

**EFFECTS OF SOYMILK, MANGO NECTAR AND SUCROSE SOLUTION
MIXES ON QUALITY OF SOYMILK BASED BEVERAGE**

M.Sc. Thesis

By

Rahel Getu

July, 2015

Jimma, Ethiopia

**EFFECTS OF SOYMILK, MANGO NECTAR AND SUCROSE SOLUTION
MIXES ON QUALITY OF SOYMILK BASED BEVERAGE**

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**In Partial Fulfillment of the Requirements for the Degree of Master of Science
in Post Harvest Management (Perishable crops)**

By

Rahel Getu

**July, 2015
Jimma, Ethiopia**

APPROVAL SHEET

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DEDICATION

I dedicate this Thesis manuscript to my mother W/ro Eineye Kerebihi and my father Ato Getu Jemere for their dedicated partnership in the success of my life.

STATEMENT OF THE AUTHOR

First, I declare that this Thesis is my original work and that all sources materials used for this Thesis have been duly acknowledged. This Thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to borrowers under the rules of the library. I solemnly declare that this is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

Rahel Getu Jemere, was born on November 3, 1988 G.C in Kolladiba Town located North Gondar Zone of Amahara Regional State. She had attended her elementary, secondary school and preparatory education at Kolladiba Elementary School, Kolladiba Comprehensive Secondary School and Aykel Preparatory School respectively. She joined then Haramaya University and graduated with Bachelor of Science Degree (B.Sc.) in Food Sciences and Post Harvest Technology in July 2009 G.C. After her Bachelor degree, she had been employed as instructor at Kolladiba Technical Vocation Education and Training Collages from September 2010 to October 2012G.C. In October 2012, she joined Jimma University College of Agriculture and Veterinary Medicine to pursue her postgraduate study leading to Master of Science Degree in Postharvest Management (Perishable crops).

LIST OF ABBREVIATIONS

AACC	American Association for Cereal Chemists
AOAC	Association of Official Analytical Chemists
AAS	Atomic Absorption Spectrophotometer
CFU	Colony Forming Unit
CSA	Central Statistics Authority
CGIAR	Consultative Group for International Agricultural Research
EPHRI	Ethiopia Public Health Research Institute
FAO	Food and Agriculture Organization
JARC	Jimma Agricultural Research Center
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
MARC	Melkasa Agricultural Research Center
MN	Mango Nectar
PEM	Protein Energy Malnutrition
RTS	Ready to Serve Beverage
SBB	Soy milk Based Beverages
SM	Soy Milk
SNNPR	Southern Nation Nationality and Peoples Region
SS	Sucrose solution

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ABSTRACT

Soybean has the main protein source among legume crops. Because of this, soymilk based beverages are presented as healthy food alternatives for human nutrition. It is similar to cow's milk both in appearance and consistency. However, the "beany" flavors, chalky mouth feel of the milk made it unacceptable by majority of soymilk consumers. The objective of this study was to develop soy-based beverages with better physicochemical and sensory quality to overcome the above limitations through mixing the milk with mango nectar and sucrose solution in different ratios. Fourteen formulations of the beverages were prepared using D-optimal mixture simplex lattice design with the aid of Design-Expert software with a ratio of 60-100% soymilk, 0-25% mango nectar and 0-15% sucrose solution. Sensory acceptability was assessed using 5-point Hedonic Scale. Total aerobic plate count was used to determine the microbial quality and shelf life stability of selected beverage. The major response variables of physicochemical, sensory and microbial quality of mix ratios were analyzed. Results of this study show that a significance difference ($p < 0.05$) in TSS, total color change, protein, ether extract, β -caroten, phytic acid, Fe, K, flavor, taste and overall acceptability were observed among the mix ratios. The overall optimum values were found in a range of SM 67-84%, MN 12-25%, and SS 0-15%. Among 14 treatment combinations considering all measured parameters soymilk based beverage with ratio of 80% soymilk, 14 % mango nectar and 6 % sucrose solution was superior in terms of attaining overall acceptability and nutritional quality . Furthermore in terms of microbial quality and shelf life stability, sample kept at 4°C was found more stable while the one kept at room temperature spoiled on the same date of preparation.

1. INTRODUCTION

Soybean (*Glycine max* L) is a species of legume crop which belongs to the large botanical *Leguminous* family (Shurtleff and Aoyagi, 2007). It is the most utilized legume as well as the most well researched and health promoting food material in the world Akubor *et al.* (2002) with the highest protein content among legumes (Lawson, 2004). It is useful as excellent nutritional supplement especially for pregnant women, lactating mothers and children (Felberg *et al.*, 2009). Soybean products are the frequently incorporated into food used for the prevention of malnutrition (Ladas *et al.*, 2005).

Njenga (2014) reported that every fourth child worldwide is affected with Protein Energy Malnutrition: - 150 million (26.7%) children are underweight while 182 million (32.5 %) are stunted. Physically, more than 70 % of PEM children live in Asia, 26 % in Africa and 4 % in Latin America and the Caribbean (Black *et al.*, 2008). Their problem can start even before birth with a malnourished mother. In Ethiopia, 52% children (6 to 60 months) are stunted and 47% underweight from the year 1995 to 2002 (UNICEF, 2006). Due to the prevailing unfavorable economic conditions in most developing countries of the world countries such as Nigeria in particular show over 40% of the populations live below poverty line (Nzeagwu and Nwaejike, 2008). The incidence of PEM varies among different age groups particularly children with an estimated 400 million to be malnourished worldwide (Agiriga and Iwe, 2009). Ethiopia had 27.8% of the populations live below poverty line 2011/12 (GTP-APR MoFED, 2011/12).

Soybean is a protein rich food crop and useful for preparation of bread, biscuits, cakes, chocolates and soymilk (Fabiya, 2006). The soymilk is prepared by soaking and grinding of soybean in water (Silva *et al.*, 2007). Soymilk is a traditional oriental food beverage introduced first in the United States and got popularity. This has been spread to many parts of the world especially the so-called developing countries, although it is still more popular in Asia than any other parts of the world (Jimoh and Kolapo, 2007).

In Ethiopian context the Faffa Food Company has been working on production of different soy products as weaning food since 2003 and became the first company which produced powdered soymilk in 2011 (Eskedar, 2012). A growing interest is also observed in processing of soybean to different products in different parts of the country. Popularization of different methods of preparation and utilization of soybean at small-scale farming level has been carried out in Jimma Agricultural Research Center. This is aimed at enhancing the use of soybean in the daily food intake of the households including soymilk (Leta *et al.*, 2007). In line with this, organized self-help groups of women were introducing commercial production of soymilk to Jimma town. It was established at cottage industry level and producing commercial soymilk, using SC-20 Indian made soymilk extraction unit (Liu and Lin, 2000). This milk is rich in plant protein as alternative source of cow milk to people who can't afford cow milk and for those with allergic reaction to cow's milk, and as a low cost source of good quality energy for human consumption (Touba *et al.*, 2013). It is white color or creamy emulsion which is similar to conventional cow milk both in appearance and consistency (Kolapo and Oladimeji, 2008). It is rich in oil, minerals and Phytochemicals (Dewell *et al.*, 2006). In addition, the proximate composition ranged from 1.99 to 2.69% total carbohydrates, 1.81 to 2.36 % fat content, 0.22 to 0.40 % ash content, moisture content 89.20 to 93.29 % (Odu *et al.*, 2012) and with no fiber content (Kolapo and Oladimeji, 2008).

Furthermore, the increasing popularity of soymilk as a beverage worldwide is accredited due to its characteristics of health benefits. For instances, its low cholesterol and lactose content, ability to reduce bone loss and menopausal symptoms, prevention and reduction of heart diseases, certain cancers, malnutrition, reduces the risk of cardiovascular diseases by reducing serum levels of low density lipoproteins (LDL) and triglycerides, reduces blood pressure and increases flexibility, and permeability of blood vessels (Adebayo *et al.*, 2008). The milk has also proven antiradical activity, through the isoflavones, which acts as an effective reducing tool of oxidative degradation of DNA, preventing premature age and the emergence of diseases like Alzheimer's disease (Hsieh *et al.*, 2009). Its isoflavones have protective effects against some types of cancer, hormone-related diseases, menopause symptoms and osteoporosis (Clerici *et al.*, 2007). However, soymilks are often characterized as having unbalanced "beany" flavors and chalky mouth feel which makes it very often

rejected by children and even adults. The beany or nutty flavor, mainly due to lipoperoxidation of polyunsaturated fatty acids (PUFA) mediated by lipoxygenase enzyme (Kale *et al.*, 2011). But the good thing is flavor can be minimized by the use of sodium bicarbonate, chemical treatment or heat treatment (Kumar *et al.*, 2002). Moreover, the soymilk beany flavor effect can also be minimized by blending the milk with different fruit or vegetable juices and other ingredient like artificial flavors (Pooneh *et al.*, 2013).

Soy milk based fruit juice beverages offer distinct nutritional advantages over the plain fruit beverage to the consumer and can mask undesirable flavors (Varzakas and Ozer, 2012). Beverages can be made from different fruits like -Mango juice. Its combination with fruit juices has been successful, indicating that consumers may be changing their attitude in relation to products made from soybeans, because it joins desirable sensory and physicochemical characteristics of fruits with good functional properties of soymilk (Zulueta *et al.*, 2007). In addition to this, fortification of mango pulp in soymilk improves the nutritional as well as therapeutic value of beverage.

Mango fruit is excellent exotic flavor, attractive fragrance, beautiful color and texture, delicious taste and nutritional properties (Arauz, 2000). The fruit contains important nutrients such as carbohydrates, minerals, and a good source of vitamin A, vitamin B₁, vitamin B₂, and vitamin C (Mamiro *et al.*, 2007). Adding mango pulp to soymilk enhances its mineral contents. It also provides sweetness and masks the beany flavor of soymilk to some extent (Sakhale *et al.*, 2012). It is also a good source of energy and rich in important minerals like potassium, magnesium, sodium, phosphorus and sulphur. Additionally, mango fruit has, moderate acidity (0.20 - 1.30%), low crude fat (0.20 g/100 g), low crude fiber (0.85 g/100 g), high reducing sugar (10.5 – 21.3%), high total sugars (10.5 – 21.3%), high soluble solids (14.2 – 26.5%) and high ascorbic acid (15.8-25.1%). Mango fruits heavy metal concentrations were very low indicating insignificant pollution of the fruits (Othman and Mbogo, 2009). It is a good source of beta-carotene which is precursor of vitamin A. Vitamin A deficiency has been known as a public health problem in more than half of all countries, especially in Africa and South-East Asia (WHO, 2006). Regarding Vitamin A deficiencies children and pregnant women are the major risk groups. Mango deliver a throng of nutrients and make healthy

eating with delightful sensory experience and a reduced risk of some types of cancer, protecting against heart disease and cholesterol build up (Gupta *et al.*, 2014). In addition, to its food value, the mango has been used for medicinal values, in Samoa; a brake infusion has been a traditional remedy for mouth infection in children (Jekayinfa and Durowoju, 2005).

Commercial sugar as a source of sucrose is an important component in the beverages. It has a sweet taste and preservation effect on microorganisms (Saddler and Stow, 2012). In beverages, sugar contributes to the mouth feel of the product by giving the good taste and texturising ingredients, such as pectin added, to slightly increase the viscosity of the product (Rachel, 2013). Sugar solution is added to gain multiple benefits of sucrose in terms of masking undesirable flavor as a source of energy, improving the rheological property of the beverages and it has great role in control of microbial growth through modifying water activity of the beverage (Miele, 2013).

Soy milk based beverage of mango nectar, sucrose solution with soy milk improves the economical, nutritional as well as sensorial value of the beverages. Furthermore, the beverage would offer several distinct nutritional advantages over the plain fruit beverage to the consumer. Perhaps the most important advantage of blending lies in its ability to utilize ingredient components to undesired sensory attributes, value adding to the beverage and reduces postharvest loss of mango fruit due to its perishable nature (Bhardwaj and Mukherjee, 2011).

Soy milk as a base for production of beverages remained disadvantage of commercial exploitation because of its low acceptability associated with unpleasant beany flavor, astringent and bitter taste. For instance, organized self-help groups of women in Jimma town are producing soy milk, but their market supply is hindered by beany flavor, astringent and bitter taste. This practice has not yet solved the sensory acceptability of soy milk by adding cinnamon and salt. Furthermore, due to new nature of the product to the local market and unusual flavor and taste the market penetration rate of their product is not as planned. In addition to these factors, information regarding nutritional contents and microbial safety of

their product need further investigation to make their production and distribution process more scientific and sustainable.

Furthermore, in Ethiopia soymilk based beverage, do not have large market share due to lack of sensory acceptability in value addition and fortification or supplementing the product with some essential sweetness, vitamin and protein sources. The market demand for high quality and value added beverages for children, pregnant and lactating women and elderly groups would be high. Moreover, soymilk based products will serve as the main protein source for economically disabled community groups who could not afford animal based products. In view of bridging up the above gaps this study was initiated to address the following objectives.

General objective

To optimize better acceptability and nutritional quality of soymilk based beverage product.

Specific objectives

- I. To assess blended components of beverage with optimal physical properties.
- II. To assess blended components of a beverage with optimal chemical properties.
- III. To assess blended components of a beverage with optimal sensory quality.
- IV. To determine microbial quality and shelf life stability of selected treatment combination under different storage condition.

2. LITERATURE REVIEW

2.1. Production, Processing and Trends in Utilization of Soybean, Mango and Sugar

2.1.1. Soybean and soymilk

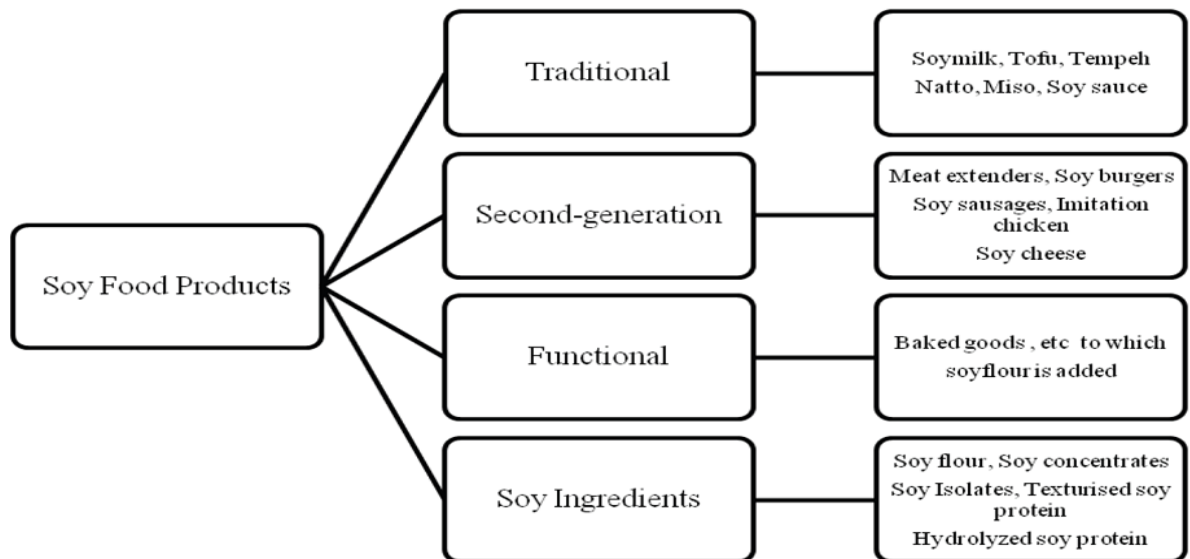
Soybean (*Glycine max* L) originated in northern China (Shurtleff and Aoyagi, 2007). It grows in tropical, subtropical and temperate climates (CGIAR, 2005). Global production of soybeans more than doubled in the period 1995-2011 to a new record volume of 263.8 million tons (Priester *et al.*, 2012).

According to Bower, (2007) soybean cultivation reached Africa in the late 1800s, although little is known of the countries to which it was first introduced. Soybean is cultivated in sub-Saharan Africa to a very limited extent (Shurtleff and Aoyagi, 2007). About Twenty-two African countries including Ethiopia are producing soybean with Nigeria, South Africa, Uganda, Zimbabwe and Malawi being the five leading soybean producers in 2006-2008 (Hailu,2010). Overall, about 19 African countries appear in the world soybean production statistics (FAO, 2008).

Soybeans was first grown in Ethiopia in 1950 (Shurtleff and Aoyagi, 2009). The crop production trend in the country showed dramatic increment between 2009/10 and 2011/12 harvesting seasons. It has been increased from 7,204.96 tons in 2009/10 to 35,880.29 tons in 2011/12 (CSA, 2012) at private peasant holdings. The major soybean producing region in Ethiopia are “Oromia (Bako, Jimma and Ambo)”, Awassa, and “Amhara” regional states. In addition, soybean seeds were grown in Pawe, Binishangul-Gumuzregion; during settlement programs in 1984 and other similar areas. Improved varieties growing in Ethiopia such as Awasa 95, Crown ford, Williams, Davis, Coker-240, and Clark-63 k, Jalale, Chere, TGX-13-3-644 and Belesa-95 (Zerihun *et al.*,2013). It has Clark 63K variety is larger size beans and produces more. It is being widely cultivated and distributed in Jimma areas and more recently in southern regions of the Gurage Zone (Fouzia, 2009).

The major processing steps of soybean products are cleaning, soaking, de-husking, milling, sieving, boiling, roasting and fermentation. Further processing depends on the type of products to be produced. Processed soybeans have been widely used in many years and countries for source of energy and protein (Kaboré *et al.*, 2011).

Food processing of products too complex from a combination of soybean and cereals or tubers to increase protein content and complement the amino acids profile in the food has been described in Nigeria by various authors including for baby foods and for breakfast cereal and for soy cassava foods. Soybean used to be made many valuable foods such as soymilk, flour, tofu, tempeh, taste soy, soy sauce, oil and margarine. These foods form an integral part of daily Asian cuisine are fast gaining popularity in western countries mainly due to their acclaimed health benefits (Lokuruka, 2010). Similarly, the utilization of soybean food products in Africa is rapidly gaining momentum. In Nigeria the private sector is playing major role in the market for soybean and soy products (Kolapo, 2011). Foods made from soybean may be divided into four classes namely soy ingredients, traditional soy foods, second generation soy foods and foods where soy is used as a functional and ingredients showed in Figure 1 (US, 2008).



Sources:-US Soybean Export Council, 2008.

