UNIVERSITY OF CAPE COAST

PHYSICOCHEMICAL, NUTRITIONAL, SENSORY AND MICROBIAL CHARACTERISTICS OF FRESH AND PASTEURIZED PINEAPPLE-CARROT-GINGER BEVERAGE

BY

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Thesis submitted to the Department of Agricultural Engineering, School of Agriculture of the College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Postharvest Technology.

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature ……..…….. Date …….

Name ……

Supervisors' Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature …….. Date:……

Name …….

Co-Supervisor's Signature …….. Date ……

Name ……

ABSTRACT

A beverage was prepared from pineapple, carrot and ginger juices using Response Surface Methodology to form a refreshing nutritional drink. Three independent variables were used to prepare the beverage: pineapple-to-carrot juice ratio (75/25-90/10), fibre size distribution (0.6-1.2 mm) and ginger concentration (2-5%). The effects of the variables were investigated and represented using quadratic models on pH, total soluble solids, beta-carotene and sensory attributes as responses. Responses for independent variables and responses were optimized by setting goals to optimize the beverage. The optimized beverage was obtained at pineapple-to-carrot juice ratio, fibre size distribution and ginger concentration of 80/20, 0.6 mm and 3% respectively. Optimized unpasteurized beverage was prepared and stored at -24, -10 and 4 ^{o}C and quality changes studied every five (5) days for 40 days. Significant decrease in ascorbic acid, beta-carotene, total antioxidant activity, total soluble solids was observed at the end of storage while total phenolic content remained almost unchanged. Minimal quality changes occurred in frozen samples. In another experiment, pasteurized (80 ^{o}C, 15 mins) optimized beverage was treated with 0.1% sodium benzoate and 0.1% citric acid and stored at 4, 28 and 38 ^{o}C and quality changes studied every 15-days for 90 days. Significant decrease in total phenolic, ascorbic acid, beta-carotene and total antioxidant activity were observed at the end of storage at rates depending on storage temperature. Frozen storage, therefore, can help slow the degradation in the quality of fresh unpasteurized beverage, while storage at low temperature can slow quality degradation in pasteurized beverage.

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DEDICATION

To my parents

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BBD	Box-Behnken Design
CFU	colony forming units
DI	Desirability index
DPPH	2,2-Diphenyl-picrylhydrazyl
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Authority
GAE	Gallic acid equivalent
HHP	High hydrostatic pressure
HIPEF	High intensity pulsed electric fields
HPP	High-pressure processing
LSD	least significant difference
OAA	Overall acceptability
OGYEA	Oxytetracycline Glucose Yeast Extract Agar
PEF	Pulsed electric fields
PME	pectin methyl esterase
PPO	polyphenol oxidase
QDA	Quantitative Descriptive Analysis
RDA	Recommended Dietary Allowance
RSM	Response Surface Methodology
SSA	Sub-Saharan Africa
TSS	Total soluble solids
X_1	pineapple-to-carrot juice ratio
X_2	Juice fibre size distribution
X_3	ginger concentration

CHAPTER ONE

INTRODUCTION

Background to the Study

Pineapple (*Ananas comosus, L. Merill*) has long been referred to as one of the most popular non-citrus fruits in tropical and subtropical regions of the world, largely because of its attractive flavour and refreshing sugar-acid balance (Bartolomé, Rupérez, & Fúster, 1996). The pineapple fruit is highly credited for adding important minerals and bioactive compounds to diet, in addition to having a delicious taste and refreshing aroma and flavour (Vasco, Ruales, & Kamal-Eldin, 2008).

Global production reached 24.79 million tonnes in 2013 with Costa Rica as the leading producer in the world with 10.8% of global output followed by Brazil (10%), Philippines (9.9%), Thailand (8.9%) and Indonesia with 7.4% (FAOSTAT, 2013). According to this FAOSTAT, Africa's total pineapple production stood at about 4.383 million tonnes in 2013, and of these, Ghana contributed about 0.637 million tonnes which was about 15% of Africa's total production. Sugarloaf and smooth cayenne are among the most prominent commercial cultivars (Thanaraj & Terry, 2011). Sugarloaf cultivar being one of the most commonly cultivated pineapple varieties is very sweet and is available almost all year round in Ghana, and its consumption is mainly limited locally, due to constraints in its shelf-life.

Many of the harvested pineapples are consumed in fresh form in Ghana and many other developing nations, yet poor postharvest management limits the shelf-life of the produce to just a few days after harvest (especially sugarloaf variety), rendering it unsuitable for human consumption. According

to Kitinoja and AlHassan (2010), pineapple ranks high among the crops that experience a high level of postharvest losses for small-scale farmers in Sub-Saharan Africa (SSA), only close to losses experienced by tomatoes, pepper, leafy greens, bananas and mangoes among others. Figure 1 shows a heap of pineapple fruits on the ground for sale under scorching sunshine, and this characterizes many situations in developing nations. It is obvious that the fruits will start to undergo quick quality deterioration under this condition.

Figure 1: Pineapple fruits for sale in an exposed environment in the sun

The perishable nature of pineapple fruit makes it difficult to store and preserve for a long time, especially given the low level of postharvest management technologies available in many developing nations. This always leads to a gradual loss of fruit flavour, nutritional quality and market value, since postharvest loss does not only encompass the physical loss of the commodity concerned (Kitinoja & AlHassan, 2010). Interventions must, therefore, be sought to reduce the postharvest losses of the fruit as one of the main tropical fruit crops.

Juicing has been one of the most commonly used technologies to process and preserve perishable fruits in order to guarantee regular supply, even during off-seasons. The manufacture of juices from fruits and vegetables

is as old as or older than agriculture (Bates, Morris, & Crandall, 2001). Juicing pineapple can be one of the ways to manage the postharvest losses that the fruit undergo. Pineapple juice is consumed and enjoyed by many people around the world, mainly in single-strength, reconstituted or concentrated form; in blends for new flavour; and in beverages as well as other products (De Carvalho, De Castro, & Da Silva, 2008). The average pineapple contains 81.2 to 86.2% moisture and 13-19% total soluble solids, of which sucrose, glucose and fructose are the main components (Dull, 1971), making the fruit very suitable for the juicing of a very tasty product.

Fruit juice is important in human nutrition far beyond its use as a refreshing source of liquid. Many fruits contain a variety of minor ingredients, particularly vitamins and minerals, as well as carbohydrates, which are the predominant solid component (Ashurst, 2008), and these become important component of juices. The health benefit of fruit juices is ascribed, in part to vitamin C (ascorbic acid), a natural antioxidant which can inhibit the development of major clinical conditions including cardiovascular diseases and cancer (Diplock, 1994; Rekha et al., 2012). According to Ashurst, there is supporting experimental evidence which indicates that ascorbic acid of natural origin is superior to that of synthetic origin. Many fruit juices are also rich in phenolic compounds and carotenoids which have antioxidant properties (Gardner, White, McPhail, & Duthie, 2000).

Pineapple fruit juice is also very rich in natural dietary fibres, and it is a well-known fact that dietary fibre plays an essential role in human health, promoting several positive physiological and metabolic effects (Raninen, Lappi, Mykkänen, & Poutanen, 2011). High consumption of dietary fibre has

been linked to reduced incidence of cardiovascular disease, diabetes, hypertension, obesity, and gastro-intestinal disorders (Anderson et al., 2009). Due to these, a tendency in the development of products enriched with fibre or with specific fibre claims has already been observed for quite some time now (Selani et al., 2014).

To improve the colour and nutritional composition, pineapple juice can be blended with carrot juice. Among common fruits and vegetables, carrots are rich in fibres, carotenoids, vitamins C and E, and phenolics (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001). Alpha and beta-carotene are the predominant carotenoids in orange carrots (Arscott & Tanumihardjo, 2010). Carrot juice is frequently blended in fruit type concoctions where only the colour and natural sweetness carry over.

Pineapple and carrot juice can form a unique fruit and vegetable juice drink with characteristic nutritional value and high appeal to the consumers' eyes. Many epidemiological studies have shown that a correlation exists between the consumption of fruits and vegetables and their products and reduced incidence of chronic diseases (Bazzano et al., 2002; Carter, Gray, Troughton, Khunti, & Davies, 2010; He et al., 2004; Hung et al., 2004; Joshipura et al., 1999; Lampe, 1999; Liu et al., 2000; Maynard, Gunnell, Emmett, Frankel, & Smith, 2003; Rissanen et al., 2003). Also, healthy dietary behaviours that avoid consumption of artificially-sweetened drinks has been associated with lower body weight and reduced incidence of obesity in children (Ludwig, Peterson, & Gortmaker, 2001). Several components with antioxidant activity are found in pineapple-carrot beverages. These include, among others, ascorbic acid, tocopherols (vitamin E), phenolic compounds,

beta-carotene and flavonoids which can quench the free radicals responsible for many body disorders.

For improving the taste, aroma, acceptability, palatability, nutritive value and to reduce bitterness, pineapple–carrot juice can be blended with spice extracts such as ginger. Ginger is a herbaceous perennial rhizome, traditionally used in culinary for its flavour and pungency. It is also used as a carminative, stimulant and for its anti-emetic properties due to gingerols and shogaols which it contains. According to Wadikar, Nanjappa, Premavalli, and Bawa (2010), ginger is also useful as an appetizer. Ginger juice extracts can be used as an additional ingredient to the pineapple-carrot beverage to add to it improved sensory and nutritional values.

The fruit and vegetables discussed above are highly valued for their characteristic nutritional, medicinal and refreshing properties, and ginger juice is also believed to have antibacterial and anti-fungal properties, implying extended shelf-life for beverages containing its content (Bhardwaj & Mukherjee, 2011). The blending of the fruit and vegetable juices for the preparation of a beverage can be a convenient approach and can provide an economic alternative for the utilization of under-utilized abundant tropical fruits and vegetables.

Despite juicing being a vital tool to check the postharvest management of pineapples to provide a refreshing and nutritional drink, the process alone cannot help retain the original quality of the raw product from which they came from for longer duration. The development and marketing of fresh fruit/vegetable juices are limited due to short shelf-life resulting from the growth of microorganisms (Chia, Shamsudin, Mohd Adzahan, & Wan Daud,

2012; Song et al., 2007). Interventions which can help maintain the quality of the juice product for a longer duration so that it is similar in quality to the raw fruit and/or vegetable must be sought.

Refrigeration and frozen storage have been used for centuries to slow down the quality degradation of stored food products and extend their shelf-life. Production of fresh and unpasteurized fruit juice products have also become common due to consumers' preference for fresh natural product with intact quality. These products are always marketed in small-scale operation, and they always have a challenge with their short shelf-life. Even under refrigeration, fruit juices still have short shelf-life, but it is longer compared to room temperature storage. Freezing of unpasteurized fruit and vegetable juices is one of the most common ways of retaining the quality of these products (Cortés, Esteve, & Frígola, 2008). Several studies have shown the effects of storing fruit and their extracts at low temperatures, but few have actually examined these effects at very low temperatures, such as the temperature of liquid nitrogen (Polinati, Faller, & Fialho, 2010). Frozen storage of unpasteurized juice products gives a longer and extended shelf-life than refrigerated storage, providing the producer or marketer an opportunity of a flexible time schedule for distribution. It has been specified that the most important nutritional changes in frozen foods are due to storage time (Sahari, Boostani, & Hamidi, 2004).

To store the juice products for long at room temperature, juice products are always pasteurized and treated with chemical preservatives. Pasteurization deactivates microorganism and enzyme activity that are responsible for degradation reactions. However, the process also destroys essential nutrients

in the product being pasteurized. Degradation in the quality of pasteurized products will also continue to take place during storage, albeit at rates dependent on the storage conditions.

Problem Statement

In many areas of Ghana where pineapple is produced, most of the harvested pineapples are consumed with little or no value addition. The inability to consume all of the fruits after harvest makes pineapple fruits experience a lot of postharvest losses. Pineapples, especially sugarloaf variety, have very limited postharvest shelf-life, especially in warm climates.

Also, the demand for fruit and vegetable juice is growing because consumers spend limited time preparing their own drinks while demanding safer, more hygienic and healthy beverage products. Postharvest management of pineapple fruits by juicing and blending with other tropical vegetable extracts is a good procedure that can help meet the needs of such consumers.

The perishable nature of fresh fruit juices necessitates immediate consumption within less than 24 hours of processing in warm climates, and extended, but still limited time in cooler environments (Bates et al., 2001). However, there are approaches to extend the shelf-life of processed fruit and vegetable products far beyond the immediate consumption stage. Fruit juice including pineapple juice is already found in most fruits and vegetable products markets, but little information exists regarding the quality changes after juicing and during storage, especially frozen storage of pineapple carrot-ginger-beverage.

Numerous researchers have studied various fruits and vegetable processing into juice (Achinewhu & Hart, 1994; Akinwale, 2000; Akinyele,

Keshinro, & Akinnawo, 1990; Rattanathanalerk, Chiewchan, & Srichumpoung, 2005; Rivas, Rodrigo, Martínez, Barbosa-Cánovas, & Rodrigo, 2006). These studies have, however, considered either a single fruit juice in isolation or in a concoction beverage, but studies have not yet considered pineapple-carrot-ginger beverages.

Objectives of the Study

Main Objective

The main objective of the study was to determine and optimize the effects of developing a pineapple-carrot-ginger beverage and to study the physicochemical, nutritional, microbial and sensory quality changes of optimized unpasteurized and pasteurized beverage during storage.

Specific Objectives

i. To determine the effect of three independent processing variables (pineapple-to-carrot juice ratios, juice fibre size distribution and ginger concentration) on the pH, TSS, beta-carotene and sensory attributes of pineapple-carrot-ginger beverage.
ii. To optimize the independent processing variables of the beverage based on pineapple-to-carrot juice ratio, fibre size distribution and ginger concentration.
iii. To determine the effects of storage temperature and time on the physicochemical, nutritional, sensory and microbial quality attributes of fresh optimized unpasteurized beverage.
iv. To determine the effects of storage temperature and time on the physicochemical, nutritional, sensory and microbial quality attributes of optimized pasteurized beverage.

Statement of Hypotheses

Null Hypotheses

1. There is no significant effect of independent variables ((pineapple-carrot juice ratios, juice fibre size distribution and ginger concentration) on pH, TSS, beta-carotene and sensory attributes.
2. There is no significant effect of storage temperature and time on the quality attributes of fresh optimized unpasteurized beverage.
3. There is no significant effect of storage temperature and time on the quality attributes of optimized pasteurized beverage.

Significance of the Study

Consumer demand for freshly squeezed and minimally processed foods is increasing (Dede, Alpas, & Bayındırlı, 2007; Varela-Santos et al., 2012). But such products are susceptible to spoilage and degradation and have limited shelf-life (Buzrul, Alpas, Largeteau, & Demazeau, 2008; Jordan, Pascual, Bracey, & Mackey, 2001). Attempts must, therefore, be made to extend the shelf-life as well as arrest the quality degradation in the nutritional composition so that consumers can still be able to benefit from their consumption.

Pineapple-carrot-ginger beverage has the opportunity to provide the necessary nutritional demands for consumers who are interested in nutraceutical products to boost their health. Bhardwaj and Pandey (2011) stated that pineapples and carrots are among the natural food products that are valued very highly for their refreshing juice, nutritional value, pleasant flavour, and medicinal properties. Optimized results can also provide the food

industry with a standard reference to be able to manufacture large scale beverages and enjoy marketability and consumer preference.

During storage, liquid foods suffer a significant number of deterioration reactions (ascorbic acid degradation, cloud loss, microbial spoilage, development of off-flavour, changes in colour, texture, appearance), with an important quality loss (Esteve & Frígola, 2007). Quality degradation studies can provide consumers with knowledge on how they can store their products in order to maximize their nutritional content while at the same time provide them with information on when to consume these products to derive maximum nutritional benefits.

Delimitations

The study was conducted mainly in Cape Coast, Central Region of Ghana. The analytical determinations were done at University of Cape Coast Chemistry Laboratory and Department of Molecular Biology and Biotechnology Laboratory.

Limitations

The raw materials (fruits and vegetables) used for the study were not bought on the same day. Since the experiments involved different phases executed at different times, the raw materials were acquired and processed at different dates.

Definition of Terms

Processing: Is the transformation of raw ingredients by physical or chemical means into food or other forms

Storage: Is the process in which cooked and raw food materials are kept in appropriate conditions for future use without any entry or multiplication of microorganisms.

Pasteurization: Is the sterilization of a food substance specially a liquid food at a temperature and for a period of exposure that destroys objectionable organisms without major chemical alteration of the substance

Organisation of the Thesis

This report is divided into Five Chapters. The organization of the report is summarized in the flowchart in Figure 2.

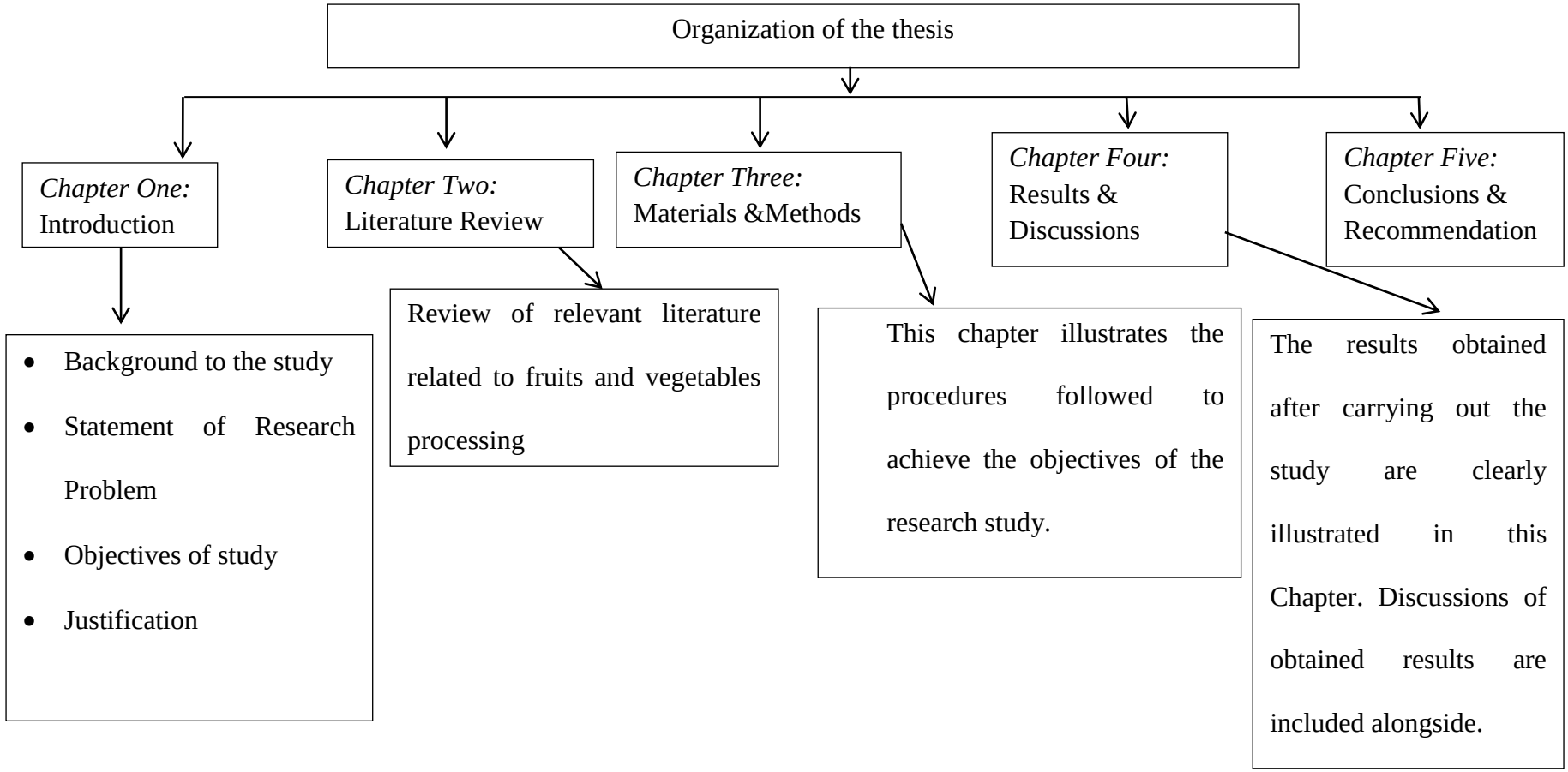

Figure 2: Flowchart showing organization of the thesis report

CHAPTER TWO

LITERATURE REVIEW

Pineapple

Pineapple Worldwide growing Status and Origin

Pineapple (*Ananas comosus* L. Merr.) is cultivated in all tropical and sub-tropical countries and ranks third among tropical fruits, only preceded by mangoes and bananas (Davey, Sripaoraya, Anthony, & Power, 2004). Figure 3 summarizes the top ten pineapple producing countries in the world in 2013. The fruit is the leading edible member of the Bromeliaceae family, which includes about 2,000 species, mostly epiphytic, and many are strikingly ornamental (Hajare et al., 2006). Nearly 70% of pineapple is consumed as fresh fruit in the fruit producing countries (De La Cruz & Garcia, 2004). It originated from Brazil and Paraguay in the Amazon basin where the fruit was domesticated (De La Cruz & Garcia, 2004).

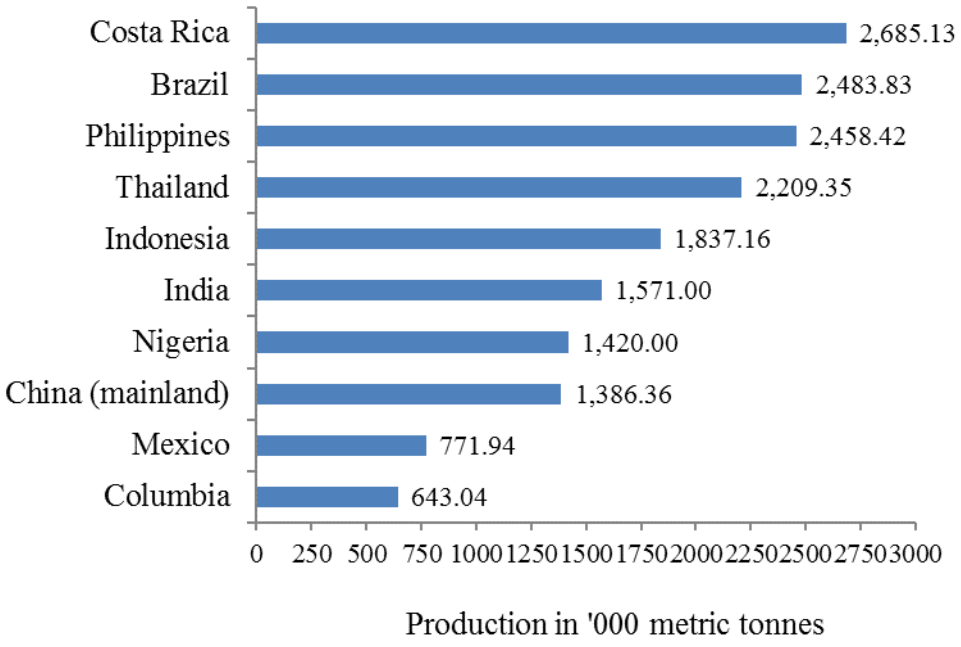

Figure 3: Ten leading countries in pineapple production worldwide in 2013

Source: FAOSTAT (2013)

Pineapple Variety

There are numerous varieties of pineapple worldwide. The most widely cultivated variety in the world is Smooth Cayenne which was first introduced in Europe from the French Guyana (Bhatia, Johri, & Ahmad, 2012). However, several new varieties have been introduced to improve the quality of the fruit that reaches international markets.

The main varieties are the following:

- Smooth cayenne; this is big, cylindrical, deep orange fruit with flat eyes and a light yellow flesh. Its taste is sweet-sour. Smooth cayenne is the most common variety worldwide, both for processing and fresh eating;
- Sugarloaf variety— this is large, heavy, and mildly sweet.
- MD2; this is the recently developed variety which is called 'Del Monte Gold'
- Other pineapple varieties are Queen or Victoria, Red Spanish.

In Ghana, the dominant variety is the sugarloaf and smooth cayenne (Osei-Kofi, Amoatey, & Lokko, 1996). Sugarloaf has high juice content followed by MD-2, and Smooth cayenne has the least juice volume. Most sugarloaf variety fruits are harvested for the local market in Ghana. It should be noted that sugarloaf pineapple variety is not suitable for exportation as it deteriorates quickly soon after harvesting.

Pineapple Physiological Maturity and Significance in Processing

Pineapple is a non-climacteric fruit and is harvested as soon as it is ready for consumption. There are a number of indices that are used to determine the commercial and physiological maturity of the fruit. Some of the

indices are variety-specific while others are general for all. For most fruits, soluble solids must fall between 11 and 18%, titratable acidity as citric acid from 0.5 to 1.6%, ascorbic acid should fall between 20 and 65 mg/100 g of fresh weight, depending on the cultivar and stage of maturity of the fruit (De La Cruz & Garcia, 2004). McGrath and Karahadian (1994) stated that these indicators have been used to determine the harvest times of pineapple fruits with acceptable flavour characteristics and structural integrities.

The proper harvest time depends on the fruit end use. Fruits for export should be cut when they are completely developed but green. Fruits for domestic market are harvested mature but not fully ripened. Dhar, Rahman and Sayem (2008) stated that pineapple fruits harvested at different maturity stages are not of uniform quality. Pineapple fruits that are destined for juicing should be harvested at the optimum maturity stage in order to obtain high-quality juice product.

