
Addis Ababa University
School of Graduate Studies
Center for Food Science and Nutrition



Physicochemical and Nutritional Evaluation of Extruded Complementary Food Developed
from Low Tannin White Sorghum and Chickpea Blends

Thesis

By: - Adil Ibrahim

Advisors: - Ato Kelbesa Urga

Ato Tilahun Bekele

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**PHYSICOCHEMICAL AND NUTRITIONAL EVALUATION OF
EXTRUDED COMPLEMENTARY FOOD DEVELOPED FROM LOW
TANNIN WHITE SORGHUM AND CHICKPEA BLENDS**

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By

Adil Ibrahim

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**SCHOOL OF GRADUATE STUDIES
ADDIS ABABA UNIVERSITY**

As Thesis research advisors, we hereby certify that we have read and evaluated this Thesis prepared, under our guidance, by Adil Ibrahim entitled: **Physicochemical and Nutritional Evaluation of Complementary Food Developed from Low Tannin White Sorghum and Chickpea Blends**. We recommend that it be accepted as fulfilling the Thesis requirement.

Mr Kelbesa Urga ----- -----
Major-Advisor Signature Date

Mr Tilahun Bekele ----- -----
Co-advisor Signature Date

As members of the Examining Board of the Final MSc Open Defense, we certify that we have read and evaluated the Thesis prepared by Adil Ibrahim and recommend that it be accepted as fulfilling the Thesis requirement for the Degree of Master of Science in Food Science and Nutrition.

----- ----- -----
Name of Chairman Signature Date

----- ----- -----
Name of Internal Examiner Signature Date

----- ----- -----
Name of External Examiner Signature Date

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BD	Bulk Density
BR	Blend Ratio
DMRT	Duncan Multiple Range Test
ER	Expansion Ratio
H	Hardness
HTST	High Temperature Short Time
SAS	Statistical Analysis System
SL	Specific Length
WAI	Water Absorption Index
WSI	Water Solubility Index

ABSTRACT

Physicochemical and nutritional evaluation of extruded complementary food developed from low tannin white sorghum and chickpea flours were studied. Sorghum and chickpea blending were designed to meet the required amount of protein from complementary foods for older infant and young children. Extrusion was performed using a pilot scale co-rotating twin-screw food extruder. One way analysis of variance were used to analyze effect of sorghum and chickpea flours blending ratio on extrudates compositions (moisture, crude protein, fat, ash, fiber and carbohydrate), specific length (SL), diametric expansion (DE), bulk density (BD), hardness (H), water absorption index (WAI), water solubility index (WSI), antinutritional factors (condensed tannin and phytate), viscosity of the gruel and sensory properties(color, flavor, taste, texture (mouth feel) and overall acceptability of the gruel). Increasing the level of chickpea flour addition to sorghum flour from 0-30% resulted in a significant increase ($P<0.05$) in moisture, crude protein, fat, ash, Ca and Zn content but decrease in carbohydrate and fiber content. Chickpea flour level showed a significant effect ($P<0.05$) on product properties. Increasing level chickpea flour from 0-30% resulted in increased BD, H, SL and WAI from 0.39 to 0.50g/cm³, 12.09 to 44.78N, 1.10 to 1.22cm/g and 4.14 to 6.91, respectively, while decrease in WSI and DE from 7.55 to 5.01% and 1.73 to 1.45cm/cm respectively. Increasing the level of chickpea flour addition to sorghum flour from 0-30% resulted in a significant decrease ($P<0.05$) on viscosity of the gruel (from 4085 to 1750cP) made from extrudates flour at 20% dry matter concentration. Extrusion cooking shows a significant reduction on tannin and phytate content of extrudates. Extrusion cooking reduced 27 to 29% of phytate and 90-91% tannin content of extrudates, when increasing the level of chickpea flour from 0 to 30 %. Chickpea flour level showed a significant decrease ($P\leq 0.05$) on phytate content but did not affect tannin content. The sensory evaluation for color, flavor and taste of the gruel prepared from extrudates at 20% dry matter revealed that significant differences ($P<0.05$) exist between 0% chickpea flour level and increasing chickpea flour level from 10-30% and there is no significant difference between 10, 20 and 30% chickpea flour level. Texture (mouth feel) and overall acceptability of the gruel has no significant difference by panelists increasing chickpea flour level from 0-30%. It was also observed that all products have a mean value of 6.33 for taste, 6.50 for color, 6.35 for flavor, 6.98 for texture (mouth feel) and 6.98 for overall acceptability on 9 point hedonic scale. This mean sensory score indicates that all the extrudates were well liked by some degree variations by the panelists. Extruded weaning food products from sorghum and chickpea flour blend provide gruels of low viscosity and antinutrient content and high energy and nutrients density, therefore, will increase food intake and bioavailability of nutrients. A serving of 100g of sorghum and chickpea flour blend extrudates produced from 10, 20 and 30% chickpea flour proportion could provide about 88,100 and >100% of protein and 88, 89 and 90% Of the total energy respectively recommended for complementary foods need to have for infants and young children.

1 INTRODUCTION

1.1 Background

Malnutrition and infectious diseases are the most widespread problems affecting infants and young children in developing countries (Mamiro *et al.*, 2005; Ouedraogo *et al.*, 2008). The importance of nutrition as a foundation for health development is underestimated. Poor nutrition leads to ill health and ill health causes further deterioration in nutritional status. Analyses indicate that as much as one half of under-five child mortality is associated with malnutrition (WHO, 2009).

Breast milk is the best and safest food for young babies. It maintains optimum growth up to the age of 4-6 months (Lutter and Rivera, 2003). Infants and young children are at increased risk of malnutrition from six months of age onwards, when breast milk alone is no longer sufficient to meet all nutritional requirements (Lutter and Rivera, 2003).

Adequate nutrition and health during the first several years of life is fundamental for child survival and the prevention of malnutrition (Lutter and Rivera, 2003). The formulation and development of nutritious complementary foods from local and readily available raw materials has received considerable attention in many developing countries. This is particularly important in countries where malnutrition is still common. In African countries, traditional foods used during the complementary feeding are frequently characterized by low nutrient density and high bulk, which can poorly affect infants' health (Omer *et al.*, 1975). Commonly-used complementary foods are prepared from flours of starchy staples, cereals and legumes, such as rice, millet, sorghum, or maize (rarely wheat). Cereals and legumes are the most common first complementary foods. Gruels/thin porridges prepared from cereals and legumes play an important role as a complementary food but their nutrient density is often low, especially deficient in essential nutrients. Cereals have low content of proteins and fat while legumes are low in fat. The presence of high concentration of crude fiber and antinutritional factors like phytic acid and condensed tannin is major factors reducing their nutritional

benefits (Hurrell *et al.*, 2003; Lalude and Fashakin. 2006; Stephenson *et al.*, 1994; Wharton 1994)

Sorghum (*Sorghum bicolor* L. Moench) is an important cereal crop grown in the semi-arid tropics of Africa and Asia due to its drought tolerance (Murty and Kumar, 1995). Sorghum ranks fifth among the world cereals, following wheat, maize, rice and barley in production area and total production (Dillon *et al.*, 2007). Sorghum grain has a proximate composition of about 7-15% proteins, 73% carbohydrate, 1.5% to 6% fat, 2.3% crude fiber and 1.6% ash (Dicko *et al.*, 2006; Leder, 2004). Sorghum is unique among cereals because some cultivars produce polymeric polyphenols known as tannins (Butler, 1990).

The food uses of sorghum are still mostly traditional and the methods of processing may involve the use of wet or dry processing. Porridges appear to be the most common types of food prepared from sorghum in Africa and Asia. Flat breads, popped snacks and alcoholic beverages are also produced from sorghum (Dicko *et al.*, 2006; Taylor *et al.*, 1997). In Ethiopia, sorghum flour is used to feed infants in the form of thick and thin porridge (Dicko *et al.*, 2006) and about 8 percent of the production is consumed as *injera*, pancake-like fluffy soft bread which is a staple food (Gebrekidan and Gebrethiwat, 1982). Beside the use of sorghum in making traditional foods, it is also used in the production of extruded products (Pelembé *et al.*, 2002).

Cereals are generally inadequate in supplying certain amino acids such as lysine and tryptophan; on the other hand legumes are inadequate in supplying sulphur-containing amino acids such as methionine and cysteine (Fashakin *et al.*, 1986; Ikujenlola and Fashakin, 2005; Marero *et al.*, 1988). Therefore cereal-legume blends are relatively high in protein (both quality and quantity) and energy because the legumes supply the lysine and tryptophan lacking in cereal and the cereals provide cysteine and methionine which are low in legumes (Annan and Plahar, 1995). Supplementation of sorghum with legumes has been advocated as a way of combating Protein-Calorie Malnutrition (Singh, 1991).

Chickpea (*Cicer arietinum* L.) is an important and cheap source of vegetable protein which can be used as a substitute for animal protein and greatly contribute to the human diet in several developing countries. Chickpea seed contains approximately 20-30% protein, 50-60% carbohydrate and 3-6% oil and are a rich source of minerals (Ibrikci *et al.*, 2003). The protein content of chickpea seeds is highly variable and determined by both genetic and environmental factors (Chavan *et al.*, 1986). Protein content in *desi* chickpea significantly varies as percentage of the total dry seed mass before (17-22%) and after (25.3-28.9%) dehulling (Badshah *et al.*, 2003; Hulse, 1991). There are two main commercially available types of chickpea grown in the world: the *desi* and the *kabuli* chickpea. *Desi* chickpea seed is small with a dark irregular-shaped seed coat whereas *kabuli* chickpeas have a larger cream-colored seed with a thin seed coat (Moreno & Cubero, 1978).

Extrusion cooking is a high temperature, short time process in which food material is cooked through the unique combination of moisture, pressure, temperature, and mechanical shear (Harper, 1981). These unique operations causes large numbers of complex changes to the food including; hydration of starches and proteins, homogenization, gelation, shearing, melting of fats, denaturation or re-orientation of proteins, plasticization and expansion of the food structure (Harper, 1981). Extrusion technology also causes considerable viscosity reduction in cereal gruels and enhances its nutrient densities (Anderson *et al.* 1969; De Muelenaere, 1989).

Extrusion cooking offers several advantages over other types of cooking processes, such as faster processing times and lower energy consumption, which consequently results in lower prices for the final products. Due to that products of extrusion are of major importance in the food and feed industries today (Wiedemann and Strobel 1987). Nutritional concern about extrusion cooking is reached at its highest level when extrusion is used specifically to produce nutritionally balanced or enriched foods, like complementary foods, dietetic foods, and meat replacers (Wiedemann and Strobel, 1987).

1.1. Statement of the Problem

In the continuous search for solution to the problem of malnutrition mainly among children of the developing countries including Ethiopia, there is need to improve the nutritive quality of locally produced foods through better processing and enrichment.

Infant malnutrition due to nutritionally inadequate diets is one of the major concerns in Ethiopia. The high cost of complementary foods, vegetables, and animal protein, together with the unavailability of nutritious foods, adds to the difficulty of providing good nutrition to infants (Asma *et al.*, 2006). Children in rural Ethiopia are especially prone to nutrient deficiencies as they eat from the family dish, which is mostly cereal (Temple *et al.*, 1996). Due to its drought tolerance and cheap cost sorghum is eaten in areas where the populations are frequently undernourished, therefore it is important to consider the quality, quantity and availability of the nutrients in the grain. The protein of sorghum, like most other cereals, is low in quantity and deficient in the essential amino acid lysine (Murty and Renard, 2001). Furthermore poor digestibility of sorghum protein, unlike other cereals, is the major problem when the grain is wet cooked as is common in porridge preparation (Duodu *et al.*, 2002).

Legumes play an important role in the agriculture and diet of many developing countries and are a major source of dietary nutrients for many people. Chickpea is a cheap source of high quantity protein in the diets of millions in developing countries, who cannot afford animal protein for balanced nutrition (Huisman and Van der Poel, 1994). In addition to proteins, it is low in fat and sodium, cholesterol free and is an excellent source of both soluble and insoluble fiber, complex carbohydrates, vitamins and minerals (Nwokolo and Smartt, 1996).

It is well known that the addition of legumes to cereals results in an increase of both quantity and quality of the protein mix. Chickpea has relatively high protein content with the concentration of lysine and tryptophan. The concentration of these two amino acids is relatively low in most cereal grains including sorghum. However, the amino acid profile of chickpea protein complements the amino acid profile of cereal grains because of the relatively high concentrations of lysine and tryptophan. It therefore, emphasized that legume seed grain

proteins are the natural supplement to cereal grain protein in producing and overall essential amino acid balance (Annan and Plahar, 1995) and has been advocated as a way of fighting Protein-Calorie Malnutrition (Singh, 1991)

Therefore in this project complementary food developed from low tannin sorghum flour supplemented with chickpea flour were extruded to investigate the nutritional, physical property, mineral content and sensory attributes.

1.2. Objective of the Study

1.2.1. General objective

- To develop extruded complementary food product from low tannin flours of white sorghum and chickpea flour and to evaluate the physical and nutritional value of the product.