Figure 1: Categories of soy food products

Soybean is in other utilization options include:- (i) weaning food, (ii) roasted soybeans (ingredients in traditional confectionery products and snacks), (iii) immature whole green soybeans (as vegetable), (iv) germinated soybean and soybean sprouts (as vegetable), (v) dehulled whole beans full fat flour, (vi) very finely ground full fat flour (as spray-dried milk alternative), (vii) oil source (shortening, margarine, cooking oil and salad dressing), paint, varnishes, printing inks, lecithin (Ahima, 2011), oil highly digestible, and (viii) soy meal for animals (soybean cake) (Bebeley ,2013). They are also an excellent source of good-quality protein, comparable to other protein foods and suitable for all ages group (Preeti *et al.*, 2007).

Soybean plant is so adaptable that virtually every part has good uses. It has been used as a human food, animal feed and industrial raw materials. As one of the world's major and fastest expanding crops, soybean contributes significantly to overall human nutrition in terms of both calorie and protein intake (FAO, 2004).

Soybean is emerging as a very important food, market and oil crop in Ethiopia. In addition to the effort made at factory level, efforts have been made in production and utilization of the crop in Ethiopia. Work on the use of defatted soy flour in traditional recipes began in about 1969 at Ethiopian Nutritional Institution, food science and technology department and the major user of the soybean in the country following soybean introduction in to Ethiopia. Traditional foods that were made with soybeans by Ethiopia Nutrition Institute were *Injera* fermented flat pancake-like bread a staple food of the highland Ethiopians, *Wots* and *Alliches* (sauces served with *Injera*), *Kitta* (unleavened bread), commonly eaten by lowland and highland Ethiopians, *Dabbo* (leavened bread), *Dabbokolo* (a 'snack food') and porridge. The recipes made from these traditional foods were reported to be highly acceptable, and efforts were made to promote their consumption by the local people. Added value soy products including soymilk, tofu and other were prepared at Pawe area for famine relief purposes. Modern soybean processing machineries were also installed by Tanana Beless project in order to support the society to combat protein energy malnutrition disease. Currently, the machineries are taken away by Faffa food share company and reinstalled in Addis Ababa so as to produce soybean based processed food products predominantly soymilk (Assefa,2008).

Soy milk is an inexpensive source of protein and calories for solving malnutrition problems in developing countries like Nigeria (Anuonye, 2011). The soy milk is used in various products like paneer, yogurt, cheese, tea and coffee whiteners, shrikh, rasogolla and various indigenous milk sweets, and confectionary (Gatade *et al.*, 2009). It is made up of various components, including lipids (such as triglycerides and phospholipids), proteins, complex carbohydrates, minerals and phytates (Malaki *et al.*, 2008).

Soy milk may contain higher levels of Na, Mn, Fe, and K (Ilana *et al.*, 2009). Rich mineral composition of soya extract, particularly calcium and iron salts, makes it a healthy product for the people suffering from cardiovascular diseases, osteoporosis, nervous system disorder and anemia. Epidemiological data have indicated that Asian populations consuming soy as a dietary staple have a lower risk for cardiovascular diseases than those consuming a western diet (Levi *et al.*, 2002). It is nutritious economic drink that attracts special attention for overcoming the problem of lack of milk in developing countries, in addition to independent use; it can be used as a supplement and ice cream (Bisla *et al.*, 2012).

2.1.2. Mango and Mango nectar

Mango is indigenous to the Indian subcontinent and Southeast Asia (Fowomola, 2010). Cultivated in many tropical regions and distributed widely in the world. Asia accounts for approximately 77% of global mango production, the Americas and Africa account for approximately 13% and 9% respectively (Evans, 2008). Mango fruits are one of the most popular fruits produced in tropical region of the world (Jasim *et al.*, 2005). The world production of mango fruits reported more than 35 million tons per year (FAO, 2012).

The amount of mango production in Africa during 2009 is 13.6 million tons (FAO, 2009) while, in Ethiopia, 97.32 thousand tons of mango fruits were produced in 2011 and mango fruits more available in Jimma area. The mango fruits are produced in West and East of Oromia, SNNPR, Benshangul and Amhara regions (Honja, 2014). Ethiopian government has a plan to expand mango production by distributing high yielding varieties for small scale farmers, especially in the Southern and Oromia region, by grafting mangos of known and

high yielding varieties. Mango fruit is high post harvest losses due to different factors (Snowdon, 2010). The exotic *Tommy Atkins* mango varieties are more suitable for different from other mango fruits by their physico-chemical characteristics and the technological qualities of their processed products (Sun-Waterhouse, 2011).

Mangoes are an important export fruit for many tropical and sub-tropical countries (Arauz, 2000). It has a source of income to farmers, traders and countries at large through their local and international markets (Rodriguez *et al.*, 2012). Due to its used vibrant colors, exotic flavors, distinctive taste, nutritional properties, and also used as food in all group age of its development (Singh *et al.*, 2013). Mango plays an important role in balancing the diet of human being by providing 64-86 calories of energy.

Mango fruits during early stages of growth are commonly used for sweet and a large number of products are prepared from ripe mango fruit. The ripened fruit is used for preparing nectar, squashes, jam, jellies, sorbets, milk shakes, mango leather and powder, sweet meat, various dehydrated products, ready-to-serve beverages and in addition canned frozen slices, purée and juice (Kittiphoom, 2012).

Ripe mango fruit is used for preparing nectar. Mango nectar is considered an excellent source of several important vitamins A, C and B complex, iron and phosphorus. It is yellow-orange in color and can be consumed by itself or mixed with other ingredients. It contains high amounts of β -carotene, a well-known carotenoid, which is responsible for the typical yellow color of the mangoes. Moreover, β -carotene is very beneficial for human consumption, as it is a provitamin A and antioxidant (Tanumihardjo *et al.*, 2010). Carotenoid is responsible for the bright yellow color of mango and are important lipophilic radical scavengers found in many fruits and vegetables (Dembitsky *et al.*, 2011).

Mango nectar beverage has a high content of fruit ingredients, sugar and acid. The production of fruit nectar has great importance in fruit juice industry. Fruit nectars can also be made from mature fruits, which cannot be used satisfactorily in standard processing (Reddy *et al.*, 2012). Mango nectars are playing a number of important roles in human health

(Tahir *et al.*, 2012). It has been reported that the consumption of mango can be linked to the prevention of cardiovascular diseases and cancer (Santhirasegaram *et al.*, 2013). It also provide foliate and potassium that are known to prevent birth defects, cancer, heart disease, hypertension and stroke and in addition rich in provitamin A (Neidhart *et al.*, 2006).

Mango nectar is regard as a valuable source of physiochemical compounds among these compounds polyphenolics are widely distributed secondary metabolites that serve as the predominant antioxidants present (Johnson, 2013). Mango juice and nectar have physical properties, such as density, specific heat and coefficient of thermal expansion, affected by their solid content and their temperature. Hence, utilization of mango nectar in soymilk based beverage can be taken as promising strategy to tackle vitamin A deficiency with a high prevalence rate among infants and young children. Vitamin A is present in the form of pro-vitamin A in plants. The most important carotenoid which is precursors of vitamin A in human beings is β -carotene, α -carotene and β - cryptoxanthin (Yahia and Ornelas, 2010). B-carotene was described as an antioxidant that muscular degeneration, Ageing and several chronic diseases in humans (Susan, 1996).

2.1.3. Sucrose (table sugar)

Sugars are categorized as carbohydrates, which are a group of compounds made up of carbon, hydrogen and oxygen. The world produced about 168 million tons of sugar in 2011. The most important sugar producing regions in Africa are north and southern regions (Sultan, 2008). It is manufacture from sugar cane or beetroot and is available in crystalline or liquid form.

Sugars are a ubiquitous component of our food supply and are consumed as a naturally occurring component of many foods and as additions to foods during processing, preparation, or at the table (Murphy and Johnson, 2003). It is added as a sweetener according to preference and regard as the ‘gold’ standard for sweet taste (Adejuyitan *et al.*, 2008). It is given an arbitrary sweetness level of one to allow its comparison with other products (Ferrari, 2011). The sweet taste is likely to improve palatability and thereby the acceptability of the food. Adding sugar may therefore help to an improved taste, an effect that may be

especially important in situations with bulky foods and poor appetite. Sugar provides a good source of calories. They act as preservatives and maintain desirable appearance, flavor, color and body in the products. However, when used for a longer period of time, there is a risk of reinforcing a preference for sweet foods and hence too high energy intake, also later in life (Bloch,2004).

Sugar has used in preparing all types of drinks such as coffee, tea, soft drinks, juices; etc. Sugar only contributes for energy and do not contain other nutrients like vitamins or minerals. The energy content of sugar is less than half of the fat. By adding sugar to foods the energy density increases. The higher energy density is likely to have a positive influence on energy intake, an effect that is worrying in high-income societies with a growing prevalence of obesity. In treatment of children with wasting who have an increased energy need, this is positive. However, if high sugar content is used in diets for children with moderate stunting who need treatment over a long period; this may impose a risk of overweight. Another important aspect of added sugar is how it affects taste.

Sugar has natural preserving properties. It inhibits the growth of micro-organisms at increased concentrations. In products such as sugar binds with water causing the water activity to fall below 0.85. This inhibits most microbial growth except yeasts and moulds (CCFRA, 2004).

2.2. Effect of processing on sensory attribute, anti-nutritional factors (ANFs) and nutrient composition of soymilk

The basic steps of preparation of soymilk include selection of soybeans, adding water, wet milling and separation of soymilk from fiber (okara), cooking to inactivate lipoxygenase and trypsin inhibitors, formulation and fortification, and packaging of the soymilk (Gandhi, 2009). Soy milk processors often want larger seeds because of easily process and desirable to minimize the amount of fiber. Larger seeds have fewer hulls and therefore less fiber can be obtained (Fouzia, 2009).

Soy milk can be made at home with traditional kitchen tool method and other with soy milk extraction machine of Illinois and Cornell methods. These methods were aimed at improving the acceptability of soy milk in terms of odor and flavor. Many processing methods have been developed for the production of good quality tasting, sweet or flavored and improve the nutritive utilization of protein of soy milk. For the sensory evaluation and anti-nutrition of soy milk indicated that soy milk Illinois method was preferred to Cornell method, while traditional method of soy milk was least preferred (Onuora *et al.*, 2007).

Upon chemical treatments, undesirable characteristic of soybean was reduced. The NaHCO_3 group would offer better advantage of easy processing of soybeans due to its effectiveness in inducing soft texture of the beans, and reducing/destroying anti-nutritional factors known to produce a highly objectionable beany or oxidized odor and flavor acceptable only to the oriental culture that have developed tolerance for it (Saidu, 2005). Blanching of soybeans for a 30 min in 0.5% NaHCO_3 following an overnight soaking result in a beverage completely free of any trypsin inhibitor (Raghuvanshi and Bisht, 2010).

Soy milk prepared from beans is heated and processes involve several stages during soy milk preparation, including the pre-treatment of beans and extraction to produce the soy milk, followed by either pasteurization or sterilization to increase its shelf life. By controlling the microbiology of the product and packaging it in appropriate containers, the shelf life of soy milk can be greatly extended and the product can be distributed over a wider area (Odu *et al.*, 2012). The soy milk must be heat treated, to ensure its safety and nutritional quality. Heating inactivates anti-nutritional factors such as trypsin inhibitors and lipoxygenases, which contribute to the beany flavor and overall increases shelf-life, physical appearance and microbial quality of the soy milk. Heating may be initiated either before or after grinding and temperatures around the boiling point are generally held for 15-20 minutes (Alexandra, 2012).

According to Dourado *et al.* (2011), anti-nutritional factor whole substance is synthesized by normal plant metabolism, which may result from different mechanisms to reduce the efficiency of utilization of the diet. Most ANFs are heat-labile, such as α -galactosides,

protease inhibitors and lectins, so cooking would eliminate any potential effects before consumption (Muzquiz and Wood, 2007). Hence, processing methods such as soaking, germination, fermentation, cooking and addition of enzymes have been reported by many researchers to alleviate the effect of ANFs and to improve the nutritional value of cereals and legume seeds (Sokrab *et al.*, 2012). Mainly, cooking and germination play an important role as they influence the bio-availability and utilization of nutrients and also improve palatability which incidentally may result in enhancing the digestibility and nutritive value (Ramakrishna *et al.*, 2006). Therefore, many food processing and preparation techniques are the main efforts made to decrease the amount of phytates in foods (Francis *et al.*, 2012).

2.3. Importance of making soy milk based beverages

Soy-based beverages are presented as healthy food alternative for human nutrition (Northon *et al.*, 2011). In Brazil, the recent growth in the non-alcoholic beverages market reported that includes bottled mineral water, soft drinks, fruit juices and soybean-based beverages is related to the fear of excessive obesity and health problems (Drunkler *et al.*, 2012). It is becoming more accepted because of their nutritional and potential health benefits. Irrespective of inherent processing hurdles, the advanced techniques in processing of soybean recognized its nutraceutical importance for prevention and treatment of certain chronic diseases, including cancers, atherosclerosis, osteoporosis, and kidney disease (Kotilainen *et al.*, 2006).

Soybean based beverages present not only the already well studied organic compounds known for their health benefits, but also, after fortification, have significant amounts of iron which are very important to maintain good bodily functions and aiming to produce healthier beverages, are more appealing to their consumers (Clark and Beale, 2010). The milk prepared from the blend of tiger nut and soybean could be used as a beverage for both the younger and older people due to the high nutrient contents such as protein and fat (Udeozor and Linda, 2012). According to Sowonola *et al.* (2005) kundu-soymilk blends could be used as a beverage for the adults and a weaning drink for children.

Soy milk based beverage is plants parts have constituted the main raw materials in non alcoholic beverages. Non-alcoholic beverages play a very important role in the dietary pattern of people in developing countries. They are regarded as after meal drinks or refreshing drinks during the dry season in rural and urban areas. The non-alcoholic nature of these beverages makes them to be readily consumed by use of Christians and Muslims as a substitute for alcoholic ones. Mango fruits have constituted the main raw materials in non alcoholic. Native cassava starch was successfully employed without any negative effects in the preparation of soy-based beverage (Northon *et al.*, 2011).

2.4. Quality of soy milk based beverages

2.4. 1. Physico-chemical property of soy milk based beverages

The orange soy milk ready to serve beverage prepared from 80% orange juice blended in soy milk was found to be best according to physico- chemical properties (Kale *et al.*, 2011). Results showed that with the increase in juice concentration percent of acidity and TSS increased while pH and fat content reduced. The increase in the soy milk amount of treatment combination in the kunnu-zaki of specific gravity decreased (Aminat *et al.*, 2013).

Chemical properties of blended beverage have direct effect on ultimate quality and storage stability of ready-to-serve beverages. In addition, the analysis values of all the fortified soy milk were improved in both quantity and quality of chemical properties (Harun *et al.*, 2011).

pH of foods is one of the most important chemical properties that determine acceptability and shelf life of blended beverages. The pH of beverage could be correlated inversely with the acidity of product and found to decrease with increase in storage (Pawar *et al.*, 2011).

Titrateable acidity is also another chemical characteristic of beverages that determine the taste and flavor of beverage products. The acidity profile should also be optimizing in combination with the carbonation level, flavor type and flavor intensity. The measurement of total titrateable acidity blended beverage and juice is required by all producers as a key quality

control indicator. Soymilk flavored with mango, guava, banana and unflavored samples were the pH and titratable acidity values were most acceptable (Rozina, 2012).

The proximate analysis values of all the fortified soymilk is improved in both quantity and quality. In addition soymilk based beverage with papaya and mango pulps are more carbohydrate and low moisture content than soymilk. Soymilk based beverage with mango pulps are moderate ash content (Geovana *et al.*, 2014). Most studies on soymilk tend to deal with blending of different fruits to increase the different nutrient (Farinde *et al.*, 2010).

Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body (Eruvbetine, 2003). Iron functions as hemoglobin in the transport of oxygen (Soetan *et al.*, 2010). Calcium is also a mineral required by the body for a variety of physiological functions and the maintenance of bone tissues throughout life (Delvalle *et al.*, 2011).

Soybean based beverages present not only recent times the already well studied organic compounds known for their health benefits, but also, after fortification, have significant amounts of iron which are very important to maintain good bodily functions and aiming to produce healthier beverages, are more appealing to their consumers (Luciula *et al.*, 2012). The iron and magnesium content of the blends increased significantly with increased addition of soymilk to kunnu. Generally the mineral content of the blends increased significantly with an increase in soymilk added to the kunnu (Sowonola *et al.*, 2005).

2.4.2. Sensory quality of soymilk based beverages

Sensory analysis is an important step in global product quality. They are the main criterion that makes the product to be liked or disliked (Falola *et al.*, 2011). Sensory evaluation is a critical stage in product development and product optimization studies, because products targeted at consumers must first appeal sensorial to them (Kpodo *et al.*, 2014). The sensory quality profile of beverage is a prime factor to consider the marketability of product (Jakhar and Pathak, 2012). Sensory tests are used for grading and pricing of products and in more

basic research to determine, exactly what chemical changes in a food affect its flavor (Drake, 2009).

Soy milk off-flavor enhanced by the ratio of soy milk in blended beverage, which decreased the total acceptability of the product (Pooneh *et al.*, 2013). The most important quality attributes of any foods to the consumer are its sensory characteristics such as texture, flavor, aroma, shape, mouth feel and color. These determine an individual's preference for specific products, and small differences between brands of similar products can have substantial influence on acceptability. Therefore, during processing great care must be taken to retain or enhance these properties. Mouth feel is the major determinants for consumer's acceptance and preference for foods (Lesschaeve and Noble, 2005).

The majority of soy milk manufacturers in the US report that the addition of flavor, and mouth-feel modifying/improving agents such as sugar, cocoa powder, vanilla flavor, malt extract, gums, etc. to improve its overall appeal. The development of soy milk formulations by adding adequate types and amounts of sweeteners, flavorings, and other products according to local market preferences is a way of increasing its acceptance (Souza and Menezes, 2008).

2.4.3. Microbial quality of soy milk based beverage

Soy milk is imitation milk similar in composition with animal milk. Its rich nutrient and moderate pH makes it an excellent culture media for the growth of microorganisms especially bacteria. Hence, the more the soy milk was available, the higher bacterial contamination. Milk products are, however, easily perishable because contaminating bacteria may multiply rapidly and render it unfit for human consumption (Ukwuru *et al.*, 2011).

