

Nutritional and functional properties of porridges from extrusion cooked  
cassava-soy composite with wheat bran or grape pomace

By

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## **DECLARATION**

I declare that the thesis, which I hereby submit for the degree PhD (Food Science) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution

**Dolapo Abimbola Oladiran**

**Date: .....**

## **DEDICATION**

**To my late supervisor, Prof. Amanda Minnaar;**

*For her patience and encouragement. I am grateful to have worked with her, albeit for a short time.*

**To my sustainer and strength;**

*For from Him, and through Him, and to Him are all things: to whom be the glory forever. Amen*

*- (Romans 11:36)*

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My sincere thanks to my co-supervisor, Prof. H.L. de Kock, for her valuable guidance, criticisms and insights.

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## ABSTRACT

Nutritional and functional properties of porridges from extrusion cooked cassava-soy composite with wheat bran or grape pomace

By

**Dolapo Abimbola Oladiran**

**Supervisor:** Prof. M.N. Emmambux

**Co-Supervisor:** Prof. H. L. de Kock

Diet related non-communicable diseases such as obesity and type-2 diabetes are on the rise in sub-Saharan Africa. The rise is related to an increasing demand for highly refined and convenience-type foods with a decline in consumption of whole grains and high fibre foods. Dietary fibre can be incorporated into locally available food crops using extrusion cooking to produce instant food products with improved nutritional and functional properties. This study therefore investigated the effect of extrusion cooking and wheat bran or grape pomace addition on the nutritional, functional and sensory properties of a cassava- soy composite. Grape pomace or wheat bran was added at 0, 10 and 20% levels to a cassava- soy composite and extrusion cooked. The composites with and without grape pomace or wheat bran were analysed for proximate composition, water absorption and solubility index, *in-vitro* starch digestibility (IVSD), soluble and insoluble dietary fibre content, *in-vitro* protein digestibility (IVPD), nitrogen solubility index, flow properties, total phenolic content and, anti-oxidant property. Descriptive sensory properties, oral processing characteristics and satiety of porridges with and without wheat bran were determined.

Extrusion cooking led to a decrease in IVPD and nitrogen solubility index of all composites. The high temperature in the extruder may have facilitated formation of covalent and non-covalent interactions between soy proteins and other food components which led to reduction in nitrogen solubility index and IVPD. Extrusion cooking led to an increase in starch digestibility of the composites compared to unextruded samples. The addition of either grape pomace or wheat bran lowered starch digestibility and estimated glycaemic index of composites. The high viscosity of composites with wheat bran may be responsible for the decrease in starch digestibility observed. High viscosity may retard enzyme diffusion to substrate for digestion and slow down the release and transit of hydrolysis products towards the absorptive surface of the mucosa. The phenolics present in grape pomace may have prevented the formation of an enzyme-substrate complex thus,

lowering starch digestibility of composites which contained grape pomace. There was an increase in soluble dietary fibre content and a decrease in insoluble dietary fibre content of all composites after extrusion cooking. High shear in the extruder may have facilitated fragmentation and depolymerization of insoluble dietary fibre.

All porridges showed shear thinning behaviour. Composites with 20% wheat bran had a higher apparent viscosity compared to the other composites. This may be due to solubilization of insoluble dietary fibre during extrusion cooking and this could be related to the decrease in IVSD and estimated glycaemic index observed in this composite. In contrast, lower apparent viscosity was observed in the composites with grape pomace upon extrusion cooking. It is possible that despite the increased solubilization of insoluble dietary fibre after extrusion cooking, the molecular weight of the soluble dietary fibre portion was low and could not promote viscosity. Although, grape pomace addition to cassava-soy composite increased the total phenolic content and anti-oxidant activity of composites, extrusion cooking decreased the total phenolic content and anti-oxidant activity of composites.

Sensory attributes such as visually perceived viscosity, presence of particles, coarseness and thickness were strongly perceived in the composite with 20% wheat bran. The addition of wheat bran reduced the cassava aroma and glossiness of composite porridges. The composite porridge with 20% wheat bran was eaten with a higher number of bites, required a longer oral processing time and total meal duration compared with porridge without wheat bran. The subjective satiety responses post ingestion of the porridges also showed the composite porridge with 20% wheat bran led to greater reduction in hunger, an increase in fullness, a decreasing desire to eat and lower prospect to consume another meal compared to the other porridges.

In conclusion, the results from this research demonstrate that extruded instant composites of cassava-soy flour with grape pomace or wheat bran have lower starch digestibility and estimated glycaemic index compared to composites without wheat bran or grape pomace. Changes in the functional properties of dietary fibre during extrusion cooking is largely responsible for the improved nutritional and sensory properties of extrudates. Thus, grape pomace or wheat bran have great potential to be incorporated as dietary fibre sources in starch-rich foods with the use of extrusion cooking to produce instant food products suitable for the management of diet-related diseases such as type-2 diabetes.

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# 1. INTRODUCTION

## 1.1 Problem Statement

There is a growing burden of diet-related non-communicable diseases in sub-Saharan Africa (SSA) that include cardiovascular diseases, cancer, diabetes and obesity (Dalal *et al.*, 2011). The prevalence of type-2 diabetes is rapidly increasing in SSA and the drivers of this epidemic are urbanization and changes in lifestyle associated with economic development (Holmes *et al.*, 2010). The changes in lifestyle include diet, physical activity, smoking and adiposity (Mbanya *et al.*, 2010).

The surge in urbanization is accompanied by nutrition transition (Popkin and Du, 2003) in SSA. Nutrition transition is referred to as change in dietary patterns (Steyn and Mschiza, 2014). Diet composition of people in developing countries is shifting rapidly towards a more energy-dense refined starch-based product, rich in total and saturated fat primarily from meat and milk consumption (Kearney, 2010), and caloric sweeteners (Lee *et al.*, 2004). There is also a decline in consumption of traditional diets high in whole grain cereal and dietary fibre (Cordain *et al.*, 2005).

Dietary management has been identified as one of the means of combatting diet related non-communicable diseases (WHO, 2003). In addressing the growing burden of type-2 diabetes in SSA, production of ready-to-eat, convenient and inexpensive foods from locally grown food crops using suitable small-to-medium scale production technologies is a viable and suitable approach (Onofiok and Nnanyelugo, 1998). Muoki *et al.*, (2012) composited high quality cassava flour with soy flour using extrusion cooking to produce an instant porridge for infants. Although the complementary porridge is able to address protein energy malnutrition amongst children, it is unsuitable for managing diet related non-communicable diseases (NCDs) such as type-2 diabetes and obesity due to its rapid starch digestion rate and high estimated glycaemic index value.

Several studies have indicated a strong inverse relationship between consumption of high dietary fibre diets with low glycaemic index and post prandial blood glucose response (Jenkins *et al.*, 2008; Cassiraghi *et al.*, 2006; Kendall, Esfahani and Jenkins, 2012). Dietary fibre is classified into insoluble and soluble for functional and technological purposes (Han *et al.*, 2017). The two dietary fibre types exert different physiological effects in the human digestive tract and in human health.

Insoluble dietary fibre is important for proper bowel function and may reduce symptoms of chronic constipation (Yamaoka *et al.*, 2014; Eswaran, Muir and Chey 2013), diverticular disease (Ünlü *et al.*, 2012), and hemorrhoids (Coffin and Shaffer, 2006). Soluble dietary fibre on the other hand is associated with reduction in cholesterol levels (Wood, 2007), ability to retard gastric emptying by increasing viscosity of stomach contents which in turn reduces the rate of digestion and nutrients uptake thus, promoting feelings of satiety (Lattimer and Haub, 2010). The increase in feeling of satiety may result from prolonged oral exposure (Fizman and Varela, 2013), increased gastric distension and longer gastric emptying time, slower nutrient absorption in the small intestine (Jansen *et al.*, 2011). The physiological functions of dietary fibres are related to their physicochemical properties such as water holding capacity, viscosity and susceptibility to bacterial degradation in the colon (Dikeman and Fahey, 2006; Kristensen and Jensen, 2011).

The health benefits associated with dietary fibre has increased the demand for fibre enriched food products (Han *et al.*, 2017). Cereals, fruits and vegetables are wholesome sources of dietary fibre. Wheat bran, a by-product of wheat milling contains about 47% dietary fibre and a larger percentage of this is insoluble with less than 3% soluble portion (Kamal-Eldin, 2009). Grape pomace is also a dense source of dietary fibre and it contains about 10 to 20% of soluble dietary fibre (Sousa *et al.*, 2014). It is also a good source of phenolic compounds (Makris *et al.*, 2007). These phenolic compounds are also of immense health benefit due to their anti-oxidant activity in scavenging free radicals in the body (Oboh and Ademosun, 2012). Phenolic compounds may also aid in lowering starch digestibility by binding to starch granule surface thus preventing enzyme-substrate complex formation (Mariano da Silva *et al.*, 2014). Phenolic compounds may also lower starch digestibility by binding to the active sites of  $\alpha$ -amylase thus, inhibiting activities of this enzyme (Forester *et al.*, 2012). Wheat bran and grape pomace are affordable and readily available and could be incorporated into cassava- soy composite to reduce the rate and extent of starch digestibility.

Recent researches on extrusion cooking of dietary fibre have shown that extrusion cooking increases solubility of dietary fibre thus improving its nutritional and functional properties (Rashid *et al.*, 2015; Andersson *et al.*, 2017). This makes extrusion cooking the processing technique of choice in producing fibre-enriched food products. The combination of thermal and mechanical energy during extrusion cooking can change the structure of dietary fibre leading to new functional properties (Dhingra *et al.*, 2012). Extrusion cooking induces changes in fibre solubility and facilitates the opening of fibre structure thus making free hydroxyl groups available to bind with

water (Sangnark and Noomhorm, 2004). There have been numerous studies on the extrusion cooking of cassava and legume food sources (Abioye *et al.*, 2016; Kareem *et al.*, 2015; Muoki *et al.*, 2012) but there are limited studies in literature on the addition of dietary fibre source to cassava and legume with extrusion cooking to produce low glycaemic index foods suitable for managing diet-related NCDs. Considering that extrusion cooking of mixed food ingredients may affect the nutritional and functional properties of the food composition consequently leading to changes in the sensory properties of extrudates. The aim of this study was thus to determine the effect of extrusion cooking and addition of wheat bran or grape pomace to cassava- soy composite on the nutritional, functional and sensory properties of extrudates.



## **2.0 LITERATURE REVIEW**

This chapter reviews the use of dietary fibre to reduce the glycaemic index of extruded starch and legume-based composites. Extruded foods are generally characterized as having rapid starch digestibility. The effect of dietary fibre addition to starch-protein based composite on the nutritional, functional, and sensory properties of extrudates will be discussed. The relative effect of extrusion cooking on the afore-mentioned properties of the extrudates is also reviewed.

### **2.1 Extrusion cooking of starch-protein based composites**

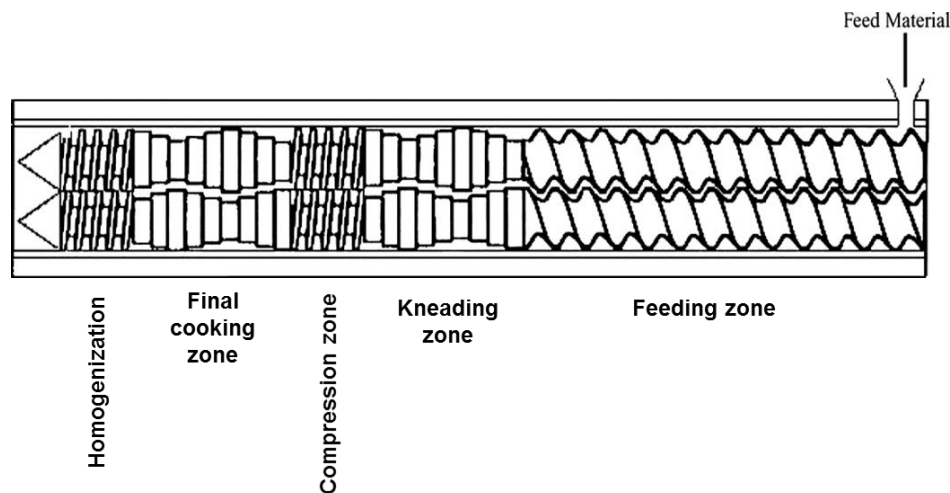
Extruded products are mostly made from starch and high starch containing food materials for example ready-to-eat snacks and porridge. However, due to increasing health concerns, there is considerable effort to fortify starchy food materials with high protein food source to increase the protein content and improve the essential amino acid balance of extruded products (Allen *et al.*, 2007; Yu *et al.*, 2012). Although extrudates from blends of starch and legumes are of immense nutritional benefit because of their high protein content, the rapid starch digestibility of some of the blends makes them a source of health concern because they induce a high rise in blood glucose post consumption (Méance, Achour and Briend, 1999).

#### **2.1.1 Extrusion process**

Extrusion is a processing technique that involves simultaneous thermal processing under pressure along with mechanical shearing to transform food raw materials. The thermal energy generated during extrusion combined with shearing effect cooks the food and the properties of the various food biopolymers are modified by changes in their physicochemical properties (Navale *et al.*, 2015).

During extrusion cooking, food materials are fed into the barrel through a hopper. The barrel is a heated hollow steel within which screws rotate. The materials fed in are propelled forward by the rotation of the screw and starch is melted due to combined effect of conducted and dissipated energy at limited moisture. The starch melt is subsequently homogenized and conveyed under high pressure through a die and the product expands to its final shape (Covas and Gaspar-Cunha, 2011). The most commonly used food extruders are single and twin-screw. The twin-screw extruders are classified into co-rotating or counter rotating based on the direction of screw rotation and the degree to which the screws intermesh.

The extruder generally has three processing zones (Figure 2.1): The feeding, kneading and final cooking zones (Harper, 1981). The feeding zone collects and pushes incoming raw materials up the screw channel to the melting zone. Screw feeding zone have relatively deep flights, large pitches and large flight angles to enhance conveying capacity. In the kneading zone, starchy food materials are compressed and melted into a continuous viscous fluid melt due to thermal energy input from the barrel wall and dissipation of mechanical energy (Fang, 2010).



**Figure 2.1.** Schematic representation of screw configuration in a co-rotating twin screw extruder (Altan *et al.*, 2009)

The viscous fluid melt is further heated in the final cooking zone and sheared by shallow flights and short pitches of screws giving rise to an elastic and amorphous slurry with steam trapped in-between at the die exit (Hauck and Huber, 1989). The die functions as a restrictive and forming device fitted at the end of the barrel. Rotating knives(s) known as cutter are mounted in front of the die plate to cut exiting extrudate into finite lengths (Fang, 2010).

Multiple unit operations (mixing, melting, degassing, cooking, and forming) are combined in an extruder into a single processing step and this potentially provides huge benefits in terms of overall equipment cost savings. The physico-chemical changes in the extrudates are mainly dependent on the parameters or processing conditions of the extrusion process. Therefore, the extruder parameters and ingredient formulation can be controlled to change characteristics of molten mass in the extruder thus changing the product properties.

## **2.1.2 Effect of extrusion cooking on nutritional and functional properties of food.**

Extrusion cooking causes chemical and structural changes in food raw materials. These chemical changes affect the functional properties of these food materials and this contributes to the nutritional properties of the extrudates. Some of these changes to nutritional (starch digestibility, protein digestibility, dietary fibre and phenolic compounds) and functional properties are discussed below.

### **2.1.2.1 Starch digestibility**

Extrusion cooking is reported to remarkably increase the digestibility of starches (Singh *et al.*, 2010). Starch undergoes several significant structural changes, which includes melting/gelatinization, dextrinization, and depolymerization during extrusion cooking (Lai and Kokini, 1991). During heating, the less ordered amorphous structure is first disrupted through its absorption of water allowing water to associate freely with the free hydroxyl group and this results in swelling and further opening of granule structure (Navale *et al.*, 2015). The combination of heating and swelling leads to destabilization of crystalline regions and this is followed by loss of starch birefringence (Jenkins and Donald, 1998).

Factors which influence the rate of starch digestion include ratio of amylose to amylopectin (Asp, 1996), degree of gelatinization (Chung *et al.*, 2006), amount of retrograded starch (Chung *et al.*, 2006), food processing conditions (Singh *et al.*, 2010) and presence of other food components (Englyst *et al.*, 1996). Table 2.1 depicts the digestibility of some extruded starch and starchy food products.

The trend in comparative starch digestibility of raw and extruded food materials in Table 2.1 shows that starch digestibility generally increased after extrusion cooking. There was an increase in RDS with a consequent decrease in SDS of normal maize starch, waxy starch, faba bean, amaranth seed and barley flour after extrusion cooking. Extrusion cooking increases availability of starch for enzymatic hydrolysis by gelatinization, inactivation of endogenous  $\alpha$ -amylase inhibitor, disruption of starch granular order and cellular structure, size reduction and increased starch surface area (Cheftel, 1985).

**Table 2.1.** Starch digestibility of different extruded starches and starch-based foods.

Sources	Processing condition	Starch digestibility		Estimated glycaemic index	References
		RDS	SDS		
Normal maize starch	Raw	24.1 <sup>a</sup>	27.8 <sup>a</sup>	ND	Robin <i>et al.</i> , 2016
	Extrusion cooking (T <sub>m</sub> = 80 - 120 °C, Feed rate = 10 kg/h, F <sub>m</sub> = 17%, Screw speed = 300 rpm)	95 <sup>a</sup>	2 <sup>a</sup>	ND	
Waxy maize starch	Raw	42.7 <sup>a</sup>	38.9 <sup>a</sup>	ND	Robin <i>et al.</i> , 2016
	Extrusion cooking (T <sub>m</sub> = 40 - 100 °C, Feed rate = 10 kg/h, F <sub>m</sub> = 18.7%, Screw speed = 250 rpm)	97.8 <sup>a</sup>	1.4 <sup>a</sup>	ND	
Barley flour	Raw	98 <sup>b</sup>	456 <sup>b</sup>	ND	Sun <i>et al.</i> , 2006
	Extrusion cooking (T <sub>m</sub> = 145 °C, Feed rate = 20 kg/h, F <sub>m</sub> = 15%, Screw speed = 200 rpm)	608 <sup>b</sup>	7 <sup>b</sup>	ND	
Faba bean	Raw	159 <sup>c</sup>	ND	ND	Alonso <i>et al.</i> , 2000
	Extrusion cooking (T <sub>m</sub> = 156 °C, Feed rate = 385 g/min, F <sub>m</sub> = 15%, Screw speed = 100 rpm)	290 <sup>c</sup>	ND	ND	
Amaranth seed	Raw	57.50 <sup>d</sup>	ND	87.19	Capriles <i>et al.</i> , 2008
	Extrusion cooking (T <sub>m</sub> = 135 °C, Feed rate = 4.4 kg/h, F <sub>m</sub> = 15%, Screw speed = 404 rpm)	61.06	ND	91.19	

T<sub>m</sub> = Temperature, F<sub>m</sub> = Feed moisture, RDS = rapidly digestible starch, SDS = slowly digestible starch, ND = not determined

<sup>a</sup> is expressed in %

<sup>b</sup> is expressed in g/kg

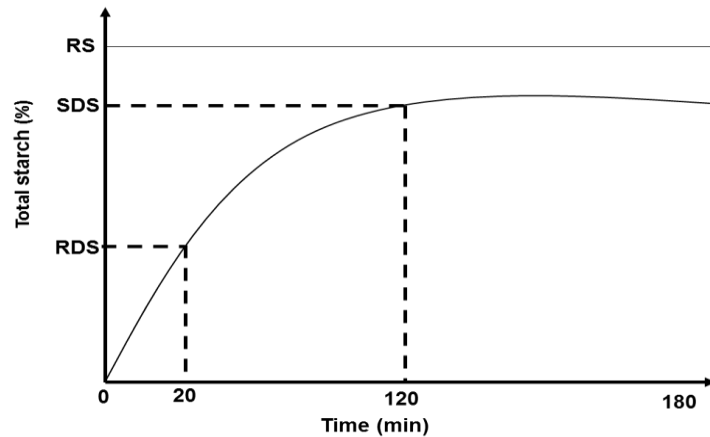
<sup>c</sup> is expressed as starch digested (mg maltose/g)

<sup>d</sup> is expressed as starch digested (g/100g)

Starch granules melt inside the extruder at relatively high temperatures combined with the presence of water, shearing effect and kneading, and these process enables starch to lose their organized molecular structure to result in increased starch susceptibility to enzymatic action (Mercier *et al.*, 1998). The increase in starch digestibility of amaranth seed after extrusion cooking also resulted in increase in estimated glycaemic index.

The trend in comparative starch digestibility of raw and extruded food materials in Table 2.1 shows that starch digestibility generally increased after extrusion cooking. There was an increase in RDS with a consequent decrease in SDS of normal maize starch, waxy starch, faba bean, amaranth seed and barley flour after extrusion cooking. Extrusion cooking increases availability of starch for enzymatic hydrolysis by gelatinization, inactivation of endogenous  $\alpha$ -amylase inhibitor, disruption of starch granular order and cellular structure, size reduction and increased starch surface area (Cheftel, 1985). Starch granules melt inside the extruder at relatively high temperatures combined with the presence of water, shearing effect and kneading, and these process enables starch to lose their organized molecular structure to result in increased starch susceptibility to enzymatic action (Mercier *et al.*, 1998). The increase in starch digestibility of amaranth seed after extrusion cooking also resulted in increase in estimated glycaemic index.

For nutritional purpose, starch is classified according to their digestibility; rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) (Englyst *et al.*, 1992). Starch digestibility and absorption is an important determinant of metabolic response following the consumption of a meal (Goni *et al.*, 1997). Figure 2.2 shows an illustration of digestion of the different starch fractions. An *in vitro* method, known as the Englyst Method (Englyst *et al.*, 1992), can be used to quantitatively measure the glucose released from a test food after digestion mimicking *in vivo* digestion (Englyst *et al.*, 1992).



RDS = rapidly digestible starch, SDS= slowly digestible starch, RS = resistant starch

**Figure 2.2.** Rate of starch digestion as a function of time (Englyst *et al.*, 1992).

As illustrated in Figure 2.2, rapidly digestible starch (RDS) is the glucose released after 20 min of enzymatic digestion; slowly digestible starch (SDS) is the glucose released between 20 and 120 min of enzymatic digestion; and resistant starch (RS) is that portion of starch which remains after the total 120 min digestion (Englyst *et al.*, 1992).

Glycaemic index (GI) is used to classify foods based on their post-prandial glucose response. Glycaemic index is the indexing of a fixed amount of available carbohydrate from a test food to the same amount of available carbohydrate from a standard food consumed by the same subject (Jenkins *et al.*, 2002). GI is classified into high (>70), medium (<70 but >55) or low (<55) (Zhang and Hamaker, 2009). The standard or reference food usually is white bread or glucose (Englyst *et al.*, 2003).

Gelatinized starch, high GI foods and RDS are rapidly digested and absorbed in the stomach and small intestine. This is subsequently followed by elevation of blood glucose level (Wong and Jenkins, 2007), whereas slowly digestible starch (SDS) such as raw starch is slowly digested into glucose. The slow rate of digestion leads to slow and prolonged release of glucose. Resistant starch (RS) on the other hand escapes digestion in the small intestine (Zhang and Hamaker, 2009).

Increasing consumption of foods with rapid starch digestibility has been identified as one of the drivers of the obesity pandemic being experienced globally (Popkin *et al.*, 2012). Obesity is identified as a risk factor for type-2 diabetes and cardiovascular diseases (Abbasi *et al.*, 2002). RDS is associated with postprandial hyperglycaemia which has been implicated in development

of these afore-mentioned non-communicable diseases (Aller *et al.*, 2011; Boers *et al.*, 2015). Slowly digested and absorbed carbohydrates are favourable for dietary management of metabolic disorders such as type-2 diabetes (Björck and Elmståhl, 2003) due to the slow response they elicit in increasing blood glucose post meal.

### 2.1.2.2 Protein digestibility

The nutritional value of protein is dependent on the quantity, digestibility and availability of essential amino acids (Singh *et al.*, 2007). Extrusion cooking variables greatly influence protein digestibility. The presence of protease inhibitors and dietary fibre can also reduce the amount of protein available for intestinal absorption (Camire, 1998). Table 2.2 shows some of the effects of extrusion processing variables on protein digestibility.

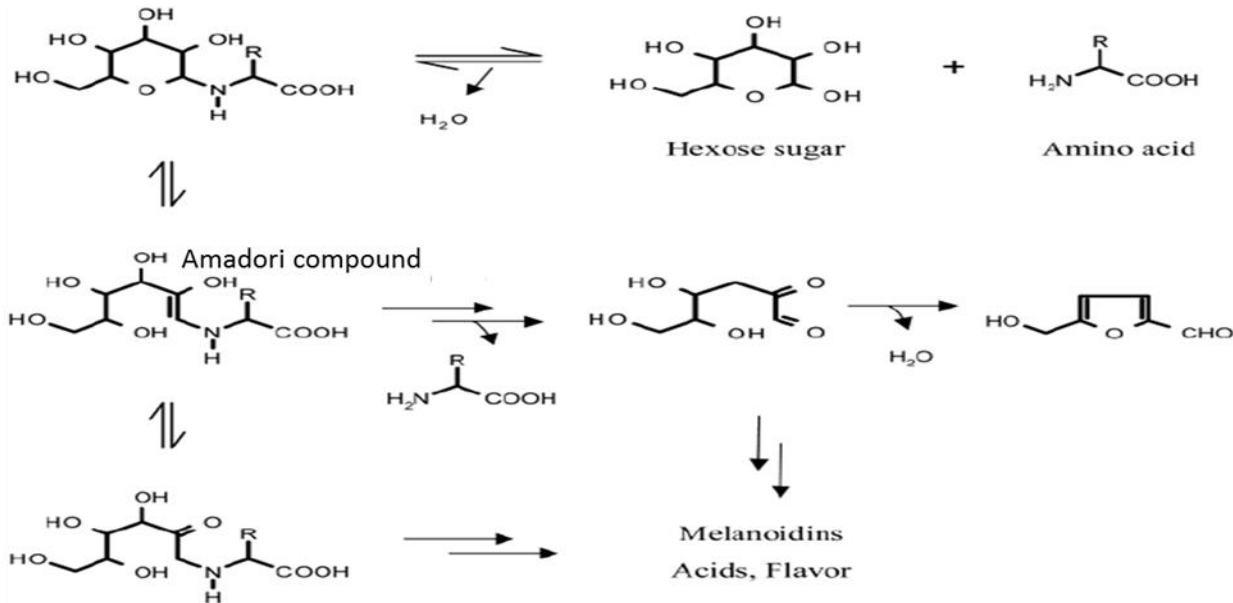
**Table 2.2.** Effect of extrusion processing variables on protein digestibility

Processing parameter	Protein digestibility	Food source	Reference
Barrel temperature	increased with increasing temperature	Lentil	Rathod and Annapure, 2015
		Lentil and horsegram	Ghumman <i>et al.</i> , 2016
Feed moisture	Decreased with lower feed moisture	Proso millet	Gulati <i>et al.</i> , 2017
Screw speed	Increased with increasing screw speed.	Corn-gluten-whey composite	Camire, 2001
Feed ratio	Increased with increasing legume source	Wheat and lentil/green pea/chick pea/yellow pea	Patil <i>et al.</i> , 2016

Increase in extrusion temperature from 100 - 150 °C promoted the inactivation of antinutritional factors that impairs digestion in lentils by 3.6% and consequently, the protein digestibility values increased (Ghumman *et al.*, 2016). Extrusion at low feed moisture of 17% significantly decreased the *in-vitro* protein digestibility of proso millet (Gulati *et al.*, 2017). Increased screw speed increased the protein digestibility of extruded corn-gluten-whey composite, because the increase in shear forces in the extruder denatures the proteins more easily, thus facilitating enzymatic

hydrolysis (Camire, 2001). Lower feed moisture in the extruder barrel is reported to initiate non-enzymatic browning reaction termed as Maillard reaction (Bastos *et al.*, 2012).

Maillard reaction is considered of great importance for flavour and colour formation in thermally processed foods. However, this reaction has detrimental effect on protein nutritional value. The initial stage in Maillard reaction involves condensation of sugars and amino acid to form a Schiff base (Moughan and Rutherford, 2008). Following condensation, the Schiff base is re-arranged to form Amadori compounds. These Amadori compounds then form cross-links with adjacent proteins and amino groups to form highly coloured polymeric heterocyclic nitrogenous compounds known as Melanoidins (Moughan and Rutherford, 2008). Melanoidins are referred to as Maillard products. A scheme showing Maillard reaction is shown in Figure 2.3.



**Figure 2.3.** Simplified scheme showing parts of the Maillard reaction (adapted from Davidek *et al.*, 2003)

Due to protein denaturation during thermal processing, previously hidden amino acid residues become exposed and are free to react with reducing sugars (Camire, 1991). The result of this is decrease in availability of amino acids involved and thus a reduction in protein digestibility (Singh



*et al.*, 2007). Lysine is the most reactive essential amino acid and it is reduced as a result of Maillard reaction (Singh *et al.*, 2007). This is because the free  $\epsilon$ -amino group of lysine can undergo reaction with many compounds including reducing sugars, fats, vitamins, polyphenols and food additives (Marrocco *et al.*, 2017). Reduction or loss in lysine results in a decrease in protein nutritional value (Singh *et al.*, 2007). Also, reduction of lysine during processing has nutritional implication because it is one of the essential amino acids that have carbon skeletons that cannot be synthesized to meet body needs from simpler molecules in humans, and therefore must be provided in the diet (National Academy of Science, 2005).

### 2.1.2.3 Phenolic compounds

Phenolic compounds are a large class of natural or synthetic organic compounds classified as secondary metabolites in plants that have a large range of structures and functions (Naczki and Shahidi, 2006). Many different phenolic compounds can be found in fruits and vegetables and, as such they form an integral part of human diet. In addition, whole grain cereals and legumes have also been studied for their antioxidant properties. This antioxidant activity is due to the presence of phenolic compounds. Table 2.3 shows effects of extrusion cooking on polyphenolic compounds.

**Table 2.3. Effects of extrusion cooking on phenolic compounds**

Food source	Extrusion parameters	Changes in polyphenols	Reference
Common bean	$T_m = 160 - 190$ °C at die exit $F_m = 45\%$	Decrease in total phenolics	Korus <i>et al.</i> , 2007
Faba and kidney beans	$T_m = 152, 156$ °C at die exit $F_m = 25\%$ Screw speed = 100 rpm	Decrease in total phenolics	Alonso <i>et al.</i> , 2000
Oat flour, dried green pea flour, fenugreek seed powder and leaf powder	$T_m = 100$ °C at die exit $F_m = 12\%$ Screw speed = 200 rpm	Decrease in total phenolics	Wani and Kumar, 2015
Wheat flour and brewer's spent grain	$T_m = 80$ °C at feed entry and 100 °C at die exit $F_m = 12 - 17$ % Screw speed = 200 rpm	Increase in total phenolics	Stojceska <i>et al.</i> , 2009
Cranberry pomace and corn starch	$T_m = 90$ °C at feed entry and 150, 170 and 190 °C at die exit $F_m = 12 - 17$ % Screw speed = 200 rpm	Increase in flavanols and procyanidins	White <i>et al.</i> , 2009

$T_m$ = Temperature,  $F_m$  = feed moisture

Phenolic compounds are heat labile. Processing at temperature above 80 °C may reduce or alter their nature (Zielinski *et al.*, 2001). The decrease observed in total phenolic contents in Table 2.3 after extrusion cooking is suggested to be because of decarboxylation of phenolic acids during extrusion cooking due to a combination of high moisture and high barrel temperature which promotes polymerization of phenols leading to a reduction in their extractability and anti-oxidant ability (Korus *et al.*, 2009; Alonso *et al.*, 2000). The increase in phenolic compounds of wheat flour and brewer's spent grain extrudate after extrusion cooking reported by Stojceska *et al.*, (2009) was attributed to wounding of cell walls of food materials by the shearing action in the extruder which then facilitated the release of phenolic acids. Thus, the increase or decrease depends on temperature and shear during extrusion cooking.

## **2.2 Composite food materials used in extrusion cooking**

Cereals and vegetable proteins have long been used in extrusion cooking but more recently, root crops have also been composited with vegetable proteins in extruded foods (Nurtama and Lin, 2009; Muoki *et al.*, 2012; Reddy *et al.*, 2014). Owing to the high protein content of leguminous proteins, they are being effectively utilized for enhancing the nutritional quality of cereal or tuber based extruded foods (Patil *et al.*, 2016; Sawant *et al.*, 2013; Navale *et al.*, 2015). Cassava and Soy will be discussed below as they were used in this study.

### **2.2.1 Cassava**

Cassava is the second most important staple food in terms of per capita calories consumed that is, over 500 million people in sub-Saharan Africa (SSA) get their calories from cassava (El-Sharkawy, 2004). Cassava is a perennial starchy, drought tolerant crop whose roots can be left in the soil and harvested when it is required (Onyenwoke and Simonya, 2014).

The world production of cassava was estimated to be about 288.4 million tonnes in 2016 (FAO, 2016) and 54% of the production was in 40 countries in SSA (Ohimain, 2014). It is estimated that about 75% of Africa's cassava output is harvested in Nigeria, the Democratic Republic of Congo, Ghana, Tanzania and Mozambique (FAO, 2005). Nigeria is the largest producer of cassava in the world with an annual production of about 50 million metric tons (FAOSTAT, 2012).

Cassava root is composed of 70% moisture, 24% starch, 2% fibre, 1% protein and 3% other substances including minerals (Tonukari, 2004). Cassava tolerance to extreme environmental conditions such as poor soil has made it an important food security crop useful in combatting hunger in developing countries (Zidenga *et al.*, 2012). However, similar to other staple starchy crops, cassava is energy dense. High energy dense foods are identified as significant risk factors driving the surge in obesity (Drewnowski and Specter, 2004; Nikolic *et al.*, 2011).

The conventional method of processing cassava roots into flour requires fermentation. The low pH and high acidity of the resultant flour makes it unsuitable for industrial use (Dziedzoave *et al.*, 2006). High quality cassava flour (HQCF) is an improvement on the flour produced from the conventional processing method. The process for manufacturing the flour is as follows: fresh cassava roots are peeled, washed, sliced, grated, pressed, dried, milled, sifted to a particle size of  $\leq 250 \mu\text{m}$ , and bagged. The production process of HQCF eliminates fermentation thus, resulting in a flour that has a pH of between 5.5 – 7.0 and acidity of up to 0.25 % (EAC, 2012). It also ensures the removal of lignin which is the major non-starch polysaccharide present in cassava. HQCF is a smooth, white/creamy, odourless and bland flour which has gained increasing use in composite flours (Maziya-Dixon *et al.*, 2005).

### **2.2.2 Soy bean**

The utilization of soy bean is rapidly on the increase in Africa because it is a low-cost protein source compared to animal protein sources and it contains significant amount of all essential amino acids (Amusat and Ademola, 2013). The seed contains 40% protein, 20% oil, 10% moisture, and around 15% minerals and ash (Osho and Dashiell, 1998). Soy bean has wide application in the reduction of malnutrition related problems particularly protein deficiencies due its nutritional value especially in sub-Saharan Africa (de Pee and Bloem, 2009). Soy bean can be processed into different products. Defatted toasted soy flour is a high protein product obtained from soy bean and it is increasingly being used as functional ingredient in the bakery and food industry (Kumar *et al.*, 2017).

Extruded cassava-full fat soy has a Protein Digestibility Corrected Amino Acid Score (PDCAAS) within recommendations for complementary foods but the kinetics of starch digestibility showed a rate of starch digestion with GI value of 90.1 (Muoki *et al.*, 2012). High GI foods are associated

with raising blood sugar level post meal (Foster-Powell *et al.*, 2002). These high GI foods have also been identified as risk factors in some diet-related non-communicable (NCDs) diseases such as cardiovascular diseases, diabetes and obesity (Cordain *et al.*, 2005).

In view of the pandemic of these NCDs, ways of reducing the rate and extent of carbohydrate digestibility are being sought. Some of the ways of reducing the digestibility of starch-based foods include the formation of amylose-lipid complex, production of enzyme-resistant starch and addition of dietary fibre to foods. The addition of dietary fibre to extruded starch and legume-based food will be focused on in this review.

### **2.3 Dietary fibre addition to food**

Dietary fibre is defined as “carbohydrate polymers with ten or more monomeric units which are not hydrolyzed by the endogenous enzymes in the small intestine of humans” (Codex, 2010). This definition classifies dietary fibre into three groups:

- 1) Carbohydrate polymers naturally occurring in the food as consumed
- 2) Carbohydrate polymers, which have been obtained from food raw materials by physiological, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities,
- 3) Synthetic carbohydrate polymers, which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

Dietary fibre is often classified based on its solubility for functional and technological purposes into soluble and insoluble dietary fibre (Han *et al.*, 2017). Dietary fibre includes insoluble fibre (lignin, cellulose and some hemicelluloses) and soluble fibre (pectins,  $\beta$ -glucans, galactomanan gums, some hemicelluloses) and a large range of non-digestible oligosaccharides including inulin (Alvarez and Peña-Valdivia., 2009; Lattimer and Haub, 2010).

There are several health claims associated with the regular intake of dietary fibre. Epidemiological studies have shown that 2 servings per day increments in whole grain intake was associated with 21% lower risk of type-2 diabetes (De Munter, 2007), lower prevalence of cardiovascular disease

in individuals with the highest dietary fibre intake (Anderson *et al.*, 2009; Threapleton *et al.*, 2013), lower rate of weight gain with increase in dietary fibre intake (Babio *et al.*, 2010; Slavin, 2005).

Many controlled intervention studies have shown that four major water soluble dietary fibre types  $\beta$ -glucan, guar gum, pectin and psyllium effectively aid in lowering carbohydrates digestion (Kaczmarczyk *et al.*, 2012). The mechanism by which dietary fibre exerts its protective effects remains to be clearly elucidated but its ability to influence nutrient absorption has been extensively explored (El Khoury *et al.*, 2011). Dietary fibre can be obtained from various food sources such as cereals, legumes, fruits and vegetables. The pericarp and pomace of these food sources are concentrated with dietary fibre and they are removed during processing as by-products. The development of extrudates with improved nutritional value by using food by-products have been the feature of several previous studies (Rashid *et al.*, 2015; Ruiz-Gutiérrez *et al.*, 2015; Stojceska *et al.*, 2008).

### **2.3.1 Effect of dietary fibre on the nutritional properties of extrudates**

The health benefits associated with dietary fibre has stimulated consumer interest on the importance of its consumption. Soluble dietary fibre is associated with trapping carbohydrate and delaying glucose absorption while insoluble dietary fibre adds bulk to diet and reduces faecal transit time through the large intestine (Dhingra *et al.*, 2012).