1.2.2. Specific objective

- To study the importance of blending ratio on functional and sensory properties of extruded products developed from low tannin flour.
- To evaluate the anti-nutritional factors of extruded products.
- To evaluate the mineral content of extruded product.

2. LITERATURE REVIEW

2.1. Complementary Food and Feeding

Child malnutrition remains a common problem in developing countries. Early growth retardation is associated with a broad range of adverse functional consequences, including delayed physical development and impaired cognitive function and school performance; malnourished children have a higher risk of infection, ill-health and death. According to Onis *et al.* (1993) the World Health Organization (WHO) defined malnutrition as “the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance and specific functions.”

Scientifically, it has been proven that breast milk is the perfect food for the growing infant during the first six months of life. It contains all the nutrient and immunological factors an infant's requires to maintain optimal health and growth (Lutter and Rivera, 2003). Breast milk maintains optimum growth up to the age of 4-6 months, thereafter faltering of growth occurs in most children. Hence, it is important that babies be given additional foods, along with breast milk at the right age and in sufficient amounts to enable them to grow and stay healthy (Lutter and Rivera, 2003).

The stage of life when foods and/or liquid milks are fed to infants and young children in addition to breast milk is known as the period of complementary feeding; non-breast-milk food items consumed at this time are defined as complementary foods (Kenneth, 1997). At this period, infants like to put things in their mouths and are interested in new tastes. The structure of the mouth in the infant is well developed to make chewing and grinding movements, teeth outburst occurs, intestinal amylase increases and the tongue reaction which pushes out non-liquid food disappears, so the infant is ready to accept semi-solid foods. Mothers also understand that babies are not satisfied with breast milk alone because frequently cry after feed (Kenneth, 1997).

The transition from exclusive breastfeeding to complementary food is a very vulnerable period. For the reason that at this stage the nutritional requirements of many infants are not met, thus leading to the beginning of malnutrition, this is prevalent in children under five years of age worldwide (Anigo *et al.*, 2009; Daelmans and Saadeh, 2003). The amount of nutrients that older infants and young children have to get from complementary foods according Codex alimentarius: Guidelines on Formulated supplementary foods for older infants and young children is shown on Table 1 below (CODEX CAC/GL 08. 1991).

Table 1. Guidelines on Formulated supplementary foods for older infants and young children

Contents	Recommended value
Protein (%)	≥15
Fat (%)	10-25
Ash (%)	≤3
Fiber (%)	≤5
CHO (%)	64±4
Energy (kcal)	400-425
Zn (mg/100g)	3.2
Ca (mg/100g)	500
Fe (mg/100g)	16

Source: CODEX CAC/GL 08. (1991)

Grantham-McGregor *et al.* (2007) suggested that poor nutrition during infancy is likely to lead to poor academic achievement, low incomes in adulthood and inadequate care for the children of subsequent generations. This cycle has contributed to the inter-generational poverty of low income countries in South Asia and Sub-Saharan Africa (Walker *et al.*, 2007).

WHO (2009) recommends that infants start receiving complementary foods at 6 months of age in addition to breast milk, initially 2-3 times a day between 6-8 months, increasing to 3-4 times daily between 9-11 months and 12-24 months with additional nutritious snacks offered 1-2 times per day, as desired. The traditional complementary foods given to young children in most developing countries are based on local staples, usually cereals. Cereals are important sources of energy in human diets. Although carbohydrates are their main dietary contribution, they provide protein and smaller amount of lipid, fiber and vitamins. It is commonly known that the main nutritional disadvantage to cereals, particularly sorghum (*Sorghum bicolor*), is their low protein content and the limited biological quality of their protein (highly deficient in

lysine and tryptophan) compared to animal protein and can be improved by combining it with other rich sources of protein (Mertz, 1970; Ortega *et al.*, 1986; Waliszewski *et al.*, 2000).

Onilude *et al.*(1999) indicated that, cereals, in order to be suitable for the feeding of young children are prepared in liquid form by diluting with a large quantity of water, thereby resulting in a large volume with low energy and nutrient density. If the concentration of solids is raised to increase the nutrient and energy density, the gruel will be too thick and viscous for an infant to eat easily. This high viscosity characteristic is referred to as dietary bulk. Various attempts have been tried to modify the starch structure to reduce bulk. These include the use of enzymes, precooking, fermentation, toasting, malting, puffing and extrusion processing of the raw materials. (Onilude *et al.*, 1999)

2.2. Production, Composition and Consumption Pattern of Sorghum and Chickpea

2.2.1. Production, composition and consumption pattern of sorghum

Doggett (1988) suggested that sorghum is domesticated and originated in the North-East quadrant of Africa, most likely in the Ethiopian-Sudan border. The presence of wild sorghums and their cultivated forms and their ecotype differentiation of sorghum into different races and their presence in different parts of the country supports that Ethiopia is one of the center of origin and diversity for sorghum. Sorghum is produced in Ethiopia on an area of 1,550,000 ha with a total production of 2,600,000 Mt and worldwide on 41,090,000 ha with a total production of 58,884,425 Mt (USDA, 2010).

The sorghum kernel is a naked caryopsis which is roughly spherical in shape and is typically 2-5mm thick at the widest point (Belton and Tyler, 2002). It is composed of three major anatomical parts, which are, the pericarp, germ and endosperm (Belton and Tyler, 2002). In general, the endosperm represents 85% of the whole grain, the germ 9%, and the pericarp only 6% (Haikerwal and Mathienson, 1971). Some varieties have a thin layer of cells underneath the pericarp called testa. This layer may contain tannins, which are phenolic compounds similar with those in fruits and red wine. The pericarp can be white, red, or yellow, and the

endosperm can be white or yellow. Pericarp thickness can vary from very thin (8 µm) to very thick (160 µm). Pericarp color and thickness, endosperm color, and presence or absence of testa determines the grain color (Rooney and Miller, 1981).

Sorghum grain has a proximate composition of about 7-15% proteins, 73% carbohydrate, 1.5-6% fat, 2.3% crude fiber and 1.6% ash (Dicko *et al.*, 2006; Leder, 2004). Sorghum is an important source of B vitamins except B12, and good source of tocopherols (Leder, 2004). The fat in sorghum grain (mainly present in the germ) is rich in polyunsaturated fatty acids. Sorghum fat has a fatty acid composition of about 49% linoleic acid, 31% oleic, 14% palmitic, 2.7% linolenic and 2.1% stearic (Dicko *et al.*, 2006).

Sorghum has some limitations, due to the presence of antinutritional factors, such as trypsin and amylase inhibitors, phytic acid, and tannins. These compounds are known to interfere with protein, carbohydrates and mineral metabolism. Removal of these undesirable components is essential to improve the nutritional quality of sorghum and effectively utilize its potential as human food or animal feed (Kumar *et al.*, 2010; Soetan and Oyewole, 2009).

Sorghum is consumed in a variety of forms that vary from region to region. In Africa, generally, it is consumed as flat bread which is mostly unleavened and prepared from fermented or unfermented dough, thin or thick fermented or unfermented porridge, boiled products similar to those prepared from maize grits or rice and fermented beverage (Leder, 2004). In Ethiopia, the milled sorghum is used for making fermented products, such as *injera* (Gebrekidan and Gebrettiwat, 1982), and for the preparation of malted and fermented beverages or sorghum beer such as *tella*. It is also used for preparation of non-fermented foods like *kitta* (flat bread), *laffiso* (pieces of sorghum injera with spices), *genfo* (porridge), *niffro* (boiled sorghum grain), and *kollo* (roasted sorghum grain). The food industry in Southern Africa has also been exploring the use of sorghum in the production of ready-to-eat products, using extrusion cooking technology and gun-puffing.

2.2.2. Production, composition and consumption pattern of chickpea

Chickpea (*Cicer arietinum*L.) is an important and cheap source of vegetable protein which can be used as a substitute for animal protein and greatly contribute to the human diet in several developing countries (Huisman and VanderPoel, 1994). In Ethiopia, the earliest finding of chickpea is reported in 1520 BC (Joshi *et al.*, 2001). Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent's production during 1994-2006. It is also the seventh largest producer worldwide and contributes about 2% to the total world chickpea production (FAO, 2008)

In Ethiopia, chickpea is widely grown across the country and serves to reduce malnutrition and improve human health especially for the poor who cannot afford animal products. Chickpea seed contains between 14.9 and 30.6% crude protein and is highly variable and determined by both genetic and environmental factors (Chavan *et al.*, 1986). Chickpea is cholesterol free and is a good source of dietary fiber, vitamins and minerals (Wood and Grusak, 2007)

There are two types of chickpea produced globally, namely *desi* and *kabuli* chickpeas. *Kabuli* chickpeas have a larger cream-colored seed with a thin seed coat whereas the *desi* type has a smaller, reddish brown-colored seed with a thick seed coat (Moreno and Cubero, 1978). The seed weight generally ranges from 0.1 to 0.3g and 0.2 to 0.6g in *desi* and *kabuli* types respectively (Frimpong *et al.*, 2009). On average, world production consists of about 75% of *desi* and 25% of *Kabuli* types (Agricultural and Agri-food Canada, 2004). While *Desi* chickpea has been widely grown in Ethiopia for household food needs and domestic markets, production of *Kabuli* types is a fairly recent practice. It is grown at the end of the main rainy season using residual soil moisture. This allows farmers to practice double cropping, which in turn increases productivity of scarce land resource and serves as an additional source of income (Joshi *et al.*, 2001)

Globally, chickpea is mostly consumed as a seed food in several different forms and preparations are determined by ethnic and regional factors (Muehlbauer and Tullu, 1997;

Ibrikci *et al.*, 2003). Especially in Asia and Africa chickpea is used in stews, soups/salads and consumed in roasted, boiled, salted and fermented forms (Gecit, 1991). Chickpea, locally known as *shimbra* is widely used in different forms as green vegetable (green immature seed), ‘*Kollo*’ (soaked and roasted) ‘*nifro*’ (boiled seeds) and ‘*wot*’ (sauces) made up of ‘*shiro*’ (powdered seeds) etc. These different forms of consumption provide consumers with valuable nutrition and potential health benefits. The traditional processing practices used to convert chickpea into consumable forms include soaking, fermentation, boiling, roasting and others. In all the forms, it may be consumed alone or mixed with cereals.

2.3. Anti-Nutritional Factors

2.2.1. Anti-nutritional factors in sorghum grain

Phytic acid (myo-inositol hexaphosphoric acid) usually occurs in seeds as mixed potassium, magnesium and calcium salts (phytins or phytate), is also associated with the bran (pericarp) of cereals. The phytate molecule is highly charged with six phosphate groups and so is an excellent chelator, forming insoluble complexes with mineral cations and proteins (Ryden and Selvendran, 1993).

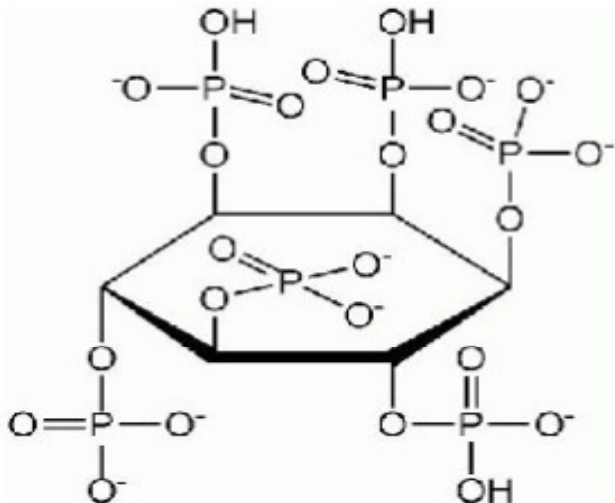


Figure 2. Structure of Phytate empirical formula= $C_6P_6O_{24}H_{18}$

The phytate content (0.300%- 0.886%) of sorghum is variable and appears to be dependent on cultivar and the highest phytate concentration is found in the germ. The inhibitory effect of phytate on protein digestibility has been demonstrated in experiments with casein and bovine serum albumin (Ali and Harland, 1991). It was observed that phytate significantly decreased in *in vitro* pepsin digestion of both casein and bovine serum albumin. This observation was attributed to the possible formation of a phytate-protein complex, which is less susceptible to enzymatic attack. Because most of phytic acid is located in the outer parts of the kernel the different products of milling contain different levels. Bran is the product having a high phytic acid content, low extraction white flours contain low phytic acid quantities (Knuckles *et al.*, 1985).

Sorghum is unique among cereals because some cultivars produce polymeric polyphenols known as tannins (Bulter, 1990). All sorghums contain phenolic compounds and most contain flavonoids, however, only cultivars with pigmented testa produced tannins. Serna-Saldivar and Rooney (1995) reported that most cultivated sorghum do not contain any condensed tannins. Sorghum tannins apparently occur only around the pericarp and testa layers (Awadelkareem *et al.*, 2009) and in some glumes (Doherty, 1987). The color of sorghum grain and flour play an important role in its acceptance. Brown sorghum includes various properties of tannin while the white sorghum has none of these anti-nutritional substances. Tabosa *et al.* (1995) assessed tannin content of 212 cultivars and found that 64% had low tannin below 0.25%, 12% had average tannin (0.26 to 0.5%), 9% had high tannin (0.51 to 0.75%) and 6% had very high tannin ($\geq 0.75\%$).

