



**College of Natural and Computational Sciences**  
**Center for Food Science and Nutrition**

Development and Optimization of Wheat-Sorghum based Bread Enhanced with  
Orange Flashed Sweet Potato

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Addis Ababa, Ethiopia

**Addis Ababa University**

**College of Natural and Computational Sciences**

**Center for Food Science and Nutrition**

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## **Declaration**

I the undersigned, declare that this is an original work and has never been presented in this of any other University and all the sources materials used for this thesis have been fully acknowledged.

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## List of acronyms

<b>AACC:</b>	<b>American Association of Clinical Chemistry</b>
<b>ANOVA:</b>	<b>Analysis of Variance</b>
<b>AOAC:</b>	<b>Association of Official Analytical Chemists</b>
<b>BDL:</b>	<b>Below Detection Level</b>
<b>BHA:</b>	<b>Butylated Hydroxyanisole</b>
<b>BHT:</b>	<b>Butylated Hydroxytoluene</b>
<b>CGIAR:</b>	<b>Consultative Group for International Agricultural Research</b>
<b>CRD:</b>	<b>Completely Randomized Design</b>
<b>CSA:</b>	<b>Central Statistical Agency</b>
<b>DP:</b>	<b>Degree of Polymerization</b>
<b>EIAR:</b>	<b>Ethiopian Institute of Agricultural Research</b>
<b>ESA:</b>	<b>East and South Africa</b>
<b>FAO:</b>	<b>Food and Agricultural Organizations</b>
<b>GI:</b>	<b>Glycemic Index</b>
<b>GNR:</b>	<b>Global Nutrition Report</b>
<b>HCl:</b>	<b>Hydrogen Chloride</b>
<b>HPLC:</b>	<b>High Performance Liquid Chromatography</b>
<b>HPMC:</b>	<b>Hydroxypropyl Methylcellulose</b>
<b>Hr.:</b>	<b>Hour</b>
<b>ICF:</b>	<b>International Classification of Functioning, Disability and Health</b>

<b>Kcal:</b>	<b>Kilo Calorie</b>
<b>MARC:</b>	<b>Melkassa Agricultural Research Center</b>
<b>Min:</b>	<b>Minute</b>
<b>Mm:</b>	<b>Millimeter</b>
<b>N:</b>	<b>Normality</b>
<b>NaOH:</b>	<b>Sodium Hydroxide</b>
<b>NI:</b>	<b>Nutrition International</b>
<b>NCDs:</b>	<b>Non-communicable Diseases</b>
<b>OFSP:</b>	<b>Orange Flashed Sweet Potato</b>
<b>ORAC:</b>	<b>Oxygen Radical Absorbance Capacity</b>
<b>RPM:</b>	<b>Revolution per minute</b>
<b>SP:</b>	<b>Sweet Potato</b>
<b>SPSS:</b>	<b>Statistical Package for Science Service</b>
<b>USA:</b>	<b>United States of America</b>
<b>USDA:</b>	<b>United States Department of Agriculture</b>
<b>V:</b>	<b>Volume</b>
<b>W:</b>	<b>Weight</b>
<b>WAC:</b>	<b>Water Absorption Capacity</b>
<b>WHO:</b>	<b>World Health Organization</b>

## Author Biography



The author was born in Arsi Zone, Ziway-dugda district from his father Kebero Jebo Dekamo and his mother Aisha Tufe Gudeta on 29 August, 1992. He has studied his Elementary, Secondary and Preparatory school at Ubo Weni Bericha, Ketar Fuafuate Senior Secondary School and Ziway-batu Preparatory School respectively. He then joined Hawassa University where he has studied a bachelor of Food Science and Post-harvest Technology. Soon after graduation, he was employed by Selale Dairy Cooperative Union where he has served as plant operator and process control for two years. He also has an experience of one and three months at Ethiopian Food, Beverage and Pharmaceutical Industry Development Institute as Junior Researcher. He was serving as Assistant Researcher II at Ethiopian Institute of Agricultural Research since June, 2017 until he joined Addis Ababa University, Center for Food Science and Nutrition for long term study. He is now a master's student of Food Science and Nutrition at Addis Ababa University, Center for Food Science and Nutrition. He has three publications with his colleagues in national and international journals and proceedings. He also has more than eight recognition of participation certificates from international and national institutes such as; Hawassa University, Ethiopian Standards Agency, Addis Ababa University (HULT Prize), Swedish University of Agricultural Sciences, Nutrition International (NI) and ISEKI Food Association (Vienna, Austria).

## **Abstract**

*Ethiopia is one of the developing countries with high prevalence of micronutrient deficiencies and protein energy malnutrition. Fortifying cereals through processing and conventional breeding can tackle micronutrient deficiency. Sorghum is a rich source of nutrients and most importantly, contains a diverse range of bioactive phenolic compounds. Wheat is a vital industrial grain for food which ranks second within the world next to rice and traded internationally. Orange Flashed Sweet Potato (OFSP) a source of food that contains useful  $\beta$ -carotene, starch, mineral, dietary fiber, and vitamins. The aim of this research is to develop and optimize sorghum and wheat based bread enhanced with orange flashed sweet potato. The main unit operations such as sorting, cleaning, weighing, washing, drying and milling were employed for raw material preparations. Standard procedures were employed to determine nutritional and anti-nutritional composition of composite flour and straight dough baking method was used to develop bread. All bread products were liked by sensory panelists. Bread sample with high ratio had high value of color and taste acceptance. Addition of OFSP to the composite flour boosted the sensory acceptance and beta carotene content of wheat-sorghum based bread. Average value of ash content of composite flour was 0.99%. As the ratio of sorghum flour in composite flour increased, the ash content of composite flour increased. All treatments were not significantly different from each other in their crude protein content. Based on our optimization, sample of 75.4% wheat: 14.6% sorghum: 10% OFSP was selected for common optimum purpose while sample of (78.3% wheat: 11.7% sorghum: 10% OFSP), (70% wheat: 22.5% sorghum: 7.5% OFSP) and (80.6% wheat: 9.4% sorghum: 10% OFSP) were selected as optimum value for proximate value, mineral, and sensory characteristics respectively. Bread developed with maximum ratio of OFSP and Sorghum flour has high sensory acceptance, ash and fiber content. Generally, formulated flour might be advantageous as a means of reducing heavy demands on importation of bread wheat and to alleviate malnutrition in Ethiopia. The researcher recommends incorporation of sorghum and OFSP in wheat flour for bread development to improve nutrient density and reduce the importation of wheat and bread cost*

**Key Words:** Bread, Baking, Optimization, Formulations, Wheat, Sorghum, Orange Flashed Sweet Potato, Sensory Analysis

## CHAPTER ONE

### 1. Introduction

#### 1.1. Background and Justification

Malnutrition refers to deficiencies, excesses, or imbalances in a person's energy intake and/or nutrients. It usually refers to several diseases, each with a specific cause related to more nutrients, such as protein, iodine, vitamin A or iron. The most crucial malnutrition in most developing countries, including Ethiopia, is micronutrient deficiencies and protein-energy malnutrition (Urigacha, 2020). According to the World Health Organization (WHO), vitamin A deficiency affects approximately 190 million children of pre-school ages and 19 million pregnant women, mainly in Africa and South-East Asia (WHO, 2019).

Cereals are climate-resilient, widely grown, accessible, and affordable to vulnerable populations. Fortifying them through processing and conventional breeding can tackle global micronutrient deficiency (CGIAR, 2021).

Sorghum is one of the leading cereal crops worldwide and ranked the fifth highest production of the cereal crops, following maize, wheat, rice and barley with 57.6 million tons of annual production globally in 2017. Ethiopia is the third largest sorghum producer in Africa after Nigeria and Sudan (FAO, 2017). Smallholder farmers cultivate 96% of the total land and take the dominant share in the production of the major crops in Ethiopia (CSA, 2015). It accounts for 19% of the domestic cereal production and 20% of the total area under cereals production (Demeke & Marcantonio, 2013). This crop also ranks third next to maize and teff, for which the total annual production is estimated to be 4.75 million tonnes (Mengistu *et al.*, 2019). Sorghum is widely grown in diverse climatic conditions and is the primary staple crop grown in the warm and dry areas where other crops can survive little, and food insecurity is widespread (Fetene *et al.*, 2011). It is gluten-free, high in resistant starch, is a rich source of nutrients, and most importantly, contains a diverse range of bioactive phenolic compounds (Dykes & Rooney, 2007). Sorghum is an important crop used to make bread and porridge; especially in under-developed and semiarid regions, it is the principal energy source and nutrition for humans (Rooney & Waniska, 2000).

Wheat is a vital industrial grain for food that ranks second within the world next to rice and is traded internationally (Falola *et al.*, 2017). Ethiopia is one of the biggest wheat producers in total area cultivated and total production (CSA, 2012). But, the assembly of wheat is tremendous of a subsistence nature and dominated by the country's smallholder farmers that cultivate more of it for home consumption and fewer of it for the commercial market (Matouš *et al.*, 2013).

Orange-fleshed sweet potato (OFSP) is an excellent source of pro-vitamin A in the diets of most people living in developing countries. It is an essential industrial crop and a source of food that contains useful  $\beta$ -carotene, starch, minerals, dietary fibre, and vitamins. Orange Flashed Sweet Potato can add natural sweetness, colour and flavour to bakery products (Edun *et al.*, 2019). Therefore, this research will address problems associated with micronutrient deficiency by developing nutritious foods and enhancing palatability based on cereal-tuber composite bread.

### **1.2.Statement of the Problem**

The main causes of Malnutrition are related to deficiencies of protein, iodine, vitamin A or iron. Vitamin A deficiency (VAD) affects approximately 190 million children of pre-school ages and 19 million pregnant women, mainly in Africa and South-East Asia (WHO, 2019). It is a major nutritional concern in poor societies, especially in lower-income countries. Bread is generally based on cereals, and the most common cereal used in producing bread is the wheat grain. But, in Ethiopia, wheat grain is scarce due to a low production of wheat is tremendously of a subsistence nature and dominated by the country's numerous smallholder farmers that cultivate more wheat for consumption and less of it for the market (Anteneh & Asrat, 2020). Over the past three years (2016/17-2019/20), Ethiopia imported on average 1.2 million metric tons of wheat commercially (excluding the informal import of significant wheat product), which accounted for about 30 percent of the domestic consumption (USDA, 2020). Ethiopia invests an average of 600 million dollars for wheat import annually (EIAR, 2019). Sorghum is the most traditional crop among cereals crops with limited product diversification practices and insignificant industrial alternative uses, as cited by Deribe and Kasa, (2020). Sorghum based products such as snack foods; bread, packed flour, cakes, relief flour, and beer are unaccustomed in Ethiopia. Therefore, this research will address problems associated with micronutrient deficiency by developing nutritious foods and enhancing palatability based on cereal-tuber composite bread.

### **1.3.Objectives**

#### **1.3.1. General Objective**

- To develop and optimize sorghum-wheat based bread enhanced with orange flashed sweet potato

#### **1.3.2. Specific Objectives**

- To enhance palatability and nutritional composition of sorghum-wheat based bread by using OFSP
- To optimize the blending ratio of sorghum-wheat-OFSP flour for bread baking

### **1.4.Research Questions**

- Does inclusion of orange flashed sweet potato in composite flour enhance the palatability of wheat-sorghum based bread?
- Does blending of sorghum and orange flashed sweet potato with wheat flour boost nutritional composition of bread?
- What is the optimum blending ratio of wheat-sorghum-orange flashed sweet potato flour to develop acceptable bread?

### **1.5.Significance of Study**

Bread with low gluten content can be developed from formulations of sorghum, wheat and OFSP. Sorghum can be used to make low glycemic index (GI) and low gluten bread by mixing with wheat and OFSP. The bread made of sorghum flour was reported to have a lower predicted GI (72) than other gluten-free bread: teff (74), buckwheat (80), and quinoa (95) bread and gluten-containing bread wheat (100). Low-GI foods are often associated with high levels of resistant starch and thus low starch digestibility; sorghum bread can be consumed by people to improve the glycemic response (Wolter *et al.*, 2014). Therefore, this research will help to develop good quality and acceptable bread product by optimizing formulations and in general developing their protocols. Also it helps to reduce the amount of wheat import for bread and enhance palatability and diversity of sorghum based foods. Based on the developed protocol one (household and commercially) can make a product of the same quality bread anywhere in the world.

### **1.6. Scope and Limitations of the Study**

The present study has several strengths and limitations. Developing and formulating composite flour to enhance palatability and enrich nutritional composition of food product is a novel technology. Developing and optimizing of wheat, sorghum and orange flashed sweet potato composite flour based food products that for food and nutrition security was also strength of this research. However, the study could not analyze all nutrients because of technical and financial constraints.

## **CHAPTER TWO**

### **2. Literature Review**

#### **2.1. Malnutrition**

Malnutrition refers to deficiencies, excesses, or imbalances in a person's energy intake and/or nutrients. According to WHO (2021), the term malnutrition addresses three broad groups of conditions: Under-nutrition, which includes wasting (low weight-for-height), stunting (low height-for-age) and underweight (low weight-for-age); Micronutrient-related Malnutrition, which provides for micronutrient deficiencies (a lack of essential vitamins and minerals) or micronutrient excess; and overweight, obesity and diet-related non-communicable diseases (such as heart disease, stroke, diabetes and some cancers).

##### **2.1.1. Malnutrition at Global Level**

It is a universal public health problem for children and adults in all continents (WHO, 2019). It is a public health concern impediment to world poverty eradication, productivity, and economic growth. By eliminating malnutrition, it is estimated that 32% of the global disease burden would be removed (WHO, 2020). As a severe widespread problem affecting children in developing countries, progress towards tackling the different forms of malnutrition remains relatively low (GNR, 2018). Malnutrition occurs due to an imbalance in the body, whereby the nutrients required by the body and the amount used by the body do not balance (WHO, 2019). There are several forms of malnutrition, and these include two main categories, namely under-nutrition and over nutrition. Undernutrition manifests as wasting or low weight for height (acute malnutrition), stunting or low height for age (chronic malnutrition), underweight or low weight for age, and mineral and vitamin deficiencies or excessiveness. Overnutrition includes overweight, obesity and diet-related non-communicable diseases (NCDs) such as diabetes mellitus, heart disease, some forms of cancer and stroke (WHO, 2019). Malnutrition is an important global issue currently, as it affects all people despite the geography, socio-economic status, sex and gender, overlapping households, communities and countries. Anyone can experience malnutrition, but the most vulnerable groups affected are children, adolescents, women, and immune-compromised people or face the challenges of poverty (GNR, 2018).

During pregnancy nutrition needs increase to meet fetal growth and development, and protect health of the mother while breast feeding. During pregnancy a women’s daily Iron requirements nearly double from 15mg per day of non-pregnant women to 27mg per day. Poor diet leads to micro nutrient deficiency. Micro nutrient deficiency during pregnancy leads to poor maternal health and adverse birth outcomes, such as pre-eclampsia, maternal mortality, low birth weight, small for gestational age and stunting. As cited by Forsido *et al.*, (2021), dietary diversity among lactating mothers is very low in low-income settings like Ethiopia. According to the report of WHO (2019), 462 million adults are underweight, while 1.9 billion adults are overweight and/or obese. In children under 5 years of age, 155 million are stunted, 52 million are wasted, 17 million are severely wasted and 41 million are overweight and/or obese.

### 2.1.2. Malnutrition in Ethiopia

In Ethiopia prevalence of stunting and acute malnutrition have decreased over the past decade but remain high, with 38 percent of children less than five years stunted and 10 percent wasted. Twenty-two percent of women of reproductive age are undernourished, leaving their children predisposed to low birth weight, short stature, low resistance to infections, and high risk of disease and death (USAID, 2018). Children in rural areas are more likely to be malnourished than those in urban areas, with variations in the severity of stunting and wasting by region.

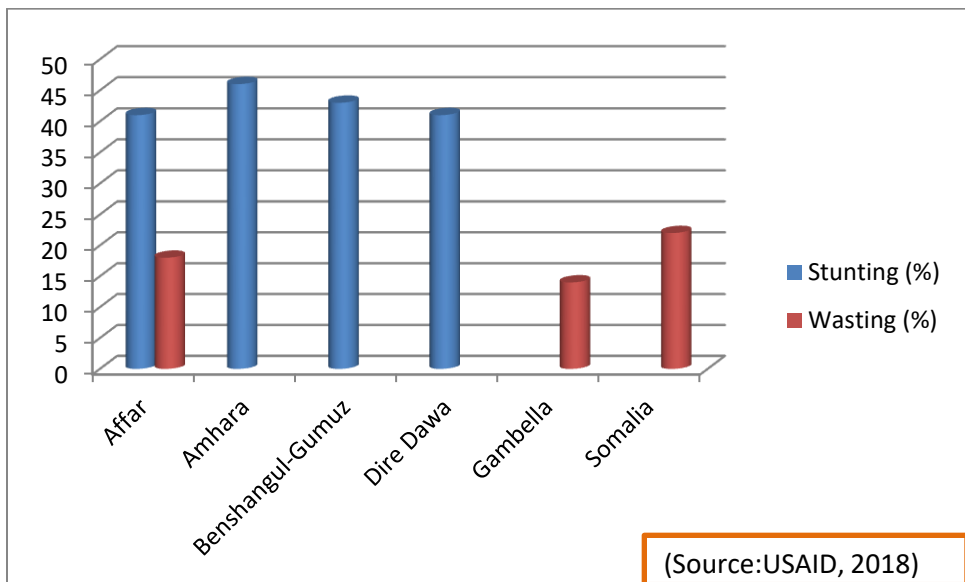


Figure 1 Severely stunted and wasted regions in Ethiopia

Stunting is highest in Amhara (46 percent), Benishangul-Gumuz (43 percent), Afar (41 percent), and Dire Dawa (41 percent), whereas wasting is highest in Somali (22 percent), Affar (18 percent), and Gambela (14 percent). Differences in stunting levels can be seen according to maternal education and wealth levels. Twenty-two percent of children whose mothers have a secondary education is stunted, while the rate rises to 42 percent of children whose mothers had no formal education. Similarly, 26 percent of children in the highest wealth quintile are stunted, while 45 percent of children in the lowest wealth quintile are stunted (CSA and ICF 2016). Intervention using blended composite flours can reduce malnutrition in sub-Saharan Africa. Prevalence of protein-energy malnutrition among vulnerable children has necessitated research on cost-effective food product development like food to food fortification of common staples like sorghum, wheat and OFSP.

## **2.2. Sorghum**

### **2.2.1. Sorghum Production**

Sorghum (*Sorghum bicolor L. Moench*) is that the sixth most planted crop within the world, and it's one amongst the foremost important cereals used as a staple food for those primarily living in arid and semiarid areas. In Africa, it's the second most vital cereal during which around 300 million people rely upon it as their daily consumptions (Zhao *et al.*, 2019). In Ethiopia, sorghum is that the third most significant staple cereal crop after teff and maize. It is also grown in most parts of Ethiopia and used as a staple food crop on which the lives of numerous poor Ethiopians depend upon it (Spielman *et al.*, 2012).

### **2.2.2. Sorghum based Traditional and Novel Foods**

Sorghum is often used for traditional, novel and functional foods and beverages either alone or in a mixture with other crops. Steamed products, that is, sorghum couscous, a dish of small balls made from steamed sorghum grain granules (often blended with other grains) is that the main staple food in North Africa (Dicko *et al.*, 2006). Boiled products, like sorghum porridge and soup, are widely consumed in Africa. Sorghum porridge comprises whole grains (often mixed with other grains like maize and millet), with or without fermentation.

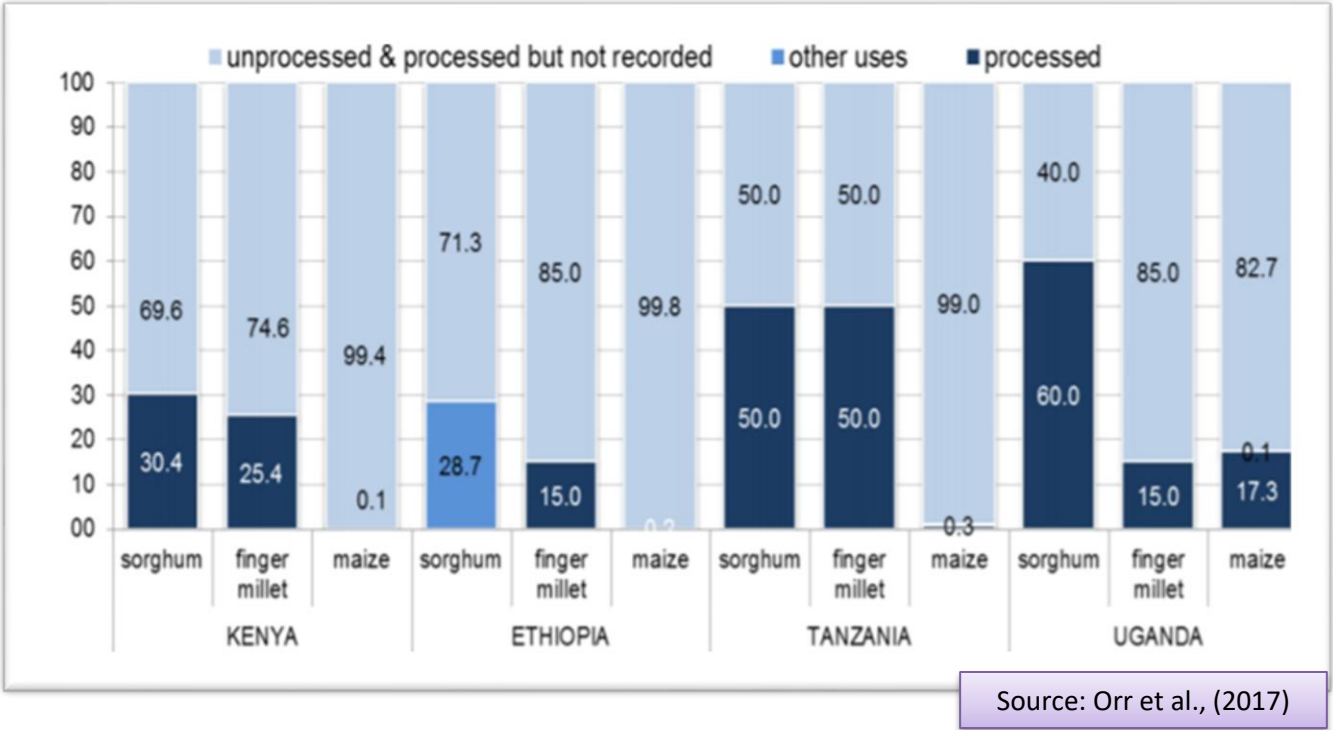


Figure 2 Sorghum food uses in ESA 2013 (percent)

Sorghum is often used to make gluten-free healthy snacks, like cookies and biscuits. Cookies made up of sorghum grain are shown to possess high phenolic contents and antioxidant activity, especially those made up of tannin sorghum, which has an antioxidant action up to twenty times above wheat cookies (Chiremba *et al.*, 2009). Porridge made from decorticated sorghum grain was reported to possess a significant slower gastric emptying rate, which was quite twice longer than other main staple foods of rice, potato, and pasta (Cisse *et al.*, 2018). Ready to eat breakfast cereals made from Sorghum whole grain, especially the tannin sorghum, were a premier source of dietary fibre and phenolic compounds with high antioxidant activity (Lopes *et al.*, 2018).

Wu *et al.* (2013) developed a sorghum grain tea using the entire grain of a red sorghum variety and traditional processing technique (soaking, steaming, and roasting). The tea exhibited antioxidant activity and  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities. Xiong *et al.*, 2019 made a sorghum grain tea employing white sorghum. The study has shown that beer made from white sorghum had quite twice higher phenolic contents than barley beer, which contributed to its high antioxidant activity (Garzón *et al.*, 2019). Blending Sorghum whole grain flour with pasta at the extent of 20% to 40% substitution of semolina significantly increased the resistant

starch, phenolic contents, and antioxidant activity of pasta (Khan *et al.*, 2015). Incorporating white or red sorghum whole grain flour into bread at a 30% substitution level for flour significantly enhanced the phenolic contents and antioxidant activity (Wu *et al.*, 2018).