Microorganisms, which are known spoilage agents of soy milk, can be controlled using a combination of preservative, pasteurization and refrigeration (Momoh *et al.*, 2011). Shelf life at ambient temperatures for all the beverages was between 1 to 2 days (Bola and Aboaba, 2004). In addition other studies indicated that the soy milk storage were shelf stable up to 2 days at room temperature (Zamal *et al.*, 2011). The use of aqueous extracts of clove guinea

pepper in combination coupled with refrigeration can appreciably prolong the shelf life of soymilk. Hence, the extracts of these spices represent an alternative source of natural antimicrobial substances for use in food systems to prevent the growth of food borne microorganisms and extend the shelf-life of the processed food (Kabiru *et al.*, 2012). Soy-Tiger Nut two blends of the milk beverage having the highest mean score on general acceptability was subjected to three storage conditions (ambient, refrigeration and freezing conditions) for four weeks. The 10:90% and 20:80% ratio soy-tiger nut milk blends were the most preferred, when compared the mean scores of the general acceptability of the sample blends. The results obtained from the effect of storage condition on the milk beverage blends showed that samples stored at freezing and refrigeration temperature condition has minimal total aerobic plate counts than those at ambient condition (Okorie *et al.*, 2014).

2.5. Optimization of soymilk based beverage

Optimization is the process of deriving the best formulations amongst several ratio mixes (Prinyawiwatkul *et al.*, 1997). The optimized beverage was characterized for its physicochemical properties, proximate composition, anthocyanins, total phenolic content and isoflavones (Murevanhema,2012). The optimized proportions of the ingredients of the product consisted of 54.0-58.5% soybeans, 37.0-42.0% peanut and 4.46 - 4.48% chocolate and had an energy value of 124.103kJ/100g. Proximate analysis of the optimized products indicated that the beverage has a protein content of 2.77%, fat content of 1.38%, carbohydrate content of 1.26%, ash content of 0.32% and water content of 94.27%. This suggests that production of an acceptable full fat soy-peanut, chocolate-flavored milk beverage is feasible through the optimization of the basic ingredients (Henry *et al.*, 2014).

3. MATERIALS AND METHODS

3.1 Description of the experimental area

The laboratory based experiment was conducted at JUCAVM in Post-harvest Management, Animal Nutrition and Plant Pathology laboratories; Mineral analysis was conducted at Food and Nutrition laboratory in Ethiopia Public Health Research Institution (EPHRI), Addis Ababa. Ambient temperature and relative humidity in JUCAVM and EPHRI were $23^{\circ}\text{C}\pm 2$ and $22^{\circ}\text{C}\pm 2$, and 47-53% and 55-58% respectively.

3.2 Experimental material

Soybean “clark 63k” variety was collected from Jimma Agricultural Research Center, freshly harvested ripe mango fruit of “Tommy Atkins” variety was obtained from Melkasa Agricultural Research Center. Commercial sugar was purchased from local market for preparation of sucrose solution.

3.3. Sample preparation

3.3.1. Preparation of soymilk

Extraction of soymilk was done according to Illinois method as indicated in Onuorah *et al.* (2007) with minor modifications of time and temperature in (Figure 2). One kg dry and clean *Clark 63k* variety soybean seed was weighed and soaked in 0.5% NaHCO_3 solution (1:3 gram/ml) for 12 hr (Belewu and Belewu, 2007). The soybean husk was removed by hand rubbing, the soaked water was drained and rinsed more than two times with clean water and blanched for 10 minutes at 100°C in 0.5% NaHCO_3 solution. Blanched sample was blended by using electric blender machine (Model of 801DEG HGBTWTG4, China) with water by keeping soybean to water ratio of 1:4 w/v for five minutes a high speed to extract the soymilk at room temperature (25°C) (Susu *et al.*, 2013). Eventually the milk was collected by filtering the slurry using sterilized double layer of cheesecloth, and filled into pre-sterilized glass bottles, packed and stored (4°C) till further use.

3.3.2. Preparation of mango nectar

Ripened Tommy Atkins mango fruits were washed, peeled, seed removed and the pulp was cut into thin slices (Figure 2). The slices were mixed with clean water in the ratio of 1:3, gram/ml (Fengxia *et al.*, 2014) and blended for two minutes at high speed by using electric blender machine used for soymilk extraction (Elbandy *et al.*, 2014). Mango nectar was collected by filtering the slurry using sterilized double layer of cheesecloth, filled into sterilized glass bottles, packed and stored at 4°C till further use.

3.3.3. Preparation of sucrose solution

As a third component for the blends sucrose solution was prepared using commercial table sugars and water in the ratio of 1:5 gram/ml blended by using blender machine for five minutes at low speed as shown in Figure 2 (Marla *et al.*, 2012). Sucrose solution was also filtered using sterilized double layer of cheesecloth to get free from impurities and filled into sterilized glass bottles, packed and stored at 4°C till further use.

3. 4. Experimental design of Soymilk Based Beverage (SBB)

Fourteen soymilk based beverage formulations were prepared containing three basic ingredients soymilk, mango nectar and sucrose solution as shown in Table 2. The proportions of these ingredients were obtained using a three component, constrained mixture design (Kpodo *et al.*, 2013). D-optimal mixture simplex lattice design was used to obtain a total of 14 design points from the three ingredients, which were obtained by considering range of 60-100% soymilk, 0-25% mango nectar and 0-15% sucrose solution using Minitab statistic software program (Table 1). Soymilk based beverage of the three basic ingredients were created different ratio by using Design-Expert ®, version 6.02 (Minnneapolis MN,USA).The range of soymilk, mango nectar and sucrose solution ingredients were determined based on the preliminary work. The design expert matrix plot for the three-component blended beverage (soymilk, mango nectar and sucrose solution) was shown in Figure 3 experimental point and experimental region. In this thesis, a multi-mix was including three basic

ingredients such as sweet source from sugar, a protein source from soybean and a vitamin and good sensory source from mango.

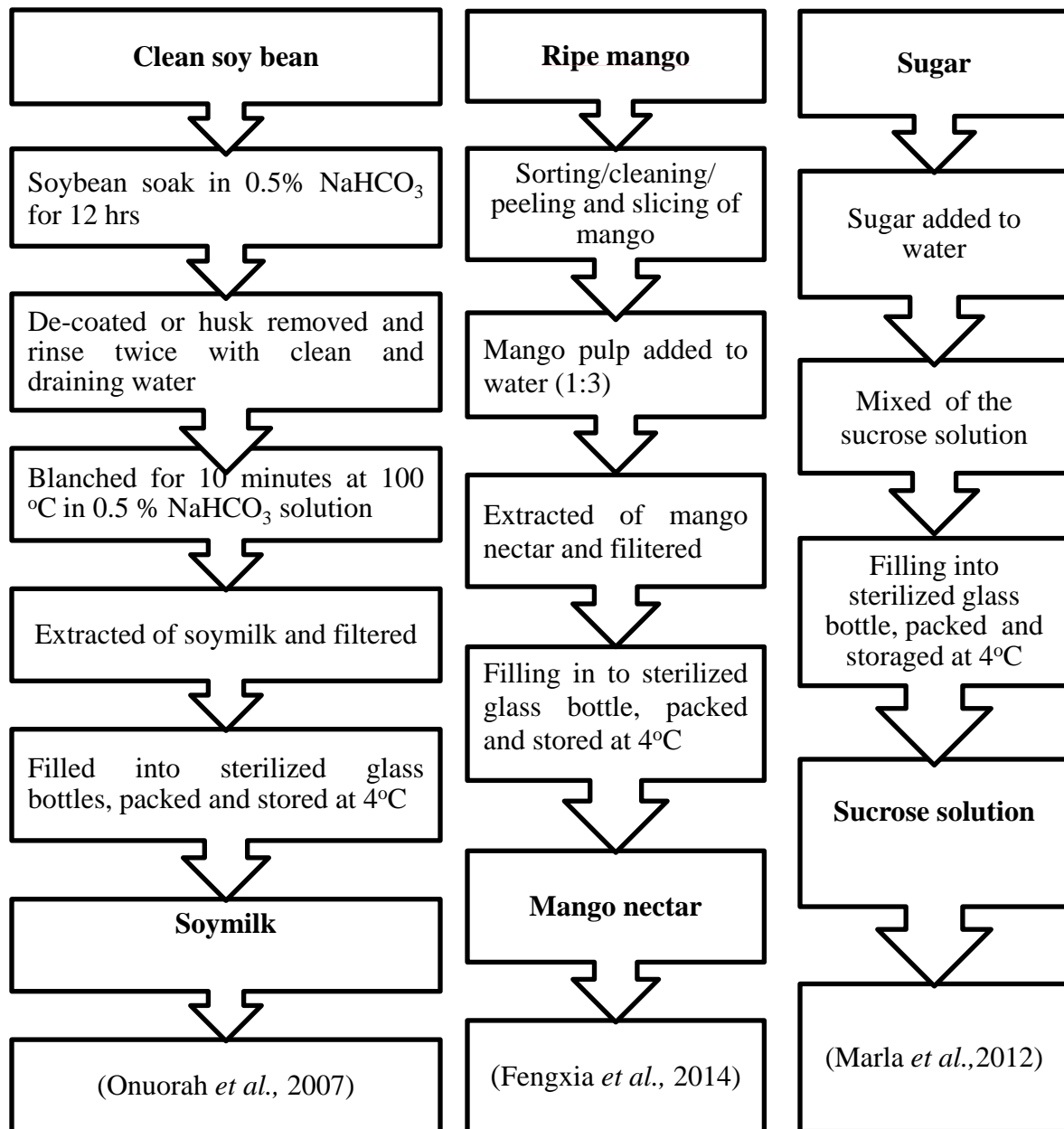


Figure 2:Flow chart of the each ingredient beverage.

Table 1: Coded lower and upper limit of each ingredient

Ingredients	Lower limit	Upper limit
Soy milk	60	100
Mango nectar	0	25
Sucrose solution	0	15

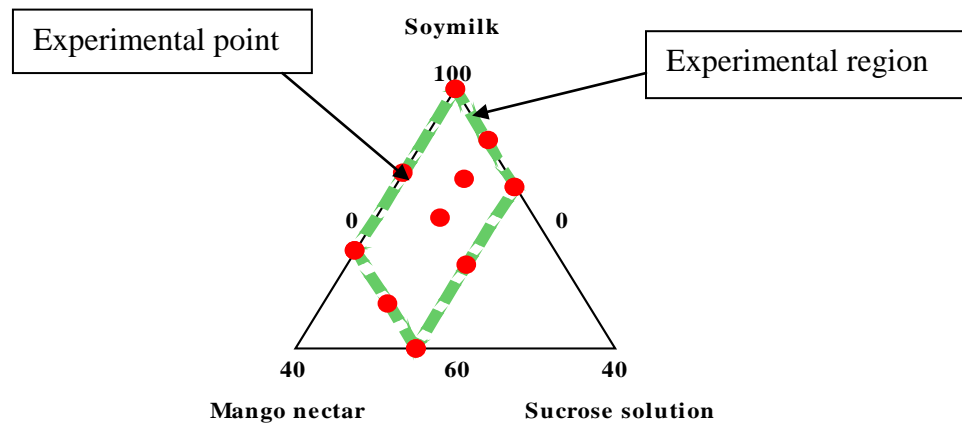


Figure 3: Simplex design plot for the three ingredients in treatment combinations of soymilk based beverage

Table 2: Proportion of different ingredients in preparation of soymilk based beverage

Run order	Soy milk (%)	Mango nectar (%)	Sucrose solution (%)
1	80	12	8
2	85	0	15
3	75	25	0
4	60	25	15
5	85	0	15
6	67	25	8
7	75	25	0
8	100	0	0

9	86	6	8
10	73	12	15
11	100	0	0
12	92	0	8
13	60	25	15
14	87	13	0

3.5. Preparation of soymilk based beverage (SBB)

The soymilk based beverages were blended according to different ratios (Table 2). Required ratios of the beverage were blended using blender machine (Model of 801DEG HGBTWTG4, China) for two minute at high speed shown in Figure 4 (Kale *et al.*, 2011). Blended samples were filled in sterilized rainy glass bottles and pasteurized for 30 minutes at $71\pm 2^{\circ}\text{C}$ in temperature controlled water bath (WB -8B Numerical Electrical Constant Temperature Water Bath Boiling, China) (Kabiru *et al.*, 2012). After pasteurization samples were kept at 4°C for further analysis. Each sample was prepared in replication of three for all parameter analysis. The best treatment combination was selected to the study microbial quality and shelf life stability of the beverage.

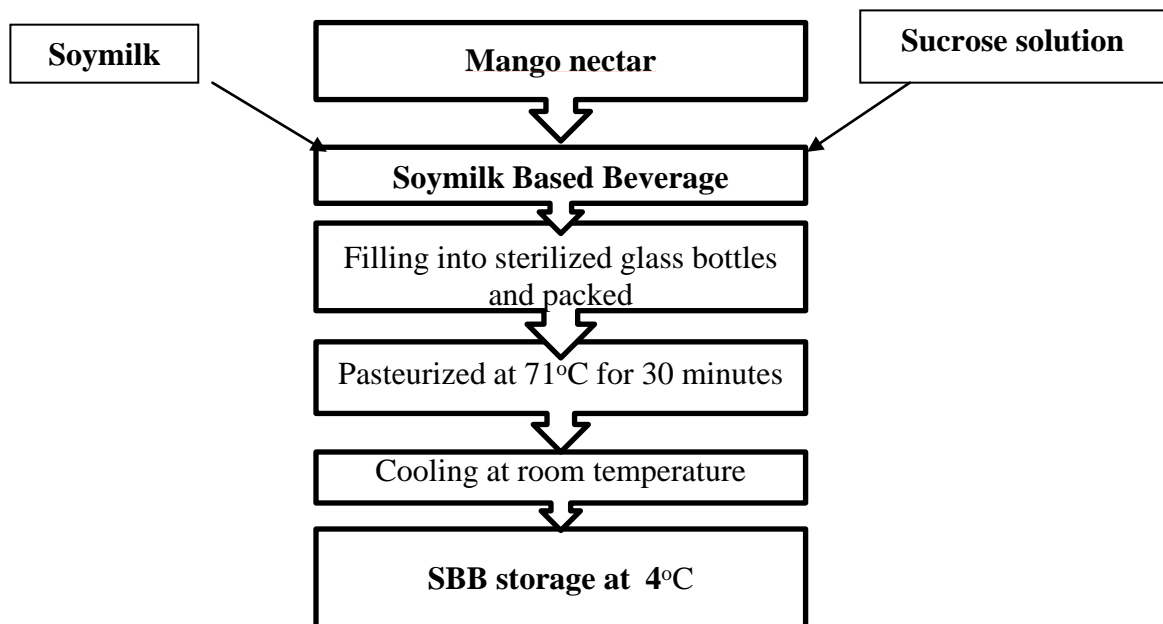


Figure 4: Flow chart of the soymilk based beverage production.

3.6. Data collected

Different response variables analysis such as physical properties, chemical properties and sensory evaluation of soymilk-based beverages were collected at different levels of the experiment. The separate analysis and determination procedures for every parameter are described below.

3.6.1. Physical properties of soymilk based beverage

3.6.1.1. Determination of specific gravity

The specific gravity of each sample was determined using pycnometer the ratio of the mass of a given sample to the mass of pure water according to equation given below (Aminat *et al.*, 2013).

$$\text{Specific gravity (g / ml)} = \frac{\text{Mass of a given sample (g)}}{\text{Mass of pure water (ml)}} \dots\dots\dots \text{Eq.1}$$

3.6.1.2. Determination of Total Soluble Solids (TSS)

Total soluble solids level of each sample was determined according to AOAC (1984) method using hand held digital refractometer (Model DR201-95, Germany).

3.6.1.3. Determination of Total color change (ΔE)

The total color change of each sample was measured using a color chromameter (Model of Accu-probe HH06M, U.S.A). Color measurement was recorded using Hunter L*, a* and b* scale (Hunter, 1996). The "L*" coordinate was a measure of lightness (white – black) and ranges from no reflection (L=0) to perfect diffused reflection (L=100), "a*" scale ranges from negative values for green to positive values for red and "b*" scale ranges from negative values for blue to positive values for yellow. Total color change (ΔE) was calculated in order to evaluate the change in color between the soymilk and soymilk based beverage samples using equation two.

$$\Delta E = \sqrt{(L_o - L^*)^2 + (a_o - a^*)^2 + (b_o - b^*)^2} \dots\dots\dots Eq.2$$

Where, ΔE = total color change, L_o, a_o, b_o are colour parameters of soymilk beverage samples, L^*, a^*, b^* colour parameters of soymilk based beverage samples.

3.6.2. Chemical properties of soymilk based beverage

The proximate analysis of the each samples were determined using wet basis as described by AOAC (2000). All chemicals and reagents used were of analytical grade.

