

**Production and characterization of mango (*Mangifera indica*) fruit
wine**

Samson Musembi Musyimi

**A thesis submitted in partial fulfillment for the degree of Master of
Science in Food Science and Technology at Jomo Kenyatta
University of Agriculture and Technology**

2013

DECLARATION

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

Sign.....Date.....

Samson Musembi Musyimi

This thesis has been submitted for examination with our approval as University supervisors:

Signature Date.....

DR. DANIEL. N. SILA

JKUAT, KENYA

Signature Date.....

PROF. CHRISTINE. A. ONYANGO

TAITA TAVETA UNIVERSITY COLLEGE, KENYA

DEDICATION

This thesis is dedicated to my family with love.

ACKNOWLEDGEMENT

My sincere thanks to the Almighty God for making this work a success. With heartfelt gratitude, I wish to acknowledge my supervisors Dr. D. N Sila and Prof. C. A Onyango, for critiquing this work for technical content and for providing their guidance, suggestions and the encouragement throughout the period of my studies. I also wish to recognize the invaluable input of Mrs. E. Okoth of the Food Science Department, Jomo Kenyatta University of Agriculture and Technology (JKUAT).

I thank (JKUAT) for providing financial support to this project. I also appreciate all the support provided by the technical staff in the Department of Food Science and Technology, Faculty of Agriculture (JKUAT); including Mr Paul Karanja, David Votha and George Mathenge.

I am forever indebted to my parents, Dr. T.M Wambua and Mrs. S.N Musyimi and family for their unrelenting support.

Finally I wish to thank my friends and colleagues for their time and moral support.

TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS	v
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF ACRONYMS	xiv
ABSTRACT	xvi
CHAPTER 1	1
1.0 INTRODUCTION	1
1.1 Background information.....	1
1.2 Problem statement	4
1.3 Justification.....	5
1.4 Objectives	7
1.4.1 Overall objective	7
1.4.2 Specific objectives	7

CHAPTER 2.....	8
2.0 LITERATURE REVIEW	8
2.1 The mango (<i>Mangifera indicus</i>).....	8
2.2 Global mango production and trade.....	11
2.3 Mango production in Kenya	12
2.3.1 Level of post harvest losses of mango fruits in Kenya	15
2.4 Post harvest value addition	17
2.4.1 Mango processing	18
2.5 Wine production from mango pulp.....	21
2.5.1 Attributes of mango fruit quality for wine production.....	22
2.5.2 Wine fermentation.....	24
2.5.3 Characterization of mango wine.....	28
2.5.4 Effect of storage bottle colour and temperature on wine colour	32
CHAPTER 3.....	33
3.0 MATERIALS AND METHODS	33
3.1 Study design	33
3.2 Extraction of pulp.....	35
Ripened mango fruits of the six varieties were sorted, washed and peeled manually using a knife.....	35

3.3 Analysis of physicochemical properties	35
3.3.1 Determination of juice recovery	35
3.3.2 Determination of simple sugars.....	35
3.3.3 Determination of pH	36
3.3.4 Determination of total soluble solids (TSS)	36
3.3.5 Determination of total titratable acidity (TTA)	36
3.4 Optimization of wine fermentation	37
3.4.1 Preparation of juice	37
3.4.2 Optimization of wine yeast from different manufacturers	37
3.4.3 Optimization of temperature and yeast inoculum size.....	38
3.4.4 Effects of fermentation temperature and yeast inoculum size on wine yeast growth.....	40
3.4.5 Effects of fermentation temperature and yeast inoculum size on substrate utilization.....	41
3.4.6 Effect of fermentation temperature and yeast inoculum size on chemical properties and volatile compounds of mango wine.....	41
3.5 Characterization of the mango wine	42
3.5.1 Determination of TTA	42
3.5.2 Determination of volatile acidity (VA).....	42

3.5.3 Determination of Ethanol	43
3.5.4 Determination of higher alcohols and major volatile compounds	43
3.6 Sensory analysis	44
3.7 Shelf stability and colour determination of the mango wine.....	45
3.7.1 Effects of storage temperature and different colour bottle on the colour of wine.	45
3.8 Statistical analysis	45
CHAPTER 4.....	46
4.0 RESULTS AND DISCUSSION	46
4.1 Screening mango varieties for their suitability for wine production	46
4.2 Optimization of fermentation process for wine production	50
4.2.1 Effects of wine yeast from different manufacturers on the fermentation rate and chemical properties of mango wine.....	50
4.2.2 Effect of temperature on wine yeast growth and substrate utilization during fermentation	52
4.2.3 Effects of temperature on chemical properties and volatile compounds of mango wine	55
4.2.4 Effects of yeast inoculum sizes on wine yeast growth and substrate utilization during wine fermentation	59

4.2.5 Effect of yeast inoculum size on chemical properties of mango wine	62
4.3 Characterization of the mango wine produced using optimized conditions.....	64
4.3.1 Chemical properties of mango wine	64
4.3.2 Volatile composition of mango wine	67
4.4 Determination of sensory properties of mango wine	71
4.5 Determination of shelf stability	75
4.5.1 Effects of storage temperatures and colour bottles on mango wine colour	75
4.5.2 Effects of wine storage on volatile acidity (VA).....	77
CHAPTER 5.....	79
5.0 CONCLUSION AND RECOMMENDATIONS	79
5.1 Conclusion	79
5.2 Recommendations	79
REFERENCES	81
PLATES	100

LIST OF TABLES

Table 1: Production of mangoes in selected Counties, 2009- 2011	14
Table 2: Physicochemical characteristics of (a) juices and (b) wines produced from different mango varieties.	23
Table 3: Composition of volatiles from three different mango varieties.....	31
Table 4: Physico-chemical characteristics of mango juice of different mango varieties.....	48
Table 5: Effects of wine yeast from different sources on the chemical properties of mango wine from Apple variety.....	51
Table 6: Chemical properties of mango wine at different temperatures at 0.05% <i>S. cerevisiae</i> inoculum size	55
Table 7: Effect of temperature on volatile compounds production (mg/L) of mango wine from Apple variety fermented at 25°C and pH 4.5 for 18 days at 0.05% inoculum size.....	57
Table 8: Chemical properties of mango wine at different inoculum sizes at 25°C	63
Table 9: Chemical characteristics of mango wine from Apple and Ng’owe varieties ...	65
Table 10: Composition of volatile compounds by Gas Chromatography–Flame Ionization Detector (GC-FID) from two mango varieties (Apple and Ng’owe) fermented at 25°C and pH 4.5 for 16 days at 0.05% inoculum size of wine yeast.	67
Table 11: Sensory evaluation of mango wine and a reference wine from grapes	72
Table 12: Effects of storage temperature and colour bottles on Apple mango wine colour	75

Table 13: Effects of storage time (12 months) on the volatile acidity of mango wine
at 15°C and in a brown bottle 77

LIST OF FIGURES

Figure 1: Mango fruits indicating (a) Apple, (b) Ngowe, (c) Kent, (d) Tommyatkins, (e) Sabine, (f) Vandyke and (g) Indigenous	10
Figure 2: Principal mango producing countries in 2010 (MT)	11
Figure 3: Mango production in metric tonnes (MT) in all the eight regions of Kenya from 2005-2009.....	13
Figure 4: The mango value chain in Kenya.	15
Figure 5: Mango fruits (Ngowe variety) in Juja open air market	16
Figure 6: Causes of postharvest losses of mango fruits in Kenya	17
Figure 7: Value added mango products including (a) mango pickles, (b) mango pulp, (c) frozen mango, (d) slices of dried mango.....	20
Figure 8: Flow chart diagram showing the study design.....	33
Figure 9: Fermentation set up at different inoculum sizes and temperatures.....	39
Figure 10: Yeast counts at different temperatures during wine fermentation at 0.05% inoculum size.....	53
Figure 11: Effects of temperature on substrate utilization and fermentation kinetics at 0.05% yeast inoculum size	54
Figure 12: Yeast growth at varying inoculum sizes at 25°C and pH of 4.5	60
Figure 13: Substrate utilization during the fermentation of mango juice from Apple variety at 25°C and pH of 4.5	61
Figure 14: Yeast growth, sugars utilization and alcohol yield at 25°C and 0.05% inoculum size.....	64

LIST OF PLATES

Plate 1: Fructose standard curve.....	100
Plate 2: Glucose standard curve	100
Plate 3: Sucrose standard curve.....	100
Plate 4: Approach during mango wine production.....	101
Plate 5: Total yeast count at maximal population (day 9)	102
Plate 6: Sensory evaluation questionnaire	102
Plate 7: Wine aging in different coloured bottles.....	103

LIST OF ACRONYMS

°Bx	Degree brix
AGRO	Agrochemical Industries
ALRMP	Arid Lands Resource Management Project
AOAC	Association of Official Analytical Chemists
CFU	Colony Forming Units
F.A.O STAT	Food Agricultural Organization Statistics
GC	Gas Chromatography
GC-FID	Gas Chromatography Flame Ionization Detector
GTZ	Gesellschaft für Technische Zusammenarbeit
HCDA	Horticultural Crop Development Authority
HPLC	High Performance Liquid Chromatography
ICRA	International Centre for Development Oriented Research in Agriculture
ICRAF	International Centre for Research in Agro forestry
IIRR	International Institute of Rural Reconstruction
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agricultural Research Institute
KENFAP	Kenya National Federation of Agricultural Producers
KHDP	Kenya Horticultural Development Program
KIRDI	Kenya Industrial Research and Development Institute
KIT	Royal Tropical Institute

KWAL	Kenya Wine Agencies Limited
MEA	Malt Extract Agar
ml	milliliters
MoA	Ministry of Agriculture
N	Normality
n	sample size
°C	degree centigrade
PSDA	Promotion of Private Sector Development in Agriculture
SMEs	Small and Medium Entrepreneurs

ABSTRACT

Mango is one of the most important tropical fruits in Kenya. Increased production has been observed over years paralleled by large postharvest losses which are partly attributed to poor value addition practices. The purpose of this study was to provide an alternative for reducing the level of postharvest losses in mango by producing mango wine. Six mature and unripe mango fruits of Apple, Ngowe, Tommy Atkins, Kent, Vandyke and Sabine varieties were harvested three times from a farm in Katheka Kai division, Machakos County of Kenya. The fruits were stored at 20°C and 85-90% relative humidity to ripen. The fruits were pulped and subjected to different fermentation conditions (varying inoculums size, temperature) to determine the optimal conditions for fermentation. The wine was analyzed for alcohol content, pH, residual °Bx, volatile acidity, titratable acidity and the major volatile compounds determined by GC-FID. Sensory evaluation and shelf life studies of the wine were also carried out. The highest recovery of juice was found in Kent and Apple (> 71 %) while the lowest was exhibited by Sabine (53%). The extracted juice had a high sugar content ranging from 17.0 - 23.9°Bx. Apple and Ngowe variety exhibited the highest sugar content (> 23°Bx) where as Sabine had the lowest (17°Bx). The optimal conditions for wine fermentation were at 25°C and 0.05% yeast concentration using wine yeast. The sensory evaluation indicated that mango wine exhibited similar sensory characteristics in terms of clarity and general acceptability as compared to a reference grape wine. This study provides evidence that mango fruits are suitable for wine processing.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background information

Mango (*Mangifera indica L.*) is one of the most important fruit trees in the tropics and subtropics. It originated from the Indian subcontinent and reached East Africa by 10th Century (Ensminger, 1994). It is commercially grown in more than 90 countries world wide and is consumed both in fresh and processed form (FAO, 2007). Over one hundred varieties are produced world wide which have similar properties but specific differences peculiar to each variety (Bally et al., 2009).

Mango is a popular commercial crop in Kenya that grows best at 0-1500m above sea level (Nakasone and Paul, 1998) but can grow at higher elevations. It's the second most important fruit in terms of area and production in the following sequence, banana (37.6%), mango (19.6%) and pineapple (12.1%), avocado (9.8%), pawpaw (5.4%), oranges (4.6%), water melon (4.2%) and passion fruit (3.7%) (Annual report HCDA, 2013). In 2007, it was estimated that the area under mango production was 14,387 Ha with an output of 280,884 metric tones (MT) (MoA, 2007). In 2008, HCDA reported 250,000Mt of mango production; however, this number greatly increased to 450,000 metric tonnes in 2010 (MoA, 2010). This is a clear indication that mango production has tremendously expanded. Gathambiri (2009) reported a percentage post harvest loss of 45% and the main reason cited was excess fruits in the market during the peak seasons.

Mango is either marketed as fresh fruit or processed into various products such as juices and mango chips to the various local, regional and international markets (International

Institute of Rural Reconstruction, IIRR, 2006). The bulk of the mangoes (over 98%) are for domestic markets and about 1-2% are for export, with a value of approximately Ksh 150 million per year (IIRR, 2006; Private Sector Development in Agriculture, PSDA, 2007). However, given the relatively small proportion of fruit exported, there is need to focus on value addition options for the remaining percentage.

Postharvest value addition technologies would reduce these losses giving farmers high returns for their crop. Processing is considered as improving the value of raw produce and an extension of storage life (Okoth et al., 2013). Mango fruits can be processed into various products: unripe mangoes are normally processed into pickles, preserves, dessert or chutneys while the ripe mangoes can be processed into dried mango chips, mango wine, mango juice, mango concentrate, mango jam, mango jelly, mango syrup and canned mango.

Middlemen, individual farmers or farmer groups sell approximately 5% of the fresh mangoes to processing industries such as Sunny Mango Processors, and Milly Processors. These are processed into mango jam, mango juice and concentrates (Gregoratti, 2009). The mango juice is sold either directly to different outlets and consumers or through distributors who in turn sell to the various consumers. Other processors (Kevian Processor Ltd) focus on processing purchased pulp in the form of concentrates mainly from Mauritius, Egypt and South Africa into juice (Gitonga; Kinuthia, 2009).

Mango wine is a potential high value added product that can be processed however there is no proper documentation of information regarding the scientific and technological approaches for its optimal production.

1.2 Problem statement

Mango production has been on the increase over the years, recording 26.0% and 29.6% increase in 2010 and 2011 respectively. The production value of mango increased by 14.3% in 2011 compared to the previous years. However, this increase in production has been accompanied by large postharvest losses of 45% due to limited value addition options during production gluts. Most of the mangoes produced are consumed as fresh fruits while the rest are sold to processing industries such as Sunny Mango Processors and Milly Processors. These companies process mango into jam, juice and concentrates. On the other hand, whole sale and retail activities do not involve any cold chain and fruits are subject to unpredictable weather thus exacerbating the problem. Other innovative value addition options which have been exploited in other countries such as India and Philipines include wine production, pickles, nectar and candies but these are largely missing in Kenya. Mango farmers remain poor due to poor prices as a result of limited knowledge on value addition options. The export market is weak due to stringent regulations governing their exportation. In Kenya, there appears to be no published information regarding the suitability of mango varieties for wine production and the fermentation conditions suitable for production of good quality wine. Furthermore, information on the characterization, safety and shelf stability of mango wine is not available.

This study explores how scientific and technological approaches can be used for optimal production of quality shelf stable mango wine towards value addition of mango produce thus reducing the major problem of post-harvest losses.

1.3 Justification

Horticulture industry in Kenya is dominated by smallholder farmers who constitute 80% of the growers and contribute about 60% to the export market. The industry plays an important role in poverty reduction by providing employment to about 2 million people annually (HCDA, 2006, 2007; MoA, 2004-2014). Fruits are a key component of the horticultural sub-sector. Kenya Agricultural Research Institute (KARI) has for the last 20 years introduced commercial mango varieties which are high yielding. Such varieties which include Tommy Atkins, Kensington, Van dyke, Haden and Apple have been widely adopted by farmers and this has led to increase in production levels especially in Eastern, Coast and Central Provinces (Gitonga *et al* 2009). This has seen the area under mango cultivation in Kenya rise from 500 ha in 1970 to approximately 30,000 ha in 2008 (MoA, 2009). Mango production has risen from approximately 254,000 tonnes with a value of Ksh 3.1 billion in 2005, to a production of 448,000 tonnes, with a value of Ksh 6.4 billion in 2008 (HCDA, 2008). Mango crop requires very low investment once they grow making them a better cash crop.

However, the high production has been accompanied by high post-harvest losses of upto 45%, (Gathambiri, 2009) which is attributed to poor utilization. The great diversity of mango fruit varieties permits its use for various purposes and markets. Mango fruits can be processed into various products: juices, jam, jelly, nectar, concentrate and wine. Mangoes can also be used as salad component, salad appetizer, pickles, candied mango pulp, ice cream component, mango scoops or tidbit, mango shake and chutney.

Mangoes processing is done for the following reasons: to decrease post-harvest losses and extend shelf life; create variety and hence widen the market; add value, thereby generating extra income; create new investment and employment opportunities and support local small-scale industry through the demand for equipment required for processing, preservation and packaging (Kenya Industrial Research and Development Institute (KIRDI) 2009).

Therefore, there is need to develop value added products to enhance mango utilization and minimize losses.

1.4 Objectives

1.4.1 Overall objective

To develop a high quality commercializable mango wine by applying scientific and technological approaches aiming at enhancing utilization of mangoes produced in Kenya.

1.4.2 Specific objectives

- 1) To determine physico-chemical properties of different mango varieties as a basis for screening suitability for wine production.
- 2) To optimize fermentation conditions of the juice obtained from the selected mango varieties during mango wine production.
- 3) To characterize chemical properties and safety of the mango wine produced.
- 4) To determine the sensory properties and shelf stability of the wine produced.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The mango (*Mangifera indica*)

Mango is a member of the family Anacardiaceae. The genus *Mangifera* includes 25 species (Mabberly, 1997) with edible fruits such as *Mangifera caesia*, *M. foetida*, *M. odorata* and *M. pajang*, although *M. indica*, the mango, is the only species that is grown commercially on a large scale.

There are two races of mango; one from India and the other from south east Asia. The Indian race is intolerant to humidity, has flushes of bright red new growth that is subject to powdery mildew and anthracnose and bears mono-embryonic fruit of high colour and regular shape (Matsuoka, 2000). The Southeast Asian race is tolerant to excess moisture, has pale green or red new growth and resists powdery mildew. It's polyembryonic fruit is pale green and has an elongated kidney shape (Karihaloo et al., 2003).

The tree is a deep-rooted, evergreen plant which can develop into huge trees, especially on deep soils. The height and shape varies considerably among seedlings and cultivars. Under optimum climatic conditions, the trees are erect and fast growing and the canopy can either be broad and rounded or more upright. Seedling trees can reach more than 20 m in height while grafted ones are usually half that size (Iyer et al., 2009).

The tree is long-lived with some specimens known to be over 150 years old and still producing fruits. The mature leaves are simple, entire, leathery, dark green and glossy;

they are usually pale green or red while young. They are short-pointed, oblong and lanceolate in shape and relatively long and narrow, often measuring more than 30 cm in length and up to 13 cm in width (Salim et al., 2002). New leaves are formed in periodic flushes about two to three times a year. In deep soil the taproot descends to a depth of 20 ft, and the profuse, wide-spreading feeder roots also send down many anchor roots which penetrate for several feet (Matsuoka, 2000).

