EXTRUSION, PHYSICO-CHEMICAL CHARACTERIZATION AND NUTRITIONAL
EVALUATION OF SORGHUM-BASED HIGH PROTEIN, MICRONUTRIENT FORTIFIED
BLENDED FOODS

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

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Abstract

The feasibility of using a wheat flour mill to refine corn, sorghum and cowpea was studied. Milling of white sorghum grain resulted in decrease in fiber content from 1.89% to 0.38% and 0.45% in raw, finely milled and coarsely milled sorghum respectively. Similarly, there was a reduction in fat (3.17% to 1.75% and 0.51%) content from raw to fine and coarse milled fractions. Starch content increased from 61.85% in raw to 69.80% in fine and 72.30% in coarse fractions. Protein content was almost unchanged at about 7.40% in all the fractions. In de-hulling and milling of cowpeas, starch and protein content increased whereas fiber, fat and ash content decreased.

There was a significant difference in expansion characteristics between whole and decorticated binary blends on account of different levels of inherent starch content. Sorghum cowpea (SC) blends had the highest specific mechanical energy (SME) range (285.74 – 361.52 kJ/kg), followed by corn soy (CS) (138.73 – 370.99 kJ/kg) and the least SME was found in sorghum soy (SS) blends (66.56 – 332.93 kJ/kg). SME was found to be positively correlated to starch content in the blends. SC blends had the most stable process followed by SSB and CSB in that order. The milling of expanded extrudates was found to be dependent on bulk density and low bulk density extrudates had bigger particle size and vice-versa. The water absorption index (WAI) for SC was 4.17 g/g to 5.97 g/g, SS ranged from 2.85 g/g to 5.91 g/g and CS ranged from 2.63 g/g to 5.40 g/g. Starch gelatinization ranged from 85.42 – 98.83% for SC, 90.70 – 96.27% for SS, and 72.57 – 95.49% for CS. The starch digestibility increased after extrusion and cooking but there was no significant change in protein digestibility. There was a significant reduction in anti-nutritional factors – phytic acid (26.06 – 44.03%), tannins (18.69 – 26.67%) and trypsin inhibitor (16.55 – 50.85%) after extrusion.

Thus, the study showed that high protein blends with superior nutrition density needed for preparation of FBFs could be produced by using existing/traditional milling capabilities and extrusion process.

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Dedication

This work is dedicated as small effort towards helping alleviate hunger and 'hidden hunger'.

Chapter 1 - Introduction

The Millenium Development Goals (Goal 1, Target 2) were set to halve the proportion of people suffering from hunger between 1990 and 2015 by United Nations (UN) in 2000. It was successful in lowering hunger by almost half from 23.3% in 1990-1992 to 12.9% in 2014-2016 (UN, 2015). However, even after this reduction, currently one in nine people in the world (795 million) are undernourished and most of them reside in developing countries. The rate of undernourishment in sub-Saharan Africa has been projected to be almost 23% for 2014-2016 period (un.org). This UN report further stated that poor nutrition caused nearly half (45%) of child deaths under five years of age (3.1 million children/year). To continue to build on the progress of MDGs, United Nations Development program (UNDP) has adopted Sustainable Development Goals (SDGs) and aims to end all forms of hunger and malnutrition (Goal 2) by 2030. It will be achieved by making sure that all people – especially children and the more vulnerable have access to sufficient and nutritious food all year round.

The inability to afford any food is the cause of hunger but another type of hunger, 'hidden hunger' which occurs due to chronic lack of vitamins and minerals in the diet has disastrous consequences. This micronutrient deficiency in diet afflicts 2 billion individuals, or one in 3 people globally (FAO, 2013) which is double the number of people who do not have enough calories to eat (FAO et al 2014). The micronutrient deficiencies have been reported to cause significant burden on the afflicted in terms of health costs and negative impacts in lost human capital and reduced economic productivity (Darnton-Hill et al., 2005). Global losses in economic productivity due to macronutrient and micronutrient deficiencies reach more than 2 to 3 percent of GDP (World Bank, 2006) at a global cost of US$1.4 to 2.1 trillion per year (FAO, 2013). Poor diet is the common cause for hidden hunger. The diets based on staples such as wheat, rice,

maize, and cassava contribute towards major share in energy intake but lack in essential vitamins and minerals. Another source of micronutrient deficiencies is impaired or reduced absorption of nutrients due to infection or parasite.

A range of interventions are needed to tackle this complex problem of hidden hunger and one of the interventions is making available food that is rich in energy, protein and micronutrients. The foods can be made effective by dietary diversification, food fortification, supplementation, and/or biofortification. This write up would focus on fortified blended foods (FBFs) as a vehicle to alleviate malnutrition.

Fortified Blended Foods

FBFs are a combination of binary blends of cereals, and legumes, with oil along with added vitamin/mineral premix and a possible source of milk based protein in it. The grains and legumes should be partially precooked in order to boost their digestibility, denature antinutritional factors, and reduce the cooking time required (Wood et al., 2008). These foods need to be energy-dense and with right micronutrient profile, easily digestible and palatable, with relatively quick cooking time (IASC, 2009).

FBFs are used in a very large scale to feed populations in low income countries, especially malnourished individuals and vulnerable groups. It was developed in the 1960s to serve as a protein-rich, micronutrient-dense food supplement for infants and young children (preschool-age children) in developing countries to improve child nutrition (Fleige et al., 2010; Pérez-Expósito & Klein, 2009). The basic recipe consisted of cereals- corn or wheat (75-80%) as source of carbohydrates and soy (20-25%) as protein source. Corn-Soy Milk (CSM) and Wheat-Soy Milk (WSM) were the first two original formulations of FBFs developed in 1967. Corn-Soy Blend (CSB) was developed in the 1980s to replace CSM and WSM because of the increasing cost and

shortage of non-fat dry milk which was a main component of CSM and WSM (Webb et al., 2011; Fleige et al., 2010; Pérez-Expósito & Klein, 2009). There were some modifications which had been made to the FBFs in the early 1990s to reflect current thinking on recommended intakes and bioavailability of nutrients, but no significant work had been done since the 1960s to incorporate advances in food science and technology into new and improved products for food aid (Fleige et al., 2010).

CSB is now the most commonly used FBF. CSB, classified as ready-to-use supplementary foods, saw changes in its macro and micronutrient profiles, proteins and energy density between 2005 and 2011 and was identified as CSB10, 11, 12, 13 & 14 and instant corn soy blend to reflect the changes. The rudimentary version of CSB was considered ineffective in addressing moderate acute malnutrition due to inadequate composition such as micronutrients, energy density, lipids and dietary fibers. CSB and its various formats is the FBF of choice for USAID implementing partners, including the World Food Program (WFP). FBFs produced in The United State have to meet the requirements from the United State Department of Agriculture (USDA), while the locally produced FBFs are controlled by organizations such as the WFP and UNICEF. The typical formulations of CSB distributed by USAID and WFP is shown in Table 1.1. The proximate composition of CSB13, CSB+, and CSB ++ is presented in Table 1.2

The benefits of FBFs are in the fact that they are carriers of essential vitamins and minerals in addition to the required calories and protein content. As they are pre-cooked, its preparation requires less time and saves on fuel which is a constraint in low resource settings. FBFs are high on digestibility and can be consumed with ease by infants and children. The cost of FBFs to the nutrition delivered is also low helping in its coverage to large extent of population in low income countries (de Pee and Bloem, 2009).

Recommendations on improvement of FBFs

Several recommendations have been put forth to improve the nutritional value of FBFs in order to cater to the requirements of different vulnerable groups.

Grillenberger et al. (2003) recommended the addition of animal source protein in addition to the protein from soy to improve the nutritional value and contributing to lean mass accretion. Fleige et al. (2010) recommended that replacing some of the soy with a dairy ingredient would improve absorption of minerals such as iron and zinc. World Food Programme (WFP) has already upgraded specifications for FBFs by adding milk powder into the blends which is called "super cereal plus". Webb et al. (2011) in their recommendation to USDA suggested an increase in protein quantity in FBFs by adding whey protein concentrate (WPC). They recommended an increase in energy density of the diet by increasing the fat content to support neuro development and absorption of fat soluble vitamins. Therefore, the recommended FBFs should be prepared and consumed with fortified vegetable oil (FVO) at defined volumes (15 g oil per 50 g dry matter and in increment of that ratio) which results in higher fat and energy content. Addition of fat improved the texture, flavor and aroma of food (Fleige et al., 2010). Upgrading the micronutrient composition of FBFs was another major recommendation in order to improve the quality of FBFs. It was further recommended by Webb et al. (2011) that a flavor enhancer might be added to formulations of FBFs. The addition of a sweet additive could enhance taste and acceptability, which was important in trying to increase the consumption among sick and undernourished children. It is also suggested by the industry that toasting the corn germ would provide and enhanced sweet flavor. de Pee & Bloem (2009) suggested to use cornmeal derived from dehulled and degermed corn and soy flour derived from dehulled soy beans in order to decrease fiber content of FBFs. Infants and young children typically eat smaller amount of high-

fiber bulky cereals, which reduces the intake amount of food and affects their nutritional status. Davidsson et al. (1996) found from their study that infants' intake of a cereal product decreased significantly from 42±23 g/d to 34±23 g/d 10 (p<0.01). The World Food Programme (WFP) also improved the specifications for their FBFs which are super cereal (CSB+) and super cereal plus (CSB++).

Additionally, the traditional cereals used in FBFs – corn and wheat was recommended in The Food Aid Quality Review (FAQR) to be replaced by other cereals such as sorghum, millets and rice.

Nutritional value of cereals and legumes

Sorghum

Sorghum (*Sorghum Bicolor* L. Moench) is an important tropical crop belonging to Poaceae family that is cultivated in many parts of Asia, Africa, and Latin America (Anglani, 1998). Sorghum is a versatile plant because it can tolerate drought, soil toxicities, a wide range of temperatures and high altitudes. The estimated annual production of sorghum in 2015/2016 season would be 67.61 million tons which is 5.82% (3.72 million tons) more than the previous crop year (world sorghum production.com). Around half of the sorghum produced is fed to livestock, and half is consumed by humans and used in other applications. Currently, most human consumption of sorghum occurs in low-income countries, while high-income countries typically use sorghum as a component in livestock feed or to produce ethanol.

Starch is the main component of sorghum grain, followed by proteins, non-starch polysaccharides (NSP) and fat (Dicko et al., 2006) and average energy value of whole sorghum flour being 356 kcal/100g (BSTID-NRC, 1996). The macromolecular composition of sorghum is similar to that of maize and wheat (BSTID-NRC, 1996). The presence of resistance starch

however, impairs its digestibility notably in infants (FAO, 1995). The protein content in whole sorghum grain is in the range of 7 to 15% (FAO, 1995; Beta et al., 1995). Jambunathan et al. (1975) classified sorghum proteins based on solubility and divided it into albumins, globulins, kafirins (aqueous alcohol-soluble prolamins), cross-linked kafirins and glutelins. The kafirins comprise about 50-70% of the proteins (Hamaker et al., 1995; Duodu et al., 2003). α- Kafirins (23 and 25 kDa) make up about 80% of the total kafirins and are considered the principal storage proteins of sorghum, whereas β-kafirins (16, 18, and 20 kDa), and γ-kafirin (28 kDa) comprise about 5% and 15% of total kafirins, respectively.

Protein digestibility of sorghum proteins is poor due it being protease resistant (Oria et al., 1995; Anglani, 1998). Further, several studies have shown that there is a decrease in protein digestibility upon cooking (Axtell et al., 1981; Taylor and Taylor, 2002). The interactions between protein protein, protein-carbohydrate, protein-(poly) phenol and carbohydrate-(poly) phenol is understood to be cause for low protein digestibility. (Axtell, 1981; Taylor and Taylor, 2002). It is also an enriched source of B vitamin that includes thiamin, riboflavin, vitamin B6, biotin and niacin (Hegedus et al., 1985) a BSTID-NRC (1996) has reported that sorghum is a good source of more than 20 minerals. Minerals like iron, zinc, potassium and phosphorous are some of the important minerals (Glew et al., 1997; Anglani, 1998). Zinc, which is an important metal for pregnant women is said to be deficient in corn and wheat (Hopkins et al., 1998). Even after being a storehouse of nutrients, sorghum based foods have continued to be nutritionally deficient and organoleptically inferior. This can be attributed largely to the presence of anti-nutritional factors (ANF) such as tannin, phytic acid, polyphenol and trypsin inhibitors which bind these food ingredients into complexes making them unavailable for human nutrition (Elsheik et al., 2000; Gilani et al., 2005; Idris et al., 2007). The presence of these anti-nutritional

factors limits the digestibility of proteins and carbohydrates by inhibiting their respective proteolytic and amylolytic enzymes (Yoon et al., 1983; Knuckles et al., 1998; Mohammed et al., 2011). They equally determine the bioavailabilty of divalent mineral elements which play key roles as enzyme stabilizers, transport co-factors in metabolic pathways and other key physiological functions. Specifically, sorghum tannins which are condensed polymeric polyphenols (proanthocyanidins) are capable of binding non-haem iron (Fe) and form complexes with proteins (Emmanbux and Taylor, 2003; Melaku et al., 2005), to inhibit enzymes of the digestive system (Ogunkoya et al., 2006). Similarly, phytic acid (myoinositol hexaphosphate), present in most plant materials as phytate salt, is the main phosphorus store in mature seeds. Its association with proteins chelates metal ions to form protein-mineral-phytate complexes which are highly insoluble at the physiological pH of human intestine (Khertapaul and Sharma, 1997; Sandberg and Andlid, 2002).

Cowpea

Cowpeas *(Vigna unguiculata),* also known as blackeye beans or southern peas, is an important grain legume in Africa and other developing countries as they are good sources of protein, energy and other nutrients (Uzogara and Ofuya, 2007). Global production of dried cowpeas in 2010 was 5.5 million metric tons of which Africa produced for 94% of this with Nigeria being the largest producer and consumer of cowpea, producing 2.2 million metric tons of dried grain in 2010 (CGIAR, 2010). Cowpea's high protein content, its adaptability to different types of soil and intercropping systems, its resistance to drought, and its ability to improve soil fertility and prevent erosion makes it an important economic crop in many developing regions. All parts of the cowpea crop are used as all are rich in nutrients and fiber. In Africa, humans consume young leaves, immature pods, immature seeds, and the mature dried seeds. 52% of Africa's production

is used for food, 13% as animal feed, 10% for seeds, 9% for other uses, and 16% is wasted (IITA).

The cowpea contains on an average 24.8% protein, 67% carbohydrates, 1.9% fat and 6.3% fiber (Davis et. al., 1991; Oyenuga, 1978). They further reported that cowpeas are valued more than cereals as nutritional supplements because of the superior amino acid profile (lysine, and tryptophan) as compared to cereals. The starch and protein digestibility of cowpeas is dependent on the level of processing and the resultant presence of anti-nutritional factors in it. Like other legumes, the extent to which the antinutritional factors present in cowpeas survive the common processing methods like germination, soaking, and cooking leads to the extent to which the digestibility can be improved (Preet and Punia, 2000). Salunkhe (1982) reported that the antinutrients present in cowpea included phytic acid, polyphenols, saponins, enzyme inhibitors, lectins etc. Phytic acid which is widely distributed in food grains reduces the bioavailabilty of minerals (Davies and Nightingale, 1975) and inhibits proteases and amylases (Singh and Krikorian, 1982). Polyphenols play an important role in the reduction of protein digestibility (Elias et al., 1979) and starch digestibility (Thompson and Yoon, 1984). Cowpeas have been shown to contain high levels of polyphenols (Laurena et al., 1987). Removal of these antinutrients, is, therefore, necessary for effective utilization of food legumes for human nutrition.

Corn

Corn (Zea mays) is the leading crop in the world followed by rice and wheat and had a global production of over 1 billion tons in 2013 (FAOSTAT, 2014). Corn is processed into a multitude of food and industrial products including starch, sweeteners, corn oil, beverage and industrial alcohol, and fuel ethanol (USDA ERS, 2016). The whole corn consists of 8-11.5% protein, 68-

74% starch, and 4-5.5% fat (Singh et al., 2014). The endosperm contains 85% starch, 8.5% protein and 1% fat. Corn starch is usually digestible after cooking or thermal processing (Ai and Jane, 2016). One of the major factors affecting the starch digestibility of corn is the amylose content and amylopectin branch length. Increased amylose content and increased amylopectin branch length causes a reduction in digestibility rate (Jane et al., 2003; Li et al., 2008). Other factors, such as surrounded by cellulose and hemicellulose fibers and/or protein matrices and interacting with lipids, can also reduce the susceptibility of starch to enzyme hydrolysis (Ezeogu et al., 2005; Ezeogu et al., 2008; Ai et al., 2013a). Corn is deficient in lysine, which is the primary limiting amino acid (Young and Pellet, 1994). The protein digestibility of corn is affected by the processing method and also by the presence of antinutritional factors. Hamaker et al. (1996) reported that cooking reduced phytic acid and that could help increase in vitro protein digestibility.

The presence of antinutritional factors in corn such as phytic acid, tannins and trypsin inhibitors has been well documented (Ejigui et al., 2005; Hotz and Gibson, 2001; Marfo et al., 1990; Chavan and Kadam, 1989) and different processing methods like fermentation, germination etc. have shown to reduce these anti-nutritional factors.

 Soy

Soybean (Glycine max) is considered an oilseed instead of pulse due to its high oil content. Nearly all soybeans are processed for the oil. Soybean protein has played an increasing role in human nutrition over the last few decades (Riaz 2001). Soy protein products are an ideal source of some of the essential amino acids used to complement cereal proteins. It supplies all 9 essential amino acids and provides many functional benefits to food processors and for a healthy diet. Three forms of soy proteins products exist as protein supplements or source of proteins and

their content ranges from 50% to over 90%, namely, soy flours/grits, soy protein concentrates, and soy protein isolates (Wolf 1970). Least refined forms of proteins are flours and grits, which have varying fat contents, particle sizes, textures, and degrees of heat treatment. Flours are prepared by grinding soybean flakes to 100 mesh (0.157 mm-sieve pore size) or finer, whereas grits are coarser than 100 mesh. Minimum protein contents of these materials range from 40% to 54% depending on the fat content. The amino acid composition of soy proteins resembles, with the exception of the sulfur containing amino acids (such as methionine), the amino acid patterns of high-quality animal protein sources (Wolf 1970). Digestibility studies in animals and humans have demonstrated that soy proteins are comparable in digestibility to other high-quality proteins such as meat, milk, fish, and egg (Wayler et al. 1983; Oste 1991). Most applications for defatted soy flours and grits involve their combination with cereals. Their addition raises both the quantity and quality of the protein in cereal products. The quality of the protein is improved in soy–cereal mixtures because soy protein is a rich source of lysine, the first limiting essential amino acid in most cereal proteins (Klein et al.1995; Lang, 1999).

The nutritional value of soybean is much lower than expected, in spite of its protein content and amino acid profile of the proteins. This is largely attributed to the presence of antinutritional factors, such as protease inhibitors, lectins, phytates and tannins (Bajpai et al., 2005). Their removal has been attempted by heat treatment with varying success (Liener, 1994; Osman et al., 2002).

Processing of FBFs

The FBFs can be made using any of the processing methods – roasting, drum drying, and extrusion. Roasting is the dry cooking of cereals, legumes or oilseeds. High processing temperatures produce a pleasant toasted flavor that improves palatability and inactivates

enzymes and antinutritional factors along with denaturation of fat-labile vitamins. However, roasting is not a sufficient treatment to pre-gelatinize starch which results in lower nutrient density in the meal (WFP). Drum drying is only applicable to food slurries or liquid food systems and the product is exposed to high temperatures for a short period of time. This technique is most expensive and sophisticated to produce FBFs.

Extrusion cooking technology is the preferred method for increasing the energy density in FBFs and decreasing the viscosity of gruels. This process has been used successfully to produce nutritious foods for distribution in dry packaged form (Bounie et al, 2010). Meance et al. (1999) found that the starch digestibility of cereal blends increased after extrusion as compared unextruded blends. Advantages of extrusion cooking include high throughput, low energy consumption, absence of process effluents and high efficiency when it comes to raw material selection, their texture and shapes (Harper, 1981). Key functions such as agglomeration, degassing, dehydration, expansion, homogenization, mixing, pasteurization, protein denaturation, shaping, shearing, texture alteration, thermal cooking and unitizing are few conditions that are generated by a food extruder for use in a variety of food uses that include food, feed and industry applications (Riaz, 2000). Further, in contrast to unprocessed sorghum which decreased protein digestibility to 20% on cooking, extruded sorghum still showed a consistent digestibility of 79% from same sorghum cultivars (Mertz et al., 1984). Hamaker et al, (1994) reported pepsin digestibility of extruded decorticated sorghum flour was 18% higher than untreated and decorticated sorghum flour. Extrusion was the best method to abolish trypsin, chymotrypsin, amylase inhibitors and haemagglutinating activity without modifying protein content. Furthermore, this thermal treatment was most effective in improving protein and starch digestibilities when compared with dehulling, soaking and germination (Alonso et al., 2000).

Objectives

This study was therefore undertaken to fill the knowledge gaps in the use of novel ingredients in developing fortified blended foods with high protein content. The following objectives were studied, -1) The feasibility of using a wheat flour mill to mill the raw materials sorghum, cowpea, and corn instead of the specialized mills these grains need to produce flour appropriate for producing FBFs; 2) To study the physicochemical properties of binary blends of cereal/legume to produce expanded extrudates with extrusion; 3) To understand the role of inherent components starch, protein and fat in binary blends in influencing the final product characteristics; 4) To study the changes in antinutritional factors in binary blends after being subjected to extrusion cooking.

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Table 1.1 Proximate analysis of corn milling streams

USAID	WFP
CSB13	**CSB (Super Cereal)**
Processed & gelatinized corn meal, 69.55%	Maize, 78.3%
Defatted and toasted soy flour, 21.85%	Whole soya, 20.0%
Soybean oil, refined deodorized, stabilized 5.5%	Dicalcium phosphate anhydrous, 1.23%
Mineral Premix, 3%	Potassium chloride, 0.27%
Vitamin antioxidant premix, 0.1%	Minerals and Vitamins, 0.2%

Source: USDA, 2008; WFP (2015); CSB = Corn Soy Blend

Table 1.2 Proximate and aflatoxin content of CSB13, CSB+ and CSB++

	CSB13	CSB+	CSB++
Energy, kcal (min)	---	380	397
Protein, % (min)	16.7	14.0	15.3
Fat, % (min)	6.0	6.0	9.59
Crude Fiber, % (max)	2.0	5.0	3.0
Moisture, % (max)	10.0	10.0	9.0
Total aflatoxin, ppb	20	20	5

Source: USDA, 2008; USDA, 2014; Webb et al (2011); CSB = Corn Soy Blend

Chapter 2 - Adaptation of Conventional Wheat Flour Mill to Refine Sorghum, Corn and Cowpea Grains

Abstract

This study was undertaken to refine sorghum, corn and cowpea grains using the flow and equipment similar to that employed in a conventional gradual reduction wheat milling system for use in developing novel fortified blended extruded foods. Decortication and milling of white sorghum grain resulted in decrease in fiber content from 1.89% to 0.38% and 0.45% in raw, finely milled and coarsely milled sorghum respectively. Similarly, there was a reduction in fat (3.17% to 1.75% and 0.51%) content from raw to fine and coarse milled fractions. Starch content increased from 61.85% in raw to 69.80% in fine and 72.30% in coarse fractions. Protein content was almost unchanged at about 7.40% in all the fractions. The high level of fat and fiber in peeler by-product fractions demonstrated that milling was effective in removing significant portions of germ and pericarp from whole white sorghum. Similar trends were observed during de-germing and milling of white corn to fine and coarse fractions. Fat content decreased from 3.30% to 1.20% and 0.59% from raw to fine and coarse milled fractions respectively. Protein content was almost unchanged at around 7.5% whereas the starch content increased from 60.10% to approximately 72%. The tip cap of the corn kernel was removed along with germ and pericarp to improve the visual appeal of the de-germinated corn products. In de-hulling and milling of cowpeas, starch and protein content increased whereas fiber, fat and ash content decreased. Milled cowpea had 26.10% protein 1.06% fiber, 1.07% fat, 3.08% ash and 40.70% starch. The milling process removed the back eye surrounding the hilum of the cowpea and improved the aesthetic value of the flour. The reduction in fat and fiber improved product stability and

extrusion properties. With adaptation of conventional wheat milling equipment, reduced fat and fiber products could be produced.

Introduction

Food aid has been defined as "the international sourcing of concessional resources in the form of, or for the provision of food" (Barrett and Maxwell, 2005). One of the key trends in food aid programs is the increase in support for local and regional procurement of food aid (Harvey et al., 2010). Fortified blended foods form an important part of the food aid ration. However, to overcome the inadequacy of current FBFs in treating young children with malnutrition (de Pee and Bloem, 2009; Skau et al., 2009), the Food Aid Quality Review (FAQR), 2011 by Webb et al. (2011) in recommendation # 18 has recommended the exploration of new products (new grains or legumes) like sorghum-soy, millet-soy, and rice-soy which could be used beyond the traditional products – corn soy blend (CSB) and wheat soy blend (WSB) in food aid programs as well as improve the quality.

Traditional grains and Alternate/New in FBFs

Corn has been the grain of choice in regular FBFs because it is a staple grain and is available in bulk for food aid programs. Corn which ranks third as staple food after wheat and rice is produced the most (Gwirtz and Garcia-Casal, 2014). It is a preferred source of starch, good source of plant-based protein, dietary fiber, B vitamins and magnesium (USAID, 2015). Corn is a highly productive crop but corn has many uses, with the least being for human consumption. 38.8% of US corn is used as feed, 30.5% for fuel ethanol production, 12.9% is exported, 3.6% is converted to high fructose corn syrup, 2.1% is utilized as sweeteners,1.8% is used for starch production, 1.5% is used for cereals and other uses, 1.0% is used in alcohol/beverage production and 0.2% is used as seed (USDA ERS, 2015). Corn is used for food as corn flour, corn meal,

hominy, grits or sweet corn. However, corn production is a highly intensive row crop production including use of large amounts of fertilizers and pesticides (Links, A., and Improvement, G., 2011). Again, due to the high demand of corn especially for fuel makes the prices increase (Tenenbaum, 2008) and it directly affects food aid donations. Thus, an alternate to corn in FBFs would help maintain a stable availability of it in food aid programs.

Soy is a legume crop that has been prolifically used in FBFs as a plant-based protein source because of its favorable content and composition of amino acids (Hoppe et al., 2008). They further reported that on comparing amino acid profile of soy to human muscle tissues, soy had limiting sulfur containing amino acid-methionine and cysteine. The soy is heat treated and used in full fat form or as defatted flour. However, soy contains high levels of anti-nutritional factors – trypsin inhibitors and phytates. The price of commodities used for FBF and relief feeding plays an important factor in determining the composition of the blends and ration (Hoppe et al., 2008). Thus soy being an inexpensive source of protein is the preferred source of proteins in FBFs.

Sorghum is looked at as a potential alternative to corn because of a number of advantages over corn and wheat. Sorghum is a drought tolerant crop and is mostly a non-genetically modified organisms (GMO) crop which allows it to be used in many countries globally that have put a ban on the use of GMO products. It is grown and consumed as a part of human food in many parts of Africa and Asia and thus is a familiar taste for the populations in these regions. Further, it is priced competitively with other food aid grains. Moreover, when it is processed properly, it contains similar levels of carbohydrates to that of CSB and also has a higher level of protein, fat and some micronutrients (Dicko et al., 2006).

Cowpea is another important alternate crop to soy that could be used in FBFs. Cowpea has high protein content, it is adaptable to different natures of soil and intercropping systems, it is drought

resistant, and it has the ability to improve soil fertility and prevent erosion. It is consumed in regions of Africa and Asia where malnutrition is prevalent and that makes it an ideal candidate for being used in FBFs. The protein in cowpea is rich in the amino acids lysine and tryptophan, when compared to cereal grains. The cowpea protein is deficient in methionine and cystine in comparison to animal protein (Davis et al., 1991).

Milling of cereal/legumes

A study from 1979 documented better nitrogen absorption and retention (indicating protein uptake) from CSB which was produced from degermed corn and dehulled soy when compared with whole cornmeal and dehulled soy bean flour as well as with whole corn meal and whole soy bean flour (Matilsky et al., 2009). The USDA specifications include the use of dehulled and degermed corn and dehulled defatted soy or optional-dehulled full-fat soy flour (de Pee and Bloem, 2009).

Corn Milling

Production wise corn has been the main cereal but ranks third as a staple food, after wheat and rice (Gwirtz and Garcia-Casal, 2014). There are two distinct methods of industrial processing of corn – wet milling and dry milling and each process generates unique co-products.

Wet Milling of Corn

According to Galitsky et al (2003), the corn wet milling industry is the most energy intensive industry within the food industry and uses 15% of the energy of food industry and contributes to 2nd largest operating cost after corn for wet millers. In the wet milling process, the steps involved are soaking or steeping whole corn (30-50 hours at 48-54°C) in dilute sulfur dioxide solution which causes softening of the kernels. Soaking leads to leaching of soluble nutrients into the water which is later evaporated to recover condensed corn fermented extractives. Then the corn

germ is removed from the soaked kernel and is further processed to obtain corn oil. The remaining portion of the germ (wet or dry) called corn germ meal is collected for feed use. The degermed corn kernel is screened to remove bran and allow passage of starch and corn gluten protein. The bran and other co-products are combined to produce corn gluten feed. The starch and gluten slurry is sent to centrifugal separators, where lighter protein fraction moves to the top and the heavier starch collects at the bottom. The gluten protein is collected and concentrated to form corn gluten meal which is a 60% protein feed. A portion of the starch is washed, dried or modified for non-food applications such as paper, textiles etc. and the remaining starch is converted into sweeteners or ethanol. On an average this milling produces 65% starch, 26% gluten feed, 5% gluten meal and 3.3% corn oil. Many of these products are destined for human consumption though they can't be consumed directly. These products require some sort of further processing to be ready for human consumption.

 Dry Milling of Corn

The dry milling consists of reduction in particle size of clean whole corn with or without screening separation, retaining some or all of the corn germ or fiber (Brubacher, 2002). The corn flour with whole or partial germ content is not shelf stable due to high fat content in corn (Gwirtz and Garcia-Casal, 2014). There are 3 types of dry milling of corn :

 1) Dry Milling: Using clean corn to reduce its particle size by using a combination of equipments like hammer mills, stone mills, roller mills, screeners, sifters, specific gravity separators, and aspirators. Specialized equipment, such as degerminators and de-hullers or peelers, may be employed in maize processing. This method is used for producing flaking grits, coarse grits, regular grits, corn meal, cones and corn flour by means of meshes ranging from 3.5 to 60. Corn flour and corn meal finds application in FBFs,

2) Nixtamalization: The nixtamilization process involves cooking and steeping (2-31 of water per kg of corn processed) the dried corn kernels in an alkaline solution preferably 1-3% of calcium hydroxide (CaOH2) for 8-16h (Mendez-Albores et al., 2004), then cooking them until tender. At this point the corn is called nixtamal and can be ground into masa for tortillas, tamales, or hundreds of other dishes. Nixtamalized corn has several benefits compared to unprocessed grains as they are more easily ground and have a higher nutritional value (increased bioavailability of niacin, improved protein quality, increased calcium) and reduced mycotoxins (Afoakwa et al., 2007).

3) Pre-cooked flour: Production of pre-cooked corn flour involves removal of hull and germ by coarse milling. After separation of germ, fiber, and fines, the endosperm is conditioned/tempered by making it to 15% moisture at 35-40°C for 1-2 h. This is followed by steam injection and flaking steps. The flakes are then dried and milled to make pre-cooked flour (Qarooni, 1996).

Sorghum Milling

Sorghum is the fifth most important cereal in terms of global production (Serna-Saldivar et al, 1988). Sorghum endosperm, unlike soft wheat endosperm, comprises starch fractions in the floury and corneous sections (Taylor and Dewar 2001). Because of these characteristics, sorghum endosperm composition with higher proportions of corneous fraction is preferred for milling because they give higher amounts of endosperm flour and better separation of the pericarp and germ (Maxson 1971; Taylor and Dewar 2001).

There are two basic principles that are used in the milling of sorghum: impact and attrition. Impact is achieved by accelerating grain against a hard surface (i.e. wood or metal) to reduce its particle size. Attrition makes use of shearing forces in rollers, rotating disks or pressurized cylinders, where, for the latter, the effect of metal to seed is compounded by seed surfaces

rubbing against each other. In practice, more than one of these principals comes in to play depending on the final product that is wanted.

Sorghum is milled in many ways and are listed below:

Tempering: Inherent anatomical structure of sorghum prevents achieving effective separation of grain germ and bran from the endosperm. Tempering of sorghum grains which is the process of increasing the moisture content of grains through the addition of water before it enters the dry milling operations makes the bran tough and the endosperm softer and more friable which facilitates their separation. The optimum condition for dry milling of sorghum is 17% moisture content in the grain and 8 hours of tempering time (Abdelrahman and Farrell, 1981).

Dry milling: After tempering the grins, they are milled to achieve a clean separation of bran, endosperm, and germ (Hahn, 1969). Grits obtained from the endosperm of the kernel, are among the most valuable products obtained from dry milling. Suitable tempering and milling would yield a large quantity of low-fat grits. Abdelrahman and Farrell (1981) observed that during dry milling of sorghum with a pre-break system produced grits with lower fat and ash content than did with a break system. Pre-breaking cracked the kernel open and increased its surface area. The end product had the grits and germ that were easily separated with sieves, and the bran was segregated by gravimetric tables.

Decortication or dehulling: This consisted of removing the outer layers of the grain, i.e. pericarp, before dry milling (Anderson et al, 1969). An abrasive mechanism such as rice decortication or debranning machines, or pearlers containing stones or resinoid disks (Rooney and Waniska, 2000) are used for decortication. This processing step produces a low fiber intermediate product which would undergo further particle size reduction and separation. A variant of this process is

decortication of the kernel, followed by degermination of tempered kernels with pin mills. This latter variant of the process would produce low fat and low fiber grits which are more stable during storage, and meet the requirements for many uses (Hahn, 1969). Abrasion and attrition machines used in processing of sorghum for food have been beneficial for rural African communities in the production of traditional food stuffs (i.e. porridges). The main difference between the two types of decorticating equipment is that attrition mills produce finer particles than the abrasion equipment (Munck 1995). Sorghum abrasive decortication sometimes precedes hammer milling. This is especially beneficial in the abrasion of sorghums with high tannin testa (Taylor and Dewar 2001).

Roller milling: It has been the most common type of dry milling operation used for production of grits and flour. Two or more consecutive breaking steps are designed for particle size reduction of the kernel into grits and flour (Hoseney et al, 1981). Roller milling is preferred on white food sorghum due to the lack of red or purple-colored pericarp and because the floury endosperm texture yields more fine particles in the flour fraction (Gomez, 1993).

Wet milling: This operation is similar to wet milling of corn; except that it is more difficult to separate protein from starch in wet-milling sorghum kernels compared to corn. The starch granules of sorghum must be bleached and sorghum oil must undergo further refining (Rooney and Waniska, 2000).

Pinilla (2010) studied the effect of different types of milling (hammer mill, roller mill and burr mill) on different types of sorghum (soft endosperm and hard endosperm) in El Salvador and found that roller milling delivered the lowest proportion of coarse particles amongst all mills tested. The author further observed that hammer mills were a practical solution in terms of lower cost than the roller mill. However, hammer milled flours had large particles that must be sifted

before being suitable for bakery applications. Burr milled flour resembled the behavior of roller milling by reaching peak viscosity and pasting temperature at similar times. Unlike roller and hammer mills, burr mills offered low cost affordable technology that could be used by small millers. Other application was its use in hammer milled flours, which resulted in production of high volume of product in a shorter time than just burr milling.

Cowpea Milling

As with all grain milling, the objective of cowpea milling is to remove the hull and reduce the endosperm to smaller particle size so that it can be used in various food preparations. However, the ease of removal of seed coat depends on how loosely or tightly the seed coat is attached to the cotyledon. Cowpea types with loosely adhered seed coat can be easily decorticated through simple operations of cracking and aspiration. However, tightly bound seed coat requires wetting/soaking to help facilitate its easy removal during subsequent milling step. In a study by Phillips et al. (2003), it was reported that the pretreatments of soaking cowpea seeds for 1 min and then drying at 100°C improved the milling efficiency. Another study by Henshaw et al. (1996) showed that presoaking affected the particle size distribution of cowpea flour and 4h of soaking produced the most medium sized particles (177-420 μm) which had the best hydration characteristics.

Dry-milling of cowpeas for flour and meal production is preferable to wet-milling to minimize microbial growth and energy input required (Phillips & McWatters, 1991). The dry milling of cowpea flour uses the machineries like dehuller, aspirator, hammer mill with cyclone. The milling in unorganized sector involves mechanically milling wet and dehulled cowpeas using plate mills that are powered by gasoline or diesel (Robinson et al., 2014). Wet milled cowpea has

to be consumed immediately otherwise expensive or laborious drying or refrigeration is required for its preservation (Fatokun et. al., 2002).

Objectives

In view of the above literature, it can be seen that different types of milling requirements needs to be taken care of when trying to mill different grains/legumes. No work has been previously attempted to mill all these grains in a single milling unit to produce dry flour. Therefore, the objectives of this study were:

1. To optimize milling conditions to mill sorghum, cowpea, and corn on a pilot roller flour mill (which is abundantly available globally).

2. To analyze the nutritional quality of the different fractions of milling stream to understand the efficacy of this milling approach.

Materials and methods

Materials

There were 3 varieties of whole sorghum grains– 2 white varieties namely -V1 (variety Fontanelle 4525) and V2 (738Y) and 1 red variety V3 (217X Burgundy) procured from Nu Life Market, Scott City, Kansas, USA. Cowpea grains (variety number 8046) were obtained from LPD Enterprises LLC (Olathe, Kansas, USA). The corn kernels were procured from Agronomy Foundation Seed, Kansas State University, Manhattan, Kansas, USA and soybeans from Kansas River Valley Experiment Field, Kansas State University, Manhattan Kansas, USA. All the raw materials used in this study were non-GMO.

Milling of sorghum and corn

Decorticated sorghum and degermed corn

Decorticated sorghum (fine and coarse) as well as degermed corn (fine and coarse) had the same milling steps. The whole grains were milled at Hall Ross Flour Mill (Kansas State University, Manhattan, KS, USA) in a Buhler conventional pilot wheat mill by using rollers (Figure 2.2). The capacity of the mill was 1000 kg/h of whole grain. The experimental milling of sorghum used 3 break rolls (BK) instead of the normal 5 BK used in conventional wheat milling as the aim of milling was to obtain only decorticated sorghum and corn flour in two particle sizes – fine (-315 µm) and coarse (+315 µm). Usually, many different types of wheat flour are extracted from the milling streams and thus 5 break rolls are used. Prior to milling the grains, they were subjected to conditioning and post conditioning processes (Figure 2.1). The sorghum grain was conditioned by adding measured quantity of water (100 l/h) to have a moisture content of 15.5% and stored in bin for tempering for 24 h. After tempering, the grains were subjected to post-conditioning processing wherein the grains passed through peeler followed by scourer and then through aspirator. For corn, water was added just before peeler. The peeler was used to remove the maximum of peelings/seed coat and the peeler throughs were discarded, the scourer helped in removing surface contamination from the grains – fines, dust including sand, clods of soil, insects and their fragments etc. The aspirator which was attached to the end of the scourer separated any detached hull particles or any other surface contaminants neatly from the grain and the cleaned grains were collected from the bottom of the aspirator into the 1 BK bin for milling. The cleaned grains from the 1 BK bins was fed to 1/2 BK double high corrugated rolls rotating at different speeds for initial coarse splitting of the grains. The broken grains and some separated bran was then sent to a vibrating sifter having screens of increasing fineness from top to bottom

(1041μm, 500μm, and 315μm). However, fragments of endosperm, bran, and germs called middlings remained after each sifting. These were sent to purifiers, where controlled flow of air lifted off the bran particles and while at the same time bolting cloth separated coarser fractions by size and quality. The coarse material was then sent to reduction rolls and again sifted. The coarse pieces from 1/2 BK screens are sized and were carried to the second set of break rolls. The second break rolls were spaced closer together, producing a finer material. This material was then sent to a sifter and the process repeated itself through the 3rd BK. After the end of 3rd BK and its sieving and reduction the decorticated sorghum flour is collected from the patent screw (fine flour) and semolina screw (coarse flour).

Milling of whole sorghum and whole corn

The milling for obtaining whole sorghum flour (fine and coarse) and whole corn flour (fine and coarse) was done at Hal Ross Flour Mill (Kansas State University, Manhattan, KS, USA) using a 18-7-301 model pilot scale circ-u-flow hammer mill (Schutte Buffalo Hammer Mill, Buffalo, NY, USA) having a width of 7 inches and rated tip speed of 16900 feet/minute (5151 m/minute). A pictorial representation of the same is provided in Fig 2.2. The cleaning and preparation of the grains followed the same steps as mentioned in milling of decorticated/degermed flour. The cleaned grain was fed to the inlet of the hammer mill fitted with a 3/64 inch (1190 μm) screen. The product is caught by the hammer rotors and the particle size is reduced till it passes from the screen jacket. This flour was sifted on a vibrating sieve assembly. The overs (+315 μm) on the screen were called whole coarse flour and the throughs were termed as whole fine flour.

