

PHYSICOCHEMICAL PROPERTIES AND BAKING QUALITIES OF BAKED WHEAT PRODUCTS  
SUPPLEMENTED WITH CASSAVA AND PIGEON PEA FLOURS

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## ABSTRACT

### PHYSICOCHEMICAL PROPERTIES AND BAKING QUALITIES OF BAKED WHEAT PRODUCTS SUPPLEMENTED WITH CASSAVA AND PIGEON PEA FLOURS

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Supplementation of wheat products with cassava and pigeon pea flours is a sustainable way to produce economical and nutritious baked products. The aims of this study were to evaluate the effects of cassava and pigeon pea flour supplementation on the physicochemical properties (chemical composition, viscosity, dough rheology) of flour blends, baking quality (bread and cookies), and digestibility of baked products from blended flour samples. Findings revealed that supplementation with pigeon pea flour increased protein content of the bread and cookie products. However, incorporation of cassava and pigeon pea flours resulted in significantly impaired bread quality, such as lowering of loaf volume and height. The quality attributes of blended flour cookies were superior to those of control (wheat cookies). The total hydrocyanic acid content of baked samples was significantly higher when added cassava flour levels increased, though still below the maximum allowable amount of 1 mg/kg. Both carbohydrate and *in vitro* protein digestibility decreased as wheat flour was substituted with cassava and pigeon pea flours, but still was above 50%.

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## CHAPTER 1

### INTRODUCTION

Wheat is the third most important cereal crop after maize and rice, with world production of 695 million metric tons annually (FAO, 2008). Among the cereal flours, wheat is extensively used for breadmaking among other uses. The unique breadmaking properties of wheat flour are due to its gluten protein that, when hydrated, forms strong, cohesive dough that retains gas and produces a light, aerated baked product (Hoseney, 1998). Malawi produces wheat on a very small scale due to its ecology, and relies on importation of wheat and wheat flour for its bakery and confectionery industry. Malawi has a domestic demand of 100,000 metric tons but only 5,000 metric tons is locally produced with 71,370 metric tons imported at a cost of about \$21.5 million a year (NSO, 2007). Such importation has led to a huge loss of currency and increased retail price of baked wheat products. Due to the high cost of and high demand for wheat flour, efforts are being directed toward developing, evaluating and providing alternative formulations of flour for bread and cookie making. The new formulation of flour for bread and cookie making is proposed to include cassava and pigeon pea flours, to be substituted for a portion of wheat.

Cassava (*Manihot esculenta*) has been studied extensively as the best raw material to partially replace wheat. Cassava flour is preferred in the bakery industry because of its good baking properties and also low production cost compared to other non-wheat flours (Falade and Akingbala, 2008). Some significant studies have been conducted to assess the feasibility of incorporating cassava flour in wheat-based baked products. Defloor et al. (1995) and Khalil et al. (2000) reported that partial replacement of cassava flour at 15% for wheat resulted in bread

with significantly improved quality and consumer acceptability. It has also been reported that substitution of wheat flour with 20% cassava flour resulted in good color, flavor, and texture of cookies (Mlingi et al., 1998). In general, the research findings have demonstrated the possibility of partial substitution of wheat flour with cassava flour and, therefore, utilization of cassava flour in baked products is suggested to minimize production costs and high retail prices of baked products.

Despite research verifying the ability of wheat and cassava flour mixtures to improve the color, flavor, and texture of baked products, and the high level of consumer acceptability, the main weakness is the low levels of protein of cassava (1–2%) compared to wheat (10 – 15%). These products could threaten the sustainability of human health, and might result in increased prevalence of malnutrition if consumed for long and in relatively huge quantities (Balagopalan et al., 1992). The nutritional status of the Malawian population remains critical. Although short-term interventions such as vitamin supplementation and food fortification with vitamins and micro nutrients have been done, the prevalence of malnutrition is still very high (FAO, 2010). Moreover, supplementation and fortification are expensive and not effective. A more effective and efficient approach to avoid chronic nutritional problems in populations consuming a large amount of cassava-based products could be protein enrichment of the cassava flour used in the preparation of such products (Graham and Archbold, 1984).

Significant studies have been implemented to improve the nutrient profile of cassava-based products. According to Graham and Archbold (1984), compared to products made from cassava flour alone, the protein-enriched products showed increase in protein content ranging from 5-27% and were all highly acceptable by the members of the taste panel. The increase in

protein content in the baked products was due to the high protein content of the leguminous flour with which cassava flour was supplemented.

The pigeon pea (*Cajanus cajan*) is an important grain legume commonly grown and consumed in tropical and sub-tropical regions of the world (ICRISAT, 1991). In Malawi, pigeon pea is underutilized as it is only consumed as relish in fresh or dry form, despite being nutritious. Mature seeds have a protein content of 18.8% (dry basis), and are a rich source of lysine but, like soybeans, are deficient in sulfur-containing amino acids such as methionine and cysteine (Singh and Diwakar, 1993). On the other hand, cassava is deficient in lysine but contains moderate amounts of methionine and cysteine (Longe, 1980). Therefore, it is envisaged a blend of cassava and pigeon pea flours would result in enriched baked products with a good balance of some of the essential amino acids.

Compared to soybeans, pigeon peas have been used little in baked foods or confectionery products despite some research studies conducted. Gayle and others (1986) reported that protein values increased from 9.2% to 13.0% as pigeon pea flour was increased in wheat-pigeon pea flour bread. They further reported that the overall acceptability of the bread was high. In an evaluation of cookies made from cocoyam and pigeon pea flour, Okpala and Okoli (2011) observed that protein content ranged from 6.40% for cookies made from 100% cocoyam flour to 12.97% for cookies made from 100% pigeon pea flour.

Utilization of local crops in baked products could lead to economic enhancement through reduction of importation costs of wheat flour. In addition, use of local crops like cassava and pigeon pea (*Cajanus cajan*) in baking will enable cheap and nutritious baked

products to become available and affordable to many people, thereby reducing prevalence of malnutrition in the country.

### **1.1 Objectives**

The objectives of the study were to;

- produce pigeon pea and cassava flours,
- evaluate the effects of supplementing wheat flour with pigeon pea and cassava flours on dough rheology using the Alveograph,
- assess the viscosity of wheat, cassava and pigeon pea blended and non-blended flours using the Brookfield viscometer,
- evaluate the physicochemical properties and baking qualities of breads and cookies made from wheat-cassava-pigeon pea flour blends, and
- evaluate the digestibility of carbohydrates and proteins in the baked products.

### **1.2 Hypothesis**

- Breads and cookies supplemented with pigeon pea and cassava flours will display different physicochemical and baking properties from breads and cookies made from wheat flour alone.

### **1.3 Justification**

For many years Malawi has been relying on importation of wheat and wheat flour for its bakery industry. Such importations have led to high production costs for baked products,

resulting in skyrocketing retail prices of baked products making the majority of the rural communities unable to purchase such products. Past research has demonstrated that cassava and pigeon pea flours can be partially substituted for wheat flour in the baking of bread and cookies. This offers great opportunity to reduce overreliance on wheat flour as the only raw material for bread and cookie making, and also should reduce retail prices of baked products. Cassava and pigeon peas are the potential crops to be evaluated for blending with wheat flour for products with increased baking qualities, human nutrition and reduced costs of production. The targeted crops for blending with wheat flour are adaptable and largely produced in Malawi. As a result, import costs for raw materials will be significantly reduced. In general, utilization of cassava and pigeon pea flours will reduce overreliance on wheat flour and result in nutritious products affordable to the rural masses, hence improving both Malawian nutrition and the national economy.

Evaluation of the physicochemical and baking qualities of baked wheat products supplemented with cassava and pigeon pea flours is a major step in facilitating utilization of the flours in baking. Knowledge of these characteristics will enhance utilization of the flours in bread and cookie making. Successful results from the study will be extended to the rural communities so that they can have skills on standalone ability to make breads and cookies from locally available raw materials. The main aim of the study, therefore, was to evaluate the physicochemical properties and baking qualities of bread and cookie samples made from wheat-cassava-pigeon pea flour blends as compared to non-blended wheat flour (control).

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Wheat

Wheat is the world's third most important crop after maize and rice. Worldwide, wheat flour is used as the raw material for baking. The gluten protein in wheat makes it a unique substrate for breadmaking. The three main types of wheat are soft, hard, and durum. Unlike hard and durum wheats, which are used mainly for bread and pasta products, respectively, soft wheat has more than one major use, including cookies, cakes, crackers and pretzels (Hoseney 1998).

The protein content varies significantly in wheat varieties and it ranges between 10 and 15% (Anjum and Walker, 2000). Wheat proteins contain albumins, globulins, gliadins and glutenins. While albumins and globulins are soluble in water and salt solution, respectively, gliadins and glutenins are collectively called gluten and are insoluble in water and salt solution. It has been shown that the gluten proteins are responsible for the cohesive, viscoelastic property of wheat flour dough and the dough's ability to retain gas during fermentation as well as dough setting during baking (Hoseney, 1998). To form cohesive and viscoelastic dough, gluten requires adequate hydration and kneading to promote cross linkages between glutenins and gliadins.

##### 2.1.2 Gluten proteins

Among the cereal flours, only wheat flour has the ability to form gluten proteins when mixed with water to form a viscoelastic material that retains gas, resulting in high quality baked

products. Hundreds of protein components which are present as either monomers or, linked by interchain disulfide bonds, as oligomers and polymers make up the gluten proteins (Wrigley and Beitz, 1998). They are unique because they are characterized by high contents of glutamine and proline, but are low in basic amino acids like lysine. The low content of basic amino acids implies that the level of electrical charges is very low. Thus, the low-charge density enhances interaction among the molecules forming the gluten, a condition that appears to be extremely necessary for dough formation (Hoseney, 1998).

The amino acid composition of gluten proteins also shows that about 35% of the total amino acids have hydrophobic side chains and hence the polar sides are not accommodated in the hydrophobic core of the protein (Hoseney, 1998). As a result, there is an increased hydrophobic interaction between gluten proteins. The hydrophobic interactions significantly contribute to the stabilization of gluten structure. In addition, the tendency of hydrophobic interactions to increase in energy with increasing temperature provides even more stability during the baking process.

Gliadin molecules have intra-molecular disulfide linkages resulting in a compact and globular shape. Compared to glutenins, gliadins have a low molecular weight of about 40,000 (Hoseney, 1998). The presence of disulfide bonds explains the need to knead the dough to break disulfide bonds between adjacent chains and realign them to form a continuous protein sheet (Stauffer 1998). When hydrated, gliadins have little or no resistance to extension, and are responsible for the dough's cohesiveness.

Glutenin proteins are a heterogeneous group of proteins and multichained, linked by interchain disulfide bonds, with average molecular weights of about 3 million (Hoseney, 1998).



The high molecular weight of glutenins has been recognized as one of the main contributing factors to wheat's desirable dough properties and baking performance. Physically, the protein is resilient and rubbery but not prone to rupture and gives the wheat dough its property of resistance to extension (Hoseney, 1998).

## **2.2 Cassava (*Manihot esculenta*, Crantz)**

Cassava is an important source of food and income throughout the tropics, including Africa, Asia and Latin America. About 600 million people in Africa, Asia and Latin America depend on the plant for their survival, deriving calories and income from the roots and leaves (IFAD, 2008). Cassava production in Africa has more than tripled since 1961, from 33 million metric tons per year to 101 million metric tons, making the continent the largest producer in the world (IFAD, 2008). Advantages of cassava as a crop include flexibility in planting and harvesting time, drought tolerance, and ability of cassava to grow and produce in low nutrient soils, where cereals and other crops do not grow well (Onwueme, 1978; Nassar, 2005).

In Malawi, cassava is the most important root crop. Cassava plays an important role as a cash crop for smallholder farmers, middlemen, as well as sellers in various markets, and is gradually becoming an important industrial crop (Benesi et al., 2001). It is grown country wide and is a staple food crop for more than 30% of the population along the central and northern lake shore areas of Lake Malawi and the Shire highlands (Moyo et al., 1998). Country wide, cassava is used as a food supplement, a main part of breakfast, and snack food (Moyo et al., 1998). Cassava leaves are an excellent source of protein compared to legumes and are commonly consumed as vegetables in many parts of the country. The fresh cassava leaves

contain 17-18% dry weight protein (FAO, 1993). The leaves are particularly important in the dry season when other green vegetables are in short supply.