The fruits can be oval, egg shaped and round depending on the variety with smooth and soft skin. When ripe, the skin is usually a combination of green, red, and yellow depending on the variety (Matsuoka, 2000). The interior flesh is bright orange and soft with a large, flat pit in the middle in all ripe varieties. The fruit has a rich luscious, aromatic flavour and a taste in which sweetness and acidity are pleasantly blended. It contains a high concentration of sugar (16–18% w/v) and many acids with organoleptic properties, and also antioxidants like carotene (Hobson et al, 1993). Sucrose, glucose and fructose are the principal sugars in ripened mango. Small amounts of cellulose, hemicellulose and pectin are also present (Reddy, 2005). Figure 1 depicts the various common mango varieties locally available in Kenya.



(a) Apple

(b) Ngowe

(c) Kent



(d) Tommy Atkins

(e) Sabine

(f) Vandyke

(g) Indigenous

Figure 1 Mango fruits indicating (a) Apple, (b) Ngowe, (c) Kent, (d) Tommy Atkins, (e) Sabine, (f) Vandyke and (g) Indigenous

Mango fruit matures in 100 to 150 days after flowering (Knight, 1997). The fruit will have the best flavor if allowed to ripen on the tree. Ripening fruit turns the characteristic color of the variety and begins to soften to the touch, much like a peach (Knight, 1997). Commercial marketability requires 13% dissolved solids (sugars). The fruit ripens best if placed stem end down in trays to prevent the sap from spreading to other parts of the fruit and also to encourage even ripening at room temperature (20-25°C) and covered with a dampened cloth to avoid shriveling (Griesbach., 1992).

2.2 Global mango production and trade

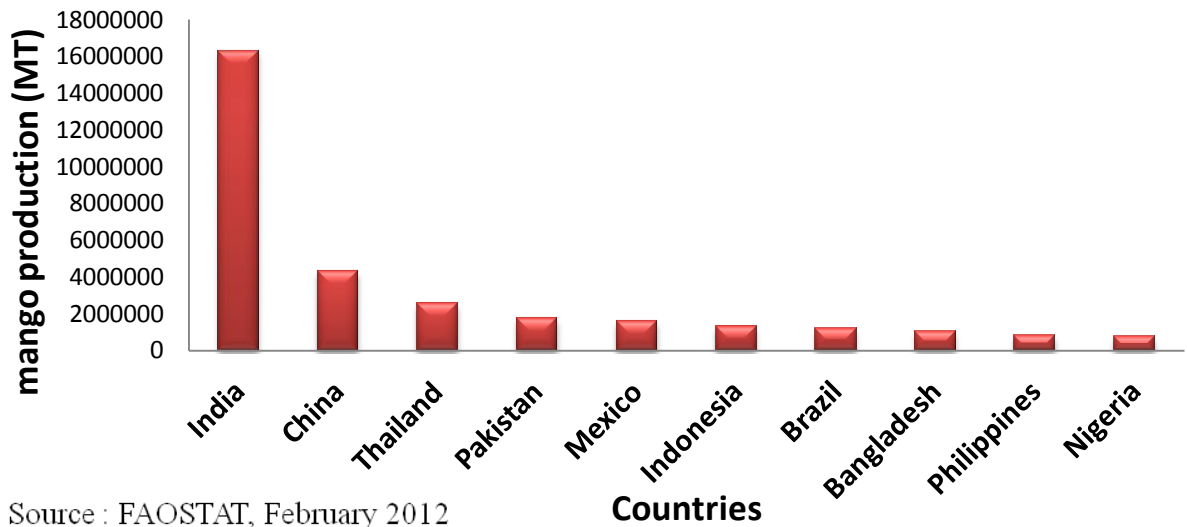


Figure 2 Principal mango producing countries in 2010 (MT)

Within international trade, fresh mango is one of the main products. It occupies the fifth place on total fruit crop production globally (Tharanathan et al., 2006), accounting for over one-third of the worldwide production on tropical fruits (Maneeapun & Yunchalad, 2004). Mangoes are grown in all continents (Galán Saúco., 2004) with Asia where its native accounting for approximately 77% of global production while America and Africa account for approximately 13% and 9% respectively (FAOSTAT, 2007). As shown in figure 2, the Asian continent, India, where the mango is considered the king of fruits, is the main global producer with 13 million to 17 million MT, followed by China (>4 million MT), Thailand (2.5 million MT), and Pakistan (1.7 million MT). In America, Mexico (1.5 million MT) and Brazil (1.2 million MT) are placed 5th and 7th respectively in the world rankings. The main three African mango producing countries

are Nigeria (795,000MT), Kenya (636,585MT) and Egypt (598,084MT) (FAOSTAT, 2012).

Global production of mango has doubled in thirty years to around 35 million (MT) in 2009 (FAOSTAT 2012). Mexico, Pakistan and the Philippines are the most important exporters for fresh mangoes with 41%, 7.6% and 7.8% of the global supply respectively (Galán Saúco., 2002; 2004).

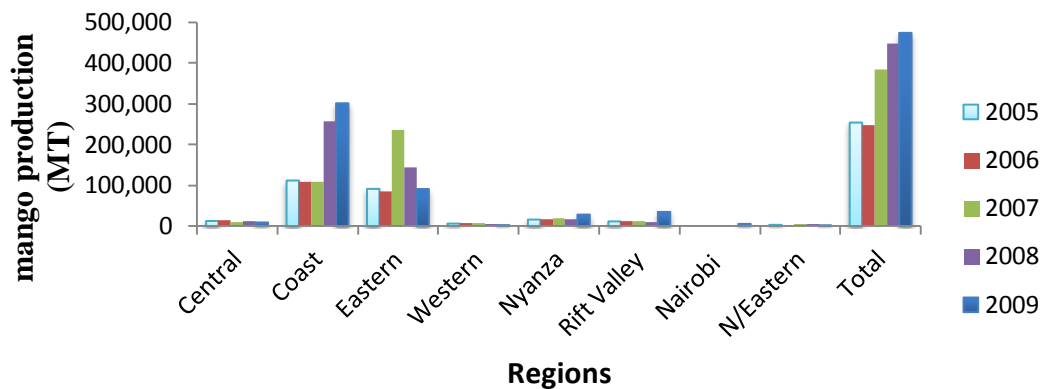
International trade in mango has risen significantly by the end of the twentieth century (Galán Saúco, 2004) which has been enabled by improved post-harvest techniques (Maneepun & Yunchalad, 2004). Large markets for fresh produce are the European Union (EU), North-America and Asia (Galán Saúco., 2002; 2004).

2.3 Mango production in Kenya

Horticulture industry in Kenya is dominated by small holder farmers who constitute 80% of the growers and contribute about 60% to the export market. The industry plays an important role in poverty reduction by providing employment to about 2 million people annually (HCDA, 2006, 2007). Fruits are a key component of the horticultural sub-sector. They generate foreign exchange earnings and provide employment opportunities and income for the rural and peri-urban communities especially women and the youth (MoA, 2004-2014, Vision 2030).

Mango is one of the high potential fruits in Kenya, suitable for different agro-ecological zones ranging from sub-humid to semi-arid (Griesbach., 2003). Generally, mango supply peaks between October and February (FAO, 2005). It is the second most

important fruit in terms of area and production in the following sequence, banana (37.6%), mango (19.6%) and pineapple (12.1%), avocado (9.8%), pawpaw (5.4%), oranges (4.6%), water melon (4.2%) and passion fruit (3.7%) (Annual report HCDA, 2013). In 2007, it was estimated that the area under mango production was 14,387 Ha with an output of 280,884 Mt (Ministry of Agriculture, 2007). In 2008, HCDA reported 250,000 Mt of mango production; however, this number greatly increased to 450,000 Mt in 2010 (Ministry of Agriculture, 2010). This is a clear indication that mango production has tremendously expanded. Figure 3 shows mango production from the eight regions of Kenya where there is an increasing trend in the total production in the respective years with Coast, Eastern and Nyanza regions exhibiting the largest production respectively.



Source: www.hcda.org.ke/statistics/2009

Figure 3 Mango production in metric tonnes (MT) in all the eight regions of Kenya from 2005-2009

In 2011, mango production constituted 19.6% of the total value of fruits produced and 5.6% of the total value of domestic horticulture (HCDA 2012). As illustrated in Table 1, the three leading County producers in terms of value in Kenya shillings are Meru,

Makueni and Kilifi. Kenya Agricultural Research Institute has for the last 20 years introduced commercial mango varieties which are high yielding resulting to increase in production in various counties.

Table 1: Production of mangoes in selected Counties, 2009- 2011

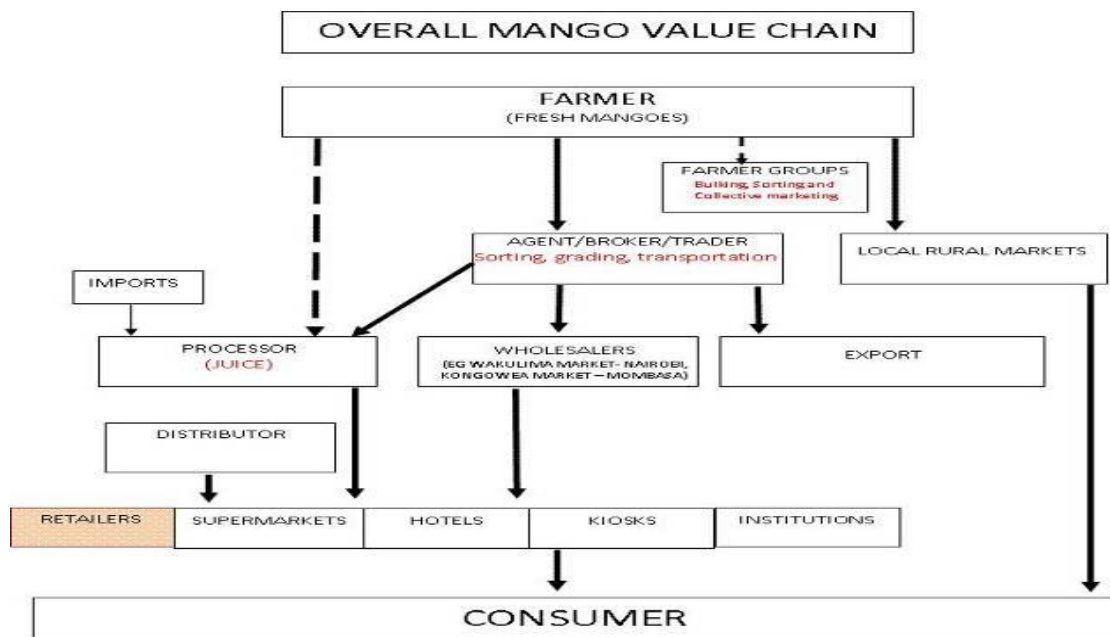
County	2009			2010			2011		
	Area-Ha	Qty (MT)	Value (kshs. Million)	Area-Ha	Qty (MT)	Value (kshs. Million)	Area-Ha	Qty (MT)	Value (kshs. Million)
Makueni	2,777	38,377	695.71	8,951	74,792	2,091.24	6,746	60,496	2,274.36
Meru	4,526	70,883	3,153.70	3,349	51,751	2,085.84	7,176	58,603	2,164.95
Kilifi	7,670	112,302	1,123.02	8,044	144,438	1,444.38	8,429	144,985	1449.85
Kwale	6,807	109,990	1,099.90	6,841	140,485	1,404.85	6,855	129,525	1,295.25
Tharaka Nithi	1,361	6,281	119.74	4,797	12,749	283.00	13,587	44,224	1,226.68
Embu	1,389	18,838	228.18	3,553	23,488	447.40	3,744	39,735	758.20
Machakos	2,537	14,115	262.40	3,108	15,639	410.93	4,078	35,345	457.90
Kitui	625	5,699	125.56	1,108	8,983	196.94	1,265	11,940	245.21
Garissa	353	3,970	24.32	420	4,020	26.74	445	4,766	130.52
Mombasa	321	5,049	50.49	238	1,470	71.46	243	4,249	43.2
Kirinyanga	160	3,815	102.80	161	3,742	92.89	161	3,891	94.62
Kisumu	149	1,989	63.40	195	3,113	89.88	210	2,750	73.45

Data source: HCDA Validated Report 2012

Griesbach (2003), although cited 31 mango cultivars available in Kenya, he classified only seven varieties (Haden, Kent, Maya, Ngowe, Sabine, Sensation, Vandyke) as important. In general, mango varieties grown in Kenya include, Tommy Atkins, Van Dyke, Keiit, Apple, Mathias, Kensington, Parvin, Azacus, Zill, Nimrod, Irwin, Haden, Ngowe, Boribo, Batawi, Dodo, Sabre, Peach, Sensation, Maya and Sabine. Six of these varieties were selected for this study.

2.3.1 Level of post harvest losses of mango fruits in Kenya

Despite this production increase, its potential in Kenya has not been fully exploited due to constraints such as diseases and insect pests, poor agronomic practices, weak marketing structure and glut during peak season (MoA, 2006). Mango production in 2006 was 163,726 tons valued at approximately KES 1Billion (MoA, 2006; HCDA 2006). Production is mainly carried out by smallholder farmers who depend largely on brokers, export agents or local traders for market information. This kind of outlet is generally unstable and offers them low and unpredictable prices. Figure 4 illustrates the flow of mangoes and their processed products from the farmer to various outlets.



Source: PSDA, 2010

Figure 4 The mango value chain in Kenya.

The farmers also lack ways of ensuring year-round income for instance minimizing post harvest losses and by employing appropriate up-to-date village-level preservation and

processing technologies. Most of these mangoes produced are consumed within the same production area or sold in local urban markets. This causes high wastage due to surplus in the market and perishability of the mango fruits.



Figure 5 Mango fruits (Ngowe variety) in Juja open air market

Figure 6 show that 51.9% of the losses that occur are due to pest and diseases while poor postharvest handling, small size, immaturity, mechanical damage, over ripeness, senescences spots caused 36.4% and poor markets (11.7%). These results are as reported by KARI (1994) where causes of postharvest losses of fruits in Kenya are cited. During harvesting, 55.8% of the farmers hand pick the mangoes and 11.7% shake the tree. The rest harvest using hooks and secateurs however, they do not treat them to control disease infections. This explains postharvest losses which occur due to the harvesting methods used resulting to mechanical damage of the fruit thus lowering their quality and shelf-

life. Most of the farmers sell their mangoes once they are ready, some of them consume them at home (3.9%), making jam (1.3%), dried mango crisps (2.6%) and wine making (1.3%). Some of the limitations that farmers encounter during mango processing include lack of equipment, (44.3%) inadequate agro processing knowledge, (30.4%) seasonal variations, (12.6%) and lack of proper packaging materials (10%) (Gathambiri, 2009).

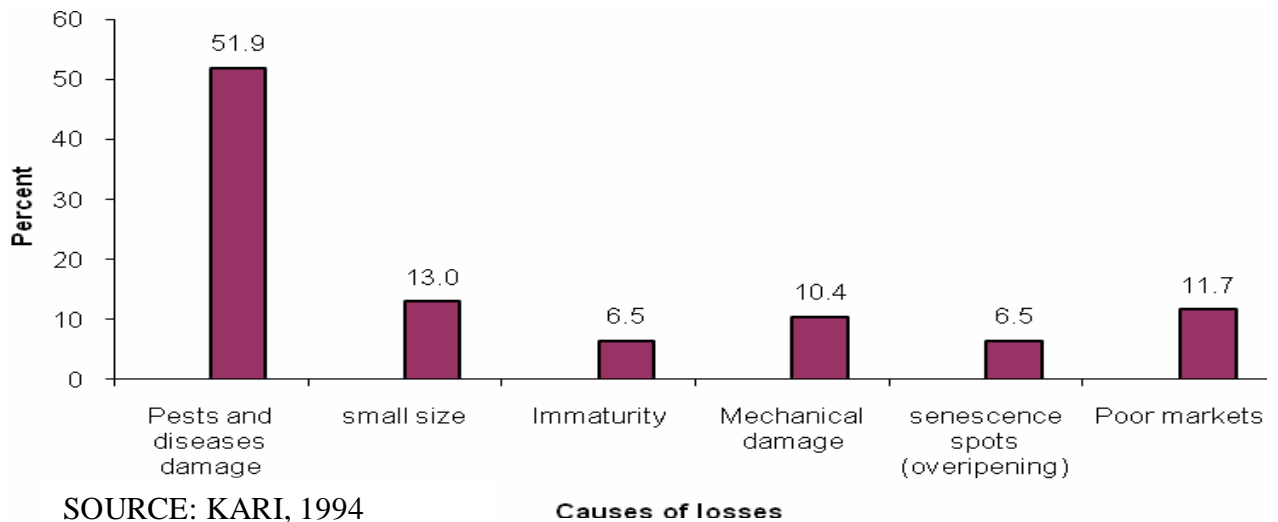


Figure 6 Causes of postharvest losses of mango fruits in Kenya

2.4 Post harvest value addition

Post harvest value addition is a process which includes primary, secondary and tertiary processing operations performed on farm produce to increase its shelf life and increase its value. Food processing increases the value of crops to farmers thus yielding higher returns, expand marketing opportunities, improve shelf-life and furthermore overcome seasonal and perishability constraints (Bachmann, 2001). Value addition involves

adoption of improved and validated processing technologies; to provide longer shelf life, maintain/improve quality and enhance form, space and time utility of the produce to reduce the problems experienced in fresh produce markets such as lack of information and market integration, reliance on spot markets, transport constraints and wastage (Abe et al., 1997). By processing the products, producers have an alternative and additional means of marketing their produce and increasing their incomes.

Mangoes are a highly perishable tropical fruit, with a shelf life of 2 to 4 weeks at 10°C to 15°C (Yahia., 1998) limiting their availability in fresh markets. The shelf stability and the physical and chemical qualities of mangoes can be threatened by pests and insects, chilling temperatures, and fungal rot, as these conditions may induce abiotic or biotic stress to the fruit (Cisneros-Zevallos, 2003).

2.4.1 Mango processing

The great diversity of mango fruit types permits its use for various purposes and markets. Mango processing entails the transformation of mango fruits into different semi-finished and or ready-to use products. Such products include: juices, jam, jelly, nectar, concentrate and wine. Mangoes can also be used as salad component, a salad appetizer, pickles, candied mango pulp, ice cream component, mango scoops or tidbit, mango shake and chutney.

Mangoes processing is done for the following reasons:

- To decrease post-harvest losses and extend shelf life
- To create variety and hence widen the market
- To add value, thereby increasing income generation options

- To create new investment and employment opportunities
- To improve the nutritional quality of mangoes e.g. through pickling
- To support local small-scale industry through the demand for equipment required for processing, preservation and packaging (KIRDI, 2009).

In Kenya, mango juice is the most common product. The fruit is first pulped into concentrate and then made into mango juice (Njiraini, 2001). Processors include Kevian Kenya Ltd, located in Thika, which sells juice under the trade name of “Pick and Peel” and Sunny Processors (Ruiru) which produce and export mainly pulp (Sunny Processors, 2010) and others such as Milly (Mombasa) and Truefoods (Nairobi). Some processors, e.g. Del Monte (Thika) import the concentrate from outside Kenya and then convert into juice for the Kenyan market. The juices are packaged in Tetra Pak carton packages and plastic bottles and sold locally (Njiraini, 2001).