Tannins form complexes with proteins, starch, minerals and digestive enzymes to cause a reduction in nutritional value of foods. Tannins have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy and protein digestibility; and also negatively affect the nutrient performance of animals and the digestibility of feeds (Ebadi *et al.*, 2005; Imik *et al.*, 2008).

2.2.2. Anti-nutritional factors in chickpea

Phytate, which is also known as inositol hexakisphosphate, is a phosphorus containing compound that binds with minerals and inhibits mineral absorption. (Walter *et al.*, 2002) Phytates are generally found in food high in fiber especially in wheat bran, whole grains and legumes (Lori *et al.*, 2001).

The presence of phytate in foods has been associated with reduced mineral absorption (zinc, iron, calcium, magnesium, manganese and copper) due to the structure of phytate which has high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing non-availability for intestinal absorption (Lopez *et al.*, 2002; Walter *et al.*, 2002).

Phytate is also known to form complexes with proteins at both acidic and alkaline pH. This interaction may affect changes in protein structure that can decrease enzymatic activity, protein solubility and proteolytic digestibility. However, the significance of protein-phytate complexes in nutrition is still under inspection. Strong evidence exists that phytate-protein interactions negatively affect protein digestibility *in vitro* and the extent of this effect depends on the protein source (Cheryan, 1980).

Tannins are polyphenol components prevalent in food legumes. Studies indicated that tannins interact with proteins, enzymes or non-enzymes, and form tannin-protein complexes, which decrease protein digestibility and protein solubility (Chavan *et al.*, 1986). Polyphenols cause either inactivation of enzyme such as trypsin and chymotrypsin or make protein insoluble. Polyphenols also inhibit several enzymes including α -amylase, lipases, pectin esterases, cellulases and β -galactosidase (Salunkhe *et al.*, 1985). In addition to this, tannins reduce the bioavailability of vitamins and minerals (Chavan *et al.*, 1986).

Chickpea seeds (whole seed) contain 78 to 272 mg tannins per 100 g seeds. Tannins are mainly located in the seed coat. There is a considerable variation in seed coat color among the various chickpea cultivars. The polyphenols in cultivars, which have darker testa color, inhibit

the digestive enzyme activity more than cultivars with lighter testa color. These components impart astringent flavors, which are not always desirable. Some processing treatments such as dehulling and cooking considerably reduce the level of tannins in legume. As such, chickpea seeds with light color are preferred for whole seed consumption (Chavan *et al.*, 1986).

2.3. Minerals

2.3.1. Calcium

Adequate calcium nutrition during childhood has important implications for bone growth and development, and is thought to reduce the incidence of osteoporosis in later life (Abbo *et al.*, 2000). Calcium is the most abundant mineral element in the body. Calcium more than 99% is located in the bones, plays an important role for structure and strength of bones and calcium regulates critical functions including nerve impulses, muscle contractions and the activities of enzymes. The body of an adult man contains about 1.2 kg calcium, accounting for about 2% of body weight. The element is present in two body parts: bone and teeth. Sufficient calcium intake is essential for bone mass in youth and for minimizing bone loss future in life (Grinder-Pedersen *et al.*, 2004; Gurr, 1999). Preterm babies are particularly at risk of not being able to absorb enough calcium for optimal bone growth. Mineral deficiencies, such as calcium, are a world-wide problem particularly in developing countries (Boukari *et al.*, 2001; Gurr, 1999)

2.3.2. Iron

Iron is a micronutrient that is most often deficient in developing countries, with children and women of reproductive age especially at risk of such deficiencies (Melaku *et al.*, 2005). Dietary iron is a core element in the synthesis of haemoglobin, plays a vital role in the transport of oxygen, and myoglobin. Age, gender and physiological status determine iron requirements. Rapid growth of infants during the first year of life requires an adequate supply of iron for synthesis of blood, muscle, and other tissues. Iron requirements are especially high in infants from the age of 6 months, in young children, and in pregnant and menstruating women. The increased iron requirement of infants and pregnant women is due to rapid growth

and new tissue formation. Menstruation and parasitic diseases cause excessive iron loss, and iron utilization is impaired during chronic infection (Domellof *et al.*, 2002; WHO 2000)

Low content and bioavailability of iron in the typical cereal-based diet is a major cause of iron deficiency (Sandberg, 2002). Weaning foods made from cereals is often low in iron content and contains significant quantities of iron absorption inhibitors, phytic acid and condensed tannins (Pynaert *et al.*, 2006).

In developing countries Fe deficiency, due to poor bioavailability, retards normal brain development in infants and affects the success of pregnancy by increasing premature deliveries, as well as morbidity of mother and child at or around child birth; it affects also working capacity, thus impairing socioeconomic development as well (Sandberg, 2002; Malenganisho *et al.*, 2007). Iron deficiency is the main cause of nutritional anaemia (Melaku *et al.*, 2005). Iron deficiencies may exist in populations that consume diets with insufficient amounts of animal-source foods (Ranum, 2001). The amount of iron absorption is inversely related to the phytic acid content of flour (Brune *et al.* 1992). Phytate: iron molar ratios > 0.15 are regarded as indicative of poor iron bioavailability (Melaku *et al.*, 2005). Absorption of iron from cereals can be increased by the degradation or removal of phytic acid with a simple processing technology (Hurrell *et al.*, 2003).

2.3.3. Zinc

Zinc is the fourth important micronutrient after vitamin A, iron and iodine. Zinc is found in all organs, tissues and body fluids, especially the bones and skeletal muscles. Zinc plays vital role in cell division, protein synthesis and growth which makes infants, children, adolescents, and pregnant women at risk for an inadequate zinc intake. Zinc contributes to reproduction, growth, taste, night vision, appetite, and the immune system functioning. Zinc also contributes to stabilize the structure of membranes and cellular components (Hambidge, 1997). Zinc nutritional status influences the absorption, transport and utilization of vitamin A. The enzyme that plays a major role in the oxidative conversion of retinol to retinal is zinc dependent, and may be adversely affected in zinc deficiency (Adeyeye *et al.*, 2000).

Zinc deficiency is a public health problem, and is associated with poor growth, decreased immune function, increased susceptibility to and severity of infections, adverse outcomes of pregnancy, and neurobehavioral abnormalities (Melaku *et al.*, 2005; Sandberg 2002). In many developing countries, zinc deficiency is due to the low consumption of animal source foods, which are rich in zinc, and a high intake of cereals and legumes, which contain substantial amounts of phytate (Romana *et al.*, 2003). Phytate-zinc molar ratio is used to estimate the likely absorption of zinc from a mixed diet (Melaku *et al.*, 2005; Walingo, 2009). Diets with a phytate-zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with phytate-zinc molar ratio between 5 and 15 have medium zinc bioavailability and those with a phytate-zinc molar ratio less than 5 have relatively good zinc bioavailability (Walingo, 2009).

2.4. Extrusion Cooking Technology

Extrusion cooking is a high temperature, short time process in which food material is cooked by a combination of moisture, pressure, temperature, and mechanical shear (Harper 1981). A (high temperature, short time) (HTST) procedure is one which uses raw material for the short residence time, high temperature, high pressure, large shear forces and intensive mixing (Zheng& Wang 1994). The process combines several unit operations including mixing, cooking, kneading, shearing, shaping, and forming. These unique operations causes large numbers of complex changes to the food including; hydration of starches and proteins, homogenization, gelation, shearing, melting of fats, denaturation or re-orientation of proteins, plasticization and expansion of the food structure (Frame, 1994). When solids and liquids are fed into the extruder, shear and kneading energy is imparted by specifically designed screws. On passing through the extrusion dies a drop in pressure causes vaporization of water, leading to product expansion. The shaped material foams and a porous structure are formed. A rotating knife cuts the shaped product. Using the extruder, raw materials can be converted, as required, into semi-cooked or finished products in 30–60 seconds (Frame, 1994).

The most basic extruder consists of a barrel with a screw fitted inside, which forces the materials fed at one end towards the other side. The material is forced to exit the chamber through a die with small holes, located at the far end. Extruders are classified according to the

method of construction as single- or twin-screw extruders. The least versatile, most simple and inexpensive to acquire is the single screw extruder (Gonzalez, 2005).

Twin-screw extruders found more and more applications in food processing because they offer high shear, high pressure and high temperature (O'Conner, 1987), and greater flexibility in controlling both, product and process parameters (Ilo *et al*, 2000). Twin screw extruders are classified according to the direction of rotation and the way in which the screws join as co-rotating and counter rotating types. In counter-rotating position, the extruder screw rotates in the opposite direction where as in the co-rotating position the screw rotates in the same direction. Twin-screw machines handle oily, sticky or very wet materials, or other products that slip in a single screw. Single screw extruders can process lipid levels of 12–17%, while twin screw extruders with proper screw configurations can handle feed lipid contents as high as 22% (Riaz, 2001).

2.5. Extruded Products

Extrusion cooked products are of major importance in the food and feed industries today. Extruders can be used for a wide range of traditional (conventional) food products, as well as in the production of numerous new products (cereal baby food, confectionery, breakfast cereals, snack foods, bakery products, flavors, pastas, pet food and meat products) (Wiedemann & Strobel 1987).

Parallel to the increased applications, interest has grown in the physico-chemical, functional and nutritionally relevant effects of extrusion processing. It significantly reduces antinutrients present in legumes (Abd El Hady and Habiba 2003; Alonso *et al*. 2000; Leontowicz *et al*. 1999; Marzo *et al*. 2002). And also, extrusion technology causes considerable viscosity reduction in cereal gruels and enhances its nutrient densities (Anderson *et al*. 1969; De Muelenaere 1989). Extrudates are microbiologically safe, can be stored for long periods because of low moisture without need for refrigeration and requires less effort for handling and less packaging materials and storage space (Filli and Nkama, 2007).

High temperature, short-time (HTST) extrusion cooking could be used to produce sorghum-based foods of high nutritional quality and in a ready-to-eat form. Much important research work on the extrusion cooking of cereals has been reported (Anderson *et al.*, 1969 ; Mercier & Feillet, 1975; Gomez & Aguilera, 1983, 1984; Björck *et al.*, 1984; Ilo *et al.*, 1996), and on the extrusion of cereal–legume composites (Maga & Lorenz, 1978; Almeida-Dominguez *et al.*, 1983; Colonna & Mercier, 1985; Falcone & Phillips, 1988) but nothing has been reported on extrusion cooking of sorghum–chickpea composites for production of ready-to-eat (instant) gruel.

2.6. Extruded Product Quality Attributes

Extruded foods are composed mainly of cereals, starches, and/or vegetable proteins. The major role of these ingredients is to give nutrients, structure, texture, mouth feel, bulkiness, and many other characteristics desired for specific finished products (Jamora *et al.*, 2002).

Water absorption index (WAI) and water solubility index (WSI), functional properties of extruded products are used as parameters for the degree of cooking of cereal products. The water absorption index (WAI), an indicator of the ability of flour to absorb water, depends on the availability of hydrophilic groups which bind water molecules and on the gel-forming capacity of macromolecules (Narbutaite *et al.*, 2008). Only gelatinized starch granules absorb water at room temperature and swell; however, starch fragmentation increases when the gelatinization degree increases, decreasing thus water absorption (Leonel *et al.*, 2009). The water solubility index (WSI) describes the rate and extent to which the component of powder material or particles dissolves in water. It depends mainly on the chemical composition of the powder and its physical state. The WSI often is used as an indicator of degradation of molecular components (Valencia *et al.*, 2009; Yu *et al.*, 2009), measures the degree of starch conversion during extrusion which is the amount of soluble polysaccharide released from the starch component after extrusion.

Expansion ratio (ER), bulk density (BD) and texture are an important quality parameter in products like breakfast cereals and ready-to-eat snack foods. In products intended for further

cooking, this may not be important; in fact, large ER, which promotes increased porosity, may result in softer texture in cooked products. Hence, choosing the optimal level depends on the intended product.

Expansion is the formation of aerated internal structure when melted feed material at high temperature and pressure suddenly release the pressure and super-heated water flashed off at the die of the extruder (Harper, 1981), and both ER and BD, represent the extent of puffing of the extrudates, depends mainly on the degree of gelatinization (Yu *et al.*, 2009). Therefore, it might be expected that these two properties would be negatively correlated, with higher ER contributing to lower BD, but Park *et al.* (1993) reported that this is not always the case. The reason could be that ER only considers the expansion in the radial direction, perpendicular to extrudates flow, whereas BD considers the expansion in all directions (Falcone and Phillips 1988).

