Baseline Survey, Biochemical, Microbial, and Technological Studies on "*Mnazi*"

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

TO MY WIFE AND CHILDREN

your life can be easier……..

more exciting……..

more rewarding!

Let prayers be your daily bread

Turn your discoveries about God into worship

Turn your discoveries about yourself into decision

Write it down lest you lose it

SHARE

Decide how you will share your discoveries with other people, both in word and practice

THANKS GIVING

Dear Lord, I thank you for the privilege of praying and receiving answers to my prayers

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ABBREVIATIONS

AA	Acetic Acid bacteria
ABABS	Agilent Basic Anion Buffer System Chromatopac
CL	Coastal Lowlands
EAT	East African Tall
GC	Gas Chromatography
GDP	Gross Domestic Products
HPLC	High Performance Liquid Chromatography
JKUAT	Jomo Kenya University of Agriculture and Technology
KCDA	Kenya Coconut Development Authority
KEBS	Kenya Bureau of Standards
KES	Kenya Shillings
LAB	Lactic Acid Bacteria
MDG	Millennium Development Goals
MOA	Ministry of Agriculture
ppm	Parts Per Million
TTA	Total Titratable Acidity
US$	Unite States of America Dollar

ABSTRACT

This thesis focused on the traditional tapping and distillation methods of *mnazi* in addition to economic importance, isolation and identification of microorganisms, preservation and chemical wholesomeness of the beverage. Educational levels and major constraints facing tappers and farmers in the coconut sub-sector were also studied.

Mnazi is an alcoholic beverage from palm tree. It is referred to as Nigerian wine, palm wine, *toddy*, *tuba*, *tuak*, *nsafufuro*. For consistency, this thesis shall adopt the word *mnazi* instead of coconut toddy or Nigerian wine as has been referred to by other scientists.

The survey involved both stratified and area-sampling methods. Specific methods for isolation, characterization and identification of bacteria and yeasts were employed including the use of API kits.

Information was obtained on identification of the right spathe, tapping process, sap collection, *mnazi* storage and distillation. The farmers interviewed were either illiterate (32.5%) or had primary education (42.0%). For tapers, 40.3% were illiterate while 53.1% had primary level of education. The major constraints faced by farmers and tappers included: lack of market, low/fluctuation of prices, poor storage/preservation facilities, pests and diseases. The total number of coconut trees was estimated as 4.6 million trees. Tappers owned 35.5% of the trees tapped while farmers owned 64.5%. The annual production and income from *mnazi* was 4.6 million decalitres and KES710 million respectively. Other coconut-based products generated KES832 million.

The newly developed products: dry, medium dry and sweet brands were stable during the first 4 weeks after production. Assimilation of sucrose and fructose were faster than glucose, with fructose being the fastest. The absence of methanol and fusel oils in fresh *mnazi* makes it possible to recommend its removal from the category of illicit brews but not its distillate because of its high levels of fusel oils.

The study identified two genera: *Acetobacter* and *Gluconobacter* as the dominant spoilage microflora in *mnazi*. Apart from spoilage, the two genera may be used for production of vinegar. A total of 15 isolates out of 86 isolates of lactic acid bacteria were identified as follows: *Lactobacillus paracasei ssp paracasei1*, *Lactobacillus paracasei ssp paracasei 2*, *Lactobacillus paracasei ssp paracasei3, Lactobacillus plantarum* and *Lactococuss lactis ssp lactis 1*. The API C AUX system identified 24 species as: *S. cerevisiae 1, S. cerevisiae 2, Candida pelliculosa*, *Candida utillis*, *Stephanoascus ciferrii*, *Kloeckera spp*, *Trichosporon asahii* and *Rhodotorula mucilaginosa*.

The findings in this study are expected to act as a base for further research and technological improvement in the *mnazi* industry.

It is also envisage that the newly established Kenya Coconut Development Authority will put in place appropriate policy measures that shall address most of the constraints faced by farmers and tappers.

Key Words: *Mnazi*, educational level, constraints, tapping, distillation, economic importance, bacteria, yeasts, chemical wholesomeness

CHAPTER ONE

GENERAL INTRODUCTION, PROBLEM STATEMENT, JUSTIFICATION AND RESEARCH OBJECTIVES

1.1 Introduction

1.1.1 Origin of coconut trees and their production in Kenya

The coconut palm (*Cocos nucifera*) is the most important palm tree in Kenya. This tree is grown in nearly 90 countries that spread along the tropical Coastal belt of the world. Other palm trees that are found in Kenya include the wild palm (*Phoenix reclinata*) and the Malaysian palm (*Elaesis guineensis*). Wild palm is commonly found along the Coastal belt of Kenya particularly in Malindi, Kilifi and Kwale districts. Although the Malaysian palm is found scattered along the Kenyan Coastal belt, it has not been produced on a commercial basis. Initial research trials however, have confirmed that this crop can be grown in the coast and western regions of Kenya.

The coconut tree is said to have originated in Asia, but this a subject of controversy (Menon and Pandalai, 1958; Thampan, 1981). There is some evidence that coconut tree originated along the Pacific coasts of Colombia and Ecuador (Menon and Pandalai, 1958; Thampan, 1981). The first European explorers found coconut in all areas of the tropics except the

Atlantic coasts of America and Africa. Coconuts appear to have been distributed by man, but possibly the floating nuts were carried to new areas by the ocean currents (Thampan, 1981; Child, 1974).

The Portuguese introduced the coconut palm in Kenya in the 16th century. Its cultivation spread rapidly along the coast and it became an industrial crop of considerable economic importance during the 20th century. Its production and marketing were handled by the Arab traders' together with the white settlers on big plantations until the 19th century when small-scale farmers started growing it. In Kenya coconut grows in the Coastal region, mainly the Coastal lowlands (CL) agro-ecological zones of CL_2, CL_3 and in some parts of CL_4. These zones are distributed in the six geographical Coastal districts namely: Kilifi, Kwale, Mombasa, Malindi, Lamu and Tana River. The coconut trees are also grown on small-scale in the arid and semi-arid areas in CL_5 along the rivers found in Malindi, Kinango and Taita Taveta areas. In Kenya, the trees are also found at the shows of Lake Victoria. The palm tree requires 800-1400 mm of rainfall per annum with an average temperature of 26 °C in the Coastal lowlands and 27 °C in the hinterland.

The coconut palm is typically a tall, graceful, unbranched tree, which can reach a height of 10-30 meters in about 100 years. It can be classified as either dwarf or tall type. The dwarf trees grow to only 10 meters, while the tall type can grow to a height of 30 meters. The tree grows from a nut

planted 60cm deep and it emerges after 3 months. It is commonly found along the Coastal strip because of its tolerance to salinity, but it can also grow in the hinterland. The nuts are large (20-30cm in diameter) with a thick, fibrous mesocarp and weigh up to one kilogram. A hard shell (endocarp) envelops the seed that has a large white, meaty endosperm. This endosperm is rich in oil and completely surrounds the internal cavity filled with coconut water. The mature tree will produce 35-70 nuts per year.

The recommended spacing of the coconut tree is 7-10 meters a part (Abdullah and Kamaruddin, 1996). This spacing is important so as to suit the large canopy of the crop as well as to minimize the overlapping of the palm leaves of adjacent trees (Magat, 1996). It is recommended to intercrop coconut with other crops such as maize or citrus fruits so as to avoid under-utilization of the soil resource (Magat, 1996). The productivity of coconut as well as the intercrops may be affected by the planting arrangement (geometry). Under monoculture system, the triangular system provides the optimum number of trees, with 15% more palms (per hectare) and minimum overlapping of leaves exists. However, in coconut-based farming system (CBFS), the square and rectangle arrangements are preferred as these facilitate the growing of intercrops (Magat, 1996). Under the rectangle system, the rows are set at right angles to one another. Spacing between the palms within the rows is closer than between rows. This provides more spaces for annual or short season intercrops. The

square system is more common than the triangle arrangement because it permits the growing of intercrops due to the larger light patches between the centres of every four palms. Coconut palms can be intercropped with grain crops, beverage crop and fruit trees (Magat, 1996).

1.1.2 Coconut-based products

The coconut tree has been grown traditionally for its copra; hence majority of the international coconut trade is in copra. Copra is a dried endosperm, containing 60-70% oil, of which over 60% is extractable (Ekpa *et al*., 1994). This oil is widely used in the production of soap, cosmetics, margarine and processed foods. The cake residue is used to make animal feed (Ekpa *et al*., 1994). Besides its industrial applications, coconut oil can be used domestically as cooking oils, body creams and, medicinally, as antidotes for poisoning and as surface protectants for minor wounds (Ekpa and Ekpe, 1996). Coconut oil is similar to palm kernel oil in chemical composition and the two are the only sources of lauric acid oil available to the world market (Berger *et al*., 1991). Recent research (Crabbe *et al*., 2001) has shown that palm oil could be used to generate environmental friendly bio-diesel which emits less unburned hydrocarbons and carbon monoxide (CO). The conversion of coconut into copra is the most popular on-farm processing activity in Kenya. First, the ripe dehusked nut is broken into two equal parts, followed by drying for 1-2 days. The kernel is then loosened and removed in a cup form after carefully breaking and shell

removal. The edible cup copra is further dried for a period of 3-5 days before being sold to the respective buyer or cooperative society. Apart from copra, the ripe kernel is used domestically to extract coconut cream (milk) by squeezing the macerated endosperm to which some water has been added. At industrial level, ripe coconut kernel is used to manufacture desiccated coconut and coconut cream (Ekpa and Ekpe, 1996). These two products are usually preserved and packed in hygienic packaging materials such as Tetra Pak for longer shelf life. In Kenya desiccated coconut is imported from Asia although there is a great potential to produce it locally. The demand for coconut cream especially to the European countries is high.

In Kenya, the tender coconuts are commonly referred to as "*madafu*". *Madafu* are used as a source of healthy drink, its gelatinous kernel can be consumed as food. Therefore in the tender nut, both the delicious beverage and the gelatinous kernel are consumed. Consumption of tender nuts by both locals and tourists increases earnings from coconut-based products as opposed to a situation where harvesting is restricted to mature nuts. Tender nuts are usually sold in mobile carts or special kiosks along the streets of the Kenyan Coastal towns and beaches. Recently the selling and consumption of tender nuts has become a common practise even in the hinterland including the streets of Nairobi.

Coconut shell is another coconut-based product that is often used as a source of fuel. The shell can also be used to make useful products such as beads, bungles, buttons, serving spoons and other decorations. When milled into flour, it can be used as a filter in thermoplastics, pesticides formulation and as a propellant for aerosols (De Silva, 1989). If burnt at controlled temperature under scanty air, it produces charcoal with average caloric value of 7.5 to 7.6 Kcal per g (dry basis). This charcoal can be used for cooking, baking ovens and by blacksmiths (Patterson and Hyman, 1991; Sanchez, 1993).

The shell can also be used to manufacture activated carbon. Charcoal briquette is another product from coconut shell. Currently in Kenya, briquettes are made from coffee husks (Patterson and Hyman, 1991; Sanchez, 1993).

The technology for the utilization of coconut husk for conversion into coir and coir products using decorticating machines is being utilized by very few operators in Kenya. Currently, the only coir fibre processing unit is Cocos Kenya at Kilifi. The other two factories which used to exist; one in Kwale and the other in Kilifi district have so far stopped production. Coir products not only enjoy both domestic and export demand but also the coconut farmers themselves are directly involved in the processing at the primary stage (Tampan, 1996). With proper utilization of the coconut husks, the coconut farmers could augment the farm level income and employment. In

Kerala, India, coir industry sustains the livelihood of nearly half a million people with women workers forming the predominant group. The industry involves both the farmers and private processors (Tampan, 1996). Coir fibre can be spined into yearn for making mats and ropes; it can be used for upholstery and for staffing mattresses. It can also be used for making brooms and brushed. When rubberized, coir fibre can be converted into rubberized coir. Bonding curled coir with natural rubber latex makes rubberized coir. With proper binding curled coir fibre could be possibly used to make ceiling boards. Fibre pads can also be made from rubberized coir. Coir dust, which is made of fine particles after the decortications process can be used for mulching as well as a medium for growing mushrooms. Coir fibre may also be used as a substitute for peat in roofing.

In Kenya, the midribs of the coconut leaflets are utilized for making brooms. These brooms are used for regular sweeping and cleaning work at the household level as well as in major cities, municipalities and county councils. Commercially, midribs are used to make toothpicks (Lorenzi *et al*., 1996; Bezerra, 1999). However, the commercialization of toothpicks from midribs is yet to take off in Kenya. Coconut leaflets are usually used to thatch residential houses, villas and beach hotels. They are also used to make baskets to carry farm produce and mats for sleeping and building of huts as well as animal shade. At the moment, there is a very high demand for brooms made from coconut midribs. The interest in coconut based

thatching material "*makuti*" in the hotel industry together with escalating cost of modern roofing materials has created a high demand for *makuti*. Many markets for brooms and *makuti* are operational in the region, the recent ones being those at Kaloleni and Bahari divisions of Kilifi district.

The coconut tree trunk is useful for construction work and in making furniture and handicrafts (Kahn and de Granville, 1993). In Kilifi district particularly Chonyi, Bahari and Kaloleni divisions it is now a common phenomenon to find people using coconut timber as building posts instead of the traditional mangrove and forest trees.

1.1.3 General information on *mnazi*

Mnazi, like any other sugary plant sap can be processed into an alcoholic beverage through fermentation of the sugars present in the sap, yielding alcohol and carbon dioxide. It is sweet, dirty brown in color, containing 10-12% sugar, mainly sucrose (Okafor, 1975). As the fermentation process continues, the sap becomes milky-white in appearance due to the presence of large numbers of fermenting bacteria and yeasts (Okafor, 1975). This product is unique in that it can be consumed without pasteurization, hence consumed with live microorganisms in it (Faparusi, 1971). Among the palms most frequently tapped for *mnazi* include: coconut palm (*Cocos nucifera*), the Malaysian oil palm (*Elaeis guineensis*), the wild date palm (*Phoenix reclinata*), the nipa palm (*Nipa fruticans*), the raphia

palm (*Raphia hookeri* or *Raphia vinifera*) and the kithul palm (Cariota *urens*) (Swings and DeLey, 1977).

Mnazi tapping for beverage is a Pan-tropical practise, but has its great historical depth in Asia and Africa (Van Oevelen, 1978). Tapping of *mnazi* involves wounding of the stem tissues or tapping from the roots or fruit bud. In West Africa, particularly in Ghana, oil palm is tapped by felling of palm trees that are ten or more years old (Essiamah, 1989). There are many variations and names including: *emu* and *ogogaro* in Nigeria, in Philippines *tuba*, *ra* or *panam culbo* in Ceylon, *tuak* or *nira* in Malaysia, *nsafufuro* in Ghana, kallu in India and *toddy* in Thailand. In Kenya it is commonly referred to as *mnazi,* uchi *wa mnazi* or *mdafu*. *Mnazi* is an important alcoholic beverage in West Africa where more than ten million people consume it. Some of the *mnazi* consuming countries in West Africa includes: Nigeria, Cameroon, Benin, Togo, Ghana and Ivory Coast (Van Oevelen, 1978). In East Africa it is consumed at the Coastal belts of Kenya and Tanzania, while in Southern Africa *mnazi* is consumed in Mozambique and South Africa. In Asia *mnazi* is popular in India, Malaysia, Thailand, Singapore, Philippines and Sri Lanka. *Mnazi* can be consumed in a variety of flavours varying from sweet unfermented to sour-fermented and vinegary alcoholic drinks. Sweet *mnazi,* for example, is a non-alcoholic drink made from fresh *mnazi*, which is pasteurized before fermentation. A similar drink known as *tembo tamu* is consumed in Lamu district of Coastal Kenya. This

drink is not pasteurized but it is consumed immediately after tapping. Mild *mnazi* is an alcoholic drink resulting from pasteurization of fermented *mnazi*, while *arrack* or *lambanog* is a spirit manufactured by distilling fermented *mnazi*. In Kenya, alcoholic spirit similar to *arrack* known as *pyuwa* (*piwa)* is distilled traditionally at the village level from *mnazi*.

Mnazi is consumed as a mildly alcoholic beverage similar to beer. Some people consume *mnazi* instead of water after meals. At the Coastal region of Kenya, *mnazi* has a special place in traditional celebrations and ceremonies such as marriage, burials and settling of disputes. In Nigeria, particularly in the Ibo speaking area, *mnazi* is a part of major preliminaries that must be prayed over and discussed before the ceremonies start (Okafor, 1977 and Odeyemi, 1977). Similar customs take place in Kenya, especially among the Miji-Kenda people in the coast. In Sri-Lanka, *mnazi* is a popular drink among the lower income people. Village folk gather in the evening to socialize and drink *mnazi* (Okafor, 1977). This practise is also common at the Coastal region of Kenya. In West Africa, *mnazi* is served as a beverage with meals and it is the main alcoholic beverage during social gatherings. The barks or stems of certain plants such as *Alstonia boonei* or *Sarcoglottis gabonensis* are added to *mnazi* and used as medicine for curing fever and other ailments (Okafor, 1977). *Mnazi* is believed to be good for health and eyesight and also serves as a sedative. It is also a mild laxative in relieving constipation (Okafor, 1977). It is prescribed as a tonic

for those recovering from diseases such as chicken pox (Okafor, 1977). Previous studies have shown that *mnazi* is an important source of nicotinic acid and vitamin C and to a lesser extent, proteins, thiamin and riboflavin (Cunningham and Wehmeyer, 1988).

Mnazi is highly perishable. It could start to deteriorate as soon as it flows out of the spadix, due to microorganisms (yeasts, lactic and acetic acid bacteria), which convert the sucrose into invert sugar and further into ethanol, acetic acid, lactate and other volatile compounds such as acetaldehyde and diacetyl. Commercially bottled *mnazi* can be preserved best in sodium benzoate at a concentration between 0.15% (m/v). Sodium metabisulphite and propionic acid could also be used as preservatives but they are less effective (Shamala *et al., 1988*). In Thailand, in almost every place, farmers use special barks such as kiam (*Cotyleobium lanceolatum*) and pra-yom (*Shorea floribunda*) for preservation. In some places, a mixture of preservatives is used instead of kiam and pra-yom. This mixture is composed of sodium metabisulphite, sodium propionate, sodium benzoate in ratio of 10:1:1, respectively (Naka, 1996). Pasteurization at 85 °C for 30 min. reduces the viable counts in mnazi to a greater extent than heat treatment at 70 °C for 35 min. and 65 °C for 40 min. (Idise and Izuagbe, 1985).

This study aims to explore into the quality aspects of both *mnazi* and its spirit (*pyuwa*) with an aim of providing a cheap alternative beverage for

both low and middle income earners. This alcoholic beverage is expected to compete effectively with beers and alcoholic drinks already in the market. This study will also exploit ways of preserving the beverage with an aim of improving its shelf life.

1.1.4 Common microorganisms in *mnazi*

The biology and habitats of acetic acid bacteria are well known due to the economic importance of these bacteria to the brewing and fermentation industries. Acetic acid bacteria are associated with wines, beers, fruits, flowers, mnazi, and beehives. These bacteria have industrial applications for they are used to manufacture vinegar and other fermentation products such as ketogluconic acid, sorbose and dihydroxyacetone (Swings and De Ley, 1981). However, these bacteria are also associated with spoilage of alcoholic beverages such as beers and wines including *mnazi*, through the production of acetaldehyde and acetic acid from ethanol. Acetic acid bacteria are divided into the genera *Gluconobacter, Acetobacter and Fratueria* (Holt *et al*., 1994). Of these, *Gluconobacter oxydans, Acetobacter aceti, Acetobacter pasteurianus, Acetobacter liquefaciens* and *Acetobacter hansenii* are normally associated with grapes and wines (Blackwood *et al*., 1969; Joyeux *et al*., 1984a). *Acetobacter* species prefer ethanol as carbon source (De Ley *et al*., 1984) and usually dominate during the later stages of wine fermentation (Drysdale and Fleet, 1985; Joyeux *et al*., 1984a).

Production of acetic acid can result from biological oxidation of ethanol by acetic acid bacteria. It involves a two-step reaction catalyzed by two membrane-bound enzymes, alcohol dehydrogenase and aldehyde dehydrogenase. Besides these two enzymes, cytochrome C and terminal oxidase are two important components for ethanol oxidation (Lu *et al*., 1999). Since Acetobacter prefers to oxidize ethanol more strongly than glucose and Gluconobacter prefers glucose more than ethanol, most strains useful in vinegar manufacture belong to *Acetobacter*, whereas *Gluconobacter* is used for industrial applications such as fermentation of ketogluconic acid, sorbose and dihydroxyacetone (Swings and De Ley, 1981). Among the *Acetobacter* species, *Acetobacter aceti, Acetobacter pasteurianus, Acetobacter polyoxogenes* and *Acetobacter europaneus* are the most popular strains for making acetic acid in vinegar factories as their oxidization of ethanol is better and they do not attack acetic acid later (Entani *et al*., 1985; Sievers et al., 1992; Lu *et al*., 1999).

Spoilage of *mnazi*, other wines and beers by acetic acid bacteria is as a result of conversion of ethanol to acetic acid in the presence of a small concentration of oxygen (Drysdale and Fleet, 1988; Bartowsky *et al*., 2003). Acetic acid is the major volatile acid in wine and is considered to be undesirable at concentration exceeding 0.4-1.5 g l^{-1}, depending on the type of wine (Davis *et al*., 1985). Many countries have imposed a strict limit on the maximum concentration of volatile acids in wine (Eglinton and

Henschke, 1999). *Acetobacter aceti* and *Acetobacter pasteurianus* are frequently associated with wines spoiled by high volatile acidity (Drysdale and Fleet, 1988). These bacteria are generally described as aerobes, but they have been routinely isolated from wine samples taken from the bottom of tanks and barrels (Joyeux *et al*., 1984a; Drysdale and fleet, 1985), which suggest that they are able to survive, and possible grow under the anaerobic to semi-anaerobic conditions that occur in these environments (Drysdale and Fleet, 1989b). In recent years, some wine manufacturers have adopted a number of practises that increase the risk of microbial spoilage of wine after bottling (Godden, 2000). These practises include the reduction of the preservative, sulphur dioxide from a level of approximately 300 mg l^{-1} to an average concentration of 74 mg l^{-1}. This reduction is due to public health related issues (Godden, 2000; Ough, 1983). In addition there are some post-bottling factors that contribute the risk of wine spoilage. These include the poor performance of bottle closures that may allow the ingress of oxygen (Zurn *et al*., 1995; Waters *et al*., 1996; Caloghiris *et al*., 1997) and the manner in which the bottles are positioned during transportation and storage (Godden, 2000). In Kenya *mnazi* spoils readily within 2-5 days of fermentation because it is stored in open containers such bottles, pots, plastic containers and gourds. In this work the issue of *mnazi* storage, preservation and packaging was also considered.

Lactic acid bacteria (LAB) are found in foods (dairy products, fermented meat, sour dough, fermented vegetables, silage, beverages- including wine), on plants, in sewage, but can also be in the genital, intestinal and respiratory tracts of man and animals (Hames *et al*., 1991). In the food industry, lactic acid bacteria act as both beneficial organisms and spoilage organisms. They are used in the production of fermented milk products such as yogurt, sour milk, cheese and butter, and in the production of sausages, pickles and sauerkraut. The result of these fermentations is more shelf-stable products with characteristic aromas and flavours. However, if the growth of lactic acid bacteria is not controlled, it can be a major cause of food spoilage. The souring of milk and the greening of meat and unfavorable flavors of *mnazi* are some of the common examples of spoilage resulting from the unchecked activity of these organisms. LAB can ferment lactose and other substrates into lactic acid and flavour compounds such as acetaldehyde, carbon dioxide and diacetyl (Marshall, 1993).

The occurrence of LAB in beers and wines including *mnazi* is usually associated with spoilage of these products. Bacterial growth in *mnazi* and beer depends on the ability to scavenge residual nutrients left following yeast growth and tolerance to the relatively hostile environment posed by fermented beverage (Pfenninger *et al*., 1979). Those nutrients that support the growth of lactic acid bacteria in wines and beer alike have been

examined by inoculating the wines or beer with LAB and following the changes in sugar and amino acid content with growth (Pfenninger *et al*., 1979). In most cases, small amounts of sucrose were metabolized along with varying amounts of maltose, maltotriose and maltotetraose. Among the amino acids absorbed by *Lactobacilli*, lysine, tyrosine and arginine predominated (Pfenninger *et al*., 1979). Spoilage of wines and beer by *Lactobacilli* is characterized by a "silky" turbidity but sometimes before this is apparent the "buttery" flavour of diacetyl may be noticed. Although lactic acid is the major metabolic end-product of LAB, its high flavour threshold in beer and wine, which contains up to 300 ppm lactate (Hough *et al*., 1982) compared with low threshold for diacetyl, about 0.15 ppm (Hough *et al*., 1982), makes the latter a potent flavour-modifying compound. Some of the LAB genera isolated from mnazi include *Leuconostoc* and *Streptococcus* (Okafor, 1972).

For thousands of years now, yeasts have provided man with products like leavened bread, beer, wine, sake, glycerol, enzymes, coenzymes and vitamins. Other products include: mono-, di-, and triphosphates of guanosine, uridine, cytidine, and adenosine. Currently, waste materials have been upgraded into utilizable form of single protein with the help of yeast cells. Yeasts, therefore, have been convenient tools for biochemists, physiologists, geneticists, cell biologists and other scientists. Indeed, the initiation of the fields of biochemistry and nutrition was based on the

discovery of enzymes and vitamins in yeast cells. Under certain circumstances, yeasts have caused food spoilage, especially at pH below 5. They may also cause infections to man and animals.

In the field of applied biotechnology, fermentation of glucose and fructose has been established through thousands of years of practise. Most ethanol produced in the world is derived from starch or sucrose (Gong *et al*., 1999), because they are readily hydrolyzed by enzymes and yeasts strains of *Saccharomyces cerevisiae* and a few other taxonomic groups with the production of wine, beer and bread (Vaughn-Martini and Martini, 1995). It has been established that plant materials that contain fermentable sugars provide suitable substrates for yeast species of *Saccharomyces*, *Candida*, *Torula* and *Hansenula* (Campbell-Platt, 1994). These yeasts, especially *Saccharomyces* are typically associated with spontaneous alcoholic fermentations of African opaque beers; *mnazi* and Asian type of beverages such as rice wine (Campbell-Platt, 1994).

1.1.5 Government policy on *mnazi* and coconut-based products

Historically, the coconut industry has operated under undefined policy in terms of development of the crop in the areas of production, processing, marketing, research and development. This is justified by the fact that all the early legislations on coconut-based products including *mnazi* did not promote the development of the palm industry. The Coconut Preservation Act, Chapter 332 of the laws of Kenya, 1915, was enacted to provide for

the improvement and regulation of the coconut planting industry, together with the Coconut Industry Act Chapter 331 of 1923, enacted to make better provision for the protection and improvement of the coconut industry have made the palm industry to be operated under ad hoc arrangements. These Acts only served to protect the palm plantations from trespass and allowed only owners of the palm to engage in marketing of coconut-based products without license. It is important to note that the Act of 1923, which is governing the palm industry development in the region, has so far outlived its appropriateness. This is because the situation of the palm industry has changed drastically from the plantation owned during the colonial period to the current small-scale family owned farms. All these changes require proper government policy that will cater for organized marketing, research and development of the industry. The situation was made even worse in 1997, when coconut and cashew nut crops were dropped from the list of protected crops that included: coffee, tea, cashew nut and coconut. Since then, the industry has operated under undefined policy making palm industry development in terms of research to lag behind due to the low priority rating of these crops given at the national level. This exposed the farmers to exploitation, demoralization and un-economic production margins.

In Kenya, restrictive laws on the mnazi dates back to the early 20th century when the colonial government enacted laws to control and regulate

tapping, sale and drinking of *mnazi*. These laws were carried out through various legal notices over the time period. The Native Liquor Regulation of November 1900 restricted the sale of native liquor in certain places. The *Mnazi* Ordinance No. 15 of 1907 defined Native Intoxicating Liquor to mean the *mnazi* such as *Tembo tamu* and *Tembo kali.* According to this Ordinance a tax was introduced to the owner of the tree tapped for *mnazi* and the District Commissioner licensed also tapping of *mnazi*. Since then *mnazi* was classified as native liquor that was prohibited. The current legislation in Kenya that deals with *mnazi* is found in the Traditional Liquor Act (Chapter 122) of the Laws of Kenya. Under this Act, *mnazi* was defined in the Act to include *Tembo tamu* and *Tembo kali* and the juice of any palm. The scope of this Act was to provide the control of African Liquor that was classified into two types, African intoxicating liquor and African spirituous liquor. *Mnazi*, other than *Tembo tamu* was classified as African Intoxicating liquor. *Tembo tamu* is a Swahili word for freshly tapped *mnazi* (unfermented *mnazi*) whereas *Tembo kali* refers to fermented *mnazi*.

Several countries in Africa, South and South East Asia, Pacific, Latin America, and the Caribbean, have had the benefit of having an Apex body for years. Such bodies are mandated to carry out the functions of marketing, processing, research and development of coconut-based products. Benin for example, has the Institute National des Rescherches Agricole du Benin (INRAB). This is a state institute with its staff being paid

from the national budget. Ivory Coast has Marc DELORME Research Station which deals with germplasm collection, characterization and evaluation. This institute operates under the National Coconut Development Program. In Nigeria, policy on improvement of coconut was introduced in National Development Plan (NDP) back in 1975-1980. In the 5^{th} development plan (1986-90), the Nigerian Institute for oil Palm (NIFOR) under the Ministry of Education, Science and Technology was mandated to provide appropriate research support to the coconut industry in the country. The government on realizing the potential of the coconut industry, particularly in the area of generating employment, increasing food production and farmers' income and for providing other sources of foreign exchange, started the Nigerian Institute for Oil Palm (NIFOR) in the late eighties. The federal government allocated funds annually for coconut breeding projects through NIFOR. In 1980, Tanzania introduced the National Coconut Development Programme (NCDP) at Mikocheni Agricultural Research Institute (MARI). The programme was vested with the mandate of implementing the research activities on coconut and coconut-based products. In Ghana there is the National Coconut Development Committee, which concentrates on the improvement of the coconut industry in Ghana, while, Jamaica has the Coconut Industry Board (CIB). This is a national institution involved in research and other activities aimed at improving the coconut industry. The institution is funded by the

British government, FAO, USAID and the Ministry of Agriculture (Jamaica). India has the India Coordinated Research Project on Palm funded by the Indian Council of Agricultural Research (ICAR). This project handles research on new coconut cultivars. In Sri Lanka, the Coconut Development Authority (CDA) has the responsibility of improvement of the coconut industry. Other institutions involved in coconut research include: the Coconut Research Institute of Sri-Lanka (CRI), which functions as a semi-government corporation. The institute is fully funded by a government grant. In South East Asia, Indonesia, which is the largest producer of coconut in the world, has in place a Research Institute for Coconut and Palm (RICP). This institute was established to solve the coconut problems and lead Indonesia towards coconut industrialization. Philippine, which is the second major producer of coconut with more than 20 million people deriving their livelihood from coconut, established the Philippines Coconut Authority (PCA) to work in conjunction with Small Coconut Farmers Development Project (SCFDP). The two institutions were mandated to oversee research and improvement of the coconut industry. Again the government of Philippine did the funding of PCA project with minor international support from UNDP/FAO. This therefore emphasizes the need to fast track the operationalization of the newly formed Kenya Coconut Development Authority so that it is able to meet her objectives.

1.2 The Problem statement

The Millennium Development Goal-1 is about eradicating extreme hunger and poverty. Approximately 75 % of the poor people in Africa live in the rural areas. Rural development is therefore essential to meeting MDG-1. Coconut being one of the rural crops has the potential to eradicate extreme poverty in the coconut growing regions in Kenya. This calls for the need to promote production and utilization of coconut-based products by ensuring a sustained flow of technologies, research and development which are suitable to the context and adequately, meet the challenges of the sub-sector.

Currently, the sub-sector is faced with challenges that hinder its development despite the enormous potential. These challenges include: lack of reliable data and/or information on the economic importance of the sub-sector to the country's GDP, in addition to lack of appropriate policy framework and development programmes for the sub-sector. Other constraints include: lack of awareness of coconut-based products along the value chain not to mention poor utilization of these products domestically. The fact that there has been low private sector investment with little or no research in the sub-sector makes it even more difficult to mitigate the challenges facing the sub-sector. The need for processing technologies along the value chain cannot be ignored. Conflicting legislations on *mnazi* has hindered its utilization and commercialization,

despite the need for a cheap and safe traditional alcoholic beverage, which, is able to compete with foreign beers, wines and spirits.

