MAKERERE UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

DEPARTMENT OF AGRICULTURAL AND BIOSYSTEMS ENGINEERING

OPTIMIZING THE PROCESSING CONDITIONS AND FORMULATION OF A BANANA-VEGETABLES INSTANT SOUP FLOUR

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DECLARATION

I, Paddy Ainebyona, declare that this research thesis is my original work and has never been submitted to any University or institution of higher learning for an academic award.

……..……

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DEDICATION

This special project report is dedicated to my parents Mr. Kamushwa George, Mrs. Birungi Lydia for their positive motivation and to their grandson Abdul AwwalAinebyona.

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LIST OF ABBREVIATIONS

&	And
µg	micrograms
AAA-EAHB	Musa, spp; East African Highland Bananas
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASLT	Accelerated Shelf Life Testing
CCD	Central Composite Design
DFA	Desirability Function Approach
Dr.	Doctor
et al.,	And others
FOSS	Free and open-source software
g	grams
Ha	Hectare
HCl	Hydrochloric Acid
IBM	International Business Machines
IITA	International Institute of Tropical Agriculture
IU	International unit
IVPD	In Vitro Protein Digestibility
kg	Kilogram
MD	Mixture Design
NaOH	Sodium hydroxide
ND	Not Defined
NZFSA	New Zealand Food Safety Authority
^{o}C	Degrees Celsius

RAE	Retinol Activity Equivalents
RCCN	Research Centers Collaborative Network
RELOAD	Reducing Losses and Adding Value
RSM	Response Surface Modeling
RTE	Ready To Eat
RVA	Rapid ViscoAnalyser
SD	Standard Deviation
SFTNB	School of Food Technology, Nutrition and Bioengineering
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
WAI	Water Absorption Index
WSI	Water Solubility Index

CHAPTER ONE: INTRODUCTION

1.1. Background

Banana fruits are commonly grown in the tropical climates (Daramola & Osanyinlusi, 2006). Global annual production of bananas stands at 72.5 million metric tons (Radha Krishna *et al.,* 2012). About eleven million metric tons of these are produced in Uganda (Tumutegyereize *et al.,* 2011; UBOS, 2016). This makes Uganda the second world's leading producer of cooking bananas after India which has an annual capacity of 27.575 million tons (Daily Records, 2018). In Uganda, production of bananas is done by 70% of farmers (Bagamba *et al.,* 2007) emanating mainly from western and central parts of the country. The western region contributesabout 68% of total bananas to the market (Daily Records, 2018). Banana business contributes 42%to rural household income (Kawongolo, 2013) and serves as a staple food for 70% of Ugandans.

Banana production and marketingfaces challenges in developing countriessuch as; delays in transportation due to poor road networks and transport means, poor storage systems, poor post-harvest technologies and fluctuating prices and market demands.These factorscontribute to ripening and senescence thuscausing post-harvest lossesin bananas. Muranga *et al.,* (2010), estimates these losses to between 22 to 45% of total production translating to annual losses of between $411.9-$842.5 millions. In an attempt to reduce banana post harvest losses most studies have focused on development of shelf stable products mainly focussing on drying to obtain flours. Muranga *et al.,*(2010)developed a raw Tooke flour for use in confectionaries and has also developed a number of products through Presidential Innitiative on Banana Industrial Development (PIBID). Most products developed from bananas have focussed on addressing issues related to malnutrition reported to be one of the rampant causes of death to children in the country.

Global health reportsattribute malnutrition due to lack of micronutrients asone of the major causes of health issues in children(Herrador *et al.,* 2014). More than 2 billion people most of whom are children lack key vitamins and minerals such as Vitamins A;C;B_6;B_{12}, Iron, zinc, and potassium (Herrador *et al.,* 2014) contributing to more than 50% of childhood mortality in developing countries (Thaoge *et al.,* 2003). In Uganda, low levels of iron has increased anemic rates to 73% (Tidemann-Andersen et al., 2011; Tumwegamire et al., 2011) while the prevalence of zinc deficiency ranges between 20–69% among children (Akande*et al.,* 2017).This indicates that commonly consumed staplesare deficient in

protein, vitaminsand nutrients required for normal body health. Development of nutrient dense foods from availableraw materials could provide means of reducing nutrient deficiencies in diets(Amegovu *et al.,* 2014). In this study, development of nutrient dense soup from bananas was explored.

Analysis of banana flour shows high starch content, extended shelf life due to low sugar and fat, high level of potassium and low tannins. Banana flour however, is deficient in key micro nutrients such as vitamins and minerals like iron and zinc. This therefore implies that there are limitations in development of products from bananas. There is need to add supplements that can improve banana nutrient levels to make it a suitable raw material for product development. Banana could be supplemented with grains, vegetables, legumes to make it possible to develop products such as instant porridges, soups, among others. In this study options for development of nutrient dense soup for children below five years of age were explored to reduce reported cases related to nutrient defficiences in current staple diets. Required nutrients were obtained from grains and vegetables.However, to reduce the time spent in cooking the soup and to improve on the nutrient concentration of the soup, extrusion cooking was used(Abdel-haleem and Omran, 2014).

Extrusion being a high temperature-short time process has numerous advantages such as versatility, high productivity, low operating costs, energy efficiencyand high quality products (Milán-Carrillo *et al.,* 2012). Extrusion increases digestibility of starch and protein (Diaz *et al.,* 2013). Extrusion also breaks down mineral-antinutrient complexes through hydrolysis thereby increasing mineral availability in extrudates, altering vitamins, and eliminating anti-nutrient factors such as phytic acid which modifies the nutritional properties of the extrudates(Pathania*et al.,* 2013; Sundarrajan, 2014).

1.2. Problem statement

Cooking banana is reported to be one of the staple foods in Uganda with the highest (220 kg) per capita consumption in the world (Kiiza, Abele, & Kalyebara, 2004). Bananas are consumed in various forms, such as cooked green, cooked ripe, cooked in the peel, steamed, made into juice, ripened for dessert, roasted, chipped and fried or dried and floured to make a host of confectionaries(Karamura *et al.,* 1998). Banana flour is utilized in the development of different products such as thin and stiff porridges, soups, bakery and confectionaries among others. Although bananas and their products are reported to be rich in starch, sugars and vitamins A and C, potassium, calcium, sodium and magnesium(Ashokkumar *et al.,* 2018), banana flour is deficient in key nutrients such as proteins, carotenes and minerals such as iron and zinc. The deficient key nutrients in bananas however, can be

obtained from vegetables which are rich in vitamins such as carotenes, folates, and thiamine and minerals; iron and zinc. The improvement of micronutrient levels of banana flour would consequently improve its nutrient levels thusa potential to reduce malnutrition levels in children below five years of age in Uganda. On the other hand, boosting the micronutrient levels of banana flour does not guarantee the availability of the nutrients. In order to improve the mineral availability, starch solubility and protein digestibility of the banana-vegetables mix, extrusion technology is the best option. However, there is limited information on the effect of extrusion conditions (barrel temperature, screw speed and moisture content) on nutritional properties, physicochemical and acceptability of banana-based flours. This studytherefore, aimed at formulating and optimizing the extrusion conditions for the production of instant banana-vegetables soup powder.

1.3. Objectives

1.3.1.Main objective

To optimize the production process for a banana-vegetables instant soup flour.

1.3.2. Specific objectives

1. To optimizethe quality ofsoup formulations fornutritional quality and physicochemical properties of banana-vegetable soup powder.
2. To optimize extrusion conditions and determine the effect of incorporation of cooked vegetable-chicken mix on sensory acceptability, nutritional composition and physicochemical properties of banana-vegetable soup powder.
3. To determine the effect of incorporation of cooked chicken-vegetable mix on shelf-stability of the instant soup powder.

1.4. Hypotheses

1. $H_0; \mu_1 = \mu_2 = \mu_3$

Where μ_1, μ_2, and μ_3 show that there are changes in Nutritional composition as a result of changing proportions of bananas-amaranths and vegetables.

2. $H_0; \mu_1 = \mu_2 = \mu_3$

Where μ_1, μ_2, and μ_3 show that there are significant changes in Nutritional quality, physicochemical properties of foods after extrusion and incorporation of chicken on nutritional quality and acceptability of the soup.

3. $H_0; \mu_1 = \mu_2 = \mu_3$

Where μ_1, μ_2, and μ_3 show that there are significant effects of using chicken on shelf stability of banana vegetable soup flours.

1.5. Justification of the study

Bananas are seasonal with high post-harvest losses of 22-45%. This results into high nutritional and economic losses amounting of $411.9 – 842.5 million annually (Muranga *et al.,* 2010). To reduce such losses, value added products are required. This was increase shelf life of bananas thus ensuring a year-round availability. Post-harvest losses amounting to 30-40% are also recorded in vegetables (Kiremire *et al.,* 2010). Pumpkins, tomatoes, mushrooms and carrots were thought important for use in the study basing on their nutrient composition. Pumpkins are rich sources of minerals such as zinc and iron and vitamins (Saeleaw & Schleining, 2011). Carrots are good sources of vitamin A and C (Arscott & Tanumihardjo, 2010). The vegetables have a potential for improving the bananas micronutrients such as zinc, iron and vitamin A. to improve the nutrient composition of the product and also make the product a feasible alternative for the fast growing population, extrusion technology was used as a good alternative to make an instant product. This will serve to reduce the micro nutrient and macro nutrients malnutrition in the country. In addition, micronutrient and macronutrient malnutrition is recognized by the Ugandan government as a potential threat to the general health and welfare of the entire population. Fortification of foods therefore is strongly recommended by the government. The developed products can potentially contribute towards reducing recorded levels of malnutrition among children below five years.

CHAPTER TWO: LITERATURE REVIEW

2.1. Bananas

The focus of this study was about utilization of Bananas in developing longer shelf stable products. Understanding bananas is therefore of paramount importance for this study. Bananas belong to *Musa acuminata* and *Musa balbisiana* (Figure 2.1) species. Bananas of the genus Musa are part of the family Musaceae. They are considered to be derived from the wild species *acuminata (AA)* and *balbisiana (BB).* The plantain, or cooking banana, is classified as *Musa paradisiacal.* Bananas are mainly tall, upright, and fairly sturdy. They are often mistaken for treesand has a pseudo-stem which stretches to a height of up to 2–8 m. The leaves can be of up to 3.5 m in length. Banana fruit grows in hanging clusters, with up to 20 fruits to a tier (called a hand) and 3-20 tiers to a bunch. The total of the hanging clusters is known as a bunch and can weigh between 30–50 kg. A single fruit averages 125 g, of which approximately 75% is water and 25% dry matter content. The edible part of the banana contains, on average, 75% water, 10% carbohydrates, and about 1% fat, 3% protein and 10% fiber.

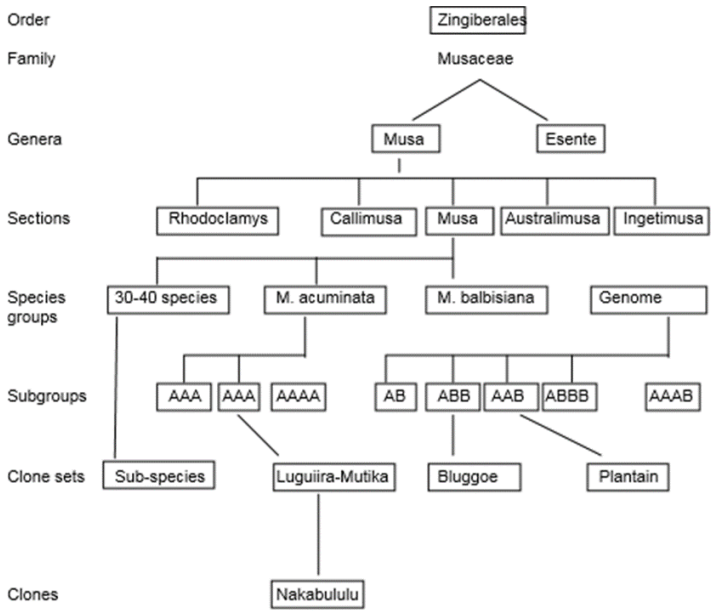


Figure 2.1: Classification of Bananas (Karamura *et al.*, 1998)

2.2.1. Banana production in Uganda

Banana is the world's fourth most widely consumed crop after maize, rice, and wheat. It is used as a staple food for most countries in the tropics and subtropics regions. The development of high yield, short-time growth and disease resistant banana varieties by institutions of agriculture has increased the

production volume of banana at harvest. At the global level, banana production is estimated at 72.5 million metric tons per year (Radha Krishna *et al.,* 2012). Six million tonnes are produced annually in Uganda (Tumutegyereize *et al.,* 2011). This production is from 70% of farmers in Uganda's major producing districts (Bagamba *et al.,* 2007) on approximately 973,340 Ha (Uganda Bureau of Statistics, 2016). These bananas are mainly transported to urban areas, where they are eaten as fruit vegetables.

2.2.2. Physical characteristics of bananas

Bananas are perennial crops with high water succulent stems that give them high resistance to seasonal droughts. Both *Musa acuminata* and *Musa balbisiana*varieties differ in physical characteristics. Both types mature in about three months from flowering time. The "*AAA-EA*" Mbidde type of bananas has compacted bunches of fruit, with starchy banana fingers, but require an expert to differentiate them from the cooking varieties. The Kisubi banana trees are usually shorter than those of cooking varieties, whereas the Sweet Ndiizi cultivar has smaller banana fingers and smaller stems in the mother crop. The average size of a banana fruit is 150 to 200 mm in length, with a diameter of 40 to 60 mm (Karamura *et al.,* 1998). The cooking variety (plantain) is usually harvested green, while the juice-yielding type can be harvested at full maturity when the first fingers near the stalk turn yellow. The ripe fruit has a unique flavor (taste and aroma).

2.2.3. Chemical characteristics of bananas

Chemical properties of cooking banana cultivars are presented in table 2.1. The chemical composition of bananas varies and the variations are reported to be the result of many factors, including ecological location, nutrition, location on the bunch from which the banana fingers are sampled for analysis, and maturity of the fruit at harvest (IITA, 1993). The different cultivars also differ considerably in terms of their chemical composition with regards to starch, sugar, fat, minerals, acidity, water content, pectin, and tannins among others. The starch content of ripe and unripe banana fruits lies in the range of 0 - 28.6% (Byarugaba-Bazirake, 2008). Kayisu, Hood, & Vansoest, (1981) reported that unripe bananas have starch contents of 20 to 25%, although no mention was made of the particular cultivar that was tested.

The average moisture content in most banana varieties is 75% with approximately 0.5-1% pectin when ripe. In the banana pulp, insoluble protopectin decreases from about 0.5% to about 0.3% fruit weight and soluble pectin shows a corresponding increase during ripening(Wills, Lim, & Greenfield, 1984).

Byarugaba-Bazirake, (2008) reported that fully ripe bananas might contain up to one-third to two-thirds of its pectin in soluble form. Tannins as chemical components of bananas are of great significance for flavor. When tannins react with proteins and glycoproteins on the surface of the tongue and buccal mucosa, they cause a drying and puckering sensation known as "astringency".

Table 2.1 Chemical composition of matooke from different cultivars

Chemical composition	**Cultivar and chemical composition (%)**		
	Nandigobe (AAA-AE)	**Bukumu (AAA-AE)**	**Embururu (AAA-AE)**
Starch (%)	81.8	82.5	82.9
Protein (%)	4.71	5.1	4.01
Fat (%)	0.87	ND	0.56
Crude fibre (%)	1.25	ND	1.33
Ash (%)	4.34	3.58	4.1
Calcium (µg/L)	0.0058	0.0044	0.0052
Potassium (µg/L)	1.9	1.82	1.84
Magnesium (µg/L)	0.09	0.09	0.01
Tannin (Abs at 500nm)	0.111	0.181	0.012

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

2.2.4. Nutritional value of banana

Bananas are excellent sources of potassium. Potassium can be found in a variety of fruits, vegetables, and even meats, however, a single banana provides 23% of the potassium that a person needs on a daily basis. Potassium benefits the muscles as it helps maintain their proper working and prevents muscle spasms. In addition, recent studies are showing that potassium can help to decrease blood pressure in individuals who are potassium deficient. Potassium also reduces the risk of stroke. Bananas are excellent source of vitamins, including vitamin A which aids in healthy teeth, bones, soft tissue, and more, B_6 which aids the body's immune system, promotes brain health, heart health, and more, C which aids in healing, growth of tissue, ligaments, and more and vitamin D which helps the body to absorb calcium. In addition, bananas aid in vitality, which makes a person have more energy both mentally and physically. Bananas are also an excellent food for people who want to lose weight.

2.2.5. Banana value chain in Uganda

Annual domestic banana consumption is between 220-460 kg/person and is the highest in the world(Kiiza *et al.,* 2004). It is the main food staple for some 13 million people and an important source of food, nutrition, and income security for smallholder producers. Producers consume about 70% of harvested bananas at home. Banana consumption (and production) is concentrated in the central and western regions with the latter having the highest consumption. Consumption is least in the northern region (figure 2.2). Processing of bananas into value added products takes the least share and stands at 3%. This can depict the amount of post-harvest losses in faced by bananas since shelf stable products out of bananas are low. Thus, processing of bananas into value added products is one area that is lacking.

Figure 2.2: Banana end markets in Uganda and consumption (Source: Bill and Melinda gates foundation report, 2009)

2.2.6. Processing of bananas into value added products

Bananas can be processed into chips, raw ripened fruit, cooked green banana, fermented and unfermented beverages, juice, puree, and dried flour for bakery and infant formula food (Mohapatra, Mishra, & Sutar, 2010) as shown in figure 2.3. Banana is also used as a starch source for various chemicals and packaging materials.

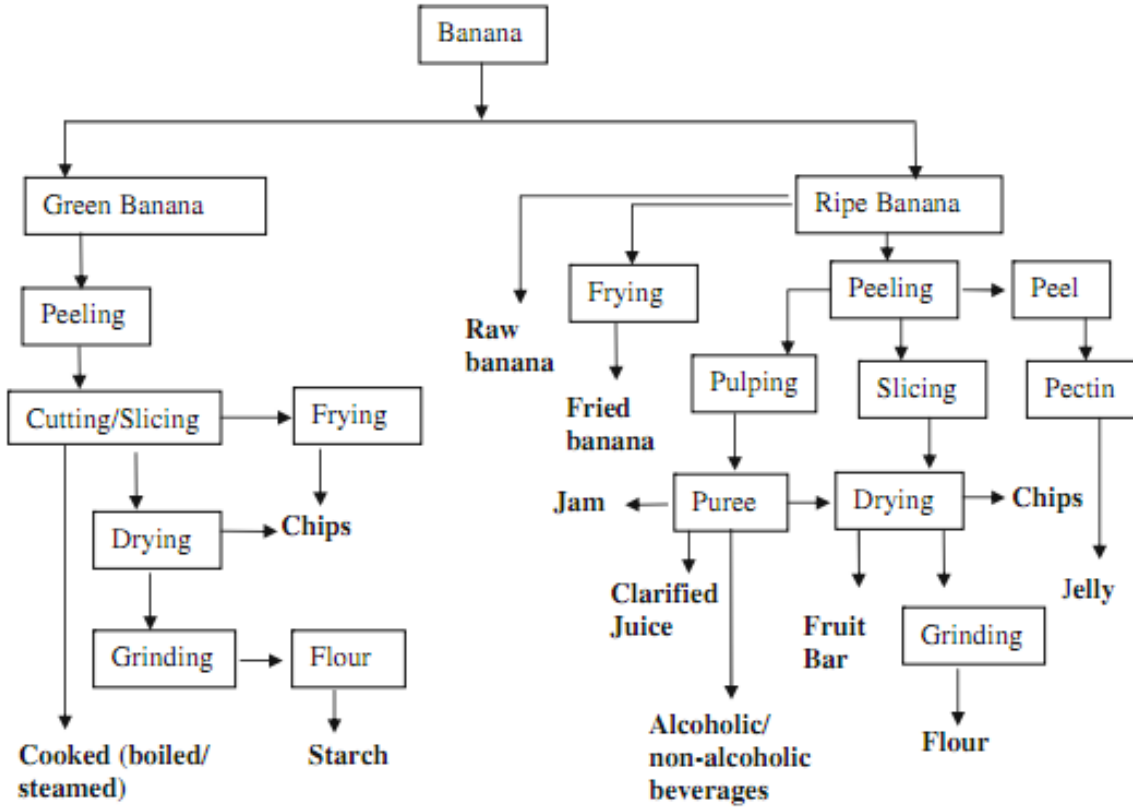


Figure 2.3: Flow chart showing processing of bananas into different products(Mohapatra et al., 2010)

In Uganda, the Presidential Initiative for Banana Industrial Development (PIBID) located in western region is the leading head in banana processing. PIBID gets banana flour from matooke to increase shelf-life of fresh bananas. According toMuranga *et al.*, (2010), Matooke (Green banana) is a very starchy food, compared to maize and potatoes, thus produces a better product. Banana flour can also be used as an alternative to wheat for its good starch composition in confectionaries.

In Kampala, a supermarket survey concluded that some available products include "Ndizi" crisps from apple bananas. These are made from locally grown bananas and plantain or "gonja". Another available productcalled Fantazee banana brunch, is manufactured by Deepa Industries in Kenya. Since most bananas are produced for traditional cooking and brewing at home, bananas are seen as subsistence fruits. Despite being the world's second largest producer of bananas (after India), Uganda does not feature among the important countries trading bananas or their products on the world market. This has been greatly contributed by low shelf life and high post-harvest losses.The feasible alternative to make products from bananas would propose improving shelf-life as well as reducing post-harvest

losses thus calling for value addition. Development ofsoups from cooking bananas is a new area of interest for research.

2.2. Description of soups

Soup is defined as a liquid food made by stewing ingredients such as vegetables, meat, fish often in a stock and with seasoning (Fatima & Al-Subhi, 2013). Hot soups are additionally characterized by boiling solid ingredients in liquids in a pot until the flavors are extracted, forming a broth.Traditionally, soups are classified into two main groups, clear soups and thick soups (Radha, *et al.*, 2015). Clear soups are based on clear un thickened broth or stock. Clear soups can be served plane or with a variety of vegetables or meat. Thick soups are classified depending upon the type of thickening agents used.Purées are made from vegetables thickened with starch.Bisques are made from puréed shellfish or vegetables thickened with cream. Cream soups can be thickened with eggs, butter, and cream. Other ingredients commonly used to thicken soups and broths include eggs, rice, lentils, flour, and grains. Other popular soups also include carrots and potatoes soup. In the interest of this study, thick soups were produced. Thick soups were made from banana flour, Amaranths supplemented with either tomatoes, mushrooms, pumpkins or carrots or all of them. The thickening agent was starch from bananas.

2.2.1. Commercial soups

Commercial soup became popular after the invention of canning in the 19th century. These soups can take two forms: canned soups and dried soups.

2.3.1.1.Canned

Canned soup can be condensed, in which case it is prepared by adding water (or sometimes milk). Canned soup can be prepared by heating in a pan, on the stovetop or in the microwave. Such soups can be used as a base for homemade soups, with the consumer adding anything from a few vegetables to eggs, meat, cream or pasta. Condensing soup allows soup to be packaged into a smaller can and sold at a lower price than other canned soups. Since the 1990s, the canned soup market has burgeoned with soups marketed as *ready-to-eat,* which require no additional liquid to prepare. Microwaveable bowls have expanded the ready-to-eat canned soup market, even more, offering convenience and are popular lunch items.

2.3.1.2.Dried

Dry soup mixes are sold by many manufacturers and are easy to reconstitute with hot water. During preparation, other fresh ingredients may be added. The first dried soup was bouillon cubes. East Asian-style instant noodle soups include ramen and seasonings. They are marketed as a convenient and inexpensive instant meal, requiring only hot water for preparation. Other dried soups include vegetable, chicken base, potato, pasta and cheese flavors.

The soups analysis shown above requires that dried soups are durable and more convenient than canned soups. They have longer shelf-life than canned soups. In this study therefore, dried soups are preferred. Dried soups were developed from dried banana flour together with other vegetables. Grain amaranth were used to improve the protein value of bananas. Vegetables chosen include carrots, pumpkins, tomatoes and mushrooms. Unnecessary vegetables wereeliminated by carrying out a response surface modelling using design expert version 11.

2.3. Choice of supplements to banana flour…for.

Provision of a nutrient-rich mixture from banana flour requires supplements. Vegetables have been chosen for their availability and a good nutrient base.Mushrooms, pumpkins, tomatoes and carrots, and amaranth grains wereused. Knowledge about these vegetables and grains is paramount.

2.3.1. Grain Amaranth

Grain amaranth belongs to order *Caryophyllales*, family *Amaranthaceae*, sub-family *Amaranthoideae*, genus *Amaranthus*, and tothe section *Amaranthus*. The genus *Amaranthus*includes approximately 60 species. Most of these species are cosmopolitan weedswhile others are cultivated amaranth species. The cultivated species can be used as food grain, leafy vegetables, forage and ornamentals(Mlakar, *et al.*, 2010). The principal species considered for grain production include *Amaranthushypochondriacus, A. cruentus and A. caudatus*.Amaranth is a highly nutritious grain; the seeds of *A. hypochondriacus* contain 15-20%of lysine-rich protein (3.2-6.4 g/100g protein compared to 2.8-3.0 g/100g protein for wheat), 58-66% of starch, 6-9% of raw fiber and 6-8% of highly unsaturated lipids. Amaranth has high concentrations of calcium (250 mg/100g) and iron (15 mg/100 g), which areten and four times higher, respectively, than those found in wheat (Milán-Carrillo *et al.*, 2012). Amaranth is an excellent source of iron (Table 2.2).