The addition of 0.25% to 0.75% high-tannin sorghum bran to meat products like pre-cooked pork and turkey patties was reported to stop the lipid oxidation during storage and was as effective because the synthetic antioxidants BHA/BHT, without compromising the meat sensory flavour attributes (Emeyer *et al.*, 2015).

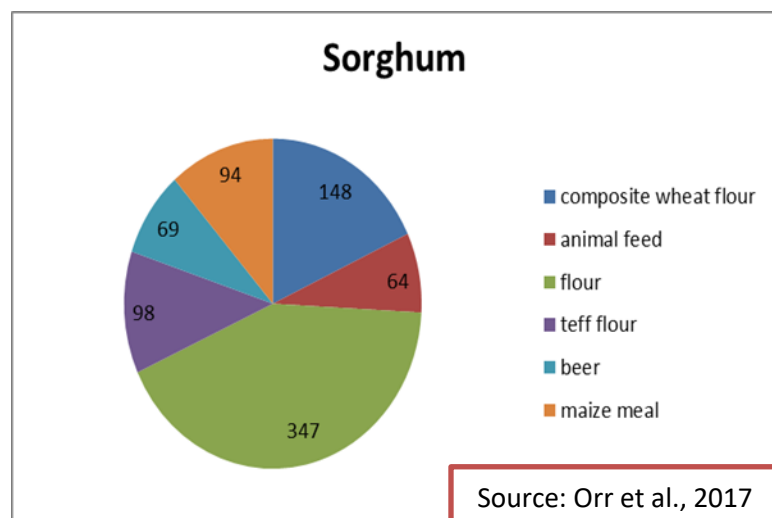


Figure 3 Value chains for sorghum in East and South Africa (ESA) countries

Sorghum is used in various ways in Ethiopia; the grain is used for human foods like injera, bread, porridge, Boiled grain, infant food, syrup, and native beverages. The principal use of sorghum in Ethiopia is for injera making either standalone product or by blending with teff. Consumption of sorghum by urban dwellers is partly explained by the supply and price of teff. Sorghum may be a substitute for making injera when the price of teff rise high (Demeke and Marcantonio, 2013).

### 2.2.3. Sorghum Nutritional Compositions

Sorghum nutritional composition varies among varieties. Generally, carbohydrates (starch and non-starch polysaccharides), proteins, and lipids are the most components of the grain (Hill et al., 2012). On the average, 100g of sorghum grain has about 72.1g carbohydrates, 12.4g water, 10.6g proteins, 6.7g fibers, and 3.5g lipids, and provides about 1, 377kJ energy (USDA, 2019).

Table 1 Nutritional Composition of Sorghum

Nutrient	Sorghum
Protein (%)	11.6
Carbohydrate (%)	77
Fat (%)	3.4
Dietary fiber (%)	9.1-11.5
Ash (%)	1.6
Calcium (mg/100g)	29
Iron (mg/100g)	4.5
Energy (KJ/100g)	1374
Vitamin A (ug retinol equivalents)	10-20
Lysine (g/100g protein)	2.0

Source: (Taylor and Emmambux, 2007)

In sorghum, the bioactive compounds are primarily the phenolic compounds. The only but abundant phenolic compound is phenolic acids. According to Girard & Awika, (2018), they are present altogether in sorghum grain, with total concentrations of 445  $\mu\text{g/g}$  to 2,850  $\mu\text{g/g}$ . Flavonoids are mainly found in sorghum bran, and therefore the types and concentrations are related to the pericarp color and thickness and presence of pigmented testa (Awika *et al.*, 2005). They're the most important class of phenolic compounds in plants and represent the foremost abundant and diverse phenolic compounds in sorghum. The flavone content in sorghum grain is about 20 to 390  $\mu\text{g/g}$ , relatively low compared to other flavonoids (Girard & Awika, 2018). The flavanone content in sorghum ranges from 0 to 2,000  $\mu\text{g/g}$ . rock bottom levels is reported in white sorghum, and therefore the highest level is found in sorghum with a yellow pericarp (Bhagwat *et al.*, 2014). The unique feature of sorghum flavonoids is its anthocyanin content. Anthocyanins are a source of natural water-soluble pigments and antioxidants (Riaz *et al.*, 2016). The most common 3-deoxyanthocyanidins in sorghum are apigeninidin and luteolinidinaglycones. Aside from providing attractive orange/red to blue/violet colors to plants, 3-deoxyanthocyanidins are potent antioxidants with antimicrobial activity and lots of other benefits (Xiong *et al.*, 2019). Sorghum is taken into account because the main dietary source for 3-deoxyanthocyanidins for humans, which might be exploited as commercial natural colorants

for foods. Sorghum tannins (condensed tannins or proanthocyanidins) are within the condensed form with high relative molecular mass and a high degree of polymerization (DP), which isn't commonly found among the main cereals (Wu *et al.*, 2013). Sorghum condensed tannins are composed of oligomers or polymers of mainly flavan-3-ol and flavan-3,4-diol and connected primarily by B-type linkages, with a mean DP of about 20 compared to other tannin-containing cereal grains of three to 10 DP (Girard & Awika, 2018).

Generally, sorghums with pigmented testa have high levels of condensed tannin content (Girard & Awika, 2018). The antioxidant activity of sorghum phenolic compounds seems to play a key role within the health promotion and disease prevention related to sorghum consumption. The phenolic compounds extracted from sorghum exhibit the very best antioxidant activity among cereal grains of wheat, rice, and corn, and also are like common fruits and vegetables (Adom & Liu, 2002). Brown sorghum bran showed the very best in-vitro antioxidant activity (oxygen radical absorbance capacity = 3,124  $\mu\text{mole TE/g}$ ) among sorghum (grain or bran), and better than most of fruits and vegetables known to possess high antioxidant activities including blueberry (ORAC = 842  $\mu\text{mole TE/g}$ ), strawberry (ORAC = 402  $\mu\text{mole TE/g}$ ), and broccoli (173  $\mu\text{mole TE/g}$ ) (Wu *et al.*, 2010).

Apart from the direct antioxidant effects, the phenolic compounds from sorghum are shown to induce endogenous detoxifying enzymes (phase II enzymes) that are liable for converting the harmful reactive oxygen or nitrogen species into non-toxic compounds, and thus indirectly enhances the body defense against oxidative stress (González-Montilla *et al.*, 2012). Allergies associated with grasses and grass pollen is extremely common. Unfortunately, Sorghum is a grass and is known to produce an allergic reaction in some people. Food allergy symptoms include tingling or itching of the mouth, swelling in and around the mouth, abdominal pain, nausea, vomiting, and even fainting. Severe allergic reaction or anaphylaxis can result in any food allergy and can be life-threatening (Brennan, 2020).

## **2.3.Wheat**

### **2.3.1. Wheat Production and Consumption**

Wheat (*Triticumaestivum L.*) is a globally produced and marketed cereal crop globally, covering about 15% of the whole sowing area of cereal crops (Kiss, 2011). It is a vital industrial and grain

for food that ranks second within the world next to rice and is traded internationally (Falola *et al.*, 2017). In sub-Saharan African countries, wheat is a strategic commodity that generates income for farmers and improves food security status (Amentae *et al.*, 2017; Minot *et al.*, 2015). Ethiopia is one of the biggest wheat producers in total area cultivated and total production (CSA, 2012).

Wheat and its products represent 14% of the whole calorie intake, which makes it the second most significant grain after maize (19%) and before teff (10%), sorghum (11%) and enset (12%) (FAO, 2014). In Ethiopia, it ranks fourth after teff, maize and sorghum in area coverage and third next maize and teff in total production (CSA, 2012). But, the assembly of wheat is tremendously of a subsistence nature and dominated by the country’s numerous smallholder farmers that cultivate more of it for home consumption and fewer of it for the commercial market (Matouš *et al.*, 2013). As cited by Minot *et al.* (2015) and CSA (2013), wheat is produced by both small-scale and large-scale commercial farms. However, in step with Demeke and Marcantonio (2013), except some government-owned large-scale and commercial farms, it's produced predominantly by smallholder farmers under rain feed conditions. It's clear that within the country small scale wheat farmers dominate large-scale commercial farms, and it has its own negative influence on production and productivity within the country and it affects the competitiveness of wheat quality at the planet market.

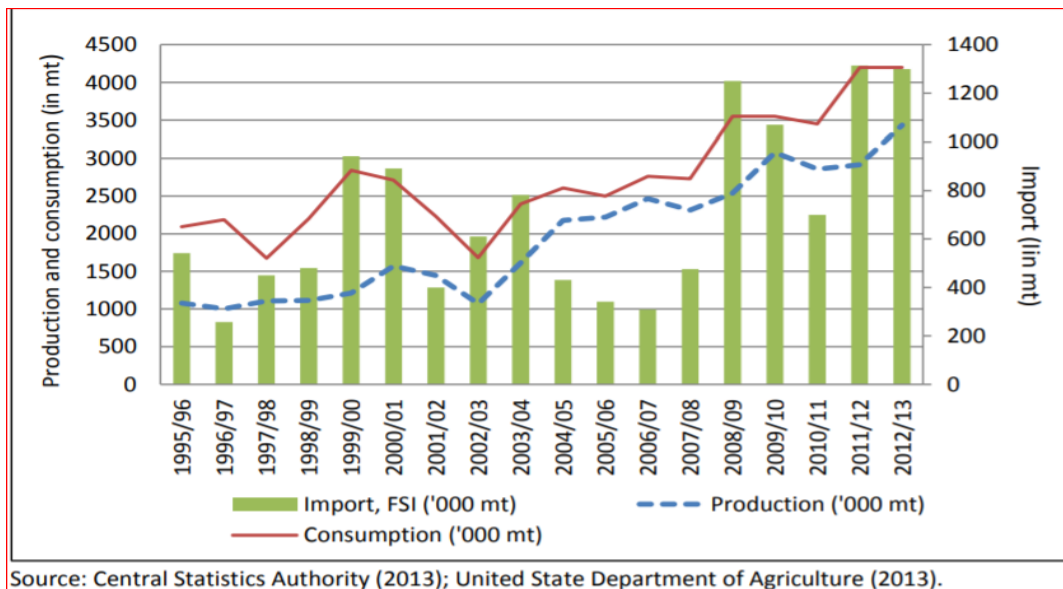


Figure 4 Wheat production and consumption in Ethiopia

### **2.3.2. Wheat Nutritional Composition**

Wheat provides nearly 55% of carbohydrate and 20% of the food calories. It contains carbohydrate 78.10%, protein 14.70%, fat 2.10%, minerals (zinc, iron) 2.10% and considerable proportions of vitamins (thiamine and vitamin-B) and other minerals. Wheat is also a vital source of traces minerals like selenium and magnesium, nutrients essential to good health (Topping D, 2007).

### **2.3.3. Wheat based Foods**

Wheat can be used to make different types of food such as bread, biscuits, confectionary products, noodles and vital wheat gluten or seitan (Kumar *et al*, 2011).

## **2.4. Orange Flashed Sweet Potato**

### **2.4.1. Orange Flashed Sweet Potato Production and Consumption**

Sweet potato (*Ipomoea batatas L.*) plays an important role in human diet and second staple food in the world. The color of sweet potato flesh varied from white, yellow, purple, and orange. Studies reported that the range in Sweet Potato flesh color and reference to nutritional and sensory acceptability. Orange-fleshed sweet potato (OFSP) is a special type of bio-fortified sweet potato that contains high levels of beta-carotene. Biofortified foods and products can be practical, affordable, and natural sources of nutrition for billions of people (GAIN, 2020). Beta-Carotene is an organic, red-orange pigment abundant in plants and fruits. OFSP has been attracting food scientists and nutritionists because of its high content of carotenoids and pleasant sensory characteristics with color (Sateesh and Workineh, 2019). It possesses the characteristic of attractive sweet taste and eye pleasing yellow to orange color to children in comparison with white-fleshed sweet potato (Neela & Fanta, 2019). OSFP have emerged as one of the most promising plant source of Vitamin A and stand to be cheaper and complementary source of to the rural and poor families (Zegeye *et al.*, 2015).

### **2.4.2. Orange Flashed Sweet Potato Nutritional Composition**

OFSP is taken into account as an upscale source of non-digestible dietary fiber, specific minerals, vitamins, and antioxidants (Endrias, Negussie, & Gulelat, 2016). Phenolic compounds and carotenoids are accountable for distinguishing flesh and skin colors (deep yellow, red to orange, purple, and pale) of Sweet Potato together with antioxidant properties (Steed & Truong,

2008). Scientists established the role of OFSP in health, and stated that it is rich in nutritional components with anti-carcinogenic and disorder preventing attributes (Chandrasekara & Josheph Kumar, 2016). Recent scientific reports concluded the ant-oxidative and radical scavenging activity of phenolic acid components in Orange Flashed Sweet Potato with beneficial health-promoting activities (Rumbaoa, Cornago, & Geronimo, 2009). Incorporation of OFSP flour to other foods appears to be the most effective way for increasing the vitamin A content of OFSP enriched food products (kidane *et al.*, 2013).

## 2.5. Bread

Bread may well be a staple food prepared from flour and water, usually by baking. It is one of the foremost vital foodstuffs consumed nowadays. The most common bread recipe contains a mixture of flour and water to supply dough or batter, perhaps fermented before baking. The top results of this modest process might be a rather versatile staple that's found today on many kitchen tables around the world (Arranz-Otaegui *et al.*, 2018).

Table 2 Main ingredients used in bread baking

No	Ingredient	Percentage
1	flour	100%
2	water	60%
3	sugar	4%
4	fat	4%
5	yeast	1%–5%
6	salt	2%

Bread is that the staple food of the center East, Central Asia, North America, Europe, and in European-derived cultures like those within the Americas, Australia, and Southern Africa, in contrast to parts of South and East Asia where rice or noodle is that the staple. It is commonly made from flour dough that's cultured with yeast, allowed to rise, and eventually baked in an oven. The addition of yeast to the bread expands the air pockets commonly found in bread. Wheat is the foremost typical grain used for the preparation of bread flour dough, due to its high levels of gluten which give the dough sponginess and elasticity (Peña *et al.*, 2016). Cereals such as rye, barley, maize, sorghum, millet and rice are also used to make bread even-though they have less gluten (Cauvain Stanley, 2015).

Source: Gisslen, (2012)

Gluten free breads are made using ground flours from a range of ingredients like almonds, rice, sorghum, corn, or legumes like beans, and tubers like cassava and sweet potato, but since these flours lack gluten they may not hold their shape as they rise and their crumb could also be dense with little aeration. Additives like xanthan gum, guar gum, hydroxypropyl methylcellulose (HPMC), corn starch, or eggs are used to compensate for the lack of gluten (Lamacchia *et al.*, 2014; Volta *et al.*, 2015). Nutritionally, bread is categorized as a source of grains within the natural phenomenon and should be an honest source of carbohydrates and nutrients like magnesium, iron, selenium, B vitamins, and dietary fiber (Mulder *et al.*, 2013).

## **CHAPTER THREE**

### **3. Materials and Methods**

#### **3.1. Study Area**

The sample preparation and experimental analysis were conducted at Melkassa Agricultural Research Center (MARC). It is found in the Great Rift Valley, 117 km away from Addis Ababa in the southeast direction and 17 km from Adama to Assela.

#### **3.2. Reagents/Chemicals and Equipment**

##### **3.2.1. Reagents/Chemicals**

Chemicals such as petroleum ether, sulphuric acid, hydrochloric acid, acetone, potassium sulphate, n-hexane, selenium metal, vanillin, catechin, hydrogen peroxide, Ortho-phosphoric acid and other analytical grade chemicals and reagents were used for proximate composition and minerals analysis.

##### **3.2.2. Equipment**

Chopin laboratory mill (Moulin CD1 mill, Chopin technology, France), Hammer mill (XFYC 810, China), Universal Centrifuge (PLC-012E, USA), Shaking water bath, Drying Oven (TR-TC-YHG-300-BS-11), Fiber-tech (Fiber tec<sup>TM</sup> 8000), Soxhlet apparatus (Soxtec<sup>TM</sup> 8000), Kjeldahl apparatus (Kjeltec<sup>TM</sup> 8100), Atomic Absorption Spectroscopy (AAS), Spectrophotometer (Genesys 10 UV, Madison, USA), HPLC, Colorimeter (Hunter lab ARS00073, China) and others were used for flour preparation, product development, and proximate and mineral analysis.

##### **3.3. Sample Collection and Preparation**

Sorghum (Melkam, white sorghum variety), five kilograms was brought from sorghum and millet improvement, Melkassa Agricultural Research Center (MARC). It was cleaned, sorted, washed with tap water, drained, de-hulled (manually), sun-dried, milled, packed in a plastic bag, and stored in a dry and cool place until further use. The sorghum sample was milled by a hammer miller (XFYC 810, China) at Food Science and Nutrition Research, a food product development laboratory. The flour was packed in a polyethylene bag and stored at room temperature until further formulation with wheat and Orange flashed sweet potato flour. Five kg

of Orange fleshed sweet potato (Vita variety) were brought from Jimma Agricultural Research Center. Then it was sorted, weighed, washed with clean tap water, peeled and cut into thin slices by stainless steel knife, spread in a tray and oven (TR-TC-YHG-300-BS-11), dried at 60°C for 10 hr. and milled into flour by hammer miller (XFYC 810, China) at MARC, Food Science and Nutrition Research, food product development laboratory. The flour was packed in a polyethylene bag and stored at room temperature for further formulation, product development, and composite flour nutritional and anti-nutritional analysis.

Wheat grain (Daka) variety was collected from Kulumsa Agricultural Research Center, National wheat breeding program. Then the wheat grain was milled using a Chopin laboratory mill (Moulin CD1 mill, Chopin technology, France). Then it was cleaned manually, picking foreign materials (stone, straw and defective seeds), winnowed, sorted and stored in a cool and dry place until milling. After a week, the cleaned wheat grain was conditioned to 16.5% of moisture level with distilled water in a plastic container and left for 18 hours to facilitate tempering situation. Then flour was extracted using Chopin laboratory mill (Moulin CD1 mill, Chopin technology, France) and sieved through a fine sieve of size 0.50 mm at Kulumsa Agricultural Research Center, Food Science and Nutrition Research laboratory. The flour was packed in a polyethylene bag and stored at room temperature for the further formulation, product development, and composite flour nutritional and anti-nutritional analysis.

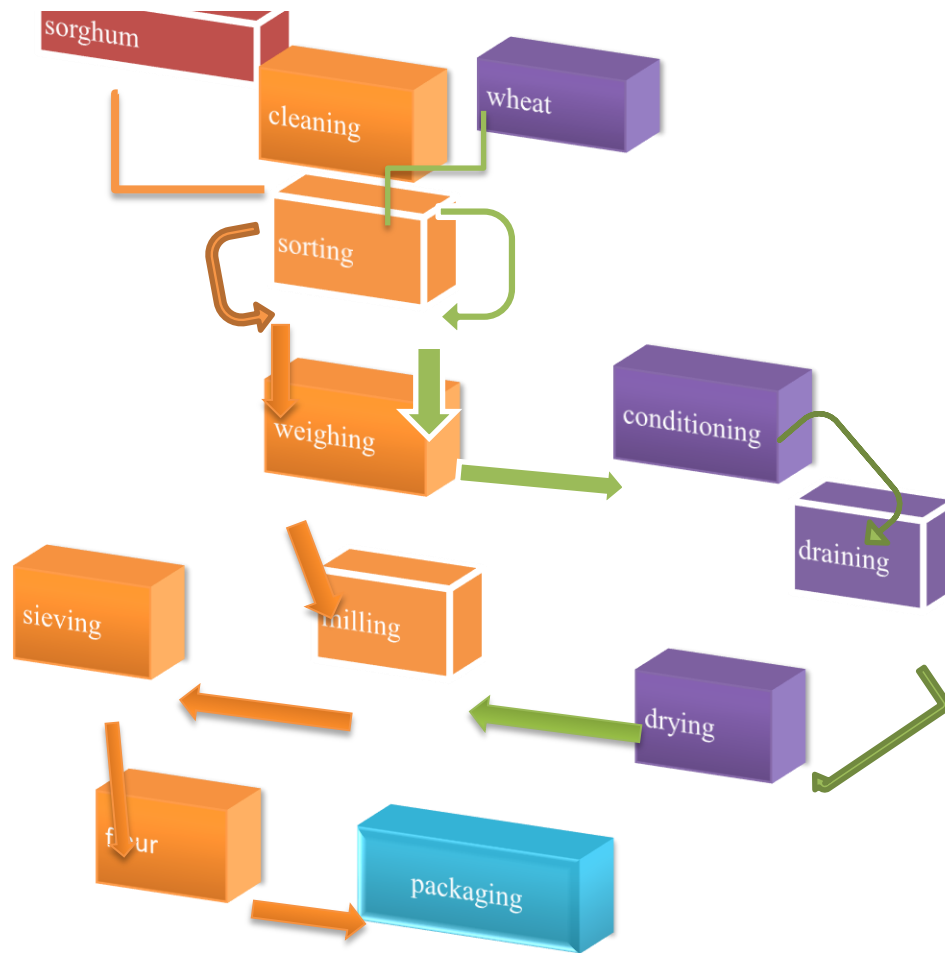


Figure 5 Flow charts of wheat and sorghum flour processing

Orange Flashed Sweet Potato flour was prepared based on Tirunch *et al.*, (2018) OFSP flour preparation procedure. The following unit operations were employed: sorting, weighing, washing, peeling and cutting into thin slices by stainless steel knife, spreading in a tray and oven drying at 60<sup>0</sup>C for 10 hr. and milling into flour. All crops (sorghum, wheat and OFSP) were produced in 2020/21 main production season.

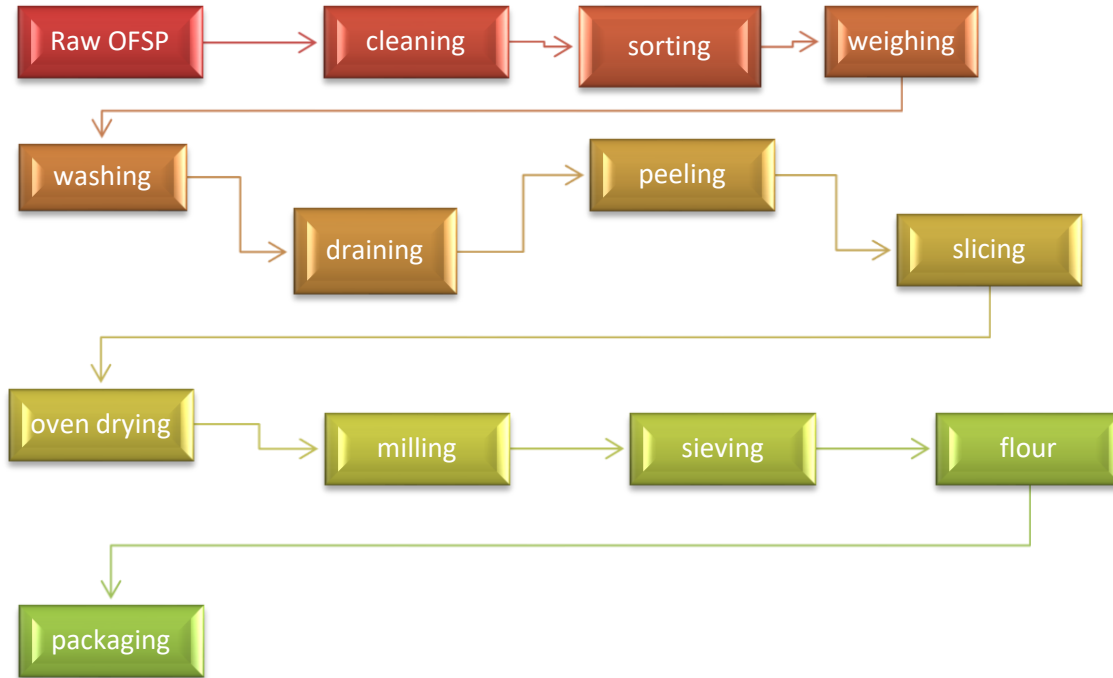


Figure 6 OFSP Flour processing flowchart

The whole flour was screened through an 80 mesh sieve, homogenized and then stored in polyethylene bags until further use. Other ingredients used in bread baking were: Water, yeast, salt, sugar, egg, and oil and baking powder



Figure 7 Sorghum, wheat grains and OFSP root

### 3.4. Experimental Design: Product Formulations and Optimization

Mixture design (D-Optimal) was used for flour formulations. Maximum and minimum levels of blending ratio were based on previous studies recommendation. According Wu *et al.* (2018) the blending ratio of Wheat to Sorghum for good quality bread was 70:30. The blending ratio of researcher interest was 60:30:10 of Wheat, Sorghum and Orange Flashed Sweet Potato respectively. Design- Expert®, version 7.0 software was used for the generation of formulations and analysis of the results. Mixture design (D-Optimal) method was employed, to formulate recipes, study the main effect of parameters, create models between the variables, and determine the effect of these variables to optimize the levels of ingredients. Eight treatments (Table 7) in random order were created and responses parameters like sensory (color, loaf volume and taste), Gluten Content, Beta-carotene, proximate (ash, protein, fiber and carbohydrate) and Minerals such as Iron (Fe), calcium (Ca), manganese (Mn) and Zinc (Zn) were evaluated.