There are several hypothesized modes of action by which dietary fibre reduces starch digestibility and post prandial glycaemia:

- (i) The addition of fibre during heat processing of the food may limit the extent of starch transformation (Ou *et al.*, 2001). Food components which readily hydrate such as dietary fibre will restrict water available to starch and reduce degree of starch gelatinization and depolymerization thus, lower starch digestibility (Chanvrier *et al.*, 2007).
- (ii) Dietary fibre is suggested to form a physical barrier around starch granules and this inhibits enzymatic activity. This physical barrier prevents enzyme substrate complex formation for digestion.
- (iii) Soluble dietary fibre mixes with food and delays gastric emptying by increasing the viscosity of gastric contents. The increase in viscosity of chyme could slow down the rate

of gastric emptying (Yu *et al.*, 2014). Based on the water retention capacity of insoluble dietary fibre, they may also alter rate of gastric emptying (Dhingra *et al.*, 2012).

- (iv) The viscous nature of soluble dietary fibre may reduce amylolysis by retarding diffusion of digestive enzymes to substrate and slow down the diffusion of digested starch thus delaying glucose absorption (Kumar *et al.*, 2012; Villemejeane *et al.*, 2016).

The effect of dietary fibre on some nutritional properties of extrudates is shown in Table 2.4. The addition of different dietary fibre sources resulted in a reduction in starch digestion and lower GI of test foods. Dietary fibre is suggested to reduce available water for starch gelatinization, thus limiting total starch gelatinization with consequent lower starch digestibility (Brennan *et al.*, 2011).

**Table 2.4.** Some nutritional properties of extruded products as affected by dietary fibre addition

Dietary fibre source	Other food materials	Extrusion conditions	Changes in nutritional properties of extrudates	Estimated glycaemic index/load	Reference
Guar gum	Wheat flour, maize grit and oat meal	T <sub>m</sub> = 40 - 180 °C, F <sub>m</sub> = 0.2 L/h, Screw speed = 315 rpm, Feed rate = 6.75 kg/h	Reduction in rate of carbohydrate digestion	ND	Brennan <i>et al.</i> , 2008
Lentils	Maize flour	T <sub>m</sub> = 80 - 110 °C F <sub>m</sub> = 25%	Reduction in glycaemic index	74.8 <sup>1</sup>	Hardacre <i>et al.</i> , 2006
Apple pomace	Corn flour	T <sub>m</sub> = 75 - 110 °C F <sub>m</sub> = 17 % Screw speed = 300 rpm, Feed rate = 80 kg/h	Reduction in rate of starch digestibility	ND	Karkle <i>et al.</i> , 2012
Chestnut mushroom stalk	Wheat flour, maize grit and oat meal	T <sub>m</sub> = 40 - 180 °C, Screw speed = 175 rpm, Feed rate = 9 kg/h	Reduction in glycaemic load	37 <sup>2</sup>	Brennan <i>et al.</i> , 2012

<sup>1</sup> is estimated glycaemic index

<sup>2</sup> is estimated glycaemic load

ND = not determined, T<sub>m</sub> = temperature, F<sub>m</sub> = feed moisture, rpm = revolutions per minute

The source of dietary fibre significantly influences its nutritional properties. Soluble dietary fibre is found in good amount in fruits (such as grapes, oranges), oats, barley and pulses such as beans. Insoluble dietary fibres are found in large amount in cereal grains such as wheat bran. Phenolic compounds which are mostly found in the bran of cereals and pulses, skin of fruits also add to the nutritional benefits associated with dietary fibre.

These phenolic compounds are reported to bind to starch granule surface and prevent the formation of enzyme-substrate complex thus lowering the rate of starch digestion (Mariano da Silva *et al.*, 2014). Both amylose and amylopectin molecules in starch granules may interact with phenolic compounds. Phenolic compounds may interact with amylose molecules through the formation of inclusion complexes (Beta and Corke, 2004) and by binding to side chains of amylopectin and the amorphous region of starch granules (Zhu *et al.*, 2009).

Phenolic compounds have also been shown to inhibit the activities of starch digestive enzymes (Hargrove *et al.*, 2011; Forester *et al.*, 2012). Depending on the type of phenolic compounds,  $\alpha$ -amylase and/or  $\alpha$ -glucosidase may be inhibited (Zhu, 2015). Also, the concentration of the inhibitor determines the type of inhibition. At low concentration of tannin, a competitive inhibition was observed and at high concentration, a non-competitive inhibition was observed (Shahidi and Chandrasekara, 2017). Competitive inhibition of tannins is suggested to be due to binding of galloylated glucose and the active sites of salivary  $\alpha$ -amylase (Shahidi and Chandrasekara, 2017) while non-competitive inhibition is attributed to interaction between tannins and secondary sites of enzymes (Zajacz *et al.*, 2007).

### **2.3.2 Dietary fibre sources**

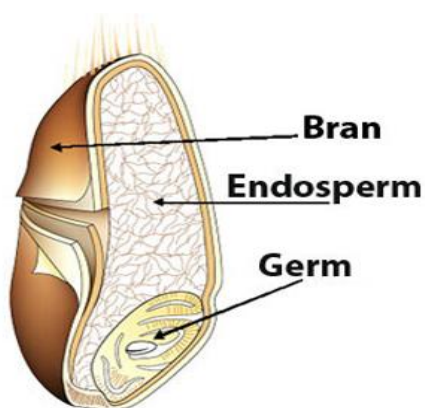
As stated earlier, dietary fibre can be obtained from various plant food sources such as cereals, legumes, fruits and vegetables. The dietary fibre sources used in this study are wheat bran and grape pomace and they are discussed in detail below.

#### **2.3.2.1 Wheat bran**

Wheat production in SSA is estimated to be about 7.1 million metric tonnes per year (FAOSTAT, 2014) and this may result in about 900 thousand tons of wheat bran generated yearly. The increasing demand for wheat in SSA has led to a widespread cultivation of this cereal in SSA.

Ethiopia and South Africa are the major producers of wheat in SSA (Shiferaw *et al.*, 2011), but substantial quantity is being grown in Kenya, Zambia (Nagassa *et al.*, 2013).

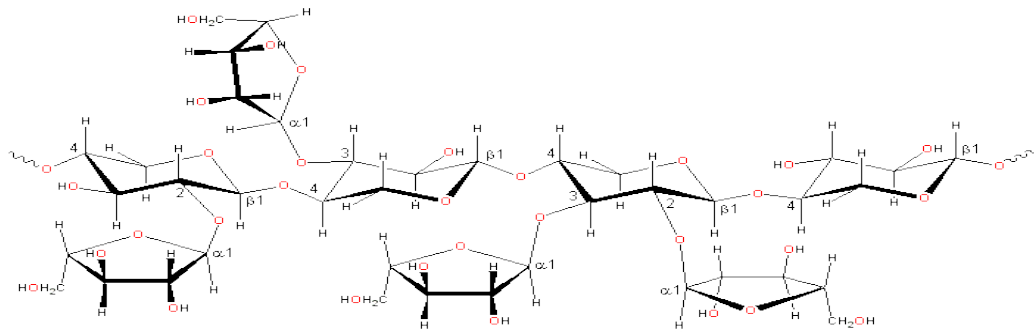
The components of wheat kernel are the germ, endosperm and bran (Figure 2.4). Wheat bran is a by-product of wheat milling and it has food and non-food applications. Wheat bran comprises of non-starch polysaccharides (NSPs) (46%), starch (10-20%), proteins (12-22%), and lignin (4-8%) (Zhang *et al.*, 2011). The components of wheat bran NSPs are arabinoxylan (17-32%), Cellulose (12%) and  $\beta$ -glucan (3%) (Van Craeyveld *et al.*, 2009; Kamal-Eldin *et al.*, 2009).



**Figure 2.4.** Wheat grain structure (World Cancer Research/ American Institute for Cancer Research, 2007).

Arabinoxylan (AX) is classified as hemicellulose and they are the major NSPs present in wheat bran (Lu *et al.*, 2000). AX are made up of pentose sugars mostly arabinose and xylose and are often referred to as pentosans (Berlanga-Reyes *et al.*, 2011). Arabinoxylans consist of linear backbone of D-xylopyranosyl units linked by  $\beta$ -1,4 glycosidic bonds to which  $\alpha$ -L-arabinofuranosyl residues are substituted at the C(O)-2 and/or C(O)-3 positions (Figure 2.5). Some arabinose residues are substituted with ferulic acid at C(O)-5 position (Frederix *et al.*, 2004; Saeed *et al.*, 2011).





**Figure 2.5.** Arabinoxylan structure (Izydorczyk and Biliaderis, 1995)

AX is classified as water extractable arabinoxylan (WEAX) and water unextractable arabinoxylan (WUAX). The extractability of AX is based on their chemical and/or physical interactions; the degree of ester linkages between ferulic acids and other cell wall components, the degree and substitution patterns of arabinose residues (Izydorczyk and Biliaderis, 1995). WEAX are loosely bound to the cell wall surface due to incomplete cross-linking and are therefore easily solubilised in water (Maes and Delcour, 2002). WUAX on the other hand, are strongly embedded in the cell wall network due to interaction with other AX through cross-linking and formation of a network matrix by covalent (e.g. ester and ether bonds, diferulic acid bridges) and non-covalent (e.g. hydrogen bonds) linkages with other cell wall components such as proteins,  $\beta$ -glucans, lignin and cellulose (Biliaderis *et al.*, 1995; Ebringerova and Heinze, 2000). Wheat bran AX contains mostly WUAX and about 4-6% is WEAX (Maes and Delcour, 2002)

The ratio of arabinose to xylose (A:X) is an indication of the degree of substitution and it is an important determinant of solubility. The ratio of A:X in wheat bran generally ranges from 0.54 to 0.71 (Izydorczyk and Biliaderis, 1995). AX fractions having a low A:X ratio are insoluble in water due to the increased tendency of aggregation in unsubstituted regions of the AX molecule. These insoluble aggregates are stabilised by hydrogen bonds and result in a more flexible configuration that can align with each other (Courtin and Delcour, 2002; Köhnke *et al.*, 2011). Along with the substitution degree, the substitution pattern also influences solubility of AX whereby long stretches of unsubstituted xylose residues promote aggregation while substitutions with arabinose residues prevents aggregation (Courtin and Delcour, 2002. Molecular weight also affects solubility. Lower molecular weight structures are more soluble compared to high molecular weight structures (Izydorczyk and Biliaderis, 1992b).

The high viscosity of some AX is a consequence of their stiff structural conformation derived from their highly-substituted backbone (Izydorczyk and Biliaderis, 1995; Izydorczyk and Biliaderis, 1992a). In this case, there is limited opportunity for aggregation due to steric hindrances because of the presence of arabinose side chains (Izydorczyk and Biliaderis (1992a).

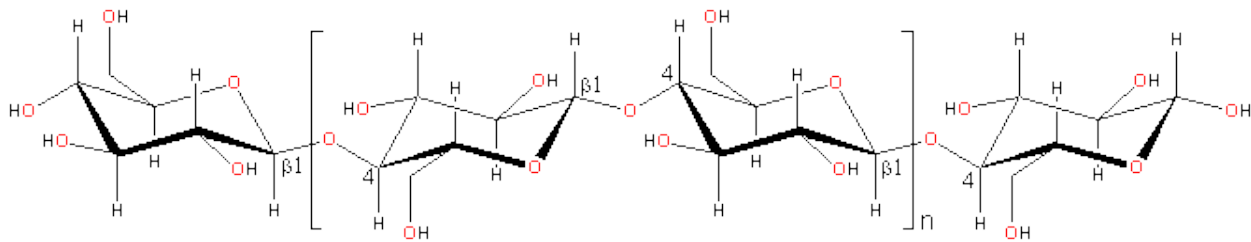
The functionality of AX is largely dependent on their physico-chemical properties such as viscosity and water solubility (Saulinier *et al.*, 2007). The physiological effects which includes laxation, blood glucose attenuation associated with dietary fibre are as a result of their functional properties.

### **2.3.2.2 Grape pomace**

Grape is one of the world's largest fruit crop with over 67 million tons produced globally every year (FAOSTAT, 2014). Grape processing generates a large number of by-products. Grape pomace is one of these by-products. Grape pomace is a rich source of dietary fibre and polyphenols (García-Lomillo and González-SanJosé, 2017). Grape pomace is composed of 51.09– 56.31 % (dry matter) dietary fibre (Bravo and Saura-Calixto, 1998; Deng *et al.*, 2011), 14 % Protein (Bravo and Saura-Calixto, 1998), 2.8 – 8.6 % fat (Karovicova *et al.*, 2015), 10 – 11% phenolic compounds (Makris *et al.*, 2007).

Grape pomace contains both soluble and insoluble dietary fibre. The insoluble dietary fibre components are mainly cellulose and hemicellulose which makes up about 63.7 -71.1 % (Llobera and Canellas, 2007; Sousa *et al.*, 2014). Pectin is the predominant soluble dietary fibre present in grape pomace (Bravo and Saura-Calixto, 1998) and it is estimated to be about 34% (Ping *et al.*, 2011).

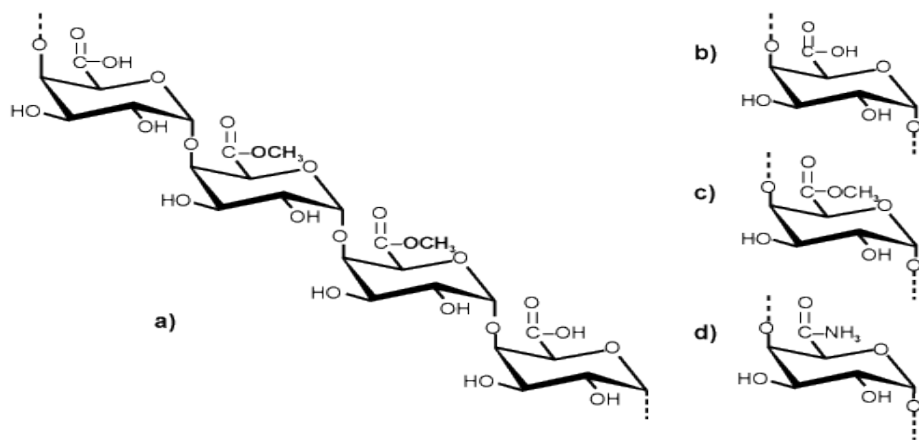
Cellulose is the world's most abundant polymer (Izydorczyk *et al.*, 2005). It is a high molecular weight linear, insoluble homopolymer of D-glucopyranosyl residues linked by  $\beta$ -1,4 glycosidic linkages (Figure 2.6). Cellulose has a very stable crystalline structure which is responsible for its low reactivity (Barasi, 2003). The  $\beta$ -1,4 linkage between glucose units holds the chain in a flat conformation which makes it possible for cellulose chains to align next to each other and form numerous hydrogen bonds between the sugar hydroxyl groups (Kumar *et al.*, 2012).



**Figure 2.6.** Cellulose structure (Nishiyama, 2009)

The human digestive system lacks the specific enzymes required to hydrolyse the  $\beta$ -1,4 linkages in cellulose and this is responsible for the main physiological function attributed to this polysaccharide being increased faecal bulking and increase in transit time (Dakhara *et al.*, 2012). Cellulose can be physically and chemically modified to improve its functionality in food systems (Izydorczyk *et al.*, 2005).

Pectin is regarded as the most structurally complex polysaccharide in nature and it is a major component of primary cell walls and all higher plants (Mohnen, 2008). Pectin comprise of two families of covalently inter-linked acid polymers, galacturonan and rhamnogalacturonan (Voragen *et al.*, 2008). Pectin exists as a linear polysaccharide made up of D-galacturonic acid linked by  $\alpha$ -1,4 linkages which are substituted by  $\alpha$ -1,2 rhamnopyranosyl residues in adjacent or alternate positions (Anderson *et al.*, 2017) (Figure 2.7). The side chains of some neutral sugars namely D-galactose, L-arabinose and D-xylose branch off from the rhamnose residues (Yapo, 2011).



**Figure 2.7.** (a) A repeating segment of pectin molecule and functional groups: (b) carboxyl; (c) ester; (d) amide in pectin chain (Sundar Raj *et al.*, 2012)

The polygalacturonic acid building blocks of pectin may have varying degrees of carboxyl groups that are methylesterified (Thakur *et al.*, 1997). Pectin can be divided into two structural groups,

high methoxyl pectins with a degree of esterification (DE) of more than 50% and low methoxyl pectin with a DE of below 50% (Haghighi and Rezaei, 2012).

Pectin has nutritional and technological importance. Its solubility in water is related to the degree of polymerization and the number of distribution of methoxyl groups. Pectin solubility generally increases with an increase in degree of esterification and decrease in molecular weight (Pomeranz, 2012). Extrinsic factors such as pH of the solution, temperature, nature and concentration of solute also influences solubility (Haghi and Razaei, 2012).

### ***2.3.3 Effect of extrusion cooking on the functional properties of dietary fibre***

The combination of thermal and mechanical energy in extrusion cooking can change the structure of dietary fibre leading to new functional properties (Dhingra *et al.*, 2012). Mechanical shear facilitates the opening of fibre structure thereby making free hydroxyl groups available to bind with water (Sangnark and Noomhorm, 2004). Extrusion cooking induces changes in fibre solubility. Processing variables such as temperature, screw speed and moisture content could influence modification of dietary fibre during extrusion cooking. Changes in insoluble to soluble dietary fibre content as a result of extrusion cooking are shown in Table 2.5. The intense mechanical shear during extrusion cooking due to low feed moisture (14%) and high temperature (160 °C) was associated with depolymerization of insoluble dietary fibre. This thereby led to an increase in soluble dietary fibre and decrease in the insoluble dietary fibre portion (Sobota *et al.*, 2010). Extrusion of oat and wheat bran at a screw speed of 200 rpm facilitated the fragmentation of insoluble dietary fibre with a consequent increase in soluble dietary fibre content.

Extrusion cooking process facilitates the breakdown of polysaccharides glycosidic bonds of insoluble dietary fibre with the release of oligosaccharides, and this may be responsible for the shift in insoluble to soluble dietary fibre ratio observed in extrudates (Stojceska *et al.*, 2009).

**Table 2.5.** Effect of extrusion cooking on dietary fibre content

Source of fibre	Extrusion conditions	Changes in dietary fibre content	Analytical method used in determining dietary fibre content	Reference
Corn grits and wheat bran	$T_m$ = die exit was varied at 160 and 130 °C, Screw speed = 72 rpm, $F_m$ = 14%	Increase in temperature led to decrease in insoluble fibre and an increase in soluble fibre	Enzymatic-gravimetric method (AACC, 2000)	Sobota <i>et al.</i> , 2010
Oats and corn bran	$T_m$ = die exit was varied at 115, 120, 130, 140 and 145 °C, Screw speed = 20 rpm, $F_m$ = 150, 200, 300 and 400 g/kg	Increasing temperature and feed moisture increased soluble dietary fibre and decreased insoluble dietary fibre.	Enzymatic-gravimetric method (AACC, 1984)	Zambrano-Zaragoza <i>et al.</i> , 2013
Oat bran	$T_m$ = 120 °C, Screw speed = 200 rpm, $F_m$ = 15 l/h	Decrease in insoluble dietary fibre and increase in soluble dietary fibre	Enzymatic gravimetric method (modification of AOAC method by Prosky <i>et al.</i> , 1985)	Gualberto <i>et al.</i> , 2000
Wheat bran	$T_m$ = 90 °C, Screw speed = 200 rpm, $F_m$ = 20%	Decrease in insoluble dietary fibre and increase in soluble dietary fibre	Enzymatic-gravimetric method (AACC, 2000)	Rashid <i>et al.</i> , 2015

$T_m$  = temperature,  $F_m$  = feed moisture

The changes in solubility as a result of extrusion cooking gives extrudates new and improved functionality. The functional properties of dietary fibre are related to their chemical composition and the process conditions used to obtain food. The functional properties of a food material could enhance its utilization in food products. The physicochemical properties of dietary fibre determine their functional properties of dietary fibre. Some of these physicochemical properties are discussed below.

### 2.3.3.1 Hydration properties

Most polysaccharides contain glycosyl units that have hydroxyl groups and each of these hydroxyl groups have the capacity to hydrogen bond to one or more water molecules (BeMiller and Huber,

2007). In aqueous systems, polysaccharides can take up water, swell and undergo partial or complete dissolution. The fate of dietary fibre in the digestive tract (induction of fermentation) is partly determined by their hydration properties and accounts for some of their physiological effects (faecal bulking of minimally fermented dietary fibre) (Dhingra *et al.*, 2012).

Hydration properties of dietary fibre can be determined through their swelling, water absorption and water holding capacities. Differences in hydration capacity of different fibre sources is related to their chemical structure and physical characteristics (Guillon and Champ, 2000). Dietary fibre from algae are reported to have a greater affinity for water than those from fruits and vegetables while cereal fibre sources present the lowest affinity (Elleuch *et al.*, 2011). The difference in affinity for water of different fibre sources is related to the number of hydroxyl groups present in their structure.

The hydration capacities of fibres containing polyelectrolytes (charged groups such as pectin rich fibres, carboxyl and sulfate groups in fibres from algae) can be influenced by environmental conditions such as pH, ionic strength, ionic form, types and concentration of ion in solution (Fleury and Lahaye, 1991; Renard *et al.*, 1994). Water affinity decreases with ionic strength, this is because ions decrease electrostatic repulsion between polysaccharides and, consequently the water affinity and expansion of charged polysaccharides (Fleury and Lahaye, 1994). A decrease in hydration capacity of sugar beet fibres was observed by increasing the concentration of NaCl in solvent (Bertin *et al.*, 1988). This was attributed to the high concentration of sodium ions that led to collapse of the pores and the micro-channels in sugar beet fibre matrix due to decrease in the electrostatic repulsion and expansion of polysaccharides (Bertin *et al.*, 1988)

The processing fibre undergoes can also modify the physical properties of fibre matrix with a consequent effect on its hydration properties (Thibault *et al.*, 1992). Extrusion cooking opens up fibre structure by mechanical shearing and makes hydroxyl groups available to bind with water (Ozyurt and Ötles, 2016). The hydration property of fibre is reported to increase in water with increase in temperature as a result of fibre solubility (Fleury and Lahaye, 1991).

Hydration properties of dietary fibre gives an indication of its potential use as an ingredient in food formulations. A knowledge on water holding and swelling capacity of dietary fibre provides information for designing fibre enriched foods. Dietary fibre with a high water holding capacity

suggests that it can be used as a functional ingredient to avoid syneresis, reduce calories, modify viscosity, and texture of formulated foods (Elleuch *et al.*, 2011). Water absorption capacity gives information on substrate pore volume and helps us in understanding the behaviour of fibre in foods or during gut transit (Guillon and Champ, 2000).

### **2.3.3.2 Solubility**

Dietary fibres are classified as soluble or insoluble, based on whether they form a solution when mixed with water (soluble), or not (insoluble). The nutritional and functional property of dietary fibre are influenced by their solubility.

The structure and stability of polysaccharide determines its solubility. If the polysaccharide structure is such that the chains have regular ordered conformation which are well packed together in a crystalline array, the polymer is likely to be more stable in solid state than in a solution (Guillon and Champ, 2000). Polysaccharides with a regular set structure are insoluble due to inter-chain hydrogen bond between hydroxyl groups and oxygen which stabilizes the linkage and results in a linear configuration (Poletto *et al.*, 2013). Polysaccharides set irregularly on the backbone or as side chains tend to be soluble (Elleuch *et al.*, 2011). This is because branching disrupts intermolecular forces, thereby preventing the formation of ordered crystalline structure (Nelson, 2001a).

The presence of ionizing group such as COOH or  $\text{SO}_4^{2-}$  in polysaccharides also increases solubility (Elleuch *et al.*, 2011). Electrostatic repulsion inhibits the polysaccharides from close packing, thus preventing the formation of ordered structures that tend to be insoluble in water (Patil 2008). The potential for inter-unit positional bonding (like  $\beta$ -glucans with mixed  $\beta$ -1-3 and  $\beta$ -1-4 linkages) increases solubility. Alterations of the monosaccharide units or their molecular form ( $\alpha$ - or  $\beta$ -form) will further increase solubility e.g. gum acacia, arabinogalactan and xanthan gum (Dhingra *et al.*, 2012). Temperature also influences stability of ordered assemblies, some material insoluble in cold water, but would dissolve at high temperature. The application of heat can promote conversion from ordered form of some polysaccharides to disordered forms. This is due to the breakage of the inter-chain hydrogen bond between hydroxyl groups and oxygen by heat. (Guillon and Champ, 2000).

### 2.3.3.3 Viscosity

Viscosity ( $\eta$ ) which is resistance to flow is described as the ratio of shear stress ( $\tau$ ) to shear rate ( $\dot{\gamma}$ ). Most polysaccharides exhibit non-Newtonian flow and an increased shear rate could either increase or decrease viscosity (Elleuch *et al.*, 2011). In relation to dietary fibre, viscosity is defined as the ability of fibre to thicken when mixed with fluid (Dikeman and Fahey, 2006).

Viscosity of polysaccharide solution or suspension depends on their intrinsic properties (molecular weight, the presence of charged groups and level of charge, shape of molecules), shear rate, temperature and its concentration (Guillon and Champ, 2000). At low concentration, molecules are more independent because they are separate from each other but as concentration increases, space between molecules reduces and they can interact with each other thereby interpenetrating one another. This interpenetration leads to the formation of an entangled network that increases viscosity (Guillon and Champ, 2000).

Generally, as the molecular weight or chain length of fibre increases, the viscosity of fibre in solution also increases. However, the concentration of the fibre in solution, processing conditions (temperature, shear rate), pH, and ionic strength all substantially depend on the fibre used. Primarily, long chain polymers, such as the gums (guar gum, tragacanth gum) bind significant water and exhibit high solution viscosity (Dhingra *et al.*, 2012).

The ability to increase the viscosity of solutions depends on the hydrodynamic volume of the polysaccharide. A large hydrodynamic volume results in increased viscosity at low concentration (Lovegrove *et al.*, 2017). Highly branched structures of polysaccharides occupy very low hydrodynamic volume and thus develops a very low viscosity, hence why soluble fibres that are highly branched or are relatively short chain polymers such as acacia gum have low viscosity (Dhingra *et al.*, 2012).

Viscosity directly relates to hydration and solubility and they make up the most important physical properties of dietary fibre from both physiological and technological point of view. Dietary fibres which can form viscous solution/gel can change the rheology of intestinal content and this impacts on starch digestion, glucose absorption and thus insulin secretion, attenuation of blood lipids, and laxation (Brennan *et al.*, 2005; Wood, 2007). Viscous fibres also have great potential for use as stabilizers and thickeners in food processing (Elleuch *et al.*, 2012).



### **2.3.4 Effect of dietary fibre addition on satiating properties of food**

Dietary fibre has been associated with enhancing post-prandial satiety (Juvonen *et al.*, 2007). Satiety is a sensation of fullness and satisfaction that continues after a meal and during the inter-meal period (Jakobsen, 2015). Satiety is modulated by physiological processes in the brain and body (Hull *et al.*, 2015), but it is also strongly influenced by social and environmental factors such as availability of food, sensory stimuli, and habitual meal times (Wood, 2009).

Foods vary in their capacity to influence satiety and appetite. This can be attributed to several properties of the food such as the macronutrient composition, physical properties and, taste sensation, among others (Kirkmeyer and Mattes, 2000). High dietary fibre foods are reported to be more satiating than high carbohydrate, fat and protein foods (Isaksson *et al.*, 2012; Chambers *et al.*, 2015).

There are several physiological mechanisms through which dietary fibre is believed to impact satiety and food intake in humans. These mechanisms are highly variable depending on the food matrix of the fibre and its chemical properties. For example, insoluble dietary fibre exerts its impact on satiety in part through bulking and increased mastication to longer oral exposure time (Burton-Freeman, 2000). Viscous fibres are thought to increase satiety through increase viscosity, gel forming in the stomach, delayed gastric emptying, delayed nutrient absorption, and fermentation in the gut (Slavin and Green, 2007).

#### **2.3.4.1 Effect of oral processing time on satiety**

Oral processing is the first stage of food digestion (Pereira *et al.*, 2007). It requires the mechanical action of the teeth and tongue together with the biochemical action of the saliva in the oral cavity to transform the ingested food into a bolus suitable for swallowing (Bilt *et al.*, 2006). Oral processing is regarded as a complex process which is controlled by the central nervous system and modulated by some input from the mouth (de Wijk *et al.*, 2011). It involves an inter-play of several factors involved in the process; food related factors which encompasses macronutrient composition, physico-chemical properties, palatability (Gaikwad, 2012), human related factors such as gender and age (Peyron *et al.*, 2004) and behavioural factors (Bilt *et al.*, 2006).

Although, smell, taste and mouthfeel/texture of food contributes to the characterization of the food's flavour, mouthfeel/texture is identified as the major sensory component of food that plays

a key role in satiety (Chambers, 2015). Through a lifetime of eating experiences, oral sensory signalling has been refined and in the process, it has been learnt that certain properties of a food's flavour are better predictors of the presence of nutrients than others (Chambers, 2015). Longer oral exposure of food gives the sensory receptors more time to respond to the food and more signals mediating satiety sensations to the brain (Lyly *et al.*, 2009).

Foods rich in dietary fibre usually requires more time and effort during mastication, and they thus have a longer oral exposure time (Lyly *et al.*, 2009). The viscosity of semi-solid foods that contain dietary fibre has been shown to be primarily related to the bulk of the intact food bolus and it usually requires high activities of compression movements which results in more chewing activity thus, prolonging oral exposure time (de Wijk *et al.*, 2011).

Increased mastication increases oral processing time and may help to decrease caloric intake by slowing consumption rate and increasing satiety hormones (Li *et al.*, 2011). The mechanism behind the higher secretion of satiety-related hormones, which appears to be induced by more chewing is unclear, but it is hypothesized that there could be a neural response in the brain regions responsible for satiety hormone secretion (Zhu *et al.*, 2013).

Hormones and regulatory factors secreted in response to movement of nutrients in the lumen are produced by the specialized enteroendocrine cells in the small intestine (Vander *et al.*, 2001). Soluble dietary fibre may prolong intestinal transit time, and this may promote the release of various satiety-related peptides, which in turn may affect gastric emptying and send signals to the brain (Slavin, 2013). The exposure of the intestinal mucosa to nutrients induces the release of appetite regulating peptides (hormones) namely; cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) (Kristensen and Jensen, 2011). These peptides mainly induce satiety, GLP-1 additionally stimulates insulin secretion (Wilcox, 2005).

The presence of dietary fibre in foods often tends to decrease palatability of the food (Juvonen *et al.*, 2007). For example, the bitterness perceived in whole grain sorghum porridge due to the presence of phenolic compounds reduces its palatability. Also, following consumption of some fibre containing porridges, the mouth still senses residue and after effects resulting from the ingested food (de Wijk *et al.*, 2011) and this might be considered as undesirable by some consumers.

#### ***2.3.4.2 Effect of dietary fibre in the physiological tract***

Dietary fibre with high water binding capacity will increase gastric distension by expanding its volume in the stomach which will increase feelings of satiety (Tan *et al.*, 2016). Dietary fibre can induce thickening of the intestinal content, which may slow down the rate by which nutrients move through the circulation and may thus, prolong the release of nutrients and lead to a longer sensation of satiety due to the release of appetitive hormones or peptides (Kristensen, 2011; Peters *et al.*, 2009). The slower uptake of nutrients is consequently followed by lower blood glucose concentrations (Hoad *et al.*, 2004; Kristensen, 2011).

The mechanism through which increased viscosity of intestinal content increases gastric emptying time is suggested as follows; viscous fluids diminish the depth of peristaltic constrictions and this results in weakened propulsion and slower movement between gastric compartments and down the gastrointestinal tract. Decreased propulsion combined with an innate resistance to flow of viscous chyme results in overall increased motility time (Ehrlein and Schemann, 2005)

Additionally, short chain fatty acids (SCFA) produced from the fermentation of dietary fibre in the intestinal microbiota are associated with stimulating the release of satiety related peptides, such as cholecystokinin (CCK) (Mathern *et al.*, 2009), peptide tyrosine-tyrosine (PYY), glucagon-like peptide 1 (GLP-1) by entero-endocrine cells (Heijboer *et al.*, 2006; Rao, 2016) thus promoting sensations of fullness.

#### ***2.3.4.3 Satiety measurement***

The measurement of quantitative effects of foods on short-term appetite enables comparison of the effects of different foods on hunger and satiety. Satiety is a subjective term, and this leads to variations in how it is perceived in food by different individuals hence why it is measured indirectly (Kristensen, 2000). The most common method used in measuring satiety is by rating subjective appetitive feelings before and after a meal and at intervals over a certain number of hours which is usually between 2 – 4 hours (Blundell *et al.*, 2010).

The appetitive feelings rated are hunger, fullness, desire to eat and prospective food consumption. A visual analog scale (VAS) is commonly used in rating the feelings of satiety. This is a 100-mm horizontal line representing a continuum of the subjective feeling in question with the opposite

ends representing extremes of the feelings being rated. The end points are anchored from not hungry at all to extremely hungry (Stubbs *et al.*, 2000).

The participants respond to a question by placing a mark on the 100-mm line at an appropriate point that best expresses the subjective feeling in question. The mark is then changed to a number by measuring the distance from the left end of the line to the mark. A graph can be drawn and the area below the curve can be used as a measure of feelings related to satiety (Flint *et al.*, 2000).

The effect of dietary fibre in increasing satiety and suppressing hunger has been reported by several studies but there are limited studies in literature on the effect of extruded foods containing dietary fibre on satiety. Lyly *et al.*, (2009), investigated the effects of three different fibre sources (wheat bran, oat and guar gum) on perceived satiety and hunger and showed that although all three fibre sources enhanced perceived satiety, guar gum had the greatest effect on perceived satiety and it was more effective in suppressing hunger and, this was attributed to the high viscosity of guar gum. Similarly, whole grain wheat bread was shown to induce increased satiety and decrease hunger compared to refined wheat bread, refined wheat pasta and whole grain wheat pasta and, the ability of fibre components of whole grain wheat bread to cause gastric distension post ingestion was associated with the feeling of increased satiety (Kristensen *et al.*, 2010).

In another study, Rao, (2016) demonstrated that guar gum is effective in increasing the perception of satiety and much helpful for appetite control. The author attributed this to the high viscosity of guar gum that facilitated slowing down the colonic transit time and production of SCFA especially propionates and butyrates which triggered the increase release of satiety hormone cholecystokin (CCK).

## **2.4 CONCLUDING REMARKS**

- Extruded cassava and defatted soy porridge have been shown to have a high protein quality. Thus, having great potential to reduce protein energy malnutrition among children.
- However, the rapid starch digestibility and high glycaemic index of extruded cassava and defatted soy porridge makes it a health concern as this makes it unsuitable for people with diet-related non-communicable diseases such as type-2 diabetes and obesity.

- Non-starch polysaccharides such as dietary fibre have been promoted as potentially useful in lowering starch digestibility of extruded foods. The modification in the functionality of dietary fibre during extrusion cooking which promotes reduction in starch digestibility requires more elucidation.
- Also, several studies have focused on the relationship between viscous fibre and satiety but there are very limited studies on the oral processing properties of extruded porridge which contains dietary fibre and how this relates to satiety.
- Food by-products such as wheat bran and grape pomace have the potential of use in reducing starch digestibility and glycaemic index of starch-protein composites with the use of extrusion cooking to modify the nutritional and functional properties of extrudate porridge.

## 2.5 Hypotheses and Objectives

### 2.5.1 Hypotheses

1. The addition of wheat bran or grape pomace to cassava-defatted toasted soy composite will lower the rate of starch digestibility of extrudates. Extrusion cooking will promote the depolymerization of insoluble dietary fibre into smaller soluble units which in turn may form a physical barrier around starch granules thereby reducing enzyme access to substrate (Brennan *et al.*, 1996). Also, phenolics compounds may inhibit the activities of starch hydrolyzing enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and thus lower starch digestion (Singh, *et al.*, 2010).
2. Extrusion cooking will promote redistribution of insoluble dietary fibre to soluble fibre and this may give extruded composites which contains wheat bran or grape pomace a higher viscosity. Mechanical stress due to high temperature and shear during extrusion cooking may weaken and promote fragmentation and depolymerization of glycosidic linkages holding polysaccharide monomers together (Zhang, Bai and Zhang, 2011). As a result, the molecular structure of wheat bran and grape pomace fibre may be altered, leading to decrease in molecular weight and subsequent increase in viscosity of solubilized lower molecular weight dietary fibre (Robin *et al.*, 2012).
3. Extrusion cooking and addition of wheat bran or grape pomace to cassava-defatted toasted soy composite will lower protein digestibility of extrudates. Extrusion cooking promotes

Maillard reaction which results in lysine loss and in extension lower protein digestibility of extruded product (Singh *et al.*, 2007). Also, the phenolic compounds present in grape pomace or wheat bran may interact with proteins through formation of indigestible complexes which results in decrease in protein digestibility (Pushparaj and Urooj, 2011).

4. The addition of wheat bran to cassava-defatted toasted soy composite will increase the oral processing time and satiety of cassava-defatted toasted soy composite. High fibre porridge will require more mastication because of its grittiness and viscosity hence will have a longer oral exposure time and this would allow more signals mediating satiety sensations to the brain (Lyly *et al.*, 2009). Also, fibre in porridges will impart a cognitive impression which will make it to be perceived as a food to reduce hunger rather than a means to reduce thirst due to the bulkiness of the porridge in the mouth (Mattes, 2005).

### **2.5.2 Objectives**

1. To determine the effect of addition of either wheat bran or grape pomace to cassava-defatted toasted soy composite and extrusion cooking on nutritional properties (*in vitro* starch digestibility, soluble dietary fibre, insoluble dietary fibre contents and *in vitro* protein digestibility) of extruded porridges.
2. To determine the effect of addition of either wheat bran or grape pomace to cassava-defatted toasted soy composite and extrusion cooking on functional properties (flow properties, water holding capacity, solubility) of extruded porridges.
3. To determine the effects of wheat bran addition at different levels (0, 10 and 20%) to a cassava-defatted toasted soy composite during extrusion cooking on the descriptive sensory properties, oral processing characteristics and subjective satiety responses of extruded instant porridges.

### **3.0 RESEARCH**

The research work is divided into three sections. The first section (3.1) is about the effects of extrusion cooking and wheat bran addition on the functional, nutritional and rheological properties of cassava-defatted toasted soy composite. The second section (3.2) deals with the effects of extrusion cooking and grape pomace addition on the nutritional, functional, and potential health promoting properties of cassava-defatted toasted soy composite. The final section (3.3) evaluated the effect of addition of wheat bran to cassava-soy extruded porridge on sensory properties, oral processing and satiety.

