

**DEVELOPMENT AND PRESERVATION OF A COCKTAIL BEVERAGE
PRODUCED FROM CARROT (*Daucus carrota*), PINEAPPLE (*Ananas comosus*)
AND MANGO (*Mangifera indica* L.)**

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IN

RADIATION PROCESSING

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DECLARATION

I, Akwesi Appiah Boateng, hereby declare that this thesis is a compilation of research results conducted by me and to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this work to Madam Adwoa Konadu Boateng and Miss Naa Anyankobea Ofori, who have been of immense help throughout my education to this level. I am very grateful for their involvement and encouragement in my life for which I know God will surely bless them plentifully.



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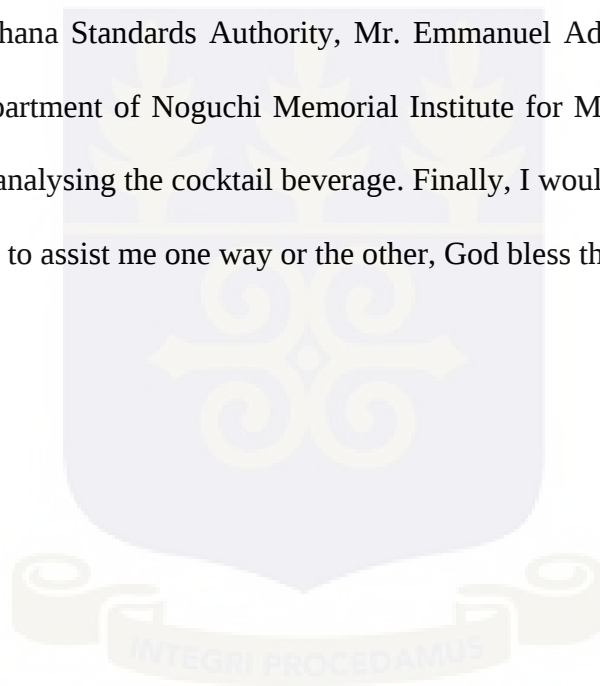
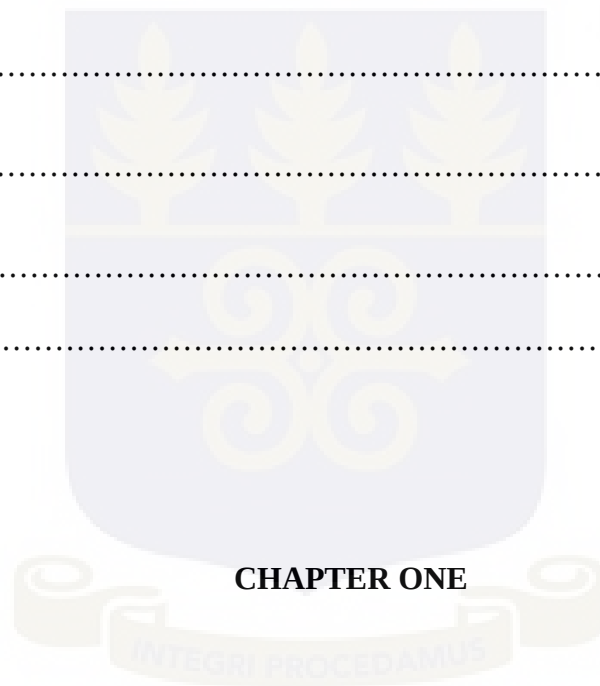


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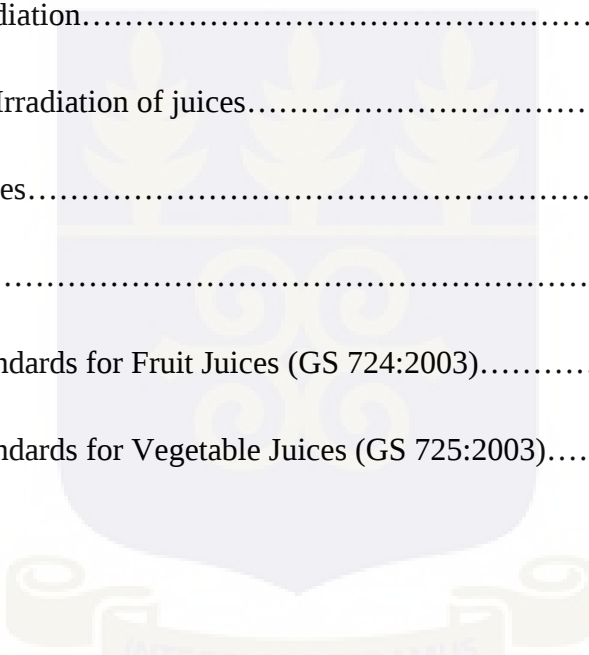
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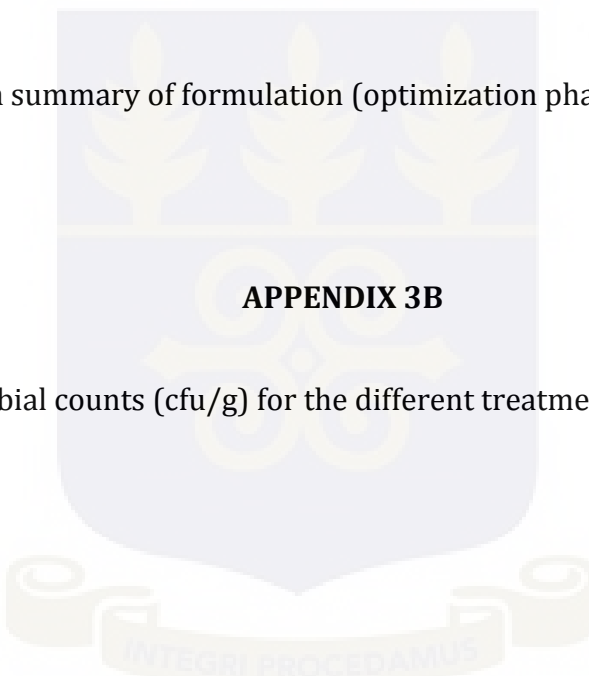
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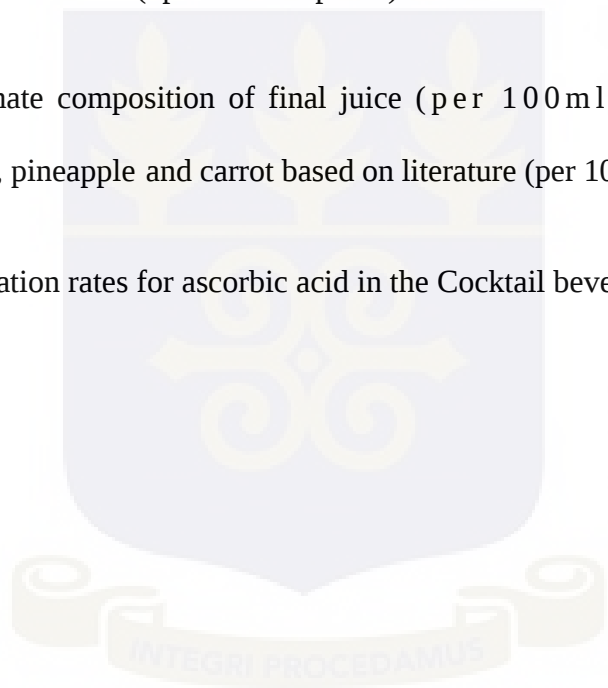


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ABSTRACT

Carrot is known to have the potential of providing vital nutrition as well as health and well-being to consumers. In this study, carrot juice was envisaged as a good vehicle for spreading its nutraceutical benefits. The study was then designed in two parts: (a) Survey to establish the beverage consumption pattern in the Anyaa-Sowutuom District in the Greater Accra Region of Ghana and (b) Carrot juice formulation and optimisation. The survey was conducted by administering a questionnaire to volunteer adult consumers. To develop an acceptable carrot juice beverage, pineapple juice and mango juice were included as components to improve taste and flavour respectively. A constrained, simplex centroid mixture design for three components was employed to optimise the proportions of the components in the final product based on sensory attributes. Chemical analyses as well as shelf stability studies were conducted for the optimised beverage preserved by chemicals and gamma radiation. Data obtained from the survey revealed that juices were very popular among consumers. The choice of a particular beverage was dependent on nutrition, taste and colour. Contour plots generated from sensory data of the products were overlaid to determine the optimum ratios of the components of the cocktail beverage. The optimised product consisted of 50-52 % Carrot, 18-20 % Pineapple and 28-30 % Mango. Analysis of the beverage revealed 223 mg/100ml Potassium, 3.92 mg/100ml Pro-vitamin A (Beta carotene) and 43 mg/100ml Vitamin C. After 8 weeks of storage 77 % Pro-vitamin A was retained in products chemically treated and as well as products irradiated at 1 kGy. However, about 60 % of this same nutrient was retained even under relatively higher dose (2.5 kGy) of gamma radiation. The microbial quality of the beverage was good under all conditions studied.

CHAPTER ONE

1.0 INTRODUCTION

High levels of fruit and vegetable consumption from epidemiological studies have suggested a reduction in the risk of developing diseases, particularly cancers, cardiovascular diseases (CVD), metabolic disorders and stroke (Sauvaget *et al.*, 2003; Joshipura *et al.*, 2001; Knekt *et al.*, 2001). Reduced overall risk of cancer mortality in Japanese populations has been associated with increased consumption of fruit and vegetables (Huang *et al.*, 2004; Suzuki *et al.*, 2002).

Fruit and vegetable juice intake has been recommended as healthy diet and has various health effects (Song *et al.*, 2006). Increased consumption of fruits and vegetable juices and a reduced consumption of sweetened beverages are some healthy dietary behaviors related to lower chronic disease risk (Pittler and Ernst, 2004) and body weight (Schulze *et al.*, 2004).

Carrot is a worldwide nutritional vegetable with immense health benefits (Karangwa *et al.*, 2010). It is a very good source of beta carotene, a vitamin A precursor (World Carrot Museum, 2012; Theodosiou *et al.*, 2010; Strube and OveDragsted, 1999; Novotny *et al.*, 1995), besides its appreciable amount of Vitamin C, Vitamin B6, thiamine, Vitamin K, Vitamin D, Vitamin E, folic acid and minerals (Dutta, 2011; Lintas, 1992). One medium size carrot provides four times the Daily Value of vitamin A (California Foundation for Agriculture in the Classroom, 2011).

Dietary consumption of foods rich in beta carotene have been reported to result in the elevation of beta carotene levels in the skin and a decreased sensitivity to stimulated sunlight (Kopcke and Krutmann, 2008; Stahl *et al.*, 2000). Increasing the beta-carotene consumption from 1.7 to 2.7 milligrams a day has been reported to reduce lung cancer risk by more than 40 % (Dutta, 2011; Stahl and Sies, 2005) and breast cancer is five to eight times less likely to develop in women who eat raw carrots (Dutta, 2011; Health Online Zine, 2010). Eating fiber rich carrots is also believed to reduce the risk of colon cancer by as much as 24 % (Carrot Nutrition Facts and Health Benefits, 2012; Dutta, 2011).

Falcarinol, a polyacetylene found in carrots has been found to reduce the rate of cancerous cells development (Purup *et al.*, 2009). Carrot is also a very good source of phytonutrient antioxidants such as alpha/beta-carotenes, lutein, ferulic acid and cyanidins which are believed to suppress lipid peroxidation (Potter, 2011), fight against free radicals to slow down the aging of cells and consequently other negative effects associated with aging (California Foundation for Agriculture, 2011).

Calcium pectate, a pectin fibre that has been found to have cholesterol lowering properties is also found in carrots (World Carrot Museum, 2012; Dutta, 2011). In a study on the therapeutic value of carrots, cholesterol levels were reduced by 11% when seven ounces of raw carrots a day were taken for three weeks (Organic Information Services, 2012). Dutta (2011) and Metzger and Barnes (2009) found that people who consumed carrots daily had one third the risks of heart attacks than those who did not.

Carrot juice has diuretic properties which help the body to eliminate excess fluid and keep infections and diseases at bay as well as preventing thread worms in children (Dutta, 2011). Consumption of this juice can pull heavy metals from fatty tissues and detoxify the body (Natural Home and Garden, 2011).

Carrot juice however is less acidic and has little flavour hence it is mostly blended with other fruits to enhance its acidity and flavour.

Pineapple (*Ananas comosus*) has long been one of the most popular of the non-citrus tropical and subtropical fruits, largely because of its immense vitamin C content, attractive flavour and refreshing sugar–acid balance (Deka, 2000). Pineapple juice is largely consumed around the world, mostly as a canning industry byproduct, concentrate and in the blend composition to obtain new flavours in beverages and other products (Awsi and Dorcus, 2012).

Mango (*Mangifera indica* L.) is a phytochemically dense fruit with high levels of carotenoids (*all-trans*-violaxanthin and *all-trans-beta*-carotene) and phenolic compounds (White gallic acids, Caffeic acids, Tannic acids) (Talcott *et al.*, 2005; Schieber *et al.*, 2000). It is a widely consumed fruit particularly appreciated for its fresh flavour, appreciable vitamins and antioxidants (Deka and Sethi, 2001).

Juice blending is one of the best methods to improve the nutritional quality of juices. It can improve the vitamin and mineral content depending on the kind and quality of the fruits and vegetables used (De Carvalho *et al.*, 2007). Aside the nutritional quality improvement,

blending can also improve the sensory and flavour characteristics of the juice as well as enhancing new product development (Jain and Khurdiya, 2004; Akinwale, 2000).

1.1 Rationale

Foods rich in beta-carotene are not only good sources of Vitamin A but may also help prevent cancer and other diet related and lifestyle diseases (Purup *et al.*, 2009). The growing consumer awareness on the influence of eating habits to their health has increased the demand for functional food products (Quarcoo, 2008). Gradually there has been a paradigm shift as there is an overall increase in natural juice consumption as an alternative to traditional caffeine containing and carbonated beverages (Adubofour *et al.*, 2010).

Food manufacturers continue to seek for product innovations geared towards the development and commercialization of functional foods. Production of fruits and vegetable juices has become an increasing interest of the beverage industry all over the world due to their low energy value and as an important source of vitamins and minerals (Awsii and Dorcus, 2012).

In recent years, a steady increase in carrot juice consumption has been reported in many countries (Schieber *et al.*, 2001). Development of this cocktail beverage will not only enhance the health of the people but create employment, wealth and also solve problems of post-harvest losses.

1.2 General Objective

The main objective of this project was to develop a consumer acceptable, beta carotene rich mixed fruit juice drink using carrots, mangoes and pineapples.

1.2.1 Specific Objectives

- i. To carry out a survey to determine the beverage consumption pattern in the Anyaa-Sowutuom district in the Greater Accra region.
- ii. To formulate a mixed fruit beverage with carrots, mangoes and pineapples.
- iii. To assess the acceptability of the product using a consumer panel.
- iv. To enhance the keeping quality of the final composite juice using chemical preservatives and gamma radiations.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Beverages

Beverages are simply drinks other than water (Encarta, 2009). They can be divided into two main categories; alcoholic (Beer, wine) and non-alcoholic beverages (Juices, tea, soda). The latter can further be subdivided into carbonated (coca cola, soda) and non-carbonated beverages (Juices, energy drink, tea). They are very essential to the physiological functions of the body as they are good sources of water and other stimulants (Ihekoronye and Ngoddy, 1985).

