

**A NOVEL MECHANISM FOR DELIVERING NUTRITION:  
SORGHUM BASED FORTIFIED BLENDED FOODS USING EXTRUSION**

by

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## Abstract

The objective of the study was to investigate extrusion as an alternative processing method and grain sorghum as a viable substitute for corn in fortified blended foods (FBFs) used in nutrition and food assistance programs around the world. In the first part of this study, sorghum-soy blend (SSB), corn-soy blend (CSB) and whole corn-soy blend (WCSB) were developed using extrusion and compared with traditional CSB13 for physico-chemical and sensory properties. After milling of extrudates, average particle size (PS) ranged between 341-447 microns, with 78-85% below 600 microns. In general, Bostwick flow rates ( $V_B=12-23$  cm/min) of rehydrated blends (11.75% solids) were within standard specifications but higher than CSB13. Descriptive sensory analysis indicated that the sorghum-based rehydrated blends were significantly less lumpy and had a more uniform texture as compared to corn-based blends and CSB13.

In the second part, the impact of decortication level and process conditions was investigated with respect to sorghum-based extruded blends. Degree of gelatinization of the whole sorghum-soy blend (WSSB) and decorticated sorghum-soy blend (DSSB) extrudates ranged from 93-97%. Expansion ratio ( $ER=3.6-6.1$ ) was correlated with specific mechanical energy input ( $SME=145-415$  kJ/kg;  $r=0.99$ ) and average particle size after milling ( $PS=336-474$  microns;  $r=-0.75$ ). Rehydrated blends at 20% solids concentration provided recommended energy density (0.8 kcal/g) for FBFs. Bostwick flow rates had high correlation ( $r=-0.91$ ) with pasting data (final viscosity) obtained using rapid visco analyzer (RVA). Addition of oil (5.5%) prior to extrusion was also studied, and resulted in process instabilities and also lower shelf-life as determined via descriptive sensory analysis (rancid and painty attributes) and gas chromatography-mass spectroscopy (hexanal, heptenal and octanal concentrations).

In conclusion, extruded sorghum-soy blends met standard specifications for energy density and consistency (Bostwick flow rate), and were superior in some aspects as compared to extruded corn-soy blends and traditional corn-soy blends (CSB13). Relationships between extrusion mechanical energy input, expansion, particle size after milling and consistency of rehydrated blends were established. Consistency of the rehydrated blends is an extremely important criterion as it affects the ease of ingestion by target consumers (children below 5 years, in this case).

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## **Dedication**

This thesis is dedicated to my beloved, late mother Mrs. Hema Padmanabhan, my father Mr. Subramanya Padmanabhan and my wife Gayathri.

## **Chapter 1 - Introduction**

The 2013 World Hunger Report highlights chronic malnutrition as a major impediment in achieving the millennium development goals (MDGs) of the United Nations. Besides overt hunger resulting from being unable to afford any food, a more insidious type of “hidden hunger” results from eating food that is sufficient but lacking in essential micro-nutrients. An estimated 30 million children continue to be born each year in developing countries with impaired growth due to poor nutrition during fetal life (UN. 2013). Throughout the developing world, malnutrition affects almost 800 million people or 20 percent of the population. Approximately one third of all child deaths in developing countries is attributed to under nutrition (WHO. 2010). Even though World Bank recently declared victory on one of the targets of the Millennium Development Goals by halving extreme poverty before 2015, United Nations declared nearly a quarter of children in developing countries under the age of five are still expected to remain undernourished (Cuesta. 2013). Chronic malnutrition poses one of the gravest threats to human development, to progress in reducing hunger by calorie intake and children weight but they include no indicator for height. On the other hand, stunting happens if a child is too short for its age and includes unseen consequences including damage to brain development and overall health. A special etiology-related report on pediatric malnutrition defines (undernourishment) as an imbalance between nutrient requirements and intake that results in cumulative deficits of energy, protein, or micronutrients. As a cause and effect mechanism they may negatively affect growth and development such as loss of lean body mass, muscle weakness, inflammation, developmental or intellectual delay, infections, and immune dysfunction, delayed wound healing and prolonged hospital stay (Mehta et al. 2013).

The resulting conditions of infant stunting, maternal ill-health and wasting of HIV/AIDS patients are being addressed using fortified blended foods (FBFs) by various feeding programs by international food-aid organizations such as USAID, WFP, USDA-FAS. FBFs are provided as food aid, used primarily as a general food ration distribution, supplementary feeding programs, maternal and child health nutrition programs, school feeding and, HIV & AIDS programmes. FBFs made from corn soy blend (CSB) and or wheat soy blend find a variety of practical use of recipe which include porridge with fried vegetables, FBF drink, roasted blended food drink, soups, unleavened bread (chapatti/roti, etc.) pancakes, tortilla, FBF roasted dough, burfi, roasted Fortified Blended Food (sweet), and Fortified Blended Food cake (baked), fermented steamed cake, sweet balls, steamed rolls/dumplings banana leaf rolls and cookies (WFP. 2009).

CSB, classified as ready-to-use supplementary foods, has evolved rapidly with identifier codes such as CSB10, 11, 12, 13 & 14 and instant corn soy blend between 2005 and 2011 with enhancements to its macro and micronutrients, protein, and energy-density profiles. The primitive version of CSB was considered ineffective in addressing moderate acute malnutrition due to inadequate composition such as micronutrients, energy density, lipids and dietary fibers. The two types of unimix, CSB+ and the most recent version implemented by USAID's partner - World Food Program is called "Super Cereal- CSB++" with the inclusion of dry skim milk and upgrades to its micronutrient profile. It is estimated that CSB++ can feed the moderate to acute malnourished infants for 16 cents to 38 cents per day. Nutrient profiles of CSB+ and CSB++ are summarized below to have a brief background and better understanding on their formulation, composition and micronutrient fortification (UNICEF. 2011).

Table 1.1 Comparative formulation, composition and contamination allowance between CSB+ and CSB++

<b>CSB+</b>	<b>CSB++</b>
Older children and adults	Infants 6-24 months
Maize 72.5-77.5%	Maize 60-65%
Whole soy 20.0-25.0%	De-hulled soy 15-20%
Vitamin/ mineral mix 0.2%	Skim Milk Powder 8%
Calcium Carbonate 1.52%	Sugar 9%
Potassium Chloride 1.763%	Soya oil 3%
	Vitamin/ mineral mix 0.2%
	Calcium Carbonate 1.52%
	Potassium Chloride 0.763%
Energy 380 kcal (min)	Energy 420 kcal
Protein 14% (min)	Protein 16% (min)
Fat 6% (min)	Fat 9% (min)
Crude fiber 5.0 (max)	Crude fiber 3.0 (max)
Moisture 10.0% (max)	Moisture 9.0% (max)
Total aflatoxin 20 ppb	Total aflatoxin 5 ppb

Table 1.2 Showing micronutrient fortification upgrades between CSB, CSB+ and CSB++

CSB		CSB+ and CSB++	
<u>Vitamins</u>		<u>Vitamins</u>	
Vitamin A	1664 IU	Vitamin A	1664 IU
Thiamine	0.128 mg	Thiamine	0.128 mg
Riboflavin	0.448 mg	Riboflavin	0.448 mg
Niacin	4.8 mg	Niacin	4.8 mg
Pantothenic acid	na	Pantothenic acid	6.7 mg
Vitamin B6	na	Vitamin B6	1.7 mg
Folate	60 mcg	Folate	60 mcg
Vitamin B12	1.2 mcg	Vitamin B12	2 mcg
Vitamin C	100mg	Vitamin C	100mg
Vitamin D	5 mcg	Vitamin D	5 mcg
Vitamin E	8.3 mg	Vitamin E	8.3 mg
Vitamin K	na	Vitamin K	100mcg
<u>Minerals</u>		<u>Minerals</u>	
Iron	8 mg	Iron	8 mg
Zinc	5 mg	Zinc	5 mg
Calcium	100 mg	Calcium	600 mg
		Potassium	400 mg
		Phosphorus	200 mg

na – not available

As a measure to improve the formulation of existing FBFs, (Webb et al. 2011) FAQR (Recommendation #18) (Webb et al. 2011) encourage blend combinations of sorghum-soy, sorghum-pea, millet-soy and rice-soy besides traditional cereals such as wheat and corn which are currently being used to produce FBFs. Nevertheless, the FAQR advocates food-processing as its key focus to address anti-nutrition factors such as the phytate in cereals which inhibit iron or zinc absorption and the enhancement of product packaging and shelf-life.

Sorghum grain is home-grown in Africa and therefore a familiar staple. The FAS-USDA Food Aid Report for fiscal year 2010 shows shipments for sorghum grain shipped as part of food aid was 510,000 metric tons at a value of \$105,585,500 (USFOODAID. 2011). In addition to

sorghum grain's flexibility in food systems and high consumer acceptability, it makes significant contributions to the nutritional value of diets of populations at risk. It is competitively priced, and a non-GMO crop. Apart from being a drought resistant crop, grain sorghum outperforms other cereals including corn, under environmental stresses and cost of cultivation. Due to a shallower rooting system in corn, preharvest insect damage, wet postharvest and poor storage practices in under developed economies increases the risk of aflatoxin concentration (Pitt et al. 2013).

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. 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 it easier for human absorption thereby improving nutraceutical value of sorghum (Awika et al. 2003). A comprehensive review by (Fleige et al. 2010a) outlines nutrition reviews on FBFs in totality covering aspects of both macro and micro-nutrient enhancement in optimizing energy density and nutrition in cereal blends to vulnerable target groups.

The objective of this study was to investigate grain sorghum as a viable alternative to corn in fortified blended foods approved by USAID in weaning and feeding programs in nutrition-challenged pockets of the world. This thesis briefly reviews grain sorghum and its processing effects using extrusion technology highlighting nutritional impact and ease of digestibility made possible by a high temperature, high shear process (Chapter 2).

The following specific objectives were investigated:

Objective 1: To develop SSB, CSB and WCSB using extrusion processing and compare their physical, nutritional and sensory attributes based on recommended standards (Chapter 3)

Objective 2: To compare the physical, chemical, microbial, sensory and shelf-life properties of extruded blends whole versus decorticated sorghum grain prepared with or without oil during extrusion (Chapter 4).

Physical properties measured included product expansion, bulk densities, particle size, viscosity and Bostwick flow rate. Chemical properties measured included proximate analyses, water activity, moisture content and the degree of gelatinization. From a food safety standpoint, microbial tests were conducted during each time interval of the shelf-life study which included plate counts of bacteria, molds, e-coli, staphylococcus and enterobactereacea. Volatiles that cause autoxidation due to vegetable oil in FBFs during extrusion and in accelerated storage conditions were detected using solid-phase microextraction (SPME) method using GC-MS.

Towards the end of the project life cycle, FBFs were subject to sensory panelists to assess aroma and flavor profiles of 19 key attributes on acceptability of FBFs during each time interval of the study.

Sorghum-Soy blend (SSB) thus developed is fortified with micronutrients based on new recommendations by FAQR in optimizing its protein profile by the inclusion of whey protein concentrate -80% (WPC-80) and energy density by addition of vegetable oil. This preliminary study indicates SSBs to be in close adherence to USDA guidelines meeting physical standards. In conclusion, extruded sorghum-soy blends met standard specifications for energy density and consistency (Bostwick flow rate), and were superior in some aspects as compared to extruded corn-soy blends and traditional corn-soy blends (CSB13) making SSBs ideal for infant consumption. Targeted as a food-aid commodity SSBs would have the potential to meet nutritional needs of vulnerable infants especially in areas around the world that are prone to food insecurity.

## **Chapter 2 - Enhancing Digestibility of Sorghum Based Foods using Extrusion Processing– A Breif Review**

### **Abstract**

The nutritive benefits of sorghum both as food and feed commodity is compromised by its composition, which may act as physical barrier to digestion when wet-cooked. Although cross-linking of protein structures is thought to be one of the major factors that influence sorghum protein digestibility, the exact reasons are yet to be fully understood. Recent research has focused on improving the digestibility of grain sorghum through extrusion processing, while retaining its nutritive and functional properties in sorghum-based foods such as tortillas, couscous, porridges and baked goods. This review will discuss the factors that affect sorghum protein digestibility including exogenous (grain organizational structure, polyphenols, phytic acid, starch and non-starch polysaccharides) and endogenous (disulphide and non-disulphide cross-linking, kafirin hydrophobicity) factors. Besides decortication, extrusion significantly reduces condensed tannins by breaking down its molecular weight and thereby increasing the cereals bioavailability. This includes optimization of extruder conditions such as the use of pre-conditioner to treat dry matter with moisture pH alteration, extruder barrel temperatures (150°C -200°C), and utilization of low process moisture (20% – 30%), with increase in screw speed (100-125 rpm) and incorporation of reducing agents have shown to enhance sorghum protein digestibility.

### **Introduction**

Grain sorghum (*Sorghum Bicolor* L. Moench) is the main staple food consumed in various traditional forms in different parts of the world. Known to be drought-resistant, sorghum is one of the four major crops cultivated for human consumption globally (Axtell et al., 1981) in semi-arid, regions like Asia and Africa, where it forms an important source of dietary energy (Correia

et al., 2010). In regions like United States, Mexico, Argentina, Brazil and Australia, grain sorghum is mainly utilized for animal feed. In recent times, sorghum has steadily gained importance as the chief nutritional component of foods used in aid programs. With more than 70% starch, an amylopectin:amylose ratio of approximately 75:25, and a crude protein value of 9.56% in vitreous endosperm flours, sorghum is an important source of calories and proteins (Ezeogu et al., 2005). It is also an enriched source of B vitamin that includes thiamin, riboflavin, vitamin B6, biotin and niacin (Hegedus et al., 1985) and minerals such as potassium and phosphorus. The nutritional benefits of sorghum are however compromised by the presence of condensed tannins, which bind proteins, enzymes and B vitamins (Anglani, 1998). This is overcome by decortication of the grain caryopsis to reduce the overall tannin content and improve its color and digestibility (Taylor et al., 2005). The major storage protein in sorghum is called kafirin that is classified as prolamins due to their soluble nature in alcohol-water mixtures. Kafirins are categorized as  $\alpha$ ,  $\beta$ ,  $\gamma$  (identified at the protein level) and  $\delta$  kafirins (identified at the gene transcript level) (Belton et al., 2006). Kafirins account for 68%-73% of total protein in whole grain flour and 77%-82% of protein in the endosperm (Hamaker et al., 1995). When wet-cooked, sorghum protein exhibits poor digestibility mainly due to two factors –(a) exogenous (grain organization structure, polyphenols, phytic acid, starch and non-starch polysaccharides) and (b) endogenous (disulphide and non-disulphide cross linking, kafirin hydrophobicity) (Duodu et al., 2003). During the cooking process, polymeric kafirin proteins form aggregates, which are insoluble due to extensive cross-linking by disulphide bonds (Belton et al., 2006). Further, polymeric kafirins are less digestible than their monomeric counterparts (Duodu et al., 2002) Novel extrusion methods such as extrusion-enzyme liquefaction produced sorghum protein concentrates (82% db) and showed enhanced in-vitro protein digestibility(66%).

Optimum extruder conditions used in this study reports 35% an in-barrel moisture content when extruded at a specific mechanical energy of 593 kJ/kg (de Mesa-Stonestreet et al., 2010).

Fapojuwo et al (1987) observed that a high temperature range between 150°C – 200°C during extrusion could improve *in vitro* protein digestibility of sorghum varieties with low tannin content.

This review attempts to evaluate published research concerning sorghum protein digestibility and whether the methods adopted impart any unique health-promoting properties. It describes our fundamental knowledge of sorghum proteins with regard to its composition, structure and functional properties. Next, it focuses on the methods adopted, such as extrusion processing, to enhance sorghum protein digestibility and the possible health benefits related to its consumption. This review will help scientists and technologists identify areas for further improvement in the processing of grain sorghum so as to enhance the nutritional and functional value of sorghum proteins in food applications.

## **Exogenous Factors: Interaction of sorghum protein with non-protein components**

### ***Grain organizational structure***

Fundamentally, proteins alone make up 70% of the starchy endosperm (Paulis and Wall, 1979) and its digestibility depends on the form in which the grain is provided as either whole with the bran component or decorticated. Proteins may also bind to dietary fiber, which are present in significant amounts in the endosperm, as shown by the association of kafirin-like proteins with acid detergent fiber in raw as well as cooked sorghum (Bach Knudsen et al., 1985). Furthermore, decorticated varieties may differ in the proportion of endosperm and germ in the grain material (Duodu et al., 2003).

As a general trend, it was observed that protein digestibility improved as the proportion of pericarp and germ material in the grain decreased during the wet cooking process (Duodu et al., 2003). The factors interfering with sorghum protein digestibility may be the presence of polyphenols and phytate found in the pericarp and germ, followed by non-starch polysaccharides. Another factor that could influence digestibility may be the endosperm cell-wall and starch in the endosperm. This structure could impact the starch-protein interaction in the grain and also act as a barrier to prevent access of proteases to proteins (Duodu, et al., 2003). The interaction of protein bodies with cell-wall components via disulphide bonds is proposed as a reason for limiting access of the protein bodies to proteases leading to low digestibility. Support for this comes from studies of a highly digestible sorghum mutant cultivar P851171, which has a unique irregularly shaped protein body compared to a smooth spherical protein body of a normal sorghum.  $\gamma$ -kafirins that is in the peripheral location of the endosperm is highly cross-linked with disulphide bonds and impedes protein body digestion of other kafirins. However, with newer cultivars being developed, the resulting surface area of protein bodies in mutant sorghum as well as its positioning of  $\gamma$ -kafirins provides greater access to centrally placed main storage protein i.e.,  $\alpha$ -kafirin to digestive proteases leading to rapid digestion (Oria et al., 2000).

### ***Cell-wall components***

The association of proteins with the pericarp and endosperm cell walls in sorghum (Glennie, 1984) could lower protein digestibility either in the form of enzyme inactivation or formation of indigestible complexes (Duodu et al., 2003). It has been observed that the cell-wall of the sorghum endosperm is associated with 46% proteins (Glennie, 1987). The nature of protein-cell wall adhesion is explained by two main modes of attachment. It is proposed that one of the factors impairing sorghum protein digestibility may be the binding of proteins to non-

starch polysaccharide components in the cell-wall, the presence of which has been shown in sorghum (Raz et al., 1991). A second factor proposed to reduce sorghum protein digestibility is ferulic acid-mediated cross-linking of proteins to cell-wall components during the cooking of sorghum in an oxidizing environment. Cooking in the presence of oxygen could lead to the formation of tyrosyl-feruloyl cross-links between proteins and arabinoxylans resulting in adhesion of proteins to the cell-wall. It is thus proposed that removal of the outer layers of the grain may improve sorghum protein digestibility by reducing the amount of cell wall material thereby reducing protein-cell wall adhesion. However, it needs to be noted that the adhesion of proteins to cell-wall may not be limited to sorghum alone and hence may not be enough to explain the low digestibility of sorghum protein as compared to other grain proteins such as maize.

### ***Anti-nutritional factors by protein-tannin complexes***

The anti-nutritional effect of tannins arises from the formation of indigestible protein-tannin complexes, composed of polar hydrogen and non-polar hydrophobic bonds, is another contributing factor in limiting protein utilization in sorghum (Chibber et al., 1980). Under optimal conditions, tannins can precipitate up to 12 times their weight of protein and inhibit digestion mechanism. Though decortication can significantly reduce the adverse effects of tannins, extrusion processing can enable the reduction of tannin levels of upto 86% present in whole sorghum when compared to an unprocessed grain (Dlamini et al., 2009).

The presence of phenolic acids and flavonoids are not known to have any adverse effects on sorghum protein digestibility (Bravo, 1998; Duodu, Taylor et al., 2003). However, the oxidation of plant phenols results in quinones which form highly reactive oxidizing agents called peroxides. The peroxides may bring about oxidation of amino acid residues and subsequently,

polymerization of protein. This is presumed to be one of the factors leading to reduced sorghum protein digestibility (Duodu, Taylor et al., 2003).

### ***Phytic acid***

Phytic acid (myo-inositol hexaphosphoric acid), containing six phosphate molecules, is highly charged and acts as a chelator to form insoluble complexes with proteins (Ryden & Selvendran 1993) thereby reducing bioavailability of trace minerals and protein digestibility. Ravindran et al.,(1999) studied the effect of the addition of microbial phytase in sorghum which led improved protein and amino acid digestibility and concluded that structural or chemical properties of phytic acid determine the degree of protein and phytate binding that influences the responses between protein and amino acid.

## **Endogenous factors**

### ***Protein Cross linking***

Figure 2.1 shows the structure of sorghum protein body with interior core composed mainly of  $\alpha$ -kafirin and the outer shell is composed of cross linked  $\beta$ - and  $\gamma$ - kafirins.

The cross linking patterns of kafirin in protein bodies influences processing, digestibility and the overall biological value (Delgadillo et al., 2006). When these proteins are cooked, they undergo physical and chemical changes that lead to thermal destruction called pyrolysis (Finley et al., 1989). Under chemical conditions, derivatives of special amino acids either in the same molecule or another protein molecule are cross linked and this cross linking decreases digestibility of food proteins. The scope of protein cross-linking is limited to a) isopeptide cross linking and b) disulphide cross linking.

### ***Disulphide cross-linking***

It is known that endosperm matrix proteins and sorghum kafirin proteins cross-link during wet cooking by disulphide bonding (Duodu, et al., 2003). Disulphide cross-linking may lower protein digestibility of sorghum in the following ways. The lowering of kafirin solubility as a result of cross-linking may affect protein digestibility when protein polymers formed through disulphide bonding of the  $\beta$ - and  $\gamma$ - kafirins become enzymatically resistant. Also, the cross-linking restricts digestion of  $\alpha$  kafirins which are centrally located in the protein body which are more digestible compared to  $\beta$ - and  $\gamma$ - kafirins (Hamaker et al., 1994). The importance of kafirin is evident from the observation of sorghum mutants with high uncooked and cooked in vitro protein digestibility show that the kafirin protein matrix and the kafirin type affect protein digestibility (Hamaker et al., 2000). Disulphide cross-linking also occurs in maize but it is not known to affect maize protein digestibility (Nunes et al., 2002). Hence the difference between sorghum and maize digestibility is not explained solely by the disulphide bonding hypothesis. On the contrary, the use of reducing agents during cooking does not seem to reverse the effect of protein digestibility completely (Oria, et al., 2000). It is observed that in cooked sorghum reduction-resistant protein oligomers are present in that does not allow easy access of disulphide bonds to reducing agent.

### **Utilizing Extrusion processing for enhanced protein digestibility**

Due to pronounced starch-protein interactions and the cross linking of disulphide bonds in proteins upon high-moisture heat conditions (Mahasukhonthachat et al., 2010), it can be concluded that conventional cooking may not improve nutritional quality of sorghum (Price et al., 1980). However, apart from agronomic and genetic approaches, suggested methods to increase digestibility under low moisture processing include pressure-cooking, popping, nixtamalisation and flaking which may include the addition of reducing agents (Ezeogu, Duodu

et al., 2005; Glennie, 1987). Other process techniques such as germination and fermentation were also found to improve protein digestibility (Correia, Nunes et al., 2010). On the other hand, extrusion cooking is an established technique gaining importance in the production of several snack and other ready-to-eat foods. 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). It appears from past work and literature study that food extrusion could be a viable option for enhancing sorghum protein digestibility by using controlled conditions of heat and moisture that would lower the formation of disulphide bonds in proteins (Mahasukhnothachat et al., 2010).

### ***Extrusion Cooking and in-vitro protein digestibility***

While the efficacy of whole grain sorghum is still being researched upon, it is known that decorticated sorghum flour extruded at higher temperatures and lower process moisture showed remarkable increase in digestibility to 81% with infant porridgereducing tannin levels. Further, in contrast to unprocessed sorghum which decreased 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. Numerous studies have been conducted to determine the key effects on in-vitro protein digestibility based on extrusion variables that include moisture, temperature and screw speeds. (Fapojuwo et al. 1987) observed

that moisture differences in a sorghum variety did not have a significant change with protein digestibility, but increase in screw speed and barrel temperature improved sorghum digestibility. The adjustments of pH values (calcium hydroxide) before extrusion, increased digestibility with increase in barrel temperature tested for two different varieties of sorghum. It was observed that that at 200 °C with pH addition before extrusion, protein digestibility increased from 44.8-74.6%(Fapojuwo, et al., 1987).

Protein digestibility of unprocessed and extruded cereals was determined using an in-vitro method of enzymatic hydrolysis. The most protein-digestible products with grain sorghum were produced at the extrusion combination of 15% feed moisture, 100 °C/150 °C product temperature and 100 rpm (Dahlin et al., 1993). Results of this study suggest that a particular combination of extrusion process conditions may be applied when extruding a wide variety of cereals, with the benefit of improving protein digestibility.

### ***Extrusion Effects on antioxidant activity***

Condensed tannins are commonly called procyanidins that are concentrated in the testa and pericarp of the grain. Sorghum procyanidins is composed of high MW(DP>10) polymers that are broken down by extrusion to lower MW constituents by increasing the procyanidin oligomers with  $DP \leq 4$  and a decrease in polymers with  $DP \geq 6$  thereby changing the relative ratios of the different molecular weights making procyanidins more bio-available (Awika et al., 2003). The oligomers and monomers of condensed tannins are more bio-available compared to polymers in both in-vivo (Deprez et al. 2001) and in-vitro (Gu et al., 2008) studies where extrusion improved bio-availability in significant levels. The quantities of condensed tannins in sorghum are affected by extrusion due to rigorous mechanical action in the extruder barrel. Dlamini et al, (2009) reported a reduction in condensed tannin levels in comparison to traditional wet cooked sorghum

porridges from 26.2 to 11.2 mg with Framida cultivars. This would mean that broken down condensed tannins are absorbed through intestinal cell mono layers which may enhance protein digestibility. During traditional cooking, there is low shear and there is tendency for sorghum kafirins to have a disulphide cross-linking (Duodu et al., 2003). Also, decortication reduced antioxidant activity by 83% due to the removal of pericarp and testa which reduces phenols. Extrusion processing generally decreased antioxidant activity when compared to conventionally cooked porridges (Duodo et al., 2002).

### ***Structural disruption with extruder conditions***

Eventhough extrusion cooking degrades starch structure, digestion is impeded in extrudates in which the degraded starch component is associated with protein bodies. In Figure 2.2, starch appears to be largely de-structured at 40% in-barrel moisture but with lower digestive properties, which may possibly be the result of retrogradation or densification effects. Intact starch granules with and without indents from protein bodies Figure 2.2, raw and 125 rpm at 25% moisture suggests that native structures were intact despite extrusion. Thus some extrusion conditions did not completely disrupt starch-protein interaction while other condition revealed separate starch and protein granules (SEM obtained for extrudates at 220 rpm at 30% moisture), yielding maximum starch digestion from animal feed. It is shown that extrusion with a reducing agent such as 2-mercaptoethanol opens up the protein matrix in hard sorghum grains where the protein sheets of kafirins are broken down to smaller fragments thereby enabling starches to gelatinize (Chandrasekar and Kirleis 1988) and also improve protein digestibility.

Early studies on prominent effects of extrusion cooking on in-vitro digestibility of sorghum protein suggests pH alteration through the addition of  $\text{Ca(OH)}_2$  prior to extrusion with a barrel temperature of  $200^\circ\text{C}$  to be a key factor in protein digestibility from 44.8% to 74.6% (Fapojuwo

et al., 1987). Further extrusion of sorghum at low process moisture showed enzyme-susceptible starch ratio, protein damage, water solubility, and thin air cell walls which facilitated protein hydrolysis. This mechanism in effect, increased protein digestibility in thin porridges (atoles) with acceptable product and sensory characteristics (Gomez et al., 1988). Sorghum protein bodies are presumably made rigid by disulfide-linked polymeric nature of the  $\gamma$  and  $\beta$  prolamins found at the periphery of the protein body which requires both thermal and shear forces to disintegrate the matrix and denature kafirin protein for enhanced bio-availability using extrusion technology.

### **Future research:**

Protein digestibility of sorghum protein is influenced by number of factors. Dependability is based on uncooked or cooked grain and its nature, which include whole grain, endosperm, protein bodies or extracted proteins. With numerous researches on sorghum grain and the effects on extrusion with sorghum, it is still unclear as to why a cooked maize protein has higher digestibility when compared to sorghum. What are puzzling between the two grains are their similarities in protein body structure and prolamins primary structure between the two grains. Some of the key areas that may need further investigations would be 1) the study of phytate and protein interactions rather than total phytate concentration. 2) It is important to know the protein binding nature with cell wall components and how cooking affects cell-wall adhesion. 3) The exact nature of pepsin-indigestible oligomeric protein species in cooked sorghum and its cross linking nature has wider scope for investigation. 4) Kafirin and zein hydrophobicity are extensive areas of research. The beta kafirins hydrophobicity when compared with beta zein is still unclear. 5) The changes in protein secondary structure between sorghum and maize through spectroscopic study for cooked and uncooked forms of isolated proteins could be related to their digestibility

(Duodu, Nunes et al., 2002). Extrusion cooking has also evolved in last decade rapidly and depending on the grain composition, extruder conditions in developing end products have been changing with physicochemical, structural and sensory aspects. Food manufacturing firms and product development companies have been constantly looking at newer scientific methods in evaluating processed foods and sorghum is now a center stage for most producers. African and Asian markets are large consumers on feeds programs for children and adults as a weaning food supplements. Sorghum is now transitioning from being a feed based commodity to a food based necessity. Hence, the scope to innovate and develop its digestibility will be of paramount interest to people involved in agricultural research, food manufactures and consumers in developing countries.

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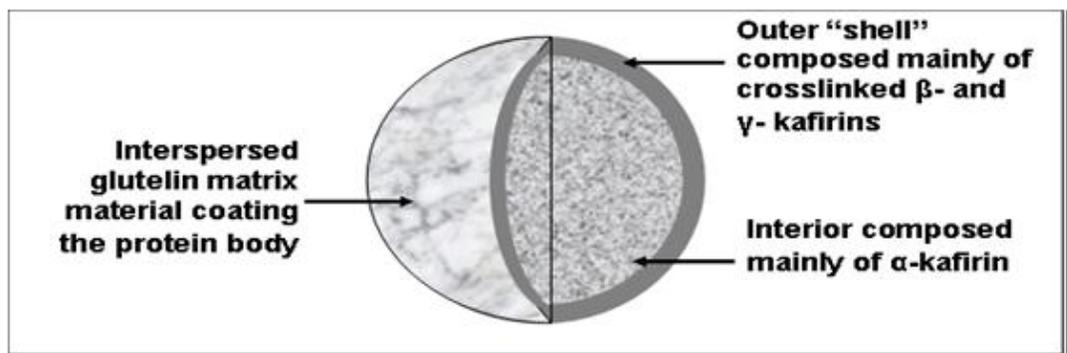


Figure 2.1 Schematic of a sorghum protein body (de Mesa-Stonestreet, Alavi et al., 2010)

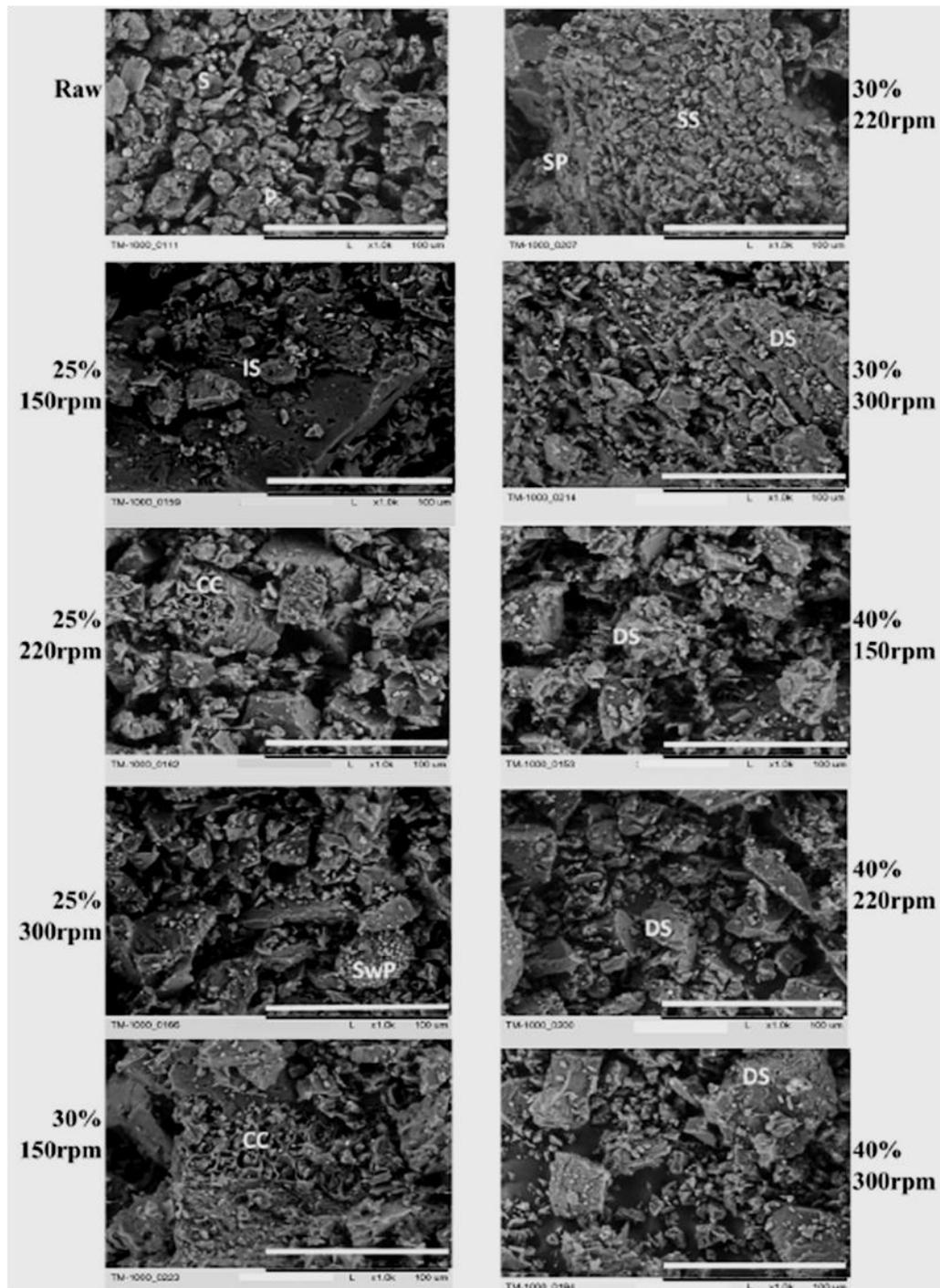


Figure 2.2 Typical scanning electron micrographs of the milled sorghum before and after extrusion conditions.

S = starch, P = Protein, IS = starch granules with indentation. DS = destructured starch. CC = cells with contents, SP = separated protein bodies. SWP = starch with protein bodies. Bar = 100 (Mahasukhonthachat, Sopade et al., 2010).

## **Chapter 3 - Development of Fortified Sorghum-Soy Blend Using Extrusion Processing**

### **Abstract**

A recommendation by United States Agency for International Development (USAID) funded study outlined new upgrades for nutrient composition and suggested use of alternative grain source for blended food production. Sorghum-soy blend (SSB), corn-soy blend (CSB) and whole-corn soy blend (WCSB) were developed by extrusion processing using high (HI) shear (450 rpm at 22% moisture) and low (LO) shear (350 rpm at 30% moisture) conditions. Physical, nutritional and sensory properties were compared with traditional food-aid formulation of CSB 13. Higher mechanical energy and lower moisture in all treatments lead to increased product expansion and lower bulk density. Pasting curves of extruded CSB showed noteworthy decrease in final viscosity from LO to HI shear (299 cP to 140 cP); while SSB had a constant viscosity (229 cP), indicating extruded blends to be thinner gruels with high Bostwick flow rates (19.5 – 23.0 cm/min). Comparatively, WCSB had higher final viscosity (329 cP & 316 cP) and lower flow rate (12.0-15.5 cm/min). SSBs final viscosity also showed good linear correlation with Bostwick flow data ( $r = -0.826$ ) at 11.75% gruel concentration meeting the set requirements for viscosity (9.0-21.0 cm/min), while CSB 13 was susceptible to lump formation and thicker gruel consistency. Descriptive sensory analysis showed significant differences in extruded SSB HI and LO shear samples at 20% blend concentration for textural attributes ( $P < 0.05$ ). The micronutrient fortified SSB developed is high energy density gruel (410 kcal/100g) with physical and viscosity flow characteristics comparable to existing fortified blended food (FBF) products currently in use for food aid delivery.