**Table 3 Mixture design (D-Optimal) coded design formulations for bread baking**

Run	Block	Component 1 A:Wheat	Component 2 B:Sorghum	Component 3 C:OFSP	Response 1 Protein	Response 2 Fiber	Response 3 Loaf Volume	Response 4 Taste	Response 5 Color	Response 6 Gluten Content	Response 7 Zinc	Response 8 Iron	Response 9 Calcium	Response 10 Manganese
1	Block 1	0.900	0.075	0.025										
2	Block 1	0.750	0.225	0.025										
3	Block 1	0.700	0.225	0.075										
4	Block 1	0.800	0.150	0.050										
5	Block 1	1.000	0.000	0.000										
6	Block 1	0.900	0.000	0.100										
7	Block 1	0.700	0.300	0.000										
8	Block 1	0.600	0.300	0.100										

Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively.

### 3.5. Flour Functional Properties

#### 3.5.1. Water Absorption Capacity (WAC)

Water absorption capacity was determined using the method of Salunkhe (1985) modified by Adebowale et al. (2002). 10 ml of distilled and deionized water was added to 1.0 g of the sample in a 15ml centrifuge tube. The suspension was mixed using vortex mixer for 5 min. The suspension obtained was centrifuged at 3500 rpm for 30 min and the supernatant was measured into a 10 ml graduated cylinder. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant.

$$\text{WAC} = 10\text{ml} - \text{volume of supernatant} \dots\dots\dots \text{Equation 1}$$

#### 3.5.2. Bulk Density

Bulk density was calculated by Narayana and Narasinga-Rao (1984) method as cited by Edema, (2005). Bulk density of flour sample was determined by measuring the weight of the flour and the corresponding volume. Ten gram of flour sample was placed in a 100-ml graduated cylinder and tapped regularly until it becomes uniform. The bulk density was calculated by dividing the mass of the powder by the volume occupied in the cylinder.

$$\text{Bulk density (g/g)} = \frac{\text{weight of sample}}{\text{Volume of sample occupied the space}} \dots\dots\dots \text{Equation 2}$$

#### 3.5.3. Dispersibility

Dispersibility was determined by the method of Kulkarni, (1991) as cited by Edema et al., (2005). Ten gram of flour sample was weighed into a 100 ml-measuring cylinder. Distilled water was added up to 100 ml volume. The sample was vigorously stirred and allowed to settle for 3 hr. The volume of settled particles was recorded and subtracted from 100 to give a difference that is taken as percentage dispersibility.

$$\text{Dispersibility} = 100 - \text{volume of settled particles} \dots\dots\dots \text{Equation 3}$$

#### 3.5.4. Swelling Power and Solubility

Swelling power and solubility of flours were assessed following the methods of Schoch, (1964). A one gram of flour ( $W_1$ ) was weighed in a centrifuge tube and weight recorded ( $W_2$ ). To this 10 ml, of distilled water was added (VE) and heated for 30 min in a water bath at 90°C with occasional stirring. The cooled content was centrifuged at 5000 rpm for 10 min. The supernatant was carefully decanted in a Petri plate ( $W_4$ ), dried at 105°C and weighed ( $W_5$ ). The inner side of

the centrifuge tube which is free from supernatant was wiped and weighed ( $W_3$ ). Values were expressed on per cent basis.

$$\text{Swelling power (g/g)} = \frac{W_3 - W_2}{W_1} \times 100 \dots \text{Equation 4}$$

$W_1$

$$\text{Per cent solubility} = \frac{W_5 - W_4}{V_E \times W_1} \times 100 \dots \text{Equation 5}$$

$V_E \times W_1$

### 3.5.5. Oil Absorption Capacity (OAC)

The method used by Adeleke and Odedeji, (2010) was used for oil absorption capacity. 10 ml ( $V_1$ ) of refined corn oil with density of 0.92 g/ml was added to one gram of flour in a 20 ml centrifuge tube. The content of the centrifuge tube was stirred for 2 min and then centrifuged at 4000 rpm for 20 min. The amount of oil separated as supernatant was decanted and measured using 10 ml cylinder ( $V_2$ ). The difference in volume was taken as the oil absorbed by the sample. Oil absorption capacity was expressed as ml of oil bound by 100 g dried flour.

$$\text{Oil Absorption Capacity (ml/g)} = (V_1 - V_2) \times 100 \dots \text{Equation 6}$$

### 3.6. Gluten Content and Quality of Flour

The gluten quantity and quality were evaluated by using perten glutomatic washing instrument (Perten, Glutomatic 2200, Sweden) according to the standard method 38-12 (AACC, 2000). Where ten grams of flour samples were weighed and placed into the glutomatic washing chamber (GM2020, Perten, Sweden) on top of the polyester screen then every sample of flour was mixed and washed with a 2% salt (NaCl) solution for 5 minutes. Then the total wet gluten was removed from the washing chamber placed in the centrifuge holder and centrifuged (CF2015, Perten, Sweden) at 6000 rpm for 1 minute. Then the gluten is weighed to obtain the total gluten and the sample was drained by putting on a sieve and weighed to get wet gluten content passed through the sieve. Finally, the wet gluten was dried in a Glutrok 2020 dryer (GT2020, Perten, Sweden) at 150°C for 4 minutes and weighed to determine the dry gluten content. In many world markets, wheat flour quality is often based upon protein content. The wheat gluten quality and quantity parameters were: Gluten strength by gluten index, Wet gluten content, Dry gluten content, and gluten water-binding capacity was calculated as follows Gluten Quality and Quantity (ICC Standard No.

155 and 158, AACC Method No. 38-12, ISO 21415).

$$\text{Gluten index (GI)} = \frac{(\text{Total wet gluten (g)} - \text{wet gluten passed through sieve})(\text{g}) \times 100\%}{\text{Total wet gluten (g)}} \dots \text{Equation 7}$$

$$\text{Wet gluten content (WGC)} = \frac{\text{Total wet gluten (g)} \times 100\%}{\text{Weight of sample (g)}} \dots \text{Equation 8}$$

$$\text{Dry Gluten Content (DGC)} = \frac{\text{Dry gluten (g)} \times 100\%}{\text{Weight of sample (g)}} \dots \text{Equation 9}$$

$$\text{Water-binding in wet gluten (WB)} = \text{WGC} - \text{DGC} \dots \text{Equation 10}$$

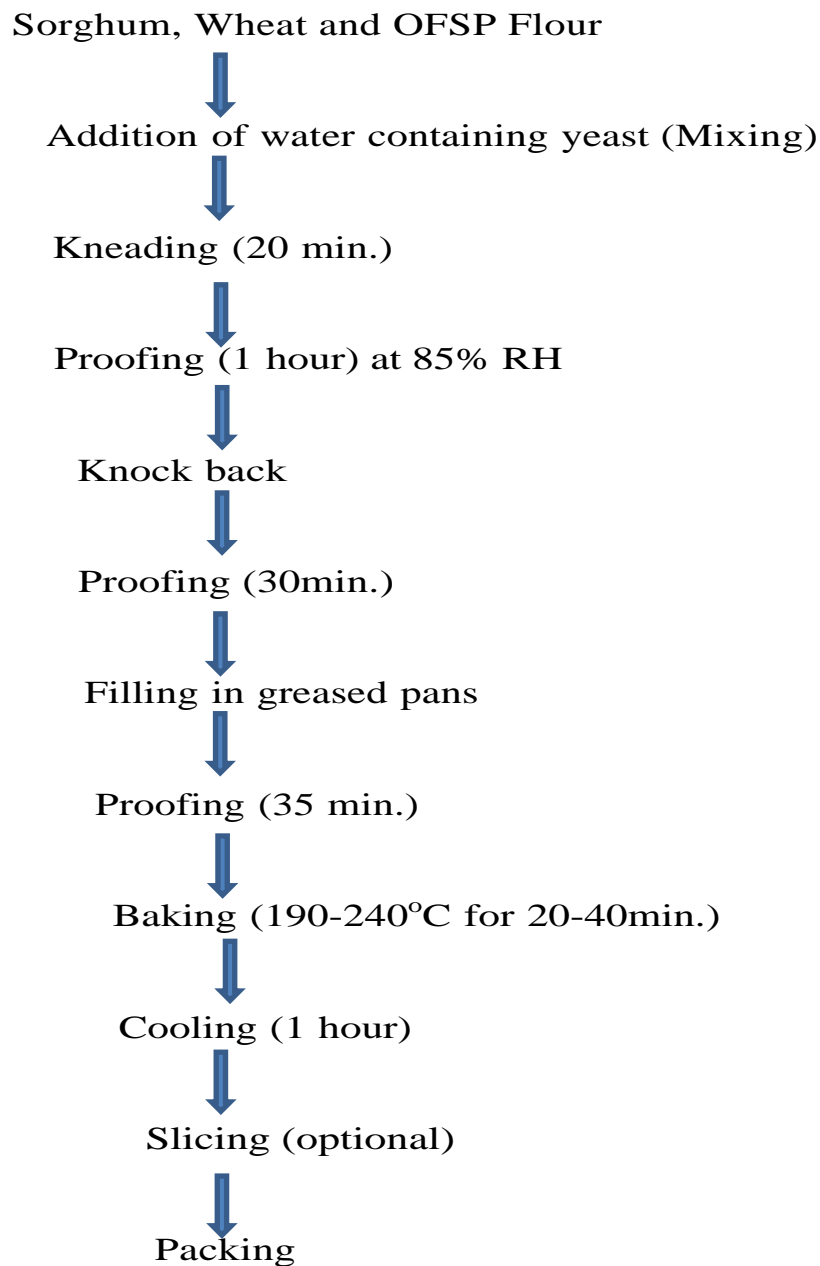
### 3.7. Dough Development Properties

Dough development properties such as flour water absorption, dough development time, dough stability and degree of softening were developed by using farinograph instrument according to the standard procedure of AACC, (2000). A flour sample of 300 g on a 14 % moisture basis was weighed and placed into a farinograph mixing bowl. Water from a burette at 30°C (Onyeike et al., 2008) was added to the flour and mixed to form dough. The water absorption and mixing characteristics of the composite flour was done using the Farinograph- instrument (Farinograph, China). As the dough was mixed the farinograph recorded a curve on the graph paper. The curve was centered on the 500 farinograph unit (FU) line  $\pm 20$  FU by adding the appropriate amount of water and was run until the curve leaved the 500 FU line. Measurements obtained from the farinograph curve were flour water absorption, dough development time, dough stability and degree of softening. The amount of water required to produce the 500 FU dough consistencies was interpreted as the percentage water absorption, while the time it took the dough to reach the threshold was interpreted as the dough development time. According to Scheeman, (2002) the stability was read as the time it took to maintain the 500 FU dough consistencies

### 3.8. Product Developments

Bread baking was performed based on method of Malik *et al.*, (2015) bread baking procedure (straight dough method). All ingredients flour (100 g blended), salt (2.5 g), water (65 ml) and yeast (2.5g) were added at mixing stage and kneaded to obtain uniform dough. The dough

samples were placed in baking pans smeared with vegetable oil and was covered for the dough to ferment resulting in gas production. The dough was then baked in an oven (Electric ovens SIMPLY 2T, China) at an average temperature of 230°C for 30 minutes. The baked loaf was carefully removed from the pans and allowed to cool and then packaged in Polyethylene bags for further analysis.



**Figure 8 Bread baking flow chart**

### **3.9.Sensory Analysis**

Bread prepared from different composite flours were evaluated based on Elia, (2011) for their sensory acceptability and preference by using experienced consumer participants. The attributes:-appearance/color, aroma, taste, texture, loaf volume and overall acceptability were assessed. The nine point hedonic scale (preference test) rated from 1 (Extremely dislike), 2 (dislike very much, 3 (Dislike moderately), 4 (Dislike), 5 (Neither like nor dislike), 6 (Like), 7 (Like moderately), 8 (Like very much) and 9 (Extremely like) for evaluating the degree of liking and disliking were employed.

### **3.10. Determination of Color of Flour and Bread**

The colour of composite flour and bread samples were analyzed by reflectance measurement using a handheld Minolta CR-300 tristimulus reflectance colorimeter (Minolta Corp., Ramsey, NJ, USA) as described by (Almeida *et al.*, 2014) and expressed in terms of the CIE: L\* (lightness/whiteness/darkness), a\* (redness/greenness), b\* (Yellowness/blueness) uniform colour space. The instrument was calibrated before starting analysis with standard white tile and black box for 100 and 0 % reflectance respectively. Composite flour and bread colour results reported in terms of 3-dimensional colour values based on the following rating scale:

L\* value whiteness 100 white, 0 black

a\* value positive values +60 red colour, negative values-60 green colour

b\* value positive values +60 yellow colour, negative values-60 blue colour

### **3.11. Bread Physical Characteristics**

The evaluated bread physical characteristics were loaf weight, loaf volume and specific loaf volume. Loaf weight was measured 30 minutes after the loaves were removed from the oven using a weighing balance. It was determined by weighing the bread loaves after cooling by using a laboratory scale electronic balance (CE- 410I, Camry Emperors, China) and the readings recorded in grams.

The loaf volume was measured using the rapeseed displacement method (AOCC, 2000, Standard 10-05) as stated by Hathorn *et al.*, (2004). Rapeseed grain was poured into a container of known volume until the bottom was covered. The loaf was placed inside

the container which was then filled to the top with more seeds. Then the extra rapeseed grain which had equal volume with the loaf was measured in a graduated cylinder and recorded as loaf volume.

Specific volume was obtained by dividing the loaf volume of bread by its corresponding loaf weight.

$$\text{Specific volume (cm}^3\text{/g)} = \frac{\text{Loaf volume (cm}^3\text{)}}{\text{Loaf weight (g)}} \dots \text{Equation 11}$$

### **3.12. Proximate Analysis**

Proximate chemical analyses of flour and all prepared bread product were determined according to AOAC, (2000) official methods and total carbohydrate was determined by difference. Total energy was determined according to Atwater and Benedict coefficients (Atwater and Benedict, 1902). All proximate analyses were conducted in triplicates and expressed as percentage in dry matter.

#### **3.12.1. Moisture Content**

Moisture content of the samples were determined according to Association of Official Analytical Chemistry (AOAC, 2000) using the official method 925.09 by oven drying method. A crucible was cleaned and dried in an oven at 105°C for 1 h and placed in desiccators to cool. The weight of the crucible (W1) was determined. A five gram samples (in triplicate) were weighed in the dry crucible (W2) and dried at 105°C for 3 h and after cooling in desiccators to room temperature it was again weighed (W3). The moisture content was determined using the following equation

$$\text{Moisture content (\%)} = \frac{W2 - W}{W2 - W1} \times 100 \dots \text{Equation 12}$$

Where, w1 = weight of sample, W2= weight of crucibles, W3 = weight of sample and crucible after oven dried.

#### **3.12.2. Determination of Crude Protein**

Protein content were determined according to AOAC, (2000) using the official method 979.09 by the Kjeldahl method.

### 3.12.2.1. Digestion

Fresh samples of 0.5000 g (in duplicate) were taken in a tector tube and 6 ml of acid mixture (5 parts of concentrated ortho-phosphoric acid and 100 parts of concentrated sulfuric acid) was added and mixed, and 3.5 ml of 30% hydrogen peroxide was added step by step. As soon as the violet reaction had ceased, the tubes were shaken and placed back to the rack. Three gram of catalyst mixture (ground 0.5000 g of selenium metal with 100 g of potassium sulfate) was added into each tube, and allowed to stand for about 10 min before digestion. When the temperature of the digester attained 370°C, the tubes were lowered into the digester. The digestion was allowed to continue until a clear solution was obtained, about 4 h. The tubes in the rack were cooled in a fume hood; 25 ml of de-ionized water was added, and shaken to avoid precipitation of sulfate in the solution.

### 3.12.2.2. Distillation

A 250 ml conical flask containing 25 ml of boric acid, 25 ml of distilled water and an indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested solution was transferred into the sample compartment of the distiller. Sodium hydroxide solution (35%) was added (40 ml) into the digested and diluted solution. The distillation process was continued for 9 min until a total volume reached between 200 ml and 250 ml. The tip of the distiller was rinsed with a few milliliter of water before the receiver was removed.

### 3.12.2.3. Titration

The distillate was titrated using 0.1N hydrochloric acid until reddish color appeared. The crude protein was determined using the following formula.

$$\text{Nitrogen (\%)} = \frac{V_{\text{HCL for sample}} - V_{\text{HCL for blank}}}{W_0} \times N_{\text{HCL}} \times 14 \times 100 \dots\dots \text{Equation 13}$$

$$\text{Protein (\%)} = 6.25 \times \% \text{ Nitrogen} \dots\dots\dots \text{Equation 14}$$

Where V is the volume of HCl in liter consumed to the end point of titration, N is the normality of HCl, W0 is sample weight on dry matter basis and 14.00 is the molecular weight nitrogen. The % of nitrogen was converted to % of protein by using appropriate conversion factor 6.25.

**3.12.3. Determination of Crude Fat**

Crude fat was determined according to AOAC (2000) using the official method 4.5.01. About 2g of flour (in duplicate) was extracted with 50 ml petroleum ether or diethyl ether for a minimum period of 4h in the soxhlet extractor. The solvent was then evaporated and the extracted fat was dried in the oven and cooled in a desiccator. The crude fat was determined according to the following equation.

Crude fat=  $\frac{W2 - W1}{W} \times 100$ .....Equation 15

Where W1 = weight of the extraction flask (g); W2 = weight of the extraction flask plus the dried crude fat (g); and W = weight of sample (g).

**3.12.4. Determination of Ash Content**

The ash content was determined by AOAC (2000) using the official method 923.03. Crucibles were placed in a muffle furnace for 30 min at 550°C. The Crucibles were then cooled in desiccators (with granular silica gel) for about 30 min at room temperature and weighed to the nearest milligram (W1). About 4.000 g of flour (in triplicate) was placed in Crucible and weighed (W2). Crucibles were placed on a hot plate under a fume-hood (AFA 1000, U.K.) and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The Crucibles with sample were placed inside the muffle furnace at 550°C for 5 h and cooled in desiccators for 1h. When cooled to room temperature, each dish with ash was reweighed to the nearest milligram (W3).

Total ash (%) =  $\frac{W3 - W1}{W2 - W1} \times 100$ .....Equation 16

Where (W2 -W1) is sample mass in g on dry base and (W3 -W1) mass of ash in g.

**3.12.5. Determination of Crude Fiber**

Crude fiber content of the flour samples was determined according to the method 962.09 of AOAC (2000) with slight modification.

**3.12.5.1. Digestion**

Flour samples of 1.000g (in triplicate) (W3 ) were placed into a 660 ml beaker; 200 ml of 1.25% sulfuric acid was added, and boiled gently for 30 min while watch glass was placed over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot

distilled water. After exactly 30 min heating, 20 ml of 28% KOH was added and boiled gently for further 30 min, with occasional stirring.

#### **3.12.5.2. Filtration**

The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with distilled water. The solution was poured into sintered glass crucible and filtered with the aid of vacuum pump (High performance vacuum pump, Robin Air way, SPX Corporation, Montpelier, USA). The wall of the beaker was rinsed with hot distilled water several times; then washings were transferred to the crucible and filtered.

#### **3.12.5.3. Washing**

The residue in the crucible was washed with hot distilled water and filtered. The residue was washed with 1% sulfuric acid and filtered, and then washed with hot distilled water and filtered; and again washed with 1% NaOH and filtered. The residue was washed with hot distilled water and filtered; and again washed with 1% sulfuric acid and filtered. Finally the residue was washed with water free acetone.

#### **3.12.5.4. Drying and Combustion**

The crucible with its content was dried in a drying oven for 2 h at 130°C and cooled for 30 min in a desiccator (with granular silica gel), and then weighed (record as W1 ). The crucible was cooled in desiccators and weighed (recorded as W2).

$$\text{Crude fiber (\%)} = \frac{W1 - W2}{W3} \times 100 \dots\dots\dots\text{Equation 17}$$

Where W1 = weight of crucible with sample after drying; W2 = weight of crucible with sample after ashing; W3 = fresh sample weight.

#### **3.12.6. Determination of Carbohydrate**

Carbohydrate content was determined by difference.

$$\text{Carbohydrate (\%)} = 100 - [\%P + \%F + \%Fb + \%A] \dots\dots\dots\text{Equation 18}$$

Where, P is protein content, F is fat content, Fb is fiber content and A is ash content.

### **3.12.7. Total Energy Determination**

The energy values were calculated using the Atwater and Benedict coefficients according to the following formula:

Energy (Kcal/100 g) = % CHO × 4 (Kcal) +% CP × 4 (Kcal) +% CF × 9 (Kcal)..... Equation 19  
(Atwater and Benedict, 1902).

Where, CHO stand for carbohydrate, CP for crude protein and CF for crude fat.

### **3.13. Analysis of Mineral Composition**

The standard method of Horwitz, W. (2000) wet digestion method was used to determine Manganese (Mn), zinc (Zn), Calcium (Ca) and iron (Fe). A 0.5 grams of flour was taken and digested with 5 ml concentrated nitric acid (HNO<sub>3</sub>) and 1 ml conc. perchloric acid (HClO<sub>4</sub>); then digested sample were filtered and made up to 100 ml in a standard flask. The atomic absorption spectrophotometer was used to determine all the minerals using appropriate lamps.

### **3.14. Analysis of Total Carotenoid and Beta-carotene**

#### **3.14.1. Beta-carotene**

Total carotenoid and beta carotene were extracted in the dim-light room. For the extraction of β-carotene, the procedure outlined in AOAC Official Method 941.15- (Pritwani & Mathur, 2017) was followed. Five grams of flour sample were measured and blended with 40 ml acetone, 60 ml petroleum ether, and 0.1 g magnesium/sodium carbonate, extracted for 5 min using mortar and pestle. Then filtration was done through the aid of a suction pump (Halstead Essex, England) and the sample was decanted into the separator funnel. The residue was washed with 25 ml portions acetone, then with 25 ml petroleum ether, and then extracts were combined. The combined extract was evaporated to dryness and residue re-dissolved in acetone. The volume made up to 5 ml using acetone depending upon the matrix. Before reading with HPLC (model) the sample was filtered through a syringe of 0.45µm pp and then 1 ml samples were put into a vial and then the sample was analyzed by high-performance liquid chromatography (Shimadzu HPLC, Japan, Kyoto). The peak responses of β-carotene were measured at wavelengths of 450 nm (Sungpuag et al., 1999).