2.1.1 Carbonated drinks

These are sweetened, non-alcoholic drinks containing carbon dioxide. It excludes tea-based products and any products meeting specific sports or energy performance-enhancing claims. Syrups for home and out-of-home carbonated soft drink dispensers can also be grouped under carbonated drinks (www.wikipedia.org, 2008).

2.1.2 Nectar

This is a diluted fruit/vegetable juice and pulp, to which sweetening agents, permitted minerals and vitamins for the purpose of fortification and permitted additives may be added. If flavours or colourings from sources other than the reconstituted fruits are included in the product, it cannot be categorized as nectar regardless of its juice content. It may contain pulp and cells from the same kind of fruit. While juice content is required to

be equal to or in excess of 25% by volume, minimum juice content varies according to the fruit in question (www.wikipedia.org, 2008).

2.1.3 Sports Drink

A sport drink is a beverage designed to help athletes rehydrate, as well as replenish electrolytes, sugars and other nutrients that are lost or depleted after strenuous exercise, training or competition (Berning *et al.*, 1998). Sports drinks are performance-enhancing products, described as isotonic (in balance with), hypotonic (lighter than) or hypertonic (heavier than) to body fluids. They are popular among athletes because they provide the necessary electrolytes to support extended exercise. They help keep the body balanced and contain the right amount of electrolyte in the fluids (Berning *et al.*, 1998). Products must contain sodium citrate (acidity regulator), sweetening agents and either glucose or dextrose, and minerals such as calcium dicitrate, potassium chloride, magnesium phosphate or zinc sulphate (Sawka, 2007; www.wikipedia.org, 2008).

2.1.4 Energy Drinks

These are energy-enhancing products, mainly carbonated and containing taurine, guarana, glucose, caffeine and other exotic herbs and substances, as well as added minerals and vitamins. The category includes still or carbonated, ready to drink products, non-ready to drink powders and concentrates (Finnegan, 2003; Reyner and Horne, 2002).

2.1.5 Juice

Codex Alimentarius (2005) defines juice as “the fermentable but unfermented juice, intended for direct consumption, obtained by the mechanical processing of sound, ripe fruits/vegetables, preserved exclusively by physical means. The juice must have the characteristic colour, flavour and taste typical of the fruit/vegetable from which it comes. It may be turbid or clear.

The juice may have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factors of the juice. The addition of sugars or acids can be permitted but must be endorsed in the individual standard (Song *et al.*, 2006; www.wikipedia.org, 2008).

2.1.5.1 Health Benefits of Juices

In many communities among the developed countries, juices are becoming an important part of their diet. They act as a nutritious beverage and play a significant part in a healthy diet as they offer good taste and a variety of nutrients found naturally in fruits and vegetables.

Hundred percent (100%) fruit and vegetable juices provide a range of nutrients including vitamins and minerals such as vitamin C, folate, potassium and antioxidants. They contain many of the nutrients of their vegetable/fruit origin but have lower fibre contents. Vegetable juices may be considered a healthier alternative to fruit juices as they have fewer kilojoules (less carbohydrate) but usually have more sodium (Deanna and Jeffrey, 2007; Franke *et al.*, 2005).

Beta carotene, the famous ingredient in carrots, is an anti-oxidant that has powerful healing virtues for many diseases and also lowers the risk of some lung and colon cancers. It is also an excellent drinkable source of potassium, which is very important in helping to maintain a healthy electrolyte balance and fluid level in the cells of the body. Lower cholesterol levels in the serum have also been attributed to pectin in carrots (World carrot museum, 2012).

Orange juice is rich in vitamin C, folic acid, potassium, and an excellent source of bioavailable antioxidant phytochemicals (Franke, *et al.*, 2005). It significantly improves blood lipid profiles in people affected with hypercholesterolemia (Reyner and Horne, 2002).

Cranberry juice has long been known to help prevent or even treat bladder infections, and it is now known that a substance in cranberries prevents bacteria from binding to the bladder (Kurowska *et al.*, 2000).

Fruit juices are known to raise serum antioxidant capacity and offset the oxidative stress and inflammation normally caused by high-fat and high-sugar meals (Ghanim *et al.*, 2010). Fruit juice intake has been consistently associated with reduced risk of many cancer types (Brock *et al.*, 1988) and delay the onset of Alzheimer's disease (Dai *et al.*, 2006).

2.1.5.2 Juice Blending

Blending of fruits and vegetable juices has proven to improve the nutritional profile of juices. Carrots are a very good source of beta carotenes, pineapples and oranges are rich in

vitamin C and other natural antioxidants (Karangwa *et al.*, 2010), mangoes are rich in fibre and moringa leaves have been reported to have high amounts of essential amino acid with the right balance, as well as high amounts of minerals and vitamins (Fuglie, 1999). Mixing two or more kinds of fruits/vegetables can result in a product with more vitamins and minerals and with different sensory and flavour characteristics when compared to the raw materials (Jain and Khurdiya, 2004; Rodrigo *et al.*, 2003; Akinwale, 2000).

2.2 The role of beverages in obesity

Obesity has been described as having a body weight more than 20 percent greater than recommended for the relevant height (Encarta, 2009). A decrease in the amount of calories consumed while maintaining or increasing physical activity has been found to control this condition (Baranowski *et al.*, 2000). The cause of obesity is not a single food or food group as often portrayed. While many factors can influence an individual's weight, the maintenance of body weight requires a balance between total energy ingested and total energy expended. Increases in body weight and body fat content occur only when chronic energy intake exceeds energy expenditures (Dietz, 1998).

The prevalence of overweight and obesity among children in Saudi Arabia has previously been reported to be between 8-14% and 6-17% respectively (El-Hazmi and Warsy, 2002; Farghaly *et al.*, 2007). Obesity is a risk factor for cardiovascular disease (Bray and Gray, 1988), diabetes (Bray *et al.*, 2008) and certain types of cancer (Fair and Montgomery, 2009). It can also be associated with non-fatal but debilitating illnesses such as respiratory difficulties and infertility (Dietz, 1998).

Non-alcoholic beverages may also contain added natural or artificial sugars such as sucrose, fructose, aspartame and sorbitol for purposes of preservation or taste enhancement. In the United States of America where obesity is on the rise, a positive correlation has been established between increased consumption of sugar sweetened beverages and high calorie content (Ebbeling *et al*, 2006). Regular soft drinks and fruit drinks contributed 7% of Americans' total caloric intake during 1999-2000 (Block, 2004). Again, caloric intake was positively associated with intake of non-diet carbonated soft drinks in 1994 (Harnack *et al.*, 1999).

As part of a prospective study conducted from 1991-1999 (Nurses' Health Study II), Schulze *et al.*, (2004) collected self-reported weight and beverage intake information multiple times from 51,603 women. Weight gains and increases in body mass index (BMI) were highest among participants who increased their sugar-sweetened carbonated soft drink intake from less than one drink per week to greater than one drink per day ($P < 0.001$). Weight gains and increases in BMI were lowest among women who decreased their intake from more than one drink per day to less than or equal to one drink per week. Similarly, increased intake of fruit punch was associated with significantly ($P < 0.01$). greater weight gain than was decreased intake. Women who reported stable beverage consumption had no significant weight gains (Schulze *et al.*, 2004).

2.3 Juice Microflora

Fruit juices contain water, sugars, organic acids, vitamins, and trace elements which provide an ideal environment for spoilage by microorganisms. They generally have a lower pH ($\text{pH} < 4.5$), thus requiring their potential spoilage agents to be acid-loving

microorganisms (Keller and Miller, 2006). The most commonly encountered microbial genera are *Acetobacter*, *Alicyclobacillus*, *Bacillus*, *Clostridium*, *Gluconobacter*, *Lactobacillus*, *Leuconostoc*, *Saccharobacter*, *Zymomonas*, and *Zymobacter* (Vasavada, 2003).

Yeast are however predominant because of their high acid tolerance and the ability of many species to grow anaerobically (Boekhout and Robert, 2003). Most spoilage yeasts are highly fermentative, forming ethanol and CO₂ from sugar, causing split cans and cartons, and explosions in glass or plastic bottles. Amongst the spoilage yeasts, *P. membranifaciens* is considered as the target microorganism for the optimization of thermal treatments of juices because it is resistant to heat as well as to moderate amounts of salt, SO₂, sorbic, benzoic and acetic acids (Boekhout and Robert, 2003).

Acid-tolerant bacteria able to grow in juices include lactic acid bacteria (*Lactobacillus* and *Leuconostoc spp.*) and acetic acid bacteria (*Acetobacter* and *Gluconobacter spp.*). *Bacillus* species are known to cause flat-sour type spoilage in acidic fruit beverages. Production of lactic acid without gas formation makes *Lactobacillus plantarum*, *Lactobacillus brevis* and *B. coagulans* resistant to thermal treatments (Vasavada, 2003; Boekhout and Robert, 2003). Most spoilage incidents consist mainly of an off-flavour or odour production, with or without sediment; sometimes discolouration or cloudiness occurs. Consumer complaints are often the only reason for companies becoming aware of the problem, since the absence of gas production make spoilage difficult to detect. The off-flavour and odour detected have been attributed to the chemical compound guaiacal, (Steyn *et al.*, 2011; Parish, 2006)

which has been described as medicinal, disinfectant-like, antiseptic, phenolic and smoky in most cases. *Alicyclobacillus acidoterrestris*, a thermo-acidophilic endospore-forming bacterium, emerged in large scale spoilage incidents in Germany, during which flat sour type spoilage with offensive smelling medicinal or antiseptic characteristics were noted in commercial pasteurized apple juice (Bevilacqua *et al.*, 2011).

Table 2.1 Microorganisms related to spoilage in fruit juices.

Microorganisms	Spoilage Effects
Highly fermentative yeasts	Production of ethanol and CO ₂ from sugar Formation of biofilm Bulging or exploding of containers
<i>Acetobacter spp.</i> , <i>Gluconobacter spp.</i>	Oxidation of ethanol Fermentation Turbidity
<i>Lactobacillus spp.</i> , <i>Leuconostoc spp.</i>	Sour or off-taste Buttermilk off-flavour Gummy slime or ropiness
<i>Clostridium spp.</i>	Production of acetic acid, CO ₂ , ethanol Production of gas Strong butyric odour Increased acidity
<i>A. acidoterrestris spp.</i>	Phenolic or antiseptic odour Off-flavour with or without light sediment or

	slight haze
<i>Bacillus spp.</i>	Flat sour
<i>Zymomonas spp.</i> , <i>Saccharobacter</i>	Ethanol production
<i>spp.</i> , <i>Zymobacter spp.</i>	
Heat resistant moulds	Off-flavour or odour like “stale” or “old”
	Development of a mycelial material
	Reduction in sugar content
	Mycotoxin production

Source: Bevilacqua *et al.*, (2011).

2.4 Safety of fruit and vegetable juices

Over 1700 people in North America fell ill in the last decade after consuming juice and cider. Most of these outbreaks involved unpasteurized juices such as apple, orange, lemon, pineapple, carrot, coconut, cane sugar, banana, acai and mixed fruit juices (Vojdani *et al.*, 2008). The most common pathogens were *Escherichia coli* O157:H7, *Salmonella sp.*, and *Cryptosporidium*. A few other outbreaks were due to *Vibrio cholerae*, *Clostridium botulinum* and yeasts (Keller and Miller, 2006).

Salmonella infections have most often been associated with animal derived foods, such as seafood, egg products, meat, and dairy foods rather than juices. However, outbreaks associated with fresh juice have occurred as far back as 1922. Early outbreaks resulting in typhoid fever were associated with poor hygiene by food handlers (Vojdani *et al.*, 2008).

Outbreaks of typhoid fever have become far less common in developed countries as disinfection of water, sanitation procedures, and hygiene practices have improved. Nonetheless, given the dramatic increase of fresh fruit imported from developing countries, typhoid fever outbreaks associated with these commodities remain a concern (Parish, 2006).

More recent outbreaks of non-typhoidal salmonellosis in fresh juice have been attributed to fecal-associated contamination of fruits and vegetables or poor processing practices. In 2005, 152 cases of *Salmonella typhimurium* infection associated with commercially distributed unpasteurized orange juice were reported in the USA (Raybaudi-Massilia *et al.*, 2009).

Several species of moulds are capable of producing different mycotoxins in fruit juices. Mycotoxins, particularly patulin, represent a potent food safety hazard. Some moulds, e.g. *Penicillium expansum*, *P. griseofulvum*, *P. roqueforti*, *P. funiculosum*, *P. claviforme*, *P. granulatatum*, and *Byssochlamys spp.*, produce patulin in apple juice, while others, such as *Neosartorya* produce fumitremorgins, terrein, verruculogen, and fischerin. *Byssochlamys* species also produce byssotoxin A and byssochlamic acid. Other mycotoxins produced in fruit juice by moulds include ochratoxin A, citrinin, and penicillic acid (Vasavada, 2003).

Although not implicated in foodborne outbreaks associated with fresh juices, another important pathogen is *Listeria monocytogenes*. It has the ability to grow at conventional refrigeration temperatures and under acidic conditions. It is ubiquitous within the environment, carried by animals, and frequently found on fruits (Keller and Miller, 2006).

The minimum pH for growth of *L. monocytogenes* is dependent on the acidulant. For malic acid, the lowest pH value for growth is from 4.4 to 4.6 depending on the strain. This pathogen causes listeriosis, a serious disease with complications including meningitis, septicemia and spontaneous abortion in pregnant women (Keller and Miller, 2006; Vasavada, 2003).

2.5 Carrot (*Daucus carota*): Distribution, Nutrient Composition and Uses

Carrot originates from the wild forms growing in Europe and southwestern Asia (Banga and Simmonds, 1984). The western type of cultivated carrot is thought to derive from the anthocyanin-containing forms found in Afghanistan. Cultivation of carrot spread to Europe in the fourteenth century. The first cultivated carrot types were purple or violet, yellow and later orange types were derived from this anthocyanin type by selection. The orange-coloured form was selected in the Netherlands in the early seventeenth century.

In 1998, 18.5 million tonnes of carrots were produced worldwide in an area of 794,000 hectares (FAO 1999). On a global scale, carrot is only a minor crop, but in northern countries of Europe, it is one of the major field vegetables. In Finland in 1995–1998, the yearly production area of carrot ranged from 1650 to 1954 hectares, which makes carrot the most common field vegetable after garden pea (Information Centre of the Ministry of Agriculture and Forestry, 1999).

2.6 Mango (*Mangifera indica*): Distribution, Nutrient Composition and Uses

The mango fruit is one of the most popular fruits for millions of people in the world. It is considered to be the choicest of all indigenous fruits in tropical areas. Mango as an emerging tropical fruit is produced in over 90 countries worldwide with production of over 28.51 million metric tonnes in 2005. Asia accounts for approximately 77% of global mango production, and the Americas and Africa account for approximately 13% and 9%, respectively (FAOSTAT, 2007). Currently, only about 3% of the world production of mango is traded globally representing a noticeable increase over the quantities traded 20 years ago (Evans, 2008).

Mango grows in a slightly acidic (5.5-7.5) and well-drained soil, whether it is sandy, loam or clay (Botting *et al.*, 1999). It is somewhat tolerant to alkalinity (Kadman *et al.*, 1976).