## **Introduction**

The number of food-insecure people from 76 countries is estimated to decline from 814 million to 802 million from 2011-2012. However, the food security rates are expected to increase by 15.1% over the next decade in 39 Sub-Saharan African (SSA) countries reaching 411 million by 2022, with a 28% rise in population in this region (Rosen et al. 2012). Food insecurity around the world has led to a significant rise in demand for food-aid commodities especially among 12 SSA countries where staple food prices increased by 71% (Minot 2011). The frequent occurrence of natural disasters has also added to the number of people with severe food insecurity especially in underdeveloped countries where effort to alleviate disaster is grossly inadequate. These factors contribute to what is now termed “the growing problem of hunger” (FAQR 2011). The United States continues to lead the rest of the world in provision with over 3 million metric tons of food supplied to approximately 50 million food-insecure people in 49 countries all over the world (USAID 2009). The US Agency for International Development (USAID) through its food aid program called “Food-for-Peace (FFP)” supported by the Public Law 480 (PL 480) provides food for overseas humanitarian aid. Another US policy in this regard is the “Title II Program” referred to as emergency feeding for combating malnutrition and to promote sustainable development. Finally, the McGovern Dole programs administered by the United States Department of Agriculture (USDA) also help support child development through school feeding, maternal and child nutrition projects. The Title II food aid program since its enactment in the 1950s has evolved consistently to improve product, programming and process quality in delivering fortified blended foods (FBFs) especially in emergency situations, to drastically reduce the high risk of mortality and severe malnutrition. In the effort to reduce child malnutrition and enrich nutritional capabilities in pregnant and lactating women, CSB has been distributed through the Food-for-Peace Program since its inception in September 1966

(Anderson et al. 1971), and have been the major ration widely distributed to address hunger in Africa and Asia. CSB consists of corn and soy flour fortified with micronutrients and it is prepared mainly as porridge (WPF 2002). Traditionally, CSB is produced from yellow corn that is dehulled and degermed, gelatinized cornmeal and is free of rancid, bitter, musty, sour and other undesirable aroma or flavor. Processing involves addition of moisture followed by partial cooking in continuous cookers or on heated flaking rolls, drying and cutting. Defatted (toasted) soy flour is produced by cleaning, cracking, dehulling, tempering, flaking, defatting with hexane, desolventising and cooling. Toasting process involves complete cooking until color change to light yellow or golden buff (USDA. 2007). The nutritive value of CSB has undergone periodic upgrades both quantitatively (calories) and qualitatively (nutrient density) by significantly increasing protein and fat levels to provide balanced intake of essential nutrients for growth and development of infants, young children and malnourished individuals, and other vulnerable groups. However, the cost of producing CSB and other soy-fortified milled cereals has been going up due to increasing utilization of main ingredients. In Title II programming, these fortified blends account for 44% of 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 the traditional cereals such as wheat and corn, in the production of FBF. Major advantages of using a non-GMO cereal such as sorghum in countries like Africa would be its acceptability to host government considering lower costs of sorghum (\$5.88-7.39 per bushel) than that of shelled corn (\$6.90-8.79 per bushel) (USDA ERS 2013). Apart from being a drought resistant crop, grain sorghum outperforms other cereals including corn, under environmental stresses and cost of cultivation. Corn experiences pre-harvest insect damage due to a shallower rooting system, wet postharvest and poor storage practices in under developed economies

increases the risk of aflatoxin concentration (Pitt et al. 2013). Grain sorghum contains phytochemicals such as phenolic compounds, plant sterols and policosanols that are rich in antioxidants and impacts human diets significantly by lowering cholesterol and promoting cardiovascular health. Condensed tannins present in phytochemicals seem to have powerful anti-carcinogenic and anti-diabetic *in-vitro* activity (Awika et al. 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 (inositol hexakisphosphate (IP<sub>6</sub>)) in cereal-legume based complementary 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). 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 it easier for human absorption thereby improving nutraceutical value of sorghum (Awika et al. 2003). Extrusion heat treatment and shear forces further inactivate 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 recommendation in the FAQR report. Sorghum soy blend as it will be called is a precooked blend using extrusion and micronutrient fortified which can be a remedy for wasted and infants with stunted growth.

The objective of this study was to develop sorghum-soy blend that conforms to the most recent recommendations from Food Aid Quality Review (FAQR) and evaluated the effects of extrusion processing on various physical characteristics of extruded sorghum-soy blend (SSB) in comparison with traditional and extruded corn-soy blends. Specific processing to product relationships were established which included extrudate expansion, post-milling particle size, viscosity profiles and their effects on Bostwick flow rate which related to gruel consistency before infant consumption. Sensory analysis was performed at 11.75% and 20% solid concentration assessing key attributes such as flavor, texture and aroma profiles. 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 lipid content by adding 15 g of vegetable oil to 50 g of FBF and 3) Upgrades to the micronutrient composition.

## **Materials and Methods**

### ***Raw Materials***

The ingredients used include the following: decorticated sorghum flour was purchased from ADM Milling Co. (Overland Park, KS., U.S.A.); defatted soy flour from ADM (Decatur, IL., U.S.A.); degermed corn flour from Bunge Milling Co. (Atchison, KS., U.S.A.); whole corn kernels was from Lortscher Agri Service Inc. (Bern, KS., U.S.A.); whey protein concentrate (WPC)-80 was purchased from Davisco Foods International Inc. (Le Sueur, MN., U.S.A.), vitamin and mineral premixes were supplied by Research Products Co. (Salina, KS., U.S.A.) and pure canola oil was purchased locally (Wesson, Omaha, NE, U.S.A.). Whole corn kernels were ground using a Hammer Mill (The Fitzpatrick Co, Model D - Comminutor, Elmhurst, IL., U.S.A.) with a sieve size of 0.38 mm reducing the particle size to ~380 $\mu$ m. CSB 13 was used as a control for all test analysis.

### ***Formulation***

Formulations were prepared according to guidelines provided by FAQR (2011). Three main base formulations consisted of sorghum, corn flour and whole corn flour (76.3%) with defatted soy flour (23.7%). Pre-weighed quantity of each formulation was blended in a ribbon mixer (Wenger Manufacturing Co., Sabetha, KS, USA) for 5 minutes before extrusion as seen in Table 3.1.

### ***Extruder Set-up***

A pilot-scale single screw extruder X-20 (Wenger Manufacturing Co., Sabetha, KS, U.S.A.) equipped with a preconditioning system was used to process sorghum-soy, corn-soy and whole corn-soy blends. The dry feed rate was set at 150 kg/h for all treatments. Two levels of mechanical energy (low and high) were obtained by varying the extruder screw speeds (350 and 450 rpm) with target in-barrel moisture of 30% and 22% wb. Steam and water injection in the

preconditioning cylinder were adjusted to 6.0 and 10.05 kg/h, respectively, for high shear treatments and 11.9 and 18.05 kg/h respectively for low shear treatments. Water in the extruder barrel for all treatments was at 4.0 kg/h. The die consisted of circular inserts of 4.1 mm diameter. As the thermocouples in the controller units were not functioning at the time of extrusion, it was not possible to record actual barrel zone temperatures. Instead, a pneumatic valve was set at P (-10) for zones 1 and 2 and (-11) for zone 3 by constantly cooling the extruder barrel with cold water. The knife speed was set at 2014 rpm. The screw configuration and barrel zone cold water set points are shown in Figure 3.1.

The extrudate was cut after exiting the extruder die with a face-mounted triple blade rotary knife. Extruded products were dried at 104 °C in a double pass dryer/cooler (Series 4800, Wenger Manufacturing Co. Sabetha, KS, USA) adjusted for 10 min retention time (5 min each for the top and bottom belts). Cooling was accomplished with room temperature air, and a 5 min retention time on the cooling belt.

***Specific mechanical energy (SME)***

Tests for specific mechanical energy (SME) were done in duplicate and mechanical energy input per unit mass of extrudate was calculated as follows:

$$SME \text{ (kJ/kg)} = \frac{\left(\frac{T}{100}\right)\left(\frac{N}{N_{\text{rated}}}\right)P_{\text{rated}}}{\dot{m}} \dots\dots\dots(1)$$

where T = net motor load percentage, N = screw speed (rpm), N<sub>rated</sub> = rated screw speed (507 rpm), P<sub>rated</sub> = rated power (37.3 kW), and  $\dot{m}$  = net mass flow rate (kg/s).

***Bulk Density***

A cylindrical steel container (V<sub>c</sub> = 1L) was filled with extrudates and the weight was recorded.

Bulk density (BD) of the extrudates was calculated as:

$$BD = \text{Wt. of sample} / V_c \dots\dots\dots(2)$$

***Expansion Ratio (ER)***

The ER is the ratio of the extrudate cross-sectional area to the die orifice cross sectional area, and was calculated as follows:

$$ER = d^2/d_{die}^2 \dots\dots\dots(3)$$

Where d is the extrudate diameter (average of 10 determinations) and  $d_{die}$  is the die diameter, which was measured using a digital caliper.

***Milling***

Dried products were ground in a single pass using a tabletop roller mill (915/ 9X6, Ross Machine and Mill Supply, Inc., Oklahoma City, OK, USA) installed with two rollers having 20 corrugations per inch and 0.2 mm clearance between the rollers. It was sieved through a 900  $\mu$ m sieve using a mechanical shaker for 5 min before micronutrient fortification.

***Fortification***

Post-extrusion, the dried extrudates were milled in 1 kilogram batches and fortified with WPC-80 (3.0%), minerals (3.0%) and vitamin premix (0.1%) and vegetable oil (5.5%) Table 4.1.

Micronutrients were added to each formulation of the ground extrudate and mixed homogeneously using a Hobart Blender – N50 (Hobart Corporation, Troy, OH, USA) for 5 minutes.

***Particle Size Analysis***

Particle size distribution of the 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.

### ***Moisture Analysis***

Product moisture of milled extrudates was conducted by oven-drying method in which 2 g  $\pm$  1 mg of sample was placed in an aluminium pan and heated at 135 °C for 2 hours based on AACC method 44-19 (AACC 2013). The lids were then replaced and the pans were transferred to a desiccator to cool for 30 minutes. The difference in weight of the sample pre and post oven-drying was calculated. Duplicate tests were conducted for each sample.

### ***Bostwick Consistometer Test***

Bostwick consistency for milled products was performed using a Bostwick Consistometer (CSC Scientific Company Inc., Fairfax, VA., USA). The Consistometer was placed on a flat surface and the leveling screws were adjusted until the bubble in the circular level was centered. Gruels of 11.75% and 20% were prepared by mixing 23.5 g and 50 g of milled product into 175 ml and of boiling distilled water, respectively. After 2 min of vigorous manual stirring while boiling, the slurry was removed from heat, stirred again for 30 s and immersed in a water bath at 30 °C for 10 min. Slurries were adjusted for water-loss through evaporation to achieve a final weight of 200 g and stirred again before placing it back in the water bath at 30 °C for 1 hr. 100 ml of this slurry was then poured into the compartment of the Bostwick Consistometer. After a settling time of 30 s, the gate was released and the slurry was allowed to flow through the graduated trough. The distance of flow was recorded after exactly 1 min (USDA 2005). Measurements for each product were obtained in triplicate.

### ***Pasting Properties***

Pasting properties were obtained using a Rapid-Visco Analyzer (RVA), (RVA4, Newport Scientific Pvt. Ltd., NSW, Australia). Sample moisture was first adjusted to 14% dry-basis (db) by adding distilled water. For testing, approximately 3.5 g of sample was added to about 25 ml of

distilled water in an aluminum test canister (Walker et al. 1988). The RVA was preheated to 50°C for 30 min prior to testing. A 13 min standard RVA temperature profile was used: 1 min holding at 50°C, 3 min 42 s temperature ramp up to 95°C, 2 min 30 s holding at 95°C, 3 min 48 s temperature ramp down to 50°C, and 2 min holding at 50°C (Batey & Curtin 2000).

### ***Bostwick simulated pasting protocol***

Simulation of Bostwick conditions was developed with change in temperature and time in the RVA protocol setting. This was done to correlate the actual Bostwick results to the controlled conditions of RVA. The following steps were followed: temperature was ramped up to 98°C and held at 98°C for approximately 3 min, followed by a temperature ramp down to 30°C in 7 min and thereafter held at 30°C for 1 hr. The speed of the paddle was adjusted to 960 rpm for the first 10 s to mix the sample homogeneously and avoid any lump formation, and then reduced to 160 rpm for the entire duration of the test. Pasting properties, such as peak, trough and final viscosities, were determined. The viscosity was recorded in Windows based ThermoLine software Version 2.0. (Newport Scientific Pvt. Ltd., NSW, Australia). Duplicate tests were conducted for each sample.

### ***Proximate Analysis***

The proximate composition of uncooked sorghum and soy flour was determined using standard methods (AOAC, 2010; AOCS, 2009). This included determination of moisture (135°C for 2h; AOAC 930.15), crude protein (based on nitrogen by combustion, 6.25X; AOAC 990.03), crude fat (petroleum ether extract method; AOAC 920.39), ash (600°C for 2h; AOAC 942.05), crude fiber (filter bag technique utilizing H<sub>2</sub>SO<sub>4</sub> and NaOH digestion for Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY); AOCS (Ba 6a-05), and total starch (aqueous alcohol

pretreatment; amyloglucosidase/ $\alpha$ -amylase method; AOAC 996.11). Protein, starch, fat, ash and crude fiber contents were reported as dry basis percentages (% db) from replicates.

### ***Descriptive Sensory Analysis***

Fortified SSB, CSB blends along with control CSB samples were subjected to quantitative descriptive sensory analysis. The method involved oil addition of 30% (15 g to 50 g of FBF) to samples prior to cooking of the gruel at 11.75 and 20% solid concentration. Cooking procedure was similar to Bostwick Consistometer test referred in earlier section.

A panel consisting of six highly-trained descriptive individuals, with a minimum 120 h of descriptive training and 1000 h of sensory testing experience on grain products, evaluated gruel samples in a randomized block design. Based on a 2 hour orientation session, the panelists developed a list of 21 appropriate attributes for texture and general flavor for samples. Attribute intensities were quantified using a 15 point scale with 0.5 increments (0.0=nothing to 15=extremely high). Products were coded with three-digit random numbers and were assigned to panelists randomly and data were analyzed in triplicates in the same session (Lawless & Heymann 1998). Samples were served in 237ml foam cups (with approximately 90ml of sample) and panelists were allocated 12 min to evaluate each sample with no more than 5 samples a day. Panelists were requested to rinse their mouth with warm water followed by the consumption of either mozzarella cheese, apples or carrots to cleanse their palate before evaluating each porridge sample. The panelists evaluated aroma, texture and mouth-feel attributes first, followed by flavor.

### ***Statistical Analysis***

Descriptive study for all attributes was analyzed by one-way ANOVA (SAS version 9.2. SAS Institute Inc., Cary, North Carolina, USA) using PROC GLM/MIXED procedure to determine

the significant effect of model and formulation on the variation observed in the different parameters. For all significant attributes, the effects were determined using pair-wise test comparisons based on SAS least square (LS) means. The criteria for significance was  $p < 0.05$ . Linear association between RVA final viscosity and Bostwick flow rate for each treatment was determined by Pearson Correlation.

## **Results and Discussions**

### ***Chemical Composition***

Proximate composition of decorticated sorghum and defatted soy flour is shown in Table 3.2. It is observed that sorghum flour had higher moisture content when compared to soy. Extrudates were dried, ground and tested for moisture in comparison with precooked CSB 13 (USDA 2007), which was used as control. The average moisture content for SSB and CSB were within the range of 4 and 6% wet weight basis (wb). However, control CSB showed higher moisture content of 9.22% compared with other blends. As expected, products produced using low in-barrel moistures with a higher mechanical energy process had lower final moisture levels. Table 3.3. Siriwardana et al. (1998) reported for cooked products with 5.8 to 6.6% moisture on dry matter basis (db), the prevention of the proliferation of microorganisms, moisture must be maintained below 5.92% at 30°C indicating a safe level of moisture to prevent fatty acid oxidation and hydrolysis and concluded that product with such low end moistures are favorable for the prevention of microbial growth and enhance shelf-life of products.

### ***Impact on expansion ratio and its effects on intermediate product characteristics***

Extrudate properties of sorghum and corn-soy blends were analyzed to establish physico-chemical relationships which bears direct consequences to final product flow characteristics with Bostwick consistency discussed in later sections. The computed SME for cereal blends ranged

from 113-196 kJ/kg. As expected, low shear treatments showed a decrease in SME values as extrusion moisture increased (Ye Sun & Muthukumarappan 2002) from 22% to 30% indicating lower melt viscosity and starch conversion in the extruder. Extruded blends experienced radial expansion between 6.08-13.59. Extrudates produced at higher specific mechanical energy with lower process moisture expanded more than treatments produced at lower screw speeds and higher moisture. This observation agrees with similar work by Onwulata & Konstance (2006), Konstance et al. (1999) & Carvalho et al. (2010). Comparing expansion of extruded blends, SSB-HI had the highest expansion ratio (13.59) with a specific mechanical energy of 167kJ/kg, signifying high degree of starch gelatinization as an effect of melt rheological characteristics. Higher SMEs aggravates greater bubble formation during extrusion followed by a mechanism of pressure release and simultaneous expansion when water vapor flashes off while product finally exits a die (Fan et al., 1996), with shorter land length (4.6mm) that attributed to extrudates of low density (49 g/L) and increased radial expansion. It is also important to note that product expansion was reduced by half from high to low shear treatment in CSB due to extruder moisture and lower mechanical energy. The relationships between ER with specific mechanical energy input, product densities and particle size is shown in Figure 3.2. As expected, high negative correlations ( $r = -0.785$ ,  $-0.902$ ) were observed with product expansion with bulk and piece densities respectively. On the contrary, a moderate correlation ( $r = 0.408$ ) was observed between ER and SME. Since SME calculations are based on mass flow rates as per equation (1), any variances with material input to the extruder could possibly alter the overall SME input values and their relationships. Single screw extruder used in this experiment had a volumetric manual fed dry recipe feed system which could have affected uniform flow due to changes in bulk density of raw materials attributing to changes in mass flow rate. No particular trend was

observed with product expansion with milled extrudate particle size ( $r = 0.185$ ). Mean particle sizes for all extruded and ground samples were found to be between 340 and 450  $\mu\text{m}$  Figure 3.3. With decrease in mechanical energy from high to low shear during extrusion, the particle size of ground extrudates for SSB and WCSB increased while it decreased marginally for CSB extrudates Table 3.4. Analysis of the cumulative particle size showed that for all extruded and ground products, approximately 80 – 85% of particles had uniform distribution, thereby approaching the recommended 95% particle distribution that passed through a 600  $\mu\text{m}$  sieve and 100% through a 1000  $\mu\text{m}$  (USDA 2005) (equivalent to U.S. mesh sizes #30 and 18, respectively). While the upper end specifications of a 100% particles passing through a 1000  $\mu\text{m}$  sieve was met, particle distribution of CSB control sample had less than 80% particles pass through a 600  $\mu\text{m}$  sieve. High-energy treatments (CSB-HI and SSB-HI) had lower peaks and a wider spread in the particle size distribution while low energy treatments had narrower peaks indicating a more uniform particle size in the distribution.

Konstance et al. (1998) reported that moisture conditions during extrusion has been indicated as being the single most determinant affecting particle size wherein mean particle size increased as feed moisture increased. Previous studies on the SME impacts with particle size on corn meal extrusion (Carvalho et al. 2010) and barley grits (Altan et al. 2009) showed an increase in particle size as SME input decreased. Though this observation agrees with SSB and WCSB, it contradicts the data in the current study with CSB. In this study, particle fractions, with same formulations in CSB showed minimal particle variances despite exposure to factors such as feed moisture and SME. Though the overall particle size variability in extruded and ground blends are small, decrease in particle size for CSB-LO could be primarily due to combined effects of high moisture and lower SME ( $\sim 139$  kJ/kg) causing higher particle degradation with enhanced

residence time in the extruder barrel. Besides macromolecular composition when cereal flours are blended at different particle specifications, the limitations in the milling process to grind extrudates to the required specifications also contributed to particle size variances. Further investigation is required given the complex thermo-mechanical nature of extrusion and rheological behavior from different starches especially with cereal-legume blended formulations and choice of a right mill such always becomes critical in meeting required particle specifications.

### ***Pasting Properties***

Pasting profiles for ground extruded blends are presented in Table 3.5, Figure 3.4 and corresponding pasting values are presented in Figure 3.4a, b shows the pasting curves for CSB and SSB high and low energy treatments compared with the control CSB sample. Products with high-energy treatments exhibited cold water swelling, indicating more damage and gelatinization of starch, induced by high shear and high particle hydration rate at ambient temperatures (Ozcan& Jackson 2005; Whalen et al. 1997). Extruded CSB samples had higher peak viscosities than other blends. Peak viscosities of extruded CSB-HI appeared very early during the heating phase and at much lower temperatures in comparison to control and its counterpart, clearly indicating faster gelatinization due to more starch available in the cereal blend (Nicole et al. 2010). Faster granular swelling with higher water absorption rates leading to starch solubilization and leaching of amylose below 90°C possibly increases viscosity of starch. With further increase in temperature and mechanical shear stress during the holding phase, there is further disruption of the starch granule and the remaining amylose is leached out and undergoes alignment (Ragae& El-Sayed 2006). This phenomenon of starch gelatinization was observed with extruded CSB and SSB high shear samples as they achieved their peak viscosity between 1.05-

2.51 min indicating shorter cooking times and lower final viscosities. These observations are consistent with the effects of extrusion kinetics on pasting properties reported by Mahasukhonthachat et al. (2010). Subsequently, pasting for low shear CSB and SSB treatments was found to occur at a much lower temperature (52°C and 54°C) indicating rapid particle hydration by larger particles that contribute to swelling of starch granules at lower temperature which contradicts with the results obtained by Onwulata & Konstance(2006). For high shear treatments pasting temperatures were significantly higher (94°C and 95°C) indicating utilization of available water by protein and starch in the slurry mix. Though CSB control took more than 4 min to achieve its peak value, it showed the highest paste stability as indicated by its lowest breakdown viscosity (91 cP), which may have potential ingredient characteristics when exposed to heat treatment at high temperature and mechanical stirring (Ragae& El-Sayed 2006).

RVA pasting profiles for SSB showed peak viscosities between 347-402 cP, which were lower to CSB (447-581cP) implying possible re-aggregation of sorghum proteins during wet cooking thereby limiting starch swelling and gelatinization (Mesa-Stonestreet et al. 2011). Further, it is shown that starch granules embedded in protein matrix slows hydration in hard sorghum kernels and reflects higher correlations with protein concentrations on peak, breakdown, trough viscosity and peak time (Griess et al. 2011).

CSB control had the highest final viscosity of 579 cP compared to all other blends indicating higher degree of starch retrogradation and re-association on starch crystals. In general, regardless of particle size, the pasting properties of all blends showed relative increase in final viscosity upon cooling which may be due to higher leaching of macromolecules and presence of ungelatinized (raw starch) in the sample which are resistant to heat and shear, that could have contributed to the final paste consistency during the cooling phase. This observed

final viscosity is correlated to texture, firmness and stickiness of the end product (Sopade et al. 2006).

### ***Assesing Bostwick Consistency with standard and modified RVA temperature profile***

Bostwick flow rates of the cooked slurry and RVA viscosities obtained at 11.75% solid content are presented in Table 3.4. The relationship between Bostwick flow rate and RVA viscosity is best described by the final viscosity values in the RVA profile. Blends with high final viscosities showed lower Bostwick flow rate on a standard RVA temperature profile. Final viscosities at 50°C and Bostwick flow rates showed high inverse correlation ( $r = -0.826$ ) shown in Figure 3.5. Both CSB and SSB showed lower end viscosities ranging between 19.5-23 cm/min. Except for CSB LO, all blends showed a decrease in flow rate with increase in final viscosity indicative of gels gelling behavior during the cooling phase. Another contributing factor on flow measurements is the effect of temperature. Mouquet et al. (2006) reported that a 10°C temperature interval (50°C and 40°C) during cooling of gels before consumption, consistency increased by 13 cm/min for high energy density multicomponent flours and recommended measurements to be performed at  $45.0 \pm 1^\circ\text{C}$ . Similar results were observed in the present study wherein, Bostwick flow rate and RVA final viscosity at 30°C showed a weak relationship ( $r = -0.13$ ) at lower temperatures, Figure 3.5. However, this study adhered to the commodity specifications of CSB 14 at 30°C as its Bostwick measurement temperature and flow rate dependency was bound to gel concentration and temperature. Notably, the final viscosity of CSB high energy treatment was the lowest among treatments and exceeded the specified flow rate range (23 cm/min), indicating possible macromolecular degradation of starch by thermal and shear forces in the extruder barrel. With shear stress applied to the starches in cereals, inhomogeneous breakdown of larger molecules in the non-crystalline region occur

causing a decrease in intrinsic viscosity and shear stress. Grandbios et al (1999) & Beyer (2000) in their fundamental approach to molecular strength of glucosidic bonds showed the effects of applied forces over time on such bonds in an amylose molecule can instantaneously exceed the bond strength thereby resulting in molecular weight reduction. Van den Einde et al (2004), in their modeling study with waxy corn starch showed that macromolecular degradation during extrusion processes can be minimized by reducing screw speeds (SME) or by increasing the depth of the gaps while keeping the shear stress lower. Mouquet & Treché (2001) in their quest to compare viscosity measurement procedures adopted for infantgruel suggested that viscosity measurements need to be made under fixed shear rate, controlled shear time and gruel temperature. With regards to these recommendations, RVA measurements were modified by matching the cooking conditions to the Bostwick flow rate protocol. Figure 3.4 illustrates the actual cooking conditions using RVA and emphasizes the importance of viscosity under controlled shear rate and time conditions. Though a thixotropic behavior was observed with a decrease in viscosity at concentrations between 9.4 and 13.4% by Mouquet & Treché(2001) our study showed under a constant shear rate of 160 rpm and 30° C for 50 minutes, viscosity profiles for all blends were stabilized and were in line with the baseline temperature for gruels at 11.75% concentration without any drastic decrease in viscosity. Flow rates were within the recommended target range of 9.0-21.0 cm/min (USDA 2005). This flow value aligns with the value obtained by Cameron et al. (2009) for CSB. Although a Bostwick flow closer to 12 cm is more typical for CSB in complementary feeding programs at 12% concentration, this would result in reduction of calories by 0.5kcal/g (Vieuet al. 2001). However, when comparing Bostwick flow rates with RVA final viscosity, it is evident that extruded CSB and SSB have higher flow rates at a given concentration confirming greater degree of starch gelatinization and protein denaturation. The

sensitivity of RVA results concur with the findings of others in distinguishing samples that were processed differently and a higher chance of consumption among infants. In this study, the actual moisture content of the milled samples was taken into account for the sample weights used for RVA analysis. Further, formula modifications and preparation methods could be used to reduce viscosity and increase energy density. It can be inferred from Bostwick values that cereal-based starches have complex rheological behavior and the accuracy of the projected gruel viscosity is hampered by the solid concentration and gruels temperature before serving. The results of this study closely adhered to USDA guidelines with regard to cooking method, time and consistency measurement of gruels.

### ***Matching Energy Densities with Porridge Consistency***

As a general practice, most cereal-based products used for complementary feeding programs are diluted to achieve a thin drinkable consistency before feeding infants, since energy-dense porridges are naturally viscous porridges at higher solids to water ratio. As a consequence, the energy density and micronutrient levels of the porridge are reduced (Stephenson et al. 1994). Several other studies reveal specific processing techniques to liquefy thick porridges by use of amylase-rich flours (ARF) also known as ‘power flour’ produced by germination of cereal grains (Gopaldas et al. 1988, Svanberg et al. 1988, Luhila & Chipulu 1988, Malleshi & Amla 1988). Considering the stomach capacity of infants (30 to 40 ml of gruel/kg of body weight), the work by Stephenson et al. (1994) rationalized a maximum of four feedings per day by mothers based on their limited time to accommodate feeding schedules among other routine household work. Though the process to liquefy thick porridge foods by advocating ARF has been encouraged by the government of Tanzania through a joint WHO/UNICEF Nutrition Support Program, this has been of no benefit to infants aged 5-12 month older when small quantities of germinated

sorghum flour was added to thick porridges (Svanberg et al.1988). When sprouting of the cereal takes place, enzymes digest a portion of the starch into dextrans and maltose that inhibit swelling when the gruel is cooked;it also reduces bulk and makes it less viscous (Ebrahim 1983). Viscosity reduction using exogenous alpha-amylase has been examined on germinated cowpea-sorghum weaning foods with lowest reconstitution time of 180 s without any modifications in sensory characteristics (Asinobi et al. 1998). On the other hand, issues of cereal malting (germination) relates to potential food safety concerns about the risk of aflatoxin contamination for corn and the risk of cyanide toxicity from sorghum that produces hydrocyanic acid upon germination(Ashworth & Draper 1992). Hence the work by Stephenson et al.(1994) concluded that thicker porridge took longer feeding time by mother but resulted in a 35% mean energy intake when compared to thin low density porridge. They suggested the compensation of thinner gruels by adding oil and peanut butter to better supplement energy density of 1 kcal/g. Cameron 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; however, the blends were excessively viscous for infant consumption. The increase in the flour to water ratio increases the viscosity significantly making it difficult for infants to swallow these gruels (WFP 2012). They suggested different cooking methods and cooking times to produce porridges with potentially lower viscosities while maintaining their caloric density. This work was further subjected to field trials by Rowe et al. (2008), where the average dry meal concentration prepared in Uganda, Malawi and Guatemala was 14% to produce spoonable / drinkableporridge gruel.

In the present study, it is evident that extruded CSB and SSB blends have an energy density of approximately 410 kcal/100g from 20% concentration based on the new

recommendations which is comparatively higher than previous FBFs used in feeding Programs. Table 3.6 shows the feeding volume and energy supply by FBF on gruel mixing ratios as recommended by FAQR (2011) for different children groups that match their gastric capacity and feeding rates. As far as the protein requirements are concerned, the CSB 13 commodity specifications of USDA 2007 recommends a minimum of 16.7% per 100 gram in the formulation 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 a test subject divided by its intake of a particular food protein during the test period. Expressed as a daily energy needs, P:E% is 6% for infants (6-24 months) with normal growth and between 6.9% to 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 the amino acid requirements of humans and their ability to digest it. The US Title II CSB provides P:E% - 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. 2010a). It is however important to note that FBFs are not intended to provide complete nutrition but only 25% dietary requirements while the balances are met by breast milk and traditional foods. Considering dilution effects of the remaining 75% of daily diets due to sharing, the efficacy of FBFs is greatly reduced. Hence substantial amount of protein supplementation on FBFs can increase protein quality. Table 3.7 shows nutrient profiles of extruded blends providing up to 420 kcal/100g, with 19 grams of protein in the formulation.

### **Whole Corn Soy Blend (WCSB)**

With several feeding programs targeted toward child nutrition, infant health can be positively impacted by increasing the intake of whole grains in their diets. The Whole Grain Council defines

whole grain (WG) products as those that contain all the naturally occurring nutrients of the whole grain kernel including the endosperm or inner core, the germ where the sprout emerges and the outer layer, called the bran (WGC2013). WG consists of resistant starch that escapes digestion in small intestine and when fermented the substrate produces butyrate which guards against colorectal cancers besides other diseases (Ahmed et al. 2000), while corn starch in particular is superior in generating butyrate along with other fermentation substrates (Weaver et al. 1992).

The dietary fiber present in corn bran and fiber refers to polymers with ten or more monomeric units which is nearly insoluble in water and is widespread in whole corn flour and a total dietary fiber of 12.8% w/w db (Serna-Saldivar 2010). Corn bran is composed of cellulose (~280 g/kg) and hemicelluloses (~700 g/kg) with small amounts of lignin (~10g/kg) and rich in ferulic acid, a phenolic antioxidant and antimicrobial agent besides residual starch, lipid, protein, ash, phenolic compounds with other trace phytochemicals (Rose et al. 2010). Hernot et al. (2008) verified that extrusion processing of Whole-corn reflected 67% digestible starch and 85% total digestible fiber in corn bran with the lowest release of glucose (9%), despite small differences among substrates, the insoluble and soluble dietary fibers in WG corn, barley, wheat and oats. The inclusion of soy flour in the formulation also contributes to total dietary fiber of 16.3% w/w db (Dreher 2001) in addition to the fiber profile from whole corn flour.

The extrusion of WCSB showed increased bulk density (130 to 140 g/L) from low to high shear indicating the consequences of insoluble dietary fiber present in the formulation. This result agreed with similar study on extrusion of insoluble fibers by other authors (Robin et al. 2012; Ainsworth et al. 2007; Andersson et al. 1981; Blake 2006; Brennan et al. 2008, Jin et al. 1995; Lue et al. 1991; Moore et al. 1990; Stojecksha et al. 2008; Yanniotis et al. 2007). Table 3.7 further confirms this result with 36% dietary fiber compared with other blends. Further, a second

reason with increase in bulk density is attributable to the addition of soy flour beyond 20% in the formulation (23.7% soy flour added in the present study) which agrees with the study by Jin et al., 1995. It is also known from several studies that insoluble fiber tends to reduce radial expansion and increase density in extruded cereals leading to harder textures. This trend on expansion ratio and density is verified with WCSB when compared to CSB treatments (Table 3.4). Quantification of this reduction of extrudate expansion is estimated when the levels of corn bran is up to 32% (Pai et al. 2009), and soy fiber of up to 20% (Jin et al. 1995).

It also becomes clear that during extrusion, the degree of starch transformation is also affected by the interactions between hard insoluble fiber particles and soft starch molecules that compete for available water besides protein bodies in the starch matrix. As seen earlier, extruded CSB and SSB showed higher peak viscosity and lower final viscosities, while the converse was true for WCSB blends Table 3.4. The hull present in WCSB and a certain degree of degraded starch during milling could have delayed particle hydration of fragmented starch granules, reflecting delay in overall cooking-time when compared to its counterpart. This result is verified with WCSB starches having lowest breakdown values and heat stable properties requiring longer time to reach its peak viscosity Figure 3.4.c, compared with other extruded blends. Further investigation may reveal if insoluble fibers in WCSB prevented starch to experience granular swelling taking longer time to attain its peak viscosity. From a gruel consistency stand point, WCSB had a spoonable consistency with lower flow rates at 30°C compared to extruded CSB and SSB that were less viscous and potable. This increase in shear viscosity with WCSB could possibly be due to interactions between larger bran particle sizes that may eventually reduce mobility of starch molecules in the gruel. Fibers in cereals have high water-retaining capacity due to their structural integrity of cell wall particles that entrapping and

absorb water (Redgwell et al.2011). Hence, it calls for a postulation that extrusion at higher specific mechanical energies can degrade this structural integrity and commensurate with the required final viscosity for specific applications. Redgwell et al.(2011) studied extrusion changes with citrus soluble fibers on their viscosity generating properties using high mechanical energies (200 to 480 kJ/kg) with extruder moisture range between 30% to 50% and reported good correlation between higher SME vales and degree of fiber solubility (up to 30%) by degrading the cell wall polymers in plant fibers. The opposite was true with WCSB as they were insoluble dietary fibers and would have required even higher SME's to disintegrate the cell wall structures compared to soluble fibers. With relatively low SME values (135to154 kJ/kg) and extruder moisture (22% and 30%), WCSB showed flow resistance and thicker gruel consistency compared to extruded CSB and SSB. This may be due to the water binding properties in the cellular matrix in corn bran fiber that include hemicelluloses bound to cellulose by hydrogen bonds (Redgwell et al. 2011) that may be responsible for initial water uptake thereby retaining its structure as they were treated considerably with lowermechanical energy. Further, research is required to understand fluid dynamics and end viscosity flow rates of gruels considering their high degrees of retrogradation which would impact delivery of required energy density per serving size consumed.

### ***Sensory Analysis comparing 11.75% and 20% solid concentration***

Results of descriptive sensory analysis are shown inTable3.8. Thekey sensory attributes are compared between 11.75% and 20% solids samples. Analysis of variance showed that there was significant effect ( $p<0.05$ ) of formulation and processing on the variations observed in the different sensory attributes. Significant differences were found in texture, aroma, and flavor in all

of the products when prepared at 11.75% and 20%, respectively. These differences illustrate the impact of sensory analysis while sorghum is used instead of corn in FBF products.

### ***Aroma***

All blends had overall grain, musty and cardboard aromas that were significantly different in the intensity of odors. At 11.75% concentration, SSB-HI samples had the highest intensity for overall grain aroma, followed by CSB Control and CSB-HI, while CSB-LO and SSB-LO had the lowest overall grain aroma. SSB-HI also had the highest musty and cardboard aroma compared to the other blends. When prepared at 20% solids, samples with highest overall grain aroma were CSB-LO and CSB-HI, followed by the CSB Control, SSB-LO and SSB-HI blends. At 11.75% solids, SSB-HI also had high scores on musty overall and cardboard odors but aroma levels reduced significantly when cooked at the 20% solids level. This drop in aroma intensity particularly with cardboard-like aroma at 20% solids could be viewed to have a positive impact due to reduced concentration of volatile compounds and indicative of delayed rancidity in grain sorghum which has scope in enhancing prolonged storages (Clemmings et al. 2010). However, at 11.75% solids, volatile compounds present in musty sorghum may have other off-odor notes, which include earthy/damp/moldy qualities (Vázquez-Araújo & Chambers 2011) that could have given rise to higher odor intensities with SSB-HI during cooking and holding at higher temperatures.

### ***Flavor***

Overall grain flavor was used as a measure for all grain attributes found within the sample, including but not limited to sorghum, oat, wheat, corn, beany, and soy. Though SSB-HI had the highest overall grain flavor at 11.75% concentration Table 3.8, the intensities were not notably significant among blends at 20% solids. As expected, sorghum flavor was higher in the SSB

samples compared to CSB samples. SSB-HI was the highest in sorghum flavor, followed by SSB-LO, followed by CSB-HI. It was not surprising that corn flavor was higher in the CSB samples compared to the SSB samples. CSB control was the highest in corn flavor, followed by CSB-LO, CSB-HI, and SSB-LO. Increasing the solids content from 11.75% to 20% also changed the flavor profile of the products. The biggest flavor difference from this change was in starch flavor. Starch flavor increased in all of the samples, from an average intensity of about 4.4 to an average intensity of about 8.1. All samples prepared at 20% concentration were significantly higher than the 11.75% samples in starch flavor.

CSB-HI was the highest in toasted flavor with both concentrations, followed by SSB-HI and CSB control. SSB-LO was lower in toasted flavor but not significantly different than CSB Control at 11.75%. The higher values seen in toasted flavors with higher input extruded samples may be due to high heat experienced by the blends during the extrusion process (Chen et al. 1991) and possibly due to oil present in fortified blends. This also confirms the possibility of maillard reaction that reflects this flavor attribute with CSB-HI. SSB-HI was the highest in musty, bitter, sour, and astringent flavor which was contrary to observations seen with odor intensity.

### ***Texture***

With increase in solid concentration from 11.75 to 20%, thickness, particle amount, and lump size increased phenomenally with all products. CSB control had the thickest consistency among concentrations and formed lumps probably it contained uncooked or partially gelatinized starch prior to cooking operation for sensory analysis, whereas extruded blends showed thinner texture when being cooked. This could be due high temperature and shear used to pre-cook starches in

the extruder that could have reflected thinner textures. SB control had high intensities with respect to adhesiveness and gumminess as low as 11.75% when compared to blends that are probably related to gelatinization of the starch during cooking resulting in a stickier product. These properties further increased dramatically throughout all blends with increase in solid percentage which was an expected phenomenon among other attributes within the textural segment. Uniformity of size decreased in all samples. The most uniform samples were again SSB samples, compared to CSB samples (although CSB-HI was not significantly different from SSB-HI). The sorghum based samples, both SSB-LO and HI, were higher in mouth drying than the CSB samples, which perhaps is related to astringent compounds in sorghum (Kobue-Lekalake al. 2007). As seen in earlier section on RVA and Bostwick data, adhesiveness was observed at a stage when starch retrogrades after cooking and during cooling phase. The values observed with gumminess also support adhesive nature of the gruel with gradual reduction in temperature. SSB samples were shown to be higher in intensities in mouth drying compared to CSB samples. One of the significant conclusions from textural studies combined with Bostwick flow rate reveals how increase in the solids concentration could affect the end consistencies of the product.