### 2.2.1 Nutritive value of cassava flour

Cassava is commonly known to be a good and cheap source of carbohydrates. After sugarcane, it is considered to be the highest producer of carbohydrates among crop plants. The protein, vitamin and mineral contents in cassava are very low. In addition, it lacks essential amino acids such as lysine (Balagopalan et al., 1992). In general, cassava is often considered inferior to maize and wheat because of its low levels of proteins, vitamins and minerals. The low protein content necessitates fortification of cassava flour with legume flour in order to improve the protein content of the end product.

**Table 1. Chemical Composition of Fresh Cassava Root**

Component	Percentage (by weight)
Water	62 - 65
Carbohydrate	32 - 35
Protein	0.7 – 2.6
Fat	0.2 – 0.5
Fiber	0.8 – 1.3
Ash	0.3 – 1.3

Source: Cited by Wenham, 1995

### 2.2.2 Cyanogen content of cassava

All cassava plant parts, apart from the seeds, contain cyanogenic glycosides (CG), a chemical substance responsible for bitterness of cassava roots and toxicity in humans. Cultivars with <math>100 \text{ mg CG kg}^{-1}</math> fresh weight cassava are called 'sweet', while cultivars with 100 to 500 mg CG  $\text{kg}^{-1}$  are 'bitter' cassava (Wheatley et al., 1993). The most abundant CG in cassava is

Linamarin (85%), with lesser amounts of Lataustralin. Total CG concentration depends on cultivar, environmental conditions during growth, agronomic practices and plant age (McMahon et al., 1995). Linamarin is synthesized in the leaf and transported to the roots. It is therefore a standard practice to process bitter cassava roots to remove CG before consumption to avoid food poisoning. Hydrogen cyanide gas (HCN) is released from CG when cassava tubers are macerated as in chewing or grating. According to O'Hair (1990), juice extraction, fermentation, frying or a combination of these processing treatments aid in reducing the HCN concentrations to safe levels. Akingbala et al. (2005) reported about 95% decrease in HCN content after grating and nearly 98% decrease after fermentation of cassava.

### **2.2.3 Cassava processing**

Due to high moisture content (~70%), fresh cassava tubers are highly perishable and become inedible within 24-72 hours after harvest. Physiological deterioration starts as soon as roots are harvested, due to enzymatic reactions, while secondary deterioration occurs 5 to 7 days after harvest. Secondary deterioration occurs due to microbial infection of mechanically damaged tissues and results in some tissue discoloration with vascular streaks spreading from the infected tissue (Wheatley and Chuzel, 1993). Therefore, processing of cassava helps to reduce postharvest losses and stabilizes seasonal fluctuations in the supply of the crop (Hahn, 2007). In Malawi, processing of cassava is done on a small scale. Cassava is processed into ingredients like flour and starch that are used in bread and doughnut making. Flour is commonly used in the food industry while starch is used in both the food and non-food industries. Industrial applications for cassava flour and starch depend on the physicochemical

properties and baking qualities of baked products. Therefore the study of physicochemical properties and baking qualities of cassava flour in baked products is important.

#### **2.2.4 Physicochemical properties of cassava flour**

Efforts are being made to partially replace wheat flour in commercial food products with non-wheat flours, as a promising means to increase utilization of indigenous crops. In view of this, physicochemical and functional properties of CF have been studied to maximize its industrial use. The functional properties of cassava and soy flour blends were studied by Akubar and Ukwuru (2003). It was observed that CF had less capacity than soy flour (SF) to bind and retain water as well as oil. The high water and oil absorption of SF was explained by the high protein content of soybeans. Kinsell (1976) reported that soy protein absorbs water up to 200% its weight whereas carbohydrate absorbs only 15% of its weight in water. Akubar and Ukwuru (2003) further reported that the water and oil absorption capacities of the SF: CF blends increased with increasing levels of SF. The flour blends had greater water and oil absorption capacities than the CF alone. The properties may give an advantage to the blends comparative to CF in baked doughs where hydration to improve handling characteristics is required.

Dough rheology studies showed that the dough development times for cassava and wheat flour blends were shorter than for the 100% wheat flour (WF), and decreased as the extent of substitution with CF increased (Eggleston, 1993). This was correlated with dilution of gluten caused by the addition of the CF, and also indicated that water uptake by the various components present in the CF was faster. Dough stability, which indicates how much additional mixing can be applied to a dough sample before it begins to break down, was much lower for

doughs made with the flour blends than with 100% WF, suggesting an overall weakening of the doughs with increased substitution of CF (Eggleston, 1993).

Evaluation of the physicochemical properties of biscuits made from wheat-cassava flour blends showed that increases in the levels of CF resulted in decreases in protein content from 13.04% in 100% WF biscuits to 8.4% in 40% CF: 60% WF biscuits (Oluwamukomi et al., 2011). This was attributed to the low protein content of the CF (1-2%) which would have lowered the protein content of the wheat-cassava flour blend. Addition of 10% soy flour (SF) to CF resulted in increases in protein and fat contents of cookies, thus improving the nutritive value of the cassava cookies (Akubar and Ukwuru, 2003). For example, the 100% CF biscuits contained 1.6% protein and 10.7% fat. These values increased to 32.2% protein and 30.5% fat, respectively, for the 20:80 (CF: SF) biscuits which had the highest level of SF incorporation (Akubar and Ukwuru, 2003).

Addition of CF to SF resulted in reduced color, crispiness, taste and flavor of biscuits, but increases in diameter, spread ratio and height of the biscuits were observed as cassava level was increased (Oluwamukomi et al., 2011). The increase in diameter and spread ratio was due to the starch polymer of cassava whose molecules is loosely connected and expands more when heated (Oluwamukomi et al., 2011). Notably, there was no significant difference in the overall acceptability between biscuits made with 100% WF and those made with the wheat-cassava-soy flour blend. The findings strengthen the possibility of using cassava flour in biscuit making.

Loaf volume of bread largely depends on the gluten content of wheat flour. This is the reason why wheat is the unique raw material for breadmaking. When gluten is hydrated, it

forms a viscoelastic network that retains more gas during baking, hence yielding an increased bread loaf volume (Hoseney, 1998). Use of composite flour, like cassava and wheat flour blend has resulted in reduced loaf volume (Eggleston, 1993, Ciacco and D'Appolonia, 1976). The reason for the reduced volume is the dilution of gluten protein as more cassava flour was added. Despite the reduced volume, differences in consumer preference for wheat bread and composite flour bread were not significant, thus acceptable bread can be made from composite wheat-cassava flour.

Hydrogen cyanide is a poisonous substance that is found in cassava roots and therefore its content in baked products made from cassava must be assessed to avoid food poisoning. Oluwamukomi et al. (2011) reported that the hydrocyanic acid content (HCN) of the biscuits increased as the level of cassava flour increased in the formulation, with 70% CF biscuits having the highest HCN value of 0.02 mg/kg of product. This observed HCN content is below the maximum allowable level of 1 mg/100 g of flour recommended by the Codex Alimentarius Commission (1985), thus making the biscuits safe for human consumption.

### **2.2.5 Pasting properties of cassava flour**

In the development of any food product from starchy crops, the knowledge of the material's pasting properties is needed to predict behavior under a given processing condition. The pasting properties of flour and/or starch relate to swelling and solubility properties of starch granules in the presence of moisture and heat. The pasting behavior of cassava flour was well studied by Eggleston (1993). The researchers observed that the typical high degree of swelling of cassava starch granules, on attaining the pasting temperature, results in a high peak

viscosity and that the subsequent paste is not very stable, readily breaking down. The pasting properties were correlated with starch damage, as samples with high starch damage had low peak viscosity values (Eggleston, 1993).

### **2.2.6 High quality cassava flour (HQCF)**

Traditionally, cassava has been processed into flour by producing peeled dried cassava chips that are then milled into flour. Alternatively, peeled roots of bitter varieties are submerged in water for 2 to 3 days to detoxify and soften them. The roots are then dewatered (pressing out the remaining water), dried, and milled into flour. Soaking and drying subjects the roots to fermentation which renders them acidic with a strong odor. This makes the roots and flour unsuitable for food utilization in industrial processing such as baking and confectionary making.

High quality cassava flour production is a technique that produces high quality cassava flour (HQCF) within 24 hours of harvesting fresh cassava roots, without the roots undergoing fermentation. This is a method that was developed by the International Institute of Tropical Agriculture (IITA) and it produces flour with low cyanide levels that is acceptable to food industry users. Cassava roots from bitter and/or sweet varieties are peeled, washed, and grated followed by adequate dewatering or pressing and immediate drying prior to milling and sieving (Diedzoave et al., 2003). The taste of HQCF depends on the cassava variety that was used to produce it. It could be white or cream in color, sweet or bland in taste, and odorless. HQCF has become an important raw material in many African countries where cassava is grown on a large

scale, because it has properties similar to cassava starch and it has no odor associated with the fermented flour.

### **2.3 Pigeon peas (*Cajanus cajan*)**

The pigeon pea belongs to the family *Leguminosae* and is among the important grain legumes grown and consumed in the tropics and the semi-arid tropics of the world (ICRISAT, 1991). The crop is well known for its ability to tolerate drought and its wide adaptability to different environmental conditions. In addition, it improves soil fertility and its biomass is a good source of organic matter. Nutritionally, pigeon peas are rich in protein particularly lysine but deficient in sulfur-containing amino acids such as methionine and cysteine. The protein content of commonly grown pigeon peas has been reported to range between 18 and 26%. This makes it a good complementary choice for use in baking of cereal and root- and tuber-based products to enhance the nutritive value of the products. Throughout Africa, including Malawi, the crop is grown for food and feed (ICRISAT, 1991). The green peas are cooked as a relish or a snack. When dried, the legumes are also cooked as a relish and eaten along with maize or cassava meal. In addition, the dried legumes are used as an ingredient in feed formulation for poultry or cattle.

#### **2.3.1 Nutritive value of pigeon peas**

Starch and protein are the major constituents of pigeon pea grains. Aleytor and Aladetimi (1989) and Oshodi and Ekperigin (1989) reported that pigeon peas contain a moderate level of crude protein (21%), about the same as in cowpeas. Pigeon peas also contain



significant levels of fat and ash, which can potentially contribute to essential fatty acids and minerals in the diet (Oshodi et al., 1993). The pigeon pea protein is extremely comparable with soybean protein in its content of essential amino acids. Comparison of amino acid content and the FAO/WHO (1985) amino acid reference values show that leucine, lysine, phenylalanine, isoleucine and valine in pigeon peas were on the high side of the recommended range of amino acid requirements for infants, and significantly higher than the minimum values recommended for preschool and school children. However, pigeon peas are usually deficient in the sulfur-containing amino acids, methionine and cysteine. They thus complement the essential amino acids in cereals which contain methionine and cysteine but lack lysine as reported by Gopalan et al. (1971) and Daniel et al. (1970).

**Table 2. Nutritive Value of Pigeon Peas**

Constituents	Green seed	Mature seed
Protein (%)	21	18.8
Protein digestibility (%)	16.8	58.5
Trypsin inhibitor (units mg <sup>-1</sup> )	2.8	9.9
Starch (%)	48.4	53.0
Starch digestibility (%)	53.0	36.2
Amylase inhibitor (units mg <sup>-1</sup> )	17.3	26.9
Soluble sugars (%)	5.1	3.1
Flatulence factors (g 100g <sup>-1</sup> soluble sugar)	10.3	53.5
Crude fiber (%)	8.2	6.6
Fat (%)	2.3	1.9
Minerals and trace elements (mg 100 <sup>-1</sup> g dry matter)		
• Calcium	94.6	120.8
• Magnesium	113.7	122.0
• Copper	1.4	1.3
• Iron	4.6	3.9
• Zinc	2.5	2.3
Vitamins (mg 100 <sup>-1</sup> g fresh weight of edible portion)		
• Carotene	469.0	
• Thiamin	0.3	
• Riboflavin	0.3	
• Niacin	3.0	
Ascorbic acid (vitamin C)	25.0	

Source: Faris et al, 1987.

