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DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

DEVELOPMENT OF *MORINGA OLEIFERA* LEAF BEVERAGE

BY

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DEVELOPMENT OF *MORINGA OLEIFERA* LEAF BEVERAGE

Thesis Submitted to the Department of Biochemistry and Biotechnology in Partial Fulfillment for the Requirement of the Award of Master of Science Degree in Food Science and Technology

BY

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JUNE, 2008

CERTIFICATION

I hereby declare that this submission is my own work towards the Master of Science and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Moringa is thought to have the potential of providing vital nutrition as well as health and well being to consumers. In this work, fresh Moringa leaves juice extract was envisaged as a good vehicle of spreading its nutraceutical benefits. To this end, the study design was in two parts: (a) surveys to establish the consumption patterns of beverages in Accra and (b) a fresh Moringa leaves' extract formulation and optimization. The survey was conducted by administering a questionnaire to volunteer adult consumers in randomly selected communities in Accra. To develop an acceptable Moringa leave extract beverage, pineapple juice, carrot extract and ginger distillate were included as components to improve taste, color and flavor respectively. A constrained, simplex centroid mixture design for three components was employed to optimize the proportions of the components in the final product based on sensory attributes. Proximate and other chemical analyses as well as shelf stability studies under three different storage conditions of temperature were done for the optimized product. Data obtained from the survey revealed that juices were the most consumed beverages, particularly among women. The choice of a particular juice drink was very much influenced by its color. Contour plots generated from sensory data of the products were overlaid to determine the optimum ratios of the juice extracts. The final composition consisting of 50-52% Moringa, 38-40% Pineapple and 10-12% carrots was validated to be adequate. The optimized beverage recorded 2.9 g/100ml of protein, 1.02 mg/100ml of iron and 159.14 mg/100ml of vitamin C. After 8 weeks of storage 78% of vitamin C was still retained even under the most severe storage conditions (sunlight). There were no microbial growths under all the conditions of storage, and the product was still acceptable. The findings show that fresh Moringa leaves could be processed into an acceptable beverage, so that its beneficial nutraceutical properties could very easily be distributed.

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CHAPTER 1

1.0 INTRODUCTION

Moringa oleifera is the most widely cultivated species of the Moringaceae, which are native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005). This rapidly-growing tree (also known as the horseradish tree or drumstick tree), was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated and has become naturalized in many locations in the tropics (Fahey, 2005). Analyses of the leaf composition have revealed them to have significant quantities of vitamins A, B and C, calcium, iron and protein (Ramachandran *et al*., 1980). In Ghana, Moringa has been grown for centuries and probably widely used by traditional herbalist for a much longer period, but it has only recently been embraced by Ghanaians for its nutritional and anecdotal medicinal properties. Many families are reportedly incorporating this herb into supplement their diets (www.info-ghana.com).

1.1 PROBLEM STATEMENT

Malnutrition is a major contributory factor to child mortality in Ghana. It is especially high in some regions, and in the Upper West Region it is reportedly at 208 per 1,000 (20.8%) (www.modernghana.com). There has been recent rise in non-communicable diseases such as cardiovascular disease, cancer, diabetes, and obesity, in the country. These are described as the greatest killers of the present time, as a result of the unhealthy lifestyle choices including diet. To prevent or minimize the occurrence of these diseases and maintain good health and well being, increase in the quality and quantity of fruit and vegetables in the Ghanaian diet which will help supply vitamins, minerals and other essential components is paramount (www.ghanahealthservice.com).

1.2 RATIONALE OF STUDY

Moringa is said to be the new hope in efforts at minimizing malnutrition and various related ailments. The leaves of this plant have been reported to have high amounts of essential amino acid with the right balance, as well as high amounts of minerals and vitamins (Fuglie, 1999). Anecdotal evidence from communities that use Moringa as food and herb, suggest that the leaves do not only provide good nutrition, but they are also believed to suppress diabetes and hypertension in adults. More definitive reports on the properties of Moringa published by Fuglie (1999), stated that, Moringa leaves contain more vitamin A than in carrots, more calcium than in milk, more iron than in spinach, more vitamin C than in oranges, and more potassium than in bananas, and that the protein quality of Moringa leaves rivals that of milk and eggs. Furthermore, Anwar *et al*., (2007) reported that, *Moringa oleifera* is very important for its medicinal value. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, anti-inflammatory, antihypertensive, antidiabetic and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia.

In Ghana, Moringa leaves are popularly distributed as a dry smooth, free flowing powder for consumers to mix as a nutritional supplement in food. For our tropical conditions, an alternative method for unleashing the great benefits of this nutritionally important plant is to incorporate it in a refreshing beverage. Such a beverage would not only add to the nutritional and health benefits that Moringa is reported to have, it will also help meet the recommendation that moderately active healthy people (including the elderly) should consume 1 liter to 1.5 liter of water per 1000 kilocalorie expended (RDA subcommittee, 1989). This is easily achievable by drinking plain water or beverages (Christian and Greger, 1994), and drinking such quantities of a Moringa leaves beverage will help avail its beneficial properties to the consumer.

From a preliminary study, aqueous extract of fresh Moringa leaves was dark green, and did not appeal to consumers as a refreshing and attractive beverage. The fresh raw Moringa extract flavor was also not a good selling point to consumers. A beverage made using Moringa leaf extract will need to have an acceptable flavor, taste and color. Appreciable amounts of pineapples are lost due to improper post-harvest handling and management. Mixing pineapple juice with the water extract of Moringa leaves would provide a natural sweetening source to the Moringa beverage and increase the utilization of a highly perishable crop. Additionally, incorporation of the aqueous extract of carrot and a natural ginger distillate to the Moringa- pineapple juice mixture will improve the color and flavor respectively of the product, and better appeal to consumers.

Objectives:

The general objective of this project was to develop an acceptable, shelf stable beverage (juice) from the water extracts of fresh *Moringa oleifera* leaves, carrot and pineapple juice.

The specific objectives are outlined as follows:

1. To carry out a survey to determine the consumption patterns of beverages in Accra.

2. To optimize the proportions of the three components (Fresh Moringa leaf extracts, pineapple juice and carrot extracts) in the beverage using response surface designs and sensory analyses.

3. To determine the nutritional composition of the fresh Moringa leaves extract and the final composite juice.
4. To assess the keeping quality of the final composite juice over a period of time under various storage conditions.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Food Beverages

Beverages are foods that are distinguished by two principal characteristics from other foods (Ihekoronye and Ngoddy, 1985). First, they are liquids or are consumed in the liquid state but the relative lack of actual food value differentiates them from others like milk and milk products. Second, they are either consumed for their thirst quenching properties or for their stimulating effects. The essential component of any beverage is the water that it contains; other components such as stimulants, coloring and flavoring ingredients may perform some useful functions but they are not essential to the proper physiological function of the body (Ihekoronye and Ngoddy, 1985). Food beverages most commonly consumed in the tropics can be divided into two; Non- alcoholic and Alcoholic beverages. The former can further be divided into non- carbonated (juices, coffee, tea, energy drink etc) and carbonated (soda, coca cola, tonic water, etc). There are also two other types of non alcoholic beverages: sports and energy drinks that need to be distinguished from each other. Sports drinks are beverages with the appropriate balance of carbohydrates and electrolytes that are designed to rehydrate athletes and adequately fuel them during exercise. On the other hand, the term energy drinks was created by companies in the beverage industry (The European Commission on Food Safety, 1999), and refers to beverages that contain caffeine in combination with other ingredients such as taurine, guarana, and B vitamins. They are purported to provide consumers with extra energy even though there is little scientific evidence to that effect (The European Commission on Food Safety, 1999).

2.1.1 Carbonated beverage

A carbonated drink is a beverage that has had carbon dioxide dissolved into it for some reason, most often to improve the taste, texture, or both. The various forms of such beverage include cola, diet soda, ginger ale etc (www.wikipedia.org).

2.1.2 Non-carbonated beverage

Non-carbonated beverages require ingredients and technique similar to those for carbonated beverage. However, since they lack the protection against spoilage afforded by carbonation, these are usually pasteurized, either in bulk, by continuous flash pasteurization prior to filling or in the bottle. The various forms of such beverage include Energy drink, Sports drink, juice etc (www.wikipedia.org).

2.1.2.1 Energy drink

There is limited evidence that consumption of energy drinks can significantly improve physical and mental performance (Scholey and Kennedy, 2004), driving ability when tired (Reyner and Home, 2002) and decrease mental fatigue during long periods of concentration (Kennedy and Scholey, 2004). It is important to realize that stimulants such as guarana and ginseng often added to energy beverages can enhance the effects of caffeine. Guarana, in particular, contains caffeine (1g guarana ≈ 40 mg caffeine) (Finnegan, 2003) and may substantially increase the total caffeine in an energy drink.

The consumption of energy drinks is very popular among consumers especially adolescents, and may have adverse affects on their health and well being. A survey of 78 youth (11-18 years) found that 42.3% of them consumed energy drinks (O'Dea, 2003); however, concern has been raised about the effects of ingredients found in energy drinks on children and adolescents (Australia New Zealand Food Authority, 2001). The caffeine content of a single serving of "energy drink" can range from 72 to 150 mg; however, many bottles contain 2-3 servings, raising the caffeine content to as high as 294 mg per bottle. In comparison, the caffeine content, per serving (8 fl. oz.), of brewed coffee, tea, and cola beverages ranges between 134-240 mg, 48-175 mg, and 22-46 mg respectively (Nawort *et al.,* 2003). While the consumption of ≤ 400 mg caffeine daily by healthy adults is not associated with adverse effects (Nawrot *et al.*, 2003), at risk groups such as women of reproductive age and children should limit their daily consumption to ≤ 300 mg caffeine and ≤ 2.5 mg caffeine/kg body weight, respectively (Nawrot *et al.*, 2003) and may need to avoid consuming energy beverages with a higher caffeine content.

Adverse effects associated with caffeine consumption in amounts ≥ 400 mg include nervousness, irritability, sleeplessness, increased urination, abnormal heart rhythms (arrhythmia), decreased bone levels, and stomach upset (Nawrot *et al.,* 2003). Furthermore, energy drinks contain added sugar, which should be limited in the diet according to the USDA Dietary Guidelines.

2.1.2.2 Sports drink

Sports drink is designed to help athletes rehydrate, as well as replenish electrolytes, carbohydrates, and other nutrients, which can be depleted after training or competition

(Casa, 2000). Electrolyte replacement promotes proper rehydration, which is important in delaying the onset of fatigue during exercise. As the primary fuel utilized by exercising muscle, carbohydrates are important in maintaining exercise and sport performance (Sawka, 2007). Sports drinks can be split into three major types. The first category is the isotonic sports drinks which contain proportions of water and other nutrients similar to the human body, and typically are six to eight percent sugar. The second category is the hypertonic sports drinks contain a lesser proportion of water, and a greater proportion of sugar, than the human body. Finally, hypotonic sports drinks contain a greater proportion of water, and a lesser proportion of sugar, than the human body.

