KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE AND RENEWABLE NATURAL RESOURCE

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DEPARTMENT OF HORTICULTURE

EFFECT OF DIFFERENT LEVELS OF INCLUSION OF SUGARCANE-PINEAPPLE COCKTAILS AND PACKAGING MATERIALS ON QUALITY OF "BISSAP"; A DRINK MADE FROM *HIBISCUS SABDARIFFA*

BY

DORCAS NAA NORLEY THOMPSON

JULY, 2019

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A THESIS SUBMITTED TO THE SCHOOL OF RESEARCH AND GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY POSTHARVEST TECHNOLOGY

JULY, 2019

DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgment is made in the thesis.

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(HEAD OF DEPARTMENT) Signature Date

DEDICATION

This work is dedicated to God who sustained me throughout this study, my family and friends for their support and concerns.

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ABSTRACT

A series of laboratory experiments were conducted to evaluate the effect of different levels of inclusion of sugarcane-pineapple cocktails and packaging materials on quality of "bissap"; a drink made from dried calyx of *Hibiscus sabdariffa*. The levels of inclusion were generated with the help of Minitab 2017 software and sensory attributes of the resulting cocktails were evaluated for their flavour, taste, sourness and sweetness. The results of the study showed that, a cocktail of 30% bissap extract, 20% pineapple and 50% sugarcane juice was the overall most acceptable cocktail. A 30% bissap extract, 40% pineapple and 30% sugarcane juice was the most acceptable in terms flavour. The most highly preferred taste was from the cocktail comprising 33% bissap extract, 33% pineapple and 34% sugarcane. In terms of sourness the most accepted cocktail was 30% bissap, 20% pineapple and 50% sugarcane juice. The sweetening effect derived from the combination of 30% bissap extract, 40% pineapple and 30% sugarcane juice was the most accepted among the other formulations. The optimization analysis derived from the Minitab 2017 software used gave the most favourable product as 30% bissap extract, 38% pineapple and 32% sugarcane juice which was significantly different ($p \leq 0.05$) among the treatments over a four week storage period. The colour of the cocktail reduced in clarity from 33.78 in plastic bottle under ambient condition to 25.08, from 31.16 in glass bottle under ambient condition to 27.13, from 31.15 in plastic bottle under refrigeration to 24.98 and from 26.29 in glass bottle under refrigeration to 27.83. The total soluble acids (TSS) reduced from 10° brix to 9.82° brix in plastic bottle and from 9.68° brix to 9.18° brix in glass bottle. Total titrable acidity (TTA) dropped from 0.45% in plastic bottle under ambient condition to 0.19%, from 0.44% in glass

bottle under ambient condition to 0.25%, from 0.45% in plastic bottle under refrigeration to 0.20% and from 0.27% in glass under refrigeration to 0.18%.

The pH levels increased from 2.96 in plastic bottle under ambient condition to 3.76, from 2.90 in glass bottle under ambient condition to 3.74, from 2.88 in plastic bottle under refrigeration to 3.74 and from 2.96 in glass bottle under refrigeration to 3.68. The vitamin C content decreased from 4.37 mg/100g to 3.16 mg/100g in plastic bottle and from 3.53 mg/100g to 2.78 mg/100g in glass bottle. The total ash dropped from 1.37 % in plastic bottle under ambient condition to 0.45 %, from 2.44 % in glass bottle under ambient condition to 1.08 %, from 0.63 % in plastic bottle under refrigeration to 0.34 % and from 0.76 % in glass bottle under refrigeration to 0.55 %. After four weeks of storage of the drinks, mould and yeast counts and aerobic plate count (APC) of microbes, were significantly high ($p \leq 0.05$) in the plastic bottles under ambient condition (22°C to 28°C) but there were no moulds and yeasts in the refrigerated bottled drinks. No coliforms were present in all treatments throughout the storage period.

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CHAPTER ONE

1.0 INTRODUCTION

Over the centuries, as the first humans began to settle in one location or the other, the need for an enhancement in what they ate and drank became a necessity. That is to say, food was needed in quantity and quality. Thus, the Egyptians in 4000BC mastered viticulture and the art of wine making. Likewise the Roman Empire, who in 300BC used honey as a preserving agent for fruit. Other innovations sprang up until in 1850 till present soft drinks were developed by combining fruit juice with other components such as sugar, carbonated water and citric acid (Jorge, 2006).

In the present age however, consumers are beginning to cherish the functionality of drinks especially with plant based (fruits and vegetables) not overlooking dairy beverages (Kausar *et al*., 2012; Davoodi *et al*., 2013). Emebu and Anyika, (2011) described the liquid extracted from fresh fruits and vegetables as juice which is a great source of vitamins, minerals, natural sugars and phytochemicals enhancing the physical and mental functioning of the body. In Ghana, one can find a variety of local beverages. The beverages go by names like "pito", palm wine, "burukutu" and "akpeteshie" which are derived from local ingredients such as millet, sugary palm sap, sorghum grains and fermented sugarcane juice respectively (WHO, 2004). A notable juice amongst them is what is popularly known as "sobolo" (*Hibiscus sabdariffa*).

Hibiscus sabdariffa from the family of *Malvaceae*, is mostly cultivated in the tropical and subtropical parts of the world. It is identified by various names such as *Guinea sorrel* or *bissap* in Senegal, *karkadé* in North Africa, *roselle* or *sorrel* in Asia and *flora of Jamaica* in Central America (Cisse, 2010). From research, the calyces have been found to be abundant with acid, pectin, crude protein and minerals such as iron, calcium, manganese and so on (Gautam, 2004). Calyces of bissap contain nine times more vitamin C than *Citrus sinensis* (Amin *et al.*, 2008). Antioxidant properties from the flavonoids and anthocyanins present decrease chronic diseases such as diabetes mellitus, dyslipidemias, hypertension and cardiovascular diseases (Cid and Guerrero, 2014).

Sobolo is usually prepared in combination with ingredients such as lemon grass, pineapple, spices and sweeteners such as sugar or honey due to its astringent properties. Pineapple (*Ananas comosus*) is from the *Bromeliaceae* family which contains a proteolytic enzyme bromelain, which digests food by breaking down protein, a good source of manganese, as well as containing substantial quantities of Vitamin C. Fresh, canned or juiced are the various forms pineapples can be utilized. Pineapples are also used as dessert, fruit salad, jam, yogurt, ice cream, candy and as a complement to meat dishes (Bhakta *et al.*, 2012).

Acids generally play a significant role in influencing the taste of both natural and processed food products by imparting a sour or sharp taste to food. Citric acid, for instance, is responsible for the sour taste of lemons, limes, grapefruits, and oranges while acetic acid is responsible for the sour taste of vinegar (Bender, 2003). The tartness of the sobolo drink is made mild by the addition of refined sugar which is extracted from sugarcane (Aslam and Khan, 2001). Sugarcane is generally identified as noble cane due to its high sucrose content and low fibre content. The sugarcane juice contains flavonoids such as apigenin, luteolin and tricin derivatives and among phenolics, hydroxycinnamic, caffeic and sinapic acid, representing a total content of around 160 mg/L (Joaquim *et al.,* 2006).

1.1 PROBLEM STATEMENT

Cardiovascular diseases (CVDs) and certain cancers could be put in check if fruits and vegetables are made a necessary part of healthy nutrition and also consumed in the right amounts. In a Joint FAO/WHO Expert Consultation on diet, nutrition and the prevention of chronic diseases, ailments such as heart disease, cancer, diabetes, obesity and micronutrient deficiencies, prevalent in less developed countries could be avoided and mitigated by consuming a minimum of 400g of fruits and vegetables (FAO/WHO, 2003). The blending together of bissap juice with tropical fruit juices is estimated to give products with high nutritional value and functional activity (Kilima *et al*., 2014).

Bissap calyx drink is widely consumed across West Africa. Roselle calyx is known to have high nutrient content and antioxidant capacity which is therefore, able to mop up free radicals and consequently control degenerative disease incidence and severity. Unfortunately, there are some potential consumers who complain about the flavour and short shelf life of existing bissap drinks and therefore abstain from it; consequently, missing its benefits. Development of an acceptable high quality bissap drink variant based on inclusion of sugar cane juice could enable such potential consumers to patronize the drink. Hence this study.

1.2 JUSTIFICATION

Hibiscus sabdariffa (bissap calyx), *Ananas comosus* (pineapple) and *Saccharum offinarum* (sugarcane) have all been acknowledged for their nutritive properties. Bissap drink containing sugarcane and pineapple could be appreciated by the section of the population who do not enjoy presently existing bissap drinks based only on pineapple. Hence benefiting from the nutritional and other phytochemicals present in the fruits and bissap calyx. Also, a new variant of the drink could help improve the incomes of producers as they could attract more consumers resulting in increased use of bissap and hence benefiting from its nutrients.

1.3 OBJECTIVES

This study seeks to formulate a consumer-acceptable shelf-stable bissap drink containing pineapple and sugarcane juice.

The specific objectives include;

- To formulate bissap drink using different levels of inclusion of bissap, pineapple and sugarcane juice.
- To evaluate sensory characteristics of the drink.
- To determine the physicochemical characteristics of the drink.
- To determine the effect of different packaging material and storage condition on quality.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF *Hibiscus sabdariffa*

There are over three hundred Hibiscus species found around the world are mostly identified as flowering plants for ornamental purposes of which *Hibiscus sabdariffa* is a member (Yadong *et al*., 2005). *Hibiscus sabdariffa* which is a shrub is found in the *Malvaceae* family and is native to Asia or Tropical Africa as a home garden crop. The plant which comes in two varieties known as *Hibiscus sabdariffa* var *altissma* and *Hibiscus sabdariffa* var *sabdariffa* is widely cultivated in the tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines (Gautam, 2004; Eltayeib and Hamade, 2014). *Hibiscus sabdariffa* is known by different names such as *Guineasorrel* or *bissap* in Senegal, *karkadé* in North Africa, *roselle* or *sorrel* in Asia and *flora of Jamaica* in Central America. In Ghana, Nigeria, Gambia and Iran it is known as *sobolo, Zobo, Wonjo* and *Chaye-Torosh* respectively (Adanlawo and Ajibade, 2006; Cisse, 2010).

As described by Mahadevan *et al*., (2009), its cylindrical dark green to red stem which is smooth or nearly smooth has a height of about 3.5m. Leaves are alternate, 7.5-12.5 cm long, green with reddish veins and long or short petioles. Leaves of young seedlings and upper leaves of older plants are simple; lower leaves are deeply 3 to 5 or even 7-lobed and the margins are toothed. Flowers, borne singly in the leaf axils are up to 12.5 cm wide, yellow or buff with a rose or maroon eye and turn pink as they wither at the end of the day. The calyx is characterized by 5 large sepals, pointed bracts around the base. The fruit is a velvety capsule, 1.25-2 cm long, which is green when immature, 5-valved, with each valve containing 3-4 seeds. The capsule turns brown and splits open when mature and dry. Seeds are kidney-shaped, light-brown, 3-5 mm long and covered with small, stout and stellate hairs. During production, for the plant to give off its maximum in terms of growth and maturity, soil conditions must be conditioned with organic materials and essential nutrients. Relatively high temperature throughout the growing and fruiting periods can be tolerated. The plant requires an optimum rainfall of approximately 45-50 cm distributed over a 90-120 day growing period (Adanlawo and Ajibade, 2006).

2.1.1 Health Benefits of Bissap

Emebu and Anyika, (2011) described the liquid extracted from fresh fruits and vegetables as juice which is a great source of vitamins, minerals, natural sugars and phytochemicals enhancing the physical and mental functioning of the body. According to Tsai *et al.*, (2002), the roselle petal extract contains anthocyanin, a flavonoid component which is a major source of antioxidant capacity. Antioxidant properties from the flavonoids and anthocyanins present decrease chronic diseases such as diabetes mellitus, dyslipidemias, hypertension, urinary tract infections and cardiovascular diseases. It is reported to be an antiseptic, sedative, digestive purgative, emollient, demulcent and astringent (Odigie *et al.*, 2003; Cid and Guerrero, 2014). The flowers and fruits are used for treatment of cough and bronchitis (Maregesi *et al.*, 2013). From research, the calyces have been found to be abundant with acid, pectin, crude protein and minerals such as iron, calcium, manganese and others. (Gautam, 2004). Calyces of bissap are said to contain nine times more vitamin C than *Citrus sinensis* (Amin *et al.*, 2008). The (anti) nutritional components of bissap calyx are shown in tables 1and 2.

Table 2.1: Anti-nutritional content of bissap calyces.

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

Source: Adanlawo and Ajibade, 2006

Table 2.2: Chemical composition of dried bissap calyces.

Serial No.	Component	Red Calyces
1	Moisture (%) (d.b.)	11.00
2	Crude protein (%)	7.88
3	Crude fibre (%)	13.20
4	Crude fat (%)	0.16
5	Ash (%)	10.60
6	Total carbohydrates (%)	57.16
7	Ascorbic acid (mg/100g)	11.00
8	Titrable acidity (mg/100g)	9.00
9	Total soluble solids (%)	5.00
10	Calcium (mg/100g)	60.00
11	Iron (mg/100g)	25.00

Source: Mohamed *et al.*, (2012).

2.1.1.1 Uses of Bissap

Throughout the world today, the calyces of *Hibiscus sabdariffa* are eminent in the production of a lot of commodities such as hot or cold beverages. This is due to its pleasing taste, provision of relief during hot weather by increasing the flow of blood to the skin surface and dilating the pores to cool the skin (Obiefuna *et al.*, 1994; Leung and Foster, 1996). The young leaves of the plant are eaten as cooked vegetable especially with soup. A meal is formed out of the pounded seeds which is used as oily soup or sauce after roasting and oil extracted from the seed is a substitute for castor oil while the residue is used in fermented form as soup or cake (Aliyu *et al.*, 2000), Again, calyces extract is also a potential source of natural colourant to replace red synthetic colouring agents for carbonated soft drinks, jams, juices, jellies, sauces, chutneys, wines, preserves and other acidic foods (Delgado and Parcedes, 2003).

2.2 PINEAPPLE

Pineapple (*Ananas comosus)* also known as the queen of fruits because its exceptional flavour and taste is from the family *Bromeliaceae*, which is one of the most important commercial fruit crops in the world (Bawura, 2013). Amongst tropical fruits in the world, pineapple comes third after banana and citrus. Major quantities of about 50% of world production come from Thailand, Philippines, Brazil and China. The remaining are from Mexico, Costa Rica, India, Nigeria, Kenya and Indonesia (Bartholomew *et al.*, 2003; FAO, 2004/2005). Between 30°N and S latitudes are found major areas of commercial cultivation with some areas considered marginal for various reasons (Bartholomew *et al.*, 2002).

With over a hundred varieties existing, the most widely cultivated include Smooth Cayenne, Queen and MD2 which was a lately introduced make up 80% of the global trade in pineapples (Ndungu, 2014). The world pineapple market has been expanding rapidly for the last twenty years showing increasing trends from 2002 to 2012, hitting 23.33 million metric tons in 2012 (UNCTAD, 2012). The pineapple is an herbaceous perennial characterized by stem, leaves, peduncle, crown, shoots and the adventitious roots and has a length of 1.0 to 1.5 meters (3.3 to 4.9 ft) (Bartholomew *et al.*, 2003; Zhang *et al.*, 2010). Its leaves are waxy, stem short and stocky which produces side shoots known as suckers in the leaf axil. About 200 or more flowers are produced in the fruit creation. Flower colours vary, depending on variety, from lavender, through light purple to red (Bartholomew *et al.*, 2003).

2.2.1 Health Benefits of Pineapple

Pineapple contains significant levels of calcium, potassium, fibre and vitamin C which acts as a water soluble antioxidant. Cholesterol and fat levels are low. Pineapples are also a good source of vitamin B1 and B6, copper and dietary fibre. A group of sulphur-containing proteolytic (protein digesting) enzymes called bromelain become very useful in aiding in digestion and also acts as an anti-inflammatory agent in conditions such as acute sinusitis, sore throat, arthritis and gout. Patients recovering from injuries and surgeries can also benefit from pineapples. Furthermore, when pineapples are ingested, blood clotting is reduced and plaque are removed from arterial walls. The enzymes present in pineapples as evident in some studies are said to improve circulation in those with narrowed arteries such as angina sufferers. Lastly, pineapple is a unique cerebral toner combating memory loss, sadness and melancholy (Joy, 2010).

2.2.2 Uses of Pineapple

Pineapples on the worldwide market are presented in varieties from whole slices to segments. Tinned pineapples are well known in canned fruit mixes and differs in terms of condiments they contain, from plain pineapple juice to sugar syrup, of varying density. The pineapple is also present on the processed sector as cut fresh fruits packed in sachets or containers. Plain or concentrated juices also signify a common use of the fruit where it is found on its own or mixed with other fruits. The containers used comprise of bottles, jars, cans, tetra packs and aluminium sachets. Fermented pineapple juice is used for wine and vinegar. The food commerce produces semi-processed pulps or frozen fruit slices used particularly for dairy products like yogurt, ice-cream and others (UNCTAD, 2016).