### 2.3.2 Protein quality

Protein quality is a main factor for pigeon pea selection for use in any food processing operation. The protein quality of pigeon peas is measured by the protein content, the levels of various amino acids, and by protein digestibility (Singh and Eggum, 1984). The sulfur-containing amino acids, methionine and cysteine, are the most limiting amino acids in legumes in general, and very low contents of these amino acids were reported by Eggum and Beames (1983) for

legumes. Cooking is the common processing operation that has significant effects on protein quality, in terms of amino acids and bioavailability of legume proteins. Cooking at high temperatures or cooking for a long time may destroy certain amino acids and make them unavailable, since each amino acid has its optimum temperature at which it is stable. Any temperature above the optimum or excessive exposure to high temperatures may destroy the amino acid and make it unavailable to the body when consumed by an individual.

### **2.3.3 Antinutritional factors**

Pigeon peas have antinutritional factors such as digestive inhibitors, oligosaccharides, phytates, and tannins. The digestive inhibitors and toxicants such as hemagglutinins inhibit the activity of the digestive enzymes such as trypsin, chymotrypsin, and amylase (Onwuka, 2006). Other antinutritional factors such as tannins and phytates decrease the absorption of divalent metal ions in the intestine. These antinutritive factors form insoluble complexes with such divalent ions as  $Fe^{++}$  and  $Zn^{++}$ , and therefore make them unavailable for absorption (Elegbede 1998). However, the inhibitors can be removed wholly or in part by suitable processing methods such as heating (Onwuka, 2006), soaking (Raw and Deosthale, 1982), fermentation (Zamora and Fielda, 1979), decortication (Rao and Deosthale, 1982) and germination (Jaya et al., 1975). Therefore, effective processing of pigeon peas before utilization is suggested to limit the effect of antinutritional factors and enhance the nutritive value.

#### **2.3.4 Physicochemical properties of pigeon peas baked products**

Utilization of legumes such as pigeon pea flour in bread and confectionery products is critical in an effort to introduce nutritious products through the creative use of indigenous crops. This also serves to reduce importation costs of wheat flour, particularly in tropical countries where environmental conditions are not suitable for wheat growing (Falade and Akingbala, 2008). Pigeon pea flour has been little used in baked foods or confectionery products despite extensive research work done on baking quality and functional properties. Physicochemical properties of pigeon pea flour are important factors in determining the role of the flour in food processing and its impact on product quality.

Significant research work has been done to study the physicochemical properties of baked products made with pigeon pea flour. Gayle et al. (1986) reported that physical, sensory, and nutritional characteristics of bread samples from wheat flour supplemented with pigeon pea flour from 0-25% had no significant differences ( $P < 0.05$ ) compared with unsupplemented breads for the characteristics tested. In their study, as pigeon pea flour was increased from 0 to 25%, protein content of the bread increased from 9.2 to 13.0%, and lysine increased from 0.3 to 171.0 mg lysine/16 g N (Gayle et al., 1986).

The effect of pigeon pea flour supplementation on wheat flour biscuits was also studied by Tiwari et al. (2011). Incorporation of pigeon pea dehulled flour (PPDF) and pigeon pea by-product flour (PPBF) to wheat flour (WF) increased the protein content of biscuits from 6.21 to 8.00 g/100 g flour and from 6.21 to 8.64 g/100 g flour, respectively. Supplementation of PPDF and PPBF affected diameter, thickness and spread ratio. In general, biscuit diameter was reduced, while thickness increased with increased substitution level (Tiwari et al., 2011).

However, the relationships among flour composition and diameter and thickness were statistically different. According to the authors, although the inclusion of PPDF and PPBF affected the physical properties, the overall acceptability of PPDF and PPBF biscuits was not statistically different from that of WF biscuits. In summary, the findings from the research study confirm the feasibility of utilizing pigeon pea flour (PPF) in biscuit making.

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Raw materials

##### 3.1.1 Pigeon pea flour preparation

The pigeon pea grains were bought from Chitedze Agricultural Research Station in Lilongwe, Malawi. The grains were graded, cleaned, and soaked in water for 38 hours, after which they were dehulled manually while still wet. The loosened seeds were washed and oven-dried at 50<sup>0</sup> C for 7 hours or until the moisture content reached 11.5%. The dried grains were milled by a hammer mill to pass through a 1 mm sieve, packaged in airtight containers, and stored at room temperature (25 to 28<sup>0</sup> C) until use.

##### 3.1.2 Cassava flour preparation

High quality cassava flour (HQCF) was used for bread and cookie baking in the study. The flour was processed at Chinangwa Mbatata Roots and Tubers Enterprise (CMRTE) in Zomba, Malawi. Cassava roots were peeled and washed before grating by a mechanical grater to form a wet mash. After grating, the grits were compressed by a hydraulic jack to remove hydrocyanic acid (HCN) and water. The lumps resulting from pressing were sundried on racks for 5 to 7 hours and then milled using a hammer mill. After milling, the flour was sieved to pass through a 0.5 mm sieve. The process took place within 24 hours of harvesting cassava to avoid fermentation that often results in bad odor. HQCF was stored in airtight containers at room temperature (25 to 28<sup>0</sup> C) until use. Below is a flow chart of the procedure (Fig. 2).

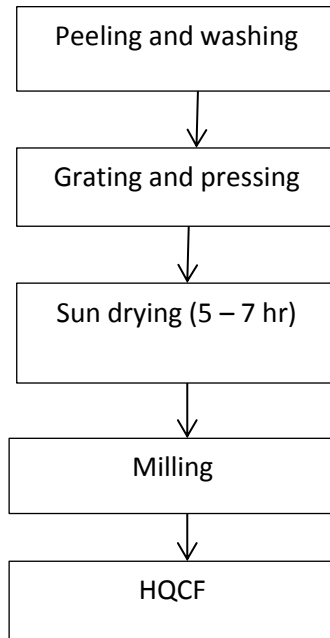


Figure 1. Flowchart for high quality cassava flour (HQCF) preparation.

### 3.1.3 Wheat flour

Biscuit wheat flour from soft wheat was obtained from Bakhresa Grain Milling Company (BGM, Blantyre, Malawi) as already-milled flour. The packaged flour samples were kept in airtight containers and stored at room temperature (25 to 28<sup>0</sup>C) until use.

### 3.2 Flour blending

The composite flours were blended as shown in Table 1. Wheat flour was used as a control flour sample. Samples A, B, C and D were wheat flour substituted with increasing amounts (5, 10, 15 and 20% w/w, respectively) of both cassava and pigeon pea flours. Samples A, B, C and control were used for breadmaking while samples B, C, D and control were used for cookie-making. Flours were mixed by a Hyaundai kitchen mixer (Beijing, China) at a speed

setting of 1 for 5 min. The flour blends were kept in labeled airtight containers at room temperature (25 to 28<sup>0</sup> C) until their use.

**Table 3. Composite Flour Formulations (% by weight)**

<b>Sample</b>	<b>Wheat flour (%)</b>	<b>Cassava flour (%)</b>	<b>Pigeon pea flour (%)</b>
A	90	5	5
B	80	10	10
C	70	15	15
D	60	20	20
E (control)	100	-	-

### **3.3 Flour tests**

The flour tests in sections 3.3.1 through 3.3.4 were performed at BGM Company in Blantrye, Malawi. The flour functional properties and viscosity studies in sections 3.3.5 through 3.3.7 were done at Chancellor College in the Zomba district of Malawi.

#### **3.3.1 Determination of flour particle size**

Particle size distributions of each of the three flour samples were determined using a Ro-tap apparatus with sieves arranged in order of decreasing mesh sizes of 330, 250, 180, 150, and 125 µm. One hundred grams of each sample was sieved by the apparatus for five minutes. For each flour sample, the overs of each sieve were collected and weighed and the respective particle size amount was expressed as g/100g.



### **3.3.2 Alveograph test**

Alveograph testing was performed by the Chopin Alveograph (Paris, France) according to AACCI Method 54-30A (AACCI, 2000) to determine gluten strength of each sample, in duplicate. A sample of 250 grams of flour was mixed with 2.5% w/w salt solution to form a dough. Circular dough patties were formed and then rested in the Alveograph in a temperature-regulated compartment at 25<sup>0</sup> C for about 20 min. The Alveograph blew air into a dough patty, which expanded into a bubble that eventually broke. From the graph, the effect of pigeon pea flour and cassava flour inclusion on the Alveostrength of wheat flour was determined.

### **3.3.3 Determination of $\alpha$ -amylase activity**

The Falling Number machine 1500 (Perten Instruments, Huddinge, Sweden) was used to determine the  $\alpha$ -amylase activity of flour and flour blends following AACCI Method 56-81B (AACCI, 2000). Seven grams of sample was added to 25 ml water in a special tube and shaken by the Falling Number machine to aid dispersion. The tube and its contents were heated in boiling water at 100<sup>0</sup> C for 60 s. The viscosity of the gel at the end of heating time was measured by allowing the stirrer-plunger to fall through the gel. The total heating time plus the time the plunger took to fall the set distance through the gel is called the Falling Number. A Falling Number value of 300 and above means low  $\alpha$ -amylase activity.

### **3.3.4 Determination of gluten content**

Gluten content was determined according to AACCI Method 38-12 (AACCI, 2000) by Glutomatic (Perten Instruments, Huddinge, Sweden). Ten grams of flour was weighed and loaded into the machine to form a dough. The dough was washed with 2% (w/v) salt solution and centrifuged in a special sieve cassette. The centrifuged dough and remainders were weighed and then weight of wet gluten was calculated.

### **3.3.5 Determination of water and oil absorption capacities**

Water and oil absorption capacities of flours and blends were determined as described by Eke and Akobundu (1993). One gram of flour sample was mixed with 10 ml distilled water or oil in a weighed centrifuge tube. The slurry was agitated on a Vortex (Scientific Industries, Inc, Bohemia, NY, USA) for 2 min, and allowed to stand at room temperature for 30 min. The slurry was then centrifuged in a high speed micro centrifuge (Seiko Co. Ltd, Tokyo, Japan) at 500 X g for 30 min. After centrifuging, the clear supernatant was decanted and discarded. The adhering drops of oil and water were removed from the tube and the tube and its contents weighed. The weight of water or oil absorbed by 1 g of flour was calculated as  $\{(W_2 - W_1/W_1) \times 100\}$  and expressed as water or oil absorption capacity.

### **3.3.6 Determination of swelling power**

Swelling power (SP) of each flour and each flour blend were determined according to Eerlingen and Declour (1997) with some modifications. The SP of each flour sample was measured at 70<sup>0</sup> C and at 80<sup>0</sup> C independently. A sample of 0.1 g was heated at 70<sup>0</sup> C or 80<sup>0</sup> C for

15 min in a water bath with intermittent shaking. The sample was then centrifuged by a high speed micro centrifuge (Seiko Co. Ltd, Tokyo, Japan) at 3000 x g for 15 min. The supernatant was decanted into a test tube and the sediment weighed. The decanted supernatant was also collected, dried and weighed. SP was calculated as  $\{(dry\ matter\ weight/sediment\ weight) \times 100\}$ .

### **3.3.7 Viscosity tests**

Flour viscosity was measured by the Brookfield viscometer (Brookfield Engineering Laboratories, Inc, Soughton, MA, USA) at 70<sup>0</sup>C and at 80<sup>0</sup>C independently. Each flour suspension (50 g in 450 ml distilled water) was heated at 70<sup>0</sup>C or 80<sup>0</sup>C. Spindle number 3 was used to stir the mixture at a shear rate of 100 rpm. A conversion factor of 20 was used to convert the dial reading. Thus, the dial reading was multiplied by 20 to come up with viscosity in centipoise (Cp).

## **3.4 Baking studies**

### **3.4.1 Breadmaking procedure**

Samples A, B, C and control were evaluated for breadmaking (Table 3) at BGM Company in Blantrye, Malawi. The ingredients are listed in Table 4 with their formula amounts. Loaves of bread were prepared from 200 g of pigeon pea-wheat-cassava flour blend or control wheat flour (Table 4), according to AACCI Approved Method 10-10B (AACCI, 2000) with some modifications. Breads were made in triplicate for each sample, with ingredients and amounts as

shown in Table 4. Yeast suspension, ascorbic acid solution, and sugar-salt solution were prepared prior to mixing. Two hundred grams of flour (14% moisture basis), fungal amylase and margarine were loaded into the mixer (MacAdams Baking Systems Pty Ltd, Cape Town, South Africa). An indentation was made where the liquid ingredients were to be placed. Sugar-salt solution, ascorbic acid, and yeast suspension were added in that order. Amount of water to be added was determined from “hand feel” during preliminary baking tests. Dough was mixed until smooth, approximately 4 to 7 minutes. Fermentation and proofing was done at 45 min by a proofer (MacAdams Baking Systems Pty Ltd, Cape Town, South Africa). Sheetting and molding was performed manually to a controlled thickness by means of gauge strips. First and second sheetings were done at  $3/16^{\text{th}}$  inch gauge strips. Panning was performed by  $5/16^{\text{th}}$  inch and then  $3/16^{\text{th}}$  inch gauge strips. The proofed doughs were baked in 18 x 8 x 6 cm baking trays at  $175^{\circ}\text{C}$  for 24 minutes. After baking, the bread was allowed to cool for 2 hours before physical measurements. A batch of four loaves of bread was baked in a day and after physical measurements, each loaf was wrapped in Ziplock bag and stored in a deep freezer at  $-4^{\circ}\text{C}$  until chemical composition analysis.