2.1.2.3 Juice

Juices are prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvents (www.wikipedia.org). They are often consumed for their nutritional and health benefits (orange juice is rich in vitamin C and cranberry juice prevents bladder infection respectively). In addition to the particular sensory and nutritive characteristics of juice, the incorporation of a proportion of another juice provides a considerable contribution to the health of the consumer. Torregosa *et al.*, (2006) combined orange juice, which has high vitamin C content, and carrot juice that contains high level of carotene. The mixture of orange juice and carrot juice was rich in antioxidants and therefore was a rich dietetic source of antioxidants. Similarly, a blended beverage composed of cashew apple juice and orange aiming to reduce the acidity of the cashew apple juice was reported by Inyang and Abah (1997). Blended non-alcoholic beverages development is a good way of improving the nutritional quality of traditional products. Mixing two or more kinds of fruits can result in a product with more vitamins and minerals and with different sensory and flavor characteristics when compared to the raw materials (Akinwale 2000; Rodrigo *et al.*, 2003; Jain and Khurdiya 2004). The nutrient composition of a typical single and blended juice is presented in Table 2.1.

Table 2.1 Nutrient composition of a single and blended juice per 100ml

Nutrient	**A***	**B****
Carbohydrate (g)	13.78	12.47
Protein (g)	0.32	0.42
Total fat (g)	0.08	0.15
Calcium (mg)	17	11
Iron (mg)	0.26	0.26
Magnesium (mg)	13	10
Potassium (mg)	134	100
Vitamin A (1U)	10.70	87
Vitamin B_2 (mg)	0.02	0.03
Vitamin C (mg)	5	25

Source: USDA Nutrient Database, Release 17 (www.nutritionanalyser.com).

A* - Pineapple juice, canned, unsweetened, without added ascorbic acid

B -** Orange – Strawberry – Banana juice

2.2 The Role of Beverages in Meeting Recommended Dietary Allowance (RDA) and As Neutraceuticals

There has been a rapid development of new products that besides sensory, overall appearance and flavor acceptance, also contain high nutritional value and functional activity. Such functional foods that have benefits of human health and well being beyond basic nutrition are often referred to as nutraceutical. The growing interest in these foods has led to the development of new beverages based on fruit juices and various blends and mixtures of them. So much is consumer awareness of the link between health and diet that the nutrient contents are an important factor that impacts on the consumer's choice. In a survey about 47% of the consumers of foodstuffs believe that fortified foods and beverages are able to supply their recommended daily vitamin intake (Sloan, 2003). De Carvalhol *et al.,* (2007) noted that commercial conventionally pasteurized or sterilized beverages based on mixtures of fruit juices are popular with consumers on the strength of their concentrations of vitamin C, vitamin A and phenolic compounds and their total antioxidant capacity.

Micronutrient are also known as essential nutrient which was defined by Whitney and Rolfes (1999) as the nutrients a person must obtain from food because the body cannot make them for itself in sufficient quantity to meet physiological needs. Micronutrient deficiencies, particularly those involving iron, zinc, folic acid, vitamin B-12 and vitamin A, remain major problems for children in many countries (Stephenson *et al.,* 2002). These deficiencies not only contribute to delays in growth and development, but are also important factors in the transmission and progression of infectious diseases (Sazawal *et* *al.,* 1998; Hambidge and Krebs, 1999). Multinutrient-fortified foods and beverages may be useful in reducing micronutrient deficiencies, especially in developing countries due to their widespread acceptability. However, pill or liquid micronutrient supplementation programs have not demonstrated long-term effectiveness, possibly due to problems in distribution or lack of public acceptance of this approach (Abrams, 2002). Also, no single fortified food is likely to provide an adequate intake of supplemental minerals, and many foods contain inhibitors of nutrient absorption. Therefore, additional strategies must be considered. A potential approach is the development of a micronutrient-fortified liquid beverage that could be introduced into both the marketplace and school feeding programs. This strategy has the dual benefit of reaching a wide cross section of consumers while providing a supplement perceived to be an enjoyable, as well as a healthy alternative to other, nutritionally inferior, beverages (Abrams, 2002).

According to Otten *et al*. (2006), fluid requirements vary widely among individuals and populations. Therefore, no estimated average requirement (EAR) has been set for water, and an adequate intake (AI) was defined instead. The AI, derived from the usual intake of total fluids in the general population, was set at 3.7 L/day for men and 2.7 L/day for women. About 80% of those daily needs are contributed by beverages, including water, and the rest by solid foods (Panel on Dietary Reference Intakes for Electrolytes and Water, 2004)

2.3 Moringa oleifera : Distribution, Nutrient Composition and Uses

Moringa oleifera has its origin in Arabia and India. Today the tree is all over the tropics, from south Asia to West Africa (von Maydell, 1986). It is most visible in parts of East and South Africa. It is now also finding its way into gardens on many Pacific islands, that is, from Kiribati to the Northern Marianas. There are about 13 species of Moringa trees in the family Moringaceae. *Moringa oleifera* is the most widely known species. Moringa oleifera has been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids (Fahey, 2005). Twenty five (25) grams daily of Moringa Leaf Powder will give the following recommended daily allowances: Protein 42%, Calcium 125%, Magnesium 61%, Potassium 41%, Iron 71%, Vitamin A 272%, and Vitamin C 22% (Ramachandran *et al.*, 1980). Price (1985) stated that for pregnant and breast-feeding women, Moringa leaves and pods can do much to preserve the mother's health and pass on strength to the fetus or nursing child. One hundred gram portion of leaves could provide a woman with over a third of her daily need of calcium and give her important quantities of iron, protein, copper, sulfur and B-vitamins. According to Fuglie (1999), the many uses for Moringa include: animal forage (leaves and treated seed-cake), biogas (from leaves), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum) and water purification (powdered seeds).

2.4 Consumption and Marketing Systems of Pineapple in Ghana

The annual production of pineapple in Ghana is 60,000 metric tonnes (www.ghanaopportunity.com). About 15,000 metric tonnes are exported whilst a further over 10,000 metric tonnes are processed into juice locally. The remainder, about 35,000 metric tonnes is marketed locally in the form of fresh fruits (www.ghanaopportunity.com).

2.5 Juice Processing

In the production of most juice types the major steps involve: extraction of the juice, clarification, juice deaeration, pasteurization, concentration, essence add-back, canning or bottling and freezing if the juice is to be marketed in this form (Ihekoronye and Ngoddy, 1985)

2.5.1 Pasteurization

According to Barclay *et al.*, (1984) swept heat exchangers or open boiling pans are used for small-scale batch pasteurization of some liquid foods. However, the large-scale pasteurization of low viscosity liquids (for milk, fruit juices) usually employs plate heat exchangers. Pasteurized food is immediately filled into cartons or bottles and sealed to prevent recontamination.

2.5.1.1 Effect of heat on juices

Pasteurization is relatively mild heat treatment that has only minor changes to the nutritional and sensory characteristics of most juices. However, the shelf-life of pasteurized juice usually only extended by a few days or weeks compared with many months with the more severe heat sterilization. The main cause of color deterioration in juices is enzymic browning by polyphenoloxidase. This is promoted by the presence of oxygen and therefore deaeration prior to pasteurization is necessary. Other pigments in plant and animal products are also mostly unaffected by pasteurization. A small loss of volatile aroma compounds during pasteurization of juices causes a reduction in quality and may also unmask other cooked flavors. Losses of vitamin C and carotene are minimized by deaeration (Fellows, 2000).

2.6 Quality issues

Quality of a given product may be looked at as its conformity to a given level of excellence which represents particular standards or specifications with minimum cost to the producer, and is believed to be satisfactory with the consumer in general (Joselyn and Heid, 1963). Factors involved in evaluating quality may be classed as appearance, kinesthetic and flavor. Appearance factors involve the sense of sight and are those which are evaluated by eyes. Kinesthetic (or textural) factors are evaluated by the sense of touch, hand or mouth feel. Finally, flavor factors involve the senses of taste and smell. These characteristics may be measured objectively – by physical or chemical procedures or subjectively – by sensory evaluation by one or more human observers (Joselyn and Heid, 1963).

According to the FAO (2008) microbial contamination of juices leads to survival or growth of pathogens and subsequent rapid spoilage. On the other hand, enzymatic activities can cause browning and flavor changes. Likewise, dissolved oxygen can also cause browning and reduction of nutrient.

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

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

2.8 Ghana Standards for Vegetable Juices (GS 725:2003)

There is also a Ghana Standard for vegetable juice, which describes vegetable juice as the liquid unfermented but fermentable product or lactic acid fermented product intended for direct consumption obtained from the edible part of one or more sound vegetables and preserved exclusively by physical means. The juice shall be free from skins, seeds and other coarse parts of the vegetables. It may be clear, turbid or pulpy. Similar to the standards for Fruit juices, the vegetable juices standards has very strict requirements for hygienic and microbial standards.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Survey on the Consumption Patterns of Beverage in Accra

A survey was conducted in randomly selected communities in Accra to determine the consumption patterns of beverages. Respondents were randomly chosen, based on consent, during visits to homes, schools and work places in the selected communities. A semi-structured questionnaire was used during the interviews to obtain information on background characteristics of respondents, frequency of consumption, attitudes and perceptions of beverages and juice consumption. The questionnaire is at Appendix 1A. The sample size of respondents required for the study was based on the confidence level and margin of error, $n = (z^*/2m)^2$, where n is the sample size, z* is the level of confidence interval (95% = 1.96) and m is the margin of error (10% = 0.10). The sample size was calculated to be 96. But because of uncertainty, it was increased to 150.

3.2 Source of Raw Materials

Fresh *Moringa oleifera* leaves were obtained from a farm in Dansoman, Accra and fresh carrots, pineapples and ginger were purchased at the Kaneshie market in Accra. These were packed into sterilized polythene bags and transported for processing and bottling at the Food Processing and Engineering Division of Food Research Institute of CSIR, Okponglo, Legon. Nutritional and quality control analyses were conducted at the Food and Agriculture Division of Ghana Standards Board, Okponglo, Legon and the Nugochi Memorial Institute for Medical Research, Legon.

3.3 JUICE AND FLAVOR EXTRACTION

3.3.1 Juice Extraction

3.3.1.1 Moringa oleifera leaf juice

One hundred gram (100 g) of fresh *Moringa oleifera* tender leaves stripped from branches (Figure 3.1) were washed thoroughly in tap water, rewashed in sterile solution (mixture of equal volumes of 5% citric acid and sodium metabisulphite) and washed again with treated water (boiled at 100^{o}C and cooled). It was then blanched in hot water, 90^{o}C for 10 minutes (Potter, 1973) and slurred using a clean commercial laboratory blender (Christison Laboratory Blender, California, U.S.A) with two hundred milliliters (200 mls) of treated water (boiled at 100^{o}C and cooled) at low speed of 18,000 rpm for 2 mins. The volume of water used in the extraction was determined after preliminary experiments on water: weight of leaves ratio for blending. Finally, the slurry was then filtered using a sterilized cheese cloth to obtain the extract. The extract was then centrifuged (refrigerated centrifuge) to obtain a clear juice extract which was pasteurized at 62^{o}C for 30 minutes (Aurand *et al.*, 1987) as shown in Figure 3.2.

Figure 3.1 Stripping of tender leaves from branches

3.3.1.2 Pineapple juice

Fresh ripe pineapples (*Ananas comosus*) were washed thoroughly in tap water, peeled and sliced (2 cm thick) with a clean knife to ensure easy blending. It was blanched in hot water, 90^{o}C for 10 minutes (Potter, 1973). The sliced pineapples were pulped using a clean commercial laboratory blender (Christison Laboratory Blender, California, U.S.A) at low speed of 18,000 rpm for 2 mins. It was then filtered using a sterilized cheese cloth to obtain the juice. The juice was pasteurized at 62^{o}C for 30 minutes (Aurand *et al.*, 1987).