Mango is also drought-tolerant, and can withstand occasional flooding (Singh, 1960). For best flowering and fruit set, good timely rainfall is necessary rather than the total rain fall.

Temperature plays an important role in mango flowering and its influence varies with cultivars (Schaffer *et al.*, 1994). Temperatures in the range of 24-30 °C are required for best flowering; however, during fruit development if sufficient water is provided the tree can withstand up to 48 °C.

In Ghana, fruits and vegetables are abundantly produced during peak seasons but due to lack of proper storage and preservation facilities, the market becomes overstocked during such seasons and a large proportion get rotten before reaching the final consumer.

Alzamora *et al.*, (2000) reported that about 30-50% of fruits and vegetables harvested in developing countries including Ghana are never consumed due to spoilage during transportation, storage and processing.

Mango is a phytochemically dense food with high levels of carotenoids and phenolic compounds (Talcott *et al.*, 2005; Schieber *et al.*, 2001; Schieber *et al.*, 2000). Gowda and Huddar, (2000) examined eight (8) tropical fruits and showed that ripe mango had the highest gallic acid content and total polyphenolics compared with the other fruits. Mercandante and Rodriquez-Amaya (1998) found *all-trans*-violaxanthin to be the predominant carotenoid in mangoes followed by *all-trans*- β -carotene. Botting *et al.*, (1999) examined the antimutagens in 25 plant foods, using the *Salmonella typhimurium* mutagenicity assay against heterocyclic amine 2-amino-3-methylimidazo. Data obtained from that study indicated strong antimutagenic properties in several plants, including mango. The presence of polyphenolics, carotenoids, and antimutagens in mango suggests significant anticancer activity.

2.7 Pineapple (*Ananas comosus*): Distribution, Nutrient Composition and Uses

Pineapple, a tropical plant with edible multiple fruit consisting of coalesced berries, is the most economically important plant in the *Bromeliaceae* family (Mynott *et al.*, 1999). It is an herbaceous perennial which grows to 1.0 to 1.5 meters (3.3 to 4.9 ft) tall, although sometimes it can be taller. The plant itself has a short, stocky stem with tough, waxy leaves. When creating its fruit, it usually produces up to 200 flowers, although some large-fruited cultivars can exceed this. The individual fruit of the flowers join together to form

what is commonly referred to as pineapple. After the first fruit is produced, side shoots (suckers) are produced in the leaf axils of the main stem. These may be removed for propagation or left to produce additional fruits on the original plant (Mynott *et al.*, 1999).

Pineapples were discovered in South America and later dispersed into other regions of the world by travelers and seafarers. It was introduced into the Philippines, Hawaii and Guam during the early 16th century by Spaniards and reached India and the east and west coasts of Africa by 1548. In 1594, it was reported growing in China and South Africa in 1655.

The successful dispersion of pineapples on a worldwide basis can be attributed to its ability to tolerate drought and the relative ease with which vegetative propagation can be established under cultivated conditions (Purseglove, 1972; Collins, 1960).

Pineapple is cultivated predominantly for its fruit that is consumed fresh or canned. It is a good source of manganese and contains significant amounts of vitamins C and B1. It is used as an ingredient in a variety of foods including pizzas, condiments, sweets, savouries, cakes, pastries and ice creams (Medina and Garcia, 2005; Bartholomew *et al.*, 2003; Rohrbach *et al.*, 2003; Purseglove, 1972).

Pineapples contain the proteolytic enzyme bromelain, which is used as a meat tenderizing agent and for medical purposes. It has been reported to have valuable biological properties such as interfering with the growth of malignant cells, inhibiting platelet aggregation, fibrinolytic and anti-inflammatory action, enhancing drug absorption and removing skin (Mynott *et al.*, 1999; Gailhofer *et al.*, 1998).

Ghana's pineapple industry has grown significantly over the past twenty years. Commercially produced pineapples are grown within a 50km radius of the capital, Accra. From here they are shipped to export markets such as Germany, Holland and Italy by sea or air. From 1994 to 1999, the total value of Ghana's pineapple exports increased nearly threefold, from US \$5.3 million to US \$15.5 million. Pineapple processing has also increased, with a number of companies involved in manufacturing single-strength juice and juice concentrate. In addition, Blue Skies Company recently established a plant for processing pineapple into slices and mixed fruit salads (www.freshplaza.com/news_detail.asp?id=704 , 2007).

2.8 Preservation

The high increase in food deterioration is due to contamination with micro-organisms since the entire environment in which we live is colonized by micro-organisms (Nelson and Tressler, 1981). Although micro-organisms are of great importance, these use human foods as source of nutrient for their growth. This of course can result in the deterioration of food. They do this by increasing their numbers, utilizing nutrients, producing enzymatic changes, contributing to flavour by breaking down products and synthesis of new components.

To prevent this, micro-organisms must be eliminated from foods as this will aid in preserving it (Ahmed *et al.*, 1991).

2.8.1 Chemical Preservation

2.8.1.1 Benzoates

Benzoates, found naturally in cranberries, are bacteriostatic and fungistatic under acidic conditions. They primarily inhibit growth of moulds, yeast and bacteria in acidic liquids and drinks, such as vinegar, fruit juice, soda and carbonated beverages. Benzoates exist as calcium, potassium and sodium salts. Sodium benzoate is used in foods with a pH below 3.6 such as soy sauce, duck sauce and salad dressings. Potassium benzoate is used to preserve foods with a pH below 4.5 such as various fruit juices and soft drinks (FDA, 2009).

The FDA currently permits a maximum of 0.1% benzoate in foods. Soft drinks are the number one source of sodium benzoate in the diet. On its own, it is not considered toxic, and studies show no adverse health effects in humans under normal conditions. They however form benzene which is carcinogenic when combined with ascorbic acid (Seager and Slabaugh, 2000).

2.8.1.2 Sorbates

Sorbic acid and its salts (collectively known as sorbates) have antimicrobial activities and also inhibit the growth of yeast and other fungi. Since the 1940s they have been found to be effective antimicrobial agents and hence are used as preservatives in a wide range of foods and drinks and, to a lesser extent, in certain cosmetics, pharmaceuticals and tobacco products. The range includes foods such as cheese products, pickles, certain fish products, carbonated beverages, margarine and certain fruit and vegetable products, including wine (Dacosta, 1994; Thakur *et al.*, 1994).

Sorbates are reported to be more efficient and less toxic than benzoate (Thakur *et al.*, 1994) and are classified as “Generally Recognized as Safe” (GRAS) additives by the United States FDA (2005). They are also found in the list of additives permitted in foods by the Food and Drugs Authority of Ghana (FDA Ghana, 2005).

Sorbic acid and its salts are practically tasteless and odourless in foods, when used at reasonable levels (< 0.3 %) and their antimicrobial activity is generally adequate (Erich *et al.*, 2000).

Potassium sorbate, a white, fluffy powder, is very soluble in water and when added to acid foods it is hydrolysed to the acid form. Sodium and calcium sorbates also have preservative activities but their application is limited compared to that for the potassium salt, which is employed because of its stability, general ease of preparation and water solubility (Tulamait *et al.*, 2005).

2.8.2 Physical preservation

2.8.2.1 Thermal Pasteurization

Pasteurization is the process of heating food, which is usually a liquid, to a specific temperature for a predefined length of time and then immediately cooling it after it is removed from the heat. This process slows spoilage due to microbial growth in the food. Unlike sterilization, pasteurization is not intended to kill all micro-organisms in the food. Instead, it aims to reduce the number of viable pathogens so they are unlikely to cause disease. Commercial-scale sterilization of food is not common because it adversely affects the taste and quality of the product (Montville and Matthews, 2005).

In the industry, fruit juices are currently treated with high temperature short time (HTST) pasteurization in order to inactivate microorganisms and enzymes which cause food deterioration. Conventional thermal pasteurization ensures safety and extends the shelf life of juice, but it often leads to detrimental changes in the sensory qualities of the product (Cortés *et al.*, 2008). The FDA (2004) suggests pasteurizing fruit juice at 71°C for 3 seconds which results in a 5-log reduction of bacteria. Actual pasteurization conditions should be chosen based on type of juice, pH, and sugar content which can all affect microbial inactivation.

2.8.2.2 Pulsed Electric Field Treatment (PEF)

Pulsed electric field treatment (PEF) is an alternative preservation method primarily used on fruit juice and milk (Heinz *et al.*, 2002). The aims of PEF treatment are to inactivate spoilage and pathogenic microorganisms, decrease the activity of enzymes, and extend the shelf life of foods without undesirable heat and chemical effects (Cserhalmi *et al.*, 2006). PEF treatment applies extremely short (microsecond) and intense electric pulses which create pores in the cell membranes of microorganisms in a process called electroporation. Electroporation in a cell membrane can occur in both protein channels and lipid domains, which lead to an osmotic imbalance and eventually, cell death (Tsong, 1991). The change in membrane permeability can be reversible or irreversible, depending on the external electric field strength (Cserhalmi *et al.*, 2006). An increase in the demand for minimally processed fresh-products has raised an interest in the development and implementation of new techniques for food processing such as high-intensity pulsed electric fields (Aguiló-Aguayo *et al.*, 2007). In general, thermal pasteurization is the standard for juice processing

as it significantly reduces microorganisms, but it can adversely affect colour, taste, aroma, appearance, and nutrient content of juice (Aguilar-Rosas *et al.*, 2007).

2.8.2.3 High Hydrostatic Pressure (HHP)

High hydrostatic pressure (HHP) processing uses pressures up to 1000 MPa, with or without heat, to inactivate harmful microorganisms in food products (Ramaswamy *et al.* 2005). High hydrostatic pressure has traditionally been used in non-food areas such as ceramic and steel production. The application of HHP in the area of food started from 1900s when Hite and other researchers applied HHP on the preservation of milk, fruits and vegetables. However, it took a long time for the commercial products to emerge in the market. In 1990, the first HHP processed fruit jams were sold in the Japanese market. Subsequently, HHP processed commercial products including fruit juices and beverages, vegetable products, among others, have been produced in North America, Europe, Australia, and Asia (Balasubramaniam *et al.*, 2008).

2.8.2.4 Irradiation

Outbreaks of food borne diseases have been associated with consumption of poor quality food. This is due to the presence of a large numbers of human pathogens in food. Irradiation effectively inactivates food borne pathogens in contaminated foods. High doses of irradiation may be required for the inactivation of some pathogens. However, higher doses of radiation could have adverse effects on sensorial and nutritional quality (Fan *et al.*, 2004).

Gamma irradiation causes oxidative stress and affects biomolecules by causing conformational changes, oxidation, rupture of covalent bonds and formation of free radicals (Cheftel *et al.*, 1985). The hydroxyl ($\text{HO}\bullet$) and superoxide anion ($\text{O}_2^{\bullet-}$) radicals that are generated by radiation could modify the molecular properties of the proteins and lipids causing oxidative modifications of the proteins and lipid peroxidation (Halliwell and Gutteridge, 1989). Chemical changes of the proteins that are caused by gamma irradiation are fragmentation, cross-linking, aggregation and oxidation caused by oxygen radicals which are generated by water radiolysis (Cho and Song, 2000).

2.8.2.4.1 Irradiation of juices

Ionizing irradiation is a non-thermal technique that effectively inactivates human pathogens and reduces spoilage in juice (Buchanan *et al.*, 1998; Thakur and Arya, 1993) and other foods (Thayur and Josephson, 1996). However, irradiated juice may develop an off-flavour and become less acceptable than non-irradiated juices (Spoto *et al.*, 1997; Thakur and Arya, 1993; Roy, 1956; Proctor and Goldblith, 1951). Much of the flavour in orange juice stems from volatile compounds, including volatile alcohols, aldehydes, esters, ketones, and hydrocarbons (Moshonas and Shaw, 1994). Among the volatile compounds important to orange juice flavour, esters and aldehydes are the primary contributors (Bruemmer, 1975).

In another study, radiation sterilization of fresh vegetable juice and the effectiveness of gamma irradiation for inactivating *Salmonella typhimurium* and *Escherichia coli* in carrot and kale juice were investigated. The test organisms were eliminated by irradiation at 3 kGy. The antioxidant capacity of the irradiated carrot juice was higher than that of the non-

irradiated control. Therefore, it was concluded that irradiation of carrot and kale juice improved the microbiological safety while maintaining or even enhancing the anti-oxidative activity (Song *et al.*, 2006).

Butchanan *et al.*, (1998) investigated the effect of irradiation on inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. It was concluded that a dose of 1.8 kGy should be sufficient to achieve the 5D inactivation of *Escherichia coli* as recommended by the National Advisory Committee for Microbiological Criteria for Foods.

2.9 Quality Issues

Quality includes all other attributes that influence a product's value to the consumer. This includes negative attributes such as spoilage, contamination with filth, discolouration, off-odours and positive attributes such as the origin, colour, flavour, texture and processing method of the food (FAO/WHO, 2008). Appearance, kinesthetic and flavour are some of the factors involved in the evaluation of the quality of a product. Appearance involves the sense of sight and is that which is perceived by the eye. Kinesthetic (or textural) factors are evaluated by the sense of touch, hand or mouth feel. Flavour also involves the senses of taste and smell. The quality of a food product can either be assessed through subjective analysis such as sensory evaluation by one or more human observers or through objective analysis by physical and chemical procedures (Joselyn and Heid, 1963).

2.10 Colour

The colour of fruit juice can have a huge influence on the type of juice products purchased by consumers. Consumers may see colour as an indicator for freshness, a specific flavour, a particular smell, and consumers may even use colour to determine the intensity of a flavour or smell. Francis (1995) stated that colour influences other sensory characteristics, which subsequently influences food acceptability, choice, and preference. Colour can be defined as the impact of the wavelengths of light in the visual spectrum from 390 to 760 nm on the human retina (Francis, 1995). According to Francis (1995), if the colour of a product is unacceptable to a consumer, the flavour and texture may not be considered at all. Colour may also be used as an indicator to determine the types and quantities of various carotenoids. Colour analysis is quick and simple and may provide more consistent results compared to carotenoid analysis (Francis, 1995).

2.11 Ghana Standards for Fruit Juices (GS 724:2003)

Fruit juices are described by the Ghana Standards Authority, (2003) as unfermented but fermentable juice, pulpy, turbid or clear, intended for direct consumption, obtained by mechanical process, from sound ripe fruit or the flesh thereof, and preserved exclusively by physical means. It is quite explicit about the strict hygienic standards expected of fruit juices and it is stringent on tolerance for microbial counts (Yeast and moulds, Coliforms).

2.12 Ghana Standards for Vegetable Juices

Ghana Standards for vegetable juices (GS 725:2003) describes vegetable juice as the liquid unfermented but fermentable product or lactic acid fermented product intended for direct

consumption obtained from the edible part of one or more sound vegetables and preserved exclusively by physical means. The juice shall be free from skins, seeds and other coarse parts of the vegetables. It may be clear, turbid or pulpy. Similar to the standards for Fruit juices, the vegetable juices standards has very strict requirements for hygienic and microbial standards.



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of Raw Materials

Fresh carrots (All season variety of average height 15 cm and weight of 200 g) and pineapples (Sugar loaf variety, 0.5 - 1.5 Kg) were obtained from Wahab Farms in Dzorwulu, Accra and Abrokwa Farms in Nsawam respectively. Mangoes (Kent variety, 500 – 600 g) were purchased at the Agbogboloshie market in Accra. These were packed and sent to Fruito Foods Processing Limited in Sowutuom for processing and bottling. Nutritional and quality control analyses were conducted at the Radiation Technology Centre of the Ghana Atomic Energy Commission, Ghana Standards Authority and the Noguchi Memorial Institute for Medical Research, Legon.

