The Potential of Sweet Sorghum [Sorghum Bicolor (L.) Moench] As A Bio- Resource for Syrup and Ethanol Production in Kenya

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented anywhere in any other university or institution either in whole or in part for the award of any degree, fellowship or any other similar title whatsoever

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DEDICATION

This work is dedicated to my late sister, Jane Kemuma Makori for her mentorship, sacrifices and counsel, you shall remain a pillar of hope in my life

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ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
AES	Atomic Emission Spectrophotometry
AOAC	Association of Official Analytical Chemists
DPPH	2, 2-Diphenyl-1-picryl hydrazyl
GAE	Gallic acid equivalent
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
ICRISAT	International Crop Research Institute for Semi-arid Tropics
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MT	Metric Tones
NARI	Nimbkar Agricultural Research Institute
QE	Quercetin equivalent
SS	Sweet Sorghum
SCS	Sugar cane syrup from Rongo
SSJ	Sweet sorghum syrup from JKUAT
SSR	Sweet sorghum syrup from Rongo
rpm	Rotation per minute
TAE	Tannic acid equivalent
TSS	Total Soluble Solids
TAA	Total Anti-oxidant Activity
TTA	Total titratable Acidity

ABSTRACT

Sweet sorghums (SS) (Sorghum bicolor L. Moench) are sorghum varieties that accumulate sugar (>8°Brix) in the stalks and produces grain. The SS crop is a multifunctional crop that can be cultivated for simultaneous production of grain for food or feed and utilization of juice from stalk in production of value-added products like syrup and ethanol. Demographic growth, diminishing arable lands, food insecurity, and climate change resulting from fossil fuels, are issues that demands attention. Sweet sorghum has shown potential to provide, food, fuel (ethanol), feed and fiber hence the need to explore its adaptability in Kenya. The objective of this study was to screen high sugar yielding SS varieties, determine their optimum harvesting time, extract the stalk juice and characterize the syrup and ethanol produced from the SS juice. Twenty five SS varieties and Mtama 1 were cultivated and evaluated in two locations, JKUAT and Awendo, Rongo District. SS stalks were harvested at three different maturity stages with an aim of obtaining the optimum harvesting time, then stripped, weighed and crushed to extract SS juice, which later was used for analysis and production of syrup (>70 °Brix) and ethanol.

Standard methods were used for analysis. Total soluble solids were analyzed by refractometry, total sugar by phenol-sulfuric acid method while sucrose, glucose and fructose content by HPLC method. The total titratable acidity was determined by titration against 0.1N NaOH to a phenolphthalein endpoint, the pH using a pH meter, while moisture and ash content by AOAC methods. Minerals composition was evaluated by spectrophotometry.

The radical scavenging activities of the SS syrup against 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) and its color were evaluated by spectrophotometry. Total phenolic acid and tannin contents were evaluated by Folin –Ciocalteu method while flavonoid content was determined by aluminum chloride method. SS syrup viscosity was evaluated using a rotational viscometer, while density and alcohol content were determined by pycnometry. GC methods with external standards were used to qualitatively and quantitatively determine acetaldehyde, propanol and ethanol concentrations in bio-ethanol.

The SS varieties that had high sugar content included IESV 91018LT, IESV 92008DL, IESV 92038/2SH, SPV 1411, IESV 930042 and Madhura which ranged between 14-23 °Brix. Optimum harvest time varied between soft and hard dough stages depending on variety. SS juice extractability varied significantly ($p=0.05$) with variety, harvest stages and location. Total soluble solids which averaged 18 °Brix varied significantly ($p=0.05$) with variety, maturity stage and location. Location significantly ($p=0.05$) influenced total sugar, sucrose and glucose, ash and moisture content while fructose, pH and total titratable acidity had insignificant effect. The fructose, glucose and sucrose concentration averaged 22.65, 28.13 and 26.4 mg/ml respectively. Moisture content averaged 81.57 %, pH 5.51, total titratable acidity 0.99 % and ash content 2.21%. SS juice minerals namely, potassium, calcium, and magnesium content averaged 0.25%, 0.16%, and 0.11% respectively, while sodium, manganese, zinc, copper, iron and phosphorous had values less than 0.1%. The syrup contained high sugar, appreciable macro and micro-minerals, bio-active compounds; total phenols, flavonoids and tannins

with the highest yield being 256.9g/kg. The syrup radical scavenging activity against DPPH radical revealed more than 50% inhibition, indication of potent anti-oxidants. Time significantly (p=0.05) influenced total residual sugar, pH and ethanol levels (13%) with variety and harvest stage being insignificant. The one-factor-at-a-time method showed temperature of 30 °C, sugar content of 20 °Brix, pH of 5 and 2 % yeast concentration for 72 hours, optimized ethanol content. In conclusion, location, variety and stage of maturity affect SS juice composition hence influencing yields and characteristics of syrup and ethanol.

CHAPTER ONE

INTRODUCTION

1.1 Background

Anthropological evidence suggests that hunters and gatherers consumed sorghum grain as early as 8000 BC (Smith, et. al., 2000). The domestication of sorghum commencing around 6000-3000 BC (Sally et. al., 2007), occurred first in the north eastern Africa, in regions that correspond to the current Southern Sudan, Ethiopia and Kenya on the southern fringe of this presumed domestication center (De Wet, 1978, Smith, et. al., 2000). This is because of the presence of different wild and diverse species in these areas (Hunter and Anderson, 1997). Later on several varieties of sorghum through disruptive selection resulted, generating improved and intermediate sorghum types. The movement of people and trade led to the spread of the improved types to other regions (Doggett, 1970).

Sweet sorghum belongs to the same species of Sorghum bicolor as do cultivated varieties of grain, forage, and broomcorn sorghum. Sweet sorghum is a C_4 plant with high growth rate, strong, branching and efficient root system with lateral shoots and taller pith filled stems. SS have leaves smaller in area as compared to maize leaves, and experience self pollination (Thabsile, 2001). SS exhibits high mineral absorption efficiency, low water requirement of 500-600 mm, tolerant to salty and alkaline soils (pH 5.0-8.5), with minimum tolerated temperature being 7-10 °C and optimal growth temperature of 27-30°C (Cocchi, 2008). Sweet sorghum contains high levels of sugars in the parenchyma juicy stems (Mamoudou et. al., 2006, Seth et. al., 2009). The stems of

sweet sorghum are not only desirable for production of syrup, ethanol, fodder and paper pulp but also for chewing as a snack by people in Southern Africa (Ritter, et. al., 2007, Thabsile, 2001).

Syrups or natural sweeteners generally refer to sweet tasting compounds with nutritional value, composed of mono and or disaccharides. They are made from sugar-rich crops such as maple trees, sugar beet, sugar cane, dates, fruit juices etc or starch-rich crops such as corn, potatoes, wheat etc where starch is hydrolyzed to simple sugars and concentrated to produce syrups. Many of these raw materials require certain environments for growth and take longer to mature, while starch hydrolysis called saccharification process increases the cost of production. Hence the need for a raw material which accumulates simple sugars in its stalks, adaptable to wider climatic and soil conditions and matures within a short period. SS syrup due to its composition, distinctive and excellent taste, has potential application in the food and pharmaceutical industry (Hunter and Anderson, 1997).

The major ethanol feedstocks world-wide include, corn, sugar cane and cellulose among others. Corn as a major feedstock faces several limitations including, requirement of good soils and high rainfall, hydrolysis process of corn starch, which involves use of special enzymes and may take several days, ultimately adds to the cost of ethanol production. Further since corn is used as human food, any diversion to ethanol production could serve only as additional stress on food security. The other major sources include sugar cane and cellulose. Sugar cane although an ideal crop for ethanol production due to its readily fermentable sugars in extracted juices takes two years to

mature and has specific growth environmental conditions e.g. requires relatively large amount of water for growth. Cellulose on the other hand requires special chemical and biological treatments before its sugars can be converted to ethanol eventually adding to the production cost.

Hence sweet sorghum looks a promising feed stock for ethanol production due to the presence of readily fermentable sugars in its stalk juice, its short maturity period and ability to tolerate abiotic and biotic stress.

With growing global concern on environmental pollution and depletion of oil supplies, energy security has become a critical issue for the global community. This has necessitated the seeking of renewable non petroleum sources of energy, such as solar, wind and biomass as a way of reducing reliance on petroleum products and mitigating environmental pollution. Ethanol a liquid fuel obtained from biomass, when blended with gasoline has shown potential as a renewable energy to be used for transportation, manufacturing, cooking and lighting purposes (Ramanathan, 2000). The objective of this study was to screen SS varieties for high sugar content, determine their optimum harvest time, extract the stalk juice and characterize the syrup and ethanol produced from the SS juice.

1.2 Statement of the Problem

The SS syrup derived from SS juice has potential uses in the food industry. Manufacturers in the food industry prefer the use sugar in the form of syrup mostly due to the ease and efficiency of liquids and the favored process economics. Sugar syrups produce pleasant flavor and occasionally cooling sensations, enhance shelf life

properties and may simultaneously provide energy, nutrients and bio active compounds. The consumption of foods containing antioxidants is now a widely considered effective strategy to reduce oxidative stress and damage while exerting beneficial effects on human health. As a result, the food industry in the recent years has been shifting its focus to antioxidant products from natural sources as a replacement for synthetic antioxidants and also as nutraceuticals. The SS syrup fits in well with these expectations as it is derived from a low-cost and of a renewable biomaterial.

Given the increasing demand for fossil fuel, depletion of the world's petroleum resources is inevitable. Ethanol produced from plant materials (biomass) is a sustainable, renewable and clean energy source. It is used for fuel or fuel additive (fuel grade ethanol) in gasoline resulting in reduced dependence on oil and mitigation of noxious emissions to the environment. Other uses include industrial applications (industrial grade-ethanol) and human consumption (food grade ethanol). In the food industry, ethanol could be blended with heating oil used in boilers and generators reducing the cost of manufacturing (Das et al., 2001, Mojovic et al., 2009).

In Kenya, sugar cane molasses the traditional feedstock for ethanol production is faced with several challenges among them diminishing arable land for cultivation, water scarcity and high cost of sugar cane production. Sweet sorghum due to its growth rate, accumulation of simple sugars and biomass, tolerance to biotic and abiotic stress, relatively low input requirements, and adaptability to a wide range of environmental conditions could prove an ideal crop for ethanol production hence the study's aim is to assess the potential of sweet sorghum m as a raw material for ethanol production in

Kenya. SS could be easily and economically converted to bio-based products (Jessup, 2009). Cultivation of sweet sorghum could be necessary since it provides both food and stalk juice (for ethanol) putting to rest the controversy of food verses fuel (Chiaramonti et al., 2002).

1.3 Justification

Sweet sorghum has many excellent characteristics making it an ideal crop for syrup and ethanol production in Kenya. They include high photosynthetic efficiency, rapid growth rate and ability to mature fast within 3 to 5 months (Almodares and Sephani, 1996), drought resistance (Tesso et al., 2005), water lodging, salinity and alkalinity tolerance (Almodares and Hadi, 2009). Sweet sorghum is efficient in water use since it requires about 8 000 m^3, as opposed to 36 000m^3 required by sugar cane per cycle. It has low fertilizer requirement as compared to other crops e.g. it requires 36 % of nitrogen needed for corn production with mechanized cultivation (Smith and Buxton, 1993).

Sweet sorghum plant has a wider adaptability to temperate, subtropical or tropical climates (Almodares and Hadi, 2009, Reddy et al., 2005), high carbon assimilation (50 g/ m^2/day) and ability to accumulate high levels of extractable sugars in the stalks (Jingshan, et al., 1997). All components of the plant have an economic value e.g. the grain can be used for food or feed, stalk for syrup and ethanol, leaves for forage, fiber for mulch, paper pulp or animal feed (Almodares et al., 2008). With population growth and diminishing arable lands, cultivation of crops adaptable to marginal conditions could become necessary in attainment of food security (Edwards, et. al., 2004, Sanchez et. al., 2002, Khush, 1999).

The syrup can be used as an ingredient in bakery products, stir fry base, in baked beans, and can be substituted in any recipe that calls for glucose syrup, maltose syrup, fructose syrup, corn syrup, molasses, maple syrup, honey. It is safe since there are no added chemicals. It also does not crystallize hence could be used as sucrose substitute in food products. Finally processing of SS juice could be a source of income for the farmers in the rural areas.

Ethanol due to its excellent properties (Gnansounou, et. al., 2004, Sheorain and Banka, 2000) has potential use as an energy source (Ratnavathi et al., 2005, Almodares and Sephani, 1997). Ethanol use as a transportation fuel has potential benefits including; reduced emission of carbon dioxide into the environment, mitigating the effects of global warming and climate change, these changes would result in substantial reduction on health costs in our society due to clean air. Ethanol production and use leads to energy diversity and security, it is also considered economically strategic since it reduces trade deficits leading to growth of economies and associated benefits. Ethanol utilization as an energy source offers dramatic advantages on the infrastructure helping develop the rural areas. The production SS syrup and ethanol would enhance the value of sweet sorghum thereby providing additional livelihood opportunities to farmers who will be involved in its cultivation.

1.4 Hypothesis

1. Extractability and composition of sweet sorghum juice are affected by location environments (location), cultivar type and maturity stage.

2. The physico-chemical, bioactive compounds and total anti-oxidant properties of SS

syrup are influenced by environment, cultivar type and maturity stage.

3. Fermentation conditions, substrates and micro-organisms influence ethanol yields.

1.5 Objectives

General objectives

To screen and identify SS cultivar with high sugar content grown in two locations (JKUAT and Rongo), extract the juice, determine the optimum harvest time and produce and characterize (physical and biochemical properties) syrup and ethanol.

Specific objectives

1. To study the effect of growth location, maturity stage and cultivar type on SS juice extractability and the total soluble solids concentration of the extracted SS juice.
2. To identify high yielding SS cultivar types based on total soluble solids of SS juice, determine the optimum harvest time and characterize the composition of SS juice from the selected cultivar types from the two location
3. To produce SS syrup by adopting standard approaches, characterize its physical, chemical and antioxidant properties, and determine the influence of the SS cultivation location.
4. To produce and characterize the physical and chemical properties of SS ethanol produced from SS juice and determine the optimal fermentation process conditions

CHAPTER TWO

REVIEW OF LITERATURE

2.1 World Sorghum Production

Sorghum is an indigenous African food crop (Mamoudou, et al., 2006, Odibo, et al., 2002). The world production of sorghum for 2010-2011 was 62.43 Million Metric Tons (USDA, 2012). Leading sorghum producing countries in 2010-2011 according to USDA (2012) were United States (14.06%), Mexico (11.84%), India (11.21%), Nigeria (10.81%), Sudan (7.38%), and Argentina (7.05%). In terms of harvested quantities, Sorghum is the fifth cereal after Wheat, Rice, Corn, and Barley (Awika and Rooney, 2004), and one of the most important food crops in Africa, Asia and Latin America (Semelsberger, et. al., 2006). Sorghum grain is utilized either directly for human food in Asia and Africa or feed and industrial purposes in developed countries (FAO, 2004, Lochte- Watson, et al., 2000, Ratvanthi and Sashidhar, 1998).

Sweet sorghum refers to a type of sorghum that store carbohydrates in form of simple sugars (Sucrose, glucose and fructose) in the stalk, with sugar concentrations of 8-20% (Rains et al., 1990). SS produces grains (3 to 7 t/ha) utilized as human food or feed for animals, its leaves and bagasse are utilized as fodder, while its stalk juice can be used in the production of ethanol and other industrial products such as syrup (Sally, et. al., 2007, Almodares and Mostafati, 2006).

2.2 Botany of Sweet Sorghum

2.2.1 Taxonomy of Sweet Sorghum

Sorghum is a plant belonging to the tribe of Andropogoneae and family of Poaceae

(Mamoudou, et al., 2006). In 1961, Clayton proposed the name *Sorghum bicolor* (L.) Moench, a name adopted as the correct name for cultivated sorghums (Doggett, 1988). The cultivated sorghums are in the sub species bicolor whose main races are; bicolor, vulgare, candatum, kafir, guinea and durra (Deu et al., 1994). Common names for sorghum most encountered include milo, sorgho, corn and guinea (Mamoudou, et al., 2006).

2.2.2 Genetic Description of Sweet Sorghum

Sorghum varieties are genetically diverse on the basis of morphological traits, differences in iso- enzyme patterns and DNA polymorphism (Deu, et al., 1994, Tao, et al., 1993). Sweet sorghum and cultivated species have a chromosome number of n = 10 while its genome has 750 Mb, twice the genome of rice (Paterson, et al., 2003). Sweet sorghum differs from grain sorghum genetically on the genes responsible for controlling plant height, presence of juice and sugars in the stalks (Mamoudou, et al., 2005).

2.3 Morphology and Physiology aspects of Sweet Sorghum

Sweet sorghum (Plate 1) is a C_4 crop with high photosynthetic efficiency attributed to the stay green gene that enables it to perform photosynthesis permanently (Almodares and Hadi, 2009). Sweet sorghum stems are sweet, juicier and taller about 1.5-3.0 m high and, their diameter ranges from 10 to 50 mm. On the stem, there is a single bud at each node, and buds on lower nodes may develop to form tillers and prop roots while those on the upper nodes may produce branches (Doggett, 1988, Isbell and Morgan, 1982). Sweet sorghum like sugar cane has sugar rich stalks (Belum, et al., 2006), the accumulation sugars starts during flowering and keeps changing in proportion with increasing maturity

(Stokes et al., 1957).

Sweet sorghum leaves, when mature reach a length of about 30 to 135 cm and a width of 1.5 to 13 cm, and have wavy margins with numerous stomata on their surfaces. SS leaves are highly resilient to drought due to waxy coating on their surface (Hunter and Anderson, 1997, Martin, et al., 1975). The total number of leaves is dependent on vegetative period, temperature and day length (Heskerth et al., 1969). The roots are deep, well spread and adventitious in nature with numerous branched lateral roots (Doggett, 1988). SS roots emerge from the coleoptile nodes and their density keep increasing till the grain filling stage, followed by a decline towards maturity (Zartman and Woyenodzic, 1979).