Milling of Cowpea

Cowpea was also milled at Hal Ross Flour Mill (Kansas State University, Manhattan, KS, USA) using the conventional Buhler pilot roller wheat milling system. This milling was shorter than

sorghum and corn milling because only one particle size of the flour was required (-315 µm) as shown in Figure 2.4. The grain was subjected to cleaning process – raw grains were passed through screener/aspirator to remove fines, brokens and black eyes of cowpea. After the first cleaning the grains were stored in 'dirty' bins. It was passed through a combination cleaner wherein the screener removed the fines and aspirator removed the light impurities. The grains were then stored in temper bins after 2nd cleaning. No tempering was done on cowpea grains because water addition which is a usual process for most of the other grains to enable ease of removal of seed coat/hull later during milling process caused cowpea fractions to become sticky and hindered in the flow of material through the milling line. From the temper bins the grains were again subject to peeling and aspiration to further remove fines and brokens (black eyes and broken cowpeas) and then finally stored in 1 BK bin till actual milling started.

The cleaned grains from the 1 BK bins was fed to 1/2 BK double high corrugated rolls rotating at different speeds for initial coarse splitting of the grains. The broken grains were then sent to a vibrating sifter having screens of increasing fineness from top to bottom (1041µm, 500µm, and 315µm). The +1041µm particles or scalp which contained mainly bran was sent to feed. The -1041µm to +500µm particles collected on top of 500µm screen was sent to purifier. Also, -500µm to +315µm particles were sent to purifier for further separation of bran and endosperm. The -315µm particles were collected as flour from 1/2 BK rolls. The throughs from the purifiers was sent to 1/2 middings reduction roll (M) and the output of 1/2 M was sent to 1/2 M sifter having 315 µm screen. The throughs from the 315 µm screen was called flour (and maximum flour was made here) and the overs were directed to feed stream.

Proximate composition

The proximate composition of raw ingredients was determined using standard methods. This included determination of moisture (135°C for 2h; AACC 44-19), crude protein (based on nitrogen by combustion, 6.25X; AOAC 920.176), crude fat (petroleum ether extract method; AOCS Ba 3-38), ash (600ºC for 2h; AOAC 942.05), crude fiber (AOAC 962.09); and total starch (glucoamylase method; AOAC 979.10). Protein, starch, fat, ash and crude fiber contents were reported as dry basis percentages (% db) from replicates

Statistical Analysis

Analysis of variance (ANOVA) was used to test whether differences occurred (p≤0.05) for each of the test parameters. Data was analyzed in duplicate. Tukey's post-hoc means separation at the 5% level of significance was used to determine which samples were significantly different for each of the measured properties. Statistical analyses were performed with SAS® statistical software (version 9.2, SAS Institute Inc., Cary, NC) using PROC GLM.

Results and Discussion

Sorghum Milling

The proximate analysis of different fractions of sorghum obtained after milling is presented in Table 2.1. It can be observed that during the production of decorticated flours (fine and coarse) there is a significant reduction (p<0.05) in fiber content of flours when compared to respective fiber content in whole grains. The fiber content decreased from 1.89% to 0.38% and 0.45% for raw V1 sorghum grain, decorticated fine V1 sorghum flour and decorticated sorghum coarse V1 flour. The same pattern was observed between whole grain and decorticated flours of V2 and V3 sorghum. The fat content also decreased significantly (p<0.05) from raw to decorticated flours in all the three varieties of sorghum. The protein content in the whole grain and in the respective

decorticated fractions were not significantly different (p>0.05). The starch content increased significantly (p<0.05) from 61.85% to 69.80%, and 72.30% in whole grain of V1 sorghum, decorticated fine flour and decorticated coarse flour respectively. Similar results were observed for V2 and V3 varieties of sorghum. The significantly high amounts of fiber and fat as compared to raw grain and decorticated fine or coarse fractions of flour in peeler by-product fractions (peeler throughs) was indicative of efficient milling. The milling was effective in removing significant portions of germ and pericarp from whole sorghum.

The protein recovery of 97.11% and 96.32% in decorticated V1 fine and decorticated V1 coarse flours as compared to raw whole grain of sorghum V1 is in accordance to previous studies that have reported similar results for decorticated sorghum flours (Abdelrahim and Mudawi, 2014; Desikachar, 1982)

It was also observed that milling of sorghum grain into whole flour of two particle sizes +315 µm and -315 µm had significantly higher (p<0.05) protein, and fiber and significantly lower (p<0.05) starch content in the larger particle size fraction. There was no significant difference in fat and ash content in both the fractions. This change in proximate analysis could have been due to less degree of milling for larger sized particles which could have removed less bran and therefore higher fiber content and lower starch content when compared to lower particle size fraction of whole sorghum. Yanez and Walker (1986) reported higher protein and fiber in coarse flour (-60, +100) as compared to fine flour of proso millet. Kerr et al. (2000) found that fat and starch content of cowpea flour increased with decrease in particle size. They attributed the higher starch content in finer particle sized flour to the occurrence of greater degradation of starch when passing through smaller sieves (and presumably higher shear conditions) that facilitate higher starch extraction.

Cowpea milling

The proximate composition of different milled fractions of cowpea is shown in Table 2.2. It was observed that the protein content of different fractions was not significantly different ($p<0.05$) except for fraction from mill by-product screening. Mill by-product screening removes the unwanted outer shell/seed coat of cowpea and thus lower protein content in that fraction was able to demonstrate the efficacy of peeling which could efficiently remove just the seed coat without taking away parts of endosperm with it that is rich in protein. The fiber content in peeler screening and mill by-product screening was found to be exceptionally high with 9.98% and 22.05% respectively. These fractions had significantly higher ($p<0.05$) fiber content than other fractions of cowpea which indicated efficient removal of seed coat from the endosperm of the grain. The milling removed the germ effectively as can be observed from significantly high ($p<0.05$) fat content of 1.73% in peeler screenings and subsequently significantly lower ($p<0.05$) fat content of 0.66% in mill by-product screening as compared to all other fractions of cowpea. Ash content was lowest in mill by-product screening and it was significantly different from other fractions of cowpea milling stream. The starch content increased significantly ($p<0.05$) after decortication of cowpea grain.

Milling does not affect the overall nutritional composition of cowpea flour to a large extent but it does reduce the fiber content (Phillips, 1982). Ward et al. (1995) reported that moisture, fat, protein, ash, and total carbohydrate composition of cowpea was not affected by milling and particle size. Reichert et al (1979) observed that on d of brown and white varieties of cowpea on barley deawner, the seed coat had least amount of protein and fat, highest amount of ash and fiber when compared to other fractions of milling. The current observations in this study was in

accordance to the above findings and clearly established that cowpea flour with low fiber and fat content could be produced on roller wheat flour mills.

Corn Milling

The proximate composition of different milled fractions of corn is given in Table 2.3. The average protein content in degermed fine (6.02%) and degermed coarse corn (7.66%) are significantly different ($p < 0.05$). The M-2 (stream) had a significantly higher ($p < 0.05$) protein content (9.51%) than the degermed fine and coarse fractions as the protein matrix surrounds the endosperm which is primarily starch (Gwirtz and Garcia-Casal, 2014). The flour stream from patent screw, 2/18 raw maize, and corn meal had lower protein content as compared to other streams and it clearly indicated that milling could remove the seed coat and would discard it through the corn meal stream. It can be observed from the table that milling was able to reduce the fiber content in degermed fractions of corn flour significantly ($p < 0.05$) from the 2/18 raw maize stream which carried most of the hull and germ. Similarly, fat and ash were also reduced in the degermed fractions. Degermination and seed coat removal significantly increased ($p < 0.05$) the starch content of the degermed corn flour fractions.

The whole corn fractions (coarse and fine) had different proximate contents as well. The coarse fraction had significantly higher ($p < 0.05$) content of protein and fiber as compared to whole fine corn flour. Fat, ash, and starch contents were found to be higher in fine fraction of whole corn as compared to whole coarse corn. In a study conducted by Bookwalter et al (1974) to characterize different fractions of dry milled high lysine corn and ordinary dent corn, they found that fat and ash content to be higher in lower particle sized corn fractions in both cases and protein and fiber to be higher in coarser fraction.

Conclusions

1. Decortication of white sorghum grain reduced the fiber content from 1.89% to 0.38% and 0.45% in raw, finely milled and coarsely milled fractions respectively. Decrease in fat content followed similar trend.

2. Starch content increased as the fiber, and fat content decreased across the flours obtained for all grains.

3. Cowpea milling was shorter than sorghum and corn milling because only one particle size (< 315 μm) was required.

4. Pre-conditioning of cowpea prior to milling was avoided as water made the cowpea fractions sticky and it obstructed the flow of cowpeas through the milling system.

5. The milling process was effective in removing the back eye surrounding the hilum of the cowpea and that improved the aesthetic value of the flour. The tip cap of the corn kernel was extracted along with germ and pericarp to enhance the visual appeal of the de-germed corn products.

5. Wheat milling system successfully produced flours of all the grains with low fiber and fat.

Acknowledgement

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Conditioning & Post Conditioning

Conditioning
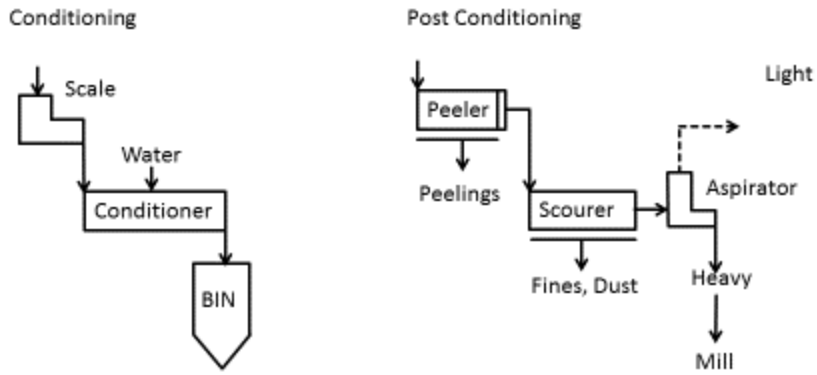
Post Conditioning

Figure 2.1 Conditioning and Post conditioning before milling sorghum and corn
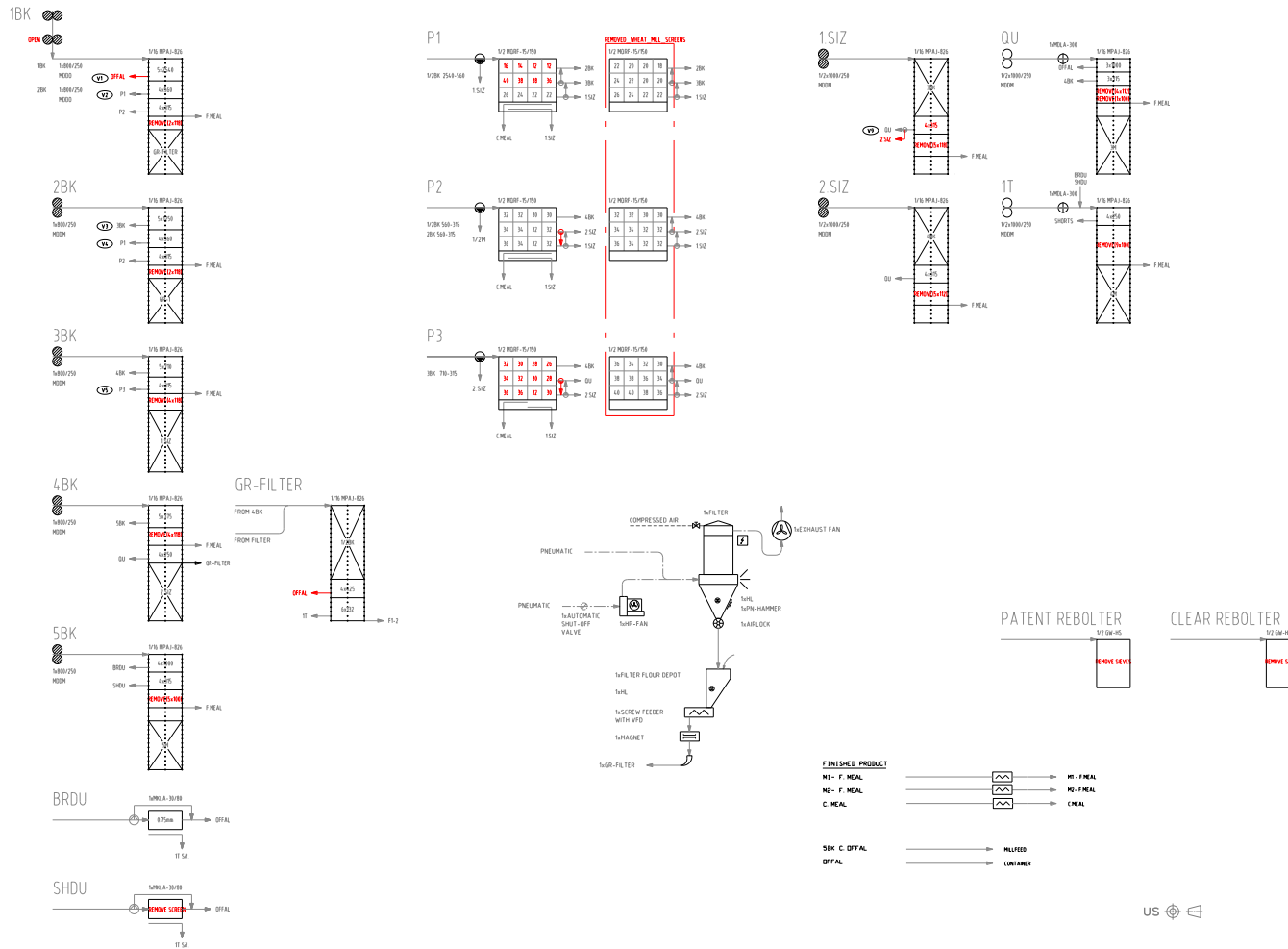
Figure 2.2 Milling Flow Chart for producing decorticated sorghum and corn flour.

BK = Break Rolls; P1, P2, P3 = Purifiers; Siz = Sizings; QU = Quality, 1T = Tailings; BRDU = Bran duster; SHDU = Shorts Duster, GR = Grader

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Whole Grain Grinding System

Corn, Sorghum

H.M.

Hammer

Screen

350 μm

Coarse

Fine
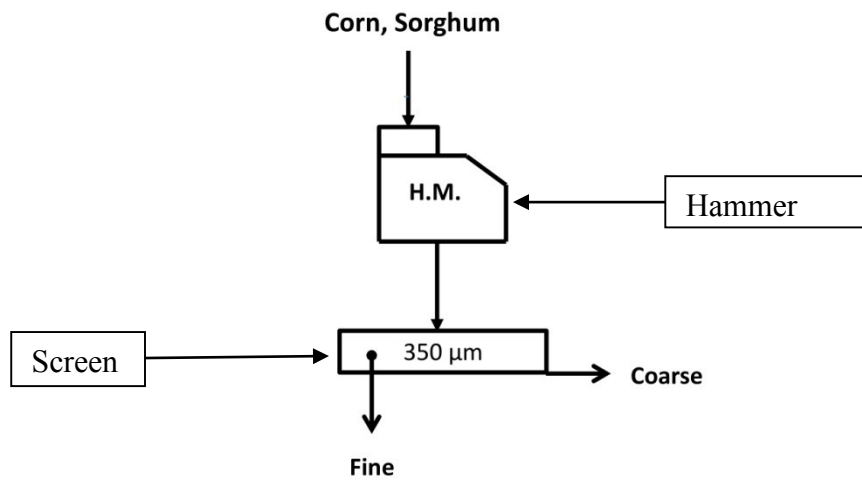
Figure 2.3 Grinding system for production of whole sorghum and whole corn.

H.M. – Hammer mill

Cowpea Milling Flow

Cowpea

1/2BK

1041 μm → FD
500 μm → P-1
315 μm → P-2

Flour

P-1

20	22	18	16	→ FD
24	22	20	18	→ FD
26	24	22	20	→ FD

1/2M   FD

P-2

38	36	34	32	→ FD
40	38	36	34	→ FD
42	40	38	36	→ FD

1/2M   FD

1/2M

315 μm → FD

Flour

BK = Break rolls, P-1 & P-2 = Triple deck purifiers, FD = To Feed, M = Middling
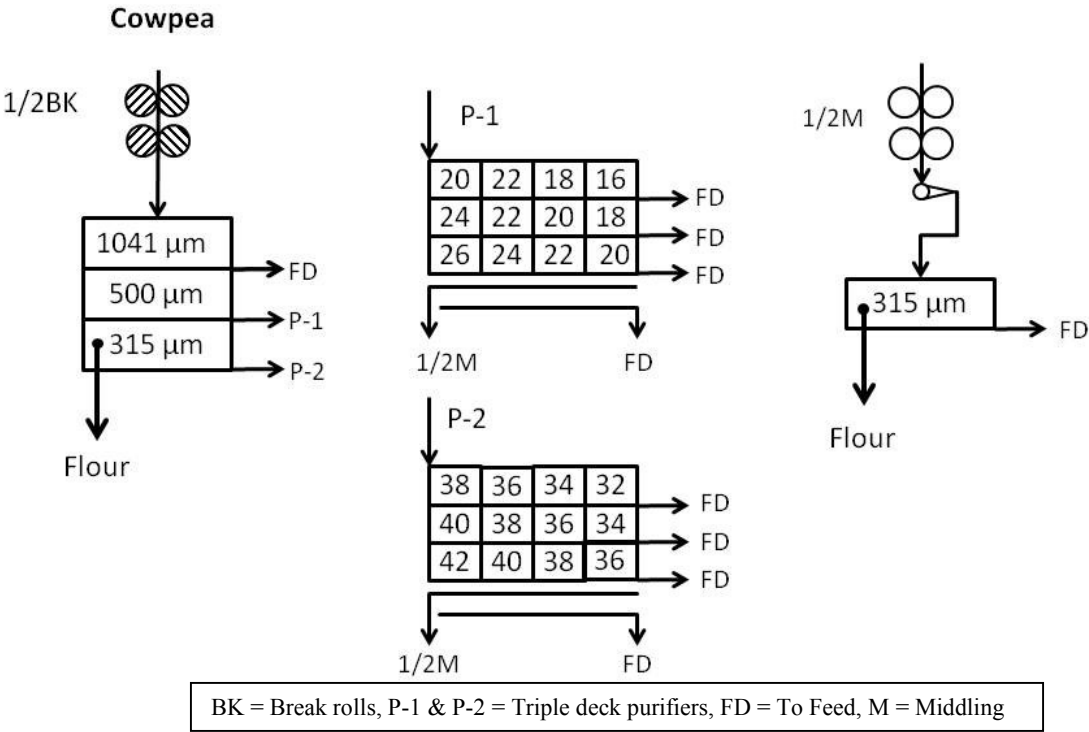
Figure 2.4 Flow chart for milling of decorticated cowpea

Table 2.1 Proximate analysis of sorghum milling streams

Milling Streams	Crude Protein (%)	Crude Fiber (%)	Crude Fat (%)	Ash (%)	Starch (%)
Poor fine	$12.3\pm0.3_b$	$1.3\pm0.1_{ae}$	$3.9\pm0.1_d$	$1.8\pm0.1_d$	$58.6\pm0.1_{dg}$
Peeler throughs	$7.8\pm0.3_a$	$2.0\pm0.2_{ci}$	$4.1\pm0.0_d$	$1.7\pm0.0_d$	$57.0\pm0.0_d$
Semolina	$7.3\pm0.3_a$	$1.0\pm0.0_{degj}$	$0.5\pm0.0_c$	$0.3\pm0.0_{ch}$	$70.5\pm0.3_{bc}$
Whole – V1	$7.6\pm0.2_a$	$1.9\pm0.1_{aci}$	$3.2\pm0.2_a$	$0.6\pm0.0_{agj}$	$61.8\pm0.6_a$
Decorticated-V1 (fine)	$7.4\pm0.1_a$	$0.4\pm0.1_b$	$1.7\pm0.0_b$	$0.7\pm0.0_{abf}$	$69.8\pm0.4_{beh}$
Decorticated-V1 (coarse)	$7.3\pm0.2_a$	$0.4\pm0.1_{bd}$	$0.5\pm0.2_c$	$0.3\pm0.0_c$	$72.3\pm0.4_c$
Whole – V2	$7.8\pm0.2_a$	$2.1\pm0.2_{chi}$	$3.4\pm0.1_a$	$0.90\pm0.0_f$	$64.2\pm0.2_f$
Decorticated-V2 (fine)	$7.5\pm0.2_a$	$0.3\pm0.1_b$	$1.5\pm0.0_b$	$0.5\pm0.0_{gh}$	$70.0\pm1.2_{bce}$
Whole – V3	$9.0\pm0.4_{cd}$	$2.3\pm0.3_i$	$3.1\pm0.1_a$	$0.8\pm0.0_{fi}$	$60.2\pm0.1_{ag}$
Decorticated-V3 (fine)	$7.9\pm0.1_{ad}$	$0.5\pm0.1_{bj}$	$1.9\pm0.1_b$	$0.7\pm0.0_{bfj}$	$70.6\pm0.2_{ch}$
Whole-V1 (+315 µm)	$9.3\pm0.3_c$	$3.1\pm0.1_f$	$3.0\pm0.1_a$	$1.3\pm0.0_e$	$57.3\pm0.6_d$
Whole-V1 (+315 µm)	$6.8\pm0.1_a$	$1.6\pm0.1_{acg}$	$3.0\pm0.1_a$	$1.3\pm0.0_e$	$68.0\pm0.8_e$

V1, V2 = white varieties of sorghum V3 = red variety of sorghum. Values in the same column not sharing the same subscript are significantly different at $p< 0.05$

Table 2.2 Proximate analysis of cowpea milling streams

Milling Streams	Crude Protein (%)	Crude Fiber (%)	Crude Fat (%)	Ash (%)	Starch (%)
Whole Cowpea	$24.96\pm0.38_a$	$2.22\pm0.18_a$	$1.22\pm0.06_a$	$3.07\pm0.24_a$	$36.15\pm0.07_a$
Peeler Screening	$25.2\pm0.21_a$	$9.98\pm0.18_b$	$1.73\pm0.00_b$	$3.52\pm0.23_a$	$18.90\pm0.42_b$
Mill by-product screening	$21.07\pm0.23_b$	$22.05\pm0.18_c$	$0.66\pm0.05_c$	$5.12\pm0.30_a$	$9.75\pm0.07_c$
From Peeler to 1/2 BK	$25.87\pm0.01_a$	$1.61\pm0.43_a$	$1.11\pm0.10_a$	$2.65\pm0.06_a$	$37.95\pm0.64_d$
Flour screening 1/2 M flour	$26.04\pm0.95_a$	$1.08\pm0.37_a$	$1.31\pm0.09_a$	$2.80\pm0.01_a$	$43.5\pm0.00_e$
Prior to 1/2 M sifter	$25.70\pm0.47_a$	$1.69\pm0.19_a$	$1.09\pm0.10_a$	$2.68\pm0.30_a$	$42.1\pm0.14_f$
Flour Screening to Bulk sack	$26.10\pm0.30_a$	$1.06\pm0.11_a$	$1.07\pm0.07_a$	$3.08\pm0.04_a$	$40.7\pm0.00_g$

BK = Break rolls, M = Middlings, Values in the same column not sharing the same subscript are significantly different at $p< 0.05$

Table 2.3 Proximate analysis of corn milling streams

Milling Streams	Crude Protein (%)	Crude Fiber (%)	Crude Fat (%)	Ash (%)	Starch (%)
M-2 Stream clear	$9.5\pm0.2_{ad}$	$0.5\pm0.08_a$	$2.0\pm0.19_a$	$1.0\pm0.04_a$	$67.5\pm0.07_a$
Flour from Patent screw	$6.1\pm0.2_b$	$0.7\pm0.21_a$	$0.6\pm0.08_b$	$0.3\pm0.03_b$	$75.5\pm1.20_b$
2/18 Raw maize	$7.8\pm0.03_c$	$1.9\pm0.2_b$	$3.3\pm0.18_c$	$1.2\pm0.07_{ac}$	$60.1\pm0.28_c$
Coarse meal	$7.9\pm0.05_c$	$0.1\pm0.06_a$	$0.4\pm0.15_b$	$0.2\pm0.04_b$	$74.5\pm0.57_b$
Degermed fine	$6.0\pm0.2_b$	$0.2\pm0.03_a$	$1.2\pm0.02_d$	$0.3\pm0.05_b$	$74.7\pm0.93_b$
Degermed coarse	$7.7\pm0.06_c$	$0.2\pm0.03_a$	$0.6\pm0.13_b$	$0.3\pm0.02_b$	$71.8\pm0.00_b$
Whole Corn (+315 μm)	$9.9\pm0.04_a$	$3.4\pm0.36_c$	$2.5\pm0.09_a$	$1.3\pm0.10_c$	$54.6\pm1.77_d$
Whole Corn (-315 μm)	$9.1\pm0.08_d$	$2.0\pm0.15_b$	$3.1\pm0.02_c$	$1.4\pm0.11_{cd}$	$63.2\pm0.07_c$

Values in the same column not sharing the same subscript are significantly different at $p < 0.05$

Chapter 3 - Characterization of physico-chemical properties of high protein extrudates from binary blends of cereal and legume flours

Abstract

Binary blends of cereal/legume flours were extruded to produce composite products of sorghum cowpea (SC), sorghum soy (SS), and corn soy (CS) with protein content ranging from 12.01-18.70%. Greater variation in motor load was observed in blends that contained whole grains and had higher inherent fat content. SME was higher in formulations with decorticated and low inherent fat content as compared to blends that contained whole grains and high fat content. Higher specific mechanical energy (SME) led to lower bulk density of extrudates across all blends. Strong positive linear correlation between SME and expansion ratio (ER) irrespective of the blend type was found. SC formulations had the least BD and highest ER (34.20 – 57.96 g/L, and 19.39-27.91) followed by CS (75.65 – 482.60 g/L, and 2.42 – 15.49) and SS (111.10 – 514.30 g/L, and 2.43-14.78) on account of higher SME generated due to higher amount of starch in SC>CS>SS.

Introduction

According to the report in The State of Food Insecurity in the World 2014 by United Nations Food and Agriculture Organization an estimated 795 million people of the 7.3 billion people in the world, which translates to one in nine were affected from chronic undernourishment in 2012-2014. There was reduction in hungry people by over 167 million since past decade and the prevalence of undernourishment has fallen from 18.7% to 11.3% globally and from 23.4% to 13.5% for developing countries (FAO et al., 2015). Cereal only formulations made from corn and sorghum would not be useful in situations of feeding to moderate malnourished kids below 5

years as these products would lack in energy and protein density (Lartey et al., 1997) and would contribute to protein–energy malnutrition (Walker, 1990). The use of composite flours has been reported to be advantageous to use in developing countries as it helps in promoting high yielding native plant species, a greater degree of supply of protein for human nutrition and better use of domestic agriculture production (Bugusu et al., 2001). In view of the above and with the current challenges of delivering more nutritional products to food aid recipients, Tufts University prepared Food Aid Quality Review (FAQR) report to United States Agency for International Development (USAID) on recommendations (1 - 18) to improve the quality of food aid products (Webb et al., 2011). The cereals only extruded products are rich in carbohydrate and fibers and relatively low in protein content, thus there is a need to enhance protein component of these products (Seth and Rajamanickam, 2012, and Devi et al., 2013). Legumes are high in nutritive value, especially proteins and their use as protein supplement in foods or as a part of composite flours are widely studied (Naivikul and D'Appolonia, 1976).

The importance of sorghum over the commonly used corn, as an alternate source of starch/calories in binary cereal blends is very relevant in today's global scenario as it is a drought tolerant crop, relatively tolerant to insect feeding, naturally gluten free, non-transgenic, and native to many countries facing challenges of undernourishment. Further, sorghum has been recommended (recommendation 18) in FAQR as a part of the new blends in the 'food aid basket'. The low protein quality of sorghum owing to high quantity of cross-linked proteins (prolamins) than other cereals (Devi et al., 2013) due to interactions of these proteins with non-protein components like lipids, starch, and polyphenols like tannins,and phytates reduces protein digestibility (de Mesa-Stonestreet et al., 2010) which necessitates a need for sorghum-based foods with higher protein content and digestibility. High protein food ingredients such as whey

protein and legume flours are available as additives that can provide both functional and nutritional value to sorghum-based snacks. Cowpea flour could be an alternate source of protein that could be blended with sorghum to form a novel cereal blend. Cowpea is a good source of energy and plant protein. Compared to other grain legumes and cereals, cowpeas are a good source of non-heme iron, which if made bioavailable could significantly contribute towards dietary iron intake in targeted interventions (Abizari et al., 2012). Soybean has been used as an effective source of plant protein from the early days of formulating cereal blends for food aid programs because of its favorable content and constitution of amino acid (Hoppe et al., 2008). Raw soy contains considerable amounts of antinutrients (antitrypsin) that potentially interfere with protein digestibility and mineral absorption. However, these and other cereals are heat treated to improve the digestibility and reduce levels of antinutrients and cooking times.

In order to reduce the low mineral bioavailability and poor digestion of cereal blends from plants due to the presence of anti-nutritional factors, special processing should be used (de Pee and Bloem, 2008). Extrusion processing can be a process that could efficiently create innovative products that might not be easily possible with other processing methods (Cisneros and Kokini, 2002, Alavi et al., 2014). Extruded products can improve the functionality in food applications, particularly in instant foods because of the changes occurring in starch such as gelatinization, or degradation that directly affects the texture of the final product (Zeng et al., 2011). Previous studies have looked into extrusion of cereals and their physico-chemical properties and rheological behavior (Ding et al 2006; Karkle et al., 2012; Devi et al., 2013). Similarly, studies have been reported about the cereal legume blends (Pelembe et al., 2002; Padmanabhan, 2013). However, not much literature is available on physicochemical properties of extruded binary blends of sorghum cowpea and sorghum soy. Therefore, it is of interest to investigate the

consequence of extrusion on physicochemical properties of novel binary blends of cereal/legume flours.

In order to address the shortcomings of current food aid blends and following the recommendations of FAQR, this study was undertaken with the objective of designing the appropriate ratio of binary blends of cereal/legume flours that could meet the nutritional requirements as recommended in FAQR for FBFs. Different granulations (fine and coarse) for two types of milled cereals (whole and decorticated) and 2 types of legumes (cowpea and soybean) with 3 different fat levels of soy were blended in appropriate ratio and physiochemical properties of these binary blends were studied after extrusion processing.

Materials and Methods

Raw Materials

Sorghum flour – variety V1 (Fontanelle 4525) as whole and decorticated flour was obtained from commercial source (Nu Life Market, Scott City, Kansas, USA). The whole grains– sorghum V1, V2 (738Y) & red variety - V3 (217X Burgundy) (Nu Life Market, Scott City, Kansas, USA), corn (Agronomy Foundation Seed, Kansas State University, Manhattan, Kansas, USA) and soybeans (Kansas River Valley Experiment Field, Kansas State University, Manhattan Kansas, USA) were used for pilot milling to obtain whole and decorticated flours in two granulations – fine and coarse. Cowpea grains (variety number 8046) were obtained from LPD Enterprises LLC, Olathe, Kansas, USA. Defatted soy flour was purchased from American Natural Soy, Cherokee, Iowa, USA. Degermed corn meal, degermed corn flour and whole corn flour was purchased from Agricor, Marion, Indiana, USA. The cereal/legume flours were blended in appropriate ratios – SC (39% sorghum, 61% cowpea), SS (75% sorghum, 25% soy), and CS (76% corn, 24% soy) using a ribbon blender and mixed for 5 minutes. The blends were mixed in

batches of 100 kg. The blends were collected in multi-walled paper bags from the bottom of the mixer by opening a sliding door.

Extrusion Processing

The binary formulations – sorghum cowpea, sorghum soy and corn soy were extruded on single screw extruder X-20 (Wenger Manufacturing Inc., Sabetha, KS, USA), equipped with a differential diameter cylinder preconditioner having a volumetric capacity of 0.056 m3 (DDC2, Wenger Manufacturing Inc., Sabetha, KS, USA) The screw diameter was 82.55 mm with an L/D ratio of 8.11. The dry feed rate was 200 kg/h for formulations made from commercially sourced flours and 166 kg/h for formulations that were obtained from flours produced from pilot milling in order to have a smooth running of extruder. Steam and water was added only in the preconditioner. Steam addition ranged 11-14 kg/h and water addition ranged from 6.9 – 16.6 kg/h across all formulations. The preconditioner discharge temperature was always maintained above 85°C with the help of right combination of water and steam in the preconditioner. The screw speed ranged from 500-550 rpm. The calculated in-barrel moisture content ranged between 18-24%. Thermocouples mounted at zones 1, 2, and 3 of the extruder barrel were used to record the respective zone temperatures from the control panel. The die temperature and pressure was also recorded directly using thermocouple and pressure gauge respectively. The die had a single circular opening of 4.1 mm. The screw configuration is shown in Fig. 3.1.The extrudates were cut at the die exit with face-mounted five blade rotary knife operating between 1300-1400 rpm. All the cut extrudates were pneumatically conveyed to Wenger Double Pass Dryer/Cooler (Series 4800, Wenger Manufacturing Inc., Sabetha, KS, USA) operating at 104°C. The retention time in the dryer was 10 minutes (5 minutes each for top and bottom belts).

Cooling was accomplished by room temperature air with 5 minutes retention time on cooling belt.

The specific mechanical energy (SME) for each treatment was calculated as follows:

$$SME = \frac{\frac{(\tau - \tau_O)}{100} \times P_{rated} \times \frac{N}{N_{rated}}}{\dot{m}} \tag{1}$$

where,

τ = operating torque (%); τ_0 = no-load torque (%); P_{rated} = rated power (37.3 kW), N = screw speed (rpm); N_{rated} = rated screw speed (507 rpm), and ṁ = net mass flow rate of extrudate at die exit (kg/s).

Bulk Density (BD)

. The raw material or the extrudate was filled in a steel cup of known volume (V= 1L) and the top of the cup was leveled to the brim and the weight (Me) was measured on a digital weighing scale which was previously tared to discount the weight of the steel cup.

Bulk Density (BD) was calculated as:

$$BD, g/L = \frac{Me}{V} \tag{2}$$

Extrudate Macrostructure

For every treatment, the length (l_e), diameter (d_e) and mass (m_e) of 20 extrudates were measured and used to obtain the radial expansion ratio (ER), specific length (lsp) and piece density (PD), as described below.

$$ER = \frac{d_e^2}{d_d^2} \quad \text{where, } d_d = \text{die diameter} \tag{3}$$

$$l_{sp}(m/kg) = \frac{l_e}{m_e} \tag{4}$$

$$PD(kg/m^3) = \frac{4m_e}{\pi d_e l_e} \tag{5}$$

Moisture analysis

The moisture content of the samples were determined by oven drying method based on AACC method 44-19 (2013). All the samples were tested for moisture in duplicate.

Particle size analysis

Particle size distribution of the raw and extruded and milled products was determined using a laser diffraction particle size analyser (LS™ 13320, Beckman-Coulter, Inc., Miami, FL, U.S.A.). Duplicate tests were conducted for each sample.

Proximate composition

The proximate composition of raw ingredients was determined using standard methods. This included determination of moisture (135°C for 2h; AACC 44-19), crude protein (based on nitrogen by combustion, 6.25X; AOAC 920.176), crude fat (petroleum ether extract method; AOCS Ba 3-38), ash (600°C for 2h; AOAC 942.05), crude fiber (AOAC 962.09); and total starch (glucoamylase method; AOAC 979.10). Starch, protein, fat, ash and crude fiber contents were reported on dry basis percentage (% db) from replicates. Total carbohydrates was calculated by the difference method (Merrill and Watt, 1973).

Statistical Analysis

The experimental designs for SC, SS and SS is shown in Fig.3.2, 3.3, 3.4 respectively. All the results were analyzed using analysis of variance (ANOVA) with general linear model procedure (SAS version 9.1, SAS Institute, Cary, North Carolina, USA). When significant effects ($p < 0.05$) were indicated by ANOVA, Tukey pairwise comparisons were conducted to distinguish which treatments differed significantly ($p < 0.05$). Pearson Correlation was determined between SME, BD, ER, PD.

Results and Discussion

Proximate composition of binary blends

The proximate composition for all binary blends were calculated from the proximate composition of the individual components in the blend are shown in Table 3.1. It can be inferred from the table that all whole blends had higher crude fiber, crude fat and ash as compared to decorticated blends. The crude fat was found to be highest in whole corn formulations as it contained the germ which has 84% (Watson, 1984) of the total oil found in corn kernel. The variation in protein content was the least in SC blends (17.24% - 18.20%) whereas the range for protein content was much wider in SS (12.87% - 19.00%) and CS (12.01% - 18.33%) blends. The large variation in protein content in SS and CS blends were due to the incorporation of soy with varying levels of fat. Full fat soy had the least protein and vice-versa and that impacted the final protein content in SS and CS blends. The starch content was lower in blends with whole flour as component whole flours had lower starch content compared to decorticated decorticated/degermed flours.

SME

The SME input during extrusion process is dependent on resistance to flow or flow temperature (T_f) of the melt inside the extruder barrel (Alavi et al., 2011). It was observed from Table 3.2, Table 3.3, and Table 3.4 that SC blends had the highest SME range (286 – 362 kJ/kg), followed by CS (139 – 371 kJ/kg) and the least SME was found in SS blends (67 – 333 kJ/kg). The higher SME in SC was due to higher starch content in it as compared to SS and CS. Soy and cowpea are the sources of protein in the blends but their respective incorporation into the blends has an impact on the SME. The starch present in the blend is the primary contributor to the viscosity of the blend and higher viscosity leads to higher T_f whereas sugar acts as a plasticizer inside the

extruder and reduces T_f. Soy also contributed towards high oil content in the blends as it acts as lubricant and decreases T_f.

Decorticated vs. Whole Blends

It can be observed from Table 3.2, Table 3.3 and Table 3.4 that decorticated blends had higher SME (163 – 362 kJ/kg) compared to whole blends (67 - 335.00 kJ/kg). Table 3.1 shows that decorticated blends had higher starch content as compared to whole blends which led to higher T_f due to increase in viscosity caused by higher starch in decorticated blends and resulted in higher SME. The presence of higher amount of fiber in whole blends also increases the Tf but this effect is overridden by the increase in temperature of die in fiber based blends. Hsieh et al. (1989) and Jin et al (1994) reported that addition of fiber in corn led to increase in temperature of die which caused lowering of viscosity of the melt and thus reduced the SME.

Low vs.medium vs. high fat

The soy with different levels of fat (low, medium and high) was incorporated in SS blends. The motor load variations and SME for these blends as observed in Table 3.3 showed that as the fat content of soy increased, SME was lowered. The stability of the extruder during the operation as reflected in the motor load changes was indicative of the fact that higher fat content in soy led to increased motor load variations. The SME of medium fat SS (273 kJ/kg) was found to higher than that of high fat SS (223 kJ/kg). Similar trend was observed for whole sorghum low fat SS (119 kJ/kg) and whole sorghum high fat SS (67 kJ/kg). The oil serves to act as a lubricant and reduces the friction between the particles in the mix and between the screw surfaces (Guy, 2001) and lowers the SME. The lubricating effect of oil contributes towards higher resistance to flow but decreases T_f and thereby lowers SME.

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Coarse vs. fine

The effect of coarse vs. fine particle size on SME was studied in SC and CS. It can be observed from Table 3.2 and Table 3.4 that SME for decorticated SC and CS was higher (362 kJ/kg and 371 kJ/kg respectively) with coarser particle size as compared to fine particle size (341 and 336 kJ/kg respectively). The opposite trend was observed for whole SC and CS with fine particle having higher SME (335 kJ/kg and 203 kJ/kg respectively) as compared to coarse particle (311 kJ/kg and 139 kJ/kg respectively). Decorticated coarse SC had higher starch content, lower fat and fiber content than decorticated fine SC (Table 3.1) which led to higher resistance to flow in coarser blends and thereby increasing the SME. In case of decorticated coarse CS which had lower starch and fat content than decorticated fine CS, the lubricating effect of oil influenced the resistance to flow. The lower fat content lowered the resistance to flow but possibly its higher T_f as compared to fine particle CS blend led to higher SME. Altan et al. (2009) showed a reduction in SME during the extrusion of barley flour when compared to that of barley grits and attributed the influence of particle size on melt viscosity during the process. Konstance and Onwulata (2006) and Desrumaux et al. (1998) reported that large particles had less contact area both between particle to particle and with that of barrel and, consequently, were less affected by barrel temperature than finer particles. The finer particles with higher contact area between itself and with the barrel would heat more rapidly causing it to reach the melt transition temperature faster as compared to coarser particles resulting in lower viscosity and hence reduce SME. In case of whole SC and CS the coarser particle blends had lower SME because the coarser particle sized blends might not have formed as viscous dough as compared to fine blends due to the overall effect of starch, oil and fiber which might have caused incomplete gelatinzation in coarser particle blends and thereby leading to less resistance to flow and thus lower SME.

Sorghum Varieties V1 vs. V2 vs. V3

It can be seen from Table 3.2 that SC had highest SME of 335kJ/kg for whole V1 variety and the lowest SME of 286kJ/kg was for whole V2 variety. The proximate composition of these blends from Table 3.1 show that V1 had highest crude fiber content (1.49%), followed by V3 (1.22%) and V2 (1.14%). Fibers pose resistance to flow and higher fiber content have higher resistance to flow and thereby highest SME is observed in blend with highest fiber content. For decorticated SC, the highest SME was observed in V3 (350 kJ/g) and the least in V2 (333 kJ/g). In decorticated blends, the role of total starch present in the blends becomes important as the fiber is removed and from Table 3.1 it can be seen that V3 had the highest total starch content (56.25%), followed by V1 (55.20%) and V2 (54.34%). The starch which is the primary contributor of viscosity in the melt (Alavi et al., 2011) increases the resistance to flow with increase in starch content and leads to an increase in SME proportional to starch content. In SS blends it can be noted from Table 3.3 that V1 blends for whole and decorticated sorghum had higher SME (67 kJ/kg and 163 kJ/kg respectively) as compared to V2 blend (51 kJ/kg and 138 kJ/kg). For whole blends of SS, the presence of higher amount of fiber and lower amount of oil in V1 was the primary cause of increase in SME whereas in decorticated SS, the higher starch content in V1 led to higher resistance to flow and thereby had higher SME as compared to V2.