3.2 Survey on the Beverage Consumption Patterns in the Anyaa-Sowutuom District in the Greater Accra Region of Ghana.

A survey was conducted in the Anyaa-Sowutuom district in the Greater Accra Region to determine their beverage consumption patterns. Respondents were randomly chosen, based on consent, during visits to homes, schools and work places. A semi-structured questionnaire was used during the interviews to obtain information on background characteristics of respondents, frequency of consumption, attitudes and perceptions of beverages and juice consumption (Appendix 1A). The sample size of respondents required for the study was based on the confidence level and margin of error, $n = (z^*/2m)^2$, where n is the sample size, z^* is the level of confidence interval (95% = 1.96) and m

is the margin of error ($10\% = 0.10$). The sample size was calculated to be 96 but because of uncertainty, it was increased to 240.

3.3 Juice Extraction

3.3.1 Carrot Juice

Fresh carrots were washed in clean water and sliced into smaller pieces to enhance easy mashing. It was blanched in hot water, $100\text{ }^{\circ}\text{C}$ for 10 minutes (Luh and Woodroof, 1975) and mashed in a plate attrition mill. Then 240 ml of treated water (Boiled at $100\text{ }^{\circ}\text{C}$ and cooled to room temperature, 26 ± 2) was added to 100 g of carrots and sent to the presser to obtain the juice. The volume of water used in the extraction was determined after preliminary experiments with different volumes (160, 180, 200, 220, 240, and 260 ml) which showed that the resultant concentration was the most accepted by consumers.

3.3.2 Pineapple juice

Fresh pineapples were washed thoroughly in clean water. They were then dipped in hot water at $90\text{ }^{\circ}\text{C}$ for 5 min followed by peeling and juicing using a juice extractor.

3.3.3 Mango juice

Fresh mangoes were cleaned thoroughly in tap water. They were then washed in hot water at 90°C for 5 min, peeled and juice extracted using a juice extractor.

3.4 Development of Composite Juice Formulations

To decide on plausible lower and upper levels for the three component mixture of the extracts, a constrained mixture design (Cornell, 1983) was used with arbitrary lower and upper bound levels (Table 3.1). For three components, the design yielded ten possible formulations (Table 3.2 and Figure 3.1) based on the lower and upper limits (Table 3.1). This was meant to be a screening design to help set realistic lower and upper limits of the components' amounts.

Table 3.1 Lower and upper limits of compositional ratio of juice (optimization)

Component name	Lower limit	Upper limit
Carrot Juice	0.5	0.7
Pineapple juice	0.2	0.4
Mango Juice	0.1	0.3

NB: (0.01=1%; 1.00=100%)

Table 3.2 Compositional ratio of ten possible formulations (Optimization)

Formulation	Proportion of Ingredient (%)		
	Carrot(X_1)	Pineapple(X_2)	Mango(X_3)
1	50.00	40.00	10.00
2	53.33	33.33	13.34
3	56.67	26.67	16.66
4	60.00	30.00	10.00
5	50.00	30.00	20.00
6	63.33	23.33	13.34
7	70.00	20.00	10.00
8	53.33	23.33	23.34
9	60.00	20.00	20.00
10	50.00	20.00	30.00

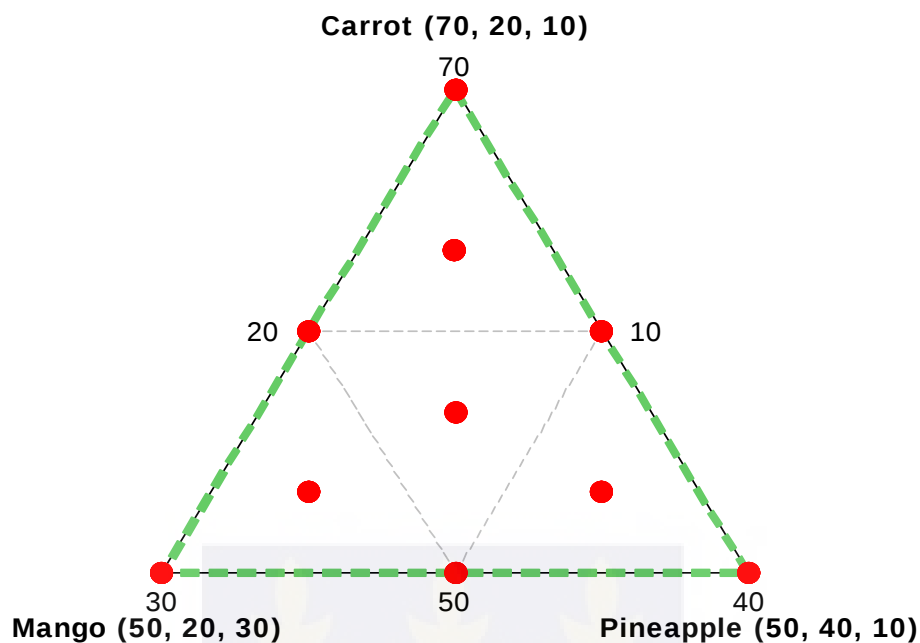


Figure 3.1 Design matrix for the 3-component mixture formulations.

3.4.1 Consumer acceptance test

Untrained consumers who were familiar with the ingredients were randomly recruited from among the staff of the Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission and students of the Department of Nuclear Agriculture and Radiation Processing, University of Ghana. The criteria for selection of panelists were that (a) they were available and willing to participate in panel tests, (b) they were regular consumers of juices, and (c) they were of sound health. A balanced incomplete block design ($t=10$, $k=4$, $r=6$, $b=15$, $\lambda=2$) (Appendix 2B) described by Cochran and Cox, (1957), was used to assign the ten (10) products to fifteen (15) panelists such that each panelist evaluated only four (4) products without the danger of fatigue. Where, t = number of formulations; b = number of panelists for each set; r = testing frequency of a formulation in each set; N = total number of panelist (4 sets); k =

number of formulations tested by each panelist; λ = maximum number of panelist testing the same formulation.

The sensory attributes considered for the evaluation were colour, taste, flavour, aftertaste and overall acceptance. Panelists assessed and assigned scores to the attributes using the 9 – point Hedonic scale where 1 represented dislike extremely and 9 represented like extremely (Appendix 2A). Responses from the products' sensory attributes were analyzed using ANOVA and mixture regression techniques.

3.4.2 Determination of the optimum components formulation

The predictive regression models developed for each of the attributes (colour, taste, flavour, aftertaste and overall acceptability) were used to generate ternary contour plots to display the effects of the components on the attributes. Contour plots of each of the attributes were overlaid based on the (consumer acceptable) limits of the attributes. The optimum region was determined by superimposing the contour plots (Palomar *et al.*, 1994) of all sensory attributes that would meet the criteria for an acceptable prototype product (Prinyawiwatkul *et al.*, 1997).

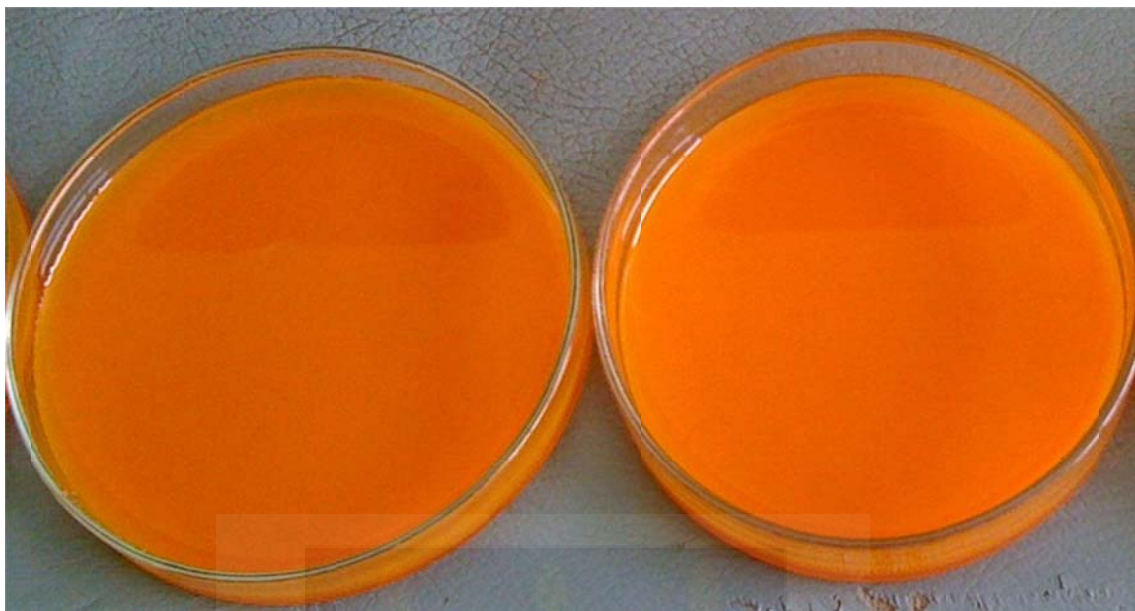


Plate 1. Photograph of the optimised juice.

3.5 Preservation treatments

The optimized final composite juice (Plate 1) was pasteurized (62 °C for 30 min), preserved, bottled and was closely monitored to determine the shelf life at room temperature (26 ± 2 °C) for eight (8) weeks. The final composite juice was preserved by chemicals and gamma radiation at different levels and doses respectively.

The parameters monitored during this period included; Ascorbic acid, Pro vitamin A, Titratable acidity, pH, Total soluble solids, Colour, Minerals and Microbial analysis. The data generated from the attributes were subjected to Analysis of Variance, and summarized in Appendix 3A.

3.5.1 Chemical preservation

Sodium benzoate and potassium sorbate were used to preserve the juice individually as

well as a combination of both. The treatments were as follows

Treatment 1 – Control (Pasteurization at 62 °C, 30 min)

Treatment 2 – Pasteurization at 62 °C, 30 min + 0.1 % Sodium benzoate

Treatment 3 – Pasteurization at 62 °C, 30 min + 0.1 % Potassium sorbate

Treatment 4 – Pasteurization at 62 °C, 30 min + 0.05 % Sodium benzoate and 0.05 % Potassium sorbate

3.5.2 Irradiation

The final composite juice after bottling and pasteurization (62 °C, 30 min) was treated with ^{60}Co gamma radiations at the Radiation Technology Centre, Ghana Atomic Energy Commission. Irradiation was carried out at 1, 1.5, 2 and 2.5 kGy at a dose rate of 2.43 kGy/hr.

Treatment 5 – Irradiation at 1 kGy

Treatment 6 – Irradiation at 1.5 kGy

Treatment 7 – irradiation at 2.0 kGy

Treatment 8 – Irradiation at 2.5 kGy

3.6 Analytical Procedure for Shelf-life Study

3.6.1 pH

The pH of ten milliliters (10 ml) of juice was determined using a pH meter (Mettler Toledo) for the eight week storage period.

3.6.2 Titratable Acidity

Ten milliliters (10 ml) of juice was mixed with 50 ml distilled water. The mixture in triplicates was then titrated against 0.1M NaOH using 1% phenolphthalein as indicator. Acidity was calculated as acetic acid (%) (AOAC, 2003).

3.6.3 Mineral Analysis

A wet digestion method (AOAC, 2003) was used to eliminate all organic matter from the sample before it was analyzed for the various minerals. About 1 ml of the sample was measured into a 250 ml beaker. Twenty five milliliters (25 ml) concentrated HNO_3 was added and the beaker was covered with a watch glass. The sample was digested with care on a hot plate in a fume chamber until all the organic matter had been oxidized (20-30 min). The pale yellow solution was cooled to room temperature ($26 \pm 2^\circ\text{C}$) and 1 ml 70% HClO_4 was added with care. Digestion was continued until the solution was almost colourless (until all the HNO_3 was removed). The solution was then cooled to room temperature after the digestion process, and about 30 ml distilled water was added and allowed to boil for about 10 mins then filtered when hot through No. 4 Whatman filter paper into a 100 ml volumetric flask. The beaker was washed well with distilled water and filtered. The flask was then cooled to room temperature and made up to the 100 ml mark. This solution was used for all the mineral analyses. The following minerals; Magnesium (Mg), Calcium (Ca), Potassium (K) and iron (Fe) were all determined using the PerkinElmer Atomic Absorption Spectrophotometer (AAS; Model AAnalyst 400, Minneapolis, U.S.A.) and results recorded in milligram (mg).

3.6.4 Pro-Vitamin A (Beta carotene)

Pro-Vitamin A analyses were determined by HPLC method as described by Pearson (1987) and results recorded in milligram (mg).

3.6.5 Vitamin C

Titration procedures as described by Pearson (1987) and results were recorded in milligram (mg).

3.6.6 Colour Determination

The colour of the juice was determined using the Minolta Chroma Meter (Minolta CR 300 series) using the L*a*b* colour system. The Chroma meter was calibrated with a standard white tile ($L^* = 97.95$, $a^* = -0.12$, $b^* = +1.64$).

3.6.7 Microbial Analyses

The beverage was tested for its microbiological safety by determining the Total Plate Count (TPC), Yeasts/Moulds, Total Coliforms, *Staphylococcus aureus*, and *Escherichia coli* using procedures outlined in the Quality Assurance Procedure Manual of the Ghana Standards Authority (2003).

3.6.7.1 Total plate Count

The total population counts of the mesophilic bacteria were determined using the Total Plate Count Method, on a plate Count Agar (pH 7.0 from Oxoid Ltd., Basingstoke, Hampshire – England). The plate was incubated at 35 °C for 48 ± 2 hr. The number of

colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g) (King *et al.*, 1986).

3.6.7.2 Yeasts and Moulds

Malt Extract Agar (pH 6.6 from Oxoid Ltd., Basingstoke, Hampshire – England) was used to determine the yeasts and moulds population in the sample. The plates were incubated at 25 °C for 5 days. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g) (King *et al.*, 1986).

3.6.7.3 Total Coliforms (Presumptive Test)

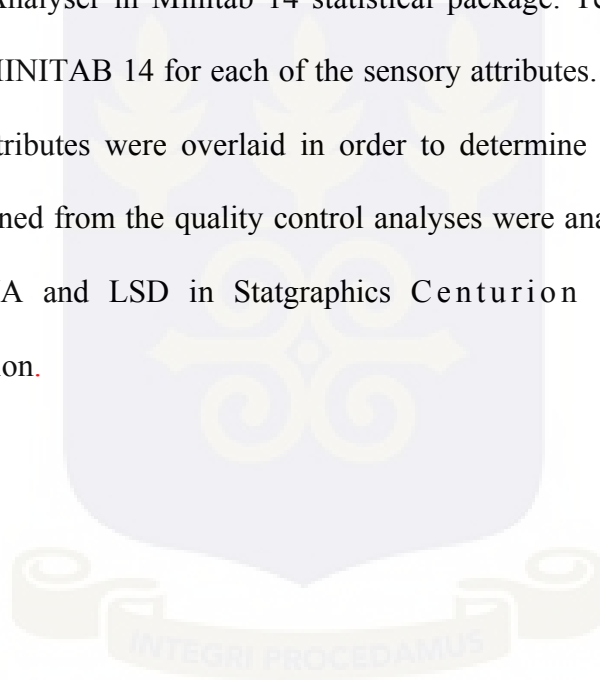
Lauryl Tryptose Broth (pH 6.8 from Oxoid Ltd., Basingstoke, Hampshire – England) was used to determine the presence of Coliforms. Fermentation tubes with inverted Durham tubes were used. The tubes were incubated at 35 °C for 48 ± 2 hr. The presence of gas trapped in the Durham tubes would indicate a positive test for Coliforms (King *et al.*, 1986).

