DEVELOPMENT AND CHARACTERISATION OF A FIBRE-RICH FOOD FROM ORANGE (*Citrus sinensis* Linn.) POMACE, SOYAMEAL AND WHEAT BRAN

BY

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A Thesis in Department of Food Technology

to the Faculty of Technology in partial fulfillment of the

requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

February 2020

CERTIFICATION

I certify that this research work was carried out by **A. O. Oduntan** in the Department of Food Technology of University of Ibadan.

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DEDICATION

This work is dedicated to the Glory of God Almighty and the memory of my late Father Pa Emmanuel Tudonu Agosa.

ACKNOWLEDGEMENTS

I give God Almighty all the Glory and Honour for the wisdom he bestowed upon me, the strength and good health in order to finish this research work.

My sincere gratitude to my Supervisor, Dr G.L. Arueya for his guidance, thoroughness, support and encouragement throughout the time of my research. A special thanks to the former and present Head of Department, Prof K.O. Falade and Prof A.A. Olapade, other lecturers in the Department; Prof O.C. Aworh, Prof G.O. Adegoke, Prof R. Akinoso, Dr P.I. Uzo-Peters, and Dr O.O. Ezekiel for their contributions and encouragement during my Postgraduate studies. To all the supporting staff of the Food Technology Department, I say a big thank you.

I am most grateful to the former Head of Department, Fruits and Spices, National Horticultural Research Institute, Ibadan, Dr B.A. Adelaja, who encouraged and facilitated my entrance into academics. My appreciation to the former Head of Programme, Product Development Programme, National Horticultural Research Institute, Ibadan, Dr O.S. Babalola, for her support and advice all the time.

This research work would not have been completed without the assistance of the technologist in the Product Development Programme, National Horticultural Research Institute, Ibadan; Mrs O.F. Adebayo, Mr R.O. Adeolu, Mr O. H. Karaki and Mrs C. B. Ojo for their support in the laboratory analysis. My sincere thanks to Mrs O. Adeola of the Chemistry Department, University of Ibadan for her assistance in the laboratory analysis.

Words cannot express how grateful I am to my darling husband, Engr Dr O.B. Oduntan, for his love, encouragement and support all the time, also to our wonderful children, Ife-Oluwa, Ore-Oluwa, Ike-Oluwa and Oluwatobiloba for their understanding, prayers and encouragement throughout the course of this research work.

I appreciate my friend and sister, Prof S.B. Fasoyiro for her encouragement, love, support and mentoring since my entrance into academics. Lastly, I am deeply indebted to my Parents, Late Pa E.T. Agosa and Deaconess J. M. Agosa for bringing me to life and their support throughout every stage of my education.

ABSTRACT

Consumers demand for high dietary fibre food products is on the increase. Food by-products such as pomace, wheat bran, and soyameal can be another veritable source of dietary fibre. Work had been done on the combination of two of the by-products (wheat bran and soyameal) while information on good fibre profile blends of the three by-products is sparse. This study was designed to develop high fibre food from orange pomace, soyameal and wheat bran.

Orange pomace (5-30%), soyameal (10-80%) and wheat bran (10-70%) were subjected to a three-component mixture design at the lower and upper ranges to meet required daily nutrient intake. Thirteen generated formulations were subjected to extrusion (5Kg per cycle) at fixed cooking temperature (110 ^{o}C) and screw speed (290 rpm). The extrudates were analysed for chemical (proximate, dietary fibre, antinutrients and antioxidant) and functional (bulk density and water absorption index) properties using standard methods. Sensory characteristics of the extruded products were determined by a panel familiar with sensory analysis (9-point hedonic scale) after which three formulations with the best organoleptic properties were selected. These were tested on forty male *Wistar* rats for toxicity for 28 days (hematological and histopathological). Shelf-life was evaluated monthly based on moisture content and fungal count under ambient conditions (indoors) for a period of five months. Data were analysed using ANOVA at $\alpha_{0.05}$.

Chemical composition of products were moisture (8.50-11.03%), ash (4.00-6.00%), protein (10.84-25.40%), fat (3.50-8.00%) and carbohydrate (39.18-59.34%). The formulations containing pomace (30%), soyameal (10%) and wheat bran (60%) had highest total dietary fibre (66.86%) and fibre fractions (insoluble 30.21% and soluble 36.65%). Lowest value of phytate (0.57 mg/g) and oxalate (0.86%) were obtained from mixture of pomace (23%), soyameal (27%) and wheat bran (50%), while lowest value of tannin (1.3 mg/g) was pomace (30%), soyameal (60%) and wheat bran (10%). Total phenol, flavonoid, carotenoid were 0.47-0.76, 2.13-8.21 and 0.02-0.14 mg/g) respectively. Bulk density and water absorption index ranged from 0.42 to 0.61 g/cm^3 and 2.91 to 3.86, respectively. Highest sensory score (5.16) for the overall acceptability was obtained in the mixture of pomace (10%) soymeal, (80%) and wheat bran (10%). Tested diets on *Wistar* rats resulted in lower weight gain which was at variance with control diet (31.55-56.21 g). White blood cells ((5283-6400) ×10^9/µL), creatine (0.63-0.70 mg/dl) and glucose (133.3-139.7 mg/dL) showed no significant difference between rats fed control and tested diets. Necrosis was not found in the kidney and liver of the rats fed with control and tested diets. The extrudates had 3-month shelf stability (moisture; 8.50-10.99%, total fungal count; 6.00-7.51 log cfu/g). Significant variations were observed among the thirteen samples. The optimal blend for the high fibre food was pomace (5%), soyameal (47.50%) and wheat bran (47.50%).

High fibre food was successfully produced from composite of orange pomace, soyameal and wheat bran. The use of orange pomace for the development of high fibre food will contribute a significant portion of fibre to human diet and also improve its disposal.

Keywords: Orange pomace, Dietary fibre, Extrusion cooking, Toxicity

Word count: 489

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CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Several food processing plants generate the majority of food by-products, many of which are thrown away or utilized at a low industrial and economic level. Indeed, food industry's waste is a promising source of useful substances like dietary fibre, antioxidants, antimicrobial agents, essential fatty acids, and minerals that could be used as a result of their inherent favourable nutritional, technical and purposeful properties. These food by-products, including orange pomace, wheat bran and soyameal have been identified as excellent sources of dietary fibre (Topping *et al*., 2007).

Citrus is the largest fruit crop worldwide, with approximately 114 million metric tons produced annually (FAO, 2020). The genius Citrus includes several important fruits, the most important species being sweet orange (*C. sinensis*). Nigeria produces about 4.1 million tonnes of citrus a year (FAO, 2020), the largest in Africa, but ninth globally. Over the years, some 15 States: Cross River, Imo, Anambra, Osun, Ondo, Lagos, Ogun, Oyo, Kwara, Benue, Abia, Plateau, Kogi, Kaduna, Enugu and Bauchi have been in the fore-front in the cultivation and consumption of sweet orange in Nigeria (Odbanjo and Sangodoyin, 2002).

A major product of orange is orange juice arising from processing while simultaneously generating various byproducts such as citrus wastes. These wastes though highly biodegradable present a serious environmental problem (Mamma and Christakopoulos, 2008). Pomace, a significant component of these wastes constitutes some 50% of the fresh fruit weight.

Orange pomace fibre has better quality than other fibre sources owing to the existence of minerals, sugars, including bioactive substances like polyphenols, flavonoids and carotenes (Fernandez-Gines *et al*., 2003). Several investigators had used citrus waste to produce enzymes (Ahmed and Mostafa, 2013), bioethanol (Wilkins *et al*., 2007), citric acid (Rivas *et al*, 2008), Xanthan gum (Stredansky and Conti, 1999) unicellular protein (De Gregorio *et al*., 2002), Prebiotics (Mandalari *et al*., 2007) and as a veritable source of natural antioxidants (Li *et al*., 2006). In most cases, the methods applied in producing these products are quite expensive and very technical making them unaffordable. The use of pomace as agent in juice extraction and clarification, waste water treatment and degumming of plant fibres have also been pursued with vigor (Kumar *et al*., 2011). Inspite of the aforementioned possibilities, waste from orange juice remains largely underutilised. Nigeria accounts for 0.3 MT of the 36.0 MMT of orange pomace generated worldwide with the possibility of producing more in years to come (Ezejiofor *et al*., 2011).

Another veritable source of fibre in the 21st century is Wheat bran (WB). Wheat bran is a dense dietary fibre source with several health gains including decrease in risk of cardiovascular disease and type 2 diabetes and maintaining the normal glucose and cholesterol level (Topping, 2007). Quite a number of studies been done in the use of wheat bran for various productions. Lebesi and Tzia (2012) reported an improvement in the nutrient of cakes when 10 to 30% WB was added. Kaur *et al*. (2012) reported satisfactory quality of pasta fortified with 15% WB. Wojtowicz and Moscicki (2011) noted that inclusion of wheat bran in an amount of 5 to 25% by weight of flour in the formulation of pasta extruded with a single screw extrusion plate improved the sensory properties. Almeida *et al*. (2013) stated that WB-fortified bread of 20% pomace had higher acceptability scores than other fibres. Sobota *et al*. (2015) established that addition of WB to pasta up to 30% gave satisfactory organoleptic properties similar to pasta produced from whole grain durum wheat. Mixtures developed from hybridization using WB and soy husks were successfully used to produce a low-fat fried donut (Lim *et al*., 2012). Nigeria's wheat milling capacity was at about 8 million tons in 2012/2013 (KPMG, 2016).

In a similar vein, soyameal is associated with some useful outcome on human health such as averting obesity, providing nutrition, reducing blood cholesterol and also plays a significant role in disease prevention. Thus its extensive use as a good protein source in animal nutrition such as layer feed (Akande *et al*., 2007), broiler production (Khan *et al*., 2018), fish feed (Attalla and Mikhail, 2008) and in various human food formulations (Ojokoh and Yimin, 2011) has long been acknowledged. Nigeria generates about 519,000 MT of soyameal in 2018 while the world production was 243.31 million metric tons (Soyameal lnfo Centre, 2018).

However, there has been no effort to harness the various unique fibre qualities of orange pomace, wheat bran and soyameal (including its inherent protein binding property) and convert same to whole foods with possible significant functional properties and attendant benefits that could contribute to reduction of non-communicable diseases in Nigeria as a developing country. New technologies like food extrusion are promising processing methods.

Food extrusion processing is known as a universal and effective technology for turning produce into products. It destroys microbes and inactivates enzymes that spoil quality as well as limit the storability of food (Aworh, 2014). Owing to its versatility, uniqueness, energy efficiency, low running cost, high quality of resultant products, high productivity, and improve protein digestibility, the technology can substitute many traditional processes in food industry.

1.2 Statement of Problem

Over the past decades, interest in dietary fibre-rich foods and antioxidants has grown and the usefulness of the raw materials has given rise to a large fibre and antioxidant market with rich process food and its components (Ajila *et al*., 2008). Cereals were the major source of dietary fibre but research has shown that dietary fibre of fruits has better quality because of the presence of sugars, minerals and polyphenols. The interest in the importance of dietary fibre to human health cannot be overlooked. Garcia-Amezuita *et al*., (2017) reported that regular consumption of fibre lowers blood cholesterol and glucose levels, reduces the risk of hemorrhoids, colorectal polyps, and diverticulitis due to its laxative properties.

Dietary fibre is also known to reduce the time of passage in the intestine, add bulkiness to stool, reduced total low-density lipoprotein and buffers excessive amounts of acid in the stomach. Dietary fiber as a functional food ingredient which can serve the techno-functional purposes necessary to develop health-promoting value-added. Other reason why dietary fibre has gain relevance included its ability to help control weight by delaying hunger. Interest in fibre sources has been renewed in drive for excellent food fibre quality, fibre-rich products, and also dense in bioactive complexes are a plus for food manufacturers, particularly in the wake of rising consumers preference of natural supplements over synthetic ingredients.

Increased consumer demand for excellent food has led to increased utilization of novel knowledge and materials. Numerous reasons that motivate consumer demand for varieties include health challenges like hypercholestereremia, obesity and cancer as well as demographic characteristics, like ethnicity, aging populations, variations in distribution systems and prices and the need for convenience (Perez-Alvarez 2010). Customer need for excellent processed food resulted to increased use of novel ingredients and technologies in the manufacture of novel foods but they also demand that it should be natural, organic or healthy food. The improved awareness amongst consumers is placing greater demand on functional foods with maximum health benefits. There is a growing awareness to replace the artificial antioxidants with normal ones from food sources (Makris *et al*., 2007).

Significant prominence is attached to functional food products which in principle, in addition to the basic nutritive roles, offer functional assistance, play a significant part such as in the inhibition of illnesses, or slowing down the development of severe sickness. Functional products are known to contain ingredients with beneficial effect without undesirable effects. The association between food and well-being has increasingly centered on usefulness of diet preferences and ailments like cardiac disease, cancer and allergies. Research supporting the relationship between diet and wellbeing has undoubtedly contributed to the swift growth in recent years of the novel food market, a purposeful food market (Viuda-Martos *et al*., 2010).

The health protective of antioxidants has become interesting components in food production. A lot of antioxidants supplement has been produced to help in preventing diseases associated with oxidative phenomena like respiratory ailments, cardiovascular diseases and cancer. There is an increasing interest to replace the synthetic antioxidants with natural ones from food sources (Oswell *et al*., 2018).

1.3 Justification

The problem of pollution arising from citrus waste will aggravate as juice production increases in the coming years. Pomace, a significant component of these wastes constitutes some 50% of the fresh fruit weight. Nigeria accounts for 0.3 million tonnes of the 36.0 million metric tonnes of orange pomace generated worldwide with the potential to produce more in years to come (Ezejiofor *et al*., 2011). Many fruit juice plants generate large amounts of residue with little or no profitable disposal options. A common nuisance associated with heaps of these waste is a fowl putrid odour, soil contaminating tendencies and a repository of cross contaminants for the food industry (Toraddo *et al*., 2011). Also, infestation by rodents and flies creates a veritable pathway for transmission of various pathogens. In these prevailing circumstances food poisoning and disease outbreak remains widespread and unavoidable.

In mitigating the aforementioned challenges, a number of researchers have used citrus waste to produce secondary products. In most cases, the methods applied in producing these products are quite expensive and very technical making them unaffordable. lt is therefore of vital importance to use such industrial by-products in order to improve the process economics in a sustainable manner. The significance of this study can therefore not be over-emphasized.

1.4 Research Questions

The following research questions guided this study:

1. What are the combinations of orange pomace, soya meal and wheat bran for the rich-fibre food?
2. What is the processing method to develop the fibre- rich food?
3. How safe are the fibre-rich food?
4. How long can the fibre-rich food keep?

1.5 Objectives of the Study

The main objective of this study was development of an acceptable extruded high fibre food from wheat bran, soya meal and orange pomace.

Specific objectives were to:

(a) To determine the proportions of orange pomace, soya meal and wheat bran in the formulations.

(b) Characterize the chemical components and evaluate the functional properties of the developed extruded product.

(c) Assess the nutritional qualities and the toxicological safety of the developed product.

(d) Determine the overall acceptability of the product and

(e) Ascertain its shelf stability under tropical conditions

CHAPTER TWO

LITERATURE REVIEW

2.1 Citrus Production

Citrus, a genus of plants belonging to the rue family (Rutaceae) and yields pulpy fruits is usually protected with fairly thick skins. The most cultivated species are pummelo, shaddock, lime, lemon, citron, grapefruit, Seville orange, mandarin, tangerine and sweet orange.

Current citrus production in the world amounts to 114 million metric tonnes of fresh fruit. Approximately 62% of this figure was sweet orange, 17% Mandarin, 11% lemon, 5% lime and 5% grapefruit. (FAO, 2020). The Food and Agriculture Organization puts China, Brazil, the India and Mexico as the dominant citrus producing countries (Table 2.1). They further posited that sweet orange is the most cultivated citrus species.

2.2 Sweet Orange Fruit Morphology

Orange fruit is a kind of berry with different colours, forms, shapes and qualities of the juice. They are in round shapes with delicate textured orange coloured skins and pulpy porridge. Their diameter ranges between two and three centimeters (Goudeau *et al.*, 2008). Sweet orange trees are medium-sized, evergreen and upright, with a scattered, slender, prickly twig. The leaves are made from aromatic, alternative, elliptical to rounded leaflets. The fruit is spherical to oval and the outer shell is bright orange and covered with small oil clips. The meat is yellow to orange and can be very sweet. Fruit centers are fixed. Citrus fruit consists of flavedo, segment, oliferois vesicles, albedo, endocarp, skin and peduncle extremity (Plate 2.1)

2.3 Varieties of Sweet Orange

Sweet orange is grouped into four different characteristics; (i) common orange (ii) pigmented orange (iii) navel orange (iv) acid less orange (Yara, 2018).

Table 2.1: Top ten Citrus fruits (tonnes) producers in 2019

Ranking	Country	Data
1	China	42,776,572
2	Brazil	19,305,081
3	India	12,763,518
4	Mexico	8,550,007
5	Spain	6,802,878
6	United States	6,662,954
7	Turkey	5,005,524
8	Egypt	4,766,378
9	Nigeria	4,114,676
10	Argentina	3,526,249
	World	**114,273,837**

Source: FAO-Stat, FAO, 2020

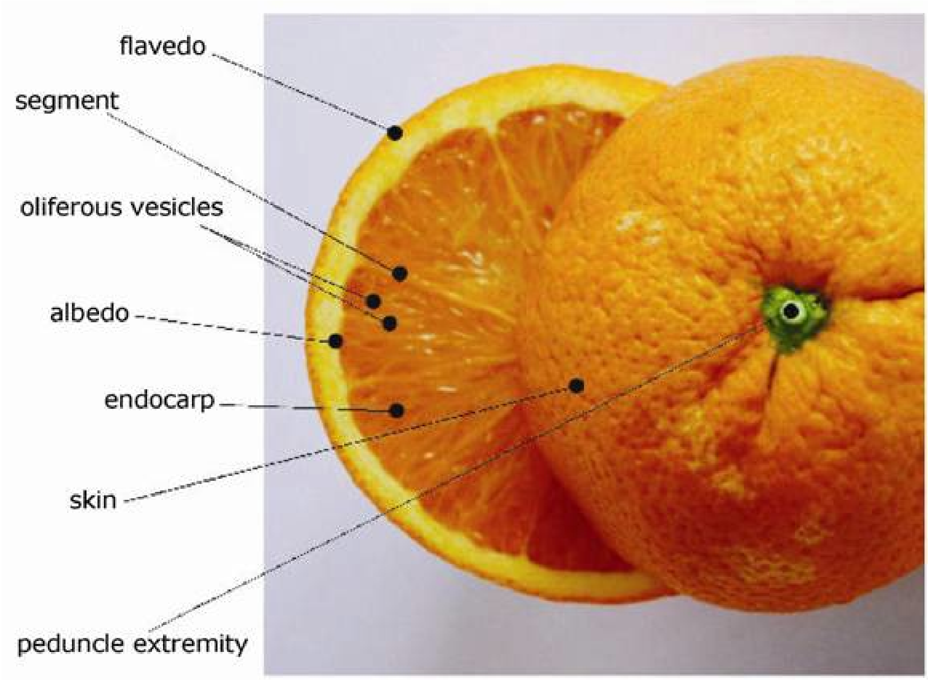


Plate 2.1: Structure of Citrus fruit. (Goudeau *et al.*, 2008).

2.3.1 Common orange

Common or round oranges are the most important group commercially; they include cultivars 'Hamlin', 'Valencia', 'Shamouti', ‘Pera’ (Plate 2.2a). They are mainly cultivated in humid climates and are often used for processing. There are various species of common orange grown widely. The most common orange varieties are Valencia, Valencia Hart's Tardiff, and Hamlin, but many other species exist (Yara, 2018).

2.3.2 Blood orange

Blood orange is a kind of sweet orange (*Citrus sinensis*) (Plate 2.2b). The most common types are *Moro*, *Tarocco* and *Sanguinello*. The red coloration is due to the high anthocyanin content (Yara, 2018). There are two types of blood oranges: the light blood and the deep blood orange. Higher amount of anthocyanin gives the fruit a deep red colour. The production of pigmented (blood) orange is limited mainly to Mediterranean climates for the fresh market (Yara, 2018). Hot days and cool nights lead to fruits with deep red flesh colour.

2.3.3 Navel orange

Navel Oranges are larger than common oranges (Plate 2.1c). They are mainly sold for fresh fruit because they contain limonin, which leads to a bitter taste after processing. Navel oranges are particularly prone to environmental pollution. For example, high temperatures of 95 to 104 ° F (Yara, 2018) during flower production, especially in humid climates, can lead to fruit loss. Moisture stress can also lead to a significant yield loss. The navel orange is of great commercial importance, as is the best-selling orange in the grocery store.

2.3.4 Acid free orange

Acid-free oranges have very little acidity and flavour (Plate 2.2d). Acid-free oranges are early fruits of the season and are also referred to as "sweet" oranges. They are usually not cultivated in huge quantities. They have very little acid, which protects against decay and is therefore unsuitable for juicing.

Plate 2.2a

Plate 2.2b

Plate 2.2c

Plate 2.2d

Plate 2.1a-d: Common varieties of orange, a - Common or round orange, b - Navel orange, c - Blood orange, d – Acid free orange

(Source: Yara, 2018)

2.4 Nutrition and Health Benefits of Sweet Orange

Sweet orange is one of the important fruit crops planted globally. Turner and Burri (2013) reported that orange fruits are rich in Vitamin C and folic acid, as well as fibre. Also, they are free of fat, sodium and cholesterol but contain calcium, niacin, potassium, vitamin B6, thiamin, phosphorus, copper and magnesium. Admittedly, they help in reducing the threat of heart diseases, certain cancer and possibly pregnant women having children with birth diseases (Muhammad-Lawal, 2007). Olatilewa *et al* (2017) reported that regular orange intake prevents frequent attacks of influenza, the common cold and bleeding while also enhancing healthy living and longevity. Citrus fruits are a significant source of bioactive complexes like vitamin C, flavonoids, pectins and phenolics which are all vital in human diet (Ebrahimzadeh *et al*., 2008). They are also rich in glycemic and glycemic saccharides. flavonols, flavons and flavanons (Calabro *et al*., 2004).

Orange fruits and some of its products are associated with anti-inflammatory, antioxidant, antigenic, anti-cancer, antimicrobial and neuroprotective properties associated with the ability to modulate hepatic lipid metabolism (Mandalari *et al*., 2017).

2.5. Processing of Sweet Orange into Various Products

2.5.1 Orange juice

Juice production essentially involves fruit sorting, washing, peeling, juicing, filtering, bottling and pasteurization. Fruit juices are divided into three based on the fruit content; fruit juice, fruit nectar and concentrate. Processing juice involves mechanical extraction of ripe fruits and pasteurization of the extracted juice. The concentrated juice is obtained by evaporation of the inherent water in the juice, restored only by reconstitution with an equivalent amount of water. This facilitates storage and transport.

On the other hand, fruit nectar is produced by diluting fruit drink or fruit puree with water, artificial sweeteners are optional. Lowest soluble solid in fruit nectar is between 25% and 50%, depending on the type of fruit (Chanson-Rolle *et al*., 2016). In general, orange juice is used in canning, including jam, marmalade and fruit butter.

2.5.2 Dietary fibre in orange

About half of fruit is made up of 50% juice and the remaining 50% constitutes the bark, albedo, sacks and seeds that constitute the pomace containing varying amounts of fibre (Fasolo *et al*., 2014). Wastes after juice extraction can be dried for future use. The waste is low in digestible carbohydrates, fat, calories and high in fibre (Romero-Lopez et al., 2011). A medium-sized orange that weighs around 154 g contains 3 g of dietary fibre. A small peeled orange has 1.8 grams of soluble fibre (Romero-Lopez *et al*., 2011). High consumption of foods rich in soluble fibre like oranges can help maintain blood cholesterol and reduce the risk of diabetes (Healthy living, 2018).

Orange is an excellent source of insoluble fibre, each small orange of 96 g contains 1.1 g of insoluble fibre, mainly from lignin, cellulose and hemicellulose from the walls of plant cells which passes through the digestive tract without breaking down. Oranges contain approximately as much insoluble fibre per serving as cooked spinach, fresh carrots and raw cranberries (Healthy living, 2018). The high fibre content of orange indicates a good dietary fibre source (Romero-Lopez *et al*., 2011).

Predominant orange fibre is pectin (65 to 70%), the remaining fibre are of trace amounts of lignin, hemicellulose and cellulose (Rao *et al*., 2016).

2.5.3 Orange pomace

Orange pomace is the waste that remains after processing oranges to juice, wine or other products. The wastes from this processing, including peel, seeds and pulp, which make up nearly 50% of unprocessed fruit, are a possible source of valuable byproducts (Gowe, 2015). Orange pulp is rich in fibre. The fibre has superior quality than others because of the occurrence of minerals, sugars, including bioactive substances like polyphenols, flavonoids and carotenes (Fernandez-Gines *et al*., 2003). Citrus pomace is sensitive to microbial and biochemical hydrolysis due to their high water content (Matouk *et al*., 2017).

Proximate properties of pomace were shown to contain 6.70 g/ 100g protein, 0.89 g/100g lipid, 2.71 g / 100g ash and 17.9 g/100g carbohydrate. Dietary fibre characterization shows

54.0 g/100g insoluble dietary fibre (IDF), 10.28 g/ 100g soluble dietary fibre (SDF), 64.3 g/100g total dietary fibre (TDF) while IDF/SDF ratio was 5.3:1.

The phenolic compounds of citrus pomace could undergo enzymatic oxidation at various stages of processing. Dehydration under suitable conditions enables reduction in water present and water activity in the products. The prevention of oxidative enzymatic reaction and the development of microorganisms prolong the storability of the products (Mhiri *et al*., 2015)

2.5.3.1 Peel

The skin constitutes 50 to 65% of the total fruit and is the main by-product. If it remains unprocessed, it becomes odoriferous, a refuge for insects, serious soil and ecological contaminants (Mandalari *et al*., 2006). It was established that orange peel extract has a good radical scavenging ability due to its richness in pectin and its accessibility (Gorinstein *et al*., 2001).

2.5.3.2 Seed

Citrus seeds, widely regarded as agricultural-industrial waste, are a possible source of oil. Orange seed oil test results in Brazil, Egypt, Nigeria and Vietnam have shown that citrus oil is a suitable avenue of unsaturated fatty acids (FA) (Saidani *et al*., 2004). A typical analysis of citrus seed oil, according to Okoye *et al*., (2011), discloses 34.0% oil content, 6.43% moisture, 51.40 acid value, 25.70 free fatty acid, saponification value 194.25 and a peroxide value of 0.36.

2.5.3.3 Utilization of orange pomace

Previous work has involved the use of pomace in the manufacture of several intermediates. Orange pomace was used in various applications, which include the production of compounds and solvents in flavours and colognes, as components in paints, cosmetics and animal supplements. Pelleted orange peel is an example of a by-product obtained from orange juice production (Cutrale, 2012).

Torrado *et al*. (2011) described the manufacture of citric acid by solid fermentation using orange pomace. Also, Ahmed and Mostafa (2013) reported the use of orange baggase and Molokhia stalk for making pectinase enzyme. Karaman *et al*. (2011) studied the use of orange-peel extracts as likely antioxidant in refined sunflower oil during storage to delay oxidation.

Zinjarde and Gampawar (2000) reported ensiling of orange pomace (OP), containing crude protein 9.82, crude fibre 7.26, ether Extract 5.34, nitrogen free extract 73.29 and total ash 4.29% (DM basis), with paddy straw (PS) in 70:30 ratio inoculated with 0.00, 0.50 and 0.75% culture of *Lactobacillus plantarum* for 60 days resulted in good quality silage.

Zaker *et al*., (2016) reported that incorporation of orange pomace up to 10% in cookies preparation increased nutritional value, especially in fibre, physical quality and general acceptance of biscuits.

2.6 Soybean

Soybean (*Glycine max*) is a legume that has a leading position as a world culture due to its almost unrivaled content of protein and edible oil. It has become a primary source of human food and a major antidote to the acute protein deficiency in the sub-humid and humid tropics where large populations of the world live. Soybeans have a crescent shape, 3-7 cm long, with 1 or 2 seeds, 1000 seeds weighing from 115 to 280 g. Immature grains are green and mature have a colour from light yellow or green to brown (Aletor *et al*., 2010). Soybeans with modern varieties have a spherical shape, yellow and green colours are the maximum desirability. The nutrient content differs considerably depending on the variety and growth conditions. It usually contains 35-40% protein, 15-20% fat, 30% carbohydrates and about 55 minerals and ash (Riaz, 2006).

Soy protein is known to decrease the threat of coronary heart disease when ingested in a low-fat food. Soy protein has recently attracted much attention, since it is known that it lowers the level of low density lipoproteins. The FDA has relevant health requirements related to soya cholesterol, suggesting that everyday ingestion of 25 g soy protein may reduce LDL cholesterol (Erdman, 2000). Its amino acid composition is close to animal proteins and usually substituted for animal protein. Soybeans are used to produce oil. Nearly

90% of soybeans consist of cotyledons and 8% of hulls. Cotyledons contain proteins, fats, carbohydrates and anti-nutritional factors (Banaszkiewicz, 2011).
Functional properties that can be attributed to soy proteins are solubility, viscosity, cohesion-adhesion, water absorption and binding, gelation, flexibility, fat absorption, emulsification, flavour binding and colour control (Jideani, 2011).

2.6.1 Bioactives in soybean

Soybean is a nutritional crop and is popular because of its nutraceutical properties; it comprises vital amino acids and secondary metabolites, like isoflavone, phytic acids, saponins, peptides, phytosterols and trypsin inhibitors (Isanga and Zhang, 2008). These complexes have antioxidant and anti-carcinogenic properties as well as positive effects against osteoporosis, menopausal symptoms, and cardiovascular disease among others (Isanga and Zhang, 2008). Additional evidence suggests that isoflavones can inhibit hormone-related cancers, such as prostate cancer and breast cancer, due to their similar estrogen structure. It has also been shown that isoflavones have antioxidant and anti-inflammatory properties (Kao *et al*., 2008). The main configuration of isoflavones is similar to 17-beta-estradiol, which makes it possible to bind to estrogen receptors, demonstrating many beneficial health effects (Gupta, 2017).

Kunitz-Trypsin inhibitor (KTI), linasin and Bowman-Birk inhibitor (BBI) are the three main and best described bioactive proteins/soybean peptides. KTI and BBI are serine protease inhibitors with molecular weights of 8 and 20.1kDa respectively (Dia *et al*., 2012). BBI inhibits trypsin and chymotrypsin while KTI inhibit only trypsin (Dia *et al*., 2012). These proteins are known to have anti-cancer-causing activity (Isanga and Zhang, 2008).
The content of soy fibre is primarily pectin polysaccharides, a type of vegetable fibre well fermented by the intestinal microflora (Rizzo and Baroni, 2018).

2.6.2 Soyameal

Soybeans are subjected to various processing methods. After the initial cleaning, grinding, conditioning, peeling, boiling or frying soybeans, the oil is obtain through mechanical or solvent extraction. Processing results in raw oil and defatted flakes. Toasting soybean at high temperature eliminates anti-nutritional substances while soybean meal is obtained. Anti-nutritional factors such as chymotrypsin inhibitor, trypsin inhibitor and alpha amylase inhibitor are denatured by processing and can be a risk if grains are eaten raw or inadequately processed. (Banaszkiewicz, 2011).

Soyameals concentrates and isolates could undergo thermal, chemical, physical and enzymatic treatments to obtain desirable structural and functional modifications. The functional activity of fine particles is generally associated to the reaction of liquid and solid. They are also linked to the properties related to the protein properties and the relationship with other food components (Moure *et al*., 2006). The functional activities are reliant on the detailed surface area, pore volume, structural parameters.
Soyameal is abundant in easily digestable amino acids, except methionine that is usually low. Moreover, soya meal is abundant in amino acids such as threonine, isoleucine, tryptophan, lysine tryptophan and valine, that are absent in crops like maize and sorghum (Nahashon and Kilonzo-Nthenge, 2012).

Soybean meal (SBM) is high in protein, and consists of many crucial and non-crucial amino acids (AA) in appreciable proportion, except two sulphurous Amino Acids. The most abundant is Glutamic acid, followed by aspartic acid, alanine, arginine, glycine serine and proline in Soyabean meal. Amongst the important AA, leucine has the uppermost concentration, followed by lysine, isoleucine, valine, threonine, tyrosine, phenylalanine and histidine. Cysteine and methionine were low in concentration (Song *et al*., 2008).

Soy protein components are related to a number of helpful effects on human welfare, like reduction in blood cholesterol, prevention of obesity, supplying nutrients and perhaps a valuable role in prevention of diseases (Mateos-Aparicio *et al*., 2008).

Soyameal contains some nutritional inhibitors such as trypsin inhibitors, lectins and lipoxygenase, which must be destroyed before or during processing into feed or food.

2.7 Wheat

2.7.1 Wheat grain morphology and composition

Wheat belongs to family Poaceae (Gramineae). With an annual harvest of more than 600 MT, it is one of the three most important cereals (FAO 2009). The total global yield was close to 607 million tons, likened to 652 MT of rice and 785 MT of corn. Wheat grains are normally oval, though the various grains are almost spherical or long, narrow and flat. Grains generally have a length of 5 to 9 mm, weight of 0.35 to 0.50 g (Belderok *et al*., 2000).

Wheat is healthful, easy to store, transport and process to different kinds of foods. Wheat contains good amount of B vitamins, protein, minerals and dietary fibre. Wheat germ makes up half of the proline flour and glutamine, but the levels of alanine, asparagine, arginine, glisine, threonine and lysine doubled (Cornell, 2012).

Wheat flour is used to make bread, cookies, sugary foods, noodles, and valuable gluten or seitan. Wheat grain is a good source of fibre, help prevent and treat certain digestive disorders.

2.7.2 Bioactives in wheat

Wheat grain is an outstanding source of naturally active phytochemicals. Biologically active phytochemicals in wheat are divided into carotenoids, phenolic acids, alkylresorcin, tocopherols and other complexes such as sterols, sterile enzymes, lignans and benzoxazinoids (Luthria *et al*., 2015). Phenolic acids can be segregated to two. The first is derivatives of hydroxybenzoic acid including vanilla, sirol, gallic acids and p-hydroxybenzoic. The second derivatives of oxycinnamic acid are ferulic, caffeic, p-coumaric, synapic acids. (Luthria and Liu, 2015). Phenolic acids in wheat ranges from 200 to 1200 mg / g dry matter. Ferulic acid is the one that occurred most among the phenolic acid in wheat grain (Liyana-Pathirana and Shahidi, 2006).

Whole grain of wheat offers an average source of vitamin E. Vitamin E includes four tocotrienols and tocopherols. Tocotrienols have an isoprenyl side chain with three double bonds whereas tocopherols have fatty chains of phytyl. Earlier studies established the occurrence of α, β, δ- and γ-tocopherol in wheat grain. The amount of tocotrienols and tocopherols in wheat grain samples ranged from 27.6 and 79.7 g / g (Moore *et al*., 2005)

In wheat, colour was a quality parameter used which is mainly associated to the occurrence of carotenoids and their esters. The amount of carotenoids in wheat is between 0.8 and 2.17g / kg (Moore *et al*., 2005). Zeaxanthin and Lutein carotenoids occurred most with concentrations of 0.5-1.44 and 0.2-0.39 g / g of grain in that order. (Moore *et al*., 2005).

