KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

CHARACTERISATION OF LOW ALCOHOL ROSE WINE PRODUCED FROM *Hibiscus sabdariffa* CALYCES AND *Sorghum bicolor* LEAF EXTRACTS

THIS DISSERTION IS PRESENTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT OF M.Sc. (Hons) DEGREE IN FOOD SCIENCE AND TECHNOLOGY.

BY:

BUAH JOSEPHINE AKUBA

PG3886409

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DECLARATION

I declare that I have undertaken the study reported herein under the supervision of Rev. Joseph Adubofour and Dr. Francis Alemawor and that except portions where references have been duly cited, this dissertation is the outcome of my own research.

……..	Buah Josephine Akuba	…….. …….
Date	Name of Student	Signature

Certified by Supervisors:

……..	Rev. Joseph Adubofour	…….. …….
Date		Signature

……..	Dr. Francis Alemawor	…….. …….
Date		Signature

Certified by Head of Department:

……..	Prof. Ibok Oduro	…….. …….
Date		Signature

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ABSTRACT

Wine is a natural fermented product from the juices of grapes and other fruits, by the action of yeast cells. Wines produced directly with alcohol contents in the range of 1.2-5.5% are low alcohol wines. Rose wines are wines that have some colour typical of red wines but only enough to turn it pink. The project was undertaken to assess the nutritional composition of *H.sabdariffa* calyces (roselle) and *Sorghum bicolor*leaves establish the optimum conditions for blends of extracts for wine production, determine the physicochemical properties of the fermented extracts aged into wine and to evaluate the sensory quality of low alcohol rose wine produced from rosellecalyces and *Sorghum bicolor* leaves. The roselle calyces and *S. bicolor* leaves were solar dried, milled separately and standard analytical methods were used to evaluate the proximate and mineral composition. Extracts were prepared from the leaves and calyces by infusion and physico-chemical analyses such as pH, titratable acidity (TA) and total soluble solids (TSS). The response surface methodology was used to establish the optimum conditions for extract blend and time of fermentation for the wine production. The optimum conditions were used to prepare bulk extracts for fermentation using *Saccharomyces cerevisiae*. Two kinds of fermented extracts were prepared. One from only *H.sabdariffa* calyces and the other from a blend of *H. sabdariffa* calyces and *S. bicolor* leaves. Physicochemical analyses were carried out on the fermented extracts and were divided into four. Two of them each from the blend and *H. sabdariffa* calyx were taken through a clarification process using egg albumin and natural sedimentation process, such that two clarified and unclarified fermented extracts were subjected to an aging period of 20 weeks. The wines were analysed during the aging period after which sensory evaluation (Affective test) was carried out using 32 panelists. Both leaves and calyces contained appreciable amounts of energy, carbohydrate, calcium, potassium, magnesium and iron. However, the amounts of nutrients determined in *H.sabdariffa* calyces were higher than those in *Sorghum bicolor* leaves. Physico-chemical properties on the extracts revealed both calyces and leaves had total soluble solids (TSS) of 0^{o} Brix. Table sugar was added to raise the TSS level to a brix of 18.62 to aid in fermentation. A fermentation time of 7.75 days and extract blend of 100% *H.sabdariffa* (HS) calyces extract as well as 75% *H.sabdariffa* and 25% *Sorghum bicolor* leaves (HS-SB) extract were the optimum conditions established through the response surface methodology. The two types of fermented extracts produced (from HS and HS-SB), had a pH of 3.61 and 3.63, TA of 7.30 g/l and 7.18 g/l respectively, which were within the pH range of 3 - 4 and TA range of 7 - 9 g/l accepted by the International Organisation of Vine and Wine. Significant differences ($p< 0.05$) were observed in the effects of clarification and blending on the total phenols (TP), total red pigments (TRP) and total colour densities (TCD) of the clarified and unclarified wines. The levels of these parameters were higher in the unclarified samples than the clarified wine samples and decreased significantly ($p<0.05$) with aging time. TP decreased from 18.21 AU to 10.16 AU in the unclarified *H. sabdariffa* wine and also from 16.58 AU to 8.19 AU for clarified wine from *H. sabdariffa* calyces. On the other hand, the decrease in TP in the blended (HS-SB) clarified and unclarified wines were from 13.97 to 7.37 AU and 16.64 to 9.15AU respectively. Panelist rated clarified wine from *H. sabdariffa* calyces as the most preferred among the four test wines in terms of aroma, taste, alcohol, acidity, sweetness and overall acceptability.

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CHAPTER ONE

1.0INTRODUCTION

1.1Background

The consumption of a variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health, in terms of preventionor cure of diseases because plants have long served as a useful and rational source of therapeutic agents (Owulade *et al.*, 2004).

Oxidative stress, which results from the imbalance between prooxidants and antioxidants in an organism, is considered to play a very important role in the pathogenesis of several degenerative diseases, such as diabetes, cancer and cardiovascular diseases, including arterial sclerosis, aging and neurodegenerative diseases (Bagchi *et al.*, 2000). However, the innate defense in the human body may not be enough for severe oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the reactive oxygen species-scavengers (ROS) (Tedesco *et al.*, 2000). Epidemiological evidence indicates that, moderate consumption of wines reduces the incidence of coronary heart disease (CHD), arterial sclerosis and platelet aggregation (Tedesco *et al.*, 2000). This greater protection may be due to the phenolic components of wines, which are particularly abundant in the red wine, since they behave as reactive oxygen species-scavengers and metal chelators (Tedesco *et al.*, 2000).

Hibiscus sabdariffa (roselle) belongs to *Malvaceae* family. It is an erect, mostly branched, annual shrub. Stems are reddish in colour and up to 3.5m tall. The calyces of *Hibiscus sabdariffa*are known to be rich in anthocyanins and contain a mixture of organic acids such as citric, malic and tartaric acids (Alobo and Offonry,

2009).Roselle calyces are edible and have been used in the preparation of tea and fermented drinks in Egypt. There are reports on the use of the red variety of roselle calyx to produce a drink in Sudan, Nigeria and West African francophone countries (Alobo and Offonry, 2009; Mahadevanand Pradeep, 2009).

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important cereal crop worldwide, both in terms of planted area and metric tons harvested. It is used among others, for various applications in food, feed, bio-fuel, paper, building materials (Berenji and Dahlberg, 2004; Taylor *et al.*,2006). Potentially, sorghum is an important source of nutraceuticals such as antioxidant, phenolics and cholesterol-lowering waxes (Gebruersa *et al.,* 2008). The anthocyanins are the major class of flavonoids studied in sorghum. Sorghums have high concentration of 3-deoxyanthocyanins (i.e. luteolinidin and apigenidin) that give stable pigments at high pH (Dykes and Rooney, 2006).

Anthocyanins vary in colour from red, pink to blue and violet. These characteristics suggest that roselle calyces and *Sorghum bicolor* leaves extracts may be suitable raw materials for the production of coloured wines (Alobo and Offonry, 2009).

1.2 Problem Statement

*Hibiscussabdariffa*calyces and *Sorghum bicolor* leaves have limited use in Ghana. *Hibiscus sabdariffa* calyces are used in the preparation of a soft domestic drink commonly called ‘bissap’ or ‘sobolo’ believed to have medicinal benefits. The *Sorghum bicolor* leaves are used in the preparation of ‘waakye’ (a local rice dish), as

a colourant, in the preparation of 'wagashie' (locally made cheese) and to feed livestock especially in northern Ghana.

Wines have become important in the life style of many people and especially Ghanaians. They are used in social gatherings like weddings, funerals, parties and in religious celebrations like Eucharistic services. All wines consumed in Ghana are imported and there is no local production. In Ghana today, consumption of alcoholic beverages has increased at an alarming rate. A report by Koranteng Adu in *The Statesman*, a local newspaper on February 25, 2008, states that Ghana was ranked 132nd out of 185 countries on the alcohol consumption chart in the year 2007. In view of this an alcohol policy draft has been made by the government which is expected to be passed to lay strict guidelines to moderate alcohol consumption with great emphasis on age, size, sex and health (Koranteng, 2008).

Wines are mostly produced from grapes, which are not available in the tropics hence the need for alternative use of plant materials and other fruits for wine production in the tropics.

A survey conducted on some churches in Kumasi metropolis to know their choice of wines (Appendix F; Figure 12) indicated that the wines used by churches for communion services were all imported wines from countries like Israel, Spain, Italy, America and South Africa, with alcohol levels below 7%.

1.3 Justification

Low alcohol rose wine produced from extracts obtained from *Hibiscus sabdariffa* calyces extract and *Sorghum bicolor* leaves extract will provide health benefits to its consumers due to its rich source of phenolic compounds and low alcohol. The production of low alcohol rose wine will help in the alcohol control measures

intended to curb alcohol consumption levels and drinking habits and reduce related social and health problems. This move is in line with the World Health Organization's (WHO) public health objective on alcohol which is to reduce the health burden caused by the harmful use of alcohol and thereby, to save lives, reduce disease and prevent injuries (Le Gales-Camus, 2004). The product will be a potential red wine which can compete with imported wines on the market and the product wouldbe beneficial to the local churches that rely on imported wines for communion services (Figure 12;Appendix F). This product could be relatively cheap because of the use of locally available raw materials and will save the government some foreign exchange for development.

There will be job creation opportunities when *Hibiscussabdariffa* calyces and *Sorghum bicolor* leaves are used for wine production.

1.4 Main objective and specific objectives

The main objective of this project work was to characterize low alcohol wine produced from *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves.

The specific objectives are outlined below:

- To determine the nutritional composition of *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves and establish the optimum conditions for blends of extracts for wine production.
- To determine the physicochemical properties of the fermented extracts aged into wine.
- To evaluate the sensory quality of low alcohol wine produced from *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 An overview of *Hibiscus sabdariffa*

Hibiscus has over three hundred species which are distributed in tropical and subtropical regions around the world. Most hibiscus species are used as ornamental plants, but many are believed to have certain medicinal properties; among them is *Hibiscus sabdariffa* (Yadong*et al*., 2005).

Hibiscus sabdariffa (Roselle) belongs to the family *Malvacea* which often survives in relatively poor soils (Oguntona, 1998). It is an annual herb cultivated for its leaves, stem, seed and calyces (Umerchuruba, 1997). The crop is native to India but was introduced to other parts of the world such as Central America, West Indies and Africa and best grown in tropical and sub-tropical regions (Umerchuruba, 1997).

Hibiscus sabdariffa is a woody-based sub shrub, growing to 2–2.5 m tall. The leavesare deeply three to five-lobed, 8–15 cm long, arranged alternately on the stems. The flowers are 8–10 cm in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, 1–2 cm wide, enlarging to 3–3.5 cm, fleshy and bright red as the fruit matures (Mohamad *et al.,* 2002). The calyx covers the seed pod which encloses the seed. The calyces mature rapidly and are ready for picking within 15 days of blossoming (McLean, 1973). Red, yellow, green and intermediate varieties of the calyces have been observed in a number of West African countries (Oguntona, 1998).The calyces are gathered for sale either fresh or dried. The seeds of roselle are fermented into a traditional alkaline condiment called bikalga (Burkina Faso), dawadawa-botso (Niger), datou (Mali) or furundu in Sudan (Parkouda*et al.*, 2009).

The Roselle is known by different names in various parts of the world. Table 1 shows some of the various names of Roselle around the world and dried Roselle calyces are shown in Plate 1.

Table 1: Various names for roselle in some parts of the world

Country	Local name(s)
Indonesia	Rosella, Rosella fruit
Ghana	Bissap, sobolo
Senegal	Bissap
France	Bissap
Mali	Dah, Dah bleni
Gambia	Wonjo
Nigeria	Zobo
Iran	Chaye-Torosh
Egypt	Karkade
Saudi Arabia	Karkade
Sudan	Karkade
Namibia	Omutete
Caribbean	Sorrel
Latin America	Sorrel
Mexico	Flor de Jamaica
Panama	Saril

(Anonymous, 2010a)

Plate 1: Dried *H.sabdariffa* calyces

(Pernmalai, 2011)

2.2 Medicinal benefits of*Hibiscus sabdariffa* and its use in food

The human body has complex systems of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Protection against free radicals can be enhanced by ample intakes of dietary antioxidants (Vertuani *et al.,* 2004). Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and activators of antioxidative defense enzyme systems to suppress free radical damage in biological systems (Aviram and Fuhrman, 2002).

Work done by many researchers show that extract from the red calyces of *H. sabdariffa* possess antioxidant properties (Ologundudu *et al.,* 2009a,b). The plant is rich in some phytochemicals as anthocyanins, and protocatechuic acid. The dried calyces of *Hibiscus sabdariffa* contain the flavonoids - gossypetin, sabdaretin, hibiscetin and anthocyanins. Flavonoids are phenolic substances that act in plants as antioxidants. The major pigment, formerly reported as hibiscin, has been identified as daphniphylline. Small amounts of myrtillin (delphinidin 3-monoglucoside), chrysanthenin (cyanidin 3-monoglucoside), and delphinidin are also present (Pietta, 2000).

Hibiscus protocatechuic acid has inhibitory and inductive effect on tumour promotion in mouse skin and in human leukemia cells respectively (Tseng *et al.,* 1997). Roselle is associated with traditional medicine and is reported to be used as treatment for several diseases such as hypertension and urinary tract infections (Odigie *et al.,* 2003).

Hibiscus sabdariffa (Roselle) has been shown to relax the uterus. It has also been used for indigestion (control of diarrhea), loss of appetite, as well as for colds, respiratory

problems and circulation disorders. Roselle has antibacterial and anti-oxidant properties; lowers the blood pressure (hypotensive effect) and possess antispasmodic effect. Roselle significantly reduces cholesterol content in blood serum and prevents oxidation of low-density lipoproteins. Roselle calyces are used as a digestive andpurgative agent and a folk remedy for abscesses (Ali *et al.*, 1991).Infusions of the calyces are regarded as diuretic, cholerectic, febrifugal and decrease the viscosity of the blood and stimulate intestinal peristalsis. The drink is also used to remedy the after effects of drunkenness, to treat coughs and as an anti-bacterial (Salah and Vierling, 2002). Roselle is potentially applicable to prevent atherosclerosis in humans due to its anti-hyper-lipidaemic effect and anti-LDL oxidation. It may therefore be useful in the prevention of a number of cardiovascular diseases in which cholesterol plays a major role (Lee *et al.*, 2002). Polysaccharides from *Hibiscus sabdariffa* flowers stimulate proliferation and differentiation of human keratinocytes (Brunold *et al.*, 2004).

Extract of roselle inhibits serum lipids and shows an anti-arterialsclerotic activity. It also has demonstrated other properties in test tube and in animal studies, such as reducing skin cancer promoted by ultraviolet light, inhibiting herpes simplex virus. Several studies have looked at the potential use of hibiscus for male and female fertility (Ali *et al.*, 2005).

2.3 Nutritional composition of *Hibiscus sabdariffa calyx*

Roselle is an important source of vitamins, minerals, and bioactive compounds, such as organic acids, phytosterols, and polyphenols, some of them with antioxidant properties. The phenolic content in the plant consists mainly of anthocyanins,

flavonoids and their respective glycosides (Oguntona, 1998; Ekwunzi, 1995). These nutrients are needed in the body for healthy growth and development. Tables 2, 3 and 4 show the nutritional and anti-nutritional composition of *Hibiscus sabdariffa* calyx.

Table 2: Proximate analysis of Roselle calyces (g/100g) dry mater

Constituent	Composition(g/100g)
Moisture	7.6
Ash	12.24
Fat	2.01
Protein	4.71
Crude Fibre	4.69
Carbohydrate	68.75

(Adanlawo and Ajibade, 2006)

Table 3: Mineral Composition of Roselle Calyces (mg/100g)

Constituent	Composition(g/100g)
Sodium	96.66
Potassium	49.35
Calcium	12.65
Magnesium	38.65
Iron	3.22
Zinc	12.22
Manganese	2.39
Nickel	1.78
Phosphorus	36.30

(Adanlawo and Ajibade, 2006)

Table 4: Anti-nutritional content and ascorbic acid composition

Constituent	Composition (%)
Phytic acid (%)	0.32
Oxalate (%)	6.15
Tannic acid (mg/100g)	2.00
Hydrocyanic acid (mg/100g)	0.16
Ascorbic acid (mg/100g)	16.67

(Adanlawo and Ajibade, 2006)

2.4 Utilization of *Hibiscus sabdariffa* in food and beverageproduction

Hibiscus sabdariffa calyx has multi-use in Africa and neighbouring tropical countries. The calyces are gathered for sale either fresh or dried. The dried calyces are utilized in Europe to make extracts for flavouring liqueurs. Roselle sauce or syrup may be prepared from the calyces and are added to puddings, cake frosting, gelatins, salad dressing, poured over gingerbread, pancakes, waffles or ice cream. The calyces possess 3.19% pectin and in West Pakistan, roselle has been recommended as a source of pectin for the fruit-preserving industry (Morton, 1987).

A beverage produced from the infusion of the calyces is called 'zobo' in Nigeria and 'bissap' in Ghana which is used for refreshment and entertainment in home and public gatherings (Schippers, 2000). The calyces are used in the production of roselle jam, jellies, cold and warm teas. The calyces are used whole in preparing melon soup and together with other soup ingredients serve as meat substitute for the poor masses who consume it. It is also used in making vegetable stews. Traditionally the calyx has been chewed to alleviate thirst on long desert trecks of Moslems (Schippers, 2000; McLean, 1973).

2.5 Origin and production of *Sorghum bicolor*

*Sorghum bicolor*is a cultivated tropical cereal grass belonging to the family *Poaceae* (*Gramineae*). It is Africa's second most important cereal in terms of tonnage. The continent produces about 20 million tonnes of sorghum per annum. The major sorghum producing countries of Africa with their production tonnage are listed in Table 5. It can be seen that sorghum production cuts across the African continent, with the northern African countries of Nigeria, Sudan, Ethiopia and Burkina Faso

accounting for nearly 70% of the continent's production. Ghana is the thirteenth major Sorghum producing country in Africa from Table 5.

Table 5: The major Sorghum producing countries of Africa.

Country	**Production (tonnes x 10^3)**
Nigeria	7 081(33.8%)
Sudan	4 470(21.4%)
Ethiopia	1 538(7.3%)
Burkina Faso	1 372(6.6%)
Egypt	862(4.1%)
Tanzania	736(3.5%)
Niger	656 (3.1%)
Mali	517 (2.5%)
Chad	497 (2.4%)
Cameroon	450 (2.1%)
Uganda	423 (2.0%)
Mozambique	314 (1.5%)
Ghana	280 (1.3%)
South Africa	211 (1.0%)
Benin	165 (0.8%)
Togo	141 (0.7%)
Senegal	140 (0.7%)
Kenya	133 (0.6%)
Somalia	100 (0.5%)

(Taylor, 2003)

Quantitatively, sorghum is the world's fifth largest most important cereal grain, after wheat, maize, rice and barley. Sorghum is believed to have originated from north-eastern Africa, possibly Nile or Ethiopian regions as early as 1000 BCand distributed all over Africa and along shipping and trade routes through the Middle East to India. World annual sorghum production is over 60 million tonnes, of which Africa produces about 20 million tonnes (Murty and Kumar, 1995).

2.6 Medicinal benefits of *Sorghum bicolor* leaves

Sorghum bicolor is used extensively in folk medicine. Sorghum bicolor is reported to contain anti-abortive, cyanogenetic, demulcent, diuretic, and emollient properties. Sorghum is used as a folk remedy for cancer, epilepsy, flux, and stomachache (Duke and Wain, 1981).

Sorghum bicolor leaves and panicles are included in plant mixture for decoctions against anaemia and measles. The red pigment is said to have antimicrobial and antifungal properties and is also used for cure against anaemia in traditional medicine. Seed extracts are drunk to treat hepatitis and decoction of twigs with melon to treat jaundice (Anonymous, 2011). *Sorghum bicolor* is rich in apigenin. Apigenin is described as a nonmutagenic bioflavonoid present in leafy plants and vegetables. Current research trials indicate that it may reduce DNA oxidative damage; inhibit the growth of human leukemia cells and induce these cells to differentiate; inhibit cancer cell signal transduction and induce apoptosis; act as an anti-inflammatory; and as an anti-spasmodic or spasmolytic as well as reduce the risk of ovarian and prostate cancers (Gutierrez, 2009).

2.7 Nutritional composition of *Sorghum bicolor*

The nutrients essential for life are proteins, fat, carbohydrates, minerals including trace elements, vitamins and water. Numerous studies including some in man have demonstrated clearly that life may be sustained by nutrient mixtures in which every component is definable chemically and soluble in water (Underwood, 1994).

Sorghum crop residues are an important potential feed resource. In 1981, 55.2 million tonnes of crop residue were produced from sorghum (Kossila, 1985). Table 6 and 7 show the proximate and mineral compositions of*Sorghum bicolor* stem flour on percentage dry basis.

Table 6: Proximate composition of *Sorghum bicolor* stem flour (%) on dry basis

Parameter	Proximate value (%)
Moisture	6.54
Protein	3.20
Crude fat	8.38
Total ash	5.34
Crude fibre	32.02
Carbohydrate	44.52

(Adetuyi *et al.*, 2007)

Table 7: Mineral composition of *Sorghum bicolor* stem flour on dry basis

Parameter	Constituent (mg/100g)
Sodium	127.61
Potassium	138.87
Calcium	151.70
Magnesium	185.33
Iron	10.98
Copper	0.47
Zinc	7.15
Manganese	2.83

(Adetuyi *et al.*, 2007)

2.8 Utilisation of *Sorghum bicolor* in food and beverage production

Sorghum in Africa is processed into a variety of attractive and nutritious traditional foods. The whole grain is boiled to produce a food resembling rice, roasted usually at the dough stage, or popped like maize. The grain is ground or pounded into flour, often after hulling. Sorghum flour is used to make thick or thin porridge, pancake,

semi-leavened bread dumplings or couscous, opaque and cloudy beers and non-alcoholic fermented beverages (Taylor and Dewar, 2000).

Sorghum grain is germinated, dried and ground to form malt, which is used as a substrate for fermentation in local beer production. Sorghum is also grown for forage, either for direct feeding to ruminants or for preservation as hay or silage (Rooney and Waniska, 2000).

Several non-edible sorghum cultivars are exclusively grown for the red dye present in the leaf sheaths and sometimes also in adjacent stem parts. A similar dye can be extracted from the grain refuse (glumes and grain wall) of several red sorghum cultivars grown for food or for beer making (Murty and Kumar, 1995). Sorghum plant residues are used extensively as material for fuel (Rooney and Waniska, 2000).

2.9Wine production and classification

Wine is a natural fermentative product of the juices of grapes and other fruits, by the action of yeast cells. This biochemical conversion of juice to wine occurs when the yeast cells enzymatically degrade the fruit sugars fructose and glucose first to acetaldehyde and then to alcohol (Akubor, 1996).

Red wines are made into a variety of styles. The stylistic differences are based on differences in wine characteristics such as grape variety, color, flavour, body, mouth feel, and aging potential. The styles range from simple, fruity, fresh, light coloured blushes and rosés to complex, full bodied, rich and dark red, with long aging potential.

Wine style produced, is influenced by the variety of grape, fruit composition, soil, climate and viticultural practices and winemaking techniques (Dharmadhikari, 2000a).

Europe is the leading wine producer and consumer of wines in the world. Europe accounted for 68% of the total wine production and consumed 66% of the total in 2009.

In Africa, South Africa (S.A) is the largest wine producer. In 2006, S.A exported 2.90% of wine to the world market (Table 9).

Table 8: The largest world wine producers in 2009

Country Ranking	Volume Produced (million hectolitres)
1 – Italy	48
2 – France	46
3 – Spain	35
4 – USA	20
5 – Argentina	12
6 – China	12
7 – Australia	11
8 – Chile	9.9
9- South Africa	9.8
10 – Germany	9.2
11 – Russia	7
12 – Romania	6.7

Source: (Per and Britt, 2009).

Report from Ghana exporter guide 2008, indicate that value of U.S. consumer oriented food exports to Ghana was $25 million in 2007, of which poultry meat, breakfast cereals, dairy products, processed fruits and vegetables, fruit and vegetable juices, wines and beer made up most of these imports. Unofficial estimates indicate that wine accounted for over 85 percent of spirits imports into West and Central Africa (Anonymous, 2010b).

Wine classification is grouped into two. A classification system by the European union and a classification system outside the European union. Outside the European union, wines are generally named for the grape variety used. European wines are named both after the place of production; e.g. Bordeaux, Rioja, Chianti, Cotnari and the grapes used, like Pinot, Riesling, Chardonnay, Merlot (Robinson, 2006). The wines of the European Union can be divided into two principal categories: Quality Wines Produced in Specified Regions (QWPSR) and Table Wine (TW) (Anonymous, 2009a).

Classification using vinification method and style refers to how the wine is made. These include classifications such as red or white wine, semi-sparkling or still wines, fortified and dessert wines, with alcohol percentage of 14 to 24 percent, by volume (ranging from slightly sweet with less than 50 g/L of sugar to incredibly sweet wines with over 400 g/L of sugar), aperitif or aperitif wines (herb or spiced wines served before meals), pop wines (sweet fruity mostly cheaper wines), sparkling wines (wine containing carbon dioxide) and table wines. Table wines may have an alcohol content that is not higher than 14% in the US. In Europe, light wine must be within 8.5% and 14% alcohol by volume. Table wines are usually classified as "white," "red," or "rosé," depending on their colour.

Rose wine is a type of wine that has some of the colour typical of a red wine, but only enough to turn it pink. This pink shade can range from a soft, subtle hue to a vibrant, hot pink, depending on the grapes and wine making techniques (Slinkard, 2012). By taste wines may be classified as dry (meaning not sweet), fruity, or sweet or wines may be classified according to specific flavours (Robinson, 2006).

