ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Effect of extrusion cooking on acceptability, nutrient bioaccessibility and anti-nutritional factors of sorghum and maize flours fortified with iron and zinc salts

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A thesis submitted to the center of Food science and Nutrition of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition.

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Declaration

I, the undersigned, hereby declare that this thesis is my original work and all sources of materials used for the thesis have been acknowledged.

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LIST OF ABBREVATIONS

DMRT	Duncan’s Multiple Range Test
EDTA	Ethylene diamine tetra acetic acid
FSSAI	Food Safety and Standard Authority of India
HTST	High Temperature Short Time
LSD	Least Significant Difference
IF	Insoluble Fiber
SF	Soluble Fiber

ABSTRACT

Food fortification stands out among public health intervention as one of the most effective method of preventing nutritional deficiency. Fortification refers to addition of nutrient to foods from which they were either absent or present in small amount to increase the intake of one or more nutrients. Effective fortification program aims to improve the bioavailable of nutrient intake of the whole population with the intent of eliminating or preventing micronutrient deficiencies in the general population and the most venerable groups. Being widely consumed, maize and sorghum flours are suitable vehicles for mass fortification. The main objective of this study was to investigate the effect of extrusion cooking on nutritional compositions, acceptability, level of anti-nutritional factors (phytic acid and tannin) and mineral bioaccessibility of sorghum and maize extrudates fortified with iron and zinc salts. Standard methods were used to analyze the proximate composition, anti-nutritional factors (phytic acid and tannin) and mineral bioaccessibility of the extrudates. The finding showed that fortification of maize and sorghum flours with iron and zinc salts prior to extrusion increased the mineral content of the extrudates produced at three extruding temperatures (110^oC, 130 ^oC and 150 ^oC). Phytic acid decreased from 20% to 28% with increasing extruding temperature ($p>0.05$).The temperature of 150^oC was found to be more effective and significantly reduced phytic acid than the lower temperature of 110 ^oC. Maize and sorghum unfortified extrudates relatively had higher soluble iron and zinc fraction. Increasing the extruding temperature caused increased the percentage of soluble iron fraction from 62.3% to 83.2% and 29.4% to 41.3% for unfortified maize and sorghum extrudates respectively. Soluble zinc percentage also increased from 17.2% to 42.6% and 65.2% to 98.4% for unfortified maize and sorghum extrudates respectively. Extrudates recorded lower sensory acceptability mainly in terms of their color and texture. Extrusion cooking is the ideal method for manufacturing a number of food products and permits the utilization and co-processing of various by products.

Keywords: Fortification, Bioaccessibility, Extrusion, Ferrous sulfate, Zinc sulfate

1. INTRODUCTION

1.1.Back ground

Cereal grains are the fruits of cultivated grasses and they provide humankind with more nourishment than any other food class. While there are about a dozen cereal crops used for food, only wheat, maize, and rice are important human food sources, accounting for 94% of all cereal consumption worldwide, (Dinga *et al.,* 2005). Magnitude of consumption of these cereals varies widely by region whereby; wheat is the major cereal being used for food in Central Asia, the Middle East, South and North America, and Europe; rice in Asia, and maize in Southern and Eastern Africa, Central America, and Mexico(FAO,2010).

The way in which maize is processed and consumed varies from one country to country, with maize flour and meal being two of the most popular products, (Pelembe *et al*., 2001). Consumption can be better estimated by adjusting the values of cereal crops used for food and human food sources by considering the extraction rate (i.e., the proportion of flour or meal produced from the whole-grain cereal, (Pelembe *et al.,*2001); (Carolina *et al,* 2013).

Maize contains about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100g,(Binata and Madhavan, 2002) and provides many of B vitamins and essential minerals along with fiber, However, maize has lower protein, vitamin B12, vitamin C, calcium, folate and iron contents, (Dinga *et al*., 2005). In countries where anemia and iron deficiency are considered moderate or severe public health problems, fortification of maize flour and maize meal with iron and other mineral as well as vitamins has been practiced to improve micronutrient intake and prevent their deficiency (Alonso *et al.*,(2001). Fortification could be an effective strategy to improve iron content of cereal based extruded products. Ferrous sulfate has been widely used to fortify various products such as chocolate drink powders, (Poltronieria *et al*., 2005), infant cereals, and was reported to be well absorbed in the body. Iron absorption enhancers such as ascorbic acid could be added along with fortificants, to improve iron bioavailability from products, (Bhumik and Kalpan., 2009). Sorghum is also the most important cereal with 22% of total cereal area, followed by millets (pearl and finger) with 19% of the total cereal land coverage. The continuing demand for these two crops is reflected in the trend for increasing area under sorghum and millets in Africa over the last fifty years (Macaule, 2015). Micronutrient deficiencies are one of the public health concerns in modern and developing societies affecting millions of people around the world and, impose a heavy burden on physical and intellectual development of at risk populations, especially women and children living in rural areas of developing countries, (Bhumik, and Kalpan., 2009). vitamin A, iron and iodine deficiencies are widespread especially in developing countries and zinc deficiency is gaining attention (Gaur and Visvanathan., 2009). Zinc deficiency is included as a major risk factor to the global and regional burden of diseases along with iron, vitamin A and iodine deficiencies since 2002, WHO/UNICEF, included zinc supplements in their recommended treatment regimen for acute diarrhea, (Pelembe *et al.*,2001), Zinc is required for the activity of more than 100 enzymes involved in most metabolic pathways, and consequently, is necessary for a wide range of biochemical, immunological and clinical functions, (Carolina *et al.,* 2013). As a result, multiple functions in the body are affected by zinc deficiency including physical growth, immune competence, reproductive functions and neurobehavioral development.

Extrusion cooking is one of the most important food processing technologies which have been used since mid 1930s for the production of cereal breakfast, ready to eat snacks, and other textured foods, (Susilowati *et al.*, 2003), where the quality of the product depends on the process conditions, such as extruder type, feed moisture, temperature profile in the barrel sections, screw speed and feed rate, (Elina *et al.,* 2013). High temperature short time extrusion technology is found to degrade the phytate content of grains effectively and increase the ionizable and soluble iron thus increasing the bioavailability of dietary iron, (Elina, *et al*.,2013); (Awika and Rooney, 2004).

Sensory analysis of snack food revealed that the product obtained under optimal conditions has the highest sensory acceptability and presented good digestibility. The major concern of food industry is to optimize the process for an optimum technological level which does not necessarily coincide with the nutritional optimum. Thus, nutritional quality of extruded products should be considered when extrusion conditions are defined. Consumer acceptance of extruded foods is mainly due to the convenience, value, attractive appearance and texture found to be particular for these foods, especially when it concerns snack products, (Awika and Rooney., 2004). Consumers want snacks that taste good, smell good, feel good, look good and in addition, are nutritionally superior and healthy.

1.2. Statement of the problem

Food industries have increased the production of ready-to-eat products using several processes. Among these processes, extrusion is a high temperature-short time treatment and well-established industrial technology, which is characterized by continuous cooking, mixing and processing and produces direct expanded materials with higher quality, (Nair and Iyengar., 2009). The major concern of industry is to optimize the process for the optimum technological levels which do not necessarily concide with the nutritional optimum. Thus, the nutritional quality of extruded products should be considered when extrusion conditions are defined. Many researchers have reported the positive and negative effects of extrusion process on nutritional quality of food and feed mixtures using different extruder conditions such as temperature, feed moisture, screw speed and screw configuration and raw-material characteristics including composition and particle size.

Most micronutrients are concentrated in the outer layers of maize and sorghum. Removing these layers in the milling process results loss of most minerals like iron and zinc. Food fortification is expected to help nutritional inadequacy in targeted population in which risk of nutrient deficiency has been identified. The effectiveness of food fortification program depends on whether or not the fortified food is accepted, purchased, and consumed by the target population. Factors such as quality, taste, and price of fortified products play important roles in determining effectiveness of fortification program. Being widely consumed in middle and low land areas of Ethiopia, maize and sorghum flours are ideal vehicles for mass fortification. Maize and sorghum serve as major staple food in several African and Latin American countries and are being fortified with one or more micronutrients including, iron, zinc, folic acid, and/or vitamin A. Although fortification of food grains with different micro nutrients has been subject to numerous studies little is known about fortification of sorghum and maize flours with different iron salts and zinc prior to extrusion. Little is also to known about the effect of extrusion cooking on sensory acceptability and nutrient bioaccessibility of maize and sorghum based products which are fortified with iron and zinc salts. Therefore, in this study the effect of extrusion process on sensory acceptability and nutrient bioaccessibility in maize and sorghum extrudates fortified with iron and zinc salt was investigated. The effect of iron and zinc fortificant on sensory acceptability of the extrudates also investigated.

1.3. Objectives

1.3.1. General objective

- To determine effect of extrusion cooking on the sensory acceptability, on antinutritional factors and bioaccessibility of iron and zinc from maize and sorghum extruded products fortified with iron and zinc salts.

1.3.2. Specific objectives

- To determine effect of the extrusion cooking on the level of antinutritional factors.
- To investigate bioaccessibility of micronutrients from sorghum and maize extruded products.
- To evaluate the sensory acceptability of iron and zinc fortified maize and sorghum extrudates.

2. LITERATURE REVIEW

2.1. Botanical description, production and utilization of sorghum and maize

2.1.1. Botanical description and production of sorghum

Sorghum [*Sorghum bicolor* (L.) *Moench*], a tropical plant belonging to family Poaceae, is one of the most important crops in Africa, Asia and Latin America. According to Awika and Rooney (2004), more than 35% of sorghum is grown directly for human consumption and the remaining is used primarily for animal feed, production of alcohol and other industrial products, (House *et al*., 2008). Several improved sorghum varieties that are adapted to semi-arid and tropic environments were released every year by sorghum breeders. Selection of varieties that meet specific local food and industrial requirements from the vast biodiversity is of paramount importance for food security.

2.1.2. Worldwide utilization of sorghum

According to Pelembe *et al*., (2001) and (Dinga *et al*., 2005), sorghum bicolor is the fifth most important cereal crop after wheat, rice, maize, and barley in terms of production. The total world annual sorghum production is about 60 million tons from cultivated area of 46 million hectare. House *et al.*, 2008) indicated that, major producers of sorghum are the United States of America, Nigeria, Sudan, Mexico, China, India, Ethiopia, Argentina, Burkina Faso, Brazil and Australia, where Burkina Faso is the world leader in sorghum production and consumption per inhabitant. Sorghum is by nature well suited to heavy soils commonly found in the tropics, where tolerance to water logging is often required. In Africa and Asia, sorghum is used both for human nutrition and animal feed. It is estimated that more than 300 million people from developing countries essentially rely on sorghum as source of energy, (Thymi, 2008).

2.1.3. Maize production and Utilization

Maize is grown throughout the world, although there are large differences in yields (Table 1). Indices of agricultural production of the Food and Agriculture Organization (FAO) of the United Nations include commodities that are considered edible and contain nutrients, and show the relative level of aggregate volume of agricultural production for each year in comparison with the base period of 1999–2001. It is estimated that in 2012, the total world production of maize was 875,226,630 tons, 27 with the United States, China, and Brazil harvesting 31%, 24%, and 8% of the total production of maize, respectively (FAO, 2011).

In the past decade, the world production of maize increased by approximately 40%, reaching about 1 billion tons in 2013 growing season (FAOSTAT, 2013). Major maize-consuming countries are located in Central America and Sub-Saharan Africa. Large scale produced maize is degermed and sold as flour or grits, and can be precooked. In several countries, maize flour is usually consumed as gruel or porridge, and in certain settings, fermentation, soaking and germination are practiced.

2.1.4. Maize and sorghum production and utilization in Ethiopia

Maize is the most widely-grown staple food crop in sub-Saharan Africa (SSA) occupying more than 33 million ha each year (FAOSTAT, 2015). The crop covers nearly 17% of the estimated and consumed by people with varying food preferences and socio-economic backgrounds. More than 300 million people in SSA depend on maize as source of food and livelihood. The top 20 countries, namely South Africa, Nigeria, Ethiopia, Tanzania, Malawi, Kenya, Zambia, Uganda, Ghana, Mozambique, Cameroon, Mali, Burkina Faso, Benin, DRC, Angola, Zimbabwe, Togo, and Cote d'Ivoire, account for 96% of the total maize production in SSA. (FAOSTAT, 2015).