The flower is erect but sometimes curved to form a goose neck. Shorter days with high temperature initiates flowering and may continue over a period of 3 to 15 days depending the panicle size, temperature and variety of SS sorghum (Quinby et al., 1973). A single panicle may carry between 800-30,000 seeds with the upper part having more outcrosses (Maunder and Sharp, 1963). Sweet sorghum seeds remain enclosed by glumes and their color varies from light brown to black (Stoskopf, 1985).

Plate 1: Sweet Sorghum crop at two stages of growth in JKUAT experimental fie

2.4 Sweet Sorghum Cultivars

The selection and development of sweet sorghum cultivars involves breeding programmes with emphasis on desirable attributes such as high sugar yielding, quality juice with excellent processing characteristics and resilience to stress (Thabsile, 2001). The two classes of sweet sorghum cultivars include syrup and sugar cultivars. The syrup cultivars contain high levels of glucose and fructose. They are high yielding, tolerant to water lodging and adaptable to wider soil and climatic conditions. Sugar cultivars are rich in sucrose and contain high purity juice, low sucrose inversion rate, low starch and aconitic acid (Almodares et al., 2008, Cowley and Lime, 1976). Sweet sorghum cultivars include; Brawley, Sart and Rio (Kangama and Rumei, 2005, Coleman, 1983).

2.5 Uses of Sweet Sorghum

Sweet sorghum is a multipurpose crop with many uses. Its stalk are chewed, and the stalk juice is used as raw material for the production of industrial products such as sugars, syrup, ethanol, starch, mono sodium glutamate, acids and vitamins, (Seetharama, et al., 2002). The sweet sorghum leaves are used for fodder while bagasse after juice extraction is used for the manufacture of pulp and paper (Hosseini et al., 2003). Sweet sorghum stalks fiber can be fermented producing methane and hydrogen (Athar 2012). World-wide sorghum grains are utilized for human food; traditionally sorghum is used in unfermented and fermented breads, porridges, snacks, tortillas, malted (traditional beers) and non-alcoholic beverages in many African and Asian countries (Jacques et al., 1999, Graham, et al., 1986). Sorghum being gluten-free, has potential for use in new foods both in the US and Europe (Athar, 2012).

2.6 Sweet Sorghum Juice Characteristics

2.6.1 Extraction of Sweet Sorghum Juice

Sweet sorghum juice is produced through pressing the sweet sorghum stalks to release the sugar rich juice (Figure 1), then the extracted juice is filtered to remove fiber (Gnansounou, et. al., 2004). The recovery of SS juice is unlikely to exceed that of sugar cane due to high fiber in the stalk. The residue (bagasse) remaining when chemically or biologically hydrolyzed, fermentable sugars may result (Woods, 2000, Chavan et al., 2009).

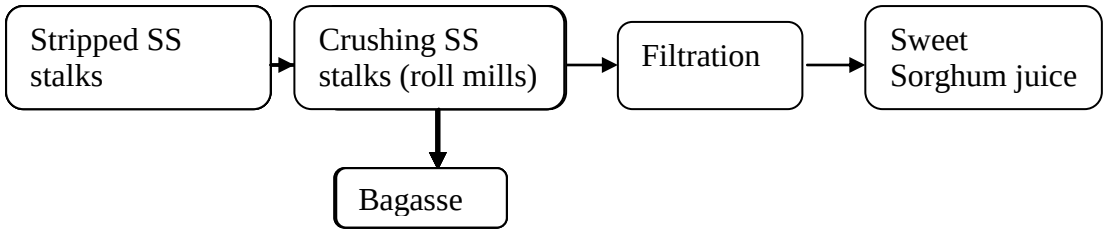

Figure 1: sweet sorghum juice extraction process

2.6.2 Chemical Composition of Sweet Sorghum Juice

The chemical composition of the sweet sorghum juice include, sugars namely, sucrose, glucose and fructose, others include, xylose, ribose, arabinose, sorbose, galactose, mannose, and polyglucose. Variety, temperature, time of the day, and maturity stage exerts an influence on the concentration of these sugars. Sucrose concentration ranges from 6.94 to 16.1 %, fructose and glucose varies from 0.18 to 4.2% and total sugar content varies from 9.19 to 23.33 % according to FAO (1994) and Muminov (1997). The mature stalks of sweet sorghum contain 73% moisture content and the solids (27%) are both divided into structural and non-structural carbohydrates. Approximately 13% are

non-structural carbohydrates composed of sucrose, glucose and fructose in variable amounts according to cultivar type, harvesting season, and other agronomic factors (Mamma et. al., 1996, Phowchinda et al., 1996).

Anglani (1998) suggested a classification of sweet sorghum based on the proportion of soluble sugars in the juice. The first group with high content of sucrose (sugary type) and the second with more monosaccharide content (syrup type). Compared to sugar cane, the main difference is that sucrose content in sugar cane is significantly higher compared to glucose and fructose (90, 4, 6% respectively), and sugars in sweet sorghum juice are very sensitive to microbial deterioration especially after harvesting and crushing (Zhang et al., 2010).

The pH according to FAO (1994) and Chavan et al., (2009) varies from 5.4 to 7.1. Phosphorous varies from 2.21 to 3.57 %, potassium 0.4 to 0.6 %, sodium 0.08 to 0.11 %, magnesium 0.05 to 0.06 % and calcium 0.11 to 0.15 %. Nitrogen varies from 0.53 to 2.18%, and moisture content varies from 83.65 to 93.10% in raw sweet sorghum juice (Batoul, 2006).

2.6.3 Effect of Harvesting Time on Sweet Sorghum

Time of harvesting and determination of maturity of sweet sorghum stalks is crucial in obtaining sweet sorghum juice with high sugar content and juice yield. Since ethanol can be obtained from juice sugar content, therefore identifying the best stage of harvesting and determining maturity could be beneficial in obtaining high ethanol yield. The maturity of sweet sorghum can be classified as early flowering, flowering, late flowering, dough and ripe (Satheesh, 2013). The total sugar content varies as the crop

approaches maturity and with the different stages of development. The early stage of development fructose is more abundant while sucrose is dominant after heading (Sipos et al., 1990). At maturity the sweet sorghum juice sugar content ranges from 10-25 °Brix (Reddy et al., 2005). Hills (1990) suggested that the sugar content in sweet sorghum juice increases between the milk stages and dough stages of the most cultivars; it starts to decline towards physiological maturity.

2.7 Syrups Production

2.7.1 Raw Materials for Syrup Production

The Codex of Alimentarius refers syrup to a concentrated sugar solution with total soluble solids of more than 65 ° Brix. Under the name sweeteners, the Food and Agricultural Organization (FAO) includes products from sugar crops, cereals, fruits, milk, or substances produced by insects with sweetening capacity. Sweeteners include several varieties of monosaccharides, disaccharides, maple syrup, date syrup, caramel, golden syrup, honey and high fructose corn syrup (Popkin and Nielson, 2003, FAO, 1994).

2.7.2 Production and Uses of Syrups

2.7.2.1 Production of syrups

Methods of syrup production include dissolution of sugars in water, evaporation of sweet juices from plants such as sugar cane, maple juice and sugar beet and finally, hydrolysis of starch to produce simple sugars followed by concentration into syrups (Zainab et al., 2011).

2.7.2.2 Uses of syrups

Types of syrups include; glucose syrup, maltose syrup, fructose syrup, sugar cane syrup, maple syrup, honey, sweet sorghum syrup. SS syrups are used as natural sweeteners either in food or pharmaceutical industries where syrups are utilized as sweetening agents, aimed at increasing palatability of substances by imparting the sweet flavor of sugars inherent in them, natural sweeteners are potent, safe, and low in calories. Natural sweeteners have the ability to impart sweet taste hence masking the unpleasant taste of a material in which it has been added. Moreover due to high sugar concentration, low water activity, anti-microbial and anti-oxidant properties sweeteners offer preservation effect by inhibiting growth of micro-organisms and increasing the shelf life of the food products. The presence of readily fermentable sugars in syrups makes them potential raw material for ethanol production, since with dilution and addition of yeasts these sugars could be converted to ethanol.

2.7.3 Syrup Production using Sweet Sorghum Juice

Production of syrup (Figure 2) involves; crushing sweet sorghum stalks between revolving serrated iron rolls followed by filtration of the expressed juice. Holding of the extracted juice for 1-2 hours help settle starch granules. Concentrating the extracted juice to 70-76 °Brix or 105 to 107 °C produced SS Syrup. The boiling should be done slowly with constant skimming to remove floating impurities such as chlorophyll, proteins, gums and waxes, and cooling to 80 °C should be fast within 10- 15 minutes before filling into sterilized bottles (Martin, 1985). Excellent syrup is possible when the sugar content of raw SS juice is above 15 °Brix hence the need for monitoring the sugar

content of the plant with maturity (Nimbkar et al., 2006).

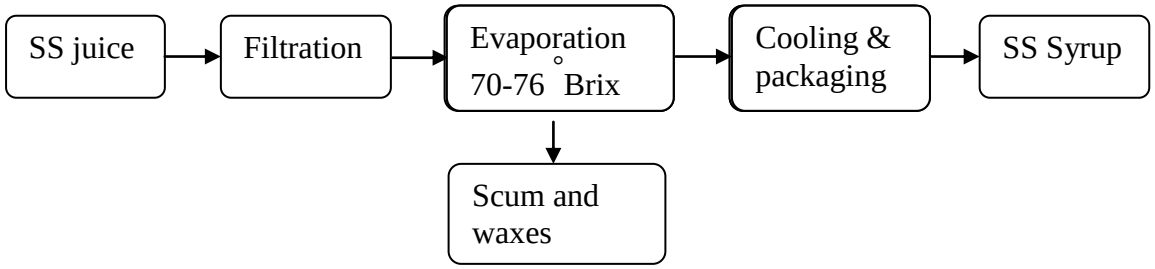

Figure 2: sweet sorghum juice conversion into syrup

2.7.4 Physico-chemical composition of SS syrup compared with Date and Honey Syrup

The table (1) herein provides a comparison of the physicochemical properties of sweet sorghum syrup with date and honey syrups; the objective was to show that sorghum syrup is comparable with other commercially available syrups like honey.

Table1: Physico-chemical composition of SS syrup compared with date and honey syrup

Parameter	**Date syrup**	**Sorghum syrup**	**Honey syrup**
Chemical properties			
Total soluble solids (%)	74.00	72.40	79.70
Moisture content (%)	16.00	22.00	17.20
Ash content (%)	6.8	4.60	0.59
pH	4.55	3.90	5.90
Total acidity (%)	0.66	0.68	0.76
Physical properties			
Density (g/cm³)	1.112	1.3915	1.425
Color properties			
L	20.39	19.07	4.455
a	30.94	4.02	1.304
b	34.99	2.18	5.280
Minerals composition (mg/100g)			
Sodium	13.00	6.95	4.70
Potassium	202.80	1393.3	90.00
Calcium	338.00	68.29	5.00
Magnesium	143.00	40.25	7.00
Manganese	0.203	0.67	1.10
Zinc	104.10	7.060	1.03
Iron	7.80	1.91	0.59
Copper	0.34	0.082	0.35
Phosphorous	67.1	3.09	4.10

SOURCE: Data on date syrup was gotten from Thabet et al., (2010), and that on honey from (Jana et al., 2012, Nimbkar et al., 2006) while that on sorghum syrup was also gotten from Akbulut and Ozcan, (2008).

2.7.5 Bio-active Compounds and Total Anti-oxidant Activity of Plants Materials

2.7.5.1 Bio-active compounds of plant materials

Bio-active compounds refer to non-essential bio-molecules present in foods that upon consumption exhibit the capacity to modulate one or more processes resulting in the promotion of health. Plants synthesize a vast range of bio-active compounds (secondary metabolites) with significant portion consisting of phenolic acids, flavonoids and tannins. There are exciting prospects that select bio-active compounds due to anti-oxidant activity will reduce the risk of many chronic diseases involving oxidative stress and damage such as cardiovascular diseases. The discoveries of novel health effects of bio-active compounds will provide scientific basis for future efforts to modify of fortify foods and food components as means of improving public health (Indu and Alan, 2010, Francesca, 2009).

Continuous efforts have been carried out to determine the presence and concentration of bio-active compounds in various plant materials, in particular agro-industrial by-products since they are renewable and abundantly available (Balasundram et al., 2006). Although bio-active compounds exist in small amounts in foods, their presence and concentration is influenced by growing environmental conditions, genetics and processing conditions (Francesca, 2009). This variability in composition and concentration and consequently their biological activity in relation to environmental,

agronomic and processing factors are seldom investigated.

The nutritional and bio-active compound characterization of sweet sorghum syrup would open the way for further research evidencing the possibility of it bearing the nutritional and health claims.

2.7.5.2 Total anti-oxidant activity of plants materials

The total anti-oxidant activity (TAA) of a plant material may result from the integrated and if any, synergistic actions of different compounds. For example, phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic di-terpenes, ascorbic acid, vitamin E and other compounds in plant materials. Anti-oxidants protect human cells against oxidative stress and or damage caused by free radicals implicated in most diseases processes such as cardiovascular diseases, cancer, asthma, inflammatory conditions, liver diseases and macular damage. It is recognized that foods with high TAA might be protective and recommendations about 'healthy' diets regard the consumption of foods rich in TAA. The concentration of TAA in foods is influenced by genetics, growing conditions, and processing conditions hence this study was aimed at determining the total anti-oxidant activity of SS syrup and its variability with cultivar type, environment and processing conditions. In conclusion, bio-active food components will play an important role in maintenance of human health in the future.

2.8 Ethanol Production

2.8.1 Raw Materials for Ethanol Production

Raw materials used for ethanol production include, simple sugars in juices extracted from sugar cane, sugar beets, sweet sorghum and fruits could be directly fermented

producing ethanol, starches from cereal grains, tuber and root crops, through hydrolysis producing simple sugars that could be fermented to produce ethanol. Cellulose from wood, agricultural residues wastes, liquor from paper mills likewise after hydrolysis could be converted to ethanol.

Fermentation of simple sugars by yeasts produces ethanol (Liu and Shen, 2008). This process depends on choice of feedstock and pre-treatment requirements, fermentation optimization and utilization of by-products (Mojolovic et al., 2009). Although ethanol has traditionally been produced from sugar cane molasses, sweet sorghum juice could compete effectively due to its readily fermentable sugars, ability to withstand marginal environments and low cost of production (Nimbkar and Rajvanshi, 2003).

2.8.2 Micro organisms used for Ethanol Production

The number of micro organisms that can ferment a wide variety of substrates producing ethanol is large, but a good micro organism for fermentation should be easy to handle, able to ferment a variety of sugars, have a high rate of fermentation, and ability to tolerate stress conditions (Zaldivar et al., 2000, Katzen, 1985). Yeasts are the main ethanol producers especially *saccharomyces cerevisiae* and *Saccharomyces carlbergensis*, due to high selectivity and specificity for ethanol production. Under anaerobic conditions, enzymes in *saccharomyces cerevisiae* are capable of converting simple sugars into ethanol (Sineriz, 1982, Harrison, 1963).

2.8.3 Production and Uses of Ethanol

2.8.3.1 Production of ethanol

Ethanol has the potential to ease both natural resource limitation and reduction of environmental pollution, its demand for both direct and indirect uses is growing significantly. Ethanol is produced both as a petrochemical through the hydration of ethylene, and biologically, by fermentation of sugars with yeast or bacteria.

Hydration of ethylene is the primary method for the industrial production of ethyl alcohol as ethanol is commonly known, where in a three-step process using sulfuric acid or by direct hydration of ethylene gas combined with water and passed through a fixed bed of reactor to form ethanol. Other methods of ethanol production involve hydrolysis of lignocelluloses, cellulose or starch either biologically (use of enzyme e.g. cellulases) or chemically (use of dilute or concentrated acids) releasing simple sugars (glucose) which are fermented to produce ethanol. Currently research is focused on the development of the simultaneous saccharification and fermentation (SSF) approach, where the intention is to perform the hydrolysis and fermentation processes simultaneously. Finally, for the simple sugars found in biomass, direct fermentation is required, since the sugars provide a ready source of carbon to be utilized by fermenting yeast or bacteria producing ethanol.

2.8.3.2 Uses of ethanol

Ethanol (ethyl alcohol) is a volatile, flammable, colorless, monohydric primary alcohol with a boiling point at 78.5 °C and miscible with water. Ethanol has many industrial

applications, they include, solvent in the manufacture of toiletries, cosmetics, detergents, disinfectants, liquors and varnish, raw material in the manufacture of organic compounds such as synthetic rubbers, a constituent component of anti- freeze mixtures and fuel substitute for gasoline in rockets propulsion. Other ethanol uses include medical wipes and hand sanitizers, human consumption (alcoholic beverages), and chemical applications (Wood et al., 1968).

With increasing oil and natural gas prices, many countries have resorted to using ethanol in its pure form or as a mixture with gasoline or diesel as fuel for transportation, manufacturing, cooking and lighting purposes (Sineriz, 1982). Ethanol properties include, low toxicity, biodegradability, renewable characteristics and oxygenate resulting in reduced carbon dioxide emissions and its effects (Almodares et al., 2008). Because ethanol is a renewable and clean fuel that can be produced locally from existing raw materials and technologies (Aslam, 1987), the food industry could benefit by blending ethanol with heating oil used in boilers and generators, reducing the cost of manufacturing.

2.8.4 Ethanol Production using Sweet Sorghum Juice

The addition of *Saccharomyces cerevisiae* to sweet sorghum juice (Figure 3), under favorable conditions result in fermentation producing ethanol, carbon dioxide, yeast biomass, and other minor components (Jacques, et al., 1999). Distillation and dehydration helped concentrate ethanol to bio-ethanol (95.6 %).