Commercial vs. Pilot milled flour

The effect of difference in milling type (commercial and pilot milled) on SME can be seen in SC and SS from Table 3.2 andTable 3.3. In SC blends, it was observed that commercially milled flours had lower SME as compared to pilot milled flour blends. Even though, the commercial milled SC had higher starch content than pilot milled flour but this difference was negated by the difference in raw material flow rate. The commercial flours were extruded at a flow rate of 200

kg/h whereas the pilot milled flour blends were extruded at 166 kg/h. The difference in flow rate was determined as the flow rates to keep the extrusion operation as smooth as possible and the motor load variations can be observed in Fig 3.5. It can be seen from equation 1 that higher flow rates lower the SME and vice-versa. In case of SS, the commercial flour blend had higher SME (223 kJ/kg) as compared to similar blend made from pilot flour (163 kJ/kg). This could be attributed to the lower particle size of commercial sorghum (74.86 μm) as compared to that of pilot milled decorticated sorghum V1 (203 μm). The lower particle size flour would degrade faster inside the extruder barrel and would form a more viscous dough than a coarser particle flour, thereby increasing the resistance to flow and causing higher SME.

An example of the effect of the blend formula on the stability of extruder while operating is shown in Figs. 3.9 and 3.10. It can be seen that the motor load variation is higher in blend that has higher fat content as compared to blend that is lower in inherent fat content. The stability of extruder is critical in producing products with consistent quality profile which in turn depends a lot on the raw material characteristics.

Expansion Characteristics - ER, BD & PD

Expansion of extrudates on exiting the die is a function of SME and extensibility of the matrix (Alavi et al., 2011). The authors explained that occurrence of expansion was due to the water vapor pressure inside nucleating bubbles as the primary contributor for expansion which in turn was a function of melt temperature. Zhu et al. (2010) had reported that higher SME led to higher melt temperatures at the die exit and thus a greater driving force for expansion.

Sorghum Cowpea

It can be observed from Table 3.2 that the ER ranged from 19.39 - 27.91. The ER was found to be higher in decorticated blends when compared to their respective whole blends and there was a

significant difference (p<0.05) between them. The higher expansion observed in decorticated blends was due to their higher SME and higher starch content than whole blends. Higher starch content provided for a more extensible matrix and thus higher expansion. The influence of varieties V1, and V2 on ER was not significant (p>0.05). The presence of higher fiber in V1 caused higher resistance to flow and thus higher SME and highest ER followed byV2 and V3. For decorticated blends V3 had the highest starch content and thus had the maximum extensible matrix and highest ER followed by V1 and V2. ER had a marked positive correlation with SME (r = 0.62) as shown in Fig. 3.6. However, it was found that there existed a high negative correlation (r = - 0.92) between ER and bulk density (OE-BD, out of extruder or just after exiting the die). Nyombaire (2007), Ding et al (2005), and Fletcher et al (1985) have all reported an inverse relation between ER and bulk density. The OE-BD for decorticated blends was lower and significantly different (p<0.05) from that of whole blends for pilot milled flours. There was no significant difference (p>0.05) between decorticated and whole blends. Due to higher ER for decorticated blends it had lower OE-BD than whole blends. Between coarse and fine grind, there was no significant difference (p>0.05) in OE-BD but coarse blend had higher bulk density. There was no significant difference (p>0.05) in OE-BD due to different varieties. The piece density has been linked with ER to describe the degree of puffing in extrudates (Asare et al., 2004). Piece density is a measure of overall or volumetric expansion (Karkle et al. 2012) and that explains the inverse relation between piece density and ER. There was an inverse correlation between ER and piece density (r = -0.98). Piece density of whole flour SC was significantly higher (0.07 - 0.08 g/cm3) than decorticated flour (0.05 – 0.06 g/cm3) because the high porosity (confirmed by low ER) of decorticated extrudates caused it to have higher volume and lower mass and that led to lower PD. There was a significant difference (p<0.05) in PD of coarse and fine whole

sorghum blends but no significant difference was found between decorticated SC of coarse and fine grind. Varietal differences of sorghum and milling type (commercial vs. pilot) did not contribute to a significant difference (p>0.05) in PD.

Sorghum Soy

Table 3.3 shows that ER for SS ranged from 2.43 – 12.23. The ER was found to be higher in decorticated blends when compared to their respective whole blends and there was a significant difference (p<0.05) between them. The higher expansion observed in decorticated blends was due to their higher SME and higher starch content than whole blends. Higher starch content provided for a more extensible matrix and thus higher expansion. The influence of varieties V1, V2 on ER was not significant (p>0.05). The presence of lipids in amounts lower than 3% does not affect expansion properties, however in amounts greater than 5% there is reduction in expansion rate (Harper, 1994). Lipids reduce the torque due to its lubricating effect which increases the melt slippage inside the extruder barrel causing insufficient pressure to be developed inside the extruder and that leads to poor/lower expansion (Singh et al., 2007). Martinez-Bustos et al (2011) reported that fiber content acted as diluents of starch and it decreased the expansion of extrudates. Guy and Horne (1988) reported that presence of fiber caused the fragmentation of cell membranes and that prevented the gas bubbles from expanding to their maximum capacity. The lack of significant difference in ER between low fat and high fat soy with pilot milled whole sorghum could be attributed to the higher variation in the readings of ER for WSS"B-V1 caused due to greater variation in motor load during extrusion. Variations in motor load cause extrudates to be of non-uniform radial expansion which can affect the inference about the process and product quality. There was a high positive correlation between SME and ER of SS binary blends (r = 0.90) (Fig. 3.7). The OE-BD for decorticated blends were found to

be lower than that of whole blends because the higher starch content created higher resistance to flow leading to higher SME and provided a more extensible matrix. There was no significant difference between the OE-BD of whole and decorticated formulations obtained from commercially milled flour. The effect of varieties of sorghum V1 & V2 on OE-BD was not statistically significant ($p>0.05$) possibly due to the large variation in the OE-BD values contributed by the presence of fat and fiber in the blends. A high inverse correlation ($r = - 0.92$) was found between ER and bulk density (OE-BD).

Piece density was significantly different in SS with whole flour blends being denser than decorticated blends (Table 3.3). The lower expansion caused whole blends to have lower volume as compared to decorticated blends and thus have higher density. The varietal difference was not observed in piece densities. Between commercial and pilot milled flour the PD was a direct reflection of ER and commercial flour based blend had significantly higher ($p<0.05$) ER and thus higher volume and that led to lower piece density as compared to pilot milled flour blend. There was a marked linear inverse correlation between SME and PD ($r = - 0.79$).

Corn Soy

Table 3.4 shows that ER ranged from 2.42 – 18.82 and was found to be higher in decorticated blends than whole blends. However, ER was affected more by the oil content in the blend. Blends with high fat soy showed lower ER. It can be observed from Fig. 3.8 that there was a high positive linear correlation between SME and ER of CS binary blends ($r = 0.92$). A moderate inverse correlation ($r = -0.62$) was found between ER and bulk density (OE-BD). The OE-BD ranged from 75.65 g/L to 482.60 g/L for C'S'B (from commercial flour) and WC'S"B (from pilot milled flour) respectively (Table 3.4). There was significant difference ($p<0.05$) between the OE-BD of whole and decorticated formulations obtained from commercially milled flour. No

significant difference was found between fine and coarse milled CSB formulations from commercially milled flours. No significant differences were found in formulations made from pilot milled flours.

Piece density for CSB formulations ranged from 0.02 g/cm³ to 0.31 g/cm³ for pilot milled WCS"B and WCS"B (aspirated) respectively (Table 3.4). There was significant difference (p<0.05) between the piece densities of whole and decorticated formulations obtained from commercially milled flour. No significant difference was observed in pilot milled flour blends except between WC'S"B and C'S"B (aspirated). There was a marked linear positive correlation between ER and PD (r = 0.70).

As discussed in previous section, a similar correlation between SME and ER was observed. As SME increases due to higher resistance to flow as well as greater extensibility of matrix on account of higher starch content in decorticated blends, it leads to a higher ER. As SME increased, it increases the expansion (Onwulata et al., 2001) causing the bulk density to decrease. The whole formulations had higher bulk density as compared to decorticated CSB blends. Higher bulk density resulted from lower SME due to the presence of extra oil content from whole corn as compared to degermed corn. The lubricating action of oil caused less mechanical shear to be transferred to the melt and thus lower ER and lower BD.

The significant difference in piece density between degermed and whole corn blends made from commercial flour was again indicative of the effect of raw material composition on the overall product characteristics A similar result was not found with blends from pilot milled flour except between whole and degermed corn with and without aspirated high fat soy because the presence of oil content contributed by corn and soy caused a large variation in bulk density measurements and that might have affected the piece density measurements as well.

Conclusions

The blends had a significant impact on the processing and product characteristics. SME, ER, OE-BD, and PD were all affected by blend constituents. Higher starch content was observed in all decorticated blends of SC, SS and CS and they created a viscous matrix inside the extruder barrel which led to higher resistance to flow and also provided more extensible matrix for expansion thereby leading to higher SME and higher ER than whole blends. The different levels of fat in soy had a significant impact on physicochemical properties of SS and CS and higher fat content led to lower SME, lower ER, higher OE-BD and higher PD. Higher oil content in formulations containing soy and/or whole corn caused a big variation in the motor load during extrusion leading to an unstable processing condition. SCB blends had the most stable process followed by SSB and CSB in that order. PD for all the three formulations was another way to understand the impact of raw materials on processing stability with PD being inversely related to ER.

Acknowledgements

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Screw element details from left to right: inlet screw = 96mm; single flight = 96 mm; small steam lock = 19 mm; single flight = 96 mm; small steam lock = 19 mm; single flight = 96 mm; medium steam lock = 19 mm; double flight = 96mm; large steam lock = 19 mm; triple flight uncut cone screw = 114 mm

Figure 3.1 Screw Profile
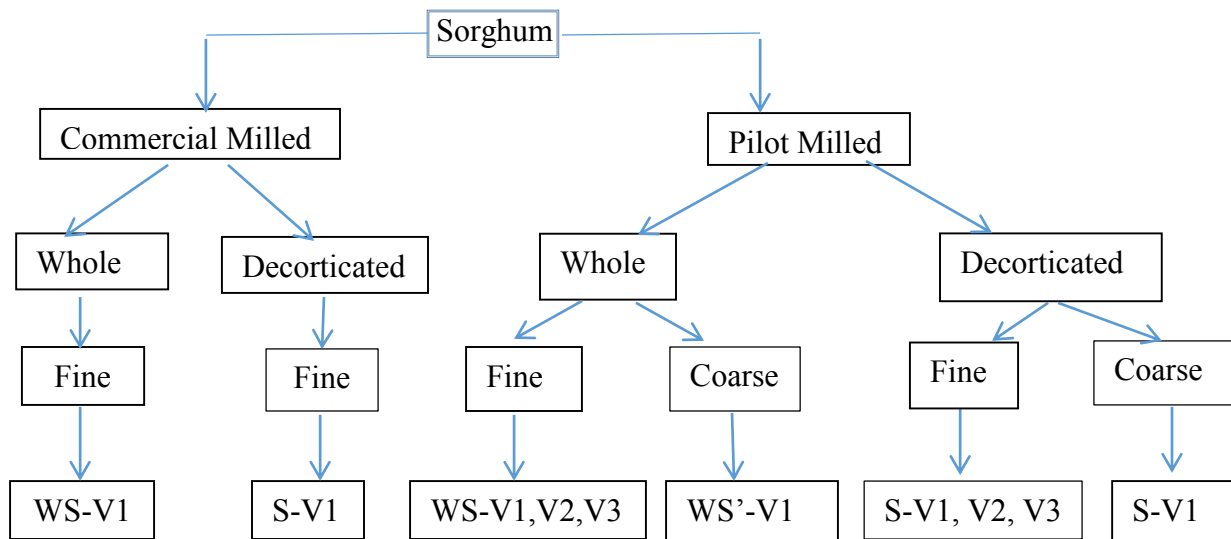
*W=Whole, S= sorghum flour, S' = coarse sorghum flour, V1, V2 = white varieties of sorghum flour , V3 = red variety of sorghum flour, Cowpea flour <315 μm was added to all final sorghum flours to get SC blends., Fine is <315 μm, Coarse is >315 μm

Figure 3.2 Experimental design for SC*
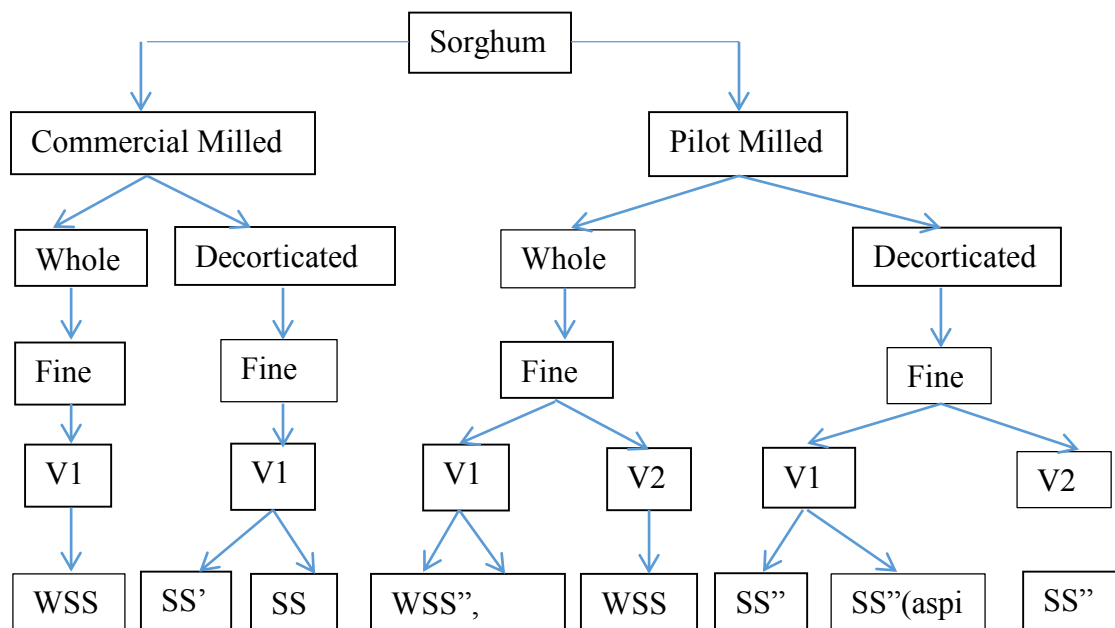
*Soy of different fat levels were added to V1 & V2 sorghum flours, Fine is <315 μm, Coarse is >315 μm, 2nd S denotes soy flour, S"= high fat soy flour, S' = medium fat soy flour, S = low fat soy flour, S"(aspi) = aspirated full fat soy flour

Figure 3.3 Experimental design for SS*
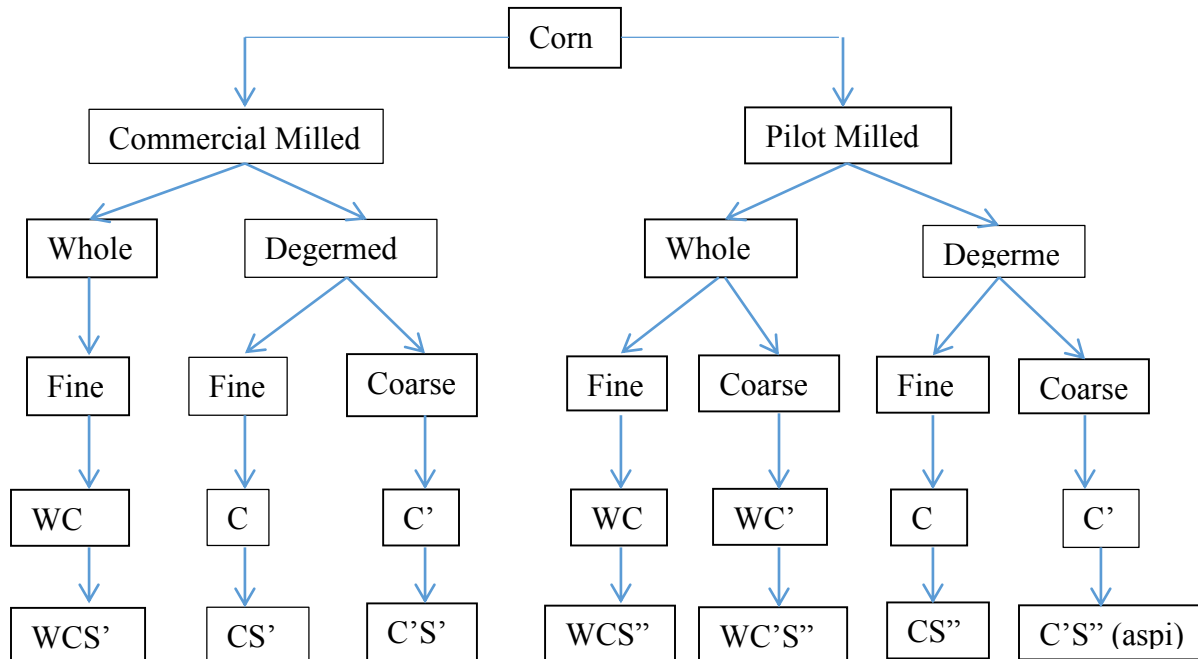
*Soy of different fat levels were added to corn flours, WC = Whole corn flour, C = Degermed corn flour, C' = Coarse corn flour, Fine is <315 μm, Coarse is >315 μm, 2nd S denotes soy flour, S"= high fat soy flour, S' = medium fat soy flour, S = low fat soy flour, S"(aspi) = aspirated full fat soy flour

Figure 3.4 Experimental design for CS*

Table 3.1 Proximate analysis of raw binary blends (dry basis)

Binary Blends	Crude protein (%)	Crude fiber (%)	Fat (%)	Ash (%)	Total Starch (%)	Total Carbohydrate (%)
			SCB - Pilot Milled			
WSC-V1	20.11	1.65	2.22	2.66	56.97	75.00
SC-V1	19.31	0.72	1.63	2.24	61.18	76.83
WS'C-V1	19.27	1.68	2.18	2.53	57.39	76.02
S'C-V1	19.13	0.71	1.08	2.26	62.16	77.53
WSC-V2	19.43	1.26	2.29	2.59	58.27	75.68
SC-V2	19.72	1.06	1.41	2.50	60.20	76.37
WSC-V3	20.07	1.35	2.05	2.61	57.70	75.27
SC-V3	19.38	0.77	1.55	2.37	62.18	76.71
			SCB - Commercial Milled			
WSC-V1	18.90	0.94	2.09	2.61	59.64	76.40
SC-V1	18.96	0.90	1.94	2.54	61.09	76.56
			SSB - Pilot milled			
WSS"-V1	16.38	3.72	7.43	2.30	54.23	73.90
SS"-V1	14.80	1.92	6.30	1.47	62.44	77.43
WSS-V1	21.23	2.67	3.19	2.85	52.93	72.73
WSS"-V2	15.05	2.98	7.60	2.16	56.76	75.18
SS"-V2	15.59	2.59	5.87	1.98	60.54	76.56
SS"-V1 (aspirated)	14.47	1.90	5.23	1.52	64.39	78.79
			SSB - Commercial Milled			
WSS'-V1	20.34	1.43	4.32	2.67	57.08	72.67
SS'-V1	20.46	1.35	4.02	2.52	59.86	73.01
SS"-V1	14.23	2.24	6.79	2.06	62.24	76.92
			CSB - Pilot Milled			
WCS"	14.92	3.26	7.01	1.65	53.88	76.42
CS"	16.74	1.76	5.68	1.49	59.99	76.09
WC'S"	14.83	3.67	7.49	2.15	54.97	75.53
C'S" (aspirated)	13.64	1.78	5.02	1.27	65.15	80.07
			CSB -Commercial Milled			
WCS'	20.50	1.80	5.29	2.78	55.82	71.43
CS'	17.94	0.88	2.68	1.82	65.37	77.56
C'S'	19.91	1.18	2.51	1.86	60.25	75.73

W = whole, 1st S = sorghum flour, 2nd S = Soy (low fat soy) flour, 1st C = degermed Corn flour, 2nd C = Cowpea flour, ' on 1st alphabet = coarse, S' = medium fat soy flour, S" = full fat soy flour, V1&V2 = white varieties of sorghum, V3 = red variety of sorghum

Table 3.2 Average motor load, SME, ER, OE-BD and PD of binary blends of SC

Formulations	Average Motor Load (%); CoV (%)	SME (kJ/kg)	Expansion Ratio	OE-BD (g/L)	PD (g/cm3)
Pilot Milled					
WSCB-V1	73.44; 5.68	335±39abg	21.39±1.68b	56.08±8.70a	0.07±0.01abg
SCB-V1	77.32; 3.89	341±16beg	27.34±1.76df	33.97±2.46b	0.06±0.01efh
WS'CB-V1	72.43; 2.61	311±14acdef	19.82±1.57bc	56.44±2.14a	0.08±0.01c
S'CB-V1	81.29; 4.92	362±29g	26.03±1.37ef	40.46±0.98bd	0.06±0.00hi
WSCB-V2	71.38; 7.02	286±32c	20.64±2.53bc	56.30±11.06ac	0.08±0.01bc
SCB-V2	76.61; 3.63	333±21bfg	23.02±1.84a	44.35±0.96bc	0.07±0.01gi
WSCB-V3	72.02; 3.54	310±43acdef	19.39±1.46c	57.57±4.65ab	0.08±0.01cd
SCB-V3	76.06; 3.09	350±22bdg	27.91±1.83d	34.48±0.99b	0.05±0.00e
Commercial Milled					
WSCB-V1	80.5; 5.84	306±31acdef	24.61±3.29ae	51.80±5.41acd	0.07±0.02afgh
SCB-V1	82.3; 1.78	322±10acdefg	24.70±2.78ae	49.80±3.05acd	0.07±0.01afgh

Different subscripts in the same column are statistically different ($p<0.05$); SME = Specific mechanical energy, OE-BD = out of extruder bulk density, PD = piece density, W = whole, S = decorticated sorghum flour, S' = decorticated coarse sorghum flour, C = cowpea flour, V1&V2 = white varieties of sorghum, V3 = red variety of sorghum
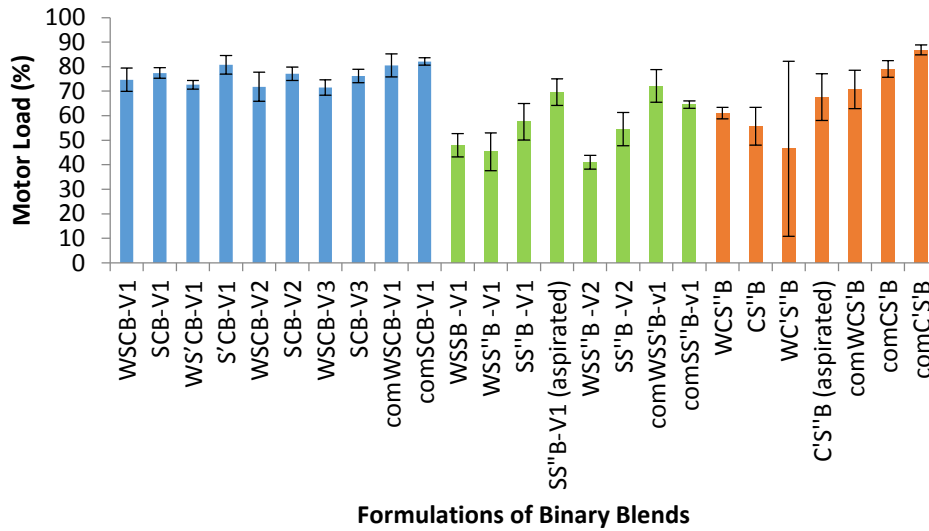
Figure 3.5 Average motor load of different binary blends

com = commercial milled and the others are pilot milled, aspirated = aspirated full fat soy, W = whole, 1st S =

decorticated sorghum flour, 2nd S = Soy (low fat soy) flour, 1st C = decorticated corn flour, 2nd C = Cowpea flour, '

on 1st alphabet = coarse, S' = medium fat soy flour, S" = full fat soy flour, V1&V2 = white varieties of sorghum, V3

= red variety of sorghum

Table 3.3 Average SME, OE-BD, Piece density and Specific Length of binary blends of SS

Formulations	Average Motor Load (%); CoV (%)	SME (kJ/kg)	Expansion Ratio	OE-BD (g/L)	Piece Density (g/cm3)
Pilot Milled					
WSS"B-V1	45.25; 16.97	67±25d	3.10±0.13c	447.80±150.42e	0.63±0.17b
SS"B-V1	57.55; 12.93	163±42de	8.21±0.64d	351.95±110.99	0.40±0.11cdef
WSSB-V1	47.97; 9.90	119±55d	4.47±1.08c	377.45±71.72eg	0.55±0.08bd
WSS"B-V2	54.52; 12.34	51±21d	2.43±0.07c	515.73±42.21e	0.69±0.09b
SS"B-V2	41.00;6.90	138±72df	9.06±2.23de	229.05±15.25agh	0.30±0.10ae
SS"B-V1 (aspirated)	69.57; 7.82	278±65ab	14.78±1.40f	107.30±6.57bcdh	0.30±0.05af
Commercial Milled					
WSS'B-V1	72.50; 8.40	333±52b	12.23±1.31b	115.95±13.20ac	0.27±0.11ac
SS'B-V1	73.40; 11.24	273±28ab	11.80±1.22ab	128.75±17.47afh	0.27±0.03ac
SS"B-V1	64.38; 2.63	223±12aef	10.36±0.61ad	163.55±8.34ab	0.19±0.01a

Different subscripts in the same column are statistically different (p<0.05), SME = Specific mechanical energy, OE-BD = out of extruder bulk density, PD = piece density, W = whole, 1st S = decorticated sorghum flour, 2nd S = low fat soy flour, S' = medium fat soy flour, S" = high fat soy flour, V1&V2 = white varieties of sorghum

Table 3.4 Average SME, OE-BD, Piece density and specific length of binary blends of CS

Formulations	Average Motor Load (%); CoV (%)	SME (kJ/kg)	ER	OE – BD (g/L)	PD (g/L)
Pilot Milled					
WCS"B	61.00; 5.31	203±18cde	3.39±0.35c	137.35±28.58ab	0.02±0.00c
CS"B	55.29; 13.82	239±112c	3.11±0.35c	148.93±49.70bce	0.08±0.00ac
WC'S"B	35.70; 49.50	139±39d	2.42±0.12c	506.30±24.08d	0.08±0.00ac
C'S"B (aspirated)	67.59; 14.06	341±80ab	18.82±1.40d	90.40±17.32ae	0.31±0.11d
Commercial Milled					
WCS'B	70.71; 11.00	262±54ac	9.42±0.94b	180.03±30.68b	0.20±0.02b
CS'B	79.10; 4.31	336±25ab	15.10±0.61a	77.15±2.77a	0.11±0.01a
C'S'B	86.80; 2.29	371±14b	15.49±0.99a	84.80±9.95ac	0.10±0.01a

Different subscripts in the same column are statistically different (p<0.05) SME = Specific mechanical energy, OE-BD = out of extruder bulk density, PD = piece density, W = whole, C= decorticated corn flour, S = low fat soy flour, S' = medium fat soy flour, S" = high fat soy flour, aspirated = aspirated full fat soy flour
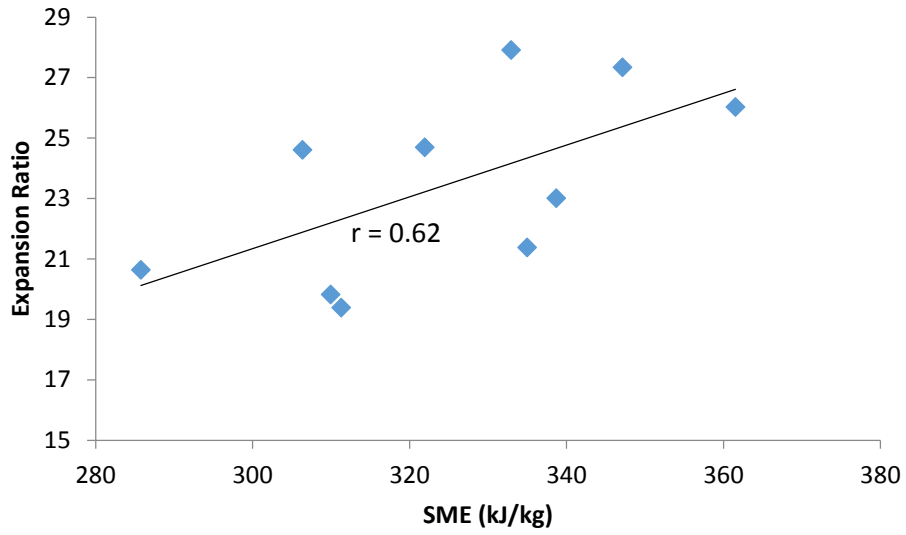
Figure 3.6 Correlation between SME & ER for binary blends of SC

SME = Specific mechanical energy, ER = Expansion ratio, SC = Sorghum cowpea
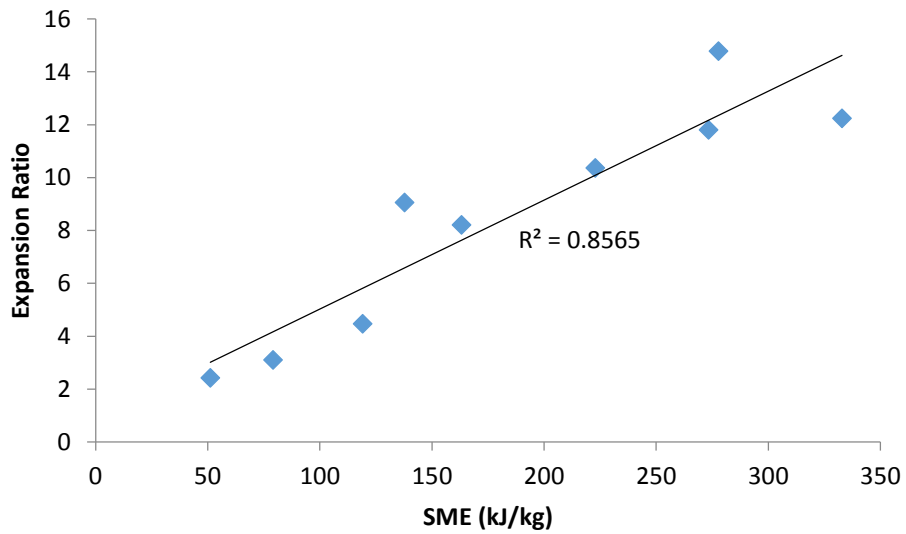
Figure 3.7 Correlation between SME & ER for binary blends of SS

SME = Specific mechanical energy, ER = Expansion ratio, SS = Sorghum soy
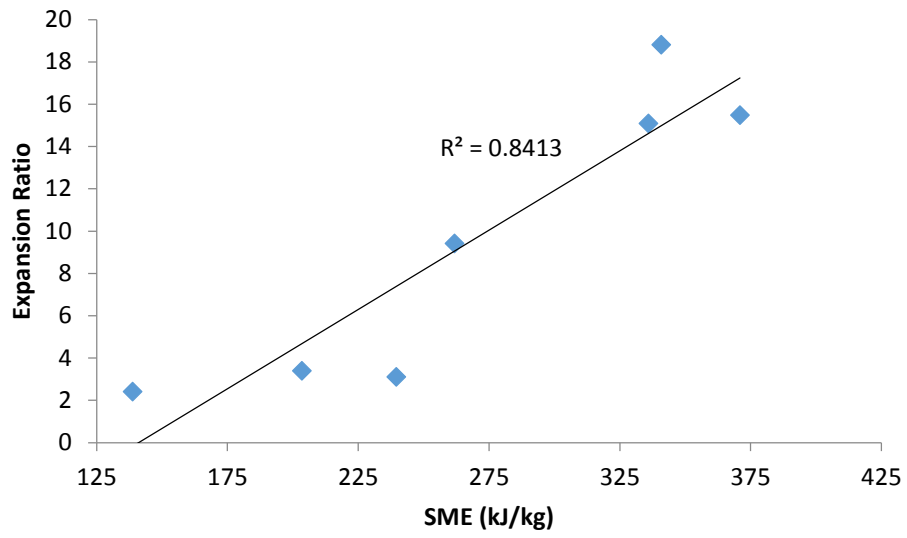
Figure 3.8 Correlation between SME and ER for binary blends of CS

SME = Specific mechanical energy, ER = Expansion ratio, CS = Corn soy
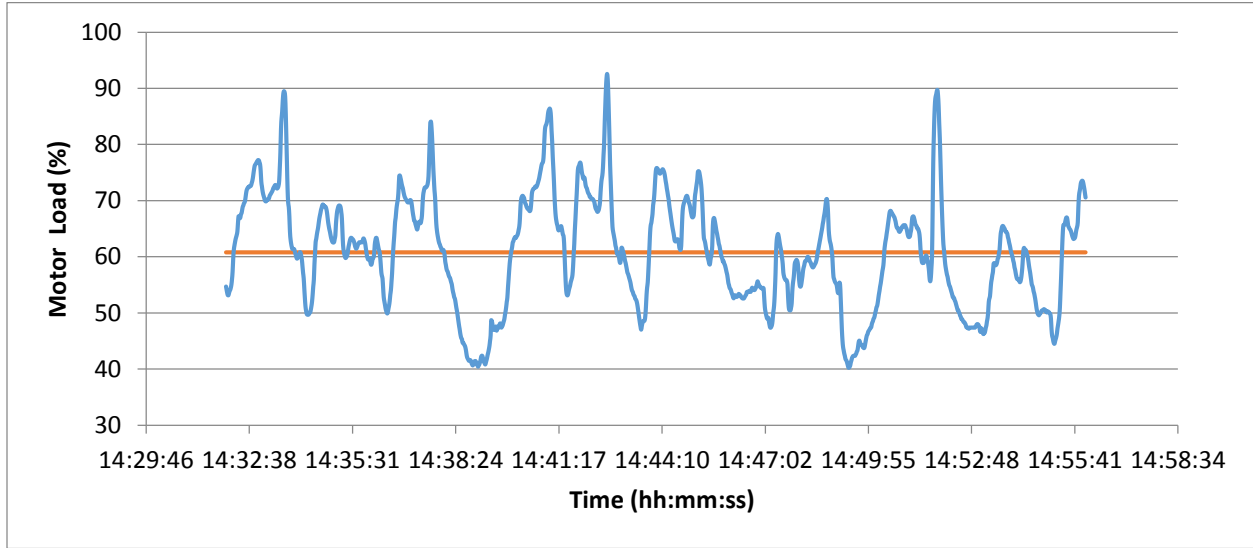
Figure 3.9 Motor load variations in full fat soy blend C'S"B as compared to average motor load.

C'S"B = Degermed corn (coarse) full fat soy blend
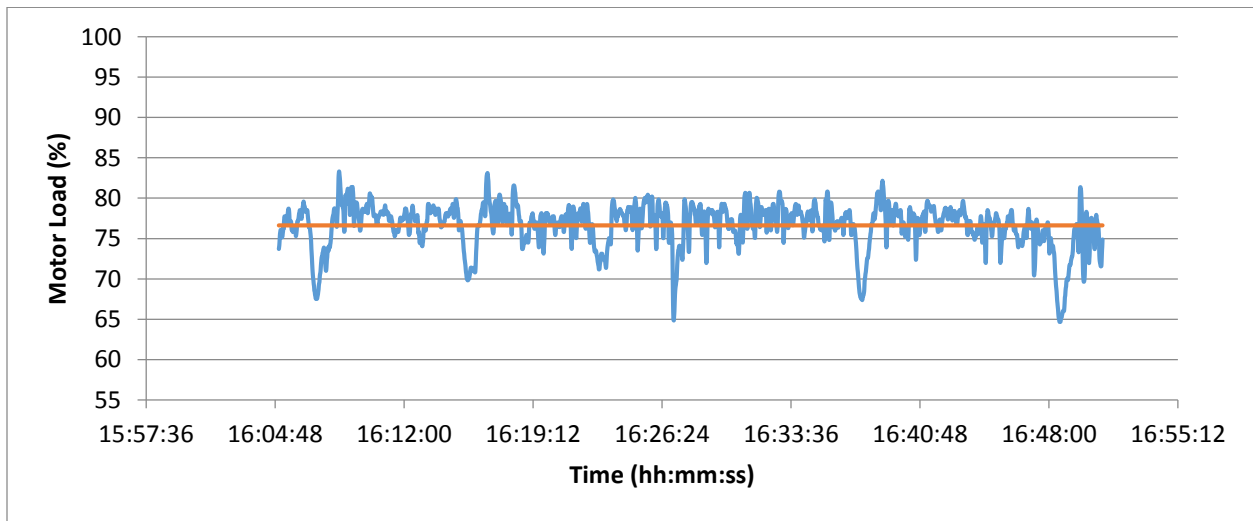
Figure 3.10 Motor load variations in SCB-V2 (pilot milled) as compared to average motor load.

SCB-V2 = Sorghum Cowpea Blend (white variety – V2) (coarse)

Chapter 4 - Characterization of extruded sorghum cowpea blends to develop pre-cooked and nutritionally dense fortified blended foods.

Abstract

Expanded formulations sorghum cowpea blend (SCB) obtained from extrusion cooking were ground using hammer mill and analyzed for changes in properties that were affected by transformation of starch and protein during the processing. Macro- and micro-nutrients were added to these milled blends to prepare fortified blended foods (FBFs) that could meet the recommendations of Food Aid Quality Review (FAQR) report on energy, protein and micronutrient content. The water absorption index (WAI) ranged from 4.17-5.97 g/g and water solubility index (WSI) ranged from 11.59-35.14% and they were affected by the formulation- whole sorghum and decorticated sorghum. Extrusion processing caused starch gelatinization in the range of 85.42-98.83% with whole sorghum formulations having lower gelatinization. The pasting properties indicated that whole grain blends of SCB had higher peak time when compared to decorticated sorghum blends and it was affected by specific mechanical energy (SME). The Bostwick flow rate of cooked porridges with 20% solids was within the recommended range of 9-21 cm/min. No significant difference in protein digestibility was observed amongst raw, extruded and cooked binary blends of SCB. Starch digestibility significantly increased after extrusion with a maximum increment of 369.49% in rapidly digestible starch (RDS). The protein digestibility did not vary significantly when subjected to extrusion and cooking. There was a significant reduction in anti-nutritional factors in extruded binary blends of SCB when compared to respective raw blends – phytic acid reduced by 16.92-29.26%, tannins were not found and trypsin inhibitors reduced by 16.55 – 22.31%. Thus,

extrusion processing of SCB with subsequent addition of macro-and micro-ingredients was effective in producing FBFs with high nutritive value.

Introduction

It has been reported in The State of Food Insecurity in the World 2014 by United Nations Food and Agriculture Organization that an estimated 795 million people of the 7.3 billion people in the world, or one in nine were suffering from chronic undernourishment in 2012-2014. The developing countries have the major share of these hungry people with one in eight or 13.5% of the population in those countries. Concerted global efforts to reduce hunger and malnutrition has resulted in reduction of hungry people by over 167 million since past decade and the prevalence of undernourishment has fallen from 18.7% to 11.3% worldwide and from 23.4% to 13.5% for developing countries (FAO et al., 2015). The least progress in reducing hunger has been in sub-Saharan region of Africa where more than one in four people remain undernourished – the highest prevalence amongst any region of the world.

Multitude of factors including population growth, poverty, conflicts, social exclusion, governance, trade policies, inequality, and natural disasters spur food insecurity in the world (Ahmed et al., 2007). Food aid can be an important tool in addressing certain food insecurity issues. It has been used as an instrument to offset food shortages in low-income countries, where fluctuations in domestic food production threaten food security (Shapouri and Rosen, 2001). The United States has been the leading contributor to international food aid with average supply of 56% of the annual total food aid donated by members of the Food Aid Committee of the International Grains Council since 1995 (Hanrahan and Canada, 2013). Food aid programs in U.S. are administered by the United States Agency for International Development (USAID) and United States Department of Agriculture (USDA) either as part of bilateral program or through

United Nation's World Food Program. The food aid has been distributed by US under four authorities: (1) the Food for Peace Act (FFPA, also known as P.L. 480); (2) the Section 216(b) program (which has been inactive since 2007); (3) the Food for Progress Act of 1985; and (4) the McGovern-Dole International Food for Education and Child Nutrition Program (Schnepf, 2015). Since late 1980s, the USAID administered FFPA Title II program has come out as the largest funding source for U.S. food aid shipments. This program provides for the donation of U.S. agricultural commodities to support specific emergency and non-emergency food needs either by monetization or direct food distribution. In 1996, the law was amended to permit the enrichment and fortification of commodities to improve their nutritional quality and included high protein blends of U.S. foods for malnourished infants, children, pregnant women, and lactating mothers. Foods donated under P.L. 480 today include whole commodities, processed foods, fortified processed foods, and blended food supplements.