Several researches demonstrated the inverse relationship between ER and BD (Yu *et al.*, 2009; Balasubramanian *et al.*, 2010; Sadik, 2010). Although feedback control of such attributes is difficult due to lack of sensors, knowing the dependence of such product attributes on processing conditions will help maintain product quality. Product bulk density is a function of the extrudate temperature and moisture entering the die (Kirby *et al.*, 1989). Thus, bulk density might be maintained by monitoring and controlling process temperature at constant moisture. Several authors have cited that lower feed moisture contents and higher barrel temperature favored the expansion of materials such as corn and whey protein concentrate (Onwulata *et al.*, 2001); Mulberry leaves (Charunuch *et al.*, 2008); cassava starch (Leonel *et al.*, 2009); teff and soybean flour (Sadik, 2010)

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 attributes (Pamies *et al.*, 2000). Several researchers agree that crispness should result from the structural properties of a food (Bouvier *et al.*, 1997).

According to Heidenreich *et al.* (2004), crispness is perceived through a combination of tactile, kinesthetic, visual and auditory sensations and represents the key texture attributes of dry snack products. Moreover, crispness is related with rapid drop of force during mastication process, attribute that is based on fracture propagation in brittle materials. Parameters regarding crispness, crackliness and hardness are the most studied, often indicating a good relationship between human perception and instrumental analysis of texture (Veronica *et al.*, 2006).

Manoh *et al.* (1978) reported a loss of color in the extrusion of corn grits. Good color must be maintained when extruded products are to be directly consumed without further processing. Predictability of discoloration during extrusion and its control are necessary for product quality assurance.

3. MATERIAL AND METHODS

3.3. Experimental Location

The experiment on the extrusion process and determination of physical properties of products were conducted at the Department of Food Technology and Process Engineering, Bahir Dar University, Bahir Dar. Determination of proximate composition of the raw materials and extrudates, functional properties (WAI and WSI) and antinutritional factors of extrudates were conducted at Center for Food Science and Nutrition Laboratory, Addis Ababa University. Determination of crude fiber composition was conducted in Ethiopian Health and Nutrition Research Institute (EHNRI) Laboratory. Sensory evaluation of the gruel was conducted using staffs and mothers who came at Ethio-Tebib hospital. Viscosity of the gruel and Instrumental texture test of the extrudates was conducted at Hawassa University; School of Nutrition, food science and post-harvest technology department.

3.4. Experimental Materials

White Sorghum grain variety (Teshale) was obtained from Melkassa Agricultural Research Center (MARC) and the kabuli chickpea grain variety (Harbu) was obtained from Debire Zeit Agricultural Research Center.

3.5. Raw Material Preparation

3.5.1. Sorghum flour preparation

Before milling, the sorghum grain was cleaned for physical impurities. About 20% water was added to temper the grain to a moisture content of 16% (Taylor & Dewar, 2001). Water is applied to toughen the pericarp and soften the endosperm, hence easing the separation of these tissues. Then the grain was mechanically dehulled using mortar and pestle to remove the bran. After dehulling the sorghum grains were sun dried and manually winnowed to separate the bran, and milled using a stone mill. The flour was then sifted to pass through 480 µm test

sieve, sealed in plastic bags and stored at room temperature until further analysis (Perez *et al.* 2008).

3.5.2. Chickpea flour preparation

Chickpea seeds were cleaned by hand-sorting to remove stones, dust, and light materials, undersized and immature seeds. Then the cleaned chickpea seeds were soaked in distilled water for 9 hrs. The proportion of seed to soaking medium was 1:3 w/v. Then soaking water was drained, and the seeds were dried at 60°C in an oven for 12 h, then dehulled and finally ground in a mill. The flour was preserved under low temperature (4°C) until further analysis (Khalil *et al.*, 2007).

3.6. Complementary Food Blending

Based on protein content of the starting, sorghum and chickpea flours were blended in order to meet the recommended amount of protein that older infants and young children have to get from complementary foods according Codex alimentarius: Guidelines on Formulated supplementary foods for older infants and young children (CODEX CAC/GL 08. 1991). The blending proportions of the four complementary foods were in the ratios of 100:0; 90:10; 80:20 and 70:30 (w/w) of white sorghum to Chickpea flour. Then each blend was extruded in three replications.

Table 2. Blending proportion

Replication	C1	C2	C3	C4
R1	C1R1	C2R1	C3R1	C4R1
R2	C1R2	C2R2	C3R2	C4R2
R3	C1R3	C2R3	C3R3	C4R3

Where, C = Chickpea flour supplementation level (C1, C2, C3 and C4 are 0, 10, 20 and 30% respectively)

R = Replication

3.7. Extrusion Operation

The formulated flours were subjected to the extrusion process. The operating conditions (barrel temperature (165°C), feed moisture content (21%), screw speed (150 rpm) and die diameter (1cm)) was kept constant throughout the experiment. The pump was adjusted to give the amount of water required to bring the moisture to 21% in the mixes at constant material feed rate by using hydration equation (Golob, 2002). These values of the parameters were set based on preliminary test.

3.7.1. Equipment for extrusion process

Extrusion was performed on a pilot scale co-rotating twin-screw food extruder (Model Clextral, BC-21 No 124, Firminy, France). The barrel has 300 mm useful length and consists of three modules each 100 mm long fitted with 25 mm diameter screws. The temperature of the modules is regulated by electrical heating and water cooling system, and the temperature at each zone was controlled by a Eurotherm controller (Eurotherm Ltd., Worthing, U.K.). The flour was fed to the extruder inlet using a twin screw volumetric feeder (type KMV-KT20), and water at ambient temperature was injected into the extruder via an inlet port by a positive displacement pump (DKM-Clextral).

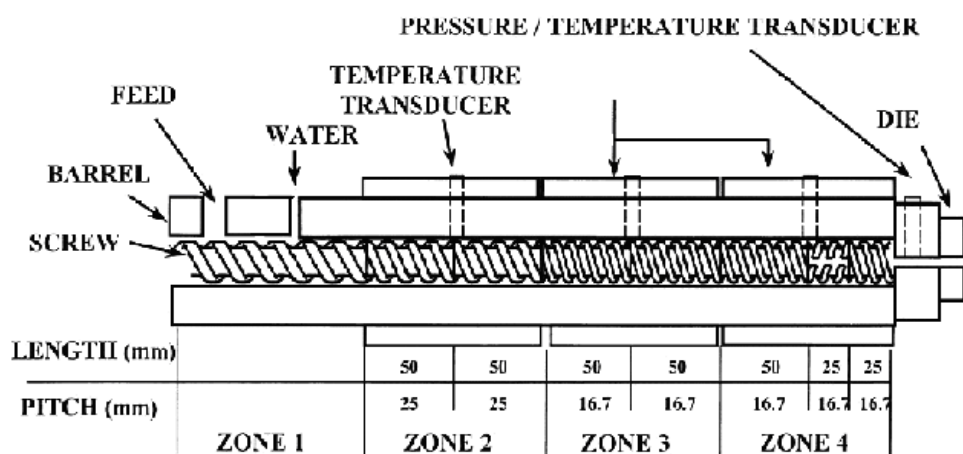


Figure 2: Screw and barrel configuration of the twin-screw BC-21 extruder

3.7.2. Extrusion of samples

Samples were extruded as straight rope (rod) for a time interval of ten seconds (Mason and Hosney, 1986). The extruded products were placed on a Table and allowed to cool for 30 minutes at room temperature for the measurement of weight, length and diameter (Ibanoglu *et al.*, 2005). Samples were collected and sealed in moisture proof plastic bags after equilibration for 24 hrs at ambient condition. Sealed samples were stored at room temperature for physicochemical determinations and sensory quality evaluation.

3.8. Proximate Composition Determination

3.8.1. Determination of moisture content (AOAC, 2000)

Empty dishes and lids (made of porcelain) were dried using air drying oven (Memment, Germany) for 1 hour at 100⁰C, transferred to the desiccator (with granular silica gel), cooled for 30 minutes, and weighed. The prepared samples were mixed thoroughly and about 5.000g of the flour and extrudates samples were transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 3 hrs at 105⁰C, and then the dishes and their contents were cooled in a desiccator to room temperature and reweighed.

Calculation

$$\text{M.C} = \left(\frac{W_2 - W_3}{W_2 - W_1} \right) \times 100$$

W₁=mass of the dish, W₂=mass of the dish and the sample before drying, and

W₃=mass of the dish and the sample after drying

3.8.2. Determination of total ash content (AOAC, 2000)

Ash was determined by incineration of known weights of the samples in a muffle furnace at 550⁰C (Gallenkamp, size 3) until a white ash was obtained. Organic matter was burned off and the inorganic material remaining is cooled and weighed. Heating was carried out in stages,

first to derive the water, then to char the product thoroughly and finally to ash at 550°C in a muffle furnace.

The ashing dishes (made of porcelain) were placed into a muffle furnace for 30 min at 550°C. The dishes were removed and cooled in a desiccator (with granular silica gel) for about 30 minutes to room temperature; each dish was weighed to the nearest g. About 2.500g of the flour and extrudates samples were added into each dish. The dishes were placed on a hot plate under a fume hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes were placed inside the muffle furnace at 550°C for 4 hours, and removed from the muffle and then placed in a desiccator for 1hr to cool. The ash was clear white in appearance when each dish cooled to room temperature. Weight of total ash was calculated by difference and expressed as percentage of sample.

Calculation:

$$\% \text{Ash} = \left(\frac{m_3 - m_1}{m_2 - m_1} \right) \times 100$$

Where m_1 = mass of crucible

m_2 = sample mass with crucible

m_3 = final mass of sample with crucible

3.8.3. Determination of crude protein (AOAC, 2000)

Protein (N×6.25) was determined by the Kjeldahl method. All nitrogen is converted to ammonia by digestion with a mixture of concentrated sulfuric acid and concentrated orthophosphoric acid containing copper sulfate and potassium sulfate as a catalyst. The ammonia released after alkalization with sodium hydroxide is steam distilled into boric acid and titrated with hydrochloric acid.

Digestion: About 0.5000g of the flour and extrudates samples were taken in a Tecator tube and 6ml of acid mixture (5 parts of concentrated ortho phosphoric acid and 100 parts of concentrated sulfuric acid) was added, mixed, thoroughly and 3.5ml of 30% hydrogen peroxide was added step by step. As soon as the violet reaction had ceased, the tubes were

shaken for a few minutes and placed back into the rack. A 3.0000g of the catalyst mixture (ground 0.5000g of copper sulphate with 100 g of potassium sulfate) was added into each tube, and allowed to stand for about 10 min before digestion. When the temperature of the digester reached 370⁰C, the tubes were lowered into the digester. The digestion was continued until a clear solution was obtained, about 1 hr. The tubes in the rack were then transferred into the fume hood for cooling; 15ml of de ionized water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation: A 250ml conical flask containing 25ml of the boric acid-indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested and diluted solution was transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml deionized water and the rinses were added into the solution. A 25ml of 40% sodium hydroxide solution was added into the compartment and washed down with a small amount of water, stoppered and the steam switched on. A 100ml solution of the sample was distilled, and then the receiver was lowered so that the tip of the condenser is above the surface of the distillate. The distillation was continued until a total volume of 150ml is collected. The tip was rinsed with a few milliliter of water before the receiver was removed.

Titration: The distilled solution was then titrated with 0.1N hydrochloric acid to a reddish color.

Nitrogen (mg) in the sample = $V \times N \times 14$

Nitrogen (g/100 g) sample = $\text{mg of nitrogen} \times 100 / \text{mg sample}$

Total nitrogen (%) = $(V - V_b) \times N \times 14 / W$

Crude protein (%) = total nitrogen (%) $\times 6.25$

Where: V = volume of hydrochloric acid consumed to neutralize the sample; V_b = the volume of acid consumed to neutralize the blank; N = normality of the acid; 14 = Eq.wt of Nitrogen; 6.25 = conversion factor from total nitrogen to crude protein.

3.8.4. Determination of crude fat content (AOAC, 2000)

Crude fat was determined by exhaustively extracting a known weight of sample in diethyl ether (boiling point, 55 °C) in a soxhlet extractor. The ether was evaporated from the extraction flask. The amount of fat was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage.

The extraction flasks were cleaned, dried in drying oven (Mettler, Germany) at 70°C for 1 hour, cooled in desiccators (with granular silica gel) for 30 minutes, and then weighed. The bottom of the extraction thimble was covered with about 2cm layer of fat free cotton. About 2.00 gram of flour and extrudates samples were added into the extraction thimbles, and then covered with about 2cm layer of fat free cotton. The thimbles with the sample content were placed into soxhlet extraction chamber. The cooling water was switched on, and a 50 ml of diethyl ether was added to the extraction flask through the condenser. The extraction was conducted for about 3 hrs. The extraction flasks with their content were removed from the extraction chamber and placed in the drying oven at 70°C for about 1hr, cooled to room temperature in the desiccator for about 30 minutes and re-weighed.

$$W=W_2-W_1$$

$$\text{Fat g/100 g fresh sample} = (W \times 100) / W_0$$

Where: W = weight of fat; W₂ = weight of extraction flask after extraction (wt. of flask and fat); W₁ = weight of extraction flask before extraction (wt. of flask);

W₀ = weight of fresh Sample.

3.8.5. Determination of crude fiber content (AOAC, 2000)

Crude fiber was determined after digesting a known weight of flour and extrudates samples by refluxing 1.25% boiling sulfuric acid and 28% boiling potassium hydroxide.