Another type of wine is the low alcohol and the reduced alcohol wines. The reduced alcohol wines have alcohol levels above 1.2% but less than 5.5%. They are normally

produced from high alcoholic wines most commonly through distillation, dialysis or reverse-osmosis to obtain the low alcohol. Low alcohol wines have alcohol percentage ranging from 0.5%-1.2%. Wines produced directly with alcohol contents in the range of 1.2-5.5% are also referred to as low alcohol wines (Erten and Campbell, 2001).

Wine classification can be made based on vintage or varietal. A vintage wine is one made from grapes that were all, or primarily, grown in a single specified year. The non-vintage wines are produced from grapes with different vintages. A varietal wine is wine made from a dominant grape such as a Chardonnay or a Cabernet Sauvignon. Plates 2, 3, 4 and 5 denote some of the different types of grapes and wines.

Plate 2: Rose wine (Anonymous, 2012)

Plate 3:Merlot grape and wine(Anonymous, 2012)

Plate 4: Red and white wine(Anonymous, 2012)

Plate 5: Bordeaux grape and wine(Anonymous, 2012)

2.10 Raw materials used in wine production

The principal raw materials used in wine making are gapes. Aside the grapes, other fruits are used in wine production especially in Africa, Asia and Latin America (Gill, 1992).

Fruit wines are fermentedalcoholic beverages made from a variety of ingredients (other than grapes) and having a variety of flavours. Fruit wines are usually named by their main ingredient fruit e.g. apple wine (Robinson, 2006).

Fruit wine can be made from virtually any plant matter that can be fermented (Harding, 2005). Most fruits and berries have the potential to produce wine. The amount of fermentable sugars is often low and need to be supplemented with sugar in a process called chaptalization in order to have sufficient alcohol levels in the finished wine (Harding, 2005).

Fruits such as strawberries, plums, watermelons, peaches, blackberries, gooseberries, boysenberries, grapes, pears, pineapples, persimmons, apples, cashew among others are all very suitable for fruit wine making (Kraus, 2002).

2.10.1 Cultured Yeast and Inoculation

Wine making from grapes is an old fermentative process which can occur spontaneously by the activity of yeasts and partly by the activity of lactic acid bacteria on grapes (Bauer and Pretorius, 2000; Zarzoso *et al.*, 2000). Yeasts of the genera *Kloeckera*, *Hansensiaspora*, *Candida*, *Pichia*, and, sometimes, *Hansenula* grow during the early stages of fermentation but eventually die off, leaving *Saccharomyces cerevisiae* as the dominant species to complete the fermentation (Phonesavard *et al.*, 2010). Collectively, these species contribute to the final quality of the wine.

The fermentation properties of *S. cerevisiae* affect acidity, ethanol content and sensory properties of the wine (Fundira *et al.*, 2002).

Yeast for enological activities should have the ability to conduct vigorous fermentation, ferment must to dryness, good tolerance to ethanol, tolerant to commonly used SO_2 levels, produce no off aromas, temperature tolerance, no or low foam formation and flocculating potential and other optional properties like killer cells' character and malic acid degradation ability (Bauer and Pretorius, 2000).

Inoculation with a starter culture is intended to establish a high population of a selected strain of *Saccharomyces cerevisiae* from the beginning of fermentation to ensure its dominance. It results in a rapid production of alcohol and consequent minimization of the growth of non-Saccharomyces yeasts present (Boulton *et al.*,1996; Dharmadhikari, 2000b). Inoculation reduces the fermentation time and

allows the formation of wines with a predictable flavour (Henick-Kling *et al.,* 1998; Comi and Croattini, 1997).

2.10.2 Primary and secondary fermentation

Fermentation is an energy yielding process in which organic molecules serve as both electron donors and electron acceptors. Alcoholic fermentation is carried out by fungi, some bacteria, algae and protozoa (Prescott*et al.,* 1999).

Primary fermentation in wine making is the conversion of sugar to ethanol, which are the foundation of the transformation of grapes into wine. The glucose, fructose and sucrose found in grape must and juice is converted into ethanol via the process of fermentation by yeast normally Saccharomyces, *S. cerevisiae*, *S. bayanus, Saccharomyces cerevisiae var. ellipsoids* or *pastorianus* (Bisson, 2001; Belitz *et al.*,1999).

Glycolysis is a biochemical pathway by which sugars are degraded in an energy yielding process to the three carbon compound pyruvate. The end products of glycolysis are pyruvate and 2 molecules of the reduced co-factor NADH. The catabolism of glucose to pyruvate in glycolysis is represented by the equation below.

Glucose + 2ADP + 2PI + $2NAD^+$ ➔ 2pyruvate + 2ATP +2NADH + $2H^+$

(Bisson, 2001; Prescott*et al.,* 1999).

In the alcoholic fermentation, pyruvate is decarboxylated to acetaldehyde, which is then reduced to ethanol by alcohol dehydrogenase with concomitant formation of NADH or NADH serving as electron donor.

CO2

Pyruvate -->Acetaldehyde -->Ethanol

In fermentation, an organic compound, in this case acetaldehyde, serves as terminal electron acceptor which leads to the production of ethanol. Yeast cells regenerate NAD^+ by transferring the hydrogen molecule (electron) to an organic molecule: acetaldehyde.

Alcoholic Fermentation (acetaldehyde final electron acceptor) is shown below.

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + 2\ ATP + heat$ (Bisson, 2001).

Secondary (malolactic) fermentation is a process by which a lactic acid bacteria Oenococcus converts L-malic acid, containing two acid groups into L- lactic acid containing one acid group. Decarboxylation of malic acid into lactic acid and carbon dioxide is aided by the enzyme malate carboxylase (Jacobson, 2006).

$COOH\text{-}CH_2\text{-}CHOH\text{-}COOH \xrightarrow{\text{malate carboxylase}} CO_2 + CH_2\text{-}CHOH\text{-}COOH.$

Secondary fermentation results in an increase in pH, of approximately 0.3 units, a decrease in titratable acidity in the range of 1-3g/L, the production of diacetyl, acetoin, acetic acid and esters (Jacobson, 2006). Secondary fermentation stabilizes wine with higher pH and low SO_2 levels microbiologically. It also improves the fruity aroma of chardonays and bouquet of white wines.

In commercial wine production, secondary fermentation is made to occur naturally to have the indigenous bacteria from their vineyards produce wine typical of the area and varietal. Concurrent primary and secondary fermentation can be advantageous

due to the decreased alcohol and increase in some nutrients such as B complex vitamins provided by yeasts autolysate (Jacobson, 2006).

2.10.3 Stages involved in red wine production

The stages involved in red wine production are presented in Figure 1.

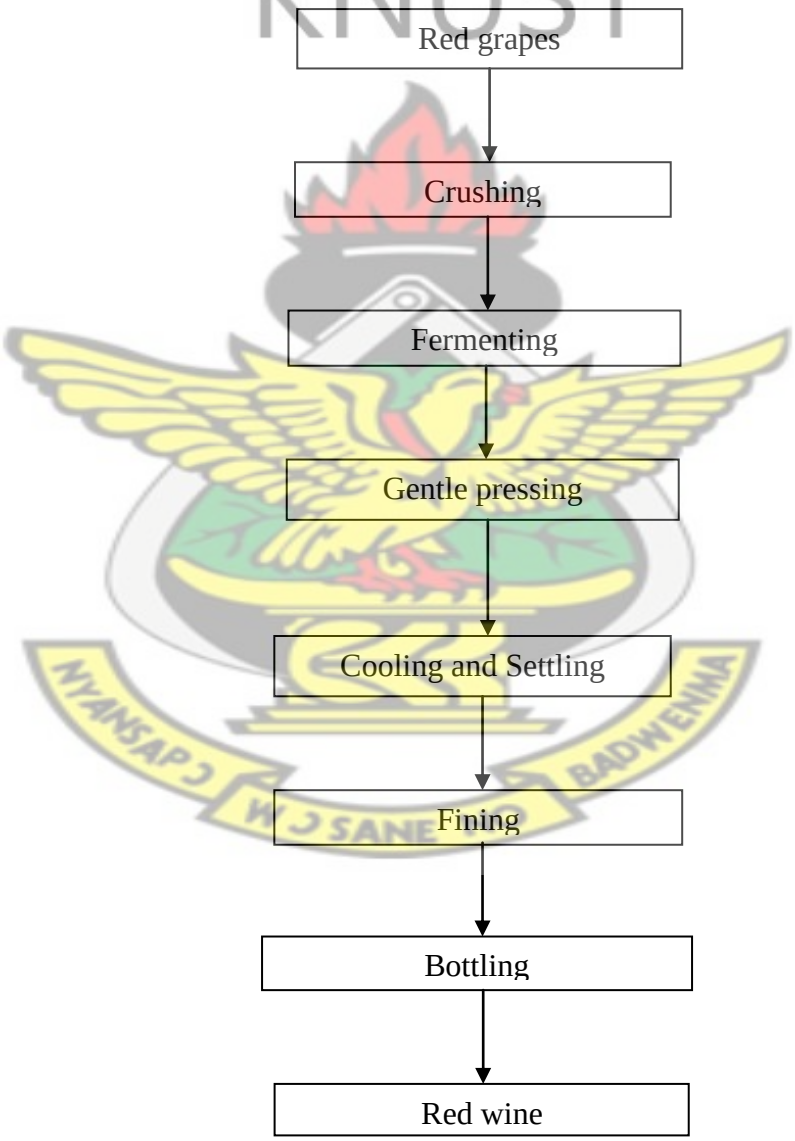

Figure 1: Flow diagram showing production of red wine (Belitz *et al.*, 1999)

Red wine grapes

Wine style, variety, and maturity criteria determines the time for maturity and harvest of the grapes. During maturation sugars accumulate, titratable acidity declines, pH rises, colour, and phenolic compounds increase and the formation of distinct varietal aroma components occur. A balance among these parameters is best for wine production (Dharmadhikari, 2000a).

Crushing and destemming of berries

This involves separating the stems from the berries and breaking the berry skin to obtain the red wine mash. About 30 mg/l of free SO_2 is added. This is to prevent the development of unwanted microbes such as indigenous yeast and harmful bacteria (Dharmadhikari, 2000a; Belitz *et al.*, 1999).

Fermenting the Must

The must (pulp of the grapes) is inoculated with the wine yeast and allowed to ferment in fermentation tanks. Red must is fermented between the temperature range of 21^{o}C- 32^{o}C. Fermentation releases heat, which increases the must temperature. Increased temperature enhances the rate of fermentation the extraction of colour and phenolic compounds. Excessively high temperature (31.6 to 35ºC) causes stuck fermentation, promote the growth of undesirable microorganisms and contribute to the formation of off-odour compounds (Dharmadhikari, 2000a).

Pressing

Pressing is the act of applying pressure to grapes in order to separate juice or wine from grapes and grape skins. Pressing completes alcoholic and malolactic fermentations after optimum extraction of colour, flavour, tannins, and other constituents, depending on the wine style (Dharmadhikari, 2000a).

Cooling and Settling

This involves wine clarification and racking. Young red wine is cloudy and the turbidity is caused by particles that remain in suspension. The particulate matter includes grape fragments, crystalline compounds (potassium bitartrate), colloidal compounds and microorganisms such as yeast and bacteria. During storage, many of the particles slowly settle to the bottom leaving the wine relatively clear. To achieve greater clarity, to make wine brilliantly clear, wine is subjected to treatments such as racking (Dharmadhikari, 2000a; Belitz *et al.*,1999). In racking, the wine is rid off the lees and off-odours (such as hydrogen sulfide) that may have developed during fermentation (Dharmadhikari, 2000a; Belitz *et al.*, 1999).

Fining

Red wine is fined to achieve greater clarity. The fining agents tend to influence the flavour of the wine. Red wines are rich in pigments and phenolic compounds such as tannins that contribute to a harsh and astringent taste. Proteinaceous fining agents such as gelatin and egg white are often used to lower the tannin level, soften the wine,

and enhance clarity. Protein fining agents have a positive charge at wine pH (3.0 - 4.0). They generally have an affinity for polyphenols and reactby forming hydrogen bonds between the phenolic hydroxyl and the peptide bonds of the protein component. Large polyphenols such as tannins and polymerized anthocyanins are preferentially removed (Zoecklein *et al.*, 1990).

For egg white fining, egg white is separated from the yolk and mixed with some water. Addition of a pinch of salt makes the solution clear. The solution is added slowly without foaming to the wine while stirring. Generally five to eight egg whites per barrel (50 gallons) are used for fining. Gelatin is used at concentrations of 30-150mg/l (Dharmadhikari, 2000a; Belitz *et al.*, 1999).

Bottling

At this stage, the wine is siphoned into wine bottles and corked securely. New and unused bottles are rinsed with hot water and dried. Used bottles are soaked in a cleaning solution (12% Benzalkonium Chloride, sodium metabisulphite) washed and dried before use. There are three different bottle colours; green, blue and clear used in bottling new wines. Coloured bottles are used to reduce light exposure to wine which reduces oxidation (Dharmadhikari, 2000a).

2.10.4 Maturation and bottle ageing of wine

Wine aging refers to the changes in the character of a wine after the fermentation period is over and involves a group of reactions that tend to improve the taste and flavour of the wine over time (Dharmadhikari, 2000a). Wine 'maturation' refers to

changes in wine after fermentation and before bottling. During this period, the wine is subjected to various treatments, such as malolactic fermentation, clarification, stabilization, and bulk storage (Butzke, 2008). The reactions during aging and maturation, may bring about noticeable changes in the composition of the wine, or may be subtle with no impact on the sensory pro/perties of the wine. The most obvious change during this stage is the colour of the wine (Butzke, 2008; Boulton *et al.,* 1996). In red wine, the purple and violet tints are progressively replaced by orange and brick red colours. The grape-derived aromas fade, and more complex and pleasing aromas develop. The taste of the wine also changes. Astringent and harsh tastes are replaced by smoother tastes. The various taste and aroma components integrate, yielding complex, rich, and delicious wines (Butzke, 2008).

In young red wines, the bright red (with purple tint) colour which is due to monomeric anthocyanin pigments are progressively replaced by the polymeric form, which results from the combination of anthocyanin pigments with tannin (Fulcrand *et al.,* 2004). The periodic exposure of the wine to air influences the changes in wine composition. During maturation, oxygen (from air) plays a role in the condensation reaction between anthocyanins and tannins, which results in the gradual loss of free anthocyanins and the formation of stable polymeric (anthocyanin tannin) pigments. As the wine matures and more polymeric pigments are formed, the colour shifts from red to orange and brick red. The condensation reaction between anthocyanins and tannins is accelerated by oxidation. If condensation continues (due to oxidation), precipitation of colouring matter occurs. The condensation reaction mechanism includes participation of acetaldehyde under aerobic conditions.

A wine well aged acquires a smoother, mellower and richer mouth feel. The improved taste is mainly due to polymerization of phenolic compounds and reduction in acidity.

Red wines are rich in flavonoid phenols, which are associated with astringency and bitterness. The monomeric flavonoids are more bitter than astringent. As flavonoid phenols polymerize, they become less bitter and more astringent (Castellari *et al.*, 1998).

During maturation, oxidative and non-oxidative polymerization and precipitation of phenolic compounds (of larger molecules) occurs. This results in a wine with reduced astringency and a smoother, softer taste. Oxidative changes affect wine maturation, as it can lead to, loss of varietal character, and the development of aldehydic aroma (Wildenradt and Singelton, 1974). The rate of oxidation depends on pH, temperature, concentration of dissolved oxygen and the phenolic composition. Oxidation is greater at high pH and high temperature. Light exposure affects wine. Exposure of light in the ultraviolet radiation range can initiate an oxidative reaction (Cano-L´opez*et al.*, 2007; Gonzales *et al.*, 1994).

During aging and maturation there is loss of yeasty aromas, retention of the varietal aroma, formation of new aromas, and integration of all flavours to produce a pleasing fragrance. Many esters and higher alcohols formed by the yeast's metabolic activity contribute to the fermentation aroma. During wine storage, the esters are hydrolyzed and the fresh and fruity aroma is lost. Concurrent with the degradation of esters, a synthesis of new esters occurs (Boulton *et al.*, 1996). Some undesirable changes occur during wine maturation and aging if not properly done. Aldehyde or nutty aromas can develop due to oxidation of wine. Many off- odours are formed due to spoilage by yeast and bacteria (Boulton *et al.*, 1996).

2.11 Physicochemical properties of wines

2.11.1 Importance of phenolic compounds

Polyphenols are natural aromatic compounds containing two or more phenolic hydroxyl groups that occur in remarkable amounts in tissues seeds and fruits of several angiosperms (Kahle *et al.,* 2006; Vinson *et al.,* 2005). Polyphenols serve as defense agents facilitating protection against microbial infection and UV radiation. The importance of phenolic compounds is related to their antioxidant activity (Jefremov *et al.,* 2007).

Phenolic compounds get into the human body as part of the diet (Avar *et al.,* 2007) and epidemiological studies have demonstrated that the consumption of phenol–rich foods and beverages contributes to the reduction of coronary heart disease mortality (Cul *et al.,*2002). Phenolic compounds also inhibit chronic inflammation and thrombosis by decreasing thrombocyte aggregation (Weisburger, 1999; Pace-Asciak, 1995) and affect apoptosis and cell proliferation through modulation of signal transduction pathways. Their antioxidant as well as anticarcinogen effects have been proven (Rusak *et al.,* 2005).

This strong antioxidant, neuroprotective and cardioprotective effect of polyphenols found in wines appears in even one thousand times diluted solutions as well. The importance of wine polyphenols lies in their good availability. In wines the polyphenols have increased digestibility. This is because during fermentation, the polyphenols get to their monomerformsand is stabilized by the alcohol in the wine (Avar *et al.,* 2007; Sun *et al.,* 2002).

Red wine phenolics contribute to the astringency, bitterness, and other tactile sensations defined as structure or body, as well as to the wine's red colour. Red wines have a reported range of 23 to 100 AU, with an average of 54 AU. Aroma and

polyphenolic compounds are important constituents of wine as they contribute to the quality of the final product (Demyttenaere *et al*., 2003).

2.11.2 Effect of organic acids and pH on wine quality and stability

Organic acids make major contributions to the composition, stability and organoleptic qualities of wines. The preservative properties of organic acids also enhance wines' microbiological and physicochemical stability (Jackson, 1994).

Red wines are stable at lower acidity, due to the presence of phenols which enhance acidity and help to maintain stability throughout aging. Total acidity influences the flavour balance of wines. The pH values of wines range from 2.8 to 4.0. Such pH values enhance the microbiological and physicochemical stability of wines (Rib´ereau-Gayon *et al*., 2006).

Low pH hinders the development of microorganisms, while increasing the antiseptic fraction of sulfur dioxide (Rib´ereau-Gayon *et al*., 2006).

2.11.3 Alcohol content of wines

Alcohol contributes to body, texture, intensity and sweetness in fruit wines. The contribution of ethanol to wine sensory properties includes enhancement of wine fullness and its effects on the headspace concentrations of many wine volatiles, contributing to sweetness (Guth and Sies, 2002).

At higher levels, it suppresses fruit aromas on the nose by secluding aroma molecules, preventing them from being released into the air. Alcohol has no taste but can overpower the palate preventing recognition of nuances in wine by obscuring

flavours. At levels of 15% or more in wine, it can cause a burning sensation in the nostrils and create a sense of bitterness and heat on the finish (Anonymous, 2010c). Also, ethanol can induce palate warmth and perceived viscosity may indirectly affect both aroma and flavour perception (Delwiche, 2004). Alcohol has seductive qualities. It supplies calories, stimulates the appetite, offers gustatory pleasure, and leads to relaxation, more social interaction and procreation (Anonymous 2010d).

2.11.4 Development of aroma compounds in wine undergoing maturation

Aroma is an important factor in quality control and quality assurance of wines. It is produced by a complex balance of more than 800 volatile compounds in different ranges of concentrations, and with different volatilities and polarities. The aroma and flavour compounds are found and released through the nascent in the grape (released only by crushing), and compounds produced by enzymaticinteractions during crushing, fermentation and the result of aging (Swiegers*et al.*, 2005).

During aging, the wine acquires aromatic complexity as a result of important modifications which results from different phenomena, such asesterification or hydrolytic reactions, redoxreactions, spontaneous clarification, CO_2elimination, slow andcontinuous diffusion of oxygen through oak wood pores and transferenceof tannins and aromatic substances from the wood into the wine. The aroma formed during the alcoholic fermentation decreases, but new compounds appear from oak wood and from the generation of primary and secondary aromas (Swiegers *et al.*, 2005; Lambrechts and Pretorius, 2000).

Qualitatively, esters are the largest constituents of wines. Esters are either cyclic (phenolic) or straight chain (aliphatic) in structure. Phenolic esters have a low

volatility and have no significant sensory impact (Rib´ereau-Gayon *et al.*, 2006; Sua´rezr *et al.,* 2006).

Aldehydes and ketones are carbonyls produced in relatively small amounts and do not play an intrinsic role in the creation of varietal wine aromas (Rib´ereau-Gayon *et al.,* 2006). The most common aldehyde found in wine is acetaldehyde which makes up more than 90% of the aldehyde content. Acetaldehyde is produced as an intermediary product of yeast metabolism during alcoholic fermentation and as a result of oxidation of ethanol during storage (Rib´ereau-Gayon *et al.,* 2006; Ebeler 1999). Normal levels in newly fermented wine are less than 75 mg/l with sensory thresholds between 100 and 125 mg/l. At levels above these values acetaldehyde imparts overripe, bruised fruit, and sherry like aromas (Zoecklein *et al.*,1999).

Quantitatively, the largest groups of alcohols found in wine are ethanol and glycerol, followed by diols, higher alcohols and esters. Ethanol contributes to the structural and textural aspects of wines and higher alcohols (fusel alcohols) are responsible for aroma due to the fact that they are found in quantities above perception threshold(Rib´ereau-Gayon *et al.,* 2006). The major compounds found in the headspace of fermenting must are typically alcohols, acetates, and ethyl esters. The higher alcohols and esters are formed in the early phase of fermentation, paralleling yeast growth (Loscos *et al.,* 2007).The aroma profile of a wine is affected by any factor that decreases the speed offermentation, specifically a decrease in temperature. Higher temperature fermentations result in larger losses of volatile components than do low temperature fermentations (Rib´ereau-Gayon *et al.,* 2006; Ebeler 1999).

2.11.5 Effect of volatile acidity /acetic acid on wine quality

Acetic acid bacteria are ubiquitous organisms that are well adapted to sugar and ethanol rich environments. They convert ethanol through acetaldehyde to acetic acid which constitutes spoilage in wines (Lonvaud-Funel, 1996; Sponholz, 1993). Wines spoiled by acetic acid bacteria is characterised by volatility, a vinegar-like sourness on the palate and a range of acetic, nutty, sherry-like, solvent or bruised apple aromas and often a reduction in fruity characters (Bartowsky *et al*., 2003).

Grapes and wine are subject to spoilage by acetic acid bacteria at many stages during the winemaking process (Drysdale and Fleet, 1988). Physically damaged grapes or those infected by fungi can become infected with acetic acid bacteria and cannot be used in wine production if the volatile acidity exceeds statutory limits (Eglinton and Henschke, 1999). Acetic acid bacteria growth can also occur in grape must or during stuck fermentation if exposed to the air. Most commonly wines are spoiled by acetic acid bacteria during maturation or storage when unintentionally exposed to air (Joyeux *et al.,* 1984a). Bacterial spoilage has also recently been reported to occur in packaged wine such as vertically upright bottles (Bartowsky *et al.,* 2003). Plate 6 shows a visually distinctive ring of bacterial biomass that is deposited on the neck of the bottle at the interface between the wine and the air headspace.

Plate 6: Bacterial ring shaped deposit at the interface between the wine and headspace in the bottle neck (Bartowsky and Henschke, 2008).

In terms of sensory, acetic acid is recognised in wine as contributing a sour flavour and at high concentration a bitter, sour flavour with a vinegar-like aroma (Peynaud, 1984).

Normal acetic acid levels formed during fermentation process is approximately 0.2-0.4 g/l. Acetic acid concentrations greater than 2-3 g/l are considered vinegar or spoiled. The legal limits for volatile acidity for red wines made of grapes less than 28 ^{o}Brix is 1.4 g/l in the USA (Jacobson, 2006).

2.11.6 Residual sugars in wines and their effect on consumer choices of wines

Sugar is an essential component in wine production as it determines the alcohol levels and the amount of residual sugars left in wines. Glucose and fructose are the main fermentable sugars in grape juice and both sugars exist approximately in equal concentrations in wines (Katharine, 2011).

Wine fermented to dryness has less residual glucose than fructose as glucose ferments at a faster rate. Residual sugar concentration is a measure of the amount of sugar solids in a given volume of wine following the end of fermentation and any sugar addition when making a sweet wine (Katharine, 2011).

Table 9:Classification of wines based on residual sugars.

Type of wine	Level of residual sugar (%)	Level of residual sugar (g/l)
Dry white	0.1-0.2%	1-2g/l
Dry red	0.2-0.3%	2-3g/l
Off dry	1.0-3.0%	10-30g/l
Sweet	>3%	30g/l
Port/sherry	5-15%	50-150g/l

Source: (Katharine, 2011)

Wine also contains very small concentrations of unfermentable sugars (such as pentose sugars) that yeast cannot convert to alcohol, which also contribute to residual sugars in wines (Katharine, 2011). Table 10 shows the different levels of residual sugars in the different types of wines. Dry wines have the least of residual sugars with dessert wines having the highest of residual sugars.

Work done by Francesco Marangon, and Stefania Troiano in Italy between 1997 and 2000 showed that 50% men and 30% women preferred dry wines. Also people preferred dry wines as they got older and had a greater knowledge of what they were drinking (Yang, 2008).