It becomes clear that at 20% solids concentration, CSB control becomes virtually a hard-dough where adjustment of water losses had no change in flow rate or end viscosity. However, SSB-HI stabilized to 9.5 cm/sec when compared to a 19.5 cm/min at lower concentration where both readings were within the recommended range of 9 – 21 cm/min (USDA 2005). The descriptive sensory results show that the CSB control was rated as the thickest in texture perception, and SSB-HI was the thinnest sample, with no difference between the other three samples. Bostwick flow rate can be used as an indication of sensory perception in gruels (Mouquet et al. 2006), and is important in the consideration of analyzing end product texture of

infant gruels. When cooked on a stove-top using the new 20% solid concentration, all blends had flow rates lesser or closer to 9.0 cm/min, meaning the new recommendations are outside of the target range set for these types of gruel where a mid-range of say 12-18 cm/min flow would have been ideal in meeting the requirements. The extruded samples were thinner, however, making them closer to the target than the control CSB sample. Considering the complexities of product dilution ratios and the end product viscosity, the role of the raw material used, processing conditions and cooking protocols could show mixed variability in matching the required specifications. Future studies may reveal newer standards in establishing a balance between desired flow rates and ideal solid concentration which may have the ideal energy density per serving size suitable to target groups.

## **Conclusions**

1. Fortified and blended foods (extruded SSB, CSB & WCSB) were developed from grains using combination of extrusion processing conditions. Extruded blends have lower end viscosities when subject to higher concentration at ambient temperature. It is noteworthy that particle variance is influenced by extrusion moisture, SME and macromolecular composition especially with blended cereals. A low RVA final viscosity indicates possible macromolecular degradation based on thermal and shear forces with time. The inclusion of dietary fiber in FBF's increases product density with reduced radial expansion. Bostwick flow rates (12-23 cm/min) of rehydrated blends (11.75% solids) were within standard specifications and higher than CSB13, also showed extruded starches (SSB, WCSB), and CSB13 to have higher thermal stabilities compared to extruded CSB.

2. Extruded SSB, CSB is less viscous, has thinner gruel consistency requiring no further dilution before feeding infant meeting USDA standards
3. Extruded and fortified SSB/CSB has comparable nutrient profile(410 kcal/100g dry wt) to the existing – CSB 13 (386 kcal/100g). With additional oil in the blend increases the total calories to provide additional 250 kcal from 30g of oil/100g of FBF.
4. Descriptive sensory analysis showed high intensity scores among attributes with SSB HI samples for 11.75% solids but was not the case with 20% solids. After cooking, SSB appears to be a thinner porridge requiring no further dilution while CSB control resists flows at 20% solids. Further research on modifying extrusion processing conditions, solid concentration and cooking methods could possibly enhance ideal consistency to suit various target consumers. Extruded and fortified SSB has a consistency comparable to the new recommendation for fortifications in Tufts report to USAID and could as well replace or compliment CSB control as FBF. Future research would evaluate starch and protein digestibility in SSB as compared to others.

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Table 3.1 Target Level of Micronutrients for FBFs

\* as modified from Webb et al. 2011.<sup>1</sup> vegetable oil providing essential fatty acids (alpha-linoleic/ $\omega$ -3), <sup>2</sup>addition of WPC 80% gives a PDCAAS score of up to 0.88.

<b>Ingredient</b>	<b>Amount (g/kg)</b>
Corn Meal/Sorghum/Whole Corn	67.27
Soy Flour	21.13
Vegetable Oil <sup>1</sup>	5.50
WPC80% <sup>2</sup>	3.00
Mineral Premix	3.00
Vitamin Premix	0.10

Table 3.2 Proximate analysis of uncooked flours (% db)<sup>1</sup>

<sup>1</sup>as specified by the Department of Animal Sciences, Kansas State University, Manhattan, KS.

\*Crude protein is calculated using a 6.25 conversion factor. “-” indicates missing or incomplete value.db: Dry basis.

<b>Ingredient</b>	<b>Moisture (%)</b>	<b>Crude Protein* (%)</b>	<b>Crude Fat (%)</b>	<b>Crude Fiber (%)</b>	<b>Ash (%)</b>	<b>Starch (%)</b>
Sorghum	11.65 ± 0.00	8.72± 0.04	1.09± 0.01	0.17± 0.02	0.55 ± 0.02	71.91 ± 0.14
Soy	8.73 ± 0.03	46.85± 0.35	1.00 ± 0.03	3.05± 0.04	6.88± 0.04	-

Table 3.3 Average moisture content of milled products  
 wb: wet basis, measurements are given as average  $\pm$  sd.

<b>Fortified Blended Foods</b>	<b>Moisture content (% wb)</b>
Sorghum soy blend; high energy (SSB-HI)	4.30 $\pm$ 0.30
Sorghum soy blend; low energy (SSB-LO)	5.83 $\pm$ 0.20
Corn Soy blend; high energy (CSB-HI)	5.22 $\pm$ 0.40
Corn Soy blend; low energy (CSB-LO)	5.69 $\pm$ 0.10
Whole corn soy blend; high energy (WCSB-HI)	5.20 $\pm$ 0.02
Whole corn soy blend; low energy (WCSB-LO)	6.83 $\pm$ 0.01
Corn Soy blend (CSB Control)	9.22 $\pm$ 0.00

Table 3.4 Mean particle size, SME Expansion ratio of extruded and milled blends

SME – specific mechanical energy, BD – bulk density, PD – piece density, MPS – mean particle size of extruded and milled blends. Measurements are given as average  $\pm$  s.d. (NA – data not available).

<b>Product</b>	<b>SME (kJ/kg)</b>	<b>Expansion Ratio</b>	<b>BD (g/L)</b>	<b>PD g/cm<sup>3</sup></b>	<b>MPS (<math>\mu</math>m)</b>	<b>% below 600 (<math>\mu</math>m)</b>
SSB-HI	167.74	13.59 $\pm$ 1.19	49	0.10 $\pm$ 0.00	442.2 $\pm$ 13.7	79
SSB-LO	113.78	6.65 $\pm$ 0.59	134	0.33 $\pm$ 0.00	437.2 $\pm$ 17.9	83
CSB-HI	196.85	7.44 $\pm$ 0.77	63	0.26 $\pm$ 0.02	340.6 $\pm$ 5.5	85
CSB-LO	138.17	6.08 $\pm$ 0.54	127	0.34 $\pm$ 0.00	436.5 $\pm$ 0.1	83
WCSB-HI	153.42	7.28 $\pm$ 2.75	130	0.31 $\pm$ 0.00	447.3 $\pm$ 10.3	81
WCSB-LO	134.88	6.24 $\pm$ 2.12	140	0.44 $\pm$ 0.15	413.0 $\pm$ 6.0	85
CSB 13	NA	NA	NA	NA	441.6 $\pm$ 7.6	75

Table 3.5 Pasting Properties

Comparison of Final viscosity and Bostwick flow rates at 11.75% solids .<sup>1</sup>PV: Peak Viscosity – the maximum viscosity that occurs prior to the initiation of the cooling phase, indicating swelling of starch. <sup>2</sup>PT: Peak Time – cooking time required for blends to reach peak viscosity, <sup>3</sup>Trough – post-peak viscosity observed either before cooling or slightly after start of cooling. <sup>4</sup>BD: Break down = PV – Trough, <sup>5</sup>Final Viscosity- the viscosity that occurs at the end of the test during the cooling phase indicating starch retrogradation. Bostwick flow rate and the final viscosity from RVA are comparable and indicate gruel consistency measured at the time of consumption. cP: centipoises (1 cP = 10<sup>-3</sup> Pa·s). Measurements are given as average ± s.d.

Standard RVA						Modified RVA		
Product	PV <sup>1</sup> (cP)	PT <sup>2</sup> (min)	<sup>3</sup> Trough	<sup>4</sup> BD (cP)	<sup>5</sup> FV 50°C (cP)	PV (cP)	FV 30°C (cP)	Bostwick flow rate (cm/min)
SSB-HI	402 ± 3	1.25	128 ± 0	274 ± 3	229 ± 0	168 ± 1	250 ± 5	19.5 ± 0.3
SSB-LO	347 ± 2	2.51	173 ± 5	174 ± 6	229 ± 10	202 ± 1	302 ± 2	20.5 ± 0.0
CSB-HI	581 ± 15	1.05	68 ± 11	514 ± 4	141 ± 11	145 ± 5	235 ± 4	23.0 ± 0.3
CSB-LO	447 ± 35	1.85	217 ± 1	229 ± 36	299 ± 1	231 ± 3	377 ± 3	19.5 ± 0.0
WCSB-HI	295 ± 22	4.05	186 ± 2	108 ± 20	316 ± 4	236 ± 55	275 ± 25	12.0 ± 0.6
WCSB-LO	286 ± 5	4.22	190 ± 4	96 ± 8	329 ± 8	237 ± 22	302 ± 15	15.5 ± 0.5
CSB 13	511 ± 12	4.98	402 ± 5	108 ± 8	943 ± 13	535 ± 53	613 ± 48	12.0 ± 0.5

Table 3.6 Targeted nutrient requirements by selected age groups

\* One-half of daily caloric requirements, # equal two-third of daily caloric requirement.

<b>Age Group</b>	<b>Mixing Ratio FBF:Oil:Water</b>	<b>Total Volume (ml)</b>	<b>Feeding rate/day</b>	<b>App.energy supplies/day (kcal)</b>
06 – 11 months	50 : 15 : 200	290	3 - 4	335*
12 – 24 months	100 : 30 : 400	580	3 - 4	670#

Table 3.7 Nutrition labeling of extruded FBFs

(literature values generated from Genesis R&D labeling software based on cereal-legume specification used in the FBF formulation)

## SSB

<b>Nutrition Facts</b>	
Serving Size (100g)	
Servings Per Container	
Amount Per Serving	
<b>Calories 410</b>	<b>Calories from Fat 90</b>
	% Daily Value*
<b>Total Fat 10g</b>	<b>15%</b>
Saturated Fat 1.5g	8%
Trans Fat 0g	
<b>Cholesterol 0mg</b>	<b>0%</b>
<b>Sodium 240mg</b>	<b>10%</b>
<b>Potassium 400mg</b>	<b>11%</b>
<b>Total Carbohydrate 62g</b>	<b>21%</b>
Dietary Fiber 8g	32%
Sugars 4g	
Protein 19g	
Vitamin A 4%	Vitamin C 70%
Calcium 50%	Iron 100%
Vitamin D 4%	Vitamin E 6%
Vitamin K 45%	Thiamin 60%
Riboflavin 60%	Niacin 60%
Vitamin B6 45%	Folate 25%
Vitamin B12 340%	Pantothenic Acid 40%
Phosphorus 70%	Iodine 20%
Magnesium 25%	Zinc 45%
Selenium 10%	Copper 8%
Manganese 45%	
*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.	
	Calories: 2,000 2,500
Total Fat	Less than 65g 80g
Saturated Fat	Less than 20g 25g
Cholesterol	Less than 300mg 300mg
Sodium	Less than 2,400mg 2,400mg
Potassium	3,500 mg 3,500 mg
Total Carbohydrate	300g 375g
Dietary Fiber	25g 30g
Calories per gram:	
Fat 9 • Carbohydrate 4 • Protein 4	

## CSB

<b>Nutrition Facts</b>	
Serving Size (100g)	
Servings Per Container	
Amount Per Serving	
<b>Calories 420</b>	<b>Calories from Fat 70</b>
	% Daily Value*
<b>Total Fat 8g</b>	<b>12%</b>
Saturated Fat 1g	5%
Trans Fat 0g	
<b>Cholesterol 0mg</b>	<b>0%</b>
<b>Sodium 240mg</b>	<b>10%</b>
<b>Potassium 250mg</b>	<b>7%</b>
<b>Total Carbohydrate 65g</b>	<b>22%</b>
Dietary Fiber 5g	20%
Sugars 3g	
Protein 18g	
Vitamin A 6%	Vitamin C 70%
Calcium 50%	Iron 90%
Vitamin D 2%	Vitamin E 4%
Vitamin K 40%	Thiamin 50%
Riboflavin 60%	Niacin 50%
Vitamin B6 35%	Folate 30%
Vitamin B12 340%	Pantothenic Acid 35%
Phosphorus 50%	Iodine 20%
Magnesium 6%	Zinc 40%
Selenium 8%	Copper 4%
Manganese 2%	
*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.	
	Calories: 2,000 2,500
Total Fat	Less than 65g 80g
Saturated Fat	Less than 20g 25g
Cholesterol	Less than 300mg 300mg
Sodium	Less than 2,400mg 2,400mg
Potassium	3,500 mg 3,500 mg
Total Carbohydrate	300g 375g
Dietary Fiber	25g 30g
Calories per gram:	
Fat 9 • Carbohydrate 4 • Protein 4	

## WCSB

<b>Nutrition Facts</b>	
Serving Size (100g)	
Servings Per Container	
Amount Per Serving	
<b>Calories 400</b>	<b>Calories from Fat 90</b>
	% Daily Value*
<b>Total Fat 10g</b>	<b>15%</b>
Saturated Fat 1.5g	8%
Trans Fat 0g	
<b>Cholesterol 0mg</b>	<b>0%</b>
<b>Sodium 240mg</b>	<b>10%</b>
<b>Potassium 400mg</b>	<b>11%</b>
<b>Total Carbohydrate 60g</b>	<b>20%</b>
Dietary Fiber 8g	36%
Sugars 3g	
Protein 18g	
Vitamin A 6%	Vitamin C 70%
Calcium 50%	Iron 100%
Vitamin D 0%	Vitamin E 6%
Vitamin K 40%	Thiamin 60%
Riboflavin 60%	Niacin 50%
Vitamin B6 45%	Folate 25%
Vitamin B12 340%	Pantothenic Acid 40%
Phosphorus 60%	Iodine 20%
Magnesium 20%	Zinc 45%
Selenium 15%	Copper 8%
Manganese 15%	
*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.	
	Calories: 2,000 2,500
Total Fat	Less than 65g 80g
Saturated Fat	Less than 20g 25g
Cholesterol	Less than 300mg 300mg
Sodium	Less than 2,400mg 2,400mg
Potassium	3,500 mg 3,500 mg
Total Carbohydrate	300g 375g
Dietary Fiber	25g 30g
Calories per gram:	
Fat 9 • Carbohydrate 4 • Protein 4	

Table3.8 Descriptive sensory analysis of CSB and SSB products

		11.75% Solids (USDA 2005)					20% Solids (Webb et al. 2011)				
		SSB Hi	SSB Lo	CSB 13	CSB Hi	CSB Lo	SSB Hi	SSB Lo	CSB 13	CSB Hi	CSB Lo
<b>Aroma</b>	Overall Grain	7.97 <sup>a</sup>	4.78 <sup>de</sup>	6.53 <sup>b</sup>	6.72 <sup>b</sup>	5.28 <sup>cde</sup>	4.56 <sup>c</sup>	4.61 <sup>e</sup>	5.42 <sup>cd</sup>	6.61 <sup>b</sup>	6.03 <sup>bc</sup>
	Musty Overall	5.83 <sup>a</sup>	3.69 <sup>cbd</sup>	3.81 <sup>cb</sup>	3.78 <sup>cbd</sup>	3.83 <sup>cb</sup>	3.64 <sup>cbd</sup>	3.67 <sup>cbd</sup>	4.03 <sup>b</sup>	3.28 <sup>d</sup>	3.44 <sup>cd</sup>
	Cardboard	6.14 <sup>a</sup>	3.36 <sup>e</sup>	4.03 <sup>cd</sup>	3.75 <sup>cde</sup>	3.78 <sup>cde</sup>	4.31 <sup>cb</sup>	4.11 <sup>cb</sup>	4.67 <sup>b</sup>	3.44 <sup>de</sup>	3.42 <sup>de</sup>
<b>Texture</b>	Thickness	4.00 <sup>de</sup>	2.56 <sup>f</sup>	5.03 <sup>cd</sup>	2.64 <sup>f</sup>	3.39 <sup>ef</sup>	5.72 <sup>c</sup>	8.31 <sup>b</sup>	12.33 <sup>a</sup>	7.89 <sup>b</sup>	8.19 <sup>b</sup>
	Particles	10.92 <sup>a</sup>	8.64 <sup>b</sup>	7.78 <sup>b</sup>	5.06 <sup>c</sup>	1.81 <sup>d</sup>	11.22 <sup>a</sup>	11.31 <sup>a</sup>	12.00 <sup>a</sup>	10.75 <sup>a</sup>	10.67 <sup>a</sup>
	Lumpy (size)	0.44 <sup>f</sup>	0.53 <sup>f</sup>	6.58 <sup>cd</sup>	3.03 <sup>e</sup>	1.00 <sup>f</sup>	5.39 <sup>d</sup>	5.19 <sup>d</sup>	11.44 <sup>a</sup>	8.39 <sup>b</sup>	7.89 <sup>b</sup> <sup>c</sup>
	Uniformity of Size	11.72 <sup>a</sup>	11.33 <sup>a</sup>	8.94 <sup>b</sup>	8.39 <sup>b</sup>	8.31 <sup>b</sup>	7.11 <sup>bc</sup>	7.50 <sup>bc</sup>	4.64 <sup>d</sup>	6.08 <sup>cd</sup>	5.41 <sup>cd</sup>
	Adhesiveness	2.22 <sup>f</sup>	0.89 <sup>g</sup>	3.61 <sup>e</sup>	1.86 <sup>f</sup>	0.89 <sup>g</sup>	9.06 <sup>d</sup>	10.14 <sup>c</sup>	11.44 <sup>a</sup>	10.50 <sup>bc</sup>	11.08 <sup>ab</sup>
	Gumminess	0.50 <sup>d</sup>	0.39 <sup>d</sup>	2.42 <sup>c</sup>	0.92 <sup>d</sup>	0.36 <sup>d</sup>	6.11 <sup>b</sup>	7.28 <sup>a</sup>	7.36 <sup>a</sup>	7.55 <sup>a</sup>	5.92 <sup>b</sup>
	Oily Mouth-feel	2.67 <sup>c</sup>	4.83 <sup>a</sup>	3.22 <sup>bc</sup>	2.86 <sup>c</sup>	3.72 <sup>b</sup>	2.94 <sup>bc</sup>	2.61 <sup>c</sup>	1.56 <sup>d</sup>	2.67 <sup>c</sup>	2.92 <sup>bc</sup>
	Residual Particles	9.31 <sup>a</sup>	9.86 <sup>a</sup>	5.86 <sup>c</sup>	4.11 <sup>d</sup>	2.81 <sup>d</sup>	7.06 <sup>bc</sup>	7.25 <sup>bc</sup>	7.86 <sup>b</sup>	6.19 <sup>c</sup>	6.11 <sup>c</sup>
	Mouth Drying	4.14 <sup>cd</sup>	3.86 <sup>d</sup>	2.61 <sup>e</sup>	2.78 <sup>e</sup>	2.92 <sup>e</sup>	5.08 <sup>ab</sup>	4.72 <sup>bc</sup>	5.47 <sup>a</sup>	5.06 <sup>ab</sup>	4.39 <sup>cd</sup>
	Mouth Coating	9.89 <sup>a</sup>	9.56 <sup>ab</sup>	5.14 <sup>d</sup>	4.47 <sup>d</sup>	4.03 <sup>d</sup>	8.56 <sup>bc</sup>	8.69 <sup>abc</sup>	8.89 <sup>abc</sup>	8.39 <sup>bc</sup>	7.67 <sup>c</sup>
	<b>Flavor</b>	Overall Grain	8.61 <sup>a</sup>	6.97 <sup>cd</sup>	6.83 <sup>d</sup>	7.06 <sup>cd</sup>	6.14 <sup>e</sup>	7.08 <sup>cd</sup>	7.36 <sup>bc</sup>	7.42 <sup>bc</sup>	7.64 <sup>b</sup>
Sorghum		6.78 <sup>a</sup>	4.83 <sup>b</sup>	1.69 <sup>ef</sup>	2.53 <sup>d</sup>	1.64 <sup>ef</sup>	3.67 <sup>c</sup>	3.42 <sup>c</sup>	2.11 <sup>de</sup>	2.06 <sup>de</sup>	1.28 <sup>f</sup>
Oat ID		0.00 <sup>e</sup>	1.28 <sup>ab</sup>	1.53 <sup>a</sup>	1.58 <sup>a</sup>	1.58 <sup>a</sup>	1.06 <sup>cd</sup>	1.08 <sup>cd</sup>	1.00 <sup>cd</sup>	0.83 <sup>d</sup>	1.31 <sup>bc</sup>
Wheat		0.33 <sup>f</sup>	1.19 <sup>c</sup>	2.08 <sup>d</sup>	2.81 <sup>ab</sup>	2.11 <sup>cd</sup>	2.50 <sup>bcd</sup>	2.39 <sup>cd</sup>	2.64 <sup>abc</sup>	2.97 <sup>a</sup>	2.44 <sup>abc</sup>
Corn		0.00 <sup>f</sup>	1.06 <sup>e</sup>	5.39 <sup>a</sup>	2.17 <sup>d</sup>	3.28 <sup>c</sup>	1.19 <sup>e</sup>	0.92 <sup>ef</sup>	4.69 <sup>b</sup>	1.11 <sup>e</sup>	3.44 <sup>c</sup>
Beany		3.17 <sup>a</sup>	2.25 <sup>bcd</sup>	2.31 <sup>bc</sup>	1.94 <sup>cd</sup>	1.72 <sup>d</sup>	2.94 <sup>ab</sup>	2.61 <sup>bc</sup>	2.58 <sup>bc</sup>	2.89 <sup>ab</sup>	2.69 <sup>b</sup>
Soy		3.14 <sup>a</sup>	1.97 <sup>b</sup>	1.17 <sup>de</sup>	1.67 <sup>bcd</sup>	1.39 <sup>cde</sup>	1.97 <sup>bc</sup>	1.81 <sup>bcd</sup>	2.28 <sup>b</sup>	2.11 <sup>b</sup>	1.08 <sup>e</sup>
Musty		5.81 <sup>a</sup>	3.36 <sup>d</sup>	3.36 <sup>d</sup>	2.89 <sup>e</sup>	3.00 <sup>de</sup>	4.08 <sup>c</sup>	4.06 <sup>c</sup>	4.67 <sup>b</sup>	4.06 <sup>c</sup>	4.44 <sup>bc</sup>
Starch		4.25 <sup>bc</sup>	3.64 <sup>c</sup>	4.94 <sup>b</sup>	4.81 <sup>b</sup>	3.97 <sup>c</sup>	8.19 <sup>a</sup>	7.94 <sup>a</sup>	8.47 <sup>a</sup>	8.28 <sup>a</sup>	8.19 <sup>a</sup>
Toasted		2.47 <sup>cd</sup>	1.92 <sup>ef</sup>	2.33 <sup>de</sup>	3.50 <sup>b</sup>	1.67 <sup>f</sup>	2.69 <sup>cd</sup>	3.00 <sup>c</sup>	3.06 <sup>c</sup>	4.25 <sup>a</sup>	3.11 <sup>c</sup>
Sweet		0.81 <sup>c</sup>	0.89 <sup>bc</sup>	1.39 <sup>ab</sup>	1.06 <sup>bc</sup>	1.17 <sup>ab</sup>	0.83 <sup>c</sup>	0.89 <sup>c</sup>	0.83 <sup>c</sup>	1.00 <sup>bc</sup>	0.97 <sup>bc</sup>
Bitter		5.06 <sup>a</sup>	4.50 <sup>b</sup>	3.44 <sup>de</sup>	3.64 <sup>cde</sup>	3.31 <sup>e</sup>	3.56 <sup>cd</sup>	3.75 <sup>c</sup>	4.42 <sup>ab</sup>	3.64 <sup>cd</sup>	3.31 <sup>de</sup>
Sour		1.89 <sup>a</sup>	1.39 <sup>cd</sup>	1.50 <sup>bcd</sup>	1.33 <sup>d</sup>	1.33 <sup>d</sup>	1.64 <sup>ab</sup>	1.67 <sup>ab</sup>	1.83 <sup>a</sup>	1.61 <sup>abc</sup>	1.42 <sup>bcd</sup>
Astringent		4.08 <sup>a</sup>	3.42 <sup>b</sup>	2.58 <sup>c</sup>	2.64 <sup>c</sup>	2.06 <sup>d</sup>	3.31 <sup>b</sup>	3.44 <sup>ab</sup>	3.86 <sup>a</sup>	3.44 <sup>ab</sup>	3.31 <sup>b</sup>

Products with different letters are significantly different from each other in that attribute ( $p < 0.05$ ).

Table 3.9 Bostwick flow rates of SSB-HI and CSB-Control.

Measurements are given as average  $\pm$  s.d.

<b>Product</b>	<b>Flow at 11.75%</b> <b>(cm/min)</b>	<b>Water loss</b> <b>adjustment</b> <b>(ml)</b>	<b>Flow at 20%</b> <b>(cm/min)</b>	<b>Water loss adjustment</b> <b>(ml)</b>
SSB HI	20 $\pm$ 0.3	20 $\pm$ 1.4	9 $\pm$ 0.4	18 $\pm$ 0.4
CSB 13	12 $\pm$ 0.5	19 $\pm$ 3.1	0 $\pm$ 0	20 $\pm$ 3.2

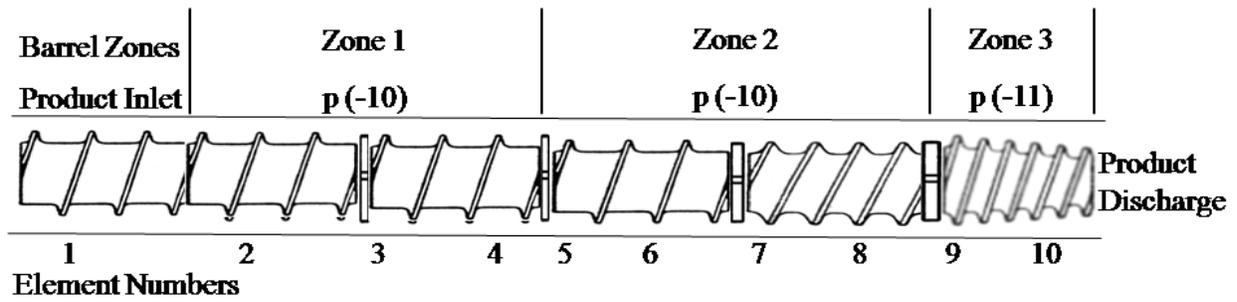


Figure 3.1 Schematic of pilot-scale single screw profile and barrel zones.

Element Numbers 1-2 =single flight screws; 3=small steam lock; 4=single flight screw; 5=small steam lock; 6=single flight screw; 7=medium steam lock; 8=double flight screw; 9=large steam lock and 10= triple flight uncut cone. P (-10, -11) in barrel zones 1,2 and 3 indicate cold water flow to cool extruder barrel for all treatments including SSB, CSB and WCSB.

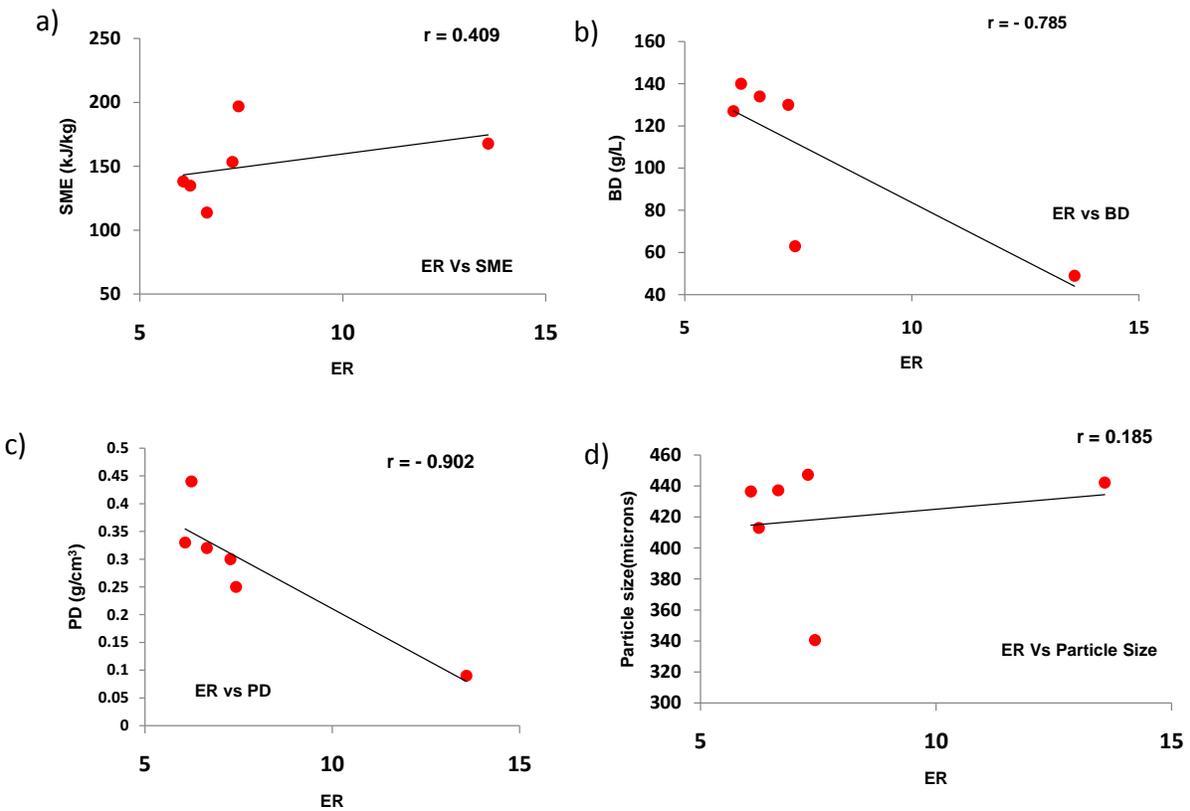


Figure 3.2 Intermediate product relationships and its impact on expansion

- a) Moderate correlation showing expansion increases at higher SME's
- b), c) - showing high inverse correlations with expansion to product bulk and piece density
- d) Expansion ratio showed weak relationship to particle size of extrudates post-milling

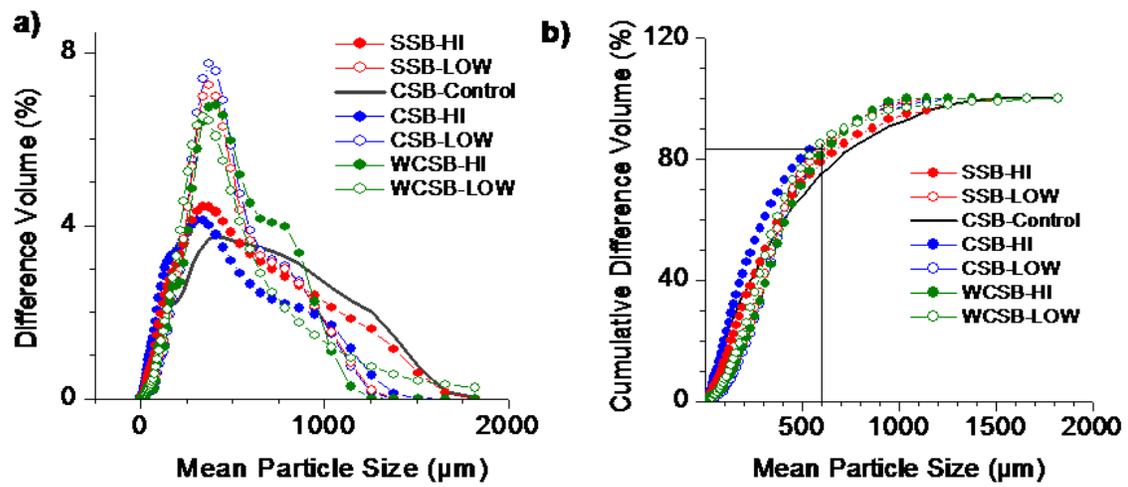


Figure 3.3 Particle size distribution of extruded and milled blends

Mean particle sizes obtained by particle size analysis as (a) the distribution of % difference volume (*left*) and (b) cumulative difference volume (*right*) of milled blends. Solid lines in (b) indicate the critical point of particle uniformity through a 600 µm sieve.

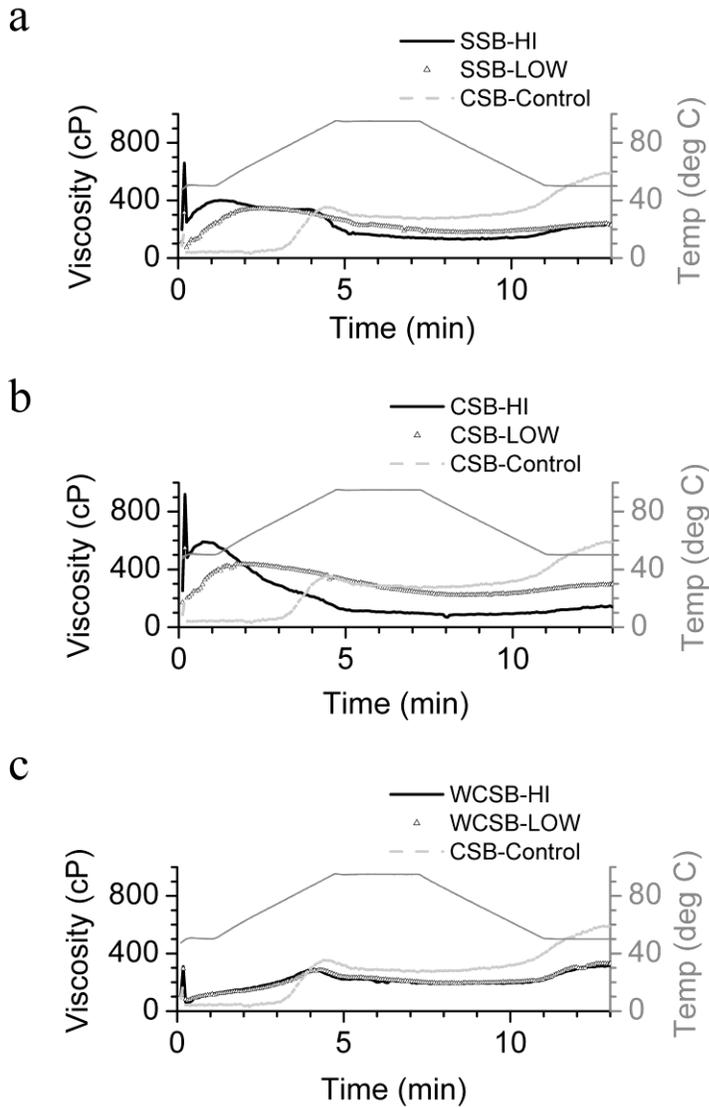


Figure 3.4 RVA pasting curves

Viscosity profiles of (a) SSB-HI (black trace) and SSB-LO (symbols), (b) CSB-HI (black trace) and CSB-LO (symbols) and (c) WCSB-HI (black trace) and WCSB-LO (symbols) in comparison with the viscosity profile of CSB Control (dashed grey trace). The temperature protocol (grey trace) applied to the samples is plotted on the right axis. Samples were ground in a roller mill with 0.2 mm roll clearance and sieved through a 900  $\mu\text{m}$  screen before RVA analysis.

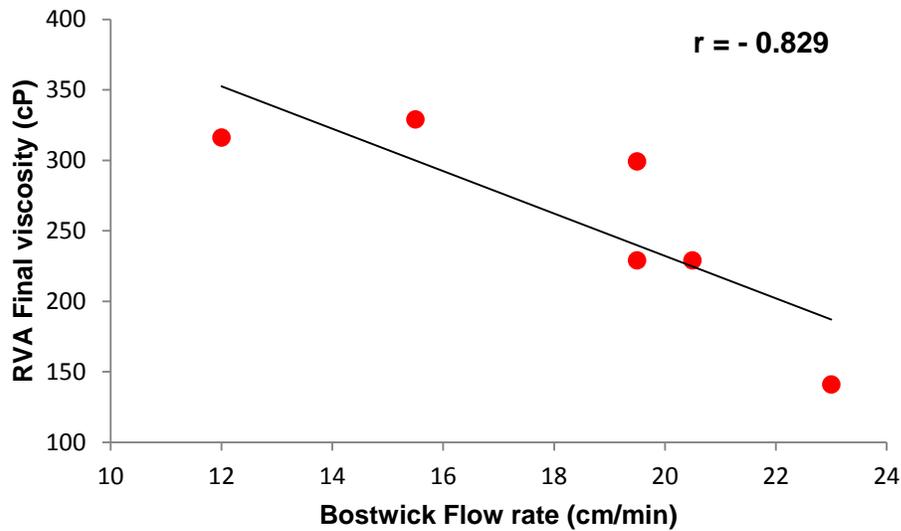


Figure 3.5 Correlation between Bostwick flow and RVA final viscosity at 50°C

Correlation coefficient was obtained by fitting the data points with the equation  $y = rx + b$ . A correlation coefficient of +1 or -1 indicates a strong linear relationship between the Bostwick flow rate and the RVA final viscosity.