Composition of the Pineapple Fruit

Nutrient content and biochemical composition of harvested produce vary with the products as they have might come from different parts of the plant (Calderón, 2010). The edible portion of the pineapple fruit, which is about 60% of the fresh weight, contains about 85% water, 0.4% protein, 14% sugar, 0.1% fat and 0.5% fibre (Py, Lacoeuilhe, & Teisson, 1987). Table 1 presents the average composition of the edible portion of the pineapple fruit. Carbohydrates make up to 85% of the total soluble solids (TSS) whereas fibre makes up 2–3%. Of the organic acids, citric acid is the most predominant. The pulp has very low ash content, nitrogenous compounds and lipids (0.1%). The composition of pineapple juice varies with geographical, cultural and seasonal

harvesting and processing. Fresh pineapple also contains minerals such as calcium, chlorine, potassium, phosphorus and sodium.

Table 1: Pineapple Composition in the Edible Portion

Component	% (wet basis)
Brix	10.8–17.5
Titratable acidity	0.6–1.62
Ash	0.3–0.42
Moisture	81.2–86.2
Fibre	0.3–0.61
Lipids	0.2
Esters (ppm)	1–250
Pigments (ppm carotenes)	0.2–2.5
Total nitrogen	0.045–0.115
Protein	0.181
Soluble nitrogen	0.079
Ammonia	0.010
Total amino acids	0.331

Source: Dull (1971)

Nutritional and Health Benefits of Pineapple

Several epidemiological studies have demonstrated a relationship between consumption of fruits like pineapples and a lower incidence of degenerative diseases such as heart disease, arthritis and aging (Blumberg, 2003). Pineapple is well known to be a rich source of vitamin C (ascorbic acid). Ascorbic acid is widely distributed in fruits and vegetables, and it has long been considered as one of the major antioxidants in fruits (Sun, Chu, Wu, & Liu, 2002). Vitamin C is also present in other fruits such as oranges, lemons, grapefruit, watermelon, papaya, strawberries, cantaloupe, mango, raspberries and cherries (Naidu, 2003) among others, as well as in certain vegetables. Vitamin C is an essential water-soluble vitamin which plays a vital

role in the formation of collagen, a primary component of much of the connective tissue in the body. Contemporary interest in vitamin C centres on its ability to perform antioxidant functions. As an antioxidant, it can help prevent the cell damage done by “free radical” molecules as they oxidize protein, fatty acids and deoxyribonucleic acid in the body. Free radical damage has been implicated in the progression of several diverse and important diseases including cancer, cardiovascular disease and cataract formation (Gershoff, 1993; Harats et al., 1998; Machlin & Bendich, 1987). Being a good source of vitamin C, if regularly consumed, pineapple juice can be an important part of a diet aimed at reducing the risk of such chronic diseases.

Humans alongside guinea pigs, bats, apes, teleost fish and some birds are unable to synthesise vitamin C and must, therefore, derive this compound from dietary intake (Nandi, Mukhopadhyay, Ghosh, Chattopadhyay, & Chatterjee, 1997). Due to this, humans have to meet their daily vitamin C requirements through fruits and vegetables and/or supplements (Phillips et al., 2010).

Pineapple is also known to contain a very important enzyme called bromelain. Bromelain belongs to a group of protein-digesting enzymes obtained commercially from the fruit and stem of pineapple. The bromelain in pineapple stimulates digestion and is essential for the proper performance of the small intestine and kidneys; it helps in detoxification, normalizes colonic flora, helps in hemorrhoid alleviation, and prevents and corrects constipation (De La Cruz & Garcia, 2004). In addition, bromelain contains several proteinase inhibitors (Maurer, 2001).

Pineapple is also a rich source of dietary fibre. Dietary fibre is a non-starch polysaccharide, which is a complex carbohydrate with many important health benefits. Dietary fibre holds water soluble nutrients in a gel matrix which delays gastric emptying and slows digestion and absorption. This tends to promote satiety, may reduce the rate of glucose uptake following consumption of glycaemic (available) carbohydrate, thus helping to prevent a surge in blood glucose levels. There are many documented health benefits of dietary fibre (Anderson, Smith, & Gustafson, 1994; Kaczmarczyk, Miller, & Freund, 2012; Kelsay, Behall, & Prather, 1978).

Description of Carrot, Nutritional Composition and Health Benefits

Carrot (*Daucus carota* subsp. sativus) belongs to the umbelliferae class and is one of the most commonly grown vegetables in the world in all seasons (Sharma, Karki, Thakur, & Attri, 2012). It is a root vegetable, usually orange in colour, though purple, red, white, and yellow varieties do exist. The orange colour is essential not only for carrots sold on the fresh vegetable market but also forms a vital significance for the food industry (Nicolle, Simon, Rock, Amouroux, & Rémésy, 2004). Consumer concern over the safety of synthetic food colourants has increased the demand for natural food colourants (Kırca, Özkan, & Cemeroglu, 2006), therefore making the orange colour of carrots vital in giving processed products like juices natural attractive colour which is well revered by consumers. Figure 4 shows the orange variety carrots, with a bright attractive orange colour. When juiced, the resultant product is a brightly attractive nutritive beverage.

Figure 4: Orange-coloured carrots

Carrot root is approximately 88.3% water, 1% protein, 9.6% carbohydrate, 0.2% fat, and 3% dietary fiber (USDA, 2015), and the carbohydrate fraction is almost exclusively simple sugars, predominantly sucrose, glucose, and fructose, with a small amount of starch. Carrots contribute significantly to dietary vitamin A intake through alpha- and beta-carotene and modestly to other nutrients. Vitamin A deficiency remains a major nutritional problem in most economically disadvantaged parts of the world (Montagnac, Davis, & Tanumihardjo, 2009; Olson, 1994), in which populations usually rely primarily on dietary sources of pro-vitamin A carotenoids to meet their vitamin A needs. Carrots are the single major source of pro-vitamin A, providing 14 to 17% of the total Vitamin A consumption (Block, 1994). Carrots also contain the B vitamins thiamin (B_1), riboflavin (B_2), and niacin (B_3) in appreciable quantities when compared with other commonly consumed vegetables. It also contains fibre, vitamin K, potassium, folate, manganese, phosphorous, magnesium, vitamin E and zinc.

Carrots in Juice Production

Carrots are often used for juice production (Yoon, Cha, Shin, & Kim, 2005), and the juice produced has a high nutritional value, as it is an excellent source of alpha-carotene, beta-carotene and ascorbic acid, and a good source of dietary fiber and minerals (Demir, Acar, & Bahçeci, 2004; Senti & Rizek, 1975). Carrot juice is also often used in fruit and vegetable juice blends. Carrot juice and blends are among the most popular non-alcoholic beverages and steady increase in their consumption has been reported in various countries (Schieber, Stintzing, & Carle, 2001), possibly due to consumers' awareness of their health benefits.

When used in juice blends, carrot gives a brightly coloured resultant product which improves consumer's sensory sense of colour. In addition to the bright colour, carrot juice is also sweet with a brix value between 6 and 9, and thus, when used in blends with higher brix helps to improve organoleptic acceptability by consumers. Several authors have reported the use of carrot juice in fruit and vegetable juice blends (Gao & Rupasinghe, 2012; Rivas et al., 2006; Saldana, Stephens, & Lime, 1976).

Carrot juice has a pH of between 6.0–6.5 (Kim, Park, Cho, & Park, 2001) and this pH conditions is capable of supporting the growth of pathogenic microorganisms. Thus, storage of raw, unprocessed carrot juice may lead to microbiological safety problems (Patterson, McKay, Connolly, & Linton, 2012). Blending untreated carrot juice with low pH fruit juices can help balance the pH and hence improve the quality of the resulting beverage product in terms of organoleptic acceptance and microbial stability.

Pigments and other Compounds in Carrots and Health Benefits

The main pigments of orange carrots are α- and β-carotene, but a few authors have described some other types of carotene (γ- and ς- carotene) and trace of lycopene (Simon & Wolff, 1987). Several studies have shown that β-carotene conversion to vitamin A occurs much faster compared to conversion of other carotenoids (van Vliet, van Schaik, Schreurs, & Van den Berg, 1995). Recently β-carotene was proven to have anti-cancer activity related to its activity as a free radical quencher and antioxidant (Miller, Sampson, Candeias, Bramley, & Rice-Evans, 1996; Polyakov, Leshina, Konovalova, & Kispert, 2001). Beta-carotene is an important biological compound because it theoretically possesses 100% vitamin A activity, while alpha-carotene possesses 50% (Chen, Peng, & Chen, 1995). Many authors have reported the health impacts of consumption of fruits and vegetables rich in carotenoids, and more particularly β-carotene (Broekmans et al., 2001; Jialal, Norkus, Cristol, & Grundy, 1991).

Moreover, carrot also contains other compounds, such as phenolic compounds (mainly caffeoyl ester) and organic acids (mainly malate and citrate), which contribute not only to the sensory qualities but also offers additional nutritional properties for human health (Nicolle et al., 2004). Phenolic compounds, especially phenolic acids and flavonoids—are ubiquitously present in vegetables, fruits, seeds, tea, wines and juices; thus they are an integral part of the human diet and have recently received much attention since many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of

cardiovascular diseases, stroke and certain forms of cancer (Klimczak, Małecka, Szlachta, & Gliszczyńska-Świgło, 2007).

Carotenoids are responsible for the yellow, orange, and red colours of carrots, while anthocyanins, a class of polyphenolic compounds, are responsible for the colour of purple carrots. All of these pigments have been studied for their health benefits, including protection from certain cancers and cardiovascular disease, and consumer interest in natural whole foods rich in these compounds, often referred to as "functional foods" is growing (Hasler & Brown, 2009).

Description of Ginger Vegetable, its Nutritional Information and Health Benefits

Ginger (*Zingiber officinale*) is a perennial plant with narrow, bright green, grass-like leaves and yellowish green flowers with purple markings. Ginger is cultivated in the tropics for its edible rhizome at approximately 10 months of age, with the root stocks serving a variety of purposes, including culinary and medicinal (Grant & Lutz, 2000).

There are a large number of nutrients in ginger such as amino acids, starch, vitamin, zingiberene, gingerol, shogaol, and ginger phenol among others. For this reason, ginger is useful for detoxification, anti–tumour operations and is also known to enhance the immune system. Recently, there has been increasing interest in the use of natural food additives and the incorporation of health promoting substances into the diet. As a natural additive due to effects of good health, ginger has been gaining much interest in a variety of foods (Yang et al., 2012). In fruit and vegetables juices, ginger is always added as a flavouring agent.

Reasons for Fruits and Vegetables Processing

Fruits and vegetables are processed for the following reasons:

- Even the very flavourful juices that may not be balanced nutritionally, or which lack particular nutrients or phytochemicals, can be blended to generate nutrients such as vitamins, minerals and nutraceuticals.
- Modern processing, packaging, ingredient technology and distribution systems insure safe, stable and appealing juice and beverage products in a convenient, economical form far different from the raw materials they came from.
- The more delicate and soft fruits cannot be kept over long periods of time and tend to deteriorate before or shortly after harvest. In this case, juicing is the only logical alternative.
- Even the more durable fruit may have poor shape, size, or blemished portions that preclude marketing them as fresh whole fruit. Such fruits can be suitably juiced.

Fruit and Vegetable Juice Blending

Two or more fruit juices/pulp may be blended in various proportions for the preparation of nectar, ready-to-serve beverages, etc. (Bhardwaj & Mukherjee, 2011). Fruit juice blends present many advantages, such as the possibility of combining different aromas and flavour, as well as the sum of nutritionally different components (Sinha, Hui, Evranuz, Siddiq, & Ahmed, 2010). Carrot for example, despite its high nutritional content, is very essential in imparting bright colour to the juice blend. The following reviews some work done on fruits and vegetables juice blending.

Inyang and Abah (1997) studied the chemical composition and organoleptic characteristics of juice from steam cashew apple juice blended with orange rich juice in the ratios of 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 and reported that before blending, orange juice had more sugars, titratable acidity and soluble solids but less ascorbic acid than cashew juice. After blending, consequently, the soluble solids, titratable acidity and total sugars of the blends increased with the proportion of orange juice while the ascorbic acid content decreased. The organoleptic evaluation revealed that a 60% cashew apple and 40% orange juice gave a good quality juice in terms of flavour, aftertaste and overall acceptability.

Akinwale (2000) studied the physicochemical properties of some tropical fruit juices (mango, pineapple, lemon, grape, orange) when blended with cashew apple juice, and reported that, while individual fruit juices contained low ascorbic acid content (54.7, 45, 14.7, 30.9 and 33.7 mg/100 ml for orange, grape, pineapple, mango and orange respectively), blending with cashew apple juice (having initial ascorbic acid content of 203.5 mg/100ml) boosted the nutritional quality of the resulting juice blends. At the same time, juice blends also had improved sensory acceptability compared to pure cashew juice alone.

Matsuura, Folegatti, Cardoso and Ferreira (2004) developed nectar based on papaya pulp and passion fruit juice enriched with acerola fruit pulp and investigated the effects on some physicochemical properties and sensory quality. The study reported that sensory acceptance of nectars was positively correlated with increase in the concentration of papaya pulp. However, the pH decreased with increase in the concentration of papaya pulp in the juice.

Mixed nectars of papaya and mango pulps were also studied (Mostafa, Abd El-Hady, & Askar, 1997), and sensory acceptance increased with increasing papaya pulp content. This information means that in juice blends, an increase in the proportion of one juice component may positively or negatively influence certain quality characteristics of the final beverage.

Jan and Masih (2012) evaluated the quality of pineapple juice with carrot and orange juice blends (with ratios 100:0:0, 80:10:10, 60:10:30, 50:20:30 of pineapple: carrot: orange) and reported that the carotenoids content (beta-carotene) of the blends increased with increase in the carrot juice content. The acceptability of the juice blends meanwhile increased with increase in the composition of orange juice in the beverage. In a related study, Yadav, Choudhary, Garhwal, Mahala and Singh (2015) found increased content of beta-carotene with increase in carrot juice content during the formulation of carrot juice blends with each of pomegranate and grapefruit juices. Carrot juice proportions of 95%, 85% and 75% with each of grapefruit and pomegranate blends resulted in 3.71, 3.63 and 3.32 mg per 100 ml and 3.64, 3.58 and 3.26 mg/100 ml of beverage beta-carotene content respectively.

Fruit and Vegetable Juice Spoilage

The shelf-life of fruit juices and concentrates is limited primarily by microbial, enzymatic, and chemical reactions that affect their nutritional quality, colour and flavour (Graumlich, Marcy, & Adams, 1986). Freshly expressed juice is highly susceptible to spoilage, in fact much more than the whole fruit from which they came from. Unprotected by skin or cell walls, fluid components are thoroughly mixed with air and microorganisms from the environment.

Thus, unheated juice is subject to rapid microbial, enzymatic, chemical and physical deterioration. Table 2 summarizes the major factors responsible for juice spoilage. The goal of processing is to minimize these undesirable reactions while still maintaining and in some cases enhancing the inherent quality of the starting fruit.

Table 2: Factors Responsible for Juice Spoilage

Hazzard	Result
Microbial contamination	Survival/growth of pathogens/ Rapid spoilage
Enzymatic activity	Browning, consistency/flavour changes
Pesticides residues	Unsafe/illegal product
Extended holding	Quality deterioration of product
Colloidal instability	Sedimentation/precipitation
Maillard reactants	Browning/quality loss
Aflatoxins on fruit	Unsafe/illegal fruit

Source: Bates et al. (2001)

Processing Technologies for Fruits and Vegetables and/or their Products

Storage and processing technologies have been utilized for centuries to transform perishable fruits and vegetables into safe, delicious and stable products (Rickman, Barrett, & Bruhn, 2007). Various processing methods are used not only to increase the edibility and palatability of fruits and vegetables, but also to prolong their shelf-life (Oey, Lille, Van Loey, & Hendrickx, 2008). This sub-section reviews some of the processing technologies used for fruits and vegetables and/or their products.

Freezing and Refrigeration

Freezing is one of the best methods for long-term storage of fruits and vegetables and their products. It preserves the original colour, flavour and

nutritive value of most fruits and vegetables. Water in fruit and fruit products constitute about 85–90% of their total composition. From a physical point of view, vegetable and fruit tissues can be considered as a dilute aqueous solution, which is the natural medium where chemical and biochemical cellular reactions take place and microorganisms grow. Crystallization of water during freezing reduces water activity in these tissues and consequently produces a decline in chemical and biochemical reactions and microbial growth. Freezing also involves the use of low temperatures and reactions take place at slower rates as temperature is reduced.

The freezing of fruits and vegetables and their products slows down but does not stop the physical, chemical, and biochemical reactions that produce deterioration. There is still a slow progressive change in sensorial and nutritional quality during frozen storage that always becomes noticeable after a period of time (De Ancos, Sánchez-Moreno, De Pascual-Teresa, & Cano, 2006). However, safe and high-quality frozen products with maximum nutritional values can be attained if diligent controls are maintained and ensured at all times. Studies have shown stable or minimal decline in phenolic contents during frozen storage of grapefruit juice at -18 ^{o}C for 2 months (Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010), vitamin C in frozen fresh-squeezed unpasteurized orange juice at -23 ^{o}C for 1 year (Lee & Coates, 1999), and beta-carotene in dill herb at -20, -30 and -40 ^{o}C for 6 months (Lisiewska, Kmiecik, & Słupski, 2004) among others.

Refrigerated storage, though essential in lowering biochemical and micro-organisms activity in stored products, can still cause degradation of the produce. Studies have shown shelf-life of produce under refrigerated storage

in the range of a few days to a few weeks, e.g. 14 days for fresh squeezed orange juice (Fellers, 1988).

Thermal Pasteurization

Thermal pasteurization methods rely essentially on the generation of heat outside the product to be heated, by combustion of fuels or by an electric resistive heater, and its transfer to the product through conduction and convection mechanisms (Pereira & Vicente, 2010). To date, the application of heat remains the most common processing method to extend the shelf-life of liquid foods due to its ability to inactivate microorganisms and spoilage enzymes (PPO, PME, etc) (Zulueta, Barba, Esteve, & Frígola, 2013). Traditionally, most preserved juices with a pH equal to or less than 4.5 are thermally processed for a few seconds at temperatures between 60 and 100 ^{o}C (Jay, 1992). During this process, a substantial amount of energy is transferred to the food which in some cases can lead to undesirable reactions and formation of sub-products (Rivas et al., 2006). The following reviews the effects of thermal pasteurization on juice characteristics.

Effect of pasteurization has been reported on pineapple juice and cashew apple juice resulting in degradation of bioactive compounds such as ascorbic acid and total carotenoids (Rattanathanalerk et al., 2005; Zepka & Mercadante, 2009), as well as the colour changes. Pasteurization also led to a decrease in the levels of vitamin A and phenolics in the transformation of mango to puree while total carotenoids and ascorbic acid were reported stable though this depended on the severity of the process (Vásquez-Caicedo, Schilling, Carle, & Neidhart, 2007). Hoffmann-Ribani, Huber and Rodriguez-Amaya (2009) reported a decrease in the levels of flavanols (quercetin,

myricetin and kaempferol) in industrially processed (pasteurized) cashew apple juice. Chen et al. (1995) showed that the more severe the heat treatment during pasteurization of carrot juice, the more the destruction of carotenoids occurred and they similarly observed a reduction in vitamin A of about 55.7% after canning at 121 ^{0}C for 30 min. Colour changes were also noted using this treatment.

Xiang et al. (2014) investigated carrot juice samples treated with thermal pasteurization for 1 min at 90 ^{o}C and reported that the process significantly affected brix and total acidity compared to the untreated carrot juice (10.33 vs 9.70 of brix for pasteurized and untreated carrot juice respectively), while the pH remained the same. At the same time, thermal pasteurization of the carrot juice resulted in reduced aerobic bacteria counts by 80%.

Saldana et al. (1976) reported that the pH of carrot juice was unaffected by thermal pasteurization. Rivas et al. (2006) also reported no change in pH of orange and carrot juices treated by thermal pasteurization, while it inactivated 100% of the total plate count in the juice blend. The total acidity was higher in the carrot juice treated with thermal pasteurization compared to the control, which also agrees with Akinyele et al. (1990).

Other Processing Technologies

Despite thermal processing being the predominant process in the food processing industry to inactivate microorganisms and enzyme activity as well as extend product shelf-life, it may under severe conditions induce several chemical and physical changes in the food that may impair the organoleptic properties and reduce the content or bioavailability of some bioactive

compounds as seen in the various literature discussed above. Therefore, there has been the emergence of mild processing technologies such as high-pressure processing, irradiation, pulsed electric fields, power ultrasound, ozone, and oscillating electric fields among others.

Non-thermal technologies have been reported to be a good way of obtaining products with fresh-like appearance while preserving their nutritional value (Martín-Belloso & Sobrino-López, 2011). The following sub-section briefly describes some of these technologies.

Pulsed Electric Fields (PEF) Processing

PEF technology is based on the application of pulses of high voltage (typically 20–80 kV/cm) delivered to the product placed between a set pair of electrodes that confine the treatment gap of the PEF chamber (Pereira & Vicente, 2010). The large field intensities are achieved through storing a large amount of energy in a capacitor bank (a series of capacitors) from a direct current power supply, which is then discharged in the form of high voltage pulses (Zhang, Barbosa-Cánovas, & Swanson, 1995). Foods can be pasteurized with pulsed electric fields at ambient or refrigerated temperatures for a short treatment time of seconds or less and this helps preserve the fresh-like quality of food.

High-intensity pulsed electric field (HIPEF) treatments is one of the non-thermal emerging technologies being studied as an alternative to thermal treatments not only to ensure safety and extend shelf-life of fruit juices but also to provide fresh-like products with high antioxidant potential (Rawson et al., 2011).

High-Pressure Processing (HPP)

High hydrostatic pressure (HHP) processing is an established non-thermal food processing and preservation technique with reduced effects on nutritional and quality parameters compared to conventional thermal processing, and the term is derived from material science in which products are treated above 100 MPa (Rawson et al., 2011). HHP processing (100–1000 MPa) is one of the most promising technologies for food treatment and preservation at room temperature.

A popular technique for HHP processing is to combine compression heating with conventional heating for food sterilization (Furukawa, Shimoda, & Hayakawa, 2003). Instantaneous adiabatic compression during pressurization provokes a quick increase in the temperature of the food products, which is reversed when the pressure is released, providing rapid heating and cooling conditions and hence short processing times (Shao, Zhu, Ramaswamy, & Marcotte, 2010).

Radiation Processing

Irradiation treatment generally involves the exposure of food products (raw or processed) to ionizing or non-ionizing radiation for the purpose of food preservation (Rawson et al., 2011). The source of ionizing radiation could be high-energy electrons, X-rays (machine generated), or gamma rays (from cobalt-60 or cesium-137), while the non-ionizing radiation is electromagnetic radiation that does not carry enough energy/quanta to ionize atoms or molecules, represented mainly by ultraviolet rays, visible light, microwaves, and infrared.

Food irradiation causes minimal change in the flavour, colour, nutrients, taste, and other quality attributes of the food (Alothman, Bhat, & Karim, 2009). However, the levels of modification (in flavour, colour, nutrients, taste etc) might vary depending on the basic raw material used, irradiation dose delivered, and on the type of radiation source employed (gamma, X-ray, UV, electron beam) (Bhat, Sridhar, & Tomita-Yokotani, 2007).

Ozone Processing

The interest in ozone as a preservation technology is based on its high biocidal efficacy and wide antimicrobial spectrum. Within the food industry, ozone has been used routinely for washing and storage of fruits and vegetables by gaseous treatment. The potential of ozonation in liquid food applications has begun to be exploited with the recent FDA approval of ozone as a direct additive to food (Cullen, Tiwari, O'Donnell, & Muthukumarappan, 2009). Ozone as an antimicrobial agent has numerous potential applications in the food industry because of its numerous advantages over traditional antimicrobial agents such as chlorine, potassium sorbates, etc. (Rawson et al., 2011).

Ultrasound Processing

Ultrasound processing on its own or in combination with heat and/or pressure is an effective processing tool for microbial inactivation and phytochemical retention (Rawson et al., 2011). Advantages of ultrasound include reduced processing time, higher throughput, and lower energy consumption (Zenker, Heinz, & Knorr, 2003). It is certainly capable of achieving the desired 5-log reduction for foodborne pathogens in fruit juices

(Salleh-Mack & Roberts, 2007), but there is accumulating evidence that it could negatively modify some food properties including flavour, colour, or nutritional value. According to Rawson, ultrasound treatment of fruit juices is reported to have a minimal effect on the ascorbic acid content during processing and results in improved stability during storage when compared to thermal treatment.

Physicochemical, Nutritional, Sensory and Microbial Quality Changes of Fruit Juice during Storage

Following processing, biochemical changes such as the concentration changes in vitamin C, sugars, soluble solids and phenols during storage of fresh-cut pineapple and juice are very important since they are used as primary quantitative parameters of quality (Gorny, 2001). Soluble solids content, total or titratable acidity, pH, water content, density and the ratio of soluble solids content to acidity are commonly used as quality attributes (Calderón, 2010).

During storage, liquid foods suffer quite a number of important deterioration reactions which include, ascorbic acid degradation, cloud loss, microbial spoilage, development of off-flavour, changes in colour, texture, appearance and quality loss alongside (Esteve & Frígola, 2007). It is on this basis that the shelf-life of the product is ascertained.