In Ethiopia, maize grows from moisture stress areas to high rainfall areas and from lowlands to the highlands (FAOSTAT, 2015). It is one of the important cereal crops grown in the country. The total annual production and productivity exceed all other cereal crops, though it is surpassed by teff in area coverage (Benti *et al*., 1997). Therefore, considering its importance in terms of wide adaptation, total production and productivity, maize is one of the high priority crops to feed the increasing population of the country.

Millions of people in Ethiopia depend on maize for their daily food especially where maize is the major crop. However, normal maize varieties cannot sustain acceptable growth and adequate health because of low content of essential amino acids. To alleviate the problem, development of quality protein maize (QPM) varieties with high lysine and tryptophan content has been enhanced in the 1990s (Worku *et al*., 2001

Maize yield is influenced by soil fertility conditions of which nitrogen and phosphorus are the most important nutrients for maize production in Ethiopia (Nigussie *et al*., 2003). However, to overcome low soil fertility problems, most of the farmers are constrained by shortage of cash to use inorganic fertilizers (Asfaw *et al*., 1997). Development of drought tolerant germ plasma for moisture stress areas was also started at Melkasa Research Center in the early 1990s under Ethiopian Agricultural Research Organization. Currently, Melkasa Research Center is responsible for development of maize technologies for moisture stress areas in collaboration with Awassa College, Alemaya University and other cooperating centers (Worku *et al.,* 2001).

Sorghum is the fifth most important cereal crop in the world after wheat, rice, corn and barley. Sorghum outperforms other cereals under various environmental stresses and is thus generally more economical to produce. More than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal feed and alcohol and industrial products. Sorghum is used in a variety of foods. The white food sorghums are processed into flour and other products, including expanded snacks, cookies and ethnic foods, and are gaining popularity in areas like Japan (Rooney, 2001).

2.2. Extrusion Technology

Processing methods tend to modify the composition and availability of nutrients in raw materials. Extrusion cooking is one of the food processing methods. It involves economical industrial procedure which combines high pressure with moderately high temperature. Extrusion results in shearing forces in addition to the thermal process applied for short period of time. Extrusion of food is an emerging technology for food industries to process and produce large number of products of varying size, shape, texture and taste, (Kaur *et al.,* 2007).Food extruders are processing machines that belong to the family of HTST with a capability to perform cooking tasks under high pressure. They provide thermo-mechanical and mechanical energy (shear) which is necessary to cause physico-chemical changes of raw materials with an intensive mixing for dispersion and homogenization of ingredients (Awika and Rooney, 2004).

Physical technological aspects like heat transfer, mass transfer, momentum transfer, residence time and residence time distribution have strong impact on food properties during extrusion-cooking and can drastically influence final product quality. Extrusion cooking is preferable to other food-processing methods in terms of continuous process with high productivity and significant nutrient retention, owing to the high temperature and short time required (Mutambuka, 2013). During extrusion cooking, the raw materials undergo different chemical and structural transformations, such as starch gelatinization, protein denaturation, complex formation between amylose and lipids, and degradation reactions of vitamins, pigments, etc.

Nowadays, extrusion-cooking is used for the production of different food products, ranging from the simplest expanded snacks to the highly-processed meat analogues. In the past, extrusion cooking has been studied extensively to produce variety of specialty foods including pasta products and ready to eat breakfast cereals, baby foods, snack foods, texturised vegetable protein, dried soups and dry beverage mixes. It improves food digestibility, (Mutambuka, 2013; Mośćicki, 2011) and bioavailability of nutrients. Compared to conventional cooking, HTST extrusion technology effectively degrades phytate of grains, which subsequently increases ionizable and soluble iron that are bioavailable.

2.3. Raw material for extrusion cooking

Extrusion cooking is characterized by low moisture content (10-40%) and high temperatures (100-180 °C). Wide range of raw materials can be utilized in the process. Extrusion is flexible in the production of new products, such as cereal baby foods, breakfast cereals, snack foods, bakery products, pastas, etc. During extrusion, food materials are thermo-mechanically cooked in a screw-barrel assembly by a combination of moisture, pressure and temperature in order to be mechanically sheared and shaped, (Awika and Rooney, 2004). Through the process, raw materials undergo different chemical and structural transformations.

2.4. Bioavailability of fortificants used for fortification

Bioavailability refers to the amount of ingested micronutrient absorbed and utilized in the body and can be influenced by chemical form of the fortificants, nutrient composition of the fortification premix, dietary composition and food matrix, overall dietary intake and physiological state of target individuals. Choice of the fortificant compound should be made considering factors such as potential for organoleptic changes to the product, bioavailability of the fortificant, cost, stability and shelf-life, (WHO, 2006). Effective fortification programs aim to improve bioavailable nutrient intake of the whole population, with the intent of eliminating or preventing micronutrient deficiencies in the general population, and the most vulnerable groups in particular. Micronutrients affect nutritional status to the extent by which they are available for absorption through the gastrointestinal tract and for systemic utilization. For this reason, bioavailability of fortified micronutrient is a key determinant factor to successful food fortification and describes the fractional amount of micronutrient absorbed and utilized.

To compare different iron compounds, the relative bioavailability is used as a critical measure to judge an iron fortification compound in relation to water-soluble ferrous sulfate, which has by definition a relative bioavailability of 100%, (WHO (2006).

2.5. Iron fortification of cereal based flours

Iron containing compounds such as ferrous sulfate, ferrous fumarate, ferric pyrophosphate and electrolytic iron powder are recommended for cereal fortification by WHO (Gaur and Visvanathan, 2015; Nair and Iyengar, 2009). Contrary to the recommendation, many cereal foods are fortified with low-cost elemental iron powders, which have lower bioavailability (WHO, 2006). The most bioavailable iron fortificants are compounds with the highest solubility in human proximal intestine. However, owing to their chemical and physical properties, water-soluble iron compounds tend to react with the food matrix, causing adverse changes during storage. To achieve an impact which is equivalent to that of highly bioavailable fortificants, larger amounts of lower bioavailable fortificants are required to be used. NaFeEDTA is an iron fortificant and is both water-soluble and iron absorption enhancer, which is related to its chelating properties, (Guy, 2001). In high-phytate cereal diets, NaFeEDTA was shown to have two- to three-fold higher absorption than ferrous sulfate (Mutambuka, 2013). Whereas, in meals prepared with low-extraction flours, it had similar bioavailability to ferrous sulfate, (Mościcki, 2011). According to the current guidelines for maize flour fortification, NaFeEDTA is the first-choice fortificant for whole-grain maize flour and maize flour, (WHO, 2006).

Ferrous sulfate is a water-soluble iron compound that is widely used with many fortification vehicles. Because it is highly water soluble, sensory changes can occur, including increased rancidity, color changes, and off-flavors during storage. Ferrous fumarate, which is poorly soluble in water and tends to cause fewer sensory changes than ferrous sulfate, has also been used for maize flour fortification in countries like Venezuela, (Mutambuka, 2013).

2.6. Zinc fortification of cereal based flours

Although the major source of zinc in human diet is animal foods, majority of the population in developing countries derive the micronutrient from plant foods, especially grains. Staple foods in developing countries include cereals and legumes, which are the main sources of zinc for most of the population. Although zinc intake appears adequate, compromised zinc status is common, (Poltronieria *et al*.,2005). Thus, fortification of staple food grains with zinc is necessitated to prevent zinc deficiency in developing countries. Zinc compounds, which are commonly used in fortification, such as water insoluble zinc oxide and zinc sulfate, are unlikely to differ in their bioavailability in zinc-fortified foods. Zinc fortification of cereal flour is safe and effective, low cost method to increase zinc intake, total absorbed zinc, and zinc status in selected population groups (WHO, 2006). Zinc fortification should be included in flour fortification programs in countries with an elevated risk of zinc deficiency, if flour is consumed in sufficient amounts by target groups. Zinc oxide is fortificant of choice because of its lower cost. The appropriate level of zinc fortification depends on the amount of flour consumption, the degree of flour extraction, and the dietary intake of zinc and phytate. Fortification of staple cereal flours could be cost-effective and sustainable way to improve zinc status in developing countries. It could be achieved by addition of suitable fortificant(s), either on a large scale to centrally processed flour or on a small scale, in local communities. In eastern Africa, maize and wheat flours are suitable vehicles for fortification as they are consumed as part of the main diet.

2.7. Maize flour fortification

Maize flour is fortified in several countries and (Table 2) provides an overview of countries currently adding one or more nutrients to maize flour under mandatory legislation.

Table1. Overview of maize fortification under mandatory legislation of different countries.

Country	Iron fortificant	Amount added Fe(ppm)	Zinc (ppm)	Folic acid (ppm)	Vit.A. IU/kg	Estimated maize flour intake g/Kg
Brazil	Ferrous fumarate	42		1.5		69
Costa rica	Unknown	22		1.3		15
Kenya	NaEDTA	5	30	0.5	0.2	219
Mexico	Ferrous Sulfate	40	40	2		337
Nigeria	Unknown	16	20		9	
South Africa	Electrolytic Fe	35	15	2	2.1	284
Tanzania	NaFeEDTA	10	30	1.5		160
Uganda	NaFeEDTA	40	30	0.5	0.5	66
Venzuela	Ferrous fumarate	50			2.8	160
USA	Unknown	28.6		1.5		

WHO, 2006

2.8. Dietary factors influencing iron and zinc absorption from fortified flours

Bioavailability of micronutrients, particularly zinc and iron, is low from plant foods, (Sandberg, 2002). Bioavailability of iron is known to be influenced by various dietary components, which include both inhibitors and enhancers of absorption. Cereals are known to contain high concentrations of one or more inhibitors of micronutrients absorption. Phytic acid, tannins, dietary fiber and calcium are the most potent absorption inhibitors of iron, while organic acids are known to promote iron absorption (Sreeramaiah et al., 2007; Sandberg, 2002). Phytate also affects zinc absorption from GIT through complaxation and precipitation. As phytate cannot be digested or absorbed, the zinc bound to phytate passes through the intestine unabsorbed. Thus, people who depend on plant based diet face risk of zinc deficiency due to interference with zinc absorbance. Antinutritional effect of phytic acid is attributed to the complexing action of phytate on divalent and trivalent metal cations. In developing countries, typical diets include whole grain flour that contains much higher phytate than low-extraction white flour. In Such cases, NaFeEDTA might be better fortificant than other fortificant for supplementation of iron, because EDTA chelate iron, and might prevent it from binding to phytates, (Guy, 2001).

3. METHODOLOGY

3.1. Sample collection and flour preparation

Sorghum and maize grains were obtained from Melkasa agricultural Research Center. White sorghum (specifically called Teshale) and white maize (MH130) were used for the investigation. The grains were converted into flour using flour miller and sieved using 0.5mm sieve and stored in air tight plastic container for further experiment.

3.2. Fortification of the flours

3.2.1. Iron salt fortification

Fortification of flours with ferrous sulfate has considered the level of native iron content of the flours. Accordingly, maize and sorghum flours were fortified prior to extrusion with 35 and 30 mg ferrous sulfate per kg of flour samples respectively according it is recommended by WHO. For both maize and sorghum samples, two kg of flour was taken based on the minimum capacity of the extruder. So that, 70 mg of ferrous sulfate was added to 2 kg of maize flour sample and 60 mg/kg of ferrous sulfate added to sorghum flour. Again, 2 kg of flour samples were taken from both maize and sorghum flour samples as control (flour with no fortificant) and the fortified samples were mixed for 1 hour using a mixer.

3.2.2. Zinc salt fortification

Based on the level of the native zinc content, maize flour samples was fortified with 30 mg of zinc sulfate per a kilogram of the flour samples and the sorghum flours were fortified with 45 mg of zinc sulfate per kg of flour samples providing that to increase the zinc content of maize and sorghum flours and to meet the daily value based on the recommendation of FAO/WHO (2002). For both maize and sorghum flour samples 2 kg of flour was taken separately and fortificants were added and mixed using a mixer.