### **3.1 EFFECTS OF EXTRUSION COOKING AND WHEAT BRAN SUBSTITUTION ON THE FUNCTIONAL, NUTRITIONAL AND RHEOLOGICAL PROPERTIES OF CASSAVA-DEFATTED TOASTED SOY COMPOSITE**

#### **Abstract**

Wheat bran was substituted with cassava-defatted toasted soy composite at 0, 10 and 20% substitution levels followed by extrusion cooking and some nutritional, functional and rheological properties of the extrudates were determined. There was a significant ( $p < 0.05$ ) increase in starch digestibility of composites after extrusion cooking but the substitution of wheat bran lowered the rate and extent of starch hydrolysis. Extrusion cooking led to a reduction in nitrogen solubility index (NSI) and *in-vitro* protein digestibility (IVPD). Substitution of cassava defatted-toasted soy composite with wheat bran reduced expansion and solubility index of extrudates. Extrusion cooking also promoted fibre fragmentation with a consequent increase in soluble dietary fibre and a decrease in insoluble dietary fibre content. All composites exhibited a shear thinning behavior and the composite with 20% wheat bran had the highest viscosity. The results indicated that substitution of starch with wheat bran together with extrusion cooking can be applied to produce products with improved nutritional and functional properties.

This phase of the study has been published:

Oladiran, D.A. and Emmambux, N.M., 2017. Effects of extrusion cooking and wheat bran substitution on the functional, nutritional, and rheological properties of cassava-defatted toasted soy composite. *Starch-Stärke*, 69(7-8).



### 3.1.1 Introduction

Extrusion cooking is a popular technology due to its versatility. It is a high temperature short time processing technique which can be used to produce a wide range of ready to eat foods (Vasanthan *et al.*, 2002) for example direct expanded products, and it is also being utilized to make fibre-rich products from food by-products (Stojceska *et al.*, 2009). Extruded products induce a high glycaemic index (Brand-miller *et al.*, 2002) due to the transformations in physico-chemical properties of raw materials during processing as a result of the combination of moisture, pressure, temperature and mechanical shear (Anton, Fulcher and Arntfield, 2009). The transformations that occur during extrusion cooking are usually accompanied by starch gelatinization and/or dextrinization which leads to high starch digestibility (Singh *et al.*, 2007); protein denaturation; enzyme inactivation (Bhattacharya and Prakash, 1994); and redistribution of dietary fibre content ratio (Stojceska *et al.*, 2009).

In a previous work, Muoki *et al.* (2012) produced an instant complimentary porridge from locally grown crops cassava and soy using extrusion cooking and the extrudate was a product of high energy density and protein quality with positive sensory attributes able to address the problem of protein energy malnutrition amongst children. However, the product was considered as having high GI due to its rapid starch digestibility as extrusion cooking led to complete starch depolymerization and as such is unsuitable for people living with non-communicable disease such as type-2 diabetes.

The use of Dietary fibre to lower glycaemic index by altering the rate and extent of starch hydrolysis has been investigated by several authors (Brennan *et al.*, 2013; Foschia *et al.*, 2013; Mudgil and Barak, 2013). Dietary fibre is classified based on its chemical, physical and functional properties into insoluble dietary fibre and soluble dietary fibre.

There are several suggestions on the mechanism through which dietary fibre lowers digestibility of starch. It has been suggested that dietary fibre competes for available water with starch during extrusion cooking and limits the extent of starch degradation thus lowering starch digestibility (Karkle *et al.*, 2012). Another probable suggestion is that soluble dietary fibre such as guar gum forms a physical barrier around starch granules which reduces enzyme accessibility to the substrate (Brennan *et al.*, 1996). Also, highly viscous dietary fibre such as guar gum and beta-glucan can increase the viscosity of food in the gut thereby slow down the diffusion of enzymes to reach the

substrate for digestion and retard diffusion of digested materials to the absorptive surface for absorption (Kim and White, 2013; Villemejeane *et al.*, 2016; Barasi, 2003). However, the mechanism relating reduced starch digestion and bran addition is not clear and this necessitates further investigation.

The thermal and mechanical energy involved in extrusion cooking processing can modify the structure of dietary fibre and impart new functionality in extrudates such as solubility and viscosity (Wolf, 2010). Brennan *et al.* (2012) reported a reduction in potential glycaemic index with increasing addition of chestnut mushroom to wheat flour in an extruded snack. Also, the replacement of corn flour with apple pomace during extrusion cooking was reported to decrease the susceptibility of starch to enzyme hydrolysis (Karkle *et al.*, 2012). Brewer's spent grain and cabbage trimmings were used to increase total dietary fibre of extrudates by Stojeska *et al.* (2009) and the shift in insoluble dietary fibre to soluble dietary fibre ratio was attributed to mechanical stress during extrusion cooking which possibly promoted the breakdown of polysaccharides glycosidic linkages and led to solubilization of insoluble dietary (Stojeska *et al.*, 2009; Brennan *et al.*, 2008a)

Wheat bran is a by-product of wheat milling and it makes up 14-19% of the wheat grain (Maes and Delcour, 2002). It is a dense source of dietary fibre and contains about 47% dietary fibre (Kamal-Eldin *et al.*, 2009). Wheat bran is a major livestock feed commodity but in the past decade its incorporation into foods has increased (Prückler *et al.*, 2014). The health benefits of wheat bran have been attributed to its dietary fibre and phytochemicals. The objective of this study was therefore to investigate the effects of extrusion cooking and the incorporation of wheat bran at 10 and 20% addition levels on some nutritional, functional and rheological properties of cassava-defatted toasted soy composite flour.

### **3.1.2 Materials and methods**

#### ***3.1.2.1 Raw materials***

High quality cassava flour produced according to FAO method (Dziedzoave, Graffham and Boateng, 2002) (fresh cassava roots were peeled, washing, grating, pressing, disintegration, sifting, drying, milling, screening, and bagging of flour) with particle size of  $\leq 250 \mu\text{m}$  was purchased from Thai Farm International (Ogun State, Nigeria). The flour contained 84.4% starch, 9.75%

moisture, pH of 5.67 and 0.5% crude fibre. Toasted defatted soy flour with particle size of  $\leq 212$   $\mu\text{m}$  was purchased from Petrow foods (Johannesburg, South Africa) and wheat bran was obtained from the Food Corp Division of Rainbow Chicken Limited foods (Pretoria, South Africa).

### ***3.1.2.2 Extrusion cooking***

Cassava and defatted toasted soy were uniformly mixed in a ratio of 65 to 35% (w/w) respectively. Wheat bran was substituted at 0, 10 and 20% (w/w) levels and mixed thoroughly with the cassava and soy composite. A co-rotating twin screw extruder (TX 32, CFAM, Potchefstroom, South Africa) was used in this study. The barrel comprised of five heating zones set at 60/80/100/140/140 °C respectively. Moisture was fed into the system at a dosing rate of 3 l/h and feed rate was 25 kg/h. A die opening of 3 mm was used and the screw speed was maintained at 200 rpm. Extrudates were immediately dried in an oven at 90 °C for 5 min and ground with an analytical mill (A11, IKA, Staufen, Germany). The ground extruded products were kept in plastic sample bottles and refrigerated at 4 °C in preparation for analysis.

### ***3.1.2.3 Analyses***

#### **3.1.2.3.1 Physico-chemical characteristics**

Expansion ratio was determined as the diameter of extrudates divided by the diameter of the die exit (3 mm) (Gujska and Khan, 1991). The Bulk density (g/ml) of the extrudate was expressed as the weight of the extrudate divided by its volume (Cauvain and Young, 1991).

Proximate analysis in terms of ash and crude fat were determined according to the AOAC methods 942.05 and 920.39A (AOAC, 2000) respectively. Protein (N x 6.25) was determined according to Dumas method (Shea and Watts, 1939) using the (Gerhardt Dumatherm) nitrogen combustion system.

Total starch was determined using the method described by Gibson and McCleary [27]. Commercial enzymes (thermostable  $\alpha$ -amylase and amyloglucosidase) from Megazyme (K-TSTA 07/11) (Megazyme International, Bray, Ireland) were used to hydrolyse starch to glucose. Glucose was then quantified colorimetrically by the glucose oxidase-peroxidase reaction and the absorbance was read at 510 nm. Total starch was then expressed as starch as a proportion (%) of

total sample weight and correction was made for moisture to determine starch on a dry weight basis.

#### **3.1.2.3.2 Water absorption capacity and Water solubility index**

The procedure described by Gujaral and Singh, (2002) was used to determine the water absorption and solubility index. Sample of 2.5 g was dispersed in 30 mL of distilled water at 30 °C for 30 min in a shaking water bath, and the mixture was vortexed every 5 min interval. The sample solution was then centrifuged at 4500 rpm for 15 min and the supernatant was decanted into an aluminium pan of known weight. Water absorption capacity was recorded as weight of gel (g) obtained per gram of dry ground sample. The amount of dry solids recovered after evaporating the supernatant in an oven at 100 °C overnight was expressed as percentage dry solid in the 2.5 g sample and defined as water solubility index.

#### **3.1.2.3.3 Nitrogen solubility index**

Nitrogen solubility index was determined according to the AACC Method 46-23 (AACC, 2000) with modification. About 1 g flour samples was dispersed in 20 mL of 0.1M NaCl solution at pH 7 and stirred continuously for 1h at 30 °C. The suspension was centrifuged (9154.3 x g, 15 min, and 4 °C) and the supernatant filtered through a Whatman No. 1 filter paper. The residue was re-washed twice in 10 mL of 0.1M NaCl solution at pH 7. The filtrate was frozen (-18 °C) over night and freeze-dried (13KL, Instruvac Lyophilizer, Midrand, South Africa) for 4 days. The nitrogen content of the freeze-dried sample was determined using a Dumatherm (DT, Gerhardt Konigswinter, Germany). Nitrogen solubility index was expressed as a percentage of the total nitrogen content of freeze-dried sample divided by total nitrogen content in flour sample on a dry basis

#### **3.1.2.3.4 In-vitro Protein digestibility**

A multi-enzyme method according to Vilakati *et al.* (2015) was used to determine the in-vitro protein digestibility of raw and extruded samples. Five (5) mL of multi-enzyme solution containing 1.6 mg trypsin (14,600 U/mg), 3.1 mg a-chymotrypsin (48 U/mg) and 1.3 mg peptidase (102 U/g) per mL kept in ice was added to sample suspension containing 6.25 mg protein/mL with pH adjusted to pH 8.0 with 0.1M NaOH and incubated at 37 °C. The solution was maintained at this temperature and stirred continuously while the pH drop in the suspension was recorded over 10

minutes at 1 min interval. The percentage in-vitro protein digestibility was calculated using the linear regression equation below;

$$Y = 210.46 - 18.10X$$

Where Y is the percentage in-vitro protein digestibility and X is the pH of sample suspension after 10 min hydrolysis.

### **3.1.2.3.5 *In-vitro* kinetics of starch digestibility (IVSD)**

The method according to Goñi *et al.* (1997) was used with slight modification. A sample (raw and extruded) containing 50 mg starch was used per assay and 1 mL of boiling water was added to each sample for easy dispersion before 10 mL of HCl-KCl buffer (pH 1.5) and 0.2 mL of solution containing 1 mg of pepsin (Sigma Aldrich P7000-100G) were added followed by incubation at 40 °C for 60 min with constant agitation. Ten (10 mL) of tris- maleate buffer (pH 6.9) was added and pH adjusted with 1M NaOH. The volume was made up to 25 mL with tris-maleate buffer and the 0 min aliquot of 0.1 mL was taken before the addition of 5 mL tris- maleate buffer (pH 6.9) containing 2.6IU of pancreatic  $\alpha$ -amylase with activity of 19.6 units/mg (Sigma-Aldrich A-3176) followed by incubation at 37 °C with constant shaking. Aliquots of 0.1 mL was taken at 5 min and then at intervals of 30 min until 3 h. The tubes containing the aliquots taken were placed in boiling water for 15 min to inactivate  $\alpha$ -amylase. Then, 1 mL of 0.4M sodium-acetate buffer (pH 4.75) and 90  $\mu$ L of amylogucosidase with an activity of 64.7 U/mg (Megazyme E-AMGDF) was added and incubated at 60 °C for 45 min. Glucose concentration was measured using glucose oxidase-peroxidase kit and the rate of *in-vitro* starch digestion was expressed as the percentage of the total starch digested at time intervals (0, 5, 30, 60, 90, 120 and 180 min).

### **3.1.2.3.6 Estimated glycaemic index (EGI)**

The first order equation proposed by Goñi *et al.* (1997) was used to describe the kinetics of starch hydrolysis:

$$C = C_{\infty} (1 - e^{-kt})$$

Where  $C$  is concentration at time  $t$ ,  $C_{\infty}$  is the percentage of starch hydrolyzed after 180 min,  $k$  is kinetic constant ( $\text{min}^{-1}$ ) and  $t$  is time (min). The parameters  $K$  and  $C_{\infty}$  were estimated for each

treatment based on the data obtained from the *in-vitro* hydrolysis procedure. The equation by Jaisut *et al.* (2008) was used to calculate the area under curve (AUC):

$$AUC = (C_{\infty} (t_f - t_0) - C_{\infty}/K) (1 - \exp(-K(t_f - t_0)))$$

Where  $t_f$  is the final time (180 min),  $t_0$  is the initial time (time 0). The hydrolysis index (HI) was defined as the area under the hydrolysis curve of sample divided by the corresponding area of white bread. Estimated glycaemic index was then estimated using the equation according to Goñi *et al.* (1997):

$$EGI = 39.71 + 0.549HI$$

Estimated glycaemic load (EGL) was calculated using:

$$EGL = \frac{EGI}{100} \times \text{available carbohydrate}$$

### **3.1.2.3.7 Nutritionally important starch fractions**

The enzymatic hydrolysis method of Goñi *et al.* (1997) was used to obtain the rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) fractions. The RDS was defined as the percentage of starch digested at 30 min, the SDS as the percentage of starch digested at 120 min and the RS was defined as the sum of RDS and SDS subtracted from the total starch.

### **3.1.2.3.8 Soluble and insoluble dietary fibre determination**

This was determined according to AOAC 991.43 (1995) method using the total dietary fibre megazyme kit (K-TDFR). Approximately 1 g of sample was dissolved in 40 mL of mes-tris (pH 8.2) buffer solution and thermostable  $\alpha$ -amylase with an activity of 3,000 U/ml (E-AMGDF) was added to hydrolyze starch to dextrins at 100 °C. Protease with an activity of 350 tyrosine U/ml (E-BSPRT) was used to solubilize protein. Amyloglucosidase with an activity of 3,300 U/ml (E-BLAAM) was used to hydrolyze starch fragments to glucose. The sample and enzyme mixture were filtered, and the residue was washed with ethanol and acetone to obtain the insoluble dietary fibre (IDF) portion. Four volumes of ethanol heated to 60 °C was added to the filtrate to precipitate the SDF and was left to stand for 1 h after which it was filtered. The soluble dietary fibre (SDF) residues were washed with 78%, 95% (v/v) ethanol and acetone. The IDF and SDF residues were

dried overnight at 100 °C. The SDF and IDF residues were corrected for protein and ash for the final calculation of SDF and IDF values.

### **3.1.2.3.9 Rheological Property**

The method according to D'Silva *et al.* (2011) was used to determine flow properties with slight modification. Flow properties were measured with a Physica MCR 101 Rheometer with Rheoplus software<sup>®</sup>, (Anton Paar, Ostfildern, Germany). Flour slurry containing 10% solid (w/v) was prepared and held at 50°C for 5 min to equilibrate and the slurry was transferred into the rheometer cup and maintained at 50°C. The sample was stirred with a vane over a shear rate range of 0.01 to 1000 s<sup>-1</sup> and the shear stress was determined. To describe the time independent flow behaviour, the experimental data (shear stress- shear rate) were fitted by Power law model;

$$\tau = K\dot{\gamma}^n$$

Where,  $\tau$  is shear stress (Pa),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>),  $K$  is the consistency co-efficient (Pa.s<sup>n</sup>) and  $n$  is the flow behaviour index.

### **3.1.3 Statistical Analysis**

The main effects of treatment (wheat bran addition level 0, 10 and 20%) were calculated by analysis of variance (ANOVA) using SPSS software version 23 (SPSS Inc., Armonk, NY). Means were compared using Fischer's least significant test (LSD) at 5% level of significance. The results of analysis were reported as mean obtained from triplicate experiments  $\pm$  standard deviation.

### **3.1.4 Results and discussion**

The effects of extrusion cooking and wheat bran substitution on some physico-chemical and functional properties of cassava-defatted toasted soy composite are shown in Table 3.1.1. Total starch and crude protein decreased with increasing substitution of wheat bran to cassava-defatted toasted soy composite. The crude protein, total starch and ash contents slightly increased after extrusion cooking. There was no significant difference ( $p>0.05$ ) in crude fat content of extruded products. When wheat bran substitution to cassava-defatted toasted soy composite increased from 0 to 20%, the expansion ratio decreased while bulk density increased significantly ( $p<0.05$ ).

Bulk density is controlled by degree of expansion as more expanded products have been reported to have larger and thinner cell walls which results in lower bulk density (Stojceska *et al.*, 2008).

**Table 3.1.1.** Effects of extrusion cooking and wheat bran addition on some chemical composition, physical and functional properties of cassava-defatted toasted soy composite

Treatment	Wheat bran addition (%)	Total starch (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Bulk density (g/ml)	Expansion ratio	WAC (g/g)	WSI (%)
Raw	0	52.8bc ± 0.4	18.5c ± 0.3	0.8b ± 0.1	2.6a ± 0.0	ND	ND	ND	ND
	10	52.0bc ± 1.1	18.5c ± 0.6	1.4c ± 0.1	2.8ab ± 0.0	ND	ND	ND	ND
	20	48.5a ± 0.1	17.8a ± 0.0	1.5c ± 0.0	3.1bc ± 0.2	ND	ND	ND	ND
Extrusion cooked	0	54.1c ± 0.7	19.5d ± 0.0	0.3a ± 0.1	2.9ab ± 0.1	0.26a ± 0.02	3.8c ± 0.2	2.9a ± 0.2	40.3c ± 2.4
	10	53.7c ± 0.2	18.8c ± 0.1	0.4a ± 0.0	3.0bc ± 0.1	0.30b ± 0.01	3.4b ± 0.2	4.0b ± 0.2	31.0b ± 1.4
	20	50.7ab ± 0.4	18.1b ± 0.0	0.5a ± 0.0	3.2c ± 0.1	0.32c ± 0.01	3.2a ± 0.5	4.3b ± 0.1	17.1a ± 1.2

Values are means ± standard deviations of 3 independent experiment. Values within the same column followed by different letters are significantly different (p<0.01).  
 ND = not determined



The degree of expansion of an extrudate is highly dependent on its size, number and distribution of air cells (Maskan and Altan, 2011). However, the presence of components such as dietary fibre which is found in wheat bran disrupts the extensibility of bubble films (Lu *et al.*, 1990). Dietary fibre may prevent air bubbles from expanding to their maximum potential by adhering to bubble structure and puncturing its cells causing premature rupture of cell walls thereby reducing cell extensibility with an increase in number of small broken cells (Van der Sman and Broeze, 2013).

During extrusion cooking, non- starch polysaccharides present in fibre have the ability to bind more water compared to protein and starch (Camire and King, 1991). The result of this binding is that water loss at the die end may be limited leading to reduced expansion and an increase in bulk density due to formation of less expanded air pockets (Chang *et al.*, 1998).

In a study on inclusion of fibrous jatobá fruit pulp to cassava starch in a puffed snack, Chang *et al.* (1998) reported similar results to those in this study and attributed the decrease in expansion ratio to the following; increasing levels of jatobá diluted starch content thereby reducing starch swelling ability. Also, fibrous jatobá ruptured cell walls of bubbles as they formed at the die end. Brennan *et al.* (2008a) also reported similar results in a study on inclusion of soluble and insoluble fibres into extruded breakfast cereal product and proposed that the increase in bulk density as soluble and insoluble fibre ratio increased may be due to formation of extrudates with denser structure of reduced average cell size and increased cell wall holes.

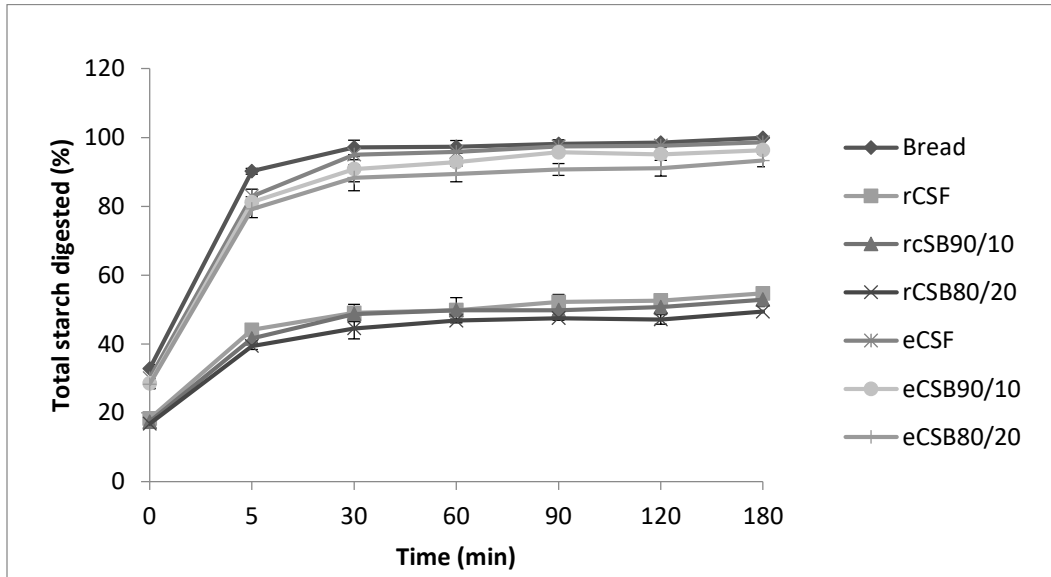
The effects of wheat bran addition on water absorption capacity and water solubility index of cassava-defatted toasted soy composite is shown in Table 3.1.1. The highest water absorption value of 4.3 g/g was observed in the composite with 20% wheat bran while the lowest value of 2.9 g/g was the composite with 0% wheat bran substitution. The substitution with wheat bran significantly ( $p < 0.05$ ) lowered the water solubility index of cassava defatted toasted soy composite. Dietary fibre has been reported to have high water absorption (Badrie and Mellowes, 1991).

The water solubility index of extruded cassava-defatted toasted soy composite with 0% wheat bran was 40.3% while the composite with 20% wheat bran was 17.1%. The presence of compounds having low molecular weight has been attributed to increase in water solubility index after extrusion cooking (Ding *et al.*, 2005). Extrusion cooking brings about starch degradation with release of low molecular weight dextrans (Maskan and Altan, 2011), and this may have raised the water solubility index of cassava-defatted toasted soy composite with 0% wheat bran. Starch content decreased as a result of wheat bran addition, thus soluble compounds released from extrusion cooking also decreased hence the lower water solubility index of extrudates with wheat bran.

The results of *in-vitro* protein digestibility and nitrogen solubility index are presented in Table 3.1.2. Extrusion cooking led to above 60% decrease in nitrogen solubility index and a slight reduction in *in-vitro* protein digestibility of extrudates. Extrusion cooking promotes covalent and non-covalent interactions among soy proteins and possibly with other food components which could result in reduction in nitrogen solubility index and *in-vitro* protein digestibility (Day and Swanson, 2013). In extrusion cooking, protein structures are disrupted by unfolding followed by their re-organization and polymerization through formation of di-sulphide bonds. Di-sulphide bonding can result in reduction in solubility (Steel *et al.*, 2012; Arêas, 1992). Prudêcio-ferreira and Arêas, (1993) reported an increase in soy protein insolubilization after extrusion cooking and they attributed this to high temperature in the extruder which facilitated di-sulphide linkages and non-covalent interactions mainly hydrophobic and electrostatic interactions. Protein insolubilization during heating have been associated with decrease in protein digestibility (Kaczmarek *et al.*, 2013). Also, protein denaturation during extrusion cooking exposes previously hidden amino acid residues thus making them free to react with reducing sugars (Camire, 2001). Muoki *et al.* (2012) reported a similar decrease in *in-vitro* protein digestibility after extrusion cooking and the decrease was attributed to the loss of a free amino acid (lysine) during extrusion cooking process due to Maillard reaction with reducing sugars. Protein digestibility is an important attribute used to assess protein quality and nutritional quality of foods. Protein insolubilization during extrusion cooking have been associated with decrease in protein digestibility (Kaczmarek *et al.*, 2013).

The effects of wheat bran substitution on starch digestibility before and after extrusion cooking on cassava-defatted toasted soy composite are shown in Figure 3.1.1. The *in-vitro* starch digestibility parameters were generally higher in extrudates than in starting raw materials. The total starch digested for cassava-defatted toasted soy flour was significantly ( $p < 0.05$ ) reduced by wheat bran substitution as shown in Figure 3.1.1. The kinetic parameters which describe the process of starch

hydrolytic digestion are shown in Table 3.1.3. The parameter  $C_{\infty}$  indicates the equilibrium concentration attained after 180 min of hydrolysis and K (kinetic constant) gives an indication of intrinsic susceptibility of starch to amylolytic digestion in the product. The substitution of wheat bran to cassava- defatted toasted soy flour significantly ( $p < 0.05$ ) lowered the  $C_{\infty}$ , hydrolysis index (HI), estimated glycaemic index (EGI) and estimated glycaemic load (EGL) of cassava-defatted toasted soy composite.



rCSF is raw cassava-toasted defatted soy flour  
 rcSB90/10 is raw cassava-toasted defatted soy flour: wheat bran (90:10)  
 rCSB80/20 is raw cassava-toasted defatted soy flour: wheat bran (80:20)  
 eCSF is extruded cassava-toasted defatted soy flour  
 eCSB90/10 is extruded cassava-toasted defatted soy flour: wheat bran (90:10)  
 eCSB80/20 is extruded cassava-toasted defatted soy flour: wheat bran (80:20)  
 White bread was used as reference

**Figure 3.1.1.** Effects of addition of wheat bran and extrusion cooking on the kinetics of starch digestion of cassava-defatted toasted soy composite

**Table 3.1.2.** Effects of wheat bran addition and extrusion cooking on IVPD, NSI starch fractions, soluble, insoluble and total dietary fibre contents of cassava-defatted toasted soy composite

Treatment	Wheat bran addition (%)	IVPD <sup>1</sup> (%)	NSI <sup>2</sup> (%)	RDS <sup>3</sup> (%)	SDS <sup>4</sup> (%)	RS <sup>5</sup> (%)	IDF <sup>6</sup> (%)	SDF <sup>7</sup> (%)	TDF <sup>8</sup> (%)
Raw	0	91.4c ± 0.1	50.8e ± 2.4	ND	ND	ND	8.4ab ± 0.1	3.2a ± 0.3	11.6a ± 0.4
	10	91.0bc ± 0.3	46.7d ± 1.2	ND	ND	ND	10.2c ± 0.2	4.2b ± 0.2	14.3b ± 0.4
	20	90.4ab ± 0.1	46.1d ± 1.4	ND	ND	ND	13.3d ± 0.1	4.9c ± 0.1	18.2c ± 0.1
Extrusion cooked	0	89.6a ± 0.5	17.1c ± 0.4	72.7c ± 0.8	26.1b ± 0.9	0.8a ± 0.0	7.2a ± 0.3	4.3b ± 0.2	11.5a ± 0.5
	10	89.2ab ± 0.4	12.4b ± 0.8	63.6b ± 1.7	35.6c ± 1.7	0.9a ± 0.0	9.3bc ± 0.9	5.1c ± 0.0	14.4b ± 0.9
	20	88.8a ± 0.3	10.8a ± 1.2	53.3a ± 1.9	45.0d ± 1.7	1.2b ± 0.1	12.3d ± 0.1	5.8d ± 0.1	18.1c ± 0.0
Bread		ND	ND	77.8d ± 0.6	20.4a ± 0.7	1.1b ± 0.0	ND	ND	ND

Values are means ± standard deviations of 3 independent experiment. Values within the same column followed by different letters are significantly different (p<0.001)

White bread was used as reference. ND = not determined

<sup>1</sup>IVPD = in-vitro protein digestibility

<sup>2</sup>NSI = nitrogen solubility index

<sup>3</sup>RDS = rapidly digested starch

<sup>4</sup>SDS = slowly digested starch

<sup>5</sup>RS = resistant starch

<sup>6</sup>IDF = insoluble dietary fibre

<sup>7</sup>SDF = soluble dietary fibre

<sup>8</sup>TDF = total dietary fibre

The substitution with wheat bran significantly ( $p < 0.05$ ) reduced the rapidly digestible starch (RDS) of cassava-defatted toasted soy composite as shown in Table 3.1.2. The slowly digestible starch (SDS) of the extrudate containing 20% wheat bran was higher than other samples (Table 3.1.2). There was no statistical ( $p > 0.05$ ) difference in the resistant starch (RS) of all products with and without wheat bran substitution.

The starting raw materials before extrusion cooking exhibited the lowest digestion curves. This is in agreement with findings which showed that native starches have a high degree of crystallinity with tightly packed  $\alpha$ -glucan chains which makes them resistant to amylolytic hydrolysis (Patel *et al.*, 2014). Due to the tight packing of  $\alpha$ -glucan chains in native starches, the ability of glucan residues to form hydrogen bonds with specific amino acid side chains within  $\alpha$ -amylase active site is limited and this impedes on starch hydrolysis (Imberty, Chanzy and Perez, 1998).

Extrusion cooking increased starch digestibility of extrudates and this could be due to gelatinization and depolymerization of starch which occurred during the cooking process thereby making starch more readily accessible for enzymatic hydrolysis. Gelatinization of starch facilitates contact between substrate and digestive enzymes thus increasing the chances of hydrolysis (Alonso, Aguirre and Marzo, 2000).

The reduction observed in starch digestibility and EGI (Table 3.1.3) with increasing wheat bran substitution might be due to fibre interaction which made less water available for starch transformation during extrusion cooking. The addition of dietary fibre has been shown to affect carbohydrate digestibility. Brennan *et al.* (2008a) illustrated that carbohydrate digestibility was decreased after wheat bran was added to wheat flour. Yağcı and Göğüş (2010) also reported a reduction in starch digestibility when various food by-products were added to rice grits and durum flour. These authors suggested that fibre may directly hinder digestion by limiting contact between starch and digestive enzymes. Similarly, Brennan *et al.*, (1996) suggested that gelatinized and disrupted starch granule could be within soluble fibre matrix and this would reduce starch digestibility due to limited access by starch degrading enzymes to the substrate.

**Table 3.1.3.** Effects of extrusion cooking and wheat bran addition on the *in-vitro* starch digestibility kinetic parameters and *in-vitro* protein digestibility of cassava-toasted defatted soy composite

Treatment	Wheat bran addition level (%)	C <sub>∞</sub> (%)	K (min <sup>-1</sup> )	HI (%)	EGI	EGL
Raw	0	54.7a ± 1.6	0.02a ± 0.01	52.4a ± 2.4	68.5a ± 1.5	30.2c ± 0.7
	10	51.0a ± 2.1	0.02a ± 0.01	50.9a ± 1.7	67.7a ± 1.0	26.0b ± 0.4
	20	49.4a ± 0.4	0.02a ± 0.01	47.6a ± 0.5	65.9a ± 0.6	21.4a ± 0.7
Extrusion cooked	0	98.6bc ± 0.5	0.03a ± 0.01	98.6b ± 1.2	93.8b ± 1.0	44.7c ± 0.6
	10	96.3b ± 2.1	0.03a ± 0.02	94.9a ± 1.7	91.8a ± 0.7	39.8b ± 1.2
	20	92.8a ± 1.0	0.02a ± 0.01	91.9a ± 1.9	90.2a ± 0.4	33.0a ± 1.1
White bread		99.7c ± 2.4	0.03a ± 0.01	100.0b ± 0.0	94.6b ± 0.8	63.4d ± 0.0

Values are means ± standard deviations of 3 independent experiment. Values within the same column followed by different letters are significantly different (p<0.05)

C<sub>∞</sub> = % starch digested after 180 min

HI, k and GI were calculated from the equation:  $AUC = \frac{C_{\infty}}{k} (1 - \exp(-k(t - t_{f0})))$  proposed by Goni *et al.* 1997

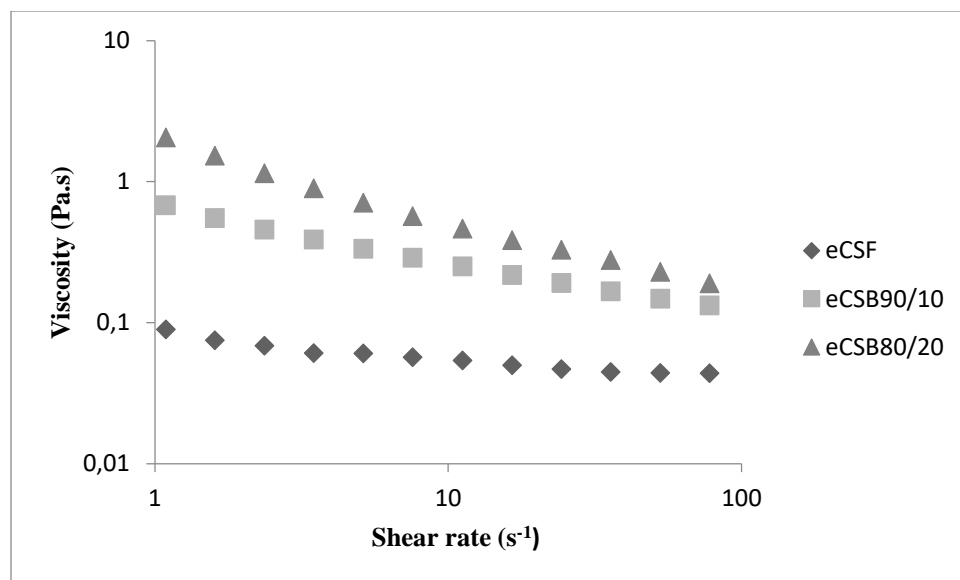
EGL per g solids was estimated as EGI\*S/100 where S is starch content (g/100g solids)

White bread was used as the reference for calculating EGI

The high RDS observed in the extrudate without wheat bran (Table 3.1.2) indicates that all the starch was practically hydrolysed within the first 30 min. The high portions of SDS recorded for cassava-defatted toasted soy composite with 20% wheat bran substitution also suggests that fibre may have limited starch digestion by forming a physical barrier around starch granule which delayed enzyme accessibility to the substrate (Brennan *et al.*, 1996; Villemejeane *et al.*, 2015).

There was a positive correlation ( $R^2 = 0.96$ ) for RDS and expansion ratio. This may further show that the extrudates had different levels of starch degradation. The control which contained no wheat bran had the highest RDS and was also more expanded as starch is the major component responsible for expansion and presence of other food components such as fibre would limit the rate of expansion and starch depolymerization (Dhingra *et al.*, 2012).

Extrusion cooking can make dietary fibre more soluble due to changes in structural and molecular properties (Dhingra *et al.*, 2012). These changes can lead to increase in viscosity and can be related to lower starch digestibility (Kim and White, 2013). The viscous properties of extrudates were thus determined. Upon extrusion, high apparent viscosity was observed with increasing wheat bran substitution (Figure 3.1.2) from 10 to 20%, while cassava-defatted toasted soy extrudate with no wheat bran recorded the lowest viscosity at all shear rates. Consistency index ( $K$ ) was also significantly higher ( $p < 0.05$ ) in the 20% wheat bran sample compared to the 0 and 10% wheat bran levels (0.1 - 0.5 Pa.s; Table 3.1.4). The  $n$ -values of all the extrudates were statistically ( $p > 0.05$ ) different and all the extrudates exhibited a shear thinning behaviour with ( $n < 1$ ). At  $50\text{s}^{-1}$ , viscosity increased with increasing levels of wheat bran substitution.



eCSF is extrusion cooked cassava-defatted soy flour  
eCSB90/10 is extrusion cooked cassava-defatted soy flour with 10% wheat bran  
eCSB80/20 is extrusion cooked cassava-defatted soy flour with 20% wheat bran

**Figure 3.1.2.** Effect of addition of wheat bran on apparent viscosity of cassava-toasted defatted soy composite at 50°C as a function of shear

The highest viscosity (444 mPa.s) at 50 s<sup>-1</sup> was recorded for 20% wheat bran substitution level to cassava-defatted toasted soy composite, but the composite with no wheat bran had the least viscosity (156 mPa.s). It was also observed that wheat bran substitution lowered the hysteresis significantly (p<0.05) even though the wheat bran which contained composites had higher viscosity.

**Table 3.1.4.** Effects of wheat bran addition on power law (*k* and *n*-values) parameters, viscosity at 50-shear and hysteresis area of cassava-defatted toasted soy composite

Treatment	<i>K</i> -value (Pa.s) <sup>a</sup>	Powerlaw index (n-value)	$\eta_{a, 50}$ (mPa.s)	Hysteresis area Pa/s
Extrusion cooked cassava-defatted toasted soy flour	0.08a ± 0.00	0.91c ± 0.01	44.4a ± 0.8	6598c ± 7
With 10% wheat bran	0.24b ± 0.01	0.88b ± 0.01	145.3b ± 3.1	5131b ± 19
With 20% wheat bran	0.47c ± 0.00	0.76a ± 0.00	239.0c ± 6.3	1765a ± 9

Values are means ± standard deviations of 3 independent experiment. Values within the same column followed by different letters are significantly different (p<0.001)



The increase observed in viscosity of composites which contained wheat bran could be a function of solubilization of insoluble dietary fibre portions during extrusion cooking or lower starch depolymerization. In a study on the effect of thermal processing (microwave and forced air oven heating) on wheat and barley flour, Căprită *et al.* (2011) attributed increase in water extract viscosity of barley and wheat flour to redistribution of the ratio of insoluble to soluble dietary fibre after heating.

The results of the current study show that substitution of wheat bran increased the dietary fibre profile of cassava defatted toasted soy composite (Table 3.1.2). Extrusion cooking decreased the IDF with a subsequent increase in SDF. The 20% wheat bran incorporation level exhibited the highest dietary fibre content.

The decrease in IDF and subsequent increase in SDF could be due to mechanical fragmentation of IDF contents during extrusion cooking. The high temperature and extreme shearing actions involved during extrusion cooking promotes fibre fragmentation and could have led to break down of IDF into smaller soluble units. Similarly, increased SDF contents of corn-wheat extrudates after extrusion cooking was reported by Sobota *et al.* (2010) and this was attributed to depolymerization of IDF fraction into soluble fractions. Rashid *et al.* (2015) also attributed increase in SDF and reduction in IDF in wheat bran extrudates to reduction in molecular weight of larger molecules which resulted in smaller soluble fragments.

Wheat bran polysaccharide contains about 70% arabinoxylan, 24% cellulose and 6%  $\beta$ -glucan (Ralet *et al.*, 1990). Arabinoxylan are complex polymers which contains  $\beta$ -1,4 linked D-xylopyranosyl backbone chain substituted with  $\alpha$ -L-arabinofuranose residues at the C-(O)-2 and/or C-(O)-3 positions (Fincher and Stone, 1986) and they are categorized into soluble water-extractable and insoluble water-unextractable arabinoxylan. Insoluble arabinoxylan contain a highly substituted population of arabinose while the soluble arabinoxylan consists of a lowly substituted population of arabinose on the xylan chain (Appeldoorn *et al.*, 2010). Extrusion has been found to increase the soluble dietary fibre due to break down of polysaccharide glycosidic bonds leading to an increase in oligosaccharides (Esposito *et al.*, 2005). Thus, it may be suggested that during extrusion insoluble arabinoxylan are broken down to form soluble fibres. An increase in arabinoxylan solubility has been reported to increase viscosity (Courtin and Delcour, 2001).