3.6.7.4 *Staphylococcus aureus*

Baird Parker Agar (pH 7.2 from Oxoid Ltd., Basingstoke, Hampshire – England) was used to determine the presence of *Staphylococcus aureus*. The plates were incubated at 37 °C for 48 ± 2 hr. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g) (King *et al.*, 1986).

3.7 Experimental Design and Statistical Analysis

Data obtained from the survey was analysed for frequencies and cross-tabulations using SPSS version 16. Formulation of the cocktail beverage was done using a Simplex Centroid Constrained Design (Cornell, 1983) for three components with the aid of Minitab 14 statistical package. A Balanced Incomplete Block Design (BIBD) was used (Cochran and Cox, 1957) to assign the 10 products obtained from the mixture design for 3 components to 15 panelists. Data for each sensory attribute were analysed using Mixture Design Analyser in Minitab 14 statistical package. Ternary contour plots were generated using MINITAB 14 for each of the sensory attributes. The ternary contour plots of each of the attributes were overlaid in order to determine the optimum formulation region. Data obtained from the quality control analyses were analysed using scatter plots in Excel, ANOVA and LSD in Statgraphics Centurion (version XVI.I) and GenStat 12th Edition.



CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Survey of Consumers about the Consumption Patterns of Beverages in the Anyaa-Sowutuom District of Ghana.

Analyses of the frequencies showed that the age distribution was very close and was made up of three age groups such that ninety-six (96) of the respondents were aged between 20-30 years, seventy-two (72) of the respondents were between 31-40 years and the remaining seventy-two (72) were between 41-50 years. The frequency distribution based on educational background of the respondents showed that only twelve (12) of them had only elementary education (Appendix 1B, Table 1.1). Two hundred and twelve (212) respondents had either secondary or tertiary education. The distribution of consumers based on family life was skewed because only eighty-four (84) out of a total of two hundred and forty (240) respondents were married.

4.1.2 Consumption of beverage

All two hundred and forty (240) of the respondents drank beverages and 238 (99.17% of respondents) specifically indicated that they drank juices, while 178 (74.17% of respondents) indicated that they drank carbonated drinks. The frequency of drinking beverages showed that, 138 (57.5%) of the respondents drank juices more than once per week, and that cost was not a consideration in their decisions to purchase juice. As many as 193 (80.4%) respondents said they were willing to try a new juice product. There were no significant differences ($p \leq 0.05$) between males and females, or between the age

groups (20 - 30, 31 - 40 year groups) in their willingness to try new fruit juices (Appendix 1B, Table 1.1).

4.1.3 Criteria for choice of juice as a beverage

4.1.3.1 Effect of gender

Juice was mostly preferred as a food supplement or nutritional requirement by both males and females. The criteria for choice and selection of juice as a food supplement did not differ significantly ($p > 0.05$) between males and females using Pearson's Chi-square (χ^2) analysis.

Taste was an important consideration for selection of juices. This was confirmed by the number of respondents, 113 (47%), that looked out for taste in juices. Females (54 % of female respondents) were however more likely to choose juices based on their taste than their counterpart males (40.3 % of male respondents). Males (26.1 % of male respondents) were also more likely to choose juices based on their colour than their counterpart females (13.2 % of female respondents) (Appendix 1C, Table 1.5). Analyses of the data using ANOVA and Pearson's Chi-square (χ^2), showed that there were significant differences ($p \leq 0.05$) between males and females in their characteristic choice of juices as beverage drinks (Appendix 1C, Table 1.5).

4.1.3.2 Effect of consumer's age

There were significant differences ($p \leq 0.05$) in the frequency of drinking juices between the age groups. Younger respondents in the age group of 20 - 30 years were less likely to

drink juice than the older age groups 31 - 50 years (Appendix 1C, Table 1.8). The reasons were not clear but could be attributed to the working status of the age groups. People of age groups 20 - 30 were mostly students and dependents, while those between 31 and 50 were usually independent and following a career and therefore more capable of purchasing exactly what they wanted.

The three age groups are most likely to choose juices for its taste (Appendix 1C, Table 1.11) than for its colour. Analyses of the data using ANOVA and Pearson's Chi-square (χ^2) showed that there are significant differences ($p \leq 0.05$) between the three age groups in their characteristic choice of juices as beverage drinks (Appendix 1C, Tables 1.8 – 1.11).

4.1.4 Summary of observation from consumer survey

Drinking of beverages was a popular practice among consumers in Anyaa-Sowutuom Constituency in Accra. Juices were particularly popular, among the working class consumers. Younger consumers (20 - 30 year olds) liked juices but did not drink as much as older people (31-50 years) probably because of purchasing capabilities. Juices were drunk as snack and as a nutritional supplement by health conscious consumers. The choice of juice as a drink was dependent on nutrition, taste and colour on one hand and gender and age of the consumer on the other hand. This information was important for determining the attributes of the cocktail beverage that made it desirable among the given target population.

4.2 Formulation of the cocktail beverage

4.2.1 Screening for proportions of components in formulation

As much as the objective was to develop a Carrot juice, the practical realities of an agreeable product taste and flavour demanded the inclusion of other ingredients to serve those functions.

4.2.2 Optimization of the components proportions in the formulations

The lower and upper percentage limits of the three ingredients were used to generate ten possible formulations (Table 3.4), which were evaluated using sensory analyses. The regression models for all sensory attributes were obtained based on means score values presented in Table 4.1

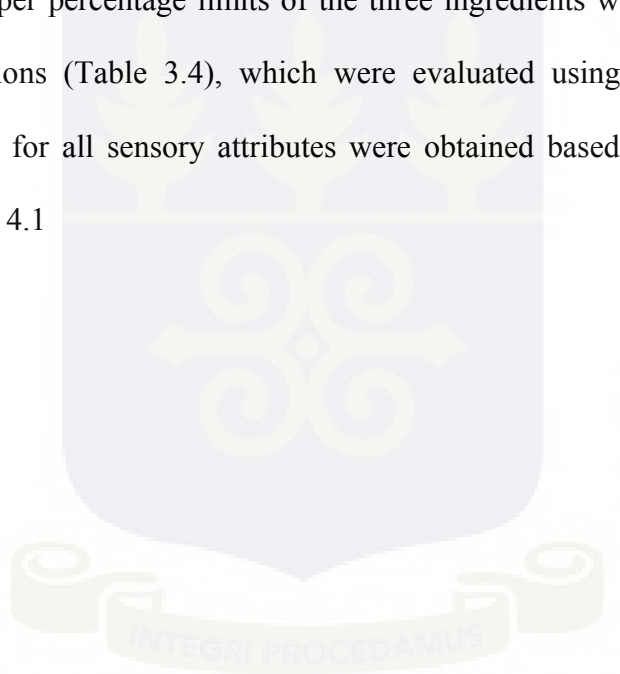


Table 4.1 Mean score value (optimization phase)

Formula number	Colour	Taste	Flavour	Aftertaste	Overall acceptability
1	7.5	6.0	6.67	6.3	6.7
2	6.3	5.5	4.8	5.7	4.8
3	6.5	6.0	6.7	4.2	5.3
4	7.3	6.5	6.0	5.0	6.0
5	6.8	6.0	6.3	4.7	5.5
6	5.8	5.8	5.2	5.2	5.5
7	5.7	4.8	6.0	5.0	4.7
8	6.3	5.7	5.2	6.2	5.3
9	5.3	6.0	4.8	5.5	5.8
10*	7.3	8.2	7.5	7.7	7.8

10* means the most acceptable formulation

4.2.3 Sensory analysis

Analysis of the sensory data from optimization showed significant differences ($p \leq 0.05$) in the sensory attributes among the ten formulations (Appendix 3A, Table 1.20). This suggested that, good variations existed between the formulations and therefore panelists were able to assess the differences in the sensory attributes of the various formulations.

4.2.3.1 Colour

Colour which is a sensation that forms part of the sense of vision, judges the appearance of a food (Jellinek, 1985). The mean score data for the various optimized formulations (Table 4.1) showed that, product number one (1), was more highly rated for colour. This formulation as shown in Table 3.2 had the minimum content of Carrots at 50%, minimum mango at 10% and maximum pineapple content at 40%. ANOVA of the regression for colour in Appendix 3A, Table 1.20 showed that the proportion of Carrot/pineapple/mango significantly influenced ($p \leq 0.05$) the colour of the juice. Ternary contour plots were generated using the predictive model to graphically display the influences of the components on colour (Figure 4.1). The figure showed that decreasing the proportion of carrot juice in the formulation to its minimum improved the colour appreciation by the panelists. This could be attributed to consumers not being familiar with beverages that are orange in colour, and hence the less orange the colour of the juice the higher it was scored.

The ingredients that were not orange, (i.e. pineapple) had a positive impact on the colour score of the juice. This was shown by the increasing acceptance by consumers when pineapple was at maximum amount (Product 1 in Table 3.2). The beverage scored the least of 5.3 for colour when the compositional ratio for pineapple was minimum and carrot was high (Figure 4.1). The highest mean score, >7.0 , was obtained when pineapple and mango were at maximum levels.

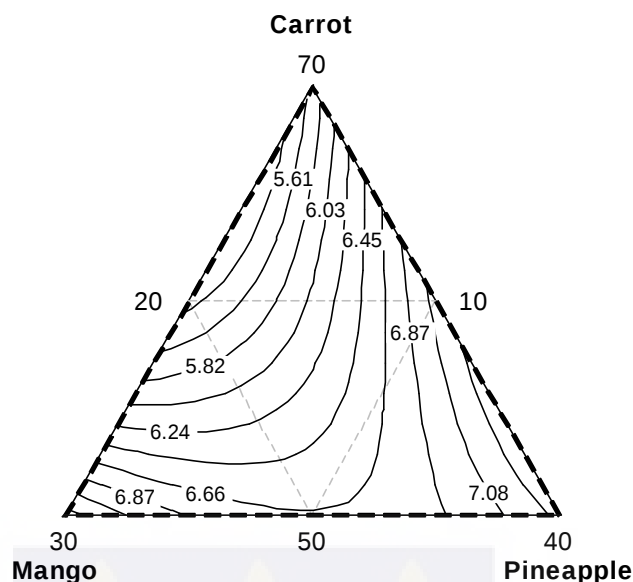


Figure 4.1 Mixture Contour Plots for Colour of the Composite Juice Formulation

4.2.3.2 Taste

The scale for rating the taste of the juices by panelists ranged from like slightly to dislike slightly. Figure 4.2 represented the ternary contour plots for taste and the model could explain 95 % of the variations due to taste. As carrot levels decreased, the mean scores gradually increased with increasing mango levels. The highest mean score, >8 , for taste was obtained when mango was at its highest level (Product number 10 in Table 3.2) whereas the least mean score, >5 , was obtained when carrots were at maximum levels (Product number 7 in Table 3.2). Again, the mean scores were increasing when the levels of pineapple were increasing with a corresponding decrease in carrot levels. The increase in mean scores were however significantly ($p \leq 0.05$) lower than when mango levels were increasing.

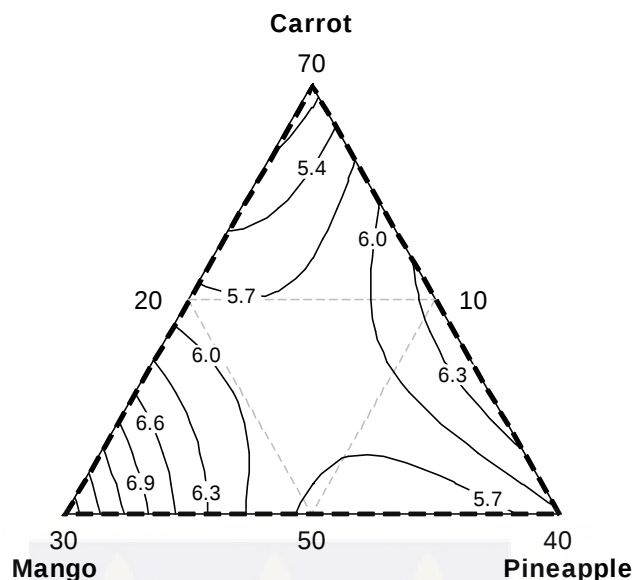


Figure 4.2 Mixture Contour Plots for Taste of the Composite Juice Formulation

4.2.3.3 Flavour

Flavour includes tastes and the aroma perceived through tasting (Jellinek, 1985). The panelists rated the flavour of the juices from like very much to neither like nor dislike. Juice made from 50 % Carrot, 10 % Pineapple and 30 % Mango (Product 10, Table 3.2) gave a flavour that was the most accepted at a mean score of 7.5 (Table 4.1).

Ternary contour plot was generated (Figure 4.3), which displays the relative influences of each of the components on the juice flavour. The figure shows that increasing the amount of carrots in the juice gave a flavour that was least liked by consumers. A reduction in the amount of carrots to the minimum with a corresponding increase in pineapple and/or mango levels registered acceptance for flavour. However, the product (product number 10, Table 3.2) with the highest mango level (30 %) gave the most acceptable mean score of 7.5 (Table 4.1).

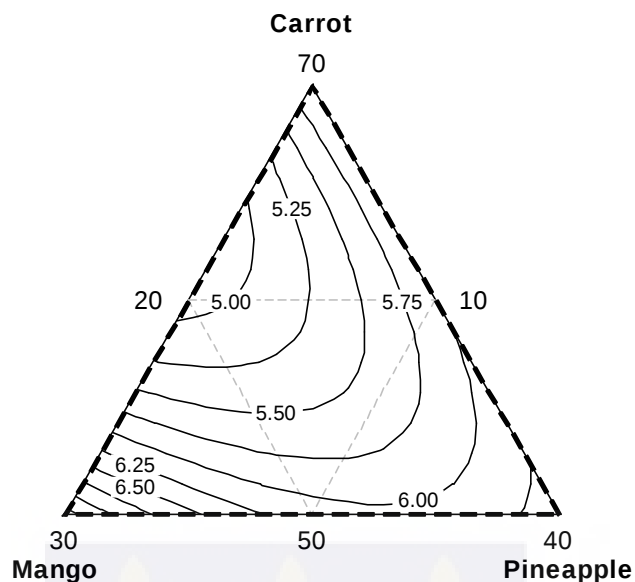


Figure 4.3 Mixture Contour Plots for Flavour of the Composite Juice Formulation

4.2.3.4 Aftertaste

Aftertaste is the lingering of the sense of taste of a product on taste buds (Quarcoo, 2008). The panelists rated the aftertaste of the cocktail beverage from dislike slightly to like very much. Ternary contour plot was generated (Figure 4.4), which displayed the relative influences of each of the components on the juice aftertaste. Mean scores were increasing as carrot proportions were decreasing. Contour plots obtained from the model for aftertaste (Figure 4.4) showed that as mango or pineapple levels increased, there were corresponding increases in the mean score values. Increase in mango levels however significantly recorded higher mean score values than increase in pineapple levels. The highest mean score for aftertaste was obtained for product 10 (Table 3.2) which had the maximum proportion of mango and minimum proportion of carrots. Thus high amounts of mangoes

and or pineapples in the product may have increased the score for aftertaste. This may be due to the sweetening nature of these fruits (Mercadante and Rodriguez-Amaya, 1998).