1.3 Justification

In order to transform the coconut sub sector from subsistence to commercial market oriented farming with an aim of improving the lives of the major stakeholders in the sub-sector, there is urgent need to increase production and utilization of coconut-based products through value addition. In order to achieve this goal, there is need to remove all the bottlenecks that impede private sector growth and development.

The work of this thesis includes a survey on production and marketing of coconut-based products with an aim of providing statistical data and economic importance of the coconut-based products. This exercise was undertaken because there was little or no information on production, and economic importance of *mnazi* and other coconut-based products by the time this work was conducted. This, information could be used by farmers, tappers, potential investors and other stakeholders in lobbying for better marketing systems and prices. The information could also be used by policy makers to formulate better and relevant policies for the coconut sub-sector.

Research on traditional tapping and distillation methods of *mnazi* and its spirit (*pyuwa*) was meant to create awareness on the concept of tapping and distillation of *mnazi* traditionally. This information could be use by scientists as the basis for further research aimed at improving the quality of *mnazi* through better taping and distillation methods.

The study on the major constraints faced by coconut farmers and tappers aimed at highlighting major challenges facing the sub-sector, hence the need to mitigate such challenges through appropriate policy guideline. This once in place is expected to enhance efficiency on production, marketing, research and development of the coconut-based products. Once implemented this will be inline with the vision of transforming Kenya into a newly industrialized country by the year 2030.

Research work on isolation and identification of native micro-flora in *mnazi* was undertaken with an aim of shedding some light to the major causes of *mnazi* spoilage within 2-3 days of fermentation. In addition the findings are expected to pave way for the possibility of utilizing these microorganisms in other fields of food and biotechnology, such as baking, brewing, manufacture of food and feed yeasts as well as the possibility of utilizing the isolated LAB in the field of probiotics. It expected that, more scientists will see the need and opportunity for further research in the coconut sub-sector.

The study on chemical wholesomeness and preservation of *mnazi* and its spirit was expected to shed some light on whether it is safe or not to drink *mnazi* and its spirit. The fact that, no harmful substances such as methanol and fusel oils were detected in *mnazi* is a positive gesture. These findings are expected to go a long way in finding lasting solution to the controversial law on *mnazi* (the Traditional Liquor Act (Chapter 122) of the Laws of Kenya). This act has been restrictive because it gives no room for further improvement and consumption of *mnazi* as an alternative alcoholic beverage to lager beers. Exemption of *mnazi* from this act is expected to provide a cheaper and high quality alcoholic drink for the low and middle income earners. The product is expected to compete effectively with the "clear" beers and spirits. This shall go a long way in improving the economic status of over 60,000 households who depend on *mnazi* with limited alternative for development.

Commercialization of *mnazi* and similar wine (palm wine) has been done with a lot of success in countries such as Nigeria, Thailand, Botswana, Malaysia, Philippines and Sri Lanka; however, Kenya is yet to realize this dream. The study on new product development was undertaken with an aim of setting benchmarks for commercialization of *mnazi*. The findings of this study could easily be scaled-up to with an aim of providing cheap but high quality alcoholic beverage (*mnazi*).

1.4 Objectives

1.4.1 General objective

To conduct a comprehensive study on traditional tapping and distillation methods of *mnazi*, investigate major constraints and educational levels of tappers and coconut farmers, identify the common microflora, and determine the chemical wholesomeness, better preservation and packaging methods for *mnazi*.

1.4.2 Specific objectives

(i) To document the traditional tapping and distillation methods of *mnazi* and its spirit (*pyuwa*).

(ii) To conduct a baseline survey on production, marketing and economic importance of *mnazi* and other coconut-based products.

(iii) To establish major constraints and educational levels of coconut farmers and tappers

(iv) To isolate, identify and characterize yeasts, acetic and lactic acid bacteria in *mnazi*

(v) To evaluate the chemical assay and biochemical quality analysis of mnazi toddy and its distillate (*pyuwa*)

(vi) To enhance the shelf life of *mnazi* through preservation and better packaging

(vii) To develop high quality *mnazi* products for commercialization

CHAPTER TWO

TRADITITIONAL TAPPING AND DISTILLATION METHODS OF *MNAZI* AS PRACTISED IN THE COASTAL REGION OF KENYA

2.1 Introduction

Mnazi is sweet exudates from tapped unopened spathe of palm trees (*Cocos nucifera*, *Phoenix reclinata* and *Hyphae conolea*) (Banzon and Velasco). It is dirty brown in color, containing 10-12% sugar, mainly sucrose (Faparusi, 1971). In Kenya, it is commonly referred to as "*mnazi*" or "*mdafu*" or coconut toddy. It is also referred to as "Nigerian wine" in West Africa (Faparusi, 1971), Toddy (Coconut sap) in Thailand and “Tuba" in the Philippines. When distilled, it is known as Lambonog or distilled wine (24-45% alcohol) (The Coconut Committee, 1992). The wine can also be converted to syrup, crude sugar or crystallized sugar (The Coconut Committee, 1992). The fermentation process makes the sap milky white in appearance due to the presence of a large number of fermenting bacteria and yeast. This product is unique in that microorganisms are alive when the wine is consumed (Faparusi, 1971). *Mnazi*, apart from being an alcoholic beverage, has some nutritive value to man. Results of a study of the wine from two species (*Hyphaene conolea* and *Phonex reclinate*) showed that it was an important source of nicotinic acid and vitamin C. To

a less extent, it is also a source of proteins, thiamine and riboflavin (Cunningham and Wehmeyer, 1988). In Thailand, *toddy* is marketed as a non-alcoholic beverage. Among the factors that affect the yield of *toddy* is age of palms and climate as well as the phenotypic yields group (Browning and Symons, 1961; Maravilla, 1975).

The tapping process of *mnazi* may involve wounding of the stem tissue, tapping from the root or fruit bud. In West Africa and particularly in Ghana, *mnazi* is tapped by felling trees of ten or more years old (Essiamah, 1989).

Indigenous beers are probably by volume the most widely consumed alcoholic beverages on the African continent (Hardy and Richet, 1933). There are two main factors, which influence the demand for traditional beers. Firstly, centuries of habit have attuned African palates to the distinctive sour taste of these beers. Secondly, these beers are affordable to a wider range of consumers, selling at a fraction of the price of other alcoholic beverages (Haggblade, 1984). In Customs Union Countries of Southern Africa (Botswana, Lesotho, Swaziland and South Africa) where aggregate economic statistics are dominated by the wealthy and westernized economy of South Africa, sorghum beer accounted for about 75% of the volume of all alcoholic beverages sold in 1977 (Deacon, 1980). In Kenya, the consumption of traditional alcoholic beverages may even be much higher due to economic hardships.

The need for high quality traditional alcoholic beverages is, of everyone's concern in Kenya. Wood (1982) pointed out that, in modernizing indigenous food processing, it should be noted that not only is a product being marketed, but also the chemical qualities of the product and its organoleptic worth are intimately integrated into the customer's philosophical and even religious views. For any meaningful modernization of a particular technology to take place, one requires adequate knowledge of the already existing technologies (Wood, 1982). Based on this fact, this study, therefore, intended to provide detailed information on the traditional tapping and distillation methods of *mnazi* as practised in Kenya. The tapping and distillation of the *mnazi* has been practised in Kenya for many years without any proper documentation of the practise. This study was, therefore, expected to educate people who have heard, seen and even drunk the mnazi but are not acquainted with its tapping and distillation processes. The study is also expected to serve as a base for comparison with other similar practises found in coconut and palm growing countries such as Philippines, Thailand and Ghana. The findings could also serve as a useful tool for further scientific research aimed at improving the tapping process.

2.2 Materials and Methods

The study covered three districts of the Coastal region of Kenya, namely Kilifi, Malindi and Kwale. The main tools used for the study included

participatory observations during the tapping and distillation processes and discussions with the concerned tappers and distillers. The other tools used were a tape recorder and a digital camera. The secondary sources of information were informal list of tappers and distillers obtained from the local chiefs' offices and that from local extension workers working with the Ministry of Agriculture. Stratified sampling was adopted for the study. Three districts with highest production of *mnazi* were chosen from the seven districts of the region. A minimum of two (2) divisions in each of the sample districts were included in the sample and this was done on Purposive Random Sampling. The selected divisions included Kaloleni and Mtwapa in Kilifi district, Malindi and Magarini in Malindi district and Matuga and Msambweni in Kwale district. A minimum of two locations were selected from each of the divisions. Finally, one village was selected from each location. The selected villages were those with experienced tappers and distillers. The last component of the study involved tappers and distillers who were again selected through Purposive Random Sampling. One tapper and one distiller were selected from every sampling village. The study was conducted in local language (Giriama) and Kiswahili. The tappers and distillers were probed through informal interview. Tappers and distillers were allowed to describe the various tapping and distillation methods using local names and terminologies, which were later, translated into technical and scientific languages. The study sought information on

different tapping and distillation methods of the *mnazi*. This information included the identification of ripe spathe (inflorescence), the tapping process, collection and storage of the wine and finally the distillation of the wine into spirit. A total of 12 tappers and 12 distillers were interviewed during the study. The data obtained was analyzed descriptively.

2.3 Results

2.3.1 Identification of ripe spathe for tapping

Ripe spathe was identified by the tappers as that which was sword like in shape, not swollen 6 -10cm from the tip, and also not swollen at the base. The length of ripe spathe ranged from 45 to 60cm long while that which followed ranged from 8 to 32cm long, depending on the phenotype of the tree. Approximately 60% of the tappers interviewed were able to identify ripe spathe as that which neither had a rough base nor showed how the spikelets (seeds) were arranged inside the sheathe. The remaining 40% of the tappers interviewed identified ripe spathe through peeling off the net like sheet (*Ndifu*) at the base of the spathe. Once this was done ripe spathe showed a clear demarcation of the spathe (florescence) from the stalk. Other tappers identified ripe spathe as that which easily shook when they climbed the tree.

2.3.2 Tapping process

The actual tapping process involved tying a rope made of sisal, creeping plant (*mbugu*) or plastic material depending on the availability around the spathe. The rope was then pushed between the spathe and the tender stem at a position 4 to 6cm from the base of the spathe; it was then tied firmly around the spathe upward up to 4 to 8cm from the tip depending on the preference of the tapper. Tying of the spathe was done so that it could be bent easily without straining or opening up to expose the spikelets. This then was followed by an incision(s) that was made on the spathe or florescence (*handa*). The incision was made at a point 3 to 4cm from the base of the spathe with the aim of allowing further gradual pulling down of the spathe without straining. The exercise of gradual bending of the spathe commenced immediately after its incision and tying of the ripe spathe. This exercise was conducted on a daily basis or after every alternate day for a period of one week. In very few cases, the spathe took as long as four weeks to reach the maximum bending position (almost horizontal). To retain the bent spathe in the inclined position, the spathe was supported either by a string tied to the spathe and a lower leaf base (*kumbi*) or a small stick inclined between the tender stem and the spathe. The incision technique differed from one tapper to another.

Over 70% of the tappers made three V-Shaped cuts along the circumference of the spathe, while the remaining less than 30% made only

one V-Shaped incision as shown in Figure 2.1A. The incision made at the base of the spathe, not only allowed easy bending of the spathe but also the exit of air and rainwater that finds its way into the spikelets of the spathe during the tapping process. Thereafter, the tip section of the spathe was cut and thrown away (4 to 8cm from the tip).

Figure 2.1 Sap collection and initial tapping process. A- Sap collection during tapping. B- Tapper making an incision at the base of the ripe spathe

This was followed by careful trimming and peeling off of the sheathe portion, 4 to 6cm from the cut end to expose the spikelets as shown in Figure 2.2B.

Figure 2.2. A -Tapper getting ready to trim off 2-3 mm of the leaflet tied section of the spathe without sheathe. B-shows the peeled and unpeeled sections of the spathe.

Tappers then tied around the exposed spikelets with leaflets plucked from the coconut tree whose midrib had been removed. The tying round or

wrapping of the exposed spikelets was made firm enough so as to avoid accumulation of air and drip back of the sap into the spikelets. It was noted that tappers practised extra care while peeling off the sheathe portion. This was done in order to avoid puncturing of the spikelets. It was reported that punctured spikelets easily rot due to accumulation of sap within them. Tappers reported that drip back of the sap into the spikelets caused souring. The leaflets used to wrap or tie around the exposed spikelets were replaced from time to time. After the replacement of the first round leaflets, the other subsequent leaflets were used without the removal of the mid-rib. Very few tappers (5%) tappers replaced the leaflets after they turned yellow, while most tappers (92%) others after three to four trimmings, while the remaining 3% of the tappers interviewed replaced the used leaflets after every two to three days.

The tapping exercise was found to be tedious for it involved climbing the tree two to three times daily. Tappers climbed the tree so as to trim the tied section of the spathe and/or collect the sap. The collected sap is normally referred to as *mnazi*. Each time the tappers climbed the tree, 2 to 3 mm of the leaflet tied section of the spathe was trimmed off. Usually the first trimmings were done without any sap collection for a period of one to six days depending on the tapper.

Thereafter, trimming was done three times a day while sap collection was conducted twice a day. Less than 2% of the tappers interviewed trimmed

the tied spathe twice a day. Every time trimming or collection of the sap was done, the collection container was put back in its collection position. The sap collection container is locally referred to as "*chiparya*". The first sap to ooze out was commonly referred to as "*mianzo*", which was thick sweet slurry and dirty brown in color. For the first one to six days of tapping, the first sap to ooze out was usually allowed to accumulate in the collection container and thereafter it was then discarded. Discarding was done daily, (morning and evening). Tappers used evidence of active fermentation (effervescence) inside the "*chiparya*" as an indicator that the sap was ripe for collection. After the first six days, the sap was collected daily using tapping containers. The collection time was between 0500 and 0900h in the morning and again between 1700 and 2200h in the evening or night time. During these two collection times, the trimming exercise was also performed in each case (that is the first and third trimming respectively). For the tappers who carried out three trimmings per day, the second trimming was done during afternoon h between 1200 and 1400h. No sap collection was done during this second trimming, but was instead allowed to accumulate, ready for the evening collection (during the third trimming). After every trimming and collection, the container was replaced as shown in Figure 2.1A. The yield of the wine from a tapped spathe increased gradually and its peak was usually reached after three weeks of continuous tapping. On average one to two liters of sap was collected daily

from one spathe. Two spathes could be tapped simultaneously if the coconut tree was high yielding. During the survey it was established that spathes were able to produce sap to the end (until the last trim); however, others (over 50%) stopped producing sap after half of their original length was trimmed off. It was also established that one spathe could be tapped continuously for a period of one to three months. It was noted that tappers kept on sharpening their tapping knives to keep them constantly sharp.

After tapping the wine collected from different trees, it was then mixed in a 20-liter container. The tappers then filtered the wine using simple sieves or traditional sieves from the coconut tree (*ndifu*), as shown in Figure 2.3**.** Filtration was done in order to remove foreign matter such as parts of leaflets, spikelets, dead bees and dead red ants. Finally, the filtered wine was stored in 20-liter containers or pots for further natural fermentation and maturation. It was noted that no other materials or reagents were added to the wine during the whole process of tapping and storage. Anything foreign including water was regarded as adulteration. Wine meant for sale was stored for one to three days depending on the demand. Majority of the people preferred blends in which fresh wine was blended with that which had been stored for one to two days. However, fresh wine was not popular because it was too sweet and was associated with constant headaches to the consumers. On few occasions storage was prolonged up to one week due to lack of customers. Wine stored for this long was later distilled into

spirit or used for vinegar production (*siki*). The vinegar from the *mnazi* was found to be very popular among the local people as a food dressing.

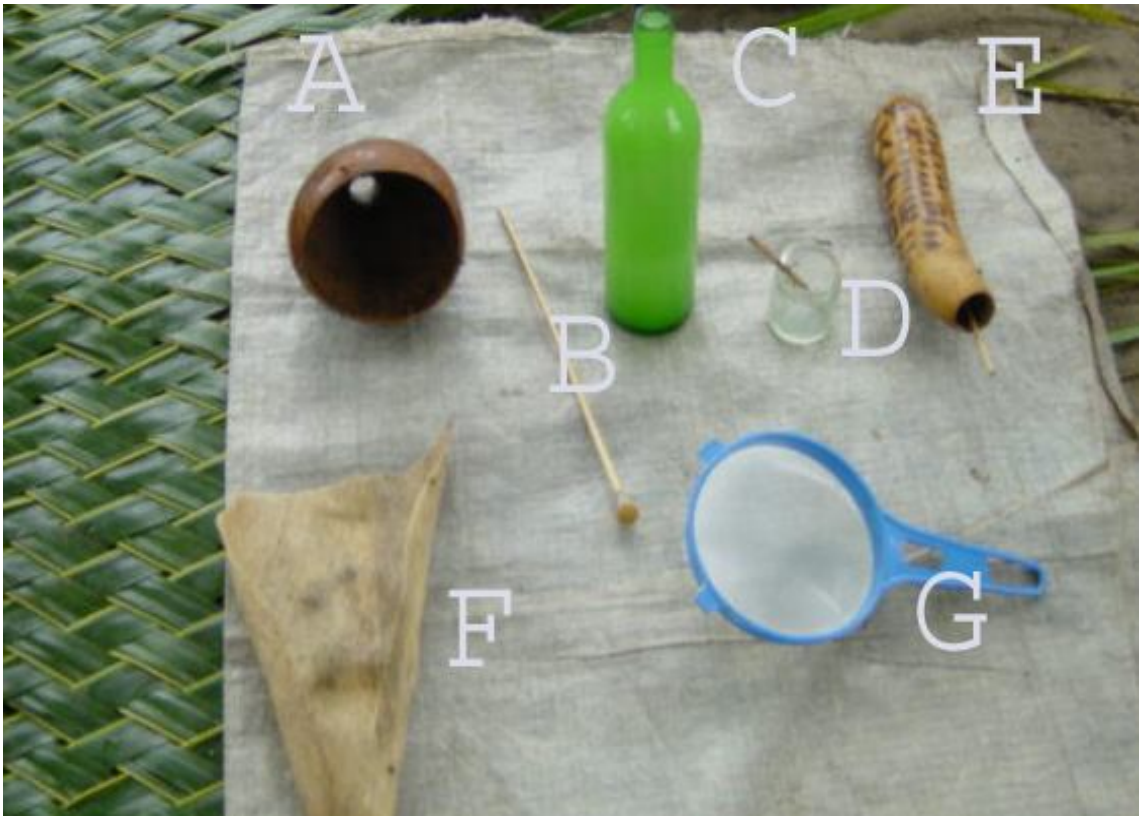


Figure 2.3: Drinking and *mnazi* filtering tools. A-traditional filtering funnel, B-drinking straw, C-measuring bottle, D-small "*mboko*", E- big "*mboko*", F-traditional filter "*ndifu*" and G-modern sieve.

2.3.3 Distillation of spirit (*pyuwa*) from *mnazi*

During the survey it was established that, wine meant for distillation into spirit (*pyuwa*), required longer storage of up to one week, although few distillers did not mind using *mnazi* that had been stored for only one to three days. However this depended heavily on experience and preference of the distiller as well as demand for the spirit. Distillation into spirit not only

raised the alcoholic content of the wine to a greater value but it was also considered as a way to preserve the wine lest it goes bad. In the distillation operation, matured wine was the only raw material required.

Maturation and storage of *mnazi* meant for distillation was usually done at ambient temperature (25-30°C). For easy control of storage time and temperature, most distillers preferred carrying out the exercise themselves. It was noted that about half of the distillers added one to two liters of the milky sediment accumulated during storage (commonly referred to as "*masimbi*" or "*sira*") to every ten liters of *mnazi* that was matured only for one or two days, but others did not did not add any "*masimbi*" to the wine. Plastic containers were not preferred as storage vessels for *mnazi* meant for distillation. Most tappers and distillers preferred the traditional pots or gourds (*vibuyu*). Most tappers and distillers regarded high quality wine for distillation as that, which was neither sour nor adulterated.

The basic assemblies of the distillation equipment used by most distillers are as shown in Figures 2.4A and 4B. The major difference between the two assemblies is the mouth of the largest pot. In Figure 2.4A the largest pot has a wider mouth in comparison to that of Figure 2.4B. The actual assembly of the equipment is as shown in Figures 2.5A and 2.5B. First the largest pot with *mnazi* in it seats at the bottom of the assembly. It acts as a heater and a reservoir of the raw material. A few distillers (less than 2%) used a twenty-liter rectangular tin (*debe*) instead of the largest pot. The

medium perforated pot with holes at its base then follows. This unit acts as a separator. The third smallest pot is then allowed to seat inside the perforated pot. This unit acts as a collector of the overhead product (distillate). Finally, a flat-based vessel made of iron or aluminum (*sufuria*) covered the perforated pot as shown in Figure 2.5.

Figure 2.4. Distillation equipment sets. In both A and B from left to right; *sufuria*, smallest pot, middle or perforated pot, and largest pot respectively. A - shows largest pot with wide mouth, while B- shows largest pot with small mouth.

The *sufuria* acted as a condenser. A small clearance between the smallest and perforated pot was made available by placing small chips of wood or smetric tones at the bottom of the perforated pot before the smallest pot was allowed to seat inside the perforated pot. Before assembling the equipment, the matured *mnazi* ready for distillation was first poured into the

largest pot or metallic container (*debe*). Thereafter, the equipment was assembled and some cold water was poured into the *sufuria*. The cold water acted as a cooling medium for the vapour or spirit that formed during the distillation process. After assembling the distillation equipment, the joint between the largest pot or *debe* and perforated pot as well as that between perforated pot and the "*sufuria*" were sealed off with some clothing (*kangas* and *lessos*) and/or mud depending on the type of the largest pot used. Distillers, who used the largest pot with a wider mouth or *debe*, sealed the joint between the largest pot and the perforated pot with some clothing followed by mud from red or loam soil (Figures 2.5A and 2.6A). However, a few distillers sealed this joint using mud only. The joint between the perforated pot and the "*sufuria*" were sealed off with some clothing (*kangas* and *lessos*) without any application of mud as shown in Figures 2.5B, 2.6A and 2.6B. Sealing of the joints was done so as to avoid escape of the vapour into the atmosphere. Distillers who used the largest pot with a small mouth did not seal the joint between the largest pot and the perforated pot. The design of this assembly was special, in that the perforated pot fitted well into the largest pot such that no sealing was required as shown in Figure 2.6B.

Figure 2.5. Two distillation equipment sets arranged vertically. A-largest pot act as a distiller (reservoir), B- metallic container (*debe*) is used in place of the largest pot.

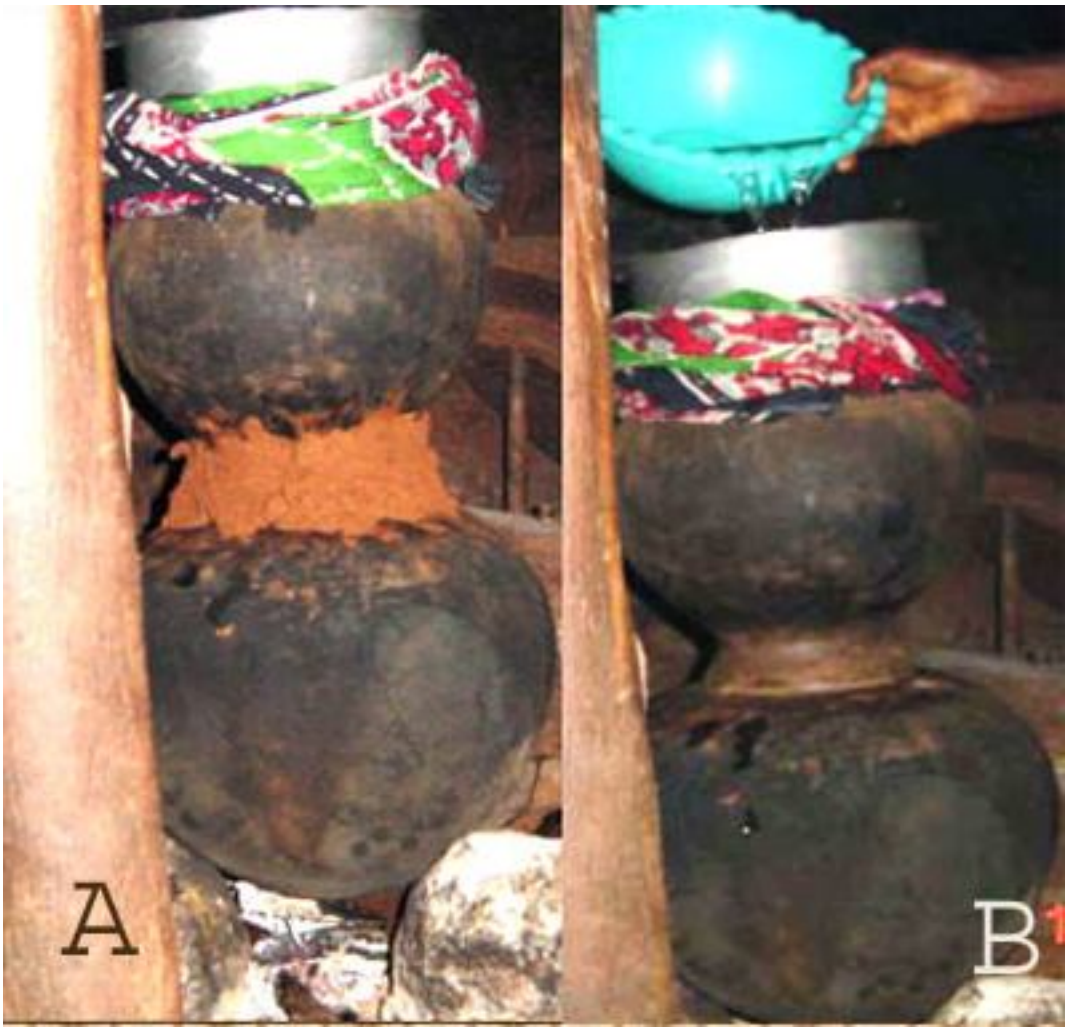


Figure 2.6. Distillation equipment sets (pots). A- a unit with the largest pot having a wide mouth; sealing between the perforated pot and largest pot is enhanced by mud from red soil. B-no mud was applied between the perforated pot and the largest pot

The distillation was carried out using a three stone traditional firewood cooker (*jiko*). The wine was then brought to boiling on the *jiko*. After boiling commenced, some of the firewood was removed to allow moderate boiling. Distillers explained that during boiling of the wine, vapor was released,

which distilled off from the largest pot, through the perforated pot and finally the same vapor condensed at the base of the flat-based vessel (*sufuria*). It was the *sufuria* that acted like a condenser while the largest pot acted like a heating vessel in the scientific flash distillation. The condensate that formed at the outside base of the *sufuria* dripped into the smallest pot with very wide mouth sitting inside the perforated pot (Figures 2.5 and 2.6).

The smallest pot, therefore, acted as a collector or reservoir for the distillate (spirit). For continuous condensation of the distillate during the distillation process, the water inside the *sufuria* was discarded after getting warm while simultaneously being replaced by more cold water. The frequency at which the warm water was being replaced depended not only on the capacity of the distillation unit, but also on the size of the condenser (*sufuria* filled with cold water). In addition, it affected the quality and quantity of distillate expected. The lesser the number of times the water was replaced, the shorter the distillation time. This in turn, gave better quality and higher percentage of alcohol, but less yields, as reported by most distillers. The warm water was replaced after it had attained a temperature of 40 to 50°C. The water temperature was determined by dipping of one's finger into the *sufuria*. Professional distillers were able to sense the correct temperature of the warm water by dipping a finger into the *sufuria*. Over 70% of the respondents (distillers) preferred replacing the warm water three times, while 26% of the respondents preferred four times

and less than 4% replaced it as many as six times. After distillation, all the firewood was removed from the "*jiko*" to allow partial cooling. Finally the equipment was dismantled, followed by quick transfer of the distillate into a clean glass bottle. The lid was screwed on before further cooling and storage at ambient temperature. The supernatant liquid that remained in the largest pot commonly referred to as "*reka*" or "*magindiza*" was discarded in a pit or toilet because it was said to be lethal to all living things including man, animals and even plants.

2.4 Discussion

The tapping process as explained in this study compares well with that of Thailand but differs with that which is practised in Ghana, where *mnazi* is tapped by felling trees that were ten or more years old (Maravilla, 1975; Essiamah, 1989). The major difference between the practise in this study and that practised in Thailand is on the section of the spathe trimmed. In this study trimming was done on the tip of the section of spathe whose sheathe was previously peeled off and the exposed spikelets tied with leaflets plucked from the tree, while in Thailand trimming of the spathe took place with its sheathe intact (Maravilla, 1975). Also found to be different was the period in which a single spathe could be tapped. In Thailand, a single spathe could be tapped for only one month, while in this study, tapping and wine collection from a single spathe continued for one to three months depending on the genotype of the tree. In this study, climate as

well as the phenotype of the coconut affected the wine yield but not the age of the tree, while in Philippines, age, phenotype of the tree and climate, all affected the wine yield (Maravilla, 1975; Essiamah, 1989). All tappers interviewed expressed the need to use very sharp tapping knives. The explanation given was that blunt knives normally gave a rough trimmed surface hence sour *mnazi*. The sour taste could have been attributed to the fact that rough surfaces allowed some drops of the oozing sap to accumulate within them. Subsequently, the microorganisms present in *mnazi* that had accumulated at the rough surface caused fermentation hence the sour taste. The microorganisms that caused fermentation included lactic and acetic bacteria (Faparusi, 1971). These bacteria caused both primary and secondary fermentation with the production of lactic, acetic and other organic acids resulting in the sour taste. While conducting this survey, tappers expressed the need to filter wine after tapping. Most tappers reported that unfiltered *mnazi* normally gave poor quality wine in terms of taste and smell. This was expected because unfiltered *mnazi* usually contained a lot of foreign matter such as parts of leaflets, fragments of spikelets, dead bees and red ants. All this mixture together with the growth and fermentation of microorganisms present in the wine (Hibbert and Barsha, 1931; Loitsyanskaya, 1951; Hehre and Hamilton, 1953) could result in a mixture of compounds such as organic acid and polysaccharides.

Acetic acid bacteria, for example, were reported to be capable of producing polysaccharides such as soluble polymers of glucose, levan and dextran (Hibbert and Barsha, 1931; Loitsyanskaya, 1951; Hehre and Hamilton, 1953). The presence of the fermentation bi-products, together with dead insects as well as parts of leaflets and spikelets could easily affect the quality of the tapped wine if not filtered. In this study, sap collection was usually done between 0500 and 0900h and again between 1700 and 2100h, which compared well with the sap collection in Thailand (Maravilla, 1975). In Thailand, the sap collection took place between 0600 and 0900h and again between 1500 and 1800h every day as reported by Maravilla (1975). Discussions with most tappers revealed that, wine and sap collection containers were not washed but instead leaflets plucked from the tree were used to remove the foreign matter in them. On the other hand, storage containers were washed but without detergent. This practise contradicted similar practises in Thailand where tapping tools were thoroughly washed with hot water (The Coconut Committee, 1992). Although no valid reasons were given to explain the practise, most tappers treated it as a taboo. Most tappers preferred the traditional gourd collection containers as opposed to plastic containers. Gourd containers were preferred because they were able to keep the wine cool. This lowered the fermentation rate; hence less wine was lost through frothing due to high fermentation rate especially when the ambient temperatures were between

25 and 30°C. During tapping, extra care was observed making sure there was minimum disturbance of the spathe. This was achieved by holding the spathe firmly while tapping it. It was established that shaking of the spathe during tapping when done regularly caused gradual drying up of the sap, hence less wine was produced. This phenomenon also requires further research to explain its validity for tappers did give any answer to its occurrence. In this study, sap was stored without adding any preservative while a similar study carried out in Thailand reported that tappers and farmers used special barks such as Kam (*Cotylobeum lanceolatum*) and pra-yom (*Shorea floribunda*) for preservation (Maravilla, 1975). They also use a mixture of chemical preservatives mainly sodium metabisulphite, sodium propionate, and sodium benzoate in the ratio of 10:1:1, respectively to preserve the tapped wine (Maravilla, 1975)**.**

The distillation process and equipment used to distil spirit from *mnazi* is similar to that described by Nout (1979) in his report about the manufacture of Nubian gin by "basin" distillation equipment**.** However, major differences between this study and that of Nout (1979)] are in the raw materials used, method of fermentation, the distillation technique and the utilization of residues left after distillation**.** In this case, mnazi was the only raw material as opposed to sugarcane or molasses mixed with components of opaque beer "*busaa*" as described by Nout (1979). Further fermentation of the mixture in that study was done for one week, whereas in this study

fermentation depended on the distiller's preference and ranged from one to seven days. During distillation, Nout (1975) indicated that distillation process was stopped after three to four replacements of the warm water, while in this study some distillers, replaced the warm water more frequently, even up to six times. Other distillers replaced the water less frequently, one to two times depending on the capacity of the condenser and the reservoir (largest pot) as well as the quantity and quality (concentration) of the expected spirit. According to Nout (1979), the residue left after distillation process was used to feed animals and in some cases it was added to the raw material for further fermentation. In this study, the residue was reported to be extremely lethal to human beings, animals and plants. It will be of great interest if a study could be conducted to confirm the allegation and establish the chemical composition of this residue. Findings of such a study could be used to manufacture poisonous chemicals to control rodents, insects, pest and even weeds. Major constraints faced by most tappers included: - provincial administration and police harassment, poor pricing and lack of market as well as lack of modern and improved tapping and processing technologies. However since the announcement by the President that *mnazi* be excluded from the list of illicit brews, and thereafter the creation of the Kenya Coconut Development Authority through a Presidential Order of 2007 which was contained in a Kenya Gazette Supplement No. 83 dated 27th August, 2007,

a legal notice no. 165, under the State Corporations Act (Cap. 446), it is therefore expected that the concerned Authority will do its best in finding lasting solutions to the major constraints facing the sub-sector as provided in this study.