**Table 4. Breadmaking Formulation**

<b>Ingredient</b>	<b>Amount per 200 g loaf</b>
Flour or flour blend	200 g
Sugar-salt solution	22 ml
Margarine (50% fat)	20 g
Fungal amylase	0.5 g
Yeast suspension	40 ml
Ascorbic acid	10 ml
Water	Variable (74 to 40 ml)

### **3.4.2 Physical analysis of bread samples**

After sufficient cooling, the weight, volume, and height of each bread loaf were measured. A digital balance (0.01 g accuracy) was used to measure bread weight. Volume was measured by seed displacement method using rapeseed. A container of known volume was put in a tray, and a bread loaf sample was placed inside the container. The container was then filled with rape seed until overfilled. A straight edge was used to scrape across the top of the container once to give a level surface. The volume of seed remaining in the container was measured in a graduated cylinder and the amount of displaced seed calculated based on the original known container volume. The procedure was repeated three times and the mean value for displaced seed volume was calculated. The mean volume of the displaced seed was equated to the volume of the bread loaf. Specific volume bread loaf was calculated as bread volume divided by bread weight.

### **3.5 Cookiemaking procedure**

Samples B, C, and D as indicated in Table 3 were evaluated alongside control (E) for cookiemaking at BGM in Blantyre, Malawi. The ingredients included fine granulated brown sugar, milk powder, iodized salt, baking powder and margarine and are listed with their formula

amounts in Table 5. Cookies were prepared according to AACCI Approved Method 10-54 (AACCI, 2000) with slight modifications. The batch size was adjusted to 200 g of flour or flour blend. A single mixing stage was performed where all the ingredients were put into a mixer (MacAdams Baking Systems Pty Ltd, Cape Town, South Africa) and mixed at low speed for a total time of 2 min, with scraping after every 30 sec. The dough was rolled to the correct thickness using gauge strips and cut using a 50 mm round cookie cutter. Cookies were baked at 182<sup>0</sup> C for 10 min in a convection oven. After cooling, cookies were analyzed for physical characteristics. Cookies were baked in triplicate for each flour sample. A batch of cookies from one flour sample was baked in a day. Physical measurements were taken after sufficient cooling of cookies and thereafter cookies were put in a ziplock bag, and then stored in a deep freezer at -4<sup>0</sup> C until chemical composition analysis.

**Table 5. Cookiemaking Formulation**

<b>Ingredient</b>	<b>Amount per batch</b>
Flour or flour blend	200 g
Sugar, fine brown granulated	80 g
Margarine, (50% fat)	100 g
Milk powder, (skim)	24 g
Baking powder	3 g
Salt	1 g
Water	Variable (45 to 55 ml)

### **3.5.1 Physical analysis of cookie samples**

Cookies were analyzed for weight, diameter, height, and spread ratio. A digital balance (0.01 g accuracy) was used to measure cookie weight. Two cookies from the same batch were laid edge to edge and the total two-cookie diameter measured. Similarly, two cookies were

stacked on top of each other and the total two-cookie height measured. Measurements were done with digital Vernier calipers. Total two-cookie diameter divided by the total two-cookie height gave the spread ratio.

### **3.6 Chemical analysis of flours, breads and cookies**

The crude protein (Kjeldahl), fat (solvent extraction), moisture (oven), ash (muffle furnace) and fiber contents were determined according to AOAC (1990) Methods.

Carbohydrate content was determined as the difference between the total sample weight and that of the moisture, protein, fat, fiber, and minerals combined. Energy value (Kcal/100 g) was calculated by the Atwater Method (Osborne and Voogt, 1998) (g of protein x 4; g of fat x 9; g of carbohydrate x 4). Analyses were performed in duplicate for the flours, their composite blends and the baked products. All the chemical analyses were done at Polytechnic in Blantyre, Malawi.

#### **3.6.1 Protein analysis**

Protein content was determined by Kjeldahl Method. Two g dried sample were added to 2 tablets of mercuric sulfate and 250 ml sulfuric acid. The mixture was heated at 450 °C for 30 min in a Kjeltec digester until a clear solution was produced. 10 ml of 0.5% w/v sodium hydroxide was added through a tube into a Kjeldhal distillation apparatus. The ammonia in the sample was steam distilled for 5 min into a receiving flask containing 5% boric acid. The sample was titrated with 0.1% v/v hydrochloric acid solution. Protein content was calculated by the equation; Nitrogen X 5.8 for CF, WF, PPF and flour blends.

### 3.6.2 Fat analysis

Fat analysis was performed using an extraction apparatus. The apparatus consists of an Extraction Unit and a Control Unit. Two g (W1) sample to be analyzed was weighed into thimbles and inserted in the Extraction Unit. 80 ml of solvent was added into the extraction unit and the thimble was closed with a cup. The cups were placed on an electrical heating plate to heat the sample with extraction solvent. The 4-step extraction procedure consisted of boiling, rinsing, recovery and pre-drying. After the extraction procedure, the sample was weighed again (W2). Percent fat was calculated as  $\{(W2 - W1)/W1 \times 100\}$ .

### 3.6.3 Moisture analysis

Five g (W1) sample was weighed into the container and placed in a vacuum oven at 130<sup>0</sup>C for 1 hour. The sample was removed from the oven and cooled in a desiccator. After cooling, the sample was weighed again (W2). Percent moisture was calculated as  $\{(W1 - W2) \times 100\}$ .

### 3.6.4 Ash analysis

Two g of flour sample was weighed in a tarred crucible and transferred to a cool Muffle Furnace before increasing the temperature stepwise to 600<sup>0</sup>C  $\pm$  5<sup>0</sup>C. The temperature was maintained for 4 hours or until a white ash was obtained. After ashing, the crucible was allowed to cool in a desiccator and weighed soon after cooling. Percent ash was calculated as  $\{(weight\ of\ crucible\ and\ ash - weight\ of\ crucible) / (weight\ of\ crucible\ and\ sample - weight\ of\ crucible) \times 100\}$ .



### 3.6.5 Fiber analysis

Two g of sample was weighed (W1) in a beaker and 150 ml of 1.25% sulfuric acid was added. The sample was boiled for exactly 30 minutes while adding water continuously to the boiling sulfuric acid to maintain the initial liquid level. After boiling, the boiled sample was washed and filtered three times with 30 ml of hot deionized water. 150 ml of 1.25% preheated potassium hydroxide (KOH) was added to the washed sample and then boiled for 30 minutes. Water was continuously added to the boiling sample and KOH to maintain the initial liquid level. After 30 minutes of boiling, the sample was washed and filtered twice using hot deionized water and then cold deionized water to cool the sample. The sample was then washed three times with 25 ml of acetone. The dry weight (W2) was determined after drying the sample in an oven at 130<sup>0</sup> C for an hour and cooling in a desiccator. Percent fiber was calculated as  $\{(W2 - W1/W1) \times 100\}$ .

### 3.7 Determination of total HCN

The HCN analyses were conducted at Chancellor College in Zomba, Malawi. Total cyanogens of each of the baked samples was determined in duplicate using the Picrate Kit Method (Bradbury et al., 1999) to assess the safety of the products for human consumption. One hundred milligrams sample was placed in a small plastic bottle. A standard linamarase-impregnated paper and 0.5 ml of water was added before the bottles were closed with a screw cap. The bottles and contents were allowed to stand for 24 h at 30<sup>0</sup> C, after which the Picrate papers were removed from the plastic bottle and 5.0 mL of water added to elute the color. The

absorbance was measured at 510 nm using a UV-Visible spectrophotometer (Shimadzu Corporation, Tokyo, Japan) and the value was used to determine the total cyanide content in mg HCN equivalents/kg fresh weight = ppm. Total cyanide content was calculated by multiplying the absorbance value by 396 (Bradbury et al., 1999); thereby giving an accurate total cyanide analysis down to a minimum of 1 ppm total cyanide.

### **3.8 Digestibility studies**

Digestibility studies were performed at Chancellor College in Zomba, Malawi.

#### **3.8.1 *In vitro* carbohydrate digestibility (IVCD)**

*In vitro* digestibility of carbohydrates of bread and cookie samples was determined according to the method described by Shekib et al. (1988) based on starch-iodine color changes. Samples of baked products were ground into powder in a blender. Starch was extracted from the baked sample using 85% orthophosphoric acid. Five ml of starch solution (obtained from a sample), 4 ml of 0.1 M phosphate buffer (pH 6.6), 1 ml of 0.003 M sodium chloride, and 1 ml of  $\alpha$ -amylase enzyme was measured and added into a test tube and the whole solution mixed thoroughly to make the reaction mixture. Appropriate blanks with enzymes were also prepared and incubated concurrently with samples at 37<sup>0</sup> C for 1 hr. Aliquots (0.2 ml) of the mixture were taken at time zero and at 1 hour after addition of the enzyme and dispensed into 10 ml Lugol's iodine solution (1:100 dilution) and the absorbance measured at 565 nm with a UV-Visible spectrophotometer (Shimadzu Corporation, Tokyo, Japan). Absorbance of the blank was subtracted from each sample reading. Samples were analyzed in

duplicates. *In vitro* carbohydrate digestibility was calculated as:  $\{(Absorbance\ at\ zero\ time - Absorbance\ at\ 1\ hour) / Absorbance\ at\ zero\ time\} \times 100$ .

### **3.8.2 *In vitro* protein digestibility (IVPD)**

Protein digestibility of bread and of cookie samples was determined in duplicate using the procedure of Mertz et al. (1984). Samples of baked products were ground into powder by a blender. Ground samples (200 mg) of bread or cookie was weighed into a flask and mixed with 35 ml of porcine pepsin (1.5 mg/ml of pepsin in 0.1 M phosphate buffer, pH 2.0). Samples were incubated for 2 h at 37<sup>0</sup> C in a shaking water bath. To stop digestion, 2 ml of 2M NaOH was added. Samples were centrifuged using a High Speed Micro Centrifuge (Seiko Co., Ltd, Tokyo, Japan) at 4900 X g, 4<sup>0</sup> C for 20 min after which the supernatants were discarded. The residues were washed and centrifuged twice, at the same centrifuge parameters, with 20 ml of 0.1 M phosphate buffer (pH 7.0). Undigested nitrogen of the residues was determined using the Kjeldahl method and multiplied by 5.8, to obtain undigested protein. The analyses were run in duplicate baked sample. Digestibility was calculated as % digestibility protein (%P) =  $\{(P\ in\ sample - undigested\ P) / P\ in\ sample\} \times 100$ .

### **3.9 Experimental design and statistical analysis**

The study was implemented in a complete block design with three replicates per sample unless otherwise stated. Breads and cookies were made from wheat flour supplemented with increasing levels of both pigeon pea and cassava flours (for total flour substitution levels of 10,

20, 30 and 40%). Pure wheat flour was used as the control for both bread and cookie samples. Flour tests were performed prior to baking tests, while the chemical composition and digestibility analysis were conducted on products after baking. All statistical analyses were performed using GeneStat version 6.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, 2002). Analysis of variance (ANOVA) was performed to determine significant differences among the samples. Means were compared using Fisher's least significant difference (LSD) procedure. Significance was defined at the 5% level.

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1. Chemical composition of flours

Highly significant differences were observed among protein contents of samples (Table 6). Within the flour category, PPF (pigeon pea flour) had the highest amount of protein, followed by the control (wheat flour, WF), and then CF (cassava flour), which was the lowest. The protein contents of PPF and CF found in this study were similar to the literature values for PPF (Eneche, 1999) and CF (Akubor and Ukwuru, 2003). The fact that PPF had the highest amount of protein is mainly attributed to the high protein content of the pigeon pea grain. Pigeon peas are legumes and are naturally rich in protein content. Among the blended samples, the blend with 5% level of PPF substitution had the lowest protein content (17.40%). Addition of PPF at high levels significantly enhanced protein content of the blended samples. Thus, the high protein content of PPF makes it a useful material for supplementation of lower protein flours, such as cassava and wheat flours, for preparation of protein-enriched products.