3.6.2.1. Determination of moisture content

The moisture contents of each sample was determined by hot air oven dry method according to AOAC (2000) 925.09. About 10 gram (M_i) of each sample was transferred to the petri-dish and then the petri-dish was placed in the oven at 105 °C for 6 hours dried in the hot air oven (Model: Leicester LE67 5FT, England), cooled in desiccators and reweighed (M_d) (Olusola and Sarah, 2014). Then moisture content was calculated as percent loss in weight using the equation three.

$$Moisture (\%) = \frac{(M_i - M_d)}{M_i} \times 100 \dots\dots\dots Eq.3$$

Where:-

M_i = intial mass and M_d = dried mass

3.6.2.2. Determination of ash content

The ash content of each sample was determined AOAC (2000) 923.03 method. The empty clean porcelain crucible with its lid was placed in the muffle furnace (Model SX-5-12, China) for 15 min and then transferred into desiccators for cooling. The mass of dried porcelain dish was measured (M_1) by analytical balance (Model:ABJ220-4M, WB1151070, Australia). Ten grams of each liquid sample was weighed (M_2) into dried crucible and a hot plate slowly increase the temperature until water fully removed and transferred the crucibles into a muffle furnace and set the temperature at 550 °C for 8 hrs. The samples were removed from the furnace and placed in the desiccators followed by the mass determination (M_3). Then ash content was calculated using equation four.

$$\text{Ash (\%)} = \frac{(M3 - M1)}{(M2 - M1)} \times 100 \dots\dots\dots \text{Eq.4}$$

Where:-

M1 = Mass of the crucible

M2 = Mass of fresh sample and crucible, and

M3 = Mass of ash and crucible

3.6.2.3. Determination of protein content

The protein content was determined based on standard AOAC (2000) method 979.09. One milliliter of each sample was added into kjeldahl digestion flask (Kjeldahl flask KF250, German) and then two grams of mixture of CuSO₄ and K₂SO₄ in (1:10 ratio) was added as catalyst to speed up digestion process. Following this five ml of 99% concentrated H₂SO₄ was added to sample flasks to initiate digestion. The mixtures were then manually shaken thoroughly to wet the sample with acid to ensure complete digestion. The flasks were then placed in digestion block and heated at 420°C for 4 hrs or until a clear colorless solution was left in the tube. The digest carefully removed and left under the fume hood for 30 minute to cool. The digested sample was then transferred into the distillation apparatus and 25ml of 40% (w/v) NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. The mixtures were then steam distilled and the liberated ammonia was collected into a 200ml conical flask containing 25ml of 4% boric acid mixed with methyl red indicator solution. Next distillation was carried out into the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the pink color solution of the receiver flask turned green indicating the presence of ammonia. Distillation was continued until the content of the flask was reaching the required amount. The green color solution was then titrated against 0.1N HCl solutions. At the end point, the green color turned to red pink color, which indicated that, all the nitrogen trapped as ammonium borate have been converted as ammonium chloride (AOAC, 1990). Eventually percentage nitrogen was calculated using equation five.

$$\text{Nitrogen (\%)} = \frac{(0.014 \times (V_s - V_b) \times M)}{W} \times 100 \dots\dots\dots \text{Eq.5}$$

Where:-

V_s = ml HCl titrant used for test portion

V_b = ml HCl titrant used for blank

M = molarities of HCl solution; and

W = test portion weight, g

$$\text{Crude protein (\%)} = 5.71 \times \% \text{ Nitrogen} \dots\dots\dots \text{Eq.6}$$

3.6.2.4. Determination of ether extracts

Ether extract is the term used to refer to the crude mixture of fat-soluble material present in a sample and determined by using soxhlet fat extraction unit (Model:SZC-C, China) according to AOAC (2000) method 45.01. The empty clean thimble and cotton were cleaned using petroleum ether and dried for 30min at 105°C in the hot air oven and cooled in desiccators and weighed (W). Then two gram of dried sample were added (W_1) into extraction flasks and were rinsed several times with petroleum ether. The sample contained in the thimble was extracted with the solvent in a soxhlet extraction apparatus for 6 hrs at a constant rate of 4-7 drops per second. After extraction each sample was transferred in to the oven for 30 minutes at 105°C. After the drying period is over the sample was removed from the oven and transferred into a desiccators. Finally the weight to the sample and container (W_2) were recorded and determined using equation seven.

$$EE (\%) = \frac{(W_1) - (W_2)}{W_1} \times 100 \dots\dots\dots \text{Eq.7}$$

Where:-

W_2 = weight of sample after extraction

W_1 = weight of sample before extraction

3.6.2.5. Determination of Carbohydrates

The total carbohydrate content was calculated based upon difference method. The mathematical expression: Total carbohydrate (%) = 100-(% P + % EE + % A + % M).The result of the subtraction was the % total carbohydrate content of each sample.

$$\text{Carbohydrate (\%)} = (100 - (\% P + \% EE + \% A + \% M)) \dots\dots\dots \text{Eq.8}$$

Where:-

P=Protein

EE=Ether extract

A=Ash

M=Moisture

3.6.2.6. Determination of gross energy

Gross energy (in Kcal) was calculated according to the method indicated in Osborne and Voogt (1978). The gross energy of each sample was calculated by multiplying each gram of protein, fat and carbohydrate obtained from laboratory analysis with their respective conversion factor as indicated in equation nine.

$$\text{Gross energy} = (\text{protein} \times 4 + (\text{carbohydrate} \times 4 + (\text{ether extract} \times 9))) \dots\dots\dots \text{Eq.9}$$

3.6.3. Other chemical properties of soymilk based beverage

3.6.3.1. Determination of pH

The pH value of each sample was determined by using digital pH meter (SET/HO11, Mauritius) in AOAC (2010) official method 981.12. The pH meter was then standardized (calibrated) using pH 4 and 7 buffer solution. The electrode of the pH meter was then washed with distilled water, blotted with tissue paper and dipped into each of beaker containing liquid samples to measure the pH value.

3.6.3.2. Determination of Titratable Acidity (TA)

TA was determined using conventional titration method. The titratable acidity content of each liquid sample was determined by taking 10 ml sample and titrated with 0.1N NaOH in the presence of 3-4 drops phenolphthalein indicator (Akhtar *et al.*, 2010). TA results were expressed as percentage citric acid, which is the main organic citric acid in mango fruit (Ueda *et al.*, 2000). Finally, titratable acidity was calculated as equation ten:

$$\% TA = \frac{(N * V1 * Eq.Wt)}{(V2 * 10)} \dots\dots\dots Eq.10$$

Where:-

TA= titratable acidity

N = Normality of titrate (NaOH) (mEq. /ml)

V1 = Volume of titrant used (ml)

Eq. Wt. = Equivalent weight of predominant acid (g)

V2 = Volume the sample (ml)

10= 1/10 is the factor relating mg/g (100/1000)

3.6.3.3. Determination of β -Carotene

β -carotene was determined according to the method of Sadler (1990). Two grams of each liquid sample was mixed with one gram of CaCl₂.2H₂O and 50 ml extraction solvent (50 % hexane, 25 % acetone, and 25 % ethanol) and shaken in five minute interval for 30 min at 4±1°C refrigeration. Again 15 ml of deionized water was added and the solution was frequently shaken in five minute interval for additional 15 min stored at 4±1°C. After the extraction phase, the organic phase, containing the β -carotenoid was separated from the water phase, using a separation funnel, and filtered using what man filter paper No.1. The extraction procedure was carried out under subdued light to avoid degradation of carotenoid. β -carotene was estimated from a standard curve(R²=0.996) of β -carotene standard product of Sigma Aldrich dissolved in the same solvent combination using double beam UV-Vis spectrophotometer (T80,China) at 450nm wavelength.

3.6.3.4. Determination of mineral (Ca and Fe) content

The dry ash procedure was used for Ca and Fe determination in soymilk based beverage measured by Atomic Absorption Spectrophotometer (AAS) (Model: Auto sampler AA 6800, Japan) according to the method of (Hernandez *et al.*, 2004). It was one gram sample for mineral were accurately weighed into porcelain crucibles and pre-ashed until the samples were completely charred on a hot plate under hood treated by few drop of deionized water until the solution boiled. The pre-ashed sample after treated, dried (removed all water) and

placed in the muffle furnace at 550 °C until the ash was obtained for about 2 hrs. Dry white ash was treated with 5 ml of 6 N HCl heated on the hot plate to wet it completely and 15 ml of 3 N HCl was added. The crucible was heated on the hot plate until the solution boiled. Then, it was cooled and filtered and again 10 ml of 3N HCl was added to the crucible and heated until the solution boils. Finally, cooled sample filtered through what man filter paper (541) in to 50 ml graduated flask and make up with distilled water to the mark. Using AAS a calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100g. The sample and standard was atomized using reducing acetylene with air gas for Ca and Fe as a source of energy for atomization. Standard stock solution of Ca and Fe were made by appropriate dilution. Ca and Fe were estimated from a standard calibration curve prepared from analytical grade CaCO₃ with a range of 0, 2, 4, 6 , 8,10,12,14 and 16 ppm, and from analytical grade iron nitrate with a range of 0, 2, 4, 6 and 8 ppm respectively. For Ca and Fe content determination absorbance was measured at 422.7 and 248.4 nm respectively. Reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption or emission values of the samples and the blank. The metal contents were calculated using equation 11:-

$$mg / 100g \text{ of } Ca \text{ or } Fe = \frac{(C_s - C_b * V)}{(10 * W)} \dots\dots\dots Eq.11$$

Where:-

C_s=Concentration of sample in ppm

C_b=Concentration of blank in ppm

V= Volume (ml) of extract and

W=Weight (g) of dried samples

3.6.3.5. Potassium analysis of soymilk based beverage

The potassium of the soymilk based beverage sample was determined by digital Flam Photometric method (Flame Photometer AJ-FP640, China) (Awonorin and Udeozor, 2014).Dried samples of 0.2g was weighed and transferred into a 250 ml conical flask.

Twenty ml of nitric acid was added from 1:1 diluted nitric acid solution and sample was boiled gently using hot plate for 10 minutes and cooled to room temperature. The digested solution of each sample was filtered using filter paper (Whatman 541) into a 100 ml volumetric flask and the volume brought to 100ml with deionized water (solution A). The blank was prepared with the same produces without sample (solution B). Five ml of solution A and B were taken into a 100 ml graduated flasks and made up to the mark with deionized water separately solution E & F respectively. Standard K solutions were prepared separately and each standard was diluted to concentrations of 0, 2, 4, 6, 8 and 10 ppm. Starting with the least concentration of two ppm, all the standard solution were sucked into the flame photometer instrument and caused to spray over the non-luminous flame. The reading were recorded and later plotted into a standard curve and used to interpolate to K level in the samples. After the standard, the beverage solutions were siphoned in turns into the instrument and their reading were recorded and potassium content was estimated using Eq.12.

$$mg / 100g \text{ of } K = \frac{((E - F) * 2000)}{W} \dots\dots\dots Eq.12$$

Where: -

E = Sample reading of the flame photometer

F = Blank reading of the flame photometer

W = Weight (g) of dried the sample

3.6.3.6. Phytic acid content analysis of soymilk based beverage

Phytic acid was determined according to (Vaintraub and Lapteva, 1988). About 0.06 gram of dried sample was extracted with 10 ml of 0.2N HCl for one hour at an ambient temperature and centrifugation at 3000 rpm for 30 min and clear supernatant sample was collected. The three ml of clear sample solution was mixed with 2 ml of wade reagent (0.03 % solution of FeCl₃.6H₂O containing 0.3% sulfosalicylic acid in deionized water) followed by centrifugation of the solution 3000rpm for 10 min. Finally, it was mixed using a vortex (Cyclone, England) mixer for 5 seconds and was measured at 500 nm using double beam

UV-Vis spectrophotometer (T80, China). Phytic acid was calculated from standard curve ($R^2=0.996$) of phytic acid standard equation.

$$\text{Phyticacid} = \frac{(\text{Absorbance} - \text{Intercept})}{(\text{Slope} * D * \text{weight of sample})} \dots\dots\dots \text{Eq.13}$$

Where: - D = Density of HCl

3.6.4. Sensory analysis of soymilk based beverage

Soymilk based beverages were prepared from three ingredients using fourteen different component proportions. Each blend was coded with three digits and presented for consumer sensory evaluation. The 5-point hedonic scale having a scoring range described in Kolapo and Oladimeji (2008) where 5= Like extremely, 4=Like moderately, 3= Neither like nor dislike, 2=Dislike moderately and 1=Dislike extremely were used to determine sensory properties of the blends. Sensory evaluation was carried out based on the sensory parameter of color, flavor, taste, aroma; mouth feel and overall acceptability. A total 50 consumers of (Linda *et al.*, 1991) were randomly selected from JUCAVM staff, students and other people gave informed about the sensory parameters and test procedures. Each sample was put at a reasonable distance between the judges to reduce halo effect. Ten ml of each freshly prepared sample was served in randomly coded white plastic cups and arranged in randomly order. After tasting each coded sample instructed to panelists that they have to rinse their mouths before moving on to the next sample. Then the score of all judges for each sample were summed up and divided by the number of panelists to find the mean value.

3.6.5. Determination of microbial quality and shelf life stability of soymilk based beverage

The total aerobic plate quality in this study has great importance in determining the quality of SBB. This study evaluated the total aerobic plate count of the best treatment combination of SBB stored at ambient (24 ± 2 °C) and 4 °C refrigeration temperature Determination of microbial quality and shelf life studies were conducted for the selected best treatment combination of optimization study. Twenty eight grams of Nutrient agar in deionized one

liter water was prepared according to manufacturer's specification. One milliliters of best treatment combination of SBB product was serially transferred into series of test tubes containing nine milliliters sterilized deionized water using a sterile pipette and shaken vigorously by using vortex mixer (Cyclone, England). Serial dilution was continued until 10^5 dilution (Udeozor and Awonorin, 2014). From serial diluted samples of 0.1 milliliter were taken and inoculated on nutrient agar (NA) media plate using spread plating technique and incubated (Heraeus, Germany) at 37°C for 24 hrs (Aminat *et al.*, 2013). After incubation, colonies on medium were counted using colony counter until no change in colony numbers were observed. The microbial quality obtained from colony forming units per milliliter (cfu/ml) in the original samples was converted to microbial load using Equation.14 (Fraizer and Westhoff, 1988).

$$ML = \left(N / V * R \right) \dots\dots\dots Eq.14$$

Where:-

ML = Microbial load

N = Number of colonies

V = Value of dilution

R = Dilution factor

3.7. Data analysis

Minitab version 16 statistical software computer package was used for ANOVA, data analysis, construction of contour plot, overlaid contour plot and response optimizer (best treatment combination) of result. The statistical significance of the terms in the regression equations was examined by analysis of variance (ANOVA) for each response and the significance test level was set at 5% ($P < 0.05$). Sweet spot of SBB products were selected using each analyzed parameters indicated in the lower and upper value. The best treatment combination of SBB products were selected using each analyzed parameters indicated in the lower and upper value, and goal of each parameter.

4. RESULTS AND DISCUSSION

The results of all the analyzed parameters for all the blends are presented and discussed in Tables and Figures indicated under each section. The data analyses of all treatment combinations were replicated for the measurement and mean values were taken for Minitab soft ware analysis. The summarized p-values of all parameters were obtained from Analysis of variance analysis (ANOVA) for each response. Model for all parameters were indicated that the lack-of-fit p-values were not significantly different at 5 % probability level. Diagnostic tools like normal and scatter plot of residuals indicated that the residuals of all response variables were normally distributed. . The regression quadratic models were used to estimate the optimum ratio mixes to get the best beverage with those parameters.

4.1. Physical properties of soymilk based beverage (SBB)

The physical properties assessed in this study were specific gravity (SG), total soluble solid (TSS) and total color change. Mixing ratio of three components of the beverage (Soymilk, Mango nectar and Sucrose solution) could make a difference in these properties. The mean values of the physical properties were summarized in contour plot Figure 5-7. The estimated regression coefficients along with their standard errors of all physical properties of SBB were shown in Appendixes Table 1. The result shows that combination ratios made a significant effect on TSS in quadratic model but no significant effect on total color change and specific gravity of SBBs (Table 3).

Table 3: Analysis of variance for the p-value of physical properties of SBB

Source	SG	TSS	ΔE
Linear	0.98	0.001	0.004
Quadratic	0.904	0.000	0.085
SM*MN	0.735	0.069	0.019
SM*SS	0.945	0.000	0.992
MN*SS	0.693	0.000	0.412
Lack-of-Fit	0.628	0.61	0.148

SM=soymilk, MN=mango nectar, SS= Sucrose solution SG=Specific gravity TSS= Total soluble solid and ΔE =Total color change.

4.1.1. Specific Gravity

The specific gravity of samples varied between 1.0-1.02 g/ml. This range is also in agreement with what were reported in different works (Bola and Aboaba, 2004; Sowonola *et al.*, 2005; Ajala *et al.*, 2012; Aminat *et al.*, 2013; Olusola and Sarah, 2014). Even though there was no significant difference observed among treatments, SG values (Table 3) were decreased with an increase in soymilk ratio. This might be associated with relative high proportion of fat in soymilk than other components.

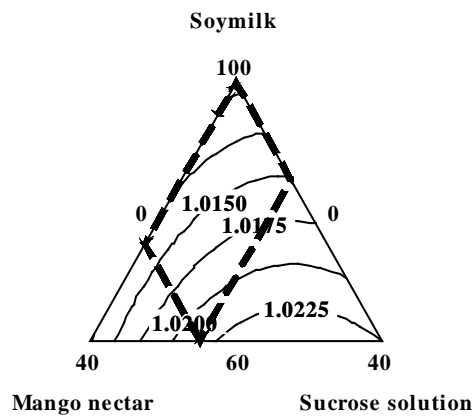


Figure 5: Mixture contour plot for Specific Gravity (g/ml)

4.1.2. Total Soluble Solids

Total soluble solids content of beverage mixes as indicated in Figure 6 ranges between 2.25 and 5.4°Brix. The soymilk based beverage of 60% SM, 25% MN and 15% SS gave the highest value of 5.4°Brix while the plain soymilk beverage had a 2.25°Brix. This indicates that with a reduction of SM ratio TSS value increased. Higher TSS values in MN and SS could contribute for an increase in this value with addition of these components. This is supported by significant ($p < 0.01$) (Table 3) interaction effects of SM and SS, and MN and

SS. Kale *et al.* (2011) in their work, showed that the TSS value of soymilk based orange juices increased from 5 to 20.2 °Brix as the soymilk ratio decreased from 90 to 10%. Different authors also reported similar results in line with the present outcome. For instance Geovana *et al.* (2014) indicated an increment of the TSS contents of SBB samples with mango pulp from 9.17 °Brix in 5% sugar content. Sakhale *et al.* (2012) also indicated that TSS value of SBB was lower than mango juices beverage, due to higher TSS value (8.46-19.83°Brix) of Mango pulp (Safina *et al.*, 2014 and Torres-Valenzuela *et al.*, 2014).

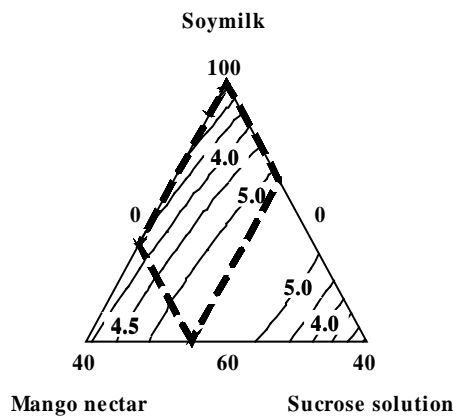


Figure 6: Mixture contour plots of Total Soluble Solids (°Brix).

The regression model equation for the Total Soluble Solid °Brix content of SBB is shown below equation 15.

$$TSS = 2.33A + 1.97B - 41.99C + 6.91AB + 74.02AC + 53.5BC \dots \dots \dots Eq.15$$

Where, TSS=Total Souble Solid, A= Soymilk,B=Mango nectar and C=Sucrose soution.