2.12 Sensory properties of wines

Aroma compounds play an important role in the quality of wine because these compounds produce an effect on sensory parameters and are crucial to determine consumer acceptability (Vilanova *et al.*, 2010; Rapp, 1990). The sensory properties of red wines, encompass multiple and interacting sensations of acidity, sweetness, bitterness, flavour, viscosity, warmth, and astringency (Gawel *et al.*, 2010). The inherent intrinsic factors of red wine affectconsumers' purchasing decision (Yang, 2008).

Yang and McCluskey have reported that flavour and astringency are significant positive sensory factors which influence consumers' willingness to pay for sensory properties in red wines and bitterness negatively affects willingness to pay for sensory properties in red wines by consumers (Yang, 2008). Flavanols are the primary sources of bitterness and astringency in red wine. Of these, the flavan-3-ol monomers (catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin

gallate) and their oligomers and polymers, which are called proanthocyanidins or condensed tannins, are the most abundant in red wines (Balentine, 1992).

2.13 Standards for wines

Standards set for wines are to ensure wholesome and quality wines from start of wine making to bottling. Labeling is important in the standardization of winebecause it provides consumers with sufficient and accurate information to help in the identification of the origin and quality status of any wine put on sale. It also prevents consumers from being misled or confused by information displayed on the label. Information displayed on the labels is grouped into compulsory information, optional information and additional optional items (Boodle, 2010).

Wine/ country of origin, bottler's details, and nominal volume of the wine and actual alcoholic strength are compulsory details which must be visibly shown on the label without having to turn the bottle.Lot Number and allergenic ingredients are also compulsory details which are required on the label. Wines containing more than 10 milligrams per litre of free SO_2 must include the term 'contains sulphites' or 'contains sulphur dioxide' on the label (Anonymous, 2010c). The optional information may include residual sugar description, wine brand name, vine variety, colour and vintage. Some legal standards for wines are total soluble solids of 4 Brix, titratable acidity (% tartaric) 0.69-0.90%, volatile acidity 0.14%, pH 3.5 to 4,alcohol 4-14% and total phenols in the range of 23- 100AU (Amerine *et al.*, 1980; Boulton *et al.*, 1999)

2.14 Spoilage of wines

Acetobacter aceti and A. pasteurianus which are acetic acid bacteria are mostly associated with wines spoiled by high volatile acidity (Joyeux *et al.*, 1984a).These bacteria are aerobes, but they have been routinely isolated from wine samples taken from the bottom of tanks and barrels (Drysdale and Fleet 1988), which suggests that they are able to survive and possibly grow under the anaerobic to semi-anaerobic conditions that occur in these environments (Drysdale and Fleet, 1989). As wine is not always sterile filtered prior to bottling, especially red wine, it often has a small resident bacterial population (10^3 cfu/ml), which under conducive conditions might proliferate. Brief aeration of red wine is sufficient to encourage the growth of acetic acid bacteria and cause wine spoilage, even when sulphur dioxide had been added (Millet and Lonvaud-Funel, 1999).

In recent years, some wine industry practitioners appear to have adopted a number of practices that increase the risk of microbial spoilage of wine after bottling (Godden, 2000).This includes reduction of sulphur dioxide levels in wines due to public health issues (Peterson *et al.*, 2000; Stockley *et al.*, 1993).

Some post bottling factors may contribute to the risk of wine spoilage. These include the poor performance of bottle closures that may allow the ingress of oxygen (Caloghiris *et al.*, 1997; Zurn *et al.*, 1995) and the manner in which the bottles are positioned during transport and storage (Godden *et al.*, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of raw materials

The *Hibiscus sabdariffa* calyces, *Sorghum bicolor* leaves and sugar were obtained from Ayigya market, Kumasi. Brewer's yeast was bought from Anchor yeast in South Africa. Plate 7 shows granulated brewer's yeast. Reagents were obtained from Biochemistry laboratory, KNUST.

Plate 7: Brewer's yeast

3.2 Sample preparation

Wholesome *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves were selected and dried to a constant weight using a solar dryer. The samples were grounded to a coarse powdery form using a kitchen blender (Binatone coffee grinder, SON NO: MPR/EE/0042) at speed 2 for 20 minutes for the proximate and nutritional analyses. Plates 8 -11 show dried roselle calyces and *S.bicolor* leaves with their grounded forms.

Plate 8: ***H. sabdariffa*** **calyces** **Plate 9:** ***S. bicolor*** **leaves**

Plate 10: **Grounded*H. sabdariffa*** **calyces** **Plate 11: Grounded*S. bicolor*leaves**

3.3 Determination of nutritional composition of *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves

3.3.1 Proximate composition of Samples

3.3.1.1 Moisture Content Determination

An amount of 2 grammes each of powdered *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves were weighed using an analytical balance (model AS260D Ohaus co-operation, USA). The weight of the petri dish and each sample were determined and recorded. The petri dish and its content were placed in a drying oven (Gallenkamp drying oven, England) at a temperature of 105 ^{O}C for six hours after which the

differences in weight were determined using the appropriate formula (Appendix A) for calculation of the moisture content. The procedure was repeated for each sample in triplicates (AOAC, 1990).

3.3.1.2 Ash Content Determination

An amount of 2 grammes of powdered *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves were weighed using an analytical balance (model AS 260D Ohaus co-operation, USA). The weight of the crucible and each sample were determined and recorded. The crucible and its content were placed in a muffle furnace (Gallenkamp drying oven, England) at a temperature of 600 ^{O}C for two hours. The crucibles were removed and allowed to cool after which it was weighed. The appropriate formula (Appendix A) was used to calculate the ash content. The procedure was repeated for each sample in triplicates (AOAC, 1990).

3.3.1.3 Crude Fat Content Determination by soxhlet extraction

An amount of 2 grammes of powdered *Hibiscus sabdariffa* calyces and *Sorghum bicolor*leaves of which the moisture content had been determined was used. The round bottom flask was weighed using an analytical balance (model AS 260D Ohaus co-operation, USA). The samples were transferred into a thimble and placed in the holding chamber of the soxhlet apparatus. An amount of hexane (100ml) was poured into the round bottom flask and the soxhlet apparatus was well connected with the heating mantle switched on. The fat extraction was carried out for sixteen hours. The hexane was recovered and the round bottom flask and its content dried, cooled and

weighed to determine the difference in weight of the flask. The appropriate formula (Appendix A) was used to calculate the fat content. The procedure was repeated for each sample in triplicates (AOAC, 1990).

3.3.1.4 Crude Protein Content Determination by the kjeldahal method

An amount of 2 grammes of powdered *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves were weighed using an analytical balance (model AS 260D Ohaus co-operation, USA) and placed in a digesting flask. Twenty five millilitres of concentrated H_2SO_4 and selenium catalyst were added. Digestion was carried out in a digestion chamber until a clear solution was obtained. The digested sample was filtered into a 100ml volumetric flask and made to the mark with distilled water and mixed well. Seventeen millilitres of NaOH and 10 ml of sample were put into the kjeldhal apparatus and heated for the distillation of ammonia. Twenty five millilitres of 4% boric acid was measured into the conical flask to receive the liberated ammonia gas.

The nitrogen content was estimated by titrating the ammonium borate formed with standard 0.096N HCl using mixed indicator and titre values recorded. The appropriate formula (Appendix A) was used to calculate the protein content. The procedure was repeated for each sample in triplicates (AOAC, 1990).

3.3.1.5Crude fibre Content Determination

The sample used for the fat determination was used for the crude fibre analysis. The defatted sample was transferred into a 500ml Erlenmeyer flask and 0.5g of asbestos and 200ml of 1.25% boiling H_2SO_4 were added and connected to a condenser and set on a hot plate. The flask boiled for thirty minutes, content filtered out and washed with boiling water till the washings were no more acidic. The residues were put back into the flask, connected to the condenser and made to boil with 200ml 1.25% NaOH for thirty minutes. It was then filtered and washed with boiling water till filtrate was no longer basic and 15ml alcohol was used to do a final washing. Residues were transferred into Gouche crucibles and dried in an electric oven for one hour at 100^{o}C. It was then cooled and the weight taken. Crucibles and contents were ignited in a muffle furnace for 30 minutes, cooled in a dessicator and weighed and loss in weight was determined. The appropriate formula (Appendix A) was used to calculate the crude fibre content. The procedure was repeated for each sample in triplicates (AOAC, 1990).

3.3.1.6: Carbohydrate / Nitrogen free extract

The total carbohydrate estimate was obtained by subtracting the sum of moisture, ash, protein, fat and crude fibre from hundred and expressed as a percentage (AOAC, 1990).

3.3.2 Determination of Minerals and Vitamin C content

3.3.2.1 Determination of K, Na, Ca, Fe, Zn, Mg and Mn by Aomic Absorption Spectrometry

The atomic absorption spectrometry was used in the determinations. Two grammes of *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves were weighed into a conditioned porcelain crucible and ashed at 550 ^{o}C for four hours. The ash was cooled and dissolved in 20 ml 10% HNO_3. The solution was filtered through an acid washed filtered paper into a 100 ml volumetric flask and made to volume with 10% HNO_3 and well mixed.

A 100 mg/l solution was prepared by pipetting 10ml of the stock standard solution into a 100ml volumetric flask and made to volume with 10% HNO_3. From the 100 mg/l solution, 10 mg/l solution was prepared by pipetting 10ml into a 100ml volumetric flask to the mark. One mg/l, 2 mg/l and 3 mg/l concentrations were prepared by pipetting 5 ml, 10 ml and 15 ml respectively into separate 100 ml flask and made to volume with 10% HNO_3.

Using a calibrated spectrometer (S Series 711239v1.27, USA) at a specific wavelength of the mineral to be analysed, and air-ethane flame type, the mineral contents for each of the samples were analysed and calculated using a standard curve. Potassium was analysed at a wavelength of 766.5 nm, sodium at 589 nm, calcium at 422.7 nm, magnesium at 285.2 nm, iron at 248.3 nm, manganese at 279.5 nm and zinc at 213.9 nm respectively (Perkin, 1982; Buchanan and Muraoka, 1964).

3.3.2.2 Determination of Phosphorus

The colorimetric method was used in this determination. The vanadate-molybdate composite reagent was used. The reagent was prepared by dissolving 20g ammonium molybdate in 400 ml distilled water at 50^{O}C and cooled. One hundred and forty (140) ml of concentrated nitric acid was gradually added to the solution and stirred. Ammonium vanadate solution was prepared by dissolving 1g of ammonium vanadate in 300ml boiling distilled water. The two solutions were combined in a 1litre flask and diluted to the mark with distilled water.

A stock solution containing 3.834g of potassium dihydrogen phosphate per litre was prepared. About 25 ml of the solution was diluted to 250 ml (1ml of the solution is equivalent to 0.2 mg P_2O_5). A standard curve was prepared by adding 5 ml, 10 ml, 20 ml, 30 ml and 40 ml of the phosphate solution to a 100 ml volumetric flask and diluted to 60 ml with distilled water. Few drops of ammonia solution was added and mixed with nitric acid in a ratio of 1:2. Twenty (20) ml vanadate molybdate reagent was added and diluted to the mark and mixed. It was allowed to stand for ten minutes and absorbances read at 470 nm in the ultra violet region.

A 10 ml sample solution *(Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf extracts) was put in a 100 ml volumetric flask. The solution was neutralized with ammonia and nitric acid solution (1:2). Twenty (20) ml of vanadate molybdate reagent was added and diluted to the mark. It was allowed to stand for ten minutes and absorbance read at 470nm in the ultra violet region (Peason, 1976).

3.3.2.3 Determination of Vitamin C content

Indophenol dye was prepared by dissolving 42 mg sodium bicarbonate, 50 mg of 2,6-dichloroindophenol sodium salt and diluted to 200 ml with distilled water. It was then filtered into an amber bottle. Metaphosphoric acetic acid was prepared by diluting 20ml acetic acid in 100ml distilled water and dissolving 7.5g metaphosphoric acid and diluting to the 250ml mark with distilled water. The solution was then filtered into a stopper bottle. Ascorbic acid standard solution was prepared by weighing 50 mg of ascorbic acid and diluted to 50 ml in a volumetric flask. The dye was standardized with the blank and the ascorbic acid. The wine samples were titrated against indophenols to a rose pink colour and titre values recorded (Nielsen, 2003). The formula for calculating the vitamin C content is indicated in Appendix F.

3.4 Preparation of extracts from *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves

Preparation of the extracts for the wine production was based on the preparation of Roselle wine by Alobo and Offonry with modifications (Alobo and Offonry, 2009). Instead of 500ml of water used for both extractions, 1000ml and 500ml of water were used for the main and re-extractions respectively. Also, the filtrates were enhanced with sugar to raise the sugar level to 18.60 Brix instead of 21 Brix and diammonium phosphate was not added.

The *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves were manually sorted and washed in cold water to remove dirt. About 100 g of the clean calyces and leaves were boiled in batches in 1000 ml of water at a temperature of 80^{O}C for 10 minutes.

They were strained using a colander and the residual calyces and leaves were further re-extracted by heating with 500 ml of water at a temperature of 80^{O}C for 5 minutes. The extracts were pooled to obtain a 1:15 *Hibiscus sabdariffa* calyx to water extract and a 1:15 *Sorghum bicolor* leaves to water extract. These extracts constituted the stock extracts.

3.5 Physicochemical analyses on *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf extracts.

The *Hibiscus sabdariffa* calyx, *Sorghum bicolor* leaf extracts were analyzed using the appropriate standard methods.

3.5.1 Determination of titratable acidity

The method described by Amerine and Ough (1980) for titratable acidity was modified by using a 10 ml sample in 100 ml distilled water instead of 5ml sample in 200 ml of distilled water. Titratable acidity was determined by the use of a 0.1N NaOH and phenolphthalein indicator. A 250 ml beaker was filled with 100ml distilled water and a few drops of phenolphthalein indicator was added and mixed. The mixture was titrated to a pale pink colour. Degassed wine sample (10ml) was measured into the mixture and titrated against 0.1 NaOH to a pale pink colour which persisted for at least 30 seconds. Degassing was done by allowing the wine sample to stand in an ultrasonic water bath for 10 minutes to remove carbon dioxide gas (Dharmadhikari,2000a).

3.5.2 Determination of pH

The digital bench top pH meter (pH/ ORP/ISE meters, Hanna Instruments, USA) equipped with an electrode was used in the determination. The electrode was washed in distilled water. It was then placed into the extracts. The pH reading was read from the recorder of the pH meter (AOAC, 1990).

3.5.3 Determination of total soluble solids.

A hand held refractometer (Atago's master series, Japan) was used. The refractometer was standardized by placing a drop of distilled water on the prism. The refractometer was placed such that it allowed entry of sunlight into the prism. The eye-piece was used to observe the standardization after adjusting the coarse and fine adjustment properly. The process was repeated for each sample and the appropriate correction factors made depending on the temperature of the sample (AOAC, 1990).

3.5.4 Determination of total colour density

The Sudraud method (Sudraud, 1958) was used in the determination. The colour density was determined directly by measuring absorbances at 420 nm and 520 nm using a 1cm optical path and the absorbances summed up (Hahn, 2009).

3.5.5 Determination of total phenols

A 100µl of extract was placed in a 10 ml volumetric flask and brought to volume with 1N HCl. The solution was incubated at $20^{O}C$ for three hours. The UV-VIS spectrometer was set at 280 nm and a blank was used to auto zero the spectrometer. The samples were placed in the spectrometer and the absorbances read and the appropriate formula (Appendix F) used to calculate the phenol content (Jacobson, 2006).

3.5.6 Determination of total red pigments

A 100µl of extract was placed in a 10 ml volumetric flask and brought to volume with 1N HCl. The solution was incubated at $20^{O}C$ for three hours. The UV-VIS spectrometer was set at 520 nm and a blank was used to auto zero the spectrometer. The wine samples were placed in the spectrometer and the absorbances read at 520nm and the appropriate formula (Appendix F) used to calculate the total red pigment (Jacobson, 2006).

3.5.7 Determination of density

Seventy five millilitres (75 ml) of extracts were measured using a measuring cylinder and put in a beaker of known weight. The beaker and its content were then weighed and the formula in Appendix F used in calculating the density (Jacobson, 2006).

3.6 Design of experiment

The response surface methodology was used to characterise the effects of pH, titratable acidity, brix, density, total colour density, total phenols and total red pigment on the production of low alcohol rose wine from extracts of *Hibiscus sabdariffa* calyces (HS) and *Sorghum bicolor* (SB) leaves. A central composite design was used to study the effects of variation in levels of fermentation time (7-10 days) and proportion of extracts used (25-100% for *Hibiscus sabdariffa* calyx extract and 0-50% for *Sorghum bicolor* leaf extract) on the various responses. Twenty eight (28) different runs were generated by a statistical tool and the responses recorded after the analysis. It was then optimized and the best formulation chosen for the wine preparation. The runs generated are shown in Appendix B – Table 1.

3.6.1 Optimum conditions for blending *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves extracts.

To establish the optimum conditions for blends of extracts for wine production, the treatment factors, responses and their respective levels were constrained. For vitamin C, total phenols, total red pigments and total soluble solids the constraints were set at maximum, total colour density and density were set at minimum. The pH was set at 3.3; alcohol at 5% and for titratable acidity, the constraints was set in a range of 7-9 g/l. The various responses were then overlaid and the Statsgraphics Centurion tool was used to select the optima based on the constraints imposed.

3.7 Fermentation of must prepared from *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf extracts.

3.7.1 Preparation of Must

The *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves were manually sorted and washed in cold water to remove dirt. About 100 g of the clean calyces and leaves were boiled in batches in 1000 ml of water at a starting temperature of 80^{O}C for 10 minutes. They were strained using a colander and the residual calyces and leaves were further re-extracted by heating with 500 ml of water at a starting temperature of 80^{O}C for 5 minutes.

The extracts from each plant material were mixed together in the ratio of 75% Roselle calyx and 25% *S.bicolor* leaf extracts and 100% Roselle calyx extracts, and diluted by a factor of ten with distilled water, to attain a rose pink colour.

3.7.2 Fermentation of Must

After the optimization, two types of wines were produced from the extracts. The first type of wine was produced from a 100% *Hibiscus sabdariffa* calyx extract and the other a combination of *Hibiscus sabdariffa* calyx extract and *Sorghum bicolor* leaf extract in a ratio of 75% and 25% respectively. Sixteen litres of each formulation was prepared.

The diluted extracts obtained were then ameliorated or enhanced, using 2.4kg of table sugar to raise the sugar level to 18.62°Brix, which is in the range of sugar levels tolerable by most yeast for alcohol production (Bisson, 2001). Granulated brewer's yeast *Saccharomyces cerevisiae* was used in the fermentation process. An amount of 0.4 g of the yeast was weighed for each eight litres of must. The yeast weighed was

propagated in 200 ml of must at a brix of 18.62 and a temperature of 30^{O}C for one hour. Sulfur dioxide was added to the must in the form of sodium metabisulphite at a concentration of 0.4 g/l to stabilize the must.

The must were poured into four different sterile plastic fermenting containers of 9 litres capacity and seeded aseptically with 200 ml yeast culture and stirred for even distribution of the yeast. A vent was made on the lid and stuffed with sterile cotton wool to allow for the exchange of gases. The musts were subjected to primary fermentation at 28 ± 2°C for eight days to produce the fermented must. The yeasts were filtered out to obtain the young wine. Plate 12 shows the must undergoing fermentation.

Plate 12: Must from *H.sabdariffa* calyces and blends of *H.sabdariffa* calyces *S. bicolor* leaves undergoing fermentation

3.7.3 Clarification of Young Wine

Eight litres of each type of the young wines were fined using egg white albumin. Egg white from one egg was whisked in 100 ml of distilled water containing 0.1442g of salt. The mixture obtained was divided into four portions. One portion was used in fining eight litres of wine. The solution was added slowly without foaming to the

wine while stirring and allowed to stand for ten days before filtering the egg albumin out using cheese cloth (Dharmadhikari, 2000a; Zoecklein*et al.*, 1990).

3.7.4 Bottle Aging of Unclarified and Clarified Fermented Must.

The unclarified and clarified fermented musts were bottled in one litre transparent glass bottles and well corked. Bottle aging was allowed for twenty weeks to allow the development of characteristic flavour of the wines (Dharmadhikari, 2000a).

3.7.5 Racking of Wine

The wines were racked off the lees every five weeks. This was done by the use of a sucker pipette to avoid contamination and ensure minimum oxygen contact with the wine. Plate 13 shows a picture of lees settlement at bottom of wine bottles.

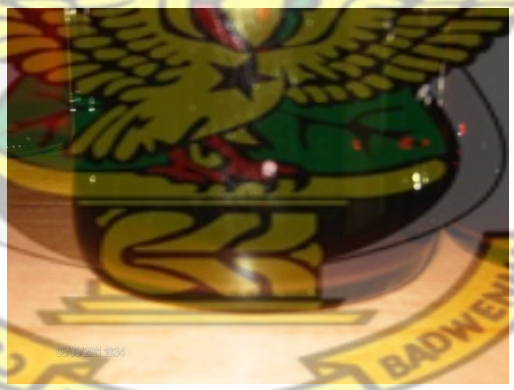

Plate 13: Lees settlement at bottom of wine bottle

3.7.6 Storage Conditions of Unclarified and Clarified Fermented Must

The bottled unclarified and clarified fermented musts were kept in a cool dry cabinet at an average temperature of 28^{o}C with no disturbances for twenty weeks. Plates 14 and 15 show wine samples under storage.

Plate 14: Wine from blend of 75% *H.sabdariffa* calyces and 25% *S. bicolor* leaves under storage in a cabinet

Plate 15: Wine from blend of 100% *H.sabdariffa* under storage in a cabinet

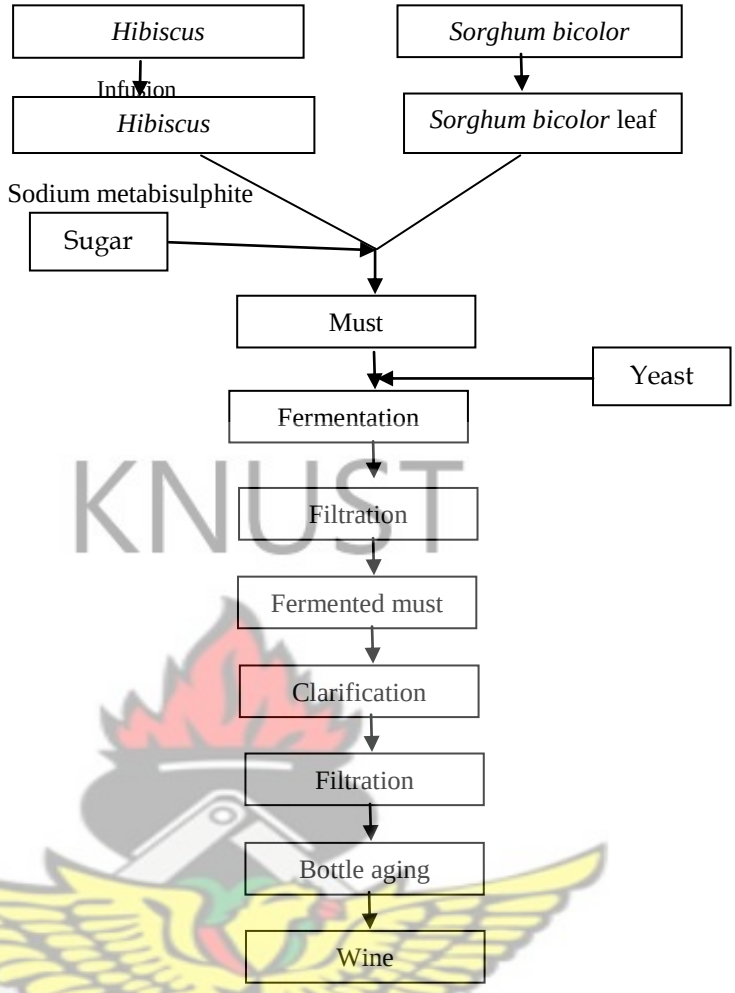

Fig. 2: Production of low alcohol rose wine from *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf.

3.8 Physicochemical analyses of fermented unclarified and clarified must and wine during aging

Analyses carried out on the unclarified and clarified must and wine during aging included total soluble solids, titratable acidity, pH, vitamin C, total phenolics, total red pigment, and total colour density. The procedures for these determinations were the same as the physicochemical analyses on *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf extracts. The rest were alcohol and volatile acidity. These parameters

were determined and monitored to assess their impact on wine characteristics, stability, quality, aroma and taste.

3.8.1 Alcohol Content

The alcohol content was obtained using the difference in potential alcohol method. In this method the alcohol contents were calculated based on the sugar contents of the must before fermentation and the final sugar level of the fermented must. The formula is seen in Appendix F (Anonymous, 2005; Jacobson, 2006).

3.8.2 Volatile Acidity

This analysis was carried out to determine the acetic acid content in the wine. The method described by Nielson (2003) for acetic acid determination was used. The wine sample was degassed by allowing the wine sample to stand in an ultrasonic water bath for ten minutes to remove carbon dioxide gas. A 25 ml of the wine sample was titrated against 1.0M NaOH solution, using three drops of phenolphthalein as an indicator and titrated to a pale pink colour. The amount of acetic acid present was then calculated per 100 ml of wine sample. The formula is shown in Appendix F.

3.9 Sensory Evaluation of Table Wine.

Wines were evaluated by a panel of 32 judges who were very familiar with wine. The judges were made up of 29 males, 3 females, ages between 18 to 80 years (final year students, university workers and lecturers in Kwame Nkrumah University of Science and Technology). Panelists were selected based on interest and availability.