3.2.3. Co- fortification process

Maize flour samples were co-fortified with 35mg of ferrous sulfate and 30 mg of zinc sulfate per kg of flour samples. Sorghum flour samples were also co-fortified with 30mg of ferrous sulfate and 45mg of zinc sulfate per kg of flour sample. Due to the working limitation of the extruder, 2 kg of flour was taken for each fortification process for both maize and sorghum flour samples. Then, the co-fortified flours were mixed by using a mixer and by a manual method to ensure the distribution of the fortificants.

3.2.4. Extrusion process

Extrusion was carried out in the Institute of Technology of Food Science and Chemical Engineering laboratory at Bahir Dar University. Moisture content of the fortified flour samples was determined before to extrusion by using Infrared Moisture Goniophotometre apparatus.

Determining the initial moisture content of the fortified flours was required to calculate and to adjust the water flour rate, and to come up with the proposed dough moisture. Twin screw co-rotating extruder (K-MV-KT20 model) was used and snack production was adjusted for maximum expansion using preliminary trials, and the optimum combination of processing variables was adjusted at barrel temperatures of 110°C, 130^{o}C and 150 ^{o}C, screw speed of extruder was adjusted at constant 200 rpm, and the moisture content of the extruder barrel was constant at 24% .Extrudates were collected and dried in hot air oven at 60˚C for 1 hour to remove any residual moisture. Extrusion water and feed rates are described in (Table 2) below. During extrusion, the barrel temperature, die pressure, feed rate, crew torque, and product temperature were recorded when stable. The extrudates were cooled to room temperature and sealed in polyethylene bags by a vacuum packager.

Table2. Extrusion water rate and feed rate combination

Sample type	Stroke level	Flour Flow rate (in rpm)	Die pressure(in Barr)	Extruding temperature (^{o}C)
S_2(zinc*)	12	7	12	110,130,150
MH(zinc*)	13	7	13	110,130,150
S3(co *)	12	7	15	110,130,150
MH (co *)	13	7	12	110,130,150
MH (Ferrous*)	17	9	13	110,130,150
S (ferrous*)	12	7	12	110,130,150
S1(con*)	12	7	12	110,130,150
MH (con*)	18	9	13	110, 130,150

S_2=sorghum fortified with zinc sulfate, MH (zinc*) = maize fortified with zinc sulfate, S3 (co*) =sorghum co-fortified, MH (co*) =Maize co-fortified, MH (Ferrous*) =Maize fortified with ferrous sulfate, S (ferrous) = Sorghum fortified with ferrous sulfate, S1 (con*) = Sorghum control, MH (con*) = maize control

3.3. Proximate composition analyses of extrudates

Proximate composition (moisture contents, ash, crude fat, crude protein, crude fiber and total carbohydrate) of targeted flours and extrudates were determined using (AOAC, 2000) method.

3.3.1. Moisture

Empty porcelain dishes were cleaned and dried using drying oven for one hour at 105 ºC. The dishes were cooled for 30 minute in desiccators with granular silica gel and weighed using a digital analytical balance to the nearest milligram (W_1). Each sample of 5.000 g was weighed (W_2) in dried and pre-weighed porcelain dish. The dishes and their contents were then placed in drying oven and dried for 3 hours at 105 ºC. The dishes and their contents were cooled in desiccators to room temperature and weighed (W_3). The procedure was repeated until a constant weight was attained (AOAC, 2000).

Moisture % $= \frac{(w2-w3)}{w2-w1} X100$ Where, W_1= weight of the crucible

W_2= Weight of crucible and fresh sample

W_3= Weight of the crucible and dry sample

3.3.2. Total ash

Ash was quantified by AOAC (2000; 923.03) as the inorganic residue present after incineration at 550 ˚C for 5 hours. Porcelain crucibles were washed with distilled water and dried in a muffle furnace for 30 min at 550 ˚C. Crucibles was cooled in desiccators for about 30 minutes at room temperature and weighed (W_1). Sample of 2.5 g was weighed to an accuracy of two decimal places in the dish (W_2). The sample was burned on a hot plate under a fume hood by gradually increasing the temperature until smoke ceases to appear. The crucibles were then placed in muffle furnace at 550 ˚C for 5hrs. . Finally, the crucibles were cooled to room temperature and reweighted (W_3).Calculation was:

Total ash % $= \frac{W3-W1}{W2-W1} X100\%$ Where, W_1=mass of the dried dish

W_2=mass of the dish and sample

W_3=mass of the dish and sample

3.3.3. Crude Fat

Crude fat was determined by AOAC (2000; 45.01). Extraction cylinder were washed with hot water and then dried in drying oven at 105 ^{0}c for one hr and cooled in desiccators. Weight of the cooled extraction cylinders were measured by analytical balance and recorded as W_1. The bottom of the extraction thimbles were covered with the layer of fat free cotton. Two gm of powdered samples were weighed into each thimble lined with cotton at their bottom and covered with layer of fat free cotton and thimbles were kept in to extraction chamber. The thimbles with their sample content were placed in to Soxhlet extraction apparatus. Then, 50 ml of petroleum ether was added in to the extraction cylinder and moved in to the heating plank and the extraction conducted for four hours. Then, the extraction cylinder was disconnected and kept in the drying oven at 70 ^{o}C for 30 minutes. Finally, the extraction cylinders were cooled in the desiccators and weighed (W_2).

$$\text{Crude fat } \% = \frac{W2-W1}{W} X100$$ Where,

W_1= mass of dried extraction flask

W_2=Weight of extraction flask and dried crude fat

W=weight of the sample

3.3.4. Crude protein

Crude protein of the flours and extrudates were determined by the Kjeldahl method (AOAC, 2000, 979.09) using Kjeltec system. Known quantity (0.5g each) of each flour or extrudate was measured and transferred to tecator tube. The tecator tube was then placed in a tecator rack; six ml of concentrated sulphuric acid was added and digested in the presence of a catalyst using a digester which was adjusted to 370 ^{o}C. The digested solution was neutralized with 25ml of 40% NaOH. A 250ml conical flask contacting 25ml of boric acid, 25ml of distilled water and indicator solution was placed under the condenser of the distiller with its tip immersed into the solution. The distillation was continued until a total volume become between 200 and 250ml. Finally, the distilled solutions were titrated with 0.1N HCl. Total nitrogen content of the samples were latter calculated using the following formula:

Calculation: Nitrogen (%) = $\frac{V_{Hcl} \text{ in L} \times \text{N Hcl (ca 0.1)} \times 14.00 \times 100}{Wo}$

Where, V- volume of HCL in L consumed to the end point of titration

N- The normality of HCL (0.1N)

W_O- weight of sample

14.00- the molar weight of nitrogen

Protein Conversion: **Protein (%) = 6.25 × % nitrogen**

3.35. Crude fiber

Crude fiber was determined by the method of AOAC (2000; 962.09), as the combustible and insoluble organic residue was obtained after the samples were subjected to acid digestion and then alkaline distillation. Clean crucible was dried with one gm celite in oven maintained at 105 °C for one hour and placed in desiccators to cool. One g of each sample was measured in the dried crucible using analytical balance (W_1). Two hundred ml of 1.25% H_2SO4 (R1) solution was added to each beaker and allowed to boil for 37 minutes. The acid was later drained using vacuum pump; sample was cooled for five minutes and then washed three times using distilled water. The same step (as H_2SO4) was followed using 1.25% NaOH solution (R2) except that column was used instead of beaker. Crucibles containing residue was dried at 130 °C for two hours by drying oven cooled in desiccators and weighed (W_2). The crucibles were transferred to muffle furnace and kept for three hours at 525 °C. Crucible containing ash was later cooled in desiccators and weighted (W_3). The crude fiber content was measured using the following formula.

Calculation was:

Crude fiber $\frac{g}{100g} = \frac{W2-W3}{W1} \text{ x } 100$ Where,

W_2=mass of the crucible
W_3=mass of the crucible and the sand
W_1 = Weight of sample

Carbohydrate content was determined by difference which was calculated as follow:

% Carbohydrates = 100 – (%protein+ % Fat + % ash + % total dietary fiber)

Gross energy determination in kilo calories

The gross energy of each extrudate samples were estimated (in Kcal/g) by multiplying the percentage of crude protein, crude fat and total carbohydrate with recommended factors.

Total energy (Kcal/100g) = (4*crude protein + 9*crude fat +4* carbohydrate).

3.4. Determination of antinutritional factors of extrudates

3.4.1. Phytate

Phytate was determined according to the method described by Latta and Eskin (1980), and as cited in Adane *et al*., (2013).

Each of the maize and sorghum extrudates (0.1gram each) were transferred to centrifuge tubes and extracted with 10ml of 0.2 N Hcl for one hr at room temperature and centrifuged at 3000 rpm for 30minutes. Clear supernatant was used for phytate determination. Two ml of wade reagent was added to three ml of the supernatant sample solution, homogenized and centrifuged at 3000 rpm for 10 seconds. Absorbance reading of the respective solution was then measured against 0.2N HCl and wade reagent (3:2 ratios) mixture blank at 500nm using UV-Vis spectrophotometer. The amount of phytic acid was calculated using phytate acid standard curve and the result was expressed as phytate in µg/g fresh weight. Serial dilution of standard phytic acid was prepared from 40 µg/g phytic acid in 0.2N HCL. The same procedure as samples was followed to read absorbance reading of the standard phytic acid.

The level of phytate was then calculated using the following formula.

$$\text{Phytate acid in } \mu g/g = \frac{\text{Ab} - \text{As} \ \ - \text{intercept} \ \ * 10}{\text{Slope} \times \text{W} \times 3}$$

Where As = sample absorbance

Ab = blank absorbance
W = weigh of sample

3.4.2. Tannin

The tannin content of the extrudates flour samples was determined by the method of Burns (1971). The extrudates flour samples were extracted with 1% Hcl in methanol for 24 hours with mechanical shaking at room temperature and centrifuged at 3000 rpm for 5 minutes. Supernatant was taken and analyzed for condensed tannin using 0.4% vanillin.

Accurately 4g of flour sample were measured in a screw cap test tube and extracted with 10 ml of 1% Hcl in methanol and allowed to shake for 24 hours at room temperature by putting on the mechanical shaker. After 24 hour of shaking time, centrifuged for 5 minutes to make clear supernatant.1ml of clear supernatant was taken in another test tube and mixed with 5 ml vanillin-Hcl reagent and waited for 20 minutes to complete the reaction. The absorbance was observed by using UV- Visible spectrometry at 500nm. D-catechin was used as standard for tannin determination where, 40mg of D-catechin was dissolved in 100 ml of volumetric flask with 1% Hydrochloric acid and used as stock solution. A serious of Standard was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1m from D-catechin stock solution in a test tube and the volume of each stock taken was adjusted to 1ml with 1%Hcl in methanol. Then, 5ml of vanillin-Hcl reagent was added to each test tube and waited for 20 minutes to complete the reaction. Finally, absorbance was observed by using UV- Visible spectrometry at 500nm.

Calculation:

$$\text{Tannin in mg/g} = \frac{[(As-Ab)-\text{Intercept}]*10}{\text{Slope x d x w}}$$

where,

As Absorbance of sample

Ab= Absorbance of blank

d = Density of the soln (0.791gm/ml)

W = Sample weight

3.5. Total iron and zinc determination of the extrudates

Mineral content of extrudates was estimated using atomic absorption spectrophotometer (AAS) by dry ashing according to AACC method AACC, (2000).

Crucibles and glass wares were washed with 10% nitric acid and placed in the oven at105^{O}C for 1hour. Crucibles were cooled in the desiccator for 30 minutes and accurately 2.5 gm extrudates flour sample was taken and charred on the hot plate under the hood until the smoke was completed. Then, the samples were ashed in the muffle furnace at 550 ^{O}C for 5 hours and taken out from the furnace and cooled in the desiccator and the total ash weight was measured. Some drops of deionized water was added to moisten it and evaporated on the hot plate and some drops of concentrated nitric acid were added and evaporated again on the hot plate and ashed once more for 30 minutes to ensure its complete ashing.