All the test flours (WF, CF and PPF) originated from starchy crops, and thus samples were analyzed for carbohydrate content (Table 6). Analysis of results shows significant differences among samples ( $P < 0.001$ ). Within the flour category, the greatest amount of carbohydrate was found in CF (84.00%), while PPF was the lowest. The results were expected since cassava is mainly composed of carbohydrate available in the form of starch. Addition of CF and PPF to WF in increasing proportions resulted in a stepwise decrease in carbohydrate content

of the flour blends. Among the flour blends, the blend with the highest proportion of CF (20%) had the lowest carbohydrate content (64.21%). This is mainly due to the low CHO content of PPF diluting the overall CHO in the blended flour samples. Olu et al. (2011) also reported a decrease in carbohydrate content as a result of supplementing wheat flour with soybean flour.

Within the flour category, WF had the highest fat content, closely followed by PPF, while CF had the lowest (Table 6). The grains of wheat and pigeon peas have considerable amounts of fat and this might have resulted in high fat content of WF and PPF. Olalekan and Bosede (2010) reported greatest amount of fat in PPF (4.78%) compared to cowpea and jack bean flours. The results also revealed that CF is poor in fat. Incorporation of PPF resulted in high fat contents of the flour blends compared to that of CF. Fat is important in product formulation to improve texture and rheology of the product. The low fat content of CF implies that, depending on the product type, fat needs to be added in the formulation if CF is to be used as the main ingredient in the formulation to improve texture and rheology as well as the overall quality of the final product.

According to results in Table 6, addition of non-wheat flours significantly influenced the ash contents of flour samples ( $P < 0.001$ ). PPF had the highest ash content of 2.40% that was significantly higher than all other flour or blended flour samples. The ash content of control (WF) was not statistically different than that of CF. As expected, supplementation of WF with CF and PPF at high levels greatly increased the ash content of the blended flour. This is mainly explained by the high ash content of PPF which could have occurred due to contamination during milling and inefficient milling. The absence of contamination during milling wheat was responsible for low ash content of WF.

Incorporation of CF and PPF influenced the moisture contents of the blends. Within the flour category, the moisture contents were 10.7%, 11.0% and 7.0% for WF, CF and PPF, respectively (Table 6). The low moisture contents of the flours were also reflected in the blends; the moisture contents of the blends ranged from 10.35% (60% WF) to 11% (90% WF). In general, the moisture content of flour samples was low, which is important for storage of flours for longer periods of time without deterioration.

Among the flour samples, the greatest amount of fiber was found in CF, whereas PPF was the lowest (Table 6). The result is ascribed to the presence of high amounts of fiber in cassava roots. The low fiber of PPF is explained mainly by the removal of the seed coat during dehulling of pigeon pea grain. Addition of CF in high proportions (15 or 20%) resulted in marked increases in fiber content of the blended flour samples. To this effect, the blended flour with 20% CF registered a fiber content of 1.45% representing a 19.4% increase in percent fiber compared with control. The presence of fiber in CF is important as a source of dietary fiber in baked products for human consumption. Dietary fiber is a non-starchy polysaccharide that is neither digested nor absorbed in the stomach. The recent increased interest in dietary fiber is related to its effects in the gastrointestinal tract. As undigested components, dietary fiber increases satiety, promotes large bowel movements, and prevents bowel inflammatory diseases and colorectal cancer (Topping and Anthony, 2003).

The energy of a food substance is a function of the total protein, fat and carbohydrates present in that food. It is estimated by multiplying the gram amounts of protein, carbohydrate and fat by their respective energy values (4, 4, and 9) and summing the three values. The results have demonstrated that CF registered the lowest energy value (346.87 Kcal/100g)

among the three single-flour samples studied. The low energy of CF was expected because cassava is a poor source of fat, which contributes the most energy by weight (9 kcal/g). A stepwise decrease in amount of energy was observed among the blends due to addition of increasing amounts of CF that diluted the fat contributed by WF and PPF. Notably, flour blends with the highest content of CF (20 %) had the lowest energy value (355.43 kcal/100 g). Still, the energy values of blended flours were above that of CF alone, implying that utilizing the flour blends would result in higher energy baked products than from CF alone.



**Table 6. Chemical Composition<sup>1</sup> (d.b.) of Wheat, Cassava, Pigeon Pea and Blended Flours**

Flour Sample <sup>2</sup>	% Protein	% CHO	% Fat	% Ash	% Moisture	% Fiber	Energy (kcal/100g)
100% WF	11.30 <sup>b</sup>	67.24 <sup>e</sup>	3.00 <sup>e</sup>	0.61 <sup>a</sup>	10.75 <sup>b</sup>	1.20 <sup>c</sup>	364.76 <sup>c</sup>
100% CF	1.50 <sup>a</sup>	84.00 <sup>g</sup>	0.80 <sup>a</sup>	0.84 <sup>a</sup>	11.00 <sup>c</sup>	1.80 <sup>d</sup>	346.87 <sup>a</sup>
100% PPF	32.65 <sup>f</sup>	54.60 <sup>a</sup>	2.40 <sup>d</sup>	2.50 <sup>c</sup>	7.00 <sup>a</sup>	0.75 <sup>b</sup>	371.00 <sup>d</sup>
90%WF:5%CF:5%PPF	17.40 <sup>b</sup>	68.30 <sup>f</sup>	1.94 <sup>c</sup>	0.57 <sup>a</sup>	11.00 <sup>c</sup>	0.80 <sup>a</sup>	360.24 <sup>bc</sup>
80%WF:10%CF:10%PPF	19.20 <sup>c</sup>	66.52 <sup>d</sup>	1.77 <sup>bc</sup>	0.77 <sup>a</sup>	10.80 <sup>b</sup>	0.95 <sup>a</sup>	358.69 <sup>b</sup>
70%WF:15%CF:15%PPF	20.35 <sup>d</sup>	65.31 <sup>c</sup>	1.58 <sup>bc</sup>	0.92 <sup>a</sup>	10.65 <sup>b</sup>	1.20 <sup>a</sup>	356.78 <sup>b</sup>
60%WF:20%CF:20%PPF	21.40 <sup>e</sup>	64.21 <sup>b</sup>	1.45 <sup>b</sup>	1.15 <sup>b</sup>	10.35 <sup>b</sup>	1.45 <sup>a</sup>	355.43 <sup>b</sup>
SEM	0.16	0.27	0.17	0.17	0.28	0.09	2.20

<sup>1</sup> Means with the same superscript in the same column are not significantly different (p<0.05).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour, CHO = carbohydrate.

#### **4.2. Particle size distribution of wheat, cassava and pigeon pea flours**

Flour samples were sieved by a Ro tap apparatus with sieves arranged in order of decreasing mesh size, and the amount of each particle size fraction was expressed as g/100g sample. The result of particle size analysis showed that the amounts of PPF and of CF particles were greatest in the large pore size category and decreased as mesh size decreased, while the opposite was observed for WF (Table 7). This implies that WF was comprised of more small particles (less than 125  $\mu\text{m}$ ) than were CF and PPF (Table 7). The presence of relatively large particles of PPF is attributed to grain hardness and inefficient milling. On the other hand, cassava roots contain some fiber which to some extent might be hard to fine mill and sieve hence resulting in more large particles of CF. It is well established that the degree of flour fineness in a milling operation depends on the type and efficiency of the applied machine (Oladunmoye et al., 2010). The small grain size and tempering of wheat before milling improves milling efficiency and resulted in fine particles. According to UNECA (1985), particle size of about 130  $\mu\text{m}$  is suitable for WF intended for baking bread, biscuits and other pastry products. From their experience with composite flours, Crabtree and James (1982) indicated that particle size of about 180  $\mu\text{m}$  and fiber-free is ideal composite flour for breadmaking.

From the current findings, WF particle size distribution was within the range expected for bread and cookie baking. Particle size is one of the most important characteristics of a flour, which may influence other physicochemical properties such as swelling, water binding capacity and pasting properties (Singh et al., 2003). The particle size distribution of a flour could be very important for specific applications. Bearing these factors in mind, PPF and CF could be best raw materials for products where lower water binding and smooth textures are not as important.

For breadmaking, the presence of fibers and large particles in flour might puncture the expanding gas cells during fermentation, thereby reducing loaf volume. Moreover the fibers and large particles of flour could result in a rough and speckled exterior bread loaf appearance. (Crabtree and James, 1982).

**Table 7. Particle Size Distribution<sup>1</sup> (g/100g) of Wheat, Cassava and Pigeon Pea Flours**

Flour Sample <sup>2</sup>	≥330 μm	330 - 250 μm	250 - 180 μm	180 - 150 μm	150 - 125 μm
100% WF	-	0.001 <sup>a</sup>	0.11 <sup>a</sup>	0.17 <sup>a</sup>	0.77 <sup>a</sup>
100% CF	11.98 <sup>a</sup>	10.65 <sup>c</sup>	8.33 <sup>b</sup>	6.76 <sup>b</sup>	1.80 <sup>b</sup>
100% PPF	11.64 <sup>a</sup>	9.30 <sup>b</sup>	8.31 <sup>b</sup>	7.45 <sup>b</sup>	2.04 <sup>c</sup>
SEM	0.30	0.16	0.09	0.25	0.05

<sup>1</sup> Means with the same superscript in the same column are not significantly different ( $p < 0.05$ ).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour.

#### 4.3. Falling Number and gluten content of flours and blends

The Falling Number machine indirectly measures  $\alpha$ -amylase activity via degree of hydrolysis of starch granules in the paste. Alpha-amylase is an enzyme that degrades starch when the grain is soaked in water. Flour with a low Falling Number (i.e., a less viscous starch paste) is deemed to have high  $\alpha$ -amylase activity and usually produces a weak bread dough that does not rise much during baking. To this effect, Falling Numbers of flours and flour blends were measured to assess the impact of CF and PPF supplementation on WF and establish its effect on baking quality (Table 8). From the results in Table 8, the control (WF) and CF had Falling Number values of 400 implying that there was very low  $\alpha$ -amylase activity (i.e., highly viscous starch paste). PPF had a mean Falling Number of 296, indicating that  $\alpha$ -amylase activity was high, resulting in a weakly viscous starch paste. The low Falling Number of PPF is ascribed

to soaking of pigeon peas during flour preparation that might have triggered the  $\alpha$ -amylase enzyme to start degrading starch. Substitution of WF with PPF alongside CF significantly lowered the Falling Number of the flour blends studied. A study has been conducted on the effect of  $\alpha$ -amylase enzyme on the Falling Number of wheat flour and it was reported that addition of malt to wheat-cassava composite flours resulted in reduced Falling Number, indicating high  $\alpha$ -amylase activity of the malt (Khalil et al., 1999). The study further revealed that doughs made from wheat-cassava composite flours supplemented with  $\alpha$ -amylase enzyme were wet and sticky. Based on Khalil's studies, use of bread improvers is encouraged where flours with low Falling Numbers are used for breadmaking.

Gluten is a type of protein that is found in WF and it develops when the flour is hydrated. Gluten content of flours and blends was measured according to AACCI Method 38-12 (AACCI, 2000) and the influence of CF and PPF supplementation on WF was established. It should be noted that no gluten was determined in CF and PPF because naturally the two have no gluten-forming protein. The gluten content of the control (28.80 %, Table 8) was significantly higher than those of the blended flours. As expected, inclusion of non-gluten forming flours (CF and PPF) resulted in dilution of gluten content of the blends compared to that of the control (WF). Among the blends, gluten content decreased from 25.1% for 90% WF to 12.05% for 60% WF. The decline in gluten content of flour blends is consistent with the reduced loaf volume of bread made from the flour blends (see details in Table 12). The quantity of gluten is responsible for extensibility and gas retention of the dough, and increased quantity results in a high quality bread product. The findings of the current study support the previous findings by Hassan et al. (2011) and Olu et al. (2011) in which the gluten content of the blended flours ranged from 32%

to 25% as WF was increasingly replaced by decorticated pigeon pea flour. Additionally, Olu et al. (2011) reported a decline in wet gluten content from 20.4% (100% WF) to 0.4% with 70%WF:30% soybean flour. According to the findings in the present study, it is suggested that where flours with low gluten content are used for breadmaking, deliberate efforts need to be made to improve bread loaf volume and quality.