3.9.1 Affective Test

Wines from extracts of *Hibiscus sabdariffa* calyces (100%) and 75% *Hibiscus sabdariffa* calyces combined with 25% *Sorghum bicolor* leaves were evaluated based on colour, aroma, sweetness, acidity, alcohol content and overall acceptability (based on overall mean value). Both acceptance and preference tests were carried out on the wines. The wines were compared among themselves to identify the best wine with the most acceptable sensory attributes. The assessment was carried out using a 7-point hedonic scale, where 1 represented like very much, 2 represented like moderately, 3 represented like slightly, 4 represented neither like nor dislike,5 represented dislike slightly, 6 represented dislike moderately and 7 represented dislike very much. The wines were stored in plain wine bottles at room temperature and were brought into the sensory laboratory one hour prior to testing to equilibrate with the environment. Samples of wine (30 ml) were served in clear disposable plastic cups. The wines were coded with three-digit random numbers and served in no particular order. Judges were instructed to chew cream cracker biscuits between tasting of different wine samples.

3.10 Data analyses

Data collected was analysed using analysis of variance (ANOVA). Tukey test was used for the multiple mean comparisons at 5% level of significance. Statsgraphics Centurion package was used for the analyses.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1Proximate and Mineral Composition of *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves

4.1.1 *Hibiscus sabdariffa* calyx (roselle)

Table 10shows the proximate composition of *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves on dry weight basis. From Table 10, the moisture content of natural roselle calyx was 10.03% which is within the range for cereals (10-12%) designed for long storage (Pomeranz and Meloan, 1987). The low moisture content is an indication of long storage ability of roselle calyces under ambient conditions because low moisture content does not support the proliferation of spoilage microbes (Pomeranz and Meloan, 1987). The high ash content of 8.92% is an indication of high mineral content of roselle calyces. In aqueous media high mineral contents are sometimes used to retard the growth of certain microorganisms due to the high osmotic pressure it exerts thereby giving extracts of roselle calyces microbial stability (Lund *et al.,* 2000). The high carbohydrate content of 65.88% of roselle calyces makes it a good source of energy and ideal for children, athletes, or those with work that are stressful and very physical (Kishore, 2012). Roselle calyx is reported to contain adequate amount of crude fibre and is useful as fodder for livestock (Anonymous, 2010a). The crude fibre content of 8.90% confirms the suitability of roselle calyx as feed for livestock. The protein content of 5.89% is within the range of protein for polished rice 5.2-7.6% (Pomeranz and Meloan, 1987) and this could be the reason why it is used in a fermented form 'bikang' in Burkina Faso as a substitute for meat by the destitute as reported by McLean (1973). The low fat content of 0.38% reduces the tendency for roselle calyces to undergo lipid oxidation, which leads to the formation of off-flavours and potentially harmful products (Pomeranz and Meloan,

1987). Adanlawo and Ajibade (2006) reportedthat the red variety of roselle calyx has a moisture content of 7.60%, protein of 4.71%, crude fibre of 4.69%, fat of 2.01% and carbohydrate content of 68.75%. The differences in the values observed can be attributed to the sources of calyces, the treatment and storage conditions of the calyces prior to analyses (Ameh *et al.*, 2009).

Table 10: Proximate composition of *Hibiscus sabdariffa* (HSC) calyces and *Sorghum bicolor* leaves (SBL) on dry weight basis

Parameter	HSC (%)	SBL (%)
Moisture	10.03±0.20	8.42±0.14
Ash	8.92 ±0.50	7.33±0.22
Protein	5.89 ±0.14	1.87±0.29
Fat	0.38 ± 0.03	0.08±0.05
Crude fibre	8.90 ± 0.50	28.77±2.09
Carbohydrate	65.88±0.61	53.53±2.17
Energy KJ/100g	1168.19	891.23

Values are means ± standard deviation of triplicate determinations

4.1.2 *Sorghum bicolor* leaves

The proximate composition of *S. bicolor* is indicated in Table 10. The moisture content of *Sorghum bicolor* leaves was 8.42% and this lies within the range for commercially dried vegetables (7-10%). This value is low such that the propensity of microorganisms to grow in *Sorghumbicolor* leaves causing microbial spoilage, food instability and deterioration is resistible (Anonymous, 2011).

Sorghum bicolor leaves had ash, protein, fat, crude fibre, carbohydrate and energy contents of 7.33, 1.87, 0.08, 28.77, 52.55% and 891.23KJ/100g respectively. The high crude fibre content makes *Sorghum bicolor* leaves suitable as feed for poultry and livestock (Pomeranz and Meloan, 1987). The lowlevel of protein, fat and high carbohydrate contents is an indication of weaker levels of glycoproteins and lipoproteins in *Sorghum bicolor* leaves (Pomeranz and Meloan, 1987). The low levels of these proteins affect the rheological properties of extracts of *Sorghum bicolor* leaves.

Energy value of 891.23KJ/100g makes *Sorghum bicolor* leaves good sources of energy. Research has shown sugars and starches to be the main storage forms of energy in the sorghum plant (Wall and Blessin, 1970). *Sorghum bicolor* leaves fairly compare with *Sorghum bicolor* stem powder, which has moisture content of 6.5%, ash of 5.34%, protein of 3.20%, fat of 8.38%, crude fibre of 32.02% and carbohydrate of 44.52% (Adetuyi *et al.*, 2007).

4.1.3 Mineral composition

Table 11 shows the mineral composition of *Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves. The predominant minerals were potassium, magnesium, calcium and phosphorus. Sodium, iron and manganese were relatively low and zinc content was the least among the minerals in roselle calyces and *Sorghum bicolor* leaves.

Table 11: Mineral composition of*Hibiscus sabdariffa* calyces and *Sorghum bicolor* leaves

Constituent	HSC *(mg/100g)*	SBL *(mg/100g)*
Sodium	13.57±0.00	11.22±0.00
Potassium	1725.25±0.02	308.31±0.00
Magnesium	289.56±0.00	131.36±0.00
Zinc	3.89±0.00	1.39±0.00
Iron	19.20±0.00	17.26±0.00
Calcium	1830.78±0.2	71.94±0.00
Phosphorus	430.00±0.00	90.00±0.00
Manganese	11.91±0.00	6.50±0.00

Values are means ± standard deviation of triplicate determination

HSC: Dried *H.sabdariffa* calyx **SBL:** Dried *S. bicolor* leaf

4.1.3.1 Potassium

The potassium content of *H.sabdariffa* calyces and *S.bicolor* leaves were 1725.251 mg/100g and 308.312 mg/100g respectively. The values were very high compared to other research work done. The reported potassium content of red roselle calyces is 49.35 mg/100g (Adanlawo and Ajibade 2006). The recommended daily intake of potassium for people of ages four years and above is 3500 mg (Lenntech, 2010). Roselle calyces and *S. bicolor* leaves provide about half and one – tenth in 100g of the leaves respectively of the RDI of potassium for humans. Incorporating roselle calyces and extracts of *S.bicolor* leaves into diets is essential for muscle contraction, in keeping acid-alkaline balance in the blood, for normal heart beat and nervous system function due to the potassium content (Shahnaz *et al*., 2003).

4.1.3.2 Calcium

The calcium content in roselle calyces and *S.bicolor* leaves were 1830.78 mg/100g and 71.94 mg/100g respectively. The calcium content in *H.sabdariffa* calyces has been stated as 12.65 mg/100g and 3.00 mg/100g (Adanlawo and Ajibade 2006; Nnam and Onyeke, 2003). The differences in the calcium content can be attributed to the variety of calyces and storage conditions of the calyces prior to analysis (Ameh *et al.*, 2009). The amounts of calcium in *S.bicolor* stem flour and sorghum grain have been stated as 151.70 mg/100g and 27 mg/100g respectively (Adetuyi *et al.*, 2007; Léder, 2004). The calcium content of*S. bicolor* leaves seem to be higher than that of sorghum grain and lower than the stem powder. Calcium is needed for bone and tooth formation, heart function, blood coagulation and muscle contraction (Wardlaw, 2003). The recommended daily intake of calcium for adolescents and adults from 19 years is between 1000 -1300 mg per day (FAO/WHO Expert Group, 1962). Roselle calyx contains high amounts of calcium so could be integrated into foods for women at menopausal age to prevent osteomalacia (FAO/WHO, 1998).

4.1.3.3 Phosphorus

Phosphorus in balance with calcium is needed for building bones and teeth. It plays a vital role in cell membrane structure, metabolic processes and it is important for production of ATP (Miller, 2008). Phosphorus content of roselle calyx and *S.bicolor*leaf were 430 mg/100g and 90 mg/100g respectively (Table 12). The phosphorus content in roselle calyces hasbeen found to be 22.0 mg/100g and 36.30 mg/100g (Nnam and Onyeke 2003; Adanlawo and Ajibade 2006). The differences could be due to soil fertility and ecological factors. The amount of phosphorus in

S.bicolor grain is 0.35 mg/100g (Leder, 2004), which does not compare well with that of the *S. bicolor* leaf. The recommended daily allowance (RDA) of phosphorus is 700 mg per day for adults, 1250 mg/day for children between 9-18 years and 460-500 mg/day for children between 1-8 years of age (Pumpkin, 2011). Consumption of adequate amounts of roselle calyces and extracts of *S. bicolor* leaves will supply the body with phosphorus for maintaining proper brain function, growth, maintenance and repair of all tissues and cells in the body (Rusher, 2011).

4.1.3.4 Iron

Iron is a trace element responsible for oxygen transport, cellular respiration and essential in the production of neurotransmitters required for healthy immune system and resistance to infections (Waters, 2010). Iron found in plant products is the non-heme form which is not easily absorbed as the heme form found in animal products (Bender, 1992). The levels of iron were 19.20 mg/100g in roselle calyces and 17.26 mg/100g in *S. bicolor* leaves. Adanlawo and Ajibade (2006) and Nnam and Onyeke (2003) reported 3.22 mg/100g and 833.00 mg/100g respectively as the iron content for roselle calyces. The variation in the iron levels in roselle could be attributed to the variety (Ameh *et al.,* 2000). The recommended daily intake for iron is 10 mg/day for males and 19 mg/day for female adults (ACU-CELL, 2004). Roselle calyces and *Sorghum bicolor* leaves can provide the body's full iron requirement in 100g of calyces and leaves. Integration of roselle calyces and extracts of *S.bicolor* leaves into diets of pregnant women is essential especially in the third trimester of pregnancy to meet the body's excessive iron demands (FAO/WHO, 1998).Addition of roselle

calyces and extracts of *S.bicolor* leaves to foods would help in the reduction of iron deficiency anaemia which is common in Africa (Nnam and Onyeke, 2003).

4.1.3.5 Magnesium

Magnesium is critical to many cell functions. It plays an important role in energy production, nerve and muscle function and cell growth and repair (Starobrat-Hermelin and Kozielec, 1997). A value of 185.33 mg/100g is reported for *S. bicolor* stem flour which compares well with the *S.bicolor* leaf of 131.36 mg/100g in this study (Adetuyi *et al.,* 2007). The magnesium content of roselle calyces has been stated to be 38.65 mg/100g and is lower than the value in this study (289.56 mg/100g) which could be due to varietal differences(Adanlawo and Ajibade, 2006). The RDI for magnesium is 310 mg/day for female adults and 400 mg/day for male adults, 80 mg for children between 1-3 years and 130 mg for children between the ages of 4 and 8 (Pumpkin, 2011). Adequate levels of Mg are needed in diets of malnourished children especially in the catch up growth associated with recovery from protein energy malnutrition (Nichols, 1978).

4.1.3.6 Manganese

Manganese is involved in the metabolism of carbohydrates, fats and proteins and also helps to nourish the brain and nerves. The RDI of manganese is 1.8 mg for adult females and 2.3 mg for adult males. The manganese content was 11.39 mg/100g in*H. sabdariffa* calyces and 6.50 mg/100g in *S.bicolor* leaves making these plants good sources of Mn. Adequate levels of manganese in the body is needed to prevent poor

bone growth, birth defects, reduced fertility and problems with blood glucose levels (Rusher, 2011).

4.1.3.7 Sodium

The content of sodium were 11.22 mg/100g and 13.57 mg/100g in *S. bicolor* leaves and roselle calyces respectively (Table 12). The sodium content in *S.bicolor* stem flour has been reported to be 127.61 mg/100g and the content in roselle calyces also found to be 96.66 mg/100g and 15.33 mg/100g (Adetuyi *et al.,* 2007; Adanlawo and Ajibade 2006; Nnam and Onyeke, 2003). The variations in the sodium contents could be attributed to the sources of calyces, the treatment and storage conditions of the calyces prior to analysis (Ameh *et al.,* 2009).

Sodium is essential for regulating muscle contractions and nerve transmissions. It helps to maintain proper balance of water and body fluids as well as the pH of blood (Anonymous, 2010e; Food and Nutrition Board, 2000). The recommended daily allowance for sodium is 1500 mg for adults between 19 years to 50 years, 1300 mg and 1200 mg for people of between 51 and 70 years and beyond 71 years respectively (Pumpkin, 2011). Hundred grammes(100g) of roselle calyces and *S. bicolor* leaves provide less than 1% of the body's sodium requirement so more of the leaves extract and calyces must be consumed to meet the required RDA.

4.1.3.8 Zinc

Zinc is a component of enzymes and insulin. It aids in wound healing, growth, tissue repair and sexual development (Underwood, 1971). The zinc contents were 3.89 mg/100g in roselle calyces and 1.39 mg/100g in *S. bicolor* leaves. In view of the

central role of zinc in cell division, protein synthesis and growth, infants, children, adolescents and pregnant women especially would be at risk if zinc intake is inadequate. The RDI for zinc is 15mg/100g. Zinc deficiency at a critical stage of development causes dyslexia (Myhill, 2010). Roselle calyces and *S. bicolor* leaves have the potential to provide children, adolescents, and pregnant women with some small amounts of zinc for a healthy growth.

4.2 Using response surface methodology (RSM) for the characterization of low alcohol rose wine production.

The response surface methodology was used to determine the effects of the factors (extracts from *S. bicolor* leaf and *H. sabdariffa* calyx) on pH, titratable acidity, brix, density, total colour density, total phenols and total red pigment in the production of low alcohol rose wine. A central composite design was used to study the effects of variation of time for fermentation (7-10 days) and percentage of extracts used (25-100% for *Hibiscus sabdariffa* calyces extract and 0-50% for *Sorghum bicolor* leaves extracts on the various responses.

The relationships between the factors and response variables are represented by a three -dimensional response surface and contour plot. $R^2 < 0.075$ indicates a significant lack of fit. This means a higher order model would be required to better describe the effects of independent variables on these responses (Alvarez and Canet, 1999). The percentages of variability explained (R^2) obtained for the responses were low ($5.27 < R^2 < 68.95$) and as such were used only for trend analysis. Titratable acidity and total soluble solids had very low R^2 values (5.27% and 17.26% respectively) so could not be modelled. The discussions are based on the significant terms in the fitted model though the trends of the visualized effects are described.

Table 12: Regression coefficients, coefficient of determination (R^2), lack of fit, and analysis of variance of regression models fitted for the 9 response variables.

Regression Coefficients	Alc. (%)	pH	TA (%)	TSS (Brix)	Den (g/ml)	TCD (AU)	TRP (AU)	TP (AU)	Vit. C (g/ml)
B_0: constant	*-57.68*	15.53	0.49	6.24	0.99	54.75	119.28	349.28	-0.01
Linear									
B_A: Days	14.92*	-3.42*	0.14	2.96	0.02	-12.00	-21.65	-83.07	0.00*
B_B : Extracts	-0.48	0.61*	-0.07	0.11	-0.03*	1.28*	-2.86*	20.52*	0.01*
Quadratic									
B_{AA}	-0.89*	0.24*	-0.07	-0.17	-0.00	0.68	0.97	4.93	-0.00
B_{BB}	-0.06	-0.00	0.00	-0.02	-0.00	-0.02	0.01	-0.52	-0.00*
Interactions									
B_{AB}	0.14*	-0.06*	0.00	0.01	0.00*	0.02	0.71	-0.92	0.00*
Variability Explained(R^2)	62.52	44.65	5.28	17.26	36.99	68.95	32.47	37.21	49.41
Lack of fit	0.25	0.00*	0.00*	0.17	0.39	0.29	0.58	0.89	0.00*

***significant at 5% level**

Legend

Alc.: Alcohol **TA**: Titratable acidity **TSS**: Total soluble solids **Den.**: Density

TCD: Total colour density **TRP**: Total red pigment **TP**: Total red pigment **Vit.C**.:VitaminC

4.2.1 Alcohol

The analysis of the coefficient estimates for the regression models (Table 12 and Appendix B -Table 1a and 1b) showed that both independent variables had an effect on the alcohol content. The response plot (Fig. 3) showed linear effects on fermentation time, quadratic effects on time of fermentation and interaction on the independent variables to be the significant terms (Table 12). The alcohol content increased as the days of fermentation gradually increased but beyond the ninth day of fermentation there was a decrease in the trend of alcohol production (Fig. 3). The fitted regression model was 62.52%. The linear effects showed that alcohol decreased or increased continuously within the experimental field (Fig. 3).

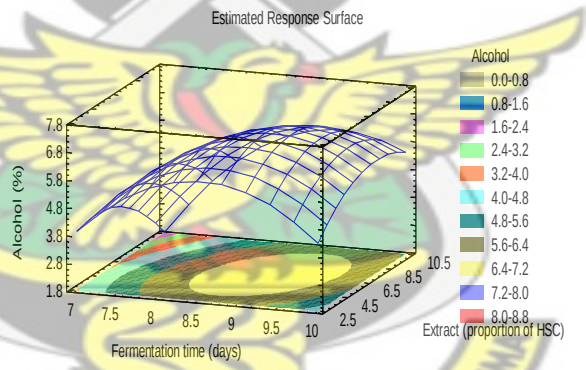

Fig. 3: Response surface plot for the effect of extract and fermentation time on alcohol. (Fitted regression model equationfor alcohol = -57.6828 + 14.9175*A* - 0.48157*B* - 0.890993A^2 + 0.137134*AB* - 0.0568658B^2)

The quadratic effect indicated the existence of an optimal level within the chosen time range for fermentation (7-10 days). The interactive effect showed that alcohol level was affected both by the time of fermentation and the proportion of extract. According to the significance test on the coefficients, fermentation time influenced alcohol production strongly (Table 13).

As fermentation progressed, alcohol increased due to the activity of yeast utilizing sugars present in the must (Bisson, 2001).

4.2.2 pH

The response plot (Fig. 4) showed linear, quadratic and interaction effects of the factors on pH. A gradual decrease was observed from the early days of fermentation to day 8.5, followed by a progressive inclination to day 10 of fermentation. The significant linear effects of the fermentation time and proportion of extract indicated pH increased or decreased continuously within the experimental field (Fig. 4).

According to the significance tests on estimated coefficients, fermentation time had a stronger effect on the pH than the extract (Table 12 and Appendix B - Table 2a and 2b). Fermentation time also showed a significant quadratic effect on the pH. This indicated the existence of an optimum level within the time range tested (7-10 days). The interaction effect of fermentation time and proportion of extract was highly significant indicating that those two factors also affected the pH of the fermented must. The fitted regression model ($R^{2)}$ was 44.64%. The significant lack of fit term showed that a higher model is needed to better explain the effect of the factors on pH. At minimum point of 7 days of fermentation time a pH value of 3.46 was obtained (Fig. 4). From the graph lower pH could be obtained by fermenting must not beyond 8.5 days. The decrease in pH during the early days of fermentation could be due to the increased activities of the wine yeast resulting in production of organic acids from available nutrients (Ojokoh *et al.,* 2002). Free SO_2 protects wine against oxidation and microbial organisms that could bring about wine spoilage. pH affects the effectiveness of SO_2 and as pH increases, free SO_2 loses its effectiveness (Pambianchi, 2011). The rise in pH beyond 8.5 days of fermentation could be an indication of loss of free SO_2

effectiveness which could bring about spoilage due to wild microorganism and oxidation.

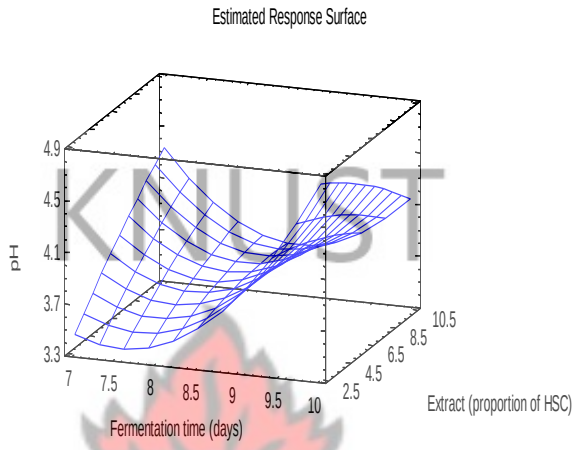

Fig. 4: Response surface plot for the effect of extract and fermentation time on pH.

(Fitted regression model equation for pH = 15.5315 - 3.45256*A* + 0.60595*B* + 0.239388A^2 - 0.0649185*AB*- 0.00302857B^2)

4.2.3 Density

The analysis of the coefficient estimates for the regression models (Table 13 and Appendix B - Table 3a and 3b) showed that both independent variables (fermentation time and proportion of extracts) had an effect on the density although in different ways. The response plot (Fig. 5) showed linear effects of extract and interaction to be the significant terms (Table 12). From the plot the densities were higher on early days of fermentation and as the proportion of HS extract increased beyond 65%, the density of the fermenting must decreased gradually. As the fermentation days increased, the densities decreased gradually. The fitted regression model (R^2) was

22.67%. The linear effects showed the density increased or decreased continuously within the experimental field (Fig. 5).

The decrease in density could be as a result of decrease in total soluble solids and an increase in alcohol content in the fermenting medium (Jacobson, 2006).

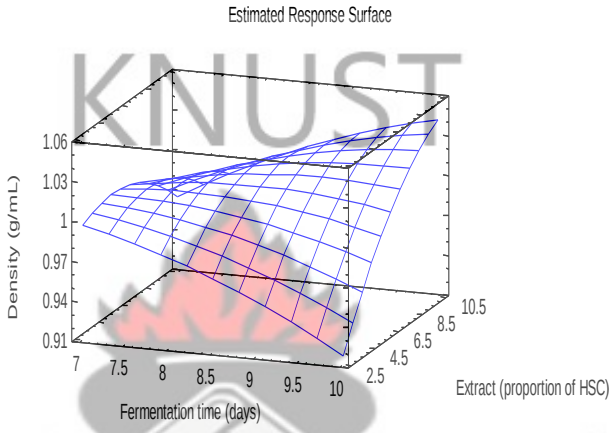

Fig. 5: Response surface plot for the effect of extract and fermentation time on density.

(Fitted regression model equation for density = 0.99461 + 0.0245892*A* - 0.0329968*B* - 0.00395588A^2+ 0.00648516*AB* - 0.00123897B^2)

4.2.4 Total colour density (TCD)

Colour is one of the most important quality attributes affecting the consumer's acceptance of food since it gives the first impression of food quality (Abou-Arab*et al.*, 2011).

Total colour density showed linear effect of extract to be the only significant term on the factors (Table 12 and Appendix B - Table 4a and 4b). The TCD increased as the proportion of HS increased irrespective of the fermentation time (Fig. 6). The fitted

regression model (R^2) was 68.89% and the linear effect indicated the total colour density increased or decreased within the experimental field (Fig. 6). From the graph HS strongly affected the TCD than SB extracts as fermenting musts which contained higher proportion of HS extracts had a higher TCD. SB is reported to have good amounts of apigenin that acts as colour stabilizer in products (Ilori and Odukoya, 2005). The expectation was for products containing SB portions to have higher colour densities than those without SB extracts. The observed trend could possibly be that the level of apigenin present in the proportion of SB extract used was not high enough for its effect to be seen.

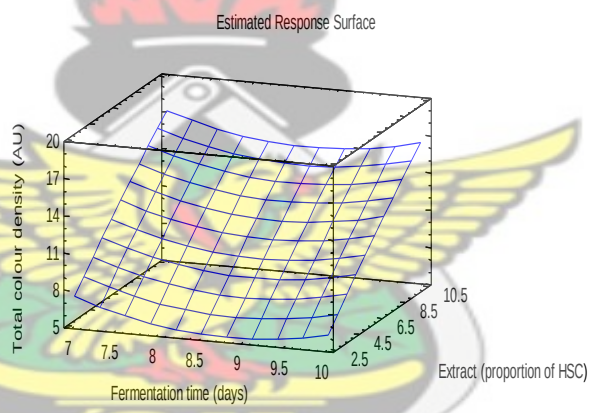

Fig. 6: Response surface plot for the effect of extract and fermentation time on total colour density.(Fitted regression model equation for TCD = 54.7466 - 12.0058*A*+ 1.27593*B*+ 0.678697A^2 + 0.0215748*AB* - 0.0150171B^2)

4.2.5 Total red pigment (TRP)

Total red pigments (anthocyanins) contribute to colour of young red wines (Rong-Rong *et al.,* 2009). The response surface plot showed linear effect of the factors on total red pigments (Table 12).

The response plot (Fig. 7) showed that higher concentrations of HS extract resulted in higher levels of TRP. Extract blend with a minimum concentration of 25% HS and 75% SB extracts had a TRP of 20.54AU and a maximum concentration of 100% HS resulted in a TRP of 37.29AU (Fig. 7). The fitted regression model (R^2 = 32.47%) had the linear effect of the extracts being the only significant term (Table 12 and Appendix B - Table 5a and 5b). This means the TRP increased or decreased constantly within the experimental field (Fig. 7). From the graph, the TRP is mainly contributed by the HS extract and fermented must with a high TRP could be obtained by using must containing higher concentrations of HS extract.