## Standard Preparation of Beta Carotene

Standard of beta carotene (1g enclosed in the vial) (Sisco Research Laboratories Pvt. Ltd. (India) was purchased. The stock solution of beta-carotene was prepared by taking 10mg in 100ml acetone with the concentration equal to 100 ppm. Then series of standard solutions were prepared from known concentrations of stock solution (15, 30, 45, and 60 ppm dilutions with 5 ml of each acetone solution. The formula to calculate the beta-carotene contents of a sample in the extract was calculated as follows

$$\text{Beta - carotene } \left( \frac{mg}{100g} \right) = \frac{\mu g \text{ of carotene per mL(HPLC result) } * \text{ dilution as read from curve}}{\text{Weight of sample} * 1000} * 100 \dots \text{Equation 20}$$

### 3.14.2. Total Carotenoids

Harvest plus crops methods were employed for total carotenoid analysis. Total carotenoids were performed spectrophotometrically (Shimadzu -UV-1800, Japan) using the method described by (Rodriguez-Amaya and Kimura, 2004). Five grams (5 g) flour sample were ground with 40 ml of cold acetone using mortar and pestle until the residue became colorless and then vacuum-filtered using a Buchner funnel. The extract was partitioned with 60 ml of petroleum ether, and then each fraction was washed with distilled water for complete acetone removal. The extracts were made up to a volume of 50 mL with petroleum ether. All of the procedures were performed in dim light. The extracted carotenoids were collected and measured at 452 nm using a UV spectrophotometer (Shimadzu -UV-1800, Japan). Total carotenoids were calculated with the following equation.

$$\text{Total Carotenoid } (\mu g/g) = [A \times \text{volume (mL)} \times 104] / [A_{1\%1\text{cm}} \times \text{sample weight (g)}] \dots \text{Equation 21}$$

Where A = absorbance; volume = total volume of extract = 50 mL; A<sub>1%1cm</sub>=absorption coefficient of β-carotene in petroleum ether (2592).

## 3.15. Analysis of anti-nutritional Factors

### 3.15.1. Tannin

Tannin was determined by using vanillin-HCL assay methods using a UV spectrophotometer (Burns, 1971) as modified by Maxson and Rooney, (1972) cereal chemistry. One gram of

the sample in a screw cap test tube was measured, and then, 10 ml of 1% HCl in methanol was added to the tube containing the sorghum sample. The sample-containing tube was placed on a mechanical shaker for 24 h at room temperature, and then, the tube was centrifuged at 1000g for 5 minutes. One milliliter (1 ml) of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent in another test tube. Then, the sample was allowed to wait for 20 minutes to complete the reaction, and then, the absorbance of the colored intensity of the sample was measured using a UV-visible spectrophotometer at 500 nm.

$$\text{Tannin (mg/g)} = \text{Equation 22}$$

Where  $A_s$  is the sample absorbance,  $A_b$  is the blank absorbance,  $d$  is the density of the solution (0.791 g/ml),  $W$  is the weight of the sample in gram, and 10 is the aliquot.

### 3.15.2. Phytate

Phytate content was estimated by undertaking phytic acid analysis using the Latta and Eskin, (1980) method as modified by Vaintraub and Lapteva (1988). UV-Vis spectrophotometer, (Lambda, 9500) was used and the series standard solution was prepared to contain 0, 5, 9, 27, and 36  $\mu\text{g/g}$  of phytic acid (sodium phytate) weighed 0.1814g in 100 ml of distilled water) in 0.2N HCl. Three ml of each standard was added into 15 ml of centrifuge tubes and 3 ml of 0.2 N HCl was used as a blank. Two ml of Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 15 seconds. The phytate concentration was calculated from the difference between the absorbance of the blank and that of the assayed sample. About 1 g of dried sample was extracted with 10 ml 0.2 N HCl for 1 hour at ambient temperature and centrifuged at 3000 rpm for 30 min. The clear supernatant was used for phytate estimation. One ml of wade reagent was added to 3 ml of the supernatant sample solution and homogenizes and centrifuged at 3000 rpm for 10 min. The absorbance at 500 nm was measured using a UV-Vis spectrophotometer ( Lambda 9500, Malaysia). The phytate concentration was calculated from the difference between the absorbance of the blank (3 ml of 0.2N HCl + 2 ml of wade reagent) and that of the assayed sample. The amount of phytic acid was calculated using a phytic acid standard curve and the result was expressed as phytic acid in  $\mu\text{g/g}$  fresh weight.

$$\text{Phytic acid } (\mu\text{g/g}) = \frac{(A_s - A_b) - \text{Intercept}}{\text{slope}} * 10 \dots \text{Equation 23}$$

$$\text{slope} * W * 3$$

Where;  $A_s$ =Absorbance of sample  $A_b$ = Absorbance of blank,  $W$ = weight of the sample.

### 3.16. Statistical Analysis

The results were subjected to Analysis of Variance (ANOVA) technique by using Completely Randomized Design (CRD) method and all pair wise comparison tests were used for mean comparisons whereas Duncan's Multiple Range test (SPSS version 21.0 for Windows, SPSS Inc, Illinois, and USA) were carried out to determine level of significance within means (at  $p \leq 0.05$ ). Both numerical optimization and graphical optimization technique were employed using the Design Expert™ version 7.0 software (State Ease Inc.).

## CHAPTER FOUR

### 4. Results and Discussion

#### 4.1. Functional Properties of Composite Flour

Functional properties are the essential physicochemical properties of foods that reflect the complex interactions between the structures, molecular conformation, compositions, and physicochemical properties of food components with the nature of the environment and conditions in which these are measured and associated (Suresh and Samsher, 2013). They are required to possibly help to predict and precisely evaluate how new proteins, fat, carbohydrates (starch and sugars), and fibre may behave in specific food systems as well as demonstrate whether or not such can be used to stimulate or replace conventional protein, fat, carbohydrates (starch and sugars), and fibre (Siddiq *et al.*, 2009).

Table 4 Functional properties composite flour

Treatment	Bulk density (g/ml)	Dispersibility (%)	Water Absorption Capacity (ml)	Oil Absorption Capacity (ml/g)	Swelling power (g/g)	Solubility (100%)
T1	0.750 ±1.216 <sup>a</sup>	77.00 ±1.286 <sup>bc</sup>	1.333 ±0.466 <sup>ab</sup>	2.433 ±54.011 <sup>a</sup>	3.367 ±64.053 <sup>a</sup>	18.802 ±12.335 <sup>b</sup>
T2	0.661 ±1.216 <sup>b</sup>	76.23 ±1.286 <sup>cd</sup>	1.667 ±0.466 <sup>a</sup>	1.9333 ±54.011 <sup>ab</sup>	3.767 ±64.053 <sup>a</sup>	16.601 ±12.335 <sup>c</sup>
T3	0.706 ±1.216 <sup>ab</sup>	75.900 ±1.286 <sup>de</sup>	1.500 ±0.466 <sup>a</sup>	2.400 ±54.011 <sup>a</sup>	3.112 ±64.053 <sup>a</sup>	20.729 ±12.335 <sup>b</sup>
T4	0.733 ±1.216 <sup>a</sup>	75.233 ±1.286 <sup>e</sup>	0.667 ±0.466 <sup>b</sup>	1.600 ±54.011 <sup>ab</sup>	3.941 ±64.053 <sup>a</sup>	15.731 ±12.335 <sup>c</sup>
T5	0.699 ±1.216 <sup>ab</sup>	79.500 ±1.286 <sup>a</sup>	1.167 ±0.466 <sup>ab</sup>	2.000 ±54.011 <sup>ab</sup>	3.417 ±64.053 <sup>a</sup>	22.515 ±12.335 <sup>b</sup>
T6	0.698 ±1.216 <sup>ab</sup>	76.667 ±1.286 <sup>bcd</sup>	1.333 ±0.466 <sup>ab</sup>	1.933 ±54.011 <sup>ab</sup>	3.560 ±64.053 <sup>a</sup>	34.983 ±12.335 <sup>a</sup>
T7	0.724 ±1.216 <sup>a</sup>	76.400 ±1.286 <sup>bcd</sup>	1.167 ±0.466 <sup>ab</sup>	1.833 ±54.011 <sup>ab</sup>	3.338 ±64.053 <sup>a</sup>	14.743 ±12.335 <sup>c</sup>
T8	0.715 ±1.216 <sup>a</sup>	77.167 ±1.286 <sup>b</sup>	1.000 ±0.466 <sup>ab</sup>	1.300 ±54.011 <sup>b</sup>	3.386 ±64.053 <sup>a</sup>	32.724 ±12.335 <sup>a</sup>
Grand mean	0.711	76.763	1.229	1.929	3.486	22.13
C.V	5.122	0.68	25.23	0.250	7.529	53.64

Values are expressed as mean ± SE (n=8). Means in the same column with different letters are significantly different based on Duncan's multiple range test ( $p < 0.05$ ). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively.

#### **4.1.1. Water Absorption Capacity**

Water absorption capacity (WAC) is the amount of water (moisture) taken up by food/flour to achieve the desirable consistency and create quality food product. It is an important functional property required in food formulations especially those involving dough handling (Oselebe *et al.*, 2008). Very low or excessive water absorption can negatively affect the quality of food products. As we have seen from Table 4, all treatments are not significantly different in their water absorption capacity. WAC values ranges from 0.667% treatment 4 (80% wheat: 15%: 5% OFSP) to 1.667% treatment 2 (75% wheat: 22.5% sorghum: 2.5% OFSP). Composite flour of treatment 2 (75% wheat: 22.5% sorghum: 2.5% OFSP) has high WAC while composite flour of treatment 4 (80% wheat: 15%: 5% OFSP) has low WAC. The higher water absorption capacity of wheat flour may be due to the higher polar amino acid residues of proteins having an affinity for water molecules (Yusuf *et al.*, 2008). The major chemical compositions that enhance the water absorption capacity of flours are proteins and carbohydrates, since these constituents contain hydrophilic parts, such as polar or charged side chains (Lawal and Adebawale, 2004). Mark *et al.*, 2018 stated that WAC increases as ratio of wheat flour to other crops increases which our experiment also verified. Kuntz (1971) reported that lower water absorption in some flours may be because of the less availability of polar amino acids in the flours as cited by Awuchi *et al.*, (2019).

#### **4.1.2. Oil Absorption Capacity**

All treatments are not significantly different in terms of their OAC except treatment 1 (90% wheat: 7.5% sorghum: 2.5% OFSP) and treatment 2 (70% sorghum: 22.5% wheat: 7.5%) which are significantly different from each other. The OAC of composite flour ranges from 1.3 ml/g (75% wheat: 22.5% sorghum: 2.5% OFSP) to 2.433 m/g (100% wheat). The possible reason for increase in the OAC of flours is the increase in the presence of non-polar side chain, which may bind the oil hydrocarbon side chains in foods and flours. The flours with high OAC are potentially beneficial in structural interactions in foods especially for improvement of palatability, extension of keeping quality, and flavor retention particularly in meat or bakery products where fat absorption is desirable (Suresh *et al.* 2015). The oil absorption of treatment 1 (90% wheat: 7.5% sorghum: 2.5% OFSP) was high which has high ratio of wheat to other composite flours. This suggested that treatment 1 (90% wheat: 7.5% sorghum: 2.5% OFSP) flour may have more hydrophobic proteins; the more hydrophobic proteins demonstrate superior

binding of lipids (Lawal and Adebawale, 2004). The major chemical component affecting oil absorption capacity is protein, which is composed of both hydrophilic and hydrophobic parts. Oil absorption capacity (OAC) is an important property since oil acts as flavor retainer and increases the mouth feel of foods (Aremu *et al.*, 2007). It has been reported that variations in the presence of non-polar side chains, which might bind the hydrocarbon side chains of oil among the flours, explain differences in the oil binding (Lawal and Adebawale, 2004).

#### **4.1.3. Bulk Density**

Bulk density (BD) gives an indication of the relative volume of packaging material required. In our experiment treatment 6 (90% wheat: 0% sorghum: 10% OFSP) has high bulk density of 0.75g/ml while treatment 2 (75% wheat: 22.5% sorghum: 2.5% OFSP) has low value of bulk density of 0.661g/ml. Alawode *et al.*, (2017) reported that, the average bulk density of sorghum and OFSP flour were 0.60g/ml which our finding also confirmed. The higher the bulk density of the flour, the denser the packaging material required for packaging. High bulk density of flours suggests their suitability for application in food preparations. On the other hand, low bulk density would be useful in the formulation of complementary foods (Suresh and Samsher, 2013). Generally, higher bulk density is desirable for the greater ease of dispersibility and reduction of paste thickness (Udensi and Okoronkwo, 2006).

#### **4.1.4. Swelling Power**

Treatment 4 (80% wheat: 15% sorghum: 5% OFSP) has high swelling power while treatment 3 (70% sorghum: 22.5% wheat: 7.5%) has low swelling power value. High starch content increases swelling capacity (index) of foods and flours, especially in starch with higher amount of the branched amylopectin (Awuchi *et al.*, 2019). This suggests that the composite flour of treatment 4 (80% wheat: 15% sorghum: 5% OFSP) has high starch content when compared with other treatments in this experiment.

#### **4.1.5. Solubility**

Flour solubility is one of the functional properties usually determined during the development and testing of a new flour or flour composite. As per our experiment, treatment 3 (70% sorghum: 22.5% wheat: 7.5%) has high level of solubility while treatment 2 (75% wheat: 22.5% sorghum: 2.5 OFSP) has low solubility. High solubility of food can show high digestibility of the food which may indicate excellent use for infant formula and food. On the other hand, insolubility is

the inability of a food to dissolve in a liquid, gaseous, or solid solvent (Rogers and Stovall, 2000). The reason why treatment 2 (75% wheat: 22.5% sorghum: 2.5 OFSP) has low solubility is maybe due to the low digestibility of sorghum.

#### 4.1.6. Dispersibility

The mean value of dispersibility ranges from 75.233 (80% wheat: 15% sorghum: 5 %) to 79.5 (100% wheat). As the ratio of wheat increased dispersibility of composite flour was increased while it was decreased as the ratio of sorghum increased. Alawode *et al.*, (2017) stated that dispersibility is a measure of the re-constitutability of flour or flour blends in water. The higher the dispersibility, the better the flour reconstitutes in water.

#### 4.2. Dough Properties

Development time is time taken for the dough to reach maximum resistance to mixing from the start of mixing. Stability is a measure of the tolerance of the flour to mixing. Degree of softening is the difference in height between the Centre of the graph at maximum resistance to mixing and the Centre of the graph at a point 12 minutes later.

**Table 5 Dough development properties**

Treatments	Water Absorption (ml/g)	Dough Development Time (min)	Dough Stability (min)	Dough Softening (Farinograh units)
T1	58.488 ±4.368 <sup>e</sup>	0.964 ±0.663 <sup>d</sup>	4.188 ±0.711 <sup>c</sup>	45.187 ±13.929 <sup>abcd</sup>
T2	60.730 ±4.368 <sup>d</sup>	2.124 ±0.663 <sup>bc</sup>	4.310 ±0.711 <sup>c</sup>	64.418 ±0.711 <sup>a</sup>
T3	60.019 ±4.368 <sup>d</sup>	2.105 ±0.663 <sup>c</sup>	4.330 ±0.711 <sup>c</sup>	63.342 ±0.711 <sup>ab</sup>
T4	63.510 ±4.368 <sup>c</sup>	2.225 ±0.663 <sup>ab</sup>	3.850 ±0.711 <sup>d</sup>	59.059 ±0.711 <sup>abc</sup>
T5	53.777 ±4.368 <sup>g</sup>	0.791 ±0.663 <sup>e</sup>	5.833 ±0.711 <sup>a</sup>	37.869 ±0.711 <sup>d</sup>
T6	56.773 ±4.368 <sup>f</sup>	0.922 ±0.663 <sup>d</sup>	4.995 ±0.711 <sup>b</sup>	44.807 ±0.711 <sup>bcd</sup>
T7	64.857 ±4.368 <sup>b</sup>	2.326 ±0.663 <sup>a</sup>	3.907 ±0.711 <sup>d</sup>	43.781 ±0.711 <sup>cd</sup>
T8	67.835 ±4.368 <sup>a</sup>	2.303 ±0.663 <sup>a</sup>	3.538 ±0.711 <sup>e</sup>	62.570 ±0.711 <sup>abc</sup>
Grand Mean	60.749	1.72	4.369	52.629
C.V	0.87	3.78	3.52	21.47

Values are expressed as mean ± SE (n=8). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively

#### **4.2.1. Water absorption**

All samples were significantly different except sample 75% wheat: 22.5% sorghum: 2.5% OFSP. The farinographic result of water absorption ranges from 53.777ml/g to 67.835ml/g. As the ratio of sorghum and OFSP increased, water absorption of composite decreased. The reason is maybe due to low protein content of sorghum and OFSP flour that holds water when compared to wheat flour. Tegegn, (2020) stated that in their thesis report the water absorption of wheat-orange flashed sweet potato ranges from 55.47 - 69.53ml/g which is nearly the same with our finding.

#### **4.2.2. Dough Development Time**

The dough development time of composite flour ranges from 0.791 to 2.326 min (table 5). Flour sample of 90% wheat: 0% sorghum: 10% OFSP has high dough development time next to sample of 100% wheat while flour sample of 70% wheat: 30% sorghum: 0% OFSP has low dough development time. As the ratio of wheat and OFSP increased, and ratio of sorghum decreased the dough development decreased. This is maybe due to the gluten properties of flour that helps the dough to quickly rise.

#### **4.2.3. Dough Stability**

Dough stability of composite flours was between 3.538 to 5.833min. Composite flour sample of 60% wheat: 30% sorghum: 10% OFSP has low dough stability while composite flour sample of 90% wheat: 0% sorghum: 10% OFSP has high dough stability. As the ratio of wheat flour increased the dough stability of composite flour were also increased (table 5).

#### **4.2.4. Dough Softening**

Dough softening value of composite flour sample ranged from 37.869 to 64.418 farinograph units. Sample of (70% wheat: 30%: 0% OFSP) has the lowest dough softening value (63.342 farinograph unit) next to the composite flour of 100% wheat flour. The sample with low softening value shows the good quality of the composite flour.

### 4.3. Proximate Composition of Composite Flour

Proximate compositions of raw materials and composite flour were listed below in Table 7 and 8 respectively in dry basis.

Table 6 Average Proximate Composition of Raw Materials (wheat, sorghum and OFSP flour) per 100g

Crops	Ash (%)	Moisture (%)	CP (%)	Crude fat (%)	Crude fiber (%)	Total carbohydrate (%)	Total energy (kcal)
Sorghum	1.672	11.871	9.873	3.823	6.431	78.201	386.703
Wheat	0.991	13.020	16.436	4.469	0.5800	77.524	416.061
OFSP	1.113	7.836	5.251	0.913	4.462	88.261	382.265

Table 7 Proximate composition of composite flour

TRT	Ash (%)	Moisture (%)	Crude protein (%)	Crude fat(%)	Crude fiber (%)	Total carbohydrate (%)	Total energy ( kcal)
T1	0.990 ±2.318 <sup>b</sup>	11.676 ±1.319 <sup>bc</sup>	10.376±3.44 <sup>b</sup>	4.496±1.610 <sup>a</sup>	1.045±0.362 <sup>bc</sup>	83.093±3.614 <sup>ab</sup>	414.34±8.416 <sup>a</sup>
T2	0.989 ±2.318 <sup>cd</sup>	11.857 ±1.319 <sup>bc</sup>	12.607±3.44 <sup>ab</sup>	0.731±1.610 <sup>d</sup>	0.880±0.362 <sup>cd</sup>	84.794±3.614 <sup>a</sup>	396.18±8.416 <sup>c</sup>
T3	0.993 ±2.318 <sup>a</sup>	13.957 ±1.319 <sup>a</sup>	12.712±3.44 <sup>ab</sup>	1.746±1.610 <sup>c</sup>	1.340±0.362 <sup>ab</sup>	83.209±3.614 <sup>ab</sup>	399.40±8.416 <sup>c</sup>
T4	0.985 ±2.318 <sup>c</sup>	10.098 ±1.319 <sup>d</sup>	16.329±3.44 <sup>a</sup>	3.270±1.610 <sup>b</sup>	1.610±0.362 <sup>a</sup>	77.806±3.614 <sup>bc</sup>	405.97±8.416 <sup>b</sup>
T5	0.991 ±2.318 <sup>b</sup>	13.020 ±1.319 <sup>ab</sup>	16.436±3.44 <sup>a</sup>	4.469±1.610 <sup>a</sup>	0.580±0.362 <sup>d</sup>	77.524±3.614 <sup>c</sup>	416.06±8.416 <sup>a</sup>
T6	0.99 ±2.318 <sup>bc</sup>	12.209 ±1.319 <sup>bc</sup>	12.064±3.44 <sup>ab</sup>	5.312±1.610 <sup>a</sup>	1.145±0.362 <sup>bc</sup>	82.884±3.614 <sup>abc</sup>	418.02±8.416 <sup>a</sup>
T7	0.988 ±2.318 <sup>d</sup>	11.511 ±1.319 <sup>c</sup>	9.67±3.44 <sup>b</sup>	4.690±1.610 <sup>a</sup>	1.010±0.362 <sup>bc</sup>	81.249±3.614 <sup>abc</sup>	415.46±8.416 <sup>a</sup>
T8	0.991 ±2.318 <sup>b</sup>	10.865 ±1.319 <sup>cd</sup>	11.723±3.44 <sup>ab</sup>	4.668±1.610 <sup>a</sup>	0.920±0.362 <sup>cd</sup>	81.699±3.614 <sup>abc</sup>	415.69±8.416 <sup>a</sup>
<b>G.</b>	0.990	11.899	12.740	3.673	1.066	81.532	410.14
<b>M</b>							
<b>CV</b>	0.08	6.54	23.47	13.57	23.29	3.84	0.68

Values are expressed as mean ± SE (n=8). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively

#### **4.3.1. Ash Content**

The ash content of composite flour ranges from 0.985% to 0.993% (Table 6). Treatment 4 (80% wheat: 15% sorghum: 5% OFSP) was significantly different from other treatments in terms of its ash content. Treatment 3 (70% wheat: 22.5% sorghum: 7.5% OFSP) has high ash content (0.993%) while treatment 4 (80% wheat: 15% sorghum: 5% OFSP) has low ash content (0.985%). Xu *et al.*, (2020) stated that as the ratio of sorghum to wheat increases the ash content of composite flour increases. As the ratio of sorghum flour in composite flour increased, the ash content of composite flour increased. Our finding also confirms their report. The high ash content indicates that the flour is rich of minerals.

#### **4.3.2. Moisture content**

The moisture content of composite flour ranges from 10.1% to 13.96%. The highest moisture content value (13.96%) was observed for treatment 4 (80% wheat: 15% sorghum: 5% OFSP) while the lowest value (10.1%) was observed for treatment 1 (90% wheat: 7.5% sorghum: 2.5% OFSP). The average value of composite flour moisture content (11.9%) was within the critical moisture (12%) content for flours and this is attributed to proper drying and handling (Tumuhimbise *et al.*, 2019). Low moisture content prevents microbial activity thus, extends the shelf life of flour (Joyce *et al.*, 2006). In our experiment, the moisture content increases as a ratio of blending of wheat flour in composite flour increases. This is maybe due to the high protein contents of wheat that holds more water when compared to sorghum and OFSP.

#### **4.3.3. Crude protein**

The crude protein content of composite flour ranges from 9.677% -16.436%. All treatments were not significantly different from each other in their crude protein content. Treatment 4 (80% wheat: 15% sorghum: 5% OFSP) has the highest value next to treatment 5 (100% wheat) while treatment 7 (60% wheat: 30% sorghum: 10% OFSP) has low protein content when compared to other composite flour. Our experiment results showed that the addition of sorghum flour resulted in the decrease of crude protein content. Sibanda *et al.*, (2015) also reported that as the ratio of sorghum flour increases the crude protein content of wheat-sorghum composite flour decreased.

#### **4.3.4. Crude fat**

Sample 2 (75% wheat: 22.5 sorghum: 2.5% OFSP), 3 (70% wheat: 22.5% sorghum: 7.5% OFSP) and 4 (80% wheat: 15% sorghum: 5% OFSP) were significantly different from other treatments

as well as each other while other samples are not significantly different from each other in terms of their crude fat content. The crude fat content ranges from 0.731% to 5.312%. The highest value scored for sample of 90% sorghum and 10% OFSP while the lowest value was scored for the sample of 75% wheat: 22.5% sorghum: 2.5% OFSP (Table 7).