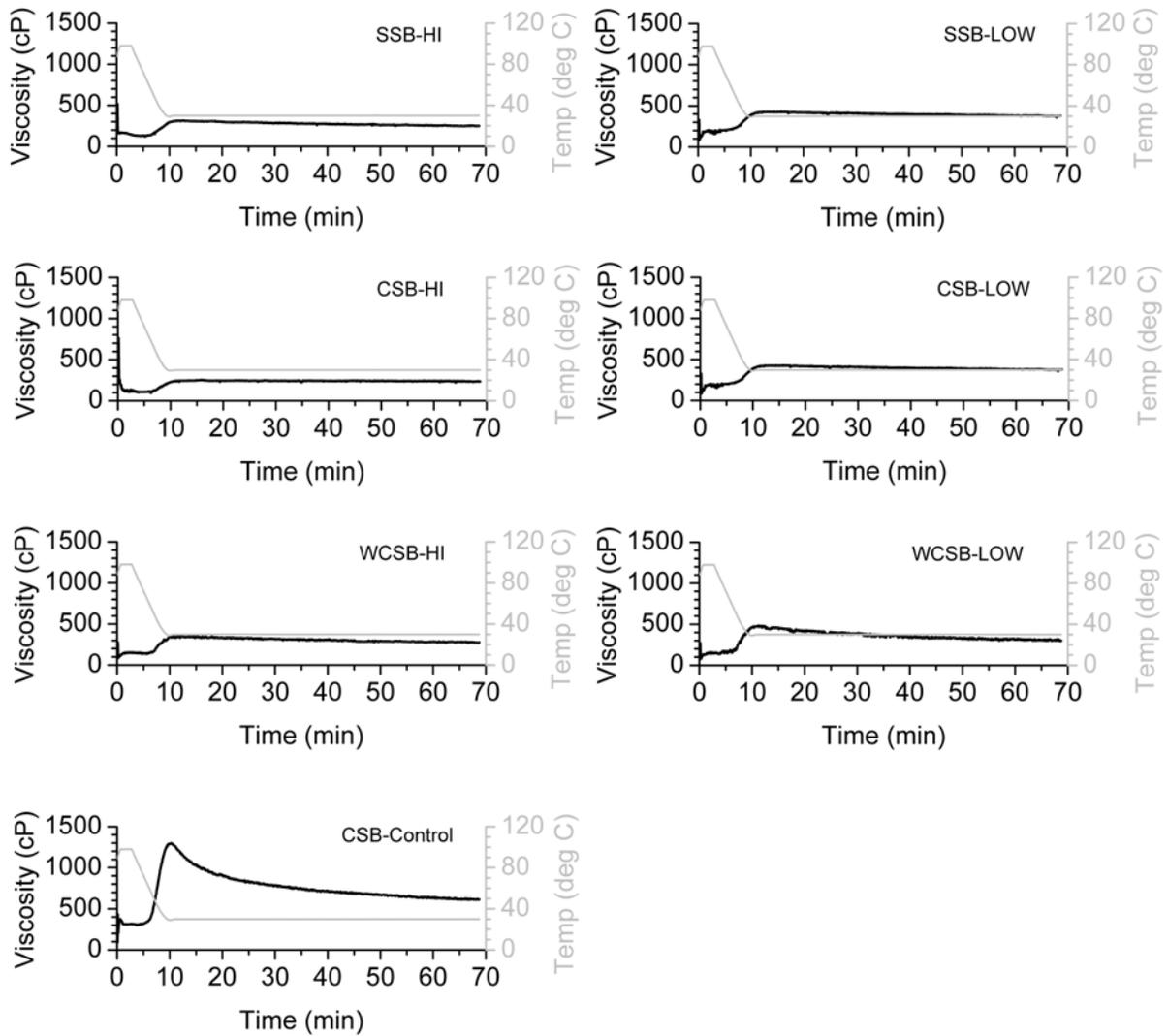


Figure 3.6 RVA pasting curves using Bostwick measurement protocols

Viscosity profiles (black traces) obtained using temperature protocols (grey traces) simulating cooking conditions of Bostwick flow measurements.

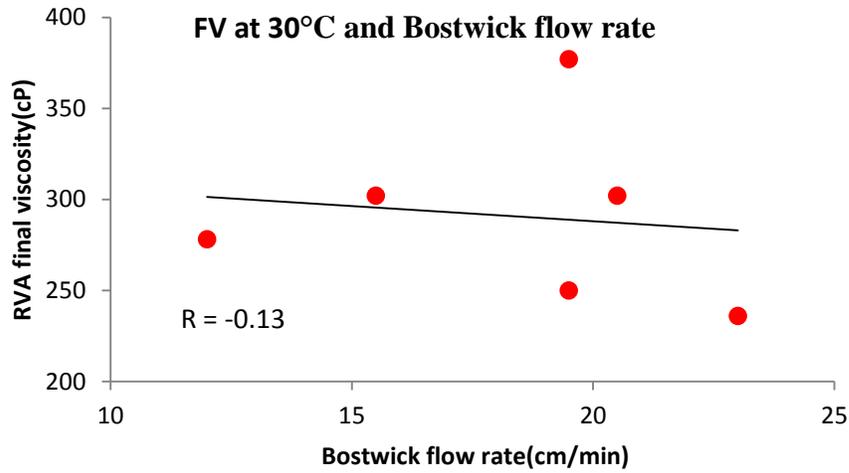


Figure 3.7 Correlation between Bostwick flow and RVA final viscosity at 30°C

Correlation coefficient was obtained by fitting the data points with the equation  $y = rx + b$ . A correlation coefficient of +1 or -1 indicates a strong linear relationship between the Bostwick flow rate and the RVA final viscosity.

## Appendix A - References used in Sensory Analysis – 15.0 point intensity scale with 0.5 increments

*Clean out: Warm water, mozzarella cheese, apples and carrots*

### **AROMA:**

Grain Overall: A general term used to describe the aromatic which includes musty, dusty, slightly brown, slightly sweet and is associated with harvested grains and dry grain stems.

Reference: 2 Tablespoons Bob's Red Mill Wheat Bran = 6.5

Preparation: Serve in a 12 oz brandy snifter covered with a watch glass.

Musty Overall: A combination of one or more aromatic impressions characterized to some degree as being somewhat dry, dusty, damp, earthy, stale, sour, or moldy. If identifiable, attribute will be listed.

Reference: 1,2,4 Trimethoxybenzene 50,000 ppm = 4.0

Geosmin 10 ppm = 8.0

Preparation: Dip an Orlandi Perfumer Strip #27995 2.2cm (second marking line) into each solution and place dipped end up in a Fisherbrand Disposable Borosilicate Glass Tubes with Threaded End (15x150mm), cap.

Cardboard The aromatics associated with cardboard or paper packaging.

Reference: Cardboard soaked in water, covered with watch glass= 7.5 (aroma)

Preparation: Place 2" square piece of cardboard in a medium snifter. Cover with ½ cup of water. Cover with a watch glass.

### **TEXTURE/ MOUTHFEEL:**

Thickness/ Viscosity: A measure of the consistency of the product when manipulate against roof of mouth with tongue.

Reference: Dillon's Whipping Cream = 4.0

Jell-O Instant Vanilla Pudding = 7.0

Highland Sour Cream = 14.0

Preparation: Place whipping cream in 1 oz cup.

Jell-O pudding: Prepare according to directions

Particles: The perception of small pieces relatively harder than surrounding product.

(amount)

Reference: Hunt's Snack Pack Tapioca Pudding = 2.0

Kozy Shack Rice Pudding = 8.0

Hunt's Snack Pack No Sugar Added Vanilla Pudding with  
Baker's Angel Flake Coconut Sweetened = 10.0

Preparation: Empty two 3.5 ounce containers of pudding into a bowl.  
Mix in ½ cup of flaked coconut until evenly distributed in  
the pudding. Fully fill in 3.5 oz. cup. Prepare the day of  
panel.

Lumpy (size) The perception of the size of lumps within sample (small to large).

Reference: Hunt's Snack Pack Tapioca Pudding = 3.0

Kozy Shack Rice Pudding = 9.0

Hunt's Snack Pack No Sugar Added Vanilla Pudding with  
Baker's Angel Flake Coconut Sweetened = 12.0

Preparation: Empty two 3.5 ounce containers of pudding into a bowl.  
Mix in ½ cup of flaked coconut until evenly distributed in  
the pudding.

Uniformity of size: The degree to which all of the particles are the same size rather than a  
mixture of different sizes.

Reference: Kroger Low Fat Cottage cheese = 6.5

Quaker Yellow Corn Meal = 12.0

Adhesiveness: Degree to which sample adheres to mouth/ palette surfaces during  
mastication.

Reference: Quaker Quick Oats (Cooked) = 3.5

Kozy Shack Rice Pudding = 10.0

Jif Peanut Butter = 13.0

Preparation: Quick Oats: For 6 panelists, boil 1 cup water on stove top.  
Stir in ½ cup oats, cook for 1 minute, stirring occasionally.

Gumminess: A sticky, gluey impression perceived in product during mastication.

Reference: Kozy Shack Rice Pudding = 6.0

Oily Mouthfeel: Related to the perceived fat content. Refers to the intensity of the oily feeling in the mouth when the product is manipulated between tongue and palate

Reference: Hellman's regular Mayo = 7.5

Babybel Regular cheese = 5.0

#### After Swallowing

Residual Particles: The amount of small pieces left around the teeth and mouth surfaces after swallowing.

Reference: Cheerios = 3.0 (4 pieces)

General Mills Wheaties = 7.0 (3 pieces)

Hunt's Snack Pack No Sugar Added Vanilla Pudding with

Baker's Angel Flake Coconut Sweetened = 9.0

Post Shredded Wheat (Spoon size) = 10.0 (1 piece)

Preparation: Empty two 3.5 ounce containers of pudding into a bowl.

Mix in ½ cup of flaked coconut until evenly distributed in the pudding.

Mouth Drying: The drying, puckering sensation on the tongue and other mouth surfaces.

Reference: 0.03% Alum solution = 1.5

0.050% alum solution = 2.5

0.100% alum solution = 5.0

Overall

**Mouthcoating:** The perception of a film left in the mouth after swallowing that maybe described as one or more of the following: slick, sticky, or starchy.

**Reference:** Hunt's Snack Pack No Sugar Added Vanilla Pudding with Baker's Angel Flake Coconut Sweetened = 5.0  
Hunt's Snack Pack Tapioca Pudding = 5.0  
Philadelphia Fat Free Cream Cheese = 9.5

**Preparation:** Empty two 3.5 ounce containers of pudding into a bowl. Mix in ½ cup of flaked coconut until evenly distributed in the pudding. Fully fill in 3.5 oz. cup. Prepare the day of panel.

## **AMPLITUDE:**

**Blendedness:** The combination of flavor notes that interact to create an equally balanced character in the product. Flavors appropriate to the product will enhance blendedness intensity as long as they are well balanced. Flavors not appropriate to the product will detract from this intensity.

**Reference:** General Mills Corn Chex = 10.0  
General Mills Wheaties = 4.0

**Fullness:** The degree of a general perception of robust flavor that rounded with body. This includes an initial high impact with multi-dimensional presentation in aromatics and flavor notes.

**Reference:** General Mills Wheaties = 8.0  
Quaker Quick Oats (cooked) = 3.0

**Preparation:** Quick Oats: For 6 panelists, boil 1 cup water on stove top. Stir in ½ cup oats, cook for 1 minute, stirring occasionally.

## **FLAVOR**

**OverallGrain:** A general term used to describe the light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice and oats.

**Reference:** Cereal Mix (dry) = 8.0 (flavor)

Preparation: Mix ½ cup of each General Mills Rice Chex, Wheaties and Quaker Quick Oats. Put in a blender and “pulse” blend into small particles.

Sorghum: A slightly sweet, musty/dusty aromatic with characteristics of chalkiness, starch and astringency. May also include slightly green and bitter  
Reference: Bob’s Red Mill sweet white sorghum flour = 5.0

Oat ID: A slightly brown, dusty aroma characteristic of oats  
Reference: Quaker Quick Oats (Uncooked) = 8.0 (flavor)

Wheat: A brown, musty/dusty, slightly nutty aromatic characteristic of products made from wheat.

Reference: Gold Medal All-Purpose Flour = 4.5 (flavor)  
Cooked American Beauty elbow macaroni = 3.5 (aroma), 5.0 (flavor)  
Gold Medal Whole Wheat Flour = 6.0 (flavor)

Preparation: All-Purpose flour: Bake 1 cup of all-purpose flour for 5 minutes at 350°F.

American Beauty macaroni: Bring 3 cups water to a rapid boil. Add 1 cup pasta & stir, returning to a rapid boil. Cook 6 minutes, stirring occasionally.  
Drain and put in cups.

Barley: A light baked barley grain aromatic.  
Reference: Quaker Quick Barley = 6.0 (flavor) (Cooked without salt)

Preparation: Soak ¼ cup barley in ½ cup water overnight (optional).  
Boil ¾ cups water, add barley (drained if pre-soaked) to the boiling water. Cover with a lid, reduce the heat to low.  
Cook 15 minutes if pre-soaked, 35 minutes if not.

Corn: Grain aromatics characteristic of corn.  
Reference: Quaker Yellow Corn Meal = 5.0 (flavor)  
General Mills Corn Chex = 7.5 (flavor)  
Toasted Corn Doritos = 8.0 (flavor)

Beany: Aromatics characteristic of beans. Most include green, pea pod, nutty, brown, soy. Also include starchy, musty/earthy, musty/dusty, sour aromatics, powdery feel, and one or more of the characteristics.  
References: Bush's Pinto Beans=5.5  
Preparation: Drain beans and rinse with de-ionized water.

Soy: Flavor associated with soybeans or soy products.  
Reference: Soy nuts (Hy-Vee Bulk) = 4.5

Musty: Aromatics associated with wet grain and damp earth.  
Reference: Cooked American Beauty elbow macaroni = 5.0  
Fresh mushroom = 10.5 (aroma)  
Preparation: Place 1 sliced button mushroom in a medium snifter.  
American Beauty macaroni: Bring 3 cups water to a rapid boil. Add 1 cup pasta & stir, returning to a rapid boil. Cook 6 minutes, stirring occasionally. Drain and put in cups.

Starch: The dry aromatic associated with starch and starch based grain product such as wheat, rice, oat and other grains  
Reference: Argo Corn Starch in Water = 3.0 (flavor)  
Cereal Mix (dry) = 7.0 (flavor)  
Cooked American Beauty elbow macaroni = 9.0 (flavor)  
3.5% Argo corn starch gel in water = 11.0 (flavor)  
Preparation: Mix 1 gram corn starch in 100ml water.  
○ Mix ½ cup of each General Mills Rice Chex, Wheaties and Quaker Quick Oats. Put in a blender and “pulse” blend into small particles.

- American Beauty macaroni: Bring 3 cups water to a rapid boil. Add 1 cup pasta & stir, returning to a rapid boil. Cook 6 minutes, stirring occasionally. Drain and put in cups.
- Starch = 11.0: Heat 250 ml of water. Dissolve cornstarch (17.5g) in 250 ml of cool water and slowly add to the heated water. Bring mix to a boil over medium-high heat, stir constantly. Let boil for 1 minutes, remove from heat and let cool. Pour in 1 oz cups.

Chicken-like	An impression of poultry flavor specifically identified as chicken. Reference: Swanson's Reduced Sodium Chicken Broth = 4.0 (flavor)
Toasted:	A moderately browned/ baked impression. Reference: Post Shredded Wheat (Spoon size) = 3.5 (flavor) General Mills Cheerios = 7.0 (flavor)
Sweet:	A fundamental taste factor of which sucrose is typical. Reference: 2% Sucrose Solution = 2.0
Bitter:	The fundamental taste factor of which caffeine or quinine is typical. Reference: 0.01% Caffeine Solution = 2.0 0.02% Caffeine Solution = 3.5 0.035% Caffeine Solution = 5.0
Sour:	A fundamental taste factor of which citric acid in water is typical. Reference: 0.015% Citric Acid Solution = 1.5
Astringent:	The drying, puckering sensation on the tongue and other mouth surfaces. Reference: 0.03% Alum solution = 1.5 0.050% alum solution = 2.5 0.100% alum solution = 5.0
Fullness:	The foundation (base) and interplay of flavor notes that give substance to the product (bloom/mouthfullness).

## Chapter 4 - Physicochemical Properties and Shelf-life of Newly Developed Sorghum-SoyBlend

### Abstract

Fortified blended foods used as porridge in supplementary feeding programs deliver improved nutrition, but there is need to diversify the range of commodities used. The impact of decortication level and process conditions at low (350 rpm), medium (400 rpm) and high (450 rpm) shear was investigated with respect to sorghum-based extruded blends. Degree of gelatinization of the whole sorghum-soy blend (WSSB) and decorticated sorghum-soy blend (DSSB) extrudates ranged from 93-97%. Expansion ratio (ER=3.6-6.1) was correlated with specific mechanical energy input (SME=145-415 kJ/kg;  $r = 0.99$ ) and average particle size after milling (PS=336-474 microns;  $r = -0.75$ ). Rehydrated blends at 20% solids concentration provided recommended energy density (0.8 kcal/g) for FBFs. Bostwick flow rates had high correlation ( $r = -0.91$ ) with pasting data (final viscosity) obtained using rapid visco analyzer (RVA). Addition of oil (5.5%) prior to extrusion was also studied, and resulted in process instabilities and also lower shelf-life as determined via descriptive sensory analysis (rancid and painty attributes) and gas chromatography-mass spectroscopy (hexanal, heptenal and octanal concentrations). Extruded sorghum-soy blends met standard specifications for energy density and consistency (Bostwick flow rate), and were superior in some aspects as compared to extruded corn-soy blends and traditional corn-soy blends (CSB13). Relationships between extrusion mechanical energy input, expansion, particle size after milling and consistency of rehydrated blends were established. Consistency of the rehydrated blends is an extremely important criterion as it affects the ease of ingestion by target consumers (children below 5 years, in this case).

## **Introduction**

Over the last four decades, the US government has continued to provide food aid primarily through the Food for Peace program, also known as US Public Law 480 (PL480). Title II is the largest of the PL480 program and deals with combating malnutrition, especially in children and mothers (7USC, Section 1721)(Fleige et al. 2010a).

Section 204 of PL480 of 7 USC Sec. 1724 requires 75% of agricultural commodities for non-emergency programs to be fortified, processed and shipped as a bagged commodity. Fortified blended foods (FBFs) are cereal-legume flour composites that undergo treatments such as blending and processing followed by micronutrient fortification with around 18 different vitamin and mineral premixes (Fleige et al. 2010b). The FBF thus developed serves as a protein-rich, micronutrient-reinvigorated food supplement suitable for children aged 6 and 24 months (pre-schooled-aged children) suffering from moderate-to-acute-malnutrition. FBFs have undergone transition from its early inception of corn soy milk with a less expensive corn soy blend (CSB) (Marchione. 2002) consumed as a weaning food. CSB, classified as ready-to-use supplementary foods, has evolved rapidly with identifier codes such as CSB10,11,12,13 & 14 and instant corn soy blend between 2005 and 2011 with enhancements to its macro and micronutrients, protein, and energy-density profiles. The most recent version implemented by USAID's partner - World Food Program is called "Super Cereal- CSB++" with the inclusion of dry skim milk and upgrades to its micronutrient profile. However, in recent years, emphasis on the inclusion of dietary fat has been pivotal for neurodevelopment, ability to absorb fat soluble vitamins and visual acuity in early childhood years. Besides increasing EFA (Essential Fatty Acid), addition of oil in FBFs increases sensory attributes and product acceptability in infants.

While the inclusion of dietary fat is estimated between 19 and 28% for infants between 6 and 23 months (Lutter and Dewey. 2003a); (Lutter and Dewey. 2003b), CSB meets only 16.7% of the required dietary fat out of the minimum 26% EFA in the formulation (Fleige et al. 2010a). As per the new recommendation in the FAQR to Title II program improvements, 15 gram of oil for every 50 grams of FBF is added in CSB 14. This intake provides two-thirds of fat requirements, 80 percent energy and 100% gross protein when combined with breast feeding. This standard is established based on earlier studies by (Dewey et al. 2004) who suggested 35 grams of oil per day for non-breastfed children aged 6-24 months. The inclusion of soybean oil in FBFs contains polyunsaturated fatty acid (PUFA) or linoleic (C18:2) and linolenic (C18:3) with fatty acid composed of 56.2 and 7.8 wt % respectively (Gunstone. 2002). This would constitute alpha-linolenic acid ( $\omega$ -3) of 1.96 g/30.0 g and linoleic acid ( $\omega$ -6) of 15.0g/30.0g ((Institute of Medicine. 2005). Consequently, the processing challenges by including vegetable oil in FBF is susceptible to oxidation due to high concentration of PUFAs in the formulation. With the highest methylene bridge number, PUFAs oxidation rates in the unsaturated fatty acid series of steric, oleic, linoleic, linolenic is in the ratio of 1:100:1200:2500 respectively (DeMan. 1999). When these fatty acids are exposed to heat during processing, they rapidly decompose by the generation of free radicals as the rate of peroxides formed increase with temperature ((Hammond et al. 2005). As a measure to improve the formulation of existing FBFs, (Webb et al. 2011)FAQR (Recommendation #18) (Webb et al. 2011) encourage blend combinations of sorghum-soy, sorghum-pea, millet-soy and rice-soy besides traditional cereals such as wheat and corn which are currently being used to produce FBFs. Nevertheless, the FAQR advocates food-processing as its key focus to address anti-nutrition factors such as the phytate in cereals which inhibit iron or zinc absorption and the enhancement of product packaging and shelf-life.

Food extruders provide thermo-mechanical energies and high shear through the screws necessary to cause the required physicochemical changes with blended cereals. It is known that food extruders provides intense mixing and shear to gelatinize starch, denature proteins, inactivate enzymes and annihilate micro-organisms making FBFs both palatable and digestible.

Five different types of whole and decorticated sorghum (Marcia-White, NK283, Red Swazi, NS 5511 and Framida (Red) showed significant reduction in total phenols and tannin content with extrusion processing. While decortication (removal of pericarp and testa) reduced 82-83% of phenols in both tannin and non-tannin sorghum, bioavailability of retained phenols is enhanced by extrusion processing in whole tannin sorghum in fermented and unfermented porridge types (Dlamini et al. 2007). From a protein digestibility stand point, sorghum, millet, quinoa, wheat, rye and corn showed significant improvement when extruded at 15% feed moisture with 100/150°C product temperature at screw speed of 100 rpm across cereals (Dahlin and Lorenz. 1993). Besides health benefits from dietary fiber, whole grains in food extrusion pose challenges due to increased levels of fat and fiber compared to refined flours and starches. This may affect degree of starch gelatinization, protein digestion and rancidity due to unstable lipids (Eastman and Lee. 2005). However, a recent study on corn starch and WPC blend with 15% added fiber extruded on a single screw showed 83-90% in vitro digestibility and a high degree of starch gelatinization (Santillan-Moreno et al. 2011).

The improvement of the newly developed sorghum-soy blend (SSB) has been geared towards the incorporation of vegetable oil to the base formulation (whole or decorticated sorghum flour, defatted soy flour and WPC80) prior to extrusion. This is done in view to enhance FBFs with lipid-based nutrient supplements (LNSs) that may offer a price benefit and consumer acceptability when designing the ration based on local programming needs (FAQR 2011).For the

first time, shelf-life procedures of FBFs have been performed based on USDAs commodity specification for CSB14 which include microbial and descriptive sensory analysis. The main focus of the study is aimed at establishing physico-chemical relationships and in assessing their raw material impacts with the decortication levels and inclusion of oil in the formulation, pre-extrusion. Post-extrusion processing and storage characteristics of FBFs in accelerated storage conditions and their sensory properties were also analyzed.

## **Materials and methods**

### ***Material procurement***

The raw materials required for producing sorghum-soy blend were procured from local suppliers in the US. Whole sorghum flour (AgVanced Enterprises, New Cambria, KS), decorticated sorghum flour (ADM milling Company, Overland Park, KS), bakers soy flour (ADM protein specialties division, Decatur, IL), whey protein concentrate (80)(Davisco Foods International, Inc, Le Sueur, MN), vitamin and mineral premixes (Research Products Company, Salina, KS), vegetable oil (soybean), (The J.M.Smucker Company, Orrville, OH).

### ***Formulations***

The base formulation consisted of whole or decorticated sorghum flour (69.42%), defatted soy flour (21.82%), and WPC-80 (3.10%) in conformance to CSB 14 (Webb et al. 2011)Table 4.1. Pre-weighed quantities of whole or decorticated sorghum flour, soy flour were mixed in a ribbon mixer (Wenger manufacturing, Sabetha, KS) for 3 minutes followed by the addition of WPC-80 for another 2 minutes. A total of 5 minutes of mixing time was accomplished in preparing the base formulation. WPC-80 was mixed separately using a Hobart Blender (Hobart Corporation, Troy, OH, USA) before being suspended into the ribbon mixer.

### ***Oil addition to selected premixed formulation prior to extrusion***

Vegetable oil (5.68%) was added to premixed formulation of each treatment of whole and decorticated sorghum-soy blends extruded at 450 rpm and 20% moisture content separately on the day of extrusion in a ribbon mixer for a total time of 8 minutes for homogeneity and avoidance of any lump formation in the premix before extrusion.

### ***Extrusion***

A pilot-scale single screw extruder (X-20, Wenger Manufacturing, Sabetha, KS) was used to process the fortified sorghum-soy blends with a length to diameter ratio (L/D) of 10 to 1. The extruder barrel consisted of 3 temperature zones with each head measuring 115 mm and the inlet head from the feed head measuring 85.72 mm. The extruder throughput was kept constant at 150 kg/hr (dry feed). The screw configuration and barrel zone temperatures are shown in Figure 4.1. The die geometry consisted of 3 die inserts with 4 circular openings in each die unit of 4.1 mm diameter wherein 3 holes in each die unit were blocked using a Teflon insert blocker to provide higher residence time and increase the degree of cook.

Three levels of mechanical energy were obtained by varying the extruder screw speeds (350, 400 and 450 rpm, respectively) and in-barrel moisture (~28%, ~24% & 20% wet basis respectively) to extrude whole and decorticated sorghum-soy blends (2 X 3 factorial design). Additionally, the experimental design included whole and decorticated sorghum-soy blends that were subject to oil addition (5.5%) prior to extrusion at high energy of 450 rpm with 20% process moisture (2 X 2 factorial design). The product was cut after exiting the extruder die with a face-mounted 3 blade rotary knife. Extruded product was dried at 104 °C (220 °F) in a double pass dryer/cooler (Series 4800, Wenger) adjusted for 10 min retention time (5 min each for the

top and bottom belts). Cooling was accomplished at room temperature, with a 5 min retention time on the cooling belt.

***Monitoring extruder conditions***

In-house data acquisition software (Lab view measurements, version 1.0) developed was administered to monitor the extruder conditions such as downspout temperature, barrel temperatures, water and steam in pre-conditioner and extruder barrel, motor load and extruder rpm.

***Specific mechanical energy (SME)***

Tests for specific mechanical energy (SME) were done in duplicate and mechanical energy input per unit mass of extrudate was calculated as shown in equation 1:

$$SME(kJ/kg) = \frac{\left(\frac{T}{100}\right)\left(\frac{N}{N_{rated}}\right)P_{rated}}{\dot{m}} \dots\dots\dots (1)$$

where T = net motor load percentage, N = screw speed (rpm), N<sub>rated</sub> = rated screw speed (507 rpm), P<sub>rated</sub> = rated power (37.3 kW), and  $\dot{m}$  = net mass flow rate (kg/s).

***Bulk density (BD)***

A cylindrical steel container (V<sub>c</sub> = 1L) was filled with extrudates and the weight was recorded. Bulk density (BD) of the extrudates was calculated as shown in equation 2 was measured in two occasions during product exit at the die end out of the extruder and after completion of drying process.

$$BD = \frac{\text{Weight of sample}}{V_c} \dots\dots\dots (2)$$

***Piece density(PD)***

(ρ) were obtained by dividing the mass of the sample (m<sub>piece</sub>) by its volume (V<sub>piece</sub>). Ten extrudate pieces were measured, and for each piece, the average of two diameter measurements was recorded with a digital caliper. The formula used as shown in equation 3;

$$\rho = \frac{m_{piece}}{V_{piece}} = \frac{3m_{piece}}{4\pi r^3} \dots\dots\dots (3)$$

***Expansion ratio (ER)***

The ER is the ratio of the extrudate cross-sectional area to the die orifice cross sectional area, and was calculated as shown in equation 4:

$$ER = \frac{d^2}{d_{die}^2} \dots\dots\dots (4)$$

Where d is the extrudate diameter (average of 10 determinations) and d<sub>die</sub> is the diameter, which was measured using a digital caliper.

***Proximate analysis***

The proximate composition of uncooked sorghum and soy flour was determined using standard methods ((AOAC International. 1999);(AOAC International. 2010); (AOAC International. 2009). This included determination of moisture (135°C for 2h; AOAC 930.15), crude protein (based on nitrogen by combustion, 6.25X; AOAC 990.03), crude fat (petroleum ether extract method; AOAC 920.39), ash (600°C for 2h; AOAC 942.05), crude fiber (filter bag technique utilizing H<sub>2</sub>SO<sub>4</sub> and NaOH digestion for Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY); AOCS (Ba 6a-05), and total starch (aqueous alcohol pretreatment; amyloglucosidase/α-amylase method; AOAC 996.11). Protein, starch, fat, ash and crude fiber contents were reported as dry basis percentages (% db) from replicates. Soluble, insoluble and

dietary fiber (TDF) content (as-is basis) in whole and decorticated flours were subjected to sequential enzymatic digestion by heat-stable  $\alpha$ -amylase, protease, and amyloglucosidase. (AACC 32-07.01. 1991).

### ***Milling***

Dried products were ground in a single pass using a tabletop roller mill (915/ 9X6, Ross Machine and Mill Supply, Inc., Oklahoma City, OK, USA) installed with two rollers having 20 corrugations per inch and 0.2 mm clearance between the rollers. It was sieved through a 900  $\mu$ m sieve using a mechanical shaker for 5 min before micronutrient fortification.

### ***Fortification***

Post-extrusion, the dried extrudates were milled and fortified with recommended levels of micronutrient premixes, vitamin(0.1) and mineral (3%) and vegetable oil (5.5%).Micronutrients were added to each formulation of the ground extrudate and mixed homogenously using a Hobart Blender – N50 (Hobart Corporation, Troy, OH, USA).

### ***Particle size analysis***

Particle size distribution of extruded and milled products was determined using a laser diffraction particle size analyzer (LS<sup>TM</sup> 13320, Beckman-Coulter, Inc., Miami, FL, USA). Duplicate tests were conducted for each sample.

### ***Product moisture***

Product moisture of milled extrudates was conducted by oven-drying method in which 2 g  $\pm$  1 mg of sample was placed in an aluminium pan and heated at 135°C for 2 hours based on air-oven method 44-19 (AACC. 2013). The lids were then replaced and the pans were transferred

to a desiccator to cool for 30 minutes. The difference in weight of the sample pre and post oven-drying was calculated. Duplicate tests were conducted for each sample.

### ***Pasting properties***

Pasting properties were obtained using a Rapid-Visco Analyzer (RVA), (RVA4, Newport Scientific Pvt. Ltd., Warriewood, NSW, Australia). Sample moisture was first adjusted to 14% dry-basis (db) by adding distilled water. For testing, approximately 3.5 g of sample was added to about 25 ml of distilled water in an aluminum test canister (Walker et al. 1988). The RVA was preheated to 50°C for 30 min prior to testing. A 13 min standard RVA temperature profile was used: 1 min holding at 50°C, 3 min 42 s temperature ramp up to 95°C, 2 min 30 s holding at 95°C, 3 min 48 s temperature ramp down to 50°C, and 2 min holding at 50°C (Batey and Curtin. 2000).

### ***Bostwick consistometer test***

Bostwick consistency for milled products was performed using a Bostwick Consistometer (CSC Scientific Company Inc., Fairfax, VA., USA). The Consistometer was placed on a flat surface and the leveling screws were adjusted until the bubble in the circular level was centered. Gruels of 20% were prepared by mixing 23.5 g of milled product into 97 ml of boiling distilled water. After 2 min of vigorous manual stirring while boiling, the slurry was removed from heat, stirred again for 30 s and immersed in a water bath at 30°C for 10 min. Slurries were adjusted for water-loss through evaporation to achieve a final weight of 117 g and stirred again before placing it back in the water bath at 30°C for 1 hr. 100 ml of this slurry was then poured into the compartment of the Bostwick Consistometer. After a settling time of 30 s, the gate was released and the slurry was allowed to flow through the graduated trough. The distance of flow was

recorded after exactly 1 min (USDA, 2005). Measurements for each product were obtained in triplicate.

### ***RVA pasting curves of modified temperature profile at 30°C***

Simulation of Bostwick conditions was developed with change in temperature and time in the RVA protocol setting. This was done to correlate the actual Bostwick results to the controlled conditions of RVA. The following steps were followed: temperature was ramped up to 98°C and held at 98°C for approximately 3 min, followed by a temperature ramp down to 30°C in 7 min and thereafter held at 30°C for 1 hr. The speed of the paddle was adjusted to 960 rpm for the first 10 sec to mix the sample homogeneously and avoid any lump formation and then reduced to 160 rpm for the entire duration of the test. Pasting properties, such as peak, trough and final viscosities, were determined. The viscosity was recorded in Windows based Thermocline software Version 2.0. (Newport Scientific Pvt. Ltd., Warriewood, NSW, Australia). Duplicate tests were conducted for each sample.

### ***Thermal Analysis - Differential scanning calorimeter (DSC)***

The thermograms for extruded and ground blends and raw blended flours of whole and decorticated sorghum soy blends were obtained with a DSC Q2000 TA (TA instruments, New Castle, DE, USA). The instrument cell constant was calibrated with indium using an empty pan as reference. The sample preparation included weighing 10 mg of ground extrudates in an aluminium pan. Distilled water was added (66.7%) to obtain solid to water ratio of 1:2 in the mixture (Zhu et al. 2010). The pans were hermetically sealed, placed in the DSC cell, and a running segment program as follows: Equilibrate at 10°C, heated from 10 to 160°C at a heating rate of 10°C/min, mark end of cycle, ramp 25°C/min to 10°C, mark end of cycle in the presence of nitrogen gas at a flow rate of 50 mL/min. The samples were again rescanned with a heating

rate of 10°C/min to 160°C as its final phase of the test. The TA Instruments Universal Analysis 2000 software(version 5.4.0) was used to analyze the resultant scans. Duplicate tests were conducted for each sample with rescan to assess degree of gelatinization, protein denaturation and amyloze-lipid complexes.Values of onset temperature ( $T_o$ ), peak gelatinization temperature ( $T_g$ ) , end-point ( $T_c$ ) and enthalpy ( $\Delta H,J/g$ ) were recordedfor melting endotherms.

### ***Shelf-life analysis***

Shelf-life study was set-up with real-time environment at 30°C and 65% relative humidity(15 months) and accelerated shelf-life test (ASLT) at 50°C and 70% relative humidity (~16 weeks). Temperature and humidity set points for real time setting were based on tropical weather in Tanzania and the average annual relative humidity in the region (SAGE. 2013). ASLT set points were based the well known  $Q_{10}$  factor (RAGNARSSON and LABUZA. 1977). The  $Q$  value is a temperature quotient and reflects the change in reaction rate for every 10°C rise in temperature expressed mathematically as  $Q_{10}$  (based on the assumption that for every 10C, deterioration factor is 2). In this study, 15 months of real time shelf-life of FBFs at 30°C equates to approximately 4 months (16 weeks) in ASLT at 50°C with a deteriorative  $Q_{10}$  reaction of 4 is calculated as shown in equation 5:

$$(Q_{10}) = \frac{\theta_{S(T_1)}}{\theta_{S(T+10)}} \dots\dots\dots (5)$$

where  $\Delta$  = temperature difference ,  $\theta_{S(T_1)}$  = shelf-life at 30°C (64 weeks); $\theta_{S(T + 10)}$  = shelf-life solved for 16 weeks at 50°C.

Table 4.2 illustrates estimated ASLT time point equivalents to its real time duration.

The design consisted comparative study of chosen four treatments with WSSB, WSSB (extruded with oil), WSSB and DSSB (extruded with oil) produced at 450 rpm and 20% process moisture. Ball glass jars (Broomfield, CL, USA) measuring 3x4.5x10.2 inches in diameter,

height and width respectively was used as a storage material for the study. The top-lids of the ball jars were replaced by actual packaging material which was crafted from a 25 kilogram paper bag manufactured as per FDA requirements for food products (21 CFR 177.1520,as amended). The packaging consisted of a multiwall paper bag of two inner walls of 50 pound nominal basis weight, natural craft paper and an outer third wall of 60 pound nominal basis weight wet strength paper in accordance with Uniform Freight Classification, Rule 40, Section 10, Table A and B. The bag also consisted of an inner plastic liner of low density polyethylene (LLDPE) film of 2.5 mm thickness with a density of 0.914 to 0.929 g/cc with a minimum heat-seal coefficient of 0.60. The film contained a minimum impact resistance of 265g in accordance with ASTM D-1709 Method A (USDA 2005).The canning jars were sanitized using 70% v/v alcohol and 150 grams of milled and fortified blends were filled under a sanitized controlled environment chamber to avoid any microbial contamination at the time of packing and storage. The study was conducted in two independent temperature and humidity controlled chambers (BIOCOLD Environmental Inc,Fenton, MO, USA) with compartment space dimensions of 11.4 x 8.10 x 8.9 ft in length, height and breadth respectively. Each chamber was sanitized and dried using a germicidal detergent (Sunflo Max 128, Kansas Correctional Industries, Lansing, KS, USA). The chambers were checked for tightly fitting doors, roof leaks, holes in walls, hard-packed floors to avoid any burrowing of rodents,and other potential risk aspects for any microbial intervention. The temperature and relative humidity logs were constantly recorded using circular recording charts from sensors inside the chambers (Honeywell, MN, USA) and every one hour data was verified with HOBO data loggers (onset, Bourne, MA, USA) that were placed in chamber beside the temperature and RH sensors.

### ***Shelf-life sample preparation***

Each of the sanitized ball jars were filled with approximately 150 grams of FBF samples based on surface area calculations per unit volume from packaging material. For WSSB and DSSB samples extruded without oil in the formulation, fresh vegetable oil (soybean oil) 5.5% (8.25 g/150g) was added and blended homogeneously in a Hobart mixer for 5 minutes before bottling and tightly sealed by screw lids and placed in shelf-life test chambers.

### ***Water activity***

Water activity of SSB samples were measured using chilled-mirror dew point hygrometer (Aqualab CX-2, Decagon Devices, Pullman, WA, USA). Samples were loaded in a disposable cup, completely covering the bottom of the cup and samples were below the half-full level to prevent sensor damage and accuracy with results. The sample drawer knob was turned to OPEN/LOAD position to pull the drawer open. The samples were placed in the drawer and checked for sample residue in the top lip of the cup. The knob was then turned to READ position to seal the sample cup with the chamber. Water activity and temperatures were recorded in less than 3 minutes for all samples and were displayed on a liquid crystal display screen after the instrument finished its read cycle accompanied by the beeper. Two replicates of each sample were tested.

