BETA-GLUCAN CONTENT IN TROPICAL MAIZE GENOTYPES: EXTRACTABILITY, STRUCTURAL ANALYSIS, RHEOLOGY AND FUNCTION

BY

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DECLARATION

I declare that, I have wholly undertaken the study reported herein under the supervision of Dr. (Mrs.) Antonia Y. Tetteh and Prof. James Henry Oldham that except portions where references have been duly cited, this thesis is the outcome of my research.

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With the greatest of respect, this thesis is dedicated to the two most important women in my life viz., Christiana and Vivian Sampson.

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ABSTRACT

Beta-glucan is a complex soluble dietary fiber with (1→3),(1→4)-β-D linkages in a repeating unit of cellotriosyl, cellotetraosyl glucose chains interspersed with β-(1→3) linkages, found mainly in the cell walls of cereal endosperm and specifically in oat and barley. Beta-glucan is known to impart some health benefits to consumers including lowering of blood glucose and cholesterol level, as well as prevention of colon cancer. Beta-glucan research has concentrated on oat, barley and wheat. The absence of these cereals in tropical West Africa necessitates an exploration into the use of cereals of tropical origin such as maize to identify their potential of being good sources of β-glucan. The objectives of this study weretoscreen tropical maize genotypes for β-glucan content,and evaluate their structure and functionality in food systems. In the present study, β-glucan contents ofseventeen Ghana maize genotypesviz., 'Mamaba', 'Suwan 1 QPM', 'Obatanpa GH', 'Omankwa','GH9', 'Catete', 'Dodzi', 'Okomasa', 'Aburohemaa', 'Abontem', 'SotuBaka', 'Dorke', 'Akposoe', 'Abeleehi', 'Safita 2', 'Ohawu Local' and 'Golden Jubilee' were analyzed using hot water, acid, alkaline and alcohol-enzyme based extraction procedures. Gum content varied by extraction method and genotype. Hot water and alkaline procedures produced low extractability demonstrating gum yield ranging from 0 to 2.29 % and 0 to 22.12 %, respectively. Significantly higher gum yields were produced by the acid and alcohol-based enzyme extraction procedures. The acid treatment produced a mean yield of 45.97 % which was not significantly different from 39.97 % yield obtained by the alcohol-enzyme treatment ($P>0.05$). With regard to genotype, hybrid maize varieties contained the highest mean gum content of 31.83 % while the landraces contained lowest value of 19.11 %. Genotypes having the highest β-glucan content were 1.4 % for

‘Obatanpa GH’, 1.68 % for ‘Abeleehi’ and 2.56 % for ‘GH 9’. Gum content of kernels had positive correlations with protein and fibre but negative correlation with nitrogen free extract. Minimal levels of impurities in the form of starch, protein and fat were present in the gum isolates. Structural analysis of maize β-glucan by ^{13}CNuclear Magnetic Resonannce produced chemical shifts similar to that of standard oat and barley spectra and characterized by slight displacements as upfield and downfield shifts. The aniline blue fluorescence microscopy revealed that β-1→3 branching in maize β-glucan was less than that in oat and was genotype dependent. ‘Abeleehi β-glucan demonstrated higher β-1→3 branching than ‘Obatanpa GH’ β-glucan. The flow behavior of 10 % maize β-glucan dispersion was pseudoplastic and non-Newtonian just as 5 % oatβ-glucan. High water binding capacity of maize β-glucan up to 232 % makes it a suitable ingredient in food products which should resist syneresis. Maize β-glucan was found to be a good fat replacer, replacing up to 15 % of fat in pie crust without compromising sensory attributes.

Chapter One

Introduction

1.0 Background

Maize (*Zea mays*) is a cereal which belongs to the tribe *Maydeae* of the grass family Poaceae (formerly known as *Gramineae*). Maize is described as the most genetically diverse among all crops and can be grown over varied geographical range in comparison to other cereal crops (Shaw, 1988). Maize was introduced to Africa in the 16th century by the Portuguese through trade routes and has since become a major staple food crop after cassava.Presently, maize makes up more than 50% of the total caloric intake (Sinha, 2007; McCann, 2005) and 53% of the protein intake of local diets (Bressani, 1991). Other uses of maize of maize include feed for livestock, raw material for many industrial products and recently, a source of biofuel. The germ serves as source of edible oil rich in vitamin E and polyunsaturated fats (Okoruwa, 1996).

Maize cultivation in West Africa is largely done with landraces which are characterized by wide genetic variability and poor nutritional characteristics regarding protein quality and vitamin A. Regarding protein quality, the landraces have low content of the essential amino acids, lysine and tryptophan, but high content of leucine, a combination, which does not satisfy Food and Agriculture Organization (FAO) requirement for human nutrition, especially for children (Sentayehu, 2008). The discovery of *opaque*-2 maize which has high lysine and tryptophan content (Mertz *et al.,* 1964) led to the development of Quality Protein Maize (QPM). Also, adding value to local maize genotypes to improve vitamin A content has also been a major objective of maize breeding programs in tropical Africa.

Value addition to agricultural commodities has over the past years received attention from both food industry and researchers, given that it increases grower returns, controls postharvest losses, satisfies demands for functional foods, and imparts health benefits to consumers. A high-value component present in cereals, which is known to impart health benefits, is β-glucan.

The major sources of β-glucan are cereals, especially oat and barley, yeasts, such as *Saccharomyces cerevisiae*, and some seaweed (Ooi, 2000). In cereals β-glucan is present in the cell wall (Vasanthan and Temelli, 2008) at levels of 3 – 11% in barley (MacGregor and Fincher, 1993), 3-7% in oats (Cui and Wang, 2009), 0.5 - 2 %in wheat and rye (Palmer and MacKenzie, 1986; Bhatty, 1992; Beresford and Stone, 1983; Skendi *et al.,* 2003; Cui and Wang, 2009) and 0.12 to 5.4 % in sorghum (Palmer and Mackenzie, 1986; Ogbonna and Egungwu, 1994; Caprita *et al.,* 2010; Darku, 2011). The wide range of values reported by these researchers suggests that β-glucan content is genotype-dependent and varies with method of isolation.

While literature reports β-glucan research on these cereals, there is dearth of information on β-glucan in maize. Cereals consumed in developing countries include maize, millet and sorghum. Investigation into the β-glucan content of maize and its functionalities may hold a promise for value-adding. As a result, many health scientists, and product developers have directed attention on β-glucan to investigate the sources, structure, physicochemical properties, and functions.

Typically, β-glucan is isolated from barley and oats and exhibits two major health benefits including lowering of serum cholesterol and moderating blood glucose level (Eastwood and Morris, 1992; Wood, 2004; Nielsen *et al.,* 2008; American Heart Association, 2003). Because lowering of cholesterol is associated with reduction in risk of cardiovascular disease, the Food and Drugs Administration (FDA) has tagged a health claim on oat and barley in the U.S.A (FDA, 1997, 2005). Other beneficial effects of cereal β-glucan include their ability to stimulate anti-tumor and anti-microbial properties and have physiological effects similar to those of soluble fibers (Brown and Gordon, 2001; Cheung and Lee, 2000; Izydorczyk *et al.,* 2000). In addition, the hydrocolloid property of β-glucan viz., water binding capacity, solubility in water, gelling ability gives an industrial value to this product.

Due to health implications regarding high intake of dietary fat, food manufacturers are replacing fats used in baked goods with fat substitutes and fat mimetics (Conforti *et al.,* 1997). Market for fat replacers has increased especially in the U.S.A. For instance, in 1991, fat replacers had a market value of 100 million dollars, and the U.S. demand for fat replacers was expected to increase at a rate of 4.5 % annually to 1.9 billion dollars as far back as1996 (Gershoff, 1995).

A true fat substitute is a substance whose physical and thermal properties resemble fat, and can be used to replace all the fat in the product (Miraglio, 1995). Baked cereal products represent one of the most consumed foodstuffs in the world (Pozo-Bayon *et al.,* 2006). Although the Ghana Heart Foundation (GHF) has not suggested a limit threshold

allowance of fat intake, other organizations have recommended limits of fat intake. A World Health Organization (WHO) report recommended that, level of fat intake should be between 15 and 30% of the total energy intake, of which saturated fatty acids should account for less than 10% energy (WHO, 2006).

The food industry has introduced a variety of fat replacers to help consumers reduce their fat intake (Gershoff, 1995). A fat replacer is an ingredient that replaces some or all the functions of fat and may or may not provide nutritional value (Miraglio, 1995). A fat substitute is an ingredient that replaces all the functions without any energy contribution (Oreopoulou, 2006). Currently, available fat replacers are fat mimics (also called mimetics) or fat analogs (Miraglio, 1995). Three categories of fat mimetics are available and encompass: starch-based replacers such as, hydrocolloids, starch derivatives, hemicelluloses, β-glucan and soluble bulking agents (Oreopoulou, 2006), lipid-based replacers including, emulsifiers, and synthetic fat substitutes, as well as protein-based replacers including; skim milk, whey, gelatin, isolates, soy protein and protein blends that also contain gum and modified starches (White, 1993). Fat mimics must have energy values from 0 to 38 KJ/g (Miraglio, 1995). These products are able to mimic the functionality of fat in baked products without compromising texture and flavor of full fat product when used as substitutes. A true fat substitute is an ingredient that replaces all the functions of fat without any energy contribution. Thus it possesses the same chemical and physical properties but is indigestible or unabsorbable.

Functional characteristics of carbohydrate-based replacers relate mostly to their ability to bind water and the resultant rheological properties. They add bulk, viscosity, structure and texture to products (Calorie Control Council, 1993; Frye and Sester, 1993; Drewnoski, 1990; Lucca and Tepper, 1994; Yackel and Cox, 1992). When used as a processing aid, β-glucan contributes to viscosity and gelling behaviour in some foods and as such has qualified for use as fat replacer in baked goods (Havrlentová *et al.,* 2012). Literature is replete with rheological properties of oat and barley β-glucan (Ahmad *et al.,* 2009; Burkus and Temelli, 1998, 2005; Dawkins and Nnanna, 1995). The function of maize β-glucan as a fat replacer in pie crust has not been investigated.

The non-availability of oat and barley in tropical regions of the world, including West Africa means that β-glucan would normally not be present in local diets in the recommended levels. Because β-glucan content of cereals is genotype-dependent, screening of several classes of maize genotypes may help reveal good sources of β-glucan. Maize germplasm commonly found in West Africa include common maize and quality protein maize of which a large number occur as landraces, few open-pollinated varieties (OPVs), few inbred lines, and even fewer hybrids. Since West Africa is considered as a secondary center of diversity of maize (Mauricio and Julien, 2004), it is expected that a wide variation in genotype will exist in the germplasm pool. Introduction of maize from International Maize and Wheat Improvement Center (CIMMYT), Mexico, for the purpose of breeding for enhanced biotic and abiotic stress tolerance, as well as breeding for Quality Protein Maize has also added to the genetic variation in maize germplasm in West Africa.

Nutritionally, maize in West Africa has been improved to enhance the levels of lysine and tryptophan to produce Quality Protein Maize, a popular example of which is 'Obatanpa GH' (Badu-Apraku *et al.,* 2006a). Other improved varieties in Ghana include 'Golden Jubilee', 'Sotu baka' and 'Abontem' whose vitamin A contents have been enhanced by the Crops Research Institute (CRI) in Ghana. It is therefore prudent to screen maize genotypes bred in Ghana for β-glucan content to enhance the uses and value of the crop.

Isolation of β-glucan presents a special problem to food manufacturers since the health benefits and functional properties derived from it depends on its viscosity, which in turn depends on its structural integrity, molecular weight and solubility. For this reason, various methods for isolation of β-glucan have been developed with the aim of preserving the natural structure as much as possible. These methods include dry and wet technologies (Vasanthan and Temelli, 2008). The wet extraction method includes aqueous-alkaline (Wood *et al.,* 1989), acid treatment, aqueous alcohol-based enzymatic process, as well as hot water treatment, whereas the dry extraction method includes pearling, dry milling/flaking and sieving and/or air classification (Ahmad *et al.,* 2009; Vasanthan and Temelli, 2008). A comparison of the two methods revealed that the dry separation method produces lower yield (25-30 %) and β-glucan of poor physicochemical properties with respect to colour and viscosity , while the wet separation results in higher yield (95 %) and relatively better physicochemical properties (Vasanthan and Temelli, 2008). Thus, the use of β-glucan concentrates from dry extraction techniques may impose some technical challenges as far as the colour and textural attributes of baked products are concerned (Vasanthan and Temelli, 2008). Notwithstanding, there is dearth of information on

application of these methods for the isolation, yield, and structure of maize β-glucan, in addition to their effect on rheological properties and functionality of the isolate.

The overall objective of the current thesis is to screen maize genotypes produced in Ghana for β-glucan content using four isolation methods and investigate the physicochemical properties and functionality of β-glucan in baked food product. The specific objectives of the research were:

1. To screen seventeen genotypes of tropical maize for β-glucan content.
2. To investigate the effect of two major isolation methods, viz., one enzymic and three nonenzymic procedures on percentage recovery of β-glucan.
3. To determine the structure of the purified maize β-glucan by ^{13}C CPMAS NMR and degree of polymerization by fluorescence technique
4. To study the functionality of the isolate regarding its water binding capacity and rheological properties.
5. To evaluate maize β-glucan as a fat replacer in pie crust.

Chapter Two

Literature Review

2.1 Origin and history of maize cultivation in Africa

Maize belongs to the tribe *Maydeae* of the grass family Poaceae (formerly known as *Gramineae*). *Maydeae* includes two species, namely, *Zea mays*, which is cultivated maize and two related groups of species known as *Zea mexicana*, which are the *teosintes*, generally believed to be the progenitor of maize, and *Tripsacum* which includes Gamma grass (Benz 1999). Maize was introduced to Africa in the 16th century by the Portuguese through trade routes and has since become a major staple food crop, making up more than 50% of the total caloric intake of local diets (Sinha, 2007; McCann, 2005).

Maize is a very diverse crop both genetically and phenotypically. Maize can be grown over diverse geographical range in comparison to other cereal crops. Maize can be cultivated from latitude 58°N to 40°S (Shaw, 1988) and grows well at sea level to an altitude as high as 3,900 m.a.s.l. (Dowswell *et al.,* 1996). Manglesdorp (1974) stated that genetic diversity in maize is the result of long selection process practiced by the Native Americans in Central America before it was spread to other parts of the world. The genetic diversity in maize in West Africa is a result of the diversity of ecological niches and the climatic stress combined with a long tradition of farmers' selection over many generations leading to differentiation of many landraces and cultivars (Sanou *et al.,* 1997). These landraces are distinguishable by their earliness and ear and kernel characteristics (Robledo, 1976; Marchand, 1976; Le Conte, 1976; Sarr, 1975), so that West Africa is considered as a secondary area of genetic diversification for maize (Brandolini, 1969).

2.1.1 Maize types in West Africa

Maize cultivation in West Africa is largely done with landraces which are characterized by wide genetic variability and are adapted to the agricultural practices in this region, viz., limited use of chemical fertilizers and intercropping practice. With the advent of maize as a cash crop in Africa, there has been introduction of open-pollinated varieties from International Maize and Wheat Improvement Center (CIMMYT) and U.S.A. (The Maize Programme, 1999) which are used as breeding material by International Institute of Tropical Agriculture (IITA) to produce open-pollinated populations and inbred lines carrying many useful traits. Collectively, these local populations and introductions which have undergone selection offer new alleles for both biotic and abiotic stress tolerance, as well as a diversity of nutritional characteristics probably, including β-glucan.

2.2 Maize production in Africa

Maize is Africa's second most important food crop, after cassava, and is grown in a wide range of environments. It has a diverse form of utilization including human food uses, animal feed formulations and as a basic raw material for many industrial processes, such as adhesive, for textile manufacture, and for pharmaceutical products (Mejia, 2005). It is predicted that by 2020 maize will surpass both wheat and rice in demand to become the number one cereal in the world (Pingali, 2001; M'mboyi *et al.*, 2010). As demand for maize increases around the world, there has been a commensurate increase in the acreage planted, as well as tremendous efforts by many countries to increase the productivity of maize (CIMMYT, 1994). Table 2.1 shows production statistics of maize in different parts of the world over a 10-year period.

According to FAO data (FAOSTAT, 2006), the area planted to maize in West and Central Africa alone increased from 3.2 million hectares in 1961 to 8.9 million ha in 2005 resulting in a corresponding increase in production from 2.4 to 10.6 million metric tonnes (IITA, 2009). Over this period, maize yield in developed countries was consistently higher than that of developing countries, the disparity attributed to use of landraces, old breeding materials, while developed countries used hybrids and improved maize varieties (Munsch, 2009).

Table 2.1 Maize production statistics in different parts of the world for 2000 and 2010.