Phenolic lipids or alkylresorcinols (ARs) are usually found in wheat (Gunenc *et al*., 2013). ARs are comparable to tocopherols, apart from a simple aliphatic hydrocarbon side chain and one phenolic ring. The alkyl chain may consist of 13 to 27 carbon atoms. 5-N-alkyl resorcinols, 5-alkenyl resorcinols, 5-oxoalkyl sorcinans, 5-oxoalkenyl resorcinos and 5-hydroxyalkenyl resorcinins are the 5 major AR classes administered in wheat. The AR values in wholemeal are in the range of 489 to 1429 g / g (Ross *et al*., 2003).

2.7.3 Bioactive phytochemicals distribution in wheat

Fractions of germ and bran contain more biologically active phytochemicals. Higher concentration of phenolic complexes was recorded in bran fractions as likened to fine grains containing the major endosperm (Liyana-Pathirana and Shahidi, 2006). Recent studies have found that most phenolic acids exist in the bind form (80%) related to a free soluble form, higher concentration of phenolic acids are identified throughout the whole grain, containing bran fractions g/ g dry weight associated to fine grain (Lu *et al*., 2014). The amount of antioxidant in wheat varies depending on the country of origin.

The concentration of carotenoids was in the order of the germ fraction, bran and endosperm fraction. Also, tocotrienols and tocopherols were differently dispersed in the grain. The concentration of tocopherols was more in the outer layers while the content of tocopherols was relatively lower in the endosperm fraction. There were also more tocotrienols in the outer layer of grain (85%, aleurone, dough and pericarp) and 15% present in the endosperm fraction (Piironen *et al*., 2009).

The concentrations of benzoxazinoids in various wheat and rye showed germ fraction had more benzoxazinoids than in the bran and endosperm. Fine bran constituted the second most enriched section. It was found that phytosterols are deposited in bran and germ of wheat grain, while sterile ferulates are mainly in bran (Nurmi *et al*., 2012). The amount of separable phytosterols like campesterol, sitosterol, stigmasterol, sitostanol and campestanol in wheat are diverse in dispersion.

2.7.4 Processing of wheat

Wheat is usually processed before consumption. Producers, processors, consumers, health and nutrition specialists studied the impact of wheat processing on bioactive phytochemical compounds. The constancy of bioactive phytochemicals is inclined by processes and their conditions. This information is dangerous for the incidence of ideal processes that can carry bioactive phytochemicals in finished wheat products.

2.7.5 Wheat bran

Wheat bran, a byproduct of normal crushing and comprises cellulose, hemicellulose, micronutrient and protein in comparatively high amount namely as 41-60% non-starch polysaccharides (26% are arabinoxylans), 15-20% protein and 10-20% residual starch (Amrein *et al*. 2003). The bran consists of numerous layers that shield the core of the grain. Bran is rich in vitamin Bs and minerals, it is parted from the starchy endosperm at the initial phase of milling. Bran safeguards the grain and endosperm constituents with the insoluble fibre. The chemical components of wheat bran are diverse, but mainly contain pentosans and polymers based on cellulose, arabinose and xylose, that are carefully linked to proteins.

Wheat bran also contains a substantial quantity of dietary fibre (DF) found in bran walls. Wheat bran has up to 47% dietary fibre, which are insoluble in water (Das *et al*., 2012). Carbohydrates and proteins account for 16% of each of the total dry weight of bran. The amount of minerals is quite high (7.2%). The two outer region of the grain is made up of lifeless vacant cells. Compartments of the aleurone layer consist of live protoplasts. This describes the higher content of protein and carbohydrates in the bran.

Wheat bran (WB) is known as a golden produce and is used in the fermentation industry as well as in pharmacy and biomedical research. WB is a residue from wheat grinding, which is produced in large quantities and not used in food in developing countries. During grinding about 50% of the wheat grain is parted (Eposito *et al*., 2005).

Minimum grinding improved the biological availability of phenolic acids in wheat bran. Scientist observed that wheat bran parts have 1.5 times greater antioxidant ability by reducing particle size from 172 to 30µm due to ultrafine grinding. The antioxidant ability is reversely proportional to the size of the wheat bran fraction. Hemery *et al*. (2010) also confirmed that the ultrafine comminution increased the surface area of wheat bran elements, which ultimately led to production of more p-coumaric acid, synapic acid and ferulic acid. Other studies showed that fine grinding led to an increase in the proportion of phenolic acids, flavonoids, anthocyanins and carotenoids compared to untreated (Brewer *et al*., 2014).

Wheat bran and germ contain nutritionally valuable ingredients such as phytochemicals and micronutrients. More phenolic compounds were found in the bran part likened to the polished grain part containing mainly endosperm (Liyana-Pathirana *et al*., 2006). Vitamins, carotenoids, phenolic acids, lignin, tocopherol and flavonoids are also concentrated in various parts of wheat bran. Wheat bran has several health effects and phytochemicals like phenolic acids, carotenoids and flavonoids. Phytochemicals are vital external sources of antioxidants in food (Ou et al., 2002).

Wheat bran as a compact spring of fibre could be a healthy food. Many health assertions have been made that combine optimal food intake with a reduction in cardiovascular disease and type 2 diabetes. The function of DF in ensuring standard glucose and cholesterol level was confirmed by some studies (Topping, 2007). Wheat bran is a good cradle of bioactive components including Dietary Fibre, and is used only to a limited extent as a food component. This is relatively due to the undesirable influence on the organoleptic and functional properties of foods enriched with bran.

2.8 Whole Foods

Food processing is termed as all procedures and technologies applied in food company in turning new produce into products (Monteiro *et al*., 2010). These categorised products into three classes: unprocessed or minimally processed food, processed cooking ingredients and ultra-processed products. The differences are in economic, social, traditional and other aspects of community health food as well as organic aspects. Whole foods are plant foods that are unprocessed, unrefined, or processed and refined slightly before consumption (Bruce *et al*., 2000). This group of products includes whole grains, tubers, legumes, fruits and vegetables.

2.9 Dietary Fibre

Dietary fibre is the remains of the eatable part of the plant and similar carbohydrates not susceptible to breakdown and assimilation in the gut of a person with full or partial fermentation in the colon. It consists of oligosaccharides, polysaccharides, lignin, and related vegetal substances. Dietary fibre has some of the following characteristics: swelling and softening of feces, lowering blood cholesterol, and glucose (AACC, 2001).

Dietary fibre comprises of many non-starch polysaccharides that include hemicellulose, cellulose, ß-glucans, pectin, lignin and gums. High-fibre diets are related to the decrease and management of certain ailments for example coronary heart diseases and diverticular (Villanueva-Suarez *et al*., 2003).

Dietary fibre in addition to its nutritive, purposeful and technical properties can be used to improve produce and by-products as food components. The physical properties are in relation to the combination of physical, biochemical and functional properties of dietary fibre. The fibres have functional properties such as water absorption, water swelling, oil retention that may be suitable in products that require water to prevent breakdown, stable high fat food, improve performance and emulsion products, as well as textures and change the viscosity (Elleuch *et al*., 2011).

Dietary fibres obtained by dissimilar processes and sources function in a different way during their passage through the digestive tract. It results from their chemical composition and physicochemical properties as well as from food processing. The recommendation for daily fibre intake is 25–30 g / day (Chau and Huang, 2003). Consumption of fibre incorporated food would help in overcoming fibre deficiency (Fernandez-Gines *et al*., 2003). When vegetable fibres are incorporated to food material, it increases water absorption and thickness of the product.

Dietary fibre is very useful in the control of human bodies. It does not break down in the human intestines and does not affect the absorption of moisture in the GIT. This increases the amount of food in the bowels and stomach, increase the feeling of fullness and contribute to weight loss (Manzoni *et al*., 2008). Dietary fibre can promote gastrointestinal motility to relieve constipation and absorb detrimental substances in the intestine, encouraging their discharge (Borycka, 2010).

Also, dietary fibre can increase duodenal flora and make available energy and nutrition for the production of probiotics. Current studies showed that dietary fibre decrease postprandial blood glucose, triglyceride concentrations and insulin (Ma and Tu, 2016) and can reduce the level of blood cholesterol. Decreased bile acid concentrations in the faeces correlate with the reduction of cancer risk (Kritchevsky, 1997). Dietary fibre reduces the period of passage in the intestine, add bulkiness to stool, reduction in total low-density lipoprotein (LDL) and buffers excessive amounts of acid in the stomach (Méndez-García *et al*., 2011).

The significance of fibres steered the growth of huge and probable marketplace for fibre-rich food and raw materials. There is now tendency to seek novel sources of DF, such as agro processing waste such as fruit pomace, which have locally been underestimated (Rodriguez *et al*., 2006). There is a predisposition to search for alternative supplies of dietary fibre for use in a manufacturing company. Commonly ingested fibre foods are grain products. However, in the last decade, high-quality fibrous matters from fruits (citrus, apples) are continually presented to the international markets. Generally, fruit fibre concentrates have a better nourishing value than the ones present in cereals because of high content of biologically active compounds present (Fernandez- Lopez *et al*. 2009).

The inclusion of fibre in usually consumed foods is a method of controlling the calorie bulkiness in food and provides the well-being related to fibre intake. Fruit and vegetable processing waste stream contains high fibre ingredients mixed with significant quantities of phytonutrients (Karle, 2012).

Lately, food producers have reacted to shopper demand for higher fibre content foods by developing food from high-fibre components. Dietary fibre can also impart certain functional properties to food, such as increased ability to retain water, ability to retain oil, emulsification and gel formation (Pakhare et al., 2016).

2.9.1 Types of Dietary fibre

Fibre is usually categorized as insoluble (IDF) and soluble dietary fibre (SDF) (Gorinstein *et al*., 2001). Solubility means water- miscible fibres. It was initially intended that the classification could be a modest way for predicting physiological function which was not the case. The SDF and IDF ratio is essential for both nutritional and purposeful properties. It is believed that fibre sources appropriate for food use should have an SDF - IDF ratio of approximately 1: 2. (Jaime *et al*., 2002). This considerably influences the sensory evaluation of the product.

Dietary fibre classified into soluble and insoluble fibres has very diverse but complementary function in the intestine. Soluble fibre consists of complexes as mucilage, beta-glucans and pectin. Soluble fibre provides important viscosity, lubrication and volume in the abdomen and gut, but is destroyed in the colon by the natural bacteria present in the colon. Alternatively, insoluble fibre includes complexes like hemicellulose, lignin and cellulose which maintain an open sponge-like structure that gives appreciated bulk and pore space, which evenly distributes pressure and pass out of the body largely unmodified. Additionally, lignin lowers the digestibility of other fibre components (Suter, 2005).

2.9.2 Significance of Dietary fibre

Currently, foods with high dietary fibre content are being developed to benefit from their dietary and nutritional functions. Dietary fibre included to food ingredients must be suitable as a dietary constituent in order for it to be acceptable. Garcia-Amezquita (2018) reported that "ideal dietary fibre" needs to meet the subsequent requirements; do not contain nutrient damaging ingredients, as concentrated as possible, as soft as possible on palate, colour and smell, have a stable components and suitable quantity of biologically active compounds. They are well stored, well suited with food processing and express physiological effects. It should be noted that the improvement of fibres not only increases the total value of foods but varied their functional properties and also considerably control the organoleptic perception of the product. The stickiness of the soluble fibre is more vital than the quantity of soluble fibre in food (Gorinstein *et al*., 2001).

Dietary fibre segments could be utilised in food processing company with exceptional results. Fibre with 15% soluble dietary fibre can bind and support the weight of water several times. Low consumption of dietary fibre is reportedly related with several disorders of the human body, including diverticulosis and colon cancer, ischemic heart disease, constipation, diabetes and other gastrointestinal tract diseases (Tungland and Meyer, 2002). It has been found that these conditions are practically absent in populations with a diet rich in fibre (Tungland and Meyer, 2002). Fibre increases water capacity and stool mass because hemicellulose and cellulose absorb water and swell. Fibre also reduces blood cholesterol (LDL) levels and improves good cholesterol levels. The incorporation of fibrous substances into the diet is vital for normal bowel function, supporting regularity, soft stools and rapid transit times, thus decreasing the possibility of colon cancer and heart disease (Suter, 2005).

Dietary fibre also increases constant blood sugar levels, reducing the insulin mechanism for converting sugar in the blood into valuable energy (Adegoke *et al*., 2006). Helps control weight by delaying relapse of hunger, improves gastric health by improving intestinal viscidness, gut motility, and shortens the passage within the digestive tract to reduce colon and rectal cancer, including diverticulosis. (Jove, 2020). It fixes bile acids and lipid constituents like cholesterol and encourages their removal in so doing decreased the plasma cholesterol level and danger of coronary disease (Jove, 2020). Excellent origin of dietary fibre includes, vegetables, whole fruits, cereal grains and legumes.

2.10 Polyphenols

Phenolic compounds are largely spread all through the vegetal kingdom and contain both simple compound like phenolic acids and difficult polymerized moiteis, Their properties, especially their antioxidant properties led to studies into their health defending characteristics and related devices. This is mainly due to their ability to act as an antioxidant and may also have anti-inflammatory effects (Barth *et al*., 2005).

Further studies have shown that polyphenols can reduce cell damage and, therefore, can be helpful to perfect human health and protect against numerous ailments related to oxidative phenomena such as cancer and diabetes, cardiovascular and respiratory ailments. Many studies showed close relationship between polyphenolic compounds, which have an antioxidant effect and reduced the risk of several diseases (Fu *et al*., 2011).

The health gains of antioxidants of biological sources are related to their function in the inhibition of many diseases known as oxidative pressure pathologies. They are associated with detrimental effects of free oxygen radicals or usually reactive oxygen species as well as substances of ordinary breakdown that becomes injurious if not nullified by cellular antioxidant defense systems. In oxidative stress, an unrestrained oxidation process can happen, injuring biotic molecules, impairing cellular roles and possibly leading to the growth of one or more ailments (Valko *et al*., 2007).

Future cohort studies have consistently shown that these phytochemicals in whole foods can provide significant defense against protracted ailments for instance cardiovascular disease and definite cancers. The complementary and synergistic properties of the bioactive phytochemicals contained in plant can be responsible for the health benefits of nutrition. In addition, phytochemicals, which are contained in various collections of plant foods, supplement each other when eaten (Okarter and Liu, 2010).

Polyphenols, a large group of chemical substances present in plants have fascinated much consideration lately for their properties and health benefits when treated as food ingredients or as a supplement. Phenolic compounds are among the widespread groups of compounds in the vegetable kingdom. It is assessed that over 8000 complexes have been segregated and labeled (Ramos, 2007). Polyphenols are polyhydroxylated phytochemicals with ordinary configurations. They are categorised to three main sub-sets, the phenolic acids, flavonoids and the stilbenoids. Many of the isolated complexes belong to the flavonoid group.

Structurally, phenolic complexes are different from modest particles, for instance phenolic acids, extremely polymerized compounds like proanthocyanidins that are found in foliage widely distributed in several foods and drinks. The most available phenols in the human food are phenolic acids, tannins and flavonoids (Dai and Mumper).

Polyphenols are good for health through a variety of means, comprising the removal of free radicals, safety and restoration antioxidants in food and the complexation of prooxidative metals. The type and content of phenolic resins are diametrically different between plants, which are mainly esterified or glycosylated. They have beneficial effects such as antioxidant, antibacterial activity, immunomodulatory effect and anti-cancer effect (Lima *et al*. 2014). Several studies have shown improved wound healing with these phytochemicals.

Some research has revealed the antioxidant property applied by polyphenols like flavonoids, especially with respect to the scavenging of several reacting species. Flavonoids are mainly regarded as the diphenylpropane moiety (C6-C3-C6) found naturally in plants and can be shared into six classes: anthocyanidins, flavones, isoflavones, flavan-3-ols, flavonols and flavanones. Flavonoids can also be used for other purposes, for example decrease in the risk of cardiovascular disease, antimutagenic activity, antiproliferative effect on tumor cells, radioprotective effect, protection against atherosclerosis, hair tonic, antimicrobial properties and hormonal storage in natural conditions. menopause women (Yao *et al*., 2004).

Numerous exact biochemical activities of polyphenols are identified. A remarkable practical application of flavonoids is the modest prevention of tyrosine kinase at its ATP joining site. The enzyme is a sheath receptor, which is described by a remarkable enzymatic action, a rise in level of manifestation can cause many proliferative diseases, like cancer and psoriasis (Lima *et al*., 2014).

Citrus peel is the richest source of biologically active phenolic compounds, especially flavonoids, with a rather high content of polyphenols compared to edible parts. Flavonoids present in citrus fruits include flavones, flavonones, flavonols, isoflavones and anthocyanidins (Senevirathne *et al*., 2009). The valued effects of citrus peel against definite degenerative diseases as an anticarcinogenic and anti-inflammatory have been observed (Imran *et al*., 2020).

2.10.1 Polyphenol as Natural and Synthetic Antioxidant

Most polyphenols are grouped as ordinary antioxidants that show their usefulness in oxidative tension. Antioxidants are complexes that prevent or slow down the oxidation of other free radicals by hindering the start or multiplication of oxidative reaction chains. Recently, research has identified potential health danger associated with the utilization of artificial antioxidants and stringent rules control their use in foods (Panicker *et al*., 2014). Some artificial phenolic antioxidants used in the food company include 2-tert-butyl-4-methylphenol (TBMP), tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and gallic acid esters. These supplements of antioxidants are believed to be unhealthy. High doses of TBHQ adversely affect the health of laboratory animals, for example, due to DNA damage that can lead to tumors in the stomach. BHA was described as a growth starter and multiplier in certain animal muscles (Dolatabadi and kashanian, 2010).

Phenolic compounds are normally used to reduce detrimental properties of compounds with high oxidative capability. The antioxidant activities of polyphenols are primarily due to their redox activities that make it possible to function as decreasing proxies, hydrogen donors and mediators of singlet oxygen. (Lima *et al*., 2014). Compound categorized with antioxidant activities included reactive oxygen species, hydrogen peroxide (H_2O_2), superoxide radical, hydroxyl radical (OH•) and singlet oxygen (O_2) (Phaniendra *et al*., 2015).

Oxidizing moieties usually called free radicals, oxidizing moieties include reactive oxygen species (ROS) and nitrogen (RNS) Reactive species are known to encourage lipids, glucose and proteins oxidation. The products formed during lipoxidation are malondialdehyde, glyoxal, acrolein, 4-hydroxy nonenal (HNE). These compounds react with amino acids leading to final aminoglycosation products (AGEs). AGES are implicated in usual events that can cause obesity, diabetes mellitus II, insulin resistance and inflammation in several tissues (Brownlee, 2005).

2.11 Extrusion cooking

The extrusion technology is a high temperature short time process (HTST) where wet material is fed into an extruder at the known temperature and pressure within residence time. (Sobowale *et al*., 2016). During the operation heat is not applied instead is obtained by the shear force in the extruder.

Extrusion processing is a useful and effective way to turn food materials into processed foods. It can substitute several indigenous processes in the foodstuff and feedstuff industry owing to its exceptionality, usefulness, high efficiency, low cost of possession, energy effectiveness, superiority of products and improved absorption and organic value of proteins. This is an extremely versatile process for food production, which allows continuous and flexible processing, which allows the manufacture of products with various textures and forms (Choton *et al*., 2020). Extrusion technology is a process that is gradually being utilised in the food industry to develop new products like cereal-based snacks, baby food, fibre modified cereal starch and breakfast cereals. The high temperature short time process reduces microbe infection and render enzyme inactive. The key technic for protecting hot and cold extrudate is the activity of water in the extrudate (0.1-0.4) (Bordoloi and Ganguly, 2014). High fibre extruded products can improve total nutrient content and taste by including high protein ingredients.

Extrudates based on flour of corn grits, rice, barley and their combinations are largely used in the extrusion industry. However, addition of by-products from various sections of fruit and vegetable processing has encouraged its novel use, in addition to the increased nutritionally advanced extruded products (Alam and Kumar, 2014).

The extrusion machine consists of a recessed screw Archimedes that revolves in a tight-fitting cylinder-shaped tube. The food materials are pre-crumpled and stirred together before being introduced into the extruder. The impact of flight on the auger propels food forward, processing and mixing ingredients into a viscous mass similar to dough (Muredzi *et al*., 2013).

Extrusion involves the following four stages (Aworh, 2014), where various ingredients are added to the extruder barrel, where the raw ingredients in the form of particles are converted into a homogeneous viscoelastic mass under high pressure and high temperature. Cooking, in which a further increase in pressure and temperature affect the product structure, colour, functional properties and expansion of the extrudates. As with other heat treatment processes for food, extruded cooking can exhibit desirable and unwanted outcome on nutritive value. Favourable outcome include gelatinization of starch, an increase in soluble fibre, the destruction of anti-nutritional factors, a reduction in lipid oxidation and contamination of microorganisms, the preservation of the natural colours and taste of food products. In addition, the extrusion process denatures undesired enzymes and sterilizes the finished product (Singh, 2007). Despite these advantages, the disadvantages of this method are the shortcomings of the Maillard reaction, leading to decrease in the nutritive value of the protein and damage of thermo labile vitamins (Nikmaram *et al*., 2015).

Regarding starch, larger molecules of fibre molecules are removed during extrusion, may combine into huge insoluble molecules or Maillard compounds, commonly referred to as lignin. The physico-chemical change can significantly affect the health advantage of extruded food. This method used in the development of strong starch (MS) for identified set of people, such as diabetics. The extrusion process contributes to the depolymerization of starch, resulting in increase in easy to digest carbohydrates with a high glycemic index (Brennan *et al*., 2013).

The cooking process in extrusion depends on two classified group factors that represent feed properties. Moisture content, particle size, feed constituents, type of feed and processing conditions of the extruder; screw speed, screw geometry and cylinder temperature (Muredzi *et al*., 2013).

a) Feed properties

Moisture is main factor in extrusion cooking. Reducing the amount of water in the feed increases viscosity, reduces productivity, increases pressure drop and increases energy consumption. However, when starch is extruded, gelatinization is more likely to occur at higher levels of moisture. The type of feed in relations to starch, protein, lipid and moisture produce a vital function in the type of the extrudates. In addition, the internal configuration of the extrudate is also influenced by difference in components (extrusionfactors.edu).

i) pH of Ingredients

Modifying pH of feed can affect the state of the proteins during extrusion thereby affecting the physical properties of the extrudates. Studies showed that increased pH in the range of 5.5 to 7.5 led to increase in unstable compounds in the starch mix during extrusion. High and low pH values led to decrease in tensile strength. A change in pH can also lead to change in the appearance and nutritive value of the extrudate (extrusionfactors.edu).

ii) Particle Size of Feeds: Starch with grain size exceeding 14 mesh is difficult to gel. Small grains (40-120 mesh) are easier to hydrate and boil than larger particles (extrusionfactors.edu).

iii) Constituencies of feed materials

Oil and emulsifier can be incorporated to the feedstock to reduce the viscidness of the feedstock. It also help in lubricating the cooking process by extruding that type of material reduced the viscous heat removed Among conditions affecting the extrusion process is the oil content in the material. Generally, it is essential that the sample has the highest average oil content of 6.5 to 8.5% before extrusion (soybeans.umn.edu).

b) Extruder Operating Parameters

Feed rates are usually maintained low to allow the extruder work under state of hunger. If the diameter of the foot of the screw increases in the modification zone and the size of the channel decreases, the crew is completely filled when the material enters the measuring zone (extrusionfactors.edu).

i) Screw Geometry

In single-screw extruder, processing factors such as screw pitch, the distance between the apex of the screw and the cylinder, the screw diameter and the number of cavities in the screw can be set.

In double screw extruder, there are many possibilities for screw geometry and configuration range. Factors to be adjusted in double screw extruder include screw pitch, root diameter, number of passes, degree of engagement of the two screws, the angle of the screw, and the mixing device.

ii) Screw speed

The screw speed influences the extent of filling inside the screw, the energy transfer rate and the machine-driven energy into the extruder, retention time of the extrudate and the cut action on the ingredients. The screw speed usually range from 100 to 500 rpm, the standard minimum screw speed is from 70 to 100 rpm.

iii) Barrel Temperature

Energy generation during friction needs the barrel to be cooled by cold water. The temperature of the extrudate can attain 180 °C. To avoid burning of material on the surface of a hot cylinder or to prevent extreme Maillard darkening or reduce protein degradation, cold water can be introduced to the cylinder. Reduction in temperature of the ingredients can be achieved by increasing the amount of fluid or reducing shear which can be achieved by reducing screw speed (Muredzi *et al*., 2013).

2.11.1 Extrusion effect on extrudate functional and physical properties

Extrudates are one of the most popular functional foods in which the potential of dietary foods and their by-products or biological residues to develop food can be used at a lower cost. The cost of producing food and other products depends on the manufacturer's ability to use all products of this process. The chemical composition of ingredient, the degree of gelation during processing and the denaturation of proteins affect the stability of the extrudate as well as other physical properties (Chevanan *et al*. 2009).

During extrusion, mixture of moisture, mechanical shear, and pressure shear can partly destroy the protein and gelatinize the starch in the feed materials. Extrusion denatured the protein, starch, fat and fibre constituents of cereals and forms complexes that can affect cholesterol levels. Extrusion cooking leads to fragmentation of starches, proteins and polysaccharides that are not cushions, forming reactive molecules that can form new bonds that increase health potential (Kahlon *et al*., 2006).

Pre-extruded flour suspensions viscosity can increase with a low possibility to form lumps, as the starch particles modified and have high absorption ability in cold and hot environments, therefore extruded flour is suggested in the provision of instant products (Adeleye *et al*., 2020).

In extrusion, expansion is the main quality index associated with fragility of the extrudate water absorption, solubility in water and crispness. During extrusion, viscoelastic materials are pushed through the die so that a swift drop in pressure evaporates part of the water and creates an expanded spongy structure. The solubility index in water (WSI) of extruded products can be many times higher in relation to raw materials and varies by 40-50% (Rzedzicki, 2005). Starch in foods becomes debilitating and therefore available for digestive enzymes. Due to this intense conversion, extruded starch products are classified by a high glycemic index (GI) (Sobota and Rzedzicki, 2009).

An important functional ingredient in extrusion operation is starch, basically accountable for the enlargement of extrudates. The biopolymer is made up of two forms of macromolecules, namely amylose and amylopectin (Brouillet-Fourmann *et al*., 2003). The amylose-amylopectin ratio is actual useful in envisaging the quality of starch-based extrudates. Amylopectin is responsible for the increase in starch at extrusion. The more the proportion of amylopectin in the mixture, the lighter the elastic and homogeneous texture with a smooth and viscous outer structure. In contrast, mixtures with more amylose contents result in tougher and less foamed the extrudate.

Starchy ingredients experience significant variations in the physical structure of starch particles during the extrusion operation. Variations in starchy properties of food from addition of lipids resulted in the development of compounds between amylose and lipids. While, it was found that the largest branched amylopectin molecules decompose under the action of mechanical forces due to shear through the extruder (Cai *et al*., 1995).

The starch expansion vastly depends on the extent of gelation in the extruder. The shear and pressure resulting from extrusion define the degree of starch gelatinization. Extrusion resulted in gelatinization of starch, inactivation of numerous inherent enzymes, a decrease in the number of microorganisms, enhancement in the digestibility and biotic value of proteins, as well as a decrease in the action of certain anti-nutritional factors (Mouquet *et al*., 2003). The gelatinization of starch during extrusion is crucial as it affects expansion and digestion and contributes to the stability of the water.

Starch gelled during processing depends on constituent size, starch and extrusion conditions. Extrusion can also improve the palatability and breakdown of the feed, deactivate nutritional inhibitory factors and destroy pathogens in the feed thereby providing producers with tools to advance the quality of their extrudates (Adeleye *et al*., 2020).

2.11.2 Extrusion cooking effect on dietary fibre

Processes such as extruder preparation, including boiling in combination with homogenization, can alter dietary fibre, both physiologically and in fibre fraction. The raw material is subjected to strong mechanical shear in the extruder under the action of rotating screws. Machining completely dislocates the initial arrangement of the fresh material. Extrusion cooking technology exposes the material to high pressure, temperature and shear, which leads to rapid gasification of internal moisture, expands and modifies the spatial structure of intermolecular and intramolecular fibres (Yang *et al*., 2017).

During processing, the molecular configuration of the ingredients changes. The thermo mechanical character of extrusion has the additional ability to redistribute soluble and insoluble fibre constituents in favour of the former. This can improve the hypocholesterolemic properties of dietary fibre and, thus, improve the fibre profile (Gajula *et al*., 2008).

Other researchers showed that extrusion has helpful reaction on total and soluble dietary fibre. Insoluble fibre reduced markedly at various processing stages because of the breakdown of covalent and non-covalent bonds in the carbohydrate and protein parts resulting to tiny more soluble molecular pieces (Rashid *et al*., 2015).

2.11.3 Effect of extrusion on the antioxidant

Different studies on extrusion technology concentrated on the development of functional products with legumes cereals (Repo-Carrasco-Valencia *et al*., 2009), fruits (Escalante-Aburto, 2014), fruit extracts and vegetables (Stojceska *et al*., 2008). Research showed that the extrusion cooking process can reduce the concentration by structurally altering antioxidant compounds such as carotenoids, anthocyanins and polyphenols (Obradevic *et al*., 2015) mainly due to high process temperature.

2.11.4 Types of Extruders

Depending on the operation, extruders are classified into two types; Hot and cold extruders and depending on the design; single screw and twin screw.

2.11.4.1 Hot extrusion

Cooking food above 100 °C is referred to as hot extrusion cooking. Friction heat and additional heat cause a rapid rise in temperature. The food then entered the barrel part with the smallest spans, where the pressure and shear are even greater. Finally, pressed from opening(s) (die) on the cylinder, the extrudate comes out under the pressure of the die, enlarge to its ultimate form and cool sharply as the water evaporates like water vapour. (Bordoloi and Ganguly, 2014).

2.11.4.2 Cold extrusion

Cold extrusion process is one in which temperature of food remained at room temperature, it is in mixing and forming food products like pasta and meat products. Extrusion at low pressure and temperatures below 100 °C is for production of licorice, surimi fish paste and pet food (Bordoloi and Ganguly, 2014).

2.11.4.3 Single screw extruder

Single screw extruder consists of a screw that revolves in a fixed cylinder and uses friction forces to increase maximum material productivity. Three categories of flows are related to single screw extruder: resistance, leak, and pressure flow (Ajita and Jha, 2017). The flow resistance results from the friction between the ingredient and the inner drum, into which the material is displaced as a result of the rotational movement of the screw. A small leak leads to reduction in the net power, because leaks occur in space between the screw and the cylinder, leaving only the necessary gap in-between the screw and the cylinder.

The damper plate, comprising of a break plate and dies has limitation with which the extruder screw thrust material. The limiters create pressure in the extruder that are important for improving mingling of products, but give rise to net loss of extruder capacity as a result of increased pressure (Ajita and Jha, 2017).

Food constituents are usually gummy during processing and likely stick to the rotating screw, which in turn reduces the output from the extruder. Single screw extruders for food process are quite easy for materials with a big resistance factor as in corn or rice grits.

Such grits could be extruded at pressure of about 15-20 mPa and they are the main material for the development of straight extruded snacks or breakfast cereals. A simple autogenous extruder is sufficient for these materials; with a small screw length (L) and diameter (D) ratio (L / D = 4-6).The major shortcoming of single-screw extruders is inadequate blending of the material. Single-screw extruders also exhibit restricted performance, particularly when using many component raw material blends. Single screw extruder is frequently believed to be a poor mixer, and no dry or liquid materials can be added to the extruder barrel. Therefore, all ingredients are mixed in a mixer before being introduced into the extruder.

2.11.4.4 Twin-Screw Extruder

Twin-screw extruders have more complicated construction. The most important features are lower energy utilization and the capacity to expand the range. The only downside is the more complicated construction and the cost (Riaz, 2012).

Currently, co-rotating food extruders are widely used because of their great efficiency, excellent mixing and screw speed (up to 700 rpm). They are typified by excellent material handling, miscibility, plasticization and extrusion efficiency.

Self-clamping and locking bolt movements successfully push the material to move thus blocking any material in the space between the cylinder surface and the bolt. That is why double screw extruders are frequently called self-scrubbing equipment. The movement of mixed materials in co-rotating twin screw extruders is trouble-free and stable without C-shaped chambers or characteristic imprints of angular waves on the surface of extrudate

(Maskan and Altan, 2011). This is a key condition in using twin screw extruders for producing crisp bread or biscuit fingers. Description of physical processes occurring in ingredients transfer mechanisms in double-screw extruders and the associated heat transfer is more complex than in single-screw extruders.

The opposite-revolving double-screw food extruders are exceptional machines. The screws revolve much slower (up to 150 rpm), but successfully mixed the ingredients and their function is similar to progressive dislocation pump, which produces high pressure in a closed C-shaped chamber on the screws (Maskan and Altan, 2011). The reflux of ingredients in the double screw extruders is negligible because of the shortest gap between the screws and the cylinder. They are mainly used to manufacture chewing gum, confectionery and to process fibrous and cellulose-containing materials. The use of double screw extruder for the manufacture of simple extruded type would be uneconomical and energy consuming.

There are many observations that are important in everyday extrusion operations. The water in the ingredients and particle size should be uniform to prevent extruder uneven operation and confirm the expected quality of extrudates. The small amount of water in the ingredients will affect the extrusion pressure, but has no significant effect on the extruder operations (Karwe, 2009). Intensive drum cooling helps to reduce the temperature and increase friction in the material associated with the standard expected of the extruded product. A decrease in material temperature increased the viscosity and progressively affects the extruder performance. Blocking several die openings ensure a swift increase in pressure and leading to strong backflow and even to machine blockage.

Modern twin-screw food extruders are designed so that raw materials can be fed through many extruder feeders, also at various positions in the barrel. Liquid components can be supplied differently to be additional gain. The base ingredient is properly introduced into the feeders of the single and double screw inlets.

2.11.5 Advantages of extrusion cooking

Extruded cooking destroys microorganisms and inactivates enzymes that alters the attribute and reduce the storage time of food. The high temperature, shear and compressive stress of extrusion processing provide a viable bioreactor environment for destroying natural thermo labile anti-nutritional factors and degrading aflatoxins in food (Aworh, 2014).

It breaks the covalent bonds in biopolymers, and forceful structure distortion and mixing enable changing the functional properties of fresh ingredients. Also, it sterilizes the processed food, preserves the original colours and aroma of the food (Saalia and Philips, 2011).

2.12 Trends in Evaluation of Food Toxicity and Safety

Toxicity tests on food or ingredients can tell us what negative effects are likely and how they can occur. However, this alone does not tell whether one can eat safely in normal quantities. In modern industrialized countries, food is generally considered to be low in risk, but some widespread food safety concerns have sparked consumer concerns about food safety.

Toxicology is a scientific discipline that studies the actual or potential threat of the detrimental effects of a substance in living organisms and environment, the connection between these harmful effects and exposure, the device of action, analysis, inhibition and management of poison (Majeed, 2017).

"Risk" means the likelihood that a substance will cause injury under certain exposure conditions. The risk concept takes into account the dose and duration of exposure as well as the toxicity of the chemical and is a better indicator of food safety. There are three main classes of hazards in food: (1) microbiological or ecological pollutants, (2) naturally occurring toxic ingredients, and (3) those caused by intentional food additives or new foods or ingredients (Rather *et al*., 2017). The most deadly contaminants are those that are caused by bacteria or mold entering the food, which produce toxins that stayed in the food after the destruction of the biological carrier.

The toxicity analysis process has changed over the past thirty mostly due to animal welfare. The development of animal mortality to determine LD50 (dose of irritant) is not the primary goal of severe toxicity studies. Currently, severe toxicity focused on the degree of severe tolerance, the type of severe toxic symptoms in the sub-lethal region, and dosage that lead to the death of several animals. The rule is applied when using 3 to 5 animals per sex and dosage group.