FBFs are not only 'ready-made' in the sense that they are nutritionally rich in their physical form with easy preparation methods, but usually they are dispensed through a standardized regime, involving registration, anthropometric measurement and cooking and hygiene training (World Health Organization, 2004; Sphere Project, 2011; Scott-Smith, 2014). The fortified foods generally combine cereals with soy, pulses, beans, oilseeds and dried skimmed milk to increase the quality and quantity of proteins. The cereals and other raw ingredients are often processed before fortification or enriched into products such as corn soy blend (CSB), wheat soy blend (WSB), fortified wheat flour, fortified cornmeal and vitamin-A fortified vegetable oil. CSB which is often used to treat moderate malnutrition and micronutrient deficiencies in underweight children is the most commonly programmed specialized product in supplementary feeding programs (GAO, 2011). CSB came into existence in the early 1970s when there was shortage of

nonfat dried milk which was used regularly in corn soy milk (CSM). The low effectiveness of CSB, which was classified as ready-to-use supplementary food in addressing moderate acute malnutrition due to inadequate compositional profile of energy density, micronutrients, lipids etc., was developed expeditiously with different variants such as CSB 10, 11, 12, 13 and CSB Plus between 2005 and 2011 with enhancements to the earlier shortcomings. In Title II programming, the fortified blends account for 44% of the commodity cost though it constitutes only 20% of the volume (FAQR, 2011). The FAQR recommends the use of other cereals namely, sorghum, millets, and rice instead of traditional cereals like corn and wheat in the production of FBFs. The major advantage of using non-GMO crops like sorghum would be its good acceptance by governments of target nations, like countries in Africa. Additionally, crops like sorghum, millets, cowpea etc. are plants that require less water and can thrive in drought like conditions (Ismail et al, 2003) which would add to the promotion of sustainable agriculture. Corn experiences pre-harvest insect damage due to shallower rooting system, wet post - harvest and storage practices in under developed economies leading to increased risk of aflatoxin contamination (Pitt et al., 2013).

Ideally, the ingredients for low-cost weaning formulations must be derived from dietary staples from the region of interest that are affordable to the section of the target population and readily available in sufficient quantity (Mensa-Wilmot et al., 2001). Therefore, in this study, sorghum and cowpea were combined in a ratio that would provide optimum protein and energy in the FBFs. Grain sorghum contains phytochemical such as phenolic compounds, plant sterols and policosanols that are rich in anti-oxidants and impacts human diets significantly by lowering cholesterol and promoting cardio-vascular health. Condensed tannins present in phytochemicals seem to have powerful anti-carcinogenic and anti-diabetic in-vitro activity (Awika and Rooney,

2004). However, the bioavailability of tannins (procyanidins and catechins) is questionable due to their bigger molecular size and susceptibility to bind food molecules into insoluble complexes making it tough for human digestion. Another anti-nutritional factor is phytic acid (inositolhexakisphosphate (IP6)) in cereal-legume based complimentary foods that inhibits iron absorption from porridges leading to a high prevalence of iron deficiency in infants. (Cook et al., 1997). Studies show that dephytinization through processing in low tannin sorghum increased iron absorption by 2-folds in sorghum reconstituted with water (Hurrell et al., 2003). Heat treatment during processing has also shown encouraging results in lowering phytates that increase iron solubility by forming iron complexes in naturally occurring plant phytates (Sandberg et al. 1989). Extrusion has been successfully used in production of low-cost cereal based weaning foods (Mustakas et al., 1964; Harper and Jansen, 1985). The processing of cereal-legume blends using extrusion has been used to completely gelatinize starch at 150-170°C extrusion temperatures with moisture range of 16-22% (Rabe et al. 1980), and denature proteins enabling a nutritious precooked blended product. When processed under ideal conditions using extrusion technology, catechins and procyanidins in tannin showed improved bioavailability of up to 50% in diets (Gu et al. 2008) and possible breakdown of high molecular weight polymers of procyanidins making is easier for human absorption thereby improving nutraceutical value of sorghum (Awika et al. 2003). Extrusion heat treatment and shear forces further inactivates trypsin inhibitors by 90% in extrudates (Nwabueze., 2007) thus retaining most of the chemically available lysine in soy flour when extruded at 100 to 115°C with 12 to 18% barrel moisture (Konstance et al., 1998). The idea behind researching other grains such as sorghum for use in FBF production is to utilize viable nutrient sources in a way to bring down production cost, increase acceptance and implement new nutrient recommendations listed in the FAQR report.

Sorghum cowpea blend (SCB) as it will be called is a precooked blend using extrusion and micronutrient fortified which can be a solution for wasted and infants with stunted growth.

The objective of this study was to develop sorghum cowpea blend that conforms to the most recent recommendations from Food Aid Quality Review (FAQR) and understand the effects of extrusion processing on starch and protein of extruded SCB in comparison with traditional CSB. Specific processing to product relationships were established which included post-milling particle size, viscosity profiles and their effects on Bostwick flow rate which related to gruel consistency before infant consumption, starch and protein digestibility and anti-nutritional factors.

Some of the key recommendations to better supplement nutritional targets along with optimal breastfeeding combined with infant feeding practices, were to:

1) Increase in quantity of protein and addition of animal protein namely WPC-80 (whey protein concentrate), so as to increase the Protein Digestibility Corrected Amino Acid Score (PDCAAS) to 0.88 (a score > 0.80 is considered good quality of protein),

2) Increase in the caloric content of FBFs by addition of oil to post extruded binary blend.

3) Upgrades to the micronutrient composition.

Materials and Methods

Materials

The materials used in this part of the study were – 1) extruded and milled binary blends of SCB, 2) sugar (C&H brand granulated white cane sugar from local store, Manhattan, Kansas, USA), 3) whey protein concentrate -WPC80 (Davisco Foods International, Inc., Eden Prarie, Minnesota, USA), 4) vitamins and minerals (Research Products Company, Salina, Kansas, USA) and non-gmo soybean oil (Zeeland Farm Services, Inc., Zeeland, Michigan, USA).

Hammer milling

The extrudates coming off the dryer was conveyed through bucket elevators and collected in large plastic bags. The extrudates were uniformly fed to the inlet of Schutte Buffalo Hammer mill (Buffalo, NY, USA) which was fitted with 3/64 inch (1190 μm) screen. The powdered extrudates were then collected directly into 50 lb 3-walled paper bags and sealed till further use.

Particle size analysis

Particle size distribution of extruded and milled binary blends were determined using a laser diffraction particle size analyzer (LSTM 13320, Beckman-Coulter, Inc., Miami, Florida, USA). Each sample was tested in duplicate.

Specific Mechanical Energy (SME)

The SME for each treatment was calculated using the formula:

$$SME = \frac{\dfrac{(\tau - \tau_O)}{100} \times P_{rated} \times \dfrac{N}{N_{rated}}}{\dot{m}} \tag{1}$$

where,

τ = operating torque (%); τ_0 = no-load torque (%); P_{rated} = rated power (37.3 kW), N = screw speed (rpm); N_{rated} = rated screw speed (507 rpm), and $\dot{m}$ = net mass flow rate of extrudate at die exit (kg/s).

Protein and Fat Analysis

The proximate composition of raw ingredients was determined using standard methods. This included determination of moisture (135°C for 2h; AACC 44-19), crude protein (based on nitrogen by combustion, (6.25X; AOAC 920.176), crude fat (petroleum ether extract method; AOCS Ba 3-38). Protein, and fat, were reported on as is basis from replicates.

Mixing protocol for addition of macro- and micro-ingredients

An increase in solid content of porridges from existing 11.75% (USDA, 2008) to 20 % was recommended in the FAQR document (Webb et al., 2011). Although this recommendation helped the porridges to be more energy dense but due to higher solid concentration, the porridge became thick and was difficult to flow. These porridges therefore could not meet the Bostwick flow requirement of 9-21cm/min. In order to meet the Bostwick flow rate requirements and make the porridge of spoonable consistency, 15% sugar was added to the blend after removing an equal quantity of the binary blend. The addition of sugar helped increase the flow rate to within Bostwick specifications due the plasticizing and viscosity reduction effect of the added sugar. It has been reported that sugar increases to the energy density FBFs with minimum increase of volume. (de Pee and Bloem, 2009).

The mixing was done in steps of decreased dilution of ingredients as the steps progressed to ensure the uniformity of mixing. All the dry ingredients – sugar, WPC80, minerals and vitamins were weighed separately to make a batch of 25 kg fortified blended food (FBF). The minerals and vitamins were mixed first in a small Hobart mixer (Model N-50, Hobart Corporation, Troy, Ohio, USA) for 1 minute till no yellow concentrates of vitamin were present. This vitamin - mineral blend was then transferred into the bowl of another Hobart mixer (Model A-200, Hobart Corporation, Troy, Ohio, USA) and 3.33 kg of milled and extruded binary blend was added to it and mixed for 3 minutes to get a uniform dilution of vitamin - mineral mix with a part of the binary blend.

The above premix was again transferred slowly to the bowl of a larger Hobart mixer (Model M802, Hobart Corporation, Troy, Ohio, USA). Then 10 kg from the remaining milled binary blend was added to it and mixed for 5 minutes on speed setting of 1. After 5 minutes of mixing

the remaining dry ingredients – milled binary blends, sugar and WPC80 were added to it and mixed for another 5 minutes on the same speed setting of 1. Once the mixing was over, 14.42 kg of the dry blend was removed from the bowl of the mixer. To the remaining 8.33 kg of dry blend remaining in the mixer, 2.25 kg of oil (for non-full fat soy formulations) and 1.375 kg of oil (for full fat soy formulations) was added and mixed for 5 minutes with 2 minutes on speed setting of 1 and 3 minutes on speed setting of 2. On completion of this step the previously removed dry blend was added back to the mixer and mixed for another 5 minutes on speed setting of 1. At the end of this step the fortified blended food was ready and is shown in Table 4.1

Bostwick Consistometer Test

Bostwick consistency is the measurement of flow of the final FBF after making a gruel/porridge with the milled extrudates and other macro- and micro-ingredients. This test was performed using Bostwick Consistometer (CSC Scientific Company, Inc., Fairfax, Virginia, USA). The Bostwick consistometer is a long trough with 0.5 cm graduations along the base. The trough is separated at one end by a spring-loaded gate. This gate forms a chamber where the sample is loaded (Fig. 4.1). The consistometer was placed on a flat surface and the levelling screws were adjusted till the bubble was placed in the center of the circular level. Gruels were prepared with 20% solids by adding 40 g of FBF to 160 ml distilled water. The solids were added to boiling water and stirred vigoursly with a fork for 1 minute and then it was removed from heat and stirred for another 30 seconds. This gruel was then covered with an aluminum foil and placed in water bath maintained at 30°C for 10 minutes. The slurries were adjusted for water loss through evaporation to the initial weight of 200g by addition of distilled water. It was again stirred and covered with aluminum foil and placed in water bath at 30°C for 1 hour. At the end of the cooling period, the water loss due to evaporation was again made up to initial weight of 200g.

The gruel was poured into the reservoir of the consistometer till it overflowed and it was levelled with flat plastic spatula. After allowing the gruel to settle for 30 s, the gate of the reservoir was opened and the gruel was allowed to flow along the graduated trough. The distance of flow was recorded exactly after 1 minute and if the gruel was between two graduations after the end of 1 minute then the higher graduation was recorded as the distance (USDA 2005). All the samples were measured in duplicate.

Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI and WSI were assessed on samples of binary blends before and after extrusion process based on the method of Anderson et al. (1970). A 2.5 g sample was dispersed in 25 g distilled water, using a glass rod to break any lumps. After stirring for 30 minutes, the dispersions were rinsed into tared centrifuge tubes and made up to 32.5g and centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted for its solid content (WSI) after evaporation of water from the supernatant. The sediment was weighed for its solid content and was used to report the WAI. The indices were calculated as:

$$\text{WAI (\%)} = \frac{Weight\ of\ sediment}{Weight\ of\ dry\ solids} \times 100$$

$$\text{WSI (\%)} = \frac{Weight\ of\ dissolved\ solids\ in\ supernatant}{Weight\ of\ dry\ solids} \times 100$$

Thermal Analysis - Differential Scanning Calorimetry (DSC)

Calorimetric measurements were carried out for different raw and extruded binary blends to understand the physical transformation of starch and proteins known as starch gelatinization and protein denaturation respectively on Q100 DSC (TA Instruments, New Castle, DE, USA). 8-10 mg of sample was weighed into large volume stainless steel DSC pans (Part no.03190029, Perkin Elmer Health Sciences Inc., Shelton, CT, USA). Distilled water was added to the sample in the pan so as to obtain a solid to water ratio of 1:2 (Stevens and Elton, 1971; Zhu et al., 2010). The

pans were hermetically sealed and the samples were allowed to equilibrate overnight. The instrument was calibrated using indium as reference material. An empty sealed pan was used as reference for all experiments. The program steps used for the test was as follows: Equilibrate at 10°C, heating the pans from 10°C to 140°C at the rate of 10°C/min, mark end of cycle, cooling down the sample from 140°C to 10°C at the rate of 25°C/min, mark the end of cycle with nitrogen gas flow rate of 50mL/min. The samples were again rescanned with heating from 10°C to 140°C at the rate of 10°C as the final phase of the test.

DSC data for each gelatinization and denaturation endotherm was analyzed for transition temperatures, onset (T_o), peak (T_p), and endpoint or conclusion (T_c) and the enthalpy (ΔH) using TA Instrument's Universal Analysis Software (version 5.4.0). All the reported data were means of two replicates.

Starch gelatinization (%) was calculated as the ratio of melting enthalpic transition difference in starches between raw and extruded binary blends and is represented as:

Starch gelatinization (%) = [(ΔH_{raw} – $\Delta H_{extruded}$)/ΔH_{raw}] x 100

Where, ΔH_{raw} = enthalpy of raw binary blend, $\Delta H_{extruded}$ = enthalpy of extruded binary blend

Total cook (%) was calculated as a ratio of the total enthalpic transition difference which includes the transition enthalpies for starch and protein fractions of the binary blends. It is represented as below:

Total cook (%) = [($\Delta H_T raw$ – $\Delta H_T extruded$)/$\Delta H_T raw$] x 100

Where, $\Delta H_T raw$ = Total enthalpy of transition of raw binary blend, $\Delta H_T extruded$ = Total enthalpy of transition of extruded binary blend

Rapid Visco Analyzer (RVA)

The RVA provides a measure of how cooked a sample is by re-cooking under relatively low shear and in excess water and measuring the pasting viscosity throughout the test. Pasting properties of the binary blends were examined using RVA. (RVA 4, Newport Scientific Pvt. Ltd.,Warriewood, NSW, Australia). The RVA was interfaced with a computer equipped with the software – Thermocline for Windows (version 3.15.2.298) for controlling the test and analyzing the results. Pasting properties were determined after running the samples on standard AACC profile (AACC 76-21.01, 1999) with a run time of 13 minutes. Peak viscosity (PV), pasting temperature (PT), trough or holding paste viscosity (HPV), breakdown (PV- HPV), final viscosity (FV) and setback (FV – HPV) were recorded. All measurements were performed in duplicate.

Starch Digestibility

Raw and extruded and milled samples of binary blends were measured by a modified *in vitro* Englyst method (Englyst et al, 1992). Briefly, samples of blends (0.6 g) and guar gum (50 mg) were placed in a centrifuge tube (45 mL). Freshly prepared pepsin solution (50 mg pepsin in 10 mL 0.01M HCl) was added to the tube, and the mixture was incubated for 2 hours at 37°C. Sodium acetate solution (0.25M) was added to the mixture to stop the digestion. Pancreatic/amyloglucosidase mixture (5 mL) and glass beads were added to the sample tube for starch digestion. The tube was incubated in a shaking water bath at 37°C and 90 strokes/min. At 20 and 120 min, an aliquot of 0.25 mL sample was pipetted into 10 ml of 66% ethanol. The glucose released at each interval was determined using glucose oxidase/peroxidase method and was converted to percentage of starch hydrolyzed by multiplying by 0.9. Starch digested at 20

min is defined as RDS, starch digested between 20 and 120 min is defined as SDS, and starch not digested after 120 min incubation is defined as RS.

Protein Digestibility

Protein digestibility of binary blends before and after extrusion was measured using a protein digestibility assay modified from Mertz et al. (1984). 200 mg of flour/milled sample was weighed and placed in 50ml centrifuge tubes. Each sample was then incubated with 35ml pepsin solution (1.5 mg pepsin in 1ml of 0.1 M phosphate buffer pH2.0) at 37°C. After two hours of incubation, 2ml of 2M sodium hydroxide was added to each tube to stop the digestion. All tubes were centrifuged at 3320g for 15 minutes at 4°C and the supernatant was discarded. The residue was washed in 10 ml of 0.1 M phosphate buffer pH 2.0, centrifuged, and supernatant was discarded. The washing steps were repeated one more time, and samples were frozen (-80°C for 30 minutes) and lyophilized. Freeze-dried samples were tested using nitrogen combustion (LECO system) to analyze the amount of undigested protein. Digested protein was calculated based on protein content of the native sorghum flour and that of the undigested fraction.

% Digestibility = {[Total N (mg) – Undigested N (mg)]/Total N (mg)}*100

Phytic Acid

Phytic acid (InsP6) was measured using Megazyme kit (Megazyme International, Ireland) for phytic acid and total phosphorous in which phytic acid is measured as phosphorous released by phytase and alkaline phosphatase. One gram of powdered sample was and transferred into 75 ml glass beaker containing 20 ml of 0.66 M hydrochloric acid. The beaker was covered with aluminum foil and stirred vigorously overnight at room temperature. 1 ml of extract was transferred to 1.5 ml microcentrifuge tube and centrifuged at 13000g for 10 minutes. 0.5 ml of the resulting extract was immediatedly transferred to fresh 1.5 ml microcentrifuge tube and

neutralized by addition of 0.5 ml of 0.75M of sodium hydroxide solution. Neutralized sample

extract solution was used in the enzymatic dephosphorylation reaction procedure. Total and free

phosphorous contents were measured for each sample. ΔA phosphorous was calculated for each

sample by subtracting the absorbance of 'free phosphorous' sample from that of the absorbance

of 'total phosphorous' sample. The phosphorous concentration is expressed as g/100g of sample

using the following calculation:

Phosphorous (g/100g) = [ΔA phosphorous x 10,000 (conversion from $\mu g\ g^{-1}$ to g/100g) x 1.0

(weight of the original sample) x 1.0 (sample volume used in colorimetric step)] / [mean M

(mean value of phosphorous standard) x 20 (original sample extract volume) x 55.6 (dilution

factor).

Mean value of phosphorous standard was obtained using a standard curve over a dynamic range

of 0-0.75 μg phosphorous. Phytic acid concentration was calculated as follows:

Phytic acid (g/100g) = Phosphorous (g/100g)/0.282. The calculation of phytic acid content was

based on the assumption that the amount of phosphorous measured was exclusively released

from phytic acid and that it comprises 28.2% of phytic acid.

 Tannins

Tannin content was measured using vanillin-HCl method (Price et al, 1978). 0.3 g of freshly

ground sample was taken in 15 ml tubes and tannins were extracted by adding 8 ml of 1% HCl in

methanol at 1 minute intervals to the sample tubes. The samples in the tube with 1% HCl in

Methanol was vortexed for 10 s and then placed in a water bath maintained at 30°C for exactly

20 minutes. At the end of 20 minutes each tube was removed from water bath and vortexed

again. The extracts were then centrifuged at 2000 rpm for 4 minutes. Two 1 ml aliquots were

taken from each tube and transferred into two separate15 ml tubes. One tube was marked for

sample determination and the other tube was marked for blank determination. The sample tubes after brief vortexing were placed in water bath maintained at 30°C after adding 5 ml of vanillin reagent to it at 1 minute intervals. The vanillin reagent was prepared by adding 1% vanillin in methanol to 8% HCl in methanol in 1:1 ratio. The blank tubes were added with 5 ml of 4% HCl in methanol and after brief vortexing was placed in the water bath at 30°C. At the end of 20 minutes the absorbance of samples and blanks were read using spectrophotometer at 500 nm. The spectrophotometer has to be zeroed using methanol blank before measuring. The difference between sample and blank would be used for final determination of phenol content and expressed as catechin equivalents (CE)/mg of sample. A standard curve using 1000 ppm of catechin standard at 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml added with 1% HCl in methanol to make volume to 1 ml and 5 ml vanillin reagent was plotted. The CE equivalent calculated as below:

CE (mg/mg of sample) = (y/m) / sample concentration (mg/ml),

Where, y and m (slope) can be calculated from the regression equation of the standard curve.

Trypsin inhibitor

Trypsin inhibitor activity was determined according to the method described by Smith et al. (1980) using BAPNA (benzoyl-DL-arginine-p-nitroanilide) as substrate (0.92 mM in 0.05 M Tris buffer/0.02 M CaCl2, pH 8.2). The sample was fine ground and 1 gm of the ground sample was used for extraction of trypsin in 50 ml of 0.01 N NaOH for 3 h. The pH was maintained within the range 8.5 to 9.0. One ml of the extract and 2 ml of trypsin solution 0.002% (Type I, of bovine pancreas, SIGMA) in 0.001M HCl were mixed with 1 ml of water. The reaction started after addition of 5 ml of substrate at 37 °C. After 10 min, the reaction was stopped by the addition of 1 ml of 30% acetic acid. The reaction mixture was filtered through filter paper (Whatman No. 3) and the absorbance due to release of *p*-nitroaniline was read at 410 nm. The

activity was interpreted as the increment of 0.01 units of absorbance at 410 nm for 10 ml of reaction mixture. Trypsin inhibitor activity is expressed in terms of mg trypsin inhibited per g of dry sample.

Statistical Analysis

All the results were analyzed using analysis of variance (ANOVA) with general linear model procedure (SAS version 9.1, SAS Institute, Cary, North Carolina, USA). When significant effects ($p < 0.05$) were indicated by ANOVA, Tukey pairwise comparisons were done to identify which treatments differed significantly ($p < 0.05$).

Results and discussion

Particle Size of Milled extrudates - Sorghum Cowpea Blend

The mean particle size of extruded SCBs ranged from 204.94 μm to 353.97 μm (Table 4.2) for SCB-V3 and SCB-V2 respectively made from pilot milled binary blends. Amongst commercial milled blends, the whole grain formulation had bigger particle size than decorticated but they were not significantly different ($p>0.05$) In the pilot milled blends, whole and decorticated formulations with fine and coarse milled raw sorghum flour showed significantly lower particle size in coarse milled blends ($p<0.05$). Further, it was observed that milled extrudates obtained from fine pilot milled blends had significant difference ($p<0.05$) in particle size between whole and decorticated formulations except for WSCB-V1 and SCB-V1 which had almost similar particle size.

No significant correlations were observed between different extrudate physico-chemical properties that would have affected the particle size. This meant that the milling of extrudates was not affected by the processing parameters and by different formulations of SCB. However,

all the blends could meet the particle size specifications of USDA commodity requirements (2014) for CSB Plus which is currently being distributed in food aid programs.

Water Absorption Index and Water Solubility Index

It was observed from Table 4.2 that the WAI for SCBs ranged from 4.17 g/g to 5.97 g/g for WS'CB-V1 (from pilot milled flour) and SCB – V1 (from commercial milled flour) respectively. Again, it can be observed from the same Table 4.2 that WSI ranged from 11.59% in WSCB-V2 to 35.14% in SCB-V3 (both from pilot milled flour). WAI measures the volume occupied by starch after swelling in excess water, which maintains the integrity of starch in aqueous dispersion (Mason and Hosney, 1986) and in case of extrudates it's a measure of intact starch after extrusion processing WAI can be used as an index of starch gelatinization (Ding et al 2005). WSI is a measure of the extent of degradation of molecular components (Kirby et al, 1988), measures the degree of starch conversion during extrusion which is the amount of polysaccharide released from starch component after extrusion.

The WAI and WSI data did not exhibit a definitive trend based on type of formulation or type of milling from which these formulations were obtained. However, the impact of formulation on the SME affected both these attributes. From Figure 4.2 it can be observed that WAI is inversely correlated to specific mechanical energy (SME) ($r = -0.46$) and positively correlated to WSI ($r = 0.74$) (Fig 4.3). SME is a measure of the energy transmitted by the screws to the material being extruded and this can be correlated to degree of starch conversion in the process (Ortiz et al., 2010). The formulations with whole sorghum flour had lower SME (Table 4.2) as compared to formulations with decorticated sorghum because decortication removed the bran and germ from the grain and that caused removal of the maximum lipids from the grain (Hubbard et al., 1950; Rooney, 1978). They reported that oil content was concentrated 76% in germ and 11% in bran of

sorghum grain. Lipids act as lubricant inside the extruder barrel and that causes a reduction in viscosity of the melt and thus lower SME. The reduction in viscosity caused lower shear rate inside the barrel and thus lower breakdown of starch molecules leading to the relationship of higher WAI with lower SME (Chang et al., 1998) and vice-versa. Similarly, an increase in SME would lead to higher starch breakdown and would cause increase in solubility of starch fraction. According to Anderson et al (1970), WSI is the amount of free polysaccharides released from starch granules after addition of excess water. The results are in confirmation with the findings of Ainsworth et al (2007), Altan et al (2008) and Sobukola et al (2013) who reported that increase in SME led to higher mechanical shear degraded macromolecules which led to a decrease in molecular weight of starch granules and thus increased WSI. Slaughter et al. (2001) also reported higher water solubility and swelling for higher gelatinized wheat, maize, and rice starches. Further, the relationship between WSI and WAI (Figure 4.4) is inverse linear (r = -0.90). This relation is a function of structural changes caused to starch subjected to mechanical shear (Smith 1992). The author showed that in this relationship, WAI increased when SME was increased due to increase in amount of swollen starch in the undamaged starch granules but on further increment of SME beyond 500 kJ/kg, the starch granules were totally damaged, resulting in decrease of WAI. The WSI kept increasing with increasing SME. In the current study, the whole grain sorghum formulations showed higher WAI due to lower SME than decorticated sorghum formulations which had lower WAI. The relationship between WAI and WSI did not show a peak after which WAI decreases because the SME for each formulation might have been high enough to degrade most of the starch granules.

Degree of Starch gelatinization

Starch transformation persists well beyond loss of birefringence and other measures of gelatinization, Patton and Sprat (1981) suggested that 'degree of cook' be used to describe the degree of starch transformation. It was observed from Table 4.2 that starch gelatinization of SCB formulations after extrusion ranged from 85.42% to 98.83% in WSCB-V2 and S'CB-V1 obtained from pilot milled flour. It was found that amongst both commercial milled flour formulations and pilot milled flour formulations, the whole sorghum blends had lower starch gelatinization than decorticated sorghum blends. These differences however were not statistically significant. The total cook of the extrudates which accounted for cook of starch and protein in the blends were lower in values than starch gelatinization values (Table 4.2) but, it too had a similar trend of whole sorghum blends having lower cook values than decorticated sorghum blends. This difference was not found to be statistically significant on comparing different blends within same variety of sorghum.

The starch gelatinization had a positive linear correlation with SME (r = 0.89) as shown in Figure 4.5 Higher SME was observed in blends of decorticated sorghum and higher mechanical energy input into the melts of these blends inside the extruder barrel caused greater gelatinization of starch as compared to whole blends of sorghum irrespective of the source of the flour. The presence of fiber and fat in whole grain formulations cause a reduction in SME and thus lower the breakdown of starch leading to lower cook. Feng and Lee (2014) reported that during extrusion lipid worked as a lubricant and reduced SME. Lin et al. (1997) also observed that increasing the fat content in extruded dry pet food decreased the degree of starch gelatinization of extrudates. The presence of fiber in the formulation increases the product temperature at die exit which is directly related higher temperature in the extruder barrel. Therefore, lower viscosity

of the dough mass inside the barrel and thus lower torque and lower SME (Jin et al., 1994) which causes less starch conversion due to breakdown or less starch gelatinization.

Pasting Properties

Off-line pasting viscometry has been shown to adequately measure degree of cook in starch (Harper 1994). The method involves re-cooking the product and monitoring viscosity changes. This has been used to quantify the cold-swelling 'cooked' component, the 'raw' component that pastes during the test, and the overall viscosity that indicates degree of starch dextrinization. Ryu et al (1993) suggested that the Rapid Visco Analyser (RVA) could be used for at-line control as it provided a relatively rapid measure of starch degradation.

The pasting properties for ground extruded SCB formulations are given in Table 4.3. It can be noted from the table that formulations containing whole grain sorghum flour had significantly higher peak time (p<0.05) compared to formulations that were constituted of decorticated sorghum flour. The decorticated sorghum formulations exhibited cold water swelling when compared to whole sorghum blends. Both these properties were directly affected by the SME input and higher energy input let to quicker peak time (r = -0.61) as shown in Figure 4.6 and cold swelling properties. Higher energy caused higher starch damage and starch gelatinization and it subsequently caused higher particle hydration at ambient temperature (Ozcan and Jackson, 2005; Whalen et al. 1997). Faster granular swelling with higher water absorption rates leading to starch solubilization and leaching of amylose below 90°C possibly increases the viscosity of starch. A further increase in temperature and mechanical stress during the holding phase causes further disruption of the starch granule and the remaining amylose is leached out (Ragaee and Abdel-Aal, 2006). Thus, the decorticated sorghum based formulations which had significantly lower peak times than whole sorghum flour based blends showed lower final viscosity with the

exception of S'CB-V1 as it did not have a significantly different peak development time as compared to WS'CB-V1. It could therefore be interpreted that higher particle size, irrespective of whole or decorticated flour led to higher starch breakdown during the extrusion process in such a way that there did not exist a difference in type of flour. These observations are consistent with the effects of extrusion kinetics on pasting properties as reported by Mahasukhonthachat et al. (2010).

The peak viscosities for binary blends containing whole sorghum flour were significantly lower ($p<0.05$) than that of blends with decorticated sorghum flour. The presence of lipids in the flours (in this case more lipids in whole sorghum flour) and starch has been reported to lower the peak viscosity due to amylose-lipid complex formation (Singh et al. 2007, Singh et al. 2003). The final viscosities of the blends with decorticated sorghum flour were found to be lower than that of whole sorghum flour blends. After the breakdown, the increase in viscosity during the cooling period is indicative not only of the normal inverse relationship between viscosity and the temperature of suspensions but also of the tendency for various constituents present in the hot paste (swollen granules, fragments of swollen granules, and colloidally dispersed and dissolved starch molecules) to associate or retrograde as the temperature of the paste decreases (Singh et al., 2007). The extruded binary blends containing decorticated sorghum flour exhibited lower setback viscosity values as compared to whole sorghum blends. The lower setback viscosity values could be due to lower extent of retrogradation/aggregation of amylose during cooling (Singh et al. 2009) as these blends contained lower fat content than whole sorghum blends.

Bostwick Consistency

The Bostwick flow rates of the cooked slurry at 20% solids concentration which had sugar, WPC 80, oil, vitamins and minerals in addition to extruded binary blend is presented in Table 4.4. It

was observed that there was no significant difference between Bostwick flow rates of formulations containing extruded binary blends of whole and decorticated sorghum flour except for that between WSCB-V2 and SCB-V2 ($p<0.05$). However, all the flow rates were within the range of 9-21 cm/min as stipulated by USDA commodity requirement for corn soy blend (CSB 13) (USDA, 2008). It can be observed from Figure 4.7 and Figure 4.8 that Bostwick flow rate is weakly correlated to peak time ($r = 0.25$) and final viscosity ($r = 0.27$). Fig. 4.9 shows an inverse correlation between SME ($r = -0.26$) and Bostwick flow rate. Analysis of all these correlations point out to the fact that Bostwick flow rate is dependent on the characteristics of starch after it has been cooked as porridge/slurry. Greater peak time in RVA is indicative of higher amount of intact starch and higher final viscosity is also an indicator of higher amount of intact starch. A positive correlation of Bostwick flow rate to these parameters therefore, points out that less degraded starch leads to higher Bostwick flow. It can be re-confirmed from the negative correlation that Bostwick flow rate has with SME.

Cold paste viscosity arises as starch becomes soluble due to cooking (Mason and Hoseney, 1986; van Lengerich, 1989). Higher SME causes greater starch degradation which leads to higher cold paste viscosity due to faster hydration (Guha et al., 1998) leading to a thicker gruel at temperatures below 50°C and thus lowers the Bostwick flow rate as observed in the flow rates of all decorticated sorghum blends except for SCB-V2 which has higher Bostwick flow rate than WSCB-V2. This discrepancy could have been possibly caused by temperature difference between the gruels of SCB-V2 and WSCB-V2 while measuring the Bostwick flow rate. Mouquet et al. (2006) reported consistency of highly concentrated gruels is highly dependent on temperature. They further stated that a 10°C temperature interval (50°C and 40°C) during

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cooling of gruels before consumption, consistency increased by 13 cm/min for high energy density multicomponent flours.

Energy Density of FBFs

The FBFs are meant to be fed to infants as complementary foods at appropriate age to meet their growth requirements. Nutritionists in pediatric divisions are of the view that complementary foods should not be introduced to infants before they are developmentally ready for them; most infants get ready for this between 4 and 6 months of age. The FAQR (Webb et al., 2011) recommended energy-dense foods with good protein content and an appropriate inclusion of essential micronutrients as necessary (albeit not always sufficient) to achieve defined nutrition goals among vulnerable populations. Staple foods must be available in sufficient quantity to ensure that nutritionally enhanced (value-added, usually processed) food products are having an additional effect instead of replacing available sources of energy in the local food supply. Therefore, they advised that the current nutritional and energy value of the FBFs, CSB or WSB be enhanced to provide in 100 g of FBF, 387 kilocalories as energy, 18g protein and 9 g fat. They further suggested use of other cereal blends than the regular CSB and WSB, use of WPC to increase the protein content in addition to other changes.

It has been recommended by many nutritionists that complementary food should be introduced to infants in semi-solid or puree state (Monte and Giugliani, 2004; Elizabeth and Vince, 2008). Therefore, as a general practice before feeding infants, most cereal-based products used for complementary feeding programs are diluted to achieve a thin drinkable consistency. Usually, energy dense porridges are naturally viscous at high solids to water ratio. Diluting of these dense porridges before feeding leads to lowering of energy density and micronutrient levels (Stephenson et al., 1994). Use of amylase-rich flours (ARF) produced by germinating the cereal

grains as an alternate method of liquefying thick porridges without addition of water has been described in many studies (Atwell et al., 1988; Ones, 1991; Gibson et. al, 1998; Mensah and Tomkins, 2003). A diluted porridge becomes less energy dense and may prevent the infants from meeting their energy requirements due to reduced stomach size (30-40 ml/kg of body weight) (Elizabeth and Vince, 2008) and therefore, Stephenson et al. (1994) rationalized a maximum of four feedings per day by mothers based on their limited time to accommodate feeding schedules along with other routine household work. However, in the work by Svanberg et al. (1988), it was found that there was no benefit to infants aged 5-12 months who were fed thick porridges added with small quantities of germinated sorghum flour (ARF source) to liquefy it. During germination of cereals, α-amylase activity is increased and this hydrolyzes amylose and amylopectin to soluble dextrins and maltose leading to a reduction in viscosity of gruels prepared from it (Ashworth and Draper, 1992). They further stated that the generated viscosity (3000 cP) is suitable for infants and child feeding without dilution with water. Hence, the study by Stephenson et al. (1994) concluded that thicker porridge resulted in 35% mean energy intake when compared to thin low density porridge even though it took longer feeding time by mothers. They suggested addition of oil and peanut butter to better supplement energy density of 1 kcal/g to compensate thinner gruels. Black et al. (2009) found that a concentration of 20% of dry meal in water was required to achieve the required energy density of 0.8 kcal/g recommended for complementary feeding programs but at this concentration the gruels were excessively thick for infant consumption. They suggested different cooking methods and cooking times to produce porridges with potentially lower viscosities while maintaining their caloric densities. In the field trials conducted by Rowe et al. (2008) in Uganda, Malawi and Gautemala, they found the average dry meal concentration of 14% produced spoonable/drinkable porridge gruel.

In the current study, it can be observed from Table 4.4 that all SCB formulations had and energy density greater than 403.62 kcal/100g from 20% concentration based on the FAQR (2011) recommendations which is comparatively higher than previous FBFs used in feeding programs. The protein requirement as specified in the 2008 CSB13 commodity requirement document, a minimum of 16.7% protein per 100 grams of product is to be used in Title II programs. Total protein requirements for infants is also evaluated by the protein efficiency ratio (PER) which is based on the weight gain of the subject being tested divided by its intake of a particular food protein during the test period. Additionally, protein quality can be evaluated by PDCASS (Protein Digestibility Corrected Amino Acid Score) based on both amino acid requirements of humans and their ability to digest it. The US Title II CSB provides P/E ratio of 17.4% with a PDCASS of 0.81 and a digestibility factor of 85% which is more than adequate for all consumers (Fleige et al., 2010 a). However, they cautioned that FBFs are intended to provide only 25% of the dietary requirements while the rest of the calories were assumed to be provided from rest of the diet. Considering dilution effects of remaining 75% of daily diets due to sharing within the family, the efficacy of FBFs is greatly reduced. In view of this, Webb et al. (2011) in their recommendation to improve the quality of FBFs, that the new CSB 14 with addition of WPC 80 would provide a PDCASS of 0.88 and protein content of 17.7g per 100g of FBF. They further added that when this would be consumed with the recommended amount of oil, the P/E ratio of CSB 14 would be 11%, in line with the recommendation that complementary foods for moderately malnourished children should have a P/E ratio of 12% (Hoppe et al., 2008).

Starch and Protein Digestibility

The in-vitro starch and protein digestibility studies were conducted on select formulations of SCB. The formulations selected were based on the superior stability i.e. relatively less changes in

operational torque during extrusion processing. This eliminated the whole blends from this part

of the study and SCB-V1 was chosen from binary blends made from commercially sourced flour

and SCB-V2 and SCB-V3 was from the blends of flour obtained after pilot milling.

It was observed from Table 4.5 that the RDS had a significant increase ($p<0.05$) from 8.16% to

22.60% in binary blends of SCB-V1 after extrusion as compared to raw blend. There was a

decrease (17.97%) in RDS for SCB-V1 after cooking. The RDS of SCB-V1 increased by

176.96% after extrusion because the maximum amount of starch breakdown/cooking occurs

inside the extruder barrel which has been discussed earlier in the DSC section. There was a

29.49% decrease in SDS which was significant ($p<0.05$) in extruded samples as compared to raw

blend of SCB-V1. Similar tends were observed for SCB-V2 and SCB-V3 blends. The starch, in

native state, is also referred to as slowly digestible starch because it can be digested in the small

intestine, albeit slowly (Englyst, et al., 1992). Starch needs to be gelatinized for efficient

hydrolysis since gelatinized starch is more susceptible to enzymatic attack (Akdogan, 1999). In

vitro starch digestibility significantly improved after the raw blend was extruded, however, the

decrease in RDS after cooking could be attributed to decrease in digestibility of sorghum when

cooked wet. Extrusion improves starch digestibility by shearing off branches on amylopectin

molecules and thereby increasing their accessibility to amylolytic enzymes (Camire, 2001). A

comparison with control samples CSB13 and CSB+ showed that the control samples after

cooking had higher RDS as compared to SC blends. The SDS was somewhat similar in both

control and SCB samples. The higher RDS of control samples could be attributed to higher

digestibility of corn starch as compared to sorghum starch during wet cooking.

The protein digestibility for SCB binary blends is shown in Table 4.6. It was observed from the

table that there was no significant difference in protein digestibilities between the three binary

blends of SCB whether raw, extruded or cooked. All the raw blends had a high digestibility of greater than 83% with SCB-V1 having the highest digestibility. After extrusion, the highest protein digestibility was found in SCB-V2 and after cooking of these blends the highest digestibility was found in SCB-V1. The digestibilities of SCB-V2 and SCB-V3 increased after extrusion whereas it remained similar for SCB-V1 when compared to digestibility of raw blend. However, after cooking the digestibility decreased, though the decrease was non-significant. The protein digestibilities of SCBs were similar to that of control CSB13 and CSB Plus as there was no significant difference ($p > 0.05$) between them in raw materials and in cooked format. Previous studies have shown a significant increase in protein digestibility after extrusion (James and Nwabueze, 2013; El-Hady and Habiba, 2003; Alonso et al. 2000; Balandran-Quintana et al.1998). The basic premise for the above studies and other similar findings has been that extrusion causes protein denaturation which increases its susceptibility to enzymatic hydrolysis and therefore improves digestibility (Amaya-Llanao et al., 2007). The results of the current study are not in confirmation with the above findings and could have been caused due to cross linking reactions between protein-protein, starch-protein and protein-lipid. During extrusion, however some interactions can reduce digestibility due to non-enzymatic browning reactions and formation of cross linking reactions (Moraru and Kokini, 2003; Camire, 2001, 2000; Ledward and Tester, 1994; Areas, 1992; Lanfer-Marquez and Lajolo, 1991; Camire et al., 1990). Onwulata et al. (2003) had reported that extrusion process does not affect the overall protein percentage. Furthermore, these researchers reported that although the amount of denatured protein increased due to extrusion temperatures, this denaturation had minimal overall effect on protein digestibility.