Country	**Year of production**						
	2000			**2010**			
	Area harvested ('000 ha)	**Production ('000 tonnes)**	**Yield (t/ ha)**	**Area harvested ('000 ha)**	**Production ('000 t)**	**Yield (t/ha)**	**% change in production**
Ghana	750	950	1.3	900	1,676	1.9	76.4
Sub- Saharan Africa	15,500	21,700	1.4	26,106	52,212	2.0	140.6
Developing countries	96,062	278,579	2.9	101,200	341,100	3.4	22.4
Developed countries	6,901	79,373	7.7	43,200	354,200	8.2	346.2
U.S.A.	28,789	256,904	8.9	32,209	333,010	10.3	29.6
World	140,182	600,000	4.3	145,759	817,110	5.2	36.2

Source: Pingali (2001) and M'mboyi *et al.* (2010).

2. 3 Maize ecotypes and their physical characteristics

Maize is characterized on the basis of growing environment, the endosperm and kernel characteristics, kernel colour, and days to maturity, and use. With regard to latitude of cultivation, two distinct classes are found, temperate and tropical maize (Dowswell *et al.*, 1996). Tropical maize grows in non-arid climate having annual mean temperature of above 18 ^{o}C (64 ^{o}F) whereas temperate maize grows at temperatures below 18 ^{o}C (McKnight and

Hess, 2000). Tropical maize is divided into three sub-classes, based on elevation of cultivation into lowland, mid-altitude, and highland maize (Munsch, 2009).

With regard to endosperm and kernel characteristics, there are seven categories of maize. These are dent, flint, sweet, floury, pop, waxy corn, and Quality Protein Maize (QPM) and each category have some unique characteristics and utilization. Similarly, on the basis of kernel colour, there can be white, yellow, red, blue, and purple maize but the predominant ones are the white and yellow types. The subsections following give a description of the various types of maize according to Brown *et al*. (1985).

(i) Dent corn (*Zea mays indentata* Sturt).

These have both soft and hard starches on top of the kernel; their kernels are predominantly white or yellow. The sides of the kernel consist of hard, so-called horny starch, and the crown contains soft starch. As the grain matures, this soft starch shrinks, forming the characteristic dent. It is the leading type of maize grown in the U.S.A and West/Central Africa.

(ii) Flint corn (*Zea mays indurate*, Sturt).

This type has soft and starchy endosperm and the horny starch extends over the top of the kernel, so there is no denting of the kernels. Also flint corn has larger kernel size with relatively small flour tissue in the endosperm.

(iii) Floury corn (*Zea mays amylacea* Sturt).

The endosperm of floury corn is predominated by soft or less densely packed starch which makes it readily ground into meal. It may also possess similar characteristics like flint corn, which is little or no indentation. Floury corn varies in coloration but white and blue are the predominant colours.

(iv) Sweet corn (*Zea mays saccharata* Sturt).

This type of maize is commonly grown in the United States for human consumption. The sugar produced by the sweet-corn plant is not converted to starch during growth unlike other types mentioned, hence the sweet taste of the kernels. The 1000 kernel weight of sweet corn is 380 g.

(v) Waxy corn (*Zea mays caretina* Sturt).

Maize is classified as waxy when it contains 100 % amylopectin. Normal maize contains 75 % amylopectin and 25 % amylose. The waxy trait is controlled by a single recessive gene, *wx*. The 1000 kernel weight of waxy corn ranges between 315 and 325 g.

(vi) Popcorn (*Zea mays everta* Sturt).

Popcorn possesses exceptional poppy qualities. It has a relatively small kernel size with hard corneous endosperm and seed coat. Kernel moisture ranges between 11 and 14 % (McGee, 1984) and when the grains are heated to about 66 ^{o}C, the starch grains are partly gelatinized. With further heating to the boiling point of water, the water vaporizes and the kernel expands rapidly in volume. The hard protein matrix holds until the pressure

becomes too great, at which point the kernel pops open and air rushes in to fill the space created by the steam (www.agclassroom.org/ok, verified July 14, 2011). This theory forms the basis of popcorn confectioneries.

2.4 Utilization of maize

Maize is used as human food; feed for livestock and as raw material for many industrial products. As food, maize finds uses as oil source, and alcoholic, and nonalcoholic beverages. Maize grits, flour and flakes constitute a variety of breakfast products (Punita, 2006). The by-products of dry milling of maize are made up of the germ and seed-coat. The germ serves as source of edible oil rich in vitamin E and polyunsaturated fats (Okoruwa, 1996) while the seed-coat or pericarp is used mainly as feed for livestock and poultry as source of dietary fibre. Dietary fibre aids intestinal motility and in recent times research has demonstrated that this class of compounds contain appreciable levels of β-glucan. Beta-glucan is now known to offer health benefits to consumers (Vasanthan and Temelli, 2008). The wet milling of maize during production of starch yields by-products such as gluten which is added to feed formulas. Maize may also be processed by nixtamalization for the production of *tortillas*, *tamales*, corn chips, t*acos*, *enchiladas*, *pozol*, and *arepa*–'maize bread without yeast' which are consumed in Latin America, or *tuo zafi*, consumed in Ghana.

Fermentation of maize also produces fermented dough and alcoholic beverages. Products of fermented doughs include *kenkey* in Ghana, *uji* in Kenya, *ogi* consumed in Nigeria, and

tamalitos consumed in Benin. Alcoholic beverages made from maize include traditionally-brewed beers such as *ahee, aliha* and *ηmeda,* prepared in Ghana.

2.5 Maize research in Ghana

Research on maize has largely focused on improvement to confer resistance to biotic and abiotic stresses through conventional breeding techniques and gene technology (Koziel *et al.*, 1993; Shepherd *et al.,* 2007), in addition to augmenting the amino acids, lysine and tryptophan (Bressani, 1975), and vitamin A content (Pilay *et al.,* 2012) . Examples of improved maize cultivars and inbred lines include *Striga-r*esistant early-maturing and extra-early maturing tropical maize inbred lines (Badu-Apraku *et al.,* 2006b; Badu-Apraku and Menkir, 2006), maize populations (Badu-Apraku and Yallou, 2009; Badu-Apraku *et al.*, 2010), and *Obatanpa* GH, a Quality Protein Maize (Badu-Apraku *et al.,* 2006a) which are also resistant to maize streak virus (MSV), *Puccinia polysora* Underw., and *Bipolaris maydis* (Shewmaker and Stalker, 1992), first-cycle tropical mid-altitude maize germplasm lines (Everett *et al.*, 1994). Other maize breeding programmes used 'Obatanpa GH' as an inbred line to produce cultivars such as 'Mamaba', 'CIDA-ba', 'Dorke', EV8766-SR, EV8363-SR, Pool-18-SR and Pool-15-SR (Akande and Lamidi, 2006). Another maize breeding objective is the enhancement of vitamin A content which has produced 'Golden Jubilee', 'Sotu baka' and 'Abontem', by the Crops Research Institute (CRI) of Ghana. To date, little attention has been given to the potential end-utilization of maize as a source of β-glucan. In recent years, there has been growing interest in β-glucan among researchers, industry and consumers because of its beneficial effects on health, leading to an upsurge of technologies for β-glucan isolation from cereals.

2.6 Structure and composition of maize grain

Cereal plants produce one-seeded dry fruit called caryopsis which is commonly called grain (Singh, 2009). Figure 2.1 depicts a schematic of the anatomical structure of maize. It is composed of three physiological parts, the outer covering, endosperm, and germ or embryo. Table 2.2 shows the chemical composition of the various parts of maize kernel, generally showing that the components are not uniformly distributed. The pericarp constitutes 5 % (w/w), the germ makes up 11 % (w/w) and the endosperm is about 80-85% of the total maize grain. The outer covering is made of two parts: an outer layer called the pericarp, and an inner layer called the testa. The pericarp is by nature highly fibrous, of which 67% is hemicellulose, 23% is cellulose and 0.1% is lignin (Landry and Moureaux, 1981; Jackson and Shandera, 1995). The endosperm is a major storage organ for starch and to a lesser extent, protein. The germ however, has rich quantities of protein, crude fat, crude fibre and sugars (Burge and Duensing, 1989).

Recently, cereal crude fibre content, composition, and structure have generated interest among cereal scientists as it contains complex carbohydrates composed of soluble and insoluble dietary fibre. Complex carbohydrate content of the maize kernel is contributed by the pericarp, the tip cap, the endosperm cell walls, and to a smaller extent, the germ cell walls. While the cell walls of endosperm and aleurone layer of oat and barley are composed of mixed linkage β-glucans and arabinoxylans (Bhatty, 1992; Marlett, 1993; Burkus and Temelli, 2000; Skendi *et al.,* 2003), there is dearth of information regarding the availability of β-glucan in maize.

The chemical composition of grains depends on genotype and environmental conditions, such as water availability, soil composition, day length, light, and temperature. The absence of oat, barley and wheat in tropical West Africa necessitates an exploration into the use of cereals of tropical origin such as maize, sorghum and millet to identify their potential as good sources of β-glucan. Table 2.2 shows the proximate composition of the major parts of maize kernel.

Table 2.2: Proximate composition of main parts of maize kernel (%).

Structure	% of kernel	Protein	Crude fat	Crude fibre	Ash	Starch	Sugar
Pericarp	5-6	3.7	1.0	86.7	0.8	7.3	0.3
Aleurone	2-3	-	-	-	-	-	-
Endosperm	80-85	8.0	0.8	2.7	0.3	87.6	0.6
Germ	10-12	18.4	33.2	8.8	10.5	8.3	10.8

Source: Watson (1987)

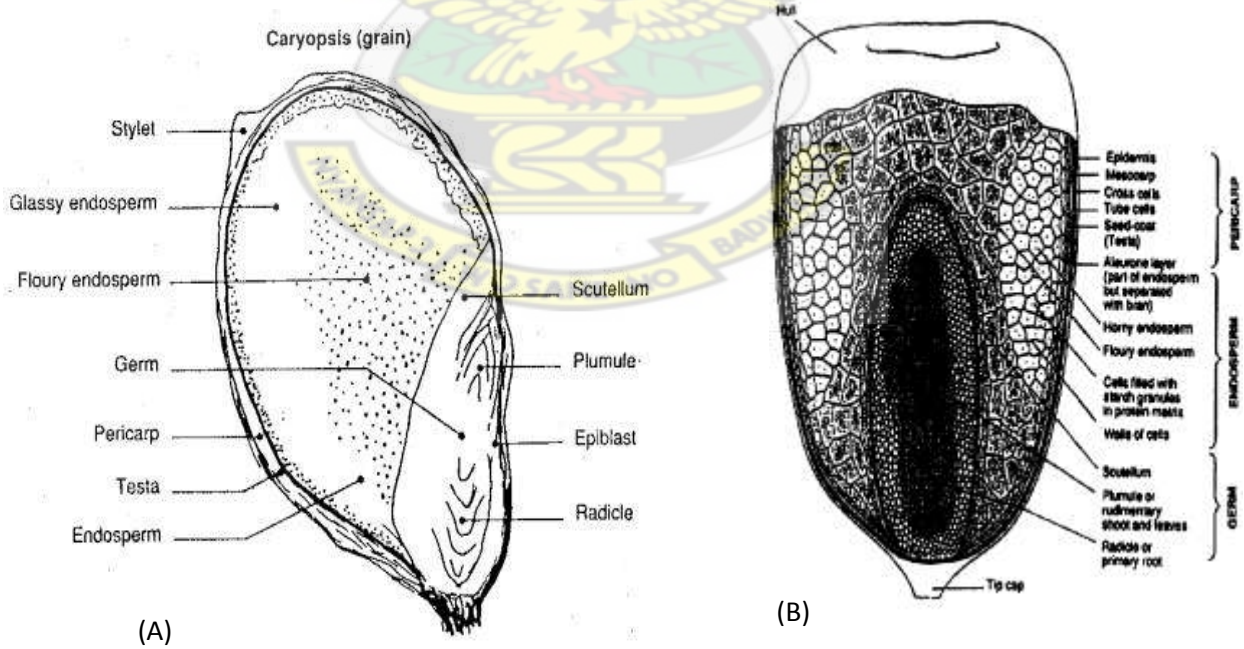
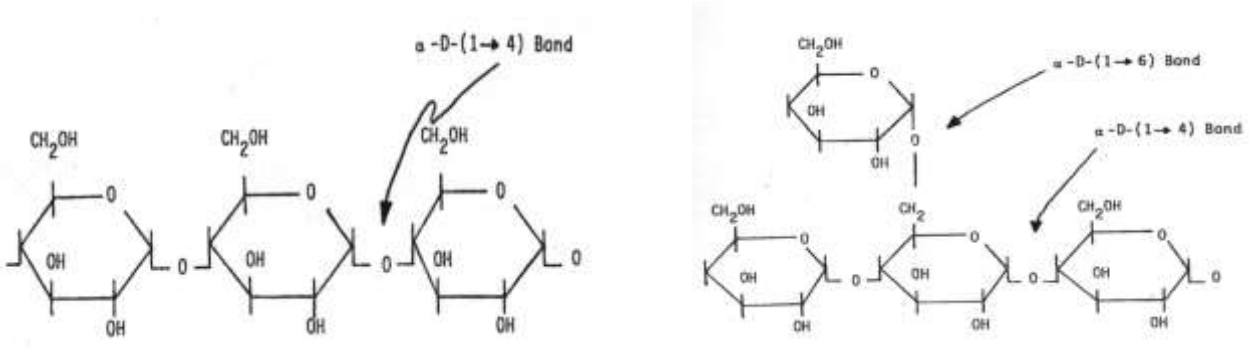


Figure 2.1 Schematic diagram of maize grain. (A) Longitudinal section. (B) Anatomical structure of maize. Source: Singh (2009).

Powell *et al.,* (1985) screened large populations of barley to determine the β-glucan content. Similarly, Lazaridou *et al.,* (2003) investigated the rheological properties of oat β-glucan in solution and gels. However literature is limited as far as the screening of maize cultivars for their β-glucan content is concerned.

2.7 Starch

Starch is a major energy reserve in plants. In cereals, starch is present in the endosperm. Each granule is a homopolysaccharide of glucose units made up of two variable portions: amylose, which is a linear glucose chain in α-(l→4)-linkages, and amylopectin, in which linear α-(l→4)-linked chains are interconnected through α-(l→6)-linkages to form irregular branches occurring approximately one per twenty-five glucose units (MacGregor and Fincher, 1993). The glucose polymers are arranged in three dimensional semi-crystalline structures called granule, having different shapes and sizes. Amylose content can vary between 11 and 37 % but on average it makes up about 30% of total glucan (Detherage *et al.*, 1955: Shannon and Garwood, 1984). Figure 2.2 shows the structures of amylose and amylopectin. Molecular mass of amylose ranges between 10^5 and 10^6 Daltons and is dependent on the degree of polymerization of the glucose units and origin of the starch. For example, wheat amylose has an average degree of polymerization of 570 glucose monomers, equivalent to a molecular mass of 10^5 Dalton, while potato amylose has an average degree of polymerization of between 1,500 and 6,000 glucose monomers and a molecular mass of 250,000 to 10^6 Daltons (Hizukuri and Takayi, 1984: Jane and Shen, 1992: Edwards *et al.*, 2002).



(A) (B)

Figure 2.2. Structure of (A) amylose and (B) amylolopectin**.** (Edwards *et al*., 2002)

On the contrary, amylopectin comprises about 70-75% of the starch and is one of the largest polysaccharides known, having molecular weight of 10^7 to 10^9 Daltons. An average amylopectin molecule is 200 to 400 nm long and approximately 15 nm wide (Kalnuma, 1988; Smith and Martin, 1993). The major physicochemical property of starch is its gelatinization property during food processing. In aqueous solution, amylose forms hydrogen bonds between the linear chains resulting in rigid gels. However, depending on the concentration, degree of polymerization, and temperature, it may crystallize and retrograde after heating (Shewmaker and Stalker, 1992).

Gelatinization of starch is essential in starch-based food production operations. Processes such as bread making, production of pasta products and starch-based snacks foods, breakfast cereals, pre-gelatinized flour, baby foods and parboiled cereals are all dependent on the proper gelatinization of starch to produce desirable organoleptic and nutritional properties in the finished product (Olkku and Rha, 1978; Lineback and Wongsrikasem, 1980; Lund, 1981). After extraction, amylopectin has more limited hydrogen bonding than

amylose in solution and is more stable, retaining fluid and giving high viscosity and elasticity to pastes and thickeners. Due to its unique textural properties, amylopectin is extensively used as gelling agent in food products such as salad dressings, ice creams, sauces, gravies, frosting, fruit gels and coatings to prevent moisture migration during processing and storage (Nisperos-Carriedo, 1994).

2.7.1 Resistant starch

Starch is generally considered as digestible. However, it contains a fraction, known as resistant starch (RS) which is not broken down by enzymes in the small intestine of humans (EURESTA GROUP, 1992). Resistant starch directly passes into the colon where it can be fermented by natural micro flora to short-chain fatty acids such as butyric acid (Baghurst *et al.,* 1996). Although RS is non-caloric ingredient and does not contribute to increase in blood glucose, it has physiological effects in the human body that are similar to that of dietary fibre, including reduction in risks for colon cancer, coronary heart disease and hyperglycemia (Cairns *et al.,* 1995; Ranhotra *et al.,* 1996). In addition, RS does not hold much water and, thus, may be a preferred fibre source for use in low-moisture products such as cookies and crackers. Unlike traditional fibre sources, RS is free of gritty mouth feel and does not alter flavor and textural properties of foods (Gao *et al.,* 2011).