The goal of non-clinical sub acute and chronic tests is to identify target toxicity organs, response to administered doses, relation to "internal exposure" and the possibility of reversal. "Internal exposure" defines as levels in the blood or tissues obtained in animal toxicological studies after the meting out of different doses of the drug.

Appraisal of 'internal' contact in animal and human experiments leads to 'safety factors' that was more reliable in assessing the toxicological danger in clinical experiments and in the pharmaceuticals / food on the market. This information is important for estimating the starting dose for human studies and for identifying specific organs and cells for clinical observation of possible side effects.

2.12.1 Rat study in toxicity test

Rats, mice and other rodents constitute over 90% of the mammals used for medical investigation. Both man and rats have many resemblances in configuration and function. Furthermore, rats are ideal for laboratory experiments due to their tiny size, low cost, ease of use and capability to reproduce in confinement.

The decision that all novel drugs and food need to be verified for animal safety before they can be tested in humans is built on the postulation that animals react to drug trials such as “small people" (Neavis, 2018).

Rats study is the central topic in product security tests. One of them is the repetitive-dose severe toxicity test that is used to test the material for the reactions of continuing exposure. Another test is a growth and procreative toxicity test that deal with the possibility of barrenness or the effect of a product on pregnancy.

2.13 Current Trends in Shelf Life of Foods

Consumers need harmless and superior products with excellent touch, appearance and taste, as well as a storage time of several weeks or months (Corrigan *et al*., 2012). Quality changes, not microbial safety alone are crucial for the shelf stability of foods (Corrigan *et al*., 2012). Food shelf stability is the time frame which the food maintains a satisfactory level quality in terms of safety.

Food preservation depends on knowledge of the procedure of reactions and the effective restriction of those who bear the greatest responsibility for the loss or damage of desired properties, and occasionally directs other responses to valuable changes. Shelf life studies could be done by (1) Real-time testing (2) accelerated shelf life testing.

2.13.1 Real time testing

Running a complete durability test for stable products during real-time testing can be very costly and time-consuming, which is why accelerated durability tests are often used. To predict the length of the preservation of perishable products, several models have been developed, most of which are based on the development of Specific Spoilage Organisms (SSOs) (Kreyenschmidt *et al*., 2010). To estimate residual shelf life at various stages of the distribution, rapid methods to determine the microbiological number of SSOs are required. Some of the rapid methods already exist, but some are not yet applicable to certain types of microorganisms.

Another possibility is to predict the remaining shelf life from the product temperature history - which requires continuous product temperature control. Currently, purchasers expect products with excellent look, texture and taste, while maintaining their nutrient Therefore, the food industry must conduct kinetic studies when a new or modified product must go on sale. There are many ways to conduct storability tests described in the literature but many of the studies are based on kinetic theory. (Kilcast and Subramanian, 2000).

Performing a full shelf life test over the total expected shelf life of an industrial product with a long shelf life could be very material intensive and greatly delayed time for sales.

To solve this problem, food scientists are undertaking expedited expiration studies where the processed food is subjected to relatively harsh storage condition. Since most denaturation process are similar to Arrhenius, the greater the temperature, the quicker the products reach fast levels of degradation (Kilcast and Subramanian, 2000).

2.13.2 Accelerated tests

Accelerated tests collect data for different keeping conditions at various times and generate kinetic diagrams (shelf life chart). By assessing the response rate profile, the order of reaction can be determined and translate the outcome from expedient expiration tests into actual sales conditions. This is determined as the proportionality constant between different storage temperatures (Kilcast and Subramanian, 2000).

An additional significant kinetic parameter that has been evaluated in shelf life studies is the activation energy (Ea), i.e. least energy required for decomposition. The equation shows that activation energy is linked to constant rates at various temperatures. Although some parameters are specified in regulations or other local market needs, usually the Scientist should determine the criteria for use. This problem turn out to be more difficult when many variables are analyzed and each of them requires its own measure and sensual features, it is difficult to determine which is the utmost significant for determining the ultimate expiry period of the manufactured goods.

2.13.3 Multivariate techniques in shelf life studies

Multivariate analysis techniques provide a number of useful durability studies tools that need to monitor many different properties. The commonly used methods is principal component analysis (PCA), the purpose is to find a new dimension in multidimensional ways that describes the data structure. These new dimension are called Principal Components (PC) and are created by linear mixtures of the source variables (Naes *et al.*, 2004).

PCA and other data decline methods have hitherto been used only as variable selection techniques for storage studies or to learn the association between sensory qualities and instrumental inquiry.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Preparation

Orange pomace was obtained after juice production from sweet oranges (Agege variety) at the juice processing pilot plant of the National Horticultural Research Institute, Jericho, Ibadan, Oyo State. The pomace was dried at 60 ± 2 ° C, ground with a laboratory mill (500 µm) and kept in an airtight 250 micron polyethylene until use. Soyameal and Wheat bran was purchased from an Agro processing supplier, Oluyole Estate, Ring Road, Ibadan, individually ground to 500 µm and packed for further use.

3.2 Experimental Design

Thirteen blends were processed by mixing the three basic ingredients; orange pomace (P) soya meal (SM) and wheat bran (WB). The proportions of these ingredients were obtained with a three component constrained mixture design (Table 3.1) (Cornell, 1983). Minitab 16 (Design-expert version 9.0.6, Stat ease Inc., Minneapolis, USA) was employed to obtain 13 design points of components (Figure 3.1). The lower limit of orange pomace, wheat bran and soyameal were 5%, 10% and 10% respectively while the upper bound constraints were 30%, 80% and 70%) were used to generate the design. The upper and lower limits were determined for orange pomace to meet the daily requirement of 30 g per day while soyameal was to contribute protein and wheat bran for its micronutrients. The response functions (Y) for the experiment model were the chemical and functional properties. The responses were related with the components ratio, the prediction equation for the model was described in Eqn. 3.1.

$$Y = AB + AC + BC + ABC + AB(A-B) + AC(A-C) + BC(B-C) + A^2BC + AB^2C + ABC^2 + AB(A-B)^2 + AC(A-C)^2 + BC(B-C)^2$$

.... (3.1)

Where A = orange pomace, B = soyameal and C = Wheat bran, AB, AC, BC, ABC, …. = interactive terms

Table 3.1: Formulations of orange pomace, soyameal and wheat bran blends as obtained in a mixture design

Run Order	Pomace (%)	Soymeal (%)	Wheat bran (%)
1	13	62	25
2	17	44	39
3	5	80	15
4	10	80	10
5	23	52	25
6	20	10	70
7	5	25	70
8	23	27	50
9	11	62	27
10	11	35	55
11	18	27	55
12	30	60	10
13	30	10	60

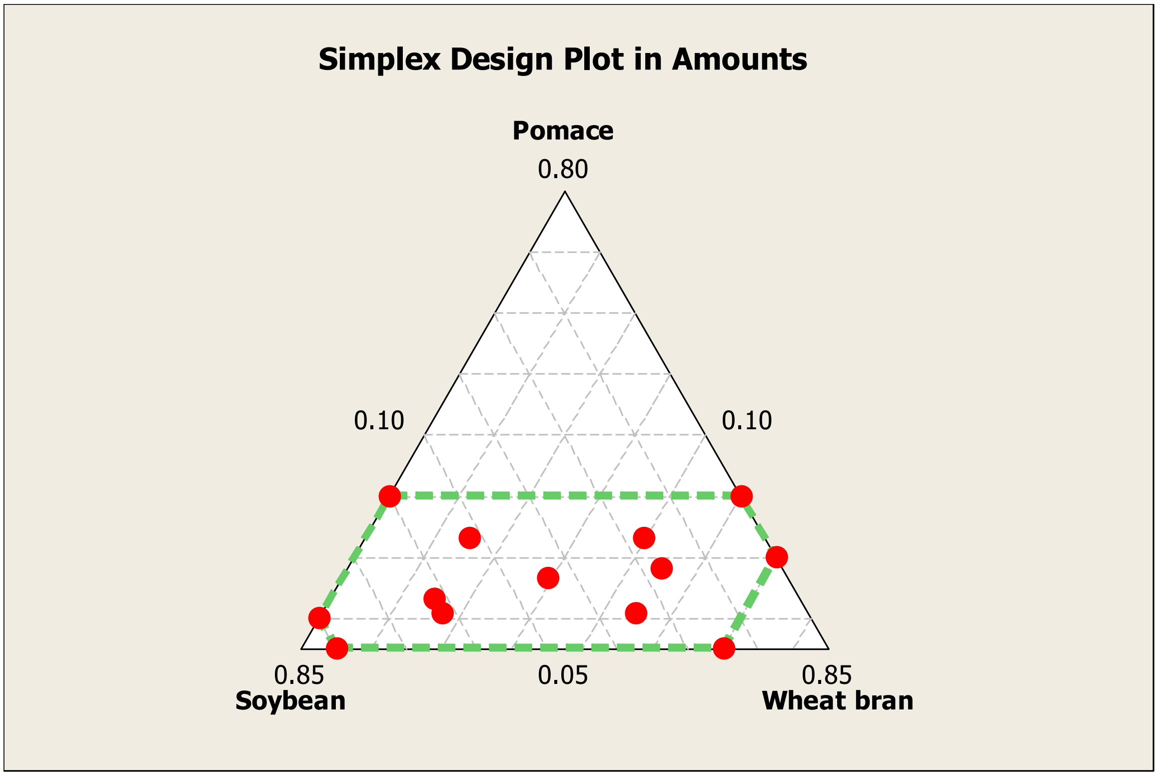


Figure 3.1: Constraint region of D optimal design plot

3.3 Extrusion cooking

Extrusion cooking was performed according to the modified Huang and Ma (2016) method. Mixtures of wheat bran, soybean meal and orange pomace with different mixing ratios (Table 3.1) were manually introduced into single screw extruder (FEXOD 302 Nigeria), with a 4 kW geared motor at a predetermined moisture content (between 20 and 22%). The extrusion conditions of 290 rpm screw speed, 110 ^{o}C temperature, and an average residence time of 2.8 minutes. The barrel diameter was 52.92 mm, the ratio of length to diameter was approximately 6:1, the compression ratio of 3: 1 and 4 mm hole on the die. Products were collected from the holes in the matrix, dried at 60 °C, cooled for 10 mins, and packed in a 250 micron plastic bag for analysis (Plate 3.1).

3.4 Chemical Composition

3.4.1 Proximate analysis of extrudate

3.4.1.1 Determination of moisture content of the blends:

Each sample (2 g) was weighed into a previously parched and assessed aluminum container. Aluminum can with the sample was moved into the oven conditioned at 100 ^{o}C to dry for 24 hrs. After 24 hours, the can with the sample was isolated from the oven, moved to a desiccator, air-conditioned for ten minutes and weighed (AOAC, 2015).

$$\%Mc = \frac{W_1 - W_2}{W_0} \times 100 \qquad \text{................ Eqn (3.2)}$$

W_1 = can and sample weight before drying

W_2 = can and sample weight after drying

W_0 = weight of sample before drying

Plate 3.1: Extruded blends of pomace, soyameal and wheat bran

3.4.1.2 Determination of ash content

AOAC method (2015) was adopted for ash determination. Samples of 2 g each were weighed in a porcelain crucible, moved to a muffle furnace set at 550 ° C for 4 hrs and ensured it turned to ash. The contents and crucible was cooled in a desiccator to around 100 ° C and weigh up. The reading was in triplicate. Ash content was obtained from the formula:

$$\% As = \frac{W_2}{W_3} \times 100 \quad (3.3)$$

Where %As = percentage ash, W2 = sample weight after ashing, W3 = sample weight before ashing

3.4.1.3 Determination of crude protein

Semi-micro Kjeldahl, procedure/technique (AOAC, 2015) was used to analyzed the protein in the extrudates. Aliquot amount (0.5 g) of finely powdered sample was carefully weighed into the Kjeldahl digestion tubes, one Kjeldahl catalyst tablet and 10ml of conc. H_2SO_4 added . The tubes were placed in a Digestion Block, heated in the hood and digested for 4 hrs to have a colourless solution. The digestion was air-cooled and cautiously poured into a 100 ml volumetric flask, the flask washed with distil water and filled to the mark with distilled water.

Markham distillation apparatus was used to carry out distillation of volatile substances, which enables the distillation of volatile substances such as ammonia. After complete distillation (10 min). The steam generator was at that point detached from the power source during the evolving emptiness to eliminate distilled water, 5 ml of the digest pipetted into the device body through a small cone opening. To this, 5 mL of 40% (w / v) NaOH was added with pipette.

The mixture was distilled for 2 min into a 50 ml Erlenmeyer flask with 10 ml of 2% boric acid solution that changed from red to green, indicating that all the ammonia released was collected. The solution was titrated against 0.01 N HCl. Nitrogen in the extrudate was evaluated by the formula:

% N = Titre value x Normality/Molarity of HCL used x Atomic mass of Nitrogen ………… (3.4)

Crude protein content calculated by multiplying the nitrogen percentage by a constant factor of 6.25…………………………..…………………………………………….. (3.5)

3.4.1.4 Determination of crude fat content

Determination of crude fat was done by AOAC (2015). Precisely, 1 g of extrudate weighed into a degreased extraction sleeve covered with cotton wool. The extractor covered and equipped with reflux condenser placed in a previously weighed 250 ml soxhlet flask. The Soxhlet flask was filled to 75% of its volume with petroleum ether and distilled for six hr. The heat source was monitored to ensure the ether boiled gently. The ether was allowed to siphon several times. Thereafter, the ether drained into stock bottle. The flask containing the oil separated, its outer part cleaned and dried to a constant weight. The percentage oil was obtained by the formula:

$$\frac{W_1 - W_0}{W_t} \text{ X } 100 \qquad \ldots\ldots\ldots(3.6)$$

Where:

Wo= the initial weight of dry soxhlet flask

W_1= the final weight of oven dried flask + oil

3.4.1.5 Determination of crude fibre content

Fibre was evaluated by the method of AOAC (2015), 2g of extrudate was weighed into fibre flask, 100 ml of 0.25 N H_2SO_4 added and refluxed for 1 h. The mixture filtered hot, the residue returned into the fibre flask, 100 ml of 0.313 NaOH added and refluxed for another 1 h. The mixture again filtered using a fibre sieve cloth. Acetone (10 ml) added to dissolve organic constituents present. The residue obtained washed with 50 ml hot H_2O and transferred to the crucible, oven dried at 105 ^oC overnight. The oven dried crucible containing the residue was cooled in a dessicator and later weighed (W_1), ashed in a muffle furnace at 550 ^oC for 4h and weighed (W_0). The percentage fibre:

$$\frac{W_1 - W_0}{W_t} \text{ X } 100 \text{ ………………………………………………………….(3.7)}$$

Wt – weight of extrudate

3.4.1.5.1 Total dietary fibre

Products were evaluated for total, soluble and insoluble dietary fibre fractions according to AOAC 991.43, and enzymatic-gravimetric procedure (Lee *et al*., 1992). Sugar in the extrudates was removed with 85% ethanol. Enzyme digests were clarified in tarred glass frit crucibles. After 1 hour the precipitates were filtered. One set each of insoluble and soluble fibre remnants was ashed in oven at 525 °C in 5 h. The total amount of fibre was calculated as the sum of the soluble and insoluble fibre.

3.4.1.5.2 Fibre fraction

Dietary fibre fractions, which include neutral detergent, cellulose, acidic detergent, lignin and hemicellulose were determined as reported by Abara *et al*. (2011).

3.4.1.5.3 Neutral detergent fibre

About 1 g of each sample was reflexed for one hour with 100 ml of neutral detergent solution prepared by mixing two solutions: one consisting of 93 g of disodium Ethylenediamine Tetra acetate Dihydrate (EDTA) and 34 g of sodium borate in distilled. After reflux, each suspension was hot filtered through a glass sintering crucible with a suction pump and the remains were cleaned with hot distilled water and acetone. Crucible was dried overnight and the weight of the neutral detergent fibre calculated.

3.4.1.5.4 Acid detergent fibre

A crucible containing a neutral detergent fibre from each sample was placed in a flask and reflexively for 60 minutes from the start of boiling (3-5 min) with an acidic detergent solution consisting of 56 ml conc. sulfuric acid and 20 g of cetyltrimethylammonium bromide in 2 liters of distilled water. The contents were filtered and residue dried overnight at 100 ^{o}C. The determination was done in triplicate.

3.4.1.5.5 Hemicellulose

The hemicellulose quantity of each product was evaluated based on the difference between Neutral and Acid Detergent Fibre of each sample.

3.4.1.5.6 Lignin

The crucible containing acidic detergent from each sample was introduced in an enameled pan and preserved for 90 min. The remaining permanganate was suctioned off from the residue and washed with hot distil water, then with ethanol and with acetone. The crucible dried and weighed hot. Loss of acidic detergent residue taken an estimate of lignin.

3.4.1.5.7 Cellulose

The crucible containing the permanganate treatment residue was put in an enameled pot partially filled with sulfuric acid and the blend was stirred to moisten the particles. The container was refilled with sulfuric acid per hour and after three hour the residual sulfuric acid was separated from the residue by pressure, carefully cleaned with hot distilled water. The loss of excess permanganate was taken as an estimate of cellulose. The determination was carried out in triplicate.

3.4.1.6 Determination of Nitrogen-free extract (NFE)

NFE was quantified by difference technique described by AOAC, (2015). This was done by subtracting the sum of ash, protein, moisture, fat and fibre from 100;

$$NFE = 100 - (\%M + \%CP + \%EE + \%CF + \%Ash) \quad \text{......... Eqn.3.8}$$

3.4.2 Antinutrients in the blends

3.4.2.1 Determination of Tannin

Tannin was quantitated as outlined by AOAC (2015). Half gramme of extrudate mixed with 10 ml of distilled water for 1 hour. Approximately, 5 ml of distilled water, two drops of $Fecl_2$ in 0.1 M HCl were added. The mixture vibrated for proper mixing, four drops of potassium ferrocyanide added and the reading taken at 620 nm by a spectrophotometer.

3.4.2.2 Determination of Phytate

Sample of 2.0 g was extracted with 40 mL of 2.4% HCl for 3 hours, sift the extract and phytate content was determined spectrophotometrically with an absorption wavelength (A) at 640 nm described in AOAC (2015).

Phytate (mg/g) =

$$phytate(mg/g)sample = \frac{conc\mu g/m/xvolxDF}{wtofsample} \quad \text{…………………} (3.9)$$

Conc ug /ml*vol*DF/wt of sample

3.4.2.3 Determination of Oxalate

Oxalate was evaluated according to Kasimala *et al* (2018). Briefly, one gramme of the sample was digested with 10 ml of 6M HCl for one hour. The filtrate treated with 10 ml of a 5% calcium chloride solution that precipitated insoluble oxalate. The total filtrate resulting from the dissolution in H_2SO_4 made up to 300ml. A 125 ml aliquot was brought to boiling point, titrated against 0.05M standardized $KMnO_4$ solution to faint pink colour that continued for approximately 30 seconds and the burette reading noted. The oxalate content was calculated with the titre value. The oxalate content was calculated according to the stoichiometric formula. The soluble oxalate was subtracted from the total Oxalate to get insoluble oxalate.

3.4.3 Antioxidants in the Blends

3.4.3.1 Total phenolic content

Total phenolic content of the extracts was determined by the method of Folin-Ciocalteu assay (Chan *et al*., 2008). Samples (0.5 mL) were placed in tubes, 2.5 mL of 10% Folin Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3 added. Tubes were allowed to stand for half an hour and absorbance read at 765 nm.

3.4.3.2 Determination of Flavonoids

Total flavonoid of the extrudate was determined by $AlCl_3$ method. Ethanol extract (1.5 mL) was added, 5 mL ddH_2O and 0.3 mL 5% $NaNO_2$ added. Reagents and blank prepared using dd H_2O, 5 minutes later, 2 mL of 1 mol dm-3 NaOH was added and marked up to 10 mL ddH_2O. The mix was briskly shaken for 5 min. at 200 rpm, incubated for 10 min and absorbance at 367 nm noted (Jagadish *et al*, 2009). Flavonoid content was estimated with the aid of a standard calibration curve prepared from Quercetin. . The content of flavonoids was stated in mg of quercetin/g.

3.4.3.3 Determination of total carotenoids

For the quantification of carotenoids, the procedure of Wang *et al*. (2009) was employed. Some quantity (1g) of extrudate and 10 ml of n-hexane –acetone –ethanol (2:1:1 volume) were kept in a flask, shaken at 200 rpm for 20 min at ambient temperature, centrifuged at 4000 rpm for 10 min at 4 ^{o}C and supernatant was combined and made up to 20 ml with extraction solvent. Thereafter, the layers were separated and the absorbance at 450 nm measured. The yield of total carotenoid (TC) (mg / 100g) was calculated according to Eqn. 3.10

$$TC(mg/100g) = Axy(mL)x10^6 / A^{\%}_{1cm} \; x1000xw \qquad \text{..........(3.10)}$$

A - Absorbance at 445 nm, y- volume of extract,

$A^{\%}_{1cm}$ - extinction coefficient of carotenoids, w- weight of sample.

3.4.3.4 Determination of ferric reducing antioxidant properties

The reducing power of the extract was evaluated by method reported by Singh *et al*., (2013). To the diluted extract was added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1% w / v). The blend incubated at 50 °C for 20 min, solution of trifluoroacetic acid (2.5 mL; 10% w / v) was added. 500 mL of ferric chloride solution (0.1% w/v) was added to each aliquot. After 30 min the absorbance at 700 nm was read. FRP was expressed as mg GAE / g**.**

3.5 Functional Properties

3.5.1 Bulk density

Bulk density (BD) was calculated by Stojcesk *et al*. (2008) expression. Ten extrudate lengths were randomly selected, the diameters of the extrudates were then quantified at 10 various points along the length of the ten samples. The bulk density (BD) was then determined using the average of the readings

$$BD = 4m/(\pi d^2 L) \qquad \text{......... (3. 11)}$$

Where m is mass, L- length ,d-diameter of extrudates

3.5.2 Determination of water absorption index (WAI) and water solubility index (WSI)

WAI and WSI were analyzed by methods of Yagci and Gogus (2008). The milled extrudates were kept in water at ambient temperature for 30 min, stirred gently and centrifuged at 3000 g for 15 minutes. The supernatant was emptied into a previously weighed vapourising dish.

$$WAI\left(\frac{g}{g}\right) = \frac{Weight\ gain\ by\ gel}{Dry\ weight\ of\ extrudate} \qquad \text{............. (3.12)}$$

$$WSI\ (\%) = \frac{Weight\ of\ dry\ solid\ supernatant}{Dry weight\ of\ extrudate}\ x\ 100 \qquad \text{...................... (3.13)}$$

3.6 Sensory Attributes of the Extruded Blends

Organoleptic evaluations were performed in the Product Development Program laboratory of the National Horticultural Research Institute, Jericho, Ibadan using a group of twenty-five (25) members, who were employees of the Institute. Extrudates were assigned codes (3-digit numbers) and served in a balanced presentation order. The semi-trained panelists were given a bottle of water and asked to rinse and drink water between samples. They were asked to rate the acceptance of products in terms of colour, aroma, crispiness, taste and overall acceptance using 9 point hedonic scale. (Meilgaard *et al.*, 2007), 9 = like extremely to 1 = dislike extremely. The average score of all 25 panelists was calculated for different characteristics.

3.7 Toxicological Studies

3.7.1 Animal requirements

Ethical consent to animals was obtained from ACUREC(UI-ACUREC/App/2016/027), University of Ibadan before study. Wistar albino rat (Rattus norvegicus) of approximate weight of 70- 80 g was used. The animals were obtained from the Department of Veterinary Pathology, University of Ibadan. The venue of the experiment was the Department of Animal Sciences, University of Ibadan. Forty (40) animals were used for the study.

3.7.2 Transportation

The animals were carried inside a well-ventilated cage from the Department of Veterinary Pathology to the Department of Animal Science where they were housed for the experiment.

3.7.3 Description of experimental design and animal procedures

Forty (40) male albino rats were kept and maintained in the metabolic cage in the Department of Animal Science, University of Ibadan. They were kept in rat cages and fed with commercial feed and provided free access to clean water in the bottle ad libitr, this was done for a week to allow the animals to get used to the new environment before treatment.

3.7.4 Animal treatment and organ examination

After one week of acclimatization, the 40 rats were separated into four groups as follows: the first group which was control received basal diet while the other three with different ratio of orange pomace, wheat bran and soyameal (17: 39:44, 5:15:80, 10:10:80)% which were the best three from the sensory analysis fed for 3 weeks. The animals received food corresponding to 10% of their body weight (10-12 g) and were given to the animals that had fasted overnight. The experimental animals were observed closely for up to 21 days, thereafter biological, hematological, biochemical and histopathological tests were carried out.

3.7.4.1 Nutritional evaluation on rats fed with the tested blends

During the experimental period (3 weeks), the consumed diets were recorded every day, and body weights were recorded every week. Nutritional evaluation of various diets was performed by determining body weight gain% (BWG%), feed efficiency ratio (FER), feed conversion ratio (FCR) according to equations described by El-Sayed *et al*.(2014) .

BWG%= Final weight (g)–initial weight (g) / Initial weight (g) × 100 ……...…. (3.14)

Feed efficiency= Body weight / Food intake ………… (3.15)

Feed conversion = feed intake/ average daily gain ……….... (3.16)

3.7.4.2 Hematological analysis

About 2 ml of blood samples was taken with heparinized capillary tubes to determine the hematocrit value according to Rodak (1995) method. Total red and white blood cells, Hemoglobin strength, Mean corpuscular volume, mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin concentration were estimated by the method reported by Lawal *et al*. (2015).

3.7.4.3 Biochemical analysis

About 2 ml of blood was collected and allowed to coagulate in pure dry centrifuge tubes at 3500 rpm for 15 minutes. A section of the pure supernatant serum taken instantly to determine glucose in accordance to the enzymatic colorimetric method (AL-Shinnawy, 2009). The actions of alanine aminotransferase (ALT) serum aspartate aminotransferase (AST), and serum alkaline phosphatase (ALP) were estimated (Buncharoen *et al*., 2012). Total cholesterol and total serum lipids were tested following method of Seneviratne *et al*. (2011). Serum total protein and albumin levels were evaluated by the procedure of Adegoke *et al*., (2012). Serum globulin was calculated by Lynons (1992). Serum urea and creatinine were determined by the methods reported by Tietz *et al* (1994).

3.7.4.4 Histopathological analysis

At the end of the study, cervical dislocation was used to sacrifice the rats for histopathological examination to help identify toxic effects on target organs (liver and kidney) according to methods of Adesiji (1999). A veterinary pathologist read the result of the Histopathology, biochemical and hematological analysis.

3.7.4.5 Method of Euthanasia/Disposition of animals

At the end of the study, animals certified fit were donated to the Zoological garden of the University of Ibadan.

3.8 Storage Studies

The three samples (3) of the products with the highest sensory scores were packed (100 g each) in 250 micron polyethylene bags and kept at ambient temperature (28 ±2 ^{o}C) and relative humidity (60 -80%) for 5 months. Evaluations were done periodically (monthly intervals) for moisture and total fungal count over a duration of five months.

3.8.1 Total fungal count evaluation

Samples were milled with a blender model Marlex Excella, 10g of each sample were diluted with 90ml of sterile distil water and shaken with orbital shaker model Gallenkamp for 2mins. 1mL was used to prepare a tenfold serial dilution to obtain a dilution range of $10^{-1} – 10^{-6}$ for each sample (AOAC, 2015), 1mL of 10^{-1}, 10^{-3}, 10^{-6} dilution were pipetted into 9cm diameter petri dish, 15mL of molten sterilized culture medium cooled to about 45 ^{O}C was poured into each plate and gently swirled to homogenize the mixture. The cultured media used was Potato Dextrose Agar (PDA) for fungi enumeration, incubated at 27 ^{o}C for 48hrs before enumeration. Colony counter of model Lapiz digital was used for the enumeration (AOAC, 2015).

3.9 Data Analysis

Analysis of extrudates was done in triplicate for statistical analysis. The collected data subjected to descriptive and ANOVA. A comparison of averages of 13 formulations was performed using variance analysis (ANOVA) followed by Duncan's multi-range test at a probability of 0.05.

3.10 Optimisation

Minitab 16 was used to generate response graphs, contour overlays, and compound component optimization. The suitability of the mathematical method was used to determine the optimal mixing component values. For multiple reactions and component variations, all targets have been combined into a desired function. Numerical optimization has found a point that maximizes the function of the desired function.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate Composition of the Extruded Blends

4.1.1 Moisture content of the extruded blends

The extrudate moisture was between 8.50 and 11.03% (Table 4.1). The lowest moisture (8.5%) was for P4 containing 10% pomace, 80% soyameal and 10% wheat bran while blend with the highest moisture (11.03%) was P1; 13% pomace, 62% soyameal and 25% wheat bran. Values were less than 11.14 – 11.87% reported by Karnopp *et al*., (2015) for grape pomace biscuits. Moisture content and water activity have a major impact on maintaining food quality and durability. It is of economic importance as it determines the dry matter in a food sample. The higher the moisture content, the faster the chance of spoilage. The extrudates moisture was less than 12%, a value that is expected to offer better prospects of shelf life (Ogundipe *et al*., 2013). Significant difference in moisture was observed between the extrudates at $p \leq 0.05$, resulting from variation in the mixture of pomace, soyameal and wheat bran. The high R^2 value of 0.6916 (Table 4.2) showed that close to 70% of the variance can be explained by the experimental interrelationship. The final equation that represents the component's effect on moisture is given in eqn. 4.1.

$$Moisture = 0.035A + 0.081B + 0.057C + 4.866AB + 1.842AC + 1.118BC \quad \text{.......... (4.1)}$$

The eqn. 4.1 for moisture content indicated positive coefficient for the components. Therefore, a unit increase in soyameal inclusion increased the moisture content at a highest value of 0.081. A lowest value was observed with a unit increase in pomace inclusion resulting in increment of 0.035 moisture. This suggests that soyameal effect was greatest on the moisture content. The combination of pomace and soyameal in the feed had highest effect on the moisture content while the interaction between the soyameal and wheat bran resulted in the lowest value of moisture.

Table 4.1: Proximate composition of extruded blends

Blend	MC (%)	Ash (%)	Protein (%)	Fat (%)	C.fibre (%)	NFE (%)
P1	11.03±0.15^{a}	5.97±0.63^{a}	22.34 ±0.04^{d}	8.09 ± 0.00^{a}	13.48 ± 0.02^{b}	39.18 ± 2.22^{f}
P2	10.57±0.15^{b}	5.76±0.05^{ab}	23.33 ± 0.12^{c}	6.50 ± 0.00^{b}	13.26 ± 0.01^{bc}	40.58 ± 2.44^{ef}
P3	9.20±0.10^{de}	5.95±0.02^{a}	25.40 ± 0.10^{a}	6.00 ± 0.00^{c}	10.47 ± 0.02^{ed}	42.98 ± 0.39^{de}
P4	8.50±0.10^{h}	5.92±0.14^{a}	24.04 ± 0.02^{b}	5.50 ± 0.00^{d}	12.37 ± 0.02^{bcd}	43.67 ± 0.38^{d}
P5	8.80±0.20^{fg}	5.05±0.02^{de}	21.63 ± 0.02^{e}	6.00 ± 0.00^{c}	12.09 ± 0.02^{bcd}	46.43 ± 0.81^{c}
P6	9.13±0.12^{de}	4.13± 0.05^{g}	13.09 ± 0.02^{l}	5.50 ± 0.00^{d}	10.55 ± 0.02^{acde}	57.59 ± 0.61^{a}
P7	9.23±0.15^{de}	4.61± 0.01^{f}	18.53 ± 0.02^{h}	3.50 ± 0.00^{h}	12.77 ± 0.02^{bcd}	51.36 ± 0.69^{b}
P8	10.20±0.20^{c}	4.76± 0.24^{ef}	19.45 ± 0.02^{g}	5.00 ± 0.00^{e}	10.54 ± 0.02^{cde}	50.05 ± 1.66^{b}
P9	9.40±0.20^{d}	4.66 ± 0.28^{f}	19.62 ± 0.01^{f}	5.00 ± 0.00^{e}	19.89 ± 0.02^{a}	41.42 ± 2.03^{def}
P10	8.60±0.20^{gh}	4.81±0.01^{def}	14.72 ± 0.02^{k}	4.00 ± 0.00^{g}	9.05 ± 0.02^{e}	58.81 ± 0.85^{a}
P11	9.07±0.12^{ef}	4.83 ± 0.02^{def}	17.78 ± 0.02^{j}	4.50 ± 0.00^{f}	13.16 ± 0.02^{bcd}	50.66 ± 0.54^{b}
P12	8.67±0.12^{gh}	5.16 ± 0.19^{cd}	18.31 ± 0.02^{i}	4.50 ± 0.00^{f}	14.22 ± 0.01^{b}	49.13 ± 0.21^{b}
P13	9.40±0.20^{d}	5.50 ± 0.00^{bc}	10.84 ± 0.02^{m}	4.50 ± 0.00^{f}	10.42 ± 0.02^{ed}	59.34± 0.84^{a}

MC – Moisture, C.fibre – Crude fibre, NFE – Nitrogen free extract.
Averages of three replicates followed by different letters in the same column are significantly different ± Standard deviation

P1= 13%pomace, 62% soyameal, 25% wheat bran,
P2 = 17% pomace, 44% soyameal, 39% wheat bran,
P3 = 5% pomace, 80% soyameal, 15% wheat bran,
P4 = 10% pomace, 80% soyameal, 10% wheat bran,
P5 = 23% pomace, 52% soyameal, 25% wheat bran,
P6 = 20% pomace, 10% soyameal, 70% wheat bran,
P7 = 5% pomace, 25% soyameal, 70% wheat bran,
P8 = 23% pomace, 27% soyameal, 50% wheat bran,
P9 = 11% pomace, 62% soyameal, 27% wheat bran,
P10 = 10% pomace, 35% soyameal, 55% wheat bran,
P11 = 18% pomace, 27% soyameal, 55 wheat bran,
P12 = 30 % pomace, 60% soyameal, 10% wheat bran,
P13 = 30% pomace, 10% soyameal, 60% wheat bran

4.1.2 Ash content of the extruded blends

The extrudates ash content was between 4.13 and 5.97% (Table 4.1). The ash content was higher than ones reported for orange pomace cookies (0.8 – 1.3%) by Zaker *et al*., (2016); and pineapple pomace based weaning food (Mishra *et al*., 2014). This implies a higher mineral content in the extrudates. The ash content indicates the minerals in the mixtures. Highest value (5.97%) of ash was observed at 13.33% pomace, 62.08 % soyameal and 24.58% wheat bran, significantly difference ($p \leq 0.05$) occurred among the 13 formulations with R^2 of 0.5997.

Predicted response plot of ash content (Fig 4.1) shows that when other components are set at the constant increase in pomace resulted in quadratic increase in ash, the same trend was observed for soyameal while increase in wheat bran led to decrease in ash from 5.2 to 4.3 % (Figure 4.1). The final equation, which represents the effect of constituents on the ash, is given in eqn. 4.2.

$$Ash = 20.70A + 6.61B + 2.38C + 28.35AB - 13.13AC + 5.04BC \quad \text{.... ..... (4.2)}$$

The equation showed that orange pomace among the components had highest unit positive increase (20.70) on ash content, followed by soyameal. One could therefore say that pomace has a major influence on the ash content of the extrudates. Interaction of orange pomace-soyameal (AB) in the mixture resulted in the largest increase in the ash content while pomace-wheat bran (AC) interaction with the negative value (-13.13) had the least effect on ash content. The negative value shows that interaction of pomace and wheat bran may reduce the ash content of the extrudates.