In Africa, young tender shoots are eaten in salads. The terminal bud or “cabbage” and the inflorescences are eaten raw or cooked. Young shoots called “hijos de pina” are sold on the vegetable markets in Guatemala. In the cleaning of machete and knife blades and with sand for scrubbing boat decks, pineapple juice can be used. Also, a whole fruit in good shape can be used as decorations such that its variegated forms are cultivated globally for their splendour indoors and out. Since 1963, thousands of potted ethylene treated pineapple plants with fruits have been shipped yearly from southern Florida to northern cities as indoor ornamentals. For the pharmaceutical industry, an enzyme extracted from the pineapple known as bromelain, is being investigated as it appears to aid in digestion. Finally, going up in the industry, pineapple leaves can be used as feed for livestock, for making paper or rope fibres. The oil cakes derived from the processing sector are also beneficial by-products for green fertiliser and compost, but also livestock feed. Fermentation of these oil cakes facilitates biogas production (Joy, 2010).

2.2.3 Varieties of Pineapple in Ghana

Pineapple is Ghana's most important single non-traditional export income earner (Mathias, 2002). Export into countries like Belgium, Switzerland, France, Italy, Luxembourg, the Netherlands, and the UK in 2005 was 46694 tonnes as against 71804 tonnes in 2004 recording values at $12,784,300 ($12.7 million) in 2005 as against $22,068,600($22 million) in 2004 (Takane, 2004; ISSER, 2006). According to Fold and Gough, (2008), there are three main types of pineapple grown in Ghana namely, MD2, Sugarloaf and Smooth Cayenne.

2.2.3.1 MD2

The MD2 pineapple is a preferred variety over the others because of its uniform bright gold colour, sweeter taste, has four times Vitamin C content, lower fibre, lower acidity, thinner skin, smaller fruits at an average of 1.5 kg each and longer shelf life. On the market, MD2 can command three times the price of other pineapple varieties. Because MD2 has longer shelf-life of 30 days compared to 21 days for other varieties, it performs better in long-distance shipping (Amar *et al.*, 2015).

2.2.3.2 Sugar Loaf

Sugarloaf leaves are dark green and smooth. The fruit is conical in shape and weighs between 2.3-2.7kg. Sugarloaf has a green skin and a cream white flesh. Soil conditions with pH between 5-6.5 in a sandy clay loam soil is suitable for its growth. Sugarloaf is very sweet, and only sold in the local market (Fold and Gough, 2008; Siddiq, 2012).

2.2.3.3 Smooth Cayenne

The leaves are dark green and spineless except at the base and tip. The fruit which is ovoid in shape weighs between 2.3-4 kg. Smooth Cayenne is also characterized by a yellow skin and a pale yellow flesh. Concerning planting conditions, it thrives well in a well-drained loam soil with pH between 5-6.5.Smooth cayenne is a sweet and juicy variety that initially had high export value but is now only sold on the local market and processing factories.(Fold and Gough, 2008; Siddiq, 2012).

2.3 SUGARCANE

Sugarcane (*Saccharum officinarum* L.*)* is a perennial, tropical and subtropical species of grass which originated from Southeast Asia and is currently being produced globally. Its characteristics comprise of rhizomes which form beneath the soil, sending secondary shoots near the parent plant with height of about 5m (16ft). The stems are unbranched, with colours ranging from green, pink to purple whereas its alternate, green leaves with thick midribs and saw-toothed edges are joined to nodes. In between the nodes is a fibrous white pith engorged with sugary sap. The leaves are of length, 30-60cm (12 to 24 in) and width of 5 cm (2.0 in). The terminal inflorescence is a panicle up to 60 cm (24 in) long, a pinkish plume that is broadest at the base and tapering towards the top. The spikelets are borne on side branches and are about 3 mm (0.12 in) long and are hidden in tufts of long, silky hair (Miraj, 2016). According to Bakker (1999), there are four domesticated species of sugarcane which are restricted to Melanesia and Indonesia. Modern cultivated variations of sugarcane are hybrids resulting from breeding between the species of former commercial importance. These breeding programmes integrate desired qualities such as vigour, hardiness and high sugar content.

2.3.1 Health Benefits of Sugarcane

In the tropics and subtropics, sugarcane juice is thought to be a healthy and nutritious drink. The sugarcane juice clearly controls host natural immunity against viral, bacterial and protozoan infections (El-Abasy *et al.*, 2003; El-Abasy *et al.*, 2002). In the medical world, India, from long ago uses sugarcane juice in their Ayurveda and Unani systems. Immuno-stimulation, anti-thrombosis activity, anti-inflammatory activity, vaccine adjuvant, modulation of acetylcholine release, anti-stress effects, raising innate immunity to infections are some biological effects of sugarcane juice as recorded by (Barocci *et al.*, 1999; El-Abasy *et al.*, 2002; Lo *et al.*, 2005). People with jaundice and liver-related disorders are encouraged to consume sugarcane juice in the traditional system of medicine (El-Abasy *et al.*, 2003; El-Abasy *et al.*, 2002).

Brash and Harve, (2002), reported that free radicals in the body which causes damage could be inhibited by supplementing antioxidants which has become an healing approach for reducing the threat of diseases. Sugarcane extract is stated to have antioxidative activities, prophylactic activities and other physiological functions (Takara *et al.*, 2002).The sugarcane juice contains flavonoids such as apigenin, luteolin and tricin derivatives and among phenolics, hydroxycinnamic, caffeic and sinapic acid, signifying a total of around 160 mg/L (Joaquim *et al.*, 2006) while, sugarcane leaves contains luteolin-8-C-(rhamnosylglucoside) as major compound which fights free radicals (Fabiana *et al.*, 2008). The roots and stems of sugarcane are used in treating skin and urinary tract infections, bronchitis, heart conditions, loss of milk production, cough, anaemia, constipation as well as general weakness (Mira *et al.*, 2011).

2.3.2 Uses of Sugarcane

Sugarcane is mainly grown for sugar production and serves as an important source of income and employment. Sugarcane by-products provide industries with sugar, chip board, paper, barrages, confectionery, chemicals, plastics, paints, synthetics, fibre, insecticides and detergents (Aslam and Khan, 2001). Also, in some areas, sugarcane is grown specifically for fresh juice production. In Malaysia, for instance, particular varieties of noble canes (*S. officinarum*), noted for their softer and less fibrous stem, are grown purposely for fresh juice production (Yusof *et al.*, 2000). Energy wise, sugarcane has been cited as a potential source of bioethanol. This has been adapted by Brazil who has expanded its cane planting program to have a successfully integrated sugar/ethanol/cogeneration business models. Also, 40% of Mauritius' electricity is generated from the bagasse. There is also the reduction of in the emission of carbon dioxide when bagasse is used instead of coal as fuel (Allen *et* al, 1997; Rogers *et al*, 2001). According to Zarrilli (2007), several countries that implemented biofuels development programs have experienced significant job creation, especially in rural areas but also along the value chain. Cheavegatti-Gianotto *et al.* (2011) further explained that during the industrial processing of sugarcane, bagasse could be transformed into paper, energy or animal feed; filter cake/vinasse to fertilizer or animal feed and molasses to animal feed.

2.4 PHYSICOCHEMICAL FACTORS RELATED TO JUICES

Processing plays a vital role in the preservation and enhanced use of fruits and vegetables. This ensures reasonable proceeds to growers to enhance their monetary condition which in turn helps to ease the problem of under-employment during off-seasons in the agricultural sectors (Vidhya and Narain, 2010). During processing and storage, quality factors of fruit products, such as colour, acidity, soluble solids, texture, pH, total acidity among others are usually affected (Wicklund *et al.*, 2005; Kvikliene *et al.*, 2006). The usable period of most fruit products are limited basically by storage temperature (Ene-Obong *et al.*, 2014). Adrian *et al.*, (2003) in their study of some fruits and vegetables revealed that vitamin C and flavonoid levels vary widely not only by species and variety, place of growth, harvesting period and storage, but also dependent on processing methods. The authors determined the concentrations of ascorbic acid and flavonoids and found significant losses during storage and especially during heat processing.

2.5 SENSORY QUALITIES OF JUICES

Zotarelli *et al.*, (2008) stated that, a combination of fruit juices could provide pleasing sensory characteristics that could earn a place for such a product on the consumer market. Before that can occur, the product must undergo a series of processes known as sensory evaluation to qualify it for the market. Stone and Sidel, (2004) described sensory evaluation as a scientific method used to induce, measure, analyse, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing.

The sensory test is set up to know how these product influences will create perceived changes to human observers. In the industrial setting, sensory evaluation offers a channel for information that is useful in management business decisions about directions for product development and deviations. Also, sensory assessment may be quite useful or even necessary in quality control (QC) or quality assurance. Reforms in traditional sensory practices may be requisite to accommodate the small panels and quick evaluations often required in online QC in the manufacturing environment. Due to the time needed to bring together a panel, prepare samples for testing, analyse and report sensory data, it can be quite challenging to apply sensory procedures to quality control as an on-line assessment (Lawless and Heymann, 2010). Extracts from bissap calyces have been noted to have a sharp and sour taste and is normally enhanced with granulated sugar and fruits depending on choice (Oboh and Elusiyan, 2004).

2.6 MICROBIOLOGICAL FACTORS AFFECTING JUICES

Microbial outbreaks occur with hefty consequences for human safety due to environmental contamination of packaging machinery in food processing entities (Parisi, 2013) and improper washing in the case of bissap leaves (Musah *et al.*,2014). The FAO in 2008 stated that, microbial contamination of juices leads to survival or growth of pathogens and ensuing quick spoilage. Signs such as browning and flavour changes are caused by enzymatic activities. Similarly, dissolved oxygen can also cause browning and reduction of nutrients. Microbial contaminants include *Escherichia coli, Salmonella sp., Shigella sp., Staphylococcusaureus* and some filamentous fungi (Barro *et al.,* 2006). The presence of polyphenols in fruits like sugarcane serve as defence agents enabling protection against microbial infection. The significance of phenolic compounds is related to their antioxidant activity (Jefremov *et al.,* 2007). Thus, Musah *et al.,* (2014) advised that for safe consumption of formulations with bissap extract, good manufacturing practices (GMPs), appropriate hygienic measures as well as infusion by boiling instead of cold water brewing should be adhered to.

2.7 PACKAGING MATERIAL FOR JUICES

According to Castberg *et al.,* (2000), the aims for packing juices is to retain a hermetic environment to prevent recontamination and to reduce extra quality degradation due to oxygen penetration. Also, to provide consumers with ingredient and nutritional information (Marsh and Bugusu, 2007). The expectations of juice consumers leads to the creation of varieties based on flavors, freshness, health, environmental stewardship, convenience, closure features, shapes and sizes. Plastic (PET (Polyethylene terephthalate) and HDPE (High-density polyethylene)) bottles layered with an oxygen barrier are becoming widespread although glass, cans and cartons are available. Active packaging, where oxygen absorbers are used to lengthen the life of a product, can improve the use of polymetric materials. Packaging materials that absorb oxygen offer barrier properties higher than other products presently on the market (Castberg *et al.,* (2000).

2.7.1 Plastic

According to Page *et al.,* (2003) plastic is classified as hybrid packaging since it is in synergy with other materials such as metals and organic components. Parisi (2012), therefore thought it best to group plastic food packages into four main groups namely;

- Rigid and semi-rigid containers: Examples are bottles, jars, trays, boxes, drums, and crates
- Flexible food package: Examples include pouches, sachets and regenerated cellulose films
- Polycoupled food package: Here, there is the combination of materials such as plastic films and paperboard foils.
- Plastic components for plastic and hybrid packages: This section is made up of enamels, gaskets, adhesive films and labels

In the production of plastic food packages, different polymers are used. The commonly existing types are Polyethylene (PE), also defined as low density polyethylene (LDPE) and high density polyethylene (HDPE), Polypropylene (PP), Polyvinyl chloride (PVC), Polyethylene terephthalate (PET) and

Polystyrene (PS) (Parisi, 2012). Plastic packaging for beverages is on the rise due to the low cost of materials and functional advantages such as chemically resistance,light weight, thermosealability, microwavability, optical properties, and unlimited sizes and shapes over traditional materials such as glass and tinplate (Marsh and Bugusu, 2007; Pimentel *et al.*, 2015).

2.7.2 Glass

Glass is appreciated for its chemical inertness, clarity and heat resistance. In food applications, its transparency is considered to be an important marketing advantage, carrying the image of a quality product. During hot filling, there is no deformation of the glass container but liable to thermal shock and may break. Failure may also occur from frictional stresses caused by repeated bottle contact along a packaging line. Glass is considered heavier than other packaging materials (McLellan and Padilla-Zakour, 2005).

Marsh and Bugusu, (2007) stated the following as advantages of glass in that, glass is reusable and recyclable. Its improved break resistance allows manufacturers to use thinner glass. It's odourless and impermeable to gases and vapours. The freshness of the product is maintained for a long period of time without impairing taste or flavour. It's also useful for heat sterilization and has good insulation. Glass can be produced in numerous different shape and variations in glass colour can protect light-sensitive contents. Disadvantages are that, there is limitation in thin glass; transportation costs; brittleness and susceptibility to breakages from internal pressure and impact.

2.7.4 Paper Carton

Marsh and Bugusu, (2007) considered paper to be lightweight; economical compared to other packaging systems; recyclable; efficient, low cost protection; available in several forms; easy handling by consumers and very good strength to weight characteristics. The disadvantages are that, it is a poor barrier properties to light, moisture; not used to protect foods for long periods of time. When used as primary packaging, it is coated or laminated to improve functional and protective properties. The combination with other materials hinders the subsequent recycling process and tears easily.

2.7.5 Cans

Cans are fabricated from aluminium or steel. Steel cans are layered to prevent corrosion from acidic juices. Loss of flavour and interaction with the can coating is a disadvantage in using cans. When properly made and closed, cans serve as an absolute barrier between the canned product and the outer environment. Cans provide high-strength containers for processed foods. Processing standards for canned foods are well defined. However, highly acidic foods such as fruits may corrode can interiors, causing extensive detinning, particularly at the product headspace interface. Aluminium cans are used for beverages while composite cans are used for frozen juice concentrate (McLellan and Padilla-Zakour, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL LOCATION

The study was carried out at the laboratories of the Food Research Institute (FRI) of the Council for Scientific and Industrial Research (CSIR), Shiashie, Accra and the Department of Horticulture, KNUST, Kumasi.

3.2 COLLECTION OF RAW MATERIALS

Dried whole bissap calyx, sugar loaf pineapples, stems of sugarcane and whole ginger *(Zingiber officinale)* spice were purchased from the Madina market in Accra. Polyethylene terephthalate (PET) bottles and clear glass bottles for packaging were also acquired.

3.3 FORMULATION PROCESS

3.3.1 Bissap calyx extract

Bissap calyces were inspected to remove any foreign material. Using the ratio of calyx: water, 1:10g/ml, 10g of calyces was weighed and washed with tap water. A 100ml of water was added to the calyces, put on low heat, stirring at intervals with a wooden spatula to attain 50°C for 30mins (Chumsri *et al.*, 2008). The temperature was intermittently checked using a thermometer. The extract was filtered with a sterilised cheese cloth.

3.3.2 Pineapple juice

The pineapples were carefully washed with 1% sodium metabisulphite and rinsed with tap water. The peels and core were cut off, pith removed and then sliced into pieces. A Panasonic Mx-AC300 mixer grinder was used to puree the slices. The juice was extracted using a sterilised cheese cloth. The juice was refrigerated.

3.3.3 Sugarcane juice

Sugarcane stems cut to length of about 20cm were thoroughly washed in a NaCl solution and peeled. The stems were passed through the stainless steel electric cylinder mill of the sugarcane juice extractor to extract the juice. 4g/100g of pineapple pulp was straightaway added to the juice to reduce pH and inhibit Polyphenol Oxidase (PPO) activity. The acidified juice was filtered, preheated to a temperature of about 50 °C and heated at a pasteurization temperature of 75°C for 30s. The pasteurized juice was cooled to about 8°C and poured into a 4L gallon sterilized with boiling water at 100°C. Bottled juice was refrigerated for further use (Kunitake*et al.*, 2014).

3.3.4 Ginger extract

Ginger was thoroughly washed with tap water, soaked in water for about 10mins, scrapped clean and chopped into pieces. 100g of ginger was blended with 100mlof water with a Panasonic Mx-AC300 mixer grinder. Ginger extract was filtered with a sterilised cheese cloth and added at 3% (w/w) (Babajide *et al.*, 2013).

