, C. 2003. Exopolysaccharide Production during batch cultures with free and immobilized *Lactobacillus rhamnosus* RW-9595M. Journal of Applied Microbiology, 95 (5): 1049 - 1057.

Beukes, E. M., Bester, B. M., Mostert, J. F. 2001. The Microbiology of South African Traditional Fermented milks. International Journal of Food Microbiology, 63: 189 -197.

Bleau, C., Monges, A., Rashidan, K., Laverdure, J P., M. Lacroix, M., Calsteren, M.R., Millette, M, Savard, R., Lamontagne, L. Intermediate chains of Exopolysaccharides from Lactobacillus rhamnosus RW-9595M increase IL-10 production by macrophages. Journal of Applied Microbiology, 108: 666 - 675.

Branca, F and Rossi, L. 2002. The role of fermented milk in complementary feeding of young children: lessons from transition countries. European J. Clinical Nutrition, 56 (4): 16 - 20.

Broadbent, J. R., McMohon, D. J., Welker, D. L., Oberg, C. J., Moineau, S. 2003. Biochemistry, Genetics, and Application of Exopolysaccharide Producing in Streptococcus thermophilus: A Review. Journal of Dairy Science, 86: 407 - 423.

Callaway, T. R., Edrington, T. S., Anderson, R. C., Harvey, R. B., Genovese, K. J., Kennedy, C. N., Venn, D.W., Nisbet, D. J. 2008. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Animal Health Research Reviews. 9 (2): 217 - 225.

Cerning, J. 1990. Exocellular Polysaccharides Produced by Lactic acid bacteria. Federation of European Microbiology Society, Microbiology Reviews, 87: 113 - 130.

Cerning, J., Bouillane, M. L., Desmazeaud, M. 1992. Isolation and characterization of Exopolysaccharides from slime producing Mesophillic Lactic acid bacteria. Journal of Dairy Science, 75: 692 - 699.

Cerning, J. 1995. Production of Exopolysaccharides by Lactic acid bacteria and dairy Propionibacteria. Lait, 75: 463 - 472.

Chichlowski, M., Croom, J., McBride, B.W., Havenstein, G.B., Koci, M.D. 2007. Metabolic and Physiological Impact of Probiotics or Direct-Fed-Microbials on Poultry: A Brief Review of Current Knowledge. Inter. J. Poultry Science.10: 694 -704.

Coeuret, V., Dubernet, S., Bernardeau, M., Gueguen, M., Vernoux, J. P. 2003. Review article - Isolation, Characterization and Identification of Lactobacilli focusing mainly on Cheeses and other Dairy products. Lait, 83: 269 - 306.

Cummings, J. H. and Macfarlane, G. T. 2002. Gastrointestinal effects of prebiotics. British J. Nutrition, 87 (2): S145 - S151.

Degeest, B., Vuyst, L. 2000. Correlation of activities of the Enzymes α-phosphoglucomutase, UDP-Galactose 4-Epimerase and UDP-Glucose Pyrophosphorylase with Exopolysaccharide Biosynthesis by Streptococcus thermophilus LY03. Applied and Environmental Microbiology, 66 (8): 3519 - 3527.

Degeest, B., Vaningelgem, F., Vuyst, L. 2001. Microbial physiology, Fermentation Kinetics, and Process Engineering of Heteropolysaccharide Production by Lactic acid bacteria. International Dairy Journal, 11: 747 - 757

Doleyres, Y., Schuab, L., Lacroix, C. 2005. Comparison of the functionality of exopolysaccharides production *in situ* or added as bioingredients on yoghurt properties. J. Dairy science, 88: 4146 - 4156.

Facklam, R., Hollis, D., Collins, D. 1989. Identification of Gram-Positive Coccal and Coccobacillary Vancomycin-Resistant bacteria. Journal of Clinical Microbiology, 27 (4): 724 - 730.

Facklam, R., Elliot, J. A. 1995. Identification, Classification, and Clinical relevance of Catalase-Negative, Gram-Positive Cocci, Excluding the Streptococci and Enterococci. Clinical Microbiology Reviews, 8 (4): 479 - 495.

Food and Agriculture Organization of the United Nations, Rome (FAO). 1990. The Technology of Traditional Milk Products in Developing Countries. FAO Animal Production and Health, Paper 85.

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). 2001. Health and Nutritional Properties of Probiotics in Food including Powder Milk and Live Lactic Acid Bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report. (http://www.who.int/foodsafety/publications/fs_management /en/probiotics.pdf.

Georgalaki, M.D., Sarantinopoulos, P., Ferreira, E.S., De Vuyst, L., Kalantzopoulos, G., Tsakalidou, E. 2000. Biochemical Properties of Streptococcus macedonicus strains isolated from Greek Kasseri cheese. Journal of Applied Microbiology, 88: 817 - 825.

Hassan, A. N., Ipsen, R. Janzen, T. Qvist, K. B. 2003. Microstructure And Rheology of Yoghurt made with Cultures Differing only in their ability to Produce Exopolysaccharides. Journal of Dairy Science, 86: 1632 - 1638.

Hassan A. N. 2008. ADSA Foundation Award: Possibilities and Challenges of EPS producing Lactic cultures in Dairy foods. Journal of Dairy Science, 91: 1282 - 1298.

Heidi, H. 2009. Differences in tree and shrub species on farms in the East Mua catchment, Kenya – considering the agro-ecological zone and ethnicity. Masters thesis, international Masters in Natural resources Management and Ecological Engineering. BOKU Vienna.

Jolly, L., Vinicent, J. F., Duboc, P., Neeser, J. 2002. Exploiting Exopolysaccharides from Lactic acid bacteria. Antonie Van Leeuwenhoek, 82: 367 - 374.

Kaplan, H. and Hutchins, W. R. 2000. Fermentation of Fructooligosaccharides by Lactic acid Bacteria and Bifidobacteria. Applied and environmental Microbiology, 66: 2682 - 2684.

Katina, K., Maina, H. N., Juvonen, R., Flander, L., Johansson, L., Virkki, L., Tenkanen, M., Laitila, A. 2009. In situ production and analysis of *Weissella confusa* dextran in wheat sourdough. Food Microbiology, 26: 734 - 743.

Khurana H. K., Kanawjia, S. K. 2007.Recent trends in Development of fermented milks. Current Nutrition and Food Science, 3: 91 - 108.