3.3.1.3 Carrot juice

Fresh carrots (*Daucus carota*) were cleaned and sliced (0.5 cm) using a clean knife to ensure easy blending. It was blanched in hot water, 90°C for 10 minutes (Luh and Woodroof, 1975). One hundred (100) grams of the sliced carrot were slurred in six hundred milliliters (600 mls) of treated water (boiled at 100°C and cooled) in a commercial laboratory blender (Christison Laboratory Blender, California, U.S.A) at low speed of 18,000 rpm for 2 mins. The volume of water used in the extraction was determined after preliminary experiments with different volumes which showed that the resultant concentration was the most accepted by consumers. The slurry was then filtered using a sterilized cheese cloth to obtain the juice. The juice was pasteurized at 62°C for 30 minutes (Aurand *et al.*, 1987).

3.3.2 Flavor Extraction

3.3.2.1 Natural ginger distillate

Ginger (*Zingiber officinale*) rhizomes was thoroughly washed, cleaned and sliced (0.5 cm) using a sterilized knife. Hundred (100) grams of the sliced ginger was slurred in two hundred milliliters (200 mls) of treated water (boiled at 100°C and cooled) in a commercial laboratory blender (Christison Laboratory Blender, California, U.S.A) at low speed of 18,000 rpm for 2 mins. The slurry was filtered using a sterilized cheese cloth to obtain extract. The extract was distilled using steam distillation. The distillate was collected and used as a natural ginger flavor (Figure 3.3).

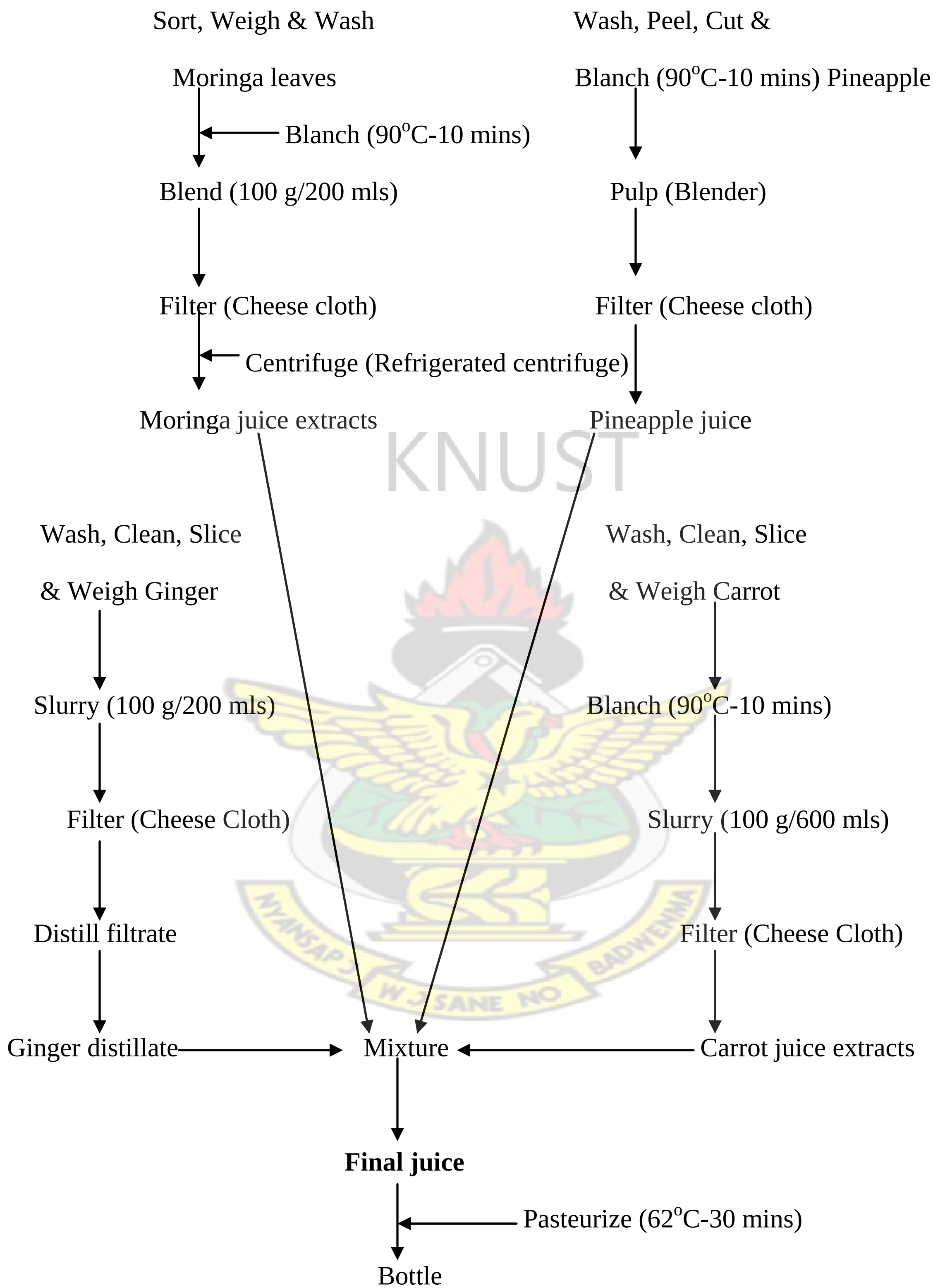


Figure 3.2 Processing of Moringa leaf beverage

Figure 3.3 Ginger distillate (flavor), carrot, pineapple and Moringa leaf juice

3.4 DEVELOPMENT OF COMPOSITE JUICE FORMULATIONS

3.4.1 Screening for component levels in a constrained 3-component formulation

Straight Moringa leaf extract did not appeal to consumers both in taste and appearance. Consequently pineapple and carrot extracts were incorporated as natural additives. To decide on plausible lower and upper levels for the three component mixture of the extracts, a constrained mixture design (Cornell, 1983) was used with arbitrary lower and upper bound levels. For three components, the design yielded ten possible formulations (Table 3.2 and Figure 3.4) based on the lower and upper limits (Table 3.1). This was meant to be a screening design to help set realistic lower and upper limits of the components' amounts.

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

Component Name	Lower Limit	Upper Limit
Moringa juice	0.40	0.50
Pineapple juice	0.30	0.40
Carrot juice	0.20	0.30

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

Table 3.2 Compositional ratio of ten possible formulations (Screening)

Formulation number	Proportion of Ingredient (%)		
	Moringa (X_1)	**Pineapple (X_2)**	**Carrot (X_3)**
1	41.7	31.7	26.6
2	40.0	30.0	30.0
3	50.0	30.0	20.0
4	40.0	35.0	25.0
5	41.7	36.7	21.6
6	46.7	31.7	21.6
7	43.4	33.3	23.3
8	45.0	35.0	20.0
9	45.0	30.0	25.0
10	40.0	40.0	20.0

The Moringa leaf beverage components (100% in mixture design) were 98% of actual formulations. All formulations contained 2% ginger distillate for a total of 100%.

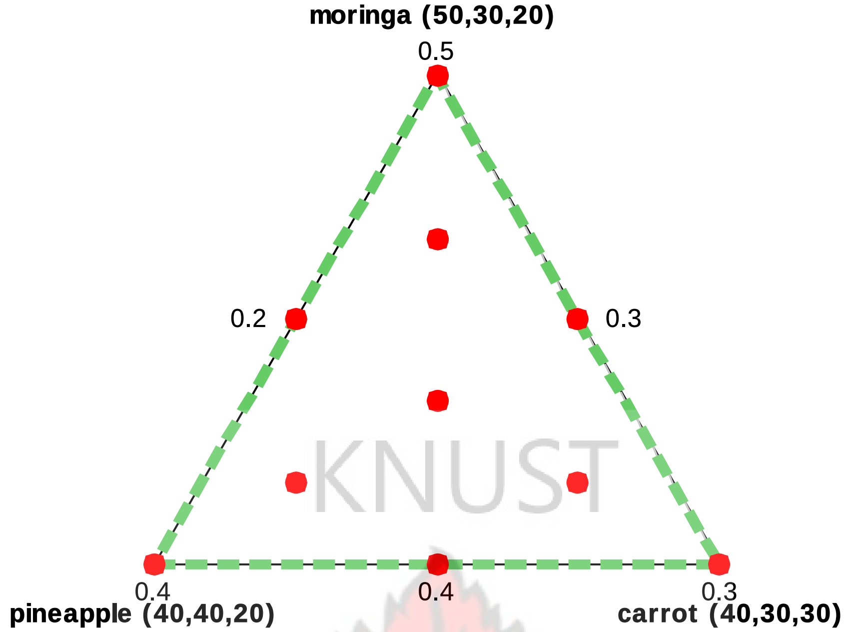


Figure 3.4 Design matrix for the 3-component mixture formulations

3.4.1.1 Consumer acceptance test

Untrained consumers (n = 60) were randomly recruited from among the staff of the Ghana Standards Board and the staff and students of the Department of Nutrition and Food Science, University of Ghana. The criteria of selection of panelists were that (a) they will be available and willing to participate in panel tests, (b) they are regular consumers of juices, and (c) they are of sound health. A balanced incomplete block design (t=10, k=4, r=6, b=15, λ=2) (Appendix 2B) described by Cochran and Cox, (1957) was used to assign the ten (10) products to fifteen (15) panelists such that each panelist evaluated only four (4) products without the danger of fatigue. The tests were replicated with two sets of 15 panelists: one by students in the Department of Nutrition and Food Science, Legon and the other by staffs of the Ghana Standard Board, Okponglo in Accra.

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

3.4.2 Optimization of the formulation of juice blends

Based on the sensory results from the screening phase, more realistic lower and upper levels of the components in the mixtures (Table 3.2) were obtained and used in a re-run of the constrained mixture design for three components. A matrix of the design for the 10 formulations is presented in Table 3.4 and Figure 3.5 based on the lower and upper limits (Table 3.3).

Table 3.3 Lower and upper limits of compositional ratio of juice (optimization)

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

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

Table 3.4 Compositional ratio of ten possible formulations (Optimization)

Formulation number	Proportion of Ingredient (%)		
	Moringa(X_1)	Pineapple(X_2)	Carrot(X_3)
1	56.7	26.7	16.6
2	50.0	20.0	30.0
3	53.4	33.3	13.3
4	63.4	23.3	13.3
5	50.0	30.0	20.0
6	53.4	23.3	23.3
7	50.0	40.0	10.0
8	60.0	30.0	10.0
9	70.0	20.0	10.0
10	60.0	20.0	20.0

The Moringa leaf beverage components (100% in mixture design) were 98% of actual formulations. All formulations contained 2% ginger distillate for a total of 100%.