Resistant starch is classified into four categories: physically inaccessible starch (RS1); RS granules (RS2); retrograded starch (RS3), and chemically modified fragments (RS4) (Englyst *et al.,* 1992; Tovar, 1992). While RS1 and RS2 are destroyed during processing, formation of RS3 is aided by gelatinization and retrogradation as interaction between

starches molecular chains increase. RS3 is the fraction that imparts desirable functional properties such as mouth feel and bulk in processed foods (Rosin *et al.,* 2002). It has been reported that, the linear chains can help increase RS content after starch de-branching (Berry, 1986).

2.7.2 Non-starch polysaccharides

Non-starch polysaccharides (NSP) are a class of carbohydrates known as dietary fibre (Caprita *et al.,* 2010). By definition, dietary fibre (DF) is the edible part of plants that are resistant to digestion and absorption in the small intestine (AACC, 2001; 2003), but moves on to the large intestine to be fermented by microorganisms (Bach-Knudsen and Jorgensen, 2001). Non-starch polysaccharides usually occur in plant cell walls where they form a heterogeneous group of non-α-glucan polysaccharides having varying degrees of water solubility, size and structure and are required for proper functioning of the human digestive system (Soma *et al.,* 2009).

There are two kinds of NSPs, the insoluble fraction and soluble fraction. Cellulose forms a major component of the insoluble fraction, while β-glucans and arabinoxylans distribute into both soluble and insoluble fractions. Hemicelluloses and lignin are associated with the insoluble fraction while pectins, gums and mucilages are also present with β-glucans and arabinoxylans (Caprita *et al.,* 2010). Cellulose, hemicelluloses and pectic substances comprise 80-90% of plant cell wall (Cummings, 1997). Southgate (1995) divided NSPs into structural and non-structural polysaccharides. Table 2.3 shows the major types of NSPs.

Table 2.3. Types of non-starch polysaccharides.

Primary Source	Major Group	Components Present	Summary of Structures	Distribution in Foods
Structural materials of plant cell wall	Cellulose		Long chain β-Glucans	All cell walls
	Non-cellulosic polysaccharides	Pectic substances	Galacturonans	Mainly in fruits and Vegetables
		Hemicelluloses	Arabinogalactans Arabinoxylans Glucuronoarabinoxylans Glucuronoxylans Xylo-glucans β-Glucans	Cereals Cereals Fruits/vegetables Fruits/vegetables Cereals
Non-structural polysaccharides	Gums, mucilages		Wide range of Heteropolysaccharides	Seeds and fruits

Source: Southgate (1995)

Cellulose confers structural support to plants and is the predominant constituent of the outer bran layers and husks of cereals. It is made up of linear glucose units in β-1→4 glycosidic linkages which are tightly packed together into microcrystalline regions (Fincher and Stone, 1986). Arabinoxylans are hemicelluloses found in the primary and secondary cell walls of cereals (Huisman *et al.,* 2000). They consist of a linear chain backbone of β-D-xylopyranosyl residues linked through (1→4) glycosidic bonds (Izydorczyk and Dexter, 2008) and can be substituted with α-L-arabinofuranose on C2 and/or C3 (Huisman *et al.,* 2000). Some of the arabinose residues are linked to fatty acids on C5 (Smith and Martin, 1993). Oligomeric sequences composed of only arabinose, or arabinose with xylose, mannose, galactose, or uronic acids have been identified in maize (Chanliaud *et al*., 1995; Saulnier *et al*., 1993: Izydorczyk and Biliaderies, 2000). Ratio of arabinose to xylose (Ara/Xyl) may differ with source and is a function of the degree of branching in the arabinoxylan polysaccharide (Verbruggen *et al.,* 1998). High ratios

indicate high degree of branching. Studies by Nyman *et al*. (1984) revealed that maize flour contained 26% L-arabinose and 24% D-xylose while whole maize kernel contained 28.1% arabinose and 32.8% xylose (Park *et al.,* 2001). The branched units of uronic and/or ferulic acid on arabinoxylan chain increases its solubility as branching reduces intermolecular forces and decreases intermolecular attraction thus reducing the nonpolar nature. To this effect arabinoxylans with high ratio of Ara/Xyl are highly soluble in water than those with low Ara/Xyl ratio.

2.8 Beta-glucan

Beta-glucan is a component of the cell wall of cereals (Miller and Fulcher, 1994) that is found in appreciable amounts in barley at 3-11% and in oats at levels of 3-7% (Bhatty, 1992). Wheat and rye are reported to have lesser quantities (<2%) of β-glucan. Cereal β-glucan is a linear polymer of glucose made up of β-(1→3) and β-(1→4) linkages. A repeating unit of three (cellotriosyl) and four (cellotetraosyl) β-(1→4) glucopyranosyl units which are separated by single β-(1→3) linkages is a predominant feature of cereal β-glucans (Fig. 2.3) (Cui and Wood, 2000). While β-(1→3) linkages occur singly, consecutive sequences of β-(1→4) linkages may vary from 3 to 14 in some cereals (Lazaridou *et al.,* 2003). The resultant structure is a polysaccharide built mainly from β-(1→3)-linked cellotriosyl (58-72%) and cellotetraosyl (20-34%) units.

Interruption of the polymer by the (1→3)-linkages creates a kink in the structure. Such kinks prevent β-glucan strands from aggregating into cellulose-like regions which are known to be insoluble. Because of this, water molecules can interact with β-glucan through

hydrogen bonding between water molecules and β-glucan's numerous hydroxyl groups (Fincher and Stone, 1986; MacGregor and Fincher, 1993).

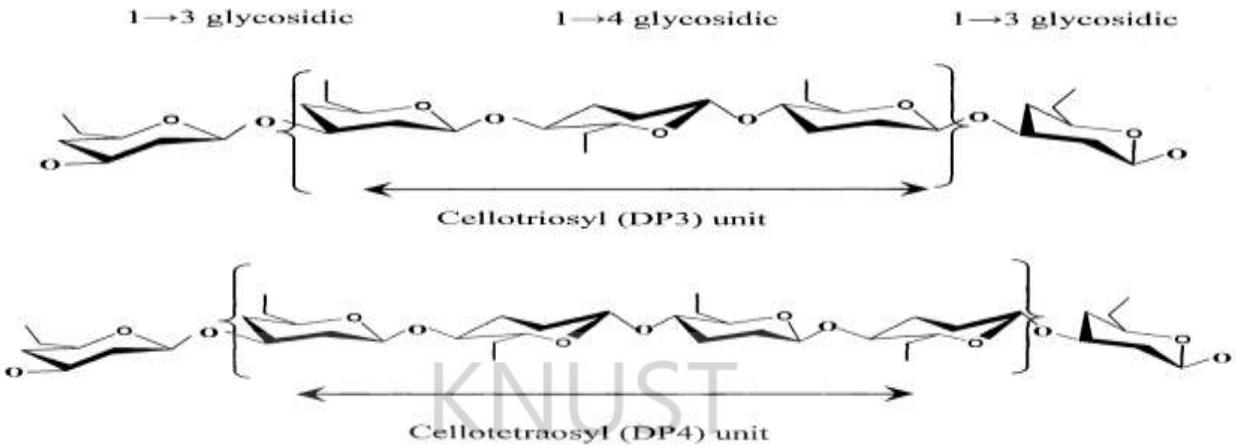


Figure 2.3 Structure of cereal β-glucan repeating units (Vasanthan and Temelli, 2008).

It has been reported that cereal β-glucans differ in the ratio of trisaccharide to tetrasaccharide and exhibits the trend, wheat>barley>oat (Lazaridou *et al.*, 2003). The molar ratio of cellotriosyl to cellotetraosyl unit is about 3:1 (Wood *et al.*, 1994). However, information on cellotriosyl/cellotetraosyl ratios in maize is unavailable. The arrangement of cellotriosyl and cellotetraosyl units and their ratio in the polymer chain are believed to be important factors controlling the solution properties of these polysaccharides (Cui and Wood, 2000: Lazaridou and Biliaderies, 2004: Tosh *et al.*, 2004a: Cui and Wang, 2006; Bohm and Kulicke, 1999) although earlier studies ascribed these to the long runs of cellulosic segments (Izawa *et al.*, 1993: Doublier and Wood, 1995).

Rapid gelation, tough gel strength and decrease in brittleness are observed in foods of high cellotriosyl/cellotetraosyl ratios and high molecular weight (Heinze and Barsett, 2005). The molecular weights of oat and barley β-glucan are variable, ranging from 20,000 to

3,000,000 Daltons (Wood *et al.,* 1991a; Cui, 2001; Yokoyama *et al.,* 2002; Lazaridou *et al.,* 2003; Åman *et al.,* 2004). Variation in molecular weight appears to be a function of the genotype, environment of cultivation and the method of β-glucan isolation (Izydorcyzk and Biliaderis, 2000).

2.9 Beta-glucan isolation and concentration

The molecular structure of β-glucan is subject to degradation by extremes of pH, temperature, organic solvents and shear. Various technologies, incorporating dry and wet separation procedures have been developed by researchers to preserve the molecular structure of β-glucan during isolation while reducing contaminants to minimum (Burrows *et al.,* 1984; Myllymaki *et al.,* 1989; Inglett, 1992; Burkus and Temelli, 1998; Vasanthan and Temelli, 2002; Vasanthan and Temelli, 2008).

2.9.1 Dry separation techniques

The dry technologies often involve the following steps in sequence: dehulling or pearling of grains, dry milling into fine flour, followed by separation of the particulates by air classification. The final product, which contains about 30% β-glucan, has a range of particle sizes, varying in shape and density, depending on the extent of size reduction (Vasanthan and Bhatty, 1995; Zheng *et al.,* 2000). When used as ingredient in food application it imparts undesirable sensory attributes resulting in low product acceptability (Vasanthan and Temelli, 2008).

2.9.2 Wet separation techniques

The wet separation process, unlike the dry method is tedious and expensive however, maximum recovery of 95 % (w/w) β-glucan can be achieved (Vasanthan and Temelli, 2008). Four wet separation techniques for β-glucan isolation are available: (a) aqueous alkali extraction, (b) aqueous alcohol-based enzymatic treatment, (c) aqueous-enzymatic treatment, and (d) aqueous-thermo mechanical treatment (Vasanthan and Temelli, 2008). The aqueous alkali isolation method involves the use of water, acidified water and/or aqueous alkali as solvents to hydrate and solubilize the β-glucan from the cell walls of cereal endosperms. Solubilization is followed by centrifugation to separate the solid particles, including starch and insoluble fibre (cellulose, lignin) from the liquid phase which contains the β-glucan and proteins. Proteins in the liquid phase are precipitated at their isoelectric point by addition of acid and subsequently removed by centrifugation. Finally, β-glucan concentrate is recovered from the liquid phase by precipitation with alcohol and dried to obtain gum (crude β-glucan).

A major drawback of the wet isolation technologies is that the reconstituted β-glucan isolate has low solution viscosity, a problem which arises from the action of the endogenous enzymes, cellulose and β-glucanase, on the solubilized β-glucan, thereby decreasing its molecular weight. In addition to this, during aqueous extraction, the hydrated β-glucan molecules become highly susceptible to shear fragmentation by the mixing and the centrifugation steps (Ghotra, 2006). Recently, a novel enzyme-aided alcohol based wet extraction technique has been developed by Vasanthan and Temelli (2009). Compared to the alkali extraction method, the enzyme-aided isolation technology

has a percentage recovery of 65% β-glucan, nevertheless, the use of alcohol in this method preserves the original molecular weight because β-glucan remains intact in the cell walls of the endosperm. Consequently, potential degradation of β-glucan by the endogenous enzymes, cellulose and β-glucanase, is minimized, and shear fragmentation does not occur. In this method, the flour is slurried with aqueous ethanol (50% v/v), followed by sequential treatment with protease and α-amylase to remove proteins and starches, respectively, from the flour. The fibre-rich β-glucan left behind is washed with absolute ethanol and recovered by filtration.

2.10 Structure of β-glucan

A number of methods for extraction of β-glucan have been developed (Johansson, 2006) which vary in extraction reagents and conditions. Extraction conditions and source of β-glucan strongly influence the structure of the purified product. For example, although the structure of barley β-glucan is believed to be similar to that of oat β-glucan (Beer *et al*., 1997; Cui *et al*., 2000; Tosh *et al*., 2004b; Papageorgiou *et al*., 2005), a comparison of the results of structural analysis of the two products is difficult because the methods of isolation and analyses differ by experiment (Johansson, 2006). For the purpose of determination of functionality and applicability in health and food manufacture, structural analysis of β-glucan is important.

2.10.1 Structural sequence analysis of β-glucan

In order to determine the structure of β-glucan, the polymer has to be broken down to oligosaccharides. The enzyme lichenase (1→3),(1→4)-β-D 4- glucanohydrolase (EC

3.2.1.73) is employed. Lichenase cleaves the (1→4)-linkage next to a (1→3)-linkage at the reducing end to produce (1→4)-linked oligosaccharides with one (1→3)-linked glucose unit as an end group at the reducing end. The action of the enzyme leads to short chains having a 3-O-β-cellotriosyl-D-glucose with DP3 as the main product and 3-O-β-cellotetraosyl-D-glucose with DP4 as a minor product. These two together make up over 90% of the total β-glucan content (Wood *et al*., 1994a). Wood *et al*. (1991b) and Izydorczyk *et al*. (1998a, b) reported that the ratio DP3:DP4 governs the structural differences of β-glucans. A high ratio indicates more cellotriosyl sequences than cellotetraosyl sequences.

Oat and barley β-glucan consist of large blocks of cellotriosyl (DP3) and cellotetraosyl (DP4) separated by single 1→3-linkages, with barley β-glucan having a higher ratio of DP3:DP4 and hence its relatively higher molecular weight (Autio, 1996). The molecular weight and structure of β-glucan is influenced by cultivar as well as isolation method (Forrest and Wainwright, 1977; Woodward *et al*., 1983; Autio, 1996; Jaskari *et al*., 1995; Beer *et al*., 1997; Goméz *et al*., 1997). Literature is replete with information on the various methods for the elucidation of the structure of β-glucan. These include enzymatic hydrolysis which make use of lichenase to break down the (1→4)-bond adjacent to a (1→3)-bond at the reducing end to produce oligosaccharides consisting of β-(1→4)-linked glucose units with one (1→3)-linked glucose at the reducing end (Wood *et al*., 1991b; Miller and Fulcher, 1994; Izydorczyk *et al.*, 1998a, b; Roubroeks *et al*., 2000; Colleoni-Sirghie *et al*., 2003). This is the basis for mixed-linkage β-glucan Megazyme assay of McCleary and Codd (1991) and McCleary and Glennie-Holmes (1985).

Other methods of β-glucan sequence analyses include methylation (Aspinall, 1982; Aspinall and Carpenter, 1984; Wood *et al.,* 1991a), liquid chromatography with reversed-phase columns for analysis of carbohydrates (El Rassi, 1995) and high-performance liquid chromatography (Jiang and Vasanthan, 2000). High performance anion-exchange chromatography with pulse-amperometric detection (HPAEC-PAD) (Izydorczyk *et al*., 1998a; Cheng and Kaplan, 2001; Panagiotopoulos *et al*., 2001; Talaga *et al*., 2002) was used in structural analysis of barley, oat and rye β-glucan (Wood *et al*., 1994c; Izydorczyk *et al*., 1998a, b; Roubroeks *et al*., 2000). Also, gas chromatography (Blakeney *et al*., 1983; Olson *et al*., 1988; Pettersen and Schwandt, 1991), capillary electrophoresis (Rydlund, 1995; Soga and Serwe, 2000; Larsson *et al*., 2001; Chiesa and Horvath, 1993; Arentoft *et al*., 1993; Hoffstetter-Kuhn *et al*. (1991), as well as Fourier transform-infrared (FT-IR) spectroscopy (Kačuráková and Wilson, 2001; Sekkal *et al*., 1995) have been used to analyse the monomers of polysaccharides. In comparison to the forgone techniques mentioned, a more powerful technique is Nuclear Magnetic Resonance (NMR).

2.10.2 Nuclear magnetic resonance

High-resolution nuclear magnetic resonance (NMR) spectroscopy is a nondestructive method that provides direct information on the chemical structure of polysaccharides (Johansson, 2006). It is a technique which finds applications in chemistry, physics, geology, biology, engineering, and the other sciences (Dybowski and Shi, 2002) where it is primarily employed to reveal the structure of a particular moiety in biological materials, the products of a reaction, the solid-state structures of compounds, the dynamics of

molecules in restricted environments, or the connection of microscopic properties to technologically important uses of materials (Bertocchi and Paci, 2008).

