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DEVELOPMENT AND QUALITY EVALUATION OF MAIZE-LUPIN (Lupinus Albus L.) EXTRUDED SNACK

Adem, Muhammed

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SCHOOL OF RESEARCH AND POSTGRADUATE STUDIES

FACULTY OF CHEMICAL AND FOOD ENGINEERING

DEVELOPMENT AND QUALITY EVALUATION OF MAIZE-LUPIN

(Lupinus albus L.) EXTRUDED SNACK

Muhammed Adem

Bahir Dar, Ethiopia

April 13, 2018
DEVELOPMENT AND QUALITY EVALUATION OF MAIZE-LUPIN (*Lupinus Albus* L.) EXTRUDED SNACK

Muhammed Adem Abdullah

A Thesis Submitted to the School of Research and Graduate Studies of Bahir Dar Institute of Technology, BDU in Partial Fulfillment of the Requirements for the Degree of Master degree in the Food Technology in the Chemical and Food Engineering faculty

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BahirDar, Ethiopia

April 13, 2018
DECLARATION

I, the undersigned, declare that the thesis comprises my own work. In compliance with internationally accepted practices, I have acknowledged and referred all materials used in this work. I understand that non-adherence to the principles of academic honesty and integrity, misrepresentation/ fabrication of any idea/data/fact/source will constitute sufficient ground for disciplinary action by the University and can also evoke penal action from the sources which have not been properly cited or acknowledged.

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This thesis is dedicated to my father and mother
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ABSTRACT

Protein rich extruded products were prepared from locally available and low cost raw materials different blends of lupin and whole grain maize flour using a twin-screw extruder. The extrusion effects on the physical, functional and proximate properties of the extruded product were evaluated as related to the different process variables. The variables includes blending ratio (10-20%), barrel temperature (120-150°C) and feed moisture content (14-18%). A three-factor, three-level Box-Behnken design (BBD) and response surfaces plot that served to evaluate the significance of independent, interaction and quadratic effects of extrusion process variables. Second order polynomial regression equations were developed to relate the product responses to blending ratio and process variables as well as to obtain the response surfaces plots. The independent variables (Blending ratio, Barrel Temperature, and feed moisture content) had significant (p ≤ 0.05) effects on physical and functional properties of extruded products. Higher blending ratio increased the bulk density and water solubility index, but decreased the expansion ratio, and water absorption index. Higher feed moisture content decreased the ER and WSI values, whereas higher feed moisture content increased BD and WAI. Higher barrel temperatures increased expansion ratio, but decreased the bulk density. In this work, the proximate compositions of the extruded material considered were product moisture content, protein content, fat, crude fiber, ash, and carbohydrate content. All these properties were significantly (P ≤ 0.05) affected by the process variables. ANOVA analysis indicated that extrudate moisture content was influenced by blending ratio and feed moisture content, protein content by linear and quadratic terms of blending ratio, fat content by linear and quadratic terms of blending ratio, (barrel temperature*feed moisture), and quadratic terms of barrel temperature, fiber, ash and starch content by blending ratio at p≤0.05. Sensory analysis was conducted using a semi trained panelists. Optimized extrusion parameters for preparation of snacks were blending ratio of 15.0611 (%), barrel temperature of 150 (°C) and feed moisture of 14.00 (%) having desirability value of 72.8%. Future research is required on the scale-up of the optimized formulation and process identified in the present study.

keywords: Extrusion cooking, Whole grain flour, physico-chemical properties, proximate composition, optimization, snack
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<tbody>
<tr>
<td>ANFs</td>
<td>Anti-nutritional factors</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BBD</td>
<td>Box-Behnken Design</td>
</tr>
<tr>
<td>BD</td>
<td>Bulk density</td>
</tr>
<tr>
<td>BR</td>
<td>Blending Ratio</td>
</tr>
<tr>
<td>BT</td>
<td>Barrel Temperature</td>
</tr>
<tr>
<td>ER</td>
<td>Expansion Ratio</td>
</tr>
<tr>
<td>MC</td>
<td>Moisture content</td>
</tr>
<tr>
<td>PEM</td>
<td>Protein-Energy Malnutrition</td>
</tr>
<tr>
<td>RER</td>
<td>Radial expansion ratio</td>
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<tr>
<td>RSM</td>
<td>Response surface methodology</td>
</tr>
<tr>
<td>SME</td>
<td>Specific mechanical energy</td>
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<tr>
<td>WAI</td>
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1. INTRODUCTION

1.1. Background

Malnutrition and undernutrition exist globally, particularly in most of the developing world as a result of the complex and widespread problem of poverty and deprivation. The most recent estimates show that more than one billion people worldwide are undernourished (FAO, 2015). According to the report in the state of food insecurity in the world in 2014 by Food and Agriculture Organization an estimated 795 million people of the 7.3 billion people in the world, or one in nine were suffering from chronic undernourishment in 2012-2014. Of these hungry people, 791 million live in developing countries making it one in eight or 13.5% of the population in those countries (FAO, 2015).

There was a reduction in hungry people by over 167 million since past decade and the prevalence of undernourishment has fallen from 18.7% to 11.3% globally and from 23.4% to 13.5% for developing countries (FAO, 2015). Even after this reduction in hunger, the least progress has been in the sub-Saharan region of Africa where more than one in four people remain undernourished – the highest prevalence amongst any region of the world. In this region, poverty causes food shortages and most vulnerable populations survive predominantly on starchy staples such as maize, wheat, rice, sorghum, millet, and cassava, with little or no meat and dairy products (Slavin, 2014). The protein nutritional quality of these staple foods is poor and lysine is the most limiting amino acid (United States Department of Agriculture (USDA) 2008).

Strategies that have been used to address protein deficiencies include food diversification (FAO, 2015), fortification of food with indispensable amino acids, supplementation with good quality protein, improvement of protein quality by plant breeding and genetic engineering, and minimising the damage to the nutritional value of protein during food processing and storage (Wrigley et al., 2004). Cereals constitute the most suitable vehicle for delivering proteins to at-risk populations because of their widespread consumption, stability, and versatility (Bulusu et al., 2007). To augment the protein quality, the concept of cereal-legume complementation by blending...
cereal and legume flours can be applied (Asare, 2002; Mensa-Wilmot et al., 2001). The optimized dietary exploitation of protein-rich plant sources, such as legume seeds, is the target of several research and development programs. Legumes are consumed by people for thousand years and they are known as poor man’s meat (Gómez et al., 2008). Indeed, legume seeds can provide valuable proteins for both nutritional and technological purposes. Among the legume seeds, lupin is an interesting one for various reasons: a very high protein content (Deshpande & Poshadri, 2011), comparable to that of soybean, a low presence of antinutritional compounds (Petterson, 1998), the functional properties of its components in food matrices (Makri, Papalamprou, & Doxastakis, 2005), pale in colour and low in odour and flavour (Clark & Johnson, 2002) and the nutraceutical potentialities of some of its proteins (Duranti, 2006). They are also known as low glycemic index foods (Gómez et al., 2008). There are many studies regarding the positive health effects of lupin in the literature. (Belski, 2010) reported that the regular consumption of legumes may help to reduce the risk of cardiovascular diseases. (Mathers, 2002) reported that the legumes contain a rich variety of compounds which if consumed in adequate quantities, may help to decrease the risk of tumor cell formation.

Lupin is a good source of nutrients not only proteins but also lipids, dietary fiber, minerals, and vitamins (Martínez-Villaluenga et al., 2006) and (Zielinska et al., 2008). It contains about twice the amount of proteins found in other legumes that are commonly consumed by humans. Lupins like other legumes have some anti-nutritional factors, which inhibits its consumption. Mainly, the presence of alkaloids (quinolizidine alkaloids) hinders its consumption without processing to remove them. Raffinose (family oligosaccharides), phytates, and tannins are also the other anti-nutritional factors found in the raw seed (Jiménez-Martínez et al., 2003) and (Sujak et al., 2006). Nevertheless, the potential of lupine in human nutrition has generally been underestimated worldwide. So, there is a need for designing studies for developing lupin-based convenient food products (Joray, Rayas-Duarte, & Mohamed, 2007a).

Some studies have investigated the extrusion process of cereal-lupin flour blends. (Jayasena & Nasar-Abbas, 2012a) studied development and quality evaluation of high-protein and high-dietary-fiber pasta using lupin flour. The same author (Jayasena et al., 2008) also studied the development and quality evaluation of lupin-fortified instant noodles. (Oliveira
et al., 2015) studied development and characterization of extruded broken Rice and Lupin (Lupinus albus). (Oliveira et al., 2015) the study aimed to obtain extrudates using a percentage of ground lupine (GL) to replace broken rice (BR) as well as to evaluate the physical and chemical characteristics of the raw materials and extrudates.

With the interest in the utilization of Lupin has increased worldwide, there are many Lupin species with significant feed, food, soil fertility maintenance (Yeheyis, Kijora, Melaku, & Girma, 2010) and industrial potential which remain underutilized. According to (Bhat & Karim, 2009), vast numbers of those underutilized species represent an enormous untouched commodity resource which can help to meet the increasing demand of food and nutrition, energy, medicines and industrial needs. Among those underutilized crops in Ethiopia, according to (Nigussie, 2012), lupin (Lupinus albus L.) is the most important traditional crop mostly produced and consumed by smallholder farmers in the North-Western part of the country. Human consumption of lupins has increased in recent years (Sánchez et al., 2005).

High-temperature short time (HTST), extrusion processing of Lupin formulated in maize flour blends at high levels has not been well studied especially in terms of the relationships between process parameters and the physical, functional, proximate and sensory properties of the extruded product and no major optimization considerations have been given. Considering the popularity of the application of maize and legume in food extrusion, such a study is considered essential for product development and process control. Therefore, this study was investigated the effect of blending ratio and extrusion conditions (extrusion temperature and feed moisture content) on the physical, functional, proximate and sensory characteristics of such composite snacks. Thus, this study will provide new insights to utilize lupin in better way to exploit their nutrient richness.

### 1.2. Problem Statement

Most developing countries experience a high burden of protein-energy malnutrition and severe micro-nutrient deficiencies, which have attracted the numerous interventions by major stakeholders, to mitigate the challenges. Ethiopia is a producer of a variety of agricultural products; it is nevertheless, one of the countries in the world with the highest number of malnourished population. According to the (Central Statistical Agency (CSA)
[Ethiopia] and ICF, 2016), about 38%, 24% and 10% of the population were identified as stunting, underweight and wasting respectively and most them suffer from severe protein deficiency. The most important documented forms of malnutrition in Ethiopia are protein-energy malnutrition and vitamin A, iodine and zinc deficiencies (Central Statistical Agency (CSA) [Ethiopia] and ICF, 2016).

Therefore, identification of relatively inexpensive high protein materials is an important task in this country. Such materials would be able to improve and upgrade the nutritional quality of the diets and the health of the people. Lupin is an undervalued grain legume that can be grown under marginal agricultural conditions. Lupin is underutilized in human foods and mainly used as an animal feed. Considering its nutritional and functional properties, it has a high potential to be used in different foods.

A combination of lupin and maize flour can also help improve the amino acid profile of the product. Maize flour proteins which are poor in lysine and relatively higher in the sulfur-containing amino acids (methionine and cysteine) can be complemented by the amino acids found in lupin protein which are high in lysine and low in sulfur-rich amino acids (Jayasena et al., 2008; Joray, Rayas-Duarte, & Mohamed, 2007).

Cereal-based foods intended for human consumption undergo some form of processing such as milling and extrusion so as to improve the palatability (Suri & Tanumihardjo, 2016). Generally, to extend the keeping quality of the flour, they are refined to remove fat rich components such as bran and germ (Schaffer-Lequart et al., 2017; van der Kamp et al., 2014). Whole grains are staple foods with a long history of human consumption and again in the recent past, consumption of whole grain products has gained more attention owing to the inherent nutritional advantages compared to refined grains. Whole grain flour essentially retains all of its bran, germ, and endosperm (Ferruzzi et al., 2014). The major limitation in the utilization of whole grain flour is its rapid deterioration of quality owing to the enzymatic breakdown of lipids, consequently leading to reduced storage period (George N. Bookwalter, Lyle, & Nelsen, 1991). Among the cereals, maize comprises of largest germ portion with high fat content. Maize grains are subjected to dry or wet milling. The dry milling involves size reduction of whole grain to obtain flour with the entire germ and fiber or further sieved to obtain degemermd flour. Due to high fat content whole or partially sieved maize flour have limited shelf stability whereas the degemermd flour is often shelf stable up
to 3 months (Gwirtz & Garcia-Casal, 2014). Thermal processing has been shown to inactivate these enzymes effectively and provide scope for shelf-life extension. One of the ways to minimize these changes is through adopting high-temperature short time processing techniques (HTST).

Lupin has a high potential of a “nonintrusive” ingredient that can be substituted or used as an alternative in foods such as cereal products and is lower in cost compared with other similar legume flours such as soybean (Jayasena et al., 2008). Therefore, the substitution of Lupin flour would improve the nutritional quality of snacks at a comparatively lower cost. The aim of the present study was to investigate the maximum incorporation of Lupin flour to improve the nutritional quality of snacks without deteriorating their physical, functional, and organoleptic properties.

Therefore, supplementation of the cereals with low cost and underutilized protein source is in need to improve their nutritional quality. Thus, blending maize with legumes could be used as a way of enhancing its protein quality and combating PEM as well.

1.3. Objective of the study

The general objective of this study was to develop high protein ready to eat extruded products from Maize-lupin (*Lupinus albus* L.) blends.

Specific objectives: Specific Objectives were to:

a. Study the effect of extrusion parameters (barrel temperature and feed moisture content) on the physical, functional, chemical and sensory qualities of extrudates,

b. Examine the effects of blending ratios (maize to lupin blend ratio) on, physico-chemical, proximate, and sensory properties of extruded snack from Maize-Lupin blend

1.4. Scope of the study

This research begins by preparing flours from Maize and Lupin and blending the two flours in different ratios then studying the proximate composition of the flours and blends of Maize and Lupin.
Then after, the different blends were extrusion-cooked and are ready for further analysis to evaluate the effect of blend ratio and extrusion operating conditions on some physical, functional properties, proximate and sensory characteristics of the extrudate. Therefore, this research covers the analysis of the proximate composition of the raw materials, blended flour, and the extrudate. Studying the effect of blend ratio and extrusion operating conditions on nutritional, physical, functional and sensory were done based on the data collected from laboratory analysis and sensory analysis. In addition to this, optimum process parameters were selected for the product with the highest overall product quality.