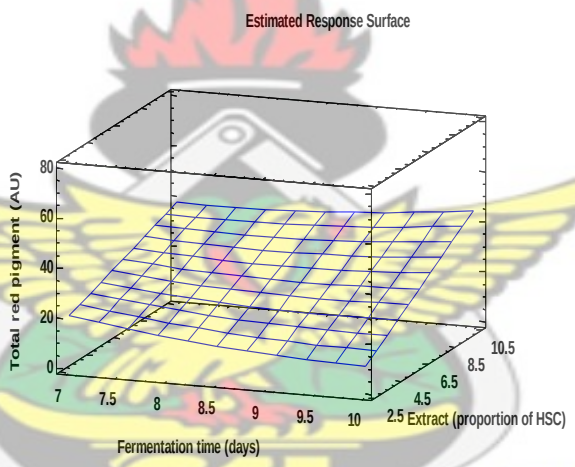

Fig. 7: Response surface plot for the effect of extract and fermentation time on total red pigment.(Fitted regression model equationfor total red pigment = 119.282 - 21.6517*A* - 2.85767*B*+ 0.97037A^2+ 0.705511*AB*+ 0.0114857B^2)

4.2.6 Total phenols (TP)

Generally, phenolic compounds in wine are divided into coloured phenols, such as anthocyanins and flavonoids, and non-colored phenols, including phenolic acids and flavan-3-ols (Rong-Rong *et al.*, 2009). Total phenols in wines contribute to the

astringency, bitterness, and other tactile sensations defined as structure or body, as well as to the wine's red colour (Rong-Rong *et al.,* 2009).

The response surface plot showed linear effect of the factors on total phenols (Table 12 and Appendix B – Table 6a and 6b). The plot in (Fig. 8) shows that irrespective of the fermentation time, levels of the total phenols increased with increasing proportion of HS extract. The higher the percentage of *Hibiscus sabdariffa* calyx extracts in the blend of extracts, the higher the total phenolic content. From the plot fermented must with higher phenolic contents could be obtained by using extract blend with a higher concentration of *Hibiscussabdariffa* calyx.

Since the fermented must containing higher percentages of HS extract had higher levels of total phenols it implied the phenols present in HS were higher in concentration than that of *Sorghum bicolor* leaf extract.

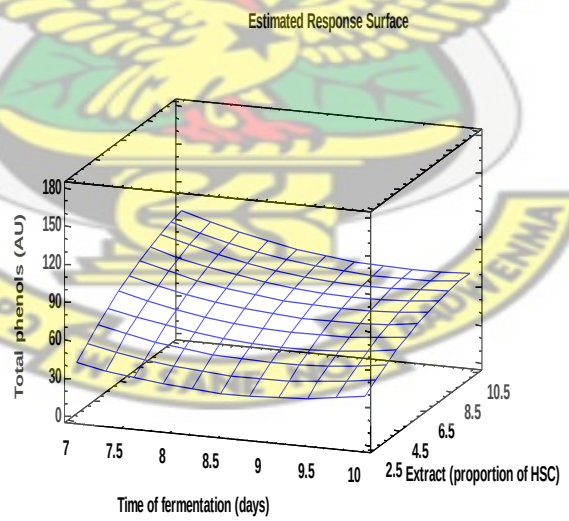

Fig. 8: Response surface plot for the effect of extract and fermentation time on total phenols.

(Fitted regression model equation for phenolic level= 349.283 - 83.0697*A*+ 20.5224*B* + 4.93227A^2 - 0.921778*AB*- 0.523857B^2)

4.2.7 Vitamin C

The response surface plot (Fig. 9) showed linear, quadratic and interaction effects of the factors on vitamin C (Table 12 and Appendix B – Table 7a and 7b). The vitamin C content decreased through the fermentation period from day 7 to day 10. The vitamin C content of the fermenting must also increase with an increasing concentration of HS extract (Fig. 9). At maximum point 0.0212 mg/ml vitamin C was obtained by fermenting the must for 7 days using 100% HSC.

The fitted regression model R^2 was 49.4172%. Linear effects of fermentation time and extract, quadratic effects of extract, interaction effect and the lack of fit were the significant terms (Table 12). The linear effect implied the vitamin C content increased or decreased constantly within the experimental field (Fig. 9). The significant quadratic term (extract) indicated the existence of an optimum level within the range of extract tested (25-100% of HS and 0-50% of SB). The significant lack of fit means that a higher model is needed to better explain the effects of the factors on vitamin C. The results obtained show that maximum vitamin C levels could be obtained by fermenting the must with a high proportion of HS extract for shorter time. The decrease in vitamin C with fermentation period is attributable to oxidationand vitamin C utilisation by yeast during fermentation (Alobo and Offonry, 2009). The increasing vitamin C content with an increasing HS proportion indicates HS as a good source of vitamin C compared to SB extract (Alobo and Offonry, 2009).

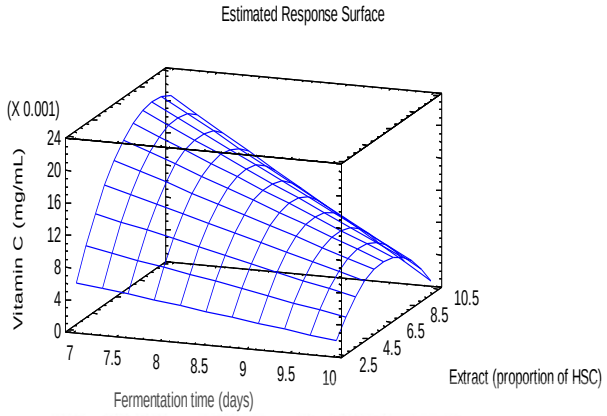

Fig. 9: Response surface plot for the effect of extract and fermentation time on vitamin C.

(Fitted regression model equation for vitamin C = -0.0109769 + 0.00039401*A* + 0.0113924*B*- 0.00001062$8A^2$ - 0.000651319*AB* - 0.00038222$9B^2$)

4.2.8 Optimization of responses

In order to optimize the process parameters for the production of the low alcohol rose wine with respect to the dependent variables considered, the contour plots were overlaid (Fig. 10). The optimum operating conditions for achieving maximum (total red pigment and total phenols), minimum (total colour density, total soluble solids and density), a pH of 3.3, titratable acidity of 0.8 andalcohol of 5.0% was fermentation time of 7.75 days and extract proportion of 100% HS and 0% SB with a desirability of 0.53 and extract proportion of 75% HS and 25% SB with a desirability of 0.50. Corresponding to these optimized values of process variables, the predicted values for alcohol, pH, density, total red pigments, total colour density, total phenolics are shown in appendix B (Table 8).

For the purpose of this study, both optimum conditions were chosen and taken through the next stage of the work. The nature of responses and optimum points of the various responses are shown in Appendix B – Table 8.

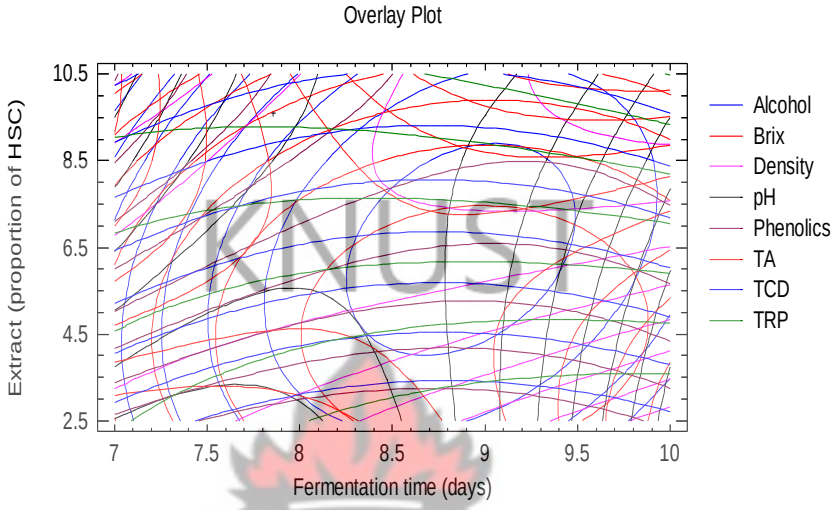

Fig. 10: Overlay of contour plots for total red pigment, total colour, total soluble solids, titratable acidity, density, total phenols, pH, density and alcohol. The + sign shows the region of optimum conditions.

4.3 Physicochemical properties of extracts, must and fermented must

4.3.1 Physicochemical properties of extracts

Table 13 shows the physico-chemical properties of extracts of *H.sabdariffa* calyces (HS), *Sorghum bicolor* (SB) leaves and a blend of *H.sabdariffa* and *S. bicolor* leaves (HS-SB). The pH was 3.69, 3.73 and 6.95 for HS, SB and HS-SB respectively. The pH of SB was almost neutral (6.95). Addition of SB to HS (in the ratio of 25%: 75%) caused an increase in the pH of the resulting blend as shown in Table 14. The pH of HS and HS-SB extracts were in the required range for wine fermentation which is 3.5 to 4.0 (Rose, 1982; Amerine *et al.*, 1980).

The low pH of the extracts enhances the effectiveness of SO_2 as an antimicrobial agent and increases microbial stability through increased inhibition of bacterial growth and enhances production of fruity esters during yeast fermentation (Rotter, 2009).

The brix of HS, HS-SB and SB were zero which indicated there was no sugar in the extracts. For wine production sugar is necessary for alcohol production during yeast fermentation. This indicates the extracts must be chaptalized or ameliorated with table sugar to increase the sugar content for the support of fermentation (Alobo and Offonry, 2009). According to the Alcohol and Tobacco Tax and Trade Bureau, sugar addition should not exceed an apparent brix of 25 (Jacobson, 2006).

Titratable acidity measures the amount of acid present in a particular product (Adeleke and Abiodun, 2010). The titratable acidity (calculated based on tartaric acid) for HS, HS-SB and SB extracts were 6.98 g/l, 6.75 g/l and 1.5 g/l respectively. A 25% addition of SB to 75% HS resulted in an acidity of 6.75 in the blend. For wine production, the titratable acidity is in the range of 6-9 g/l (Boulton *et al.*, 1999) and the acidities of HS and HS-SB are within the acceptable range. Must with high pH and low titratable acidity producewines which are flat and unstable to oxidation and microbial spoilage (Demyttenaere *et al.*, 2003). The high pH and low acidity of SB indicate extracts from SB has to be acidified by adding more tartaric acid to make the extract stable to oxidation and microbial spoilage. Titratable acidity affects the taste of the extract and the resulting wine (Anonymous, 2009b).

Red wine colour measurements are based on the absorbance of monomeric anthocyanin pigments and polymeric pigment forms in the visible and ultra-violet regions. Red wine phenolics contribute to astringency, bitterness and other tactile sensations defined as structure or body of the wine. The total phenolic content of the

extracts were 16.81 AU, 14.73 AU and 13.53 AU for HS, HS-SB and SB extracts respectively. These phenol levels were lower than the amounts normally found in red wine which is between 23 to 100 AU with an average of 54 AU. The lower phenolic levels are due to the dilution made in the extract preparation.

Total red pigment of the extracts was 5.85 AU for HS, 5.56 AU for HS-SB and 2.69 AU for SB. Colour densities of the extracts were 1.84 AU, 1.61 AU and 1.76 AU for HS, HS-SB and SB respectively. The total red pigment, colour densities and phenolic contents were high in HS products relative to the blend as shown in Table 13. The total red pigments, colour densities and phenolic contents contribute to the colour; quality and mouth feel of the extract as well as the health promoting properties through their ability to act as antioxidants (Kinsella *et al*. 1993).

Table 13: Physicochemical properties of extracts of *Hibiscus sabdariffa* calyx, blend of *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf (75% HS and 25% SB)

Parameter	HS Extract	HS-SB Extract	SB Extract
pH	3.69±0.01	3.73±0.02	6.95±0.00
Brix	0.00±0.00	0.00±0.00	0.00±0.00
Density(g/ml)	0.98±0.00	0.99±0.00	0.89±0.00
Titratable acidity (tartaric acid, g/l)	6.98±0.08	6.75±0.00	1.5±0.04
Total red pigment (AU)	5.85±0.08	5.56 ±0.51	2.69±0.08
Total phenols (AU)	16.81 ±0.42	14.73 ±1.09	13.53±0.26
Colour intensity (AU)	1.84±0.03	1.61±0.02	1.76±0.02

[a-b] mean values with superscripts bearing different alphabets in the same row are significantly different

4.3.2 Physicochemical properties of must and fermented must

4.3.2.1pH and Titratable acidity (TA)

The physicochemical properties of the must and fermented must of *Hibiscus sabdariffa* calyces (HS) and *Sorghum bicolor* leaves (SB) are shown in Table 14 and Appendix C.

The pH values of 3.65 and 3.69 for the must of HS and HS-SB respectively falls within the optimal limits of 3.5-4.0 for wine fermentation (Rose, 1982; Amerine *et al.*, 1980). The fermentation was carried out for eight days. Significant differences ($P < 0.05$) were observed in the pH of the must and the fermented must (Table 14) for each of the two products at the end of fermentation. The pH decreased through the fermentation period. For the fermented must produced, the drop in pH was from 3.65 to 3.61 for HS and 3.69 to 3.63 for HS-SB.

Titratable acidity measures the amount of acid present in a particular product (Adeleke and Abiodun, 2010). Significant differences ($P<0.05$) were observed between the TAs of the must and fermented must of the two products (Table 14). The TA values of 7.08 g/l and 6.90 g/l for the must of HS and HS-SB respectively are within the normal ranges (6 and 9 g/l) for grapes and wine (Boulton *et al.*, 1999; Ough and Amerine 1988). There was an increase in the titratable acidity at the end of fermentation (Table 14). The decrease in pH or increase in titratable acidity was as a result of utilization of nutrients by yeast and the production of organic acids during fermentation (Ojokoh *et al.*, 2002). This observation is similar to work done on roselle wine in which the TA increased from 0.69 to 0.75% and the pH decreased from 3.72 to 3.43 at the end of 30 days aging after fermentation (Alobo and Offonry, 2009).

4.3.2.2 Total soluble solids (TSS), density and alcohol

Total soluble solids measures the sugars present in the extract, fermented must and wine. As shown in Table 14, the extracts of HS and SB had no sugars (TSS= 0.00^{O}Brix) which necessitated amelioration to raise the levels of sugar to18.62°Brix for adequate fermentation by yeast to take place. Significant differences existed between the TSS of the must and the fermented must of the products. At the end of the fermentation, the sugar content dropped from 18.62 to 15.62 and 14.62 for HS and HS-SB fermented must respectively. The drop in sugar was as a result of yeast activities utilizing the sugars for alcohol production.

The density of the must slightly decreased at the end of fermentation (1.04-1.03 g/l and 1.06-1.02g/l) for HS and HS-SB. These changes between the densities of the must and fermented must were significant ($p< 0.05$). The decrease in density was as a result of the alcohol produced as alcohol is less dense (Jacobson, 2006). At the end of the fermentation process, analcohol of 1.78 and 2.37% were produced for HS and HS-SB wines. The alcohol produced at the end of fermentation was low. Work done by Yokotsuka and co - workers reported that Hibiscus *sabdariffa* calyces contained small amounts of acetaldehyde, pyruvic acid, and alpha- keto glutaric acid, which normally react with SO_2 to form bisulphate complexes in fermentation, and hence the SO_2 added in the fermentative process in the form of sodium metabisulphite left in free form suppressed the growth of yeast, leading to the low amount of alcohol produced at the end of fermentation (Yokotsuka *et al.*, 1997).

4.3.2.3 Total colour density, total red pigment and total phenols

Phenolics contribute to the red pigmentation, the brown forming substrates, and the bitter and astringent taste and give wines their colour and quality (Jacobson, 2006). Through the fermentation period, the total phenols decreased and significant changes ($p < 0.05$) between the must and the fermented must were established. The phenolic content of HS must and fermented must (21.06 and 18.21 AU respectively) were higher compared to that of HS-SB must and fermented must (19.23 and 16.64 AU respectively) as shown in Table 14.

Total red pigment (anthocyanins) contributes to wine colour and at the end of the eight days fermentation, the values decreased from 6.39 AU to 6.31 AU for HS must and fermented must and 6.07 to 5.33 AU for HS-SB must and fermented must. The decrease in the values of anthocyanins between the must and fermented must of each of the two products was significant.

The decrease in total phenols and total red pigments at the end of fermentation could be attributed to the bleaching effect of the sulphur dioxide added to the fermenting must in the form of sodium metabisulphite, hydrolysis and polymerization of monomeric anthocyanins during the fermentative process (Jacobson, 2006).

The colour density of the fermented must decreased after fermentation. The change was from 1.65 to 1.36 AU for HS fermented must and 1.43 to 1.28 AU for HS-SB fermented must. The decrease in the values of colour densities between the must and fermented must of each of the two products was significant, $p < 0.05$. The decrease in the colour density may be due to the alcohol build-up during fermentation as a rise in alcohol decreases the hydrogen – ion bonds (Margalit, 1997). The total phenols, total red pigments and total colour densities were higher for HS products compared to HS-

SB products. This could be due to the different anthocyanin concentration in the different plant species (Jackman andSimith, 1996).

Table 14: Physicochemical properties of *Sorghum bicolor* leaf and *Hibiscus sabdariffa* calyx musts and fermented musts.

Parameter	HS		HS-SB	
	Must	**Fermented Must**	**Must**	**Fermented Must**
pH	3.65±0.01[a]	3.61±0.01[b]	3.69±0.01[c]	3.63±0.04[d]
Brix	18.62±0.0[a]	15.62±0.5[b]	18.62±0.0[c]	14.62±0.5[d]
Density(g/ml)	1.04±0.00[a]	1.03±0.00[b]	1.06±0.00[c]	1.02±0.00[d]
Titratable acidity (tartaric acid g/l)	7.08±0.04[a]	7.30±0.04[b]	6.90±0.08[c]	7.18±0.04[d]
Colour density (AU)	1.96±0.01[a]	1.36±0.09[b]	1.71±0.48[c]	1.28±0.03[d]
Total Anthocyanins (AU)	6.39±0.04[a]	6.31±0.02[b]	6.07±0.11[c]	5.33±0.05[d]
Total phenols (AU)	21.06±0.1[a]	18.21±0.1[b]	19.23±0.3[c]	16.64±0.6[d]
Alcohol (%)	0	1.78±0.30	0	2.37±0.30
Volatile acidity (g/100ml)	0	0.06±0.01	0	0.06±0.01

[a-b, c-d] mean values with superscripts bearing different alphabets in the same row are significantly different. Comparison pertains to only two treatments(must and its corresponding fermented must)

4.4 Physicochemical properties of wines from *Hibiscus sabdariffa* calyx and blend of *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf extracts during aging.

Legend

HSU: Unclarified wine from *Hibiscus sabdariffa* calyx extract

HSC: Clarified wine from*Hibiscus sabdariffa* calyx extract

HS-SBU: Unclarified wine from blends of *Hibiscus sabdariffa* calyx extract and *Sorghum bicolor* leaf.

HS-SBC: Clarified wine from blends of *Hibiscus sabdariffa* calyx extract and *Sorghum bicolor* leaf.

Tables 15 to 23 indicate measurement of physicochemical properties of the wine samples from week 0 to week 20.

The effect of aging on total soluble solids of wine samples is presented in Table 15. Significant ($p<0.05$) decrease in the total soluble solids were established in the wine samples during aging (Appendix D, Table 1a and 1b). HSU and HSC both had an initial total soluble solids of 15.62 ^{o}Brix and the value decreased to final values of 11.12and 11.78 ^{o}Brix at week 20 respectively (Table 15). Similarly, HS-SBU and HS-SBC both had initial total soluble solids of 14.62 ^{o}Brix (week 0) and decreased to 11.22 and 11.67^{o} Brix at week 20 respectively. The reduction in TSS during the aging period indicated secondary fermentation and the observation confirms similar work done on roselle wine in which the sugars dropped from 21.00 to 4.90^{o}Brix at the end of 30 days of aging (Alobo and Offonry, 2009) as well as on pear wine in which the decrease in TSS was from 24 to 8-13.20 ^{o}Brix at the end of aging for one month (Maragatham and Panneerselvam, 2011).

Slightly higher sugar levels were found in the clarified wines compared to the unclarified wines during aging. This may be attributed to slow rate of fermentation in

the clarified wines. (Maragathamand Panneerselvam, 2011). This is due to the agglomerates formed between the negatively charged yeast cells and the positively charged egg albumin which eventually drop to the bottom of the bottle as lees (Anonymous, 2002). The reduced total soluble solids at the end of aging are due to the utilization of sugars in the production of alcohol (Maragatham and Panneerselvam, 2011).

Table 15: Effect of aging on total soluble solids of wine samples

	0	5	10	15	20
HSU	15.62±0.50^{a}	14.613±0.29^{a}	14.28±0.50^{a}	12.78±0.50^{b}	11.12±0.50^{b}
HS-SBU	14.62±0.50^{a}	14.28±0.50^{a}	14.12±0.50^{a}	12.12±0.50^{b}	11.22±0.09^{b}
HSC	15.62±0.01^{a}	14.53±0.35^{a}	13.12±0.50^{ab}	12.62±0.50^{b}	11.78±0.50^{c}
HS-SBC	14.62±0.01 ^{a}	14.28±0.50^{a}	13.62±0.50^{ab}	12.28±0.50^{bc}	11.67±0.09^{c}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p< 0.05$).

The effect of aging on density of the wine samples is shown in Table 16. The wine samples decreased significantly ($p<0.05$) with aging time (Appendix D, Table 2a and 2b). The densities varied from 1.03 to 0.99 g/ml in HSU and 1.02 to 0.99 g/ml in HS-SBU samples. In the case of HSC and HS-SBC samples, the densities ranged from 1.03 to 1.00 g/ml and 1.02 to 0.99 g/ml respectively. The decreases in the densities can be attributed to the removal of unwanted wine components that affect clarification, astringency, colour, bitterness, and aroma in the form of sediments and the utilization of sugars by residual yeast for alcohol production during the aging period (Harbertson, 2009; Jacobson, 2006).

HSC had the highest density among the wine samples. Through blending and clarification, the combined phenolics from both extracts and the effect of the clarifying agent could have affected the rate of precipitation of colloidal wine particles. This gave HSC a different precipitation rate from the rest of the wine samples and this could have contributed to the slightly high density in HSC wine (Guerra, 2011).

Table 16: Effect of aging on density(g/ml) of wine samples

	0	5	10	15	20
HSU	1.03±0.00^{a}	1.02±0.001^{ab}	1.01±0.01^{abc}	1.00±0.07^{bc}	0.99±0.01^{c}
HS-SBU	1.02±0.01^{a}	1.02±0.02^{a}	1.01±0.06^{ab}	1.00±0.01^{ab}	0.99±0.04^{b}
HSC	1.03±0.03^{a}	1.02±0.02^{a}	1.02±0.06^{a}	1.01±0.01^{a}	1.00±0.00^{b}
HS-SBC	1.02±0.04^{a}	1.02±0.01^{a}	1.02±0.02^{a}	1.01±0.04^{ab}	0.99±0.02^{b}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p < 0.05$).

The alcohol content of wine samples during the 20 week period of aging are represented in Table 17. The alcohol contents of the wine samples increased significantly ($p < 0.05$) with time of aging (Appendix D, Table 3a and 3b).The alcohol contents increased during the aging period from 1.78 to 4.44% in HSU wine samples and from 1.78 to 4.25% in HSC wine samples. With regards to HS-SBU and HS-SBC wines, the alcohol levels increased from 2.37 to 4.38% and from 2.37 to 4.11% respectively (Table 17). Low alcohol wines are wines directly produced with alcohol contents of more than 1.2 % and less than 5.5%. The alcohol content of the samples in this study falls within the range for low alcohol wines (Erten and Campbell, 2001).

Minimum levels of alcohol was observed in the clarified wines as a result of the effect of the egg albumin binding to some yeast cells in the wines, bringing about slow rate of fermentation, yielding lower levels of alcohol in the clarified wines compared to the unclarified wines (Anonymous, 2002).The low alcohol content of the samples at the end of fermentation could be attributed to the influence of free SO_2 from dissociation of the added sodium metabisulphite. The SO_2 in free form suppress yeast growth due to its antimicrobial effect (Yokotsuka *et al.*, 1997).

Table 17: Effect of aging on alcohol content (%) of wine samples

	0	5	10	15	20
HSU	1.78±0.29^{a}	2.37±0.17^{a}	3.26±0.17^{ab}	3.55±0.29^{bc}	4.44±0.29^{c}
HS-SBU	2.37±0.29^{a}	2.57±0.29^{a}	2.96±0.29^{a}	3.85±0.29^{b}	4.38±0.06^{b}
HSC	1.78±0.29^{a}	2.42±0.67^{a}	2.57±0.21^{a}	3.46±0.29^{ab}	4.25±0.29^{b}
HS-SBC	2.37±0.29^{a}	2.57±0.29^{a}	2.66±0.29^{ab}	3.75±0.29^{bc}	4.11±0.37^{c}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p < 0.05$).

The acetic acid contents of the wine samples during the 20 week aging period are shown in Table 18. Acetic acid is the main component of volatile acidity and is critical in assessing wine quality. Its concentration in wines is approximately 0.5 g/l and must remain below 1.1 g/l according to current legislation (Vilela-Moura, 2008). Blending and clarification had no significant effect ($p > 0.05$) on the volatile acidities of the wines produced from HS and HS-SB (Appendix D, Table 4a and 4b). The volatile acidities of unclarified wines ranged from 0.056 to 0.064 g/100ml and for the clarified wines, the range was from 0.056 to 0.067 g/100ml (Table 19). The normal acetic acid levels formed during the fermentation process are approximately 0.2-0.4

g/l. The legal limits for volatile acidity of red wines made from grapes with total soluble solids less than 28^{o}Brix exclusive of sulphur dioxide is 1.4 g/l in the United States of America (Jacobson, 2006). In Australia, the maximum possible limit for volatile acidity (VA) is 1.5 g/l expressed as acetic acid (Boodle, 2010). In the European Community, the maximum limit for red wines is 1.2 g/l, for white wines it is 1.08 g/l and for sweet botrytis infected wines 1.5 g/l (Anonymous, 2010). The VAs determined in this study were within the acceptable limits as stated above. Wines containing acetic acid contents beyond the legal or acceptable limits are considered spoilt because they impart a bitter, sour flavour with vinegar like aroma (Jacobson, 2006; Peynaud, 1984).