Several types of NMR are available for structural determination either in the liquid state or the solid state, one-dimensional or two-dimensional mode which utilize ^{1}H, ^{13}C or both, including Magnetic Angle Spinning (MAS) NMR, High Resolution Magnetic Angle Spinning (^{1}H HRMAS) . In the field of food science, Cross polarization–magneticic angle spinning (CP-MAS) NMR spectroscopy and MAS spectroscopy was first employed in the study of cellulose but has now extended to structural determination of starch and other polysaccharides. Cross polarization–magic angle spinning NMR (^{13}C CP-MAS/NMR) - enables the investigation of conformations and static interactions. The effect of ball milling on maize and wheat starch was studied by ^{13}C CPMAS NMR which revealed the pattern of damage during milling (Morrison *et al*., 1994; Lewen *et al*., 2003). The macromolecular structure and digestibility of products of Maillard browning involving potato starch, amylose and amylopectin with L-lysine was investigated by ^{13}C CPMAS NMR spectroscopy (Pizzoferrato *et al*., 1999). Other applications of ^{13}C CP NMR in food polymers include studies on ageing of starch in bread during storage (Calucci *et al*., 2004), a study of polymorphism in cellulose (Gast *et al*., 1980; Atalla *et al*., 1980; Earl and VanderHart, 1980; Horii *et al*., 1982; Maciel *et al*., 1982; VanderHart and Atalla, 1984; Isogai *et al*., 1989; Waigh *et al*., 2000), pectin (Synytsya *et al*., 2003) distribution of arabinans and hemicellulosic polymers in cell wall of sugar beet, *B. vulgaris* (Renard *et al*., 1999).

The molecular structure of wheat proteins during processing (Zhang *et al*., 2005; Alberti *et al*., 2002) and changes in post-mortem muscle of meat were studied by ^{13}C CPMAS NMR. The structure of glucagel, a mixed-linked (1→3),(1→4)-β-D-glucan isolated from barley showed dual distinct conformation upon analysis with ^{13}C CPMAS NMR spectroscopy (Morgan *et al*., 1999). Also, ^{13}C CP/MAS has been used to elucidate specific bonding between barley β-glucan and bile acid salt, glycocholic acid through a similarity in chemical shift values for both the anhydrous and hydrated states. This explains the role β-glucan plays in reducing blood cholesterol levels (Bowles *et al*., 1996). Studies by Virki *et al*. (2005) using ^{13}C CP/MAS NMR demonstrated similar spectra of soluble oat and barley β-glucan with slight differences in structures of extracted fractions from the water-insoluble material.

Dudley *et al*. (1983) used CPMAS NMR for analyses of cellulose oligomers and the structure of cellulose. Morgan *et al*. (1999) analyzed the structure of β-glucan extract from barley with CPMAS NMR. CPMAS NMR was also for analysis of whole grain oat and barley (Johansson *et al*., 2004), for oat bran (Johansson *et al*., 2000) and for non-starchy polysaccharides of both oats and barley (Virki *et al*., 2005) and oat bran (Aspinall and Carpenter, 1984). However, there is no information on the elucidation of the maize β-glucan by CPMAS NMR.

2.11 Aniline blue fluorescence microscopy

Aniline blue is the most widely used fluorochrome in botany. This technique makes use of the aniline blue dye at low concentrations of dye to impart a yellow fluorescence to various

parts of the cell wall, notably sieve plates, pit fields, as well as cell plates. These areas are considered to contain the (1→3)-β-D-glucan known as callose (Currier and Strugger, 1956; Smith and McCully, 1977). The binding of the fluorochrome to (1→3)-β-D-glucan in cells permits their quantification.

This technique has been extensively used in the determination of (1→3)-β-D-glucan in yeast (Ishihara *et al*., 2007; Sekiya-Kawasaki *et al*., 2002; Watanabe *et al*., 2001) and has been successfully used in sorghum (Smith and McCully, 1977). The fluorochrome binds to isolated samples of a variety of β-(1→3) and (1→4) linked-glucans. The purified fluorochrome binds strongly to all regions of primary cell walls, lignified walls, as well as to regions known to contain a high concentration of callose. Crude aniline blue also binds albeit weakly to all cell walls but the intense fluorescence in areas rich in callose may be due to more ready penetration of the fluorochrome into those regions. The apparent specificity for callose may be due to interferences with the fluorochrome by other components of the crude dye which binds to cell wall regions not containing callose. This can be eliminated by prior treatment of the sample with periodic acid-schiff (PAS) reaction (Smith and McCully, 1977). Non-specific binding of fluorochrome to lignified cell wall is eliminated by prior staining with toluidine blue (Smith and McCully, 1977).

2.12 Rheological properties of β-glucan

Beta-glucan, like other polysaccharides, such as xanthan gum, guar gum, gum arabic, and carbohydrate hydrocolloids exhibits rheological properties. The parameters used to characterize the rheological behavior of gum dispersions include viscosity, flow behavior

index, consistency coefficient, pseudoplasticity, viscoelasticity, and thixotropy. The rheological properties of β-glucan in oat and barley have been investigated in the past (Skendi *et al.,* 2003; Lazaridou *et al.,* 2003; Ghotra 2006; Ahmad *et al.,* 2009). Cereal β-glucan solutions are non-Newtonian fluids which exhibit a flow behavior characterized as pseudoplasticity or shear thinning (Doublier and Wood, 1995; Lazaridou *et al*., 2000; Ghotra 2006).

Non-Newtonian fluids demonstrate different viscosities under different shear rates, whereby the ratio of shear stress to shear rate is a function of shear rate. This ratio, termed "apparent viscosity (η)" is presented in equation (1) (Zhong and Daubert, 2007):

$$\eta = f(\dot{\gamma}) = \frac{\sigma}{\dot{\gamma}} \qquad (1)$$

where $\dot{\gamma}$ is the shear strain rate, and σ is the shear stress.

Various models have been generated by rheologists to describe both Newtonian and non-Newtonian fluids. The Hershey-Bulkley model (Sharoba *et al.,* 2005) (equation 2) is of useful significance as it describes the rheological behavior of a wide range of fluids.

Hershey-Bulkley model $\sigma = \sigma_o + K\dot{\gamma}^n$ (2)

where σ is the shear stress (Pa), σ_o is the yield stress (Pa s), $\dot{\gamma}$ is the shear strain rate (s^{-1}), K is the consistency coefficient (Pa s^n), and n is the flow behavior index (dimensionless). The term "yield stress" describes the stress required to initiate flow in some materials. The pseudoplastic flow behavior of dispersions of β-glucan can be

adequately explained by the Power Law model (Marcotte *et al.*, 2001; Sahin and Ozdemir, 2004) in equation 3, where yield stress is zero.

Power law model $$\sigma = K\dot{\gamma}^{n} \quad (3)$$

Gum dispersions having n =1 are considered to be Newtonian, those forming highly viscous solutions have n <1 and are termed pseudoplastic and n> 1 are classified as dilatant (shear thickening) (Marcotte *et al.*, 2001). While the rheological properties of oat and barley β-glucan dispersions have been reported in literature (Ghotra, 2006; Ahmad *et al.*, 2009; Skendi *et al.*, 2003), there is dearth of information on β-glucan in maize and its rheological properties. Typical rheograms describing rheological properties of fluids are provided (Fig. 2.4).

A B

Figure 2.4. Typical rheograms of fluids measured at 20 ^{o}C. (A) Shear stress against shear rate. (B) Apparent viscosity as a function of shear rate for different types of fluids: 1) Newtonian; 2) shear-thinning (pseudo-plastic); 3) shear-thickening (dilatants): 4) Bingham plastic; and 5) Herschel–Bulkley. Source: Zhong and Daubert (2007).

The viscosity of β-glucan solution ranges from a random coil nature at low concentrations, followed by formation of junction zones at increased concentration, and then to a micellar

structure at high concentration. Junction zones are formed when individual chains associate with one another through hydrogen bonding. The formation of many junction zones above a critical concentration produces a micellar structure (Grimm *et al.*, 1995), which encompasses inter- and intra-molecular hydrogen bonding between regular cellulosic regions of β-glucan chains (Doublier and Wood, 1995; Bohm and Kulicke, 1999). At high viscosity, inter-micelle associations through hydrogen bonding leads to the formation of three-dimensional network of β-glucan chains to produce a gel. Figure 2.3 shows the various stages of rheological properties of β-glucan solutions.

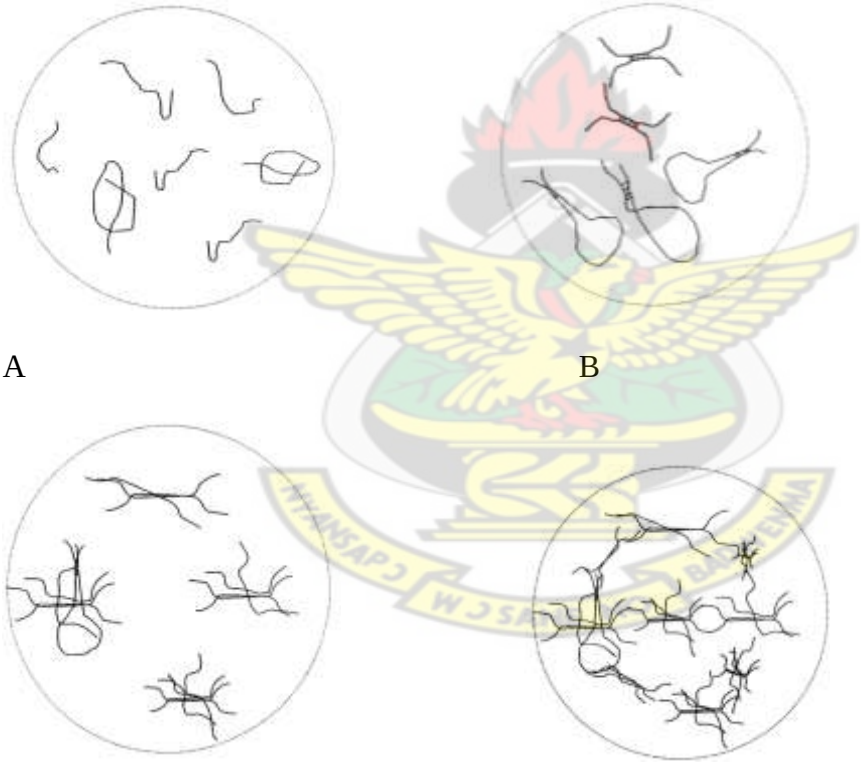


Figure 2.5: Schematic of rheological properties exhibited by β-glucan at increasing concentrations (A) Random coil. (B) Junction zone formation. (C) Individual micelle structure. (D) Micelle network forming a gel. Source: Ghotra (2006).

Viscosity of β-glucan in solution is influenced by its concentration, temperature and length of the β-glucan chain, with the longest chains resulting in the highest viscosity (Vasanthan and Temelli, 2008). At concentrations above 0.27 % cereal β-glucan solutions typically exhibit non-Newtonian behavior, whereby the viscosity changes with shear rate, temperature, pressure and time of shearing (Barnes, 1997; Ren *et al.,* 2003). Such behaviour of fluids is described as pseudoplastic flow behavior (Doublier and Wood, 1995; Lazaridou *et al.,* 2003), meaning viscosity decreases when the solution is subjected to increasing rates of shear. Isolation of β-glucan presents a special problem to food manufacturers as the derived health benefit and functional properties in food products depend on its viscosity, which in turn depends on its molecular weight and solubility of the β-glucan.

2.13 Fat replacers

Natural fats have many useful functions in the diet. They are among nutrients essential for proper growth, development and maintenance of good health. They furnish fat-soluble vitamins A, D, E, and K and assist in their absorption in the small intestine. Fats are the only sources of essential fatty acids (EFAs), such as linoleic and linolenic acids, and they contribute energy of 9 kcal/g, compared to 4 kcal/g each for proteins and carbohydrates (Senanayake and Shahidi, 2005). In addition, fats contribute favorably to the taste, consistency, stability, texture, colour and palatability of foods.

However, it is widely recognized that, high amount of fat in diets may result in obesity and cardiovascular disease. It is often recommended that people choose foods with low fat and

high complex carbohydrate as prescribed in the new Food Guide Pyramid published by the United States Department of Agriculture (Goldie, 2005). Although the Ghana Heart Foundation (GHF) has not suggested a threshold allowance of fat intake, other organizations have recommended limits of fat intake. A recent World Health Organization (WHO) report recommended that the level of fat intake should be between 15 and 30 % of the total energy intake, of which saturated fatty acids should account for less than 10 % energy (WHO, 2006). The food industry has responded to this demand by introducing a variety of fat replacers to help consumers reduce their fat and caloric intake (Gershoff, 1995).

A fat replacer is an ingredient that replaces some or all the functions of fat and may or may not provide nutritional value (Miraglio, 1995). Fat replacers are generally categorized into two groups: fat substitutes and fat mimetics. Fat substitutes are macromolecules that physically and chemically resemble triglycerides and which can theoretically replace fat in foods on a one-to-one, basis (Peters and Lawson, 1997). Often referred to as fat-based fat replacers, fat substitutes are either chemically synthesized or derived from conventional fats and oils by enzymatic modification, and have chemical structures and physiochemical properties somewhat close to that of fats (Lipp and Anklam, 1998; Kosmark, 1996; Peters and Lawson, 1997). Many fat substitutes are stable at cooking and frying temperatures (Duflot, 1996; Harrigan and Breene, 1989; Kosmark, 1996). They are usually either indigestible or contribute lower calories on a per gram basis. On the other hand, fat mimetics are ingredients that have distinctly different chemical structures from fat. They are usually carbohydrate and/or protein-based. They have diverse functional properties that

mimic some of the characteristic physiochemical attributes and desirable eating qualities of fat, viz. viscosity, mouthfeel and appearance (Johnson, 2000; Duflot, 1996; Harrigan and Breene, 1989).

2.13.1 Carbohydrate-based fat mimetics

These fat replacers are made from carbohydrates, such as cellulose, dextrins, maltodextrins, polydextrose, gums, fibre, and modified starch. Carbohydrate-based fat replacers provide up to 4 kcal/g of energy but, because they are often mixed with water, they typically provide only 0.5 to 2 kcal/g, calories. They are used mainly as thickeners, stabilizers in a variety of foods, such as dairy products, frozen desserts, sauces, salad dressings, processed meats, baked goods, spreads, chewing gums, and sweets.

The most widely used carbohydrate-based fat replacers are dextrins, gums and modified starches, which absorb water and form gels that impart a texture and mouth feel similar to fat. The solubility, gelation and water binding potential of fat mimetics are related to the particulate surface area (Wylie-Rosett, 2002). When hydrated, polydextrose forms a gel that mimics some of the functional characteristics of fat. Maltodextrins, a nutritive polysaccharide derived from hydrolysis of corn starch, can function as fat mimetics in flour-based dry mixes, baking systems, fillings, and icings (Swanson *et al.*, 2002). Maltodextrins can be substituted for 25 to 35 % of fat in cookies, while cellulose and indigestible fibre is able to replace up to 50% of fat in baked products without compromising physical and sensory characteristics (Conforti *et al.*, 2001). Thus, the diverse properties of these plant-based carbohydrates and their derivatives are used when

developing fat mimetics. Inulin, a non-digestible natural fructo-oligosaccharide, is considered to have functional properties that enable it to act as a fat mimetic without adversely affecting flavor, based on its ability to stabilize the structure of the aqueous phase, creating an improved and creamy mouth feel (Aryana and Haque, 2001; El-Nager *et al.*, 2002).

2.13.2 Protein-based fat mimetics

To date the major source of protein-based fat mimetics is whey protein, milk and egg protein, with caloric value between 1 to 4 kcal/g. Micro-particulate protein products are tiny spherical particles, which can provide a creamy mouth feel similar to fats. They often incorporate water due to their wide surface area. The major advantage of a protein-based fat mimetic is that, a gram of protein-based fat mimetic can replace 3 g of fat in cream. Protein-based fat mimetics are not suitable for use in fried foods but can be used in dairy products, such as fat-free ice creams, frozen desserts, and milkshakes. In addition protein-based fat mimetics are used in reduced-fat versions of butter, sour cream, low-fat cheese, yogurt, low-fat baked goods, salad dressing, margarine, mayonnaise, coffee creamers, soups and sauces (Cheung *et al.*, 2002). Other protein-based fat mimetics include gelatin, isolated soy protein, protein blends that combine animal and/or vegetable protein and carbohydrate gums. A combination of protein, starches, and hydrocolloids has been suggested to have synergistic effects for lowering fat and retaining textural characteristics of the products (Ordonez *et al.*, 2001; Ruthing *et al.*, 2001).

2.13.3 Fat-based substitutes

Mono- and diacylglycerol, propylene glycol mono- and diesters and sodium or calcium stearoyl are common fat-based emulsifiers used to manipulate the structure of fat in traditional products and to enhance the functionality of reduced-fat food systems (Lucca and Tepper, 1994; Duxbury, 1992). Mono- and diacylglycerol are subject to the same hydrolysis as triacylglycerol and therefore, yield 8 -9.1 Kcal/g. Blends of various emulsifiers and other ingredients yield similar energy values as do the individual components. A commercially available fat substitute, Caprenenin, which is a triacylglycerol esterified with caprilyic, capric and behenic saturated fatty acids contributes 21 KJ/g calories in chocolate due to the poor absorption capacity of behenic acid. Other fat-based fat substitutes, such as Olestra have properties similar to naturally occurring fat but provide zero calories and pass through the body unabsorbed. Olestra is a sucrose polyester consisting of a mixture of hexa-, hepta-, and octa- esters of sucrose with long-chain fatty acids. It has organoleptic and thermal properties of fat but cannot be hydrolyzed by gastric or pancreatic lipase and is unabsorbed in the gut (U.S. Food and Drug Administration, 2003). Unlike carbohydrate and protein-based fat mimetics, fat substitutes are heat stable and can withstand frying temperatures. Several studies have shown that their application in fried snack foods and yogurt leads to decreased energy and macronutrient intakes in consumers (Burns *et al.*, 2001, 2002).