Digestion: About 1.6000g of flour and extrudates samples were placed into a 600ml beaker, 200ml of 1.25% H₂SO₄ was added, and boiled gently for 30 minutes placing a watch glass

over the mouth of the beaker. During boiling, the level of the sample solution was kept constant with hot distilled water. After 30 minute boiling, 20ml of 28% KOH was added and boiled gently for a further 30 minute, with occasional stirring.

Filtration: The bottom of a sintered glass crucible was covered with 10 mm sand layer and wetted with a little distilled water. The solution was poured from beaker into sintered glass crucible and then the vacuum pump was turned on. The wall of the beaker was rinsed with hot distilled water several times; washings were transferred to crucible and filtered

Washing: The residue in the crucible was washed with hot distilled water and filtered (repeated twice). The residue was washed with 1% H₂SO₄ and filtered, and then washed with hot distilled Water and filtered; and again washed with 1% NaOH and filtered. The residue was washed with hot distilled water and filtered; and again washed with 1% H₂SO₄ and filtered. Finally the residue was washed with water- free acetone.

Drying and combustion: The crucible with its content was dried for 2 hours in an electric drying oven at 130⁰C and cooled for 30 min in the desiccator (with granular silica gel), and then Weighed. The crucible was transferred to a muffle furnace and incinerated for 30 min at 550⁰C. The crucible was cooled in the desiccator and weighed.

Then the fiber was calculated as a residue after subtraction of the ash.

$$\text{Crude fiber g /100 g} = (W_1 - W_2) \times 100 / W_3$$

Where: W₁ = weight of (crucible +sample) after drying

W₂ = weight of (crucible + sample) after ashing W₃ = weight of fresh sample

3.8.6. Determination of carbohydrate

Utilizable carbohydrate content was determined by difference. It was determined by subtracting the crude protein, crude fiber, total ash and fat from the total dry weight of the sample.

3.8.7. Determination of gross energy

Gross energy was determined by calculation from fat, carbohydrate and protein contents using the Atwater's conversion factors; 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates and expressed in calories (Guyot *et al.*, 2007).

3.8.8. Minerals Analysis

The mineral contents were determined by the procedure of AOAC (1984). Calcium, iron, and zinc were determined using an Atomic Absorption Spectrophotometer. After removal of organic material by dry ashing the residue was dissolved in dilute acid. The solution was sprayed into the flame of Atomic Absorption Spectrophotometer (Varian SpectraAA-20Plus, Varian Australia Pty., Ltd., and Australia) and the absorption of the metal to be analyzed was measured at a specific wavelength.

Mineral determination: Ashes were obtained from dry ashing. The ash was wetted completely with 5ml of 6N HCl, and dried on a low temperature hot plate. A 7ml of 3N HCl was added to the dried ash and heated on the hot plate until the solution just boils. The ash solution was cooled to room temperature at open air in a hood and filtered through a filter paper (Whatman 42, 125mm) into a 50ml graduated flask. A 5ml of 3N HCl was added into each crucible dishes and heated until the solution just boiled, cooled, and filtered into the flask. The crucible dishes were again washed three times with de-ionized water; the washings were filtered into the flask. A 2.5ml of 10% Lanthanum chloride solution was added into each graduated flask. Then the Solution was cooled and diluted to the mark (50ml) with de-ionized water. A blank was prepared by taking the same procedure as the sample

$$M \text{ content (mg/100g)} = (a-b) \times V / 10W$$

$$M \text{ content (mg/kg)} = ((a-b) \times V) / W$$

Where: M= Mineral content W= Weight (g) of samples; V= Volume (V) of extract; a = Concentration (μ g/ml) of sample solution; b = Concentration (μ g/ml) of blank solution.

3.9. Determination of Antinutritional Factors

3.9.1. Determination of phytate content

Phytate was determined by the method of Latta and Eskin (1980) and later modified by Vantraub and Lapteva (1988). About 0.1000g of flour and extrudates samples were extracted with 10ml 0.2N HCl in a mechanical shaker for 1hour at an ambient temperature and centrifuged at 3000rpm for 30 minute. The clear supernatant was used for phytate estimation. A 2ml of Wade reagent (containing 0.03% solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.3% of sulfosalicylic acid in water) was added to 3ml of the sample solution (supernatant) and the mixture was mixed on a Vortex (Maxi Maxi II) for 5 seconds. The absorbance of the sample solutions were measured at 500 nm using UVVIS spectrophotometer (Beckman DU-64- spectrophotometer, USA).

A series of standard solution were prepared containing 0, 5, 9, 18, 27 and 36 $\mu\text{g}/\text{ml}$ of phytic acid (analytical grade sodium phytate) in 0.2N HCl. A 3ml of standard was added into 15ml of centrifuge tubes with 3ml of water which were used as a blank. A 1ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 seconds. The mixtures were centrifuged for 10 minutes and the absorbances of the solutions (both the sample and standard) were measured at 500nm by using deionized water as a blank. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation:

Calculation: Phytic acid in $\text{mg}/100\text{g} = (\text{absorbance}-\text{intercept})3/(\text{slope} \times \rho \times \text{wt. of Sample} \times 10)$

Where, ρ is density

3.9.2. Condensed tannin determination

Tannin content was determined by the method of Burns (1971) as modified by Maxson and Rooney (1972). About 1.0000 gram of flour and extrudates sample was weighed in a screw cap test tube. The chickpea flour was extracted with 10ml of 1% HCl in methanol for 24 hours

at room temperature with mechanical shaking. After 24 hours shaking, the solution was centrifuged at 1000rpm for 5 minutes. A 1ml of supernatant was taken and mixed with 5 ml of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol).

D-catechin was used as standard for condensed tannin determination. A 40mg of D-catechin was weighed and dissolved in 100 ml of 1% HCl in methanol, which was used as stock solution. A 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of stock solution was taken in test tube and the volume of each test tube was adjusted to 1ml with 1% HCl in methanol. A 5ml of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample solutions and the standard solution were measured at 500nm by using water to zero the spectrophotometer, and the calibration curve was constructed from the series of standard solution using SAS. A standard curve was made from absorbance versus concentration and the slope and intercept were used for calculation.

Concentration of tannin was read in mg of D-catechin per 100g of sample

Tannin in mg/100g = (absorbance-intercept)/ (slope x density x weight of samplex10)

3.10. Determination of Product Properties

3.10.1. Specific length, Degree of expansion and Bulk density

Five pieces of extrudates samples were taken randomly from each treatment to measure the length, diameter and weight of the extrudates. The average values were used to calculate specific length, expansion ratio and bulk density of extrudates. Length of the extrudates samples were measured by a pocket size steel tape of 1mm accuracy. The diameter of the extrudates were measured by a vernier caliper (СДЕЛИАНО, cccp, Russia) having 0.05 mm accuracy and the weight were measured by a digital balance (ADAM, AFP1200, South Africa) of 0.01g sensitivity. Specific length, degree of expansion and bulk density of extrudates samples were calculated as follows (Mason and Hosney, 1986):

$$SL = L/W \qquad DE = \frac{D}{d} \qquad BD = \frac{4W}{\pi D^2 L}$$

Where: SL is specific length (cm/g), DE is expansion ratio (cm/cm), BD is bulk density (g/cm³), L is length (cm), W is weight of extrudates (g), D is diameter of extrudates (cm) and d is diameter of die hole (cm).

3.10.2. Water absorption and water solubility index

The Water absorption index (WAI) and water solubility index (WSI) were determined by the method described by Anderson *et al.* (1969). Ground product passing through a 60 mesh screen was suspended in 30 ml of water at 30 °C for 30 min, and then centrifuged at 3000rpm for 10 min. The supernatant was decanted into an evaporating dish of known weight. The WAI was calculated as the total moisture absorbed divided by the dry weight of sample.

$$WAI = \frac{W_s}{W_o}$$

Where: W_s - weight of sediment (g) and W_o -weight of sample (g)

The WSI was determined by dividing the amount of solids recovered from evaporation of the supernatant by the weight of the original sample, expressed as a percentage.

$$WSI = \frac{W_r}{W_o} \times 100$$

Where: W_r is the weight (g) of residual supernatant after evaporation

W_o is weight of sample (g)

3.10.3. Instrumental texture test

Ten pieces of extrudates having 5 cm were randomly selected and placed on the loading cell of a TA-XT2 texture analyzer (Ametek, Lloyd Instruments, UK). General purpose compression test was conducted on the extrudates. The changes in the force required to break the sample

during compression were recorded in the form of peak and the texture was expressed as hardness, peak force (N) of the first compression required for sample's breakage (Veronica *et al.*, 2006).

3.10.4. Viscosity determination

The gruels were prepared in a glass beaker by mixing 15, 18 and 20 g of flour and 100 ml of water. The gruel was placed in a water bath maintained at 40°C (heating temperature) and its viscosity was measured at this temperature. The paste viscosity was measured using a Brookfield Viscometer (Model DVII Rheometer V2.0 RV; Middleboro, Massachusetts, USA). The gruel was poured into the viscometer beaker, cooled to 40°C and viscosity was measured (in centipoises, cP) using spindle number 6 at ashear rate of 50 revolution per minute (RPM). Within 10 minute, the average of the maximum and minimum viscosity reading was recorded (Alvina *et al.*, 1990).

3.11. Sensory evaluation

The panelists were selected from the staff s and mothers who came at Ethio-Tebib hospital to follow their childes health status. The panel members were assigned individually to well brighten room.

The panelists were instructed about the purpose and objective of this test, so the selection of panelists was based on interest and willing to serve. The health status of the panelists was also considered during panelist selection (not suffering from colds and allergic that affect their sensitivity to the product). The weaning food thin porridge (gruels) was served to the panelists in white and transparent glass cups at about 40°C. Panelists were asked to rinse their mouth with tap water that was provided to them, before the next serving. The containers with the samples were coded in three digits and kept far apart to avoid biases and for independent judgment. The panelists were asked to rank the gruel on the basis of appearance (color), flavor, taste, and texture (mouth feel) using a nine point hedonic scale, (where 1 = dislike

extremely and 9 = like extremely). Overall acceptability of the samples was also rated on same scale with 9 = extremely acceptable and 1 = extremely unacceptable (Inyang and Idoko, 2006).

3.12. Statistical analysis

The analysis was carried out in duplicates for all determinations. The mean and standard deviation of means were calculated. The data were analyzed by one way analysis of variance (ANOVA) using SAS software (SAS system for windows 9.0). Duncan Multiple Range Test (DMRT) was used for multiple mean separations at 5% probability level.

4. RESULTS AND DISCUSSIONS

4.1. Proximate Composition of Sorghum and Chickpea Flour

Proximate compositions of sorghum and chickpea flour used in this study are presented in Table 3. The moisture content of sorghum flour obtained in this study was 8.24% which is below 10%. Such low moisture content of flours prevents microbial activity and extends the shelf life of flours (Kikafunda *et al.*, 2006). The protein content of sorghum was found to be 12.69 % (Table 3). The amount of protein in the sorghum sample was higher than other sorghum cultivars, name gobiye (10.8%) reported by Mihiret (2009) but it was in the range (7-15%) reported by Leder (2004). The crude fat content (4.58%) obtained in this study was higher than 3.2% of sorghum flour reported by Mihiret (2009) but it was within the range (1.5% to 6%) reported by Dicko *et al.* (2006). Ash content (1.75%) was comparable with values reported by Leder (2004). The amount of Ca, Fe and Zn was 17.44, 8.13, 2.08 mg/100g respectively (Table 3). In contrast, the values of iron obtained in the present investigation were higher than values (5.7 mg/100g) reported by Dicko *et al.* (2006) and Ca content appeared to be comparable with the amount indicated by the same researchers. Both Ca and Fe content are in the range of Ca (11.8-24.1mg/100g) and Fe (4.7-8.6mg/100g) reported by Kruger *et al.* (2012). Zinc content was comparable with values reported by Mihiret (2009).