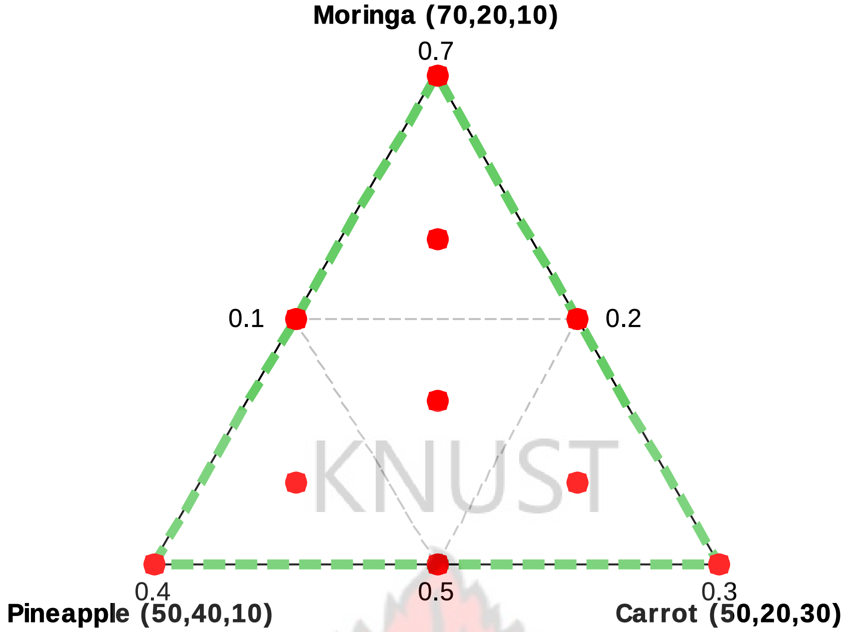


Figure 3.5 Design matrix for the 3-component mixture formulations

3.4.2.1 Consumer acceptance test

The 10 products obtained from the optimization experiments were evaluated using sensory techniques as described in Section 3.4.1.1. The sensory attributes considered for the evaluation were color, taste, flavor, aftertaste and overall acceptance. Data for the responses for the sensory attributes were analyzed using ANOVA and mixture regression techniques.

3.4.2.2 Determination of the optimum components formulation

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

3.4.3 Validation of formulations from the optimum region

With the optimum formulations design determined, two (2) formulations from that region and two from outside of the region were selected. The products were made and subjected to two sets of fifteen (15) panelists. Comparisons of the mean values of the sensory attributes of products made from formulations from the predicted optimum region and outside of the region were made.

3.5 Proximate Analysis

3.5.1 Moisture content

Moisture contents of the samples were determined in triplicate, at 103 ± 2^{o}C for 2 hours using the air oven drying method in accordance with AOAC (1990) method 977.11 and results recorded in grams (g).

3.5.2 Fat content

The fat content were determined in duplicate by the gravimetric Werner-Schmid process as described in Pearson's Chemical Analysis of foods (1987) and results recorded in grams (g).

3.5.3 Protein content

The macro kjeldahl procedure based on the AOAC (1990) method 984.13 was used. The protein content of samples was determined in duplicates by analyzing for total nitrogen and then converting it to protein using the conversion factor 6.25. The results were recorded in grams (g).

3.5.4 Carbohydrate

Carbohydrates were determined by difference and results recorded in grams (g).

3.5.5 Ash

The dry ashing method in accordance with AOAC (1990) was used in this determination using Gallenkamp Muffle Furnace, England and results recorded in grams (g).

3.6 Analytical procedure for shelf-life study

3.6.1 pH

The pH of ten milliliters (10 mls) of juice was determined using a pH meter (Model pHep3, µicropHep).

3.6.2 Titratable Acidity

Ten milliliters (10 mls) of juice was mixed with 100ml distilled water. The mixture in triplicate was then titrated against 0.1M NaOH using 1% phenolphthalein as indicator. Acidity was calculated as acetic acid (%).

3.6.3 Alcohol Content

The pycnometer procedure based on AOAC (1984) method 9.037 was used. The corresponding percentage of alcohol was determined by weight in distillate from table 52.005 (AOAC, 1984). The result was multiplied by weight of distillate and divided by weight of sample and recoded in percentages (%).

3.7 Chemical Analysis

3.7.1 Mineral Analysis

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

3.7.2 Provitamin A

Provitamin A analysis were determined by HPLC method as described in Pearson's Composition and Analysis of Foods (1987) and results recorded in milligram (mg).

3.7.3 Vitamin B_2

HPLC techniques were used as described in Pearson's Composition and Analysis of Foods (1987) and results recorded in milligram (mg).

3.7.4 Vitamin C

Titration procedures as described in Pearson's Composition and Analysis of Foods (1987) and results were recorded in milligram (mg).

3.8 Color Determination

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

3.9 Microbial Analyses

The juice was tested for their microbiological safety by determining the Total Plate Count TPC), Yeasts /Moulds, Total Coliforms and *Staphylococcus aureus* using procedures outlined in the Quality Assurance Procedure Manual of Ghana Standards Board, Okponglo, Legon.

3.9.1 Total Plate Count

The total population counts of the mesophilic bacteria were determined using the Total Plate Count Method, on a plate Count Agar (pH 7.0 from Oxoid Ltd., Basingstoke, Hampshire – England). The plate was incubated at 35^oC for 48 ± 2 hrs. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g).

3.9.2 Yeasts and Moulds

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

3.9.3 Total Coliforms (Presumptive Test)

Lauryl Tryptose Broth (pH 6.8 from Oxoid Ltd., Basingstoke, Hampshire – England) was used to determine the presence of Coliforms. Fermentation tubes with inverted Durham tubes in them were used. The tubes were incubated at 35^oC for 48 ± 2 hrs. The presence of gas trapped in the Durham tubes would indicate a positive test for Coliforms.

3.9.4 Staphylococcus aureus

Baird Parker Agar (pH 7.2 from Oxoid Ltd., Basingstoke, Hampshire – England) was used to determine the presence of *Staphylococcus areaus.* The plates were incubated at $37^{o}C$ for 48 ± 2 hrs. The number of colonies developed were counted and recorded as colony forming units per gram of sample (cfu/g).

3.10 Experimental Design and Statistical Analysis

Data obtained from the survey was analyzed for frequencies, cross-tabulations and Pearson's Chi-squared tests of associations using SPSS 11.5. Formulation of the Moringa juice beverage was done using a Simplex Centroid Constrained Design (Cornell, 1983) for three components with the aid of Minitab 14 statistical package. A Balanced Incomplete Block Design (BIBD) was used (Cochran and Cox, 1957) to assign the 10 products obtained from the mixture design for 3 components to 15 panelists. Data for each sensory attribute were analyzed using ANOVA (Statgraphics plus 3.0). Predictive multiple regression models (response surface design) were obtained for each of the sensory attributes, from which ternary contour plots were generated using MINITAB 14. The ternary contour plots of each of the attributes were overlaid in order to determine the optimum formulation region. Data obtained from the quality control analyses were analyzed using scatter plots in Excel, ANOVA and LSD in Statgraphics plus (version 3.0).

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Survey of Consumers about the Consumption Patterns of Beverages in Accra.

4.1.1 Demographics of respondents

The survey covered sixteen (16) communities in Accra. One hundred and fifty (150) respondents were interviewed using questionnaires (Appendix 1A), and they were made up of 81 male and 69 female. The respondents were drawn from all walks of life (Students, Bankers, Nurses, Teachers, Hairdressers, Traders, Laboratory technicians, etc). Analyses of the frequencies showed that the age distribution was very close and was made up of two age groups such that seventy-five (75) of the respondents were aged between 20-30 years, and the other seventy-five (75) were between 30-40 years. The frequency distribution based on educational background of the respondents showed that only four (4) of them had no formal education, Appendix 1B, Table 1.1. One hundred and twenty four (124) respondents had either secondary or tertiary education. It appears that the distribution of consumers based on family life was skewed because only forty three (43) out of a total of 150 respondents were married.

4.1.2 Consumption of beverage

One hundred and forty-nine (149) of the respondents drink beverages and 139 (93% of respondents) specifically indicated that they drink juices, while 101 (67%) indicated that they drink carbonated drinks. When the responses were normalized according to relative frequencies of favorite drinks, the results were as shown in Figure 4.1. Coffee was the least favorite (beverage) among respondents (8.5%). On the other hand, juice was the top favorite at 19% followed closely by food drinks (milo, soy drink, etc) at 18%. The frequency of drinking beverages showed that, 50 (33%) of the respondents drink juices more than once per week, and that cost was not a consideration in their decisions to purchase juice. As many as 132 (88%) respondents said they were willing to try a new juice product. There were no significant differences ($p \leq 0.05$) between males and females, or between the age groups (20-30, 31-40 year groups) in their willingness to try new fruit juices.

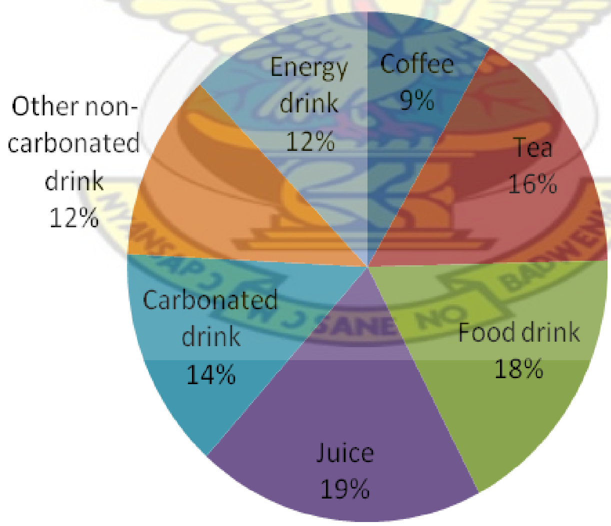


Figure 4.1 Patterns of various beverage consumption in Accra.

4.1.3 Criteria for choice of juice as a beverage

4.1.3.1 Effect of gender

Taste is an important consideration for selection of juice. This was confirmed by the number of respondents, 69 (46%), that looked out for taste in juices. The criteria for choice and selection of juice differed between males and females. Analyses of the data using Pearson's Chi-squared (χ^2), showed that there are significant differences ($p \leq 0.05$) between males and females in their choice of beverage drinks. The odds ratio analyses showed that females are 10 times more likely to choose juice as drink compared to males. The criteria for choosing juices were also significantly different between females and males. Females were 3 and 2 times more likely to consider color and taste respectively in choosing juices. Males on the other hand are 3 times more likely than females to choose juice based on the entirety of its quality and not on a particular sensory attribute (Appendix 1B, Table 1.2). In ascribing reasons for drinking juices, females significantly differed from males. Females were two times more likely to drink juices as snack and as food supplements for nutritional benefit.

4.1.3.2 Effects of consumers age

There were significant differences ($p \leq 0.05$) in the frequency of drinking juices between the age groups. Respondents in the age group of 31-40 years were two times more likely to drink juice than the younger age group 20-30. The reasons were not clear but could be attributed to the working status of the age groups. People of age groups 20-30 are mostly students and dependents, while those between 31 and 40 are usually independent and following a career and therefore more capable of purchasing exactly what they want. The criterion for choosing juice was significantly different between the age groups. While both groups considered taste as an important criteria for choosing a fruit juice, the older group (31-40 years) were 2 times more likely to use color as a criterion.