**Table 8. Falling Number<sup>1</sup> and Gluten Content<sup>1</sup> of Flours and Blended Flour Samples**

Flour Sample <sup>2</sup>	Falling Number (seconds)	% Gluten
100% WF	400.00 <sup>f</sup>	28.80 <sup>e</sup>
100% CF	400.00 <sup>f</sup>	ND
100% PPF	296.00 <sup>e</sup>	ND
90%WF:5%CF:5%PPF	283.00 <sup>d</sup>	25.10 <sup>d</sup>
80%WF:10%CF:10%PPF	266.00 <sup>c</sup>	23.50 <sup>c</sup>
70%WF:15%CF:15%PPF	261.00 <sup>b</sup>	19.10 <sup>b</sup>
60%WF:20%CF:20%PPF	257.00 <sup>a</sup>	12.05 <sup>a</sup>
SEM	0.76	0.12

<sup>1</sup> Means with the same superscript in the same column are not significantly different (p<0.05).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour, ND= not determined

#### 4.4. Work and Alveostrength

Work and Alveostrength of flours and blended flours were measured to determine the effect of CF and PPF supplementation on wheat dough rheology. Due to the inherent lack of gluten, work and Alveostrength of CF and PPF were not measured. Work and Alveostrength significantly decreased with addition of non-wheat flours to WF (Table 9). Work refers to the amount of energy required to inflate a piece of dough into a balloon before it ruptures, thereby giving information about dough strength. From the results, more work was required to inflate the control dough balloon than those of blends, resulting in significantly higher Alveostrength

values for WF. Consequently, the control exhibited the greatest work (221 kJ) and Alveostrength (33.79) values, which were statistically different from the respective values for the blends. Work and Alveostrength are greatly reduced by addition of CF and PPF, even at only 5% substitution levels, due to the dilution effect on gluten. The values of the measured parameters (work and Alveostrength) displayed by WF are related to gluten content that is responsible for dough extensibility. Similar results were reported by Wu et al. (2009) who found that work and Alveostrength decreased notably as wheat flour was supplemented with increasing proportions of sweet potato paste. Again, the authors attributed the result to dilution of gluten in WF.

Gluten stretches enormously when hydrated and it needs a substantial amount of energy to be inflated into a balloon before rupturing. The lower gluten content in the flour blends resulted in reduced work and Alveostrength. The relatively low work values of blended doughs might also be attributed to the presence of large flour particles in PPF and fibers in CF that might have punctured the dough as it was being inflated, resulting in rupture of the dough before full expansion. For the purpose of breadmaking, the results suggest that doughs made from blended flours will be weak and not rise much during baking, hence producing reduced volume loaves.

**Table 9. Work<sup>1</sup>, and Alveostrength<sup>1</sup> of Flours and Blended Flour Samples**

Flour Sample <sup>2</sup>	Work (Kj)	Alveostrength
100% WF	221.00 <sup>e</sup>	33.79 <sup>e</sup>
100% CF	ND	ND
100% PPF	ND	ND
90%WF:5%CF:5%PPF	216.00 <sup>d</sup>	33.14 <sup>d</sup>
80%WF:10%CF:10%PPF	210.00 <sup>c</sup>	32.11 <sup>c</sup>
70%WF:15%CF:15%PPF	201.00 <sup>b</sup>	30.81 <sup>b</sup>
60%WF:20%CF:20%PPF	197.50 <sup>a</sup>	30.12 <sup>a</sup>
SEM	0.81	0.12

<sup>1</sup> Means with the same superscript in the same column are not significantly different ( $p < 0.05$ ).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour, ND= not determined.

#### 4.5. Water and oil absorption capacities of flours and blends

Supplementation of WF with CF and PPF significantly influenced the water absorption capacity (WAC) and oil absorption capacity (OAC) of samples ( $P < 0.001$ ) (Table 10). Among the samples, CF displayed the highest WAC (12.9%), followed by PPF, while the control had the lowest (2.1%). The flour blends had moderate WAC values that were also higher than that of the control (WF), and these increased as levels of non-wheat flours were increased in the blends. Thus incorporation of CF and PPF at high levels significantly improved the WAC of flour blends. A similar trend was observed in the OAC of the flour samples within the flour category. The greatest OAC was observed for CF, while the control had the lowest. The OAC of the flour blends decreased upon addition of CF and PPF, as compared to CF alone. To this effect, the high WAC and OAC of CF might be attributed to the molecular structure of cassava starch. Molecules of cassava starch are loosely linked, allowing for more penetration of liquid materials. The presence of high levels of fat and protein in WF and PPF could explain the low

OAC of the flours. As a result, OAC values of flour blends were significantly lower than that of CF alone.

These absorption capacity attributes may give advantage to the blends relative to the single flours (CF, PPF and WF) in baked products where hydration to improve dough handling is a preferred characteristic. The blends could also be used in formulations for products such as ground meat, doughnuts and pancakes, where oil absorption is not of paramount importance. It should be noted that the WAC and OAC of PPF found in this study are much lower than those reported by Olalekan and Bosede (2010). The authors reported WAC and OAC values of 14.8% and 18.9%, respectively, for PPF. Based on the results, the lower WAC of WF confirms the low charge density and relative hydrophobicity of wheat protein that gives it a tendency to interact more with itself. Therefore, WF could be more useful than CF and PPF as a functional ingredient in viscous foods, like baked products, gravies and soups, to increase viscosity (Abulude et al., 2006).

**Table 10. Water and Oil Absorption Capacities<sup>1</sup> of Flour and Blended Flour Samples**

Flour Sample <sup>2</sup>	% WAC	% OAC
100% WF	6.1 <sup>a</sup>	7.0 <sup>b</sup>
100% CF	12.9 <sup>f</sup>	9.0 <sup>f</sup>
100% PPF	10.4 <sup>e</sup>	7.1 <sup>c</sup>
90%WF:5%CF:5%PPF	4.7 <sup>b</sup>	7.5 <sup>d</sup>
80%WF:10%CF:10%PPF	6.0 <sup>c</sup>	7.1 <sup>c</sup>
70%WF:15%CF:15%PPF	6.0 <sup>c</sup>	7.7 <sup>e</sup>
60%WF:20%CF:20%PPF	6.6 <sup>d</sup>	6.4 <sup>a</sup>
SEM	0.1	0.0

<sup>1</sup> WAC = water absorption capacity, OAC = oil absorption capacity. Means with the same superscript in the same column are not significantly different ( $p < 0.05$ ).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour.



#### 4.6. Swelling power and viscosity of flours and blended flours

The swelling power (SP) was assessed to observe the nature of bonds within starch granules of flours and flour blends. Heating of samples in the presence of water at 70<sup>0</sup> C or 80<sup>0</sup> C, resulted in samples with different SP values (Table 11). Results indicated that SP values of the flours and flour blends are influenced by temperature. Among the flours, CF had the highest SP at both temperatures studied compared to those of WF and PPF. The SPs of the blended flours were significantly lower than those of the pure flour samples. The variation in SP is indicative of the differences in bonding forces within the starch granules of the samples (Nwokocha et al., 2008). According to Balagopalan et al. (1988), the bonds relax with increases in thermal agitation, causing the starch granules to imbibe water and swell. In the present study, CF displayed weaker bonds within starch granules as indicated by the high SP values at 70<sup>0</sup> C and 80<sup>0</sup> C. Conversely, the low SPs of WF and PPF reflected their stronger bonding within their starch granules.

In the development of any food product from starchy crops, knowledge of physicochemical properties like viscosity or pasting properties of the starch, which is a main component of flour, is needed to predict behavior under a given processing condition. In the present study, flour viscosity was measured by the Brookfield viscometer (Brookfield Engineering Laboratories, Inc. Stoughton, MA, 02702, USA) at 70<sup>0</sup> C and 80<sup>0</sup> C. The analysis of results revealed that the viscosity of flour samples increased with increases in temperature. Cassava flour had an initial viscosity of 1180 Cp at 70<sup>0</sup> C and it reached a maximum viscosity of

1380 Cp at 80<sup>0</sup> C that was significantly higher than control and flour blends. The viscosity displayed by flour samples is related to their SP and the trend in viscosity results was almost the mirror image of the SP results. The viscosity patterns may be related to the differences in chemical composition of the various starches (amylose: amylopectin) and the nature of the bonding within the starch structure. The starch polymer of CF has molecules that are loosely linked (Oluwamukomi et al., 2011) and they easily absorb water and expand more when heated, thereby increasing viscosity (Caicco and D'Applonia, 1976). PPF registered the lowest viscosity at both temperatures, probably due to its low carbohydrate content. The flour blends had intermediate viscosities but higher than those of PPF at both temperatures. This is ascribed to incorporation of CF in the blends. Consequently, the viscosity of blends increased as the proportion of CF was increased. According to Nuwamanya et al. (2010), the relatively low viscosities of WF, PPF and flour blends would indicate better cooking properties. The results of Nuwamanya et al. (2010) study further suggest that CF flour needs to be modified to change the water holding properties of the starch granules and thus improve its application in the food industry.

**Table 11. Swelling Power<sup>1</sup> and Viscosity<sup>1</sup> of Flours and Blended Flour Samples**

Flour Sample <sup>2</sup>	% SP (70 <sup>0</sup> C)	% SP (80 <sup>0</sup> C)	Viscosity 70 <sup>0</sup> C (Cp)	Viscosity 80 <sup>0</sup> C (Cp)
100% WF	4.2 <sup>d</sup>	4.9 <sup>d</sup>	36.5 <sup>b</sup>	235.0 <sup>d</sup>
100% CF	5.6 <sup>f</sup>	6.7 <sup>e</sup>	1180.0 <sup>e</sup>	1380.0 <sup>f</sup>
100% PPF	4.3 <sup>e</sup>	4.7 <sup>d</sup>	22.0 <sup>a</sup>	30.0 <sup>a</sup>
90%WF:5%CF:5%PPF	3.3 <sup>b</sup>	3.8 <sup>c</sup>	44.0 <sup>c</sup>	76.0 <sup>b</sup>
80%WF:10%CF:10%PPF	3.3 <sup>b</sup>	3.6 <sup>b</sup>	44.0 <sup>c</sup>	140.0 <sup>c</sup>
70%WF:15%CF:15%PPF	3.6 <sup>c</sup>	3.8 <sup>c</sup>	46.0 <sup>c</sup>	165.5 <sup>c</sup>
60%WF:20%CF:20%PPF	3.0 <sup>a</sup>	3.2 <sup>a</sup>	55.0 <sup>d</sup>	335.0 <sup>e</sup>
SEM	0.1	0.1	2.0	3.4

<sup>1</sup> WAC = water absorption capacity, OAC = oil absorption capacity, SP = swelling power. Means with the same superscript in the same column are not significantly different (p<0.05).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour.

#### **4.7. Physical characteristics of bread made from wheat flour supplemented with cassava and pigeon pea flours**

Physical characteristics of breads were measured to determine the effect of supplementation of CF and PPF on baked loaf weight, height, and volume. In the present study, breads made from the flour blends were denser compared to the control bread (WF) (Table 12, Figure 2). In contrast, loaf height, volume and specific volume were higher for the control than the composite breads. According to Wu et al. (2009), bread loaf weight is determined by amount of baked dough, moisture, and carbon dioxide diffused out of the loaf during baking. It should be known that bread loaf volume is affected by protein quality and quantity. The presence of gluten protein in wheat flour was presumed responsible for the increased loaf volume and height. Gluten develops when WF is mixed with water and it forms a matrix that retains more gas. As the dough is baked, it expands more, thereby increasing loaf volume and

height. As a consequence of gas retention the bread loaf is lighter. However, addition of CF and PPF to WF lowered the amount of gluten in the blends, causing poor gas retention and thus reduced loaf volume and height of the blended flour breads.

The presence of fiber in CF and relatively large particles of PPF might also have played significant roles in reducing loaf volume by puncturing gas cells as the dough was expanding. The results are in agreement with findings by Eggleston et al. (1993) and Defloor et al. (1993). Eggleston et al. (1993) reported a decrease in volume and specific volume of baked bread samples as WF was substituted with cassava flour and starch at increasing levels. The reduction in loaf volume as a result of increased supplementation of non-wheat flours is an undesirable quality economically, and poses a threat to the baking industry. It is reasonable that bread with a high loaf volume attracts more customers who believe that it is more valuable than a smaller loaf of the same weight for the same price. It is therefore suggested that more research work needs to be done to explore alternatives to improve breads made from blended flours. Such efforts will attract bakers to use blended flours for breadmaking.