Table 18: Effect of aging on volatile acidity (g/100ml) of wine samples

	0	5	10	15	20
HSU	0.06±0.012^{a}	0.061±0.005^{a}	0.069±0.020^{a}	0.067±0.005^{a}	0.064±0.014^{a}
HS-SBU	0.056±0.014^{a}	0.059±0.005^{a}	0.064±0.014^{a}	0.068±0.014^{a}	0.056±0.014^{a}
HSC	0.064±0.014^{a}	0.064±0.014^{a}	0.067±0.017^{a}	0.068±0.018^{a}	0.064±0.014^{a}
HS-SBC	0.056±0.014^{a}	0.061±0.009^{a}	0.064±0.008^{a}	0.068±0.007^{a}	0.067±0.017^{a}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p< 0.05$).

Table 20 shows the effect of aging on total phenols of wine samples. Significant differences ($p<0.05$) were established during the aging period from week 0 to week 20 (Table 19, Appendix D, Table 5a and 5b). Initially, the total phenolic content of HSU was 18.21 AU and the value decreased to 10.16 AU at the end of week 20. HSC had an initial total phenolic content of 16.58 AU and at week 20 the value had decreased to 8.19 AU. On the other hand, HS-SBU had an initial total phenolic content of 16.64

AU and decreased progressively to 9.15 AU at the end of the aging period while HS-SBC had an initial value of 13.97 AU at week 0 and decreased significantly to 7.37 AU at the end of week 20.Throughout the aging process, the values of the clarified wines were lower compared to the unclarified wines. This could be attributed to the fact that egg albumin has an affinity for polyphenols in acidic medium and reacts by forming hydrogen bonds between the phenolic hydroxyl and the carbonyl oxygen of the peptide bond of the protein component. This causes coagulation with higher polymeric phenols such as tannins to form insoluble complexes which settle at the bottom of the aging wine bottle as lees (Harbertson 2009; Zoecklein et al., 1997). Egg-whites are often used to reduce astringency by removing small quantities of phenolic materials from red wines (Morris and Main, 1995). Red wines have a phenolic content of 23 to 100 AU, with an average of 54 AU and white wines have an average corrected absorbance of 4 AU, with a range of 1 to 11 AU (Waterhouse, 2002). In this study, the total phenolic content of the wines were lower than that reported in literature for red wines but at the end of the 20 week aging, the phenolic contents were within the range for white wines.

A progressive decline in the total phenolic contents of the wines occurred through the aging period due to polymerization of phenolic compounds (Garciafalcon, 2007). HSU had an initial value of 18.21 and 10.16 AU at the end of week 20, whereas HS-SBU had a value of 16.64 and 9.15 AU at week 0 and week 20 respectively (Table 19). The total phenols in HSC were 16.58 AU initially and decreased significantly to 8.19 AU at the end of the aging period. At week 0, the phenolic content of HS-SBC was 13.97 AU and also decreased up to 7.37 AU at week 20. The total phenolic content of the wines made from only *Hibiscus sabdariffa* calyx extracts had higher values compared to those from a blend of extracts from *Hibiscus sabdariffa* calyces

and *Sorghum bicolor* leaves.The difference in the phenolic contents of the wines is attributed to the initial quantitative levels of phenols present in *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf extracts. From the results shown in Table 13, the amounts of phenols present in *Hibiscus sabdariffa* calyx extract was more than the amounts determined in *Sorghum bicolor* leaf. Substituting 25% of *Hibiscus sabdariffa* calyx extract with *Sorghum bicolor* leaf extract could have caused a decrease in the total phenols of the resulting blend.

Table 19: Effect of aging on total phenols (AU) of wine samples

	0	5	10	15	20
HSU	18.21±0.17^{a}	16.67±0.67^{b}	14.51±1.05^{c}	13.73±0.05^{c}	10.16±0.02^{d}
HS-SBU	16.64±0.66^{a}	14.38±0.44^{b}	13.85±0.09^{b}	12.41±0.17^{c}	9.15±0.03^{d}
HSC	16.58±0.51^{a}	15.61±0.21^{ab}	14.39±0.28^{b}	10.35±0.09^{c}	8.19±0.57^{d}
HS-SBC	13.97±0.65^{a}	12.55±0.44^{b}	12.01±0.39^{b}	9.62±0.53^{c}	7.37±0.18^{d}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p < 0.05$).

The effect of total red pigment on aging of wine samples is shown in Table 20. Anthocyanins are the main compounds responsible for the colour of young wines. They are relatively unstable and tend to form new compounds, some of them being more stable than pure anthocyanins, during maturation and aging (Cano-L´opez *et al.*, 2007).

Total red pigment declined significantly ($p<0.05$) during the twenty week aging period (Appendix D, Table 6a and 6b). The percentage decrease in TRP levels were 50.71, 53.62, 58.16 and 59.91% for HSU, HSC, HS-SBU and HS-SBC wines respectively (Table 20).

The total red pigments of the unfined wines were higher than the fined wines. A similar trend was reported by Castillo-Sa´nchez *et al.* (2006) by using casein, egg albumin, gelatin and polyvinylpolypyrrolidine as fining agents on Vinha˜o wines in which the fined wines had lower anthocyanin levels compared to the unfined wines. The loss in total red pigment is due to the decrease in amount of oligomeric flavanols as egg albumin significantly decreases the amount of oligomeric flavanols in wines (Cosme *et al.*, 2009).

Wines from blends of *Sorghum bicolor* leaf extracts and *Hibiscus sabdariffa* calyx extract had relatively lower values of total red pigments compared to those prepared from only *Hibiscus sabdariffa* calyx extract (Table 20). The initial amounts of total red pigments in the *Sorghum bicolor* leaf extracts were lower compared to *Hibiscus sabdariffa* calyx extracts. Consequently, the substitution caused a further decrease in the total red pigment of the blended wine. The progressive decline in the total red pigment may be attributed to the anthocyanins undergoing hydrolytic and degradation reactions during aging (Santos-Buelga *et al.*, 1999), participation of monomeric anthocyanins in various forms of condensation reactions with flavanols, or their combination with other small molecules possessing a polarizable double bond (pyruvic acid, acetaldehyde), to form pyranoanthocyanins (Monagas *et al.*, 2006). The ideal temperature for wine aging is 13 ^{o}C (Pandell, 2006). It is also possible that the high aging temperature (28^{o}C) could have accelerated polymerization of anthocyanins with each other and other phenolics (Gomez plaza, 2000).

Table 20: Effect of aging on total red pigment (AU) of wine samples

	0	5	10	15	20
HSU	6.31±0.02^{a}	5.549±0.19^{b}	4.94±0.16^{c}	4.01±0.04^{d}	3.21±0.06^{e}
HS-SBU	5.33±0.06^{a}	4.52±0.02^{b}	4.27±0.06^{bc}	4.01±0.04^{c}	3.10±0.09^{d}
HSC	5.67±0.31^{a}	5.44±0.05^{a}	4.39±0.14^{b}	3.93±0.12^{c}	3.04±0.05^{d}
HS-SBC	4.84±0.07^{a}	4.22±0.08^{b}	4.08±0.08^{b}	3.598±0.02^{c}	2.90±0.08^{d}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p< 0.05$).

The colour density of wine samples during the 20 week period of aging is represented in Table 21. The colour densities decreased significantly ($p< 0.05$) over the aging period (Appendix D, Table 7a and 7b). Unclarified *Hibiscus sabdariffa* calyx extract wine (HSU) had an initial total colour density of 1.36 AU and decreased to 0.89 AU at the end of aging at week 20. Wine from clarified*Hibiscus sabdariffa* calyx extract (HSC) had an initial total colour density of 1.20 AU and at week 20 the value had decreased to 0.67 AU. On the other hand, at week 0, the unclarified wine from a blend of*Hibiscus sabdariffa* calyx extract and*Sorghum bicolor* leaf extract (HS-SBU), had an initial value of 1.28 AU and at week 20 the value had decreased to 0.68 AU (Table 21). The clarified blended wine (HS-SBC) had an initial value of 1.13 AU and at the end of the 20 weeks the value had decreased to 0.63 AU. Throughout aging, the total colour densities of the clarified wines were relatively lower than the unclarified wines. A similar observation was made by Castillo-Sa´nchez *et al,* (2006) on the effect of various fining agents on Vinha˜o wines. The researchers found the colour densities of the unclarified wines to be relatively higher than the clarified wines. Egg albumin cause precipitation of some phenolics, removes partially soluble compounds that makes wine cloudy or form precipitates at bottom of bottles andincreases the rate

of settling process, thereby adding brilliance to the fined wines (Harbertson ,2009; Castillo-Sa´nchez *et al.*,2006, Sims*et al.,* 1995).

The percentage decrease in the total colour density over the aging period was 65.44, 53.13, 55.83 and 55.75% for HSU, HS-SBU, HSC and HS-SBC respectively. (Table 21). The actual colour of rose wines vary depending on the grapes involved, and often may seem to be more orange than pink or purple (Anonymous, 2003).

The colour densities of the blended wines were lower compared to the wines from only *Hibiscus sabdariffa* calyx extracts. The difference is attributed to the initial amounts of phenolics which were present in the two extracts with levels in *Hibiscus sabdariffa* calyx extracts being higher. This trend is similar to that observed by Auw *et al* (1996) in their study on the effect of processing on phenolics and the colour of some wines and juices. They reported the colour of blended wines to be relatively lower than the unblended wines. The progressive decline in the colour density through the ageing period could be attributed to the replacement of monomeric anthocyanins with polymeric anthocyanins which resulted from the combination of anthocyanins pigments with tannins. This condensation reaction is accelerated by oxidation leading to the precipitation of coloured matters in the wine and polymerisation of pigments under anaerobic conditions (Castillo-Sa´nchez *et al.,* 2006).

Table 21: Effect of aging on total colour density (AU) of wine samples

	0	5	10	15	20
HSU	1.36±0.04^{a}	1.12±0.14^{b}	1.07±0.21^{b}	0.95±0.01^{b}	0.89±0.00^{c}
HS-SBU	1.28±0.01^{a}	1.08±0.08^{ab}	0.96±0.04^{b}	0.91±0.01^{bc}	0.68±0.01^{c}
HSC	1.20±0.04^{a}	1.10±0.06^{a}	0.78±0.02^{b}	0.76±0.01^{b}	0.67±0.01^{b}
HS-SBC	1.13±0.05^{a}	1.02±0.19^{a}	0.72±0.03^{b}	0.68±0.04^{b}	0.63±0.03^{b}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p< 0.05$).

Table 22 shows the effect of aging on titratable acidity of the wine samples. Significant incremental changes were established in the titratable acidities through the aging period (Table 22, Appendix D, Table 8a and 8b). The TAs varied from 7.30 to 8.10 g/l for HSU and 7.26 to 8.57 g/l for HSC wines at the end of the 20 - week aging period. In the case of the blended wines, HS-SBU had a titratable acidity range of 7.18 to 8.30 g/l and HS-SBC had a range of 7.15 to 8.40 g/l at the end of the 20 weeks (Table 22). Also, Table 22 shows the TA of HS-SBC to be higher during aging compared to HS-SBU though its initial TA was lower than that of HS-SBU. The differences due to clarification could be attributed to the fact that the egg albumin causes much removal/ loss ofhydrogen tartrate (e.g. potassium hydrogen tartrate or calcium tartrate) from the wine, resulting in the lowering of pH and the significant increase in the TAs (Eggers, 2006).Increases in TA of the wines during aging may be due to large production of succinic acid in the wine (Coulter, 2005; De Klerk,2010). The titratable acidities were within the normal ranges of 6 and 9 g/l for grapes and wine (Boulton *et al.*, 1999; Ough and Amerine, 1988).

Table 22: Effect of aging on titratable acidity (g/l) of wine

	0	5	10	15	20
HSU	7.30±0.04^{a}	7.42±0.04^{a}	7.48±0.03^{ab}	7.75±0.08^{b}	8.10±0.15^{c}
HS-SBU	7.18±0.04^{a}	7.20±0.03^{ab}	7.36±0.04^{ab}	7.43±0.09^{c}	8.30±0.12^{d}
HSC	7.26±0.04^{a}	7.39±0.04^{b}	7.55±0.04^{b}	7.58±0.15^{b}	8.57±0.03^{c}
HS-SBC	7.15±0.08^{a}	7.46±0.04^{ab}	7.50±0.08^{b}	7.60±0.02^{b}	8.40±0.04^{c}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p< 0.05$).

Table 23 show the effect aging on pH of wines. Significant decrement was established in the pH value over the 20 week aging period (Appendix D, Table 9a and 9b). HSU and HSC both had an initial pH of 3.61 at week 0 and the pH decreased to 3.14 and 3.01 respectively at week 20. HS-SBU and HS-SBC also both had pH of 3.63 at week 0 and at the end of week 20 the values were 3.08 and 3.10 respectively (Table 23). The decrease in pH indicated increase in acidity.

Wines are buffered by two systems malic acid/hydrogen malate (H_2M/ HM) and tartaric acid/hydrogen tartrate (H_2T/HT) (Eggers, 2006). Extracts of *H.sabdariffa*calyx contained high amounts of tartaric acid (6.98 g/l, Table 14) and also high amounts of calcium and potassium 1830.78 and 1725.25 mg/100g respectively (Table 11). Tartaric acid can precipitate these two main salts as either potassium hydrogen tartrate (KHT) and calcium tartrate (CaT) which are insoluble and forms lees at the bottom of wine bottles at a typical wine pH of 3 to 4. Due to these buffering systems the pH of the wines are maintained. However, too much loss of the hydrogen tartrates lowers the wine pH (Eggers, 2006).Clarification stabilizes wine by removing components from wine including KHT and CaT precipitates. HSC

had the least pH indicating too much removal of the hydrogen tartarates by the egg albumin. The pH varied between 3.01 and 3.63 whichis within the range of 3 to 4 typical for wines (Jacobson, 2006).

Table 23: Effect of aging on pH of wine samples

	0	5	10	15	20
HSU	3.61±0.06^{a}	3.55±0.02^{ab}	3.48±0.018^{abc}	3.35±0.01^{c}	3.14±0.01^{d}
HS-SBU	3.63±0.01^{a}	3.58±0.01^{a}	3.52±0.01^{ab}	3.40±0.10^{b}	3.08±0.01^{c}
HSC	3.61±0.01^{a}	3.46±0.07^{ab}	3.32±0.02^{b}	3.29±0.20^{b}	3.01±0.01^{c}
HS-SBC	3.633±0.06^{a}	3.433±0.01^{a}	3.37±0.04^{ab}	3.293±0.01^{b}	3.107±0.01^{c}

Values are expressed as mean ± SD of triplicate determinations. Mean values in the same row with different superscripts are significantly different at ($p< 0.05$).

4.5: Sensory evaluation

Affective test

The results of the sensory evaluation (affective test) of wines from *Hibiscus sabdariffa* calyces, and a blend of *Hibiscus sabdariffa* calyces and *Sorghum bicolor*leaves are presented in Table 24 (acceptance test), Fig.11(preference test) and Appendix E.

The sensory panelists comprised 90% males and 10% females between the ages of 18 - 80 years, though it is generally known that women can smell and taste wine better than men(Wilf, 2011). Most females declined to partake in the sensory exercise on religious grounds because of the alcohol content. Panelists between the ages of 18-30 years were 67.67%, 16.67% of the panelists were between the ages of 31-45 years, 13.33% between the ages of 46-60 years and 3.33% between the ages 61-80 years.

Different age groups were used in the sensory evaluation because considerable diversity exist in all age groups regarding wine tasting as wine judging ability may decline with age, experience and mental concentration may compensate for sensory loss (Jackson, 2009).

Among the test wines, no significant differences ($p>0.05$) existed in the colour, aroma, sweetness, acidity, alcohol content and overall acceptability of the wine samples. HSC had the lowest scores (most acceptable) for all the attributes except acidity where HSU had the lowest score. There were virtually no significantdifference ($p>0.05$) between the clarified and unclarified wines in terms of colour. HSC was the most acceptable wine to the panelists in terms of the sensory attributes analysed among the test wines.

In terms of the product preference, 33.33% of the panelists preferred HSC wine most, followed by HSU wine with 26.67% and 20% of the panelists preferred the HS-SBU and HS-SBC wines. Among the same products 33.33% of the judges least preferred HSU and HS-SBU wines, 29.63% least preferred HS-SBC wines and only 3.70% least preferred HSC wine as shown in Fig. 11.

Table 24: Mean sensory scores of wines from *Hibiscus sabdariffa* calyx, a blend of *Hibiscus sabdariffa* calyx and *Sorghum bicolor* wine samples.

Product	**HSU**	**HS-SBU**	**HSC**	**HS-SBC**
Sensory attribute				
Colour	2.63±1.314[a]	2.50±1.295[a]	2.44±1.189[a]	3.06±1.435[a]
Aroma	3.69±1.749[a]	3.31±1.730[a]	3.06±1.435[a]	3.59±1.583[a]
Sweetness	3.09±1.889[a]	3.16±1.743[a]	2.97±1.694[a]	3.16±1.851[a]
Acidity	3.31±1.645[a]	3.94±1.865[a]	3.66±1.789[a]	3.44±1.759[a]
Alcohol content	3.22±1.930[a]	3.56±1.625[a]	3.00±1.524[a]	3.31±1.655[a]
Overall acceptability	3.19±1.127[a]	3.29±1.052[a]	3.03±0.978[a]	3.31±1.012[a]

Lower values indicate greater acceptance on a 7-point hedonic scale.

Mean values in the same row with same superscripts are non -significantly different at ($p< 0.05$).

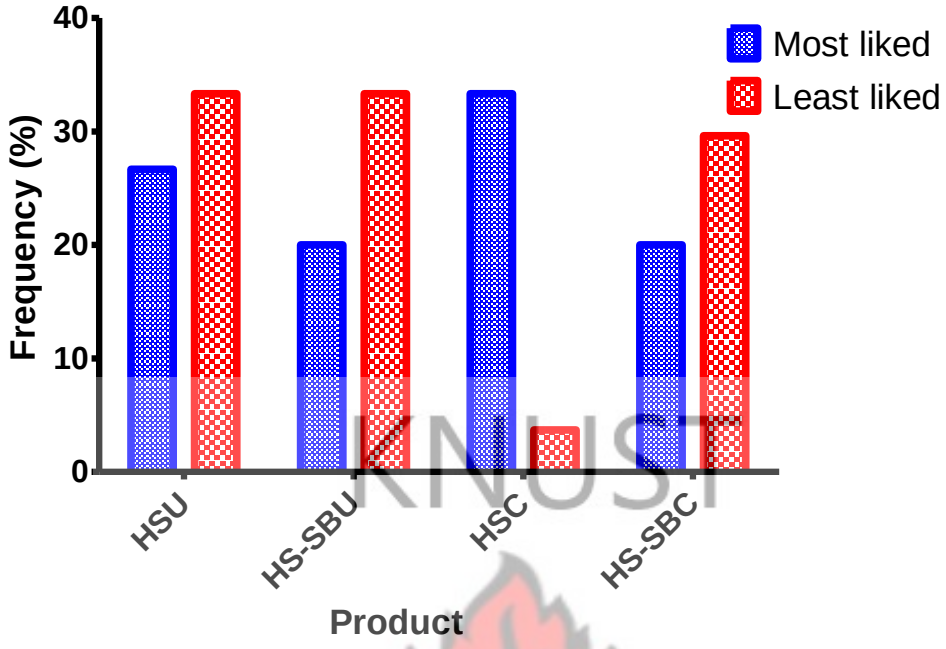

Fig. 11: Most preferred and least preferred wines

Panelists' comments on the most preferred wines were the attractive colour, smooth taste and harmonious blend of alcohol, aroma, sweetness and acidity in those wine samples felt in the mouth during tasting. However, panelists least preferred HSU and HS-SBU wines because of a sharp aroma and taste.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1: CONCLUSION

The study showed *H.sabdariffa* calyces and *S. bicolor* leaves to be good sources of nutrients. Both contained appreciable amounts of nutrients, with*S.bicolor* leaves containing high amounts of crude fibre than *H.sabdariffa* calyces. The response surface methodology established that 100% *H.sabdariffa* calyx extract and a blend of 75% *H.sabdariffa* calyx plus 25% *S. bicolor* leaf extractswith a fermentation time of 7.75 days were found to be the optimal conditions for the wine production.

The composition of the low alcohol rose wines produced from *H.sabdariffa* calyces and blends of S.*bicolor* leaves and *H.sabdariffa* calyces showed acceptable physicochemical properties. At the end of week 20, alcohol contents of 4.44%, 4.25%, 4.38% and 4.11% were obtained for HSU, HSC, HS-SBU and HS-SBC wines respectively, all of which fall within the internationally acceptable range of 1.2 – 5.5% for low alcohol wines. The pHs of the wines prepared were in the acceptable pH range of 3-4 for wines. HSU, HSC, HS-SBU and HS-SBC wines had titratable acidities (TA) of 8.10 g/l, 8.57 g/l, 8.30 g/l and 8.40 g/l respectively. These levels were in the normal range of 7-9 g/l for wine titratable acidity.

Acceptable low alcohol rose wines were prepared from *H.sabdariffa* calyces and blends of *H.sabdariffa* calyces and *S.bicolor* leaves based on panel responses. Although there were no significant differences in the sensory attributes of the wines produced, clarified wine made from *H.sabdariffa* calyces extracts (HSC) had the most preferred sensory qualities in terms of colour, aroma, taste, sweetness, acidity and overall acceptability.

5.2: RECOMMENDATION

It is recommended that:

Different fining agents aside egg albuminsuch as gelatin and Polyvinyl polypyrrolidone (PVPP) should be used in the clarification process to determine the fining agent that has a minimal effect on the loss of phenolic and anthocyanin contents of the wines.

Comprehensive analysis should be done on the residues of the leaves and calyces after the extracts have been taken out to identify the potential use of the residues in bioconversion and waste technology applications such as substrates for mushroom and food enzyme production.

Sensory evaluation should be carried out on the most preferred test wine which is HSC,using a commercial wine with similar sensory characteristics such as colour and alcohol content as a reference wine to identify the competitiveness of HSC with other accepted commercial wines on the market.

The effect of mycoderma should be monitored and investigated to identify its effect on wines during aging.

Further studies should be carried out on *H.sabdariffa* calyces and *S. bicolor* leaves to determine and quantify the anthocyanin and phenols present and their antioxidant activities in the wine.

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APPENDICES

APPENDIX A: Nutritional composition of *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf.