Anti-nutritional factors

The levels of phytic acid in the raw and extruded SCB blends are presented in Table 4.7. It was found that there was a significant difference ($p < 0.05$) between phytic acid content in raw blends as well among extruded blends. The highest mean phytic acid content in raw blends was found in SCB-V1 (1138.50 mg/100g) followed by SCB-V3 (942.05 mg/100g) and SCB-V2 (804.22 mg/100g) in that order. The same trend was observed in extruded blends also. Thus, varietal differences of sorghum attributed the differences in phytic acid content in raw blends which is consistent with previous studies that have reported differences in phytic acid content due to different varieties of sorghum (Saravanabavan et al. 2013; Afify et al., 2011). Further, it was observed that there was a reduction in phytic acid content in all the three blends after extrusion. This reduction ranged from 16.92% - 29.26% in SCB-V2 and SCB-V3 respectively. Batista et al. (2010) reported a similar level of reduction in phytic acid content (17-26%) after extrusion of common beans (phaseolus vulgaris L.). Other studies have also reported a significant reduction in phytic acid content after extrusion. Alsonso et al. (2000) reported a decrease in phytic acid content in both faba beans and kidney beans as compared to raw after extrusion. High performance liquid chromatography (HPLC) analysis performed by Sandberg et al. (1987) showed that during extrusion, some molecules of inositol hexaphosphate were hydrolysed to penta-, tetra-, and triphosphates due to thermal degradation. Barroga et al. (1985) and Kataria et al. (1989) stated that changes due to thermal degradation of these molecules, as well as changes in their chemical reactivity or the formation of insoluble complexes could explain about the reduction in the anti-nutrient content due to thermal processing. Thus, the heat and shear inside the extruder can be used to improve the nutritional value of cereal blends by reducing phytic acid levels. CSB+ had lower phytic acid content than all the SCBs after they were extruded.

No tannin content was found in raw blends of SCB and therefore none was found in extruded blends as shown in Table 4.7. The tannins were estimated in raw individual ingredients (not shown) and they did not show any tannin content and therefore the raw blends and extruded blends also did not show any presence of tannins. It has been reported (Awika and Rooney, 2004) that more than 99% of sorghum grown in USA are tannin free. This has been made possible by decades of breeding efforts to improve the feed value of tannin sorghums.

The changes in trypsin inhibitor are shown in Table 4.7. It was observed that there was a significant reduction ($p<0.05$) in trypsin inhibitor levels between raw and extruded blends. The reduction in trypsin inhibitor levels ranged from 16.55% - 22.31% with maximum reduction in SCB-V1 and least in SCB-V2 blends. Trypsin inhibitor is heat labile and degrades significantly with extrusion processing (Kaur et al., 2015). However, all SCBs had higher trypsin inhibitor levels than the control CSB+ . Probably, the use of heat treated soy in CSB+ could have caused a lower level of trypsin inhibitor in it.

Conclusions

1. The particle size of milled extrudates of SCB were not affected by the processing parameters and by also by different formulations of SCB.

2. WAI and WSI were not affected by formulation or the milling but it was influenced by SME. WAI was negatively correlated to SME and the converse was true for WSI.

3. Degree of gelatinization of starch was affected by the formulation and whole sorghum based blends had lower degree of gelatinization. Though the difference was statistically not significant.

4. The pasting properties of the blends were affected by the formulation and whole sorghum based formulations had lower peak viscosity and greater peak time than blends with

decorticated sorghum flour which indicated greater degree of breakdown of starch molecules in decorticated formulations.

5. The Bostwick flow rates of all the formulation were within the USDA stipulated range for FBFs between 9-21 cm/min. The flow rates differed amongst different formulations but no statistically significant difference was found between them.

6. All the FBFs made from SCB had an energy density above the FAQR recommended value of 387 kcal/100gm.

7. Starch digestibility increased significantly after extrusion. Protein digestibility increased after extrusion but it was statistically non-significant and it decreased by a small amount which was also found to be statistically non-significant. This occurred possibly due to the interactions between protein-protein, starch-protein and protein-lipid during extrusion processing.

8. Extrusion was effective in reducing the anti-nutritional factors – phytic acid, tannins and trypsin inhibitors significantly in binary blends of SCB.

Acknowledgement

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Table 4.1 Composition of FBFs

Ingredients	Amount (%)
Milled extrudates (SCB)	63.40
Sugar	15.00
WPC80	9.50
Oil	9.00
Mineral Premix	3.00
Vitamin Premix	0.10

FBFs = Fortified blended foods, WPC80 = Whey protein concentrate with 80% protein content

Table 4.2 Mean values of SME, particle size, WAI, WSI, starch gelatinization and total cook of SCB formulations

Formulation	SME (kJ/kg)	Particle size (µm)	WAI (g/g)	WSI (%)	Starch gelatinization (%)	Total cook (%)
WSCB-V1com	$306\pm31_{acdef}$	$250.33\pm12.53_a$	$5.52\pm0.66_{ab}$	$12.81\pm0.31_a$	$89.90\pm4.95_a$	$77.84\pm2.85_{abc}$
SCB-V1com	$322\pm10_{acdefg}$	$243.43\pm2.43_a$	$5.97\pm0.29_a$	$13.77\pm0.88_a$	$95.16\pm0.46_a$	$89.00\pm1.59_{adf}$
WSCB-V1	$335\pm39_{abg}$	$315.41\pm4.39_b$	$5.36\pm0.31_{ab}$	$16.52\pm0.79_a$	$93.17\pm0.94_a$	$89.02\pm6.83_{adf}$
SCB-V1	$341\pm16_{beg}$	$318.98\pm2.38_b$	$4.25\pm0.07_{bd}$	$31.47\pm1.10_{bc}$	$95.97\pm0.68_a$	$95.04\pm0.07_{de}$
WS'CB-V1	$311\pm14_{acdef}$	$256.29\pm1.24_a$	$4.17\pm0.14_b$	$26.32\pm3.01_b$	$93.04\pm5.05_a$	$91.93\pm4.61_{adf}$
S'CB-V1	$362\pm29_g$	$239.38\pm3.92_a$	$4.41\pm0.04_{bf}$	$32.72\pm0.13_{bc}$	$98.83\pm0.25_a$	$93.91\pm3.11_{dg}$
WSCB-V2	$286\pm32_c$	$236.16\pm4.95_a$	$5.21\pm0.27_{ab}$	$11.59\pm0.21_a$	$85.42\pm6.24_a$	$70.26\pm0.83_c$
SCB-V2	$333\pm21_{bfg}$	$353.97\pm1.84_d$	$4.24\pm0.12_{be}$	$30.97\pm0.93_{bc}$	$96.81\pm0.30_a$	$78.55\pm0.53_{bcf}$
WSCB-V3	$310\pm43_{acdef}$	$335.85\pm4.93_{bd}$	$5.10\pm0.44_{ab}$	$15.81\pm3.05_a$	$91.40\pm1.22_a$	$91.72\pm2.64_{adf}$
SCB-V3	$350\pm22_{bdg}$	$204.94\pm3.59_c$	$4.18\pm0.05_{bc}$	$35.14\pm2.48_c$	$98.71\pm1.82_a$	$97.97\pm1.54_d$

com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. Values in the same column not sharing the same subscript are significantly different at $p< 0.05$, SME = Specific mechanical energy, WAI = Water absorption index, WSI = Water solubility index, W = whole, S = decorticated sorghum flour, S' = decorticated coarse sorghum flour, C = cowpea flour, V1 & V2 = white varieties of sorghum, V3 = red variety of sorghum

Table 4.3 Mean pasting properties of SCB

Formulation	PV (cP)	Peak Time (min)	PT (°C)	Trough (cP)	Breakdown (cP)	FV (cP)	Setback (cP)
WSCB-V1com	$399.50\pm13.44_{be}$	$4.08\pm0.04_{b}$	$70.12\pm5.76_{b}$	$268.50\pm10.61_{b}$	$131.000\pm24.04_{bd}$	$247.50\pm0.71_{bc}$	$116.50\pm24.75_{bcde}$
SCB-V1com	$140.50\pm4.95_{a}$	$0.81\pm0.05_{a}$	$50.35\pm0.49_{a}$	$86.00\pm11.31_{a}$	$54.50\pm16.26_{a}$	$99.00\pm24.04_{a}$	$44.75\pm7.78_{a}$
WSCB-V1	$334.50\pm3.54_{c}$	$4.11\pm0.00_{b}$	$68.15\pm0.64_{b}$	$176.00\pm1.41_{cdef}$	$158.50\pm2.12_{bd}$	$243.50\pm19.09_{bc}$	$76.50\pm33.23_{ab}$
SCB-V1	$432.50\pm4.95_{bef}$	$1.31\pm0.00_{a}$	$50.00\pm0.00_{a}$	$310.00\pm2.83_{b}$	$122.50\pm2.12_{d}$	$166.50\pm2.12_{fg}$	$43.00\pm4.24_{a}$
WS'CB-V1	$31750\pm6.36_{bcd}$	$1.45\pm0.00_{a}$	$50.00\pm0.00_{a}$	$124.50\pm9.19_{adg}$	$193.00\pm15.56_{b}$	$185.5\pm4.95_{cefh}$	$61.00\pm14.14_{ad}$
S'CB-V1	$446.50\pm19.09_{f}$	$1.21\pm0.14_{a}$	$50.00\pm0.00_{a}$	$144.00\pm24.04_{cg}$	$302.50\pm4.95_{ce}$	$202.00\pm16.97_{cgi}$	$58.00\pm7.07_{ae}$
WSCB-V2	$319.50\pm2.12_{cd}$	$4.38\pm0.00_{b}$	$73.35\pm1.27_{b}$	$183.00\pm4.24_{c}$	$136.50\pm2.12_{bd}$	$290.50\pm3.54_{b}$	$154.00\pm1.41_{c}$
SCB-V2	$391.50\pm6.36_{e}$	$1.21\pm0.33_{a}$	$50.00\pm0.00_{a}$	$122.50\pm6.36_{afg}$	$269.00\pm0.00_{e}$	$168.50\pm0.71_{gh}$	$46.00\pm5.66_{a}$
WSCB-V3	$279.50\pm3.54_{d}$	$3.98\pm0.00_{b}$	$70.60\pm1.77_{b}$	$122.50\pm19.09_{aeg}$	$157.00\pm15.56_{bd}$	$209.50\pm28.99_{cdfh}$	$87.00\pm9.90_{abc}$
SCB-V3	$434.50\pm13.44_{bf}$	$1.18\pm0.28_{a}$	$50.00\pm0.00_{a}$	$98.50\pm12.02_{ag}$	$336.00\pm25.46_{c}$	$140.00\pm0.00_{aeg}$	$41.50\pm12.02_{a}$

com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. PV = Peak Viscosity; PT = Pasting Temperature; FV = Final Viscosity. Values in the same column not sharing the same subscript are significantly different at $p< 0.05$

Table 4.4 Bostwick flow rate, protein, fat and energy density of SCB

Formulation	Bostwick Flow Rate (cm/min)	Binary Blend Protein (%)	FBF Protein (%)	Binary blend Fat (%)	FBF Fat (%)	Energy density (kcal/100g)	FBF Energy density (kcal/100g)
WSCB-V1com	15.25±1.06$_{acd}$	17.24	18.53	1.91	10.97	363.25	408.05
SCB-V1com	15.50±0.00$_{acd}$	17.33	18.58	1.77	10.88	363.47	407.91
WSCB-V1	14.25±1.06$_{abc}$	18.2	19.14	2.01	11.03	359.39	405.47
SCB-V1	15.75±0.35$_{acd}$	17.42	18.65	1.47	10.69	358.92	405.17
WS'CB-V1	15.50±0.71$_{acd}$	17.44	18.65	1.97	11.01	359.68	405.66
S'CB-V1	12.75±0.35$_{bd}$	17.29	18.56	0.98	10.38	357.01	403.97
WSCB-V2	11.50±0.71$_{b}$	17.55	18.73	2.07	11.07	359.98	405.85
SCB-V2	17.25±1.06$_{c}$	17.8	18.88	1.27	10.57	356.46	403.62
WSCB-V3	14.00±0.71$_{ab}$	18.14	19.10	1.85	10.93	358.86	405.14
SCB-V3	15.50±0.00$_{acd}$	17.53	18.72	1.4	10.64	358.86	405.14

FBF = Fortified Blended Food, com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. Values in the same column not sharing the same subscript are significantly different at $p < 0.05$. The protein, fat and energy values have been reported on as is basis based on data reported in FAQR (2011). FAQR guidelines for FBFs: Protein $\geq$ 18g, Fat $\geq$ 9g, Energy $\geq$ 387 Kcal

Table 4.5 Starch Digestibility of SCB

Binary Blend	RDS (%)	SDS (%)	RS (%)
SCB-V1 RM	$8.16\pm0.58_a$	$66.25\pm0.68_a$	$25.59\pm0.10_a$
SCB-V1 ME	$22.60\pm1.81_{bg}$	$46.71\pm0.98_b$	$30.70\pm0.83_{ab}$
SCB-V1 Cooked	$17.97\pm0.20_{bc}$	$54.63\pm1.16_{cd}$	$27.40\pm1.36_{ab}$
SCB-V2 RM	$8.37\pm1.49_a$	$64.93\pm2.21_a$	$26.70\pm3.69_a$
SCB-V2 ME	$20.68\pm1.27_{bg}$	$45.98\pm0.06_b$	$33.34\pm1.33_{ab}$
SCB-V2 Cooked	$13.56\pm0.85_c$	$55.33\pm2.04_c$	$31.11\pm2.89_{ab}$
SCB-V3 RM	$6.13\pm0.55_a$	$62.64\pm0.79_a$	$31.23\pm1.35_{ab}$
SCB-V3 ME	$28.78\pm2.48_d$	$46.16\pm1.11_b$	$25.06\pm3.58_a$
SCB-V3 Cooked	$14.31\pm0.91_{cef}$	$50.19\pm0.86_{bd}$	$35.50\pm1.77_b$
CSB13 RM	$18.72\pm0.18_{bf}$	$54.66\pm0.07_c$	$26.62\pm0.14_a$
CSB13 Cooked	$21.42\pm0.31_{bg}$	$49.79\pm0.64_b$	$28.79\pm0.35_{ab}$
CSB+ RM	$23.28\pm0.50_{gh}$	$47.17\pm0.98_b$	$29.55\pm0.64_{ab}$
CSB+ Cooked	$27.37\pm0.26_{dh}$	$41.46\pm0.39_f$	$31.17\pm0.47_{ab}$

SCB= Sorghum Cowpea Blend; V1 & V2 = white variety of sorghum; V3 = red variety of sorghum; RM = Raw material; ME = Milled extrudate; RDS = Rapidly digestible starch; SDS = Slowly digestible starch; RS = Resistant starch; CSB= Corn soy blend

Table 4.6 Protein digestibility of SCBs, CSB13, and CSB Plus

Formulation	RM-Digestibility (%)	ME-Digestibility (%)	Cooked digestibility (%)
SCB-V1com	$87.93\pm0.01_a$	$87.34\pm0.01_a$	$85.86\pm0.03_a$
SCB-V2	$86.34\pm0.04_a$	$92.46\pm0.04_b$	$84.08\pm0.02_a$
SCB-V3	$83.10\pm0.05_a$	$88.31\pm0.01_a$	$83.56\pm0.01_a$
CSB13	$80.72\pm0.10_a$	N/A	$88.35\pm0.04_a$
CSB Plus	$85.80\pm0.04_a$	N/A	$89.22\pm0.05_a$

com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. RM = Raw material; ME = Milled extrudate. Values in the same row not sharing the same subscript are significantly different at $p< 0.05$.

Table 4.7 Changes in phytic acid, tannins and trypsin inhibitor before and after extrusion of SCBs

Formulation	Phytic Acid (mg/100g)		Tannins (mg/100mgCE)		Trypsin Inhibitor- TIA (mg/g)	
	RM	ME	RM	ME	RM	ME
SCB-V1com	$1138.50\pm5.60_a$	$832.07\pm5.68_b$	0.00 ± 0.00	0.00 ± 0.00	$228.54\pm8.27_a$	$177.55\pm1.04_b$
SCB-V2	$804.22\pm1.74_a$	$560.72\pm3.24_b$	0.00 ± 0.00	0.00 ± 0.00	$229.79\pm4.23_a$	$191.75\pm1.13_b$
SCB-V3	$942.05\pm4.40_a$	$689.21\pm8.06_b$	0.00 ± 0.00	0.00 ± 0.00	$233.73\pm2.00_a$	$186.59\pm9.48_b$
CSB PLUS	884.54 ± 4.60	---------	0.00 ± 0.00		55.39 ± 1.01	--------

com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. RM = Raw material; ME = Milled extrudate; CE = Catechin equivalents; TIA = Trypsin inhibitor activity. Values in the same row not sharing the same subscript are significantly different at $p<0.05$.

Figure 4.1 Image of Bostwick Consistometer
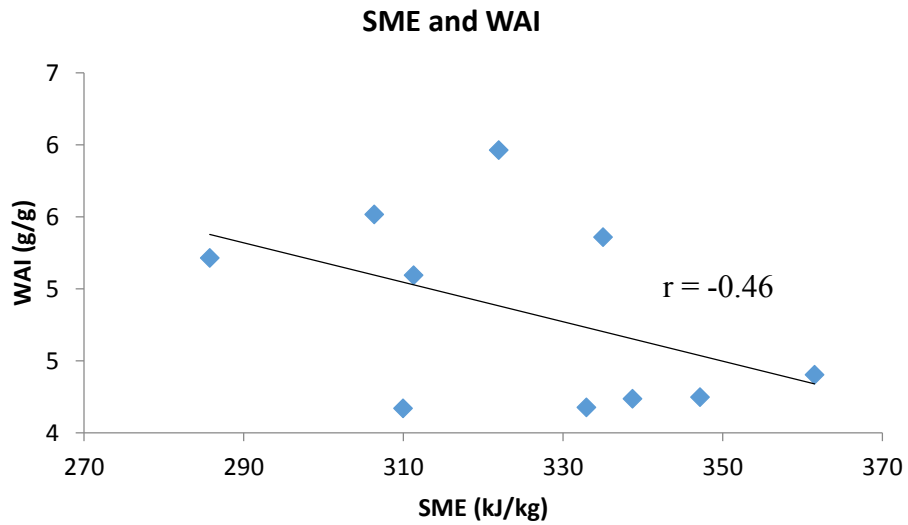
Figure 4.2 Correlation between SME and WAI for SCBs

SME = Specific mechanical energy, WAI = Water absorption index, SCB = Sorghum cowpea
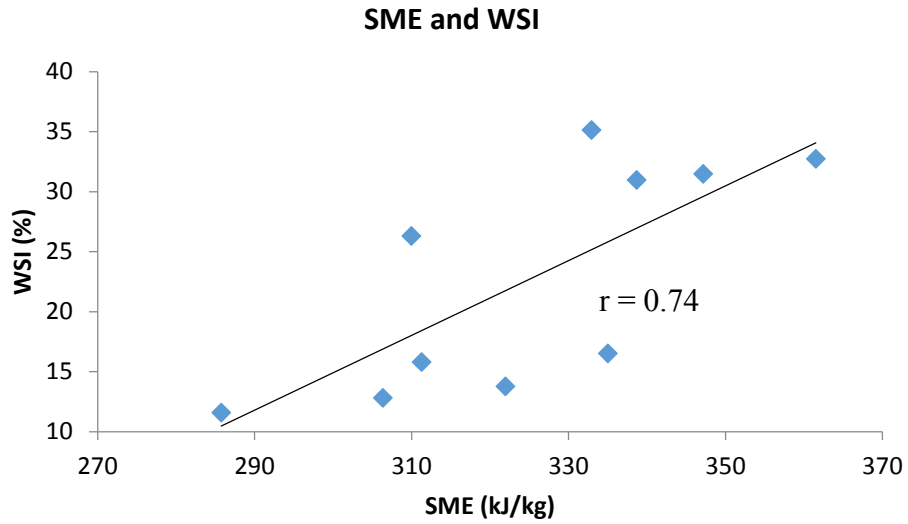
Figure 4.3 Correlation between SME and WSI for SCBs

SME = Specific mechanical energy, WSI = Water solubility index, SCB = Sorghum cowpea blend
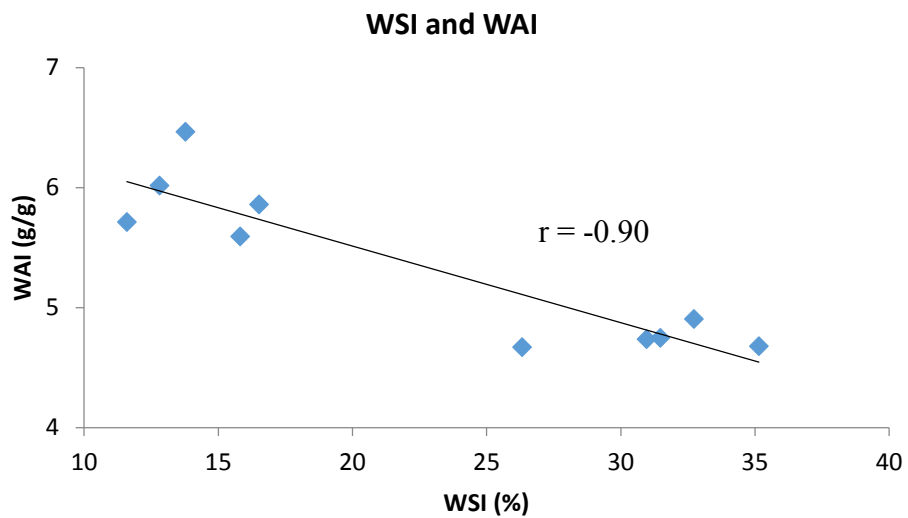
Figure 4.4 Correlation between WSI and WAI for SCBs

WSI = Water solubility index, WAI = Water absorption index, SCB = Sorghum cowpea blend
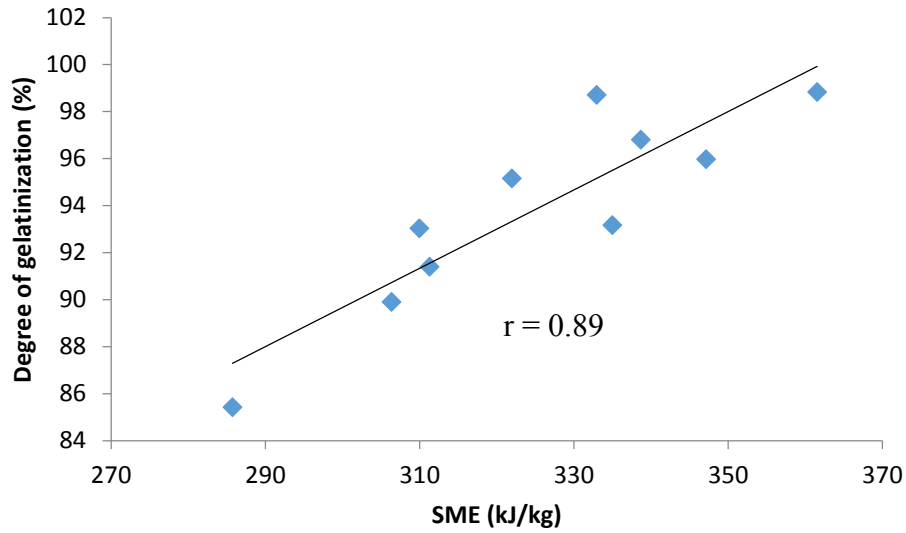
Figure 4.5 Correlation between SME and degree of gelatinization for SCBs

SME = Specific mechanical energy, SCB = Sorghum cowpea blend
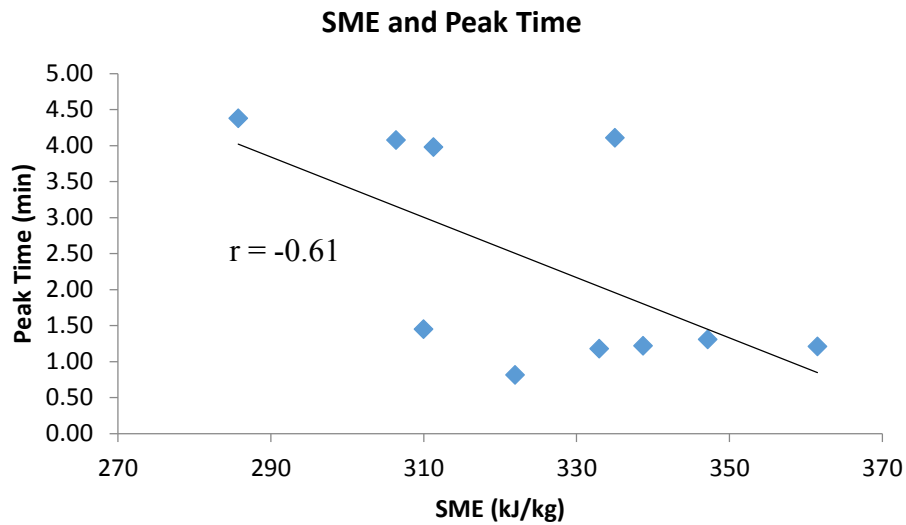
Figure 4.6 Correlation between SME and Peak time for SCBs

SME = Specific mechanical energy, SCB = Sorghum cowpea blend
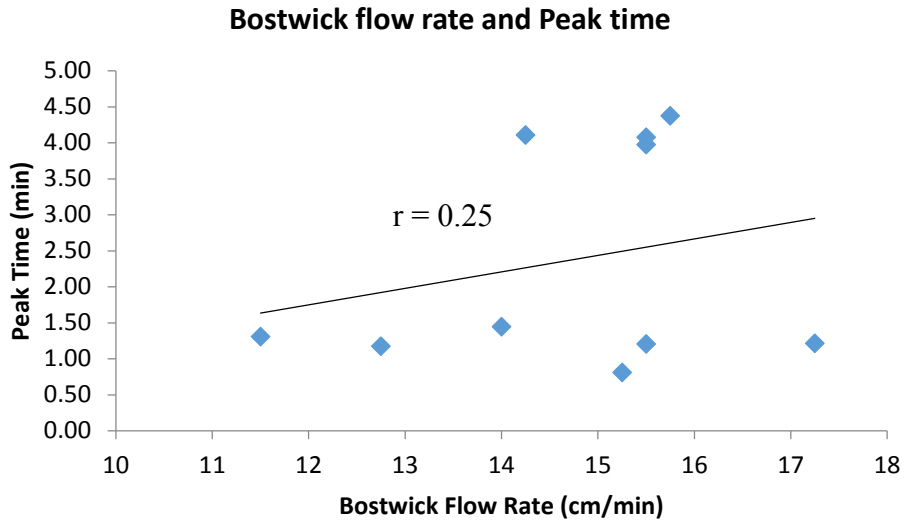
Figure 4.7 Correlation between Bostwick flow rate and Peak time for SCBs

SCB = Sorghum cowpea blend
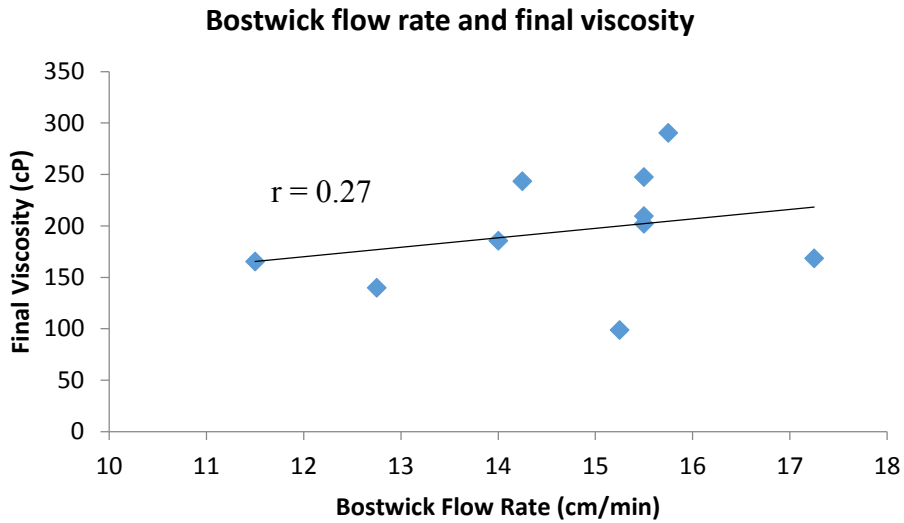
Figure 4.8 Correlation between Bostwick flow rate and final viscosity for SCBs

SCB = Sorghum cowpea blend
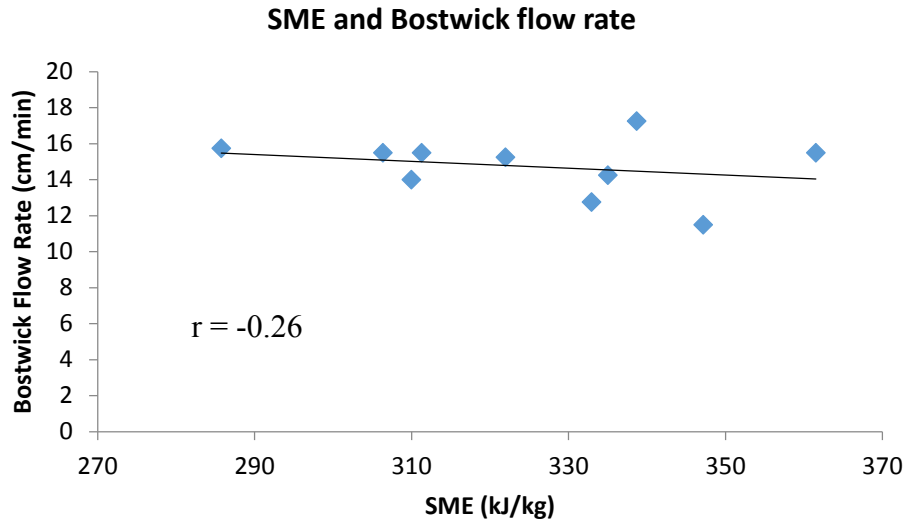
Figure 4.9 Correlation between SME and Bostwick flow rate for SCBs

SME = Specific mechanical energy, SCB = Sorghum cowpea blend

Chapter 5 - Characterization of extruded sorghum soy blends to develop pre-cooked and nutritionally dense fortified blended foods.

Abstract

Expanded formulations sorghum soy blend (SSB) obtained from extrusion cooking were ground using hammer mill and analyzed for changes in properties that were affected by transformation of starch and protein during the processing. Macro- and micro-nutrients were added to these milled blends to prepare fortified blended foods (FBFs) that could meet the recommendations of Food Aid Quality Review (FAQR) report on energy, protein and micronutrient content. The water absorption index (WAI) ranged from 2.82-5.90 g/g and water solubility index (WSI) ranged from 6.22-18.50% and they were affected by the formulation- whole/decorticated sorghum and different levels of fat. Extrusion processing caused starch gelatinization in the range of 90.69 - 96.26%.The pasting properties indicated that whole grain blends of SSB had lower peak time and higher final viscosity when compared to decorticated sorghum blends. The Bostwick flow rate of cooked porridges with 20% solids was within the recommended range of 9-21 cm/min. No significant difference in protein digestibility was observed amongst raw, extruded and cooked binary blends of SSB. Starch digestibility significantly increased after extrusion with a 149.65% increment in rapidly digestible starch (RDS). The protein digestibility did not vary significantly when subjected to extrusion and cooking. There was a significant reduction in anti-nutritional factors in extruded binary blends of SSB when compared to respective raw blends – phytic acid reduced by 25.33%, tannins were not found, and trypsin inhibitors reduced by 19.50%.Thus, extrusion processing of SSB with subsequent addition of macro-and micro-ingredients was effective in producing FBFs with high nutritive value.

Introduction

Hunger is a pervasive problem in emerging economies, undermining people's health, productivity, and often their very survival (Smith et al. 2006). According to the report in The State of Food Insecurity in the World 2014 by United Nations Food and Agriculture Organization an estimated 795 million people of the 7.3 billion people in the world, or one in nine were suffering from chronic undernourishment in 2012-2014. Of these hungry people, 791 million live in developing countries making it one in eight or 13.5% of the population in those countries. There was reduction in hungry people by over 167 million since past decade and the prevalence of undernourishment has fallen from 18.7% to 11.3% globally and from 23.4% to 13.5% for developing countries (FAO et al., 2015). Even after this reduction in hunger, the least progress has been in sub-Saharan region of Africa where more than one in four people remain undernourished – the highest prevalence amongst any region of the world.

Population growth, poverty, conflicts, social exclusion, governance, trade policies, inequality, and natural disasters are some of the causes of food insecurity in the world (Ahmed et al., 2007). Therefore, the importance of food aid in addressing certain food insecurity issues becomes paramount. Food aid has been used as an instrument to offset food shortages in low-income countries, where fluctuations in domestic food production threaten food security (Shapouri and Rosen, 2001). FBFs are not only 'ready-made' in the sense that they are nutritionally rich in their physical form with easy preparation methods, but usually they are dispensed through a standardized regime, involving registration, anthropometric measurement and cooking and hygiene training (World Health Organization, 2004; Sphere Project, 2011; Scott-Smith, 2014). The United States is the leading contributor to international food aid with average supply of 56% of the annual total food aid donated by members of the Food Aid Committee of the International

Grains Council since 1995 (Hanrahan and Canada, 2013). Food aid programs in U.S. are administered by the United States Agency for International Development (USAID) and United States Department of Agriculture (USDA) either as part of bilateral program or through United Nation's World Food Program. These food aids have been distributed by US under four authorities: (1) the Food for Peace Act (FFPA, also known as P.L. 480); (2) the Section 216(b) program (inactive since 2007); (3) the Food for Progress Act of 1985; and (4) the McGovern-Dole International Food for Education and Child Nutrition Program (Schnepf, 2015). Since late 1980s, the USAID administered FFPA Title II program has emerged as the largest funding source for U.S. food aid shipments. This program provides for the donation of U.S. agricultural commodities to support specific emergency and non-emergency food needs either by monetization or direct food distribution. In 1996, the law was amended to permit the enrichment and fortification of commodities to improve their nutritional quality and included high protein blends of U.S. foods for malnourished infants, children, pregnant women, and lactating mothers. Foods donated under P.L. 480 today include whole commodities, processed foods, fortified processed foods, and blended food supplements.

The fortified foods routinely combine cereals and soybean meal products to increase the quality and quantity of proteins. Wheat, corn, sorghum, rice and soy are often processed, fortified or enriched into products such as corn soy blend (CSB), wheat soy blend (WSB), fortified wheat flour, fortified cornmeal and vitamin-A fortified vegetable oil. CSB is often used to treat moderate malnutrition and micronutrient deficiencies in underweight children. CSB is the most commonly programmed specialized product in supplementary feeding programs (GAO, 2011). In the early 1970s when there was shortage of nonfat dried milk which was used regularly in corn soy milk (CSM) till then was reformulated to CSB. The ineffectiveness of CSB, which is

classified as ready-to-use supplementary food in addressing moderate acute malnutrition due to inadequate compositional profile of energy density, micronutrients, lipids etc., CSB, has developed expeditiously with different variants such as CSB 10, 11, 12, 13 and CSB Plus between 2005 and 2011 with enhancements to the earlier shortcomings. In Title II programming, the fortified blends account for 44% of the commodity cost though it constitutes only 20% of the volume (FAQR, 2011). The FAQR recommends the use of other cereals namely, sorghum, millets, and rice instead of traditional cereals like corn and wheat in the production of FBF. The major advantage of using non-GMO crops like sorghum is that it would be well received by governments of target nations, like countries in Africa. Additionally, crops like sorghum, millets, cowpea etc. are plants that require less water and can thrive in drought like conditions (Ismail et al, 2003) which would add to the promotion of sustainable agriculture. Corn experiences pre-harvest insect damage due to shallower rooting system, wet post - harvest and storage practices in under developed economies leading to increased risk of aflatoxin contamination (Pitt et al., 2013).

Ideally, the ingredients for low-cost weaning formulations must be derived from dietary staples from the region of interest that are affordable to the section of the target population and readily available in sufficient quantity (Mensa-Wilmot et al., 2001). Therefore, in this study, sorghum and cowpea were combined in a ratio that would provide optimum protein and energy in the FBFs. Grain sorghum contains phytochemical such as phenolic compounds, plant sterols and policosanols that are rich in anti-oxidants and impacts human diets significantly by lowering cholesterol and promoting cardio-vascular health. Condensed tannins present in phytochemicals seem to have powerful anti-carcinogenic and anti-diabetic in-vitro activity (Awika and Rooney, 2004). However, the bioavailability of tannins (procyanidins and catechins) is questionable due

to their larger molecular size and tendency to bind food molecules into insoluble complexes making it difficult for human digestion. Another anti-nutritional factor is phytic acid (inositolhexakisphosphate (IP6)) in cereal-legume based complimentary foods that inhibits iron absorption from porridges leading to a high prevalence of iron deficiency in infants. (Cook et al., 1997). Studies show that dephytinization through processing in low tannin sorghum increased iron absorption by 2-folds in sorghum reconstituted with water (Hurrell et al., 2003). Heat treatment during processing has also shown encouraging results in lowering phytates that increase iron solubility by forming iron complexes in naturally occurring plant phytates (Sandberg et al. 1989). Extrusion has been successfully used in production of low-cost cereal based weaning foods (Mustakas et al., 1964; Harper and Jansen, 1985). The processing of cereal-legume blends using extrusion has been used to completely gelatinize starch at 150-170°C extrusion temperatures with moisture range of 16-22% (Rabe et al. 1980), and denature proteins enabling a nutritious precooked blended product. When processed under ideal conditions using extrusion technology, catechins and procyanidins in tannin showed improved bioavailability of up to 50% in diets (Gu et al. 2008) and possible breakdown of high molecular weight polymers of procyanidins making is easier for human absorption thereby improving nutraceutical value of sorghum (Awika et al. 2003). Extrusion heat treatment and shear forces further inactivates trypsin inhibitors by 90% in extrudates (Nwabueze., 2007) thus retaining most of the chemically available lysine in soy flour when extruded at 100 to 115°C with 12 to 18% barrel moisture (Konstance et al., 1998). The idea behind researching other grains such as sorghum for use in FBF production is to utilize viable nutrient sources in a way to bring down production cost, increase acceptance and implement new nutrient recommendations listed in the FAQR report.

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Sorghum soy blend (SSB) as it will be called is a precooked blend using extrusion and micronutrient fortified which can be a solution for wasted and infants with stunted growth. The objective of this study was to develop SSB that conforms to the most recent recommendations from Food Aid Quality Review (FAQR) and understand the effects of extrusion processing on starch and protein of extruded SSB in comparison with traditional CSB. Specific processing to product relationships were established which included post-milling particle size, viscosity profiles and their effects on Bostwick flow rate which related to gruel consistency before infant consumption, starch and protein digestibility and anti-nutritional factors.

Some of the key recommendations to better supplement nutritional targets along with optimal breastfeeding combined with infant feeding practices, were to:

1) Increase in quantity of protein and addition of animal protein namely WPC-80 (whey protein concentrate), so as to increase the Protein Digestibility Corrected Amino Acid Score (PDCAAS) to 0.88 (a score > 0.80 is considered good quality of protein),

2) Increase in the caloric content of FBFs by addition of oil to post extruded binary blend.

3) Upgrades to the micronutrient composition.

Materials and Methods

Materials

The following material components were used – 1) extruded and milled binary blends of SSB, 2) sugar (C&H brand granulated white cane sugar purchased from local store, Manhattan, Kansas, USA), 3) whey protein concentrate -WPC80 (Davisco Foods International, Inc., Eden Prarie, Minnesota, USA), 4) vitamins and minerals (Research Products Company, Salina,

Kansas, USA) and non-gmo soybean oil (Zeeland Farm Services, Inc., Zeeland, Michigan, USA).

Hammer Milling

The extrudates were cooled and then conveyed through bucket elevators and collected in large plastic bags. The extrudates from these bags were then fed into the inlet of Schutte Buffalo Hammer mill (Buffalo, NY, USA) fitted with 3/64 inch (1190 µm) screen. The powdered extrudates were filled directly into 50 lb 3-walled paper bags and sealed till further use.

Particle size analysis

Particle size of extruded and milled binary blends were determined using laser diffraction particle size analyzer (LSTM 13320, Beckman-Coulter, Inc., Miami, Florida, USA). The samples were tested in duplicate.

Specific Mechanical Energy (SME)

The SME was calculated as follows:

$$SME = \frac{\dfrac{(\tau - \tau_O)}{100} \times P_{rated} \times \dfrac{N}{N_{rated}}}{\dot{m}} \tag{1}$$

where,

τ = operating torque (%); τ_0 = no-load torque (%); P_{rated} = rated power (37.3 kW), N = screw speed (rpm); N_{rated} = rated screw speed (507 rpm), and ṁ = net mass flow rate of extrudate at die exit (kg/s).

Protein and Fat Analysis

Standard methods were used to determine the proximate content of raw ingredients. The moisture content was determined using AACC 44-19 method (135°C for 2h), crude protein was

determined based on nitrogen by combustion, (6.25X; AOAC 920.176), crude fat (petroleum ether extract method; AOCS Ba 3-38). Duplicates were run for each sample and the results were reported on as is basis.

Mixing protocol for addition of macro- and micro-ingredients

It was recommended by Webb et al. (2011) in the FAQR that the solid content in the porridges made from FBFs be increased. The increase suggested by them was 20% solids as compared the current 11.75% (USDA, 2008) to have energy dense porridge. However, the porridges became thicker when the solids content was increased and it had difficulty in flowing. The thicker gruels therefore had a Bostwick flow rate lower than the recommendation of 9-21cm/min. In order to satisfy the Bostwick flow rate criteria, sugar was added at 15% after removing an equal quantity of the binary blend. Plasticizing and viscosity reducing properties of sugar helped increase the Bostwick flow rate to within the recommendation. It has been reported that addition of sugar increases to the energy density FBFs with minimum increase of volume. (de Pee and Bloem, 2009).