Temperature management is the most important tool a food technologist or engineer can apply to extend shelf-life and maintain the quality of fruits and vegetables and their products. The degradation rate in quality is usually mitigated by reducing the temperature of product storage (Kramer, 1977) since nutritional quality of food during storage has become increasingly an important problem (Burdurlu, Koca, & Karadeniz, 2006). This important

fact is of great significance to the consumer and processor who must have awareness on how to store the juice containers and when to consume them in order to derive maximum benefit from them. The following sub-section reviews effects of storage on the quality attributes of fruit and vegetable juices.

Effect of Storage on Vitamin C (Ascorbic Acid)

Vitamin C (ascorbic acid) is an important component of our nutrition and is used as an additive in many foods because of its antioxidant capacity (Burdurlu et al., 2006), and thus increases the quality and technological properties of food as well as nutritional value (Larisch, Groß, & Pischetsrieder, 1998; Solomon, Svanberg, & Sahlström, 1995). However, ascorbic acid is an unstable compound and easily decomposes under less desirable conditions (Lee & Coates, 1999). The compound is least stable during processing and it is highly sensitive to oxidation and leaching into water-soluble media during processing, storage and cooking of fresh, frozen and canned fruits and vegetables (Franke, Custer, Arakaki, & Murphy, 2004; Lathrop & Leung, 1980; Saldana et al., 1976). According to Davey et al. (2000) and Murcia, López-Ayerra, Martinez-Tomé, Vera and García-Carmona (2000), the retention of ascorbic acid is often used as an estimate for the overall nutrient retention of food products.

The nutrient is most sensitive to destruction when the commodity is subjected to adverse handling and storage conditions. Studies have shown that ascorbic acid content decreases during storage depending on various storage conditions such as temperature, oxygen and access to light (Esteve, Frígola,

Rodrigo, & Rodrigo, 2005; Kabasakalis, Siopidou & Moshatou, 2000). The following articles review the effects of storage on ascorbic acid content.

Achinewhu and Hart (1994) studied the ascorbic acid contents of pineapple juice of four different pineapple species grown in the Rivers State of Nigeria and reported that ascorbic acid of pasteurized pineapple juice stored in plastic bottles at room temperature (30-32 ^{o}C) for two months was reduced to between 10 and 21 percent of its original fresh juice content. The ascorbic acid content was initially reduced by thermal pasteurization process which continued to undergo degradation during storage of the pineapple juice at room temperature.

Klimczak et al. (2007) studied the effect of storage time and temperature on the vitamin C content in two commercial orange juices both fresh and after storage at 18, 28 and 38 ^{o}C for 2, 4 and 6 months, and reported that vitamin C was the most content affected by both duration and temperature of storage. The study reported that after 6 months of storage at 18, 28 and 38 ^{o}C, vitamin C content of the juice decreased by 21%, 31% and 81%, respectively. In a related study, Kabasakalis et al. (2000) studied the ascorbic acid degradation of different commercial fruit juices stored in closed containers and reported ascorbic acid losses ranging between 29-41% after four months of storage at room temperature.

Lee and Coates (1999) carried out a storage study of frozen, fresh-squeezed, unpasteurized, polyethylene-bottled orange juice to determine vitamin C loss on a monthly basis for 24 months, and reported vitamin C loss of 19.2% over the entire storage period at -23 ^{o}C. The orange juice studied initially had 40.6 mg/100 ml vitamin C content, but this reduced to 32.8

mg/100 ml after 2 years of storage, losing about 0.8 % of its vitamin C content every month. Fellers (1988) also conducted a shelf-life study and quality evaluation of freshly squeezed, unpasteurized polyethylene-bottled citrus juices stored at -1.7, 1.1, 4.4 and 7.8^{o}C and reported that ascorbic acid retention after 2 weeks of storage at the three lowest storage temperatures was about 91–93% for two orange juices and 86–88% for the grapefruit juice.

Igual et al. (2010) studied grapefruit juice stored at 4 and -18 ^{o}C for 2 months and reported that after 12 days of storage, ascorbic acid content decreased for juice samples irrespective of whether stored under refrigeration or frozen, and thereafter maintained constant ascorbic acid content till the end of frozen storage. In the refrigerated juice, the proportion of ascorbic acid decreased significantly throughout storage.

Spínola, Mendes, Câmara and Castilho (2013) studied the stability of L-ascorbic acid in passion fruit extracts during storage at 4, -20 and -80 ^{o}C and reported that at 4 ^{o}C, ascorbic acid remained stable for at least 24 h with ascorbic acid recovery of 97.8% for extract solutions, but thereafter registered a notable decline throughout the entire study. During 1 week storage at -20 ^{o}C, ascorbic acid was stable with a recovery of 96.7%, while storage at -80 ^{o}C resulted in a minimal loss for up to 4 weeks (<2%). This study revealed that the best storage temperature to slow degradation for the fruit extract was -80 ^{o}C, followed by -20 ^{o}C, while 4 ^{o}C was not suitable for long-term storage in order to preserve ascorbic acid content.

Effect of Storage on Carotenoids Content

Similar to anthocyanins, carotenoids exist as plant pigment responsible for the orange, yellow and red colour and have health promoting effects. Beta-

carotene, β-crytoxanthin and alpha-carotene are the main precursors of vitamin A, which cannot be synthesised within the body and must be supplemented by daily intake, hence are considered to help in reducing the incidence of cancer and other diseases (Block, Patterson, & Subar, 1992; Omenn et al., 1996). It is important to bear in mind that carotenoids are highly unstable in nature, being both photo- and thermolabile, which tend to oxidise easily if not protected from light and the atmosphere.

Carotenoids have been reported to deteriorate during storage of food products, at rates depending on the storage conditions (Provesi, Dias, and Amante, 2011; Chen et al., 1995; Lin & Chen, 2005).

Effect of Storage on Total Phenolic Content and Antioxidant Activity of Fruit Juice

The total antioxidant activity of fruit juices is contributed by a group of phytochemicals, among them being vitamin C, total phenolics, carotenoids, flavonoids, etc. The change in the contents of these phytochemicals will result in the change in the total antioxidant activity of the product. The following reviews some studies on total phenolic and antioxidant changes during storage.

Klimczak et al. (2007) studied the effect of storage on the content of polyphenols and antioxidant activity of orange juices stored at 18, 28 and 38 ^{o}C and reported a decrease in the content of polyphenols at the end of storage and this was reflected by the decrease in the antioxidant capacity of the juices. After storage of the juices at 18, 28 and 38 ^{o}C for 4 months, the total phenolic content of the juices as determined by the Folic-Ciocalteu assay decreased by 7%, 11% and 20% respectively. However, after two months of further storage,

the juices showed a significant increase in the total phenolic content. The respective decrease of total antioxidant activity was 18%, 45% and 84% after 6 months of storage at the respective storage temperature regimes.

Igual et al. (2010) studied the effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice and reported that frozen (-18 ^{o}C) unpasteurized juice and conventionally pasteurized ones preserved about 75% and 20% of the total phenols and antioxidant capacity, respectively after 2 months of storage. The total antioxidant activity of the stored grapefruit decreased during storage for all kinds of treatment to the samples in this study.

Laorko, Tongchitpakdee and Youravong (2013) studied the effect of storage of micro-filtered non-pasteurized pineapple juice at 4, 27 and 37 ^{o}C on some phytochemical properties (vitamin C, total phenol content, antioxidant activity), and reported that the phytochemical properties and total phenolic content of the juice significantly decreased as storage time and temperature increased. The best storage temperature for non-thermally pasteurized and clarified pineapple juice was found to be 4 °C since this allowed the best pineapple juice quality preservation.

Arena, Fallico and Maccarone (2001) studied the effect of storage on the total antioxidant capacity of blood orange juices and reported that the total antioxidant capacity of freshly squeezed juices remained unchanged during storage at 2 ^{o}C for 60 days. In comparison, juice reconstituted from concentrate had decreased antioxidant activity.

Babsky, Toribio and Lozano (1986) investigated the composition of clarified apple juice concentrates during storage at 37 ^{o}C for 111 days and

reported an increase in the phenolic compounds from 149 mg/100 ml to 215 mg/100 ml at the end of storage. Del Caro, Piga, Vacca and Agabbio (2004) studied antioxidant activity in some cultivars of grapefruit juice during storage at 4 ^{o}C for 15 days and reported that antioxidant activity significantly decreased in juices made from some cultivar while it increased in another. It seems from this that antioxidant activity may increase or decrease during storage depending on the characteristics of the juice being stored.

Mgaya-Kilima, Remberg, Chove and Wicklund (2014) studied the effect of storage time and temperature on the total phenols and antioxidant activity of pasteurized (82.5 ^{o}C, 20 minutes) roselle-fruit juice blend (with mango, papaya, and guava juice) and reported that total phenols and antioxidant activity of the juice blends significantly decreased during storage at 4 and 28 ^{o}C for 6 months.

Effect of Storage on TSS, pH and Titratable Acidity of Fruit Juice

pH is one of the important quality characteristics that describes the stability of bioactive compounds in fruit juice (Sánchez-Moreno, Plaza, de Ancos, & Cano, 2006). Organic acids and sugar ratios primarily create a sense of taste that is perceived by specialized taste buds on the tongue. Sweetness is due to sugar and sourness is due to organic acids and these are dominant components in the taste of fruits (Stanley, 1991). The pH, titratable acidity and TSS of fruit juice may increase, decrease or remain statistically unchanged during storage as is reviewed below.

Chia et al. (2012) evaluated the effect of storage of thermally pasteurized (80 ^{o}C, 10 minutes) pineapple juice at 4 ^{o}C for 13 weeks on the juice quality attributes and reported that pasteurized juice maintained a higher

TSS during the whole period of storage compared to the untreated juice. The pH and TSS, however, did not significantly change for the entire duration of storage for pasteurized samples while pH increased and TSS decreased significantly for unpasteurized juice samples.

Kaanane, Kane, and Labuza (1988) evaluated the physicochemical quality changes of pasteurized orange juice stored at 4, 22.5, 35, and 45 °C for 14 weeks and reported that the pH, TSS, titratable acidity and total sugars did not significantly change during storage of the juice for all temperatures. However, a significant change was observed for ascorbic acid content, reducing sugars and furfural production, with temperature of storage having a profound effect on these changes. Laorko et al. (2013) also studied the effect of storage of micro-filtered non-pasteurized pineapple juice at 4, 27 and 37 ^{o}C on the physicochemical properties and reported that the TSS and pH of the juice were not affected by storage time and temperature after 6 months of juice storage.

Mgaya-Kilima et al. (2014) investigated the effect of storage time and temperature on the physicochemical properties of pasteurized (82.5 ^{o}C, 20 minutes) roselle-fruit juice blend (with mango, papaya, and guava juice) and reported that TSS, pH and reducing sugars of the juice blends significantly increased during storage at 4 and 28 ^{o}C for 6 months. However, during storage of the juice blends, titratable acidity significantly decreased under the same storage conditions.

Nisar, Baba, Masoodi and Yildiz (2015) studied the effect of thermal treatments (65 ^{o}C, 30 minutes) of preservative treated apple pulp on the physicochemical characteristics during storage at 25 ^{o}C for 90 days and

reported a significant increase in acidity and a simultaneous decrease in pH. The TSS of the juice also increased with increase in storage time. Acidity and pH are always interdependent in that the lower the pH, the higher is the acidity during storage at room temperature.

Cortés et al. (2008) studied the physicochemical quality changes of pasteurized orange juices during 7 weeks of refrigerated storage at 2 and 10 ^{o}C and reported a significant increase in pH values during storage while the brix values in pasteurized orange juice (11.4) did not change significantly during storage. Similar observation of increase in pH values of fruit juice has been reported by Del Caro et al. (2004) for citrus segments and juices stored at 4 ^{o}C, while contrary result of no significant change in pH values has been reported by Esteve et al. (2005) for pasteurized orange and orange-carrot juice during storage at 4 and 10 ^{o}C for 12 weeks. Stability of brix values in fruit juices during storage has been reported by some other authors such as Rivas et al. (2006) on pasteurized orange-carrot juice and Bull et al. (2004) on pasteurized orange juice.

Effect of Storage on Microbial Quality Changes of Fruit Juice

The spoilage caused by microorganisms in juices includes cloud loss, development of off-flavours, carbon dioxide production, and changes in colour, texture, and appearance resulting in degradation of product (Sperber & Doyle, 2009). The critical factors affecting the spoilage of juices include juice pH, oxidation reduction potential, water activity, availability of nutrients, presence of antimicrobial compounds, and competing microflora (Aneja, Dhiman, Aggarwal, Kumar & Kaur, 2014). Among these factors, pH and water activity are the most influential factors affecting the spoilage of juices.

Lavinas, Almeida, Miguel, Lopes and Valente-Mesquita (2006) reported reduced total aerobic bacteria as well as total fungi in cashew apple juice during frozen storage at -22 ^{o}C for 120 days while refrigerated juice stored for 1 week at 4 ^{o}C experienced reduced aerobic bacteria and increased yeast and mould during storage. Patterson et al. (2012) studied the microbiological quality and safety of carrot juice during refrigerated storage and found that in untreated juice, the total microbial counts increased rapidly and reached counts of 7 log CFU per ml within 4, 3 and 1 day from an initial counts of 5.8 log CFU per ml during storage at 4, 8 and 12 ^{o}C respectively. In comparison, high pressure processed carrot juiced had reduced log reduction in microbial counts and there was little growth of the survivors during storage at 4 ^{o}C for 22 days.

Effect of Storage on Sensory Quality of Juice

From the point of view of consumers, the flavour, colour and organoleptic taste of fruit juice is very important because it determines the marketability of juice. Organoleptic quality like colour, flavour, and nutritive value of fruit products generally reduces with increase in storage period (Bhardwaj & Pandey, 2011). Foods are known to develop undesirable characteristics in storage such as off-flavour, undesirable taste, odour, etc. that may have a significant effect on the consumer who can either accept or reject the product.

Ayranci and Tuetuencueler (1993) prepared pasteurized carrot juice from each of three different cultivars: yellow-, orange-, and purple-coloured carrots and stored at 5, 20 and 27 ^{o}C. In this study, they reported that each of the 3 juices kept at 27 ^{o}C deteriorated organoleptically in the first 2 weeks of

storage, while juices stored at 20 and 5 ^{o}C were stable for up to 7 and 12 weeks respectively. Oliveira, Ramos, Minim and Chaves (2012) studied the sensory changes of whole mango juice stored at 25, 35 and 45 ^{o}C and found that the flavour, aroma and colour were the most affected by storage temperature and time and significantly decreased at the end of storage.

Sensory Analysis in Fruit Juice Beverage

Sensory evaluation is a scientific method that evokes, measures, analyses and interprets responses to products as perceived through the senses of sight, smell, touch, taste, and sound (Stone & Sidel, 2004). Sensory analysis in the food industry is an important tool used to make decisions, evaluate the quality of new products, redesign and reduce food costs and to analyze the relationship between the processes/ingredients and the sensory attributes.

Like other scientific methods of taking measurements, sensory evaluation is concerned with precision, accuracy, and sensitivity and aim as much as possible in avoiding false–positive results (Meiselman, 1993). Reliable sensory evaluation is based on the skill of the sensory analyst in optimizing four factors: definition of the problem, test design, instrumentation (if any), and interpretation of the results (Meilgaard, Carr, & Civille, 2006).

The most promising methodologies which are applied in sensory study are quantitative descriptive analysis (QDA; also known as sensory profiling) and acceptance sensory analysis (Stone & Sidel, 2003). The sensory profiling technique provides a detailed sensory description and quantification of the product's characteristics. Affective tests, such as the acceptance test, are used when it is necessary to know the consumers attitude towards the product.

Sensory analysis is based on choosing a panel of specific numbers, training them on the parameters of the food to be evaluated, and then carrying out a test and thereafter analyzing. Many authors carried out sensory analysis of various food products in this way (Allgeyer, Miller, & Lee, 2010; Bayarri, Calvo, Costell, & Durán, 2001; McEwan & Colwill, 1996; Schiffman, Crofton, & Beeker, 1985).The sensory attributes of importance in fruit juice are appearance, aroma, consistency, texture and flavour (Meilgaard et al., 2006).

Panel Management

Two general types of panels are used in sensory evaluation. A descriptive panel is commonly used to determine differences between food samples. The descriptive panelist is experienced in the type of food being tested and receives expensive training prior to testing. Meanwhile a consumer or acceptance panel is selected from the public according to the demographics necessary to taste-test a product.

Panel selection

The sensory analyst must recruit people who can make a reliable commitment of time and who also know what is expected of them during the test. Generally, taste panels usually consist of people who meet the following criteria:

- They are in good health and free of illness related to sensory properties, such as chronic colds, food allergies, etc.
- They are nonsmokers (Smoking is known to dull the olfactory and gustatory sensations).
- They are not colour blind.
- They have no strong likes or dislikes for the food being tested.

CHAPTER THREE

MATERIALS AND METHODS

Introduction

This chapter outlines the procedures followed to achieve the objectives of the study. It outlines the procedures for raw materials acquisition, processing, storage and step-by-step methods followed to do the analytical determinations of the beverage quality attributes.

Location of Study

This study was performed at Cape Coast, Central Ghana. Analytical determinations of quality attributes were conducted at the Laboratories at Chemistry Department and Department of Molecular Biology and Biotechnology of the University of Cape Coast.

Raw Materials Collection

Pineapple fruits (sugarloaf variety) were purchased from Elmina Market, Cape Coast, Central region, Ghana, while fresh orange variety carrots and ginger rhizomes were purchased from Kotukuraba market, Cape Coast, Central region, Ghana.

Pineapple fruits were selected based on fruit colour as maturity index. The selected pineapple fruits were of eating quality and were harvested few days before selling and allowed to undergo full ripening. The fresh orange variety carrots and ginger selected were of firm texture, with no surface bruises or other physical injuries. The raw materials were transported to a cottage industry site at Elmina (Mavern Foods Ltd), Central region, Ghana for processing.

Raw Materials Preparation

The obtained raw materials were processed following Hazzard Analysis Critical Control Points (HACCP) guidelines (Food & Drug Administration, 2001). Briefly, according to the guidelines, all raw fruits and vegetables selected were of high-quality class, harvested at the right physiologically mature stage, cleaned before processing in a chlorinated water solution for about 2 minutes, and rinsed thoroughly with potable water. The carrots leaf portions attached to the root were trimmed off to leave only the edible portions desired for juice processing.

Juice Extraction

Pineapple Juice Extraction

- The cleaned pineapple fruits were manually peeled using stainless steel knife and chopped into small pieces and the juice extracted by use of a commercial juice machine (FT–0.5, China) after blending with commercial type juice blender (Philips electric blender).
- The obtained juice was immediately stored at -24 ^{o}C prior to blending with other juices.

Carrot/ginger Juice Extraction

- The carrot roots were chopped into smaller pieces and the juice extracted using potable water (Voltic Ghana Water) in the ratio of 1:1.6 (w/w of carrot to water) using a commercial juice blender. The obtained product was passed through juice extraction machine (FT-0.5, China) to sieve it using different sieve sizes.

- Ginger juice was extracted in a similar manner to carrots and filtered using a muslin cloth and all juices obtained immediately stored at -24 ^{o}C prior to blending.

Experimental Design

Response surface methodology (RSM) was used to design and optimize the pineapple-carrot-ginger beverage experiment using Design Expert software, Version 9.0.6.2 (Stat-Ease, Inc., Minneapolis, USA). Box-Behnken Design (BBD) was used to study the combined effect of three independent variables – pineapple-to-carrot juice ratio, juice fibre size distribution (mm), and ginger concentration (%)–coded as X_1, X_2, and X_3 respectively on the dependent variables (responses). The minimum and maximum values for the independent variables were 3–9 (75/25 v/v – 90/10 v/v) for pineapple-to-carrot juice ratios (X_1), 0.6–1.2mm for juice fibre size distribution (X_2), and 2-5% for ginger juice concentration (X_3) in the beverage. Table 3 shows a summary of the independent variables and their levels used in the design. The levels of independent variables were chosen based on pre-experiment trials and cost considerations of raw materials to produce the beverage.

Table 3: Independent Variables and their Levels Used to Design the Experiment

Factors	-1 (Minimum)	0 (Middle)	+1 (Maximum)
X_1	75/25	86/14	90/10
X_2	0.6mm	0.9mm	1.2mm
X_3	2%	3.5%	5%

The complete design consisted of 15 treatment combinations (including 3 replicates of the centre point) and was carried out in random order (Table 4).

Table 4: Complete Design Showing Independent Variables and their Levels

Sr. No.	Independent variables		
	X_1	X_2 (mm)	X_3 (%)
1	9 (1)	0.9 (0)	5 (1)
2	9 (1)	0.9 (0)	2 (-1)
3	6 (0)	0.9 (0)	3.5 (0)
4	3 (-1)	0.9 (0)	5 (1)
5	6 (0)	1.2 (1)	5 (1)
6	3 (-1)	0.9 (0)	2 (-1)
7	3 (-1)	1.2 (1)	3.5 (0)
8	3 (-1)	0.6 (-1)	3.5 (0)
9	6 (0)	1.2 (1)	2 (-1)
10	9 (1)	0.6 (-1)	3.5 (0)
11	6 (0)	0.6 (-1)	5 (1)
12	9 (1)	1.2 (1)	3.5 (0)
13	6 (0)	0.9 (0)	3.5 (0)
14	6 (0)	0.6 (-1)	2 (-1)
15	6 (0)	0.9 (0)	3.5 (0)

The effects of each factor were assessed on the responses and partitioned into linear, quadratic and interactive components as represented in equation (1):

$$Y = b_0 + \sum_{i=1}^{j} b_i x_j + \sum_{i=1}^{j} b_{ii} x_i^{\,2} + \sum\sum b_{ij} x_i x_j \qquad (1)$$

Where Y= the dependent variable (response), b_0 =constant, b_i=linear coefficient, b_{ii} the quadratic coefficient and b_{ij} the interaction coefficient. x_i and x_j are the levels of the independent variables. Three-dimensional plots were generated by keeping one variable constant at the centre point and varying the other two variables within the experimental range. The independent variables were investigated for their effects on the dependent variables (responses). The responses measured were pH, TSS, beta-carotene,

and the sensory attributes (colour, aroma, taste, consistency, and overall acceptability) of the beverage.

Optimization of the Juice Processing Variables

The optimization of the beverage production process was performed using Design Expert DX9 using a multivariate response method called Overall Desirability index, DI, as described by Myers and Montgomery (2002) using Equation (2).

$$DI = [\prod_{i=1}^{n} d_i]^{1/n} \quad (2)$$

Where n is the number of responses, d_i represents the desirability index for each response variable, and n is the number of response variables.

The desirability index ranges between 0 to 1, with 0 being the least desirable and 1 the most desirable. The goal of optimization studies is to maximize the desirability index. The optimization process incorporates goals and criteria for the independent and dependent variables. For this study, the goals for the independent variables and responses were one of these five criteria: minimize, target, maximize, in range, and none.

Storage Study of Optimized Juice Samples

Fresh Unpasteurized Beverage Storage

The optimized beverage was prepared and packaged in pre-sterilized plastic packaging bottles and corked tightly without pasteurization prior to storage. The packaged juice samples were immediately stored at -24, -10 and 4 ^{o}C and investigated for quality changes at 5-day intervals for 40 days. Three (3) samples were prepared for each storage temperature and time.

Pasteurized Beverage Storage

Prior to packaging, the optimized juice samples were prepared and pasteurized at 80 ^{o}C for 15 minutes, treated with 0.1% sodium benzoate and 0.1% citric acid preservatives and then cooled to room temperature before packaging in pre-sterilized plastic bottles and corked. The samples were stored at the respective temperature regimes of 4, 28 and 38 ^{o}C for further investigation of the juice quality attributes at 15-day intervals and at monthly intervals for sensory quality for a total of up to 90 days. Three (3) samples were prepared for each storage temperature and time. Figure 5 gives a detailed overview of how the beverage was prepared and summarizes the whole experimental study.