#### **4.3.5. Fiber content**

The crude fiber content of composite flour ranges from 0.58% to 1.34% (table 8). Treatment 4 (80% wheat: 15% sorghum: 5% OFSP) was significantly different from other treatments except treatment 3 (70% wheat: 22.5% sorghum: 7.5% OFSP). The lowest value scored for treatment 5 (100% wheat) while the highest value was scored for treatment 3 (70% wheat: 22.5% sorghum: 7.5% OFSP). This experiment finding correlates with the report of Tegegne, (2020), which states that as the ratio of inclusion of OFSP decreased, the fiber content of wheat-OFSP flour decreased.

#### **4.3.6. Total carbohydrate**

Total carbohydrate of composite flour ranges from 77.806% to 82.884% (Table 8). Ibrahim and Ani, (2018) also reported that the average carbohydrate content of 60% wheat and 40% sorghum were 76.27% which was nearly close to our finding. Treatment 4 (80% wheat: 15% sorghum: 5% OFSP) has the lowest value while treatment 6 (90% wheat: 0% sorghum: 10% OFSP) has the highest value. Our experiment result shows that, the composite flour with high amount of wheat and OFSP flour has high content of total carbohydrate.

#### **4.3.7. Available energy**

Total energy of wheat: sorghum: OFSP composite flour ranges from 399.40kcal to 418.02kcal (table 7). Treatment 2 (90% wheat: 7.5% sorghum: 2.5% OFSP), 3 (70% wheat: 22.5% sorghum: 7.5% OFSP) and 4 (80% wheat: 15% sorghum: 5% OFSP) are significantly different from each other and all treatments in terms of their total energy contents. Treatment 6 (90% wheat: 0% sorghum: 10% OFSP) has the highest energy value of 418.02Kcal while treatment 3 (70% wheat: 22.5% sorghum: 7.5% OFSP) has the lowest energy value of 399.40Kcal. The highest total energy value of composite flour is due to high content of protein and carbohydrate content of flour samples (Mugalavai *et al.*, 2020).

#### 4.4. Color of Composite Flour

Color of flour is one of important parameter that indicates the quality of flour.

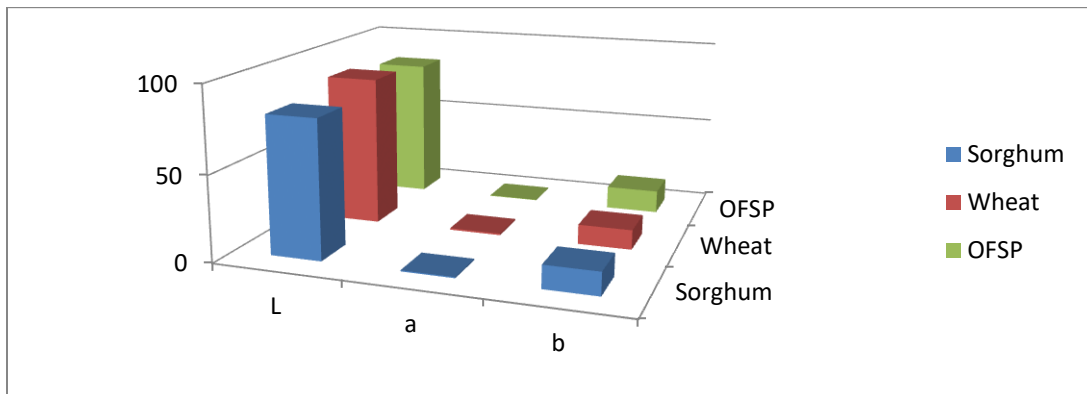


Figure 9 Color value of Raw Materials

The color of composite flour depends on the color of individual crops.

Table 8 Color of composite flour

Treatment	L*	a*	b*
T1	86.897 ± 2.333 <sup>b</sup>	0.987 ± 0.556 <sup>de</sup>	13.100 ± 2.052 <sup>c</sup>
T2	88.030 ± 2.333 <sup>b</sup>	1.860 ± 0.556 <sup>b</sup>	14.073 ± 2.052 <sup>b</sup>
T3	89.623 ± 2.333 <sup>a</sup>	0.633 ± 0.556 <sup>f</sup>	9.9267 ± 2.052 <sup>e</sup>
T4	87.650 ± 2.333 <sup>ab</sup>	2.163 ± 0.556 <sup>a</sup>	16.960 ± 2.052 <sup>a</sup>
T5	87.820 ± 2.333 <sup>ab</sup>	0.897 ± 0.556 <sup>e</sup>	11.603 ± 2.052 <sup>d</sup>
T6	82.503 ± 2.333 <sup>c</sup>	1.400 ± 0.556 <sup>c</sup>	13.263 ± 2.052 <sup>bc</sup>
T7	86.460 ± 2.333 <sup>b</sup>	1.150 ± 0.556 <sup>d</sup>	12.833 ± 2.052 <sup>c</sup>
T8	87.707 ± 2.333 <sup>ab</sup>	0.550 ± 0.556 <sup>f</sup>	11.443 ± 2.052 <sup>d</sup>
Grand mean	87.086	1.205	12.900
C.V	1.70	10.14	4.26

Values are expressed as mean ± SE (n=8). Means in the same column with different letters are significantly different based on Duncan's multiple range test ( $p < 0.05$ ). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively

#### 4.4.1. L\* value

All treatments were not significantly different in terms of their lightness except treatment 6 (90% wheat: 0% sorghum: 10% OFSP). The L\*-value of composite flour ranges from 82.503 to 89.623. As the value of L\* increases the product acceptance increases.

#### 4.4.2. a\* and b\* value

The composite flour lowest “a\* and b\*” value was observed for treatment 8 while the highest value observed for treatment 4. As the ratio of sorghum and OFSP flour increased, a\* and b\* value of composite flour increased. This is due to low color characteristics of sorghum and OFSP flour when compared with wheat flour.

#### 4.5. Wet and Dry Gluten of Composite Flour

Gluten is the complex protein that helps the bread to expand and enhance extensibility. It was not detected in both sorghum (100%) and OFSP (100%) flour. This might be due to the low gluten content of both crops.

Table 9 Wet and dry gluten content of individual crops

Crops	Wet gluten (%)	Gluten index (%)	Dry gluten (%)	WBCG (%)
Wheat	37.549	82.688	9.750	19.117
Sorghum	ND	ND	ND	ND
OFSP	ND	ND	ND	ND

Where ND: Not Detected

Table 10 Wet and dry gluten content of composite flour

Treatments	Wet gluten (%)	Gluten index (%)	Dry gluten (%)	WBCG (%)
T1	28.65 ±5.297 <sup>d</sup>	88.124 ±3.696 <sup>b</sup>	11.150 ±2.077 <sup>c</sup>	17.500 ±3.361 <sup>e</sup>
T2	35.300 ±5.297 <sup>b</sup>	83.711 ±3.696 <sup>c</sup>	13.200 ±2.077 <sup>b</sup>	22.100 ±3.361 <sup>bc</sup>
T3	28.600 ±5.297 <sup>d</sup>	84.381 ±3.696 <sup>c</sup>	14.650 ±2.077 <sup>a</sup>	22.900 ±3.361 <sup>ab</sup>
T4	33.463 ±5.297 <sup>c</sup>	84.433 ±3.696 <sup>c</sup>	12.647 ±2.077 <sup>b</sup>	21.430 ±3.361 <sup>c</sup>
T5	37.549 ±5.297 <sup>a</sup>	82.688 ±3.696 <sup>c</sup>	9.750 ±2.077 <sup>c</sup>	19.117 ±3.362 <sup>d</sup>
T6	37.050 ±5.297 <sup>a</sup>	85.225 ±3.696 <sup>c</sup>	13.200 ±2.077 <sup>b</sup>	23.850 ±3.361 <sup>a</sup>
T7	26.450 ±5.297 <sup>e</sup>	91.661 ±3.696 <sup>a</sup>	8.150 ±2.077 <sup>e</sup>	14.100 ±3.361 <sup>g</sup>
T8	22.250±5.297 <sup>f</sup>	92.064 ±3.696 <sup>a</sup>	10.537 ±2.077 <sup>c</sup>	16.200 ±3.361 <sup>f</sup>
Grand mean	31.164	86.536	11.660	19.650
C.V	2.31	1.72	3.49	2.81

Values are expressed as mean ± SE (n=8). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively. WBCG stands for

#### 4.5.1. Wet gluten

The wet gluten content of composite flour ranges from 22.25% to 37.549% (table 10). Treatment 8 (60% wheat: 30% sorghum: 10% OFSP) has the lowest value of wet gluten content while treatment 6 has the highest value next to 100% wheat sample. As have observed from the result (Table 11), the wet gluten content increased as the addition of sorghum decreased. This is due to the low gluten content of sorghum grain.

#### 4.5.2. Gluten index

The Gluten Index is a measure of the gluten characteristics, which indicates whether the gluten is weak, normal or strong. The gluten index of composite flour in our finding ranged from 82.688

to 92.064%. Treatment 5 (100% wheat) has the lowest value of gluten index while treatment 8 (60% wheat: 30%: 10% OFSP) has highest value. This was maybe due to the low ratio of wheat flour and maximum level of sorghum.

#### 4.5.3. Dry gluten

The dry gluten content of composite flour ranges from 8.15 to 14.65%. Treatment 7 has lowest value of dry gluten while treatment 3 has the highest value (table 10).

#### 4.5.4. Water holding capacity of gluten

The water binding capacity of gluten (WBCG) of composite flour ranges from 14.1-23.85%. Treatment 7 has the lowest value while treatment 6 has the highest value.

#### 4.6. Anti-nutritional Factors of Composite Flour and bread product

The bioavailability of minerals in Sorghum and wheat bran is under debate because of the presence of the anti-nutrient factors such as: tannin and phytic acid.

Table 11 Anti-nutritional factors of composite flour and Bread

S code	Anti-nutritional factors			
	Tannin (%)		Phytate (%)	
	Composite Flour	Bread Product	Composite Flour	Bread Product
T1	0.0670 ± 0.1465 <sup>a</sup>	BDL	83.148 ± 7.501 <sup>a</sup>	82.685 ± 7.6276 <sup>a</sup>
T2	0.1387 ± 0.1465 <sup>a</sup>	BDL	76.821 ± 7.501 <sup>a</sup>	72.654 ± 7.6276 <sup>c</sup>
T3	0.2583 ± 0.1465 <sup>a</sup>	BDL	62.315 ± 7.501 <sup>b</sup>	60.957 ± 7.6276 <sup>c</sup>
T4	0.1863 ± 0.1465 <sup>a</sup>	BDL	84.815 ± 7.501 <sup>a</sup>	80.988 ± 7.6276 <sup>b</sup>
T5	0.1987 ± 0.1465 <sup>a</sup>	BDL	83.148 ± 7.501 <sup>a</sup>	82.222 ± 7.6276 <sup>b</sup>
T6	0.1267 ± 0.1465 <sup>a</sup>	BDL	77.593 ± 7.501 <sup>a</sup>	75.741 ± 7.6276 <sup>bc</sup>
T7	0.1507 ± 0.1465 <sup>a</sup>	0.055 ± 0.2677 <sup>a</sup>	85.309 ± 7.501 <sup>a</sup>	83.482 ± 7.6276 <sup>a</sup>
T8	0.1747 ± 0.1465 <sup>a</sup>	BDL	79.907 ± 7.501 <sup>a</sup>	73.272 ± 7.6276 <sup>c</sup>
Grand mean	0.1626	BDL	79.132	76.500
C.V	100.38	BDL	9.4788	9.9707

Values are expressed as mean ± SE (n=8). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively

#### 4.6.1. Tannin

As per our experiment, tannin concentrations of most composite flour bread were below the detection limit (BDL). This might be due to the complete tannin reduction during the de-hulling of sorghum and milling process. Also, the below detection limit of the anti-nutritional factors might be linked with the formation of insoluble complexes due to thermal degradation and denaturation during the bread baking process (Kataria *et al.*, 1998). They are concentrated in the external covers in the pericarp and aleurone layer of the grain at lower levels, in the germ as well (Cheryan,1980); 90% of the phytic acid in the grain is in the aleurone layer with 10% in the embryo (Dost and Tokul, 2005). Consequently, the amount of phytic acid is greatly determined by the fractions removed during milling: white flour has almost no phytate (Anonymous, 1979).

#### 4.6.2. Phytate

As per our experiment, the value of phytate in composite flour and bread product were 62.315mg/100g to 85.309mg/100g and 60.957 to 83.482mg/100g respectively. Both flour and bread product phytate content was very low when compared with grains. The amount of phytic acid is greatly determined by the fractions removed during milling: white flour has almost no phytate (Anonymus, 1979). The phytate content was decreased in bread product when compared with the composite flour. This is maybe due to process of dough development and baking temperature

#### 4.7.Total carotenoid and beta-carotene content of composite flour and bread

Total and beta carotene content of composite flour were listed and discussed below in table 13

Table 12 Total carotenoid and beta-carotene content of composite flour and bread

Sample Code	Total Carotenoids (mg/100g)		Beta-carotene(mg/100g)	
	Composite Flour	Bread Product	Composite Flour	Bread Product
T1	12.669±12.368 <sup>cd</sup>	2.893 ±1.24ab	7.362 ±8.381 <sup>c</sup>	BDL
T2	6.917 ±12.368 <sup>d</sup>	1.954 ±1.24 <sup>bc</sup>	6.228 ±8.381 <sup>c</sup>	BDL
T3	27.905 ±12.368 <sup>b</sup>	2.725 ±1.24 <sup>b</sup>	13.166 ±8.381 <sup>b</sup>	BDL
T4	16.527 ±12.368 <sup>c</sup>	2.635 ±1.2 <sup>4b</sup>	8.401 ±8.381 <sup>c</sup>	0.056 ±0.8757 <sup>bc</sup>
T5	BDL	BDL	BDL	BDL
T6	32.890 ±12.368 <sup>ab</sup>	2.816 ±1.24 <sup>b</sup>	21.300 ±8.381 <sup>d</sup>	0.430 ±0.8757 <sup>b</sup>
T7	6.223 ±12.368 <sup>d</sup>	0.154 ±1.24 <sup>c</sup>	0.140 ±8.381 <sup>d</sup>	BDL
T8	35.904 ±12.368 <sup>a</sup>	3.998 ±1.24 <sup>a</sup>	23.217 ±8.381 <sup>a</sup>	0.880 ±0.8757 <sup>a</sup>
<b>Grand mean</b>	18.054	2.3061	10.014	BDL
<b>C.V</b>	21.65	27.96	14.27	BDL

Values are expressed as mean ± SE (n=8). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively. BDL: Below detection level.

#### 4.7.1. Total carotenoid and beta-carotene of composite flour

The highest value of total carotenoid and beta carotene content of composite flour were 35.904mg/100g and 23.217mg/100g respectively. As the ratio of OFSP in composite flour increased, the total carotenoid and beta-carotene content were increased. Nzamwita *et al.*, (2017) found in their previous study that, the composite flour of OFSP (10%) and wheat (90%) has the average value of 29.171 mg/100g beta carotene which was higher than our finding while that of 100% wheat has BDL which our experiments also confirmed.

#### 4.7.2. Total carotenoid and beta-carotene of bread

The beta carotene content of bread product was below detection level in all treatments except treatment 8 (0.88 mg/100g), treatment 6 (0.43 mg/100g) and treatment 4 (0.056 mg/100g). The total carotenoid content of bread product ranged from 0.154mg/100g to 3.998mg/100g. The high content of total carotenoid and beta carotene in sample of 60% sorghum: 30% sorghum: 10% OFSP when compared to other samples was maybe due to the maximum level of OFSP added to composite flour. The levels of total carotenoid and beta-carotene may be negatively affected during heat processing such as baking and dough preparation stage (Nzamwita *et al.*, 2017). Neither beta-carotene nor other forms of carotenoids were detected in bread containing 100% wheat flour and bread product even though the presence of lutein and lutein esters in whole grain wheat flours has been reported by Ziegler et al. (2015) and this was maybe due to the degree of refinement in the flours used in the current study. Our experiment also shows that the total carotenoid and beta-carotene content of composite flour was higher than that of bread product. This might be due to the high heating temperature during bread baking.

#### 4.8. Mineral Composition of Composite Flour and Bread

Mineral composition of raw materials and composite flour were listed and discussed below in table 13 and 14 respectively.

Table 13 Mineral composition of individual crops flour

Treatment	Iron	Zinc	Calcium	Manganese
Sorghum	13.9	3.5	2.17	1.95
Wheat	4.71	41.2	2.14	2.45
OFSP	7.47	1.23	18.7	0.97

Table 14 Mineral composition of composite flour

Sample code	Zinc(mg/100g)	Iron (mg/100g)	Calcium (mg/100g)	Manganese (mg/100g)
T1	18.830 ±9.270 <sup>c</sup>	2.481 ±4.141 <sup>c</sup>	1.803 ±3.208 <sup>c</sup>	2.5993 ±0.752 <sup>a</sup>
T2	24.040 ±9.270 <sup>cd</sup>	11.851 ±4.141 <sup>a</sup>	1.629 ±3.208 <sup>c</sup>	1.6447 ±0.752 <sup>c</sup>
T3	17.337 ±9.270 <sup>ef</sup>	8.418 ±4.141 <sup>b</sup>	1.042 ±3.208 <sup>c</sup>	0.5823 ±0.752 <sup>e</sup>
T4	12.293 ±9.270 <sup>f</sup>	4.757 ±4.141 <sup>c</sup>	7.359 ±3.208 <sup>b</sup>	1.0693 ±0.752 <sup>d</sup>
T5	40.557 ±9.270 <sup>a</sup>	4.524 ±4.141 <sup>c</sup>	2.154 ±3.208 <sup>c</sup>	2.441 ±0.752 <sup>a</sup>
T6	34.714 ±9.270 <sup>b</sup>	3.367 ±4.141 <sup>c</sup>	1.803 ±3.208 <sup>c</sup>	2.599 ±0.752 <sup>a</sup>
T7	27.373 ±9.270 <sup>c</sup>	13.518 ±4.141 <sup>a</sup>	2.296 ±3.208 <sup>c</sup>	2.311 ±0.752 <sup>ab</sup>
T8	21.626 ±9.270 <sup>de</sup>	8.423 ±4.141 <sup>b</sup>	10.026 ±3.208 <sup>a</sup>	1.926 ±0.752 <sup>bc</sup>
<b>Grand mean</b>	24.59	7.1674	3.5140	1.8967
<b>C.V</b>	12.15	24.80	20.73	14.54

Values are expressed as mean ± SE (n=4). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively

Treatment 6 (90% wheat: 0% sorghum: 10% OFSP) has high zinc content next to treatment 5 (100% Wheat) while treatment 4 (80% wheat: 15% sorghum: 5% OFSP) has low value (table 14). Treatment 8 (70% wheat: 30% sorghum: 0% OFSP) has high Iron content while treatment 1 (90% wheat: 7.5% sorghum: 2.5 OFSP) has low value. Iron content of composite flours increased when the level of sorghum flour supplementation was increased. Taha (2000) studied the minerals content of two wheat variety and found that amount of calcium content were 0.51% which nearly correlates with our finding. The calcium and manganese content of composite ranged from 1.042mg/100g to 10.026mg/100g and 0.5823 to 2.599mg/100g respectively.

#### 4.9. Color of Bread

Bread colour is an important quality parameter that influences consumer acceptability. The colour of bread is dependent on flour and chemical reactions like Maillard reaction that occurs during baking (Barros *et al.*, 2018). The lightness (L\*), redness (a\*) and yellowness (b\*) values of the crust and crumb varied significantly ( $p < 0.05$ ) among the various wheat-OFSP composite bread formulations (table 15).

Table 15 Color of sorghum-wheat based bread

Sample code	L*	a*	b*
T1	50.687 $\pm$ 5.491 <sup>ab</sup>	6.570 $\pm$ 1.989 <sup>a</sup>	27.227 $\pm$ 3.090 <sup>ab</sup>
T2	54.337 $\pm$ 5.491 <sup>a</sup>	8.017 $\pm$ 1.989 <sup>a</sup>	30.800 $\pm$ 3.090 <sup>a</sup>
T3	47.413 $\pm$ 5.491 <sup>ab</sup>	8.603 $\pm$ 1.989 <sup>a</sup>	29.277 $\pm$ 3.090 <sup>ab</sup>
T4	54.433 $\pm$ 5.491 <sup>a</sup>	6.720 $\pm$ 1.989 <sup>a</sup>	26.243 $\pm$ 3.090 <sup>bc</sup>
T5	54.743 $\pm$ 5.491 <sup>a</sup>	6.663 $\pm$ 1.989 <sup>a</sup>	27.497 $\pm$ 3.090 <sup>ab</sup>
T6	50.850 $\pm$ 5.491 <sup>ab</sup>	9.243 $\pm$ 1.989 <sup>a</sup>	27.373 $\pm$ 3.090 <sup>ab</sup>
T7	52.903 $\pm$ 5.491 <sup>ab</sup>	6.157 $\pm$ 1.989 <sup>a</sup>	26.597 $\pm$ 3.090 <sup>abc</sup>
T8	44.893 $\pm$ 5.491 <sup>b</sup>	7.500 $\pm$ 1.989 <sup>a</sup>	22.517 $\pm$ 3.090 <sup>c</sup>
Grand Mean	51.283	7.434	27.191
CV	10.03	14.793	9.09

Values are expressed as mean  $\pm$  SE (n=6). Means in the same column with different letters are significantly different based on Duncan's multiple range test ( $p < 0.05$ ). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively

The color (L\*) value of bread ranges from 44.893% (60% wheat: 30% sorghum: 10% OFSP) to 54.743% (100% wheat). Treatment 5 (100%) has the highest (L\*) value of 54.743% while Treatment 8 (60% wheat, 30% sorghum and 10% OFSP) has the lowest color (L\*) value of

44.893%. The average “a\*” and “b\*” value of bread samples were 7.434 and 27.191 respectively. As the ratio of OFSP and sorghum increased, the L\* value were decreased while a\* and b\* were increased. This is maybe linked to the high beta-carotene and other pigments in OFSP flours (Chikpah *et al.*, 2020) and due the high content of tannins and phenolic compounds from sorghum (Vargas-Solorzano *et al.*, 2014).

Nzamwita *et al.*, (2017) reported that the average color (L\*) value of OFSP (10%) and wheat (90%) composite flour bread were 50.7% while “a\*” and “b\*” were 6.3% and 25.4% respectively which was nearly the same with our finding.

#### 4.10. Bread Proximate Composition

Proximate composition of bread products were listed in table 17 and discussed below

Table 16 Proximate composition of wheat-sorghum based bread

TRT	Ash (%)	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Carbohydrate (%)	Total energy (Kcal/100 g)
T1	0.979±0.447 <sup>b</sup>	6.900±1.573 <sup>a</sup>	9.050±1.919 <sup>d</sup>	4.613±1.175 <sup>a</sup>	0.772±0.258 <sup>c</sup>	97.907±2.868 <sup>a</sup>	469.35±15.668 <sup>a</sup>
T2	0.979±0.447 <sup>b</sup>	5.321±1.573 <sup>ab</sup>	12.848±1.919 <sup>ab</sup>	4.502±1.175 <sup>a</sup>	0.934±0.258 <sup>bc</sup>	93.567±2.868 <sup>cd</sup>	466.18±15.668 <sup>a</sup>
T3	0.977±0.447 <sup>b</sup>	5.847±1.573 <sup>ab</sup>	12.863±1.919 <sup>ab</sup>	1.785±1.175 <sup>c</sup>	1.062±0.258 <sup>abc</sup>	90.671±2.868 <sup>e</sup>	430.20±15.668 <sup>c</sup>
T4	1.032±0.447 <sup>b</sup>	3.798±1.573 <sup>b</sup>	13.140±1.919 <sup>ab</sup>	3.308±1.175 <sup>b</sup>	0.893±0.258 <sup>bc</sup>	92.092±2.868 <sup>de</sup>	450.70±15.668 <sup>b</sup>
T5	0.980±0.447 <sup>b</sup>	7.350±1.573 <sup>a</sup>	11.462±1.919 <sup>bc</sup>	4.248±1.175 <sup>ab</sup>	0.935±0.258 <sup>bc</sup>	94.700±2.868 <sup>bc</sup>	462.88±15.668 <sup>ab</sup>
T6	0.982±0.447 <sup>b</sup>	7.023±1.573 <sup>a</sup>	13.854±1.919 <sup>a</sup>	2.107±1.175 <sup>c</sup>	1.198±0.258 <sup>ab</sup>	90.297±2.868 <sup>c</sup>	435.57±15.668 <sup>c</sup>
T7	0.979±0.447 <sup>b</sup>	4.272±1.573 <sup>b</sup>	9.706±1.919 <sup>cd</sup>	4.500±1.175 <sup>a</sup>	0.918±0.258 <sup>bc</sup>	96.691±2.868 <sup>ab</sup>	466.09±15.668 <sup>a</sup>
T8	1.9780±0.447 <sup>a</sup>	4.321±1.573 <sup>b</sup>	13.393±1.919 <sup>a</sup>	3.948±1.175 <sup>ab</sup>	1.365±0.258 <sup>a</sup>	93.731±2.868 <sup>cd</sup>	464.03±15.668 <sup>a</sup>
G m	1.111	5.609	12.040	3.627	1.009	93.707	455.62
cv	31.84	18.80	8.70	15.95	21.70	1.60	1.58

Values are expressed as mean ± SE (n=8). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively.