Dissolution of the ash was started by treating the ash with 7ml of 6N Hcl to wet it completely and carefully taken to the dryness on a lower temperature hot plate. Then, 15ml of 3N Hcl and heated on the hot plate until the solution just boils and cooled and filtered through the filter paper in to a 50 ml volumetric flask. Again, 10 ml of 3N Hcl was added to the crucible and heated until the solution just boil and cooled, filtered in to the graduated flask. Crucibles were washed with deionized water three times and the washing was filtered in to the flask. The filter paper was washed thoroughly with deionized water and the washing was collected in to the flask. Finally, the contents of the flask was diluted and marked to 50 ml with deionized water. The sample solutions were transferred in the urine cap bottle until the time of analysis. Afterwards Fe and Zn contents of the extrudates were determined by using AAS. The blank was prepared by taking the same amount of the reagents following the same instruction used for the sample. Calculation was:

$$\text{Metal content } \frac{\text{mg}}{100\text{g}} = \frac{[(\text{CS} - \text{Cb}) * \text{V}]}{10 * \text{w}}$$

Where,

C_s: concentration of sample in ppm

C_b: Concentration of blank in ppm

V: Volume (ml) of the extract

W: weight (g) of sample

3.6. Mineral Bioaccessibility of Extrudates using In-Vitro Enzyme Digestion

Extrudates were subjected to in-vitro enzymatic digestion with pepsin and pancreatin according to the method described by Ikeda (1990).

Enzyme solution containing 16 mg pepsin and 3.5 ml of 0.06 N Hcl, 1.0 g sodium chloride made up to 100 ml with deionized water was prepared. Another solution containing 1.6 g of pancreatin was dissolved in 7.5 PH phosphate buffer and made up to 100 ml with same buffer was also prepared. In a test tube, 10 ml of pepsin enzyme solution was added to 0.25 g of the sample. The test-tubes were closed and shakened and incubated at 37 °C for 3 h. After peptic digestion, the PH of the samples was adjusted to 8.0 using phosphate buffer. Toluene was added to the buffer to prevent the growth of micro organisms. 12.5 ml of Pancreatin solution was then added to the digestion mixtures and samples were subsequently incubated for 20 h at 37 °C. After digestion, the suspensions were placed in ice-cold vessel and then clarified by centrifugations at 3000 rpm for 20 min. The supernatants obtained were subjected to mineral analysis using atomic absorption spectrophotometer (AAS). The results obtained were used in calculating the percentage of minerals in the extrudates that are bioavailable in soluble fractions.

Calculation was:

$$\text{Mineral in soluble fraction } \% = \frac{X}{Y} X100\%$$

Where X = Minerals in soluble forms after digestion

Y = Total minerals

3.7. Sensory Analysis

The extruded products were evaluated for their sensory acceptability by 10 panelist graduate students at center of Food science and Nutrition, Addis Ababa University. The samples were coded with three random digit numbers and presented to the panelists. A total of twenty four blind coded extrudates were delivered to each panelist with two rounds of sensory analyses days. The panelists were delivered with 12 sorghum and maize based extrudates each to evaluate the sensory properties and over all acceptability of the extrudates fortified with ferrous sulfate, zinc sulfate and co-fortified with ferrous sulfate and zinc sulfate, extruded with three different extrusion temperatures (110 ^{o}C,130 ^{o}C and 150 ^{o}C). Informed consent to participate in the study was obtained from each of the panelists. Score record sheets were prepared based on seven point hedonic scale. Descriptive terms (color/appearance, flavor, texture, taste, overall acceptability) were provided to the panelists and they were requested to rank all products on seven hedonic scale (1 = extremely dislike, 2= dislike very much, 3= moderately dislike, 4 = neither like nor dislike, 5= like moderately, 6 = like very much and 7= extremely like). Sensory parameters like color, hardness, texture, flavour and over all acceptability were compared with control sample. Mean values of the scores from all 10 panelists for each of the attributes were analyzed.

3.8. Statistical Analysis

The raw data was interred in to Microsoft Excel sheet and transported to SPSS version 20 for statically analyses using appropriate tool. One way ANOVA was used to test level of statistical significance difference mean among dependent variables. Mean comparison between variables was done by Duncan's Multiple Range Test (DMRT). A 'p' value of less than 0.05 was considered as significant.

4. Results and Discussion

4.1. Proximate composition

The proximate composition of raw maize and sorghum flour samples and extrudates (both maize and sorghum extrudates) fortified with ferrous sulfate, Zinc sulfate and co-fortified with ferrous and zinc sulfate were summarized in Table 3 and Table 4 respectively.

Table3. Proximate composition of raw maize and maize extrudate.

Maize extrudates	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Fiber (g/100g)	Total CHO(g/100g)	G/Energy (Kcal/g)
MH130	10 ± 0.1^{cd}	11.2 ± 0.2^{f}	1.6 ± 0.00^{a}	5.5 ± 0.4^{f}	1.56 ± 0.04^{ab}	80.34 ± 0.27^{a}	414.86 ± 0.75^{e}
MC 110	9.8 ± 0.5^{c}	10.3 ± 0^{cd}	1.4 ± 0.3^{a}	3.5 ± 0^{e}	2.14 ± 0.06^{d}	83.08 ± 0.15^{b}	386.1 ± 1.95^{ab}
MC 130	9.4 ± 0^{cd}	10.5 ± 0.0^{d}	1.6 ± 0^{a}	2.75 ± 0.3^{bcd}	1.73 ± 0.13^{b}	83.76 ± 0.16^{cd}	391.24 ± 0.90^{cd}
MC150	9.4 ± 0.3^{c}	10.6 ± 0.1^{e}	1.4 ± 0.3^{a}	2.5±0bc	1.44 ± 0.163^{a}	83.37 ± 0.07^{bc}	388.46 ± 1.67^{abcd}
MF 110	9.5 ± 0.1^{c}	10 ± 0.3^{b}	1.6 ± 0^{a}	2.5 ± 0.0^{bc}	1.69 ± 0.13^{b}	84.21 ± 0.56^{de}	391.22 ± 3.37^{cd}
MF 130	10.1 ± 0^{d}	10.1 ± 0.1^{bc}	1.2 ± 0^{a}	2.5 ± 0^{bc}	2.14 ± 0.03^{de}	84.1 ± 0.11^{de}	387.44 ± 0.11^{abc}
M F 150	9.9 ± 0.1^{cd}	9.7 ± 0.1^{a}	1.2 ± 0^{a}	3 ± 0^{cde}	1.87 ± 0.03^{bc}	84.24 ± 0.17^{de}	386.52 ± 0.11^{ab}
MZ110	9.6 ± 0^{cd}	10 ± 0^{b}	1.4 ± 0.3^{a}	1.76 ± 0.3^{a}	2.31 ± 0.42^{e}	84.54 ± 0.11^{ef}	388.92 ± 0.39^{abcd}
MZ130	9.7 ± 0.1^{cd}	10 ± 0.0^{bc}	1.4 ± 0.3^{a}	2.75 ± 0.4^{b}	1.79 ± 0.07^{b}	85.01 ± 0.07^{f}	391.96 ± 1.86^{d}
MZ150	8 ± 0.3^{a}	9.7 ± 0.1^{a}	1.2 ± 0^{a}	2.25 ± 0.4^{ab}	2.34 ± 0.49^{e}	84.52 ± 0.54^{ef}	387.66 ± 1.61^{abc}
MCO110	9.4 ± 0.1^{b}	10.2 ± 0^{bc}	1.2 ± 0^{a}	2.5 ± 0^{bc}	1.43 ± 0.19^{a}	83.68 ± 0.19^{bcd}	390.3 ± 0.76^{bcd}
MCO130	9.4 ± 0^{c}	10.7 ± 0.1^{e}	1.4 ± 0.3^{a}	3.25 ± 0.4^{dc}	1.34 ± 0.57^{a}	83.31 ± 0.13^{bc}	388.64 ± 3.05^{abcd}
MCO150	8.7 ± 0.5^{ab}	10.0 ± 0.5^{bc}	1.4 ± 0.3^{a}	2.17 ± 0.7^{e}	2.06 ± 0.01^{cd}	81.74 ± 0.41^{a}	384.76 ± 1.47^{a}

Results were expressed as Mean ± standard deviation, n=2.
Means in the same column with same letters are not significantly different ($P < 0.05$)

MH130= Raw maize , M C110=Maize control extruded at110°C, M C130= Maize control extruded at 130°C, M C150=Maize control extruded at 150°C, M F110 =maize fortified with ferrous sulfate and extruded at110°C, M F130=Maize fortified with ferrous sulfate and extruded at130°C, M F150=Maize fortified with ferrous sulfate and extruded at150°C, M Z 110= Maize fortified with zinc sulfate and extruded at110°C, M Z130= Maize fortified with zinc sulfate and extruded at130°C, MZ150= maize fortified with zinc sulfate and extruded at150°C, M Co110 °C= Maize co fortified with iron and zinc salts and extruded at110°C, MCo130= Maize co fortified with iron and zinc salts and extruded at130°C, MCo150= Maize co fortified with iron and zinc salts and extruded at150°C.

4.1.1. Moisture

The moisture content of raw maize and sorghum flour samples is 10.0 and 10.4 g/100g respectively. This result is comparable with that of reported by (Binata and Madhavan, 2002). Reduced moisture content of the extrudates was recorded upon increasing the extruding temperature.

This study showed that, the moisture content of maize extrudate fortified with ferrous sulfate is 9.5, 10.1and 9.9 g/100g for MF110, MF130 and MF150 respectively, however there is no significance difference within extrudates. Even though, the moisture content of the extrudates decrease as the extruding temperature increase, the decrease was not significant ($p<0.05$).

For maize extrudates with no fortificants the moisture content obtained is 9.8, 9.4 and 9.4 for MC110, MC13 0and MC150 respectively ($p>0.05$). Extrudates produced at 150°C relatively has lower moisture content than extrudate produced at 110 °C and 130°C. Similar trend was observed for maize extrudates fortified with zinc sulfate and co-fortified with ferrous sulfate and zinc sulfate.

For sorghum extrudates (SF, SZ, SCo and SC) the moisture content ranges from 8.2 g/100g to 11.2g/100g for minimum and maximum value as indicated in Table 4. Adding iron and zinc fortificants to flours has no effect on the moisture content of the extrudates. However, during extrusion process upon increasing the extruding temperature, decreased moisture contents of extrudates was observed, but the decrease was not significant within the extruding temperatures ($p>0.05$). This might be due to the range between the extruding temperature (110°C, 130°C and 150°C) is small so that, significant difference was not observed.

Fig.1 Extruder Machine and extrusion feeder with twin screw

Fig.2. Extruding process and collecting the extrudate

4.1.2. Protein

Data on the protein content of the raw and extrudate flour samples are presented in Table 3.The protein content of the raw maize and sorghum flour samples is 11.2 and 11.05 g/100g respectively. The protein content of unfortified maize extrudates is 10.3, 10.5 and 10.6 for MC110, MC130 and MC150 respectively so that, the extrudates has lower protein content than the raw samples. Camire, (2001) explained that the reduction in protein content during extrusion is due to loss of amino acids during Millard reaction.

For ferrous iron fortified extrudates (MF110, MF130 and MF150) the protein content is 10, 10.1 and 9.7 g/100g was result obtained in respective to their extruding temperatures and there was no significant difference between MF110 and MF130 but, MF150 was significantly decreased and different than MF110°C and MF130°C ($p<0.05$). Similar protein reduction effect was observed for zinc sulfate fortified and co-fortified extrudates.

For unfortified sorghum extrudates the recorded protein content was 10.8, 8.6 and 10.8 g/100g for SC110, SC130 and SC150 respectively. Relatively the same protein result was also obtained for ferrous sulfate fortified extrudates and zinc sulfate extrudates with slightly decreased protein value was obtained but, the decrease in protein content within the extrudates is not significant ($p>0.05$). In general observation, there was no significant difference ($p>0.05$) between unfortified and fortified extrudate samples within the extruding temperature.