1.5. Significance of the study

This research work has the capacity to address the twin problems of protein-energy malnutrition and micronutrient deficiencies, which pose a challenge to meeting nutrition, related goals set under the Growth and Transformation Plan (GTP) and that is also closely aligned to Millennium Development Goals (MDGs). It will stimulate the establishment of facilities for production of nutrient-dense extruded snacks, which could be useful for dieting and readily employed in nutrition intervention programs like school feeding programmes, community nutrition activities, nutrition support in emergency situations and promotion of food security for vulnerable groups/households.
2. LITERATURE REVIEW

2.1. General aspects of lupin

Grain legumes are important sources of significant amounts of proteins, carbohydrates, fiber, vitamins and some minerals. They are used in many parts of the world for both animal and human nutrition (Osman, 2007).

The name Lupin is derived from the Latin word Lupus, meaning ‘wolf’. The Romans believed that lupins robbed the soil nutrients in the same way that wolf would steal domestic animal (Gresshoff, 2003). It is known as lupines in the United States, as turmus in the Middle East, Tawari in Latin America and Gibto in Ethiopia. The plant is characterized by having various flowering spikes in the large range of colors (Getachew, Umeta, & Retta, 2012).

2.1.1 Origin of lupin

Lupin, a leguminous seed originated during the pre-Incas civilization more than 3500-4000 years ago and is being increasingly used as human food due to its nutritional and functional properties (Duranti et al., 2008). (Torres et al., 2007) reported that seeds of lupin varieties (L.angustifolius, L.albus) are considered as a rich source of protein content in the human nutritional diet which can be replaced for foods of high protein soy diet.

2.1.2 Traditional uses and current utilization of the lupin seeds

The Lupin seeds are used traditionally in many different ways. In Ethiopia, it is consumed as a roasted bean “kolo” (snacks) and lupin powder for preparation of stews/sauce (‘Shiro’) (Habtie, Admassu, & Asres, 2009) like other common legumes such as pea, bean, etc. Lupin seed is used to prepare local alcoholic drink “katikala” or ‘gibto areke’ in the north - western part of Ethiopia (Tizazu & Emire, 2010). The local community used gibto areke locally made antihypertensive medicinal preparation.

Nowadays generally plant proteins are increasingly used as food ingredients because they improve nutritional profile, stabilize the texture and optimize recipe costs. Lupin seeds are commonly used to prepare edible refined oil. The application of the lupin seeds is gradually increasing with its introduction as an ingredient in the extruded products.
It is an excellent ingredient for vegetarian food, owing to a combination of nutritional, technological and sensory characteristics (as highlighted by European projects such as Healthy Pro Food, Lupicarp, Healthy Lupin, Plants Pro Food, and Like Meat). There is growing evidence that lupin-based diets can contribute to prevent and treat a number of diseases, including type-2 diabetes mellitus (T2DM), hypertension, cardiovascular diseases, and metabolic syndrome. Finally, Lupin grain contains a moderate amount (10-15%) of oil featuring a particularly high ω3/ω6 ratio (Annicchiarico, Harzic, & Carroni, 2010).

Nutritional properties Lupin has a distinct chemical composition compared to that of cereals like wheat, rye, and corn. However, the lupin crops can also be used as suitable substitutes for cereals in providing the highly nutritious product. The nutritional quality of a product depends on the quality and quantity of the nutrients present (Repo-Carrasco-Valencia et al., 2010). The Lupins are reported to have a high content of protein, dietary fiber and specific bioactive compounds such as tocopherols and phenolics. The distribution of chemical constituents in the seed varies according to species and the cultivars (Repo-Carrasco-Valencia & Cruz, 2009).

2.1.3 Protein and amino acid composition

Proteins are complex organic biomolecules consisting of a chain of amino acid molecules which plays an important role as a principal constituent of the protoplasm of the cell structure thereby considered essential to life (Morris, 1992). The main biological functions of protein are replication of DNA, building blocks of cells, formation, and stabilization of foams and emulsions (Guerrieri & Yada, 2004).

Generally, plant proteins are increasingly used as food ingredients because they improve nutritional profile, stabilize the texture and optimize recipe costs. Analyses of nutritional values of *Lupinus albus* have shown that the bio-availability of the constituents is comparable to those of processed soybeans (Joray et al., 2007b).

Grain legumes are main sources of vegetable protein, among which *Lupinus albus* is known to have seeds with the highest protein content like soybean (Sujak et al., 2006a) and (Martínez-Villaluenga et al., 2006). Based on this fact *Lupinus albus* seeds have been employed as a protein source for animal and human nutrition in various parts of the world (Sánchez et al., 2005).
The requirements with regard to chemical composition, nutritional value, and product safety were laid down by the Advisory Committee on Novel Foods and Processes (ACNFP) in 1996 for certified lupins (sweet lupins). Based on the strength of this certification, these products were recommended as feedstuffs and food ingredients (e.g. lupin flours for baked goods) (Sipsas, 2008).

As a member of legume family, lupin bean protein is rich in lysine and deficient in sulfur-containing amino acids (Jiménez-Martínez et al., 2003) and (Sujak et al., 2006a). In contrast, its arginine content is markedly higher (Zraly et al., 2007). And also, the value of leucine is satisfactory for most of the species of Lupinus. Apart from the highest level of amino acids within the crude protein, it was found to have a better and nutritionally more beneficial amino acid composition and the highest essential amino acid level (EAA) (Sujak et al., 2006a).

It is also characterized by a higher essential amino acid index (EAAI) as well as chemical score(CS) of restrictive amino acids, and the highest protein efficiency ratio (PER), expressed in terms of the availability of leucine and tyrosine as compared to blue and yellow lupine varieties (Sujak et al., 2006a).

2.1.4 Total fat and fatty acid composition

The fat level in lupin is ranked third after groundnut (Arachis hypogaea L.) and soybean (Glycin max) among legumes (Joray et al., 2007a). The lipid contents of Lupinus albus are similar to other species of the genus Lupinus like Lupinus campestris (Jiménez-Martínez et al., 2003). The mean value of crude fat in Lupinus albus grown in different parts of the world is 13% (Jiménez-Martínez et al., 2003).

The oil extracted from Lupinus albus seed consist various types of fatty acids. The fatty acids of the oil from the raw seed are composed of more of unsaturated fatty acid and a small percentage of saturated fatty acids. This means Lupinus albus can be a potential source of a considerable amount of useful vegetable fat. Among the unsaturated fatty acids, majorly oleic and linolenic acids are found (Uzun, Arslan, Karhan, & Toker, 2007). The high content of unsaturated fatty acids and a desirable ratio of ω6 and ω3 fatty acids, make the crop a healthy alternative edible oil source (Joray et al., 2007a).
Different studies have been undertaken regarding the proximate composition of various cultivars of Lupinus albus. In Ethiopia moisture, protein, ash, fat, crude fiber, ADF, NDF oligosaccharides and lignin contents were reported to be 8.6, 35.8, 3.3, 9.4, 10.6, 14.6, 17.6, 6.6 and 0.6% respectively (Getachew et al., 2012). This report has shown that Lupinus albus has comparable protein content with other legumes like soybean. In Spain protein, ash, fat, sucrose, crude fiber contents and starch contents for the crop were found to be 30.6, 3.65, 14.64, 2.58, 39.42 and 3.27% respectively (Erbaş, Certel, & Uslu, 2005). In Turkey, similar studies have investigated that Lupinus albus has 8.32, 32.2, 2.65, 5.95 and 16.2% of moisture, protein, ash, fat and crude fiber respectively (Sujak et al., 2006).

2.1.5 Carbohydrates and dietary fiber content

Lupin is considered to be rich sources of dietary fiber which are generally above 10%, but are not as good sources of carbohydrates when compared to soya bean (Joray et al., 2007a). Starch, a carbohydrate is composed of amylose (α- (1→ 4) glycosidic linkage), amlopectin (α- (1→ 6) glycosidic linkage) and α-glucan which accounts to about 99% total weight. The digestible form of starch called the resistant starch that can be easily digested by the human small intestine and is highly beneficial for better glycemic control, lower the risk of cardiovascular diseases and maintaining the bowel health (Fuentes-Zaragoza & Sánchez-Zapata, 2011).

According to (Champ et al., 2003), “Dietary fiber is an edible part of the plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin and associated plant substances”. The soluble dietary fiber (e.g. pectin) are class of carbohydrates which are absorbed by the small intestine whereas the insoluble dietary are the carbohydrates (e.g. hemicellulose) which cannot be absorbed and less metabolized by the small intestine (Englyst et al., 2007). Like soybean, Lupinus albus has high dietary fiber content (mean value 30%). It has the lowest Glycemic Index of any commonly consumed grain (Zraly et al., 2007).

2.1.6 Bioactive components and micronutrients

Bioactive compounds are secondary metabolites that are present abundantly in plants and plant foods possessing biological activity. Some class of bioactive compounds (eg.
polyphenols) play an important role as antioxidant and anti-inflammatory effects in the human diet. Recent studies have suggested that bioactive compounds, especially polyphenols help in reducing the risk of neurodegenerative and diabetic diseases and regulation of apoptosis in tumor cells (Ferrazzano et al., 2011). Polyphenols, a class of bioactive compounds in the food, attributes to the bitterness, color, and flavor of the products (Han et al., 2007). (Moghadasian & Frohlich, 1999) reported that phytosterols are an important class of bioactive compounds that help in lowering the cholesterol absorption in the human intestine. In addition, phytosterols have also shown antiviral and anti-tumor properties. Lupin also possessed desirable composition of micronutrients (vitamins and minerals) (Repo-Carrasco-Valencia & Cruz, 2009). Only traces of tannins, trypsin inhibitors, and phytates were detected in the seeds of lupin (Jiménez-Martínez et al., 2003).

2.2. General aspects of Maize

Maize (Zea mays L.), also known as Corn to many people, is the leading most valuable cereal crop in the world followed by rice and wheat (FAOSTAT, 2016). Global production of corn exceeded 1 billion metric tons in 2013, and it is a staple food for over 1.2 billion people (Lozano-Alejo et al., 2007). Corn is a staple for large populations in Latin America, Africa, and Asia, where it is consumed as “corn on the cob” or corn kernels and used to prepare various kinds of traditional foods. A whole corn kernel is composed of 4 different parts: endosperm (82% to 84% of whole kernel mass, db), germ (10% to 12%, db), bran (5% to 6%, db), and tip cap (1%, db), as shown in Figure 2-1 (Ai & Jane, 2016) and (Watson et al., 2003).
Normal maize kernels consist of 6% to 12% (db) protein, with the majority located in the endosperm and germ (Figure 2-1). Although the corn endosperm has a lower concentration of protein (7% to 10%, db) than the germ (17% to 19%, db), the total amount of protein in the endosperm (70% to 79% protein of whole kernel) is higher than that of the germ (18% to 28%). Maize is an important source of protein in human diets, particularly for those populations who consume corn as a staple. However, like many other cereal grains, maize is deficient in lysine, which is the primary limiting amino acid. The protein digestibility of corn is affected by the processing method and also by the presence of antinutritional factors. (Ai & Jane, 2016) reported that cooking reduced phytic acid and that could help increase in vitro protein digestibility.

Kernels of normal maize contain 3% to 6% lipids (db), with the majority located in the germ (Figure 2-1, 81% to 85% of total lipids, db; (Watson et al., 2003)). Maize oil extracted from the kernel contains 79% triglycerides, 9% polar lipids (phospholipids and glycolipids), 5% sterols, 4% mono- and diglycerides, 3% hydrocarbons-sterol esters, 1% free fatty acids, and
trace amounts of waxes, tocopherols, and carotenoids. Triglycerides from maize oil have a high level (about 60%) of linoleic acid (C18:2), which is nutritionally valuable because linoleic acid is an essential polyunsaturated fatty acid for humans (Watson et al., 2003). In the past decades, research has been performed on the development of high-oil maize (containing 7% to 15% lipids, db) for improved nutritional values.

The whole maize consists of 68-74% starch. Cornstarch is usually digestible after cooking or thermal processing. One of the major factors affecting the starch digestibility of corn is the amylose content and amylopectin branch length. Increased amylose content and increased amylopectin branch length cause a reduction in digestibility rate. Other factors, such as surrounded by cellulose and hemicellulose fibers and/or protein matrices and interacting with lipids, can also reduce the susceptibility of starch to enzyme hydrolysis (Ai & Jane, 2016).

The presence of antinutritional factors in corn such as phytic acid, tannins, and trypsin inhibitors has been well documented (Ai & Jane, 2016) and different processing methods like fermentation, germination etc. have shown to reduce these anti-nutritional factors.

2.2.1 Food Uses and shelf life of Whole Maize Flour

Maize is consumed across the world in a variety of whole and processed products. Whole-grain maize is consumed boiled or roasted on the cob, canned or frozen sweetcorn, and as popcorn.

Different processing methods result in changes to the nutritional profile of maize products, which vary by nutrient and strain of maize. The most common processing method is milling, which grinds the maize into coarse whole-grain pieces or fine flour and removes much of the bran and germ, as in the case of refined maize flour. Milled maize can be processed by heat into foods such as porridge, polenta, grits, baked goods, and other locally named dishes. Other maize products include snack foods, such as deep-fried maize chips, and industrially extruded, puffed, or flaked products. Extrusion is a quick, high-temperature technology used for processing maize that results in chemical and nutritional changes in the resulting maize products (Suri & Tanumihardjo, 2016).
2.2.2 Whole-grain Maize Composition

Whole grains, as defined by the U.S. Food and Drug Administration (FDA), are “intact, ground, cracked, or flaked fruit of the grain whose principal components, the starchy endosperm, germ, and bran, are present in the same relative proportions as they exist in the intact grain” (O’Neil et al., 2011). The European HEALTHGRAIN Forum defined whole grains as consisting of the intact, ground, cracked, or flaked kernel after the removal of inedible parts such as the hull and husk (Gong et al., 2018). This definition still holds even if the grain components are initially separated and later recombined. Whole grains have received much attention recently because of the health benefits linked to their consumption. Health and nutrition policies (Food and Agriculture Organization of the United Nations, Food and Drug Administration and Whole Grains Council in the United States of America) have encouraged the increase of dietary fiber in foods, mainly grain based products such as expanded ready-to-eat (RTE) snacks. In this context, the use of whole grains, such as whole grain maize flour in extruded products can be an effective alternative for obtaining healthier extruded snacks (Carvalho-Wells et al., 2010). Many large cohort studies have examined the relationship between whole grain intake and health outcomes and found whole grains are associated with a lower body mass index and less weight gain (Bellisle et al., 2014; Rose, 2014). Whole-grains maize are also associated with lower blood lipid values and decreased risk of cardiovascular disease (Gong et al., 2018).