4.1.3 Protein content of the extruded blends

The extrudates had protein in the range of 10.84 and 25.40% (Table 4.1). The values obtained were higher than the ones reported for snacks enriched with mango peel powder (Korkerd *et al*., 2016) and fortified wheat biscuit with citrus peel (Yousseff and Mousa, 2012. The blends with the highest protein content (24.04%) contained 10% pomace, 80% soyameal and 10% wheat bran while the lowest (10.84%) was found for blends of 30% pomace, 10% soyameal and 60% wheat bran.

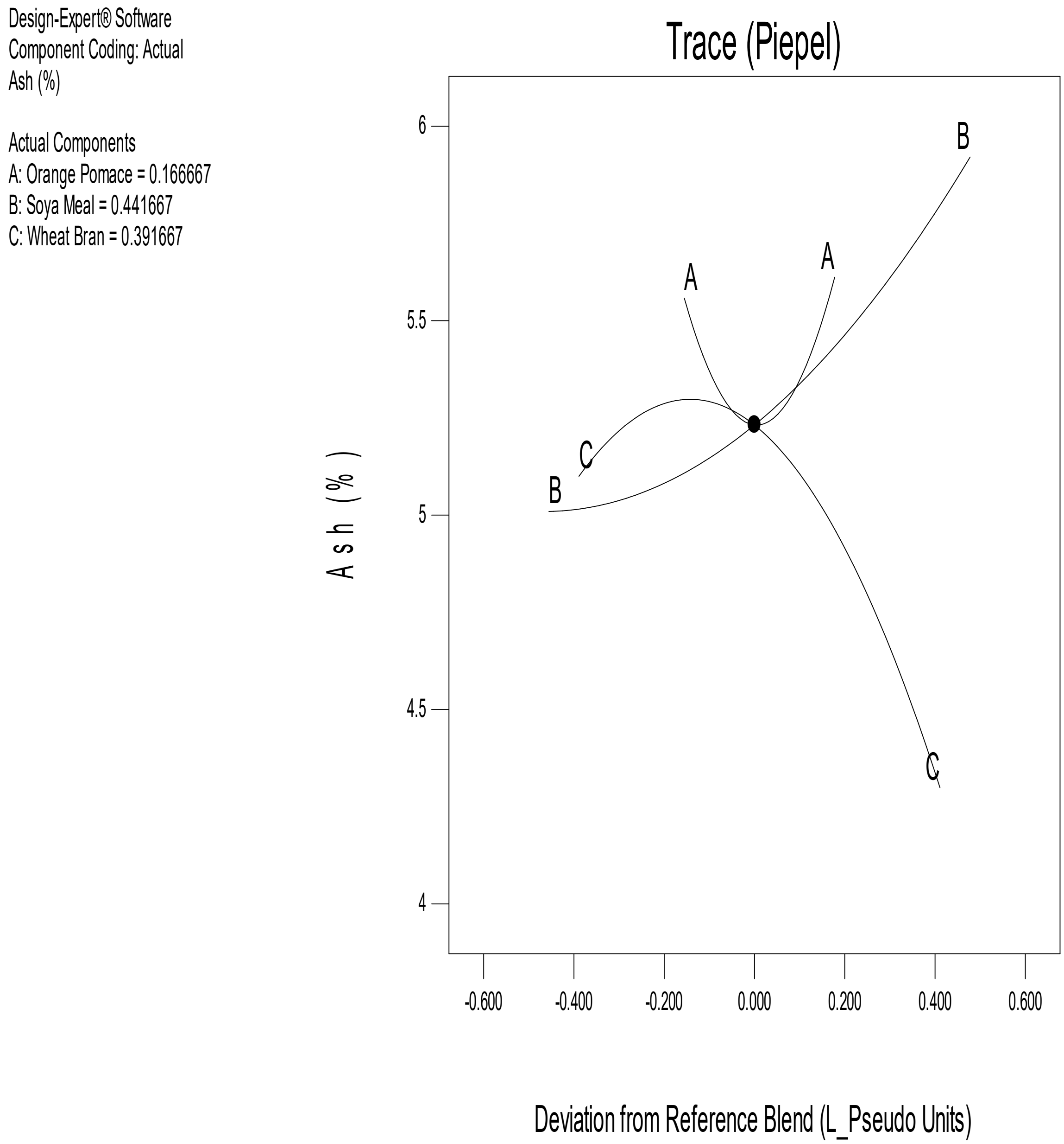


Figure 4.1: Predicted ash plot of components

The protein requirement is the amount of protein or its amino acid components or both that metabolic demand and nitrogen balance must be met in the diet (FAO, 2012).
The requirements are usually higher than metabolic demand due to digestion and absorption factors that affect digestibility and the resulting nitrogen loss (FAO, 2012). High protein content in extrudates could be due to soyameal which is a rich source of protein.

Significant variation was observed in samples ($p < 0.05$) while F and p values are shown in Table 4.2. Predicted protein trace plot shows that further addition of pomace and wheat bran from the reference blend led to quadratic decrease in protein while increase in soyameal led to increase in protein from 20 to 24% (Figure 4.2).

4.1.4 Fat content of the extrudates

The fat content (3.5–8.0%) was low in the extrudates compared to 17.12% reported for orange pomace fortified biscuit (Khule *et al*., 2019) and can be a fascinating meal for people who care about their weight. Moreover, fat content was highest (8.09%) for the blend containing 13% pomace, 62% soyameal and 25% wheat bran while lowest fat content (3.50%) was observed for blend with 5% pomace, 25% soyameal and 70% wheat bran. The blends were significantly different ($p < 0.05$) from each other while F and R^2 values were 3.54 and 0.7165 respectively.
Dietary fats are vital for maintaining health. In addition to providing vitality, they contain fat-soluble vitamins, are mechanical components of the body which take part in important physical processes such as growth, development, inflammation and brain functions. Foods low in fat tends to be better stored because they are less prone to rancidity and contribute to low energy value of food products (Gbenyi *et al*. 2016).

The increase in pomace and wheat bran from the reference mixture showed a quadratic reduction in the fat content, the addition of soyameal content resulted in an increase in fat from 5.9% to 6.1% before a decrease to 6.0 % (figure 4.3).

Table 4.2: Summary of regression analysis (proximate and antinutrient) of the models

Parameter	Moisture	Ash	Protein	Fat (%)	Crude fibre	Nitrogen free extract	Tannin	Phytate	Oxalate
R^2	0.6916	0.5997	0.7165	0.5269	0.5093	0.5616	0.7098	0.4676	0.5646
Adjusted R^2	0.4739	0.3137	0.5140	0.1890	0.1588	0.4739	0.5026	0.0873	0.2536
F-value	0.58	2.10	3.54	1.56	1.45	6.40	3.42	1.23	1.82
Adeq Precision	4.79	4.94	5.58	4.39	4.13	6.46	5.32	3.21	3.53

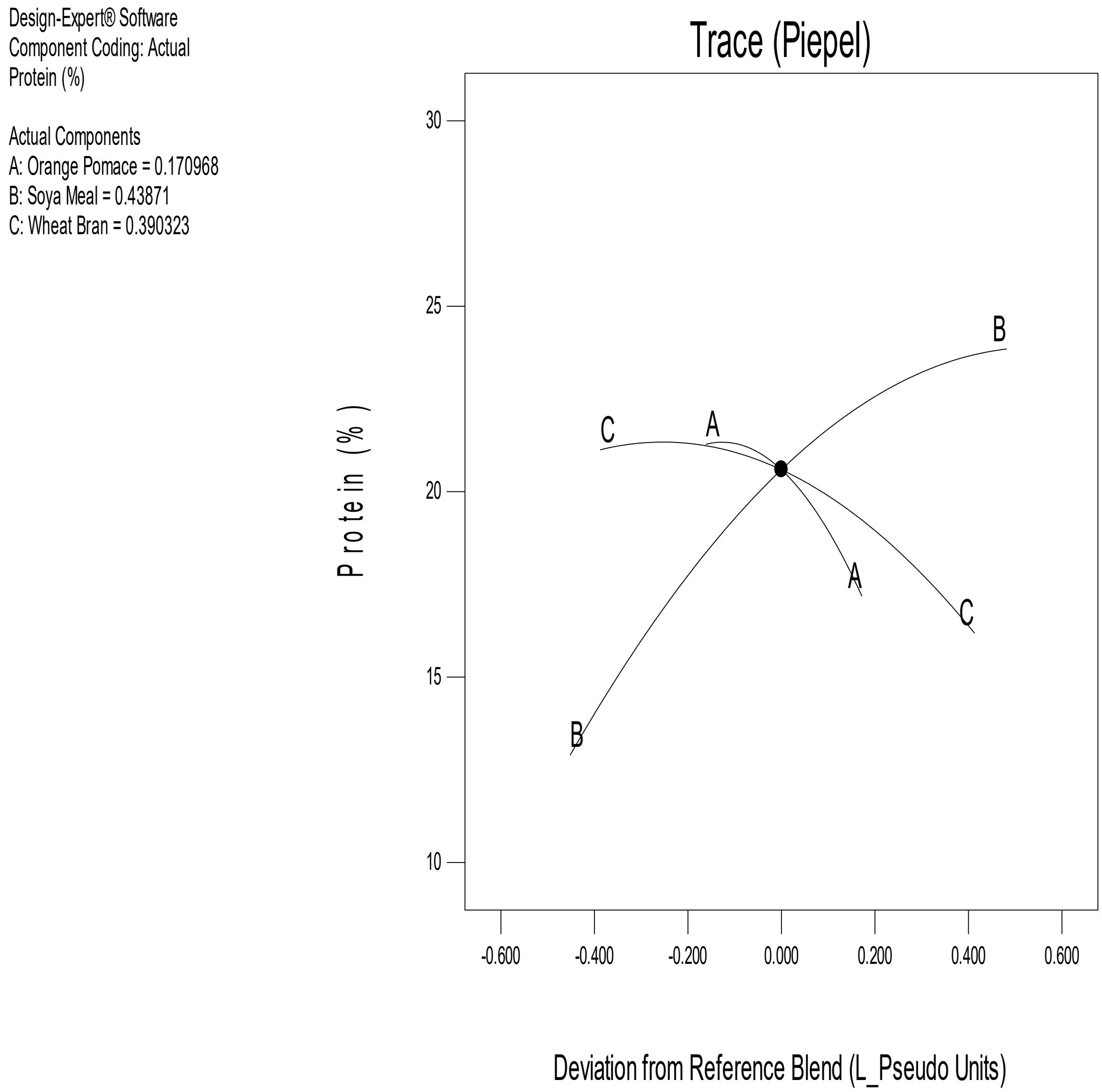


Figure 4.2: Predicted protein trace plot

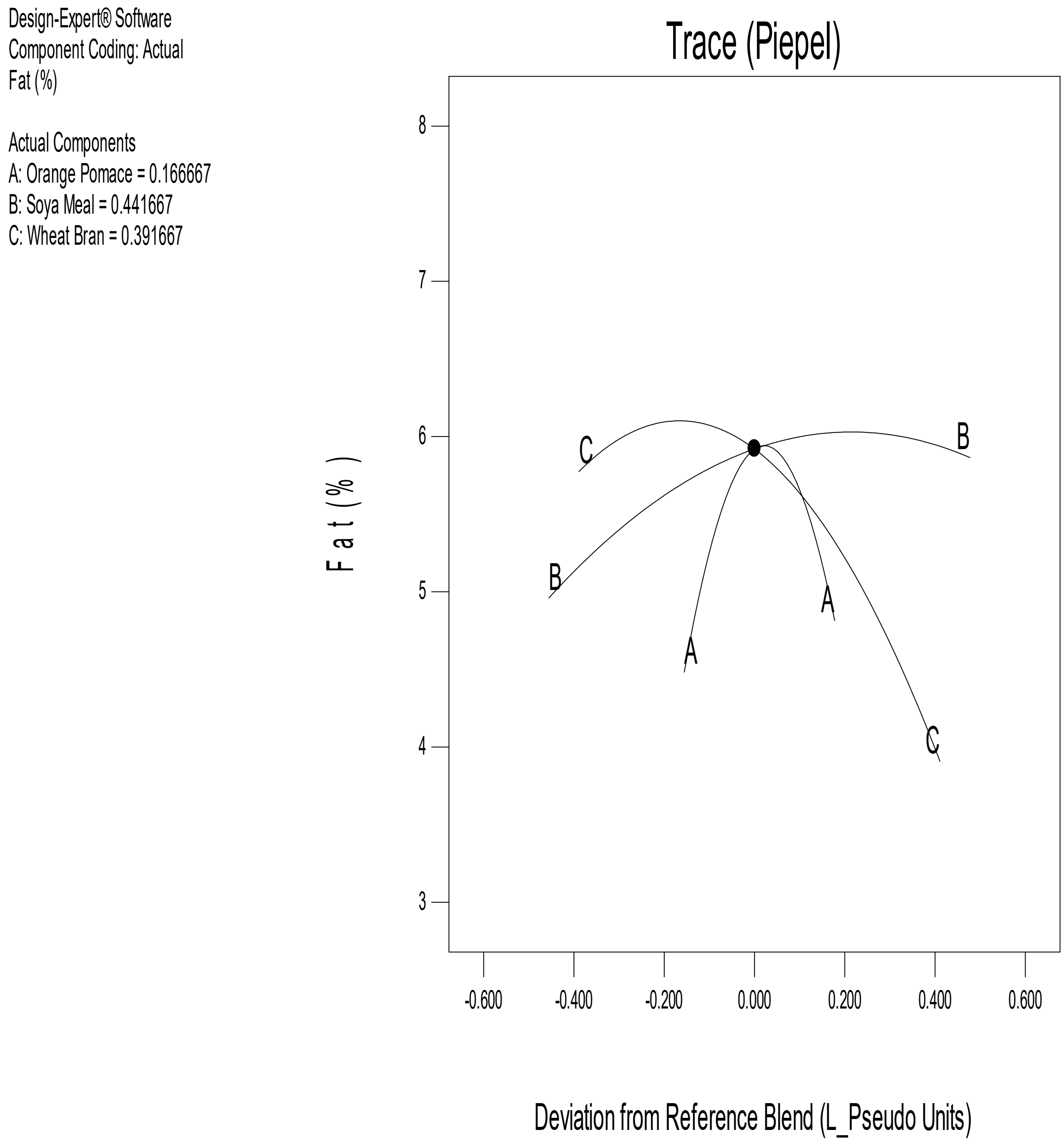


Figure 4.3: Predicted fat trace plot of products

4.1.5 Crude fibre of the extruded blends

A quick inspection of crude fibre (9.05-19.89%) (Table 1) of the extrudates showed values above 0.24-4.75% reported for tomato by-product extrudates (Karthika *et al*. (2016). The extrudates high in fibre may be due to the ingredients of pomace, wheat bran and soyameal, which have high fibre. The products can certainly function as a good source of fibre that meets the Codex standard. Codex Alimentarius states that each product called a "fibre source" must have 3 g of fibre value per 100 g (Codex, 2009). Fibre value was higher when pomace and soyabean concentration were increased while the highest value (19.89%) was obtained at the mixture of 11% pomace, 62% soyameal and 27% wheat bran. The values differ significantly ($p > 0.05$) between all 13 mixtures. Fibre is vital in the diet for breakdown and disposal of waste. The contraction of the intestinal muscle walls is stimulated by the fibre and thus counteracts constipation (Mohan and Kalidass (2010).

The predicted crude fibre trace plot showed increase in soyameal and wheat bran led to an increase in crude fibre, until the optimum point was reached, after which a drop occurred, while increase in the orange pomace led to a sharp drop in the crude Fibre (Figure 4.4).

The high fibre of the product was due to the interaction between orange pomace and wheat bran as shown in Figure 4.5. The addition of fibre-rich ingredients affects the texture of product hardness; this effect was enhanced by heat treatment during cooking by extrusion due to the large amount of protein in the ingredients (Karthika *et al*., 2016).

4.1.5.1 Total dietary fibre of the extruded blends

Total dietary fibre of raw ingredients use for the formulations were: pomace; 27.69% (TDF), 15.23% (IDF), 12.46% (SDF), soyameal; 37.69 (TDF), 13.88 (IDF), 23.81 % (SDF), wheat bran 31.08% (TDF), 10.67% (IDF), 20.41% (SDF), which added to the total fibre, soluble and insoluble fibre content of the food. The contour plot of the Total Dietary Fibre Mixture (TDF) showed TDF in a limited area within 43.08% and 66.86% (Figure 4.5), the mixture that had the lowest TDF were P (13.33%), SM (62.08%) and WB (24.58%), while the maximum TDF was for the mixtures of P (30%), SM (10%) and WB (60%). The total fibre model (linear) was not significant at $p > 0.05$. Higher dietary fibre was observed along the high mix of wheat bran and orange pomace and increased with more orange pomace and

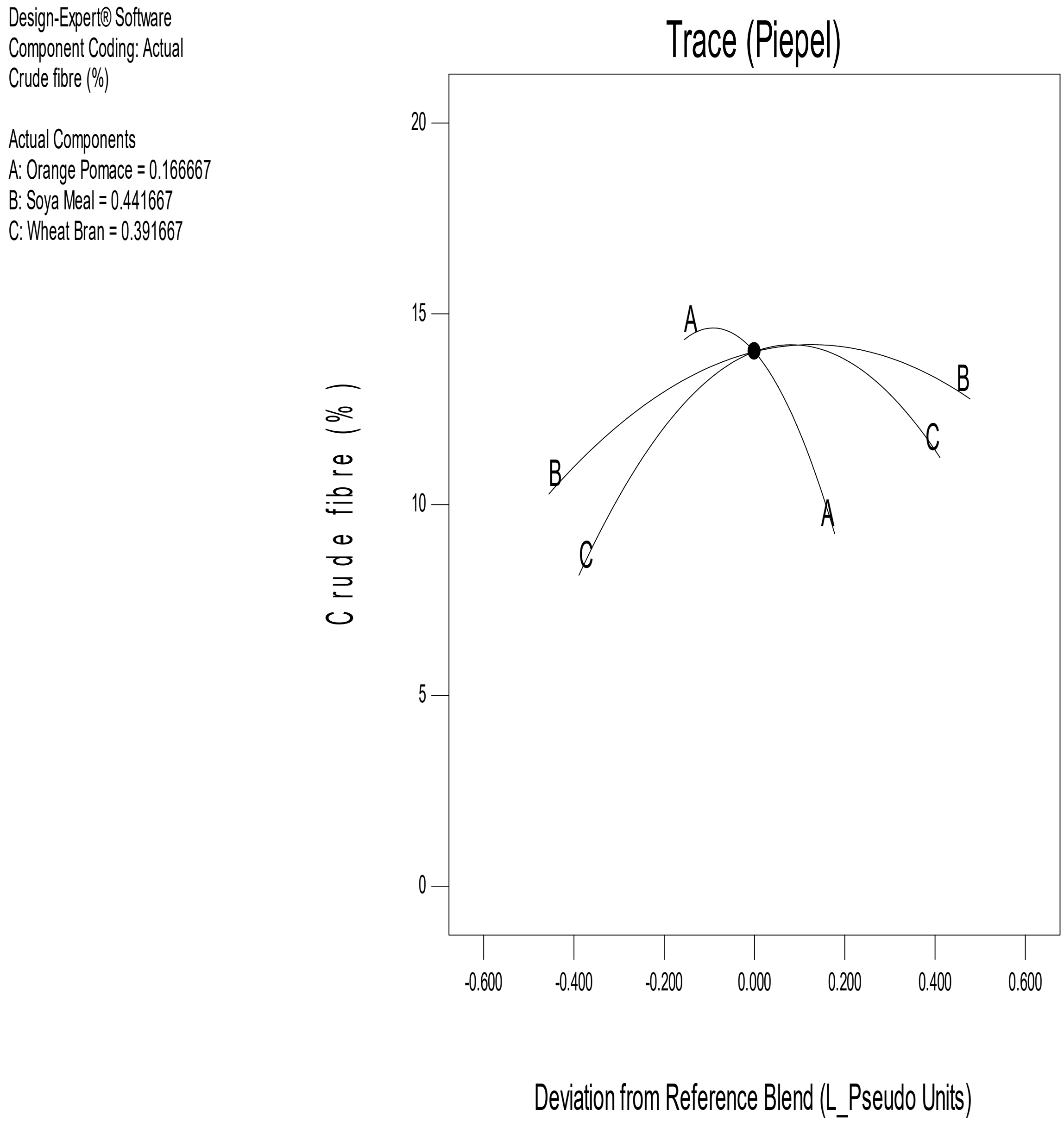


Figure 4.4: Predicted crude fibre trace plot of extruded blends

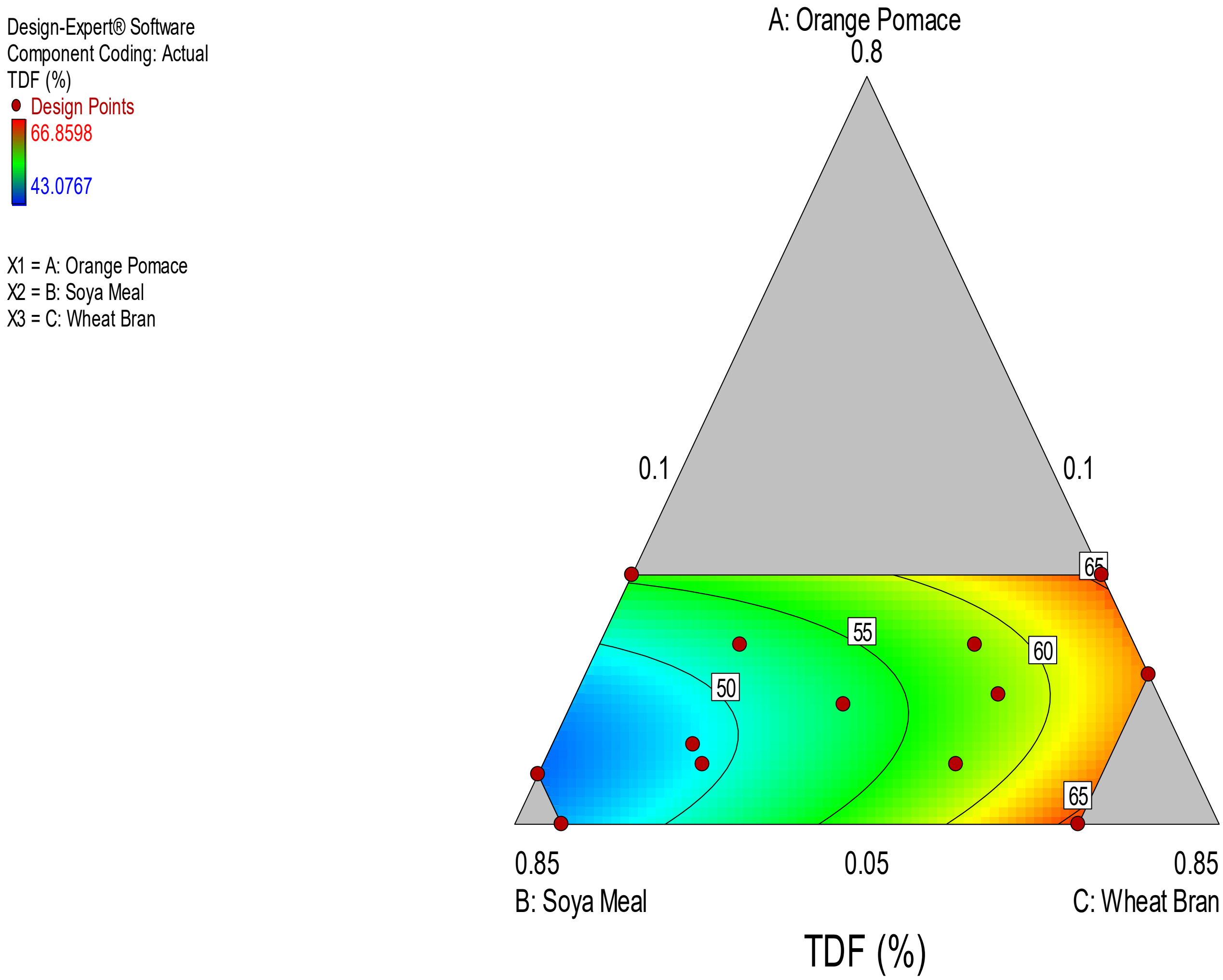


Figure 4.5: Mixture contour plot total dietary fibre of the extruded blends

less soyameal. Dietary fibre also increased with high mixture of wheat bran and less pomace. Significant difference in the dietary fibre of the extrudates was observed ($p < 0.05$). Figure 4.6 showed relationship of the components on the total dietary fibre. An increase in the amount of orange pomace and wheat bran resulted to an increase in the total content of dietary fibre, while an increase in the soyameal reduced the total amount of dietary fibre. The value of R^2 and adjusted R^2 were 0.6082 and 0.5298 respectively.

Eqn. (4.3) reflects the effects of fibre components and interactions.

$$TDF = 251.29A + 45.90B + 85.26C - 249.89AB - 315.40AC - 20.59BC \quad \text{...eqn. 4.3}$$

The effect of orange pomace was highest on the (coefficient of +251) dietary fibre while interaction of orange pomace and wheat bran (AC) gave the minimum effect (coefficient of - 315.40). The products value was between 43% and 67%. Four of the thirteen samples were rated as low, while the remaining nine were rated as medium (Rodríguez *et al*., 2006). The authors classified total fibre as low (30 to 50%), intermediate (50 to 70%); and high (70 to 90%). Dietary fibre is an important constituent of low-fat diet which supports maintenance of sugar level in blood and physical weight (Hong *et al*., 2012). The usefulness of fibre intake is mainly in defense against the onset of coronary ailment and several cancers and lowering of blood cholesterol (Cui *et al*., 2011). It is also established to have positive effects on insulin levels and protection against type 2 diabetes (Mościcki and Wójtowicz, 2013). The production and consumption of these high-fibre foods will certainly help consumers comply with dietary fibre standards of 25 to 35 grams per day. Other possible functional properties that such products would have included decreased post-prandial blood glucose response and laxation, modulation of blood lipid profiles, (Viuda-Martos, 2010).

4.1.5.2 Insoluble and soluble dietary fibre of the extruded blends

The products insoluble dietary fibre contour plot (IDF) was between 13.08 to 39.78%. The quantity of insoluble fibre was more in the mix of high soyameal and orange-pomace, also at the low quantity of wheat bran and high concentration of orange pomace (Figure 4.7). Insoluble Dietary Fibre increased with higher amount of orange pomace, confirming that pomace had greatest influence on insoluble fibre as given in Eqn. 4.4.

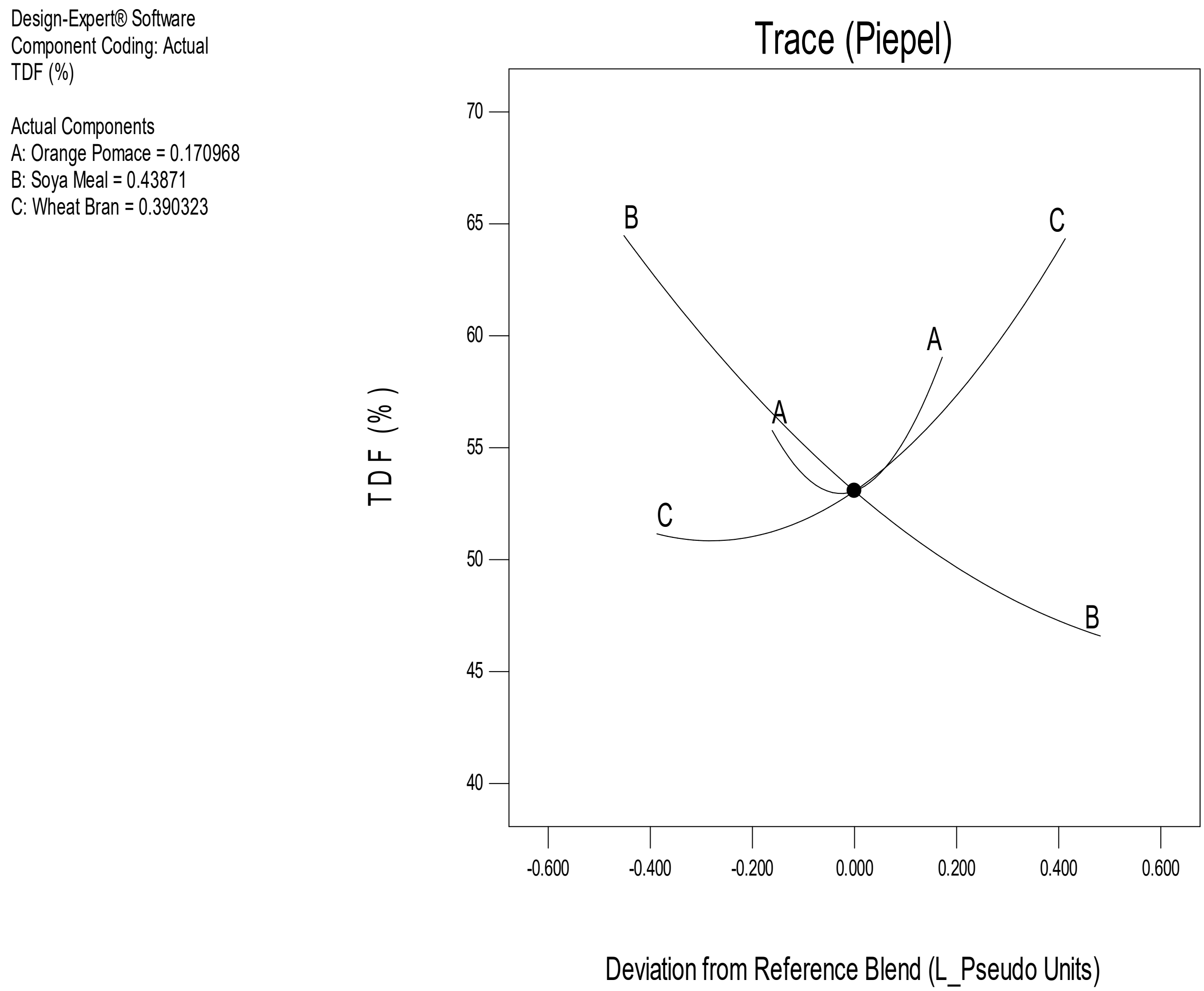


Figure 4.6: Components interaction effects on the total dietary fibre of extruded blends

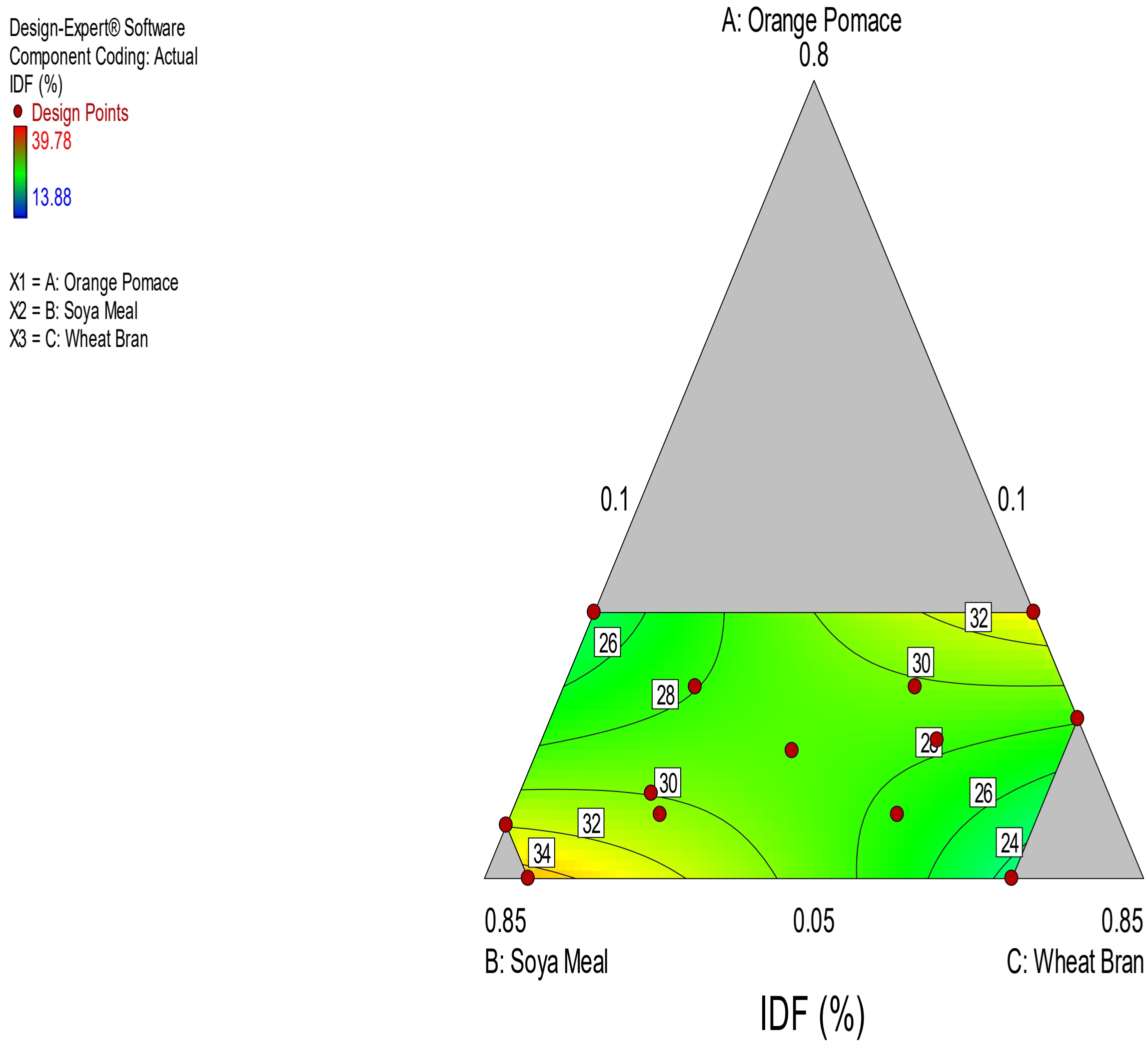


Figure 4.7: Mixture contour plot of insoluble dietary fibre of extruded blends

Figure 4.8 expresses the effect of the interface of the components. There was a net increase in IDF with orange pomace and soyameal. IDF decreased with increasing wheat bran.

Equation 4.4 shows the interaction effects of components on the IDF; Orange pomace soyameal (AB) had greatest influence on the IDF with coefficient of 124.32, while wheat bran (10.97) had the least influence.

$$IDF = 64.24A + 41.3B + 10.97C + 124.32AB + 36.16AC + 14.5BC \quad ..... (4.4)$$

The quantity of insoluble fibre in the extrudates was more and similar to that of Kucerova *et al*. (2013) baked products. Insoluble fibre is identified to increase the bulkiness of food, shorten passage through digestive tract and improve intestinal peristalsis (Grundy *et al*., 2016).

Alternatively, contour plot of soluble dietary fibre (SDF) were between 3.3 and 45.58% (figure 4.9). It was observed that an increase in SDF is related to high wheat bran and a lower concentration of orange pomace. The relations of components with respect to soluble dietary fibre (Figure 4.10) seemed to be that an increase in orange pomace and wheat bran led to an increase in SDF, while an increase in soyameal led to a decline in SDF.