Extrusion process decreased the protein content of the extrudates but, the decrease was not significant between 110°C and 130°C extruding temperature ($p<0.05$). Extruding at higher temperature (150°C) significantly decreased the protein content of the extrudates ($p<0.05$).This trend was also observed by Anuonye *et al*., (2009). The protein reduction during extrusion might be due to the gelatinization and protein denaturation effect of the extrusion (Rampersad *et al.*, 2003, Yaqoub *et al*., 2009). Iwe *et al.,* (2001), also reported that, effect of extrusion cooking on proteins is mainly due to loss of amino acids during Millard reactions and protein denaturation.

Table 4. Proximate composition of sorghum extrudates.

Sorghum extrudate	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Fiber(g/100g)	CHO(g/100g)	G/Energy (Kcal)
S1	10.4±0.4^{d}	11.6±0.1^{b}	1.6±0.1^{a}	3.6±0.1^{c}	2.02±0.02^{c}	81.18±0.6^{a}	403.52±0.24^{e}
SC110	9±0.3^{bc}	10.8±0.4^{a}	1.5±0.4^{a}	1.5±0^{a}	1.67±0.2^{a}	84.53±0.19^{e}	394.82±0.23^{bc}
SC130	8.6±0^{ab}	10.6±0.4^{a}	1.6±0^{a}	1.5±0^{a}	2.05±0.0^{c}	84.25±0.14^{de}	392.9±0.00^{ab}
SC150	8.2±0^{a}	10.8±0.4^{a}	1.4±0.3^{a}	1.8±0.4^{a}	2.12±0.0^{c}	83.94±0.18^{cde}	394.67±3.04^{bc}
SF110	11.4±0^{e}	10.7±0^{a}	1.6±00^{a}	1.5±0^{a}	2.7±0.0^{e}	83.49±0.00^{abc}	390.26±0.00^{a}
SF130	9±0.3b^{c}	10.7±0^{a}	1.3±0.1^{a}	1.4±0.1^{a}	2.1±0.0^{c}	84.49±0.01^{e}	393.32±1.27^{ab}
SF150	8.4±0.1^{a}	10.7±0.3^{a}	1.6±0^{a}	1.5±0^{a}	1.75±0.07^{ab}	84.46±0.36^{e}	394.12±0.31^{b}
SZ110	10.4±0^{d}	11.4±0^{bc}	1.4±0.3^{a}	1.5±0^{a}	2.5±0.2^{d}	83.25±0.49^{ab}	392.08±1.95^{ab}
SZ130	9.5±0.4^{c}	11.7±0.3^{c}	1.6±0^{a}	1.8±0.4^{a}	1.72±0.1^{ab}	83.23±0.55^{ab}	395.79±1.65^{bcd}
SZ150	10.2±0^{d}	11.4±0.0b^{c}	1.5±0.1^{a}	1.5±0^{a}	1.89±0.1^{abc}	83.76±0.03^{bcd}	393.94±0.16^{b}
SCO110	11.2±0.3^{e}	10.8±0.1^{a}	1.6±0^{a}	2.5±0^{b}	1.89±0.1^{abc}	83.21±0.21^{ab}	398.54±0.28^{d}
SCO130	11.2±0.4^{e}	11.3±0.1^{b}	1.6±0^{a}	2.3±0.4^{b}	1.95±0.0^{bc}	82.91±0.21^{a}	397.91±0.55^{cd}
SCO150	9.1±0.1^{bc}	11.5±0.1^{bc}	1.4±0.3^{a}	1.8±0.4^{a}	1.99±0.04^{c}	83.36±0.17^{abc}	395.19±3.06^{bcd}

Results were expressed as Mean ± standard deviation, n=2.
Means in the same column with same letters are not significantly different (*P* < 0.05)

S1= sorghum raw, S C110=sorghum control extruded at110^{o}C, S C130= sorghum control extruded at 130^{o}C, SC150=sorghum control extruded at 150^{o}C, S F110 =sorghum fortified with ferrous sulfate and extruded at110^{o}C, S F130=sorghum fortified with ferrous sulfate and extruded at130^{o}C, S F150= sorghum fortified with ferrous sulfate and extruded at150^{o}C, S Z 110= sorghum fortified with zinc sulfate and extruded at110^{o}C, S Z130= sorghum fortified with zinc sulfate and extrude at130^{o}C, SZ150= sorghum fortified with zinc sulfate and extrude at150^{o}C, S Co110 ^{o}C= sorghum co fortified with iron and zinc salts and extruded at110^{o}C, SCo130= sorghum co fortified with iron and zinc salts and extruded at130^{o}C, SCo150= sorghum co fortified with iron and zinc salts and extruded at150^{o}C.

4.1.3. Ash

Ash gives an indication of inorganic elements that are present in a food as minerals. The ash content of raw flour samples is 1.6 g/100g for both maize and sorghum flour samples. The ash content of the maize extrudates ranges from 1.6g/100g to 1.2g/100g for maximum and minimum ash value respectively. The ash content of the extrudates decreased as compared to the raw flour samples. The ash content of sorghum extrudates ranges from 1.6g/100g to 1.4g/100g maximum and minimum ash value respectively, but significant difference was not observed within and among the extrudates. Adding different fortificants to the sorghum flour samples prior extrusion has not affected the total ash content of the extrudates. Extrusion has no significant effect on ash contents of the extrudates of iron salt fortified, zinc salt fortified, co-fortified and on unfortified extrudate samples. This might be due to organic matters could not be affected at lower temperature. Simons *et al.,* (2012), also did not find a significant effect of extrusion on ash composition of kidney beans and faba beans.

4.1.4. Fat

The fat content of the raw maize and sorghum flour samples were 5.5g/100g and 3.5g/100g respectively. Both maize and sorghum extrudates has lower fat content than the raw samples. The fat content of the maize extrudates ranges from 3.5g/100g to 1.76g/100g maximum and minimum fat content of the extrudates. Sorghum extrudates recorded fat content ranging from 1.5 g/100g to 2.5g/100g for minimum and maximum fat value. For unfortified sorghum extrudates the fat content was 1.5, 1.5 and 1.8 but there is no significant difference within extrudates ($p>0.05$). Addition of different iron and zinc salt to flour samples prior to extrusion has no effect on the fat content of the samples.

4.1.5. Fiber

Fiber content of raw maize and sorghum flour samples 1.56 and 2.02g/100g respectively as indicated in Table 3 and Table 4. The fiber content of maize extrudates ranges from 1.34 to 2.34g/100g minimum and maximum values respectively and there is significant difference within the extrudates varying with extruding temperature. For unfortified maize extrudates the fiber content recorded was 2.14, 1.73, and 1.44g/100g for MC110, MC130 and MC150 respectively, and there is significant difference among the extrudates ($p<0.05$).

As compared to the raw flow samples, both maize and sorghum extrudates has increased dietary fiber. This is considered due to during extrusion the decrease in insoluble fiber and increase in soluble fiber, showing that extrusion process caused a redistribution of the IF and SF fractions Mutambuka., (2013). Vasanthan.,(2002), also explained that change in dietary fiber profile during extrusion of barley flour might be attributed primarily a shift from insoluble dietary to soluble dietary fiber.

As compared to maize extrudates sorghum extrudates has lower fiber content with significant difference within the extrudates ranging from 1.67g/100g to 2.5g/100g for minimum and the maximum fiber content of the sorghum extrudate samples respectively. As indicated in this study, increasing the extruding temperature result increased fiber content of the extrudates. This is due to extruding temperature causes conversion of insoluble fiber into soluble fiber, as a result the total fiber content of the extrudates increased.

4.1.6. Total carbohydrate and energy

The total carbohydrate content of raw maize and sorghum flour samples was 80.34g/100g and 81.18g/100g respectively. The total carbohydrate of the maize extrudates (MC, MF, MZ and MCo) was ranges from 80.34g/100g to 85.01g/100g however there is no significant difference within the extrudates ($p>0.05$). Extrudate has relatively higher total carbohydrate than the raw flour samples. The sorghum extrudates total carbohydrates ranging from 82.91g/100g to 84.53g/100g for minimum and maximum values respectively, however there is no significant difference within the extrudates.

The gross energy content of maize and sorghum raw flour samples was 414.86Kcal/100g respectively. This finding was similar to the result reported by paulos (2009). The gross energy of maize extrudates ranges from 384.76 to 391.96Kcal/100g for maximum and minimum gross energy values and for sorghum extrudates gross energy ranging from 398.54Kcal/100g to 390.26 Kcal/100g maximum and minimum gross energy values respectively, but, there is no significant difference among the extrudates ($p<0.05$).

4.2. Anti-nutritional factors

4.2.1. Phytic Acid

Data on phytic acid of raw and extruded maize and sorghum flours are presented in Table 5.

The mean value for phytic acid content of the raw maize and sorghum flour samples (MH130 and S1) is 276.7 and 271.16mg/100g respectively.

Table5. Phytic acid and Tannin content of the raw and extrudate samples

Maize extrudates	Phytic acid(mg/100g)	Tannin(mg/100g)	Sorghum extrudate	Phytic acid (mg/100g)	Tannin (mg/g)
MC110	219.17±0.30^{b}	Absent	SC110	243.83±0.11^{c}	0.65±0.01^{a}
MC 130	212.83±0.63^{ab}	Absent	SC130	232.17±0.11b^{c}	Absent
MC150	210.49±0.10a^{b}	Absent	SC150	230.67±0.94^{ab}	Absent
MF110	220.67±0.47^{b}	Absent	SF110	234.50±0.94^{ab}	Absent
MF130	210.23±0.01^{ab}	Absent	SF130	233.67±0.61^{bc}	Absent
MF150	197.17±0.11^{a}	Absent	SF150	231.10±0.30^{bc}	Absent
MZ110	210.1±0.14^{ab}	Absent	SZ110	233.67±0.42^{bc}	0.62±0.04^{a}
MZ130	210.0±0.42^{ab}	Absent	SZ130	227.99±0.32^{ab}	Absent
MZ150	203.5±0.35^{ab}	Absent	SZ150	226.83±0.02^{ab}	Absent
M Co110	216.3±0.12^{b}	Absent	S Co110	219.84±0.16^{a}	Absent
M Co 130	203.49±0.82^{ab}	Absent	S Co130	225.17±0.10^{ab}	Absent
M Co 150	202.83±0.83^{ab}	Absent	S Co-150	225.67±0.28^{ab}	Absent
MH130	276.7±0.37^{d}	Absent	S	271.16±0.05^{d}	0.68±0.01^{a}

Results were expressed as Mean ± standard deviation, n=2.
Means in the same column with same letters are not significantly different (*P* < 0.05)

MH130=Raw maize, M C110=Maize control extruded at110°C, M C130= Maize control extruded at 130°C, M C150=Maize control extruded at 150°C, M F110 =maize fortified with ferrous sulfate and extruded at110°C, M F130=Maize fortified with ferrous sulfate and extruded at130°C, M F150=Maize fortified with ferrous sulfate and extruded at150°C, M Z 110= Maize fortified with zinc sulfate and extruded at110°C, M Z130= Maize fortified with zinc sulfate and extruded at130°C, MZ150= maize fortified with zinc sulfate and extruded at150°C, M Co110 °C= Maize co fortified with iron and zinc salts and extruded at110°C, MCo130= Maize co fortified with iron and zinc salts and extruded at130°C, MCo150= Maize co fortified with iron and zinc salts and extruded at150°C. S C110=sorghum control extruded at110°C, S C130= sorghum control extruded at 130°C, SC150=sorghum control extruded at 150°C, S F110 =sorghum fortified with ferrous sulfate and extruded at110°C, S F130=sorghum fortified with ferrous sulfate and extruded at130°C, S F150=sorghum fortified with ferrous sulfate and extruded at150°C, S Z 110= sorghum fortified with zinc sulfate and extruded at110°C, S Z130= sorghum fortified with zinc sulfate and extrude at130°C, SZ150= sorghum fortified with zinc sulfate and extrude at150°C, S Co110 °C= sorghum co fortified with iron and zinc salts and extruded at110°C, SCo130= sorghum co fortified with iron and zinc salts extruded at 130 °C,SCo150= Sorghum co-fortified with iron and zinc salt extruded at 150°C

Extrusion process caused a significant reduction in phytic acid content of the extrudates produced at three different extruding temperatures (110°C, 130 °C and 150 °C) as compared to the raw samples but, the reduction was not significant within the extruding temperature. This is considered due to the range between extruding temperatures is small.