Chapter Three

Materials and Methods

3.1 Maize genotypes

Seventeen genotypes of maize were supplied by the Crops Research Institute (CRI) of the Council for Scientific and Industrial Research (CSIR), Fumesua, Kumasi in the Ashanti Region of Ghana. Maize samples were chosen to represent different classes of maize viz., Quality Protein Maize (QPM), landraces, hybrids and Open Pollinated Varieties (OPV). The genotypes used for the study were: 'Mamaba', 'Suwan 1 QPM', 'Obatanpa GH', 'Omankwa', 'GH 9', 'Catete', 'Dodzi', 'Okomasa', 'Aburohemaa', 'Abontem', 'Sotu Baka', 'Dorke', 'Akposoe', 'Abeleehi', 'Safita 2', 'Ohawu Local' and 'Golden Jubilee'.

3.2 Materials

Analytical grade reagents comprising; hexane, sulphuric acid, hydrochloric acid, sodium hydroxide, citric acid, sodium bicarbonate, glycine, ethanol, sodium acetate, glacial acetic acid, morpholino propanesulfonic (MOPS) acid, glucose oxidase/ peroxidase (GOPOD), D-glucose, sodium phosphate, lichenase, aniline blue, were purchased from Pakus Ventures, Amakom, Kumasi. Bromelain (E.C. 3.4.22.32, pineapple stem), amyloglucosidase (E.C. 3.2.1.3, *Aspergillus niger*) and thermostable α-amylase (E.C. 3.2.1.1, *Bacillus subtilis*) were purchased from Sigma-Aldrich (St. Loius, MO, U.S.A.). Mixed linkage β-glucan and total starch assay kits were purchased from Megazyme International Limited (Bray, Ireland). Distilled water was used for all experimentations.

3.3 Characterization of maize

Maize samples were evaluated for morphological characteristics. These included hundred kernel weight, kernel texture and colour.

3.4 Maize flour preparation

Two kilograms of each maize genotype were air-dried and milled using a Laboratory Scale Hammer Mill (Schutte-Buffalo Hammermill, Buffalo NY, U.S.A.) into flour and sieved through a screen of 0.2 mm. The maize flour were kept in high density polyethylene bags and stored in a cool and dry environment until ready for use. Maize flour was used for all chemical analyses. The chemical analysis on maize flour included proximate analyses, total starch determination, isolation of gum followed by purification to β-glucan isolate. The gums served as raw material for determination of rheological properties, composition of the (1→3)-β-D-glucan fraction by fluorescence spectrofluorometry , structural analysis by NMR, and finally for studies on the functionality of the gums as fat replacers in pie crust.

3.5 Chemical analyses

3.5.1 Proximate analysis

3.5.1.1 Determination of moisture content

Moisture content was determined by the method of A.O.A.C. No. 945.38 (A.O.A.C., 2005). Two grams of maize flour were weighed and transferred into a previously dried and weighed glass crucible and placed in a hot air oven to dry at 105 °C for 5 h. Samples were

cooled in a desiccator, weighed, and returned to the oven to dry to constant weight. Loss in weight was calculated as percentage moisture (Appendix 1).

3.5.1.2 Determination of ash content

Ash content was determined by the method of A.O.A.C. No. 936.07 (A.O.A.C., 2005). In this method, 2.0 g of dried maize flour from 3.5.1.1 were transferred into a pre-ignited and pre-weighed porcelain crucible and combusted in a muffle furnace (XD- 1200 N, China)) at 600 °C for 2 h. The crucibles containing ash were cooled and re-weighed. Loss in weight was calculated as percentage ash content (Appendix 2)

3.5.1.3 Determination of crude fat content

Crude fat determination followed the method of A.O.A.C. No. 2003.05 (A.O.A.C., 2005). Two grams of sample were transferred into a 22 × 80 mm paper thimble and capped with glass wool, dropped into a thimble holder and attached to a pre-weighed 500 ml round bottom flask containing 200 ml hexane and assembled on a semi-continuous soxhlet extractor. Contents of the thimble were refluxed for 16 h after which the hexane was recovered on a steam water bath. The flask containing the fat was heated for 30 min in an oven at 103 ^{o}C, cooled in a desiccator and weighed. Increase in weight of flask was recorded from which the percentage crude fat was calculated (Appendix 3).

3.5.1.4 Determination of protein content

Protein content was measured following the Kjeldahl nitrogen determination of A.O.A.C. No.2001.11 (A.O.A.C., 2005). In this method, to 2.0 g of dried maize flour in a Kjedahl

flask was added 25 ml concentrated (98 %) H_2SO_4 and digested till the colour of the solution turned clear. The solution was transferred into a 100 ml volumetric flask and the volume made up to the mark with distilled water. Ten milliliters of the solution were distilled and titrated against 0.1 M hydrochloric acid against a blank. Titre values of duplicate samples were recorded and percentage nitrogen calculated (Appendix 4). Percentage nitrogen (% N) was converted to percent crude protein by multiplying by a factor of 6.25.

3.5.1.5 Determination of crude fibre content

Crude fibre was determined according to the procedure of A.O.A.C. 920.86 (A.O.A.C. 2005). To 2.0 g of defatted maize flour in a 750 ml Erlenmeyer flask was added 200 ml of boiling 1.25 % H_2SO_4 and refluxed for 45 min. The mixture was screened with cheese cloth and residue washed with large volumes of boiling water till filtrate was no longer acidic. The reflux was repeated with 1.25 % sodium hydroxide (NaOH), screened and washed to remove all alkali. The residue was transferred to a previously weighed porcelain crucible (M1), dried for 1 h at 100°C, cooled in a desiccator and re-weighed (M2). The crucible was ignited in the muffle furnace at 600 ^{o}C for 30 min and re-weighed after cooling in a desiccator (M3). Increase in weight was calculated as percentage crude fibre (Appendix 5).

3.5.1.6 Determination of available carbohydrate content

Available carbohydrate content was calculated by difference [(100- total of M)] where M is moisture + crude fat + ash + crude fibre + crude protein (A.O.A.C. No. 986.25) (A.O.A.C., 2005). All proximate determinations were done in triplicate.

3.6 Beta-glucan isolation

Beta-glucan isolates from cereals are commonly referred to as gums. Gum was isolated from maize flour using four wet extraction methods viz., aqueous alkali process (Wood *et al*., 1989), hot water, acid extraction (Ahmad *et al*., 2009) and alcohol-based enzyme extraction (Vasanthan and Temelli, 2002) procedures.

3.6.1 Aqueous alkali extraction

Alkali is used to solubilize β-glucan and proteins in the maize flour, while starch and other forms of fiber remain insoluble. Centrifugation of the mixture, followed by protein precipitation of the liquid phase at the isoelectric point leaves β-glucan.

To 50 g of maize flour was added 300 ml of 80 % ethanol and refluxed for six hours. The slurry was sieved with a cheese cloth and the filtrate was discarded. The residue was dissolved in 7 parts of 1 M NaOH in a 1 L beaker and heated at 55 ^{o}C for 90 min with constant stirring. The slurry was centrifuged (Centrikon T-42K, UK) at 3,400 r.p.m. for 20 min at 40 ^{o}C. The supernatant was mixed with 3 parts of 1 M NaOH and centrifuged at 3,700 r.p.m. for 20 min at 40 ^{o}C. The residue was discarded and the pH of the supernatant was adjusted to 7 with 8 M citric acid to precipitate the protein. This was followed by

centrifugation at 4,100 r.p.m. for 25 min. The supernatant was then mixed with equal volume of 80 % ethanol and held for 20 min. Finally, the mixture was centrifuged at 1,900 r.p.m. for 25 min at 40 ^{o}C. After discarding the supernatant the residue was dried on a petri dish in an oven at 55-60 ^{o}C for 36-48 hours to obtain gum. Gum was weighed and expressed as percentage recovery (Ahmad *et al*., 2009).

3.6.2 Aqueous acid extraction

In this method, acid is used to solubilize β-glucan and proteins in the maize flour, while starch and other forms of fiber remain insoluble. Centrifugation of the mixture, followed by protein precipitation of the liquid phase at the isoelectric point leaves β-glucan.

To 50 g of maize meal was added 300 ml of 80 % ethanol and refluxed for 6 h. The slurry was sieved in a cheese cloth and the filtrate discarded. The residue was dissolved in 7 parts 1 M citric acid and heated at 55 ^{o}C for 90 min with constant stirring. The slurry was centrifuged at 3,400 r.p.m. for 20 min at 40^{o}C. The supernatant was mixed with 3 parts of 1 M citric acid and centrifugation was repeated at 3,700 r.p.m. The residue was discarded and the pH of the supernatant was adjusted to 7 with 8 M NaOH to precipitate the protein. This was followed by centrifugation at 4,100 r.p.m. for 25 min. The supernatant was then mixed with equal volume of 80 % ethanol and held for 20 min. The mixture was centrifuged at 1,900 r.p.m. for 25 min at 40 ^{o}C. The supernatant was discarded and the residue dried on a petri dish in an oven at 55-60 ^{o}C for 36-48 h to obtain gum. Gum was weighed and expressed as percentage recovery (Ahmad *et al*., 2009). The gum was analyzed for its proximate composition as outlined previously.

3.6.3 Hot water extraction method

To 50 g of maize meal was added 300 ml of 80 % ethanol and refluxed for 6 h. The slurry was sieved in a cheese cloth and the filtrate was discarded. The residue was dissolved in 10 parts hot water (80 ^{o}C) and heated at 55^{o}C while mixing for 90 min in a 1 L beaker. The slurry was centrifuged at 3,400 r.p.m. for 20 min at 40 ^{o}C after which the supernatant was adjusted to pH 8.5 using sodium bicarbonate. Following centrifugation at 3,700 r.p.m. for 20 min at 40 ^{o}C, the residue was discarded and the pH of the supernatant adjusted to 4 with 2 M citric acid to precipitate the protein. This was followed by centrifugation at 4,100 r.p.m. for 25 min. The supernatant was then mixed with equal volume of 80 % ethanol and held for 20 min. The mixture was centrifuged at 1,900 r.p.m. for 25 min at 40 ^{o}C and the residue was dried in an oven as previously stated. Gum was weighed and expressed as percentage recovery (Ahmad *et al*., 2009).

3.6.4 Alcohol-based enzyme extraction

The alcohol-based enzyme extraction was performed on three genotypes having the highest gum yield from the acid isolation method. The genotypes were 'Abeleehi', 'Obatanpa GH' and 'GH9'. In the alcohol-enzyme based extraction technique, the non β-glucan components represented by starches, dextrins and protein are removed with alcohol and β-glucan is retained in the fiber that is left after filtration. To 50 g of maize flour was added 300 ml of ethanol (50 %) to form a slurry and refluxed for six hours. The slurry was filtered through a cheese cloth to separate the fibre concentrate and the filtrate was discarded. This process was repeated to allow for maximum removal of the starches and proteins from the fibre concentrate on the screen. The residue was then slurried

sequentially in 8 parts of aqueous ethanol (50 % v/v) containing Bromelain (E.C. 3.4.22.32, Sigma-Aldrich, St. Louis, MO, U.S.A.) (1 % protein dry weight basis) and α-amylase (E.C. 3.2.1.1, Sigma-Aldrich, St Loius, MO, U.S.A.) (1 %, starch dry weight basis). After each enzyme addition, the mixture was incubated at 35 ^{o}C for 20 h to hydrolyze proteins and starches, respectively, filtered and washed with ethanol (96.1 %) to recover β-glucan concentrate. The wet concentrate was oven-dried at 50-55^{o}C for 24-48 h to obtain a gum. Gum was weighed and expressed as percentage recovery (Vasanthan and Temelli, 2009). The gum was analyzed for its proximate composition as outlined previously.

3.7 Starch

Total starch content in the maize flour and gum were determined by the A.O.A.C. No. 996.11 method which is based on Megazyme kit (Megazyme International, Wicklow Ireland.). In this method, 100 mg of gum was transferred to a glass test tube (16 × 120 mm). The tube was tapped to ensure that the entire sample settled at the bottom of the tube. About 0.2 ml of aqueous ethanol (80 % v/v) was added to the tube to aid gum dispersion. About 3 ml of thermostable α-amylase (1:30 in Reagent 4; 50 mM 3-(N-morpholino) propanesulfonic acid (MOPS buffer), pH 7.0) was added and stirred on a vortex mixer for 2 min. The tube was incubated in boiling water bath for 6 min with intermittent stirring. The tube was transferred to a water bath at 50 °C and 4 ml sodium acetate buffer was added (200 mM, pH 4.5), followed by 2 drops of amyloglucosidase (20 U). The tube was stirred on a vortex mixer for 1 min and incubated at 50 °C for 30 min. The entire content of the test tube was transferred to a volumetric flask and made up to the 100 ml mark with

distilled water. Ten milliliter of the solution was centrifuged at 3,000 r.p.m. for 10 min and the supernatant was used for the assay. Duplicate aliquots (0.1 ml) were transferred to the bottom of glass test tubes (16 × 100 mm) followed by addition of 3.0 ml of glucose oxidase/peroxidase (GOPOD) reagent to each test tube (including the D-glucose controls and reagent blanks) and incubated for 20 min at 50°C. Standard solutions of D-Glucose controls consisting of 0.1 ml of D-glucose standard solution (1 mg/ml) and 3.0 ml of GOPOD reagent were prepared, while reagent blank solutions consisted of 0.1 ml of distilled water and 3.0 ml of GOPOD reagent. The absorbance was read for each sample and the D-glucose control at 510 nm against the reagent blank (A.O.A.C., 2005).

3.8 Purification of β-glucan

Purified β-glucan was prepared from gum samples using a non-alkaline and non-enzymatic procedure of Ghotra (2006). Twenty grams of gum isolated from alcohol-based enzyme extraction method was solubilized in 300 ml millipore water at 82 ^{o}C for 2 min with constant stirring. The mixture was centrifuged at 4,100 r.p.m. for 3 min and the supernatant collected. Beta-glucan was precipitated from the supernatant by addition of equal parts of concentrated ethanol (96.1 %). The precipitate was dried in the oven at 50 ^{o}C to constant mass. Percentage recovery of purified β-glucan was calculated (Appendix 6).

3.9 Beta-glucan assay

Beta-glucan assay was performed using the Beta-Glucan (Mixed Linkage) Assay Kit K-BGLU of Megazyme (Megazyme International, Ireland) based on the McCleary method. About 0.5 g of gum from alcohol-based enzyme extraction was transferred into three

polypropylene 50 ml centrifuge tubes and 1.0 ml of aqueous ethanol (50 % v/v) was added to each tube. This was followed by the addition of 5 ml of sodium phosphate buffer (20 mM, pH 6.5) to the tubes and vortexed to obtain a homogeneous mixture. The tubes were incubated in boiling water for 2 min, re-stirred and heated for 3 min. The tubes were cooled to 40 ^{o}C and 0.2 ml of lichenase [specific, endo-(1-3)(1-4)-β-D-glucan 4-glucanohydrolase] added to the tubes and incubated at 40 ^{o}C for 1 h. The volume of the mixture was adjusted to 30.0 ml with distilled water and stirred for 3 min. The solution was centrifuged at 1,000 r.p.m. for 8 min and aliquots (0.1 ml) from the filtrate carefully transferred to the bottom of three test tubes. An aliquot of sodium acetate buffer (50 mM, pH 4.0) was added to one of the three test tubes (reaction blank) while 0.1 ml of β-glucosidase (0.2 U) in 50 mM acetate buffer pH 4.0 was added to the other two test tubes and incubated at 40 ^{o}C for 15 min. Three milliliter GOPOD reagent was added to each tube and further incubated at 40^{o} C for 20 min. Absorbance was measured at 510 nm for each reaction using a spectrophotometer (UVC 121108, England) (McCleary and Codd, 1991).

3.10 Colour measurement

The colour of gums were measured using the L*, a* and b* colour space (CIELAB space) with Colorimeter CR-Minolta 200, Osaka, Japan). The L* value indicates lightness, where L* = 0 is completely black and L* = 100 is completely bright. The a* value represents red-green with positive a* and negative a* depicting red and green, respectively. The b* values on the other hand represent yellow-blue, with positive b* representing yellow and negative b* representing blue. The meter was calibrated with white tile (L* = 93.30, a* = 0.32 and b* 0.33). Gums were placed in a transparent petri dish and the measuring head of the meter

was carefully placed on three different locations on the petri dish. The measurements were determined in triplicates and mean and standard deviations determined.