Eqn. 4.5 showed components influence on the SDF. It specified that orange pomace with a coefficient of 187.17 had the maximum influence on SDF. Soyameal had minimum influence. The relations between pomace and wheat bran had lowest influence on the SDF with a coefficient of -351.76.

$$SDF = 187.17A + 4.87B + 74.29C - 125.71AB - 351.76AC - 35.10BC \quad ....4.5$$

Soluble dietary fibre was found to successfully reduce serum cholesterol and increase glucose breakdown and insulin reaction (Lattimer and Haub, 2010). Several water-soluble fibres are viscid in the gut. Increased viscosity is known to inhibit bile acids, cholesterols, other lipids, and the production of micelles (Jesch and Carr, 2017). This reduces cholesterol intake and promotes excretion of cholesterol from the body (Carr and Jesch, 2006).

Initial studies on soluble fibre showed prolonged gastric emptying and reduced macronutrient intake led to reduced postprandial blood sugar and insulin quantities

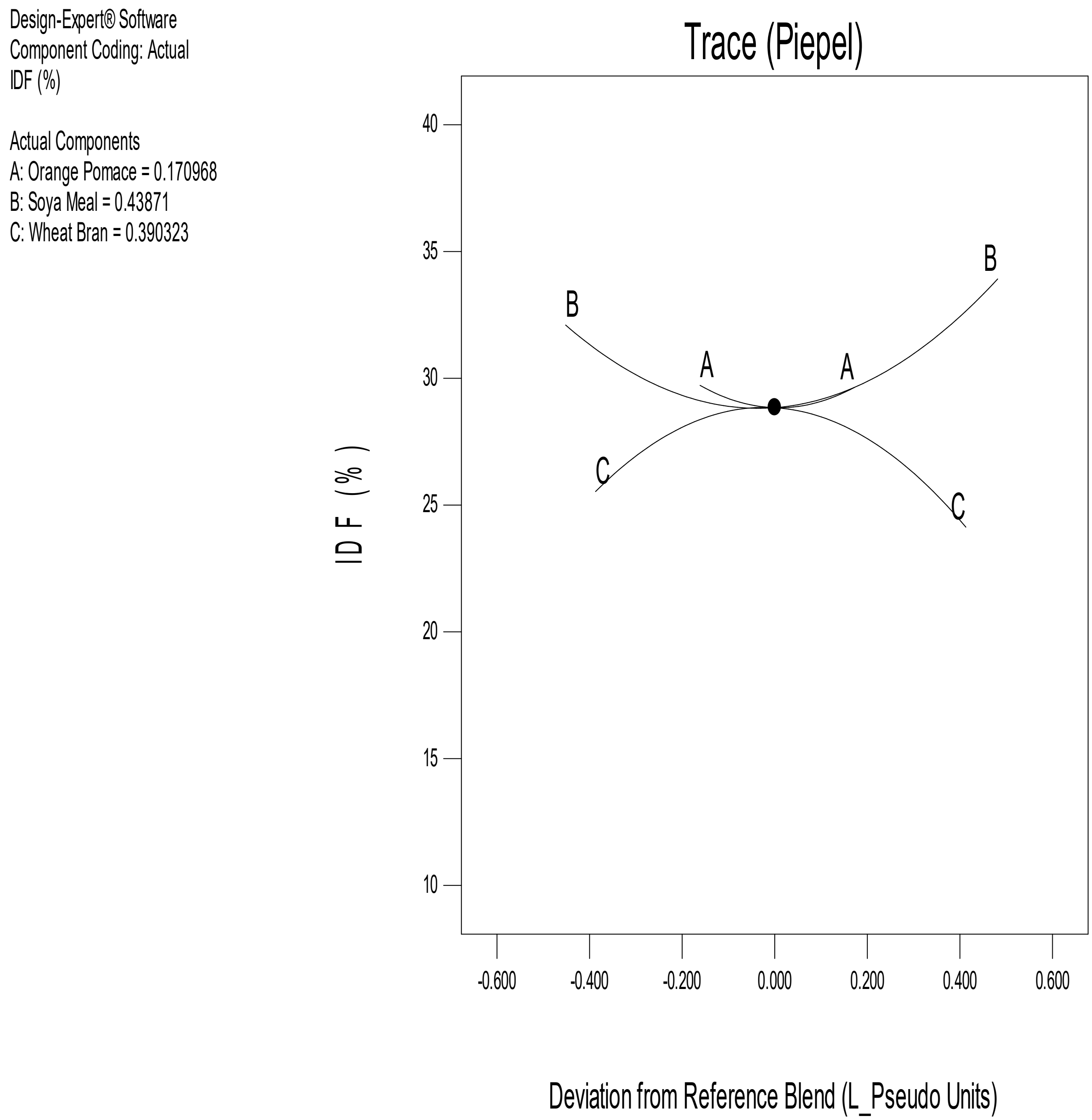


Figure 4.8: components interaction effects on the insoluble dietary fibre

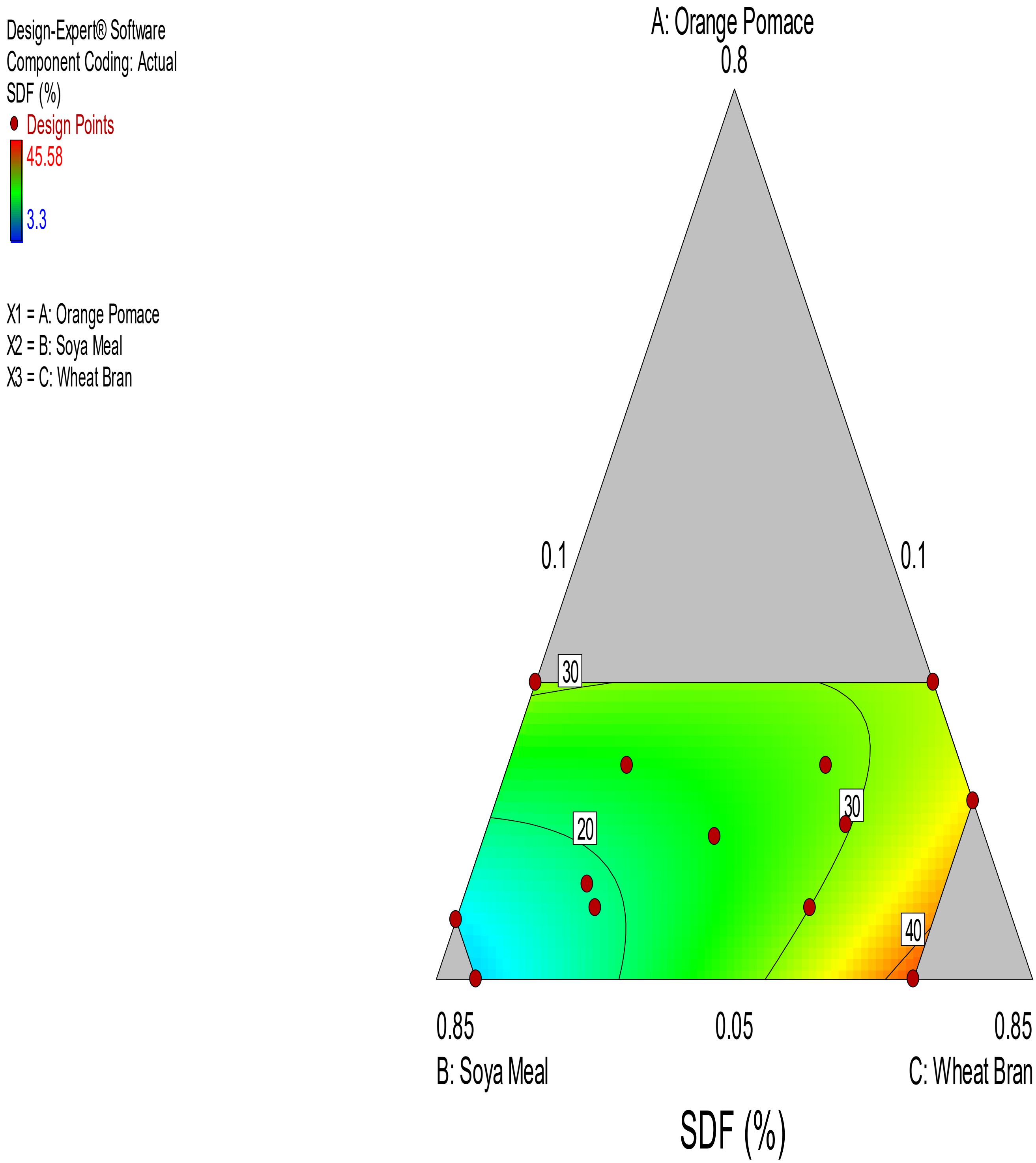


Figure 4.9: Mixture contour plot of soluble dietary fibre of extruded blends

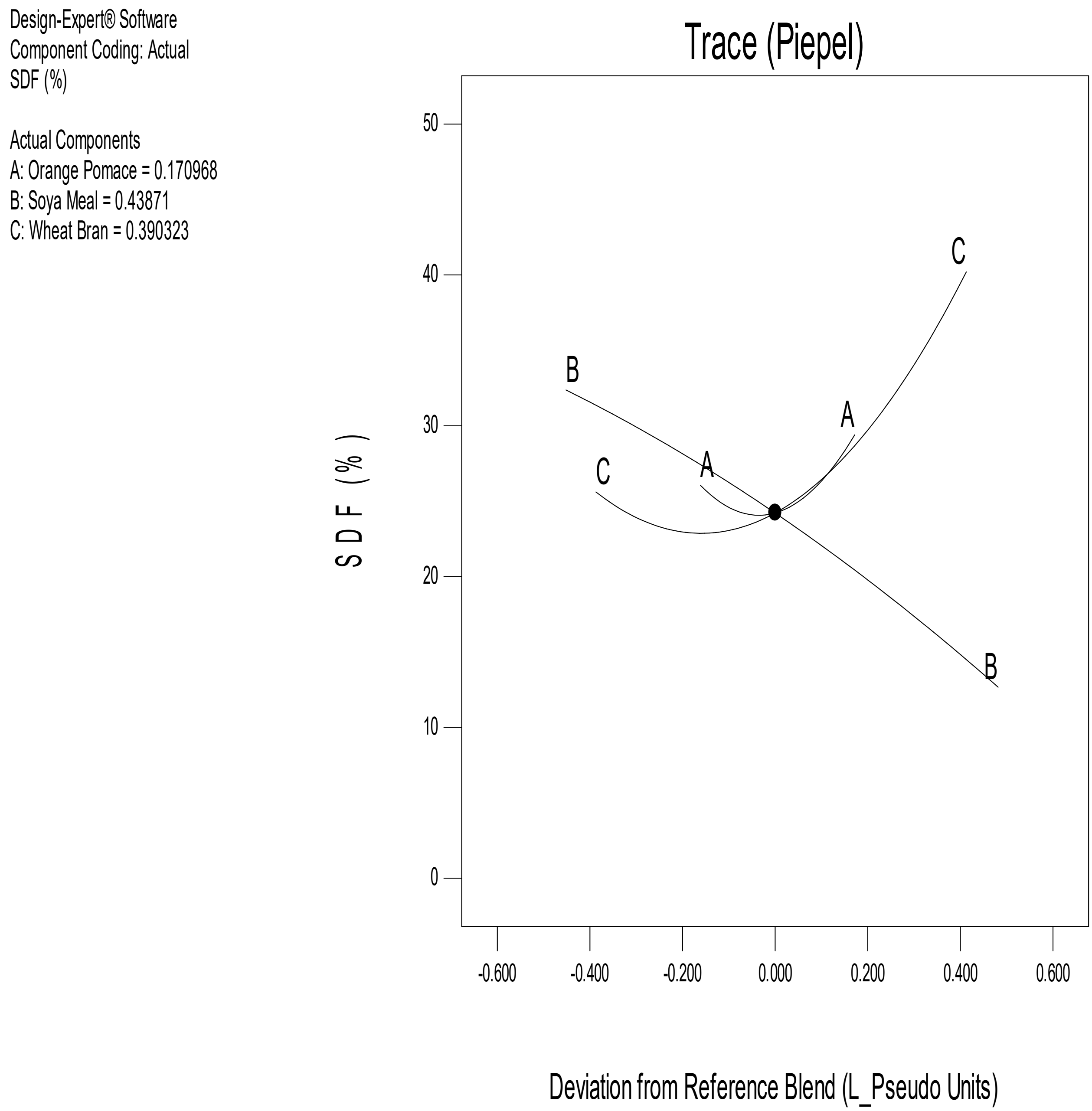


Figure 4.10: Components interaction effects on the soluble dietary fibre

Table 4.3: Insoluble and soluble dietary fibre relative proportion

Blend S/N	Orange pomace (%)	Soyameal (%)	Wheat bran (%)	Insoluble dietary fibre (%)	Soluble dietary fibre (%)	IDF/SDF ratio
1	13	62	25	39.78	3.30	12:05:1
2	17	44	39	31.39	15.11	2.08:1
3	5	80	15	37.21	9.39	3.96:1
4	10	80	10	24.69	22.01	1.12:1
5	23	52	25	13.88	45.58	1:3.28
6	20	10	70	32.15	29.77	1.08:1
7	5	25	70	23.05	42.92	1:1.86
8	23	27	50	36.33	16.64	2.18:1
9	11	62	27	33.89	19.73	1.72:1
10	11	35	55	19.65	38.24	1:1.95
11	18	27	55	27.39	38.50	1:1.41
12	30	60	10	29.65	24.31	1.22:1
13	30	10	60	30.21	36.65	1:1.21

Results are average of three replicates

(Lattimer and Haub, 2010). There is indication that more intake of insoluble and soluble fibre may affect the danger of experiencing cardiovascular disease (CVD) by taking into account threat like elated serum LDL cholesterol (Kendall *et al*., 2010).

Table 4.3 showed the ratio of IDF and SDF, the highest ratio was 12.05:1(blend 1) which was significantly higher than 2:1. Two (2) of the extrudates met the standard 2: 1 ratio, a proportional ratio that was stated to be good for the food ingredient (Jaime *et al*., 2002). The inequality in the ratio of IDF to SDF can be due to the different proportion of ingredients used for the formulation.

4.1.6 Carbohydrate content of the extruded blends

The carbohydrate content (NFE) of the extruded blends (39.18 to 59.34%) (Table 4.1) was below 66.86 – 81.5 g/100g which were previously stated for some snacks (Navarro-Cortez *et al*., 2016; Ayala-Rodrıguez *et al.,* 2009). This may be due to low carbohydrate content in soyameal and orange pomace. In addition to fat, carbohydrates also determine the energy storage of food. The present dietary guidelines are in support of everyday consumption of macronutrients, with carbohydrates up to 55% dietary energy (Layman *et al*., 2003). However, three independent groups reported benefits effects of carbohydrates in adults diet (Parker *et al*., 2002). The food under consideration may contribute to meeting this need. The product was low in carbohydrate and fat while it was high in protein and fibre for the middle age who is interested in maintaining/ reducing weight gain to meet the prevailing global health challenges.

The effect of the components interaction showed that a rise in pomace and wheat bran resulted in linear increase in carbohydrate content, addition of soyameal resulted in a linear reduction in carbohydrate of the products (Figure 4.11).

4.1.7 Tannin content of the extruded blends

Tannin content of the extrudates was between 1.30 and 2.14 mg/g (Table 4.4). Tannin in high amount means the possibility of poor digestibility of proteins caused by the formation of tannin complexes of proteins that irreversibly bind digestive enzymes. This inhibits the activity of enzymes, which makes the breakdown of proteins impossible. This deprives

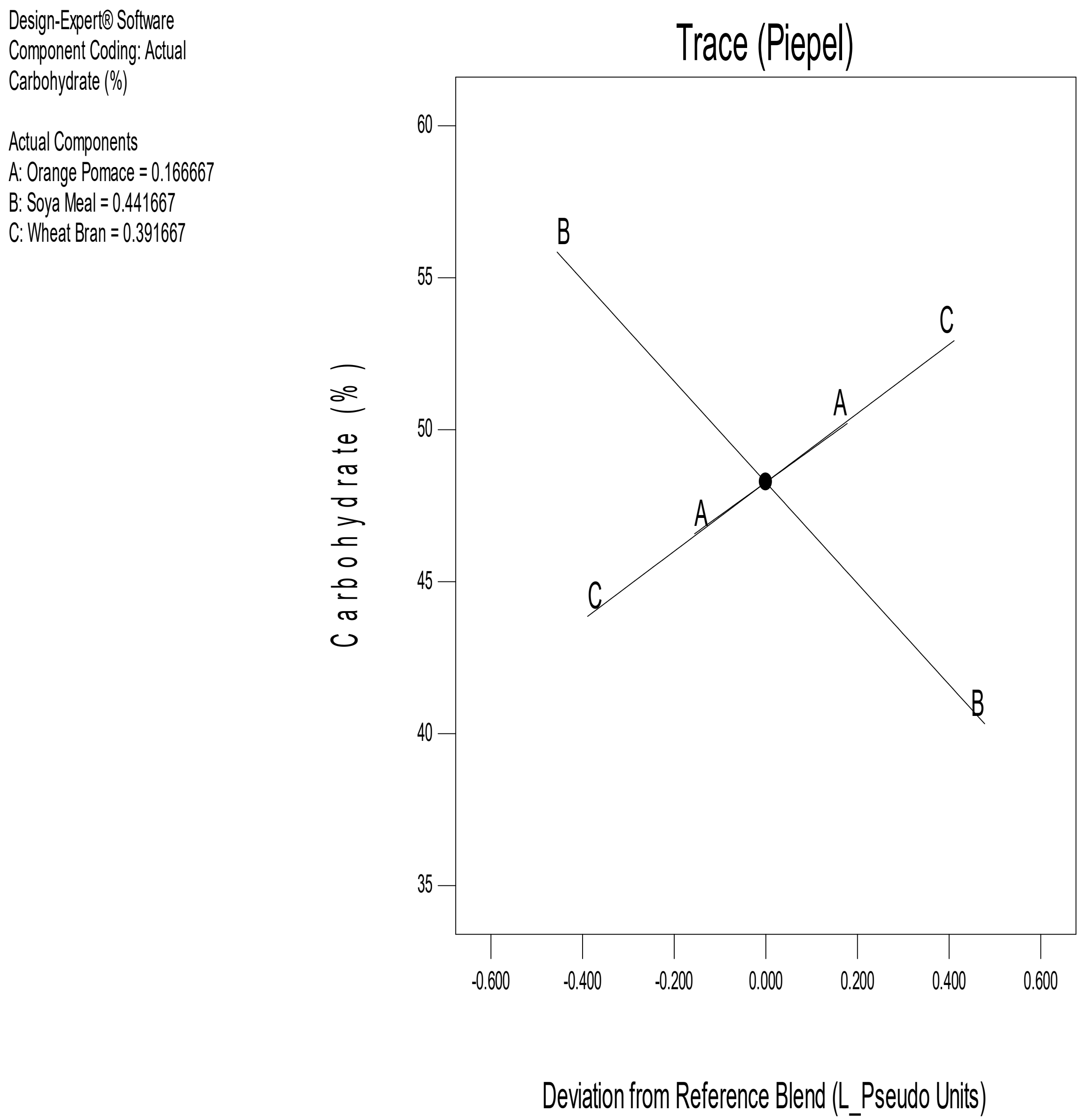


Figure 4.11: Predicted carbohydrate trace plot of extruded blends

Table 4.4: Antinutrients content in extruded blends

Blend (P:S:W)	Tannin (mg/g)	Phytate (mg/g)	Oxalate (%)
13:62:25	2.12±0.01^{a}	1.54±0.01^{a}	3.27±0.02^{a}
17:44:39	1.97±0.03^{ab}	1.48±0.01^{a}	2.92±0.01^{b}
5:80:15	1.63±0.01^{cde}	1.26±0.02^{bc}	2.05±0.01^{d}
10:80:10	1.59±0.02^{cdef}	1.23±0.01^{bcd}	1.97±0.03^{de}
23:52:25	1.74±0.01^{bcd}	1.05±0.01^{gh}	1.81±0.01^{def}
20:10:70	2.14±0.04^{a}	1.16±0.03^{def}	2.47±0.02^{c}
5:25:70	1.44±0.02^{ef}	0.99±0.01^{h}	1.42±0.01^{g}
23:27:50	1.49±0.01^{def}	0.57±0.02^{i}	0.86±0.02^{h}
11:62:27	2.02±0.01^{ab}	1.28±0.01^{b}	2.58±0.01^{c}
10:35:55	1.55±0.03^{def}	1.08±0.02^{fg}	1.67±0.03^{efg}
18:27:55	1.87±0.01^{a}	1.10±0.03^{efg}	2.06±0.02^{d}
30:60:10	1.30±0.01^{f}	1.18±0.01^{cde}	1.52±0.01^{fg}
30:10:60	1.43±0.01^{ef}	1.03±0.02^{gh}	1.48±0.01^{g}

Averages of three replicates followed by different letters in the same column are significantly different ± Standard deviation.

O – orange pomace, S – soyameal and W – Wheat bran

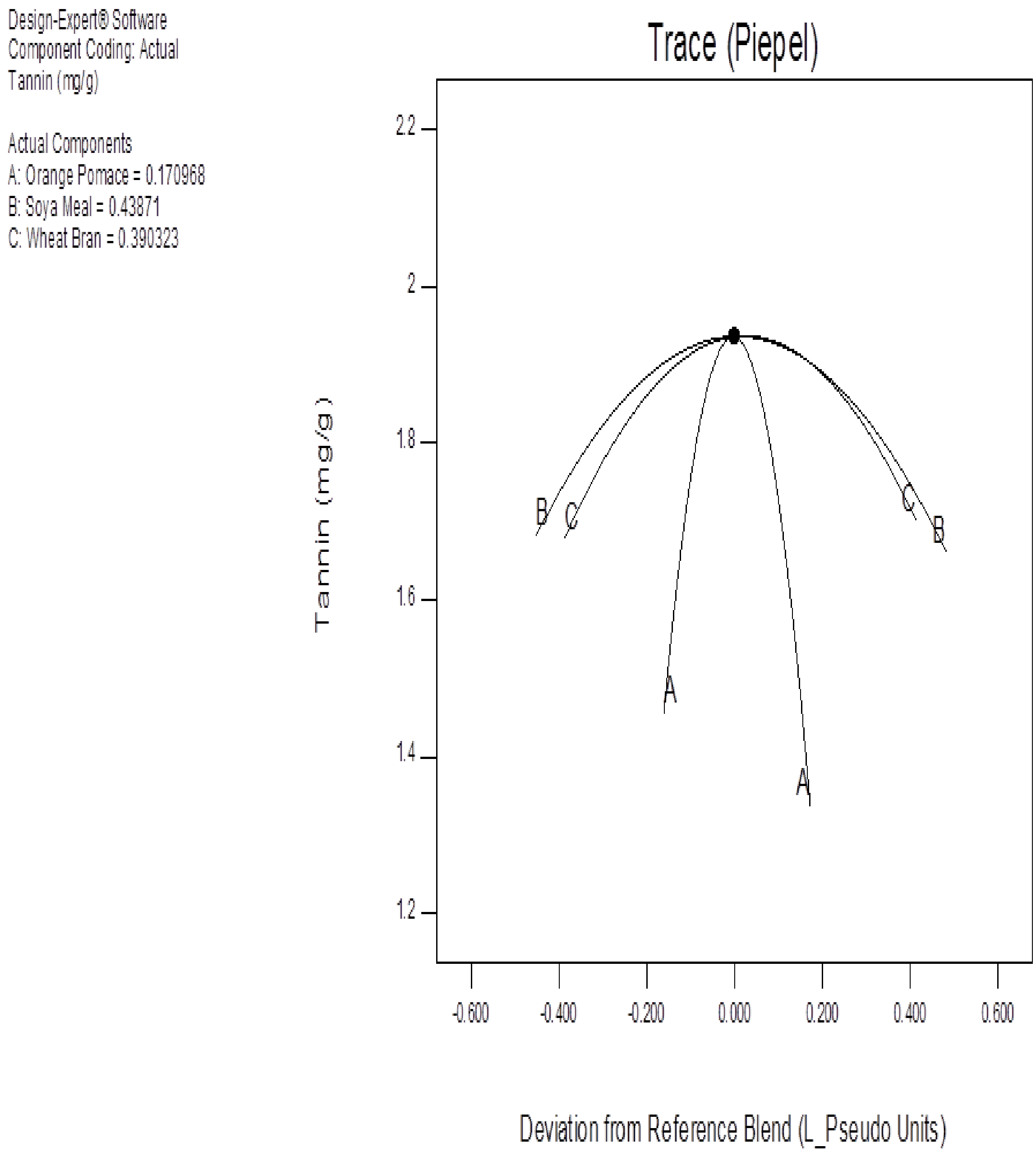


Figure 4.12: Predicted Tannin trace plot of components

proteins and other nutrients of digestion. The presence of a high concentration of tannins also indicates the possibility of poor palatability of the food due to the bitter taste, thereby reducing food intake.

Akinmutimi *et al*. (2009) observed high tannin content lowers cellulase action by joining fibres, which affects digestibility. Tannin levels in extrudates were below the harmful level of 76 - 90 g per kg Dry matter reported by Ifile *et al*. (2011), thereby suggesting product safety. Blends containing 30% pomace, 60% soyameal and 10% wheat bran had least tannin content while significant variations ($p < 0.05$) were observed among the 13 blends. F and R^2 values are presented in Table 4.2. The predicted trace plot of tannins showed increase in pomace, soyameal and wheat bran after the reference experience decrease in tannin, but a rapid drop in the pomace was observed (Figure 4.12)

The decrease may be the result of degradation of tannins caused by heat, enzymatic or non-enzymatic oxidation, which causes the extraction of more phenols (Sultana *et al*., 2012).

The equation showing influence of components and their interaction on tannin is shown in eqn. (4.6). Soyameal had the highest effect (1.21) on the tannin, increase in pomace may cause a reduction of tannin as shown by its negative coefficient (-29.93) while the interaction between the pomace and wheat bran which was positive (AC) had the highest increase on tannin.

$$Tannin = 29.93A + 1.21B + 0.84C + 33.25AB + 35.72AC - 0.29BC \quad \text{....... (4.6)}$$

4.1.8 Phytate content of the extruded blends

The extruded blends contained phytate in the range of 0.57 and 1.54 mg/g (Table 4.4). The values of phytate in the extrudates were low to be of nutritional concern when compared with the permissible level of 5 g/kg (Oduntan *et al*., 2014). Blend of 23% pomace, 27% soyameal and 50% wheat bran had the least amount of phytate while blends of 13% pomace, 62% soyameal and 25% wheat bran contained highest phytate content.

Variations in the phytate content were significant ($p< 0.05$) among the 13 blends. The F and R^2 values are shown in Table 4.2. The acid has the potential to reduce the physiological availability of calcium, zinc, iron and magnesium in the diet (Oduntan *et al*., 2014). The

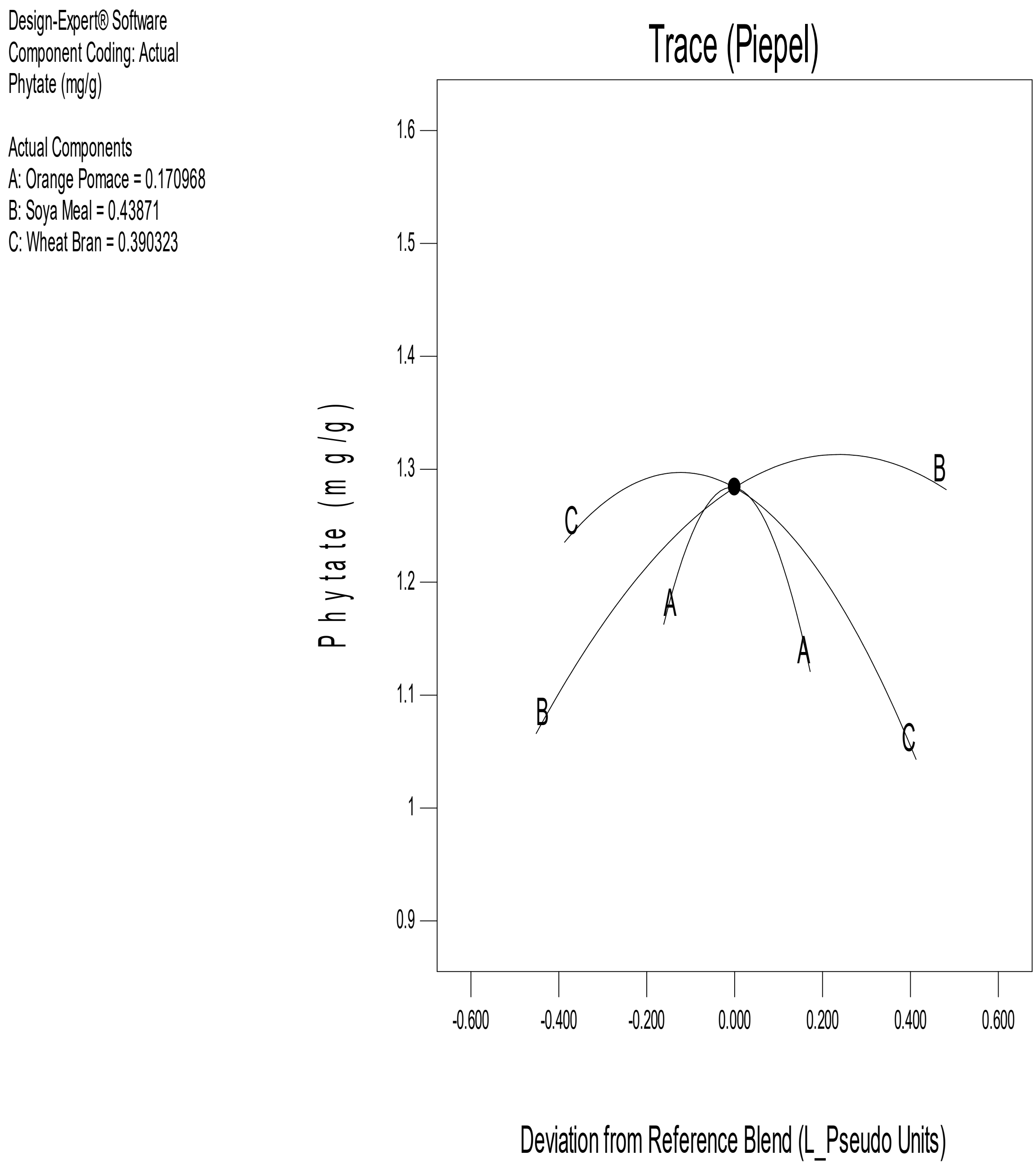


Figure 4.13: Predicted phytate trace plot of components

predicted trace plot of phytate showed a quadratic increase in phytate as the amount of soyameal increased, while phytate decreased with the increase of pomace and wheat bran (Figure 4.13).

4.1.9 Oxalate content of the extruded blends

The amount of oxalate present in the extrudates ranged from 1.42 to 3.27% (Table 4.4). Oxalate effects its action by joining to certain divalent minerals, such as copper, iron and calcium, which reduces their bioavailability by reducing their absorption. Oxalate binds excess calcium, forms calcium oxalate which is insoluble and not absorbed by the body. It is regarded toxic at higher concentration of above 250 mg/100g (Musa *et al*., 2014), but not hurtful when available in small quantity. The amounts in this study were lower than those harmful to humans. The components and their interaction are shown in equation (4.7), in which pomace with the lowest coefficient (-35.97) had least effect on the amount of oxalate present in the blends, increase in pomace may result in decrease in oxalate while interaction of soyameal and wheat bran (1.61) had least effect on the oxalate content.

$$Oxalate = -35.97A + 1.36B - 0.30C + 52.85AB + 59.02AC + 1.61BC \quad \text{....... (4.7)}$$

4.2 Antioxidant Components

4.2.1 Total phenolic content of the extrudates

Total phenolic content (TPC) of the extrudate ranged from 0.41 to 0.76 mg/g (Table 4.4). Maximum content was observed in blend 2 and 6(P17 S44 WB39, P20 S10 WB70) while blend 8 (P23 S27 WB50) had the minimum phenol content. There were significant variations ($p< 0.05$) among most of the blends. Model coefficients and other statistics are given in Table 4.5. The values were lower than ones reported for biscuit enriched with mango peel powder by Ajila *et al*. (2008). The existence of phenol in small quantities in food is believed to have nutraceutical properties, inhibit proliferation of cancer cells and protect neurons against oxidative stress (Roleira *et al*., 2015).

Table 4.5: Antioxidant contents in the extruded blends

Blend S/N	Orange pomace (%)	Soymeal (%)	Wheat bran (%)	Total phenol (mg/g)	Flavonoid (mg/g)	Carotenoid (mg/g)	FRAP (mg/g)
1	13	62	25	0.72± 0.01^{bc}	5.97±0.43^{a}	0.10±0.00^{b}	24.33±2.45^{a}
2	17	44	39	0.76 ±0.02^{ab}	8.21±1.32^{de}	0.06±0.00^{de}	9.07±1.92^{de}
3	5	80	15	0.55 ± 0.01^{fa}	4.09±0.20^{cd}	0.04±0.00^{ef}	12.67±0.67^{cde}
4	10	80	10	0.60± 0.01^{de}	4.71±0.28^{cd}	0.12±0.00^{ab}	2.13±2.12^{gf}
5	23	52	25	0.57± 0.01^{ef}	3.19±0.16^{defg}	0.10±0.00^{b}	9.27±1.14^{de}
6	20	10	70	0.76 ± 0.02^{a}	4.01±0.37^{def}	0.10±0.00^{bc}	22.69±4.33^{ab}
7	5	25	70	0.47 ± 0.02^{i}	2.69±0.89^{efg}	0.14±0.00^{a}	16.24±4.27^{bc}
8	23	27	50	0.41 ± 0.03^{j}	1.57±0.24^{g}	0.06±0.0^{de}	13.00±5.59^{cd}
9	11	62	27	0.72 ± 0.03^{c}	6.87±3.65^{ab}	0.02±0.00^{f}	5.93±3.55^{efg}
10	11	35	55	0.51 ± 0.02^{h}	2.19±0.29^{efg}	0.11±0.00^{ab}	8.73±1.88^{def}
11	18	27	55	0.52± 0.01^{gh}	3.16±0.87^{defg}	0.07±0.05^{cd}	6.27±1.66^{defg}
12	30	60	10	0.61 ± 0.03^{d}	3.41±0.51^{defg}	0.02±0.00^{f}	0.44±3.74^{g}
13	30	10	60	0.62 ± 0.02^{d}	2.13±0.34^{fg}	0.02±0.00^{f}	9.69±9.63^{cde}

Averages of three replicates followed by different letters in the same column are significantly different ± Standard deviation

FRAP –Ferric reducing antioxidant property

The antioxidant property of phenolic moieties is primarily as a result of their redox properties, which could play a vital function in the absorption and removal of free radicals, the decomposition of singlet oxygen and triplet oxygen or peroxide (Oduntan *et al*., 2014). These natural antioxidants work by removing harmful free radicals associated with advanced cancer and other deteriorating diseases, including poor brain function (Dillard *et al*., 2000). Normal plot of residuals of TPC shows good linear relationship (Figure 4.14) and confirmed the adequacy of the model. Phenolics are present in plants, and when phytochemicals are ingested in plant foods such as these, phytochemicals add to the absorption of normal antioxidants in man diet. Numerous findings showed that antioxidants inhibit the incidence of deteriorating diseases like certain cancers, neurodegradative and cardiovascular diseases, cataracts, aging and oxidative stress dysfunctions (Hollman, 2001). The predicted TPC trace graph shows that the increase in total phenolic compounds occurred with an increase in pomace to the optimum point, after which a further increase led to its decrease, while an increase in soyameal and wheat bran reduced the total phenol content, as shown in Figure 4.15.