However some programmes have begun funding and sensitizing farmers on the need of value addition. GTZ/PSDA is supporting low-income mango producers in mango producing areas to improve the quality of mangoes they produce, reduce postharvest losses, access new and more reliable markets and ultimately increase their incomes from increased mango sales.

In principle, therefore, there is potential for increasing the processing of mangoes into products of high value and long-shelf life as a way of mitigating against losses due to gluts in production and at the same time diversifying utilization and markets. In countries like Philippine and India, products have been developed from mango fruits for

instance, mango pickles, dried mango, frozen mango and mango pulp as shown in figure 7.



(a) Mango pickles



(b) Mango pulp



(c) Frozen mango

Source: Griesbach, 1992



(d) Slices of dried Mango

Figure 7 Value added mango products including (a) mango pickles, (b) mango pulp, (c) frozen mango, (d) slices of dried mango

Despite this great diversity in mango products, in Kenya most are consumed fresh or channeled towards production of only juices and concentrates. One of the alternatives and profitable methods of using mangoes would be in mango wine production. More so, in contrast to most foods and beverages that spoil quickly or that can spread diseases, wine does not spoil if stored properly. The alcohol in wine, ethanol, is present in sufficient concentration to kill pathogenic microorganism, which makes wine to be considered safer to drink than water and milk (Bisson and Butzkc, 2007).

In Kenya, over the last six years, wine consumption in restaurants and at corporate and other functions has risen (Country report, 2013). This has been largely attributable to growing demand from the middle classes. Their higher disposable incomes along with company advertising have led to the greater appreciation of wine and consequently the proliferation of wine brands within the market. This trend has continued as wine is continually perceived as a symbol of refined taste and status. The wine industry is inextricably linked to the tourism industry in Kenya. Kenyan tourism has made great strides in recent years, particularly driven by domestic tourism. This has led to many international hoteliers opening outlets within the country (Country report, 2013). The country has a potential of consuming 6.2 million litres annually, with an average revenue of about Kshs 67 billion (Country report, 2013). This bodes well for local wine suppliers who anticipate continued healthy category growth over the period.

2.5 Wine production from mango pulp

Wine production and consumption is popular all over the world and is one of the ancient practices. Historians believe that wine was being made in Caucasus and Mesopotamia as early as 6000 BC (Robinson, 1994). Although grapes are the main raw material used for wine production, there is an increasing interest in the search for other fruits, such as apricot, apple and palm sap, suitable for wine making. In countries where grapes are not abundantly available, local fruits that are cheap and readily available are used as an alternative (Onkarayya 1986; Reddy 2005).

Wine production from mango pulp was first reported in 1963 (Anonymous, 1963). In 1966, Czyhrinciwk reported the technology involved in mango wine production. However, Kulkarni et al. (1980), Onkarayya and Singh (1984), and Onkarayya (1985, 1986) screened some varieties of mango for wine making and found that mango wine had similar characteristics to that of grape wine. Their work was inadequate, particularly in giving details on the effects of temperature and yeast inoculum on the chemical characteristics of mango wine.

2.5.1 Attributes of mango fruit quality for wine production

The suitability of mango variety for wine production is generally screened on the basis of juice quality. This has not been reported in Kenya. However, it has been done in India. Fully ripe mangoes contain three types of sugars: glucose, fructose, and sucrose and 70% to 80% of water by weight. As shown in Table 2 (Reddy and Reddy 2005), the total soluble solid of mango juice was between 14.2% and 20.5%. The sugar content of mango juice ranged from 15% to 18.5% (w/v), whereas the titratable acidity as malic acid ranged from 0.31% to 0.47% (w/v). The pH of the juices was between 3.8 and 4.5.

Table 2: Physicochemical characteristics of (a) juices and (b) wines produced from different mango varieties.

Variety	Sugars % (w/v)	Titration acidity % (TA)	pH	TSS %	
(a) Juices					
A. Banaesha	18	0.320	4.5	20.1	
Alphonso	16.3	0.350	4.1	16	
Banglora	16.0	0.310	4.2	16.5	
Banginapalli	18.5	0.326	4.0	20.5	
Neelum	15.0	0.471	3.8	15.5	
Raspuri	15.5	0.430	3.9	14.2	
(b) Wines					
Variety	Alcohol % (w/v)	Titration acidity%	Volatile acidity%	pH	Residual °Bx
A. Baneshan	8	0.610	0.0100	4.0	2.0
Alphonso	7.5	0.650	0.0100	3.8	2.1
Banglora	7	0.622	0.0201	4.0	2.0
Banginapalli	8.5	0.600	0.0108	3.7	2.0
Neelum	6.5	0.826	0.234	3.6	2.5
Raspuri	7	0.735	0.210	3.8	2.4

Source: Reddy and Reddy (2005)

Generally, fructose represents approximately 20% to 30% of total sugars during mango fruit development and may be considered as a storage sugar. Moreover, fructose sweetness is more pronounced than the other sugars, and it may thus account for a significant part of the sweet taste of mango. The pH increases during the maturation and ripening of the fruit. According to this, at low pH values, such as during the early stages of development, pH increase result in a decrease in malic acid content, whereas at higher pH values in the final stages of fruit development in mango flesh (pH from 3 to 4.5 during fruit growth), the pH results increase in malic acid content. Citric acid is synthesized when fruit growth becomes slower and when energy demand is low (Selvaraj and Kumar 1994). At maturation, however, malic acid concentration is linked to total cation concentrations of ripe fruit.

2.5.2 Wine fermentation

Wine fermentation is a process where wine yeasts, belonging mainly to *Saccharomyces cerevisiae* species, catalyze the conversion of sugars (from fruit juice) into ethanol, carbon dioxide and minor metabolites that will give flavor to the final product (Fleet and Heard, 1993). Virtually all alcoholic beverages are produced using different species of *Saccharomyces*. *Saccharomyces* spp. are generally used because they are comparatively efficient in alcohol production and can tolerate higher levels of ethanol than other fungi (Fleet and Heard, 1993). Wine fermentation is either performed conventionally without inoculation or by the addition of selected wine yeast into grape juice in stainless steel tanks, which is common with many white wines like Riesling, in an open wooden vat, inside a wine barrel and inside the wine bottle itself as in the production of many

sparkling wines (Fleet & Heard, 1993; Couto et al., 2005; Vilanova et al., 2005; Gadre-Cerdan & Ancin-Azpilicueta, 2006). Batch fermentation is preferable by inoculating the actively growing yeast into must contained in fermentors or conical flasks fitted with a rubber cork fixed with a bent glass tube for CO₂ release under stationary conditions for a period of 20 days. Wine production involves several stages.

Wine can be categorized as sweet or dry depending on the conditions during alcoholic fermentation. The subjective sweetness of a wine is determined by the interaction of several factors, including the amount of sugar in the wine, but also the relative levels of alcohol, acids and tannins (Johnson, 1989). In general, sugars and alcohol enhance a wine's sweetness while acids and tannins counteract it leading to a dry wine (Peynaud, 1987). Dry wines have less residual sugars relative to sweet wines although they still contain some traces of unfermentable sugars such as pentose (Johnson, 1989). Because the sugar in a dry wine has been allowed to ferment along with the yeast, it tends to have higher alcohol content than sweet wine. Dry wines are popularly referred to as table wines because they are taken with most foods. One of the fundamental methods of differentiating dry wine from sweet wine is by determining the residual sugar. Residual sugar typically refers to the sugar remaining after fermentation stops, or is stopping, but it can also result from the addition of unfermented must (a technique practiced in Germany and known as Süssreserve) or ordinary table sugar (Rapp 1988; Nobel 1994). Residual sugar is usually measured in grams of sugar per litre of wine, often abbreviated to g/l or g/L. Even among the driest wines, it is rare to find wines with a level of less

than 1 g/L, due to the unfermentability of certain types of sugars, such as pentose (Verzera et al. 2008). By contrast, any wine with over 45 g/L would be considered sweet, though many of the great sweet wines have levels much higher than this. For example, the great vintages of Château d'Yquem contain between 100 and 150 g/L of residual sugar. The sweetest form of the Tokaji, the Eszencia – contains over 450 g/L, with exceptional vintages registering 900 g/L. A sweet wine such as a Vouvray can actually taste dry due to the high level of acidity. A dry wine can taste sweet if the alcohol level is elevated (Verzera et al. 2008). Medium and sweet wines have a perception among many consumers of being of lower quality than dry wines. However, many of the world's great wines, such as those from Sauternes (including Barsac) or Tokaj, have a high level of residual sugar, which is carefully balanced with additional acidity to produce a harmonious result.

Fermentation conditions highly affect the quality of wine. It is known that fermentation conditions like temperature and pH will have great impact on the aroma quality of wine (Gerbaux et al., 2002). Many investigators reported the modified temperature on wine fermentation (Killian & Ough, 1979; Renolds et al., 2001; Gerbaux et al., 2002). Increasing fermentation temperature from 15°C to 30°C tends to increase black currant flavour, reduce herbaceous aroma and increase wine colour (Renolds et al., 2001). At low temperatures, alcohol yield was found to be higher while secondary metabolites to alcoholic fermentation increased as the temperature increased (Torija et al., 2003). The variation in alcohol levels could be due to the difference in their optimal physico-

chemical conditions. Temperature has a positive influence on fermentation—to some extent, temperature increases the yeast growth, the speed of enzyme action and the cell sensitivity to the toxic effect of alcohol, which increases with temperature because of increased membrane fluidity (Renolds et al., 2001). This may partially explain the rapid decline in yeast viability at temperatures higher than 20°C during wine fermentation (Torija et al. 2002). On the other hand, fermentations conducted in excessively acidic media began too slow due to the low growth rate of the yeast. In another study, the fermentation rate was improved with higher amounts of yeast, but ethanol production was not affected (Erten et al. 2006). Kumar et al. (2009) optimized the fermentation conditions, temperature, pH, and inoculum size using the response surface methodology. Reddy and Reddy (2010) investigated the effect of fermentation conditions such as pH, sulfur dioxide (SO₂), and aeration on mango wine composition and yeast growth and found out that they greatly influenced the quality of wine.

Differences in composition between mango varieties have been previously reported which may affect wine production. Of interest to the winemaker are the major fruit components: sugar, organic acids, aroma and flavor compounds, phenolic compounds/tannins, certain amino acids, and certain metallic compounds such as potassium (Torija et al., 2002). Flavor is a combination of taste and aroma, and is of particular importance in determining food preferences.

2.5.3 Characterization of mango wine

As shown in table 3, mango wine has been characterized in terms of ethanol concentration, higher alcohols and volatiles present (Reddy et al., 2009; Varakumar et al., 2010). Reddy (2005) reported the composition of mango wines produced from three different cultivars. He suggested that the concentration of ethanol, organic acids, tannins, and aromatic volatile compounds produced differed with each mango variety.

Ethanol is the principal metabolite produced during wine fermentation. From the fermentation of mango juice, ethanol is produced in highest concentration than other metabolites. In general, the concentration of ethanol contributes to the whole characteristic quality and flavor of the produced wine (Bauer and Pretorius., 2000). The percentage of ethanol produced in mango wines is between 7% and 8.5% (w/v), comparable with moderate grape wines (Reddy et al., 2009). Another parameter, which highly influences the quality of wine, is acidity. The main organic acid present in mango musts and produced wine is malic acid; the other acids were less than 1 g/L (Erten, et al., 2006).

Mango fruit is known to have good aroma and flavor. The flavor of mango fruit is due to the volatile components that are present. Volatile compounds in the wine are responsible for aroma because they have greater vapor pressure (Bauer and Pretorius., 2000). These compounds include several higher alcohols and their esters that are synthesized during fruit ripening, juice fermentation or later, during storage of the wine in wooden casks or

in bottles (Reddy et al., 2009; Varakumar et al., 2010). Among all flavor compounds present in wine, higher alcohols occupies first place on quantity basis and also influence certain sensory characteristics of wine. Higher alcohols are synthesized during fermentation from oxo-acids that are derived as by-products from amino acid and glucose metabolism (Mangas et al. 1994).

The flavor of wine depends on many varietal or fermentative compounds, which are present in highly variable amounts and are mainly alcohols, esters, terpenes, sulfur compounds, acids, and lactones (Rapp 1988; Nobel 1994). Volatile aroma compounds are present in fruit juices, and many are synthesized by wine yeast during wine fermentation. As far as consumers are concerned, the aroma and the flavor of wine are among the main characteristics that determine its quality and value (Mauricio et al. 1997; Swiegers et al. 2005; Molina et al. 2007). The aroma complexity dramatically increases during alcoholic fermentation because of the synthesis of important volatile compounds by the wine yeast and the release of some varietal aroma precursors (Mauricio et al. 1997; Swiegers et al. 2005). The nature and the amount of the synthesized volatile compounds depend on multiple factors, such as the nitrogen content of the must, the temperature of fermentation, and the yeast strain (Lambrechts and Pretorius 2000; Swiegers et al., 2006). The volatile compounds synthesized by wine yeasts include higher alcohols (fusel, marzipan, and floral aromas), medium and long-chain volatile acids (fatty, cheesy, and sweaty aromas), acetate esters and ethyl esters

(fruity and floral aromas), and aldehydes (buttery, fruity, and nutty aromas), among others (Stashenko et al., 1992).

Table 3: Composition of volatiles from three different mango varieties

Compound name	Banginapalli (mg/L)	Alphonso (mg/L)	Totapuri (mg/L)	Source: Reddy and Reddy (2009)
Alcohol				ND: not detected
Ethanol	8.5	7.5	7	
1-Propanol	54.11	42.32	47.13	
Isobutyl alcohol	102.40	115.14	98.87	
Isoamyl alcohol	125.2	108.40	140.44	
Pentan-2-one	1.43	1.15	1.51	
Hexane-1-ol	1.42	1.02	ND	
Phenethyl alcohol	22.15	24.15	20.48	
Ester				
Ethyl acetate	35.15	30.42	27.48	
Ethyl hexanoate	0.942	0.671	0.552	
Ethyl decanoate	2.34	1.86	1.43	
Acid				
Acetic acid	0.201	0.163	0.155	
Propanoic acid	0.145	0.217	0.184	
Butanoic acid	0.932	0.745	0.874	
Benzoic acid	1.08	1.21	1.43	

2.5.4 Effect of storage bottle colour and temperature on wine colour

The browning of bottled wines during storage has been reported by several researchers (Fabios et al., 2000; Selli et al., 2002; Benítez et al., 2003). The effect of bottle colour and storage conditions on mango juice chemical attributes have been reported by Alaka et al., 2003. According to these authors, glass bottles will give greater protection against the degradation of the chemical attributes of the mango juices. Packaging Ogbomoso mango juices in glass bottles and storing them at 6°C will give good protection against phytochemical of mango juices. Reddy (2005) examined the change in the colour of the mango wine at 8°C, 16°C, and 25°C stored in white, green, and brown storage on the basis of the browning index. Wine stored in darker brown bottles at low temperature showed low browning indices when compared with wine stored in clear bottles suggesting that wines should be kept in dark bottles to prevent browning.

The contents of ascorbic acid and sugars together with storage temperatures, sunlight, and colour of bottles affect the browning in orange juice and orange wines (Moll., 1999). Sunlight can cause undesired changes in wines. Therefore, in winemaking, the use of bottles that will prevent the transmission of shortwave lights such as ultraviolet, is recommended because shortwave lights have high energy and can initiate some chemical reactions (Boulton et al. 1996). In the brewing industry, brown bottles are commonly used to prevent the transmission of lights with a wavelength between 350 and 550 nm and the formation of undesired aroma compounds (Moll 1999).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study design

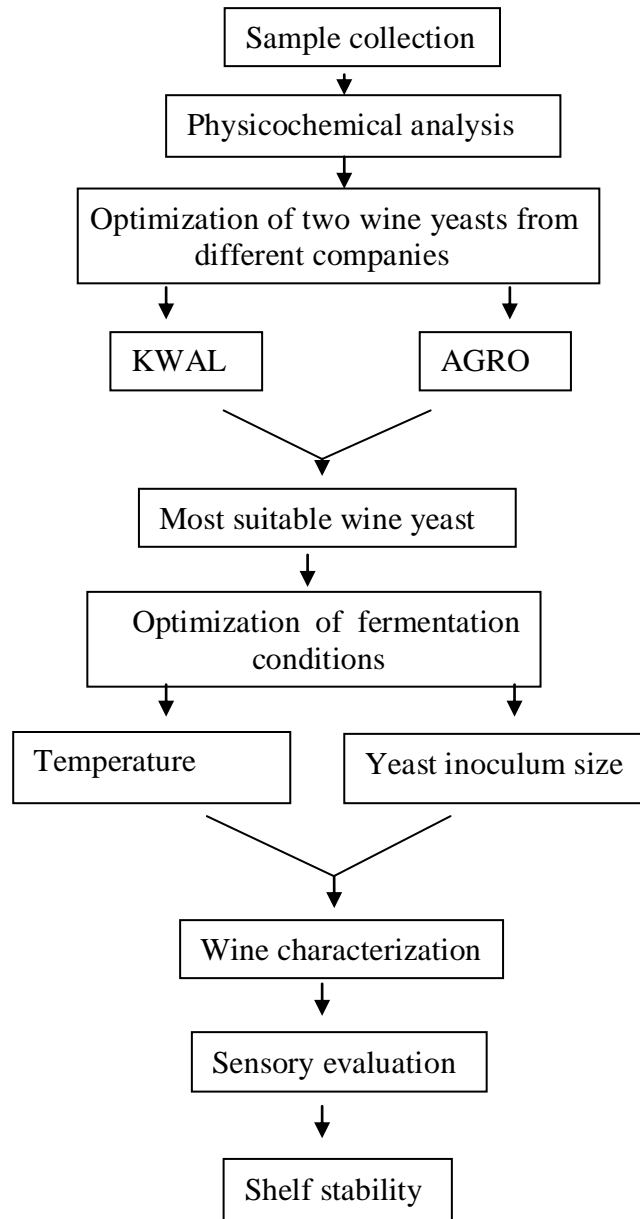


Figure 8 Flow chart diagram showing the study design

Six mature and unripe mango fruits of Apple, Ngowe, Tommy Atkins, Kent, Vandyke and Sabine varieties were harvested three times from a farm in Katheka Kai division, Machakos County of Kenya. They were then packed in crates and transported to Jomo Kenyatta University of Agriculture and Technology, Department of Food Science and Technology. The fruits were then washed with tap water and a detergent (easy foam) and stored in a cold room (controller, MCU-2260C-S, Sanyo Electric Co., Ltd. Japan) at 20°C and 85-90% relative humidity to ripen. No pretreatment of the fruits was done prior to ripening. Ripeness was determined by firmness using a rheometer (Model CR-100D, Sun Scientific Co. Ltd Japan) after 7-10 days.