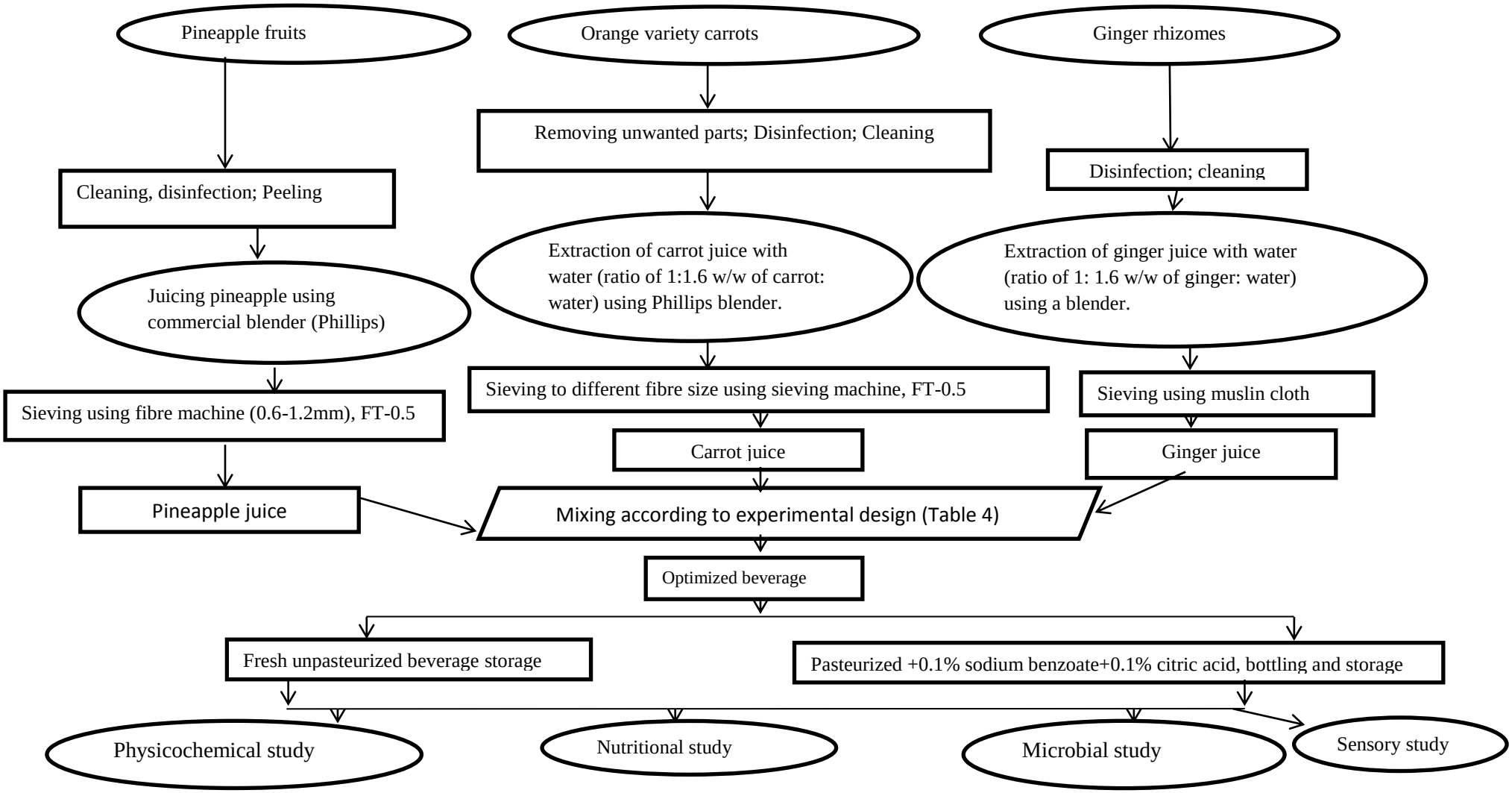

Figure 5: Flowchart showing Overview of the production and analysis of pineapple-carrot-ginger beverage

Physicochemical, Nutritional, Sensory and Microbial Quality Determinations

The pH, TSS, titratable acidity, ascorbic acid, total phenolic content, total antioxidant activity, beta-carotene, and microbial quality as well as the sensory quality of the optimized samples during storage were monitored. The procedures used to carry out these quality determinations are described below.

Determination of TSS (obrix)

A refractometer measures the TSS or sugar as ^{o}brix in 0.1% graduations. The TSS of the juice samples were determined using a hand held refractometer (RHB-32ATC, SYSTEM ANATECH, ^{o}brix 0–32%) with an automatic temperature adjustment after calibration with distilled water.

pH Determination

The pH of the juice samples was determined by the use of a pH meter (model 3510, Wagitech International, Jenway) after calibration with buffer solutions of pH 4.0 and 7.0 respectively. The juice sample was put in a 100 ml beaker, thoroughly stirred, and the electrodes of pH meter immersed in and direct reading taken after the reading stabilized. Samples were measured when their temperature was averagely 20 ^{o}C.

Determination of Titratable Acidity

The juice titratable acidity was determined as in the method of Rekha et al. (2012) using titration method up to pH 8.1. 10 ml fruit juice sample was diluted to 100 ml using distilled water and homogenized by shaking. The resulting solution was filtered through Whatman filter paper to obtain a clear filtrate. 10 ml of the filtrate was pipetted into a 200 ml conical flask and titrated against standardized 0.1N NaOH solution from the burette using

phenolphthalein as indicator. The end point of the titration was reached when the filtrate changed to a permanent pink colour. The volume of NaOH solution required for titration was noted.

Titratable acidity (TA) of the juice sample was calculated according to Equation (3).

$$\text{TA}\left(\% \frac{w}{w}\right) = \frac{\text{Net ml of titrant x Normality of titrant x 6.4}}{\text{sample weight}} \quad (3)$$

Ascorbic Acid Determination

The content of ascorbic acid was determined by titration with 2,6-dichlorophenolindophenol, according to AOAC (1984) and with the changes proposed by Abano, Ma, and Qu (2014). In brief, analytical reagents used were weighed with electronic weighing balance (model LE623P, Sartorius AG Germany) having ± 0.0001 g accuracy. A DCPIP dye solution was prepared by dissolving 52 mg of 2,6–dichlorophenolindophenol in small volume of distilled water containing 42 mg of sodium bicarbonate ($NaHCO_3$) and the solution made to 200 ml with distilled water. The standard stock solution of concentration (1 mg/ml) was prepared by dissolving 100 mg of standard ascorbic acid in 100 ml of 4% oxalic acid solution in a volumetric flask.

10 ml of the stock solution was taken and diluted to 100 ml with oxalic acid solution. To 5 ml of the standard working solution was added 10 ml oxalic acid solution in a 100 ml conical flask and titrated against the 2, 6-dichlorophenol indophenol dye and the volume of titre noted (V_1 ml). The appearance of the pink colour which persists for few minutes indicated the endpoint of the titration and the dye consumed is equivalent to the amount of ascorbic acid.

Ascorbic acid of the juice sample was extracted using 50 ml oxalic acid by taking 5 ml juice sample, weighing and thoroughly agitating to ensure uniform mixing, followed by filtration using Whatmann filter paper. To 5 ml of the supernatant was added 10 ml oxalic acid, and the mixture titrated against the dye (V_2 ml). The ascorbic acid in mg/100 ml was calculated using Equation (4):

$$Ascorbic\ acid, in\ mg\ per\ 100\ ml = \left(\frac{0.5}{V1} \text{x} \frac{V2}{5\ ml} x \frac{50\ ml}{weight\ of\ sample}\right) \text{X}100 \qquad (4)$$

Estimation of Total Phenolic Content

The content of total phenolic was measured using Folin–Ciocalteu method (Singleton & Rossi, 1965). Each juice sample (5 ml) was diluted to 50 ml using distilled water and thoroughly agitated to ensure uniform mixing, then filtered through Whatman filter paper to obtain a clear filtrate. An aliquot (500 µL) of the extract, blank or standard was placed in a test tube, where the Folin-Ciocalteu reagent (2.5 ml), previously diluted 10-fold was added and the mixture allowed to react for about 3 minutes under continuous shaking before 2 ml of saturated sodium carbonate (75 g/L) was added and shaken well.

The samples were then left standing for 90 minutes at room temperature and the absorbance read at 765 nm in a Shimadzu UV mini-1240 UV-VIS Spectrophotometer (Kyoto, Japan). Blank was concomitantly prepared with methanol solution instead of extract solution. A standard curve was plotted using different concentrations of gallic acid (standard 0-200 µg/ml) and the results of the phenolics determinations were expressed as mg gallic acid equivalents (mg GAE) per 100 ml.

Determination of Juice Total Antioxidant Activity

The total antioxidant activity was determined as proposed by Brand-Williams, Cuvelier, and Berset (1995) with slight modifications by measuring the radical scavenging activity and electron donating ability of juice extracts through bleaching a purple solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in methanol . To determine the antioxidant activity of the juice samples, 1 ml of the sample was added to 4 ml of methanol and thoroughly agitated for about 15 minutes. The resulting solution was filtered using Whatman filter paper to obtain a clear filtrate. 100 µL of extract was added to 2 ml of DPPH (0.04 g/L in methanol) and mixed by vortex for 5 minutes and incubated for 30 minutes in the dark. Against a blank of methanol without DPPH, the sample absorbance was read at 517 nm in a spectrophotometer (UV mini-1240 UV-VIS Spectrophotometer, Shimadzu, Japan). The antioxidant activity was determined as the percentage decline of the absorbance, relative to the control, corresponding to the percentage of DPPH that was scavenged. The percentage of DPPH scavenged was determined from equation (5) as:

$$\%\ \text{Inhibition of DPPH} = \frac{A_{CON} - A_{SAMPLE}}{A_{CON}} x100 \qquad (5)$$

Where A_{CON} is the absorbance of the control

And A_{SAMPLE} is the sample absorbance

Determination of Beta-Carotene in the Juice Sample

This was carried out according to AOAC (1980), with slight modifications. In to a tube containing 50 ml of 95% ethanol, 10 ml (measured and weighed) of the juice sample was placed and left for about 20 minutes with periodic shaking. The resulting solution was filtered using filter paper and 15 ml of distilled water added to the resultant filtrate.

25 ml of petroleum ether (pet-ether) was added to the filtrate and shaken gently to obtain a homogenous mixture and it was left to stand until two separate layers are obtained. To fully extract the carotenoids, additional volumes of pet ether were added until the extract became fairly yellow at the top layer and completely colourless at the bottom layer. The absorbance of the extracts was measured using a spectrophotometer (UV mini-1240 UV-VIS, Shimadzu, Japan) at 460 nm against a blank of pet-ether.

The concentration of β-carotene was calculated using Beer-Lambert Law, which states that the absorbance is directly proportional to the concentration of the pigment, as represented by equation 6:

$$Total\ carotenoids\ (\mu g/ml) = \frac{ABS\ X\ V\ (ml) X 10{,}000}{2592 * W\ (g)} \qquad (6)$$

Where ABS is the sample absorbance read from the spectrophotometer; V (ml) is the volume of solvent used for extraction; W (g) is the weight/volume of sample initially taken; 2592 is the extinction coefficient of beta-carotene in pet- ether.

Microbial Load Determinations

10 ml of each sample was first homogenized in 90 ml of sterile peptone water and this was used as a stock solution (first dilution) from which subsequent dilutions was obtained. Subsequent serial dilutions were made by taking 1 ml from the first dilution (10^{-1}) until (10^{-9}) and shaken properly. The pour plate technique was used where the serial dilutions were put into petridishes and plate count molten agar were used for bacteria and OGYEA with oxytetracycline antibiotics for mould and yeast and incubated for 24 hours at 35 ^{o}C and 3 – 4 days at 25 ^{o}C for total plate count and total fungi respectively. Growth of colonies were identified and counted using SC6

digital colony counter and mean number of colonies obtained. Results were expressed as $\log_{10}$CFU per ml of sample.

Sensory Analysis

Consumers were randomly selected from among the staff and students of the School of Agriculture, University of Cape Coast, Ghana and semi-trained on the important aspects of conducting the sensory evaluation. The criteria of selection of the panelists were that (a) they were available and willing to participate in the sensory analysis tests, (b) they were regular consumers of juices, and (c) they were of sound health. Sensory panel committees of 20 members (both male and female) were selected for the analysis. After selection, the panelists were trained to recognize and score different quality attributes of the juice samples including appearance, colour, aroma, taste, consistency and overall acceptability.

The juice samples were served at between 1– 4 ^{o}C to the panelists. The panelists were instructed not to ingest any food for at least an hour prior to sensory testing. The instructions provided to the panelists were precise and clear, both verbally before the test and then also in written form on the score sheet. The prepared juice samples were served in transparent plastic cups and the sensory evaluation room was well lit in order to allow genuine observation of the colour of the sample. The panelists were required to take salted biscuits in between samples and rinse their mouth with water before going on to the next sample to avoid the effects of the previous sample.

The samples were assessed using a 9 point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like). Like extremely 9; like very much

8; like moderately 7; like slightly 6; neither like nor dislike 5; dislike slightly 4; dislike moderately 3; dislike very much 2; dislike extremely 1.

Statistical Analysis

In this study, two methods of statistical analysis were used to complete the whole experiment. In the first experiment to investigate and optimize the effects of pineapple-to-carrot juice ratio, fibre size distribution and ginger concentration on pH, TSS, β-carotene and sensory attributes, a second-order polynomial model was fitted to the mean values of the experimental results to get the regression equations with Design Expert Software version 9.0.6.2 (Stat-Ease, Inc., Minneapolis, USA). Analysis of variance (ANOVA) was performed to find out the statistical significance of the model terms at a probability of 5%. The accuracy of the model to describe the response variables was diagnosed against the coefficients of determination (R^2) values. One independent variable was kept constant and the 3D plots for 2 factors generated for the various responses.

For storage study of the beverage, ANOVA for repeated measures (performed in GenStat Discovery Edition 4) was used to determine whether the effects of storage temperature and time on physicochemical, nutritional, microbial and sensory characteristics were statistically significant. Values of means represent the means for three independent determinations. Differences at $p<0.05$ were considered to be statistically significant according to the least significant difference test (LSD).

CHAPTER FOUR

RESULTS AND DISCUSSION

Effects of Independent Variables on pH, TSS, Beta-Carotene and Sensory Attributes of Pineapple-Carrot-Ginger Beverage

Introduction

In this section, a 3-factor Box-Behnken Design (BBD) was used to establish and optimize the effects of independent processing variables to make a pineapple-carrot-ginger beverage. The beverage was prepared using 3 different factors as pineapple-to-carrot juice ratios (75/25–90/10), fibre size distribution (0.6–1.2 mm) and ginger juice concentration of beverage (2–5%). These 3 factors were used as independent variables whose effects on beverage pH, TSS, beta-carotene content and sensory attributes (colour, aroma, taste, consistency, and overall acceptability) were investigated.

The results of the 15 experiments performed according to BBD are shown in Table 5. Table 6 shows the regression coefficients and coefficient of determination (R^2) values for quadratic models of 8 dependent variables of pineapple-carrot-ginger beverage. When R^2 approaches unity, the better the empirical model fits in describing the effect of independent variables on responses.

Table 5: Results of pH, TSS, Beta-Carotene and Sensory Attributes of Pineapple-Carrot-Ginger Beverage

Run No.	Actual values (Coded values)			Experimental values of responses							
	X_1	X_2 (mm)	X_3 (%)	pH	TSS (brix)	β-carotene (µg/ml)	Colour	Aroma	Taste	Consistency	Overall acceptability
1	9 (1)	0.9 (0)	5 (1)	4.79	15.0	2.29913	5.90	6.57	6.48	6.71	7.1
2	9 (1)	0.9 (0)	2 (-1)	4.74	15.4	2.13439	5.48	6.71	7.00	6.71	7.14
3	6 (0)	0.9 (0)	3.5 (0)	4.77	14.6	2.27122	5.90	6.62	6.81	6.81	7.29
4	3 (-1)	0.9 (0)	5 (1)	4.84	13.4	3.98701	7.90	7.10	6.86	6.86	7.67
5	6 (0)	1.2 (1)	5 (1)	4.78	15.2	2.92567	6.38	6.38	6.19	6.43	6.71
6	3 (-1)	0.9 (0)	2 (-1)	4.79	12.1	4.44792	8.00	7.19	7.71	7.19	7.81
7	3 (-1)	1.2 (1)	3.5 (0)	4.79	12.4	3.86098	7.86	6.90	7.00	7.00	7.67
8	3 (-1)	0.6 (-1)	3.5 (0)	4.82	13.4	4.51813	8.38	7.24	7.57	7.14	7.71
9	6 (0)	1.2 (1)	2 (-1)	4.75	15.2	2.44946	6.43	6.62	6.67	6.62	7.05
10	9 (1)	0.6 (-1)	3.5 (0)	4.74	15.4	1.81211	6.29	6.81	7.14	7.05	7.29
11	6 (0)	0.6 (-1)	5 (1)	4.76	15.0	2.88876	6.29	7.14	7.19	6.76	7.43
12	9 (1)	1.2 (1)	3.5 (0)	4.72	15.4	2.21991	5.48	6.10	6.48	6.43	6.33
13	6 (0)	0.9 (0)	3.5 (0)	4.75	14.0	2.54044	6.05	6.52	6.67	7.10	7.24
14	6 (0)	0.6 (-1)	2 (-1)	4.69	15.0	2.54128	7.10	6.86	7.05	7.05	7.38
15	6 (0)	0.9 (0)	3.5 (0)	4.76	15.0	2.39995	6.43	7.00	7.33	7.10	7.71

Table 6: Regression Coefficients and R^2 values for the Model Terms in the Quadratic RSM for the Various Responses of Pineapple-Carrot-Ginger Beverage

Response	b_o	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2	R^2
pH	4.76	-0.0313*	0.0038	0.025*	0.0025	-9.32E-19	-0.01	0.02625	-0.01875	0.00375	0.8698
p=		0.0119	0.6629	0.0273	0.8359	1	0.4227	0.079	0.1767	0.7659	
TSS	14.53	1.2375**	-0.0750	0.1125	0.25	-0.425	-1.87E-17	-0.75417*	0.370833	0.195833	0.9503
p=		0.0004	0.6296	0.4762	0.2805	0.0949	1	0.0172	0.1453	0.4043	
Beta-carotene	2.40	-1.04356**	-0.03803	0.06594	0.266236*	0.156411	0.032182	0.607367**	0.091549	0.205875	0.9803
p=		< 0.0001	0.6242	0.4073	0.0493	0.1898	0.7676	0.0024	0.4326	0.1132	
Colour	6.13	-1.1238**	-0.2388	-0.0675	-0.0725	0.13	0.19	0.572917*	0.302917	0.120417	0.969
p=		< 0.0001	0.0612	0.5266	0.6275	0.3968	0.2338	0.0112	0.0928	0.4473	
Aroma	6.71	-0.2800**	-0.2563**	-0.02375	-0.0925	-0.0125	-0.13	0.095833	-0.04667	0.0833333	0.9023
p=		0.0055	0.0079	0.7083	0.325	0.8885	0.1857	0.327	0.6195	0.3883	
Taste	6.94	-0.255*	-0.32625*	-0.21375	-0.0225	0.0825	-0.155	0.174167	-0.06333	-0.0983333	0.8430
p=		0.0473	0.0204	0.0797	0.8767	0.5754	0.3117	0.2788	0.6772	0.5234	
Consistency	7.00	-0.16125**	-0.19**	-0.10125*	-0.12	0.0825	0.025	0.027083	-0.12542	-0.162917*	0.9329
p=		0.0085	0.0043	0.0462	0.0782	0.1892	0.6646	0.6521	0.0773	0.0345	
Overall acceptability	7.41	-0.375**	-0.25625*	-0.05875	-0.23	0.025	-0.0975	0.062083	-0.22542	-0.0454167	0.927
p=		0.0022	0.0109	0.4074	0.0543	0.7965	0.3372	0.5449	0.065	0.6549	

*significant at $p<0.05$, **significant at $p<0.01$, b_o is the intercept of the model

Effect of Independent Variables on pH

The pH values of the juice blend ranged from 4.69 to 4.84 in the range of the independent variables used in making the beverage (Table 5). As can be seen from Table 6 and Figure 6A-C, pH was significantly affected by the pineapple-to-carrot juice ratio and ginger concentration. Increasing the pineapple-to-carrot juice ratio significantly ($p<0.05$) decreased the pH of the beverage while increase in the concentration of ginger significantly increased the beverage pH. The quadratic and interactive effects of the independent variables were, however, non-significant ($p>0.05$) on beverage pH.

Pineapple juice has a pH value between 4 to 4.5, while carrot juice has a pH value between 6.0 to 6.5 (Kim et al., 2001). Many fruit or vegetable juices show their pH in the range of low acid, contrary to carrot juice that exhibits a high pH value close to neutral. The blending of carrot juice with pineapple and ginger juice, therefore, decreased and balanced the high pH of carrot juice to a lower pH value of the juice blend.

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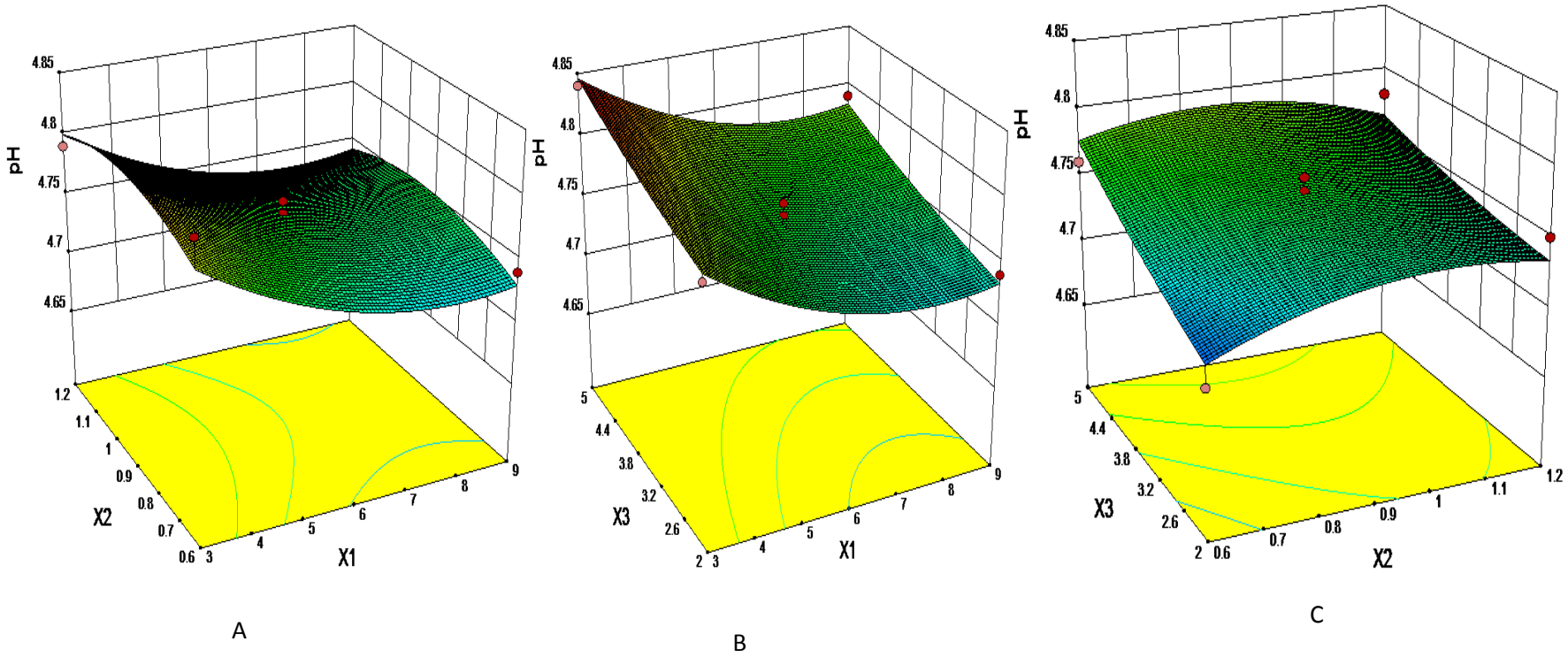
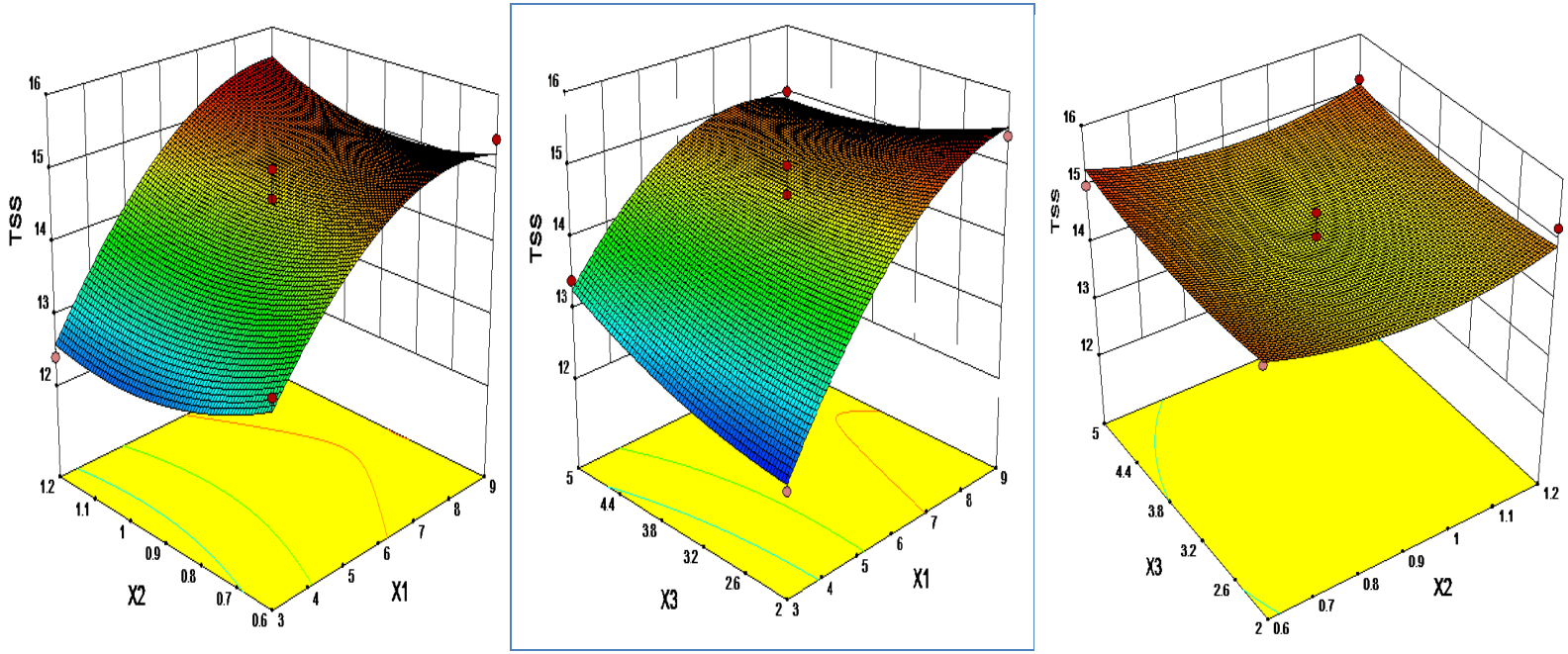


Figure 6: Effects of A) pineapple-to-carrot juice ratio and fibre size distribution B) pineapple-to-carrot juice ratio and ginger concentration and C) fibre size distribution and ginger concentration on pH

Effect of Independent Variables on the TSS

The TSS is a very important parameter in fruit juice and is often used for grading its quality (McAllister, 1980). The TSS of the beverage ranged from 12.1 to 15.4 ^{o}brix in the range of the independent variables used in the study (Table 5). As shown in Table 6 and Figure 7A-C, increase in the pineapple-to-carrot juice ratio significantly ($p<0.01$) increased the TSS of the beverage. On the other hand, increase in ginger concentration insignificantly increased the TSS while increase in fibre size distribution insignificantly decreased the TSS of the beverage. The quadratic effect of the pineapple-to-carrot juice ratio on TSS was very profound and showed statistical significance ($p<0.05$). The interaction effects between pineapple-to-carrot juice ratio and juice fibre size distribution resulted in insignificant increase in TSS whereas TSS insignificantly decreased on the interaction of pineapple-to-carrot juice ratio with ginger concentration.