2.2.3 Whole-grains maize flour shelf-life

Cereal-based foods intended for human consumption undergo some form of processing such as milling so as to improve the palatability (Suri & Tanumihardjo, 2016). Generally, to extend the keeping quality of the flour, they are refined to remove fat rich components such as bran and germ. In the recent past, consumption of whole grain products has gained more attention owing to the inherent nutritional advantages compared to refined grains. Whole grain flour essentially retains all of its bran, germ and endosperm (Van der Kamp et al., 2014). Clean, undamaged grains essentially store longer time, but the tissue disruption during milling process can initiate degradation reactions. The major limitation in the utilization of whole grain flour is its rapid deterioration of quality owing to the enzymatic
breakdown of lipids, consequently leading to reduced storage period (Chassagne-Berces et al., 2011).

The major ways of lipid degradation are by hydrolytic or oxidative reactions or sometimes combination of both. Hydrolysis of lipids generates free fatty acids (FFAs) which in turn serve as substrates for further oxidation. Lipases are the enzymes responsible for hydrolytic degradation of lipids. Lipoxygenase and peroxidase are known to initiate the oxidative breakdown of lipids in cereals (Galliard, 1983).

Among the cereals, maize comprises of largest germ portion with high fat content. Maize grains are subjected to dry or wet milling. The dry milling involves size reduction of whole grain to obtain flour with the entire germ and fiber or further sieved to obtain degermed flour. Due to high fat content whole or partially sieved flour have limited shelf stability whereas the degermed flour is often shelf stable up to 3 months (Gwirtz & Garcia-Casal, 2014).

Enzymes resulting in deterioration of food quality need to be controlled to extend product shelf-life. Various techniques have been developed for controlling the undesirable activities of enzymes in foods. These include thermal processing, chemical treatments, and control of water activity involving dehydration and salting. These techniques also have certain limitations, such as changing the quality attributes of certain foods, and health risks associated with certain chemical treatments (Ashie et al., 1996).

Thermal processing has been shown to inactivate these enzymes effectively and provide scope for shelf-life extension. Inactivation of lipase activity through wet and dry heat processing in oats, millet flour, rice bran, and sorghum have been reported (Bookwalter et al., 1974). Most of the thermal processing employed involve longer processing time that in turn affects product quality. One of the ways to minimize these changes is through adopting high-temperature short time processing techniques (HTST).

2.3. Protein Energy Deficiency in Developing countries

Protein Energy Malnutrition (PEM) refers to a group of diseases that result from under nutrition and is a major public health problem in developing countries. It is a macronutrient deficiency disease resulting from an inadequate intake and/or utilization of protein and energy, and affects children most because of their higher needs for protein and energy per
kilogram body weight compared to adults (McGuire & Beerman, 2012). Globally, about 793 million people were undernourished, while Southern Asia and sub-Saharan Africa accounted for 63% of undernourished people worldwide in 2014-2016. It is estimated that approximately 27% of children younger than five years in developing countries are underweight and in sub-Saharan Africa 38% have stunted growth while 28% are underweight (UNSCN, 2011; Yalew, Amsalu, & Bikes, 2014). Ethiopia being one of these countries malnutrition is an important public health problem; stunting, underweight and wasting were identified as 44%, 29% and 10% (Central Statistical Agency (CSA) [Ethiopia] and ICF, 2016). PEM is associated with the deaths of approximately 5 million children each year (WHO, U. & Mathers, 2017). The main cause of PEM in developing countries is dependence on a single starchy staple for virtually all the protein and energy requirements (UNSCN, 2011; Yalew et al., 2014).

2.3.1 Functions of proteins in human nutrition

Proteins contribute to cell growth, repair and maintenance, act as enzymes and hormones, maintain fluid, electrolyte and acid base balance and also maintain a strong immune system (McGuire & Beerman, 2012; Slavin, 2014). When fats and carbohydrates are not provided in adequate amounts in the diet, proteins also serve as an energy source, limiting their availability for the functions stated earlier (McGuire & Beerman, 2012). Additionally, proteins act as carriers for other nutrients that include lipids, Vitamin A, iron, sodium and potassium. Consequently, protein deficiency in children is also accompanied by other nutrient deficiencies including micronutrient deficiency (Müller & Krawinkel, 2005). Acute malnutrition causes wasting, low weight-for-height, while chronic malnutrition causes stunting, low height-for-age. Underweight, low weight-for-age reflects both stunting and wasting (McGuire & Beerman, 2012). Protein helps maintain a strong immune system by supporting the increased production of antibodies in response to common infections such as colds, flu or allergic reactions (Thompson et al 2008). Especially, children who have PEM have greatly increased susceptibility to life-threatening infectious diseases such as HIV/AIDS, tuberculosis and malaria (Pulfrey, 2006). There is also evidence that chronic PEM in 5 to 10-year olds impairs cognitive development (Hoffer, 2001).
2.3.2 Importance of lysine in the diet

L-lysine was discovered as an indispensable amino acid by Osborne and Mendel using a rat model as a measure of nutritional adequacy in 1914. These workers also showed that rats required lysine for growth by using wheat gliadin as the protein source in place of casein. The biological functions of lysine include; synthesis of connective tissues such as bone, skin, collagen, and elastin; synthesis of carnitine and resultant conversion of fatty acids to energy; support for healthy growth and development and maintenance of healthy immune function, particularly with regard to antiviral activity. The structure of L-lysine characterized by the presence of an amino group at the end of a 4-carbon aliphatic side chain [-(CH$_2$)$_4$-NH$_3$] makes it a relatively reactive component in different chemical reactions including carbonyl-amine interactions (Walsh, 2002).

2.4. Food Extrusion

Extrusion is defined as "shaping by force through a specially designed opening often after previous heating of the material" (Sundarrajan, 2014). Extrusion is the continuous formation of semi-solid materials through a die. Several types of extruders are available in the market including ram or piston types and screw or worm types. Extrusion cooking combines the heating of food products with the act of extrusion to create a cooked and shaped food product. It is a process in which moistened, starchy, proteinaceous foods are cooked and worked into viscous, plastic-like dough. The results of cooking the food ingredients during extrusion are gelatinization of starch, denaturation of protein, inactivation of raw food enzymes, destruction of naturally-occurring toxic substances, diminishing of microbial counts in the final product, etc. Upon discharge through the die, the hot, plastic extruded product expands rapidly with loss of moisture and heat because of the sudden decrease in pressure. After expansion cooling, and drying, the extruded product develops a rigid structure and maintains a porous texture. Advantages of food extrusion are versatility, high productivity, low cost, ability to shape the product, high product quality, energy efficiency, production of new foods, and no effluents or waste. Extrusion cooking has been used in a large number of food applications as it has some unique positive features compared with other heat processes.
Nowadays, the food extruder is considered as a high-temperature and short-time (HTST) bioreactor that transforms raw ingredients into a variety of modified intermediate and finished products. During the extrusion process, the material is treated not only by heating, but also by intense mechanical shearing, compression, and torque, which are able to break the covalent bonds in biopolymers (Singh et al., 2007). Thus, the functional properties of the food ingredients are rapidly modified due to the combined influence of temperature, pressure, shear and time (Carvalho & Mitchell, 2001). Food extrusion also permits the inactivate the undesirable enzymes that can affect the quality and eliminate several anti-nutritional factors, such as trypsin inhibitors, haemagglutinins, tannins and phytates (Singh et al., 2007).

Maize and wheat are widely consumed throughout the world as they are a good source of starch and several micronutrients, such as vitamin B1, vitamin B5, folate (vitamin B9), dietary fiber, vitamin C, phosphorus, and manganese. Widely used in making breakfast cereals and snacks through extrusion processes, corn provides products that present an attractive crunchy texture, golden color, and desirable flavor, but are limited in protein content. Lupin grain has a unique combination of high protein and dietary fiber content with very little available carbohydrate. Moreover, lipid in lupin grain is mainly composed of “healthy” fatty acids, e.g. linoleic, linolenic k2 and oleic acids. The grain also contains vitamins and antioxidants including tocopherols; carotenoids; B-vitamins and phenolic compounds. In addition, Lupin is low in anti-nutritional factors such as trypsin inhibitors and saponins compared to many other legumes (Jiménez-Martínez et al., 2003). Studies have demonstrated that lupin flour can be used to formulate acceptable baked products, as well as other foods such as pasta (Martínez-Villaluenga et al., 2006), meat products (Drakos et al., 2007) and dairy products (Jiménez-Martínez et al., 2003).

2.4.1 Twin-screw extruders

The term ‘twin-screw’ applies to extruders with two screws of equal length placed inside the same barrel (Harper, 1994). Twin-screw extruders are more complicated than single-screw extruders, but at the same time provide much more flexibility and better control. Twin-screw extruders are generally categorized according to the direction of screw rotation and to the degree to which the screws intermesh (Figure 2-2).
1) Counter-rotating twin-screw
2) Co-rotating twin-screw
3) Non-intermeshing counter-rotating

The twin-screw extruder was originally developed for processing plastics. In the food industry, twin-screw extruders were widely used from the mid-1980s to the mid-1990s. Food companies began using twin-screw extruders for producing products like sticky caramels and candies that could not be made with single-screw machines. Very soon, twin-screw extruders became popular with the food manufacturers for specialized food items.

**Figure 2-2 Schematic diagram of a typical twin-screw extruder and its three common types**

Adopted from (Guy, 2016; Riaz, 2016)

(a) Intermeshing co-rotating;
(b) Intermeshing counter-rotating;
(c) Non-intermeshing counter-rotating.

### 2.4.2 Snack Foods Production Technology

Snack foods are foods which can be taken in place of or between meals. They are convenient because they are quick and easy to eat. The snack foods industry is a vibrant sector and future for the industry looks promising and bright. Snack foods have become a significant part of the diet of many individuals and can influence overall nutrition (Sharma et al., 2016). The most widely consumed extruded snacks are made primarily with cereals/grains due to
their good expansion characteristics; however, they tend to be low in protein and many other nutrients. Development in the snack food industry is numerous and ever changing (Kumar et al., 2010; Sharma et al., 2016).

However, the main sector, which is defined clearly as snack foods, contains the major snack products such as popcorn, potato chips or crisps and baked or fried snacks and starch-based snacks. There are many ingenious variations in the processes used by the industry, which serve to increase the range of products manufactured. A number of the most important processes are carried out using extrusion cookers as part of the production line.

In extruding snack foods, grain and other ingredients are mixed and cooked under pressure, shear at high temperature in a tube, which is also called barrel. The resulting mass is forced through a die, cut into individual pieces and assumes the various shapes that consumers have come to expect in the snack food aisles of markets (Harper, 1994). Novel ingredients, cutting-edge extrusion technology, and innovative processing methods are combined to yield new snack products with ever widening appeal to health-conscious consumers that are seeking different textures and mouth feeling with convenience (Chanvrier et al., 2013). In addition, several extrusion processing conditions are accounted for the quality of finished products. The control of feed rate, screw speed, barrel temperature and barrel pressure, together with the above-mentioned critical parameters, will determine the crispness, hardness, and various other characteristics that will influence the success of the product (Harper, 1994).

The success or failure of a new extruded snack food product is directly related to sensory attributes, where texture plays a major role. In such foods, where expansion is desired and puffed products are expected, texture is of major importance, with crispness being one of the most important attribute (Valles Pamies et al., 2000). Several researchers agree that crispness should result from the structural properties of a food (Altan, McCarthy, & Maskan, 2008; Chakraborty et al., 2011; Valles Pamies et al., 2000) crispness is perceived through a combination of tactile, kinesthetic, visual, and auditory sensations and represents the key texture attributes of dry snack products.

(Guzmán-Ortiz et al., 2015) developed and characterized a snack food utilizing soybean and maize flour as its main ingredient in single screw and showed low density and high expansion degree. Extruded flours from Maize, sorghum, Bengal gram, rice and soya
chunks were prepared and had higher values of expansion degree, water absorption index, dispersible and lower Hunter L value, particle size index, and water solubility index than conventional flours (Sharma et al., 2016). Sweet whey solids (SWS) or whey protein concentrate (WPC) were added at concentrations of 250 and 500 g/kg to corn meal, rice, or potato flour to make snack products (Onwulata et al., 2001). High-temperature short time (HTST) air puffing was used for production of potato–soy ready-to-eat snack food as it ideally produced highly porous and light texture viz. puffing temperature (185–255 °C) and puffing time (20–60 s) for potato–soy blend with varying soy flour content from 5 to 25% (Nath & Chattopadhyay, 2008). In another study by (Rodríguez-Miranda et al., 2011) extruded snacks were prepared from flour blends made with taro and nixtamalized (TFeNMF) or non-nixtamalized maize (TFeMF) using a single-screw extruder with taro flour proportion in formulations (0–100 g/100 g) and extrusion temperatures (140–180 °C) and flour mixtures made from taro and nixtamalized maize flour produced puffed extruded snacks with good consumer acceptance.

2.4.3 Operation parameters and effects on the physical, functional and nutritional properties of the extruded products

Extrusion cooking technology serves the food industry as a method of high temperature-short and used for the development of new products such as cereal-based snacks including dietary fiber, baby foods, breakfast cereals and modified starch from cereals. The place of extrusion foods in the snack food market has grown rapidly in the past 30 years mainly because it can economically produce a variety of products with an attractive texture, size, and shape.

Maize flour is a major ingredient in extruded foods, such as ready-to-eat breakfast cereals and snacks. Numerous studies have investigated maize flour and the extrusion process. In recent years, products combining maize and other legume grains are getting more attention in the food industry. (Duranti, 2006) mentioned that of the approximately 13,000 known species of legume grains, only a small number are used directly as a food item. The increased interest vegetable proteins for health reasons may lead to a greater consumption of legumes, particularly in fortified foods and traditional dishes produced through extrusion technology.
The many independent and response variables involved in the extrusion process make it a complex process to investigate. The independent variables including feed rate, raw material combination, barrel temperature, raw material moisture content, screw speed, screw profile, die shape, die size, barrel length and barrel diameter. Of these variables, the independent variables can be controlled before the process; in other words, they are either a feature of the machine or something the machine allows it to control. Feed composition is a dominant parameter that influences the intermediate process variables. Extrusion conditions, characteristics of the starch granule, and presence of other components such as protein, fibers, and sugars directly affect the degree of transformation (Chanvrier et al., 2007). (Arivalagan et al., 2018) reported that a soft texture product resulted from a coconut (Cocos nucifera L.) haustorium based extrudates. Studies showed that incorporation of legume proteins to starch-rich ingredients such as maize flour significantly decreased mechanical energy input, and resulted in products with reduced expansion and increased hardness (Zare & Pletch, 2010).