The physico-chemical properties determined included juice yield, °Bx, pH, reducing sugars and titratable acidity. For optimal wine production, fermentation temperature was varied at 20°C, 25°C, 30°C and 35°C and yeast inoculum at 0.0065%, 0.01%, 0.05% and 0.1%. Wine yeasts (*Saccharomyces cerevisiae*) obtained from KWAL and Agrochemical Industries was used. The wine produced was analyzed for alcohol content, pH, residual °Bx, volatile acidity and titratable acidity. Characterization of the major volatile compounds, 1- propanol, isobutyl alcohol, phenethyl alcohol, methanol, acetaldehyde, ethyl acetate and acetic acid, found in wines were determined by GC-FID. Shelf life studies of the wine were carried by monitoring the browning index and volatile acidity respectively. Sensory evaluation was done using a nine point hedonic scale based on colour, mouthfeel, aroma, clarity, and general acceptability.

3.2 Extraction of pulp

Ripened mango fruits of the six varieties were sorted, washed and peeled manually using a knife.

Ripeness was indicated by a rapid decline in fruit firmness using a rheometer (Model CR-100D, Sun Scientific Co. Ltd Japan). The flesh was cut away from the seed using a knife and then homogenized using a pulp extractor. Juice was obtained by passing the pulp through a muslin cloth. Juice obtained in this manner was then subjected to physicochemical analysis.

3.3 Analysis of physicochemical properties

3.3.1 Determination of juice recovery

Three mango fruit samples from each variety were weighed and the juice extracted measured. The percentage juice recovery was calculated as:

$$(\%) \text{ Juice recovery} = \frac{\text{juice yield (ml)}}{\text{weight of sample (g)}} \times 100 \dots\dots\dots \text{Equation 1}$$

3.3.2 Determination of simple sugars

Quantification of reducing sugars present was determined using High Performance Liquid chromatography (HPLC) method as outlined in AOAC (1996). A sample of 10 g each of fruit pulp was refluxed in 96% ethanol for 1 hour. The extract was filtered using cotton wool and concentrated by rotary evaporator. This was then diluted with 75% acetonitrile in the ratio of 1:1. Standard solutions of sucrose, fructose and glucose were prepared at varying concentrations, 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml and injected

together with the sample extracts into HPLC (LC- 10AS, Shimadzu Corp., Kyoto, Japan) fitted with Refractive Index Detector (RID) having the following conditions: oven 35°C, flow rate: 0.5-1.0 ml/min, injection volume – 20 µl, column- (NH₂P-50 E). The standard curves were drawn and used to quantify the reducing sugars of the samples. (See plates 1, 2 and 3).

3.3.3 Determination of pH

pH determination was done by the method of Ofori and Hahn (1994). The pH meter (TOA pH Meter HM-7B, Tokyo, Japan) was calibrated at 25°C using buffer solutions of acidic and basic values of 4.01 and 8.06 respectively.

3.3.4 Determination of total soluble solids (TSS)

The TSS were determined as total sugars in the juice using an Atago hand refractometer (RX 5000, Atago, Tokyo, Japan). One ml of a well homogenized mango pulp was placed at the screen of a calibrated hand refractometer. The readings were taken and results expressed in °Bx.

3.3.5 Determination of total titratable acidity (TTA)

Total titratable acidity analysis was done using AOAC, 1995 method. Approximately 10 ml of homogenized sample was pipetted into a 100ml conical flask and 2 drops of 1% phenolphthalein indicator used. Titration was done using 0.1N NaOH to a persistent faint pink colour compared against a white background. The titre volume was noted and used for calculations of TTA which was expressed as percentage malic acid which is the

main organic acid in mango fruit (Ueda et al, 2000). Calculations of TTA was determined and expressed as follows;

$$\% \text{ malic acid} = A \times 0.009 \times \frac{100}{V} \dots\dots\dots \text{Equation 2}$$

Where: A = ml of 0.1 NaOH required for the titration

V = ml of sample taken for the test. 0.009 is a Constant`

3.4 Optimization of wine fermentation

3.4.1 Preparation of juice

Mango variety with the most suitable physicochemical characteristics for wine production was selected for this study. After extraction, the juice was pasteurized in a stainless steel container at 65°C for 10 minutes and cooled immediately with cold tap running water to 27±2°C. The pH of the mango juice was adjusted to 4.5 by addition of CaCO₃ (food grade) and C₆H₈O₇ (food grade) respectively. The mango juice was not ameliorated with fermentable sugars prior to fermentation.

3.4.2 Optimization of wine yeast from different manufacturers

Wine yeast was obtained from two different manufacturing companies, Kenya Wine Agencies Limited (KWAL) and Agrochemical Industries and tested for their variability during fermentation. The treated juice was divided into four portions of 500 ml and put in sterile fermentation jars. Two portions were inoculated with wine yeast from KWAL

and the other two inoculated with wine yeast from Agrochemicals Industries and allowed to ferment at 25°C and a pH of 4.5.

3.4.3 Optimization of temperature and yeast inoculum size

Active dried wine yeast obtained from Kenya Wine Agencies Limited (KWAL) was used. For optimal yeast inoculum size determination, the yeast was varied in concentrations of 0.0065% (control), 0.01%, 0.05% and 0.1% levels. Prior to inoculation, the yeast strain was rehydrated by adding it in 200ml of the mango juice at $37\pm 2^{\circ}\text{C}$ for 10 minutes. After 10 minutes, the slurry was allowed to cool and attain the same temperature as of the juice ($27\pm 2^{\circ}\text{C}$) and then poured into the fermentation jars respectively.

For optimal temperature determination, the treated juice was divided into different portions of 500 ml each and put in 1000 ml sterile fermentation jars at varying fermentation temperatures as illustrated in Figure 9. Mango wine production from sample collection to development of a high quality mango wine is also illustrated. (See **plate 4**).

Yeast inoculum sizes (%)				Temperature (°C)
0.0065	0.01	0.05	0.1	
▲	▲	▲	▲	20
▲	▲	▲	▲	
▲	▲	▲	▲	
▲	▲	▲	▲	25
▲	▲	▲	▲	
▲	▲	▲	▲	30
▲	▲	▲	▲	
▲	▲	▲	▲	35
▲	▲	▲	▲	

▲ - Fermentation jars

Figure 9 Fermentation set up at different inoculum sizes and temperatures

The experiments were carried out by incubating the appropriate number of inoculated flasks at different temperatures; 20°C (controller, MCU-2260C-S, Sanyo Electric Co., Ltd. Japan) 25°C, 30°C, and 35°C (Incubator IS62, Yamato Scientific Co., Ltd. Japan) as illustrated in the figure. At the start of fermentation, fermentation jars were shaken intermittently to evolve dissolved CO₂ thus facilitating the fermentation process. The jars were closed using a rubber stopper fitted with a bend tube to release carbon dioxide. Fermentation rate was monitored every 24 hours by checking the changes in TSS (°Bx) while yeast growth was monitored by taking samples every 24 hours for the determination of total yeast count. End of fermentation was determined when the °Bx could not change any further.

After fermentation, the wine samples were centrifuged (Centrifuge Model H-2000C Shimadzu Corp., Kyoto, Japan) at 7,000g for 5 minutes prior to analysis. The clear supernatant samples were stabilized with the addition of 30 mg SO₂/l and kept in air tight glass jars and stored at -8°C (Chester freezer, model: K302) until the physicochemical analyses were completed.

3.4.4 Effects of fermentation temperature and yeast inoculum size on wine yeast growth

During fermentation, samples were taken aseptically for yeast counts after every 24 hours. The yeast counts were determined by use of malt extract agar (MEA) and peptone water (AOAC, 1996). The MEA was prepared by weighing 20 g and dissolving it in 500 ml of distilled water in a 1000 ml conical flask and sterilizing in an autoclave for 30 min at 121°C. The peptone water was prepared by dissolving 1 g in 100 ml distilled water; 9 ml of the solution was then transferred into each test tube appropriately and sterilized in an autoclave for 30 min at 121°C. The samples were cooled to 25°C±2; then 1 ml portion of each was serially diluted in the test tubes containing 9 ml of 0.1% sterile peptone water and mixed well. About 20 ml of the MEA was poured into petri dishes and allowed to set under aseptic conditions. Three dilutions were considered and 0.1 ml of each diluted sample from the three dilutions was spread on the set MEA and incubated at 25°C for 48 hours. Three appropriate plate counts between 20-200 colony range were considered to compute the average count per gram and reported as CFU/ml (See plate 5).

3.4.5 Effects of fermentation temperature and yeast inoculum size on substrate utilization

The fermentation rate of mango wine was determined at varying temperatures and yeast inoculum sizes by calculating °Bx utilization per day using first order kinetics during the fermentation process.

$$\text{Substrate utilization: } -\frac{d[x]}{dt} = k[x] \dots \dots \dots \text{Equation 3}$$

Rearrangement yields the following: $\frac{d[x]}{[x]} = -k dt \dots \dots \dots \text{Equation 4}$

Where x = product

t = time

3.4.6 Effect of fermentation temperature and yeast inoculum size on chemical properties and volatile compounds of mango wine.

The effect of temperature and yeast inoculum size on alcohol content, pH, TTA, residual °Bx and volatile acidity of mango wine was determined. The influence of temperature on acetaldehyde, ethyl acetate, 1-propanol, isobutanol, isoamyl alcohol, phenyl ethanol, acetic acid and methanol as the major volatile compounds was also determined as outlined in 3.5.4.

3.5 Characterization of the mango wine

Characterization of mango wine was based on the two most suitable mango varieties for wine production. The chemical properties analyzed were: alcohol content, pH, TTA, residual °Bx and volatile acidity and the major volatile compounds were: acetaldehyde, ethyl acetate, 1-propanol, isobutanol, isoamyl alcohol, phenethyl alcohol, acetic acid and methanol.

3.5.1 Determination of TTA

This was determined according to Lum Eisenman (1992) where 5 ml of wine was drawn into a pipette and the sample transferred into a flask. About 50 ml of distilled water and 3 drops of 1% phenolphthalein added into the solution. A 10-ml pipette was filled with 0.1 N NaOH solution and titrated while mixing the wine sample by rocking the flask. Titration was stopped when the sample turned to a faint pink. The pipette scale was read and the quantity of NaOH solution used recorded.

TTA Calculations

When a 5-milliliter sample and 0.1N sodium hydroxide are used, titratable acid is calculated using the following formula.

$$TTA (\%) = 0.15 \times ml \text{ of } NaOH \text{ used} \dots\dots\dots \text{Equation 5}$$

3.5.2 Determination of volatile acidity (VA)

This was determined by taking 10ml of the wine sample and collecting 75ml of the distillate in a 250 ml conical flask. The distillate was titrated against 0.1N NaOH in the

presence of 1% phenolphthalein until a pink colour persisted. The amount of NaOH used was noted and used for calculation as described using AOAC (2000) method as follows:

$$VA \text{ in g/l of acetic acid} = (\text{ml NaOH used}) \times (0.06) \dots\dots\dots \text{Equation 6}$$

3.5.3 Determination of Ethanol

Ethanol was determined by putting 100 ml of the sample into a flask and adding 50ml of distilled water and then connected to distillation apparatus AOAC (1995) methods. A portion of 100ml of the distillate was collected under ice. The distillate was filled into a pre-weighed pycnometer (25ml) and cooled to 14°C. The lid of the thermometer was replaced and the pycnometer wiped with a paper towel. The reading of the balance was tarred and the pycnometer weighed at 15.5°C. This measurement was repeated using distilled water. The specific gravity of the distillate was determined using water as the reference at 15.5°C as follows:

$$Sg = \frac{\text{weight of sample}}{\text{weight of an equal volume of water}} \dots\dots\dots \text{Equation 7}$$

Where Sg = specific gravity

Using the conversion tables, the specific gravity value was converted to the corresponding percentage alcohol content.

3.5.4 Determination of higher alcohols and major volatile compounds

Higher alcohols and major volatile compounds were determined according to AOAC (1995) methods. The distillates were prepared by putting 100 ml of the sample into a flask and adding 50ml of distilled water and connecting to distillation apparatus. A

portion of 100ml of the distillate was collected under ice. Standard solutions of known alcohols were prepared and 0.2 µl injected in a Gas Chromatograph fitted with a Flame Ionization Detector (FID) (GC-9A, Shimadzu Corporation, Kyoto, Japan). The conditions were as follows: glass packed column: diethyleneglycol succinate 15% (3m x 3 mm i.d); injector/detector temperature: 220°C and N₂ was the carrier gas with a flow of 20 ml/min. The eluted compounds were detected by FID where the fuel gas was hydrogen with a flow rate of 40 ml/min and the oxidant was air with a flow rate of 40ml/min. Appropriate dilutions were made were the curves were too big. One µl of the distilled samples was injected into the GC-FID under the same conditions as of the known standards. Esters as ethyl acetate and volatile acids were also determined using GC – FID according to established methods (AOAC, 1995). Identification of the sample peaks was done by comparing the retention time of the samples with those of authentic standard compounds injected under the same conditions. The compounds to be identified were preliminarily identified from (Reddy and Reddy, 2005; 2009). All the analyses were determined in triplicate and the mean ± SD calculated.

$$Conc.(ppm) = \frac{\text{peak area of compound}}{\text{total area of peaks}} \times \frac{(\text{alcohol content})}{100} \times \text{density at } 20^{\circ}\text{C} \times 10^6$$

...Equation 8

3.6 Sensory analysis

Mango wine was compared with a commercialized grape wine (Changli Chardonnay wine 2010, Yueqiannian Winemaking Company, Changli County, China) as the reference wine for colour, clarity, mouth feel, aroma and general acceptability. A panel

of 30 untrained panelists was selected randomly in groups of ten. Sensory evaluation was done using a nine point hedonic scale where 9 denoted like extremely and 1; dislike extremely (See **plate 6**). The ratings for the sensory attributes were analyzed as described by (Ihekoronye, et al., 1985).

3.7 Shelf stability and colour determination of the mango wine

Shelf stability was determined by monitoring the volatile acidity (3.5.2) by drawing the sample through a syringe and colour change (3.7.1) of the mango wine after every four months for a period of one year at different storage temperatures.

3.7.1 Effects of storage temperature and different colour bottle on the colour of wine.

Mango wine from the most suitable variety was stored in different bottles; brown, green and clear (See **plate 7**) at temperatures of 10°C, 15°C, 20°C and 25°C for 12 months and the wine colour determined after every 4 months during storage. Wine colour was determined by optical density measurements using a spectrophotometer UV-VIS (UV-1601 PC model, Shimadzu Corp., Kyoto, Japan) by adding absorption values at 520 nm and 420 nm.

3.8 Statistical analysis

Sample collection was done three times and analyses done in duplicates. Data was assessed using Genstat 12th edition by one way analysis of variance and a t-test for two samples analysis. Duncan multiple range test was used to determine significant means. Significance was defined at $p \leq 0.05$.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Screening mango varieties for their suitability for wine production

The results of juice yield and chemical composition of mango juice is presented in Table 4. The fruits of different varieties were found to vary in sugar concentrations and other chemical characteristics.

Kent variety had the highest juice yield whereas Sabine had the lowest. Juice yield is an important parameter in wine production as it reflects on the final quantity of the wine. Fruits with high juice yield are preferred for wine production relative to low yielding fruits for economical purposes.

The main prerequisite character of juice for fermentation is sugar content. The total soluble solids (TSS) of the mango ranged from 17.0 ± 0.12 (Sabine) to $23.9 \pm 0.21^\circ\text{Bx}$ (Apple). For wine production, a minimum of 18.0°Bx is required thus Apple, Ng'owe, Vandyke and Kent were within the acceptable range. The variability in total soluble solids of different cultivars at different stages of ripeness are attributed to the alteration occurring in structure during ripening processes at various hydrolytic processes enzymatic instigated breakdown of complex carbohydrates to smaller ones like sucrose, glucose and fructose (Saeed, et al., 2009; Rathore, et al., 2007)

The simple sugars found in mango fruit are glucose, fructose and sucrose. These were in the range of $16.95 \pm 1.13\%$ (Sabine) to $23.78 \pm 1.24\%$ (Apple). Fructose and glucose are

the products of sucrose hydrolysis, glucose also being produced by starch hydrolysis (Saeed et al., 2010). Fructose sweetness is more pronounced than the other sugars, and thus accounts for a significant part of the sweet taste of mango. (Rathore, et al., 2007). The sugar content of a given fruit is essential for wine production as these sugars are utilized during fermentation to yield alcohol. The higher the sugar content the higher the alcohol yield.

Table 4: Physico-chemical characteristics of mango juice of different mango varieties

Mango variety	Juice yield (%)	°Bx	Simple sugars (% w/v)	pH	Titrateable acidity (%)
Apple	71.34±1.59 ^c	23.90±0.21 ^e	23.78±1.24 ^{bc}	4.25±0.04 ^c	0.46±0.04 ^c
Ng'owe	67.64±5.70 ^{bc}	23.10±0.42 ^d	22.23±0.23 ^c	4.89±0.03 ^f	0.35±0.03 ^a
Tommy Atkins	67.28±0.94 ^{bc}	17.00±0.12 ^a	16.94±1.73 ^a	4.54±0.04 ^d	0.39±0.02 ^b
Vandyke	58.92±7.27 ^{ab}	21.80±0.06 ^c	21.68±0.06 ^b	4.74±0.05 ^e	0.37±0.01 ^a
Kent	72.83±7.04 ^c	18.00±0.15 ^b	17.91±0.15 ^a	4.03±0.03 ^b	0.55±0.03 ^d
Sabine	52.93±4.93 ^a	17.00±0.14 ^a	16.95±1.13 ^a	3.92±0.03 ^a	0.82±0.04 ^e
p value	<0.004	<0.001	<0.001	<0.001	<0.001
d.f	5	5	5	5	5
Cv%	8	0.7	8.2	0.6	3.4
l.s.d	9.26	0.2585	2.963	0.04398	0.02965

Means within the same column sharing the same superscripts were not significantly different ($p>0.05$)

Values are presented as mean \pm SD.

n = 3

The titratable acidity as malic acid ranged from 0.35 (Ng'owe) to 0.82% (Sabine) whereas the pH ranged from 3.92 (Sabine) to 4.89 (Ng'owe). Mango contain significant amount of organic acids. The major ones are tartaric, malic and citric acid. Total titratable acidity refers to the sum of these three organic acids (Amerine, 1950). Of these three, tartaric and malic account for 90% in the juice (Amerine, 1950). Malic acid which is the main acid in ripe mango fruit ((Ueda et al, 2000) is synthesized when fruit growth becomes slower and when energy demand is low (Selvaraj and Kumar 1994). The acceptable range of titratable acidity for wine production according to Amerine, 1950 is 0.3-0.6%.

The pH in the fruit pulp plays an important role in flavor promotion as well as a preservation factor (Saeed et al., 2010). The pH of all the varieties was within the acceptable range for wine production except for Sabine variety. According to Reddy (2005), the optimal pH for juice must to produce wine is 4.00- 4.90. The author further suggested that these pH values offer optimal conditions for yeast proliferation during fermentation.

These results suggest that some varieties of mango juice have a potential for producing quality wine based on its chemical composition. From the six varieties, Apple and Ng'owe exhibited the most suitable properties for wine production and as a result, were selected for the subsequent experiments.