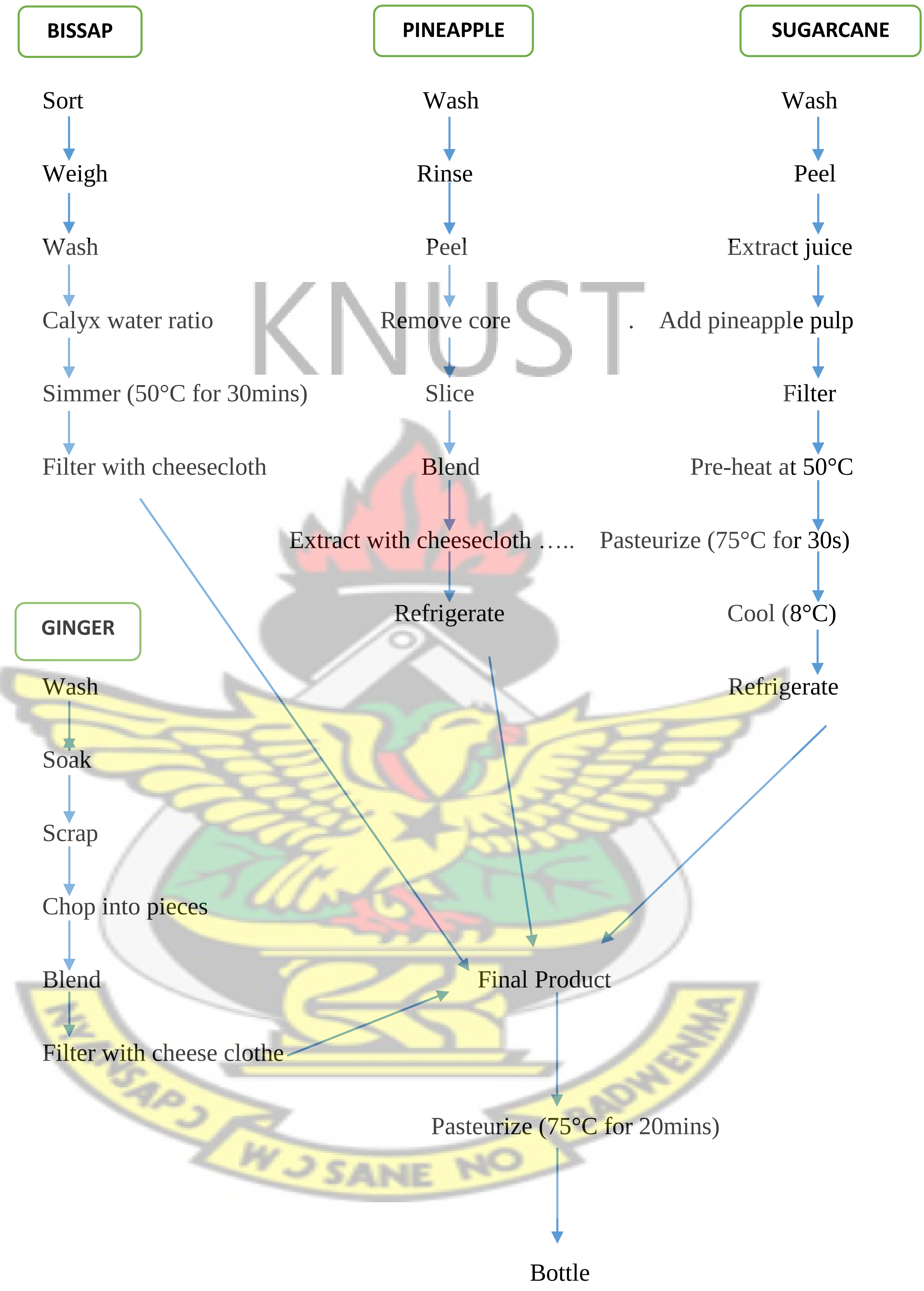

Figure 3.1: Flow diagram of production of bissap, pineapple and sugarcane cocktail.

3.4 SENSORY EVALUATION

3.4.1 Setting of arbitrary levels for preliminary test

A preliminary test was conducted with upper and lower arbitrary levels set using the Minitab 2017 software. This was done to aid in setting realistic upper and lower limits. The mixture design resulted in ten formulations which were subjected to sensory analysis in the sensory lab of the Food Research Institute.

Table 3.1: Arbitrary Lower and upper limits of the three component juice

Component	Lower (%)	Upper (%)
Bissap extract	50	70
Pineapple Juice	10	30
Sugarcane Juice	20	40

Table 3.2: Formulations derived from arbitrary lower and upper limits.

Formulation Code	Bissap Extract	Pineapple Juice	Sugarcane Juice
F579	54	23	23
F909	70	10	20
F776	64	13	23
F686	50	20	30
F205	60	10	30
F190	58	16	26
F332	50	30	20
F813	50	10	40
F611	60	20	20
F792	54	13	33

3.4.2 Optimization of formulated juice blend

Table 3.3: Optimized lower and upper limits of the three component juice

Component	Lower (%)	Upper (%)
Bissap Juice	30	50
Pineapple Juice	20	40
Sugarcane Juice	30	50

3.4.3 Consumer acceptance test

The consumer acceptance evaluation took place in individual booths under white light and with adequate ventilation in the sensory laboratory of CSIR-FRI. Sensory panellist made up of staff from CSIR-FRI numbered 30. Redness, clarity, flavour, bissap taste, sourness, sweetness and overall acceptance were the sensory attributes measured for the evaluation. Samples were labelled with random three digit codes and the sample order was randomized. Panellists assessed by rinsing their palates with room temperature water between samples and allotted scores to the attributes using the 9 – point Hedonic scale where 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much and 1=dislike extremely. Responses received from the questionnaires (Appendix 1) were analysed using ANOVA and mixture regression techniques. This was done for both preliminary and optimization phases of the experiment.

3.5 PHYSICOCHEMICAL ANALYSIS

3.5.1 Colour

The colour meter (Konica Minolta) was used to determine the “L”, “a” and “b” values of the sample solution.

3.5.2 Total Soluble Solids (TSS)

The TSS was measured with a LCD Digital bench type (HI 96801) refractometer. A25% sucrose solution and distilled water was used to calibrate the refractometer. A 10*ul* of the prepared sample solution was placed on the prism surface. Readings were taken and recorded as °brix.

3.5.3 Total Titrable Acidity (TTA)

Total titrable acidity was determined as citric acid. Ten millilitres of juice was measured into a conical flask. With the addition of three drops of phenolphthalein indicator, the conical flask was swirled. A burette was filled with1.0M sodium hydroxide (NaOH) solution and titrated against the content in the conical flask. The end point was calculated when the initial colour changed to pink. This was done in triplicates.

Calculations

$$\text{The \%TTA} = \frac{\text{Average titre} \times \text{citric acid factor} \times 100}{\text{Volume of solution (10ml)}}$$

3.5.4 pH

The pH was measured using pH meter (ELICO 617) which was calibrated with two standard buffers 4.0 and 7.0. Ten (10) millilitres of each of the sample was measured into 100ml beaker. The electrode of the pH meter was placed inside the sample and the pH was read directly from the screen of the meter.

3.5.5 Vitamin C

Ten millilitres of the sample was measured with a test tube into a conical flask. A hundred millilitres of distilled water was added. Using starch complex as indicator, the diluted sample was titrated against iodine solution held in a burette. End-point values were recorded when initial colour changed to faint pink after standardizing with ascorbic acid. The experiment was carried out in triplicates.

<u>Calculation</u>

$C_1V_1 = C_2V_2$

Where C_1 = Concentration of standard ascorbic acid

V_I = Volume of ascorbic acid

C_2 = Unknown concentration of iodine

V_2 = Volume of iodine

3.5.6 Ash Content

Silica crucibles were ignited, cooled and weighed. Five grams of the test sample was weighed into the crucible. Sample was placed into the furnace and was ashed for 8 hours at 550°C (±10°C). The resulting ash was cooled in a desiccator to room temperature and weighed to a constant weight (AOAC, 2005).

Calculations

(A +B) –A = B

(A + C) -A = C

% Ash = C/B x 100where A = crucible weight, B = sample weight and C = ash weight.

3.6 SHELF LIFE STUDIES

Microbial studies and physicochemical parameters were undertaken. The accepted optimized formulation was subjected to storage under two conditions namely temperature and packaging material. Temperature comprised of room temperature and relative humidity at 22°C-28°C, 72.4% respectively and refrigerated temperature and relative humidity 4-11°C, 58.3% while packaging material comprised PET and clear glass bottle.

3.6.1 Microbiological Analyses

3.6.1.1 Aerobic Plate Count (APC)

1ml of sample was measured and homogenised with 9ml of Salt Peptone Solution (SPS). Serial dilutions were made of which 1ml was inoculated into sterile petri dishes. 10ml of Plate Count Agar (PCA) was added, mixed and allowed to set. Incubation of the set dishes occurred at 30°C for 48-72 hours (NMKL.No.86, 2013).

3.6.1.2 Yeasts and Molds

1ml of sample was measured and homogenised with 9ml of Salt Peptone Solution (SPS). Serial dilutions were made of which 1ml was inoculated into sterile petri dishes. 10ml of DRBC was poured. The inoculated plate was swirled and allowed to set. The dishes were incubated at 25°C for 5 days. Colonies were counted after 5days of incubation. Yeasts and molds were distinguished through microscopic examination (ISO 21527-1:2008).

3.6.1.3 Total Coliforms

1ml of sample was measured and homogenised with 9ml of Salt Peptone Solution (SPS). Serial dilutions were made of which 1ml was pipetted into sterile petri dishes. 5ml of Tryptone Soy Agar (TSA) which was previously cooled at 45°C was added, mixed and allowed to solidify. The dishes were pre-incubated at 25°C for 2 hours. 10ml of Violet Red Bile Salt Lactose Agar (VRBLA) was poured on top, allowed to set and incubated at 37°C for 24±3 hours (NMKL.No.44, 2004).

3.7 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The bissap drink formulations were derived using the Minitab v.17 statistical package. The serving order of the three component formulation was carried out using a Balanced Incomplete Block Design to 30 panellists. Sensory attributes were analysed using ANOVA from the Minitab v.17 statistical package. Contour plots for each sensory attribute were derived and overlaid to define the optimum region of the formulation. Data derived from the physicochemical and microbial analysis were subjected to ANOVA to ascertain the significant differences of the storage factors using Statistix 9 software.

CHAPTER FOUR

4.0 RESULTS

4.1 FORMULATION PROCESS

The bissap, pineapple and sugarcane juices were selected for the red colour, flavour and sweetness respectively. Sugarcane was selected as a sweetener to bring variation among the sweeteners used in already existing bissap drinks. This new variation was appreciated by the sensory panelists.

4.2 SENSORY EVALUATION OF BISSAP COCKTAIL

4.2.1 Preliminary Phase

Arbitrary levels that were set for preliminary sensory evaluation showed no significant differences among the attributes of the ten formulations when analysed. The output of the overlaid contour plots of the regression model from the mean score values resulted in a wide optimized area (Figure 4.1). Consequently, new upper and lower limits were generated for the various components.

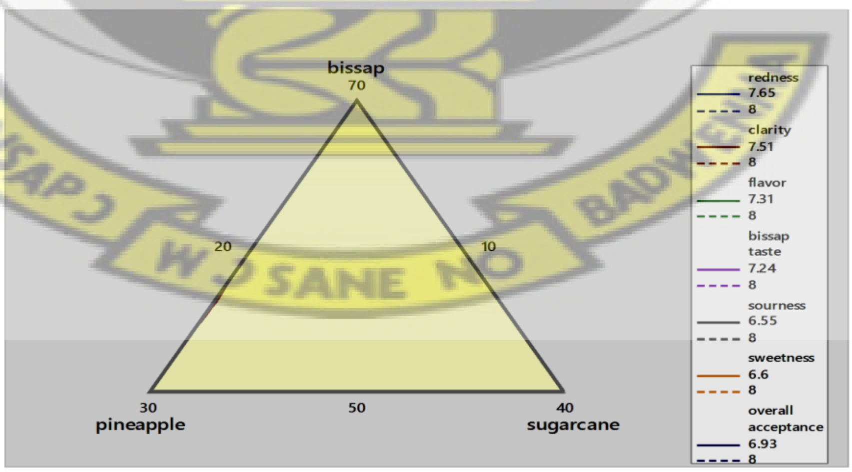

Figure 4.1: Overlaid contour plot for preliminary phase

4.2.2 Optimization Phase

The new lower and upper limits in Table 4.1 were used to generate ten formulations as reported in Table 4.2. These were subjected to the sensory panel assessment and the acquired data on the various attributes were subjected to one-way ANOVA analysis where mean score values were obtained (Table 4.3). Two optimal regions (Figure 4.2) were gained after superimposing contour plots of flavour, bissap taste, sourness and sweetness which were used as the determinants for judging overall acceptability. An optimal formulation which was prepared and subjected to physicochemical and microbial analysis was selected from the optimal area on the left (Figure 4.2) having inclusion levels for bissap, pineapple and sugarcane at 0.30, 0.38 and 0.32 respectively. This is because, the availability of raw materials, ease of processing and ultimately, the overall acceptability of 7.5 compared to the right side which gives and overall acceptability of 7.3 was considered.

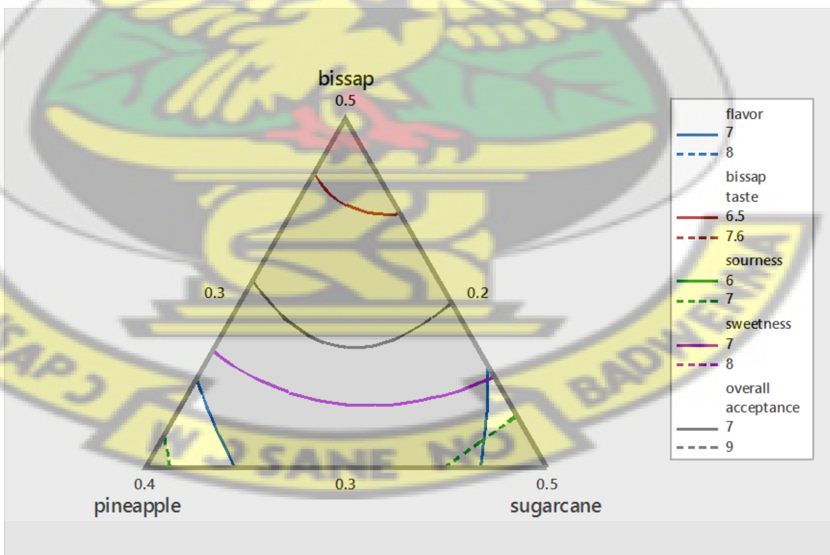

Figure 4.2: Overlaid contour plot for optimal regions

Table 4.1: Optimized lower and upper limits of the three component juice

Component	Lower (%)	Upper (%)
Bissap Juice	30	50
Pineapple Juice	20	40
Sugarcane Juice	30	50

Table 4.2: Formulations derived from optimized lower and upper limits.

Formulation Code	Bissap Juice	Pineapple Juice	Sugarcane Juice
F579	50	20	30
F909	30	20	50
F776	30	40	30
F686	30	30	40
F205	33	33	34
F190	33	23	44
F332	40	30	30
F813	43	23	34
F611	36	26	38
F792	40	20	40

Table 4.3: Mean values for optimized bissap, pineapple and sugarcane drink.

FORMULATION	REDNESS	CLARITY	FLAVOR	TASTE	SOURNESS	SWEETNESS	OVERALL ACCEPTANCE
F579	7.70 ±0.83^{a}	7.50 ±1.38^{a}	6.80 ±1.62^{a}	6.53 ±1.87^{a}	6.23 ±2.11^{ab}	6.16 ±1.78^{c}	6.63 ±1.52^{ab}
F909	7.40 ±1.07^{a}	7.60 ±1.03^{a}	7.20 ±1.34^{a}	7.33 ±1.44^{a}	7.33 ±1.26^{a}	7.53 ±1.30^{ab}	7.63 ±1.12^{a}
F776	7.16 ±1.20^{a}	7.36 ±1.12^{a}	7.30 ±1.46^{a}	7.20 ±1.12^{a}	7.03 ±1.27^{ab}	7.56 ±1.27^{a}	7.50 ±0.97^{ab}
F686	7.60±0.89^{a}	7.53 ±0.93^{a}	6.63 ±1.37^{a}	6.96 ±1.21^{a}	6.66 ±1.18^{ab}	7.03 ±1.24^{abc}	7.10 ±1.06^{ab}
F205	7.76 ±0.77^{a}	7.46 ±0.68^{a}	7.20 ±1.03^{a}	7.46 ±1.00^{a}	6.93 ±1.17^{ab}	7.40 ±1.27^{abc}	7.43 ±1.00^{ab}
F190	7.70 ±0.70^{a}	7.40 ±0.89^{a}	7.16 ±1.51^{a}	7.33 ±1.42^{a}	6.93 ±1.33^{ab}	7.23 ±1.35^{abc}	7.33 ±1.34^{ab}
F332	7.70 ±0.70^{a}	7.33 ±1.02^{a}	6.70 ±1.23^{a}	6.66 ±1.15^{a}	6.66 ±1.39^{ab}	6.60 ±1.81^{abc}	7.06 ±1.20^{ab}
F813	7.76 ±0.62^{a}	7.33 ±0.95^{a}	6.66 ±1.29^{a}	6.46 ±1.47^{a}	5.86 ±1.59^{b}	6.30 ±1.70^{bc}	6.53 ±1.50^{b}
F611	7.50 ±1.30^{a}	7.20 ±1.27^{a}	6.60 ±1.73^{a}	6.36 ±1.54^{a}	6.76 ±1.40^{ab}	6.90 ±1.62^{abc}	6.96 ±1.49^{ab}
F792	7.80 ±0.66^{a}	7.36 ±0.89^{a}	6.80 ±0.96^{a}	6.66 ±1.32^{a}	6.40 ±1.42^{ab}	6.50 ±1.71^{abc}	7.03 ±1.24^{ab}

Mean and standard deviation values with common letters in the same column indicate that there is no significant difference among samples ($P \leq 0.05$) from Tukey's mean test.