The mixing was done in steps of decreased dilution of ingredients as the steps progressed to ensure the uniformity of mixing. All the dry ingredients – sugar, WPC80, minerals and vitamins were weighed separately to make a batch of 25 kg fortified blended food (FBF). The minerals and vitamins were mixed first in a small Hobart mixer (Model N-50, Hobart Corporation, Troy, Ohio, USA) for 1 minute till no yellow concentrates of vitamin were present. This vitamin - mineral blend was then transferred into the bowl of another Hobart mixer (Model A-200, Hobart Corporation, Troy, Ohio, USA) and 3.33 kg of milled and extruded binary blend was added to it and mixed for 3 minutes to get a uniform dilution of vitamin - mineral mix with a part of the binary blend.

The above premix was again transferred slowly to the bowl of a larger Hobart mixer (Model M802, Hobart Corporation, Troy, Ohio, USA). Then 10 kg from the remaining milled binary blend was added to it and mixed for 5 minutes on speed setting of 1. After 5 minutes of mixing the remaining dry ingredients – milled binary blends, sugar and WPC80 were added to it and mixed for another 5 minutes on the same speed setting of 1. Once the mixing was over, 14.42 kg of the dry blend was removed from the bowl of the mixer. To the remaining 8.33 kg of dry blend remaining in the mixer, 2.25 kg of oil (for non-full fat soy formulations) and 1.375 kg of oil (for full fat soy formulations) was added and mixed for 5 minutes with 2 minutes on speed setting of 1 and 3 minutes on speed setting of 2. On completion of this step the previously removed dry blend was added back to the mixer and mixed for another 5 minutes on speed setting of 1. At the end of this step the fortified blended food was ready and is shown in Table 5.1

Bostwick Consistometer Test

The test was done using a Bostwick Consistometer (CSC Scientific Company, Inc., Fairfax, Virginia, USA). Bostwick consistometer measures the flow of any viscous material which in this case is the the final FBF after making a gruel/porridge with the milled extrudates and other added macro- and micro-ingredients. The Bostwick consistometer is made of stainless steel and has a trough with precise 0.5 cm markings engraved along the base. At one end of the trough is a spring loaded gate called the reservoir and the sample is loaded here after closing the gate (Fig. 5.1). The consistometer was placed on a flat surface and the twisting screws provided at the same end as that of the reservoir were adjusted till the levelling bubble provided on the front of the instrument was placed in the center. Gruels containing 20% solids was made by adding 40 g of FBF to 160 ml distilled water in a glass beaker. The solids (FBF) were added to boiling water and stirred vigoursly with a fork for 1 minute and thereafter it was removed from heat and stirred

for another 30 seconds. This hot gruel was covered with an aluminum foil and placed in water bath maintained at 30°C for 10 minutes. At the end of 10 minutes the gruel was removed from the water bath and the slurry was adjusted for water loss through evaporation to the initial weight of 200g by addition of distilled water. It was again stirred and covered with aluminum foil and placed in water bath at 30°C for 1 hour. At the end of the cooling period, the water loss due to evaporation was again made up to initial weight of 200g. The gruel was then poured into the reservoir of the consistometer till it overflowed and it was levelled with a flat plastic spatula. After allowing the gruel to settle for 30 s, the gate of the reservoir was opened and the gruel was allowed to flow along the graduated trough. The distance of flow was recorded exactly after 1 minute and if the gruel was between two graduations after the end of 1 minute then the higher graduation was recorded as the distance (USDA 2005). All the samples were measured in duplicate.

 Water Absorption Index (WAI) and Water Solubility Index (WSI)

A method based on Anderson et al. (1970) was used to measure WAI and WSI of binary blends before and after extrusion process. A 2.5 g sample was dispersed in 25 g distilled water, using a glass rod to break any lumps. After stirring for 30 minutes, the dispersions were rinsed into tared centrifuge tubes and made up to 32.5g and centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted for its solid content (WSI) after evaporation of water from the supernatant. The sediment was weighed for its solid content and was used to report the WAI. The indices were calculated as:

$$\text{WAI (\%)} = \frac{Weight\ of\ sediment}{Weight\ of\ dry\ solids} \times 100$$

$$\text{WSI (\%)} = \frac{Weight\ of\ dissolved\ solids\ in\ supernatant}{Weight\ of\ dry\ solids} \times 100$$

Thermal Analysis - Differential Scanning Calorimetry (DSC)

Calorimetric measurements were carried out for different raw and extruded binary blends to understand the physical transformation of starch and proteins known as starch gelatinization and protein denaturation respectively on Q100 DSC (TA Instruments, New Castle, DE, USA). 8-10 mg of sample was weighed into large volume stainless steel DSC pans (Part no.03190029, Perkin Elmer Health Sciences Inc., Shelton, CT, USA). Distilled water was added to the sample in the pan so as to obtain a solid to water ratio of 1:2 (Stevens and Elton, 1971; Zhu et al., 2010). The pans were hermetically sealed and the samples were allowed to equilibrate overnight. The instrument was calibrated using indium as reference material. An empty sealed pan was used as reference for all experiments. The program steps used for the test was as follows: Equilibrate at 10°C, heating the pans from 10°C to 140°C at the rate of 10°C/min, mark end of cycle, cooling down the sample from 140°C to 10°C at the rate of 25°C/min, mark the end of cycle with nitrogen gas flow rate of 50mL/min. The samples were again rescanned with heating from 10°C to 140°C at the rate of 10°C as the final phase of the test.

DSC data for each gelatinization and denaturation endotherm was analyzed for transition temperatures, onset (To), peak (Tp), and endpoint or conclusion (Tc) and the enthalpy (ΔH) using TA Instrument's Universal Analysis Software (version 5.4.0). All the reported data were means of two replicates.

Starch gelatinization (%) was calculated by comparing the enthalpic transition difference in starches between raw and extruded binary blends. Calculations were made using the equation below:

Starch gelatinization (%) = [(ΔHraw − ΔHextruded)/ΔHraw] x 100

Where, ΔHraw = enthalpy of raw binary blend, ΔHextruded = enthalpy of extruded binary blend

Total cook (%) was calculated as a ratio of the total enthalpic transistion difference which includes the transition enthalpies for starch and protein fractions of the binary blends. It is represented as below:

Total cook (%) = [(ΔH_Traw − ΔH_Textruded)/ΔH_Traw] x 100

Where, ΔH_Traw = Total enthalpy of transition of raw binary blend, ΔH_Textruded = Total enthalpy of transition of extruded binary blend

Rapid Visco Analyzer (RVA)

The RVA provides an index of how cooked a sample is by re-cooking under relatively low shear in excess water and measuring the pasting viscosity throughout the test. Pasting properties of the binary blends were examined using RVA. (RVA 4, Newport Scientific Pvt. Ltd.,Warriewood, NSW, Australia). The RVA was interfaced with a computer equipped with the software – Thermocline for Windows (version 3.15.2.298) for controlling the test and analyzing the results. Pasting properties were determined after running the samples on standard AACC profile (AACC 76-21.01, 1999) with a run time of 13 minutes. Peak viscosity (PV), pasting temperature (PT), trough or holding paste viscosity (HPV), breakdown (PV- HPV), final viscosity (FV) and setback (FV – HPV) were recorded. All measurements were performed in duplicate.

Starch Digestibility

Raw and extruded and milled samples of binary blends were measured by a modified *in vitro* Englyst method (Englyst et al, 1992). Briefly, samples of blends (0.6 g) and guar gum (50 mg) were placed in a centrifuge tube (45 mL). Freshly prepared pepsin solution (50 mg pepsin in 10 mL 0.01M HCl) was added to the tube, and the mixture was incubated for 2 hours at 37°C. Sodium acetate solution (0.25M) was added to the mixture to stop the digestion.

Pancreatic/amyloglucosidase mixture (5 mL) and glass beads were added to the sample tube for starch digestion. The tube was incubated in a shaking water bath at 37°C and 90 strokes/min. At 20 and 120 min, an aliquot of 0.25 mL sample was pipetted into 10 ml of 66% ethanol. The glucose released at each interval was determined using glucose oxidase/peroxidase method and was converted to percentage of starch hydrolyzed by multiplying by 0.9. Starch digested at 20 min is defined as RDS, starch digested between 20 and 120 min is defined as SDS, and starch not digested after 120 min incubation is defined as RS.

Protein Digestibility

Protein digestibility assay modified from Mertz et al. (1984) was used to measure the digestibility of binary blends before and after extrusion. Flour samples (200mg/sample) were weighed and placed in 50ml centrifuge tubes. Each sample was incubated with 35ml pepsin solution (1.5 mg pepsin in 1ml of 0.1 M phosphate buffer pH2.0) at 37°C. After two hours of incubation, 2ml of 2M sodium hydroxide was added to each tube to stop the digestion. All tubes were centrifuged at 3320g for 15 minutes at 4°C and the supernatant was discarded. The residue was washed in 10 ml of 0.1 M phosphate buffer pH 2.0, centrifuged, and supernatant was discarded. The washing steps were repeated one more time, and samples were frozen (-80°C for 30 minutes) and lyophilized. Freeze-dried samples were tested using nitrogen combustion (LECO system) to analyze the amount of undigested protein. Digested protein was calculated based on protein content of the native sorghum flour and that of the undigested fraction.

% Digestibility = {[Total N (mg) – Undigested N (mg)]/Total N (mg)}*100

Phytic Acid

Phytic acid (InsP6) was measured using Megazyme kit (Megazyme International, Ireland) for phytic acid and total phosphorous in which phytic acid is measured as phosphorous released by

phytase and alkaline phosphatase. One gram of powdered sample was and transferred into 75 ml glass beaker containing 20 ml of 0.66 M hydrochloric acid. The beaker was covered with aluminum foil and stirred vigorously overnight at room temperature. 1 ml of extract was transferred to 1.5 ml microcentrifuge tube and centrifuged at 13000g for 10 minutes. 0.5 ml of the resulting extract was immediatedly transferred to fresh 1.5 ml microcentrifuge tube and neutralized by addition of 0.5 ml of 0.75M of sodium hydroxide solution. Neutralized sample extract solution was used in the enzymatic dephosphorylation reaction procedure. Total and free phosphorous contents were measured for each sample. ΔA phosphorous was calculated for each sample by subtracting the absorbance of 'free phosphorous' sample from that of the absorbance of 'total phosphorous' sample. The phosphorous concentration is expressed as g/100g of sample using the following calculation:

Phosphorous (g/100g) = [ΔA phosphorous x 10,000 (conversion from $\mu g\ g^{-1}$ to g/100g) x 1.0 (weight of the original sample) x 1.0 (sample volume used in colorimetric step)] / [mean M (mean value of phosphorous standard) x 20 (original sample extract volume) x 55.6 (dilution factor).

Mean value of phosphorous standard was obtained using a standard curve over a dynamic range of 0-0.75 μg phosphorous. Phytic acid concentration was calculated as follows:

Phytic acid (g/100g) = Phosphorous (g/100g)/0.282. The calculation of phytic acid content was based on the assumption that the amount of phosphorous measured is exclusively released from phytic acid and that this comprises 28.2% of phytic acid.

 Tannins

Vanillin-HCl method (Price et al., 1978) was used to measure the tannins. The test samples were ground and 0.3 g of it was taken for assessing the tannins. The ground samples were transferred

into 15 ml tubes. The extraction of tannins was performed by adding 8 ml of 1% HCl in methanol at 1 minute intervals to the sample tubes. The samples in the tube with 1% HCl in Methanol was vortexed for 10 s and then placed in a water bath maintained at 30°C for exactly 20 minutes. The sample tubes were removed from the water bath after 20 minutes and vortexed again. The extracts were then centrifuged at 2000 rpm for 4 minutes. Two 1 ml aliquots were taken from each tube and transferred into two separate15 ml tubes. One tube was marked for sample determination and the other tube was marked for blank determination. The sample tubes after brief vortexing were placed in water bath maintained at 30°C after adding 5 ml of vanillin reagent to it at 1 minute intervals. 1% vanillin in methanol was added to 8% HCl in methanol in 1:1 ratio to prepare the vanillin reagent. The blank tubes were added with 5 ml of 4% HCl in methanol and after brief vortexing was placed in the water bath at 30°C. After completion of 20 minutes the absorbance of samples and blanks were read using spectrophotometer at 500 nm. The spectrophotometer was zeroed using methanol blank before measuring. The difference between sample and blank was used for final determination of phenol content and expressed as catechin equivalents (CE)/mg of sample. A standard curve using 1000 ppm of catechin standard at 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml added with 1% HCl in methanol to make volume to 1 ml and 5 ml vanillin reagent was plotted. The CE equivalent was calculated as below:

CE (mg/mg of sample) = (y/m) / sample concentration (mg/ml),

Where, y and m (slope) can be calculated from the regression equation of the standard curve.

 Trypsin inhibitor

Trypsin inhibitor activity was determined, according to the method described by Smith et al. (1980) using BAPNA (benzoyl-DL-arginine-p-nitroanilide) as substrate (0.92 mM in 0.05 M Tris buffer/0.02 M CaCl2, pH 8.2). The sample was fine ground and 1 gm of the ground sample

was used for extraction of trypsin in 50 ml of 0.01 N NaOH for 3 h. The pH was maintained within the range 8.5 to 9.0. One ml of the extract and 2 ml of trypsin solution 0.002% (Type I, of bovine pancreas, SIGMA) in 0.001M HCl were mixed with 1 ml of water. The reaction started after addition of 5 ml of substrate at 37 °C. After 10 min, the reaction was stopped by the addition of 1 ml of 30% acetic acid. The reaction mixture was filtered through filter paper (Whatman No. 3) and the absorbance due to release of p-nitroaniline was read at 410 nm. The activity was interpreted as the increment of 0.01 units of absorbance at 410 nm for 10 ml of reaction mixture. Trypsin inhibitor activity is expressed in terms of mg trypsin inhibited per g of dry sample.

Statistical Analysis

All the results were analyzed using analysis of variance (ANOVA) with general linear model procedure (SAS version 9.1, SAS Institute, Cary, North Carolina, USA). When significant effects ($p < 0.05$) were indicated by ANOVA, Tukey pairwise comparisons were done to identify which treatments differed significantly ($p < 0.05$).

Results and discussion

Particle size of milled extrudate - Sorghum Soy Blend

The mean particle size of extruded binary blends of SSBs ranged from 196.93 μm to 424.28 μm (Table 5.2) with the least being for SS"B-V1 (commercial) and SS"B-V2 (pilot) respectively. Amongst commercial milled blends, the whole grain formulation had significantly ($p<0.05$) bigger particle size than decorticated. In the pilot milled blends, whole formulations had significantly lower ($p<0.05$) particle size as compared to decorticated blends. No significant correlations were observed between different extrudate physico-chemical properties that would have affected the particle size. However, the effect of overall fat content in the blend seemed to

affect the particle size when commercial and pilot blends were analyzed separately. Higher fat

content produced extrudates with higher density due to low expansion and on being subjected to

hammer milling these extrudates needed more force than other expanded blends and thus

produced lower particle size. Inspite of differences in particle sizes of different blends, all the

blends could meet the particle size specifications of USDA commodity requirements (2014) for

CSB Plus which is currently being distributed in food aid programs.

Water Absorption Index and Water Solubility Index

It was observed from Table 5.2 that the WAI for SSBs ranged from 2.85 g/g to 5.91 g/g for

WSS"B-V1and SS"B – V2 (both pilot milled) respectively. Again, it can be observed from the

same Table 5.2 that WSI ranged from 6.23% in WSSB-V1to 21.45% in WSS"B-V1 (both pilot

milled). The WAI and WSI data did not exhibit a definitive trend based on type of formulation or

type of milling from which these formulations were obtained. The impact of formulation affected

the WAI. From Figure 5.2 it can be observed that WAI was positively correlated to starch

content (r = 0.78). Higher starch content leads to higher water absorption due to higher

gelatinization and increase in SME showed increase in WAI as shown in Figure 5.3 (r = 0.66).

WAI increased when SME was increased due to increase in amount of swollen starch in the

undamaged starch granules (Smith, 1992). The fiber content in the blend was inversely

correlated to WAI (r = -0.76) as shown in Figure 5.4. Chevanan et al. (2007) reported that

increasing DDGS content which is rich in fiber from 20 to 60% reduced the WAI of extrudates

by 25.7%. There was an inverse correlation between WAI and WSI and higher WAI is indicative

of less starch degradation and thus it lowers the WSI. Besides the starch gelatinization, which

results in the release of amylose and amylopectin, there can also occur dextrinization and other

reactions that lead to the formation of low molecular weight compounds, influencing the WSI (Camire & Krumhar, 1990).

Pasting Properties

The pasting properties for ground, extruded binary blends of SSB are given in Table 5.3. It can be observed from the table that blends containing whole grain sorghum flour had higher peak time compared to formulations that were constituted of decorticated sorghum flour though not significantly different The high fiber content in whole grain blends compete with starch in water absorption and therefore delays the swelling of starch leading to higher peak time. Also, higher SME, lowered the peak time (Fig. 5.5) due to its effect in breaking down starch molecules ($r = -0.46$). The peak viscosity was higher in blends with decorticated sorghum flour though the difference was not significant. The whole sorghum based blends had higher protein and fat that affected the peak viscosity. Egouletey and Aworh ([1991](#)) reported that protein and fat interaction in the blend of African yam, beans, and cassava starch lowers the peak viscosity. Sandhu and Singh (2007) also attributed lower peak viscosity to difference in protein contents of blends. The breakdown viscosity ranged from 17.50 – 272.50 cP. Trough and breakdown are pasting properties that indicate the ability of a food material to remain undisrupted on being subjected to long periods of constant high temperature and ability to withstand breakdown during cooking (Normita and Cruz, 2002). It is regarded as the measure of the degree of disintegration of the granules or "paste stability" (Dangate, 1984). It was observed that blends with whole sorghum had lower breakdown viscosities than decorticated blends. Even though the troughs did not generally show any significant difference ($p>0.05$) between blends with whole and decorticated, and with different levels of fat, but it was observed that fiber and fat had an impact on the breakdown viscosities. SS"B-V1 (aspirated) showed the highest breakdown viscosity which

meant it was the least stable during the holding phase and whole blends were more stable during the holding phase as compared to decorticated blends owing to their low peak viscosities and lower starch content. The final viscosity ranged from 153.50 – 260.00 cP. Shimels et al. (2006) reported that final viscosity was used to indicate the ability of starch to form paste or gel after cooling. Higher final viscosities were observed in blends containing whole flour and/or high fat content though the difference was not significant (p>0.05). The low peak viscosity and breakdown viscosity and high final viscosity of whole grain blends suggested to an influence by the amount of amylose leaching, amylose–lipid complex formation, friction between swollen granules, granule swelling, and competition for free water between leached amylose and remaining ungelatinized granules (Ahmadi-Abhari et al., 2013) . This result appeared to suggest that the starch granules became more resistant to thermal treatment and mechanical shearing (BeMiller, 2011). A decrease in breakdown viscosity might be due to less swelling of the starch granules in the presence of fiber (Kaur et al., 2008) and fat. The higher final viscosities in whole grain blends may have been the combined effect of retrogradation of amylose molecules as well as higher water absorption by the inherent fiber content in the blend. The peak viscosities for binary blends containing whole sorghum flour were significantly lower (p<0.05) than that of blends with decorticated sorghum flour. The extruded binary blends containing decorticated sorghum flour exhibited lower setback viscosity values as compared to whole sorghum blends.

Bostwick Consistency

The Bostwick flow rates of the cooked slurry at 20% solids concentration which had sugar, WPC 80, oil, vitamins and minerals in addition to extruded binary blend is presented in Table 5.4. It was observed that whole formulations and blends with higher fat content had lower Bostwick value. Further, it was observed from Figure 5.6 that there was a positive correlation between ER

and Bostwick Flow rate (r = 0.81). ER overall effect on the extrudate due to the effect of the formulation and the SME. Higher ER results from higher SME and lower fat content in the formula. Therefore, higher ER is indicative of higher starch transformation due to its breakdown during the process of extrusion. The starch breakdown produces lower molecular fractions which do not absorb as much water as an intact starch granule and therefore, flows more freely during the Bostwick flow test. The final viscosity of blends during RVA did not correlate with Bostwick flow rate possibly because Bostwick flow rate was conducted at 30°C whereas final viscosity was recorded at 50°C and the difference in the temperature might have further changed the viscosity profile of the blends. Inspite of the differences in flow rates between all the blends they were within the range of 9-21 cm/min as stipulated by USDA commodity requirement for corn soy blend (CSB 13) (USDA, 2008) except for WSS"B-V2 which had the least SME of 51.20 kJ/kg and an therefore least ER of 2.43. Analysis of all these correlations point out to the fact that Bostwick flow rate is dependent on the characteristics of starch after it has been cooked as porridge/slurry.

Degree of Starch gelatinization

The starch gelatinization levels of SSB formulations are summarized in Table 5.2. The extrusion processing aided in starch gelatinization that ranged from 90.70 – 96.27%. There was no statistically significant difference (p>0.05) in gelatinization values across different blends. However, it was noted that blends with higher oil content (whole blends) had lower values of gelatinization. Similarly, the total cook of the blends after taking into consideration the total enthalpy (starch gelatinization and protein denaturation) ranged from 64.46 – 78.50% as shown in Table 5.2. Although, all blends had starch gelatinization above 90% but possibly the differences of this in blends could be attributed to different levels of starch in the blends. The

starch content in whole blends ranged from 47.36 – 54.71% and that in decorticated blends ranged from 53.71 – 57.28%. Thus higher starch content could have contributed towards higher transition enthalpy during the starch gelatinization process and also, lower inherent fat content in decorticated blends might have formed lower amylose –lipid complex during the thermal transition process and thereby increasing the gelatinization when compared to whole blends. Similarly, whole blends had higher protein content (13.37 – 19.00%) and higher fat content (1.30 – 3.32%) as compared to decorticated blends (12.87 – 18.70% protein) and 2.85 – 6.62% inherent fat. This accounted for lower energy transfer to whole blends during processing because oil provides a lubricating effect (Feng and Lee, 2014) and that would have caused the protein which requires higher energy to be less denatured than starch to cook as compared to decorticated blends. Harper et al (1981) reported that the heat of reaction for protein ranged between 90 – 100 kJ/kg and 10-19 kJ/kg for cereal starches (Stevens and Elton, 1971).

Energy Density of FBFs

The FBFs are aimed towards infants as complementary foods provided at suitable age to meet their growth requirements. The FAQR (Webb et al., 2011) recommended energy-dense foods with good protein content and an appropriate inclusion of essential micronutrients as necessary (albeit not always sufficient) to achieve defined nutrition goals among vulnerable populations. Therefore, they advised that the current nutritional and energy value of the FBFs, CSB or WSB be enhanced to provide in 100 g of FBF, 387 kilocalories as energy, 18g protein and 9 g fat. They further suggested use of other cereal blends than the regular CSB and WSB, use of WPC to increase the protein content in addition to other changes. In addition to it, the availability of staple foods must be ensured so that nutritionally enhanced foods add to it rather than replacing them as sources of energy and nutrition in the local food supply.

There are several recommendations by nutritionists that complementary food should be introduced to infants in semi-solid or puree state (Monte and Giugliani, 2004; Elizabeth and Vince, 2008). Therefore, as a general practice most cereal-based products used for complementary feeding programs are diluted to achieve a thin drinkable consistency before being fed to infants. However, dilution causes lowering of energy density and micronutrient levels (Stephenson et al., 1994) in these porridges. Numerous studies have described the use of amylase-rich flours (ARF) produced by germinating the cereal grains as an alternate method of liquefying thick porridges without addition of water (Atwell et al., 1988; Ones, 1991; Gibson et. al, 1998; Mensah and Tomkins, 2003). The reduced stomach size (30-40 ml/kg of body weight) of infants may prevent them from meeting their energy requirements if they are eating a low-energy diet (Elizabeth and Vince, 2008) and therefore, Stephenson et al. (1994) rationalized a maximum of four feedings per day by mothers based on their limited time to accommodate feeding schedules along with other routine household work. However, in the work by Svanberg et al. (1988), it was found that there was no benefit to infants aged 5-12 months who were fed thick porridges added with small quantities of germinated sorghum flour (ARF source) to liquefy it. Hence, the study by Stephenson et al. (1994) concluded that thicker porridge resulted in 35% mean energy intake when compared to thin low density porridge even though it took longer feeding time by mothers. They suggested addition of oil and peanut butter to better supplement energy density of 1 kcal/g to compensate thinner gruels. Black et al. (2009) found that a concentration of 20% of dry meal in water was required to achieve the required energy density of 0.8 kcal/g recommended for complementary feeding programs but at this concentration the gruels were excessively thick for infant consumption. They suggested different cooking methods and cooking times to produce porridges with potentially lower viscosities while maintaining their

caloric densities. In the field trials conducted by Rowe et al. (2008) in Uganda, Malawi and Gautemala, they found the average dry meal concentration of 14% produced spoonable/drinkable porridge gruel.

In the current study, it can be observed from Table 5.4 that all SSB formulations had energy density in the range of 394.36 – 413.63kcal/100g from 20% concentration based on the FAQR (2011) recommendations which is comparatively higher than previous FBFs used in feeding programs. The protein requirement as specified in the 2008 CSB13 commodity requirement document, a minimum of 16.7% protein per 100 grams of product is to be used in Title II programs. Protein efficiency ratio (PER) is another way to evaluate the protein requirements for infants. When expressed at a daily energy need, P/E ratio is 6% for infants (6-24 months) with normal growth and between 6.9 - 8.9% for infants with stunted growth in the same category (World Health Organization, 2007). Additionally, protein quality is evaluated by PDCASS (Protein Digestibility Corrected Amino Acid Score) based on both amino acid requirements of humans and their ability to digest it. The US Title II CSB provides P/E ratio of 17.4% with a PDCASS of 0.81 and a digestibility factor of 85% which is more than adequate for all consumers (Fleige et al. 2010 a). However, they cautioned that FBFs are intended to provide only 25% of the dietary requirements while the rest of the calories were assumed to be provided from rest of the diet. Considering dilution effects of remaining 75% of daily diets due to sharing within the family, the efficacy of FBFs is greatly reduced. In view of this, Webb et al. (2011) in their recommendation to improve the quality of FBFs, that the new CSB 14 with addition of WPC 80 would provide a PDCASS of 0.88 and protein content of 17.7g per 100g of FBF. They further added that when this would be consumed with the recommended amount of oil, the P/E ratio of

CSB 14 would be 11%, in line with the recommendation that complementary foods for moderately malnourished children should have a P/E ratio of 12% (Hoppe et al., 2008).

Starch and Protein Digestibility

The in-vitro starch and protein digestibility studies was conducted on a select formulation of SSB. The formulation was selected based on the energy density of binary blend and superior stability i.e. relatively less changes in operational torque during extrusion processing. This eliminated the whole blends and low and high fat blends from this part of the study SS'B-V1 was chosen from binary blend made from commercially sourced sorghum flour.

It was observed from Table 5.5 that the RDS had a significant increase ($p<0.05$) from 10.29% to 25.69% in binary blends after extrusion as compared to raw blend. The RDS increased by 149.65% after extrusion because the maximum starch breakdown/cooking occurs inside the extruder barrel which has been discussed earlier in the DSC section. There was a 21.53% decrease in SDS which was significant ($p<0.05$) in extruded samples as compared to raw blend. Further, it was observed that there was a decrease in RDS after cooking of extruded samples whereas the SDS increased. It has been reported that wet cooking of sorghum reduces its digestibility (Duodu et al., 2003; Hamaker et al., 1986). The starch, in native state, is also referred to as slowly digestible starch because it can be digested in the small intestine, albeit slowly (Englyst, et al., 1992). Starch needs to be gelatinized for efficient hydrolysis since gelatinized starch is more susceptible to enzymatic attack (Akdogan, 1999). In vitro starch digestibility significantly improved after the raw blend was extruded. Cooking of extruded SSB reduced the RDS as compared to blend after extrusion but was significantly higher ($p<0.05$) than raw blend. SDS was further significantly higher after cooking. As the processing/cooking progressed, there was a decline in SDS and then increased and it could be either seen as in

increase in RDS or RS depending on the nature of the starch under study. In SSB, higher SDS after cooking resulted in higher RS. This could have been possibly the result of higher degree of retrogradation on wet cooking. On comparison to control samples CSB13 and CSB+, it was found that raw controls had higher RDS and SDS than SSB. The lower RS in CSB13 and CSB+ after cooking it as porridge could have been due to the lower amount retrogradation in the control samples as compared to SSB.

The protein digestibility for SSB binary blends is shown in Table 5.6. It was observed from the table that there was no significant difference in protein digestibility between the raw and extruded binary blends of SSB. The raw blend had a high digestibility of than 87.21% and the extruded blend had a digestibility of 86.69%. There was no significant difference ($p>0.05$) between the protein digestibility of raw SSB, CSB13, and CSB+. However, after cooking the digestibility of SSB decreased significantly ($p<0.05$) to 82.17%. The digestibilities of CSB13 and CSB+ increased after cooking to 88.35% and 89.22%.though the increase was non-significant. It was found that the decrease in protein digestibility of SSB after cooking was lower than that of CSB13 and CSB+ but the difference was not significant ($p>0.05$). Previous studies have shown a significant increase in protein digestibility after extrusion (James and Nwabueze, 2013; El-Hady and Habiba, 2003; Alonso et al. 2000; Balandran-Quintana et al.1998). The basic premise for the above studies and other similar findings has been that extrusion causes protein denaturation which increases its susceptibility to enzymatic hydrolysis and therefore improves digestibility (Amaya-Llanao et al., 2007). The results of SSB are not in confirmation with the above findings and could have been caused due to cross linking reactions between protein-protein, starch-protein and protein-lipid. During extrusion, however some interactions can reduce digestibility due to non-enzymatic browning reactions and formation of

cross linking reactions (Moraru and Kokini, 2003; Camire, 2001, 2000; Ledward and Tester, 1994; Areas, 1992; Lanfer-Marquez and Lajolo, 1991; Camire et al., 1990). Onwulata et al. (2003) had reported that extrusion process does not affect the overall protein percentage. Furthermore, these researchers reported that although the amount of denatured protein increased due to extrusion temperatures, this denaturation had minimal overall effect on protein digestibility.

Anti-nutritional factors

The levels of phytic acid in the raw and extruded SSB blends are presented in Table 5.7. It was found that there was a significant difference ($p < 0.05$) between phytic acid content in raw blends as well among extruded blends. The mean phytic acid content in raw and extruded blend of SSB was found to be 752.80 mg/100g and 556.6 mg/100g respectively. Thus there was a significant ($p < 0.05$) reduction of 26.06% in phytic acid levels after extrusion. A significant reduction in phytic acid and tannins after extrusion of acha/soybean flours was reported by Anuonye et al (2010). Batista et al. (2010) reported a similar level of reduction in phytic acid content (17-26%) after extrusion of common beans (phaseolus vulgaris L.). High performance liquid chromatography (HPLC) analysis of low fiber diet of bran-gluten-starch mixture after extrusion showed that some molecules of inositol hexaphosphate were hydrolysed to penta-, tetra-, and triphosphates due to thermal degradation Sandberg et al. (1987). Barroga et al. (1985) and Kataria et al. (1989) determined thermal degradation changed these molecules along with their chemical reactivity or the formation of insoluble complexes could explain about the reduction in the anti-nutrient content due to thermal processing. Thus, the heat and shear inside the extruder can be used to improve the nutritional value of cereal blends by reducing phytic acid levels.

Tannin, a phenolic derivative of flavone, that occurs as glycosides in the natural states and forms complexes with available protein (Singh et al. 2007; Nikmaram et al., 2015) and inhibit enzymes (Scalbert et al., 2000). No tannins were found in raw binary blends (Table 5.7). Individual analysis of each raw component of the binary blend also did not show any presence of tannins. Most of the US grown sorghums do not contain tannins (USAID, 2016; Awika and Rooney, 2004). Less than 2% of the sorghum grown in US have high tannins (Balota, 2012). Egounlety and Aworh (2003) reported non detection of tannins in dehulled soybeans.

Trypsin inhibitor levels were reduced significantly ($p < 0.05$) after extrusion in SSB when compared to raw blends and is presented in Table 5.7. A reduction of 19.50% in trypsin inhibitor levels was observed after extrusion. Trypsin inhibitor is heat labile and degrades significantly with extrusion processing (Kaur et al., 2015). Other studies have also found that trypsin inhibitors are thermolabile and their inhibitory activity can be reduced by an appropriate thermal treatment (Anton et al., 2008; Shimelis and Rakshit, 2007; Alonso et al., 2000). Balandran-Quintana et al. (1998) observed that extrusion cooking was one of the best processing methods for improving the protein quality of legumes.

Conclusions

The results of this study showed that raw material composition and processing conditions affect the properties of the final product. The particle size of extrudates after hammer milling was influenced by the inherent fat content in the blend. Higher fat content produced lower particle size after milling. WAI was positively correlated to higher starch content and inversely correlated to fiber content. WAI and WSI were inversely correlated. Lower peak viscosity and higher final viscosity was observed in blends containing whole sorghum flour. The energy density of the FBFs were above the USDA recommended standard of 387kcal/100g. Blends with

high fat content and whole sorghum flour had lower Bostwick flow rate as compared to low/medium fat and decorticated sorghum flour blends. Extrusion processing helped achieve a high degree of starch gelatinization of 90% and more in all the blends and also increased the starch and protein digestibility. The ant nutritional factors phytic acid, tannins and trypsin inhibitors were significantly reduced after extrusion.

Acknowledgement

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Table 5.1 Composition of FBFs

Ingredients	Amount (%)
Milled extrudates (SSB)	63.40
Sugar	15.00
WPC80	9.50, 13.00 (for high fat blends)
Oil	9.00, 5.50% (for high fat blends)
Mineral Premix	3.00
Vitamin Premix	0.10

FBFs = Fortified blended foods, WPC80 = Whey protein concentrate with 80% protein content

Table 5.2 Mean values of SME, particle size, WAI, WSI, starch gelatinization and total cook of SSB formulations

Formulation	SME (kJ/kg)	Particle size (µm)	WAI (g/g)	WSI (%)	Starch gelatinization (%)	Total cook (%)
SS'B-V1com	$273\pm28_{ab}$	$208.52\pm0.83_a$	$5.17\pm0.31_{ac}$	$7.79\pm0.99_a$	$93.62\pm3.90a$	$77.51\pm3.76a$
SS"B-V1com	$223\pm12_{aef}$	$196.93\pm4.98_a$	$5.08\pm0.26_{ac}$	$8.72\pm0.39_a$	$92.10\pm2.21a$	$71.57\pm4.91a$
WSS'B-V1com	$333\pm52_b$	$224.02\pm0.25_b$	$5.23\pm0.24_{ac}$	$7.23\pm0.48_a$	$93.06\pm2.44a$	$68.50\pm1.37a$
WSS"B-V1	$67\pm25_d$	$230.27\pm1.14_b$	$2.85\pm0.18_b$	$21.45\pm2.06_b$	$90.69\pm0.43a$	$64.46\pm1.87a$
WSSB-V1	$119\pm55_d$	$255.43\pm3.85_c$	$4.11\pm0.41_{ab}$	$6.23\pm0.62_a$	$94.47\pm1.42a$	$69.34\pm4.16a$
SS"B-V1	$163\pm42_{de}$	$388.15\pm3.22_d$	$5.85\pm0.23_c$	$6.82\pm0.88_a$	$94.06\pm0.17a$	$78.44\pm6.13a$
WSS"B-V2	$51\pm21_d$	$255.08\pm2.63_c$	$3.20\pm0.13_b$	$9.13\pm0.41_a$	$92.84\pm1.44a$	$66.82\pm4.09a$
SS"B-V2	$138\pm72_{df}$	$424.28\pm2.18_e$	$5.91\pm0.18_{cd}$	$7.30\pm0.93_a$	$96.26\pm0.31a$	$78.50\pm0.88a$
SS"B-V1 (aspi)	$278\pm65_{ab}$	$375.59\pm1.85_f$	$5.64\pm0.41_{ce}$	$18.51\pm2.84_b$	$95.08\pm0.56a$	$78.22\pm0.05a$

com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. Aspi is aspirated full fat soy. Values in the same column not sharing the same subscript are significantly different at p< 0.05, SME = Specific mechanical energy, WAI = Water absorption index, WSI = Water solubility index, 1st S = decorticated sorghum flour, 2nd S = low fat soy flour, S' = medium fat soy flour, S" = high fat soy flour, W = whole, V1 & V2 are white varieties of sorghum

Table 5.3 Mean pasting properties of SSB

Formulation	PV (cP)	Peak Time (min)	PT (°C)	Trough (cP)	Breakdown (cP)	FV (cP)	Setback (cP)
SS'B –V1com	$232.00\pm1.41_{ade}$	$4.31\pm0.09_a$	$72.55\pm1.20_a$	$108.50\pm10.61_a$	$123.50\pm9.19_a$	$156.00\pm33.94_a$	$47.50\pm23.33_{acde}$
SS"B –V1com	$257.50\pm7.78_{ade}$	$4.78\pm0.00_{ab}$	$73.40\pm0.07_a$	$151.50\pm2.12_{ab}$	$106.00\pm5.66_{ab}$	$170.00\pm7.07_{ab}$	$18.50\pm9.19_{ab}$
WSS'B–V1com	$191.50\pm10.61_{abc}$	$4.68\pm0.24_{ab}$	$72.20\pm2.90a$	$135.00\pm28.28_a$	$56.50\pm38.89_{ab}$	$177.50\pm30.41_{ab}$	$42.50\pm2.12_{acde}$
WSS"B -V1	$144.00\pm18.38_c$	$5.11\pm0.19_{bce}$	$68.37\pm4.77_a$	$127.50\pm10.61_a$	$16.50\pm7.78_b$	$260.50\pm35.36_b$	$127.50\pm31.82_c$
WSSB -V1	$277.50\pm17.68_d$	$5.48\pm0.04_c$	$70.52\pm4.00_a$	$211.50\pm20.51_b$	$66.00\pm2.83_{ab}$	$239.50\pm19.09_{ab}$	$28.00\pm1.41_{bd}$
SS"B -V1	$193.50\pm12.02_{bcef}$	$5.08\pm0.04_{bcde}$	$56.80\pm9.62_{ab}$	$150.50\pm14.85_{ab}$	$43.000\pm26.87_{ab}$	$243.50\pm16.26_{ab}$	$93.00\pm31.11_{acde}$
SS"B -V2	$233.00\pm19.80_{adf}$	$4.18\pm0.10_a$	$50.00\pm0.00_b$	$139.00\pm14.14_a$	$94.000\pm5.66_{ab}$	$194.50\pm17.68_{ab}$	$75.00\pm24.04_{acde}$
WSS"B -V2	$147.50\pm6.36_e$	$4.64\pm0.16_{ae}$	$66.85\pm2.05_{ab}$	$130.00\pm2.83_a$	$17.50\pm3.54_{bc}$	$222.50\pm17.68_{ab}$	$92.50\pm20.51_{acde}$
SS"B-V1 (aspirated)	$397.50\pm24.75_h$	$2.84\pm0.19_f$	$50.00\pm0.00_{bc}$	$125.50\pm11.31_a$	$272.50\pm36.06_d$	$153.50\pm7.78_a$	$28.50\pm3.54_{be}$

com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. PV = Peak Viscosity; PT = Pasting Temperature; FV = Final Viscosity. Values in the same column not sharing the same subscript are significantly different at $p< 0.05$, 1st S = decorticated sorghum flour, 2nd S = low fat soy flour, S' = medium fat soy flour, S" = high fat soy flour, W = whole, V1 & V2 are white varieties of sorghum, aspirated = aspirated full fat soy flour.

Table 5.4 Bostwick flow rate, protein, fat and energy density of SSB

Formulation	Bostwick Flow Rate (cm/min)	Binary Blend Protein (%)	FBF Protein (%)	Binary Blends Fat (%)	FBF Fat (%)	Binary Blend Energy density (kcal/100g)	FBF Energy density (kcal/100g)
SS'B-V1com	$17.50\pm0.71_{ae}$	18.70	19.45	3.67	12.09	372.27	413.63
SS"B-V1com	$13.50\pm0.71_{bc}$	12.93	18.60	6.17	10.45	382.75	402.93
WSS'B-V1com	$16.30\pm0.35_{a}$	18.53	19.35	3.94	12.25	371.84	413.36
WSS"B-V1	$12.80\pm0.35_{c}$	14.60	19.66	6.62	10.74	374.90	397.94
WSSB-V1	$14.00\pm0.00_{bcd}$	19.00	19.65	2.85	11.57	357.20	402.97
SS"B-V1	$15.80\pm0.35_{ad}$	13.12	18.72	5.59	10.09	373.99	397.36
WSS"B-V2	$8.30\pm0.35_{f}$	13.37	18.88	6.75	10.82	376.04	398.66
SS"B-V2	$19.00\pm0.00_{e}$	13.83	19.17	5.21	9.84	369.27	394.36
SS"B-V1 (aspi)	$21.80\pm0.35_{g}$	12.87	18.56	4.65	9.49	370.32	395.03

FBF = Fortified blended food, com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. 1st S = decorticated sorghum flour, 2nd S = low fat soy flour, S' = medium fat soy flour, S" = high fat soy flour, W = whole, V1 & V2 are white varieties of sorghum, Asp i= aspirated full fat soy flour. Values in the same column not sharing the same subscript are significantly different at p< 0.05. FAQR (2011) guidelines for FBFs: Protein ≥ 18g, Fat ≥ 9g, Energy ≥ 387 Kcal

Table 5.5 Starch Digestibility of SSB

Binary Blend	RDS (%)	SDS (%)	RS (%)
SS'B - RM	$10.29\pm0.23_{a}$	$41.48\pm1.11_{a}$	$48.23\pm0.92_{a}$
SS'B - ME	$25.69\pm0.39_{b}$	$32.55\pm0.80_{b}$	$41.76\pm1.05_{b}$
SS'B-Cooked	$18.48\pm0.17_{c}$	$36.87\pm1.03_{c}$	$44.65\pm0.95_{c}$
CSB13 RM	$18.72\pm0.18_{c}$	$54.66\pm0.07_{d}$	$26.62\pm0.14_{d}$
CSB13 Cooked	$21.42\pm0.31_{d}$	$49.79\pm0.64_{e}$	$28.79\pm0.35_{de}$
CSB+ RM	$23.28\pm0.50_{e}$	$47.17\pm0.98_{f}$	$29.55\pm0.64_{ef}$
CSB+ Cook ed	$27.37\pm0.26_{f}$	$41.46\pm0.39_{a}$	$31.17\pm0.47_{f}$

SS'B = Sorghum soy blend (commercial milled), CSB = Corn soy blend, RM = Raw material, ME = Milled extrudate, RDS = Rapidly digestible starch, SDS = Slowly digestible starch, RS = Resistant starch, Values in the same column not sharing the same subscript are significantly different at p< 0.05.