Of all the three ingredients used in making the beverage, pineapple juice had the highest brix content (14-17) and it therefore had the most significant impact on beverage TSS. The TSS of the formulated beverages varied mainly as a function of the amount of pineapple juice added. Increase in the level of pineapple juice resulted in significant increase in beverage brix content. Zulueta, Esteve, Frasquet, and Frígola (2007) also reported a positive correlation in brix with the presence of pineapple juice in a beverage composed of pineapple juice and skimmed milk.



A B C

Figure 7: Effects of A) pineapple-to-carrot juice ratio and fibre size distribution B) pineapple-to-carrot juice ratio and ginger concentration and C) fibre size distribution and ginger concentration on TSS

Effect of Independent Variables on Beta-Carotene Content

The beta-carotene content ranged from 1.812 to 4.518 µg/ml in the range of the independent variables used in formulating the beverage (Table 5). The variation in beta-carotene content of the beverage as affected by the independent variables is shown in Table 6 and the response surface plots in Figure 8A-C. The main effect of pineapple-to-carrot juice ratio significantly affected the beverage beta-carotene content. Increase in the pineapple-to-carrot juice ratio resulted in significant ($p<0.01$) decrease in beta-carotene content of beverage, while increase in fibre size distribution and ginger concentration insignificantly decreased and increased respectively the beverage beta-carotene content. The interaction effects between pineapple-to-carrot juice ratio and fiber size distribution resulted in significant ($p<0.05$) increase in beta-carotene content. The quadratic effect of the pineapple-to-carrot juice ratio was also very profound and showed statistical significance ($p<0.01$).

The highest beta-carotene content of beverage was obtained at a pineapple-to-carrot juice ratio of 75/25, while the lowest was obtained at a ratio of 90/10 (Table 5). Thus, as the proportion of carrot juice increased in the beverage, the content of beta-carotene increased significantly. Yadav et al. (2015) also found increased beta-carotene content with increase in the proportions of carrot juice in carrot-grapefruit and carrot-pomegranate juice blends. Jan and Masih (2012) also reported increased beta-carotene content of pineapple-carrot-orange beverage with increase in the proportion of carrot juice. It has been reported that beta-carotene constitutes a large proportion of the carotenoids in carrot (Chen et al., 1995). Increase in the proportion of

carrot juice therefore significantly increased the beta-carotene content of the beverage.

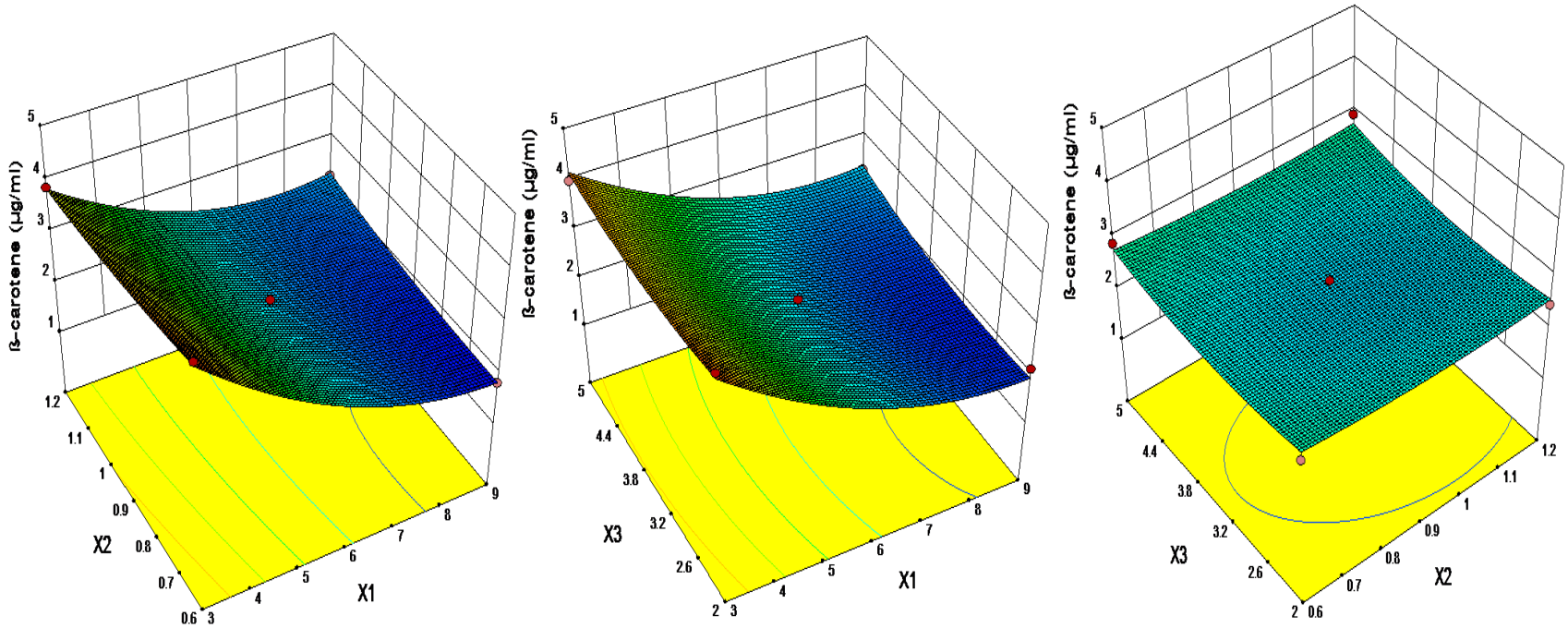


Figure 8: Effects of A) pineapple-to-carrot juice ratio and fibre size distribution B) pineapple-to-carrot juice ratio & ginger concentration, and C) fibre size distribution and ginger concentration on beverage beta-carotene content

Effect of Independent Variables on the Sensory Attributes

Effect on Beverage Colour

The sensory score for colour ranged from an average value of 5.48 (corresponding to X_1 value of 90/10 pineapple to carrot juice ratio) to 8.38 (corresponding to X_1 value of 75/25) in the range of the independent variables used (Table 5). As can be seen from Table 6 and Figure 9A-C, beverage colour was significantly affected by the pineapple-to-carrot juice ratio. Colour of beverage significantly ($p<0.01$) decreased with increase in the pineapple-to-carrot juice ratio of the beverage. The quadratic effect of the pineapple-to-carrot juice ratio was also profound on colour and showed statistical significance ($p<0.05$). The panelists' rating for colour was affected only by pineapple-to-carrot juice ratio (X_1). As the pineapple-to-carrot juice ratio decreased in the beverage, the average score for colour significantly ($p<0.01$) increased.

As was expected, this result shows that the sensory score for colour was affected only by the proportion of carrot juice in the beverage. Increase in the proportion of carrot juice resulted in significant increase in colour. The increase in colour of beverage with increase in carrot juice was as a result of the increase in beta-carotene content contributed by carrot juice. The trend obtained for beta-carotene was the same as the one for colour, as both depended on the pineapple-to-carrot juice (Table 6). Munsch, Simard, and Girard (1983) demonstrated that the colour change of carrot juice during processing correlated well with the carotenoid content. Colour is an important quality attribute of foods (Chen et al., 1995). This result is promising for using natural colours in food products since consumer concerns over the safety of

synthetic food colourants have increased hence, the demand for alternative natural ones (Kırca et al., 2006). The bright orange colour of carrots is very attractive in processed beverage products, and this has a high product appeal to consumers.

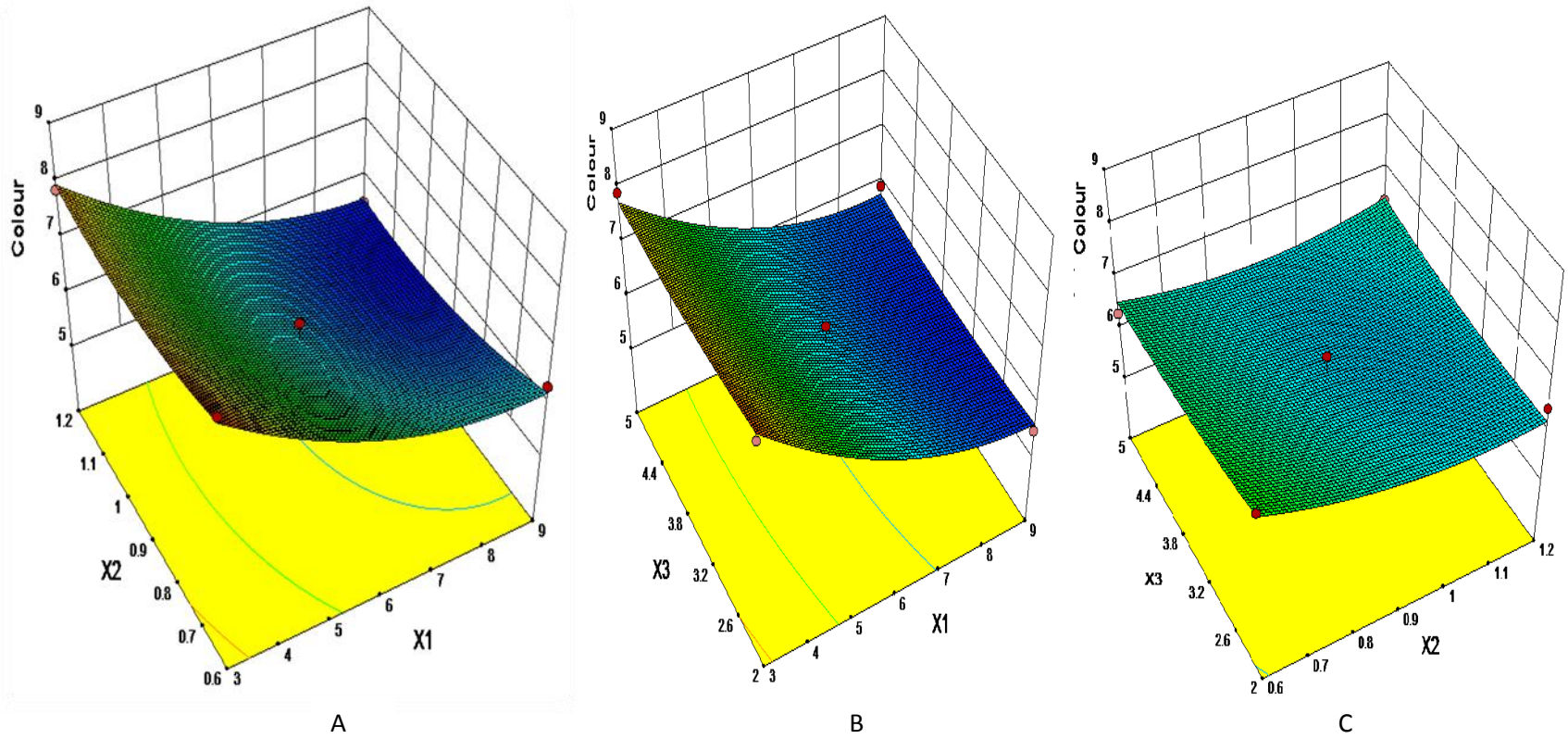


Figure 9: Effects of A) pineapple-carrot juice ratio and fibre size distribution B) pineapple-to-carrot juice ratio and ginger concentration, and C) fibre size distribution and ginger concentration on beverage colour

Effect on Aroma

The aroma of the beverage was significantly affected by the pineapple-to-carrot juice ratio and fibre size distribution (Table 6). Increase in the pineapple-to-carrot juice ratio and juice fibre size distribution significantly ($p<0.01$) decreased the average score for aroma of the beverage (Table 6 & Figure 10A-C). The result indicated that the rating for aroma of the formulated beverages in the range of the independent variables used improved with increase in the content of carrot juice. Carrot juice has a sweet aromatic flavour that imparts important flavour to the juice blend. Sulaeman and Driskell (2010) stated that not only are carrots universally relished for their taste, digestibility, fibers, and high contents of provitamin A but also have very delicious flavour. Saldana et al. (1976) reported that taste testers consistently rated the carrot-orange puree plus pineapple juice beverage as having the most desirable flavour and plain carrot juice the least desirable flavour.

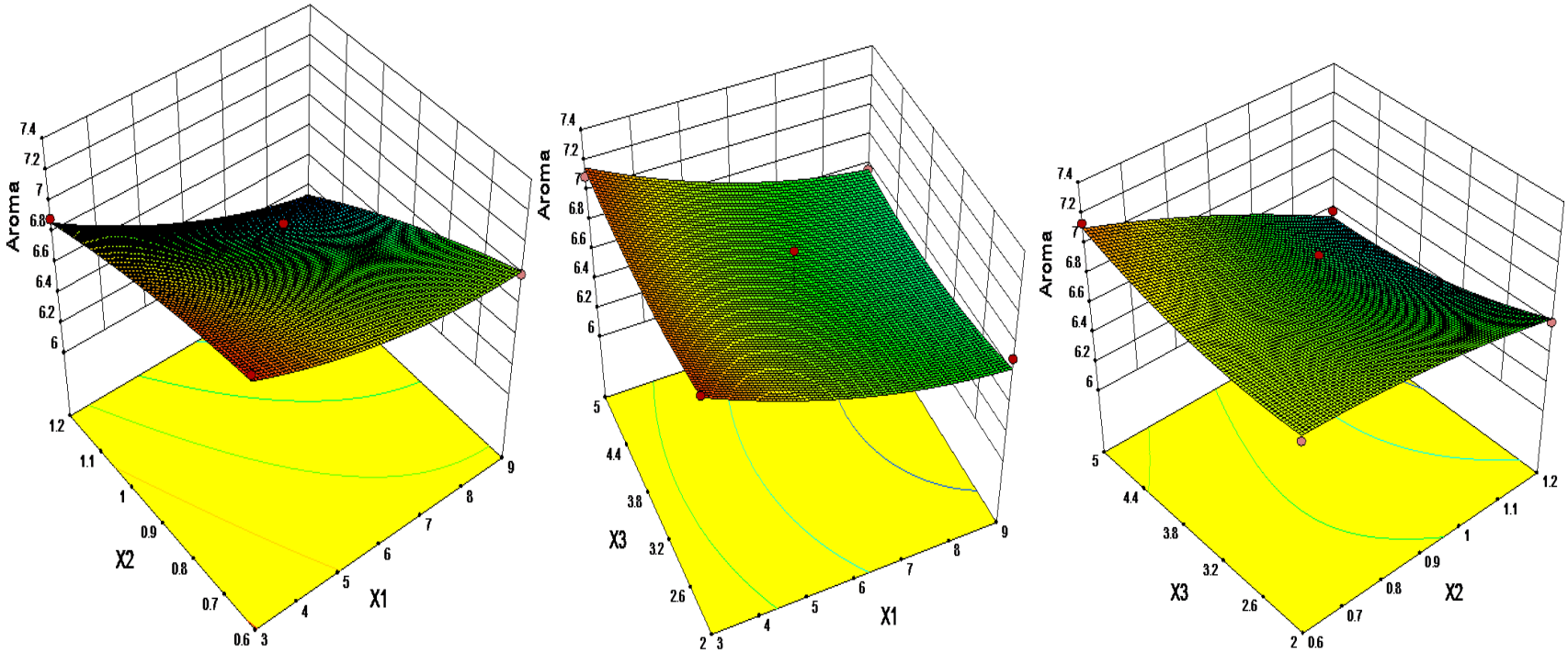


Figure 10: Effects of A) pineapple-to-carrot juice ratio and fibre size distribution B) pineapple-to-carrot juice ratio and ginger juice concentration, and C) fibre size distribution and ginger juice concentration on beverage aroma

Effect on Beverage Taste and Consistency

The average values for taste response ranged from 6.48 to 7.57, indicating all products were acceptable in terms of taste (Table 5). The pineapple-to-carrot juice ratio and juice fibre size distribution significantly affected the taste of the beverage as rated by the sensory panelists (Table 6). Increase in the levels of pineapple-to-carrot juice ratio and fibre size distribution significantly ($P<0.05$) decreased the average taste score of the beverage (Figure 11A-C & Table 6). The effect of pineapple-to-carrot juice ratio and fibre size distribution had the same effect on taste as was on aroma of the formulated beverages. It therefore implies that the proportion of carrot juice, in comparison to the pineapple juice, had a great effect on these responses. Panelists also gave better scores for taste as the fibre size distribution decreased.

All the three independent variables (pineapple-to-carrot juice ratio, fibre size distribution and ginger concentration) significantly affected the consistency of the beverage (Table 6 and Figure 12A-C). Increase in the levels of pineapple-to-carrot juice ratio, juice fibre size distribution and ginger concentration resulted in significant decrease in the beverage consistency.

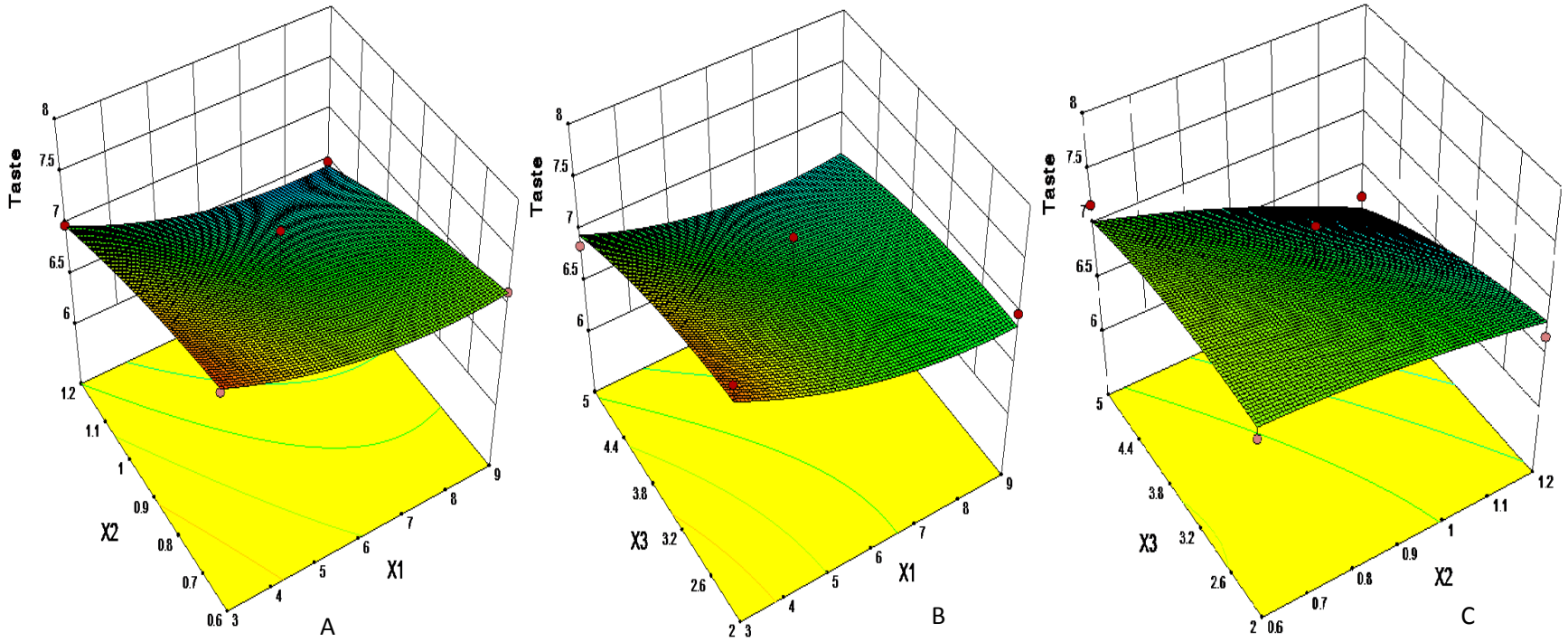


Figure 11: Effects of A) pineapple-to-carrot juice ratio and fibre size distribution B) pineapple-to-carrot juice ratio and ginger juice concentration, and C) fibre size distribution and ginger juice concentration on beverage taste

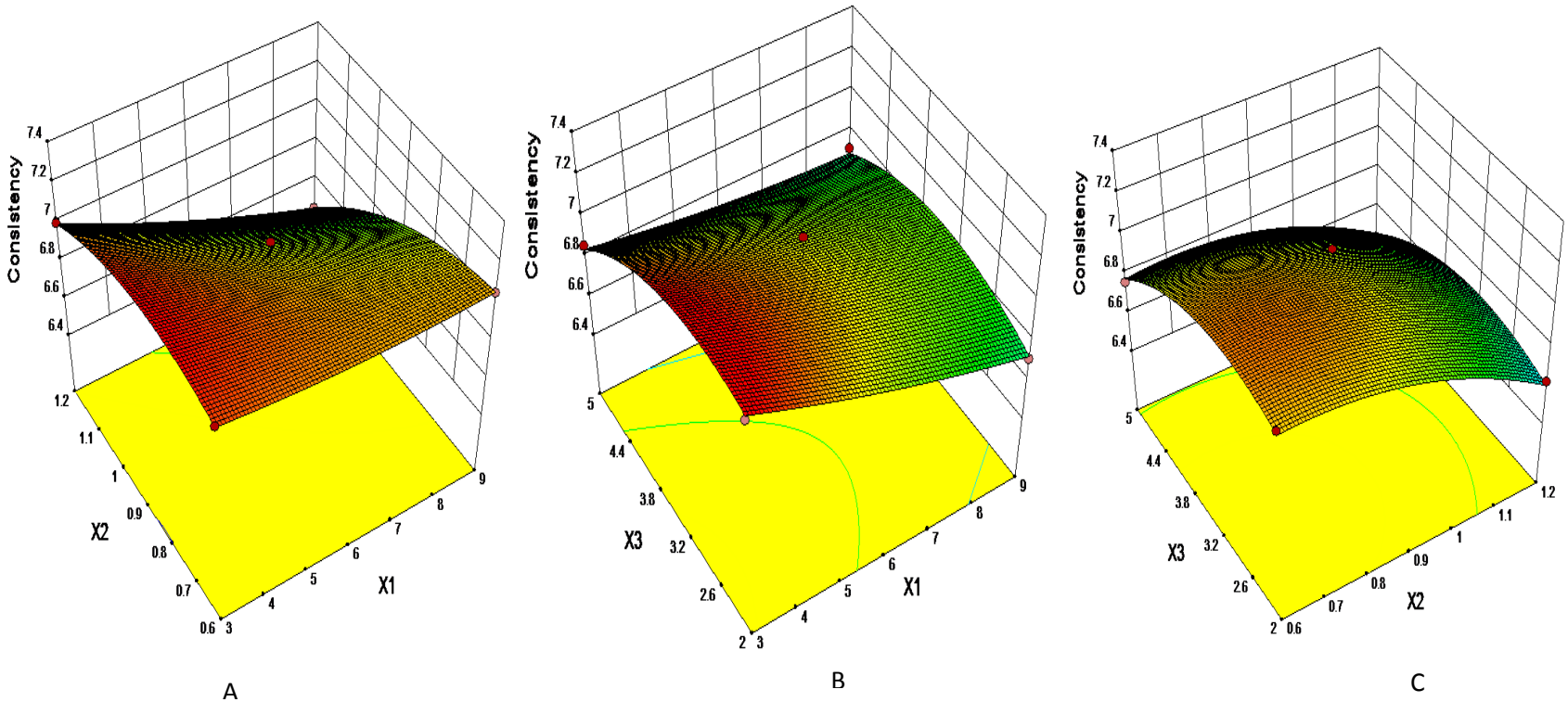


Figure 12: Effects of A) pineapple-to-carrot juice ratio and fibre size distribution B) pineapple-to-carrot juice ratio and ginger juice concentration, and C) fibre size distribution and ginger juice concentration on beverage consistency

Effect on Beverage Overall Acceptability

Average overall acceptability of the beverage ranged from 6.33 to 7.81 in the range of the independent variables, indicating all the products were highly acceptable. Average overall acceptability was lowest at pineapple-to-carrot juice ratio of 90/10 and juice fibre size of 1.2 mm, and highest at a pineapple-to-carrot juice ratio of 75/25 and fibre size of 0.9 mm (Table 5). The pineapple-to-carrot juice ratio and juice fibre size distribution significantly affected the overall acceptability of the beverage. Increase in the levels of pineapple-to-carrot juice ratio and fibre size distribution resulted in significant decrease in the beverage overall acceptability (Table 6 and Figure 13A-C).