Soluble fibres form viscous fluid with water and increase in viscosity is dependent on the concentration of polysaccharides and their chemical composition (Schneeman, 2008). The increase in viscosity as a result of fibre fragmentation and solubilization is a physiological benefit. High viscosity in the gut lumen can significantly reduce post prandial glucose response and nutrient levels by reducing glucose absorption (Dartois *et al.*, 2010). SDF acts like a sponge and absorbs water in the intestine where it mixes with the food to form an entangled network and thereby retard rate of absorption of glucose (Dartois *et al.*, 2010). It is suggested that under the condition of increased viscosity, there is also less interactions between substrates and digestive enzymes hence the reduction in starch digestion (Kim and White, 2013).

### **3.1.5 Conclusions**

The reduction observed in the starch digestibility and EGI of cassava-defatted toasted soy composite substituted with wheat bran is as a result of changes in functional and rheological properties of wheat bran during extrusion cooking. The fragmentation and depolymerization of IDF in wheat bran to produce more SDF will promote increase in viscosity of cassava-defatted toasted soy composite and the increase in viscosity can delay the digestion of starch and absorption of glucose. This study therefore demonstrates that dietary fibre could be added to locally available food crops to produce food products suitable for the management of nutritional related diseases such as type 2 diabetes.

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### **3.2 EFFECTS OF EXTRUSION COOKING AND GRAPE POMACE ADDITION ON THE NUTRITIONAL, FUNCTIONAL AND POTENTIAL HEALTH PROMOTING PROPERTIES OF CASSAVA-SOY COMPOSITE.**

#### **Abstract**

Cassava- soy composite was extruded with grape pomace at 0, 10 and 20% addition levels. Some of the nutritional, functional, rheological properties, total phenolic contents and anti-oxidant activity of the extruded products were analysed. Extrusion cooking significantly led to a significant ( $p<0.05$ ) increase in starch digestibility of composites but the addition of grape pomace lowered the rate and extent of starch hydrolysis. The water absorption capacity of composites decreased while the solubility index increased with increasing addition of grape pomace. FTIR-ATR spectra showed an increase in  $\beta$ -sheets formation as the addition of grape pomace increased. The composite with 20% grape pomace had the lowest viscosity and all composites exhibited a shear thinning behaviour. Extrusion cooking led to depolymerization of dietary fibre and redistribution of insoluble dietary fibre to soluble dietary fibre ratio. The total phenolic contents and anti-oxidant activity of cassava-soy composite was significantly ( $p<0.05$ ) increased with the addition of grape pomace. The results indicate that grape pomace can be added to starch-rich foods with extrusion cooking to produce instant products with potential health promoting properties.

### 3.2.1 Introduction

Grape pomace is a by-product of wine and grape juice production. It is estimated that more than half of grapes cultivated worldwide are used in the wine industry (Martin-Carrón *et al.*, 2000) leaving behind a vast proportion of waste (Dwyer, Hosseinian, and Rod, 2014). Grape pomace comprises of the skin, pulp and seed (Sousa *et al.*, 2014). Grape pomace is mostly used in animal feed (Baumgärtel *et al.*, 2007) and as fertilizer (Bekhit *et al.*, 2016). It is characterized with having a high dietary fibre content (Kumar *et al.*, 2010) and phenolic compounds (Altan *et al.*, 2008). The red grape pomace contains between 51.09 – 56.31% total dietary fibre (Ding *et al.*, 2011) and about 28 – 35% polyphenols (Pascariu *et al.*, 2015). The phenolic compounds are mainly condensed tannins, anthocyanins and resveratrol, and they are known to possess anti-oxidant and radical scavenging properties (Yu and Ahmedna, 2013). The dietary fibre components of grape pomace are pectin, cellulose and hemicelluloses (Deng *et al.*, 2011)

Dietary fibre and phenolic compounds have been proven to be of immense health benefits (Rudra *et al.*, 2015). The benefits include their ability to influence carbohydrate metabolism (Rudra *et al.*, 2015). Dietary fibre is classified into soluble and insoluble dietary fibre. Soluble dietary fibre is mostly associated with ability to influence post-prandial glucose response due to their viscous nature by slowing down gastric emptying (Yu *et al.*, 2014), lower rate of starch hydrolysis (Hardacre *et al.*, 2015), slow down diffusion of starch hydrolysis products to the intestinal gastric mucosa and reduction in glucose absorption (Villemejeane *et al.*, 2016). Insoluble dietary fibre adds bulk to diet and reduces faecal transit time (Dhingra *et al.*, 2012).

Several authors have reported that phenolic compounds can influence carbohydrate hydrolysis (Hanhineva *et al.*, 2010; Zhu, 2015). A variety of phenolic compounds have been shown to inhibit the activities of  $\alpha$ -amylase and/or  $\alpha$ -glucosidase. The inhibitory phenolic compounds include flavonoids (flavonones, catechins, anthocyanins, flavonols, isoflavones, and flavones), phenolic acids and tannins (proanthocyanidins and ellagitannins). Phenolic compounds are postulated to bind to active or secondary sites of digestive enzymes (Barrett *et al.*, 2013) and/or bind to substrate thus reducing starch hydrolysis (Zaj acz *et al.*, 2007).

Extrusion cooking is used to better utilize food by-products (Stojceska *et al.*, 2009) and produce dietary fibre enriched foods (Brennan *et al.*, 2008a). Extrusion cooking is an efficient and versatile processing technique which combines high temperature and pressure within a short time to cook food materials (Ilo *et al.*, 2000). Extrusion cooking is advantageous because of its ability to blend varieties of ingredients to produce a wide range of food products with improved nutritional qualities, many of which cannot be produced easily by any other process (Obatolu *et al.*, 2005). Food ingredients and their components undergo several transformations during extrusion cooking giving rise to extrudates with improved functionalities. The changes in the functional properties is related to the nutritional properties of extrudates (Alam *et al.*, 2016).

There have been studies carried out on improving on the nutritional properties of extrudates by addition of fruit by-products. Fruits blend waste (orange peel, grape seed and tomato pomace) was extruded with rice grits by Yagci and Gogus, (2009). The authors reported that starch digestibility decreased with increasing addition of the fruit blend waste. This was attributed to the presence of non-starch polysaccharides in the fruit blend waste that limited contact between digestive enzyme and starch. In another study, Dehghan-Shoar *et al.*, (2011) extruded tomato waste (tomato skin, seed and paste) and corn grits. The authors reported a decrease in starch digestibility of extrudates with the highest ratio of tomato waste. It was suggested that this might be due to complex formation between starch and pectin and the starch-pectin complex might have limited accessibility of  $\alpha$ -amylase to starch for digestion.

The effect of added functional ingredients on nutritional and quality parameters of extrudates is important in order to create a platform for developing new food products from food by-products. The objective of this study was therefore to investigate the effects of extrusion cooking and grape pomace addition at 0, 10 and 20% level on some of the nutritional, functional, and potential health promoting properties of cassava- soy composite.

### **3.2.2 Materials and methods**

#### **3.2.2.1 Raw materials**

The raw materials cassava and defatted toasted soy flour described in section 3.1.1.2 were used in this experiment. Grape pomace was kindly donated by Brenn-O-Kem (Pty) Ltd (Wolseley, South Africa).

### **3.2.2.2 Extrusion cooking**

Cassava and defatted toasted soy were uniformly mixed in a ratio of 65 to 35% (w/w db) respectively. Grape pomace was added at 0, 10 and 20% (w/w) levels and mixed thoroughly with the cassava and soy composite. A co-rotating twin screw extruder (TX 32, CFAM, Potchefstroom, South Africa) was used in this study. The barrel comprised of five heating zones set at 60/80/100/140/140 °C respectively. Moisture was fed into the system at a dosing rate of 3 l/h and feed rate was 25 kg/h. A die opening of 3 mm was used and the screw speed was maintained at 200 rpm. Extrudates were immediately dried in an oven at 90 °C for 5 min and ground with an analytical mill (A11, IKA, Staufen, Germany). The ground extruded products were kept in plastic sample bottles and refrigerated at 4 °C in preparation for analysis.

### **3.2.2.3 Analyses**

#### **3.2.2.3.1 Physico-chemical characteristics**

Expansion ratio was determined as the diameter of extrudates divided by the diameter of the die exit (3 mm) (Gujska and Khan, 1991). Twenty extrudates were measured for each treatment. The Bulk density ( $\text{g/cm}^3$ ) of the extrudate was expressed as the weight of the extrudate divided by its volume (Cauvain and Young, 2009)

Proximate analysis in terms of ash and crude fat were determined according to the AOAC methods 942.05 and 920.39A (AOAC, 2000) respectively. Protein ( $\text{N} \times 6.25$ ) was determined by Dumas combustion method according to the AACC International (2000) crude protein combustion method 46 – 30. Total starch was determined using the method described by McCleary, Solah and Gibson (1994) Commercial enzymes (thermostable  $\alpha$ -amylase and amyloglucosidase) from Megazyme (K-TSTA 07/11) (Megazyme International, Bray, Ireland) were used to hydrolyse starch to glucose. Glucose was then quantified colorimetrically by the glucose oxidase-peroxidase reaction and the absorbance was read at 510 nm. Total starch was then expressed as starch proportion (%) of total sample weight and correction was made for moisture to determine starch on a dry weight basis.

#### **3.2.2.3.2 Water absorption capacity and Water solubility index**

The procedure described by Gujaral and Singh, (2002) was used to determine the water absorption and solubility index. Raw and extruded flours (2.5 g) were dispersed in 30 mL of distilled water

at 30 °C for 30 min in a shaking water bath, and the mixture was vortexed every 5 min interval. The sample solution was then centrifuged at 4500 rpm for 15 min and the supernatant was decanted into an aluminium pan of known weight. Water absorption capacity was recorded as weight of pellet (g) obtained per gram of dry ground sample. The amount of dry solids recovered after evaporating the supernatant in an oven at 100 °C overnight was expressed as percentage dry solid in the 2.5 g sample and defined as water solubility index.

#### **3.2.2.3.3 Nitrogen solubility index**

Nitrogen solubility index was determined according to the AACC Method 46-23, (2000) with modification. About 1g flour samples was dispersed in 20 mL of 0.1M NaCl solution at pH 7 and stirred continuously for 1h at 30 °C. The suspension was centrifuged (9154.3 x g, 15min, and 4 °C) and the supernatant filtered through a Whatman No. 1 filter paper. The residue was re-washed twice in 10mL of 0.1M NaCl solution at pH 7. The filtrate was frozen (-18 °C) over night and freeze-dried (13KL, Instruvac Lyophilizer, Midrand, South Africa) for 4 days. The nitrogen content of the freeze-dried sample was determined using a Dumatherm (DT, Gerhardt Konigswinter, Germany). Nitrogen solubility index was expressed as a percentage of the total nitrogen content of freeze-dried sample divided by total nitrogen content in flour sample on a dry basis

#### **3.2.2.3.4 *In-vitro* Protein digestibility**

A multi-enzyme method according to Vilakati *et al.* (2015) was used to determine the *in-vitro* protein digestibility of raw and extruded samples. Five (5) mL of multi-enzyme solution containing 1.6 mg trypsin (14,600 U/mg), 3.1 mg a-chymotrypsin (48 U/mg) and 1.3 mg peptidase (102 U/g) per mL kept in ice was added to sample suspension containing 6.25 mg protein/mL with pH adjusted to pH 8.0 with 0.1M NaOH and incubated at 37 °C. The solution was maintained at this temperature and stirred continuously while the pH drop in the suspension was recorded over 10 minutes at 1 min interval. The percentage *in-vitro* protein digestibility was calculated using the linear regression equation;

$$Y = 210.46 - 18.10X$$

Where Y is the percentage *in-vitro* protein digestibility and X is the pH of sample suspension after 10 min hydrolysis (Vilakati *et al.* 2015)

### 3.2.2.3.5 FTIR-ATR Spectroscopy

The secondary structure of protein secondary structure of samples (raw and extruded) was determined by FTIR spectroscopy as described by Anyango *et al.*, (2011). Samples were stored in a desiccator containing silica gel for 72 h at ambient temperature to ensure minimal moisture content before spectroscopic analysis. Absorbance spectra were recorded using a Perkin Elmer Spectrum 100 with a universal attenuated total reflectance accessory (Perkin Elmer, Connecticut, USA). Samples were scanned over a wavelength range of 4000  $\text{cm}^{-1}$  to 600  $\text{cm}^{-1}$  and averaged from a total of 32 scans with a resolution of 8  $\text{cm}^{-1}$ . Spectra measurements were corrected for background. The FTIR-ATR spectra were deconvoluted using Lorentzian filter with a resolution enhancement factor of 2 and a bandwidth of 8  $\text{cm}^{-1}$ . A rubber band correction of 64 baseline points was used for correcting absorbance spectra. The relative proportions of  $\alpha$ -helical conformations and the  $\beta$ -conformation were determined using the equation below. The  $\alpha/\beta$  ratio were calculated:

$$\% \alpha\text{-helical conformation} = \frac{\text{Abs } \alpha\text{-helix peak}}{\text{Abs } \alpha\text{-helix} + \beta\text{-sheet peak}} \times 100\%$$

$$\% \beta\text{-sheet conformation} = \frac{\text{Abs } \beta\text{-sheet peak}}{\text{Abs } \alpha\text{-helical peak} + \text{Abs } \beta\text{-sheet peak}} \times 100\%$$

$$\alpha:\beta \text{ ratio} = \frac{\% \alpha\text{-helical conformation}}{\% \beta\text{-sheet conformation}}$$

Where:

Abs  $\alpha$ -helix peak = absorbance at  $\approx 1647 \text{ cm}^{-1}$  after baseline correction (Anyango *et al.*, 2011)

Abs  $\beta$ -sheet peak = Absorbance at  $\approx 1620 \text{ cm}^{-1}$  after baseline correction (Anyango *et al.*, 2011).

### 3.2.2.3.6 *In-vitro* kinetics of starch digestibility (IVSD)

The method according to Goñi *et al.* (1997) was used with slight modification. A sample (raw and extruded) containing 50 mg starch was used per assay and 1 mL of boiling water was added to each sample for easy dispersion before 10 mL of HCl-KCl buffer (pH 1.5) and 0.2 mL of solution containing 1 mg of pepsin (Sigma Aldrich P7000-100G) were added followed by incubation at 40  $^{\circ}\text{C}$  for 60 min with constant agitation. Tris-maleate (10 mL) at pH 6.9 was added and pH adjusted with 1M NaOH. The volume was made up to 25 mL with tris-maleate buffer and the 0 min aliquot of 0.1 mL was taken before the addition of 5 mL tris- maleate buffer (pH 6.9) containing 2.6IU of

pancreatic  $\alpha$ -amylase with activity of 19.6 units/mg (Sigma-Aldrich A-3176) followed by incubation at 37 °C with constant shaking. Aliquots of 0.1 mL was taken at 5 min and then at intervals of 30 min until 3 h. The tubes containing the aliquots taken were placed in boiling water for 15 min to inactivate  $\alpha$ -amylase. Then, 1 mL of 0.4M sodium-acetate buffer (pH 4.75) and 90  $\mu$ L of amylogucosidase with an activity of 64.7 U/mg (Megazyme E-AMGDF) was added and incubated at 60 °C for 45 min. Glucose concentration was measured using glucose oxidase-peroxidase kit and the rate of in-vitro starch digestion was expressed as the percentage of the total starch digested at time intervals (0, 5, 30, 60, 90, 120 and 180 min).

### 3.2.2.3.7 Estimated glycaemic index

The first order equation proposed by Goñi *et al.*, (1997) was used to describe the kinetics of starch hydrolysis:

$$C = C_{\infty}(1 - e^{-kt})$$

Where C is concentration at time t,  $C_{\infty}$  is the percentage of starch hydrolyzed after 180 min, k is kinetic constant ( $\text{min}^{-1}$ ) and t is time (min). The parameters K and  $C_{\infty}$  were estimated for each treatment based on the data obtained from the *in-vitro* hydrolysis procedure. The equation by Jaisut *et al.*, (2008) was used to calculate the area under curve (AUC):

$$AUC = C_{\infty} (t_f - t_0) - \left(\frac{C_{\infty}}{K}\right) \left(1 - \exp\left(-k(t_f - t_0)\right)\right)$$

Where  $t_f$  is the final time (180 min),  $t_0$  is the initial time (time 0). The hydrolysis index (HI) was defined as the area under the hydrolysis curve of sample divided by the corresponding area of white bread. Estimated glycaemic index was then estimated using the equation according to Goñi *et al.*, (1997):

$$EGI = 39.71 + 0.549HI$$

Estimated glycaemic load = estimated glycaemic index/100  $\times$  Available carbohydrate

### 3.2.2.3.8 Nutritionally important starch fractions

The enzymatic hydrolysis method of Goñi *et al.*, (1997) was used to obtain the rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) fractions. The RDS was



defined as the percentage of starch digested at 30 min, the SDS as the percentage of starch digested at 120 min and the RS was defined as the sum of RDS and SDS subtracted from the total starch.

### **3.2.2.3.9 Soluble and insoluble dietary fibre determination**

This was determined according to AOAC 991.43, (1995) method using the total dietary fibre megazyme kit (K-TDFR). A sample of 1 g of sample was dissolved in 40 mL of mes-tris (pH 8.2) buffer solution and thermostable  $\alpha$ -amylase with an activity of 3,000 U/ml (E-AMGDF) was added to hydrolyze starch to dextrans at 100 °C. Protease with an activity of 350 tyrosine U/ml (E-BSPRT) was used to solubilize protein. Amylogucosidase with an activity of 3,300 U/ml (E-BLAAM) was used to hydrolyze starch fragments to glucose. The sample and enzyme mixture were filtered, and the residue was washed with ethanol and acetone to obtain the insoluble dietary fibre (IDF) portion. Four volumes of ethanol heated to 60 °C was added to the filtrate to precipitate the SDF and was left to stand for 1 h after which it was filtered. The soluble dietary fibre (SDF) residues were washed with 78%, 95% (v/v) ethanol and acetone. The IDF and SDF residues were dried overnight at 100 °C. The SDF and IDF residues were corrected for protein and ash for the final calculation of SDF and IDF values.

### **3.2.2.3.10 Dynamic Viscosity**

The method according to D'Silva, Taylor and Emmambux, (2011) was used to determine flow properties with slight modification. Flow properties were measured with a Physica MCR 101 Rheometer with Rheoplus software<sup>®</sup>, (Anton Paar, Ostfildern, Germany). Flour slurry containing 10% solid (w/v) was prepared and held at 50 °C for 5 min to equilibrate and the slurry was transferred into the rheometer cup and maintained at 50 °C. The sample was stirred with a vane over a shear rate range of 0.01 to 1000 s<sup>-1</sup> and the shear stress was determined. To describe the time independent flow behaviour, the experimental data (shear stress-shear rate) were fitted by Power law model;

$$\tau = k\dot{\gamma}^n$$

Where,  $\tau$  is shear stress (Pa),  $\dot{\gamma}$  is the shear rate (s<sup>-1</sup>),  $K$  is the consistency co-efficient (Pa.s<sup>n</sup>) and  $n$  is the flow behaviour index.

#### **3.2.2.3.11 Particle size determination by wet sieving**

Approximately 10% (w/v) sample suspension was prepared with distilled water and passed through a stack of sieves with aperture sizes (500, 212, 180, 75, 38  $\mu\text{m}$ ). The residue retained on each sieve was dried at 60°C to constant weight in an oven. The dried residue was expressed as percentage mass retained and the smallest fraction was calculated as difference between initial sample weight and sum of residues retained in sieves.

#### **3.2.2.3.12 Preparation of extracts for total phenolic content and antioxidant capacity assays**

The method described by Kayitesi (2013) was used to prepare extracts from raw and extruded cassava- soy composite substituted with 0, 10 and 20% grape pomace using acidified methanol (1% conc. HCl in methanol) as solvent. Each sample (3 g) was extracted with 30 ml of solvent in three steps; 10 ml of solvent was initially added to 3 g of sample in a conical flask, stirred for 3 h to allow diffusion of phenolics from cellular matrix after which they were centrifuged at 3500 rpm for 10 min at ambient temperature (25 °C). The supernatant was decanted and the residue was rinsed with 10 ml of solvent, stirred for 20 min and centrifuged using conditions described earlier. The supernatant was decanted, and the rinsing was repeated. The supernatants recovered were stored in an air tight glass bottle covered with aluminium foil and stored in the cold room at -20 °C within 1 week of analysis.

#### **3.2.2.3.13 Total phenolics determination**

The total phenolics content of extracts of cassava- soy composite with and without grape pomace was determined spectrophotometrically using the Folin Ciocalteu procedure modified for 96-well microplate described by Apea-Bah *et al.*, (2014). Extracts or catechin standard were dissolved in 1M HCl in methanol. About 18.2  $\mu\text{L}$  was added to microplate, after which 36.4  $\mu\text{L}$  of 10% (v/v) Folin-Ciocalteu reagent in water added. Thereafter, 145.4  $\mu\text{L}$  of 700 mM sodium carbonate was added and the plate incubated for 2 h in the dark at 25 °C. A standard catechin calibration curve was obtained by using concentrations of 0.1 to 0.5 mg/mL in acidified methanol. The absorbance of the extracts and catechin standards were read at 750 nm using a microplate reader (Multiskan FC, Thermo Fisher Scientific, Shanghai, China) and the results were expressed as mg catechin equivalents (CE)/g sample on dry weight basis.

#### **3.2.2.3.14 Determination of anti-oxidant capacity**

The antioxidant capacity (radical scavenging capacity) of extracts was determined using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS<sup>+</sup>) assay. The ABTS<sup>+</sup> radical scavenging activity of the extracts was determined according to the protocol described by Awika *et al.*, (2003). A working solution was prepared from the addition of 58 mL phosphate buffer at pH 7.4 to 2 mL of ABTS mother solution (prepared by adding equal volumes of 3 mM potassium persulphate and 8 mM ABTS salt and left in the dark for 16 h). Working solution (190  $\mu$ L) was added to 10  $\mu$ L of the extract in a 96-well microplate and the mixture was incubated in the dark for 30 min at ambient temperature (25 °C). Absorbance was read at 750 nm. Trolox was used as the standard and the results were expressed as micromole Trolox equivalent per gram ( $\mu$ mol TE/g) on dry weight basis.

#### **3.2.3 Statistical Analysis**

The main effects of treatment (grape pomace addition level 0, 10 and 20%) were calculated by analysis of variance (ANOVA) using SPSS software version 20 (SPSS Inc., Armonk, NY). Means were compared using Fisher's least significant test (LSD) at 5% level of significance. The results of analysis were reported as mean obtained from triplicate experiments  $\pm$  standard deviation.

#### **3.2.4 Results and Discussion**

The effects of grape pomace addition and extrusion cooking on some physico-chemical and functional properties of cassava-soy composite are shown in Table 3.2.1. The addition of grape pomace to cassava-soy composite led to a decrease in total starch and crude protein content. This is expected as starch and protein content in grape pomace is low. Crude fat and ash content of cassava- soy composite increased with increase in level of grape pomace addition. After extrusion cooking, crude fat was decreased and ash content was significantly ( $p < 0.05$ ) increased.

The variations in addition level of grape pomace significantly ( $p < 0.05$ ) effected on bulk density and expansion ratio of cassava-soy composite. It was observed that as the level of addition of grape pomace increased from 0 to 20%, the bulk density of extrudates increased and the expansion ratio decreased. Altan *et al.*, (2008b) also reported an increase in bulk density and decrease in expansion ratio when grape pomace addition level to barley increased during extrusion cooking. This was associated with increasing fibre content in the food ingredient mix and sugar present in grape

pomace which may have lowered melt temperature and thus reduce the vapor pressure of water. The dilution of starch as grape pomace level increased could influence the macro-structure of extrudates by causing a reduction in the amount of extensible polymers in the bubble cell walls (Altan *et al.*, 2008a).

Bulk density is an important parameter in the production of expanded and formed foods and it is closely associated with expansion ratio (Asare *et al.*, 2004). The presence of inert materials such as dietary fibre is reported to disrupt the stretching and setting of bubble films (Altan and Maskan, 2012). Fibre particles could hinder the growth of air cells by puncturing them before they reach their maximum expansion limits (Lue *et al.*, 1990) and this leads to formation of extrudates with a dense structure and smaller air cells (Ainsworth *et al.*, 2007).

Extrusion cooking significantly ( $p < 0.01$ ) increased the water absorption (WAI) and solubility index (WSI) of extrudates (Table 3.2.1). The increase was probably due to starch gelatinization/depolymerization which led to an increase in availability of hydrophilic groups in the food system (Menegassi *et al.*, 2011). WAI decreased significantly ( $p < 0.001$ ) as the addition of grape pomace in cassava-soy composite increased in extrudates (Table 3.2.1).

**Table 3.2.1.** Effects of extrusion cooking and grape pomace addition on some chemical composition, physical and functional properties of cassava-soy composite

Treatment	Grape pomace addition (%)	Moisture (%)	Crude fat (%)	Protein (%)	Crude fibre (%)	Ash (%)	Starch (%)	Bulk density	Expansion ratio	WAI <sup>2</sup> (g/g)	WSI <sup>3</sup> (%)
Raw	0	8.3 <sup>d</sup> ± 0.1	1.0 <sup>d</sup> ± 0.0	18.4 <sup>c</sup> ± 0.2	4.0 <sup>a</sup> ± 0.1	2.1 <sup>a</sup> ± 0.0	53.9 <sup>b</sup> ± 0.5	ND <sup>1</sup>	ND	1.77 <sup>a</sup> ± 0.04	7.9 <sup>a</sup> ± 0.1
	10	8.6 <sup>e</sup> ± 0.1	1.8 <sup>e</sup> ± 0.0	17.4 <sup>a</sup> ± 0.2	8.8 <sup>b</sup> ± 0.1	3.2 <sup>c</sup> ± 0.1	47.2 <sup>c</sup> ± 0.4	ND	ND	1.78 <sup>a</sup> ± 0.02	9.7 <sup>b</sup> ± 0.3
	20	8.1 <sup>c</sup> ± 0.1	2.1 <sup>f</sup> ± 0.0	17.0 <sup>a</sup> ± 0.1	11.6 <sup>c</sup> ± 0.0	4.1 <sup>e</sup> ± 0.1	43.0 <sup>a</sup> ± 0.2	ND	ND	1.80 <sup>a</sup> ± 0.02	10.6 <sup>b</sup> ± 0.1
Extrusion cooked	0	5.8 <sup>b</sup> ± 0.0	0.3 <sup>a</sup> ± 0.0	19.4 <sup>d</sup> ± 0.1	4.0 <sup>a</sup> ± 0.0	2.4 <sup>b</sup> ± 0.0	54.7 <sup>b</sup> ± 0.3	0.26 <sup>a</sup> ± 0.01	3.7 <sup>c</sup> ± 0.2	2.44 <sup>d</sup> ± 0.01	45.7 <sup>c</sup> ± 0.8
	10	4.2 <sup>a</sup> ± 0.1	0.4 ± 0.0	17.9 <sup>b</sup> ± 0.2	8.8 <sup>b</sup> ± 0.1	3.4 <sup>d</sup> ± 0.1	47.9 <sup>c</sup> ± 0.4	0.31 <sup>b</sup> ± 0.00	2.8 <sup>b</sup> ± 0.0	2.21 <sup>c</sup> ± 0.01	47.6 <sup>d</sup> ± 0.3
	20	4.1 <sup>a</sup> ± 0.0	0.6 <sup>c</sup> ± 0.0	17.8 <sup>b</sup> ± 0.1	11.4 <sup>c</sup> ± 0.1	4.2 <sup>e</sup> ± 0.0	43.8 <sup>a</sup> ± 0.6	0.34 <sup>c</sup> ± 0.01	2.5 <sup>a</sup> ± 0.1	2.05 <sup>b</sup> ± 0.02	48.1 <sup>d</sup> ± 0.1

Values are means ± standard deviations of 3 independent experiment. Values within the same column followed by different letters are significantly different (p<0.05).

<sup>1</sup> Not determined in raw composites

<sup>2</sup>Water absorption index.

<sup>3</sup>Water solubility index

The WSI values of extruded composites were between 45.7 to 48.1% and it was observed that that WSI increased as grape pomace addition level increased from 0 to 20% (Table 3.2.1). Similar trend in result was reported by Altan *et al.*, (2008a) in the study on incorporation of tomato or grape pomace to barley flour. The decrease in WAI as by-products (tomato and grape pomace) increased was attributed to the induced stress during extrusion cooking which may have opened the structure of fibre thus allowing more water to enter into contact with hydrophilic groups (Altan *et al.*, 2008a). Depolymerization and dextrinization of starch which occurs during extrusion cooking may also have contributed to the increase in WAI after extrusion cooking (Sarawong *et al.*, 2014).

The increase observed in the WSI as grape pomace addition increased could be related to fibre modification during extrusion cooking as a result of high shear which led to the release of low molecular weight compounds (Altan and Maskan, 2012). Similarly, Larrea *et al.*, (2005b) reported an increase in WSI of extruded orange pulp and wheat, and this increase was largely attributed to degradation of components of fibre during extrusion cooking. The highest value of WSI was found at the highest level (20%) of grape pomace addition to cassava-soy composite (48.1%). This result may be related to availability of more sugar in the system due to sugar content of grape pomace in addition to the fibre content. Onyango *et al.* (2004b) suggested that an increase in WSI with increasing sugar concentration was a sign of increased solubilization of starch.

The addition of grape pomace to cassava-soy composite lowered the NSI and IVPD of cassava-soy composite (Table 3.2.2). Extrusion cooking also slightly decreased IVPD of extruded products and significantly ( $p < 0.05$ ) lowered the NSI of extrudates. Nitrogen solubility can be related to protein digestibility as an increase in soluble nitrogen is often accompanied by an increase in protein digestibility. Di-sulphide bonds are cleaved under high temperature and this results in the unfolding of protein structure. The unfolded protein polypeptides can interact through covalent inter-molecular disulphide bonds between polypeptide chains to form cross-links (Ljøkjel *et al.*, 2004). Cross-linking of proteins may prevent enzyme access to peptide bonds by blocking sites of enzyme attack (Heck *et al.*, 2013).

**Table 3.2.2.** Effects of extrusion cooking and grape pomace addition on the *in-vitro* protein digestibility, nitrogen solubility, protein secondary structure, soluble and insoluble dietary fibre content of cassava- soy composite.

Treatment	Grape pomace addition (%)	Amide I		Amide II		Amide I	Amide II	Ratio ( $\alpha:\beta$ )	Ratio ( $\alpha:\beta$ )	IDF (%)	SDF (%)
		IVPD (%)	NSI (%)	$\alpha$ -helix wave number ( $\text{cm}^{-1}$ )	$\beta$ -sheet wave number ( $\text{cm}^{-1}$ )	$\alpha$ -helix wave number ( $\text{cm}^{-1}$ )	$\beta$ -sheet wave number ( $\text{cm}^{-1}$ )				
Raw	0	91.4 <sup>de</sup> ± 0.1	50.0 <sup>e</sup> ± 0.5	ND	1637	1547	1516	ND	1.11 <sup>e</sup> ± 0.01	8.4 <sup>b</sup> ± 0.1	3.2 <sup>a</sup> ± 0.3
	10	88.3 <sup>c</sup> ± 0.7	16.8 <sup>cd</sup> ± 0.1	ND	1638	1551	1516	ND	1.01 <sup>d</sup> ± 0.01	13.1 <sup>d</sup> ± 0.2	4.4 <sup>b</sup> ± 0.1
	20	85.0 <sup>b</sup> ± 0.5	16.2 <sup>c</sup> ± 0.2	ND	1627	1544	1515	ND	0.95 <sup>c</sup> ± 0.02	18.7 <sup>f</sup> ± 0.2	6.1 <sup>c</sup> ± 0.2
Extrusion cooked	0	90.8 <sup>d</sup> ± 0.3	17.2 <sup>d</sup> ± 0.1	1643	1625	1542	1514	1.04 <sup>c</sup> ± 0.01	0.84 <sup>b</sup> ± 0.01	7.2 <sup>a</sup> ± 0.2	4.3 <sup>b</sup> ± 0.2
	10	86.2 <sup>b</sup> ± 0.2	11.5 <sup>b</sup> ± 0.1	1647	1627	1543	1514	0.98 <sup>b</sup> ± 0.01	0.80 <sup>a</sup> ± 0.01	9.7 <sup>c</sup> ± 0.4	6.8 <sup>d</sup> ± 0.4
	20	82.8 <sup>a</sup> ± 0.7	9.9 <sup>a</sup> ± 0.2	1647	1620	1543	1512	0.95 <sup>a</sup> ± 0.01	0.77 <sup>a</sup> ± 0.02	14.4 <sup>e</sup> ± 0.3	10.8 <sup>e</sup> ± 0.4

Values are means ± standard deviations of 3 independent experiment. Values within the same column followed by different letters are significantly different ( $p < 0.001$ )

Protein secondary structure was determined by ATR-FTIR spectra of amide I and amide II region

IVPD = *in-vitro* protein digestibility

NSI = nitrogen solubility index

ND = not detected in the composites

IDF = insoluble dietary fibre

SDF = soluble dietary fibre

ND = not detected in the samples

The polyphenols present in grape pomace may also have contributed to the reduction observed in IVPD and NSI as polyphenols are known to interact with proteins to form indigestible complexes which are less susceptible to proteolytic digestion (Alonso *et al.*, 2000). Arimboor and Arumughan, (2011) similarly reported that interactions of sea buckthorn polyphenols with proteins led to decrease in the *in-vitro* protein digestibility of sea buckthorn. Also, Labuckas *et al.*, (2007) found that the presence of polyphenols decreases the protein solubility of walnut flour.

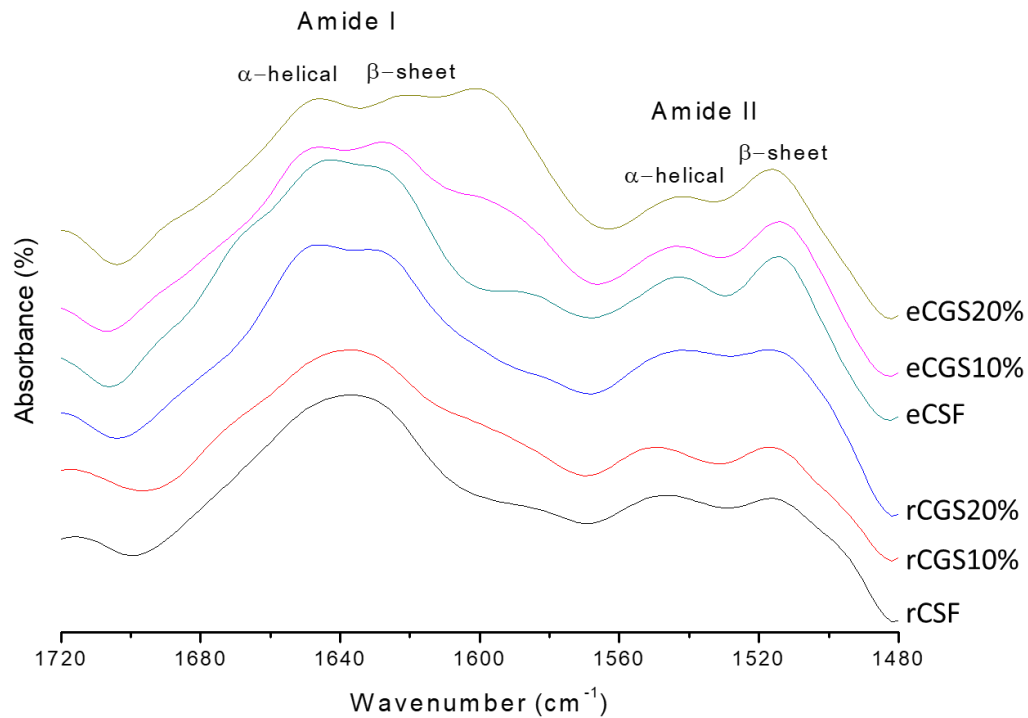
Hydrogen bonding and hydrophobic interaction are reported to be the primary attractive forces between protein molecule and phenolic group (Bartolome *et al.*, 2000). The aromatic nuclei and hydroxyl group of the aromatic ring of the phenolic compounds provide the principal binding sites for protein-phenolic complexation (Ali *et al.*, 2012). It has been suggested that hydrogen bonding between the phenolic hydroxyl group and the NH- and CO- groups of proteins are involved in protein-phenolics interaction (Haslam *et al.*, 1999). These interactions result in formation of insoluble complexes which ultimately reduces the digestibility of proteins. Phenolic compounds may also directly bind to proteolytic enzymes. Covalent attachment between phenolics and the reactive nucleophilic sites in the enzyme have been reported to result in the inhibition of actions of proteolytic enzymes (Rohn *et al.*, 2002) and this results in decrease in *in-vitro* protein digestibility.

The ATR- FTIR deconvoluted spectra of raw and extruded composites of cassava-soy with 0, 10 and 20% grape pomace was determined to examine the effect of grape pomace addition and extrusion cooking on the protein secondary structure of cassava-soy composites (Figure 3.2.1). The amide I band was observed at 1627 – 1637 for the raw composites and at 1620 to 1647  $\text{cm}^{-1}$  for the extruded composites. The amide I band which appears between 1680 and 1600  $\text{cm}^{-1}$  primarily results from CN stretching, CCN deformation, and in-plane NH bending modes of groups in the polypeptide chain (Bandeka, 1992; Byaruhanga *et al.*, 2006). The amide II vibrations are reported to be an out of phase combination of mainly the NH bending vibrations and CN stretch (Barth, 2007) and they were observed between 1550 and 1500  $\text{cm}^{-1}$  (Byaruhanga *et al.*, 2006).

There were no clear peaks of  $\alpha$ -helical conformation in the raw composite without grape pomace and that which contained 10% grape pomace. Only the raw composite which contained 20% grape



poma0ce showed a visible peak at  $1647\text{ cm}^{-1}$  and this is associated with the  $\alpha$ -helix of the protein secondary structure.