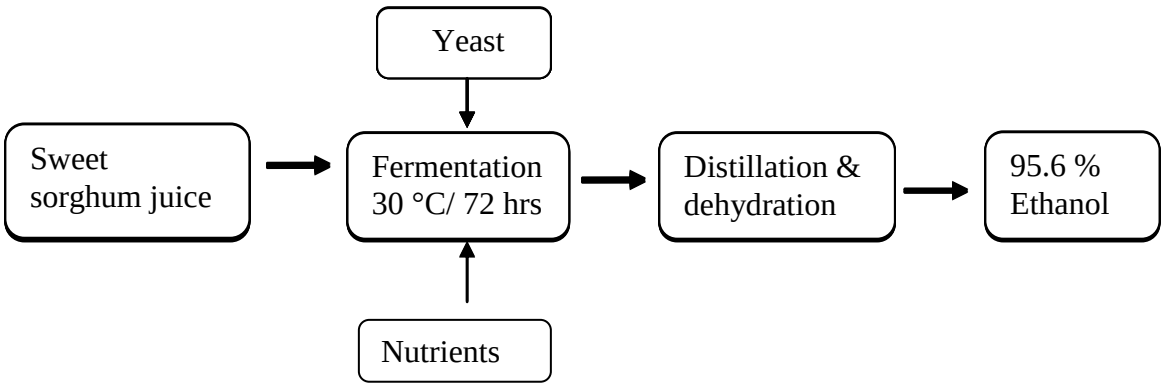

Figure 3: sweet sorghum juice conversion into ethanol (bio-ethanol) adapted from Almodares et al., (2009).

2.9 Optimization of Fermentation Process Conditions using Sweet Sorghum Juice

The interaction of factors such as yeasts, substrate, and environmental conditions namely, temperature, nutrients, pH and time do influence fermentation. Most of these factors govern the fermentation efficiency and resultant ethanol yield hence the effect of optimizing these factors in relation to ethanol production and recovery should be studied. The one-factor-at-a-time classical method of optimization can be applied to optimize medium components and or process conditions. The method involves changing one independent variable while fixing the other variables to a certain level. This strategy is simple, easy and allows the individual effects of process conditions to be seen on graphs (Bibhu et al., 2007).

2.9.1 Effect of Temperature on Ethanol Production

The optimal temperature for ethanol production while using yeast could be between 28 and 32 °C. Conversion of sugars to ethanol requires yeast cells that are tolerant to high ethanol concentrations and relatively high temperature (Prescott and Dunn, 1959).

2.9.2 Effect of Sugar Concentration on Ethanol Production

High sugar concentration acts adversely on yeast while low concentration is uneconomical, hence optimum sugar concentration is desirable for maximum ethanol production. Prescott and Dunn (1959) suggested a 10 to 18 % sugar concentration as satisfactory due to variations among yeasts. For industrial purposes, sugar concentrations I n excess of 20 % result in substrate inhibition reducing ethanol yield (Thomas, 1990).

2.9.3 Effect of pH level on Ethanol Production

The initial pH of the fermentation medium is crucial hence adjusted to favor growth of yeast and inhibit growth of bacteria, optimal pH of 5 favor higher ethanol production.

2.9.4 Effect of Yeast Concentration on Ethanol Production

High fermentation efficiency requires a strong, pure and vigorous yeast strain; reports indicate that a pure growing yeast strain in its logarithmic phase and at 2 % v/v is sufficient to initiate rapid growth and efficient fermentation.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Site, Varieties and Experimental Design

The objective of the study was to determine whether location (environment) has a significant effect on sweet sorghum performance compared to established variety (Kari Mtama 1). The study also attempts to select promising varieties for recommendation to farmers.

3.1.1 Study Site

The experiment was carried out at JKUAT experimental farm in Thika and Rongo District. JKUAT is situated at 1° 10' S latitude, 37° 7' E longitude, with an altitude of 1416 m above sea level. The annual rainfall is 856mm, with a bimodal distribution having a major peak in April and a minor peak in November with a dry period between June and October. The soils found in this area are rhodic ferralsols with pH of 6.2. Rongo is situated at 0° 44' S latitude, 34° 37' E longitude and has an altitude of 1440 m above sea level. The annual rainfall is 1250 mm with 2 cropping seasons. The soils found in these areas are the humic acrisols with pH of 5.81.

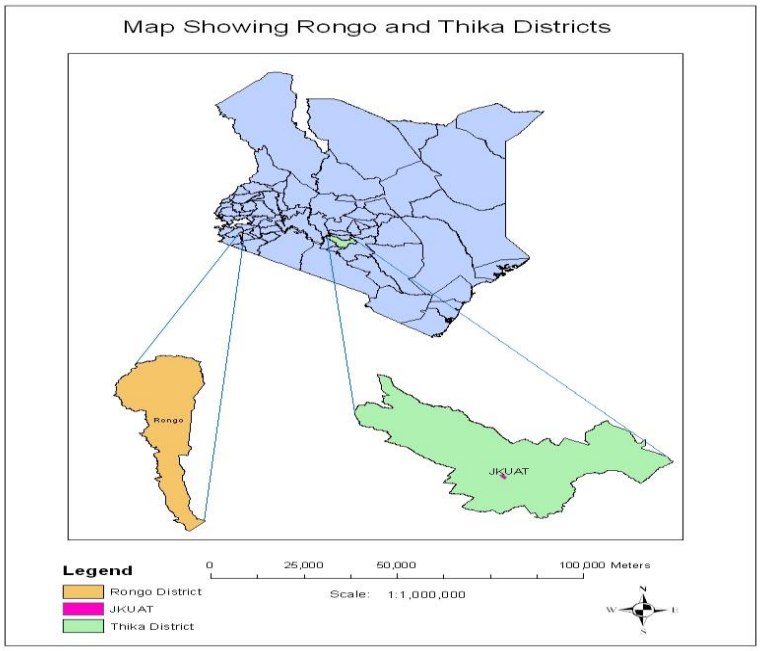

Figure 4: map of Thika and Rongo

3.1.2 Sweet Sorghum Varieties

The varieties used in this study were sourced from the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) Nairobi office and one hybrid variety obtained from Nimbkar Agricultural Research Institute (NARI) Maharashtra India (Table 2). The SS varieties were planted in November 2009, April and August 2010 and April 2011. Kari Mtama 1 a grain sorghum variety sourced from ICRISAT was used as a control in this study.

Table 2: Varieties of sweet sorghum and grain sorghum (Kari Mtama 1) studied

VARIETY	VARIETY
KARI MTAMA 1(Control)	IESV 93042 SH
NTJ 2	Ent 64 DTN
SPV 422	ICSB 654
IESV 92001 DL	ICSR 93034
ICSV 93046	IESV 92038/ 2SH
IESV 91018LT	104 GRD
IS 2331	IESV 92028DL
IESV 92008DL	ICSB 324
LC	SPV 1411
ICSV 700	IESV 92021DL
SDSL 90167	IESV 930042
E36-1	S 32
IESV 91104DL	MADHURA

3.1.3 Experimental Design

The study involved two field trials at JKUAT experimental farm and Awendo division in Rongo District respectively. Twenty five sweet sorghum varieties and one control were evaluated in a completely randomized block design (CRBD) with three replications. Each plot consisted of 4 rows, 5 m long and 3 m wide ($15m^2$), the spacing was 75cm by 30 cm and cultural practices such as weeding were done to assume optimal stalk and sugar yields. Laboratory experiments were performed at food science laboratories in JKUAT.

3.2 Sweet Sorghum Stalks

Harvesting of sweet sorghum stalks was carried out at three different maturity stages of the grain namely; harvest stage 1(soft dough stage), harvest stage 2 (hard dough stage) and harvest stage 3 (physiological maturity stage), the three were selected because maximum sugar yields occurs around this period. Harvesting was done manually, where

ten SS plants were randomly selected from the middle rows and cut as close to the ground as possible, after which the panicles and leaves were removed, then the fresh stalks were weighed and crushed or squeezed using roll mills to extract SS juice. The SS juice was filtered using filter cloth before storage at -20 °C until use.

3.3. Processing Methods

The objective of the study was to extract SS juice from the sweet sorghum stalks, afterwards use the filtered SS juice to process SS syrup and SS ethanol.

3.3.1 Sweet Sorghum Juice Extraction Process

The first processing step, juice extraction involves the use of roller mills to squeezes the sugar rich juice out of the sweet sorghum stalks. The juice is further filtered using a filter cloth before use and storage at – 20 °C. SS juice yield is unlikely to be as high as that of sugar cane due to the relatively high fiber content in sweet sorghum stalks.

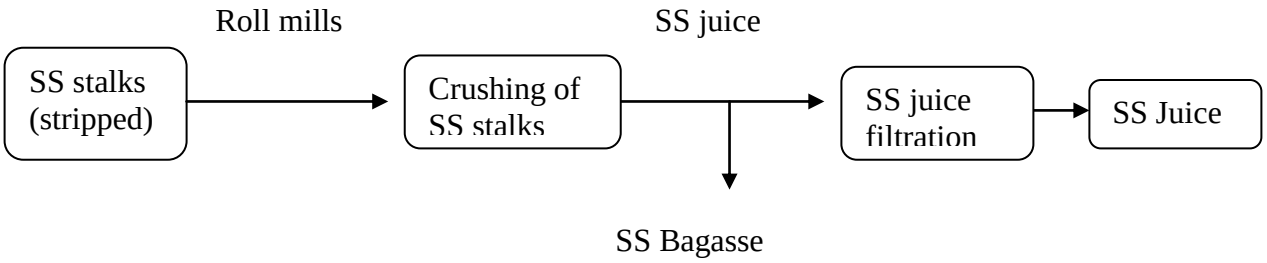

Figure 5: Typical sweet sorghum juice extraction process (Gnansounou et al., 2005)

Juice extraction percentage

Juice Recovery refers to the proportion of SS juice produced to the weight of SS stalks crushed. The traditional method to extract sugar from sweet sorghum stalks is to squeeze them through a roller mill, releasing the sugar rich juice in a process derived from sugar cane sugar extraction. Juice recovery as a percentage may be referred to as juice

extraction percentage (JEP).

The juice extraction percentage is determined using the equation.

JEP = Juice weight / stripped stalk weight× 100

The stalk and juice weight were determined through weighing using a weighing balance and reported as stalk or juice weight per plant.

3.3.2 Sweet Sorghum Syrup Production

3.3.2.1 Preparation of syrup

The initial mean ° Brix of the clarified raw juice was 18% (w/w). After filtration of sap, the juice was subjected to slow heating in a stainless steel pan using a hotplate, with continuous agitation (Figure 6). During concentration, there was formation of foam due coagulation of suspended particles, which was continuously removed. The concentration of the sap was terminated when the total soluble solids content reached 74-76 °Brix. The syrup was then cooled to ambient conditions and packaged in sterilized containers and stored at 4°C until further analyses.

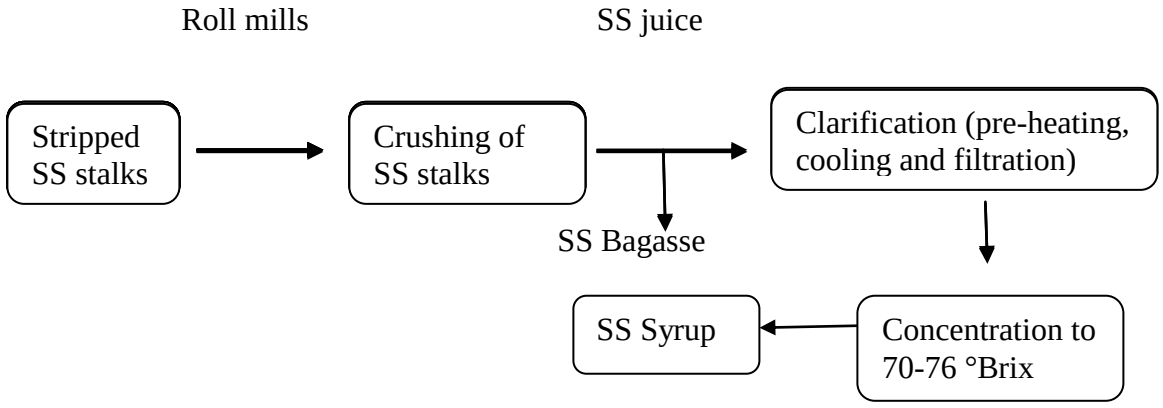

Figure 6: Typical sweet sorghum syrup production process (Saikat et al., 2012)

3.3.2.2 Syrup extraction percentage and syrup yields

Syrup extraction percentage refers to the proportion of syrup recovered from SS juice as a percentage while syrup yield was calculated based on 100 kg of SS juice. The aim was to find out the quantity of syrup likely to be recovered and the influence of maturity stage on syrup extraction and yield. Syrup extraction percentage (SEP) and Syrup yields (SY) were determined using the following equations:

$$\text{Syrup Yield (SY)} = \text{SEP} \times \text{Juice weight}$$

$$\text{Syrup Extraction Percentage (SEP)} = \frac{\text{Syrup weight}}{\text{Juice weight}} \times 100$$

3.3.3 Sweet Sorghum Ethanol Production

The aim of the experiment was to ferment unpasteurized SS juice using *S. cerevisiae* yeast at pre-set conditions.

3.3.3.1 Activation of yeasts (Saccharomyces cerevisiae)

The dry yeast was activated using pre-culture broth, whereby 1.0g of dry yeast was added into 19 mls of pre-culture broth. The pre- culture broth contained; 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extract, 1.0 KH_2PO_4, and 0.5g $MgSO_4.7H_2O$ per liter. Then the mixture was shaken at 200 rpm for 25 to 30 minutes (Xiaorong et al., 2010).

3.3.3.2 Fermentation of sweet sorghum juice

Fermentation involved placing 400 mls of SS juice in 500 mls flask and the adding activated yeast at 2 % v/v. Fermentation conducted in incubators pre- set at 30±1 °C for 72 hours. To monitor fermentation, 70 mls of the fermenting broth were aseptically drawn at 24 hour intervals. From the 70 mls, 20mls were centrifuged at 5000 rpm for 15

minutes at 4 °C to remove cells, and the supernatant was used for pH and total residual sugar determination, while the remaining 50 mls were subjected to distillation to recover ethanol.

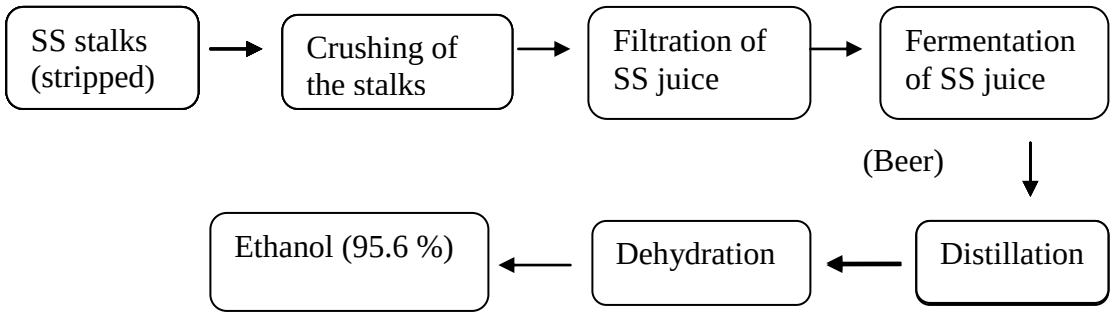

Figure 7: Typical sweet sorghum ethanol production process (Jia et. al., 2013).

3.4 Analytical Methods

This was performed in order to characterize the properties of the SS juice, sweet sorghum syrup and sweet sorghum ethanol.

3.4.1 Chemical Methods

The aim was to characterize the chemical properties of SS juice, syrup and ethanol.

3.4.1.1 Determination total soluble solids

Total soluble solids content of a solution is determined by the index of refraction. This is measured using a refractometer, and is referred to as the degrees Brix. The total soluble solids of sweet sorghum juice, SS syrup and fermentation broth (total residual sugar) was determined using a hand refractometer (Atago, 31617 Model).

3.4.1.2 Determination of moisture content

The experiment was aimed at determining the moisture content of SS juice and SS

syrup. Moisture content was determined according to the method of association of official's analytical chemists (AOAC 1990) as follows: Two grams of the sample were weighed in a clean dry and pre-weighed crucible, and then placed in an oven at 105 ± 2°C for 3 hours to vaporize water. Then the crucible was transferred into a desiccator and allowed to cool before determining its weight. Further placement in the oven was carried out until constant weight was obtained.

Moisture content (M.C) was calculated using the formula;

$$M.\ C\ (\%) = (W_2\text{-}W_1) - (W_3\text{-}W_1)/\ (W_2\text{-}W_1) \times 100$$

M.C: Moisture content W_2: weight of crucible with sample before drying

W_1: weight of empty crucible W_3: weight of crucible and sample after drying

3.4.1.3 Determination of pH

The pH of SS juice, and fermentation broth was measured using pH/conductivity meter, (Denver, model 20), according to Meade and Chen (1977), who reported that the pH of juices was to be determined without dilution while the SS syrup required dilution before determination. The standard buffer solutions and samples were cooled to 25 °C while the electrode and receptacle were rinsed using a portion of the solution to be tested. The beaker was filled to a depth that would be covered by the bulb of the glass electrode. The temperature of the solution was recorded, the system was allowed to come to equilibrium and the pH was recorded.

3.4.1.4 Determination of total titratable acidity

The total titratable acidity of SS juice and SS syrup were determined using the following procedure. An aliquot sample (10 ml) was titrated with a standard alkali (0.1 NaOH) to a phenolphthalein end point, and the titer was recorded. The following formula was used to calculate the total titratable acidity;

% acid (wt/wt) = N×V ×Eq. wt ×100/ W×1000

N is normality of the titrant usually NaOH W is mass of sample (g)

V is volume of titrant (mls) 1000 is the factor relating mg to g

Eq. wt is the equivalent weight of predominant acid (mg/ mEq)

3.4.1.5 Determination of total sugar using phenol-sulfuric acid method

The total sugar of SS juice was determined using the following procedure; One milliliter of the sample was added to 1 milliliter of phenol solution (50 g/L) in a tube, and the contents were mixed, a blank was also prepared. Then 5 ml of concentrated sulphuric acid was added rapidly so that the stream produced a good mixing and the tube was agitated. After 10 minutes the sample was placed in a water bath at 25-30 °C for 20 minutes, then the absorbance of the sample was measured at 490 nm, using glucose as standard. The amount of sugar was determined using the reference standard curve.