Calculations for proximate composition

Moisture

Weight of crucible = W1

Weight of crucible and wet sample = W2

Weight of crucible and dry sample = W3

$$\% \text{ Moisture} = \frac{W2-W3}{W2-W1} * 100\%$$

SD: Standard deviation

***Hibiscus sabdariffa* calyx**

	1	2	3
W1	34.47	37.09	36.83
W2	36.47	39.09	38.83
W3	36.27	38.89	38.63
% Moisture	9.80	10.12	10.16
Average =10.03%		**SD = 0.20**	

***Sorghum bicolor* leaf**

	1	2	3
W1	33.70	24.37	23.63
W2	35.70	26.34	25.63
W3	35.53	26.17	25.46
% Moisture	8.26	8.50	8.49
Average=8.42%		**SD= 0.14**	

Ash content

Weight of crucible = A1

Weight of crucible and sample = A2

Weight of crucible and ashed sample = A3

% Moisture = $\frac{A3-A1}{A2-A1} * 100\%$

***Hibiscus sabdariffa* calyx**

	1	2	3
A1	31.59	29.28	32.41
A2	33.59	31.29	34.41
A3	31.77	29.45	32.57
% Ash	8.92	9.19	8.23
Average = 8.92%		**SD = 0.50**	

***Sorghum bicolor* leaf**

	1	2	3
A1	33.18	33.40	30.44
A2	35.19	33.41	32.45
A3	33.34	33.54	30.59
% Ash	7.58	7.24	7.18
Average =7.33%		**SD= 0.22**	

Protein

% Protein = $\frac{100*N(HCl)*0.01401*F*(V-V1)*100}{(100*\text{Weight of sample})}$

N(HCl) = 0.0957

Weight of roselle calyx (RP) = 2.9180g

Weight of Sorghum bicolor leaf (SP) = 2.9180g

F = factor used = 6.25

V = titre value of sample

V1 = titre value of blank = 0.15mL

***Hibiscus sabdariffa* calyx**

	1	2
Initial volume	10.40	12.70
Final volume	12.70	14.90
Volume used	2.30	2.20
% Protein	6.03	5.75
Average = 5.89%		**SD= 0.20**

***Sorghum bicolor* leaf**

	1	2
Initial volume	9.50	6.80
Final volume	10.40	7.50
Volume used	0.90	0.7
% Protein	2.15	1.58
Average=1.87		**SD= 0.41**

Crude fibre

$$\% \text{ Crude fibre} = \frac{(C1 - C2)}{(C3)} * 100$$

C1= Weight of dried sample

C2 = Weight of ashed sample

C3 = Weight of defatted sample

***Hibiscus sabdariffa* calyx**

	1	2	3
C1	22.09	33.30	30.21
C2	21.92	33.12	30.00
C3	2.05	2.06	2.14
% Crude fibre	8.42	8.92	9.36
Average = 8.90%		**SD= 0.47**	

***Sorghum bicolor* leaf**

	1	2	3
C1	30.48	32.97	33.95
C2	29.82	32.39	33.38
C3	2.14	2.03	2.11
% Crude fibre	30.95	28.56	26.79
Average=28.77%		**SD= 2.09**	

Fat

$$\% \text{ Fat} = \frac{F2-F1}{F3} * 100$$

F1 = Weight of flask

F2 = Weight of flask and sample

F3 = Weight of sample

Hibiscus sabdariffa calyx

	1	2	3
F1	120.01	118.39	120.84
F2	120.02	118.40	120.85
F3	2.05	2.06	2.14
% Fat	0.39	0.44	0.33
Average = 0.38%		**SD= 0.06**	

Sorghum bicolor leaf

	1	2	3
F1	119.49	177.73	122.09
F2	119.49	177.73	122.09
F3	2.14	2.03	2.11
% Fat	0.08	0.05	0.14
Average= 0.08%		**SD= 0.05**	

Carbohydrate

Carbohydrate = 100 – (M + A + P + F + C)

M = Moisture

A = Ash

P = Protein

Fat = Fat

C = Crude fibre

Energy

Energy (kJ) = (17* Protein) + (37* Fat) + (16* carbohydrate)

MINERAL ANALYSIS

Calculation for Zn, Mn, K, Na, Fe, Ca, Mg (mg/Kg) = $\frac{\text{Weight of sample result (mg)}}{\text{Weight taken(g)}}$ *100

Calculation for phosphorus

P_2O_5 % = $\frac{(A * 100ml*100\%)}{(B*10ml*1000)}$

A: Weight of sample result (mg)

B: Weight of sample taken (g)

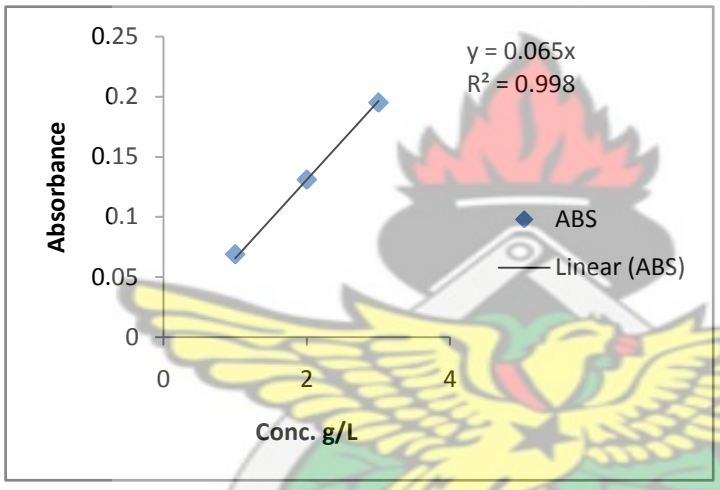

Fig. 1: Calibration curve for potassium

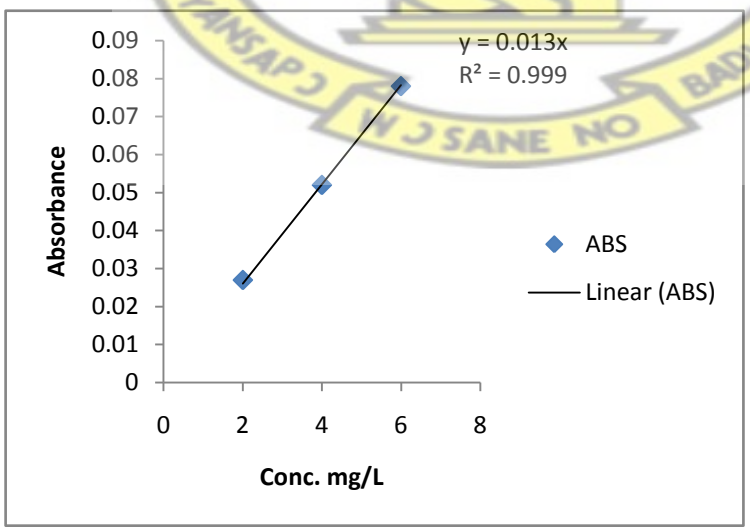

Fig. 2: Calibration curve for calcium

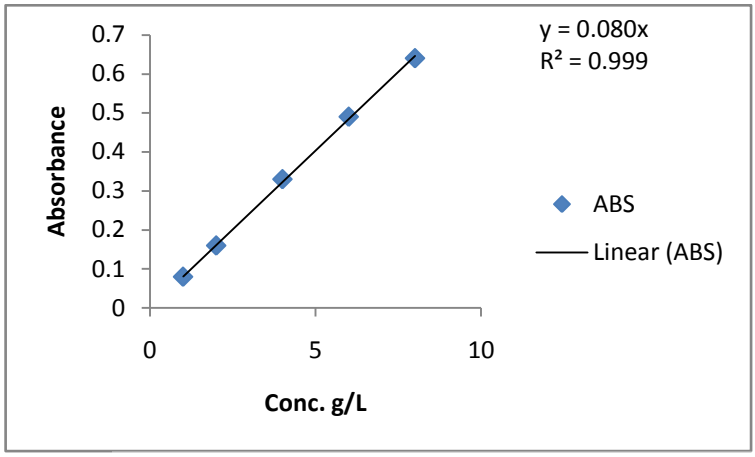

Fig. 3: Calibration curve for phosphorus

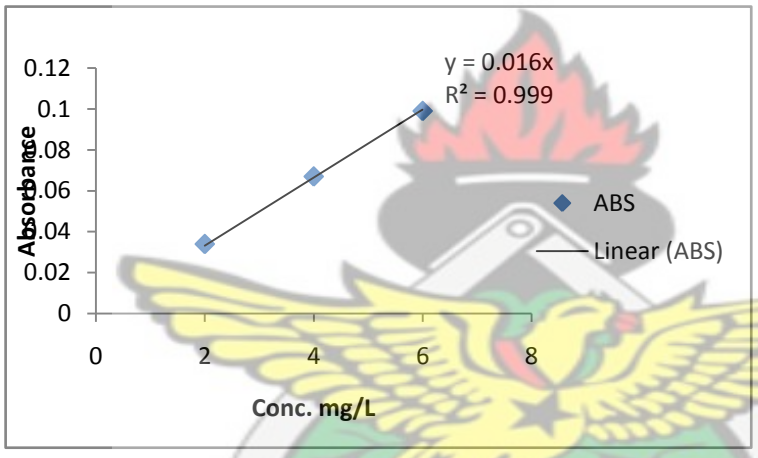

Fig. 4: Calibration curve for iron

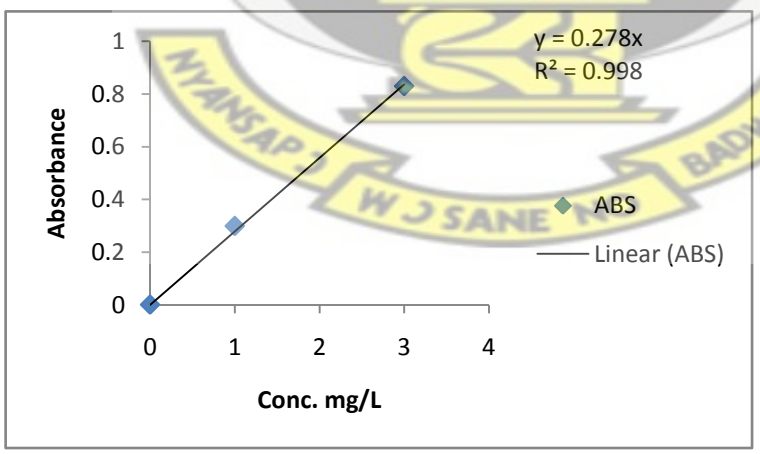

Fig. 5: Calibration curve for magnesium

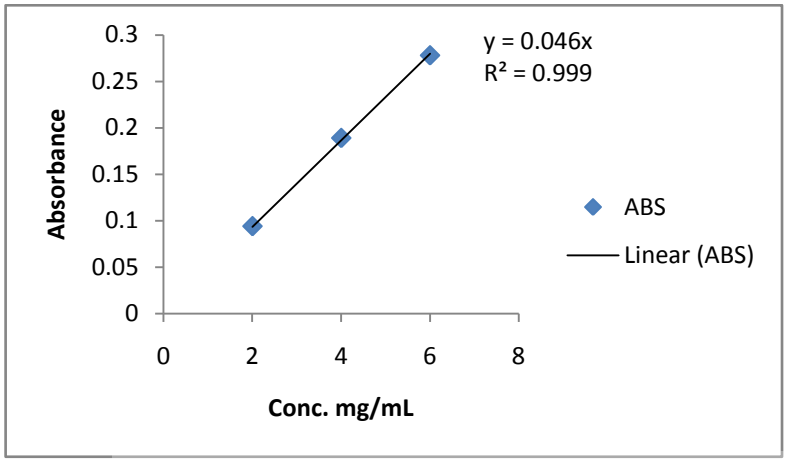

Fig. 6: Calibration curve for manganese

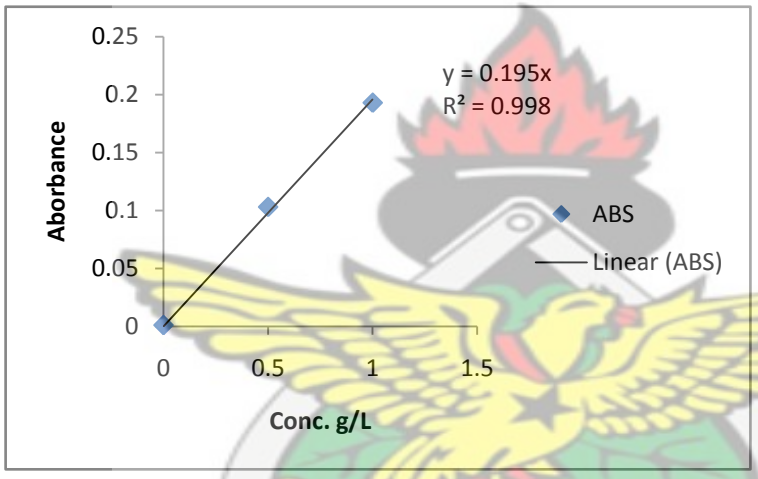

Fig. 7: Calibration curve for sodium

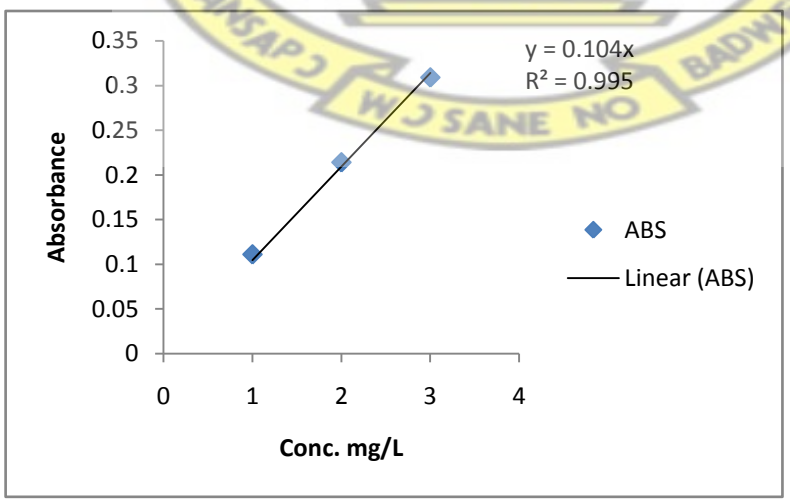

Fig. 8: Calibration curve for zinc

APPENDIX B: Analysis of variance and regression coefficients for parameters under response surface methodology.

Table 1: 28 runs developed for the various responses

Run	F1	F2	R1	R 2	R3	R4	R5	R6	R7	R8	R8
	FT	EP	Alc.	pH	TA	TSS	Den.	TCD	TP	TRP	Vit.C
	Days		%		%	^{o}Brix	g/ml	AU	AU	AU	mg/ml
1	7	H10S0	4.08	4.42	0.75	11.71	1.00	15.24	92.33	27.45	0.015
2	7	H10S0	0.52	4.4	0.71	17.74	0.94	17.89	100.91	47.88	0.016
3	7.75	H10S0	3.49	4.03	0.97	12.71	0.97	15.53	88.08	33.13	0.039
4	9.25	H10S0	7.22	4.25	0.79	6.62	0.98	15.38	109.03	63.65	0.004
5	10	H10S0	5.32	4.4	1.01	9.62	1.05	14.12	69.54	45.43	0.002
6	10	H10S0	5.32	4.4	1.01	9.62	1.05	14.12	69.54	45.43	0.002
7	8.5	H10S0	5.98	2.94	0.88	2.94	1.03	16.74	52.79	19.45	0.004
8	7	H7.5S2.5	4.08	4.3	0.79	11.71	0.98	14.10	74.48	29.42	0.019
9	7	H7.5S2.5	4.08	4.3	0.79	11.71	1.04	14.97	74.49	29.92	0.019
10	7.75	H7.5S2.5	5.27	2.87	0.98	9.72	1.00	8.62	49.51	18.34	0.010
11	9.25	H7.5S2.5	5.32	4.32	0.95	9.62	0.99	20.54	91.73	52.41	0.013
12	10	H7.5S2.5	7.81	4.33	0.71	5.62	1.03	11.26	57.98	19.07	0.011
13	10	H7.5S2.5	6.62	4.29	0.64	7.62	1.03	14.31	50.30	18.18	0.009
14	8.5	H7.5S2.5	5.44	2.88	0.75	9.62	1.06	10.75	65.99	27.29	0.019
15	7	H5S5	4.20	4.25	1.05	11.62	0.98	9.80	36.21	15.37	0.019
16	7	H5S5	4.20	4.22	0.86	11.62	1.03	9.24	172.57	75.65	0.029
17	7.75	H5S5	6.03	2.98	0.98	8.62	0.94	9.85	41.33	13.84	0.008
18	9.25	H5S5	5.68	4.44	0.58	9.62	1.06	6.91	32.39	11.89	0.009
19	10	H5S5	4.49	4.49	0.86	11.00	0.98	10.06	44.475	15.83	0.003
20	10	H5S5	4.79	4.57	0.96	10.62	0.88	8.72	59.43	23.24	0.004
21	8.5	H5S5	7.81	4.35	0.9	5.62	1.00	8.02	54.85	25.38	0.002
22	7	H2.5S7.5	3.32	3.03	0.83	13.00	1.03	13.53	25.035	10.54	0.003
23	7	H2.5S7.5	3.02	3.07	0.77	13.62	0.94	5.03	27.68	10.95	0.003
24	7.75	H2.5S7.5	5.44	3.15	0.9	9.62	1.03	4.78	24.57	9.56	0.004
25	9.25	H2.5S7.5	5.39	4.53	0.97	9.51	0.87	4.23	27.72	12.54	0.002
26	10	H2.5S7.5	4.20	4.37	0.83	11.62	0.94	7.34	37.49	12.15	0.002
27	10	H2.5S7.5	4.20	4.59	0.83	11.62	0.95	6.70	51.74	14.91	0.004
28	8.5	H2.5S7.5	8.40	4.48	0.75	4.62	0.99	3.87	37.62	24.75	0.009

Legend

EP: Extract proportion **FT**: Fermentation time **Alc**.: Alcohol
TA: Titratable acidity **TSS**: Total soluble solids **Den**.: Density
TCD: Total colour density **TRP**: Total red pigment **TP**: Total phenols
Vit. C: Vitamin C **H**: H.sabdariffa calyx extract **S**: Sorghum bicolor leaf extract

Table 1a: Analysis of variance for alcohol

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:Fermentation time(days)	15.9593	1	15.9593	17.88	0.0029
B: Extract	0.156628	1	0.156628	0.18	0.6863
AA	19.8077	1	19.8077	22.20	0.0015
AB	5.95021	1	5.95021	6.67	0.0325
BB	3.53688	1	3.53688	3.96	0.0817
Lack-of-fit	20.0826	14	1.43447	1.61	0.2534
Pure error	7.13902	8	0.892378		
Total (corr.)	72.6324	27			
R^2 = 62.52%					

Table 1b: Regression coefficients for alcohol

Coefficient	Estimate
Constant	-57.6828
A: Fermentation time (Days)	14.9175
B:Extract	-0.48157
AA	-0.890993
AB	0.137134
BB	-0.0568658

Table 2a: Analysis of variance for pH

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:Fermentation time(days)	1.80817	1	1.80817	487.87	0.0000
B: Extract	0.0580179	1	0.0580179	15.65	0.0042
AA	1.42985	1	1.42985	385.79	0.0000
AB	1.33347	1	1.33347	359.79	0.0000
BB	0.0100321	1	0.0100321	2.71	0.1385
Lack-of-fit	5.72282	14	0.408773	110.29	0.0000
Pure error	0.02965	8	0.00370625		
Total (corr.)	10.392	27			
R^2 = 44.64%					

Table 2b: Regression coefficients for pH

Coefficient	Estimate
Constant	15.5315
A: Fermentation time (Days)	-3.45256
B:Extract	0.60595
AA	0.239388
AB	-0.0649185
BB	-0.00302857

Table 3a: Analysis of variance for density

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:Fermentation time(days)	0.000183489	1	0.000183489	0.11	0.7507
B: Extract	0.00964427	1	0.00964427	5.68	0.0443
AA	0.000390456	1	0.000390456	0.23	0.6443
AB	0.0133072	1	0.0133072	7.84	0.0232
BB	0.00167896	1	0.00167896	0.99	0.3490
Lack-of-fit	0.0293617	14	0.00209727	1.24	0.3939
Pure error	0.0135731	8	0.00169664		
Total (corr.)	0.0681392	27			
R^2 = 36.99%					

Table 3b: Regression coefficients for density

Coefficient	Estimate
Constant	0.99461
A: Fermentation time (Days)	0.0245892
B:Extract	-0.0329968
AA	-0.00395588
AB	0.00648516
BB	-0.00123897

Table 4a: Analysis of variance for total colour density

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:Fermentation time(days)	4.49375	1	4.49375	0.78	0.4022
B: Extract	353.715	1	353.715	61.60	0.0001
AA	11.4931	1	11.4931	2.00	0.1949
AB	0.147278	1	0.147278	0.03	0.8767
BB	0.246657	1	0.246657	0.04	0.8410
Lack-of-fit	120.713	14	8.62239	1.50	0.2869
Pure error	45.9403	8	5.74254		
Total (corr.)	536.749	27			
R^2 = 68.95%					

Table 4b: Regression coefficients for total colour density

Coefficient	Estimate
Constant	54.7466
A: Fermentation time (Days)	-12.0058
B:Extract	1.27593
AA	0.678697
AB	0.0215748
BB	-0.0150171

Table 5a: Analysis of variance for total red pigment.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:Fermentation time(days)	22.5344	1	22.5344	0.09	0.7748
B: Extract	2357.34	1	2357.34	9.17	0.0164
AA	23.4942	1	23.4942	0.09	0.7702
AB	157.49	1	157.49	0.61	0.4564
BB	0.144289	1	0.144289	0.00	0.9817
Lack-of-fit	3268.91	14	233.494	0.91	0.5827
Pure error	2057.38	8	257.173		
Total (corr.)	7887.29	27			
R^2 = 32.47%					

Table 5b: Regression coefficients for total red pigment

Coefficient	Estimate
Constant	119.282
A: Fermentation time (Days)	-21.6517
B:Extract	-2.85767
AA	0.97037
AB	0.705511
BB	0.0114857

Table 6a: Analysis of variance for total phenols.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:Fermentation time(days)	1005.31	1	1005.31	0.84	0.3863
B: Extract	8244.25	1	8244.25	6.88	0.0305
AA	606.988	1	606.988	0.51	0.4967
AB	268.842	1	268.842	0.22	0.6483
BB	300.154	1	300.154	0.25	0.6301
Lack-of-fit	8010.09	14	572.15	0.48	0.8916
Pure error	9579.44	8	1197.43		
Total (corr.)	28015.1	27			
R^2 = 37.21%					

Table 6b: Regression coefficients for total phenols

Coefficient	Estimate
Constant	349.283
A: Fermentation time (Days)	-83.0697
B:Extract	20.5224
AA	4.93227
AB	-0.921778
BB	-0.523857

Table 7a: Analysis of variance for vitamin C

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:Fermentation time(days)	0.000602623	1	0.000602623	87.37	0.0000
B: Extract	0.000254368	1	0.000254368	36.88	0.0003
AA	2.81832E-9	1	2.81832E-9	0.00	0.9844
AB	0.000134225	1	0.000134225	19.46	0.0023
BB	0.000159795	1	0.000159795	23.17	0.0013
Lack-of-fit	0.00112299	14	0.0000802133	11.63	0.0008
Pure error	0.0000551776	8	0.00000689721		
Total (corr.)	0.00232918	27			

R^2 = 49.42%

Table 7b: Regression coefficients for vitamin C

Coefficient	Estimate
Constant	-0.0109769
A: Fermentation time (Days)	0.00039401
B:Extract	0.0113924
AA	-0.000010628
AB	-0.000651319
BB	-0.000382229

Table 8: Optimum point and nature of response

Response	Optimum point	Nature of optimum
Alcohol	5.0	Set at 5%
Density	1.00	Minimum
pH	3.82	Set at 3.3
Phenolics	80.30	Maximum
TCD	14.81	Minimum
TRP	35.90	Maximum

APPENDIX C: T-test on physicochemical properties of must and fermented must of *Hibiscus sabdariffa* calyx and *Sorghum bicolor* leaf.

Legend

HS: *Hibiscus sabdariffa* calyx

HS-SB: *Sorghum bicolor* leaf

Table 1: Summary statistics for total soluble solids

	HS Must	HS Fermented must
Count	3.00	3.00
Average	18.62	15.62
Standard deviation	0.00	0.50
Coefficient of variation	0.00%	3.20%
Minimum	18.62	15.12
Maximum	18.62	16.12
Range	0.00	1.00
Stnd. skewness		0.00
Stnd. Kurtosis		
Alpha = 0.05,	**p-value= 0.00**	

Table 2: Summary statistics for density

College	HS Must	HS Fermented Must
Count	3.00	3.00
Average	1.04	1.03
Standard deviation	0.00	0.00
Coefficient of variation	0.01%	0.00%
Minimum	1.04	1.03
Maximum	1.04	1.03
Range	0.00	0.00
Stnd. Skewness	0.28	1.19
Stnd. kurtosis		3.00
Total	**Alpha = 0.05**	**p-value=7.50E-10**

Table 3: Summary statistics for titratable acidity

College	HS Must	HS Fermented Must
Count	3.00	3.00
Average	7.08	7.18
Standard deviation	0.04	0.04
Coefficient of variation	0.61%	0.60%
Minimum	7.05	7.13
Maximum	7.13	7.20
Range	0.08	0.08
Stnd. Skewness	1.22	-1.22
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.0474201**

Table 4: Summary statistics for total red pigment

	HS Must	HS Fermented Must
Count	3.00	3.00
Average	6.39	6.31
Standard deviation	0.04	0.02
Coefficient of variation	0.55%	0.32%
Minimum	6.35	6.29
Maximum	6.42	6.33
Range	0.07	0.04
Stnd. Skewness	-0.30	0.00
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.03**

Table 5: Summary statistics for total phenols

	HS Must	HS Fermented Must
Count	3.00	3.00
Average	21.06	18.21
Standard deviation	0.12	0.17
Coefficient of variation	0.57%	0.91%
Minimum	20.96	18.06
Maximum	21.19	18.39
Range	0.23	0.33
Stnd. Skewness	0.89	0.44
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

Table 6: Summary statistics for pH

	HS Must	HS Fermented Must
Count	3.00	3.00
Average	3.65	3.61
Standard deviation	0.01	0.01
Coefficient of variation	0.16%	0.16%
Minimum	3.65	3.61
Maximum	3.66	3.62
Range	0.01	0.01
Stnd. Skewness	1.22	1.22
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

Table 7:Summary statistics for total colour density

	HS Must	HS Fermented Must
Count	3.00	3.00
Average	1.97	1.36
Standard deviation	0.01	0.09
Coefficient of variation	0.29%	6.62%
Minimum	1.95	1.29
Maximum	1.96	1.46
Range	0.01	0.17
Stnd. Skewness	-0.17	0.99
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

Table 8: Summary statistics for pH

	HS-SB Must	HS-SB Fermented Must
Count	3.00	3.00
Average	3.70	3.64
Standard deviation	0.01	0.01
Coefficient of variation	0.16%	0.16%
Minimum	3.69	3.63
Maximum	3.70	3.64
Range	0.01	0.01
Stnd. Skewness	-1.22	-1.22
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

Table 9: Summary statistics for total soluble solids

	HS-SB Must	HS-SB Fermented Must
Count	3.00	3.00
Average	18.62	14.62
Standard deviation	0.00	0.50
Coefficient of variation	0.00%	3.42%
Minimum	18.62	14.12
Maximum	18.62	15.12
Range	0.00	1.00
Stnd. Skewness		0.00
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

Table 10: Summary statistics for density

	HS-SB Must	HS-SB Fermented Must
Count	3.00	3.00
Average	1.06	1.02
Standard deviation	0.00	0.00
Coefficient of variation	0.00%	0.08%
Minimum	1.06	1.02
Maximum	1.06	1.02
Range	0.00	0.00
Stnd. Skewness	0.66	-1.13
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 2.41E-7**

Table 11:Summary statistics for titratable acidity

	HS-SB Must	HS-SB Fermented Must
Count	3.00	3
Average	7.06	7.30
Standard deviation	0.04	0.04
Coefficient of variation	0.61%	0.59%
Minimum	7.05	7.28
Maximum	7.13	7.35
Range	0.08	0.08
Stnd. Skewness	1.22	1.22
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

Table 12:Summary statistics for total colour density

	HS-SB Must	HS-SB Fermented Must
Count	3.00	3.00
Average	1.71	1.28
Standard deviation	0.00	0.03
Coefficient of variation	0.05%	2.67%
Minimum	1.70	1.24
Maximum	1.71	1.31
Range	0.00	0.07
Stnd. Skewness	-1.22	-0.79
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

Table 13:Summary statistics for total red pigment

	HS-SB Must	HS-SB Fermented Must
Count	3.00	3.00
Average	6.07	5.33
Standard deviation	0.11	0.06
Coefficient of variation	1.78%	1.07%
Minimum	5.98	5.28
Maximum	6.19	5.39
Range	0.21	0.11
Stnd. Skewness	0.81	0.85
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

Table 14: Summary statistics for total phenols

	HS-SB Must	HS-SB Fermented Must
Count	3.00	3.00
Average	19.23	16.64
Standard deviation	0.31	0.66
Coefficient of variation	1.63%	3.96%
Minimum	19.01	16.19
Maximum	19.59	17.40
Range	0.58	1.21
Stnd. Skewness	1.14	1.15
Stnd. kurtosis		
Total	**Alpha = 0.05**	**p-value= 0.00**

APPENDIX D: Multifactor ANOVA for physico-chemical properties during aging.