In a study conducted by (Gujska & Khan, 1990), navy bean high protein fraction (43.75% protein) (HPF) and high starch fraction (HSF), pinto bean HPF (44.66% protein) and HSF blends and maize processed using air classification were extrusion-cooked and the functional properties of the extrudates were studied. Addition of HPF to the HSF decreased the expansion of the extruded blends and the extruded products were also softer and more fragile. Lightness and yellowness increased with increasing protein content. Water absorption index and oil emulsification capacities were better for the corn high protein fractions blended up to the 20% level. In general, extruded blends had lower expansion index and oil absorption capacity and higher protein density and oil emulsification capacities than extruded maize or HSFs. Expansion index, however, increased with increasing protein content.

The extrusion barrel temperature is one of the other important parameters which determine the quality of extruded product. (Sacchetti et al., 2004) studied the effects of extrusion temperature and feed composition on the functional, physical, and sensory properties of chestnut and rice flour-based snack-like products. Chestnut flour was found to be suitable for the extrusion-cooking process adopted if properly mixed with rice flour. Chestnut flour (30%) along with rice flour was processed at 120°C producing a snack-like product with
limited density and browning. Since the material is exposed to high temperature and pressure due to shearing force for short period, its nutrients are preserved and the desirables changes like starch gelatinization, protein denaturation, food enzymes inactivation and reduction of microbial counts occurs (Arivalagan et al., 2018).

The structural property changes of maize starch material during extrusion as a function of feed moisture content was studies by (Saini, 2015). It was found that higher feed moisture decreased the radial expansion ratio of extrudates, resulting in a higher apparent density and lower porosity values.

The response variables will be affected by the independent variables and directly influence the raw material as it is processed into the final product (Repo-Carrasco-Valencia & Cruz, 2009). All these variables are important to the process and need to be examined.

Physical properties are important factors in extruded products and will directly affect the customer’s acceptability of the final products. In order to develop more information regarding the relationship between processing variables and the physical properties of the extruded products, numerous studies have been done on the different raw materials and extrusion variables. (Tumuluru et al., 2013) studied the secondary extrusion variables and physical properties of fish- and rice-based snacks. (Yu et al., 2012) studied the effect of extrusion conditions on the functional and physical properties of corn flour and soy protein isolate (SPI) blends snacks. (Onwulata et al., 2001) studied the effect of dairy proteins in extrusion processing and other applications.

The literature includes many reports of research on extrusion cooking of cereals (maize, rice, and wheat) and the addition of legumes to these cereal-based extruded products (Ali et al., 2016; Asare et al., 2012; Kayacier et al., 2014). However, only a limited number of extruded products have been prepared with lupin, (Bhat & Karim, 2009; Jayasena & Nasar-Abbas, 2012a; Zhang et al., 2012) especially expanded snack products, possibly because of its low expansion, bitterness, and anti-nutritional factors.

Several physical, functional and nutritional parameters have been selected to describe the properties of extruded products, and the relationship between these physical, functional and nutritional parameters and certain important extrusion process variables has been studied. Hence, this study was conducted to develop maize based extrudates with the incorporation of varying proportion of Lupin and to evaluate physical (expansion ratio and bulk density),
functional parameters, (water absorption index and water solubility index) and nutritional (including moisture, protein, fat, fiber, ash and total carbohydrate content) characteristics of extrudates.

A response surface methodology (RSM) is generally used to study the relations between extrusion variables and these physical, functional, nutritional and sensory properties. Response surface methodology (RSM) is a statistical and mathematical method that uses quantitative data in an experimental design to determine and simultaneously solve multivariate equations to optimize processes and products (Myers, Montgomery, & Anderson-Cook, 2016) and (Khuri & Mukhopadhyay, 2010).

2.5. Concluding Remark

PEM malnutrition is the major nutritional deficiency disease among developing world. This review presented a comprehensive and critical analysis of published scientific work on the nutritional, health and technological functionality of lupin flour addition to snack and other extruded products. Evidence has been presented that supplementing extruded products with lupin flour improves their nutritional profile mainly through increased protein and dietary fibre. Maize is the leading most valuable cereal crop in the world and is suitable vehicles for proteins.

Investigations on lupin flour incorporation into extruded products have demonstrated that a 10% rate substitution of maize flour resulted in equal or better quality compared to maize - only extruded products. Technological drawbacks such as lowered expansion and denser pore structure in the final product are common when lupin substitution was beyond 10%. Lupin incorporation has also some other positive features in nutritional and very satisfactory sensory characteristics and is a necessary step toward the fight against PEM. Lastly, numerical optimisation of the formulation and processing parameters of lupin-maize, to maximise lupin incorporation rate and nutritional benefits whilst maintaining snack quality is lacking.
3. METHODOLOGY

3.1. Raw Materials

Maize (*Zea mays* L.) BH-540 variety was procured from the Adet Agricultural Research Center North Western, Ethiopia. Lupin (*L. albus* L.) seeds were procured from Bahir Dar local market. All samples were cleaned manually to remove foreign matters, immature, and damaged seeds.

3.2. Sample and Snack preparation

3.2.1 Flour preparation from maize and lupin

Maize (*Zea mays* L.) BH-540 variety was carefully cleaned to remove foreign materials and ground into fine flour using the stone commercial mill to pass through a 0.5mm mesh sieve and packed in polyethylene bags.

Lupin flour was obtained after the debittering process consisted of cleaning, boiling, and debittering stages. Extraneous material, and immature and damaged seeds were removed first. Five kg of chosen samples of lupin seeds were washed and presoaked in water for 12 hours, then the soaked seeds were boiled for one hour (1:3, seeds: water) to destroy thermo-labile anti-nutritional factors, such as trypsin inhibitors and to soften the seeds. The boiled seeds were debittered with tap water for five days at room temperature (~25 °C); the debittering water was changed every 6 hours (Erbas, 2010; Ertaş & Bilgiçli, 2012).

Afterwards, the whole seed was de-hulled manually and the kernel were oven dried at 65 °C for 24 hours. The dried samples were milled using laboratory sample mill with a sieve size of 0.5mm meshes and packed in polyethylene bag until analysis and extrusion were conducted (Erbas, 2010; Getachew et al., 2012).

3.2.2 Blend Formulation

Maize flour (MF) and lupin flour (LF) were mixed at various weight ratios of 10:90, 15:85, 20:80 and 100:0%, respectively. The three-different flour mixture were prepared in a ribbon
blender (Model AB, Alvan blanch Type, England) for 20 minutes. They were packed in polyethylene bags and stored at room temperature until ready for extrusion.

3.2.3 Extrusion apparatus and adjustment of process parameters

The extrusion process is conducted by setting the process parameters in accordance with the experimental design using a twin-screw extruder (model Clextral, BC-21 N0 194, Firminy, France). Prepared samples were subjected to extrusion test at all combinations of the operating conditions (blend ratios, barrel temperature, and feed moisture).

![Figure 3-1 Structure of the research experiment](image)

Necessary calibration and adjustment of the flour feed rate and water flow rate were performed prior to the extrusion process. Extrusion was then performed at 60 g/min (3.6 Kg/hr) flour feed rate and 200 revolutions per min (rpm) screw speed (Filli et al., 2012).
3.2.4 Barrel temperature adjustment

Barrel temperature of zone three of the extruder barrel was varied at 120, 135 and 150 °C. These ranges of temperatures were selected based on the results found by the preliminary extrusion trials. Temperature measurement is done with a thermocouple for the zone, set deep into the barrel walls. These signals are sent to controllers which regulate the corresponding heaters (Ali et al., 2016; Filli et al., 2014).

3.2.5 Feed moisture adjustment

The moisture content of the dough inside the barrel was adjusted by varying the water injection rate of the pump. Water injected into the extruder at a point close to the material feed port. Feed moisture was adjusted by injecting water at a level of 14, 16 and 18% (selected based on the results of the preliminary extrusion trials) in the mixes for a constant material feed rate of 60 g/min by using hydration equation (3.1) (Filli et al., 2011).

\[ Wa = Sw \left( \frac{m - m_o}{100 - m} \right) \]  

(3.1)

Where:

- \( Wa \) - Weight of water added (g)
- \( Sw \) - Sample flour weight (g)
- \( m_o \) - Original flour moisture content (% weight base)
- \( m \) - Required dough moisture level (% weight base)

Extruded samples were collected when the extrusion process parameters reach steady states. Steady state will reach when there was no visible drift in torque and die pressure. The extruded products were then labeled and placed on a table and allowed to cool for 30 minutes at room temperature for the measurement of weight, length, and diameter. Following these samples were sealed in plastic bags, labeled and stored at room temperature until physical, functional and nutritional properties were analyzed and sensory evaluation was conducted.
3.3. Experimental design and statistical analysis

Experiments were designed using the software package of RSM (Design-Expert, version 7.0.0, Stat-Ease, Inc., Minneapolis, MN). Response surface Methodology was used to investigate the effects of extrusion condition on the responses. Response surface methodology (RSM) is a statistical method used to describe the relationship between process variables and product quality characteristics. In statistics, the response surface methodology (RSM) explores the relationships between multiple explanatory variables and one or more response variables. The method was introduced by G. E. P. Box and K. B. Wilson in 1951 (Myers et al., 2016). The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response. RSM model gives an approximation result, but it is widely used because such a model is easy to estimate and apply, even when little is known about the process.

Box-Behnken for a three-factor, three-level combinations were used for this study. Box-Behnken was selected because of its economical design i.e., uses a lower number of total runs and hence economizes resource and time. Moreover, it is used to estimate parameters of the full second-degree model in all scientific research areas with more accuracy (Myers et al., 2016). The independent variables considered were a percentage of corn and lupin blend ratio (10-20 g/100g blend), barrel temperature (120-150°C) and feed moisture content (14-18%). The upper and lower levels of variables were fixed based on different cereal-legume composite extrusion studies and preliminary tests. Operating variables and blending ratio ranges and the three standardized levels were modified during preliminary studies of each variable. According to the Box-Behnken design, the experimental plan comprised of 15 experimental runs with one blocking and three center points. The independent variables are coded in terms of coded values, -1, 0, and 1 as given in Table 3-1(below).
Table 3-1 Process variable and their levels to be used in the Box-Behnken Design

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Codes</th>
<th>Variables actual and coded levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blending ratio (%)</td>
<td>B</td>
<td>-1  10  15  20</td>
</tr>
<tr>
<td>Barrel temperature (°C)</td>
<td>T</td>
<td>-1  120 135 150</td>
</tr>
<tr>
<td>Feed moisture (%)</td>
<td>M</td>
<td>-1  14  16  18</td>
</tr>
</tbody>
</table>

Different blends were prepared by mixing the weighed amount of Maize flour with lupin flour for 15 minutes using laboratory scale mixer (Ribbon Universal mixing blender) (Model AB, Alvan blanch Type, England). The three different blend ratios on their weight basis were **100:0 (control), 90:10, 85:15 and 80:20** Maize and Lupin flour respectively. After blending, the flours were packed in polyethylene bags and stored until the extrusion process.

The statistical analysis was performed according to the set of 16 treatments. The extrusion variables studied was feed moisture content, barrel temperature, and legume/cereal (Maize-Lupin) blend ratio. The data were analyzed and modeled using Design-Expert 7 to generate second-degree polynomial models with response surface effects (Myers et al., 2016). The significant terms in the models were identified by analysis of variance (ANOVA) for each response. Significance was judged by determining the probability level that the $F$-statistic calculated from the data was less than 5%. The model adequacy was checked by $R^2$. Data analysis for each response variable was analyzed using multiple regression procedures with Design Expert version 7.0 (Statease Inc., Minneapolis, MN, USA). In order to determine the effect of each independent variable in the responses, the experimental data were fitted to the selected models and regression coefficients obtained, the model proposed for each response ($Y$) was:

$$Y = b_0 + b_1 B + b_2 T + b_3 M + b_{11} B^2 + b_{22} T^2 + b_{33} M^2 + b_{12} BT + b_{13} BM + b_{23} TM \quad \text{(3.2)}$$

Eqn. (3.2). Second order polynomial equation.

Where $Y=$the response, $B=$ blending ratio (%), $T=$barrel temperature (°C), $M=$ feed moisture (,), $b_0=$intercepts, $b_1$, $b_2$, $b_3$ are linear, $b_{11}$, $b_{22}$, $b_{33}$ = are quadratic and $b_{12}$, $b_{13}$ and $b_{23}$ are...
interaction regression coefficient terms, respectively. Also, the coefficients of determination (R²) were calculated. The adequacy of the models was tested by separating the residual sum of squares into pure error and lack of fit.

For each response, a response surface plot was generated from the regression equations by holding the one variable at its middle value and changing the other two variables. A numerical multi-response optimization was used to determine the optimal processing conditions to further evaluate the physical (RER and BD), functional (WAI and WSI), chemical (moisture content, protein content, fat, crude fiber, ash and carbohydrate profiles) properties of the extrudate. Data were expressed as mean±standard deviation (SD) of triplicate determinations. When significant effects (p ≤ 0.05) were indicated by ANOVA, Tukey pairwise comparisons were done by SAS 9.1 to identify which treatments differed significantly (p ≤ 0.05).

### 3.4. Physical properties analysis

Two physical parameters (Radial Expansion Ratio and Bulk Density) were used to describe the properties of the extruded products.

#### 3.4.1 Radial expansion ratio (RER)

Radial expansion ratio (RER) is defined as the ratio of the diameter of the extrudate to the diameter of the die (Coulter & Lorenz, 1991). The diameters of the extrudates were measured by an Electronic Digital Caliper having 0.01 mm accuracy. In order to determine the RER, the diameters of three randomly selected samples were measured, from each run and their average was taken. Equation (3.2) was used to calculate the RER:

\[
ER = \frac{D_e}{D_d}
\]

Where: - \(ER\) - Expansion ratio
- \(D_e\) - Diameter of extrudates (mm)
- \(D_d\) - Diameter of the die (mm)
3.4.2 Bulk density (BD)

The length was measured by an Electronic Digital Caliper (СДЕЛАНО, СССР, Russia) having 0.01 mm accuracy. Weight was measured by an electronic balance of 0.01 g sensitivity.