3.4.1.6 Determination of sucrose, fructose and glucose of sweet sorghum juice (HPLC Method)

The simple sugars namely, fructose, glucose and sucrose of SS juice were determined using the following procedure; Twenty mls of the sample were centrifuged at 10,000

rpm for 10 minutes at 25°C, the supernatant then filtered using a 0.45µm filter, and diluted in the ratio 1:1 with acetonitrile-water mixture (1:1). The separation of sugars was accomplished by aminopropylsilyl column (NH_2P-5O 4Ɛ, 4.6×250mm), with a mobile phase of 75 % acetonitrile and 25 % de-ionized water. The flow rate was 0.6 mL/min with oven temperature of 35°C and a refractive index detector (RID) for detecting the separated sugars. 20 µl of both sample and standard were injected with quantification being accomplished by use of standards calibration curves (Almodares et al., 1997).

3.4.1.7 Determination of ash content

Ash content refers to the inorganic residue remaining after water and organic matter have been removed by heating from foods. Ash provides a measure of the total amount of minerals since they are not destroyed by heating. The ash content of the SS juice and SS syrup were determined according to AOAC Method (1990) as follows; Two grams of the sample were placed in a clean pre-weighed crucible, and then the crucible with its content was ignited in a muffle furnace at about 550 °C for over 3 hours, until light grey ash was obtained. The crucible was removed from the furnace, cooled in a desiccator and weighed. The procedure was repeated till a constant weight was obtained. Ash content was calculated using the following equation:

Ash Content % = W_2-W_1/W_3× 100

W_1 is the weight of crucible W_2 is the weight of crucible with ash

W_3 is the weight of the sample

3.4.1.8 Determination of mineral composition

The Minerals content was determined according to the AOAC methods. Two grams of syrup samples were dried in the oven, ashed in muffle furnace and diluted with 1% HCl. Mineral constituents (Ca, Mg, Mn, Zn, Cu and Fe) were determined using Atomic Absorption Spectrophotometry, while Atomic Emission Spectrophotometry was used to analyze Na and K respectively in using Atomic Spectrophotometer (Model AA 6200, Shimadzu, Kyoto, Japan).

3.4.2 Bio-active Compounds and Total Anti-oxidant Activity SS syrup

The objective of the study was to characterize and evaluate the influence of location on bio-active compounds and total anti-oxidant activity in SS syrup.

3.4.2.1 Sample preparation

The ethanolic extracts of syrups were prepared by dissolving 100g of syrup in 1 liter of ethanol at room temperature for 48 hours; then the extracts were filtered through a filter paper (Whatman No. 42) and finally concentrated using a rotary evaporator with a water bath set at 40 °C. The recovery of the extract ranged from 9-20 % w/w.

3.4.2.2 Total phenolic acid content determination

Total phenolic content was determined according to the Folin-Ciocalteu procedure (Singleton and Ross, 1965). A 100-µL aliquot of the extracted sample was added to 500 µL of 0.2N Folin-Ciocalteu reagent and 6 ml of distilled water. After mixing the contents for 1 min, 4 mL of saturated Na_2CO_3 was added. Samples were left to stand at room temperature for 90 min and absorbance measurements taken at 725nm using a

UV-VIS 1601 Shimadzu spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid was used as a reference standard, and the results expressed as milligram Gallic acid equivalents (mg GAE) per 100 g of syrup.

3.4.2.3 Total flavonoid content determination

Aluminium Chloride method was used for the determination of total flavonoid according to Chang et al., (2000) with slight modification. 2 ml of syrup extracts was mixed with 0.1 ml of 10% aluminum chloride (m/v), 0.1 of 1 mol/L potassium acetate and 2.8 ml distilled water. A volume of 10 % (m/v) aluminum chloride was substituted by the same amount volume of distilled water in blank. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm using a Shimadzu 1601 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Quercetin was used as reference for the calibration curve and the results were expressed as quercetin acid equivalents (mg QAE/100 g) per 100 g syrup.

3.4.2.4 Total tannin content determination

Five grams of the sample was dispersed in 50 ml of distilled water and shaken. The mixture was then allowed to stand for 30 minutes at room temperature before it was filtered using a filter paper (Whatman No. 42). Two milliliters sample and Folin-Ciocalteu reagent were added to each test tubes followed by 2.5 ml of saturated sodium carbonate solution. The content of each tube was made up to 50 ml mark and incubate at room temperature for 90 minutes. The absorbance was measured using a UV-Vis spectrophotometer (Shimadzu 1601, Japan) at 760 nm. Tannin content was expressed as

tannic acid equivalent (TAE) according to Kirk and Sawyer, (1998).

3.4.2.5 Total anti oxidant activity determination

The scavenging capacity of the syrup extracts against 2, 2-Diphenyl-1-picryl hydrazyl radical (DPPH) were determined using UV-Vis spectrophotometer (Shimadzu 1601, Japan) at 517 nm. The extracts were prepared by dissolving 10g of sample in 100mls of ethanol overnight, and then concentrated using a rotary evaporator with a water bath at 40 °C. The extract concentrations were prepared at 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 mg/ml in methanol. 1ml of sample extract was placed in a test tube, and 3 ml of methanol added followed by 0.5 ml of 1mM DPPH in methanol, a blank solution was prepared containing the same amount of methanol and DPPH. Ascorbic acid was used as a standard. Inhibition of free radical DPPH in percent (%) was calculated using the formula: according to Ayoola et al., (2008).

Percentage inhibition (%) = $[(A_{control} - A_{sample})/A_{control})] \times 100$

$A_{control}$ is the absorbance of the control reaction (containing all reagents except test samples); and As_{ample} is the absorbance of the test samples. Synthetic antioxidant L-ascorbic acid was used as a positive control.

3.4.3 Determination of Alcohol Content, Acetaldehyde and Alcohol Profile

The goal of the experiment was to determine the alcohol content and the composition of the ethanol (bio-ethanol) produced after distillation and dehydration.

3.4.3.1Determination of alcohol content

A top-filled pycnometer allows for obtaining a given volume of measured and/or

working liquid with a high accuracy. First the pycnometer was filled with distilled water and then the unknown liquid. The volume of water that was used to fill the pycnometer and the stopper was compared with that of the sample (alcohol distillate), thereafter, the mass of water compared to the mass of distillate, the ratio obtained thereof was used to determine alcohol content from the alcohols tables.

3.4.3.2 Determination of acetaldehyde and alcohol profile

Acetaldehyde and alcohols concentration profile were determined using gas chromatography (Shimadzu, GC-14B, Japan), with a stainless column, polyethylene glycol (PEG-20M), an auto headspace samples injection and a flame ionization detector (FID). The chromatogram was run at 220 °C detection column and 150 °C injection column temperatures respectively, using N_2 as a carrier gas and H_2 as a combustion gas. Standards (external) of different alcohols were used to calibrate standard curves for each alcohol. A sample of 1 µl was injected, and all determinations were done in duplicates. Concentrations of different alcohols were determined from peak areas of the obtained chromatograms.

3.4.4 Physical methods

The study aimed at characterizing the physical properties of SS syrup, these physical characteristics included, color, viscosity and density.

3.4.4.1 Color characteristics of SS syrup

The color of the syrup was determined using a NF-333-Color spectrophotometer (Nippon Denshoku Industries, Japan). Results were expressed according to CIELAB

color coordinates system, L*, a* and b*; where L* represents the perceived lightness, a* and b* indicate the change in hue from red to green and from yellow to blue, respectively.

3.4.4.2 Viscosity characteristics of SS syrup

The apparent viscosity of the syrup was measured according to the procedure using a Brookfield viscometer (model HBT Brookfield Eng. Lab., USA) at 25 ± 1°C using spindle No.2 , RPM of 30 and in a 250-ml capacity glass beaker (60 mm diameter), (Akbulut and Ozcan, 2008).

3.4.4.3 Density characteristics SS syrup

Density was determined at 25 °C, by weighing the sample in a 25 ml pycnometer. The pycnometer was filled with syrup and incubated at 20 ºC for 1 hour for equilibration before determination (AOAC, 1990).

3.4.5 Determination of Optimal Fermentation Process Conditions

The objective was to investigate the optimal fermentation process conditions necessary to achieve high ethanol yields. These fermentation process conditions included temperature, pH, total soluble solids, yeast concentration and time which have influence ethanol productivity.

3.4.5.1 Effect of temperature on ethanol production

To study the effect of temperature on ethanol productivity, fermentation was carried out at temperatures 25, 30 and 35 °C. The initial sugar content was 17 °Brix and pH of 5.86. The total residual sugar, pH changes and alcohol content were monitored at intervals of

24 hours.

3.4.5.2 Effect of sugar concentration on ethanol production

To study the effect of sugar concentration on ethanol production during fermentation, the sugar concentration of SS juice was adjusted to 15, 20 and 25 °Brix; the experiment was conducted at temperature level of 30 °C and pH of 5.86. The total residual sugar, pH changes and alcohol content, were monitored at intervals of 24 hours

3.4.5.3 Effect of pH on ethanol production

To study the effect of pH on ethanol production, fermentation was conducted at pH levels of 5, 6, and 7 at 30°C and 20 °Brix. The pH of the medium was adjusted by gradually adding 2N H_2SO_4 and 2N NaOH (if required). The total residual sugar, pH changes and alcohol content were monitored at intervals of 24 hours.

3.4.5.4 Effect of yeast concentration on ethanol production

To study the effect of yeast concentration on ethanol production, fermentation conducted at yeast concentration levels of 2, 4 and 6 %, at 30 °C and 20 °Brix. The total residual sugar, pH changes and alcohol content were monitored at intervals of 24 hours.

3.5 Statistical Analyses

Statistical analyses were performed using Genstat Programme version 14. The means were compared using least significant difference (LSD) and p- value (at 5 %) and the standard error of difference of means (s.e.d) was determined as a measure of precision.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Sweet Sorghum Juice Extractability

The experiment was conducted to study the effect of variety, maturity stage and agro-ecological zone (environment) on SS juice extractability characteristics. Twenty five SS varieties were studied alongside one grain sorghum variety Kari Mtama 1 for comparison with the SS varieties. To investigate the effect of environment on juice extraction properties the 26 varieties were planted in JKUAT and Rongo respectively. However in Rongo only three varieties were able to grow, the rest failed, hence only the results for the three varieties are represented hereunder.

4.1.1 Stalk weight

Characteristics stalk weight was significantly ($p=0.05$) influenced by the varieties and harvest stages (Table 3 and 4). Although varieties SPV 422, IESV 91018 LT, IS 2331, IESV 92008 DL, LC, SDSL 90167, E 36-1, IESV 93042 SH, Ent 64 DTN, ICSR 93034 and ICSB 324 showed no significant difference. The highest stalk weight was 370.30 g/plant for variety ICSV 93046 while the lowest was 11.20 g/plant for variety NTJ 2. Stalk weight decreased with maturity and in addition JKUAT zone produced heavier stalks than the Rongo zone. Stalk weight differences could be attributed to varietal differences, cultural practices, diseases, time of harvest, soil moisture and cultivation conditions (Freeman et al., 1973, Purseglove, 1975).

4.1.2 Juice weight

The juice weight was significantly influenced by variety and harvest stages (Table 3 and 4). The highest juice weight was 146.5g/plant for variety ICSV 93046 while the lowest was 1.90 g/plant for SPV 1411. Juice weight decreased with maturity, e.g. the harvest stage 1 recorded the highest means for varieties grown in JKUAT while the harvest stage 2 produced the highest mean for varieties from the Rongo environments. SS juice characteristics are influenced by cultivation location, cultivar type and crop harvesting time. This suggests that sorghum cultivar selection is a very important factor to consider prior to site selection when cultivating sorghum crops for stalk juice production.

4.1.3 Juice extraction percentage (JEP)

The juice extraction percentage showed significant ($p=0.05$) differences for both variety and harvest stages except variety SPV 1411 (Table 3 and 4). The highest juice extraction percentage was 49.10 % for Madhura variety, while the lowest was 16.04 % for variety SPV 1411 respectively. Juice extraction percentage decreased with maturity showing maximum extractability at the soft and hard dough harvest stages for SS juice from JKUAT and Rongo respectively. JKUAT zone produced higher juice extractabilities compared to Rongo zone.

Table 3: Effect of maturity stage and variety on stalk weight, juice weight and juice extraction percentage (JKUAT) (n=10)

Variety	Stalk weight/plant (g)			Juice weight / plant (g)			Juice extraction percentage (%)		
	Harvest Stage 1	Harvest Stage 2	Harvest Stage 3	Harvest Stage 1	Harvest Stage 2	Harvest Stage 3	Harvest Stage 1	Harvest Stage 2	Harvest Stage 3
Kari Mtama 1	191.9	178.0	184.4	68.50	60.00	59.50	37.27	35.60	34.00
NTJ 2	132.9	85.80	93.80	53.50	31.50	29.00	38.11	41.50	26.40
SPV 422	206.8	180.5	191.5	82.50	57.00	59.00	42.38	33.90	29.40
IESV 92001 DL	214.4	249.4	217.3	79.50	56.40	66.50	40.86	34.30	27.60
ICSV 93046	370.3	273.2	276.7	146.5	105.5	103.0	39.89	40.80	37.10
IESV 91018 LT	182.6	158.8	160.1	59.50	49.00	42.50	35.14	34.10	26.80
IS 2331	243.5	185.8	216.1	92.50	58.00	70.50	38.93	33.20	35.00
IESV 92008 DL	196.5	130.5	170.2	74.50	38.00	43.50	39.59	33.20	26.40
LC	231.6	194.0	212.9	90.50	66.50	74.00	41.09	40.30	36.20
ICSV 700	303.7	289.5	246.8	126.5	110.0	99.00	44.87	41.70	42.20
SDSL 90167	172.8	148.2	156.7	68.00	52.00	47.00	40.38	37.60	30.70
E 36-1	160.5	184.5	186.0	65.00	57.50	60.50	41.00	33.40	34.30
IESV 91104 DL	259.9	239.2	176.7	100.0	94.00	63.50	40.50	37.20	36.20
IESV 93042 SH	179.2	128.5	204.4	69.50	42.00	97.50	41.02	32.60	44.50
Ent 64 DTN	158.5	143.0	226.2	67.50	40.50	67.50	44.87	29.90	29.50
ICSB 654	92.00	102.0	73.30	31.50	33.50	19.00	40.38	34.10	26.40
ICSR93034	208.5	176.8	148.6	80.00	57.50	50.00	41.00	34.80	34.80
IESV92038/2SH	121.7	128.5	84.50	45.50	39.50	25.50	39.72	34.00	30.50
104 GRD	277.0	192.8	200.4	95.50	50.50	59.00	33.29	26.20	26.10
IESV92028 DL	242.9	257.5	290.3	37.50	102.5	118.5	41.91	43.80	41.50
ICSB 324	191.5	132.8	141.8	60.50	41.00	44.50	42.81	34.50	33.50
SPV 1411	203.9	185.0	211.3	69.00	40.00	51.00	30.42	23.40	22.30
IESV92021 DL	118.6	141.8	93.80	40.00	41.50	22.50	36.00	36.70	21.50
IESV930042	152.3	135.0	126.5	57.00	42.00	42.50	41.08	36.20	33.80
S 32	139.0	93.50	77.50	53.00	31.00	29.50	39.49	34.70	27.40
Madhura	295.5	362.7	262.1	118.5	124.0	127.0	41.12	36.00	49.10
Mean	201.8	179.9	178.1	74.30	58.50	60.40	39.44	35.10	32.40
Range	92.00-370.30	85.80-362.7	73.30-290.30	31.50-146.50	31.00-124.00	19.00-127.00	30.42-44.87	23.40-43.80	21.50 49.10
LSD $_{(0.05)}$	61.65	47.05	59.8	62.02	47.66	88.98	8.651	15.17	21.29
p-value	<0.001	<0.001	<0.001	0.11	0.01	0.62	0.40	0.78	0.65
s.e.d	31.38	23.95	30.44	30.17	23.19	43.29	4.21	7.38	10.36
Harvest stage	Stalk weight			Juice weight			Juice extraction percentage (JEP)		
LSD $_{(0.05)}$	11.93			2.65			2.66		

Table 4: Effect of maturity stage and variety on stalk weight, juice weight and juice extraction percentage (Rongo)
(n=10)

Variety	Stalk weight / plant(g)			Juice weight/plant (g)			Juice extraction percentage (%)		
	Harvest Stage 1	Harvest Stage 2	Harvest Stage 3	Harvest Stage 1	Harvest Stage 2	Harvest Stage 3	Harvest Stage 1	Harvest Stage 2	Harvest Stage 3
NTJ 2	54.00	37.40	11.20	10.00	11.50	2.60	18.40	31.50	23.12
SPV 1411	79.00	88.00	12.00	20.00	17.00	1.90	25.16	19.20	16.04
Madhura	94.00	164.00	71.50	18.00	32.00	9.00	19.05	20.00	12.56
Mean	75.70	96.50	31.57	16.00	20.17	4.50	20.87	23.60	17.24
$LSD_{(0.05)}$	10.82	42.56	5.06	6.92	4.35	1.25	5.73	9.80	3.21
P- value	0.001	0.001	0.001	0.03	0.001	0.001	0.05	0.04	0.001
s.e.d	0.83	17.39	2.07	2.83	1.78	0.51	2.34	4.00	1.31
Harvest stage	Stalk weight			Juice weight			Juice extraction percentage		
$LSD_{(0.05)}$	21.12			4.29			4.74		

4.2. Sweet Sorghum Juice Characteristics

The experiment was conducted to determine the composition of SS juice. The varieties with the highest Brix concentration and common in both JKUAT and Rongo were selected, and their extracted juice was used for analysis and utilization in syrup and ethanol production. For Rongo only three varieties grew of which two were selected. Total soluble solids are important since it is an indicator of the sugar contents of varieties, influences syrup and ethanol yields, and also with TTAs the syrup sensory properties.