3.11 Structural analysis of β-glucan

3.11.1 Quantification of (1→3)-β-D-Glucan by the aniline blue method

To 2 g of gum in a test tube was added 25 ml of distilled water to solubilize β-glucan by incubation of the mixture in a water bath at 80 ^{o}C for 30 min. The supernatant was decanted into a separate test tube and 2.1 ml aniline blue mix consisting of 0.03 % aniline blue (Wako, Osaka, Japan), 0.18 N HCl and 0.49 M glycine/NaOH, pH 9.5 was added. The resulting mixture was incubated at 50 ^{o}C for 30 min and incubation was repeated at 29 ^{o}C to allow reaction with the fluorochrome and subsequent decolorization. Fluorescence was quantified using a spectrofluorometer (RF-5300PC, Shimadzu, Kyoto). The excitation wavelength was 400 nm/slit having width of 5 nm, and the emission wavelength 460 nm/slit of width 5 nm. The fluorescence intensity was measured in triplicate and reported as mean percentage (Wantanabe *et al.*, 2001).

3.11.2 ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy

A one-dimensional liquid ^{13}C NMR spectroscopy was performed on maize β-glucan with a Bruker AV 500 NMR Spectrometer (Germany) at 125.76 MHz using a 5 mm BBO probe. Maize β-glucan was dispersed (2 % w/v) in pure deuterated methylsulphoxide (d6-DMSO) by heating and continuous stirring at 90 ^{o}C for 3 h. The proton-decoupled spectra were recorded at 70 ^{o}C overnight by applying 12,800 pulses with a delay time of 2 s and a radio

frequency tip angle of 30^{o}. Chemical shifts were expressed in parts per million (ppm) relative to d6-DMSO at 39.5 ppm and reported relative to tetramethylsilane (TMS).

3.12 Rheological behaviour of maize β-glucan

Maize genotypes which produced highest gum yield, as well as minimum impurities, following the alcohol-enzyme isolation technique (Vasanthan and Temelli, 2008; Sampson *et al*., 2013) were evaluated for their rheological behaviour. The maize genotypes were 'Abeleehi' a normal maize, and, 'Obatanpa GH' and 'GH9', both of which are QPMs. Oat gum was isolated from rolled oats, purchased from the local market was used as control.

3.12.1 Rheological characterization of maize β-glucan

Five percent (w/v) and 10 % (w/v) dispersions of maize gum were used for rheological measurements. These were prepared by gently stirring β-glucan samples in deionized water at 90 ^{o}C until complete solubilization, followed by cooling to room temperature. Apparent viscosity (Pa s) was determined at six intervals of fixed shear rates ranging from 0.0 to 12,800 s^{-1} by use of a Brookfield digital viscometer (DV-I+, U.S.A). All viscosity measurements were taken at 27 ^{o}C using a 600 ml beaker. Shear rates were quoted in s^{-1} following multiplication of the speed in revolution per minute by a conversion factor assigned to each LV spindle (LV1=6.4, LV2=32, LV3=128) by the manufacturer (Koocheki *et al*., 2009). Shear stress (Pa) was calculated by multiplying apparent viscosity and shear rate.

3.12.2 Rheological models

The rheological models used to describe the consistency and flow behaviour of the gums were designated:

Power-law model $\sigma = K\dot{\gamma}^n$ (1)

and

Hershey-Bulkley model $\sigma = \sigma_o + K\dot{\gamma}^n$ (2)

Where σ is the shear stress (Pa), σ_o is the yield stress (Pa), $\dot{\gamma}$ is the shear strain rate (s^{-1}), K is the consistency coefficient (Pa s^n), and n is the flow behavior index (dimensionless). Two normal plots were generated from the data, viz., apparent viscosity versus shear rate and shear rate versus shear stress. From the plot of shear stress versus shear rate it was identified that yield stress was zero, hence the power law was applied to measure the rheological parameters. A logarithmic conversion of the model parameters was computed. Logarithmic plots of apparent viscosity (ŋ) versus shear rate were used to determine the flow behavior and consistency coefficient of maize β-glucan (Koocheki *et al*., 2009).

3.12.3 Water Binding Capacity (WBC) of maize β-glucan

To 2 g of gum, 20 ml distilled water was added and vigorously mixed on a vortex mixer for 15 min. The tubes were centrifuged at 3,000 r.p.m. for 30 min. The supernatant was discarded, and the amount of water held in the hydrated sample was determined by heating the pre-weighed pellet in a hot air oven at 120 °C for 2 h. The WBC of each sample was expressed as the mass of water held by 1.0 g of β-glucan sample (Wong and Cheung, 2005).

3.13 Incorporation of β-glucan into pastry preparations

The ingredients and quantities used in each pastry variation are shown in Table 3.1. A pie crust formulation derived from Dwyer and Gallagher (2001) was used as formula for the control. Levels chosen for fat replacement were 0 % (control), 15 % and 20 % to conform to standard methods using carbohydrate-based products as fat replacers in baked goods. Carbohydrate-based replacers derived from of maize β-glucan, hereinafter referred to as *MaiLean* were used. Three types of *MaiLean* originating from the three maize genotypes in the current study were evaluated for their performance as fat replacers in pie crust. These were designated *MaiLean OB* (from maize genotype 'Obatanpa GH'), *Mailean AB* (from maize genotype 'Abeleehi') and *MaiLean GH* (from maize genotype 'GH9'). Equal levels of the individual carbohydrate-based fat replacer were used throughout for each pastry variation.

3.13.1 Mixing and baking of pie crust

The dry ingredients were weighed using a top loading kitchen scale with precision of 0.1 g, and mixed in a plastic mixing bowl. The solid shortening was added to the dry ingredients and mixed between the fingers until no lumps of shortening were observed. The water was added to the mixture and turned rapidly with a wide blade metal kitchen spatula until a smooth and uniform ball of pastry was obtained. The ball of pastry was rolled out to a thickness of 3 mm by use of a wooden rolling pin, after which discs of 60 mm × 60 mm were cut out with a biscuit cutter to make 25 pieces. The discs were arranged on a metal baking sheet and placed on the middle rack in a preheated 215 ^{o}C in a Chandley Deck oven (JKP14W0T1WW, Singapore). After baking for 12 min, pie crusts were removed from the

oven and allowed to cool to room temperature. Pie crusts were evaluated for moisture and crude fat as previously indicated in sections 3.5.1.1 and 3.5.1.3 respectively within 24 h after baking.

Table 3.1 Formulations for full-fat and reduced-fat pastry

Ingredient	**Control**	**15 %**	**20 %**
Flour[1] (g)	200	200	200
Shortening[2] (g)	100	85	80
Fat replacer[3] (g)	0	15	20
Salt[4] (g)	0.5	0.5	0.5
Water (ml)	10	10	10

[1]All Purpose wheat flour (Takoradi Mills, Ghana);[2]Cook Brand Margarine, Roml SmilFood bv, Holland; [3]Three carbohydrate-based fat replacers isolated from maize were used: *MaiLean OB*, *MaiLean AB*, and *MaiLean GH* (Sampson *et al*. 2013); [4]Iodated Salt

3.13.2 Sensory evaluation

Sensory evaluation was conducted by eighteen panelists recruited from the Departments of Food Science and Technology and Department of Biochemistry and Biotechnology of Kwame Nkrumah University of Science and Technology, Kumasi. Panelists were introduced to the control pie crust and individual seven-point hedonic rating scale, a score of 1 represented dislike extremely and 7 represented like extremely, for five attributes, namely, colour, taste, crispiness, brittleness and overall acceptability (Appendix 6). Crispiness was defined as force required to break crust when placed between the incisors. Brittleness was the force required to break crust when picked with the fingertips. When panelists became familiar with the control, ballot sheet for each pie crust variation were administered. A 3×3 factorial experiment laid out in a randomized complete block design with three replications was used for this study. There were three types of carbohydrate-

based fat replacers and three levels of fat replacements. Panelist received samples in a random order with rinsing of the mouth between samples with crackers and water to remove residual fat. The sensory data was subjected to one way analysis of variance. Location of differences in mean was determined by the least significant difference test (LSD) at the 0.05 level of probability.

3.14 Statistical analyses

A descriptive analyses encompassing means, median, standard errors and coefficient of variation were computed. Analysis of variance (ANOVA) was computed and differences in means were located using the Least Significant Difference (LSD) test. A paired comparison t-test was performed on the means of yields of β-glucan isolation methods at a confidence interval of 95 %. This was done to evaluate the efficiency of the acid, alkaline and alcohol-enzyme based isolation methods. Correlation coefficients among β-glucan content, 100-kernel weight, moisture, fat, protein, ash and nitrogen free extract were computed. SPSS version 17 and SAS 9.1.3 (SAS Institute, Cary, NC) were used for all computations.

Chapter Four

Results and Discussion

4.1 Morphological characterization of maize

In the current study, seventeen maize genotypes bred and released in Ghana from 1961 to 2007 were evaluated for kernel characteristics. This set comprised 3 hybrid varieties, 11 open pollinated varieties, and 3 landraces. Maize kernels were classified on the basis of their colour and texture. Table 4.1 shows the characterization of the maize genotypes. Twenty-nine percent of the kernels were yellow and 71 % were white. The predominant kernel texture was dent. About 35 % of the maize genotypes screened were QPM while 65 % were common maize. Approximately 18% of the maize screened was hybrid genotypes, 18% were landraces and 64 % were OPVs.

4.2. 100-kernel weight

One-hundred kernel weight is a measure of the compactness of kernel with respect to starch, protein and other macro components of the seed (Osborne *et al*., 1997). In simple terms, it is an estimate of the density of the kernels, and determines the economic value of maize variety. The 100-kernel weight varies between maize varieties, from year to year and from field to field of the same variety. A wide variation in 100-hundred kernel weight was observed among the maize varieties, covering a range of 17.33 g for 'Dodzi' to 34.04 g for 'Mamaba' (Table 4.1).

4.3 Proximate composition

A wide variation in moisture content of kernels was observed. 'Mamaba' recorded the lowest moisture (3.97 %) while Aburohemaa had the highest moisture content (8.00 %). Fat content of maize varieties varied between a low value of 2.10% for 'Dodzi' and the highest value of 5.82 % for 'Obatanpa GH'.

Table 4.1 Characterization and proximate composition of maize genotypes. All values are expressed on dry matter basis.

No.	Name	Variety	Kernel colour	100-Kernel weight (g)	Moisture %	Fat %	Protein %	Ash %	Fibre %	NFE[2] %
1	'*Mamaba*'	Hybrid Common	White	34.04^{c}± 0.05	3.97^{a} ±0.82	4.46^{b} ±0.87	12.59^{a} ±0.70	0.76^{a} ±0.20	2.18^{b} ±0.47	76.04^{b}± 0.57
2	'Suwan1 QPM'	Hybrid QPM	Yellow	27.94^{ba} ±0.24	5.62^{b} ±0.44	3.81^{a} ±0.59	12.09^{a} ±0.14	1.36^{a} ±0.12	3.98^{c} ±0.89	73.14^{b}± 0.75
3	'Obatanpa GH'	OPV[1] QPM	White	18.13a ± 0.01	5.72^{b} ±0.23	5.82^{b}± 0.67	17.28^{c} ±0.67	1.36^{a} ±0.10	2.11^{b} ±0.11	67.71^{a} ± 0.96
4	'Omankwa'	OPV Common	White	26.81^{b} ± 0.01	5.69^{b} ±0.57	2.83^{a}± 0.48	9.41^{a} ±0.76	0.99^{a,} ±0.22	2.24^{b} ±0.33	70.73^{a} ±1.45
5	'GH9'	Hybrid QPM	Yellow	24.36^{a} ± 0.01	6.38^{b} ±0.56	5.67^{b} ±0.05	12.90^{a} ±0.21	1.03^{a,} ±0.30	1.83^{a} ±0.08	72.19^{a} ±0.42
6	'Catete'	Landrace Common	White	20.02^{a} ± 1.80	6.62^{b} ±0.03	5.51^{b} ±0.46	13.69^{b} ±0.47	2.25^{b} ±0.68	1.39^{a} ± 0.12	70.54^{a} ±1.35
7	'Dodzi'	OPV Common	White	17.33^{a} ± 0.01	6.40^{b} ±0.43	2.10^{a} ±0.27	13.30b ±0.75	1.34^{a} ±0.24	1.92^{b} ±0.33	74.96^{b} ±0.35
8	'Okomasa'	OPV Common	White	25.84^{b} ± 0.01	5.85^{b,c} ±0.06	3.97^{a} ±0.23	10.48^{a} ±0.03	0.54^{a} ±0.25	0.57^{a} ±0.14	78.59^{b} ±0.75
9	'ABH'	OPV Common	White	27.72^{b} ± 0.02	8.00^{c} ±0.33	5.70^{b} ±0.50	9.77^{a} ±0.12	1.22^{a} ±0.06	1.64^{a} ±0.11	73.67^{b} ±1.81
10	'Abontem'	OPV QPM	Yellow	21.38^{a} ± 0.01	6.78^{b} ±0.01	5.04^{b} ±0.78	8.98^{a} ±0.75	0.99^{a,} ±0.08	1.67^{a} ±0.22	76.54^{b} ±0.43
11	'Sotubaka'	Landrace Common	Yellow	20.32^{a} ± 0.03	6.63^{b} ±0.17	5.59^{b} ±0.21	11.62^{a} ±0.67	1.16^{a,} ±0.07	1.47^{a} ±0.50	73.53^{b} ±0.43
12	'Dorke'	OPV Common	White	28.52 ^{b} ± 0.10	5.04^{a} ±0.51	4.75^{b} ±0.12	13.07^{b} ±0.11	0.71^{a} ±0.32	1.87^{b} ±0.06	74.56^{b} ±0.77
13	'Akposoe'	OPV QPM	White	24.38^{a} ± 0.02	5.50^{a} ±0.14	4.86^{b} ±0.12	13.87b ±1.21	0.99$^{a,\}$ ±0.18	1.59^{a} ±0.35	73.19 ^{b} ±0.94
14	'Abeleehi'	OPV Common	White	24.97^{a} ± 0.05	4.96^{a} ±0.10	5.23^{b} ±0.16	10.37^{a} ±0.53	1.47^{a} ±0.55	0.33^{a} ±0.27	77.64^{b} ±1.14
15	'Safita 2'	OPV Common	White	19.23^{a} ± 0.01	5.38^{a} ±0.13	3.30^{a} ±0.27	13.67^{b} ±1.80	1.04^{a} ±0.55	0.78^{a} ±0.09	75.83^{b} ±0.86
16	'Ohawu local'	Landrace Common	White	22.33^{a} ± 0.20	7.68^{c} ±0.07	4.38^{b} ±0.35	12.31^{a} ±0.34	1.00^{a} ±0.15	1.71^{a} ±0.03	73.92^{b} ±0.72
17	'Golden Jubilee'	OPV QPM	Yellow	28.46^{b,} ± 0.12	6.65^{b} ±0.07	4.91^{b} ±1.54	9.12^{a} ±0.01	0.79 ±0.15	0.99^{a} ±0.33	77.54^{b} ±0.11
			Mean	**24.30**	**6.05**	**4.57**	**12.01**	**1.11**	**1.62**	**74.71**

Numbers in columns followed by different superscripts are significantly different at P<0.05. [1]Open pollinated variety. [2]Nitrogen Free Extract; [3]Quality Protein Maize; Kernel weight $LSD_{.05}$ = 7.8; Moisture $LSD_{.05}$ = 1.57; Crude fat $LSD_{.05}$=2.08; Crude protein $LSD_{.05}$=3.95; Ash $LSD_{.05}$=0.77; Fibre $LSD_{.05}$= 1.50; NFE $LSD_{.05}$= 5.37. ABH = 'Aburohemaa'.

The fat content for 'Obatanpa GH' was higher than that of sorghum (3.9%), wheat (1.4 %) and barley (3.4 %) but comparable with oat (5.9 %) (FAO, 1999). Maize with fat content exceeding 6 % is regarded as high-oil maize. Currently, maize breeding programs around the world have the objective of enhancing fat content in maize to levels above 4 %, for use as livestock and poultry perform better on such genotypes with less feed.

The crude protein content of maize varied between 8.00% for 'Abontem' and 17.10 % for 'Obatanpa GH' (Table 4.1). The maize genotypes used in this study had higher protein content than sorghum (8.3 %) but comparable to that of barley (11.0%), oat (9.3%) and wheat (10.7 %) (FAO, 1999). Total ash content estimates the total mineral composition of food. The ash content of maize varied between 0.55% for 'Okomasa' and 2.55 % for 'Catete', with a mean of 1.55%. The predominant minerals in maize are designated: Iron (Fe), Zinc, Copper (Cu) and Cobalt (Co) according to Sokrab *et al*. (2011).The available carbohydrate or Nitrogen Free Extract (NFE) ranged between 67.71 and 78.59 %. In this research NFE was representing starch while the fibre represents non starch polysaccharides such as beta-glucan, celluloses, arabinoxylans, pectin, hemicelluloses and lignin (Caprita *et al.*, 2010).

The genotypes were grouped into two categories, the first by variety: as landraces, OPVs, and hybrids, and the second category by kernel type: as QPM and common maize. Moisture content of the landraces was significantly higher than that of hybrid at 95 % confidence interval (Table 4.2). Mean moisture content of OPVs and landraces were not

different. This observation was not unexpected as enhancement of landraces by genetic improvement into genotypes is aimed at improving the dry matter content.