2.5 Conclusion and recommendations

Traditional methods for tapping and distillation of coconut sap have evolved over a long period in the Coastal region. These methods are used in the identification and tapping of ripe spathe. They are similarly applied in the collection, storage and distillation of the sap. It is necessary to formulate a clear policy concerning tapping and consumption of *mnazi*. In addition, other issues related to germplasm development, technology transfer, marketing and distribution of coconut and coconut products need to be addressed. The need for better tapping methods and proper management to replace the already existing traditional methods in order to increase *mnazi* yields should also be considered.

CHAPTER THREE

EDUCATIONAL LEVEL AND MAJOR CONSTRAINTS FACED BY FARMERS AND TAPPERS IN PRODUCTION AND MARKETING OF COCONUT-BASED PRODUCTS IN KENYA

3.1 Introduction

In Kenya, the coconut tree is found at the Coastal region and it is grown traditionally mainly for its tender nut, copra and oil. Coconut farming in most of the coconut growing countries is dominantly based on smallholdings, which are beset with problems of low productivity and steady decline in acreage (Banzon and Velasco 1982). In some parts of the Coastal Kenya, where the soil is too poor to sustain other crops, coconuts and cashewnuts represents the only form livelihood for hundreds of thousands of people. Coconut in Kenya, therefore, is more than a commercial crop, in the sense that it is also a social crop. This is attributed to the fact that, coconut-based products are the major income earners for the rural poor, at the same time, the coconut farms are usually family owned and passed from one generation to the next.

Despite the fact that coconut is the main cash and social crop at the Coastal region of Kenya, very little work on coconut research and development has been conducted. According to a survey conducted by Warui and Gethi (1980), Bole rot disease was identified as the main cause of many trees standing dead in the coconut field. This is a fungal disease

which is capable of wiping out the whole coconut plantation if control measures are not put in place. According to Warui and Gethi (1980), among the insect pests that are responsible for decline of coconut production were Rhinoceros beetle (*Orctes monoceros*) and Coriel bug (*Pseudtheraptus wayi*) (Warui and Gethi, 1980). According to Kadere et al. (2004) lack of market, low prices, pests and diseases, poor infrastructure as well as lack of credit to farmers as some of the major constraints faced by coconut farmers in Kenya. This study was conducted with the aim of providing information on major constraints and educational levels of coconut farmers and tappers.

3.2 Methodology

3.2.1 Research design

Methodology used in this study was largely quantitative. However, participatory approaches were used for gathering information that was used in guiding the survey and enriching analysis and interpretation of the survey results. Both cases were simultaneously and sequentially utilized to collect the required information. A simple cross-section survey design was applied to collect the data, where households of the farmers and tappers in the projected areas (all the six districts) were given an equal chance of being selected for the survey.

3.2.2 Population

According to the population census of Kenya (1999), the six survey districts had 469,792 number of households out of which only 208,362 were indicated as farmers' households. Since not all the farmers' households were involved in coconut production. The survey targeted households in areas with high concentration of coconut population whereas areas with no or little activities were isolated from the survey. This exercise of area identification was done with the help of local provincial administration (Sub-chiefs) together with area staff from the Ministry of Agriculture (MoA). Survey targeted all the heads of households practicing coconut farming while the questionnaire for tappers targeted the individual tappers in the selected areas. For families living in homesteads, it was the homestead head that was targeted, since according to *Mijikenda* customs/traditions it is the homestead head that can claim ownership of all the coconut trees owned by members of his homestead. This trend is passed from generation to generation.

3.2.3 Sample

The survey was conducted in the Coastal region of Kenya between June 2002 and April 2003 by trained enumerators. The survey covered 6 districts of the coast province of Kenya namely; Kwale, Mombasa, Kilifi, Malindi, Tana River and Lamu, with major focus on coconut growing areas. The

focal point was sub location. However, areas with no coconut farming or very little of it were left out the survey.

During the survey a total of 2,814 tappers and 9,155 heads of household/homestead were interviewed based on areas with significant concentration of coconut farming across the region.

3.2.5 Sampling method

Both the stratified and area-sampling methods were employed for the purpose of this survey. First the population was divided into homogeneous sub-parts (strata), which were mainly the tappers and coconut farmers. Area sampling then followed in which the project area (coast province) was divided into small administrative areas (districts). The districts were further sub-divided into divisions and locations. This method assisted in identifying the exact locations/sub-locations where the actual survey was to be conducted. In the selected areas, all the farmers and tappers were then interviewed.

3.2.6 Data collection tool

The main tool used for the survey was a questionnaire. Primary data was collected from both farmers and tappers using two-structured questionnaires. One questionnaire was designed specifically for farmers while the other targeted tappers only. The study was conducted in local language (Giriama) and Kiswahili. The tappers and farmers were probed

through simple questions found in the questionnaires. Information sought under these instruments related to annual production of various coconut-based products as well as earnings from these products, prices and quantities of mnazi tapped at low and high season as well as ownership of trees being tapped. The secondary sources of information were informal list of tappers and coconut farmers obtained from the local chiefs' offices and that from local extension workers working with the Ministry of Agriculture.

3.2.7 Pre-testing of the data collection tool

The appropriateness of the data collection tool(s) was studied during stakeholders seminar held at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) where 26 participants participated in this exercise. Out of the 26 participants, 6 of them were enumerators picked from all the six districts where survey was to be conducted. The main aim of the seminar was to refine the instrument and improve in overall planning process. After the seminar the 6 enumerators undertook the actual pre-testing exercise in 5 days at the following sites- Mombasa- Kisauni, Kilifi-Mtwapa, Kwale- Matuga, Malindi- Gede, Lamu- within the island, and Tana River-Kipini. After the pre-testing, the data from the six sites was analyzed with the major focus on problems encountered in getting information from the respondents and the ability to achieve the set goals. Finally all the questions that proved difficult to administer were revised accordingly in line

with the findings of the pre-testing exercise while important information that was not captured in the pre-tested survey questionnaire(s) was included in the revised version.

3.2.8 Data collection

The data collection exercise was carried out by a team of over 700 enumerators identified at the sub-location level with the help of both provincial administration and extension workers of the ministry of agriculture. This method of selection was done to make sure each enumerator had full knowledge of the areas of interest and was acceptable to all farmers and tappers in the area. The Principal Investigator trained each team over a 1day period on the survey approach and how to accurately administer the questionnaires to the respondents. In the actual implementation of the data collection exercise, each team of enumerators was supervised on a day-to-day basis by the Principal Investigator assisted by his two assistant and the MoA staff based at divisional level. To authenticate and cross check quality of work done by the enumerators, the supervisors made frequent spot checks among farmers and tappers alike. This was done to ensure all the tappers and farmers in the selected areas were visited and the information given was correct. The spot check was done on randomly selected zone of each sub-location on 10 farmers and 10 tappers in a row. All the questions in the questionnaires were asked in local language (Giriama) and Kiswahili.

3.2.9 Data processing

Data entry, processing and analysis were done using SPSS for Windows (Version 8.0) spreadsheet program and Microsoft Excel 2000. Descriptive statistics (frequencies, scores, mean, maximum, minimum) were determined. A team of trained data input and coding clerks managed the whole exercise of data processing. The actual processing and analysis started with data cleaning to remove the gaps and ensure consistency.

3.3 Results

3.3.1 Survey

During the survey a total of 9,155 farmers were interviewed out of which, Kilifi had 4,142, Kwale - 2,568, Malindi- 776, Mombasa- 754, Lamu- 791, and Tana- River- 124. On the other hand a total of 2,814 tappers were interviewed out of which Kilifi had 1,784, Mombasa- 435, Malindi- 313, Kwale- 224, Tana-River- 56 and Lamu- 2.

3.3.2 Farmers

On average, 32.5% of the 9,155 farmers interviewed were found to be illiterate, while 42.0% had primary school level of education. Only 11.2% had secondary school education while 2.1% had college or university certificate. Others fall under the category of informal education (Figure 3.1).

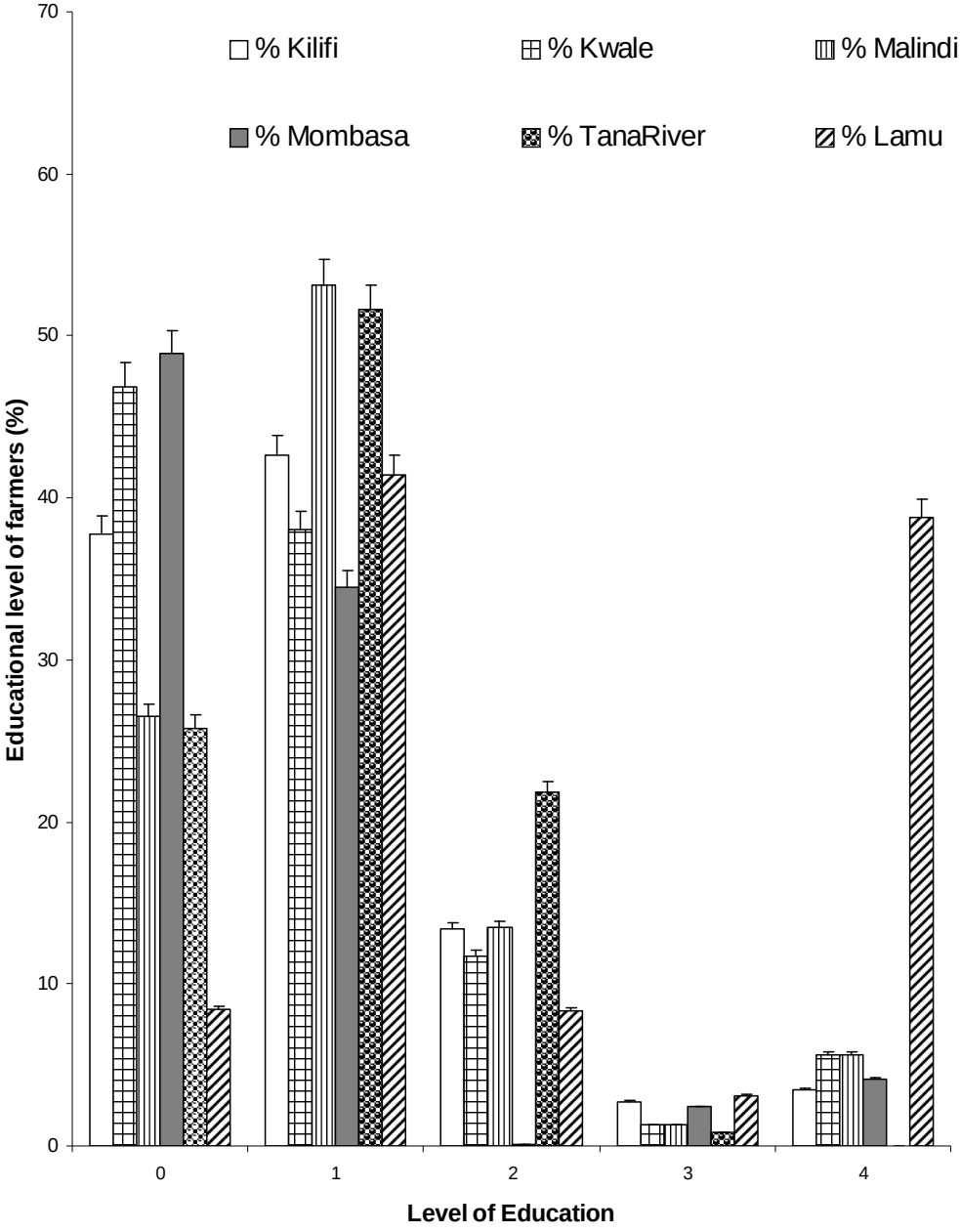


Figure 3.1: Education level of farmers in all the six districts in the Coastal province of Kenya. 0- No education, 1- Primary, 2- Secondary, 3- Tertiary & University, 4- Informal education Bars represent mean value + SDE of the data.

Changamwe division of Mombasa district had the highest number of farmers who were illiterate (64.3%) followed by Likoni division still in Mombasa district (49.8%) and Matuga division of Kwale district (48.7%) respectively (Table 3.1).

Table 3.1: Education level of farmers at the divisional level of each district

		Educational Levels (%)				
Districts	**Divisions**	**0**	**1**	**2**	**3**	**4**
Kilifi	Bahari	35.9	41.5	14.8	3.8	4.0
	Chonyi	39.7	43.8	11.0	3.9	1.6
	Kaloleni	38.7	42.5	12.9	2.0	3.9
	Kikambala	29.9	43.0	2.8	1.7	4.7
Kwale	Matuga	48.7	36.9	11.0	1.9	1.4
	Msambweni	46.2	38.5	11.9	1.0	2.4
Malindi	Magarini	22.2	66.5	9.7	1.6	0.0
	Malindi	27.9	48.9	14.7	1.3	7.4
Mombasa	Changamwe	64.3	25.8	7.2	3.6	0.0
	Kisauni	47.5	38.1	10.5	2.7	90.0
	Likoni	49.8	28.2	9.5	1.4	11.3
Tana River	Kipini	25.8	51.6	21.8	0.8	0.0
Lamu	Amu	3.7	28.8	13.6	2.1	51.8
	Faza	12.4	26.8	3.7	5.5	51.6
	Kizingitini	26.4	22.7	13.2	0.0	37.7
	Mpeketoni	0.6	88.3	10.0	0.6	0.6

Legend: 0 -No education; 1 -Primary; 2 -Secondary; 3 -University/College; 4 -Informal education

3.3.3 Tappers

Results of the questionnaires presented to the respective respondents showed that on average majority of the tappers in all the five districts were found to be either illiterate (40.3%) or had only primary school certificates (53.1%). Only a few had secondary school education (4.5%) and less than 0.4% had tertiary level of education (Figure 3.2).

The divisions that were leading in terms of illiterate tappers were Likoni and Kisauni in Mombasa district, Matuga in Kwale and Kaloleni in Kilifi. Bahari, Chonyi and Kikambala all of Kilifi district and Matuga of Kwale district were the leading divisions with tappers that had attained secondary school education. On the other hand Malindi division of Malindi district was found to be leading in terms of tappers that had attained tertiaty level of education (Table 3.2)

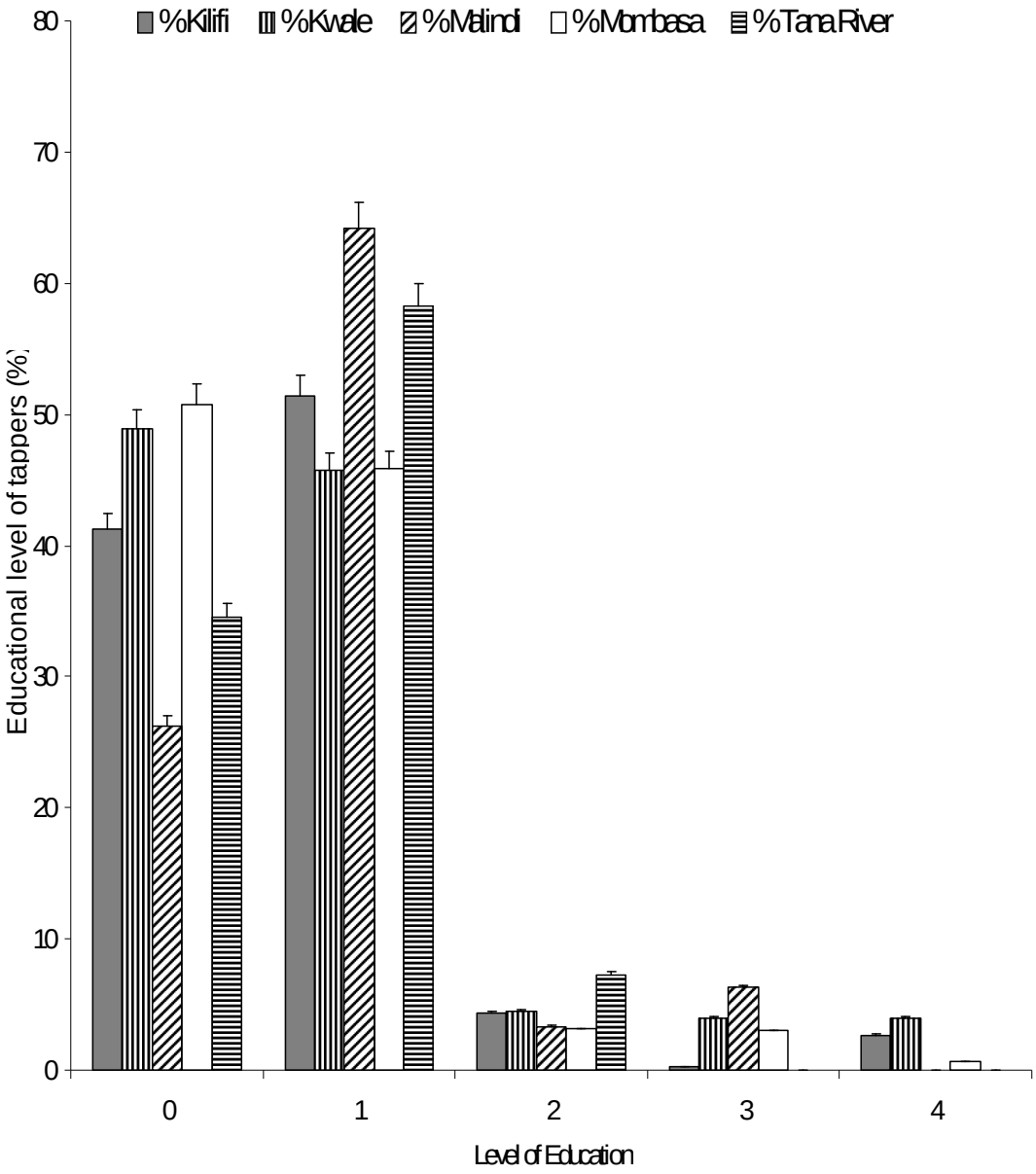


Figure 3.2: Education level of Tappers in all the six districts of the Coastal region of Kenya
0 - No education, 1- Primary, 2- Secondary, 3- Tertiary & University, 4- Informal education
Bars represent mean value + SDE of the data.

Table 3.2: Education level of tappers at the divisional level of each district

		Educational levels (%)				
Districts	**Divisions**	**0**	**1**	**2**	**3**	**4**
Kilifi	Bahari	28.4	60.6	9.7	0.0	1.3
	Chonyi	35.2	54.2	6.4	1.4	2.5
	Kaloleni	50.4	44.7	1.7	0.0	3.2
	Kikambala	24.2	67.3	6.1	0.0	2.4
Kwale	Matuga	51.0	38.8	6.1	2.0	2.0
	Msambweni	48.3	47.7	4.0	0.0	0.0
Malindi	Magarini	2.4	95.1	2.4	0.0	0.0
	Malindi	29.9	59.4	3.4	7.3	0.0
Mombasa	Changamwe	35.7	60.7	3.6	0.0	0.0
	Kisauni	51.8	44.5	3.3	0.4	0.0
	Likoni	56.0	36.0	0.0	0.0	8.0
Tana River	Garsen	39.4	57.6	3.0	0.0	0.0
	Kipini	27.3	59.1	13.6	0.0	0.0

Legend: 0 -No education; 1 -Primary; 2 -Secondary; 3 -University/College; 4 -Informal education

3.3.4 Major constraints faced by the farmers

Among the major constraints faced by the farmers in the entire six districts are shown in Figure 3.3. Lack of market for the coconut-based products was ranked highest (28.6%) followed by low/fluctuation of prices (20.3%).

Pests and diseases (12.4%), poor transport/infrastructure (9.4%) and lack of credit/loans (7.7%) were ranked third, fourth and fifth respectively. Other constraints included destruction of young and old coconut trees by fire and wildlife (6.9%), provincial administration and police harassment (5.3%), drought/poor soil/low production (6.4%), lack of storage facilities (1.6%), lack of technical know-how (1.4%) and lack of regulatory body (0.5%) as shown in Figure 3.3.

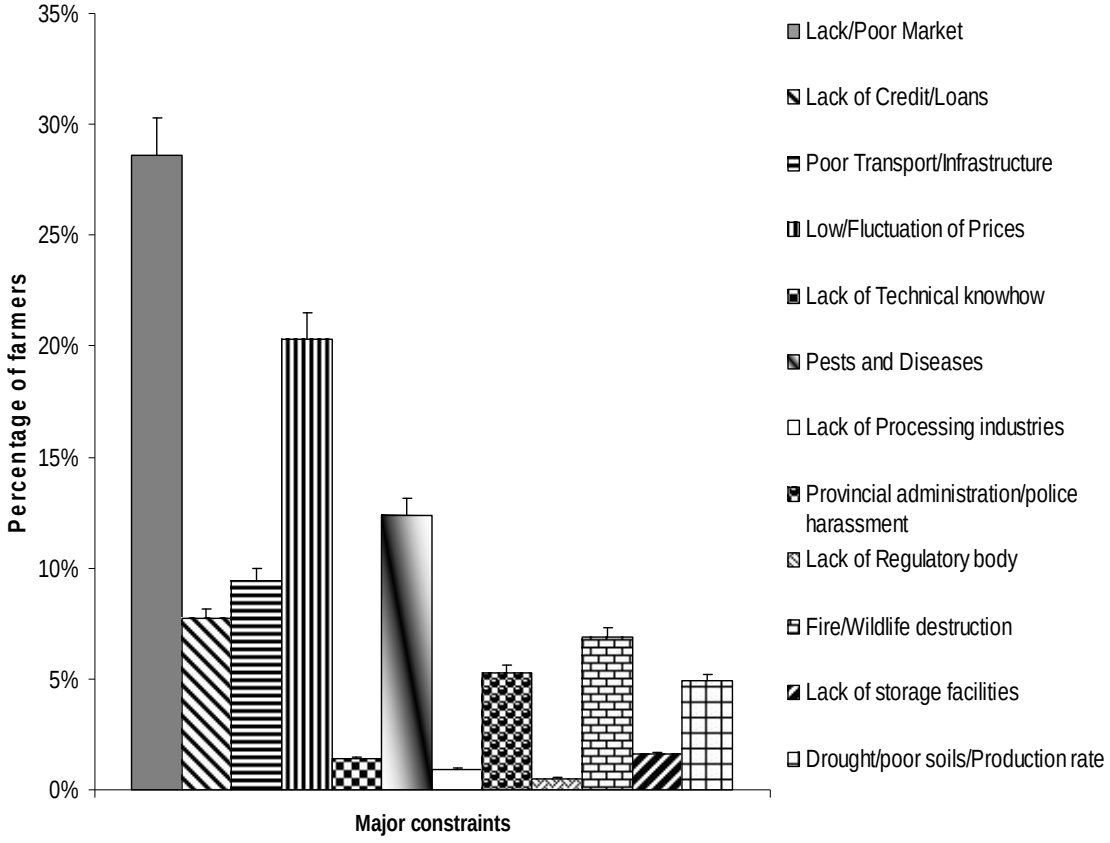


Figure 3.3: Summary of constraints of farmers in the six districts of the Coastal Province of Kenya. Bars represent mean value + SDE of the data.

3.3.5 Major constraints faced by the tapper

Major constraints faced by the tappers in all the five districts compared well with those given by the farmers. Lack of market or poor marketing was ranked highest (31.1%); the constraint of police and/or provincial administration harassment (20.8%) was ranked second unlike in the case of farmers where it was ranked seventh. Third in the series was low/fluctuation of prices (15.7%), followed by poor storage/preservation facilities (8.7%). Fifth in the series was risk of falling from the coconut tree due to snake-bites/strong winds/slippery trees (5.7%). Other constraints included: pests and diseases (4.3%), lack of regulatory body (3.8%), lack of technical know-how (3.2%), poor transport and infrastructure (1.8%), lack of payment by unfaithful vendors and drunkards (1.7%), destruction by fire and wild animals (1.2%) as well as lack of credit facilities (2.0%), see Figure 3.4.

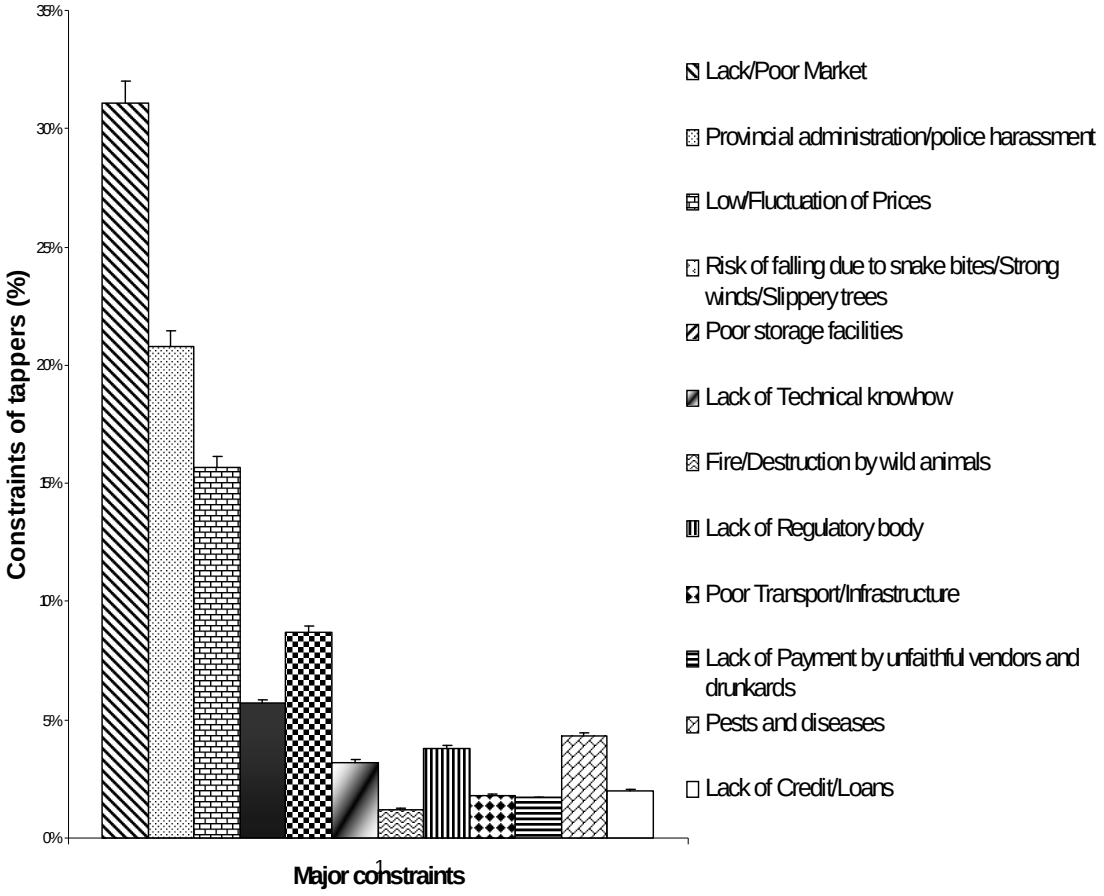


Figure 3.4: Summary of constraints of tappers in the six districts of the Coastal province of Kenya. Bars represent mean value + SDE of the data

3.4 Discussion

Majority of the tappers were found to be either illiterate (40.3%) or had only primary school certificate (53.1%). The same applied to the farmers of whom 32.5% were illiterate while 42.0% had primary school certificate of

education. During the survey it was observed that lack of adequate education was a major contributory factor to lack of general understanding of the marketing structure facing the coconut industry. This in turn left most farmers and tappers with no solution to the major constraints facing their most cherished crop (the coconut tree), the so-called “tree of life”. Although most respondents (farmers and tappers) were able to articulate the problems surrounding the coconut industry, most of them were pessimistic of any possible solutions in the near future. Farmers and tappers alike believed that any person who will come up with solutions to the problems surrounding the industry would be like a God sent “Messiah” to them. The study established that the marketing system of the sub-sector was not efficient. As a result of this, unscrupulous middlemen and private traders were taking advantage of the situation, where farmers and traders alike were being exploited. This was evident in a situation where one bundle broom that sold at KES5 at farm gate, was subdivided into 2 to 3 bundles of broom by the so-called middlemen and later each bundle was sold at a price of KES20 in the streets of Nairobi and its environs. This means a broom that was bought for KES5 at farm gate ended up fetching as much as KES60. This study established that most of the problems currently facing coconut tree farmers and tappers could be traced as far back as 16th century (over five centuries back). The fact that no solutions have been found for the myriads of problems facing the coconut industry in Kenya is

partly due to the level of illiteracy facing the farmers and tappers as well as lack of clear government policy on the coconut industry. As a result of all this, the communities in the region are faced with many other poverty related problems such as malnutrition, lack of school fees, poor and inadequate of health facilities, poor shelter and lack of safe drinking water. Among the major constraints that were highly ranked by most farmers and tappers alike are: lack of market and low prices for the coconut-based products. In Kenya, the amorphous marketing structure in the coconut industry is responsible for the low prices of most coconut-based products. In addition, mismanagement and collapse of most farmers' cooperative societies in the region in 1980's, and consequently, liberalization and privatization, resulted in uncoordinated marketing structure that placed marketing and production of coconut-based products wholly under the private sector. As a result, farmers have had a free hand to sell their products to buyers or agents of their choice. Under such circumstances, the marketing structure became amorphous; thereby subjecting most coconut growers and tappers to unfair tactics of private traders with middlemen taking the center stage. It is, therefore, necessary for farmers and tappers to be organized in order to create competitive marketing of their coconut-based products. There are many achievements that can be made by having organized marketing structures. Firstly, farmers stand to benefit by collective action in getting their agricultural inputs, selling their

produce, getting and disseminating information, or sharing expertise. Secondly, farmers' organizations likewise may serve as an organizational framework for the efficient delivery of credit and extension services. This is expected to increase productivity and income generation.

Other constraints such as lack of credit and loans, poor infrastructure and/or transportation systems, lack of regulatory bodies and provincial administration/ police harassment are all directly or indirectly related to lack of proper government policy on the coconut industry. This in turn has rendered the coconut dependant communities in a state of hopelessness, pathetic and abject poverty. Lack of proper government policy on the coconut industry in Kenya can be traced back as early as 1915 in our history. In 1915, the Coconut Preservation Act Chapter 332 of the laws of Kenya was enacted generally to protect coconut plantations from trespass and theft. In 1923, the Coconut Industry Act Chapter 331 of the laws of Kenya was developed. Under this Act, coconut plantation owners were given authority to market their coconut products without license. This, however, did not give provision for other stakeholders in the coconut industry the autonomy to do so. The dropping of coconut and cashew nut trees from the list of protected crops in 1997, as well as the restrictive laws such as the Traditional Liquor Act (Chapter 122) of the laws of Kenya hampered the use, development and exploitation of coconut-based

products. All these laws have had a negative impact on production and marketing of products from the coconut tree.

Lack of small and medium-scale enterprises (SMEs) as well as large industries to process coconut-based products in Kenya; contribute greatly to the constraints related to lack of technical know-how and lack of proper storage facilities faced by both farmers and tappers. In view of this, the government should put in place all the necessary mechanisms that will address this issue as a matter of urgency. It is envisaged that the newly established Kenya Coconut Developemnt Authority (KCDA) will move with speed in finding lasting solutions to the problems of production, processing, and marketing as well as research and development of the coconut sub-sector.

Constraints related to poor soil and low production or yields were also reported by some of the farmers. This study established that low yields were attributed to use of genetically inferior cultivars, aging palms and poor management practises particularly on crop nutrition and population density. Although mixed cropping of coconut has been proven economically viable, very few farmers actually practise this system. During the survey, it was observed that research activities based on coconut were minimal. Research should, therefore, be given first priority so as to identify high yielding coconut varieties for establishment of new orchards. The approach in research should, therefore, bring together farmers, breeders, genetic

resource scientists as well food technologists to define a wider range of utilization of coconut and other bi-products from the coconut tree.