Kim, Y., Oh, S., Yun, H.S., O h, S., Kim, S.H. 2010. Cell-bound Exopolysaccharide from Probiotic bacteria Induces Autophagic cell death of Tumour cells. Letters in Applied Microbiology, 51: 123–130

Kleerebezem, M., Boels, C. I., Groot, M. N., Mierau, I., Sybesma, W., Hughenholtz, J. 2002. Metabolic Engineering of *Lactococcus lactis*: The impact of genomics and metabolic modeling. Journal of Biotechnology, 98: 199 - 123.

Kojic, M., Vujcic, M., Banina, A., Cocconcelli, P., Cerning, J., Topisirovic, L. 1992. Analysis of Exopolysaccharide Production by Lactobacillus casei CG11, Isolated from Cheese Applied And Environmental Microbiology, 58 (12): 4086 - 4088

Kolida, S., Gibson. R.G. 2007. Prebiotic Capacity of Inulin-Type Fructans 1–3. J. Nutr. 137: 2503S - 2506.

Korakli, M., Ganzle, M.G., Vogel, R.F. 2002. Metabolism by Bifidobacteria and Lactic acid bacteria of Polysaccharides from Wheat and Rye, and Exopolysaccharides produced by Lactobacillus sanfranciscensis. Journal of Applied Microbiology, 92. 958 - 965

Korakli, M., Pavlovic, M., Ganzle, M. G., Vogel, R. F. 2003. Exopolysaccharide and Kestose Production by *Lactobacillus sanfranciscensis* LTH2590. Applied and Environmental Microbiology: 2073 - 2079.

Korakli, M., Vogel, R. F. 2006. Structure/function relationship of homopolysaccharide producing glycansucrases and therapeutic potential of their synthesized glycans. Applied Microbiol. Biotechn. 71: 790 - 803.

Kumar, A. S., Mody, K., and Jha, B. 2007. Bacterial exopolysaccharides – a perception. J. Basic Microbiology. 47: 103 - 117.

Lebeer, S., Claes, I. J. J., Verhoeven, T. L. A., Vanderleyden, J., Keersmaecker, S. C. J. 2010. Exopolysaccharides of Lactobacillus Rhamnosus GG form a Protective Shield against Innate Immune Factors in the Intestine. Microbial Biotechnology 4(3): 368 - 374

Looijesteijn, P. J., Boels, I. C., Kleerebezem, M., Hugenholtz, J. 1999. Regulation of Exopolysaccharide production by Lactococcus lactis subsp.cremoris by sugar source. Applied and Environmental Microbiology, 65. (11): 5003 - 5008.

Lore, T.A., Samuel K. Mbugua, S.K., Wangoh, J. 2005. Enumeration and identification of microflora in *suusac*, a Kenyan traditional fermented camel milk product. Lebensm-Wiss. Technol. 38: 125 - 130

Macfarlane, S., Macfarlane, G. T., Cummings, J. H. 2006. Review article - Prebiotics in the gastrointestinal tract. Alimentary pharmacology and Therapeutics, 24: 701 - 714.

Macfarlane, G. T., Steed, H., Macfarlane, S. 2008. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. J. Applied Microbiology, 104: 305 - 44.

Mackey, T., Lejeune, V., Janssens, M., Wauters, G. 1993. Identification of Vancomycin-Resistant Lactic acid bacteria Isolated from Human. Journal of Clinical Microbiology, 31(9): 2499 - 2501.

Mathara J.M., Miyamoto T., Koaze H., Kiiyukia C., and Yoneya T. 1995. Production of Traditional Fermented Milk in Kenya, a Review: 257 - 263.

Mathara J.M., Schillinger, U., Kutima, P.M., Mbugua, S.K., Guigas, C., Franz. C., Holzapfel, W. H. 2008. Functional properties of *Lactobacillus plantarum* strains isolated from Maasai Traditiona1 Fermented Milk products in Kenya. Current Microbiology. 56: 315 - 321.

Miles, L. 2007. Are probiotics beneficial for health? J. compilation, 32: 2 - 5.

Milliere, J. B., Mathot, A., Schmitt, P., Divies, C. 1989. Phenotypic Characterization of Leuconostoc Species. Journal of Applied Bacteriology, 67: 529 – 542.

Mokua, R.A. 2004. Effect of Kenyan fermented milk on *Eschericia coli*. M.SC.dissertation, University of Wisconsin-Stout, Menomonie.

Moosavi-Nasab, M.; Gavahian, M.; Yousefi, A.R; Askari, H. 2010. Fermentative production of Dextran Using Food industry wastes. World academy of Science, Engineering and Technology 68: 1231- 1233.

Mozzi, F., Rollan, G., Giori, G. S., Valdez, G. F. 2001. Effect of Galactose and Glucose on the Exopolysaccharide Production and Activities of Biosynthetic Enzymes in *Lactobacillus casei* CRL 87. Journal of Applied Microbiology, 91: 160 – 167.

Mozzi, F., Vaningelgem, F., Herbert. E. M., Van der Meulen, R., Moreno, M. R. F., De Valdez, G. F., De Vuyst, L. 2006. Diversity of Heteropolysaccharide- Producing Lactic Acid Bacterium Strains and their Polymers. Applied and Environmental Microbiology: 4431- 4435

Mureith, W., Biggelaar, C. D., Wesekania, E. W., Kamau, K., Gatundu, C. 2000. Management of trees used in Mursik (Fermented milk) Production in Tranzoia District, Kenya. Journal of Ethnobiology, 20 (1): 75 – 91.

Nakamura, T., Sugai, M., Ozawa, A. N., Ariga, H., Koaze, H., Kiiyukia, C., Arai, I., Urashima, T. (1999). Microbiological properties of Maziwa Lala, a Kenyan Traditional Fermented Milk of Maasai Community, Kenya. Milk science, 48 (1)

O'Connor, E. B., Barrett, E., Fitzgerald, G., Hill, C., Stanton, C., Ross, R. P. 2005. Production of Vitamins, Exopolysaccharides and Bacteriocins by Probiotic Bacteria.Ch.8 in; Probiotic Dairy Products, Tamime, A (Ed.). Blackwell Publishing Ltd: 167 – 194.

Olano-Martin, E., Mountzouris, K. C., Gibson, G. R., Rastal A. R. 2000. In Vitro fermentability of dextran, oligodextrin and Maltodextrin by Human gut bacteria. British Journal of Nutrition, 83: 247 – 255.