2.3.2. Pumpkin

Pumpkin belongs to genus *Cucurbita*of the family *Cucurbitaceae,*whichis one of the largest families of the plant kingdom(Bhat & Bhat, 2013). Pumpkinsare widely grown and consumed in many tropical and sub-tropical countries around the world. Pumpkins are found in many shapes, sizes and colors. Pumpkins are consumed in a variety of ways such as fresh or cooked vegetables. Pumpkin can be processed into flour which has a longer shelf-life. Pumpkin flour is used because of its highly-desirable flavor, sweetness and deep yellow-orange color. It has been reported to be used to supplement cereal flours for soups, sauces, instant noodle and spice as well as a natural coloring agent in pasta and flour mixes(Kuchtová et al., 2016). Pumpkin is rich in β-carotene (Table 2.2), vitamins, minerals, pectin and dietary fiber(Saeleaw & Schleining, 2011). Consumption of foods containing carotene helps prevent vitamin deficiencies such as skin diseases, eye disorders etc. Incorporation of β-carotene rich materials in human diet is therefore considered a cost-effective approach to vitamin-A related health problems (Pongjanta et al., 2006).

Pumpkin flours have been utilized to produce vitamin A rich soups(Ravi, Lakshmi, & Ranjani, 2010a) reported an increase in the β-carotene content of complementary weaning mix based on sorghum, green-gram and rice flour on incorporation of pumpkin flour. Swar, Alhaj, & Osman, (2014) reported that a vitamin A rich porridge can be obtained from blends of sorghum flour and pumpkin pulp to address vitamin A deficiency in Sudan. In Ethiopia, Abebe et al., (2011) found out that addition of pumpkin to corn-legume blend and *Kocho* legume blend increased the vitamin A content of the mixes by 25 and 180-fold respectively.

2.3.3. Carrot

Carrot (*Daucuscarota* L) is one of the popular root vegetables grown throughout the world. China is the major carrot producing country in the world (Sharma, Karki, Thakur, & Attri, 2012). Carrot is an economically important horticultural crop that has gained popularity in recent decades due to increased awareness of its nutritional value (Arscott & Tanumihardjo, 2010). Carrots contribute significantly to dietary vitamin A intake through α- and β-carotene (Table 2.2) and modestly to other nutrients (Arscott & Tanumihardjo, 2010). Carotenoids are important micronutrients for human health. The total carotenoids content in the edible portion of carrot range from 6000 to 54,800µg/100g(Sharma *et al.,* 2012).In India, carrot powder has been incorporated into dal soup to address vitamin A deficiency

(Pandey & Kulshrestha, 2003).Onabanjo *et al.,* (2008) blended carrot powder in addition to other ingredients to develop a complementary food.

Table 2.2: The nutritional composition of grain amaranth and some selected vegetables (Value per 100g)

Nutrient	Grain amaranth	Mushrooms	Tomatoes	Carrot	Pumpkin
Energy (Kcal)	371.00	328	302	341	364
Protein (g)	13.56	10.0	12.91	8.10	14.29
Lipids (g)	7.02	4.0	0.44	1.49	3.57
Carbohydrate* (g)	65.25	64.66	74.68	79.57	71.43
Iron (mg)	7.61	1.00	4.56	3.93	4.50
Zinc (mg)	2.87	1.54	1.71	1.57	0.32
Vitamin A (µg RAE)	0.00	0.00	862	3423	0.00
Sugars (g)	1.69	3.26	43.9	38.82	28.57
Fiber, dietary (g)	6.70	4.8	16.5	23.6	14.3
Vitamin C (mg)	4.20	7.0	116.7	14.6	0.00

*Content determined by difference **Source:** USDA (2018)

2.3.4. Mushrooms

Mushrooms are healthy foods. They are poor in energy content and fat but are rich in vegetable proteins, chitin, vitamins and minerals (Manzi *et al.,* 1999). Mushrooms have a great nutritional value since they are quite rich in protein. They are sources of important content of essential amino acids and fiber, with poor fat content(Reis *et al.,* 2012). Edible mushrooms are rich sources of vitamins (B1, B2, B12, C, D and E) (Reis *et al.,* 2012).Mushrooms have also been reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer (Manzi, Aguzzi, and Pizzoferrato, 2001). The nutritional content of mushrooms is indicated in table 2.2 above.

2.3.5. Tomatoes

Known scientifically as *Solanumlycopersicum*, tomato is the berry of a plant from the nightshade family. It is native to South America. Despite technically being a fruit, tomato is generally categorized as a vegetable.Tomatoes are the major dietary source of antioxidant lycopene, which has been linked to

many health benefits, including reduced risk of heart disease and cancer.They are also a great source of vitamin C, potassium, folate and vitamin K.They are usually red when mature, but can come in a variety of colors, including yellow, orange, green and purple.Then there are many subspecies of tomatoes, with different shapes and flavor.

The combinations of different compositions of soups are thought to supply the nutritional demands of children aged 1-5 years shown in table 2.3.

Table 2.3: Recommended daily Nutritional requirements for 2-5 years old children

Category	Age (yrs)	Protein (g)	Fat (g)	Energy (kCal)	Calcium (mg)	Iron (mg)
Children	2-3	16.7	27	1060	600	9
Children	4-6	20.1	25	1350	600	13

The correct quantity of different proportions that can give the daily nutritional requirements of children aged 2-5 years wasobtained by carrying out response surface modeling. Response surface modeling can be effective when the process is carefully thought about through optimization of different ingredients. There is need to understand the process of optimization.

2.4. Process optimization

2.3.6. Background

Optimization improves performance of a system, process, or product with an objective of obtaining maximum benefit(De Aguiar *et al.*, 1995). Issues with optimization usually arise in food production and processing while designing, developing and managing quality of food products. Optimization of food productionseeks to provide answers to the following questions.

1. How changes in one set of variables affect overall food quality? How are they correlated with each other?
2. Which food raw materials and recipes should be used to produce an optimal quality food product?
3. Which processing conditions must be used to getthe best food product?
4. How to determine the cause of food product defects and further control them?

5. Are the recipes and processing conditions currently used for an existing food product the best?
6. What measures can be taken to increase production yield?
7. Is it possible to have a successful food product with cost-effective raw materials and procedures?

2.3.7. Optimization methods

There is a myriad optimization approaches which can be employed in process design. These include fractal analysis, Response Surface Methodology (RSM), generic algorithms, multivariate analysis, fuzzy logic and linear programming (Sablani, 2008). Majority of the conventional approaches are arduous and time-consuming since they involve numerous runs. Interaction factors are also ignored and thus chances of obtaining optimum conditions are uncertain (Mu, *et al.*, 2009). Additionally, some of these conventional methods like generic algorithms and fuzzy logic are very complex and not user-friendly. Additionally, there is limited knowledge on the use of these optimization approaches hence their limited application (Sablani, 2008)

2.3.8. Choice of an optimization method

Response Surface Methodology (RSM) is the commonest and most effective tool used in the food industry and biotechnology for optimizing processes (Bas and Boyaci, 2007). RSM involves a collection of statistical and mathematical techniques that are useful for developing, improving, and optimizing processes in which a response of interest is influenced by several variables (Montgomery, 2001). The objective is to simultaneously optimise levels of the variables to generate best system performance (Box & Draper, 2007)).

RSM enables rapid, effective and efficient development of new products and process at a reduced cost and time (Hu, 1999; Peričin*et al*, 2008). RSM reduces the number of experiments required even when a myriad of variables is involved and this makes it less laborious and time-consuming than other methods (Wang *et al*, 2014). RSM also facilitates identification of operating conditions that favour high yields and production of certain products. RSM is flexible and widely used in production and optimization of different products (Singh *et al*., 2009).

2.3.9. Experimental designs in RSM

It is very important to select an experimental design before applying RSM. There are many types of experimental designs. The choice depends on the complexity of the model and whether models sufficiently represent the experimental data adequately. Examples of these experimental designs include three level factorials, Box–Behnken, central composite, optimal and Doehlert designs (Myers *et al.,* 2009). The designs differ from one another with respect to the number of levels of variables, runs, and blocks. However, Central Composite Design (CCD) and d-optimal are the most popular among the RSM designs (Bezerra*et al.,* 2008). The d-optimal design is commonly used in designing experiments with two quantitative factors having three levels each. D-optimal design minimizes the generalized variance of the estimated regression coefficients (Unal*et al*, 1998). Thus, offers an added advantage compared to CCD.

2.3.10. Steps involved in the application of response surface methodology

According to Montgomery, (2001)the steps followed during optimization using RSM include:

1. Selection of independent variables of major effects on the system through reviewing literature and the restriction of the experimental region, according to the objective of the study.

2. Choosing the experimental design and carrying out the experiments according to the selected experimental matrix.

3. Mathematical–statistical treatment of the obtained experimental data through the fit of a polynomial function.

4. Evaluating the model's fitness and model adequacy.

5. Verifying the requirement and possibility of performing a displacement in the direction of the optimal region.

6. Obtaining the optimum values for each studied variable.

2.3.11. Determination of optimum conditions for multiple variable responses in soups.

Several optimization methods can be used to optimize multi-response systems. The selection of an optimization method depends on the complexity, merits of the method and recommendations by other researchers. The different optimization methods include conventional graphics method, nonlinear programming methods, improved graphics method, extended response surface and desirability function

approach (Sablani, 2008).Desirability Function Approach (DFA) is the most important and widely used in optimization. DFA is an analytical technique for optimizing multiple responses simultaneously. DFA calculates individual desirability associated with each response and then an overall desirability (ranging between 0 and 1) can be calculated as the geometric mean of individual desirability. According to Banga et al. (2003), desirability level indicates the closeness of the response to the set target value. DFA is simple and quick to apply and allows subjective judgment on the importance of response variables compared to other techniques including comparing responses of different scales (Guillou&Floros, 1993). The different formulations were developed from which optimization of the responses could be determined. The processes used to make instant products is extrusion. There is need to understand the process of extrusion.

2.5. Extrusion

Extrusion cooking, as a multi-step, multi-functional and thermal/mechanical process has permitted a large number of food applications (Singh *et al.,* 2007). It has created a huge impact in the food industries towards shaping and deriving ready to eat products (Fellows, 2000). The use of extrusion in the food processing has increased its popularity due to its versatility, cost-effectiveness, environmental friendliness and better product output (Sundarrajan, 2014). The principle of extrusion involves loading of raw materials in the feeding hopper from where the screw conveys them through a barrel under pressure. The materials are compressed into a semi-solid, plasticized mass as they pass down the barrel. The selection of right extruder for the production of ready to eat (RTE) or cereal snacks depends on the nature of raw materials used, bulk density and type of product to be produced (Fellows, 2000).

2.3.12. Types of extruders

Two types of extruders are used for food production: single-screw extruders and twin-screw extruders. Single-screw extruders are the most common extruders used in the food industry. Twin-screw extruders are used for high-moisture extrusion, products that include higher quantities of components such as fibers, fats, etc. and more sophisticated products (Steel *et al.,* 2012).

2.3.12.1. Single-screw extruders

Single-screw extruders are the most common extruders applied in the food industry. The classification of single-screw extruders can be defined based on processes involved or equipment parameters such as conditioning moisture content (dry or wet), solid or segmented screw, desired degree of shear and heat source. However, practically, the main classification used considers the degree of shear and the heat

source (Steel *et al.,* 2012). Single-screw extruders can be grouped into four different types based on the degree of shear, as follows: cold forming extruders, high-pressure forming extruders, low-shear cooking extruders and collet extruders (Steel *et al.,* 2012)

2.3.12.2. Twin screw extruders

Twin-screw extruders are composed of two axes that rotate inside a single barrel; usually, the internal surface of the barrel of twin-screw extruders is smooth. Depending on the position of the screws and their direction of rotation, four different types of configurations are possible: (i) co-rotating intermeshing screws; (ii) co-rotating non-intermeshing screws; (iii) counter-rotating intermeshing screws; and (iv) Counter-rotating non-intermeshing screws. Conical intermeshing extruders also exist (Steel *et al.,* 2012). The advantage of using twin screw extruders is versatility to process a wide range of products like tortillas, cereal snacks, extruded corn snacks, and multigrain snacks. Due to high capital and maintenance costs, single screw extruders are considered to be cost-effective when compared to twin screw extruders (Sundarrajan, 2014). Figure 2.4 shows a schematic diagram of a single screw extruder

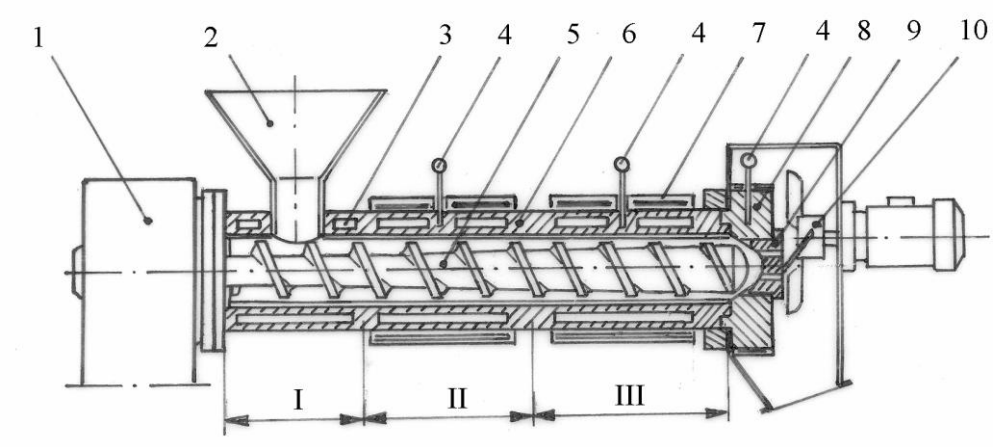


Figure 2.4: A cross-section of a single-screw extrusion-cooker:

1 - engine, 2 - feeder, 3 - cooling jacket, 4 - thermocouple, 5 – screw, 6 - barrel, 7 - heating jacket, 8 - head, 9 - net, 10 -cutter, I - transport section, II – compression section, III –melting and plasticization section (Moscicki *et al.,* 2013).

2.3.13. Processing parameters affecting the nutritional quality of extruded products

Different processing parameters have effects on the nutritional quality of extruded products. The primary process parameters include feed rate, screw speed, barrel temperature, moisture content, feed formulation, screw and die configuration. The secondary process parameters include dye temperature,

pressure, and torque (Sundarrajan, 2014). Razzaq *et al.,* (2012) studied the effect of barrel temperature, screw speed and moisture content on the chemical characteristics of maize (*Zea mays.* L) extrudates. The author found out that these extruder variables (150 °C, 170 rpm and 18% moisture content) considerably enhances apparent absorption of sodium, potassium, calcium, and iron. Increase in barrel temperature and feed moisture content causes an increase in the water absorption index (Agathian *et al.,* 2015). Several other workers have studied the effect of extrusion variables such as feed moisture, barrel temperature, screw speed, feed rate (Albarracín, *et al.,* 2015; Dias-Paes and Maga, 2004; Filli *et al.,* 2010; Ilo *et al.,* 1999).

2.3.13.1. Moisture content

Moisture acts as a plasticizer in extrusion cooking. Several workers have investigated the effect of feed moisture content in extrusion cooking especially its effect on functional properties of the extrudate. Bhise *et al.,* (2013) while studying the optimization of extrusion conditions for production of texturized flaxseed defatted meal by response surface methodology discovered that increase in feed moisture content causes an increase in fat absorption capacity, water holding capacity, water solubility index and water absorption index of the flour. According to Chávez-Jáuregui *et al.,* (2000), the study of on the extrusion cooking process for amaranth (*Amaranthuscaudatus*L.) showed that increase in feed moisture caused an increase in shearing stress, density, and a decrease in texture acceptability of the amaranth flour extrudate. Investigations carried out by Gbenyi, *et al.,* (2017) to evaluate the effect of feed moisture, barrel temperature and single screw extrusion on physicochemical properties and acceptability of grain sorghum showed that increase in feed moisture content and die temperature cause an increase in water absorption index, water solubility index and swelling power.

2.3.13.2. Barrel Temperature

The process temperature is an important parameter in extrusion cooking process. Ilo *et al.,* (1999) while investigating the extrusion cooking and product properties of rice flour and banana-amaranth blends discovered that increasing extrusion barrel temperature had a positive effect in the texture softening, but it increased the browning of the extrudate and probably the extent of nutritional loss due to Maillard reactions. Singh, *et al.,* 2007 analyzed the effects of extrusion temperature (164, 182, 200°C) levels (0-30%) and grits moisture content (g/100 g sample) (18-24%) on textural and physicochemical properties of rice grits.

2.3.14. Effect of extrusion cooking on nutritional quality

2.3.14.1. Protein retention and digestibility

Extrusion improves protein digestibility through denaturation, which exposes enzyme-access sites (Zarzycki *et al.,* 2015). Most proteins such as enzymes and enzyme inhibitors lose activity due to denaturation. The extent of denaturation is typically assessed as change in protein solubility in water or aqueous solutions. These changes are more pronounced under high shear extrusion conditions (Zarzycki *et al.,* 2015). Koeppe *et al.,* (1987) found a slight increase (from 82.1% to 85.4%) in the *in-vitro* protein digestibility of banana-amaranth-maize gluten meal extrudates. Extrusion cooking increased the *in-vitro* protein digestibility (IVPD) of African bread fruit-defatted soybean-corn flours by 22.77% (James & Nwabueze, 2013). Rampersad *et al.,* (2003) investigated the effect of extrusion cooking (barrel temperature of 130 °C, screw speed of 150 rpm and feed moisture 15%) and roasting on protein digestibility using the *in-vitro* method. The authors discovered extrusion cooking improved the *in-vitro* protein digestibility (IVPD) of the pearl millet based weaning foods.

2.3.14.2. Vitamin A retention

Vitamin A deficiency is a major cause of blindness in many less-developed nations, and the vitamin is important for healthy immune system function. Unfortunately, oxygen and heat destroy vitamin A and related carotenoids. Increasing barrel temperatures from 125 to 200ºC resulted in more than 50% destruction of trans β-carotene in wheat flour 50% (Zarzycki *et al.,* 2015). Extrusion cooking (25 % moisture content, 120 rpm and 100 °C) caused a significant decrease of vitamin A contents of extruded blend of pigeon pea and unripe plantain blends (Rampersad *et al.,* 2003). James and Nwabueze, (2013) reported 65.95% vitamin A retention of an extruded full-fat blend of African breadfruit soybean-corn snack at 21% moisture content and 140 °C barrel temperature.

2.3.14.3. Mineral bioavailability

Extrusion can improve the absorption of minerals by reducing other factors that inhibit absorption. Extrusion increases the apparent absorption of iron in extruded meal-based diets (Alonso, Rubio, Muzquiz, & Marzo, 2001). Poltronieri *et al.,* (2000) assessed the effect of extrusion cooking on *the in-vitro* iron availability of African weaning foods; their results show that extrusion increases the available iron.

2.6. Instant products

Instant products are a group of dried foods which play an important role in the nutrition of people because they can fulfill present and future social consumer requirements. Instant products come in diverse forms which aid easy access to nutrients (Usha, 2007). Pelembe *et al.,* (2002) developed a protein-rich composite sorghum–cowpea instant soups by extrusion cooking process. Instant grain base for use in weaning foods was made from a blend of wheat, mung bean and groundnut (Pathania *et al.,* 2013). Milán-Carrillo *et al.,* (2012) produced high antioxidant instant banana-amaranth flour suitable to elaborate a nutraceutical beverage. Shadan, *et al.,* (2014) formulated, prepared and evaluated low-cost instant products based on cereals and pulses.

2.7. Sensoryevaluation

Sensory evaluation refers to the science of judging and evaluating quality of food by the use of senses. It is of practical application in product development and helps in product matching, improvements, grading as well as establishing the worth of acceptance of a product(Meilgaard, Sc, Civille, & Carr, 1999). Sensory attributes of a food product are important in its overall acceptance (Cardello, 1994). The acceptability of any food is largely influenced by its organoleptic properties. These sensory attributes are a function of the physico-chemical composition of food product. They are influenced by quality and composition of raw materials (Arvanitoyannis *et al.,* 2007).In the process of sensory evaluation, food is uniformly prepared and presented to panelists in isolated booths (Hashmi, 2007). Panelists evaluate the food and recordscores for parameters as required in the study on a sensory evaluation sheet that is decoded and analyzed by statistical procedures. The parameters commonly evaluated include taste, mouth feel, flavor, texture, color, aftertaste, appearance and overall acceptability.

A consumer acceptability taste using a 9-point hedonic scale is used to assess and compares the sensory attributes of the samples provided. The commonly used hedonic scale is; “1” Dislike extremely, “2” Dislike very much, “3” Dislike moderately, “4” Dislike slightly, “5” Neither like nor dislike, “6” Like slightly, “7” Like moderately, “8” Like very much and “9” Like extremely

2.8. Shelf life studies of food products

Food is inherently perishable and depending on its physical and chemical properties and storage conditions, there is a point when either its quality becomes unacceptable or harmful to the consumer.

At this point, it is said to have reached the end of its shelf-life and the ability to predict this is of great value to the food industry. According to NZFSA (2005), the shelf life of a product begins from the time the food is manufactured. Most consumers on daily basis not only make demands as to how to obtain high-quality foods but also have some expectations that such qualities obtained from these foods was maintained at a high level during the period between purchase and consumption (Kilcast and Subramaniam, 2000)

2.3.15. Definition of shelf life

IFST (1993) defines product shelf-life as the time during which the product was remain safe, be certain to retain desired sensory, chemical, physical and microbiological characteristics and comply with any label declaration of nutritional data when stored under the recommended conditions. Thus, shelf life of a dried soup would be the time during which the product remains safe, retains its color, flavor, and aroma, have a stable peroxide value, free fatty acid content as well as be microbiologically safe and stable.

2.3.16. Factors affecting shelf life

Product shelf life is controlled by three major factors; product characteristics, environment to which the packaged product is exposed during distribution and properties of the package (Robertson, 1993)

2.3.16.1. Product characteristics

According to Robertson (1993), most foods fall into one of these three main classes; perishable (very short shelf life products), semi-perishable (short to medium shelf life products) and ambient temperature shelf stable (medium to long shelf life products). These are based on different characteristics associated with different food products as well as kinds of changes that occur during their storage. Ambient temperature shelf-stable foods include some natural foods such as cereal grains, nuts and confectionery products and processed food products such as canned foods, soft drinks and cake mixes.Thus, soups fall into this category.

2.3.16.2. Distribution environment

Packaged foods may lose or gain moisture; they could also reflect the temperature of their environment because very few food packages are good thermal insulators. Thus, the environment has an important influence on the rate of deterioration of packaged foods.According to IFST (1993), factors that

influence or control the shelf-life of food products can be again grouped into *intrinsic* and *extrinsic* factors.

Intrinsic factors

Intrinsic factors are properties of the final product which include water activity (*a*w); pH value and total acidity (type of acid); available oxygen; natural micro-flora and surviving microbiological counts; natural biochemistry of the product formulation (enzymes, chemical reactants) and the use of preservatives in product formulation (for example salt or sodium bicarbonate).

Extrinsic factors

These are factors which the final product encounters as it travels through the food chain. They include time-temperature profile during processing; temperature control during storage and distribution; environmental microbial counts during processing, storage, distribution and consumer handling.

2.3.17. Predicting shelf-life/shelf life testing

Introducing a new food product on the market usually does not take a day. Certain measures especially concerning shelf-life need to be established before certification to be put on shelves for consumers. Thus, food products require a date or labeling indicating when the product is likely to become unacceptable for consumption. As stated by Kilcast and Subramaniam (2000), while determining shelf life of products, products which may be expected to have a longer shelf life may require knowledge of their storage characteristics over the intended shelf-life period and this can introduce unacceptable delays in putting the product on the shelf.

Generally, the shelf life testing of food products falls into one of three categories (Gacula & Kubala, 1975)

- experiments designed to determine the shelf life of existing products
- experiments designed to study the effect of specific factors and combinations of factors such as storage temperature, package materials, or food additives on product shelf life
- tests designed to determine the shelf life of prototype or newly developed products

2.3.18. Selection criteria to assess shelf life

Every food product is associated with specific attributes either in their raw state or after certain conditions have been applied. Depending on the attributes, certain changes are noticed especially during storage and these changes help to detect if products have gone bad or not. According to Fuller *et al.,* (2006), in the selection of criteria for shelf life assessment, two main steps are needed. First, changes that can be measured and appropriate for the product are selected. These criteria normally change gradually with time so the onset of the change can be measured. Example of such criteria includes microbiological, nutritional change, undesirable change (loss or change of color, textural changes like loss of crispness, staling) and change in a functional property. Second, there must be some decision made about how much loss of quality characteristics can be accepted. In other words, how much loss of a quality characteristic can be accepted before spoilage is declared

2.3.19. Types of shelf life testing

According to NZFSA, (2005), there are two main methods of shelf life analysis. The two methods need to be exploited for comparison.

2.3.19.1. Direct method:

This is the most commonly used testing method. It involves storing the product under the normal or pre-selected conditions for a period of time longer than the expected shelf life. The product is checked at regular intervals to see when it begins to spoil. The exact procedure is unique for each product. This method is usually referred to as real-time studies. Shelf life of food samples can be determined according to the flow chart illustrated in figure 2.5 below.