4.2 Optimization of fermentation process for wine production

4.2.1 Effects of wine yeast from different manufacturers on the fermentation rate and chemical properties of mango wine

There was no significant difference ($p>0.05$) on the chemical properties and fermentation rates of the wine yeasts from KWAL and Agrochemicals (table 5). The two types of wine yeasts had similar fermentation characteristics in terms of utilization of sugars, acidity development, alcohol yield and yeast growth. The pH values fluctuated from 4.09 to 4.04 and residual °Bx values were 5.4 for all the mango wines during fermentation (Table 5). Based on these results Agrochemical yeast was used for subsequent experiments together with Apple mango variety.

Table 5: Effects of wine yeast from different sources on the chemical properties of mango wine from Apple variety

Parameters	KWAL	AGRO
Ethanol content (% v/v)	9.46±0.02 ^c	9.44±0.02 ^c
pH	4.09±0.02 ^b	4.04±0.02 ^b
TTA	0.93±0.02 ^a	0.93±0.02 ^a
Residual °Bx	5.4±0.02 ^a	5.4±0.02 ^a
Volatile acidity (g/l as acetic acid)	0.37±0.02 ^a	0.37±0.02 ^a
Fermentation rate (<i>k</i>) °Bx/day	1.16±0.02 ^c	1.18±0.02 ^c

Means within the same row with the same superscript were not significantly different ($p>0.05$)

mean±S.D

n= 3

4.2.2 Effect of temperature on wine yeast growth and substrate utilization during fermentation

As shown in figure 10, the growth in yeast counts varied according to temperature. The growth curve was characterized by a series of short-lag, exponential, stationary and decline phases for all temperatures. Fermentation at 20°C was the slowest. This caused a delay in reaching the maximal population (10^8 cfu /ml), but once it was reached there was a long stationary phase. Fermentations at 25°C and 30°C reached similar maximal populations, although the initial fermentation rates were faster than at 20°C. Fermentations at 35°C had a very short lag phase, but a quick exponential phase, and reached the maximal population first. However, this fermentation proceeded with a very short stationary phase. At 35°C the decline phase began on day seven of fermentation. Yeast cell viability therefore decreased as temperature increased from 35°C.

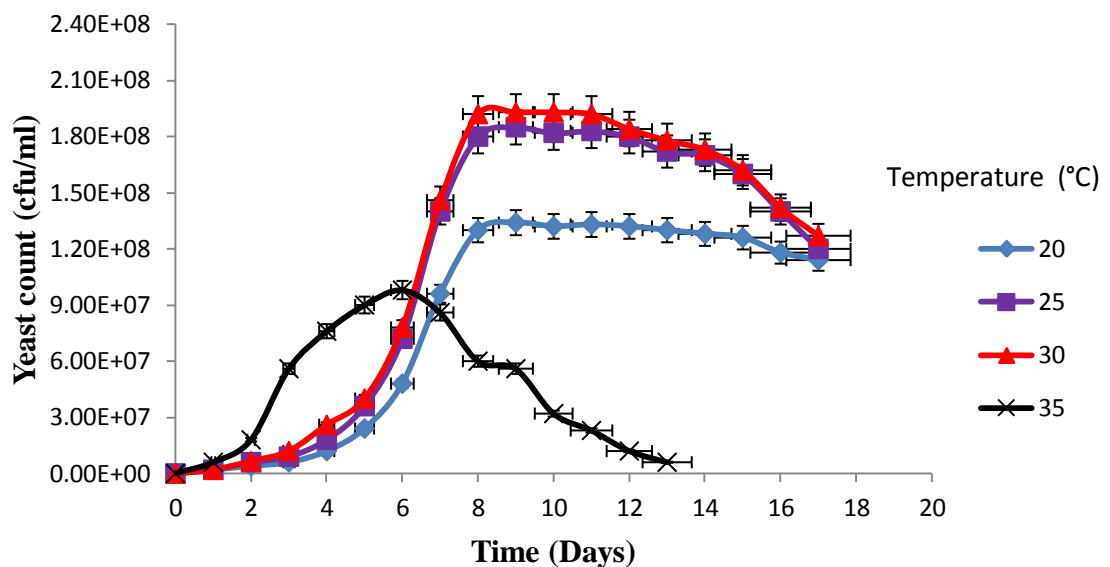
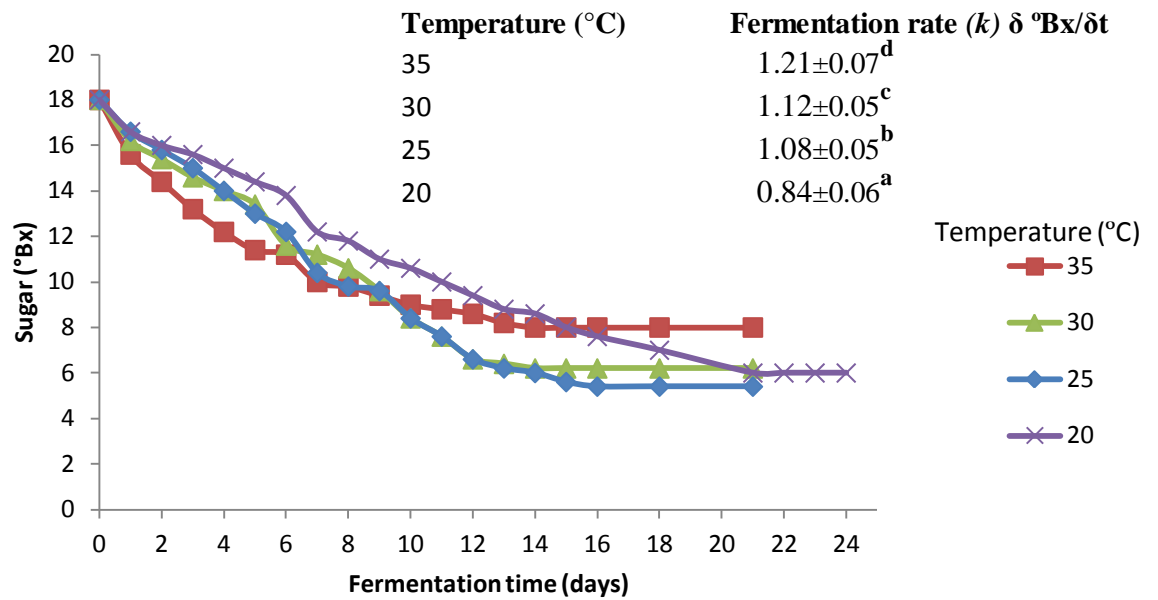


Figure 10 Yeast counts at different temperatures during wine fermentation at 0.05% inoculum size

The increase in the total yeast count during the first few days of fermentation can be attributed to the presence of utilizable sugar and the favorable environment for yeast growth (Berry, 2000). During anaerobic growth the yeast utilizes intermediate products like acetaldehydes as hydrogen acceptors for alcohol production (Okafor, 1987; Prescott et al., 2008).

Fig. 11 shows the effects of temperature on the fermentation kinetics of apple mango wine at 0.05% yeast inoculum size. At 35°C, initial fermentation rate was high although towards the end of fermentation, day 10, the rate decreased leading to high residual sugars. This was because of the decrease in yeast count at this temperature hence fermentation could not be completed. At 30°C and 25°C, fermentation rate was not significantly different ($p>0.05$) although towards the end of fermentation, at 30°C, the residual sugars were higher. Fermentation rate was slowest at 20°C as shown in the figure. At this temperature, the residual sugars were of the same concentration as fermentation at 30°C.



Fermentation rate (k) with the same superscripts were not significantly different ($p > 0.05$)

Figure 11 Effects of temperature on substrate utilization and fermentation kinetics at 0.05% yeast inoculum size

Generally, high temperatures increased the rate of fermentation but the sugars were not completely utilized as shown in figure 11. Temperature affected not only the fermentation kinetics (rate and length of fermentation) but also the yeast metabolism, which determined the chemical composition and in turn the quality of wine. High temperatures increased the enzyme activity during the initial phase therefore, increasing the rate of fermentation. High temperatures, 30°C and 35°C, decrease the stability of enzymes and other biomolecules therefore decreasing the enzyme activity hence the decrease in the use of available sugars (Sevda, 2011). In contrast, low fermentation temperatures, which started more slowly, had the lowest concentration of residual sugars because the high yeast biomass was maintained throughout the process.

4.2.3 Effects of temperature on chemical properties and volatile compounds of mango wine

Table 6: Chemical properties of mango wine at different temperatures at 0.05% *S. cerevisiae* inoculum size

Parameters	20°C	25°C	30°C	35°C
Ethanol content (% v/v)	8.07±1.42 ^c	9.44±1.74 ^d	7.20±1.69 ^b	6.93±1.72 ^a
pH	4.01±0.04 ^b	4.01±0.03 ^b	3.99±0.03 ^b	3.99±0.04 ^b
TTA	0.93±0.23 ^a	0.93±0.22 ^a	0.94±0.24 ^a	0.94±0.22 ^a
Residual °Bx	6.0±0.11 ^b	5.4±0.10 ^a	6.0±0.10 ^b	8.0±0.10 ^c
Volatile acidity (% v/v as acetic acid)	0.37±0.17 ^a	0.39±0.17 ^a	0.48±0.18 ^b	0.51±0.17 ^c
p value	<0.004	<0.001	<0.001	<0.001
d.f	5	5	5	5
Cv%	8	0.7	8.2	0.6
l.s.d	9.26	0.2585	2.963	0.04398

Means within the same row with different superscripts were significantly different ($p < 0.05$)

Values are presented as mean ± SD

n=3

Alcohol yield was highest when fermentation was conducted at 25°C for all the inoculum sizes. At the same temperature, there was maximum conversion of sugars and fermentation took 16 days to utilize the available sugars in the mango juice at 0.05% yeast inoculum size.

Fermentation temperatures of 20°C and 25°C had the highest alcohol yield whereas 30°C and 35°C had the lowest yield. The concentration of alcohol decreased as the temperature increased from 25°C which has been related to a drop in the ethanol yield and a reduced use of substrate (Casey and Ingledew, 1986). This difference in ethanol yield at different temperatures could also be related to biomass production (Majesu's, et al., 2003).

The content of volatile acids, which measures the degree of sourness of the wine, should be as low as possible. About 90% of the volatile acid consists of acetic acid (Yannam, et al, 2009). Volatile acidity increased as the temperature increased although these values were within the range of 0.3 to 0.6% reported for wines (Amerine et al., 1980). From this study, low temperatures, 25°C and 20°C, resulted to low residual sugar which is attributable to these temperatures as suitable for the proliferation of yeast cells during fermentation. There was no significant difference ($p>0.05$) on pH and TTA at different fermentation temperatures.

The effect of temperature on the volatile composition of mango wine is presented in Table 7. All the secondary metabolism products varied with different temperatures but at 35°C, all the compounds experienced a low concentration.

Table 7: Effect of temperature on volatile compounds production (mg/L) of mango wine from Apple variety fermented at 25°C and pH 4.5 for 18 days at 0.05% inoculum size

Metabolite (mg/l)	20°C	25°C	30°C	35°C	P value	d.f	l.s.d	Cv%
Ethanol (% v/v)	8.07± 0.53 ^c	9.44± 0.42 ^d	7.20± 0.65 ^b	6.93± 0.51 ^a	<0.001	8	0.3216	2.2
Acetaldehyde	25.0 ± 1.15 ^d	23.2 ± 0.51 ^c	19.4 ± 0.41 ^b	17.4 ± 0.72 ^a	<0.001	8	1.144	2.9
Ethyl acetate	51.7 ± 1.28 ^c	56.7 ± 1.28 ^d	32.1 ± 0.78 ^b	19.8 ± 0.63 ^a	<0.001	8	1.962	3.9
1-Propanol	43.9 ± 2.45 ^b	44.6 ± 2.45 ^c	52.3 ± 1.53 ^d	41.9 ± 3.60 ^a	<0.001	8	1.366	1.6
Isobutanol	91.4 ± 1.31 ^a	93.9 ± 1.31 ^c	110.7 ± 1.98 ^d	93.2 ± 1.88 ^b	<0.001	8	2.587	1.4
Isoamyl alcohol	123.8 ± 5.78 ^a	124.2 ± 5.78 ^b	204.1 ± 5.68 ^d	154.6 ± 7.49 ^c	<0.001	8	4.139	1.5
Phenyl ethanol	28.1 ± 1.23 ^c	28.8 ± 1.23 ^d	26.5 ± 0.58 ^b	22.2 ± 1.83 ^a	<0.001	8	2.130	4.4
Acetic acid (g/l)	0.31 ± 0.04 ^a	0.37 ± 0.04 ^b	0.47 ± 0.03 ^c	0.54 ± 0.05 ^d	<0.001	8	0.0417	5.3
Methanol	126.8 ± 6.12 ^c	129.3 ± 6.12 ^d	118.1 ± 5.42 ^a	123.7 ± 6.86 ^b	<0.001	8	2.371	1

Means within the same row sharing the same superscript were not significantly $p>0.05$ different.

Values are presented as mean ± SD

n=3

The concentration of acetaldehyde decreased as the temperature increased. This was contrary to what had previously been reported (Ribéreau-Gayon et al., 1975). The concentration of ethyl acetate ranged from 19.8 to 56.7 mg/L, the lowest values being observed in experiments with high temperature (35°C). The methanol concentration was in the range of 118.1-129.3 mg/l in all the temperatures although it was higher than in grape wine (<100mg/l). The maximum threshold for methanol content is in the range of 600 mg/l-800 mg/l (Craig 1998; Soufleros *et al.* 2001). Acetic acid was significantly different at all the fermentation temperatures, although its concentration was within the acceptable limits. Acetic acid in high concentrations (>0.7 g/ L) might give a taste and odour of vinegar (Whasley, et al., 2010).

The acceptable range of acetaldehyde and ethyl acetate is 13 to 40 g/L and 50 to 75 mg/L respectively (Adsule, 1992). The concentration of higher alcohols, 1-propanol, isobutanol, isoamyl and phenyl ethanol increased with temperature but at 35°C, their concentration decreased significantly.

Several studies have described the influence of temperature on the production of secondary metabolites during alcoholic fermentation and the final quality of wine (Aragon et al., 1998; Antonelli et al., 1999; Reynolds et al., 2001). It was previously described that low fermentation temperatures, 20°C and 25°C enhanced the aromatic characteristics of wines, possibly because of greater synthesis and a greater retention of higher alcohols (Ribéreau-Gayon et al., 2000). The results obtained showed similar

trends in levels of higher alcohols in wines. Fermentation conducted at low temperatures (<15°C) leads to more aromatic and paler wines (Walker 1998).

The alcohol concentration decreased as the temperature increased, which is due to the increase in the concentration of products from other metabolic pathways such as glycerol and acetic acid (Reddy and Reddy, 2005).

In the present study, it was found that ester formation was greatly influenced by temperature. The formation and retention of fermentation esters, such as ethyl acetate, were reported to be affected by fermentation temperature (Killiam and Ough, 1979). Killiam and Ough, 1979 found greater amounts of ethyl acetate in wines fermented at 10°C than at 20°C. In this study, concentration of ethyl acetate was highest at 25°C compared to 20°C, 30°C and 35°C indicating a deviation from what (Killiam and Ough, 1979) had previously reported. Ethyl acetate adversely affects the quality of wine due to its unpleasant flavor in high concentrations (>100 mg/L). On the other hand, at very low concentrations (50-80 mg/L) it has a positive impact on the flavor of wines (Tesevic et al., 2009).

4.2.4 Effects of yeast inoculum sizes on wine yeast growth and substrate utilization during wine fermentation

The multiplication of *Saccharomyces cerevisiae* during fermentations at different inoculum levels is given in Fig. 12. The fresh mango juice exhibited zero yeast count before inoculation. As the inoculum level rose, the yeast count in the fermenting mango must increased and the maximum yeast count was obtained at 1.98E+08 CFU/ml with

inoculum level of 0.1% at 25°C and a pH of 4.5 on day 7. Experiments with the highest yeast count, 0.05% and 0.1% showed a short stationary growth during fermentation where viable counts varied from 1.80E+08 CFU/ml to 1.92E+08 CFU/ml.

From figure 12, it can be shown that during fermentation, higher counts were observed with higher inoculum sizes of wine yeast.

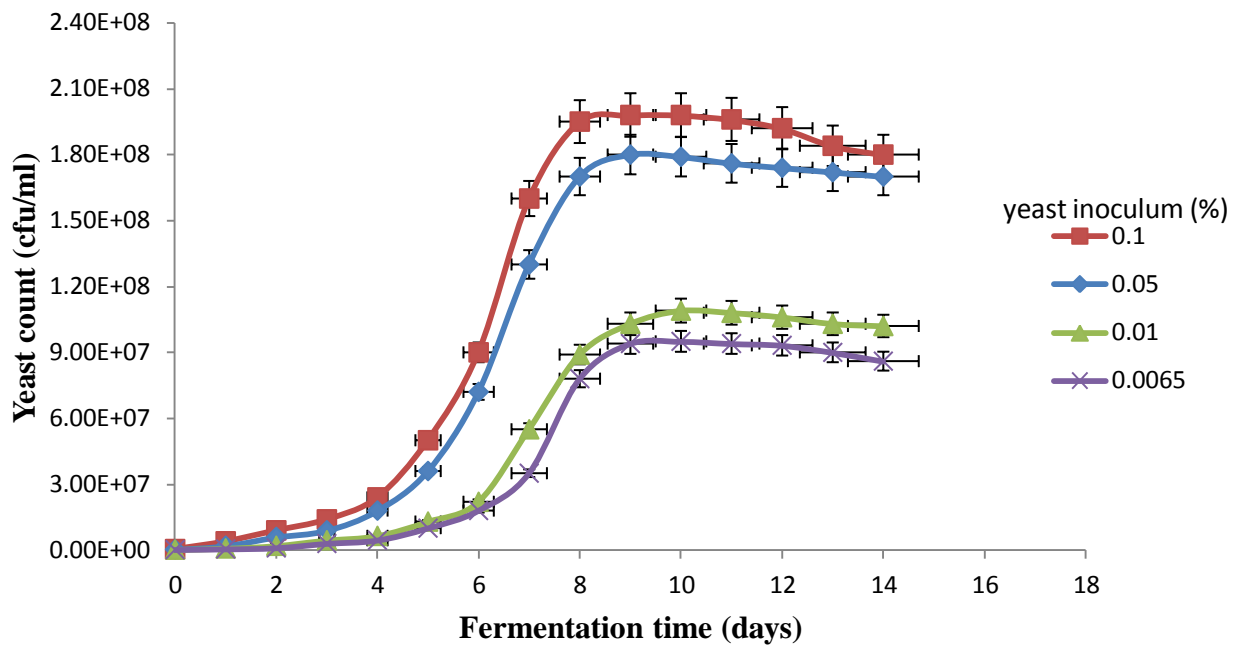
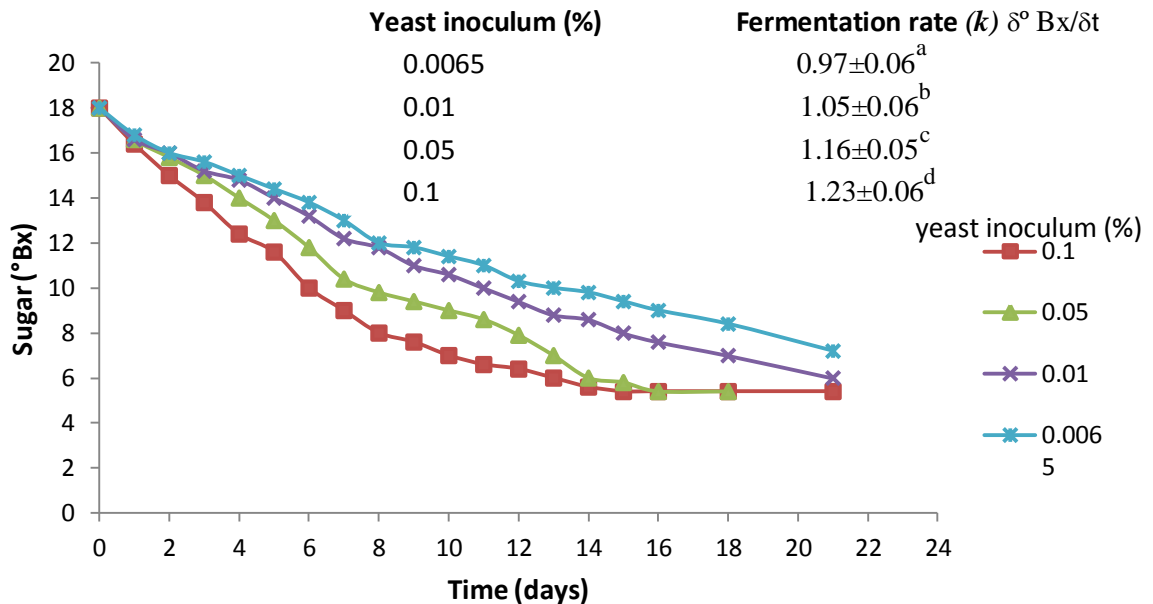