4.1.4 Summary of observation from consumer survey

Drinking of beverages is a popular practice among consumers in Accra. Juices are particularly popular, among females and working class consumers. Younger consumers (20-30 year olds) like juices but do not drink as much as older people (31-40 years) probably because of purchasing capabilities. Juices are drunk as snack and as a nutritional supplement by health conscious consumers. The choice of juice as a drink is dependent on taste and color on one hand, gender and age of the consumer on the other hand. This information is important for determining the attributes of the Moringa leaves beverage that will drive it among a given target population.

4.2 Formulation of Moringa beverage

4.2.1 Screening for proportions of components in formulation

As much as the objective was to develop a Moringa leaf juice, the practical realities of an agreeable product color and the need for an appropriate sweetener demanded the inclusion of other ingredients to serve those functions. Similarly, carrot extracts were also used to add color to the product, since preliminary tests indicated that the green Moringa juice color was not acceptable to consumers.

4.2.1.1 Sensory analysis

Analysis of the sensory data from the screening study showed no significant differences ($p \geq 0.05$) (Appendix 2C, Table 1.4) in all the attributes among the products. This suggests that, there was very little variation among the ten formulations. The implication is that the experimental space (that is the minimum and maximum values of the ingredient ratios) was too narrow and resulted in products that were very similar. The overlaid contour plots of the regression models obtained from the mean score values (Appendix 2C, Table 1.3) for all the sensory attributes showed a very wide optimized region (Figure 4.2). This provided information for re-defining and widening the lower and upper limits for the ingredients in an optimization study using the same constrained mixture design for the juice formulations.

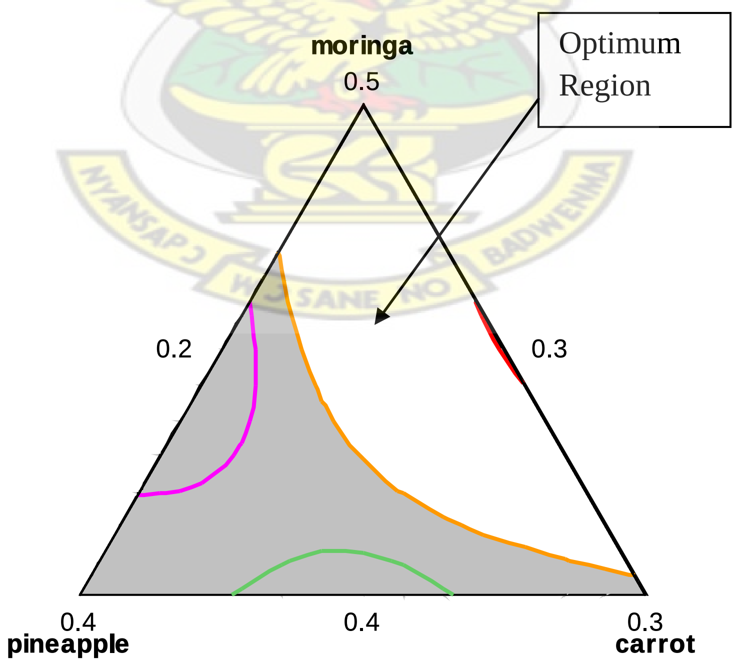


Figure 4.2 Overlaid Contour Plot (Screening)

4.2.2 Optimization of the components proportions in the formulations

In the optimization study, Moringa leaf juice which was the focus of the work and thus, the main ingredient was allocated between 50-70 percent, Pineapple juice which served as a sweetener was allocated between 20 – 40 percent, and carrot as coloring of the final juice was allocated between 10 – 30 percent of the volume of the total mixture. The lower and upper percentage limits of the three ingredients were used to generate ten possible formulations (Table 3.4), which were evaluated using sensory analyses. The regression models for all sensory attributes were obtained based on means score values presented in Table 4.1.

Table 4.1 Means score value (optimization phase)

FORMULA NUMBER	COLOR	TASTE	FLAVOR	AFTERTASTE	OVERALL ACCEPTABILITY
1	5.96	5.29	6.21	5.17	5.33
2	6.50	4.83	5.63	4.75	4.83
3	6.75	5.96	6.08	5.54	6.38
4	5.00	4.75	6.08	5.04	5.13
5	6.33	5.67	6.42	5.83	6.58
6	6.42	5.13	6.00	5.33	5.08
7	7.00	6.17	6.33	6.33	6.25
8	5.92	5.63	6.00	5.29	5.79
9	5.21	4.42	5.13	4.75	4.63
10	5.92	5.08	5.54	5.17	5.29

4.2.3 Sensory analysis

Analysis of the sensory data from optimization showed significant differences ($p \leq 0.05$) in the sensory attributes, color and overall acceptance among the ten formulations (Appendix 2C, Table 1.5). This suggests that, good variations existed between the formulations and therefore panelists were able to assess the differences in the sensory attributes of the various formulations.

4.2.3.1 Color

Color which is a sensation that forms part of the sense of vision, judges the appearance of a food (Jellinek, 1985). The mean score data for the various optimized formulations (Table 4.1) shows that, product number seven (7), was more highly rated for color. This formulation as shown in Table 3.4 had the minimum content of Moringa at 50%, minimum carrot at 10% and maximum pineapple content at 40%. The sensory data for color was fitted to a regression model and it was reasonably accurate, showing R-squared (adjusted) of 70%. ANOVA of the regression for color in Appendix 2C, Table 1.5, shows that the proportion of Moringa/pineapple/carrot significantly influenced ($p \leq 0.05$) the color of the juice. Ternary contour plots were generated using the predictive model to graphically display the influences of the components on color (Figure 4.3). The figure showed that decreasing the proportion of Moringa juice in the formulation to its minimum improved the color appreciation by the panelists. This could be attributed to consumers not being familiar with green beverages, and hence the less green the color of the juice the higher it was scored.

The ingredients that were not green, (i.e. pineapple) had a positive impact on the color score of the juice. This was shown by the increasing acceptance by consumers when pineapple was at maximum amount (Product 7 in Table 3.4). The level of acceptance reduced to 6.0 when pineapple moved towards their minimum compositional ratio. The highest mean score, >6.5, was obtained when pineapple and carrots were at maximum levels. This confirms the likeness for lighter colored juices by consumers.

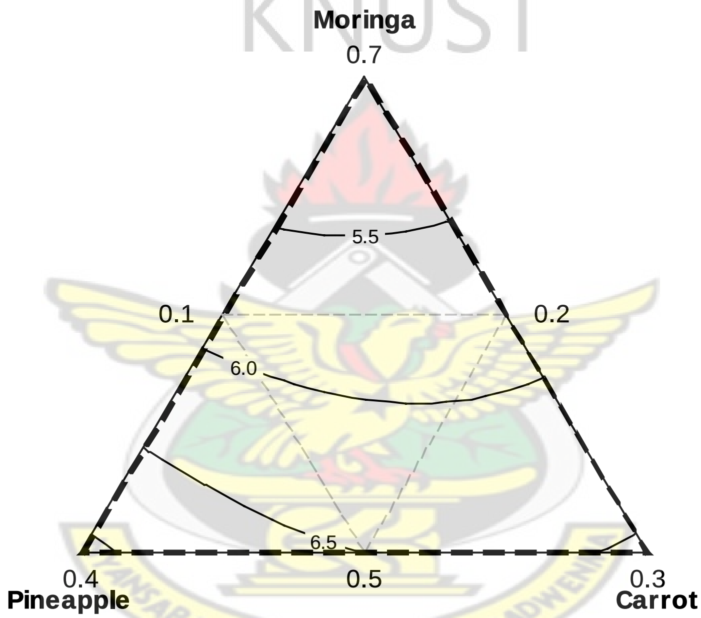


Figure 4.3 Mixture Contour Plots for Color

Regression model for color

$Y = 3.81X_1 + 24.39X_2 + 13.71X_3 - 22.77X_1X_2 - 0.04X_1X_3 - 34.13X_2X_3$

Where, X_1 = Moringa X_2 = Pineapple X_3 = Carrot

$R^2 = 86.68\%$

R^2 adjusted = 70.03%

4.2.3.2 Taste

The taste of the juices was rated by panelist from like slightly to dislike slightly. Figure 4.4 represents the contour plots for taste and the model could explain 95% of the variations due to taste. The highest mean score, >6, for taste was obtained when pineapple was at its highest level (product number 7 in Table 3.4). The taste of the juice was scored the highest at minimum Moringa levels and was disliked slightly at maximum level. A mean score of >5 was scored when carrot was at minimum and reduced to 5.0 at maximum.

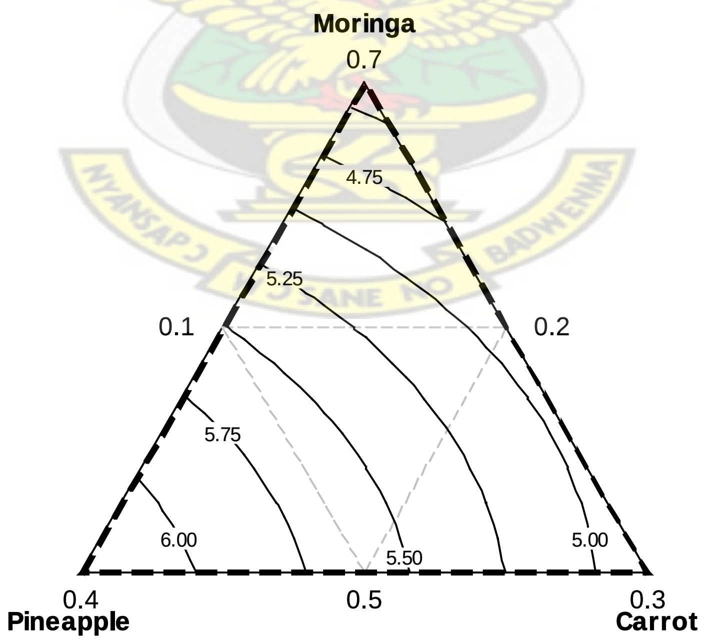


Figure 4.4 Mixture Contour Plots for Taste

Regression model for taste

$$Y = -1.147X_1 + 4.393X_2 - 8.066X_3 - 20.423X_1X_2 - 30.082X_1X_3 - 6.787X_2X_3$$

Where, X_1 = Moringa X_2 = Pineapple X_3 = Carrot

R^2 = 94.92%

R^2 adjusted = 88.58%

4.2.3.3 Flavor

Flavor includes tastes and the aroma perceived through tasting (Jellinek, 1985). Flavor of the juices was rated from like moderately to neither like nor dislike. Juice made from 50% Moringa, 30% pineapple and 20% carrot (Product 5, Table 3.4) gave a flavor that was the most accepted at a mean score of 6.42 (Table 4.1). The least acceptable flavor (mean score = 5.4) was found in product number 9 that had the highest amount 70% of moringa, the lowest proportion of pineapple 20% and carrot 10%. The predictive model for flavor could explain 85% of the variations in the flavor of the juices (R-square = 85%). Since the regression model did not suffer from lack of fit, contour plots was generated (Figure 4.5), which displays the relative influences of each of the components on the juice flavor. The figure shows that increasing the amount of Moringa in the juice gave a flavor that was least liked by consumers. A reduction in the amount of Moringa to the minimum level gave an acceptable mean score of 6.2 (Figure 4.5).