Scale: 9=like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely

4.2.3 Sensory Attributes from Optimized Phase

4.2.3.1 Redness

From Table 4.3, the level of redness of all the three digit coded formulations were equally accepted. The R^2obtained from the regression model (Appendix 2) signified the effect of the independent variables on redness. The mixture contour plot (Figure 4.3) showed that the higher the amount of bissap, the redder the colour. This was observed with F579, F332 and F792 who had the highest mean score. The influence of pineapple and sugarcane caused a dip in acceptance of the red colour at their maximum levels as identified with F776 and F909 respectively.

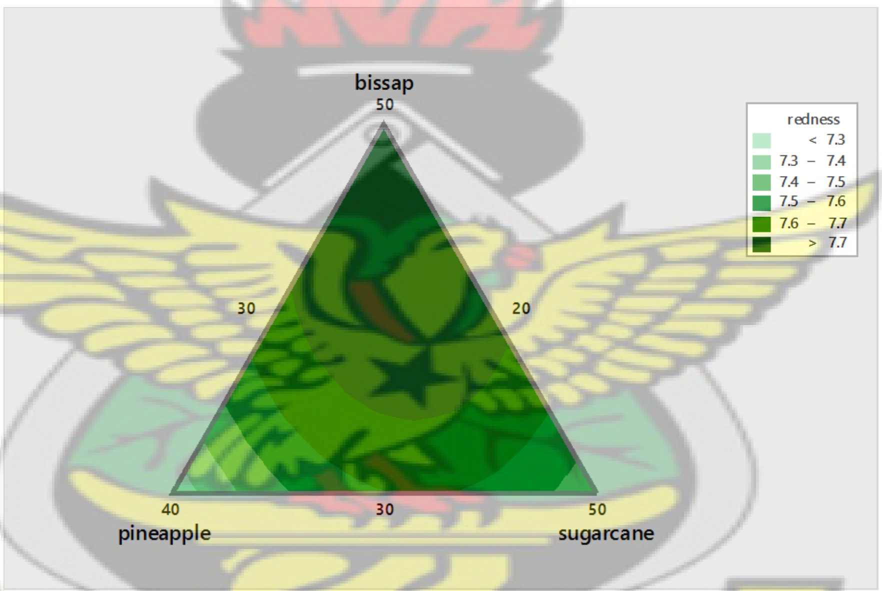

Figure 4.3: Mixture contour plot of redness

4.2.3.2 Clarity

The most preferred product was F909 with mean score 7.60 though there were no significant differences between the formulations. The panellist rated clarity as “like moderately” on the 9- point hedonic scale. The mixture contour plot (Figure 4.4) suggests that higher concentrations of sugarcane gives the drink a hazy nature as F909 had proportions of bissap, pineapple and sugarcane at 30%, 20% and 50% respectively.

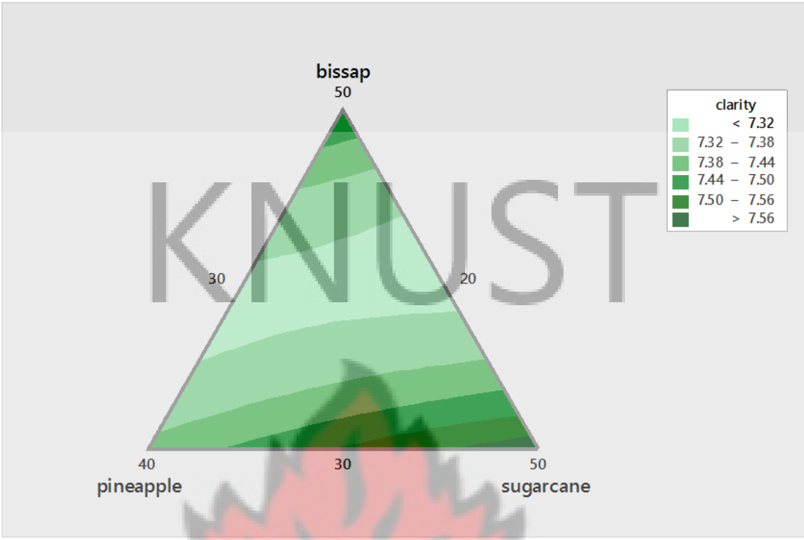

Figure 4.4: Mixture contour plot of clarity

4.2.3.3 Flavour

According to the mixture contour plot (Figure 4.5), flavour was pronounced in product F776 which had a high concentration of pineapple at 40%. However, there were no significant differences between the products. Panellists rated their response between "like slightly" and "like moderately". The regression model (Appendix 2) explains that a variation in the independent variables will have an effect on flavour.

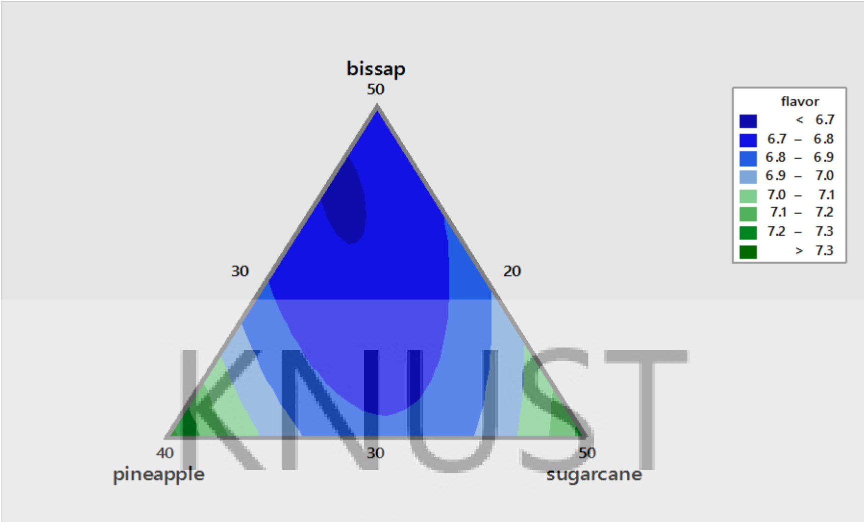

Figure 4.5: Mixture contour plot of flavour

4.2.3.4 Taste

The mixture contour plot suggests that taste was more pronounced when there were almost equal concentrations of pineapple or sugarcane as observed with product F205 which had concentrations of bissap, pineapple and sugarcane at 33%, 33% and 34% respectively. Bissap taste was acceptable to consumers at "like slightly" to "like moderately". Minimum amounts of pineapple and sugarcane reduced acceptability.

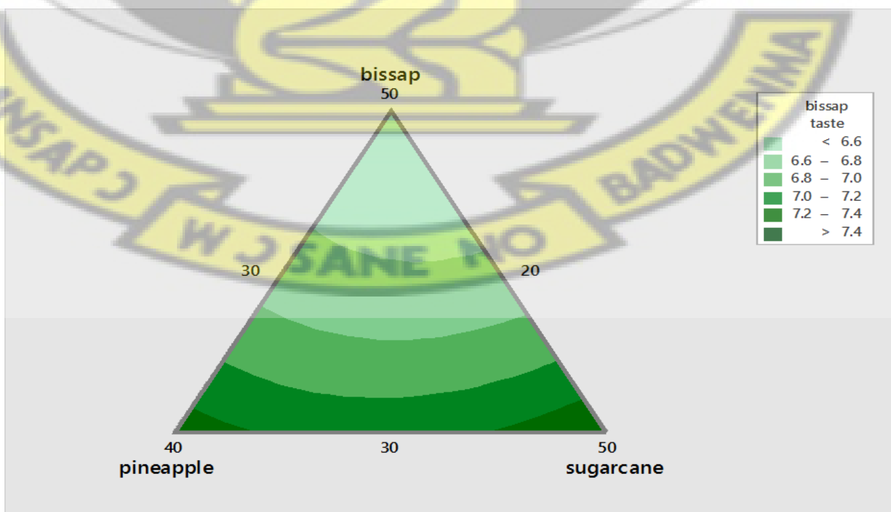

Figure 4.6: Mixture contour plot of taste

4.2.3.5 Sourness

There were significant differences between the formulations. Product F909 was the most liked with mean score 7.33. This could be attributed to the high concentration of sugarcane which masks the sour taste associated with bissap. An R^2 of 79.44% indicating a significance means that the model is good enough to describe the effect of sourness on the formulation. Panellists rated sourness between "neither like nor dislike" to "like moderately".

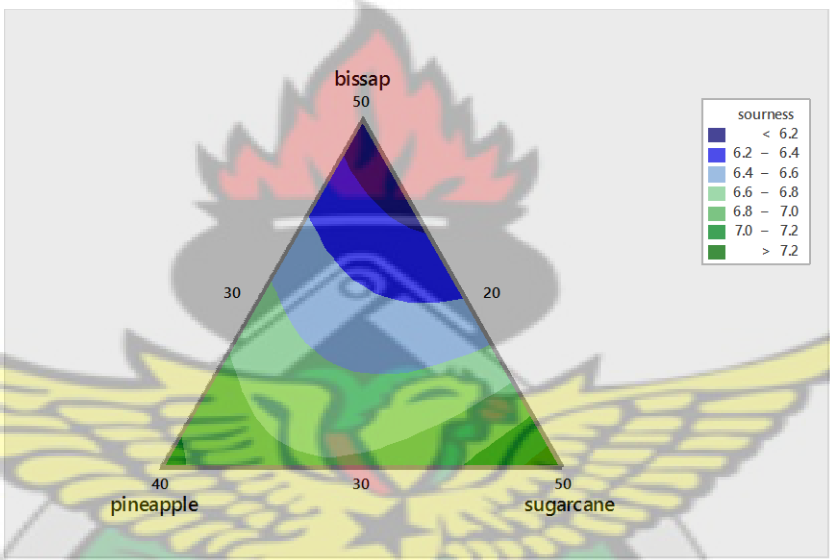

Figure 4.7: Mixture contour plot of sourness

4.2.3.6 Sweetness

Product F776 had a mean score of 7.56 rating it as the most accepted. The least accepted was product F579 with a mean score of 6.16. The higher the concentration of bissap, the less sweet the product. From the mixture contour plot, sweetness is more pronounced when there are high concentrations of pineapple and sugarcane. With an R^2 value of 91.04%, sweetness is considered to be an important factor in the acceptability of the formulation.

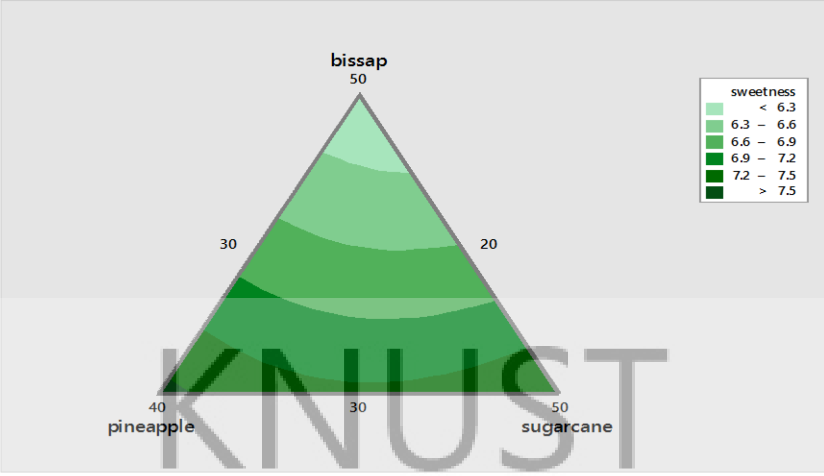

Figure 4.8: Mixture contour plot of sweetness

4.2.3.7 Overall Acceptance

The formulations were acceptable on the scale of "like slightly" to "like moderately". The most preferred product F909 at a mean score of 7.63 followed by F776. The least accepted was F813. From the mixture contour plot, additions of pineapple and/or sugarcane with low levels of bissap can merit the acceptance of a bissap formulated drink. Recording an R^2 of 86.39% means that there could be modelling since the independent variables had an effect on the acceptability of the formulation.

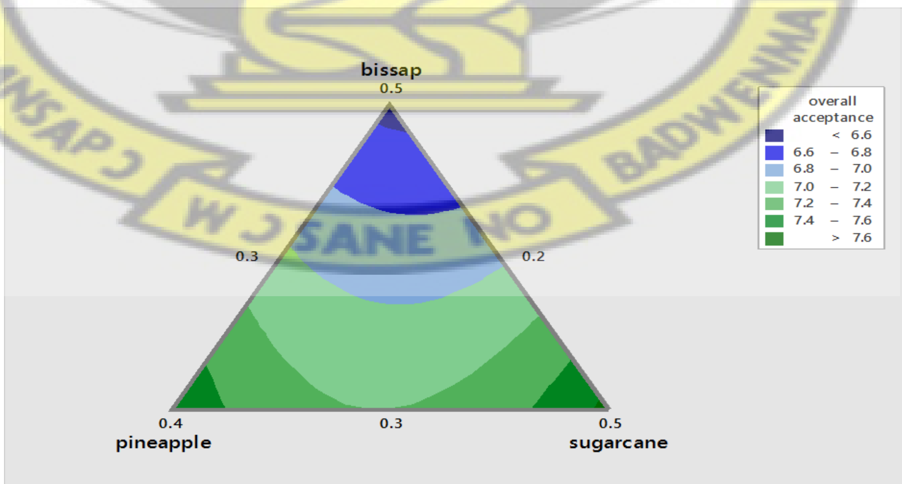

Figure 4.9: Mixture contour plot of overall acceptance

4.3 PHYSICOCHEMICAL ANALYSIS OF ACCEPTED OPTIMIZED FORMULATION OVER A FOUR WEEK PERIOD

All significant differences were pegged at 95% significance level.

4.3.1 Week 0

4.3.1.1 Colour

***L**

Significant differences were observed between packaging material and storage condition interaction (Table 4.4). Bissap drink stored at ambient condition in plastic bottle recorded the significantly highest "L" value (33.78). The least was glass bottle at refrigeration condition (26.29). The "L" value of the bissap drink in the packaging material differed significantly. The highest was in plastic bottle (32.47) and the least was in glass bottle (28.73). With the storage conditions, the highest was at ambient condition (32.48) and the least was at refrigeration condition (28.72).

Table 4.4: Effect of packaging materials and storage conditions on "L" of bissap drink

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	31.16b	26.29c	28.73b
Plastic	33.78a	31.15b	32.47a
means	32.48a	28.72b	

HSD (0.01) PM=0.18, SC=0.18, PM×SC=0.32

***a**

There was an interactive effect of the packaging materials and storage conditions (Table 4.5). Bissap drink stored under refrigeration condition in glass bottle (22.78) was significantly highest. Those stored in plastic bottle under refrigeration condition was the least (15.92). Glass bottle and plastic bottle were significantly different with regards to packaging material in that, glass bottle was highest (21.84) and plastic bottle was the least (17.20). Ambient condition recorded the highest (19.69) while refrigeration was the least (19.35) in terms of storage conditions.

Table 4.5: Effect of packaging materials and storage conditions on "a" of bissap drink

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	20.90b	22.78a	21.84a
Plastic	18.47c	15.92d	17.20b
means	19.69a	19.35b	
HSD (0.01)	PM=0.10, SC=0.10, PM×SC=0.18		

***b**

There were significant differences between packaging material and storage condition interaction (Table 4.6). Significantly highest "b" value was recorded by bissap drink stored in glass bottle at refrigeration condition (3.97). The least "b" value was plastic bottle at refrigeration condition (2.02). Across the packaging material, the highest "b" value was in glass bottle (3.68) and the least was in plastic bottle (2.31). Across the storage conditions, the highest "b" value which was at refrigeration (3.00) was not significantly different from ambient condition (2.99).

Table 4.6: Effect of packaging materials and storage conditions on "b" of bissap drink

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	3.38b	3.97a	3.68a
Plastic	2.60c	2.02d	2.31b
Means	2.99a	3.00a	
HSD (0.01) PM=0.04, SC=0.04, PM×SC=0.07			

4.3.1.2 Total Soluble Solids (TSS)

There were significant differences in the packaging material for bissap drink stored (Figure 4.10). Significantly highest TSS (10° brix) was recorded by bissap drink stored in plastic bottle and the least was in glass bottle (9.68° brix).

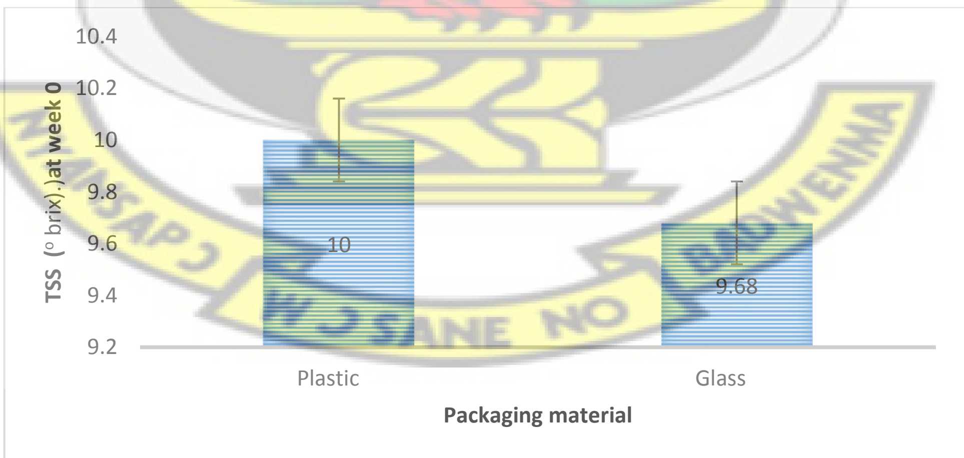

Figure 4.10: Effect of packaging material on TSS (° brix) of bissap drink at week 0

4.3.1.3 Total Titrable Acid (TTA)

There were significant differences between packaging material and storage condition interaction (Table 4.7). Significantly highest TTA was recorded by bissap drink stored in plastic bottle at ambient condition which was similar to those stored in plastic bottle at refrigeration condition and in glass bottle at ambient condition. The least TTA was glass bottle at refrigerator condition (0.27%). Across the packaging material, the highest TTA was in plastic bottle (0.45%) and the least was in glass bottle (0.36%). Across the storage conditions, the highest TTA was at ambient condition (0.45%) and the least was at refrigeration (0.36%).