3.5 Conclusion and recommendations

In conclusion, the coconut industry in Kenya requires to be revitalized through proper government policy on coconut sub-sector that shall make it possible to repeal some of the outdated Acts in the laws of Kenya, with the purpose of introducing new laws that will make processing and marketing of coconut-based products more viable and competitive. The establishment of KCDA by the government should the commended. The authority should be provided with adequate funding and support by both government and private sector if it is expected to transform the industry from semi-subsistence, low-input, low-productivity system into a full-fledged commercial cash crop. Solutions to problems facing the coconut industry shall be found once appropriate promotion; development and research institutions are established. Taking all this into consideration, Kenya as a country will benefit a lot once diversified processing industries and coconut research institutions are established.

CHAPTER FOUR

PRODUCTION, MARKETING AND ECONOMIC IMPORTANCE OF *MNAZI* AND OTHER COCONUT-BASED PRODUCTS

4.1 Introduction

In Kenya, coconut products are sold with little or no value addition. This makes the prices of such products too low for farmers to break even. Currently there are only three copra-milling companies are operational, situated in Mombasa, Malindi and Lamu respectively. The extracted oil from copra is used in soap industries and candle manufacturing. However, the oil can be further refined into cooking fat and oil. Conversely, it is worthy noting that, technologies developed in other parts of the world have seen countries like Malaysia taking a leading role in the processing of products like pared kernel, desiccated coconut, coconut cream, coconut shell, coconut flour, and activated carbon through the SMEs and large industries (Severio, 1996). In Thailand, where the coconut industry is well developed, the fruit of the coconut palm is the main source of many food products such as coconut milk/cream, desiccated coconut, coconut chip, coconut water, nata de coco, coconut oil, copra plasticizers, resins, non-soap detergents, food preparations and confectioneries, handicraft, vinegar and alcoholic beverages, culture media and others such as carpets and rugs. The tapped palm sap *(mnazi*) is used for sugar production and making of toddy beverages (Severio, 1996). Recent research carried by

Crabbe *et al*., (2001) has shown that bio-diesel can be generated from palm oils, with an aim of substituting N0.2 diesel fuel. This fuel is environmental friendly because there is substantial reduction of unburned hydrocarbons, Carbon Monoxide (CO) and particulate matter emission when it is used in conventional diesel engines (Sharp, 1996). Moreover, it contains no sulphur, so the sulphate fraction in the fuel is eliminated and since the oil originates from vegetable matter, the CO_2 produced is sequestered and the net CO_2 released into the atmosphere would be reduced greatly.

Based on the fact that there are many different categories of products that can be derived from the coconut tree, the crop can therefore be regarded as one of the high value cash crop with the potential for intensification. Despite this, coconut farmers are suffering due to low farm productivity, unstable and poorly developed markets for their products. Evidence from other parts of Africa, indicates that farm incomes and productivity can benefit from engaging in cash crops with well-developed channels for procuring inputs on credit and marketing the crop (Poulton *et al*., 1998; Dorward *et al*., 1998).

Economists have long advocated for specialization and commercialization as part of a broader strategy of comparative advantage. The underlying premise is that markets allow households to increase their incomes by producing that which provides the highest returns to the land and labour,

and the using cash to buy household consumption items, rather than being constrained to produce all various goods needed for consumption (Timmer, 1997). With proper policy and management of the sub-sector, coconut farming should be considered for the comparative advantage strategy, considering the fact that coconut does well in soils where other food crops perform poorly. It is important to note that cash-generating crops can help farmers overcome capital constraints on the purchase of lumpy assets and inputs, which can be used to expand food crops as well as cash crop production (Von Braun and Kennedy, 1994). Coconut as a cash generating crop can play this role if well promoted with proper marketing channels.

4.2 Methodology

Research design, Sample and sapling methods, data collection and processing were carried out as described in Section 3.2.

4.3 Results

4.3.1 Survey

The number of both famers and tappers interviewed at district level is as provided in Section 3.3.1.

4.3.2 Production and marketing

According to the survey a total of 41.9 million units of coconuts were produced annual. Kilifi district was leading with a production of 24.3 million units, followed by Kwale (9 million units) and Malindi (4.4 million units). The

least was Tana River with 0.68 million units followed by Mombasa and Lamu with 1 million and 2.4 million units respectively. The copra production was 14 million metric tons. Kilifi was leading with 12.2 million metric tons followed by Malindi (0.9 million metric tons) and Kwale (0.55 million metric tons), the least being Tana River with 31 thousand metric tons. The results for other products are as provided in Table 4.1.

Table 4.1: Annual production of coconut-based products per district ('000 units)

	Districts covered during the survey						
Products	Kilifi	Kwale	Malindi	Mombasa	Lamu	Tana River	Totals
Coconut	24,344.85	8,973.75	4,419.83	1,038.58	2,405.69	684.73	41,867.43
Copra[a]	12,240.12	551.89	915.99	133.52	140.34	31.09	14,012.95
Makuti	11,671.38	2,558.37	6,602.51	440.93	253.43	562.45	22,089.07
Kanja	7,585.33	974.39	207.88	85.09	12.55	na	8,865.24
Fagio	2,809.15	55.38	159.41	20.43	2.86	0.64	3,047.87
Madafu	1,009.55	325.68	730.92	144.14	115.56	93.01	2,418.86
Shell	14,634.77	2,263.99	444.01	470.65	0.75	23.49	17,837.66
Tree trunk	5.65	5.95	1.03	2.03	13.01	0.06	27.73
Totals	**74,300.80**	**15,709.40**	**13,481.58**	**2,335.37**	**2,944.19**	**1,395.47**	**110,166.81**

[a] Figures in metric tons

According to the survey a total of KES405 million (US$ 5.2 million) was earned The highest earnings were realised from the sale of copra, followed by coconut, *makuti*, *kanja*, *fagio* and *madafu* (Table 4.2).

Table 4.2: Annual earnings from coconut-based products per district

(Kshs '000)[1]

	Districts covered during the survey						
Products	Kilifi	Kwale	Malindi	Mombasa	Lamu	Tana River	Totals
Coconuts	65,731.10	20,190.94	14,452.84	4,060.85	5,581.20	2,862.17	112,879.10
Copra	109,449.07	6,418.48	9,196.54	1,295.14	1,206.92	274.14	127,840.29
Makuti	43,650.96	17,703.92	32,022.17	2,654.40	1,272.22	3,384.45	100,688.12
Kanja	22,452.58	3,030.35	463.57	262.08	50.20	na	26,258.78
Fagio	16,208.80	493.44	1,753.51	174.88	18.39	0.32	18,649.34
Madafu	6,582.27	1,465.56	3,391.47	699.08	486.51	651.07	13,275.96
Shells	947.20	161.62	18.34	156.88	0.25	na	1,284.29
Trunks	203.68	209.02	2,994.77	62.46	607.18	na	4,077.11
TOTALS	**265,225.66**	**49,673.33**	**64,293.21**	**9,365.77**	**9,222.87**	**7,172.15**	**404,952.99**

na, data not available because production was not for sale but for home use

[1]values obtained from annual production multiplied by mean prices (US$1= KES70)

According to survey findings, a total of 44,663 trees were tapped, out of which 15,839 were owned by the tappers while the remaining 28,824 were owned by farmers. Kilifi district had the highest number of trees tapped (26,858), followed by Malindi (7,878), Mombasa (6,080), Kwale (3,350), Tana River (470) and Lamu 27 trees. On average 35.5% of the coconut tapped were owned by the tappers while the remaining 64.5% were owned by the farmers (Table 4.3).

Table 4.3: Number of coconut trees tapped and percentage ownership per district

District	Trees tapped	Trees tapped (owned by tappers)	Trees tapped (owned by farmers)	Trees tapped (%) (owned by tappers)	Trees tapped (%) (owned by farmers)
Kilifi	26,858	11,652	15,206	43.4	56.6
Kwale	3,350	378	2,972	11.3	88.7
Lamu	27	na	27	na	100
Malindi	7,878	1,070	6,808	13.6	86.4
Mombasa	6,080	2,637	3,443	43.4	56.6
Tana River	470	102	368	21.7	78.3
Totals	**44,663**	**15,839**	**28,824**	**35.5[a]**	**64.5[a]**

[a] Overall percentage in all the six districts

During low season, the mean prices of *mnazi* per litre ranged from KES15.2 to KES25.0, while the prices at high season ranged from KES8.0 to KES20.0. Kilifi district recorded highest quanties of *mnazi* both during low and high saesons (26,666.3 and 52,281.7 litres/day, respectively). During low season, a total of 9.2 million litres, while 13 million litres were realized during high season (Table 4.4).

Table 4.4: Prices and quantities of *mnazi* tapped during low and high seasons of the year per district

	Low season[1]				High season[1]			
District	Quantity / Day (litres)	Quantity/ Season (litres)[a]	Mode price/litre (Kshs)	Mean price/litre (Kshs)	Quantity / Day (litres)	Quantity/ Season (litres)[a]	Mode price/litre (Kshs)	Mean price/litre (Kshs)
Kilifi	26,666.30	5,706,588	15	15.2	52,281.70	7,894,537	8	10
Kwale	3,573.00	764,622	10	16.8	7,300.50	1,102,376	9.4	10
Lamu	42	8,988	25	25	135	20,385	20	20
Malindi	7,915.50	1,693,917	15	17	14,808.00	2,236,008	9.2	10
Mombasa	4,008.80	857,883	20	23.2	9,509.00	1,435,859	15.8	15
Tana River	950	203,300	20	19	1,842.00	278,142	13.3	15
Total	**43,155.60**	**9,235,298**			**85,876.20**	**12,967,307**		

[1]Low season: March to October((214 days), High season: November to February (151 days)
[a]Figures obtained by multiplying quantity per day by the number of days in the respective season

Percentage survey coverage in Kwale, Kilifi, Malindi and Lamu districts were 29.68, 52.53, 45.29 and 85.75%, respectively. However the coverage in Mombasa and Tana River districts were more than 3 times the acrearage provided by the ministry of Agriculture (304.38 and 491.41%, respectively). Since the acreage covered in Mombasa and Tana River districts were higher than that documented by the ministry of agriculture (Table 4.5).

Table 4.5: Number of coconut trees based on acreage covered during the survey per district

District	Survey coverage (%)	Acrerage[1]	Acreage[2]	No. coconut trees	No. coconut trees (estimated)[b]
Kilifi	52.53	44,288.60	23,263.56	1,220,172	2,322,810
Kwale	29.68	44,768.51	13,285.92	401,158	1,351,611
Malindi	45.29	10,531.62	4,770.23	264,262	583,489
Mombasa	304.38[a]	1,786.82	5,430.50	236,399	236,399
Lamu	85.72	6,102.75	5,231.49	94,090	109,764
Tana River	491.41[a]	506.57	2,489.50	15,503	15,503
Totals		**107,984.87**	**42,514.20**	**2,231,584**	**4,619,576**

[1] Source: Ministry of Agriculture, Kenya 1999

[2] Source: Survey conducted in this study

[a] Figures higher than those provided by the Ministry of Agriculture

Ministry value converted into acres using : 1 Hectares = 2.47105 acres

For economic analysis purposes, the percentage coverage in these two districts was therefore estimated based on survey data only while that provided by the ministry of agriculture was ignored. From Table 4.5, the estimated number of coconut trees was therefore found to be 4.62 million. According to Table 4.5, Kilifi is leading with 2.32 million trees, followed by Kwale with 1.35 million trees. Others were Malindi (0.58 million trees), Mombasa (0.24 million trees), Lamu (0.11 million trees) and Tana River (15 thousand trees).

4.3.3 Economic analysis of coconut-based products

Using the values of the annual earnings per district provided in Tables 4.2 and 4.6, the income from coconut-based products per farmer was calculated by taking the annual earnings divided by the percentage acreage covered during the survey all divided by the estimated total number of farmers in the district. These results are provided in Table 4.6. Table 4.6 therefore shows the annual income from coconut-based products per household. Malindi recorded the highest annual income/household of followed by Kilifi, Tana-River, Kwale, Mombasa and Lamu. A total of KES832 million was realized from the sale of coconut-based products. Kilifi led followed by Kwale, Malindi, Lamu, Mombasa.

Table 4.6: Annual income from coconut-based products per farmer
(For conversion US$1=KES78 as at April, 2003)

District	Survey coverage (%)	No. farmers[a]	Products earnings (KES)[b]	Total annual earnings (KES) per district[c]	Annual Income/household (Kshs)[d]
Kwale	29.68	8,652	49,673,330	167,362,972	19,344
Kilifi	52.53	7,885	265,225,660	504,903,217	64,033
Malindi	45.29	1,713	64,293,210	141,958,953	82,872
Mombasa	304.38[1]	1,414	9,365,770	9,365,770	12,421
Lamu	85.72	923	9,222,870	10,759,298	11,657
Tana River	491.41[1]	233	7,172,150	7,172,150	57,840
Totals		**20,820**	**404,952,990**	**832,156,590**	

[a]Figures obtained by dividing the number of farmers interviewed divide by survey coverage (%)

[b]Survey values obtained from Table4.2

[c]Figures calculated by taking products earnings divided by the survey coverage (%) given in Table4.6

[d]Values calculated by dividing the total annual earnings with the number of farmers/district

[1]Since the percentage coverage were too high they were ignored during calculation of estimates

4.3.4 Economic analysis of *mnazi*

The annual income from *mnazi* per tapper per district is provided in Table 4.7 below. The annual production was calculated by adding the total quantities of *mnazi* produced during low and high season. The overall mean price per litre of *mnazi* was calculated by finding the mean prices during low season and high season. Finally the Annual incomes were calculated by taking the annual production multiplied by the mean prices. Incomes per tapper were calculated by taking the annual earnings divided by the number of tappers per district. The results show that a total of 22

million litres were produced annual (Table 4.7), while KES710 million was earned annual through the tapping of *mnazi*. Kilifi recorded the highest production (13 million litres). Second in production was Malindi (4 million litres) followed by Mombasa (3 million litres) and Kwale (1.9million litres). In terms of earnings, Kilifi led with KES 393 million while Malindi was second followed by Kwale, Mombasa, Tana River and Lamu (KES147.5; 105.7; 53.2; 9.1 and 856.6 million respectively) as shown in Table 4.7.

Table 4.7: Annual incomes from *mnazi* per tapper per district

District	Coverage (%)	Annual Production (litres)[a]	Mean Price (KES)	Estimated Annual Earnings (KES)[b]	Tappers Interviewed (estimated)[c]	Annual Income/ tapper[d]
Kwale	29.68	1,866,998	16.8	105,679,132	755	139,972
Kilifi	52.53	13,601,125	15.2	393,560,061	3,396	115,889
Malindi	45.29	3,929,925	17.0	147,513,193	691	213,478
Mombasa	304.38[1]	2,293,742	23.2	53,214,814	435	122,333
Lamu	85.72	29,373	25.0	856,655	2	428,328
Tana-River	491.41[1]	481,442	19.0	9,147,398	56	163,346
Total		**22,202,605**		**709,971,254**	**5,765**	

[a]Values are sum of quantities produced during low and high season.

[b]Values calculated by multiplying annual production with mean prices divided by the fraction of survey coverage

[c]Values calculated by dividing tappers interviewed (survey) by survey coverage (%)

[d]Values calculated by dividing from annual earnings divided by number of tappers interviewed (estimated)

[1]Since the percentage coverage were too high they were ignored during calculation of estimates

4.4 Discussion

Coconut tree, despite being an economically important cash crop at the Coastal region of Kenya (tree of life), there has been very little effort to maximize the production and use of coconut-based-products, for both domestic and industrial applications due to poor government policy and lack of incentives. From the results (Tables 4.1 and 4.2), coconut, copra, *makuti*, *kanja*, *fagio* and *madafu* are the main coconut-based products produced in large quantities at the farm gate. These products are generating more income than other coconut-based products. In Malindi the top income earners were *makuti* and *madafu* as opposed to the other districts in which coconut, copra and *makuti* are taking leading role in quantities produced and incomes generated. This is attributed to the fact that Malindi is one of the leading tourist towns in the region hence the high demand of thatching material (*makuti*) for the tourist hotels, villas and resorts as well as the tender nuts (*madafu*) by tourists. *Madafu* are consumed because of their natural sweet water. At the industrial level, there are only three copra-milling companies that are operational, situated in Mombasa, Malindi and Lamu respectively while the two coir fibre factories which used to make fibre from husks (one in Kwale and the other in Kilifi) have closed down. This showed that the coconut industry was being operated below the expected capacity in terms of development of the crop in the areas of production, processing, marketing, research and

development. Lack of small and medium scale enterprices (SMEs) and large-scale industries to process coconut-based products, has contributed greatly to the under development of the coconut industry in Kenya. For Kenya to take her position in terms of production, processing, marketing and research of coconut-based products, the government should come up with an appropriate strategy for the development of coconut sub-sector through the newly established Kenya Coconut Development Authority. Since coconut has a vital role to play in rural development of the Coastal region of Kenya, any development and exploitation of other economic uses of coconut-based products will not only benefit the Coastal communities but also the Kenyan government through saving of the foreign exchange that the country spends annually on the importation of vegetable oils and fats. The country currently imports 95% of its edible oil requirements (250,000 metric tones) at an estimated cost of KES11.1 billion (US$14.23 million). About 30% of the total import (75,000 metric tones) is used annually in the manufacture of soap. Since the extracted oil from copra can be used in soap industries, candle manufacturing and in some cases the oil can be further refined for making cooking fat and oil (Sanchez, 1992), with proper policy therefore, the country can cut down its oil imports through substitution of some of its imported oil with coconut oil. Apart from coconut palm, Kenya is suitable for the growth of wild palm (*Phoenix reclinata*) and Malaysian palm (*Elaeis guineensis*). Initial research trials have confirmed

that Malaysian palm can grow in the coast and western regions of Kenya. Although the crop is doing very well in western Kenya, trials at the coast show that it is not doing all that well at the coast as expected due to inadequate rainfall. However, with irrigation using Ramisi, Tana and the Athi rivers, Kwale and Tana River districts could serve as the major producers of Malaysian Palm in Kenya.

Most of the trees tapped are owned by the farmers (64.5%) while the tappers themselves owned the remaining 35.5%. In Lamu district, only two tappers were interviewed, however the number is suspected to be even much higher. It was reported by the research assistants that, most tappers declined to be interviewed because tapping and drinking of any alcoholic beverage were considered as being against the Islamic teachings and principles. Most of the people living in Lamu district are Muslims (over 90%), so these results were justifiable. The fact that people in Lamu drink sweet coconut toddy (*tembo tamu*), which is a non-alcoholic drink from fresh mnazi before active fermentation commences, confirms the fact that tapping of coconut toddy takes place in Lamu. Out of 2,231,584 trees counted (Table 4.5) only 44,663 trees were being tapped (Table 3); this gives a mere 2% of the number of trees tapped in the region. According to Kadere et al., (2004) some of the constraints faced by tappers and farmers were based on lack of proper incentives. Once a permanent solution is found to these constraints tappers could easily find themselves doubling

the production of *mnazi* from the current projected figure of 22.2 million litres to 44.4 million litres.

Generally, it is believed that coconut trees used for *mnazi* production cannot be used for nut production, but research strongly shows that it is practical, feasible and economically viable to produce both *mnazi* and nuts in the same spathes/spadices of coconut through a sequential *mnazi* tapping and nut production scheme (SMWTNP). This technique involves the taping for sap (*mnazi*) during the first half of the spathe, followed by allowing the remaining half to develop normally producing mature nuts (Maravilla, 1975 and Naka, 1996). Tapping and consumption of *mnazi* when well controlled under proper government policy, may lead to the production of cheap and high quality traditional alcoholic beverage that may serve as an alternative drink to the clear beers such as lager beers. The fact that many Kenyans are dying due to consumption of “illicit brews” with others being inflicted with permanent injuries such as loss of sight, show the need for safe and cheap alcoholic drink that will cater for the low income earners, who go for the illicit brews because they cannot afford the lager beers.

The surveyed area in Mombasa and Tana River districts is far much bigger than that estimated by the Ministry of Agriculture. The higher Figures could have been attributed to the fact that the survey covered parts that had been omitted by the Ministry of Agriculture officials. Some of the constraints

faced by extension officers on the ground that impacted negatively on service delivery and data collection were given as: under staffing, lack of adequate financial allocation and means of transport. The fact that local research assistants were engaged in this study made it possible to cover areas that could not be reached by the Agricultural Extension Officers. In Mombasa district for example the survey covered fifteen (15) different sub-locations, while in Tana River district, a total of four (4) sub locations were covered.. In addition most officers regard Tana River district as a security risk district. This makes most of them being confined to their offices with little activity on the ground. The officers therefore were unable to give accurate statistical estimates on coconut-based products. For our study however, local research assistants were engaged hence we were able to cover areas that could not be covered by the Agricultural Extension Officers (AEOs) on the ground.

During the survey it was established that most beach hotels and holiday resorts at the Coastal region of Kenya utilized various coconut-based products such as *madafu*, coconut, shell and leaves for various uses. *Madafu* drinks for example are used to welcome tourists on arrival. They are also used to make drinks such as Pina Colada commonly known as *nazi* milk juice during international cocktails. The coconut shells are used as decoration in hotels. They are also used to make serving spoons (*upawa*) and other products such as bangles, beads and combs. The

coconut branches are weaved for decorations inside the hotels. The coconut branches (*makumbi*) are used for fencing and partitioning. Finally the trees apart from providing beauty (scenario) they also serve as a windbreaker.

The results show that *mnazi* earnings are higher than those from other coconut-based products except in Kilifi district where other coconut-based products fetched more money than *mnazi*. These earnings could even triple with value addition.

From this study, it is clear that coconut farming in Kenya requires to be transformed from semi-subsistence, low-input, low-productivity system into a full-fledged commercial cash crop. To achieve this objective, both the government and the private sector should come with a strategy of supporting the sub-sector.

4.5 Conclusion and recommendations

In Kenya, the need to transform coconut farming from semi-subsistence, low-input, low-productivity system into a full-fledged commercial cash crop requires proper policy on production, processing, marketing, trading and warehousing of coconut-based products including *mnazi*. Among the coconut based-products, *mnazi* has more annual income than other coconut-based products. Value addition is therefore required for better utilization and marketing hence increased income.

CHAPTER FIVE

PRESERVATION, PRODUCT DEVELOPMENT AND CHEMICAL ANALYSIS OF *MNAZI* AND ITS DISTILLATE (*PYUWA*)

5.1 Introduction

Tapping and consumption of *mnazi* as a mildly alcoholic beverage at the Coastal region of Kenya has been going on for the last four centuries. Its popularity is evident during traditional celebrations and ceremonies such as marriage, burials and settling of disputes. A similar practise is found in Nigeria. In the Ibo speaking area for example, *mnazi* is a part of major preliminaries that must be prayed over and discussed before the ceremonies start (Okafor, 1977 and Odeyemi, 1977). In Sri-Lanka, it is a popular drink among the lower income people. Village folk gather in the evening to socialize and drink mnazi (Okafror, 1977). *Mnazi* is believed to be good for health and eyesight and also serves as a sedative; it is also a mild laxative in relieving constipation (Okafor, 1977). It is prescribed as a tonic for those recovering from diseases such as chicken pox. Previous studies have shown that *mnazi* is an important source of nicotinic acid and vitamin C and to a lesser extent, proteins, thiamine and riboflavin (Cunningham and Wehmeyer, 1988). Apart from direct consumption as an alcoholic beverage, *mnazi* could also be used for leavening of dough (Somiari and Udoh, 1993) or distilled into local gin, popularly known as

chang`aa (Kadere, *et al*., 2004). The tapping process of wine used in this study was as explained by Kadere *et al*., (2004) while its distillate was traditionally distilled as explained by Kadere, *et al*., (2004). This paper is part of this thesis (see Chapter Two).

Generally, spoilt wine affects its taste, odour and visual sensation. This is mainly caused by cork related problems, growth of spoilage microorganisms (yeast or bacteria), sulphur off-odours, exposure to sunlight and temperature extremes (Thomas, 1993). A wide variety of contaminating yeast species have been implicated in wine spoilage as they may dominate when competition by bacteria is hampered by high sugar or alcohol concentrations, low pH values or the presence of some preservatives (Thomas,1993).

In this study, new products were developed that addressed the quality issue, based on existing information on wine preservation. In previous studies, scientists were able to preserve commercially bottled *mnazi* in sodium benzoate (C_6H_5.COONa) at a concentration of 0.15% (m/v). Sodium metabisulphite ($Na_2S_2O_5$) and propionic acid were also found suitable as preservatives but they are less effective (Shamala *et al., 1988*). In Thailand, in almost every place, farmers use special barks such as kiam (*Cotyleobium lanceolatum*) and pra-yom (*Shorea floribunda*) for preservation. In some places, a mixture of preservatives is used instead of kiam and pra-yom. This mixture is composed of sodium metabisulphite,

sodium propionate ($CH_3.CH_2.COONa$), sodium benzoate in ratio of 10:1:1, respectively (Naka, 1996). Pasteurization at 85°C for 30min. reduces the viable counts in mnazi to a greater extent than heat treatment at 70°C for 35min. and 65°C for 40min. (Idise and Izuagbe, 1985). European regulations do permit the use of sorbic and ascorbic acids to increase antimicrobial and antioxidant potential in wine. Other products such as diethyl pyrocarbonate, pimaricin and nisin have been tested but abandoned due to their toxicity or undesirable side effects (Broughton *et al*., 1996; Marcillaud and Doneche, 1997).

According to the laws of Kenya (Cap. 122), *mnazi* and its distillate are still being categorized as an “illicit brews”. This not withstanding, the two alcoholic beverages have been consumed for the last four centuries. It is envisaged that the findings of this study, especially the chemical wholesomeness of *mnazi* and its distillate will provide the appropriate recommendations on categorization of *mnazi* and its distillate.

5.2 Methodology

5.2.1 Samples for analysis

Samples of *mnazi* and its distillate that were used for chemical analysis were obtained from Chonyi and Kikambala areas of the Coastal region of Kenya. The samples were collected in sterile sampling tubes. The pH of the sample was determined at the sampling site using a portable pH meter.

The samples were kept at 4°C and transported in cool boxes packed with dry ice to the Food Science and Technology Laboratory at the Jomo Kenyatta University of Agriculture and Technology (JKUAT). To ensure consistency, three tappers and three distillers were selected as sources of the required samples. Their selection was based on their consistency in the way they conducted their tapping and distillation processes. Another factor that was used in the screening exercise was variation in execution of the technology of tapping and distillation. Since earlier survey had indicated little or no variation in the two technologies (Kadere, *et al*., 2004), samples collected from three tappers and three distillers were found to be adequate to provide conclusive results.

5.2.2 Chemical wholesomeness of raw and preserved *mnazi* and its distillate

5.2.2.1 Chemical quality of fresh and preserved *mnazi* and its distillate

The pH was measured using a digital pH-meter after calibration with standard buffers at pH 4.0 and 9.18 respectively. Total Titratable acidity (TTA) was determined by adding 10g of the sample into 100ml of alcohol 70% (v/v) previously neutralized, followed by 0.5ml of phenolphthalein solution. The mixture was shaken for 1 h followed by filtration. Then 50ml of the filtrate was titrated with 0.1N sodium hydroxide. Fixed acidity was determined by evaporating 50ml of the sample using water bath followed by titration as for TTA. Volatile acidity was determined by calculating the difference between TTA and fixed acidity.

5.2.2.2 Determination of volatile compounds in the fresh and preserved mnazi and its distillate

The alcohol content of the samples was determined by distillation method. Specific gravity of the distillate was determined using a pycnometer. The distillate/water ratio was determined and the index corresponding to alcohol percentage (v/v) was determined using the AOAC alcohol tables.

The relative concentrations of volatile compounds: acetaldehyde, ethyl acetate, methanol, 1-propanol, isobutanol and amyl alcohols were determined by gas chromatography (Shimadzu GC-9A) using a glass packed column (15% DEGS, 3m length x 3mm internal diameter). The detector used was FID. Nitrogen was used as carrier gas at a flow rate of 50ml/min. The column initial temperature was held at 50°C for 2min. while the final column temperature was held at 150°C for 5min. The programme rate was 5°C/min. During ignition, the air supply was set at 0.2Kg/cm and maintained at 0.5Kg/cm during operation. Samples of 1µl of wine were directly injected into the column and the concentrations of the above mentioned volatile compounds were determined using standard samples. All samples were analysed in duplicates. 0.1µl each of the standards were injected and the elution time determined. The column temperature was programmed at 3°C/min. The temperatures of the injector and FID detector were each at 220°C. The standards used were: methanol, butan-1-ol, 1-propanol, 2-methyl-1-propanol (isobutyl alcohol), 2-methyl-1-butanol and 3-methyl-1-butanol (Isoamyl alcohol), diethyl ether, ethoxy ether,

acetaldehyde and 4-propanol. The absence of methanol in the tested samples was confirmed using the Deniges test Method. Using Schiff's reagent, the presence of methanol was determined through colour change the colour of the resulting mixed solution. Presence of violet colour within a few minutes was considered positive result, while delayed colour change up to about 30min. indicated presence of methanol but only in traces.

The following formula was used to determine the concentration of volatiles in each sample using the gas chromatographic method:

$$C = \left[\left(\frac{A_p C_a}{A_t \times 100} \right) \rho \times 10^{6} \right]$$

Where: C - concentration [ppm]; A_p - peak area of the compound; C_a - alcohol content; A_t - total area of all peaks; ρ - the density of the solvent.

The presence of sugars such as sucrose, glucose, and fructose was analyzed using HPLC chromatographic method. Standard solutions dissolved in acetonitrile were each injected (10µl each) and the elution time of each standard was noted. The prepared samples were mixed with acetonitrile in the ratio of 1:1, stored under refrigeration before injection. The column used for analysis was Shodex NH2 P-50 4E, the mobile phase used for elution was acetonitrile: water (75:25). The oven temperature was maintained at 35°C with a flow rate of 0.8ml/min.; the pressures were maintained at 98-100 Kilogramme-forces (Kgf) during analysis.

The presence or absence of anions in fresh *mnazi* was confirmed using the Agilent Basic Anion Buffer System (ABABS). The ABABS uses the principle of that allows migration of both the anion and Electroosmotic flow (EOF) in the same direction. First, a highly alkaline pH condition is used to confer and promote migration of a negative charge of inorganic and organic anions as well as amino acids and carbohydrates towards the anode and past the detector. To reverse the EOF so that migration can be towards the anode, a quaternary ammonium salt was used. The the sample was diluted with deionized water in the ratio of 1:50 and an indirect UV detection was employed to visualize anions which had little or no chromophore. A capillary made of fused silica with id = 50µm, l=104cm and L =112.5cm (G1600-64211) was used. The capillary temperature was 15°C, while the applied voltage was at -30 kV, injection conditions were: 1). Pressure: 50 mbar for 6 seconds from sample vial; 2). Post-injection of buffer from InHome vial, 50 mbar for 4 seconds. The detection wavelength was: Signal 350/20nm, reference 230/10nm. Preconditioning was done using buffer flush for 4min at 1bar prior each run.

5.2.3 Preservation of *mnazi*

5.2.3.1 Method 1: Preservation by pasteurization and chemical preservatives

5.2.3.1.1 Preservation technique for developed products

Freshly tapped *mnazi* toddy was blended with that which had been allowed to ferment for a period of 8-12h so as to enhance quality of the final product. The blended *mnazi* was then pasteurized at different time-temperature combinations (65, 75 and 80°C for 40, 35 and 30min., respectively). After pasteurization, the coded samples were cooled in stainless steel vessels that were immersed in a basin of cold water to a temperature between 40 and 60°C. Finally, the samples were poured in clean sterilized plastic bottles with similar codes which contatined sodium benzoate (0.25, 0.5, 0.75% (m/v) depending on the sample) or or a mixture of sodium benzoate and sodium metabisulphite, as shown in the Table 5.1. The filled bottles were then closed and sealed, followed by mixing through thorough shaking of the contents in sealed bottles for 5-10min. so as to dissolve the preservatives. For the control, some samples of the blended *mnazi* were neither pasteurized nor preserved by chemicals. Finally the samples were transported to the laboratory at JKUAT for the determination of shelf life.