Patel, S., Goyal, A. 2012. The current trends and future perspectives of prebiotics research: A review. 3 Biotech, (2): 115 – 125.

Parvez, S., Malik, K.A., Ah Kang, S., Kim, H. Y. 2006. Probiotics and their fermented food products are beneficial for health. J. Applied Microbiology, 100: 1171–1185

Ramos, A., Boels, I. C., De Vos W. M., Santos, H. 2001. Relationship between glycolysis and exopolysaccharide biosynthesis in *Lactococcus lactis*. Applied and Environmental Microbiology, 67 (1): 33 – 41.

Rattanachaikunsopon, P., Phumkhachorn, P. 2010. Lactic Acid Bacteria: Their Antimicrobial Compounds and Their Uses in Food Production. Annals of Biological Research, 1 (4): 218-228

Ricciardi, A., Parente, E., Crudele, M. A., Zanetti, F., Scolari, G., Mannazzu, I. 2002. Exopolysaccharide Production by Streptococcus thermorphilus SY: Production and Preliminary characterization of the polymer. Journal of Applied Microbiology, 97 (2): 297 – 306.

Ruas-Madiedo, P., Reyes-Gavilan, G. C. 2005. Invited review**:** Methods for the screening, isolation and characterization of exopolysaccharides produced by Lactic acid Bacteria. J. Dairy science, 88: 1843 - 1856.

Ruas-Madiedo, P., Gueimonde, M., Reyes-Gavilan, G.C., Salminen, S. 2006. Short communication: Effects of polysaccharides isolated from "Viili" on adhesion of Probiotics and pathogens to the intestinal mucus. J. Dairy science, 89: 2355 - 2358.

Russo, P., Lopez, P., Capozzi, V., Palencia, P. F., Duenas, T.M., Spano, G., Fiocco, D. 2012. Beta-Glucans Improve Growth, Viability and Colonization of Probiotic Microorganisms. International Journal of Molecular Sciences, 13: 6026 - 6039.

Saija, N., Welman, A.D., Bennett, R. J. 2010. Development of a Dairy based Exopolysaccharide Bioingredient. International Dairy Journal, 20: 603 - 608.

Salazar, N., Gueimonde, M., Hernandez-Barranco,A. M., Ruas-Madiedo, P., Clara G. de los Reyes-Gavilan, C.G. 2008. Exopolysaccharides Produced by Intestinal *Bifidobacterium* Strains Act as Fermentable Substrates for Human Intestinal Bacteria. Applied and Environmental Microbiology, 4737 - 4745

Sanni A. I. ; Onilude A.A. ; Ogumbanwo S.T.; Fadahunsi I. F.; Afolabi R.O. 2002.Production of Exopolysaccharides by LAB isolated from traditional fermented foods in Nigeria. Eur. Food Res Technol. 214. pp: 405 - 407

Savadogo, A., Ouattara C. A. T., Savadogo, P. W., Barro, N.., Ouatarra, A. S., Traore, A. S. 2004. Identification of exopolysaccharide-producing Lactic Acid Bacteria from Burkina Faso fermented milk samples. African J. Biotechnology, 3(3): 189 - 194

Shih, F. 2003. Probiotics and Prebiotics as Functional Food Ingredients. Nahrung/Food, 47 (5): 285- 285.

Sneath P. H.A., Mair, N. S., Sharpe, M. E., Holt, J. G., 1986. Berge's Manual of Systematic Bacteriology, Volume 2. Williams and Wilkins, Baltimore, U.S.A.

Simpson, W. J., Hammond, J. R. M., Miller, R. B. 1989. Avoparcin and Vancomycin; Useful antibiotics for isolation of brewery Lactic acid bacteria. Journal of bacteriology, 64: 299 – 309.

Skriver, A., Karychev, R. 2007. Modifying yoghurt texture by exopolysaccharide producing Lactic acid bacteria. CHR, HANSEN, A/S: 1 -5.

Tayuan, C.; Tannok, G. W.; Rodtong, S.; 2011. Growth and Exopolysaccharide production by Weissella species from low cost substitutes for sucrose. African Journal of Microbiology research, Vol. 5(22); pp: 3693-3701.

Thamaraj, N., Shah. N. P. 2003. Selective Enumeration of *Lactobacillus delbrueckii ssp.bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus*, and *Propionibacteria*. Journal of Dairy Science Association, 86: 2288 - 2296.

Thunnel, R. K. 1995. Symposium: The Dairy Leuconostoc, Taxonomy of the Leuconostoc. Journal of Dairy Science, 78 (11): 2514 - 2522.

Tieking, M., Korakli, M., Ehrmann, M. A., Ganzle, M. G., Vogel, R. F. 2003. *In situ* production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of Lactic Acid Bacteria. Applied and Environmental Microb. 69: 945 - 952.

Toma, M. M., Pokrotnieks J. 2006. Probiotics as functional foods: Microbiological and Medical aspects. Acta Universitaillustis Latviencis, Vol. 710, Biology: 117 -129.

Van den Broek, L. A. M., Hinz, S. W.A., Beldman, G., Vincken,J., Voragen, A. G. J. 2008. Bifidobacterium carbohydrases - their role in breakdown and synthesis of (potential) prebiotics. Mol. Nutr. 52: 146 - 163

Vaningelgem, F., Zamfir, M., Mozzi, F., Andriany, T., Vancanneyte, M., Swings, J., De Vuyst, L. 2004. Biodiversity of exopolysaccharides produced by *Streptococcus thermophilus* strains is reflected in their production and their molecular and Functional characteristics. Applied and Environmental Microb.70(2): 900 - 912.

Vaningelgem, F., Zamfir, M., Andriany, T., De Vuyst, L. 2004. Fermentation conditions affecting the bacterial growth and exopolysaccharide production by *Streptococcus thermophilus* ST 111 in milk based medium. J. Applied Microb. 97(6): 1257 - 1273

Vasilean, L., Segal, R. 2011. The influence of biosynthesized exopolysaccharides on some characteristics of fermented dairy products. The Annals of the University Dunarea de Jos of Galati, Fascicle VI-Food Technology, 35 (1): 71 - 76.

Vasileva, T., Ivanova, I., lliev, I. 2010. Glucooligosaccharides synthesis by glycotransferases from mutant strain *Leuconostoc Mesenteroides* M2860 and their prebiotic potential. Biotechnol. and Biotechnol. Eq. 24: 553 - 557.