The bulk density of the extrudate samples were determined according to the method described by (Coulter & Lorenz, 1991). It was determined as weight divided by volume assuming a cylindrical shape of the extrudate. The equation for the determination of bulk density is shown below.

\[ \rho = \frac{4w}{L \cdot \pi d^2} \]  

(3.4)

Where:

- \( \rho \) - Bulk density (g/cm\(^3\))
- \( d \) - Diameter of the extrudate (cm)
- \( L \) - Length of extrudate (cm)
- \( w \) - Weight of extrudate (g)

3.5. Functional properties

Two functional parameters (Water Absorption Index (WAI) and Water Solubility Index (WSI)) were used to describe the properties of the extruded products. WAI and WSI were measured using the method of (Anderson et al., 1970)

3.5.1 Water Absorbing Index (WAI)

Water absorption index of the flour, formulated flour and extrudate were determined according to (Anderson et al., 1970). About 3g of the sample was ground and sieved through a standard sieve (#50), the sample was placed into a centrifuge tube and then soaked with 25ml distilled water at 30°C for 10 minutes with intermittent shaking every 2 min. The subsequently sample was centrifuged at 3000 rpm for 15 min (Sorvall GLC-2B General Laboratory Centrifuge, Du Point Instrument).
The clear supernatant of the centrifugation was transferred into pre-dried and weighed glass baker for the estimation of the water solubility index (WSI). The gel remaining in the centrifuge tube was weighed and WAI was calculated as follows:

\[
WAI = \frac{W_g}{W_{ds}}
\]  

(3.5)

Where:

- \textit{WAI} - water absorption Index
- \textit{Wg} - weight of the gel
- \textit{Wds} - weight of dry sample

**3.5.2 Water Solubility Index (WSI)**

The supernatant preserved from WAI measurement was evaporated at 96 °C temperature for overnight. The WSI was calculated as:

\[
WSI = \frac{W_r}{W_s} \times 100
\]  

(3.6)

Where:

- \textit{WSI} - Water solubility index (%)
- \textit{W_r} - Weight of residual supernatant after evaporation (g)
- \textit{W_s} - Weight of sample (g)

**3.6. Proximate composition analysis and experimental procedures**

The proximate analyses of the pure ingredients and the designed points/ proportions were analyzed by standard (AOAC, 2000) methods.
3.6.1 Moisture content

The determination of moisture content of the raw materials and extrudates were performed gravimetrically according to hot air oven method. The water content in the sample by oven method was determined by evaporation of water lost in the sample.

A clean crucible was dried in an oven at 105 °C for an hour and placed in desiccators to cool, and the weight of crucible (W$_1$) was determined. About 5g of samples was weighed in a dry crucible (W$_2$) and dried at 102 °C for 5 hrs. after cooling to room temperature and was weighed (W$_3$) again.

At the end the moisture content was determined by the equation:

$$Moisture \ content \ in \ percent \ (%) = \left(\frac{W_2 - W_3}{W_2 - W_1}\right) \times 100$$ (3.7)

Where:

$W_1 = \text{Weight of the drying crucible},$

$W_2 = \text{Mass of the drying crucible and the sample before drying},$

$W_3 = \text{Mass of the drying crucible and the sample after drying}.$

3.6.2 Crude protein

Protein content was determined according to (AOAC, 2000) using the official method 979.09. A digestion flask containing a sample, concentrated acid (sulphuric acid) and catalyst mixture (K$_2$SO$_4$ and CuSO$_4$) are added and exposed to a temperature in a Kjeldahl digester (VELP Scientifica, DK, Italy). Next, the sample was cooled and 40% NaOH was added to it as an indicator. Then the sample was distilled UDK Series DISTILLATION UNITS and the distillate titrated with standardized 0.1N Hydrochloric acid to a reddish color.

$$Nitrogen \ (%) = \left(\frac{[V_2 - V_1] - B \times N \times 14.007}{W_2}\right) \times 100$$ (3.8)

Where

$V_1 = \text{Volume of the standard hydrochloric acid solution used in the titration of the sample}$
\[ V_1 = \text{Volume of the standard hydrochloric acid solution used in the titration of the blank} \]

\[ B = \text{Volume of hydrochloric acid consumed blank} \]

\[ N = \text{Normality of standard hydrochloric acid} \]

\[ W_c = \text{Weight of the sample, g} \]

\[ \text{Protein content (\%, w/w) = \% Nitrogen*Factor specific for different product} \quad (3.9) \]

### 3.6.3 Crude Fat

To measure the fat content of the samples, (AOAC, 2000) method was followed. Empty extraction flasks were cleaned and dried at 92 °C for at least an hour and then kept in the desiccator for at least half an hour. The mass of cooled flasks was weighed (\(W_1\)). About 5 g of the sample was weighed (\(W_2\)) in to each of the thimbles lined with fat free cotton at their upper and bottom. The thimbles with their sample content were placed in to the soxhlet extraction chamber.

A 40 ml of petroleum ether was added in to each flask used for the extraction. The extraction process was done for about 4 hr. Then the flasks with their contents were removed from the soxhlet and placed in drying oven at 92 °C for 1 hr. The flasks were then placed in desiccators for 30 min. The masses of each flask together with its fat contents were weighed immediately after it is taken out of the desiccator (\(W_3\)).

The crude fat content was calculated from the equation:

\[ \text{Weight of fat } W = W_3 - W_1 \quad (3.10) \]

\[ \text{Fat } \frac{g}{100g} \text{ fresh sample} = \frac{W}{W_2} *100 \quad (3.11) \]

Where:

\[ W = \text{weight of fat} \]

\[ W_3 = \text{weight of fat + flask after extraction and drying} \]
\[ W_1 = \text{weight of empty extraction flask} \]
\[ W_2 = \text{weight of the sample} \]

### 3.6.4 Dietary fiber

To measure the dietary fiber content of the samples, (AOAC, 2000) method was followed. About 2.0 g of defatted sample was weighed in each of 600 ml beaker. A 200 ml of 1.25% sulfuric acid solution was added to each beaker and allowed to boil on hot plate for 30 min by rotating and stirring periodically. During boiling the level was kept constant by addition of hot distilled water. After 30 min, 20 ml of 28% potassium hydroxide solution was added into each beaker and again allowed to boil for another 30 min. The level was still kept constant by addition of hot distilled water. The solution in each beaker was then filtered through crucibles containing sand by placing each of them on Buchner funnel fitted with a rubber stopper. During filtration, the sample was washed with hot distilled water. The final residue was washed with 1% sulphuric acid solution, hot distilled water, 1% sodium hydroxide solution and finally with acetone. Each of the crucibles with their contents was dried for 2 hrs. at 130 °C and cooled in desiccators and weighed (\( W_1 \)). Then again, they were ashed for 30 min at 550 °C in furnace and were cooled in desiccators. Finally, the mass of each crucible was weighed (\( W_2 \)).

The crude fiber was calculated from the equation:

\[
\text{Total Crude fiber} = \left( \frac{W_1 - W_2}{W_3} \right) \times 100
\]

(3.12)

Where:

\( W_1 = \text{Crucible weight after drying} \)
\( W_2 = \text{Crucible weight after ashing} \)
\( W_3 = \text{Sample dry weigh} \)

### 3.6.5 Total ash

Ash was determined by the method of the Association of Official Analytical Chemists (AOAC, 2000), using the official method 923.03. Clean porcelain crucible, dried at 120 °C in an oven was ignited at about 550 °C in a muffle furnace for 3 hours was cooled in
desiccators and weighed \((M_1)\). Then about 2.0 g samples were weighed into a previously dried and weighed \((M_2)\) porcelain crucible. These samples were dried at 120 °C for 1 hour and carbonized by oven until the contents turn black. The crucible with the contents was placed in a Muffle furnace set at 550°C for 1 hour to ignite until ashing was completed. After this period the crucible with its content was removed and cooled in desiccators. The crucible with the residue was weighed \((M_3)\). The weight of the ash was expressed as a percentage of the initial weight of the samples. The total ash was expressed as percentages on dry matter basis as follows: -

\[
\text{Total Ash} (\%) = \left( \frac{M_3 - M_1}{M_2 - M_1} \right) \times 100
\]

(3.13)

Where: \((M_2-M_1)\) is sample mass in g on dry base and \((M_3-M_1)\) mass of ash in gm

3.6.6 Total Carbohydrates

Total carbohydrates (%) was determined by difference that was by subtracting the sum of the percentages of moisture (M), crude protein (P), lipid (L), fiber (F), and ash content (A) from 100.

\[
\% \text{Total CHO} = 100 - [\% \text{Moisture} + \% \text{Protein} + \% \text{Fat} + \% \text{Fiber} + \% \text{Ash}]
\]

(3.14)

3.7. Sensory Evaluation of the products

Sensory evaluation has been widely used in the evaluation of snacks. Organoleptic properties of the samples were evaluated by 20 semi-trained panelists recruited from the staff of Faculty of Chemical and Food Engineering, Bahir Dar Institute of Technology for various sensory attributes using a 9-point hedonic scale questionnaire, where “9” represents like extremely while “1” represents dislike extremely score. In the questionnaire, the panelists were asked to evaluate the intensity of the following attributes of maize-lupin snacks: color, flavor, texture, taste and overall acceptability. Data were analyzed statistically using analysis of variance (ANOVA) and mean separation was done by Duncan (1955) multiple range tests at 5% level of probability.
4. RESULTS AND DISCUSSION

4.1. Chemical Composition of Raw Materials and the Extrudate

The significant influences of blending ratio and extrusion cooking processing variable (barrel temperature and feed moisture content) to produce maize-lupin based extrudates were studied. The effect of these variables on the quality attributes of response variables of the extrudates such as physical properties (bulk density and expansion), functional properties (water absorption index and water solubility index), proximate composition (moisture, protein, fat, crude fiber, ash and starch) of the extrudate flours, and sensory quality attributes (visual colour, appearance, flavour, texture, taste, and overall acceptability) of the extrudates were investigated.

4.1.1 Proximate Composition of raw and blend Maize and Lupin Flour

The chemical compositions of maize flour and lupin flour are shown in Table 4-1. The moisture content of maize flour was 10.50% while that of milled lupin flour was 7.77%. Maize exhibited typical proximate compositional values, similar to those reported by (Reyes-Moreno et al., 2003). Similarly, the crude protein, fat, crude fiber, ash, and starch of milled maize flour were 10.22%, 4.26%, 2.03%, 2.22%, and 70.76%, respectively. The crude protein, fat, crude fiber, ash, and starch of lupin flour was 39.95%, 14.54%, 2.62%, 2.55%, and 32.54%, respectively. (Getachew et al., 2012) observed the similar result for moisture, protein, fat, crude fiber, ash, and carbohydrate for white Lupin in case of traditionally processed Lupin flour. Proximate composition implies that Lupin was having higher nutritional value as compared to milled maize. Variation in nutrient composition of maize and lupin from previous studies may be due to varietal effects, environmental factors such as geographic location and growing season, as well as differences in the techniques used for analysis.

The moisture content of LF is significantly \( p \leq 0.05 \) lower than the moisture content of MF, it could be attributed to LF composition contains oligosaccharides and polysaccharides which characterized as high water holding capacity (Sujak et al., 2006).
Table 4-1 Proximate composition of Maize and Lupin flour

<table>
<thead>
<tr>
<th>Component (g/100 g dry basis)</th>
<th>Maize (MF)</th>
<th>Lupin (LF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>10.502±0.947&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.772±0.204&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein</td>
<td>10.225±0.912&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.958±0.863&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>4.260±0.044&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.543±0.396&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.027±0.189&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.627±0.150&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>2.219±0.508&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.552±0.191&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Carbohydrates*</td>
<td>70.768±0.333&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.548±0.361&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values presented as means±standard deviation, MF-Maize flour, LF-Lupin flour, means with the same superscript within a row are not significantly different (P ≤ 0.05).

Table 4-2 Proximate composition of Maize and Lupin flour blends

<table>
<thead>
<tr>
<th>Component (g/100 g dry basis)</th>
<th>MF90:10LF</th>
<th>MF85:15LF</th>
<th>MF80:20LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>6.75±0.50</td>
<td>6.89±1.20</td>
<td>7.65±1.73</td>
</tr>
<tr>
<td>Protein</td>
<td>9.36±0.53</td>
<td>14.09±0.93</td>
<td>19.22±0.71</td>
</tr>
<tr>
<td>Fat</td>
<td>3.60±0.19</td>
<td>4.05±0.24</td>
<td>5.40±0.19</td>
</tr>
<tr>
<td>Fiber</td>
<td>1.89±0.16</td>
<td>2.34±0.12</td>
<td>2.61±0.23</td>
</tr>
<tr>
<td>Ash</td>
<td>1.88±0.69</td>
<td>2.00±0.33</td>
<td>2.12±0.18</td>
</tr>
<tr>
<td>Total Carbohydrates*</td>
<td>76.52±0.41</td>
<td>70.64±0.56</td>
<td>62.99±0.61</td>
</tr>
</tbody>
</table>

Values presented as means±standard deviation, MF-Maize flour, LF-Lupin flour, Means with the same superscript within a row are not significantly different (P ≤ 0.05).

Figure 4-1 Extrudates developed from maize lupin with three different blending ratios
4.2. Effect of Blending Ratio and Extrusion Processing Parameters on Physical and Functional Properties of the Maize-Lupin Snack

Response surface methodology (RSM) was used to analyze the relationship between the dependent and independent variables. Extrudates of different physical and functional properties (radial expansion ratio, bulk density, water absorption index and water solubility index) were obtained at different processing conditions (blending ratio, barrel temperature, and moisture content) as shown in (Table 4-3 below).

Table 4-3 Effect of extrusion condition on product physical and functional responses

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Responses (Physical &amp; Functional properties)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B</strong></td>
<td><strong>T</strong></td>
</tr>
<tr>
<td>20</td>
<td>135</td>
</tr>
<tr>
<td>20</td>
<td>135</td>
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<tr>
<td>15</td>
<td>120</td>
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<td>10</td>
<td>120</td>
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<td>20</td>
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<tr>
<td>20</td>
<td>150</td>
</tr>
<tr>
<td>10</td>
<td>150</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

Values presented as means±standard deviation. Means with the same superscript within a column are not significantly different (P≤0.05). B, blending ratio; T, barrel temperature; M, feed moisture; RER, radial expansion ratio; BD, bulk density; WAI, water absorption index; WSI, water solubility index.
4.2.1 Radial Expansion Ratio (RER)

Radial expansion ratio (RER) is the most important physical property and indicates the extent of puffing of extruded products as it exits the extruder. The effects of process variables on the RER of extrudates presented in Figure 4-2 below exhibit that an increase of lupin flour level and feed moisture content during extrusion decrease the RER values. It was suggested that increase in the level of lupin flour means an increase in the level of protein, fat and fiber contents and with lower starch content, as with the results of this study, discussed within “Bulk Density” section.

Starch was the main ingredient responsible for the dough development inside the extruder barrel and consequent expansion at the die exit (Pęksa et al., 2016). Amount of starch in the ingredient decreased as the lupin flour level increased since the lupin flour was added in place of maize flour. (Saini, 2015) reported that RER increased with increasing maize starch level. That might be due to the easy-to-expand properties of maize starch.

Blending ratios, barrel temperature, and moisture content significantly affected the radial expansion ratio (RER) through a linear model. The analysis of variance (ANOVA) of the models showed that the quadratic effects from blending ratio and feed moisture content were significant with ER. The interaction effect between the three independent variables (blending ratio, moisture content, and barrel temperature) were insignificant with ER.