With respect to varietal differences there was no significant difference ($P>0.05$) between the crude fat content of OPV and hybrid (Table 4.2). The experimental fat contents are corroborated by the FAO (1999). Mean protein content for hybrid maize genotypes (12.53 %) was comparable to the landraces (12.41 %) but was significantly higher ($P<0.05$) than that of OPVs (11.62 %). Similarly, the protein content for QPMs was higher than common maize and this could be due to the increased lysine and tryptophan content through gene biotechnology (Table 4.2). In a previous study, protein content of QPM was reported to be 9.8% which was not significantly different from 9.1 % of common maize (Miko *et al*., 2001). Our observation in this study was in contrast to previous report of QPM protein content being equivalent to that of common maize.

Table 4.2 shows the ash content of the various groups. There was no significant difference ($P>0.05$) in the ash content among the landraces, OPVs and hybrids used in current study. The mean ash content of about 1.0 % was lower than that of sorghum (2.6 %) and oat (2.3 %) (FAO, 1999).

Table 4.2: Variation in proximate composition of individual maize genotypes

Parameter						
	% Moisture	**% Fat**	**% Protein**	**% Ash**	**% Fibre**	**% NFE**
Min.	3.97	2.83	8.98	0.54	0.57	70.91
Max.	8.00	5.82	17.10	2.25	3.98	78.84
Comparison among Maize category						
Breeding type						
Landrace	6.98^{a}±0.61	5.28^{a}±0.81	12.41^{a}±0.84	1.17^{a}±0.17	1.71^{a}±0.23	74.14^{a}±0.74
OPV	6.00^{a}±0.90	4.36^{b}±1.26	11.62^{b}±1.97	1.07^{a}±1.07	1.47^{b}±0.67	74.34^{a}±3.25
Hybrid	5.23^{b}±1.23	4.64^{b}±0.94	12.53^{a}±0.41	1.05^{a}±0.30	2.66^{c}±1.15	73.79^{a}±2.00
Maize type						
Normal maize	6.02^{a}±1.20	4.35^{a}±1.19	11.82^{a}±1.63	1.13^{a}±0.46	1.46^{a}±0.64	74.46^{a}±2.52
QPM	6.10^{a}±0.56	5.02^{b}±0.72	12.39^{a}±3.10	1.09^{b}±0.23	2.03^{b}±1.03	73.39^{a}±3.49

Numbers in columns followed by different superscripts are significantly different at P<0.05.

4.4 Gum yield and β-glucan recovery

Gum is the product recovered after solubilization and separation of non-β-glucan components from the grain. It can be referred to as crude β-glucan as it is expected to contain some fat, protein, starch and ash. Table 4.3 shows the descriptive statistics of gum yield of maize using non-enzymic isolation procedures.

Table 4.3: Descriptive statistics of the data on the three extraction methods of β-glucan

Statistic	**Hot water**	**Alkaline**	**Acid**
Number of varieties screened	10	17	17
Mean yield (%)	0.66	6.59	26.28
Median yield (%)	0.18	5.82	21.33
Variance	0.77	28.80	165.17
Standard Deviation	0.88	5.37	12.85
[1]SEM	0.28	1.30	2.91
[2]CV (%)	132.3	81.6	48.9
Min.	0	0	7.34
Max.	2.59	22.12	56.25

[1]Standard Error of the Mean [2]Coefficient of Variation

Among the three methods of extraction, hot water method recorded lowest β-glucan yield followed by alkaline method, while the acid extraction method produced the highest gum yield (Table 4.3). For hot water extraction, approximately 50 % of the β-glucan yield was in the interval 0 to 0.18% and 50% of the β-glucan yield was located in the interval 0.18 to 2.59 %. A coefficient of variation of 132 % obtained for hot water extraction method shows a very low precision demonstrating that this method for isolation of β-glucan from maize may not be effective. For alkaline extraction method, a mean gum yield of 6.59% was recorded. Fifty percent of the data were in the range of 0 and 5.82 % and 50% was located in the interval 5.83 and 22.12 % gum yield. The high values of coefficient of variation could be attributed to differences in genetic composition of the accessions. On the basis of coefficient of variation and gum yield, the acid extraction method gave the highest precision (48.9 %) and highest mean yield (26.28 %), regardless of the variation in the genotypes (Table 4.3). Table 4.4 shows the yield of gum from different maize genotypes using non-enzymic methods. Gum yield from the acid extraction method ranged between 8.3 for 'Dorke' and 51.72% for 'Abeleehi'. Among the non-enzymic methods for β-glucan isolation from maize, acid extraction is the method of choice. The low efficiency of β-glucan extraction by hot water was also reported by Symons and Brennan (2004). Conversely, Ahmad *et al*. (2009) reported highest gum yield from barley by hot water extraction (5.4 %), followed by enzyme extraction (5.2 %), then acid process (4.65%), with alkaline extraction recording the lowest gum yield of 3.94 %.

With the acid extraction method, genotypes having gum yields exceeding 41 % were selected for further studies. These were 'Obatanpa GH', 'Abeleehi' and 'GH9' (Table 4.5).

A paired comparison test on mean gum yield from acid and alkaline extraction procedures demonstrated significant difference at the 95 % confidence interval (Table 4.3). The three varieties were evaluated for their β-glucan content using the enzyme-based alcohol extraction method, which is reported to be an improved isolation method with regard to yield and removal of impurities (Vasanthan and Temelli, 2009). Table 4.5 shows descriptive statistics of gum yield from the three genotypes using the enzymic method of isolation. These statistics were compared to the acid extraction method.

Table 4.4 Mean yield of gum from maize genotypes by non-enzymic isolation methods

No.	Variety	Yield of gum (%)		
		Hot water	**Alkaline**	**Acid**
1	'*Mamaba*'	-	7.34^{a} ±0.34	15.63^{a} ±0.76
2	'Suwan 1 QPM'	-	6.33^{a}±0.34	35.07^{b} ±3.11
3	'Obatanpa GH'	2.33^{e} ±0.33	7.14^{a}±0.58	41.42^{c} ±3.04
4	'Omankwa'	0.55^{b} ±0.03	4.43^{c} ±0.42	14.35^{a} ±2.76
5	'GH9'	-	18.35^{d}±0.58	44.79^{c}±9.30
6	'Catete'	0.40^{b} ±0.01	4.28^{c} ±0.28	18.56^{a}±2.63
7	'Okomasa'	0.003^{d} ±0.03	0.00^{d}	18.91^{a} ±2.92
8	'ABH'	0.002^{d} ±0.0	5.67^{bc} ±0.47	16.44^{a}±1.39
9	'Abontem'	0.18^{c} ±0.01	20.67^{e} ±1.26	19.13^{a} ±1.54
10	'Sotubaka'	0.10^{c} ±0.01	8.20^{f} ±0.38	25.80^{b} ±0.47
11	'Akposoe'	2.08^{a} ±0.13	0.02^{d} ±0.00	39.29^{bc}±4.14
12	'Abeleehi'	0.004^{d} ±0.02	5.30^{c} ±0.42	51.72^{d} ±4.11
13	'Safita 2'	1.23^{a} ±0.03	7.52^{f} ±0.43	26.22^{b} ±2.02
14	'Ohawu local'	-	5.13^{bc} ±0.69	13.47^{a} ±1.58
15	'Golden Jubilee'	-	7.88^{af}±1.27	40.28^{c}±9.70
16	'Dorke'	-	3.79^{c} ±1.17	8.33^{d} ± 1.13
17	'Dodzi'	-	0.00^{d}	14.78^{a}± 1.44

Numbers in columns followed by different superscripts are significantly different ($P<0.05$), followed by their respective standard deviation. 'ABH'= 'Aburohemaa'. Hot water isolation $LSD_{.05}$ =1.59; Alkaline isolation $LSD_{.05}$=9.46; Acid isolation $LSD_{.05}$=6.07

Table 4.5 Descriptive statistics of gum yield extracted from the three genotypes using enzymatic and non-enzymic method

Cultivar	**Enzymic gum yield (%)**	**Non-enzymic gum yield (%)**
'Abeleehi'	33.20±1.24	51.72 ±4.11
'Obatanpa GH'	41.89±3.18	41.42 ±3.04
'GH9'	38.18±2.00	44.79±9.3
LSD	14.93	6.07
Statistic		
N	9	9
Mean	39.97	45.97
Median	40.31	44.74
Variance	43.03	48.79
Standard deviation	6.56	6.98
[1]SEM	2.19	2.33
[2]CV (%)	16.42%	15.20
Min	28.91	37.07
Max	50.00	56.25

[1]Standard error of mean [2]Coefficient of variation

Gum recovery was expressed as the percentage ratio of gum in flour. Using the enzymic method, the recovery of gum ranged between 33.2 % for ''Abeleehi' to 41.9% for 'Obatanpa GH' (Table 4.5) which corresponds to recovery rate of 42.8 to 61.9 %, respectively. Statistically there was no significant difference (P>0.05) among the maize genotypes with respect to alcohol-based enzyme extraction method. Mean gum yield from enzymic extraction was 39.97 %. A paired comparison test at a confidence level of 95 % showed no significant difference (P>0.05) between the alcohol-based enzyme and acid extraction methods. On the basis of the standard deviation and coefficient of variation recorded in Table 4.5, the enzymic extraction method shows high precision and accuracy for the determination of gum from maize. Generally, gum yield from maize (36.39 to 43.92

%) was lower compared to those of barley (81.4 %) and oat (86.8 %) (Ahmad *et al*., 2009, 2010). Vasanthan and Temelli (2008) indicated that efficiency of β-glucan extraction was enhanced with reducing particle size. Lazaridou *et al*. (2003) reported that, thicker cell walls show greater resistance to extraction of high molecular weight polymers.

Maize genotypes were grouped into hybrids, OPVs and landraces. Gum yield differed ($P<0.05$) in the various groups (Table 4.6). Hybrid varieties exhibited the highest gum yield regardless of the isolation method, while landraces gave lowest gum yield. The higher concentration of gum in the hybrids may probably have been an indirect benefit arising from linkage of the trait of interest for which the hybrids were developed to β-glucan.

Table 4.6: Variation in gum yield as a function of cultivar type (pooled data)

	% Gum yield		
Cultivar type	**Number screened**	**Alkaline**	**Acid**
Hybrid	3	10.41^{b}±3.46	31.83^{b}±13.72
OPV	11	5.71^{a}±2.01	26.72^{c}±13.42
Landrace	3	5.09^{a}±1.77	19.11^{a}±5.38

Numbers in columns followed by different superscripts are significantly different ($P<0.05$), followed by their respective standard deviation.

Another factor which may have contributed to the high gum yield may also be the result of heterotic effect from combining inbred lines into hybrids. The lowest gum yield in landraces, being unimproved genotypes confirms the phenomenon of heterosis.

4.5 Correlation for β-glucan concentration with proximate composition

A correlation analysis was performed on quality of β-glucan with 100-kernel weight, moisture, fat, protein, ash, fibre and nitrogen free extract. Table 4.7 shows the correlation coefficients and probability values. There was a negative but significant correlation ($P<0.05$) between nitrogen free extract with protein (Eulis *et al*., 1997), ash, and fibre. Also there was a negative correlation (Table 4.7) between 100-kernel weight and moisture content and this observation is corroborated by Reddy and Daynard (1983) who demonstrated that maize kernel weight is genetically determined unlike in soybean where a direct relationship between 100-kernel weight and moisture content existed (Deshpande *et al*., 1993). A significant and positive correlation was observed for β-glucan with protein, ash and fibre (Table 4.7) signifying that as protein and fibre content increase there is a corresponding increase in the β-glucan content of maize. An *r*-square value of 0.40 and 0.24 indicate that 40 % and 24 % of the variation in the β-glucan concentration in maize is explained by variation in protein and crude fibre content, respectively. Two of the genotypes which showed high beta-glucan content were QPMs. This suggests that more work is needed to confirm the relationship between QPM and β-glucan content in maize. In contrast, the correlation between fibre and nitrogen free extract was negative but significant demonstrating that, as fiber increases non soluble polysaccharides, particularly starch decreases. Similar work on starch and β-glucan concentration in maize showed a significant ($P= 0.04$) but negative correlation ($r = -0.77$) between starch and β-glucan (Tetteh *et al*., 2013a, b).

Table 4.7. Correlation matrix of β-glucan content with kernel weight, moisture, fat, protein, ash, fibre and nitrogen free extract.

	[1]KW	Moisture	Fat	Protein	Ash	Fibre	[2]NFE
[1]KW							
Moisture	-0.35 (0.16)						
Fat	0.07 (0.80)	0.21 (0.43)					
Protein	-0.43 (0.09)	-0.29 (0.26)	0.09 (0.70)				
Ash	-0.45 (0.07)	0.17 (0.50)	0.22 (0.40)	0.30 (0.24)			
Fibre	0.21 (0.43)	-0.09 (0.74)	-0.17 (0.50)	0.21 (0.42)	0.11 (0.67)		
[2]NFE	0.33 (0.20)	-0.11 (0.66)	-0.21 (0.40)	-0.58 (0.01)	-0.52 (0.04)	-0.48 (0.04)	
[3]BGLU	-0.32 (0.22)	0.03 (0.91)	0.20 (0.42)	0.63 (0.006)	0.45 (0.06)	0.49 (0.04)	-0.98 (<0.01)

[1]100-kernel weight; [2]Nitrogen Free Extract; [3]β- glucan. Values in parenthesis are probability levels. (P<0.05)

4.6 Chemical composition of acid extracted isolates

For the purpose of determining the efficiency of the acid extraction method, gum isolates were analyzed for impurities, such as fat, protein, and ash. Table 4.8 shows the chemical constituents of gum. One hundred percent minus impurities was considered as carbohydrate content of gum which is expected to contain β-glucan plus other non-soluble polysaccharides. The chemical composition of the isolates is an index of the efficiency of the extraction method. Residual mean concentrations of the impurities ranged from moisture, 2.53 to 4.99 %, fat, 0.5 to 2.79 %, ash, 0.17 to 1.15 % and protein, 0.41 to 1.73 %. Residual fat content across the samples was not significantly different (P>0.05) with the exception of 'Abontem' whose fat content (2.73 %) was highest and different from the samples. With regards to residual protein all samples recorded

Table 4.8 Chemical composition of gum from acid isolation procedure

No.	Variety	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Gum (%)
1	'*Mamaba*'	3.08^{a} ±0.04	1.38a±0.13	0.40^{c,e} ±0.02	1.06^{a} ±0.00	94.08
2	'Suwan 1 QPM'	3.39^{a} ±0.04	0.73^{a} ±0.03	0.25^{a} ±0.02	1.20^{b} ±0.00	94.43
3	'Obatanpa GH'	3.49^{a,c} ±0.03	1.13^{a} ±0.13	0.54^{c,e} ±0.02	0.64^{a} ±0.02	94.20
4	'Omankwa'	3.79^{a,c} ±0.23	0.65^{a} ±0.05	0.42^{c} ±0.02	1.27^{b}±0.00	93.87
5	'GH9'	3.94^{c}±0.57	0.70^{a} ±0.05	0.53^{b} ±0.38	0.74^{a} ±0.33	94.09
6	'Catete'	3.62^{a,c}±0.03	0.95^{a} ±0.05	0.36^{a} ±0.02	1.28^{b} ±0.00	93.79
7	'Okomasa'	4.22^{c} ±0.19	1.10^{a} ±0.10	0.18^{d} ±0.01	1.28^{b} ±0.01	93.22
8	'Aburohemaa'	3.73^{a,c} ±0.14	0.75^{a} ±0.05	0.48^{e} ±0.03	0.95^{a} ±0.12	94.09
9	'Abontem'	3.81^{a,c} ±0.09	2.73^{b} ±0.06	0.37^{a} ±0.00	1.18^{b} ±0.05	91.91
10	'Sotubaka'	4.99^{b}±0.08	0.70^{a} ±0.05	0.23^{a} ±0.02	1.20^{b} ±0.03	92.88
11	'Akposoe'	3.27^{a} ±0.03	0.88^{a} ±0.03	0.35^{a} ±0.02	0.63^{a} ±0.21	94.87
12	'Abeleehi'	2.53^{d}±0.02	1.08^{a} ±0.08	0.41^{c} ±0.02	1.28^{b} ±0.01	94.70
13	'Safita 2'	4.26^{c}±0.20	0.75^{a} ±0.05	0.34^{a} ±0.02	1.51^{b}±0.23	93.14
14	'Ohawu local'	4.67^{b}±0.09	0.88^{a} ±0.13	0.23^{a} ±0.02	1.28^{b} ±0.01	92.94
15	'Golden Jubilee'	4.60^{b} ±0.03	0.70^{a} ±0.08	0.31^{a} ±0.00	0.43^{a} ±0.03	93.96
16	Dorke	3.56^{a} ±0.01	0.86^{a} ±0.03	0.24^{a} ± 0.04	1.23^{b} ±0.11	94.11
17	Dodzi	3.88^{a}±0.03	0.95^{a} ±0.21	0.41^{c} ±0.01	1.33^{b} ±0.21	93.43
	Mean	**3.82**	**0.98**	**0.39**	**1.07**	**93.74**
	Median	**3.69**	**0.87**	**0.36**	**1.21**	**93.93**
	Variance	**0.40**	**0.24**	**0.04**	**0.10**	**0.60**
	SD	**0.63**	**0.49**	**0.19**	**0.31**	**0.77**
	[1]SEM	**0.15**	**0.50**	**0.49**	**0.08**	**0.01**
	[2]CV	**16**.54	**50.32**	**49.41**	**28.92**	**18.86**
	Min.	**2.51**	**0.50**	**0.17**	**0.41**	**91.80**
	Max.	**5.07**	**2.79**	**1.15**	**1.73**	**95.04**

Numbers in columns followed by different superscripts are significantly different (P<0.05), followed by their respective standard deviations.[1]Standard error of mean.[2]Coefficient of variation; Moisture $LSD_{.05}$ = 1.11; Crude fat $LSD_{.05}$= 1.06; Crude protein $LSD_{.05}$= 0.66; Ash $LSD_{.05}$=0.34.

substantially low and similar values (Table 4.8). The genotypes, 'Abeleehi', 'Obatanpa GH' and 'GH9' which had been selected for further work on the basis of their high gum concentrations (Tables 4.4 and 4.5) also recorded very low impurities in their gum isolates. Residual mean moisture, fat, ash and protein concentrations in the gum were compared to the original concentrations in the flour (Table 4.1). For all genotypes, impurities decreased. Moisture was reduced by 36 %, fat by 78.5 %, ash by 64 % and protein by 91 %. Similar observation was reported by Ahmad *et al*. (2009) on barley gum isolated with acid, where fat was reduced by 76.86 %, ash by 56.84 %, and protein by 51.69 %. For oat gum

isolation by the acid extraction method, fat was reduced by 80.91 %, ash by 44.04 %, and protein by 47.80 % (Ahmad *et al*., 2010). The high efficiency in removal of total nitrogen can be attributed to the iso-electric point of protein being favoured in the acidic range, hence thorough removal of proteinaceous matter from the flour during the extraction process. Residual fat is detrimental to the quality of gum isolate as it can undergo oxidation to produce compounds having rancid flavor in products containing β-glucan isolates (Molteberg *et al*., 1995). The impurities in the alcohol-based enzyme-extracted gums from 'Abeleehi', 'Obatanpa GH' and 'GH9' were determined (Table 4.9) and compared to their corresponding acid-extracted gums (Table 4.10).