Vuyst, L., Vanderveken, F., Ven, S.V. Degeest, B. 1998. Production by and Isolation of Exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth associated biosynthesis. Journal of Applied Microbiology, 84: 1059 – 1068.

Vuyst, L., Degeest, B. 1999. Heteropolysaccharides from Lactic Acid Bacteria. Federation of European Microbiology Societies Microbiology Reviews, 23: 153 - 177.

Walstra, P., Wouters, J. T. M., Geurts, T. J. 2006. Dairy Science and Technology. Second Edition. Taylor and Francis group, LLC.1 - 763.

Woodmansey, E. J. 2007. Intestinal bacteria and ageing. J. Applied Microb. 102: 1178 - 1186

Zisu, B. and Shah, N. P. 2003. Effects of pH, Temperature, Supplementation with whey protein concentrates, and Adjunct cultures on the production of exopolysaccharides by *Streptococcus thermophilus* 1275. J. Dairy science, 86: 3405 - 3415.

7.0 APPENDICES

Appendix 1: *Mursik* Samples that were collected for analysis from Njoro District

	Sample code	Household (Owner's home	Division	Sample particulars
1	MH1	Scholar Koech	Mauche south	3 days old, boiled milk, made in gourd smoked with *Senna didymobotrya* (Senetwet).
2	MH2	Alice Milgo	Mauche North	3 days old, boiled milk, made in gourd smoked with '*Sumbeywe*t' (botanical name not found).
3	MH3	Rhoda Malel	Mauche North	3 days old, boiled milk, made in gourd smoked with *Senna didymobotrya.*
4	MH4	Ann Langat	Mauche North	3 days old, boiled milk, made in gourd smoked with *Senna didymobotrya.*
5	MH5	Susan Manyei	Mauche North	3 days old, boiled milk, made in gourd smoked with *Senna didymobotrya*.
6	MH6	Rebecca Taputany	Mauche South	3 days old, Raw milk, made in gourd smoked with *Senna didymobotrya.*
7	MH7	Grace/James Mutai	Mauche South	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*
8	MH8	Rael Kipyegen	Mauche South	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*
9	MH9	Mrs. Ruto	Mauche South	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*
10	MH10	Lilian Rono	Mauche South	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*
11	MH11	Rhoda Langat	Mauche North	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
12	MH12	Ann Cheptoo	Mauche North	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*
13	MH13	Alice Milgo	Mauce North	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*
14	MH14	Julius Ruto	Mauche North	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*
15	MH15	Nancy Yebei	Mauche North	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*
16	MH16	Rhoda Malel	Mauche North	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
17	MH17	Ann Langat	Mauche North	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya.*

	Sample code	Household (Owner's home)	Location	Sample particulars
18	MH18	Mauche Hotel	Mauche	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
19	MH19	Evalyn Kiprop	Njoro	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
20	MH20	Mary Langat	Njoro	3 days old, Raw milk, made in gourd smoked with *Senna didymobotrya*.
21	MH21	Margaret Kiptui	Njoro	3 days old, Raw milk, made in gourd smoked with *Senna didymobotrya*.
22	MH22	Alice Koech	Njoro	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
23	MH23	Sarah Cherogony	Njoro	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
24	MH24	Francis Wendot	Njoro	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
25	MH25	Vivian Ruto	Njoro	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
26	MH26	Margaret	Njoro	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
27	MH27	Rael Ngetich	Njoro	3 days old, Boiled milk, made in gourd smoked with '*Uswet*' (botanical name not found).
28	MH28	Mary Langat	Njoro	3 days old, Boiled milk, made in gourd smoked with '*Uswet*' (botanical name not found).
29	MH29	Alice Koech	Njoro	3 days old, Boiled milk, made in gourd smoked with '*Uswet'* (botanical name not found).
30	MH30	Sara Rogony	Njoro	3 days old, Boiled milk, made in gourd smoked with '*Uswet'* (botanical name not found).
31	MH31	Evalyn Kiprop	Njoro	3 days old, Boiled milk, made in gourd smoked with '*Uswet*' (botanical name not found).
32	MH32	Salama Hotel	Mukungugu	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
33	MH33	Chamgei Hotel	Mukungugu	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
34	MH34	Baraka Hotel	Mukungugu	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
35	MH35	Mama Esther Kiosk	Mukungugu	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.

	Sample code	Household (Owner's home)	Location	Sample particulars
36	MH36	Mama Kayai	Mukungugu	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
37	MH37	Mama Esther	Mukungugu	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
38	MH38	Belbur milk bar	Mukungugu	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.
39	MH39	Wileli Hotel	Mukungugu	3 days old, Boiled milk, made in gourd smoked with *Senna didymobotrya*.

Appendix 2: One-way ANOVA table for Lactic acid bacteria (Log cfu/ml) in *Mursik* from Njoro District.

Source of Variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
LAB Genus	1	34.954	34.9536	67.45	0.0001
Error	74	38.348	0.5182		
Total	75	73.301			

Appendix 3: Two-way ANOVA table for Lactic acid bacteria (Log cfu/ml) in *Mursik* per Location in Njoro District

Source of Variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Genus	1	66.708	66.70845	236.11	0.0001
Location	2	15.3770	7.68851	27.21	0.0001
Location x Genus	2	15.4518	7.72593	27.35	0.0001
Error	146	41.2498	0.28253		
Total	151	138.7872			

Appendix 4: One-way ANOVA table for the EPS levels in *Mursik* from different Household in Njoro District.

Source of Variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Household	25	9370.7936	374.8317	28.53	0.0001
Error	26	341.6291	13.1396		
Total	51	9712.4227			

Appendix 5: One-way ANOVA table for the EPS levels in *Mursik* from different locations in Njoro District.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Locations	2	1052.9658	526.4829	3.35	0.0529
Error	23	3614.5623	157.1549		
Total	25	4667.5281			

Appendix 6: Two-way ANOVA table for the effects of growth medium and incubation time on exopolysaccharide production by *Mursik* and Mesophillic milk culture.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Growth medium (G)	3	22386.844	7462.281	210.35	0.0001
Incubation time (T)	4	14828.851	3707.213	104.50	0.0001
Culture **(**C**)**	1	10816.831	10816.831	304.91	0.0001
GT interaction	12	9444.237	787.020	22.19	0.0001
GC interaction	3	23548.279	7849.426	221.26	0.0001
TC interaction	4	2005.232	501.308	14.13	0.0001
GTC interaction	12	5725.952	477.163	13.45	0.0001
Error	40	1419.011	35.475		
Total	79	90175.236			