The regression equation for the relationship between expansion ratio (ER) and independent variables in terms of coded form can be shown by the following equation;

\[
ER = +0.91 - 0.16B + 0.051T - 0.013M - 5.492E-003B^2 + 0.054T^2 + 0.034M^2 
\]

(R\(^2\)=99.54) \hspace{1cm} (4.1)

Quadratic equation (4.1) showed that the negative significant (p < 0.05) linear coefficients of blending ratio (B) and feed moisture (M) indicated that their effects on ER were a decrease. The coefficient of T is indicating the positive effect on expansion ratio, which means expansion ratio increased with increasing the barrel temperature. In linear terms, blending ratio (B), barrel temperature (T) and moisture content (M) were found to be significant (P<0.05). F-value for linear terms of, blending ratio (B), barrel temperature (T)
and moisture content ($M$) were 81.15, 11.19 and 9.23 and P-value was found to be 0.0003, 0.0204 and 0.0288 (P<0.05) respectively, validating that terms were significant.

Blending ratio (Lupin content) was the most significant factor affecting ER. Starch content, size, number and distribution of air cells within the extruded material determine the degree of expansion. Blends with a higher maize proportion can be expected to exhibit greater expansion since cereals have excellent expansion properties and are known to be the most adequate for extrusion processes. In contrast, decrease in starch content and increase in protein and fat content in the blend due to the addition of lupin significantly affected the expansion ratio (ER) of the extrudates. Expansion is highly dependent on chemical composition of the raw ingredients used. When materials containing starch are extrusion cooked, expansion is dependent on formation of a starch matrix that traps water vapor and results in the formation of bubbles. According to (Oliveira et al., 2017) increasing the level of corn flour increased the expansion ratio and decreased bulk density, which may be related to the properties of lupin protein in the lupin flour. The majority of Lupin albus proteins—the legumin-like storage proteins are generally not thought of as functional proteins and might impede full starch gelatinization (Froidmont et al., 2008; Jayasena & Nasar-Abbas, 2012a; Young & Pellett, 1994). (Chinnaswamy & Hanna, 1988) noted that the expanded volume of cereal flour decreased with increasing amounts of protein and lipid but increased with starch content. In addition, the decrease in expansion may also due to the usage of whole grain maize flour as a base flour for complementation.

(Pelembe, Erasmus, & Taylor, 2002) reported that ER decreased as the percentage of cowpeas increased in composites and indicated that the structure of extrusion-expanded products depended on starch gelatinization.

With the increase in temperature, there was an increase in radial expansion ratio (RER). The expansion increased due to the higher degree of superheating of water in the extruder encountering the bubble formation (Bhattacharya & Prakash, 1994). A similar effect of extrusion temperature on expansion ratio has been reported by other researchers (Kumar et al., 2017; Suksomboon et al., 2011).

Moisture plays a key role in the mechanism responsible for expansion. The 3D plot (Figure 4-2) shows that increasing feed moisture resulted in decreased ER. The same plot indicated that increasing the amount of blending ratio (lupin flour) indicated a decrease in the ER.
Considering the elastic properties of the amylopectin network as being responsible for diametral expansion is helpful in explaining such role of moisture. This is a commonly observed phenomenon in many extruded foods, which can be attributed to the fact that the amount of expansion in a food material depends on the pressure differential between the die and the atmosphere (Kumar et al., 2017). (Saini, 2015) reported a decrease in ER with an increase in feed moisture of maize flour based extrudates.

Figure 4-2 Response surface plot of radial expansion ratio (RER) of Maize-Lupin blend extrusion for the effect of blending ratio, barrel temperature and feed moisture content
4.2.2 Bulk density (BD)

Bulk density is a very important parameter in the production of extruded products. Bulk density has been linked with the expansion ratio in describing the degree of puffing in extrudates. The bulk density (BD) of the composite flours extrudates ranged from 0.328 to 0.919 g/cc, which generally is high. It has also been observed that higher bulk density is desirable for greater ease of dispensability and reduction of paste thickness (Awolu et al., 2015). The obtained values of BD were higher than those reported by (Suksomboon et al., 2011) and similar to those obtained by (Awolu et al., 2015). It is evident that the observed differences between the experimentally determined values and that of the preliminary study were caused by the difference in blending ratio, crop types, and whole grain maize flour used during the formulation.

The effects of process variables on the bulk density of extrudates are presented in (Figure 4-3) exhibiting that increase of blending ratio (lupin flour level) and feed moisture content during extrusion increase the bulk density values.

The increase in bulk density with increasing lupin flour level could be due to the addition of increasing amounts of fiber and protein to the blend which might affect the extent of starch gelatinization and the rheological properties of the melted material in the extruder (Yağcı & Göğüş, 2008). The non-starch polysaccharides in fiber may bind water more tightly during extrusion than do protein and starch. This binding may inhibit water loss at the die and thus reduce expansion and so probably increased bulk density. The increasing bulk density caused by less expansion and more impact was because the presence of fiber particles tended to rupture the cell walls before the gas bubbles had expanded to their full potential (Altan et al., 2008).

At feed high moisture levels, bulk density is also high. This is because the extrusion cooking is not enough to cause vaporization of the moisture leading to the retention of moisture and hence the reduced puffing of the product. As a result, a denser product was obtained (Asare et al., 2012). This is what was observed with products from runs that had high feed moisture levels. The effect of feed moisture on Bulk density (BD), observed in this experiment similar to several previous studies. Increased feed moisture content during extrusion may reduce the elasticity of the dough through plasticization of the melt, resulting in reduced SME and
therefore reduced gelatinization, decreasing the expansion and increasing the density of extrudate (Ding et al., 2005). It was depicted that bulk density increased with increase in moisture as higher water content produced extrudates denser than those produced with low water content (Omwamba & Mahungu, 2014; Saini, 2015).

The regression model for bulk density as a function of the variables analyzed can be described by the following equation in terms of coded values:

\[ BD = +0.59 + 0.12B - 0.075T + 0.067M - 0.018BT - 3.908E -0.004BM + 7.702ET - 0.003TM + 0.13B^2 - 0.13T^2 - 0.021M^2 \]  

\[ (R^2 = 97.03) \]  

(4.2)

Bulk density was significantly affected by the linear (P < 0.05) effects of blending ratio, barrel temperature, and feed moisture, and quadratic (P < 0.05) effects of blending ratio and barrel temperature (ANOVA table). The regression model fitted to experimental result of bulk density showed model F-value of 18.19 which was significant (P<0.05) whereas the lack of fit F value is 7.96, which was not significant. The models showed a good fit with \( R^2 = 0.97 \) for BD. This suggests a very good fit to the experimental data and the model could be used to describe the process.

The response surface plots (Figure 4-3) showed that product bulk density increased with increasing blending ration and feed moisture, whereas decreased with increasing barrel temperature.

(Hagenimana et al., 2006) reported that the BD increased with increase in moisture content during the extrusion of rice flour. Bulk density is a measure of how much expansion has occurred as a result of extrusion. The heat developed during extrusion can increase the temperature of the moisture above the boiling point so that when the extrudate exits from the die, a part of the moisture would quickly flash-off as steam and result in an expanded structure with large alveoli and low bulk density.

On the other hand, if not enough heat is generated to flash-off enough of the moisture (either through low process temperature or high feed moisture), less expansion occurs resulting in a high bulk density product with collapsed cells which usually disintegrates on cooling. High bulk density
product is an indication of more uniform and continuous protein matrix and, therefore, the extrudate is dense with parallel layers, no air pockets and is not spongy upon hydration (Taranto et al., 1978).

Interpretation of regression equation can be improved by response surface plot presenting constant values of dependent variables as a function of two independent variables studied over their range of variation.

**Figure 4-3** Effect of blending ratio (BR), barrel temperature (BT) and feed moisture content (MC) on BD
The suitability of extrudates for application depends on their functional properties such as water absorption and water solubility indices. Functional properties of the extrudates are, generally, related to the molecular modifications that occur during extrusion cooking process. Results obtained for WAI and WSI of extrudate as function of independent variables are given in Table 4-3 above.

4.2.3 Water Absorption Index (WAI)

This index describes the weight of water that is bound to one gram of dry sample. WAI, an index of starch gelatinization and measures the amount of water absorbed by starch.

The water absorption index (WAI) of the Maize-Lupin extruded snack ranged from 4.350 to 6.403 Table 4-3 above. (Pardhi et al., 2017; Sajad Ahmad Wani & Kumar, 2016) and (Yağcı & Göğüş, 2008) reported the WAI was in the range of 4.72 to 7.81; 4.11 to 5.86, and 3.65±0.06 to 5.59±0.05 g/g dry solid respectively. WAI depends on the availability of hydrophilic groups that bind water molecules. The predicted model from regression analysis for WAI is shown in Eq. (4.3) (in terms all independent variables in coded values) was developed as follows.

\[ WAI = +5.52 - 0.55B + 0.31T + 0.22M - 0.038BT + 0.012BM - 0.26TM + 0.19B^2 - 0.12T^2 - 0.059M^2 \]  
\[ R^2 = 95.42 \]  

The significance of coefficient of the fitted quadratic model was evaluated by using f-test and p-value. Regression model fitted to experimental results of WAI showed the p-value for lack of fit of 0.1726 which implies the lack of fit was non-significant. The value of \( R^2 \) was found to be 0.9542. Quadratic equation 4.3) showed that the coefficient of \( T \) and \( M \) was positive. Therefore, water absorption index increased with increasing, barrel temperature and moisture content. The coefficient of \( B \) indicates the negative effect on water absorption index, which means water absorption index increased with decreasing blending ratio.

ANOVA analysis demonstrated that the linear model was significant (\( P < 0.05 \)). Increasing the blending ratio (content of lupin) from 10% to 20%, there was a significant decrease of in water absorption index. This could be due to decreased availability of starch in the lupin flour which has the higher water absorption capacity. Relative decrease in starch content
with addition of Lupin may affect the extent of starch gelatinization in barrel and caused reduced water absorption. Similarly, (Singh, Sekhon, & Singh, 2007) reported a decrease in WAI with addition of pea grits in extrusion of rice was due to the dilution of starch in rice pea blends.

Water absorption index was found to increase when the temperature was raised from 120ºC to 150ºC. At higher temperature, the starch granule is disrupted and more water is bound to the starch molecule resulting in increased WAI (Kumar et al., 2010). (Kumar et al., 2010) examined the increased effect of WAI with increased temperature which is in support of our current findings. With extruded corn grits, a similar trend was observed by (Anderson et al., 1970).

Furthermore, water absorption index also increased when moisture content level of the feed was increased from 14% to 18%. This was in agreement with (Ding et al., 2005; Seth, Badwaik, & Ganapathy, 2015) who observed that, at higher moisture content, the viscosity of starch would be low, allowing the starch molecules to move freely and thereby enhancing the penetration of heat as a result greater gelatinization. Because the water acts as a lubricant in the extrusion medium, resulting in reduction of friction of the screw and the inner wall of the extruder barrel on the starch molecules, thereby resulting in less degradation of the amylose and amylopectin and, consequently, in an increase in WAI.
Figure 4-4 shows the response surface plot of WAI vs two independent variables at a time with the third taken at the midpoint level.

4.2.4 Water Solubility Index (WSI)

WSI often used as an indicator of degradation of molecular components (Filli et al., 2011), measures the amount of soluble components released from the starch after extrusion (Anderson et al., 1970). High WSI is an in vitro indicator of good starch digestibility as it implies the extent of gelatinization and dextrinization (Anton et al., 2009). It can also measure the degree of starch conversion during extrusion, which corresponds to the amount of soluble polysaccharide released from the starch granule (Ding et al., 2005).
The results of water solubility index (WSI) for different experimental conditions are showed in Table 4-3 above. The regression model fitted to experimental result of water solubility index shows model F-value of 9.51 which was significant, whereas the lack of fit F value is 0.89, which was not significant. The $R^2$ was found to be 0.9448 and Adj $R^2$ was 0.8454. The quadratic model obtained from regression analysis for water solubility index in terms of coded levels of the variables is as:

$$WSI = +13.36+1.27*B+0.84*T-0.31*M+0.090*B*T$$
$$-0.041*B*M-0.17*T*M-0.35*B^2+0.28*T^2-5.651E-003*M^2$$ (R$^2$=94.48) (4.4)

Quadratic equation (4.4) showed that the coefficient of $B$ and $T$ is positive. Therefore, water solubility index increased with increasing the blending ratio and barrel temperature. The coefficient of $M$ indicates the negative effect on water solubility index, which means water solubility index increased with decreasing feed moisture content. Water solubility index is not only due to starch content but also due to water-soluble components like protein which are present in raw material i.e., maize and lupin.
Figure 4-5 shows the response surface plot of WSI vs two independent variables at a time with the third taken at the midpoint level.

WSI increased as a function of blending ratio (Lupin content) (Figure 4-5 above). WSI is a parameter that reflects the degradation suffered by the components of the fiber (Larrea, Chang, & Martinez-Bustos, 2005). The increased WSI found in extruded products can be related to the lower molecular weight components, which can be separated quite easily from each other when the processing conditions are more severe (Yağcı & Göğüş, 2008). Lupin is relatively high in fiber, protein and fat content. Above critical concentration, the fiber molecules disrupt continues structure of the melt in extruder, hindering elastic deformation during extrusion (Moraru & Kokini, 2003). So, the greatest WSI values may due to
disintegration of starch granules and low molecular compounds from extrudate melt during extrusion process, this may cause in an increase in soluble material. Figure 4-5 above indicates that water solubility index increased with increasing the die temperature. The influence of high temperature, pressure and shearing forces intensifies the starch chain depolymerization process which in turn contributes to an increase in water solubility index value of the extrudates (Guzmán-Ortiz et al., 2015). (Omohimi et al., 2014) reported that, increased barrel temperature increases WSI, due to increased solubility of starch molecules. This was also in consistent with the results of (Ding et al., 2005).

The response surface plot for the extrudates (Figure 4-5 above) showed the WSI decreased with increasing feed moisture content. The increase in moisture may have contributed to the reduction of friction, because water acts as a lubricant, leading to lower values of WSI. Similar effects have been reported in literature for rice-based extrudates (Ding et al., 2005), fish and rice flour coextrudates (Tumuluru et al., 2013), and maize-mungbean extrudates (Ali et al., 2016). It was suggested that increasing WSI is caused by greater shear degradation of starch during extrusion at low moisture conditions.