Figure 12 Yeast growth at varying inoculum sizes at 25°C and pH of 4.5

Yeast inoculum level significantly affected wine fermentation with higher inoculum levels shortening the fermentation time. Yeast inoculum size of 0.1% gave the highest yeast count during fermentation. The process offers better control of alcoholic fermentation by establishing a high population of wine yeast and affects wine quality (Erten, et al. 1996). The inoculation of wine yeast with low inoculum levels (0.0065%

and 0.01%) resulted to a long lag phase which is attributed to stuck fermentations hence development of wine with poor qualities (Macrae, et al., 1993). As it can be seen from Fig. 13, the higher the inoculum size, the higher was the initial fermentation rate. At 0.0065% inoculation the rate of utilization of sugars was lowest (18-15 °Bx) after initial four days of fermentation whereas at 0.1% inoculum size, it was fastest (18-12.4 °Bx) after initial four days. Experiments with higher inoculum size, 0.1% and 0.05%, rapidly reached the completion of fermentation relative to lower inoculum which resulted to high residual sugars. Faster fermentations were observed with an increase in inoculum size. Similar results are reported by other authors with brewer's and wine yeast strains (Mateo and Edelen, 1996, 2001) although these researchers did not mention the specific concentrations of the inoculum sizes.



The rate of fermentation at varying inoculum sizes was significantly different ($p < 0.05$) as shown by the different superscripts

Figure 13 Substrate utilization during the fermentation of mango juice from Apple variety at 25°C and pH of 4.5

4.2.5 Effect of yeast inoculum size on chemical properties of mango wine

From Table 8, it can be observed that the inoculum concentration had no effect on pH, titratable acidity and volatile acidity of the mango wine. Yeast inoculum concentration of 0.05% gave the highest alcohol yield as compared to the rest of the inoculum concentrations. There was no significant difference ($p>0.05$) in the chemical properties of mango wine produced from 0.1% and 0.05% yeast inoculum concentrations except for the alcohol yield.

Alcohol yield increased with increase in inoculum concentration up to 0.05%. Higher concentrations of inoculum did not give significantly higher alcohol content. This implies that as the concentration of yeast inoculum increases, yeast converts more sugars to alcohol, while at higher concentrations yeast could not utilize more sugar for conversion as in the case of 0.1%.

Table 8: Chemical properties of mango wine at different inoculum sizes at 25°C

Parameters	0.0065% (control)	0.01 %	0.05 %	0.1%	P value	d.f	l.s.d	Cv%
Ethanol content (% v/v)	6.93±0.04 ^a	7.20±0.04 ^b	9.44±0.04 ^d	8.67±0.04 ^c	<0.001	3	0.1106	0.7
pH	4.08±0.02 ^a	4.05±0.02 ^a	4.08±0.02 ^a	4.09±0.02 ^a	<0.375	3	0.4949	6.3
TTA	0.93±0.07 ^a	0.94±0.06 ^a	0.93±0.07 ^a	0.93±0.06 ^a	<0.160	3	0.01087	0.6
Residual °Bx	7.4±0.10 ^c	6.0±0.20 ^b	5.4±0.10 ^a	5.4±0.10 ^a	<0.001	3	0.3026	2.6
Volatile acidity (g/l as acetic acid)	0.29±0.13 ^a	0.28±0.13 ^a	0.27±0.13 ^a	0.27±0.14 ^a	<0.001	3	0.01034	0.5
Fermentation rate(<i>k</i>) ° Bx/day	0.97±0.06 ^a	1.05±0.06 ^b	1.16±0.05 ^c	1.23±0.06 ^d	<0.001	3	0.02771	1.3

Means within the same row with the same superscript were not significantly different ($p>0.05$)

Values are presented as mean ± SD

n=3

A temperature of 25°C and inoculum size of 0.05% were chosen as optimal conditions for wine production as summarized in figure 14.

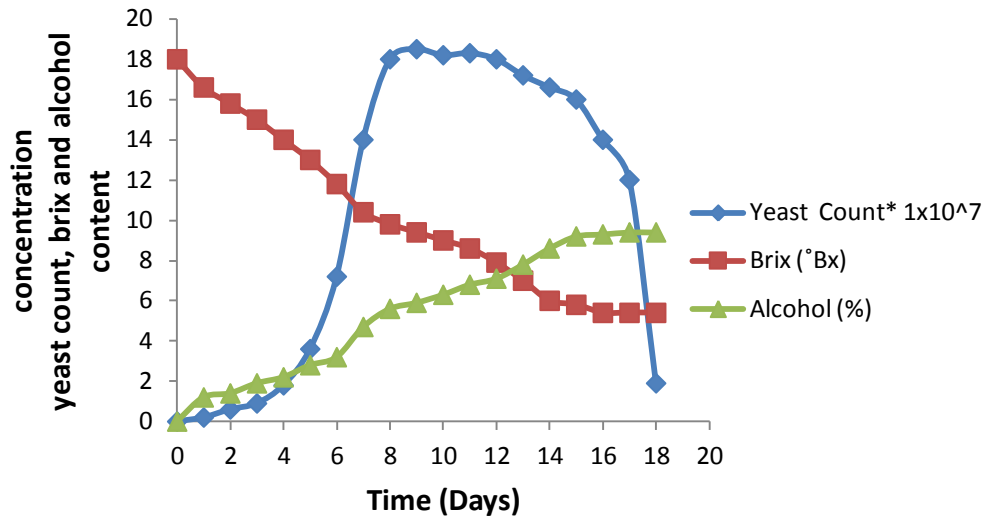


Figure 14 Yeast growth, sugars utilization and alcohol yield at 25°C and 0.05% inoculum size

4.3 Characterization of the mango wine produced using optimized conditions

4.3.1 Chemical properties of mango wine

The chemical properties of Apple and Ng’owe mango wine fermented with wine yeast at 25°C, pH 4.5 and inoculum size of 0.05% are shown in Table 9. The percentage of ethanol produced from the mango wines was between 8.89 and 9.47% w/v, comparable with moderate grape wines (Reddy, 2005). The TTA increase corresponded with the fall in pH value as fermentation progressed. The final acidity ranged from 0.81 to 0.96% (v/v) as tartaric acid. Volatile acids measure the degree of sourness of wine. The volatile acidity (as acetic acid) of the wine was between 0.37 and 0.49% v/v which is within the acceptable range of 0.3 to 0.6% reported for wines (Amerine et al, 1980).

Table 9: Chemical characteristics of mango wine from Apple and Ng'owe varieties

Mango variety	Alcohol content(% v/v)	Residual °Bx	pH	TTA (%)	Volatile acidity (g/l)
Apple	9.47±1.24 ^b	5.4±0.04 ^a	3.98±0.21 ^a	0.96±1.24 ^b	0.37±0.04 ^a
Ng'owe	8.89±1.46 ^a	5.4±0.03 ^a	4.06±0.42 ^a	0.81±0.23 ^a	0.49±0.03 ^b

Means within the same column with the same superscript were not significantly different ($p>0.05$)

Values are presented as mean ± SD.

n=3

The principal metabolite produced from the wine is ethanol. In general, the concentration of ethanol contributes to the whole characteristic quality and flavour of the produced wine. According to Michael (2000), a good table wine must have alcohol content ranging from 8 to 14%. The mango wines produced had ethanol contents in this range. Acidity plays a vital role in determining wine quality by aiding the fermentation process and enhancing the overall characteristics of the wine. Lack of acidity will mean a poor fermentation (Berry, 2000). On the other hand, the decrease in pH has been observed for wines produced from other tropical fruits (Aderiye, et al., 1990). This decrease in pH was desirable as it helped to maintain the acidity of the wine high enough to inhibit the growth of undesirable micro-organisms. Fermentation of the juice resulted in residual °Bx of 5.4. The sugars were utilized for alcohol and organic acid production (Akubor, 1996).

4.3.2 Volatile composition of mango wine

Table 10: Composition of volatile compounds by Gas Chromatography–Flame Ionization Detector (GC-FID) from two mango varieties (Apple and Ng’owe) fermented at 25°C and pH 4.5 for 16 days at 0.05% inoculum size of wine yeast.

Name of the Compound	Apple	Ng’owe	Acceptable limits*
Metabolites (mg/l)			
Ethanol (% v/v)	9.47± 1.24 ^b	8.89± 1.46 ^a	8-13
1-Propanol	66.11± 1.21 ^b	61.32± 0.54 ^a	<400
Isobutyl alcohol	105.40± 0.87 ^a	111.14± 0.76 ^b	<400
Isoamyl alcohol	119.2± 0.62 ^b	112.40± 0.25 ^a	<400
Phenethyl alcohol	24.15± 0.41 ^a	26.15± 0.31 ^b	<400
Methanol	129.23± 5.34 ^a	126.15± 6.11 ^a	<100
Acetaldehyde	18.2 ± 0.51 ^b	21.42± 0.33 ^a	<100
Ethyl acetate	73.15± 0.73 ^b	62.42± 0.82 ^a	<100
Acetic acid (g/l)	0.37± 0.96 ^a	0.46± 0.55 ^b	0.3-0.7

Means within the same row sharing the same superscript were not significantly $p>0.05$ different.

Values are presented as mean ± SD.

n=3

< - less than

*source: Chemistry in wine making; Heather Wansbrough (1998)

From Table 10, it was observed that the wine produced had their methanol content varying between 126 mg/l to 129 mg/l. According to previous reports these methanol levels are not potentially injurious to health (Craig 1998; Soufleros *et al.*, 2001) although they were significantly higher than what is reported for grape wine (<100mg/ml) (Reddy, 2005).

Methanol is formed from the enzymatic hydrolysis of the methoxy groups of pectin during fermentation, and its content depends on the extent to which the solids, especially the skins, which have high pectin content, are macerated (Peinado, Moreno, Muñoz, Medina, & Moreno, 2004). Therefore, the differences in the concentrations of methanol between Apple and Ng'owe varieties could be related to the pectin content of each fruit.

Isoamyl alcohol is the major higher alcohol found in wines (>50%), and its concentration has been reported in the range of 90 to 292 mg/L (Useeaglio-Tomasset 1975; Boulton *et al.*, 1996).

In this study, isoamyl alcohol was in the range of 112.4 to 119.2 mg/l. The other higher alcohols like 1-propanol concentrations were in the medium range, as in the case of grape wine 60–80 mg/l (Reddy, 2005).

Acetaldehyde content varied between 18 to 21mg/l. This was relative to acetaldehyde content in wine produced from grapes which is usually in the range of 13–30 mg/ l. (Longo *et al.* 1992). At low levels, acetaldehyde gives a pleasant fruity aroma to wines,

but in higher concentrations, it has a pungent, irritating odor (Miyake & Shibamoto, 1993). The other aldehydes were not identified in the mango wine.

The concentration of esters (ethyl acetate) was between 27 and 33 mg/l. Ethyl esters are one of the most important groups of aroma compounds in wine, and their concentrations depend on several factors, such as yeast strain, fermentation temperature, aeration, and sugar content. These compounds contribute positively to the overall wine quality, and most of them have a mature flavor and fruity aroma that contribute to the “fruity” and “floral” sensory properties of wines (Perestrelo, Fernandes, Albuquerque, Marques, & Camara, 2006). Consequently, ethyl acetate adversely affects the quality of wine due to its unpleasant flavor in high concentrations. On the other hand, at very low concentrations (50-80 mg/L) it has a positive impact on the flavor (Tesevic et al., 2009). The concentration of this compound varied significantly among the two varieties.

Acetic acid was in the range of 0.37 to 0.46 in the two varieties. Acetic acid in high concentrations (>0.7 g/ L) might give the taste and odour of vinegar (Whasley, et al, 2010).

Phenethyl alcohol was in the range of 24.15 and 26.15 in the two varieties. Phenethyl alcohol is an aroma carrier and its presence may contribute to the floral nuance of wines (Wondra & Berovic, 2001). The aroma character of this compound changes with its oxidation from rose to a hyacinth bouquet. Further oxidation produces esters with a fine honey nose.

The main groups of compounds that form the *fermentation bouquet* are organic acids, higher alcohols, and esters and, to a lesser extent, aldehydes (Lambrechts and Pretorius, 2000).

Mango fruit is known to have good aroma and flavour. The flavour of mango fruit is due to the volatile components that are present. The flavour of wine depends on many varietal or fermentative compounds, which are present in highly variable amounts and are mainly alcohols, esters, sulfur compounds and acids (Rapp 1988; Nobel 1994). Volatile aroma compounds are present in fruit juices, and many are synthesized by wine yeast during wine fermentation. The aroma and flavor of wine are among the main characteristics that determine its quality and value (Mauricio et al. 1997; Swiegers et al. 2005; Molina et al. 2007).

The volatile profile is important in wine, as it contributes to the quality of the final product. It is due to the combined effects of several volatile compounds, mainly alcohols, aldehydes, esters, acids and other minor components already present in the grapes as well as those that are being formed during the fermentation and maturation process (Verzera et al., 2008). The aroma complexity dramatically increases during alcoholic fermentation because of the synthesis of important volatile compounds by the wine yeast and the release of some varietal aroma precursors (Mauricio et al., 1997; Swiegers et al., 2005). The nature and the amount of the synthesized volatile compounds depend on multiple factors, such as the nitrogen content of the must, the temperature of fermentation, and the yeast strain (Lambrechts and Pretorius 2000; Swiegers et al.,

2006). The volatile compounds synthesized by wine yeasts include higher alcohols, medium and long-chain volatile acids, acetate esters, ethyl esters and aldehydes (Stashenko et al., 1992).

Statistical analysis of the concentrations of the major volatile compounds in the two varieties showed significant differences ($p < 0.05$) in their concentrations except for methanol. This implied that different mango varieties have varying concentrations of volatile compounds.

4.4 Determination of sensory properties of mango wine

The results for sensory evaluation of mango wine are presented in Table 11. There was no significant difference ($p \geq 0.05$) in clarity and general acceptability between the mango wine and the reference wine from grapes. However, the reference wine received higher ratings for clarity than the mango wine.

Table 11: Sensory evaluation of mango wine and a reference wine from grapes

	Sensory parameters				
	Colour	Mouth feel	Aroma	Clarity	General acceptability
Mango wine (Apple variety)	7.2 ^b	6.8 ^a	8.2 ^b	6.8 ^a	6.8 ^a
Grape wine (Chardonnay)	6.9 ^a	7.2 ^b	7.4 ^a	7.0 ^a	7.0 ^a

Means within the same column with the same superscript were not significantly different (p>0.05)

The preliminary test investigation characterized the new wine as a novel, special type of wine, with a pleasant soft aroma and fruity taste. This wine had light golden yellow colour with clarity and possessed good body. The results have shown that the wine produced from mango is very similar to the commercial grape wine in terms of aroma and taste.

The aroma of mango wine was significantly higher ($p \leq 0.05$) than that of grape wine. Significant changes in wine aroma occur during maturation and aging. These include the loss of certain grape or yeasty aromas, retention of the varietal aroma, formation of new aromas, and above all, integration of all flavours to produce a harmonious and pleasing fragrance (Buglione and Lozan, 2002). Many esters and higher alcohols formed by the yeast's metabolic activity contribute to the fermentation aroma. During wine storage, the esters are hydrolyzed and the fresh and fruity aroma is lost. This was evidenced in the produced mango wine. Concurrent with the degradation of esters, a synthesis of new esters occurs. For example, the formation of isoamyl acetate and diethyl succinate (Coulter, et al., 2005). During maturation and aging, the concentration of monoterpene alcohol declines and monoterpene oxides are formed. This leads to the loss and alteration of floral aroma. The terpene compound gives a floral aroma to the wine (Coulter, et al. 2005). The oxide terpene derivatives, such as alphaterpineol, have a pine like odor; whereas, its precursor linalool has a floral fragrance. In an acidic medium, such as wine, the bound terpenes are slowly converted to free volatile terpenes over

time. When these reactions occur, the fruity aroma of a wine is enhanced during maturation (Coulter, et al. 2005).

Colour is one of the most appealing properties of a wine. The colour of mango wine is light yellow. During maturation, when the wine is exposed to air, the colour becomes darker, and with over-aeration, becomes brown (Castellari, et al. 1998). Several phenolic compounds are involved in oxidative reactions. To minimize oxidation and browning, mango wines are generally treated with minimum oxygen exposure. Besides phenolic oxidation, other reactions such as the Maillard reaction and sugar caramelization contributed to colour in mango wine during fermentation (Buglione and Lozan, 2002).

Many compositional changes contribute to the taste and mouthfeel of mango wine. The important changes include polymerization of phenolic compounds and reduction in acidity (Godden, 2001). Phenolic compounds play an important role in the taste and flavour of wine. Mango wines contain mostly non-flavonoid phenols. Bitterness is primarily attributed to flavonoid phenols (Lubbers, et al., 2001). As flavonoid phenols polymerize, they become less bitter and more astringent (Lubbers, et al., 2001). During maturation, oxidative and non-oxidative polymerization and precipitation of phenolic compounds occurs. This results in a wine with a smoother and softer taste (Buglione and Lozan, 2002). Another factor which contributed to improved mouthfeel was loss of acidity. This occurred as a result of acid precipitation and ester formation. Acidity enhances the taste and loss of acidity makes wine taste mellower (Coulter, et al., 2005).