**Table 12. Physical Characteristics<sup>1</sup> of Bread Made from Wheat Flour Supplemented with Cassava and Pigeon Pea Flours**

Bread Sample <sup>2</sup>	Loaf weight (g)	Loaf height (cm)	Loaf volume (cm <sup>3</sup> )	Specific volume
100% WF	283.30 <sup>a</sup>	16.17 <sup>d</sup>	1500 <sup>d</sup>	5.29 <sup>d</sup>
90%WF:5%CF:5%PPF	281.00 <sup>a</sup>	12.10 <sup>c</sup>	1367 <sup>c</sup>	4.86 <sup>c</sup>
80%WF:10%CF:10%PPF	286.00 <sup>a</sup>	7.67 <sup>b</sup>	917 <sup>b</sup>	3.20 <sup>b</sup>
70%WF:15%CF:15%PPF	296.00 <sup>b</sup>	5.80 <sup>a</sup>	717 <sup>a</sup>	2.42 <sup>a</sup>
SEM	4.08	0.58	39.10	0.13

<sup>1</sup> Means with the same superscript in the same column are not significantly different ( $p < 0.05$ ).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour.

#### **4.8. Physical characteristics of wheat cookies supplemented with cassava and pigeon pea flours**

Supplementation of WF with CF and PPF significantly improved the diameter and weight of blended flour cookies (Table 13, Figure 2). The diameter and weight of the control cookies were significantly lower than cookies with a high proportion of CF and PPF. However, increasing supplementation with CF and PPF had no effect on cookie height and a non-significant increasing trend for spread ratio. In general, the results showed that cookie diameter, weight, height, and spread ratio increased as the level of non-WF supplementation was increased. The elastic properties of gluten in WF to extend and contract when hydrated were likely responsible for the smaller diameters of 100% WF cookies. Gluten tends to stretch more when hydrated and during baking as the sugar melts. However, the stretching does not go far and it retracts back, thereby reducing the cookie diameter. The dilution of gluten in the blended flours resulted in increased cookie diameters. The weak connections of starch polymer molecules in CF might have also contributed to increased expansion of blended flour cookies, yielding larger diameters.

The current findings conform to findings of previous researchers. Enrichment of wheat-cassava biscuits (cookies) with soy flour resulted in increases in weight, diameter and spread ratio (Olowamukomi et al., 2011). As the proportion of cassava and soy flour increased in the supplementation, the mean weight, diameter and spread ratio of the biscuits increased considerably. The authors attributed the low spread ratio of wheat biscuits to the stronger bonding of starch polymer molecules that resulted in limited expansion of the biscuits during baking (Olowamukomi et al., 2011). The heavier weight and larger diameter of the blended

flour cookies are added advantages because such properties could attract higher prices at the market. This implies that if baking industries adopt use of non-wheat flours, such as CF and PPF, in cookie formulations, they are likely to increase their profits, while also improving nutrition of the local population.

**Table 13. Physical Characteristics<sup>1</sup> of Cookies Made from Wheat Flour Supplemented with Cassava and Pigeon Pea Flours**

Cookie Sample <sup>2</sup>	Diameter (cm)	Height (cm)	Weight (g)	Spread ratio
100% WF	9.18 <sup>a</sup>	0.96 <sup>a</sup>	21.37 <sup>a</sup>	9.59 <sup>a</sup>
80%WF:10%CF:10%PPF	9.46 <sup>ab</sup>	0.98 <sup>a</sup>	25.70 <sup>bc</sup>	9.65 <sup>a</sup>
70%WF:15%CF:15%PPF	9.56 <sup>bc</sup>	0.96 <sup>a</sup>	23.17 <sup>ab</sup>	9.94 <sup>a</sup>
60%WF:20%CF:20%PPF	10.11 <sup>c</sup>	1.00 <sup>a</sup>	26.10 <sup>bc</sup>	10.11 <sup>a</sup>
SEM	0.24	0.02	1.20	0.35

<sup>1</sup> Means with the same superscript in the same column are not significantly different ( $p < 0.05$ ).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour.

#### **4.9. The effect of cassava and pigeon pea flours supplementation on chemical composition of breads and cookies**

Analysis of the chemical composition of breads and cookies revealed that the protein content was significantly higher among samples made from blended flours (Tables 14 and 15) as compared to their respective control samples. The high protein contents of blended flour breads and cookies are due to high protein content of the blended flour samples from supplementation with PPF. The findings are consistent with findings by Tiwari et al. (2011) and Hassan et al. (2011) who reported that addition of PPF to WF increased the protein contents of cookies and bread. Generally, it is clear that the protein content increased with increasing level of PPF. In Malawi, pigeon peas are commonly consumed although pigeon pea as flour has not

been tested as a marketable commodity. If PPF is used to make protein-enriched products that are acceptable for consumption, protein intake will increase and thereby may alleviate malnutrition problems related to low protein intake.

Carbohydrate contents among the blended flour bread samples were not statistically different from each other (Table 14). However, it is important to note that the carbohydrate contents of blended flour breads were higher than that of the control. Similarly, the carbohydrate contents among the blended flour cookies were not significantly different from each other and were slightly higher as compared to the control (Table 15). There was no consistent trend among the carbohydrate results for the baked products, and the carbohydrate contents of the baked products did not correspond to the blended flours from which they were made (Table 6). This could be due to differences in moisture of the baked breads as compared to their respective flour samples from which they were made. Compared to breads, cookies had the greatest amount of carbohydrate, due to the fact that baked cookies had a much lower moisture level, i.e., they are a drier product than bread.

There were significant differences between the control and the blended flour samples in fat content for breads and for cookies. Addition of non-wheat flours resulted in lower fat contents in both products. The highest contents of fat were found in control bread (4.55%) and control cookies (25.40%). It is worth noting that cookies had a significantly higher amount of fat content compared to bread, due to the higher amount of fat in the cookie formulation. The results are similar to findings by Gayle et al. (1986) where supplementation of WF with PPF in bread resulted in a decrease in fat content from 5.93% (100% WF) to 5.0% (75% WF). In a different study, replacement of WF with pigeon pea byproduct flour (PPBF) raised the fat

content of cookies (Tiwari et al., 2011). Although both WF and PPF are rich in fat, the fat content of the products in the current study did not increase significantly because CF diluted the fat present in WF and PPF. The fat content of the baked products was therefore greatly dependent upon flour blend composition, since the amount of formulation fat added was held constant for all bread samples and for all cookie samples.

According to results on Tables 14 and 15, the ash contents of bread and cookie samples were significantly different between control and blended flour products. It is clear from the results that the highest ash contents were found in blended products with the highest proportion of CF and PPF. The high ash contents of blended flour bread and cookie samples are mainly due to incorporation of PPF at high levels. Based on the results, supplementation with PPF in the product formulation is encouraged to improve intake of minerals through consumption of the products.

Moisture contents of breads and cookies were assessed to determine storability of the products. The results indicated that the moisture contents among bread samples made from blended flours were not statistically different from each other (Table 14), and that WF bread had a significantly higher amount of moisture (37.20%) compared to the blended flour breads. Within the blend breads, 80% WF bread had the lowest moisture content (34.00%). On the contrary, the lowest moisture content of cookies was observed in 80% WF cookies (3.10%) compared to 60% WF cookies which had the highest moisture content (4.80%, Table 15). Similar findings were reported by Eneche (1999). The author found that the moisture content of 100% maize flour cookies was 6.6% and it significantly decreased to 5.0% with 35% PPF



supplementation. The low moisture content of the products is important for prolonging their shelf life.

After baking, bread has an initial crumb moisture content that is between 35% and 45% (Strausser, 2004). From the result (Table 14), the moisture content of composite breads is slightly below this range. Staling of bread starts soon after baking as the bread loaf cools due to migration of moisture from crumb to crust resulting in crystallization of amylopectin. As the process continues, the texture of the crust increases (Strausser, 2004). Bread with more moisture content than required, will have most of the moisture migrate to the crust creating a favorable condition for the growth of molds hence staling.

Fiber contents of the blended flour bread and cookie samples increased as a result of increased proportion of CF and PPF (Tables 14 and 15). The high fiber content of the blended flour products was mainly contributed to by CF since the fiber content of CF was significantly higher than that of WF and PPF (Table 6). Similar results were reported by Olowamukomi et al. (2011) where incorporation of CF resulted in an increase in the fiber content from 0.2% in the control (wheat flour) to 0.64% with 70% CF. Recently, inclusion of fiber in food has attracted much attention due to its physiological benefits such as promoting large bowel movements and preventing diseases like inflammatory bowel disease and colorectal cancer.

Energy values of the baked products were estimated to determine the amount of energy to be gained from consumption of the products. The energy of all bread samples were not significantly different from each other, indicating that supplementation of WF with CF and PPF did not influence energy (kcal/100 g), and that the breads at any level of substitution studied could give almost the same amount of energy when consumed (Table 14). Contrary to

the results for bread, the energy of cookies was significantly different among samples, with the control cookies having the highest value compared to those of the blended flour cookies. Again, the results imply that supplementation of WF with CF and PPF had a negative effect on the energy of the baked products. Supplementation with CF, which is low in fat content, was responsible for the relatively low energy values of the blended flour breads and cookies. Results obtained in the present study show a general deviation from the previous literature by Tiwari et al. (2011), Hassan et al. (2011) and Akunor and Onimawo (2003). For example Tawiri et al. (2011) reported a small but statistically significant increase in calorific value of wheat bread supplemented with decorticated pigeon pea flour (DPPF), from 386 kcal/100g (control) to 390 kcal/100g (25% DPPF). The energy values of cookies were much higher than those of bread samples and were probably due to the high levels of fat and sugar in the cookie formulation.

Total hydrocyanic acid content (HCN) of baked samples significantly increased as the level of CF increased in the formulation ( $P \leq 0.001$ ) (Tables 14 and 15). Thus, bread and cookie samples with highest amounts of cassava had the greatest HCN contents. As anticipated, the control (100% WF) bread and cookie samples had the lowest HCN contents, of 0.06 and 0.03 mg/kg, respectively. It is clear from the results that the HCN of breads were higher than that of cookies, probably due to the baking of cookies at high temperature ( $182^{\circ}\text{C}$ ). The low HCN content of the products could be partially explained by the pressing steps of cassava wet mash during preparation of high quality cassava flour (HQCF). Most of the HCN was eliminated alongside excess water as the wet mash was being pressed. Almazan (1990) reported a decline in HCN of bread as a result of flour dilution by non-cassava flour and baking at high temperature. Lower maximum levels of HCN are recommended for some products. According

to Codex Alimentarius Commission (1985) 1 mg HCN/ 100 g is the maximum limit allowable for cassava flour. There is no maximum allowable HCN level for bread. However, 1 mg/kg, which is one tenth that recommended by the Codex Alimentarius Commission and the maximum allowable amount per RMRDC (2004), is probably safe for consumption. Although HCN levels were slightly high in the study samples with high proportions of CF, the HCN contents were still below 1 mg/kg. This implies that the products are safe for human consumption, without causing health problems due to HCN.

**Table 14. Chemical Composition<sup>1</sup> (d.b.) of Bread Made From Wheat Flour Supplemented with Cassava and Pigeon pea Flours**

<b>Bread Sample<sup>2</sup></b>	<b>% Protein</b>	<b>% CHO</b>	<b>% Fat</b>	<b>% Ash</b>	<b>% Moisture</b>	<b>% Fiber</b>	<b>Energy (kcal/100g)</b>	<b>Hydrocyanic acid (mg/kg)</b>
100% WF	10.35 <sup>a</sup>	46.15 <sup>a</sup>	4.55 <sup>c</sup>	1.40 <sup>a</sup>	37.20 <sup>c</sup>	0.35 <sup>a</sup>	266.95 <sup>a</sup>	0.06 <sup>a</sup>
90%WF:5%CF:5%PPF	10.95 <sup>a</sup>	47.90 <sup>b</sup>	3.75 <sup>b</sup>	1.40 <sup>a</sup>	35.60 <sup>ab</sup>	0.40 <sup>a</sup>	269.15 <sup>a</sup>	0.16 <sup>b</sup>
80%WF:10%CF:10%PPF	11.70 <sup>b</sup>	48.50 <sup>b</sup>	3.35 <sup>ab</sup>	1.70 <sup>b</sup>	34.00 <sup>a</sup>	0.75 <sup>b</sup>	270.90 <sup>a</sup>	0.19 <sup>c</sup>
70%WF:15%CF:15%PPF	12.00 <sup>b</sup>	47.50 <sup>b</sup>	3.15 <sup>a</sup>	1.80 <sup>b</sup>	34.75 <sup>ab</sup>	0.80 <sup>b</sup>	266.35 <sup>a</sup>	0.21 <sup>c</sup>
SEM	0.23	0.34	0.14	0.07	0.44	0.06	2.15	0.01

<sup>1</sup> Means with the same superscript in the same column are not significantly different (p<0.05).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour, CHO = carbohydrate.