### ***Mold and bacteria***

Mold and Bacterial counts were estimated using Dichloran-glycerol (DG-18) agar base (Oxoid, Basinkstoke, Hampshire, England) and plate count agar (PCA) (Beckton, Dickinson and Company, Sparks, MD, USA). Each of the media prepared was for a capacity of 750 ml distilled water to which 23.62 grams of DG-18 and 17.62 grams of PCA were added and placed on a

heater cum shaker (Thermolyne, Dubuque IA, USA) for 5 minutes with a magnetic stirrer placed inside each of the prepared media for homogenous mixing. The media were placed in an autoclave high pressure steam sterilizer (Yamato Scientific America Inc. Orangeburg, NY, USA) set to 121°C and a pressure of 29.0 psi. After 2 hours, both mediums were removed from the autoclave and were placed in the stirrer for 10 minutes. Thereafter to the DG-18 media, 75 mg of Chloramphenicol (Genlantis, San Diego, CA, USA) dissolved in 0.5 ml of ethyl alcohol (Decon Labs, King of Prussia, PA) was mixed and stirred again for 5 minutes. Both mediums were then poured into 100 x 15mm petri plates (Fisher Scientific, Pittsburg, PA) and were inverted after two hours after solidification. Thereafter, inoculums consisting of 100 ml distilled water for all samples were prepared by dissolving 0.1% (1 gm to 1 liter distilled water) of peptone (Beckton, Dickinson and Company, Sparks, MD, USA) and were placed in the autoclave at 121°C and removed after 2 hours. 9ml test tube samples were also prepared separately and autoclaved along with the inoculums. The blanks/inoculums were then treated with 10 grams of each sample (WSSB HI, DSSB HI, WSSB HI (Oil) & DSSB HI (Oil)), and contents were transferred into sterilized bags and placed in a stomacher (Seaward Medical Ltd, OGN, London, UK) for 120 seconds for a homogenous mixture. Serial dilutions ( $10^{-2}$  to  $10^{-3}$ ) were made and 0.1 mL aliquots were inoculated in duplicates onto the culture media and evenly spread using inseeded glass rods in a controlled environment chamber for all replicates. Incubation time, temperature and techniques are listed in Table 4.3

### ***E.coli/coliform, enterobactereacea and staphylococcus aureus***

Products that were stored under accelerated shelf-life conditions were further subject to additional microbial tests during each of the five time points fore.coli/coliform, enterobactereacea

and staphylococcus aureus. Each procedure was performed in duplicates before incubation and interpretation as recommended by plate manufacturer.

### ***Sample preparation***

To 100 ml blanks prepared as detailed in earlier section, 10 grams of sample from storage during each time interval was added and mixed manually for 1 minute. The mixture was transferred into a sterile container and was blended for 120 seconds to homogenize sample. Thereafter, 1 ml of the inoculate solution was pipette out into blanks containing 9ml test tubes making to make-up the required dilution ( $10^{-2}$  to  $10^{-3}$ ).

### ***Inoculation***

Petriefilm plates (3M, St.Paul, MN, USA) were placed on a level surface and the top film was lifted exposing the media. 1 ml of the sample solution was pipette out from 10 ml of the plating solution in a perpendicular position to the center of the bottom film. The top film was carefully rolled down to avoid any entrapment of air bubbles. To ensure even spread, a spreader was used with its flat side on top of the film over the inoculums and gentle pressure was applied on the ridge side of the spreader. The spreader was removed and after one minute of waiting, petrifilms were then incubated.

### ***Incubation***

Petriplates were placed with clear side up incubators (Tritech Research Inc. Los Angeles, CA, USA) with different incubation temperatures as shown in Table 4.3.

### ***Volatile extraction procedure using gas chromatography***

Headspace solid phase micro extraction (HS-SPME) method was chosen for assessing oil rancidity levels in FBFs at each time point of the shelf-life study. 0.25 g of the milled SSB

samples were suspended in a 10 mL screw-cap vial equipped with a polytetrafluoroethylene/silicone septum. The internal standard used was 2ppm of 1,3-dichlorobenzene dissolved in hexane (mixture of isomers, optima grade, Fisher Scientific; Pittsburgh,PA, USA). Exactly 990  $\mu$ L of deionized water was added to ground sample in the vial along with 10 $\mu$ L of the internal standard. The vials were equilibrated for 10 min at 50°C in the autosampler (Pal system, model CombiPal, CTC Analytics, Zwingen, Switzerland) and agitated at 250 rpm. After equilibration, a 50/30  $\mu$ m divinylbenzene/carboxen/polydimethylsiloxane fiber was exposed to the sample headspace for an extraction time of 30 min at 50°C. After sampling, the analyte were desorbed from the SPME fiber coating to the injection port of gas chromatography (GC) at 270°C for 3 min in split less mode. After each extraction, SPME fibers were baked out for 20 min at 270°C.

### ***Chromatographic analyses***

The isolation, tentative identification, and semi-quantification of the volatile compounds were performed on a gas chromatograph (GC) (Varian GC CP3800; Varian Inc., Walnut Creek, CA, USA), coupled with a Varian mass spectrometer (MS) detector (Saturn 2000). The GC-MS system was equipped with an RTX-5MS (Crossbond® 5% diphenyl/95% dimethyl polysiloxane) column (Restek, U.S., Bellefonte, PA, USA; 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m film thickness). The initial temperature of the column was 40°C and was held at that temperature for 4 min; the temperature was then increased by 5°C per min to 260°C, and held at this temperature for 7 min. All samples were analyzed in triplicates. Volatiles were identified using two different analytical methods: (1) mass spectra and (2) Kovats indices (NIST/EPA/NIH Mass Spectral Library, Version 2.0, 2005). The retention times for a C7-C40 saturated alkane mix (Supelco Analytical,

Bellefonte, PA, USA) was used to determine experimental Kovats indices for the volatile compounds detected.

### ***Descriptive sensory analysis***

#### ***Sample preparation***

Stored shelf-life samples from each time points were tested separately for aroma and flavor attributes. 50 g of whole or decorticated blended sample was suspended to 230 ml of deionized boiling water (200 ml plus an additional 30 ml to compensate for evaporation losses based on initial trials) brought back to a boil, and cooked for 2 minutes while vigorously stirring with a wooden spoon. Thereafter, the gruel was weighed to confirm its final weight of 250g (20% solids) and placed in a 400 ml beaker until they reached the serving temperature range of 30-35°C.

#### ***Panel and testing***

Fortified WSSB and DSSB for all 5 time points of the shelf-life study were subjected to quantitative descriptive sensory analysis. A panel consisting of six highly-trained descriptive individuals, with a minimum 120 h of descriptive training and 1000 h of sensory testing experience on grain products, evaluated gruel samples in a randomized block design. Based on a 2 hour orientation session, the panelists developed a list of 18 appropriate attributes for aroma and flavor for samples. Attribute intensities were quantified using a 15 point scale with 0.5 increments (0.0 = nothing to 15 = extremely high). Approximately 30 g of each sample was served in a 4 oz foam cup and were coded with three-digit random numbers and assigned to panelists and data were analyzed in duplicates in the same session (Lawless and Heymann, 1998). Each sample was evaluated individually in the temperature range between 30°C to 35°C during a 12 minute time period. Four samples were evaluated per day. Deionized water,

carrots and unsalted top crackers were used by the panelists to cleanse their palate before evaluating each sample.

### ***Statistics***

Linear physico-chemical relationships were determined by Pearson Correlation. Descriptive sensory attributes were being tested for interaction effect between time point and product by two-way ANOVA using the PROC GLIMMIX procedure (SAS version 9.2. The SAS Institute Inc., Cary, NC, USA). For all significant attributes differences were determined using pair-wise test comparisons based on SAS least square (LS) means. The criteria for significance were  $p < 0.05$ . Principal component analysis was performed using Unscrambler version 10.2 (Camo Software Inc., Woodbridge, NJ, USA).

## **Results and Discussions**

### ***Proximate composition***

Proximate composition for whole sorghum flour, decorticated sorghum flour, defatted soy flour and WPC80 is shown in Table 4.4. Whole sorghum flour showed higher percentages of crude fat, crude fiber, total dietary fiber and ash in comparison to decorticated sorghum flour. As anticipated, higher values are made available from the bran present in the flour along with kernel endosperm of sorghum signifying its flour grade quality. Protein source comparisons showed higher protein and fat fractions with WPC80 though it constitutes only 3% of the overall formulation. On the other hand, defatted soy flour had higher values with fiber and its corresponding ash content. It is significant to note that protein sources in blends are provided proportionately from overall formulation from cereal (6.7 to 8.7%) in 67.27 g/kg, legume (46.8%) in 21.13 g/kg and whey protein concentrate (WPC-80) (82.0%) in 3 g/kg in the ingredient mixture.

Apart from the cereal-legume protein source, the inclusion of WPC in the formulation contains essential amino acid profile and increases protein quality score i.e., Protein Digestibility Corrected Amino Acid Score (PDCAAS) up to 0.88 while foods with a PDCAAS above 0.80 is considered as vital nutrient source as per FAQR 2011 (Webb et al. 2011). Though proximate values for defatted soy flour aligns with a previous study, crude protein and fiber levels in sorghum flours were considerably present in lower fractions when referenced to a minimum 10-11% and 7% respectively (Ilo et al. 2000). Lower protein and fiber fractions could possibly relate to the variety, hardness, and post harvest storage handling of the grain. Final product moisture of extruded and dried blends were between 4.30 and 6.83% wet basis (w.b.) for low, medium and high screw rpm conditions Table 4.5 while there was significant increase in moisture content with products extruded with oil in the formulation at high shear rates (9.22 and 9.34%) as seen in Table 4.6. Oil in the formulation also impacts particles in the recipe and the internal components of the extruder. As a result, thicker cell walls were observed with extrudates. With more internal fat in the product, drying the product also becomes a limitation with higher moisture levels in contrast to other treatments. Nevertheless, all extruded and dried FBFs were well within its microbial safety range of < 12% moisture content, which would have otherwise called for extended drying time at an increased temperature.

### ***Extrudate as an intermediate product impacting porridge consistency***

Although FBFs are milled products that are reconstituted with boiling water before consumption, this study evaluated extrudate properties prior to milling and how relationships impact the end product flow rates of the newly developed FBFs (Figure 4.2). Specific mechanical energy (SME) of extruded blends ranged between 144-414 kJ/kg (Table 4.7). As expected, SME versus process in-barrel moisture showed moderate to marked degree of

negative correlation ( $r = -0.63$ ) (Figure 4.3). Besides process moisture, the relationship is also depended on the flour composites, in this case, starch and protein which may have direct influence on motor loads and available moisture utilization of the ingredient mixture inside the extruder barrel. Product expansion ratio for extruded WSSB and DSSB ranged between 3.65 and 6.10 for low to high shear treatments. DSSB showed steady increase in expansion at higher screw speeds compared to WSSB. In general, ER increased with higher SME and lower process moisture as seen in Table 4.7 with a high positive correlation ( $r = 0.99$ ), however, with the exception of WSSB HI. Product densities also showed high negative correlation with SME ( $r = -0.98$ ) for both piece and bulk densities Figure 4.3. A key factor affecting product expansion could be the presence of bran with high fiber which are hard outer layers of grain sorghum constituting both aleurone and pericarp layers (Robin et al. 2012) in WSSB that had direct influence on vapor pressure, bubble growth and cell structures. Also, extrudates produced at a low extrusion temperature (30 to 70° C) could experience hard texture and larger bulk density thereby reducing product expansion (Plahar et al. 2003a). Product expansion is further influenced by protein levels in the formulation including sorghum, soy and WPC80. As expected, with increase in ER, piece density of extrudates decreased (0.46 to 0.21 g/cm<sup>3</sup>) which agree with the work by (Devi et al. 2013), with sorghum based cereals treated with whey protein. Despite proximate variances with whole-sorghum flour with increased fat and fiber levels, it is interesting to observe extruded WSSB expanded fairly well at low and medium shear conditions. ER also had significant impact on extrudate properties showing high negative correlations ( $r = -0.97$ ,  $r = -0.96$ ) with bulk and piece densities respectively (Figure 4.4). With increase in ER, extrudate densities are lowered and are predisposed to fracturability due to thinner cell wall thickness. This relationship was further established moderate to high relationship with both SME

and ER on particle size ( $r = - 0.61$ ,  $r = - 0.72$ ) respectively. Similarly, SMEs for WSSB (Oil) and DSSB (Oil) high shear treatments with oil in the formulation prior to extrusion showed high correlations ( $r = 0.90$ ,  $r = - 0.86$ ,  $r = - 0.98$ ) with ER, piece density and particle size respectively with representative extrudate samples. (Figure 4.5). Due to additional oil in the formulation, the bulk density however showed a moderate correlation ( $r = - 0.60$ ). Following the SME relationships, ER also showed high negative correlations ( $r = - 0.92$ ,  $r = - 1.00$ ) for piece density and particle size (Figure 4.6). Extrudates thus produced and analyzed were milled and expected to meet the required USDA specifications of 95% particles to pass through a 600  $\mu\text{m}$  sieve and 100% through a 1000  $\mu\text{m}$  sieve. Mean particle size of ground extrudates were distributed between 336 and 474.2  $\mu\text{m}$  for FBFs extruded at different energy levels Table 4.7, Figure 4.7. Particle size generally decreased with increase in process moisture for WSSBs (449 to 413  $\mu\text{m}$  from high to low shear). It is evident that water acts as a plasticizer providing molecular mobility which is supplemented by shear provided by the screw enabling lower melt viscosity in the extruder barrel. Conversely, DSSB HI showed the lowest particle size at 20% process moisture. Smaller particle size for DSSB high shear is attributed to higher SME (414.55 kJ/kg) relating to greater product expansion that lead to fracturability of the extrudate to smaller fragments and further refinement in particle dimension was made possible with milling. Oil in the formulation showed mixed effects between WSSBs and DSSBs at 20% process moisture due to motor load fluctuations and the absence of product stability which lead to particle variances (Figure 4.8). In the present study, the choice of a table top roller mill could achieve only 80-85% particle passage below 600  $\mu\text{m}$  level and a 100% through 1000  $\mu\text{m}$  sieve. Though strong relationships between process and intermediate product characteristics were established, it is evident that oil in the formulation experienced surging and product inconsistencies Table 4.8. Wide motor load

fluctuations (55-90%) were observed with oil treated samples during data collection and treatment change over due addition of 5.5% level of oil that influenced the functioning of the screw and extruder barrel (Figure 4.9). Contrary to expectations, motor loads and SME increased with oil in the formulation. This fluctuation is directly attributed to additional oil added in the premix with single screw extruders. As an effect of material back-up, drastic head pressure build-up (400 - 1100 psi) was observed with WSSB and DSSB oil treated samples while treatments without oil showed a consistent head pressure between 400-600 psi. Similar work on producing high protein weaning foods using peanut, maize and soybean yielded oily extrudates with poor physical consistency due to high fat levels in peanuts (Plahar et al. 2003b). With a drag flow mechanism in single screw extrusion, it is suspected that high frictional forces of the material against barrel walls and lack of adequate kneading could have caused high pressure build-ups and demanded more power. Additionally, the recipe characteristics in FBFs containing high protein levels in the formulation could have competed with feed moisture contributing to the variability in the melt viscosity. Another attribute to increased SME with oil treated samples could be the restricting to die open area giving more residence time could have led to clogging at the final cooking zone and the die area of the extruder barrel. Variability with extrudate density attributes to oil in the formulation that could have possibly affected melt viscosity due to uneven mixing and conveying of materials at high speeds with low process moisture. However, average values motor load percentages were obtained at critical points based on run time from data acquisition software to calculate SME values for oil treated samples. The reversal behavior of increasing SME with oil in the formulation is still unclear and requires further research. Proper choice of milling such as the use of hammer mill with appropriate screen size could optimize

particle size meeting required specifications of 95% passage through a 600 micron sieve for commercial scale production of FBFs.

### ***Starch paste viscosity profiles***

Starch pasting profiles of extruded and milled blends are shown in Figure 4.10. Milled particles suspended in water during RVA test exhibited cold swelling peaks indicating higher degree of starch damage brought about by processing conditions (Ozcan and Jackson, 2005); (Whalen et al. 1997). Peak viscosity was higher for DSSBs treatments than WSSBs with shorter cooking time to reach its peak viscosity indicating high starch swelling capacity. On the other hand WSSB showed gradual increase in peak viscosity from low to high shear (371 – 420 cP) indicating greater granular swelling capabilities exhibited as a result mechanical shear induced during extrusion. A high peak viscosity observed in DSSB in comparison to WSSB is likely due to its functionality to experience more hydration, surface area and heat absorption as an effect of decortication. Except for WSSB medium shear treatment; pasting temperature for all blends was between 50-60 °C. Higher pasting temperature for WSSB MED at 71°C relate to starch paste requiring more heat to undergo gelatinization by enabling swelling and solubility with available water and under constant shear in RVA. Additionally, leaching of amylose in the slurry mixture could have inhibited starch swelling by forming complexes with lipids, resulting in a lower peak viscosity (Sang et al. 2008), when compared to DSSB medium shear treatment. It is also important to note that fortification of cereal-legume blend and the addition of added protein can also reduce peak and cold paste viscosities. ((Anyango et al. 2011) showed that fermented porridges had lower peak and cold paste viscosities due to lower starch and high protein content in cowpea fortified sorghum foods (70:30 ratio). Break down viscosity for all WSSB treatments consistently showed lower break down values with higher thermal and shear

stabilities. The opposite was true for DSSB starches. This feature with WSSB starches may be attributed to the insoluble dietary fiber components such as cellulose, hemicelluloses and lignin resisting high shear and temperature in RVA. Lower break down values are associated with setback region which relates to realignment of the crystalline structure of amyloze and amylopectin during the cooling phase of RVA. Though amyloze plays a prominent role with short term effects of retrograded starch, amylopectin plays an equally important role with earlier stages of processing (Gudmundsson. 1994). The results of RVA for all WSSB treatments aligned with the work by (Ragae and Abdel-Aal. 2006), wherein heat treated whole grain sorghum has the lower peak viscosity, but showed paste stability with lower break down values.

Peak viscosity of WSSB HI (Oil) and DSSB HI (Oil) was lower in comparison to WSSB HI and DSSB HI respectively Table 4.10, Figure 4.10. According to (Ilo et al. 2000), the addition of vegetable oil acts as a plasticizer cum lubricant and increases the mobility of the flour composite polymer by decreasing the melt viscosity of the biopolymer. Again, as anticipated, pasting temperatures were higher indicating amyloze-lipid complexes with oil treated FBFs requiring more time to cook with an effort to enhance granular swelling. It is however noteworthy to see DSSB HI (Oil) to have a fair paste stability with lowest breakdown value in comparison to other DSSB treatments at different screw speeds.

### ***Bostwick flow rate determining gruel consistency***

With a constant volume of liquid released in Bostwick consistometer, three flow regimes act on fluids such as inertia and gravity in short term followed by viscous forces that balances surface tension forces (Mccarthy and Seymour. 1993) which affects flow resistance of cooked gruels. As an extension to RVA's quantitative assessment on viscosity, empirical methods from Bostwick Consistometer gives us a fair indication about flow resistance of rehydrated blends. Field trials of

CSB typically prepared at 9 and 14% solids have shown very thick porridge consistency, making it difficult for infant consumption requiring dilution either during or after cooking (Fleige et al. 2010a). The thickness is attributed to starch granules (93% carbohydrate in cornmeal is starch (Pomeranz. 1988) absorbing 10 their weight in water when cooked during the process of gelatinization (Hoseney. 1994). The current study showed Bostwick flow rates for extruded FBFs from 7.5 cm/min up to 12.0 cm/min when reconstituted at 20% solid concentration at 30°C. This flow range aligns with optimum consistency of complementary foods used in Title II programs (8.0 to 12.0 cm/min) (Black et al. 2009); (Vieu et al. 2001)), while it is significant to note that CSB has a Bostwick flow rate of only 2.0 cm/min at 19% solid concentration (Black et al. 2009). Optimal flow rates for extruded FBF could possibly be due to higher breakdown of starch granules or dextrinization of long branched of starch chains through extrusion. High mechanical shear induced to FBFs gelatinizes starch and denatures proteins to higher degrees. While only marginal changes were seen in flow rates at low shear treatments, flow rates varied significantly among medium, high shear and oil treated samples. WSSB medium shear showed the highest flow of 12.0 cm/min followed by DSSB HI (11.5 cm/min). Initial study of sorghum soy blend flow characteristics showed that with lower RVA final viscosity at 50°C during the cooling phase, Bostwick flow rates increased considerably with thinner gruel consistency. A strong correlation ( $r = - 0.91$ ) between RVA final viscosity and the Bostwick rates was established, however, moderate relationships were seen with particle size with standard RVA profile ( $r = 0.43$ )Figure 4.11. On the other hand, oil treated WSSB showed an increase in flow rate by about 2.0cm/min, DSSB showed a reversal trend Table 4.6wherein flow rate decreased drastically with DSSB (Oil) by 4.5 cm/min. This decrease possibly explains the re-association of starch molecules tending to aggregate and crystallize out of the solution due to the governance of

amyloze. At high shear rates during extrusion and at constant shear and temperature, DSSB treated with oil could have possibly had a higher degree of amyloze leaching which could have contributed to a higher final viscosity observed from RVA. Based on viscosity measurements studies of weaning foods and their densities by various authors in recent past (Black et al. 2009);(Mouquet et al. 2006); (Mouquet and Treche. 2001);(Stephenson et al. 1994); (Vivas et al. 1987), the current study confirms extrusion processing can produce thinner gruel consistency which can match optimum flow conditions and energy density (0.8 kcal/g) required for feeding infants. A recent study with extruded cassava starch blend with soy flour used as complementary porridge also reported the gruel to be energy dense with enhanced protein profile in addressing protein energy malnutrition in sub-Saharan Africa (Muoki et al. 2012). Here again, the relationship between RVA final viscosity and Bostwick flow rates showed high negative correlation ( $r = - 0.82$ ) as seen in Figure 4.12. Nevertheless, feeding temperature of gruels before consumption is to be considered to validate the actual flow rates of extruded FBFs which need further investigation. The effects of retrograded starch and its relationship with flow rates of extruded blends under conditioned time, temperature and shear is further discussed in the following section.

#### ***Modified RVA at 30°C with extended time at a constant shear rate***

The RVA temperature, shear and time protocols were modified based on the cooking procedure recommended in the commodity specifications for CSB 13 (USDA 2008). The current study postulated, change in viscosity profiles and starch paste final viscosity with rapid heating and extended cooling at a constant temperature (30°C) using this novel extended RVA protocol. Pasting profiles were reproduced to compare starch paste viscosity of gruels extruded at different conditions over an extended period under constant shear and temperature.

The viscosity of low and medium shear WSSB and DSSB were consistent with stable viscosity (Figure 4.13a,b) when compared with FBFs produced at high shear and with oil in the formulation (pre-extrusion)(Figure 4.13c, d). Interestingly, the viscosity profile for WSSB HI showed higher peak viscosity and final viscosity with lower paste stability when subject to prolonged shearing compared to DSSB HI treatments (Figure 4.13c). With oil addition (pre-extrusion), WSSB showed a decrease in its viscosity profile and reflected a reversal behavior in comparison to WSSB HI extruded without oil. The decrease in viscosity explains complexation of amylose with lipids which reduces starch solubility in water affecting rheological properties by retarding retrogradation process (Salman and Copeland. 2010). On the other hand DSSB HI (Oil) remained fairly constant while experiencing extended shear under constant temperature. Despite the relative increase in RVA final viscosity at 30°C, it is noteworthy to observe strong relationships with gruel consistency ( $r = - 0.87$ ) for treatments without oil (Figure 4.14) and ( $r = - 0.91$ ) for blends with premixed oil in the formulation (Figure 4.15). However, as in standard RVA profile, the particle size and final viscosity showed moderate relationship ( $r = 0.32$ ).

### ***Thermal transitions of FBFs***

Endothermic transitions including onset, gelatinization or denaturation peak, and end points with corresponding enthalpies are summarized in Table 4.11, Figure 4.16. Starch gelatinization or protein denaturation of individual flours was represented by the first endothermic peak and  $T_g$  of whole sorghum flour, decorticated sorghum flour, soy flour, WPC-80 were between 66 and 83°C. Transition enthalpies for starch were between 2-3 J/g whereas for proteins with negligible starch fractions had enthalpies less than 0.1 J/g. For raw blends of WSSB and DSSB,  $T_g$  was 77.09 and 79.49 °C respectively, and for extruded blends,  $T_g$  was observed at a higher temperature range of 88-97 °C. Gelatinization temperature for individual sorghum starch (73.71 and 77.38 °C) falls in

close proximity with  $\pm 3$  deviation reported previously (Yoo et al. 2013); (Aboubacar and Hamaker. 1999); (Akingbala et al. 1988) and enthalpy for raw sorghum of 2.24-2.74J/g were also similar to reported values by (Claver et al. 2010). The denaturation temperatures for soy protein (82.98 °C) also showed similar data reported (Qi et al. 2011); (Sobral et al. 2010). While starch gelatinization peak is irreversible, a second endothermic peak was observed for all flour categories at a peak temperature ( $T_g$ ) between 83.53 – 123.97 °C, which represents the reversible dissociation of type II (amorphous) amyloze-lipid complexes naturally present in flours (Putseys et al. 2010) which is seen at higher temperatures. Supposedly, the gelatinization peaks for extruded blends showed higher temperature range between 109.67 and 123.97 °C. There were incremental temperature rise of nearly 10 to 15°C observed between onset and peak on the first and second endothermic peak (amyloze-lipid complex) of raw blends versus extruded flours. This could possibly be due to the functionality of flour composites with the interaction of a starch source from sorghum and three other protein sources in the blend which include kafirin protein bodies embedded in sorghum starch, major constituents of soy protein, namely the 7S and 11S globulins and lastly  $\alpha, \beta$  – lactalbumins in WPC at 80% strength. Literature indicates incremental temperature rise associated with protein aggregation at the onset and unfolding on its structure in the event of complete denaturation (Beveridge et al. 1985). At the onset point of the first endothermic peak shoulder, starch and protein source compete for available water for gelatinization or denaturation. The onset and peak temperature and enthalpy for WSSB & DSSB extruded at low shear and high shear with oil showed similarities despite high peak temperature. The addition of fat (Gimenez et al. 2013) in the formulation could have had a type I starch-lipid interaction at lower temperature (Gimenez et al. 2013) and protein-lipid interactions wherein lipid bilayers become more stable with hydrophobic oil environment (Dael & Cauwelart 1988;

Mohammed 2002) increasing the mobility of oil molecules. Secondly, with decreasing process moisture, fusion enthalpies increased with increase in the lipid-amyloze complex (Gimenez et al. 2013);(Pilli et al 2008). This is attributable to the formation of crystalline amyloze-lipid complex when material temperature is positioned between its melting ( $T_m$ ) and glass transition ( $T_g$ ) temperatures (Slade,L., & Levine,H. 1987). Further, water in extruder generates plasticizing effect and any decline in process moisture could elevate ( $T_m$ ) and ( $T_g$ ) of the material extruded (Ollett et al. 1990); (vanSoest et al. 1996).Starch-lipid complexes thus heated cooled and reheated showed higher enthalpy values during the second scan probably due to enhanced conditions after the first heating cycle when starch is completely gelatinized and amyloze leaching occurs with complexation. However, the amyloze lipid complex overlaps between the gelatinization peak and lipid-amyloze complex peak after the first heating cycle at much lower temperatures. This observation varies from the work by (Eliasson. 1994) who showed the lipid-amyloze complex to occur well above 100°C in wheat and potato and maize starches with increase in enthalpies two-fold. Assuming that the first endothermic peak is still a gelatinization peak with protein interference, we still see a 93-97% degree of gelatinization as FBFs have experienced high SMEs that strongly affect starch gelatinization by rupturing intermolecular hydrogen bonds. However, the mechanism of protein interference with starch in gelatinization process resulting in the overlap of the endothermic peaks typically with extruded flour composites will help to understand the degree of gelatinization more accurately.

### ***Shelf-life study***

.Besides formulation and processing, this section of the study involves storage of extruded, milled and fortified FBFs under elevated temperature and relative humidity conditions. Extrudates produced were subject to storage in liner bags for a period of 14 months before

milling and fortification prior to shelf-life studies. During each time interval of the study, FBFs are analyzed for physicochemical, microbial and sensory aspects to identify mechanisms responsible for lipid oxidation causing rancidity and assess the food safety aspects of SSBs under accelerated conditions.

### ***Water activity and its effects on bacterial and mold growth***

FBFs produced by extrusion processing followed by milling and fortification with micronutrients and vegetable oil were packaged and stored at 50°C and 70% RH. Water activity and product moisture was analyzed during each time interval as shown in Figure 4.17a, b. Water activity of FBFs ranged between 0.37 and 0.68 with moisture content between of 5 to 12%. WSSB HI showed a decrease in  $a_w$  during week 6 and 9, while DSSB HI (Oil) showed continuous increase with time. With an exception of WSSB HI, all treatments showed increase in water activity from week 1 to week 13 and a drop trend with week 16. DSSB-HI (Oil) had the highest  $a_w$  (0.68) and moisture (12%) among treatments getting closer to the minimum  $a_w$  required for microbial growth at 0.60 for salt-tolerant microorganisms. Other microbial infestations occurs at a  $a_w$  of 0.80 for most molds, 0.87 for yeasts, and 0.91 for most pathogens (Beuchat. 1981). Though gradual increase in  $a_w$  is related to higher temperature and relative humidity, it does not necessarily mean a faster reaction rate for microbial intervention in this case. This postulation is confirmed by lower or absence of microbial colonies as seen in Figure 4.17c specifically for week 13. The sparsely found colonies could be attributed low temperature extrusion of FBFs that may have eliminated spores at constant temperature and residence time under low moisture conditions. Further, a recent study showed bacterial inactivation with e-coli of corn meal and WPC 80 below its detection limits (<20 cfu/g) when extruded at 75 and 95°C respectively (Ukuku et al. 2012). In the present study, e-coli were however tested negative

during each time point of the shelf-life study. Water acts as a plasticizer in low and intermediate moisture foods, and related to water activity ( $a_w$ ), which is a measure of water availability for the microbial growth. With increase in  $a_w$  and moisture, the rate of crystallization increases with molecular mobility from glassy to a rubbery state (Labuza and Hyman. 1998) allowing reaction to occur more freely during weeks 6, 9 and 13. However, comparing the microbial traces between bacteria and molds, there seems more occurrence of bacterial growth from week 6 onwards among all treatments, whereas molds traces in small colonies showed up during week 6, 9 and 13. Microbial colonies across the 16 weeks period for all FBFs were less than 5000 cfu/g which meets the USDA guideline allowance of a maximum of 50,000 cfu/g each for bacteria and mold. However, there has been no trace of mold growth with oil treated samples across the first three time points. In anticipation to assess variability between oil treated samples WSSB and DSSB, there were no significant differences seen in bacterial growth across time points.

### ***Assessing oxidative stability using solid-phase microextraction (SPME) – Gas Chromatography***

GC-MS data of major volatiles was identified during each time interval of the accelerated shelf-life study using SPME. A total of 35 aromatic compounds were tentatively identified among WSSB, DSSB, WSSB (O), DSSB (O) food samples extruded at high shears summarized in appendix b&c. These aromatic compounds were grouped as: alcohols (6 compounds), aldehydes (12 compounds), ketones (5 compounds), amine (1 compound), esters (2 compounds), pyrazines (4 compounds), furans (1 compound), terpenes (2 compounds), benzene derivatives, (1 compound) and alkenes (1 compound). It is observed that the majority of volatiles found in samples were aldehydes (Figure 4.18) that are known to have low odor threshold values and are

found in abundance in cereal grains (Liu et al. 2012), and in particular are responsible for oxidized flavor of fats in cooking oils (Katsuta et al. 2008).

Hexanal produced the largest GC peak and was the major volatile involved in oxidative degradation as an effect of autoxidation of polyunsaturated fatty acids (PUFAs) which limited shelf-life of FBFs. According to Ho et al 1988, the cleaving of 13-hydroperoxides lead to formation of hexanal in linoleates. Hexanal concentrations for WSSB were highest during week 13 at 62.54 ppm and DSSB was at 97.93 ppm during week 9 of the test. On the other hand, WSSB (O) had the highest concentration of 49.38 ppm during week 9 and DSSB (O) at 91.62 ppm during week 6. It is evident that DSSBs had higher hexanal concentrations when compared to WSSBs. Higher hexanal concentration is directly related to accelerated test conditions affecting the stability of fatty acids due to their degree of unsaturation. Edible soy bean oil contains triglycerides, essential free fatty acids, pigments, phospholipids (Garcia et al 1997) used in fortification of FBFs. The fatty acids comprises of C18:2 (linoleic acid) (54%), C18:1 (oleic acid) (23%), C18:3 (linolenic acid) (7.8%), and C16 (palmitic acid) (11.0%) (Gunstone. 2002). Hence linoleic acid with 2 or more double bonds is susceptible to rapid decomposition in the lipid oxidation pathway and a high iodine value further increases the rate of oxidation (Shahidi. 2003). Additionally, PUFAs require lowest activation energy (65 kcal/mol) for the hydrogen atom to dissociate from the C-H bond to initiate free radical formation, by biallylic hydrogen (Shahidi and Zhong. 2010). Besides hexanal, other compounds in the aldehydes group that contributed to rancidity were 2 hexanal (upto 3 ppm), heptenal (upto 14.72 ppm), 2-heptenal (upto 16.22 ppm), octanal (upto 27.31 ppm) and 2-octenal (upto 40.41 ppm). Figure 4.18b-f. In the ketone group, 2-heptanone (upto 9.72 ppm) and 2-nonanone (<1ppm) were observed (Figure 4.19). Alcohols including 1-pentanol, 2-nonen-1-ol were detected along with 1-octen-3-ol which

is considered to have strong mushroom flavor with high odor threshold values, and hence not a contributing factor in the flavor or lipid in foods (Ho. 2005). Another important volatile detected was 2-pentyl furan (upto 35.52 ppm) which is well known as an autoxidation compound responsible in the flavor reversion of soybean oil (Ho et al 1978). Other minor compounds detected as an effect of Maillard reaction were furfural which is formed during possessing caramel-like and fruity characteristics and pyrazines containing nitrogen that are characterized by nutty and roasted aromatic compound (Mottram. 1994). (Vazquez-Araujo et al. 2011) listed compounds such as geosmin, dimethoxybenze, 3-octanone and 1,24-trimethoxybenze as potential volatiles causing musty odor notes in a shelf-life study among 27 different sorghum grain types, which were not detected in the present study.

Interestingly, the study shows a pattern to understand deterioration rates with oil added to the formulation pre verses post extrusion. From Figure 4.18, Figure 4.19, it is evident that the onset of flavor reversion begin during week 6 and spiked to its highest degree of decomposition during week 9, and thereafter the decomposition rates decrease rapidly by week 13 followed by termination in week 16. This pattern is attributable to the widely accepted lipid autoxidation pathways through a free radical chain mechanism which has three distinct phases – initiation, propagation and termination (Shahidi and Zhong. 2010). Though the initiation process of lipid oxidation is not well understood, we observe flavor reversion begins in week 6 but not rancid to flavor reversed and FBFs becoming completely rancid by week 9. Overall this trend is predominant with WSSB (O) and DSSB (O) where oil was added pre-extrusion in the formulation. This explains reactions of extrusion cooking whereby lipid oxidation with higher peroxide value, destruction on antioxidants and cis-trans isomerization of unsaturated fatty acids play crucial role in the shelf-life of a product (Ilo et al. 2000). Further, deterioration during early

shelf-life cycle is activated by accelerated temperature and humidity conditions. In this study, the only antioxidant detected was butylated hydroxytoluene (BHT) for all samples but at lower concentrations of upto 22 ppm while the maximum limit of BHT, BHA and TBHQ either singly or in combination is upto 200 ppm in vegetable oils (Ding and Zou. 2012). It is noteworthy that no external antioxidants were added in the formulation but for the detection of BHT which could be from soybean oil. Finally, the last analyte detected was diethyl phthalate (DEP) with a maximum of 3.82 ppm (Figure 4.20). DEPs are ubiquitous environmental contaminants with low molecular weight present in foods at various proportions. DEPs are also used in polyethylene food packaging materials as plasticizers (Cao. 2008) and migration of plasticizers from the packaging material is also a vital path for phthalates to come in contact with FBFs due to the effects of temperature and humidity. The safe levels of DEP consumption in infant foods is however not clear with very limited information due to packaging options and manufacturing practices. Nevertheless proposing hexanal thresholds as a precursor to stability of FBFs were subject to the results obtained from sensory panelists during each time interval of the study.

### ***Descriptive Sensory Analysis***

#### ***Aroma***

Aroma is a quality that can be perceived by the olfactory sense with agreeable odor. The sensory interaction scores between WSSB verses DSSB with oil added pre and post extrusion are shown in respectively. Aroma profiles for rancid and painty attributes passed through with acceptable odor for the first three time intervals (week 0, week 6, week 9) for WSSB and DSSB with oil added post extrusion and were tested completely rancid with off-odor notes released by week 13 and 16. Since products turned from not being rancid during week 9 to completely rancid during week 13, it could be inferred that the shelf-life decomposition should have occurred between

week 9 and week 13. Hence it is appropriate to infer that shelf-stability was at least 9 weeks in accelerated conditions equating to 9 months in real time. Comparatively, pre-extruded FBF samples turned completely rancid as early as week 9 averaging highest rancid and painty scores directly linking lipid oxidation with key volatiles detected at higher concentration in GC-MS during week 9. Here again, shelf-stability relates to at least 6 weeks in accelerated equating to 6 months in real-time. Other attribute scores related to grain overall (associated with dusty and dry grain), musty overall associated with one or more aromatic impressions such as stale or moldy and cardboard were possible impacts of prolonged storage at extreme temperature and humidity conditions.