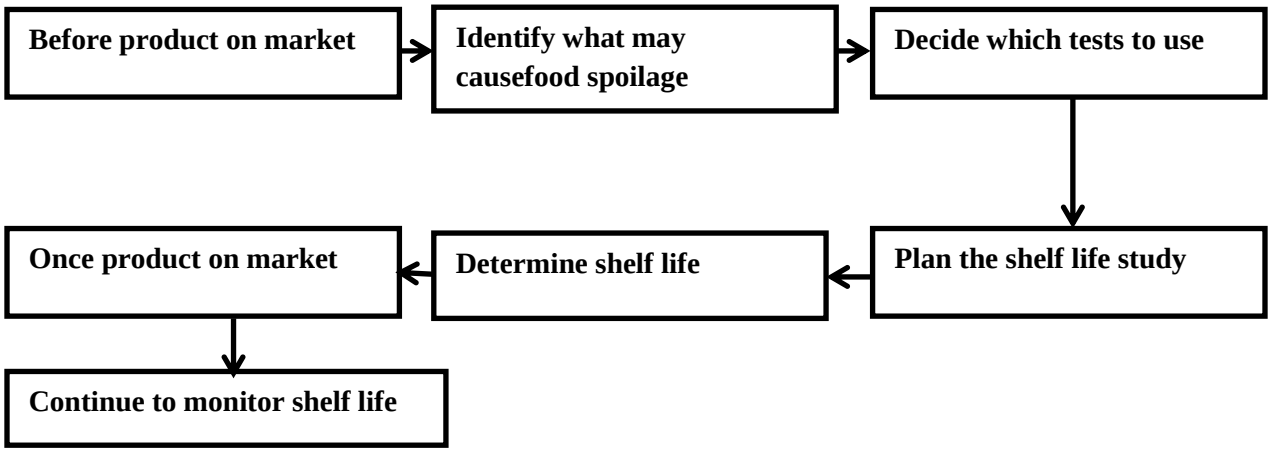


Figure 2.5: Flow chart illustrating steps for calculating the shelf life of food products by direct method (Source: NZFSA, 2005)

2.3.19.2. Accelerated shelf life studies

This attempts to predict the shelf life of a product without running a full-length storage trial. This typeof study is usually used for a product with a longer shelf life. Acceleration factors such as temperatureare applied to the product to attempt to increase the rate of deterioration.The experimentaldetermination of shelf-life using direct method can require a considerable amount ofexperimentation, with consequent costs and demands on time. When the actual storage time islong, shelf-life studies are based on accelerated shelf life testing (ASLT). This techniqueshortens the process of obtaining theexperimental data (Kebede*et al.,* 2015; Mizrahi, 2000). With accelerated shelf life testing the trialperiod is shortened by deliberately increasing the rateof deterioration (Martins and Silva, 2004). This is usually done by increasing the storagetemperature. The results are then used to estimate the shelf life under normal storage conditions(Yang *et al.,* 2013).With accelerated shelf life testing the trial period is shortened by deliberately increasing the rate of deterioration (Martins and Silva, 2004). This isusually done by increasing the storage temperature. The results are then used to estimate the shelf life under normal storage conditions(Yang *et al.,* 2013).

Accelerated shelf-life testing (ASLT) methods have been used to predict shelf-life at normalconditions (Rao *et al.,* 2012) based on data collected at high temperature or high humidityconditions (Achour*etal.,* 2001). The data from higher testing temperatures in food quality lossare used to determine shelf-life at regular storage conditions through the use of Arrhenius orLinear equations (Gulla and Waghray, 2012). Food products are conditioned and stored atelevated temperature and/or humidity and the quality changes of the product are evaluated at aspecific sampling rate (Achour*et al.,* 2001). ASL study could significantly shorten the durationof shelf life study to half or quarter of the direct method (Cardelli and Labuza, 2001). One of theprincipal methods of predicting the shelf life of processed food products is to monitor the level oflipid degradation in fat containing foods stored at elevated temperatures. The quantitative approach to shelf life prediction requires that the deteriorativemechanisms limiting shelf life of the specific food be identified and that the index of deteriorativereaction be measured as a function of time. Since the chemical reactions in food can be very complex it is usually easier to

examine a reaction from purely mathematical or semi empirical approach based onchemical laws rather than on a mechanistic approach in which each step must be known(Labuza and Kamman, 1983).

The loss of quality for most foods can be presented by a mathematical equation of the followingform:

$\pm \frac{dA}{d\theta} = KA^n$ where A= Quality factor measured

θ=time

K = a constant which depends on temperature and water activity

n = a power factor called order of the reaction which defines whether the rate isdependent orindependent.

$\pm \frac{dA}{d\theta}$ = rate of change of time. A negative sign is used if the deterioration is loss of A andpositive if itis for production of undesirable products.

For quality a change in foods, the reaction order has generally been shown to be either 0 or 1depending on the reaction involved (Labuza, 1982; Pope, 1980). If n = 0 the reaction is said to bezero order with respect to A. This means that the rate of loss of A is constant and independent ofthe concentration of A. Zero order reaction is mainly applicable to non-enzymatic browning indried foods and lipid oxidation in dried foods (Labuza, 1982).

When n = 1 the reaction is first order whereas the loss of quality is dependent on the amount of A left. The deterioration which falls in first order reaction includes vitamin and protein loss in dried foods and vegetable rancidity in dried foods (Labuza, 1982).In some studies reactions were describedby nonlinear equations such as polynomial equations (Smoot and Nagy, 1980).

The principle factor affecting the kinetics of reactions in processed and dried foods istemperature. Increases in temperature are known to accelerate deteriorative reactions in food andthus reduce shelf life of food. According to Labuza (1982), temperature is assumed to follow Arrhenius equation aspresented in equation 2.1.

$$K = K_o \exp^{(Ea/RT)} \qquad 2.1$$

Where

K= rate constant

K_o= pre exponential constant

E_a= Activation Energy

R = Gas constant

T = Absolute temperature

Arrhenius equation is the best approach in modeling temperature dependence (Saguy and Karel, 1980), since unlike other temperature dependent models, it based on theories of thermodynamics (Labuza and Kamman, 1983). The activation energy is generally derived from the slope of a plot of natural logarithm of rate of constant (k) versus the inverse of absolute temperature and depends on compositional factors such as water activity, moisture content and solid concentration. When the reaction mechanism changes with temperature the activation energy may vary substantially.

Large statistical errors are commonly associated with the calculations of the temperaturedependence of the reactions. Some studies have suggested methods of analyzing kinetic datawhich provide statistically more reliable results (Cohen and Saguy, 1985). Arrhenius plot canalso be used to establishshelf life plots of specific products based on a known end point qualitydeterioration value (Labuza and Kamman, 1983). There could be many limitations besidesstatistical errors in using the Arrhenius and shelf life plots to predict shelf life at lowertemperature. Generally the problem occurs because some reactions which predominate at highertemperature do not pre dominate at lower temperature (Labuza and Riboh, 1982).

Another important prediction for accelerating the effect of E_A called the Q10 Value is also used (Leeand Krochta, 2002). The relationship between Q10 and E_A is described in equation 2.2 below

$$\log Q_{10} = \frac{2.189 E_A}{T(T+10)} = \log\left[\frac{shelf\quad life\quad at\, T+10}{Shel\quad life\quad at\quad T}\right] \qquad 2.2$$

Where Q_{10} Increase in rate or decrease in shelf life for a 10^oC rise in T

T is in K

E_A is in Cal/mole

2.3.20. Measurement of product shelf-life

2.3.20.1. Sensory panels

Sensory techniques are required in determinations of changes in food quality as they are subjected to storage (Kilcast and Subramaniam, 2000). Trained panelists are commonly used to measure the sensory changes in a product over time. The panelists should be highly skilled at describing the appearance, aroma, flavor, and texture of the product. For most shelf life studies, three to five trained sensory panelists evaluate the samples at each time points. A sensory panel leader would lead the group discussion and record their consensus of opinion. However, the final decision on the acceptability of the product is reserved to the project head or the company (National foods lab, 2013).

2.3.20.2. Physical measurements

Changes in color and texture are the common physical measurements used to monitor changes in food product attributes during storage. Thus, a change in product color or texture can be used to detect whether the product has gone bad or is still in good condition for human consumption. These changes may be as a result of some chemical reactions occurring in the product, such as those caused by interaction of ingredients or by environmental influences, such as moisture migration through the packaging and non-enzymatic reaction which may alter the color of the product Kilcast and Subramaniam, (2000)

2.3.20.3. Chemical measurements

Chemical analyses play a vital role in shelf-life testing. This is because they can be used either to measure the endpoints of chemical reactions occurring in food during storage or to confirm the results obtained by the sensory panel Kilcast and Subramaniam, (2000). Even though there are many chemical reactions occurring concurrently in any food product especially during storage, only the major ones influencing changes in the product quality are monitored and measured during shelf-life testing Kilcast and Subramaniam, (2000). Some chemical tests used in determining changes in a particular quality characteristic include free fatty acid and peroxide value measurements which serve as markers for the level of rancidity of products.

2.3.20.4. Microbiological stability determinations.

According to Kilcast and Subramaniam, (2000), important aspects considered in the determining of microbiological stability of food product are;

1. Microbial growth, which leads to the spoilage of a food product

2. The growth of microbial pathogens that affect the safety of the product

The water activity, storage temperature, time and pH are used to predict to a large extent the micro-organisms that are likely to grow in a given food product. The time to spoilage is usually determined by storing the product at the appropriate temperature and measuring the microbial load at regular intervals. The time to reach a pre-determined level of microbial count (total count and level of individual microbes) is considered to be the end-point. Since it is advisable to leave a safety margin in setting the

shelf-life, generally 70% of the time to spoilage is taken to be the storage life (Gonzallez-Ferrero., 2015).

CHAPTER THREE: Optimization of the effect of formulation on nutritional quality, texture and physicochemical properties of banana-vegetable soup powder

Abstract

There are reported cases of malnutrition in developing countries due to low levels of nutrients in staple diets consumed in homes. Uganda being a developing country has been a victim of such cases. The largest composition of the population consume cooking bananas at an annual rate of 220 kg/person which is among the world's largest consumption rate. Uganda being a large consumer of cooking bananas is also among the largest producers of the fruit. The availability of the fruit with little being sent to the international market due to low shelf life and high post-harvest losses has initiated value addition to the fruit. This has seen several products developing from cooking bananas, among which include raw tooke flour for use in confectionaries, tooke porridges, snacks and cakes.In this research, a nutrient rich soup flour is developed from banana flour by enriching it with amaranth flour, pumpkins, tomatoes, mushrooms and carrots……The purpose for this study was to optimizethe effect of formulation of banana-amaranth and vegetables mixon texture,nutritional and physicochemical properties of the soup. Response surface methodology (RSM) was carried out using Design expert (Stat Ease Software, Version 11.0.0.1) to give an optimal composition of the soup. The mixture design in design expert gave an optimum product composition consisting of banana-amaranths, pumpkins, carrots and mushrooms at 82%, 9%, 5% and 4% respectively. The optimal product had an energy composition of 409.39kCal/100g, peak viscosity of 2631.41Cp while the holding viscosity, breakdown viscosity, final viscosity, peak time, pasting temperature, carbohydrates, proteins and zinc contents of 1430.11Cp, 1209.57Cp, 2495.29Cp, 4.9 minutes, 78.41^{o}C, 65.38%, 14.86% and 13.50g/100g respectively.Mathematical models for predicting nutritional composition, viscosity, physicochemical properties were significant ($p<0.05$). The coefficient of determination R^2 ranged from 0.40 (Peak time) to 0.99 (fat content). Fat content, Protein Content, moisture content, in vitro protein digestibility, Fiber content and Zinc with R^2 values of 0.99, 0.93, 0.93, 0.91, 0.9, and 0.9 respectively. The lack of fit test was not significant ($p<0.05$).

Key words: Banana, Amaranth, vegetables, soup, optimization; response surface methodology

3.1.Introduction

Children malnutrition remains a big challenge in low income and middle-income countries (Black *et al.,* 2008). Nutritional improvement of staple foods has been explored to reduce these deficiencies in

recent product developments. Bananas are highly available on the Ugandan market with a potential for utilization to provide for nutrition demands of Uganda's population. Grain amaranth (*Amaranthus*) is considered as one of the fastest growing andhigh yielding cropswith a potential to contribute to the nutritional availability in bananas(Akande *et al.,* 2017; Tibagonnzeka, *et al.*,2014). It contains superior quality protein but has marginal levels of leucine (an essential amino-acid) and is limited in vitamin A (0 µg RAE/100 g or 2 IU/ 100 g) (USDA, 2014). This implies that complementing the mix with vegetables may enhance the quality of banana based soups as is the goal of this study. Pumpkins are considered as good sources of zinc and iron and so are the carrots. Mushrooms and tomatoes were considered as good sources of vitamins A and C as well as good supplements for minerals(Manzi *et al.,* 2001).Amaranth flour, Pumpkins, mushrooms and carrots were used to enrich matookeflour in the development of a nutrient enriched soup.

3.2.Methods and materials

3.2.1. Raw material selection for use in this study

The plantain used in this study were the cooking type also locally known as matooke, a *Musa sp*. triploid acuminate genome group (AAA-EAHB). The species used in this study were chosen due to their better nutritional properties than other banana species(Kawongolo, 2013; Nyombi *et al.,* 2009). To improve the protein content of banana flour, grain amaranth flour was added because it is reported to be a rich source of proteins (Soriano-García*et al.,* 2018). The vegetables were chosen basing on their nutrient values; carrots are rich in beta carotene (124.28 mg/kg) (Bystrická*et al.,* 2015), pumpkins are rich in zinc(Ravi, Lakshmi, & Ranjani, 2010b) while mushrooms are rich in most minerals(Manzi *et al.,* 1999).

3.2.2. Sample preparation

Green cooking bananas were peeled under cold water treated with 1 percent sodium metasulphite to avoid browning and sliced to about 2 mm thickness. The slices were laid for drying in air oven driers at Jakana foods ltd, Kawempe, Kampala Uganda. The slices were dried at 60°C for 6 hours in an air ovendriers (SAVER TRAY DRIER, Model: R-5A). Fresh Mushrooms were purchased from Capital shopper's supermarket and were dried for 4 hours at 60^{o}C using the same driers at Jakana. Carrots were purchased from Kalerwe market in Kampala, chopped, sliced and laid on steel trays to dry for 12 hours at 50^{o}C in air oven driers(Pandey & Kulshrestha, 2003b). Pumpkins, and tomatoes were purchased

from Kalerwe market, Kampala transported in nylon bags chopped, sliced and laid on trays for drying for 6 hours at 60^{o}C. The dried samples were milled using a wonder mill. The flour was sieved using a laboratory test sieve (Wagtech international, Ltd., 300 MIC, BS 410), packed in polythene papers, and kept in plastic buckets for further analysis.

3.2.3 Experimental Design

The proportions of vegetables and banana powder used in this study were determined basing on the nutritional requirements of children aged 6-59 months. The ingredients were entered into the Nutri-Survey for windows (SEMEO-TROPMED RCCN, University of Indonesia) and adjustments in the proportions of the ingredients made to meet the target percentages of proteins (16 g), energy (1060 kCal). The resultant ratios were used in the Design expert (Stat Ease, Version 11.1.0.1 Minneapolis) to obtain runs for use during optimization process. Thirteen formulations were generated and presented in table 3.1.

Table 3.1: Proportions of bananas and selected vegetables obtained using Nutri-survey

Formulation code	**Amounts of ingredients g per 100 g of flour**					
	Banana	**Grain Amaranths**	**Pumpkins**	**Tomatoes**	**Mushrooms**	**Carrots**
S0 (Control)	50	50	0	0	0	0
S1	42.5	42.5	15	0	0	0
S2	42.5	42.5	0	15	0	0
S3	42.5	42.5	0	0	15	0
S4	42.5	42.5	0	0	0	15
S5	40	40	20	0	0	0
S6	40	40	0	20	0	0
S7	40	40	0	0	20	0
S8	40	40	0	0	0	20
S9	45	45	10	0	0	0
S10	45	45	0	10	0	0
S11	45	45	0	0	10	0
S12	45	45	0	0	0	10

$S_{0\text{-}12}$ refers to samples with respective Banana, Amaranths, Pumpkins, Tomatoes, Mushrooms and Carrot combinations.

3.2.4 Determination of nutritional properties

The moisture, crude protein, ash, total fat, minerals (iron, potassium and zinc), total carbohydrate, dietary fiber, total fat and gross energy contents of the banana-based flours were determined using standard methods.

3.2.4.1 Determination of moisture content

The moisture content of the samples was determined by oven drying overnight at 105°C (AOAC, 1999). Dried aluminum dishes were weighed and their respective weights recorded as W_1. About 2 g of the ground sample was weighed using an analytical electronic balance into already weighed dishes (W_2) and dried in a fan oven at 105 °C overnight. After evaporation of moisture from the sample, it was left to cool in a desiccator and weighed again to obtain weight after drying (W_3). The experiment was carried out in triplicate. The percentage average difference in the weights of three samples of flour, before and after oven drying was calculated to give the moisture content.

$$\%\ moisture\ content = \frac{Loss\ in\ weight}{Weight\ of\ sample}\ x\ 100$$

3.2.4.2 Determination of crude protein content

Protein determination was carried out using the method as described by AOAC, (1990). The determination of protein by micro Kjeldahl method involves three stages, the digestion stage, the distillation stage and the titration stage.Weighed sample (1.0g) was digested with concentrated sulphuric acid (20 ml) and digestion mixture (10.0 g) in Kjeldahl digestion flask. The contents were cooled and transferred to 250 ml volumetric flask. The volume was made up to the mark with distilled water and mixed. Measured aliquot (5 ml) was poured in distillation flask followed by 40% sodium hydroxide and ammonium borate was collected through a condenser in a flask containing 10 ml of 4.0% boric acid solution. The distillate was titrated with 0.1 N sulphuric acid. A blank sample was also run along with the samples(Bhat & Bhat, 2013).

$$\%\ \boldsymbol{Nitrogen} = \frac{Titrevaluex\ 0.0014\ x\ \text{volume made}}{\text{Aliquote taken (g)}x\ \text{Weight of sample (g)}} X100$$

$$\%\ Protein = \%\ Nitrogen\ x\ 6.25$$

Determination of fat content

Total fat content was determined using the Soxhlet method (AOAC, 1999). About 4g of the sample was weighed into thimbles; 40 ml of extraction solvent (Petroleum Ether) was measured into beakers, the thimbles containing the samples placed in the beaker containing the petroleum ether which was later fixed on a Soxtec equipment. Fat extraction was done by boiling the samples for about an hour. The solvent was distilled off and the fat dried in an air oven at 100 °C for about 30 minutes. The oil collected in the beaker was weighed. Percentage fat content was determined using the following formula:

$$Fat\ content = \frac{W_3 - W_2}{W_1}\ X\ 100$$

Where: W_1 = weight of sample, W_2 = weight of empty beaker, W_3 = weight of beaker + fat

3.2.4.3. Determination of Ash content

The ash content was determined as described by AOAC, (1990). The crucibles for ashing were properly washed and dried in a hot air oven and allowed to cool in desiccators. The crucibles were weighed and their weight recorded as W_1. About 2g of the flour sample (W_2) was weighed into the crucible and the weight noted (W_3). The crucibles and their content were transferred into the muffle furnace set at and maintained at 550°C before timing commences for six hours. The ashing was complete when there is no black speck in the ash that is the sample turned grey. The crucibles containing the ash were taken out and transferred to the desiccators to cool after which the weights were taken. The difference in weight between the weight of the crucible and the crucible plus the ash was calculated as ash content. The percentage of ash was calculated as follows:

$$\%\ Ash\ content\ = \frac{Weight\ of\ ash}{Weight\ of\ sample}\ x\ 100$$

3.2.4.4. Dietary fiber content

The dietary fiber content of the mixed flours was determined using the FOSS Fibertec 2010. About 1.0g of the sample was weighed into the crucible. The mixture for dietary fiber determination was prepared from 20g of cetyltrimethylammonium bromide, concentrated sulphuric acid (28 ml) and 400ml of distilled water and made up to 1L with distilled water. The glass crucibles containing the samples were fixed into the Fibertec machine. The temperature was set at 50°C and the mixture boiled

for 45minutes after which it was washed severally with distilled water. The glass crucibles were taken to the oven maintained 100^{o}C for 45 minutes to drive off the moisture. Dietary fiber was obtained as the difference between the weight of the empty glass crucible and that after removal from the oven.

$$\%\ Crude\ fibre = \frac{Loss\ in\ weight}{Weight\ of\ sample} x100$$

3.2.4.5. Determination of total carbohydrates content

The carbohydrate content was determined by difference on dry weight basis. The total percentages of the fat, crude protein, ash, dietary fiber was deducted from 100%, giving the amount of nitrogen-free extract otherwise known as carbohydrate.

On dry weight basis

$$\%\ \mathrm{CHO} = 100\ \% - (\%\ \mathrm{Fat} + \%\ \mathrm{Ash} + \%\ \mathrm{dietary\ Fibre} + \%\ \mathrm{Crude\ Protein})$$

3.2.4.6. *In vitro* protein digestibility

In vitro protein digestibility (IVPD) was determined according to the method by Khalil *et al.,* 1984. Approximately 0.2 grams of the sample was weighed into two 50 milliliter centrifuge tubes. To determine digestibility, two milliliters of distilled water was added to the 0.2g samples, shaken and the tubes was placed in a boiling water bath for 20 minutes. To each of the sample, 20 milliliters of 0.1 M phosphate buffer with pepsin (1:3000 IU Hog pepsin/L, pH 2.0) was added. A blank was prepared in a similar manner without the sample. The tubes were incubated in a shaking water bath at 37 ^{o}C, for two hours and then centrifuged at 6000 rpm for 15 minutes. The supernatant was removed with a dropper and discarded. Ten milliliters of Phosphate buffer were added to each tube and centrifuged at 6000 rpm and the supernatant discarded. The residue was removed and placed in the center of a filter paper on a Buchner funnel. Suction was applied to the filter flask and the remaining residue rinsed from the tube into the funnel using five milliliters of the buffer. The filter papers were rolled and inserted into Kjeldahl flasks. The flasks were dried in the oven at 100 ^{o}C for a minimum of 15 minutes. Into the Kjeldahl flasks containing the filter paper and sample, ten milliliters of concentrated Sulphuric acid, one gram of Potassium Sulphate and one milliliter of ten percent Copper Sulphate solution was added. Digestion, distillation, and titration was done as described in the determination of initial protein content. Percentage protein digestibility was calculated as follows:

% In vitro protein digestibility = $\frac{\mathrm{A}-\mathrm{B}}{\mathrm{A}}$

Where;

A = % Protein in sample before digestion

B = % Protein in sample after pepsin digestion

3.2.4.7. Determination of gross energy content

Gross energy content was determined by oxygen bomb calorimeter (GallenkampAutobomb) (AOAC, 1999). About 1 g of the sample was weighed, pelleted and placed in a clean combustion crucible. A platinum wire (10cm) was attached between the electrodes of the bomb. The combustion crucible containing the sample was set in place in the loop electrode. A cotton thread was tied in the middle of the platinum wire. The thread was adjusted until it touches the sample. Distilled water (200 ml) was put in the calorimeter bucket and placed in the calorimeter. The bomb was filled with oxygen to 30 atmospheres gauge pressure. The cover of the calorimeter was closed, the thermometers lowered and the circulating motor was started. The temperature of the water in the outer jacket was adjusted to equal that of the calorimeter. The initial temperature of the calorimeter was recorded as T_i. The sample was then ignited. The final temperature was read and recorded as T_f. The calorimeter was then opened and the bomb was taken out of the bucket. The acid correction was done by rinsing all inner bomb surfaces with distilled water and collecting all the washings in a clean beaker. The washings were titrated against 0.1 N sulphuric acid with methyl orange indicator. Gross energy was calculated using the following formula:

$$Gross\ energy\ (Kcal\ per\ g\ DM) = ((Tf - Ti) - (correction\ factor)\ x\ He)/(W\ x\ DM\%)$$

Where: T_f = final temperature, T_i = initial temperature, W = weight of sample, He = hydrothermal equivalent, correction factor = w + titer, titer = amount of sulphuric acid used, w = length of wire left.

3.2.5 Determination of Physicochemical properties of the flours

3.2.5.1 Bulk density

A 50-gram sample of flour was poured from a constant height into an empty pre-weighed 100 milliliters measuring cylinder up to a certain height. The cylinder was tapped continuously and carefully until no further decrease in the level of flour is observed at the graduation mark and the weight of the cylinder taken. Bulk density was expressed as grams per milliliters. For each formulation, three replicates were made.

3.2.5.2 Water absorption capacity

The water absorption capacity (WAC) of flours was measured using the centrifugation method reported by (Kaur and Singh, 2006). Each sample of three grams was dissolved in 25 milliliters of distilled water and placed in a 50 mL pre-weighed centrifuge tube. The mixture was stirred at five minutes interval, held for 30 minutes, followed by centrifugation for 30 minutes at 3,000×g. The supernatant was decanted, excess moisture removed by drying at 50 ^{o}C for 25 minutes and then the sample was reweighed.

3.2.4.8. Water absorption index and water solubility index

The water absorption index (WAI) and water solubility index (WSI) of samples was determined by methods reported by Kaur and Singh, 2006. A sample of three grams was dissolved in 30 milliliters of distilled water and heated in a water bath at 90 ^{o}C for 15 minutes. The cooked paste was cooled to room temperature, transferred to pre-weighed centrifuge tubes, and centrifuged at 3,000×g for ten minutes. The supernatant was decanted into a pre-weighed evaporating dish to determine its solid content and the sediment was weighed. The weight of dry solids was recovered by evaporating the supernatant overnight at 105 ^{o}C. The WAI and WSI was calculated as follows:

$$\text{WAI} = \frac{\text{Weight of sediment}}{\text{Weight of flour sample}}$$

$$\text{WSI} = \frac{\text{Weight of dissolved solids in supernatant}}{\text{Weight of flour sample}}$$

Pasting properties of soups

Pasting properties of instant soups were determined using a Rapid Visco Analyzer (RVA-4, Newport Scientific). The pasting temperature (PT), peak viscosity (PV, the maximum hot paste viscosity), holding strength or trough viscosity (the trough at the minimum hot paste viscosity), final viscosity, breakdown (BD, peak viscosity-holding strength or trough viscosity) and setback (SB, final viscosity-holding strength) were obtained using Thermocline for Windows software. The viscosities were presented in Centipoises. The pasting time were presented in minutes.

3.2.6 Sensory evaluation of formulations for acceptability of the formulations.

Sensory evaluation of samples was carried out to choose the most acceptable formulation. To improve on sensory acceptability of the soup, the samples were prepared using chicken broth prepared from chicken legs, parsley, thyme, bay leaves, onions, garlic, salt and water. The samples were prepared by

mixing 50g of the samples together with 800mls of chicken broth, boiled and allowed to simmer for five minutes. The prepared samples were served to 30 panelists from the school of food science and technology. The most acceptable combination of flours basing on the 9-point hedonic scale were chosen.