### 4.3. Effect of Blending Ratio and Extrusion Processing Parameters on Proximate composition of Maize-Lupin snack

Studies were carried out using the response surface method in modeling the chemical composition of extruded maize – lupin blends as affected by the feed composition level of lupin added to maize (B), and process variables barrel temperature (T) and feed moisture (M) for the manufacture of snack. Proximate composition data on maize and lupin flour extrudates produced based on Box Behnken design are given in Table 4-4 below. The data reported is an average of three measurements. The product moisture content, crude protein, crude fat, crude fiber, ash, and starch content of the extruded food flour formulations ranged between 5.63–7.15%, 8.05–18.12%, 1.71–5.87%, 1.57–2.72%, 1.55–2.22%, and 64.79–81.49% respectively (Table 4-4).
Table 4-4 Effect of blending ratio and extrusion condition on proximate composition and product responses

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<th>Independent variables</th>
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Results are expressed as means±SD, n=3. * determined by difference; Means with different superscripts within a column are significantly different (P ≤ 0.05).

4.3.1 Product moisture content

The moisture content of the Maize-Lupin snack ranged between 5.63% and 7.15%. This index is presumed as one of the most important determinants of the shelf-stability of food products. High-moisture (>14%) products usually have shorter shelf-stability compared with lower-moisture (<14%) products (Asare, 2002; Michael Vadakekara Joseph, 1995),
and all cases should require further processing or drying so as to allow for extended storage time.

This is because the former has higher water activity, which enhances microbial activity. Therefore, there is the need for this further cost operation to bring down the levels of moisture to allow for easy handling, storage, and improved general acceptability.

The regression model for predicting moisture content could explain 91.73% of the observed variations and a non-significant $F$-value of 0.6001 as lack-of-fit. The ANOVA shows that the linear terms blending ratio and feed moisture are significant ($P \leq 0.05$). However, barrel temperature as a linear independent variable, blending ratio and feed moisture as interaction and quadratic, terms had no significant effect ($P \leq 0.05$).

The regression equation for the relationship between product moisture content and independent variables in terms of coded form is shown in equation (4.5).

\[
MC = +6.89+0.16 \cdot B - 0.012 \cdot T + 0.11 \cdot M + 0.036 \cdot B \cdot T \\
- 0.071 \cdot B \cdot M - 0.055 \cdot T \cdot M - 0.072 \cdot B^2 - 0.034 \cdot T^2 + 0.083 \cdot M^2
\]  
\( (R^2 = 91.73) \) (4.5)

From the response surface plot (Figure 4-6 below) obtained from the model, the moisture content of the Maize-Lupin extrudates increased with increasing levels of feed moisture and Lupin at all levels. When compared with the data on extruded maize alone, the maize/lupin blends had higher moisture values. The damaged starch and incorporation of the legumes could be the reason for the observed higher values in moisture content.

This result is in agreement with other researches such as (Asare, 2002) and (Asare et al., 2012) who reported similar findings. Cowpea proteins are known to have water-binding effects, hence its significant influence in the models. This explanation can also be extended to the influence of lupin proteins. (Prinyawiwatkul, Beuchat, & Phillips, 1995), in their studies on extrusion of peanut flour, indicated that higher feed moisture resulted in higher moisture content of the extruded samples and that high protein increased the moisture content of the extrudates, as it results in decreased moisture flash-off and high fissuring. The present results corroborate these
findings, where an increase in feed moisture increased the moisture content of the extrudates.

Figure 4-6 Response surface plot for Moisture content (as a function of blending ratio, barrel temperature and moisture content)

Our finding is in the range of (Tiwari et al., 2011) who reported 8.5% to 10% of moisture content as a quality factor for the deep-fried snack prepared from Rice Brokens and Legumes By-Product.
4.3.2 Protein Content

Cereal grains are known to be low in protein (both in quantity and quality) and hence the need for complementation with legumes. This combination would help make complete proteins because the legume would provide lysine and other limiting amino acids. The protein content measured for all possible runs of Maize-Lupin extrudates ranged from 8.46% - 18.12%. It is important to note that protein derived from the balance of these cereals with the legumes can supply essential amino acids needed for growth and maintenance. With these significant protein increases, the consumption of this ready-to-eat cereal-legume snack would contribute protein to a person’s diet especially in the developing world.

Models developed for each run could explain 98.80% of the variations and a non-significant F-value of 0.17 (as lack-of-fit). The variable which showed significant influence on protein content is the blending ratio (levels of Lupin content) ($P \leq 0.05$). The regression analysis results indicated that the linear and quadratic terms of blending ratio (Lupin content) had a significant ($P \leq 0.05$) positive effect on protein content.

The predicted regression model can be described by the following equation:

$$
protein = +14.04 + 3.42 \cdot B - 0.25 \cdot T - 0.054 \cdot M - 0.23 \cdot B \cdot T + 0.022 \cdot B \cdot M \\
+ 0.37 \cdot T \cdot M + 0.26 \cdot B^2 - 0.33 \cdot T^2 + 0.13 \cdot M^2
$$

(R$^2$=98.80) (4.6)

The response surface plot for Maize-Lupin extrudates (Figure 4-7) also revealed the additions of Lupin as increasing the protein content of the extrudates. The level of moisture added and barrel temperature showed no significant effect ($P \leq 0.05$).

Lupin flour addition, blending ratio (10-20%), produced a great impact on proximate properties of maize flour based extrudates (Table 4-4 above). As grain legume flour contains more proteins than cereal starch (Duranti, 2006), levels of crude protein increased as a function of increasing rate of lupin addition. It is noteworthy that although we did not characterize the amino acids present in our snacks, it can be assumed that the amino acid profile of extrudates containing legume flour has changed from almost non-existent (maize snack control) to a relevant source of lysine (Duranti, 2006). In extruded goods, addition of legume flour to cereal-based formulations has proven to positively impact their essential
amino acid balance (Gujska & Khan, 1991; Jayasena et al., 2008; Lazou & Krokida, 2010; Silva et al., 2014; Sundarajah, 2014). Addition of high protein-high lysine material is known to positively affect the protein quality of cereal foods, therefore, with respect to lysine and Sulphur amino acid contents, legume and cereal proteins are nutritionally complementary.

**Figure 4-7** Response surface plot for Protein content (as a function of blending ratio, barrel temperature and moisture content)

The result is similar with (Kayacier et al., 2014) who reported 15.51% of crude protein from wheat and legumes (chickpea, soy and pea) blend; (Awolu et al., 2015) 15.91-24.00% crude protein from rice, cassava, and kersting’s groundnut blends, and (Tiwari et al., 2011) 15.3%
to 18.8% of crude protein from rice brokens and legumes by-product blend. But, the result is less than that of (Wani & Kumar, 2016) report (27.75–29.28%) who produced ready-to-eat snack from rice, cassava and kersting's groundnut composite flours but, higher than that of (Obadina et al., 2013) report (8.28±0.16 to 9.22±0.455) who formulated from wheat, lentil and chickpea flour (10–50%) and (Pastor-Cavada et al., 2011) who reported (7.38 to 12.13%) protein from whole maize, brown rice and wild legumes (Lathyrus annuus and Lathyrus clymenum). The difference could be due to the difference in blending ratio and crop types used during the formulation. According to (Joseph, 2016) blending of cereal-based foods and their processing methods can improve the protein content of the flour.

4.3.3 Fat content

Fat provides lubrication effect in the compressed polymer mix as well as modifies the eating quality of extrudates. The fat content of the extrudates ranged from 3.17% - 5.87%.

The germ and bran of cereals contain some lipids because we use the whole-grain maize flour; nevertheless, cereals are naturally low-fat foods. It is expected that relatively high fats contents are obtained in the cereal-legume blends as compared to results from the study involving only the cereals. The addition of the Lupin caused the considerable increase in the fat content in the extruded snack. This is because Lupin is known to contain considerably high amounts of fat (14.54%). However, it was observed that the fat content also decreased notably after sample processing. This is due to lipid degradation from the high processing temperatures, the screw speed used for extrusion, and that the fatty acids in the raw material form complexes with amylose, making extraction more difficult according to (Pérez-Navarrete et al., 2006). A disadvantage is that high fat content could affect the shelf life of the composite flour due to oxidative activities of the fat, which is leading to the development of unpleasant and odorous compounds (Obadina et al., 2013).

The regression models developed to predict the fat content explained 99.16% of the variations and a non-significant F-value of 0.1045 (as lack-of-fit). The model for Fat content as a function of the independent variables analyzed is shown below:

\[
Fat = +4.14 + 1.06B + 0.11T - 0.064M - 0.052BT + 0.083BM + 0.064TM - 0.21TB + 0.18B^2 + 0.39T^2 + 0.14M^2
\]

\( \text{R}^2 = 99.16 \)  \( \text{(4.7)} \)
Analysis of variance (ANOVA) results showed that blending ratio (the level of lupin addition) was the most significant variable. These findings suggest that a 10% - 20% level of lupin addition to any cereal system would considerably raise the overall fat content of the system. Other variables, which showed a significant effect in the maize-lupin blend snack were the quadratic effects of the blending ratio and barrel temperature and the interaction of barrel temperature and feed moisture (at p≤0.05) which had a negative effect Equation ((4.7)).

From (Figure 4-8), the response surface plot for the fat content in maize-lupin extrudates showed a significant increase as the level of lupin increased.
The result is similar with that of (Sharma et al., 2016) who reported 3.19% crude fat in expanded snack food processed from Maize, sorghum, Bengal gram, rice and soya chunks, and (Tumuluru et al., 2013) observed that 0.19 to 1.30% crude fat from Rice flour and fish coextrudates. However, the result is lower than that of (Awolu et al., 2015b) report (8.14–15.75%) who processed ready-to-eat snack food from rice, cassava and kersting's groundnut composite flours. This is may be due to the differences in crop types used and the processing methods employed during the formulation of the flour. For instance, the processing methods i.e., using of the whole-grain maize flour (without removal of outer part of maize and germ) have significant effect on increasing in fat content. In case of our finding, increasing the lupin content can improve the fat content of blended extruded snack food.

4.3.4 Crude Fibre

A highly expanded cereal product is usually difficult to achieve due to the high fiber and fat content (Kirkwood, 1984). Fibre particles usually decrease a product’s expansion by rupturing the cell walls before the gas bubbles could expand to its full potential. Consequently, extruded products with a high fiber content are usually compact, tough, not crisp, and with undesirable texture (Liu et al., 2000; Sadik, 2015).

From the experiment, crude fiber content determined ranged from 1.73% - 2.72%. These values showed an increase over the control (Maize snack) value (1.57%) (Table 4-4 above). These differences are because of the effects of the levels of blending ratios and use of whole-grains maize flour. The results suggest that producing high fiber snack with high nutritional and health benefits is possible by adding LF (Jayasena & Nasar-Abbas, 2012; Levent & Bilgiçli, 2012).

The predicted model for crude fiber can be described by the following equation:

\[
Fiber = +2.24+0.35* B +0.11* T +0.040* M +0.076* B * T -6.667E-003 * B * M -0.054* T * M -0.058* B^2 +0.048* T^2 -0.038* M^2 \quad (R^2=93.93) \quad (4.8)
\]

Based on the statistical analysis of the data, from independent variables blending ratio significantly (p≤0.05) affected the crude fiber of Maize-lupin snack. The ANOVA showed that the model of crude fiber was significant (p≤0.05) with a coefficient of determination of
R²= 93.93, which indicated that 94 % of the changes on crude fiber could be explained by the model. Also, the ANOVA indicated no lack of fit for this model.

![Figure 4-9 Effect of blending ratio (BR), barrel temperature (BT) and feed moisture content (MC) on crude fiber](image)

This is similar with the investigations of (Guzmán-Ortiz et al., 2015) who reported fiber content from 17.7 to 32.3 g kg⁻¹ in a blend of maize and Lima bean flour snack food. However, the result is lower than that of (Chanvrier et al., 2013) report (5.4–16.9%) who processed ready-to-eat Starch-based extruded cereals snack food from Whole wheat (%), Wheat flour (%) and Corn flours. The author also stated that increasing the added fibers
content, regardless of the types of fibers, also induces a loss of expansion and produce most compacted product.

4.3.5 Ash Content

Crude ash content gives an indication of inorganic elements that are present in a food as minerals. The ash content showed an increasing trend and ranged between (1.66–2.23%) at 20% incorporation level of lupin. The extrudates with the addition of Lupin showed higher ash content, and the increase was directly proportional to the amount of lupin incorporated. The ash content in extruded snack was significantly affected by the level of lupin flour in linear terms. Barrel temperature and moisture content had no significant effect on ash content of resultant snacks. No significant quadratic and interactions were noted for ash content. At linear level enhancement in the concentration of lupin flour resulted in higher ash content in snacks. Similar result (1.51 to 3.83%) was reported by (Awolu et al., 2015) when Ready-to-eat extruded snack was produced from composite flour comprising rice, cassava and kersting’s groundnut flours. (Alonso et al., 2001) also reported less influence of extrusion cooking in ash content.

Multiple regression equations generated to predict the ash as affected by different factors in terms of coded factors is as follows:

\[
\text{Crude Ash} = +1.85 + 0.16 B + 0.063 T + 4.066 E - 0.005 M - 0.014 B \times T - 0.055 B \times M - 0.056 T \times M + 0.081 B^2 + 0.025 T^2 + 0.011 M^2
\]

\[ \text{(R}^2=91.18) \] (4.9)
4.3.6 Total Carbohydrates

Carbohydrate is the main source of energy in human nutrition. The carbohydrate contents in the maize-lupin blended extruded food had ranged from 64.79–75.60%. The highest carbohydrate content was detected in the blended ratio of 90% maize and 10% lupin. The least carbohydrate content was seen in the blended ratio of 80% maize and 20% lupin (Table 4-4). With the increasing of lupin content in the blend, the carbohydrate content was found to be decreased. This is due to the fact that contents of carbohydrate present in lupin is low.
The quadratic model describing the carbohydrate content of snacks could be written as:

\[
Total - CHO = +70.84 - 5.15B + 0.16T - 0.056M + 0.085B \times T \\
+0.027B \times M + 0.045T \times M - 0.42B^2 - 0.073T^2 - 0.31M^2
\]

\[(R^2 = 99.33) \quad (4.15)\]

The lack of fit (LoF) of the selected regression model was not significant \((p \leq 0.05)\). This model may be considered appropriate for predicting the effect of scaled variables on carbohydrate content due to obtained high regression coefficient \((R^2 > 0.99)\). Carbohydrate content of extrudates was significantly affected by the linear terms of blending ratio \((B)\) \((p \leq 0.05)\). In this case, the coefficient of blending ratio \((B)\) was negative, indicating that the available carbohydrate decreased versus the increasing of blending ratio.