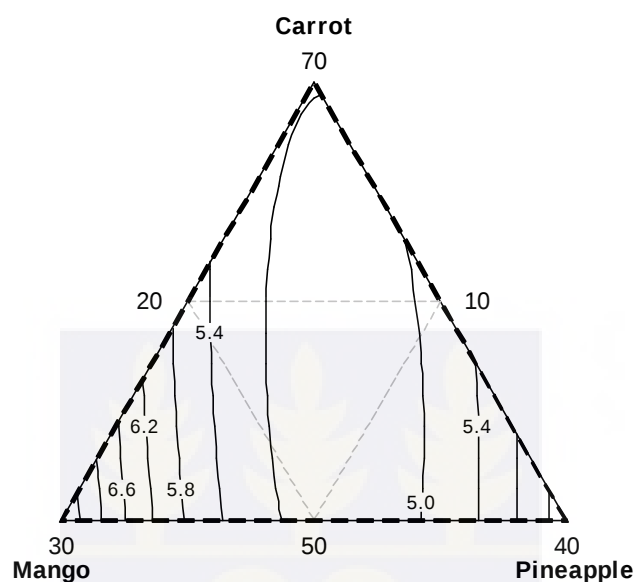


Figure 4.4 Mixture Contour Plots for Aftertaste of the Composite Juice Formulation

4.2.3.5 Overall acceptability

Carrot being the main ingredient was accepted by consumers when a minimum amount (that is 50 % of the total volume of the mixture) was used. Juice made from the minimum amount of carrots had the highest mean score (>7) for overall acceptability. Contour plots obtained from the model for overall acceptability (Figure 4.5) showed that as mango or pineapple levels increased, there were corresponding increases in the mean score values. The highest mean score for overall acceptability was obtained for product 10 (Table 3.2) which had the highest level of mango and least level of carrots. Similar results were recorded when Quarcoo (2008) obtained lower mean scores when moringa levels were high in her cocktail beverage.

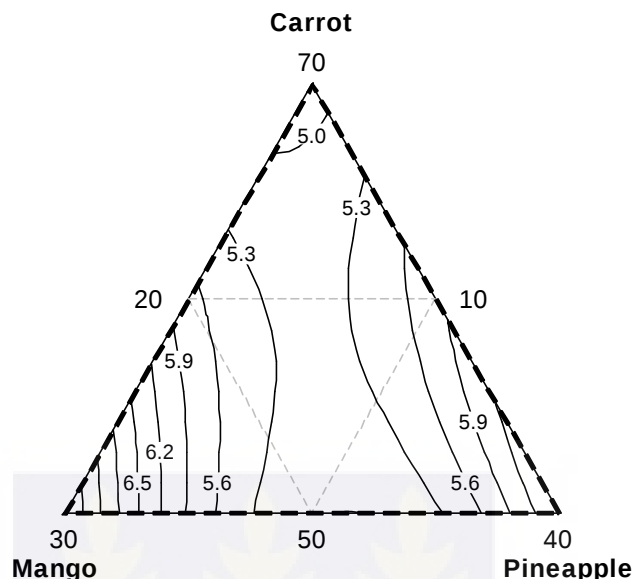


Figure 4.5 Mixture Contour Plots for Overall Acceptability of the Composite Juice Formulation

4.2.4 Region of Optimum formulation

Sidel and Stone (1983) defined optimization in sensory evaluation as a procedure for developing the best possible product in its class. It is impossible to develop a product with all sensory qualities that would satisfy consumers although an optimal formation should maximize consumer acceptance (Moskowitz, 1994). To obtain the optimum region therefore, and hence the ingredient formulation that would obtain optimum colour, taste, flavour, aftertaste, overall acceptability for the cocktail beverage, the contour plots for all the attributes (colour, taste, aftertaste, flavour and overall acceptability) were overlaid in a single graph on same axis as shown in Figure 4.6. The optimum region in this overlaid plot was where the criteria for all the five response variables were satisfied.

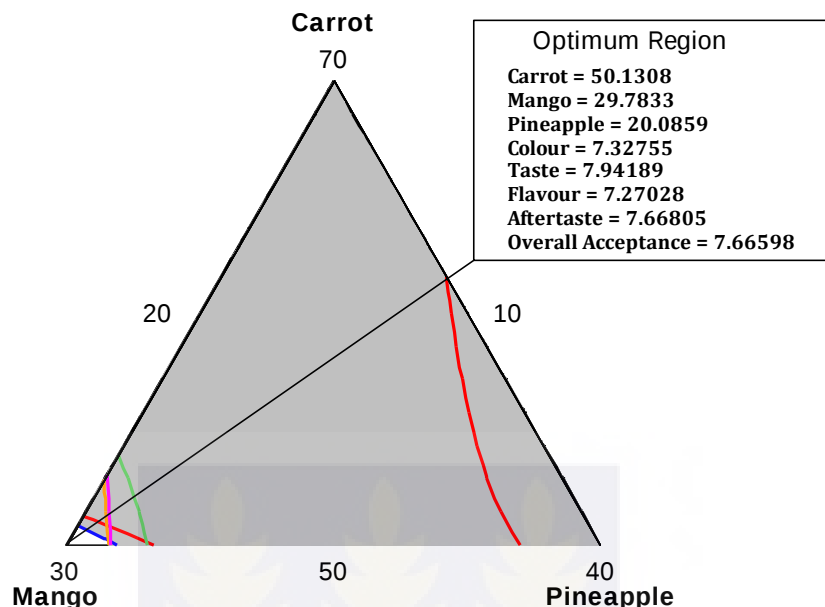


Figure 4.6 Overlaid Contour Plot (Optimization) of the Composite Juice Formulation

4.3 Shelf-Life Analysis

4.3.1 Minerals

Storage time had no significant ($p > 0.05$) effect on the minerals (calcium, magnesium, iron and potassium) analyzed. Neither irradiated nor chemically treated beverages significantly ($p > 0.05$) differed from the control in mineral content.

The mineral analysis (magnesium, potassium, calcium and iron) revealed that there was general reduction after the extraction of juice from the carrots. This was due to the elimination of fibre and also dilution to obtain the juice. However, from literature (USDA Nutrient Database, 2009 and www.nutritionanalyser.com, 2009) a good amount of potassium, 115 mg/100 g and 240 mg/100 g, has been found in raw pineapple and carrot respectively which helped boost the content of the minerals in the juice. This resulted in

potassium recording 223.39 mg/100 g, the highest amount among the minerals of the final composite juice.

Table 4.2 Proximate composition of final juice (per 100 ml) and corresponding values for mango, pineapple and carrot based on literature (per 100 g)

Composition	Carrot juice extract	Final composite juice	Literature on carrot ¹	Literature on pineapple ¹	Literature on mango ²
Magnesium (mg)	10.46	7.68	18.0	12.0	90.0
Potassium (mg)	231.17	223.39	240.0	115.0	156.0
Calcium (mg)	15.50	10.40	33.0	13.0	10.0
Iron (mg)	0.48	0.35	0.66	0.28	0.13
Pro-vitamin A (mg)	6.21	3.92	8.29	0.03	0.06
Vitamin C (mg)	9.03	43.01	7.0	36.20	27.70

¹www.nutritionanalyser.com

²USDA Nutrient Database (<http://www.nal.usda.gov/fnic/foodcomp/search>, 2009)

4.3.2 Vitamin C

The data for ascorbic acid in the beverage stored under room temperature for eight weeks with different treatments is displayed in (Figure 4.7). Analysis of variance and multiple

range tests by LSD (Appendix 3A, Table 1.15) showed that storage period as well as treatments significantly ($p \leq 0.05$) affected the ascorbic acid content of the final cocktail beverage.

Dionisio *et al.*, (2009) reported that ascorbic acid in mangoes and many other fruits are highly sensitive to gamma radiation. Ascorbic acid content in the beverages was constantly degrading with time for all the different treatments. The data was fitted into linear trend line model and the trends showed that the rates of ascorbic acid degradation were lower (≤ 2.8 mg/week) when the beverage was treated with chemical preservatives and with lower gamma radiation doses (1 and 1.5 kGy). The rate of degradation increased to 3.5 mg/week when the beverage was preserved with relatively higher doses of gamma radiation (2 and 2.5 kGy).

Beverages with chemical preservatives as well as those treated with lower radiation doses preserved ascorbic acid levels better than those preserved with higher radiation doses. Ascorbic acid under all treatments degraded with time, but chemical preservatives as well as lower radiation doses could retain about 50 % of the original ascorbic acid content of the beverage after eight weeks of storage (Potter, 2011).

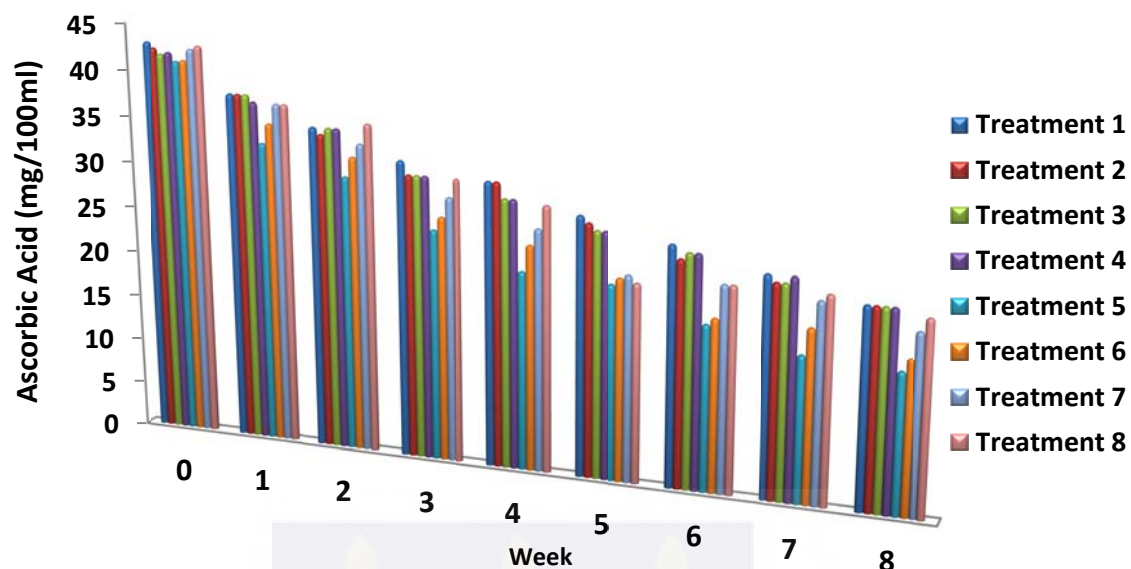


Figure 4.7 Ascorbic Acid Content of Beverage Given Different Treatments during storage period

Treatment 1 – Control (Pasteurization at 62 °C, 30 min)

Treatment 2 – Pasteurization at 62 °C, 30 min + 0.1 % Sodium benzoate

Treatment 3 – Pasteurization at 62 °C, 30 min + 0.1 % Potassium sorbate

Treatment 4 – Pasteurization at 62 °C, 30 min + 0.05 % Sodium benzoate and 0.05 % Potassium sorbate

Treatment 5 – Irradiation at 2.5 kGy

Treatment 6 – Irradiation at 2.0 kGy

Treatment 7 – irradiation at 1.5 kGy

Treatment 8 – Irradiation at 1.0 kGy

Table 4.3 Degradation rates for ascorbic acid in the Cocktail beverage during storage.

Treatment	Linear model for ascorbic acid
Treatments 1,2,3,4,7 and 8	$Y = -2.78X + 43$
Treatments 5 and 6	$Y = -3.5X + 43$

Where, X= Storage time

Y= Total amount of ascorbic acid

4.3.3 Pro – Vitamin A

The analysis of the data for pro-vitamin A showed significant effects ($p \leq 0.05$) for the interaction of both treatments and time (Figure 4.8). Analysis of variance and multiple range tests by LSD (Appendix 3A, Table 1.19) showed that pro – vitamin A was constantly degrading with time for all the treatments.

The results indicated that irradiation had higher deteriorative effects on the pro - vitamin A content of the juice beverage. The lowest dose (1 kGy) of gamma radiation used in the preservation of the juice had significantly ($p \leq 0.05$) higher levels of deterioration than any of the chemical preservatives used as at week 6 of storage. However, it retained same amount of this nutrient as the chemically treated products at the end of storage (week 8). Although irradiation at the highest dose (2.5 kGy) had the highest deteriorative effect on the pro-vitamin A content, it however retained about 60 % of this nutrient at the end of storage (Andress *et al.*, 1998).

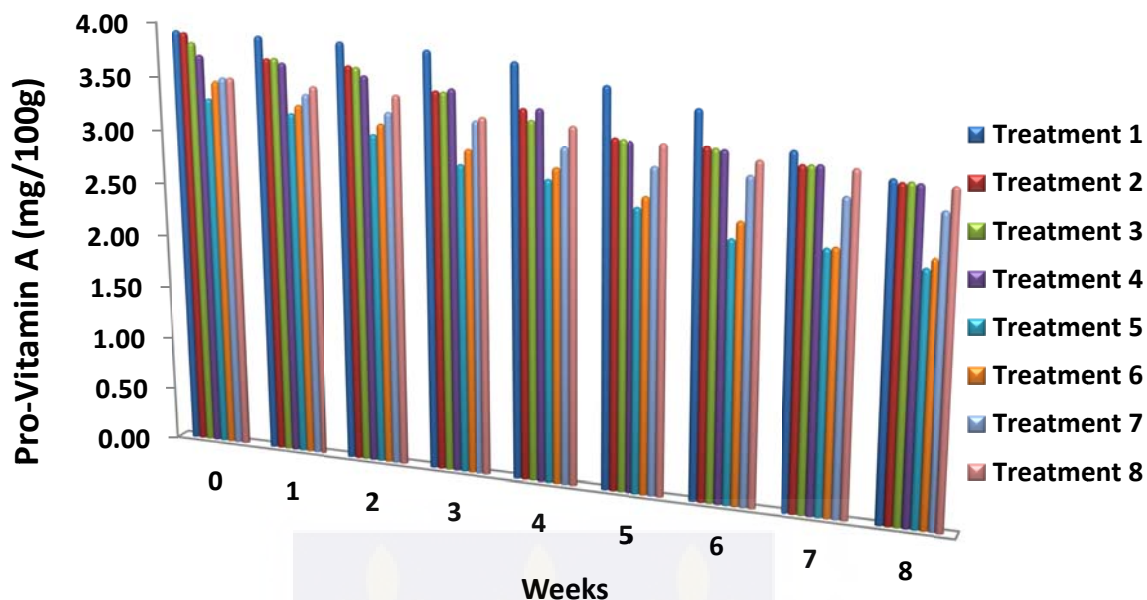


Figure 4.8: Pro-Vitamin A Content of Beverage Given Different Treatments during storage period

4.3.4 pH and Titratable acidity

Titrateable acidity and pH of the juice is a measure of the sourness of the product and it also reflects on the stability of the product with regards to deterioration during storage (Potter, 2011).