Table 4.7: Effect of packaging materials and storage conditions on TTA (%) of bissap drink at week 0

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	0.44a	0.27b	0.36b
Plastic	0.45a	0.45a	0.45a
Means	0.45a	0.36b	
HSD (0.01)	PM=0.06, SC=0.06, PM×SC=0.12		

4.3.1.4 pH

There were no significant differences among the treatments.

4.3.1.5 Vitamin C

The packaging materials showed significant differences for bissap drink stored at week 0 for Vitamin C (Figure 4.11). Significantly highest Vitamin C was recorded by bissap drink stored in plastic bottle (4.37mg/100g) while the least was in glass bottle (3.53mg/100g).

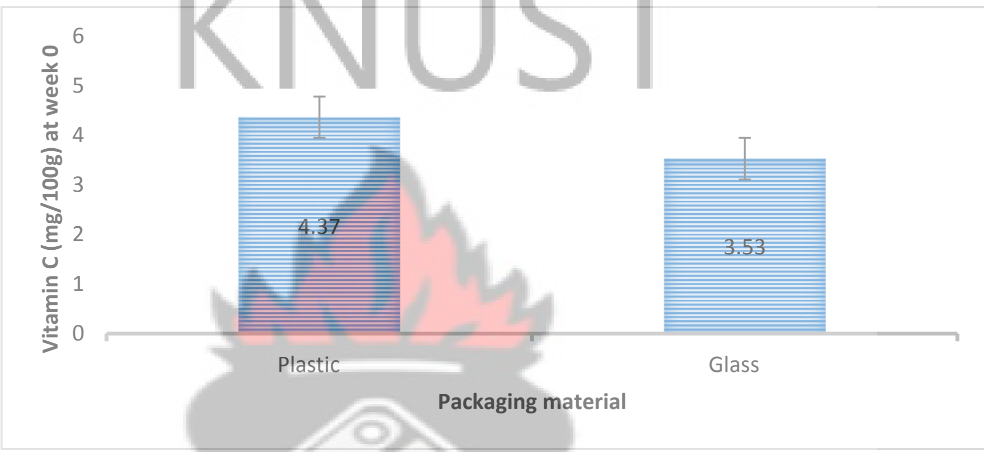

Figure 4.11: Effect of packaging material on Vitamin C (mg/100g) of bissap drink at week 0

4.3.1.6 Ash Content

There was an interactive effect of the packaging materials and the storage conditions (Table 4.8). Bissap drink stored under ambient condition in glass bottle was significantly highest (2.44%). Those stored in glass bottle under refrigeration (0.76%) was the same as those stored in plastic bottle under refrigeration (0.63%), thus recording the least. Glass bottle was significantly highest (1.60%) compared to plastic bottle which was the least (1.00%) with regards to packaging material. Refrigeration was the least (0.70%) compared to ambient condition which was significantly higher (1.91%) in terms of storage conditions.

Table 4.8: Effect of packaging materials and storage conditions on Ash (%) of bissap drink at week 0

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	2.44a	0.76c	1.60a
Plastic	1.37b	0.63c	1.00b
means	1.91a	0.70b	
HSD (0.01) PM=0.10, SC=0.10, PM×SC=0.18			

4.3.2 Week 1

4.3.2.1 Colour

***L**

Significant differences were observed between packaging material and storage condition interaction (Table 4.9). Bissap drink stored at refrigeration condition in glass bottle recorded the significantly highest “L” value (30.68). The least “L” value was glass bottle at ambient condition (26.57). The “L” value of the bissap drink in the packaging material differed significantly. The highest was in plastic bottle (29.26) and the least was in glass bottle (28.62). With the storage conditions, the highest was at refrigeration (30.50) and the least was at ambient condition (27.38).

Table 4.9: Effect of packaging materials and storage conditions on "L" of bissap drink at week 1

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	26.57d	30.68a	28.62b
Plastic	28.20c	30.32b	29.26a
Means	27.38b	30.50a	

HSD (0.01) PM=0.03, SC=0.03, PM×SC=0.05

***a**

There was an interactive effect of the packaging materials and the storage conditions (Table 4.10). Bissap drink stored under ambient condition in plastic bottle was significantly highest (19.89). Those stored in glass bottle under refrigeration condition was the least (15.06). Glass bottle and plastic bottle were significantly different with regards to packaging material in that, plastic bottle was highest (19.11) and glass bottle was the least (16.55). Ambient condition recorded the highest (18.96) while refrigeration was the least (16.70) in terms of storage conditions.

Table 4.10: Effect of packaging materials and storage conditions on "a" of bissap drink at week 1

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	18.03c	15.06d	16.55b
Plastic	19.89a	18.34b	19.11a
means	18.96a	16.70b	

HSD (0.01) PM=0.03, SC=0.03, PM×SC=0.06

***b**

There were significant differences between packaging material and storage condition interaction (Table 4.11). Significantly highest "b" value was recorded by bissap drink stored in glass bottle at ambient condition (2.89). The least "b" value was glass bottle at refrigeration condition (1.70). Across the packaging material, the highest "b" value was in plastic bottle (2.59) and the least was in glass bottle (2.30). Across the storage conditions, the highest "b" value was at ambient condition (2.89) and the least was at refrigeration (2.00).

Table 4.11: Effect of packaging materials and storage conditions on "b" of bissap drink at week 1

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	2.89a	1.70d	2.30b
Plastic	2.86b	2.30c	2.59a
Means	2.89a	2.00b	
HSD (0.01)	PM=9.69, SC=9.69, PM×SC=0.02		

4.3.2.2 Total Soluble Solids (TSS)

Significant differences were observed between packaging material and storage condition interaction (Table 4.12). Bissap drink stored at refrigeration condition in plastic bottle recorded the significantly highest TSS and was comparable to those stored in glass bottle at refrigeration and ambient condition. The least TSS was plastic bottle at ambient condition (9.37^{o} brix). The TSS level of the bissap drink in the packaging material differed significantly. The highest was in glass bottle (9.75^{o} brix) and the least was in plastic bottle (9.60^{o} brix). With the storage conditions, the highest was at refrigeration (9.82^o brix) and the least was at ambient condition (9.53^o brix).

Table 4.12: Effect of packaging materials and storage conditions on TSS (^{o} brix) of bissap drink at week 1

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	9.70a	9.80a	9.75a
Plastic	9.37b	9.83a	9.60b
Means	9.53b	9.82a	
HSD (0.01) PM=0.08, SC=0.08, PM×SC=0.15			

4.3.2.3 Total Titrable Acid (TTA)

There were significant differences in the storage condition for bissap drink stored at week 1 (Figure 4.12). Significantly highest TTA (0.25%) was recorded by bissap drink stored at refrigeration and the least was at ambient condition (0.16%).

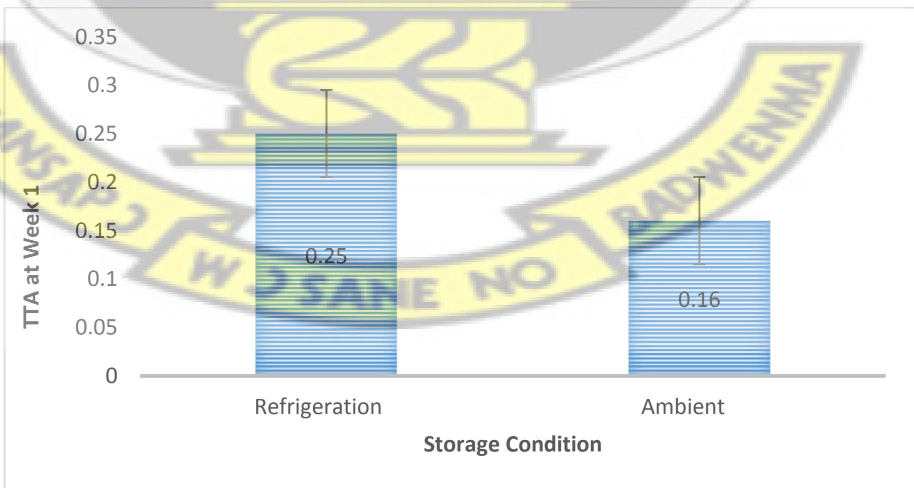

Figure 4.12: Effect of storage conditions on TTA (%) of bissap drink at week 1

4.3.2.4 pH

There was an interactive effect of the packaging materials and the storage conditions (Table 4.13). Bissap drink stored under ambient condition in plastic bottle was significantly highest (2.96) similar to those in glass bottle under refrigeration condition (2.96). Those stored in glass bottle under ambient condition was the least (2.90) and was the same as those stored in plastic bottle under refrigeration condition (2.88). Glass bottle and plastic bottle were significantly the same with regards to packaging material whiles refrigeration and ambient conditions were also significantly equal in terms of storage conditions.

Table 4.13: Effect of packaging materials and storage conditions on pH of bissap drink at week 1

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	2.90b	2.96a	2.93a
Plastic	2.96a	2.88b	2.92a
means	2.93a	2.92a	

HSD (0.01) PM=0.02, SC=0.02, PM×SC=0.03

4.3.2.5 Vitamin C

There were significant differences in the packaging materials and storage conditions for bissap drink stored at week 1 (Figure 4.13). Significantly highest Vit. C in the packaging material was recorded by bissap drink stored in plastic bottle (3.75mg/100g) and the least was in glass bottle (2.78mg/100g). Significantly highest Vit. C in storage conditions was recorded by bissap drink stored under refrigeration condition (3.58mg/100g) whiles the least was under ambient conditions (2.96mg/100g).

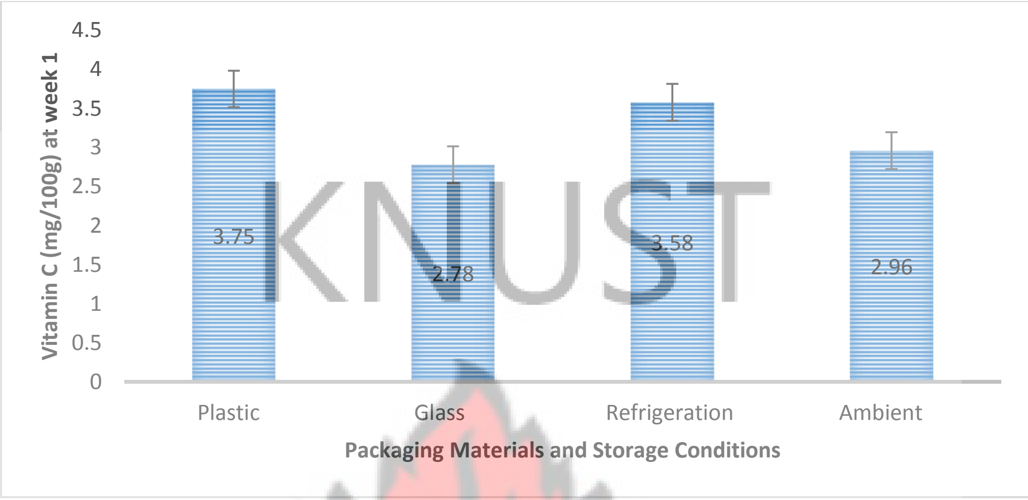

Figure 4.13: Effect of packaging materials and storage conditions on Vitamin C (mg/100g) of bissap drink at week 1

4.3.2.6 Ash Content

There were significant differences between packaging material and storage condition interaction (Table 4.14). Significantly highest ash content was recorded by bissap drink stored in plastic bottle at ambient condition (2.72%) which was similar to glass bottle at refrigeration condition (2.54%). The least ash content was recorded by plastic bottle at refrigeration condition (1.89%). Across the packaging material, there was no significant difference. Across the storage conditions, the highest ash content was at ambient condition (2.45%) and the least was at refrigeration (2.21%).

Table 4.14: Effect of packaging materials and storage conditions on Ash (%) of bissap drink at week 1

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	2.18b	2.54a	2.36a
Plastic	2.72a	1.89c	2.30a
means	2.45a	2.21b	

HSD (0.01) PM=0.10, SC=0.10, PM×SC=0.19

4.3.3 Week 2

4.3.3.1 Colour

***L**

Significant differences were observed between packaging material and storage condition interaction (Table 4.15). Bissap drink stored at ambient condition in glass bottle recorded the significantly highest "L" value (27.32). The least "L" value was glass bottle at refrigeration condition (24.71). The "L" value of the bissap drink in the packaging material differed significantly. The highest was in glass bottle (26.06) and the least was in plastic bottle (25.18). With the storage conditions, the highest was at ambient condition (26.22) and the least was at refrigeration condition (25.00).

Table 4.15: Effect of packaging materials and storage conditions on "L" of bissap drink at week 2

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	27.32a	24.71c	26.06a
Plastic	25.12b	25.24b	25.18b
Means	26.22a	25.00b	
HSD (0.01) PM=0.11, SC=0.11, PM×SC=0.21			

***a**

There was an interactive effect of the packaging materials and the storage conditions (Table 4.16). Bissap drink stored under refrigeration and ambient condition in plastic bottle was significantly highest though not significantly different. Those stored in glass bottle under ambient condition was the least (15.29). Glass bottle and plastic bottle were significantly different with regards to packaging material in that, plastic bottle was highest (20.73) and glass bottle was the least (16.70). Refrigeration condition recorded the highest (19.46) while ambient condition was the least in terms of storage conditions (17.97).

Table 4.16: Effect of packaging materials and storage conditions on "a" of bissap drink at week 2

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	15.29c	18.11b	16.70b
Plastic	20.65a	20.80a	20.73a
Means	17.97b	19.46a	
HSD (0.01) PM=0.15, SC=0.15, PM×SC=0.27			

***b**

There were significant differences between packaging material and storage condition interaction (Table 4.17). Significantly highest "b" value was recorded by bissap drink stored in plastic bottle at ambient condition (3.58). The least "b" value was glass bottle at ambient condition (2.06). Across the packaging material, the highest "b" value was in plastic bottle (3.46) and the least was in glass bottle (2.52). Across the storage conditions, the highest "b" value was at refrigeration (3.15) and the least was at ambient condition (2.82).

Table 4.17: Effect of packaging materials and storage conditions on "b" of bissap drink at week 2

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	2.06d	2.98c	2.52b
Plastic	3.58a	3.33b	3.46a
Means	2.82b	3.15a	

HSD (0.01) PM=0.05, SC=0.05, PM×SC=0.09

4.3.3.2 Total Soluble Solids (TSS)

From Table 4.18, the interactions recorded significant differences. Bissap drink stored at ambient condition in glass bottle recorded the significantly highest TSS and was similar to glass bottle at refrigeration condition and plastic bottle at ambient condition. The least TSS was plastic bottle at ambient condition (9.57^o brix). Across storage conditions, the highest TSS was at refrigeration (9.92^o brix) and the least was at ambient condition (9.75^o brix). Across, packaging material, the highest TSS was in glass bottle (9.93° brix) and the least was in plastic bottle (9.73° brix).

Table 4.18: Effect of packaging materials and storage conditions on TSS (° brix) of bissap drink at week 2

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	9.93a	9.93a	9.93a
Plastic	9.57b	9.90a	9.73b
Means	9.75b	9.92a	
HSD (0.01) PM=0.10, SC=0.10, PM×SC=0.18			

4.3.3.3 Total Titrable Acid (TTA)

Significant differences were recorded for both storage conditions and packaging materials stored at week 2 (Figure 4.14). With packaging materials, glass bottle was significantly higher (0.24%) while plastic bottle was the least (0.21%). Bissap drink stored at ambient condition recorded a significantly higher TTA (0.25%) while refrigeration scored the least (0.2%).

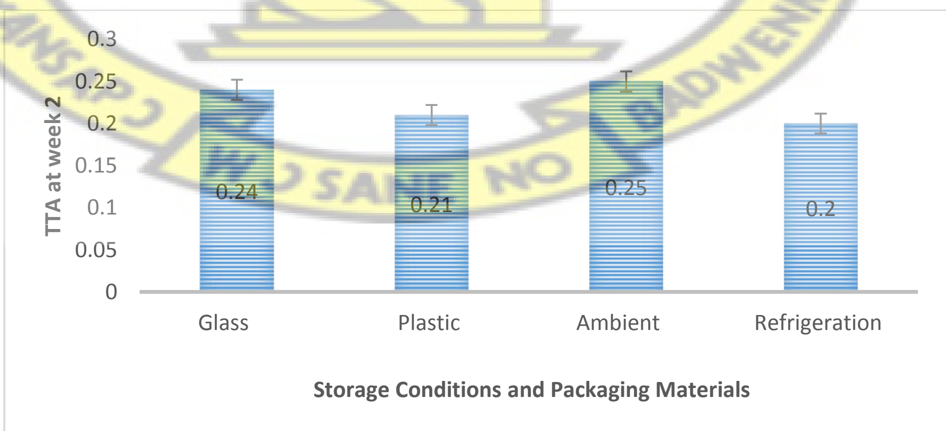

Figure 4.14: Effect of storage conditions and packaging materials on TTA (%) of bissap drink at week 2

4.3.3.4 pH

There were no significant differences among the treatments.