Table 3. Proximate composition of sorghum and chickpea flours

Proximate compositions	*Sorghum	*chickpea
Moisture (%)	8.24 ±0.12	7.38±0.02
Protein (%)	12.69±0.01	25.86± 0.17
Fat (%)	4.58± 0.08	6.93 ±0.03
Ash (%)	1.75± 0.01	3.13 ±0.03
Fiber (%)	2.65 ±0.04	2.26±0.05
¹ CHO (%)	70.09± 0.09	54.44± 0.20
Energy (kcal)	372.39±1.06	383.58± 0.11
Zn (mg/100g)	2.08 ±0.03	3.50 ±0.05
Ca (mg/100g)	17.44 ±0.30	105.58± 0.74
Fe (mg/100g)	8.13 ±0.01	5.42± 0.24
Condensed tannin (mg/100g)	78.50 ±4.59	164.85± 1.95
Phytate(mg/100g)	350.05± 2.42	80 ±1.71

* = mean±SD of three replications; ¹ = Obtained by difference; CHO = carbohydrate (%);

Ca = calcium (mg/100g); Fe = Iron (mg/100g); Zn = Zinc (mg/100g)

Condensed tannins and phytate contents of sorghum flour were found to be 78.50mg/100g and 350.05mg/100g, respectively (Table 3). The tannin content was within the range of tannin free sorghum value 50-380 mg/100g reported by Awika (2000) and lower than the value 200-500mg/100g reported by Cheng *et al.* (2009). Phytate content of sorghum was higher than the value (246-300mg/100g) reported by Mahgoub *et al.* (1997) and lower than other sorghum cultivars, named gobiye (432.66mg/100g) and meko (464.94) reported by Mihiret (2009) and Tizazu (2010) respectively

The protein content of chickpea sample 25.86% (Table 3) was higher than values (23.14 % and 24.0 %) reported by Zhao *et al.* (2005) and Iqbal *et al.* (2006) respectively but it was within the range (14.5 to 30.6%) reported by Chavan *et al.* (1986). The crude fat content of chickpea (6.93%) obtained in the present investigation was higher than the fat content (5.2 %) reported by Iqbal *et al.* (2006) and (5.69 %) reported by Zhao *et al.* (2005) and comparable with values (6.70-7.60 %) and (5.12 - 8.57 g/100g) reported by Sanjeeva *et al.* (2010) and Patane (2006), respectively.

The moisture content of chickpea flour in the present study was 7.38% (Table 3) and similar to the studies undertaken by Patane (2006). The ash content in the present study of chickpea samples was higher (3.13%) than the ash content (2.4%) reported by Sabanis *et al.* (2006). Similar or higher ash levels were reported by Zhao *et al.* (2005) (3.0 %), and Iqbal *et al.* (2006) (3.6 %).

Calcium, Iron and Zinc contents of chickpea flour in the present study were 105.58, 5.42 and 3.50mg/100g respectively (Table 3). Calcium content was comparable with values (106.60mg/100g) reported by Wang and Daun (2004) while zinc content was lower than the values (4.40mg/100g) indicated by the same researchers. Zinc content were comparable with values (3.5mg/100g) reported by Ibanez *et al.* (1998). Iron content was higher than (4.46mg/100g) reported by Ibanez *et al.* (1998) and Similar to values (5.50mg/100g) reported by Wang & Daun. (2004). Condensed tannin and phytate contents of chickpea flour were found to be 164.85mg/100g and 80mg/100g, respectively. Both phytate and tannin are lower

than the values 97.46mg/100g and 175.23mg/100g respectively reported by Dejene (2010) and phytate content was similar to the value reported by Malunga (2012)

4.2. Proximate Composition of Extrudates

4.2.1. Moisture contents of extrudates

The effect of chickpea flour proportion on proximate composition of extrudates was presented in Table 4. The moisture content of the sorghum-chickpea blend extrudates ranged between 9.35% - 9.94%. The products had moisture contents low enough to have an extended shelf life. In dry food systems, moisture content of between 6-10% has been established to prolong the shelf life of foods, beyond which the storability of the system could be impeded by chemical and microbiological agents (Brncic *et al.*, 2006). The moisture content of the products was significantly increased ($P<0.05$) from 9.35 to 9.94% with increase in level of chickpea flour at 30% but increased with no significance at 10 and 20% chickpea flour level (Table 4). This could be due to the increased water hydration capacity of the blend with increased chickpea flour. Sadik (2010) reported similar findings by increasing soy flour level.

Table 4. Proximate composition of extrudates (%)

Variable Chickpea %	n	Moisture	Protein	Fat	Ash	Fiber	¹ CHO	Energy(kcal)
0	3	9.35±0.00 ^c	12.10±0.10 ^d	3.85±0.02 ^d	1.73±0.01 ^d	2.42±0.14 ^a	70.55±0.17 ^a	365.23±0.62 ^c
10	3	9.40±0.15 ^{bc}	13.18±0.11 ^c	4.13±0.03 ^c	1.86±0.01 ^c	2.26±0.06 ^{ab}	69.16±0.14 ^b	366.53±0.48 ^c
20	3	9.58±0.07 ^b	14.98±0.03 ^b	4.60±0.06 ^b	1.99±0.01 ^b	2.20±0.08 ^b	66.66±0.16 ^c	367.95±0.18 ^b
30	3	9.94±0.15 ^a	16.05±0.09 ^a	5.18±0.03 ^a	2.19±0.08 ^a	1.98±0.05 ^c	64.66±0.31 ^d	369.47±1.15 ^a
Mean	12	9.57	14.08	4.44	1.94	2.22	67.76	367.30
CV		1.14	0.83	0.61	2.04	4.03	0.30	0.19

Values followed by different letters within a column indicate significant difference ($p<0.05$); n = number of observation; * = mean ±SD; ¹ = obtained by difference; CHO = carbohydrate (%); CV = coefficient of variation

4.2.2. Protein content of extrudates

Cereal grains are known to be low in protein (both in quantity and quality) and hence the need for complementation with legumes help the protein content to be complete as the legume would provide both quality and quantity lacking in cereals. The protein content of the extrudates significantly increased ($P < 0.05$) from 12.10% to 16.05% with increasing level of chickpea flour from 0 to 30% (Table 4). This could be due to the higher amount of protein content in chickpea flour. At 10, 20, 30% level of chickpea supplementation the extrudates met 87.86, 99.86 and $>100\%$ respectively of the recommended protein content ($\geq 15\text{g}$) that complementary foods need to have for older infants and young children (CODEX CAC/GL 08. 1991)

4.2.3. Fat contents of extrudates

Fat provides lubrication effect in the compressed polymer mix as well as modifies the eating quality of extrudates (Guy, 1994). From the study it was found that chickpea flour proportion had significant ($P < 0.05$) effect on fat content of the product. Increasing the level of chickpea flour from 0 to 30 %, resulted in a significant increase ($p < 0.05$) in fat content of extrudates from 3.85 to 5.18g/100g. Lipids which have been recommended to be around 10-25 g for 100 g (Table 1) (CODEX CAC/GL 08. 1991) were approximately 4.13, 4.60 and 5.18 g for 10, 20 and 30% of chickpea flour level respectively (Table 4). These values are lower than the recommended values. This might be due to lower fat content of the starting material and due to partial decomposition and volatilization of lipid components during extrusion (Smith and Singh 1996). Another explanation for the lower lipid level is the formation of complexes with amylose or protein (Camire, 2000).

4.2.3. Ash content of extrudates

Ash content of extrudates at 0% chickpea level didn't show any change because of less influence of extrusion cooking on ash content (Marzo *et al.*, 2002). However ash content of the extrudates significantly increased ($p < 0.05$) from 1.73 to 2.19% with increasing level of

chickpea flour supplementation from 0 to 30% (Table 4) since chickpea flour contains relatively higher amount of ash than sorghum. Reported literature findings have shown that legumes contain significant quantities of ash than cereals (Onwueme and Sinha, 1991; Uzogara and Ofuya, 1992). The ash content of extrudates is within the range of ash content ($\leq 3\%$) recommended for complementary foods (CODEX CAC/GL 08. 1991)

4.2.4. Carbohydrate content of extrudates

Carbohydrate content of the extrudates significantly decreased ($p < 0.05$) from 70.55% to 64.66% with increasing level of chickpea flour supplementation from 0 to 30% (Table 4). Decrease in carbohydrate and fiber contents and increase in protein, fat and ash contents could be due to the higher amount of protein, fat, ash and lower fiber contents of chickpea flour. At 10, 20, 30% level of chickpea flour supplementation the extrudates met 100% of the recommended carbohydrate content ($64 \pm 4\%$) of complementary foods for older infants and young children (Table 1) (CODEX CAC/GL 08. 1991).

4.2.5. Total Energy of extrudates

Energy value of the extrudates significantly increased ($p < 0.05$) from 366.53 to 369.47 kcal/100g with increasing level of chickpea flour supplementation from 10 to 30% (Table 4). The energy value of the extrudates were 366.53, 367.97 and 369.47 kcal/100g at 10, 20 and 30% chickpea flour supplementation level respectively and these provide 88.8, 89.2 and 89.6% of the average energy (400-425 kcal) recommended for complementary foods for older infants and young children (CODEX CAC/GL 08. 1991) (Table 1).

4.3. Mineral Contents of Extrudates

Ca and Zn content of extrudates significantly increased ($P < 0.05$) from 17.15 to 43.18, and 2.06 to 2.41 mg/100g, respectively (Table 5), with increase in level of chickpea flour supplementation from 0 to 30% for Ca and from 10 to 20% for Zn. Fe content of extrudates was significantly decreased ($p < 0.05$) from 9.58 to 8.07 mg/100g with an increase in level of chickpea flour supplementation from 0 to 20%. However, the proportion of chickpea flour did

not show significant difference ($p \geq 0.05$) on the Fe content of the extrudates between levels 20-30% proportions (Table 5). The increase in Ca and Zn contents and decrease in Fe contents of extrudates were expected as chickpea flour contains relatively higher levels of Ca and Zn and lower level of Fe than sorghum. Iron content of the row flour is increased after extrusion cooking and it is most likely to the result of the wear of metallic pieces, mainly screws, of the extruder (Alonso et al., 2001). As level of chickpea flour supplementation increased from 0 to 30% the Ca, Zn and Fe content of the extrudates met from 3.4-8.6, 64-75 and 60-49% respectively of the recommended amount of minerals that complementary foods need to have for older infants and young children (CODEX CAC/GL 08. 1991) (Table 1).

Table 5. Mineral contents of extrudates (mg/100g)

Chickpea (%)	n	Ca	Fe	Zn
0	3	17.15±0.23 ^d	9.58±0.29 ^a	2.05±0.02 ^c
10	3	26.02±0.43 ^c	8.48±0.12 ^b	2.06±0.01 ^c
20	3	36.01±0.27 ^b	8.07±0.06 ^c	2.27±0.01 ^b
30	3	43.18±0.53 ^a	7.82±0.11 ^c	2.41±0.03 ^a
Mean	12	30.59	8.49	2.20
CV		1.26	1.96	0.96

Values followed by different letters within a column indicate significant difference ($p < 0.05$); n = number of observation; * = Mean±SD; Ca = calcium content (%); Zn = zinc content (%); Fe = iron content (%); CV = coefficient of variation

4.4. Antinutritional Contents of Extrudates

4.4.1. Condensed tannin content

Proportion of chickpea flour did not show a significant effect ($p \geq 0.05$) on the tannin content of extrudates (Table 6). However tannin content of the extrudates was lower (90-91%) than tannin content of row sample. This could be due to destruction of tannin at high temperature (Anton *et al.*, 2009). This result supports the finding by Nwabueze (2007) while extruding African breadfruit based spaghetti, observed 89% reduction in tannin content. Aseffa (2008) reported 73.68% and 62.50% reduction in the amounts of tannins in Awassa and Belessa soybean varieties, respectively, during extrusion cooking.

Table 6.* Antinutritional content of extrudates

Chickpea (%)	n	Tannin (mg/100g)	Phytate (mg/100g)
0	3	7.16±0.47 ^a	255.32±0.71 ^a
10	3	8.43±0.32 ^a	237.13±0.79 ^b
20	3	7.68±0.65 ^a	215.00±0.27 ^c
30	3	7.84±1.44 ^a	193.68±0.32 ^d
Mean	12	7.78	225.28
CV		10.76	0.25

Values followed by different letters within a column indicate significant difference ($p < 0.05$); * = mean±SD; n = number of observations; CV = coefficient of variation

4.4.2. Phytic acid content

Increasing the level of supplementation of chickpea flour from 0 to 30% caused a significant ($P < 0.05$) decrease in phytate content of extrudates from 255.32 to 193.68mg/100g (Table 6). This could be due to the lower amount of phytate in chickpea flour. Extrusion cooking showed 27 to 29% reduction on phytate content of extrudates from the row sample as level of chickpea flour supplementation increased from 0 to 30 %. This could be due to degradation of phytate to simple and lower molecular weight components at higher temperature (Anton *et al.*, 2009). Nwabueze, (2007) reported 27.7-32.7% reduction in phytic acid by extrusion cooking. Aseffa (2008) also reported that extrusion cooking caused the reduction of phytic acid levels in Awassa and Belessa soybean varieties by 49.13% and 38.69%, respectively.

4.4.2. Bioavailability of iron, zinc and calcium of extrudates (Molar ratios of phytate:iron and phytate:zinc and calcium:phytate)

Phytate: Mineral molar ratio used to estimate the absorption of minerals from foods. In present study phytate: calcium, phytate: iron, phytate: zinc molar ratio was evaluated to estimate their absorption from extrudates prepared from sorghum supplemented with chickpea flour level from 0-30%.