4.5 Determination of shelf stability

4.5.1 Effects of storage temperatures and colour bottles on mango wine colour

Table 12 shows the changes observed in mango wine colour as optical density after a twelve month storage period. Wine stored in brown bottles at low temperatures of 10°C and 15°C showed low browning indices when compared to wine stored in green and clear bottles. The storage temperature of 20°C and 25°C significantly increased ($p \leq 0.05$) the colour intensity of the mango wines in all the different coloured bottles. Wine colour intensity increased with time and storage temperatures. Mango wine in clear bottles stored at 25°C had the highest browning index.

Table 12: Effects of storage temperature and colour bottles on Apple mango wine colour

Temperature (°C)	Colour intensity (browning index)		
	Brown bottle	Clear bottle	Green bottle
10	0.23±0.01 ^a	0.26±0.01 ^b	0.24±0.01 ^a
15	0.23±0.01 ^a	0.26±0.01 ^b	0.24±0.01 ^a
20	0.26±0.01 ^b	0.29±0.01 ^c	0.27±0.01 ^b
25	0.30±0.01 ^c	0.34±0.01 ^d	0.31±0.01 ^c

Means sharing the same superscripts were not significantly different ($p \geq 0.05$)

Values are represented as mean±S.D

n=3

Some metal ions, SO₂, organic acids, ethanol, and phenolic compounds present in must and wine, are susceptible to oxidation, and among them phenolic compounds are considered the major substances to cause browning (Junquera et al., 1992; Macheix et al., 1991; Toit et al., 2006; Vivas et al., 2001). Phenols' regenerative capacities in

coupled oxidation with other compounds and their non-oxidative browning reactions constitute the major reason for browning (Boulton et al., 2001).

The browning of wine can be classified into enzymic browning and non-enzymic browning in terms of the initiative mechanism: the former almost entirely occurs in grape must; the latter can happen both in grape must and wine, while as a result of fermentation and some operations it prevails in wine (Es-Safi et al., 2003; Main, 1992; Spagna et al., 2000; Sullivan, 2002). The enzymic oxidation of phenols, particularly in the presence of atmospheric oxygen and polyphenoloxidase (PPO), takes place in the early stages of processing and is well known to be a cause of browning in foodstuffs (Wang, 1990).

Non-enzymic oxidation, also called chemical oxidation, prevails in wine with the characteristics of regeneration and autocatalysis, and it may also occur through the direct reaction with light (Main, 1992). *o*-Diphenols, mainly including caffeic acid and its esters, catechin, epicatechin, anthocyanins and their derivatives, and gallic acid, are considered to be the most susceptible to oxidation in non-enzymic browning process, and the levels of flavan-3-ols are most significantly correlated to the browning degree of most white wines (Fernández-Zurbano et al., 1995, 1998; Lopez-Toledano et al., 2002; Oszmianski et al., 1996; Saucier & Waterhouse, 1999).

4.5.2 Effects of wine storage on volatile acidity (VA)

Table 13: Effects of storage time (12 months) on the volatile acidity of mango wine at 15°C and in a brown bottle

	Months			
	0	4	8	12
Volatile acidity (g/l)	0.3700±0.01 ^a	0.3867±0.006 ^b	0.3967±0.006 ^b	0.4133±0.006 ^c

Means sharing the same superscripts were not significantly different ($p \geq 0.05$)

Values are represented as mean±S.D

n=3

Where;

d.f=8

n=sample size

cv%=1.8

d.f=degrees of freedom

l.s.d=0.01331

cv%=coefficient of variation

p<0.001

l.s.d=least significant difference

As shown in table 13, the volatile acidity of the wine ranged from 0.37 g/l after processing to 0.41 g/l after a storage period of twelve months. There was a significant difference in the volatile acidity of the wine after twelve months storage. This increase in the volatile acidity is thought to have been generated during racking of the wine when the wine came in contact with oxygen. Although, according to (Gonzales, et al. 1994) a typical good table wine has a volatile acidity of 0.3–0.4 g/L. (Duncan and Kleinig, 1999) reported that values higher than 0.6 g/l as an indication of acetic spoilage.

High VA levels in a wine indicate past or current oxidative spoilage, usually through the action of acetic acid bacteria, the bacteria responsible for wine turning to vinegar (Godden, 2001). Monitoring VA during the wine's aging phase helps the winemaker to spot and address spoilage issues before they become more significant.

Acetic acid bacteria are very prevalent in nature and are well adapted to growth in sugar-rich and alcohol rich environments. Their metabolism is strictly aerobic: cellular oxidation of sugars and ethanol are coupled with the citric acid cycle and respiratory chain electron transport mechanisms (Godden, 2001). They belong to the *Acetobacteraceae* family. The bacteria of this family are separated into two genera: *Acetobacter* and *Gluconobacter*. The principle property of both genera is to oxidize ethanol to acetic acid (Iland, et al. 2000).

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study demonstrates that mango juice can be used successfully in the production of wine. The best varieties for wine production are Apple and Ngowe due to the high juice content, low acidity and high sugar content. Contrary, Sabine variety is not suitable for wine production, it is suggested that alternative uses should be sought for its utilization.

Based on this study, it can be concluded that a 16 day fermentation at 25°C and yeast concentration of 0.05% at a pH of 4.5 yields optimal fermentation characteristics for mango wine production. Sensory evaluation results indicated that mango wine possesses a pleasant aroma and mouthfeel and is comparable to grape wine. The wine is stable for about 1 year when stored in dark (brown and green) bottles and low temperatures of 15°C. This work provides an alternative pathway for utilization of mango during glut periods.

5.2 Recommendations

It is recommended that the output of this research be disseminated to relevant stakeholders within Kenya including farmers, cooperatives, processors and policy makers. Further work is needed to refine the quality of the wine for commercial production.

The results also indicate that other tropical fruits might have the potential to produce high quality wine of commercial viability. This work needs to be extended to other fruits and vegetables.

More work needs to be done on the chemical and microbial stability of the wine over longer periods. Other value added products derived from the wine can be obtained including vinegar.

REFERENCES

- Abe K, Mathooko, F.M., Kiiyukia, C., Mwaniki, M.W., Mungai, C. W. and Koaze, H. (1997). Surveillance of Postharvest Handling of Perishable Food Crops part I- case study of Kenya. JICA technical report- department of food science and Postharvest Technology, Jomo Kenyatta University of agriculture and technology Nairobi, Kenya 235-240.
- Aderiye B. I, Akpapunam MA, Akubor P. I (1990). Effects of fermentation variables on the quality of cashew wine. *Journal of Agriculture Science and Technology* 1, 16-69.
- Adsule R.N, Kotech P.M, Kadam S.S. (1992). Preparation of wine from pomegranate. *Beverage Food World* 19:13–16.
- Akubor P. I (1996). The suitability of African bush mango juice for wine production. *Plant Foods for Human Nutrition* 49: 213-219.
- Alaka O, Aina JO, Falade KO. (2003). Effect of storage conditions on the chemical attributes of Ogbomoso mango juice. *European Food Research and Technology* 218:79–82.
- Amerine, M.A, Berg, H.W., Kunkee, R.E., Ough, C.S., Singletons, V.L. and Webb, A.D. (1980). *The technology of wine making*. AVI publishing company, West port, Connecticut.
- Amerine, M.A and M.A Joslyn (1950). *Table wines: the technology of their production*. University of California press.

- Anonymous. (1962). *Wealth of India—raw materials 6 (L–M)*. CSIR. New Delhi
- Antonelli, A., Castellari, L., Zambonelli, C., Carnacini, A. (1999). Yeast influence on volatile composition of wines. *Journal of Agriculture and Food Chemistry* 47 (3): 1139–1144.
- AOAC. (1995). Association of Official Analytical Chemists. *Official Methods of Analysis*, 16th Edition. Washington DC, USA.
- AOAC. (1996). Association of Official Analytical Chemists. *Official Methods of Analysis*. 17th Edition. Washington DC, USA.
- AOAC. (2000). Association of Official Analytical Chemists. *Official Methods of Analysis*. 17th Edition, 955.24, 962.12, 968.08 and 967.21, Washington DC, USA.
- Aragon, P., Atienza, J., Climent, M.D. (1998). Influence of clarification, yeast type and fermentation temperature of the organic acid and higher alcohols of Malvasia and Muscatel wines. *American Journal of Enology and Viticulture*. 49 (2): 211–219.
- Bachmann. J, (2001). Adding value to farm produce: An overview. *Appropriate Technology*
- Bally, I., Lu P. and Johnson, (2009). *Mango breeding in Jain, S.M and Priyadarshan P.M (eds), reeding plantation tree \crops: Tropical Species Springer Science; Business Media.*
- Baisya, R.K. (2004). Post harvest management of fruits and vegetables – A technology management perspective. *Indian Food Pack*. (July–Aug), 78–82.

- Bauer, F.F. and Pretorius, I.S. (2000). Yeast stress response and fermentation efficiency: How to survive the making wine. *South Africa Journal of Enology and Viticulture*. 21, 27–51.
- Benítez P, Castro R, García Barroso C. (2003). Changes in the polyphenolic and volatile contents of ‘fino’ sherry wine exposed to ultraviolet and visible radiation during storage. *Journal of Agriculture and Food Chemistry* 51(22): 6482–7.
- Berry, C.J.J. (2000). *First Steps in Wine Making*. Published by G.W. Kent, Inc. 3667 Morgan Road, Ann Arbor MI 48108, pp: 235.
- Bisson, L.F. and C.E. Butzkc, 2007. *History of Wine making*. WINE, Microsoft Encarta.
- Boulton RB, Singleton VL, Bisson LF, Kunkee RE. (1996). *Principles and practices of wine making*. Aspen, New York
- Brock, D. T., Madiga, T. N., Martinko, M. J., and Packer, J. (1994), *Industrial Microbiology/ Biocatalysis*, in *Biology of Microorganisms*, 7th ed., Benjamin/Cummings, Corey, P. F. and Bozik, T., eds., Prentice-Hall, Inc., New Jersey, pp. 386–390.
- Buglione, M., and Lozano, J. (2002). Nonenzymatic browning and chemical changes during grape juice storage. *Journal of Food Science* 67(4): 1538–1543.
- Campbell, A. N., Mitchell, G. L., and Reece, B. J. (1997), *Biology, Concepts and Connections*, 2nd ed., Benjamin/Cummings, pp. 89–91.
- Casey, G.P., Ingledew, W.M., (1986). Ethanol tolerance in yeasts. *CRC Critical Reviews in Microbiology* 13 (3), 219– 280.

- Castellari, M., Arfelli, G., Ripon, C and Amati, A. (1998) Evolution of Phenolic Compounds in Red Winemaking as Affected by Must Oxygenation. *American Journal of Enology and Viticulture*. 49:1, 91 – 94.
- Cheirslip, B. and Umsakul, K. (2008). Processing of banana based wine product using pectinase and α -amylase. *Journal of Food Process Engineering*, 31, 78–90.
- Cisneros-Zevallos. (2003). The use of controlled post harvest abiotic stresses as a tool for enhancing the nutraceutical content and adding value of fresh fruits and vegetables. *Journal of Food Science* 68:1560–1565.
- Coulter, A. D., Godden, P.W., and Pretorius, I. S. (2004). Succinic acid-how is it formed, what is its effect on titratable acidity, and what factors influence its concentration in wine? *The Australian and New Zealand Wine Industry Journal*, 19(6) 22-25.
- Country report (2013). Wine in Kenya. Accessed 2013.
- Couto, M.M.B., Reizinho, R.G. and Duarte, F.L., (2005). Partial 26S rDNA restriction analysis as a tool to characterize non-Saccharomyces yeasts present during red wine fermentations. *International Journal for Food Microbiology*. 102, 49–56.
- Craig, A. (1998). Comparison of the headspace volatiles of kiwifruit wine with those wines of *Vitis vinifera* variety Muller-Thurgau. *American Journal of Enology and Viticulture* 39, 321–324.
- Czyhrinciwk N. (1966). The technology of passion fruit and mango wines. *American Journal of Enology and Viticulture*. 17:27–30.

- Duncan, B., and Kleinig, A. (1999). Oxygen Transmission Analysis of Wine Bottle Closures. Southcorp Wines Internal Project P9703.
- Erten Huseyin, Hasan Tanguler, Turgut Cabaroglu and Ahmet Canbas. (1996). The Influence of Inoculum Level on Fermentation and Flavour Compounds of White Wines Made from cv. Emir. *J. Inst. Brew.* 112(3), 232–236.
- Es-Safi, N. E., Cheynier, V., & Moutounet, M. (2003a). Effect of copper on oxidation of (+)-catechin in a model solution system. *International Journal of Food Science and Technology*, 38(2), 153–163.
- Fabios M, Lopez-Toledano A, Mayen M, Merida J, Medina M. (2000). Phenolic compounds and browning in sherry wines subjected to oxidative and biological aging. *Journal of Agriculture and Food Chemistry* 48(6):2155–9.
- FAO, 2001. The world trade in mangoes and mango products and opportunities for Kenya partnerships for food industry development - fruits and vegetables (PFID-FV). <http://www.allaboutmangoes.com/mango%20production/MangoKenya>.
- FAO (Food and Agriculture Organization of the United Nations). (2005). FAOSTAT 2005 database.
- Fernańdez-Zurbano, P., Ferreira, V., Escudero, A., & Cacho, J. (1998). Role of hydroxycinnamic acids and flavanols in the oxidation and browning of white wines. *Journal of Agricultural and Food Chemistry*, 46(12), 4937–4944.
- Fernańdez-Zurbano, P., Ferreira, V., Peña, C., Escudero, A., Serrano, F., & Cacho, J. (1995). Prediction of oxidative browning in white wines as a function of their

- chemical composition. *Journal of Agricultural and Food Chemistry*, 43(11), 2813–2817.
- Fleet, G.H., Heard, G.M. (1993). Yeasts: growth during fermentation. In: Fleet, G.M. (Ed.), *Wine Microbiology and Biotechnology*. Harwood Academic Publishers, Chur, Switzerland, pp. 27– 54.
- Food and Agriculture Organization of the United Nations. 2007. FAOSTAT Statistic database agriculture, Rome, Italy. <http://faostat.fao.org/faostat/collection>.
- Galán Saúco, V., 2002 Mango: Emerging tropical export crop. *Chronica Horticulturae* 42, 4: 14-17.
- Galán Saúco, V., 2004 Mango production and world market: Current situation and future prospects. *Proceedings of the VIIth International Symposium on Mango*. Editors Pinto, A.C.Q. et al. *ActaHorticulturae* 645: 107-116.
- Gathambiri C.W., Muchui, M. N., Njuguna, J. K., Wepukhulu, S B., Kiiru, S. N., Wanjala, S. N. and Kariuki, D. N, (2006) Evaluation of postharvest characteristic of mango fruit: 10th KARI Biennial Scientific Proceedings.
- Giri, K.V., KrishnaMurthy, D.V. & Narashimha Rao, P.L. 1953 Separation of organic acids. *Journal of Indian Institute of Sciences* 35A, 77–98.
- Gitonga K. J., Chitere, P. O., Odegi, C. (2009). The effects of agricultural extension services on farmer performance. 12th KARI Biennial proceedings.
- Godden, P., Francis, L., Field, J., Gishen, M., Coulter, A., Valente, P., Hoj, P. and Robinson, E. (2001) *Wine Bottle Closures: physical characteristics and effect on*

- composition and sensory properties of a Semillon wine 1. Performance up to 20 months post-bottling. *Australian Journal of Grape and Wine Research* 7, 64 – 105.
- Gonzales Cartagena, L., Perez-Zuniga, F.J. and Bravo Abad, F. (1994) Interactions of Some Environmental and Chemical Parameters Affecting the Colour Attributes of Wine. *American Journal of Enology and Viticulture* 45:1, 43 – 48.
- Gregoratti, C. 2009. Global nuts and local mangoes: a critical reading of the UNDP Growing Sustainable Business Initiative in Kenya. Published online in *Agriculture and Human Values*. <http://www.springerlink.com/content>
- Griesbach J. 1992. A guide to propagation and cultivation of fruit trees in Kenya. Schriftenreihe der GTZ no. 230. Eschborn, Germany. 180 pp.
- Griesbach J. (2003). *Mango Growing in Kenya*. ICRAF, Nairobi, Kenya.
- HCDA (2006). *Horticultural news magazine*.
- HCDA (2007). *Marketing Statistics*.
- HCDA (2010) *HCDA validated report*. HCDA, Nairobi, Kenya.
- HCDA (2012) *HCDA validated report*. HCDA, Nairobi, Kenya.
- Hobson, G. E.; Grierson, D. In *Biochemistry of Fruit Ripening*; Seymour, G. B., Ed.; Chapman and Hall: London, 1993, pp 405–442.
- Horticultural Crops Development Authority (2008) *Horticultural Data 2005-2007. Validation Report*. HCDA, Nairobi, Kenya.
- Huang, S. M., Chuang, H. C., Wu, C. H. and Yen, G. C., (2008). Cytoprotective effects of phenolic acids on methylglyoxal-induced apoptosis in neuro-2A cells. *Molecular and Nutritional Food Research*. 52, 940-949.

- Ihekoronye, A.I. and P.O. Ngoddy (1985). *Integrated Food Science and Technology for the Tropics*. Macmillan Publishers, London, UK., ISBN: 9780333388839, Pages: 386.
- Iland, P.G., Ewart, A., Sitters, J.H., Markides, A. and Bruer, N. (2000) *Techniques for chemical analysis and quality monitoring during winemaking*. WineTitles, Adelaide.
- Iyer CPA and Schnell R.J. (2009). *Breeding and Genetics*. In: Litz RE (ed.) *The Mango*, 2nd Edition. Botany, Production and Uses. CABI, Oxfordshire, UK, pp. 67-96.
- Joshi, V.K. & Bhutani, V.P. (1991a) The influence of enzymatic clarification in fermentation behavior and qualities of apple wine. *Science des Aliments* 11, 491–498.
- Joshi, V.K. & Sharma, S.K. (1995) Comparative fermentation behavior and physico-chemical sensory characteristics of plum wine as effected by the type of preservatives. *Chemical and Microbial Technology (Lebensmittel)* 17, 65.
- Joshi, V.K., Bhutani, V.P. & Sharma, R.C. (1990) Effect of dilution and addition of nitrogen source in chemical, mineral and sensory qualities of wild apricot wine. *American Journal of Enology and Viticulture* 41, 229–231.
- Junquera, B., Gonzalez-San Jose, M. L., and Diez, C. (1992). Enzymic browning of grapes and wines. *Revista Espanola de Ciencia y Tecnologia de Alimentos*, 32(5), 481–491.