4.3.3.5 Vitamin C

There were significant differences in the packaging material for bissap drink stored at week 2 (Figure 4.15). Significantly highest Vit. C (3.16mg/100g) was recorded by bissap drink stored in plastic bottle and the least was in glass bottle (2.61mg/100g).

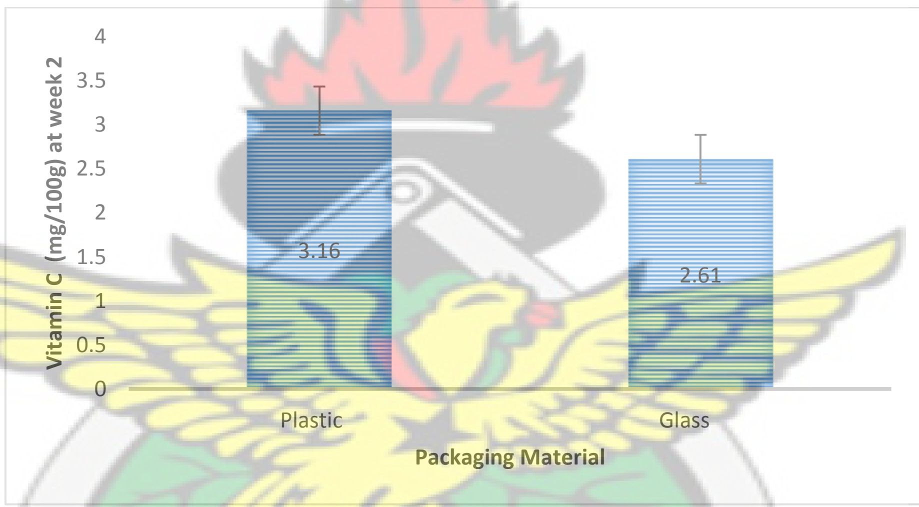

Figure 4.15: Effect of packaging material on Vitamin C (mg/100g) of bissap drink at week 2

4.3.3.6 Ash Content

There was an interactive effect of the packaging materials and the storage conditions (Table 4.19). Bissap drink stored under ambient condition in glass bottle was significantly highest (2.07%). Those stored in plastic bottle under ambient condition (0.42%) was the same as those stored in plastic bottle under refrigeration (0.46%), thus recording the least. Glass bottle was significantly highest (1.36%) compared to plastic bottle which was the least (0.44%) with regards to packaging material. Refrigeration was the least (0.56%) compared to ambient condition which was significantly higher (1.25%) in terms of storage conditions.

Table 4.19: Effect of packaging materials and storage conditions on Ash (%) of bissap drink at week 2

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	2.07a	0.65b	1.36a
Plastic	0.42c	0.46c	0.44b
means	1.25a	0.56b	

HSD (0.01) PM=0.06, SC=0.06, PM×SC=0.11

4.3.4 Week 3

4.3.4.1 Colour

***L**

Significant differences were observed between packaging material and storage condition interaction (Table 4.20). Bissap drink stored at refrigeration condition in glass bottle recorded the significantly highest "L" value (27.83). The least "L" value was plastic bottle at refrigeration condition which was similar to plastic bottle at ambient condition. The "L" value of the bissap drink in the packaging material differed significantly. The highest was in glass bottle (27.48) and the least was in plastic bottle (25.03). With the storage conditions, the highest was at refrigeration (26.40) and the least was at ambient condition (26.10).

Table 4.20: Effect of packaging materials and storage conditions on "L" of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	27.13b	27.83a	27.48a
Plastic	25.08c	24.98c	25.03b
Means	26.10b	26.40a	

HSD (0.01) PM=0.18, SC=0.18, PM×SC=0.33

***a**

There was an interactive effect of the packaging materials and the storage conditions (Table 4.21). Bissap drink stored under refrigeration condition in plastic bottle was significantly highest (20.15). Those stored in glass bottle under refrigeration condition was the least (14.75). Glass bottle and plastic bottle were significantly different with regards to packaging material in that, plastic bottle was highest (19.19) and glass bottle was the least (16.15). Ambient condition recorded the highest (17.89) while refrigeration was the least (17.45) in terms of storage conditions.

Table 4.21: Effect of packaging materials and storage conditions on "a" of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	17.55c	14.75d	16.15b
Plastic	18.23b	20.15a	19.19a
Means	17.89a	17.45b	
HSD (0.01) PM=0.23, SC=0.23, PM×SC=0.43			

***b**

There were significant differences between packaging material and storage condition interaction (Table 4.22). Significantly highest "b" value was recorded by bissap drink stored in glass bottle at ambient condition (3.21). The least "b" value was glass bottle at refrigeration condition (1.64). Across the packaging material, the highest "b" value was in plastic bottle (3.00) and the least was in glass bottle (2.43). Across the storage conditions, the highest "b" value was at ambient condition (3.08) and the least was at refrigeration (2.35).

Table 4.22: Effect of packaging materials and storage conditions on "b" of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	3.21a	1.64c	2.43b
Plastic	2.94b	3.06b	3.00a
means	3.08a	2.35b	
HSD (0.01) PM=0.06, SC=0.06, PM×SC=0.11			

4.3.4.2 Total Soluble Solids (TSS)

There were significant differences between packaging material and storage condition interaction (Table 4.23). Significantly highest TSS was recorded by bissap drink stored in glass bottle at refrigeration condition which was similar to those stored in plastic bottle at refrigeration condition and in plastic bottle at ambient condition. The least TSS was glass bottle at ambient condition (8.47^o brix). Across the packaging material, the highest TSS was in plastic bottle (9.82^o brix) and the least was in glass bottle (9.18^o brix). Across the storage conditions, the highest TSS was at refrigeration (9.83^o brix) and the least was at ambient condition (9.17^o brix).

Table 4.23: Effect of packaging materials and storage conditions on TSS (^{o} Brix) of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	8.47b	9.90a	9.18b
Plastic	9.87a	9.77a	9.82a
Means	9.17b	9.83a	

HSD (0.01) PM=0.10, SC=0.10, PM×SC=0.18

4.3.4.3 Total Titrable Acid (TTA)

Significant differences were observed between packaging material and storage condition interaction (Table 4.24). Bissap drink stored at ambient condition in glass bottle recorded the significantly highest TTA (0.25%). The least TTA was glass bottle at refrigeration condition which was similar to those stored in plastic bottle at ambient/refrigeration condition and glass bottle at refrigeration condition. Across storage conditions, the highest TTA was at ambient condition

(0.21%) and the least was at refrigeration (0.19%). Across, packaging material, the highest TTA was in glass bottle (0.22%) and the least was in plastic bottle (0.19%).

Table 4.24: Effect of packaging materials and storage conditions on TTA (%) of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	0.25a	0.18b	0.22a
Plastic	0.19b	0.20b	0.19b
means	0.21a	0.19b	

HSD (0.01) PM=0.02, SC=0.02, PM×SC=0.03

4.3.4.4 pH

There were significant differences between packaging material and storage condition interaction (Table 4.25). Significantly highest pH was recorded by bissap drink stored in plastic bottle at ambient condition (3.76) which was similar to those stored in plastic bottle at refrigeration condition (3.74) and in glass bottle at ambient condition (3.74). The least pH was glass bottle at refrigeration condition (3.68). Across the packaging material, the highest pH was in plastic bottle (3.75) and the least was in glass bottle (3.71). Across the storage conditions, the highest pH was at ambient condition (3.75) and the least was at refrigeration (3.71).

Table 4.25: Effect of packaging materials and storage conditions on pH of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	3.74a	3.68b	3.71b
Plastic	3.76a	3.74a	3.75a
means	3.75a	3.71b	

HSD (0.01) PM=0.02, SC=0.02, PM×SC=0.03

4.3.4.5 Vitamin C

There were no significant differences among the treatments.

4.3.4.6 Ash Content

From Table 4.26, the interactions recorded significant differences. Bissap drink stored at ambient condition in glass bottle recorded the significantly highest ash content (1.08%). The least ash content was in plastic bottle at refrigeration condition (0.34%) which was similar to those stored in plastic bottle at ambient condition (0.45%) and glass bottle at refrigeration condition (0.55%). Across storage conditions, the highest ash content was at ambient condition (0.75%) and the least was at refrigeration (0.45%). Across, packaging material, the highest ash content was in glass bottle (0.81%) and the least was in plastic bottle (0.40%).

Table 4.26: Effect of packaging materials and storage conditions on Ash (%) of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	means
Glass	1.08a	0.55b	0.81a
Plastic	0.45b	0.34b	0.40b
means	0.75a	0.45b	

HSD (0.01) PM=0.18, SC=0.18, PM×SC=0.34

4.4 SHELF LIFE STUDIES ON MICROBIAL LOAD OVER A FOUR WEEK PERIOD

4.4.1 Week 0

4.4.1.1 Aerobic Plate Count (APC)

There were no significant differences among the treatments.

4.4.1.2 Total Coliform

There were no significant differences among the treatments.

4.4.1.3 Mould Count

There were no significant differences among the treatments.

4.4.1.4 Yeast Count

There were no significant differences among the treatments.

4.4.2 Week 1

4.4.2.1 Aerobic Plate Count (APC)

There were significant differences in the storage conditions for bissap drink stored at week 1 (Figure 4.16). Significantly highest APC (125.5×10^3cfu/ml) was recorded by bissap drink stored under ambient condition and the least was at refrigeration (151×10^2 cfu/ml).

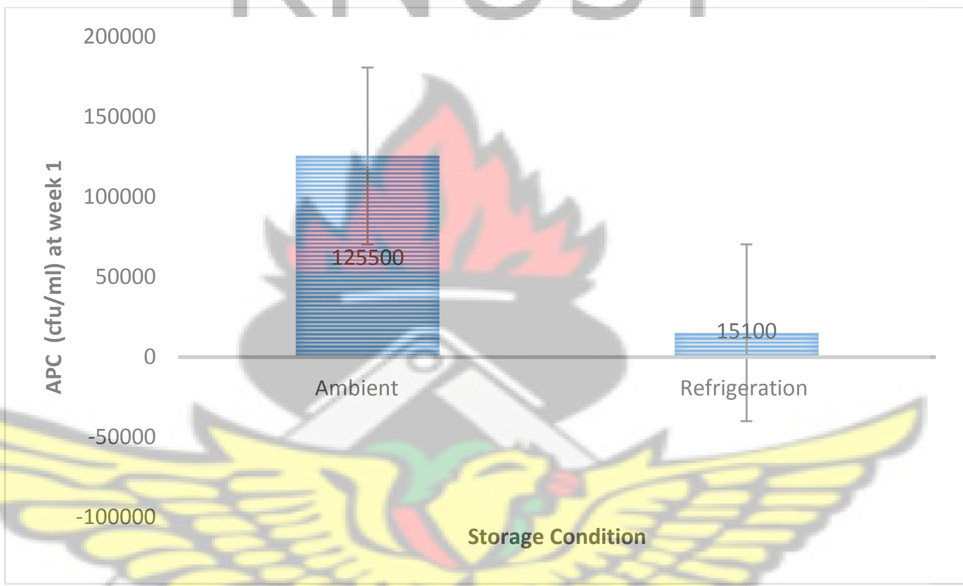

Figure 4.16: Effect of storage conditions on APC (cfu/ml) of bissap drink at week 1

4.4.2.2 Total Coliform

There were no significant differences among the treatments.

4.4.2.3 Mould Count

There were significant differences in the storage conditions for bissap drink stored at week 1 (Figure 4.17). Significantly highest mould count (6.3×10^2cfu/ml) was recorded by bissap drink stored under ambient condition and the least was at refrigeration (0 cfu/ml).

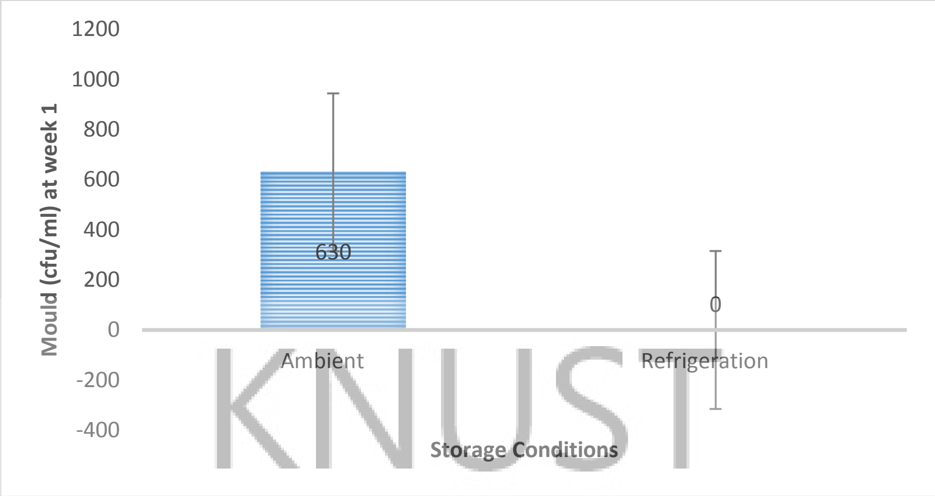

Figure 4.17: Effect of storage conditions on mould content (cfu/ml) of bissap drink at week 1

4.4.2.4 Yeast Count

There were no significant differences among the treatments.

4.4.3 Week 2

4.4.3.1 Aerobic Plate Count (APC)

There were significant differences in the storage conditions for bissap drink stored at week 2 (Figure 4.18). Significantly highest APC (17×10^4 cfu/ml) was recorded by bissap drink stored under ambient condition and the least was at refrigeration (295×10^1 cfu/ml).

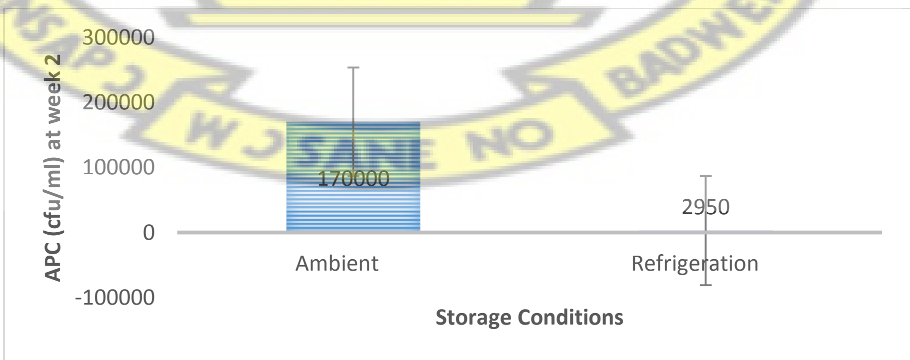

Figure 4.18: Effect of storage conditions on APC (cfu/ml) of bissap drink at week 2

4.4.3.2 Total Coliform

There were no significant differences among the treatments.

4.4.3.3 Mould Count

There were no significant differences among the treatments.

4.4.3.4 Yeast Count

There were no significant differences among the treatments.

4.4.4 Week 3

4.4.4.1 Aerobic Plate Count (APC)

There were significant differences in the storage conditions for bissap drink stored at week 3 (Figure 4.19). Significantly highest APC (2745×10^2 cfu/ml) was recorded by bissap drink stored under ambient condition and the least was at refrigeration (10.55×10^2 cfu/ml).

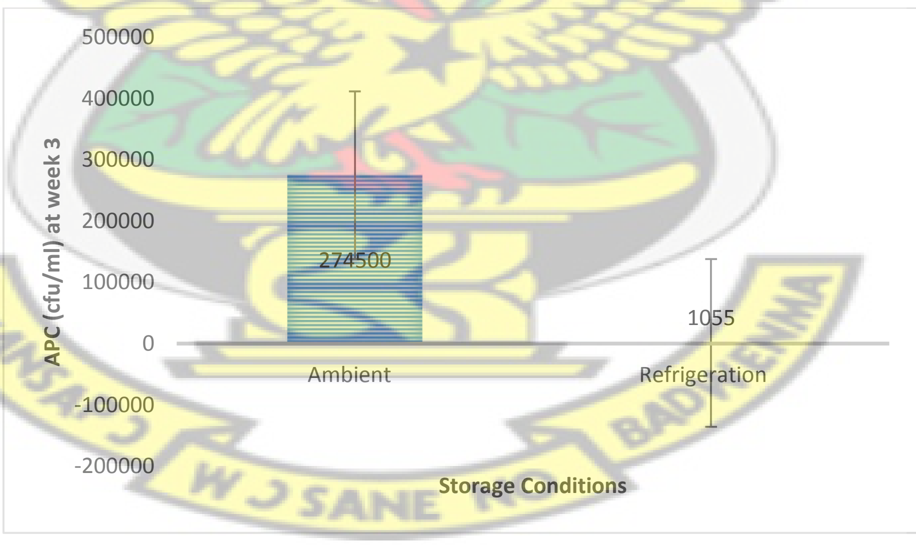

Figure 4.19: Effect of storage conditions on APC (cfu/ml) of bissap drink at week 3

4.4.4.2 Total Coliform

There were no significant differences among the treatments.