As indicated in (Table 5) for maize extrudates reduced phytic acid was observed as extruding temperature increased as compared to the raw flour samples. For unfortified maize extrudates the phytic acid content is 219, 212 and 210 mg/100g for MC110, MC130 and MC150 respectively where the phytic acid content was reduced through the extruding process. But, the reduction was not significant within the extrudates ($p>0.05$). The phytic acid content reduction percentage of unfortified extrudates (control extrudates) was 20%, 23% and 23.9% respectively within the extruding temperature. The phytic acid content of the ferrous sulfate fortified extrudates also reduced with 20.2%, 24% and 28% respectively for MF110, MF130 and MF150 as compared to the raw flour samples.

Zinc sulfate fortified and co-fortified maize extrudates also observed for reduced phytic acid content within the extruding temperature and in comparison to the raw flour samples. The reduction percentage is 24.1%, 24.3% and 26.5% for zinc sulfate fortified maize extrudates (MZ110, MZ130 and MZ150°C) and 21.8%, 26.5%, and 26.8% for co-fortified maize extrudatesMCo110, MCo130 and MCo150 respectively. Extruding at higher temperature (150°C) shows a significant reduction in phytic acid content of the extrudates.

Sorghum extrudates relatively has higher phytic acid content that maize extrudates as indicated in (Table 5) and their phytic acid content varies from 219.84 to 243.83 mg/100g minimum and maximum values respectively. The phytic acid content of unfortified sorghum extrudates is reduced with 10%, 14% and 15% for SC110, SC130 and SC150 respectively as compare to the raw samples.

For ferrous sulfate fortified extrudates, extrusion caused reduced phytic acid content in 13.5%, 13.8% and 14.8% for SF110, SF130 and SF150 respectively. The same effect was observed for Zinc sulfate fortified and co-fortified extrudates. Increasing the extruding temperature significantly increased the percent of phytic acid reduction. Generally, this study indicated that, extruding at higher temperature (150^{o}C) has significant effect on reducing the phytic acid content of the extrudates ($p<0.05$) than the lower extruding temperature.

The reduction in this anti- nutrient can be explained by partial degradation of the phosphate molecule and it is also, considered during extrusion part of inositol hexaphosphate was hydrolysed to penta-phosphates and tetra phosphates. Thermal hydrolysis is credited for effects of extrusion cooking on phytates. Karla *et al*., (2010) were reported that, during extrusion process the inositol hexaphosphate is hydrolyzed to lower weight intermediaries that form insoluble complex with other components reducing phytate availability.

Alonso *et al*., (2000) also, studied the comparative effect of extrusion cooking, dehulling, soaking, and germination on reduction of phytic acid and tannin in kidney beans and faba beans. They reported that, extrusion cooking effectively inactivated the phytic acid in the two beans under study. Extrusion hydrolyses phytate to release phosphate molecules. Similar phytate reduction has been observed by Ummadi *et al*., (1995) in various types of extruded legumes. Extrusion cooking of peas and kidney beans causes phytate hydrolysis that clarifies the increased availability of minerals after extrusion cooking (Alonso *et al*., 2001).

4.2.2. Tannin

In the present study, tannin was not detected in raw sample of maize flour and hence, it was not detected in maize extrudates produced at 110°C, 130 °C and 150 °C after extracting the flour samples with 1% HCL in methanol for 24 hours. But, lower amount of tannin was observed in raw sorghum flour sample and in SC110 and SZ110 which were sorghum control extrudate and sorghum extrudate with zinc sulfate and extruded at lower temperature as indicated in (Table 5) above. Extrusion became more effective treatment in the reduction of tannin content. This reduction might be due to destruction of condensed tannins at high temperature. Thermal treatments such as extrusion imply a qualitative change in the chemical structure of tannins. Thermal degradation of these molecules as well as change in their chemical reactivity or the formation of insoluble complexes could explain a significant reduction of the anti- nutrients by thermal processing (Rahul *et al.*, 2016).

4.3. Total Iron and Zinc analysis

The total iron and zinc content of the raw samples and extrudates (both maize and sorghum extrudates) were summarized in the Table 6

Table 6. Iron and zinc content of maize and sorghum extrudates (mg/100g).

Maize extrudates	Fe	Zn	Sorghum extrudates	Fe	Zn
MC110	2.36±0.21^{a}	1.98±0.05^{c}	SC110	4.76±0.13^{cd}	1.23±0.13^{a}
MC130	1.91±0.05^{a}	1.83±0.17^{bc}	SC130	3.96±0.22^{abc}	1.06±0.06^{a}
MC150	2.47±0.24^{a}	1.88±0.03^{bc}	SC150	4.16±0.12^{bcd}	1.95±0.01^{a}
MF110	12.45±0.9^{b}	1.56±0.01^{abc}	SF110	7.96±0.98^{e}	1.29±0.28^{ab}
MF130	15.4±1.38^{c}	1.51±0.01^{abc}	SF130	7.70±0.29^{e}	1.69±0.18^{b}
MF150	11.92±0.82^{b}	1.38±0.13^{ab}	SF150	11.98±0.79^{f}	1.45±0.14^{ab}
MZ110	2.64±0.1^{a}	5.51±0.67^{d}	SZ110	5.2±0.08^{d}	1.43±0.92e
MZ130	2.46±0.28^{a}	7.9±0.15^{fg}	SZ130	3.74±0.10^{b}	4.46±0.24^{e}
MZ150	2.14±0.07^{a}	8.03±0.83^{g}	SZ150	4.6±0.13^{bcd}	4.41±0.01^{e}
MCo110	12.55±0.3^{b}	7.57±0.36^{f}	SCo110	13.28±0.18^{g}	4.31±0.11^{e}
MC0130	12.65±0.17^{b}	6.24±0.05^{e}	SC0130	13.8±0.11^{g}	2.28±0.46^{c}
MCo150	12.73±0.08^{b}	6.29±0.07^{e}	SCo150	12.39±0.01^{f}	3.01±0.14^{d}
MH130	2.48±0.016^{a}	1.51±0.32^{abc}	S1	3.51±0.25^{b}	1.23±0.23^{a}

Results were expressed as Mean ± standard deviation, n=2.
Means in the same column with same letters are not significantly different (*P* < 0.05)

MH130= Raw maize , M C110=Maize control extruded at110°C, M C130= Maize control extruded at 130°C, M C150=Maize control extruded at 150°C, M F110 =maize fortified with ferrous sulfate and extruded at110°C, M F130=Maize fortified with ferrous sulfate and extruded at130°C, M F150=Maize fortified with ferrous sulfate and extruded at150°C, M Z 110= Maize fortified with zinc sulfate and extruded at110°C, MZ130= Maize fortified with zinc sulfate and extruded at130°C, MZ150= maize fortified with zinc sulfate and extruded at150°C, MCo110 °C= Maize co fortified with iron and zinc salts and extruded at110°C, MCo130= Maize co fortified with iron and zinc salts and extruded at130°C, MCo150= Maize co fortified with iron and zinc salts and extruded at150°C, S1= sorghum control, SC110=sorghum control extruded at110°C, SC130= sorghum control extruded at 130°C, SC150=sorghum control extruded at 150°C, SF110 =sorghum fortified with ferrous sulfate and extruded at110°C, SF130=sorghum fortified with ferrous sulfate and extruded at130°C, SF150=sorghum fortified with ferrous sulfate and extruded at150°C, S Z 110= sorghum fortified with zinc sulfate and extruded at110°C, SZ130= sorghum fortified with zinc sulfate and extrude at130°C, SZ150= sorghum fortified with zinc sulfate and extrude at150°C, S Co110 °C= sorghum co fortified with iron and zinc salts and extruded at110°C, SCo130= sorghum co fortified with iron and zinc salts and extruded at130°C, SCo150= sorghum co fortified with iron and zinc salts and extruded at150°C.

The total iron content of raw maize and sorghum flours observed was 2.48 and 3.51 mg/100g respectively, indicating that low percentage of iron both in maize and sorghum. The finding is comparable with sule *et al*., (2014).

Fortifying maize flour with 35mg of ferrous sulfate per kg of flour prior to extrusion increased the iron content of the extrudates by 80%, 83.8% and 79.9 % which are extruded at 110, 130 and 150 ^{o}C extruding temperature respectively, whereas fortifying sorghum flour with 30mg of ferrous sulfate per kg of flour sample increased the iron content of sorghum extrudates by 55.54%, 54.4% and 70.7% which are extruded at 110, 130 and 150 ^{o}C extruding temperature respectively, which is an indication for fortification of flours prior to extrusion with iron salt increase the level of iron the extrudates. Hence fortification could be a good solution to minimize iron deficiency. However, there is no significant difference within the extrudates. This is comparable with the report by Anton *et al*., (2009).

The iron content of co-fortified extrudates also increased by 80%, 80.4% and 80.5% for maize and 73.4, 74.6 and 71.6 for sorghum extrudates respectively which are extruded at the same extruding temperature but, there is no significant difference within extruding temperature. This indicates that extrusion process has no significant on iron content.

The iron content of unfortified (control) flour samples also increased after extrusion process, but significant difference was not observed ($p>0.05$). The increment iron content of the extrudates during extrusion is considered to be due to wear and tear of extruder metal parts and metallic contamination of the flours during extrusion process. Similar study was reported by Alonso *et al*., (2001), iron content of pea and kidney bean seed flours is increased after extrusion processing and it is most likely to the result of the wear of metallic pieces, mainly screws, of the extruder and extrusion does not significantly affect mineral composition of pea and kidney bean seeds, except for iron.

Zinc content of maize and sorghum flour samples is 1.51 and 1.23 mg/100g respectively. Extrusion process has no effect on zinc con tent of unfortified maize and sorghum extrudates. Fortifying maize flour with 30mg zinc sulfate per kg of flour samples prior to extrusion increased the zinc content of the extrudates by 72.6%, 80%, 81.2% of maize and 13.9%, 72.4% and 72.1% sorghum extrudates flour samples respectively, which are extruded at 110, 130 and 150°C but there is no significant difference within the extrudate. Extrusion cooking improves the mineral absorption by decreasing the factors that hinders the absorption. For example, phytate forms the insoluble complexes with the minerals and eventually influences the absorption of mineral badly (Alonso *et al*., 2001).

4.4. Bioaccessibility of iron and zinc

Table7. Soluble fractions (minerals) after in-vitro digestion of maize and sorghum extrudates with pepsin and pancreatin.

Maize extrudates	Soluble Fe (mg/100g)	Soluble Fe (%)	Soluble Zn (mg/100g)	Soluble Zn (%)	Sorghum extrudate	Soluble Fe (mg/10g)	Soluble Fe (%)	Soluble Zn (mg/100g)	Soluble Zn (%)
MC110	1.47 ± 0.01^{ef}	62.3	0.34 ± 0.05^{a}	17.2	**SC110**	1.40 ± 0.08^{de}	29.4	0.802 ± 0.08^{a}	65.2
MC130	1.59 ± 0.03^{f}	66.4	0.71 ± 0.11^{b}	38.8	**SC130**	1.53 ± 0.07^{e}	38.6	0.83 ± 0.67^{a}	78.3
MC150	1.64 ± 0.01^{f}	83.2	0.80 ± 0.03^{b}	42.6	**SC150**	1.72 ± 0.11^{ef}	41.3	0.94 ± 0.16^{a}	98.4
MF110	0.86 ± 0.01^{a}	8.3	0.72 ± 0.04^{b}	46.2	**SF110**	0.87 ± 0.79^{a}	10.9	1.27 ± 0.07^{ab}	83.4
MF130	0.97 ± 0.05^{ab}	8.5	0.89 ± 0.09^{bc}	58.9	**SF130**	1.05 ± 0.11^{b}	13.6	1.47 ± 0.38^{ab}	89
MF150	1.21 ± 0.09^{cd}	10.2	1.22 ± 0.20^{cd}	88.4	**SF150**	1.08 ± 0.01^{b}	11.3	0.92 ± 0.19^{a}	63.4
MZ10	1.06 ± 0.03^{bc}	51	0.81 ± 0.01^{b}	14.7	**SZ110**	1.16 ± 0.00^{ab}	22.3	1.43 ± 0.58^{ab}	53.8
MZ130	1.16 ± 0.00^{c}	47	0.86 ± 0.06^{bc}	10.9	**SZ130**	1.18±0.01ab	31.6	1.47 ± 0.44^{ab}	32.9
MZ150	1.65 ± 0.23^{f}	77.1	1.18 ± 0.01^{cd}	14.7	**SZ150**	1.31 ± 0.07^{bc}	28.5	1.89 ± 0.18^{b}	42.9
MCo110	1.25 ± 0.00^{cd}	10	1.30 ± 0.26^{d}	17.2	**SCo110**	1.16 ± 0.01^{ab}	8.7	1.49 ± 0.14^{ab}	34.6
MCo130	1.19 ± 0.01^{cd}	9.4	1.73 ± 0.01^{e}	27.7	**SCo130**	1.29 ± 0.06^{bc}	9.4	1.86 ± 0.21^{b}	81.5
MCo150	1.38 ± 0.04^{de}	10.8	2.37 ± 0.34^{f}	37.7	**SCo150**	1.38 ± 0.05^{d}	11.1	1.98 ± 0.02^{ab}	65.8

Results were expressed as Mean ± standard deviation, n=2.