Appendix 7: Phenotypic and Biochemical characterization of EPS Producing Lactic acid bacteria Isolated from *Mursik*

Genus		Cocci			Rods / Coccobacilli				
		Lactococcus lactis	*Pediococcus*	*Enterococcus*	*Lactobacillus* (Homofermentative)	*Lactobacillus* (Heterofermentative)	*Weissella* Arginine Negative	*Weissella* Arginine positive	*Leuconostoc*)
No of isolates		94	0	8	13	0	0	1	6
Cell morphology		Cocci	Cocci	Cocci	Rod	Rod	Rod	Coccobacilli	Coccobacilli
Cell arrangement		Single, paired, chained cells	paired tetrads	Single cells, paired, chains	Single cells, paired, chains	Single cells, paired, chains	Single cells, paired, chains	Single cells, paired, chains	Single cells, paired, chains
Phenotypic Characteristics									
1. Gas production from MRS broth		-	-	-	-	+	+	+	+
2. Arginine hydrolysis		+[(a)]	+	+	±	+	-	+	-
3.Growth Temperature	10 ºC	+	± (Most -)	+	±	±	±	±	+
	30 ºC	+	+	+	+	+	+	+	+
	35 ºC	+	+	+	+	+	+	+	+
	40 ºC	+	+	+	+	+	+	+	±
	45 ºC	-	± (Most +)	±	-[(b)]	±	±	+	-
4.Tolerance to Sodium Chloride (NaCl)	4%	+	+	+	+	±	±	±	±
	6.5%	±	±	±	±	±	±	±	-
5. Vancomycin resistance (30 mcg disc): S- susceptible R- resistant		S	R	S	R/S	R/S	R/S	R	R
6. Litmus milk Test: A- acid production, R- reduction of litmus colour, C- coagulum formed		+ (ARC)	+(ARC)	(+)ARC	± (ARC)	± (ARC)	± (ARC)	± (ARC)	± (ARC)

Characterization based on Garvie *et al.*, 1986; *Simpson et al.*, 1988; Millere *et al.*, 1989; ; Mackey *et al.*, 1993; Facklam and Elliot; 1995; Thunnel, 1995; Facklam *et al.*, 1989.

[(a)] *Lactococcus lactis subsp. cremoris* are negative arginine hydrolysis test.

Appendix 8: EPS levels produced by the Exopolysaccharide Producing Lactic acid bacteria isolated from *Mursik*.

EPS producing LAB isolate (Code)		LAB Genus / species	EPS level (mg/L Glucose equivalent) in Whey medium
1.	MH1 (1) M17	*Lactococcus lactis subsp.lactis*	57.00
2	MH1 (4) M17	*Leuconostoc mesenteroides*	193.55
3	MH2 (3) M17 -1	*Lactococcus lactis subsp.lactis*	nd
4	MH2 (3) M17 -2	*Lactococcus lactis subsp.lactis*	142.25
5	MH 3 (5) M17	*Lactobacillus species (Homofermentative).*	188.30
6	MH4 (1) M17	*Lactococcus lactis subsp.lactis*	114.00
7	MH4 (2) M17	*Enterococcus species*	100.35
8	MH4 (3) M17	*Lactococcus lactis subsp.lactis*	76.05
9	MH4 (4) M17	*Lactococcus lactis subsp.lactis*	87.50
10	MH4 (5) M17	*Lactococcus lactis subsp.lactis*	104.05
11	MH5 (1) M17	*Lactobacillus species (Homofermentative)*	268.65
12	MH5 (4) M17	*Lactococcus lactis subsp.lactis*	215.70
13	MH6 (2) M17	*Lactococcus lactis subsp.lactis*	218.90
14	MH6 (3) M17	*Lactococcus lactis subsp.lactis*	71.95
15	MH6 (5) M17	*Lactococcus lactis subsp.lactis*	199.50
16	MH7 (1) M17	*Lactococcus lactis subsp.lactis*	244.25
17	MH7 (2) M17	*Lactococcus lactis subsp.lactis*	134.45
18	MH7 (3) M17	*Lactococcus lactis subsp.lactis*	247.85
19	MH8 (2) M17	*Lactobacillus species (Homofermentative)*	44.30
20	MH8 (3) M17	*Leuconostoc mesenteroides*	nd
21	MH9 (1) M17	*Lactococcus lactis subsp.lactis*	167.25
22	MH9 (2) M17	*Lactococcus lactis subsp.lactis*	232.95

EPS producing LAB isolate (Code)		LAB Genus / species	EPS level (mg/L Glucose equivalent) in Whey medium
23	MH9 (3) M17	*Lactococcus lactis subsp.lactis*	182.15
24	MH9 (4) M17	*Lactococcus lactis subsp.lactis*	209.60
25	MH10 (1) M17	*Lactococcus lactis subsp.lactis*	162.70
26	MH10 (2) M17	*Lactococcus lactis subsp.lactis*	215.35
27	MH11 (4) M17	*Lactococcus lactis subsp.lactis*	191.00
28	MH11 (7) M17	*Lactococcus lactis subsp.lactis*	73.15
29	MH12 (1) M17	*Lactococcus lactis subsp.lactis*	155.65
30	MH12 (2) M17	*Lactococcus lactis subsp.lactis*	157.25
31	MH12 (5) M17	*Lactococcus lactis subsp.lactis*	169.83
32	MH13 (1) M17	*Leuconostoc mesenteroides*	nd
33	MH13 (4) M17	*Lactococcus lactis subsp.lactis*	171.80
34	MH13 (5) M17	*Leuconostoc mesenteroides*	nd
35	MH13 (7) M17	*Leuconostoc mesenteroides*	nd
36	MH13 (8) M17	*Leuconostoc mesenteroides*	nd
37	MH14 (2) M17	*Lactococcus lactis subsp.lactis*	nd
38	MH14 (4) M17	*Lactococcus lactis subsp.lactis*	293.78
39	MH15 (4) M17	*Lactococcus lactis subsp.lactis*	165.50
40	MH15 (6) M17	*Lactococcus species*	nd
41	MH16 (1) M17	*Lactococcus species*	nd
42	MH16 (4) M17	*Lactococcus species*	nd
43	MH16 (5) M17	*Lactococcus species*	nd
44	MH16 (6) M17	*Lactococcus species*	nd