4.2.2 Flavonoids content of the extruded blends

Flavonoids in the extrudates was from 1.57 to 8.21 mg/g (Table 4.5). Blend 2 had the highest value for flavonoids while blend 8 had the lowest value. Significant variations ($p < 0.05$) were observed among the blends while R^2 was 0.4485. Flavonoids were shown to have many beneficial properties including antimicrobial, anti-inflammatory enzymatic inhibition, estrogenic, antiallergic, antioxidant, and antitumor effects (Harbone and Williams, 2000). The existence of substantial quantity of bioactive complexes like flavonoid in these pomace based food assures of their substantial health benefit value (Saura-Calixto *et al*., Goni, 2007). Higher concentration of flavonoid was observed with higher soyameal quantity and small quantity of orange pomace. The predicted trace plot of flavonoid shows increase in pomace and wheat bran resulted in a quadratic reduction in the flavonoid quantity and increase in the soyameal value of flavonoid until it reached the optimal point after which further increase led to decrease in the flavonoid content (Figure 4.16).

Table 4.6: Summary of regression analysis of (antioxidant) models

Response	Total phenol	Flavonoid	Carotenoid	Frap
R^2	0.3822	0.4485	0.4995	0.2276
Adjusted R^2	-0.0590	0.0546	0.1420	0.0731
F-value	0.87	1.14	1.40	0.50
Adeq Precision	3.33	2.94	3.83	2.75

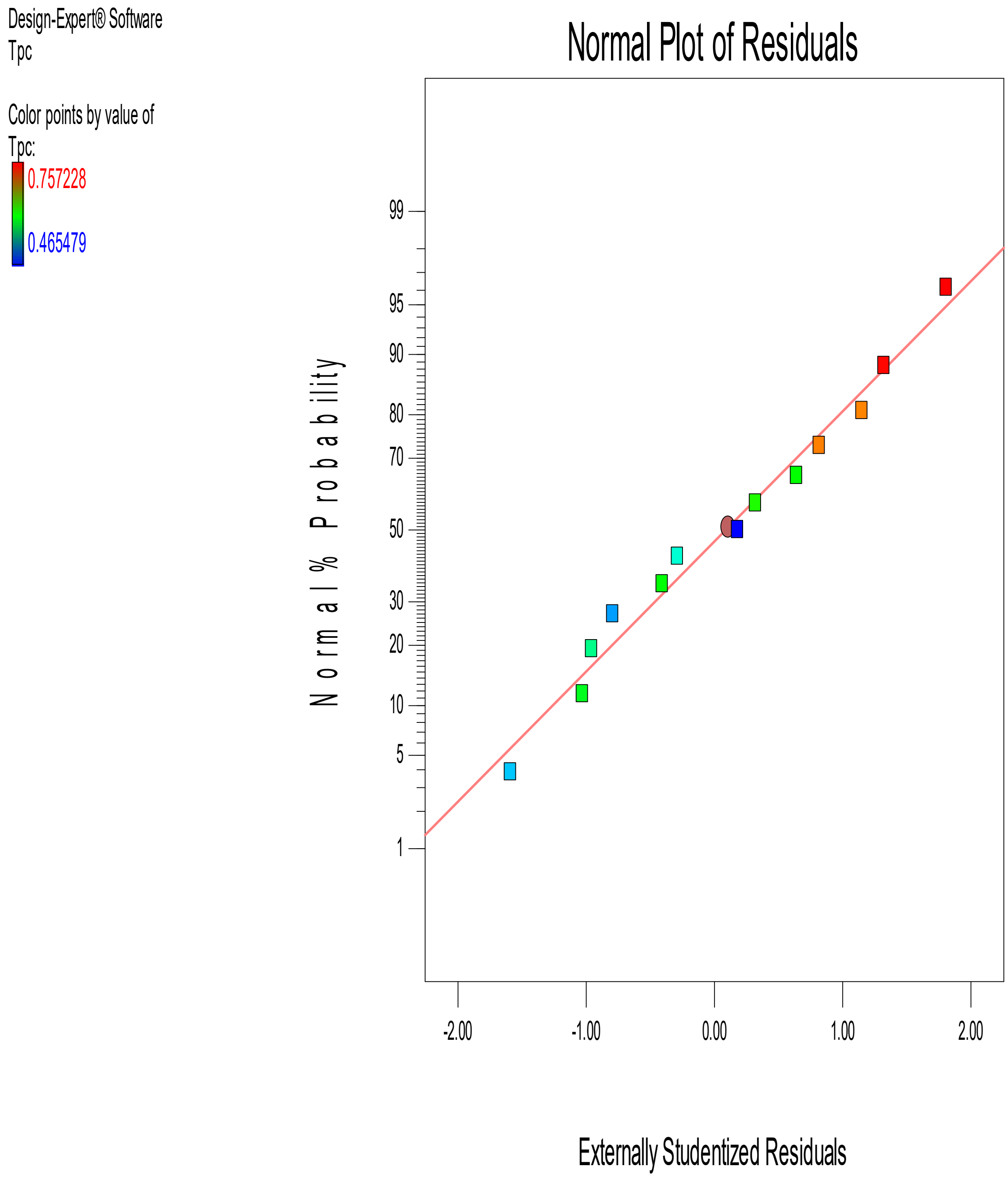


Figure 4.14: Normal plot of residuals of total phenolic content

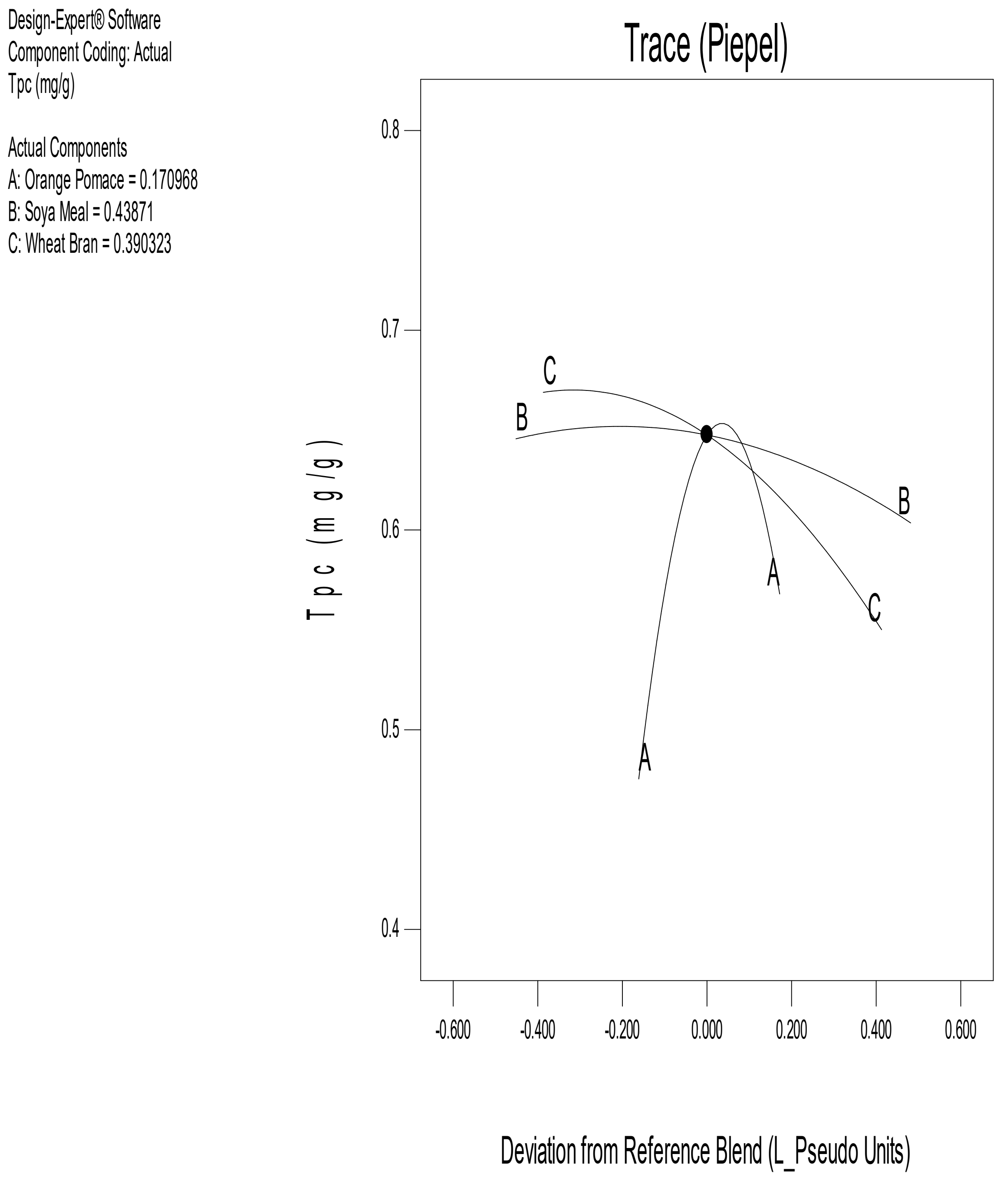


Figure 4.15: Predicted TPC trace plot of components

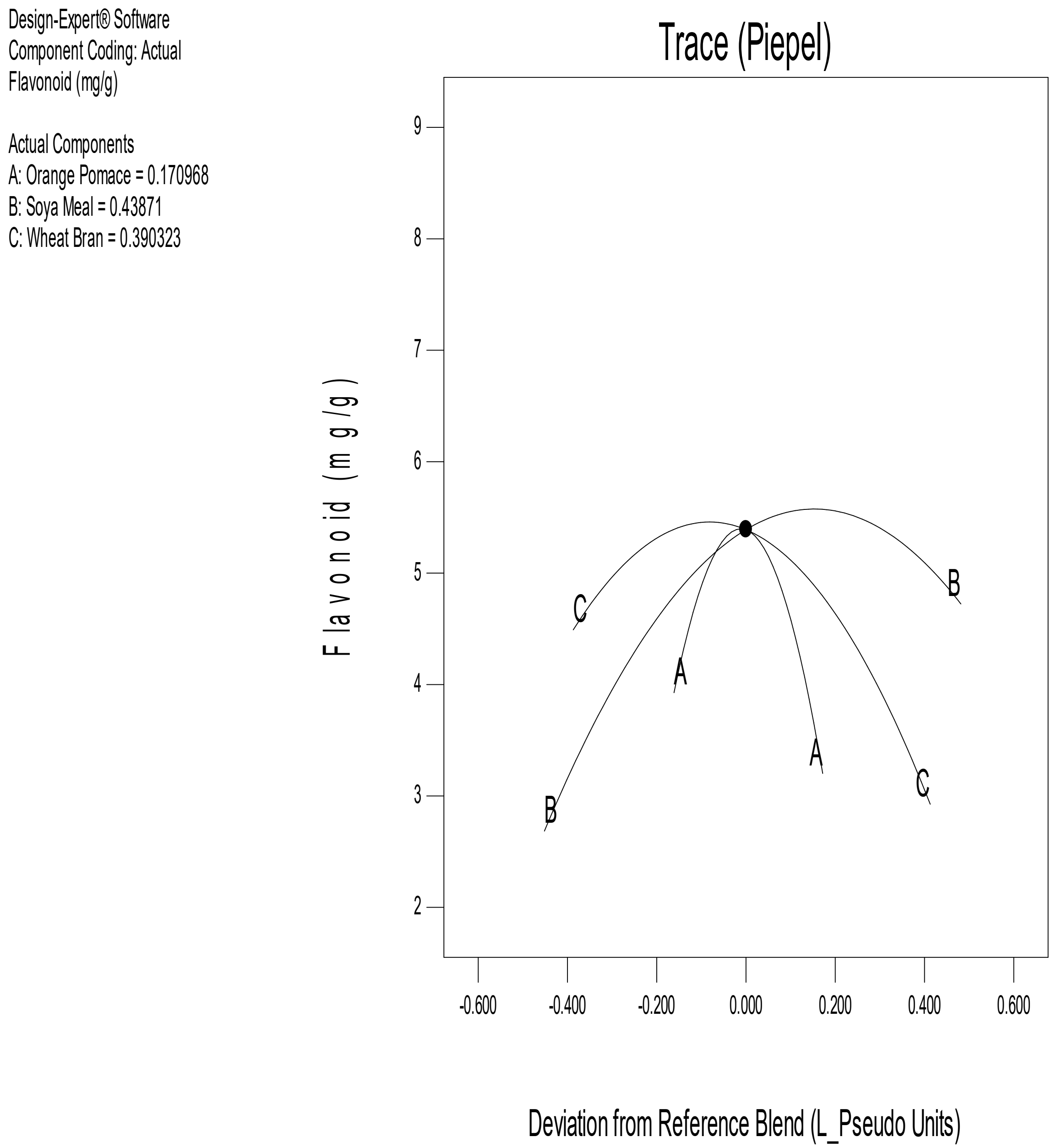


Figure 4.16: Predicted flavonoid trace plot of components

4.2.3 Carotenoids content of the extruded blends

The carotenoid content of the extrudates ranged from 0.02 to 0.14 mg/g (Table 4.5). This was higher than the value of biscuit enriched with mango peel powder stated by Ajila and Rao (2013) but smaller than values stated for crispbreads with carrot and pumpkin by-products. The highest value was observed (0.14 mg/g) at blend of 5% pomace, 25% soyameal and 70% wheat bran while the least (0.02 mg/g) was observed for three blends with higher amount of pomace and soya meal. Carotenoids are a photosynthetic auxiliary pigment that cannot be produced by the human body. These colorants that are converted by the human body into vitamin A must be supplemented by ingestion (Van den Berg *et al*., 2000).

An increase in carotenoid concentration was observed with a high quantity of wheat bran and a small quantity of pomace. This suggested an increase in the nutraceutical properties of the products. Significant variation ($p < 0.05$) was observed among most of the blends. Carotene in addition to be a safe source of vitamin A is also a healthy food colour. Evidence showed that β-carotene as a highly active singlet oxygen scavenger can be useful in the avoidance of free radical diseases (Obradović, *et al*., 2014). For example, a food rich in beta-carotene may protect against the threat of developing certain cancers, prevent skin diseases and vision problems (Konrade *et al*., 2018). The total daily consumption of carotenoids in the western diet is about 6 mg (Leitzmann, 2016).

Predicted carotenoid trace plot of the components is shown in Figure 4.17; increase in the pomace content after the reference mix led to a sharp decrease in carotenoid, increase in soyameal also led to a reduction in the amount of carotenoid while further addition of wheat bran resulted in an increase in carotenoids.

4.2.4 Ferric reducing antioxidant properties (FRAP) of the extruded blends

The value of FRAP of the extrudates was between 0.44 to 24.33 mg/g. This value was higher than 5.2 – 12.8 µmol TE/g reported for snacks enriched with powdered tomato by Wojtowicz *et al*. (2018).

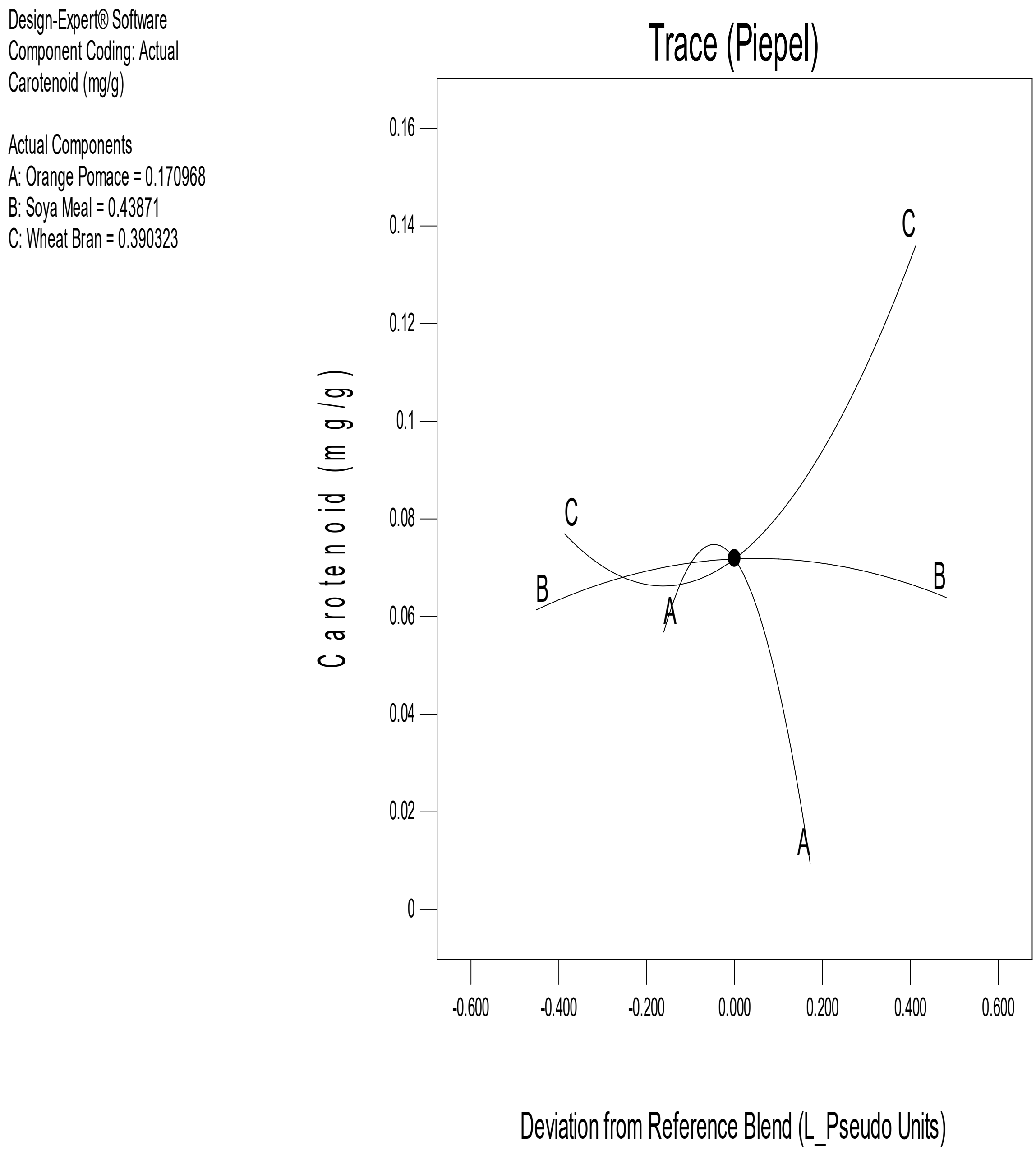


Figure 4.17: Predicted carotenoid trace plot of components

The ferric reducing antioxidant power (FRAP) is a widely acknowledged indicator of phenolic activity similar to the reducing power (Dong *et al*., 2014). Higher FRAP was observed at higher quantity of wheat bran and lower quantity of orange pomace. The predicted FRAP trace of the components showed that increase in pomace brought about a sharp reduction in FRAP, also rise in soyameal decreased FRAP, while increase in wheat bran led to the increase of FRAP (Figure 4.18). The components and their interaction shows that pomace had least impact on FRAP, increase in pomace may lead to decrease in ferric reducing antioxidant power of the blends. Pomace wheat bran (AC) with the highest coefficient (192.80) had the greatest impact on FRAP (eqn. 4.8).

$$FRAP = 140.08A + 9.69B + 22.82C + 183.97AB + 192.80AC - 30.24BC \quad \text{..... (4.8)}$$

4.3 Functional Properties

4.3.1 Bulk density of the extruded blends

The extrudate bulk density (BD) was from 0.42 to 0.61 g/cm^3 (Table 4.7). The value were higher than the one reported for guava pomace and rice flour extrudate by Tangirala *et al*., (2012) but comparable to that of Sweet potato and tomato pomace extrudate (Dhungana *et al*. 2014). Bulk density is usually a measure of the physical properties and characteristics of extrudates (Escalante-Aburto *et al*. 2014). It describes the expansion of products in all directions. Bulk density was higher with high soyameal concentration and low orange pomace concentration. This corroborated the previous studies that when materials high in fibre and protein are added to starch products, density of the product increased (Veronica *et al*., 2006).

The predicted bulk density trace diagram of the components (Figure 4.19) shows that a boost in pomace and wheat bran led to a decrease in bulk density, while an increase in soyameal led to an increase in bulk density.

The more the soyameal, the higher the bulk density of the extrudates which may be the result of high content of lipids and protein of soyameal which influenced the rheological characteristics of the blend as supported by Yagci and Gogus (2008). Equation (4.9) shows that pomace had lowest contribution to the bulk density with coefficient of -0.45 while pomace wheat bran interaction (1.60) contributed most to the bulk density.

Table 4.7: Values of functional variables of extruded blends

Blend S/N	Orange pomace (%)	Soymeal (%)	Wheat bran (%)	Bulk density (g/cm^3)	Water solubility index (%)	Water absorption index (g/g)
1	13	62	25	$0.58±0.00^{b}$	$4.67±2.31^{e}$	$2.93±0.16^{e}$
2	17	44	39	$0.61±0.01^{a}$	$9.33±1.15^{bcd}$	$2.91±0.01^{e}$
3	5	80	15	$0.59±0.01^{b}$	$10.00±0.00^{bcd}$	$3.19±0.14^{de}$
4	10	80	10	$0.53±0.01^{c}$	$12.67±1.15^{ab}$	$3.18±0.07^{de}$
5	23	52	25	$0.53±0.01^{c}$	$8.67±2.31^{bcde}$	$3.61±0.06^{bcd}$
6	20	10	70	$0.44±0.00^{g}$	$8.00±0.00^{cde}$	$3.86±0.05^{b}$
7	5	25	70	$0.46±0.01^{f}$	$8.00±2.00^{cde}$	$3.52±0.20^{bcd}$
8	23	27	50	$0.46±0.01^{f}$	$8.67±1.15^{bcde}$	$3.69±0.20^{b}$
9	11	62	27	$0.48±0.01^{e}$	$12.67±3.05^{ab}$	$3.67±0.11^{b}$
10	11	35	55	$0.48±0.01^{e}$	$11.33±3.05^{abc}$	$3.49±0.14^{bcd}$
11	18	27	55	$0.51±0.01^{d}$	$14.67±4.16^{a}$	$3.62±0.18^{bc}$
12	30	60	10	$0.53±0.01^{c}$	$6.00±2.00^{de}$	$3.24±0.06^{cde}$
13	30	10	60	$0.42±0.01^{h}$	$8.00±2.00^{cde}$	$4.29±0.24^{a}$

Averages of three replicates followed by different letters in the same column are significantly different ± Standard deviation

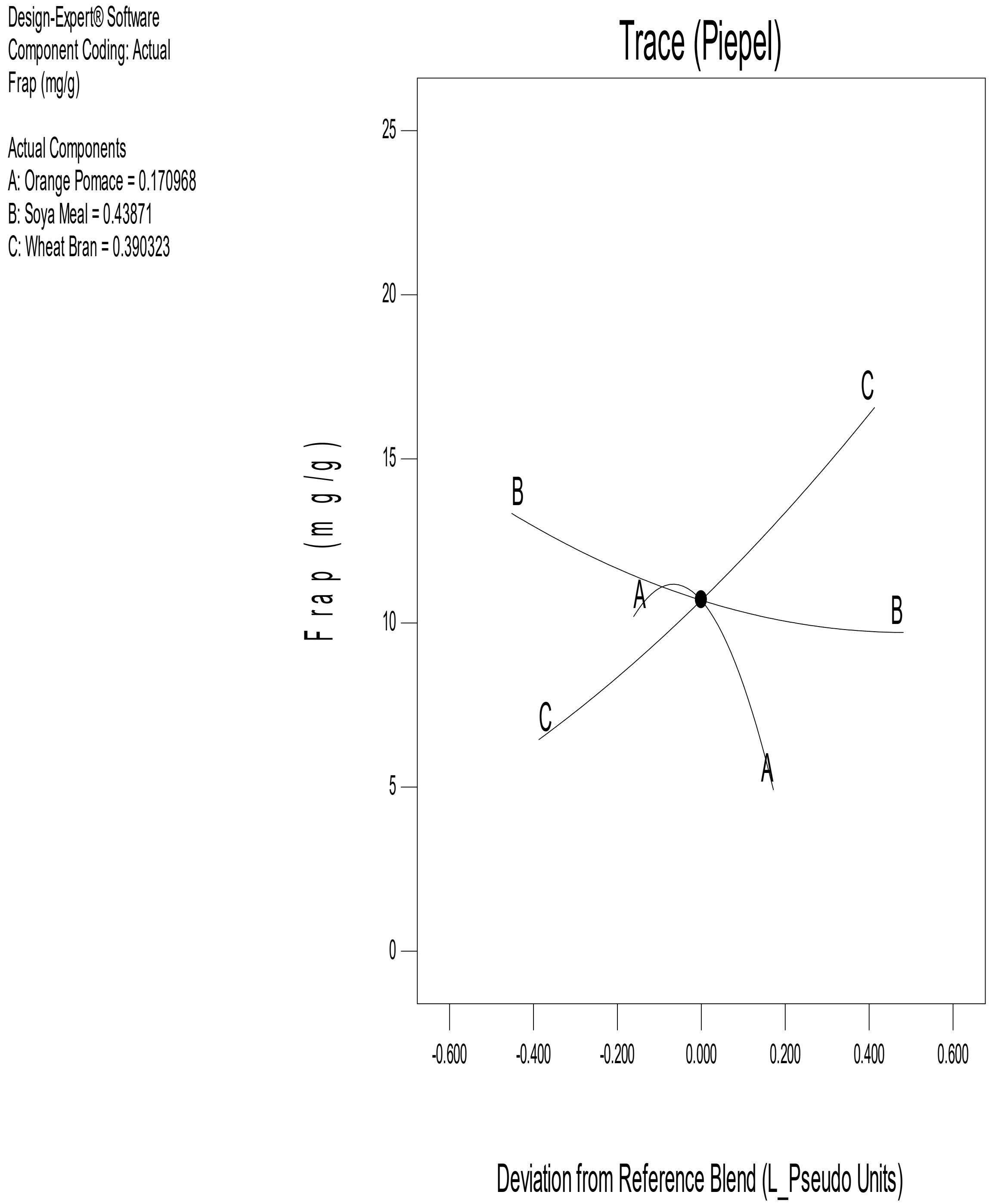


Figure 4.18: Predicted FRAP trace plot of components

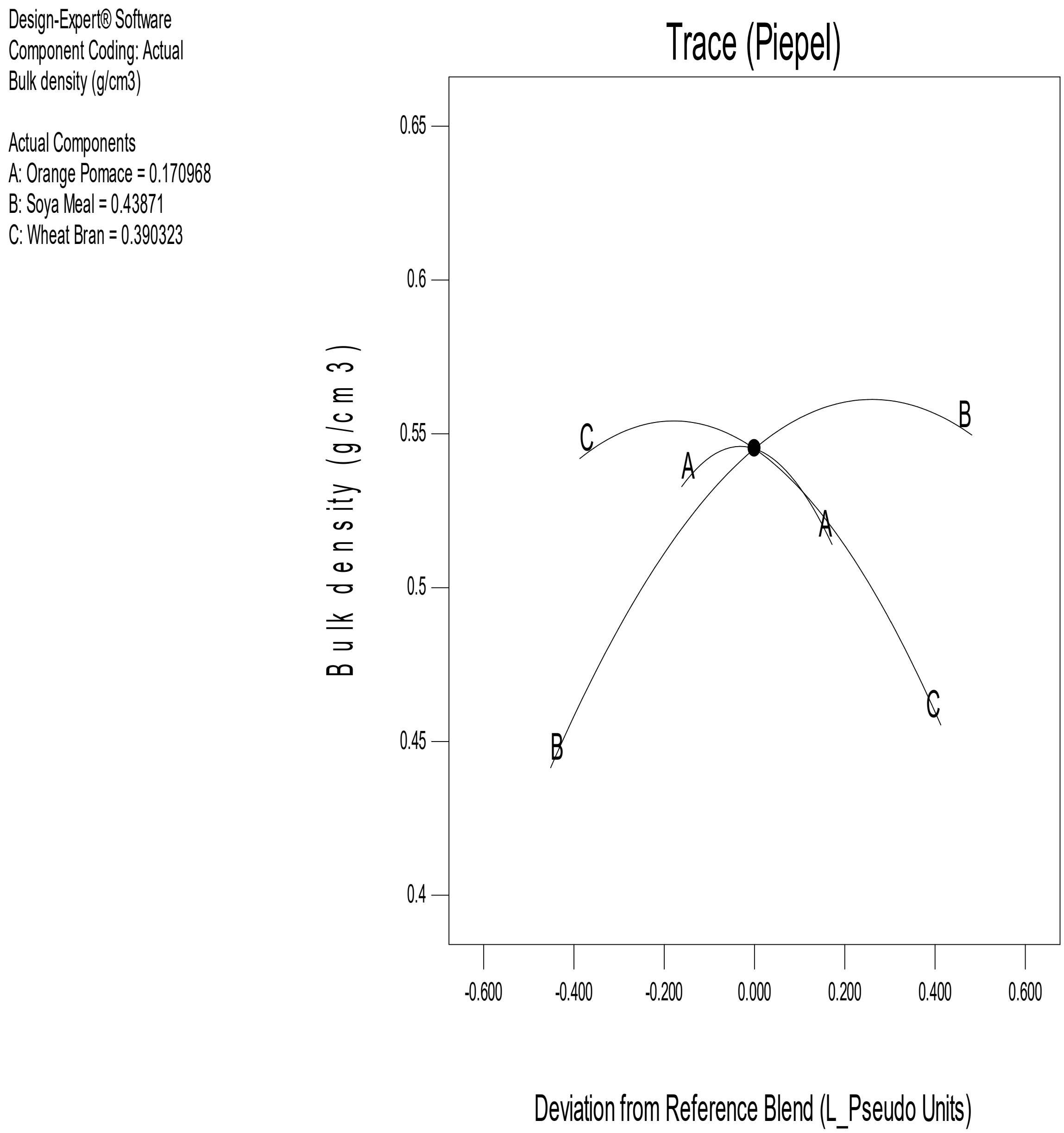


Figure 4.19: Predicted bulk density trace plot of components

$$Bulk\ density = -0.45A + 0.50B + 0.28C + 1.40AB + 1.60AC + 0.48BC \quad \text{....... (4.9)}$$

4.3.2 Water solubility index of the extruded blends

The values for the water solubility index (WSI) for the extrudates were 4.67 to 14.67 % (Table 4.7). This is higher than for sweet potato and tomato pomace (Dhungana *et al* (2014) and red lentil-carrot pomace snack. Water solubility and absorption parameters were used to describe the extruded product and are useful to predict the behavior of the extruded material during further processing. Solubility in water also specifies the number of small molecules dissolved in water. (Ajita *et al*., 2017).

Low WSI is associated with occurrence of water insoluble compounds formed by the macromolecules of amylose, proteins and lipids compounds of the raw materials (Wojtowicz *et al*., 2018). It is an indicator of extent of starch degradation. This means that at lower WSI, the starch degrades only slightly and such a condition results in a lower soluble particle in the formulation (Hernandez-Diaz *et al.,* 2007). Higher WSI values were observed with increased soyameal and low pomace concentration, also at high concentrations of wheat bran and low pomace. This is due to low carbohydrate content of the blends. Predicted WSI trace plot of components indicated increase in soyameal and wheat bran led to an increase in water solubility index until it reached its maximum, when further increase led to a decrease in WSI (Figure 4.20).

WSI serves as a sign of the breakdown of molecular compounds such as starch, fibre and protein (Suksomboon *et al*., 2011, Seth and Rajamanickam 2012). The increase in soyameal content may be in relation to the solubilisation of proteins through extrusion caused by the mechanical cutting (Ghumman *et al*., 2016).

4.3.3 Water absorption index of the extruded blends

The water absorption index (WAI) of the extrudate was between 2.91 and 4.29 (Table 4.5). Some of the value was lower and some were within the values (3.2 – 5.9) reported for snacks supplemented with tomato powder by Wojtowicz *et al*., (2018).