3.2.7 Data analysis

All experiments were conducted in triplicate. Statistical analysis of the data was performed by analysis of variance (ANOVA), using SPSS software (IBM statistics, version 20). A probability value of difference $p \leq 0.05$ was considered to denote statistical significance. All data are presented as mean values ± standard deviation (SD). Regression analysis was performed to indicate the relationship between the tested parameters and formulations.

3.3 Results

3.3.1 Effect of variation of „„„„„…formulations on the Nutritional composition of the soups

The formulations presented different values for the tested parameters.Each of the formulation has 50% Banana, 50% Amaranths grains mixed with any of 10%, 15%, and 20% for pumpkins, tomatoes, mushrooms and carrots. The samples were formulated and different tests carried whileoptimizing the ingredients of the formulation. Results from proximate composition of the formulations are presented in table 3.2.

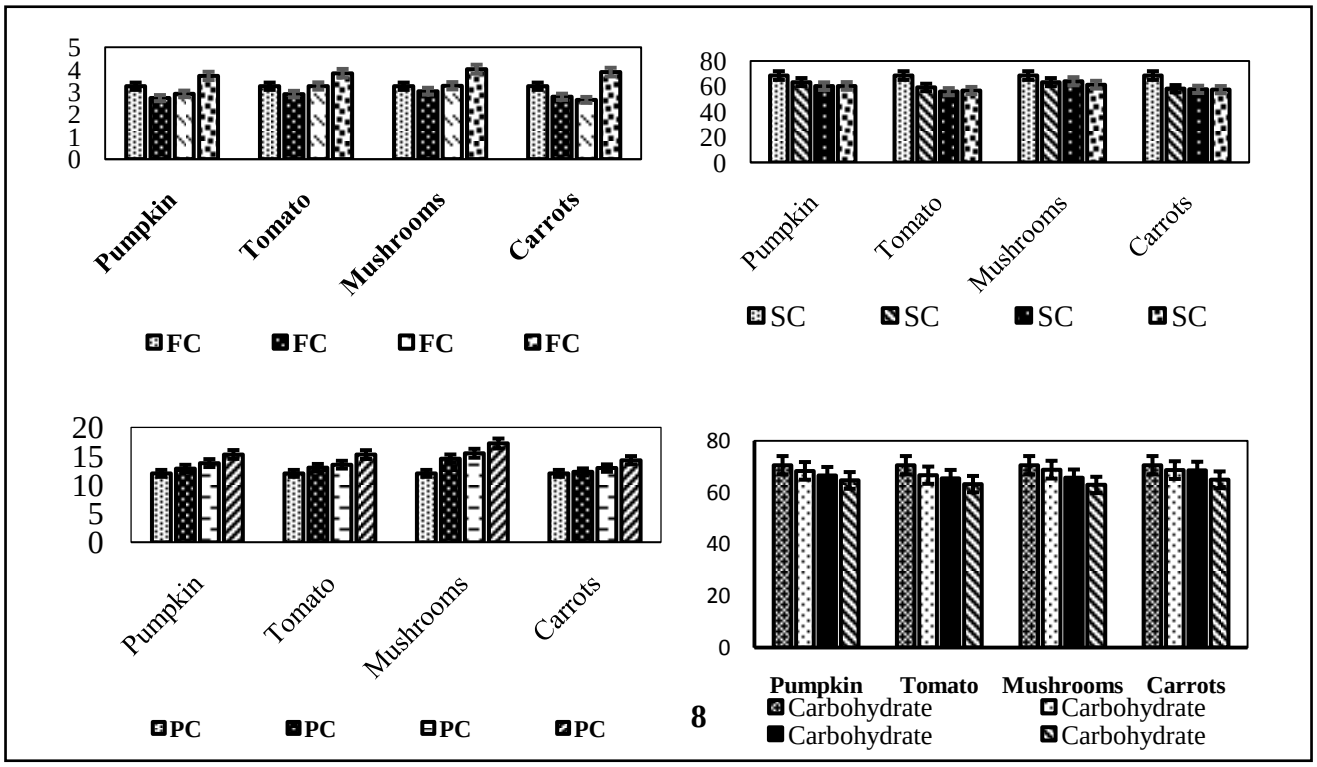


Figure 3.1: Variation of nutrition values in the formulation;Fat content (FC), Protein content (PC), Carbohydrates and Starch content (SC).

The gross energy content (GE) varies between 369.41±11.16 and 441.98±5.17kCal/100g. The formulation with pumpkin at 10%, 15% and 20% have a GE of 390.53, 410.50 and 411.31 respectively, The formulation with tomatoes at 10%, 15% and 20% have a GE of 410.59, 386.93 and 414.07 kCal/100g respectively.The formulation with mushrooms in the order of 10%, 15% and 20% have a GE of 416.38, 386.93, 391.69 kCal/100g respectively. The formulation with carrots at 10%, 15% and 20% have a GE of 414.19, 441.98, and 401. 55 kCal/100g respectively. However, the formulation with 15% pumpkins, 20% pumpkins and 10% carrots are not significantly ($p=0.05$) different from each other.The IVPD content varies between 58.90 and 72.71%. The formulation with pumpkin at 10%, 15% and 20% have an IVPD content of 72.56, 66.70 and 71.16% respectively. The formulation with tomatoes at 10%, 15% and 20% have IVPD composition of 71.73, 65.09 and 66.27% respectively. The formulation with mushrooms in the order of 10%, 15% and 20% have an IVPD composition of 58.90, 71.70 and 62.63% respectively. The formulation with carrots at 10%, 15% and 20% have an IVPD content of 67.35, 69.88 and 72.71% respectively. The addition of carrots improved the IVPD content relative to the control. There is no statistical significance between variations of IVPD in the formulations. The fat content of the formulations vary between 2.61±0.10 and 4.01±0.10 %. The formulation with pumpkin at 10%, 15% and 20% have a fat content of 2.72, 2.91, and 3.71 respectively. The formulation with tomatoes at 10%, 15% and 20% have a fat content of 2.90, 3.26 and 3.83 respectively. The formulation with mushrooms in the order of 10%, 15% and 20% have a fat content of 3.03, 3.27 and 4.01 % respectively. The formulation with carrots at 10%, 15% and 20% have a fat content of 2.61, 2.64 and 3.88% respectively. However, the formulation with 10% composition of pumpkins, tomatoes, mushrooms and carrots are not significantly different from each other. The protein content of the formulations varies between 12.00±0.65 and 17.20±0.53%. The formulation with pumpkin at 10%, 15% and 20% have a protein content of 12.85, 13.79, and 15.29% respectively. The formulation with tomatoes at 10%, 15% and 20% have a protein content of 12.99, 13.51 and 15.27% respectively. The formulation with mushrooms in the order of 10%, 15% and 20% have a protein content of 14.56, 15.50 and 17.20 %

respectively. The formulation with carrots at 10%, 15% and 20% have a protein content of 12.25, 12.92 and 14.29% respectively. However, the formulation with 10% carrots and the control are not significantly different from each other.The carbohydrates of the formulations were determined by a method of difference. The carbohydrates content of the formulations varies between 63.02 and 70.67%. The formulation with pumpkin at 10%, 15% and 20% have a carbohydrates content of 68.46, 66.64 and 64.76% respectively.The formulation with tomatoes at 10%, 15% and 20% have a carbohydrates content of 66.79, 65.55 and 63.33% respectively. The formulation with mushrooms in the order of 10%, 15% and 20% have a carbohydrates content of 68.93, 65.71 and 63.02% respectively. The formulation with carrots at 10%, 15% and 20% have a carbohydrates content of 68.76, 68.57, and 65.00% respectively.The starch content of the formulations varies between 55.83±0.56 and 68.52±0.74%. The formulation with pumpkin at 10%, 15% and 20% have a starch content of 63.38, 60.18 and 60.33% respectively. The addition of pumpkins thus reduces starch content in the formulations. The formulation with tomatoes at 10%, 15% and 20% have a starch content of 59.13, 55.83 and 56.70% respectively. The formulation with mushrooms in the order of 10%, 15% and 20% have a starch content of 63.35, 64.03 and 61.31% respectively. The formulation with carrots at 10%, 15% and 20% have a starch content of 58.11, 57.63 and 57.37% respectively, thus the change in starch content due to carrots is in the order of Control>10%>15%>20%.

Table 3.2: Proximate composition of different flour formulations for making soups

Formulation Code*	GE (kCal/100g)	MC (%)	Fat (%)	Fiber (%)	Protein (%)	IVPD (%)	% Ash	Carbohydrates (%)	Starch content (g/100g)
S0	414.75±0.21^{cd}	9.69±0.19^{a}	3.25±0.12^{bc}	1.45±0.14^{a}	12.00±0.65^{a}	67.20±9.97^{a}	2.94±0.02^{a}	70.67	68.52±0.74^{g}
S1	410.50±5.53^{bcd}	10.96±0.29^{bc}	2.91±0.13^{a}	2.59±0.04^{bcd}	13.79±0.18^{bc}	66.70±5.99^{a}	3.11±0.03^{bc}	66.64	60.18±2.10^{de}
S2	386.93±4.48^{ab}	11.09±0.64^{c}	3.26±0.08^{ab}	3.34±0.39^{f}	13.51±0.67^{bc}	65.09±10.00^{a}	3.25±0.07^{de}	65.55	55.83±0.56^{a}
S3	369.41±11.16^{a}	9.56±0.27^{a}	3.27±0.13^{ab}	2.90±0.11^{bcde}	15.50±0.70^{e}	71.70±5.94^{a}	3.06±0.04^{b}	65.71	64.03±1.10^{f}
S4	441.98±5.17^{e}	10.02±0.24^{ab}	2.64±0.80^{a}	2.43±0.36^{bc}	12.92±0.64^{ab}	69.88±7.16^{a}	3.42±0.04^{f}	68.57	57.63±1.30^{abc}
S5	411.31±11.28^{bcd}	10.52±0.19^{abc}	3.71±0.06^{bc}	2.79±0.10^{bcd}	15.29±0.90^{de}	71.16±7.29^{a}	2.93±0.02^{a}	64.76	60.33±0.61^{de}
S6	414.07±1.15^{cd}	11.24±0.36^{c}	3.83±0.05b^{c}	3.02±0.17^{def}	15.27±0.64^{de}	66.27±1.54^{a}	3.31±0.02^{e}	63.33	56.70±0.41^{ab}
S7	391.69±3.13^{abcd}	9.66±0.47^{a}	4.01±0.10^{c}	3.17±0.18^{ef}	17.20±0.53^{f}	62.63±5.14^{a}	2.94±0.04^{a}	63.02	61.31±1.79^{e}
S8	401.55±6.62^{bcd}	10.68±0.42^{bc}	3.88±0.19^{bc}	2.95±0.05^{bc}	14.29±0.64^{bc}	72.71±5.61^{a}	3.20±0.09^{cd}	65.00	57.37±1.30^{abc}
S9	390.53±4.77^{abc}	10.67±0.25^{bc}	2.72±0.05^{a}	2.25±0.18^{b}	12.85±0.84^{ab}	72.56±8.44^{a}	3.05±0.02^{b}	68.46	63.38±0.99^{f}
S10	410.59±0.35^{bcd}	11.15±0.43^{c}	2.90±0.03^{a}	2.54±0.23^{bcd}	12.99±0.35^{ab}	71.73±7.94^{a}	3.63±0.02^{g}	66.79	59.13±0.74^{cd}
S11	416.38±7.86^{d}	9.67±0.26^{a}	3.03±0.04^{a}	3.81±0.04^{g}	14.56±0.65^{cbd}	58.90±9.84^{a}	3.03±0.05^{g}	68.93	63.35±1.10^{f}
S12	414.19±7.48^{cd}	10.43±0.23^{abc}	2.78±0.10^{a}	2.61±0.10^{bcd}	12.25±0.62^{a}	67.35±10.15^{a}	3.17±0.03^{cd}	68.76	58.11±2.11^{bc}

Values represent mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different ($p < 0.05$). *Formulation codes represent samples in the respective formulations indicated in table 4-1.

Effect of formulations on micro nutrients composition of the soups

The formulations presented different values for the tested parameters. Each of the formulation has 50% Banana, 50% Amaranths grains mixed with any of 10%, 15%, and 20% for pumpkins, tomatoes, mushrooms and carrots. The samples were formulated and different tests carried on to optimize the ingredients of the formulation. Results from nutrient composition of the formulations are presented in table 3.3.

Table 3.3. Micronutrients compositions of the samples

Formulation code*	Iron (mg/100g)	Zinc (mg/100g)	K (mg/100g)	Vit C (mg/100g)	Vit A RAE (mg/100g)
S0	27.3	9.93	1115.2	10.61±2.34[a]	0.017±0.00[a]
S1	29.4	12.74	1017.2	14.25±1.04[a]	0.064±0.00[a]
S2	27.1	9.8	1098.4	24.52±2.67[a]	0.437±0.02[d]
S3	26.96	19.64	1134.4	16.19±1.25[a]	0.020±0.00[a]
S4	39.3	12.52	1188.8	16.64±1.14[a]	0.157±0.00[b]
S5	38.4	12.26	923.4	15.84±2.35[a]	0.062±0.00[a]
S6	42	13.28	1660.4	27.69±2.18[a]	0.449±0.02[d]
S7	45.8	16.81	1233.2	18.41±2.80[a]	0.014±0.00[a]
S8	38.1	13.92	1092	11.95±1.37[a]	0.205±0.02[b]
S9	21.4	8.13	1211.6	28.47±39.61[a]	0.063±0.00[a]
S10	26.2	10.76	1366	23.71±6.76[a]	0.321±0.14[c]
S11	32.7	15.98	1146.4	14.14±1.20[a]	0.019±0.00[a]
S12	16	9.09	1123.6	12.27±1.13[a]	0.167±0.01[b]

Values represent mean ± standard deviation (n=3). *Formulation code represent samples in the respective formulations as indicated in table 4-1.

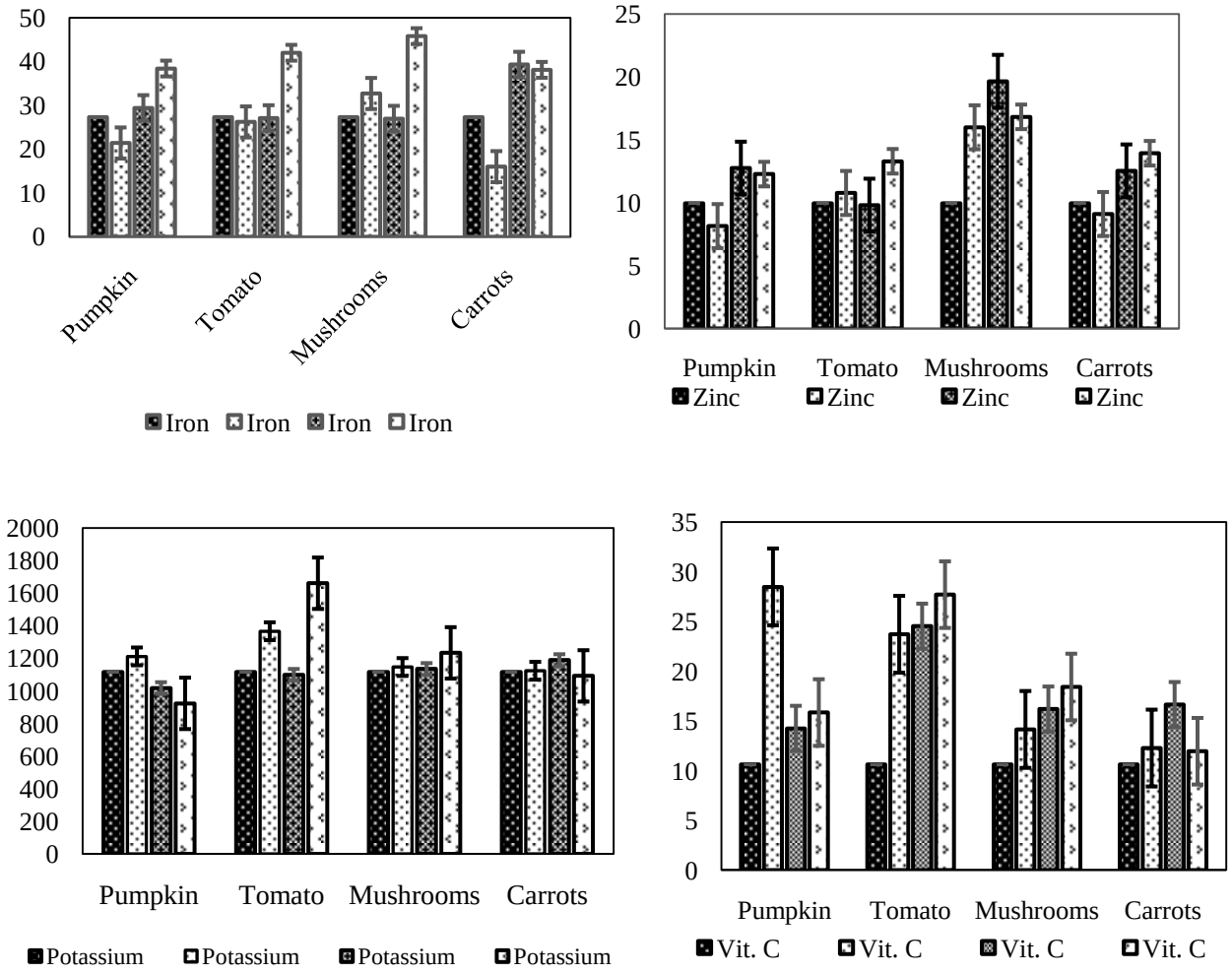


Figure 3.2: Variation in mineral composition and micronutrients; Iron content, Zinc content, Potassium content and Vitamin C in the formulations

The mineral and micronutrient composition of the soups formulations has been illustrated in figure 3.2 above. Iron content varies between 16 and 45.8 mg/100g. The formulation with pumpkin at 10%, 15% and 20% have an iron content of 21.4, 29.4 and 38.4 mg/100g respectively. The formulation with mushrooms in the order of 10%, 15% and 20% have an iron composition of 32.7, 26.96 and 45.8 mg/100g respectively. The formulation with carrots at 10%, 15% and 20% have an iron content of 16, 39.3 and 38.1 mg/100g respectively. The addition of vegetables improved iron content. Zinc varies between 8.13 and 19.64 mg/100g. The formulation with pumpkin at 10%, 15% and 20% have a zinc

content of 8.13, 12.74 and 12.26 mg/100g respectively. The formulation with tomatoes at 10%, 15% and 20% have a zinc composition of 10.13, 9.8 and 13.28 mg/100g respectively. The formulation with mushrooms in the order of 10%, 15% and 20% have a zinc composition of 15.98, 19.64 and 16.81 mg/100g respectively. The formulation with carrots at 10%, 15% and 20% have a zinc content of 9.09, 12.52 and 13.92 mg/100g respectively. The Vitamin C content varies between 10.61 and 28.47 mg/100g. The control sample has a Vitamin C content of 10.61 mg/100g. The formulation with pumpkin at 10%, 15% and 20% have a Vitamin C content of 28.47, 14.25 and 15.84 mg/100g respectively.

Effect of formulations on physicochemical properties of the soups

The formulations presented different values for the tested parameters. Each of the formulation has 50% Banana, 50% Amaranths grains mixed with any of 10%, 15%, and 20% for pumpkins, tomatoes, mushrooms and carrots. The samples were formulated and different tests carried on to optimize the ingredients of the formulation. Results from physico-chemical composition are presented in table 3.4.

Table 3.4: Physicochemical properties

Formulation code*	Bulk density (kgm^{-3})	FC (%)	FS (%)
S0	774.03 ± 9.81^{ef}	3.55±0.33	ND
S1	813.57 ± 19.99^{f}	4.98±0.09	1.25±0.02
S2	705.00 ± 16.55^{cd}	1.27±0.02	0.84±0.73
S3	754.73 ± 1.55^{de}	1.22±0.03	ND
S4	645.40 ± 0.87^{ab}	5.07±0.19	ND
S5	753.93 ± 15.44^{de}	2.45±0.06	ND
S6	710.63 ± 5.35^{cd}	ND	ND
S7	681.67 ± 0.61^{bc}	3.74±1.39	ND
S8	682.30 ± 5.20^{bc}	6.08±0.26	3.65±0.15
S9	810.33 ± 2.49^{f}	1.25±0.05	ND
S10	769.70 ± 1.39^{ef}	ND	ND

S11	725.10±2.95^{cde}	5.54±0.84	ND
S12	631.37±59.93^{a}	2.51±0.14	ND

Values represent mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different ($p < 0.05$). *Formulation codes represent samples in the respective formulations indicated in table 4-1.

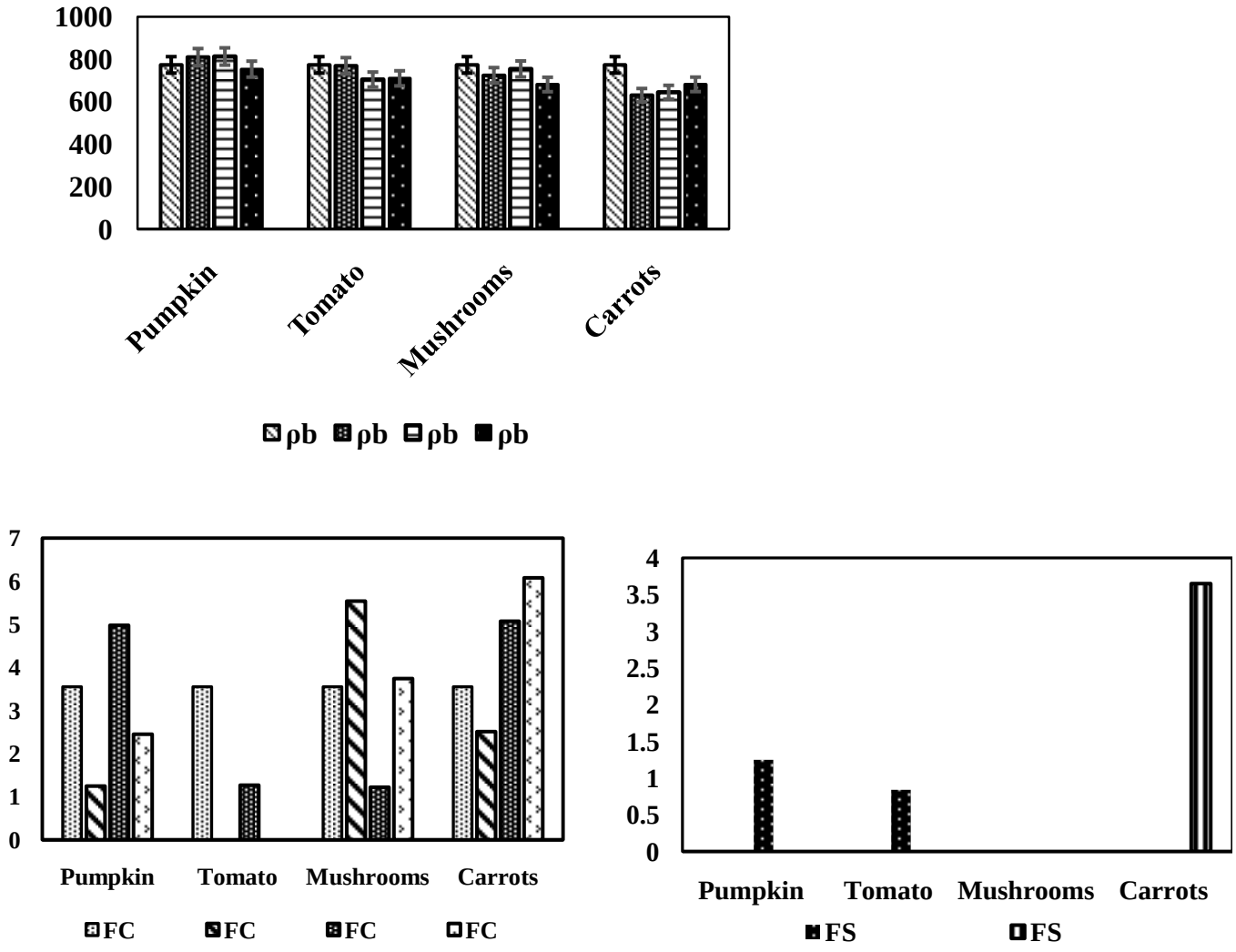


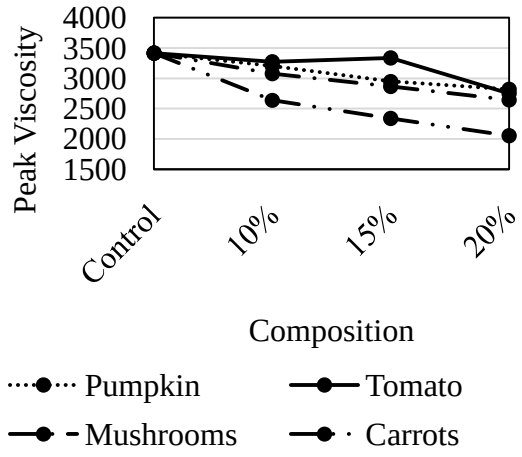
Figure 3.3: Variation of physicochemical properties in the formulation; bulk density (ρb), Foaming Capacity (FC) and foaming stability (FS)

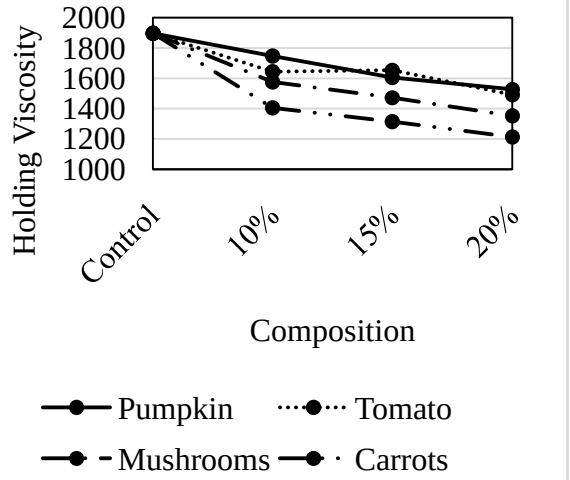
The variations in the physicochemical composition of the soups has been illustrated in figure 3.3 above. Bulk density content varies between 631.37±59.93 and 813.57±19.99 kgm^{-3}. The control sample has a bulk density of 774.03 kgm^{-3}. The formulation with pumpkin at 10%, 15% and 20% have a bulk

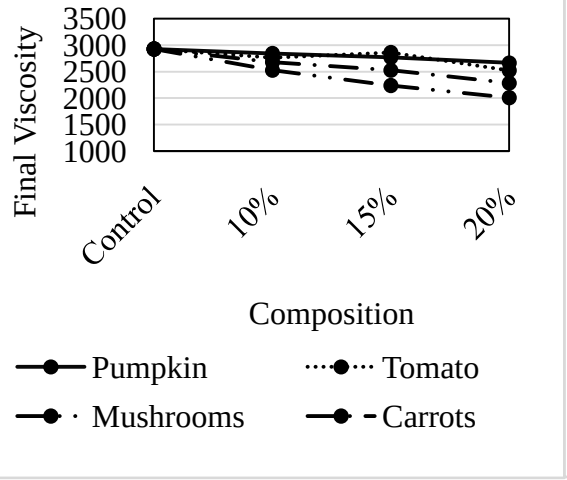
density content of 810.33, 813.57 and 753.93 kgm^{-3} respectively. The formulation with tomatoes at 10%, 15% and 20% have bulk density of 769.70, 705.00 and 710.63 kgm^{-3} respectively. The formulation with mushrooms in the order of 10%, 15% and 20% have a bulk density of 725.10, 754.73 and 681.67 kgm^{-3} respectively. The formulation with carrots at 10%, 15% and 20% have a bulk density of 631.37, 645.40 and 682.30 kgm^{-3} respectively. There isstatistical significance between variations of bulk density in the formulations. The formulations could foam in some cases and not stable at some cases. The foaming capacity varies between 1.22±0.03 and 6.08±0.26% while foaming stability for the formulations is highly un predictable. The control sample has a foaming capacity of 3.55±0.33% with no stability. The formulation with pumpkin at 10%, 15% and 20% have a foaming capacity of 1.25, 4.98 and 2.54% respectively. The only stable formulation is one where pumpkin is at 15%. The formulation with tomatoesat 15% is at 1.27% with a stability of 0.84% while the foaming capacity and stability of tomatoes at 10% and 20% are not defined.