EPS producing LAB isolate (Code)		LAB Genus / species	EPS level (mg/L Glucose equivalent) in Whey medium
45	MH17 (1) M17	*Lactococcus lactis subsp.lactis*	nd
46	MH17 (3) M17	*Lactococcus lactis subsp.lactis*	162.2
47	MH17 (7) M17	*Lactococcus lactis subsp.lactis*	nd
48	MH 18 (1) M17	*Lactococcus lactis subsp.lactis*	nd
49	MH 18 (3) M17	*Lactococcus species*	nd
50	MH 18 (6) M17	*Lactococcus lactis subsp.lactis*	218.45
51	MH 19 (2) M17	*Lactococcus lactis subsp.lactis*	55.70
52	MH 19 (4) M17	*Lactococcus lactis subsp.lactis*	166.6
53	MH 19 (6) M17	*Lactococcus lactis subsp.lactis*	178.95
54	MH 20 (1) M17	*Lactococcus lactis subsp.lactis*	80.35
55	MH 20 (2) M17	*Lactococcus lactis subsp.lactis*	182.85
56	MH20 (3) M17	*Lactococcus lactis subsp.lactis*	128.25
57	MH20 (4) M17	*Lactococcus lactis subsp.lactis*	nd
58	MH20 (5) M17	*Enterococcus species*	245.90
59	MH20 (6) M17	*Lactococcus lactis subsp.lactis*	138.65
60	MH21 (1) M17	*Lactococcus lactis subsp.lactis*	204.20
61	MH21 (2) M17	*Enterococcus Species*	62.20
62	MH21 (3) M17	*Lactococcus lactis subsp.lactis*	140.75
63	MH21 (4) M17	*Lactococcus lactis subsp.lactis*	114.20
64	MH21 (5) M17	*Lactococcus lactis subsp.lactis*	134.60
65	MH21 (6) M17	*Lactococcus lactis subsp.lactis*	130.9
66	MH22 (3) M17	*Lactococcus lactis subsp.lactis*	nd
67	MH22 (4) M17	*Lactococcus lactis subsp.lactis*	nd

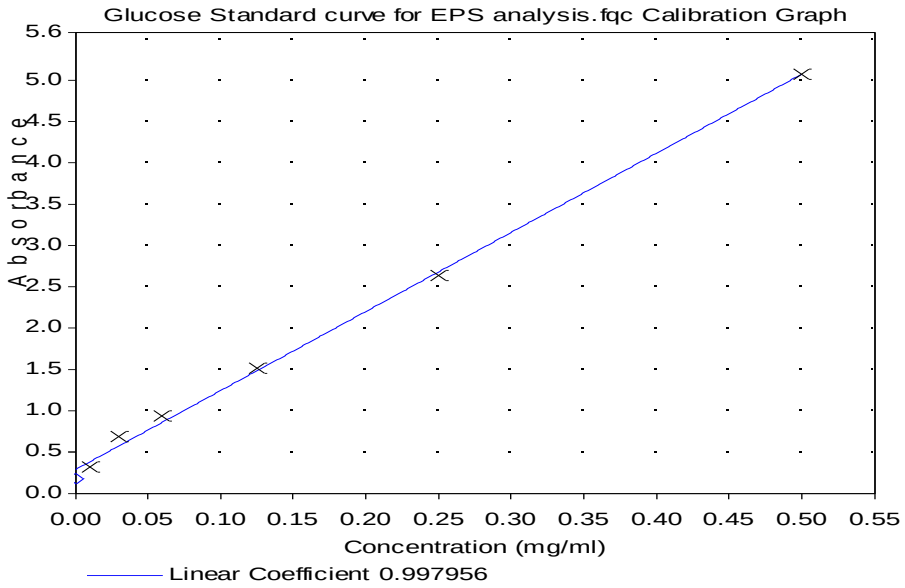
EPS producing LAB isolate (Code)		LAB Genus / species	EPS level (mg/L Glucose equivalent) in Whey medium
68	MH23 (3) M17	*Lactococcus lactis subsp.lactis*	nd
69	MH23 (4) M17	*Weissella species*	244.5
70	MH23 (5) M17	*Enterococcus species*	237.60
71	MH24 (1) M17	*Lactobacillus species (Homofermentative)*	nd
72	MH 24 (3) M17	*Lactobacillus species (Homofermentative)*	149.0
73	MH 24 (6) M17	*Lactococcus lactis subsp.lactis*	nd
74	MH25 (1) M17	*Lactococcus lactis subsp.lactis*	187.80
75	MH25 (2) M17	*Lactococcus lactis subsp.lactis*	185.40
76	MH25 (3) M17	*Lactococcus lactis subsp.lactis*	nd
77	MH25 (4) M17	*Lactococcus lactis subsp.lactis*	190.40
78	MH25 (6) M17	*Lactococcus lactis subsp.lactis*	180.00
79	MH26 (6) M17	*Lactococcus lactis subsp.lactis*	100.4
80	MH27 (2) M17	*Lactococcus lactis subsp.lactis*	186.90
81	MH27 (3) M17	*Lactococcus lactis subsp.lactis*	135.20
82	MH27 (5) M17	*Lactococcus lactis subsp.lactis*	181.9
83	MH28 (2) M17	*Lactococcus lactis subsp.lactis*	369.80
84	MH28 (3) M17	*Lactococcus lactis subsp.lactis*	264.60
85	MH28 (5) M17	*Lactococcus lactis subsp.lactis*	224.60
86	MH28 (6) M17	*Lactococcus lactis subsp.lactis*	nd
87	MH29 (1) M17	*Lactococcus lactis subsp.lactis*	108.95
88	MH29 (2) M17	*Lactococcus lactis subsp.lactis*	283.93
89	MH29 (3) M17	*Lactococcus lactis subsp.lactis*	250.8
90	MH29 (4) M17	*Lactococcus lactis subsp.lactis*	122.70