4.1.3. Total Color Change

The total color change (ΔE) content of beverage mixes varied from 0 to 29.51. Pure soymilk (100%) taken as a base or target color and total color change of beverages were assessed against with the pure soymilk color. The p-value was shown highly significant ($p < 0.01$) difference in the linear model and significance difference ($p < 0.05$) in the interaction of

soymilk and mango nectar shown in Table 3. Based upon this treatment combination of 60% SM, 25% MN, and 15% SS provided the highest color change (29.51). The total color change was changed positively as mango nectar percentage increased. This might be because of mango nectar intense yellow color which imparts significant color change as compared to target value of pure soymilk (Brecht and Yahia, 2009).

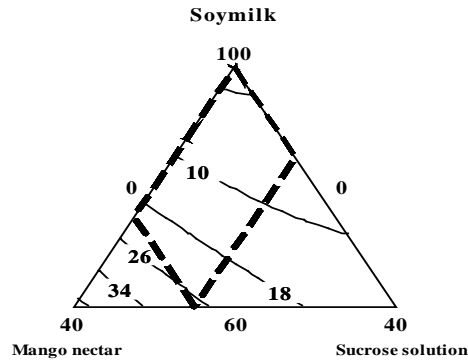


Figure 7: Mixture contour plot of Total Color Change

4.2. Chemical properties of soymilk based beverage (SBB)

4.2.1. Proximate analysis

When non alcoholic beverages are formulated, apart from sensorial property its proximate composition is another factor to be considered. Combining different composition of beverage at different ratio will result variation in proximate composition. Among 14 combination ratios, treatments showed significant differences ($p < 0.05$) in terms of protein and ether extracts, but other proximate parameters showed no significant differences ($p > 0.05$) including gross energy value (Table 4). The estimated regression coefficients along with their standard errors for all proximate compositions and gross energy are summarized in Appendix Table 2.

Table 4: Analysis of variance (ANOVA) p-value for the proximate composition and gross energy

Source	MC	Ash	Protein	EE	CHO	GE
Linear	0.258	0.438	0.295	0.002	0.062	0.701
Quadratic	0.832	0.467	0.026	0.021	0.588	0.974
SM*MN	0.509	0.364	0.578	0.031	0.519	0.824
SM*SS	0.685	0.331	0.604	0.204	0.754	0.854
MN*SS	0.577	0.346	0.592	0.178	0.372	0.721
Lack-of- fit	0.431	0.504	0.078	0.592	0.096	0.594

SM= soymilk, MN= mango nectar and SS= sucrose solution; MC=Moisture content, EE= ether extract and CHO= Carbohydrate

Mean moisture content (%) of beverage mixes are presented in Figure 8 and varied in range of 88- 90 %. Higher moisture content associated with high SM ratio (without MN and SS) as compared to other components. Similarly result more than 90% moisture content also reported on pure soymilk beverage (Gatade *et al.*, 2009; Aminat *et al.*, 2013; Ikya *et al.*, 2013 and Geovana *et al.*, 2014). The lower value associated with lowest value of SM (60%) and higher values of MN (25%) and SS (15%).

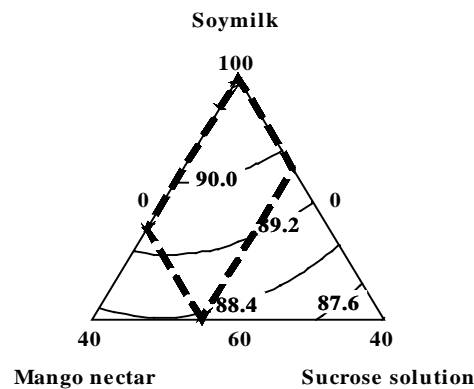


Figure 8: Mixture contour plots of moisture content (percent)

Ash content of a beverage roughly used as an indicator to estimate total mineral content (Neha and Ramesh, 2012). The variation in ash content was due to variation in inorganic compounds present in SBB extracted from soybean, mango and sugar. The mean ash content of samples varied from 0.12-0.17% as indicated in Figure 9. The average higher value was for pure soymilk and result of this study is also in agreement with others work (Geovana *et al.*, 2014 and Onuorah *et al.*, 2007) due to higher portion of soymilk (Gesinde *et al.*, 2008). Okoye and others (2008) also stated that ash contents of soymilk based beverage were increased as the level of soybean increased. In similar work, Northon *et al.* (2011), reported that, the ash content of SBB decreased from 0.48,0.37 and 0.17% as the amount of soy extract decreased from 8, 6 and 4 % respectively for a beverage prepared from soy extract and cassava starch.

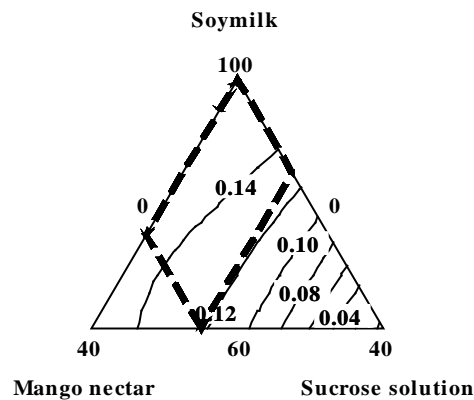


Figure 9: Mixture contour plots of Ash content (percent).

The mean protein content of the SBB was shown in Figure 10 and p-value presented in Table 4 with a significant difference at $p < 0.05$ in the quadratic model. The protein content of samples varied from 2.75 to 5.43 %.The highest value for 100%SM and the lowest value for SM, MN and SS ratio of 60%, 25% and 15% respectively as shown in Figure 10. The 100% soymilk protein content was similarly reported of Ikay *et al.* (2013) in the range of 3-5.91%. This result is in line with different literature information for high protein of soymilk (Anonymous, 2012) and low protein content of mango (0.44-0.58%) (Safina *et al.*, 2014). In

addition, to this Shakle *et al.*, (2012) observed that an increased in protein content (2.75-4.04 %) of SBB with an increased in soymilk ratio from 50-80%. However, protein values of SBB in this study slightly lower than values reported in Bahareh (2009); Ezekiel and Fapohunda (2012), with protein content of 8% and 6.73% respectively. These variations in protein content might be associated with variation on varieties and growth environment conditions (Hobbs *et al.*, 1998).

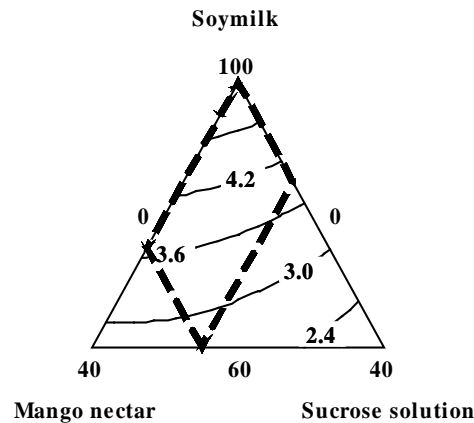


Figure 10: Mixture contour plots of Protein content (percent).

The mean ether extract of the SBB varied from 1.45 to 2.37 % as shown in Figure 11. The p-value of ether extract content was different at $p < 0.05$ with the presence of SM and MN as shown in Table 4. Soymilk 100% had the maximum ether extract and likewise protein content the lowest value was for 60% SM, 25% MN and 15% SS. Similar result in soymilk beverage reported by different authors having 2.6% (Onuorah *et al.*, 2007), 2.35% (Salim *et al.*, 2007) and 2.6% (Gatade *et al.*, 2009). This associated with higher oil content of SM as compared to SM based product made from addition of MN and SS (Augustine *et al.*, 2013 and Gatade *et al.*, 2009). Results reported in this study were higher than values reported in Shakle *et al.* (2012), an increase in fat content from 0.62 to 0.81% as the soymilk amount increased from 50% to 80%, but lower than what indicated in Awonorin and Udeozor (2014) in tiger nut soymilk product increased from 8.82% to 10.51% as the amount of soymilk increased from 10% to 50%. The lower the fat content of the beverage might be associated

with lower fat concentration in fresh (Mudau *et al.* (2013) and dried mango pulp (Mamiro *et al.*, 2007).

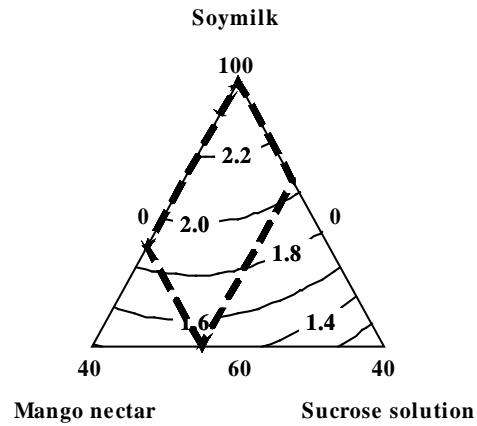


Figure 11: Mixture contour plots of Ether extract (percent).

The mean carbohydrate content of soymilk based beverage was founded between 1.214 to 7.318% as shown in Figure 12 with non significant values as shown in Table 4. Even though, there was no significant difference in carbohydrate content among mixes, the higher value was registered for a sample having lower protein and fat content. The highest (7.31 %) value was for a sample with SM 60%, MN 25% and SS 15%. This might be because of added MN and SS which contributed higher carbohydrate value as compared to pure soymilk (Shobana and Rajalakshmi, 2010). Similarly study reported by Mamiro *et al.* (2007) showed that mango pulp had high carbohydrate content of 15.56%. Edith and Paul (2012) also reported that carbohydrate content of the soymilk fortified beverages had higher carbohydrate content than the unfortified beverage. The least amount of carbohydrate determined in plain soymilk results were comparable with the report of Marina *et al.* (2013) presented the soy extract carbohydrate content of 1.6%.

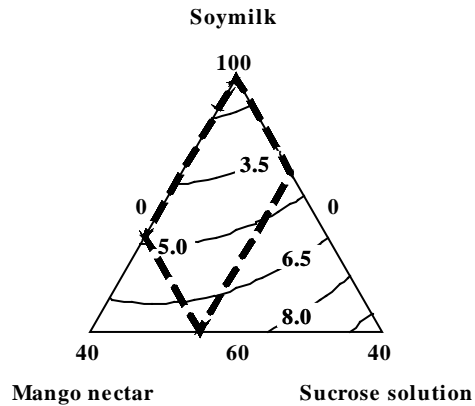


Figure 12: Mixture contour plots of Carbohydrate content (percent).

Gross energy content of different SBB, varied from 47.19 to 54.17 Kcal/100g as shown in Figure 13 and Table 4 despite there was no significant difference ($p < 0.05$). The lowest energy content (47.19) was recorded in the pure SM but the highest (54.17 %) was SBB samples with SM 60%, MN 25% and SS 15% combination. This might be attributed to the high carbohydrate content of MN and SS. The current study is in comparable with SBB gross energy values increased the same as the increased carbohydrate contents (Awonorin and Udeozor, 2014).

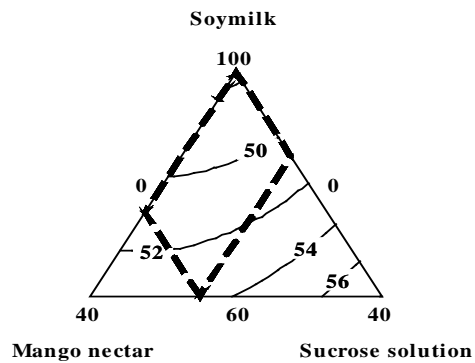


Figure 13: Mixture contour plots of Gross Energy content (percent).

Table 5: Best fit quadratic model equations for different proximate composition response

No.	Response	Quadratic model equations
1	Protein	5.460A+0.385B+0.595C-2.673AB-6.547AC+7.037BC
2	Ether extract	2.358A-2.288B-4.014C+3.685AB+5.114AC+5.680BC

A=Soymilk, B=Mango nectar and C=sucrose solution

4.2.2. TA and pH

TA and pH analysis of estimated regression coefficients along with their standard error are summarized in the Appendix Table 3. There were no significant differences in blend ratios in terms of TA and pH as indicated Table 6. The TA and pH obtained from SBB were shown in the mixture contour plots of Figure 14. An increase in TA or decrease in pH values were observed with an increase in MN.

Table 6: Analysis of variance p-value of TA and pH

Source	TA (%)	pH
Linear	0.998	0.647
Quadratic	0.600	0.163
SM*MN	0.277	0.222
SM*SS	0.808	0.830
MN*SS	0.415	0.660
Lack-of- fit	0.078	0.893

SM=Soymilk, MN=Mango nectar, SS=Sucrose solution, TA=Titrateable acidity

The TA of the SBB varies from 0.038% to 0.064% as shown in Figure 14a. The highest value was the treatment combination of SM 60%, MN 25% and SS 15% and the lowest for 100% SM. Addition of SS with MN resulted in considerable increase in the TA of the beverage, this might be due to high percentage of titrateable acidity (0.72 %) of mango pulp (Mamiro *et al.*, 2007). The effects of fruit juices to boost TA of different SBB are also indicated in different authors (Kale *et al.*, 2011; Rozina *et al.*, 2012 and Shakel *et al.*, 2012).

The pH of the beverage varied from 6.62 to 6.98 as shown in Figure 14b. The pH value was measured with lowest value after mango nectar was added. Similar findings were reported by several works (Kale *et al.*, 2011; Rozina *et al.*, 2012; Shakel *et al.*, 2012 and Geovana *et al.*, 2014). In general, increasing in pH was observed, which is generated by the decline in acidity (Kazuhiro, 2011) due to increased SM portion in the mixes.

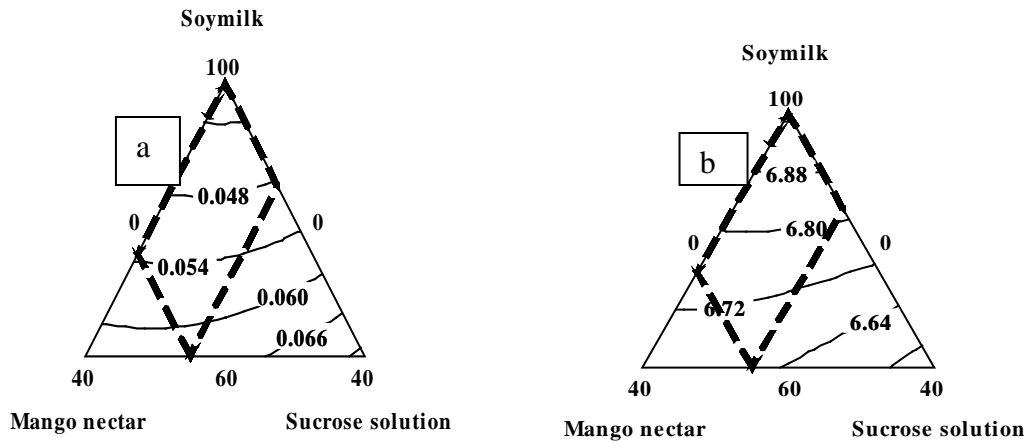


Figure 14: Mixture contour plots Titratable Acidity (a) and pH (b).

4.2.3. β -carotene

Estimated mean β -carotene contents varied between 0.0002 to 0.00127 mg / 100 ml of beverage (Figure 15). In terms of β -carotene content blend ratios of SBBs showed highly significant differences ($P < 0.01$) except interaction effect of SM and SS as shown in Table 7. An increment in β -carotene content was in parallel proportion of mango nectar added in the mix due to high β -carotene content of mango pulp. The treatment combination of 75% SM, 25% MN and 0% SS has shown the higher β -carotene content than pure 100% SM. The intense yellow-orange color of mango fruit pulp may be a good indicator of β -carotene (Vásquez *et al.*, 2002). This study is in agreement with Madukwe *et al.* (2013) soymilk blend with carrot powder blended were the higher β carotene content.

Table 7: Analysis of p-value of β -carotene content

Source	β -carotene
Linear	0.001
Quadratic	0.000
SM*MN	0.000
SM*SS	0.321
MN*SS	0.001
Lack-of- fit	0.323

SM= Soymilk, MN=mango nectar and SS= Sucrose solution.

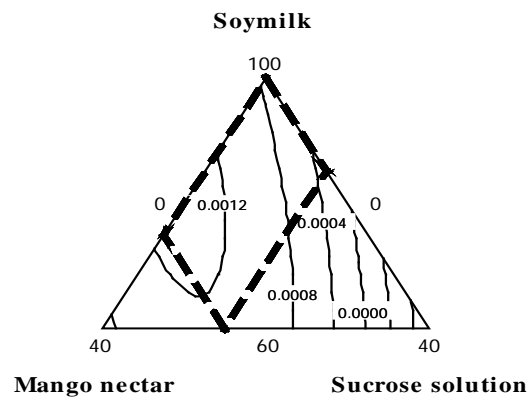


Figure 15: Mixture contour plot of β - carotene (mg/100g) content.

The regression model equation for the β - carotene content of SBB is shown in Eq. 26(All independent variable in coded values) indicating quadratic effects with variables.

$$\beta\text{-caroten} = 0.000735A - 0.00807B - 0.00715C + 0.014457AB + 0.005566AC + 0.029716BC. \text{Eq.26}$$

Where, A=Soymilk, B= Mango nectar and C= Sucrose solution.

4.2.4. Minerals (Ca, Fe and K)

K contents of beverage mixes showed significant difference ($p < 0.05$) in quadratic model unlike to that of Fe and Ca concentration (Table 8). The estimated regression coefficients along with their standard errors as shown in Appendix Table 5 and standard curve of mineral value of SBB were summarized as shown in Appendix Figure 2- 4.

Table 8: Analysis of variance p-value of mineral content of soymilk based beverage

Sources	Mineral		
	Ca	Fe	K
Linear	0.591	0.011	0.004
Quadratic	0.484	0.066	0.000
SM*MN	0.762	0.551	0.476
SM*SS	0.996	0.046	0.000
MN*SS	0.691	0.014	0.001
Lack-of-fit	0.072	0.07	0.066

Where:-Ca=Calcium, Fe=Iron, K=Potassium, SM=Soymilk MN=Mango nectar, SS=Sucrose solution

Literature data showed that freshly prepared SM is rich in Fe, Ca and K (Ezekiel and Fapohunda, 2012) as compared to MN and SS. Mango had low Ca and Fe (Sarkiyayi *et al.*,2013) but with high K concentration (Othman and Mbogore, 2009).