Table 5.6 Protein digestibility of SSB, CSB13, and CSB Plus

Formulation	RM-Digestibility (%)	ME-Digestibility (%)	Cooked digestibility (%)
SS'B-V1com	$87.21\pm6.60_a$	$86.69\pm1.74_a$	$82.17\pm3.61_a$
CSB13	$80.72\pm1.00_a$	N/A	$88.35\pm4.06_a$
CSB Plus	$85.80\pm0.40_a$	N/A	$89.22\pm3.96_a$

SS'B = Sorghum soy blend (commercial milled), CSB13 = Corn soy blend13, CSB Plus = Corn soy blend plus, RM = Raw material, ME = Milled extrudate, com = blend that had raw materials procured commercially, V1 = white sorghum variety

Table 5.7 Changes in phytic acid, tannins and trypsin inhibitor before and after extrusion of SSB

Formulation	Phytic Acid (mg/100g)		Tannins (mg/100mg CE)		Trypsin Inhibitor - TIA (mg/g)	
	RM	ME	RM	ME	RM	ME
SS'B-V1com	$752.80\pm0.02_a$	$556.6\pm0.03_b$	$0.00\pm0.00_a$	$0.00\pm0.00_b$	$210.60\pm14.60_a$	$169.53\pm0.93_b$
CSB Plus	884.54 ± 4.60	---------	0.00 ± 0.00	----------	55.39 ± 1.01	-----------

SS'B = Sorghum soy blend, com = blend that had raw materials procured commercially; CSB = Corn Soy Blend; RM = Raw material; ME = Milled extrudate; CE = Catechin equivalents; TIA = Trypsin inhibitor activity. Values in the same row not sharing the same subscript are significantly different at $p < 0.05$.

Figure 5.1 Image of Bostwick Consistometer

Figure 5.2 Correlation between Starch content and WAI for SSB

WAI = Water absorption index, SSB = Sorghum soy blend
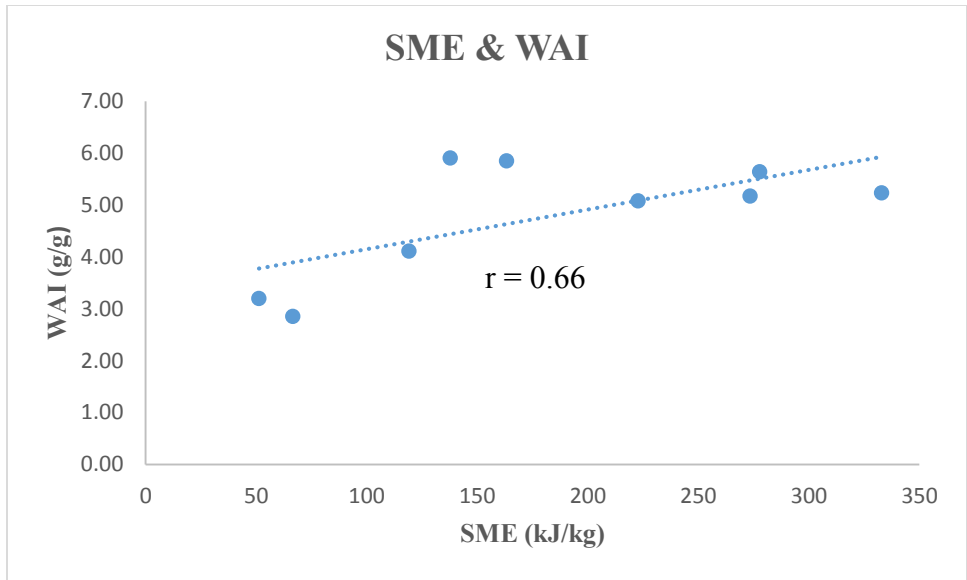
Figure 5.3 Correlation between SME and WAI for SSB

SME = Specific mechanical energy, WAI = Water absorption index, SSB = Sorghum soy blend
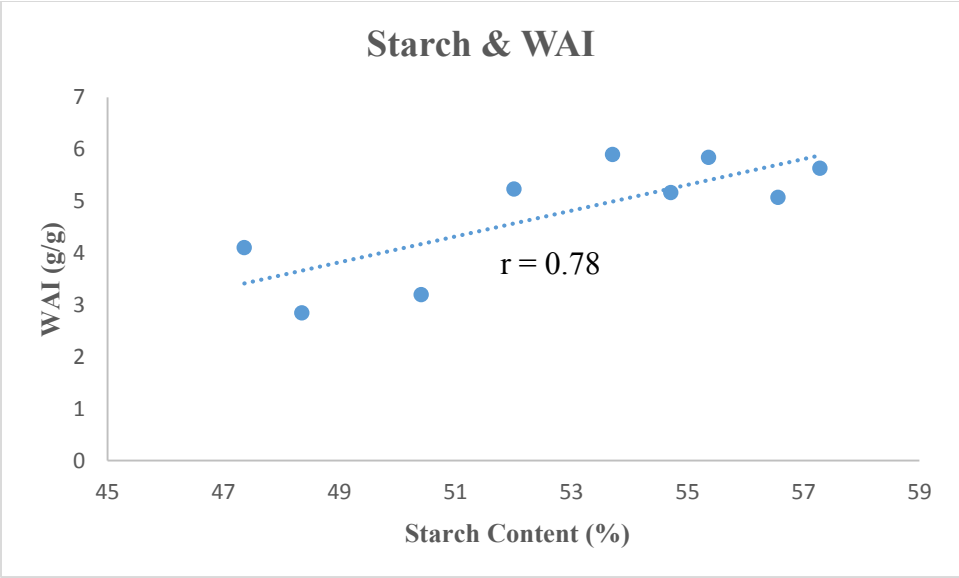

Figure 5.4 Correlation between crude fiber and WAI for SSBs
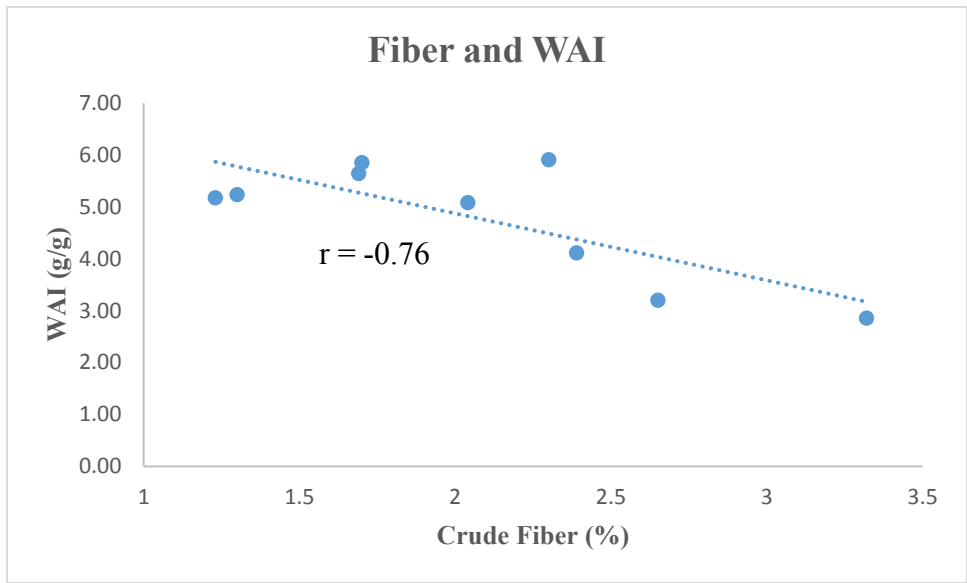
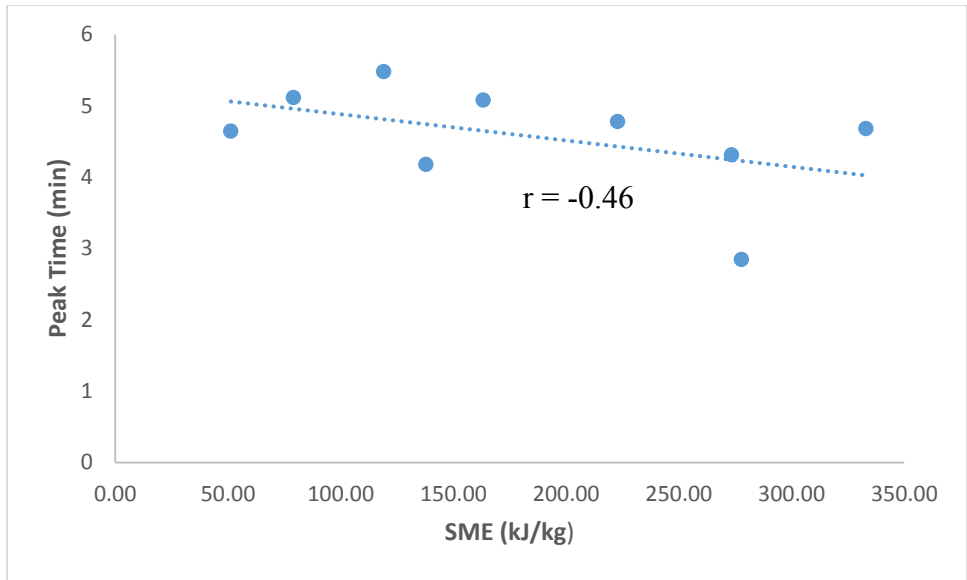WAI = Water absorption index, SSB = Sorghum soy blend

Figure 5.5 Correlation between SME and Peak Time for SSB

SME = Specific mechanical energy, SSB = Sorghum soy blend
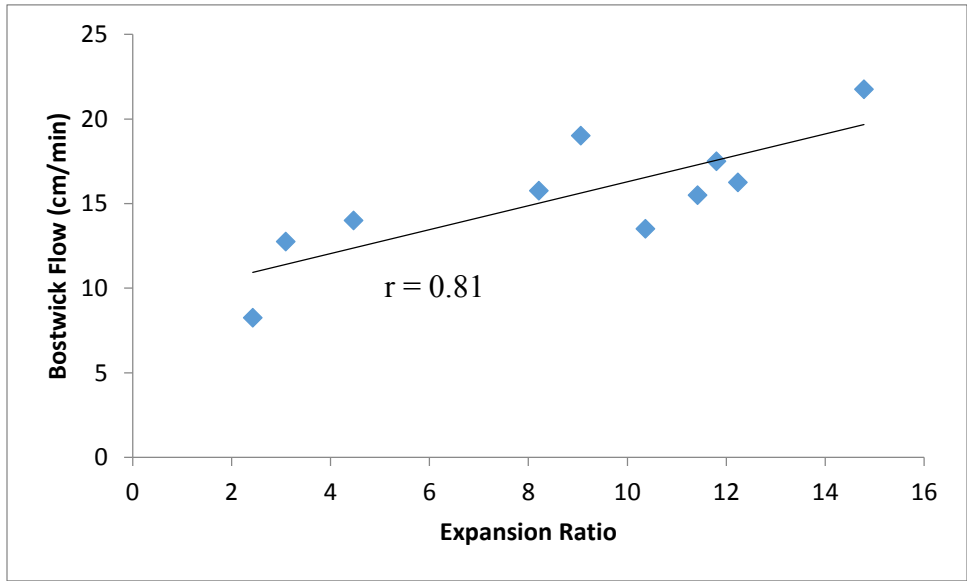
Figure 5.6 Correlation between ER and Bostwick flow rate for SSB

ER = Expansion ratio, SSB = Sorghum soy blend

Chapter 6 - Characterization of extruded Corn Soy blends to develop pre-cooked and nutritionally dense fortified blended foods.

Abstract

Expanded formulations corn soy blend (CSB) obtained from extrusion cooking were ground using hammer mill and analyzed for changes in properties that were affected by transformation of starch and protein during the processing. Macro- and micro-nutrients were added to these milled blends to prepare fortified blended foods (FBFs) that could meet the recommendations of Food Aid Quality Review (FAQR) report on energy, protein and micronutrient content. The water absorption index (WAI) ranged from 2.63-5.40 g/g and water solubility index (WSI) ranged from 7.81-24.42% and they were affected by the formulation- whole/decorticated corn and different levels of fat. Extrusion processing caused starch gelatinization in the range of 72.57 – 95.49%.The pasting properties indicated that whole grain blends of CSB had lower peak viscosity and higher peak time when compared to decorticated corn blends. The Bostwick flow rate of cooked porridges with 20% solids was within the recommended range of 9-21 cm/min. No significant difference in protein digestibility was observed amongst raw, extruded and cooked binary blends of CSB. Starch digestibility significantly increased after extrusion with a 226.81% increment in rapidly digestible starch (RDS). The protein digestibility did not vary significantly when subjected to extrusion and cooking. There was a significant reduction in anti-nutritional factors in extruded binary blend of CSB when compared to respective raw blend – phytic acid reduced by 44.03%, tannins were not found, and trypsin inhibitors reduced by 28.26%.Thus, extrusion processing of CSB with subsequent addition of macro-and micro-ingredients was effective in producing FBFs with high nutritive value.

Introduction

Malnutrition and undernutrition exists globally, particularly in emerging economies as a result of complex and widespread problem of poverty and deprivation. According to the report in The State of Food Insecurity in the World 2014 by United Nations Food and Agriculture Organization an estimated 795 million people of the 7.3 billion people in the world, or one in nine were suffering from chronic undernourishment in 2012-2014. Of these hungry people, 791 million live in developing countries making it one in eight or 13.5% of the population in those countries. There was reduction in hungry people by over 167 million since past decade and the prevalence of undernourishment has fallen from 18.7% to 11.3% globally and from 23.4% to 13.5% for developing countries (FAO et al., 2015). Even after this reduction in hunger, the least progress has been in sub-Saharan region of Africa where more than one in four people remain undernourished – the highest prevalence amongst any region of the world. . There has been a major global effort to tackle childhood malnutrition (Duggan, 2016).

Therefore, the importance of food aid in addressing certain food insecurity issues and malnourishment becomes paramount. Food aid has been used as an instrument to offset food shortages in low-income countries, where fluctuations in domestic food production threaten food security (Shapouri and Rosen, 2001). FBFs are not only 'ready-made' in the sense that they are nutritionally rich in their physical form with easy preparation methods, but usually they are dispensed through a standardized regime, involving registration, anthropometric measurement and cooking and hygiene training (World Health Organization, 2004; Sphere Project, 2011; Scott-Smith, 2015). The recipes are developed to provide a balanced intake of essential nutrients for growth and development of young children and for malnourished individuals (Hoppe et al., 2008).The United States is the leading contributor to international food aid with average supply

of 56% of the annual total food aid donated by members of the Food Aid Committee of the

International Grains Council since 1995 (Hanrahan and Canada, 2013). Food aid programs in

U.S. are administered by the United States Agency for International Development (USAID) and

United States Department of Agriculture (USDA) either as part of bilateral program or through

United Nation's World Food Program. These food aids have been distributed by US under four

authorities: (1) the Food for Peace Act (FFPA, also known as P.L. 480); (2) the Section 216(b)

program (inactive since 2007); (3) the Food for Progress Act of 1985; and (4) the McGovern-

Dole International Food for Education and Child Nutrition Program (Schnepf, 2015). Since late

1980s, the USAID administered FFPA Title II program has emerged as the largest funding

source for U.S. food aid shipments. This program provides for the donation of U.S. agricultural

commodities to support specific emergency and non-emergency food needs either by

monetization or direct food distribution. In 1996, the law was amended to permit the enrichment

and fortification of commodities to improve their nutritional quality and included high protein

blends of U.S. foods for malnourished infants, children, pregnant women, and lactating mothers.

Foods donated under P.L. 480 today include whole commodities, processed foods, fortified

processed foods, and blended food supplements.

The fortified foods routinely combine cereals and soybean meal products to increase the quality

and quantity of proteins. Wheat, corn, sorghum, rice and soy are often processed, fortified or

enriched into products such as corn soy blend (CSB), wheat soy blend (WSB), fortified wheat

flour, fortified cornmeal and vitamin-A fortified vegetable oil. CSB is often used to treat

moderate malnutrition and micronutrient deficiencies in underweight children. CSB is the most

commonly programmed specialized product in supplementary feeding programs (GAO, 2011). In

the early 1970s when there was shortage of nonfat dried milk which was used regularly in corn

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soy milk (CSM) till then was reformulated to CSB. The ineffectiveness of CSB, which is classified as ready-to-use supplementary food in addressing moderate acute malnutrition due to inadequate compositional profile of energy density, micronutrients, lipids etc., CSB, has developed expeditiously with different variants such as CSB 10, 11, 12, 13 and CSB Plus between 2005 and 2011 with enhancements to the earlier shortcomings. In Title II programming, the fortified blends account for 44% of the commodity cost though it constitutes only 20% of the volume (FAQR, 2011). In Malawi, CSB based porridge is promoted both as a complementary food for primary prevention of undernutrition as well as supplemental feeding for moderately undernourished (Thakwalakwa et. al. 2010). Ideally, the ingredients for low-cost weaning formulations must be derived from dietary staples from the region of interest that are affordable to the section of the target population and readily available in sufficient quantity (Mensa-Wilmot et al., 2001). The ingredients (cereals and legumes) are heat treated before milling to improve digestibility and to reduce levels of antinutrients and cooking times. Heat treatment during processing has also shown encouraging results in lowering phytates that increase iron solubility by forming iron complexes in naturally occurring plant phytates (Sandberg et al. 1989). However, the bioavailability of tannins (procyanidins and catechins) is questionable due to their larger molecular size and tendency to bind food molecules into insoluble complexes making it difficult for human digestion. Another anti-nutritional factor is phytic acid (inositolhexakisphosphate (IP6)) in cereal-legume based complimentary foods that inhibits iron absorption from porridges leading to a high prevalence of iron deficiency in infants. (Cook et al., 1997). Studies show that dephytinization through processing in low tannin sorghum increased iron absorption by 2-folds in sorghum reconstituted with water (Hurrell et al., 2003). Extrusion has been successfully used in production of low-cost cereal based weaning foods (Mustakas et

al., 1964; Harper and Jansen, 1985). The processing of cereal-legume blends using extrusion has been used to completely gelatinize starch at 150-170°C extrusion temperatures with moisture range of 16-22% (Rabe et al. 1980), and denature proteins enabling a nutritious precooked blended product. When processed under ideal conditions using extrusion technology, catechins and procyanidins in tannin showed improved bioavailability of up to 50% in diets (Gu et al. 2008) and possible breakdown of high molecular weight polymers of procyanidins making is easier for human absorption thereby improving nutraceutical value of sorghum (Awika et al. 2003). Extrusion heat treatment and shear forces further inactivates trypsin inhibitors by 90% in extrudates (Nwabueze., 2007) thus retaining most of the chemically available lysine in soy flour when extruded at 100 to 115°C with 12 to 18% barrel moisture (Konstance et al., 1998).

The objective of this study was to develop CSB that conforms to the most recent recommendations from Food Aid Quality Review (FAQR) and understand the effects of extrusion processing on starch and protein of extruded CSB in comparison with traditional CSB13 and CSB Plus. Specific processing to product relationships were established which included post-milling particle size, viscosity profiles and their effects on Bostwick flow rate which related to gruel consistency before infant consumption, starch and protein digestibility and anti-nutritional factors.

Some of the key recommendations to better supplement nutritional targets along with optimal breastfeeding combined with infant feeding practices, were to:

1) Increase in quantity of protein and addition of animal protein namely WPC-80 (whey protein concentrate), so as to increase the Protein Digestibility Corrected Amino Acid Score (PDCAAS) to 0.88 (a score > 0.80 is considered good quality of protein),

2) Increase in the caloric content of FBFs by addition of oil to post extruded binary blend.

3) Upgrades to the micronutrient composition.

Materials and Methods

Materials

The materials used are described – 1) extruded and milled binary blends of CSB, 2) sugar (C&H brand granulated white cane sugar from local store, Manhattan, Kansas, USA), 3) whey protein concentrate -WPC80 (Davisco Foods International, Inc., Eden Prarie, Minnesota, USA), 4) vitamins and minerals (Research Products Company, Salina, Kansas, USA) and non-gmo soybean oil (Zeeland Farm Services, Inc., Zeeland, Michigan, USA).

Hammer milling

The cooled extrudates of binary blends were conveyed through bucket elevators and collected in large plastic bags. These bags of extrudates were then fed to the inlet of Schutte Buffalo Hammer mill (Buffalo, NY, USA) fitted with 3/64 inch (1190 μm) screen. The powdered extrudates were then collected directly into 50 lb 3-walled paper bags and sealed till further use.

Particle size analysis

Laser diffraction particle size analyzer (LSTM 13320, Beckman-Coulter, Inc., Miami, Florida, USA) was used to test the particle size of milled extrudates. Each sample was tested in duplicate.

Specific Mechanical Energy (SME)

The SME for each treatment was calculated as follows:

$$SME = \frac{\frac{(\tau - \tau_O)}{100} \times P_{rated} \times \frac{N}{N_{rated}}}{\dot{m}}$$ (1)

where,

τ = operating torque (%); τ_0 = no-load torque (%); P_{rated} = rated power (37.3 kW), N = screw

speed (rpm); N_{rated} = rated screw speed (507 rpm), and ṁ = net mass flow rate of extrudate at die

exit (kg/s).

Protein and Fat Analysis

The proximate composition of raw ingredients was analyzed the following methods:

determination of moisture (135°C for 2h; AACC 44-19), crude protein based on nitrogen by

combustion, (6.25X; AOAC 920.176), crude fat (petroleum ether extract method; AOCS Ba 3-

38). Protein, and fat, were reported on as is basis from replicates.

Mixing protocol for addition of macro- and micro-ingredients

In order to increase the energy density of FBF based porridges, Webb et al. (2011) recommended

to increase the solids content in porridges from existing 11.75% (USDA, 2008) to 20% but the

porridge became thicker and was difficult to flow. The increased concentration of porridges

caused the Bostwick flow rate of these gruels to be below the recommended flow rate of 9-

21cm/min. In order to meet the Bostwick flow rate requirements, 15% sugar was added after

removing an equal quantity of the binary blend. Sugar has a plasticizing and viscosity reducing

effect and that helped in increasing the Bostwick flow rate to the prescribed standards. It has

been reported that sugar increases to the energy density FBFs with minimum increase of volume.

(de Pee and Bloem, 2009).

The mixing was done in steps of decreased dilution of ingredients as the steps progressed to

ensure the uniformity of mixing. All the dry ingredients – sugar, WPC80, minerals and vitamins

were weighed separately to make a batch of 25 kg fortified blended food (FBF). The minerals

and vitamins were mixed first in a small Hobart mixer (Model N-50, Hobart Corporation, Troy,

Ohio, USA) for 1 minute till no yellow concentrates of vitamin were present. This vitamin -

mineral blend was then transferred into the bowl of another Hobart mixer (Model A-200, Hobart Corporation, Troy, Ohio, USA) and 3.33 kg of milled and extruded binary blend was added to it and mixed for 3 minutes to get a uniform dilution of vitamin - mineral mix with a part of the binary blend.

The above premix was again transferred slowly to the bowl of a larger Hobart mixer (Model M802, Hobart Corporation, Troy, Ohio, USA). Then 10 kg from the remaining milled binary blend was added to it and mixed for 5 minutes on speed setting of 1. After 5 minutes of mixing the remaining dry ingredients – milled binary blends, sugar and WPC80 were added to it and mixed for another 5 minutes on the same speed setting of 1. Once the mixing was over, 14.42 kg of the dry blend was removed from the bowl of the mixer. To the remaining 8.33 kg of dry blend remaining in the mixer, 2.25 kg of oil (for non-full fat soy formulations) and 1.375 kg of oil (for full fat soy formulations) was added and mixed for 5 minutes with 2 minutes on speed setting of 1 and 3 minutes on speed setting of 2. On completion of this step the previously removed dry blend was added back to the mixer and mixed for another 5 minutes on speed setting of 1. At the end of this step the fortified blended food was ready and is shown in Table 6.1

Bostwick Consistometer Test

Bostwick Consistometer (CSC Scientific Company, Inc., Fairfax, Virginia, USA) was used for this test. This instrument is used to measure the viscosity/consistency of viscous products. The final FBF after making a gruel/porridge with the milled extrudates and other macro- and micro-ingredients is a viscous product and therefore the consistometer is apt to conduct the flow test. The Bostwick consistometer has a long trough with 0.5 cm graduations engraved on the base. The trough is separated at one end by a spring-loaded gate which is called the reservoir. This gate forms a chamber where the sample is loaded (Fig. 6.1). The consistometer unit was placed

on a flat surface and levelled by adjusting the screws provided at the end having the reservoir. Level adjustment was stopped when the levelling bubble provided at the other end of the unit was at the center. 40 g of FBF was added to 160 ml distilled water in a beaker to make gruel with 20% solids. The solids were added to boiling water and stirred vigoursly with a fork for 1 minute and then it was removed from heat and stirred for another 30 seconds. This gruel was then covered with an aluminum foil and placed in water bath maintained at 30°C for 10 minutes. The gruel was adjusted for water loss through evaporation at the end of 10 minutes of cooling to the initial weight of 200g by addition of distilled water. It was again stirred and covered with aluminum foil and placed in water bath at 30°C for 1 hour. At the end of the cooling period, the water loss due to evaporation was again made up to initial weight of 200g. The gruel was poured into the reservoir of the consistometer until it overflowed and it was levelled with flat plastic spatula. After allowing the gruel to settle for 30 s, the gate of the reservoir was opened and the gruel was allowed to flow along the graduated trough. The distance of flow was recorded exactly after 1 minute of opening the reservoir gate and if the gruel was between two graduations after the end of 1 minute then the higher graduation was recorded as the distance (USDA 2005). All the samples were measured in duplicate.

Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI and WSI were assessed on samples of binary blends before and after extrusion process based on the method of Anderson et al. (1970). A 2.5 g sample was dispersed in 25 g distilled water, using a glass rod to break any lumps. After stirring for 30 minutes, the dispersions were rinsed into tared centrifuge tubes and made up to 32.5g and centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted for its solid content (WSI) after evaporation of water

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from the supernatant. The sediment was weighed for its solid content and was used to report the

WAI. The indices were calculated as:

$$WAI\ (\%) = \frac{Weight\ of\ sediment}{Weight\ of\ dry\ solids} \times 100$$

$$WSI\ (\%) = \frac{Weight\ of\ dissolved\ solids\ in\ supernatant}{Weight\ of\ dry\ solids} \times 100$$

Thermal Analysis - Differential Scanning Calorimetry (DSC)

Calorimetric measurements were carried out for different raw and extruded binary blends to understand the physical transformation of starch and proteins known as starch gelatinization and protein denaturation respectively on Q100 DSC (TA Instruments, New Castle, DE, USA). 8-10 mg of sample was weighed into large volume stainless steel DSC pans (Part no.03190029, Perkin Elmer Health Sciences Inc., Shelton, CT, USA). Distilled water was added to the sample in the pan so as to obtain a solid to water ratio of 1:2 (Stevens and Elton, 1971; Zhu et al., 2010). The pans were hermetically sealed and the samples were allowed to equilibrate overnight. The instrument was calibrated using indium as reference material. An empty sealed pan was used as reference for all experiments. The program steps used for the test was as follows: Equilibrate at 10°C, heating the pans from 10°C to 140°C at the rate of 10°C/min, mark end of cycle, cooling down the sample from 140°C to 10°C at the rate of 25°C/min, mark the end of cycle with nitrogen gas flow rate of 50mL/min. The samples were again rescanned with heating from 10°C to 140°C at the rate of 10°C as the final phase of the test.

DSC data for each gelatinization and denaturation endotherm was analyzed for transition temperatures, onset (To), peak (Tp), and endpoint or conclusion (Tc) and the enthalpy (ΔH) using TA Instrument's Universal Analysis Software (version 5.4.0). All the reported data were means of two replicates.

Starch gelatinization (%) was calculated as the ratio of enthalpic transition difference in starches between raw and extruded binary blends and is represented as:

Starch gelatinization (%) = [(ΔHraw − ΔHextruded)/ΔHraw] x 100

Where, ΔHraw = enthalpy of raw binary blend, ΔHextruded = enthalpy of extruded binary blend

Total cook (%) was calculated as a ratio of the total enthalpic transistion difference which includes the transition enthalpies for starch and protein fractions of the binary blends. It is represented as below:

Total cook (%) = [(ΔH$_T$raw − ΔH$_T$extruded)/ΔH$_T$raw] x 100

Where, ΔH$_T$raw = Total enthalpy of transition of raw binary blend, ΔH$_T$extruded = Total enthalpy of transition of extruded binary blend

Starch Digestibility

Raw and extruded and milled samples of binary blends were measured by a modified *in vitro* Englyst method (Englyst et al, 1992). Briefly, samples of blends (0.6 g) and guar gum (50 mg) were placed in a centrifuge tube (45 mL). Freshly prepared pepsin solution (50 mg pepsin in 10 mL 0.01M HCl) was added to the tube, and the mixture was incubated for 2 hours at 37°C. Sodium acetate solution (0.25M) was added to the mixture to stop the digestion. Pancreatic/amyloglucosidase mixture (5 mL) and glass beads were added to the sample tube for starch digestion. The tube was incubated in a shaking water bath at 37°C and 90 strokes/min. At 20 and 120 min, an aliquot of 0.25 mL sample was pipetted into 10 ml of 66% ethanol. The glucose released at each interval was determined using glucose oxidase/peroxidase method and was converted to percentage of starch hydrolyzed by multiplying by 0.9. Starch digested at 20 min is defined as RDS, starch digested between 20 and 120 min is defined as SDS, and starch not digested after 120 min incubation is defined as RS.

Rapid Visco Analyzer (RVA)

The RVA provides an index of how cooked a sample is by re-cooking under relatively low shear in excess water and measuring the pasting viscosity throughout the test. Pasting properties of the binary blends were examined using RVA. (RVA 4, Newport Scientific Pvt. Ltd.,Warriewood, NSW, Australia). The RVA was interfaced with a computer equipped with the software – Thermocline for Windows (version 3.15.2.298) for controlling the test and analyzing the results. Pasting properties were determined after running the samples on standard AACC profile (AACC 76-21.01, 1999) with a run time of 13 minutes. Peak viscosity (PV), pasting temperature (PT), trough or holding paste viscosity (HPV), breakdown (PV- HPV), final viscosity (FV) and setback (FV – HPV) were recorded. All measurements were performed in duplicate.

Protein Digestibility

Protein digestibility binary blends before and after extrusion was measured using a protein digestibility assay modified from (Mertz et al., 1984). Flour samples and extruded and milled samples (200mg/sample) were weighed and placed in 50ml centrifuge tubes. Each sample was incubated with 35ml pepsin solution (1.5 mg pepsin in 1ml of 0.1 M phosphate buffer pH2.0) at 37°C. After two hours of incubation, 2ml of 2M sodium hydroxide was added to each tube to stop the digestion. All tubes were centrifuged at 3320g for 15 minutes at 4°C and the supernatant was discarded. The residue was washed in 10 ml of 0.1 M phosphate buffer pH 2.0, centrifuged, and supernatant was discarded. The washing steps were repeated one more time, and samples were frozen (-80°C for 30 minutes) and lyophilized. Freeze-dried samples were tested using nitrogen combustion (LECO system) to analyze the amount of undigested protein. Digested protein was calculated based on protein content of the native sorghum flour and that of the undigested fraction.

% Digestibility = {[Total N (mg) – Undigested N (mg)]/Total N (mg)}*100

 Phytic Acid

Phytic acid (InsP6) was measured using Megazyme kit (Megazyme International, Ireland) for phytic acid and total phosphorous in which phytic acid is measured as phosphorous released by phytase and alkaline phosphatase. One gram of powdered sample was and transferred into 75 ml glass beaker containing 20 ml of 0.66 M hydrochloric acid. The beaker was covered with aluminum foil and stirred vigorously overnight at room temperature. 1 ml of extract was transferred to 1.5 ml microcentrifuge tube and centrifuged at 13000g for 10 minutes. 0.5 ml of the resulting extract was immediatedly transferred to fresh 1.5 ml microcentrifuge tube and neutralized by addition of 0.5 ml of 0.75M of sodium hydroxide solution. Neutralized sample extract solution was used in the enzymatic dephosphorylation reaction procedure. Total and free phosphorous contents were measured for each sample. ΔA phosphorous was calculated for each sample by subtracting the absorbance of 'free phosphorous' sample from that of the absorbance of 'total phosphorous' sample. The phosphorous concentration is expressed as g/100g of sample using the following calculation:

Phosphorous (g/100g) = [ΔA phosphorous x 10,000 (conversion from μg g^{-1} to g/100g) x 1.0 (weight of the original sample) x 1.0 (sample volume used in colorimetric step)] / [mean M (mean value of phosphorous standard) x 20 (original sample extract volume) x 55.6 (dilution factor).

Mean value of phosphorous standard was obtained using a standard curve over a dynamic range of 0-0.75 μg phosphorous. Phytic acid concentration was calculated as follows:

Phytic acid (g/100g) = Phosphorous (g/100g)/0.282. The calculation of phytic acid content was based on the assumption that the amount of phosphorous measured is exclusively released from phytic acid and that this comprises 28.2% of phytic acid.

Tannins

Vanillin-HCl method (Price et al., 1978) was used to measure the tannins. The test samples were ground and 0.3 g of it was taken for assessing the tannins. The ground samples were transferred into 15 ml tubes. The extraction of tannins was performed by adding 8 ml of 1% HCl in methanol at 1 minute intervals to the sample tubes. The samples in the tube with 1% HCl in Methanol was vortexed for 10 s and then placed in a water bath maintained at 30°C for exactly 20 minutes. The sample tubes were removed from the water bath after 20 minutes and vortexed again. The extracts were then centrifuged at 2000 rpm for 4 minutes. Two 1 ml aliquots were taken from each tube and transferred into two separate15 ml tubes. One tube was marked for sample determination and the other tube was marked for blank determination. The sample tubes after brief vortexing were placed in water bath maintained at 30°C after adding 5 ml of vanillin reagent to it at 1 minute intervals. 1% vanillin in methanol was added to 8% HCl in methanol in 1:1 ratio to prepare the vanillin reagent. The blank tubes were added with 5 ml of 4% HCl in methanol and after brief vortexing was placed in the water bath at 30°C. After completion of 20 minutes the absorbance of samples and blanks were read using spectrophotometer at 500 nm. The spectrophotometer was zeroed using methanol blank before measuring. The difference between sample and blank was used for final determination of phenol content and expressed as catechin equivalents (CE)/mg of sample. A standard curve using 1000 ppm of catechin standard at 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml added with 1% HCl in methanol to make volume to 1 ml and 5 ml vanillin reagent was plotted. The CE equivalent was calculated as below:

CE (mg/mg of sample) = (y/m) / sample concentration (mg/ml),

Where, y and m (slope) can be calculated from the regression equation of the standard curve.

Trypsin inhibitor

Trypsin inhibitor activity was determined, according to the method described by Smith et al. (1980) using BAPNA (benzoyl-DL-arginine-p-nitroanilide) as substrate (0.92 mM in 0.05 M Tris buffer/0.02 M CaCl2, pH 8.2). The sample was fine ground and 1 gm of the ground sample was used for extraction of trypsin in 50 ml of 0.01 N NaOH for 3 h. The pH was maintained within the range 8.5 to 9.0. One ml of the extract and 2 ml of trypsin solution 0.002% (Type I, of bovine pancreas, SIGMA) in 0.001M HCl were mixed with 1 ml of water. The reaction started after addition of 5 ml of substrate at 37 °C. After 10 min, the reaction was stopped by the addition of 1 ml of 30% acetic acid. The reaction mixture was filtered through filter paper (Whatman No. 3) and the absorbance due to release of p-nitroaniline was read at 410 nm. The activity was interpreted as the increment of 0.01 units of absorbance at 410 nm for 10 ml of reaction mixture. Trypsin inhibitor activity is expressed in terms of mg trypsin inhibited per g of dry sample.

Statistical Analysis

All the results were analyzed using analysis of variance (ANOVA) with general linear model procedure (SAS version 9.1, SAS Institute, Cary, North Carolina, USA). When significant effects ($p < 0.05$) were indicated by ANOVA, Tukey pairwise comparisons were done to identify which treatments differed significantly ($p < 0.05$).

Results and discussion

Particle Size of Extruded and Milled Corn Soy Blend

The mean particle size of extruded binary blends of CSBs ranged from 186.54 µm to 248.58 µm (Table 6.2) with the least being for WC'S"B and highest being for CS"B respectively from pilot milled binary blended flours. In extruded blends from commercial flour, it was observed that the whole flour blend (WCS'B) had a significantly lower ($p<0.05$) particle size as compared to decorticated blend CS'B. Therefore, it could be seen that whole grain flour blends had lower particle size than decorticated blends. This could be attributed to the effects of extrusion processing on these blends. The whole grain blends had lower SME due to the presence of higher inherent fat content in the whole blends. Low SME caused lower expansion which resulted in extrudates with higher bulk density when compared to decorticated blends. On feeding this high density extrudates through hammer mill, the impact of the hammers might have been more as compared to more expanded and low density decorticated blend extrudates which would have been comminuted far easily than dense extrudates. Higher impact thus caused the whole blends to be broken into smaller particles. On comparing the particle sizes of milled extrudates produced from coarse or fine raw flours, it was observed that there was a significant difference ($p<0.05$) between them and extrudates from coarse flour (decorticated or whole) had lower particle size as compared to extrudates from fine flour. The out of extruder (OE) bulk density of extrudates from coarser flour was higher and thus on milling had greater impact of hammer mill on them and thus produced lower particle size. There was an inverse linear correlation ($r = -0.68$) between OE-BD and particle size (Figure 6.2). The blend made from aspirated soy (C'S"B) had a particle size similar to that of blends made from decorticated fine flour even though it had decorticated coarse corn in the blend probably because of its lower inherent fat content as

compared to high fat soy and that helped in this blend having a similar SME as that of decorticated fine blends. Thus, it could be inferred that particle size of extrudates when subjected to hammer milling was dependent on the OE-BD of the extrudates to an extent. Inspite of differences in particle sizes of different blends, all the blends could meet the particle size specifications of USDA commodity requirements (2014) for CSB Plus which is currently being distributed in food aid programs.

Water Absorption Index and Water Solubility Index

It was observed from Table 6.2 that the WAI for CSBs ranged from 2.63 g/g to 5.40 g/g for pilot milled blend C'S''B (aspirated) and CS'B (commercial milled) respectively. Again, it can be observed from the same Table 6.2 that WSI ranged from 7.81% in WCS'B (commercial milled) to 24.42% in pilot milled blend C'S''B (aspirated). Both WAI & WSI were found to be non-significant (P>0.05) amongst all blends. The pilot milled blends showed a negative linear correlation between WAI and WSI (r = -0.71). The WAI is an estimator of amount of water absorbed by starch and can be an indicator of starch gelatinization as it is known that disrupted starch granules bind more water (Ding et al., 2006). Particle size of milled extrudate blends had a positive linear correlation with WAI (r = 0.76) and that indicated higher degradation of starch granules in bigger sized particles. As discussed in previous section, larger particle sized extrudates resulted from blends that had higher expansion (low OE-BD) which was due to presence of higher starch content in these blends. Higher starch content leads to higher water absorption due to higher gelatinization and increase in SME showed increase in WAI. These results differ from those of Carvalho et. al. (2010) who reported that WAI values decrease with increase in particle size. On the other hand Figure 6.3 showed that WSI had a positive linear correlation with SME (r = 0.71) which was indicative that higher mechanical energy input

caused higher degradation/dextrinization of starch and thereby releasing more amount of soluble polysaccharides. Camire and Krumhar (1990) reported that apart from starch gelatinization, which results in the release of amylose and amylopectin, there could also occur dextrinization and other reactions that lead to the formation of low molecular weight compounds, influencing the WSI.

The WSI was found to be higher in decorticated blends because these blends had higher SME on account of lower inherent fat and fiber content as compared to whole blends. On comparing the fine vs. coarse blends, it was found that WSI was higher in decorticated coarser blend as compared to decorticated fine blend on account of higher SME. However, the result was opposite for whole blends with fine particle size showing higher WSI because of higher SME. C'S"B (aspirated) was different from all other blends because it had a lowest WAI (2.63 g/g) but had the highest WSI (24.42%).