Means in the same column with same letters are not significantly different (*P* < 0.05)

M C110=Maize control extruded at110°C, M C130= Maize control extruded at 130°C, M C150=Maize control extruded at 150°C, M F110 =maize fortified with ferrous sulfate and extruded at110°C, M F130=Maize fortified with ferrous sulfate and extruded at130°C, M F150=Maize fortified with ferrous sulfate and extruded at150°C, M Z 110= Maize fortified with zinc sulfate and extruded at110°C, M Z130= Maize fortified with zinc sulfate and extruded at130°C, MZ150= maize fortified with zinc sulfate and extruded at150°C, M Co110 °C= Maize co fortified with iron and zinc salts and extruded at110°C, MCo130= Maize co fortified with iron and zinc salts and extruded at130°C, MCo150= Maize co fortified with iron and zinc salts and extruded at150°C, S C110=sorghum control extruded at110°C, S C130= sorghum control extruded at 130°C, SC150=sorghum control extruded at 150°C, S F110 =sorghum fortified with ferrous sulfate and extruded at110°C, S F130=sorghum fortified with ferrous sulfate and extruded at130°C, S F150=sorghum fortified with ferrous sulfate and extruded at150°C, S Z 110= sorghum fortified with zinc sulfate and extruded at110°C, S Z130= sorghum fortified with zinc sulfate and extrude at130°C, SZ150= sorghum fortified with zinc sulfate and extrude at150°C, S Co110 °C= sorghum co fortified with iron and zinc salts and extruded at110°C, SCo130= sorghum co fortified with iron and zinc salts and extruded at130°C, SCo150= sorghum co fortified with iron and zinc salts and extruded at150°C.

As indicated in (Table 7), maize and sorghum extrudates fortified with ferrous sulfate and co-fortified extrudates has lower iron bioaccessibility after invitro digestion by pancreatic and pepsin enzymes. Relatively maize unfortified extrudates has higher iron bioavailability. For zinc sulfate fortified maize extrudates the percentage of soluble fraction of iron recorded is 51%, 47% and 77.1%, respectively for MZ110, MZ130 and MZ150 and there was a significant difference in percentage soluble iron within group of the extrudates ($p<0.05$). Sorghum extrudates fortified with ferrous sulfate and co-fortified extrudates were observed for lower percentage of soluble iron fraction. As extruding temperature increases, increased soluble fraction of iron was observed.

Andrews, (2015) discussed that, the increased in mineral absorption by extrusion may be, to some extent attributed to the reduction phytic acid and destruction of tannins during thermal processing so that, extrusion cooking improves mineral absorption by decreasing the factors those hinder absorption.

Unfortified sorghum extrudates and extrudates fortified with zinc sulfates also have higher percentage of soluble iron fraction as compared to other extrudates. The percentage of soluble iron fraction of sorghum unfortified samples is 29.4%, 38.6% and 41.3% for SC110, SC130 and SC150respectively. As extruding temperature increased, percentage of soluble iron fraction was also increased. This is considered due to during thermal processing or as extruding temperature increases there is a reduction or total destruction of anti-nutritional factors like phytic acid and tannin that can bind iron and inhibit absorption (Andrews, 2015).

The percentage of soluble zinc fraction for iron fortified maize extrudate was 46.2%, 58.9% and 88.4% for MF110, MF130 and MF150 respectively and there was significant difference within extrudates. It was observed that extrudates with higher total iron with lower percentage of soluble iron fraction has higher percentage of soluble zinc. This is considered due to the nature characteristics of iron and zinc interacting each other might cause one inhibit the absorption of the other.

The unfortified maize extrudates has higher percentage of soluble zinc with significant difference within extrudates as indicated in (Table 7). Zinc fortified and co- fortified extrudates has relatively lower percentage of soluble zinc. In general, for maize extrudates, the maximum percentage of soluble iron was 83.2% observed for maize unfortified extrudate produced at higher extruding temperature (MC150) and the minimum observed soluble iron was 8.3% (MF110) through all the extrudates and the percentage of soluble zinc was 88.4% and 10.9% the maximum and minimum values. This finding is comparable with Saha *et al.*, (1994) indicated that phytate degradation occurring during thermal processing allows iron and zinc invitro availability of pearl millet flour to be double.

According to Mamiro *et al.*, (2004*),* the reduction of phytic acid and tannin content of complementary cereal based food from 1150 to 660 mg/100g led to an increase in invitro iron and zinc solubility from 4.8% to 18.8%. Invitro studies are use full to provide knowledge on mineral and antinutritional factor interactions or on efficiency of technological process related to another in improving mineral solubility, but they cannot be substituted for in vivo studies.

Alonso *et al.*, (2000) also reported that, extrusion enhanced apparently the absorption of iron and zinc in extruded products. This increased absorption explained by the positive effect of extrusion in phytate content reduction, chemical alteration induced by heat in other compounds of the products, as fiber could be responsible for the higher mineral absorption observed in the extrudates.

4.5. Sensory evaluation

Table 8.Sensory score mean values of maize and sorghum extrudates fortified with iron and zinc salts.

	Sensory parameters				
Extrudates	Color	Odor	Texture	Taste	Over all acceptability
S C110	4.4 ± 1.5^{b}	4.3 ± 1.4^{c}	4.1 ± 1.3^{bcde}	4.7 ± 1.9^{d}	4.7 ± 1.9^{cd}
S C 130	3.8 ± 1.3^{ab}	4.4 ± 1.8^{c}	5 ± 1.2^{e}	4.4 ± 1.9^{cd}	4.7 ± 1.1^{cd}
S C 150	3.8 ± 1.1^{ab}	4±1.1bc	4 ± 1.8^{bcde}	4.3 ± 1.8^{bcd}	$4.5\pm1.^{8cd}$
S F 110	3.7 ± 1.4^{ab}	2.9 ± 1.4^{ab}	2.7 ± 1.9^{a}	3.1 ± 1.7^{a}	3.1 ± 1.4^{a}
S F 130	3.6 ± 1.3^{ab}	3.2 ± 1.6^{abc}	3.7 ± 1.8^{abcd}	3.6 ± 1.6^{abcd}	3.8 ± 1.9^{abc}
S F150	2.6 ± 1.4^{a}	3.2 ± 1.7^{abc}	$4\pm2.0b^{cde}$	3.2 ± 1.6^{ab}	3 ± 1.5^{a}
S Z110	2.8 ± 1.2^{a}	3.3 ± 1.2^{abc}	3.9 ± 1.5^{bcde}	3.4 ± 1.3^{abc}	3 ± 1.3^{ab}
S Z130	3.9 ± 1.1^{ab}	3.9 ± 1.2^{bc}	4.7 ± 1.5^{de}	4.2 ± 1.2^{abcd}	4.3 ± 1.3^{bcd}
S Z 150	2.6 ± 1.3^{ab}	4.2 ± 1.9^{c}	4.3 ± 1.1^{bcde}	4.2 ± 1.1^{abcd}	4.5 ± 1.8^{cd}
S CO 110	2.9 ± 1.1^{a}	2.2 ± 1.6^{a}	3.1 ± 1.0^{ab}	3.1 ± 1.2^{a}	$3.1\pm1.^{9a}$
S CO 130	2.6 ± 1.7^{a}	3.7 ± 1.3^{bc}	4.6 ± 1.5^{cde}	4.3 ± 1.4^{bcd}	4.5 ± 1.5^{cd}
S CO150	3.4 ± 1.4^{a}	3.5 ± 1.7^{bc}	3.4 ± 1.9^{abc}	3.9 ± 1.7^{abcd}	5 ± 1.6^{d}
M C110	4.4 ± 1.2^{abcd}	4.5 ± 1.9^{bcd}	4.2 ± 1.1^{cd}	4.1 ± 1.7^{ab}	4.4 ± 1.8^{bcd}
M C 130	5.3±1.8cd	4.4 ± 1.8^{bcd}	4.7 ± 1.9^{d}	4.2 ± 1.7^{ab}	4.4 ± 1.6^{bcd}
M C150	5.4 ± 1.3^{d}	4.7 ± 1.2^{cd}	4.4 ± 1.9^{cd}	4.3 ± 1.5^{b}	4.6 ± 1.8^{cd}
MF110	4.8 ± 1.7^{abcd}	4.4 ± 1.8^{bcd}	3.9 ± 1.2^{bcd}	4 ± 1.1^{ab}	4.2 ± 1.7^{bcd}
M F 130	4 ± 1.5^{ab}	3.5 ± 1.9^{abc}	3.2 ± 1.0^{abc}	3.4 ± 1.1^{ab}	3.5 ± 1.9^{ab}
M F150	4 ± 1.2^{abc}	3.5 ± 1.1^{abc}	3.3 ± 1.2^{abc}	3.6 ± 1.1^{ab}	3.8 ± 1.2^{abc}
M Z110	5.1 ± 1.7^{bcd}	3.1 ± 1.5^{a}	3.2 ± 1.0^{abc}	3.4 ± 1.8^{ab}	3.5 ± 1.7^{ab}
M Z130	3.7 ± 1.9^{a}	3.1 ± 1.6^{a}	2.3 ± 1.6^{a}	3.2 ± 1.3^{a}	3.2 ± 1.3^{a}
M Z150	3.9 ± 1.1^{ab}	4 ± 1.4^{abcd}	4.1 ± 1.4^{bcd}	3.8 ± 1.6^{ab}	4.3 ± 1.6^{bcd}
M CO 110	4.3 ± 1.4^{abcd}	3.4 ± 1.4^{ab}	2.9 ± 1.1^{ab}	3.5 ± 1.3^{ab}	4.2 ± 1.9^{bcd}
M CO130	4.2 ± 1.8^{abcd}	3.5 ± 1.5^{abc}	3.2 ± 1.5^{abc}	3.6 ± 1.4^{ab}	3.6 ± 1.9^{abc}
M CO150	5.1 ± 1.8^{bcd}	4.1 ± 1^{abcd}	2.9 ± 1.4^{ab}	3.8 ± 1.8^{ab}	4.7 ± 1.4^{d}

Results are means ± SD of duplicate analyses. n=2

Means within the same column followed by different letters are significantly different by a nonparametric test for independent samples (*$p<0.05$).*

S C110=sorghum control extruded at110°C, S C130= sorghum control extruded at 130°C, SC150=sorghum control extruded at 150°C, S F110 =sorghum fortified with ferrous sulfate and extruded at110°C, S F130=sorghum fortified with ferrous sulfate and extruded at130°C, S F150==sorghum fortified with ferrous sulfate and extruded at150°C, S Z 110= sorghum fortified with zinc sulfate and extruded at110°C, S Z130= sorghum fortified with zinc sulfate and extrude at130°C, SZ150= sorghum fortified with zinc sulfate and extrude at150°C, S Co110 °C= sorghum co fortified with iron and zinc salts and extruded at110°C, SCo130= sorghum co fortified with iron and zinc salts and extruded at130°C, SCo150= sorghum co fortified with iron and zinc salts and extruded at150°C, M C110=Maize control extruded at110°C, M C130= Maize control extruded at 130°C, M C150=Maize control extruded at 150°C, M F110 =maize fortified with ferrous sulfate and extruded at110°C, M F130=Maize fortified with ferrous sulfate and extruded at130°C, M F150=Maize fortified with ferrous sulfate and extruded at150°C, M Z 110= Maize fortified with zinc sulfate and extruded at110°C, M Z130= Maize fortified with zinc sulfate and extruded at130°C, MZ150= maize fortified with zinc sulfate and extruded at150°C, M Co110 °C= Maize co fortified with iron and zinc salts and extruded at110°C, MCo130= Maize co fortified with iron and zinc salts and extruded at130°C, MCo150= Maize co fortified with iron and zinc salts and extruded at150°C.