- Karihaloo J.L., Dwivedi Y.K., Archak S. and Gaikwad A.B. (2003). Analysis of genetic diversity of Indian mango cultivars using RAPD markers. *Journal of Horticultural Science and Biotechnology*. 78, 285-289.
- Kenya Agricultural Research Institute (KARI) -Thika (1994) Centre Annual Report.
- Killiam, E., Ough, C.S., (1979). Fermentation esters — formation and retention as affected by fermentation temperature. *American Journal of Enology and Viticulture* 30 (4), 301–305.
- Knight RJ (Jr.). 1997. Important mango cultivars and their descriptors. Homestead, Florida, USA: Tropical Research and Education Center, University of Florida.
- Kinuthia, Z. Warui, D. and Karanja, F. (2009). Mapping and characterising water points in Mbeti South location, Mbeere District. Institute of Economic Affairs, Nairobi.
- Kulkarni JH, Singh H, Chada KL. 1980. Preliminary screening of mango varieties for wine making. *Journal of Food Science and Technology* 17:218–21.
- Kumar YS, Prakasam RS, Reddy OVS. 2009. Optimization of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology. *International Journal of Food Science and Technology* 44:2320–7.
- KIT, Faida MaLi and IIRR, 2006. Chain empowerment: Supporting African farmers to develop markets. Royal Tropical Institute, Amsterdam; Faida Market Link, Arusha; and International Institute of Rural Reconstruction, Nairobi. 212 pp.
- Lambrechts MG, Pretorius IS. 2000. Yeast and its importance to wine aroma—a review. *South Africa Journal of Enology and Viticulture* 21:97–129.

- Larue, F., Lafon-Lafourcade, S., Ribe´reau-Gayon, P., 1980. Relationship between the sterol content of yeast cells and their fermentation activity in grape must. *Applied and Environmental Science*
- Longo, E., Vlazquez, J.B., Siero, C., Ansado, C.J. & Villa, T.G. (1992). Production of higher alcohols, ethyl acetate, acetaldehyde and other compounds by *S. cerevisiae* wine strains isolated from the same region (Salnes N.W Spain). *World Journal of Microbiology and Biotechnology* 8, 539–541.
- Lopez-Toledano, A., Mayen, M., Merida, J., & Medina, M. (2002). Yeast induced inhibition of (+)-catechin and (–)-epicatechin degradation in model solutions. *Journal of Agricultural and Food Chemistry*, 50(6), 1631–1635.
- Lubbers, S., Verret, C., & Voilley, A. (2001). The effect of glycerol on the perceived aroma of a model wine and white wine. *LWT e Food Science and Technology*, 34 (4), 262e265.
- Lucero, P., Pen˜alver, E., Moreno, E., Lagunas, R., 2000. Internal trehalose protects endocytosis from inhibition by ethanol in *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology* 66 (10), 4456– 4461.
- Ma. Jesu´s Torija, Nicolas Roze`s, Montse Poblet, Jose´ Manuel Guillamo´n, Albert Mas. (2003). Effects of fermentation temperature on the strain population of *Saccharomyces cerevisiae*. *International Journal of Food Microbiology* 80, 47– 53
- Macheix, J. J., Sapis, J. C., & Fleuriet, A. (1991). Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. *Critical Reviews in Food Science and Nutrition*, 30(4), 441–486.

- Macrae, R., Robinson and Sadler M.J (1993). Alcohol fermentation: Encyclopedia for food technology and Nutrition. Vol. 3, 1735-1842. Academic Press Limited, London
- Main, G. L. (1992). Juice fining treatments to reduce browning and use of sulfur dioxide in white wine production. Ph.D. Thesis, University of Arkansas, AR, United States.
- Maneeapun, S. and Yunchalad, M., (2004). Developing processed mango products for international markets. *ActaHorticulturae* 645: 93-105.
- Mangas, J.J., Cabranes, C., Moreno, J. And Gomis, D.B. (1994). Influence of cider making technology on cider taste. *Lebensm.-Wiss. Technol.* 27, 583–586.
- Marín, M.R., Alcoholic fermentation modeling: current state and perspectives. *American Journal of Enology and Viticulture.*, 1999, 50(2), 166–178.
- Mateo, J.J., Jimenez, M., Huerta, T., and Pastor, A., (1991). Contribution of different yeasts isolated from musts of monastrell grapes to aroma of wines. *International Journal of Food Microbiology* 14, 153– 160.
- Matsuoka T (2000). Non-destructive techniques for quality evaluation of intact fruits and vegetables. *Food Science and Technology Research*, 6, 248–251
- Mauricio JC, Moreno J, Zea L, Ortega JM, Medina M. 1997. The effects of grape must fermentation conditions on volatile alcohols and esters. *J Sci Food Agric* 75:155–60.

- Michael, P. (2000). Foods of the Gods: Part 1-Wine in Ancient Egypt. Retrieved from: <http://www.touregypt.net/Egypt-info/magazine-mag11012000-magf.htm>, (Accessed on: August 12, 2010). *Microbiology* 39, 808.
- Miyake, T., & Shibamoto, T. (1993). Quantitative analysis of acetaldehyde in foods and beverages. *Journal of Agricultural and Food Chemistry*, 41(11), 1968-1970.
- MoA (Ministry of Agriculture) and Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH (2006). Report on the Mango Value Chain Stakeholder Workshop, Nairobi/ Thika, 6-8 March 2006. Promotion of Private Sector Development in Agriculture (PSDA) Project. GTZ, Nairobi, Kenya.
- Molina AM, Swiegers JH, Varela C, Pretorius IS, Agosin E. 2007. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Applied Microbiology and Biotechnology* 77:675–87.
- Moll M. 1991. *Beers and coolers*: Berlin: Springer-Verlag.
- Nakasone H.Y. and Paul R.E. (1998). *Crop Production Science in Horticulture*.
- Nester, W. E., Roberts, C. E., Lindstorm, E. M., Pearsall, N. N., and Nester, T. M.(1983), Wine, in *Microbiology*, 3rd ed., CBS College Publishing, New York, pp. 766–773.
- Njuguna J.K., Wepukhulu S.B. and Wanjala S. (2009). Mango cultivar evaluation programme in Kenya. *Acta Horticulturae*. 820, 133-135.
- Nobel AC. (1994). Wine flavor. In: Piggott JR, Patterson A, editors. *Understanding natural flavors*. Glasgow: Blakie Academic and Professional. p. 226–42.

- Ofori, F. and Hahn, S.K. (1994). Tropical Root Crops. A developing economy. Proceedings of the 9th symposium of the international Society for Tropical root Crops, Pg 20–26. Accra, Ghana. Pg31, 249-254.
- Okafor, N., 1987. Industrial Microbiology. University of Ife Press Ltd, pp: 413.
- Okoth E. M, Sila D.N, Onyango C. A, Owino W.O, Musembi S.M, Mathooko F.M. (2013). Evaluation of physical and sensory quality attributes of three mango varieties at three stages of ripeness, grown in lower eastern province of Kenya - part 1. Journal of Animal and Plant Sciences. 17, Issue 3: 2608-2618.
- Onkarayya, H. & Singh, H. (1986). Screening of mango varieties for dessert and madeira- style wine. American Journal of Enology and Viticulture, 35, 63–65.
- Onkarayya, H. 1986. A rapid modernization process to improve mango dessert wines. J. Food Sci. Technol. 23, 175–176.
- Oszmianski, J., Cheynier, V., & Moutounet, M. (1996). Iron-catalyzed oxidation of (+)-catechin in model systems. Journal of Agricultural and Food Chemistry, 44(7), 1712–1715.
- Peinado, R. A., Moreno, J. A., Muñoz, D., Medina, M., & Moreno, J. (2004). Gas chromatographic quantification of major volatile compounds and polyols in wine by direct injection. Journal of Agricultural and Food Chemistry, 52(21), 6389e6393.
- Perestrelo, R., Fernandes, A., Albuquerque, F., Marques, J., & Camara, J. (2006). Analytical characterization of the aroma of Tinta Negra Mole red wine:

- identification of the main odorants compounds. *Analytica Chimica Acta*, 563(1e2), 154e164.
- Prescott, L.M., J.P. Harley and D.A. Klein, 2008. *Microbiology*. 7th Edn., Mc Graw-Hill Companies, Inc., 1221 Avenue of the Americas, New York.
- PSDA, 2007. Report on the mango value chain development stakeholders workshop held at Wida Highway Motel, 21st – 23rd March 2007. Promotion Of Private Sector Development in Agriculture.
- Kenyan Ministry of Agriculture and Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Germany.
- Rapp A. 1998. Volatile flavor of wine: correlation between instrumental analysis and sensory analysis. *Nahrung* 42:351–63.
- Reddy and Reddy (2005). Production and characterization of wine from mango fruit (*Mangifera indica* L). *World Journal of Microbiology & Biotechnology* 21:1345–1350.
- Reddy and Reddy (2009) Production, optimization and characterization of wine from mango (*Mangifera indica* Linn.). *Natural Product Radiance*, Vol. 8(4), 2009, pp.426-435.
- Reynolds, A., Cliff, M., Girard, B., Kopp, T.G., 2001. Influence of fermentation temperature on composition and sensory properties of Semillon and Shiraz wines. *Am. J. Enol. Vitic.* 52 (3), 235–240.

- Ribe´reau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). Handbook of enology (2nd ed.). The chemistry of wine stabilization and treatments (Vol. 2). John Wiley and Sons Ltd, Chichester, England:
- Rib´ereau-Gayon, P., Dubourdieu, D., Don`eche, B., Lonvaud, A., 2000. Handbook of enology. The Microbiology of Wine and Vinifications, vol. I. Wiley, West Sussex, England.
- Robinson K, The Oxford Companion to Wine, Oxford University Press, London, 1994.
- Salim AS, Simons AJ, Orwa C, Chege J, Owuor B and Mutua A. 2002. Agroforestry database: a tree species reference and selection guide. Version 2.0 CD-ROM International Centre for Research in Agroforestry. Nairobi, Kenya
- Saucier, C. T., & Waterhouse, A. L. (1999). Synergetic activity of catechin and other antioxidants. *Journal of Agricultural and Food Chemistry*, 47(11), 4491–4494.
- Selli S, Canbas A and Unal U (2002). Effect of bottle colour and storage conditions on browning of orange wine, *Nahrung*, 2002, 46, 64-67.
- Selli S, Kurkcuoglu M, Kafkas E, Cabaroglu T, Demirci B, Baser KHC, Canbas A. 2004. Volatile flavour components of mandarin wine obtained from clementines (*Citrus reticula* Blanco) extracted by solid-phase micro extraction. *Flavour Fragrance Journal* 19:413–6.
- Selvaraj Y, Kumar R. 1994. Enzymatic regulation in ripening mango fruit. *Indian Journal of Horticulture* 51:316–23.

- Sevda SB, Rodrigues L (2011). Fermentative Behavior of *Saccharomyces* Strains during Guava (*Psidium Guajava* L) Must Fermentation and Optimization of Guava Wine Production. *Journal of Food Processing Technology* 2, 118.
- Sinclair, C.G. and Kristiansen, B., (1987). *Fermentation kinetics and modelling*. Taylor & Francis: New York.
- Soufleros EH, Pissa I, Petridis D, Lygerakis M, Mermelas K, Boukouvalas M, Tsimitakis E. 2001. Instrumental analysis of volatile and other compounds of Greek kiwi wine; sensory evaluation and optimization of its composition. *Food Chemistry* 75:487–500.
- Spagna, G., Barbagallo, R. N., & Pifferi, P. G. (2000). Fining treatments of white wines by means of polymeric adjuvants for their stabilization against browning. *Journal of Agricultural and Food Chemistry*, 48(10), 4619–4627.
- Stashenko H, Macku C, Shibamoto T. 1992. Monitoring volatile chemicals formed from must during yeast fermentation. *J Agric Food Chem* 40:2257–9.
- Sullivan, P. J. (2002). *The effects of microoxygenation on red wines*. M.S. Thesis, California State University, Fresno, CA, United States.
- Swiegers JH, Bartowksy EJ, Henschke PA, Pretorius IS. 2005. Yeast and bacterial modulation of wine aroma and flavor. *Australian Journal of Grape Wine Research* 11:127–38.
- Te_sevi_c, V., Niki_cevi_c, N., Milosavljevi_c, S., Baji_c, D., Vajs, V., Vu_ckovi_c, I., et al. (2009). Characterization of volatile compounds of “Drenja”, an alcoholic

- beverage obtained from the fruits of cornelian cherry. *Journal of the Serbian Chemical Society*, 74(2), 117-128.
- Tharanathan, R.N., Yashoda, H.M. & Prabha, T.N., 2006 Mango (*Mangifera indica* L.), the king of fruits—An overview. *Food Reviews International* 22: 95–123
- The Technology of Winemaking, 4th edition. Westport, CT: AVI Publishing Co., Inc.
- Toit, W. J. d., Marais, J., Pretorius, I. S., & Toit, M. d. (2006). Oxygen in must and wine A review. *South African Journal of Enology and Viticulture*, 27(1), 76–94.
- Toriija MJ, Roes N, Pblet M, Guillamon MJ, Mas A. 2002. Effects of fermentation temperature on the strain population of *Saccharomyces cerevisiae*. *International Journal for Food Microbiology* 80:47–53.
- Ueda M., Sasaki, K., Utsunomiya, N. Inaba, K and Shimabayashi, Y. 2000. Changes in physical and chemical properties during maturation of the mango fruit (*Mangifera indica* L. Irwin) cultured in plastic green houses. *Journal of Food Science*, 50: 1646-1648
- Useglio-Tomasset L. 1975. Volatiles of wine dependant on yeast metabolism. *Proceedings of the 4th International Oenological Symposium, Valencia, Spain*, p. 346–70.
- Varakumar S, Kumar YS, Reddy OVS. 2011. Carotenoid composition of mango (*mangifera indica* L.) wine and its antioxidant activity. *Journal of Food Biochemistry* 35:1538–47.

- Verzera A, Ziino M, Scacco A, Lanza CM, Mazzaglia A, Romeo V, Conduro C. 2008. Volatile compound and sensory analysis for the characterization of an Italian white wine from “Inzolia” grapes. *Food Analytical Methods* 1(2):144–51.
- Verzera A, Ziino M, Scacco A, Lanza CM, Mazzaglia A, Romeo V, Conduro C. 2008. Volatile compound and sensory analysis for the characterization of an Italian white wine from “Inzolia” grapes. *Food Analytical Methods* 1(2):144–51.
- Vivas, N., Vivas de Gaulejac, N., & Nonier, M. F. (2001). Scavenging and antioxidant properties of different soluble compounds in hydroalcoholic media. Comparison with SO₂ efficiency. *Bulletin de L’O.I.V.*, 74(843/844), 331–346.
- Walker GM. (1998). *Yeast physiology and biotechnology*. New York: Wiley.
- Wallace, R. A., King, J. L., and Sanders, G. P. (1981), *Diversity in Fungi*, in *Biology*, 2nd ed., Scott Foresman and Company, pp. 495–496.
- Wang, Z. (1990). *Food enzymology*. Beijing: China Light Industry Press.
- Whasley F. Duarte Disney R. Dias, José M. Oliveira José A. Teixeira João B. de Almeida e Silva Rosane F. Schwan (2010). Characterization of different fruit wines made from cacao, cupuassu, gabirola, jaboticaba and umbu. *LWT - Food Science and Technology* 43, 1564-1572.
- Wondra, M., & Berovic, M. (2001). Analyses of aroma components of Chardonnay wine fermented by different yeast strains. *Chemical Analysis*, 39(2), 141e148.
- Yahia EM. 1998. Modified and controlled atmosphere for tropical fruits. *Horticultural Review* 22:123–83.

Yannam Sudheer Kumar, Reddy Shetty Prakasam and Obulam Vijaya Sarathi Reddy (2009). Optimisation of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology. *International Journal of Food Science and Technology*. 44, 2320–2327.

PLATES

Plate 1 Fructose standard curve

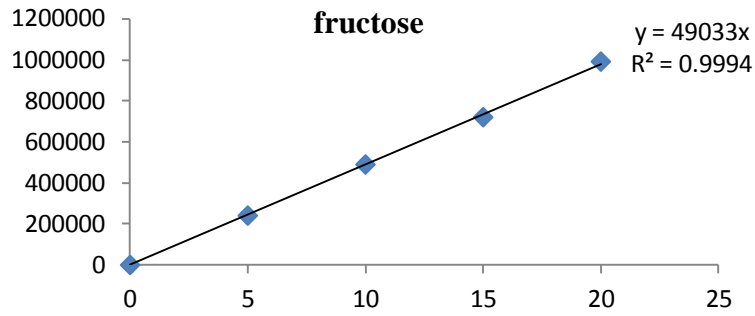


Plate 2 Glucose standard curve

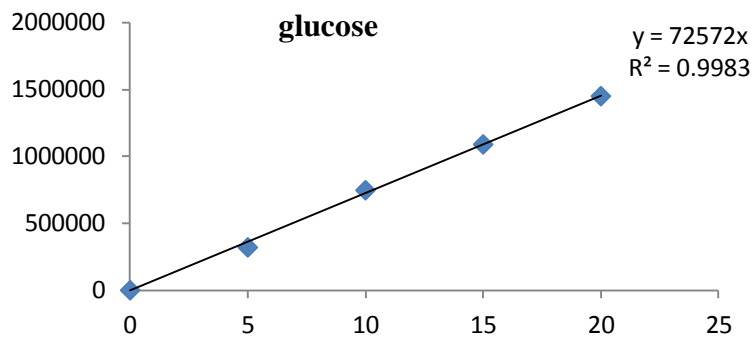


Plate 3 Sucrose standard curve

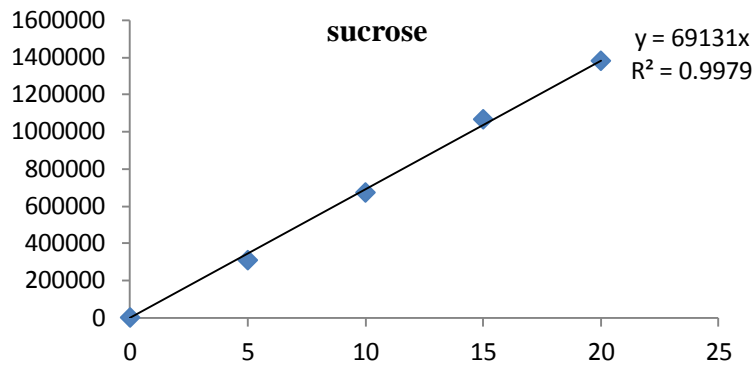


Plate 4 Approach during mango wine production



Sample collection

Collection of mango fruit samples



Preparation of the mango pulp



Fermentation of the mango



Wine filtration and centrifugation



Active Fermentation (25°C)



Racking and Maturation



Chemical analysis



Mango wine



Mango wine from Apple and Ngowe varieties



Juice preparation



Wine filtration



Exhibitions

Plate 5 Total yeast count at maximal population (day 9)



Plate 6 Sensory evaluation questionnaire

Date.....Time.....

Instructions

You are provided with four batches of different coded samples of mango wines and a reference wine to carry out sensory evaluation on them. You are also provided with water to rinse your mouth after tasting each sample. Please taste and rate them on the following quality parameters; colour, clarity, mouthfeel, aroma and general acceptability of each of the samples by inserting the appropriate score in the table below.

You are also requested to give any comments about the products and please be honest.

Thank you.

Hedonic score:

Like extremely- 9; Like very much- 8; Like moderately -7; Like slightly -6; Neither like nor dislike -5; Dislike slightly- 4; Dislike moderately -3; Dislike very much -2; Dislike extremely -1

Sample	Colour	Clarity	Mouth feel	Aroma	General acceptability
SNW					
SAW					
DNW					
DAW					
DCW					

General comments on the products.....
.....
.....
.....
.....

Plate 7 Wine aging in different coloured bottles