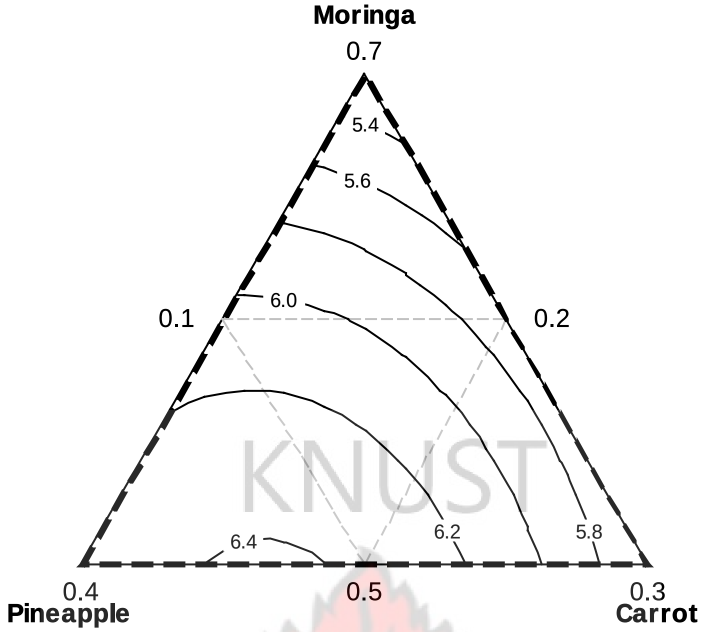


Figure 4.5 Mixture Contour Plots for Flavor

Regression model for flavor

$Y = 0.26X_1 - 6.27X_2 - 10.70X_3 - 32.90X_1X_2 - 26.65X_1X_3 - 44.27X_2X_3$

Where, X_1 = Moringa X_2 = Pineapple X_3 = Carrot

R^2 = 85.25%

R^2 adjusted = 66.81%

4.2.3.4 Aftertaste

Aftertaste is the lingering of the sense of taste of a product on taste buds. The least score for aftertaste was obtained for products 2 and 9 (Table 3.4) with high amounts of Moringa while the highest mean score (6.33) was obtained for product 7, which had maximum amounts of pineapple. Thus high amounts of pineapple in the product may have increased the score for aftertaste. This may be due to the sweetening nature of the pineapple. Regression model for aftertaste showed R-square of 87%. Contour plots obtained from the regression model for aftertaste (Figure 4.6) showed that carrot and Moringa at their maximum levels gave juices that were neither liked nor disliked by consumers. As the compositions of Moringa and carrot were reduced, high mean scores for aftertaste in the juice were recorded.

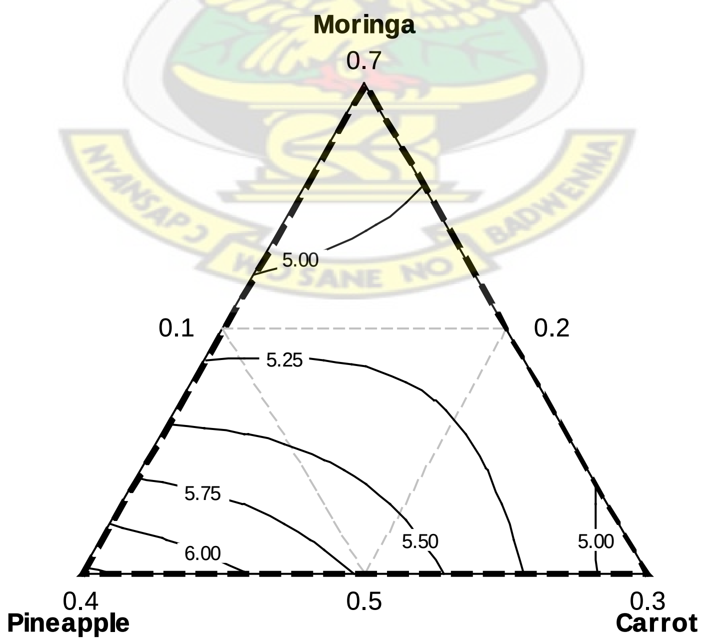


Figure 4.6 Mixture Contour Plots for Aftertaste

Regression model for aftertaste

$$Y = 5.88X_1 + 26.80X_2 - 18.07X_3 - 39.18X_1X_2 + 32.41X_1X_3 + 16.50X_2X_3$$

Where, X_1 = Moringa X_2 = Pineapple X_3 = Carrot

R^2 = 94.34%

R^2 adjusted = 87.28%

4.2.3.5 Overall acceptability

The contour plots for overall acceptability is represented in Figure 4.7 and the model could explain 69% (R^2adjusted) of the variations. Products with an amount of pineapple between the ranges of 30-40 percent and carrot between 10-20 percent were the most accepted by the consumers. On the other hand, Moringa juice being the main ingredient was accepted by consumers when a minimum amount (that is 50% of the total volume of the mixture) was used. Juices made with the minimum level of Moringa had the highest mean score (>6) for overall acceptability.

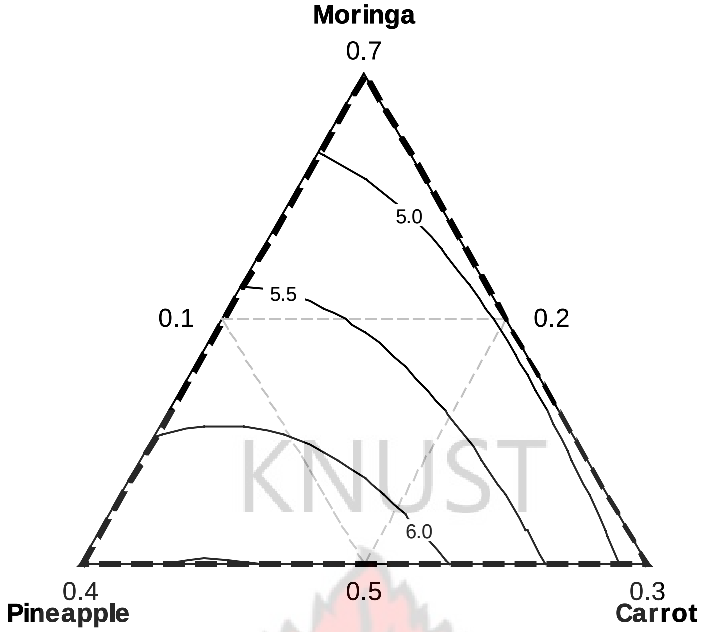


Figure 4.7 Mixture Contour Plots for Overall acceptability

Regression model for overall acceptability

$Y = 2.47X_1 + 3.18X_2 - 17.99X_3 + 8.72X_1X_2 + 20.65X_1X_3 + 72.36X_2X_3$

Where, X_1 = Moringa X_2 = Pineapple X_3 = Carrot

$R^2 = 86.01\%$

R^2 adjusted = 68.53%

4.2.4 Region of Optimum formulation

Optimization in sensory evaluation is defined as a procedure for developing the best possible product in its class (Sidel and Stone, 1983). Although an optimal formation should maximize consumer acceptance, it is impossible to develop a product with all sensory qualities that would satisfy consumers in most applications. It should only be possible to approach that result (Moskowitz, 1994). To obtain the optimum region therefore, and hence the ingredient formulation that would obtain optimum color, taste, flavor, after taste, overall acceptability for Moringa leaf juice, the contour plots for these attributes were overlaid in a single graph on same axis as shown in Figure 4.8. The optimum region in this overlaid plot is where the criteria for all the five response variables (color, taste, flavor, after taste, overall acceptance) were satisfied.

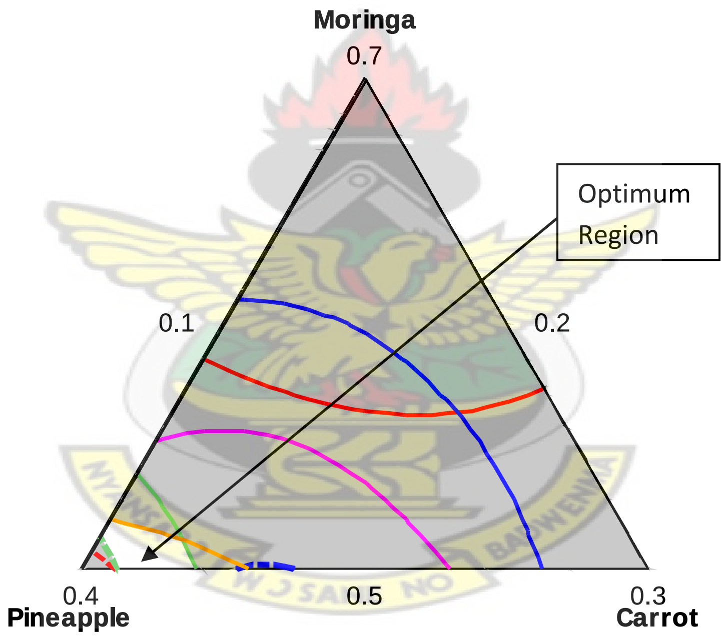


Figure 4.8 Overlaid Contour Plot (Optimization)

4.2.5 Validation for formulations in the optimum region

To determine whether the predictive models obtained were accurate in practical terms, two formulations from the (optimum region) and two from the non optimal region (outside the optimum region) were produced (Figure 4.9) and subjected to the panelists for sensory evaluation. The formulations that were selected are shown in Table 4.2.

Table 4.2 Compositional ratio of formulations for validation of optimum region

Formula number	**Moringa $_{(X1)}$**	**Pineapple $_{(X2)}$**	**Carrot $_{(X3)}$**
Formulations within the optimum region			
1	51.5	38.1	10.4
2	50.7	36.7	12.6
Formulations from outside of the optimum region			
3	68.0	20.9	11.1
4	54.8	27.7	17.5

The Moringa leaf beverage components (100% in mixture design) were 98% of actual formulations. All formulations contained 2% ginger distillate to make up a total of 100%.

Results from the validation study showed that, there were significant differences ($p \leq 0.05$) in all the attributes (Appendix 2C, Table 1.6). Comparison of the predictive and experimental mean values of the formulations from the optimum region and outside of the region is shown in Table 4.3. The results indicate that the predicted and experimental values showed good agreement with the predicted values. Any of the recipes in the optimized region therefore is a preferred formulation by consumers. The predicted data from outside of the optimum region were usually not in good agreement with the experimental data.

Table 4.3 Predicted and validated mean values of selected formulations

Formula Number /Region	**Predicted / Experimental model**	**Color**	**Taste**	**Flavor**	**After taste**	**Overall acceptance**
1	Predicted	6.83	6.12	6.28	6.05	6.31
Optimum	Experimental	6.57	7.03	6.73	6.73	6.83
2	Predicted	6.82	6.07	6.34	6.10	6.43
Optimum	Experimental	7.07	6.93	6.60	6.43	7.07
3	Predicted	5.17	4.59	5.41	4.83	4.84
Non-optimum	Experimental	4.70	4.20	4.80	4.17	4.17
4	Predicted	6.12	5.47	6.22	5.43	5.87
Non-optimum	Experimental	6.03	5.27	5.27	5.30	5.80

Figure 4.9 Juices obtained using formulation from optimum region (samples 721 and 356) and from outside the optimum region (sample 673 and 481)

4.4 Proximate Analysis

The composition of Moringa leaves and the final composite juice is as presented in Table 4.4. The data of Moringa leaves from literature (Campden and Chorleywood, 1998), pineapple and carrot (www.nutritionanalyser.com) are presented alongside for easy comparison. Moringa leaves clearly have significant amounts of protein and minerals, and can be a good source of nutrients if incorporated as an ingredient in food product formulation.