The water absorption index showed the amount of water restrained by the extrudate. WAI is ascribed to the distribution of starch in excess water and the concentration is increased

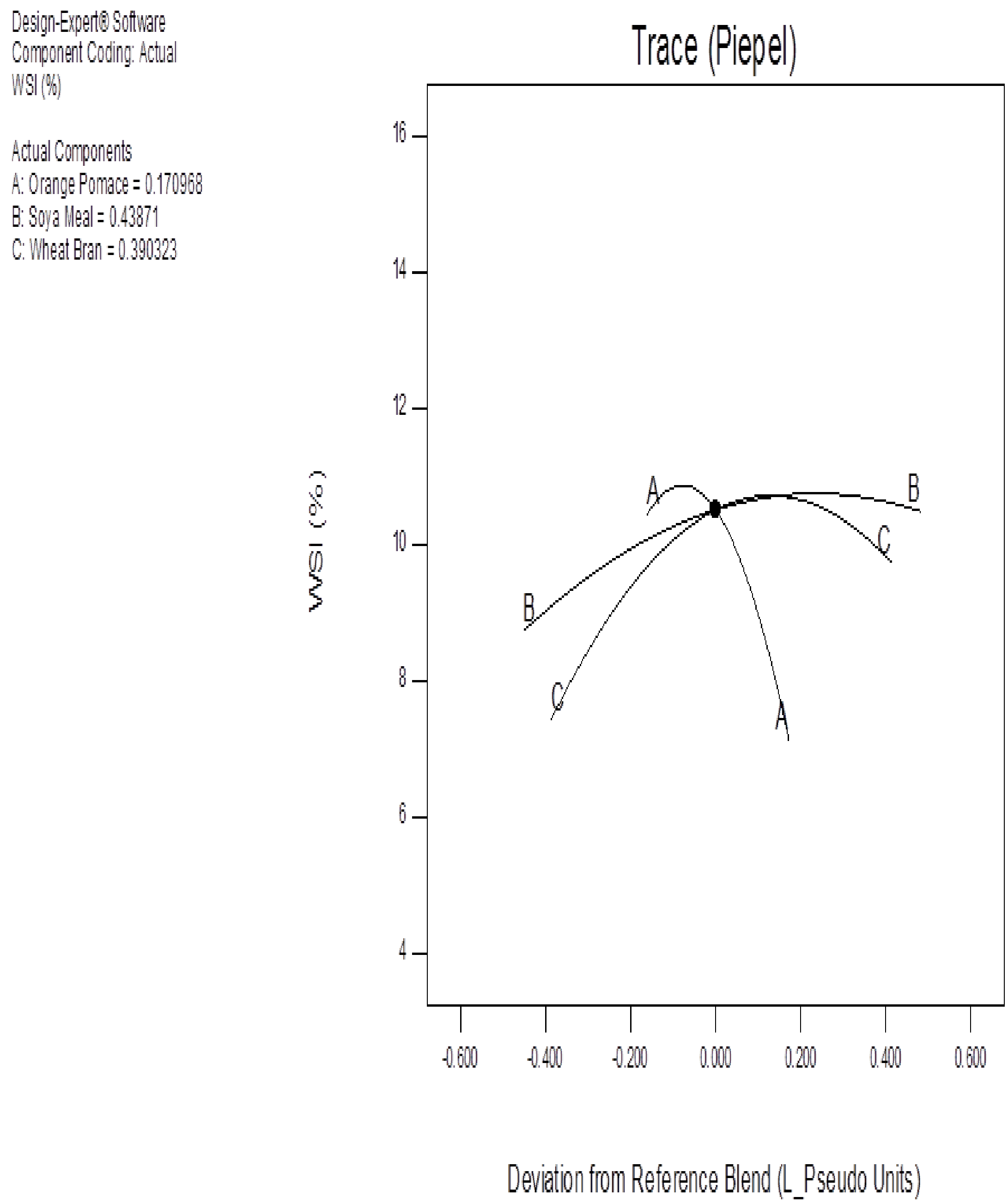


Figure 4.20: Predicted water solubility index trace plot of components

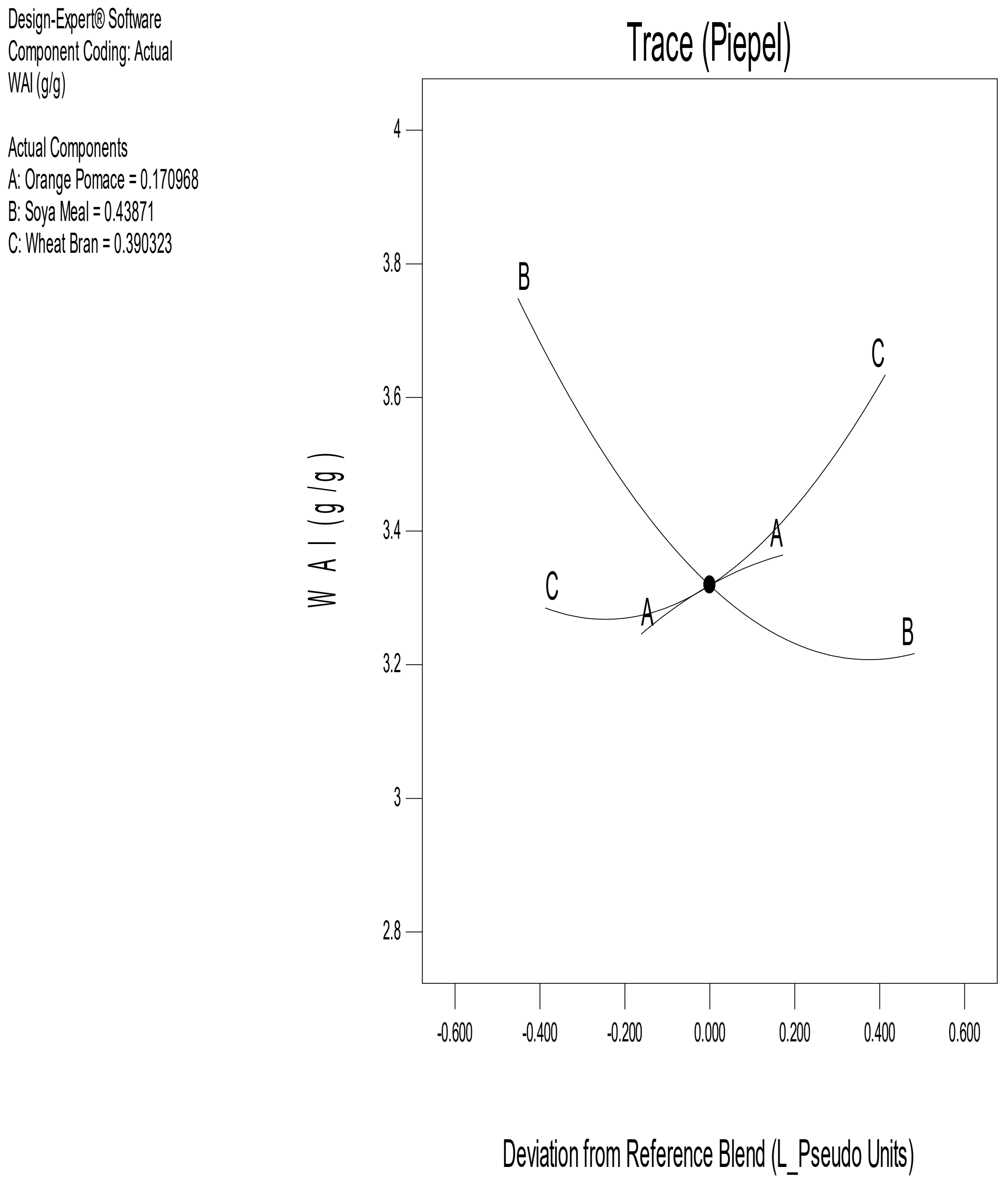


Figure 4.21: Predicted WAI trace plot of components

by the extent of starch destruction due to gelatinization and extrusion-induced breakup. It corresponds to molecular weight decrease of amylose and amylopectin molecules (Yagci and Gogus, 2008). A decrease in the water absorption index with increasing amount of soy flour can be linked to the lubricating effect of its lipids (Navarro-Cortez *et al*., 2016) which makes the oil in soybean to interfere with the water uptake. Predicted WAI trace plot shows increase in wheat bran and pomace led to increase in WAI while increase in soyameal led to reduction in WAI (Figure 4.21). The high value of the water absorption index in products refers to their dietary fibre content and their ability to retain water in their matrix. This property may increase the fecal weight and possibly reduce the absorption of nutrients from the gastrointestine after ingestion (Gallaher and Schneeman, 2001).

Soyameal wheat bran (BC) interaction had least effect on WAI while wheat bran contributed most to the water absorption index of the products (eqn. 4.10).

$$WAI = 3.02A + 3.31B + 4.19C + 0.51AB + 0.47AC - 2.07BC \quad \text{.........} \ (4.10)$$

4.4 Sensory Attributes of the Extruded Blends

The sensory assessment scores for colour, aroma, crispiness, taste and overall acceptability are presented in Figure 4.22a-e. The highest level of acceptance in the organoleptic assessment considering the overall acceptability (5.16) was found for the blend of 10% pomace, 80% soymeal and 10% wheat bran and the lowest one for the blend of 13% pomace, 62% soymeal and 25% wheat bran (3.2). The highest level for aroma, crispiness and taste acceptance was also reported for the blend of 10% pomace, 80% soymeal and 10% wheat bran (5.56, 5.12 and 5.36 points respectively). Colour was best scored for blend of 17% pomace, 44% soyameal and 39% wheat bran (5.72) and the lowest for blend of 13% pomace, 62% soyameal and 25% wheat bran.

The acceptable samples were those with average ratings for the overall acceptance of more than 5.0, that means neither like nor dislike (Sabanis *et al*., 2009).

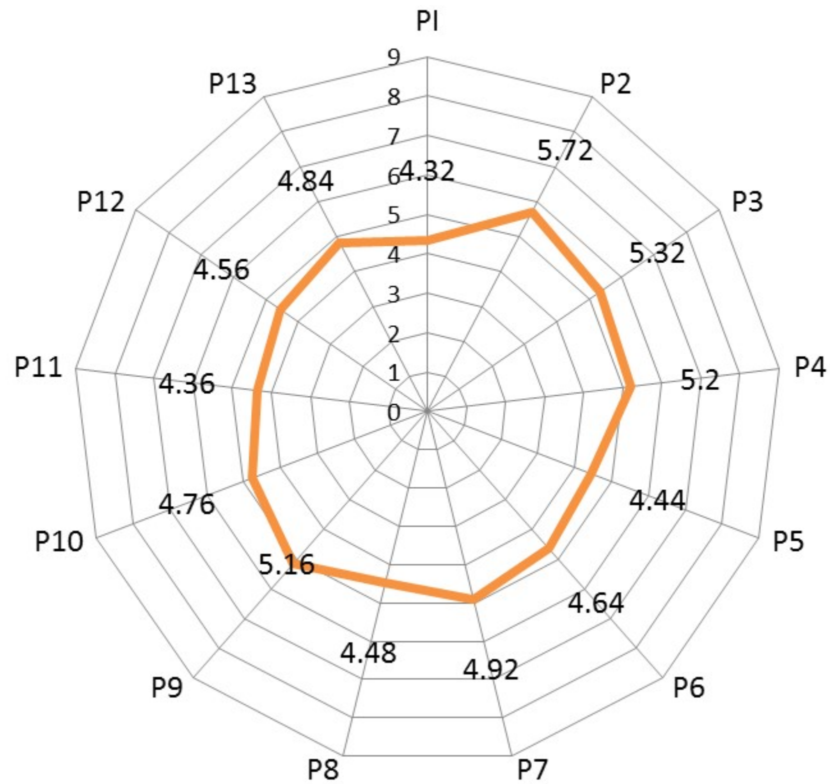


Figure 4.22a: Colour ratings of the extruded blends

P1= 13%pomace, 62% soyameal, 25% wheat bran,

P2 = 17% pomace, 44% soyameal, 39% wheat bran,

P3 = 5% pomace, 80% soyameal, 155 wheat bran,

P4 = 10% pomace, 80% soymeal, 10% wheat bran,

P5 = 23% pomace, 52% soyameal, 25% wheat bran,

P6 = 20% pomace, 10% soyameal, 70% wheat bran,

P7 = 5% pomace, 25% soyameal, 70% wheat bran,

P8 = 23% pomace, 27% soyameal, 50% wheat bran,

P9 = 11% pomace, 62% soyameal, 27% wheat bran,

P10 = 10% pomace, 35% soyameal, 55% wheat bran,

P11 = 18% pomace, 27% soyameal, 55 wheat bran,

P12 = 30 % pomace, 60% soyameal, 10% wheat bran,

P13 = 30% pomace, 10% soyameal, 60% wheat bran

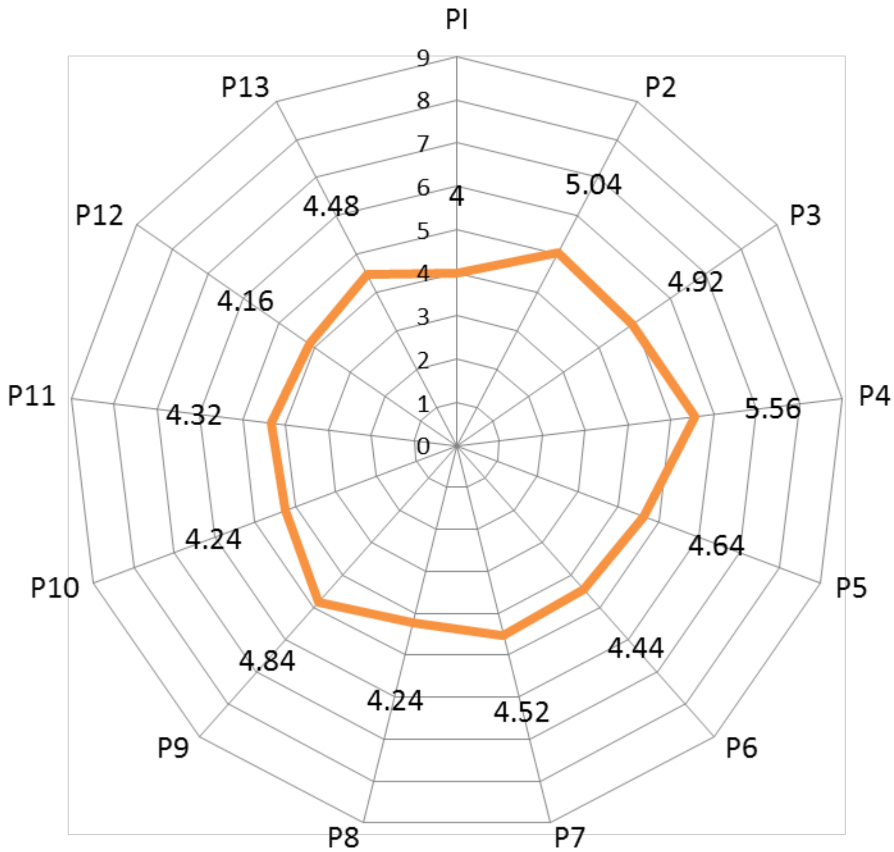


Figure 4.22b: Aroma ratings of the extruded blends

P1= 13%pomace, 62% soyameal, 25% wheat bran,
P2 = 17% pomace, 44% soyameal, 39% wheat bran,
P3 = 5% pomace, 80% soyameal, 155 wheat bran,
P4 = 10% pomace, 80% soymeal, 10% wheat bran,
P5 = 23% pomace, 52% soyameal, 25% wheat bran,
P6 = 20% pomace, 10% soyameal, 70% wheat bran,
P7 = 5% pomace, 25% soyameal, 70% wheat bran,
P8 = 23% pomace, 27% soyameal, 50% wheat bran,
P9 = 11% pomace, 62% soyameal, 27% wheat bran,
P10 = 10% pomace, 35% soyameal, 55% wheat bran,
P11 = 18% pomace, 27% soyameal, 55 wheat bran,
P12 = 30 % pomace, 60% soyameal, 10% wheat bran,
P13 = 30% pomace, 10% soyameal, 60% wheat bran

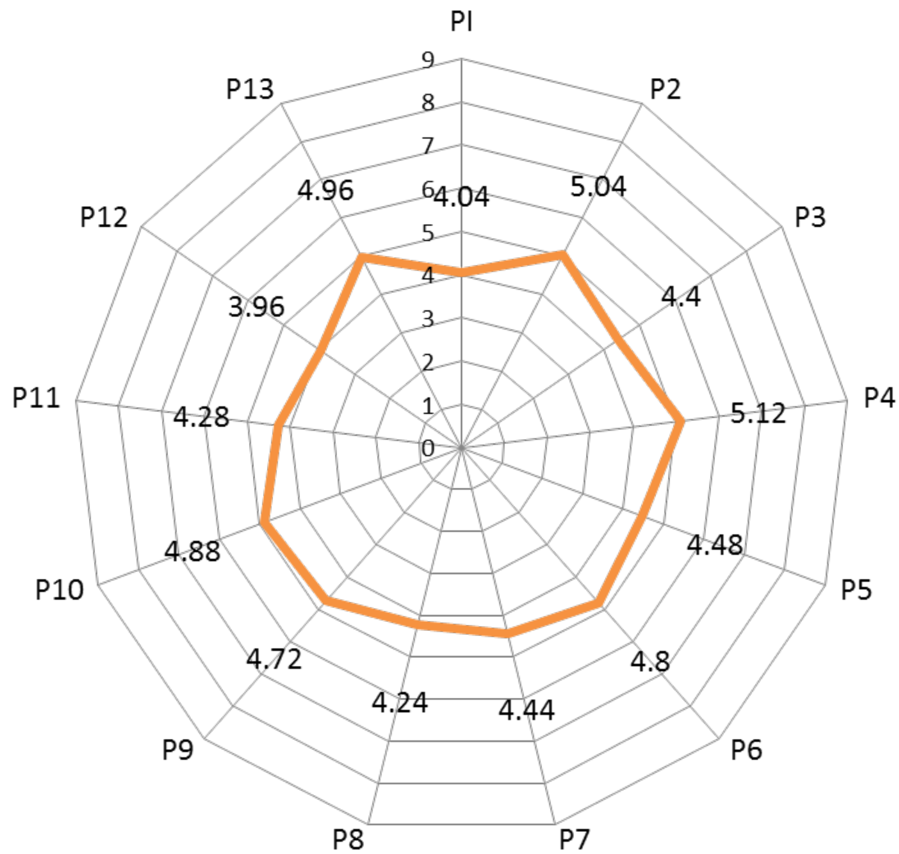


Figure 4.22c: Crispiness ratings of the extruded blends

P1= 13%pomace, 62% soyameal, 25% wheat bran,
P2 = 17% pomace, 44% soyameal, 39% wheat bran,
P3 = 5% pomace, 80% soyameal, 155 wheat bran,
P4 = 10% pomace, 80% soymeal, 10% wheat bran,
P5 = 23% pomace, 52% soyameal, 25% wheat bran,
P6 = 20% pomace, 10% soyameal, 70% wheat bran,
P7 = 5% pomace, 25% soyameal, 70% wheat bran,
P8 = 23% pomace, 27% soyameal, 50% wheat bran,
P9 = 11% pomace, 62% soyameal, 27% wheat bran,
P10 = 10% pomace, 35% soyameal, 55% wheat bran,
P11 = 18% pomace, 27% soyameal, 55 wheat bran,
P12 = 30 % pomace, 60% soyameal, 10% wheat bran,
P13 = 30% pomace, 10% soyameal, 60% wheat bran

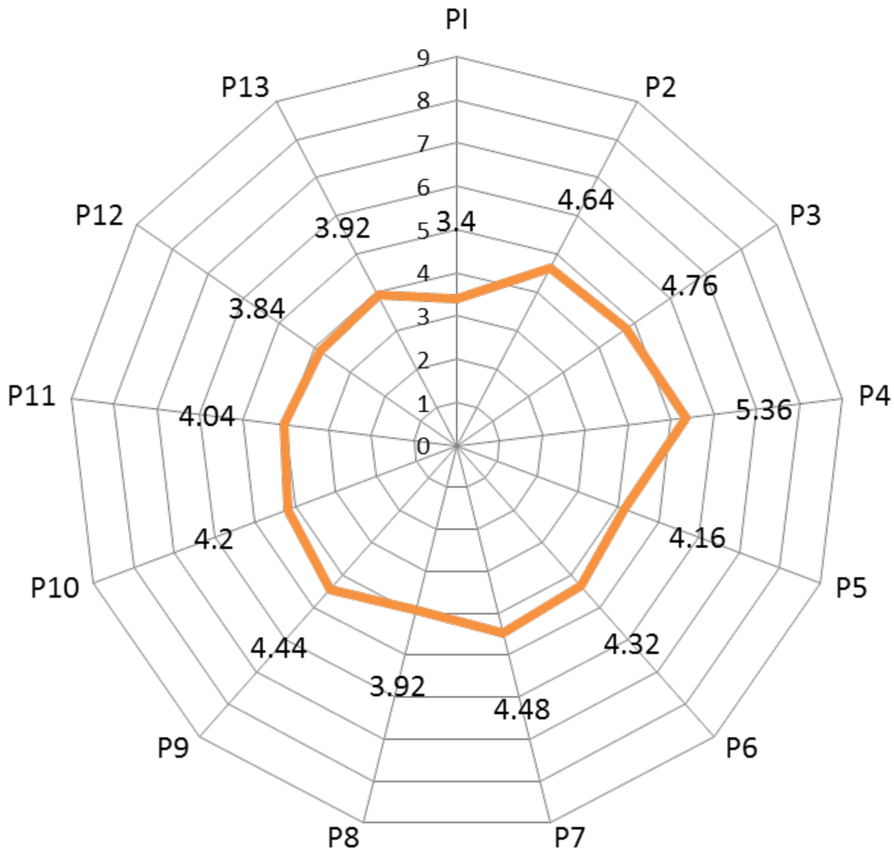


Figure 4.22d: Taste ratings of the extruded blends

P1= 13%pomace, 62% soyameal, 25% wheat bran,
P2 = 17% pomace, 44% soyameal, 39% wheat bran,
P3 = 5% pomace, 80% soyameal, 155 wheat bran,
P4 = 10% pomace, 80% soymeal, 10% wheat bran,
P5 = 23% pomace, 52% soyameal, 25% wheat bran,
P6 = 20% pomace, 10% soyameal, 70% wheat bran,
P7 = 5% pomace, 25% soyameal, 70% wheat bran,
P8 = 23% pomace, 27% soyameal, 50% wheat bran,
P9 = 11% pomace, 62% soyameal, 27% wheat bran,
P10 = 10% pomace, 35% soyameal, 55% wheat bran,
P11 = 18% pomace, 27% soyameal, 55 wheat bran,
P12 = 30 % pomace, 60% soyameal, 10% wheat bran,
P13 = 30% pomace, 10% soyameal, 60% wheat bran

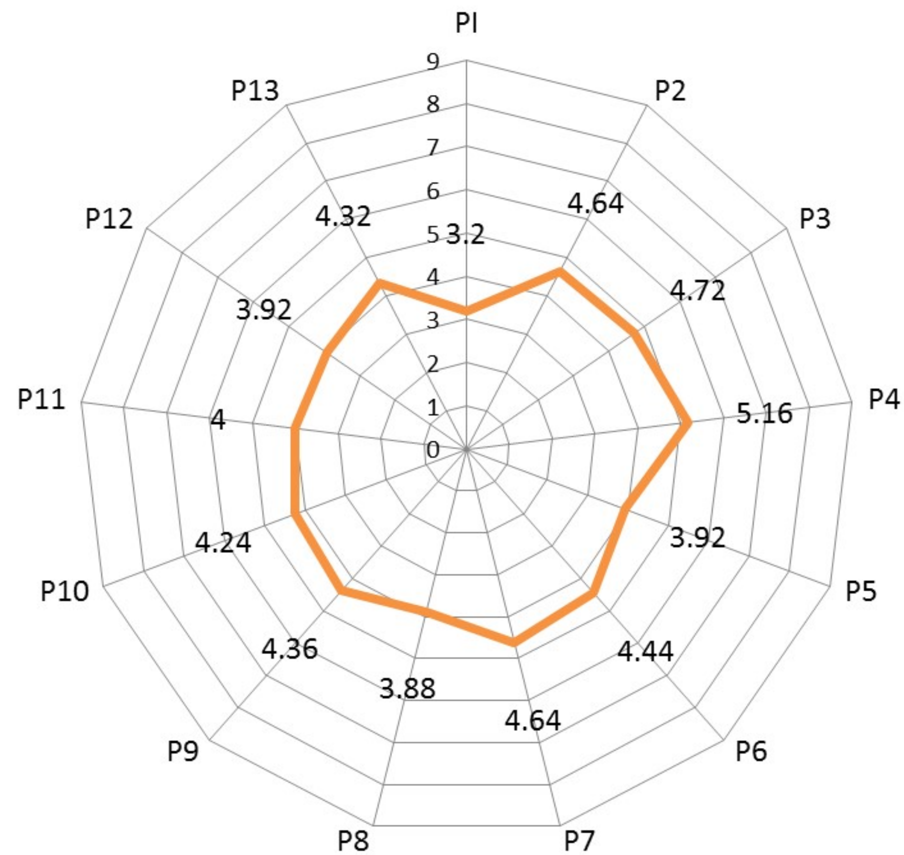


Figure 4.22e: Overall acceptability of the extruded blends

P1= 13%pomace, 62% soyameal, 25% wheat bran,
P2 = 17% pomace, 44% soyameal, 39% wheat bran,
P3 = 5% pomace, 80% soyameal, 155 wheat bran,
P4 = 10% pomace, 80% soymeal, 10% wheat bran,
P5 = 23% pomace, 52% soyameal, 25% wheat bran,
P6 = 20% pomace, 10% soyameal, 70% wheat bran,
P7 = 5% pomace, 25% soyameal, 70% wheat bran,
P8 = 23% pomace, 27% soyameal, 50% wheat bran,
P9 = 11% pomace, 62% soyameal, 27% wheat bran,
P10 = 10% pomace, 35% soyameal, 55% wheat bran,
P11 = 18% pomace, 27% soyameal, 55 wheat bran,
P12 = 30 % pomace, 60% soyameal, 10% wheat bran,
P13 = 30% pomace, 10% soyameal, 60% wheat bran

(9-point scale, where 1 point – the lowest level of acceptance and 9 points – the highest one).

Out of the 13 samples 9 had average score of 4.5 and above for the overall acceptability. Therefore, it can be concluded that the addition of pomace not more than 10%, soymeal 80% and wheat bran 10% had best organoleptic acceptance among the extrudates. This agrees with the observation of Zaker *et al*. (2016) who worked on orange pomace biscuit and that of Ajila *et al*. (2008) who studied enriched mango peel powder biscuit that 10% fortification was adjudged best for organoleptic acceptance.

4.5 Nutritional Properties of the Products

4.5.1 Feed Efficiency/weight gain

The best three samples with the highest overall acceptability score from sensory evaluation were used for rat studies along with a commercial feed as control f. At the expiration of the experiment, weight gain and food consumed by the rats fed on the experimental diets were lower than the control (Figure 4.23). There were no significant differences in food intake between control and tested diets, but weight gain was significantly different. Rat fed with food blend of 17% pomace, 44% soyameal and 39% wheat bran had the highest weight gain while those fed with 5 % pomace, 80% soyameal and 15% wheat bran had lowest weight gain. Lower weight gain from the tested food suggests that the food could be considered to make a difference in the onset and progression of overweight and obesity. This is in line with findings of Keenan *et al*., (2006) that fibre is able to reduce body weight gain or decrease weight gain. This has been attributed to various factors such as soluble fibre, which produces glucagon-like peptide (GLP-1) and peptide TY when fermented in the colon. The two intestinal hormones take part in inducing satiety, decrease energy intake and also decrease metabolizable energy.

Rat fed with control diet were able to digest the diet better than the other tested diets which resulted in increased digestion and absorption of nutrients. Soluble dietary fibre in form of neutral detergent fibre was more in the control diet than the tested diets (Table 4.8) leading to more absorbed nutrients and weight gain. The same trend was observed in the Feed efficiency ratio and conversion ratio (Figure 4.24 and 4.25).

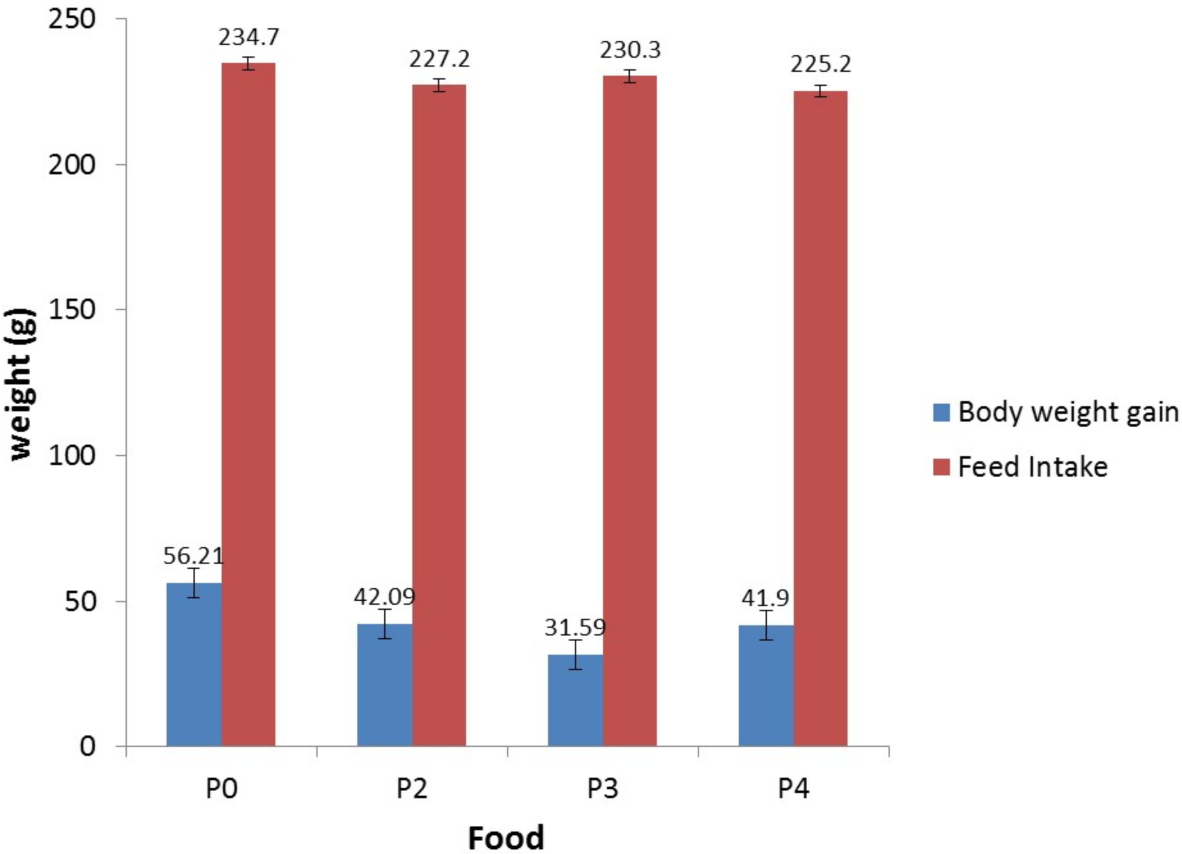


Figure 4.23: Food intake and Body weight gain by rats fed with control and tested diets

P0 = control,

P2 = 17 % pomace 44% soyameal 39 % wheat bran,

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 = 10 % pomace 80 % soyameal 10 % wheat bran

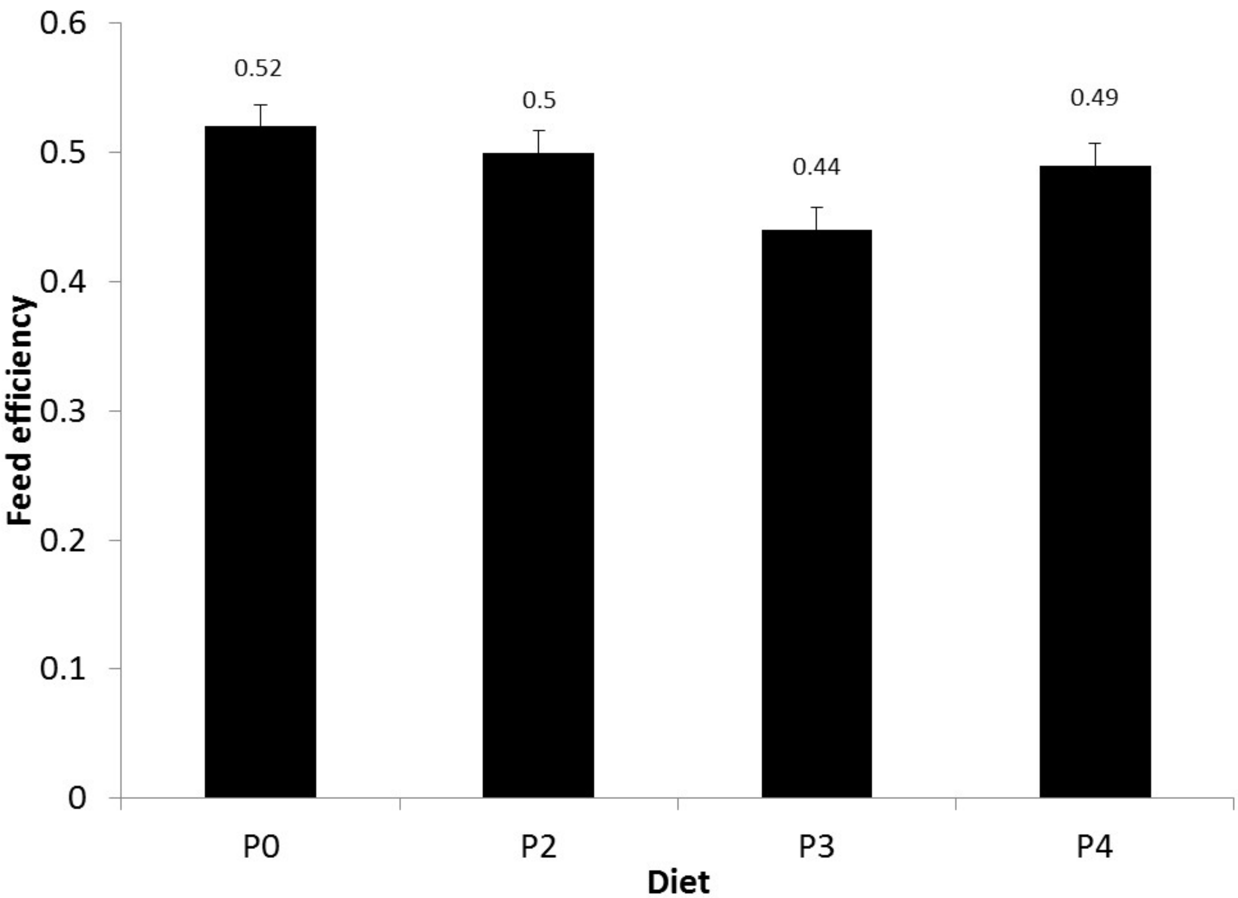


Figure 4.24: Feed efficiency of control and tested diets

P0 = control,

P2 = 17 % pomace 44% soyameal 39 % wheat bran,

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 = 10 % pomace 80 % soyameal 10 % wheat bran

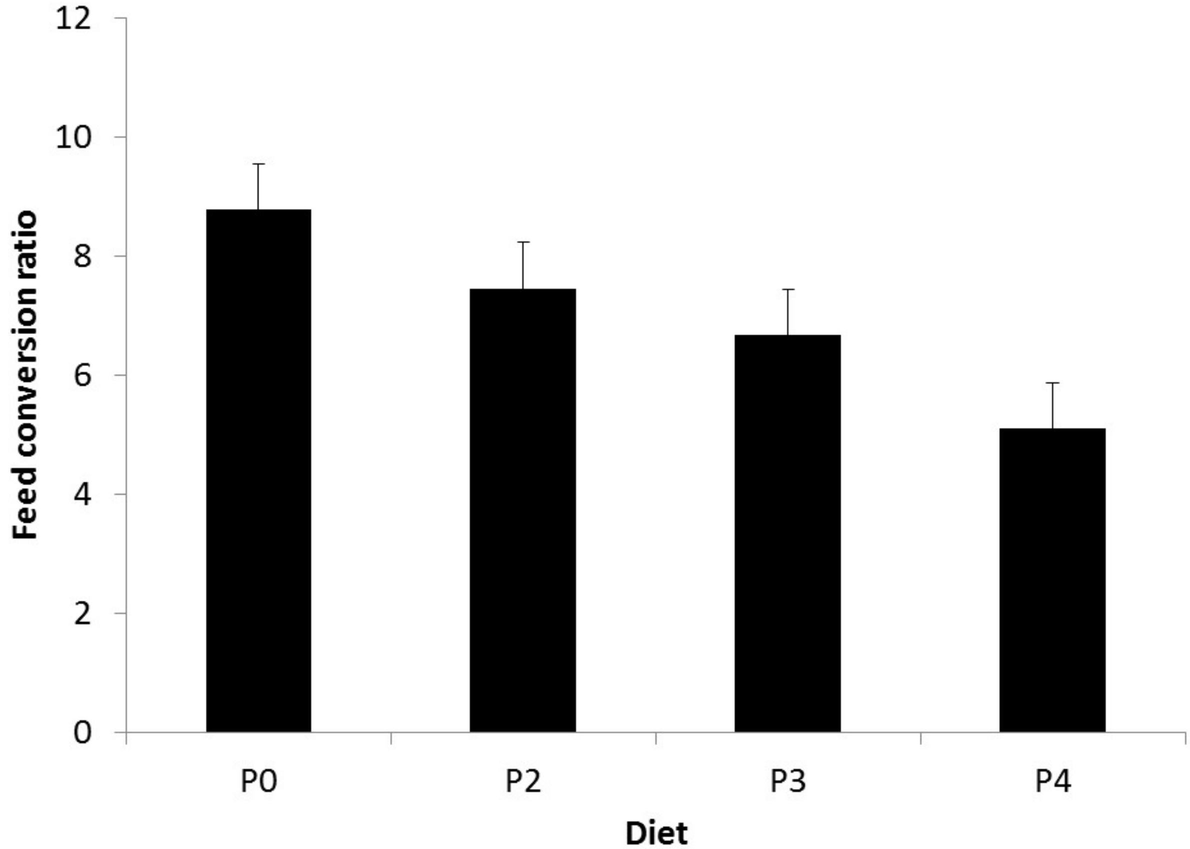


Figure 4.25: Feed conversion ratio of control and tested diets on rat

P0 = control,

P2 = 17 % pomace 44% soyameal 39 % wheat bran,

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 = 10 % pomace 80 % soyameal 10 % wheat bran

Table 4.8: Fibre fraction of control and experimental diets fed to rat

Diet	%NDF	%ADF	%ADL	%Hemicellulose	%Cellulose	%Lignin
P0	62.19	33.70	15.90	28.49	17.80	15.90
P2	39.67	18.85	2.76	20.82	16.09	2.76
P3	46.28	29.61	5.89	16.67	23.72	5.89
P4	41.63	26.48	3.36	15.15	23.12	3.36

Results are average of three replicates

NDF – Neutral detergent fibre

ADF – Acid detergent fibre

ADL – Acid detergent lignin

P0 = control,

P2 = 17 % pomace 44% soyameal 39 % wheat bran,

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 = 10 % pomace 80 % soyameal 10 % wheat bran.