Increase in the levels of ginger concentration insignificantly decreased the overall acceptability of the beverage. The quadratic and interactive effects of variables were insignificant on OAA. The result obtained for OAA seemed to have been contributed by the responses for aroma and taste of the formulated beverage products as they both relied on pineapple-to-carrot juice ratio and fibre size distribution as the significant main effects in the range of the independent variables used in the study.

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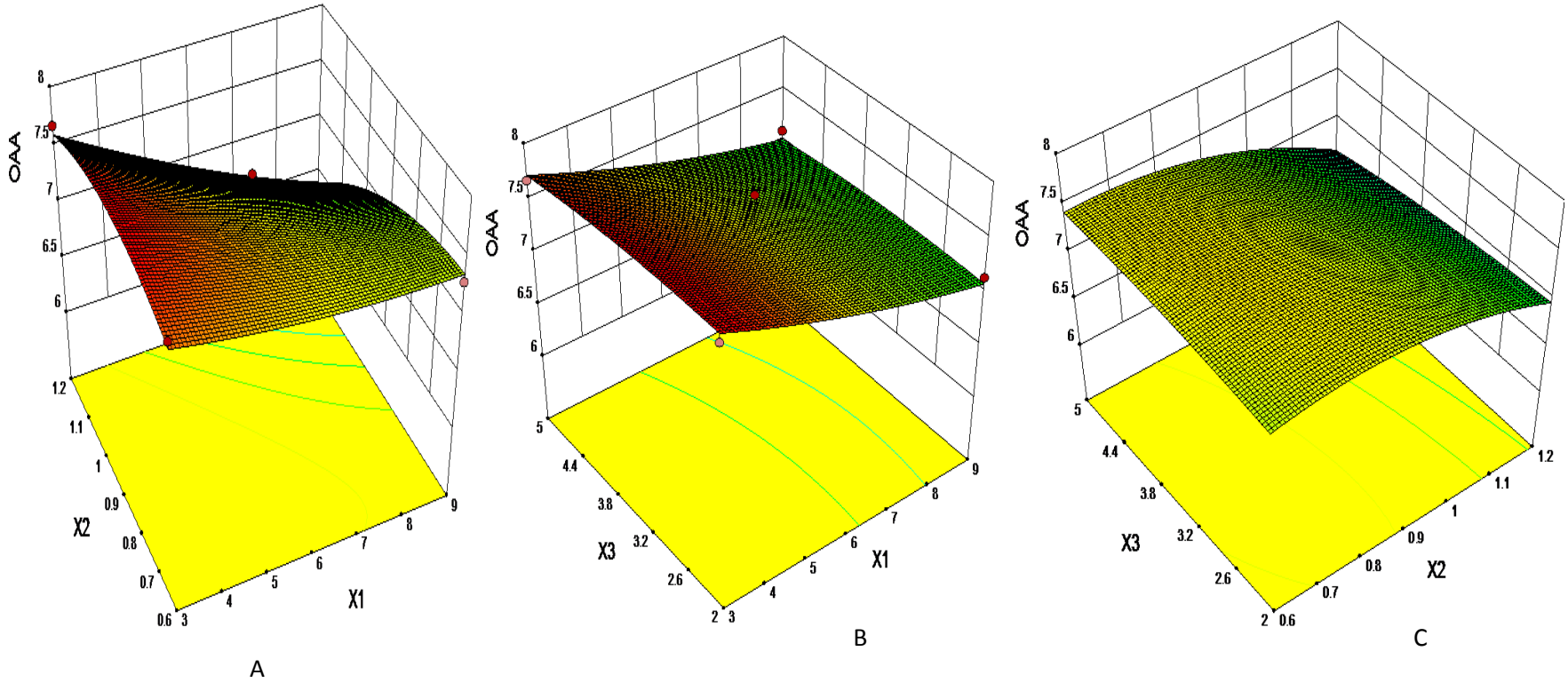

Figure 13: Effects of A) pineapple-to-carrot juice ratio and fibre size distribution B) pineapple-to-carrot juice ratio and ginger juice concentration, and C) fibre size distribution and ginger concentration on overall acceptability (OAA)

Optimization of the Variables and Responses

The levels of variables were optimized using Design Expert DX9 software (Stat-Ease, Inc., Minneapolis, USA) using the concept of Desirability Index in Equation (2). The criteria were to set goals for the independent variables and responses to maximize the desirability index. The criteria applied for optimization are summarized in Table 7 to justify the choice of optimal conditions.

Table 7: Criteria Applied for Optimization of the Variables

Constraint	Goal	Lower Limit	Upper Limit
Pineapple to carrot juice ratios	is in range	3	9
Fibre size distribution	is in range	0.6	1.2
Ginger Concentration	is target = 3	2	5
pH	minimize	4.69	4.84
TSS	maximize	12.1	15.4
Colour	maximize	5.48	8.38
Aroma	maximize	6.1	7.24
Taste	maximize	6.19	7.71
Consistency	maximize	6.43	7.19
Overall acceptability.	maximize	6.33	7.81
Betacarotene	maximize	1.81211	4.51813

Therefore by overlaying all the responses, pineapple-to-carrot juice ratio (X_1) was 3.469, fibre size distribution (X_2) was 0.6 mm and ginger concentration (X_3) as targeted was 3% and the desirability index for this

choice was 0.770. X_1 was rounded off to the nearest whole number = 4. Figure 14 shows the desirability plot of the result of optimization. The optimized levels of independent variables were therefore as follows: Pineapple-to-carrot juice ratio (X_1) = (80/20), Fibre size distribution (X_2) = 0.6mm, and Ginger concentration (X_3) = 3%.

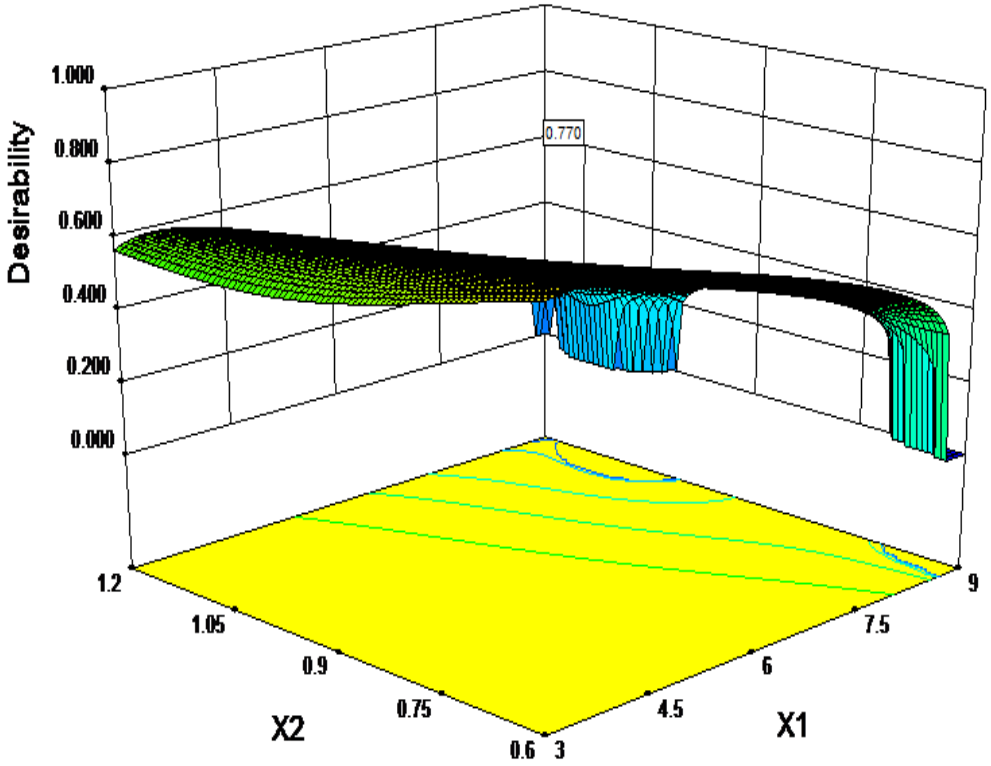

Figure 14: Desirability graph for the variables after optimization

Storage of Optimized Fresh Unpasteurized Pineapple-Carrot-Ginger Beverage

Following optimization, fresh pineapple-carrot-ginger beverage was prepared at the optimized levels of pineapple-to-carrot juice ratio, fibre size distribution and ginger concentration to study the changes in physicochemical, nutritional, microbial and sensory quality in storage. The beverage was stored in plastic packaging bottles at -24, -10 and 4 ^{o}C for 40 days and assayed at 5-

days intervals. 3 samples were prepared for each storage temperature and time and determinations of quality attributes carried out in duplicate.

Effect of Storage Temperature and Time on the Total Phenolic Content of Fresh Unpasteurized Beverage

The mean initial total phenolic content of the fresh pineapple-carrot-ginger beverage was 51.647 mg GAE/100 ml. Figure 15 shows the variation in the total phenolic content of fresh unpasteurized beverage during the 40-day storage at -24, -10, and 4 ^{o}C. Storage temperature did not significantly ($p>0.05$) affect the total phenolic content of the beverage, while the effect of storage time was significant ($p<0.05$) (Table B-1 in Appendix B for ANOVA).

The total phenolic content remained significantly unchanged during storage at -24 ^{o}C for the whole storage duration while it increased slightly during the last 10 days of storage at -10 and 4 ^{o}C. Klimczak et al. (2007) similarly observed a significant increase in the total phenolic content of orange juices at the end of storage for 6 months at 18, 28 and 38 ^{o}C. Piljac-Žegarac, Valek, Martinez, and Belščak (2009) also reported a significant increase in the total phenolic content of pomegranate and cherry juices during 29-day storage at 4 ^{o}C. It is likely that during storage of the beverage, some compounds are formed that react with the Folin-Ciocalteu reagent to enhance the phenolic content.

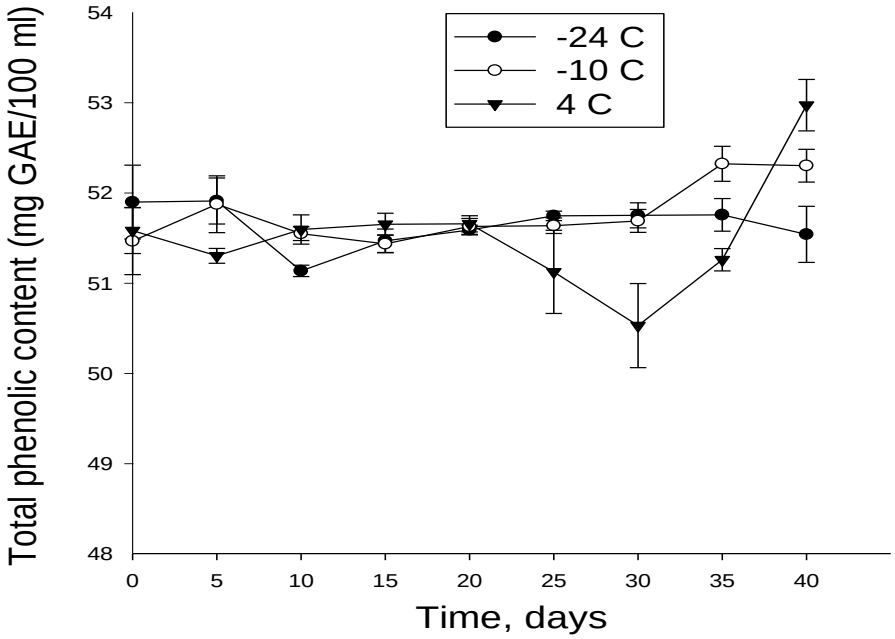

Figure 15: Effect of storage temperature and time on total phenolic content of fresh unpasteurized pineapple-carrot-ginger beverage

At the end of the 40-day storage period, storage temperature and time resulted in very minimal changes in total phenolic content of the beverage (Table A-1 in Appendix A). The finding obtained from this study is in agreement with the findings of other authors who reported the stability of total phenolic content during storage of freshly squeezed grapefruit juice at -18 and 4 ^{o}C for 2 months (Igual et al., 2010), kiwifruits at 0 ^{o}C for 2 months (Tavarini, Degl'Innocenti, Remorini, Massai, & Guidi, 2008) and frozen raspberry fruit at -20 ^{o}C for 365 days (de Ancos, González, & Cano, 2000). It, therefore, seems that frozen and refrigerated storage does not influence the total phenolic content of beverage samples. Freezing is one of the most important methods used to retain the sensorial and nutritional characteristics of fruits and vegetables and/or their products.

Effect of Storage Temperature and Time on the Ascorbic Acid Stability of Unpasteurized Pineapple-Carrot-Ginger Beverage

Temperature is described to be one of the main factors that significantly influence vitamin C stability in solution (Hernández, Lobo, & González, 2006; Phillips et al., 2010). Vitamin C (ascorbic acid) is often used as a reference in different industrial food processes since its presence indicates a high nutritional quality of the final product due to its ability to undergo easy degradation (Klimczak et al., 2007). Figure 16 shows the variation in the ascorbic acid content of fresh unpasteurized pineapple-carrot-ginger beverage during storage at -24, -10, and 4 ^{o}C.

The mean initial ascorbic acid content of the beverage was 20.522 mg per 100 ml. During storage, storage temperature and time significantly ($p<0.05$) affected the ascorbic acid content of the beverage (Table B-2 in Appendix B). The ascorbic acid decreased in all the samples during the 40-day storage period, with the highest decrease occurring in samples stored at 4 ^{o}C, and the least decrease for those at -24 ^{o}C. The ascorbic acid retention was about 95%, 83% and 17% at -24 ^{o}C, -10 ^{o}C and 4 ^{o}C respectively at the end of storage.

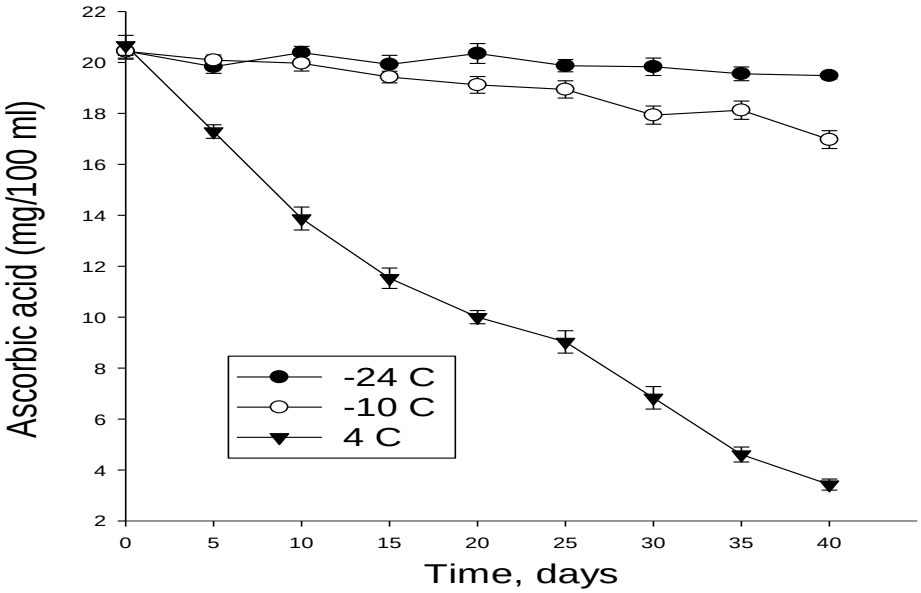

Figure 16: Effect of storage temperature and time on ascorbic acid content of pineapple-carrot-ginger beverage

These results indicate that storage of the beverage samples at -24 ^{o}C was the most effective in slowing down ascorbic acid degradation. At 4 ^{o}C there was a notable continuous decline in ascorbic acid content for the assayed samples throughout the whole study period, something indicative that this condition was not suitable for the storage of the unpasteurized samples for long. The result for storage at 4 ^{o}C is in agreement with the observations made by other authors who similarly reported continuous degradation in ascorbic acid during storage of untreated fruit juice samples of passion fruit extracts at 4 ^{o}C for 9 weeks (Spínola et al., 2013), pineapple juice at 4 ^{o}C for 13 weeks (Chia et al., 2012) and grapefruit at 4 ^{o}C for 2 months (Igual et al., 2010).

The result obtained for frozen storage of beverage samples was in conformity with the results obtained by other authors who similarly reported minimal decline in ascorbic acid during frozen storage of freshly squeezed, unpasteurized orange juice at -23 ^{o}C for 1 year (Lee & Coates, 1999), and grapefruit juice at -18 ^{o}C for 2 months (Igual et al., 2010). Cortés, Esteve,

Frígola, and Torregrosa (2005) also reported 4.1% decrease in the ascorbic acid content of orange-carrot juice after 132 days of storage at -40 ^{o}C.

According to data available from literature, ascorbic acid degradation follows both aerobic and anaerobic pathways and is a result of many factors which include, among others, heat, oxygen and exposure to light (Kabasakalis et al., 2000; Robertson & Samaniego, 1986), storage temperature and storage time (Fellers, 1988; Robertson & Samaniego-Esguerra, 1990). Degradation of ascorbic acid via aerobic pathway occurs mainly during processing of fruit juices, whereas the anaerobic degradation occurs mainly during juice storage (Burdurlu et al., 2006; Johnson, Braddock, & Chen, 1995).

Effect of Storage Temperature and Time on the TSS of Fresh Unpasteurized Pineapple-Carrot-Ginger Beverage

According to McAllister (1980), brix is often used for grading the quality of fruit juices. Figure 17 shows the variation in TSS of pineapple-carrot-ginger beverage during 40 days storage at -24, -10 and 4 ^{o}C. Storage temperature and time significantly ($p<0.05$) affected the TSS of fresh beverage (Table B-3 in Appendix B).

The TSS decreased by about 2.5%, 3.6%, and 15.1% for samples stored at -24, -10 and 4 ^{o}C respectively at the end of 40 days. During storage at 4 ^{o}C, the samples maintained nearly stable TSS content between 0 and 5 days, thereafter increasing insignificantly between day 5 and day 10, followed by a significant decrease for the remaining days in storage. There was very minimal decline in TSS for samples stored at frozen temperatures (-24 and -10 ^{o}C).

The result for TSS at 4 ^{o}C is in agreement with that of other authors who also reported a decline in TSS during storage of unpasteurized pineapple

juice (Chia et al., 2012). According to Rivas et al. (2006), fermentation of sugars in fruit juice may be caused by micro-organisms which can cause a decline in brix content. Fermentation of the fresh unpasteurized beverage samples at 4 ^{o}C could have occurred due to chemical breakdown of the sugars by bacteria, yeasts or other microorganisms, consequently leading to the decline in brix content. Microorganisms that cause fermentation of sugars can utilize the soluble solids present in the juice samples and change the brix content. The increase in the TSS of juice samples stored at 4 ^{o}C between the 5th and 10th day could be attributed to the breakdown of complex carbohydrates into simple carbohydrates. The minimal decrease in TSS at frozen temperatures could be due to the inactivity of the microorganisms' activity due to frozen storage.

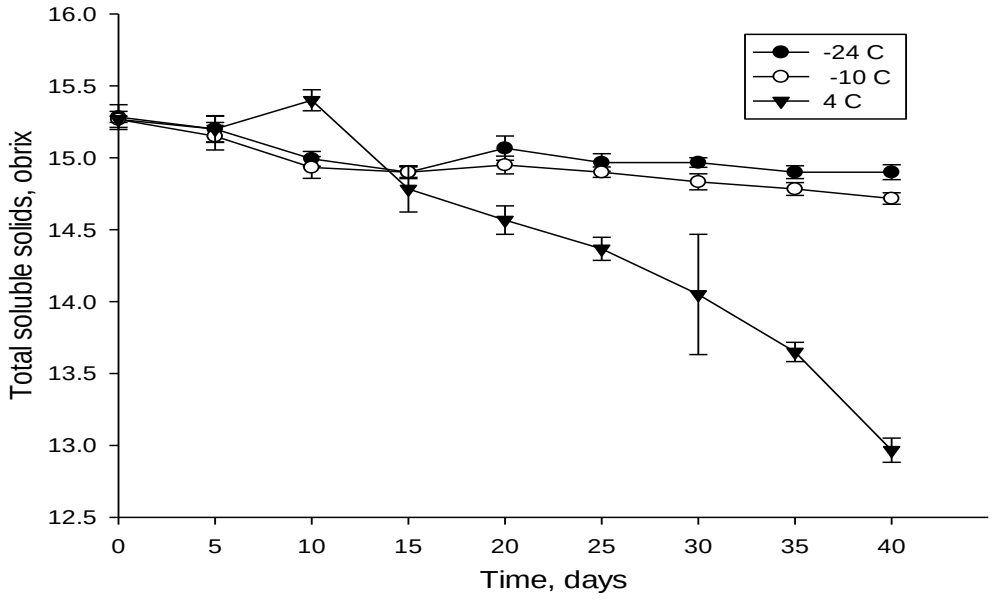

Figure 17: Effect of storage temperature and time on the TSS of unpasteurized pineapple-carrot-ginger beverage

Effect of Storage Temperature and Time on the Total Antioxidant Activity of Fresh Unpasteurized Beverage

Storage temperature and time significantly ($p<0.05$) affected the total antioxidant activity of pineapple-carrot-juice beverage as determined by the DPPH radical scavenging assay (Table B-4 in Appendix B). Samples lost 6.73%, 7.26% and 28.86% of their total antioxidant activity at -24, -10 and 4 ^{o}C respectively at the end of storage.

Figure 18 shows the variation in total antioxidant activity of fresh unpasteurized pineapple-carrot-ginger beverage during storage at -24, -10 and 4 ^{o}C. The total antioxidant activity increased slightly between day 0 and day 5 at -24 and -10 ^{o}C and further increased for samples stored at -24 ^{o}C up to day 10 and then decreased for the rest of the storage period. While at 4 ^{o}C, the total antioxidant activity of the samples decreased during the whole duration of storage. In general, the total antioxidant activity of samples in terms of %inhibition of DPPH radical decreased during storage.

Similar results of decrease in total antioxidant activity of fruit juices have been reported during storage of grapefruit juice at -18 and 4 ^{o}C (Igual et al., 2010) and commercial orange juices at 18, 28 and 38 ^{o}C (Klimczak et al., 2007). The minimal decrease in total antioxidant activity at frozen temperature storage could be attributed to the minimal decreases in antioxidant compounds like phenolic content, beta-carotene and vitamin C as compared to 4 ^{o}C where some of these compounds decreased rapidly during storage.

The role of antioxidant compounds in reducing the risk of many chronic diseases such as cancer, coronary heart disease, and immune system decline has been well documented (Kaur & Kapoor, 2001). Some of the most

exciting research in the last decade has been the discovery of a group of nutrients, which have protective effects against cell oxidation. These naturally occurring compounds impart bright colour to fruits and vegetables and act as antioxidants in the body by scavenging harmful free radicals, which are implicated in most degenerative diseases (Kaur & Kapoor, 2001).

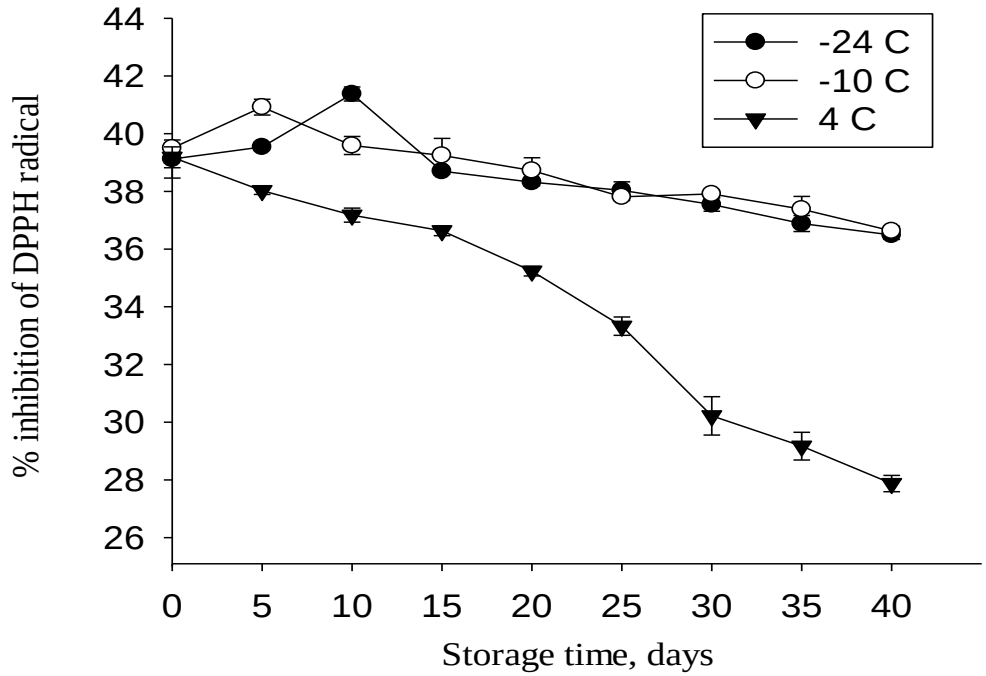

Figure 18: Effect of storage temperature and time on the total antioxidant activity of pineapple-carrot-ginger beverage

Effect of Storage Temperature and Time on the pH and Titratable Acidity of Fresh Unpasteurized Beverage

Acidity and pH are always interdependent, and the lower the pH, the higher is the acidity and vice versa during storage (Nisar et al., 2015). Flavour promotion and preservation are mainly affected by pH and titratable acidity of fruit juices and every fruit product has a specific range of pH and acidity for which consumers prefer. Figures 19 and 20 show the effect of storage on pH and titratable acidity of fresh unpasteurized beverage respectively. Storage temperature and time significantly ($p<0.05$) affected the pH and titratable

acidity of the beverage (Table B-6 & B-7 in Appendix B). During storage at -24 ^{o}C, acidity showed insignificant changes during storage, while at -10 ^{o}C it showed a significant decrease on the 5th and 15th days and thereafter showed insignificant changes for the rest of storage. At 4 ^{o}C, the acidity of fresh unpasteurized beverage significantly ($p<0.05$) decreased during storage.