4.2.1 Total Soluble Solids of SS juice

Total soluble solids (TSS) was significantly (p=0.05) influenced by variety and harvest stage for SS juice from Rongo, while for SS juice from JKUAT harvest stages 1 and 3 showed significant difference with variety and harvest stage 2 had insignificant effect

(Table 5 and 6). The highest Brix values were 23.00, 22.50, 22.00 and 21.5°Brix, for varieties IESV 91018LT, SPV 1411, IESV 930042, IESV 92008DL, IESV 92038/2SH and Madhura respectively. These varieties were selected as the best for growth at harvest stage 1 under tested conditions (Kenya). The range 13.5 to 23.0 °Brix is similar to the findings of Hunter and Anderson (1997) who reported a TSS range of 10 to 25 %, Almodares et al. (1997) also reported a soluble solids concentration range of 13 to 24 °Brix. Total soluble solids decreased with maturity due to the transfer of synthesized sugars from the stem to the grain for development e.g. growth stage is a factor affecting carbohydrate content according to Tsuchihashi and Goto (2004). For the three harvest stages SS juice from JKUAT produced higher Brix values compared to SS juice from Rongo, this is could be attributed to growing conditions (Freeman, et al., 1973).

Table 5: Effect of maturity stage and variety on sweet sorghum juice total soluble solids (°Brix) (JKUAT) (n=10)

Variety	Harvest Stage 1	Harvest Stage 2	Harvest Stage 3
Kari Mtama 1	21.00	19.50	18.00
NTJ 2	19.00	20.50	15.00
SPV 422	21.00	21.50	19.50
IESV 92001 DL	20.50	20.00	21.00
ICSV 93046	21.50	23.00	19.50
IESV 91018 LT	23.00	22.50	18.50
IS 2331	20.00	21.00	16.50
IESV 92008 DL	22.50	21.00	19.00
LC	21.50	17.50	16.50
ICSV 700	19.50	19.50	15.00
SDSL 90167	21.00	22.50	18.50
E 36-1	21.50	20.50	18.00
IESV 91104 DL	20.00	21.00	16.00
IESV 93042 SH	22.00	20.50	17.50
Ent 64 DTN	21.50	22.50	20.50
ICSB 654	19.00	20.00	13.50
ICSR 93034	21.00	21.00	18.00
IESV 92038/2SH	22.00	21.00	19.00
104 GRD	21.00	21.50	20.00
IESV 92028 DL	19.50	21.50	16.00
ICSB 324	19.50	19.50	18.00
SPV 1411	23.00	21.00	19.00
IESV 92021 DL	21.50	21.00	20.50
IESV 930042	23.00	21.50	17.00
S 32	21.00	20.00	17.00
Madhura	21.50	20.00	19.50
Mean	21.06	20.81	17.94
Range	19.00-23.00	17.50-23.00	13.50-21.00
LSD (5%)	3.057	3.537	6.98
p-value	0.29	0.57	0.89
s.e.d	1.49	1.72	3.39
Harvest stage	Total soluble solids (°Brix)		
$LSD_{(0.05)}$	0.81		

Table 6: Effect maturity stage and variety on sweet sorghum juice total soluble solids (°Brix) (Rongo) (n=10)

Variety	Harvest Stage 1	Harvest Stage 2	Harvest Stage 3
NTJ 2	16.00	19.00	17.00
SPV 1411	16.00	18.00	15.00
Madhura	14.00	17.00	14.00
Mean	15.33	18.00	15.33
LSD (5%)	3.46	2.83	2.83
P-value	0.33	0.30	0.10
s.e.d	1.41	1.16	1.16
Harvest stage	Total soluble solids (°Brix)		
$LSD_{(0.05)}$	1.40		

4.2.2 Chemical Characteristics of Sweet Sorghum Juice

The goal of the experiment was to characterize and determine the effect of location, harvesting stage and cultivar type on the chemical properties of sweet sorghum juice.

4.2.2.1 Moisture content of juice

Moisture content showed no significant (p=0.05) difference among the varieties and harvest stages (Table 7 and 8). The highest moisture content was 89.15 % for Madhura variety while the lowest was 73.99 % for variety SPV 1411. The range observed was smaller as compared to the findings of Batoul (2006) who found a moisture range of 83.65 to 93.10 % for sweet sorghum juice; this could be due to the presence of more solids in the SS juice. In explaining this Hunter and Anderson (1997) noted that sweet sorghum varieties as opposed to grain sorghum retained water in their stalks with maturity as a way to balance the sugar concentrations. High moisture content could be associated to reduced storage period of raw SS juice under normal conditions.

4.2.2.2 The pH of SS juice

The pH showed no significant (p=0.05) difference among the varieties and harvest stages (Table 7 and 8). The highest pH value was 5.74 for variety IESV 91018LT while the lowest pH value was 5.27 for Madhura variety. This pH range is similar to the findings of FAO (1994) where the pH varied from 5.4 to 6.0 in raw SS juice. The sweet sorghum juice from JKUAT had a higher pH value as compared to that from Rongo, which probably could be due to low acid content. The pH of sweet sorghum juice during fermentation could influence the rate of yeast growth, metabolism and ethanol production.

4.2.2.3 Total titratable acidity of juice

Total titratable acidity (TTA) the sum of titratable acids in the SS juice showed no significant (p=0.05) difference for the varieties and harvest stages (Table 7and 8), this trend could be due the action of natural yeasts on SS juice. The highest total acidity content was 1.36% for variety IESV 91018LT while the lowest content was 0.44 % for Madhura variety. Elena (2007) while determining total titratable acidity of sweet sorghum juice found a TTA content of 0.24 % (as % malic acid), also Saikat et al., (2011) found a TTA content of 0.28 % (as % citric acid) for SS juice. SS juice from JKUAT had higher total titratable acidity content when compared to SS juice from Rongo. Total titratable acidity content is influenced by the presence of organic and inorganic acids such as citric, malic, tartaric, and acetic and phosphoric acids in SS juice. The variation between the 2 zones could be attributable to differences in the environment conditions (Suzanne, 2010).

4.2.2.4 Ash content of SS juice

The goal was to determine the ash (inorganic) content of SS juice. The ash content showed no significant (p= 0.05) difference among the varieties and harvest stages (Table 7 and 8). The highest ash content was 2.87% for variety SPV 1411 while the lowest was 0.85 % for Madhura variety. The findings were similar to those of Vasilica et al., (2010) who reported a maximum ash content of 3 % for SS juice. The SS juice from JKUAT exhibited higher ash content as than that of Rongo this could be due to difference in growing conditions.

Table 7: Effect of maturity stage and variety on SS juice characteristics (JKUAT) n=10

	Harvest Stage 1				**Harvest Stage 2**				**Harvest Stage 3**			
Variety	M.C (%)	pH	TTA (%)	A.C (%)	M. C (%)	pH	TTA (%)	A.C (%)	M.C (%)	pH	TTA (%)	A.C (%)
IESV 91018 LT	82.39	5.66	1.36	2.37	81.30	5.72	0.87	1.96	82.17	5.74	0.77	2.03
IESV 92008 DL	82.34	5.70	0.91	2.07	80.37	5.72	0.93	2.31	84.47	5.70	0.78	1.65
IESV 92038 /2SH	82.14	5.62	0.92	2.56	80.37	5.59	1.02	1.74	82.68	5.59	0.82	2.46
SPV 1411	81.13	5.60	0.98	2.40	80.80	5.62	0.80	2.87	73.99	5.66	1.16	2.03
IESV 930042	78.69	5.62	0.72	1.90	82.43	5.60	1.15	2.08	83.03	5.65	1.34	2.00
Madhura	85.56	5.63	0.66	2.16	82.12	5.46	0.95	2.17	80.34	5.60	1.04	1.95
Mean	82.04	5.64	0.92	2.24	81.23	5.62	0.95	2.19	81.11	5.66	0.99	2.02
Range	78.69-85.56	5.60 - 5.70	0.66-1.36	1.90-2.56	80.37 - 82.43	5.46-5.72	0.80-1.15	1.74-2.87	73.99-84.47	5.59-5.74	0.77-1.34	1.65 - 2.46
$LSD_{(0.05)}$	4.095	1.57	0.68	3.30	4.88	1.30	0.79	3.03	4.70	1.44	1.17	2.60
p-value	0.08	1.00	0.29	0.84	0.84	0.99	0.90	0.95	0.01	1.00	0.78	0.98
s.e.d	1.67	0.64	0.28	1.35	1.99	0.53	0.32	1.24	1.92	0.59	0.48	1.06
Harvest stage	Moisture Content (M.C)			pH			Total titratable acidity(TTA)			Ash Content(A.C)		
$LSD_{(0.05)}$	1.89			0.34			0.26			0.75		

Table 8: Effect of maturity stage and variety on sweet sorghum juice characteristics (Rongo) (n=10)

Variety	Harvest Stage 1				Harvest Stage 2				Harvest Stage 3			
	M.C (%)	pH	TTA (%)	A.C (%)	M.C (%)	pH	TTA (%)	A.C (%)	M. C (%)	pH	TTA (%)	A.C (%)
SPV 1411	86.25	5.35	0.80	1.60	85.40	5.61	0.84	1.54	84.36	5.60	1.00	1.23
Madhura	87.51	5.34	0.79	1.00	88.10	5.27	0.44	0.85	89.15	5.41	0.50	1.28
Mean	86.68	5.34	0.80	1.30	86.80	5.44	0.64	1.20	86.76	5.50	0.75	1.26
LSD (0.05)	4.35	1.01	0.32	0.64	7.77	1.76	0.20	0.25	4.44	1.40	0.16	0.32
P-value	0.47	0.98	0.94	0.06	0.39	0.62	0.01	0.002	0.04	0.72	0.001	0.68
s.e.d	22.57	0.36	0.12	0.23	2.80	0.63	0.07	0.09	1.60	0.50	0.06	0.11
Harvest stage	Moisture Content (M.C)			pH			Total titratable acidity(TTA)			Ash Content (A.C)		
LSD (0.05)	5.39			0.50			0.79			1.23		

4.2.3 Sugar Characteristics of Sweet Sorghum Juice

The objective of the study was to characterize and determine the effect of location, maturity stage and variety on total sugar, fructose, glucose and sucrose characteristics of SS juice.

4.2.3.1 Total sugar content in raw SS juice

Total sugar content showed significant (p=0.05) difference among the varieties and harvest stages except the harvest stages for SS juice from JKUAT (Table 9 and 10). The highest total sugar content was 21.84 % for variety SPV 1411 while the lowest was 11.94 % for Madhura variety. The range obtained was slightly higher than the findings

of Batoul (2006) who found a range of 9.19 to 18.47%, Sir Elkhatim (2003) found a range of 13.64 and 18.78 %, while Cowley and Lime (1976) found a range of 13-17%. The total sugar content of SS juice varied with varieties and maturity stage. However, as the crop matured the total sugar content decreased as a result of the transfer of accumulated photosynthates from the stalk sap to the developing grains (Almodares et al., 1994 and 2008). SS juice from JKUAT had higher sugar content than SS juice from Rongo due to differences in soil and climatic conditions (Freeman, et al., 1973).

4.2.3.2 Fructose, glucose and sucrose contents of juice

Fructose, glucose and sucrose, were insignificantly ($p=0.05$) influenced by harvest stage, but variety significantly influenced fructose and sucrose (Table 9 and 10). The highest fructose content was 40.50 mg/ml for variety IESV 92038/2SH while the lowest was 4.80 mg/ml for Madhura variety. The highest glucose content was 49.70 mg/ml for IESV 930042 variety while the lowest was 6.55 mg/ml for Madhura variety.

The highest sucrose content was 52.55 mg/ml for SPV 1411while the lowest was 0.3 mg/ml for Madhura variety. According to the study done by Almodares et al., (2008) the hard dough stage produced the highest fructose and glucose content attributable to high invertase activity. The SS juice from JKUAT had higher simple sugar content as compared to SS juice from Rongo this could be attributed to soil and climatic conditions that influence plant development (Tsuchihashi and Goto, 2004).

Table 9: Effect of maturity stage and variety on total sugar, fructose, glucose, and sucrose content of SS juice (JKUAT) (n=10)

Variety	Harvest Stage 1				Harvest Stage 2				Harvest Stage 3			
	TS (%)	**Fructose mg/ml**	**Glucose mg/ml**	**Sucrose mg/ml**	**TS (%)**	**Fructose mg/ml**	**Glucose mg/ml**	**Sucrose mg/ml**	**TS (%)**	**Fructose mg/ml**	**Glucose mg/ml**	**Sucrose mg/ml**
IESV 91018 LT	20.74	21.0	22.6	20.2	21.29	24.70	25.70	41.50	16.89	20.60	41.40	43.00
IESV 92008 DL	21.29	27.2	29.3	4.40	19.64	33.70	39.50	9.30	17.44	29.80	36.80	4.10
IESV 92038 /2SH	20.74	35.0	38.9	12.4	19.64	40.50	49.10	4.70	17.44	34.70	42.70	4.90
SPV 1411	21.84	16.9	25.2	16.8	19.64	26.20	29.70	22.90	17.44	22.90	33.00	52.50
IESV 930042	21.84	32.8	39.9	18.1	20.19	31.60	49.70	13.20	15.24	19.10	32.50	23.00
Madhura	20.19	23.0	31.9	11.1	18.54	36.40	42.20	10.90	17.99	30.70	38.50	13.10
Mean	21.11	26.0	31.3	13.9	19.82	32.20	39.30	17.10	17.07	26.30	37.50	23.40
Range	20.19 - 21.84	16.90 - 35.0	22.6 - 39.9	4.40 - 20.20	18.54 - 21.29	24.70 - 40.50	25.70 - 49.70	4.70 - 41.50	15.24 - 17.99	19.10 - 34.70	32.50 - 42.70	4.10 - 52.50
$LSD_{(0.05)}$	5.49	40.72	38.41	16.72	3.6	58.31	62.76	29.65	8.00	47.58	62.86	40.28
p-value	0.96	0.86	0.83	0.34	0.63	0.98	0.90	0.15	0.96	0.95	1.00	0.11
s.e.d	2.25	16.64	15.70	6.83	1.489	23.83	25.65	12.12	3.27	19.44	25.69	16.46
Harvest stage	Total sugar (TS)			Fructose			Glucose			Sucrose		
$LSD_{(0.05)}$	1.66			11.88			13.65			9.43		

Table 10: Effect of maturity stage and variety on juice total and simple sugars content (Rongo) (n=10)

Variety	Harvest stage 1				Harvest stage 2				Harvest stage 3			
	TS (%)	**fructose (mg/ml)**	**Glucose (mg/ml)**	**Sucrose (mg/ml)**	**TS (%)**	**fructose (mg/ml)**	**Glucose (mg/ml)**	**Sucrose (mg/ml)**	**TS (%)**	**fructose (mg/ml)**	**Glucose (mg/ml)**	**Sucrose (mg/ml)**
SPV 1411	14.14	8.70	10.80	6.00	17.44	18.70	23.20	1.70	15.24	14.30	24.80	2.00
Madhura	11.94	4.80	6.55	2.10	15.24	10.10	14.55	1.50	11.94	7.85	9.70	0.30
Mean	13.04	6.75	8.68	4.05	16.34	14.40	18.88	1.60	13.59	11.08	17.25	1.15
LSD (5%)	3.18	1.58	2.74	3.21	2.45	5.95	4.60	0.68	1.39	2.37	3.11	0.17
P value	0.13	0.002	0.013	0.03	0.07	0.02	0.01	0.46	0.003	0.002	<0.001	<0.001
s.e.d	1.14	0.57	0.99	1.16	0.88	2.14	1.66	0.25	0.50	0.85	1.12	0.06
Harvest stage	Total sugar (TS)			Fructose			Glucose			Sucrose		
$LSD_{(0.05)}$	1.93			7.16			16.60			5.66		

4.2.4 Mineral Characteristics of Sweet Sorghum Juice

The study of the SS juice mineral composition (macro and micro-elements) was necessitated due to their influence on the nutritional value of the SS syrup and being required by the fermenting yeasts during ethanol production. Sodium was insignificantly ($p=0.05$) influenced by variety and harvest stage for SS juice from JKUAT, but didn't show a significant difference for SS juice from Rongo (Table 11 and 12). The highest Sodium content was 40.10 mg/100g for a variety IESV 930042 while the lowest content was 7.44 mg/100g for variety Madhura. The range obtained was lower compared to the findings of Sir Elkhatim (2003) and FAO (1994) that reported a range of 0.05 to 0.15%

and 0.08 to 0.11% respectively. SS Juice from JKUAT had higher sodium content as compared to SS juice from Rongo. This low sodium content could be attributed to soil, climatic and cultural conditions.

Potassium was insignificantly (p=0.05) influenced by variety and harvest stage for the two zones except for variety that significantly influenced juice from Rongo (Table 11 and 12). The highest potassium content was 348.70 mg/100g for variety SPV 1411 while the lowest content was 141.00 mg/100g for variety IESV 930042 respectively. The obtained range was lower compared to the findings of Sir Elkhatim (2003) and FAO (1994), which found a range of 0.4 to 0.6 %. Juice from Rongo had higher potassium content compared to that from JKUAT.

Calcium showed no significant (p=0.05) difference for variety and harvest stage for the two zones (Table 11 and 12). The highest calcium content was 235.70 mg/100g for variety IESV 92008 DL while the lowest content was 82.6 mg/100g for variety Madhura. The range obtained was similar to the findings of FAO (1994) whose range was 0.11 to 0.15% and Sir Elkhatim (2003) whose range was 0.10 to 0.18%. Juice from JKUAT had higher calcium concentration as compared to juice from Rongo. This could be due to genetic, soil, climatic and cultural factors (Belitz et al., 2009).