Table 7. Bioavailability of Minerals

		*Phytate : minerals molar ratio		
Chickpea (%)	n	^α Phytate:Ca	^β Phytate:Fe	^ϖ Phytate:Zi
0	3	0.90±0.01 ^a	2.25±0.06 ^b	1.47±0.02 ^a
10	3	0.55±0.01 ^b	2.36±0.03 ^a	0.90±0.02 ^b
20	3	0.36±0.00 ^c	2.25±0.02 ^b	0.59±0.01 ^c
30	3	0.27±0.01 ^d	2.09±0.03 ^c	0.45±0.01 ^d
Mean	12	0.52	2.24	0.85
CV		1.35	1.69	1.59

* = mean±SD

^β = (mg of Phytate/MW (molecular weight) of Phytate: mg of iron/MW of iron

^ϖ = (mg of Phytate/MW (molecular weight) of Phytate: mg of Zinc/MW of Zinc

^α = (mg of Calcium/MW of Calcium: mg of phytate/MW of phytate).

4.4.2.1. Phytate:zinc molar ratios

Phytate:zinc molar ratio is used to estimate the likely absorption of zinc from food (Melaku *et al.*, 2005; Walingo, 2009). Increasing chickpea flour level from 0-30%, phytate:zinc molar ratio significantly decreased ($p < 0.05$) this shows that supplementation of sorghum with chickpea causes significant reduction on phytate contents of extrudates which enhance zinc absorption. Phytate:zinc molar ratio of the extrudates is 1.47, 0.9, 0.59 and 0.45 at 0, 10, 20 and 30% chickpea flour supplementation respectively (Table 7). Phytate:zinc molar ratio of the extrudates were less than 5 indicating good zinc bioavailability. Diets with a phytate:zinc molar ratio greater than 15 have relatively low zinc bioavailability, those with phytate:zinc molar ratio between 5 and 15 have medium zinc bioavailability and those with a phytate:zinc molar ratio less than 5 have relatively good zinc bioavailability (Walingo, 2009).

4.4.2.2. Phytate:iron molar ratios

Phytate: iron molar ratios of extrudates prepared from sorghum supplemented with 0, 10, 20 and 30% chickpea flour level were 2.25, 2.36, 2.25 and 2.09 respectively (Table 7). The obtained value shows that increasing chickpea flour supplementation level at 30% improves

iron bioavailability even if all extrudates have low iron bioavailability. Phytate: iron molar ratios > 0.15 are regarded as indicative of poor iron bioavailability (Melaku *et al.*, 2005).

4.4.2.3. Phytate:calcium molar ratios

Phytate:calcium molar ratio of extrudates prepared from sorghum supplemented with chickpea were significantly decreased with increasing chickpea flour level from 0-30%. This might be due to that increasing chickpea flour supplementation level from 0-30% significantly decreased phytate content whereas increased calcium content. Phytate:calcium molar ratio of the extrudates is 0.9, 0.55, 0.36 and 0.27 at 0, 10, 20 and 30% chickpea flour supplementation respectively (Table 7). The values obtained show that extrudates may have good calcium bioavailability. The calcium:phytate molar ratio of <6 is indicative of favorable for calcium absorption (Melaku *et al.*, 2005).

4.5. Extrudates Physical Properties

4.5.1. Expansion ratio

Expansion is the formation of aerated internal structure when melted feed material at high temperature and pressure suddenly release the pressure and super-heated water flashed off at the die of the extruder (Harper, 1981).

The Expansion ratio(ER), measured as diameter of expansion, decreased from 1.73 to 1.45 as the level of chickpea flour supplementation increased from 0 to 30 % (Table 8). It is due to the increase of protein as a result of chickpea flour. This might be due to the higher starch content of sorghum and/or to the higher protein content of chickpeas. Pelembe *et al.* (2002) and Sadik (2010) showed that increasing protein content in the mixture may decrease ER during extrusion cooking. The addition of protein to starchy extrudates reduced the expansion of products by reducing the extensibility of the starch polymer during its expansion at the die exit (Chiyakul *et al.*, 2009; Paton and Spratt., 1984). Chinnaswamy and Hanna (1988) also noted that the expanded volume of cereal flour decreases with increasing amounts of protein and lipid but increases with starch content. The lower ER in the mixtures of higher chickpea

content may also be related to the lower ratio of amylose over amylopectin in sorghum. Amylopectin exerts a positive and amylose a negative influence on ER (Feldberg, 1969).

Table 8. Extrudates physical properties

*Product physical property						
Chickpea (%)	n	ER	SL	BD	Texture(H)	Viscosity
0	3	1.73±0.06 ^a	1.10±0.07 ^b	0.39±0.01 ^d	12.09±1.93 ^b	4085.33±15.70 ^b
10	3	1.58±0.05 ^b	1.24±0.05 ^a	0.41±0.01 ^c	16.70±2.19 ^b	5120.33±172.93 ^a
20	3	1.51±0.03 ^{bc}	1.25±0.03 ^a	0.45±0.01 ^b	18.20±3.08 ^b	2788.67±177.85 ^c
30	3	1.45±0.02 ^c	1.22±0.03 ^a	0.50±0.01 ^a	44.78±8.57 ^a	1750.67±8.33 ^d
Mean	12	1.57	1.20	0.44	22.94	3436.25
CV		2.74	3.88	2.07	20.85	3.62

Values followed by different letters within a column indicate significant difference ($p < 0.05$); n = number of observation; * = mean±SD; SL = specific length (cm/g); ER = expansion ratio (cm/cm); BD = bulk density (g/cm³); H = hardness (N)=newton; CV = coefficient of variation viscosity = viscosity in centipoise (cP)

4.5.2. Specific length

Extrudates specific length (SL) is related to the expansion volume. The more the extrudates expands in either the axial and radial direction, the less dense they become indicating a higher proportion of starch gelatinization (Hsieh et al, 1993). The amount of chickpea added to the blend had negative correlation with the radial expansion ratio but positively correlated with the longitudinal expansion ratio. Increasing the level of chickpea flour supplementation from 0 to 10 %, resulted in a significant increase ($p < 0.05$) in specific length 1.10-1.24cm/g but while increasing chickpea flour supplementation level to 20 and 30% there is no significant difference on specific length of the extrudates and decreased from 1.25 to 1.22cm/g (Table 8). This might be due to the increase in the bulk density with the increase in the protein content of the mixture. Yogci and Gogus (2008) reported a similar observation for the radial expansion ratio when different combinations of ingredients were used. The decrease in the radial expansion ratio has been attributed to the dilution effect of protein on starch gelatinization with the increased firmness of plasticized extrudates (Yogci and Gogus, 2008). Hsieh *et al* (1988) reported that increasing wheat bran content in cornmeal up to 30%, resulted in 20% increase in longitudinal expansion and bulk density, but decreased radial expansion.

4.5.3. Bulk density

The bulk density (BD) is an index of extent of puffing. The extrudates having lower expansion showed higher density and vice versa (Sadik, 2010). From this study, it was found that chickpea flour proportion had significant ($p < 0.05$) effect on the density of the product. Increasing the level of chickpea flour from 0 to 30 %, resulted in a significant increase ($p < 0.05$) in BD of extrudates from 0.39 to 0.50g/cm³ (Table 8). Starch-protein interactions probably played an important role in affecting the density by disrupting the continuous starch matrix and thus reducing the extensibility (Shi *et al.*, 2011). Direct relationships between legume content and density in cereal-legume extrusion have been previously reported (Bhattacharya and Prakash, 1994; Ilo *et al.*, 1999 and De Mesa *et al.*, 2009).

4.5.4. Instrumental texture

Hardness is the force required to compress a substance between the molar teeth or between the tongue and the palate. It is measured as force necessary to attain a given deformation (Szczesniak, 1975). An increase in chickpea flour level from 0 to 30 % resulted in a significant ($p < 0.05$) increase in instrumental texture (hardness) from 12.09 to 44.78N (Table 8). An increase in hardness with the level of chickpea flour could be attributed to reduction in expansion due to starch-protein interaction. Rayas-Duarte *et al.* (1998) reported that decreased breaking strength of extrudates was associated with high expansion index and low bulk density. Similar findings have been reported by Pelembe *et al.* (2002) and Sadik (2010). De Mesa *et al.* (2009) also found out that increase in soybean concentration (5–20%) increased hardness of corn extrudates.

4.5.5. Viscosity

The reduced viscosity was very beneficial for infant feeding. The high bulk (low nutrient density) of cereal weaning porridges is a major cause of infant malnutrition in Africa, since it limits nutrient intake (Da *et al.*, 1982). The addition of chickpeas not only improves the protein content and quality of the extruded complementary gruel but additionally reduced

viscosity; these would enable the gruel to be fed to infants at higher concentration, hence increasing food energy intake. At 20% dry matter concentration, the viscosity of the gruel significantly ($p<0.05$) reduced from 4085-1750cP with increasing the level of chickpea flour from 0-30% (Table 8). When the chickpea flour proportion was 20 and 30% the viscosity of the gruel was within the range of a semi-liquid consistency (1000-3000 cP) which has been proposed as being suitable for cereal-based porridges intended for use in infant feeding (Mosha and Svanberg., 1983). Pelembe *et al.* (2002) reported that, reduced viscosity of protein rich extrudates could be very beneficial for infant feeding.

4.6. Functional Properties of Extrudates

4.6.1. Water solubility index

Water solubility index (WSI) determines the amount of free polysaccharide or polysaccharide release from the granule on addition of excess water (Sriburi and Hill, 2000). Increasing the level of chickpea flour from 0 to 30 %, resulted in a significant decrease ($p<0.05$) in WSI of extrudates from 7.55 to 5.01% (Table 9). Extrusion is known to decrease protein solubility, which has been related to the formation of insoluble aggregates, extrudates of high legume content contain more starch aggregates or microgels which will be suspended in water (Gomez & Aguilera, 1983; Zhu *et al.*, 1996 and Liu and Hsieh, 2008). Singh *et al.* (2007) found out WSI decreased with increase pea grits when investigating the effect of addition of pea grits on rice extrudates. Chang *et al.* (2001) also reported lower WSI with increasing levels of soy protein concentrate in cassava starch-soy protein concentrate blend extrusion.

Table 9. Extrudates functional properties

Chickpea (%)	*Functional properties		
	n	WAI (g/g)	WSI (%)
0	3	4.14±0.08 ^d	7.55±0.07 ^a
10	3	5.41±0.16 ^c	6.59±0.02 ^b
20	3	6.54±0.13 ^b	6.10±0.01 ^c
30	3	6.91±0.02 ^a	5.01±0.04 ^d
Mean	12	5.75	6.31
CV		1.90	0.69

Values followed by different letters within a column indicate significant difference ($p<0.05$); * = mean±SD; n = number of observations; CV = coefficient of variation; WAI= Water absorption index WSI= Water solubility index

4.6.2. Water absorption index

Water absorption index (WAI) measures the amount of water absorbed by starch and can be used as an index of gelatinization (Anderson *et al.*, 1969). Gelatinization is the conversion of raw starch into a cooked and digestible material by the application of water and heat. Also, gelatinization is one of the important effects that extrusion has on the starch component of foods. Increasing the level of chickpea flour from 0 to 30 %, resulted in a significant increase ($p < 0.05$) in WAI of extrudates from 4.14 to 6.91g/g (Table 9). This might be due to relatively higher amylose content of chickpea than sorghum. Sorghum starch has more amylopectin than amylose (Serna-Saldivar and Rooney, 1995), while legume starch has more amylose than amylopectin (Guilbot & Mercier, 1985). Plembe *et al.* (2002) also showed that addition of cowpea to sorghum caused a significant increase on WAI of extrudates due to the higher amylose/amylopectin ratio of the cowpeas. Mercier and Feillet (1975) observed that higher amylose results in a higher WAI.

4.7. Sensory

A number of organoleptic features, such as flavor, taste, appearance and texture, may affect infant's intake of transitional foods (Birch, 1998) and also which may results in increased consumption (Hofvander and Underwood, 1987).

4.7.1. Appearance (color)

Vision plays a major role in sensory analysis and the appearance of food can have a major effect on its acceptability (Kikafunda *et al.*, 2006). The color of the gruel made from extrudates having 0% chickpea flour level was relatively most preferred (liked moderately) by the panelists, while the gruels prepared from extrudates increasing the level of chickpea flour supplementation from 10 to 30 %, were preferred with no significant difference ($p < 0.05$) in between, liked slightly (Table 10). The presence of sugars from starch hydrolysis might also lead to color changes in the presence of proteins upon exposure to high temperature as reported by Kikafunda *et al.* (2006)

Table 10. Sensory mean scores of extruded infant gruel

Chickpea (%)	n	* Sensory quality attributes				
		Color	Taste	Flavor	Mouth feel	Over all acceptance
0	30	7.50±0.82 ^a	7.00±0.64 ^a	7.40±0.93 ^a	7.10±0.71 ^a	7.10±0.71 ^a
10	30	6.20±1.00 ^b	6.20±0.89 ^b	6.00±0.79 ^b	7.00±0.79 ^a	7.00±0.64 ^a
20	30	6.40±0.81 ^b	6.00±0.64 ^b	5.90±0.71 ^b	6.80±0.89 ^a	7.00±0.79 ^a
30	30	6.40±1.22 ^b	6.10±0.96 ^b	6.10±1.16 ^b	7.00±0.64 ^a	6.80±0.89 ^a
Mean	120	6.63	6.33	6.35	6.98	6.98
CV		14.75	12.59	14.37	10.94	10.94

Values followed by different letters within a column indicate significant difference ($p < 0.05$). * = Mean±SD, n = number of judges; CV = coefficient of variation

4.7.2. Flavor

For flavor, highest scores were obtained for gruels prepared from extrudates at 0% level of chickpea (liked moderately) and is significantly different from other gruels prepared from extrudates having 10, 20 and 30% chickpea flour level, which are preferred relatively less (liked slightly) by the panelists and there is no significant difference ($p < 0.05$) on flavor of the gruel prepared from extrudates increasing chickpea flour level from 10 to 30% (Table 10).