At 4 ^{o}C, the pH increased significantly ($p<0.05$) while the titratable acidity decreased. Other authors have reported an increase in the value of pH with a simultaneous decrease in acidity during storage of rosette-fruit juice blend at 4 and 28 ^{o}C (Mgaya-Kilima et al., 2014), orange juices at 2 and 10 ^{o}C (Cortés et al., 2008), and citrus segments and juices at 4 ^{o}C (Del Caro et al., 2004). Contrarily, in other studies, no significant change in pH and acidity during storage was reported in pineapple juice at 4 ^{o}C (Chia et al., 2012; Laorko et al., 2013) and orange juice at 4, 22.5, 35 and 45 ^{o}C (Kaanane et al., 1988).

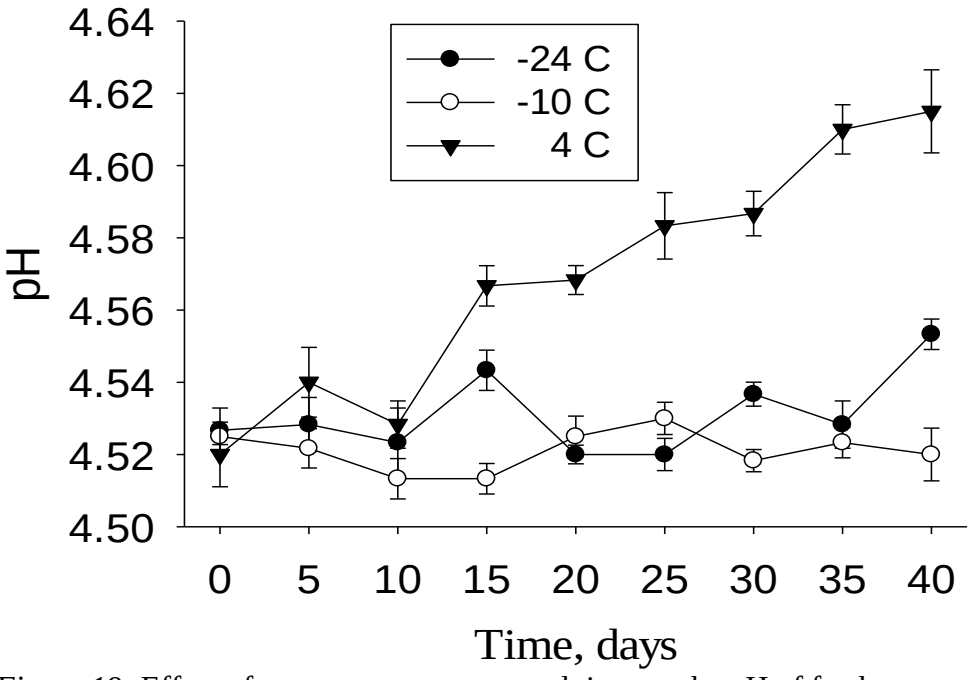

Figure 19: Effect of storage temperature and time on the pH of fresh unpasteurized pineapple-carrot-ginger beverage

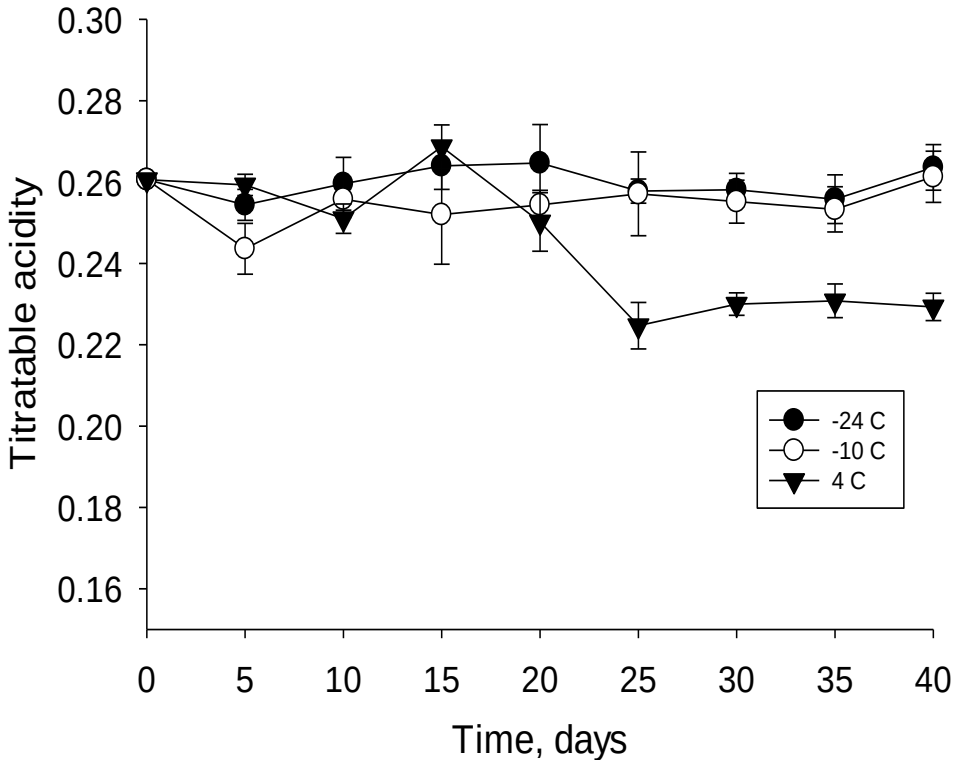

Figure 20: Effect of storage temperature and time on the titratable acidity of unpasteurized pineapple-carrot-ginger beverage

Effect of Storage Temperature and Time on the Beta-Carotene Content of Fresh Unpasteurized Pineapple-Carrot-Ginger Beverage

Figure 21 shows the effect of storage temperature and time on the beta-carotene content of the beverage. Storage temperature and time significantly ($p<0.05$) affected the beta-carotene content of the unpasteurized beverage (Table B-5 in Appendix B). Storage at 4 ^{o}C as opposed to -24 ^{o}C and -10 ^{o}C facilitated faster degradation of beta-carotene. Significant decrease, amounting to about 28% of beta-carotene content for samples stored at 4 ^{o}C was observed` at the end of storage, while frozen storage nearly maintained the beta-carotene content constant during storage. At the end of 40 days storage, samples stored at -24 and -10 ^{o}C lost about 5% and 7% beta-carotene content respectively, which are considered minimal decrease.

This result is in agreement with Lisiewska et al. (2004) who found that the level of beta-carotene was stable in all samples of dill herb during frozen storage (-20, -30 and -40 ^{o}C). Similarly, Wu, Perry, and Klein (1992) also found no significant change in the beta-carotene content of green beans and broccoli during US retail market simulation and frozen storage at -20 ^{o}C for 16 weeks. Freezing and frozen storage generally preserves the level of carotenoids. The significant decrease during the first few days of frozen storage could have resulted due to processing.

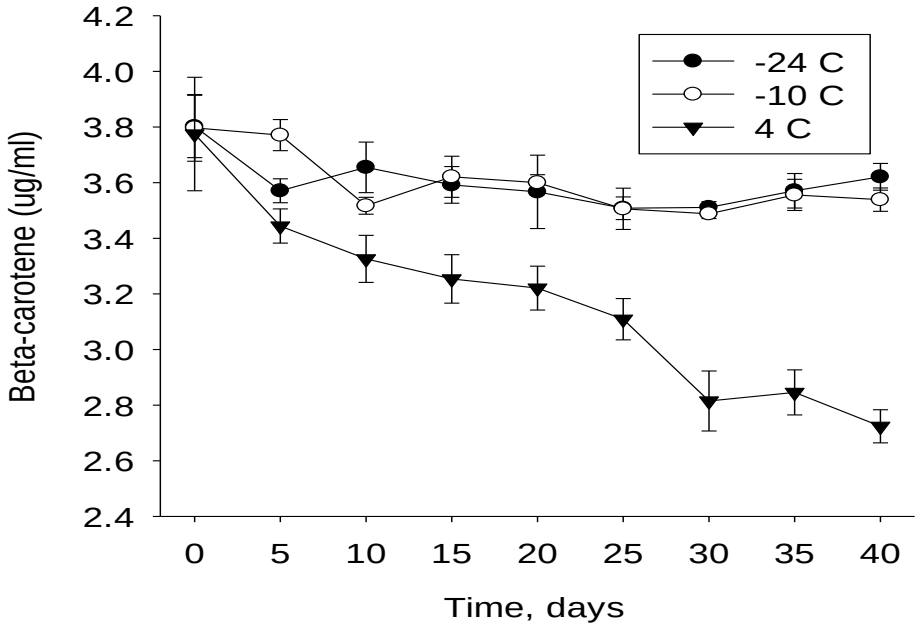

Figure 21: Effect of storage temperature and time on the carotenoids content of unpasteurized beverage.

The continuous degradation of beta-carotene during storage at 4 ^{o}C could have resulted due to the decline in ascorbic acid content since ascorbic acid is reported to offer some protection to degradation of carotenoids. Processing and storage are known to cause the instability of the polyene chain (the distinctive structural feature of carotenoids which consists of alternating double and single carbon-carbon bonds) of carotenoids (Plaza et al., 2011). As

a consequence, the compounds may undergo geometric isomerization processes (promoted in the presence of light, heat and acids) and oxidation processes (promoted by light, heat, metals, enzymes and peroxides) and inhibited by antioxidants, which are the main causes of degradation (Rodriguez-Amaya, 1997).

Effect of Storage Temperature and Time on Microbial Quality of Fresh Unpasteurized Beverage

Figures 22 and 23 show the effect of storage on the total plate counts and total fungal counts respectively for unpasteurized pineapple-carrot-ginger beverage as determined using the pour plate technique. Storage temperature and time significantly ($p<0.05$) affected the total plate count and total fungal count of the stored beverage samples (Table B-8 and B-9 in Appendix B). The mean initial populations of total aerobic bacteria and total fungal count in the fresh beverage were 4.44 and 4.34 $\log_{10}$CFU ml^{-1}, respectively. The total plate count safe limit for fruit juice is 6 log CFU/ml, beyond which the product becomes dangerous to consume (Elliott & Michener, 1961).

Frozen storage brought about 0.5 $\log_{10}$ reductions in the total plate count after the first 5 days of storage and remained insignificantly unchanged for the rest of the days in storage. The result for frozen storage is in agreement with the work of other authors who reported reduction in total plate count of rosette-fruit drinks during frozen storage for 2 weeks (Fasoyiro, Ashaye, Adeola, & Samuel, 2005), freshly squeezed, unpasteurized, polyethylene-bottled citrus juice during frozen storage at -1.7 ^{o}C for 3 weeks (Fellers, 1988). Frozen storage causes denaturation of cellular proteins, and induces temperature shock in some microorganisms especially the thermophiles and

mesophiles (Fennema, 1985).The microbial cells which are still viable after freezing gradually die when stored in the frozen condition and this could be the reason for the reduction of total microbial count of frozen samples. A most important part of any bacteriological standards is the total count of aerobic bacteria (total plate count). Elliott and Michener (1961) reported that the survival of some microorganisms has been found to differ when they are subjected to adverse conditions such as frozen storage, as some of them do die, while others survive.

During storage at 4 ^{o}C, the total plate count and total fungal count increased significantly during storage and approximated exponential growth characteristic of microorganism's growth. Given the fact that the samples were unpasteurized and were stored at 4 ^{o}C, this means the microorganism population initially in the samples continued to grow, albeit at a slow pace for the first 10 days, after which their number grew exponentially.

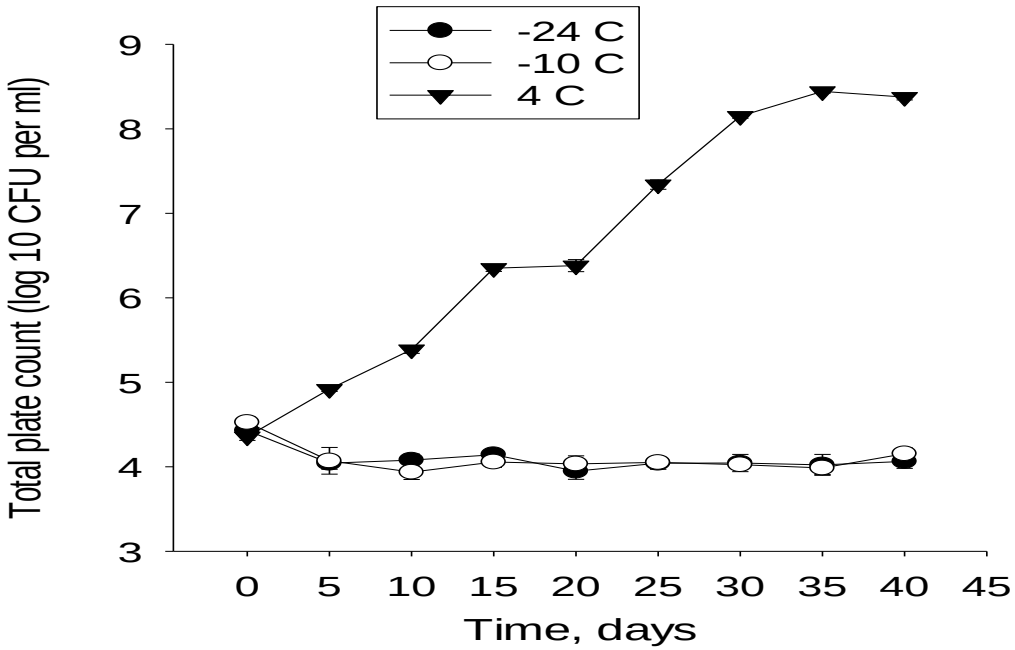

Figure 22: Effect of storage temperature and time on the total plate count of fresh unpasteurized beverage

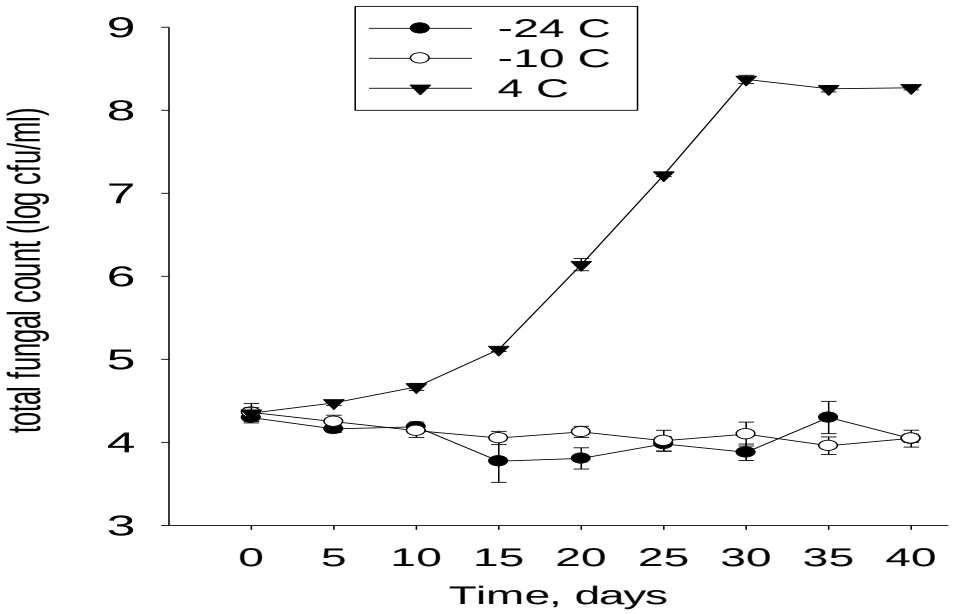

Figure 23: Effect of storage temperature and time on the total fungal count of fresh unpasteurized beverage

Effect of Storage Temperature and Time on the Sensory Characteristics of Unpasteurized Beverage during Storage

The sensory analysis result of fresh unpasteurized beverage is presented in Table 8. Storage temperature and time had no significant effect on aroma, taste and overall acceptability of pineapple-carrot-ginger beverage. Samples stored at 4 ^{o}C had gone bad after 10 days of storage and therefore was not served for sensory evaluation after the 10th day. The results of the sensory analysis indicated that the samples remained acceptable since they all averaged above 5 (which is neither like nor dislike of product).

Table 8: Sensory Quality Results of Unpasteurized Pineapple-Carrot-Ginger Beverage during Storage at -24, -10 And 4 ºC

Storage time (days)	Colour			Aroma			Taste			Consistency			Overall acceptability		
	-24ºC	-10ºC	4 ºC	-24ºC	-10ºC	4 ºC	-24ºC	-10ºC	4 ºC	-24ºC	-10ºC	4 ºC	-24ºC	-10ºC	4 ºC
0	7.00	6.00	6.06	7.06	6.44	7.44	6.94	6.78	7.61	6.64	6.33	7.22	6.89	6.89	7.72
5	7.22	7.78	6.95	6.17	6.61	6.48	6.67	6.83	6.75	6.50	7.28	6.91	6.72	7.56	7.14
10	6.72	6.44	5.88	6.50	6.28	5.89	6.67	6.61	5.89	6.94	6.31	5.94	7.06	6.61	6.00
15	7.22	7.39	NA	4.89	6.00	NA	4.67	5.61	NA	6.39	6.44	NA	5.28	6.50	NA
20	7.39	7.33	NA	7.06	7.39	NA	7.28	7.17	NA	7.39	7.44	NA	7.67	7.83	NA
25	7.59	7.88	NA	7.12	7.94	NA	7.35	7.82	NA	7.65	8.18	NA	8.06	8.47	NA
30	6.12	5.88	NA	6.29	7.29	NA	6.12	5.82	NA	6.88	6.47	NA	6.82	6.18	NA
35	7.88	7.71	NA	6.88	7.29	NA	6.65	7.47	NA	7.06	7.65	NA	7.18	7.59	NA
40	7.06	7.65	NA	6.88	7.06	NA	6.76	6.65	NA	7.35	7.65	NA	7.71	7.18	NA

NA = not applicable as the sample got spoiled to the extent that it could not be served for sensory evaluation

Storage of Pasteurized Optimized Pineapple-Carrot-Ginger Beverage

In this section of the study, optimized pineapple-carrot-ginger beverage was prepared and thermally pasteurized at 80 ^{o}C for 15 minutes, treated with 0.1% sodium benzoate and 0.1% citric acid preservatives and stored at 4, 28 and 38 ^{o}C in plastic packaging bottles. Investigations on physicochemical, nutritional, and microbial quality changes were done every 15 days and at monthly intervals for sensory quality changes for a total of 90 days on the stored beverage

Effect of Storage Temperature and Time on Total Phenolic Content of Pasteurized Pineapple-Carrot-Ginger Beverage

As shown in Table 9, storage temperature and time significantly ($p<0.05$) affected the total phenolic content of the pasteurized beverage. There was a significant decrease in the total phenolic content during the first 45 days of storage in all the samples under investigation. After 45 days of storage at 4, 28 and 38 ^{o}C, the contents of total phenolic decreased by 15.5%, 16% and 14.8% respectively.

In the subsequent storage after 45 days, total phenolic content of the beverage increased significantly. It is possible that during storage some compounds are formed that react with the Folin-Ciocalteu reagent and significantly enhance total phenolic content. This result is in agreement with other authors who also found significant increase in the total phenolic content at the end of storage of commercial orange juice brands at 18, 28 and 38 ^{o}C for 6 months (Klimczak et al., 2007).

Table 9: Changes in the Content of Ascorbic Acid, Total Phenolic and Beta-Carotene during 90 days Storage of Pasteurized Pineapple-Carrot Beverage at 4, 28 And 38 °C

Days of storage	Content (mg/100 ml)								
	Ascorbic acid (mg/100 ml)			total phenolic content (mg/100 ml)			Beta-carotene (µg/ml)		
	4 °C	28 °C	38 °C	4 °C	28 °C	38 °C	4 °C	28 °C	38 °C
0	9.927a	9.927a	9.927a	60.09a	60.09a	60.09a	2.864a	2.864a	2.864a
15	8.928b	8.309b	6.329b	58.97ab	55.77b	56.56b	2.587b	2.336bc	2.363b
30	8.429b	6.776c	5.162c	57.35b	52.43c	55.14c	2.681ab	2.373b	2.267b
45	8.218bc	6.182c	3.868d	50.75c	50.45c	51.16d	2.546b	2.439b	2.174bc
60	7.793c	4.952d	3.227de	55.18b	56.16b	53.18cd	2.384b	2.151c	2.244b
75	7.126c	4.299de	2.36e	56.77b	57.14b	57.79b	2.563b	2.182c	2.085c
90	7.11c	3.947e	1.793e	59.66a	59.56a	56.1bc	2.394b	2.168c	2.151c
$LSD_{0.05}$	0.9062			2.292			0.2039		

Values in each column in each temperature of storage with different letters indicate significant difference between each other according to least significant difference test.

Effect of Storage Temperature and Time on Beta-Carotene Content of Pasteurized Beverage

Storage temperature and time significantly ($p<0.05$) affected the beta-carotene content of pasteurized beverage sample (Table C-4). There was significant decrease in the content of this compound, the magnitude of the decrease increasing with increase in storage temperature. The gradual decrease is probably due to the fact that beta-carotene is sensitive to heat and increase in storage temperature resulted in a faster rate of decline in their content.

The faster degradation in content of beta-carotene at 28 and 38 ^{o}C could have been as a result of higher temperature of storage as well as less protection from ascorbic acid since ascorbic acid also showed continuous decline at these temperatures.

Effect of Storage Temperature and Time of Pasteurized Beverage on Ascorbic Acid Content

According to literature, ascorbic acid decreases in juices during storage at rates depending on storage conditions such as temperature, oxygen and access to light (Kabasakalis et al., 2000). The mean initial ascorbic acid content of pasteurized samples was 9.927 mg per 100 ml (Table 9).

Storage temperature and time significantly ($p<0.05$) affected the ascorbic acid content of the pasteurized pineapple-carrot-ginger beverage (Table C-3). Ascorbic acid decreased significantly for all samples, but the decrease was more rapid at higher storage temperatures. Retention of ascorbic acid was about 72, 40 and 18% at 4, 28 and 38 ^{o}C respectively at the end of the 90-day storage. At storage temperature of 4 ^{o}C, the loss of ascorbic acid in the pineapple-carrot-ginger beverage was lowest compared to other

temperatures of storage. Ascorbic acid was almost undetected at the end of storage for samples stored at 38 ^{o}C.

The result of this study is in agreement with those of other authors who similarly found ascorbic acid decline during storage of citrus juice concentrates at 28, 37 and 45 ^{o}C for 8 weeks (Burdurlu et al., 2006), grapefruit juice stored at 30 and 50 ^{o}C for 6 weeks (Lee & Nagy, 1988), orange-carrot juice stored at 2 and 10 ^{o}C (Torregrosa, Esteve, Frígola, & Cortés, 2006), orange juice stored at 10 ^{o}C (Tiwari, O'Donnell, Muthukumarappan, & Cullen, 2009) and commercial fruit juices stored at refrigerated and room temperature for 4 months (Kabasakalis et al., 2000) among many other studies.

Effect of Storage Temperature and Time on the Total Antioxidant Activity of Pasteurized Pineapple-Carrot-Ginger Beverage

Table 10 shows the results of total antioxidant activity of pineapple-carrot-ginger beverage measured by the DPPH radical scavenging assay during storage at 4, 28 and 38 ^{o}C. Storage temperature and time significantly affected the total antioxidant activity of the beverage (Table C-2). During storage at 4 ^{o}C, there was a slight initial increase in the antioxidant activity for the first 15 days in storage, followed by subsequent decrease for the rest of the storage. The result obtained at 4 ^{o}C agrees well with the study of Piga, Agabbio, Gambella, & Nicoli (2002) that reported an increase in the total antioxidant activity in the DPPH antioxidant activity during storage of mandarin juices at 4 ^{o}C for 15 days.

At 28 and 38 ^{o}C, the total antioxidant decreased significantly for the whole duration of storage. Samples lost about 21, 47 and 65% of their total antioxidant activity at 4, 28 and 38 ^{o}C respectively at the end of storage. The

decrease in total antioxidant activity could be attributed to the decrease in antioxidant compounds like phenolics, ascorbic acid, beta-carotene, etc.