EPS producing LAB isolate (Code)		LAB Genus / species	EPS level (mg/L Glucose equivalent) in Whey medium
91	MH29 (5) M17	*Lactococcus lactis subsp.lactis*	200.20
92	MH29 (6) M17	*Lactococcus lactis subsp.lactis*	nd
93	MH1 (2) MRS	*Lactococcus lactis subsp.lactis*	368.50
94	MH7 (2) MRS	*Lactobacillus species (Homofermentative)*	262.30
95	MH7 (3) MRS	*Enterococcus species*	119.60
96	MH7 (4) MRS	*Lactococcus lactis subsp.lactis*	236.80
97	MH8 (2) MRS	*Lactobacillus Plantarum*	226.7
98	MH8 (3) MRS	*Lactobacillus species (Homofermentative)*	258.1
99	MH8 (4) MRS	*Lactobacillus species (Homofermentative)*	298.40
100	MH8 (5) MRS	*Lactobacillus species (Homofermentative)*	228.50
101	MH9 (2) MRS	*Lactobacillus species (Homofermentative)*	285.80
102	MH9 (4) MRS	*Lactococcus lactis subsp.lactis*	281.00
103	MH9 (5) MRS	*Enterococcus species*	345.90
104	MH14 (3) MRS	*Lactococcus lactis subsp.lactis*	192.80
105	MH15 (4) MRS	*Lactococcus lactis subsp.lactis*	301.80
106	MH15 (5) MRS	*Enterococcus species*	195.00
107	MH17 (1)MRS	*Lactobacillus species (Homofermentative)*	171.60
108	MH18 (1)MRS	*Lactococcus lactis subsp.lactis*	423.60
109	MH18 (2)MRS	*Lactococcus lactis subsp.lactis*	428.90
110	MH18 (3)MRS	*Lactococcus lactis subsp.lactis*	396.60
111	MH18 (4)MRS	*Lactococcus lactis subsp.lactis*	449.90
112	MH20 (1) MRS	*Lactococcus lactis subsp.lactis*	nd

EPS producing LAB isolate (Code)	LAB Genus / species	EPS level (mg/L Glucose equivalent) in Whey medium
113 MH21 (1) MRS	*Lactococcus lactis subsp.lactis*	215.1
114 MH21 (2) MRS	*Lactococcus lactis subsp.lactis*	319.30
115 MH21 (3) MRS	*Lactococcus lactis subsp.lactis*	339.90
116 MH21 (4) MRS	*Lactococcus lactis subsp.lactis*	312.50
117 MH21 (6) MRS	*Lactococcus lactis subsp.lactis*	422.50
118 MH22 (1) MRS	*Enterococcus species*	nd
119 MH23 (1) MRS	*Lactococcus lactis subsp.lactis*	242.90
120 MH20 (1) Rog	*Lactobacillus species (Homofermentative*	289.50
121 MH21 (1) Rog	*Lactococcus lactis subsp.lactis*	358.50
122 MH22 (1) Rog	*Lactococcus lactis subsp.lactis*	308.80

nd – not done

Appendix 9: Calibration Curve and EPS levels from *Mursik* samples during traditional fermentation process



Batch : carbohydrate analysis in EPS extracted from Mursik samp (1).qre

	Sample	Rep.	490.0nm	Conc. (mg)	Errors
1	GIDI	1	0.9357	0.0678	None
2		2	0.8965	0.0637	None
3		Mean:	0.9161	0.0657	None
4	G2D1	1	0.6785	0.0409	None
5		2	0.4188	0.0138	None
6		Mean:	0.5487	0.0273	None
7	G3D1	1	0.6296	0.0358	None
8		2	0.6709	0.0401	None
9		Mean:	0.6502	0.0379	None
10	G1D2	1	1.3945	0.1157	None
11		2	1.4504	0.1215	None
12		Mean:	1.4224	0.1186	None
13	G2D2	1	1.9418	0.1729	None
14		2	1.3239	0.1083	None
15		Mean:	1.6329	0.1406	None
16	G3D2	1	0.7311	0.0464	None
17		2	0.7988	0.0535	None
18		Mean:	0.7650	0.0499	None
19	G1D3	1	0.7093	0.0441	None
20		2	0.6837	0.0414	None
21		Mean:	0.6965	0.0428	None
22	G2D3	1	0.9420	0.0684	None
23		2	0.8474	0.0585	None
24		Mean:	0.8947	0.0635	None
25	G3D3	1	1.3023	0.1061	None
26		2	0.8150	0.0552	None
27		Mean:	1.0587	0.0806	None

Amount of EPS (mg/L Glucose Equivalents) = Conc. (mg) from the table x 5 x 1000/10. Sample size = 10 ml. Dilution factor = X5.

Appendix 10: Copy of study Survey Questionnaire

SALOME CHELAGAT MUIGEI,

DEPT.DAIRY AND FOOD SCIENCE
AND TECHNOLO
GY,
EGERTON UNIVERSITY,
P.O. BOX 536**,**
EGERTON
DATE:

Dear Sir /Madam,

RE: RESEARCH QUESTIONNAIRE

I am a student Egerton university pursuing Master Degree in Food Science and Technology. My research title is '**Production and Prebiotic Properties of Exopolysaccharides in Kenyan Fermented Milk, *Mursik*'**. Samples of *Mursik* will be collected from Njoro District and analyzed at Egerton University Food Science Laboratories. Prior to the laboratory analysis, a study survey is being done to collect the general information of the traditional technology for the *Mursik* production.

I am therefore requesting you to complete the attached Questionnaire and return it to the undersigned as soon as possible.

The information gathered by the questionnaire will be used for academic purposes only. All the completed questionnaires will be treated with treated with utmost confidentiality.

Thank you for giving your valuable time to make a contribution towards this research.

Yours Faithfully,

Salome C. Muigei

QUESTIONNAIRE

A STUDY SURVEY FOR TRADITIONAL TECHNOLOGY FOR *MURSIK* PRODUCTION IN NJORO DISTRICT

PREAMBLE

Please answer the following questions by ticking in the box for the correct answer and filling in the answers where applicable. All the responses in this questionnaire will be treated with confidentiality and will only be used for purposes of the study.

A. SOCIO-DEMOGRAPHIC DATA

1. (a) Household number: ……
   (b) Name (Optional)……..
   (c) District……. ……..
   (d) Division …….
   (e) Village……

2. Sex: Male ☐ Female ☐

3. Age (years):
   20 – 35 ☐ 36 – 45 ☐ 46 – 55 ☐ 0ver 56 ☐

4. Level of Education:
   - No formal education ☐
   - Primary ☐
   - Secondary ☐
   - Secondary and Certificate/Diploma ☐
   - University ☐

5. Occupation: ……..

B. Traditional production Technology for *Mursik*

6. What type of milk do you use for *Mursik* making (please tick where appropriate)

   Raw milk ☐ Boiled milk ☐

   Other(s) (please state):

   1.……

   2.…….

   3.……..