#### **4.10.1. Ash Content**

Ash is an inorganic residue in any food product, which directly denotes the mineral content. As we have seen from table 19, the ash content of bread product ranged from 0.977% (70% wheat: 22.5% sorghum: 7.5% OFSP) to 1.978% (70% wheat: 30% sorghum: 10% OFSP). As the ratio of sorghum increased (Table 16), the ash content of bread product increased. This shows that sorghum is relatively rich in total mineral contents when compared with wheat. Our finding also showed that at maximum level of OFSP inclusion, the ash content was high when compared with other treatments. Edun *et al.*, (2019) also reported that as the inclusion of OFSP increased, the ash content of OFSP and wheat composite flour bread were increased.

#### **4.10.2. Moisture content**

The moisture content of bread product ranges from 3.798% (80% wheat: 15% sorghum: 5% OFSP) to 7.35% (100% wheat). The lowest moisture content of bread product is an indication of low water activity which shows the relatively higher keeping quality.

#### **4.10.3. Protein content**

Proteins are very important macro-nutrients for structural, functional performers of different biomolecules in human body, and they provide the essential amino acids required for metabolism. The crude protein content of composite bread decreased gradually ranging from 13.854% (90% wheat and 10% OFSP) to 9.05 % (90% wheat: 7.5% sorghum: 2.5% OFSP). Xu *et al.*, (2020) reported a similar trend with a decrease from 100% wheat bread protein content of 11.55% to 11.26% for the 25% sorghum-wheat bread. This is maybe due to the relatively higher content of crude protein in wheat grain when compared to sorghum and OFSP.

#### **4.10.4. Crude Fiber content**

Crude fiber is one of the non-digestible carbohydrates, which provides the fecal bulkiness, less intestinal transit, role in cholesterol level reduction, and trapping dangerous substance like cancer-causing agents, and also encourages the growth of natural microbial flora in gut (Slavin, 2013). The fiber content of wheat-sorghum-OFSP composite flour bread was increased as the addition of OFSP (10%) and sorghum flour (30%) increased and that of minimum level wheat (60%). Edun *et al.*, (2019) also reported that inclusion of OFSP and sorghum increases the fiber

content of bread. This is due to the higher fiber content of OFSP and sorghum when compared to wheat.

#### **4.10.5. Fat content**

The fat concentrations directly influence the energy density of the food product. They are important in the body as energy suppliers and essential components of cell membranes. The fat content of bread product ranged from 1.785% to 4.613% (table 16). As the ratio of sorghum flour decreased and addition of wheat flour increased, the fat content of bread increased. This is due to the low fat content of sorghum grain and high fat content of wheat grains. Mtelisi *et al.*, 2020 reported that the fat content of sorghum-wheat composite flour ranges from 1.25% to 2.36% which has no big difference with our finding.

#### **4.10.6. Total Carbohydrate**

The carbohydrates content of sorghum-wheat composite flours shown in Table 16 ranged from 90%–97%. The bread sample of 90% wheat: 7.5% sorghum: 2.5% OFSP and 70% wheat: 30% sorghum: 0% OFSP has high content of carbohydrate value (97.907%) and (96.697%) respectively while sample of 90% wheat: 0% sorghum: 90% OFSP has low value of total carbohydrate (90.297%). Our finding showed that the inclusion of sorghum in bread increased the carbohydrate content of bread samples.

#### **4.10.7. Available energy**

The total energy content of sorghum-wheat composite flours shown in (Table 16) ranged from 430 kcal to 469kcal. The highest total energy value of bread product might be due to high content of protein and carbohydrate content of bread samples (Mugalavai *et al.*, 2020).

### **4.11. Sensory Characteristics of Bread**

Sensory evaluation is important in the development of new foods, the improvement of existing products and quality control in the food industry and research and development. Even though the preference rates differs among samples, the results for sensory evaluation (Table 18) reveals that all formulated bread samples were acceptable by panelists.

Table 17 Sensory characteristics of sorghum-wheat based bread

<b>TRT</b>	<b>Color</b>	<b>Texture</b>	<b>Aroma</b>	<b>Taste</b>	<b>Appearance</b>	<b>Overall acceptance</b>
T1	5.950 ±0.8632 <sup>cd</sup>	5.8500 ±0.699 <sup>d</sup>	6.150 ±0.5769 <sup>b</sup>	5.850 ±0.886 <sup>ab</sup>	5.800 ±0.85 <sup>cd</sup>	6.000 ±0.748 <sup>cd</sup>
T2	7.050 ±0.8632 <sup>ab</sup>	6.700 ±0.699 <sup>bc</sup>	6.450 ±0.5769 <sup>b</sup>	6.800 ±0.886 <sup>ab</sup>	6.800 ±0.85 <sup>ab</sup>	6.850 ±0.748 <sup>ab</sup>
T3	6.100 ±0.8632 <sup>cd</sup>	6.250 ±0.699 <sup>cd</sup>	6.600 ±0.5769 <sup>ab</sup>	6.000 ±0.886 <sup>ab</sup>	6.250 ±0.85 <sup>abcd</sup>	6.200 ±0.748 <sup>bcd</sup>
T4	6.750 ±0.8632 <sup>bc</sup>	6.700 ±0.699 <sup>bc</sup>	6.450 ±0.5769 <sup>b</sup>	6.400 ±0.886 <sup>ab</sup>	6.500 ±0.85 <sup>abc</sup>	6.750 ±0.748 <sup>abc</sup>
T5	7.300±0.8632 <sup>ab</sup>	7.150 ±0.699 <sup>ab</sup>	6.550 ±0.5769 <sup>ab</sup>	6.400 ±0.886 <sup>ab</sup>	7.100 ±0.85 <sup>a</sup>	7.050 ±0.748 <sup>a</sup>
T6	7.850 ±0.8632 <sup>a</sup>	7.600 ±0.699 <sup>a</sup>	7.250 ±0.5769 <sup>a</sup>	7.000 ±0.886 <sup>a</sup>	7.100 ±0.85 <sup>a</sup>	7.400 ±0.748 <sup>a</sup>
T7	5.650 ±0.8632 <sup>d</sup>	6.050 ±0.699 <sup>d</sup>	6.500 ±0.5769 <sup>ab</sup>	5.650 ±0.886 <sup>b</sup>	5.300 ±0.85 <sup>d</sup>	5.700 ±0.748 <sup>d</sup>
T8	6.500 ±0.8632 <sup>bc</sup>	5.950 ±0.699 <sup>d</sup>	6.000 ±0.5769 <sup>b</sup>	5.900 ±0.886 <sup>ab</sup>	6.000 ±0.85 <sup>bcd</sup>	5.9000±0.748 <sup>d</sup>
Grand Mean	6.644	6.531	6.494	6.250	6.356	6.481
CV	8.53	6.55	8.02	13.80	10.57	8.22

Values are expressed as mean ± SE (n=6). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000) and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively.

Color of bread crust is an important sensory characteristic, which can enhance acceptability (Udofia *et al.*, 2013). All bread samples were liked by panelists in terms of their color characteristics.

Taste is an important sensory attribute of any food. As cited by Udofia *et al.*, (2013), bread consumption is often enhanced by taste (Sim and Tam, 2001). Treatment 6 (90% wheat: 0% sorghum: 10% OFSP) has high score (7.850) of color, taste, aroma, texture, appearance and overall appearance while treatment 1 (90% wheat: 7.5% sorghum: 2.5% OFSP) and 7 (70% wheat: 30% sorghum: 0% OFSP) has low values. As the ratio of addition of OFSP flour increased, the color, taste, aroma, texture, appearance and overall acceptability of bread products were increased while inclusion of sorghum decreased sensory acceptance of bread (Table 18). Bread developed from maximum level of OFSP inclusion and minimum level of sorghum flour (90% wheat: 0% sorghum: 10% OFSP) has gotten highest score of all sensory attributes by panelists when compared with other composite flour.

Ombaka, J.O, (2018) reported that, as the ratio of inclusion of OFSP increases, the color, taste and overall acceptance of composite bread increases. This is maybe due to it's the natural sweetness and high level of pigmented beta-carotene content.

Bread developed from maximum level of OFSP inclusion has gotten highest score of taste and color by panelists when compared with other composite flour. This is due to the natural sweetness of OFSP which enhances the sweetness of food products. This experiment also confirms the hypothesis which states the inclusion of OFSP flour in baked product increases the taste and color of the food products. Edun *et al.*, (2019) also stated that Orange Flashed Sweet Potato can add natural sweetness, color and flavor to bakery products. This confirms the hypothesis which states that inclusion of OFSP flour in baked product increases the sensory attributes of food products. Samples with maximum level of wheat and sorghum had low degree of liking when compared to other bread samples. Keregero & Mtebe, (1994) also reported that in the case of composite flour bread, preference for the product improved as the amount of sorghum flour decreased.

#### 4.12. Characterization of Bread Physical Characteristics

The bread-making quality parameters measured for the pup loaf straight-dough procedures baking were color, loaf volume, loaf weight, and specific loaf volume. For the improvements in physical and sensory properties and shelf life of bread product, quality is very important. The quality of bread is normally defined based on its volume, color, texture, and flavor of bread (Quilez *et al.*, 2006).

Table 18 Loaf volume, loaf weight and specific volume of sorghum-wheat based bread

Sample code	Loaf volume (cm <sup>3</sup> /100g)	Loaf weight (g)	Specific volume (cm <sup>3</sup> /100g)
T1	225.94 ±49.094 <sup>a</sup>	87.077 ±6.4796 <sup>a</sup>	2.5970 ±0.639 <sup>ab</sup>
T2	223.67 ±49.094 <sup>a</sup>	76.044 ±6.4796 <sup>b</sup>	2.943 ±0.639 <sup>ab</sup>
T3	232.54 ±49.094 <sup>a</sup>	67.854 ±6.4796 <sup>c</sup>	3.429 ±0.639 <sup>a</sup>
T4	232.51 ±49.094 <sup>a</sup>	73.948 ±6.4796 <sup>b</sup>	3.096 ±0.639 <sup>a</sup>
T5	290.97 ±49.094 <sup>a</sup>	84.728 ±6.4796 <sup>a</sup>	3.434 ±0.639 <sup>a</sup>
T6	232.45 ±49.094 <sup>a</sup>	75.104 ±6.4796 <sup>b</sup>	3.160 ±0.639 <sup>a</sup>
T7	224.79 ±49.094 <sup>a</sup>	74.944 ±6.4796 <sup>b</sup>	3.011 ±0.639 <sup>ab</sup>
T8	152.14 ±49.094 <sup>b</sup>	73.792 ±6.4796 <sup>b</sup>	2.063 ±0.639 <sup>b</sup>
Grand Mean	226.88	76.687	2.967
CV	17.78	3.97	18.97

Values are expressed as mean ± SE (n=4). Means in the same column with different letters are significantly different based on Duncan's multiple range test (p < 0.05). Where T1 (0.900:0.075:0.025), T2 (0.75:0.225:0.025), T3 (0.700:0.225:0.075), T4 (0.800:0.150:0.050), T5 (1.000:0.000:0.000), T6 (0.900:0.000:0.100), T7 (0.700:0.300:0.000), and T8 (0.600:0.300:0.100) of wheat, sorghum and OFSP respectively

#### **4.12.1. Loaf volume**

Loaf volume is used as criteria to measure the quality of fresh bread in research quality control in industry and by consumers. The bread loaf volume of our experiment ranged from 152.14cm<sup>3</sup>/100g to 290.97cm<sup>3</sup>/100g (Table 18). The samples were not significantly different from each other except sample 60% wheat: 30% sorghum: 10% OFSP. Bread developed from 100% wheat has high bread volume while sample 60% wheat: 30% sorghum: 10% OFSP has low bread volume. As we have observed from our result, as the ratio of wheat decreased, the bread volume decreased. Even though there was a decrease in bread volume as the ratio of sorghum and OFSP increased, acceptable bread was developed. This might be due to high gluten content of wheat grain when compared to sorghum and OFSP. Our finding is in agreement with the Hugo *et al.*, (2003) who concluded that the substitution of wheat flour with up to 40% sorghum flour decreased the bread volume and Mahendran & Hariharan, (2018) who reported that the addition of sorghum to wheat flour negatively influence the loaf volume.

#### **4.12.2. Loaf weight**

Bread loaf weight was ranged from 67.854g to 84.728g (Table 18). There were no significance difference between samples except sample of (100%) wheat and sample of (70% wheat: 22.5% sorghum: 7.5% OFSP). Sample of 100% wheat has high loaf weight while sample of (70% wheat: 22.5% sorghum: 7.5% OFSP) has low loaf weight when compared to other samples.

#### **4.12.3. Specific Loaf volume**

Specific volume is an indication of the gluten content of the bread but, other constituents such as starch and fiber also contribute to the specific volume of bread. It ranged from 2.06cm<sup>3</sup>/g to 3.434cm<sup>3</sup>/g in our research finding. The highest and lowest level of specific volume was observed in sample of (100% wheat) and sample of (60% wheat: 30% sorghum: 10% OFSP) respectively. Aprodu, (2020) also reported in their finding that, the composite flour of 70% wheat and 30% sorghum had the specific loaf volume of 2.87cm<sup>3</sup>/g which is in range with our finding. This was due to the highest and lowest ratio of loaf volume and loaf weight of samples respectively. Edun *et al.*, (2018) also reported that a decrease in bread specific volume when wheat flour was replaced with 10-30% OFSP flour.

#### 4.13.Optimization of Formulations and Point Predictions

Formulations and predictions point for optimization of crops were listed in table 10 below.

Figure 10 design summary of formulation and results

Run	A: wheat	B: sorghum	C: OFSP	Protein	Beta-carotene	Fiber	Loaf Volume	Color	Taste	Gluten	Iron	Zinc	Overall acceptability	Calcium
1	0.9	0.075	0.025	10.376	7.362	1.045	225.94	5.95	5.85	28.65	2.481	18.830	2.599	1.803
2	0.75	0.225	0.025	12.607	6.228	0.88	223.67	7.05	6.8	35.3	11.851	24.040	1.645	1.629
3	0.7	0.225	0.075	12.712	13.166	1.34	232.54	6.1	6	28.6	8.418	17.337	0.582	1.042
4	0.8	0.15	0.05	16.329	8.401	1.61	232.51	6.75	6.4	33.463	4.757	12.293	1.069	7.359
5	1	0	0	16.436	0.00	0.58	290.97	7.3	6.4	37.549	4.524	40.557	2.441	2.154
6	0.9	0	0.1	12.064	21.3	1.145	232.45	7.85	7	37.05	3.367	34.714	2.599	1.803
7	0.7	0.3	0	9.67	0.14	1.01	224.79	5.65	5.65	26.45	13.518	27.373	2.311	2.296
8	0.6	0.3	0.1	11.723	23.217	0.92	152.14	6.5	5.9	22.25	8.423	21.626	1.926	10.026

### 4.13.1. Analysis

To find the optimum set of formulations and to describe the behavior of the system, a linear model were used for describing each response

Response = A+B+C, where; A- wheat, B- sorghum and C- OFSP

#### 4.13.1.1. Protein

Table 19 Model summary statistics of Protein

Source	Std Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	2.66	0.1801	-0.1479	-1.3097	99.47

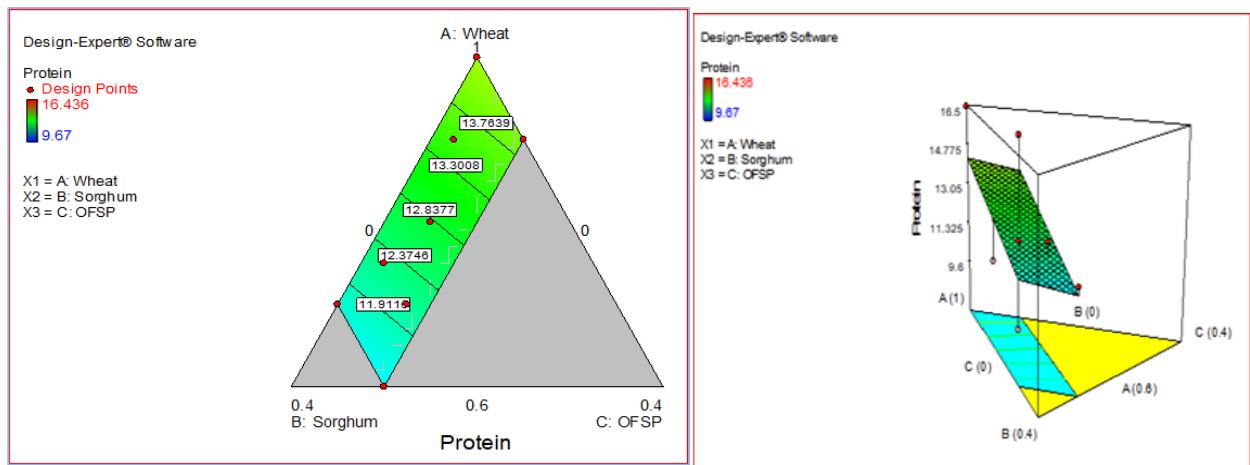
#### Equation for protein

Final Equation in Terms of Actual Components:

$$\text{Protein} = +14.22693 * \text{Wheat} + 5.80402 * \text{Sorghum} + 11.13566 * \text{OFSP}$$

Final Equation in Terms of L\_Pseudo Components:

$$\text{Protein} = +14.23 * A + 10.86 * B + 12.99 * C$$



a) Contour plot representation of protein      b) 3D representation of protein

Figure 11 Graphical presentation of protein optimization

#### 4.13.1.2. Fiber

Table 20 Model summary statistics of fiber

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.33	0.176	-0.153	-1.105	1.42

A negative "Pred R-Squared" implies that the overall mean is a better predictor of response than the current model.

"Adeq Precision" measures the signal to noise ratio. A ratio of 1.91 indicates an inadequate signal and we should not use this model to navigate the design space.

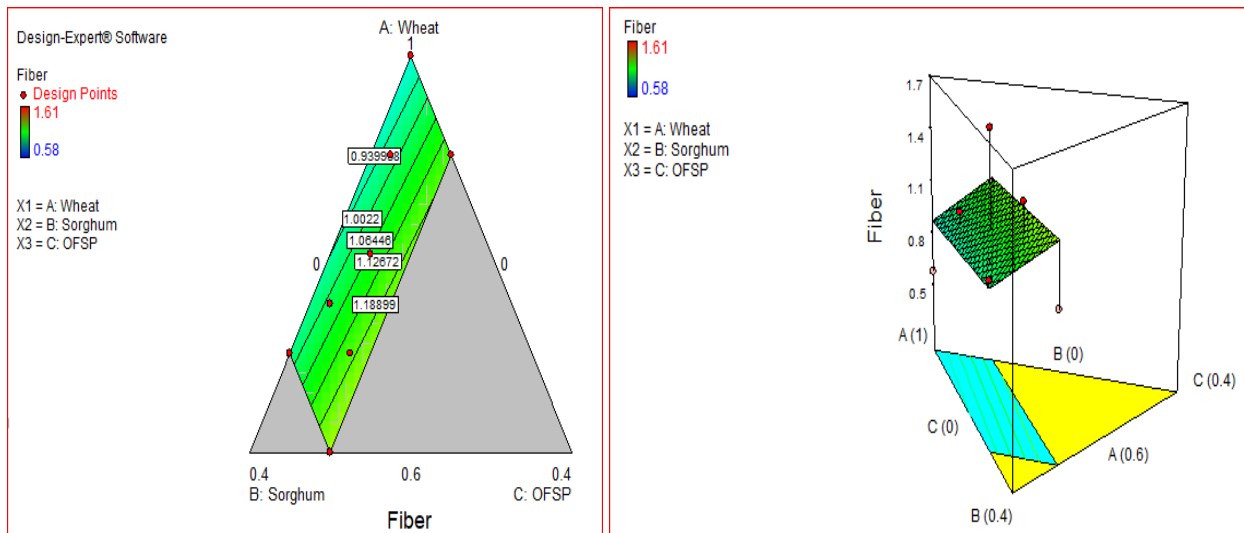
### Equation for Fiber

Final Equation in Terms of L\_Pseudo Components:

$$\text{Fiber} = +0.88 * A + 1.00 * B + 2.07 * C$$

Final Equation in Terms of Real Components:

$$\text{Fiber} = +0.87768 * \text{Wheat} + 1.18495 * \text{Sorghum} + 3.85585 * \text{OFSP}$$



a) Contour plot representation of fiber

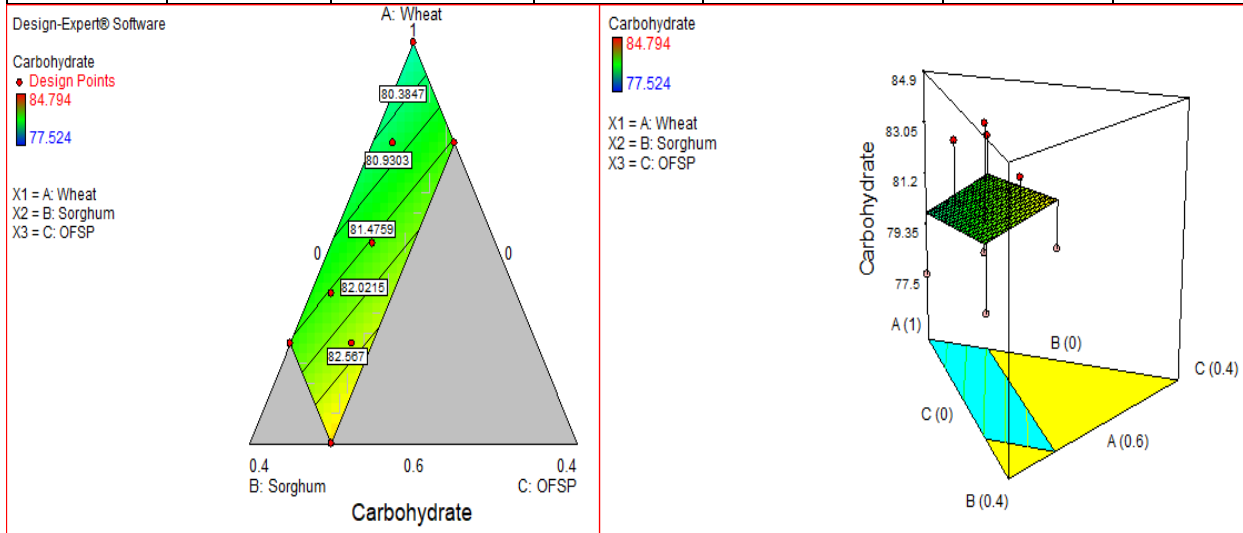
b) 3D representation of fiber

Figure 12 Graphical representation of fiber optimization

### 4.13.1.3. Carbohydrate

Table 21 model summary statistics of carbohydrate

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	2.85	0.15	-0.19	-0.977	94.43	suggested



a) Contour plot representation of carbohydrate

b) 3D representation of carbohydrate

Figure 13 Graphical representation of carbohydrate optimization

### 4.13.1.4. Color

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	predicted R-Squared	PRESS
Linear	0.61	0.5218	0.3305	0.0259	3.75