There was a general reduction in pH levels of the cocktail beverage for all the treatments during storage (Figure 4.9). The reduction could have been due to storage environment induced degradation of other nutrients such as proteins leading to changes in pH (Quarcoo, 2008). It could not have been due to microbial activity since growth was within acceptable limits (< 10).

The analysis of the data for pH showed significant effects ($p \leq 0.05$) for the interaction of both treatments and time (Appendix 3A, Table 1.12). The multiple range analysis showed

that while there were no significant difference ($p \geq 0.05$) in pH between the highly irradiated (2.0 and 2.5 kGy) beverages (4.24 and 4.23), the pH of the beverages chemically treated were significantly higher (4.31 - 4.32). Irradiation therefore had no effect on the pH but the chemicals used for the treatment altered the ionic concentrations to affect the pH.

Results from the two-way analysis of variance revealed that treatment and storage time had significant effects ($p \geq 0.05$) on the total titratable acids content of the cocktail beverage. There was significant ($p \geq 0.05$) interaction between storage time and treatments on the percentage titratable acidity of the beverages (Appendix 3A, Table 1.13).

Titratable acidity at the end of storage of the final composite beverage for all treatments was significantly lower than the initial. This might be due to conversion of acids into salts and sugars by enzymes particularly invertase (Bhardwaj and Mukherjee, 2010, Kumar *et al.*, 1992). Similar results were recorded by Dhaliwal and Hira (2001) in carrot juice blends and Tiwari (2000) in guava and papaya blends. Though beverages treated with gamma radiation recorded the least titratable acidity at the end of storage, its corresponding pH was also the least. Quarcoo, (2008) also recorded similar results and concluded that the trend is difficult to explain, but could be due to some buffering effects of the beverage proteins.

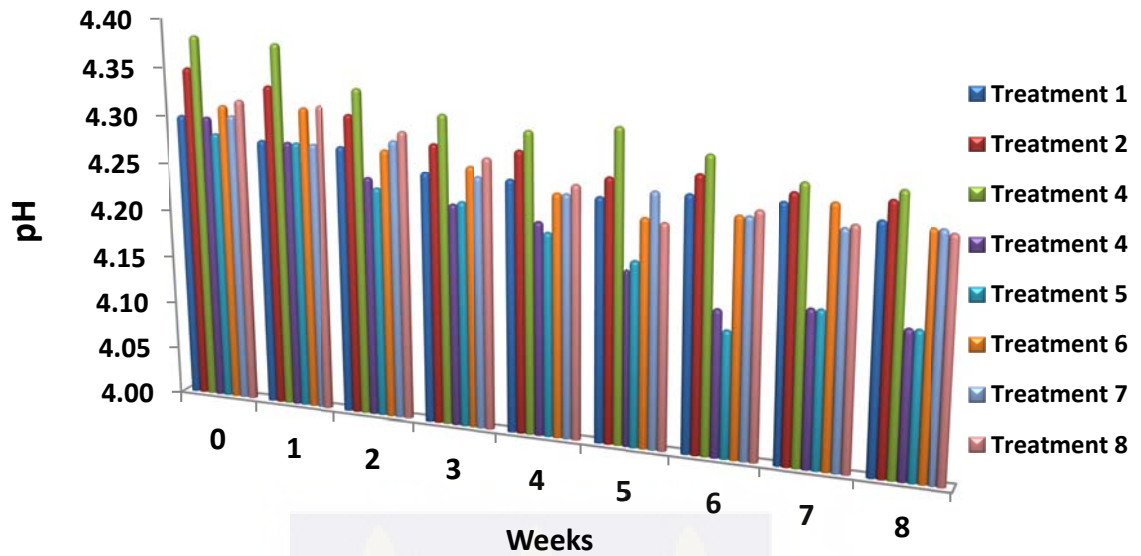


Figure 4.9: pH of Beverage Given Different Treatments during storage period

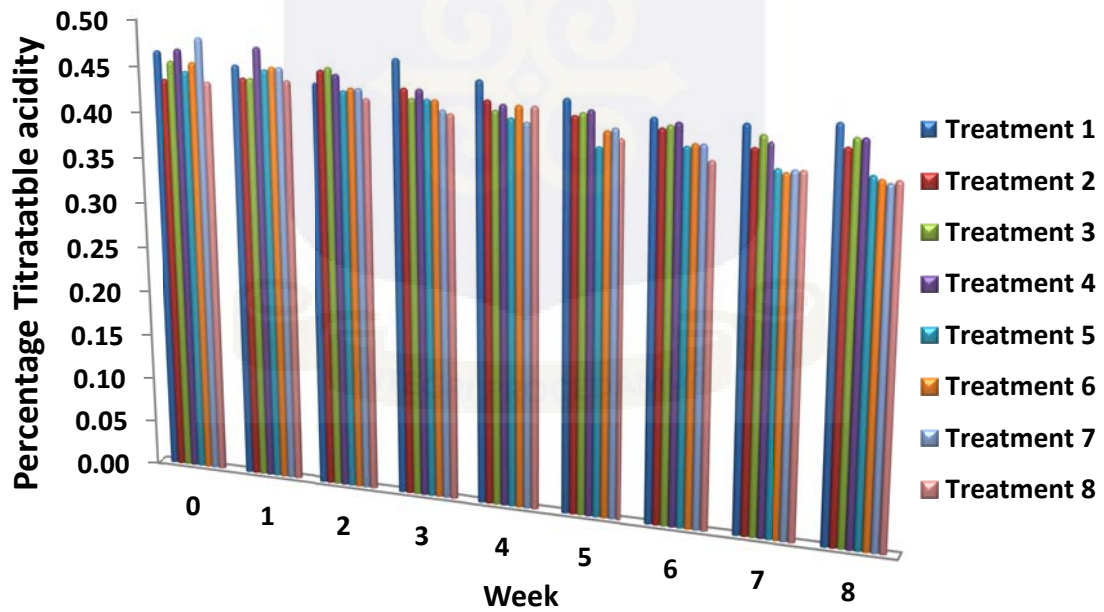


Figure 4.10: Percentage Titratable Acidity of Beverage Given Different Treatments during storage period

4.3.5 Total Soluble Solids (TSS)

Analysis of variance and multiple range tests by LSD (Appendix 3A, Table 1.14) showed that storage period as well as treatments significantly ($p \leq 0.05$) affected the total soluble solids of the final cocktail beverage. The TSS of the beverage increased with gradual passage of storage time. This gradual increase might have been due to hydrolysis of polysaccharides into monosaccharides and oligosaccharides. Similar results were reported by Deka (2000) in juice blends as he found an increasing trend in total soluble solids during storage at ambient temperatures. At the end of the eighth (8th) week of storage, T1, T2 and T3 recorded the least (12.50, 12.58 and 12.54 respectively) TSS with T5 and T6 recording the highest (13.38 and 13.34 respectively) (Figure 4.11).

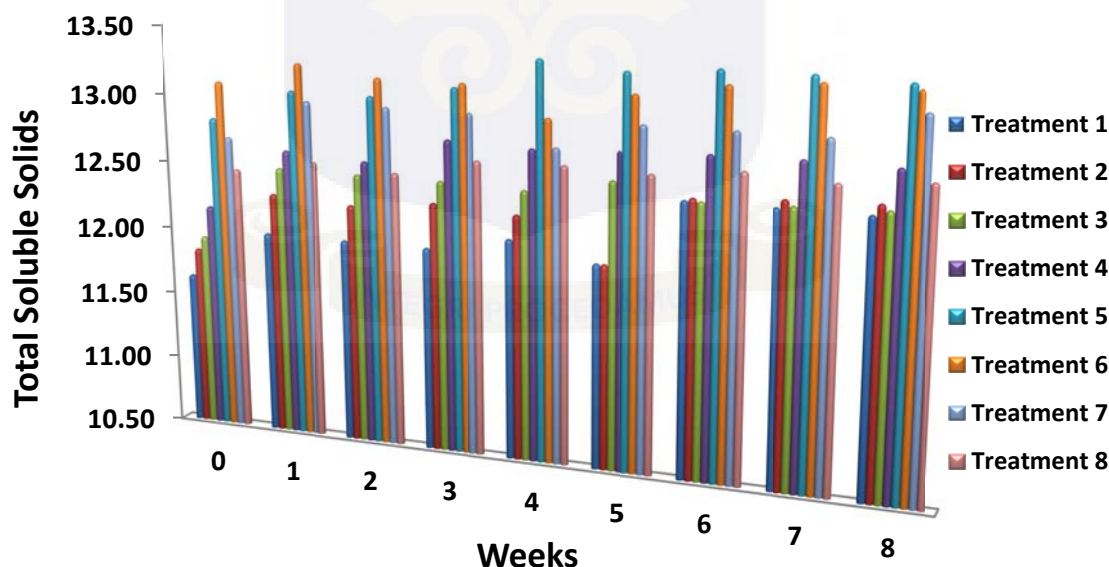


Figure 4.11: Total Soluble Solids of Beverage Given Different Treatments during storage period

4.3.6 Colour

One of the most important quality attributes of food is its colour. The first impression of the quality and acceptability of a particular food is judged upon its appearance (Neilsen, 1998). Results of the consumer survey (Appendix 1B, Table 1.1) indicated that some consumers make choices of juices based on the colour.

The juices were yellowish-orange in colour. The Minolta Chroma Meter (Minolta CR 300 series) measures $L^*a^*b^*$. L^* is a colour parameter that measures the extent of lightness, thus L^* when zero (0) would indicate black, and when one hundred (100) would indicate white; a^* when positive signifies reddish colour coordinate and when negative signifies greenish colour coordinate; b^* value when positive signifies yellowish colour coordinate and bluish colour coordinate when negative (Quarcoo, 2008).

4.3.6.1 Colour L^*

The L^* values for the juice were significantly ($p \leq 0.05$) affected by the treatments as well as storage time. The Multiple Range Test by LSD at the end of storage showed that the control treatment and irradiation at 2.0 kGy significantly ($p \leq 0.05$) preserved the L^* value (37.20 and 37.21 respectively) of the juice better than the other treatments.

L^* values for the different treatments were gradually decreasing with storage time. This gradual darkening observed could be due to non-enzymatic browning in an aqueous environment that has proteins and reducing sugars (Theodosiou *et al.*, 2011). Though the decrease observed was across all treatments, beverages treated chemically had significantly lower L^* values. Thus chemically treated beverages turned darker as

compared to the control and irradiation at 2 kGy.

4.3.6.2 Colour a*

Storage time as well as treatments had significant ($p \leq 0.05$) effects on the a* values of the beverage. A positive a* value suggested reddish colouration, and this for all treatments was gradually decreasing with storage time. However, the reddish colour of the beverage was significantly ($p \leq 0.05$) better preserved by irradiation (a* range 7.72-8.34) than with chemicals (a* range 7.10-7.16) at the end of storage (Theodosiou *et al.*, 2011) (Appendix 3A, Table 1.17).

4.3.6.3 Colour b*

The positive b* values (12.56 – 12.59) were a measure of the yellowness of the product. Analysis of variance showed that neither storage time nor treatments significantly affected the yellowness of the juices (Theodosiou *et al.*, 2011).

4.3.7 Microbial analyses

The results for growth of Coliforms, *Staphylococcus aureus*, *Escherichia coli*, Yeast /Moulds and mesophilic bacteria (Appendix 3B, Table 1.21) revealed that microbial growths for all the treatments during the eight weeks period of study were within acceptable limits (0 to < 10) hence insignificant. Quarcoo, (2008) recorded similar results when Moringa juice blends were pasteurized under same conditions as the former during storage period.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Juice was very popular among beverage consumers in the Anyaa-Sowutuom Constituency. It is particularly popular, among the working class consumers. The choice of juice as a beverage was usually determined by its nutrition, colour and taste, and consumers will try a new juice product if it meets their criteria.

High amounts of carrot juice in the composite juice impacted negatively on its sensory acceptability. On the other hand high amounts of mango and/or pineapple juice strongly improved the scores of the sensory attributes. Using constrained mixture optimization techniques it was possible to obtain an acceptable juice that had a ratio of 50:20:30 for Carrot: Pineapple: Mango.

Comparing the nutrient composition of some blended juices from literature with the final composite juice, the latter is a more nutritious beverage considering the Pro-vitamin A (3.01mg/100ml), Iron (0.35mg/100ml) and potassium content (223mg/100ml).

The keeping quality of the cocktail beverage at room temperature ($26 \pm 2^{\circ}\text{C}$) for eight weeks was good. Vitamin C and Pro-Vitamin A were better retained when the beverage was preserved chemically and at lower gamma radiation doses (1 and 1.5 kGy).

Colour of the juice was however best preserved by irradiation at 2.0 kGy. The rate of degradation of vitamins C and A were slow even at lower gamma radiation doses, when almost 50 % of vitamin C and 71 % of vitamin A were retained after 2 months of storage.

In sum, no one particular treatment was the most efficient during the shelf stability study period. In terms of most of the parameters, the chemical preservatives (T1 – T4) as well as irradiation at 1kGy were the best preservatives.

5.2 Recommendation

It is hereby recommended that:

- i. A survey for the acceptability, competitiveness and consumer perceptions of the optimized cocktail beverage is carried out.
- ii. Further studies may be carried out to verify the medicinal potential of the juice, particularly for diabetic and hypertensive conditions.
- iii. Further studies of shelf-life beyond two months should be carried out to ascertain the exact quality keeping period of the product.
- iv. Study on packaging effect on storability to determine the type of packaging that can best prevent interaction between the environment and product.

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APPENDIX 1A

**UNIVERSITY OF GHANA
SCHOOL OF NUCLEAR AND ALLIED SCIENCES
DEPARTMENT OF NUCLEAR AGRICULTURE AND RADIATION PROCESSING**

Questionnaire to establish the beverage consumption pattern in the Anyaa-Sowutuom District of the Greater Accra Region.

1. Date.....

A. RESPONDENTS

3. Sex..... M/F

4. Age ☐ 21-30 ☐ 31-40 ☐ 41-50 ☐ 51-60

5. Educational status

a) None

b) Sec/Tech/Comm.

c) Tertiary

d) Elementary

e) Vocational

f) other (specify)

6. Marital status.....S/M/D/W

7. Family size.....

8. Number of children/dependent.....

9. Major occupation.....

B. PRODUCT

10. Do you drink beverages?.....Y/N?

If yes, indicate the form of beverage(s) you **drink**.

Product	N/Y
Coffee	
Tea	
Food drinks (e.g. Milo, Soya milk)	
Juices (fruits, vegetables)	

Energy drink	
Carbonated drinks	



If you drink fruit/vegetable juices, please answer the following:

11. How often do you drink fruit/vegetable juices?

- a) Once a week b) More than once a week c) Occasionally d) Hardly

12. Why do you drink fruit/vegetable juices?

- a) Snack b) Nutrient requirement c) As food supplement d) Inexpensive
e) Other (specify).....

13. Are you willing to try new fruit/vegetable juice products?

.....Y/N

14. What characteristic attributes do you look for in a fruit/vegetable juice?

- a) Flavor b) Color c) Taste d) After-taste e) Overall acceptance
f) All of the above

C. HEALTH

15. Do you know of any health problems associated with the consumption of fruit/vegetable juices?Y/N

If yes list them

.....
.....