Pasting Properties

The pasting properties for ground, extruded binary blends of CSB ranged from 210 – 529 cP for WCS'B (commercial) and WC'S"B (pilot milled) respectively and are given in Table 6.3. It can be observed from the table that blends containing whole grain corn flour had lower peak values compared to formulations that were constituted of decorticated flour though not significantly different. This was due to the higher amount of starch content in decorticated blends (53.55 – 59.71%) as compared to whole blends (47.96 – 49.91%). In the context of fine vs. coarse corn flour, it was observed that in decorticated blends, the peak was higher in fine blend on account of lower SME (33595 kJ/kg) as compared to non-significantly higher SME in coarser blend (370.99 kJ/kg). Both the peak values were cold paste viscosity. The cold viscosity value was indicative of the starchy fraction being converted such that the hydroxyl groups on the surface of

the sheared starch were exposed, enabling the starch to absorb much more water (Duarte et al., 2009). Bouvier and Campanella (2014) had reported that an increase in SME during extrusion cooking, led to development of lower molecular weight starch granules and that led to decreasing viscosity in starch rich solutions. However, the peak values were opposite in case of whole blends where coarse blend had higher peak value as compared to fine blend. This could be explained by higher level of starch degradation in coarser blend which is reflected by higher WSI and WAI values for this blend as compared to fine blend. It can be observed that the peak viscosity increases with peak time. The higher peak time indicates presence of more intact starch and thus more time to swell and develop the peak. Amongst all the blends, the lowest peak time was observed in C'S'B (commercial) and it was significantly different ($p<0.05$) for the same blend made from fine corn CS'B. In case of whole blends, fine flour blend (WCS"B) had lower peak time than coarse blend WCS"B. In both cases lower peak time was observed with high SME .The pasting temperature was negatively correlated with SME ($r = -0.79$) as shown in Figure 6.4. It was also observed that breakdown viscosity was higher in blends with lower peak time ($r = -087$). High levels of breakdown are likely associated with high degree of collapse of swollen starch granules (indicated by lower trough viscosity) leading to a greater release of solubilized starch (Highley et al., 2003). The higher breakdown is characterized by lower trough viscosity and they had an overall negative linear correlation ($r = -0.60$). The whole and decorticated blends as well as fine and coarse flour blends showed the same trend. The final viscosity had a negative linear correlation (Figure 6.5) with SME ($r = -0.85$) and also it correlated with breakdown viscosity ($r = -0.56$) indicating that increased breakdown viscosity lowered the final viscosity (Highley et al.,2003). The effect of SME on the final viscosity was more evident in fine vs. coarse blends as compared to decorticated vs. whole blends indicating

that raw blend particle size was impacted to a greater extent than the compositional differences due to decorticated and whole flours during extrusion and its effect on final viscosity. High levels of breakdown would promote greater reassociation between the solubilized starch granules and would show an increase in setback viscosity($r = 0.49$).

Bostwick Consistency

The Bostwick flow rates of the cooked slurry at 20% solids concentration which had sugar, WPC 80, oil, vitamins and minerals in addition to extruded binary blend is presented in Table 6.4. Overall, it was observed that SME had a positive linear correlation with Bostwick flow rate ($r = 0.75$). Higher SME promotes more dextrinization of starch and causes formation of maltodextrins which reduces the viscosity of gruels (Mouquet, 1998). WSI was also positively correlated to Bostwick flow rate ($r = 0.86$) which again confirms that higher breakdown of starch leads to higher flow rates of gruels. Bostwick flow rate had a negative linear correlation with final viscosity (Figure 6.6) observed during changes in pasting properties ($r = -0.73$). Final viscosity is dependent on the reassociation of starch molecules during the cooling phase of the RVA cycle and higher reassociation would lead higher viscosity and thus lower the Bostwick flow rate of porridges. Padmanabhan (2013) reported a similar correlation between final viscosity and Bostwick flow rate in sorghum soy blends.

It was found that the Bostwick flow rate of decorticated blend was significantly higher ($p<0.05$) than that of whole blends produced from commercial flour. As explained earlier whole blend (WCS'B) had lower SME as compared to decorticated blend (CS'B) and thus would have less dextrinized starch and thus was thicker. Another factor could be the presence of higher inherent fat in whole blend (4.73%) as compared to decorticated blend (2.45%). The overall effect of low SME and high fat caused the whole blend to have a lower WSI and thus a lower Bostwick. Also,

it might have been possible that higher lipids could have complexed more with amylose and that could have increased the viscosity of whole blends. Tang and Copeland (2006) reported that increase in fatty acids led to increase in complexation with starch in dispersions used for RVA analysis. On comparing fine vs. coarse blends, it was observed in decorticated recipes from commercial flour, coarse blend had significantly higher (p<0.05) Bostwick flow rate but the result was opposite in similar comparison of blends made from whole flour (pilot milled). In both scenarios, it was observed that Bostwick flow rate was higher in blend that had higher SME and higher WSI. However, C'S"B (aspirated) had the highest Bostwick flow rate of 24.0 cm/min amongst all blends as it had the highest WSI of 24.42%.that Inspite of the differences in flow rates between all the blends they were within the range of 9-21 cm/min as stipulated by USDA commodity requirement for corn soy blend (CSB 13) (USDA, 2008) except for CS"B which had the least flow rate of 8.50 cm/min. Analysis of all these correlations point out to the fact that Bostwick flow rate is dependent on the characteristics of starch after it has been extruded and cooked as porridge/slurry with 20% solids.

Degree of Starch gelatinization

The starch gelatinization levels of CSB formulations are summarized in Table 6.2. Extrusion caused starch gelatinization in binary blends that ranged from 72.57 – 95.49%. There was statistically significant difference (p<0.05) between decorticated blends and whole blends. The decorticated blends had higher degree of starch gelatinization as compared to whole blends. It was found that starch gelatinization had a positive linear correlation (r = 0.78) with SME. As shown in Figure 6.7. The inherent fat content in whole blends was higher than that of decorticated blends and thus it had lower SME causing it to have lower gelatinization as compared to decorticated blends. The total cook of the extrudates which accounted for cook of

starch and protein in the blends were lower in values than starch gelatinization values (Table 6.2) and they were not statistically different (p>0.05) between whole and decorticated blend or fine and coarse blend. Whole blends had higher protein content (13.25 – 18.33%) and higher fat content (4.73 – 6.69%) as compared to decorticated blends (12.01 – 18.02% protein) and 2.27 – 5.07% inherent fat. This accounted for lower energy transfer to whole blends during processing because oil provides a lubricating effect (Feng and Lee, 2014) and that would have caused the protein which requires higher energy to be less denatured than starch to cook as compared to decorticated blends. There was no statistically significant difference (p>0.05) in gelatinization values between decorticated blends as well as between whole blends. However, it was noted that blends with higher oil content (whole blends) had lower values of gelatinization. The starch content in whole blends ranged from 47.96 – 49.91% and that in decorticated blends ranged from 53.55 – 59.71%. Thus higher starch content could have contributed towards higher starch gelatinization process and also, lower inherent fat content in decorticated blends might have formed lower amylose –lipid complex during the thermal transition process and thereby increasing the gelatinization when compared to whole blends. Harper et al (1981) reported that the heat of reaction for protein ranged between 90 – 100 kJ/kg and 10-19 kJ/kg for cereal starches (Stevens and Elton, 1971).

Energy Density of FBFs

The FAQR (Webb et al., 2011) recommended energy-dense foods with good protein content and an appropriate inclusion of essential micronutrients as necessary (albeit not always sufficient) to achieve defined nutrition goals among vulnerable populations. They, thus proposed changes in the current nutritional and energy profile of FBFs (CSB or WSB) so that 100 gm of it could provide 387 kilocalories as energy, 18g protein and 9 g fat. They further suggested inclusion of

other cereal blends, use of WPC to increase the protein content and quality amongst other changes. In addition to it, the availability of staple foods must be ensured so that nutritionally enhanced foods add to it rather than replacing them as sources of energy and nutrition in the local food supply.

As a general practice most cereal-based products used for complementary feeding programs are diluted to achieve a thin drinkable consistency before being fed to infants. This has been also put forth by several nutritionists (Monte and Giugliani, 2004; Elizabeth and Vince, 2008). The porridges when diluted causes a lowering of its energy density and micronutrient levels (Stephenson et al., 1994). A number of studies have described the use of amylase-rich flours (ARF) produced by germinating the cereal grains as an alternate method of liquefying thick porridges without addition of water (Atwell et al., 1988; Ones, 1991; Gibson et. al, 1998; Mensah and Tomkins, 2003). The reduced stomach size (30-40 ml/kg of body weight) of infants may prevent them from meeting their energy requirements if they are eating a low-energy diet (Elizabeth and Vince, 2008) and therefore, Stephenson et al. (1994) suggested a maximum of four feedings per day by mothers based on their limited time to accommodate feeding schedules along with other routine household work. However, in the work by Svanberg et al. (1988), it was found that there was no benefit to infants aged 5-12 months who were fed thick porridges added with small quantities of germinated sorghum flour (ARF source) to liquefy it. Hence, the study by Stephenson et al. (1994) concluded that thicker porridge resulted in 35% mean energy intake when compared to thin low density porridge even though it took longer feeding time by mothers. They suggested addition of oil and peanut butter to better supplement energy density of 1 kcal/g to compensate thinner gruels. Black et al. (2009) found that a concentration of 20% of dry meal in water was required to achieve the required energy density of 0.8 kcal/g recommended for

complementary feeding programs but at this concentration the gruels were excessively thick for infant consumption. They suggested different cooking methods and cooking times to produce porridges with potentially lower viscosities while maintaining their caloric densities. In the field trials conducted by Rowe et al. (2008) in Uganda, Malawi and Gautemala, they found the average dry meal concentration of 14% produced spoonable/drinkable porridge gruel.

In the current study, it can be observed from Table 6.4 that all CSB formulations had energy density in the range of 392.86 – 411.79 kcal/100g from 20% concentration based on the FAQR (2011) recommendations which is comparatively higher than previous FBFs used in feeding programs. The protein requirement as specified in the 2008 CSB13 commodity requirement document, a minimum of 16.7% protein per 100 grams of product is to be used in Title II programs. Protein efficiency ratio (PER) is another way to evaluate the protein requirements for infants. When expressed at a daily energy need, P/E ratio is 6% for infants (6-24 months) with normal growth and between 6.9 - 8.9% for infants with stunted growth in the same category (World Health Organization, 2007). Additionally, protein quality is evaluated by PDCASS (Protein Digestibility Corrected Amino Acid Score) based on both amino acid requirements of humans and their ability to digest it. The US Title II CSB provides P/E ratio of 17.4% with a PDCASS of 0.81 and a digestibility factor of 85% which is more than adequate for all consumers (Fleige et al. 2010 a). However, they cautioned that FBFs are intended to provide only 25% of the dietary requirements while the rest of the calories were assumed to be provided from rest of the diet. Considering dilution effects of remaining 75% of daily diets due to sharing within the family, the efficacy of FBFs is greatly reduced. In view of this, Webb et al. (2011) in their recommendation to improve the quality of FBFs, that the new CSB 14 with addition of WPC 80 would provide a PDCASS of 0.88 and protein content of 17.7g per 100g of FBF. They further

added that when this would be consumed with the recommended amount of oil, the P/E ratio of CSB 14 would be 11%, in line with the recommendation that complementary foods for moderately malnourished children should have a P/E ratio of 12% (Hoppe et al., 2008).

Starch and Protein Digestibility

The in-vitro starch and protein digestibility studies was conducted on one select formulation of CSB. The formulation was selected based on the energy density of binary blend, Bostwick flow rate, and superior stability i.e. relatively less changes in operational torque during extrusion processing. This eliminated the whole blends as well as high fat blends from this part of the study. C'S'B was chosen from binary blend made from commercially sourced flour.

It was observed from Table 6.5 that the RDS had a significant increase (p<0.05) from 8.13% to 26.57% in binary blends after extrusion as compared to raw blend. The RDS increased by 226.93% after extrusion because the temperature and shear inside the extruder barrel breaks down and cooks the starch. There was significant increment (65.14%) in RDS after it was cooked A significant decrease (p<0.05) of 15.95% was observed in SDS after extrusion when compared to raw blend mainly due to the conversion of SDS present in raw blend being converted to RDS during extrusion processing. A further significant decrease (p<0.05) of 19.64% of SDS was observed after the binary blend was cooked. A higher SDS content indicates a lower starch gelatinization index in cereal products (*Englyst et al., 2003*). As the processing/cooking progresses, there is a steady decline in SDS and it could be seen as in increase in RDS. The starch, in native state, is also referred to as slowly digestible starch because it can be digested in the small intestine, albeit slowly (Englyst, et al., 1992). Starch needs to be gelatinized for efficient hydrolysis since gelatinized starch is more susceptible to enzymatic attack (Akdogan, 1999). In vitro starch digestibility significantly improved after the raw blend

was extruded. The resistant starch (RS) showed a significant (p<0.05) decrease from 38.17% in raw blend to 8.30% in extruded blend. Cooking of extruded CSB reduced the RS significantly (p<0.05) as compared to blend after extrusion. On comparison to control samples of CSB13 and CSB+, it was found that raw CSB13 and CSB+ had significantly higher (p<0.05) higher RDS and lower RS than C'S'B whereas SDS was similar in raw C'S'B and CSB13 and it was lower in CSB+. The higher RDS values for CSB13 and CSB+ could have been due to the raw corn and soy in them being heat treated prior to be used. On cooking, it was found that C'S"B had the least RS as compared to controls possibly due to higher conversion of starch into cooked starch due to the effect of extrusion.

The protein digestibility for CSB binary blends is shown in Table 6.6. It was observed from the table that there was no significant difference (p>0.05) in protein digestibility between the raw and extruded binary blends of CSB. The raw blend had a digestibility of than 79.53% and the extruded blend had a digestibility of 80.63%. There was no significant difference (p>0.05) between the protein digestibility of raw CSB, CSB13, and CSB+. However, after cooking the digestibility of raw C'S'B was found to be unchanged from that of extruded C'S'B at 80%. The digestibilities of CSB13 and CSB+ increased after cooking to 88.35% and 89.22%.though the increase was non-significant (p>0.05) when compared to respective raw blends. Previous studies have shown a significant increase in protein digestibility after extrusion (James and Nwabueze, 2013; El-Hady and Habiba, 2003; Alonso et al. 2000; Balandran-Quintana et. al.1998). The basic premise for the above studies and other similar findings has been that extrusion causes protein denaturation which increases its susceptibility to enzymatic hydrolysis and therefore improves digestibility (Amaya-Llanao et al., 2007). The results of CSB are not in confirmation with the above findings and could have been caused due to cross linking reactions between protein-

protein, starch-protein and protein-lipid. During extrusion, however some interactions can reduce digestibility due to non-enzymatic browning reactions and formation of cross linking reactions (Moraru and Kokini, 2003; Camire, 2001, 2000; Ledward and Tester, 1994; Areas, 1992; Lanfer-Marquez and Lajolo, 1991; Camire et al., 1990). Onwulata et al. (2003) had reported that extrusion process does not affect the overall protein percentage. Furthermore, these researchers reported that although the amount of denatured protein increased due to extrusion temperatures, this denaturation had minimal overall effect on protein digestibility.

Anti-nutritional factors

The levels of phytic acid in the raw and extruded CSB blends are presented in Table 6.7. It was found that there was a significant difference ($p<0.05$) between phytic acid content in raw blends as well among extruded blends. The mean phytic acid content in raw and extruded blend of C'S'B was found to be 567.54 mg/100g and 317.64 mg/100 g respectively. Thus there was a significant ($p<0.05$) reduction of 44.03% in phytic acid levels after extrusion. The levels of phytic acid in CSB was significantly lower ($p<0.05$) than the control, CSB Pus which had a phytic acid level of 1885 mg/100 g. CSB Plus is made from heat treated but unextruded flours and thus it had higher phytic acid levels. Several studies have reported a reduction in phytic acid content of cereals and legumes after extrusion (Kaur et al., 2015; Sahig et. al., 2015; Anuonye et al. 2010). Sandberg et al. (1987) reported that during extrusion, some molecules of inositol hexaphosphate were hydrolysed to penta-, tetra-, and triphosphates due to thermal degradation. Barroga et al. (1985) and Kataria et al. (1989) stated that changes due to thermal degradation of these molecules, as well as changes in their chemical reactivity or the formation of insoluble complexes could explain about the reduction in the anti-nutrient content due to thermal

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processing. Thus, the heat and shear inside the extruder can be used to improve the nutritional value of cereal blends by reducing phytic acid levels.

No tannins were detected in raw and extruded blends of C'S'B as shown in Table 6.7. The individual components of raw binary blend of C'S'B also did not show any tannin content. There was 23.98% reduction in tannin levels after extrusion. Tannin, a phenolic derivative of flavone, that occurs as glycosides in the natural states and forms complexes with available protein (Singh et al. 2007; Nikmaram et al., 2015) and inhibit enzymes (Scalbert et al., 2000). Most of the US grown sorghums do not contain tannins (USAID, 2016; Awika and Rooney, 2004). Less than 2% of the sorghum grown in US have high tannins (Balota, 2012). Egounlety and Aworh (2003) reported non detection of tannins in dehulled soybeans.

Trypsin inhibitor levels were reduced significantly (p<0.05) after extrusion in C'S'B when compared to raw blends and is presented in Table 6.7. A reduction of 28.26% in trypsin inhibitor levels was observed after extrusion. Trypsin inhibitor is heat labile and degrades significantly with extrusion processing (Kaur et al., 2015). The trypsin levels in CSB Plus was found to be 105.25 TIU/g which was significantly lower (p<0.05) than C'S'B. Probably, initial heat treatment of CSB Plus raw materials might have caused the reduction in trypsin inhibitor levels. Many studies have also found that trypsin inhibitors are thermolabile and their inhibitory activity can be reduced by an appropriate thermal treatment (Anton et al., 2008; Shimelis and Rakshit, 2007; Alonso et al., 2000). Balandran-Quintana et al. (1998) observed that extrusion cooking was one of the best processing methods for improving the protein quality of legumes.

Conclusions

The study showed that raw material composition affected the final properties of the product after extrusion. The amount of starch and changes to its properties during extrusion was the major

contributor affecting the product characterization. The level of inherent fat content in the blend was another factor which influenced the final product behavior. The particle size of extrudates after hammer milling was influenced by the bulk density of the extrudates. Higher bulk density extrudates produced lower particle size. WAI and WSI were dependent on amount of starch content in the blend and higher starch content led to higher SME and thus higher WAI and WSI. Lower starch content in whole blends caused them to have lower peak viscosities as comapared to decorticated blends. Higher SME caused more starch gelatinization and thus it reduced the peak time. Bostwick flow rate was also dependent on SME and whole blends had lower Bostwick flow on account of lower SME. Coarse blend had higher Bostwick flow rate as they had higher SME during processing. The energy density of the FBFs were above the USDA recommended standard of 387kcal/100g. The starch gelatinization was higher in decorticated blends (>88%) because of higher SME. There was an increase in RDS and decrease in SDS as the processing/cooking steps progressed for C'S'B. Raw CSB13 and CSB Plus had higher RDS as compared to C'S'B as they were heat treated before being used in the blend. The ant nutritional factors phytic acid, tannins and trypsin inhibitors were significantly reduced after extrusion.

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Table 6.1 Composition of FBFs

Ingredients	Amount (%)
Milled extrudates (CSB)	63.40
Sugar	15.00
WPC80	9.50, 13.00 (for high fat blends)
Oil	9.00, 5.50% (for high fat blends)
Mineral Premix	3.00
Vitamin Premix	0.10

FBF = Fortified blended food, CSB = Corn soy blend, WPC80 = Whey protein concentrate with 80% protein content

Table 6.2 Mean values of SME, particle size, WAI, WSI, starch gelatinization and total cook of CSB formulations

Formulation	SME (kJ/kg)	Particle size (µm)	WAI (g/g)	WSI (%)	Starch gelatinization (%)	Total cook (%)
CS'Bcom	$336\pm25_{ab}$	$242.15\pm1.98_a$	$5.40\pm1.01_a$	$10.80\pm1.46_a$	$95.49\pm0.29a$	$76.44\pm1.25a$
WCS'Bcom	$262\pm54_{ac}$	$204.58\pm1.83_b$	$4.17\pm0.69_a$	$7.81\pm0.93_a$	$74.91\pm3.95b$	$73.31\pm0.69ab$
C'S'Bcom	$371\pm14_b$	$204.04\pm0.88_b$	$4.67\pm0.02_a$	$23.38\pm1.53_a$	$91.58\pm1.95a$	$73.62\pm2.10ab$
CS"B	$239\pm112_c$	$248.58\pm3.80_a$	$4.52\pm0.60_a$	$8.93\pm0.40_a$	$88.64\pm0.73a$	$72.45\pm1.07ab$
WC'S"B	$139\pm39_d$	$186.54\pm1.52_c$	$3.44\pm0.63_a$	$8.51\pm1.16_a$	$76.56\pm2.60b$	$70.31\pm0.41b$
WCS"B	$203\pm18_{cde}$	$232.49\pm0.26_d$	$4.78\pm0.52_a$	$11.99\pm2.65_a$	$72.57\pm2.54b$	$69.00\pm1.41bc$
C'S"B (aspi)	$341\pm80_{ab}$	$247.30\pm1.20_a$	$2.63\pm1.35_a$	$24.42\pm13.24_a$	$91.05\pm0.80a$	$74.42\pm1.12ab$

C= degermed corn, C' = degermed coarse corn, W = whole, S' = medium fat soy, S"= high fat soy, com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. Aspi is aspirated full fat soy. Values in the same column not sharing the same subscript are significantly different at $p< 0.05$

Table 6.3 Mean pasting properties of CSB

Formulation	PV (cP)	Peak Time (min)	PT (°C)	Trough (cP)	Breakdown (cP)	FV (cP)	Setback (cP)
CS'Bcom	$310.50\pm3.54_a$	$3.98\pm0.28_a$	$50.00\pm0.00_a$	$122.00\pm14.14_{aef}$	$188.50\pm17.68_a$	$214.00\pm1.41_a$	$92.00\pm12.73_a$
WCS'Bcom	$210.00\pm4.24_{ab}$	$4.32\pm0.19_a$	$50.00\pm0.00_a$	$130.50\pm0.71_{aef}$	$79.50\pm4.95_b$	$212.50\pm7.78_a$	$82.00\pm7.07_a$
C'S'Bcom	$274.00\pm25.46_{ab}$	$1.82\pm0.05_b$	$50.00\pm0.00_a$	$75.00\pm0.00_{ab}$	$199.00\pm25.46_a$	$145.50\pm3.54_b$	$70.50\pm3.54_a$
CS"B	$323.50\pm33.23_a$	$5.25\pm0.09_c$	$71.70\pm5.73_b$	$265.50\pm33.23_c$	$58.00\pm0.00_b$	$403.00\pm4.04_c$	$137.50\pm9.19_b$
WC'S"B	$529.00\pm28.28_c$	$5.32\pm0.09_c$	$72.60\pm1.27_b$	$431.50\pm27.58_d$	$97.50\pm0.71_b$	$811.00\pm12.73_d$	$379.50\pm14.85_c$
WCS"B	$293.00\pm1.41_{ab}$	$4.38\pm0.10_a$	$58.03\pm0.74_a$	$192.00\pm4.24_{ce}$	$101.00\pm2.83_b$	$278.00\pm5.66_e$	$86.00\pm3.41_a$
C'S"B (aspi)	$462.50\pm26.16_c$	$1.35\pm0.14_b$	$50.00\pm0.00_a$	$60.00\pm11.31_{bf}$	$402.50\pm14.85_c$	$71.50\pm7.78_f$	$11.50\pm3.54_d$

C= degermed corn, C' = degermed coarse corn, W = whole, S' = medium fat soy, S"= high fat soy, com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. PV = Peak Viscosity; PT = Pasting Temperature; FV = Final Viscosity.

Values in the same column not sharing the same subscript are significantly different at $p< 0.05$

Table 6.4 Bostwick flow rate, protein, fat and energy density of CSB binary blend and FBF

Formulation	Bostwick flow rate (cm/min)	Binary Blend protein (%)	FBF Protein (%)	Binary Blend Fat (%)	FBF Fat (%)	Binary Blend Energy density (kcal/100g)	FBF Energy density (kcal/100g)
CS'Bcom	$16.00 \pm 0.00_a$	16.39	19.22	0.80	12.76	368.16	411.03
WCS'Bcom	$14.30 \pm 0.35_a$	18.33	17.99	1.61	11.31	369.36	411.79
C'S'Bcom	$19.50 \pm 0.71_b$	18.02	19.03	1.07	11.2	364.61	408.78
CS"B	$8.50 \pm 0.71_c$	19.88	19.88	1.57	9.75	373.95	397.41
WC'S"B	$11.30 \pm 0.35_d$	13.25	18.81	3.28	10.78	376.47	399.01
WCS"B	$15.00 \pm 0.00_a$	13.28	18.82	2.90	10.49	375.54	398.42
C'S"B (aspi)	$21.00 \pm 0.00_b$	12.01	18.02	1.57	9.35	366.76	392.86

FBF = Fortified Blended food, C= degermed corn, C' = degermed coarse corn, W = whole, S' = medium fat soy, S"= high fat soy, com represents the blends that had raw materials procured commercially and for other blends the raw flours were made by milling at pilot mill. . Aspi is aspirated full fat soy. Values in the same column not sharing the same subscript are significantly different at $p < 0.05$. FAQR (2011) guidelines for FBFs: Protein $\geq$ 18g, Fat $\geq$ 9g, Energy $\geq$ 387 Kcal

Table 6.5 Starch Digestibility of CSB

Binary Blend	RDS (%)	SDS (%)	RS (%)
C'S'B - RM	$8.13\pm0.33_a$	$53.70\pm0.15_a$	$38.17\pm0.47_a$
C'S'B - ME	$26.57\pm0.26_b$	$45.43\pm1.12_b$	$28.30\pm1.03_{bd}$
C'S'B-Cooked	$43.88\pm1.65_c$	$36.23\pm0.44_c$	$19.89\pm1.44_c$
CSB13 RM	$18.72\pm0.18_d$	$54.66\pm0.07_a$	$26.62\pm0.14_b$
CSB13 Cooked	$21.42\pm0.31_e$	$49.79\pm0.64_d$	$28.79\pm0.35_{bd}$
CSB+ RM	$23.28\pm0.50_e$	$47.17\pm0.98_e$	$29.55\pm0.64_{de}$
CSB+ Cooked	$27.37\pm0.26_b$	$41.46\pm0.39_f$	$31.17\pm0.47_e$

C= degermed corn, C' = degermed coarse corn, W = whole, S' = medium fat soy, CSB13 = Corn soy blend 13, CSB+ = Corn soy blend plus, RM = Raw material; ME = Milled extrudate; RDS = Rapidly digestible starch; SDS = Slowly digestible starch; RS = Resistant starch, Values in the same column not sharing the same subscript are significantly different at $p< 0.05$.

Table 6.6 Protein digestibility of CSB, CSB13, and CSB Plus

Formulation	RM-Digestibility (%)	ME-Digestibility (%)	Cooked digestibility (%)
C'S'B-com	$79.53\pm0.00_a$	$80.63\pm0.73_a$	$79.72\pm1.15_a$
CSB13	$80.72\pm5.00_a$	N/A	$88.35\pm4.06_a$
CSB Plus	$85.80\pm0.40_a$	N/A	$89.22\pm3.96_a$

C' = degermed coarse corn, S' = medium fat soy, CSB13 = Corn soy blend 13, CSB+ = Corn soy blend plus, RM = Raw material; ME = Milled extrudate. com = blends that had raw materials procured commercially, Values in the same row not sharing the same subscript are significantly different at $p< 0.05$.

Table 6.7 Changes in phytic acid, tannins and trypsin inhibitor before and after extrusion of CSB and comparison with CSB Plus

Formulation	Phytic Acid (mg/100g)		Tannins (mg/100mg CE)		Trypsin Inhibitor –TIA(mg/g)	
	RM	ME	RM	ME	RM	ME
C'S"Bcom	$567.54\pm8.52_a$	$317.64\pm2.85_b$	0.00 ± 0.00	0.00 ± 0.00	$190.21\pm4.44_a$	$136.67\pm8.33_b$
CSB Plus	884.54 ± 4.60	---------	0.00 ± 0.00	----------	55.39 ± 1.01	-----------

C' = degermed coarse corn, S' = medium fat soy, com = blends that had raw materials procured commercially, CSB+ = Corn soy blend plus, RM = Raw material; ME = Milled extrudate. Values in the same row not sharing the same subscript are significantly different at p< 0.05.

Figure 6.1 Image of Bostwick Consistometer
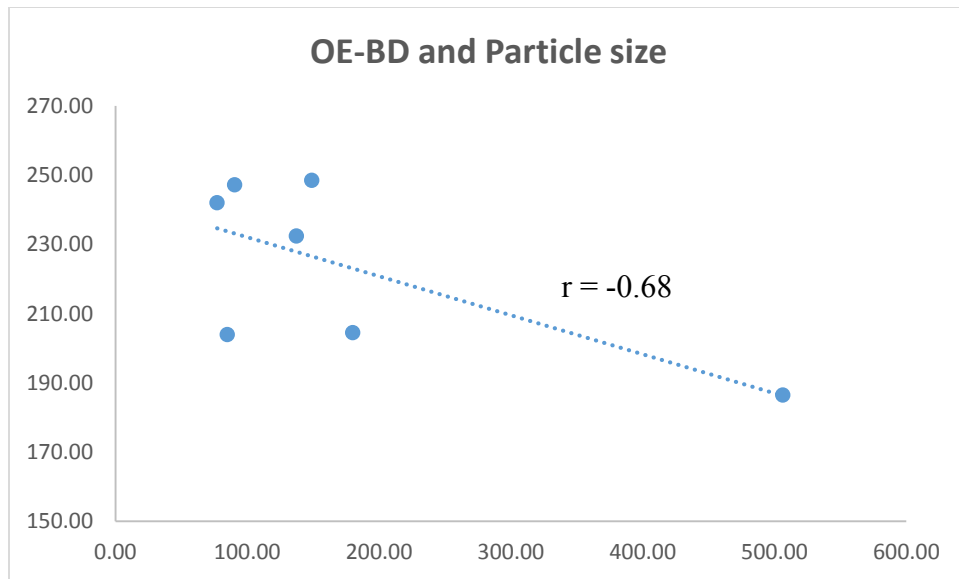
Figure 6.2 Correlation between OE-BD and particle size for CSB

OE-BD = Out of extruder bulk density, CSB = Corn soy blend
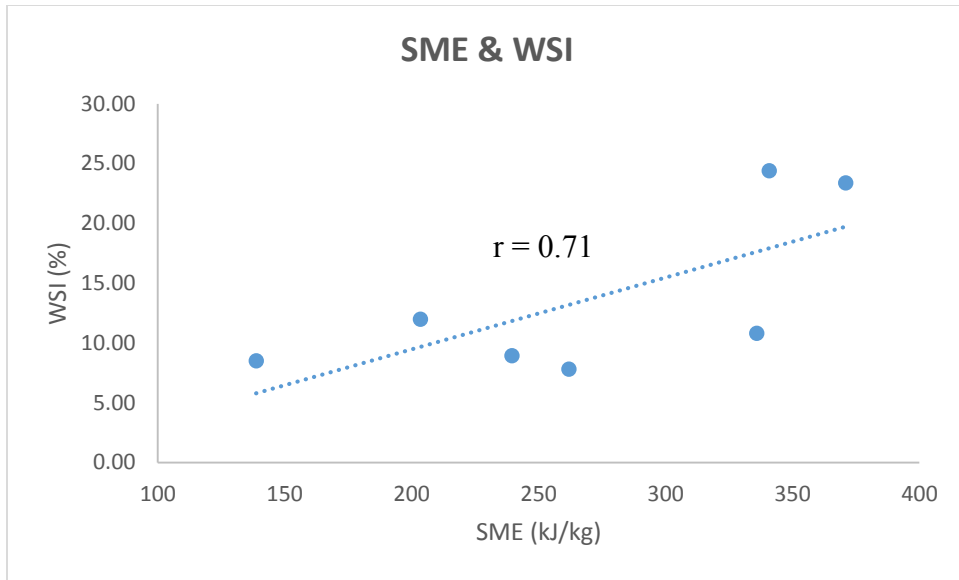
Figure 6.3 Correlation between SME and WSI for CSB

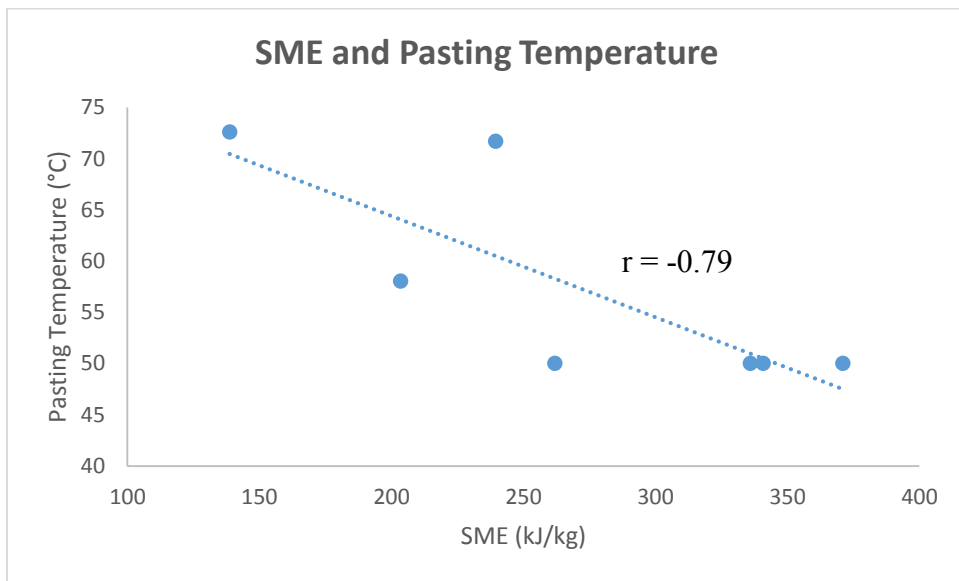SME = Specific mechanical energy, WSI – Water solubility index, CSB = Corn soy blend

Figure 6.4 Correlation between SME and Pasting Temperature for CSBs

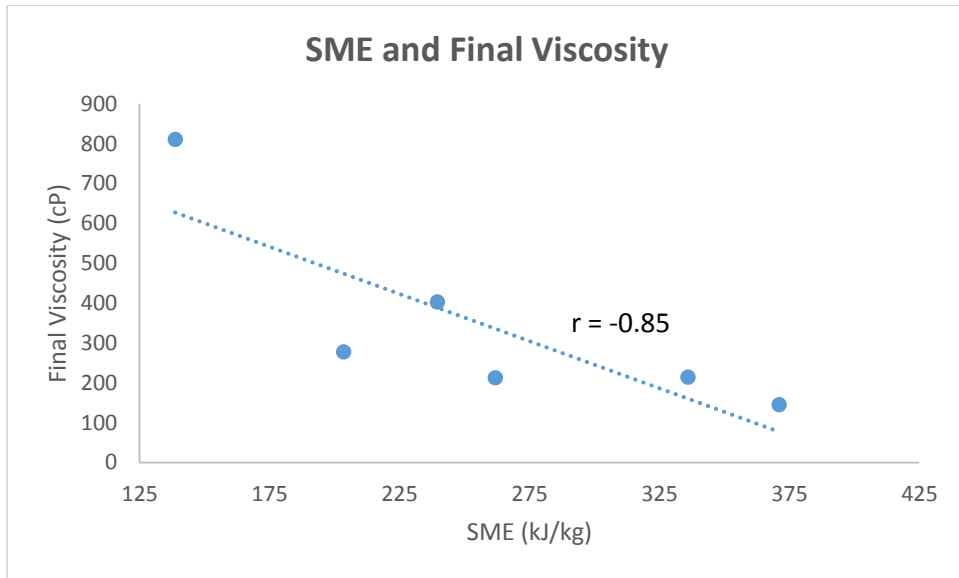SME = Specific mechanical energy, CSB = Corn soy blend

Figure 6.5 Correlation between SME and final viscosity for CSB

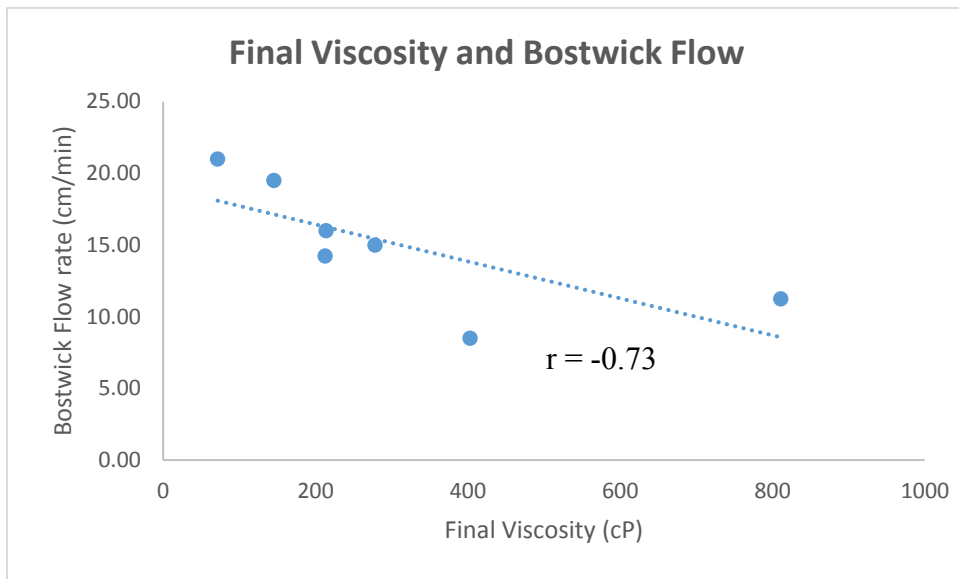SME = Specific mechanical energy, CSB = Corn soy blend

Figure 6.6 Correlation between final viscosity and Bostwick flow rate for CSB
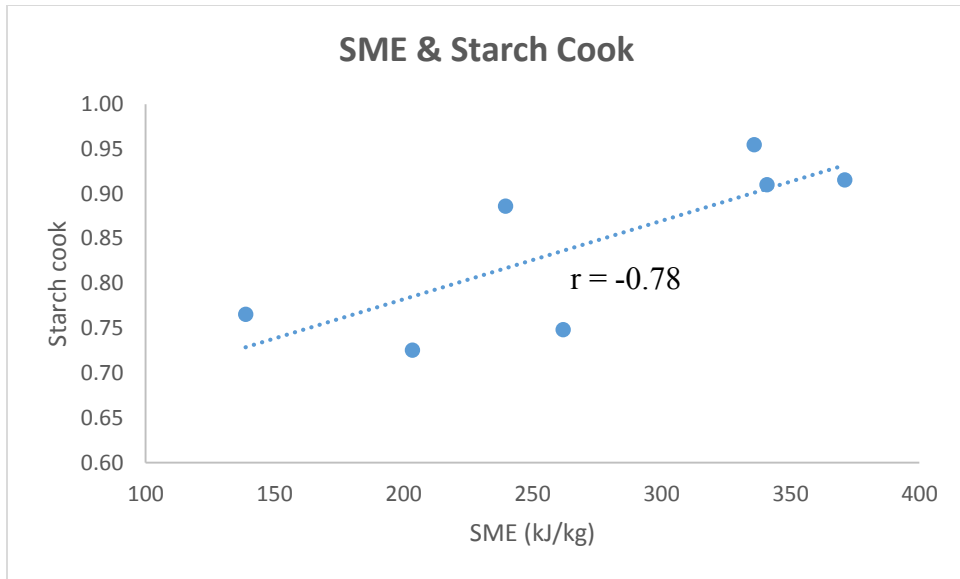
CSB = Corn soy blend

Figure 6.7 Correlation between SME and starch cook for CSB

SME = Specific mechanical energy, CSB = Corn soy blend

Chapter 7 - Conclusions and Future Work

1. Conventional wheat flour mill could be successfully adapted to mill sorghum, corn and cowpea grains. Depending on the type of grain and particle size needed, the length of the milling process could be adjusted to get the desired flour.

2. The expansion characteristics of cereal/legume blends was dependent on the inherent starch, protein and fat content. Higher starch content was observed in decorticated blends when compared to whole blends.

3. Higher oil content in formulations containing soy and/or whole corn caused a big variation in the motor load during extrusion leading to unstable processing conditions. SC blends had the most stable process followed by SS and CS in that order.

4. Extrusion processing led to a high degree of starch gelatinization in all the blends. The gelatinization ranged from 72.57% - 98.83% with the least gelatinization occurring in CS and highest in SC blends.

5. The peak values observed during RVA showed that whole blends had lower peak viscosity and higher peak time as compared to decorticated blends. This showed that decorticated blends had higher starch breakdown during extrusion.

6. There was a significant increase in starch digestibility of the blends but the protein digestibility did not have significant changes after extrusion and cooking.

7. The formulation of FBFs and the processing conditions were able to achieve the required energy density of >387 kcal/100g and a Bostwick flow rate between 9 – 21 cm/min as recommended in the FAQR.

8. A significant reduction in antinutrients – phytic acid, tannins and trypsin inhibitors was possible due to extrusion and that would increase the nutritional quality of FBFs.

Future Work

1. A deeper look on the effect of SME and thermal energy on the changes in amylose/amylopectin ratio and to correlate it to the physico-chemical characteristics like Bostwick flow rate and degree of cooking in FBFs. Instruments like gel permeation chromatography (GPC), and size exclusion chromatography (SEC) could be used to ascertain the changes in amylose/amylopectin ratio.

2. The synergistic effect of starch and protein after extrusion and its effect on gelling properties could be studied with the help of viscometry to further understand the influence of these on the Bostwick flow rate of binary blends/FBFs.