The calcium content in fourteen soymilk based beverage samples ranged from 154.67 - 242.65 mg per 100 g as shown in Figure 16.The Ca contents of SBB was increased with the increment of soymilk ratio in SBB which is in agreement with Sowonola *et al.*(2005).

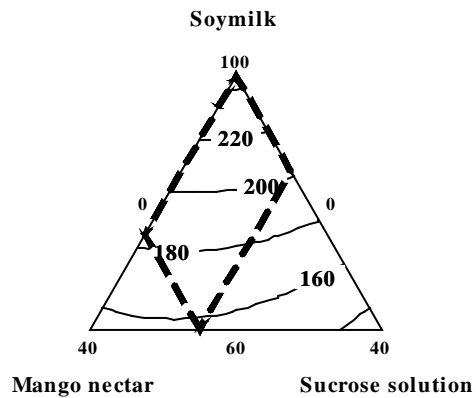


Figure 16: Mixture contour plots of Ca (mg/100g)

The Fe content of SBB was presented in Figure 17 ranged from 0.97-1.84 mg/100g which is in the range of soy based fruit juices as indicated in Luciula *et al.* (2012) (0.08 to 1.38 mg 100mL⁻¹).

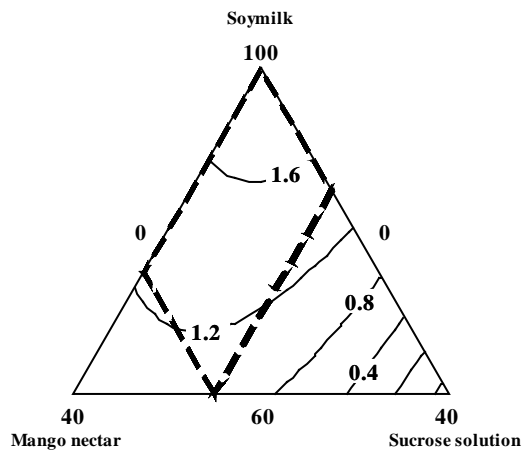


Figure 17: Mixture contour plots Fe (mg/100g)

The potassium content varies from 959.45-1640.44 mg/100g and this range is in agreement to results indicated in Ilana *et al.* (2009) for soymilk with Brazil nut beverage and soymilk

with K values of 995.42 and 1297.38 mg/100 g respectively. The K content obtained from SBB was indicated in contour plot (Figure 18).

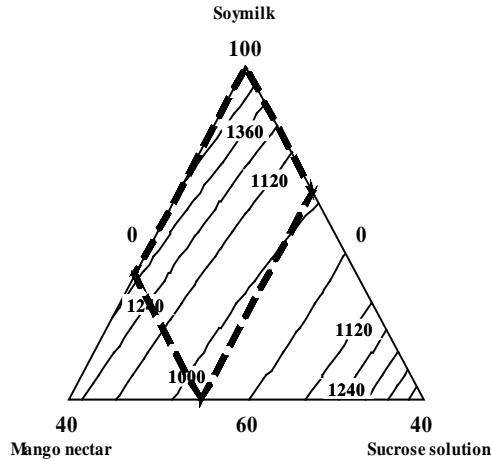


Figure 18: Mixture contour plots K contents (mg/100g).

The regression model for K is indicated in the following equation.

$$K = 1617 A + 1253 B + 11879 C - 697 AB - 16776 AC - 12611 BC \dots \dots \dots Eq.16$$

Where, K= potassium, A= Soymilk, B=Mango nectar and C=Sucrose solution. K=potassium

Generally, Ca and Fe levels in this study were found higher than reported by other authors (Ilana *et al.*, 2009; Aminat *et al.*, 2013 and Nezife, 2013). These variations might be associated with geographical variation and agricultural conditions to grow soybeans (Dinize-Filho *et al.*, 2008).

4.2.5. Phytic acid content

Phytic acid is widely distributed in commonly consumed foods (Alabaster *et al.*, 1996). Most of Anti Nutritional Factors (ANFs) are protease inhibitors and lectins are heat labile, but others as phytic acid and polysaccharides non starch (PNS) only decrease with addition of enzyme in diet (Dourado *et al.*, 2011). The mean phytic acid significantly different at 5% level as shown in Table 9. The corresponding contour plot is shown in Figure 19. The estimated regression coefficients along with their standard errors and standard curve of

phytic acid are presented in Appendix Table 6 and Figure 5 respectively. Phytic acid naturally occurs in soybeans can make up to 1–1.5 g per 100 g of the dry weight (Liener, 1994) and also most soybean products have 1 to 3% phytic acid (Esteves *et al.*, 2010). The phytic acid contents of beverages ranged from 1.001-3.31mg/100g as indicated. This can be due to the contribution of different ingredients ratio with different phytates content. The plain SM pytic acid content in this study is lower than what is reported in Susu *et al.* (2013) and Soybean contains 1% to 3% phytic acid (Isiguro *et al.*, 2005). However, as indicated in Figure 19, pure SM (100%) had the highest value (more than 3 mg/ 100 g sample) among beverage mixes and the lowest was for beverage enriched with MN (25%) and SS (15%). This indicates that, with addition of mango nectar and sucrose solution, in addition of masking the undesirable flavor of the beverages, enhances the absorption of mineral nutrients through reducing effects of ANFs.

Table 9: Analysis of variance p-value of phytic acids

Sources	Phytic acids
Linear	0.01
Quadratic	0.106
SM*MN	0.044
SM*SS	0.597
MN*SS	0.34
Lack-of-fit	0.063

Where:-SM=Soymilk MN=Mango nectar, SS=Sucrose solution

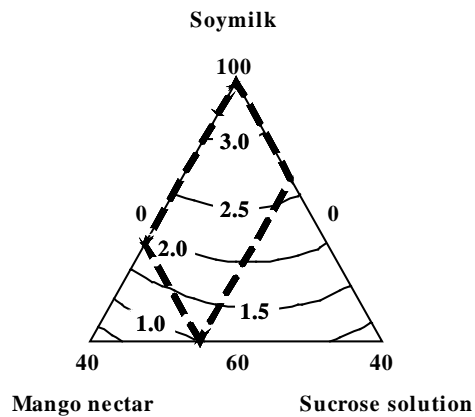


Figure 19: Mixture contour plot of Phytic acid content.

4.3. Sensory analyses of Soymilk Based Beverage (SBB)

The sensory evaluation mean scores of a 5- point hedonic scale results are also presented in Appendix Table 8. Mixture contour plots for all the sensory attributes are presented in Figure 20(a-f). As proportion of MN and SS were increased; consumer over all preference was increased. This might be associated with masking effect of MN and SS on unwanted favor of SM. Mean scores for color, flavor, taste, aroma, mouth feel and overall acceptability were varied from 3 (neither like nor dislike) to 4 (Like moderate). There was significant difference ($p < 0.05$) in the interaction of SM and MN to alter taste, flavor and mouth feel of beverages, and overall acceptability shown in Table 10.

Table 10: Analysis of variance for the p-value of sensory evaluation

Source	Color	Flavor	Taste	Aroma	Mouth feel	Over all acceptability
Linear	0.703	0.146	0.083	0.789	0.703	0.203
Quadratic	0.991	0.05	0.016	0.192	0.002	0.144
SM*MN	0.944	0.028	0.007	0.261	0.03	0.046
SM*SS	0.966	0.216	0.29	0.631	0.578	0.597
MN*SS	0.934	0.082	0.09	0.52	0.793	0.258
Lack-of- fit	0.218	0.502	0.262	0.252	0.151	0.227

SM=Soymilk, MN=Mango nectar, SS=Sucrose solution

Generally, acceptability of flavor, taste, aroma, mouth feel and overall preference increased as proportion of SM decreased as presented in Figure 20. Sucrose is added to improve the sensory acceptance of many foods, increasing the sweetness of the final products and masking the undesirable ‘green bean flavor’ of soybean (Favaro, 2001). In other words, all the sensory attributes increased as volume of MN and SS increased except effect of color change due yellow- orange color of mango pulp (Jorge *et al.*, 2006). In the other works indicated in Anchan and Adisak (2012); Edith and Paul (2012) color content of plain soymilk had the highest value while the soymilk fortified had the least value. Based upon this, beverage containing 60% SM, 25% MN and 15% SS gave excellent sensorial quality than other treatment combinations. This might be linked with the decreasing concentration of soymilk contributing in reduction of unpleasant beany flavor of soybean as a limiting factor of acceptability of the beverage in soya based food markets. Shakle *et al.* (2012) also indicated that the beany flavor in soymilk commonly reduced the acceptability of soymilk blended mango juices beverage product. In other study, decreasing soymilk amount and blending some amount fruit and chocolate increased in the sensory quality which result in to

improved flavor, mouth feel and overall acceptability (Gatade *et al.*, 2009; Kale *et al.*, 2011 and Rozina, 2012). Therefore, blending changes and enhances overall flavor and acceptance of the beverage and this in turn enhances soy consumption (Rodriguez *et al.*, 2014).

Table 11: Best fit quadratic model equations for different sensory quality response

No	Response	Quadratic model equations
1	Flavor	$3.613A+1.040B-1.302C+5.560AB+7.331AC+11.269BC$
2	Taste	$3.7332A+0.861B+0.8765C+5.988AB+5.015AC+8.87BC$
3	Mouth feel	$3.354A+1.603B+4.54C+7.095AB+4.117AC-1.998BC$

Where, A= Soymilk, B= Mango nectar and C= Sucrose solution.

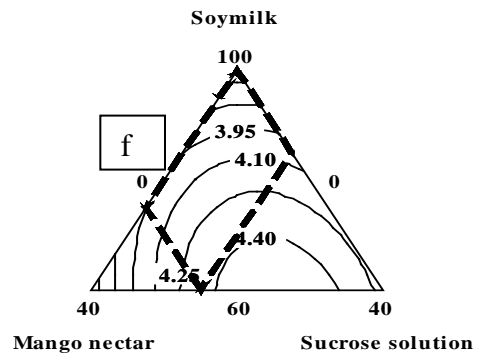
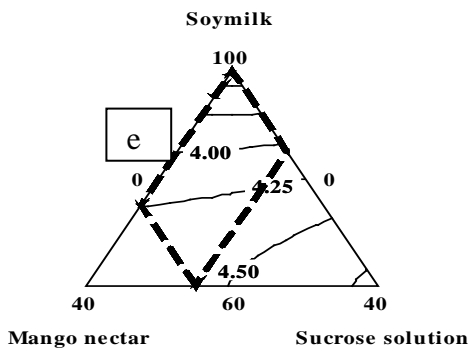
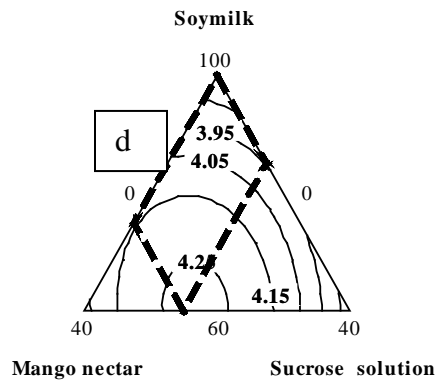
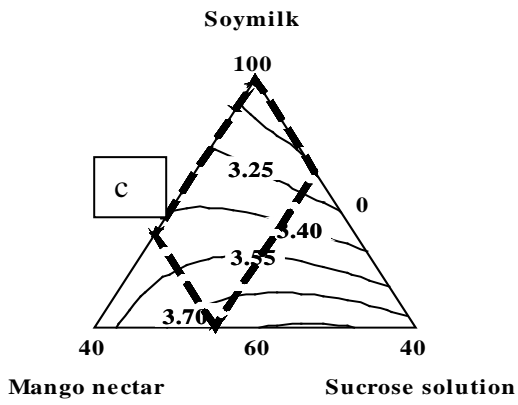
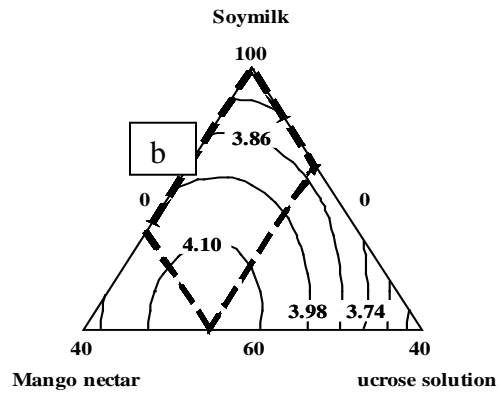
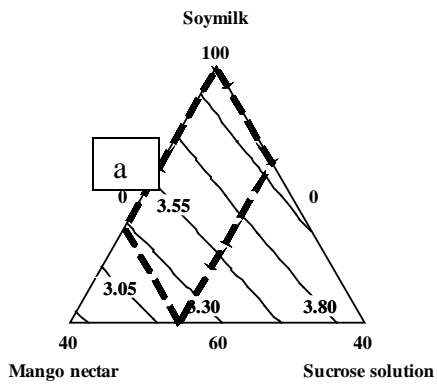


Figure 20: Mixture contour plots; color (a), flavor (b), taste (c), aroma (d), mouth feel (e) and overall acceptability (f).

4.4. Optimal mixture compositions

All parameters were analyzed using response optimizer to choose the overlaid contour plot and best treatment combination of Minitab software for final recommendation and use.

4.4.1. Region of optimum mixture composition of physical properties

The white area indicates the optimum point of formulation to develop soymilk based beverage with good physical properties which can serve the expected purpose. Best treatment combination of physical properties SBB obtained from SM, MN and SS at different ratios were determined through minimizing specific gravity and total color change, and maximizing total soluble solid value of beverages. The optimum point for specific gravity, total soluble solid and total color change varied between 1.01-1.02 g/ml, 3.6-5.4°Brix and 6-22.93 respectively. The region of optimum point physical properties of SBB samples was obtained through graphical optimization, in which the proportion ranged between 73-86% SM, 0-25% MN and 8-15% SS as (Figure 21). However with a specific value of 79% SM, 6% MN and 15% SS. Predicted best response values, desirability and composite desirability of each parameter are presented in Table 12.

Table 12: The response optimizer analysis of the best treatment combination values physical parameter analysis.

Parameters	Predict response	desirability	Composite desirability
SG (g/ml)	1.02	0.7	
TSS (°brix)	5.23	0.95	
Total color change	8.53	0.71	
Total			0.78

SG=Specific gravity, TSS=Total soluble solid

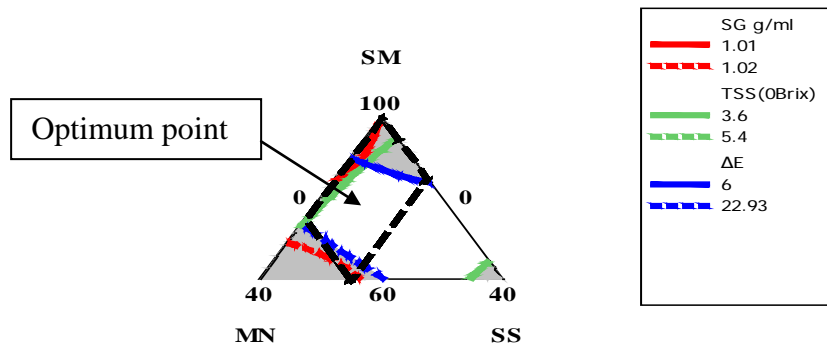


Figure 21: Overlaid contour graph of Physical properties

4.4.2. Region of optimum mixture composition of chemical properties

Optimized spot which includes all the optimum points for different chemical parameters (protein 2.76-5.4%, EE 1.45-2.37% , carbohydrate 3.38-7.32%, gross energy 47.19-54.17 K cal/100gm,pH 6.62-6.98,Beta-carotene 0.0005-0.00127 mg/100gm, calcium 154.67-242.65 mg/100gm and iron 0.97-1.84mg/100gm indicated in Figure 22. The region of optimum mixture for chemical parameters was found in the samples prepared within the range of 60-84% SM, 6-25% MN and 0-15 % SS (Figure 22). From the optimal value it can be seen that sucrose solution can be used from the lowest to the maximum value without affecting the chemical properties, where as the optimum content was found at the maximum amount of soymilk and mango nectar.

Moreover, the best treatment combination was selected based on the lower and upper values of all chemical parameter results. The best chemical parameters were obtained for treatment combination of 84% SM, 16 % MN and 0%SS. This combination gave relatively better moisture, ash, protein, ether extract, carbohydrate, gross energy, TA, pH, β - carotene, with moderate phytic acids and mineral content. In addition, the predicted best response values, desirability and composite desirability of each parameter are indicated in Table 13.

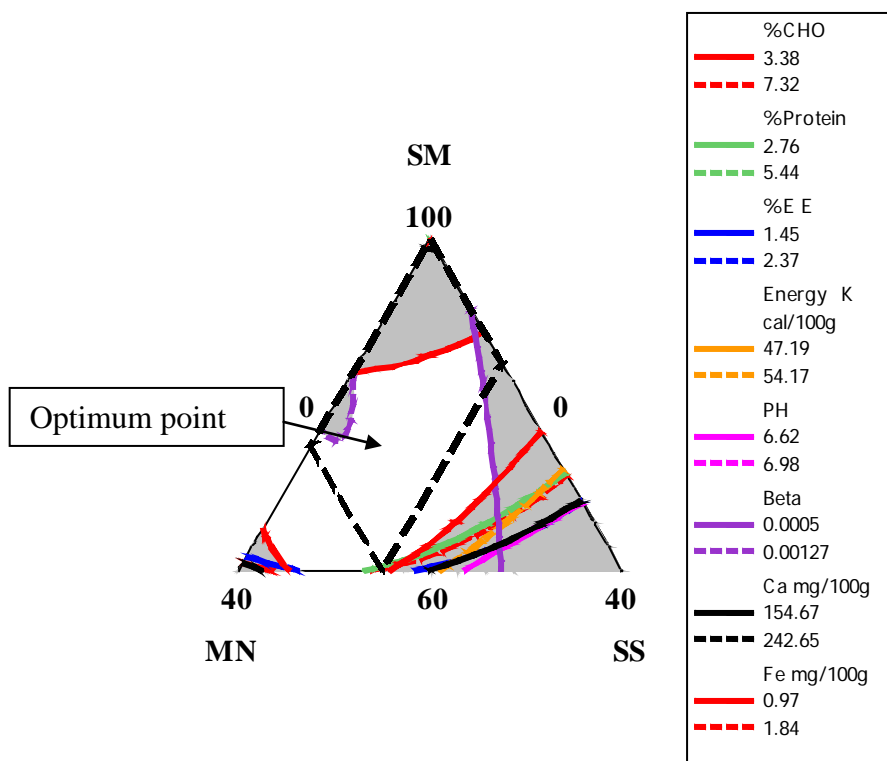


Figure 22: Overlaid contour graph of chemical parameters

Table 13: The response optimizer analysis of the best treatment combination for all-chemical properties SBB

Parameters	Predicted response	Desirability	Composite desirability
MC (%)	90.07	0.32	
Ash (%)	0.15	0.5	
Protein (%)	4.28	0.58	
EE (%)	2.11	0.71	
CHO (%)	3.4	0.36	
Energy (Kcal/100g)	49.64	0.35	
TA %	0.05	0.53	
pH	6.82	0.55	
β-carotene (mg/100ml)	0.001	0.95	
Ca (mg/100g)	204.94	0.57	
Fe (mg/100g)	1.48	0.58	
K (mg/100g)	1463.53	0.74	
Phytic acid (mg/100g)	2.57	0.32	
Total			0.52

MC=Moisture content, EE=Ether extract, CHO=Carbohydrate, TA=Titrateable acidity

4.4.3. Region of optimum mixture composition of all physicochemical parameters

The sweet spot which includes all the optimum points (total color change 6-22.93, protein 2.76-5.4%, EE 1.45-2.37%, carbohydrate 3.38-7.32%, gross energy 47.19-54.17 Kcal/100gm, pH 6.62-6.98, β -carotene 0.0005-0.00127 mg/100gm, calcium 154.67-242.65 mg/100gm and iron 0.97-1.84mg/100gm) are indicated in Figure 23. The sweet spot was found in the samples prepared within the range of 68-86 % SM, 6-25 % MN and 0-15 % SS.