4.4.4.3 Mould Count

There were significant differences between packaging material and storage condition interaction (Table 4.27). Significantly highest mould count was recorded by bissap drink stored in plastic bottle at ambient condition (7×10^2cfu/ml). The least mould count was in glass bottle at refrigeration (0cfu/ml) which was similar to those stored in plastic bottle at refrigeration (0cfu/ml). Across the packaging material, the highest mould count was in plastic bottle (3.5×10^2cfu/ml) and the least was in glass bottle (1.55×10^2cfu/ml). Across the storage conditions, the highest mould count was under ambient condition (5.05×10^2cfu/ml) and the least was under refrigeration (0cfu/ml).

Table 4.27: Effect of packaging materials and storage conditions on mould content (cfu/ml) of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	310.00b	0.00c	155.00b
Plastic	700.00a	0.00c	350.00a
means	505.00a	0.00b	

HSD (0.01) PM=117.12, SC=117.12, PM×SC=234.46

4.4.4.4 Yeast Count

There were significant differences between packaging material and storage condition interaction (Table 4.28). Significantly highest yeast count was recorded by bissap drink stored in plastic bottle at ambient condition (6×10^2cfu/ml) and similar to glass bottle at ambient condition (1.7×10^2cfu/ml). The least yeast count was glass at refrigeration (0cfu/ml) which was similar to those stored in plastic at refrigeration (0cfu/ml). Across the packaging material, the yeast count was not significantly different. Across the storage conditions, the highest yeast count was under ambient condition (3.85×10^{2}cfu/ml) and the least was under refrigeration (0cfu/ml).

Table 4.28: Effect of packaging materials and storage conditions on yeast content (cfu/ml) of bissap drink at week 3

Packaging material (PM)	Storage condition (SC)		
	Ambient	Refrigeration	Means
Glass	170.00ab	0.00b	85.00a
Plastic	600.00a	0.00b	300.00a
Means	385.00a	0.00b	

HSD (0.01) PM=217.90, SC=217.90, PM×SC=436.22

CHAPTER FIVE

5.0 DISCUSSION

5.1 FORMULATION OF DRINK

The mixture of the three fruit is essential because it provides an alternative use of fruit species and offers a new way for fruit consumption while providing raw materials for the agro-industry (Oludemi and Akanbi, 2013). Calyces from the bissap, pineapple peels and bagasse obtained after extraction of sugarcane juice can be incorporated in animal feed. Mixed fruit juices add nutritional value and provide pleasant sensory attributes that is capable of gaining space in the consumer market (Zotarelli *et al.,* 2008).

5.2 SENSORY CHARACTERISTICS

5.2.1 Colour

From the study, all ten formulations were not significantly different from one another. Panellists rated the redness of the samples from "like moderately" to "like very much". The redness could be accounted for by the presence of a natural red colourant colour which may be due to the presence of high anthocyanin in the bissap calyces (Tsai and Ou, 1996; Delgado and Parceedes, 2003). Also, the high ratings of colour influenced the acceptability of flavour as described by Garber and Hyatt, (2000); Bayarri *et al*., (2001). Foods such as red beverages can be presumed to be sweet, thus, accounting for the high likeability of the formulations (Garder and Hyatt, 2000).

5.2.2 Clarity

Clarity as defined by Carrasco and Siebert, (1999) is how clear versus how cloudy beverages appear. From Table 4.3, panellists rated clarity from "like moderately" to "like very much". The mixture contour plot (Figure 4.4) suggests that higher concentrations of sugarcane gives the drink a hazy nature as F909 had proportions of bissap, pineapple and sugarcane at 30%, 20% and 50% respectively. The hazy nature of the sample is due to polyphenol-protein interactions which is commonly present in sugarcane (Jefremov *et al.*, 2007; Siebert, 2009).

5.2.3 Flavour

With respect to flavour, the ten samples did not differ significantly. On the hedonic scale, the samples ranged from "like slightly" to "like moderately". F776 which recorded the highest mean score (Table 4.3) had the highest proportion of pineapple. Pineapple which is an acidic fruit may have contributed to the enhanced flavour perceived by the panellists as indicated by Curi *et al.,* (2017).

5.2.4 Taste

There were no significant differences between the formulations. The samples were scored between "like slightly" to "like moderately". The taste was more pronounced when there were almost equal concentrations of pineapple or sugarcane as observed with product F205 which had concentrations of bissap, pineapple and sugarcane at 33%, 33% and 34% respectively. Lawless and Heymann, (2010) stated that the addition of flavour or other ingredients in formulating products produces estimated sensory matches to a near target which could be a standard formula or an existing successful product.

5.2.5 Sourness

Sourness is a taste sensation attributed to the presence of acid (Chambers and Lee, 2007). The most liked was F909 with mean score of 7.33 while the least like was F813 with mean score of 5.86 (Table 4.3). This could be attributed to the high concentration of sugarcane in F909 which masks the bitter taste associated with bissap (Table 4.2). This claim is supported by Lawless and Heymann, (2010) who stated that in fruit beverages and wines, the sourness of acids can be somewhat concealed by sweetness from sugar.

5.2.6 Sweetness

Product F776 had a mean score of 7.56 rating it as the most accepted. The least accepted was product F579 with a mean score of 6.16. The higher the concentration of bissap, the less sweet the product. Sweetness was scored high in F776 and F909 due to the high concentrations of pineapple and sugarcane compared to the other formulations. Pineapple is known to impart some level of sweetness (Bhakta*et al.*, 2012).

5.2.7 Overall acceptance

Overall acceptance has to do with the degree of satisfaction with the sensory qualities or performance of the product (Lawless and Heymann, 2010). The formulations were acceptable on the scale of “like slightly” to “like moderately”.

The most preferred was product F909 at a mean score of 7.63 followed by F776. The least accepted was F813. From the mixture contour plot (Figure 4.9), additions of pineapple and/or sugarcane with low levels of bissap can merit the acceptance of a bissap formulated drink. The high preference could be attributed to the level of flavour, bissap taste, sourness and sweetness of the formulations.

5.3 PHYSICOCHEMICAL PARAMETERS

5.3.1 Colour

***L**

Over the three week period, plastic in both refrigeration and ambient condition got darker. Glass bottle in ambient condition looked clearer while glass bottle in refrigeration fluctuated in clarity

***a**

Plastic bottle in refrigeration showed an increase in its redness over the storage period. Plastic bottle in ambient condition increased steadily but declined by week 3. Glass bottle in ambient declined by week 2 but started rising by week 3. Glass bottle in refrigeration experienced fluctuations over the period. The positive increasing and decreasing trend among the treatments showed the different levels of redness over the storage period. This could be due to the presence of red pigment in the bissap calyces. Chumsri *et al.,* 2008 and Cisse *et al.,* 2011 confirmed this by saying that factors such as light, pH, temperature, oxygen, ascorbic acid and sugar could result in the degradation or stability of anthocyanins. Also, the presence of polyphenoloxidase (PPO) could be a cause of colour change when it oxidises o-diphenols to o-quinones, which subsequently polymerise and produce brown pigments (Buta and Moline, 2001).

***b**

The yellow component of the drink in plastic bottle under refrigeration declined by week 3. A similar trend occurred for plastic bottle in ambient condition. Glass bottle in refrigeration declined by week 2 and rose in week 3. Fluctuations were observed for glass bottle under refrigeration. The positive values of all the treatments over the storage period showed the level of yellowness and this could be due to the presence of pineapple and sugarcane juices which are characterised by a yellow colour.

5.3.2 Total Soluble Solids (TSS)

At week 0, plastic recorded the highest TSS. Both in week 1 and 2, there was the observation that though TSS was reducing, plastic bottle stored in refrigeration had the highest TSS. By week 3, the trend was the same but plastic bottle in ambient condition also had the highest while glass bottle in ambient condition reduced. All the same, refrigeration for both glass bottle and plastic bottle had higher scores. Bhardwaj and Pandey, (2011) pointed out that a reduction in TSS of fruit drinks during storage is not a good quality that would aid in the preservation of the drink. On the other hand, sugarcane which was used as a sweetener is claimed by Chen *et al.*, (2013) to be appropriate for diabetics in place of aerated drinks and Cola.

5.3.3 Total Titrable Acidity (TTA)

The TTA analysis showed that the drink stored in plastic bottle and glass bottle under ambient condition and in plastic bottle under refrigeration (Table 4.19) had the highest TTA in week 0. Glass bottle at refrigeration recorded the lowest. By the first week, TTA reduced in both storage conditions though it was still high under refrigeration. Into the second week, TTA had decreased in both packaging material as well as under refrigeration but increased steadily under ambient condition. TTA was observed to have dropped further among the interactions though glass bottle at ambient condition was higher compared to the others. According to Bhardwaj and Pandey (2011), a decrease in acidity is possible because of acidic hydrolysis of polysaccharides where non-reducing sugars are converted into reducing sugars by the use of acid.

5.3.4 pH

The pH value obtained for week 0 showed no significant differences among the treatments. At week 1, drink in plastic bottle under ambient condition and glass bottle at refrigeration had the highest pH compared to drink in plastic bottle at refrigeration and glass bottle at ambient condition. Week 2 showed no significant differences among the treatments. By the end of the study, pH was shown to have risen in all treatments though glass bottle at refrigeration was the least. The increase in pH could be due to the fact that fruit juices are relatively rich in organic acids which tend to decrease over time (Tasnim *et al.,* 2010).

5.3.5 Vitamin C

Drink in plastic bottle was seen to have had the highest vitamin C content (4.37mg/100g) at week 0. Into the following week, a dip was observed in both packaging materials but was high under refrigeration (3.58mg/100g). Vitamin C continued to decrease in both packaging materials by the second week till there were no significant differences observed in the third week. The decrease in vitamin C may be due to the fact that, the packaging materials were susceptible to light, temperature, oxygen and other environmental factors (Gordon, 2006).

5.3.6 Ash content

Ash analysis saw drink in glass under ambient condition (2.4%) have a higher ash content compared to plastic bottle at ambient condition, glass bottle at refrigeration and plastic bottle at refrigeration. As storage period increased, there was further reduction in plastic bottle under refrigeration in week 1 and in week 2 in both plastic bottle on refrigeration and plastic bottle in ambient condition. By week 3, there was reduction in all treatments except plastic at ambient condition which increased slightly. The ash content recorded fell between 0.3 and a little above 2% which has been stipulated as the recommended range (Belitz and Grosch, 1999). Thus, the treatments did not have a significant effect on the ash content of the drink.

5.4 MICROBIAL STUDIES

5.4.1 Aerobic Plate Count (APC)

APC was not significant at the start of study. There was an indication of higher count under ambient condition compared to refrigeration in week 1. A similar trend was observed for week 2 and 3. APC at ambient condition continued to increase while APC decreased under refrigeration as the weeks progressed. This is deemed true by Wareing (2007) who reported that a heat resistant spore-forming aerobic organism known as *Alicyclobacillus acidoterrestris* can survive fruit juice heat treatments and grow in juices at temperatures of 26–50°C and a minimum pH of 2.0. Chilling of juice to below 20°C after pasteurization can control its growth and survival.

5.4.2 Total Coliforms

Analysis indicated no trace of coliforms over the storage period. This is in conformity with what Andres *et al.,* (2004) said stating that, the Safe Food Consumption Standard disallows coliforms in fruit juices.

5.4.3 Mould count

No mould count was recorded initially but was present under ambient conditions in week 1 while refrigeration recorded nothing. But by week 2, there was no trace of mould. Mould re-emerged at week 3 at a higher population under ambient conditions. As evidently confirmed by Rivas *et al*., (2006) who ascribed the presence of moulds to low pH values and high sugar content. Also, the presence of chitosan, which exhibits antimicrobial properties could have accounted for the suppression and re-emergence of mould population as observed by Roller and Covill (1999).

5.4.4 Yeast count

From week 0 to 2, there was the absence of yeast. However, yeast count was observed in the third week under ambient condition in both packaging materials where plastic bottle had higher count compared to glass bottle. The presence of yeast is due to their resistance to organic acid and defensive influence of sugars to especially heat resistant species as stated by Stratford *et al.*, (2000).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

A series of laboratory experiments were conducted to evaluate the effect of different levels of inclusion of sugarcane-pineapple cocktails and packaging materials on quality of "bissap"; a drink made from dried calyx of *Hibiscus sabdariffa*. The levels of inclusion were generated with the help of Minitab 2017 software and sensory attributes of the resulting cocktails were evaluated for their flavour, taste, sourness and sweetness. The results of the study showed that, a cocktail of 30% bissap extract, 20% pineapple and 50% sugarcane juice was the overall most acceptable cocktail. A 30% bissap extract, 40% pineapple and 30% sugarcane juice was the most acceptable in terms flavour. The most highly preferred taste was from the cocktail comprising 33% bissap extract, 33% pineapple and 34% sugarcane. In terms of sourness the most accepted cocktail was 30% bissap, 20% pineapple and 50% sugarcane juice. The sweetening effect derived from the combination of 30% bissap extract, 40% pineapple and 30% sugarcane juice was the most accepted among the other formulations.

The optimization analysis derived from the Minitab 2017 software used gave the most favourable product as 30% bissap extract, 38% pineapple and 32% sugarcane juice which was significantly different ($p \leq 0.05$) among the treatments over a four week storage period. The colour of the cocktail reduced in clarity from 33.78 in plastic bottle under ambient condition to 25.08, from 31.16 in glass bottle under ambient condition to 27.13, from 31.15 in plastic bottle under refrigeration to 24.98 and from 26.29 in glass which was significantly different ($p \leq 0.05$) among the treatments under refrigeration to 27.83. The total soluble acids (TSS) reduced from 10° brix to 9.82° brix in plastic bottle and from 9.68° brix to 9.18° brix in glass bottle. Total titrable acidity (TTA) dropped from 0.45% in plastic bottle under ambient condition to 0.19%, from 0.44% in glass bottle under ambient condition to 0.25%, from 0.45% in plastic bottle under refrigeration to 0.20% and from 0.27% in glass under refrigeration to 0.18%.

The pH levels increased from 2.96 in plastic bottle under ambient condition to 3.76, from 2.90 in glass bottle under ambient condition to 3.74, from 2.88 in plastic bottle under refrigeration to 3.74 and from 2.96 in glass bottle under refrigeration to 3.68. The vitamin C content decreased from 4.37 mg/100g to 3.16 mg/100g in plastic bottle and from 3.53 mg/100g to 2.78 mg/100g in glass bottle. The total ash dropped from 1.37 % in plastic bottle under ambient condition to 0.45 %, from 2.44 % in glass bottle under ambient condition to 1.08 %, from 0.63 % in plastic bottle under refrigeration to 0.34 % and from 0.76 % in glass bottle under refrigeration to 0.55 %. After four weeks of storage of the drinks, mould and yeast counts and aerobic plate count (APC) of microbes, were significantly high ($p \leq 0.05$) in the plastic bottles under ambient condition (22^{o}C to 28^{o}C) but there were no molds and yeasts in the refrigerated bottled drinks. No coliforms were present in all treatments throughout the storage period.

6.2 RECOMMENDATION

The study on bissap cocktail can be enhanced by focusing on

- A survey to ascertain the consumer acceptance of this optimized product with other already existing bissap formulations.
- Other types of packaging material that could prolong the storability of the cocktail.
- The addition of preservatives to increase the shelf-life of the cocktail.
- The effect of the cocktail on the health of diabetics.

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APPENDICES

APPENDIX 1

SENSORY EVALUATION FORM

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF HORTICULTURE

SENSORY EVALUATION SHEET

DATE:……………………………

PRODUCT: Bissap, Pineapple and Sugarcane Drink

INSTRUCTION:

Please, you have been provided with different formulatedBissap, Pineapple and Sugarcane Drinks. Please assess the following sensory attributes given below for each coded product, using the scale below. Please rinse your mouth in between samples with the water provided.