4.4.1 Carbohydrate

Results of the proximate composition of carbohydrate of the fresh Moringa leaf extract showed 8.7 g/100g was extracted, which is more than half the total amount in the fresh leaves reported in literature. However, both carrots and pineapple juices have lower amounts of carbohydrate (Table 4.4) and therefore caused a reduction of carbohydrates in the final composite juice to 4.91 g/100g. The centrifugation of the Moringa leaf extract during the processing also took out the insoluble fiber which is a form of carbohydrate.

4.4.2 Protein

According to Aurand and Woods (1973) the colloidal dimensional structure of proteins makes it uneasy to pass through semi permeable membranes. Also some proteins are not water soluble and therefore could not have been extracted in the aqueous medium. As much as 4.8 g/100g of proteins was extracted from the fresh leaves. The relatively very low protein composition of the pineapple and carrot of 0.54 g/100g and 1.0 g/100g respectively (www.nutritionanalyser.com) further decreased the total composition in the final mixture to 2.9 g/100g.

4.4.3 Fat

Fat is soluble in organic solvents like petroleum ether. Since water was used in extraction, only 0.83 g/100g was extracted from the fresh leaves. The addition of fat from the pineapple and carrot accounts for the increase in fat content of the final composite juice of 1.81 g/100g.

4.4.4 Water

The total water content of the final juice was 86.82 g/100g. Though the water content of fresh Moringa leaves reported in literature was 75 g/100g, the use of water in slurring of the leave increased the water content to 81.30 g/100g. The water composition of raw pineapple and carrot are 86 g/100 g and 87.5 g/100 g respectively (www.nutritionanalyser.com). The pineapple was pressed and pulp and was allocated almost 40 percent, whiles the carrot which extracted with 600 mls of water per 100 g was allocated 10 percent of the final juice. The increment of water recorded in the composition of the final juice was thus expected.

4.4.5 Minerals

The mineral analysis (magnesium, potassium, calcium and iron) revealed that there was general reduction after extraction from leaves. However, from literature (Campden and Chorleywood, 1998) a good amount of potassium, 115 mg/100 g and 240 mg/100 g, has been found in raw pineapple and carrot respectively which helped boost the content of the minerals in the juice. This resulted in potassium recording 77 mg/100 g, the highest amount among the minerals of the final composite juice.

4.4.6 Vitamins

Adequate absorption of fat – soluble vitamins (vitamin A) depends on efficient fat absorption (Wardlaw and Insel, 1996). A total of 48.8% fat extracted from the leaves was able to extract along 5.98 mg/100 g of provitamin A. The level of provitamin A in carrot made a positive impact on the Moringa juice by increasing the total amount of the provitamin A to 6.64 g/100 g.

Water – soluble vitamins, like riboflavin and particularly ascorbic acid is easily destroyed by heat, light, exposure to air, cooking in large amounts of water and alkalinity (Wardlaw and Insel, 1996). The extraction medium (i.e. water) for riboflavin and ascorbic acid strongly reflected in the values recorded. A total amount of 215 mg/100 g of riboflavin and 220 mg/100 g of ascorbic acid were extracted from the fresh leaves. However, exposure to air during preparation and the use of heat for pasteurization destroyed some of the water- soluble vitamins and hence reducing the amount of ascorbic acid to 159.14 mg/100 g in the final juice (Table 4.4). The appreciable amount of vitamin C present in the pineapple juice was an additional source in the final product.

Table 4.4 Proximate composition of fresh Moringa leaves extract, final juice and also literature on fresh Moringa leaves[1], pineapple[2] and carrot[2] (per 100g)

Composition	Fresh Moringa leaves extract	Final composite juice	Literature on Fresh Moringa leaves[1]	Literature on pineapple[2]	Literature on carrot[2]
Carbohydrate (g)	8.7 ± 0.06	4.91 ± 0.02	13.4	12.63	9.0
Protein (g)	4.8 ± 0.08	2.9 ± 0.06	6.7	0.54	1.0
Fat (g)	0.83 ± 0.03	1.81 ± 0.01	1.7	0.12	0.20
Water(g)	81.30 ± 0.04	86.82 ± 0.05	75.0	86.0	87.5
Magnesium(mg)	24.44 ± 0.02	12.30 ± 0.03	24.0	12.0	18.0
Potassium(mg)	98.16 ± 0.15	77.01 ± 0.28	259.0	115.0	240.0
Calcium(mg)	124.4 ± 0.03	60.65 ± 0.05	440.0	13.0	33.0
Iron(mg)	3.05 ± 0.01	1.02 ± 0.05	7.0	0.28	0.66
Pro-vitamin A(mg)	5.98 ± 0.01	6.64 ± 0.02	6.8	0.03	8.29
Vitamin B_2(mg)	0.05 ± 0.01	0.03 ± 0.03	0.05	0.03	0.05
Vitamins C(mg)	215 ± 0.27	159.14 ± 0.12	220	36.20	7.0

[1]Campden and Charleywood (1998)

[2] www.nutritionanalyser.com

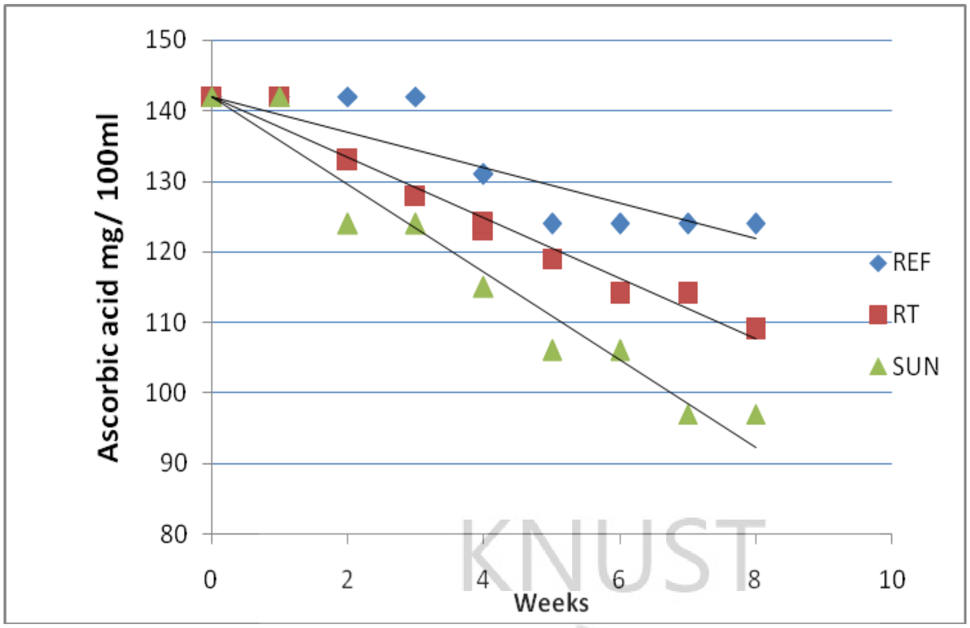
4.5 SHELF-LIFE ANALYSIS

The optimized final composite juice was pasteurized (62^{o}C for 30 mins), bottled and was closely monitored to determine the shelf – life under three different storage conditions; refrigerator (5^{o}C), room temperature (26 ^{o}C) and sunlight (above 37 ^{o}C) for eight (8) weeks. The parameters monitored during this period included; ascorbic acid, titrable acidity, pH, alcohol content, color and microbial analysis. The data generated from the attributes were subjected to Analysis of Variance, and summarized in Appendix 3A, Table 1.7.

4.5.1 Vitamin C

The data for ascorbic acid in the beverage stored under different conditions is displayed in (Figure 4.10). The figure shows that the rates of ascorbic acid degradation were lower when the beverage was stored in the refrigerator than at ambient conditions or in the sun. The data was fitted into linear trendline models and the trends show that when stored under refrigerator condition, the rate of ascorbic acid degradation was 2.5mg/week. The rate of ascorbic acid degradation in the juices increased to 4mg/week when it was stored at room temperature and 6mg/week when stored in the sun, as shown in Table 4.5.

Analysis of variance and multiple range tests by LSD (Appendix 3A, Table 1.7 and 1.8 respectively) showed that the storage conditions had significant ($p \leq 0.05$) effects on the Ascorbic acid. Storage of the juice in the refrigerator preserved ascorbic acid content better than at room temperature conditions. The room temperature conditions also significantly ($p \leq 0.05$) preserved ascorbic acid in the juices better than when stored in the sun. The effect of storage time on the ascorbic acid content of the juices was also significant ($p \leq 0.05$). Ascorbic acid under all storage condition degraded with time, but even under sunlight, 78% of the original amount of ascorbic acid was retained in the juice after eight weeks of storage.



REF= Refrigerator RT= Room temperature SUN= Sunlight

Figure 4.10 Ascorbic acid content under different storage conditions and time

Table 4.5 Degradation rates for ascorbic acid in Moringa juice during storage.

Storage condition	**Linear model for ascorbic acid**	**R – Squared**
Refrigeration (15°C)	Y = -2.51X + 142	0.79
Room Temperature (25°C)	Y = -4.29X + 142	0.97
Sun (above 35°C)	Y = -6.22X + 142	0.95

Where, X= Storage time

Y= Total amount of ascorbic acid

4.5.2 pH and Titratable acidity

Titratable acidity and pH of the juice is a measure of the sourness of the product and it also reflects on the stability of the product with regards to deterioration during storage.

The analysis of the data for pH showed significant effects ($p \leq 0.05$) of storage conditions and time. The multiple range analysis showed that while there were no significant difference ($p \geq 0.05$) in pH between refrigerator and room temperature storage (4.02 and 4.04), the pH of the beverage stored under sunlight was significantly higher (4.09). This could be due to heat and/or sunlight induced degradation of some components like protein that will affect the pH. Such a reaction or degradation could not have been due to microbial activity because there was no microbial growth.

Storage time did not show significant effects on the titratable acidity of the juices. On the other hand, there were significant differences between the titratable acidity of the juices stored at different conditions. Titratable acidity at the end of storage of the final composite juice in the refrigerator (0.27%) was significantly lower than that stored at room temperature (0.28%) and sunlight (0.29%). Though juice stored in the sun recorded the highest titratable acidity its corresponding pH was also the highest. This trend is difficult to explain, but could suggest some buffering effects of the juice proteins.

4.5.3 Color

Color is one of the most important quality attributes of food. The first impression of the quality and acceptability of a particular food is judged upon its appearance (Neilsen, 1998). Results of the consumer survey (Section 4.1.3.1) indicated that some consumers make choices of juices based on color. The juices were all yellowish-green in color. L* is a color parameter that measures the extent of lightness, thus L* when zero (0) would indicate black, and when one hundred (100) would indicate white; a* when positive signifies reddish color coordinate and when negative signifies greenish color coordinate; b* value when positive signifies yellowish color coordinate and bluish color coordinate when negative.