Harvest stage and variety significantly (p=0.05) influenced the concentration of magnesium except the maturity stage for SS juice from JKUAT, while the harvest stage and variety insignificantly influenced magnesium concentration of SS juice from Rongo (Table 11and 12). The highest magnesium concentration was 157.0 mg/100g for Madhura variety while the lowest was 55.90 mg/100g for variety IESV 92038/2SH. This

range was higher than the findings of FAO (1994) who found 0.05 to 0.06% this could be attributed to cultural practices, soil, genetic and climatic factors (Belitz et al., 2009). SS Juice from Rongo had higher magnesium content as compared to juice from JKUAT.

Manganese was significantly (p=0.05) influenced by harvest stage while variety was insignificant for the two zones (Table 11and 12). The highest manganese content was 23.40 mg/100g for variety IESV 92008 DL while the lowest was 3.20 mg/100g for Madhura variety. The SS juice from JKUAT had higher manganese content as compared to SS juice from Rongo.

Zinc showed insignificant (p=0.05) difference for varieties and harvest stage for the two zones except variety significantly influenced SS juice from Rongo (Table 11 and 12). The highest Zinc content was 33.6 mg/100g for Madhura variety while the lowest was 16.0 mg/100g for IESV 930042 variety. SS Juice from Rongo had higher zinc content as compared to the SS juice from JKUAT.

Iron showed significant (P=0.05) difference for both variety and harvest stage for SS juice from Rongo while it was insignificant for SS juice from JKUAT (Table 11 and 12). The highest iron content was 22.21 mg/100g for variety SPV 1411 while the lowest was 7.36 mg/100g for Madhura variety. The SS juice from Rongo had higher iron content compared to SS juice from JKUAT. Copper had no significant (P=0.05) difference for both variety and harvest stages for the two zones (Table 11 and 12). The highest copper content was 7.71 mg/100g for variety SPV 1411 while the lowest was 1.80mg/100g for variety IESV 92038/2SH. SS juice from Rongo had higher copper content as compared to SS juice from JKUAT.

Phosphorous content was significantly (P=0.05) influenced by variety and harvest stages for SS juice from Rongo while being insignificant for SS juice from JKUAT (Table 11 and 12). The highest phosphorous content was 44.79 mg/100g for variety SPV 1411 while the lowest was 11.80mg/100g for variety IESV 91018LT. The range obtained was lower in comparison to the findings of Sir Elkhatim (2003), who reported a range of 1.31 to 4.63 %, while FAO (1994) reported a range of 2.21 to 3.57 %. SS Juice from Rongo had higher phosphorous content as compared to SS juice from JKUAT, the variation could be attributable to soil, climatic and genetic factors (Belitz et al., 2009). The experiment revealed that agro- ecological zones had an effect on SS juice mineral composition.

Table 11: Effect of maturity stage and variety on juice mineral composition in mg/100g (JKUAT) (n=10)

Variety	Na	K	Ca	Mg	Mn	Zn	Fe	Cu	P
Harvest Stage 1									
IESV 91018 LT	25.0	157.0	145.40	70.80	14.4	16.30	13.90	5.4	14.70
IESV 92008 DL	28.4	163.0	120.40	56.80	5.8	16.70	13.90	5.3	15.90
IESV 92038 /2SH	33.4	179.0	108.50	55.90	5.3	19.10	11.80	1.8	22.70
SPV 1411	31.6	157.0	187.00	81.70	5.0	20.00	5.70	1.8	21.20
IESV 930042	29.5	141.0	167.00	66.20	6.9	17.00	2.80	2.1	25.00
Madhura	25.2	143.4	179.00	84.40	3.2	16.80	14.70	2.0	21.10
Harvest Stage 2									
IESV 91018 LT	37.2	216.0	144.70	75.00	6.67	25.60	4.70	3.5	19.80
IESV 92008 DL	29.2	207.0	119.90	70.50	11.78	18.80	4.30	3.4	21.00
IESV 92038 /2SH	26.3	167.0	177.60	79.80	8.97	22.50	7.10	3.6	26.40
SPV 1411	35.0	178.0	138.30	71.70	7.46	22.60	2.50	3.0	20.30
IESV 930042	33.7	224.0	145.90	68.30	7.06	18.60	5.10	3.5	15.80
Madhura	20.2	151.0	82.60	55.90	7.47	19.50	3.10	4.0	14.90
Harvest Stage 3									
IESV 91018 LT	35.3	244.0	143.20	65.80	10.90	18.50	4.80	1.92	11.80
IESV 92008 DL	35.8	229.0	235.70	80.90	23.40	18.30	18.50	1.88	34.60
IESV 92038 /2SH	34.8	183.0	218.00	56.70	14.90	18.60	3.80	2.15	16.40
SPV 1411	29.5	265.0	198.40	64.80	14.00	17.30	3.30	2.12	22.40
IESV 930042	40.1	161.0	150.50	65.90	9.30	16.00	4.30	2.59	13.40
Madhura	34.8	185.0	130.90	63.70	8.50	17.10	6.10	3.08	18.80
Mean	31.4	186.0	1552.0	68.60	9.50	18.80	7.30	2.96	19.80
Range	**20.2-37.2**	**141.0-265.0**	**82.60-235.70**	**55.90-84.40**	**3.20-23.4**	**16.00-25.60**	**2.50-18.50**	**1.80-5.40**	**11.80-34.60**
$LSD_{(0.05)}$	8.87	212.3	90.29	21.75	6.752	23.20	9.83	3.347	18.81
P –value	0.634	0.997	0.932	0.975	0.349	1.00	0.548	0.956	0.95
s.e.d	4.34	103.9	44.21	10.65	3.3	11.36	4.81	1.64	9.21
Harvest stage									
$LSD_{(0.05)}$	6.01	15.4	63.9	15.88	4.12	16.9	6.84	2.41	13.8

Table 12: Effect of maturity stage and variety on juice mineral composition in mg/100g (Rongo) (n=10)

Variety	Na	K	Ca	Mg	Mn	Zn	Fe	Cu	P
Harvest Stage 1									
SPV 1411	26.02	279.00	107.90	119.20	10.52	19.83	18.33	7.00	44.79
Madhura	18.59	265.00	170.80	123.80	12.80	31.09	9.65	7.12	35.26
Mean	22.30	272.00	139.40	121.50	11.66	25.46	13.99	7.06	40.03
LSD $_{(0.05)}$	3.40	18.65	14.88	18.38	0.94	1.981	1.43	1.41	1.79
P- value	0.004	0.11	0.001	0.53	0.003	<0.001	<0.001	0.82	<0.001
s.e.d	1.22	6.72	5.36	6.62	0.34	0.713	0.52	0.51	0.65
Harvest Stage 2									
SPV 1411	22.31	348.70	144.70	126.00	9.59	16.09	22.21	7.71	24.83
Madhura	7.44	156.90	94.50	157.60	9.89	32.25	7.36	7.25	34.95
Mean	14.87	252.80	119.60	141.80	9.74	24.17	14.78	7.48	29.89
LSD $_{(0.05)}$	0.61	26.13	16.41	15.99	0.74	3.26	1.62	0.17	1.34
P- value	<0.001	<0.001	0.001	0.005	0.32	<0.001	<0.001	0.02	<0.001
s.e.d	0.22	9.41	5.91	5.76	0.27	1.17	0.59	0.062	0.48
Harvest Stage 3									
SPV 1411	24.16	317.80	126.30	122.60	10.20	17.96	20.26	7.36	34.80
Madhura	11.15	265.00	132.90	120.90	6.38	33.62	20.50	7.50	32.26
Mean	17.66	291.40	129.60	121.80	8.29	25.79	20.38	7.43	33.52
LSD $_{(0.05)}$	2.06	19.84	25.29	19.28	0.81	3.33	1.51	1.99	2.86
P- value	<0.001	0.002	0.51	0.82	<0.001	<0.001	0.68	0.86	0.086
s.e.d	0.74	7.15	9.11	6.94	0.29	1.2	0.04	0.72	1.03
Harvest stage									
LSD $_{(0.05)}$	11.77	28.44	17.21	53.6	9.46	8.21	23.06	10.4	15.6

4.3. Production and Characterization of Sweet Sorghum Syrup

The objective of the study was to produce SS syrup from the six selected varieties and determine the influence of maturity stage and variety on syrup extraction percentage and syrup yields.

4.3.1 Syrup Production Using Sweet Sorghum Juice

The harvest stage significantly (p=0.05) influenced syrup extraction percentage and

syrup yield while variety had an insignificant effect for SS juice from JKUAT (Table 13). The highest syrup extraction percentage was 29.22 % for variety SPV 1411 while the lowest was 17.56 % for Madhura variety respectively. The highest syrup yield was 292.2 g/kg for variety SPV 1411 while the lowest was 175.6g/kg for Madhura variety respectively. The average syrup extraction percentage for the three harvest stages was 20.98, 25.69 and 23.38% for harvest stage 1, 2, and 3 respectively. Hence harvest stage 2 produced the highest yield of syrup. The interaction between harvest stage and variety also significantly influenced syrup extractability and yield. SS syrup yield is influenced by application of nitrogen and climatic conditions, variety, stalk diameter, juice composition e.g. aconitic acid and total soluble solids concentrations (for quality syrup >15 °Brix), processing equipment and conditions, (Nimbkar et al., 2006).

Table 13: Effect of maturity stage and variety on syrup extraction percentage (SEP) and syrup yield (n=10)

Variety	Harvest Stage 1				Harvest Stage 2				Harvest Stage 3			
	Juice weight (g)	Syrup weight (g)	SEP (%)	Syrup yield (g/kg)	Juice weight (g)	Syrup weight (g)	SEP (%)	Syrup yield (g/kg)	Juice weight (g)	Syrup weight (g)	SEP (%)	Syrup yield (g/kg)
IESV 91018 LT	95.60	18.70	19.09	190.90	73.40	18.40	25.04	250.4	53.70	12.50	22.80	228.0
IESV 92008 DL	100.20	26.00	25.81	258.10	77.70	22.20	28.15	281.5	57.70	10.80	18.21	182.1
IESV 92038 /2SH	42.60	9.30	21.57	215.80	69.80	19.10	26.77	267.8	31.30	7.80	24.71	247.2
SPV 1411	53.60	10.90	20.63	206.30	72.80	18.8	25.6	256.0	48.10	14.30	29.22	292.2
IESV 930042	91.50	19.60	21.26	212.60	57.60	14.20	25.08	250.8	65.20	14.90	22.58	225.8
Madhura	68.40	12.00	17.56	175.60	73.00	17.20	23.48	234.9	90.10	20.60	22.74	227.4
Mean	75.30	16.10	20.98	209.90	70.70	18.30	25.69	256.9	57.70	13.50	23.38	233.7
Range	42.6-100.2	9.30-26.0	17.56-25.81	175.6-258.1	57.60-77.70	14.20-22.20	23.48-28.15	234.9-281.5	31.30-90.10	7.80-20.6	18.21-29.22	182.1-292.2
LSD $_{(0.05)}$	52.94	14.05	5.126	51.24	44.89	15.05	7.143	71.42	54.18	16.23	6.75	67.46
p-value	0.15	0.15	0.08	0.08	0.91	0.86	0.70	0.70	0.30	0.56	0.09	0.09
s.e.d	21.63	5.74	2.095	2.094	18.34	6.15	2.919	29.19	22.14	6.63	2.757	27.57
Harvest stage	Juice weight			Syrup weight			Syrup extraction percentage		Syrup yield			
LSD $_{(0.05)}$	17.82			5.31			2.24		22.42			

4.3.2 Chemical Characteristics of Sweet Sorghum Syrups

The aim of the study was to investigate the chemical characteristics of SS syrups made from Madhura juice at the physiological stage of maturity (harvest stage 3) grown in the two environments. This was achieved by determining the moisture content, ash content, total soluble solids, total titratable acidity and pH of SS syrup. Sugar cane was used as a control since it is grown in Rongo, and its molasses are used for ethanol production.

4.3.2.1 Moisture content of sweet sorghum syrups

The moisture content differed significantly among the syrup types and region (p=0.05), the moisture content of the syrups were 24.67, 28, and 32 % for sorghum syrup from JKUAT, sorghum syrup from Rongo, and sugar cane syrup from Rongo respectively

(Table 14). Moisture content variations were attributable to processing conditions e.g. they were concentrated while ensuring the sensory properties are enhanced hence resulting in different total soluble solids dependent on raw SS juice composition. Moisture content affects the ability of syrup to flow, storage stability, processing behavior, quality and appearance of syrups (Nimbkar, et al., 2006).

4.3.2.2 Ash content of sweet sorghum syrups

The ash content showed a significant (p=0.05) difference among the syrup types and region. Ash content values were 5.14, 5.16 and 0.47 % for sorghum syrup from JKUAT, sorghum syrup from Rongo, and sugar cane syrup from Rongo respectively (Table 14). The results obtained were comparable with those of Akbulut and Ozcan, (2008), and Saikat et al., (2011) whom reported ash content of 4.6 and 4.17% respectively for SS syrup. The ash content variation could be attributed to soil, climatic and genetic factors, cultural practices, and harvesting stage. Higher mineral absorption efficiency for sweet sorghum may be the cause of high ash content as compared to sugar cane plant (Belitz et al., 2009).

4.3.2.3 Total titratable acidity and pH of sweet sorghum syrups

The pH showed a significant difference (p=0.05) among the syrup types and regions (Table 14). The pH values were 4.95, 5.39 and 5.04 for SS syrup from JKUAT, SS syrup from Rongo, and sugar cane syrup from Rongo respectively. The pH values showed that the syrups studied are slightly acidic an indicator of their composition is a complex mixture of sugars, organic acids and minerals. The pH is likely to be influenced by

seasonal effects, varietal and maturity variations, and also processing conditions. Due to removal or conversion of organic acids the syrupping process lower pH, but the concentration effect may lead to higher levels.

Total titratable acidity (TTA) showed a significant difference among the syrup types (p=0.05) while the effect of region was insignificant (Table 14). TTA values were 0.39, 0.43 and 0.2 for SS syrup from JKUAT, SS syrup from Rongo, and sugar cane syrup from Rongo respectively. The TTA values were influenced by genetic differences. Total acidity gives the syrups their distinctive taste and flavor (Suzanne, 2010).

4.3.2.4 Total soluble solids of sweet sorghum syrups

The total soluble solids (TSS) showed a significant difference (P=0.05) among the syrup types and regions (Table 14). The TSS values were 76, 72 and 68 °Brix for SS syrup from JKUAT, SS syrup from Rongo, and sugar cane syrup from Rongo respectively. TSS values of syrups could be influenced by soil and climatic conditions, SS juice composition and processing conditions. Total soluble solids enable the syrups to be utilized as sweeteners or preservatives in food products such as juices, drinks, dairy, bakery and confectionery products (Elena, 2007).

Table 14: chemical characteristics of sweet sorghum syrups (n=3)

Syrup type	Moisture content (%)	Ash content (%) (d.b)	pH	Total Acidity (%)	Total soluble solids (°Brix)
Sweet Sorghum Syrup (JKUAT)	24.67	5.14	4.95	0.39	76.00
Sweet Sorghum Syrup (Rongo)	23.12	5.16	5.39	0.43	72.00
Sugar Cane Syrup (Rongo)	19.62	0.47	5.04	0.20	68.00
Mean	22.47	3.59	5.13	0.34	72.00
$LSD_{(0.05)}$	0.02	0.61	0.14	0.03	2.00
P-value	0.001	0.001	0.001	0.001	0.001
S.e.d	0.01	0.25	0.06	0.01	0.82

4.3.3 Mineral Composition of Sweet Sorghum Syrups

The minerals; potassium, sodium, calcium, magnesium, manganese, zinc, iron and copper showed a significant difference (p=0.05) among the syrup types and region (Table 15). The average concentrations of K, Na, Ca, Mg, Mn, Zn, Cu and Fe were 80.78, 107.22, 190.40, 103.90, 86.07, 8.47, 5.69, and 18.48 mg/100g respectively. The results obtained showed sweet sorghum syrups as excellent source of macro and micro elements contributing to biological processes in the human body. Potassium a common cation in the intracellular fluid regulates the osmotic pressure within the cell, sodium an extracellular constituent maintains the osmotic pressure of the extracellular fluid, and hence both help maintain blood pressure. The roles of calcium include involvement in the muscular system and controls processes such as muscle contraction, blood clotting, brain cells activity and cell growth.

Magnesium helps to relieve fatigue, relaxing muscles, nerves and blood vessels, while manganese is a co-factor in many enzymatic reactions especially in energy production and anti oxidant defenses. Iron is essential for respiration at the cellular level by

synthesizing hemoglobin that helps carry oxygen to the cells. Zinc and copper are components of a number of enzymes, essential for metabolic activities of the body (Belitz et al., 2009). Also if, the syrups could be utilized as raw material for ethanol production the mineral elements could provide the fermenting yeast with the necessary macro and micro nutrients required for their growth and ethanol production.

Table 15: mineral composition of sweet sorghum syrups (n=3)

Syrup type	Mineral composition in mg/100g							
	Na	K	Ca	Mg	Mn	Zn	Cu	Fe
Sweet Sorghum Syrup (JKUAT)	84.25	145.00	272.33	124.60	68.90	8.42	3.53	15.43
Sweet Sorghum Syrup (Rongo)	153.13	133.20	190.87	118.50	87.93	11.62	5.86	19.74
Sugar Cane Syrup (Rongo)	4.95	43.46	108.01	68.70	101.39	5.36	7.69	20.27
Mean	80.78	107.22	190.40	103.90	86.07	8.47	5.69	18.48
Range	4.95-153.13	43.46-145.00	108.01-272.33	68.70-124.6	68.9-101.39	5.36-11.62	3.53-7.69	15.43-20.27
$LSD_{(0.05)}$	2.46	5.18	4.36	7.10	3.39	1.01	0.97	1.02
P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
s.e.d	1.01	2.12	1.78	2.90	1.39	0.41	0.40	0.42

4.3.4 Bio-active Compounds and Total Anti-oxidant Activity Characteristics of SS syrup

The objective of this experiment was to characterize the bio-active compounds and total anti-oxidant properties of sweet sorghum syrups.