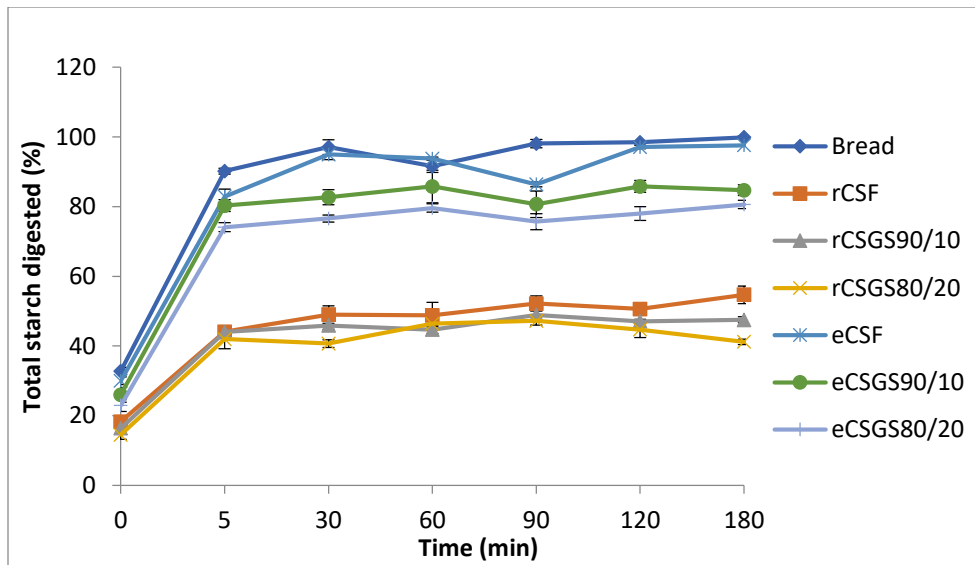
**Figure 3.2.1.** FTIR spectra of raw and extruded cassava-soy composite with and without grape pomace. rCSF is raw cassava-soy flour, rCGS10% is raw cassava- soy flour with 10% grape pomace, rCGS20% is raw cassava-soy flour with 20% grape pomace, eCSF is extruded cassava-soy flour, eCGS10% is extruded cassava-soy flour with 10% grape pomace, eCGSS20% is extruded cassava-soy flour with 20% grape pomace

Due to the absence of clear peaks showing the  $\alpha$ -helical conformation in the amide I region of the raw composites, the ratio of  $\alpha/\beta$  conformation could not be determined in these composites. The extruded composites on the other hand all showed visible peaks of  $\alpha$ -helical and  $\beta$ -sheet conformation both in the amide I and amide II regions (Table 3.2.2). There was a significant decrease in  $\alpha$ -helix to  $\beta$ -sheet ratio from 1.04 to 0.95 in the amide I region and from 0.84 to 0.77 in the amide II region of the extruded composites as the level of addition of grape pomace increased from 0 to 20% (Table 3.2.2). This indicates that grape pomace addition promoted an increase in  $\beta$ -sheet formation with a consequent decrease in  $\alpha$ -helical conformation. Mehanna *et al.*, (2014) reported shifts in the ratio of  $\alpha/\beta$  conformation in the amide I and II regions of whey isolate after addition of tannic acid and this was attributed to binding between tannic acid and whey protein.

The formation of  $\beta$ -sheet is reported to be indicative of protein aggregation (Byaruhanga *et al.*, 2006). The increase in proportion of  $\beta$ -sheets has been linked to decrease in *in-vitro* protein digestibility due to the highly hydrophobic nature of  $\beta$ -sheets (Carbonaro *et al.*, 2012). Wang *et al.*, (2014) reported increase in surface hydrophobicity of soy protein with increasing  $\beta$ -sheets conformation. High hydrophobicity has been suggested to reduce solubility of legume proteins through facilitating protein-protein interaction and aggregate formation which thereby limits enzyme access to susceptible sites (Yang *et al.*, 2016)

Starch digestion was generally higher in extrusion cooked porridges compared to the starting raw materials (Figure 3.2.2). Total starch digested (TSD) of cassava- soy composites was significantly ( $p<0.001$ ) reduced by addition of grape pomace. The porridge with 20% grape pomace exhibited the lowest TSD (%). Starch digestibility kinetic parameters ( $C_{\infty}$ , HI, EGI and EGL) were significantly ( $p<0.01$ ) increased by extrusion cooking (Table 3.2.3) but the addition of grape pomace lowered the values of these parameters. The EGL of extrusion cooked porridges were 44.1, 35.6 and 26.5 for cassava-soy porridge, porridges with 10 and 20% grape pomace respectively.

The estimated rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) fractions of the raw and extrusion cooked composites are shown in Table 3.2.3. These starch fractions (RDS, SDS and RS) determined from *in-vitro* starch digestion can be used as a prediction tool of *in-vivo* glucose response. The raw composites had lower RDS while SDS and RS were high. The addition of grape pomace significantly ( $p<0.001$ ) increased the SDS of cassava-soy porridge. The reference (white bread) had the highest RDS and least SDS while the extruded composite which had 20% grape pomace composite had the highest SDS and least RDS amongst the extruded samples. The estimated resistant starch (RS) of the reference was significantly lower to that of extruded composites with and without grape pomace.



rCSF is raw cassava- soy flour  
rCSGS90/10 is raw cassava- soy flour with 10% grape pomace  
rCSGS80/20 is raw cassava- soy flour with 20% grape pomace  
eCSF is extruded cassava- soy flour  
eCSGS90/10 is extruded cassava- soy flour with 10% grape pomace  
eCSGS80/20 is extruded cassava- soy flour with 20% grape pomace  
White Bread was used as reference

**Figure 3.2.2.** Effects of grape pomace addition and extrusion cooking on the kinetics of starch digestion of cassava-soy composite

The type of starch, physical state of starch, rheological characteristics of food, its texture, the presence of other non-starch components and the interactions that occur between starch and these non-starch components are all factors that could influence enzymatic digestibility (Singh *et al.*, 2007). Native starches are reported to have a high resistance to enzymatic hydrolysis due to their highly organized and intact crystalline structure (Rocha *et al.*, 2010) hence the lower digestibility observed in the starting raw materials.

Extrusion cooking may promote physical disruption of the organized granular structure of native starches and this leads to gelatinization and disintegration of starch (Hagenimana *et al.*, 2006). Starch gelatinization and disintegration facilitates amylolytic hydrolysis by making starch more accessible to digestive enzyme thus increasing starch digestibility (Mishra *et al.*, 2012) and this may be responsible for significant increases in digestibility observed in extrudates after extrusion cooking.

**Table 3.2.3.** Effects of extrusion cooking and grape pomace addition on in-vitro starch digestibility kinetic parameters, starch fractions and pH of cassava-soy composite and reference sample.

Treatment	Grape pomace addition (%)	$C_{\infty}$ (%)	K ( $\text{min}^{-1}$ )	HI (%)	EGI	EGL	RDS (%)	SDS (%)	RS (%)	pH
Raw	0	55.8 <sup>b</sup> ± 2.4	0.020 <sup>a</sup> ± 0.00	53.8 <sup>c</sup> ± 1.4	69.3 <sup>c</sup> ± 1.2	30.3 <sup>d</sup> ± 0.4	13.5 <sup>a</sup> ± 0.5	45.0 <sup>cd</sup> ± 0.7	41.6 <sup>c</sup> ± 0.2	6.30 <sup>d</sup> ± 0.01
	10	48.6 <sup>a</sup> ± 0.5	0.017 <sup>a</sup> ± 0.00	49.4 <sup>b</sup> ± 1.5	66.9 <sup>b</sup> ± 2.1	22.5 <sup>b</sup> ± 0.5	11.1 <sup>a</sup> ± 1.1	46.0 <sup>cd</sup> ± 0.4	43.0 <sup>c</sup> ± 0.9	5.28 <sup>b</sup> ± 0.00
	20	46.4 <sup>a</sup> ± 1.1	0.015 <sup>a</sup> ± 0.01	47.2 <sup>a</sup> ± 0.4	65.6 <sup>a</sup> ± 0.8	16.3 <sup>a</sup> ± 0.7	8.5 <sup>a</sup> ± 0.7	48.6 <sup>d</sup> ± 0.5	43.9 <sup>c</sup> ± 1.2	4.53 <sup>a</sup> ± 0.01
Extrusion cooked	0	98.6 <sup>e</sup> ± 0.7	0.031 <sup>c</sup> ± 0.01	98.2 <sup>f</sup> ± 0.7	93.6 <sup>f</sup> ± 1.3	44.1 <sup>f</sup> ± 1.1	70.1 <sup>d</sup> ± 0.9	28.9 <sup>a</sup> ± 1.0	1.3 <sup>a</sup> ± 0.1	6.15 <sup>c</sup> ± 0.00
	10	85.2 <sup>d</sup> ± 2.9	0.027 <sup>bc</sup> ± 0.00	86.4 <sup>e</sup> ± 0.5	87.2 <sup>e</sup> ± 0.6	35.6 <sup>e</sup> ± 0.4	62.0 <sup>c</sup> ± 1.3	35.2 <sup>b</sup> ± 2.6	2.8 <sup>b</sup> ± 0.2	5.28 <sup>b</sup> ± 0.01
	20	80.2 <sup>c</sup> ± 1.5	0.020 <sup>a</sup> ± 0.00	79.6 <sup>d</sup> ± 0.7	83.4 <sup>d</sup> ± 0.3	26.5 <sup>c</sup> ± 0.6	53.4 <sup>b</sup> ± 1.9	43.4 <sup>c</sup> ± 1.3	3.2 <sup>b</sup> ± 0.2	4.55 <sup>a</sup> ± 0.00
	Bread	99.1 <sup>e</sup> ± 0.4	0.029 <sup>bc</sup> ± 0.01	100.0 <sup>g</sup> ± 0.1	94.6 <sup>g</sup> ± 0.6	64.8 <sup>g</sup> ± 0.1	73.1 <sup>d</sup> ± 1.1	25.7 <sup>a</sup> ± 0.7	1.1 <sup>a</sup> ± 0.2	nd

Values are means ± standard deviations of 3 independent experiment. Values within the same column followed by different letters are significantly different (p<0.01)

$C_{\infty}$  = % starch digested after 180 min

HI, k and GI were calculated from the equation:  $AUC = \int_0^{\infty} C_{\infty} (t_{f_0} \infty/k) (1 - \exp(-k(t_{f_0} - t) - (Ct)))$  proposed by Goni *et al.* 1997

EGL per g solids was estimated as  $EGI * S / 100$  where S is starch content (g/100g solids)

White bread was used as the reference for calculating EGI

nd = not determined in bread because it was not needed

The reduction observed in EGI and EGL when grape pomace was added to cassava- soy may be attributed to the inhibitory effects polyphenols present in grape pomace have on starch digestive enzymes. The ability of polyphenols to inhibit activities of alpha amylase and alpha glucosidase is well reported in literature (Hanhineva *et al.*, 2010; Sales *et al.*, 2012). Phenolic compounds bind to the active and secondary sites of digestive enzymes therefore making them inactive (Kandra *et al.*, 2004). The activities of alpha amylase are reported to be inhibited by tannins while smaller phenolics such as phenolic acids inhibit the activities of alpha-glucosidase (Guzar *et al.*, 2012).

The inhibitory effects of tannins on alpha-amylase is reported to be concentration dependent. Polymers have higher inhibitory activity against alpha-amylase than oligomers while reverse is the case for alpha-glucosidase (Cires *et al.*, 2017). Miao *et al.* (2014) in a study on grape skin extract inhibition of alpha-amylase *in-vitro* reported a non-competitive inhibition of pancreatic alpha-amylase by grape skin extract. The authors reported that grape extract polyphenol occupied one of the binding sites of alpha-amylase, interacted with side chains of alpha-amylase and formed hydrogen bonds and Van der Waal forces with residues of the catalytic site (Miao *et al.*, 2014).

In a study on effects of anti-oxidant of grape pomace extract on post-prandial hyperglycaemia in diabetic mice, Hogan *et al.*, (2010) reported that grape pomace extract inhibited alpha glucosidase activity and promoted decrease in post prandial glucose response. Also, Barrett *et al.*, (2013) suggested that tannins in grapes had a high inhibitory effect on alpha amylase and alpha glucosidase. It is important to note that pH could not have had an effect on starch digestibility because buffers were used to ensure that all the treatments were at the same pH throughout the assay.

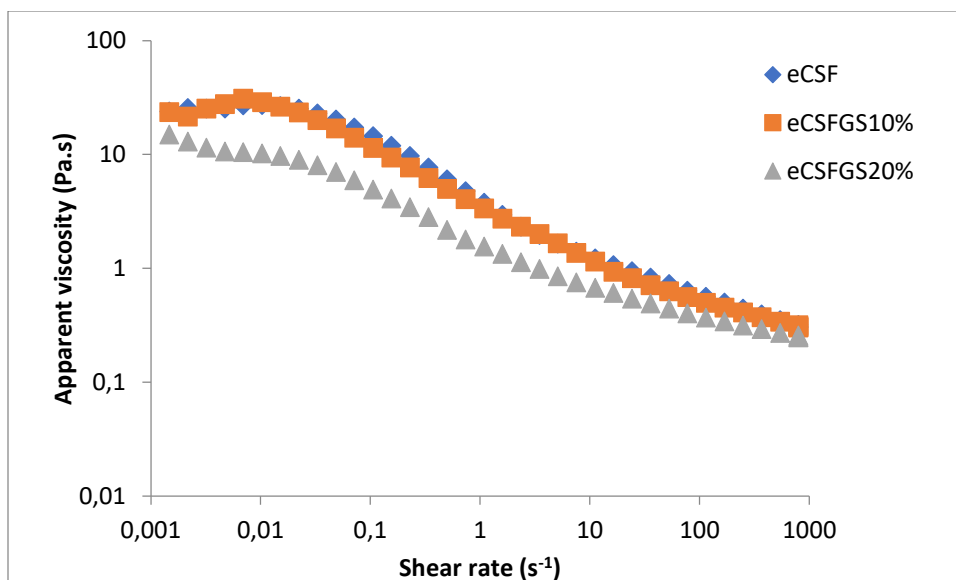
Grape pomace addition significantly ( $p < 0.01$ ) increased the insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) of cassava -soy composite (Table 3.2.2). Soluble dietary fibre (SDF) contents of cassava-soy composite with and without grape pomace increased with a decrease in insoluble dietary fibre (IDF) after extrusion cooking (Table 3.2.2). There was about 54.5% increase in SDF content of composite with 10% grape pomace and the highest increase was observed in cassava-soy composite with 20% grape pomace addition level with about 77% increase. Redistribution in IDF to SDF ratio was also reported in extrusion cooking of lemon fibre by Méndez-García *et al.*, (2011). Martínez-Boustos *et al.*, (2011) also reported that extruded blends of sugar bagasse fibre, whey protein concentrate, and corn starch had higher SDF and decreased

IDF content compared to the unextruded blends. The authors suggested that solubilization and fragmentation of dietary fibre during extrusion cooking led to the increase in soluble dietary fibre.

Mechanical stress during extrusion cooking has been associated with the breakdown of polysaccharide glycosidic bonds giving rise to the release of oligosaccharides hence the increase in SDF contents (Esposito *et al.*, 2005). According to Ralet *et al.*, (1991), extrusion cooking leads to solubilization of IDF and it also promotes the reduction in molecular weight of non-cellulosic polysaccharides and pectin. Grape pomace dietary fibre primarily consists of cellulose, hemicellulose and pectin (Sousa *et al.*, 2014). Several authors have shown that extrusion cooking solubilizes insoluble pectic polysaccharide (Thibault *et al.*, 1992; Waldron *et al.*, 1994). Pectin consists of  $\alpha$ -(1,4)- linked homogalacturonan and rhamnogalacturonan which are highly branched with various neutral sugars (Endres *et al.*, 2006). The high shear during extrusion cooking may result in breakage of  $\alpha$ -(1,4)-glycosidic linkages in the galacturonan backbone of pectic polysaccharides and this would lead to increase in solubility (Voragen *et al.*, 1995).

The cassava-soy composite with 20% grape pomace had the lowest apparent viscosity (Figure 3.2.3). The zero-shear viscosity,  $K$  and  $n$ -value of the cassava-soy (the control) and the composite with 10% grape pomace level statistically ( $p < 0.001$ ) differed from the composite with 20 % grape pomace (Table 5). All samples exhibited a shear thinning behaviour with  $n < 1$ . The addition of grape pomace significantly ( $P < 0.001$ ) lowered the hysteresis area of cassava-soy composite (Table 5).

Apparent viscosity of non-starch polysaccharides depends on ionically charged groups, molecular weight, concentration of dietary fibre, surrounding structures (Caprita and Caprita, 2011) and pH (Guillon and Champ, 2000). The low viscosity recorded for the composites containing grape pomace despite the increase in soluble dietary fibre after extrusion cooking could be as a result of breakdown of high molecular weight polysaccharides to low molecular weight in the soluble fractions (Svanberg *et al.*, 1995) which are less viscous. Another contributing factor to low viscosity as suggested by Nyman and Haska, (2013) is degradation of attached side chains of dietary fibre during storage by endogenous pectinases. In this case, the grape pomace might have been stored for a long period after crushing of grape fruits to expel juice hence the lower viscosity observed in the composites which had grape pomace.



eCSF is extrusion cooked cassava- soy flour

eCSB90/10 is extrusion cooked cassava-soy flour with 10% grape pomace

eCSB80/20 is extrusion cooked cassava- soy flour with 20% grape pomace

**Figure 3.2.3.** Effect of grape pomace addition on apparent viscosity of extruded cassava-soy composite at 50 °C as a function of shear

Pectin is estimated to be about 34% in grape pomace and it is one of the major component of grape pomace dietary (Ping *et al.*, 2011). Pectin is reported to be a process-sensitive non-starch polysaccharide which can undergo depolymerization at relatively intermediate heating temperature ( $\geq 50$  °C) (Lovegrove *et al.*, 2015). Heating is found to change the conformational properties of pectin in solution and this directly impacts on its functionality (Shpigelman *et al.*, 2014). pH also significantly influences viscosity. The pH values recorded for the extruded composites with and without grape pomace is shown in Table 3.2.3. The cassava-soy composite with 20% grape pomace had the lowest pH value of 4.55 while that with 10% grape pomace was 5.28 and the control without grape pomace had a pH value of 6.15. Pectin depolymerization is reported to be dominated by acid hydrolysis at pH 4.5 (Renard and Thibault, 1996; Lovegrove *et al.*, 2015) and this may also have contributed to the low viscosity recorded for the composite with 20% grape pomace.

**Table 3.2.4.** Effect of grape pomace addition on power law ( $k$  and  $n$ -values) parameters, zero-shear viscosity, viscosity at shear rate  $50 \text{ s}^{-1}$  and hysteresis area of extruded cassava-soy flour

Treatment	$K$ -value (Pa.s) <sup>n</sup>	Power law index ( $n$ -value)	$\eta_{a, 50}$ (mPa.s)	$\eta_0$ ( $\times 10^3 \text{ mPa.s}$ )	Hysteresis area Pa/ (S· ml)
Extrusion cooked cassava-soy flour	$3.78b \pm 0.49$	$0.56a \pm 0.1$	$728b \pm 15$	$24b \pm 2$	$17357c \pm 10$
With 10% grape pomace	$3.14b \pm 0.70$	$0.56a \pm 0.0$	$629b \pm 28$	$23b \pm 2$	$14682b \pm 33$
With 20% grape pomace	$2.15a \pm 0.06$	$0.61b \pm 0.1$	$473a \pm 1$	$14a \pm 3$	$13365a \pm 18$

Values are means  $\pm$  standard deviations of 3 independent experiment.

Values within the same column followed by different letters are significantly different ( $p \leq 0.05$ )

Svanberg *et al.*, (1995) reported a reduction in apparent viscosity of carrots after boiling compared to microwave heating. The authors suggested that the reduction may be due to intensity of treatment associated with boiling that caused more glycosidic linkages breakage and loss of intermolecular association of water-soluble polysaccharides. Also, the viscosity of Brussels sprouts was reported to be lower than that of green beans after heat processing and the authors attributed this to depolymerization of high molecular weight polysaccharides with a consequent increase in low molecular weight polysaccharides (Nyman *et al.*, 1994).

Extrusion cooking makes use of high shear during processing and this facilitates extensive degradation of dietary fibre polysaccharides and cleavage of some glycosidic linkages that results in increased solubility and possibly also in reduced viscosity (Poutanen, 2001). It can thus be suggested that despite the higher concentration of SDF in the composite with 20% grape pomace, the molecular weight of these SDF portion was very low and as such could not promote viscosity hence why the apparent viscosity was the lowest.

The effects of extrusion cooking and grape pomace addition on particle size distribution of cassava-soy composites are shown in Table 3.2.5.



**Table 3.2.5.** Effects of extrusion cooking and grape pomace addition on percentage (%) mass of particles retained in sieves of cassava- soy composite

<b>Treatment</b>	<b>Grape pomace addition (%)</b>	<b>&lt; 38 <math>\mu\text{m}</math></b>	<b>38 - 75 <math>\mu\text{m}</math></b>	<b>75 – 180 <math>\mu\text{m}</math></b>	<b>180 - 212 <math>\mu\text{m}</math></b>	<b>212 – 500 <math>\mu\text{m}</math></b>	<b>&gt; 500 <math>\mu\text{m}</math></b>
<b>raw</b>	0	70.8d $\pm$ 0.4	3.0b $\pm$ 0.6	3.5a $\pm$ 0.5	2.2ab $\pm$ 0.3	12.3c $\pm$ 0.7	8.4ab $\pm$ 0.6
	10	55.6b $\pm$ 0.5	1.0a $\pm$ 0.0	3.2a $\pm$ 0.0	2.3ab $\pm$ 0.0	21.4d $\pm$ 0.1	16.6c $\pm$
	20	50.8a $\pm$ 0.5	2.8ab $\pm$ 0.3	2.6a $\pm$ 0.6	3.1b $\pm$ 0.3	22.7d $\pm$ 0.8	17.9c $\pm$ 0.3
<b>Extruded</b>	0	79.8e $\pm$ 0.8	4.1bc $\pm$ 0.7	5.1a $\pm$ 0.2	1.4a $\pm$ 0.1	3.2a $\pm$ 0.9	6.4a $\pm$ 0.2
	10	72.6d $\pm$ 0.7	2.5ab $\pm$ 0.4	3.6a $\pm$ 0.6	5.8c $\pm$ 0.3	5.6ab $\pm$ 0.8	9.8b $\pm$ 0.8
	20	65.3c $\pm$ 1.5	5.7c $\pm$ 0.4	4.7a $\pm$ 0.5	5.7c $\pm$ 0.4	7.7b $\pm$ 0.7	10.9b $\pm$ 0.8

Values are means  $\pm$  standard deviations of 3 independent experiment.

Values within the same column followed by different letters are significantly different ( $p \leq 0.001$ )

The addition of grape pomace increased the mass retained in the higher mesh sieves while the smallest portion retained in the sieve size below 38  $\mu\text{m}$  was highest in the control (cassava-soy composite). Extrusion cooking significantly ( $p < 0.001$ ) decreased the particle size of extrudates as percentage mass of extrudates retained in sieves 500 and 212  $\mu\text{m}$  were significantly ( $p < 0.01$ ) lower after extrusion cooking compared to the starting raw materials particularly in the composites which contained grape pomace.

The addition of grape pomace resulted in an increase in the total phenolics and anti-oxidant activity of cassava-soy composite (Table 3.2.6). As the level of addition of grape pomace increased, the total phenolic content and anti-oxidant activity also increased. Extrusion cooking significantly ( $p < 0.05$ ) lowered the total phenolic content and anti-oxidant activity of cassava-soy composite. Similar findings were reported by Wani and Kumar, (2016) in extrusion cooking of a nutritious snack made from a combination of fenugreek seed flour, fenugreek leave flour, oat, dried green pea and corn flour and the reduction in total phenolic content was attributed to a decrease in amount of free phenolics. In another study, Caltinogu *et al.*, (2013) ascribed the reduction in total phenolic content and anti-oxidant activity to increasing temperature from 100 to 120  $^{\circ}\text{C}$ .

**Table 3.2.6.** Effects of extrusion cooking and grape pomace addition on the total phenolics contents (TPC) and anti-oxidant activity (AA) of cassava-soy composite

Treatment	Grape pomace addition (%)	Total phenolics contents (mg CE/g)	Antioxidant activity ( $\mu\text{mol TE/g}$ )
			ABTS assay
<b>raw</b>	0	2.1b $\pm$ 0.2	7.7b $\pm$ 0.2
	10	5.5d $\pm$ 0.2	10.6c $\pm$ 0.3
	20	7.7f $\pm$ 0.1	13.5d $\pm$ 0.3
<b>Extruded</b>	0	0.9a $\pm$ 0.0	5.5a $\pm$ 0.6
	10	3.5c $\pm$ 0.2	8.5b $\pm$ 0.1
	20	5.9e $\pm$ 0.1	9.8c $\pm$ 0.3

Values are means  $\pm$  standard deviations of 3 independent experiment.

Values within the same column followed by different letters are significantly different ( $p \leq 0.001$ )

The high temperature in the extruder barrel facilitates degradation of bioactive phenolic compounds with consequent loss of their antioxidant capacity (Shahidi and Yeo, 2016). Also, high temperature could alter the molecular structure of phenolic compounds thereby leading to either a decline in chemical reactivity or reduction in extractability of these phenolic compounds and loss of anti-oxidant activity because of a certain degree of polymerization (Altan *et al.*, 2009). Dietary phenolics have proven antioxidant activity (Zhang *et al.*, 2015). They help in the prevention of some processes involved in the development of degenerative diseases such as cancer by protecting the DNA from oxidative damage (Gonzalez-Aguilar *et al.*, 2008), CVDs by inhibiting oxidation of LDL cholesterol (Prahalthan *et al.*, 2012) and type-2 diabetes through their hypoglycaemic effect (Prabhakar *et al.*, 2013).

### **3.2.5 Conclusions**

The reduction observed in the starch digestibility of cassava- soy composite as grape pomace addition increased may be related to the dietary fibre and phenolic compounds present in grape pomace. These phenolics can inhibit the activities of starch hydrolyzing enzymes thereby delaying the digestion of starch and absorption of glucose. Although extrusion cooking reduced the total phenolics and anti-oxidant activity of extrudates however, significant amounts were still retained. This study indicates that grape pomace can be added to starch-rich foods as a functional ingredient with the use of extrusion cooking to produce fibre-rich extrudates which has the potential to lower glycaemic index.

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### **3.3 EFFECTS OF ADDITION OF WHEAT BRAN TO CASSAVA-SOY EXTRUDED PORRIDGE ON SENSORY PROPERTIES, ORAL PROCESSING AND SATIETY**

#### **Abstract**

This study evaluated the effect of adding wheat bran to extrusion cooked cassava- soy porridge on descriptive sensory properties, oral processing characteristics and subjective satiety responses. A cassava-defatted toasted soy composite and wheat bran (100:0, 90:10, 80:20 ratios) were extruded. Fifteen subjects (23 - 47 years, mean BMI 22.6 kg/m<sup>2</sup>) consumed 250g of each porridge type (in duplicate) over 8 breakfast meals. Subjects were video-recorded while eating the porridges. Oral exposure time and number of bites were recorded, and eating rates and bites taken were determined. Subjects rated hunger, fullness, desire to eat and prospective consumption before meal, post meal and periodically over 3 h post consumption. A separate panel profiled the descriptive sensory attributes of the porridges. The porridge with 20% wheat bran was eaten at a slower rate and with more bites compared to all the other porridges. Also, consuming the porridge with 20% wheat bran led to greater reduction in hunger, more fullness, a lower desire to eat and lower prospect to consume another meal compared to the other porridges. The cassava-soy porridges with wheat bran appeared more viscous and had more visible particles. These results indicate that wheat bran as a source of dietary fibre has the potential to be incorporated as a component of extruded starch-rich foods to produce instant products which can promote satiety.

### 3.3.1 Introduction

In recent years, the health benefits associated with dietary fibre has increased consumer demand for fibre-rich products. Studies have shown the benefits of diets rich in dietary fibre in reducing the risks of developing type-2 diabetes (Anderson, 2004; Liu *et al.*, 2000) and cardiovascular diseases (McKeown *et al.*, 2002; Sahyoun *et al.*, 2006), controlling serum lipid concentration (Jenkins *et al.*, 2001; Marlett *et al.*, 1994) and weight management (Papathanasopoulos and Camilleri, 2010; Boaz, Leibovitz and Wainstein, 2013).

Dietary fibres are conventionally classified as water soluble or insoluble (Chawla and Patil, 2010). Soluble dietary fibre is associated with delaying glucose absorption (Rodriguez *et al.*, 2006). It can also be fermented by the colonic micro flora to produce short-chain fatty acids (butyrate, propionate and acetate) (Rodriguez *et al.*, 2006) while insoluble dietary fibre adds bulk to the diet and reduces faecal transit time through the large intestine (Dhingra *et al.*, 2012).

In a previous study on the extrusion cooking of a cassava-defatted toasted soy composite with wheat bran substitution, Oladiran and Emmambux, (2017) demonstrated that extrusion cooking promotes the fragmentation of wheat bran insoluble dietary fibre. The fragmentation leads to an increase in soluble dietary fibre content and consequent increase in viscosity of extrudates. Dietary fibre has been reported to suppress hunger and increase satiety through its ability to add bulk to the diet and increase viscosity of foods (Slavin and Green, 2007). Increase in viscosity has been shown to influence average bite size, eating rate (consumption of volume per minute) and overall *ad-libitum* energy consumed to satiation (Ziljstra *et al.*, 2008).

There are several factors which directly impinge on food intake regulation and satiety. These factors include food form (McCrickerd *et al.*, 2012; Leidy, Bales-Voelker & Harris, 2011), food texture (Chambers, 2016), food composition (Fizman and Varela, 2013; Clark and Slavin, 2013) and individual difference in endocrine levels (De Silva and Bloom, 2012; Austin and Marks, 2009).

Differences in appetitive and satiety responses have been demonstrated for liquid, semi-solid and solid foods. Liquid foods are reported to induce weaker suppressive appetitive response compared to semi-solid and solid foods (Mattes and Rothacker, 2001; Tsuchiya *et al.*, 2006). The duration of oro-sensory exposure was highlighted as the major contributory factor to the response elicited. It is hypothesized that when food stays longer in the mouth, the exposure to sensory receptors is



longer in the oral cavity, thus allowing more opportunity for exposure to smell, texture, taste and other properties of the food thus leading to earlier sensory satiation (Zijlstra *et al.*, 2009).

The texture of food will determine the extent to which it requires oral processing. Foods with a chewy, hard or viscous texture usually require more mastication and are eaten more slowly (Ferriday *et al.*, 2016). These foods have longer oro-sensory exposure time and this triggers satiety responses because of signals sent to the body in the process of oral processing that nutrients have been consumed (De Graaf, 2012; Bolhuis *et al.*, 2014). Forde *et al.* (2017) also reported that the structure of foods determines the degree of mastication required to form a swallow-able bolus as observed in a study of oral processing behaviour across a wide range of Asian foods.

There is also increasing evidence on the impact of some macronutrients on satiety. Foods high in dietary fibre were reported to have a more suppressive effect on hunger compared to other macronutrients (Bellissimo and Akhavan, 2015). Dietary fibre reduces energy density by adding bulk to diet and its presence in food also leads to prolonged oral processing time. This is because dietary fibre increases chewing and limits eating rate by promoting the secretion of saliva and gastric juice thereby leading to the expansion of the stomach with a consequent increase in satiety (Slavin and Green, 2007).

The ability of some fibres to increase viscosity also contributes to the strong suppressive appetitive response they elicit post ingestion (Chambers, McCrickerd and Yeomans, 2015). Hoad *et al.*, (2004) reported greater satiety for guar gum and high viscosity alginates compared to lower viscosity alginates and this was attributed to distention in the gastric antrum together with altered transport of nutrients to the small intestine. Similarly, a systematic review by Wanders *et al.*, (2011) reported that viscous fibres promoted feelings of satiety more than less viscous fibres. Also, Harrold *et al.*, (2014) found a reduced subjective rating of hunger and prospective food consumption 3 h after consumption of a breakfast meal containing viscous fibre compared to another breakfast meal containing resistant starch.

In the study of the effects of dietary fibre on satiety, most studies focus on addition of viscous fibres such as guar gum and beta-glucan directly to foods (Tosh *et al.*, 2013; Ames and Storsley, 2015). There are limited studies on the effect of dietary fibre as part of extruded foods on oral processing and how this relates to satiety. Extrusion cooking is a versatile cooking process which

has gained increasing use due to its ability to blend different food ingredients to produce a wide range of food products with improved nutritional and functional properties. There is also growing interest in the use of food by-products as dietary fibre sources due to their potential health promoting properties. This study therefore seeks to determine the effects of wheat bran addition to a cassava-soy composite that are then extrusion cooked to an instant porridge on sensory properties, oral processing and subjective satiety responses when consuming the porridge.

### **3.3.2 Materials and methods**

#### ***3.3.2.1 Raw materials***

High quality cassava flour with particle size of  $\leq 250 \mu\text{m}$  produced according to FAO method (Dziedzoave, Graffham and Boateng, 2003) was purchased from Thai Farm International (Ogun State, Nigeria). The process for manufacturing the flour was as follows: fresh cassava roots were peeled, washed, sliced, pressed, grated, dried, milled, sifted to a particle size of  $\leq 250 \mu\text{m}$ , and bagged. The flour contained 84.4% starch, 9.8% moisture, a pH of 5.7 and 0.5% crude fibre. Toasted defatted soy flour with particle size of  $\leq 212 \mu\text{m}$  was purchased from Petrow Foods (Johannesburg, South Africa) and wheat bran was obtained from Food Corp (Pretoria, South Africa) and milled to a particle size of  $\leq 500 \mu\text{m}$ .

#### ***3.3.2.2 Extrusion cooking***

Cassava and defatted toasted soy flours were uniformly mixed using a Talsa mixer (90 ST, Xirivella, Spain) in a ratio of 65:35 (w/w). Wheat bran was added to the cassava-defatted toasted soy mixture at 0, 10 and 20% (w/w) levels and mixed thoroughly. Water at a dosing rate of 3 l/h and feed rate at 25 kg/h was fed into a co-rotating twin screw extruder (TX 32, CFAM, Potchefstroom, South Africa) with a barrel comprised of five heating zones set at 60/80/100/140/140 °C respectively. A die opening of 3 mm was used and the screw speed was maintained at 200 rpm. Extrudates were immediately dried in an oven at 90°C for 5 min and milled after cooling using a hammer mill. The ground extruded products were kept in air tight plastic buckets and refrigerated at 4 °C in preparation for analysis.

### 3.3.2.3 Sample preparation

Four soft porridges (20% solids) were prepared by adding boiling water to the extrudates and a commercial instant sorghum porridge product used as standard. Xylitol (1.5%) was added to sweeten the porridges. The porridges were stirred to get products of uniform consistency devoid of lumps. Prior to sensory evaluation, the porridges were kept at 50 °C on a warming tray (Sunbeam SWT-250, Johannesburg, South Africa) for a maximum duration of 5 min. The dietary composition of the extruded instant and commercial porridges is shown in Table 3.3.1. A portion size of 250 g with energy content of between 179 to 192.6 Kcal was served to each subject because the Australian national dietary guideline recommends a breakfast porridge portion size of 140g (170 Kcal) for adults. The 250 g portion size was therefore used in this study to get similar content.

**Table 3.3.1.** Dietary characteristics of 250 g portions of cassava-soy composite with and without wheat bran and an instant sorghum based commercial product

	Extruded cassava-soy composite	composite with 10% wheat bran	composite with 20% wheat bran	Commercial product
Carbohydrate (g)	34.3	33.5	32.1	37.4
Protein (g)	9.75	9.40	8.55	3.55
Fat (g)	0.15	0.20	0.23	1.25
Dietary fibre (g)	5.75	7.20	9.05	3.00
Sweetener (g)	3.75	3.75	3.75	3.75
Energy content (Kcal)	192.6	188.4	179.7	190.1

### 3.3.2.4 Descriptive sensory analyses

The generic descriptive analysis method according to Einstein, (1991) was used. A separate trained sensory panel (n=11) participated in 8 h of training which took place over 4 days. The four prepared porridges were served in glass ramekins (40g portions) covered with aluminium foil and with stainless steel teaspoons. Through consensus, descriptive terms (Table 3.3.2) and scale anchors for the evaluation of the porridges were developed and defined by panellists. Reference standards were used to ensure that all panellists agreed on the various sensory descriptors identified (Table 3.3.2).

Thirty descriptors were used to differentiate amongst the porridges. Each panellist evaluated eight porridges within a 1 h 30 min session and one porridge was presented at a time to avoid fatigue with a 10 min delay between each serving. Analysis was done in duplicate over two days giving 44 data points per porridge. The order of sample presentation was randomized over the panel using the Williams Latin square design. Filtered tap water was used to rinse the mouth before and between samples. Tests were conducted in a sensory evaluation laboratory equipped with individual booths. The panellists entered their responses directly on to Compusense Five software (Compusense Five release 4.6, Compusense, Guelph, Ontario, Canada).

#### ***3.3.2.5 Dynamic viscosity***

The dynamic viscosity of the porridges was measured with a Physica MCR 101 rheometer with Rheoplus software<sup>®</sup>, (Anton Paar, Ostfildern, Germany). Porridge containing 20% solid (w/v) was prepared and held at 50 °C for 5 min to equilibrate. The porridge was then transferred into the rheometer cup and maintained at 50 °C. The porridge was stirred with a vane and the viscosity was recorded over a shear rate range of 0.01 to 1000 s<sup>-1</sup>.