Table 10: Changes in the Total Antioxidant Capacity of Pasteurized Beverage (Measured by DPPH Assay) during 90 Days Storage at 4, 28 and 38 ºC

Days of storage	DPPH (%inhibition)		
	4 ºC	28 ºC	38 ºC
0	40.07b	41.97a	40.6a
15	43.34a	37.99b	35.08b
30	36.69c	30.61c	30.35c
45	34.78d	30.28c	29.21c
60	32.84e	27.51d	20.15d
75	31.77e	24.38e	16.75e
90	31.48e	22.2f	14.32f
$LSD_{0.05}$	1.554		

Values in each column in each temperature of storage with different letters indicate significant difference between each other according to least significant difference test.

Effect of Storage Temperature and Time on TSS, pH, and Titratable Acidity of Pasteurized Pineapple-Carrot-Ginger Beverage

Table 11 shows the physicochemical quality changes of pasteurized beverage during 90-day storage. Compared to the fresh unpasteurized sample, the beverage had a higher brix content following thermal pasteurization treatment. This difference could have been as a result of evaporation of water from the beverage following the thermal pasteurization process, and hence higher brix content. The TSS of the pasteurized beverage, however, was not significantly affected by storage temperature and time (Table C-6). This result is in agreement with other authors who reported no significant change in the TSS of pasteurized pineapple juice during storage at 4 ºC for 13 weeks (Chia et al., 2012), pasteurized orange juice stored at 4 °C, 22.5 °C, 35 °C, and 45 °C (Kaanane et al., 1988).

Table 11: Changes in Physicochemical Quality of Pasteurized Pineapple-Carrot-Ginger Beverage during Storage at 4, 28 And 38 ºC

Days of storage	Physicochemical characteristics								
	TSS (brix)			pH			Titratable acidity (g per 100 ml)		
	4 ºC	28 ºC	38 ºC	4 ºC	28 ºC	38 ºC	4 ºC	28 ºC	38 ºC
0	15.633a	15.733a	15.767a	4.055a	4.055a	4.055a	0.315b	0.315c	0.315c
15	15.683a	15.717a	15.717a	4.06a	4.052ab	4.0533a	0.322a	0.319c	0.325bc
30	15.633a	15.683a	15.683a	4.062a	4.032b	4.0517a	0.329a	0.325bc	0.3302b
45	15.667a	15.75a	15.717a	4.067a	4.055a	4.06a	0.330a	0.332b	0.336ab
60	15.733a	15.767a	15.667a	4.068a	4.055a	4.048a	0.328a	0.328b	0.332b
75	15.667a	15.733a	15.767a	4.062a	4.048b	4.055a	0.322ab	0.331b	0.338a
90	15.733a	15.717a	15.683a	4.077a	4.073a	4.063a	0.331a	0.344a	0.346a
$LSD_{0.05}$	0.13172			0.02407			0.011574		

Values in each column in each temperature of storage with different letters indicate significant difference between each other according to least significant difference test.

The effects of storage temperature and time were not significant ($p<0.05$) on pH (Table C-5). The pH of pasteurized beverage was, however, lower compared to the fresh unpasteurized one, possibly resulting from the addition of citric acid as a preservative. The results obtained for pH during storage was in agreement with the study of other authors who similarly reported no significant changes in pH during storage of micro-filtered non-pasteurized pineapple juice at 4, 27 and 37 ^{o}C after 6 months storage (Laorko et al., 2013), pasteurized carrot-orange juice blend at 2 and 12 ^{o}C for 10 weeks (Rivas et al., 2006).

Titratable acidity during storage was significantly ($p<0.05$) affected by storage temperature and time (Table C-7). Acidity increased during storage and the least increase was observed during storage at 4 ^{o}C. Nisar et al., (2015) similarly found increased titratable acidity during storage of apple pulp at 25 ^{o}C for 90 days.

Effect of Storage on Sensory Characteristics of Pasteurized Pineapple-Carrot-Ginger Beverage

Table 12 shows the fluctuations in the sensory characteristics of the beverage during storage at 4, 28 and 38 ^{o}C. Storage temperature and time significantly affected the colour of the beverage during the 90 days storage. Mean scores for colour decreased significantly for samples during storage, with samples stored at 28 and 38 ^{o}C having the most decrease in scores of colour change in the beverage. Figure 24 shows the colour of samples at the end of 90-day storage showing how storage temperature affected the colour. There was better colour retention for samples stored at 4 ^{o}C while browning of samples occurred during storage at 28 and 38 ^{o}C. The browning of the juices

stored at higher temperatures can be explained by the degradation in ascorbic acid at these higher temperatures since ascorbic acid degradation in fruit juices is reported to result in the formation of products that lead to browning (Wang et al., 2006).

The aroma and consistency of the beverage were insignificantly affected by storage temperature and time at the end of the storage. Taste was significantly affected by storage temperature and time. The average score for taste decreased significantly during the 90 –day storage period. The minimum decrease in taste was observed in samples stored at 4 ^{o}C. The decrease in taste score may be attributed to the increase in titratable acidity as panelists indicated some sort of bitterness for samples at the end of storage. Nisar et al. (2015) similarly observed a decrease in taste score of apple pulp during 90 day storage at 25 ^{o}C. Beverage overall acceptability (OAA), meanwhile, was significantly affected by storage temperature and time. The OAA decreased significantly during storage for all the samples (Table 12). The decline in OAA with storage time and temperature might have resulted from the decrease in colour of the beverage, and the increase in titratable acidity of the beverage, thus considerably influencing the panelists' overall response towards the product. Bitterness was reported by most panelists for samples stored for longer durations.

Table 12: Sensory Analysis Results of Pasteurized Pineapple-Carrot-Ginger Beverage during Storage at 4, 28 and 38 ºC

Days of storage	Sensory quality														
	Colour			aroma			Taste			Consistency			OAA		
	4 ºC	28 ºC	38 ºC	4 ºC	28 ºC	38 ºC	4 ºC	28 ºC	38 ºC	4 ºC	28 ºC	38 ºC	4 ºC	28 ºC	38 ºC
0	6.9a	6.9a	6.9a	6.05a	6.05a	6.05a	5.7a	5.7a	5.7a	6.05a	6.05a	6.05a	6.05a	6.05a	6.05a
30	6.4b	5.05b	5.6b	5.85a	5.9a	5.85ab	5.6a	5.65ab	5.35a	6.55a	6.3a	6.25a	5.75b	4.5b	4.75b
60	6.35b	4.6c	5c	5.75a	6.1a	6.3a	5.85a	6.2a	5.3a	6.4a	6.55a	6.55a	5.3c	4.55b	4.75b
90	6.3b	4.3c	4.05d	5.7a	5.7a	5.2b	5.4a	4.85b	4.15b	5.85a	6.05a	6.05a	5.35bc	4.85b	4.35b
$LSD_{0.05}$	0.4366			0.872			1.023			0.7861			0.7216		

Values in each column in each temperature of storage with different letters indicate significant difference between each other according to least significant difference test.

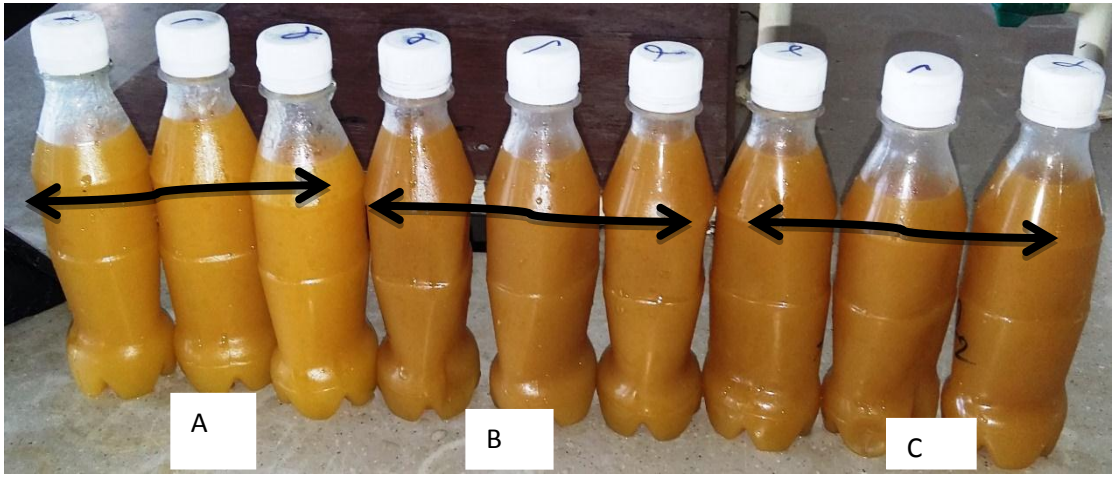

Figure 244: Colour of beverage at the end of storage: From left to right: 3 samples each stored at A) 4 ^{o}C, B) 28 ^{o}C and C) 38 ^{o}C.

Effect of Storage Temperature and Time on the Microbial Quality Changes of Pasteurized Pineapple-Carrot-Ginger Beverage

Table 13 shows the microbial quality changes of pasteurized pineapple-carrot-ginger beverage during storage at 4, 28 and 38 ^{o}C. Storage temperature and time had no significant effect on the microbial quality characteristics of the beverage. There were no significant microbial growth of counts during storage and this could be because they were inactivated during pasteurization or their growth was inhibited by the preservatives applied to the beverage.

Table 13: Microbial Quality Changes of Pasteurized Pineapple-Carrot-Ginger Beverage during Storage at 4, 28 and 38 ^{o}C

Days of storage	Microbial counts (Log_{10} CFU/ml)					
	Total plate count			Total fungal count		
	4 ^{o}C	28 ^{o}C	38 ^{o}C	4 ^{o}C	28 ^{o}C	38 ^{o}C
0	1.301a	1.852a	1.492a	1.905a	1.619ab	1.764ab
15	1.742a	1.418a	1.607a	1.661a	1.602b	1.651b
30	1.301a	1.56a	1.864a	1.807a	2.04a	2.092a
45	1.689a	1.46a	1.333a	1.989a	1.864a	1.869a
60	1.392a	1.492a	1.607a	1.888a	2.001a	1.988a
75	1.46a	1.577a	1.36a	1.854a	1.911a	1.982a
90	1.752a	1.783a	1.774a	2.03a	1.937a	2.079a
$LSD_{0.05}$	0.5695			0.434		

Values in each column in each temperature of storage with different letters indicate significant difference between each other according to least significant difference test.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The effects of 3 independent variables (pineapple-to-carrot juice ratio, fibre size distribution and beverage ginger concentration) on 8 response variables (pH, TSS, β-carotene, colour, aroma, taste, consistency, and overall acceptability) of pineapple-carrot-ginger beverage were investigated. Within the range of the independent variables studied, the main effects of increasing the pineapple-to-carrot juice significantly increased the TSS of beverage. On the other hand, increasing the pineapple-to-carrot juice ratio significantly decreased the beta-carotene content and colour of the beverage. Increase in the levels of pineapple-to-carrot juice ratio and fibre size distribution resulted in significant decrease in scores for aroma, taste, consistency and overall acceptability. The optimum levels of independent variables were obtained as pineapple-to-carrot juice ratio 80/20, fibre size distribution 0.6 mm, and beverage ginger concentration of 3%.

Minimal decreases were observed in the total antioxidant activity, ascorbic acid content, TSS and beta-carotene content during frozen storage of fresh optimized unpasteurized beverage. Bacterial counts were significantly reduced during the first 5 days of frozen storage (-24 and -10 ^{o}C) of fresh unpasteurized beverage and remained insignificantly unchanged for the rest of storage days.

For pasteurized optimized beverage, significant decreases in ascorbic acid content, total phenolic content, beta-carotene and total antioxidant activity were observed during storage at 4, 28 and 38 ^{o}C. Acidity significantly

increased, while insignificant changes were observed for TSS and pH. Colour, taste and overall acceptability decreased significantly during storage while aroma and consistency were insignificantly affected by storage temperature and time. No significant microbial growth was observed during storage of pasteurized beverage

Conclusions

Statistical analysis using RSM appeared to be a valuable tool for studying the effects of production of pineapple-carrot-ginger beverage using the different levels of independent variables. The response surface quadratic models and graphical representation of effects led to a better understanding of the effects of the different variables on the beverage characteristics.

During storage of unpasteurized beverage, storage temperature of -24 ^{o}C was found to be the best since it preserved the beverage nutritional quality as well as the microbial and sensory quality for the whole study period. Storage at -10 ^{o}C was also better in terms of quality preservation, but inferior compared to -24 ^{o}C for nutritional quality degradation. Storage at 4 ^{o}C was effective only for 10 days, after which the microbial beverage quality reached unsafe limits.

For pasteurized beverage storage, storage at 4 ^{o}C helped maintained better quality of beverage compared to 28 and 38 ^{o}C. However, the sensory quality of beverage became inferior at all temperatures at the end of pasteurized beverage storage.

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Recommendations

In light of the results of this study, the following recommendations are made with some suggestions for further study.

- Frozen storage is very vital in retaining the quality characteristics of fresh beverages and can therefore be used if it is able to meet the economic costs of electricity.
- Storage of fresh unpasteurized beverage at refrigerated temperature (4 ^{o}C) extends the shelf-life, but only for a few days. It is recommended to be used to store up to a maximum of 10 days for fresh unpasteurized pineapple-carrot-ginger beverage.
- Fortification of pasteurized beverage with synthetic ascorbic acid can be done to add value to the beverage since pasteurization drastically affects the ascorbic acid.
- Novel processing techniques can be investigated on pineapple-carrot-ginger beverage to study their effects on quality degradation in storage. Techniques such as pulsed electric fields, high-pressure processing, etc. can be studied on pineapple-carrot-beverage since they have been shown to retain essential nutrients during processing of food.

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APPENDICES

APPENDIX A

Quality changes during storage of fresh unpasteurized beverage

Table A-1: *Changes in Ascorbic Acid, Total Phenolics, Beta-Carotene and Total Antioxidant Activity of Fresh Unpasteurized Beverage during Storage At -24, -10 And 4 °C*

Days of storage	Content											
	Ascorbic acid (mg/100 ml)			Total polyphenols (mg/100 ml)			Beta-carotene (µg/ml)			Total antioxidant activity (%)		
	-24 °C	-10 °C	4 °C	-24 °C	-10 °C	4 °C	-24 °C	-10 °C	4 °C	-24 °C	-10 °C	4 °C
0	20.451a	20.442a	20.672a	51.896a	51.464b	51.580b	3.802a	3.797a	3.775a	39.122b	39.499b	39.183a
5	19.835a	20.091a	17.289b	51.908a	51.872a	51.3b	3.571ab	3.771a	3.444b	39.541b	40.921a	38.027b
10	20.388a	19.971ab	13.874c	51.133b	51.546b	51.592b	3.655a	3.517b	3.326b	41.377a	39.593b	37.177bc
15	19.924a	19.429b	11.529d	51.467ab	51.433b	51.65b	3.592a	3.621a	3.254b	38.700bc	39.248b	36.636c
20	20.353a	19.120b	10.002e	51.586a	51.626ab	51.655b	3.567b	3.6ab	3.221bc	38.321c	38.723bc	35.235d
25	19.874a	18.942bc	9.029e	51.743a	51.634a	51.122bc	3.508b	3.506b	3.109c	38.037c	37.811c	33.329e
30	19.832a	17.934cd	6.835f	51.749a	51.687a	50.527c	3.511b	3.488b	2.815d	37.546cd	37.910c	30.221f
35	19.555a	18.127c	4.608g	51.754a	52.32a	51.257b	3.571ab	3.556b	2.846d	36.887d	37.383cd	29.169g
40	19.483a	16.973d	3.428h	51.538a	52.299a	52.970a	3.621a	3.539b	2.724d	36.488d	36.631d	27.873h
$LSD_{0.05}$		0.9908			0.7133			0.2335			1.0196	

Means in each column within each treatment having different letters are significantly different according to LSD test at $p<0.05$.

Table A-2: *Changes in TSS, Titratable Acidity and pH of Fresh Unpasteurized Beverage during Storage at -24, -10 and 4 °C*

Days of storage	TSS (brix)			Titratable acidity (g/100 ml)			pH		
	-24 °C	-10 °C	4 °C	-24 °C	-10 °C	4 °C	-24 °C	-10 °C	4 °C
0	15.283a	15.267a	15.267a	0.261a	0.261a	0.261a	4.527b	4.525a	4.520e
5	15.200a	15.150a	15.200a	0.254a	0.244b	0.260ab	4.528b	4.522a	4.540 d
10	14.993a	14.933ab	15.400a	0.260a	0.256a	0.251b	4.523b	4.513a	4.528 de
15	14.900b	14.900b	14.783b	0.264a	0.252ab	0.269a	4.543a	4.513a	4.567c
20	15.067a	14.950a	14.567bc	0.265a	0.254a	0.250b	4.520b	4.525a	4.568bc
25	14.967ab	14.900b	14.367c	0.258a	0.257a	0.225c	4.520b	4.530a	4.583b
30	14.967a	14.833b	14.050c	0.258a	0.255a	0.230c	4.537ab	4.518a	4.587b
35	14.900b	14.783b	13.650d	0.256a	0.253a	0.231c	4.528b	4.523a	4.610a
40	14.900b	14.717b	12.967e	0.264a	0.261a	0.229c	4.553a	4.52a	4.615a
$LSD_{0.05}$		0.3603			0.01705			0.01937	

Means in each column within each treatment having different letters are significantly different according to LSD test at $p<0.05$.

Table A-3: *Changes in Total Plate Count and Total Fungal Count of Fresh Unpasteurized Beverage during Storage at -24, -10 And 4 °C.*

Days of storage	Total plate count (log CFU per ml)			Total fungal count (log CFU per ml)		
	-24 °C	-10 °C	4 °C	-24 °C	-10 °C	4 °C
0	4.43a	4.526a	4.357g	4.299a	4.361a	4.352e
5	4.043b	4.071b	4.919f	4.162a	4.25a	4.475e
10	4.079b	3.937b	5.388e	4.187a	4.141a	4.668e
15	4.143b	4.057b	6.351d	3.774b	4.053a	5.119d
20	3.949b	4.035b	6.381d	3.807b	4.126a	6.141c
25	4.043b	4.052b	7.34c	3.982ab	4.02b	7.22b
30	4.045b	4.025b	8.15b	3.881b	4.1a	8.372a
35	4.023b	3.986b	8.443a	4.3a	3.96b	8.258a
40	4.064b	4.155b	8.375ab	4.045a	4.049ab	8.27a
$LSD_{0.05}$		0.2402			0.3400	

Means in each column within each treatment having different letters are significantly different according to LSD test at $p<0.05$.

APPENDIX B

ANOVA for fresh unpasteurized beverage storage

Table B-1: *ANOVA of the Effects of Storage Temperature and Time of Fresh Unpasteurized Beverage on Total Phenolic Content*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	1.6547	0.8273	2.89	0.086
Residual	15	4.2871	0.2858	0.9	
Time	8	10.8217	1.3527	4.27	0.003
Time*Temperature	16	17.8486	1.1155	3.52	0.002
Residual	120	38	0.3167		
Total	161	72.6121			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table B-2: *ANOVA of the Effects of Storage Temperature and Time of Fresh Unpasteurized Beverage on Ascorbic Acid*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	2735.625	1367.813	1168.37	<.001
Residual	15	17.5605	1.1707	2.1	
Time	8	799.8201	99.9775	179.34	<.001
Time* Temperature	16	839.1086	52.4443	94.07	<.001
Residual	120	66.897	0.5575		
Total	161	4459.011			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table B-3: *ANOVA of the Effects of Storage Temperature and Time of Fresh Unpasteurized Beverage on TSS*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	9.40553	4.70276	33.24	<.001
Residual	15	2.12187	0.14146	2.36	
Time	8	18.0324	2.25405	37.58	<.001
Time*Temperature	16	15.88465	0.99279	16.55	<.001
Residual	120	7.19833	0.05999		
Total	161	52.64278			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table B-4: *ANOVA of the Effects of Storage Temperature and Time of Fresh Unpasteurized Beverage on Total Antioxidant Activity*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	712.6935	356.3468	550.38	<.001
Residual	15	9.7118	0.6475	1.02	
Time	8	713.3089	89.1636	139.8	<.001
Time* Temperature	16	299.5276	18.7205	29.35	<.001
Residual	120	76.5351	0.6378		
Total	161	1811.777			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table B-5: *ANOVA of the Effects of Storage Temperature and Time of Fresh Unpasteurized Beverage on Beta-Carotene Content*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	6.69519	3.34759	94.57	<.001
Residual	15	0.53095	0.0354	1.15	
Time	8	3.94138	0.49267	16	<.001
Time* Temperature	16	2.50496	0.15656	5.08	<.001
Residual	120	3.69551	0.0308		
Total	161	17.36799			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table B-6: *ANOVA of the effects of storage temperature and time of fresh unpasteurized beverage on pH*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	0.068009	0.034004	170.76	<.001
Residual	15	0.002987	0.000199	0.8	
Time	8	0.026724	0.00334	13.44	<.001
Time*Temperature	16	0.036136	0.002259	9.09	<.001
Residual	120	0.02983	0.000249		
Total	161	0.163685			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table B-7: *ANOVA of the effects of storage temperature and time of fresh unpasteurized beverage on titratable acidity*

Source of variation	df	MV	SS	MS	v.r.	p-value
Temperature	2		0.006188	0.003094	20.52	<.001
Residual	15		0.002262	0.000151	0.86	
Time	8		0.004697	0.000587	3.36	0.017
Time* Temperature	16		0.01023	0.000639	3.66	0.002
Residual	114	-6	0.019934	0.000175		
Total	155	-6	0.042377			

df =degree of freedom; SS = Sum of squares; MS = Mean square; MV = missing value

Table B-8: *ANOVA of the effects of storage temperature and time of fresh unpasteurized beverage on total plate count*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	116.2654	58.13272	3266.17	<.001
Residual	6	0.10679	0.0178	1.14	
Time	8	15.89926	1.98741	127.8	<.001
Time*Temperature	16	42.03439	2.62715	168.94	<.001
Residual	48	0.74644	0.01555		
Total	80	175.0523			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table B-9: *ANOVA of the effects of storage temperature and time of fresh unpasteurized beverage on total fungal count*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	90.08549	45.04275	4217.28	<.001
Residual	6	0.06408	0.01068	0.34	
Time	8	21.17799	2.64725	84.8	<.001
Time*Temperature	16	52.3401	3.27126	104.79	<.001
Residual	48	1.49837	0.03122		
Total	80	165.166			

df =degree of freedom; SS = Sum of squares; MS = Mean square

APPENDIX C

ANOVA for pasteurized beverage

Table C-1: *ANOVA of the Effects of Storage Temperature and Time of Pasteurized Beverage on Total Phenolic Content*

Source of variation	df	MV	SS	MS	v.r.	p-value
Temperature	2		37.274	18.637	6.39	0.01
Residual	15		43.735	2.916	0.89	
Time	6		984.704	164.117	50.08	<.001
Time*Temperature	12		150.67	12.556	3.83	0.003
Residual	89	-1	291.634	3.277		
Total	124	-1	1494.508			

df =degree of freedom; SS = Sum of squares; MS = Mean square; MV = missing value

Table C-2: *ANOVA of the Effects of Storage Temperature and Time of Pasteurized Beverage on Total Antioxidant Activity*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	1792.616	896.308	570.74	<.001
Residual	15	23.557	1.57	1.12	
Time	6	5268.378	878.063	624.24	<.001
Time*Temperature	12	739.436	61.62	43.81	<.001
Residual	90	126.596	1.407		
Total	125	7950.582			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table C-3: *ANOVA of the Effects of Storage Temperature and Time of Pasteurized Beverage on Ascorbic Acid Content*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	265.2755	132.6377	168.79	<.001
Residual	15	11.7873	0.7858	1.72	
Time	6	426.6219	71.1036	155.57	<.001
Time* Temperature	12	64.2822	5.3569	11.72	<.001
Residual	90	41.1337	0.457		
Total	125	809.1006			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table C-4: *ANOVA of the Effects of Storage Temperature and Time of Pasteurized Beverage on Beta-Carotene Content*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	1.68605	0.84302	40.95	<.001
Residual	15	0.3088	0.02059	0.78	
Time	6	5.01045	0.83508	31.76	<.001
Time* Temperature	12	0.68776	0.05731	2.18	0.057
Residual	90	2.36618	0.02629		
Total	125	10.05924			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table C-5: *ANOVA of the Effects of Storage Temperature and Time of Pasteurized Beverage on pH*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	0.003054	0.001527	2.89	0.087
Residual	15	0.007933	0.000529	1.5	
Time	6	0.005354	0.000892	2.53	0.052
Time* Temperature	12	0.002746	0.000229	0.65	0.728
Residual	90	0.0317	0.000352		
Total	125	0.050787			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table C-6: *ANOVA of the Effects of Storage Temperature and Time of Pasteurized Beverage on TSS*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	0.05571	0.02786	2.07	0.161
Residual	15	0.20214	0.01348	1.26	
Time	6	0.03857	0.00643	0.6	0.646
Time*Temperature	12	0.10762	0.00897	0.84	0.562
Residual	90	0.95952	0.01066		
Total	125	1.36357			

df =degree of freedom; SS = Sum of squares; MS = Mean square

Table C-7: *ANOVA of the Effects of Storage Temperature and Time of Pasteurized Beverage on Titratable Acidity*

Source of variation	df	SS	MS	v.r.	p-value
Temperature	2	0.000933	0.000466	6.32	0.01
Residual	15	0.001106	7.37E-05	0.93	
Time	6	0.006966	0.001161	14.65	<.001
Time*Temperature	12	0.001017	8.47E-05	1.07	0.395
Residual	90	0.007133	7.93E-05		
Total	125	0.017154			

df =degree of freedom; SS = Sum of squares; MS = Mean square