4.3.4.1 Bio-active Compounds Characteristics of SS syrup

The aim of this experiment was to determine the total phenolic, flavonoid and tannin content (bio-active compounds) present in sweet SS syrup, due to their health benefits. The total phenolic and total flavonoid showed a significant difference among the syrup types and regions (p=0.05), while the total tannin content showed a significant

difference among the syrup types but not regions (Table16). The total phenolic content ranged from 184.70 to 261.31 mg gallic acid equivalent (GAE) /100 ml of SS syrup. This range was higher compared to honey whose total phenolic content varied from 21.6 to 181.0 mg GAE/ 100g (Saba et al., 2011, Nikolett, 2010). The total flavonoid content ranged from 75.62 to 197.50 mg quercetin equivalent (QE) /100 ml of SS syrup, this was higher than the total flavonoid content of honey that varied from 1.97 to 58.74 mg QE/100g (Saba et al., 2011). Total tannin content ranged from 41.03 to 45.26 mg tannic acid equivalent (TAE) /100 ml of SS syrup. The study showed that SS syrups could be a potential source of biologically active compounds. The bio-active compounds have health benefits that include, anti oxidant activity, anti-microbial effects, immune system boosters, improve blood circulation and cardiac health, slow down loss of bone tissues especially at menopause, anti -ulcer effects, and anti- inflammatory effects in vivo. Presence of bio-active compounds in SS syrups allows for its exploitation as possible functional food (Jay, 2008).

Table 16: Bioactive compounds of sweet sorghum syrups (n=3)

Syrup type	Total phenolic acid content (mg GAE/100ml)	Total flavonoid content (mg QE/100ml)	Total tannin content (mg TAE/100ml)
Sweet Sorghum Syrup (JKUAT)	261.31	197.50	45.26
Sweet Sorghum Syrup (Rongo)	184.70	75.62	42.99
Sugar Cane Syrup (Rongo)	216.10	115.00	41.03
Mean	220.70	129.37	43.09
$LSD_{(0.05)}$	8.86	2.34	1.41
P-value	0.001	0.001	0.001
s.e.d	3.62	0.95	0.58

4.3.4.2 Total Anti-oxidant Activity of Sweet Sorghum Syrups

The scavenging activity of SS syrups against 2, 2-Diphenyl-1-picryl hydrazyl (DPPH), was used for the determination of anti oxidant activity (Suresh et al., 2008, Braca et. al. 2002). Ascorbic acid was used as a standard. The ascorbic acid concentration of 0.008 mg/ml was able to inhibit 50 % of DPPH radical. This is attributed to the presence of hydrogen atoms on the phenolic group of the ascorbic acid standard that helps inhibit DPPH radical by breaking the chain of reaction. SS syrups exhibited potent anti- oxidant activity (Table 17), comparable to honey from different regions whose DPPH inhibition varied from 28 to 76 % (Saba et al., 2011).

The syrup anti- oxidant activity could be attributable to the presence of polyphenols such as phenolic acids, flavonoids, and tannins, which are known natural free radical scavengers. DPPH gives a strong absorption band at 517 nm in the visible region, when its electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the solution's color changes from deep violet to light yellow. The reduction of absorbance is a measure of the radical scavenging capacity or antioxidant power of the syrup extract (Ayoola et al., 2008). The syrup produced in this study contained bio-active compounds, which if developed and studied further could find application in the food and pharmaceutical industry as natural preservative and sweetener, also as raw material for ethanol production (Halliwell, 1994).

Table 17: percentage inhibition of SS syrups against DPPH radical (n=3)

Syrup concentration (mg/ml)	Sweet sorghum syrup (JKUAT)	Sweet sorghum syrup (Rongo)	Sugar cane syrup (Rongo)
0.05	78.02±0.018	79.97±0.0006	78.99±0.0006
0.1	78.02±0.008	82.85±0.0006	79.47±0.002
0.5	80.92±0.013	82.61±0.0006	81.40±0.0006
1.0	84.54±0.007	87.92±0.0006	83.33±0.0006
2.0	88.41±0.005	92.75±0.001	84.06±0.00
5.0	93.72±0.02	96.14±0.001	92.75±0.0006

4.3.5. Physical Characteristics of Sweet Sorghum Syrup

The objective of the study was to produce SS syrup, characterize its physical, chemical and anti-oxidant properties while investigating the influence of environment on these properties, i.e. JKUAT and Rongo environments were used. SS juice from Madhura variety at physiological maturity stage (harvest stage 3) for SS syrup production because it grew and produced enough juice in both environments. SS syrup was compared with sugar cane syrup a common feedstock for ethanol production in Kenya. Sugar cane syrup was obtained from Rongo, a sugar producing region in Kenya.

4.3.5.1 Color properties of sweet sorghum syrups

The aim was to characterize the color of the SS syrup, since color is influenced by the juice composition, processing conditions it could be used as an indicator of quality of SS syrup. The parameters L, a, b were used in the description of the color of SS syrup. The **L** parameter showed no significant difference (p=0.05) among the syrup types and region, **a** parameter showed a significant difference (p=0.05) for both the syrup types

and region, and also the **b** parameter showed a significant difference (p=0.05) for syrup types and region (Table 18). The **L** values varied from 21.11 to 22.87, **a** values varied from – 0.28 to 0.68, while **b** values varied from 0.33 to 1.82. The averages values for **L**, **a**, **b**, were 22.26, +0.24, and +0.75. Therefore, a low **a** value and a high **L** value are indicative of satisfactory quality syrup. Higher **a** value could result from excessive sugar caramelization, while higher **L** values are indicative of lightly color as opposed to dark color.

4.3.5.2 Density of sweet sorghum syrups

The determined density showed a significant difference (p=0.05) among the syrup types and region (Table 18). Density values were 1.41, 1.31 and 1.30 g/cm³ for SS syrup from JKUAT, SS syrup from Rongo, and sugar cane syrup from Rongo respectively. Density routinely is used to determine the carbohydrate concentration in syrups, juice and beverages in the food industry (Akbulut and Ozcan, 2008).

4.3.5.3 Viscosity of SS syrups

Viscosity showed a significant difference (p=0.05) among the syrup types and region. Viscosity varied from 26.90- 81.0 pa.s (Table 18). Sweet sorghum syrups exhibited a shear thinning behavior which could be attributed to the entangled highly asymmetric particles present in the syrups. Inter- particle or intermolecular interactions decreases with increasing shear rate resulting in decreased viscosity. Viscosity determination is critical because it is a significant factor in determining the overall quality and stability of a food system, energy usage, process design and control, and equipment selection

(Akbulut and Ozcan, 2008).

Table 18: The color, density and viscosity characteristics of sweet sorghum syrup (n=3)

Syrup type	Color properties			Density	Viscosity properties (pa.s)			
	L	a	b	g/cm³	6 rpm	12 rpm	30 rpm	60 rpm
Sweet Sorghum Syrup (JKUAT)	22.87	-0.28	1.82	1.41	40.00	40.50	45.30	43.80
Sweet Sorghum Syrup (Rongo)	21.80	0.68	0.11	1.31	30.00	30.50	31.20	26.90
Sugar Cane Syrup (Rongo)	22.11	0.32	0.33	1.30	75.00	78.50	81.00	73.80
Mean	22.26	0.24	0.75	1.34	48.33	49.83	52.50	48.17
Range	**21.80-22.87**	**0.28-0.68**	**0.11-1.82**	**1.30-1.41**	**30.0-75.0**	**30.5-78.5**	**31.2-81.0**	**26.9-73.8**
$LSD_{(0.05)}$	2.62	0.04	0.26	0.02	4.76	4.08	4.70	3.45
P-value	0.62	0.001	0.001	0.001	0.001	0.001	0.001	0.001
s.e.d	1.07	0.02	0.11	0.01	1.94	1.67	1.92	1.42

*rpm-refers to rotations per minute

4.4 Fermentation of Sweet Sorghum Juice to Produce Ethanol

4.4.1 Changes during Fermentation of Sweet Sorghum Juice

The changes in the pH, total residual sugars and ethanol content were estimated during the fermentation process, with the aim of determining the time when optimum ethanol concentration is generated by the fermenting yeasts. The sweet sorghum juice was unpasteurized since the goal of the study was to replicate the fermentation in the field and minimize the processing costs to guarantee maximum benefits to the farmers.

4.4.1.1 The pH changes during fermentation process

The harvest stage and time significantly influenced the pH, while variety was insignificant (p=0.05). The highest initial pH of sweet sorghum juice was 5.6 for variety IESV 91018LT, while after fermentation, the lowest pH observed was 3.6 for variety IESV 92038 /2SH (Table 19). The pH decline to around 4.0 at the end of fermentation

could be attributed to the production of organic acids such as acetic acid, which lowers the pH of the fermentation medium. These results are similar to the findings of Dombek and Ingram (1987), which reported that the pH of fermenting sweet sorghum juice declined to 3.5 due to the production of organic acids by the acetobacter bacteria. Laopaiboon et al., (2007) also reported similar decline from an initial pH of 4.7 to 4.1 after 14 hours and relatively remained constant until the end of the experiment.

4.4.1.2 Total residual sugar changes during fermentation process

Total residual sugar was significantly influenced by time (p=0.05) while harvest stage and variety were insignificant (Table 19). The highest initial sugar was 20.0 °Brix for varieties IESV 92008 DL, SPV 1411 and Madhura while the lowest total residual sugar was 7 °Brix for variety IESV 91018 LT. The total residual sugar of all varieties decreased with time this is similar to the findings of Siriyotha et al., (2006) who found out that sugar content decreased during fermentation. The residual sugar decline could be due to utilization by yeasts to produce ethanol. The reduced fermentative activity is reduced by ethanol accumulation.

4.4.1.3 Ethanol changes during fermentation process

Ethanol content as significantly (p=0.05) influenced by the time while variety and harvest stage were insignificant (Table 19). The alcohol content increased with time to a high of 13.8 % for Madhura variety, this is in agreement with Pramanik (2003) who reported an increase from 4.5 to 9.28 % and 1.78 to 8.05% after 48 to 72 hours respectively. While Xiaorong et al., (2010) also reported an increase of alcohol content to 13 %. To produce ethanol *S. cerevisiae* utilizes sugars from sweet sorghum juice as a

source of both carbon and energy. After absorption, glucose is metabolized into pyruvate through a series of reactions catalyzed by a set of enzymes in the glycolytic pathway. Further pyruvate is decarboxylated to acetaldehyde which is reduced to ethanol (Dickinson et al. 1998). Ethanol at levels above 12 % becomes inhibitory to yeast by disrupting the protein-lipid interactions in the plasma membrane, allowing more protons from the medium into the cell, acidifying the cytoplasm, and at high levels it causes cell death (Dombek and Ingram, 1987).

Table 19: Effect of maturity stage, variety and time on pH, total residual sugar and ethanol content during fermentation of sweet sorghum juice (n=10)

Variety	Total residual sugars (°Brix)				The pH level				Ethanol content (%)			
Time (hrs)	0	24	48	72	0	24	48	72	0	24	48	72
Harvest Stage 1												
IESV 91018 LT	17	16.5	11.5	7.0	5.5	3.9	3.8	3.7	0.0	2.3	8.1	12.3
IESV 92008 DL	18.5	18.0	12	9.5	5.5	3.9	3.8	3.8	0.0	3.1	7.2	11.2
IESV 92038 /2SH	19.0	18.5	13.5	10.5	5.4	3.8	3.8	3.7	0.0	2.9	8.4	12.5
SPV 1411	17.5	17.0	12.5	10.0	5.4	3.9	3.8	3.7	0.0	3.2	7.8	10.8
IESV 930042	18.5	17.5	12.5	10.0	5.4	3.9	3.7	3.7	0.0	3.1	7.5	13.6
Madhura	15.5	14.5	12.0	9.5	5.4	4.1	3.8	3.8	0.0	2.2	6.2	9.1
Means	17.67	17.0	12.3	9.42	5.43	3.92	3.78	3.73	0.0	2.8	7.53	11.58
Harvest stage 2												
IESV 91018 LT	18.5	17.5	13.0	11.5	5.5	3.8	3.7	3.6	0.0	4.0	7.5	13.5
IESV 92008 DL	20.0	18.5	11.5	9.0	5.5	3.9	3.8	3.8	0.0	3.4	7.9	12.4
IESV 92038 /2SH	19.0	18.5	16.0	10.0	5.4	3.8	3.6	3.6	0.0	3.5	8.6	13.6
SPV 1411	18.5	17.5	13.0	9.0	5.4	3.9	3.8	3.6	0.0	2.8	6.4	10.3
IESV 930042	18.0	17.0	11.0	7.5	5.5	3.9	3.8	3.8	0.0	3.2	8.3	10.1
Madhura	19.5	18.5	13.5	10.0	5.3	3.9	3.7	3.7	0.0	2.8	7.1	12.9
Means	18.9	17.9	13	9.5	5.43	3.87	3.73	3.68	0.0	3.28	7.63	12.1
Harvest stage 3												
IESV 91018 LT	17.5	15.0	12	8.5	5.6	4.2	3.9	3.9	0.0	3.7	9.9	12.7
IESV 92008 DL	18	16.5	9.0	7.5	5.5	4.5	3.9	3.8	0.0	3.4	8.2	13.0
IESV 92038 /2SH	19	16.0	9.0	8.5	5.5	3.9	4.0	3.8	0.0	3.5	7.9	10.0
SPV 1411	20	19.0	12.0	10.5	5.5	4.1	4.0	4.0	0.0	4.5	8.5	11.0
IESV 930042	18.5	16.0	10.0	8.0	5.5	4.1	4.0	4.0	0.0	3.2	7.0	13.1
Madhura	20.0	17.0	11.0	9.5	5.4	3.9	3.9	3.8	0.0	2.8	9.6	13.8
Means	18.83	16.1	10.5	8.75	5.5	4.1	3.95	3.88	0.0	3.52	8.52	12.27
Time												
[LSD($_{0.05}$)	1.23				0.11				0.55			
Harvest stage												
LSD$_{(0.05)}$	1.06				0.09				0.47			

4.4.2 Acetaldehyde and Alcohol Profile

The objective of this experiment was to investigate the types of alcohols and their concentration in the distillate obtained through the distillation of the fermented sweet

sorghum juice. The study showed that only ethanol and propanol were present while other alcohols like methanol were absent. Acetaldehyde an important sensory carbonyl compound giving producing a distinctive fruity odor at low levels was also found to be present.

4.4.2.1 Acetaldehyde concentration

Acetaldehyde concentration showed no significant difference for variety and harvest stage (Table 20). The highest acetaldehyde level was 0.07 % for Madhura variety while the lowest was 0.01% for IESV 92008 DL variety. Acetaldehyde may have been produced by yeasts (film yeasts) as a leakage product excreted during growth or through the oxidation of ethanol by acetic acid bacteria. According to Liu and Gordon, 1998, acetaldehyde is usually converted to ethanol, although this may take longer in beers with high alcohol content.

4.4.2.2 Ethanol concentration

Ethanol showed no significant difference ($p=0.05$) for both variety and harvest stage (Table 20). The highest ethanol concentration was 99.60 % while the lowest was 98.22% for Madhura variety in the harvest stage 2 and 3 respectively. The process of glycolysis through Embden- Meyerhoff – Parnas pathway produces pyruvate, decarboxylation of pyruvate leads to the formation of acetaldehyde. Thereafter, reduction of acetaldehyde forms ethanol, carbon dioxide, glycerol and organic acids as principal compounds (Varnam et al., 1994).

4.4.2.3 Propanol concentration

Propanol was significantly (P= 0.05) influenced by harvest stage while variety had an insignificant influence (Table 20). The highest propanol concentration was 0.24% for variety IESV 92008DL while the lowest was 0.10 % for Madhura variety. Propanol synthesized from carbohydrates or amino acids in the fermentation broth is one of the aliphatic alcohols produced during fermentation (Varnam et al., 1994).

Table 20: Effect of maturity stage and variety on acetaldehyde, ethanol and propanol at 72 hrs of fermentation of sweet sorghum juice (n=10)

Variety	Harvest Stage 1			Harvest Stage 2			Harvest Stage 3		
	Acetaldehyde (%)	Ethanol (%)	Propanol (%)	Acetaldehyde (%)	Ethanol (%)	Propanol (%)	Acetaldehyde (%)	Ethanol (%)	Propanol (%)
IESV 91018 LT	0.02	99.42	0.10	0.03	99.51	0.16	0.02	99.36	0.23
IESV 92008 DL	0.01	99.11	0.14	0.05	99.56	0.13	0.03	99.30	0.24
IESV 92038 /2SH	0.03	99.44	0.12	0.06	99.05	0.13	0.01	99.41	0.18
SPV 1411	0.04	99.38	0.11	0.06	99.46	0.13	0.02	99.34	0.16
IESV 930042	0.05	99.47	0.12	0.04	99.48	0.14	0.02	99.38	0.21
Madhura	0.07	98.99	0.15	0.02	98.22	0.13	0.02	99.60	0.10
Mean	0.04	99.42	0.12	0.04	99.21	0.13	0.02	99.40	0.18
Range	0.01-0.07	98.99-99.47	0.10-0.15	0.02-0.06	98.22-99.56	0.13-0.16	0.01-0.30	99.30-99.60	0.10-0.24
LSD (5%)	0.08	0.61	0.06	0.06	2.06	0.04	0.02	0.32	0.08
P-value	0.61	0.34	0.51	0.53	0.61	0.45	0.44	0.37	0.03
s.e.d	0.03	0.25	0.03	0.02	0.84	0.02	0.01	0.13	0.03
	Acetaldehyde			Ethanol			Propanol		
Harvest stage LSD($_{0.05}$)	0.02			0.41			0.06		

4.5 Optimization of the Fermentation Process Conditions using Sweet Sorghum Juice

4.5.1 Effect of Temperature on Ethanol Production

Temperature is one of the significant factors that influence alcohol production (Figure 8). The optimal temperature, determined by fermenting SS juice at 25, 30 and 35 °C with an initial sugar content of 17 °Brix and pH of 5.86. Alcohol content increased with time and after 72 hours, the alcohol content was 10.8, 13.72 and 11.21 % for 25, 30 and 35 °C respectively. Therefore, 30 °C produced higher alcohol content than 25 and 35 °C. This could be attributed to enzymatic activity involved in ethanol production where temperature exerts a profound effect on all aspects of yeast growth, metabolism and fermentation (Kadambini, 2006).