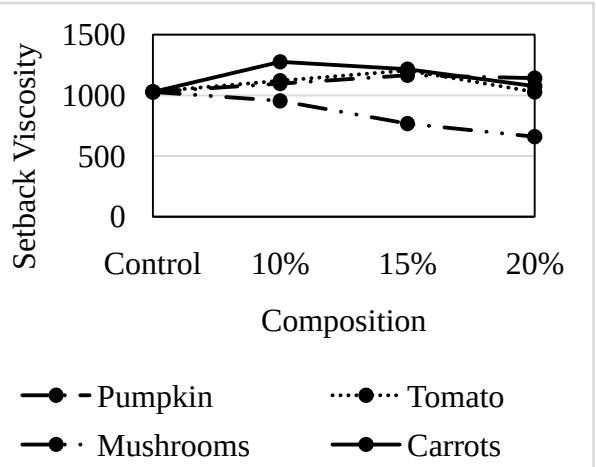
Effect of formulations on pasting properties of the soups

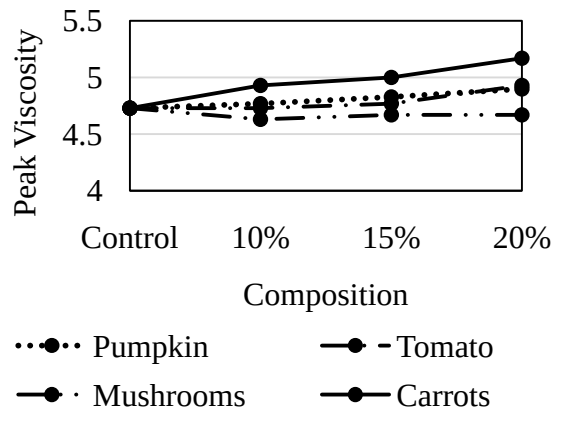
Pasting properties of the banana-vegetables soups presented in Table 4-3 were obtained using standard procedures presented byShimelis, et al.,(2006). A rapid Visco Analyzer (Model: RVA-4, New Port scientific, Pty. Ltd., Australia) was used to evaluate testing properties of banana flours. 3.5g of flour was weighed and put into a canister from which 25 mls of distilled water was added (Cheok et al., 2018). The canister was inserted into the pasting cell and the run was started.The test runs included 1 minute of mixing, stirring and warming up to 50^{o}C, 3 minutes and 42 seconds of heating at 12^{o}C/min up to 95^{o}C, 2.5 minutes of holding at 95^{o}C, 3 minutes and 42 seconds for cooling back to 50^{o}C at the same rate as heating and 2 minutes holding at 50^{o}C. The process ends after 13 minutes.Significant differences were observed in terms of pasting properties fordifferent formulations against control formulation. The control formulation had the highest significant ($p<0.05$) peak viscosity, holding viscosity and final viscosity compared to the rest of the formulations and significantly lower for breakdown viscosity, setback viscosity, peak time and pasting temperature.


Peak Viscosity
4000
3500
3000
2500
2000
1500
Control
10%
15%
20%
Composition
Pumpkin
Tomato
Mushrooms
Carrots


Holding Viscosity
2000
1800
1600
1400
1200
1000
Control
10%
15%
20%
Composition
Pumpkin
Tomato
Mushrooms
Carrots


Final Viscosity
3500
3000
2500
2000
1500
1000
Control
10%
15%
20%
Composition
Pumpkin
Tomato
Mushrooms
Carrots


Setback Viscosity
1500
1000
500
0
Control
10%
15%
20%
Composition
Pumpkin
Tomato
Mushrooms
Carrots


Peak Viscosity
5.5
5
4.5
4
Control
10%
15%
20%
Composition
Pumpkin
Tomato
Mushrooms
Carrots

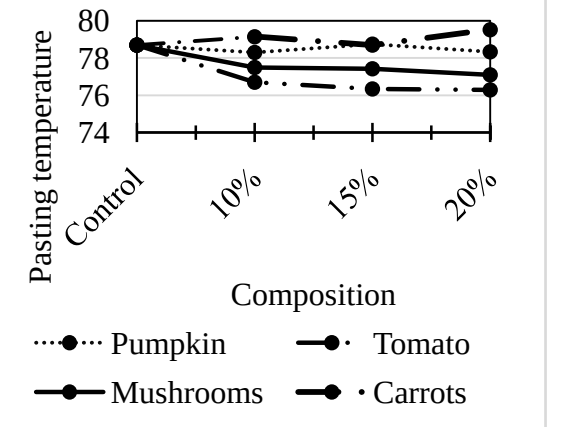

Pasting temperature
80
78
76
74
Control
10%
15%
20%
Composition
Pumpkin
Tomato
Mushrooms
Carrots

Figure 3.4: Pasting properties of the soup samples; Peak Viscosity, holding viscosity, final viscosity, setback viscosity, peak time and pasting temperature.

General pasting properties of soup flour samples were determined using the RVA technology at the department of food technology and nutrition of Makerere University. The results are vary as shown in figure 3.4 above. RVA results indicate that the control formulation has the highest viscosities (final viscosity, peak viscosity, holding viscosity, and setback viscosity) compared to other formulations and that as the percentage composition of pumpkins, tomatoes, mushrooms and carrots increase, there is a general decline in viscosities. In all the formulations, the peak viscosity lies in the range of 2338.5 to 3413.5 Cp, Holding Viscosity lies between 1212 to 1896.5 Cp, Breakdown Viscosity lies between 842.0 to 1628 Cp. The final Viscosity lies between 766.5 to 1276.5 Cp, the peak time lies between 4.63 to 5.17 minutes while the pasting temperature lies between 76.28 to 79.53 ^{o}C.General pasting properties have been indicated in table 3.5 below.

Table 3.5: Pasting properties of flour samples

Formulation code*	Peak Viscosity (Cp)	Holding viscosity (Cp)	Breakdown Viscosity (Cp)[1]	Final Viscosity (Cp)	Setback Viscosity (Cp)[2]	Peak Time (Minutes)	Pasting Temp. (^{o}C)
S0	3413.50±45.96^{g}	1896.50±51.62^{h}	1517.00±97.58^{cde}	2925.50±12.02^{e}	1029.00±63.64^{cd}	4.73±0.09^{abc}	78.70±0.57^{cde}
S1	2952.00±26.87^{cdef}	1605.50±17.68^{ef}	1346.50±44.55^{bcde}	2771.00±9.90^{de}	1165.50±7.78^{cde}	4.83±0.05^{abcd}	78.75±0.49^{de}
S2	3336.00±288.5^{fg}	1654.50±23.33^{fg}	1681.50±265.17^{e}	2858.50±200.11^{e}	1204.00±176.78^{de}	4.77±0.14^{abcd}	76.33±0.53^{a}
S3	2865.50±10.61^{cde}	1473.00±38.18^{cd}	1392.50±27.58^{cde}	2239.50±2.12a^{b}	766.50±36.06^{ab}	4.67±0.00^{ab}	77.43±0.04^{abcd}
S4	2338.50±12.02^{ab}	1313.50±10.61^{ab}	1025.00±1.41^{ab}	2530.50±17.68^{cd}	1217.00±7.07^{de}	5.00±0.00^{de}	78.70±0.57^{cde}
S5	2817.00±14.14^{cde}	1528.00±12.73^{de}	1289.00±1.41^{bcd}	2670.50±3.54^{de}	1142.50±9.19^{cde}	4.90±0.05^{bcd}	78.33±0.04^{bcde}
S6	2749.00±132.94^{cd}	1492.50±4.95^{cde}	1256.50±127.99^{bc}	2520.00±91.92^{cd}	1027.50±86.97^{cd}	4.93±0.00^{cde}	76.28±0.53^{a}
S7	2646.00±28.28^{bc}	1352.50±6.36^{b}	1293.50±21.92^{bcd}	2010.50±17.68^{a}	658.00±11.31^{a}	4.67±0.00^{ab}	77.10±0.64^{abc}
S8	2054.00±22.63^{a}	1212.00±46.67^{a}	842.00±24.04^{a}	2288.50±19.09^{bc}	1076.50±27.58^{cde}	5.17±0.05^{e}	79.53±0.53^{e}
S9	3206.00±8.49^{efg}	1746.00±15.56^{g}	1460.00±7.07^{cde}	2842.50±0.71^{e}	1096.50±14.85^{cde}	4.77±0.05^{abcd}	78.30±0.14^{bcde}
S10	3271.50±140.71^{fg}	1643.50±54.45^{fg}	1628.00±86.27^{de}	2764.00±66.47^{de}	1120.50±12.02^{cde}	4.73±0.09^{abcd}	76.70±0.00^{ab}
S11	3075.00±0.00de^{fg}	1576.50±2.12d^{ef}	1498.50±2.12^{cde}	2530.50±10.61^{cd}	954.00±8.49^{bc}	4.63±0.05^{a}	77.50±0.14^{abcd}
S12	2641.00±9.90^{bc}	1404.00±0.00^{bc}	1237.00±9.90^{bc}	2680.50±53.03^{de}	1276.50±53.03^{e}	4.93±0.00^{cde}	79.15±0.00^{e}

Values represent mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different ($p < 0.05$). *Formulation codes represent samples in the respective formulations indicated in table 4-1.[1]Break down Viscosity= Peak – Holding Viscosity, [2]Setback Viscosity=Final-Holding Viscosity.

2.9. Optimization of responses

Fitting the response surface models for Nutritional composition of the enriched banana vegetable soup.

The ‘‘fitness’’ of the models and their suitability to accurately predict the variation were studied using coefficients of determination (R^2) and F-values as shown in Table 3.6. All the model terms were significant ($p<0.05$). The coefficient of determination R^2 ranged from 0.40 (Peak time, pasting property) to 0.99 (fat content). The R^2 is the proportion of variability in the response values explained or accounted for by the model. The closer the value of R^2 to unity, the better the empirical models fit the actual data and the more relevant the dependent variables in the model have in explaining the behavior of variations. Responses whose coefficients of determination were so close to unity were analyzed. These included Fat content, Protein Content, moisture content, Overall acceptability, in vitro protein digestibility, Fiber content and Zinc with R^2 values of 0.99, 0.93, 0.93, 0.91, 0.91, 0.9, and 0.9 respectively. The lack of fit test was not significant ($p<0.05$).

Model reduction was carried out by dropping the insignificant terms based on the p-value >0.05 in order to improve the model equations using the automatic selection approach.

Table 3.6: Coefficients for the fitted first order and second-order polynomials representing the relationship between the response and formulations of vegetable chicken soup mixture

	Responses									
	GE	**MC**	**Fat**	**FC**	**PC**	**Vit C**	**Carb**	**IVPD**	**Fe**	**Zn**
$\mathbf{X_1}$	41.49	0.1	0.03	0.02	0.11	0.09	0.71	0.74	0.27	0.1
$\mathbf{X_2}$	210.91	-0.46	0.8	0.08	0.21	1.79	-2.96	-0.18	9.25	1.52
$\mathbf{X_3}$	121.8	0.19	0.58	0.1	1.01	1.47	2.33	6.39	8.78	1.33
$\mathbf{X_4}$	25.65	-0.37	0.66	-1.03	0.44	1.55	2.76	-3.09	6.3	-2.14
$\mathbf{X_5}$	-93.11	-0.22	0.68	0.08	0.27	1.49	0.41	-2.4	10.02	2.1
$\mathbf{X_1X_2}$	-2.08	0.01	-0.01	-	-	-0.02	0.04	-	-0.11	-0.02
$\mathbf{X_1X_3}$	-1.04	-	-0.01	-	-0.01	-0.02	-0.02	-0.07	-0.1	-0.01
$\mathbf{X_1X_4}$	0	0.01	-0.01	0.01	-	-0.02	-0.03	0.05	-0.07	0.03
$\mathbf{X_1X_5}$	1.66	0	-0.01	-	-	-0.02	-	0.04	-0.11	-0.02
$\mathbf{R_2}$	0.57	0.93	0.99	0.9	0.93	0.54	0.82	0.91	0.75	0.9
Model (F-value)	13.08	139.58	605.8	130.16	188.83	9.66	44.91	98.72	25.26	76.29

X_1= Banana and Amaranth. X_2= Pumpkins X_3= Tomatoes X_4= Mushrooms X_5= Carrots, $X_{1\text{-}5}$ predicts single effects while X_1X_2, X_1X_3, X_1X_4, and X_1X_5 predicts interaction effects.

3.2.4.9. Model graphs

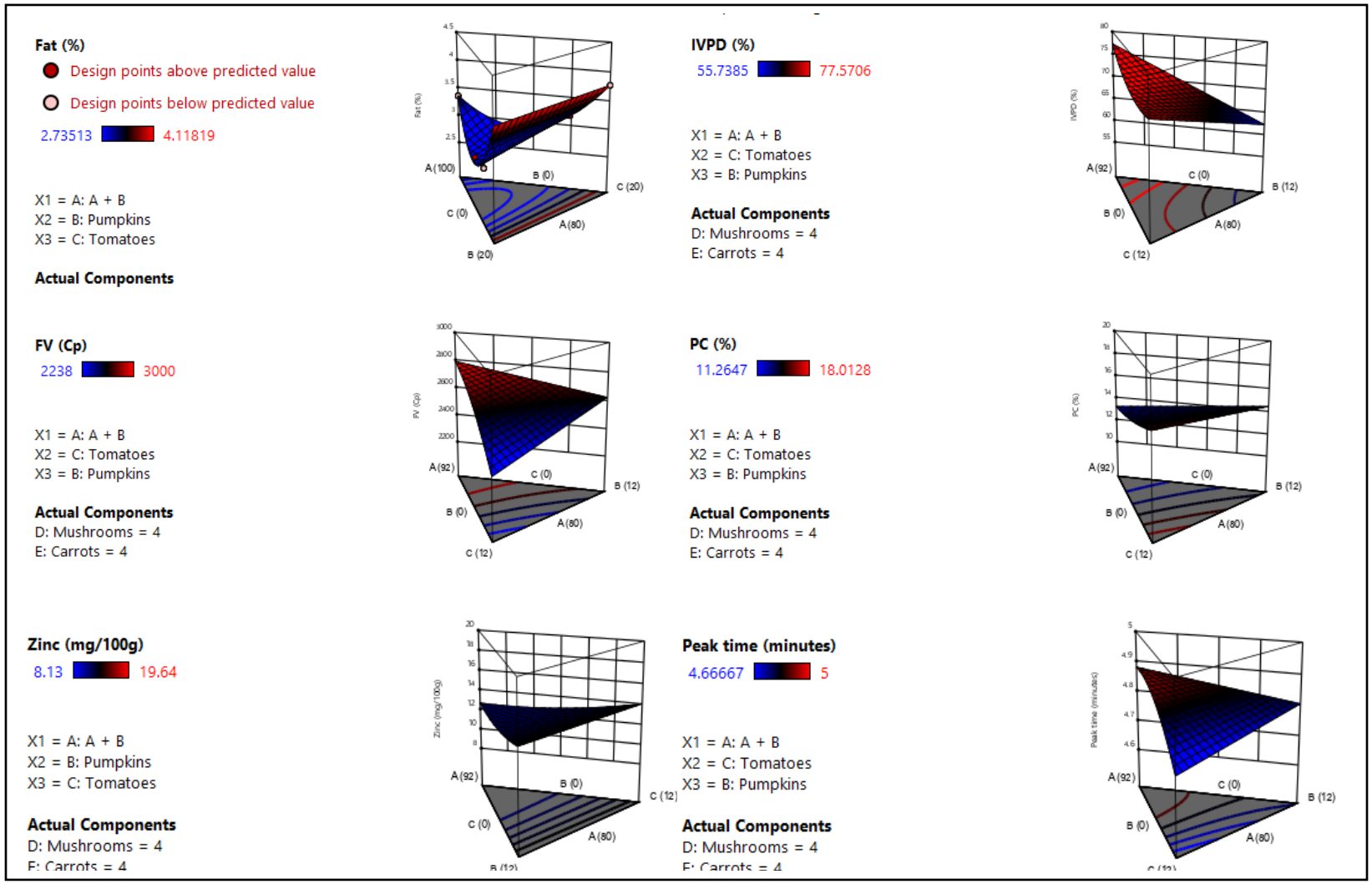


Figure 3.5: 3-D response surface plots showing the effect of ingredient mixes on Zinc content, IVPD, Fat and protein Content.

3.2.4.10. Predictive models for nutrition composition of the formulations

Models predicting the variability of textural properties were developed in addition to models predicting Variability of nutritional properties and physicochemical properties.A mathematical model presented in equation 3.1 was built through regression based on the coefficient data in Table 3.6 for predicting the variability of zinc in the formulation. Zinc content ranges between 8.13 and 19.64 mg/100g. The R^2 of the second order polynomial model was good and explained 90% of the variability.

$$Zn = 0.1X_1 + 1.52X_2 + 1.33X_3 - 2.14X_4 + 2.10X_5 - 0.02X_1X_2 - 0.01X_1X_3 + 0.03X_1X_4 - 0.02X_1X_5 (R^2 = 0.90) \quad (3.1.)$$

The interaction and single variations were all significant terms of the model ($p<0.05$). The model explains that changes in X_2, X_3 and X_5 causes a significant changes in zinc content with X_5 causing the most significant change. The coefficients for the model terms shows high positive changes in the model. The variations of pumpkin, carrots and tomatoes in figure 3.5 ranges between 0 and 12, with the highest effects in the response identified when Pumpkins, carrots and tomatoes are at the maximum. It can therefore be concluded that Zinc content of the soup increased with increase in the proportion of carrots, pumpkin and tomatoes respectively in the formulation. The interaction factor for mushrooms was negative implying that Zn reduces with increase in the proportion of mushrooms in the formulation. The Quadratic interaction between Banana-Amaranth with Pumpkin, Tomatoes, Carrots had a significant negative effect on Zn content while the interaction effect between Banana-Amaranth and Mushrroms had a positive significant effect on Zinc content of the sample. It can be concluded that mushrroms do not contribute significantly to the zinc content of the samples. The curve indicated in 3.5 indicates that the Quadratic effect was negative. The R^2 of the predicted model was high and attained high significance in relation to other models ($R^2 = 0.90$, $p = 0.0001$). The model had a non significant lack of fit which suggests a good fit to the mathematical model in Equation (3.1). This means that this model is valid and can be used in subsequent prediction and optimisation stages.

A mathematical model in equation 3.2 for In vitro Protein digestibility (IVPD) of the sample was built based on coefficient data in Table 3.6. Equation 4.2predicts variability of IVPD in the soup. IVPD ranges between 55.7 and 77.6% in the samples. The R^2 of the second order polynomial model was good and explained 91% of the variability.

$$IVPD = 0.74X_1 - 0.18X_2 + 6.39X_3 - 3.09X_4 - 2.40X_5 - 0.07X_1X_3 + 0.05X_1X_4 + 0.04X_1X_5 (R^2 = 0.91) \quad (3.2.)$$

The interaction and single variations were all significant terms of the model ($p<0.05$). The model explains that changes in X_3 causes a significant positive change in IVPD while X_4 produces a significant negative change in IVPD. The variations of pumpkin, carrots and tomatoes from the curve ranges between 0 and 12, with the highest effects in IVPD occurs when Pumpkins, carrots and tomatoes are at the maximum. It can therefore be concluded that IVPD of the soup increased with increase in the proportion of tomatoes in the formulation. The interaction factor for pumpkins, mushrooms and carrots were negative implying that IVPD reduces with increase in the proportion of pumpkins, mushrooms and carrots in the formulation. The Quadratic interaction between Banana-Amaranth with Tomatoes had a significant negative effect on IVPD content while the interaction effect between Banana-Amaranth with Mushrroms and carrots had a significant negative effect on IVPD of the sample. It can be concluded that mushrooms, pumpkins and carrots do not contribute significantly to the IVPD in the formulation. The curve indicated in figure 3.5 indicates that the Quadratic effect was positive.

The R^2 of the predicted model was high and attained high significance in relation to other models (R^2 = 0.91, p = 0.0001). The model had a non significant lack of fit which suggests a good fit to the mathematical model in Equation (3.2). This means that this model is valid and can be used in subsequent prediction and optimisation stages.

A mathematical model in equation 3.3 predicts variability of Fat Content (Fat) content in the sample based on the coefficient data in Table 3.6. The R^2 of the second order polynomial model was good and explained 99% of the variability.

$$Fat = 0.03X_1 + 0.80X_2 + 0.58X_3 + 0.66X_4 + 0.68X_5 - 0.01X_1X_2 - 0.01X_1X_3 - 0.01X_1X_4 - 0.01X_1X_5 (R^2 = 0.99) \quad (3.3.)$$

The interaction and single variations were all significant terms of the model ($p<0.05$). The model explains that changes in X_1, X_2, X_3, X_4 and X_5 causes a positive significant change in fat content with X_2 causing the most significant change. This indicates that fats in the sample are greatly contributed by pumpkins in the sample. The variations of pumpkin, carrots, mushrooms and tomatoes from the curve ranges between 0 and 12, with the highest effects in the response identified indicates identified when Pumpkins, carrots and tomatoes are at the maximum. It can therefore be concluded that fat content of the soup increased with increase in the proportion of carrots, mushrooms, pumpkin and tomatoes respectively in the formulation. The Quadratic interaction between Banana-Amaranth with Pumpkin, Tomatoes, Mushrooms and Carrots had a significant negative effect on Fat content. The curve indicated

in figure 3.5 indicates that the Quadratic effect was positive. The R^2 of the predicted model was high and attained the highest significance in relation to other models ($R^2 = 0.99$, $p = 0.0001$). The model had a non significant lack of fit which suggests a good fit to the mathematical model in Equation (3.4). This means that this model is valid and can be used in subsequent prediction and optimisation stages.

A mathematical model in equation 3.4 for Protein Content (PC) content of the sample was built through regression based on the coefficient data in Table 3.6. Equation 3.4 describes the relationship between PC and soup formulation. PC content ranges between 11.26 and 18.01%. The R^2 of the second order polynomial model was good and explained 93% of the variability.

$$PC = 0.11X_1 + 0.21X_2 - 1.01X_3 + 0.44X_4 + 0.27X_5 - 0.01X_1X_3 (R^2 = 0.93) \quad (3.4.)$$

The interaction and single variations represented were all significant terms of the model ($p<0.05$). The model explains that changes in X_2, X_3 and X_5 causes a significant changes in PC with X_5 causing the most significant change. The coefficients for the model terms shows high positive changes in the model. The variations of pumpkin, carrots and tomatoes from the curve ranges between 0 and 12, with the highest effects in the response identified indicates identified when Pumpkins, carrots and tomatoes are at the maximum. It can therefore be concluded that PC of the soup increased with increase in the proportion of carrots, pumpkin and tomatoes respectively in the formulation. The interaction factor for mushrooms was negative implying that PC reduces with increase in the proportion of mushrooms in the formulation. The R^2 of the predicted model was high and attained high significance in relation to other models ($R^2 = 0.93$, $p = 0.0001$). The model had a non significant lack of fit which suggests a good fit to the mathematical model in Equation (6). This means that this model is valid and can be used in subsequent prediction and optimisation stages.