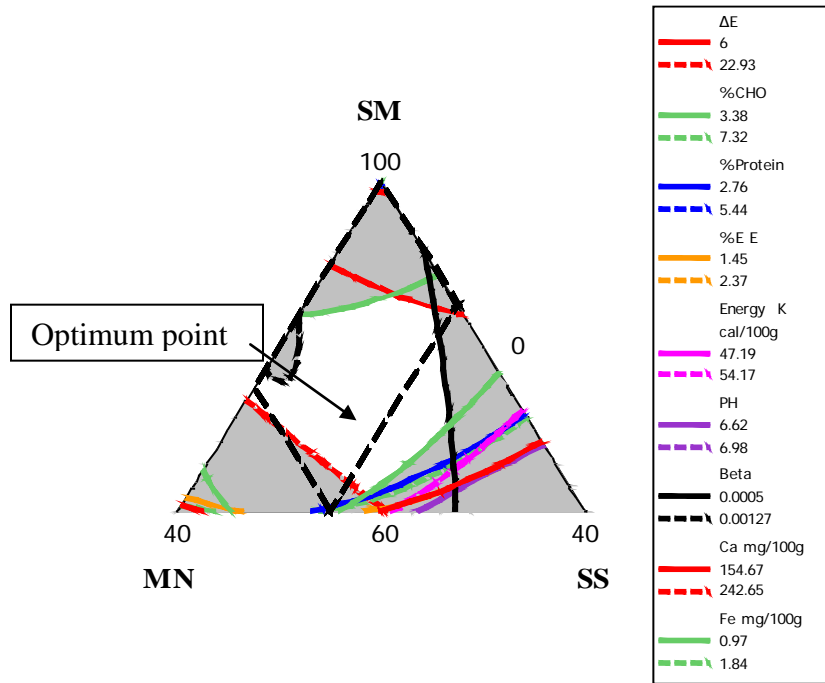


Figure 23: Overlaid contour plot of all physicochemical properties

In addition, the best treatment combination of fourteen formulations containing SM, MN and SS were selected based on all physicochemical properties using lower and upper values. It was found from blend ratio of 84 %SM, 11 % MN and 5%SS. Predicted response values of each physicochemical property and their corresponding desirability values are presented in Table 14.

Table 14: The response optimizer analysis of the best treatment combination value for physicochemical properties

Parameters	Predicted responses	Desirability	Composite desirability
SG(g/ml)	1.01	0.5	
TSS (°brix)	4.05	0.57	
Total color change	8.78	0.7	
MC (%)	90.05	0.33	
Ash (%)	0.14	0.49	
Protein (%)	4.19	0.54	
EE (%)	2.12	0.72	
CHO (%)	3.5	0.37	
Energy (Kcal/100g)	49.8	0.37	
TA (%)	0.05	0.56	
pH	6.82	0.56	
β-carotene (mg/100ml)	0.00111	0.83	
Ca (mg/100g)	205.5	0.58	
Fe (mg/100g)	1.55	0.67	
K (mg/100g)	1267.87	0.45	
Phytic acid (mg/100g)	2.67	0.28	
Total			0.51

SG=Specific gravity, TSS=Total soluble solid, MC=Moisture content, EE=Ether extract, CHO=Carbohydrate, TA=Titrateable acidity

4.4.4. Region of optimum mixture composition of sensory evaluation

Optimization for sensory evaluation is defined as a procedure for developing the best possible product in its class to be accepted by consumers (Stone *et al.*, 2012). Although an optimal formation should maximize consumer acceptance, it is impossible to develop a product with all five sensory qualities that would satisfy consumers in most applications (Moskowitz, 1994). To obtain the optimum region, the ingredient formulation was obtained based on lower and upper value of color, flavor, taste, aroma, mouth feel and overall acceptability for soymilk based beverage. Based upon this, overlaid contour plots for these attributes are shown in Figure 24. The sweet spot was obtained by optimal value placing a range of color 3.02-4.3, flavor 3.8-4.18, taste 3.9-4.28, aroma 3.5-3.78, mouth feel 3.4-4.48 and overall acceptability 3.48-4.44. The optimum region in this overlaid plot was where the criteria for all the five response variables (color, flavor, taste, aroma, mouth feel and overall

acceptability) were contented and this region was found in the range of 60-74% SM, 12-25% MN and 8-15% SS.

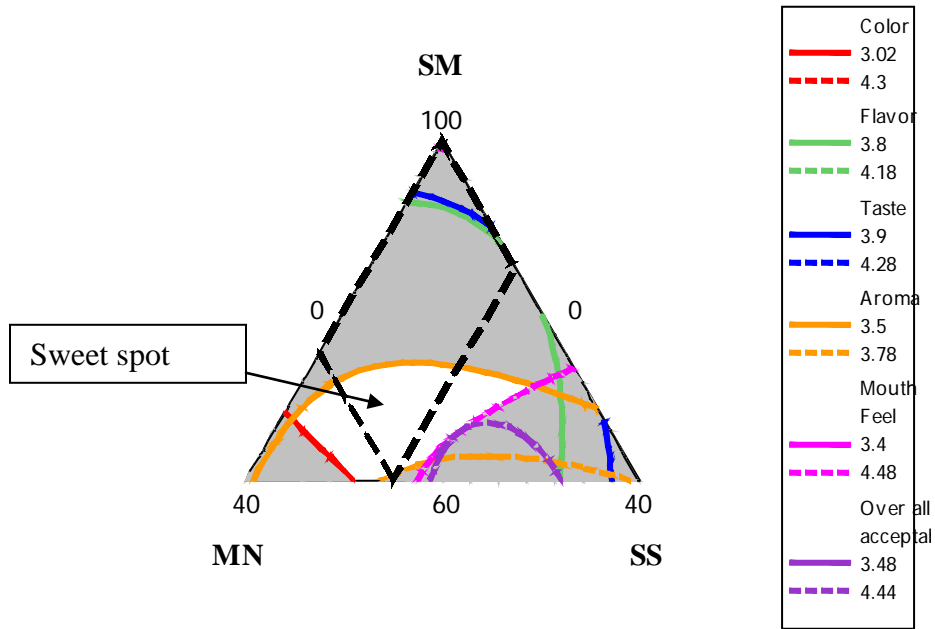


Figure 24: Overlaid contour plot of sensory parameters

The best treatment combination of SBB in sensory acceptance was found for beverage mix with 68 % SM, 17% MN and 15% SS. The higher overall acceptability was 4.4 which suggested that best accepted by judges compared with unblended soymilk samples. The preference order was also influenced by sugar portion, in which the samples with more SS were more preferred than samples with small or no sucrose. This study shows that addition of MN to SM improved the flavor attributes as comparing with plain SM. In addition, the predicted responses value and desirability are presented in Table 15. These days' beverage manufacturers have targeted sensory as an extremely important marketing tool. Therefore the results of the present study show that formulation of SBB with MN and SS provides great competitive advantages to enhance overall acceptability and market opportunity through masking of 'undesirable beany flavor of pure SM.

Table 15: The response optimizer analysis of the best treatment combination value for sensory quality

Parameter	Predicted responses	Desirability	Composite desirability
Color	3.44	0.33	
Flavor	4.12	0.90	
Taste	4.25	0.94	
Aroma	3.64	0.82	
Mouth feel	4.42	0.95	
Overall acceptable	4.38	0.94	
Total			0.77

4.4.5. Region of optimum mixture composition of Physicochemical and Sensory Quality

The study focused on determining the optimal blend ratio of individual food source that are suited to produce SBB with desirable physicochemical and sensory compositions. In order to determine the optimum formulation, the regions of acceptability in the contour plot for total color change, protein, EE, carbohydrate, calorie, pH, Beta-carotene, calcium, iron and overall sensory attribute were superimposed. Superimposition of contour plot regions (total color change 6-22.93, protein 2.76-5.44%, EE 1.45-2.37%, carbohydrate 3.38-7.32%, gross energy 47.19-54.17%, pH 6.62-6.98, β -carotene 0.0005-0.00127% mg/100gm, Ca 154.67-242.65 mg/100gm, Fe 0.97-1.84 mg/100gm and over all acceptability received hedonic ratings 3.48-4.48) resulted in optimum regions as shown in Figure 25. The white region in this figure indicates that any point within this region represents an optimum combination of SM, MN and SS, which results in desirable physicochemical and sensorial attributes. The overall optimum values for these properties were found in a range of 67-84% SM, 12-25% MN and 0-15% SS.

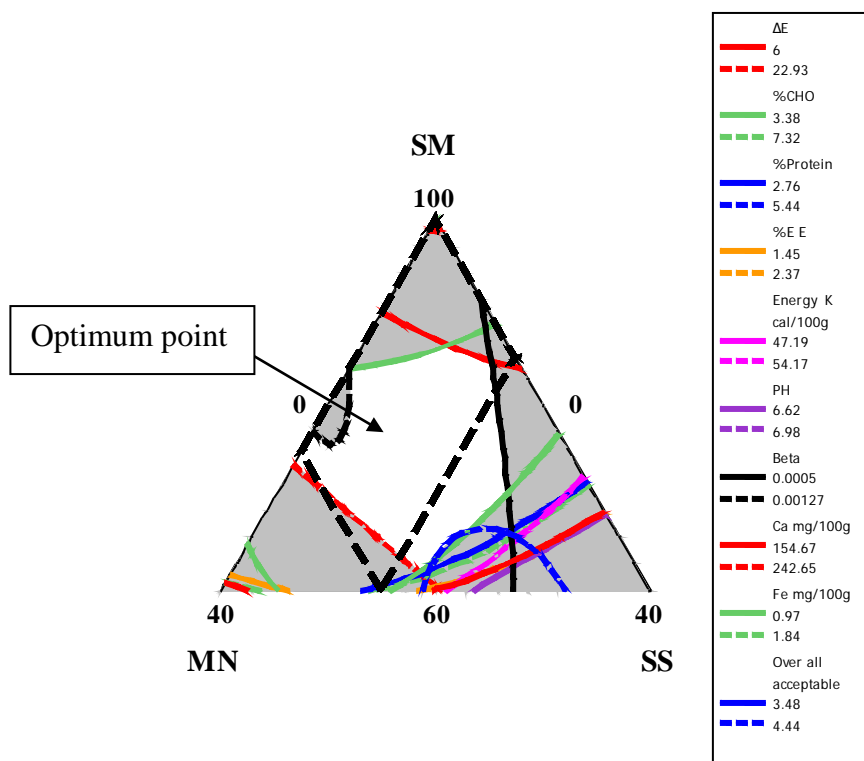


Figure 25: Overlaid contour plot of all physicochemical quality and sensory acceptability

Soymilk based beverage with a combined use of SM, MN and SS is resulted in improved physicochemical and sensory quality of pure SM. Thus, soybean, mango and sugar could be used as a beverage mix for all age persons due to nutrient potentials (high protein, fat, and carbohydrate, β -carotene contents, mineral and sensory) diversify diet choices with acceptable sensorial properties. Addition of mango nectar in the beverage increases TSS, TA, β - carotene, potassium content, and sensory quality. The TSS, calorie and sensory quality was also increased as the quantity of sucrose solution increased. Those were attributed to the high sensory quality of SBB, will make the beverage to be suitable for consumers. Predicted responses values all parameters through best treatment combination study and their corresponding, desirability are indicated in Table 16. Physicochemical and sensory quality of 80 % SM beverage was best improved when 14% MN and 6% sucrose solutions were added respectively. Such type of soymilk improvement through addition of nutrient enriched and flavor masking components will enhance consumption of soymilks by different social groups.

Table 16: The response optimizer analysis of the best treatment combination value for all parameter

Parameters	Predicted responses	Desirability	Composite desirability
SG (g/ml)	1.02	0.57	
TSS (°brix)	4.38	0.68	
Total color change	11.43	0.61	
MC (%)	89.81	0.42	
Ash (%)	0.14	0.43	
Protein (%)	3.92	0.44	
EE (%)	2.04	0.64	
CHO (%)	4.09	0.47	
Energy (Kcal/100g)	50.35	0.45	
TA (%)	0.05	0.47	
pH	6.791	0.47	
β-carotene (mg/100 ml)	0.001	0.87	
Ca (mg/100g)	196.68	0.48	
Fe (mg/100g)	1.47	0.58	
K (mg/100g)	1196.56	0.35	
Phytic (mg/100g)	2.45	0.37	
Color	3.64	0.49	
Flavor	4.02	0.75	
Taste	4.14	0.76	
Aroma	3.38	0.49	
Mouth feel	4.15	0.72	
Overall acceptability	4.13	0.68	
Total			0.54

SG=Specific gravity, TSS=Total soluble solid, MC=Moisture content, EE=Ether extract, CHO=Carbohydrate, TA=Titrateable acidity

4. 5. Microbial and shelf life stability of selected SBB

Soy milk is an excellent medium for microbial growth making it a suitable vehicle for various food-borne diseases (Frazier and Wsethoff, 1988). This study evaluated the total aerobic plate count of the best treatment combination of SBB stored at ambient (24 ± 2 °C) and refrigeration temperature (4 °C). The total aerobic plate count of best treatment combination of SBB stored sample at room temperature stayed acceptable for one day but samples stored at refrigerated remained shelf stable up to 11 days as shown in Figure 26. Similar studies reported that

soymilk with NaHCO_3 and Na_2CO_3 when treated with propyl gallate, Ascorbylplamitate and both propyl gallate and Ascorbylplamitate and stored at room temperature, couldn't stored for more than one day (Odu and Egbo, 2012). The same is reported in Gesinde *et al* (2008). The soymilk-based juice samples treated with *Aframomumdanielli* powder were better preserved than untreated ones (control) (Dauda and. Adegoke, 2014). In different study addition of 0.1% sodium benzoate and 0.1% potassium sorbate, 2% sucrose and propyggallate and Ascorbic pamitate at the ratios: 100ppm Ascorbic palmitate and 100ppm propyl gallate, 200ppm Ascorbic palmitate, 200ppm propyl gallate reduced microbial count at both ambient and refrigeration temperatures than the control (without preservative and antioxidant) (Odu and Egbo, 2012). This is the reason why several workers are exploring the use of chemical preservatives for prolonging the shelf life of soymilk products (Uriah and Iwagbe, 1990).

The effect of certain preservatives at various concentrations within their maximum permissible levels along with pasteurization and refrigeration storage on the microbial keeping quality of soymilk was used with the intention of determining which combination is best for a prolonged shelf life (Osuji and Anyaiwe, 2010). In this study, the best treatment combination of SBB sample was pasteurized at 71 °C for 30 minutes, stored at 4 °C refrigeration temperature and maintained with good microbial quality without use of any preservative. In similar studies it is reported that soymilk samples treated sing Na_2CO_3 and NaHCO_3 , and pasteurized and stored at refrigerated temperature exhibited no growth in aerobic population from day 0 to day 6 (Odu and Egbo, 2012). In other similar work it is reported that pasteurized and refrigerated soymilk showed no change on taste, odor and microbial count on sixth day of storage (Bola and Ababa, 2004 and Onurah *et al.*, 2007). These works confirmed that combination of pasteurization and refrigeration treatments were found most suitable for prolonging shelf life of soymilk beverages with minimal nutrient loss (Kabiru *et al.*, 2012).

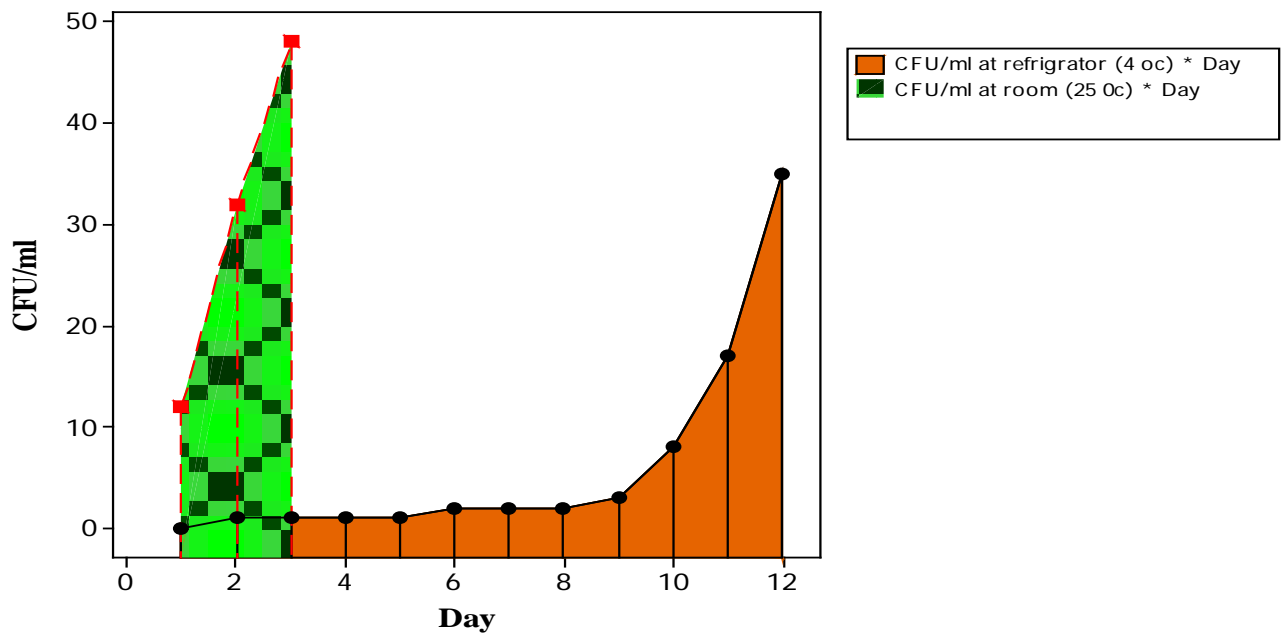


Figure 26: Scatter plot of total aerobic plate count of room and refrigerator temperature Vs day.