### ***Flavor***

Similar sensory scores were recorded for flavor profiles in comparison to aroma with rancid and painty attributes. Flavor, a sensory impression is determined by chemical senses of both taste and smell becomes vital in evaluating acceptability of FBFs affecting these senses. Rancidity scores were significantly different for week 6 with WSSB and DSSB samples whilst the opposite was true for oil treated samples throughout the shelf-life cycle. From a panelist point of view, test scores indicate significant spike in rancidity levels especially during week 13 for post extrusion oil treated samples and at week 9 for pre-extrusion oil treated samples in comparison to their preceding time intervals. This is a possible indication that flavor attributes scores provide insights to consumer acceptability and in deciding the actual detection point of deterioration of FBFs in validation of the shelf-life from a food safety standpoint. Figure 4.21 shows a principle component bi-plot with 90 and 3% variances. Overall the aroma and flavor profiles of DSSB samples are influenced by toasted and brown attributes while all samples during week 13 and 16 are characterized by rancid and painty attributes. With the exception of pre-extruded oil samples,

the first time intervals showed a high score on grain overall aroma, overall grain flavor and starch flavor.

### ***Relating volatiles to descriptive sensory analysis***

Several studies indicate different hexanal thresholds based on food type, storage conditions and time interval to determine the oxidative stability in foods (Plahar et al. 2003a);(Park. 1993);(Fritsch and Gale. 1977, Garzon et al. 2004)(Frankel. 2005, Elisia and Kitts. 2011), however it was not possible to establish a benchmark applicable to flour composites typically with sorghum-soy blends. Hexanal thresholds in this study were matched with values obtained from GC-MS and descriptive sensory analysis as seen in Figure 4.21 Principal component analysis of the samples and attributes. Figure 4.21 which tested both aroma and flavor focusing on rancid and painty attributes by panelists.

### **Conclusions**

In conclusion, extruded sorghum-soy blends met standard specifications for energy density and consistency (Bostwick flow rate), and were superior in some aspects as compared to extruded corn-soy blends and traditional corn-soy blends (CSB13). Relationships between extrusion mechanical energy input, expansion, particle size after milling and consistency of rehydrated blends were established. At 20% solids, extruded SSBs still showed flow rates meeting the minimum required USDA standard whereas CSB13 resisted flows at this concentration. Though extrudate properties of FBFs behaved as expected with different energy levels, formulations consisting oil had a mixed effect on extrusion parameters and posed processing challenges during extrusion. FBFs had minimal microbial intervention during the shelf-life period, but had short shelf-life stability. FBFs with oil added pre-extrusion turned

completely rancid at week 9 (9 months in real time) equating to atleast 6 months shelf-stability, while FBFs with oil added post-extrusion deteriorated at week 13 (12 months in real time) equating to atleast 9 months shelf-stability. Further investigation on oil-addition methods, extruder hardware configuration and process control is required to enhance the production of energy dense FBFs. Choice of natural anti-oxidants, post-harvest handling and storage of ingredients before processing and better manufacturing practices can enhance product shelf-stability.

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Table 4.1 Target levels of ingredient formulation for FBFs

\*as modified from Webb et al. 2011. 1 rapeseed oil providing essential fatty acids (alpha-linoleic/ $\omega$ -3), 2 addition of WPC 80% gives a PDCAAS score of up to 0.88

Ingredient	Amount (g/kg)
Whole or Decorticated Sorghum Flour	67.27
Soy Flour	21.13
Vegetable Oil <sup>1</sup>	5.50
WPC 80% <sup>2</sup>	3.00
Mineral Premix	3.00
Vitamin Premix	0.10

Table 4.2 Scheduled Accelerated Vs Real Time Points

\*ASLT – Accelerated shelf-life test, # - 52 weeks/4 = 13 weeks, 64 weeks/4 = 16 weeks

<b>ASLT*</b> <b>(Weeks)</b>	<b>Real time</b> <b>(Weeks)</b>
0	0
6	24
9	36
13 <sup>#</sup>	52
16 <sup>#</sup>	64

Table 4.3 Microbial Test Procedures

Microbial Test	Incubation Time/Temperature/Method
Mold/Fungi	48-72h at 29°C ± 1°C
Bacteria	48h ± 2h at 32°C ± 1°C (AOAC-990.12)
E.coli	48h ± 2h at 35°C ± 1°C (AOAC-991.14)
Enterobactereacea	24h ± 2h at 35°C ± 1°C (AOAC-2003.01)
Staphylococcus aureus	24h ± 2h at 37°C ± 1°C (AOAC-2003.07)

Table 4.4 Proximate analysis of raw materials (% db)<sup>1</sup>

<sup>1</sup>as specified by the Department of Animal Sciences, Kansas State University, Manhattan, KS.

\*Crude protein is calculated using a 6.25 conversion factor. “-” indicates missing or incomplete value. WSF – Whole Sorghum Flour, DSF – Decorticated Sorghum Flour, WPC – whey protein concentrate. db: Dry basis.<sup>2</sup> – as-is basis. TDF – Total Dietary Fiber, SDF – Soluble Dietary Fiber, IDF – Insoluble Dietary Fiber

Ingredient	Moisture (%)	Crude Protein* (%)	Crude Fat (%)	Crude Fiber (%)	TDF <sup>2</sup> (%)	SDF <sup>2</sup> (%)	IDF <sup>2</sup> (%)	Ash (%)	Starch (%)
WSF	9.98±0.00	6.73±0.02	2.75± 0.34	0.69± 0.09	3.48±0.96	3.06±1.16	0.41±0.19	1.17± 0.02	69.4±0.70
DSF	11.65±0.00	8.72±0.04	1.09 ±0.01	0.17± 0.02	2.56±0.78	2.24±0.77	0.33±0.00	0.55±0.02	71.91±0.14
Soy Flour	8.73±0.03	46.85±0.35	1.00 ±0.03	3.05± 0.04	n.d.	n.d.	n.d.	6.88± 0.04	n.d.
WPC 80	4.60±0.20	82.00±1.00	5.50 ±1.00	n.d.	n.d.	n.d.	n.d.	3.00±0.50	n.d.

Table 4.5 Average moisture content of milled FBFs

Different energy levels and sorghum type -wb: wet basis, measurements are given as average  $\pm$  sd.

Fortified Blended Foods	Moisture content (% wb)
Whole sorghum-soy blend (WSSB) LO	4.30 $\pm$ 0.00
Whole sorghum-soy blend (WSSB) Medium	5.22 $\pm$ 0.60
Whole sorghum-soy blend (WSSB) HI	5.20 $\pm$ 0.00
Decorticated sorghum-soy blend (DSSB) LO	5.83 $\pm$ 0.10
Decorticated sorghum-soy blend (DSSB) Medium	5.69 $\pm$ 1.30
Decorticated sorghum-soy blend (DSSB) HI	6.83 $\pm$ 0.00

Table 4.6 Average moisture content of milled FBFs at 450 rpm showing oil effects sorghum type -wb: wet basis, measurements are given as average  $\pm$  sd.

Fortified Blended Foods	Moisture content (% wb)
Whole sorghum-soy blend (WSSB) HI	5.20 $\pm$ 0.00
Whole sorghum-soy blend (WSSB) HI (Oil)	9.22 $\pm$ 0.00
Decorticated sorghum-soy blend (DSSB) HI	6.83 $\pm$ 0.00
Decorticated sorghum-soy blend (DSSB) HI (Oil)	9.34 $\pm$ 1.00

Table 4.7 Extrudate properties of different energy and decortication levels

Lo-low shear 350 rpm, Med – medium shear 400 rpm, HI – high shear 450 rpm, (Oil) – added pre-extrusion, MC – moisture content, SME – specific mechanical energy, ER – expansion ratio, PD – piece density, BD – bulk density, MPS – mean particle size, n.d. – data not available

Treatment ID	MC (%)	Torque (%)	SME (kJ/kg)	ER	PD (g/cm <sup>3</sup> )	BD (g/L)	MPS (µm)	% below 600 µm
WSSB LO	28	56	144.00	3.65±0.46	0.46±0.00	260±2.12	413.0 ± 2.1	84.3
WSSB MED	24	58	191.28	3.84±0.75	0.42±0.01	247±4.24	442.0 ± 2.3	82.9
WSSB HI	20	52	172.73	3.80±0.59	0.48±0.01	247±4.24	449.3 ± 2.8	81.8
DSSB LO	28	58	154.50	3.69±0.53	0.46±0.02	259±5.66	407.1 ± 2.0	86.2
DSSB MED	24	68	254.26	4.26±0.79	0.36±0.00	176±5.92	474.2 ± 0.1	78.4
DSSB HI	20	80	414.55	6.10±0.71	0.21±0.01	100±0.96	336.0 ± 3.4	89.3

Table 4.8 Extrudate properties produced at 450 rpm showing oil effects

Lo-low shear 350 rpm, Med – medium shear 400 rpm, HI – high shear 450 rpm, (Oil) – added pre-extrusion, MC – moisture content, SME – specific mechanical energy, ER – expansion ratio, PD – piece density, BD – bulk density, MPS – mean particle size.

Treatment ID	MC (%)	Torque (%)	SME (kJ/kg)	ER	PD g/cm <sup>3</sup>	BD g/L	MPS (µm)	% below 600 µm
WSSB HI	20	52	172.73	3.80±0.59	0.48±0.01	247±4.24	449.3 ± 2.8	81.8
WSSB HI (Oil)	20	67	249.27	4.19±1.15	0.55±0.04	328±61.98	433.3 ± 2.8	84.5
DSSB HI	20	80	414.55	6.10±0.71	0.21±0.01	100±0.96	336.0 ± 3.4	89.3
DSSB HI (Oil)	20	67	293.77	5.78±2.62	0.34±0.08	357±18.38	358.9 ± 6.7	92.9

Table 4.9 Pasting Properties – Impact of energy levels

<sup>1</sup>PV: Peak Viscosity – the maximum viscosity that occurs prior to the initiation of the cooling phase, indicating swelling of starch. <sup>2</sup>PT: Peak Time – cooking time required for blends to reach peak viscosity, <sup>3</sup>Trough – post-peak viscosity observed either before cooling or slightly after start of cooling. <sup>4</sup>BD: Break down = PV – Trough, <sup>5</sup>Setback = FV – Trough. \* - Bostwick flow rate of cooked Gruels correlated with final viscosity. Measurements are given as average ± s.d.

Treatment ID	RVA						FV (cP)	Bostwick* cm/min
	PV <sup>1</sup> (cP)	PT <sup>2</sup> (min)	P Temp (°C)	<sup>3</sup> Trough	<sup>4</sup> BD (cP)	<sup>5</sup> Setback (cP)		
WSSB LO	371 ± 12	3.62±0	51.85±1.2	165 ± 0	206 ± 12	94 ± 25	259 ± 25	10.5 ± 0
WSSB MED	396 ± 2	3.75 ± 0	71.00±1.4	221 ± 1	175 ± 3	48 ± 4	269 ± 4	12.0 ± 2
WSSB HI	420 ± 3	3.95 ± 0	53.90±5.3	253 ± 11	167 ± 9	112 ± 1	365 ± 11	7.5 ± 0
DSSB LO	478 ± 6	2.72 ± 0	57.65 ±15	217 ± 16	262 ± 22	85 ± 18	301 ± 1	10.0 ± 0
DSSB MED	474 ± 13	2.72 ± 0	50.95 ± 0	211 ± 9	263 ± 22	70 ± 26	281 ± 35	10.0 ± 0
DSSB HI	484 ± 11	2.72 ± 0	50.95 ± 15	185 ± 3	299 ± 9	70 ± 2	255 ± 1	11.5 ± 0

Table 4.10 Pasting Properties – Impact of Oil

Treatment ID	RVA						Bostwick*	
	PV <sup>1</sup> (cP)	PT <sup>2</sup> (min)	P Temp (°C)	<sup>3</sup> Trough	<sup>4</sup> BD (cP)	<sup>5</sup> Setback (cP)	FV (cP)	cm/min
WSSB HI	420 ± 3	3.95 ± 0	53.90 ± 5	253 ± 11	167 ± 9	112 ± 1	365 ± 11	7.5 ± 0
WSSB HI (Oil)	381 ± 3	3.79 ± 0	61.53 ± 12	175 ± 11	206 ± 8	70 ± 1	246 ± 2	9.5 ± 0
DSSB HI	484 ± 11	2.72 ± 0	50.95 ± 15	185 ± 3	299 ± 9	70 ± 2	255 ± 1	11.5 ± 0
DSSB HI (Oil)	460 ± 12	3.89 ± 0	59.58 ± 1	239 ± 7	221 ± 5	88 ± 18	327 ± 11	7.0 ± 0

Table4.11 Thermal analysis of individual flours, raw and extruded blends

I – individual flours, WSF – Whole sorghum flour, DSF – Decorticated sorghum flour, SF – Soy flour, WPC – Whey protein concentrate. <sup>RB</sup> – Raw blends, <sup>Ex</sup> – Extruded blends. T<sub>o</sub> – onset temperature, T<sub>g</sub> – gelatinization/denaturation temperature, T<sub>c</sub> – end point temperature, DG- Degree of gelatinization. na – not applicable

Treatment ID	Endothermic Peak - 1				Endothermic Peak-2				DG (%)
	Transition Temperature			Enthalpy	Transition Temperature			Enthalpy	
	T <sub>o</sub> (°C)	T <sub>g</sub> (°C)	T <sub>c</sub> (°C)	ΔH (J/g)	T <sub>o</sub> (°C)	T <sub>g</sub> (°C)	T <sub>c</sub> (°C)	ΔH (J/g)	
WSF <sup>I</sup>	68.04 ± 0.41	73.71 ± 0.46	85.58 ± 0.93	2.74 ± 0.21	94.10 ± 2.34	102.74 ± 1.66	112.55 ± 0.86	0.15 ± 0.11	na
DSF <sup>I</sup>	70.94 ± 0.81	77.38 ± 0.65	91.80 ± 1.25	2.27 ± 0.29	96.93 ± 0.18	104.08 ± 0.41	114.57 ± 0.89	0.17 ± 0.03	na
SF <sup>I</sup>	77.76 ± 0.65	82.98 ± 0.99	90.29 ± 2.15	0.07 ± 0.01	96.62 ± 0.79	102.46 ± 1.01	110.96 ± 1.23	0.90 ± 0.23	na
WPC-80 <sup>I</sup>	63.67 ± 0.94	66.51 ± 0.34	72.92 ± 0.09	0.04 ± 0.01	75.77 ± 1.35	83.53 ± 1.78	101.60 ± 4.10	1.35 ± 0.04	na
WSSB RAW <sup>RB</sup>	70.89 ± 0.63	77.09 ± 0.68	94.10 ± 0.38	2.10 ± 0.08	97.38 ± 0.62	102.44 ± 0.69	114.85 ± 0.55	0.21 ± 0.02	na
DSSB RAW <sup>RB</sup>	73.66 ± 1.68	79.49 ± 1.57	94.33 ± 0.37	1.91 ± 0.03	99.23 ± 1.32	104.88 ± 1.36	115.65 ± 1.24	0.16 ± 0.02	na
WSSB LOW <sup>Ex</sup>	81.01 ± 0.40	89.51 ± 0.04	98.71 ± 0.66	0.08 ± 0.00	103.63 ± 1.17	109.67 ± 1.53	121.40 ± 3.00	0.06 ± 0.03	97.08
WSSB MED <sup>Ex</sup>	86.53 ± 3.56	93.17 ± 0.52	102.42 ± 0.11	0.08 ± 0.09	104.34 ± 1.15	116.82 ± 0.21	127.32 ± 2.71	0.22 ± 0.01	97.08
WSSB HI <sup>Ex</sup>	76.43 ± 2.91	88.14 ± 1.43	98.34 ± 0.72	0.15 ± 0.04	100.58 ± 0.46	111.25 ± 8.82	133.38 ± 6.23	0.44 ± 0.09	94.52
WSSB HI (Oil) <sup>Ex</sup>	81.16 ± 1.55	89.11 ± 0.56	97.83 ± 0.35	0.07 ± 0.03	102.01 ± 2.42	111.96 ± 2.45	123.97 ± 1.60	0.14 ± 0.04	97.44
DSSB LOW <sup>Ex</sup>	83.07 ± 1.70	91.91 ± 0.15	99.52 ± 0.67	0.06 ± 0.02	105.70 ± 0.47	118.17 ± 0.71	137.26 ± 3.84	0.41 ± 0.03	97.35
DSSD MED <sup>Ex</sup>	83.48 ± 2.39	91.75 ± 1.19	100.12 ± 0.20	0.10 ± 0.03	102.86 ± 0.24	117.47 ± 2.34	127.24 ± 3.64	0.18 ± 0.07	95.59
DSSB HI <sup>Ex</sup>	88.37 ± 2.78	96.54 ± 4.16	106.54 ± 6.60	0.14 ± 0.11	112.87 ± 1.06	123.48 ± 1.51	135.43 ± 3.01	0.23 ± 0.12	93.83
DSSB HI (Oil) <sup>Ex</sup>	81.84 ± 2.25	91.03 ± 1.58	99.27 ± 0.51	0.09 ± 0.04	104.64 ± 0.55	115.70 ± 0.33	127.51 ± 0.25	0.24 ± 0.02	96.03

Table 4.12 Descriptive analysis of oil added post-extrusion to FBFs

Time Point		Day 1		Week 6		Week 9		Week 13		Week 16		<i>p</i> -value
Sample		WSSB	DSSB	WSSB	DSSB	WSSB	DSSB	WSSB	DSSB	WSSB	DSSB	
Aroma	Rancid	0.46 <sup>d</sup>	0.50 <sup>d</sup>	1.79 <sup>c</sup>	0.54 <sup>d</sup>	0.67 <sup>d</sup>	0.58 <sup>d</sup>	8.63 <sup>a</sup>	7.38 <sup>b</sup>	6.86 <sup>b</sup>	8.43 <sup>a</sup>	<0.0001
	Painty	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.13 <sup>d</sup>	0.00 <sup>d</sup>	0.42 <sup>d</sup>	0.50 <sup>d</sup>	6.67 <sup>a</sup>	5.42 <sup>b</sup>	4.56 <sup>c</sup>	5.24 <sup>bc</sup>	0.0211
	Musty Overall <sup>ns</sup>	2.83	2.50	3.75	2.75	3.71	3.71	5.13	5.00	4.68	4.47	0.2639
	Cardboard	3.08 <sup>e</sup>	3.04 <sup>e</sup>	4.58 <sup>bc</sup>	3.21 <sup>e</sup>	4.83 <sup>abc</sup>	3.92 <sup>cde</sup>	3.58 <sup>de</sup>	4.96 <sup>ab</sup>	4.43 <sup>bcd</sup>	5.59 <sup>a</sup>	0.0001
	Toasted	1.75 <sup>cd</sup>	4.08 <sup>a</sup>	2.29 <sup>bc</sup>	4.71 <sup>a</sup>	3.04 <sup>b</sup>	4.29 <sup>a</sup>	1.50 <sup>de</sup>	1.54 <sup>cde</sup>	1.63 <sup>cde</sup>	0.88 <sup>e</sup>	<0.0001
	Brown	1.17 <sup>c</sup>	2.54 <sup>b</sup>	0.88 <sup>c</sup>	2.54 <sup>b</sup>	2.08 <sup>b</sup>	3.29 <sup>a</sup>	1.13 <sup>c</sup>	1.08 <sup>c</sup>	1.23 <sup>c</sup>	0.65 <sup>c</sup>	<0.0001
	Grain,Overall	6.75 <sup>b</sup>	7.13 <sup>ab</sup>	6.63 <sup>b</sup>	6.71 <sup>b</sup>	6.54 <sup>b</sup>	7.63 <sup>a</sup>	2.83 <sup>d</sup>	3.58 <sup>d</sup>	4.52 <sup>c</sup>	3.10 <sup>d</sup>	<0.0001
	Rancid	0.54 <sup>f</sup>	0.54 <sup>f</sup>	3.42 <sup>d</sup>	2.08 <sup>e</sup>	1.88 <sup>e</sup>	1.25 <sup>ef</sup>	8.50 <sup>bc</sup>	9.50 <sup>ab</sup>	8.44 <sup>c</sup>	9.95 <sup>a</sup>	0.0008
Flavor	Painty	0.00 <sup>e</sup>	0.08 <sup>de</sup>	0.79 <sup>d</sup>	0.75 <sup>d</sup>	0.50 <sup>de</sup>	0.50 <sup>de</sup>	5.13 <sup>c</sup>	6.08 <sup>b</sup>	5.99 <sup>b</sup>	7.18 <sup>a</sup>	0.0431
	Overall Grain	7.08 <sup>ab</sup>	7.38 <sup>a</sup>	6.88 <sup>ab</sup>	7.08 <sup>ab</sup>	6.67 <sup>b</sup>	6.71 <sup>b</sup>	3.96 <sup>d</sup>	3.75 <sup>d</sup>	4.87 <sup>c</sup>	3.04 <sup>e</sup>	<0.0001
	Sorghum <sup>ns</sup>	4.38	3.54	4.25	3.75	4.04	4.04	3.58	3.38	4.03	2.99	0.3410
	Soy <sup>ns</sup>	2.58	2.92	2.67	2.83	2.46	2.71	1.25	1.79	1.68	1.28	0.1159
	Starch	7.83 <sup>a</sup>	7.79 <sup>a</sup>	7.71 <sup>a</sup>	7.58 <sup>a</sup>	6.21 <sup>b</sup>	6.00 <sup>b</sup>	5.71 <sup>b</sup>	3.25 <sup>d</sup>	6.23 <sup>b</sup>	4.53 <sup>c</sup>	<0.0001
	Toasted	2.58 <sup>cd</sup>	3.38 <sup>ab</sup>	2.33 <sup>d</sup>	3.50 <sup>ab</sup>	3.00 <sup>bc</sup>	3.75 <sup>a</sup>	1.58 <sup>e</sup>	1.67 <sup>e</sup>	2.12 <sup>de</sup>	0.93 <sup>f</sup>	<0.0001
	Brown	1.67 <sup>cd</sup> e	2.04 <sup>bcd</sup>	0.79 <sup>g</sup>	2.21 <sup>bc</sup>	2.33 <sup>b</sup>	3.04 <sup>a</sup>	1.38 <sup>ef</sup>	1.46 <sup>ef</sup>	1.55 <sup>de</sup>	0.93 <sup>fg</sup>	<0.0001
	Cardboard	3.25 <sup>c</sup>	3.08 <sup>c</sup>	4.50 <sup>a</sup>	3.29 <sup>c</sup>	3.17 <sup>c</sup>	3.21 <sup>c</sup>	4.67 <sup>a</sup>	4.79 <sup>a</sup>	4.68 <sup>a</sup>	3.95 <sup>b</sup>	0.0021
	Musty	4.67 <sup>e</sup>	4.71 <sup>e</sup>	5.21 <sup>de</sup>	4.67 <sup>e</sup>	5.75 <sup>cd</sup>	5.50 <sup>d</sup>	6.17 <sup>bc</sup>	8.13 <sup>a</sup>	6.53 <sup>b</sup>	8.43 <sup>a</sup>	<0.0001
	Astringent <sup>ns</sup>	3.00	2.96	3.25	3.42	3.13	2.96	4.00	4.38	4.08	3.93	0.3797

Products with different letters are significantly different from each other in that attribute ( $p < 0.05$ ). (ns) -means there is no interaction effect in that attribute.

Table 4.13 Descriptive analysis of oil added pre-extrusion to FBFs

Products with different letters are significantly different from each other in that attribute ( $p < 0.05$ ). (ns) - means there is no interaction effect in that attribute

Time Point		Week 0		Week 6		Week 9		Week 13		Week 16		p-value
Sample		WSSB(O)	DSSB(O)	WSSB(O)	DSSB(O)	WSSB(O)	DSSB(O)	WSSB(O)	DSSB(O)	WSSB(O)	DSSB(O)	
Aroma	Rancid	0.58 <sup>ef</sup>	0.50 <sup>f</sup>	0.79 <sup>ef</sup>	1.50 <sup>e</sup>	9.29 <sup>a</sup>	8.38 <sup>ab</sup>	7.46 <sup>b</sup>	4.67 <sup>d</sup>	6.28 <sup>c</sup>	6.36 <sup>c</sup>	<0.0001
	Painty <sup>ns</sup>	0.13	0.00	0.00	0.29	5.29	5.13	4.04	3.00	4.58	4.79	0.3761
	Cardboard	2.92 <sup>e</sup>	3.29 <sup>cde</sup>	4.04 <sup>bc</sup>	3.88 <sup>bcd</sup>	4.50 <sup>b</sup>	4.42 <sup>b</sup>	5.79 <sup>a</sup>	4.63 <sup>b</sup>	3.21 <sup>de</sup>	5.98 <sup>a</sup>	<0.0001
	Toasted	1.96 <sup>bc</sup>	2.17 <sup>bc</sup>	2.54 <sup>ab</sup>	2.42 <sup>ab</sup>	2.00 <sup>bc</sup>	2.04 <sup>bc</sup>	1.46 <sup>cd</sup>	3.04 <sup>a</sup>	1.10 <sup>d</sup>	0.97 <sup>d</sup>	0.0048
	Brown	1.13 <sup>cd</sup>	1.25 <sup>bcd</sup>	0.46 <sup>e</sup>	0.92 <sup>de</sup>	1.71 <sup>b</sup>	1.58 <sup>bc</sup>	1.25	2.38 <sup>a</sup>	0.90 <sup>de</sup>	0.50 <sup>e</sup>	0.0024
	Grain,Overall	7.08 <sup>a</sup>	7.04 <sup>ab</sup>	6.83 <sup>abc</sup>	6.13 <sup>bc</sup>	4.25 <sup>d</sup>	3.63 <sup>d</sup>	3.63 <sup>d</sup>	6.08 <sup>c</sup>	3.64 <sup>d</sup>	4.01 <sup>d</sup>	<0.0001
	Musty Overall	2.83 <sup>cd</sup>	2.79 <sup>d</sup>	3.08 <sup>cd</sup>	3.46 <sup>c</sup>	4.38 <sup>b</sup>	3.21 <sup>cd</sup>	4.88 <sup>b</sup>	4.50 <sup>b</sup>	3.05 <sup>cd</sup>	5.67 <sup>a</sup>	<0.0001
Flavor	Rancid <sup>ns</sup>	0.88	0.75	2.42	2.58	9.25	9.50	8.29	7.79	7.87	7.68	0.7330
	Painty	0.08 <sup>e</sup>	0.00 <sup>e</sup>	0.92 <sup>d</sup>	1.29 <sup>d</sup>	5.38 <sup>ab</sup>	5.79 <sup>a</sup>	5.46 <sup>ab</sup>	2.88 <sup>c</sup>	5.37 <sup>ab</sup>	5.11 <sup>b</sup>	<0.0001
	Overall Grain	6.96 <sup>a</sup>	7.04 <sup>a</sup>	7.17 <sup>a</sup>	6.92 <sup>a</sup>	4.46 <sup>cd</sup>	4.79 <sup>c</sup>	4.13 <sup>cd</sup>	5.79 <sup>b</sup>	3.91 <sup>d</sup>	5.98 <sup>b</sup>	0.0005
	Sorghum <sup>ns</sup>	4.13	4.42	4.50	4.58	3.96	4.08	3.63	3.75	3.21	3.85	0.8534
	Soy	2.58 <sup>a</sup>	2.29 <sup>abc</sup>	2.50 <sup>a</sup>	2.67 <sup>a</sup>	1.79 <sup>d</sup>	1.79 <sup>d</sup>	2.00 <sup>cd</sup>	2.04 <sup>bcd</sup>	1.63 <sup>d</sup>	2.47 <sup>ab</sup>	0.0120
	Starch <sup>ns</sup>	7.88	7.71	7.29	7.63	5.79 <sup>b</sup>	5.75	5.75	6.13	5.14	5.97	0.2685
	Toasted <sup>ns</sup>	2.63	2.54	2.17	2.58	2.21	2.25	1.46	2.54	1.60	2.30	0.0618
	Brown	1.67 <sup>ab</sup>	1.25 <sup>bcd</sup>	0.79 <sup>d</sup>	0.96 <sup>cd</sup>	1.42 <sup>bc</sup>	1.63 <sup>ab</sup>	1.29 <sup>bcd</sup>	2.04 <sup>a</sup>	0.93 <sup>cd</sup>	1.68 <sup>ab</sup>	0.0275
	Cardboard	3.17 <sup>f</sup>	3.38 <sup>ef</sup>	3.88 <sup>cd</sup>	3.67 <sup>de</sup>	3.63 <sup>def</sup>	3.92 <sup>cd</sup>	4.33 <sup>bc</sup>	4.08 <sup>cd</sup>	5.36 <sup>a</sup>	4.60 <sup>b</sup>	0.0225
	Musty	4.63 <sup>d</sup>	4.58 <sup>d</sup>	5.04 <sup>d</sup>	5.08 <sup>d</sup>	5.67 <sup>c</sup>	5.92 <sup>bc</sup>	6.13 <sup>bc</sup>	6.13 <sup>bc</sup>	7.53 <sup>a</sup>	6.47 <sup>b</sup>	0.0198
Astringent <sup>ns</sup>	3.29	3.08	3.50	3.08	3.50	3.63	4.13	3.50	3.98	3.49	0.2249	

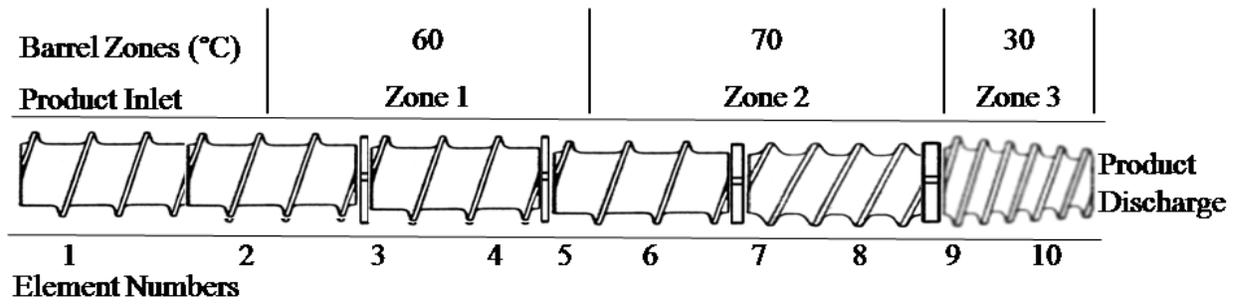


Figure 4.1 Schematic of pilot-scale single screw profile and barrel zone temperatures. Element Numbers 1-2 =single flight screws; 3=small steam lock; 4=single flight screw; 5=small steam lock; 6=single flight screw; 7=medium steam lock; 8=double flight screw; 9=large steam lock and 10= triple flight uncut cone.

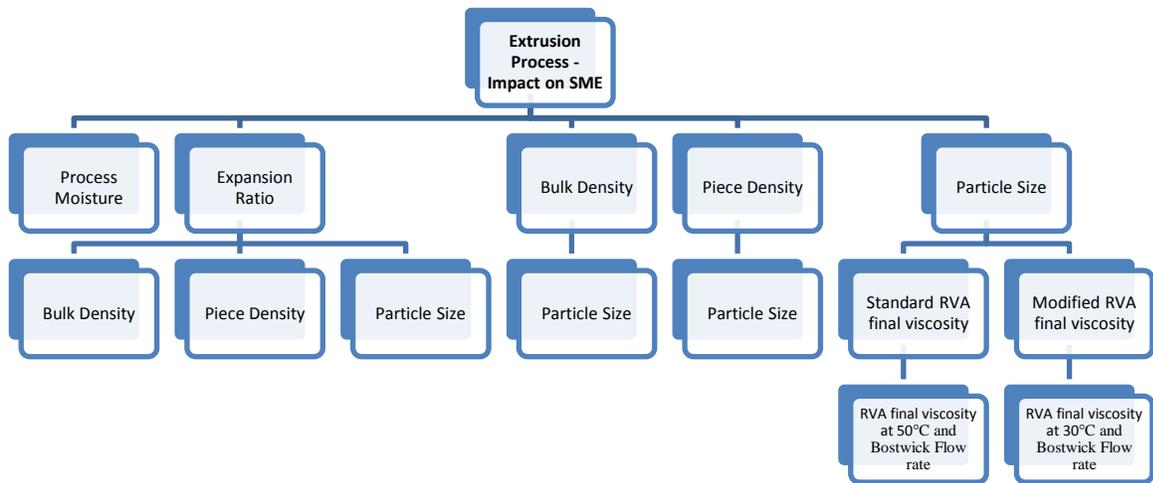


Figure 4.2 Relationship Heirarchy: Extrusion - Intermediate Product - Final product

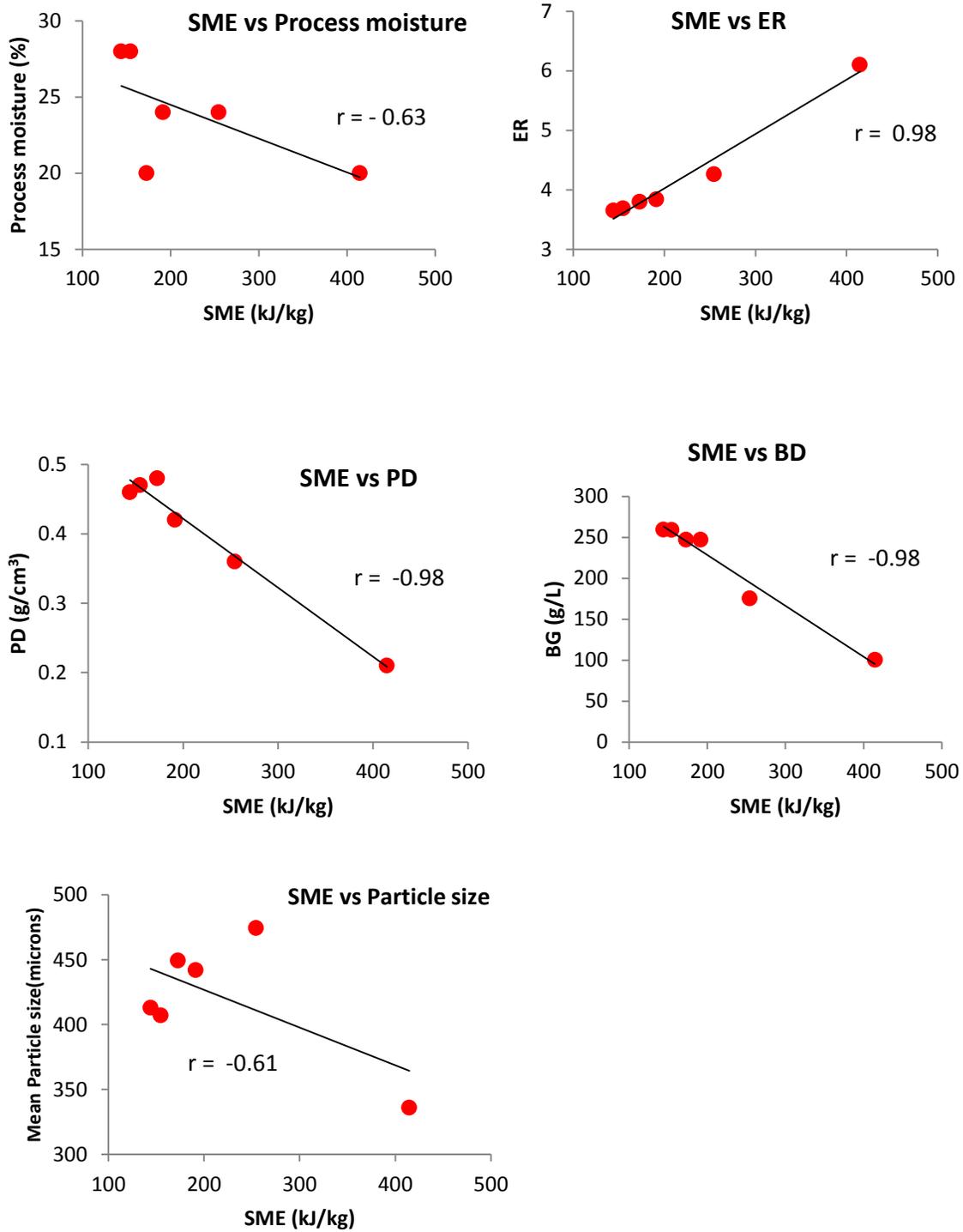


Figure 4.3 Impact of SME on Extrudate properties for treatments without oil in formulation

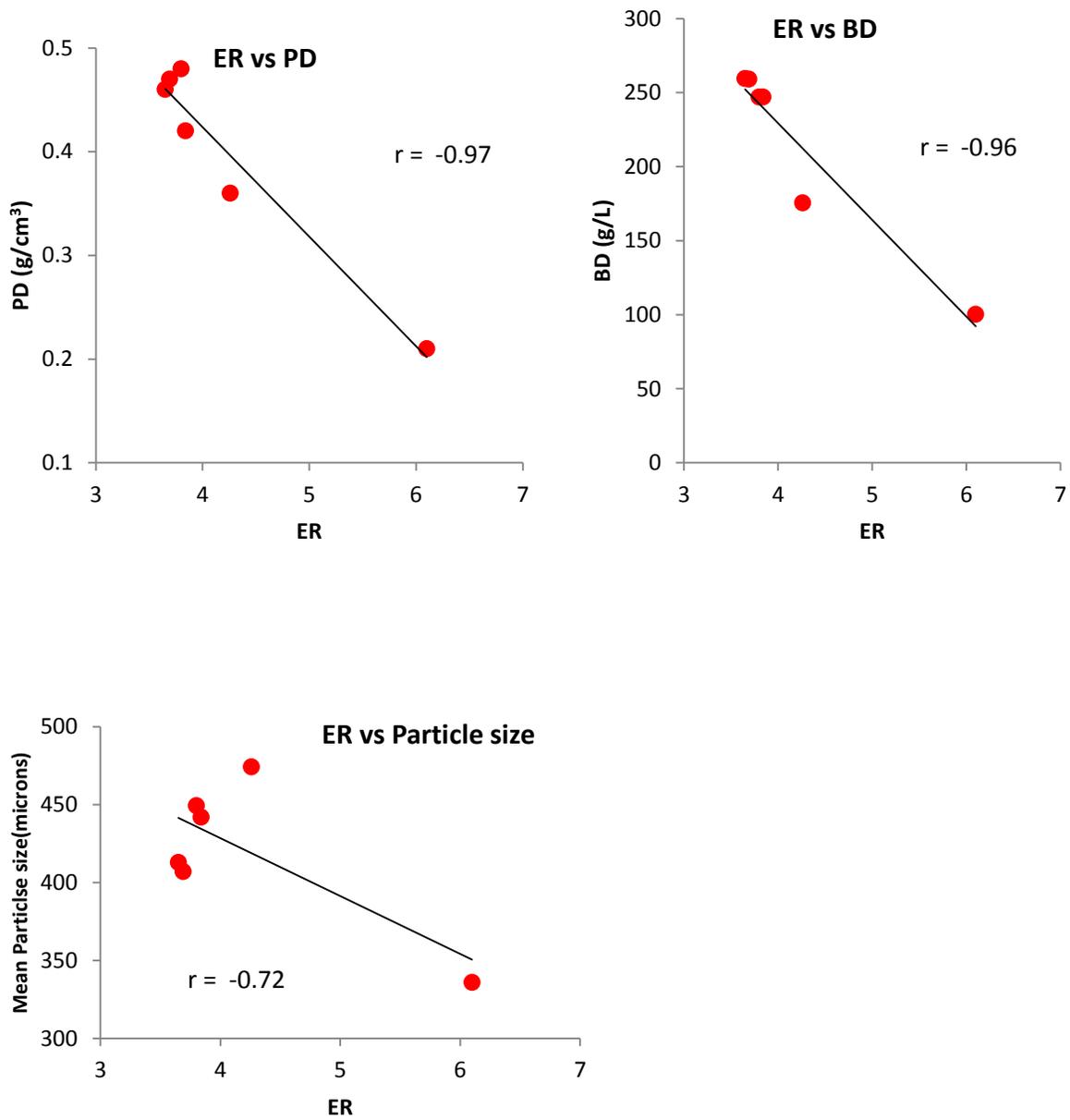


Figure 4.4 Impact of product expansion on extrudate properties without oil in formulation