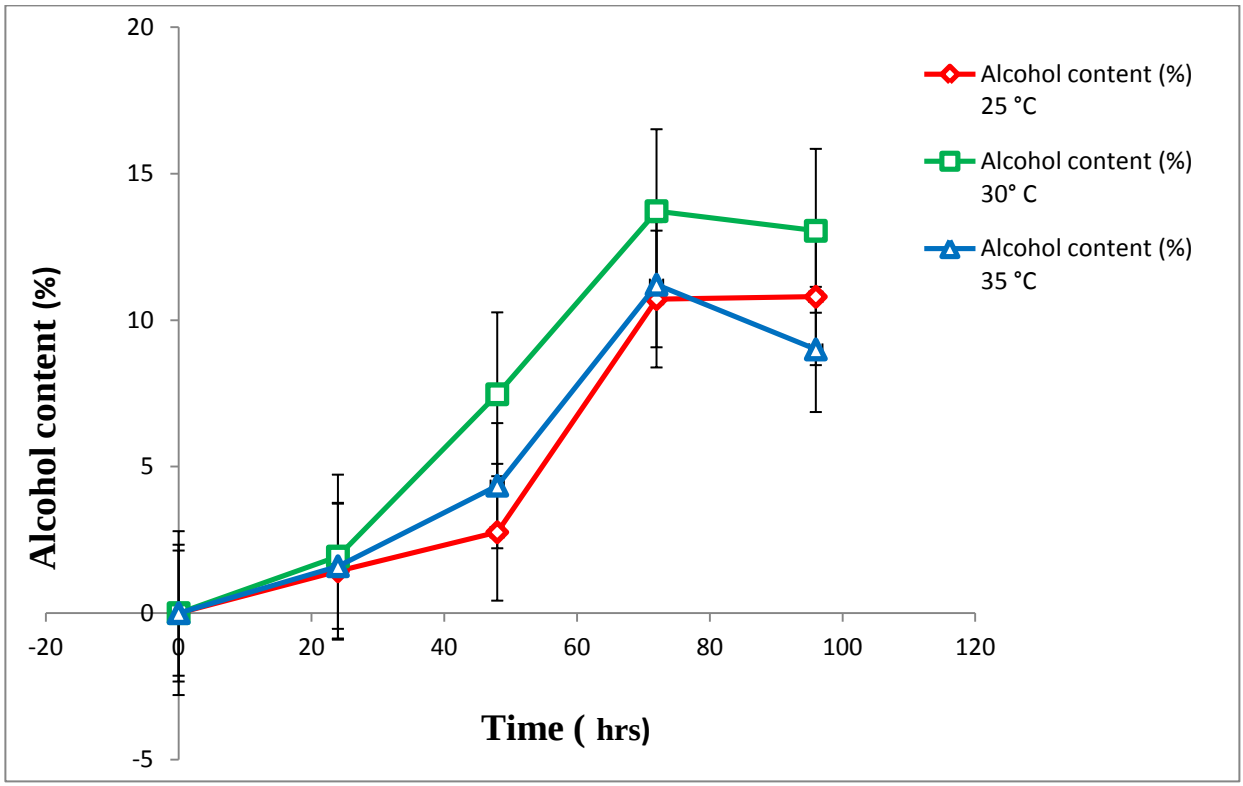

Figure 8: Effect of temperature on ethanol production

4.5.2 Effect of Sugar Concentration on Ethanol Production

Sugar concentration is a significant factor that influences ethanol production. Optimal sugar concentration was determined by fermenting SS juice at 15, 20 and 25 °Brix sugar concentrations at temperature level of 30 °C (Figure 9). Alcohol content increased with time, and after 72 hours, the highest alcohol content was 8.71, 12.8 and 10.19 % for 15, 20 and 25 °Brix respectively. Therefore, 20 °Brix produced higher alcohol content than 15 and 25 °Brix. Jones et al., (1981), observed that high ethanol yield resulted from high substrate concentration. Inhibition of fermentation resulting from osmotic stress may be caused by too high substrate concentrations. The optimum sugar concentration for ethanol production varies considerably among yeasts depending on species, strain and

the conditioning of the yeast (Batoul, 2006).

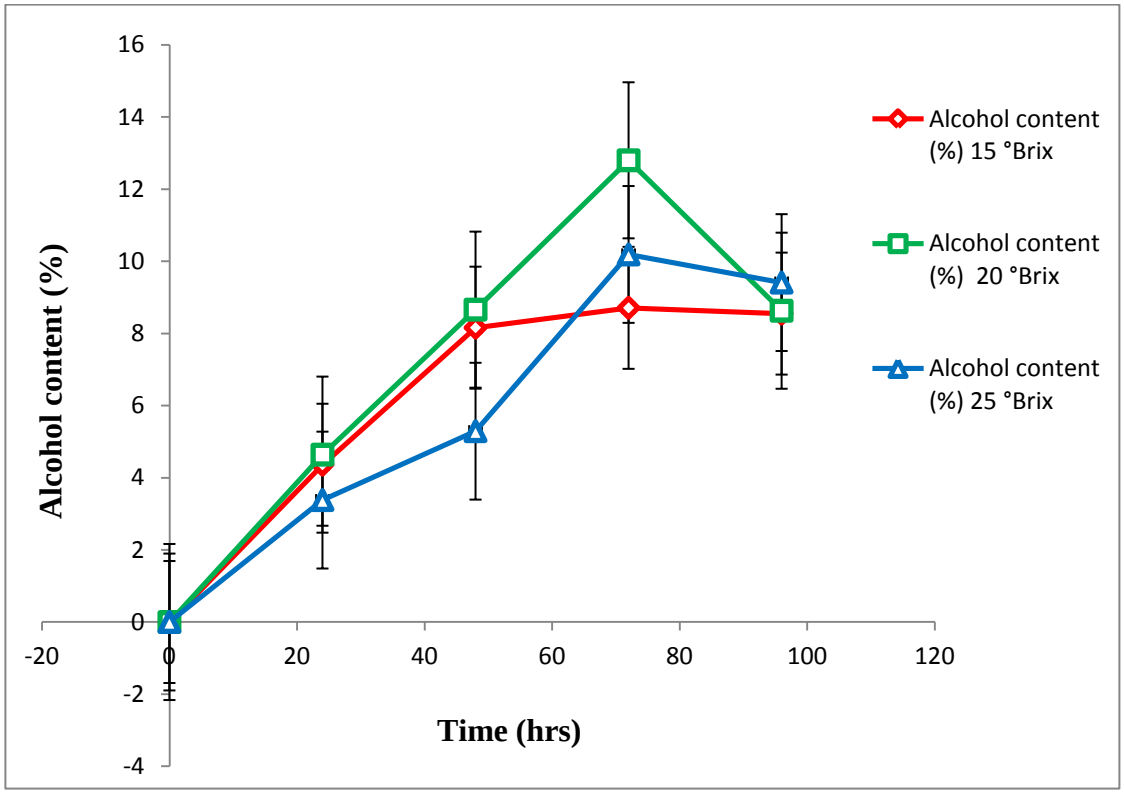

Figure 9: Effect of sugar concentration on ethanol production

4.5.3 Effect of pH on Ethanol Production

The optimal pH, determined by fermenting SS juice at 5, 6, and 7 pH levels and at 30°C temperature level (Figure 10). The pH of the medium was adjusted by gradually adding 2N H_2SO_4 and 2N NaOH (if required). Alcohol content increased with time and after 72 hours of fermentation, the alcohol content reported was 12.75, 10.02, and 10.27% for 5, 6, and 7 pH levels respectively. Therefore, pH level 5 produced higher alcohol content as compared to 6 and 7 pH levels. The initial and dynamic changes of pH level during

fermentation have an effect on optimum alcohol yield, since the activity of zymase enzymes produced by *Saccharomyces cerevisiae* is pH dependent (Batoul, 2006).

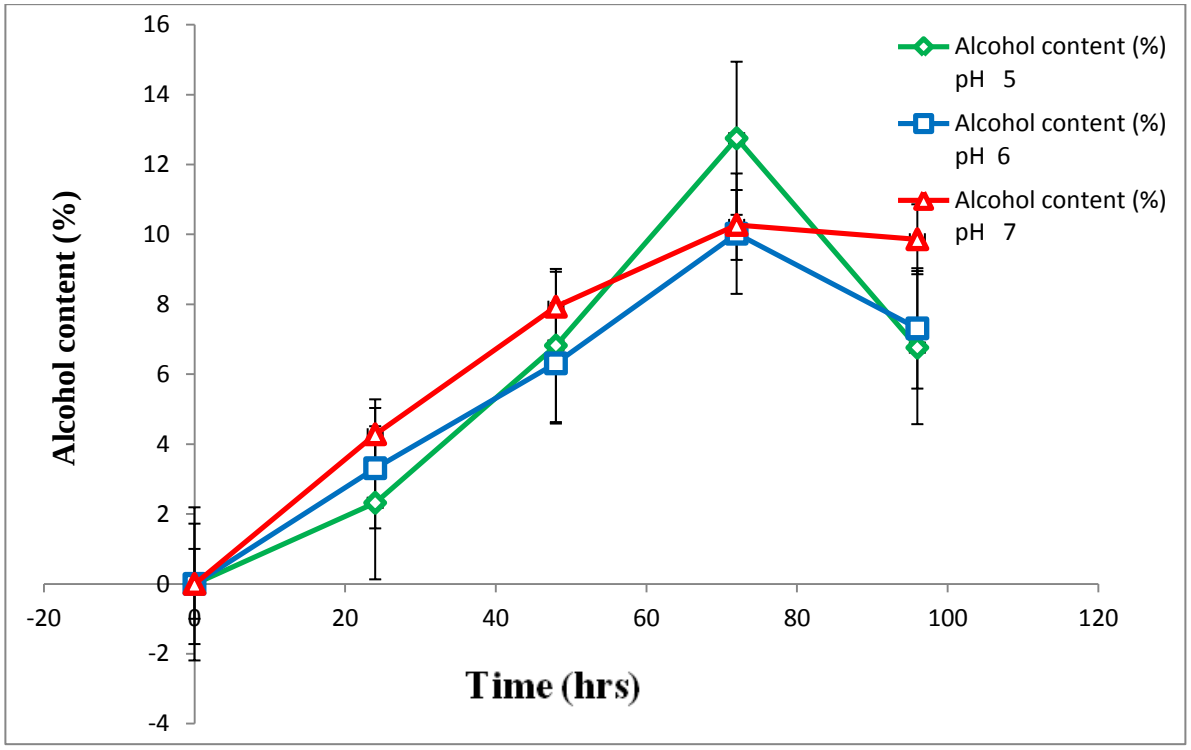

Figure 10: Effect of pH on ethanol production

4.5.4 Effect of Yeast Concentration on Ethanol Production

The optimal yeast concentration was determined by fermenting SS juice at 2, 4 and 6 % yeast concentration levels at temperature of 30 °C (Figure 11). Alcohol content increased with time and after 72 hours of fermentation, the alcohol content reported was 13.88, 10.8 and 9.8 % for 2, 4, and 6 % yeast concentrations respectively. Therefore, 2 % yeast concentration resulted in higher ethanol yield as compared to 4 and 6 %. The

results are similar to the findings of Batoul (2006), who found out that 2 % of actively growing yeast when inoculated in SS juice, rapid fermentation and optimum ethanol yield resulted. Reduced ethanol yield with increasing yeast concentration resulted from increased biosynthesis of glycerol which is non-fermentable (Brumm and Hebeda, 1988).

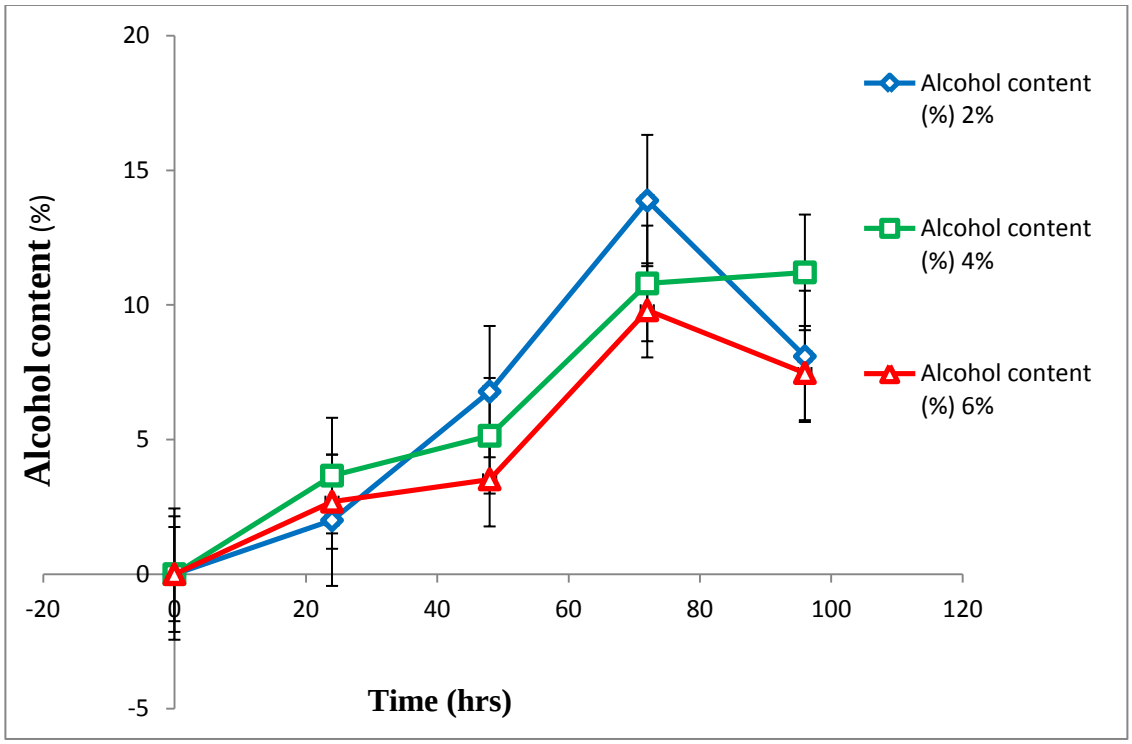

Figure 11: Effect of yeast concentration on alcohol production

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study revealed that juice extractability was significantly influenced by variety maturity stage and location. Optimum harvest time based on high juice extractability, juice yield and sugar concentration, was at the hard dough stage for SS juice from Rongo, and soft dough stage for SS juice from JKUAT. However to obtain both the stalk and grain, harvesting should be conducted at the maturity stage. The selected SS varieties with the highest biomass and sugar content were IESV 91018LT, IESV 92008DL, IESV 92038/2SH, SPV 1411, IESV 930042 and Madhura.

SS juice characteristics included; juice extraction percentage of 16.04-49.10, TSS content of 13.5-23.0 °Brix, pH of 5.27-5.74, total titratable acidity of 0.44-1.36%, ash content of 0.85-2.87%, also rich in both macro and micro-elements, making it a potential low-cost substrate for syrup and ethanol production.

SS syrup extraction percentage varied with harvest stage, with the hard dough stage producing the highest syrup yield at 256.9g/kg of SS juice. It was found to contain high total soluble solids, appreciable minerals amounts, and bio-active compounds, namely total phenolic, flavonoids and tannins. The total anti-oxidant activity for the tested concentrations was more than 50% inhibition of the DPPH radical.

The fermentation process using S cerevisiae yielded ethanol concentrations which varied significantly with time and maximized at around 13%. Optimum ethanol content using the classical method of one-factor- at- time was obtained at temperature of 30 °C, sugar

content of 20 °Brix, pH of 5 and 2 % yeast concentration for 72 hours. Hence efforts should be made to establish and promote small scale ethanol and syrup making enterprises in the rural areas with the goal of reducing poverty.

5.2 Recommendations

The traditional method of extracting SS juice is to squeeze the stalks through the roller mill, releasing the sugar rich juice. The extraction and sugar recovery increases with reduced roll gap, although smaller roll gaps may lead to frequent mill blockages. The main drawback of this method is that substantial fermentable sugars are not recovered with a single crushing. Hence to increase the extraction efficiency (95%), other methods such as, shredding of stalks before crushing and adding water during the squeezing process, use of multi-staged crushing process, and application of immobile extraction technologies should be adopted.

To prevent spoilage of foods and extend their shelf life, synthetic additives (preservatives) are added. However there is a growing concern among consumers on synthetic additives, this has led to the search for natural additives of plant origin with preservative effect on foods, hence the need to study the stability and anti -microbial activity of SS syrup.

During batch fermentation process the rate of ethanol production is maximized only for a brief period and thereafter declines progressively. There is need to study the effect of other fermentation methods (fed-batch and continuous fermentation methods) on ethanol production using SS juice, with the aim of adopting SS juice for industrial ethanol production.

The one- factor- at - a time method of fermentation process optimization, is based on changing one independent variable while fixing the others at a certain level. This method is applied for media components and process conditions optimization. Although the method is simple and easy it does not show the interactions between the components. The interactions could be assessed using factorial design methods which also allows for the estimation of the effects of each factor and interaction.

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