4.5.3.1 Color L*

The L* values for the juice were significantly ($p \leq 0.05$) affected by the storage conditions as well as storage time. The Multiple Range Test by LSD at the end of storage showed that refrigerator significantly preserved the L* value (44.19) of the juice better than the other storage conditions. L* values for the juices stored at room temperature (43.79) and sunlight (42.95) were all significantly different. Thus samples stored in the sun turned darker compared to those stored at ambient and refrigerator conditions. The darkening observed under sunlight could be due to degradation of the chlorophyll by UV light and temperature or non-enzymatic browning in an aqueous environment that has proteins and reducing sugars.

4.5.3.2 Color a*

The analysis of variance for a* values showed significant effects of storage conditions and storage time. A negative a* value suggests green coloration, and this was significantly higher in absolute terms for juices stored in the refrigerator (-33.63) than at room temperature (-33.53) and sunlight (-33.30) at the end of storage.

4.5.3.3 Color b*

Positive b* values are a measure of the yellowness or blueness of the product. Analysis of variance showed that storage time and storage temperature significantly affected the yellowness of the juices. Samples at the end of storage in the refrigerator had significantly higher b* values (12.99), than those stored at room temperature (12.66) and sunlight (12.23). Thus storage temperature significantly influences color of the juice with time.

4.5.4 Alcohol content

The three different storage conditions were not significantly different in alcohol production with storage time. There were no detectable amounts of alcohol in the juice under any of the storage conditions, for the entire eight weeks shelf life study period. Indeed the microbial analysis confirmed that there were no growths at any of the storage conditions.

4.5.5 Microbial analysis

The results for growth of Total Coliform, *Staphylococcus areaus*, Yeast /Mould and Total Plate Count (Appendix 3B, Table 1.9) revealed that there was no microbial growth under all conditions for the eight weeks period of study.

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Juice is very popular among consumers in Accra, and it is the leading form of beverage consumed. Females are particularly interested in juices, and are far more likely to choose juice for beverage than their male counterparts. The choice of juice as a beverage is usually determined by its color and taste, and consumers will try a new juice product if it meets their criteria.

High amounts of Moringa extracts in the composite juice impacted negatively on its sensory acceptability. On the other hand high amounts of pineapple juice strongly improved the scores of the sensory attributes. Using constrained mixture optimization techniques it was possible to obtain an acceptable juice that had a ratio of 50:38:12 for Moringa: Pineapple: Carrot.

Comparing the nutrient composition of some blended juices from literature with the final composite juice, the latter is a more nutritious beverage considering the protein, Iron, vitamin A and C content.

The keeping quality of the Moringa juice in the refrigerator (5^oC), at room temperature (25^oC), or even when exposed to the sun (above 37^oC) for eight weeks, was good. All attributes were better preserved when the juice was stored in the refrigerator. The rate of degradation of vitamin C was slow even under harsh conditions, when almost 80% of the vitamin was retained after 2 months of storage.

5.2 Recommendation

It is hereby recommended that:

i. A survey for the acceptability, competitiveness and consumer perceptions of the optimized Moringa juice drink is carried out.

ii. Further studies may be carried out to verify the medicinal potential of the juice, particularly for diabetic and hypertensive conditions.

iii. Further studies of shelf-life beyond two months should be carried out to ascertain the exact quality keeping period of product.

iv. Study on packaging effect on storability to determine the type of packaging that can best prevent interaction between the environment and product.

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APPENDIX 1A

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

BEVERAGE DEVELOPMENT PROJECT

Questionnaire to establish the consumption pattern of beverage in Accra.

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1. Date………………. 2. Town……………… 3. Region…………………………….

A. RESPONDENTS

4. Sex…………. M/F 5. Age [ ] 20-30 [ ] 31-40 [ ] 41-50 [ ] 51-60

6. Educational status

a) None
b) Sec/Tech/Comm.
c) Tertiary
d) Elementary
e) Voc
f) Other (specify)

7. Marital status…………S/M/D/W 8. Family size……………………

9. Number of children/dependent………… 10. Major occupation……………………

B. PRODUCT

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

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

Product	**N/Y**
Coffee	
Tea	
Food drinks (e.g. Milo, Soya milk)	
Juices (fruits, vegetables)	
Energy drink	
Other non-carbonated drinks	
Carbonated drinks	

If you drink juices, please answer the following:

13. How often do you drink juices?

a) Once/week b) More than once/week

c) Occasionally d) Hardly

14. Why do you drink juices?

a) Snack b) Nutrient requirement c) As food supplement

d) Inexpensive e) Other (specify)……………………………………………

15. Are you willing to try new juice product? ....Y/N

16. What characteristic attributes do you look for in a juice?

a) Flavor b) Color c) Taste d) After-taste e) Overall acceptance

f) All of the above

C. HEALTH

17. Do you know of any health problems associated with the consumption of juices? ……….Y/N

If yes list them

……………………………………………………………………………………………
……………………………………………………………………………………………

18. Do you know of any good health claims attributed to juices? ………….... Y/N

If yes list them

……………………………………………………………………………………………
……………………………………………………………………………………………

19. Do those good health claims influence your choice of juice? ……………Y/N

APPENDIX 1B

Table 1.1 Frequencies of some responses of survey

Parameters	**Category**	**Percent (%)**
Gender	Male Female	54.0 46.0
Age (years)	20-30 31-40	50.0 50.0
Marital status	Single Married Divorced	68.7 28.7 2.7
Educational status	No basic education Elementary Sec/Tech/Comm. Vocational Tertiary	2.7 6.7 18.7 8.0 64.0
Beverage consumer	Yes No	99.3 0.7
Juice consumer	Yes No	92.7 7.3
Carbonated drink consumer	Yes No	63.7 32.3
Reason for juice consumption (Inexpensive)	Yes No	0.0 100.0
New juice recipe	Yes No	88.0 12.0

Table 1.2 Association between age, sex and some responses of survey

Parameter	**Estimate (χ^2)**	**Odds ratio (male : female)**
Consumption of juice as a snack	2.99	0.57
Consumption of juice as a food supplement	2.31	0.53
Choice of juice based on color	3.09	0.35
Choice of juice based on taste	2.99	0.57
Choice of juice based on the entirety of juice	5.62	3.40
Juice as a choice of beverage	6.51**	0.10
Parameter	**Estimate (χ^2)**	**Odds ratio (20-30 : 31-40)**
Choice of juice based on color	0.76	0.60
Juice as a choice of beverage	0.88	0.55
Non-carbonated drink as a choice of beverage	5.34*	0.46

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

APPENDIX 2A

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY & BIOTECHNOLOGY

SENSORY EVALUATION FORM

NAME: ……………………………………………………………………………………………………

PRODUCT: Vegetable-Fruit Juice **DATE: ..………………………………**

INSTRUCTION:

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

A NINE POINT HEDONIC SCALE

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

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

Any other comment (s)……………………………………………………………………………………...

………

………

………

………

Thanks for your cooperation

APPENDIX 2B

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

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

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

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

Where, t= no. of formulations; b= no. of panelists for each set;

r= testing frequency of a formulation in each set; N= total no. of panelist (4 sets);

k= no. of formulations tested by each panelist;

λ= maximum no. of panelist testing the same formulation.

APPENDIX 2C

Table 1.3 Means score value (Screening phase)

FORMULA NUMBER	COLOR	TASTE	FLAVOR	AFTERTASTE	OVERALL ACCEPTABILITY
1	6.60	6.55	6.85	6.20	6.45
2	6.58	6.46	6.42	5.92	6.50
3	6.54	6.08	6.42	6.38	6.58
4	6.83	5.75	6.04	5.75	6.00
5	6.54	6.77	6.77	6.38	6.54
6	6.46	6.08	6.54	5.67	6.04
7	6.29	5.63	6.04	5.88	5.92
8	6.86	6.23	7.00	5.96	5.91
9	6.04	6.46	6.88	6.38	6.58
10	6.08	6.21	6.08	5.79	6.04

Table 1.4 Anova summary of formulation (screening phase)

Source of variance	Parameter				
	Color	Taste	Flavor	Aftertaste	Overall acceptance
Between groups	0.81	1.30	1.48	0.61	0.92

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

Table 1.5 Anova summary of formulation (optimization phase)

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

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

Table 1.6 Anova summary of formulation (Validation phase)

Source of variance	Parameter				
	Color	Taste	Flavor	Aftertaste	Overall acceptance
Between groups	10.55**	20.09**	8.98**	15.91**	17.68**

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

APPENDIX 3A

Table 1.7 Anova summary for shelf-life study

Source of variance	Parameters					
	Vitamin C	pH	Titrable acidity	Color L*	Color a*	Color b*
Storage condition	56.96**	7.74**	48.00**	56.16**	83.82**	60.55**
Storage time (week)	48.43**	51.97**	53.66**	122.26**	68.91**	343.300**

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

Table 1.8 Summary table for multiple range test by storage condition for shelf-life

Parameter	Storage condition	LS Mean	Nutritional content (week 8)
Vitamin C (mg/100ml)	Refrigerator	132.78^{a} ±0.05	124
	Room temp.	124.94^{b} ± 0.04	109
	Sunlight	117.00^{c} ±0.05	97
pH	Refrigerator	4.02^{a} ±0.01	4.24
	Room temp.	4.04^{a} ±0.01	4.44
	Sunlight	4.09^{b} ±0.02	4.47
Titrable acidity (%)	Refrigerator	0.27^{a} ±0.01	0.26
	Room temp.	0.28^{b} ±0.02	0.28
	Sunlight	0.29^{c} ±0.01	0.29
Color L*	Refrigerator	44.19^{a} ±0.08	42.38
	Room temp.	43.79^{b} ±0.08	42.03
	Sunlight	42.95^{c} ±0.07	41.70
Color a*	Refrigerator	-33.63^{a} ±0.02	-33.22
	Room temp.	-33.53^{b} ±0.02	-33.11
	Sunlight	-33.30^{c} ±0.01	-33.03
Color b*	Refrigerator	12.99^{a} ±0.05	11.88
	Room temp.	12.66^{b} ±0.05	11.33
	Sunlight	12.22^{c} ±0.04	11.02

NB: Different alphabets (a, b, c) assigned to LS Mean values for the 3 different conditions of a parameter represents significant differences ($p \leq 0.05$); whiles same alphabets represents insignificant differences ($p \leq 0.05$).

APPENDIX 3B

Table 1.9 Microbial analysis for shelf-life study

STORAGE CONDITION	**STORAGE TIME (WEEK)**	**YEAST AND MOULDS (10^{-1})**	**TOTAL COLIFORMS (10^{-1})**	**TOTAL PLATE COUNT (F/S)**	***STAPHYLOCOCCUS AREAUS*** **(10^{-1})**
Refrigerator	0	< 10	0	1	<10
Room Temp.	0	< 10	0	1	<10
Sunlight	0	< 10	0	1	<10
Refrigerator	2	< 10	0	2	<10
Room Temp.	2	< 10	0	1	1
Sunlight	2	< 10	0	<10	<10
Refrigerator	4	< 10	0	<10	<10
Room Temp.	4	< 10	0	<10	1
Sunlight	4	< 10	0	<10	<10
Refrigerator	6	< 10	0	<10	<10
Room Temp.	6	< 10	0	3	<10
Sunlight	6	< 10	0	3	<10
Refrigerator	8	< 10	0	2	<10
Room Temp.	8	< 10	0	1	<10
Sunlight	8	< 10	0	1	<10