Table 5.1: Preservation conditions of various developed products

Sample codes	Sodium benzoate (% m/v)	Temperature (°C)	Pasteurization time (Min)
A1	0.25	85	30
A2	0.25	75	35
A3	0.25	65	40
B1	0.5	85	30
B2	0.5	75	35
C1	0.75	85	30
C3	0.75	65	40
C-65	Non	65	40
C-75	Non	75	35
D1*	10:1	75	35
D2**	1:1	75	35

*Sodium benzoate and sodium metabisulphite in the ratio of 10:1 (0.25% (m/v):0.025% (m/v))

**Sodium benzoate and sodium metabisulphite in the ratio of 1:1 (0.25% m/v)

5.2.3.1.2 Determination of shelf life of the preserved products

For shelf life, eight bottles were picked from each preserved and coded samples. Four bottles of each coded sample were stored at 04°C while the remaining four bottles were stored at room temperature for a period of 2 months. During storage, acidity, pH and sugar levels were monitored for a period of 2 months.

Similarly, 8 bottles of the control sample (non preserved freshly tapped *mnazi*) were preserved as explained above, four under refrigeration and the other four at a room temperature. The parameters (acidity, pH and sugar levels) were monitored for period of one week.

5.2.3.2 Method 2: Preservation by chemical preservatives

5.2.3.2.1 Preservation technique for developed products

In addition to the combined preservation technique (preservation by both chemical and pasteurization), a method to preserve *mnazi* by chemical preservatives alone was developed based on the fact that fruit wines may be preserved without undergoing pasteurization. Initial trials of the preserved products were done, in which four (4) samples of each product were stored at both refrigeration and room temperatures. After observing that the products were very stable within the two weeks of trials, three brands were developed namely: “Dry”, “Medium Dry” and “Sweet” brands based on the level of fermentation. To improve on the packaging material, translucent glass bottles were used. Labels for the products were designed, that incorporated culture and the Coastal Kenya background. A different design was used for each brand as shown in Appendix 2.

Procedures for the development of three preserved toddy brands:- Sweet, Medium Dry and Dry

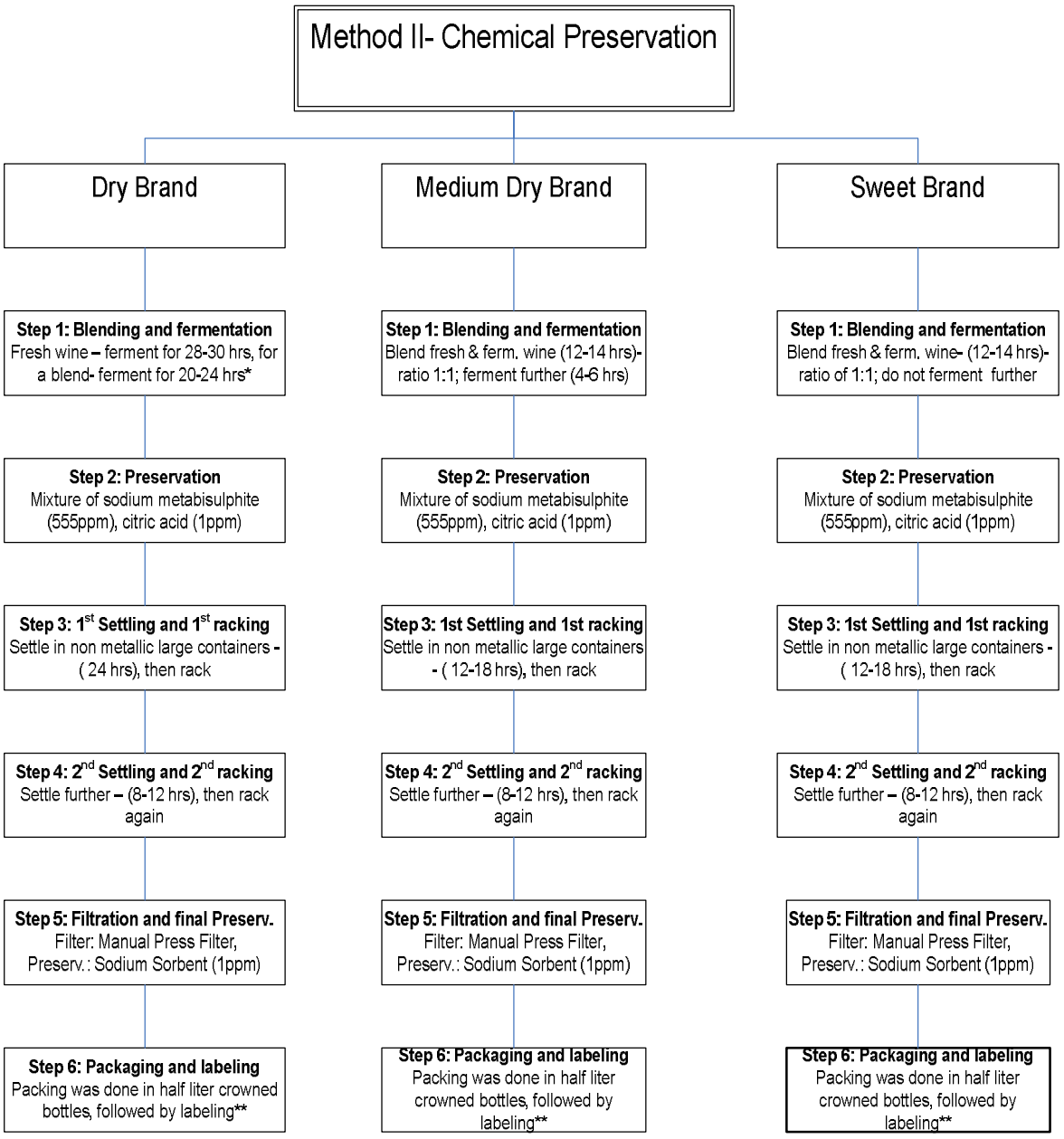


Figure 5.1: Systematic steps for the development of three brands: sweet, medium dry and dry *mnazi* products (using chemical preservatives)

5.2.3.2.2 Determination of shelf life of the preserved products

The shelf-life of the three brands was determined as given in section 5.2.2.1.2 above. Just like in section 5.2.2.1.2, parameters such as acidity, pH and sugar levels were monitored during storage for a period of 2 months.

5.3 Results

5.3.1Changes of alcohol content, pH and acidity of freshly tapped *mnazi* during storage

The changes in alcohol content, pH and acidity in freshly tapped *mnazi* during storage at room and refrigeration temperatures are shown in Tables 5.2 and 5.3, respectively.

Table 5.2: Changes of alcohol content, acidity and pH of freshly tapped *mnazi* during storage at room temperature (25 °C)

Storage Time (Days)	Tapper 1			Tapper 2		
	Alcohol Cont. (v/v)	Acidity % (v/v)	pH	Alcohol Cont. (v/v)	Acidity % (v/v)	pH
1	5.80	0.24	4.00	5.90	0.25	3.90
2	6.80	0.31	3.80	6.90	0.39	3.85
3	7.00	0.39	3.75	7.32	0.41	3.77
4	7.10	0.42	3.61	7.50	0.49	3.69
5	6.80	0.48	3.50	7.30	0.52	3.54
6	6.75	0.59	3.36	7.0	0.61	3.39

Table 5.3: Changes of alcohol content, acidity and pH of freshly tapped *mnazi* during storage at 04°C

Storage Time (Days)	Tapper 1			Tapper 2		
	Alcohol Cont. (v/v)	Acidity % (v/v)	pH	Alcohol Cont. (v/v)	Acidity % (v/v)	pH
1	5.80	0.24	4.00	5.90	0.25	3.90
2	5.90	0.28	3.85	6.00	0.30	3.86
3	6.13	0.28	3.86	6.32	0.31	3.86
4	6.30	0.28	3.80	6.40	0.31	3.86
5	6.40	0.29	3.80	6.52	0.32	3.79
6	6.60	0.33	3.70	6.70	0.35	3.72

From the results, freshly tapped *mnazi* had an alcohol content of about 6% (v/v). The alcohol content and acidity increased gradually with time during storage while pH decreased (Tables 5.2 and 5.3). The alcohol content in freshly tapped *mnazi* stored at room temperature reached a maximum (7.1 and 7.5 % (v/v) for tappers 1 and 2 respectively) on day 4, before gradual decline. However, acidity kept on increasing without a maximum, making pH to decrease gradually too. Freshly tapped *mnazi* collected from tapper 1 and stored at 25°C, had its alcohol content increase from 5.8 to 7.1% (v/v), while that from tapper 2 increased from 5.1 to 7.5% (v/v) as shown in Table 5.2

Freshly tapped *mnazi* stored at 04°C had its alcohol content increase gradually from 5.8 to 6.6% (v/v) and 5.9 to 6.7% (v/v) for tappers 1 and 2, respectively (Table 5.3).

5.3.2 Changes of alcohol content, acidity and sugar levels of preserved *mnazi* during storage

Changes in alcohol content, acidity and sugar levels in the newly developed wine products during storage are provided in Tables 5.4 and 5.5. From the results; there is a correlation between increase in alcohol content and decrease of common sugars found in *mnazi*. Samples prepared using the first method (Method1) showed stability only during the first 4 weeks of storage irrespective of the storage conditions (Table 5.4). On further storage, significant changes were observed between 6^{th} and 8^{th} week of storage. However, products such as A2, A3 and C65 showed significant changes, including gas formation within 4^{th} week of storage (Table 5.4). The results show that sucrose and fructose were assimilated faster than glucose, with assimilation of fructose being the fastest (Table 5.4 and 5.5). All samples produced gas at the end of 8^{th} week except D2 and indicator that fermentation was taking place during storage. The gradual increase in alcohol content throughout the storage period confirms this assumption.

Table 5.4: Alcohol content, pH and sugar levels during room temperature storage of the preserved *mnazi* alcoholic products in plastic bottles

Sample codes	pH Value				Alcohol (%)				Glucose (mg/g)			Fructose (mg/g)			Sucrose (mg/g)			Storage Condn.	
	0w	4w	6w	8w	0w	4w	6w	8w	4w	6w	8w	4w	6w	8w	4w	6w	8w	4w	8w
A1	4.24	4.23	4.10	3.80	3.98	3.99	4.52	6.07	41.74	10.60	7.48	3.52	0.00	0.00	43.14	8.88	0.01	NG	G++
A2	3.71	3.70	3.69	3.67	3.77	3.77	4.24	5.01	61.23	30.23	27.05	17.23	1.34	6.08	266.00	36.00	0.00	G+	G++
A3	4.31	4.30	3.90	3.73	3.27	3.27	3.55	4.64	37.05	9.96	7.54	4.35	2.00	1.32	106.34	15.65	0.01	G+	G++
B1	4.37	4.37	4.00	3.70	4.64	4.64	4.73	4.97	37.96	34.6	33.60	19.06	1.09	0.00	0.22	0.00	0.00	NG	NG
B2	4.61	4.60	4.11	4.03	3.77	3.77	4.12	5.46	67.92	37.95	29.09	14.16	8.23	7.54	372.55	1.00	0.67	NG	G++
C1	4.50	4.47	4.00	3.97	3.34	3.34	3.86	5.46	53.86	30.76	20.98	3.81	0.00	0.00	0.06	0.00	0.00	NG	G++
C3	4.27	4.27	3.99	3.77	3.77	3.77	4.64	6.07	55.81	8.90	4.24	0.29	0.00	0.00	0.00	0.00	0.00	NG	G++
C-65	3.89	3.87	3.64	3.17	8.65	9.15	9.92	10.93	26.76	1.00	0.00	2.77	0.00	0.00	0.00	0.00	0.00	G++	G++
C-75	4.04	4.03	3.99	3.90	5.20	5.23	5.99	6.38	48.59	38.96	33.49	0.00	0.00	0.00	0.01	0.00	0.00	NG	G+
D1	4.20	4.20	4.08	3.90	3.74	3.77	4.32	6.30	5.64	0.59	0.00	0.38	0.00	0.00	0.00	0.00	0.00	NG	G++
D2	4.48	4.47	4.11	3.80	3.56	3.56	3.88	4.42	56.31	15.09	7.53	18.67	4.08	6.03	105.45	5.89	2.05	NG	NG

0w - Tests conducted 2 days after bottling 4w, 6w, 8w - Tests conducted after 4, 6 and 8 weeks respectively

NG - No gas production, G+ - Little gas produced, G++ - high quantity of gas produced

Table 5.5: Alcohol content, pH and sugar levels during storage at room and refrigeration temperatures of the preserved *mnazi* alcoholic products in glass bottles

Samp codes	pH Value				Alcohol (%)				Glucose (mg/g)			Fructose (mg/g)			Sucrose (mg/g)			Storage Condn.	
	0w	4w	6w	8w	0w	4w	6w	8w	4w	6w	8w	4w	6w	8w	4w	6w	8w	4w	8w
SW04	4.10	4.09	4.09	4.00	5.01	5.22	5.25	5.33	65.00	64.66	55.70	6.00	5.99	5.77	264.06	264.00	263.90	NG	G+
SW25	4.10	4.00	4.00	3.98	5.01	5.30	5.36	5.43	65.00	63.99	60.09	6.00	5.45	5.00	264.06	263.88	263.00	NG	G+
MD04	3.90	3.89	3.83	3.80	5.84	5.89	5.94	5.99	40.38	39.00	37.00	3.56	3.00	3.00	110.93	110.23	110.02	NG	NG
MD25	3.90	3.86	3.80	3.77	5.84	5.95	5.99	6.01	40.38	37.88	332.99	3.56	2.56	2.50	110.93	109.98	109.88	NG	NG
D04	3.38	3.37	3.36	3.30	7.91	7.99	8.00	8.03	25.00	24.67	23.89	0.00	0.00	0.00	45.12	45.00	44.09	NG	NG
D25	3.38	3.36	3.30	3.22	7.91	8.02	8.12	8.33	25.00	23.99	20.24	0.00	0.00	0.00	45.12	44.79	40.99	NG	NG

0w – Tests conducted 2 days after bottling; 4w, 6w, 8w – Tests conducted after 4, 6 and 8 weeks respectively; NG - No gas production; G+ - Little gas produced

SW04, SW25-Sweet brands stored at 04 ºC and 25 ºC respectively MD04, MD25- Medium Dry brands stored at 04 ºC and 25 ºC respectively

D04, D25 – Dry brands stored at 04 and 25 ºC respectively

5.3.3 Volatile compounds in *mnazi* and its distillate (pyuwa)

Tables 5.6 and 5.7 show how acidity and other volatile components in *mnazi* and its distillate vary depending on the source. From the results fresh *mnazi* did not register any fusel oil. Whereas *mnazi* that had stayed for at least four days showed some traces of fusel oils.

Table 5.6: Composition of volatile substance and relative concentrations of fusel oils in *mnazi* distillate (Pyuwa) from three different distillers

Parameter	Dist. 1	Dist. 2	Dist. 3	Mean	STDEV	SKEW
Volatile acidity % (v/v)	0.03	0.03	0.03	0.03	0.001	1.732
Acetic acid % (v/v)	0.05	0.05	0.04	0.05	0.006	-1.732
Esters (mg/100 ml ethanol)[1]	0.06	0.07	0.07	0.07	0.004	-1.293
Aldehydes (mg/100 ml ethanol)[2]	0.02	0.05	0.05	0.03	0.154	-1.090
Ethanol % (v/v)	44.62	37.81	36.33	39.59	4.421	1.513
Methanol[3]	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.
Acetaldehyde[3]	16.85	34.41	n.d	25.63	12.42	n.a
Propanol[3]	n.d	83.15	58.34	70.75	17.54	n.a
Isoamyl ethanol[3]	881.30	682.09	346.12	636.50	270.49	0.74
Butanol[3]	n.d	n.d	n.d	n.a	n.a	n.a
Iso-butanol[3]	222.37	130.04	101.00	151.14	63.38	1.33
*RT 17.64[3]	139.79ρ	175.31ρ	218.74ρ	n.a	n.a	n.a

[1]at temperature 70-80 °C, results expressed as acetyl acetate

[3] ppm on the basis of absolute alcohol

*unknown- unable to determine based on standards used, ρ= desity of compound to be identified

'n.a' =not applicable, 'n.d'= Too low to be detected by this method

Table 5.7: Relative composition of volatile substance and fusel oils in freshly tapped *mnazi* based on samples collected from three different tappers

Parameter	20- 24 hrs after tapping			4 days after tapping					
	Tap. 1	Tap. 2	Tap. 3	Tap. 1	Tap. 2	Tap. 3	Mean	STDEV	SKEW
Total acidity % (v/v)	0.59	0.58	0.60	0.69	0.63	0.70	0.63	0.047	0.624
Fixed acidity % (v/v)	0.33	0.34	0.34	0.43	0.44	0.48	0.39	0.059	0.315
Volatile acidity % (v/v)	0.26	0.24	0.26	0.32	0.34	0.36	0.30	0.045	0.125
Acetic acid % (v/v)	0.63	0.57	0.62	0.66	0.67	0.69	0.64	0.039	-0.746
Ethanol % (v/v)	6.18	6.30	6.25	6.88	6.90	6.70	6.54	0.030	0.104
Methanol[3]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	n.a	n.a
Acetaldehyde[3]	n.d.	n.d.	n.d.	0.01	0.01	n.d.	0.00	0.006	-1.732
Propanol[3]	n.d.	n.d.	n.d.	0.03	0.01	0.02	0.01	0.010	0.855
Isoamyl ethanol[3]	n.d.	n.d.	n.d.	0.23	0.25	0.12	0.1	0.076	0.000
Butanol[3]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a	n.a	n.a
RT8.05[3]	n.d.	n.d.	n.d.	0.04	0.06	0.04	0.02	0.016	0.000
RT 17.64[3]	n.d.	n.d.	n.d.	0.05	0.03	0.06	0.02	0.007	Infin.

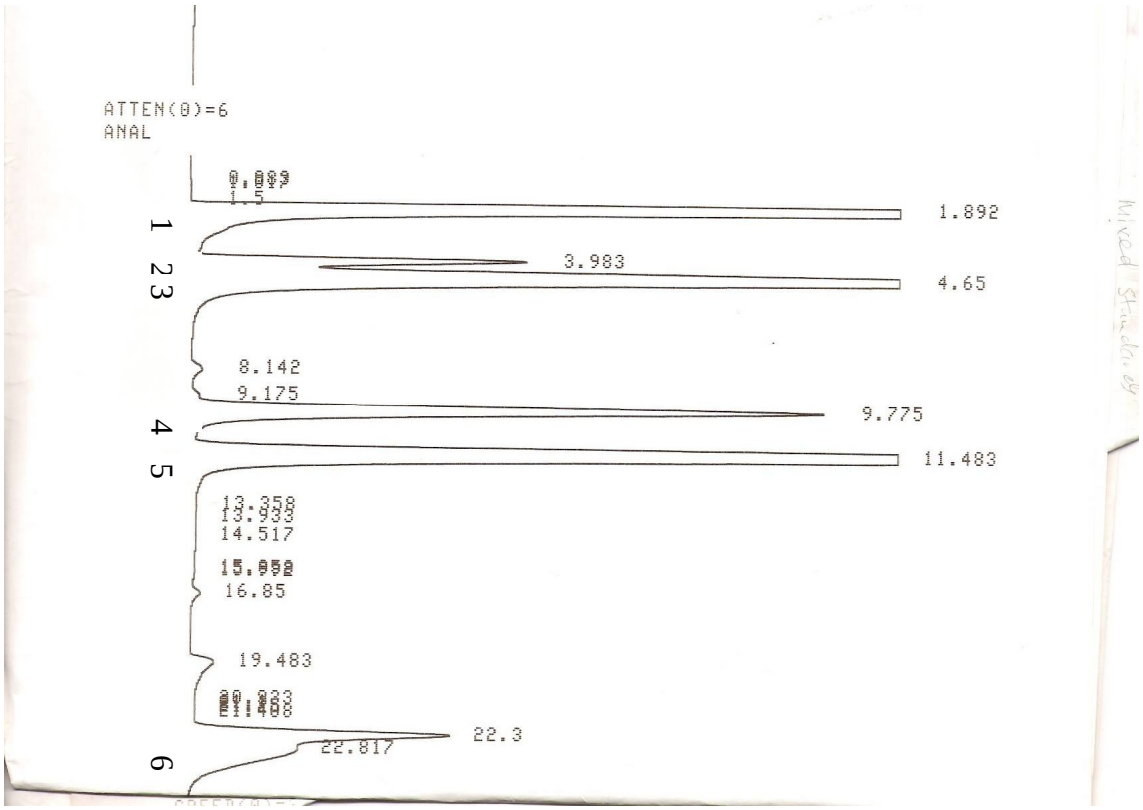
[1]at temperature 70-80°C, results expressed as acetyl acetate

[2] experiment done at and the results are expressed as acetaldehyde

[3] ppm on the basis of absolute alcohol

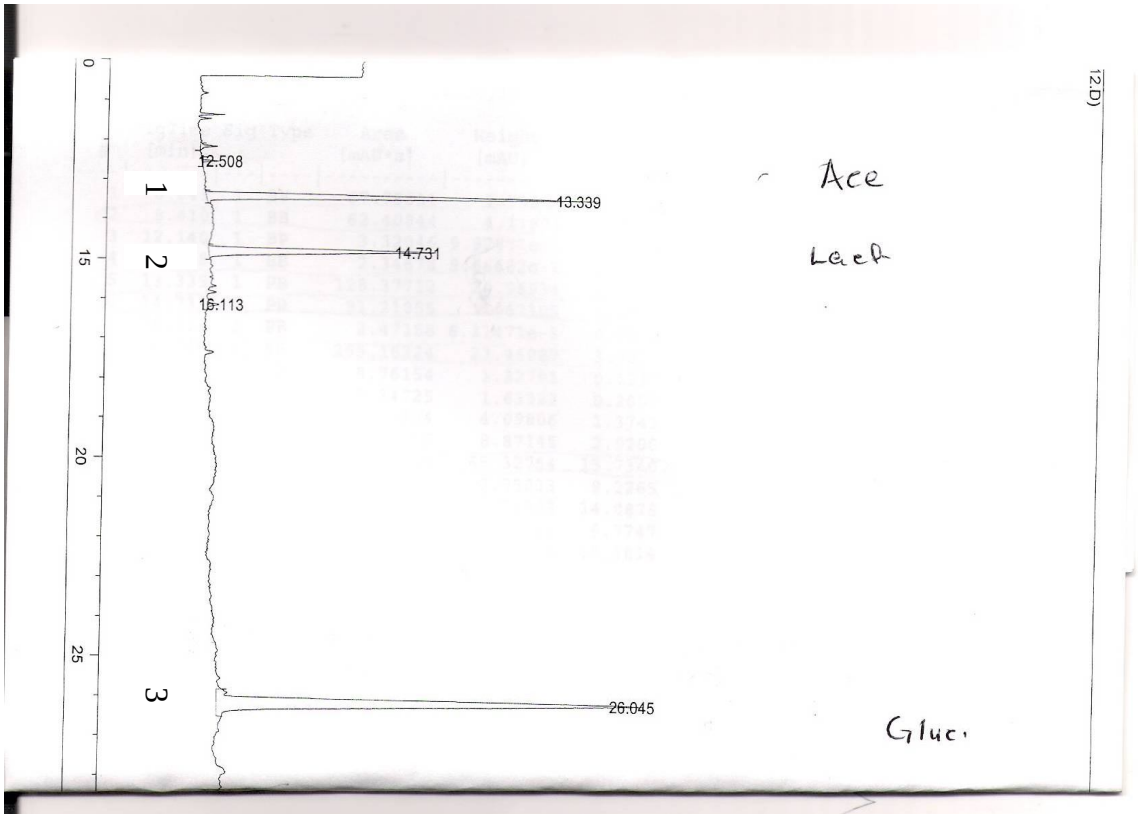
'n.a' =not applicable, 'n.d'= Too low to be detected by this method; infin- #Div/0

There were significant levels in the distillate (Figure 5.2). A quick profile by the Basic Anion Buffer System (ABABS) method confirmed the presence of glucose, lactose, fructose and sucrose in *mnazi*. Other anions whose presence was confirmed included: chlorides, acetate and lactate (Figure 5.3).



1:	Acetyaldehyde	2:	Not identified (lack of stds)
3:	Ethanol	4:	Butanol
5:	isoamyl alcohol	6:	Not identified (lack of stds)

Fugure 5.2: Gas chromatography eluting profile of the volatile compounds in *mnazi* distillate



1: Acetone 2: Lactose 3: Glucose

Figure 5.3: Agilent Basic Anion Buffer System Chromatopac of anionic compounds present in *mnazi*

5.4. Discussion

Mnazi has for a long time been consumed as a traditional alcoholic drink at the Coastal region of Kenya, without proper hygienic packaging and preservation. As a result of this practise tappers and traders (*wachuuzi*) alike have incurred a lot of losses in terms of wastage and poor prices due to short shelf life of *mnazi*. Under special circumstances, some local distillers tried to distill it into spirit as explained in Chapter 2 of this study.

However, since *mnazi* is still considered as an “illicit brew”, the challenges and constraints explained in Chapter 3 of this study act as impediment to promotion and utilization of *mnazi* and its spirit.

This study revealed that *mnazi* had a very short shelf life (1-2 days) if proper preservation techniques are not employed. This could be attributed by the vast different types of micro-flora found naturally in *mnazi* as established in this thesis (Chapters 6, 7 and 8 of the thesis). In addition, its chemical composition makes it a suitable substrate for the natural micro flora. These microorganisms are capable of fermenting sugars and other bi-products of fermentation with the release of volatile and flavour compounds such as organic acids, esters, ethers and fusel oils. Romano *et al*., (1997a), reported that both *Saccharomyces* and non-*Saccharomyces* yeasts contribute significantly to the flavour and quality of wine. According to Pretorius, (2000), volatile profile of wines is dominated by those components that are formed and retained most during fermentation, since these compounds are present in the highest concentrations

In this study, *mnazi* fermentation by both methods was allowed to take place at room temperature except when it was stored at refrigeration temperature. Previous studies, however, have shown that wines produced at low temperatures (10-15°C) have a tendency to develop certain characteristics of taste and aroma (Feuillat et al., 1997), in addition to improved quality due to fewer higher alcohols and a greater proportion of

acetate and ethyl esters among the total volatile compounds (Argiriou *et al*., 1996). In addition, low temperatures reduce the growth of acetic and lactic acid bacteria and this can make it easier to control alcoholic fermentation. However, the optimal growth temperature for *Saccharomyces cerevisiae* is 25°C, while 13°C is restrictive and increases the risks of stuck or sluggish fermentations (Meurgues, 1996). Low temperatures increase the duration of alcoholic fermentation, decrease the rate of yeast growth and modify the ecology of wine fermentation. The main volatile compounds that were found in both *mnazi* and its spirit include propanol, isoamyl ethanol butanol and acetic acid. In *mnazi*, the levels of the volatiles were far much less than 600 mg/l, which is considered the threshold value of acceptability in wine (Romano, 1990). The fact that no traces of methanol were detected in *mnazi* qualifies it further as safe drink. As shown in Table 5.7, *mnazi* meets all the Kenya Bureau of Standards specifications for fortified wines (KS1122:2007), still table wine (KS 05-609:1990) and sparking wines (KS 05-1121:1994). These findings can therefore be used as a justification to policy change that aims at exclusion of *mnazi* from the category of “illicit brews” hence making it a legal product. Once this is achieved, promotion of this product could be given priority with emphasis on hygienic packaging and preservation. As for the *mnazi* distillate (*pyuwa*), all the volatile compounds analyzed were within the 600ppm threshold except isoamyl alcohol, which was above 600ppm in two out of

the three samples analyzed. Methanol was not detected in all the three samples of *mnazi* distillate. According to International Programme on Chemical Safety (IPCS), iso-butanol is considered slightly toxic and has been shown to cause liver damage in mice and human at a threshold of 100 ppm. Propanol causes drowsiness, gastrointestinal pains, and nausea, and may be even lethal if the threshold is above 400ppm. Iso-amyl alcohol has similar health effect including irritation on the eye, nose, throat, skin and mild nacrosis if the exposure is above 100ppm. Based on these findings consumption of *mnazi* distillate commonly referred to as *pyuwa* is not recommended on the basis of these alcohols.

In this study, two methods were employed with an aim of prolonging the shelf life of *mnazi* and at the same time ensuring hygienic packaging and handling. The products developed using method-2 above were more stable compared to those developed using method-1 (Tables 5.4 and 5.5). Preservation by method-1 used a combination sodium benzoate and pasteurization. Pasteurization at 65 °C was found to be less effective than that at 75 °C (Table 5.4). Similarly use of sodium benzoate and sodium metabisulphite in the ratio of 10:1 was found to be less effective than a ration of 1:1 (Table 5.4). The differences in in shelf-life between Method-1 and Method-2 could be attributed, partly by the packaging material (translucent glass bottles) and partly by the method employed. The fact that our products could not keep longer than 2 months (normally preserved

and well packaged wines and beers have a shelf life of more than three months) could be attributed to the type and conditions of filter unit employed. In this study, a leaky manual press filter was used because others were not available. In addition, the filter medium (membrane) was used repeatedly because new supplies were not available locally. However, the results obtained were convincing enough.

5.5. Conclusion and recommendations

From the finding, glass bottles were found to be better packaging materials for *mnazi* than plastic bottles. Preserved *mnazi* was able to keep for more than two months without spoilage. On the wholesomeness of both the *mnazi* and its distillate, only the *mnazi* was found to be successful, hence the author recommends it. This was based on the fact that it was free from methanol and the levels of other volatile compounds (fusel oils) were less than 100ppm. However, its distillate was found to be unsafe for human consumption despite the fact that methanol levels were insignificant. Based on these findings, only *mnazi* meets the required specifications for beers, wines and spirits according to the specifications of KEBS. This therefore calls for the need to de-gazette it from the category of “illicit brews”. *Mnazi* distillate (*pyuwa*) however registered higher content of isoamyl alcohol than the recommended limits. It is against this background that the author doe not recommend de-gazetting of *mnazi* distillate (pyuwa) from the category of “illicit brews” unless the distillation technology is improved.

CHAPTER SIX

ISOLATION AND IDENTIFICATION OF THE GENERA ACETOBACTER AND GLUCONOBACTER IN *MNAZI*

6.1 Introduction

Mnazi is a typical tropical alcoholic beverage, produced by fermentation of sugary coconut sap. The coconut sap is tapped from palm trees grown at the Coastal region of Kenya. The trees commonly used for this purpose are *Elaeis guineensis, Raphia vinifera, Cocos nucifera and Arenga pinnata. Mnazi* is a whitish, effervescent, acidic alcoholic beverage (Swings and De Ley, 1977). The tapping process of the toddy used in this study was as explained by Kadere *et al*., (2004). It is a product of a mixed alcoholic, lactic and acetic fermentation. As a first step, the sugar of the sap is fermented to ethanol within 8-12 h by yeasts and bacteria, thus creating a highly suitable medium for the development of acetic acid bacteria. During fermentation, the acetic acid bacteria appear after 2-3 days. Acetic acid bacteria utilizing the glucose and/or sucrose might be present in earlier stages of the *mnazi* fermentation (Okafar, 1975).

Since their first discovery and reporting as a unique group, the acetic acid bacteria have been labeled with numerous genetic names, which have been the subject of extensive discussion and revision. The eighth edition of

Berger's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) recognized only two genera, *Acetobacter* (motile by petrichous flagella or non-motile) and *Gluconobacter* (motile by polar flagella or non-motile), and placed the genus *Gluconobacter* with the family *Pseudomonadaceae*; however, the genus *Acetobacter* was not assigned to any particular family and was grouped within the genera of uncertain affiliation. The Approved List of Bacterial Names, (Skerman, *et al*., 1980) acknowledged both the genera *Acetobacter* and *Gluconobacter*. The ninth edition of Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons, 1984) recognized the fact that the genera *Gluconobacter* and *Acetobacter* were closely related; hence they were placed within the family *Acetobacteraceae*. Members of the family are united by their unique ability to oxidize ethanol to acetic acid. Under this family we have genera *Acetobacter*, *Gluconobacter* and *Frateuria* (Holt *et al*., 1994). Of these, *Gluconobacter oxydans, Acetobacter aceti, Acetobacter pasteurianus, Acetobacter liquefaciens* and *Acetobacter hansenii* are normally associated with grapes and wines (Blackwood *et al*., 1969, Joyeux *et al*., 1984b).

According to Ruiz *et al*., (2000), acetic acid bacteria are divided into the genera *Acetobacter, Acidomonas, Gluconobacter* and *Gluconacteobacter*. Acetobacter species prefer ethanol as carbon source (De Ley *et al*., 1984) and usually dominate during the later stages of wine fermentation (Drysdale and Fleet, 1985; Joyeux *et al*., 1984a; Du Toit and Lambrechts,

2002). *Acetobacter* species were earlier isolated from *mnazi* (Faparusi, 1973; Faparusi and Bassir, 1972; Okafar, 1975) and from immature spadix of palm tree (Faparusi, 1973*). A. pasteurianus* was isolated from *mnazi* (Simmonart and Laudelout, 1951), and *A. aceti subsp. Xylinium* from the leaflets of the palm tree and the surrounding air (Faparusi, 1973). *Gluconobacter oxydans subsp. Suboxydans* was found on the floret of palm tree (Faparusi, 1973); in the tap holes and in palm sap (Faparusi, 1974).