**Table 3.3.2.** Sensory descriptors and evaluation guidelines used by sensory panel to evaluate cassava- soy porridges with 0, 10 and 20% wheat bran addition

<b>Descriptor</b>	<b>Definition</b>	<b>References</b>	<b>Rating scale</b>
<b><i>Aroma</i></b>			
Overall aroma	Intensity of the overall aroma of the porridge		Not intense = 0 Very intense = 10
Earthy aroma	Intensity of aroma associated with the earth or damp soil,	Damp soil = 10	Not earthy = 0 Very earthy = 10
Toasted nut aroma	Intensity of aroma associated with toasted peanuts	Toasted peanuts = 10	Not nutty = 0 Very nutty = 10
Starchy aroma	Intensity of aroma associated with under-cooked maize porridge	35% ACE maize flour in boiling water =10	Not starchy = 0 Very starchy = 10
Bran aroma	Intensity of aroma associated with whole grain products	20% ground Kelloggs All bran flakes in boiling water = 10	Not bran-like = 0 Very bran-like = 10
Soy aroma	Intensity of aroma associated with soya beans or soy products	35% defatted toasted soy flour in boiling water = 10	Not soy-like = 0 Very soy-like = 10
Cassava aroma	Intensity of the aroma characteristic of cassava porridge	10% high quality cassava flour in boiling water = 10	Not cassava = 0 Very intense cassava = 10
<b><i>Appearance</i></b>			
Brown colour	Degree to which porridge appears brown	Chocolate milk = 10	Not brown = 0 Very brown = 10
Glossy	Amount of shine or gloss perceived on the surface of the product	Egg white = 10	Not glossy = 0 Very glossy = 10
Viscosity	Perceived thickness of product when it is stirred	Filtered water = 0 Hullet's golden syrup = 10	Not viscous = 0 Very viscous = 10

**Table 3.3.2 Cont.**

Sticky	How the spoon adheres to the product when it is used to pull the porridge from the sides of the bowl	10% high quality cassava flour in boiling water = 10	Not sticky = 0 Very sticky = 10
Particles	Visible specks present in product. Particles can be different colours	Cooked sorghum porridge (Monati super mabele) = 7	No particles = 0 Many particles = 10
<b>Flavour</b>			
Sweet	Intensity of the sweet taste of which sucrose is typical	2% sucrose solution = 5	Not sweet = 0 Very sweet = 10
Sour	Intensity of the sour taste associated with citric acid	Mageu No 1 beverage = 7	Not sour = 0 Very sour = 10
Umami	Intensity of the umami taste of monosodium glutamate	10% aromat seasoning in boiled water = 10	Not umami = 0 Very umami = 10
Starchy flavour	Intensity of the flavour associated with under-cooked maize porridge	35% ACE maize meal in boiling water = 10	Not starchy = 0 Very starchy = 10
Earthy flavour	Intensity of the flavour associated with the earth or soil, natural	Damp soil = 10	Not earthy = 0 Very earthy = 10
Toasted nut flavour	Intensity of the typical flavour of peanuts	Toasted peanuts = 10	Not nutty = 0 Very nutty = 10
Beany flavour	Intensity of the flavour associated with under-cooked legumes	20% cowpea flour in boiled water = 10	Not beany = 0 Very beany = 10
<b>Mouthfeel and Texture</b>			
Coarse	The degree of grittiness or graininess in porridge as a result of small particles	Iwisa stiff maize porridge (35% flour in water) = 5	Not coarse = 0 Very coarse = 10
Thickness	A measure of consistency of the porridge in the mouth	Water = 0 Thick cooked maize porridge = 10	Not thick = 0 Very thick = 10

**Table 3.3.2 Cont.**

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Adhesiveness	Degree to which product adheres to the palate surface during mastication	Water = 0 Cooked Jungle Oats = 5 Peanut butter = 10	Not adhesive = 0  Very adhesive = 10
Astringent	Intensity of the dry puckering sensation on the tongue and other mouth surfaces	Strong black tea = 10	Not astringent = 0 Very astringent = 10
<i><b>Aftertaste</b></i>			
Sour aftertaste	Intensity of the sour taste associated with citric acid	Mageu No 1 beverage = 7	Not sour = 0 Very sour = 10
Astringent aftertaste	Intensity of the dry puckering sensation on the tongue and other mouth surfaces	Strong black tea = 10	Not astringent = 0 Very astringent = 10
Beany aftertaste	Intensity of the flavor associated with under-cooked legumes	20% cowpea flour in boiled water = 10	Not beany = 0 Very beany = 10
Sweet aftertaste	Intensity of the sweet taste of which sucrose is typical	2% sucrose solution = 5	Not sweet = 0 Very sweet = 10

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### ***3.3.2.6 Subjects and experimental procedure – oral processing and satiety related perceptions test***

Volunteers (n=15) were recruited from the Department of Food Science, University of Pretoria. Participant eligibility was based on age of 18-50 years, body mass index (BMI) of between 18 and 30 kg/m<sup>2</sup>, and a low dietary restraint  $\leq 8$  based on the three-factor eating questionnaire-R18 (TFEQ-R18) (Karlsson *et al.*, 2000), no food allergies or intolerances and not following a special or restricted diet at the time of the study. The qualified panel had a mean age of  $34.1 \pm 8.7$  y and BMI of  $22.6 \pm 2.6$  kg/m<sup>2</sup>. Based on the TFEQ-R18, the mean cognitive restraint, emotional and uncontrolled eating scores of the subjects were  $2.5 \pm 1.4$ ,  $1.4 \pm 0.6$ , and  $1.9 \pm 0.8$  respectively. For  $p = 0.05$ , a treatment difference of 10 mm on the satiety scale, and completing the study with data from 15 participants was estimated to provide a power level of approximately 0.9 (Flint, Raben and Astrup, 2000). Participants were informed about the purpose of the study and written consent was obtained from each participant. Ethical approval for the study was granted by the Ethics Committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa (reference EC 160713-054).

Participants were instructed to fast for at least 8 h before evaluation of porridges. They arrived at the sensory laboratory at 07:30 am daily and each session began with ratings of hunger level (how hungry do you feel right now?), fullness (how full do you feel right now?), desire to eat (how strong is your desire to eat right now?) and prospective food consumption (how much food do you think you could eat?) recorded on 100 -mm visual analog scales (VAS) on paper. The scales were anchored from “not much at all” on the left to “extremely much” on the right. Each participant evaluated the food privately in a sensory booth fitted with a web camera that was placed below the computer monitor. The video recording on the monitor was minimized to prevent distractions and eliminate factors that might influence eating behaviour.

At each breakfast session, each participant was served a 250g portion of one of the breakfast porridges and a stainless-steel teaspoon and was instructed to eat the full portion at their normal eating rate while being video recorded. Hunger, fullness, desire to eat and prospective food consumption as before were rated immediately after eating and at intervals of 30, 60, 90, 120, 150 and 180 min from end of consumption of the porridge.



The order of consuming the four porridges on different days was randomized over the panel. There was a total of eight sessions to obtain 2 replicates per panellist for each porridge type. The method by Forde et al. (2013a) was used for video-data collection. A total of 120 video recordings were coded for oral processing characteristics using the linguistic annotator software (ELAN 4.9.1, Max Planck Institute for Psycholinguistics, The Language Archive, Nijmegen, The Netherlands). The number of bites was coded as a key point event while total oral exposure time was coded as a continuous event. Eating rate (g/min) was calculated by dividing the mass of food consumed by the total oral exposure time. Bite size (g/bite) was calculated by dividing the mass of porridge served by the number of bites it took to consume the food. Bite rate ( $s^{-1}$ ) was calculated by dividing the number of bites taken to consume the porridge by the total oral exposure time. Calorie velocity was estimated as eating rate multiplied by energy density. All videos were coded by a single coder. Each participant received ZAR 70.20/ h for the time (total 8 h) spent to eat the breakfast porridge in the sensory laboratory.

### **3.3.3 Statistical analyses**

All data were analysed using IBM SPSS Statistics 20 for Windows (Armonk, NY, USA). Significance was set at  $P < 0.05$  and means were separated using Fisher's least significant difference (LSD) test. Mean descriptive panel ratings per replicate was subjected to analysis of variance (ANOVA) with porridge type as the independent variable. Panel mean scores of the attributes were subjected to principal component analysis (PCA) using the correlation matrix described by Borgognone *et al.*, (2001). One-way ANOVA was used to analyse differences in oral processing characteristics of porridges. The satiety related measures (hunger, desire to eat, fullness and prospective consumption) were assessed by a mixed model repeated measures ANOVA with treatment (porridge type) and time as repeated factors. Graphical curves were drawn as a function of time for each satiety measure. The total area under curve was calculated from the post meal consumption time points of each satiety measure using the trapezoid rule. Associations between satiety related perceptions and oral processing characteristics were evaluated by calculating Pearson correlation co-efficient.

### 3.3.4. Results

#### 3.3.4.1 Descriptive sensory evaluation

Thirty descriptors describing aroma, appearance, mouthfeel and aftertaste were generated to characterize the sensory properties of cassava-soy porridges with and without wheat bran (Table 3.3.2). The effect of wheat bran addition (0, 10 and 20%) in the porridges on descriptive sensory ratings is shown in Table 3.3.3.

Bran aroma was perceived more in the composite porridge with 20% wheat bran and the commercial product compared to the porridges with no or 10% wheat bran. Starchy aroma was strongly but equally perceived in all four porridges. The addition of wheat bran reduced cassava aroma in the porridge. The intensity of earthy, soy and toasted nut aroma was not significantly ( $p>0.05$ ) different in porridges with and without wheat bran. In terms of appearance, the addition of wheat bran increased the visually perceived viscosity and presence of particles but reduced the glossiness of the porridges. There was no significant ( $p>0.05$ ) difference in the brown colour and stickiness of the porridges including the commercial product.

As expected the intensity of flavour attributes of the commercial product differed significantly ( $p>0.05$ ) from the experimental porridges. The experimental porridges with and without wheat bran did not differ in flavour attributes. It has been reported that the addition of thickener can only modify flavour of foods if added at a concentration higher than its critical concentration  $c^*$ . Below the  $c^*$  value, the presence of macromolecules did not affect flavour perception (Tournier *et al.*, 2007). Wheat bran addition increased the coarseness and thickness of porridges. However, the intensity of other mouthfeel and texture attributes were not significantly ( $p>0.05$ ) different among the composite porridges and commercial product.

Bran aroma was perceived more in the composite porridge with 20% wheat bran and the commercial product compared to the porridges with no or 10% wheat bran. Starchy aroma was strongly but equally perceived in all four porridges. The addition of wheat bran reduced cassava aroma in the porridge. The intensity of earthy, soy and toasted nut aroma was not significantly ( $p>0.05$ ) different in porridges with and without wheat bran. In terms of appearance, the addition of wheat bran increased the visually perceived viscosity and presence of particles but reduced the glossiness of the porridges. There was no significant ( $p>0.05$ ) difference in the brown colour and stickiness of the porridges including the commercial product.

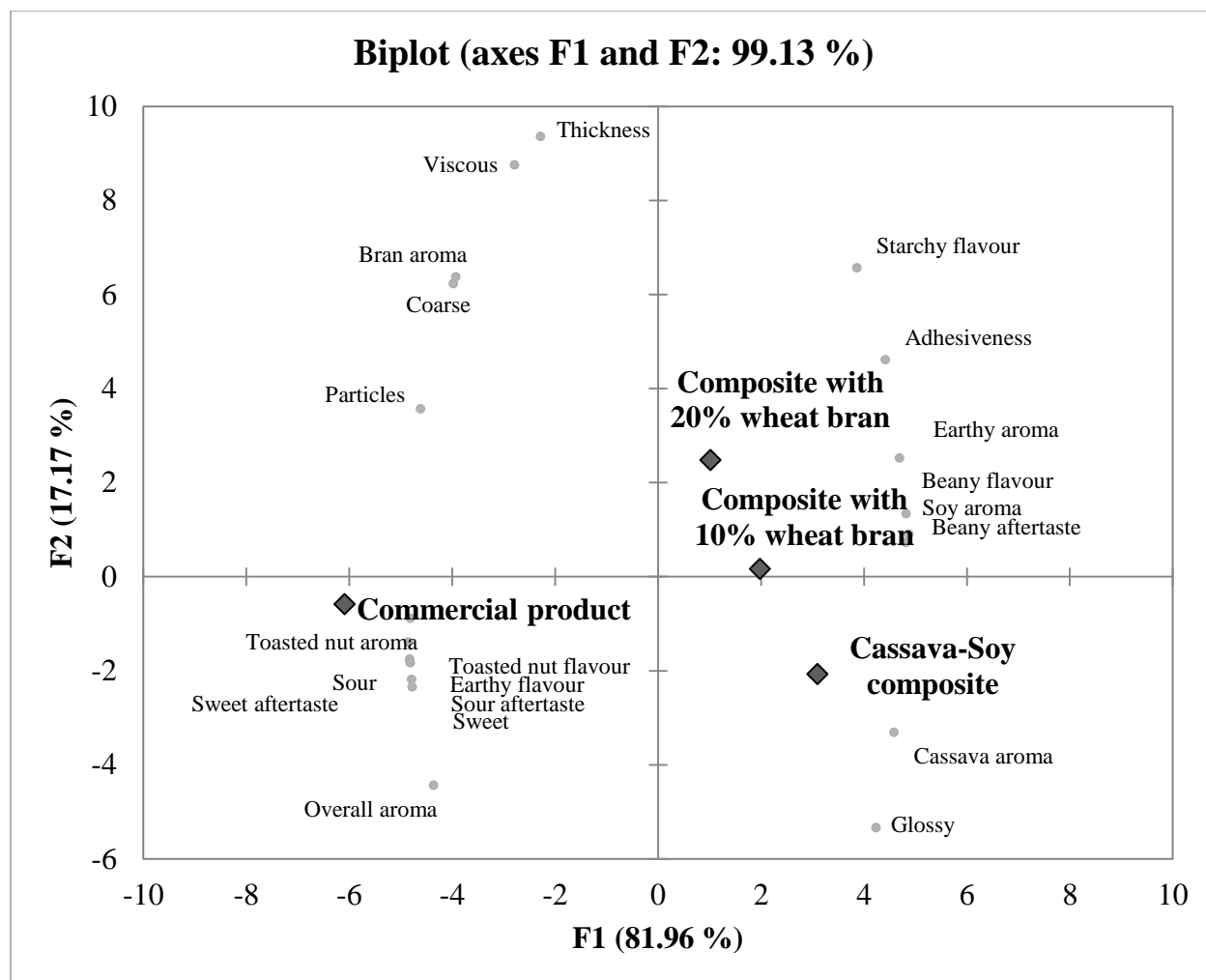
**Table 3.3.3.** Effect of wheat bran addition at 0, 10 and 20% levels to extrusion cooked cassava- soy composites (20% solids) on mean ( $\pm$  standard deviation) descriptive sensory ratings of prepared porridges

Sensory attribute		Cassava-soy composite	Composite with 10% added wheat bran	Composite with 20% added wheat bran	Commercial product	<i>P</i> -value
Aroma	Overall aroma	6.5 $\pm$ 0.0 ab	6.6 $\pm$ 0.1 ab	6.1 $\pm$ 0.5a	7.8 $\pm$ 0.5 b	0.033
	Earthy aroma	4.2 $\pm$ 0.0 b	4.6 $\pm$ 0.1 b	4.3 $\pm$ 0.1 b	2.1 $\pm$ 0.1 a	0.000
	Toasted nut aroma	2.6 $\pm$ 0.4 a	3.3 $\pm$ 0.4 a	3.0 $\pm$ 0.7 a	5.6 $\pm$ 0.3 b	0.010
	Starchy aroma	3.8 $\pm$ 0.0 a	4.6 $\pm$ 0.7 a	4.6 $\pm$ 0.2 a	3.0 $\pm$ 0.3 a	0.051
	Bran aroma	2.7 $\pm$ 0.0 a	3.8 $\pm$ 0.2 a	5.0 $\pm$ 0.4 b	5.5 $\pm$ 0.4 b	0.002
	Soy aroma	5.2 $\pm$ 0.3 b	5.0 $\pm$ 0.1 b	4.6 $\pm$ 0.4 b	0.9 $\pm$ 0.2 a	0.000
	Cassava aroma	4.2 $\pm$ 0.8 b	2.7 $\pm$ 0.6 ab	2.3 $\pm$ 0.6 ab	0.4 $\pm$ 0.1 a	0.013
Appearance	Brown colour	6.5 $\pm$ 1.1 a	6.3 $\pm$ 0.1 a	6.2 $\pm$ 0.1 a	6.2 $\pm$ 0.1 a	0.926
	Glossy	8.2 $\pm$ 0.0 d	6.3 $\pm$ 0.3 c	4.7 $\pm$ 0.2 b	3.2 $\pm$ 0.0 a	0.000
	Viscosity	3.4 $\pm$ 1.6 a	6.4 $\pm$ 0.7 b	8.5 $\pm$ 0.1 d	8.0 $\pm$ 1.1 c	0.007
	Sticky	5.5 $\pm$ 0.2 a	5.5 $\pm$ 0.5 a	6.5 $\pm$ 0.6 a	5.6 $\pm$ 1.1 a	0.416
	Particles	0.7 $\pm$ 0.0 a	3.0 $\pm$ 0.5 b	4.9 $\pm$ 0.3 c	8.5 $\pm$ 0.2 d	0.000
Flavour	Sweet	1.3 $\pm$ 0.2 a	1.3 $\pm$ 0.1 a	1.2 $\pm$ 0.2 a	6.6 $\pm$ 0.6 b	0.000
	Sour	1.6 $\pm$ 0.1 a	1.9 $\pm$ 0.2 a	1.8 $\pm$ 0.2 a	5.7 $\pm$ 1.2 b	0.007
	Umami	2.7 $\pm$ 0.8 a	3.1 $\pm$ 0.9 a	2.7 $\pm$ 0.4 a	2.0 $\pm$ 0.4 a	0.292
	Starchy flavour	4.6 $\pm$ 0.6 ab	5.2 $\pm$ 0.1 ab	5.5 $\pm$ 0.1 b	3.2 $\pm$ 0.9 a	0.049
	Earthy flavour	2.6 $\pm$ 0.5 a	2.7 $\pm$ 0.1 a	2.7 $\pm$ 0.6 a	4.8 $\pm$ 0.0 b	0.011
	Toasted nut flavour	2.5 $\pm$ 0.6 a	2.7 $\pm$ 0.1 a	2.7 $\pm$ 0.6 a	4.8 $\pm$ 0.0 b	0.016
	Beany flavour	5.4 $\pm$ 1.1 b	5.7 $\pm$ 0.7 b	5.0 $\pm$ 0.1 b	1.2 $\pm$ 0.6 a	0.009
Mouthfeel And Texture	Coarse	0.6 $\pm$ 0.1 a	2.8 $\pm$ 0.2 b	5.0 $\pm$ 0.8 c	6.1 $\pm$ 0.3 c	0.001
	Thickness	2.7 $\pm$ 0.7 a	5.0 $\pm$ 0.2 b	8.7 $\pm$ 0.1 d	6.9 $\pm$ 0.1 c	0.000
	Adhesiveness	5.1 $\pm$ 0.1 b	5.4 $\pm$ 0.0 b	5.7 $\pm$ 0.1 b	3.4 $\pm$ 0.8 a	0.019
	Astringent	3.3 $\pm$ 0.4 a	3.9 $\pm$ 0.3 a	4.0 $\pm$ 0.3 a	3.5 $\pm$ 0.8 a	0.430
Aftertaste	Sour aftertaste	1.7 $\pm$ 0.4 ab	1.6 $\pm$ 0.0 a	1.8 $\pm$ 0.2 a	4.2 $\pm$ 1.1 b	0.031
	Astringent aftertaste	2.9 $\pm$ 0.8 a	3.5 $\pm$ 0.2 a	3.4 $\pm$ 0.2 a	3.4 $\pm$ 1.0 a	0.780
	Beany aftertaste	4.3 $\pm$ 1.3 b	4.6 $\pm$ 0.6 b	3.7 $\pm$ 0.6 ab	0.6 $\pm$ 0.1 a	0.022
	Sweet aftertaste	1.0 $\pm$ 0.1 a	1.0 $\pm$ 0.0 a	1.0 $\pm$ 0.0 a	5.0 $\pm$ 0.8 b	0.002

Values are mean ratings for a trained panel (n=12)  $\pm$  standard deviation. The definition of attributes is shown in Table 3.3.2. Values within the same row followed by the same letter are not significantly different (p<0.05)

Only the intensity of beany aftertaste was rated significantly higher in cassava-soy with and without wheat bran compared to the commercial sorghum product used as standard. All the other aftertaste attributes were rated low in the composite porridges while the commercial sorghum product was rated significantly ( $p>0.05$ ) higher.

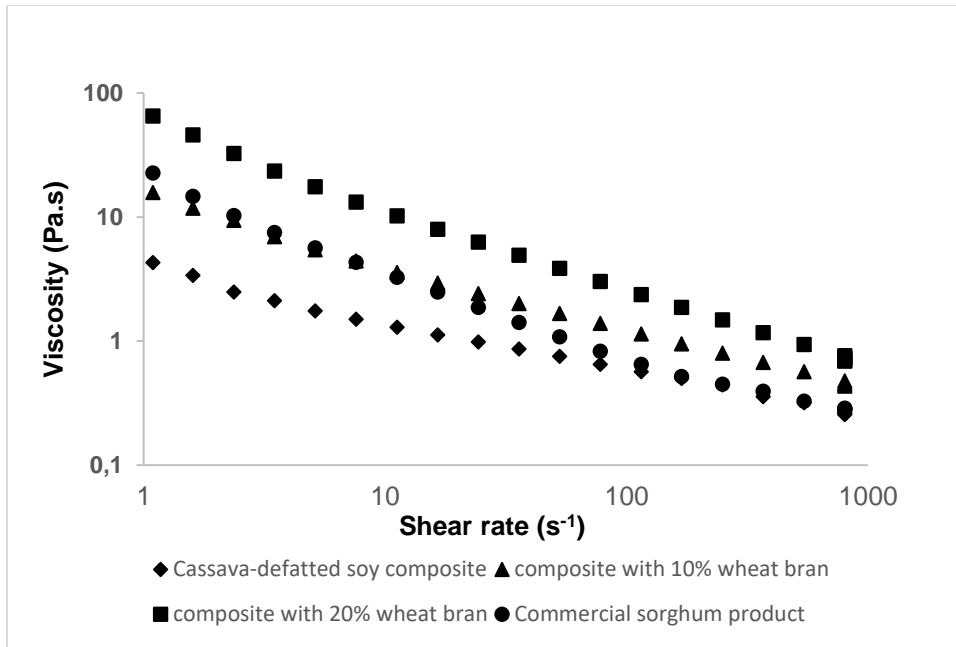
Principal component analysis (PCA) was used to summarize the variations in descriptive sensory attributes of the porridges (Figure 3.3.1). The first 2 PCs described 99% of the variation in the porridges. The first PC explained 82% of the variation. The commercial sorghum product located on the left was differentiated from the cassava-soy composites with and without wheat bran on the right. The porridge on the left was characterized as sweet, with sour aftertaste, with earthy and toasted nut flavour. The cassava composite with and without wheat bran on the right were characterized as more starchy, beany, adhesive, with more soy, cassava and earthy aroma. The second PC accounted for the other 17% of the variation. Here, porridges with 10 and 20% wheat bran at the top of the plot were separated from the commercial product and the composite with no wheat bran at the bottom by attributes thickness, viscosity, coarseness, with many particles characterizing the former and stronger overall aroma particularly characterizing the commercial product.



**Figure 3.3.1.** Principal component analysis (PCA) loading of extruded cassava-soy composite porridge with and without wheat bran and commercial sorghum product

### 3.3.4.2 Dynamic viscosity

The viscosity measurements of the porridges is shown in Figure 3.3.2. Upon reconstitution of extrudates, high viscosity was observed with increasing wheat bran addition from 10 to 20%, while cassava-soy extrudate with no wheat bran was the least viscous at all shear rates. The commercial sorghum product used as standard was more viscous than the porridge with no wheat bran but less viscous compared to the porridges with wheat bran.



**Figure 3.3.2.** Apparent viscosity of porridges at 50 °C as a function of shear rate

### 3.3.4.3 Variability in oral processing characteristics across porridges

Table 3.3.4 summarizes the main oral processing characteristics for each porridge. There was no significant difference ( $p>0.05$ ) in the average number of bites, bite sizes and bite rates used to consume a 250g portion of all the breakfast porridges. It took significantly more time ( $p<0.05$ ) to orally process and complete the consumption of the porridge with 20% wheat bran compared to the other porridges. This suggests that the porridge with 20% wheat bran had a slower eating rate.

The calorie velocity which provides an estimate of the rate of calorie intake within a meal was between 59.3 and 83.2 kcal/g/min. The porridge with 20% wheat bran had the lowest calorie velocity. This suggests that the rate of calorie intake while eating the porridge with 20% wheat bran was low compared to the other porridges.

**Table 3.3.4.** Means ( $\pm$  standard deviation) of oral processing characteristics of composite porridges and commercial sorghum product

Treatment	Bites	Oral Processing time (s)	Total meal duration (s)	Average bite size (g/bite)	Eating rate (g/s)	Bite rate ( $s^{-1}$ )	Calorie velocity (Kcal/g/min)
Cassava-Soy composite	17.0a $\pm$ 2.1	111.1a $\pm$ 33.0	145.4a $\pm$ 39.9	16.0a $\pm$ 4.4	1.9b $\pm$ 0.5	0.12a $\pm$ 0.03	83.2b $\pm$ 23.4
Composite with 10% wheat bran	17.4a $\pm$ 5.0	132.5a $\pm$ 40.9	156.1a $\pm$ 44.7	15.0a $\pm$ 3.1	1.7b $\pm$ 0.5	0.12a $\pm$ 0.02	76.9b $\pm$ 22.2
Composite with 20% wheat bran	19.5a $\pm$ 3.7	162.5b $\pm$ 43.0	189.0b $\pm$ 44.6	13.6a $\pm$ 3.5	1.4a $\pm$ 0.3	0.11a $\pm$ 0.02	59.3a $\pm$ 15.1
Commercial product	17.2a $\pm$ 4.4	119.7a $\pm$ 35.4	147.3a $\pm$ 39.0	15.4a $\pm$ 3.2	1.8b $\pm$ 0.5	0.12a $\pm$ 0.03	81.6b $\pm$ 24.2
<i>F</i> value	2.1	10.3	6.9	2.3	5.8	5.7	7.7
df	3	3	3	3	3	3	3
<i>P</i> value	0.107	0.000	0.000	0.082	0.001	0.001	0.000

Values are means  $\pm$  standard deviation. Values within the same column followed by different letters are statistically different ( $p < 0.05$ )

### 3.3.4.4 Inter-relationships between the various oral processing characteristics

Table 3.3.5 shows the correlations between oral processing variables. Oral processing time correlated significantly but weakly with number of bites ( $r= 0.60, p = 0.01$ ) and strongly with total meal duration ( $r= 0.97, p = 0.01$ ) such that as the oral processing time increased the number of bites and total meal duration also increased. A higher oral processing time (s) was negatively and strongly associated with a slower eating rate (g/min) ( $r= -0.91, p = 0.01$ ), weakly with a lower bite rate ( $s^{-1}$ ) ( $r= -0.53, p = 0.01$ ) and smaller average bite size (g/bite) ( $r= -0.61, p = 0.01$ ). As the bite rate ( $s^{-1}$ ) increased the total meal duration also decreased ( $r= -0.60, p = 0.01$ ). The results obtained are in line with previous reports of inter-relationships between oral processing parameters (Forde *et al.*, 2013, 2017; Ferriday *et al.*, 2016; Bolhuis *et al.*, 2014).

**Table 3.3.5.** Inter-relationships (Pearson’s partial correlations) between oral processing variables

	Bites	Oral processing time (s)	Total meal duration (s)	Average bite size (g/bite)	Eating rate (g/min)
Bites	-				
Oral processing time (s)	0.60**	-			
Total meal duration (s)	0.62**	0.97**	-		
Average bite size (g/bite)	-0.96**	-0.61**	-0.61**	-	
Eating rate (g/min)	-0.62**	-0.91**	-0.95**	0.65**	-
Bite rate ( $s^{-1}$ )	0.25	-0.53**	-0.60**	-0.25	0.60**

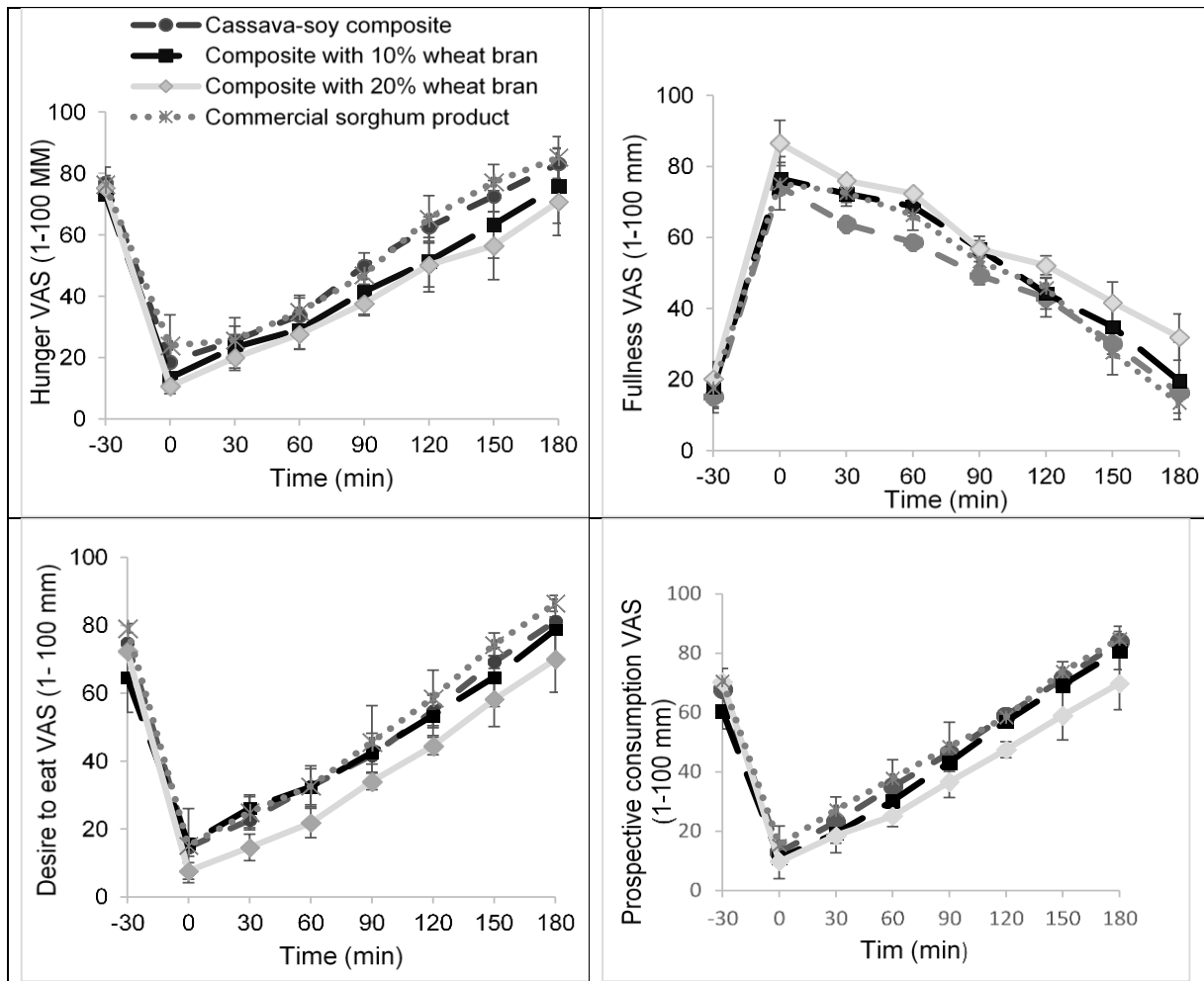
\*\*Correlation is significant at 0.01 level. All values are rounded to two decimal places

### 3.3.4.5 Satiety related ratings

The mean visual analog scale (VAS) scores for hunger, fullness, desire to eat and prospective consumption post porridge ingestion completed every 30 min throughout the test period are shown in Figure 3.3.3. All test porridges led to significant changes from baseline ( $p<0.01$ ) for all satiety measures rated. Hunger decreased as a result of consuming the porridge and then gradually increased for all test porridges from after consumption up to 3 h. Overall, the area under curve (AUC) of hunger and prospective consumption were influenced by porridge type as the composite



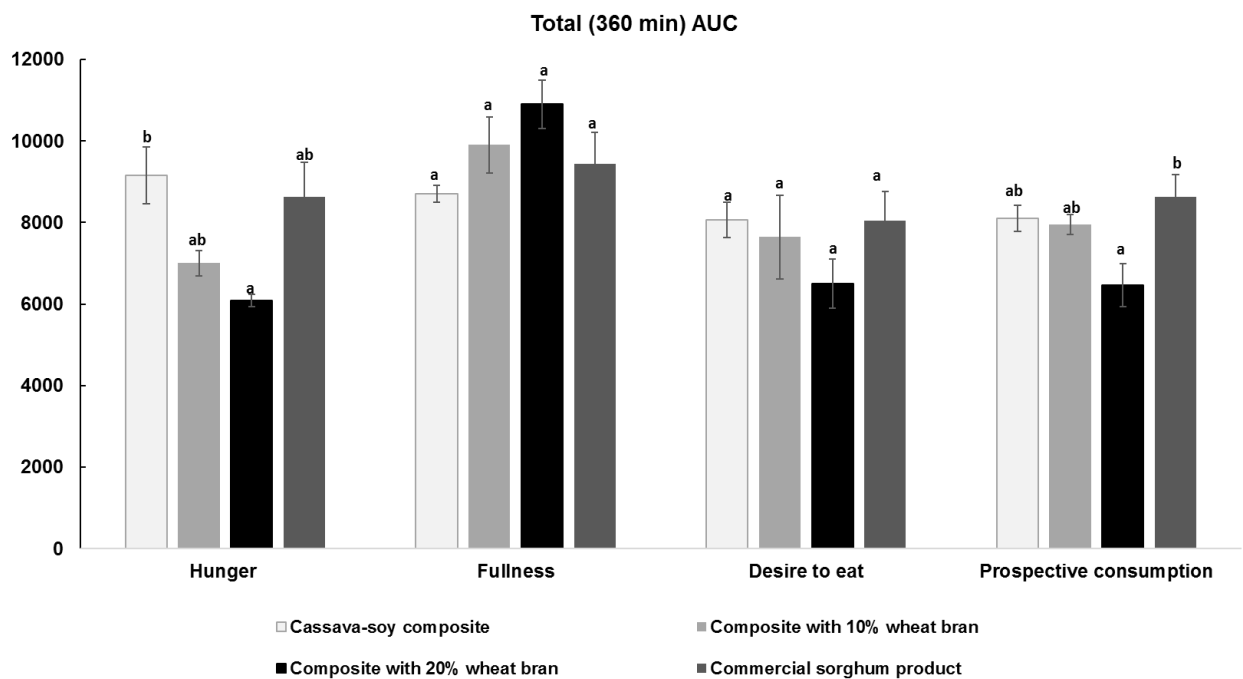
with 20% wheat bran led to greater reductions in hunger ( $p = 0.029$ ) and prospective consumption ( $p = 0.030$ ) post consumption compared to the other porridges.



**Figure 3.3.3.** Mean VAS scores of hunger, fullness, desire to eat and prospective consumption following the consumption of porridges

Statistically, there was no difference ( $p > 0.05$ ) in the total AUC for fullness and desire to eat post consumption of all porridges (Figure 3.3.4). Repeated measures ANOVA revealed a significant treatment effect for hunger ratings ( $p < 0.008$ ) wherein hunger ratings were lower after ingestion of the porridge with 20% wheat bran compared to the other porridges. Fullness ratings were higher after consuming the porridges with 10 and 20% wheat bran compared to the other porridges ( $p < 0.022$ ), the desire to eat was lower after consuming the porridges with 10 and 20% wheat bran ( $p < 0.013$ ) and prospective consumption was lower after the ingestion of the composite porridge

with 20% wheat bran ( $p < 0.022$ ) compared to the other porridges. A post consumption effect of time was also observed for all the satiety measures. There was a change in the ratings for all satiety measures over the three hours after eating the meal; hunger ratings ( $p < 0.0001$ ) and fullness ratings decreased ( $p < 0.0001$ ), while the desire to eat increased ( $p < 0.0001$ ) with time. As expected, when asked, “how much food do you think you could eat?”, panellists were more eager to consume more as the time lapsed post consumption of any of the porridges ( $p < 0.0001$ ). There were no significant porridge type  $\times$  time interactions for all the satiety related measures.



**Figure 3.3.4.** Total area under curve (AUC) for hunger, fullness, desire to eat and prospective consumption of duplicate porridge ingestion. Different letters above each bar represent statistically significant differences between porridges ( $p < 0.05$ )

### 3.3.4.6 Relationship between oral processing characteristics and satiety related measures.

Table 3.3.6 summarizes the correlations between some oral processing characteristics and satiety related measures. The subjective hunger ratings reported correlated negatively and strongly with fullness ( $r = -0.81$ ,  $p = 0.013$ ), weakly with desire to eat ( $r = 0.63$ ,  $p = 0.05$ ) and strongly with prospective food consumption ( $r = 0.79$ ,  $p = 0.02$ ). Oral processing time was negatively correlated with hunger ( $r = -0.80$ ,  $p = 0.05$ ), fullness ( $r = -0.91$ ,  $p = 0.01$ ), desire to eat ( $r = -0.84$ ,  $p = 0.01$ ) and prospect to consume another meal ( $r = -0.85$ ,  $p = 0.01$ ). Porridges that had longer oral exposure

were associated with lower hunger ratings post consumption ( $r = -0.80, p = 0.05$ ). Porridges eaten at a slower rate (g/min) were associated with greater post meal fullness ( $r = -0.87, p = 0.01$ ), led to lower desire to eat post ingestion ( $r = 0.92, p = 0.01$ ) and lower prospect to consume another meal post ingestion of porridges ( $r = -0.86, p = 0.01$ ).

**Table 3.3.6.** Correlations (Pearson’s partial correlations) between oral processing variables and satiety measures (hunger, fullness, desire to eat and prospective consumption)

	Hunger	Fullness	Desire to eat	Prospective consumption
Hunger	-			
Fullness	-0.81*	-		
Desire to eat	0.63*	-0.75*	-	
Prospective consumption	0.79*	-0.83*	0.82*	-
Oral processing time (s)	-0.80*	-0.91**	-0.84**	-0.85**
Eating rate (g/min)	0.74*	-0.87**	0.92**	-0.86**

\*Correlation is significant at 0.05 level

\*\*Correlation is significant at 0.01 level

### 3.3.5 Discussion

The bran aroma associated with composites with 10 and 20% wheat bran was as a result of the wheat bran that was added. The toasted nut aroma perceived in the composites (both with and without wheat bran) might be attributed to Maillard reaction products formed during extrusion cooking. Similarly, the brown colour observed in the cassava-soy containing porridges could be due to Maillard type reactions during extrusion cooking. Maillard reaction occurs between the free amino groups of proteins and the carbonyl group of reducing sugars at temperature between 140 – 165 °C (Singh, Gamlath and Wakeling, 2007) leading to browning and flavour development (Millward, 1999).

The porridge with 20% wheat bran was more viscous compared to the other porridges and the composite with no wheat bran was the least viscous. Extrusion cooking has been reported to promote depolymerization of insoluble dietary fibre and this leads to an increase in soluble dietary fibre portions (Brennan *et al.*, 2008a). Soluble dietary fibre in water forms a viscous fluid

(Schneeman, 2008). The more soluble dietary fibre in the extrudates with wheat bran may be responsible for the higher viscosity observed in the porridges (Oladiran and Emmambux, 2017).

This study assessed the short-term satiating effects and oral processing characteristics of extruded cassava-soy porridge with and without wheat bran. The oral exposure when consuming the porridge with 20% added wheat bran was significantly longer and consuming that meal took much longer. Oral exposure time of a 250 g porridge portion with 20% wheat bran was 46% longer than porridge with no wheat bran.

The porridge with 20% wheat bran had the highest viscosity and the descriptive sensory panel also described this porridge as being more viscous and thicker compared to the other porridges. A thicker product is eaten more slowly and stays longer in the oral cavity resulting in a longer oral exposure time (Zijlstra *et al.*, 2008). This was corroborated by the rate of eating the porridge with 20% added wheat bran which was also significantly lower than the other porridges.

The result of this study is in agreement with that of Zhu, Hsu and Hollis, (2013) who reported that increasing the viscosity of a semi-solid food by the addition of guar gum resulted in a slower eating rate and longer oral exposure time compared to a test product without guar gum. Also, Mattes and Rothacker, (2001) reported that the longer oral exposure time observed for the thick shake used in their study compared to the thin shake was due to the more viscous texture of the thick shake.