The result was comparable with the findings of Rashad and Moharib (2003) of lowered nutritional parameters as a result of high fibre feed fed to rat. The trend could be due to the distribution of soluble and insoluble fibre fraction; hemicellulose, cellulose and lignin present in the tested and control diets as seen in Table 4.8. The high content of hemicellulose and cellulose which are digestible led to higher conversion ratio of the nutrient for body utilization while low amount of lignin which is indigestible was observed in the tested diets.

4.5.2 Hematological parameters

The result of the hematological parameters shows that Packed Cell Volume (PCV) count to be from 35.67 to 37.33% (Table 4.9) and is within clinical range for rat. The highest PCV was obtained for diet P2 followed by diet P3. No significant difference was observed between the control (P0) and the tested diets. The PCV known as hematocrit (Ht) or (HCT) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in the blood. According to Isaac *et al*. (2013) PCV is involved in the transportation of oxygen and absorbed nutrients. Increased PCV shows better transport and therefore increases the primary and secondary polycythemia.

A higher PCV value for rats fed with the study diet shows that diets are suitable for transporting oxygen and nutrients. The hemoglobin (HGB) value of the albino rat fed the diet P2 (12.63) was the highest, followed by P3, P0 and finally P4. No significant variation ($p > 0.05$) was observed among HGB of rat fed with the food. The result suggests that hemoglobin is sufficient for the albino rats for its physiological function of transporting oxygen to the animal tissues to oxidize red blood cells (RBC). Rats fed control and experimental feed (P0, P2, P3 and P4) were not significantly different. Diet P2 had the highest mean RBC count (6.00×10^{12}) while P4 had the least (5.66×10^{12}). These values are

Table 4.9: Hematological variables of rat fed control and experimental diets

Diet	PCV (%)	HGB (g/dl)	RBC (x 10^{12})	WBC (x 10^9)	Plt	Lym	Neu	Mo	Eos
P0	36.33[a]	12.07[a]	5.95[a]	5283[a]	171667[a]	65.33[a]	30.33[a]	1.67[a]	2.67[a]
P2	37.33[a]	12.63[a]	6.00[a]	5950[a]	191000[a]	66.33[a]	31.00[a]	1.33[a]	1.33[a]
P3	37.00[a]	12.33[a]	5.96[a]	6400[a]	156000[a]	70.33[a]	25.33[a]	2.00[a]	2.33[a]
P4	35.67[a]	11.97[a]	5.66[a]	6100[a]	133333[a]	66.67[a]	30.67[a]	1.33[a]	1.33[a]

Value are mean of three replicates followed by different letters in the same column are significantly different.

PCV – packed cell volume, HGB – Heamoglobin, RBC –red blood cell, WBC – white blood cell, plt – platelet, lym - Lymphocyte, Neu –Neutrophils, Mo -Monocycte, Eos –Eosinophils.

P0 = control,

P2 = 17 % pomace 44% soyameal 39 % wheat bran,

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 = 10 % pomace 80 % soyameal 10 % wheat bran.

in the laboratory range for rat (Sharp *et al*., 1998) and suggested no toxic effect of experimental diet on the rat.

Red blood cells are used for transportation of oxygen and carbon dioxide in the body (Isaac *et al.* 2013); hence, low red blood cell count signifies a decrease in the level of oxygen transported to the tissues and carbon dioxide returned to the lungs (Soetan *et al*., 2013).

Highest white blood cell (WBC) was recorded for rats fed with diet P3 (6400×10^{9}) which was not significantly different ($p > 0.05$) from other diets. The main roles of white blood cells are defending the body by phagocytosis against attack by foreign organisms, combating infections, production, transportation and distribution of antibodies in the immune response. The result of the study is within the clinical range suggesting that the albino rats were able to generate antibodies in the course of phagocytosis with high degree of resistance to diseases (Soetan *et al*., 2013). It will also encourage their compliance to natural ecology and disease dominant conditions as supported by Iwuji and Herbert (2012).

Platelet count for rat fed with the diets was between 133333 and 191000, the highest from diet P2 while the lowest was from diet P4. There was no significant variation observed among the control and the test diets ($p > 0.05$) The difference in the level could be as a result of variation in the type (dose, structure, soluble, insoluble) of dietary fibre in the diets (Rashad and Moharib, 2003). Table 4.8 also corroborates the findings showing difference in the Dietary fibre fraction. Platelets are involved in blood clotting, the platelet concentration was within the clinical range, suggesting that the process of clot formation (blood clotting) will be quick in the event of an injury in the control diet and on tested diets. Lymphocyte value for the control and test diets were from 65.33 % to 70.33 %. The highest from Diet P3 and lowest from diet P1. No significant variation ($p > 0.05$) obtained from the diets.

Maximum neutrophilis were recorded for the blood samples of rats fed P2 (31%), followed by diet P4 (30.67%) while diet P3 had the least (25.33%). No significant variation ($p > 0.05$) was observed among the diets. The result shows no toxic effect on the blood samples of rat fed the experimental diets.

4.5.3 Biochemical parameters

The estimates of biochemical indices of liver and kidney in the study were summarized in Table 4.10. No significant variation observed ($p > 0.05$) in AST, ALT, ALP, BUN and creatinine of rat fed with control and tested diets. The low value of AST for the tested diets indicated proper functioning of the liver. All serum biochemical parameters were normal except AST which is slightly lower and are in the control range for rats. Reference ranges for AST, ALP, ALT, BUN and creatinine are between 50 and 150, 30 and 130 IU / l, 10 and 40, 12.0 and 25.8 and 0.4 and 2.3 mg / dl respectively (Buncharoen *et al*., 2012). These suggest that the tested diets had no toxic effect on the liver and kidney of the albino rats. Livers and kidneys are internal organs that have different functions. An important role of these organs is the disposal of waste products and toxic substances. The dysfunction of these organs can lead to biochemical substances entering the bloodstream (Atangwho *et al*., 2007). The effect of the diet on serum total protein was highest for the rat fed with diet P3 (7.83 g/dl) while lowest was found for diet P4 (Table 4.11), no significant variation ($p > 0.05$) was found among the control and tested diets. Albumin was between 2.60 and 3.10 for the diets, P3 also had the highest while the lowest was for diet P4. Globulin was highest with diets P2 and P3 (4.73), the lowest for P4 with a value of 4.47. However, Albumin/Globin ratio (A/G) was highest (0.67) for the diet P0 (control) followed by P3 (0.66) with the lowest 0.58 for diet P4. No significant variation ($p> 0.05$) was observed among the diets.

Triglyceride (TG) ranges from 46.30 – 55.0 (Table 4.12), total cholesterol (TC) was from 59.30 to 70.0. Highest value was observed for diet P4 while P0 (control) had the lowest value. High density lipoprotein (HDL), low density lipoprotein (LDL) and glucose (Glu) were highest for diet P4, while lowest value was observed for P0 (HDL and LDL) and P3 for glucose. No significant variation ($p > 0.05$) was observed among the values. This indicated that the diet had no toxic effects on the rats used in this study. It can therefore be concluded that the formulated food are safe for human consumption.

Table 4.10: Effect of diet on biochemical parameters of rat fed with control and tested diets

Diet	AST	ALT	ALP	BUN	CRE
P0	44.33[a]	32.00[a]	116.0[a]	16.53[a]	0.70[a]
P2	44.33[a]	30.67[a]	117.0[a]	16.70[ab]	0.70[a]
P3	42.67[a]	31.67[a]	117.3[a]	17.70[a]	0.70[a]
P4	41.67[a]	30.00[a]	114.0[a]	16.70[ab]	0.63[a]

Averages of three replicates followed by different letters in the same column are significantly different

AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, ALP – Alkaline phosphatase, BUN – Blood urea nitrogen, CRE - Creatinine

P0 = control,

P2 = 17 % pomace 44% soyameal 39 % wheat bran,

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 = 10 % pomace 80 % soyameal 10 % wheat bran

Table 4.11: Effect of diet on the serum total protein, albumin, globulin and AG ratio on rat fed with control and tested diet

Diet	T.PROT (g/dl)	ALBUL (g/dl)	GLOB	AG.r
P0	7.50[a]	3.03[a]	4.53[a]	0.67[a]
P2	7.60[a]	2.87[a]	4.73[a]	0.61[a]
P3	7.83[a]	3.10[a]	4.73[a]	0.66[a]
P4	7.07[a]	2.60[a]	4.47[a]	0.58[a]

Averages of three replicates followed by different letters in the same column are significantly

T.PROT – Total protein, ALBUL – Albumin, GLOB – Globulin, AG.r – Albumin globulin ratio
P0 = control, P2 = 17 % pomace 44% soyameal 39 % wheat bran, P3 = 5 % pomace 80% soyameal 15 % wheat bran, P4 = 10 % pomace 80 % soyameal 10 % wheat bran

Table 4.12: Effect of diet on serum lipids and glucose of rat fed with control and tested diets

Diet	TRIG	CHOL	HDL	LDL	GLU
P0	46.7a	59.3a	30.0a	20.0a	137.0a
P2	47.0a	64.7a	31.3a	23.9a	135.7a
P3	46.3a	67.7a	35.0a	23.4a	133.3a
P4	55.0a	70.0a	35.0a	24.0a	139.7a

Averages of three replicates followed by different letters in the same column are significantly

TRIG = Triglyceride, CHOL – Total cholesterol, HDL – High density lipoprotein, LDL – Low density lipoprotein, GLU - Glucose

P0 = control,

P2 = 17 % pomace 44% soyameal 39 % wheat bran,

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 = 10 % pomace 80 % soyameal 10 % wheat bran

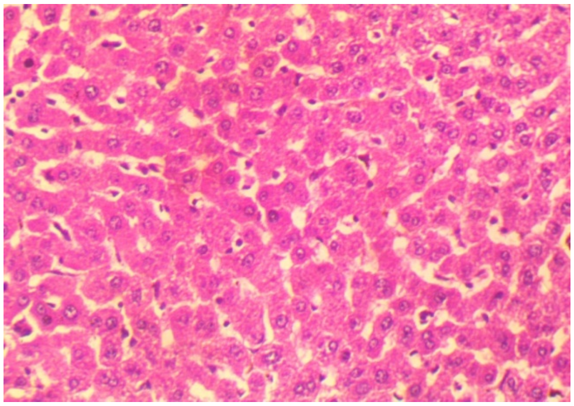
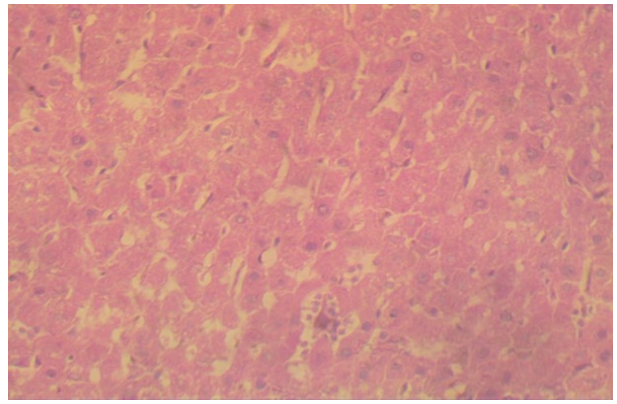
4.5.4 Histopathology

On sections of liver tissue in the treated groups there were no visible injuries that could be caused by feed toxicity. (Plate 4.1A to D). Sections of the kidney of the rat fed on the tested diets show no toxicity effect (Plate 4.2 A - D) on the control and the tested diets. The liver and kidney status of the rats fed on control and tested diets did not differ significantly, which shows no toxicity due to the feed.

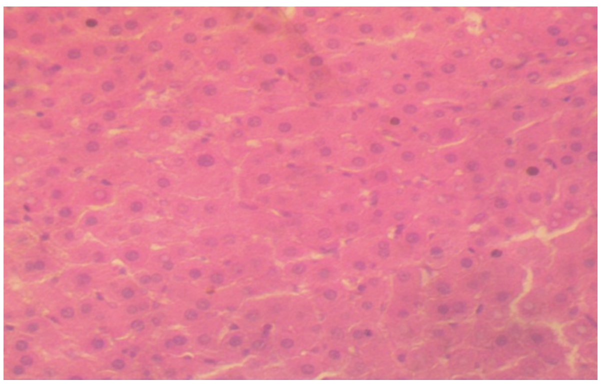
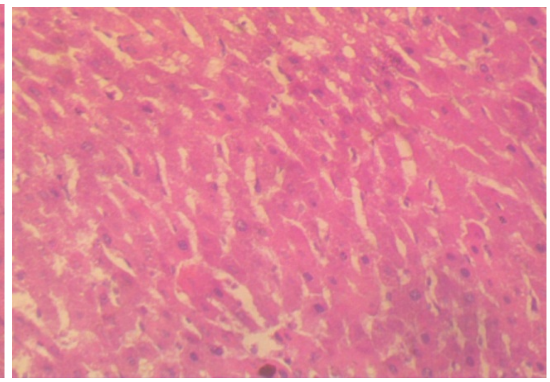
4.6 Storage Studies on the Extruded Blends

Moisture content of the extruded blends increased during a shelf life of 150 days (Table 4.13). P2 had highest moisture (10.59%) at the beginning of the study while P4 had the lowest moisture (8.50%), the same trend was observed at the end of the storage studies. Increase in moisture content will encourage microbial growth resulting in faster rate of deterioration of the extrudates. The increase in moisture content might be due to the absorption of moisture from the storage environment due to its hygroscopic nature. Similar results were obtained for the storage stability of extruded rice-based snack with apricot (Nazir *et al*., 2017).

The total fungal count of the extruded blend during 5 month storage showed increase in the total count from the month of production to the fourth (Table 4.13) month but decreased in the fifth month without significant variation. This suggested that there could be other chemical reaction taking place which inhibited fungi growth or depletion of the nutrients for fungal growth. The growth of fungi might lead to production of some toxins in the extrudates which might be toxic if consumed by man and a risk to human's health.

P0 = Control 400x B = Diet P2

C = Diet P3 400x D = Diet P4 400x

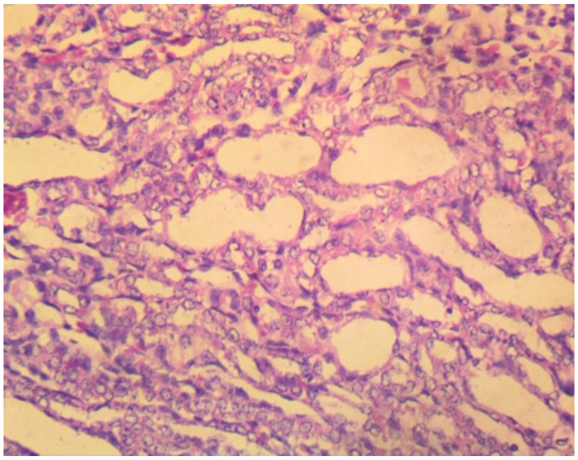
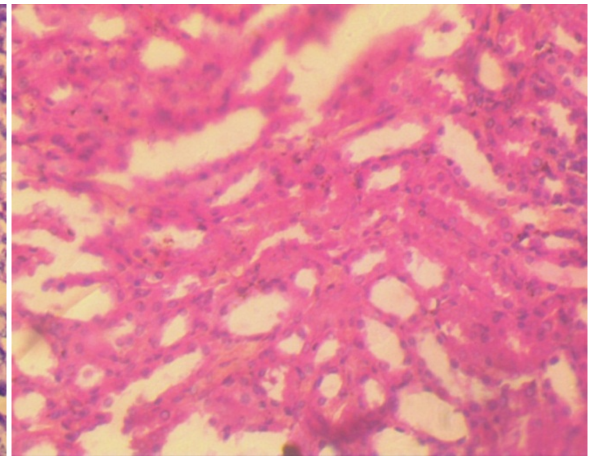
Plate 4.1A to D: Photomicrographs of liver sections of rats fed with the control and tested diets

P0 = control,

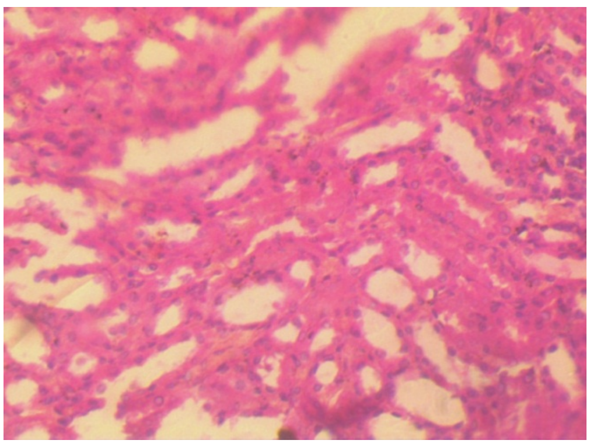
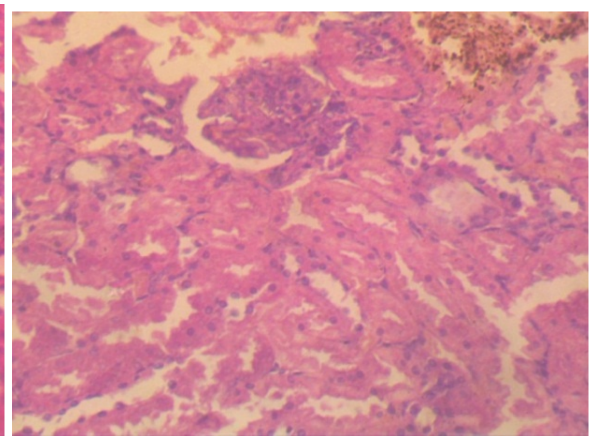
P2 = 17 % pomace 44% soyameal 39 % wheat bran,

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 = 10 % pomace 80 % soyameal 10 % wheat bran

A= Diet P0 (Control)   x 400   B = Diet P2 x400

C = Diet P3   x400   D = Diet P4   x400

Plate 4.2A to D: Photomicrograph of kidney sections of the rat fed with the control and tested diets. P1 = control, P2 = 17 % pomace 44% soyameal 39 % wheat bran, P3 = 5 % pomace 80% soyameal 15 % wheat bran, P4 = 10 % pomace 80 % soyameal 10 % wheat bran

Table 4.13: Storage effect on moisture and total fungi count of extruded blends

Period (Month)	Sample	Characteristic	
		Moisture (%)	TFC X 10^6 (CFU)
1	P2	10.59^a	13.00^b
	P3	10.59^a	13.50^b
	P4	10.59^b	41.60^a
2	P2	10.69^a	34.70^a
	P3	9.32^a	34.30^a
	P4	8.63^b	33.23^a
3	P2	10.99^a	7.280^a
	P3	9.64^a	7.51^a
	P4	8.65^b	6.52^b
4	P2	11.33^a	86.67^a
	P3	10.99^a	80.67^b
	P4	9.00^b	82.00^b
5	P2	11.71^a	23.67^b
	P3	11.37^a	6.00^c
	P4	9.39^b	72.00^a

Values are mean of replicates determination,

P2 = 17 % pomace 44% soyameal 39 % wheat bran

P3 = 5 % pomace 80% soyameal 15 % wheat bran,

P4 =10 % pomace 80 % soyameal 10 % wheat bran

4.7 Optimization and Validation

4.7.1 Optimization

The optimization of components was done to achieve the best blend. The general-purpose graphic optimization technique was used to determine the optimal process able component for the development of food based on orange pomace using the design expert software version 9.0.6 (Statease Inc., Minneapolis, USA). The contour plots for all answers were overlaid, and regions that best met all constraints were chosen as the optimal mix. The main criteria for constraints optimization were minimum pomace, maximum soyameal and wheat bran.

This objective was described in terms of desirability function which combines the overall effect of the components. Figure 4.26 represent the mixture contour plot of optimized desirability where it indicates the highest mixture blend of the graph. The solution generated for optimal blends for high fibre orange pomace based food specified limits were orange pomace 5%, soyameal 47.5% and wheat bran 47.5%.

4.7.2 Validation

The optimum component was positioned in the numerical optimization to validate the results predicted by the regression model. The optimum blends were subjected three times to the same experimental and analytical procedures applied at the beginning of this study. The experimental values for the solutions were close to the predicted values. The confirmation report for the altered experiment is given in table 4.12. The experiment errors for all attributes were non-significant and are less than $p<0.05$. Thus the optimum component predicted by the model was found suitable.

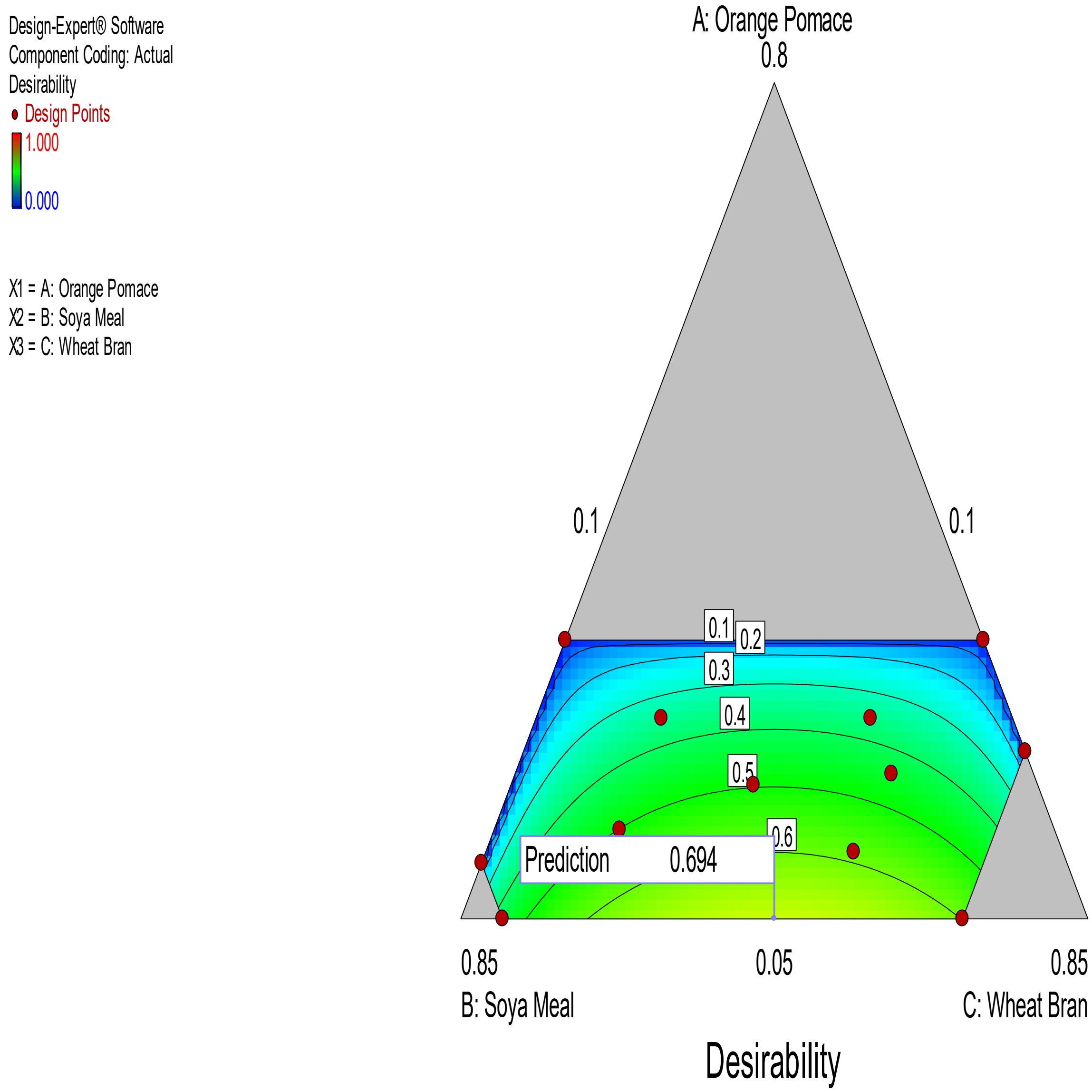


Figure 4.26: Mixture contour plot of desirability of pomace, soyameal and wheat bran blend

Table 4.14: Validity values of Experimental and predicted

Response	Experiment	Predicted	Error %
Moisture (%)	9.26	8.90	0.24
Ash (%)	4.61	5.87	0.04
Protein (%)	24.82	22.40	0.22
Fat (%)	3.5	4.79	0.36
Crude fibre (%)	12.87	14.34	0.39
Carbohydrate (%)	44.94	43.70	0.10
Tannin (%)	1.42	1.01	0.13
Phytate (%)	0.89	1.24	0.31
Oxalate (%)	1.32	0.99	0.03
TDF (%)	55.79	60.0	0.32
IDF (%)	39.13	40.4	0.20
SDF (%)	16.66	19.6	0.39
TPC (mg/g)	0.35	0.41	0.01
Flavonoid (mg/g)	2.60	3.10	0.37
Carotenoid (mg/g)	0.70	0.50	0.21
Frap (mg/g)	13.89	13.01	0.04
Bulk density (g/cm^3)	0.64	0.40	0.19
Water absorption index	3.58	4.76	0.15
Water solubility index	10.4	9.1	0.29

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study has shown that it is possible to develop rich-fibre foods from orange pomace, soyameal and wheat bran. Mixture design modeling has been effective in quantifying the effects of orange pomace, soybean and wheat bran on several extrudate quality attributes and can be used as a tool to optimize extrudate formulation.

The most preferred blend with the highest sensory score was 10% pomace, 80% soyameal and 10% wheat bran. The diets induced a reduction in weight gain and had no toxic effect on blood and organ of rat fed with them. The blends had three months of good storability.

Extrusion of these raw materials yielded products which could contribute significant portion of fibre to human diet. Transformation of this waste to wealth makes it possible for citrus processing companies to reduce their cost of treatment, generate additional profits from what was previously considered waste and thus improve their competitiveness as well as reduce its environmental pollution.

Utilization of orange pomace for the development of rich-fibre food will be an emerging area in the food industry.

5.2 Recommendation

For further studies, it is recommended that:

1) Studies on different packaging materials such as high density polyethelene, polystyrene and skillts should be carried out to determine the best package for longer storage to protect the colour of the products from oxidation and loss of appearance.
2) Natural colourants to improve the colour of the products should be investigated.

3) Accelerated storage studies should be carried out whereby different storage temperature and relative humidity considered for longer shelf life.

4) Accelerated storage studies should be carried out during which temperatures and relative humidity is properly evaluated to see their impact on the shelf life.

5) Epidemiological studies should also be conducted to further confirm the health benefits.

5.3 Contributions to Knowledge

1) The technical feasibility of orange pomace hitherto unknown in the development of antioxidant dense and fibre rich food has been established.

2) Optimal blend combination for high fibre food from orange pomace, soyameal and wheat bran was established.

3) This work has contributed to the growing body of research on the development of unconventional sources of dietary fibre) like food products) into innovative food products that could contribute significantly to fibre intake of consumers in Nigeria and beyond.

4) This is of public health benefit in the wake of increasing global and local risks prevalence and mortality from non-communicable diseases which are associated with inadequate dietary fibre intake.

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Appendix A : ANOVA

Response 3 **Protein**
ANOVA

Analysis of variance table

	Sum of	Mean	F	p-value

Source	Squares	df	Square	Value	Prob > F	
Model	163.85	5	32.77	3.54	0.0649	not significant
Linear Mixture	*151.31*	*2*	*75.66*	*8.17*	*0.0148*	
AB	*2.54*	*1*	*2.54*	*0.27*	*0.6170*	
AC	*3.26*	*1*	*3.26*	*0.35*	*0.5718*	
BC	*3.34*	*1*	*3.34*	*0.36*	*0.5668*	
Residual	64.83	7	9.26			
Cor Total	228.68	12				

Response Crude fibre

	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**Df**	**Square**	**Value**	**Prob > F**	
Model	73.28	5	14.66	1.45	0.3146	not significant
Linear Mixture	*29.41*	*2*	*14.71*	*1.46*	*0.2956*	
AB	*4.32*	*1*	*4.32*	*0.43*	*0.5337*	
AC	*12.32*	*1*	*12.32*	*1.22*	*0.3055*	
BC	*11.27*	*1*	*11.27*	*1.12*	*0.3255*	
Residual	70.60	7	10.09			
Cor Total	143.88	12				

Response 10 Oxalate

	Sum of	**Mean**	**F**	**p-**

Source	Squares	Df	Square	Value	value Prob > F	
Model	2.20	5	0.44	1.82	0.2285	not significant
Linear Mixture	*0.25*	*2*	*0.12*	*0.51*	*0.6232*	
AB	*0.97*	*1*	*0.97*	*3.99*	*0.0860*	
AC	*1.41*	*1*	*1.41*	*5.82*	*0.0467*	
BC	*0.026*	*1*	*0.026*	*0.11*	*0.7551*	
Residual	1.70	7	0.24			

Response 11 Tpc

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.042	5	8.389E-003	0.87	0.5472	not significant
Linear Mixture	*8.785E-003*	*2*	*4.392E-003*	*0.45*	*0.6528*	
AB	*0.020*	*1*	*0.020*	*2.12*	*0.1891*	
AC	*0.029*	*1*	*0.029*	*3.00*	*0.1269*	
BC	*2.035E-003*	*1*	*2.035E-003*	*0.21*	*0.6606*	
Residual	0.068	7	9.684E-003			
Cor Total	0.11	12				

Response 14 Bulk density

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.025	5	4.940E-003	2.20	0.1666	not significant

Linear Mixture	*0.019*	*2*	*9.378E-003*	*4.17*	*0.0641*
AB	*6.832E-004*	*1*	*6.832E-004*	*0.30*	*0.5985*
AC	*1.034E-003*	*1*	*1.034E-003*	*0.46*	*0.5192*
BC	*2.259E-003*	*1*	*2.259E-003*	*1.01*	*0.3494*
Residual	0.016	7	2.246E-003		

Response 15 WSl

	Sum of		Mean	F	p-value	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	27.29	5	5.46	0.49	0.7750	not significant
Linear Mixture	*14.09*	*2*	*7.05*	*0.63*	*0.5589*	
AB	*3.05*	*1*	*3.05*	*0.27*	*0.6169*	
AC	*6.54*	*1*	*6.54*	*0.59*	*0.4685*	
BC	*0.80*	*1*	*0.80*	*0.072*	*0.7964*	
Residual	77.92	7	11.13			
Cor Total	105.22	12				

Response 18 TDF

	Sum of		Mean	F	p-value	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	567.48	5	113.50	3.23	0.0790	not significant
Linear Mixture	*494.69*	*2*	*247.34*	*7.04*	*0.0211*	
AB	*21.64*	*1*	*21.64*	*0.62*	*0.4583*	

AC	*40.30*	*1*	*40.30*	*1.15*	*0.3197*
BC	*4.16*	*1*	*4.16*	*0.12*	*0.7409*
Residual	245.94	7	35.13		
Cor Total	813.42	12			

Appendix B

Desirability

Solutions

Number	**Orange Pomace**	**Soya Meal**	**Wheat Bran**	**Desirability**	
1	0.050	0.475	0.475	0.694	Selected

1 Solutions found

Number of Starting Points: 113

Orange Pomace	**Soya Meal**	**Wheat Bran**
0.18	0.27	0.55
0.05	0.8	0.15
0.11	0.35	0.54
0.23	0.27	0.5
0.05	0.25	0.7
0.23	0.52	0.25
0.2	0.1	0.7
0.11	0.62	0.27
0.3	0.6	0.1
0.17	0.44	0.39
0.3	0.1	0.6

0.1	0.8	0.1
0.13	0.62	0.25
0.198009	0.412752	0.389239
0.204573	0.154102	0.641324
0.0587245	0.643646	0.29763
0.0585714	0.461293	0.480135
0.3	0.298767	0.401233
0.3	0.176293	0.523707
0.282202	0.264381	0.453418
0.3	0.443318	0.256682
0.245681	0.596859	0.15746
0.234567	0.116365	0.649068
0.0810671	0.444436	0.474497
0.170293	0.45824	0.371467
0.19775	0.461033	0.341217
0.1198	0.600189	0.280011
0.252198	0.502738	0.245064
0.0657607	0.728784	0.205456
0.189577	0.309183	0.501241
0.131017	0.331908	0.537075
0.282059	0.243347	0.474595
0.3	0.341315	0.358685
0.139977	0.284523	0.5755
0.110127	0.257776	0.632097
0.211597	0.615613	0.17279
0.295815	0.150231	0.553954

0.266916	0.225059	0.508026
0.114709	0.404481	0.480809
0.23683	0.441366	0.321804
0.119616	0.653127	0.227256
0.103999	0.310867	0.585134
0.195856	0.477331	0.326813
0.229165	0.147713	0.623122
0.193098	0.206196	0.600706
0.207606	0.559491	0.232903
0.111662	0.446762	0.441576
0.264668	0.512923	0.222409
0.25221	0.413621	0.334168
0.121777	0.746656	0.131568
0.137531	0.618563	0.243906
0.242715	0.130806	0.62648
0.0960803	0.658262	0.245658
0.143075	0.277011	0.579913
0.275026	0.212165	0.512809
0.267561	0.383635	0.348804
0.0735788	0.438131	0.48829
0.132861	0.224427	0.642712
0.3	0.440854	0.259146
0.192299	0.146499	0.661202
0.3	0.354114	0.345886
0.3	0.407353	0.292647
0.20703	0.381181	0.411789

0.10375	0.19625	0.7
0.20201	0.65406	0.14393
0.190441	0.692657	0.116902
0.124063	0.661555	0.214382
0.3	0.357591	0.342409
0.224758	0.41603	0.359212
0.268538	0.458693	0.272768
0.141165	0.281024	0.577811
0.28113	0.530853	0.188017
0.195755	0.681112	0.123133
0.193862	0.656628	0.14951
0.3	0.380648	0.319352
0.0793325	0.650165	0.270502
0.286693	0.170696	0.542611
0.200531	0.115333	0.684136
0.243831	0.634457	0.121712
0.230992	0.616113	0.152895
0.0872205	0.652251	0.260529
0.294139	0.155622	0.550239
0.0673765	0.8	0.132624
0.0571375	0.784592	0.158271
0.219241	0.293192	0.487567
0.22897	0.482954	0.288075
0.0548917	0.343432	0.601676
0.3	0.334616	0.365384
0.135543	0.645534	0.218923

0.154933	0.676807	0.16826
0.152864	0.444496	0.40264
0.0715596	0.411741	0.5167
0.297106	0.115895	0.586998
0.099354	0.216199	0.684447
0.208117	0.647878	0.144004
0.134583	0.264666	0.600751
0.128043	0.590361	0.281596
0.22323	0.411849	0.364921
0.232791	0.101103	0.666107
0.3	0.478335	0.221665
0.195365	0.594105	0.21053
0.0712609	0.453988	0.474751
0.0844296	0.28041	0.635161
0.3	0.171129	0.528871
0.253253	0.139194	0.607553
0.133701	0.403921	0.462378
0.140619	0.568311	0.291071
0.209246	0.387557	0.403198
0.113958	0.329876	0.556166
0.1063	0.652216	0.241483
0.0993798	0.598085	0.302535
0.3	0.289596	0.410404
0.140275	0.631382	0.228343

Appendix C: Orange pomace

Appendix D: Rat Disection

Appendix E: Orange pomace based food


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