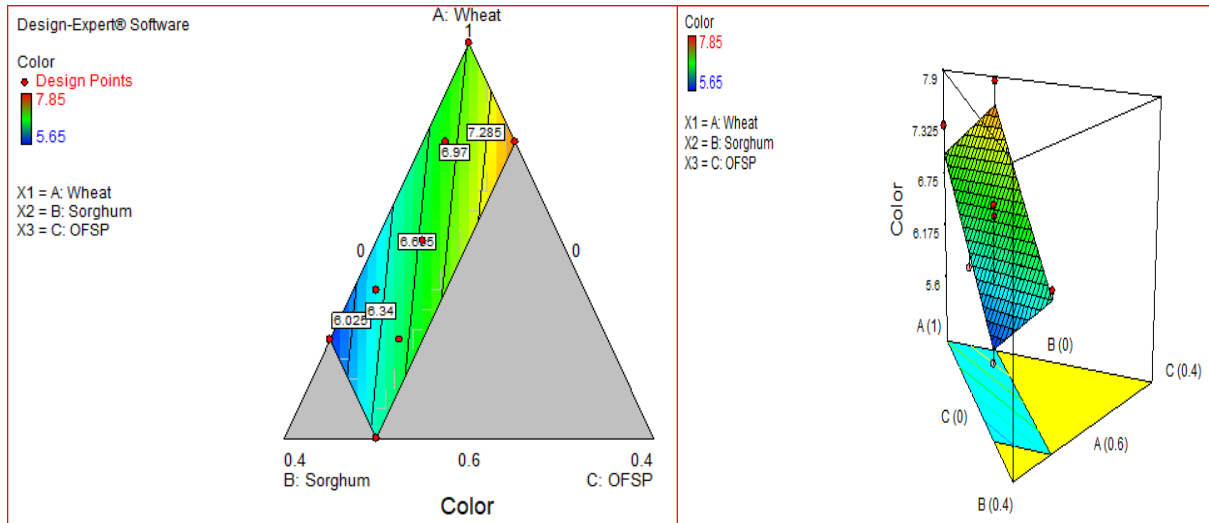
Equation of color

Final Equation in Terms of L\_Pseudo Components:

$$\text{Color} = +6.99 * A + 5.40 * B + 9.43 * C$$

Final Equation in Terms of Real Components:

$$\text{Color} = +6.99000 * \text{Wheat} + 3.02333 * \text{Sorghum} + 13.09000 * \text{OFSP}$$



a) Contour plot representation of color

b) 3D representation of color

Figure 14 Graphical representation of color optimization

#### 4.13.1.5. Taste

##### Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.45	0.3672	0.1141	-0.3461	2.17

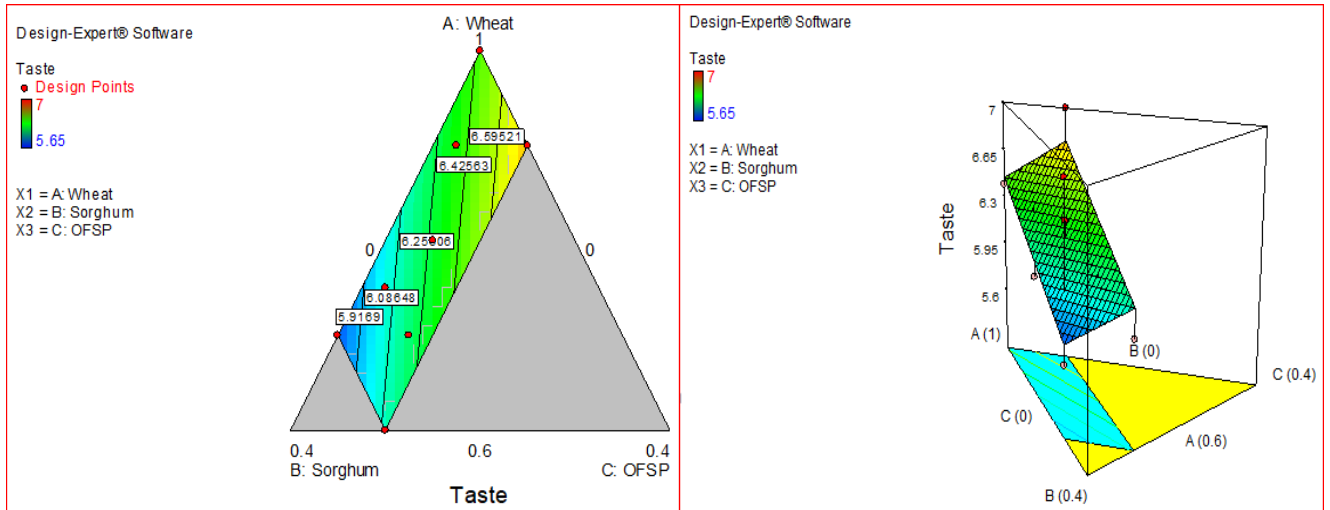
Equation of Taste

Final Equation in Terms of L\_Pseudo Components:

$$\text{Taste} = +6.45 * A + 5.58 * B + 7.71 * C$$

Final Equation in Terms of Real Components:

$$\text{Taste} = +6.45028 * \text{Wheat} + 4.26859 * \text{Sorghum} + 9.59535 * \text{OFSP}$$



a) Contour representation of Taste

b) 3D representation of Taste

Figure 15 Graphical representation of taste optimization

#### 4.13.1.6. Overall acceptability

Model summary statistics

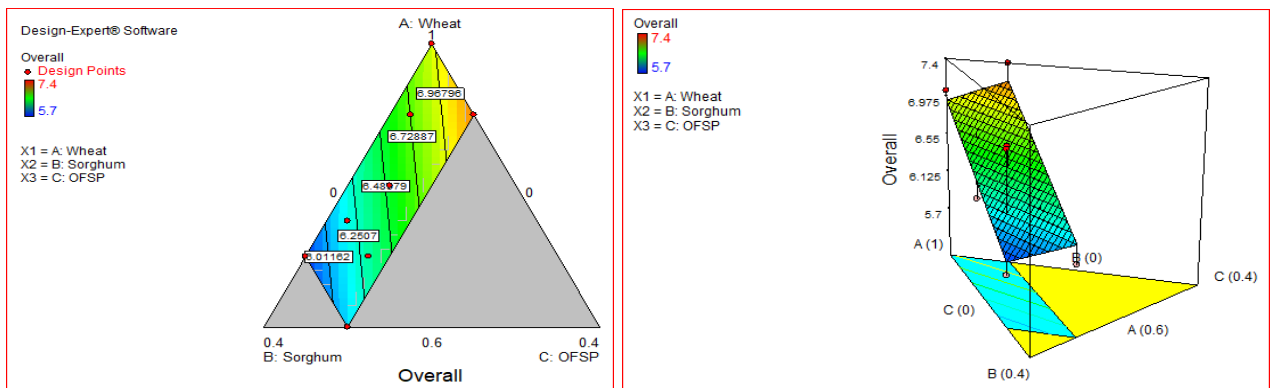
Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.48	0.5603	0.3845	0.1247	2.31

Equations for overall acceptability

Final Equation in Terms of L\_Pseudo Components: Overall = +6.94 \* A+ 5.47\* B +8.00\* C

Final Equation in Terms of Real Components:

Over all = +6.94394 \* Wheat +3.26695\* Sorghum +9.57493\* OFSP



a) Contour representation of overall acceptability b) 3D representation of overall acceptability

Figure 16 Graphical representation of overall acceptability

### 4.13.1.7. Bread loaf volume

#### Model Summary Statistics

Source	Std. Dev.	Adjusted R-Squared	Predicted R-Squared	R-Squared	PRESS
Linear	21.99	0.7534	0.6547	0.4375	5514.87

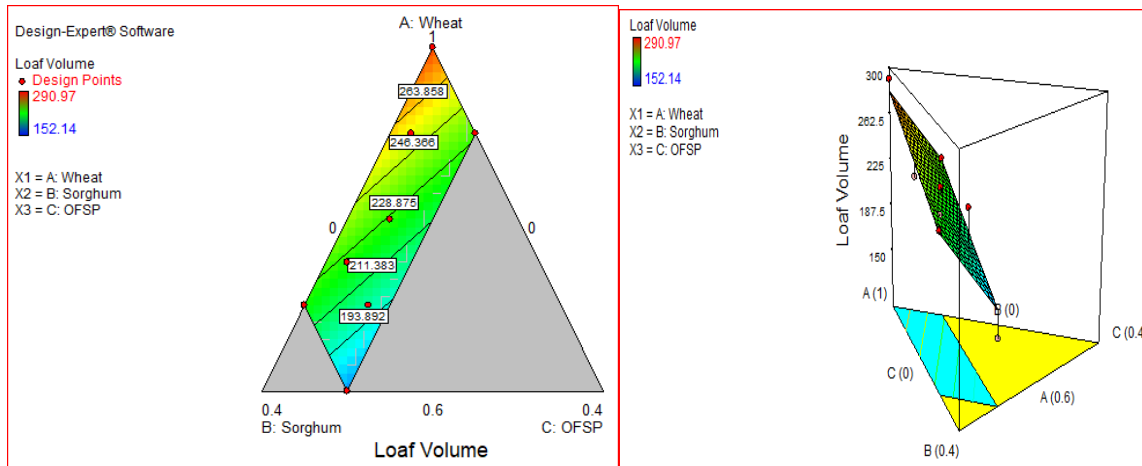
Equation for loaf volume

Final Equation in Terms of L\_Pseudo Components:

$$\text{Loaf Volume} = +281.35 * A + 203.50 * B + 81.20 * C$$

Final Equation in Terms of Real Components:

$$\text{Loaf Volume} = +281.34918 * \text{Wheat} + 86.72608 * \text{Sorghum} - 219.02152 * \text{OFSP}$$



a) Contour representation of loaf volume

b) 3D representation of loaf volume

Figure 17 Graphical representation of bread loaf volume optimization

### 4.13.1.8. Bread loaf weight optimization

#### Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Square	Predicted R-Squared	PRESS
Linear	4.55	0.6202	0.4683	-0.0078	274.58

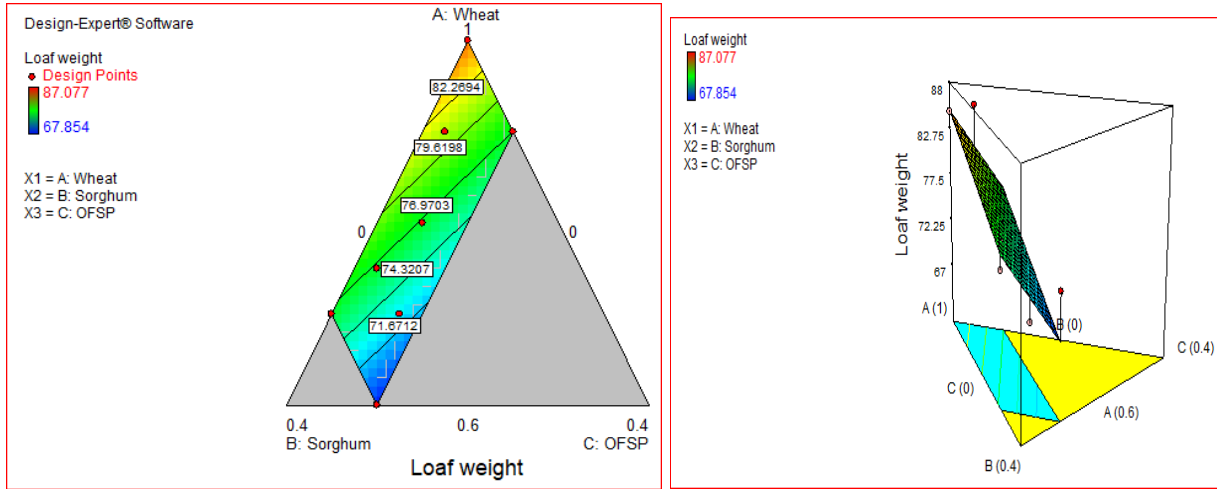
Equation for loaf weight

Final Equation in Terms of L\_Pseudo Components:

$$\text{Loaf weight} = +84.92 * A + 73.67 * B + 52.91 * C$$

Final Equation in Terms of Real Components:

$$\text{Loaf weight} = +84.91893 * \text{Wheat} + 56.79935 * \text{Sorghum} + 4.89766 * \text{OFSP}$$



a) Contour plot representation of bread loaf weight

b) 3D representation of bread loaf weight

Figure 18 Graphical representation of bread loaf weight optimization

#### 4.13.1.9. Beta-carotene optimization

Model Summary Statistics

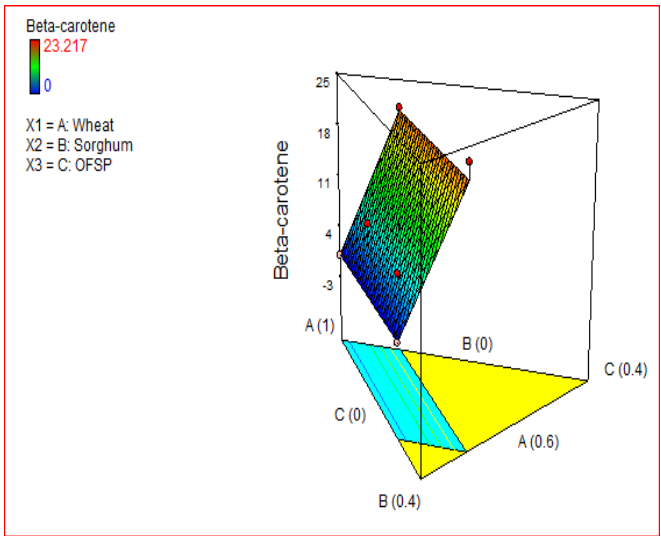
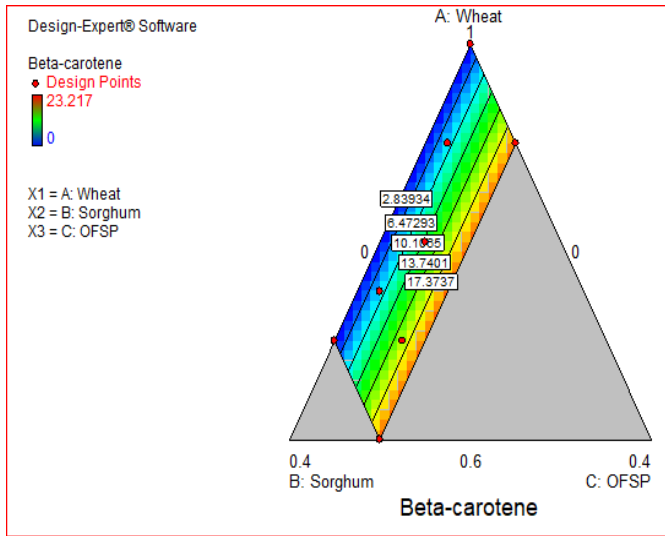
Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	4.55	0.6202	0.4683	-0.0078	274.58

Equation for beta-carotene optimization

$$\text{Final Equation in Terms of L_Pseudo Components: Beta-carotene} = +0.18 * A + 0.27 * B + 83.50 * C$$

Final Equation in Terms of Real Components:

$$\text{Beta-carotene} = +0.17801 * \text{Wheat} + 0.39771 * \text{Sorghum} + 208.47091 * \text{OFSP}$$



a) Contour plot representation of beta-carotene

b) 3D representation of beta-carotene

Figure 19 Graphical representation of beta-carotene

#### 4.13.1.10. Gluten

##### Model Summary Statistics

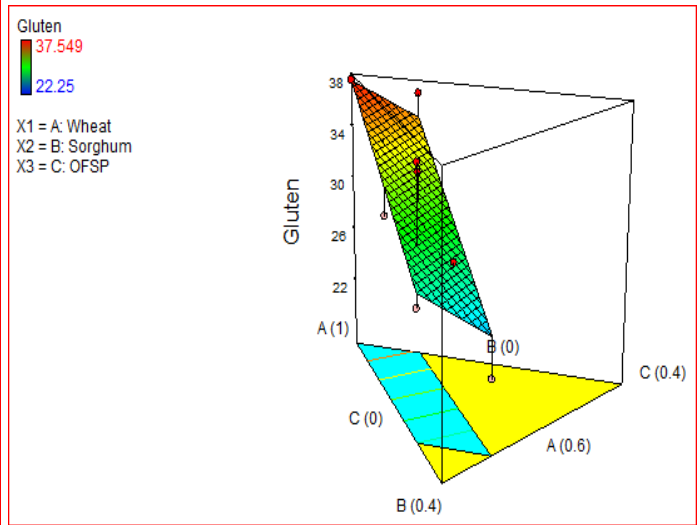
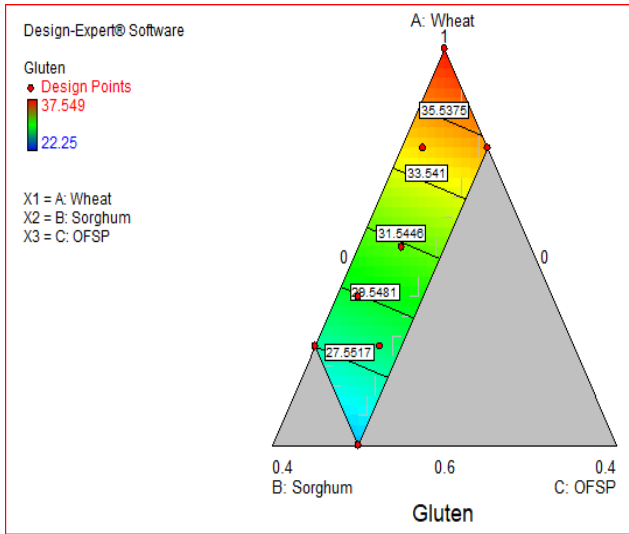
Source	Std. Dev.	Adjusted R-Squared	Predicted R-Squared	R-Squared	PRESS
Linear	4.14	0.5968	0.4355	0.1190	187.11

Equation for Gluten

Final Equation in Terms of L\_Pseudo Components:  $\text{Gluten} = +37.53 * A + 24.23 * B + 28.40 * C$

Final Equation in Terms of Real Components:

$\text{Gluten} = +37.53394 * \text{Wheat} + 4.28391 * \text{Sorghum} + 14.69202 * \text{OFSP}$



a) Contour plot representation of gluten optimization

b) 3D representation of gluten optimization

Figure 20 Graphical representation of Gluten optimization

#### 4.13.1.11. Iron

##### Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	2.25	0.7784	0.6897	0.3415	75.47

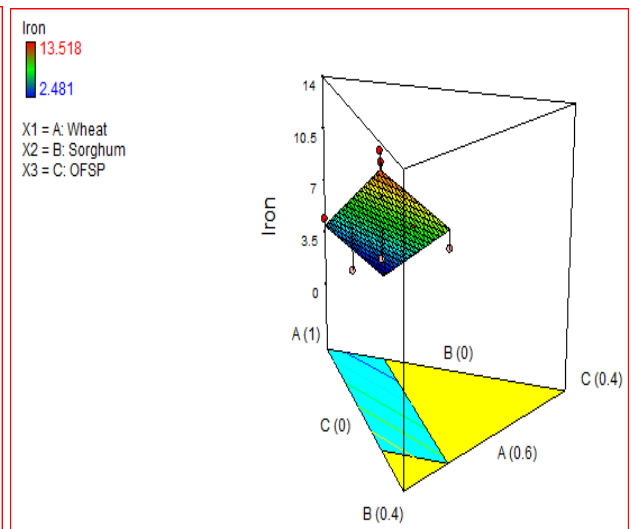
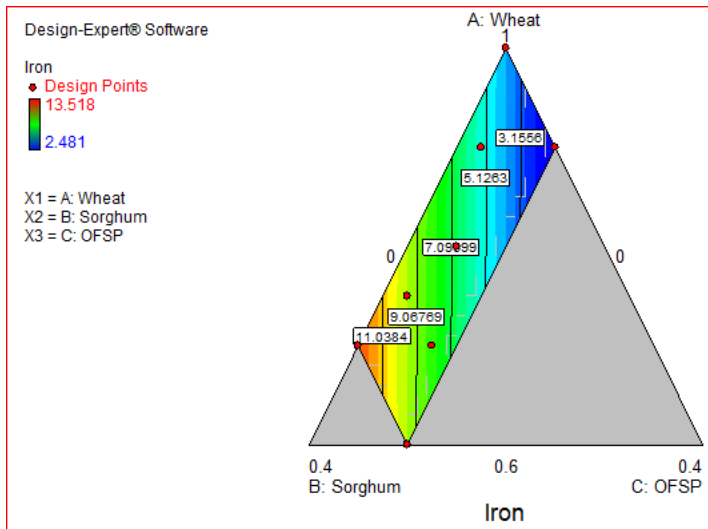
#### Equation of Iron

Final Equation in Terms of L\_Pseudo Components:

$$\text{Iron} = +4.07 * A + 15.24 * B - 7.47 * C$$

Final Equation in Terms of Real Components:

$$\text{Iron} = +4.06846 * \text{Wheat} + 31.99368 * \text{Sorghum} - 24.76717 * \text{OFSP}$$



a) Contour plot representation of iron

b) 3D representation of iron

Figure 21 Graphical representation of Iron optimization

#### 4.13.1.12. Zinc

##### Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	9.28	0.2962	0.0146	-1.0223	1236.17

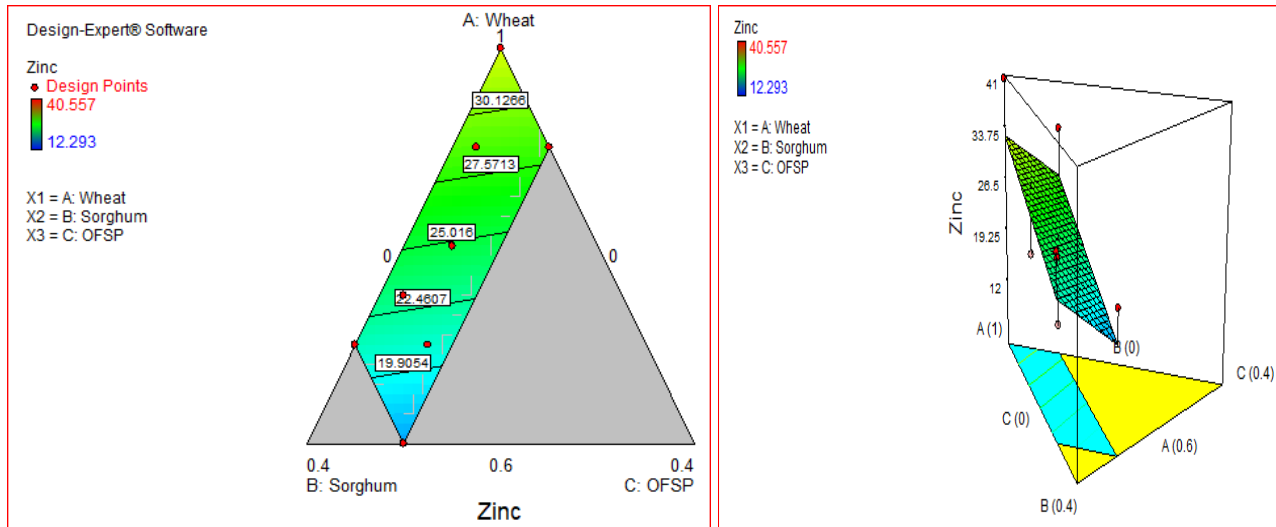
##### Equation for Zinc

Final Equation in Terms of L\_Pseudo Components:

$$\text{Zinc} = +32.68 * A + 17.61 * B + 14.93 * C$$

Final Equation in Terms of Real Components:

$$\text{Zinc} = +32.68187 * \text{Wheat} - 5.00137 * \text{Sorghum} - 11.68841 * \text{OFSP}$$



a) Contour plot representation of zinc

b) 3D representation of zinc

Figure 22 Graphical representation of zinc optimization

#### 4.13.1.13. Calcium

##### Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	3.27	0.2964	0.0150	-0.9713	149.