Legend

HSU: Unclarified wine from *Hibiscus sabdariffa* calyx extract

HSC: Clarified wine from *Hibiscus sabdariffa* calyx extract

HS-SBU: Unclarified wine from blends of *Hibiscus sabdariffa* calyx extract and *Sorghum bicolor* leaf.

HS-SBC: Clarified wine from blends of *Hibiscus sabdariffa* calyx extract and *Sorghum bicolor* leaf.

Table 1a: Analysis of variance (ANOVA) for the effect of aging on Total soluble solids

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	590.006	19	31.053	165.31	0.0000
Within groups	7.32593	39	0.187844		
Total (Corr.)	597.332	58			

Table 1b: Multiple Range Tests for Total soluble solids using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HSC-wk.20	3	3.61333	X
HS-SBU-wk.20	3	3.63333	X
HS-SBU-wk.20	3	11.12	X
HSU-wk.20	3	11.2267	X
HSC-wk.15	3	11.6733	XX
HS-SBC-wk.15	3	11.78	XXX
HSC-wk.10	3	12.12	XXX
HSU-wk.15	3	12.28	XXXX
HS-SBC-wk.10	3	12.62	XXX
HS-SBC-wk.05	3	12.78	XXXX
HSC-wk.05	3	13.12	XXXX
HSU-wk.10	3	13.62	XXXX
HS-SBU-wk.10	3	14.12	XXX
HSU-wk.05	3	14.28	XXX
HS-SBU-wk.05	3	14.28	XXX
HSC-wk.0	3	14.28	XXX
HSU-wk.0	2	14.53	XXX
HS-SBC-wk.0	3	14.6133	XX

Table 2a: Analysis of variance (ANOVA) for the effect of aging on density

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00818902	19	0.000431001	12.45	0.0000
Within groups	0.00138499	40	0.0000346247		
Total (Corr.)	0.00957401	59			

Table 2b: Multiple Range Tests for density using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HS-SBC-wk20	3	0.988435	X
HSC-wk20	3	0.98848	XX
HS-SBU-wk20	3	0.993936	XXX
HSU-wk20	3	0.99791	XXXX
HS-SBC-wk15	3	1.00438	XXXXX
HS-SBU-wk10	3	1.00664	XXXXX
HS-SBU-wk15	3	1.00718	XXXX
HSU-wk15	3	1.00749	XXXX
HSC-wk15	3	1.01097	XXXXX
HSU-wk10	3	1.01332	XXXX
HS-SBC-wk10	3	1.01558	XXXX
HS-SBC-wk05	3	1.01614	XXX
HSC-wk10	3	1.01836	XXX
HS-SBU-wk05	3	1.01841	XXX
HSC-wk05	3	1.0186	XXX
HS-SBC-wk0	3	1.02151	XXX
HS-SBU-wk0	3	1.0222	XXX
HSU-wk05	3	1.02329	XX

Table 3a: Analysis of variance (ANOVA) for the effect of aging on alcohol

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	37.0339	19	1.94915	20.04	0.0000
Within groups	3.79303	39	0.0972571		
Total (Corr.)	40.8269	58			

Table 3b: Multiple Range Tests for alcohol using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HSU-wk0	3	1.776	X
HSC-wk0	3	1.776	X
HS-SBC-wk0	3	2.368	XX
HS-SBU-wk0	3	2.368	XX
HSU-wk05	3	2.37195	XX
HS-SBC-wk05	3	2.56928	XXX
HS-SBU-wk05	3	2.56928	XXX
HS-SBU-wk10	3	2.664	XXXX
HSU-wk10	3	2.66795	XXXX
HSC-wk05	3	2.79819	XXXX
HS-SBC-wk10	3	2.96	XXXXX
HSC-wk10	2	3.108	XXXXXX
HSU-wk15	3	3.45728	XXXXX
HSC-wk15	3	3.552	XXXXX
HS-SBC-wk15	3	3.75328	XXXX
HS-SBU-wk15	3	3.848	XXX
HSU-wk20	3	4.04928	XX
HS-SBU-wk20	3	4.11243	XX

Table 4a: Analysis of variance (ANOVA) for the effect of aging on volatile acidity

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	0.00101867	19	0.000053614	0.31	0.9958
Within groups	0.00685867	40	0.000171467		
Total (Corr.)	0.00787733	59			

Table 4b: Multiple Range Tests for volatile acidity using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HS-SBU-wk0	3	0.056	X
HS-SBU-wk20	3	0.056	X
HS-SBC-wk0	3	0.056	X
HS-SBU-wk05	3	0.0586667	X
HSU-wk0	3	0.06	X
HS-SBC-wk05	3	0.0613333	X
HSU-wk05	3	0.0613333	X
HS-SBC-wk10	3	0.064	X
HSC-wk05	3	0.064	X
HSC-wk20	3	0.064	X
HSC-wk0	3	0.064	X
HSU-wk20	3	0.064	X
HS-SBU-wk10	3	0.064	X
HSC-wk10	3	0.0666667	X
HS-SBC-wk20	3	0.0666667	X
HSU-wk15	3	0.0666667	X
HSC-wk15	3	0.068	X

HS-SBU-wk15	3	0.068	X

Table 5a: Analysis of variance (ANOVA) for the effect of aging on Total phenols

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	538.59	19	28.3469	140.59	0.0000
Within groups	8.06489	40	0.201622		
Total (Corr.)	546.655	59			

Table 5b: Multiple Range Tests for total phenols using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HS-SBC-wk20	3	7.365	X
HSC-wk20	3	8.19	XX
HS-SBU-wk20	3	9.15	XX
HS-SBC-wk15	3	9.62	X
HSU-wk20	3	10.1633	X
HSC-wk15	3	10.35	X
HS-SBC-wk10	3	12.0083	X
HS-SBU-wk15	3	12.4067	XX
HS-SBC-wk05	3	12.5517	XXX
HSU-wk15	3	13.73	XXX
HS-SBU-wk10	3	13.8478	XX
HS-SBC-wk0	3	13.9667	X
HS-SBU-wk05	3	14.3778	XX
HSC-wk10	3	14.3783	XX
HSU-wk10	3	14.5089	XX
HSC-wk05	3	15.6083	XX
HSC-wk0	3	16.5833	X
HS-SBU-wk0	3	16.6433	X

Table 6a: Analysis of variance (ANOVA) for the effect of aging on Total red pigment

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	52.7001	19	2.77369	229.22	0.0000
Within groups	0.484028	40	0.0121007		
Total (Corr.)	53.1842	59			

Table 6b: Multiple Range Tests for total red pigment using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HS-SBC-wk20	3	2.9	X
HSC-wk20	3	3.03667	X
HS-SBU-wk20	3	3.09667	X
HSU-wk20	3	3.20667	X
HS-SBC-wk15	3	3.59833	X
HSC-wk15	3	3.93	XX
HSU-wk15	3	4.00667	XX
HS-SBU-wk15	3	4.00667	XX
HS-SBC-wk10	3	4.075	XXX
HS-SBC-wk05	3	4.22333	XXXX
HS-SBU-wk10	3	4.27111	XXX
HSC-wk10	3	4.39833	XX
HS-SBU-wk05	3	4.52333	XX
HS-SBC-wk0	3	4.84	XX
HSU-wk10	3	4.94111	X
HS-SBU-wk0	3	5.32667	X
HSC-wk05	3	5.44333	XX
HSU-wk05	3	5.54889	XX

Table 7a: Analysis of variance (ANOVA) for the effect of aging on Total colour density

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	2.74076	19	0.144251	25.97	0.0000
Within groups	0.222167	40	0.00555417		
Total (Corr.)	2.96293	59			

Table 7b: Multiple Range Tests for total colour density using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HS-SBC-wk20	3	0.630383	X
HSC-wk20	3	0.668033	XX
HS-SBU-wk20	3	0.677933	XXX
HS-SBC-wk15	3	0.6809	XXX
HS-SBC-wk10	3	0.718611	XXX
HSC-wk15	3	0.7608	XXXX
HSC-wk10	3	0.7783	XXXX
HSU-wk20	3	0.885067	XXXX
HS-SBU-wk15	3	0.905033	XXXX
HSU-wk15	3	0.954867	XXX
HS-SBU-wk10	3	0.959133	XXX
HS-SBC-wk05	3	1.02157	XXX
HSU-wk10	3	1.06721	XXXX
HS-SBU-wk05	3	1.08937	XXXX
HSC-wk05	3	1.1049	XXXX
HSU-wk05	3	1.11679	XXX
HS-SBC-wk0	3	1.12433	XXX
HS-SBU-wk0	**3**	**1.20303**	**XXX**

Table 8a: Analysis of variance (ANOVA) for the effect of aging on Titratable acidity

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	9.8673	19	0.519332	103.33	0.0000
Within groups	0.201042	40	0.00502604		
Total (Corr.)	10.0683	59			

Table 8b: Multiple Range Tests for Titratable acidity using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HSC-wk0	3	7.15	X
HSU-wk0	3	7.175	XX
HSU-wk05	3	7.2	XXX
HS-SBC-wk0	3	7.275	XXXX
HS-SBU-wk0	3	7.3	XXXXX
HSU-wk10	3	7.35833	XXXXXX
HS-SBC-wk05	3	7.3875	XXXXXX
HS-SBU-wk05	3	7.41667	XXXXX
HSU-wk15	3	7.425	XXXX
HSC-wk05	3	7.4625	XXXX
HS-SBU-wk10	3	7.48333	XXXX
HS-SBC-wk10	3	7.5	XXX
HSC-wk10	3	7.55	XXX
HSC-wk15	3	7.575	XXX
HS-SBC-wk15	3	7.6	XX
HS-SBU-wk15	3	7.75	X
HSU-wk20	3	8.1	X
HS-SBU-wk20	3	8.3	XX

Table 9a: Analysis of variance (ANOVA) for the effect of aging on pH

Source	Sum of squares	Df	Mean Square	F-Ratio	P-Value
Between groups	2.15558	19	0.113452	40.27	0.0000
Within groups	0.104241	37	0.00281732		
Total (Corr.)	2.25982	56			

Table 9b: Multiple Range Tests for pH using 95% Tukey HSD method

Product	Count	Mean	Homogenous groups
HSC-wk.20	3	3.01	X
HS-SBU-wk.20	3	3.08333	X
HS-SBU-wk.20	3	3.08333	X
HSU-wk.20	3	3.14333	XX
HSC-wk.15	3	3.29	XX
HS-SBC-wk.15	3	3.29333	XX
HSC-wk.10	2	3.315	XXX
HSU-wk.15	3	3.35	XX
HS-SBC-wk.10	2	3.37	XXX
HS-SBC-wk.05	3	3.43333	XXXX
HSC-wk.05	2	3.455	XXXXX
HSU-wk.10	3	3.48444	XXXX
HS-SBU-wk.10	3	3.52333	XXX
HSU-wk.05	3	3.55	XXX
HS-SBU-wk.05	3	3.58	XX
HSC-wk.0	3	3.61333	X
HSU-wk.0	3	3.61667	X
HS-SBC-wk.0	3	3.63333	X
HS-SBU wk. 0	3	3.63667	X

APPENDIX E: SENSORY EVALUATION

Legend

352*: H.sabdariffa* unclarified wine (HSU)

983*: H.sabdariffa* and *S.bicolor* unclarified wine (HS-SBU)

154: *H.sabdariffa* clarified wine (HSC)

604: H.sabdariffa and S.bicolor clarified wine (HS-SBC)

SENSORY FORM (ACCEPTANCE TEST)

AFFECTIVE TEST

Male/ Female……

Age 18-30___ 31-45___ 46-60___ 61-80___

In front of you are five coded products. Evaluate the samples starting from left to right. In between each product you taste, eat some biscuits to mask the taste before you taste the next product.

Legend

1: Like very much 2: Like moderately

3: Like slightly 4: Neither like nor dislike

5: Dislike slightly 6: Dislike moderately

7: Dislike very much

Sensory parameters	352	983	154	604
Colour				
Aroma				
Sweetness				
Acidity				
Alcohol content				

Comments

1. Which of the products do you like most in terms of the parameters assessed and why?

……
……
……

2. Which of the products do you like the least in terms of the parameters assessed and why?

.....
.....

RESULTS FROM SENSORY EVALUATION

Table 1: Age distribution

SEX	18-30 YEARS	31-45 YEARS	46-60 YEARS	61-80 YEARS
	(%)	(%)	(%)	(%)
MALE	60	13.33	13.33	3.33
FEMALE	6.67	3.33	0	0
TOTAL	67.67	16.67	13.33	3.33

Table 2: Results for unclarified *H.sabdariffa* wine

Product	Colour	Aroma	Sweetness	Acidity	Alcohol	Overall acceptability
352	2	5	3	1	2	2.6
352	3	2	1	1	3	2
352	1	7	3	3	5	3.8
352	6	7	1	2	1	3.4
352	2	3	1	2	2	2
352	3	2	2	3	4	2.8
352	2	5	1	4	1	2.6
352	3	5	7	5	2	4.4
352	1	2	6	2	6	3.4
352	1	5	2	2	2	2.4
352	4	2	3	5	3	3.4
352	3	3	4	3	2	3
352	5	6	4	6	7	5.6
352	2	5	4	1	6	3.6
352	3	2	4	3	1	2.6
352	2	5	3	3	2	3
352	3	2	1	2	1	1.8
352	1	3	5	5	4	3.6
352	3	3	3	5	3	3.4
352	1	6	2	2	1	2.4
352	4	5	6	6	5	5.2
352	2	4	3	3	3	3
352	5	4	3	3	3	3.6
352	4	2	2	6	6	4
352	2	7	7	5	5	5.2

352	3	2	3	3	5	3.2
352	1	2	1	1	1	1.2
352	3	2	3	4	5	3.4
352	1	1	1	3	1	1.4
352	2	3	2	2	2	2.2
352	4	3	7	7	7	5.6
352	2	3	1	3	2	2.2

Table 3: Results for unclarified *H.sabdariffa* and *S.bicolor* wine

Product	Colour	Aroma	Sweetness	Acidity	Alcohol	Overall acceptability
983	1	2	3	6	3	3
983	2	2	3	6	6	3.8
983	3	7	5	7	4	5.2
983	1	4	7	2	5	3.8
983	1	3	1	1	2	1.6
983	2	2	2	3	4	2.6
983	2	1	1	4	1	1.8
983	2	5	7	5	2	4.2
983	1	2	6	3	6	3.6
983	1	2	4	1	1	1.8
983	5	1	2	5	3	3.2
983	2	2	3	3	3	2.6
983	5	6	3	6	5	5
983	2	4	3	3	5	3.4
983	2	2	2	3	1	2
983	2	5	1	5	2	3
983	5	3	2	5	3	3.6
983	2	3	4	3	3	3
983	3	3	3	3	4	3.2
983	2	7	1	1	1	2.4
983	4	3	6	7	5	5
983	2	4	3	3	4	3.2
983	3	3	2	2	3	2.6
983	5	4	2	7	5	4.6
983	2	7	6	7	7	5.8
983	5	5	5	5	3	4.6
983	2	2	3	3	3	2.6
983	2	2	3	2	4	2.6
983	1	1	2	5	6	3
983	2	3	2	3	2	2.4
983	3	2	2	5	5	3.4
983	3	4	2	2	3	2.8

Table 4: Results for clarified *H.sabdariffa* wine

Product	Colour	Aroma	Sweetness	Acidity	Alcohol	Overall acceptability
	3	2	3	3	2	2.6
	3	4	3	6	4	4
154	1	3	3	2	2	2.2
154	2	1	6	5	1	3
154	4	3	2	3	2	2.8
154	2	2	2	5	4	3
154	1	1	1	4	1	1.6
154	3	3	7	5	2	4
154	1	3	5	3	6	3.6
154	1	2	4	7	4	3.6
154	2	3	2	5	3	3
154	3	3	3	3	3	3
154	3	6	3	6	5	4.6
154	1	4	2	6	6	3.8
154	2	3	1	3	1	2
154	2	3	2	1	2	2
154	3	3	1	2	2	2.2
154	5	4	2	3	4	3.6
154	2	3	3	2	3	2.6
154	3	3	1	1	2	2
154	4	1	4	3	5	3.4
154	2	4	3	2	6	3.4
154	3	2	2	1	2	2
154	3	7	4	7	1	4.4
154	6	6	7	6	4	5.8
154	2	5	6	5	3	4.2
154	1	2	1	2	2	1.6
154	2	2	3	5	4	3.2
154	1	1	3	4	2	2.2
154	3	3	3	3	3	3
154	2	3	2	2	4	2.6
154	2	3	1	2	1	1.8

Table 5: Results for clarified *H.sabdariffa* and *S. bicolor* wine

Product	Colour	Sweetness	Alcohol	Acidity	Alcohol	Overall acceptability
604	5	2	1	2	3	2.6
604	5	3	3	5	5	4.2
604	2	5	1	1	3	2.4
604	1	4	7	5	2	3.8
604	5	5	2	3	2	3.4
604	3	2	1	5	4	3
604	1	5	2	4	1	2.6
604	3	3	7	4	2	3.8
604	1	3	5	3	6	3.6
604	5	5	7	5	4	5.2
604	2	2	3	6	6	3.8
604	3	2	2	2	1	2
604	3	7	3	6	5	4.8
604	3	5	1	7	7	4.6
604	2	2	2	3	1	2
604	2	4	3	1	2	2.4
604	2	3	3	3	5	3.2
604	2	4	1	2	2	2.2
604	4	4	4	2	4	3.6
604	3	5	2	2	2	2.8
604	4	2	5	2	5	3.6
604	2	4	5	3	5	3.8
604	4	5	4	2	3	3.6
604	4	6	5	5	2	4.4
604	7	7	6	7	4	6.2
604	3	4	3	2	3	3
604	3	3	2	1	1	2
604	5	2	2	4	3	3.2
604	1	2	3	4	5	3
604	3	2	3	5	2	3
604	3	1	1	1	4	2
604	2	2	2	3	2	2.2

ANALYSIS OF VARIANCE FOR THE SENSORY ATTRIBUTES

Table 6:Analysis of variance (ANOVA) for colour of the wines.

Source	Sum of squares	Df	Mean square	F-Ratio	P-value
Between groups	7.625	3	2.54167	1.48	0.2238
Within groups	213.25	124	1.71976		
Total (Corr.)	220.875	127			

Table 7:Analysis of variance (ANOVA) for aroma of the wines.

Source	Sum of squares	Df	Mean square	F-Ratio	P-value
Between groups	7.71094	3	2.57031	0.97	0.4103
Within groups	329.344	124	2.656		
Total (Corr.)	337.055	127			

Table 8:Analysis of variance (ANOVA) for sweetness of the wines.

Source	Sum of squares	Df	Mean square	F-Ratio	P-value
Between groups	0.75	3	0.25	0.08	0.9721
Within groups	400.125	124	3.22681		
Total (Corr.)	400.875	127			

Table 9:Analysis of variance (ANOVA) for acidity of the wines.

Source	Sum of squares	Df	Mean square	F-Ratio	P-value
Between groups	7.21094	3	2.40365	0.77	0.5138
Within groups	387.844	124	3.12777		
Total (Corr.)	395.055	127			

Table 10:Analysis of variance (ANOVA) for alcohol of the wines.

Source	Sum of squares	Df	Mean square	F-Ratio	P-value
Between groups	5.21094	3	1.73698	0.61	0.6110
Within groups	354.219	124	2.8566		
Total (Corr.)	359.43	127			

Table 11:Analysis of variance (ANOVA) for overall acceptability of the wines.

Source	Sum of squares	Df	Mean square	F-Ratio	P-value
Between groups	1.66844	3	0.556146	0.51	0.6758
Within groups	135.089	124	1.08943		
Total (Corr.)	136.757	127			

Table 12: Most liked and least liked products.

Product	Frequency Most liked	Most liked (%)	Frequency Least liked	Least liked (%)
HSU	4	26.67	9	33.33
HS-SBU	3	20.00	9	33.33
HSC	5	3.33	1	3.70
HS-SBC	3	20.00	8	29.63
Total	**34**	**100**	**32**	**100**

APPENDIX F: FORMULA USED

Titratable acidity

Titratable acidity (TA) = 75 * N * T/ S

N = Normality

T= Titre value

S = Sample volume

75 = constant

Volatile acidity (acetic acid)

Moles of base added = Moles of acid initially present

Moles of base added = Molarity of base * volume of base used

Amount of sample used = 25 ml

Moles of acid initially present (n)= Molarity (M) mol/L* Titre value (T) ml*1L/(1000 ml)

Molar mass of acetic acid = 60g/mol

Grammes of acetic acid = 60g/mol *n mol

Grammes of acetic acid in 100 ml sample = 60g/mol*n mol*100ml

Vitamin C

Ascorbic acid (mg/ml) = (X- B) * (F/E) * (V/Y)

X = Vol. of sample for titration

B = Vol. of blank

F = Titre of dye

E = Vol. assayed (2 ml)

V = Vol. of initial assay solution (7 ml)

Y= Vol. of sample aliquot titrated (7 ml)

F = Ascorbic acid (mg) in vol. of standard solution titrated

(Average vol. of dye used to titrate standard solution) – (average vol. of dye used to titrate blank)

Phenols = Total phenolics (A.U.) = $^{HCl}A_{280}$ - 4

Wine colour density (A.U.) = $A_{420} + A_{520}$

Total red pigment (A.U.) = A_{520} *100

Density = $\frac{\text{Weight}}{\text{Volume}}$

Alcohol = 0.592* [(Initial Brix -3) – (Final Brix - 3)]

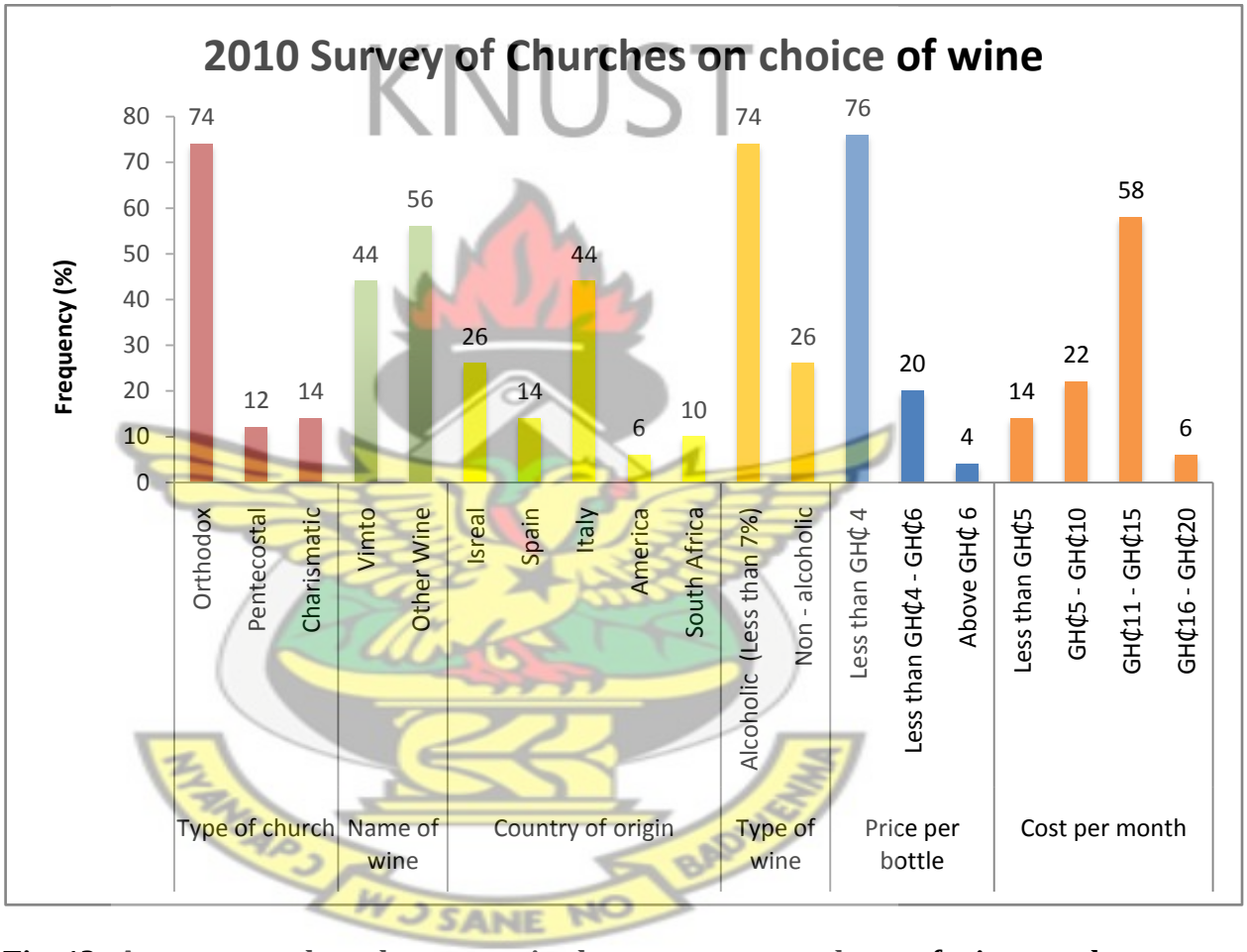

Fig. 12: A survey conducted to ascertain the source, type and cost of wines used for communion services by some churches in Kumasi metropolis.