**Table 15. Chemical Composition<sup>1</sup> (d.b.) of Cookie Made From Wheat Flour Supplemented with Cassava and Pigeon pea Flours**

Cookie Sample <sup>2</sup>	% Protein	% CHO	% Fat	% Ash	% Moisture	% Fiber	Energy (kcal/100g)	Hydrocyanic acid (mg/kg)
100% WF	8.75 <sup>a</sup>	59.31 <sup>a</sup>	25.40 <sup>b</sup>	1.35a	3.15 <sup>a</sup>	0.20 <sup>a</sup>	508.20 <sup>c</sup>	0.03 <sup>a</sup>
80%WF:10%CF:10%PPF	8.80 <sup>a</sup>	61.80 <sup>a</sup>	21.40 <sup>a</sup>	1.65a	3.10 <sup>a</sup>	0.55 <sup>b</sup>	485.80 <sup>b</sup>	0.10 <sup>b</sup>
70%WF:15%CF:15%PPF	9.75 <sup>a</sup>	60.90 <sup>a</sup>	19.85 <sup>a</sup>	1.85ab	3.80 <sup>a</sup>	0.70 <sup>bc</sup>	473.20 <sup>ab</sup>	0.14 <sup>c</sup>
60%WF:20%CF:20%PPF	11.05 <sup>a</sup>	59.28 <sup>a</sup>	19.70 <sup>a</sup>	1.90b	4.80 <sup>a</sup>	0.75 <sup>bc</sup>	468.70 <sup>a</sup>	0.16 <sup>c</sup>
SEM	0.82	0.98	0.69	0.16	0.68	0.06	4.52	0.01

<sup>1</sup> Means with the same superscript in the same column are not significantly different (p<0.05).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour, CHO = carbohydrate.

#### **4.10. Carbohydrate and protein digestibility of breads and cookies supplemented with cassava and pigeon pea flours**

The quality and bio-availability of any nutrient is determined by its digestibility. Thus, *in vitro* digestibility studies of carbohydrates and proteins were conducted to determine the quality and bio-availability of the nutrients in the products. Both carbohydrate and protein digestibility decreased as WF was replaced with cassava and pigeon pea flours (Table 16). It is clear from the results that the highest *in vitro* carbohydrate digestibility (IVCD) of bread was found for the control (66.8%), whereas 70% WF bread registered only 60.5%. The trend was similar to the IVCD of cookies, for which cookies with the lowest proportion of WF (60%) had the lowest IVCD (59.4%). The low rate of carbohydrate digestion in relation to high levels of non-wheat flours is most likely explained by the high fiber content contributed by CF. The fibers in CF are less susceptible to  $\alpha$ -amylase hydrolysis in the analyses and might have contributed to low IVCD of blended flour breads and cookies. The findings are in conformity with observations by previous researchers. Mais (2008) reported that inclusion of fiber significantly reduced the IVCD due to lower rates of sugar being released by  $\alpha$ -amylase enzyme in breads and cookies.

Baked samples with lowest protein contents (controls, Tables 14 and 15) had the greatest *in vitro* protein digestibility (IVPD) whereas the samples with highest protein contents had the lowest IVPD values (Table 16). The result found in the current study supports previous findings by Okpala (2011) who reported a decline in IVPD of cookies as supplementation with PPF and cocoyam was increased. Digestion of protein is reported to be reduced by antinutritional factors such as trypsin inhibitors (Onwuka, 2006). Perhaps CF and PPF contained

higher levels of such antinutritional factors, and therefore it is possible that increasing levels of flour substitution resulted in decreasing IVPD in the blended flour samples. However, antinutritional factor analysis was not conducted in the present study to support the aforementioned factor.

*In vivo* digestion of protein and carbohydrates is a complex process, affected by a number of factors, including temperature, chemical bonding and many more, hence a comprehensive study to better understand the factors affecting digestibility is suggested. The results could be used to control for or even reduce the adverse effects of the factors in dough formulations and on digestibility of baked products.

**Table 16. *In vitro* Digestibility<sup>1</sup> of Carbohydrate and Protein of Bread and Cookie Samples Supplemented with Cassava and Pigeon Pea Flours**

Bread			Cookies		
Bread Sample <sup>2</sup>	% IVCD	% IVPD	Cookie Sample <sup>2</sup>	% IVCD	% IVPD
100% WF	66.8 <sup>b</sup>	70.2 <sup>c</sup>	100% WF	63.4 <sup>d</sup>	73.0 <sup>c</sup>
90%WF:5%CF:5%PPF	65.8 <sup>b</sup>	69.6 <sup>b</sup>	80%WF:10%CF:10%PPF	61.2 <sup>c</sup>	70.2 <sup>b</sup>
80%WF:10%CF:10%PPF	61.5 <sup>a</sup>	66.3 <sup>a</sup>	70%WF:15%CF:15%PPF	60.6 <sup>b</sup>	69.6 <sup>a</sup>
70%WF:15%CF:15%PPF	60.5 <sup>a</sup>	66.1 <sup>a</sup>	60%WF:20%CF:20%PPF	59.4 <sup>a</sup>	67.6 <sup>a</sup>
SEM	0.50	0.14	SEM	0.04	0.32

<sup>1</sup> Means with the same superscript in the same column are not significantly different ( $p < 0.05$ ).

<sup>2</sup> WF = wheat flour, CF = cassava flour, PPF = pigeon pea flour, IVCD = *in vitro* digestibility of carbohydrate, IVPD = *in vitro* digestibility of protein.



100% WF



80%WF: 10%CF: 10%PPF



70%WF:15%CF:15%PPF



60%WF:20%CF:20%PPF



100%WF



90%WF:5%CF:5%PPF



80%WF:10%CF:10%PPF



70%WF:15%CF:15%PPF

Figure 2. Bread and cookie photographs. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.



## CHAPTER 5

### 5.1. CONCLUSIONS

The studies demonstrated that incorporation of cassava flour (CF) and pigeon pea flour (PPF) influenced physical and chemical properties of wheat bread and cookies. From the results, the protein content of wheat flour (WF) alone was significantly lower than those of blended flours, and increased as the levels of CF and PPF were increased. Supplementation with CF and PPF negatively affected the carbohydrate content and energy value of the flour blends. Thus, the carbohydrate content and energy values of WF were higher than those of the flour blends.

Particle size analysis of the flours and flour blends has revealed that WF had a greater proportion of small particles ( $\leq 125 \mu\text{m}$ ) than CF and PPF. On the other hand, CF and PPF were comprised more of large particles ( $\geq 330 \mu\text{m}$ ). The particle sizes of the control flour (WF) were within the recommended particle size range for breadmaking. Therefore, fine milling and sieving of CF and PPF is suggested to achieve the recommended particle size for bread, biscuit and pastry products ( $130 \mu\text{m}$ , UNECA, 1985).

The Falling Numbers of WF and CF were substantially higher than that of PPF (296). The low Falling Number of PPF indicates high  $\alpha$ -amylase activity (low paste viscosity). The result could be due to soaking of pigeon pea grains during flour preparation that stimulated  $\alpha$ -amylase enzyme to start degrading starch leading to low paste viscosity. As expected, WF had the highest gluten content compared to flour blends. Increasing quantities of CF and PPF at high proportions progressively reduced the gluten contents of the flour blends. Similarly, work and Alveostrength of the control flour (WF) were higher than of the flour blends. This was explainable by the low gluten content of the flour blends.

The water absorption capacity (WAC) of CF was significantly higher than WAC values of control (WF) and flour blends. WAC of CF was approximately six times higher than that of the control flour. Supplementation with CF resulted in a slight increase in WAC of the flour blends. In the same line, the oil absorption capacity (OAC) of CF was higher than of control and blended flours. In addition, the swelling power (SP) of CF was significantly higher than those of control and flour blends. The behavior exhibited by flour samples on WAC and OAC reveal their molecular structure.

At each of the temperatures studied (70 and 80 degrees C); the flour viscosities among samples were statistically different. CF exhibited the greatest flour viscosity at both temperatures compared to control and flour blends. As the levels of supplementation were increased, the viscosity of flour blends slightly increased. This was due to addition of CF at increased levels. The low viscosities of WF and PPF are related to the cohesiveness of the starch molecules of the flours.

Among the physical properties of bread, loaf volume was negatively affected due to the dilution of gluten as CF and PPF were added at high levels. Conversely, bread loaf weight increased with the supplementation of non-wheat flours and became denser as the proportion of CF and PPF were increased in the formulation. In general, wheat bread had superior baking quality attributes compared to blended flour breads. More studies on the use of non-wheat flours alongside wheat flour (WF) are suggested to explore alternatives on how best to improve the baking quality of blended flour bread. On the other hand, supplementation of WF with non-wheat flours greatly improved the physical properties of cookies, including weight, diameter, height, and spread ratio. The greatest cookie weight, diameter and spread ratio were found for

cookies with the highest amounts of CF and PPF, whereas the control cookies had the lowest values for all parameters.

Supplementation of WF with CF and PPF increased protein content of the baked bread and cookie products as compared to the counterpart control WF products. The increase in protein content is explained by the addition of PPF at increased levels. The higher protein contents of the products are important to improving the nutrition of people consuming such products. However, the supplementation also notably decreased the fat and energy values of the products. Although the fat content and energy values were lower than those of the studied control, they were still higher than those of the typical bread consumed in Malawi and could lead to improved nutrient intake. In addition, the *in vitro* digestibility of carbohydrates and *in vitro* digestibility of protein of breads and cookies slightly decreased with increased supplementation of CF and PPF but still, above 50%. This implies that consumption of large quantities of CF- and PPF-enriched products might contribute to alleviating protein-energy malnutrition in at-risk populations.

## **5.2. RECOMMENDATIONS AND SUGGESTED FUTURE STUDIES**

The findings of the present study have clearly demonstrated the possibility of utilizing CF and PPF in wheat breads and cookies. This would support industrial utilization and the consumption of under-utilized crops such as pigeon peas. Therefore, large scale production of the crops is encouraged to support industrial use.

Replication of the study is suggested to validate the findings. For example the results for OAC, carbohydrate content and energy of the products were not consistent, so replication

would assist with validating the findings and coming up with concrete results. Expansion of the study is also suggested to evaluate additional flour blend proportions to facilitate optimum utilization of the flours in baking.

The upcoming studies should focus more on using specialized and appropriate equipment like the Brabender Amylograph and Rapid Visco Analyzer (RVA) to get comprehensive and accurate results about the properties of the flours being evaluated. For example, there is the need to study flour's viscosity at different temperatures and times to establish gelatinization, setback and retrogradation properties with regards to starch present in that flour sample. The information is critical to understanding factors that affect flour viscosity and better control of food processing.

The *in vitro* digestibility studies only considered temperature, pH and enzyme concentration as factors that affect carbohydrate and protein digestion. Biologically, *in vivo* digestion is a complex process affected by many factors, including antinutritional factors, chemical bonding and many more. Therefore it is suggested to conduct a detailed study on digestibility of the products to establish their quality. The study should also combine amino acid (AA) analysis with the enzyme assay to determine the available AA in the baked sample.

Quality of blended flour bread was compromised at higher levels of non-wheat flour substitution, so there is a need to explore use of bread improvers to enhance quality of blended flour bread thereby promoting use of the non-wheat flours in breadmaking. Texture analysis of bread and cookies is also a recommended future study. Sensory studies are another important area to assess for palatability and acceptability of the baked products, and thus encourage

increases in industrial production and consumption of the baked products made from the blended flour materials.

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