A mathematical model in equation 3.5 for Fibre Conctent (FC) content of the sample was built based on the coefficient data in Table 3.6. Equation 3.5 describes the relationship between FC and soup formulation. The R^2 of the second order polynomial model was good and explained 90% of the variability.

$$FC = 0.02X_1 + 0.08X_2 + 0.1X_3 - 1.03X_4 + 0.08X_5 + 0.01X_1X_4 (R^2 = 0.90) \quad 3.5.$$

The model explains both single effects and interaction effects for X_1X_4 . The interaction and single variations were all significant terms of the model ($p<0.05$). The model explains that changes in X_2, X_3 and X_5 causes a significant changes in PC with X_5 causing the most significant change. The coefficients for the model terms shows high positive changes in the model. The variations of pumpkin, carrots and

tomatoes from the curve ranges between 0 and 12, with the highest effects in the response identified indicates identified when Pumpkins, carrots and tomatoes are at the maximum. It can therefore be concluded that PC of the soup increased with increase in the proportion of carrots, pumpkin and tomatoes respectively in the formulation. The interaction factor for mushrooms was negative implying that FC reduces with increase in the proportion of mushrooms in the formulation. The Quadratic interaction between Banana-Amaranth with Pumpkin, Tomatoes, Carrots had a significant negative effect on FC content while the interaction effect between Banana-Amaranth and Mushrroms had a positive significant effect on FC of the sample. It can be concluded that mushrroms do not contribute significantly to the FC of the samples. The curve indicated in figure 3.5 indicates that the Quadratic effect was positive. The R^2 of the predicted model was high and attained high significance in relation to other models ($R^2 = 0.90$, $p = 0.0001$). The model had a non significant lack of fit which suggests a good fit to the mathematical model in Equation (3.5). This means that this model is valid and can be used in subsequent prediction and optimisation stages.

3.2.4.11. Optimal solutions for selection of nutrient enriched soup flour

The Optimal formulation was obtained basing on the behavior of starch (pasting properties) and nutritional composition of the formulation. The selection of the model to use was based on the value with the greatest desirability using Desirability Function approach (DFA). The final composition of flour with the best combination according to the criteria was 81.67% of bananas mixed with Amaranth in the ratio of 1:1, 4% Mushrooms and 5.34% Carrots at a desirability index of 0.51. The formulation had an energy composition of 409.39 kCal/100g), with peak, holding (hot paste), breakdown, final and setback viscosities of 2631.41, 1430.11, 1209.57, 2495.29 and 1056.92 Cp respectively. The peak time, Pasting Temperature, Carbohydrates, Protein content,IVPD and Zinc content are 4.9 minutes, 78.41 ^{o}C, 65.38%, 14.86%, 67.83%, and 13.5 respectively.

Table 3.7: Optimal solutions for nutritious and viscous soup formulations.

Number	**1**	**2**	**3**	**4**	**5**
Banana + Amaranths	**81.67**	80.00	81.58	80.00	80.00
Pumpkins (%)	**9.24**	10.41	12.53	12.89	13.74
Tomatoes (%)	**0.00**	0.00	0.19	0.85	0.00
Mushrooms (%)	**3.76**	3.62	0.00	0.00	0.00
Carrots (%)	**5.34**	5.97	5.71	6.26	6.26
GE (kCal/100g)	**409.39**	413.45	415.93	420.03	420.75
Peak Viscosity (cP)	**2631.41**	2557.80	2645.15	2574.98	2575.85
Holding viscosity (cP)	**1430.11**	1403.42	1459.55	1433.72	1434.81
Breakdown Viscosity (cP)	**1209.57**	1176.56	1195.13	1164.21	1164.36
Final Viscosity (cP)	**2495.29**	2468.71	2612.53	2579.33	2584.79
Setback Viscosity (cP)	**1056.92**	1043.98	1148.06	1132.38	1137.29
Peak Time (Minutes)	**4.90**	4.94	4.95	4.98	4.98
Pasting Temp. (^{o}C)	**78.41**	78.45	78.66	78.60	78.69
Carbohydrates (%)	**65.38**	64.56	65.62	64.86	64.91
Protein content (%)	**14.86**	15.31	14.48	14.95	14.96
IVPD (%)	**67.83**	67.68	68.71	68.09	68.08
Zinc (g/100g)	**13.50**	13.80	12.38	12.96	13.00
Desirability	**0.51**	0.50	0.50	0.49	0.49
	Selected				

2.10. Discussion and Conclusion

The results indicated that there is a significant change (p=0.05) in texture as well as nutritional composition and physico-chemical properties.The pasting properties of bananas-amaranth mixture indicated the highest Viscosities due to the starch content in Bananas being higher (68.52%) as indicated in table 2.1. Viscosities tend to go down as the starch content goes down and this is due to the

association of starch with other components such as proteins, fats among others(Cheok et al., 2018; Shimelis et al., 2006). The selected formulation presented in table 3.7 has a gross Energy Content of 409.39 kCal/100g which is greater than the energy reported for bananas. The protein content of 14.86% is higher than that reported by Muranga et al., (2010) of 5.57%. The peak Viscosity (219.28RVU), holding viscosity (119.18 RVU), and breakdown viscosity (100.80 RVU) are lower than the pasting properties exhibited by 100% banana flour by Muranga et al., (2010)of 375.92 RVU, 201.29 RVU and 174.63 RVU for peak, holding and breakdown viscosities respectively which are good properties for use in the baking industries (Daramola & Osanyinlusi, 2006). This is explained by the reduction in the starch content of flours when the starch rich banana flour is replaced with the vegetable flours (Adeniji et al., 2010; Shimelis et al., 2006). The reduction in the viscosities brings this to the viscosity range for children below five years of between 2000 – 3000 Cp (1Cp=12RVU). This indicates that the soup is palatable to the target group. It can be concluded that the addition of vegetables in bananas improves the nutrient composition of banana flour as well as the texture, physicochemical and mineral composition of the samples thus allowing for the development of a good quality soup from banana-vegetable mixes. Further developments to improve acceptability of the soup and also make the product a user friendly product were adopted and agrees to extrusion as a legit technology for producing instant products. Chicken being one of the flavors consumed by majority of people was incorporated into the soup samples and its effect analyzed.

2.11. References

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CHAPTER FOUR: RESEARCH MANUSCRIPT TWO

Optimization of extrusion conditions and determination of the effects of incorporation of cooked vegetable-chicken mixture on sensory acceptability, nutritional composition and physico-chemical properties of the instant soup powder.

Abstract

The effect of feed moisture content and barrel temperature on product responses which include; vitamin A retention, vitamin C, viscosity, total fats, were studied using response surface methodology. The mixture of 50:50 banana-Amaranth was extruded at different moisture content (12–20%) and barrel temperature (120-180°C).The results were analyzed for easy reconstitution using boiled water and a flour rate of 8% (80g of flour into 1L of water) from which some samples which could not reconstitute were eliminated. To improve acceptability, the samples were blended with chicken-vegetables mixture that was cooked conventionally and dried to get flour. There is therefore, a need to develop nutritious and instant foods. However, little information exists on the production of instant soup from banana based composite flour and the effect of extrusion variables on banana-amaranths based flour is not known. This study was therefore aimed at producing nutritious instant banana-amaranth based soups and to determine the effect of extrusion variables (feed moisture content and barrel temperature) on the nutritional quality and overall acceptability of the products. This study achieved the objective of contributing to the nutrition status of children under-five years of age.

4.1. Introduction

The global lifestyle of people, characterized by limited free time and increased working hours, has led to an increased demand of RTE products(Filli, Nkama, & Jideani, 2013). Extrusion cooking technology has been employed in developing RTE products from raw materials ranging from cereal flour, tubers and legumes. Examples of extruded products include breakfast cereals, snacks, porridges, soups, flakes and quickcooking pasta(James & Nwabueze, 2013).Like other thermal processing methods, extrusion has effects on nutritional and physicochemical properties(Nayak *et al.,* 2011).The destruction of pro-vitamin Awas reported to be low compared to conventional traditional methods due to HTST technology explored during extrusion(Singh *et al.,* 2007). The destruction of pro-vitamin A depends onthe extrusion condition; moisture content, screw speed, feed rate and temperature(Pathania *et al.,* 2013). Extrusion cooking is a known technology used to produce instant foods of good sensory quality

for children(Muoki, 2013). Extrusion offers hope for improving nutrition in less-developed nations(Camire, 2001). The process enhances protein digestibility of foods through protein denaturation, unfolding of polypeptide bonds and reduction in anti-nutritional factors (Björck & Asp, 1983). It also improves mineral bioavailability(Alonso *et al.,* 2001) and results in higher vitamin A retention. Previous research on extrusion cooking of instant soups has been explored to analyze the effect of extrusion on vitamin A retention (Pathania *et al.,* 2013; Pelembe *et al.,* 2002).

4.2. Materials and methods

4.2.1. Materials

The materials presented in table 4.1were purchased from Capital shoppers' super market, garden city branch, Kampala Uganda. The materials were parked and transported in paper bags and polythene papers. The materials were first stored in a refrigerator to be prepared later in the afternoon.

Table 4.1: Formulations for preparation of chicken vegetable mix

	Formulation		
Component	**1**	**2**	**3**
Chicken	0.814kg	0.858kg	0.888kg
Water	2500 mls	2500 mls	2500 mls
Pumpkin	600g	600g	600g
Carrots	20g	20g	20g
Cooking time	2h	2h	2h
Onion	15g	15g	15g
Rosemary	0.5g of leaves	0.5g, leaves	0.5g, leaves
Thyme	1 sprig	1 sprig	1 sprig
Basil	0.5g of leaves	0.5g of leaves	0.5g of leaves
Dill pp	1 stalk	1 stalk	1 stalk
Bay leaves	5g	5g	5g
Parsley	5g	5g	5g
Gallic	7g	7g	7g
Chicken left after cooking	0%	10%	20%

4.2.2. Methods

4.2.2.1. Method of preparation

The samples are weighed in the respective amounts using a Newal Kitchen Scale weighing scale (Model No: NWL-7001, Turkish Origin) and put in an Aluminium source pan. The samples are boiled using fire set using total gas for one hour. The pumpkin and carrot samples are then added to continue the boiling for about 30 minutes. The boiled samples are left to simmer for 45 minutes. The samples are then laid on an Aluminium foil and dried in an air Oven drier for about 48 hours. The dried samples are then milled using a Pigeon mixer grinder (Classical lite, Stove Kraft Pvt. Ltd, India made). They are then sieved using a 450 MIC sieve. In sample set 1, all the chicken pieces were removed while in sample set 2 and 3, a significant amount of chicken was left to account for the 10% and 20% of the total mass content respectively.

Table 4.2: Coding of flour vegetable-chicken mixes.

		Chicken composition		
Flours		**0%**	**10%**	**20%**
S1	50:50	X1	X2	X3
	60:40	X11	X22	X33
S2	50:50	Y1	Y2	Y3
	60:40	Y11	Y22	Y33

The ratios are in the form of F:C where F refers to extruded flour and C refers to chicken flour. Chicken composition refers to the percentage of chicken meat dried with the vegetables. Samples S1 and S2 are extruded flour samples at different extrusion conditions. Sample, S1 was extruded at an outlet temperature of 120^{o}C and moisture content of 10.17%. Sample, S2 was extruded at an output temperature of 140^{o}C and a moisture content of 14.3%. In all cases the screw speed, federate and cutting rate were kept constant at 45Hz, 40Hz and 50Hz respectively. The produced samples shown in table 4.2 were taken to the laboratory at SFTNB for nutritional, textural and physicochemical analysis.

4.2.2.2. The central composite design used to optimize levels of feed moisture content, screw speed and the barrel temperature of the extruded flours

A central composite design (Table 4.3) were used to determine the effect of feed moisture content and barrel temperature on the nutritional quality and consumer acceptability of Banana based flours. Each

combination of feed moisture content and barrel temperature were investigated first for reconstitution and were then evaluated.

Table 4.3: Central Composite Design used to optimize feed moisture, screw speed and barrel temperature for the production of instant banana-based soup flours

Run	A: Barrel Temperature (^oC)	B: Feed Moisture (%)
1	120	10.12
2	140	14.3
3	160	17.86
4	180	15.71

The other parameters of the extruder were kept constant include the feed screw speed, feeding rate, cutting rate maintained at 45Hz, 40Hz and 50Hz respectively.

4.2.2.3. Reconstitution experiment to determine the most acceptable flours

Samples extruded at conditions in table 4.3 were prepared by boiling water and mixing with extruded flour at a flour rate of 8g in 100mls of water (8%). The samples were thoroughly mixed and poured on Aluminium foils for observation. Sample S1 and S2 were easy to reconstitute compared to samples S3 and S4. The pictorial effects have been illustrated as shown in figure 4.1 below

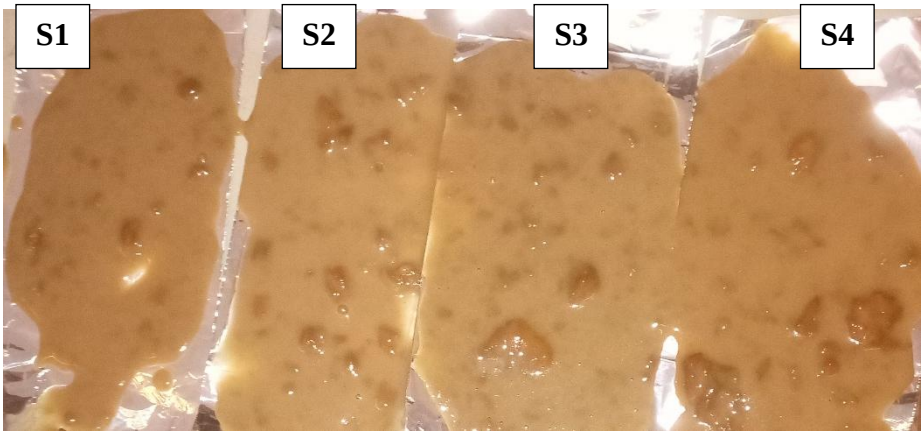

Figure 4.1: Reconstitution of extruded samples at different conditions

4.2.2.4. Effect of extrusion

Effect of extrusion on the dependent parameters were analyzed. Data were presented in form of models and graphs while using the Desirability Function Approach, the best models were selected and the effects of the parameters discussed.The dependent variables were expressed individually as a function of the independent variables. The data was fitted to a second-order approximation model shown as equation 4.1(Pathania *et al.*, 2013):

$$Y = B_0 + \sum_{i-1}^{k} B_i X_i + \sum_{i-1}^{k} B_{ii} X_i^{\,2} + \sum_{i-1}^{k} B_{ij} X_i X_j + \varepsilon \qquad (4.1)$$

Where Y is the response function, X_i is the feed moisture content, $X_j$is the barrel temperature, ε is the random error, B_o the center point of the system, B_i, B_{ii}, and B_{ij} represent the coefficients of the linear, quadratic and interactive effects of the independent variables respectively, and X_i, $X_i^{2,}$ and X_iX_j represent the linear, quadratic and interactive effects, respectively of the independent variable

4.2.2.5. Preparation of composite flour soups for acceptability screening

Three different soups extruded at different conditions were prepared by mixing 50g of the flour in 800mls of water under constant stirring. Each composite flour was cooked until it boils up and then it was allowed to simmer for five minutes. This was ready for serving to a panelist of about 30 people.

4.2.2.6. Sensory acceptability of the composite flour soups.

The soup mix was evaluated for its color, appearance, aroma and texture on a 9-point hedonic scale by a trained panel of 30 people. The 9-point hedonic scale grading was as follows: “1" Dislike extremely, "2" Dislike very much, "3" Dislike moderately, "4" Dislike slightly, "5" Neither like nor dislike, "6" Like slightly, "7" Like moderately, "8" Like very much and "9" Like extremely. The samples were coded and then presented to the panel for evaluation (Rodrigues *et al.,* 2012).;

4.2.2.7. Nutritional and physicochemical properties

In order to determine the change in nutritional physicochemical and textural changes in the soup. The generated samples were analyzed using standard methods described in section 3.6. Viscosity measurements were carried out using the Brookfield viscometer.

4.3. Results

Table 4.4: Consumer acceptability scores of soups from different flour formulations

Formulation code*	Appearance	Color	Texture	Aroma	Mouth feel	Taste	Overall acceptability
X1	6.53±1.94[a]	6.77±2.18[a]	6.53±1.41[a]	6.33±1.94[a]	6.80±1.65[a]	6.60±1.71[a]	7.20±1.58[a]
X11	6.53±1.83[a]	6.70±1.76[a]	6.57±1.45[a]	6.70±1.62[a]	6.43±1.63[a]	6.00±1.93[a]	6.97±1.77[a]
X2	6.93±1.11 [a]	6.67±1.49[a]	6.07±1.41[a]	6.43±1.48[a]	6.57±1.79[a]	6.93±1.57[a]	6.80±1.73[a]
X22	6.53±1.63[a]	6.13±1.55[a]	6.47±1.36[a]	7.13±1.81[a]	7.47±1.25[a]	7.17±1.42[a]	7.13±1.78[a]
X3	7.30±1.47[a]	7.00±1.44[a]	6.87±1.53[a]	6.73±1.17[a]	7.00±1.58[a]	7.43±1.19[a]	7.93±1.11[b]
X33	6.87±1.17[a]	6.30±1.24[a]	6.87±0.86[a]	7.13±1.41[a]	7.23±1.28[a]	7.40±1.16[a]	6.77±1.22[a]
Y1	6.67±1.27[a]	6.67±1.42[a]	6.63±1.45[a]	6.93±1.26[a]	7.07±1.60[a]	7.07±1.41[a]	7.30±1.02[ab]
Y11	7.17±1.37[a]	6.70±1.34[a]	6.70±1.29[a]	6.83±1.42[a]	7.07±1.44[a]	6.87±1.28[a]	7.20±1.67[ab]
Y2	7.00±1.62[a]	7.17±1.53[a]	6.63±1.47[a]	6.97±1.27[a]	6.43±1.33[a]	6.80±1.61[a]	6.77±1.10[a]
Y22	7.40±1.04[a]	6.37±1.19[a]	6.20±1.54[a]	6.60±1.50[a]	6.77±1.63[a]	7.17±1.21[a]	7.33±1.21[a]
Y3	6.50±1.98[a]	6.83±1.21[a]	6.37±1.45[a]	6.53±1.50[a]	6.77±1.57[a]	7.07±1.31[a]	7.07±1.41[a]
Y33	6.70±1.84[a]	6.73±1.36[a]	6.37±1.73[a]	6.00±1.26[a]	6.37±1.19[a]	6.90±1.95[a]	6.97±1.25[a]

Values represent mean ± standard deviation (n=30). Means in the same column with different superscripts are significantly different ($p < 0.05$). *Formulation codes represent samples in the respective formulations indicated in table 4.2

There is no significant difference between the sample formulations and appearance. Sample S10, has the largest appearance of 7.4 while S0 has the least appearance of 6.13. There is no significant difference between the sample appearance ($p=0.05$).The results for sensory analysis of soups made from different flour mixtures of banana, grain amaranths and any of mushrooms, tomatoes, carrots, pumpkins are presented in Table 4-6. For each sample, banana and amaranth flours were used fixed at a proportion of 50:50. The formulation composition are as represented in table 4.2. The Control sample S0 had the lowest scores on all the attributes. Sample S4 had the best scores on aroma and mouth feel of 7.13 and 7.47 respectively which represents like moderately on the hedonic scale. The sample formulation S5 had the best scores on taste and Overall acceptability of 7.43 and 7.93 which represents like very much on the hedonic scale, thus the most preferred sample. The sample formulation S10 had the best scores for appearance (7.40) while the sample formulation S9 had the best scores for color (7.00) which represent like moderately on the hedonic scale. The test attributes all had the same

superscripts for all the formulations which depicts that all the attributes are not significantly different from each other ($p \leq 0.05$). However, for appearance, color, texture, aroma, mouth feel, taste and overall acceptability there is no statistically significant difference between the formulations at $p \leq 0.05$.

4.3.1. Overall acceptability of the formulation

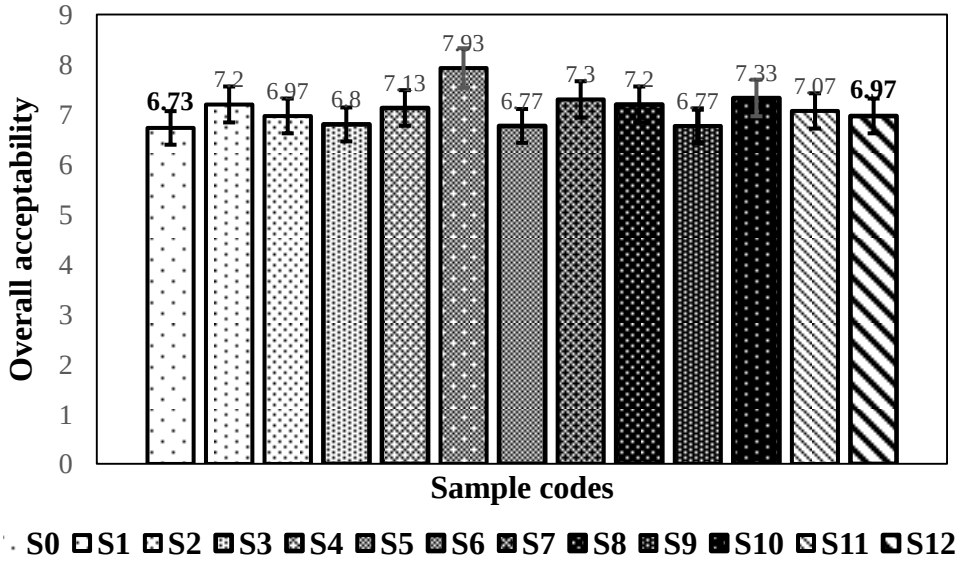

Figure 4.2: Overall acceptability of the sample formulations

The overall acceptability of the samples was plotted against the sample codes as shown in figure 4.2above. The formulation code S5 has the most overall acceptability of 7.93 which represents like very much on the hedonic scale. There are no significant difference between the attributes and Overall acceptability of the samples. This means that the formulations are appealing to the humans for consumption as a palatable food. The most preferred sample according to table 4.4 has a flour composition of 40%, 40% and 20% banana, amaranth grains and pumpkins respectively. The next preferred sample S10 has a composition of 45%, 45% and 10% respectively for banana, amaranth and tomatoes while the next preferred S2 has a formulation of 42.5%, 42.5% and 15% respectively for banana, amaranth and pumpkins. It can be concluded that formulations containing mushrooms are the least acceptable followed by tomatoes, carrots and pumpkins respectively. Referring to the analysis of variance table in the appendix section B, there is no statistical significance between groups of overall acceptability and within the groups at $p \leq 0.05$. This shows that all the formulations can be considered acceptable in front of the customer.

Table 4.5: Proximate composition of extruded samples

Sample code*	MC (%)	Fiber (%)	Fat (%)	Ash (%)	Vit C (mg/100g)	Vit A RAE mg/100g
X1	9.32±0.01de	5.11±0.16bc	13.61±0.43cde	3.93±0.00a	9.26±0.96ab	0.74±0.02ab
Y1	8.53±0.03a	5.22±0.06c	12.72±0.82bc	4.14±0.03abcd	11.45±0.87cde	1.18±0.03e
X11	8.88±0.01bc	5.34±0.28cd	12.41±0.51bc	4.26±0.64abcd	10.71±0.85bcd	0.84±0.03abc
Y11	8.82±0.03ab	4.08±0.08a	13.01±0.82bcd	4.01±0.00ab	13.14±0.82e	0.97±0.01cd
X2	9.25±0.03d	3.67±0.21a	14.74±0.08ef	4.15±0.06abcd	9.79±1.16abc	0.76±0.02ab
Y2	9.20±0.04cd	3.68±0.01a	14.50±0.37de	4.36±0.18abcd	10.65±0.60bcd	0.76±0.00ab
X22	9.37±0.19de	5.76±0.26de	12.49±0.38bc	4.03±0.05abc	10.11±0.61bc	0.70±0.01a
Y22	9.47±0.10de	7.59±0.07f	12.25±0.96bc	4.19±0.26abcd	7.83±0.96a	0.98±0.25cd
X3	10.52±0.07g	4.61±0.12b	11.89±0.72b	4.75±0.11d	21.98±0.73g	1.08±0.04de
Y3	9.62±0.26ef	5.81±0.07de	16.38±0.45f	4.64±0.03cd	12.13±1.37de	1.12±0.07de
X33	10.21±0.16g	5.09±0.02bc	9.18±0.17a	4.57±0.14bcd	18.17±1.49f	1.08±0.27de
Y33	9.83±0.04f	6.02±0.32e	9.12±0.23a	4.42±0.00abcd	12.88±1.21e	0.87±0.01bc

Values represent mean ± standard deviation (n=3). Means in the same column with different superscripts are significantly different ($p<0.05$). *Sample codes Xi (50:50) and Xii (60:40) represent Sample 1 (S1)for Extruded flour: Vegetable Flour respectively while Yj (50:50) and Yjj (60:40) represent Sample 2 (S2) for Extruded flour: Vegetable Flour respectively.

Generally, there are large variations in the results for the final developed product compared to those that were obtained in objective one. A comprehensive comparison was desired to have a broader picture of how this became an important variation for consideration.