5. SUMMARY AND CONCLUSION

The mango nectar flavored and sucrose solutions sweetened SBB can be produced to overcome limitation of unwanted flavor of SM. Differences in proportion of SM, MN and SS alter physico-chemical and sensory quality of SBB. The nutritious beverage with better quality can be developed by addition of MN and SS to certain portion. When all measured parameters were optimized together, the best blend ratio for best parameters combination was obtained from mix of 80 % SM, 14 % MN and 6% SS. This ratio resulted in better physicochemical and sensory quality for specific gravity (1.01g/ml), total soluble solid (4.38°brix), total color change(11.43), MC (89.81%), Ash (0.14%),Protein (3.92%),EE(2.04%),Carbohydrate (4.09%), Gross energy (50.35 kcal/100g), TA (0.05%),pH (6.79), β -carotene (0.001mg/100 ml), Calcium (196.68 mg/100g), Iron (1.47 mg/100g), Potassium (1196.56 mg/100g), Phytic acid (2.45mg/100g), and Overall sensory acceptability (4.13).The microbial load (total aerobic count) in the samples was within the acceptable range when the product stored at 4 °C(for 11 days) but it was spoiled after first day when stored at room temperature. From this study it can be, concluded that possibility of developing a product with good physicochemical and sensory quality when soymilk blended with MN and SS. It is suitable for all age groups in developing countries to supply nutritious food where hunger and protein energy malnutrition is prevalent. Furthermore the study is beneficial for cow milk intolerant groups (due to low cholesterol level and lactose intolerance) as an alternative source of cow milk. The study of the outcomes would be used to generate scientific baseline information for subsequent studies that focus on SBB value added products improvement and related programs. Today food and beverage manufacturers have targeted functional food as an extremely important marketing tool. Therefore, this result is beneficial to most of the society who use soymilk based beverage as important soymilk based non-alcoholic beverage with low cost as compared to dairy based products. However, care should be taken not to add as much quantities of mango nectar (more than 25 %) which would result in coagulation of the beverage might be due to reduced pH because of addition of more MN.

6. FUTURE LINE OF WORK

As future line of work the following points are recommended:

- i. The shelf life of pasteurized soy based beverage is short as proved in this work. To sustain efficient market and availability of soy based beverage a further microbial control and shelf life extension mechanisms should be sought to produce and distribute soymilk based beverages.
- ii. Further fortification study to enrich the beverage with important vitamins and minerals is an important point to be considered as future area of work to produce nutrient enriched beverage.

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Appendices Table 1. Estimated regression coefficients of physical parameter

Term	TCH		SG		T.S.S	
	RC	SE	RC	SE	RC	SE
A	0.5	0.991	1.00935	0.00213	2.33	0.05252
B	217.6	46.505	0.98917	0.09999	2	2.46504
C	33.4	138.135	1.02743	0.297	-41.88	7.32205
A*B	-180.0	61.468	0.04631	0.13216	6.86	3.25821
A*C	1.8	161.732	0.02467	0.34773	73.89	8.57282
B*C	-145.4	168.028	0.14784	0.36127	53.45	8.90659
R-sq	98.63		71.46		99.67	

A= Soymilk, B=Mango nectar, C= Sucrose solution, RC= Regression coefficient SE= Standard error R²= Determination coefficient

Appendices Table 2. Estimated regression coefficients of proximately analysis

Term	MC		Ash		Protein		Ether extract		CHO		Calorie	
	RC	SE	RC	SE	RC	SE	RC	SE	RC	SE	RC	SE
A	90.864	0.1653	0.1609	0.0058	5.459	0.0742	2.359	0.023	1.16	0.1944	47.69	0.677
B	79.977	7.7573	0.2416	0.2737	0.385	3.4842	-2.253	1.072	21.65	9.122	67.86	31.774
C	74.082	23.042	-0.7418	0.8128	0.595	10.349	-4.105	3.184	30.17	27.096	86.11	94.38
A*B	7.143	10.253	-0.2065	0.3617	-2.672	4.6053	3.619	1.417	-7.88	12.057	-9.65	41.998
A*C	10.922	26.978	0.807	0.9517	-6.545	12.117	5.202	3.727	-10.39	31.724	-20.91	110.5
B*C	16.964	28.029	0.9454	0.9887	7.036	12.589	5.879	3.873	-30.82	32.959	-42.25	114.8
R-sq	94.49		78.35		98.98		99.14		98.64		84.3	

Appendices Table 3. Estimated regression coefficients of TA and P^H

Term	TA		PH	
	RC	SE	RC	SE
A	0.03876	0.001213	6.9737	0.1201
B	0.03609	0.056941	6.8358	0.56363
C	0.03387	0.169136	5.5686	1.67418
A*B	0.08783	0.075263	-0.9865	0.74499
A*C	0.04965	0.198028	0.4349	1.96017
B*C	0.17672	0.205738	0.9298	2.03648
R-sq	96.81		98.13	

A= Soymilk, B=Mango nectar, C= Sucrose solution, RC= Regression coefficient SE= Standard error R²= Determination coefficient

Appendices Table 4. Estimated regression coefficients of β -carotene

Term	Beta carotene	
	RC	SE
A	0.00073	0.000032
B	-0.00807	0.001514
C	-0.07158	0.004496
A*B	0.014457	0.002001
A*C	0.005566	0.005264
B*C	0.029716	0.005469
R-sq	98.86	

A= Soymilk, B=Mango nectar, C= Sucrose solution, RC= Regression coefficient SE= Standard error R²= Determination coefficient

Appendices Table 5. Estimated regression coefficients of mineral content

Ca		Fe		K	
RC	SE	RC	SE	RC	SE
245.91	4.616	1.84	0.03384	1617	15.03
67.52	216.636	-1.5	1.58806	1253	705.54
-41.07	643.487	-11.94	4.71711	11879	2095.71
-89.59	286.343	1.31	2.09905	-697	932.56
-3.74	753.409	13.04	5.5229	-16776	2453.7
322.94	782.752	18.01	5.73793	-12611	2549.23
96.52		98.02		99.45	

A= Soymilk, B=Mango nectar, C= Sucrose solution, RC= Regression coefficient SE= Standard error R²= Determination coefficient

Appendices Table 6. Estimated regression coefficients of phytic acid

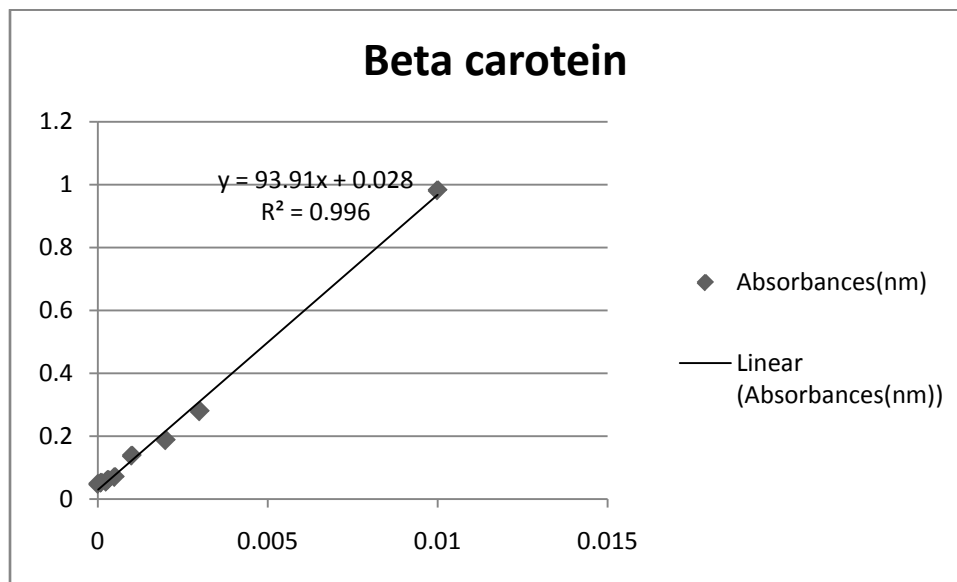
Term	Phytic acid	
	RC	SE
A	3.31	0.0906
B	-12.5	4.2533
C	-8.046	12.6338
A*B	13.41	5.6219
A*C	8.15	14.7919
B*C	15.6	15.3678
R-sq	98.17	

A= Soymilk, B=Mango nectar, C= Sucrose solution, RC= Regression coefficient SE= Standard error R²= Determination coefficient

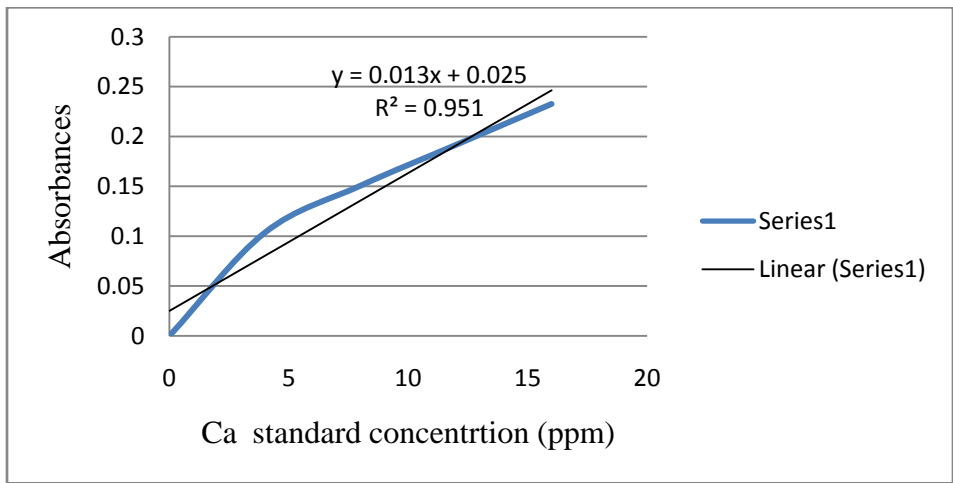
Appendices Table 7. Estimated regression coefficients of sensory quality

Term	Color		Flavor		Taste		Aroma		Mouth feel		Overall acceptable	
	RC	SE	RC	SE	RC	SE	RC	SE	RC	SE	RC	SE
A	4.208	0.09	3.613	0.0334	3.73	0.027	3.002	0.0489	3.354	0.043	3.56	0.0687
B	0.776	4.26	1.04	1.569	0.86	1.27	1.991	2.295	1.603	2.042	-2.41	3.2231
C	3.098	12.669	-1.302	4.66	0.87	3.78	7.31	6.817	4.54	6.07	1.47	9.57
A*B	-0.407	5.637	5.56	2.07	5.98	1.68	3.665	3.033	7.095	2.7	10.023	4.26
A*C	0.662	14.83	7.331	5.457	5.015	4.426	-3.989	7.98	4.115	7.1	6.166	11.2
B*C	-1.325	15.41	11.269	5.669	8.87	4.598	5.581	8.292	-1.998	7.38	14.196	11.64
R-sq	94.24		95.59		97.04		95.67		98.05		90.88	

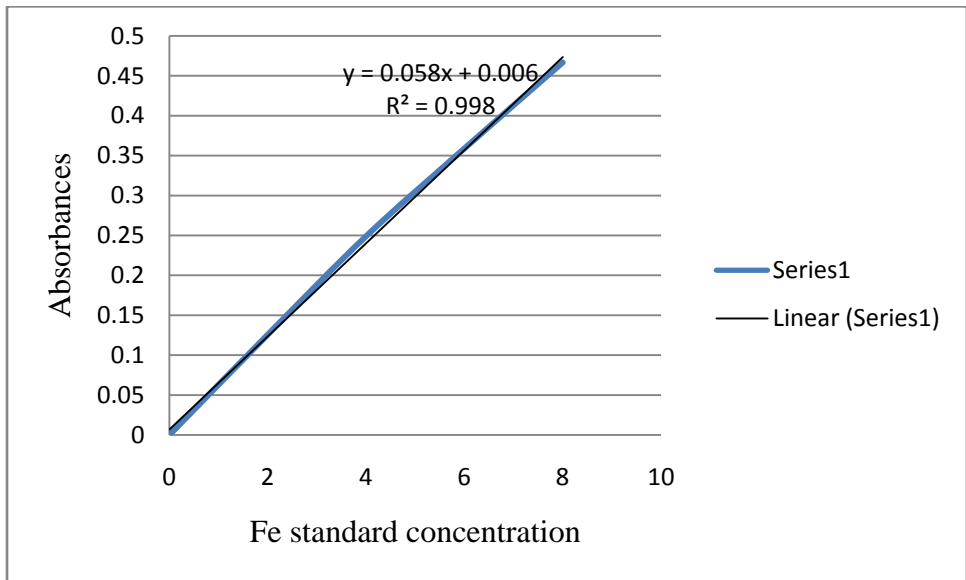
A= Soymilk, B=Mango nectar, C= Sucrose solution, RC= Regression coefficient SE= Standard error R²= Determination coefficient



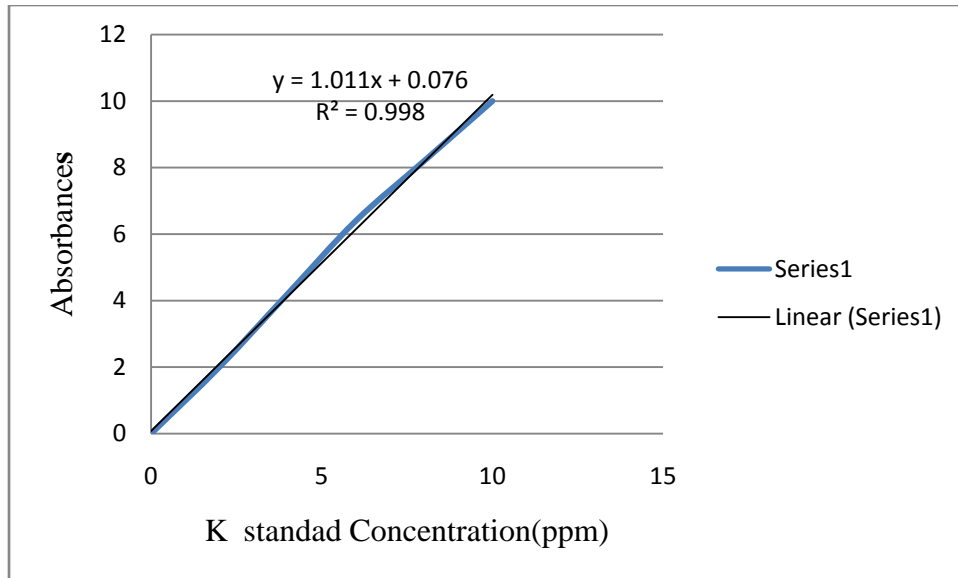
Appendices Figure 1. Beta carotene standard curve of soymilk based beverage



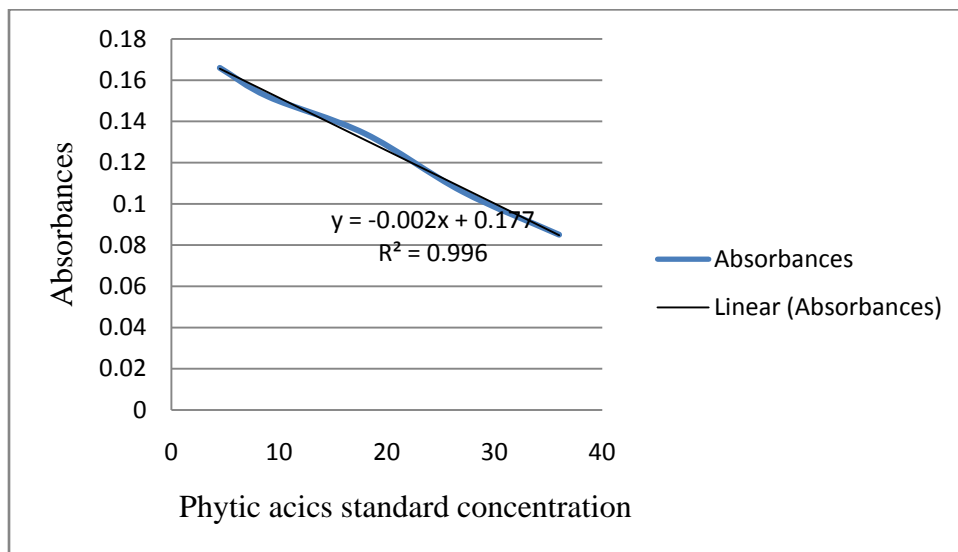
Appendices Figure 2. Calcium standard curve of soymilk based beverage



Appendices Figure 3. Iron standard curve of soymilk based beverage.



Appendices Figure 4. Potassium standard curve of soymilk based beverage.



Appendices Figure 5. Phytic acid standard curve of soymilk based beverage

Sensory evaluation questioner form

Date: _____

Time: _____

You are provided with a total of soymilk based beverage samples each with a three digit code. Please assesses the samples in the given order and rate your evaluation in accordance with the following scale.

N.B Please clean your mouth with water before you proceed from one sample to the next.

Scale:

1=Dislike Extremely

2=Dislike Moderately

3=Neither like nor dislike

4=Like Moderate

5= Like Extremely

Appendices Table 8. Sensory Evaluation Form

S. No	Sample Code	Color	Flavor	Taste	Mouth feel	Aroma	overall acceptability
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							

Suggestions and Comments:

Thank you very much for your participation