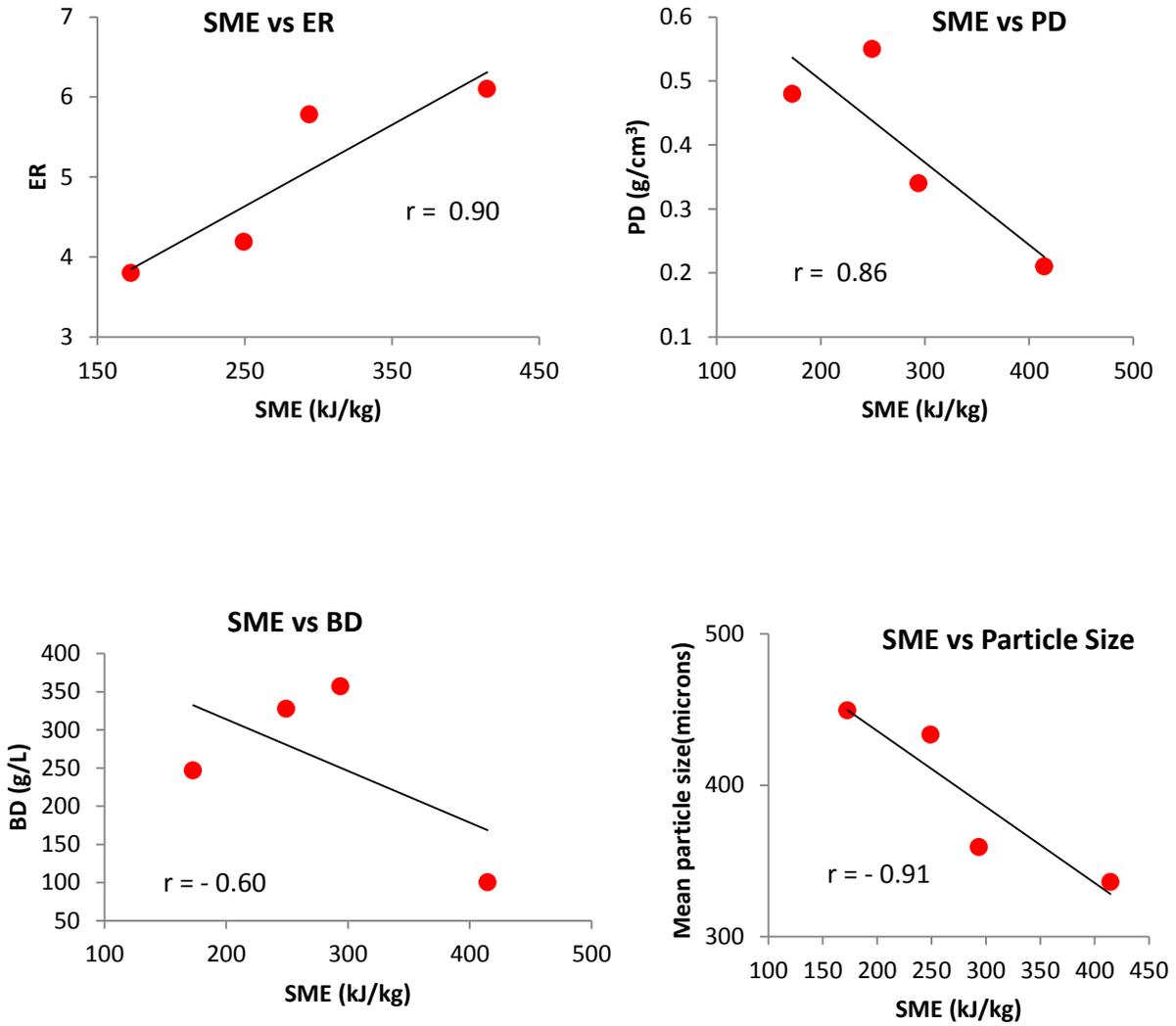


Figure 4.5 Impact of SME on Extrudate properties for treatments with premixed oil in formulation

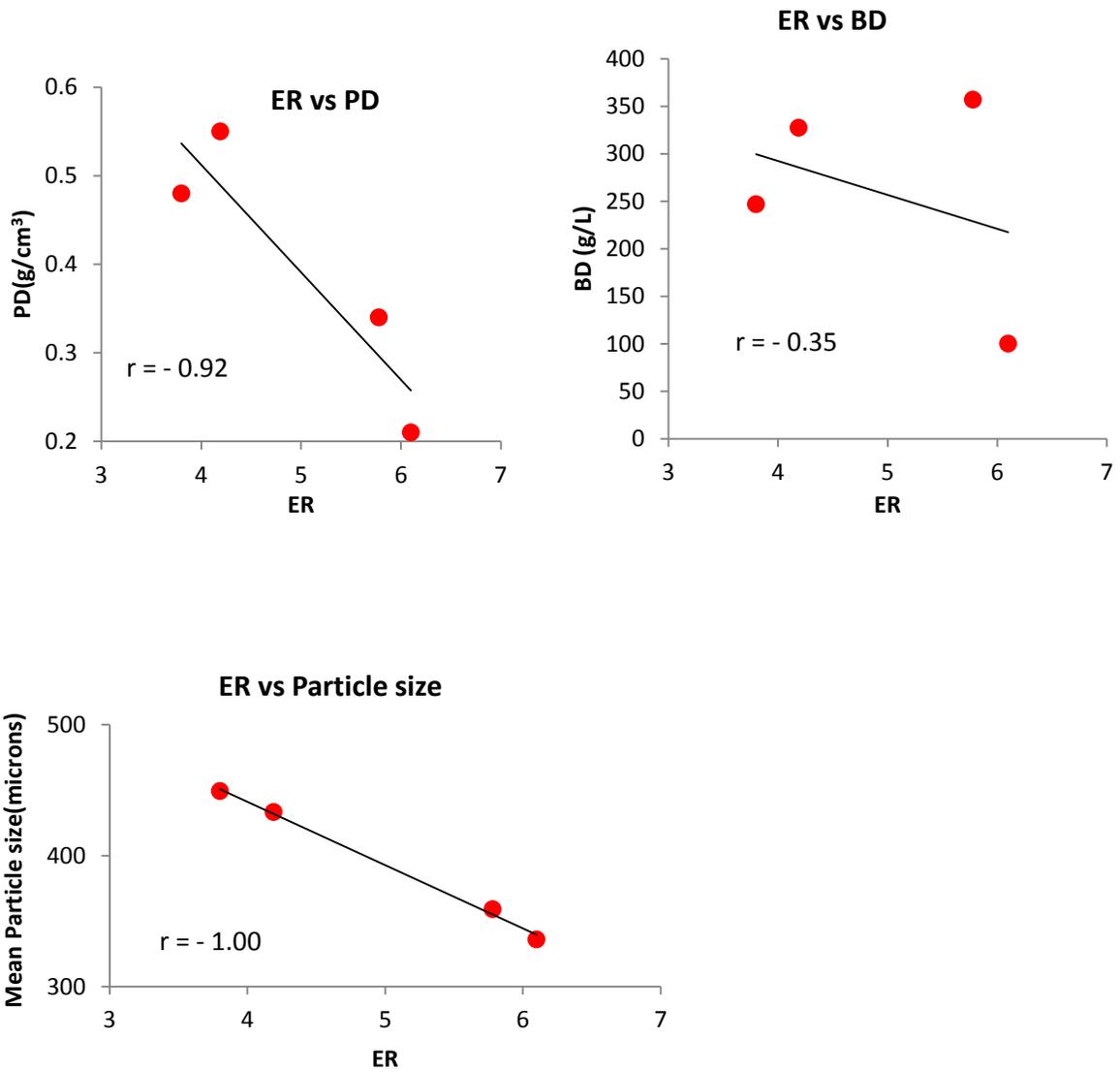


Figure 4.6 Impact of product expansion on extrudate properties with premixed oil in formulation

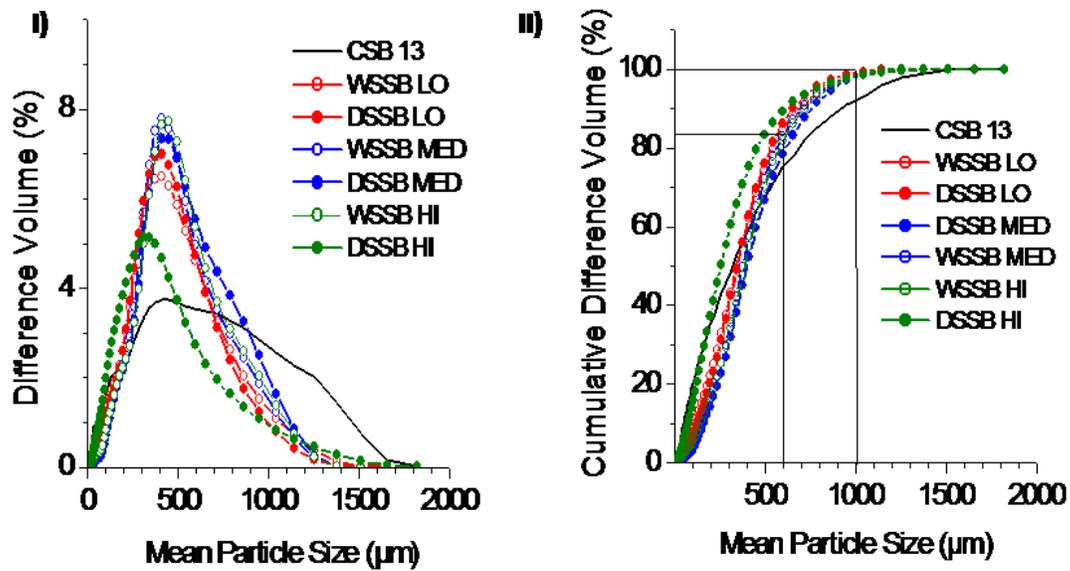


Figure 4.7 Particle size distributions of extruded and milled blends

– Impact on energy levels. Mean particle sizes obtained by particle size analysis as (I) the distribution of % difference volume (left) and (II) cumulative difference volume (right) of milled blends. Solid lines in (II) indicate the critical point of particle uniformity through a 600 µm sieve.

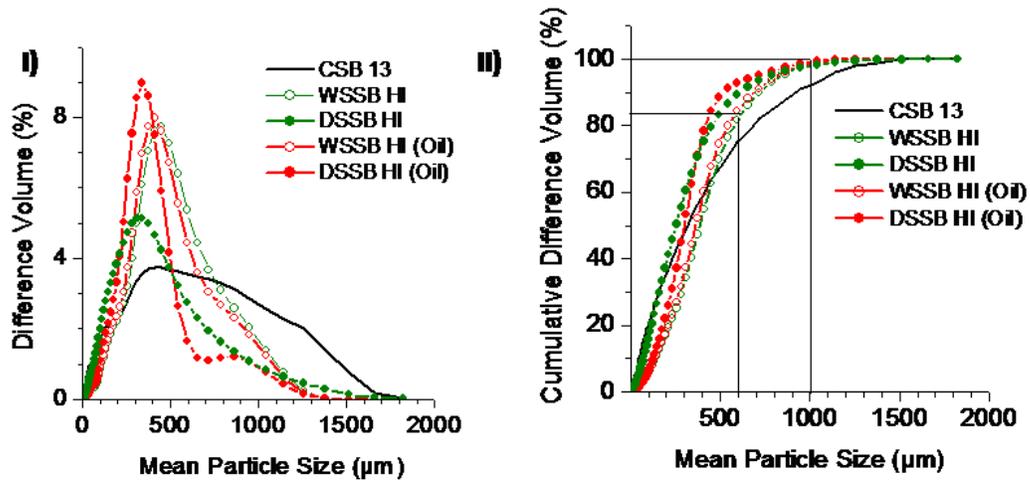
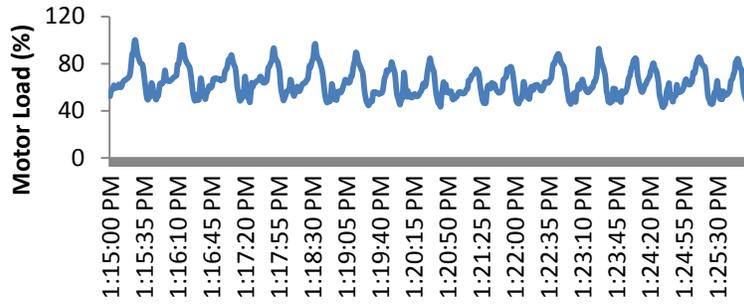


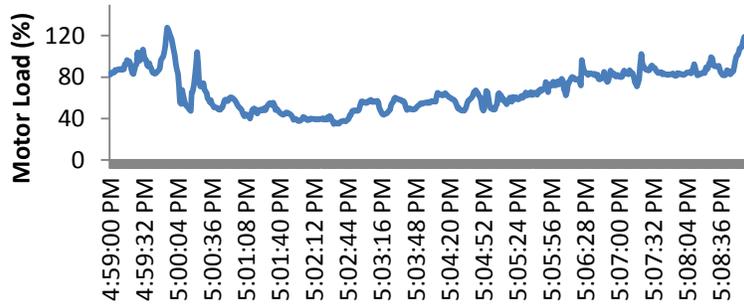
Figure 4.8 Particle size distributions of extruded and milled blends

– Impact on oil Mean particle sizes obtained by particle size analysis as (I) the distribution of % difference volume (left) and (II) cumulative difference volume (right) of milled blends. Solid lines in (II) indicate the critical point of particle uniformity through a 600 µm sieve.



7) WHOLE SORGHUM SOY BLEND (WSSB)  
Extruded with 5.5% vegetable

High shear – 450 RPM, 20% extrusion moisture



8) DECORTICATED SORGHUM SOY BLEND (DSSI)  
Extruded with 5.5% vegetable

High shear – 450 RPM, 20% extrusion moisture



Figure 4.9 Motor load fluctuations with oil added in formulation

WSSB (top), DSSB (bottom) showing product surging and extrudate instability due to unstable SMEs from DAQ data

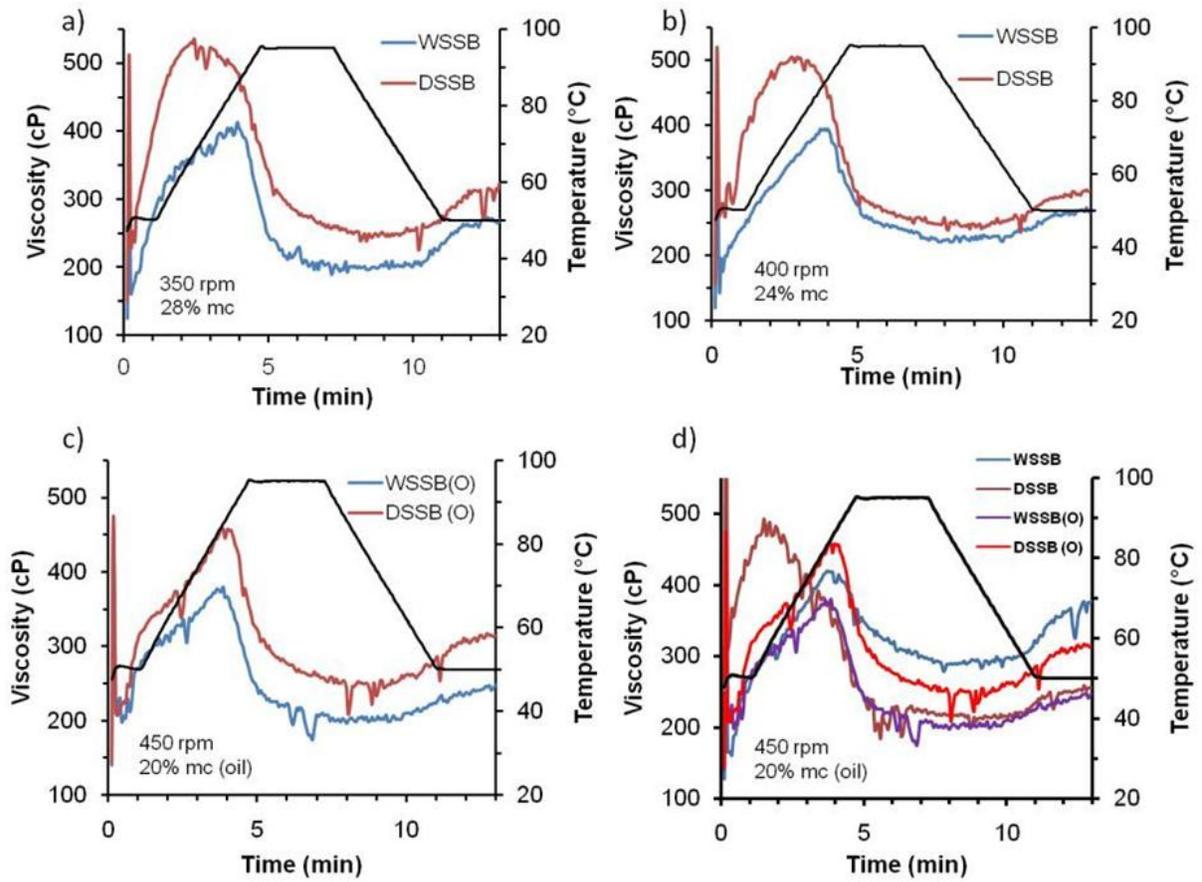


Figure 4.10 RVA pasting curves.

a), b), c) – RVA pasting curves showing comparative viscosity profiles of WSSB versus DSSB blends extruded with Low (LO), Medium (MED) and High (HI) screw speeds. d) – pasting profiles of FBFs with oil added pre-extrusion

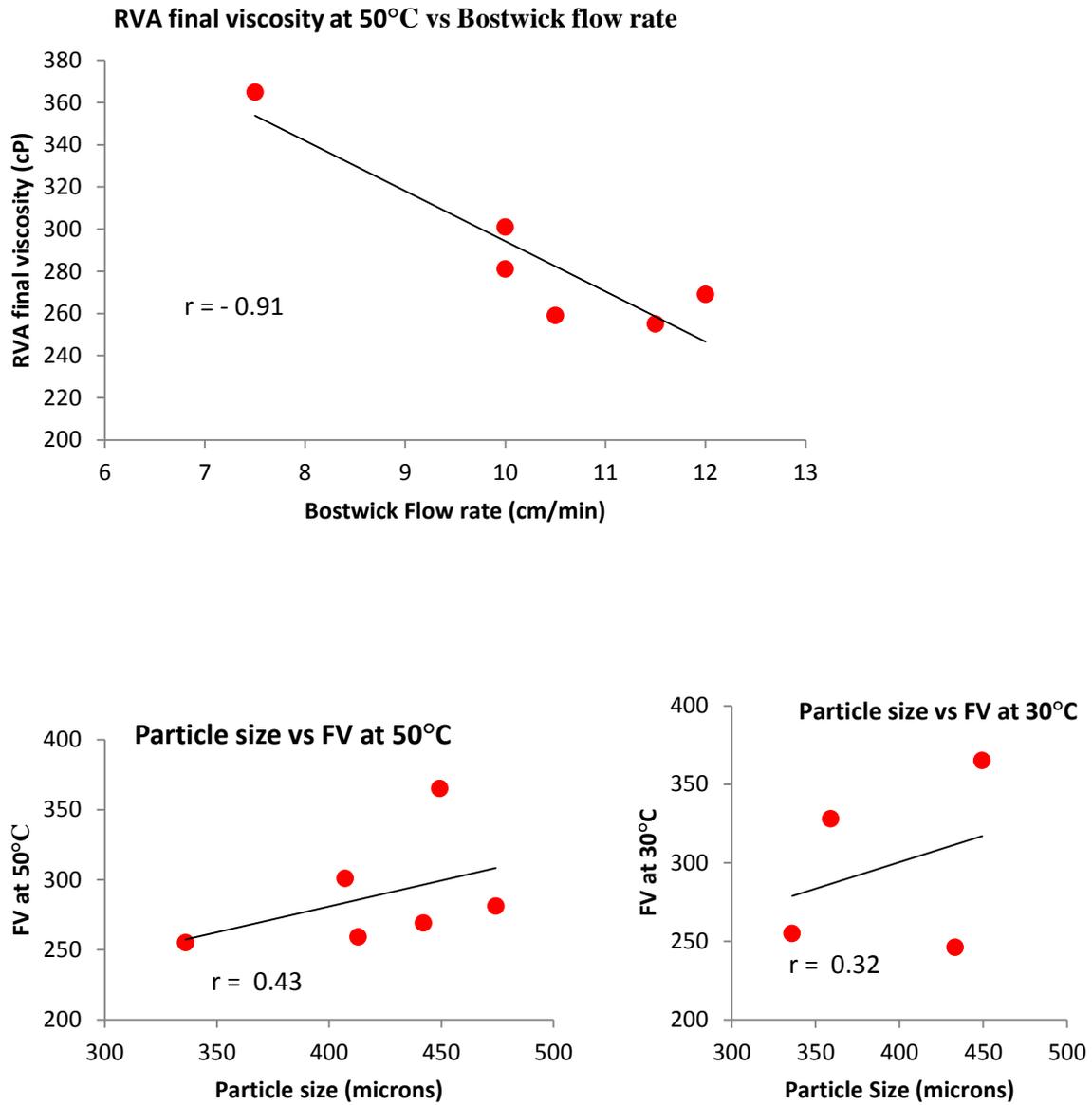


Figure 4.11 Correlations

Between RVA final viscosity at 50°C and Bostwick flow rate (top), Relationships between particle size and final viscosity at 50°C (left) and 30°C (right)

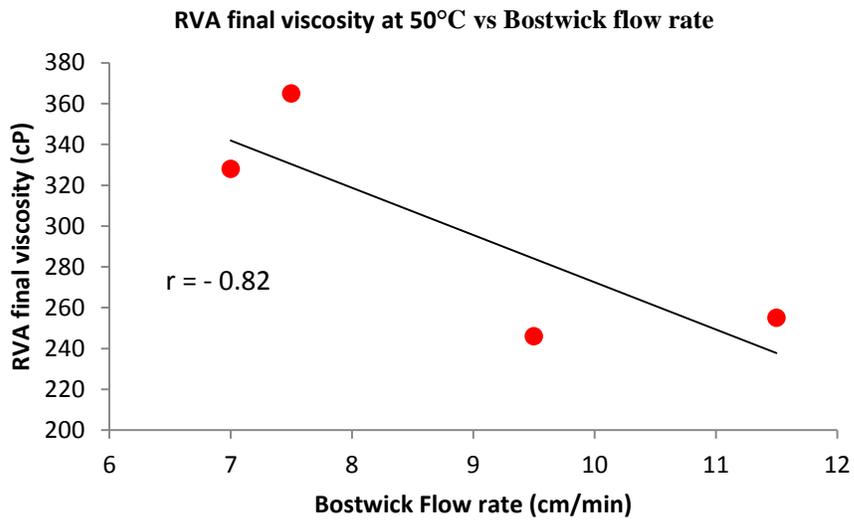


Figure 4.12 RVA final viscosity at 50°C and Bostwick correlation for premixed oil in formulation

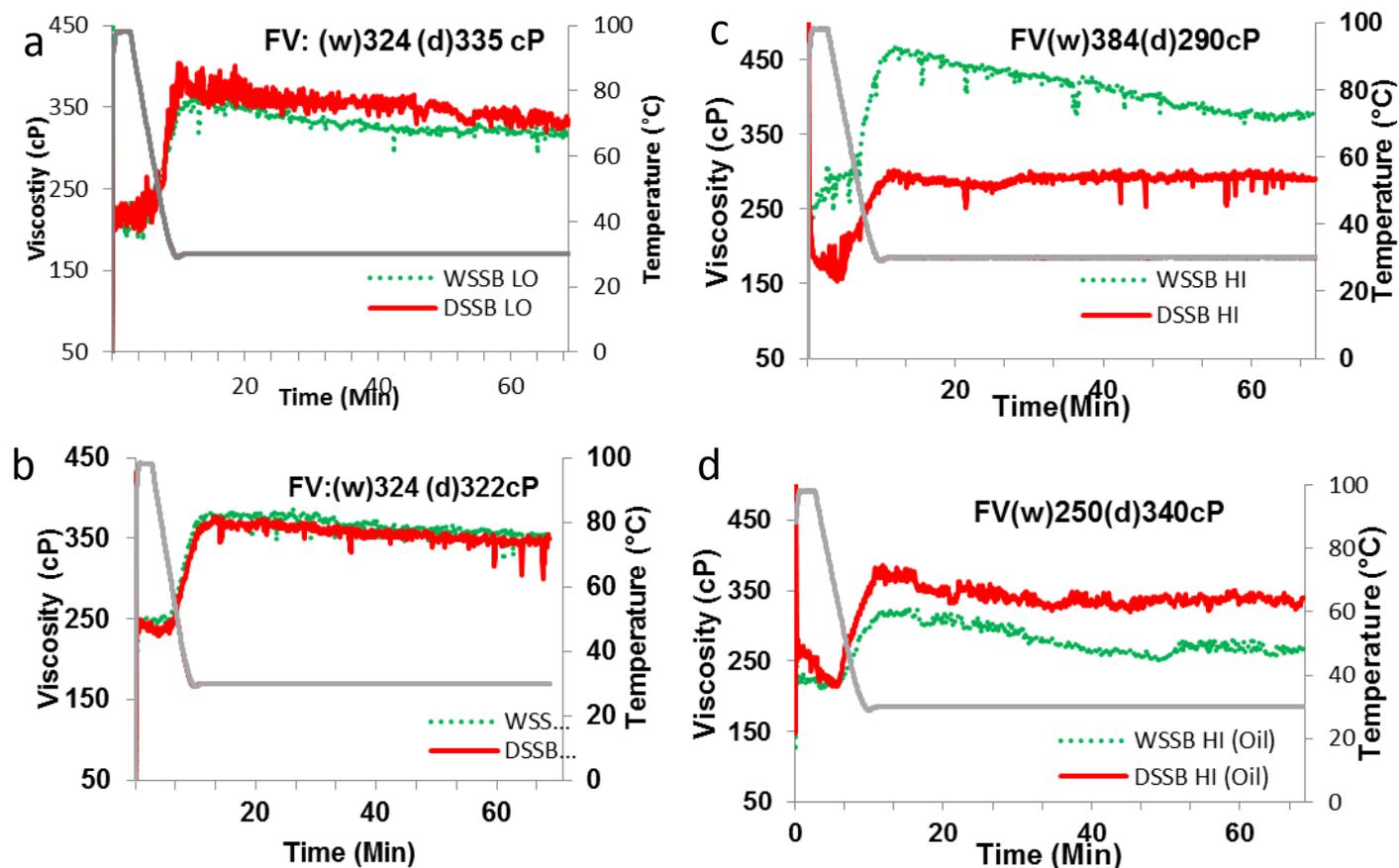


Figure 4.13 Simulated RVA profiles with extended time, at constant shear and temperature  
 a),b) – Showing consistent and stable viscosity profiles of low and medium shear conditions  
 c), d) – Showing comparative changes in viscosity profiles at high screw speed due to effects of oil added pre-extrusion (temperature maintained at 30°C for all treatments ). (w) – whole sorghum soy blend, (d) – decorticated sorghum soy blend, FV – final viscosity

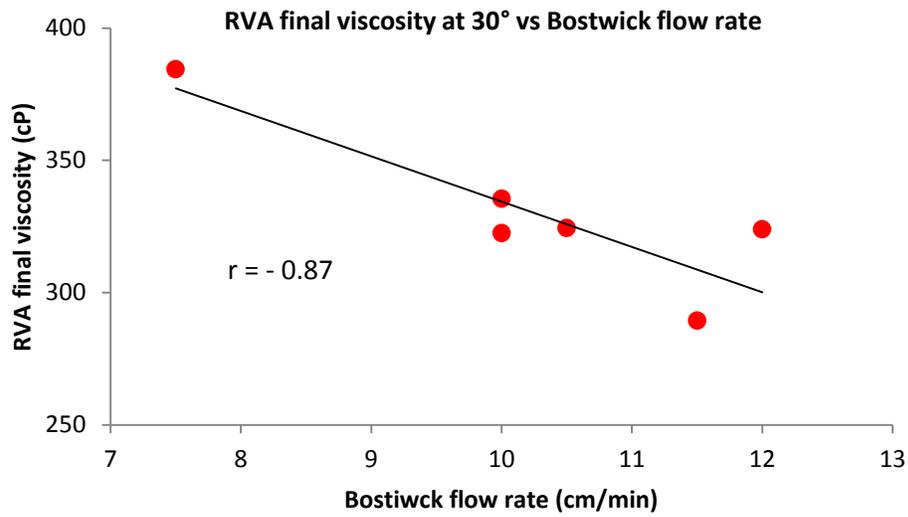


Figure 4.14 RVA final viscosity at 30°C and Bostwick correlation without oil in formulation

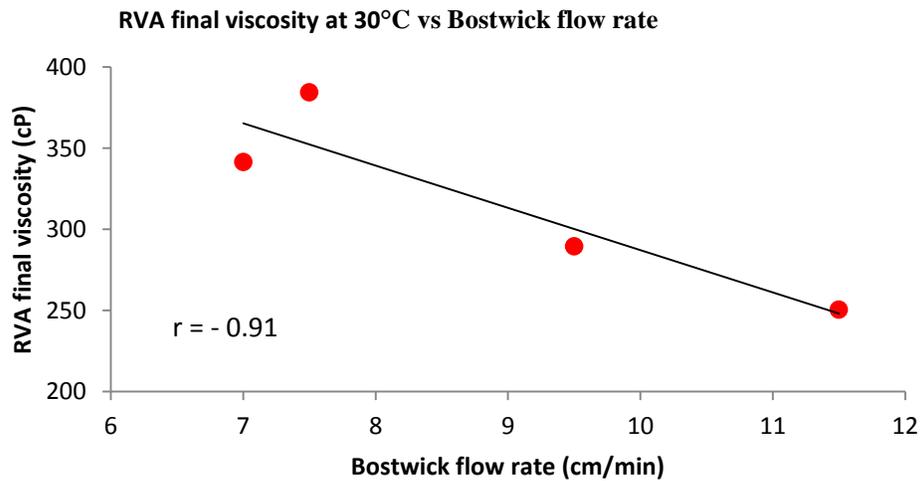


Figure 4.15 RVA final viscosity at 30°C and Bostwick correlation for premixed oil in formulation

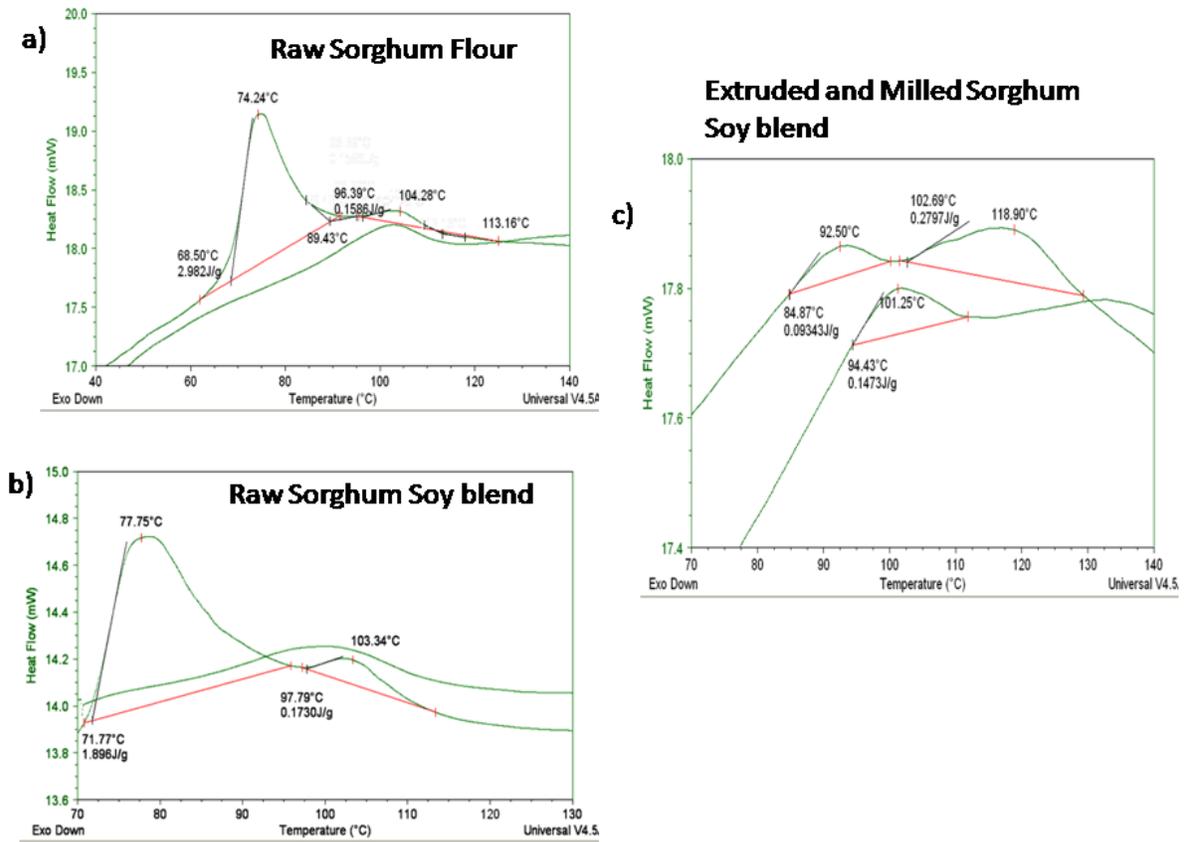


Figure 4.16 Differential Scanning Calorimetry (DSC) thermograms of aqueous dispersions (66.7% water) of a) Raw sorghum flour, b) raw sorghum soy blend, c) extruded and milled sorghum soy blend

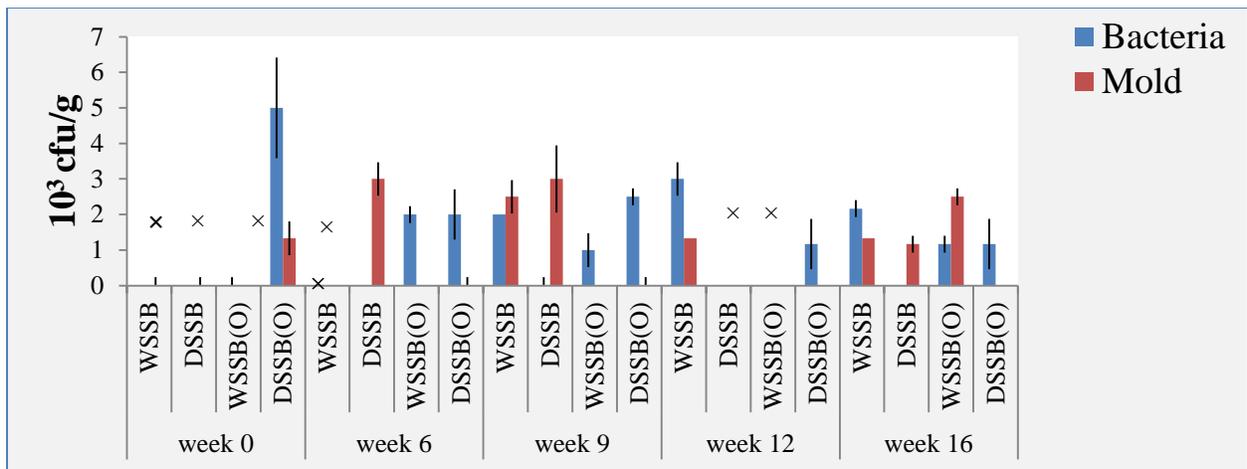
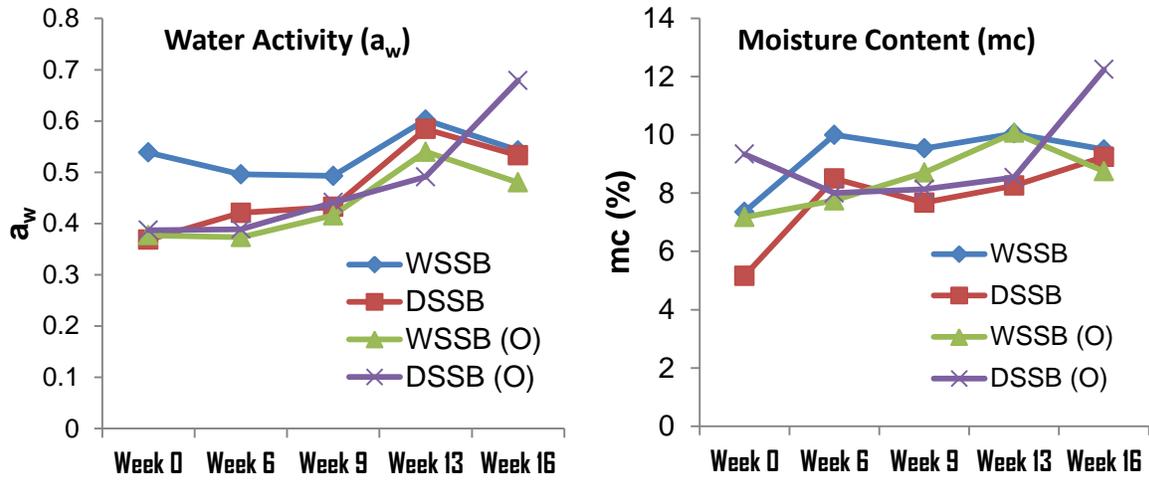


Figure 4.17 Water activity, Moisture, Microbial growth

(top left) Water activity at 25°C, (top right) Moisture content (readings in duplicate were similar) (bottom) Bacteria and mold growth, across time points of the shelf-life study. (x) – means no microbial colonies detected. *e-coli*, *enterobactereacea* and *staphylococcus aureus* was tested negative during each time interval of the study.