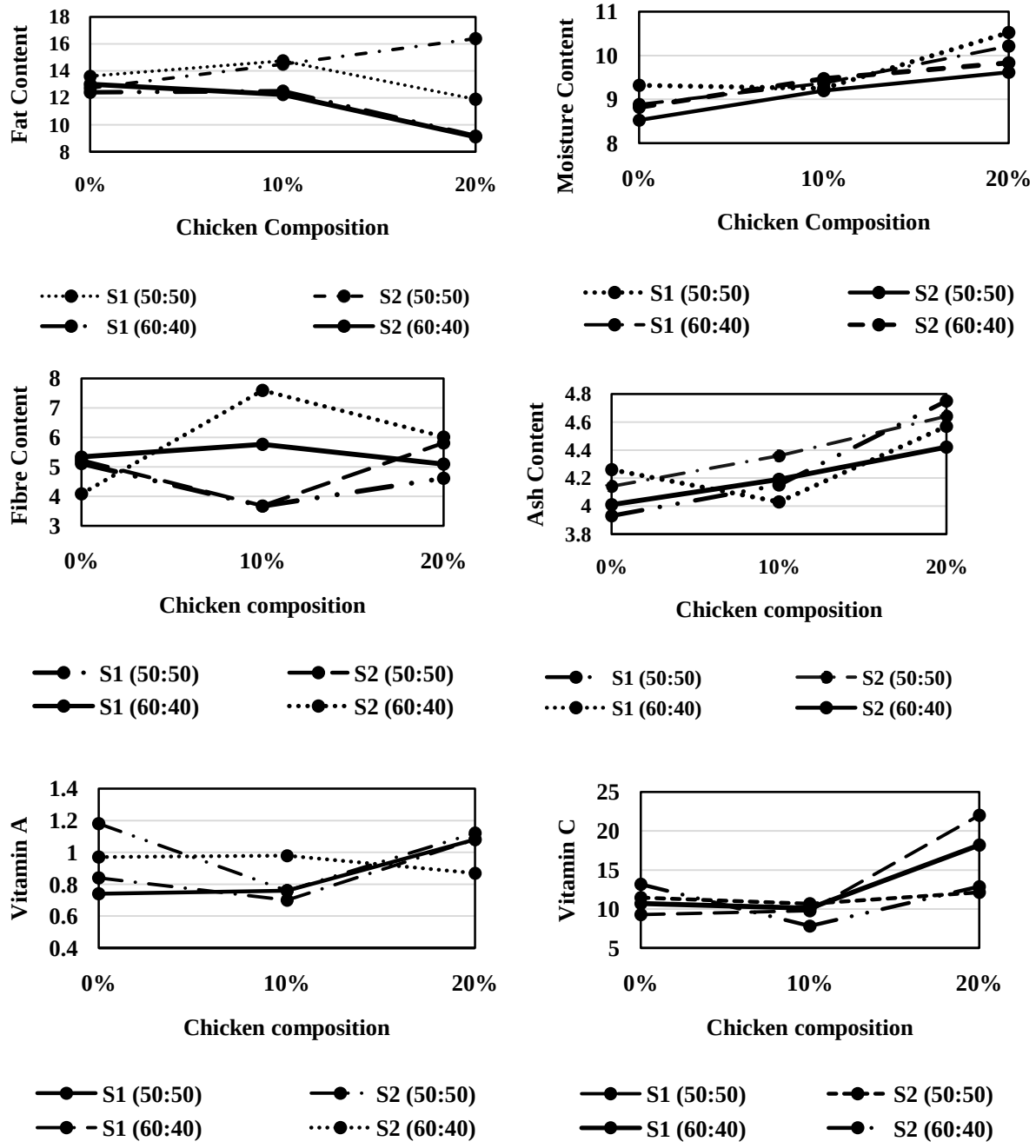

Figure 4.3: Variations in nutrient composition; moisture content, fiber content, fat content, ash content, Vitamin A and Vitamin C in the formulations

The moisture content varies between 8.53±0.03 and 10.52±0.07%. The graphical representation explains the trend in the variation of moisture content with the samples (Figure 4.3). Moisture content increases with composition of chicken in the samples. The addition of chicken thus leads to the storage of more water molecules in the samples. The fiber content varies between 3.67±0.21 and 6.02±0.32%. The graphical representation explains the trend in the variation of fiber content within the samples.

Fiber content improves with composition of chicken in the samples and is greater compared to unextruded samples. The fat content varies between 9.12±0.23 and 16.38±0.45%. The graphical representation explains the trend in the variation of fat content within the samples. Fat content increases with composition of chicken in the samples. The addition of chicken thus leads to storage of more fat molecules in the samples. There is a general increase in the fat content of the samples compared to unextruded flour samples.The ash content varies between 3.93±0.00 and 10.52±0.07%. The graphical representation explains the trend in the variation of ash content within the samples. Ash content increases slightly with composition of chicken in the samples. The addition of chicken thus improves on the quantity of ashes in the samples. The vitamin A content of the samples varies between 0.70±0.03 and 1.18±0.03 RAE mg/100g. The graphical representation explains the trend in the variation of Vitamin A within the samples. Vitamin A increases with increases with composition of chicken in the samples. There is a general improvement in Vitamin A related to the vitamin A that was obtained with unextruded samples. The vitamin C content of the samples varies between 7.83±0.96 and 21.13±1.37 mg/100g. The graphical representation explains the trend in the variation of Vitamin C with the samples. Vitamin C increases with increases with composition of chicken in the samples. There is a general increase in Vitamin C related to the vitamin C that was obtained with unextruded samples.

4.4. Optimization of responses

Predictive models describing variability of dependent variables in the soups.

Responses	**Model**		**Lack of fit**
Overall acceptability	$7.12-0.02X_1+0.05X_3-0.06X_4+0.10X_1X_3+0.11X_1X_4$	R^2 = 0.89, p=0.0001	0.1785
Fat content	$12.72+0.33X_1+0.2207X_3-1.26X_4-0.40X_1X_3-0.23X_1X_4$	R^2 = 0.90, p=0.0001	6.85
Ash content	$4.28-0.20X_3-0.04X_4-0.01X_1X_3-0.05X_1X_4$	R^2 = 0.96, p=0.0001	0.0414
Fibre	$5.08-0.15X_1-0.14X_3+0.39X_4-0.43X_1X_3-0.07X_1X_4$	R^2 = 0.86, p=0.0001	2.20
Vitamin C	$12.34-X_1-0.12X_3-0.20X_4+2.15X_1X_3+0.14X_1X_4$	R^2 = 0.96, p=0.0001	9.93
Vitamin A	$0.92+0.05X_1+0.01X_3-0.02X_4+0.1X_1X_3-0.03X_1X_4$	R^2 = 0.84, p=0.0001	0.0722

X_1,=barrel temperature X_2,=Moisture content, X_3=Chicken vegetables mixand X4= banana-amaranth flour

Barrel temperature (*X*1) and banana-amaranthflour (X4) had a negative linear effect on acceptability whereas chicken composition (X3) had a positive linear effect on Overall acceptability of the soup. The interactive effects were positive. The increase in temperature caused a significant reduction in acceptability of the soup. Increase in chicken composition produces the soup by increasing the flavor in the soup. Barrel temperature (*X*1) and chicken composition (X3) had a positive linear effect in the composition of fats whereas banana-amaranth flour (X4) and interactions had a negative linear and Quadratic effects on fat content. The increase in temperature and chicken composition had a significant increase in the fat composition of the soup. Increase in chicken composition increases the soup's fat content because of its high fat content. Barrel temperature (*X*1), Chicken composition (X3) and banana-amaranth flour (X4) had significant linear and interactive effects on the ash content of the soups. The increase in temperature and chicken composition caused a significant reduction in Ash content of the soup. Barrel temperature (*X*1) and chicken composition (X3) had a negative linear and interactive effects on fiber content of the soup whereas banana-amaranth flour (X4) had a positive linear effect on Vitamin C composition of the soup. The increase in temperature caused a significant reduction in fiber content of the soup. Barrel temperature (*X*1), chicken composition (X3) and banana-amaranth flour (X4) had a negative linear effect and a positive interactive effectson Vitamin C composition of the soup. The increase in temperature, chicken composition and banana flours reduces the Vitamin C composition of the soup. Barrel temperature (*X*1) and chicken composition (X3) had a positive linear and interactive effects on Vitamin a composition of the soup whereas banana-amaranth flour had a negative linear and interactive effects on the soup. Increase in temperature increases the vitamin A availability (Singh et al., 2007).

Table 4.6: Optimal solutions for nutritious and viscous soup formulations.

No.	Temp	MC	CC	BF	VF	OA	FC	Fat	Ash	Vit C	Vit A	Desirability	
1	**120.00**	**14.30**	**20**	**50**	**50**	**7.80**	**4.81**	**12.70**	**4.72**	**21.09**	**1.11**	**0.51**	**Selected**
2	120.00	14.30	20	50	40	7.80	4.81	12.70	4.72	21.09	1.11	0.51	
3	120.33	14.30	20	50	40	7.79	4.82	12.75	4.72	20.95	1.11	0.51	
4	120.98	14.30	20	50	40	7.77	4.85	12.84	4.71	20.69	1.11	0.50	
5	120.00	14.02	20	50	40	7.80	4.81	12.70	4.72	21.09	1.11	0.50	

MC=moisture content, CC= Chicken level in the vegetable; BF=Banana-amaranth flour; VF=Vegetable flour, OA=Overall acceptability, FC= Fiber content.

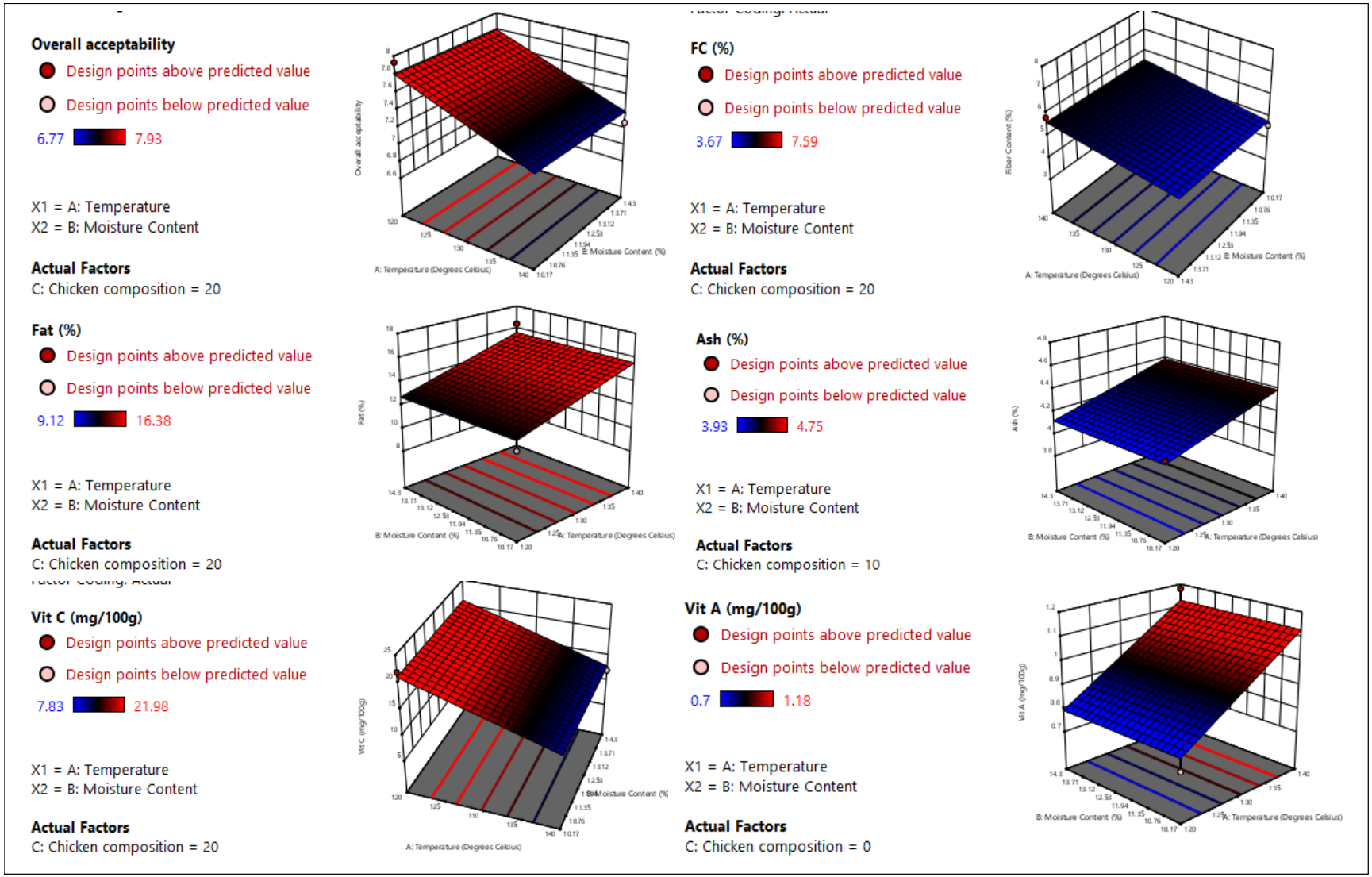

Figure 4.4: Response surface plots showing effect of varying different levels of moisture and temperature and chicken composition on Vitamin A, Vitamin C, Ash content, Fat, Fiber content and Overall acceptability.

4.5. Conclusion and recommendations

The results indicated that there is a significant change (p=0.05) in texture as well as nutritional composition and physico-chemical properties. The optimum extrusion conditions were temperature; moisture combinations of 120^{o}C and 14.3% respectively as shown in table 4.6.The banana flour was at 50% and a vegetable with chicken composition of 20% at 50%. The Overall acceptability, fiber content, fat content, Ash content, Vitamin C and Vitamin A composition of the optimum flour was at 7.80, 4.81%, 12.70%, 4.72%, 21.09 g/100g and 1.11 mg/100g respectively.

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CHAPTER FIVE: RESEARCH MANUSCRIPT THREE

Estimating effect of incorporation of cooked chicken-vegetable mix on shelf-stability of the instant soup flours using accelerated shelf-life testing.

Abstract

Most foods deteriorate with storage time thus the need to determine the time for which the flours are going to remain fit for human consumption. The effect of incorporating chicken has been analyzed. In order to reduce on the time spent in carrying out the experiment, ASLT was opted. This was done by elevating the storage temperature to 37^{o}C and then project the effect to room temperature.Twelve (12) formulations extruded at two different temperature (120^{o}C and 140^{o}C): Moisture content combinations mixed with carrot and pumpkin soup prepared with different ingredients and chicken wings at different levels (0%, 10% and 20%) were subjected to same storage conditions. Microbial analysis of the flours indicated that all samples contained recommendable counts for yeasts and molds, total coliforms and total plate count, and were therefore considered good for human consumption for the storage period.

Keywords: Shelf life, microbial load, yeasts and molds, total plate count, total coliforms

5.1. Introduction

It is common for all processed foods to deteriorate during storage from their first date of production. Determining the time for which the developed product shall remain acceptable for consumption without causing harm to the consumer isvery important in new development. Before setting theexperiment for shelf life testing, it is required to identify the indices of quality loss of a particularproduct. It is important to study the deteriorative factors of the food product in order to predict shelf life(Villota *et al.,* 1992).Shelf life determination of a product normally involvesstoring the product under pre-selected conditions for a period of time longer than the expectedshelf life and checking the product at regular intervals to see when it begins to spoil(Labuza *et al.,* 2002). This method requires aconsiderable amount of experimentation making theprocedure costly and time consuming. Accelerated Shelf Life Testing (ASLT) can be used toshorten the time of experimentation. With ASLT, food products are stored at elevatedtemperatures to accelerate the deterioration process(Shalmany, 2012). Data fromelevated temperatures are converted to regular storage conditions using Arrhenius or Linear equations(Waghray,*et al.,* 2017). The estimation of shelf lifeusing ASLT requires identification of the major spoilage agent or reactions and selection of appropriate indices of spoilage. Therefore the

objective of this study was todetermine the effect of incorporating different levels of chicken to the shelf-life of instantbanana-amaranths soups mixed with cooked vegetables.

5.2. Materials and methods

Accelerated shelf life testing was used to determine the shelf life of different formulatedsoups. Portions (10g) of each sample were packed and sealed in transparent polyethylene bagsand stored at an elevated temperature of 37^{o}C.Sampling for analysis was doneat 0, 14, and 28 days(Waghray *et al.*, 2017).Microbial load was used as a basis to estimate the shelf life of the soup samples. Total coliforms, yeasts and molds and total plate counts were determined and used as indices of quality loss during storage (AOAC, 1995: method 965.33). The dataobtained from elevated temperatures was used to estimate the shelf life at room temperature which are expected to vary between 23 °C and 27 °C usingboth Arrhenius and Linear models as suggested by Lee & Krochta, (2002).

5.2.1. Yeasts and molds

Yeasts and molds were enumerated using the surface spread technique in ISO 21527 – 2:2009. About 15.8 g of Dichloran Rose Bengal Chloramphenicol (DRBC) agar (M588-500G, Hi-Media Laboratories Pvt. Ltd.) was weighed and mixed with distilled water (500 ml). The mixture was sterilized by autoclaving at 121^{o}C for 15 minutes and rapidly cooled in a water bath to about 47^{o}C. Molten agar (20 ml) was aseptically poured into the petri dish and allowed to set. The petri dishes were inverted to avoid the dripping back of condensed water onto the solidified agar. Inocula (0.1 ml) from the serial dilutions (10^{-1} to 10^{-7}) of the soup flour samples were aseptically transferred onto the center of the solidified agar and evenly spread over the surface of the agar using a sterile wire loop. This set up was incubated at 37^{o}C for 5 days. Plates with colonies between 30 and 300 were taken and counted using the colony counter and results expressed as colony forming units per gram(cfu/g).

5.2.2. **Total** plate**count**

The total plate count was enumerated using the pour plate technique, ISO 4833:2013 method. Plate count agar was prepared by dissolving 11.75 g of the agar powder (1056.00, CONDA Pronodisa Laboratories Conda U.S.A) into distilled water (500 ml) and sterilized by autoclaving at 121°C for 15 minutes. It was then allowed to cool to about 47°C using a water bath (Grants instrument Ltd, Shepreth, England). Serial dilutions (10^{-1} to 10^{-7}) of the sample solutions were made. Inocula (1 ml) from each selected dilution were aseptically transferred to petri dishes and about 20 ml of molten agar poured into

each petri dish containing the inoculum. This inoculum was carefully mixed with the agar by rotating the petri dishes and then allowed to solidify. The dishes were then inverted after solidification of the agar and incubated at 37°C for 24 hours. Plates with colonies ranging from 30 to 300 were considered for counting using the colony counter (Stuart SC6, UK) and the results expressed as colony forming units per gram (cfu/g).

5.2.3. Total coliforms count

The total coliforms were determined using the pour plate technique in ISO 4832:2006. Violet Red Bile Lactose (VRBL) agar (M581-500G. Hi-Media Laboratories Pvt. Ltd.) was prepared by weighing 20.75 g of media powder into 500ml of distilled water. The mixture was heated on a Bunsen burner flame until boiling and allowed to boil for 2 minutes then rapidly cooled to 47°C using a water bath. Serial dilutions (10^{-1} to 10^{-7}) of the soup flour samples was made and 1 ml of inoculum from each dilution transferred aseptically to petri dishes. Molten agar (20 ml) was poured into each of the petri dishes containing inocula and carefully mixed by rotating the petri dishes. After solidification, agar (5 ml) was poured over the surface of the previously solidified mixture and left to solidify again to create an anaerobic atmosphere. The dishes were then inverted and incubated at 37°C for 24 hours. After 24 hours, the purplish colonies formed which were considered to be typical coliform colonies. Petri dishes with counts ranging from 30 to 300 were counted using the colony counter and results expressed in colony forming units per gram (cfu/g).

5.2.4. Calculations

The microbial counts, represented as colony forming units per gram (cfu/g), were calculated using equation 5.1 below

$$C = \frac{\sum X}{V\left[n_1 + n_2(0.1)\right]d} \tag{5.1}$$

Where: C =microbial counts, $\sum X$ = sum of all counted colonies, n_1 = number of petri dishes at which the first counting was done, n_2 = number of petri dishes at which the second counting was done, d = dilution factor at which the first counting was done.

5.3. Results and discussion

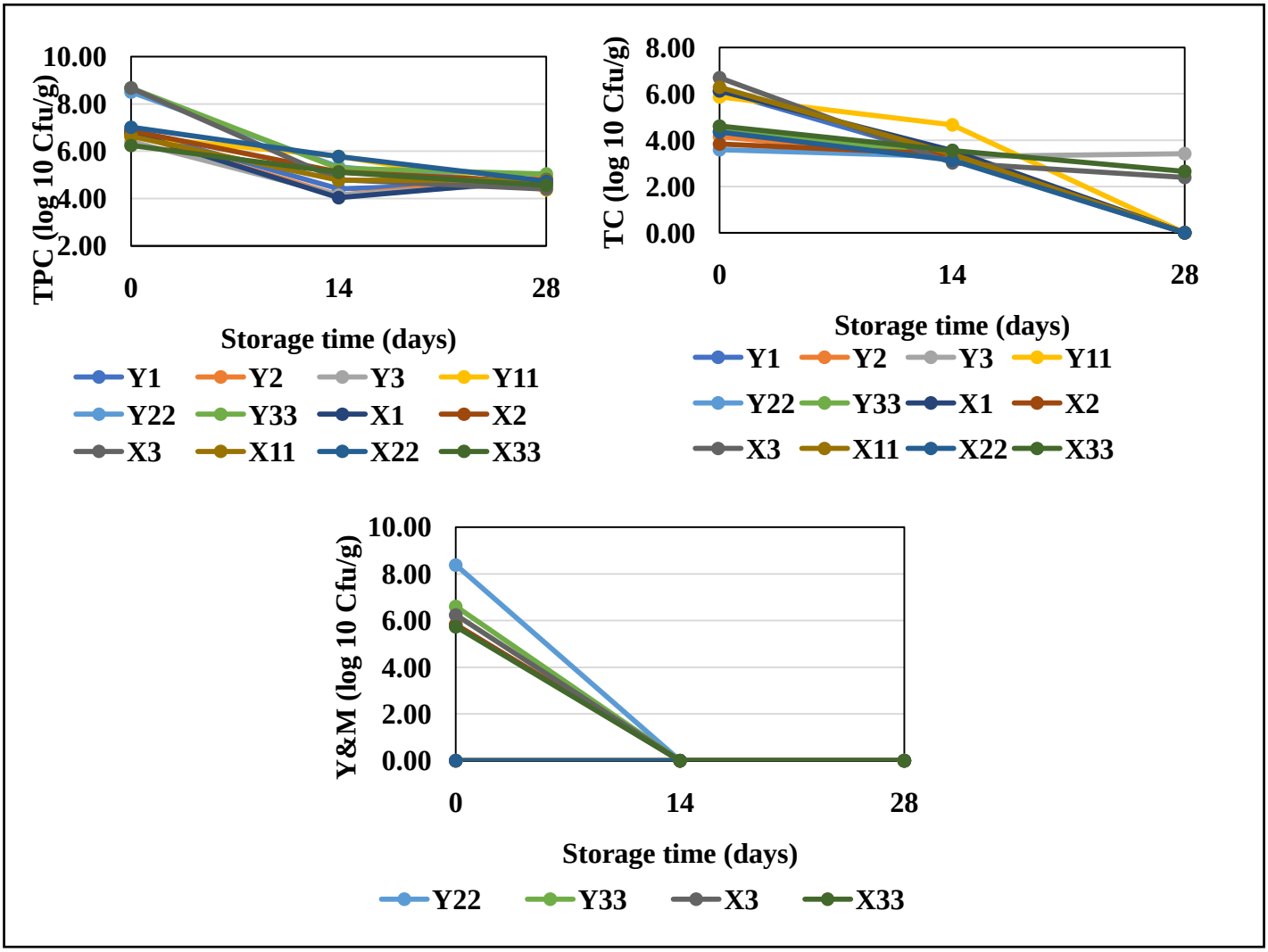

Figure 5.1: Microbial changes in the soup samples during storage

5.3.1. Microbial load in the flours

5.3.1.1. Total plate count

There were significant differences in the total plate count of the flour samples during the high temperature storage ($P<0.05$) as illustrated in figure 5.1. Generally, the total plate count in all the soup flours decreased from day 0 to day 28. The reduction could have been contributed by the packaging method and the high temperature storage. The differences in the microbial load could be due to the different components in the soup. Microbial growth and metabolism is a major cause of flour spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors (Dalgaard*et al*., 2006; Emborg*et al*., 2005; Gram and Dalgaard, 2002). The observed total plate counts were all within acceptable limits; since 10^6cfu/g is the maximum permissible level for aerobic plate counts in meat products (UNBS, 2015; ICMFS 2002). Therefore, all the soup flours analyzed over the period of four weeks of storage at high temperature can be considered acceptable for human consumption

5.3.1.2. Total coliform

There were significant differences in the total coliforms in the storage of the flour samples during high temperature storage for the entire period of 6 weeks storage as shown in figure 5.1. The population of total coliforms in the soup flour samples were less than 6 log cfu/g at both day 0 and day 28. The total coliforms decreased throughout the high temperature storage. The presence of total coliforms is an indication of contamination by humans, contaminated water used during processing or ingredients used during processing. Some of the spices used in the flours could also contain some potential contaminants (Gungor & Gokoglu, 2010). However, spices such as garlic, and ginger used contain antimicrobial agents such as allicin and gingerol respectively and therefore form natural preservatives in the food.

5.3.1.3. Yeasts and Molds

There were no yeasts and molds detected in most of the flours. Yeasts and molds in most of the flours were in acceptable limits.

The Arrhenius model has been used very often to predict the storage time of most foods at different temperatures (Dube, 2015). This was used to predict the storage time at any time.