Acetobacter cells and *Gluconobacter* alike are Gram-negative or Gram variable, ellipsoidal to rod-shaped, straight or slightly curved, 0.6 - 0.8µm X 1.0 - 0.4µm, occurring singly, in pairs or chain. Pleomorphic form occurs which may be spherical, elongated, swollen, club shaped, curved or filamentous. *Acetobacter* cells are non-motile or motile, if motile, petrichous or lateral flagella are present. However, motile strains of *Gluconobacter* have three and eight polar flagella; a single flagellum is rarely observed. In liquid media, *Acetobacter* forms a ring, film or pellicle, uniform turbidity of the medium and a cell deposit is sometimes observed (De Ley, Swings and Gosselé, 1984). Some strains produce a pink, non-diffusible pigment whereas others may produce a soluble, dark brown pigment and ɣ-pyrone. The pathway for ɣ-pyrone formation has been elucidated (Asai, 1968) and it is believed that the product of brown pigments is related to ɣ-pyrone synthesis (Rainbow, 1981).

The strains of acetic acid bacteria are useful for vinegar production; however, lack of defined pure starter cultures is due to problems in strain isolation, cultivation and preservation of vinegar bacteria (Kittelmann *et al*., 1989; Sievers *et al*., 1992; Sokollek and Hames, 1997). Acetic acid bacteria are able to produce high amounts of acetic acid from alcohol. Further more, these bacteria can produce other compounds, apart from acetic acid, that can influence wine quality (Drysdale and Fleet, 1989a). Earlier research has also shown that acetic acid bacteria (genera *Acetobacter* and *Gluconobacter*) were able to produce some polysaccharides such as cellulose, levan and dextran (Hibbert *et al*., 1931; Loitsyanskaya, 1965 and Hehre *et al*., 1951). Valla and Kjosbakken (1981) showed that cellulose-negative strain of *Acetobacter xylinum* obtained by spontaneous mutation produced an extra-cellular polysaccharide composed of glucose, rhamnose, mannose and glucuronic acid in a molar ratio of 3:1:1:1 (Hibbert *et al*., 1931; Loitsyanskaya, 1965 and Hehre *et al*., 1951).

The objective of this study was to investigate the occurrence and identification of the dominant spoilage genera of acetic acid bacteria in *mnazi* tapped by traditional methods at the Coastal region of Kenya.

6.2. Methods

6.2.1. Samples

The *mnazi* samples for the isolation of the Acetic acid bacteria (AA) were obtained from Mtwapa and Kikambala areas of the Coastal region of Kenya.

Details of sample collection, transportation and storage are as described in section 5.2.1 of this thesis write-up.

6.2.2. Isolation and identification of acetic acid bacteria (AAB)

The strains were isolated by plating one milliliter of each dilution series previously pre-enriched in a basal medium (5% glucose, 1% yeast extract, 100ppm cycloheximide) onto GYP agar {glucose (2% m/v), Na-acetate.$3H_2O$ (0.5% m/v), Tryptone (0.5% m/v), Yeast extract (0.5% m/v), Potassium phosphate (0.1% m/v), Tween 80 solution (0.5% v/v) and agar (1.7% m/v)} and Mannitol medium {mannitol (2.5% m/v), yeast extract (1% m/v), and agar (1.5% m/v)}. The pH for GYP was adjusted to pH 6.8 whereas mannitol medium was adjusted to pH 7.0. Growth of lactic acid bacteria was inhibited by addition of 50mg/l primaricin and 50mg/l nisin to GYP agar and Mannitol medium respectively. The dilutions were plated in triplicate and incubated at 25, 30 and 37°C for a period of 3-5 days. Representative colonies of the isolates (98 strains) were Gram stained using the conventional method. Isolates that were Gram negative/positive (variable), oxidase and catalase positive were stored on GYP agar slants at

4°C and transferred monthly until identification. The ninety eight (98) acetic acid bacterial isolates were reduced further to 17 isolates based on physiological and morphological similarities. The 17 isolates were identified up to genera using the following biochemical and physiological tests: Oxidase test (Kovacs, 1956; Steel, 1961) was carried out using test strips (Difco). Oxidase positive colonies developed pink colour, which became successively dark red, purple and black in 5-10 seconds. A delayed positive was indicated by purple colouration within 10-60 seconds, any later reaction was regarded as negative. Growth in gelatin and gelatin liquefaction was done using the gelatin infusion broth (Gelatin- 40g, Beef heart, solids from infusion-500g, Tryptose-10g, NaCl- 5g, distilled water-1000ml, pH 7.4). Any liquefaction of the medium was considered positive result for gelatin liquefaction. Peptone broth (Peptone-10g/l) was used to determine growth in peptone. Motility observation was conducted using the motility test by soft agar medium (Glucose-0.5g, yeast extract-0.5g, peptone-0.5g, meat extract-0.5g, Tween 80-0.05g, agar-0.15g, distilled water-100ml and pH 6.8). Incubation of previously stabbed medium was done at 30°C for 2-3 days. Cultures that showed positive growth only at the stabbed areas were regarded as non-motile while those that showed growth all over the medium were regarded as motile. Oxidation of ethanol and acetic acid at pH 7.0 and pH 4.5 was determined as explained by Frateur (1950). Over oxidation of ethanol into acetic acid and finally into

CO_2 and H_2O was done at pH 4.5 and 7.0 respectively by the method explained by Carr (1968). The medium used for these tests contained 3% Difco yeast extract, 2% ethanol, 0.0022% bromocresol blue (green) and 2% agar. Oxidation of lactate was done using the method explained by Frateur (1950) using yeast water agar {yeast extract (3% m/v), calcium lactate (2% m/v), agar (2% m/v)}, while 2% sodium acetate was used instead of calcium lactate for the oxidation of acetate**.** Growth at temperatures 15, 25, 30, 37, 40 and 45°C and that at pH 2.5, 3.0, 4.0, 7.0, 8.0 and 8.5 was also conducted using GYP broth (Frateur, 1950). This was then followed by production of a brown pigment on GYP medium (Drysdale and Fleet, 1988). Biochemical tests, included fermentation of carbohydrates using 22 different sugars. The basal medium for these tests was GYP broth incorporated with 0.5 ml of 5% tested sugar as the sole source of carbon. For Esculine 2.5% was used instead. Tests preparations were incubated at 30°C and readings were done after 1-10 days.

6.3. Results

6.3.1. Identification of the genera *Acetobacter* isolated from *mnazi*

All the strains that were Gram negative or Gram variable, oxidase negative, catalase positive ellipsoidal to rod-shaped, straight or slightly curved were preliminarily identified as acetic acid bacteria according to the biochemical and physiological tests described in materials and methods (Table 6.1). All the isolated *Acetobacter* strains were Gram variable, oxidase negative and

catalase positive. They all showed positive growth at pH 7.0 and pH 4.5. Growth at 25 and 30 and 40°C was positive for all the *Acetobacter* strains, while growth at 45°C, pH 8.5 and pH 2.5 was negative for all. Most of them were unable to grow at 15°C, pH 3.0 and pH 8.0. Motility on molten agar was positive, growth on peptone medium and formation of brown pigment on GYP medium were all negative. Most strains showed positive growth on gelatin, but gelatin liquefaction was negative. These strains registered positive growth on lactate; in addition they were able to oxidize lactate to CO_2 and H_2O with deposit of $CaCO_3$ around the inoculated zones. All strains suspected to fall under the genera *Acetobacter* were able to oxidize acetate to CO_2 and H_2O except TYC4031, TP3051 and AYC4031. All *Acetobacter* strains over-oxidized ethanol to acetic acid and finally to CO_2 and H_2O in neutral (pH 7.0) and acidic conditions (pH 4.5). The acetic acid produced by *Acetobacter* strains changed the indicator from blue to yellow and upon further incubation, the acetic acid was further oxidized to CO_2 and H_2O; the indicator then reverted to the blue color.

Most *Acetobacter* strains were able to ferment the following sugars: arabinose, xylose, ribose, glucose, galactose, mannose, melibiose and trehalose (Table 6.2). All the strains were unable to ferment the following sugars: Amylagdine, cellibiose, esculine, fructose, lactose, maltose, mannitol, melezitose, Na-gluconate, raffinose, Rhamnose, salicine, sorbitol, sucrose.

Table 6.1: Physiological and Biochemical characteristics of *Acetobacter* Strains

Bacterial Strains	**TYC 4031**	**TP 3051**	**AYC 4031**	**GY 541**	**GY 542**	**GY 553**	**GY 554**	**GY 555**	**GY 557**	**GY 558**
G. Stain	v	v	v	v	v	v	v	v	v	v
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-
growth in gelatin	+	+	+	+	+	-	+	+	+	+
Gelatin Liquefaction	-	-	-	-	+	-	-	-	-	-
Growth pH 2.5	-	-	-	-	-	-	-	-	-	-
Growth pH 3.0	-	-	+	-	-	-	-	+	-	-
Growth pH 4.5	+	+	+	+	+	+	+	+	+	+
Growth pH 7.0	+	+	+	+	+	+	+	+	+	+
Growth pH 8.0	+	+	+	-	-	-	-	-	-	-
Growth pH 8.5	-	-	-	-	-	-	-	-	-	-
Growth at 15 °C	-	+	+	-	-	-	+	-	-	-
Growth at 25°C	+	+	+	+	+	+	+	+	+	+
Growth at 30°C	+	+	+	+	+	+	+	+	+	+
Growth at 40°C	+	+	+	+	+	+	+	+	+	+
Growth at 45°C	-	-	-	-	-	-	-	-	-	-
Growth in peptone	-	-	-	-	w	-	-	-	-	-
[1]Growth in Lactate	+	+	+	+	+	+	+	+	+	+
[1]Growth in Acetate	-	-	-	+	+	w	+	+	+	+
Oxid. Ethanol at pH 7.0	+	+	+	+	+	+	+	+	+	+
Oxid. Ethanol at pH 4.5	+	+	+	+	+	+	+	+	+	+
Over oxid. Ethanol at pH 7.0	+	+	+	+	+	+	+	+	+	+
Over oxid. Ethanol at pH 4.5	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	w	+
Brown pigment on GYP	-	-	-	-	-	-	-	-	-	-

+= positive result, -= negative result, v=variable, w= weak

[1]Strains did not only show positive growth but were also able to oxidize the compounds to CO_2

Table 6.2: Fermentation of common sugars by *Acetobacter* species isolated from *mnazi*

GENERA	*ACETOBACTER*									
Bacterial Strains	TYC 4031	TP 3051	AYC 4031	GY 541	GY 542	GY 553	GY 554	GY 556	GY 557	GY 558
Sugars										
Amylagdine	-	-	-	-	-	-	-	-	-	-
Arabinose	-	+	±	±	±	±	±	±	±	±
Cellibiose	-	-	-	-	-	-	-	-	-	-
Esculine	-	-	-	-	-	-	-	-	-	-
Fructose	-	±	-	-	-	-	-	-	-	-
Galactose	-	+	±	+	±	±	+	+	-	-
Glucose	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-	-	-	-
Mannose	-	±	-	±	±	±	±	±	±	±
Melezitose	-	-	-	-	-	-	-	-	-	-
Melibiose	±	+	-	+	+	±	±	±	+	+
Na-gluconate	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-
Ribose	±	+	-	±	±	±	-	-	±	±
Salicine	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-
Trehalose	-	-	-	±	+	±	±	±	±	±
Xylose	+	+	+	+	+	+	±	±	±	±

+, positive result; -, negative result; ±, variable result

6.3.2. Identification of the genera *Gluconobacter* isolated from *mnazi*

As indicated in Table 6.3, all the strains that were Gram negative (variable), catalase positive, oxidase negative, ellipsoidal to rod-shaped, straight or slightly curved were preliminarily identified as acetic acid bacteria according to the biochemical and physiological tests described in materials and methods. All isolated strains of *Gluconobacter* were Gram-variable rods, oxidase negative and catalase positive. They were strictly aerobic and motile on molten agar medium (Table 6.3). They also showed positive growth at pH 4.5 and 7.0 while that at pH 2.5, 8.0 and 8.5 were negative. All strains showed negative growth at pH 3.0 except TYC4032. Growth at temperatures 15, 25 and 30°C was positive while that at 45°C was negative; at 40°C growth was either positive or negative depending on the strain. Most strains were able to grow in peptone medium. Although most strains showed positive growth on gelatin, liquefaction of gelatin on the other hand was negative. *Gluconobacter* strains registered positive growth on lactate but they were not able to oxidize it to CO_2 and H_2O with deposit of $CaCO_3$ around the inoculated zones. Similarly, most of them were unable to oxidize acetate. All *Gluconobacter* strains oxidized ethanol to acetic acid in neutral (pH 7.0) and acidic conditions (pH 4.5), but upon further incubation, the acetic acid was not over-oxidized to CO_2 and H_2O hence the color of the medium changed from blue to yellow without reverting back to blue. Some of the *Gluconobacter* strains were able to

form brown pigmentation on GYP medium, while others were unable to form brown pigmentation on GYP medium.

Table 6.3: Physiological and Biochemical characteristics of *Gluconobacter* Strains

Bacterial Strains	TP3 052	TYC 3031	TYC 4032	TYC 3034	TYC 3041	GY 201	GY 203
Gram Stain	v	v	v	v	v	v	v
Catalase	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-
Growth in gelatin	+	+	+	+	+	+	+
Gelatin liquefaction.	-	-	-	-	-	-	-
Growth at pH 2.5	-	-	-	-	-	-	-
Growth at pH 3.0	-	+	-	-	-	-	-
Growth at pH 4.5	+	+	+	+	+	+	+
Growth at pH 7.0	+	+	+	+	+	+	+
Growth at pH 8.0	-	-	-	-	-	-	-
Growth at pH 8.5	-	-	-	-	-	-	-
Growth at 15°C	+	+	+	+	+	+	+
Growth at 25°C	+	+	+	+	+	+	+
Growth at 30°C	+	+	+	+	+	+	+
Growth at 40°C	+	+	-	-	+	+	+
Growth at 45°C	-	-	-	-	-	-	-
Growth in peptone	+	w	+	+	+	-	-
[1]Growth in Lactate	+	+	+	+	+	+	+
Growth in Acetate	-	-	-	-	-	w	+
Oxid. Ethanol at pH 7.0	+	+	+	+	+	+	+
Oxid. Ethanol at pH 4.5	+	+	+	+	+	+	+
Over oxid. Ethanol at pH 7.0	-	-	-	-	-	-	-
Over oxid. Ethanol at pH 4.5	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+
Brown pigment on GYP	w	w	+	-	-	+	-

+= positive result, -= negative result, v=variable, w= weak

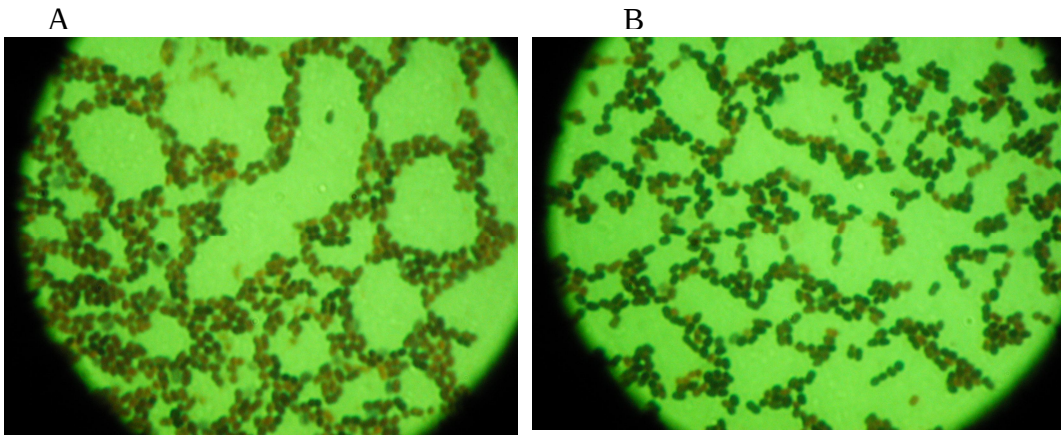
[1]Strains registered positive growth; however they were unable to oxidize lactate to CO_2 with deposit of $CaCO_3$ around the inoculation areas

Table 6.4: Fermentation of common sugars by *Gluconobacter* species isolated from *Mnazi*

GENERA	*GLUCONOBACTER*						
	TP	TYC	TYC	TYC	DCY	GY	GY
Bacteria Strains	3052	3031	4032	3034	3031	201	203
Sugars							
Amylagdine	-	-	-	-	-	-	-
Arabinose	+	+	+	+	-	±	-
Cellibiose	-	-	-	-	-	-	-
Esculine	-	-	-	-	-	-	-
Fructose	±	+	-	+	-	+	-
Galactose	+	+	+	+	+	+	-
Glucose	+	+	+	+	+	+	-
Lactose	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-
Mannitol	-	+	-	+	-	-	-
Mannose	±	-	-	±	-	-	-
Melezitose	-	-	-	-	-	-	-
Melibiose	+	+	±	+	±	±	-
Na-gluconate	-	-	-	-	-	±	-
Raffinose	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-
Ribose	+	-	±	+	±	±	-
Salicine	-	-	-	-	-	-	-
Sorbitol	-	+	-	±	-	-	-
Sucrose	-	+	-	-	-	-	-
Trehalose	-	-	-	-	-	-	-
Xylose	+	+	+	+	±	-	-

±, variable

Figure 6.1 shows the morphological appearance of Gram stained acetic acid bacteria strains as observed under a microscope.



X 400 Maginification
A & B : Isolates of Gram stained Acetic acid bacteria under the microscope

Figure 6.1: Isolates of Acetic Acid Bacteria as observed under microscope at X400 magnification

Fermentation of the most common sugars by *Gluconobacter* strains are shown in Table 6.4. Like Acetobacter strains, most *Gluconobacter* strains were able to ferment the following sugars: arabinose, xylose, ribose, glucose, galactose, mannose and melibiose. However, unlike *Acetobacter* they were unable to ferment mannose and trehalose. All the strains of *Gluconobacter* were unable to grow in the following sugars: Amylagdine, cellibiose, esculine, lactose, maltose, mannitol, melezitose, Na-gluconate, raffinose, Rhamnose and salicine; however, unlike *Acetobacter*, some of

the *Gluconobacter* strains were able to ferment fructose and sorbitol. All *Gluconobacter* strains were unable to ferment sucrose except TYC3031.

6.4. Discussion

Today, acetic acid bacteria have been classified into 24 different genera. The major genera involved in vinegar production include: *Acetobacter, Gluconobacter, Gluconacetobacter, Asaia, Neoasaia, Saccharibacter, Frateuria* and *Kozakia* (De Vero and Giudici, 2008). From the results (Tables 6.1and 6.3) all the isolated strains were found to be Catalase positive, Oxidase negative, Gram negative (variable), obligate aerobic and grew at pH 4.5. According to the eighth edition of Bergey's manual of determinative Bacteriology (Buchanan and Gibbons, 1974), these strains should be classified into the genera *Acetobacter* or *Gluconobacter*.

Classification of the isolated strains under the genus *Acetobacter* was based on the ninth edition of Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons, 1974), as well as the reports of Leifson, (1954) and Gosselé *et al*., (1983). The strains that were classified under the genus *Acetobacter* were Gram negative (variable), obligatory aerobic rods, motile, growth at pH 4.5, oxidizing lactate and acetate to CO_2 and H_2O, no or very poor growth on peptone, and able to grow on gelatin but unable to liquefy it. The optimum temperature for these strains as given by De Ley and Swings (1984a and 1984b) was reported to be in the range of 25°C to 35°C. According to Holt *et al*., (1994) both *Acetobacter* and *Gluconobacter*

alike were unable to grow at 37°C, however, in this study, *Acetobacter* strains showed positive growth not only at 37°C but also at 40°C. Positive growth was also registered at pH 4.5 and 7.0 with negative growth at pH 2.5, 8.5, while only a few were able to grow at pH 8.0 and 3.0. In the acid formation test, all the *Acetobacter* isolates were positive for glucose, xylose, while some were able to produce acids from ribose, trehalose, melibiose, mannose, galactose and glucose. This again is confirmed by the Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons, 1984). According to this study, strains of *Acetobacter* were unable to form acid from the following sugars: lactose, cellibiose, fructose, mannitol, sorbitol, esculine, maltose and melezitose (Tables 6.2). This again confirms the findings by Minakami *et al*., (1984) and those provided by the Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons, 1984). Finally, *Acetobacter* strains as provided in this study, were confirmed and differentiated from *Gluconobacter* strains by the method described by Carr, (1968). Based on this method, *Acetobacter* strains were able to over-oxidize ethanol to acetic acid and finally to CO_2 and H_2O through tricarboxylic acid cycle in neutral and acidic conditions (pH 7.0 and 4.5 respectively). Whereas due to non-functional tricarboxylic acid cycle in *Gluconobacter*, the genera is unable to oxidize most organic acids such as acetic, citric, lactic, malic, pyruvic and succinic (Holt *et al*., 1994). Upon incubation, all *Acetobacter* strains were able to change the medium from

blue to yellow and upon further incubation; it reverted back to blue indicating that the acetic acid was converted into CO_2 and H_2O. This not only confirms the presence of *Acetobacter* strains, but also differentiates them from the *Gluconobacter* strains.

The classification of the genus *Gluconobacter*, isolated in this study was also based on the ninth edition of Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons, 1984), as well as the reports of Leifson, (1954) and Gosselé *et al*., (1983). The physiological properties of *Gluconobacter* strains were similar to those of *Acetobacter*. However, most *Gluconobacter* were able to grow in peptone agar as opposed to *Acetobacter*, which were unable to grow on this medium. *Gluconobacter* strains showed positive growth at 15°C while most *Acetobacter* strains were unable to grow at this temperature. While all *Acetobacter* strains were unable to produce brown pigmentation on GYP, some of the *Gluconobacter* were able to produce brown pigment on GYP. Almost all *Gluconobacter* strains were unable to oxidize acetate while some of the *Acetobacter* strains did not oxidize acetate to CO_2 and H_2O. This, however, contradicts the suggestion that all *Gluconobacter* strains were unable to oxidize lactate and acetate while all *Acetobacter* strains were able to oxidize the same (Swings and De Ley, 1981). Most of the sugars that gave positive fermentation with *Acetobacter* strains did the same with *Gluconobacter*. However, trehalose and mannose showed positive fermentation with

Acetobacter but negative with *Gluconobacter* while, fructose and mannitol had negative fermentation with most *Acetobacter* but gave positive results with most *Gluconobacter*. As expected *Gluconobacter* strains oxidize ethanol to acetic acid but were unable to over-oxidize it to CO_2 and H_2O in neutral and acidic conditions (pH 7.0 and 4.5 respectively). This test served as one of the major differences between *Gluconobacter* and *Acetobacter* during classification of *Gluconobacter*. This again confirms the findings by Minakami *et al*., (1984); Swings and De Ley, (1981) and those provided by the Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons, 1984). The other difference between *Acetobacter* and *Gluconobacter* is inability of the former to oxidize lactate while the latter was unable to do so. This phenomenon also differentiates *Gluconobacter* from *Gluconacetobacter* (Navarro and Komagata, 1999).

Acetic acid bacteria, though for a long time have been believed to play little, if any, role during winemaking operations due to their aerobic nature (Drysdale and Fleet, 1988), recent findings have shown that acetic acid bacteria do contribute significantly to volatile acidity in must and wine, hence the spoilage associated with most wines (Joyeux *et al*., 1984b; Drysdale and Fleet, 1989b). Wine spoilage by acetic acid bacteria is through the production of acetaldehyde and acetic acid from ethanol. Acetic acid is one of many chemical components found in wine and is produced in low concentrations by yeasts during alcoholic and by lactic

acid bacteria during the malolactic fermentation. However, the concentrations of this acid can be significantly increased through the action of spoilage yeasts, spoilage species of lactic acid bacteria, and, in particular, by acetic acid bacteria. Acetic acid constitutes the major volatile acid in wine (especially *mnazi*) and is considered objectionable at levels above 1.2 to 1.4g/L (Margalith, 1981 and Ribéreau-Gayon, *et al*., 1975). The inability of acetic acid bacteria to grow under the anaerobic conditions that generally prevail in wine has been recognized by wine-makers for many years, and as such, the control of these bacteria has not been viewed as a major problem to the wine industry. However in recent years, the ability of the acetic acid bacteria to affect wine quality has been the subject of renewed interest and research. For example, there is speculation that these bacteria may survive and grow under the semi-anaerobic to anaerobic conditions that occur in stored wine to affected wine quality by mechanisms other than the production of acetic acid (Drysdale and Fleet, 1985; Joyeux *et al*., 1984a).

6.5. Conclusion and recommendations

Mnazi when freshly tapped is sweet, oyster white. This alcoholic drink when not preserved turns into vinegar (acetic acid) in 2-5 days of continuous fermentation. Vinegar therefore is one of the value added bi-products of *mnazi*. The fact that acetic acid bacteria of the genera *Acetobacter* and *Gluconobacter* were abundantly in *mnazi* tapped by

traditional methods provide enough explanation as to why *mnazi* spoils readily 2-5 days after tapping. As pointed early, acetic acid bacteria tend to appear after 2-3 days after the onset of coconut sap fermentation. This again confirms the fact that *mnazi* spoilage is mainly caused by the presence of acetic acid bacteria in the wine. The significant populations of acetic acid in *mnazi* may influence the composition of *mnazi*, the growth of yeasts during alcoholic fermentation and the growth of lactic acid bacteria during malolactic fermentation (Joyeux *et al*., 1984a, 1984b; Sponholz and Dittrich, 1985a, 1985b). This therefore, accelerates the spoilage process through significant increase of acid mainly acetic acid and other volatile components. Some of the volatile substances associated with acetic acid include: gluconic acid and ketogluconic acid (Holst *et al*., 1982, Izuo *et al*., 1980, Seiskari *et al*., 1985 and Tramper *et al*., 1983). The *Acetobacter* and *Gluconobacte*r strains isolated in this study are responsible for the spoilage of *mnazi*; however, they may be utilized as useful microorganisms in the production of vinegar. Further research should be conducted with an aim of incorporating the strains isolated in this study into the vinegar manufacturing industry. Success in this line of research will go a long way in improvement of the vinegar industry in Kenya. The fact that substances of industrial importance such as sorbose, dihydroxyacetone, gluconic acid and ketogluconic acid were obtained through oxidation of sugars and sugar alcohols by *Gluconobacter oxydans* qualifies the need for further research

on the isolated strains to establish their usefulness in industrial application (Holst *et al*., 1982, Izuo *et al*., 1980, Seiskari *et al*., 1985 and Tramper *et al.*, 1983). I therefore recommend the use of other procedures such as 16S and/or 23S rRNA gene sequencing, DNA-DNA similarity tests, DNA base composition as well as DNA relatedness and Quinone analysis. These procedures are expected to help in identification of the respective species in the two genera before serious research on industrial application of the identified species is embarked on.

CHAPTER SEVEN

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA IN *MNAZI*

7.1 Introduction

Lactic acid bacteria (LAB) comprise a group of bacteria that are united by a constellation of morphological, metabolic and physiological characteristics. The general description of the bacteria included in the group is Gram-positive, non-spore forming, non-respiring (lack catalase) cocci or rods, which produce lactic acid as a major end product during fermentation of carbohydrates. The core group of LAB includes *Lactobacillus, Lueconostoc, Pediococcus and Streptococcus*. Others include *Tetragenococcus* and *Vagococcus*. Members of the genus *lactobacillus* are characterized as Gram-positive, non-motile, non-spore forming; rod shaped catalase-negative, auxotrophic, aciduric, facultative anaerobes (Batt, 2000). The lactic acid bacteria are found in foods (dairy products, fermented meat, sour dough, fermented vegetables, silage beverages-including wine), on plants, in sewage, but can also be in the genital, intestinal, and respiratory tracts of man and animals (Hames *et al*., 1991). In the food industry, lactic acid bacteria act as both beneficial organisms and spoilage organisms. They are used in the production of fermented milk products such as yoghurt, sour milk, cheese, and butter, and in the

production of sausages, pickles, and sauerkraut. The result of these fermentations is more shelf-stable products with characteristic aromas and flavours, however, if the growth of lactic acid bacteria is not controlled, they can be a major cause of food spoilage.

Previous studies have shown that the presence of LAB in beers and wine is responsible for the spoilage of such alcoholic drinks (Pfenninger *et al*., 1979); however, no attempts has been made to isolate, identify and exploit the utilization of wide range of native microorganisms from *mnazi* including LAB for food and alcoholic beverage processing. This work aims at isolation and identification of common LAB species in *mnaz*i responsible for its spoilage and to avail them for future economic use.

7.2. Material and Methods

7.2.1 Sample

Sample collection, preservation and transportation were carried out as described in section 6.2.1 of this thesis write-up.

7.2.2 Isolation and identification of LAB

Before isolation, the sample was enriched in litmus milk medium (0.5% yeast extract and 0.5% glucose). The actual isolation and identification of suspected LAB was carried out in Bromo-Crystal Purple (BCP) and De man Rogosa and Sharpe (MRS) agar. To discourage the growth of yeasts, 10ppm of cycloheximide was added in both media. Triplicate pour plates

were prepared for each dilution and the plates were incubated at 25, 30 and 37°C, respectively for a period of 2-3 days. Isolates were picked from plates with less than 30 colonies. Pure colonies of the selected isolates were obtained by transferring three times from the BCP agar into Tryptone-Yeast extract-Lactose- Glucose (TYLG) broth (yeast extract 0.5%, tryptone 1%, glucose 0.5%, lactose 0.5%, tween-80 0.1%, L-cysteine 0.01%). Thereafter one loopful of inoculums from each tube, which showed positive growth, was streaked onto plates with BCP agar for further isolation of pure cultures. After incubation for 1-2 days, the same exercise was repeated until three transfers were made. The isolated pure culture colonies were Gram stained and catalase test was performed on each of the colony. Representative colonies of the isolated bacteria that were Gram positive rods or cocci and catalase negative colonies were stored in litmus milk medium at 4°C and transferred every 2 months or stored in skim milk medium (skim milk 10%, L-glutamic acid monosodium salt 0.1%) at -20°C until identification. Based on morphological observation of the isolated strains after Gram stain, and catalase test, a total of eighty-six (86) different colonies were picked. Gram stain was conducted using conventional methods, while catalase test was conducted by adding 3% of freshly prepared hydrogen peroxide on the colonies on MRS agar plates previously incubated at 30°C for 48 h. The presence of catalase was indicated by the production of bubbles.

Based on morphological and physiological similarities, further screening was conducted and twenty seven (27) representative strains were picked out of the eight six (86) isolates for further taxonomy. The 27 isolates were identified using the following physiological and biochemical tests: The ability to grow at 10, 15 and 45°C incubation temperatures and pH 4.4 and 9.6 was conducted using TYLG broth incorporated with BCP dye (0.006%) as an indicator. Any colour change from purple to yellow was regarded as positive results, while purple colour indicated negative result. The pH of the media was adjusted using 5N NaOH (for pH 9.6) and 5N H_2SO_4 (for pH 4.4). Growth in 6.5 and 18% saline solution was done in TYLG broth, previously sterilized by membrane filtration. After incubation at 30°C for 10 days, any bottom sedimentation was regarded as positive result. Production of ammonia from L-arginine was tested as described by Schillinger and Lüke (1987) using 1.25% L-arginine in TYLG broth. Hydrolysis of sodium hippurate with production of benzoic acid was conducted using TYLG consisting of 1% sodium hippurate. After incubation for 10 days at 30°C, the culture was centrifuged at 3000 rpm for 15min. Thereafter 1ml of 50% sulphuric acid was added to 1 ml of the super natant, followed by shaking for 30min. Any formation of benzoic acid was regarded as positive results. Homo-Hetero fermentation was conducted to establish the ability of LAB to produce gas in Gibson medium consisting of three parts mainly: broth A (distilled water-180 ml, skim milk-20 g, glucose-

0.9 g and litmus indicator); broth B (distilled water- 50ml, agar- 0.75g, peptone- 0.5g, yeast extract- 0.7g and 2.5ml of 0.4% manganese sulphate) and water agar (distilled water- 50ml and agar- 0.75g). To a 9ml mixture of A and B broths in a tube, previously sterilized at 110°C for 20min. and maintained at 50°C in a hot air oven, a pure culture of the strain under investigation was inoculated. 2-4ml of agar water was then added into the inoculated mixture of A and B broths followed by incubation at 30°C for 10 days. Tubes that produced gas, hence creating a gap between the water agar and the mixture of A and B broths were regarded as hetero fermenters, while those that did not produce gas were considered as homo fermenters.