Sensory analysis of both maize and Sorghum extrudates fortified with ferrous sulfate, zinc sulfate, co-fortified extrudates and extrudates with no fortificants which were extruded at 110°C, 130 °C and 150 °C was conducted to identify if there is a significant difference on acceptability between the fortified and unfortified extrudates samples and investigate the effect of extrusion on sensory acceptability of the extrudates. Sensory attributes for which analyses done were color, odor, Texture, Taste and over all acceptability.

One of the major problems in iron fortification has been the development of unacceptable color changes in fortified foods.

Fig.3. Maize and sorghum extrudates

In iron fortified foods, change in color is the main concern because the iron compound frequently induces organoleptic changes in the food vehicle, especially in color.

Cereal flours such as maize and sorghum flours are currently the most frequently used vehicles for iron and zinc fortification. As pointed out by Hurrell, (2004), fortifying these products present two main disadvantages for iron fortification: they contain high levels of phytic acid (potential inhibitor of iron absorption) and they are extremely sensitive to fat Oxidation. In iron-fortified foods, change in color is the main concern because the iron compound frequently induces organoleptic changes in the food vehicle, especially in color.

The result for sensory analyses of sorghum extrudates for color attribute was recorded on average 2.6 and 5.4 minimum and maximum response respectively. This indicates there were the panelists those moderately disliked the color of the sorghum extrudates irrespective of their extruding temperature and also, there were panelists those moderately liked the color of the extrudates. This sensory score is comparable with the finding report by Sacchetti *et al.*, (2003).

Both maize and sorghum products those were fortified with ferrous sulfate had more yellow tone. For unfortified extrudate samples, significant difference was not observed ($p>0.05$) between 130 ^{o}C and 150 ^{o}C extruding temperature. The color acceptance response recorded for the control sorghum extrudates by the panelists were 3.8 and 4.4 for minimum and maximum values which is 70% of the panelists responded neither liked nor disliked the color of the extrudates.($p>0.05$).

Sorghum extrudates fortified with ferrous sulfate, zinc sulfate, co- fortified with ferrous sulfate and zinc sulfate was recorded for lower color acceptance by panelists. In over all observation, sorghum extrudates has lower color acceptance than maize extrudates due to its having slightly darker color.

Odor is one important sensory parameter in ferrous sulfate and zinc sulfate fortified products. In this study the recorded sensory score ranges from 2.2 to 4.7 minimum and maximum values respectively. Relatively unfortified extrudates was scored with better odor acceptance but, there is no significant difference within extrudates ($p>0.05$). Snacks produced at 110 ^{o}C extruding temperature, expected for higher oxidation where it contributes the products to have unpleasant odor and have unacceptable odor by the panelists. For maize extrudates better response was recorded regarding to odor. Products fortified with ferrous sulfate and zinc sulfate was neither liked nor disliked ($p>0.05$).

Texture is another important sensory parameter attribute investigated in this study. It was observed differing extruding temperature has significant effect ($p<0.05$) on the texture of extrudates. For sorghum extrudates, an increased extruding temperature (130 ^{o}C and 150 ^{o}C) was caused decreased moisture content of the final product. As a result the extrudates has crunchy texture and responded as accepted texture than extruded at 110 ^{o}C. In relative to sorghum extrudates, maize extrudates has better acceptance in terms of texture. The unfortified extrudates moderately liked with no significant difference within the extrudates. Ferrous sulfate fortified extrudates moderately disliked with no significant difference between MF130 ^{o}C and MF150^{o}C. Co- fortified extrudates also received lower texture acceptance.

Generally, maize extrudates has harder texture in relative to sorghum extrudates and harder to break it, also difficult to chew. Therefore, maize extrudates recorded lower sensory response for sensory parameter texture parameter.

Taste is very important and unforgatable sensory parameter in sensory study of ferrous sulfate fortified products. Sorghum extrudate fortified with ferrous sulfate responded moderately disliked taste score as compared with unfortified products. Ferrous sulfate most likely has adverse effect on organoleptic quality of foods in particular on color and taste of the products. The extrudate with no fortificant moderately liked with no significant difference with in group of extruding temperatures where as ferrous sulfate fortified extrudates disliked slightly by the panelists but, there was no significant difference between 130oC and 150 oC extruding temperature. Walter *et al.*, (2002) Examined the sensory qualities or acceptability of cereal products fortified with iron or zinc fortificants and noted that the level of fortificants affected the acceptability of noodles made from wheat flour fortified with iron and zinc. Noodles with a high level of fortification (100 mg/kg flour) were in general liked less than those with a lower level of fortification (60 mg/kg flour).

Zinc sulfate fortified extrudates neither liked nor disliked with no significance difference within extrudate ($p>0.05$). Co-fortified sorghum extrudates extruded at lower temperature (SCo110 ^{o}C) moderately disliked for its taste whereas, Sco130 and Sco150 relatively neither liked nor disliked. For maize extrudate, all were recorded slightly disliked taste and there is no significant difference within and among the extrudates ($p>0.05$). Extrusion processing has not improved the sensory acceptability of the extrudates

Comparison of mean of sensory scores indicates that extrudates fortified with ferrous sulfate and zinc sulfate received lower score for over all acceptability. Generally, both maize and sorghum extrudates have scored lower overall acceptability.

5. Conclusion and recommendations

5.1. Conclusion

In conclusion, this study indicated that extrusion process significantly affected the nutritional, anti nutritional and mineral bioaccessibility of the extrudates. Extrusion caused a significant reduction of phytic acid appeared to be the most effective process with improving mineral bioaccessibility. The increase in mineral absorption observed after extrusion could be partly attributed to the total destruction of tannin and significant reduction of phytic acid. Fortifying the flours with ferrous sulfate and zinc sulfate significantly increased the iron and zinc content of both the maize and sorghum extrudates. But, flour fortification with ferrous sulfate caused lower sensory acceptability of the extrudates especially the taste and colors of the extrudates are affected.

At increased extruding temperature, due to the Millard reaction in the process the yellowish color of the ferrous sulfate fortified extrudates slightly covered with light brown and causing lower moisture content of the extrudates at higher extruding temperature significantly contributed to the odor and texture of the extrudates. It can be concluded that extrusion increases absorption of iron and zinc of extruded snacks. Such increased can be described by the positive effect of extrusion toward the decreased effect on antinutritional factors such as phytates and tannins.

5.2. Recommendations

The current study with its own limitation has investigated the effects of extrusion cooking process on sensory acceptability and nutrient bioaccessibility of sorghum and maize flours fortified with iron and zinc salts. But the following issues should also be considered in the future based on the outcomes of the current study.

- Further study on storage stability of the fortified micronutrients and effect of extrusion on oxidation reaction of the fortificants during long storage.
- Further study on effect of extrusion on different phytochemicals of sorghum and maize extrudates to ensure the health benefit.
- It should be important studying the effect of extrusion on the amino acids retention to study the effect of extrusion on the protein and nutritional composition of the extrudates.

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7. ANNEXES

Annexes1. Sensory Evaluation score sheet

Sample code: _______________

Date: _____________________

Sex: ________________

Instruction

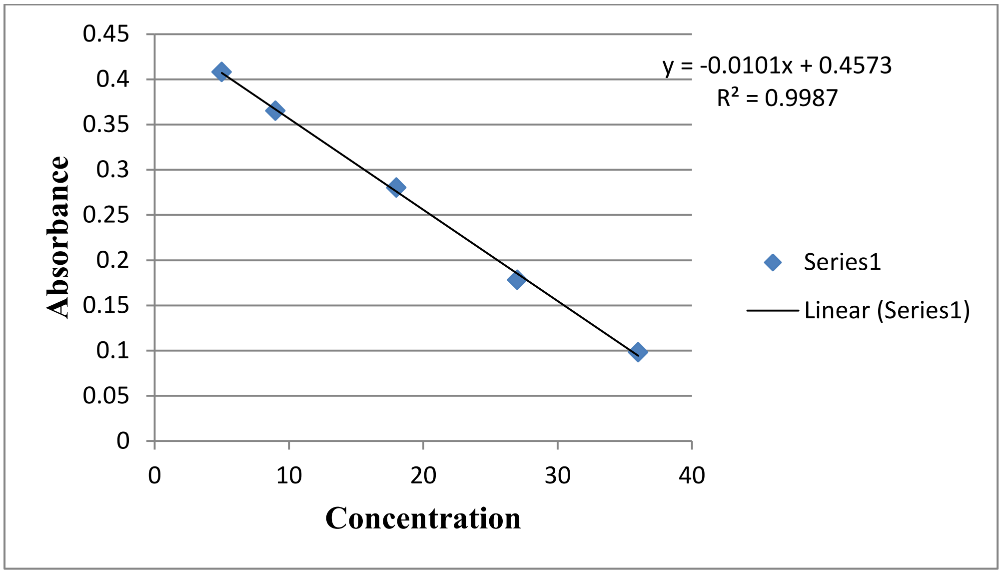
Please rinse your mouth with some water before and taste the given samples for the given sensory parameters. Please tick the box in the table below to indicate your attitude about the product.

Procedure

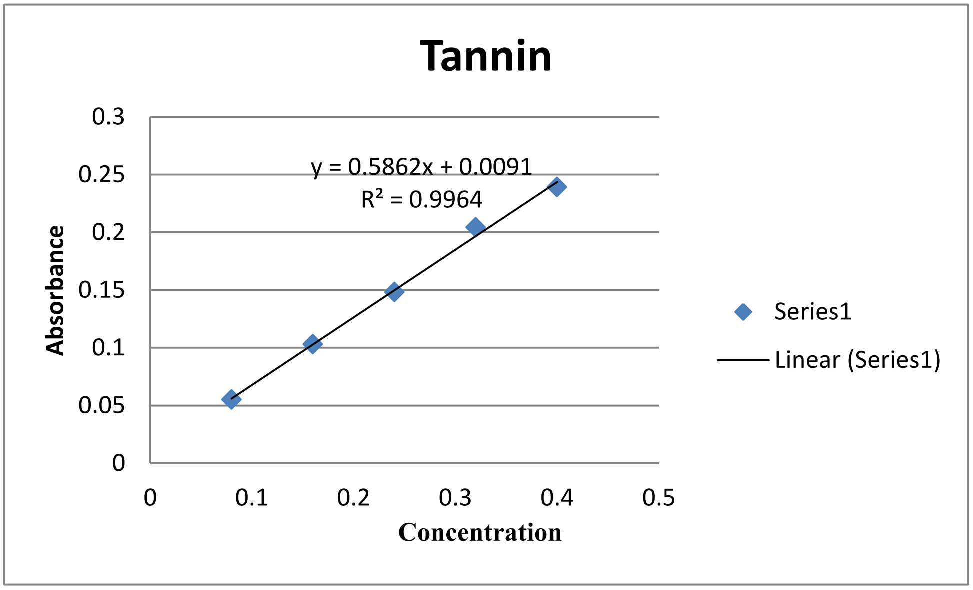
Assessors are presented with different samples and asked to rank in ascending order using ranking test according to their preference.

Attributes	Extremely dislike	Dislike very much	Dislike moderately	Neither like nor Dislike	Like moderately	Like very much	Like extremely
Taste							
Color							
Odor							
Texture							
Over all acceptability							

Annex 2. Phytic acid standard calibration curve



Annex 3. Tannin standard calibration curve



Annex 4. Iron Standard calibration curve