The data obtained in this study is consistent in showing that wheat bran addition in cassava-soy porridge can increase satiety. The porridge with 20% wheat bran suppressed hunger more and led to a greater decrease in prospect to consume food post consumption and over a 3 h period thereafter compared to the other porridges. In a similar study, Isaksson *et al.*, (2012), reported that consuming whole grain rye breakfast porridge increased feelings of fullness and decreased hunger compared to white wheat bread 4 h after consumption. The effect rye has on satiety was suggested to be mediated through stomach distention and delayed gastric emptying. Mathern *et al.*, (2009) also found that addition of fenugreek fibre to a breakfast porridge led to reduction in hunger and more fullness compared to a placebo breakfast and this was attributed to the viscosity of fenugreek which slowed down the rate of gastric emptying.

It has been indicated that a viscous beverage gives a different mouthfeel which may induce a sensation of fullness and satiety (Lyly *et al.*, 2009; Mattes and Rothacker, 2001). It can be

suggested that the thick and coarse mouthfeel of the porridge with 20% added wheat bran as described by the sensory panel may have contributed to its longer oral exposure time thus promoting feelings of satiety. The effects wheat bran had on satiety as observed in this study may be explained by several mechanisms, this includes; that the high water holding capacity of dietary fibre may have increased stomach distention thus triggering signs of satiety (De Graaf *et al.*, 2004). Another probable explanation could be that arabinoxylan, which is the water-soluble component of wheat bran increased the viscosity of intestinal content which delayed gastric emptying (Lafiandra, Riccardi, and Shewry, 2014). This consequently slowed down the rate of nutrient absorption in the small intestine thus giving rise to protracted feelings of fullness. Also, viscous fibres may form a barrier around undigested nutrients. This barrier inhibits or delays the activities of digestive enzymes by limiting their accessibility to the substrate for digestion and by also slowing down diffusion of nutrients. As a result, digestion and absorption would occur at a much slower rate (Brennan *et al.*, 1996) and this is consequently followed by a decline in feelings of hunger.

The satiating properties of the porridge with 20% wheat bran may also be attributed to cognitive influences during consumption of the porridge driven by sensory properties whereby the thicker porridge is expected to be more filling based on past eating experience. According to Chambers (2016), before food arrives in the gut, pre-ingestive signals from the consumer's expectations about that food (involving attention and memory processes), the pleasure they experience while eating it and the sensory appraisal of the food will influence not only how much is eaten at that eating episode (satiation) but also in the period after consumption. The satiety cascade model predicts that early pre-ingestive signals from cognitive and sensory processes are the main drivers of satiation and that cognitive, sensory, post-ingestive and post-absorptive signals are combined to determine the experience of satiety (Chambers *et al.*, 2015).

In this study, strong correlations were observed between oral exposure and the satiety measures. De Graaf and Kok, (2010) suggested that oral exposure is essential to achieve optimal satiety. A longer oral exposure of food was found to decrease food intake (Wijlens *et al.*, 2015; Bolhuis *et al.*, 2011). The feelings of satiety are increased through longer oro-sensory exposure through inhibition of gastric emptying rate (Smeets, Erkner and De Graaf, 2010).

The result of this study is in support of similar studies (Chambers *et al.*, 2015) which reported that dietary fibre has more satiating effects compared to other food macronutrients. The porridge with 20% wheat bran contained more dietary fibre hence the suppressive effect elicited on hunger. The *ad libitum* intake at lunch post 3 h of porridge consumption and time to the next meal were unfortunately not measured. Future work on the effect of porridge that contains wheat bran on subsequent food intake should be carried out as this would help to elucidate on the effect of dietary fibre on *ad libitum* energy intake at the following lunch meal.

The palatability of the porridges was not assessed before or during the oral processing test and this may be a limitation of this study. It has been demonstrated that macronutrient composition influences palatability (McCrary *et al.*, 2006), and eating rate has been reported to be increased by palatability (Yeomans *et al.*, 1996). One can therefore not rule out a possible effect of taste pleasantness of porridge on eating rate. In future studies, the palatability and consumer acceptability of extruded porridges with wheat bran should be conducted to assess how the instant porridge is perceived by consumers and how its hedonic properties may be influencing the ingestion of the porridge.

### **3.3.6 Conclusions**

Extruded cassava-soy porridge that contains wheat bran prolongs oral exposure time and increases satiety. The variations in oral processing of a fixed mass portion of extrusion cooked cassava-soy porridge with and without wheat bran is probably related to the difference in viscosity of the porridges. The solubilization of insoluble dietary fibre in wheat bran during extrusion cooking increases viscosity of porridge, the high viscosity prolongs oral exposure time and this in turn promotes feelings of satiety. This study therefore shows that wheat bran as a source of dietary fibre has the potential to be incorporated into starch-rich foods with the use of extrusion cooking to produce instant products useful for appetite control.

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## **4.0 General Discussion**

This section will first provide a critical review of some of the methodologies used in this study. Subsequently, the main findings in this research will be examined. This will comprise of the effect of extrusion cooking and addition of either wheat bran or grape pomace on the nutritional, functional, potential health promoting and sensory properties of cassava-composite. A mechanism by which the changes in functionality of extrudates occurred during processing and how this relates to the nutritional properties and physiological functions of the extrudates is proposed.

### **4.1 Methodology**

#### **4.1.1 Selection of raw materials**

The use of food crops indigenous and locally available could be ideal to address the problem of diet-related NCDs. This is because they are generally available in sufficient quantity. Cassava, soy bean, wheat bran and grape pomace were used in this study. Cassava is an important crop in Africa. The FAO (2013) reported that sub-Saharan Africa produces more cassava than any other crop. About 140 million metric tonnes of cassava was produced in 2011 compared to 65 million metric tonnes of maize produced in the same year (FAO, 2011). Additionally, cassava is relatively easy to grow and its tolerance to adverse environmental condition such as drought and poor soil makes it an important food security crop useful in combatting hunger in developing countries (Zidenga *et al.*, 2012). Cassava utilization has advanced from subsistence and household utilization to being used in industrialized commodities (UNIDO, 2006). High quality cassava flour (HQCF) is mostly used for industrial purposes. HQCF is unfermented cassava flour with a pH of between 5.5 – 7.0 and acidity of  $\leq 0.25\%$  (EAC, 2012). The flour is without flavour nor odour and has a particle size of between 250 – 500  $\mu\text{m}$  (Dziedzoave *et al.*, 2006).

Defatted toasted soy flour was used in this study due to its minimal fat content compared to industrial full fat soy flour that has a fat content of about 18 -21%. The high fat content in the full fat flour could promote rancidity in extruded products during storage prior to analysis. Wheat bran is also a major by-product of wheat milling which is used in food supplement and livestock feeding (Onipe *et al.*, 2015). Wheat is the second most cultivated crop in South Africa after maize. About 1.8 million tonnes of wheat was produced in South Africa in 2011 (DAFF, 2015) and South Africa

is the 4<sup>th</sup> largest producer of wheat in Africa (FAO, 2016). Around 90% of wheat bran is used in livestock feeding while only 10% is used in food industry as fibre source (Hossain *et al.*, 2013). The wine industry generates a large amount of grape by-product such as grape pomace that is regarded as food waste. About 25% of the grape weight results in by-product or waste (Dwyer, Hosseina and Rod, 2014). There is a huge potential for the use of food by-products that are regarded as functional ingredients to be incorporated into food products. The use of these mentioned food materials would ensure and promote the use of food raw materials that are locally and readily available for utilization in Africa.

#### **4.1.2 Analytical methods**

##### ***4.1.2.1 Protein digestibility***

Protein digestibility is determined using biological or enzymatic methods. The pepsin and the multi-enzyme methods are commonly applied in *in-vitro* protein digestibility determination. The pepsin method is a single enzyme assay. This method requires initial protein content determination in the test food. This is followed by digestion using pepsin at specific conditions that simulate the stomach condition. The residual protein content as insoluble protein in the digested test food is determined and expressed as a percentage of the initial protein content (Hamaker, 1987). One of the drawbacks of this method is that it could underestimate protein digestibility for proteins that are resistant to pepsin because of their primary structure or due to acid stability of their tertiary structure (Swaisgood and Catignani, 1991).

The multi-enzyme method was used in this study. The multi-enzyme method closely mimics protein digestion in humans (Hsu *et al.*, 1977). This method involves the combined use of 3 enzymes; trypsin, chymotrypsin and porcine intestinal peptidases in determination of protein digestibility (Hsu *et al.*, 1977). The principle behind the multi-enzyme assay is that protons are released from cleaved peptide bonds during proteolysis and this results in decrease of pH of the *in-vitro* system. This method assumes that the rate of change in pH is correlated with protein digestibility and there is a direct relationship between the observed pH drop and protein hydrolysis (Moughan *et al.*, 1989).

The multi-enzyme technique requires short time, it has a high degree of sensitivity and is very effective in predicting apparent digestible protein of food samples and products (Hsu *et al.*, 1977).

Contrary to the pepsin method, multi-enzyme technique estimates protein hydrolysis in the stomach, small intestine and hind gut of simple stomach of animals. This infers that more bonds will be hydrolysed by the multi-enzyme than the pepsin method. The limitation associated with the multienzyme method is that components of food materials may interfere with the pH drop due to their buffering capacity (Urbano *et al.*, 2005). However, good correlations ( $r > 0.8$ ) have been found with the multi-enzyme method and *in-vivo* digestibility (rat true faecal) method particularly when plant protein sources were analysed (Butt *et al.*, 2012). Therefore, *in-vitro* assay gives an estimate of protein digestibility and *in-vivo* assay would be required for absolute values.

#### ***4.1.2.2 Nitrogen solubility index***

Nitrogen solubility index (NSI) was determined to assess the soluble nitrogenous compounds in the extruded products. The reduction in nitrogen solubility observed upon extrusion cooking may be attributed to formation of di-sulphide and hydrophobic linkages due to high temperature in the extruder (Prudêncio-Ferreira and Arêas, 1993). The major storage proteins in legumes are globulins and they are soluble in salt solution (Kiosseoglou and Paraskevopoulou, 2011). The samples (raw and extruded) were dispersed in water at 30 °C and stirred at a low speed of 100 rpm for 30 min after which they were centrifuged twice with NaCl to extract all solubilized proteins. The filtrate was then freeze dried to concentrate soluble protein. This was done because preliminary studies on liquid extracts showed variations in result obtained possibly due to the dilution of soluble protein and this made it below the detection limit of the dumatherm (DT, Gerhardt Königswinter, Germany). The detection limit of the dumatherm is 0.01 mg N absolute. Freeze drying helped to improve the detection level as it concentrated the nitrogenous compounds and it also ensured repeatability of results.

#### ***4.1.2.3 Starch digestibility***

Starch is hydrolysed by amylolytic enzymes to produce glucose for energy supply in plants, animals and humans. Starch is successively hydrolysed by salivary and pancreatic  $\alpha$ -amylase in the mouth and small intestine, respectively, before being absorbed as glucose in the small intestine. Starch is hydrolysed by  $\alpha$ -amylase into maltose and malto-triose as end products as well as  $\alpha$ -limit dextrins, which contain branch points resistant to  $\alpha$ -amylase. The  $\alpha$ -amylase products are further

hydrolysed to glucose by the combined action of two brush border exo-hydrolase double-headed enzymes, maltase-glucoamylase (MGAM) and sucrase-isomaltase (SIM) (Nichols *et al.*, 2003).

There are several methods used in *in-vitro* digestibility of starch determination but the most common are the Englyst *et al.*, (1992) and Goni *et al.*, (1997) methods. The Goni *et al.*, (1997) method was used in this study. This method involves the use of  $\alpha$ -amylase and amyloglucosidase to digest starch while protein was digested using pepsin. The  $\alpha$ -1,4 glycosidic bonds in starch were hydrolysed by  $\alpha$ -amylase into dextrans and oligosaccharides. These were further broken down by amyloglucosidase into glucose. The use of  $\alpha$ -amylase and amyloglucosidase in conjunction closely stimulates the process of starch digestion in the small intestine where a larger percentage of starch are digested (Hasjim *et al.*, 2010).

The process of starch digestion in humans starts orally before the gastric and intestinal phases of digestion. The Goni *et al.* (1997) method used excludes the oral phase of digestion where the food is made into a bolus by mixing with saliva and the salivary  $\alpha$ -amylase begins the process of starch break down. The exclusion of this step may be due to certain concerns and which are for good reasons. Chewing is reported to raise practical issues when used in routine testing such as the differences in chewing rate, enzyme activity and saliva volume. All these variations may limit ability to obtain reproducible *in-vitro* digestion results (Englyst *et al.*, 1992; Germaine *et al.*, 2008).

The human chewing phase can be simulated using mechanical means (Germaine *et al.*, 2008). In this study, the extrudate was already milled and glass beads were included to mechanically disrupt the physical structure of the test food samples. Although, the form of the test food samples used in this study may not necessarily be a cause of concern due to the exclusion of the chewing phase of digestion. This is because the samples are instant porridges which requires minimal chewing compared to solid foods.

Certain food and human physiological factors such as digesta viscosity, gastric emptying rate and transit time through the gastro intestinal tract that affect starch digestion are not considered in the Goni *et al.*, (1997) method. Several *in-vivo* studies have shown that these afore-mentioned factors influence the rate of starch digestion (Turnbull *et al.*, 2005) and as such their oversight may be a limitation of the Goni *et al.*, (1997) method. However, the Goni *et al.*, (1997) is well correlated ( $R^2 = 0.952$ ) with *in-vivo* studies (Goni *et al.*, 1997).



The GI of the extruded products was estimated in order to give an indication of the effect of consuming cassava-defatted toasted soy porridge with and without wheat bran addition on postprandial glycaemia. The estimated glycaemic index (EGI) prediction equation based on a first order rate equation proposed by Goni *et al.*, (1997) was used in estimating glycaemic index of samples. The EGI values are based on their glycaemic effect compared with that of a standard food (Venter *et al.*, 2003). In this method, glycaemic index is calculated from the hydrolysis index. The hydrolysis index is calculated as the area under curve of test food as a percentage of the corresponding area under curve of reference sample. The Goni *et al.*, (1997) glycaemic index prediction model has been reported to give good EGI estimates (Germaine *et al.*, 2008).

#### **4.1.2.4 Soluble and insoluble dietary fibre**

There are two methods used in analysing dietary fibre: enzymatic - gravimetric and enzymatic-chemical methods. The food components vary depending on the method used. The enzymatic-chemical method involves extraction steps followed by hydrolysis of polysaccharides and subsequent colorimetric, gas chromatography (GC) or high-performance liquid chromatography (HPLC) use in analysing components of monosaccharides.

The AACCI approved method 32-05.01/AOAC methods 985.29 (Prosky *et al.*, 1985) and 32-07.01/AOAC method 991.43 (Lee *et al.*, 1992) consolidated by Megazyme (AOAC, 2007) was used in this study. Samples were cooked at  $\sim 100^{\circ}\text{C}$  with heat stable  $\alpha$ -amylase to give gelatinisation, hydrolysis and depolymerisation of starch. This was followed by enzymatic digestion with protease and amyloglucosidase to solubilize proteins and hydrolyse starch fragments to glucose respectively. The sample suspension was filtered, rinsed with water and the filtrate was kept for soluble dietary fibre determination. The residue was further washed with ethanol and acetone then dried overnight for insoluble dietary fibre determination. The filtrate was treated with four volumes of ethanol to precipitate soluble fibre and remove depolymerized protein and glucose. The precipitate was also dried overnight. A correction is then made for undigested protein and ash, and the result is expressed as a proportion of the starting material. This method allows for quantification of both soluble and insoluble dietary fibre separately. Some modifications were made to this method in this study; fibre-cap capsules were used for filtration instead of celite to retain insoluble dietary fibre. The fibre-cap capsule is a polypropylene container with a sieve size of 25  $\mu\text{m}$ . The membrane used in the capsule allows free flow of solvent through the sieve

during filtration, but not insoluble dietary fibre. The filtration of precipitate after ethanol addition for the soluble dietary fibre determination step was replaced with centrifugation.

The enzymatic-chemical method has been criticized as not very robust especially where low levels of dietary fibre are present, being expensive to set up especially with regards to GC and HPLC use and time consuming (Greenfield and Southgate, 2003). The enzymatic-gravimetric method is thought to give good precision with high fibre foods and whole grain products but with limitation of requiring great skill when measuring low levels of dietary fibre (Champ *et al.*, 2003). The components of dietary fibre measured by the AOAC 991.43 and AOAC 985.29 methods as used in this study are cellulose,  $\beta$ -glucan, galactomannan, arabinoxylan, pectin, arabinogalactan, some resistant starch, some inulin and some polydextrose (McCleary, 2013). There may be underestimation of soluble dietary fibre due to some low molecular weight soluble fibre not being able to precipitate in ethanol. Some of these soluble dietary fibres include galacto-oligosaccharides, fructo-oligosaccharides (McCleary, 2013).

#### ***4.1.2.5 Total phenolics and anti-oxidant activity***

Acidified methanol was used for extraction of phenolics from composite samples. There is no ideal extraction method to recover 100% of phenolics present in the food matrix (Naczki and Shahidi, 2006). The solubility of phenolics is dependent on several factors which includes; the polarity of solvent used, degree of polymerization of phenolics, interaction of phenolics with other food constituents and formation of insoluble complexes (Waterman and Mole, 1994). Acidified methanol was used due to its suitability in extracting bioactive compounds such as phenolic acids, anthocyanins, and flavonoids (Dykes *et al.*, 2005; Awika and Rooney, 2004).

The Folin-Ciocalteu method (Singleton and Rosi, 1965) was used to determine total phenolic content in this study. It is widely used for measuring phenolics content because of its simplicity and reproducibility (Macdonald-Wicks *et al.*, 2006). This assay measures concentration of phenolic hydroxyl group in extract (Waterman and Mole, 1994). Phenolic compounds react with the Folin-Ciocalteu reagent under basic conditions, through the dissociation of a proton from the phenolic hydroxyl group which leads to the formation of a phenolate anion (Macdonald-Wicks *et al.*, 2006). The phenolate ion reduces the Folin-Ciocalteu reagent (a yellow acidic solution

containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids) to a blue molybdenumtungsten complex (Abu Bakar, *et al.*, 2009)

The drawback associated with the Folin-Ciocalteu method is that it is not specific because it detects all phenols with varying sensitivity (Sun *et al.*, 1998b). It is subject to interference from non-phenolic reducing compounds such as ascorbic acid, reducing sugars, sulphur dioxide, amino acids with phenolic rings and some inorganic compounds (Phipps *et al.*, 2007). This interference could result in over-estimation of total phenolic content of the extracts. However, the Folin- Ciocalteu method is useful in that it gives a rough estimate of total phenolic content in plant-based samples (Everette *et al.*, 2010)

The ABTS radical cation decolourization assay was used to determine the antioxidant activity in terms of radical scavenging capacity of the extracts from composite with grape pomace. This assay measures the relative ability of an antioxidant to scavenge the ABTS<sup>•+</sup> radical compared to Trolox a water-soluble vitamin E analog (Awika *et al.*, 2003). This method is preferred for its rapid reaction time and can be used over a wide range of pH values (Arnao, Cano and Acosta, 1995). The ABTS assay is recommended for samples with pigmentation as there would be no interference of ABTS with spectra of coloured compounds (Almeida *et al.*, 2011). This makes this method suitable for use in this study

#### ***4.1.2.6 Apparent/dynamic viscosity***

The vane with cup was used to measure the apparent/dynamic viscosity of extruded porridge at different shear rate. The porridge (20% solids content) was cautiously poured into the cup to prevent formation of air bubbles that may interfere with viscosity measurement. A layer of paraffin oil was spread on top of the sample in the cup to prevent moisture loss. The porridge in the cup was allowed to equilibrate to ensure uniformity in temperature of porridge before the test was started. The vane probe was used because it is recommended for heterogenous compounds or material that may contain some suspended solids (Barnes and Nguyen, 2001) such as fibre particles. Advantages of using the vane probe includes that it allows measurements to be made in the absence of slips (Barnes and Nguyen, 2001). The geometry of the vane does not confine sample to a narrow angular gap that limits the largest acceptable particle in the sample (Barnes, 1995).

#### 4.1.2.7 Sensory properties

Sensory properties were only determined on the extruded composites with added wheat bran. This is because the grape pomace used in this study were sun dried and stored in an open space and its suitability for human consumption could not be guaranteed by the supplier. In future studies, grape pomace would be sourced directly from a winery and collected immediately after juice extraction. This would ensure the drying and storage of grape pomace are monitored and its suitability for human consumption can be guaranteed such that it can be used in sensory studies.

The descriptive sensory evaluation was used to determine the sensory attributes of composite porridges substituted with wheat bran. According to Einstein (1991), descriptive sensory evaluation is defined as ‘the identification, description and quantification of sensory attributes of a food product using human subjects who have been specifically trained for this purpose’. This infers that the ability of the panellists to precisely rate the sensory attributes of the food product is crucial to the success of the method. The panellists were trained to familiarize themselves with the test porridges, they generated the sensory attributes that best differentiated between the porridges. The intensity of the sensory attributes generated were rated in all the porridges. One of the major strengths of descriptive sensory evaluation is that it allows relationships between descriptive sensory and instrumental measurements to be determined (Murray *et al.*, 2001).

Oral processing is an important factor that contributes to satiety. The method by Forde *et al.* (2013a) was used in determining oral processing characteristics. Human subjects were video recorded while eating to determine the role of oral sensory exposure on satiety in this study. Number of bites was coded as a key event while oral exposure time was coded as a continuous event. Other oral processing characteristics such as bite rate, eating rate were calculated.

Satiety is a complex process influenced by a number of factors which includes food form, macronutrient composition, texture (Chambers, 2015), differences in individual endocrine levels (Austin and Marks, 2009) and environmental cues (De Graaf *et al.*, 2004). Despite these differences and factors which affects satiety, various studies have established that most people can relate to and identify variations in satiety sensations thus, enabling subjective assessments with a simple set of questions (Stubbes *et al.*, 2000). The visual analog scale (VAS) is commonly used in measuring subjective satiety sensations and it was used in this study. The four satiety related

measures namely; hunger, fullness, desire to eat, and prospective consumption developed by Roger and Blundell (1979) were assessed on a 100-mm VAS over a 3 h period at 30 min interval. The major drawback of this method is that the same four questions are repeated at every time interval and this could bore the participants. Therefore, boredom may influence how subjects rate satiety sensations. Some of the advantages though are the method is easy to use and the subjective rating has been shown to be repeatable and sensitive to exposure of food components (Flint *et al.*, 2000; Stubb *et al.*, 2000).

## **4.2 Research findings and future work**

This section would first discuss the effects of addition of wheat bran or grape pomace during the extrusion cooking of cassava- defatted toasted soy composite on the nutritional and functional properties. Secondly, how these changes in nutritional and functional properties influenced sensory properties of instant porridges will be discussed.

### **4.2.1 Nutritional and functional properties**

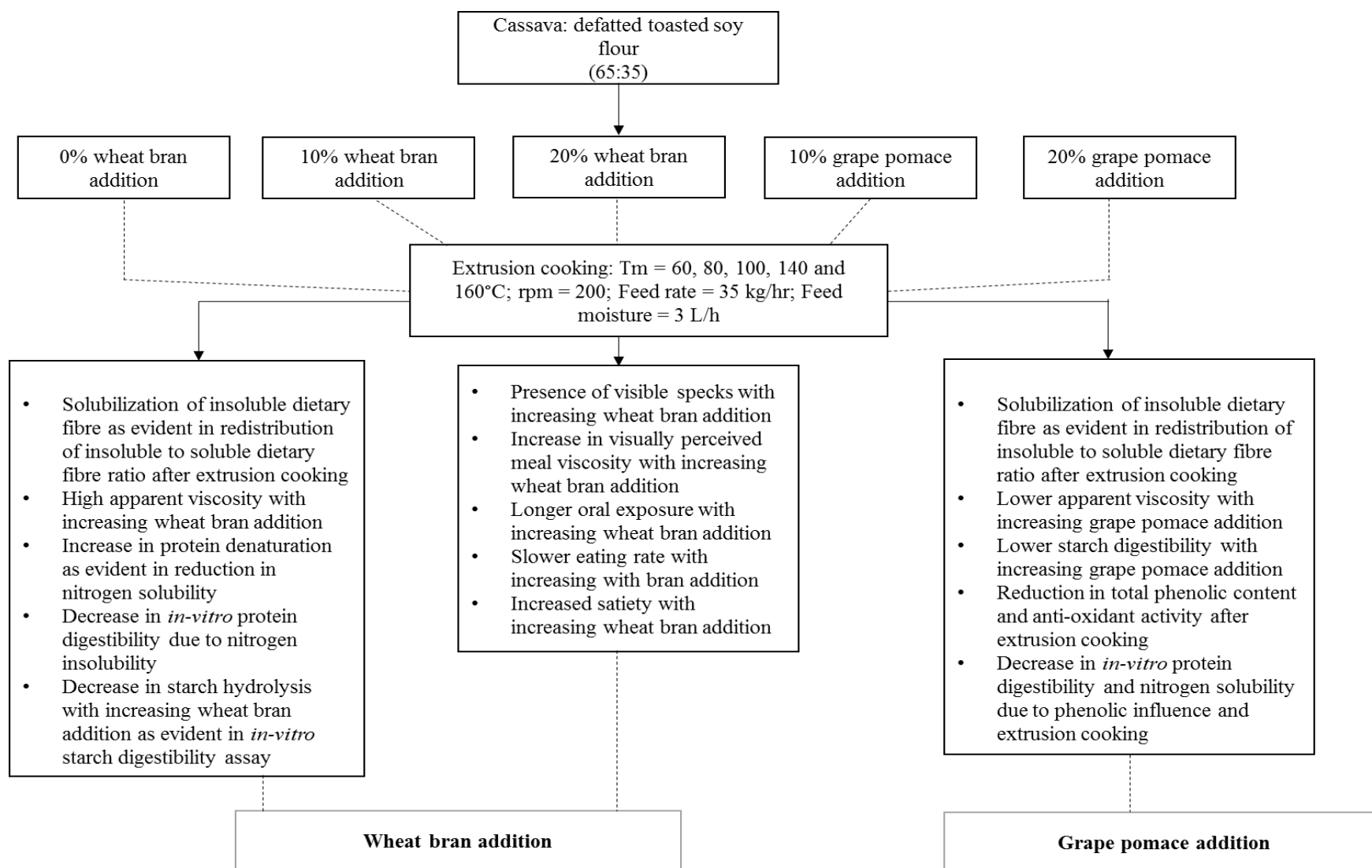
The comparison of decrease in *in-vitro* starch digestibility (IVSD) and *in-vitro* protein digestibility (IVPD) of extruded cassava-defatted toasted soy composites due to the addition of different dietary fibre sources used in this study is shown in Table 4.1. Although, there is really no basis of comparison between the two dietary fibre sources because grape pomace and wheat bran are made of different components, a comparison might help to suggest the mechanism of action of these fibre sources on IVSD and IVPD. The mechanism of action of decrease in IVSD and IVPD due to the different components of wheat bran and grape pomace on cassava-defatted toasted soy composite will be discussed in detail in a later part of this text.

**Table 4.1.** Comparison of percentage decrease in IVSD and IVPD of extruded cassava-defatted toasted soy composite with addition of either wheat bran or grape pomace.

	Addition level (%)	IVSD (% decrease)	IVPD (% decrease)
Wheat bran	10	2.3	0.4
	20	6.0	0.9
Grape pomace	10	13.6	5.1
	20	18.7	8.8

IVSD = *in-vitro* starch digestibility  
IVPD = *in-vitro* protein digestibility

A general representation of the effects of extrusion cooking and wheat bran or grape pomace addition on some nutritional, functional and sensory properties of extrudates is depicted in Figure 4.1.



**Figure 4.1.** Representation of effects of extrusion cooking and wheat bran or grape pomace addition on some nutritional, functional and sensory properties\* of cassava-soy extrudate. \*only wheat bran addition.

In this study, extrusion cooking led to redistribution of insoluble to soluble dietary fibre content of all composites with and without wheat bran or grape pomace. The mechanical shear in the extruder led to breakage of polysaccharide glycosidic linkages. The alteration in composition of dietary fibre in extrudates could be related to changes in molecular structure of dietary fibre during extrusion cooking. While solubilization of insoluble dietary fibre promoted an increase in dynamic viscosity of composites with added wheat bran, the composites with grape pomace had a lower dynamic viscosity compared to the control with no grape pomace despite the increase in soluble dietary fibre after extrusion cooking. This may be due to the differences in the dietary fibre composition, molecular configuration and amount of phenolic compounds present in wheat bran and grape pomace. The low viscosity in composites with grape pomace may be due to low molecular weight of soluble dietary fibre after extrusion cooking. It is possible that despite the solubilization of insoluble dietary fibre in grape pomace, the molecular weight was low and unable to promote an increase in viscosity of composites.

Svanberg *et al.*, (1995, 1997) reported that severe heat treatment of carrots led to depolymerization and loss of intermolecular association of water soluble polysaccharides. The consequent effect of this was low viscosity due to low molecular weight of water-soluble polysaccharides. In another study on the effects of guar gum flours of different relative molecular weight on viscosity in solutions and digesta, Roberts *et al.*, (1989) reported that low molecular weight guar gum had a much lower viscosity compared to the medium and high molecular weight guar gum solutions. It is suggested that there is a positive relationship between molecular weight and viscosity of dietary fibre in solution. The low molecular weight guar gum and water-soluble components of carrots may be short chain polymers which have low hydrodynamic volume hence the low viscosity.

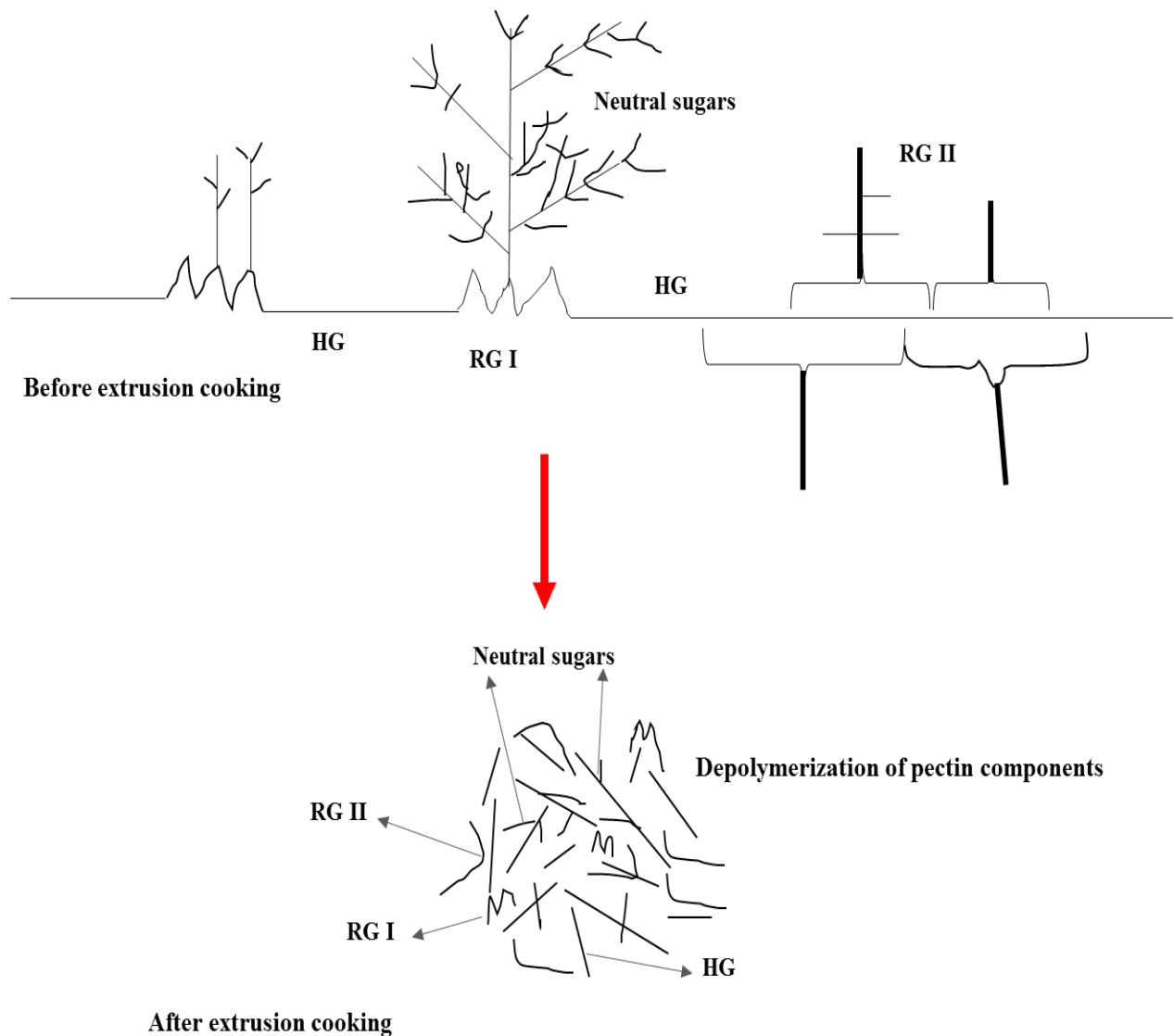
Pectin is one of the main soluble dietary fibre components of grape pomace. The primary structural feature of pectin is a linear chain of  $\alpha$ -(1,4) linked D-galacturonic acid units with varying degrees of methyl esterification (Sayah *et al.*, 2016). The pectin network is generally believed to be formed by three blocks of co-polymers, namely, homogalacturonan, rhamnogalacturonan I and the highly substituted rhamnogalacturonan II (O'Neil and York, 2003). Grapes have more rhamnogalacturonan I backbone than rhamnogalacturonan II. The rhamnogalacturonan I region consists of disaccharide repeating units of  $\alpha$ -(1,4)-D-galacturonosyl ( $\alpha$ GalA) and  $\alpha$ -(1,2)-L-rhamnogalacturonosyl ( $\alpha$ Rha) residues with side chains and branching comprised primarily neutral



sugars;  $\alpha$ -L-arabinofuranosyl ( $\alpha$ Araf) and  $\beta$ -D-galactopyranosyl ( $\beta$ Galp) residues (Hanlin *et al.*, 2010). Other glycosyl residues including  $\alpha$ -L-fucosyl (Fucp),  $\beta$ -D-glucuronosyl (GlcPA), 4-O-methyl- $\beta$ -D-glucuronosyl (4-O-Me-GlcPA) and phenolic acids such as ferulic acid may also be present in some of the sidechains (O'Neil *et al.*, 2001).

Heat treatment has been reported to promote degradation of side chains (mainly neutral sugars) of pectic substances (Zhang *et al.*, 2013). Ideally, presence of side branches ( $\alpha$ -L-arabinofuranosyl ( $\alpha$ Araf) and  $\beta$ -D-galactopyranosyl ( $\beta$ Galp) residues) would enhance entanglements with increase in concentration. This would lead to increase in viscosity due to significant intermolecular interactions (Hwang and Kokini, 1992). It is speculated that thermo-mechanical processing such as extrusion cooking as used in this study may lead to breakage of glycosidic linkages between neutral sugars that make up the branches on the rhamnogalacturonan I region of pectin in grape pomace. The breakage of glycosidic linkages may have led to the reduction in molecular weight of pectin molecules and a subsequent reduction in viscosity observed in composites with grape pomace. Figure 4.2 shows a pictorial representation of the pectin components of grape pomace before extrusion cooking and fragmented components after extrusion cooking.

Non-enzymatic degradation of pectin through the  $\beta$ -elimination reaction may also have occurred during extrusion cooking. This reaction leads to removal of the activated hydrogen atom at the C-5 position of galacturonic acid residues by suitable proton acceptors and this leads to the breakage of the glycosidic linkage at C-4 in the  $\beta$ -position (Diaz *et al.*, 2007). This results in formation of unstable intermediary anions stabilized by losing the C-O linkage in the  $\beta$ -position consequently followed by a double bond between C-4 and C-5 at the non-reducing end (Sila *et al.*, 2006).  $\beta$ -elimination is reported to be prevalent at above pH 4.5 and elevated processing temperature (Kravtchenko *et al.*, 1993).



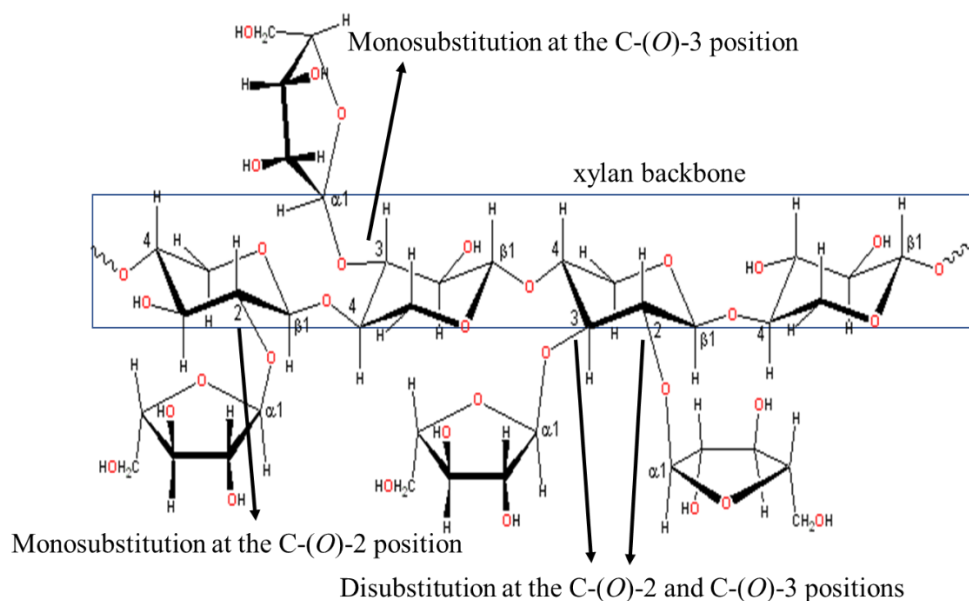
**Figure 4.2.** Structural model for grape pomace pectin (adapted from Deco *et al.*, 1995) and depolymerization of pectin components after extrusion cooking. HG, homogalacturonan; RG I (rhamnogalacturonan I); RG II (rhamnogalacturonan II)

The structural makeup of the dietary fibre sources (wheat bran and grape pomace) used in this study differs. Table 4.2 shows the non-starch polysaccharides present in grape pomace and wheat bran. Cellulose is present in both wheat bran and grape pomace.