After the initial identification, isolates that showed some similarities were grouped together and further screening followed. In the second phase of identification, fifteen (15) representative isolates were picked out of the 27 isolates and acid production from carbohydrates (amygdaline, L-arabinose, D-cellobiose, esculine, D-fructose, D-galactose, D-glucose, lactose D-mannitol, D-mannose, D-maltose, D-melezitose, D-melibiose, Na-gluconate, D-raffinose, L-rhamnose, D-ribose, salicine, D-sorbitol, sucrose, D-trehalose and D-xylose) was evaluated as explained by Parente *et al*. (1997). The lactic acid isomer test was conducted using the lactic isomer kit as previously described (von Krusch and Lompe, 1982; Parente *et al*. 1997) using the UV-spectrophotometer at wavelength of 340nm using a

cuvette size of 1.00cm light path. The reading of the absorbance was done at 20 to 25°C. Finally the concentration of D- and L-lactic acid was calculated as follows:

$$C = \left[\left(\frac{V \times MW}{\varepsilon \times \delta \times v \times 1000} \right) \Delta A\,(g\ /\ l) \right]$$

where C is concentration [g lactic acid $/l$ sample solution], V is final volume $[ml]$, v is sample volume $[ml]$, MW is molecular weight of the substance to be assayed $[g/mol]$, ε is extinction coefficient of NADH at 340 nm- 6.3 $[l mol^{-1} cm^{-1}]$, it therefore follows that:

It follows for D-lactic acid:

$$C = \frac{2.240 \times 90.1}{\varepsilon \times 1.00 \times 0.100 \times 1000} \Delta A[g/l]$$

$$= 0.3203 \times \Delta A = \frac{2.260 \times 90.1}{\varepsilon \times 1.00 \times 0.100 \times 1000} \Delta \mathrm{A}[g/l]$$

$$= 0.3232 \times \Delta \mathrm{A}\ \ [g/l]$$

The selected 15 strains were further confirmed for production of acids from carbohydrates and related compounds by use of the API 50CHL system (BIOMÉRIEUX SA, France). All LAB identification procedures were conducted in accordance with manufacture's instructions. Portions of growth of each isolate were aseptically transferred from a freshly inoculated stock culture using a swab to an ampule of API 50 CHL basal

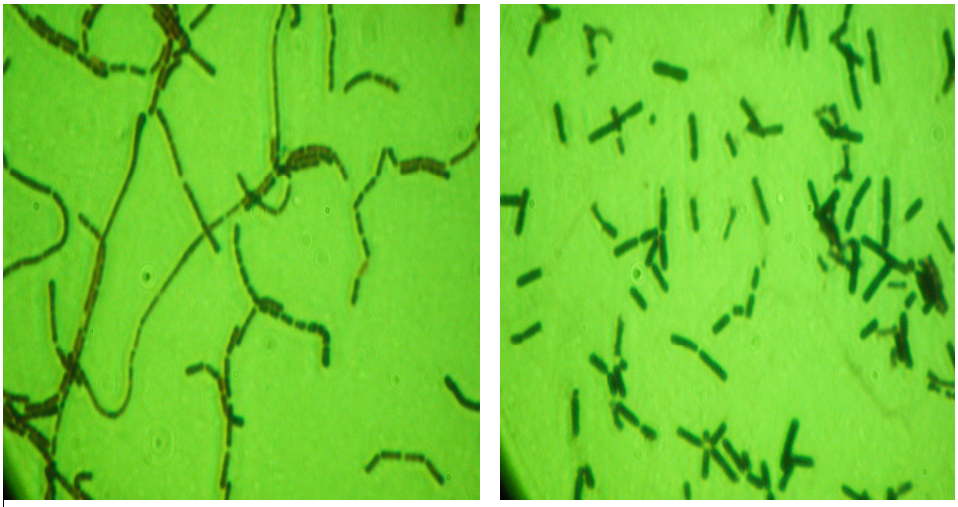
medium and the emulsified to give a final turbidity equivalent to McFarland standard #2. Each tube of the API 50 CHL strip was inoculated with the bacterial suspension using a sterile pipette. The strip was placed in the incubation tray with honeycombed wells each filled with distilled or demineralised water according to the instructions of the manufacturer. The tray with the strips in it was covered loosely with a lid, and incubated at 30°C for 72h. Reactions were visually examined after 24, 48 and 72h and determined to be positive or negative based on colour change in the tube caused by anaerobic production of acid and detected by the pH indicator present in the chosen medium. The results, which form biochemical profiles, were identified using an apiweb^{TM} software version 5.1. These profiles were then compared to those listed in the API 50 CHL Analytical Profile Index. Identifications listed in the index as excellent, very good or acceptable were accepted as correct. This together with other supplementary tests such as growth at different pH levels, incubation temperatures, and NaCl concentrations were used to confirm the strains. In contrast, further supplementary tests, such as; degradation of urea and starch, motility, Voges Proskauer, growth in MRS and Acetate media were used to confirm the remaining presumptive or questionable identifications.

7.3. Results

Figure 7.1 shows, the morphological appearance under Gram stain technique. Initial screening was based on morphological appearance and

catalase test. A total of 86 strains, were screened and the number was reduced to twenty seven (27). After conducting some preliminary physiological tests, the number was reduced further to 15 isolates.

A B



X 400 Maginification
A &B: Isolates of Gram stained Lactic acid bacteria under the microscope

Figure 7.1: Gram stained isolates lactic acid bacteria as observed under the microscope

All the 15 strains were found to be Gram positive (Figure 7.1) and catalase negative, non-spore forming rods or cocci. These strains were preliminarily identified for further biochemical and physiological tests as described in materials and methods. The physiological and biochemical characterization of the isolates from *mnazi* are presented in Tables 7.1 and 7.2. All the 15 isolates were considered LAB based on their positive Gram reactions, non

motility, absence of catalase activity and spore formation, and their rod or coccal shape. All the strains grew at 15 and 30°C but not at 45°C (Table 7.1). However, there were variations in growth at 10°C. Almost 50% of the 15 isolates were able to grow at 6.5% NaCl but none was able to grow at 18% NaCl. All the 15 isolates were unable to produce cabondioxide from glucose except TB405 (Table 7.1). They all showed positive growth at pH 7.0 and pH 4.4 with an exception of TB405 which was not able to grow at pH 4.4. Very few registered positive growth at pH 9.6. All the isolated strains were ADH- (negative arginine hydrolysis) while most of them were hippurate positive.

A total of 15 strains were positively identified after isolation using API 50 CHL kit (BioMėrieux® SA, France). Table 7.3 shows the LAB species positively identified in which five different species were identified. The predominant species were *Lactobacillus paracasei ssp paracasei 2* (7 strains), *Lactobacillus paracasei ssp paracasei 1* (4 strains) and *Lactobacillus plantarum 1* (2 strains). As indicated in Table 7.3, all the 15 strains were able to ferment ribose, glucose, fructose, N-Acetyl-glucoside, arbutine, esculine and salicine.

All the strains identified as *Lactobacillus paracasei spp paracasei 2* (CM201, CM203, CB301, TB302, TB405, TB402 and TM302) showed positive reaction for acid production from ribose, galactose, glucose, fructose, mannose, mannitol, Sorbitol, N-Acetyl-glucosamine, amydaline,

arbutine, esculine, salicine, cellibiose, maltose, Saccharose, Trehalose, inuline, Melezitose, turanose, tangarose and gluconate. Identification of these strains by API 50 CHL was extremely good. According to analytical profile index, all the strains identified as *Lactobacillus paracasei ssp paracasei 2* were listed in the identification index as excellent except for CM203, which was listed as very good identification. The identification % was very high (greater or equal to 96.2%), while the T value was approximately 0.9. All the strains had α-Methyl-D-glucoside as the only test against.

The *Lactobacillus paracasei ssp paracasei 1* (CM4081, CM4091, CB204 and CB4041) showed similar trends as those of *Lb. Paracasei ssp paracasei 2* with an exception of CM4081, which registered negative results on sorbitol, maltose, saccharose, inuline, melezitose and gluconate. The strain CB204 also showed a peculiar trend in that unlike other strains, it produced negative results with Sorbitol. In addition, all the strains belonging to this category were able to ferment lactose except for CM4081. Strains belonging to this group were positively identified as very good with an exception of CB204 that was rated as an excellent identification. The identification percentage was over 99%, while the T value was over 0.8 for all except CM4081 that had a T value of 0.63.

Strains that were positively identified as *Lb. plantarum 1* were CB303 and CM402. The pattern of fermentation for these two strains was similar to that

of *Lb. paracasei ssp paracasei 2*, except that they showed positive results on raffinose, β-gentobiose, lactose and melibiose. In addition negative results were recorded on insuline. CM402 registered negative results on Trehalose, gluconate, and turanose. Just like *Lb. paracasei ssp paracasei 1* and 2. The identification index was extremely good, in that one had excellent level of identification (CB303) while the other one (CM402) had very good index of identification. The identification % was more than 99% for both strains with a T value of approximately 0.8%.

The strain CB3021 was identified as *Lb. paracasei ssp paracasei 3*. The fermentation pattern of this strain is similar to that of *Lb. paracasei app paracasei 2*; however, this strain was unable to ferment sorbitol, maltose, saccharose, inuline, melezitose and gluconate. The identification however was categorized as doubtful with identification % of 96.1% and a T value of 0.61. This shows those further tests need to be done to validate this result.

Finally, the strain CM303 was identified as *Lactococcus lactis ssp lactis 1*. This strain registered positive results on ribose, glucose, fructose, α-Methyl-D-glucoside, N-Acetyl-glucosamine, arbutine, esculine, salicine, cellibiose, maltose, saccharose, trehalose and gluconate. This stain was different from all the other 14 isolates in that is was unable to produce acid from galactose, mannose, mannitol, sorbitol, amygdaline, inuline, melezitose turranose and tangarose. According the identification index, the

strain was identified as doubtful, with identification % of 94.6% while the T value was given as 0.42.

Table 7.1: Characterization of Lactic Acid Bacteria based on morphological, physiological and biochemical tests

S/N	Strain Ref.	Morphol	Gram Stain	Cat. Test	CO_2 from gluc.	Hippur.	NH_3 from arginine	Lactate isomer	Growth at pH			Growth at temp. (°C)				Growth at saline sol. (%)	
									4.4	7.0	9.6	10	15	30	45	6.5	18
1	CB303	Rods	+	-	-	+	-	L-(100%)	+	+	-	-	+	+	+	+	-
2	CM402	Rods	+	-	-	-	-	L- (D)-(79%)	+	+	-	-	+	+	-	-	-
3	CM4081	Rods	+	-	-	+	-	L-(100%)	+	+	VW	-	+	+	+	+	-
4	CM4091	Rods	+	-	-	+	-	L- (98%)	+	+	+	+	+	+	-	-	-
5	CB204	Rods	+	-	-	+	-	L- (97%)	+	+	-	W	+	+	-	-	-
6	CB4041	Rods	+	-	-	-	-	L- (95%)	+	+	-	W	+	+	-	-	-
7	CM201	Rods	+	-	-	-	-	L- (D)-71%)	+	+	-	-	+	+	-	+	-
8	CM203	Rods	+	-	-	+	-	L-(96%)	+	+	VW	-	+	+	-	-	-
9	CB301	Rods	+	-	-	-	-	L-(98%)	W	+	VW	+	+	+	-	+	-
10	TB302	Rods	+	-	-	+	-	L- (97%)	+	+	-	+	+	+	-	-	-
11	TB405	Rods	+	-	-	+	-	L- (96%)	-	+	-	W	+	+	-	+	-
12	TB402	Rods	+	-	-	+	-	L-(100%)	+	+	VW	+	+	+	+	-	-
13	TM302	Rods	+	-	-	+	-	L-(99%)	VW	+	-	W	+	+	-	+	-
14	CB3021		+	-	-	+	-	L-(99%)	+	+	W	-	+	+	-	W	-
15	CM303		+	-	-	+	-	L-(100%)	+	+	-	-	+	+	-	-	-

W, Weak growth; vw , Very weak; +, All strains positive; -, All strains negative;

Table 7.2: Fermentation profiles of LAB isolated from *mnazi* by convectional method

Strain Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Strain reference		CB 303	CM 402	CM 4081	CM 4091	CB 204	CB 4041	CM 201	CM 203	CB 301	TB 302	TB 405	TB 402	TM 302	CB 3021	CM 303
S/N	Substrates (Tests)															
1	Amylagdine	+	+	-	+	+	+	+	-	+/-	+	-	+	-	-	+
2	L-Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Cellibiose	+	+	-	+	+	+	+	+	+/-	-	+/-	+	-	-	-
4	Esculine	+	+	+	+	+	+	+	-	+/-	+	+	+	-	+	+
5	D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Galactose	+	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+
7	D- Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Lactose	-	+	+	+	+	-	+	-	-	-	-	+	-	-	-
9	Maltose	+	+	-	-	-	-	+	-	+/-	-	-	+	+	-	-
10	Mannitol	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+	+
11	D-Mannose	-	-	-	+	+	-	+	+	+	+	-	+	+	+	+
12	Melezitose	+	+	-	+	+	-	+	+	+	+	+	-	+	-	+
13	Melibiose	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
14	N- Acetyl - glucosamine	+	-	-	-	+	-	+/-	+	+	+	+	+	+	-	+
15	D-Raffinose	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Ribose	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
18	Salicine	+	+	+/-	+	+	+	+	+/-	+	+	+	+	+	+	+/-
19	Sorbitol	+	+	+	+	-	-	+	+	+	+	+	+	-	-	+/-
20	Saccharose	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-
21	Trehalose	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+
22	D-Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+, All strains positive; -, All strains negative; +/-, varible results

Table 7.3: Fermentation profiles of LAB isolated from *mnazi* by API 50 CH kit grown 30 °C

Strain Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Strain reference		CB 303	CM 402	CM 4081	CM 4091	CB 204	CB 4041	CM 201	CM 203	CB 301	TB 302	TB 405	TB 402	TM 302	CB 3021	CM 303
S/N	Substrates (Tests)															
0	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	Glycerol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Erythritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D-Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	L-Arabinose	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	D-Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	L-Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Adonitol	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
9	β- Methyl-xyloside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
11	D- Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	D-Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
14	L-Sorbose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
19	Sorbitol	+	+	-	+	-	+	+	+	+	+	+	+	+	-	-
20	α-Methyl-D-mannoside	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	α-Methyl-D-glucoside	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+
22	N- Acetyl - glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	Amygdaline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
24	Arbutine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	Esculine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

26	Salicine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
27	Cellibiose	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
28	Maltose	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+
29	Lactose	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-
30	Melibiose	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
31	Saccharose	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+
32	Trehalose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
33	Inuline	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-
34	Melezitose	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-
35	D-Raffinose	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
36	Amidon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	Glycogene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
38	Xylitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	β- Gentiobiose	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
40	D-Turanose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
41	D-Lyxose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	D-Tagatose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
43	D-Fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	L-Fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45	D-Arabitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	L-Arabitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	Gluconate	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+
48	2 ceto-gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	5 ceto-gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Level of Identification		E.I.	V.G.I.	V.G.I.	V.G.I.	E.I.	V.G.I.	E.I.	V.G.I.	E.I.	E.I.	E.I.	E.I.	E.I.	D.F.	D.F.
Identification %		99.9	99.3	99.2	99.9	99.9	99.0	96.2	99.3	96.2	96.2	96.2	96.2	96.2	96.1	94.6
Test		0.86	0.78	0.63	0.86	0.81	0.8	0.9	0.88	0.9	0.9	0.9	0.9	0.9	0.61	0.42
Test against		TAG 7%	TRE 96%	AMY 98%,	NON	SOR 86%	ADO 13%,	MDG 83%	MDG 83%	MDG 83%	MDG 83%	MDG 83%	MDG 83%	MDG 83%	MAL 80%,	GAL 90%,
Identified as:		**Lb. plantarum 1**		**Lb. paracasei ssp paracasei 1**				**Lb. paracasei ssp paracasei 2**							**LPSP 3**	**LLSL 1**

+, All strains positive; -, All strains negative; E.I, Excellent identification; V.G.I, Very god identification; D.F., Doubtful identification; Lb.- *Lactobacillus*; LPSP 3- *Lb. paracasei ssp paracasei* 3; LLSL 1- *Lactococcus lactis ssp lactis* 1

7.4 Discussion

LAB species are of paramount importance in food industry, both as beneficial organisms and as spoilage organisms. They are used in the production of fermented milk products such as yoghurt, sour cream, cheese and butter, and in the production of sausage, pickles and sauerkraut. In all these products the fermentation process gives characteristic aromas and flavours. Previous studies were able to isolate LAB species in different ecological niches such as milk, meat, vegetables as well as mouth, intestine, and vagina of mammals. Despite all these efforts there have been very few attempts targeting the isolation of LABS from traditional wines such as *mnazi*. The presence of LAB in *mnazi* is more of spoilage rather than beneficial organisms.

The lactic microflora of *mnazi* sampled at the Coastal region of Kenya was dominated by homofermentative *lactobacilli*. In general, over 80% of all the isolates belonged to the species *Lactobacillus paracasei ssp paracasei*. Specifically 47% were identified as *Lactobacillus paracasei ssp paracasei2*, 27% *Lactobacillus paracasei ssp paracasei1* while 7% were identified as *Lactobacillus paracasei ssp paracasei3.* From the results it is clear that the species *Lactobacillus paracasei ssp paracasei* is the most common LAB in *mnazi.* Other LAB species that were isolated include *Lactobacillus plantarum*, 13%, and *Lactococuss lactis ssp lactis1*, 6%.

Although all the strains were Gram negative, with *Lactococcus* cells being oval cooci commonly arranged in pairs or chain while *Lactobacillus* cells were rod-shaped arranged in chains. It is worthy pointing out that in characterizing the morphology of lactic acid bacteria, it may be difficult, at times, to distinguish a short rod from an ovoid coccus. Determining the arrangement (pairs versus tetrads of cocci, for example) can be challenging as well. Observation should be done carefully and repeatedly before making a final decision. Different LAB strains differed in their temperature requirements, but in general, the results obtained in this study agreed well with the data published earlier (Collins *et al*., 1989). As expected all strains registered positive growth at 15°C but were not able to at 18% NaCl. Most of them were unable at 45°C and at pH 9.6. All strains were found to be L (+) lactic isomer except for CM201 that was L (D) isomer. This confirms the preliminary identification that the strains belong to the genera *Lactobacillus*. This work confirms the fact that many of the recently described LAB species do not fit into the traditional classification scheme based on morphology and growth temperatures but instead one should rely on other method of classification such as those based on biochemical and physiological criteria (Kandler and Weiss, 1986).

API 50 CH fermentation system enables strain characterization in terms of growth and metabolism on a wide range of individual substrates and thus

fulfils an important role in the taxonomic identification of *Lactobacilli* (Vandamme, *et al*., 1996). Our results, based on BioMérieux software version 3 on carbohydrates fermentative pattern clearly showed that the isolates that were positively identified as *Lactobacillus paracasei ssp paracasei* were quite uniform in their biochemical and physiological characteristics. The only marked difference observed was that strain CM4081 did not produce acid from saccharose and gluconate. This phenomenon is therefore regarded as an inconsistent attribute of this species. In this study The API 50 CHL carbohydrates fermentative profile of *Lb. paracasei ssp paracasei 2* did not agree with the findings published by Charteris, *et al*., (2002) in that all the strains in this study showed the ability to produce acid from dulcitol and inositol while the control of the same species published by Charteris, *et al*., (2002) were unable to produce alcohol from these two alcohols (dulcitol and inositol). In addition, the *Lb. paracasei ssp paracasei 2* in this study were able to ferment α-Methyl-D-glucoside as opposed to those published by Charteris, *et al*., (2002). In this study the fermentation profile of all the strains identified as *Lb. paracasei ssp paracasei* were almost identical. The major distinction between *Lb. paracasei ssp paracasei 1* and *Lb. paracasei ssp paracasei 2* is the ability of *Lb. paracasei ssp paracasei 1* to ferment lactose and *α*-Methyl-D-glucoside as opposed to *Lb. paracasei ssp paracasei 2,* which was unable

to ferment these two sugars. On the other hand, the isolate that was identified as *Lb. paracasei ssp paracasei 3* was significantly different from the other two species of *Lb. paracasei ssp paracasei 1 and 2,* in that it was unable to ferment gluconate, melezitose, inuline, saccharose, sorbitol and maltose. However, this species was classified as doubtful, meaning more tests need to be conducted to ascertain it's really identity. The presence of large number of *lactobacilli paracasei ssp paracasei* calls for further research to acertain whether *mnazi* could be having the properties of a functional food. This is because previous studies have shown the ability of *lactobacillus paracasei* species to have probiotic properties (Lindberg, *et al*., 1996; Gardiner, *et al*., 1998). Previous works have shown that probiotics are able to modulate immune responses, lower biomarkers such as harmful faecal enzymes activities, show positive effects against superficial bladder cancer and cervical cancer (MacFarland, 2000). Other benefits associated with probiotic include alleviation of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) symptoms, infection control and eradication of multi drug-resistance microbes, lowering of cholesterol in the blood and antimutagenic/anticarcinogenic activities (Haenel and Bendig, 1975; Mitsuoka, 1982 and Salminen, *et al*., 1998). Although at the moment there is no scientific data that has been availed to prove that *mnazi* is actually a functional food, general observations has it

that most people who are daily drinker of *mnazi* seem to be less susceptible to diseases such as malaria, cholera, typhoid influenza and other opportunistic diseases caused by common bacteria and viruses. This therefore calls for an urgent need to study the possibility of probiotic properties of the LAB isolates from *mnazi.*

Based on carbohydrates fermentation pattern two homofermentative *lactobacilli* strains (CB303 and CM402) were identified as *Lb. plantarum*. These two strains were different from the *Lb. paracasei ssp paracasei* strains in that they were able to ferment lactose, melezitose, raffinose and gentobiose while *Lb. paracasei ssp paracasei* were unable to ferment these carbohydrates. Previous studies were able to isolate *Lb. planturum* from fermented sausages (Parente, *et al*., 2001), naturally fermented sicilian green olives (Randazzo, *et al*., 2004). *Lactobacillus plantarum* is not only used as a starter culture in cheese making but can as well be used as a probiotic LAB (Gomes, *et al*., 1995; Gardiner, *et al*., 1998; Vinderola, *et al*., 2000). *Lactobacillus plantarum* is distinguished by its biochemical ability to dissimilate hexoses exclusively through the Embden-Meyerhof pathway. Gluconic acid and pentoses, however are dissimilated through the oxidative pentose-phosphate pathway. This gives a total percentage of LAB in *mnazi* with strains that could be probibiotic as 94%, hence, making them the most predominant LAB species in mnazi.

Lactococcus lactis is widely used by the dairy industry for the manufacture of fermented milk products. The primary role of *L. lactis* during fermentations is to the production of lactic acid from milk sugar lactose.

7.5 Conclusion and recommendations

The presence of Lactic acid bacteria in *mnazi* is an indication that LAB species are one of the major groups of microorganisms that are responsible for the spoilage of *mnazi*. However the presence of bigger percentage of the *Lactobacillus paracasei spp paracasei, Lactobacillus plantarum* and *Lactococcus lactis spp lactis* calls for further research on ability of the isolated strains to produce lactic acid using different types of substrates, ability to produce bacteriocins and volatile compounds and their potential as starter cultures of different food products.

CHAPTER EIGHT

ISOLATION AND IDENTIFICATION OF YEASTS IN *MNAZI*

8.1 Introduction

Mnazi is a fermented coconut sap obtained from coconut tree. It is sweet, dirty brown in colour, containing 10-12% sugar, mainly sucrose (Faparusi, 1971). As the fermentation process continues, the sap becomes milky-white in appearance due to the presence of large numbers of fermenting bacteria and yeasts (Okafor, 1975).

Yeasts are a group of intercellular microorganisms most of which belong to the fungi division of *Ascomycota* and *Fungi imperfecti*. Humans have known yeasts for thousands of years as they have been used in fermentation processes like in the production of alcoholic beverages and bread leavening. Yeasts have been isolated from the fermenting sap of *mnazi*, e.g. *Elaesis guineansis* (Basir, 1962).

In the field of applied biotechnology, fermentation of glucose and fructose has been established through thousands of years of practise. Most ethanol produced in the world is derived from starch or sucrose (Gong, *et al*., 1999). These carbohydrates are readily hydrolyzed by enzymes present in yeasts especially *Saccharomyces cerevisiae* and a few other yeasts with

the production of alcohol (beer) and gas (bread leavening) (Vaughn-Martini and Martini, 1995). It has been established that only plant material that contains fermentable sugars provides suitable substrates for yeast species of *Saccharomyces*, *Candida*, *Torula* and *Hansenula* (Campbell-Platt, 1994). These yeasts, especially *Saccharomyces* are typically associated with spontaneous alcoholic fermentation of African opaque beers; *mnazi* and Asian type of beverages such as rice wine (Campbell-Platt, 1994). Food grade yeasts are also used as sources of high nutritional value proteins, enzymes and vitamins, with application in the health food industries as nutritional supplements, as food additives, conditioners and flavouring agents. They are also used in the production of microbiology media, as well as livestock feeds. Yeast are included in starter cultures, for the production of specific types of fermented foods like cheese, bread, sour dough, fermented meat and vegetable products, vinegar, etc.

The significance of yeasts in food technology as well as in human nutrition, as alternative sources of protein to cover the demands in a world of low agricultural production and rapidly increasing population, makes the production of food grade yeasts important. A large part of the earth's population is malnourished, due to poverty and inadequate distribution of food. Scientists are concerned whether the food supply can keep up with the pace of the world population increase, with the increasing demands for

energy, the ration of land area required for global food supply or production of bio-energy, the availability of raw materials, as well as the maintenance of wild biodiversity (Bekatorou, *et al*., 2006).

Various microorganisms are used for human consumption worldwide as single cell protein (SCP) or as components of traditional food starters, including algae (*Spirulina*, *Chlorella*, *Laminaria*, *Rhodymenia*, etc), bacteria *(Lactobacillus*, *Cellulomonas* and *Alcaligenes* etc), fungi (*Aspergillus, Penicillium*, etc) and yeasts (*Saccharomyces, Candida, Kluyveromyces, Pichia and Torulopsis*) (Jay, 1996 and Ravindra, 2000). Among the yeast species, *Saccharomyces cerevisiae* and *Candida utilis* are fully accepted for human consumption, but very few species are commercially available. This study was undertaken, to isolate, enumerate and identify the common yeast strains in *mnazi* tapped by traditional methods by the Coastal people of Kenya so as to avail them for future economic use in the food and biotechnology industries.

8.2 Materials and Methods

8.2.1 Sample

Sample collection, preservation and transportation were carried out as described in section 7.2.1 of this thesis write-up.

8.2.2 Isolation of yeasts

Before isolation, the samples were enriched in litmus milk medium (0.5% yeast extract, 0.5% glucose and 100ppm chloramphenicol). The actual isolation and identification of suspected yeasts was carried out in Yeast extract-Malt extract (YM) agar (0.3% yeast extract, 3% malt extract, 0.5% peptone 1% glucose and 1.8% agar). Triplicate pour plates were prepared for each dilution and the plates were incubated at 25, 30 and 37°C, respectively for a period of 1-2 days. Isolates were picked from plates with less than 30 colonies. Pure colonies of the selected isolates were obtained by transferring three times from the YM agar into Tryptone-Yeast extract-Lactose- Glucose (TYLG) broth (yeast extract 0.5%, tryptone 1%, glucose 0.5%, lactose 0.5%, tween-80 0.1%, L-cysteine 0.01%) or YM broth (0.3% yeast extract, 3% malt extract, 0.5% peptone and 1% glucose). Thereafter, one loopful of inoculums from each tube, which had shown positive growth, was streaked onto plates with YM agar for further isolation of pure cultures. After incubation for 1-2 days, the same exercise was repeated until three transfers were made. The isolated pure culture colonies were Gram stained before the representative colonies of the isolated yeasts were stored in agar slants of YM agar at 4°C and transferred every 2 months or stored in skim milk medium (skim milk 10%, L-glutamic acid monosodium salt 0.1%) at -20°C until identification. Based on morphological observation of the

isolated strains after Gram stain, a total of one hundred and ninety eight (198) different colonies were picked. Based on distinct morphological differences such as colour, shape and size, this number was reduced further to thirty five (35). The 35 isolates were picked and purified further by streaking at least three times on YM agar. The purified isolates were stored in agar slants of YM agar at 4°C and transferred every 2 months or stored in skim milk medium at -20°C until required for identification.

8.2.3 Identification of yeasts

Yeast isolates were Gram stained for morphological observation. Identification of the isolated yeasts based on their morphological, physiological and biochemical properties was done as described by Deak and Beuchat (1996), Van der Walt and Yarrow (1984), and also by using API 20 C AUX (BioMérieux, SA, France). In addition API ID32 C test strips for yeast were used to compare the results obtained using API 20 C AUX system.

Growth on 50% (w/v) glucose-yeast extract agar (glucose 50%, yeast 5% and agar 3%) was carried out by transferring 1 straight wire of culture previously transferred three times in YM broth onto the 50% glucose-yeast extract agar. Incubation was done at 25°C for one month, any growth was considered as positive growth.

Determination of hydrolysis of urea was done using the method of Van der Walt and Yarrow (1984). Commercially produced Christensen's urea agar base (Merck) was used. The slants were inoculated from a suspension of the actively growing yeast culture using a sterile wire loop and incubated at 25°C for 4 days. The development of a deep pink colour in the agar was considered as a positive reaction.

To determine growth at 37°C, 1% of a fresh culture previously transferred three times on YM broth was inoculated into a special broth containing yeast extract (0.5%), glucose (2%) and peptone (1%). Incubation was done at 37°C for 3 weeks and the presence of any growth in form of gas production, turbidity or sedimentation was considered a positive result.

For growth in cycloheximide, 0.5ml of glucose assimilation medium was added into 4.5ml of filter-sterilized solution of cycloheximide (10mg of cycloheximide was dissolved in 90ml of distilled water). 1% of fresh culture previously transferred three times into YM broth was inoculated into the prepared medium and incubated at 25°C for 3 weeks. Any growth within one week was considered as positive result, while growth after 2-3 weeks was considered as positive/negative result.

Formation of mycelium was examined on corn meal agar (Merck). About 7 ml of the medium was poured onto the top of a sterile glass slide and this was allowed to solidify. By making two streaks on the surface of with a

straight wire containing fresh culture previously prepared three times on the slide with corn meal agar. The slides were then placed on top of a horse shoe type of glass rod immersed in a Petri dish containing 7-10ml of distilled water. The Petri dish was covered before incubation at 25°C for 5-7 days. Microscopic observations of the wet mount were done after every three days for any formation of mycelium or pseudo-hyphae.

Fermentation of D-glucose, sucrose, D-galactose, lactose, maltose and raffinose was tested according to the description of Van der Walt and Yarrow (1984). A positive result was indicated by accumulation of gas in the Durham tubes.

Liquid assimilation of carbon compounds was carried out by adding 4.5ml of distilled water into small test tubes with caps previously oven sterilized at 175°C for a period of 1.5h. Each tube with distilled water was sterilized by autoclaving at 121°C for 15min. An aliquot (0.5ml) of filter sterilized yeast nitrogen base (Difco laboratories, Detroit, MI, USA) containing 5% of the compound under test was aseptically added to the tubes. The tubes were inoculated by aseptically adding 0.1ml of a viable suspension in Ringers solution (Oxoid) of an actively growing culture. The carbon compounds tested were galactose, glucose, sucrose, lactose, L-arabinose, maltose, D-mannitol, melibiose, raffinose, soluble starch, Trehalose, xylose, α-methyl-D-glucoside, Cellobiose, Erythritol, Xylitol, citrate and DL-lactate. The tubes

were inoculated as in the sugar fermentation tests. A positive reaction was detected by visual inspection for an increase in the turbidity of the solution.