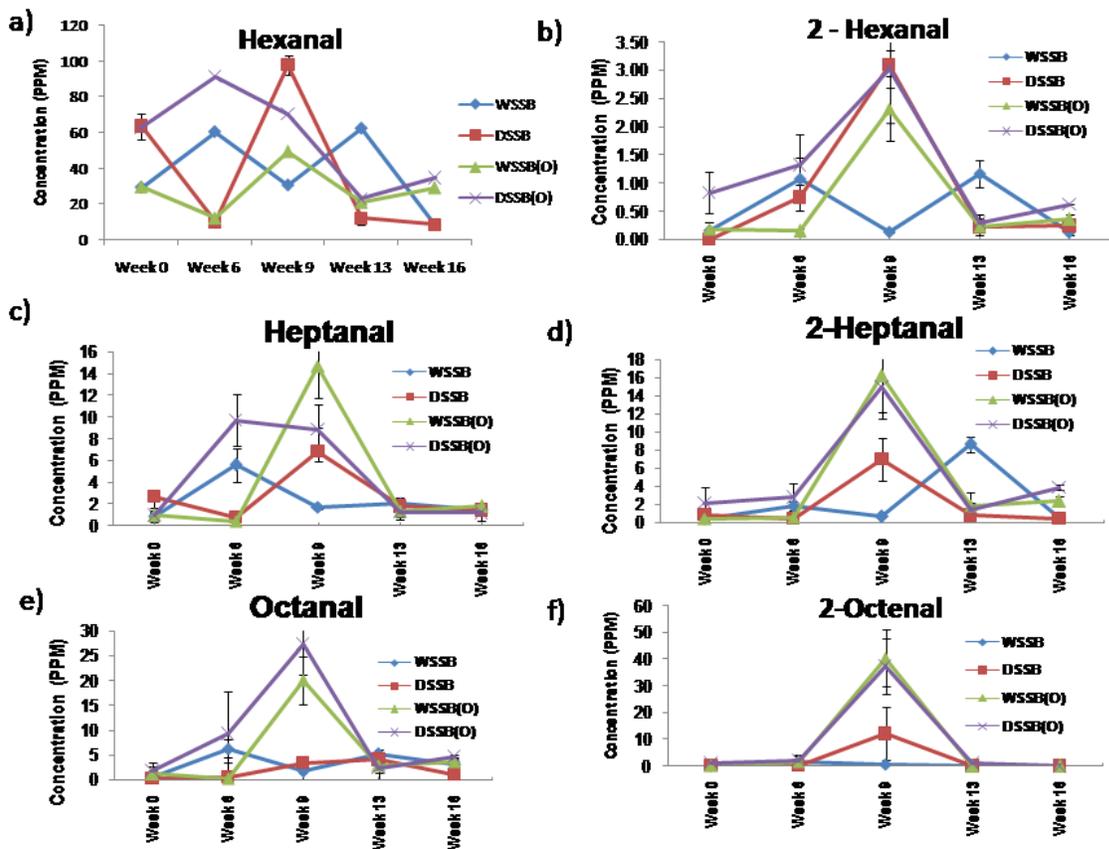


Figure 4.18 Key volatiles detected from aldehydes by autoxidation of unsaturated fatty acids. Concentration in PPM from area under the peaks of volatiles that were precursors in flavor reversion and oxidation of vegetable oil (soy bean) stored at 50°C and 70% RH across 16 weeks. a), b), c), d) originated from Linoleic acid, e), f) from Oleic acid.

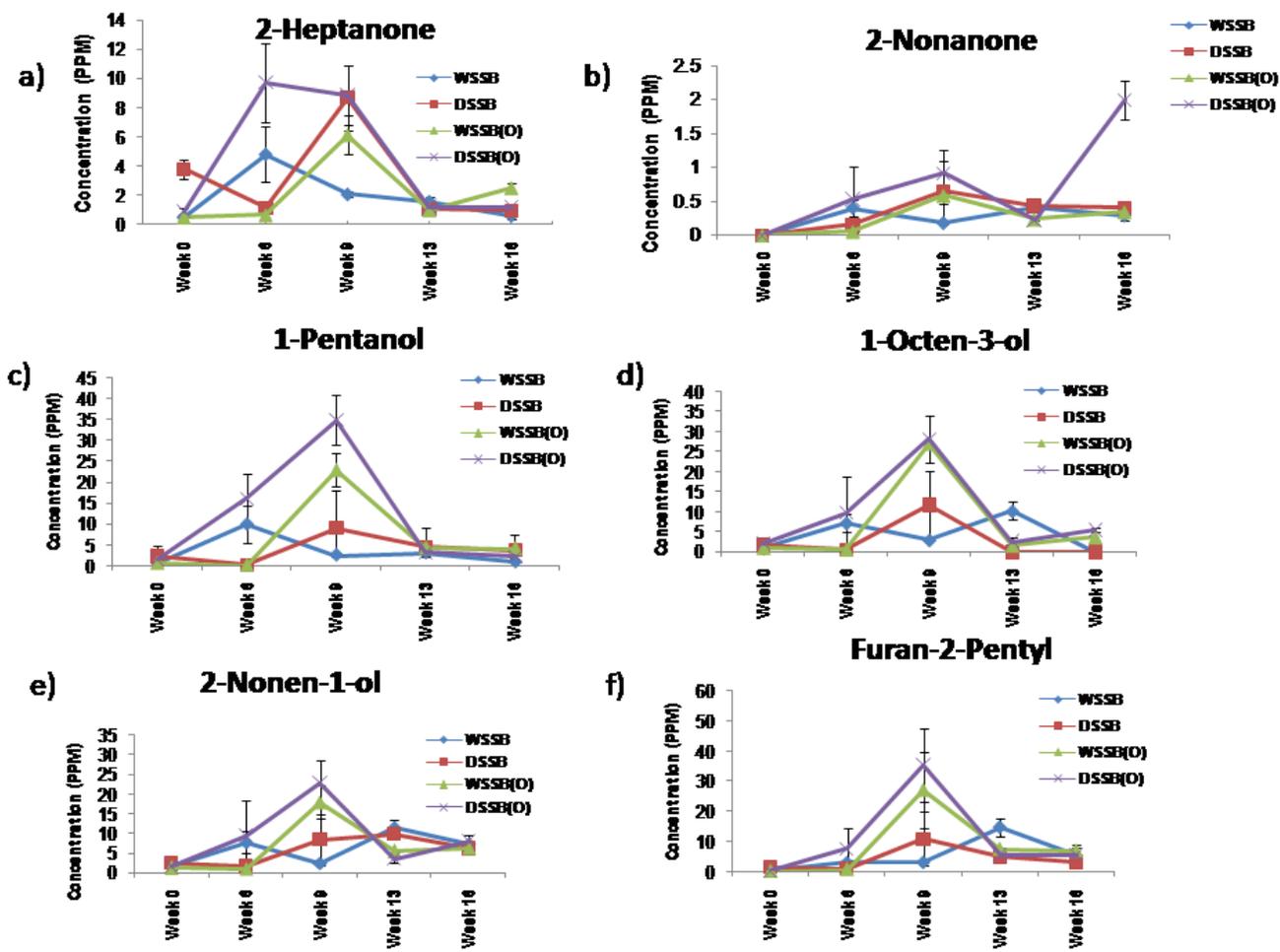


Figure 4.19 Ketones, Alcohols

a),b) -Aliphatic Ketones formed by autoxidation. c), d), e) Alcohols , f) – Furan-2-Pentyl (A well known autoxidation product of linoleate involved in the reversion of soybean oil).

## Diethyl Pthalate

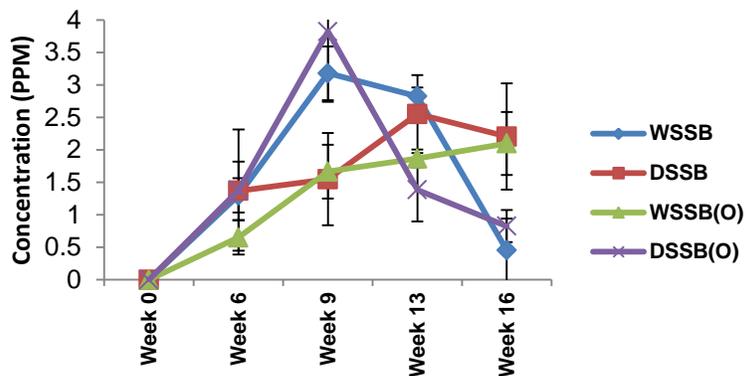


Figure 4.20 Diethyl phthalate (DEP)

– A group of diesters or orthophthalic acid found in foods, a migrant compound from packaging materials, mostly plastics.

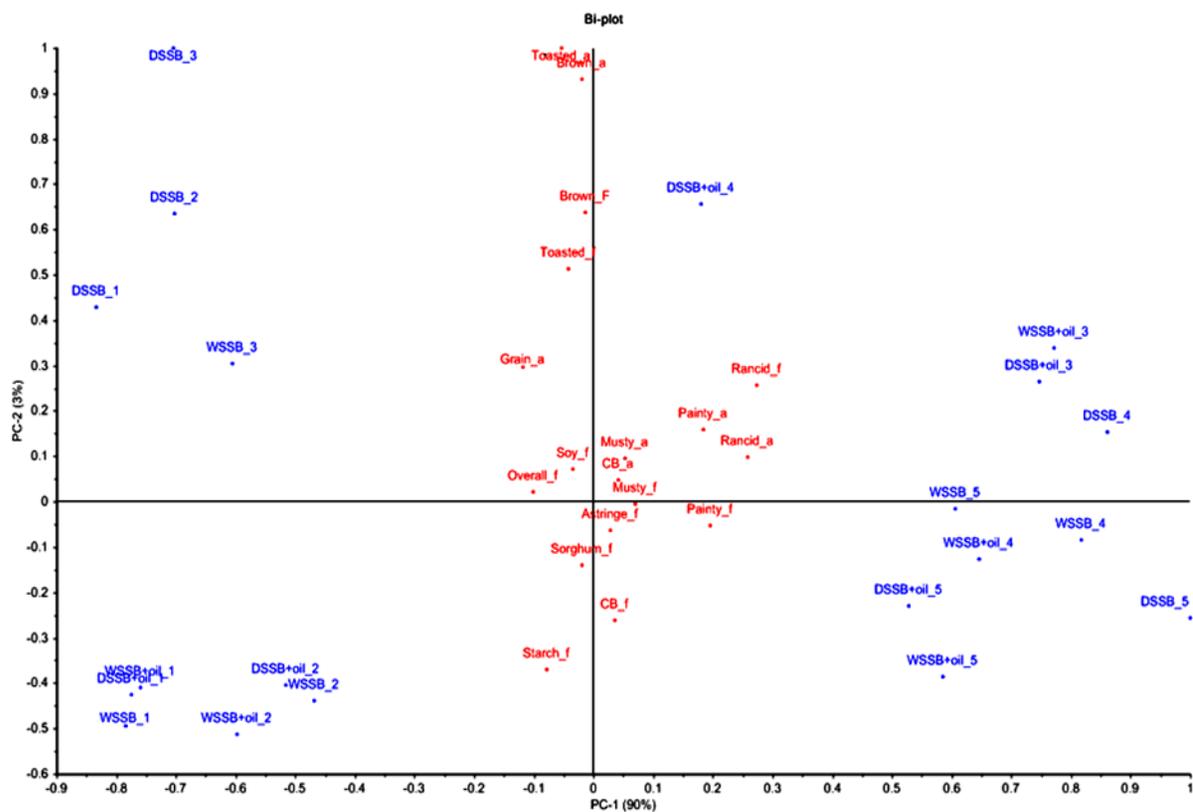


Figure 4.21 Principal component analysis of the samples and attributes.

For the samples, “a” indicates aroma attributes, “f” indicates flavor attributes, “1”, “2”, “3”, “4” and “5” indicates day 1, week 6, week 9, week 13, week 16 respectively

## Appendix B - Volatile Aromatic Composition detected in FBFs

Compound Name	Week 0				Week 6				Week 9				Week 13				Week 16			
	WSSB		DSSB		WSSB		DSSB		WSSB		DSSB		WSSB		DSSB		WSSB		DSSB	
	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A
Butanoic Acid Methyl Ester	0.42	0.16	0.68	0.43	0.46	0.11	0.03	0.01	0.29	0.12	0.37	0.18	nd	nd	nd	nd	nd	nd	nd	nd
1H-Pyrrole, 1-methyl	nd	nd	nd	nd	3.13	0.42	0.52	0.29	nd	nd	1.21	1.41	nd	nd	nd	nd	nd	nd	nd	nd
2-Pentyn-1-ol	nd	nd	nd	nd	nd	nd	nd	nd	0.09	0.01	0.71	0.57	0.30	0.22	0.13	0.04	0.08	0.03	0.09	0.02
1-Pentanol	0.87	0.47	2.40	1.23	9.97	4.57	0.26	0.07	2.66	0.46	9.06	6.62	3.20	0.62	4.52	0.88	1.03	0.25	3.78	0.54
2-Hexanone	0.14	0.04	0.53	0.07	0.81	0.16	0.42	0.54	0.38	0.08	1.05	0.73	nd	nd	0.15	0.04	0.11	0.05	0.11	0.02
Hexanal	29.38	18.14	63.68	7.19	60.59	3.56	9.88	2.63	30.64	3.53	97.93	5.39	62.54	12.79	12.21	3.73	8.27	3.13	8.55	1.19
3-Pentenal, 4-methyl	nd	nd	nd	nd	nd	nd	nd	nd	0.09	0.01	0.51	0.53	0.22	0.04	0.09	0.03	0.08	0.03	0.03	0.01
Pyrazine, methyl-	nd	nd	5.47	1.16	1.34	0.37	1.09	0.37	0.20	0.04	3.91	3.06	nd	nd	0.42	0.11	nd	nd	nd	nd
Furfural	0.12	0.06	2.62	0.54	1.65	0.41	0.53	0.21	0.08	0.01	0.86	0.65	nd	nd	nd	nd	0.01	0.00	0.06	0.02
2-Hexenal	0.17	0.14	nd	nd	1.08	0.38	0.75	0.24	0.14	0.03	3.10	4.02	1.16	0.24	0.22	0.10	0.13	0.06	0.24	0.10
2-Heptanone	0.52	0.25	3.81	0.66	4.83	3.02	1.15	0.40	2.10	0.17	8.70	5.93	1.61	0.33	1.10	0.25	0.63	0.25	1.00	0.06
Heptanal	0.83	0.47	2.69	0.39	5.59	1.55	0.79	0.21	1.68	0.19	6.87	4.51	2.09	0.51	1.78	0.48	1.43	0.23	1.41	0.17
2-Hexenal, 2 methyl	nd	nd	nd	nd	0.35	0.08	nd	nd	0.17	0.01	0.37	0.38	0.28	0.05	0.83	0.22	0.51	0.18	0.87	0.11
Pyrazine, 2,5 dimethyl	0.09	0.04	4.53	0.97	0.78	0.23	1.37	0.44	0.09	0.01	4.57	3.90	0.03	0.00	0.16	0.04	0.01	0.01	0.20	0.06
Pyrazine ethyl	nd	nd	1.23	0.84	0.72	0.19	0.15	0.12	0.04	0.03	0.80	0.34	nd	nd	nd	nd	0.02	0.01	0.04	0.01

Compound Name	Week 0				Week 6				Week 9				Week 13				Week 16			
	WSSB		DSSB		WSSB		DSSB		WSSB		DSSB		WSSB		DSSB		WSSB		DSSB	
	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S
2-Heptenal	0.48	0.33	0.87	0.17	1.82	0.49	0.42	0.12	0.68	0.06	6.99	5.42	8.64	1.27	0.82	0.30	0.60	0.23	0.45	0.08
Benzaldehyde	1.69	0.92	7.43	1.37	6.28	2.04	3.28	1.05	3.86	1.40	11.09	6.96	4.66	1.09	2.56	0.53	1.37	0.48	2.78	0.62
1-Octen-3-ol	0.95	0.52	1.83	0.41	7.27	2.30	0.61	0.56	3.02	0.05	11.72	8.48	10.24	2.23	nd	nd	nd	nd	nd	nd
5-Hepten-2-one,6 methyl	0.47	0.23	1.01	0.14	1.18	0.43	0.18	0.05	0.52	0.05	1.27	0.80	nd	nd	nd	nd	nd	nd	nd	nd
Furan, 2-pentyl-	0.56	0.31	1.54	0.18	3.23	0.76	1.22	0.42	3.38	0.25	11.04	9.03	14.95	2.90	5.16	0.89	5.56	2.59	3.32	0.33
1-Nonen-3-ol	nd	nd	nd	nd	0.47	0.02	nd	nd	0.20	0.16	0.62	0.48	nd	nd	nd	nd	nd	nd	nd	nd
Octanal	0.55	0.34	0.37	0.09	6.32	1.77	0.59	0.19	1.69	0.15	3.38	2.32	5.14	0.93	4.08	1.03	3.03	1.09	2.30	0.11
1,3 Dicholorben(IS)	5.46	4.40	8.00	0.00	8.00	0.00	7.99	0.00	8.00	0.00	8.00	0.00	8.00	0.02	8.00	0.00	8	0	8	0
1-Hexanol-2-ethyl	0.34	0.18	0.40	0.24	0.25	0.06	0.03	0.03	0.16	0.01	0.44	0.35	0.05	0.03	nd	nd	nd	nd	0.05	0.01
D-Limonene	1.06	1.00	1.14	0.57	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Eucalyptol	0.10	0.07	0.18	0.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dodecene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Benzeneacetaldehyde	0.46	0.28	3.47	0.60	1.49	0.47	0.47	0.19	0.37	0.06	1.51	1.02	1.46	0.17	0.08	0.05	0.46	0.15	0.10	0.03
2-Octenal	0.29	0.18	0.24	0.16	1.41	0.43	0.43	0.15	0.67	0.07	12.16	9.97	nd	nd	nd	nd	0.35	0.10	nd	nd
Pyrazine 2 ethyl 3,5 dimethyl	nd	nd	2.38	0.52	0.57	0.20	1.23	0.40	0.21	0.02	7.42	5.59	nd	nd	0.91	0.16	nd	nd	nd	nd
2-Nonanone	nd	nd	nd	nd	0.40	0.12	0.15	0.06	0.18	0.03	0.65	0.45	0.41	0.05	0.44	0.07	0.29	0.08	0.41	0.04
3,5 Octadien - 2-one	nd	nd	nd	nd	3.10	1.32	1.98	0.63	2.51	0.41	17.52	12.73	nd	nd	nd	ND	1.05	0.31	2.44	0.32
2-Nonen-1-ol	1.62	0.89	2.58	0.54	7.86	2.88	1.59	0.52	2.45	0.34	8.45	6.50	11.72	1.83	9.97	1.43	7.38	2.38	6.17	0.62
BHT	3.21	3.12	4.10	2.60	3.75	1.95	0.63	0.18	1.62	0.71	1.04	0.47	0.88	0.28	1.48	0.52	0.50	0.21	4.52	5.10
DEP	N/A	N/A	nd	nd	1.30	0.55	1.37	0.45	3.18	0.66	1.55	0.71	2.83	0.26	2.55	0.60	0.46	0.19	2.20	0.82

Compound Name	Week 0				Week 6				Week 9				Week 13				Week 16			
	WSSB (OIL)		DSSB (OIL)		WSSB (OIL)		DSSB (OIL)		WSSB (OIL)		DSSB (OIL)		WSSB (OIL)		DSSB (OIL)		WSSB (OIL)		DSSB (OIL)	
	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S
Butanoic Acid Methyl Ester	0.40	0.10	0.44	0.02	0.04	0.02	0.79	0.77	0.31	0.07	0.41	0.07	nd	nd	nd	nd	nd	nd	nd	nd
1H-Pyrrole, 1-methyl	nd	nd	nd	nd	nd	nd	nd	nd	0.53	0.11	0.64	0.13	nd	nd	nd	nd	nd	nd	nd	nd
2-Pentyn-1-ol	0.12	0.11	0.46	0.20	nd	nd	nd	nd	3.58	4.66	1.16	0.17	0.17	0.06	0.17	0.07	0.18	0.04	0.20	0.02
1-Pentanol	0.73	0.39	1.67	0.39	0.19	0.05	16.21	15.36	22.97	3.97	34.99	6.02	4.41	0.42	3.36	1.20	4.30	0.42	2.18	0.20
2-Hexanone	0.12	0.04	0.21	0.06	0.09	0.02	1.39	1.35	0.56	0.16	0.95	0.18	0.15	0.01	0.16	0.06	0.22	0.03	0.10	0.01
Hexanal	29.65	18.2	63.08	2.74	12.12	2.46	91.62	0.79	49.38	12.53	70.85	12.21	20.98	2.72	23.39	9.63	29.22	4.99	35.11	26.42
3-Pentenal, 4-methyl	nd	nd	nd	nd	nd	nd	nd	nd	0.70	0.18	1.04	0.20	0.14	0.01	0.16	0.06	0.18	0.03	0.11	0.01
Pyrazine, methyl-	nd	nd	0.29	0.15	0.07	0.03	1.77	1.65	1.41	0.40	1.57	0.35	nd	nd	0.21	0.08	0.37	0.05	0.09	0.01
Furfural	0.06	0.02	0.26	0.01	0.06	0.02	0.62	0.55	0.55	0.10	0.56	0.21	0.01	0.00	0.02	0.01	0.03	0.01	0.07	0.01
2-Hexenal	0.19	0.12	0.83	0.37	0.15	0.04	1.32	1.45	2.32	0.57	3.02	0.32	0.23	0.16	0.30	0.14	0.35	0.09	0.62	0.00
2-Heptanone	0.50	0.21	0.94	0.22	0.65	0.17	9.72	9.06	6.13	1.32	8.87	2.01	1.02	0.05	1.25	0.48	2.51	0.34	1.19	0.12
Heptanal	0.96	0.44	2.14	1.08	0.42	0.35	6.78	5.98	14.72	3.00	21.96	3.87	1.39	0.22	1.50	0.63	1.77	0.26	3.05	0.21
2-Hexenal, 2-methyl	nd	nd	nd	nd	0.03	0.01	0.68	0.66	0.25	0.04	0.83	0.19	0.74	0.08	2.10	2.00	0.58	0.08	0.08	0.01
Pyrazine, 2,5-dimethyl	0.06	0.02	0.28	0.02	0.06	0.03	1.41	1.23	0.03	0.00	0.25	0.12	0.01	0.00	0.09	0.04	0.02	0.00	0.10	0.00
Pyrazine ethyl	0.04	0.01	0.14	0.00	nd	nd	0.66	0.67	0.18	0.17	0.24	0.13	nd	nd	0.05	0.02	0.05	0.01	0.01	0.00

Compound Name	Week 0				Week 6				Week 9				Week 13				Week 16			
	WSSB (OIL)		DSSB (OIL)		WSSB (OIL)		DSSB (OIL)		WSSB (OIL)		DSSB (OIL)		WSSB (OIL)		DSSB (OIL)		WSSB (OIL)		DSSB (OIL)	
	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S	A	S
2-Heptenal	0.48	0.33	2.18	1.78	0.58	0.15	2.78	2.22	16.22	4.07	14.91	3.50	1.83	0.61	1.47	0.75	2.36	0.57	3.89	0.32
Benzaldehyde	1.68	0.72	2.64	0.46	2.50	0.71	9.71	11.81	3.45	2.85	7.99	1.49	2.35	0.36	4.83	1.83	2.74	0.39	2.39	0.09
1-Octen-3-ol	1.11	0.53	2.19	0.72	0.66	0.15	9.84	9.14	26.99	5.61	28.25	5.95	1.66	0.55	2.64	1.05	3.69	0.42	5.46	0.43
5-Hepten-2-one,6 methyl	0.60	0.25	0.46	0.04	0.19	0.06	2.05	1.86	1.31	0.30	1.49	0.34	nd	nd	nd	nd	0.29	0.03	0.15	0.02
Furan, 2-pentyl-	0.34	0.14	0.62	0.18	0.97	0.28	7.92	6.84	27.14	12.49	35.52	12.12	7.61	0.33	5.71	1.25	7.11	1.74	5.76	0.68
1-Nonen-3-ol	0.05	0.02	0.16	0.08	0.08	0.03	0.59	0.50	2.72	0.66	2.09	0.44	nd	nd	nd	nd	0.20	0.04	0.23	0.03
Octanal	1.21	0.67	1.83	0.86	0.34	0.14	9.27	8.49	20.08	4.87	27.31	6.03	2.85	0.45	2.33	0.88	3.73	0.69	4.69	0.50
1,3 Dichlorobenzene (IS)	8.00	0.00	8.00	0.00	8.00	0.01	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00
1-Hexanol-2-ethyl	0.44	0.16	1.94	0.31	0.10	0.03	1.21	1.22	0.16	0.06	0.21	0.04	nd	nd	0.05	0.02	0.06	0.01	0.08	0.02
D-Limonene	0.40	0.17	0.23	0.11	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Eucalyptol	0.05	0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dodecene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Benzeneacetaldehyde	0.32	0.17	0.58	0.10	0.14	0.05	1.46	1.29	0.62	0.11	0.74	0.07	0.14	0.02	0.28	0.10	0.32	0.05	0.15	0.01
2-Octenal	0.26	0.15	1.11	0.73	1.76	2.54	2.09	1.81	40.44	10.71	37.32	10.27	nd	nd	0.94	0.43	nd	nd	nd	nd
Pyrazine 2 ethyl 3,5 dimethyl	nd	nd	0.19	0.03	0.05	0.02	0.83	0.02	0.73	0.24	1.45	0.25	0.18	0.02	0.30	0.11	nd	nd	nd	nd
2-Nonanone	nd	nd	nd	nd	0.06	0.02	0.54	0.47	0.59	0.13	0.92	0.34	0.24	0.01	0.22	0.07	0.34	0.05	2.00	0.29
3,5 Octadien - 2-one	nd	nd	nd	nd	1.55	0.53	7.52	6.67	25.58	6.21	31.08	6.16	2.30	0.31	2.04	0.80	2.97	0.48	11.12	1.42
2-Nonen-1-ol	1.21	1.30	1.59	1.35	1.04	0.33	9.61	8.80	18.04	4.25	22.84	5.82	5.45	0.61	3.64	1.19	6.29	1.05	8.17	1.58
Butylated Hydroxytoluene	5.29	4.25	22.73	36.88	2.35	1.17	18.74	19.17	3.32	0.89	4.53	2.91	2.24	1.14	2.70	1.93	2.29	0.74	0.49	0.33
Diethyl Phthalate	N/A	N/A	nd	nd	0.65	0.26	1.38	0.93	1.66	0.41	3.82	1.08	1.87	0.14	1.38	0.48	2.10	0.48	0.83	0.25

nd.-not detected; N/A-not available; A-average; S-standard deviation.

## Appendix C - References used in Sensory Analysis:

*Clean out: Cracker and carrots*

### Aroma

Grain Overall: A general term used to describe the aromatic which includes musty, dusty, slightly brown, slightly sweet and is associated with harvested grains and dry grain stems.

Reference: Cereal Mix(dry) = 7.5(a)

Preparation: Serve 2 Teaspoons in a 12 oz brandy snifter covered with a watch glass.

Musty Overall: A combination of one or more aromatic impressions characterized to some degree as being somewhat dry, dusty, damp, earthy, stale, sour, or moldy. If identifiable, attribute will be listed.

Reference: 1,2,4Trimethoxybenzene 50,000 ppm = 4.0(a)

Preparation: Dip an Orlandi Perfumer Strip #27995 2.2cm (second marking line) into solution and place dipped end up in a Fisherbrand Disposable Borosilicate Glass Tubes with Threaded End (15x150mm), cap.

Cardboard: The aromatics associated with cardboard or paper packaging.

Reference: -Cardboard soaked in water, covered with watch glass= 7.5 (a)

Preparation: -Place 2" square piece of cardboard in a medium snifter. Cover with ½ cup of water. Cover with a watch glass.

Toasted: A moderately browned/baked impression

Reference: General Mills Cheerios crushed = 7.0 (a)

Preparation: ¼ cup crushed Cheerios in a medium snifter

Brown: A rich full round aromatic impression always characterized as some degree of darkness generally associated with attributes such as toasted nutty, roasted, sweet

Reference: Bush Pinto Beans (canned) = 6.0 (a)

Preparation: Drain beans and rinse with de-ionized water. Place one table spoon in a medium snifter at room temperature.

Rancid: A somewhat heavy aromatic characteristic of old, oxidized, decomposing fat and oil. The aromatics may include painty, varnish, or fishy.

Reference: Microwaved Wesson vegetable oil (3 min at high) = 6.0(a)  
Microwaved Wesson vegetable oil (5 min at high) = 8.0(a)

Preparation: -Microwave ½ cup oil on high power for 3minutes.  
Let cool andServe ¼ cup in a 12 oz brandy snifter covered with a watch glass.

-Microwave ½ cup oil on high power for 5minutes.

Let cool andServe ¼ cup in a 12 oz brandy snifter covered with a watch glass.

Painty: The aromatic associated with rancid oil and fat, typically in the late stages of rancidity.

Reference: Microwaved Wesson vegetable oil (3 min at high) = 3.5 (a)  
Microwaved Wesson vegetable oil (5 min at high) = 4.5(a)

Preparation: -Microwave ½ cup oil on high power for 3minutes.  
Let cool andpour into 1 oz cups. Serve covered.-Microwave ½ cup oil on high power for 5 minutes. Let cool and Pour into 1 oz cups. Serve covered.

## **Flavor**

OverallGrain: A general term used to describe the light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice and oats.

Reference: Cereal Mix (dry) = 8.0 (f)

Preparation: Mix ½ cup of each General Mills Rice Chex, Wheaties and Quaker Quick Oats. Put in a blender and “pulse” blend into small particles. Serve in 1 oz cup.

Sorghum: A slightly sweet, musty/dusty aromatic with characteristics of chalkiness, starch and astringency. May also include slightly green and bitter

- Reference: Bob's Red Mill sweet white sorghum flour = 5.0(f)
- Preparation: Serve sorghum flour in 1 oz cup
- Soy: Flavor associated with soybeans or soy products.
- Reference: Soy nuts (Hy-Vee Bulk) = 4.5(f)
- Preparation: Serve Soy nut in 1 oz cup
- Starch: The dry aromatic associated with starch and starch based grain product such as wheat, rice, oat and other grains
- Reference: Cereal Mix (dry) = 7.0 (f)  
Cooked American Beauty elbow macaroni = 9.0 (f)
- Preparation: -Mix ½ cup of each General Mills Rice Chex, Wheaties and Quaker Quick Oats. Put in a blender and “pulse” blend into small particles and serve in 1 oz cups.  
-American Beauty macaroni: Bring 3 cups water to a rapid boil. Add 1 cup pasta & stir, returning to a rapid boil. Cook 6 minutes, stirring occasionally. Drain and place into 3.25 oz cups.
- Toasted: A moderately browned/ baked impression.
- Reference: Post Shredded Wheat (Spoon size) = 3.5 (f)  
General Mills Cheerios = 7.0 (f)
- Preparation: Serve Shredded Wheat in 3.25oz cup  
Serve Cheerios in 3.25oz cup
- Brown: A rich full round aromatic impression always characterized as some degree of darkness generally associated with attributes such as toasted nutty, roasted, sweet
- Reference: Bush Pinto Beans (canned) = 3.0 (f)
- Preparation: Drain beans and rinse with de-ionized water. Serve in 3.25 oz cups.
- Cardboard: packaging
- The flat aromatics that may be associated with cardboard or paper
- Reference: Mama Mary's Pizza Crust = 3.0 (f)
- Preparation: Cut pizza crust into 2” square piece and place in 3.25 oz cups
- Musty: Aromatics associated with wet grain and damp earth.
- Reference: Cooked American Beauty elbow macaroni = 5.0

Preparation: American Beauty macaroni: Bring 3 cups water to a rapid boil. Add 1 cup pasta & stir, returning to a rapid boil. Cook 6 minutes, stirring occasionally. Drain and place into 3.25oz cups

Rancid: A somewhat heavy aromatic characteristic of old, oxidized, decomposing fat and oil. The aromatics may include painty, varnish, or fishy.

Reference: Microwaved Wesson vegetable oil (3 min at high) = 7.0 (f)  
Microwaved Wesson vegetable oil (5 min at high) = 9.0(f)

Preparation: -Microwave ½ cup oil on high power for 3minutes.  
Let cool andpour into 1 oz cups. Serve covered.-Microwave ½ cup oil on high power for 5 minutes. Let cool and Pour into 1 oz cups. Serve covered.

Painty: The aromatic associated with rancid oil and fat, typically in the late stages of rancidity.

Reference: Microwaved Wesson vegetable oil (3 min at high) = 2.5 (f)  
Microwaved Wesson vegetable oil (5 min at high) = 5.0(f)

Preparation: -Microwave ½ cup oil on high power for 3minutes.  
Let cool andpour into 1 oz cups. Serve covered.-Microwave ½ cup oil on high power for 5 minutes. Let cool and Pour into 1 oz cups. Serve covered.

Astringent: The drying, puckering sensation on the tongue and other mouth surfaces.

Reference: 0.050% alum solution = 2.5  
0.100% alum solution = 5.0

## Chapter 5 - Conclusions and Future Work

- 1) One of the most critical aspects when it comes to an intermediate or final product assessment is the detailed information about raw materials used in the project. It is very important to document proximate values from ingredient companies along with details on grain type (hard or soft endosperm), harvest time, milling methods used and the time between milling and shipping before being processed for research purposes. If whole grains are used in the project, it becomes all the more critical to record total dietary fiber (TDF) values and compare them with decorticated sorghum as they directly relate to product expansion, color and texture of extrudates. Verification of TDF and crude fiber with literature values would be informative to discuss results.
- 2) Extrusion hardware- When it comes to flour composites such as cereal-legume mixtures, single screw extruders have issues with mixing due to lower process moisture used during extrusion which could pose challenges with SME fluctuations and data inconsistency. Additionally, starch and protein interactions compete with available moisture and there could be possible ingredient agglomeration which may cause inconsistent data with intermediate product. Hence, assessment of ideal screw profile with forward and reverse kneading block combinations could be an option. In this current project, only small, medium and large steam locks were used during extrusion.
- 3) Panel Board, No load Torque and DAQ – There has always been issues when it comes to recording the right reading when product is stabilized in the panel board. It is advisable to check the functioning of panel board to ensure steady reading. No load torque with actual screw profile is always overlooked before extrusion. It will be a good practice to match

DAQ data and panel board data and finally the manual entry from run sheets before the actual run to ensure data accuracy.

- 4) Extrusion day – One of the most essential planning activity on extrusion day is sample and data collection as follows:-
- An eye for details is required to legibly label raw samples and have enough inventories for future tests/analysis is pivotal for any project.
  - This is another opportunity to make sure that supplier has provided detailed information about the dry recipe for future analysis or discussion
  - Take pictures of entire work flow process including ingredient supply bags, screw profile picture, die, knives, dryer conditions, panel board, NLT and raw samples collected
  - During extrusion, it is a good practice to obtain mass flow rates at 3 or 4 different occasion (in triplicates for one minute) for a single treatment to ensure even and steady flows which would relate accuracy with SME calculation. The same practice to be adopted for product bulk densities.
  - Assessing moisture content of extrudates out of extruder and comparing them out of dryer would give a good understanding on moisture losses and end product moisture.
- 5) Extrudate milling in a conventional method such as wheat milling or pin milling poses challenges in getting to the right particle size specifications. Expanded extrudates have difficulties to enter between smaller roll clearances and show inconsistent milling patterns. Choice of the mill is very crucial when it comes to scale-up projects and to meet standard guidelines. Hammer mill as an option has been tried out in the Hall Ross mill

and initial trials indicate particle size meet the required specifications of 95% below 600 microns. However fine tuning on the screen size and mesh number could come closer in meeting the specifications.

- 6) Proximate analyses data can be verified with supplier data in ensure accuracy with numbers and in discussing results.
- 7) In chapters 3 and 4, the relationships discussed with regression lines are pertaining to actual experimental design conditions and hence the number data points are fewer. Further studies could possibly include a wider range of extrusion conditions which would enable more data points and eliminate the occurrence of an outlier or discuss outliers based on actual process conditions.
- 8) When it comes to adding oil in the formulation, it is recommended that oil is pumped into the extruder barrel at the end of the run to assess oil effects in the extruder. Premixing oil prior to extrusion has not created a significant impact with melt viscosity or SME values in anticipation of lubrication effects. Assessment of homogeneity of mixing (distributive vs dispersive) is vital to study extrudate properties, end product flow rate and oxidative rancidity.
- 9) From a shelf-life stand point, it is important to note that no anti-oxidants were used in the formulation. Though very small amounts were present ( 5ppm) by the addition of vegetable oil, suggested amounts of upto 5000ppm could enhance shelf-stability and also prevent rancidity during extrusion. Since the FAQR does not specify the use of approved anti-oxidants, future work can consider the use of different natural anti-oxidants to improve shelf-life periods and also suggest ideal proportions to be used in the formulation pre/post-extrusion.