**Table 4.2.** The percentage composition of non-starch polysaccharides in wheat bran and grape pomace.

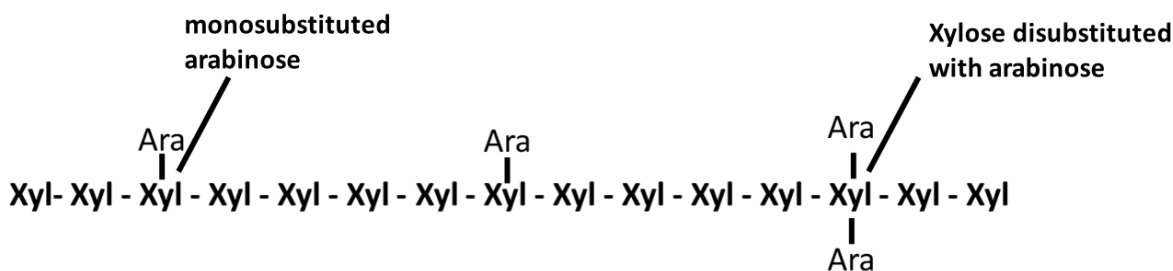
	Arabinoxylan (%)	Cellulose (%)	Pectin (%)	Hemicellulose (%)	Reference
Wheat bran	70	19	Not reported	6	(Bergmans <i>et al.</i> , 1996; Kamal-Eldin, 2009)
Grape pomace	Not reported	36	34	24	(Bravo and Saura-Calixto, 1998; Ping <i>et al.</i> , 2011)

Arabinoxylan, the major non-starch polysaccharide present in wheat bran consists of a linear xylan backbone which may be unsubstituted, monosubstituted or disubstituted at the C-(O)-2 and/or C-(O)-3 positions with  $\alpha$ -L-arabinofuranosyl side chains (Ordaz-Ortiz and Saulinier, 2005). It also contains some phenolic acids such as ferulic and  $p$ -coumaric acids which may be esterified to arabinofuranosyl residues (Merali *et al.*, 2015). A structural unit of arabinoxylan is shown in Figure 4.3.



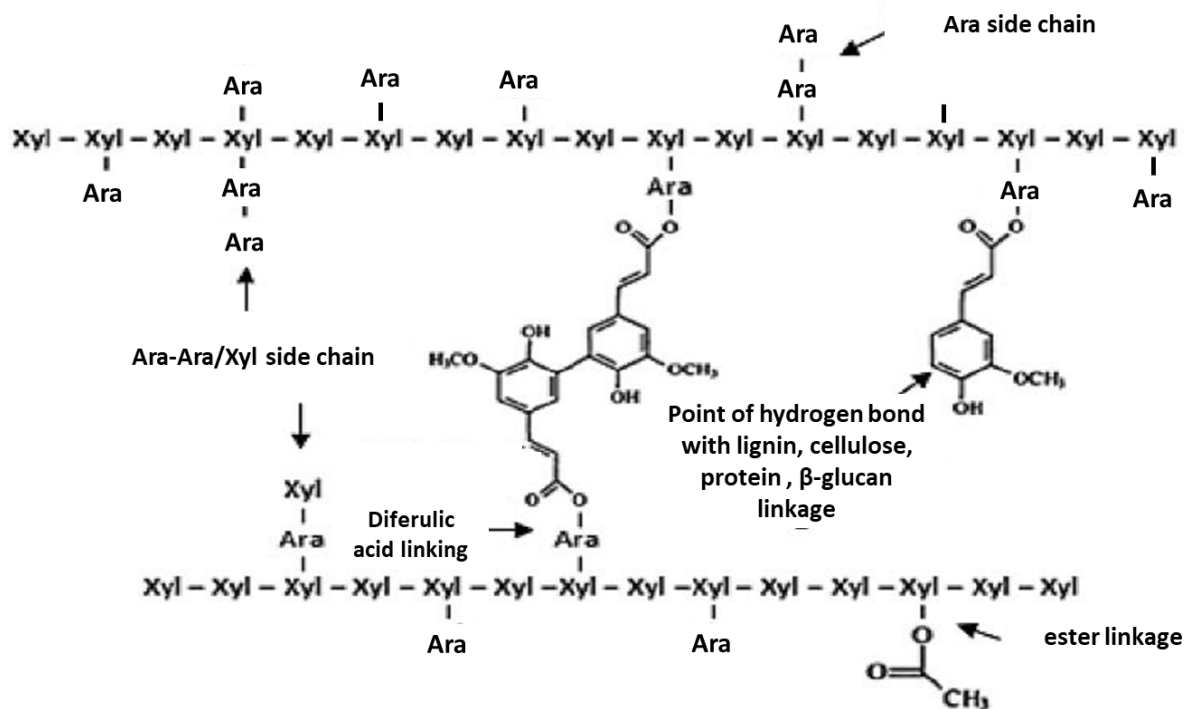
**Figure 4.3.** Structural unit of arabinoxylan (Izydorczyk and Biliaderis, 1995)

There are two types of arabinoxylan; water extractable (WEAX) and water unextractable arabinoxylan (WUAX) (Maes and Delcour, 2002). The WEAX in wheat bran are less substituted with arabinose because it has higher proportion of un-substituted xylose residues and a lower proportion of di-substituted xylose residues (Delcour, Van Win and Grobet, 1999) while the WUAX on the other hand are highly substituted with arabinose on the xylan backbone (Maes and Delcour, 2002). The schematic representations of the different features of WEAX and WUAX is presented in Figure 4.4 and 4.5 respectively.



**Figure 4.4.** Schematic representation of water extractable arabinoxylan (WEAX)

The WUAX are reported to be strongly embedded in the cell wall network by physical and chemical association with other WUAX through cross linking. They readily form a network matrix of covalent (e.g. ester and ether bonds and diferulic acid bridges) and non-covalent (e.g. hydrogen bonds) linkages with other cell wall components such as  $\beta$ -glucans, protein, lignin and cellulose (Biliaderis *et al.*, 1995; Ebringerova and Heinze, 2000). These interactions make it impossible to extract WUAX with water except when they are physically, chemically or enzymatically treated to render them water-soluble (Schooneveld-Bergmans *et al.*, 1998). It is possible that the changes in solubility observed in this study with composite of cassava-defatted toasted soy with wheat bran may be due to disruption of WUAX association with other cell wall components as a result of the thermo-mechanical energy in the extruder. It is probable that the disruption led to release of WUAX from wheat bran cell wall and detachment from other cell wall components to make them soluble.



**Figure 4.5.** Schematic representation of the features of water unextractable arabinoxylan (WUAX) (Adams *et al.*, 2004). Ara, arabinose; Xyl, xylose.

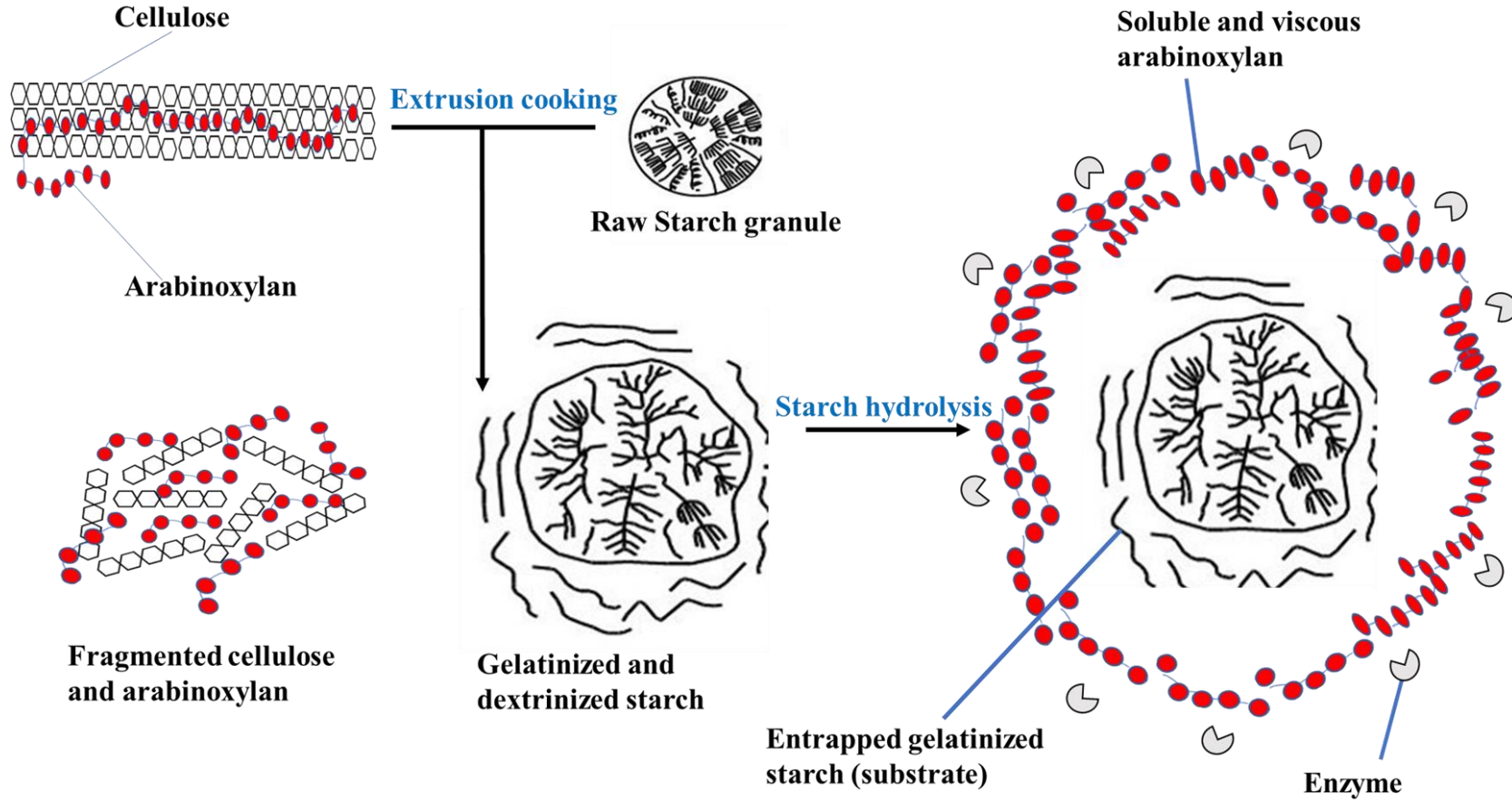
Cellulose is the common non-starch polysaccharide to both grape pomace and wheat bran. Cellulose is made up of long chains of repeating glucose units linked by  $\beta$ -(1,4) glycosidic bonds to form a linear structure (Nishiyama, 2009). Cellulose molecules lie close together to form rigid structures (Barasi, 2003). The rigid structure of cellulose makes them less prone to depolymerization by thermal treatment compared to pectin in grape pomace and arabinoxylan in wheat bran. Although, cellulose can be depolymerized by high temperature and shearing action in the extruder, they are insoluble in nature except when they are enzymatically or chemically modified. This infers that cellulose depolymerization during extrusion cooking probably does not promote their solubility compared to pectin and arabinoxylan.

Extrusion cooking led to rapid *in-vitro* starch digestibility of all extrudates, but the addition of dietary fibre in the form of grape pomace or wheat bran lowered *in-vitro* starch digestibility and estimated glycaemic index (Figure 3.2.1), though these composites still classify as high EGI foods. The addition of wheat bran increased the viscosity of composite while the addition of grape pomace lowered viscosity and the latter composite had higher phenolic compounds. The mechanism of action of wheat bran and grape pomace on starch digestibility differed. Wheat bran

may have lowered starch digestibility by means of trapping gelatinized starch within soluble dietary fibre matrix which prevented enzyme-substrate complex formation. Extrusion cooking may have solubilized wheat bran arabinoxylan into smaller soluble components that are viscous. Figure 4.6 depicts a schematic representation of how soluble dietary fibre influences starch digestion. It can be implied that the means through which soluble dietary fibre attenuates post-prandial blood glucose rise is by entrapping starch so that digestion of starch and absorption of nutrients take place at a slower rate.

On the other hand, despite the solubilization of insoluble dietary fibre during extrusion cooking, the low apparent viscosity of extrudates with grape pomace indicates that another factor was responsible for the lower starch digestibility observed. Grape pomace is rich in phenolic compounds and it may be possible that a starch-phenolics complex was formed which lowered starch digestibility. Zhu (2015) suggested that phenolic compounds can be tightly complexed inside the cavity of amylose helices through hydrophobic interactions. The complex prevents enzyme-substrate complex formation, and this lowers starch digestibility.

The interactions between starch and phenolic compounds have been postulated to be based on hydrogen linkages (Bordenave *et al.*, 2014). The disruption of starch granules during extrusion cooking will increase swelling and opening of amylose and amylopectin chains and this may enable phenolic compounds to bind to specific sites on the amylose and amylopectin molecules via hydrogen bonds (Barros *et al.*, 2012). Also, the high molecular weight of procyanidins present in grape pomace would provide more hydroxyl groups for hydrogen bonding and possibly some hydrophobic domains for interaction with amylose (Barros *et al.*, 2012). Phenolics are also known to inhibit activities of starch hydrolyzing enzymes by binding to active sites of these enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) (Adefegha *et al.*, 2015) thus lowering starch digestibility.



**Figure 4.6.** Schematic illustration of probable effect of extrusion cooking on starch, cellulose and arabinoxylan and the probable effect of soluble arabinoxylan on starch digestibility

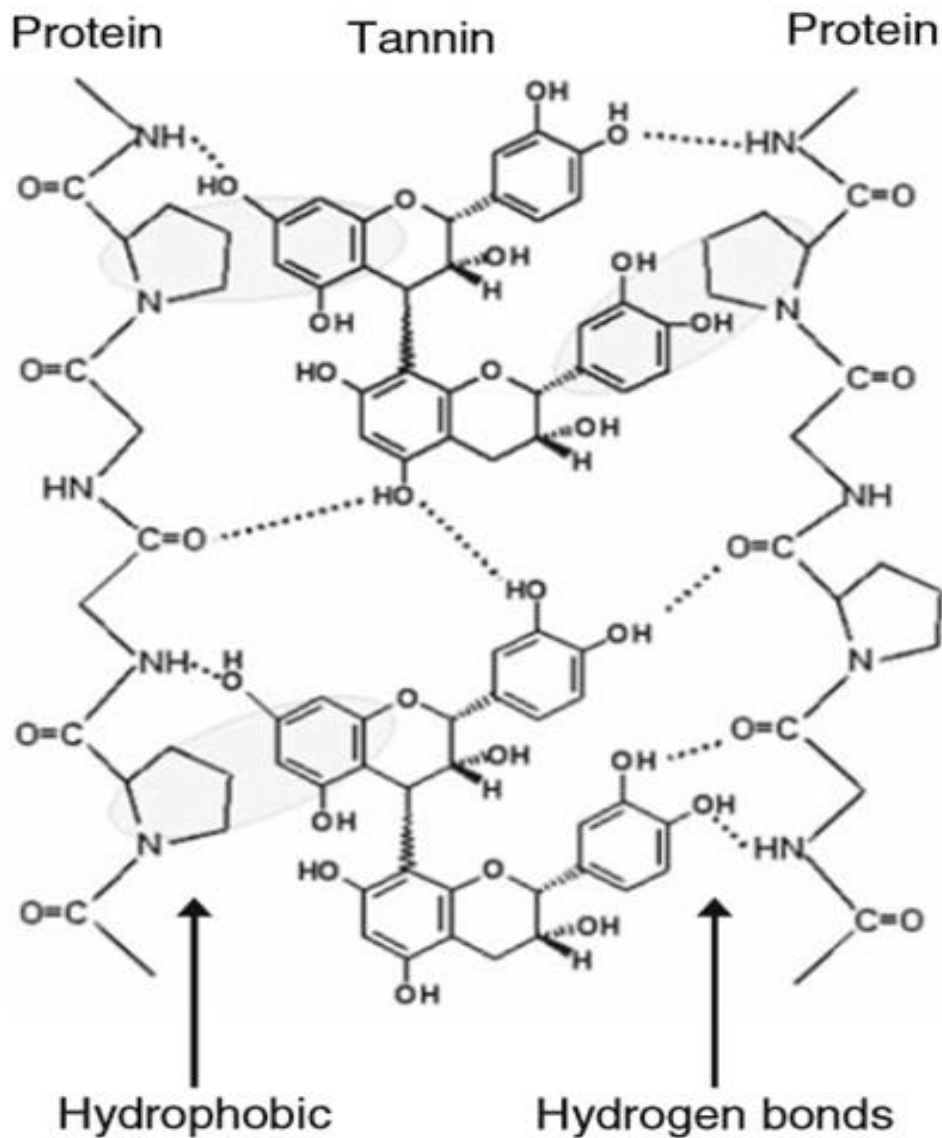
The interaction between phenolic compounds and digestive enzymes involves formation of hydrogen bonds between the hydroxyl group of the phenolic compound and the catalytic residues of the enzyme binding site and formation of a conjugated  $\alpha$ -system that stabilizes the interaction with the active site (Lo Piparo *et al.*, 2008). Yilmazer-Musa *et al.*, (2012) reported that grape procyanidins can inhibit the activities of both  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes. According to Miao *et al.*, (2014), the kinetics result obtained in their study showed grape skin exerted a non-competitive mode inhibition against  $\alpha$ -amylase. Non-competitive inhibition involves a molecule, in this case a phenolic compound binding to a site other than the active site of the enzyme. This site is known as the allosteric site. The binding of the inhibitor (phenolic compound) to the allosteric site would cause a conformational change to the enzyme's active site. This would then result to loss of specificity of the active site and substrate i.e. the enzyme and substrate can no longer bind to form a complex (Martinez-Gonzalez *et al.*, 2017). Non-competitive inhibition interferes with the conversion of the enzyme-substrate complex into reaction products (Tadros *et al.*, 2014).

The reduction in total phenolics and antioxidant activity of composites after extrusion cooking may be attributed to decarboxylation of free phenolics during extrusion cooking (Dlamini *et al.*, 2007). Additionally, the high temperature in the extruder may have promoted polymerization, oxidation and thermal degradation of phenolic compounds (Ruiz-Gutierrez *et al.*, 2015). The degradation and oxidation during extrusion cooking may have transformed phenolic compounds into oxidative products having low anti-oxidant activity (Arts *et al.*, 2001). The formation of a protein-phenolics complex could also account for reduction in total phenolics and as this would reduce extractability of phenolic compounds thus leading to lower anti-oxidant activity (Rawel *et al.*, 2005). Hydrogen bonding and hydrophobic interactions are mainly responsible for chemical reaction between proteins and phenolic compounds (Ozdal *et al.*, 2013). An illustration of hydrogen bonding and hydrophobic interactions between tannins and protein is shown in Figure 4.7.

Extrusion cooking has been associated with protein aggregation and this results in decrease in nitrogen solubility (Li and Lee., 1997). The high temperature involved in extrusion cooking promotes formation of di-sulphide bonds between protein chains and this leads to cross-linking of



protein polypeptides to form insoluble complexes (Zink *et al.*, 2016). These insoluble complexes prevent enzyme access to peptide bonds or they mask the sites of enzyme attack. This implies that cross-linking of proteins through formation of di-sulphide bonds may lead to protein aggregation and these aggregates hinders enzyme access for proteolysis and this results in decrease in protein digestibility and solubility.



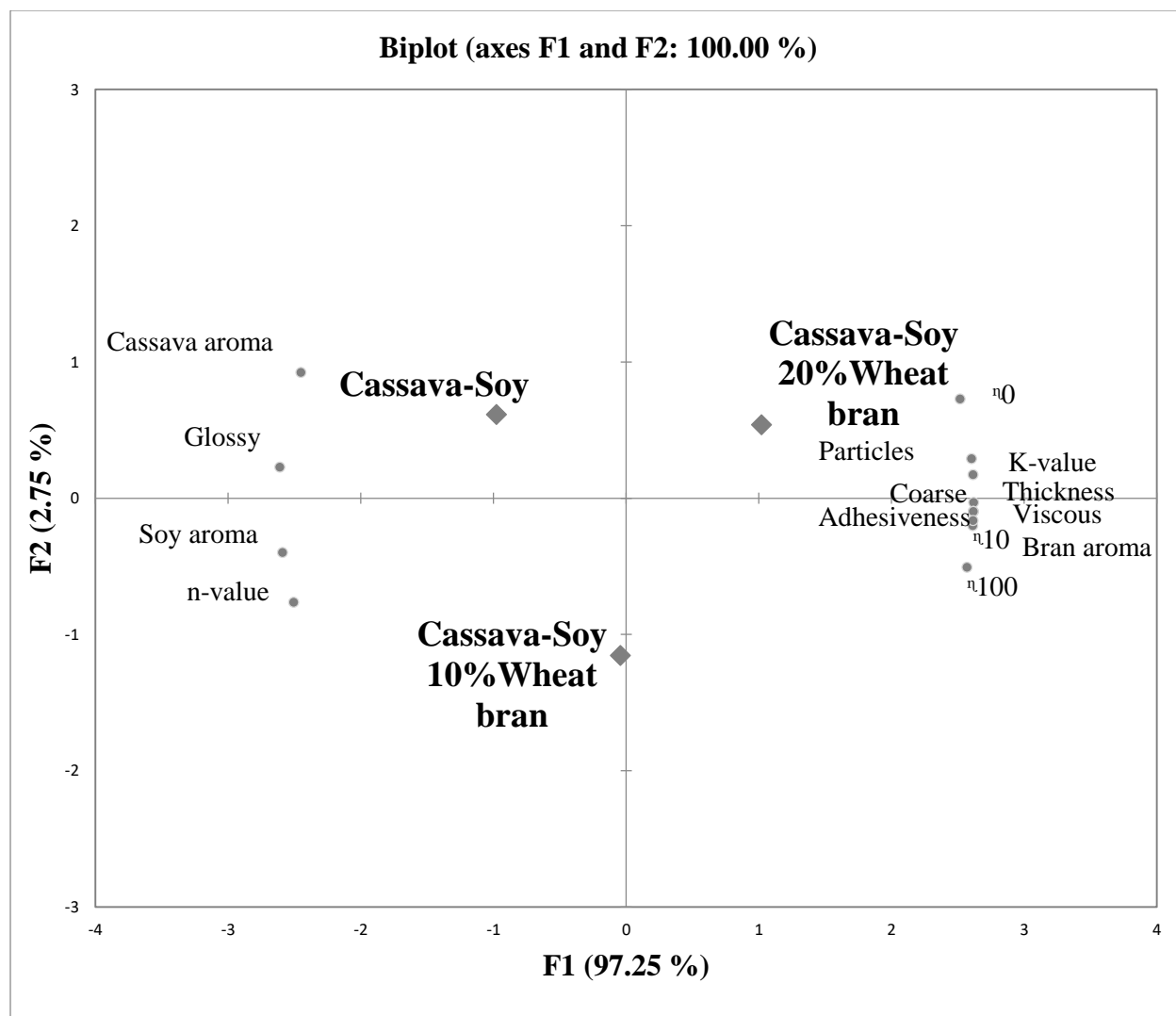
**Figure 4.7.** Hydrogen bonds and hydrophobic interactions between tannin and protein (Santos-Buelga and De Freitas 2009).

As stated earlier, protein-phenolics interaction may have occurred during extrusion cooking and this could also be responsible for the lower nitrogen solubility observed in composites with grape

pomace. Heat treatment have been found to promote the formation of insoluble protein complexes with phenolic compounds which results in decrease nitrogen solubility (Yagoub *et al.*, 2004). Enzyme-phenolic complex formation may also contribute to decrease in *in-vitro* protein digestibility of composites which contained grape pomace. The highly reactive nature of phenolic compounds due to the presence of multiple hydroxyl groups (Figure 4.7) makes them available to form hydrogen bonds with active site of digestive enzymes (Pereanez *et al.*, 2011) and this consequently reduces the activity of the digestive enzyme.

#### 4.2.2 Sensory Properties

The result of this study shows that oral sensory exposure is an important factor that contributes to satiety. The increased solubility of wheat bran arabinoxylan during extrusion cooking led to an increase in viscosity of composite porridges that contained wheat bran. High meal viscosity has been associated with increased satiety (Marciani *et al.*, 2000; Zhu *et al.*, 2013). The porridge with 20% wheat bran was described as having the highest viscosity by the descriptive sensory panellists and this was also further substantiated by the apparent viscosity measurement. Some of the descriptive sensory and rheological properties of the porridge were included in a principal component analysis (PCA). The first 2 factors accounted for 100% of the explained variation among the porridges (Figure 4.8) with factor 1 accounting for 97%. The porridge with 20% wheat bran was clearly separated from the porridge with 0 and 10% wheat bran by factor 1. The descriptive sensory attributes; coarse, adhesive, presence of particles, bran aroma, viscous, thick and rheological properties;  $\eta_0$ , zero shear viscosity;  $\eta_{10}$ , viscosity at shear rate  $10 \text{ s}^{-1}$ ;  $\eta_{100}$ , viscosity at shear rate  $100 \text{ s}^{-1}$ , consistency value (*K*-value) were strongly associated with the porridge with 20% wheat bran. The power law index (*n*-value), glossy, cassava and soy aroma were associated with the porridge with 0% wheat bran. The more viscous porridge was also eaten more slowly compared to the other porridges thus having a higher oral sensory exposure. The porridge also led to greater reductions in hunger, increased fullness, lower desire to eat and lower prospect to consume another meal post consumption of the porridge compared to the other porridge.



**Figure 4.8.** Principal component analysis (PCA) showing the effects of wheat bran addition on the sensory and rheological properties of cassava-defatted toasted soy porridge.

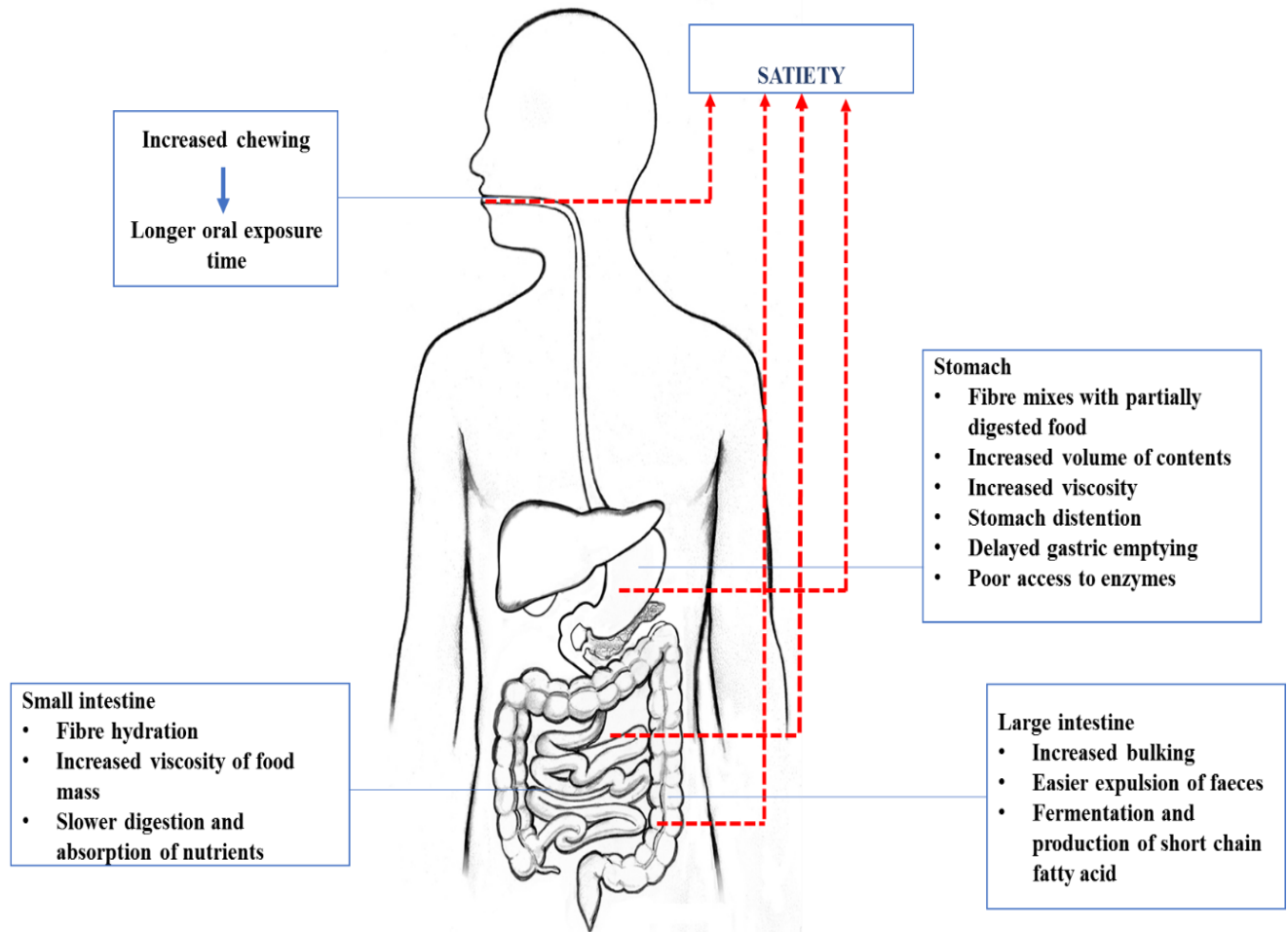
Sensory exposure has been reported to be dependent on the exposure duration and the intensity of the stimulus (Ruijschop *et al.*, 2008). A slower eating rate would lead to prolonged oral exposure time and this would in turn increase the overall exposure to sensory stimulation in the oral and retro-nasal cavity, this is subsequently followed by increased satiety or suppressed appetite (Ruijschop *et al.*, 2011; Bolhuis *et al.*, 2011).

The coating of food in the oral mucosa may contribute to the sensory sensations that lead to a longer oral exposure time (Prinz, Huntjens and Wijk, 2006). After a semi-solid food such as porridge is swallowed, a viscous salivary coating is retained on the back of the tongue (Prinz,

Huntjens and Wijk, 2006). In this study, viscous porridge may have formed a coating that contained some bran particles in the oral mucosa thereby prolonging oral exposure time of porridge. Residue from the semi-solid food may also be retained on the surface of the tongue and teeth (Kashket *et al.*, 1991; Heath and Prinz, 1991). The retained coating contains residues or particles of food and this may lead to a prolonged perception of food aroma after the food bolus has been swallowed (Buettner *et al.*, 2001). It is possible that a food that leaves coating and residues behind in the oral mucosa after food bolus has been swallowed may lengthen the time of oral sensory exposure. Although residues retained in the oral mucosa after eating was not investigated in this study, it can be speculated that more residues of the porridge that contained 20% wheat bran was probably retained in the oral mucosa after food bolus was swallowed and this may have contributed to the longer oral sensory exposure time observed for this porridge compared to the other porridges.

There is increasing evidence of a direct relationship between increased meal viscosity and increased satiety (Chambers *et al.*, 2015). It is hypothesized that the potential effect viscous fibre has on satiety are mediated by physiological changes in the gut. These changes include increase in gastric distention and decrease in gastric emptying rate (Wanders *et al.*, 2013). From the mouth through to the large intestine, viscous dietary fibre promotes satiety and some of the ways through which it affects the digestive process of food is shown in Figure 4.9.

According to Erhlein and Schemann, (2005), the gastric emptying of viscous content is slower compared to non-viscous content partly due to the enhanced resistance to flow of the viscous chyme. Additionally, viscous chyme would lead to a diminished peristaltic constriction followed by reduced propulsion and slower movement of the chyme between gastric compartments down the gastrointestinal tract (Erhlein and Schemann, 2005). In addition to slow gastric emptying rate, viscous food content may also entrap undigested nutrient thus preventing access of pancreatic  $\alpha$ -amylase for amyolytic hydrolysis. This would lead to a delay in digestion and limited diffusion of nutrients from the lumen to the mucosa epithelium (Grundy *et al.*, 2016).



**Figure 4.9.** Physiological effect of dietary fibre in the human digestive tract

Studies have shown that the nutrients released during mastication in the oral cavity and subsequent sites of the gastrointestinal tract may trigger neuronal and humoral signals that have an impact on digestive processes (Maljaars *et al.*, 2008, Juvonen *et al.*, 2009). The release of gut appetite hormones such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY), Cholecystokinin (CCK) may be triggered by specific nutrient sensing enteroendocrine cells that are present throughout the gastrointestinal tract or through neuro-endocrine signalling pathways (Grundy *et al.*, 2016; Feltrin *et al.*, 2004). The results from this study suggest that increased viscosity of food is directly related to increased satiety and inversely related to postprandial glucose response as reported by some other authors (Zhu *et al.*, 2013; Mattes and Rothacker, 2001, Schroeder *et al.*, 2013).

One major limitation to this study is not determining how palatability may influence consumption of porridge in terms of eating rate. Viscosity and presence of particles in the porridge with 20% wheat bran may be viewed as either pleasant, less pleasant or unpleasant by subjects who participated in the study and this may possibly have influenced their eating behaviour. Food texture together with other sensory properties such as taste, appearance and aroma are important factors that contribute to consumer enjoyment and appreciation of food products (Koç *et al.*, 2013). This infers that consumers would enjoy and rather prefer palatable foods. Palatability of a food has been linked with changes in eating behaviour and increased eating rate (Yeomans, 1998). Future studies should address the effect of food palatability on eating behaviour. This would help understand if slower eating rate observed in porridge with 20% wheat bran was solely due to the viscous property of the food or if some palatability factors were at play.

Also, *ad libitum* intake at lunch post 3 h of porridge consumption was unfortunately not carried out. Future work should be considered to determine the effect of porridge that contained wheat bran on subsequent food intake as this would help to elucidate better on the effect of dietary fibre on *ad libitum* energy intake at the following lunch meal.

## 5.0 CONCLUSIONS AND RECOMMENDATION

In this study, instant products were made by incorporating either wheat bran or grape pomace in cassava-defatted toasted soy composite. The addition of either wheat bran or grape pomace with extrusion cooking changes the nutritional and rheological properties of cassava-defatted toasted soy composite. Extrusion cooking promotes solubilization of insoluble dietary fibre. The redistribution of insoluble to soluble dietary fibre may be due to depolymerization of non-starch polysaccharides because of thermal and mechanical shear in the extruder. The addition of dietary fibre and changes from insoluble to soluble dietary fibre impacts the nutritional and potential health benefits of extruded cassava-defatted toasted soy composite. The composites with either wheat bran or grape pomace contain dietary fibre content that can partly contribute to the recommended average intake of dietary fibre for adults.

The addition of wheat bran or grape pomace can reduce glycaemic load of food consumed as measured *in-vitro* and may likely reduce post-prandial blood glucose. The viscosity of composite porridge with added wheat bran were higher after extrusion cooking as a result of the solubilization of insoluble dietary fibre. The high viscosity of these composites can lower starch hydrolysis by limiting the access of  $\alpha$ -amylase to starch during digestion and promote slower absorption of nutrients. The phenolic compounds in grape pomace can lower starch digestibility and EGI, by inhibiting the activities of  $\alpha$ -amylase. This is beneficial especially for people with type-2 diabetes as the consumption of a viscous porridge would attenuate post-prandial blood glucose and insulin levels. This beneficial effect would require further research by conducting *in-vivo* glucose determination to clearly illustrate relationships between dietary fibre source and glycaemic response.

The addition of grape pomace increased the total phenolic content and anti-oxidant activity of cassava-defatted toasted soy composite. Although, a decrease in total extractable phenolics and anti-oxidant activity were observed upon extrusion cooking probably due to thermal degradation, the porridge may still possess some radical scavenging properties with potential health benefit. Dietary phenolics have proven antioxidant activity and their beneficial role in altering chronic diseases (cardiovascular diseases, cancers) risk factors are associated to this anti-oxidant activity.

The instant porridge made from cassava-defatted toasted soy composite and wheat bran has potential satiety promoting properties. This is related to the longer oral exposure time of this porridge that may have increased the overall sensory stimulation in the oral and retro-nasal cavity consequently, increasing the satiety signals sent to the brain. The subsequent effect of this is increased satiety and suppressed appetite. The presence of particles in the wheat bran composite and its viscous nature may have contributed to the longer oral exposure time and increased satiety.

Future studies on the satiety promoting potential and oral processing properties of the composites with grape pomace is needed to elucidate on the mechanisms by which it may promote satiety despite its low viscosity. Also, further research to evaluate the consumer acceptability of the porridge with grape pomace or wheat bran is required as this would provide valuable information on the commercial potential of an instant porridge made from cassava-soy composite with either wheat bran or grape pomace.

Indirect measurement of faecal or plasma short chain fatty acids (SCFA) are recommended in future research in order to provide a good indication of the fermentability of the non-starch polysaccharides present in grape pomace and wheat bran. This will ascertain their health benefits.

It is also recommended that future studies to investigate the effect of polymer length and molecular weight of dietary fibre sources on viscosity and other rheological properties before and after extrusion cooking is carried out. This would help relate some of the rheological properties to the nutritional properties.

This study demonstrates that instant food products with nutritional and potential health promoting properties can be produced with the use of extrusion cooking to incorporate dietary fibre to locally available starch-rich foods. The use of locally sourced raw materials will reduce dependence on imported food products and create new uses for these indigenous food crops. Also, extrusion cooking can be an alternative way of better utilizing food by-products such as wheat bran and grape pomace.



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## 7.0 PUBLICATION, PRESENTATIONS AND POSTERS FROM THIS RESEARCH

### *Publication*

Oladiran, D.A. and Emmambux, N.M., 2017. Effects of extrusion cooking and wheat bran substitution on the functional, nutritional, and rheological properties of cassava-defatted toasted soy composite. *Starch-Stärke*, 69(7-8).

### *Conference poster*

Oladiran, D.A., Emmambux, N.M. and de Kock, H.L. The effect of addition of wheat bran to cassava-soy extruded porridge on oral processing and satiety. 3<sup>rd</sup> International Conference on Global Food Security Cape Town, South Africa. 3<sup>rd</sup> – 6<sup>th</sup> December 2017.

Oladiran, D.A., Emmambux, N.M. and de Kock, H.L. The effect of addition of wheat bran to cassava-soy extruded porridge on oral processing and satiety. 22<sup>nd</sup> Biennial International SAAFoST Congress and Exhibition, Cape Town, South Africa. 3<sup>rd</sup> – 7<sup>th</sup> September 2017.

Oladiran, D.A. and Emmambux, N.M. Effects of incorporation of grape pomace and extrusion cooking on the nutritional and functional properties of cassava-defatted soy porridges. 2<sup>nd</sup> World Nutrition Congress, 30<sup>th</sup> August – 2<sup>nd</sup> September 2016. Cape Town, South Africa.

### *Conference oral presentation*

Oladiran, D.A., Emmambux, N.M., and Minnaar, A. Effect of incorporation of wheat bran and extrusion cooking on the nutritional and functional properties of cassava-defatted soy porridges. 21<sup>st</sup> Biennial International SAAFoST Congress and Exhibition, 6<sup>th</sup> - 9<sup>th</sup> September 2015. Durban, South Africa.

Oladiran, D.A., de Kock, H.L. and Emmambux, N.M. Effects of extrusion cooking and wheat bran addition on the rheological properties and oral processing characteristics of cassava-defatted toasted soy porridge. 2<sup>nd</sup> Cereal Science and Technology-South Africa New Voices Symposium. 11<sup>th</sup> May 2017. *Winner of best PhD oral presentation.*