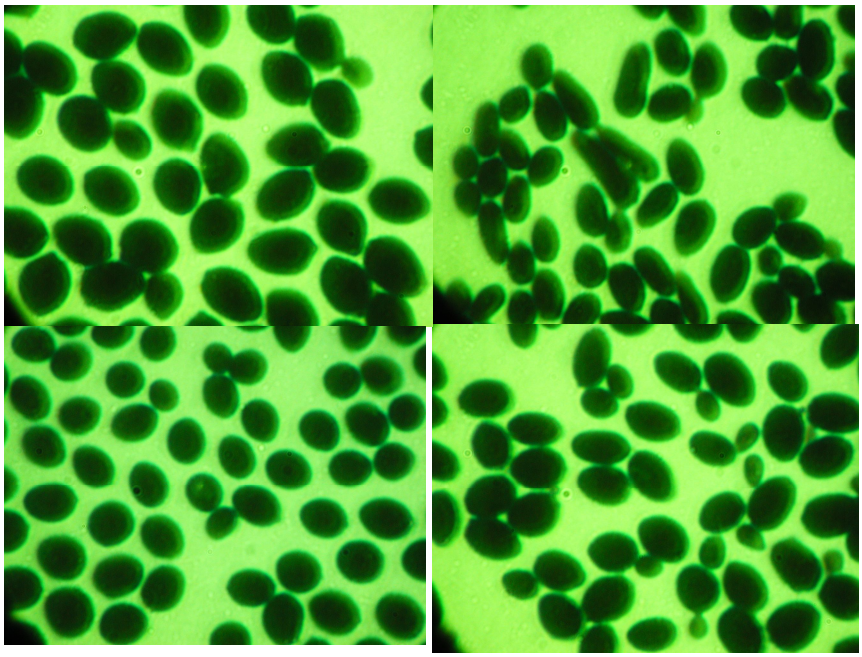
Portions of growth of each isolate were aseptically transferred from a freshly inoculated stock culture to an ampule of API 20 C AUX basal medium and the emulsified to give a final turbidity equivalent to McFarland standard #2. Each well of the API 20 C AUX strip was inoculated with the suspension, and the strip was placed in the incubation tray provided by the manufacturer, covered loosely with a lid, and incubated at 30°C for 72h. Reactions were visually examined at 24, 48 and 72h and determined to be positive or negative based on the presence or absence of turbidity in the carbohydrates wells. A seven -digit biocode was generated on the basis of these observations by assigning a weighted score to positive reactions. These codes were then compared to those listed in the API 20 C AUX Analytical Profile Index. The results, which form biochemical profiles, were identified using an apiweb^{TM} software version 4.0. Identifications listed in the index as excellent, very good or acceptable were accepted as correct. This together with other supplementary tests described above such as presence of nuclei, presence of hyphae or pseudohypha were used to confirm the strains.

Further confirmation of the isolates positively identified as *Saccharomyces cerevisiae* was conducted using an API ID 32 C kit. Identification was

accomplished as directed by the manufacturer (bioMèrieux). The molten API basal medium ampoules were inoculated with yeast cells picked from individual colonies and the resulting suspension was standardized to turbidity equal to McFarland standard #2. Each ampoule was inoculated and trays were incubated for 72h at 30°C. Ampoules showing turbidity significantly greater than that of the negative control were considered positive. A ten-digit biocode was generated on the basis of these observations by assigning a weighted score to positive reactions. These codes were then compared to those listed in the API ID 32 C Analytical Profile Index. The results, which form biochemical profiles, were identified using an apiweb^{TM} software version 3.0. Morphology on cornmeal agar (Difco) was also evaluated as suggested by the manufacturer.

8.3 Results

A total of thirty five (35) yeast strains out of the isolated one hundred and ninety eight (198) isolates from *mnazi* were identified in this study (Tables 8.1, 8.2 and 8.3). The morphological observation yeasts under Gram stain are shown in Figure 8.1, while the ability to hydrolyze urea is as shown in Figure 8.2. A deep pink colour indicates positive test.



Top left- MM2533; Top right-MM3062; Bottom left-3081; Bottom right-MM3752
All X 400 Maginification

Figure 8.1: Gram stained yeast cells under microscopic observation

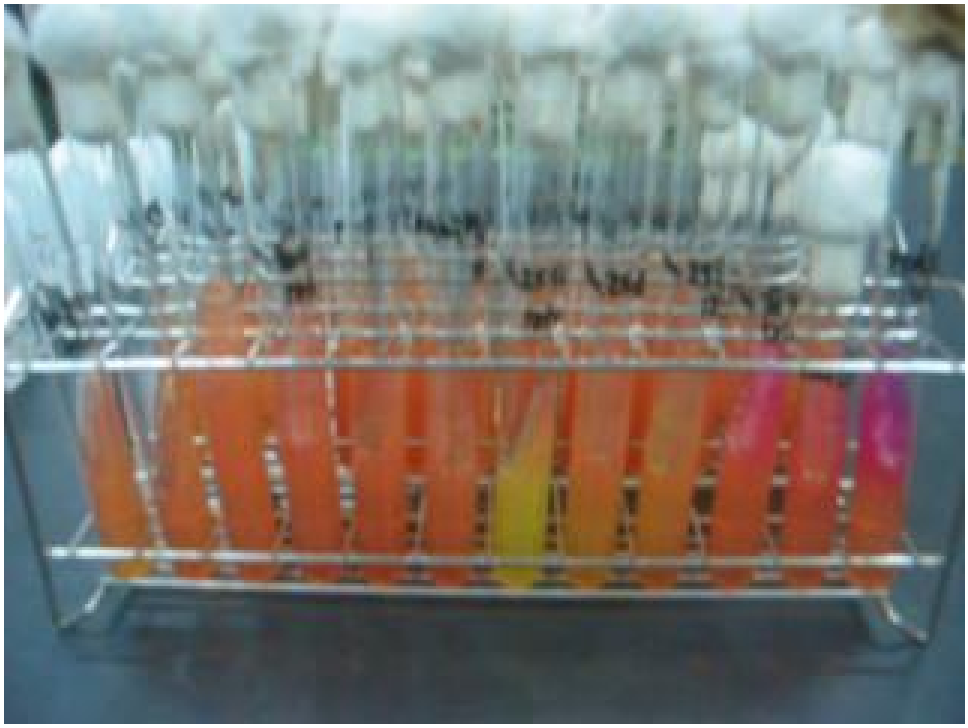


Figure 8.2: Hydrolysis of urea by yeasts isolated from *mnazi*

The API C AUX system identified twenty-four (24) species as *S. cerevisiae 1* (68.6%), two (2) species belonged to *S. cerevisiae 2* (5.7%). Almost 75% of all the isolated strains belonged to the genera *Saccharomyces*. The other isolates were identified as *Candida pelliculosa* (5.7%), *Candida utillis* (5.7%), *Stephanoascus ciferrii* (2.8%), *Kloeckera spp* (2.8%), *Trichosporon asahii* (2.8%) and *Rhodotorula mucilaginosa* (2.8%). While API C AUX system was able to identify at least nine (9) different species from the coconut toddy, API ID32 C on the other hand classified all the 35 strains

into only two different species, namely: *Saccharomyces cerevisiae* and *Saccharomyces exeginuus* (*C. holmii*). According to API ID32 C all the species isolated from *mnazi* belonged to the genera *Saccharomyces*.

From the results, all the twenty four (24) strains of yeasts that were successfully identified as *S. cerevisiae* by API 20 C AUX could ferment glucose and sucrose, while most of them were able to utilize galactose, raffinose, and maltose. They were not able to utilize cellobiose, trehalose, melezitose, lactose, inositol, sorbitol, arabinose, xylose and 2- ceto-gluconate. As for API ID32 C all strains that were successfully identified as *S. cerevisiae* were able to ferment sucrose and raffinose in addition to fermenting glucose and galactose (with an exception of three strains - MM3751, MM3759, MM3062). Two strains wereidentified as *C. Holmii* by API ID 32C. These strains were able to ferment glucose, galactose and sucrose but they were unable to ferment maltose, lactose and raffinose. In addition to positive growth at 37 °C and 50% glucose but they were unable to degrade urea (Table 8.1). These results are compatible with those published by Van der Walt and Yarrow (1984). The physiological and biochemical characteristics listed in Table 8.1 seem to confirm the identification of *S. cerevisiae*, some of the obvious tests include: positive growth at 37°C, ability by most species to grow at 50% glucose, negative growth in cycloheximide (most of the species) and inability by most species

to degrade urea (Van der Walt and Yarrow, 1984). However further study such as RapID Yeast Plus system (Remel), Seminested PCR (snPCR), Antigen Detection may however be necessary so as to support this assumption. Detailed results on identification of different yeast species are provided in Tables 8.1, 8.2 and 8.3.

Table 8.1: Identification of yeasts isolated from *mnazi* using physiological and morphological characteristics as well as fermentation and assimilation of carbon compounds

S. No.	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Stain Reference No.	MM 2532	MM 2536	MM 2565	MM 3035	MM 3053	MM 3741	MM 3759	MM 2546	MM 2551	MM 2561	MM 3083	MM 3042	MM 3751	MM 3055	MM 3052	MM 3034	MM 3557	MM 3061	MM 3084	MM 3758
Fermentation of:																				
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	+	-	+	-	+	-	+	-	+	+	-	+	-	-	+	-	-	-
Assimilation of:																				
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	-	+	-	+	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+
Cellobiose	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Trehalose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melibiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	+	-	+	+	+	+	-	+	-	+	-	+	+	+	+	-	+	+	-
Erythritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MDG	-	-	-	-	-	+	-	+	+	-	-	+	+	+	+	+	-	-	-	-
Succinic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Other Tests:																				
At 37 °C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50 % Glucose	+/-	+	+	+/-	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+	+	+
In Cycloheximide	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Urea utilization	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Pseudohyphae	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-

Table 8.1: (Continued)

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
	MM	MM	MM	MM	MM	MM	MM	MM	MM	MM	MM	MM	MM	MM	MM
Strain Reference No.	3762	3051	2554	3761	3754	3041	2534	3062	3075	3081	2533	2547	3074	2543	3745
Fermentation of:															
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
Assimilation of:															
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trehalose	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+
Melibiose	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-
Raffinose	-	+	-	-	-	+	-	+	+	+	+	+	+	+	+
Erythritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MDG	-	+	+	+	-	+	-	-	+	-	+	+	-	+	+
Succinic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Other Tests:															
At 37 °C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
50 % Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
In Cycloheximide	+/-	-	-	+/-	+/-	-	-	-	-	-	-	+	-	-	-
Urea utilization	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-
Pseudohyphae	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+

+/- = weak, + = positive test, - = negative test

Table 8.2: Identification of yeast isolates with the API 20 C AUX System

Test Nos.	S. NO.	01 – 07	08 – 09	10 – 11	12	13 – 14	15	16	17	18	19	20	21	22	23	24
	Strain Reference NO.	MM2532, MM2536, MM2565, MM3035, MM3053, MM3741, MM3759	MM2546, MM2551,	MM2561, MM3083	MM 3042	MM3051, MM3751	MM 3055	MM 3052	MM 3034	MM 3557	MM 3061	MM 3084	MM 3758	MM 2554	MM 3762	MM 3761
0	Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Glycerol	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-
3	2 ceto- gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Xylose	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
6	Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Xylitol	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
8	Galactose	+	+	+	+	+	+	+	-	-	+	+	-	+	-	+
9	Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	α-Methyl-D-glucoside	-	+	-	+	+	+	+	-	-	-	+	-	+	-	-
12	N-Acetyl–glucosamine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	Cellobiose	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
14	Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Maltose	-	-	+	+	+	-	+	-	+/-	+	+	-	+	+	-
16	Saccharose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	Trehalose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	Melezitose	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
19	Raffinose	-	+	-	+	+	-	+	-	-	-	+	-	-	-	-
20	Hyphae/ Pseudo-Hyphae	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
	Level of Identification	LD	VGI	VGI	GI	GI	VGI	GI	GI	GI	VGI	GI	LD	VGI	GI	LD
	Identification %	91.6	99.7	99.5	97.9	97.9	99.8	94.0	91	97.5	99.5	98.1	74.7	99.2	97.5	74.7
	Test	0.89	0.79	0.97	0.86	0.86	0.75	0.82	0.89	0.81	0.97	0.69	0.97	0.83	0.81	0.97
	Test against	MAL	MAL	NIL	GLY	MDG	MGD	GLY	MAL	GAL	NIL	GLY	MAL	MDG	GAL	MAL
	Identified as:	*Sacharomyces cerevisiae*														

Table 8.2: (Continued)

Test No.	*S.No.* / *Strain Reference No.*	25 MM 3754	26 MM 3041	27 MM 2534	28 MM 3062	29 MM 3075	30 MM 3081	31 MM 2533	32 MM 2547	33 MM 3074	34 MM 2543	35 MM 3745
0	Control	-	-	-	-	-	-	-	-	-	-	-
1	D-Glucose	+	+	+	+	+	+	+	+	+	+	+
2	Glycerol	-	-	-	+	+	+	+	+	+	+	+
3	2 ceto- gluconate	-	-	+	-	-	-	-	+	+	-	+
4	Arabinose	+	-	-	-	-	-	-	+	+	-	+
5	Xylose	+	-	-	+	+	+	-	+	+	+	+
6	Adonitol	+	-	-	-	-	-	-	-	+	-	+
7	Xylitol	-	-	-	-	-	-	-	-	+	-	-
8	Galactose	+	+	-	-	+	+	+	+	+	+	+
9	Inositol	-	-	-	-	-	-	-	+	-	-	+
10	Sorbitol	-	-	-	-	-	-	-	-	+	-	-
11	α-Methyl-D-glucoside	-	+	-	-	-	+	+	-	-	+	+
12	N-Acetyl–glucosamine	-	-	-	-	-	-	-	+	+	-	+
13	Cellobiose	-	-	-	-	+	+	+	+	-	-	+
14	Lactose	-	-	-	-	-	-	-	-	+	-	+
15	Maltose	+	-	-	+	+	+	+	+	+	+	+
16	Saccharose	+	+	-	+	+	+	+	+	+	+	+
17	Trehalose	-	+	-	-	-	+	+	+	+	+	+
18	Melezitose	-	-	-	-	-	+	-	-	-	-	-
19	Raffinose	+	+	-	+	+	+	+	+	+	+	+
20	Hyphae/ Pseudo-Hyphae	-	-	-	-	-	-	-	+	-	-	+
	Level of Identification	VGI	VGI	GI	VGI	VGI	GI	VGI	EI	VGI	EI	EI
	Identification %	99.3	72.6	95.2	99.4	99.4	99.8	98.7	99.9	99.4	99.3	99.9
	Test	1.0	0.53	0.79	0.88	0.63	0.83	0.83	0.91	0.91	0.72	0.70
	Test Against	NIL	MAL	CEL	NIL	GAL	Nil	MLZ	NIL	MLZ		GLY
	Identified as	*S. cerevisiae2*		*Klo spp*	*C. utilis*		*C. pelliculosa*		*Steph. ciferrii*	*S. clor.*	*Rh. Mucilaginosa 2*	*Tr. asahii*

+ = positive test, - = negative test, EI = excellent identification, VGI = very good identification, GI = good identification,
S. = Saccharomyces, C. = Candida, Klo spp = Kloeckera species, Steph. = Stephanoascus, Rhodotorula. = Tr- = Trichosporon

Table 8.3: Identification of yeast isolates with the API 20 C AUX System

Test Nos	*S.No.* / *Strain Ref. No.*	01 – 19 MM2532, MM2536, MM2546, MM2551, MM2565, MM3035, MM3042, MM3051,MM3053, MM3055, MM3052, MM3083, MM3758, MM2561, MM3557, MM3761, MM3034, MM2554, MM2534	20 – 22 MM3751, MM3759, MM3062	23 MM 2543	24 – 32 MM3041*, MM3754*, MM3074*, MM3075*, MM3081*, MM3745*, MM2533, MM2547*, MM3762*	33 – 35 MM3061, MM3084, MM3741
0	GAL	+	-	+	+	+
	SOR	-	-	-	-	-
1	ACT	-	-	-	-	-
	XYL	-	-	-	-	-
2	SAC	+	+	+	+	+
	RIB	-	-	-	-	-
3	NAG	-	-	-	-	-
	GLY	-	-	-	-	-
4	LAT	-	-	+	-	-
	RHA	-	-	-	-	-
5	ARA	-	-	-	-	-
	PLE	-	-	-	-	-
6	CEL	-	-	-	-	-
	ERY	-	-	-	-	-
7	RAF	+	+	+	+	+
	MEL	-	-	-	-	-
8	MAL	-	-	-	-	-
	GRT	-	-	-	-	-
9	TRE	-	-	+	-	-
	MLZ	-	-	-	-	-
A	2KG	-	-	-	-	-
	GNT	-	-	-	-	-
B	MDG	+	+	+	+	-
	LVT	-	-	-	-	-
C	MAN	-	-	-	-	-
	GLU	+	+	+	+	+
D	LAC	-	-	-	-	-
	SBE	-	-	-	-	-
E	INO	-	-	-	-	-
	GLN	-	-	-	-	-
	Level of Identific.	Good Identification	Good Identific.	Good Identific.	Good Identification	Low Discrim in.
	Identific. %	94.2%	95.5%	98.1%	94.2%	95.8%
	Test	0.73	0.69	0.72	0.73	0.95
	Test Against	MAL	MAL	MAL	MAL	TRE
	Identified as:	Saccharomyces cerevisiae				C. holmii

+ = positive test, - = negative test

8.4 Discussion

Mnazi which is commonly referred to as coconut *toddy* or Nigerian wine is produced and consumed in very large quantities in the Coastal region of Kenya.

In recent years, several identification methods have been proposed as alternatives to cumbersome classical yeast identification techniques. Among these methods, commercial miniaturized systems such as Vitek, API 32C, API 20C AUX (bioMèrieux), Yeast Star (Clarc Laboratories, Heerlen, The Netherlands), Auxacolor (Sanofi, Paris, France), and RapID Yeast Plus system (Remel) were designed to shorten the identification.

The physiological, morphological and biochemical characteristics listed in Tables 8.1, 8.2 and 8.3 seem to confirm this identification, but further study may be necessary to support this assumption. However, it should be noted that the API kit were developed primarily for identification of clinical yeasts (Heard, *et al*., 1998).

In this study we observed that over 75% of all the isolates from *mnazi* belonged to the genera *Saccharomyces*. Our results (both with DI 32C and API 20C) are compatible with those of other published reports (Faparus, 1971; Okafor, 1972). For example, Okafor (1972) reported that *Saccharomyces cerevisiae* constitutes about 70% of the total population of yeasts in *mnazi*.

The fact that this study successfully isolated and identified some of the most

important yeasts notably *Saccharomyces cerevisiae* and *Candida* utilis raises very strong hope for future use of these species in local biotechnology related industries such as the Muhoroni- based Agro-Chemical and Food Company and the East African Breweries limited. Previous studies have shown that among the yeast species, *S. cerevisiae* and *C. utilis* are fully accepted for human consumption as a single cell protein (SCP) or as component of traditional starters but very few species of these yeasts are commercially available (Jay, 1996; Ravindra, 2000). In addition *S. cerevisiae* has been used as a top fermentation starter culture in the production of ales, stouts and wheat beers (Goldammer, 2000).

According to Salminen *et al*. (1999), probiotic properties of yeasts, like *S. cereviciiae* have been reported and displayed as the ability to survive through gastrointestinal (GI) tract and interact antagonistically with GI pathogens such as *Escherichia coli*, *Shigella* and *Salmonella*. It will be interesting if further study can be conducted to confirm any probiotic properties of these yeast species, since unconfirmed reports indicate that heavy consumers of *mnazi* at the Coastal region of Kenya are more resistant to common and opportunistic diseases such as typhoid, diarrhoea, malaria, common flu and fever.

In the animal feed industry, *Torula* or *Candida* yeast refers to products containing *Candida utilis*, which have been used commercially for more than 60 years as nutritional supplements in feeds. Food grade *Torula* yeast is

cultivated in mixtures of sugars and minerals, usually containing molasses, cellulosic wastes (e.g. spruce wood) or brewing by-products (Lezcano, 2005; Kuzela *et al*. 1976; Weatherholtz and Holsing, 1975). Once thermolyzed and spray dried, *Torula* yeast can be used as a meat substitute or food additive in many processed foods, in seasonings, spices, sauces, soups, in baby food, meat product or diet and vegetarian food (Lezcano, 2005; Kuzela *et al*. 1976; Weatherholtz and Holsing, 1975). The findings of this study can also be exploited for related work in the animal feed industry.

As for the other yeast strains isolated and identified in this study *Candida pelliculosa*, *Candida utillis*, *Stephanoascus ciferrii*, *Kloeckera spp*, *Trichosporon asahii* and *Rhodotorula mucilaginosa* have also been reported in yoghurt, cheese and *makamo* (Tzanetakis *et al*., 1998; Viljoen, 1998; Sserunjogi, 1999). *Candida holmii* has been reported in milk due to its ability to utilize galactose. This yeast has an inducible hexokinase (which phosphorylates glucose) and a constitutive galactokinase; galactose will be used first even in the presence of glucose (Marshall, 1993). *Rho. rubra* (*mucilaginosa*) is associated with products based on milk facts (Jakobsen and Narvhus, 1996).

8.5 Conclusion and recommendations

During this study, it was found that the identification of all common yeast isolates by the two systems (API ID 32C and API 20C) were comparable in their overall efficacy; however, the interpretation of test results obtained with

ID 32C system was more difficult and required greater experience than did interpretation of those obtained with API 20C. This, therefore, explains the reason why a mismatch of about 11 strains was detected between the two systems. This calls for further study using modern and more accurate methods such as the RapID Yeast Plus system (Remel), Seminested PCR (snPCR), Antigen Detection and other Biochemical Methods to confirm the mismatched species and those which were successfully identified by the two systems.

However, it is worthy to note that this study successfully identified twenty four (24) species of *Saccharomyces cerevisiae* which can be exploited further for local industrial application such as baking, making of wine, beer, production of portable fuel ethanol and single cell protein. This therefore, calls for further work on ethanol tolerant, osmo-tolerant, acid tolerant as well as flocculating properties of these species. There is also need to conduct further work on suitability and optimum conditions for their use in the baking and fermentation industries. In addition, this study highly recommends further work to be conducted so as to ascertain the probiotic properties of the isolated and identified yeast species belonging to *S. cerevisiae*.

CHAPTER NINE

GENERAL CONCLUSION

9.1 General conclusion

Studies on traditional tapping and distillation methods, revealed the need for a clear policy on tapping, distillation and consumption of *mnazi* and its distillate. In addition, future work should exploit the possibility of finding better tapping methods and proper management to replace the already existing traditional tapping and distillation methods with an aim of increasing yields, efficiency and income. In this study, the distillation residue was reported to be extremely lethal to human beings, animals and plants. It will be of great interest if a study could be conducted to confirm the allegation and establish the chemical composition of this residue for further use in the manufacture of poisonous chemicals to control rodents, insects, pest and even weeds.

Major constraints faced by most tappers included: - provincial administration and police harassment, poor pricing and lack of market as well as lack of modern and improved tapping and processing technologies. As a way forward, appropriate strategies on production, processing and marketing need to be put in place.

This study shows that preserved mnazi was able to keep for more than two months without spoilage instead of the normal 2-3 days. In addition it was

found to be free from methanol and the levels of other volatile compounds (fusel oils) were less than 100ppm, hence meeting KEBS specifications for beers, wines and spirits. It will be therefore prudent, to de-gazette it from the category of “illicit brews”. *Mnazi* distillate (*pyuwa*) however registered higher content of isoamyl alcohol than the recommended limits hence not advisable to de-gazette it from the category of “illicit brews” unless the distillation technology is improved.

The presence of acetic and lactic acid bacteria in *mnazi* is enough evidence why *mnazi* has a short shelf-life of 1-2 days. The *Acetobacter* and *Gluconobacte*r strains isolated in this study are therefore responsible for the spoilage of *mnazi*; however, they may be utilized as useful microorganisms in the production of vinegar. However, further research need to be conducted with an aim of identifying the species of the two genera. The author therefore recommends the use of other procedures such as 16S and/or 23S rRNA gene sequencing, DNA-DNA similarity tests, DNA base composition as well as DNA relatedness and Quinone analysis.

The presence of Lactic acid bacteria in *mnazi* is an indication that LAB species are one of the major groups of microorganisms that are responsible for the spoilage of *mnazi*. However the presence of bigger percentage of the *Lactobacillus paracasei spp paracasei, Lactobacillus plantarum* and *Lactococcus lactis spp lactis* calls for further research on ability of the isolated strains to produce lactic acid using different types of substrates,

ability to produce bacteriocins and volatile compounds and their potential as starter cultures of different food products.

This study successfully identified yeasts species as follows: *S. cerevisiae 1, S. cerevisiae 2, Candida pelliculosa*, *Candida utillis*, *Stephanoascus ciferrii*, *Kloeckera spp*, *Trichosporon asahii* and *Rhodotorula mucilaginosa*. These yeasts can be exploited further for local industrial application such as baking and fermentation industries. This therefore, calls for further work on ethanol tolerant, osmo-tolerant, acid tolerant as well as flocculating properties of these species. There is also need to conduct further work on suitability and optimum conditions for their use in the baking and fermentation industries and probiotic properties yeast species belonging to *S. cerevisiae*.

CHAPTER TEN

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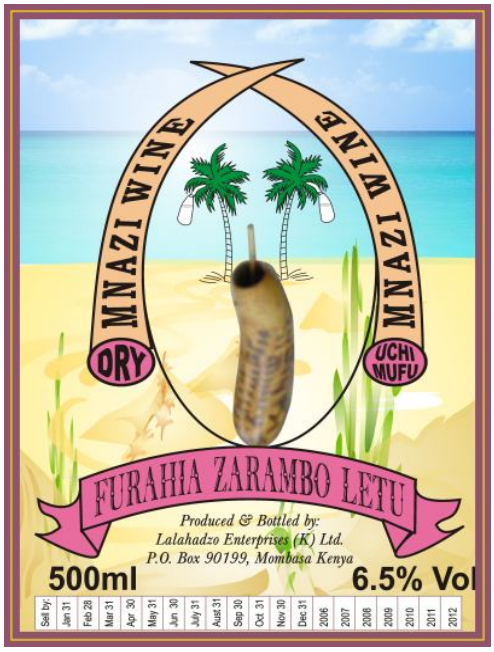
11. APPENDICES

Appendix 1: Identification of important microorganisms in mnazi using API kit system

Appendix 2: Design models of potential labels of the developed products showing different brands and different packaging materials



Appendix 3: Questionnaire 1- mnazi/mkoma farmer

MNAZI AND MKOMA ANALYSIS (SURVEY) IN THE COASTAL REGION OF KENYA

SURVEY OF OWNER OF MNAZI/MKOMA (FARMER)

1 Social - Demography (SD)

District …… Name of Enumerator ……..……..

Division …….. ID No. of Enumerator ……

Location …… Time of interview ……..

Sub location …….. Date of interview ……..……

Village ……..…..…..…..

CHARACTERISTIC OF THE HOUSEHOLD (FARMER) (SDI)

SDI 1	Name of Respondent ……..…….. ID No. ……..….	
SDI 2	Name of head of household …….. ID No. ……..	
SDI 3	Sex of Respondent 1 = Male ……..…. 2 = Female (SEX) 1…….	
SDI 4	Sex of household head 1 = Male …….. 2 = Female……	
SDI 5	Age of Respondent (AGE) 1……..…..….	
SDI 6	Age of Head of household (Age) 2……..……	
SDI 7	Schooling Respondent (EDUc)1……..…… Schooling Head of household (EDUC) 2……. 0 = No education 1 = Primary 2 = Secondary 3 = University/College 4=Others (specify)	
SDI 8	Number of family members of farmer Male …….. Female ……..	
SDI 9	Major source of income of farmer…….. 1 = Farming 2 = Formal employment 3 = Labour/Casual work 4 = Others specify	

2 AGRICULTURE PRODUCTION (AGPRO)

AGPRO 1	Tenure of land (Ten)……..	
	1 = Inherited 2 = Family land 3 = Bought 4= Rented 5 = Gift 6 = allocated 7 = Others (specify)	
AGPRO 2	Ownership document (TITLE)…….. 1 = Title deed 2 = Letter of allotment 3 = No document 4 = Others specify	
AGROP 3	Acreage under Coconut/Mkoma (ACREUE)…..…..….	
AGROP 4	Total number of coconuts (NOCOT)……..	

COCONUT TREE PRODUCTS (COPRO)

Name of product	Quantity (kg)/ No products per year **(QTY**) (kg/No	Utilization **(USE)** 1 = Domest. 2 = Sold 3 = Both	If, sold, price (KES) per Kg/item **(PP KG)** Item/Kg Indicate	Total amount per year **(AMT**) (KES)	Where sold **(MKT)** 1 = Farm gate 2 =Local Mkt 3 = Others
Coconut - peeled Un-peeled					
Copra					
Makuti (thatching material)					
Coconut shell					
Madafu					
Fagio (broom)					
Tree (trunk)					

MNAZI (MNAZI) - (COWI)

COWI 1	Name of the Tapper ……	
COWI 2	Sex of the Tapper …… 1 = Male, 2 = Female	
COWI 3	Age of the Tapper ……..	
COWI 4	Schooling of Tapper…… 0 = No education 1 = Primary 2 = Secondary 3 = College/University 4=Others specify	
COWI 5	Total No. of Trees owned…… No. of trees tapped…… No. of trees not tapped……	
COWI 6	• Quantity tapped: Low season…….. High season…….. • Quantity retained by farmer: Low season…….. High season..……..….. • Quantity retained by tapper per day (litres)……..……..	
COWI 7	Price per litre of Mnazi (KES) Low season …….. High season……..……	

MAJOR CONSTRAINTS (PROBLEM) FACED BY MNAZI FARMERS (CONST)

CONSTRAINTS (PROB)	POSSIBLE SOLUTION (SOL)
(1) (2) (3) (4) (5) (6) (7) (8) (9)	

Appendix 4: Questionnaire 2- mnazi/mkoma tapper

MNAZI AND MKOMA ANALYSIS (SURVEY) IN THE COASTAL REGION OF KENYA

SURVEY OF TAPPERS OF COCNUT/MKOMA TREES

1 Social - Demography (SD)

District … …… Name of Enumerator ……..……..

Division ……..….. ID No. of Enumerator ……..…….

.

Location ……..…… Time of interview ……..……..

Sub location ……..…….. Date of interview ……..……..……..

Village ……..……

CHARACTERISTIC OF TAPPER (SODEI)

SODEI 1	Name of Respondent …….. ID No. ……	
SODEI 2	Name of head of household …… ID No. ……..……..……..	
SODEI 3	Sex of Respondent 1 = Male 2 = Female (SEX) 1	
SODEI 4	Sex of household head 1 = Male 2 = Female	
SODEI 5	Age of Respondent (AGE) 1…….	
SODEI 6	Age of Head of household (Age) 2……	
SODEI 7	Schooling Respondent (EDUc)1……..…… Schooling Head of household (EDUC) 2……..…… 0 = No education 1 = Primary 2 = Secondary 3 = University/College 4= Others Specify	
SODEI 8	Number of family members of the Tapper Male …… Female ……..	
SODEI 9	Major source of income of Tapper…… 1 = Farming 2 = Tapping 3 = Labour/Casual work 4 = Others specify	

MAJOR CONSTRAINTS (PROBLEM) FACED BY MNAZI FARMERS (CONST)

CONSTRAINTS (PROB)	POSSIBLE SOLUTION (SOL)
(1) (2) (3) (4) (5) (6) (7) (8) (9)	

MNAZI (MNAZI) - (COMKO)

COMKO 1	Total number of trees tapped ……	
COMKO 2	No. of trees tapped owned by Tapper…….	
COMKO 3	No. of trees tapped not owned by Tapper……..	
COMKO 4	State high & low tapping season (months) Low season……. High season…….	
COMKO 5	Quantity tapped per day (litres) Low season …… High season…….	
COMKO 6	Quantity reserved for the owner if tapper not the owner of coconut tree per day (litres) Low season …… High season …….	
COMKO 7	Quantity retained by the tapper per day (litres) Low season …… High season …….	
COMKO 8	Quantity for domestic consumption by the tapper per day (litres) Low season …… High season …….	
COMKO 9	Price per litre of Mnazi (KES) Low season ……High season ……	

COMKO 10	Mnazi tapped is sold to whom 1-Vendor 2-Drinker 3-Others (specify)	
COMKO 11	Quantity not sold per day due to lack of buyers (litres) Low season …… High season …..……..	
COMKO 12	How is the unsold mnazi utilized…… 1- Give it free 2- Discard it 3-Distill into pyuwa 4- Others specify……..	
COMKO 13	Given the right motivation or conducive environment, state the Max number of trees you can tap per day…….Trees	
COMKO 14	State the frequency of climbing the tree per day during the tapping season…… 1- Once 2- Twice 3-Thrice	
COMKO 15	State the maximum number of days Mnazi toddy and/or Pyuwa can be stored without going bad Mnazi toddy….……days Pyuwa……..days	
COMKO 16	How long have you been tapping…….Yrs	