16. Do you know of any good health claims attributed to fruit/vegetable juices?

.....Y/N If yes list them

.....

17. Do those good health claims influence your choice of fruit/vegetable juice?

.....Y/N

APPENDIX 1B

Table 1.1 Frequencies of some responses of survey

Parameters	Category	Percent (%)
Gender	Male	49.60
	Female	50.40
Age (years)	21-30	40.0
	31-40	30.0
	41-50	30.0
Marital status	Single	58.3
	Married	35.0
	Widowed	1.2
	Divorced	5.4
Educational status	Elementary	5.0
	Sec/Tech/Comm.	20.8
	Tertiary	67.5
Major occupation	Unemployed	21.2
	Student	20
	Worker	58.8
Beverage consumer	Yes	100
	No	0
Juice consumer	Yes	99.17
	No	0.83
Carbonated drink consumer	Yes	74.17
	No	26.83

Reason for juice consumption (Inexpensive)	Yes	0.0
	No	100.0
Try new juice recipe	Yes	80.4
	No	19.6
Frequency of drinking juices	Occasionally/Hardly	40.8
	At least once a week	57.5
Reason for drinking juice	Snack	36.2
	Nutrient requirement/Food supplement	62.1
Characteristic attributes looked for	Aftertaste	1.2
	Flavour	3.8
	Overall acceptance	26.8
	Colour	20
	Taste	47.1

APPENDIX 1C

Cross tables of respondents against product characteristics

Table 1.2 Gender * How Often Do You Drink Fruit/Vegetable Juices

		HOW OFTEN DO YOU DRINK FRUIT/VEGETABLE JUICES		Total
		OCCASIONALLY/H ARDLY	AT LEAST ONCE A WEEK	
SEX	MALE	38	80	118
	FEMALE	60	58	118
Total		98	138	236

Table 1.3 Gender * Why Do You Drink Fruit/Vegetable Juices

		WHY DO YOU DRINK FRUIT/VEGETABLE JUICES		Total
		SNACK	NUTRIENT REQUIREMENT/F OOD SUPPLEMENT	
SEX	MALE	44	74	118
	FEMALE	43	75	118
Total		87	149	236

Table 1.4 Gender * Are You Willing To Try New Fruit/Vegetable Juice Products

		ARE YOU WILLING TO TRY NEW FRUIT/VEGETABLE JUICE PRODUCTS			Total
		YES	NO	5	
SEX	MALE	97	21	0	118
	FEMALE	95	20	1	116
Total		192	41	1	234

Table 1.5 Gender * What Characteristic Attributes Do You Look For In Juices

		WHAT CHARACTERISTIC ATTRIBUTES DO YOU LOOK FOR IN JUICES					Total
		AFTER TASTE	FLAVOU R	OVERALL ACCEPTA NCE	COLOUR	TASTE	
SEX	MALE	2	6	30	31	48	117
	FEMA LE	1	3	33	16	65	118
Total		3	9	63	47	113	235

Table 1.6 Gender * Do You Know Of Any Health Problems with Juice Consumption

		DO YOU KNOW OF ANY GOOD HEALTH CLAIMS WITH JUICE CONSUMPTION		Total
		YES	NO	
SEX	MALE	88	30	118
	FEMALE	97	21	118
Total		185	51	236

Table 1.7 Gender * Do You Know Of Any Good Health Claims With Juice Consumption

		DO YOU DRINK BEVERAGES	Total
		YES	
SEX	MALE	119	119
	FEMALE	121	121
Total		240	240

Table 1.8 Age Group * How Often Do You Drink Fruit/Vegetable Juices

		HOW OFTEN DO YOU DRINK FRUIT/VEGETABLE JUICES		Total
		OCCASIONALLY/ HARDLY	AT LEAST ONCE A WEEK	
AGE GROUP	21-30	37	58	95
	31-41	17	53	70
	41-50	34	37	71
Total		98	138	236

Table 1.9 Age Group * Why Do You Drink Fruit/Vegetable Juices

		WHY DO YOU DRINK FRUIT/VEGETABLE JUICES		Total
		SNACK	NUTRIENT REQUIREMENT/ FOOD SUPPLEMENT	
AGE GROUP	21-30	36	59	95
	31-41	31	38	69
	41-50	20	52	72
Total		87	149	236

Table 1.10 Age Group * Are You Willing To Try New Fruit/Vegetable Juice Products

		ARE YOU WILLING TO TRY NEW FRUIT/VEGETABLE JUICE PRODUCTS			Total
		YES	NO	5	
AGE GROUP	21-30	84	10	0	94
	31-41	59	8	1	68
	41-50	49	23	0	72
Total		192	41	1	234

Table 1.11 Age Group * What Characteristic Attributes Do You Look For In Juices

		WHAT CHARACTERISTIC ATTRIBUTES DO YOU LOOK FOR IN JUICES					Total
		AFTER TASTE	FLAVOU R	OVERALL ACCEPTA NCE	COLOUR	TASTE	
AGE GROUP	21-30	1	4	29	15	46	95
	31-41	2	4	14	21	27	68
	41-50	0	1	20	11	40	72
Total		3	9	63	47	113	235

APPENDIX 2A

UNIVERSITY OF GHANA LEGON

SENSORY EVALUATION FORM

NAME:

PRODUCT: Cocktail Beverage (Vegetable-Fruit)

DATE:.....

INSTRUCTION:

Please, you are provided with various formulations of Carrot-Pineapple-Mango cocktail beverage. You are requested to make independent and fair judgment on the following sensory attributes given below for each coded product. Using the 9-point Hedonic scale with numbers 1, 2, 3 ...9 (as shown below); please indicate your preference by matching each attribute with an appropriate score or number.

A NINE POINT HEDONIC SCALE

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

CODE	COLOUR	TASTE	FLAVOUR	AFTER TASTE	OVERALL ACCEPTANCE
.....					
.....					
.....					
.....					

Any other comment (s).....

Thanks for your cooperation

APPENDIX 2B

PROTOCOL FOR SENSORY EVALUATION OF TEN FORMULATIONS USING BALANCED INCOMPLETE BLOCK DESIGN

Panelist BLOCK Treatment

1	1	1
2	1	2
3	1	3
4	1	4
5	2	1
6	2	2
7	2	5
8	2	6
9	3	1
10	3	3
11	3	7
12	3	8
13	4	1
14	4	4
15	4	9
16	4	10
17	5	1
18	5	5
19	5	7
20	5	9
21	6	1
22	6	6
23	6	8
24	6	10
25	7	2
26	7	3
27	7	6
28	7	9
29	8	2
30	8	4

Panelist	BLOCK	Treatment
31	8	7
32	8	10
33	9	2
34	9	5
35	9	8
36	9	10
37	10	2
38	10	7
39	10	8
40	10	9
41	11	3
42	11	5
43	11	9
44	11	10
45	12	3
46	12	6
47	12	7
48	12	10
49	13	3
50	13	4
51	13	5
52	13	8
53	14	4
54	14	5
55	14	6
56	14	7
57	15	4
58	15	6
59	15	8
60	15	9

NB: ($t=10$, $b=15$, $r=6$, $N=60$, $k=4$ and $\lambda=2$)

Where, t = no. of formulations; b = no. of panelists for each set; r = testing frequency of a formulation in each set; N = total no. of panelist (4 sets); k = no. of formulations tested by each panelist; λ = maximum no. of panelist testing the same formulation.

Appendix 3A**Anova Summary for Shelf-Life Study****Table 1.12 Analysis of Variance for pH - Type III Sums of Squares**

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
MAIN EFFECTS					
A:Storage week	0.65002	8	0.0812525	1281.06	0.0000*
B:Treatment	0.301281	7	0.0430402	678.59	0.0000*
INTERACTIONS					
AB	0.104069	56	0.00185837	29.30	0.0000*
RESIDUAL	0.00913333	144	0.0000634259		
TOTAL (CORRECTED)	1.0645	215			

All F-ratios are based on the residual mean square error.

*Denotes significant difference ($p \leq 0.05$)

Table 1.13 Analysis of Variance for TTA - Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
MAIN EFFECTS					
A:Storage week	0.0820333	8	0.0102542	152.75	0.0000*
B:Treatment	0.0264	7	0.00377143	56.18	0.0000*
INTERACTIONS					
AB	0.0198333	56	0.000354167	5.28	0.0000*
RESIDUAL	0.00966667	144	0.0000671296		
TOTAL (CORRECTED)	0.137933	215			

All F-ratios are based on the residual mean square error.

*Denotes significant difference ($p \leq 0.05$)

Table 1.14 Analysis of Variance for TSS - Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
MAIN EFFECTS					
A: Week	41.0687	8	5.13359	1624.70	0.0000*
B: Treatment	3.4673	7	0.495329	156.76	0.0000*
INTERACTIONS					
AB	1.91384	56	0.0341758	10.82	0.0000*
RESIDUAL	0.455	144	0.00315972		
TOTAL (CORRECTED)	46.9049	215			

All F-ratios are based on the residual mean square error.

*Denotes significant difference ($p \leq 0.05$)

Table 1.15 Analysis of Variance for Vitamin C - Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
MAIN EFFECTS					
A:Storage week	11708.9	8	1463.61	1494.15	0.0000*
B:Treatment	918.277	7	131.182	133.92	0.0000*
INTERACTIONS					
AB	257.534	56	4.59881	4.69	0.0000*
RESIDUAL	141.057	144	0.979561		
TOTAL (CORRECTED)	13025.8	215			

All F-ratios are based on the residual mean square error.

*Denotes significant difference ($p \leq 0.05$)

Table 1.16 Analysis of Variance for colour L* - Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
MAIN EFFECTS					
A:Storage week	6.21546	8	0.776933	11.19	0.0000*
B:Treatment	30.2923	7	4.32747	62.34	0.0000*
INTERACTIONS					
AB	3.91072	56	0.0698342	1.01	0.4762
RESIDUAL	9.99587	144	0.0694157		
TOTAL (CORRECTED)	50.4144	215			

All F-ratios are based on the residual mean square error.

*Denotes significant difference ($p \leq 0.05$)

Table 1.17 Analysis of Variance for color a* - Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
MAIN EFFECTS					
A:Storage week	4.94515	8	0.618144	1628.28	0.0000*
B:Treatment	39.2429	7	5.60613	14767.36	0.0000*
INTERACTIONS					
AB	5.9412	56	0.106093	279.46	0.0000*
RESIDUAL	0.0546667	144	0.00037963		
TOTAL (CORRECTED)	50.1839	215			

All F-ratios are based on the residual mean square error.

*Denotes significant difference ($p \leq 0.05$)

**Table 1.18 Analysis of Variance for color b* - Type III Sums of Squares**

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
MAIN EFFECTS					
A:Storage week	1.63425E7	8	2.04281E6	1.00	0.4386
B:Treatment	1.43014E7	7	2.04306E6	1.00	0.4336
INTERACTIONS					
AB	1.14375E8	56	2.0424E6	1.00	0.4869
RESIDUAL	2.94105E8	144	2.04239E6		
TOTAL (CORRECTED)	4.39123E8	215			

All F-ratios are based on the residual mean square error.

*Denotes significant difference ($p \leq 0.05$)

Table 1.19 Analysis of Variance for Pro-Vitamin A - Type III Sums of Squares

<i>Source</i>	<i>Sum of Squares</i>	<i>Df</i>	<i>Mean Square</i>	<i>F-Ratio</i>	<i>P-Value</i>
MAIN EFFECTS					
A: Week	1.70131E7	8	2.12664E6	527994.21	0.0000*
B:Treatment	1.58169E7	7	2.25956E6	560993.43	0.0000*
INTERACTIONS					
AB	1.35915E6	56	24270.6	6025.79	0.0000*
RESIDUAL	580.0	144	4.02778		
TOTAL (CORRECTED)	3.41898E7	215			

All F-ratios are based on the residual mean square error.

*Denotes significant difference ($p \leq 0.05$)

Table 1.20 Anova summary of formulation (optimization phase)

Source of variance	Parameter				
	Colour	Taste	Flavor	Aftertaste	Overall acceptance
Between groups	3.17**	2.44**	1.20	1.49	1.76

* Significant at 95 confidence interval (CI); **significant at 99% CI

APPENDIX 3B

Table 1.21 Microbial counts (cfu/g) for the different treatments during storage.

Treatment	Storage time	Yeast & Moulds	<i>Staph aureus</i>	Total Coliforms	<i>E. coli</i>	Total plate count
1	0	< 10	0	0	0	1
2	0	< 10	0	0	0	0
3	0	< 10	0	0	0	0
4	0	< 10	1	0	0	1
5	0	< 10	0	0	0	1
6	0	< 10	0	0	0	1
7	0	< 10	0	0	0	1
8	0	< 10	0	0	0	1
1	1	< 10	0	0	0	1
2	1	< 10	0	0	0	1
3	1	< 10	0	0	0	1
4	1	< 10	0	0	0	1
5	1	< 10	0	0	0	1
6	1	< 10	0	0	0	1
7	1	< 10	0	0	0	1
8	1	< 10	0	0	0	1
1	2	< 10	0	0	0	1
2	2	< 10	0	0	0	1

3	2	< 10	0	0	0	1
4	2	< 10	0	0	0	1
5	2	< 10	0	0	0	1
6	2	< 10	0	0	0	1
7	2	< 10	0	0	0	1
8	2	< 10	1	0	0	1
1	3	< 10	0	0	0	1
2	3	< 10	0	0	0	1
3	3	< 10	0	0	0	1
4	3	< 10	0	0	0	1
5	3	< 10	0	0	0	1
6	3	< 10	0	0	0	1
7	3	< 10	0	0	0	1
8	3	< 10	0	0	0	1
1	4	< 10	0	0	0	1
2	4	< 10	0	0	0	1
3	4	< 10	0	0	0	1
4	4	< 10	0	0	0	1
5	4	< 10	0	0	0	1
6	4	< 10	0	0	0	1
7	4	< 10	0	0	0	1
8	4	< 10	0	0	0	1

1	5	< 10	0	0	0	1
2	5	< 10	0	0	0	0
3	5	< 10	0	0	0	1
4	5	< 10	0	0	0	1
5	5	< 10	0	0	0	1
6	5	< 10	0	0	0	1
7	5	< 10	1	0	0	1
8	5	< 10	1	0	0	1
1	6	< 10	1	0	0	1
2	6	< 10	0	0	0	1
3	6	< 10	0	0	0	1
4	6	< 10	0	0	0	1
5	6	< 10	0	0	0	1
6	6	< 10	0	0	0	1
7	6	< 10	0	0	0	1
8	6	< 10	0	0	0	1
1	7	< 10	0	0	0	1
2	7	< 10	0	0	0	1
3	7	< 10	0	0	0	1
4	7	< 10	0	0	0	1
5	7	< 10	0	0	0	1
6	7	< 10	0	0	0	1

7	7	< 10	0	0	0	0
8	7	< 10	0	0	0	0
1	8	< 10	2	0	0	1
2	8	< 10	0	0	0	0
3	8	< 10	0	0	0	1
4	8	< 10	0	0	0	1
5	8	< 10	0	0	0	0
6	8	< 10	0	0	0	0
7	8	< 10	0	0	0	0
8	8	< 10	0	0	0	0

< 10 Means no growth recorded. The method and the medium used, Malt Extract Agar (pH 6.6 from Oxoid Ltd., Basingstoke, Hampshire – England) only records growth when there are 10 or more viable counts.