Processing method like using whole grain flour may increases carbohydrate content in the diet \((Robin et al., 2015)\). Similar results were reported by \((Oliveira et al., 2017)\) who reported 57.27 and 83.76 mg/100 mg of sample; and thus, claimed that carbohydrate was the major compound of the extruded cereals, produced from maize flour and whole grain wheat flour.

For the graphical representation of the functions of this design, graphs are used which describe the individual and cumulative effects of the variables tested and their effect on the response. Figure 4-11 shows the response surface graph in a three-dimensional plane for the regression model fitted to the data.
4.4. Effect of blending ratio and extrusion processing parameters on sensory characteristics of Maize-Lupin snack

The nutritional characteristics of the extrudates are a key consideration, but the extrudates need to have satisfactory organoleptic properties, which are deciding factors for overall acceptability. The effects of blending ratio, barrel temperature and moisture content on consumer acceptability of colour, flavor, texture, taste, and overall acceptability of the snacks expressed as their mean scores in Table 4-5 below.

The sensory scores for all the sensory properties indicate that all products have a mean value greater than ~5, indicating that the products are well liked by the panel judges. Scores for
colour, flavour, texture, taste and overall acceptance revealed that significant (p<0.05) differences exist between the products.

Addition of lupin flour exhibited a substantial effect on the sensory properties of maize-lupin extruded snacks. The sensory scores for colour (consumer likeness of colour) of snacks were improved by the incorporation of lupin flour. A light yellow colour imparted by natural yellow pigments present in lupin flour was attractive to the judges (up to 20% lupin substitution) as compared with the whitish colour of the control sample. Similar improvements in colour of different foods by lupin incorporation have been reported in other studies. Colour scores of spaghetti containing 15–30% lupin flour was much higher than those prepared by adding 15–30% light buck wheat or amaranth (Rayas-Duarte et al., 1996)

**Table 4-5 Mean scores for different sensory attributes of extruded snacks**

<table>
<thead>
<tr>
<th>Extrusion Conditions</th>
<th>Sensory properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR BT MC</td>
<td>Colour</td>
</tr>
<tr>
<td>20 135 18</td>
<td>6.5±1.67&lt;sup&gt;bde&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 135 14</td>
<td>6.7±0.86&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 120 14</td>
<td>6.7±0.98&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 135 16</td>
<td>6.5±1.15&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 135 16</td>
<td>6.9±0.83&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 120 16</td>
<td>5.95±1.19&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 135 16</td>
<td>7.1±0.79&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 135 18</td>
<td>6.2±1.24&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 120 18</td>
<td>7.2±1.20&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 135 14</td>
<td>5.45±1.00&lt;sup&gt;fg&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 150 14</td>
<td>6.7±0.86&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>15 150 18</td>
<td>7.45±0.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 120 16</td>
<td>7.35±1.14&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 150 16</td>
<td>6.80±1.32&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 150 16</td>
<td>5.95±1.00&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>4.9±1.33&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as means±SD, n=20.
Means with different superscripts within a column are significantly different (P≤ 0.05).

The flavor acceptance of the blended extruded snack food was found in between 5.70 and 6.95%. The highest flavor acceptance was scored in 85% maize, and 15% lupin blended and the least was scored in 80% maize and 20% lupin blended ratio Table 4-5 above.

The addition of lupin flour at 20% had no effect on the flavor acceptability of Maize-Lupin snacks. Evidence of beany aftertaste was not noted by panelists as an undesirable flavor
they observed during tasting. This may be due to higher cooking temperatures (120 °C to 150 °C), which could be key in modifying and eliminating off-flavors to improve their sensory properties (Simons et al., 2015; Yanez, Lobos, Diaz, & Ballester, 1986). However, it’s expected that as lupin incorporation increased beyond 20%, the flavor scores decreased. Hall and Johnson (2006) state that there was a significant difference in flavor between the control pasta sample and samples containing 28% lupin flour. The main reason might be that lupin flour had a natural “beany” flavor.

The textural properties of snack play an important role in terms of quality and consumer acceptability. The results of texture profile analysis are shown in Table 4-5 above.

Lupin incorporation substantially affected the texture/mouthfeel of the Maize-lupin snacks. However, all the samples containing 10 to 20% lupin flour were rated just above 5 and were significantly different. Nevertheless, some of the sensory panelists described the maize-lupin snack as hard and dry, which was reflected in a reduced textural rating. Similar trends were observed in study conducted by (Hall & Johnson, 2006) using ASL (Australian Sweet Lupin (Lupinus angustifolius)) flour in bread, muffins, pasta, chocolate chip, cookies, and breakfast bars.

The taste of the extruded snack was also affected by the lupin incorporation. Up to 20% addition of lupin flour had significant effect on the taste; however, the addition of 40 or 50% of lupin flour significantly decreased the taste of the final product and resulted in lower score for taste as reported by (Jayasena & Nasar-Abbas, 2012). At 40 and 50% lupin flour addition, the beany flavor might have a prominent negative effect on the taste of the product as reported by the same author. Liu et al., (2000) described the high-fiber extruded cereal product as (1) low expansion with hard texture and (2) rough and uneven “shark skin.” These features make products of this type not appealing to the potential end users. Extrudate with a high percent lupin flour in this study exhibited similar characteristics due to high fiber content. Bearing in mind that we also used the whole-grain maize flour.

Overall acceptability scores demonstrate significant effect of lupin flour addition up to 20% (Table 4-5). However, Hall and Johnson (2004) claim that the addition of 40 and 50% lupin flour significantly decreased the overall acceptability of the final product. So, this study results are in agreement to those of Hall and Johnson (2004), which showed nearly the same mean scores for overall acceptability between the control pasta sample and samples
containing lupin flour. Even though higher color scores were recorded for lupin-containing samples, 40 and 50% lupin-incorporated samples had lower scores for overall acceptability. This might be because of the poor ability of lupin to form a strong protein matrix, resulting in a poorer texture product. In addition, the beany flavor of lupin flour affected the flavor and taste of the final products. This trend has been reported for bean extrudates (Gujska & Khan, 1991; Jayasena et al., 2008).

Studies done by (Johnson et al., 2003) measured consumer acceptability of some food products containing novel legume flours Australian sweet lupin (ASL) at the same 30% inclusion. They reported a similar overall acceptability of the product using a 9-point hedonic scale.

4.5. **Optimum condition for maize-lupin extruded snack product**

Numerical optimization was carried out in Design Expert v7 considering four dependent (response) variables. In accordance with Bas and Boyaci (2007), from numerous factors (physical, functional, chemical, and sensory), it is necessary to select those ones that have governing and major effects for optimization purpose. The variables were kept in range during optimization. The goals were assigned to each response parameters as shown in the Table 4-6 below. Overlay plots were obtained by superimposing the contour graphs of all estimated responses using RSM.
Table 4-6 Conditions during optimization of extrusion process parameters

<table>
<thead>
<tr>
<th>Name</th>
<th>Goal</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Lower Weight</th>
<th>Upper Weight</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A:B</td>
<td>is in range</td>
<td>10</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>B:T</td>
<td>is in range</td>
<td>120</td>
<td>150</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>C:M</td>
<td>is in range</td>
<td>14</td>
<td>18</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>RER</td>
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<td>0.705967</td>
<td>1.223</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>WSI</td>
<td>maximize</td>
<td>11.4692</td>
<td>15.3071</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Protein</td>
<td>maximize</td>
<td>10.619</td>
<td>18.1167</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Texture</td>
<td>maximize</td>
<td>5.15</td>
<td>6.7</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Predicted values of RER (1.1875), WSI (15.462), Protein Content (14.9695), and texture (6.3012) were used to overlay plot graphical optimization (Figure 4-12). Best extrusion conditions were blending ratio of 15.0611 (%), barrel temperature of 150 (°C) and feed moisture of 14.00 (%) having desirability value of 0.728 (Table 4-6).
Figure 4-12 Point predictions of process parameters and responses using numerical optimization for extrusion process parameters

Graphical multi-response optimization technique was adopted to determine the workable optimum conditions for the development of extruded product using design expert software (Statease, DE 7.0). The process parameters were optimized for higher RER, WSI, protein and texture parameters. These constraints resulted in “feasible zone” of optimum conditions (shaded area in the superimposed contour plots). Superimposed contour plots having common superimposed area of all the responses for extrusion processes are presented in Figure 4-13 below.
Figure 4-13 Overlay contours of different responses for optimization of extrusion process parameters for extruded snack.
5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Extrusion provides a high-volume, low-cost alternative to conventional food processing methods. Improving the nutritional status of the maize based extrudates is prime important to make them nutritionally acceptable. Experiments were conducted in a twin-screw extruder to develop, model and optimize the effect of extrusion processing parameters on physical, functional and nutritional properties of high protein rich extrudates based on whole grain maize and lupin flour. The parameters studied under the process: blending ratio, barrel temperature and feed moisture levels. They are important and significant process variables and interrelated in affecting the physical, functional, nutritional, and the sensory characteristics of the end products.

Physical and functional indices such as RER, BD, WAI, and WSI were significantly affected by the blending ratios, barrel temperature, and feed moisture. Low feed moisture content and blending ratio resulted in good expansion, and less bulk density. Increasing the feed moisture decreased both the water solubility index and increase the water absorption index. High lupin additions (blending ratio) reflected in the increases in protein, fat, crude fiber, and ash contents of maize and lupin blend extrudates. For instance, there was a remarkable increase in protein content by as much as 55.57%. High protein foods are required in many developing countries where a considerable proportion of the population lives under protein malnutrition.

The addition of lupin rather improved the color by making it more attractive to the consumers. Based on the overall sensory acceptability it possible to incorporate up to a certain level without affecting their consumer acceptability.

In the end, the models developed, showed that extrusion condition optimal to produce puffed or direct expanded extrudates. The optimization of parameters resulted in blending ratio of 15.0611%, barrel temperature of 150°C, and feed moisture of 14.00%. The optimized value obtained for the final product was 1.187% ER, 14.9695% water solubility index, 13.314% protein, and 6.3012 % texture of extruded product.
The findings of this study demonstrate the feasibility of developing lupin garnished extruded products from maize flour with high protein quality and nutrient density, positive sensory characteristics. Hence, the blended snacks have great potential to be used as a protein-rich complementary food, to prevent Protein Energy Malnutrition among vulnerable population in Africa and other developing countries.

5.2. Recommendations

Hence if protein dense products are desired, such studies should be extended to higher protein (Lupin) levels to assess their influence on the physical and sensory properties of the extruded product. Further studies should also be carried out to find the effect of extrusion cooking on the levels of anti-nutrients and the physicochemical properties of the maize–lupin extrudates. Microbial analysis and shelf-life studies of the Maize-Lupin snack is recommended to assess the stability and microbial safety of the product.
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APPENDIX

Appendix 1 Analysis of variance for physical and functional properties of maize-lupin blend snack

Appendix 1:1 Analysis of variance showing the linear, quadratic, interaction and lack of fit of the RER, BD, WAI and WSI.

<table>
<thead>
<tr>
<th>Response</th>
<th>df</th>
<th>source</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>9</td>
<td>Model</td>
<td>0.345672</td>
<td>0.038408</td>
<td>17.22872</td>
<td>0.0030*</td>
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<tr>
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<td>Residual</td>
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<td>0.002229</td>
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<td></td>
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<tr>
<td></td>
<td>3</td>
<td>Lack of Fit</td>
<td>0.008743</td>
<td>0.002914</td>
<td>2.425472</td>
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</tr>
<tr>
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<td>Pure Error</td>
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<td>0.001202</td>
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</tr>
<tr>
<td></td>
<td>14</td>
<td>Cor Total</td>
<td>0.356818</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BD</td>
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<td>Model</td>
<td>0.329552</td>
<td>0.036617</td>
<td>18.19023</td>
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<td>Cor Total</td>
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<tr>
<td>WAI</td>
<td>9</td>
<td>Model</td>
<td>4.071482</td>
<td>0.452387</td>
<td>11.56619</td>
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<td>Residual</td>
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<td>Cor Total</td>
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</tr>
</tbody>
</table>

Where: *Significant at *P < 0.05, df, degrees of freedom; ER, Expansion ratio; BD, bulk density; WAI, water absorption index; WSI, water solubility index
Appendix 2 post ANOVA tables for physical and functional properties

Appendix 2: Post ANOVA statistics for ER, BD, WAI and WSI

<table>
<thead>
<tr>
<th>Regression</th>
<th>Response Variables</th>
<th>ER</th>
<th>BD</th>
<th>WAI</th>
<th>WSI</th>
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<tr>
<td>C.V. %</td>
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<td>R-Squared</td>
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<tr>
<td>Pred R-Squared</td>
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</table>

Where - ER, expansion ratio; BD, bulk density; WAI, water absorption index; WSI, water solubility
Appendix 3 Analysis of variance for proximate composition of maize-lupin blend snack

Appendix 3:1 Analysis of variance (ANOVA) for product moisture content, protein, fat, fiber, crude ash, and total carbohydrate

<table>
<thead>
<tr>
<th>Response</th>
<th>df</th>
<th>source</th>
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<th>Mean squares</th>
<th>F value</th>
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<td>Lack of Fit</td>
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</table>

*Significant at P<0.05, df: degrees of freedom; CHO, carbohydrate
Appendix 4 post ANOVA tables for proximate composition

Appendix 4:1 Post ANOVA statistics for product Moisture, Protein, Fat, Fiber, Ash and Carbohydrate content

<table>
<thead>
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<th>Regression</th>
<th>Response Variables</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Fiber</th>
<th>Ash</th>
<th>Total CHO</th>
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<tr>
<td>C.V. %</td>
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</table>

*C.V.%; CHO, carbohydrate*
Appendix 5 Sensory analysis score card

Hedonic Rating Scale for Extruded Products

Panelist Name: ______________________________

In front of you there are 16 samples of extruded product. Please evaluate and rate the samples for each attribute. Starting in any order, choose a sample, taste the sample and **Put the number values how much you like or dislike each of the sensory characteristics.** You can taste the samples more than ones. Proceed through the other samples in a similar manner, rinsing your mouth between each.

<table>
<thead>
<tr>
<th>Sensory Characteristics</th>
<th>Samples code</th>
</tr>
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<tbody>
<tr>
<td>Color</td>
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<tr>
<td>Flavor</td>
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</tr>
<tr>
<td>Texture</td>
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</tr>
<tr>
<td>Taste</td>
<td></td>
</tr>
<tr>
<td>Overall Acceptability</td>
<td></td>
</tr>
</tbody>
</table>

1 = Extremely Dislike     6 = Slightly Like
2 = Dislike Very Much     7 = Moderately Like
3 = Moderately Dislike    8 = Like Very Much
4 = Slightly Dislike      9 = Extremely Like
5 = Neither Like nor Dislike

Any additional comments

______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________

Thank you