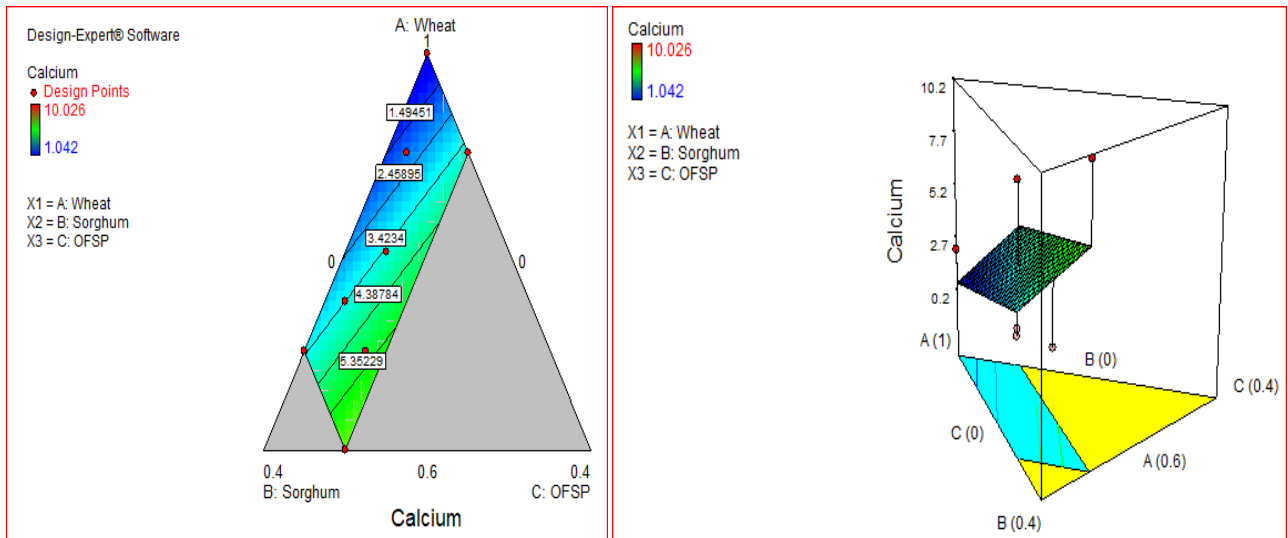
##### Equation for Calcium

Final Equation in Terms of L\_Pseudo Components:

$$\text{Calcium} = +0.53 * A + 4.26 * B + 13.33 * C$$

Final Equation in Terms of Real Components:

$$\text{Calcium} = +0.53006 * \text{Wheat} + 9.84342 * \text{Sorghum} + 32.52198 * \text{OFSP}$$



a) Contour plot representation of calcium

b) 3D representation of calcium

Figure 23 Graphical representation of calcium optimization

### 4.13.2. Optimization

There are three choices for optimizations. These are numerical, graphical and point optimization which discussed below

#### 4.13.2.1. Numerical Optimization

##### 4.13.2.1.1. Proximate (Protein, Fiber & carbohydrate) Numerical Optimization

Solutions

Wheat	Sorghum	OFSP	Protein	Fiber	Carbohydrate
0.783	0.117	0.100	12.934	1.211	82.192

##### 4.13.2.1.2. Mineral (Iron, Zinc and Calcium) Optimization

Solutions

Wheat	Sorghum	OFSP	Iron	Zinc	Calcium
0.700	0.225	0.075	8.189	20.875	4.516

#### 4.13.2.1.3. Sensory (Color, Taste and Overall acceptability) Optimization Solutions

Wheat	Sorghum	OFSP	Color	Taste	Overall acceptability
0.806	0.094	0.100	7.228	6.56	6.86

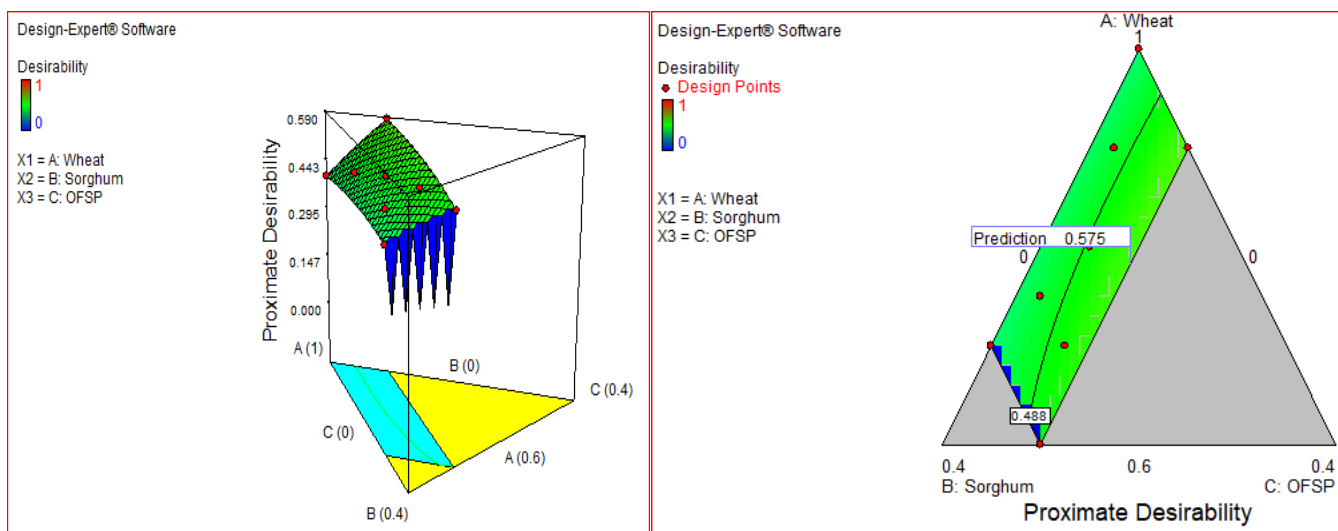
#### 4.13.2.1.4. Common (Main Parameters) Optimization Solutions

Wheat	Sorghum	OFSP	Protein	Carbohydrate	Beta-carotene	Loaf volume	Overall	Gluten	Color	Taste	Iron	Zinc
0.754	0.146	0.100	12.69	82.36	21.04	202.90	6.67	30.40	7.02	6.45	5.26	22.74

#### 4.13.2.2. Graphical Optimization

##### 4.13.2.2.1. Proximate composition optimization

Sample of 78.3% wheat: 11.7% sorghum: 10% OFSP was selected as optimum value for proximate value optimization



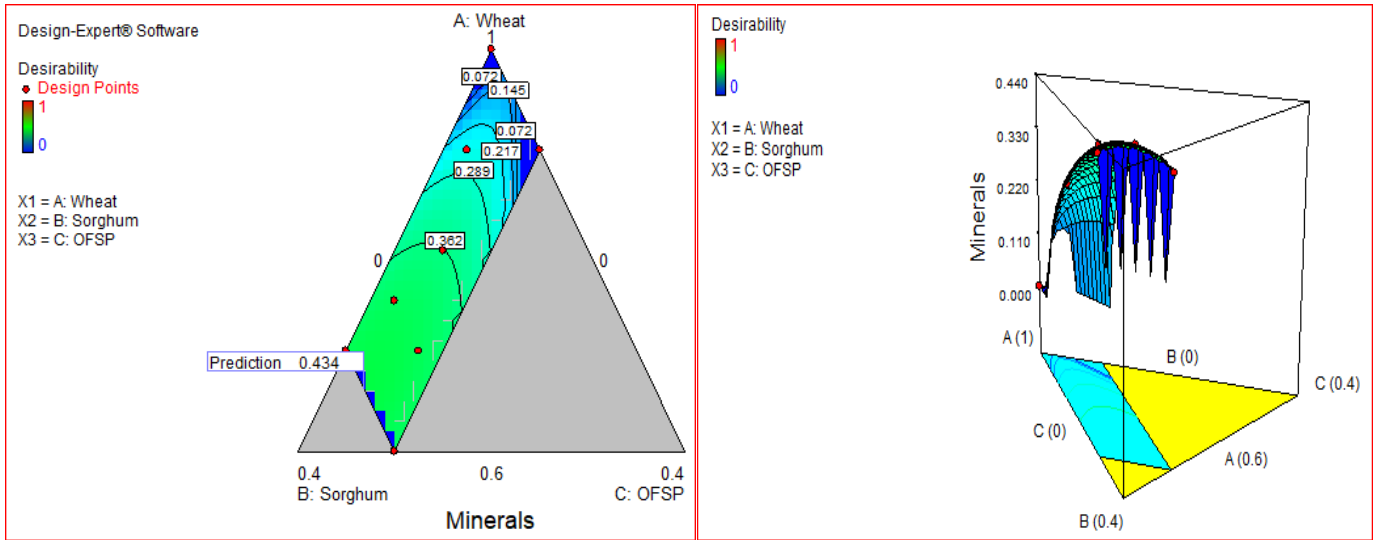
A) 3D representation of proximate

B) Contour plot representation of proximate

Figure 24 Graphical representation of proximate optimization

#### 4.13.2.2.2. Mineral composition optimization

Sample of 70% wheat: 22.5% sorghum: 7.5% OFSP was selected for the purpose of mineral optimization



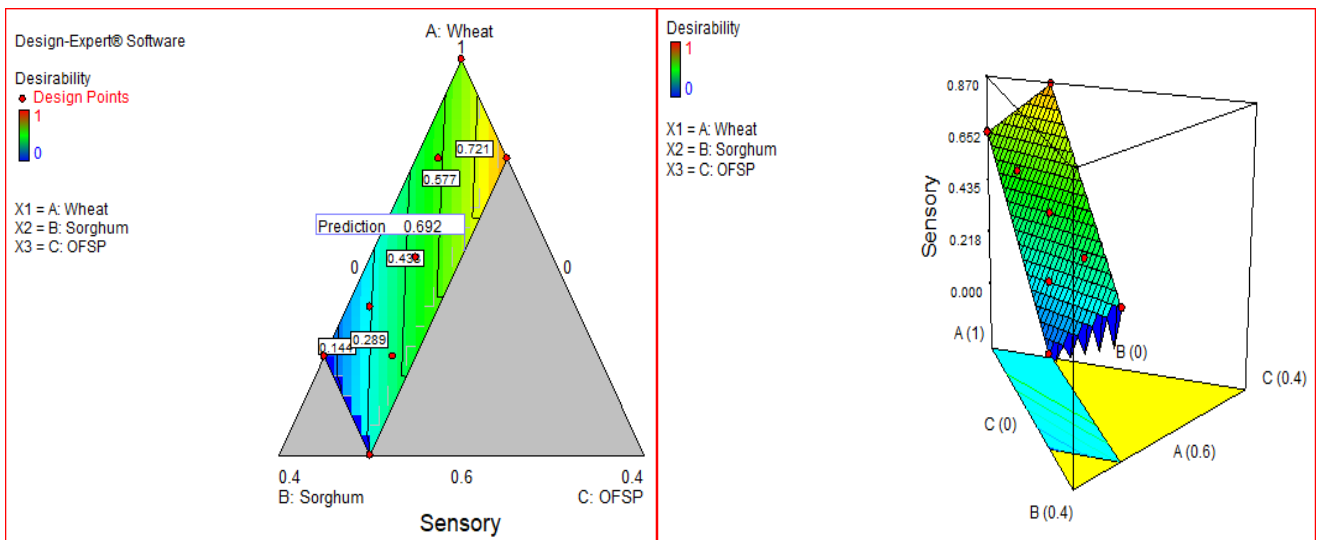
A) Contour plot representation of minerals

B) 3D representation of minerals

Figure 25 Graphical representation of minerals optimization

#### 4.13.2.2.3. Sensory Optimization

Sample of 80.6% wheat: 9.4% sorghum: 10% OFSP was selected as optimum value for sensory characteristics



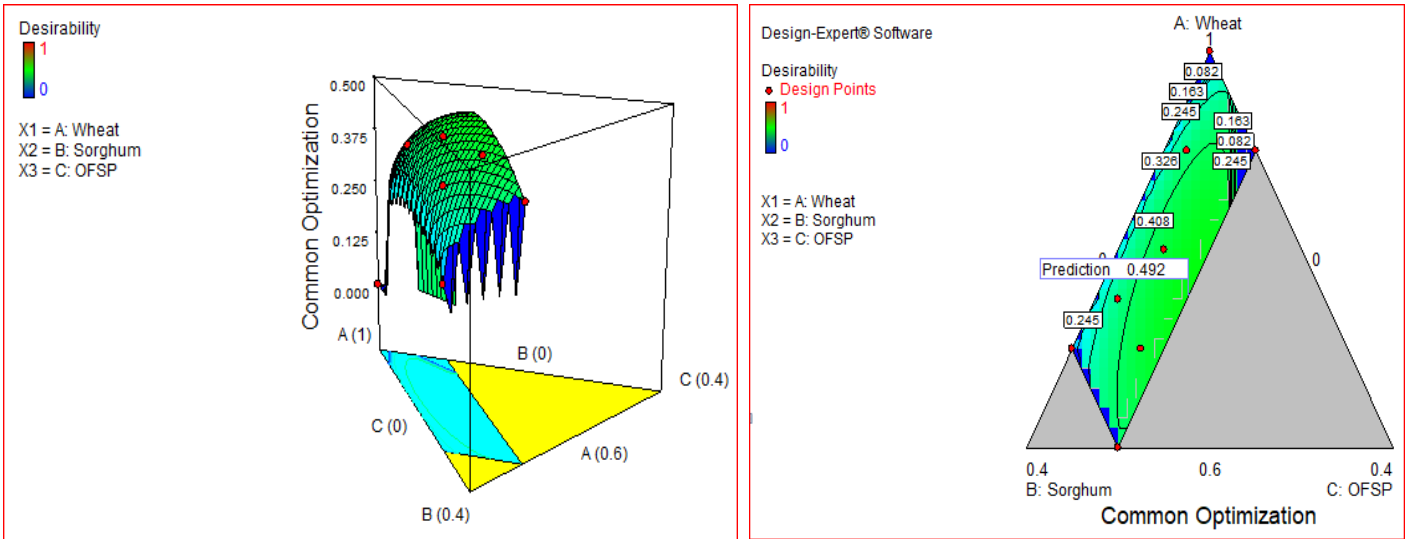
A) Contour plot representation of sensory

B) 3D representation of sensory

Figure 26 Graphical representation of sensory optimization

#### 4.13.2.2.4. Common Optimum

Sample of 75.4% wheat: 14.6% sorghum: 10% OFSP was selected for common optimum purpose



A) 3D representation of common optimum    B) Contour plot representation of common optimum

Figure 27 Graphical representation of common optimum

#### 4.13.2.3. Point Prediction

Point prediction is one mode of expression for optimization point. Based on our result the optimized point for optimum value of our product was wheat (75%), sorghum (15%) and OFSP (10%).

Table 22 Point prediction of common optimum

Component	Name	Level	Low Level	High Level	Std. Dev.	Coding	
A	Wheat	0.75	0.60	1.00	0.000	Actual	
B	Sorghum	0.15	0.000	0.30	0.000	Actual	
C	OFSP	0.100	0.000	0.100	0.000	Actual	
	Total =	1.00					
Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Protein	12.6883	1.61	8.54	16.84	3.11	4.70	20.68
Fiber	1.22034	0.20	0.70	1.74	0.39	0.22	2.22
Carbohydrate	82.3572	1.73	77.91	86.81	3.33	73.79	90.93
Color	7.02096	0.37	6.07	7.97	0.71	5.20	8.85
Taste	6.44632	0.27	5.74	7.15	0.53	5.09	7.81
Overall	6.6703	0.29	5.92	7.42	0.56	5.22	8.12
Loaf Volume	202.904	13.36	168.56	237.25	25.73	136.76	269.05
Loaf weight	72.8123	2.76	65.71	79.92	5.32	59.13	86.50
Beta-carotene	21.0389	1.26	17.81	24.27	2.42	14.82	27.25
Gluten	30.3962	2.51	23.93	36.86	4.84	17.95	42.84
Iron	5.26128	1.37	1.74	8.78	2.64	-1.52	12.04
Zinc	22.7442	5.64	8.26	37.23	10.85	-5.16	50.65
Calcium	5.08868	1.99	-0.020	10.20	3.83	-4.75	14.93

## CHAPTER FIVE

### 5. Conclusions

As per this experiment, bread developed with maximum ratio of OFSP and Sorghum flour has high sensory acceptance and ash contents respectively. Composite flour and bread of sample 100% wheat has high protein content among all samples. All developed bread was accepted by panelists even though their preference was different. Addition of OFSP to composite flour boosted sensory acceptance and beta carotene content of composite flour. Wet gluten content increased as the addition of sorghum decreased. This might be due to the low gluten content of sorghum grain. Based on our optimization, sample of 75.4% wheat: 14.6% sorghum: 10% OFSP was selected for common optimum purpose while sample of (78.3% wheat: 11.7% sorghum: 10% OFSP), (70% wheat: 22.5% sorghum: 7.5% OFSP) and (80.6% wheat: 9.4% sorghum: 10% OFSP) were selected as optimum value for proximate value, mineral, and sensory characteristics respectively. Such information is important for creating new standards and alternative formulations. Generally, formulated flour might be advantageous as a means of reducing heavy demands on importation of bread wheat and alternative source to alleviate malnutrition in Ethiopia.

## **6. Recommendation**

The researcher recommends incorporation of sorghum and OFSP in wheat flour for bread development; to improve nutrient density of bread, enhance food and nutrition security, to reduce the importation of wheat and bread cost. More efforts will also be needed to raise awareness among processors, consumers, extension experts, development agents and farmers on the bread developed from wheat-sorghum enhanced with OFSP in the cultural, social and dietary habits, and then to create market demand. The Ethiopian Government should incorporate sorghum in wheat flour for bread and other confectionaries for improved nutrient diversity of bread and reduced food insecurity.

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## 8. Appendixes

### Appendix 1 Raw materials preparation





Appendix 2 Functional properties, Nutritional and Anti-nutritional Analysis of Composite Flour





Color Data Table [D65/10]

Name	Date	Time	L*	a*	b*	Distance (mm)	Height (mm)	Turntable	Interval(s)
14	2/11/2021	03:11	87.62	2.25	17.23	82.6	18.0	On	5
14	2/11/2021	03:10	87.71	1.99	16.49	82.6	18.0	On	5
19	2/11/2021	03:09	87.99	1.04	12.47	82.6	18.0	On	5
19	2/11/2021	03:09	87.98	1.04	12.47	82.6	18.0	On	5
19	2/11/2021	03:09	83.41	1.37	13.56	82.6	18.0	On	5
16	2/11/2021	03:07	82.54	1.40	13.27	82.6	18.0	On	5
16	2/11/2021	03:07	82.51	1.40	13.26	82.6	17.9	On	5
16	2/11/2021	03:06	82.46	1.40	13.26	82.6	18.0	On	5
18	2/11/2021	03:03	90.59	0.51	9.23	82.6	17.9	On	5
18	2/11/2021	03:02	90.58	0.51	9.23	82.6	17.9	On	5
18	2/11/2021	03:01	90.32	0.67	10.09	82.6	17.9	On	5
Sample	2/11/2021	02:58	89.22	-1.02	0.51	82.6	17.9	On	5
Sample22	2/8/2021	08:01	85.01	-1.04	0.88	82.6	17.9	On	5
Sample21	2/8/2021	08:01	84.77	-1.03	0.88	82.6	17.9	On	5



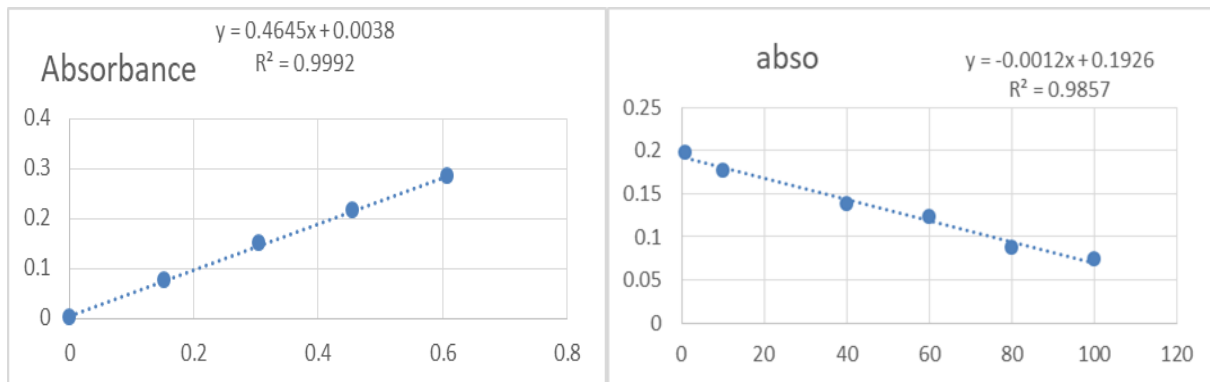
Appendix 3 Product Development and Sensory Analysis



#### Appendix 4 Sensory analysis template

Sample code	Sensory Characteristics					
	Color	Texture	Aroma	Taste	Appearance	Overall acceptance

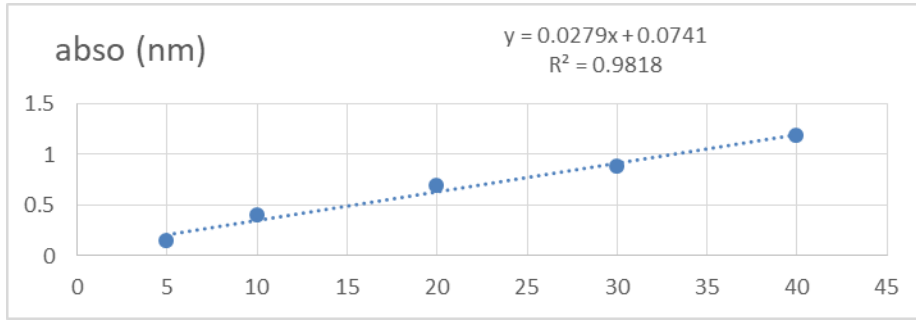
#### Appendix 5 Tannin and phytate calibration curve



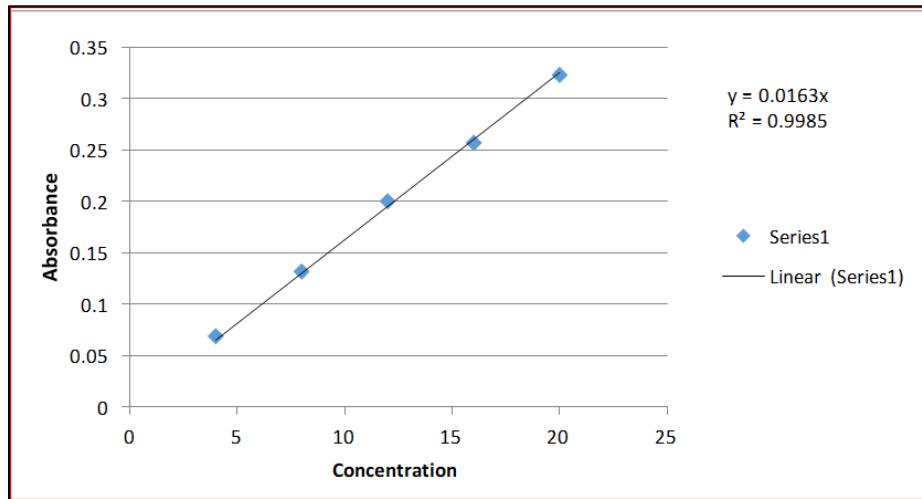
a) Tannin calibration curve

b) phytate calibration curve

Appendix 6 Standard Beta-carotene calibration curve



Appendix 7 Calibration curve for mineral (Iron)



Appendix 8 Standard calibration curve for Zinc (mg/l)