7. What type of containers do you Use for *Mursik* fermentation(please tick the appropriate answer(s))

Gourds ☐ Plastic containers ☐ Metallic containers ☐

Others (please List)

1……
2……..
3……

8. How is the *Mursik* fermentation container prepared prior to filling the milk for fermentation? (please explain)

……
……
……
……
……

9. Are there any substances added to the milk before *Mursik* fermentation? (please tick where appropriate)

Yes ☐ No ☐ Don't know ☐

10. If the answer to Question 10 is yes, please list down the additives and their roles;

Additive	Role
1……	…….
2……	……
3……	…….
4……..	……

11. Where do you store the fermenting *Mursik* during the fermentation process (Please explain)

……
……
……
……

12. How long does the *Mursik* take from the 1st day of fermentation to the day when it is ready for consumption? (Please tick the appropriate answer).

3 days ☐ 7 days ☐ 10 days ☐ more than 10 day ☐

13. Is there a way that *Mursik* fermentation process can be accelerated (speeded) (please tick the appropriate answer)

Yes ☐ No ☐ Don't Know ☐

14. If the answer to Question 13 is yes, please explain how this is or can be done;

……
……
……
……

15. How long can you store the ready to consume *Mursik* (please state)

……

16. How is *Mursik* utilized? (Please tick the appropriate answer(s).
    1. as a food
    2. as refreshment
    3. as a nutraceutical product
    4. 1, 2, and 3 above
    5. Only 1and 3 above
17. *Mursik* is believed to cure and prevent certain diseases when consumed (please tick where appropriate)

True ☐ Not true ☐ Don't Know ☐

18. If the answer to Question 18 is True, please list some of the conditions cured or prevented by *Mursik.*
    1……..
    2……
    3……
    4……
19. How is *Mursik* used to achieve the above health promoting effects? (please briefly explain)
    1……..
    2……
    3……
    4……
20. Is *Mursik* produced in your village for sale? (please tick where appropriate)

Yes ☐ No ☐

21. If the answer to Question 20 is yes, please explain how it is produced and sold.

……..……
……
……
……

22. Please give your opinion about production of *Mursik* for sale (Commercial) purposes

……
……

23. Please give a general comment that will contribute to the success of this research

……
……
……
……

Appendix 11: Copy of the letter for Provision of Research grants by the National Council for Science and Technology (NCST).

REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telegrams: "SCIENCETECH", Nairobi
Telephone: 254-020-241349, 2213102
254-020-310571, 2213123.
Fax: 254-020-2213215, 318245, 318249
When replying please quote

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: **NCST/5/003/PG/118**

Date: **15th February 2010**

Salome Chelagat Muigei,
P.O. Box 536,
NJORO

Email: smuigei@yahoo.com

RE: ST &I COMPETITIVE GRANT (M.Sc)

You have been awarded the Science, Technology and Innovation Grant for your M.Sc Research Proposal.

The Research Committee of the National Council for Science Technology has approved an amount of Kenya shillings **200,000** towards your proposal titled ***'Production and prebiotic properties of exopolysaccharides in a Kenyan fermented milk 'Mursik'.***

Find the enclosed ***Research Grant Contract Form (RIG/03A)*** that should be duly completed and sent back to the National Council for Science & Technology. You should attach a copy of your ***National Identity Card, details of the Work plan, breakdown of the half yearly Budget and an Acceptance letter.***

Your acceptance letter and Contract Form should reach us not later than **1st March 2010** for further action.

PROF. SHAUKAT A. ABDULRAZAK, Ph.D, FIBiol, MBS
SECRETARY/CEO

Cc: Vice Chancellor,
Egerton University,
Box 536-20115,
NJORO

Encl. Research Grant Contract Form (RIG/03A)

Appendix 12: Copy of the Letter of Permission to carry out research in Njoro District from the District Commissioner.

OFFICE OF THE PRESIDENT
PROVINCIAL ADMINISTRATION

Telegrams: **"DISTRICTER" Njoro**
Telephone: Njoro
When replying please **Quote**

THE DISTRICT COMMISSIONER
NJORO DISTRICT
P.O BOX 500
NJORO

4th March 2010

Ref. No ED.12/10 VOL.I/11

Salome Chelagat Muigei
Department of Dairy and Food Science & Technology
Egerton University
PO Box 536
EGERTON

RE: PERMISION TO CARRY OUT ACADEMIC RESEARCH

Your letter dated 1st March 2010 on the above refers.

This is to inform you that this office has no objection in you carrying out the research within Njoro District. Ensure you report to the respective District officers on the ground.

By a copy of this letter, all District officers are asked to assist the student.

Thank you.

MWACHIDUDU CHIMERA
For: District Commissioner
NJORO DISTRICT

Copy to:
All District Officers
Njoro District

Appendix 13: copy of the letter of provision of research grants by the board of Post graduate studies, Egerton University

EGERTON UNIVERSITY
Tel: Pilot: 254-51-2217620
254-51-2217877
254-51-2217631
Dir.line/Fax: 254-51-2217847
Cell Phone
P.O. Box 536 - 20115
Egerton, Njoro, Kenya
Email: eugradschool@wananchi.com
www.egerton.ac.ke

OFFICE OF THE DIRECTOR, GRADUATE SCHOOL

Ref: KM162/1986/07 Date: 11th May, 2010

Ms. Salome Chelagat Muigei,
Egerton University,
Department of Daftec

Thro' COD, Daftec forwarded and Congratulations

Thro' Dean, Agriculture Forwarded with congratulations. 13/05/2010

Dear Ms. Muigei,

RE: AWARD FOR POSTGRADUATE RESEARCH FUNDS

I am pleased to inform you that at a Board of Postgraduate Meeting held on 14th April, 2010, you were awarded Kes.150,000.00 (Kenya Shillings One Hundred and Fifty Thousand only) to assist you in your Masters Research Programme.

Please arrange to apply for the funds immediately form DVC(R&E). Your request should pass through your major supervisor, through your COD, through Dean of your Faculty and through the Director Graduate School.

Congratulations for winning the award and I wish you success in your research.

Thank you.

Yours sincerely,

Prof. Michael A. Okiror,
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

c.c. VC
DVC (A&F)
DVC (AA)
DVC (R&E) - note for action
Director (R&E)

MAO/qma

DEPT. OF DAIRY AND FOOD SCIENCE
EGERTON UNIVERSITY

EGERTON UNIVERSITY
RECEIVED
12 MAY 2010
FACULTY OF AGRICULTURE