5.4. Conclusion

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APPENDIX

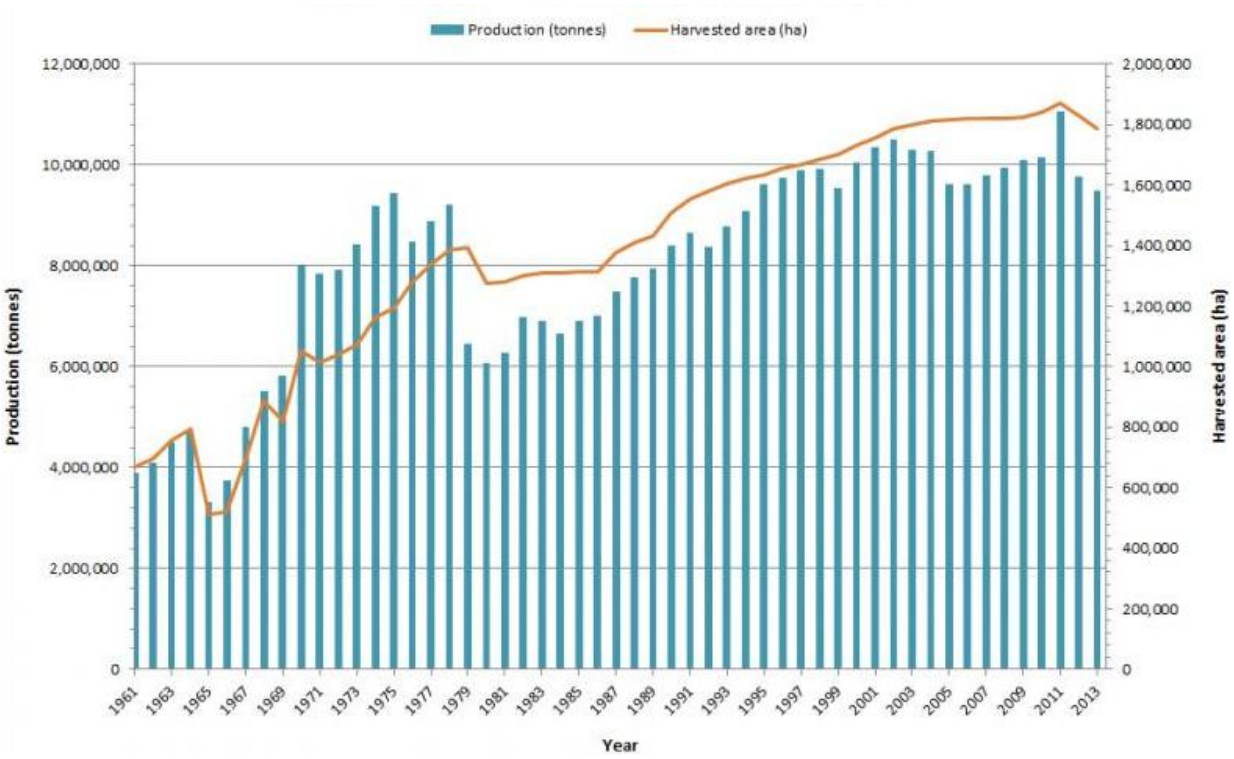
A. ANOVA FOR SENSORY EVALUATION

		Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	13.400	2	6.700	2.399	.097
	Within Groups	243.000	87	2.793		
	Total	256.400	89			
Color	Between Groups	3.622	2	1.811	1.150	.321
	Within Groups	137.000	87	1.575		
	Total	140.622	89			
Texture	Between Groups	.556	2	.278	.112	.895
	Within Groups	216.733	87	2.491		
	Total	217.289	89			
Aroma	Between Groups	6.489	2	3.244	1.598	.208
	Within Groups	176.667	87	2.031		
	Total	183.156	89			
Mouth feel	Between Groups	3.200	2	1.600	.734	.483
	Within Groups	189.700	87	2.180		
	Total	192.900	89			
Taste	Between Groups	1.089	2	.544	.234	.792
	Within Groups	202.733	87	2.330		
	Total	203.822	89			
Overall acceptability	Between Groups	2.156	2	1.078	.644	.527
	Within Groups	145.500	87	1.672		
	Total	147.656	89			

B. ONE WAY ANOVA FOR PROXIMATE COMPOSITION

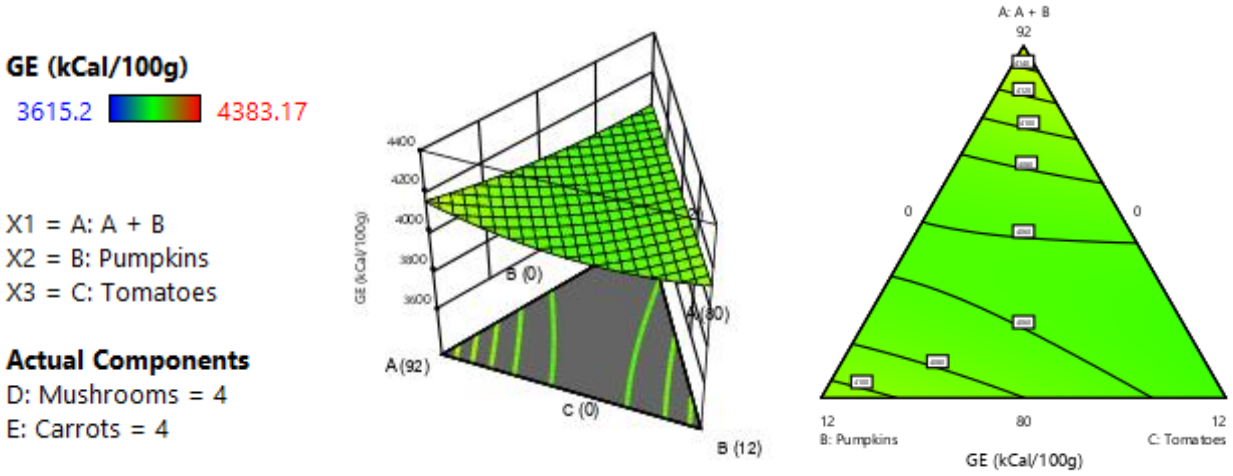
		Sum of Squares	df	Mean Square	F	Sig.
MC	Between Groups	13.998	12	1.167	9.567	.000
	Within Groups	3.170	26	.122		
	Total	17.168	38			
Fat	Between Groups	7.979	12	.665	11.269	.000
	Within Groups	1.534	26	.059		
	Total	9.513	38			
Fiber content	Between Groups	11.721	12	.977	26.234	.000
	Within Groups	.968	26	.037		
	Total	12.689	38			

C. Production of dessert and Cooking Bananas in Uganda (Source FAOSTAT, 2015)

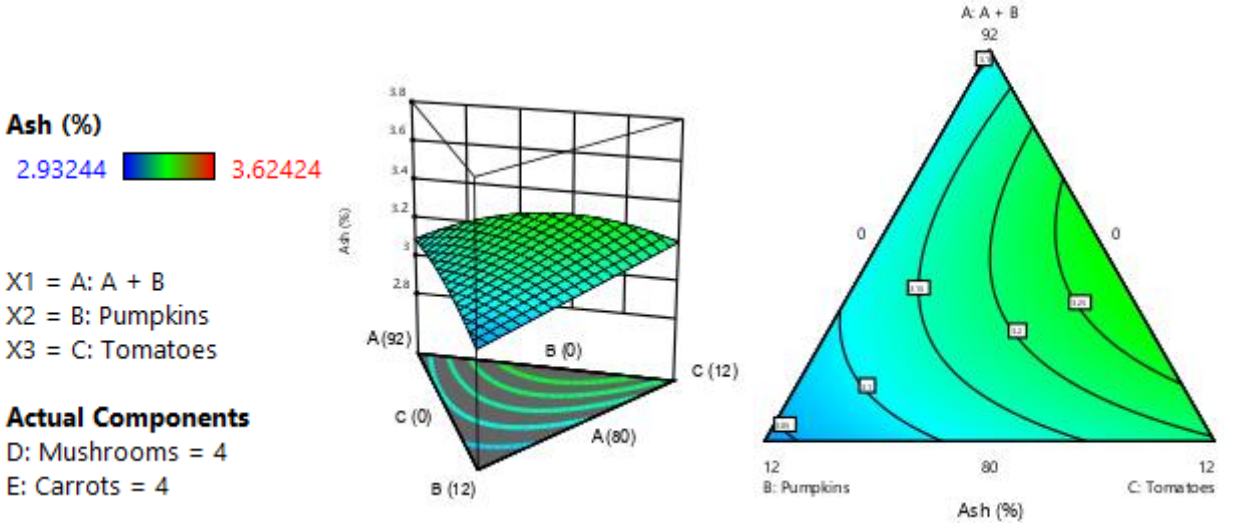


D: 3D PLOTS AND 2D CONTOURS FOR RSM

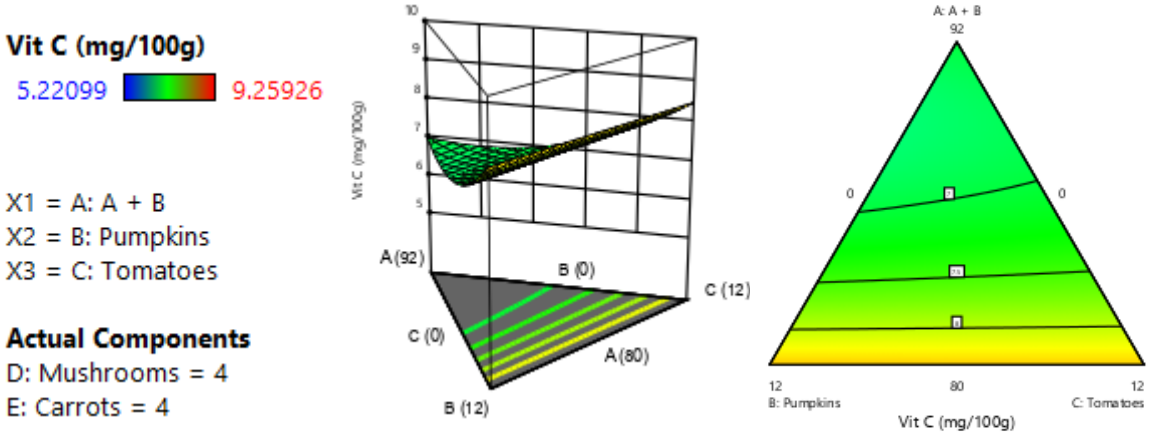
4.1.8 Gross Energy Content



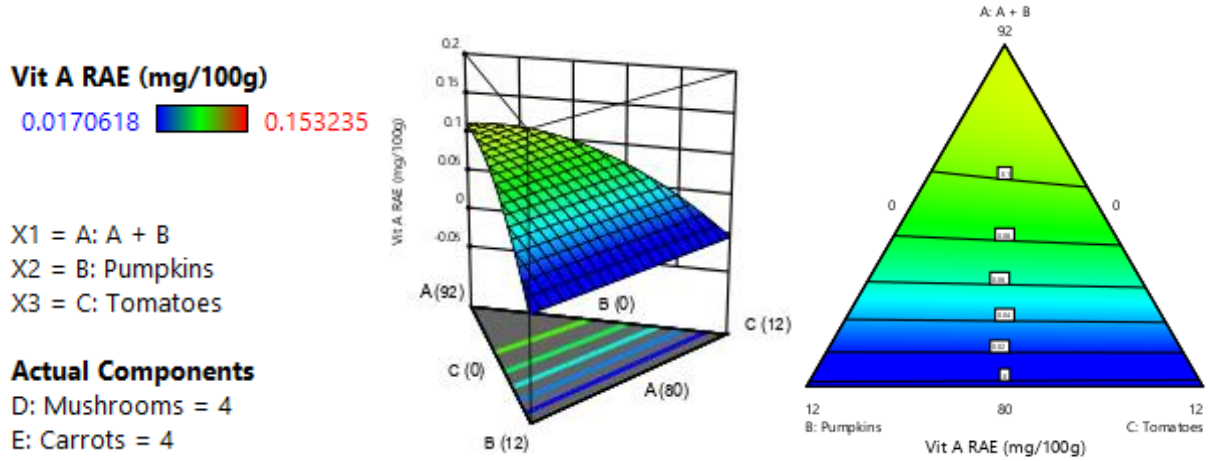
4.1.9 Ash Content



4.1.10 Vitamin C



4.1.10 Vitamin A



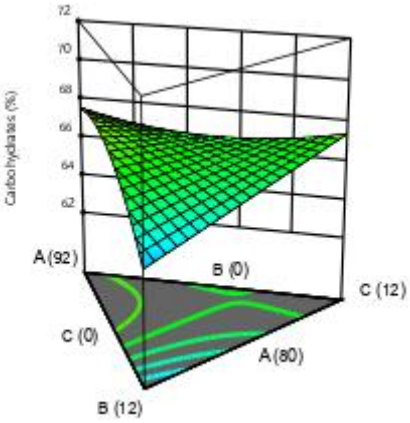
4.1.10 Carbohydrates

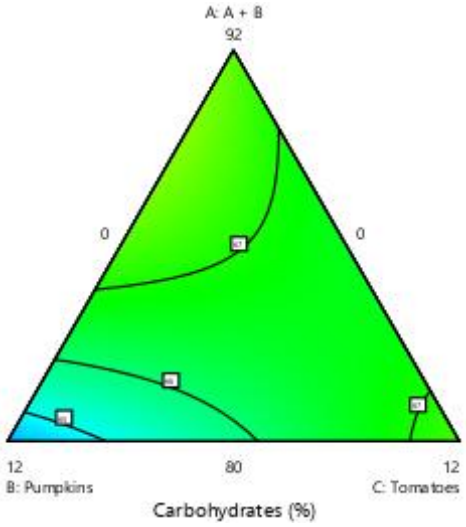
Carbohydrates (%)

63.02 70.67

X1 = A: A + B
X2 = B: Pumpkins
X3 = C: Tomatoes

Actual Components
D: Mushrooms = 4
E: Carrots = 4





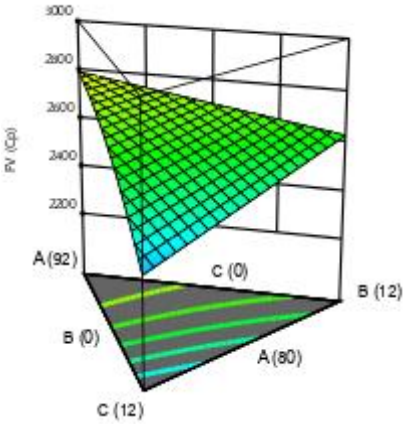
4.1.10 Final Viscosity (Cp)

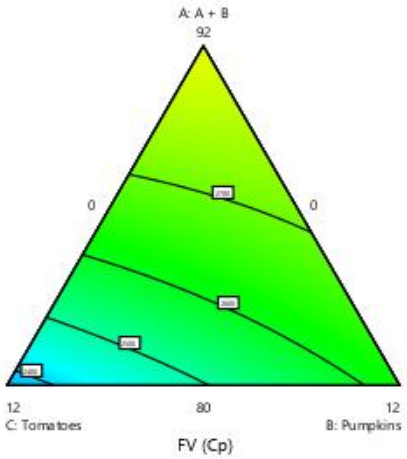
FV (Cp)

2238 3000

X1 = A: A + B
X2 = C: Tomatoes
X3 = B: Pumpkins

Actual Components
D: Mushrooms = 4
E: Carrots = 4





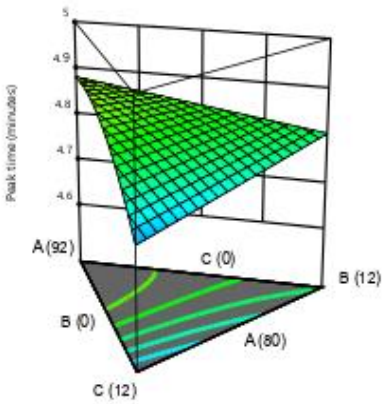
4.1.10 Peak time

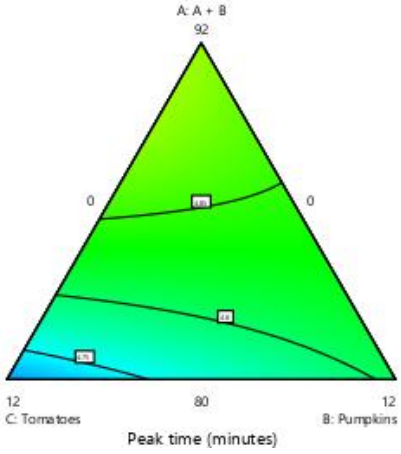
Peak time (minutes)

4.66667 5

X1 = A: A + B
X2 = C: Tomatoes
X3 = B: Pumpkins

Actual Components
D: Mushrooms = 4
E: Carrots = 4





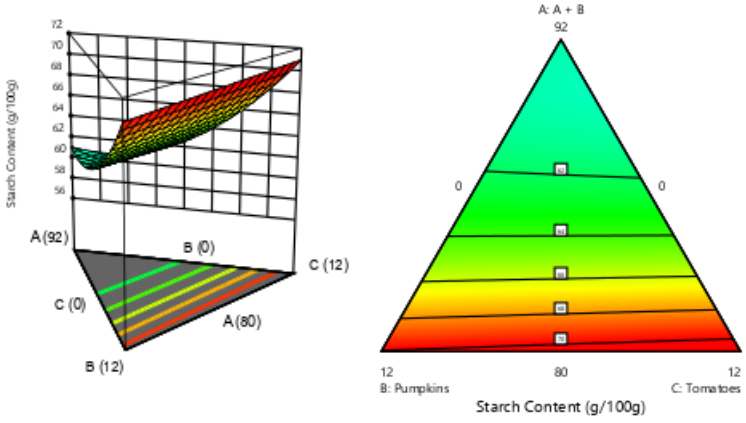
4.1.10 Starch Content

Starch Content (g/100g)

56.5534 69.9168

X1 = A: A + B
X2 = B: Pumpkins
X3 = C: Tomatoes

Actual Components
D: Mushrooms = 4
E: Carrots = 4



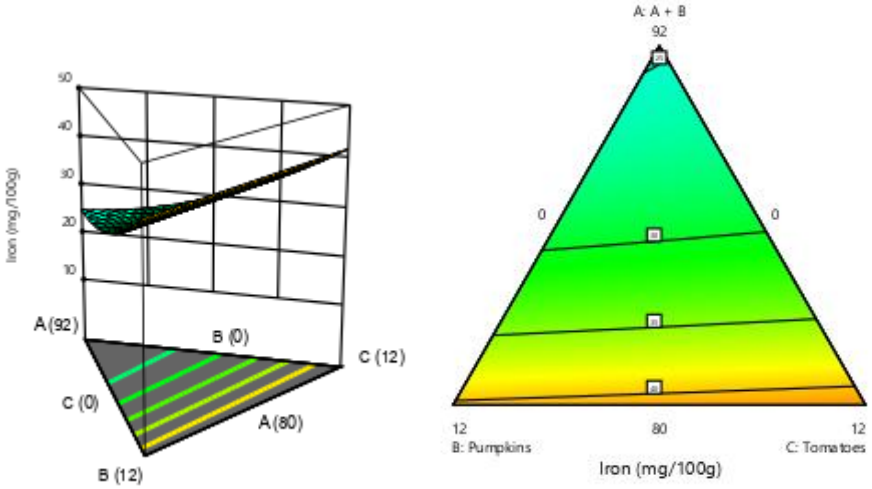
4.1.10 Iron Content

Iron (mg/100g)

16 45.8

X1 = A: A + B
X2 = B: Pumpkins
X3 = C: Tomatoes

Actual Components
D: Mushrooms = 4
E: Carrots = 4



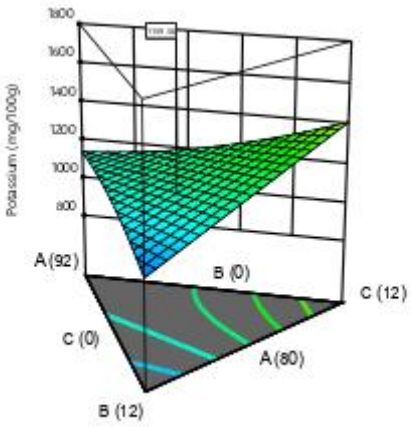
4.1.10 Potassium

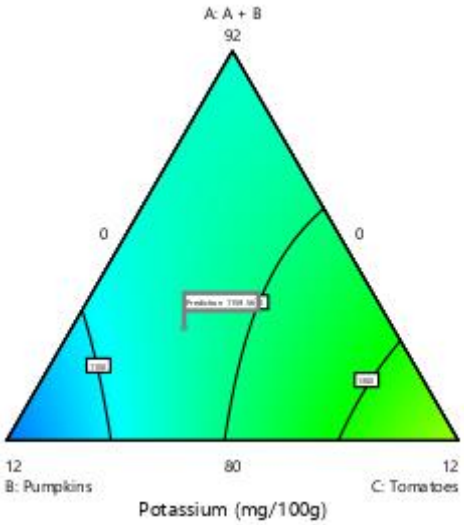
Potassium (mg/100g)

923.4 1660.4

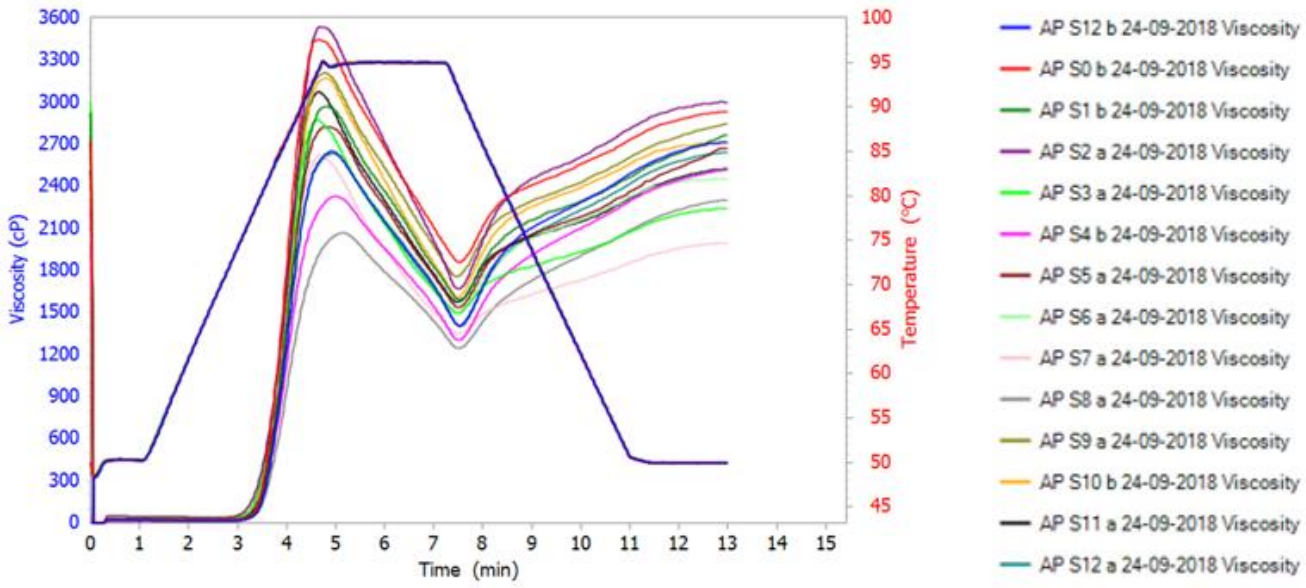
X1 = A: A + B
X2 = B: Pumpkins
X3 = C: Tomatoes

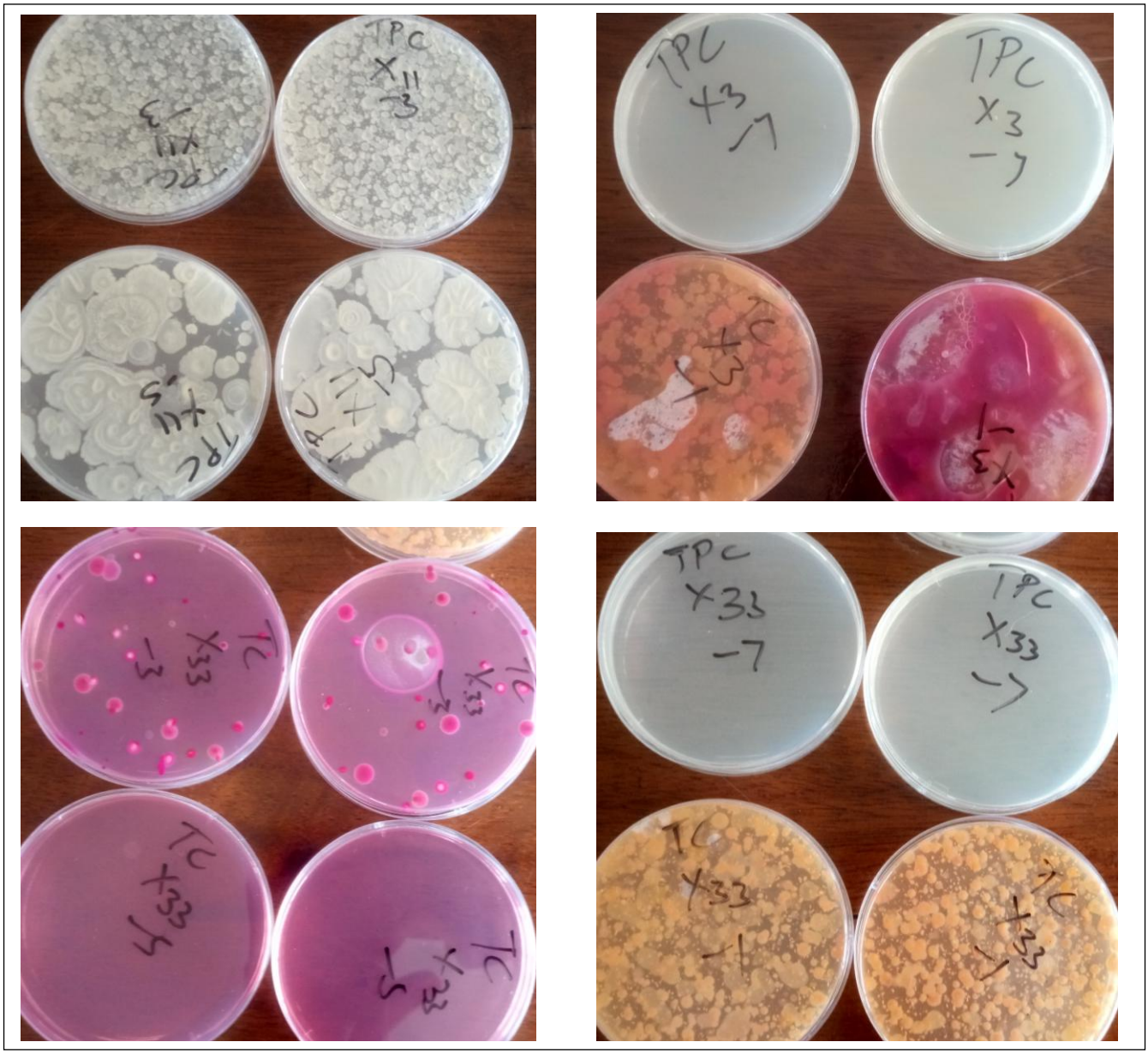
Actual Components
D: Mushrooms = 4
E: Carrots = 4





Pasting profile for the soup flours




TPC
X11
-3
TPC
X3
-7
TC
X33
-7
TC
X33
-1