4.7.3. Taste

The taste of gruel prepared from 0% chickpea flour supplementation level extrudates was most preferred (liked moderately) and is significantly different ($p < 0.05$) from gruels prepared from 10, 20, 30% chickpea flour supplementation level extrudates, which are relatively less preferred (liked slightly) by the panelists and there is no significant difference on taste of the gruel which were prepared from extrudates with increasing chickpea flour level 10 to 30%.

4.7.4. Texture and over all acceptability

Increasing the level of chickpea flour supplementation from 0 to 30 % didn't show significance difference on preference of the texture (mouth feel) of the gruel prepared from extrudates. The gruels were liked moderately by panelists (Table 10). Overall acceptability of

gruels prepared from extrudates also didn't show significant difference with increasing chickpea flour level from 0 to 30% and all products were moderately accepted by panelists.

5. SUMMARY AND CONCLUSIONS

The major objective of this study was to develop extruded complementary food product and investigate the effect of blending ratio on physicochemical and sensory properties of extruded products from low tannin flours white sorghum and chickpea flour. Extrusion of samples was carried out using a pilot scale co-rotating twin-screw food extruder at four levels of chickpea flour supplementation (0, 10, 20 and 30%).

Proportions of chickpea flour were found to have significant effect on extruded product properties and were found the most dominant factor to chemical compositions. The crude protein, fat, ash, Ca, Zn and total energy contents of extrudates were significantly increased with increase in the level of chickpea flour to sorghum but the carbohydrate and fiber content were found decreased. The proportion of chickpea flour had significant effect on physical and functional properties of the extrudates. Specific length of the extrudates made from 10% chickpea flour level was significantly increased from specific length extrudates made from sorghum alone. However, further increasing the level chickpea flour didn't show any significant difference on specific length of extrudates. Increasing the level of chickpea flour from 0-30% was significantly increased the bulk density of the extrudates and hardness of the extrudates significantly increased only at 30% chickpea flour level, whereas the specific length and viscosity was decreased. On the other hand, increasing the level of chickpea flour significantly increased the WAI of extrudates and decreased the diametric expansion and WSI of extrudates.

The condensed tannin and phytate content of extrudates was significantly reduced by extrusion cooking. Increasing the level of chickpea flour caused a significantly decrease in the phytate content of extrudates. However, proportion of chickpea flour had no significant effect ($P \geq 0.05$) on the condensed tannin content.

The sensory evaluation for color, flavor and taste revealed that a significant difference exist between the gruels prepared from 0% and 10% chickpea flour level extrudates and there is no significant difference on gruels prepared with increasing chickpea flour level from 10-30%.

The gruel texture (mouth feel) and overall acceptance revealed that there is no a significant difference between the gruels prepared from extrudates with increasing chickpea flour level from 0-30%. It was also observed that all products have a mean value of 6.33 for taste, 6.50 for color, 6.35 for flavor, 6.98 for texture (mouth feel)and 6.98 for overall acceptability on 9 point hedonic scale. The mean sensory score indicates that all the extrudates were well liked by some degree variations by the panelists.

Extruded complementary food products from sorghum and chickpea flour blend provide gruels of low viscosity and antinutrient content and high energy and nutrients density, therefore, will increase food intake and bioavailability of nutrients and can have some potential to solve the problem of malnutrition in its various forms that are mainly common among the people of the developing countries like Ethiopia. A serving of 100g of sorghum and 30% chickpea flour blend extrudates could provide higher amount of protein than cerelac and mameal and met >100% of protein and 90% of the total energy recommended for complementary foods need to have for infants and young children.

Table 11 comparisons of optimum blend with other products and standards

Products	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	CHO (%)	Energy(kcal)
Mameal	4.35	10.76	1.49	2.12	0.17	81.28	380.89
Cerelac	---	15.50	9.0	2.8	1.7	68.50	417
CODEX CAC/GL 08. 1991	---	≥15	10-25	≤3	≤5	64±4	400-425
Sorghum + 30%chickpea	---	16.05	5.18	2.19	1.98	64.66	369.47

Where= CHO = carbohydrate (%); Mameal= corn and rice instant baby meal; cerelac= banana & wheat infant cereal

Recommendation

- Further research works like fortification is needed to enhance the mineral and vitamin content of sorghum-chickpea flour extrudates in order to meet the required amount by infants and young children.

- The effects of extrusion parameters such as barrel temperature, screw speed, feed rate and die diameter on physicochemical, nutritional and antinutritional factors of sorghum and chickpea flour extrudates should be investigated.
- The effect of chickpea flour proportion on vitamins and minerals content of sorghum-chickpea extrudates should be studied.
- Adding other preprocessing like germination before extrusion cooking of sorghum-chickpea blend in order to reduce phytate content more and to enhance minerals bioavailability should be investigated.

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7. APPENDIX

Appendix Table 1. ANOVA for Moisture Contents

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.6390	0.2130	17.81	0.0007
Error	8	0.0960	0.0120		
Corrected Total	11	0.7350			

Appendix Table 2. ANOVA for Crude Protein

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	28.2073	9.4024	1287.32	<.0001
Error	8	0.0584	0.0073		
Corrected Total	11	28.2657			

Appendix Table 3. ANOVA for carbohydrate content

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	61.68	20.56	493.60	<.0001
Error	8	0.33	0.04		
Corrected Total	11	62.01			

Appendix Table 4. ANOVA for Total Energy

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	30.08	10.03	20.38	0.0004
Error	8	3.94	0.49		
Corrected Total	11	34.02			

Appendix Table 5. ANOVA for Crude Fat

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	3.07	1.02	749.58	<.0001
Error	8	0.01	0.00		
Corrected Total	11	3.08			
Total					

Appendix Table 6. ANOVA for Crude Fiber

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.30	0.10	12.58	0.0021
Error	8	0.06	0.01		
Corrected Total	11	0.36			

Appendix Table 7. ANOVA for Ash Content

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.3413	0.1138	72.62	<.0001
Error	8	0.0125	0.0016		
Corrected Total	11	0.3538			

Appendix Table 8. ANOVA for Calcium Content

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	1168.3174	389.4391	2610.04	<.0001
Error	8	1.1937	0.1492		
Corrected Total	11	1169.5111			

Appendix Table 9. ANOVA for Zinc Content

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.2746	0.0915	207.21	<.0001
Error	8	0.0035	0.0004		
Corrected Total	11	0.2781			

Appendix Table 10. ANOVA for Iron Contents

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	5.48	1.83	66.11	<.0001
Error	8	0.22	0.03		
Corrected Total	11	5.70			

Appendix Table 11. ANOVA for Tannin content

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	2.4481	0.8160	1.17	0.3811
Error	8	5.5991	0.6999		
Corrected Total	11	8.0473			

Appendix Table 12. ANOVA for Phytate Content

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	6441.729	2147.243	6561.01	<.0001
Error	8	2.618	0.327		
Corrected Total	11	6444.348			

Appendix Table 13. ANOVA for Water Solubility Index

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	10.0874	3.3625	1757.01	<.0001
Error	8	0.0153	0.0019		
Corrected Total	11	10.1028			

Appendix Table 14. ANOVA for Water Absorption Index

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	14.0317	4.6772	390.41	<.0001
Error	8	0.0958	0.0120		
Corrected Total	11	14.1276			

Appendix Table 15. ANOVA for Expansion Ratio

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.1271	0.0424	23.02	0.0003
Error	8	0.0147	0.0018		
Corrected Total	11	0.1418			

Appendix Table 16. ANOVA for Specific Length

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.0450	0.0150	6.88	0.0132
Error	8	0.0174	0.0022		
Corrected Total	11	0.0624			

Appendix Table 17. ANOVA for Bulk Density

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.0196	0.0065	80.17	<.0001
Error	8	0.0007	0.0001		
Corrected Total	11	0.0203			

Appendix Table 18. ANOVA for Texture

Source	DF	Squares	Mean Square	F Value	Pr > F
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Model	3	1968.4184	656.1395	28.69	0.0001
Error	8	182.9399	22.8675		
Corrected Total	11	2151.3583			

Appendix Table 19. ANOVA for Viscosity

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	19554003.58	6518001.19	421.52	<.0001
Error	8	123704.67	15463.08		
Corrected Total	11	19677708.25			

Appendix Table 20. ANOVA for Color

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	31.425	10.475	10.98	<.0001
Error	116	110.700	0.954		
Corrected Total	119	142.125			

Appendix Table 21. ANOVA for Taste

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	18.825	6.275	9.90	<.0001
Error	116	73.500	0.634		
Corrected Total	119	92.325			

Appendix Table 22. ANOVA for Odor

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	44.7000	14.9000	17.89	<.0001
Error	116	96.6000	0.8328		
Corrected Total	119	141.3000			

Appendix Table 23. ANOVA for Mouth feel

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	1.4250	0.4750	0.82	0.4874
Error	116	67.5000	0.5827		
Corrected Total	119	68.9250			

Appendix Table 24. ANOVA Over All Acceptability

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	1.425	0.475	0.82	0.4874
Error	116	67.500	0.582		
Corrected Total	119	68.925			

Appendix Table 25. ANOVA for Phytate: Calcium Molar Ratio

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.7034	0.2345	4689.50	<.0001
Error	8	0.0004	0.0001		
Corrected Total	11	0.7038			

Appendix Table 26. ANOVA for Phytate: Zinc Molar Ratio

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	1.8596	0.6199	3381.14	<.0001
Error	8	0.0015	0.0002		
Corrected Total	11	1.8611			

Appendix Table 27. ANOVA for Phytate: Iron Molar Ratio

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	0.1112	0.0371	25.87	0.0002
Error	8	0.0115	0.0014		
Corrected Total	11	0.1227			

Appendix Table 28. Sensory evaluation questionnaire format

Date -----

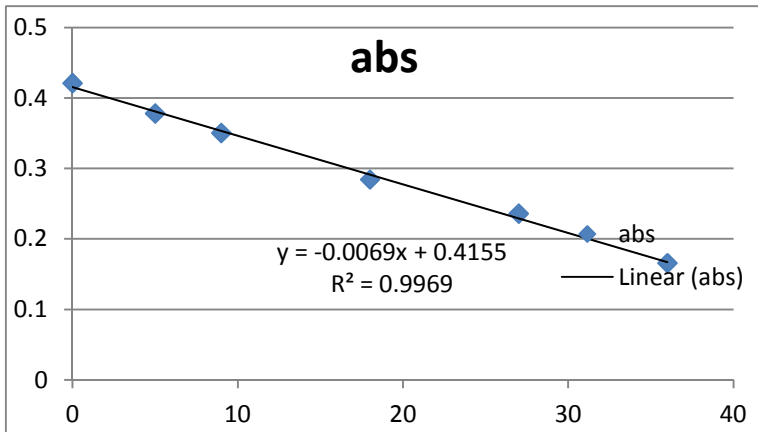
Panelist Name -----

Age -----

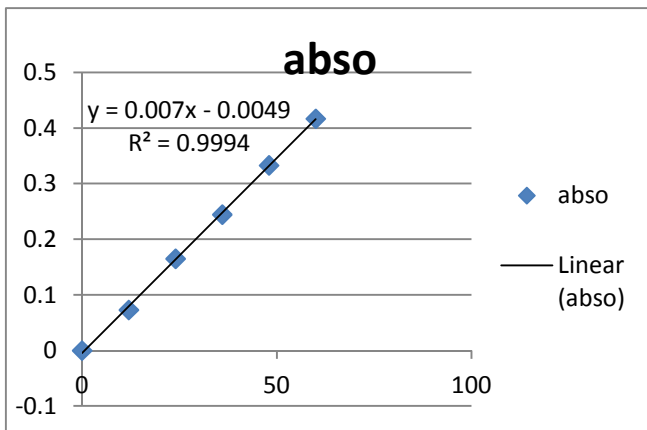
Sex -----

Please read the description part before rating the products.

Sensory perception					
(score)					
Sensory quality attributes					
	Appearance				
	(Color)	Taste	Odor	Mouth feel	Overall acceptability
1=dislike extremely					1=Extremely unacceptable
2=dislike very much					2=very much unacceptable
3=dislike moderately					3=moderately unacceptable
4=dislike slightly					4=Slightly unacceptable
5=neither like nor dislike					5=neither acceptable nor unacceptable
6=like slightly					6=Slightly acceptable
7=like moderately					7=moderately acceptable
8=like very much					8=highly acceptable
9=like extremely					9=Extremely acceptable



Appendix Figure 1. Calibration curve for phytate determination



Appendix Figure 2. Calibration curve for tannin determination