SCALE INTERPRETATION

9=like extremely 8=like very much 7=like moderately 6=like slightly

5=neither like nor dislike 4=dislike slightly 3=dislike moderately

2=dislike very much 1=dislike extremely

	SAMPLE CODE		
ATTRIBUTES			
Redness			
Clarity			
Flavor			
Bissap taste			
Sourness			
Sweetness			
Overall Acceptance			

Comments………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………
……………*Thanks for cooperating*

APPENDIX 2 - Regression co-efficient of the regression models of the various sensory attributes

Regression co-efficient	**Redness**	**Clarity**	**Flavor**	**Bissap taste**	**Sourness**	**Sweetness**	**Overall Acceptance**
Bissap	0.03	0.15	0.09	0.11	0.07	0.06	0.04
Pineapple	0.03	0.08	0.25	0.16	0.13	0.18	0.18
Sugarcane	0.01	0.12	0.15	0.17	0.22	0.18	0.18
Bissap and Pineapple	0.00	0.00	0.00	0.00	0.00	0.00	1.84
Bissap and Sugarcane	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pineapple and Sugarcane	0.00	5.76	0.00	0.00	0.00	0.00	0.00
R^2 (%)	71.04	73.18	63.94	62.43	79.44	91.04	86.39

Significance level at $p \leq 0.05$

APPENDIX 3 – ANOVA FOR THE VARIOUS SENSORY PARAMETERS

APPENDIX 3A - Analysis of Variance for Redness (component

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	0.258705	0.258705	0.051741	1.96	0.267
Linear	2	0.138133	0.022103	0.011051	0.42	0.683
Quadratic	3	0.120572	0.120572	0.040191	1.52	0.338
bissap*pineapple	1	0.036340	0.037276	0.037276	1.41	0.300
bissap*sugarcane	1	0.023192	0.023655	0.023655	0.90	0.397
pineapple*sugarcane	1	0.061040	0.061040	0.061040	2.32	0.203
Residual Error	4	0.105455	0.105455	0.026364		
Total	9	0.364160				

APPENDIX 3B- Analysis of Variance for Clarity

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	0.090018	0.090018	0.018004	2.18	0.235
Linear	2	0.023544	0.017937	0.008969	1.09	0.420
Quadratic	3	0.066473	0.066473	0.022158	2.69	0.182
bissap*pineapple	1	0.015571	0.015907	0.015907	1.93	0.237
bissap*sugarcane	1	0.050875	0.050859	0.050859	6.17	0.068
pineapple*sugarcane	1	0.000027	0.000027	0.000027	0.00	0.957
Residual Error	4	0.032992	0.032992	0.008248		
Total	9	0.123010				

APPENDIX 3C - Analysis of Variance for Flavor

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	0.439784	0.439784	0.087957	1.42	0.378
Linear	2	0.189411	0.109707	0.054854	0.88	0.481
Quadratic	3	0.250373	0.250373	0.083458	1.35	0.378
bissap*pineapple	1	0.054069	0.055598	0.055598	0.90	0.397
bissap*sugarcane	1	0.010624	0.011176	0.011176	0.18	0.693
pineapple*sugarcane	1	0.185680	0.185680	0.185680	2.99	0.159
Residual Error	4	0.248066	0.248066	0.062016		

Total	9	0.687850

APPENDIX 3D- Analysis of Variance for Bissap taste

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	0.94528	0.945277	0.189055	1.33	0.403
Linear	2	0.81441	0.024029	0.012014	0.08	0.921
Quadratic	3	0.13087	0.130866	0.043622	0.31	0.820
bissap*pineapple	1	0.02847	0.029406	0.029406	0.21	0.673
bissap*sugarcane	1	0.06251	0.063124	0.063124	0.44	0.542
pineapple*sugarcane	1	0.03988	0.039882	0.039882	0.28	0.624
Residual Error	4	0.56877	0.568773	0.142193		
Total	9	1.51405				

APPENDIX 3E - Analysis of Variance for Sourness

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	1.29525	1.295245	0.259049	3.09	0.148
Linear	2	1.07741	0.195979	0.097990	1.17	0.398
Quadratic	3	0.21783	0.217834	0.072611	0.87	0.528
bissap*pineapple	1	0.00442	0.003908	0.003908	0.05	0.840
bissap*sugarcane	1	0.09361	0.094912	0.094912	1.13	0.347
pineapple*sugarcane	1	0.11980	0.119799	0.119799	1.43	0.298
Residual Error	4	0.33524	0.335245	0.083811		
Total	9	1.63049				

APPENDIX 3F- Analysis of Variance for Sweetness

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	2.14986	2.149863	0.429973	8.13	0.032
Linear	2	2.03021	0.121842	0.060921	1.15	0.403
Quadratic	3	0.11965	0.119652	0.039884	0.75	0.575
bissap*pineapple	1	0.00787	0.008383	0.008383	0.16	0.711
bissap*sugarcane	1	0.03308	0.033706	0.033706	0.64	0.469
pineapple*sugarcane	1	0.07870	0.078701	0.078701	1.49	0.290
Residual Error	4	0.21163	0.211627	0.052907		
Total	9	2.36149				

APPENDIX 3G - Analysis of Variance for Overall acceptance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	1.01127	1.011266	0.202253	5.08	0.070
Linear	2	0.85703	0.169130	0.084565	2.12	0.235
Quadratic	3	0.15423	0.154233	0.051411	1.29	0.392
bissap*pineapple	1	0.00018	0.000275	0.000275	0.01	0.938
bissap*sugarcane	1	0.01349	0.014028	0.014028	0.35	0.585
pineapple*sugarcane	1	0.14056	0.140559	0.140559	3.53	0.134
Residual Error	4	0.15933	0.159334	0.039834		
Total	9	1.17060				

APPENDIX 4 – ANOVA FOR PHYSICOCHEMICAL PARAMETERS

APPENDIX 4A – COLOUR

a. Week 0

- L

Source	DF	SS	MS	F	P
PM	1	41.9628	41.9628	5086.40	0.0000
SC	1	42.2625	42.2625	5122.73	0.0000
PM*SC	1	3.7408	3.7408	453.43	0.0000
Error	8	0.0660	0.0082		
Total	11	88.0322			

Grand Mean 30.598   CV 0.30

- a

Source	DF	SS	MS	F	P
PM	1	64.7281	64.7281	24736.8	0.0000
SC	1	0.3300	0.3300	126.12	0.0000
PM*SC	1	14.7187	14.7187	5624.97	0.0000
Error	8	0.0209	0.0026		
Total	11	79.7977			

Grand Mean 19.519   CV 0.26

- b

Source	DF	SS	MS	F	P
PM	1	5.58968	5.58968	14271.5	0.0000
SC	1	0.00021	0.00021	0.53	0.4866
PM*SC	1	1.01501	1.01501	2591.51	0.0000
Error	8	0.00313	0.00039		
Total	11	6.60803			

Grand Mean 2.9925   CV 0.66

b. Week 1

- L

Source	DF	SS	MS	F	P
PM	1	1.2033	1.2033	6876.19	0.0000
SC	1	29.1408	29.1408	166519	0.0000
PM*SC	1	2.9800	2.9800	17028.8	0.0000
Error	8	0.0014	0.0002		
Total	11	33.3256			

Grand Mean 28.940   CV 0.05

- a

Source	DF	SS	MS	F	P
PM	1	19.7633	19.7633	74112.5	0.0000
SC	1	15.2776	15.2776	57291.1	0.0000
PM*SC	1	1.5123	1.5123	5671.13	0.0000
Error	8	0.0021	0.0003		
Total	11	36.5554			

Grand Mean 17.830   CV 0.09

- b

Source	DF	SS	MS	F	P
PM	1	0.23520	0.23520	9408.00	0.0000
SC	1	2.28813	2.28813	91525.3	0.0000
PM*SC	1	0.30083	0.30083	12033.3	0.0000
Error	8	0.00020	0.00002		
Total	11	2.82437			

Grand Mean 2.4383   CV 0.21

c. Week 2

- L

Source	DF	SS	MS	F	P
PM	1	2.0917	2.09168	603.37	0.0000
SC	1	4.6252	4.62521	1334.19	0.0000
PM*SC	1	5.5624	5.56241	1604.54	0.0000
Error	8	0.0277	0.00347		
Total	11	12.3070			

Grand Mean 25.598   CV 0.23

- a

Source	DF	SS	MS	F	P
PM	1	48.6421	48.6421	8350.58	0.0000
SC	1	6.6305	6.6305	1138.29	0.0000
PM*SC	1	5.3600	5.3600	920.18	0.0000
Error	8	0.0466	0.0058		
Total	11	60.6793			

Grand Mean 18.715   CV 0.41

- b

Source	DF	SS	MS	F	P
PM	1	2.63203	2.63203	4577.45	0.0000
SC	1	0.32670	0.32670	568.17	0.0000
PM*SC	1	1.02083	1.02083	1775.36	0.0000
Error	8	0.00460	0.00058		
Total	11	3.98417			

Grand Mean 2.9883 CV 0.80

d. Week 3

- L

Source	DF	SS	MS	F	P
PM	1	17.9341	17.9341	2135.01	0.0000
SC	1	0.2670	0.2670	31.79	0.0005
PM*SC	1	0.4840	0.4840	57.62	0.0001
Error	8	0.0672	0.0084		
Total	11	18.7523			

Grand Mean 26.254 CV 0.35

- a

Source	DF	SS	MS	F	P
PM	1	27.6337	27.6337	1899.22	0.0000
SC	1	0.5764	0.5764	39.62	0.0002
PM*SC	1	16.6852	16.6852	1146.75	0.0000
Error	8	0.1164	0.0146		
Total	11	45.0117			

Grand Mean 17.669 CV 0.68

- b

Source	DF	SS	MS	F	P
PM	1	0.98613	0.98613	986.13	0.0000
SC	1	1.59870	1.59870	1598.70	0.0000
PM*SC	1	2.13363	2.13363	2133.63	0.0000
Error	8	0.00800	0.00100		
Total	11	4.72647			

Grand Mean 2.7133 CV 1.17

APPENDIX 4B - TSS

a. Week 0

Source	DF	SS	MS	F	P
PM	1	0.24083	0.24083	72.25	0.0000
SC	1	0.00750	0.00750	2.25	0.1720
PM*SC	1	0.00750	0.00750	2.25	0.1720
Error	8	0.02667	0.00333		
Total	11	0.28250			

Grand Mean 9.8250 CV 0.59

b. Week 1

Source	DF	SS	MS	F	P
PM	1	0.06750	0.06750	40.50	0.0002
SC	1	0.24083	0.24083	144.50	0.0000
PM*SC	1	0.10083	0.10083	60.50	0.0001
Error	8	0.01333	0.00167		
Total	11	0.42250			

Grand Mean 9.6750 CV 0.42

c. Week 2

Source	DF	SS	MS	F	P
PM	1	0.12000	0.12000	48.00	0.0001
SC	1	0.08333	0.08333	33.33	0.0004
PM*SC	1	0.08333	0.08333	33.33	0.0004
Error	8	0.02000	0.00250		
Total	11	0.30667			

Grand Mean 9.8333 CV 0.51

d. Week 3

Source	DF	SS	MS	F	P
PM	1	1.20333	1.20333	481.33	0.0000
SC	1	1.33333	1.33333	533.33	0.0000
PM*SC	1	1.76333	1.76333	705.33	0.0000
Error	8	0.02000	0.00250		
Total	11	4.32000			

Grand Mean 9.5000 CV 0.53

APPENDIX 4C – TTA

a. Week 0

Source	DF	SS	MS	F	P
PM	1	0.02613	0.02613	23.76	0.0012
SC	1	0.02253	0.02253	20.48	0.0019
PM*SC	1	0.02083	0.02083	18.94	0.0024
Error	8	0.00880	0.00110		
Total	11	0.07830			

Grand Mean 0.4050 CV 8.19

b. Week 1

Source	DF	SS	MS	F	P
PM	1	0.00101	0.00101	4.48	0.0671
SC	1	0.02521	0.02521	112.04	0.0000
PM*SC	1	0.00101	0.00101	4.48	0.0671
Error	8	0.00180	0.00023		
Total	11	0.02903			

Grand Mean 0.2075 CV 7.23

c. Week 2

Source	DF	SS	MS	F	P
PM	1	0.00441	4.408E-03	15.11	0.0046
SC	1	0.00908	9.075E-03	31.11	0.0005
PM*SC	1	0.00008	7.500E-05	0.26	0.6258
Error	8	0.00233	2.917E-04		
Total	11	0.01589			

Grand Mean 0.2242   CV 7.62

d. Week 3

Source	DF	SS	MS	F	P
PM	1	1.408E-03	1.408E-03	16.90	0.0034
SC	1	2.408E-03	2.408E-03	28.90	0.0007
PM*SC	1	5.208E-03	5.208E-03	62.50	0.0000
Error	8	6.667E-04	8.333E-05		
Total	11	9.692E-03			

Grand Mean 0.2042   CV 4.47

APPENDIX 4D – pH

a. Week 1

Source	DF	SS	MS	F	P
PM	1	0.00030	0.00030	4.50	0.0667
SC	1	0.00013	0.00013	2.00	0.1950
PM*SC	1	0.01470	0.01470	220.50	0.0000
Error	8	0.00053	0.00007		
Total	11	0.01567			

Grand Mean 2.9233   CV 0.28

b. Week 3

Source	DF	SS	MS	F	P
PM	1	0.00563	5.633E-03	61.45	0.0001
SC	1	0.00480	4.800E-03	52.36	0.0001
PM*SC	1	0.00120	1.200E-03	13.09	0.0068
Error	8	0.00073	9.167E-05		
Total	11	0.01237			

Grand Mean 3.7317   CV 0.26

APPENDIX 4E – VITAMIN C

a. Week 0

Source	DF	SS	MS	F	P
PM	1	2.07501	2.07501	47.50	0.0001
SC	1	0.20021	0.20021	4.58	0.0647
PM*SC	1	0.22141	0.22141	5.07	0.0545
Error	8	0.34947	0.04368		
Total	11	2.84609			

Grand Mean 3.9492   CV 5.29

b. Week 1

Source	DF	SS	MS	F	P
PM	1	2.80333	2.80333	65.36	0.0000
SC	1	1.15320	1.15320	26.89	0.0008
PM*SC	1	0.05880	0.05880	1.37	0.2754
Error	8	0.34313	0.04289		
Total	11	4.35847			

Grand Mean 3.2667   CV 6.34

c. Week 2

Source	DF	SS	MS	F	P
PM	1	0.91301	0.91301	37.34	0.0003
SC	1	0.09188	0.09188	3.76	0.0886
PM*SC	1	0.00021	0.00021	0.01	0.9287
Error	8	0.19560	0.02445		
Total	11	1.20069			

Grand Mean 2.8808   CV 5.43

APPENDIX 4F – ASH

a. Week 0

Source	DF	SS	MS	F	P
PM	1	1.08000	1.08000	412.74	0.0000
SC	1	4.36813	4.36813	1669.35	0.0000
PM*SC	1	0.66270	0.66270	253.26	0.0000
Error	8	0.02093	0.00262		
Total	11	6.13177			

Grand Mean 1.3017   CV 3.93

b. Week 1

Source	DF	SS	MS	F	P
PM	1	0.00907	0.00907	3.34	0.1050
SC	1	0.17041	0.17041	62.73	0.0000
PM*SC	1	1.06208	1.06208	390.95	0.0000
Error	8	0.02173	0.00272		
Total	11	1.26329			

Grand Mean 2.3308   CV 2.24

c. Week 2

Source	DF	SS	MS	F	P
PM	1	2.52083	2.52083	2775.23	0.0000
SC	1	1.44213	1.44213	1587.67	0.0000
PM*SC	1	1.61333	1.61333	1776.15	0.0000
Error	8	0.00727	0.00091		
Total	11	5.58357			

Grand Mean 0.9017   CV 3.34

d. Week 3

Source	DF	SS	MS	F	P
PM	1	0.51667	0.51667	56.93	0.0001
SC	1	0.30401	0.30401	33.50	0.0004
PM*SC	1	0.13441	0.13441	14.81	0.0049
Error	8	0.07260	0.00908		
Total	11	1.02769			

Grand Mean 0.6042 CV 15.77

APPENDIX 4G – APC

a. Week 0

Source	DF	SS	MS	F	P
PM	1	1.818E-25	1.818E-25	1.78	0.2533
SC	1	1.307E-24	1.307E-24	12.78	0.0233
PM*SC	1	4.481E-59	4.481E-59	0.00	1.0000
Error	4	4.089E-25	1.022E-25		
Total	7	1.897E-24			

Grand Mean 9600.0

b. Week 1

Source	DF	SS	MS	F	P
PM	1	9.248E+07	9.248E+07	0.64	0.4690
SC	1	2.438E+10	2.438E+10	168.32	0.0002
PM*SC	1	9680000	9680000	0.07	0.8088
Error	4	5.793E+08	1.448E+08		
Total	7	2.506E+10			

Grand Mean 70300 CV 17.12

c. Week 2

Source	DF	SS	MS	F	P
PM	1	9245000	9245000	0.06	0.8135
SC	1	5.581E+10	5.581E+10	383.19	0.0000
PM*SC	1	6845000	6845000	0.05	0.8390
Error	4	5.826E+08	1.457E+08		
Total	7	5.641E+10			

Grand Mean 86475 CV 13.96

d. Week 3

Source	DF	SS	MS	F	P
PM	1	3.311E+09	3.311E+09	3.24	0.1461
SC	1	1.495E+11	1.495E+11	146.46	0.0003
PM*SC	1	3.251E+09	3.251E+09	3.18	0.1489
Error	4	4.084E+09	1.021E+09		
Total	7	1.602E+11			

Grand Mean 137778 CV 23.19

APPENDIX 4H – MOLD

Week 1

Source	DF	SS	MS	F	P
PM	1	7200	7200	1.36	0.3086
SC	1	793800	793800	149.77	0.0003
PM*SC	1	7200	7200	1.36	0.3086
Error	4	21200	5300		
Total	7	829400			

Grand Mean 315.00   CV 23.11

a. Week 3

Source	DF	SS	MS	F	P
PM	1	76050	76050	58.50	0.0016
SC	1	510050	510050	392.35	0.0000
PM*SC	1	76050	76050	58.50	0.0016
Error	4	5200	1300		
Total	7	667350			

Grand Mean 252.50   CV 14.28

APPENDIX 4I – YEAST

a. Week 3

Source	DF	SS	MS	F	P
PM	1	92450	92450	20.54	0.0106
SC	1	296450	296450	65.88	0.0013
PM*SC	1	92450	92450	20.54	0.0106
Error	4	18000	4500		
Total	7	499350			

Grand Mean 192.50   CV 34.85