Table 4.9. Impurities in alcohol-based enzyme extracted gum of three maize genotypes.

Gum source	**Moisture (%)**	**Fat (%)**	**Ash (%)**	**Protein (%)**	**Starch (%)**	**Gum (%)**
'Obatanpa GH'	4.58±1.75	2.16±0.22	0.46±0.44	1.00±0.09	0.17±0.04	92.69±1.70
'Abeleehi'	5.05±0.35	2.58±0.14	0.50±0.05	0.71±0.02	0.27±0.07	90.87±0.29
'GH 9'	4.05±0.06	1.30±0.17	0.42±0.02	0.88±0.03	0.18±0.02	93.23±0.16
Mean	**4.56**	**2.01**	**0.47**	**0.86**	**0.21**	**91.94**

Table 4.10. Efficiency of gum isolation method measured as residual proximate composition of gum.

Parameter		**Moisture/%**	**Ash/ %**	**Fat/ %**	**Protein/ %**	**NFE/ %**
Maize flour		5.69^{c}±0.70	1.28^{c}±0.39	5.57^{b}±0.43	13.48^{b}±3.02	73.98^{b}±4.23
Mean Acid extracted gum		3.32^{a}±0.76	0.66^{a}±0.30	1.97^{a}±0.23	0.90^{a}±0.34	94.16^{a}±0.83
Mean Enzyme/alcohol gum		4.56^{b}±0.56	0.47^{b}±0.08	2.01^{a}±0.59	0.86^{a}±0.57	92.15^{a}±0.46
Percent removal of Component	Acid	42	48	65	93	21
	Enzyme	20	63	64	94	20

Numbers in rows followed by different superscripts are significantly different ($p<0.05$), followed by their respective standard deviations.

Removal of ash, protein and fat by both methods were appreciably equal (Table 4.10). Working with barley, Ahmad *et al*. (2009) reported 70.25 % removal of fat, 51.36 % removal of ash, and 51.4 % removal of protein by the enzyme extraction method. For oat gum isolation fat was reduced by 77.2 %, ash by 38.1 %, and protein by 56.71 % (Ahmad *et al*., 2010). Beer *et al*. (1996) reported a relatively higher residual protein in oat gum from oat bran concentrate by the same method. In the present study, removal of protein from maize flour by acid and alcohol-enzyme isolation procedures was much more efficient than that reported for oat and barley.

4.7 Starch content of gum and β-glucan assay

Starch is a major impurity in gum isolates (Ahmad *et al*., 2010). The original starch content of the maize flour of 'Obatanpa GH' and 'Abeleehi' were found to be 85.14 ±1.07 and 75.02 ±0.92 %, respectively. In order to ascertain the efficiency of the extraction method, starch content of the alcohol-based enzyme extracted isolates were determined. In addition, pure β-glucan content of the gums was assayed for. Figure 4.1 shows residual starch and pure beta-glucan content in the gum samples. The alcohol-based enzyme method removed essentially all starch from the flour samples. Residual starch content ranged from a least value of 0.17 % for 'Obatanpa GH', 0.18 % for 'GH 9', and 0.27 % in 'Abeleehi'. Isolation of small amounts of starch during β-glucan extraction from cereals is often encountered (Burkus and Temelli, 2005; Xu *et al*., 2007). The efficiency of starch removal from 'Abeleehi' and 'Obatanpa GH' were 99.76 and 99.79 %, respectively. For oat and barley, starch was reduced by 94.1 % (Ahmad *et al*., 2010) and 97.7 %, respectively (Ahmad *et al*., 2009).

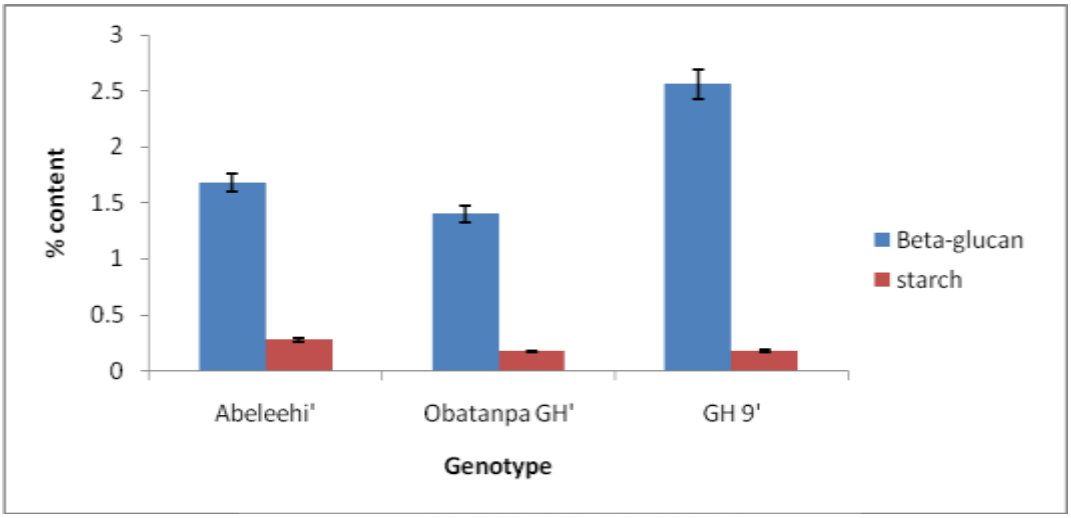


Figure 4.1. Residual starch and β-glucan recovery from maize

Although the gum yields of the three selected genotypes were not significantly different (Table 4.5), the actual β-glucan content from the assay showed differences ($P<0.05$) as was also observed by (REFSSS). Pure β-glucan content of maize, expressed as percentage recovery in 100 g of flour, ranged from a least value of 1.4 % for 'Obatanpa GH', 1.68 % for 'Abeleehi', and 2.56 % in 'GH 9'. In the current study, β-glucan content in maize grown in Ghana was higher compared to Turkey maize (0.5 to 1.3 %) (Demirbas, 2005), sorghum (0.12 %) (Niba and Hoffman, 2003; Darku, 2011), and wheat (0.52 to 1.0 %) (Beresford and Stone, 1983; Cui and Wang, 2009). However, higher concentrations of β-glucan have been reported for oat (2 to 6 %) by (Cui and Wang, 2009; Ahmad *et al.*, 2010; Bhatty, 1992) with barley recording the highest of 4 to 11 % (MacGregor and Fincher, 1993; Ahmad *et al*., 2009).

4.8 Colour

The data on surface colour of maize gum isolates was recorded using the L*a*b* colour space and are presented in Table 4.11. In the current study, the L* values for maize gums ranged between 79.67 and 83.95. This range of L* values are higher than those reported by Ahmad *et al*. (2009) for barley β-glucan but similar to those of barley dietary fibre (Wong and Cheung, 2005) and oat β-glucan (Ahmad *et al*., 2010). The L* values for 'Abeleehi' and 'Obatanpa GH' were comparable but varied significantly ($P<0.05$) from GH9. The high degree of lightness (L*) of maize gums makes them good ingredient for baked products as well as transparent food systems (Ahmad *et al*., 2009, 2010) as they will not impart colour to the product in which they are used.

The b* values ranged from +11.57 to +21.57 indicating slightly yellowish appearance and suitability for incorporation of the gum in soup, dough and dips where it would impart the desirable cream to yellow colour. A b* value of +23.45 for oat gum (Ahmad *et al*., 2010) and +19.32 for barley gum (Ahmad *et al*., 2009) was similar to the +21.57 of 'GH 9' gum. The degree of yellowness is represented by positive b* values. The closer the b* value to 60, the deeper the yellow intensity. 'GH 9' recorded the highest b* value of 21.57 which was significantly different ($P<0.05$) from 'Obatanpa GH' and 'Abeleehi' which recorded 13.33 and 11.57, respectively.

Table 4.11 Colour parameters of gum isolates from three maize genotypes

Sample	L*	a*	b*
'Obatanpa GH'	83.95±0.30 [b]	-2.31±0.04[b]	13.33±0.03[a]
'GH9'	79.68±0.13 [a]	-3.75±0.03[c]	21.57±0.12[b]
'Abeleehi'	79.67±0.08 [a]	-0.96±0.07 [a]	11.57±0.07[a]

Numbers in columns followed by different super scripts are significantly different ($P<0.05$), followed by their respective standard deviations. $LSD_{.05}$ for L*= 0.16; $LSD_{.05}$ for a*= 0.04; $LSD_{.05}$ for b*= 3.92.

4.9 ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy

A ^{13}C NMR spectroscopy was used to study the structure of maize β-glucan. 'Obatanpa GH' and 'Abeleehi' β-glucan were used for structural analysis. The two maize gum isolates produced identical spectra (Fig. 4.2). Comparison of maize gum isolates spectrum with conventional oat and barley β-glucan spectra revealed similar and analogous chemical shifts, however slight displacement of peak signals occurred at the glycosidic linkages and at C1, C2, C5 and C6 (Table 4.12). It was expected that resonances due to anomeric β-Glucopyranosyl carbon (C-1) would occur slightly downfield (>100 ppm; i.e 102-105 ppm) (Cui *et al*., 2000; Ghotra *et al*., 2008) whereas the alpha configuration of the anomeric carbons which resonate at 100 ppm should be absent. Their presence indicates contamination of the isolate with starch. Previous works on structural characteristics of carbohydrate polymers have revealed that displacement in peak signals may arise from variation in conformation caused by hydration or dehydration (Saito *et al*., 1989), methylation and acetylation (Synytsya *et al*., 2003), as well as formation of mixtures and complexation with other compounds (Wu *et al*., 2011).

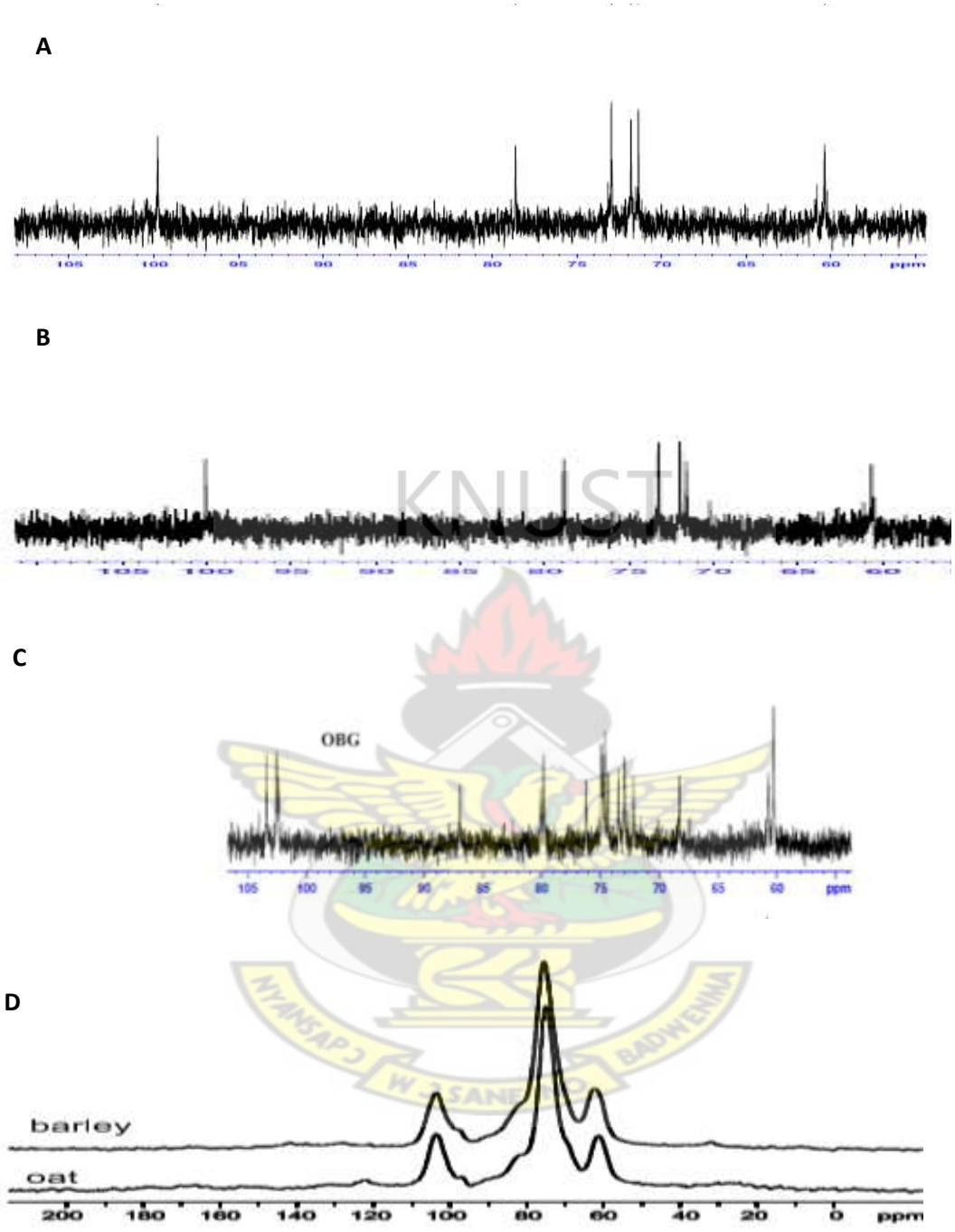


Figure 4.2 ^{13}C CPMAS NMR spectra of the soluble maize β-glucan in current study, (A) 'Obatanpa GH' (B) 'Abeleehi' and (C/D), oat and barley β-glucan. (Johannson *et al*., 2006; Agbenorhevi *et al*., 2011).

Table 4.12. Chemical shifts (ppm) of three sources of β-glucan and modified β-glucans.

Carbon Number	Oat β-glucan[1,2]		Oat β-glucan/tea polyphenols mixture[2]	Barley[1,3]	Curdlan[4,5]		Maize	
	1	2			Anhydrous	Hydrated	'Obatanpa GH'	'Abeleehi'
C1	105	103.20	102.70	105	104.50	104.30	100.00	100.00
C2	69-72	71.70	-	69-72	73.50	73.80	72.97	72.97
C3	69-72	69-72	81.20	69-72	89.80	87.30	71.80	71.80
C4	69-72	69-72	71.70	69-72	68.80	69.10	71.30	71.30
C5	79	81.60	-	79	75.50	75.80	78.62	78.62
C6	60.50	62.50	62.40	60.50	61.80	61.20	60.30	60.30

[1]Johannson, 2006; [2]Wu *et al.* (2011); [3]Morgan (1999); [4]Polymer of β-1→3 glucan; [5]Saitô *et al.* (1989).

Displacements in peak signals were displayed as upfield chemical shifts for C1, C5 and C6, and as downfield shifts for C2 (Table 4.12). In the current study, maize β-glucan exhibited upfield displacements at C1, C5 and C6, as well as downfield shifts for C2 (Table 4.12). Hydration has been recognized as a very important process in conformational stabilization of a variety of biological macromolecules. Previous ^{13}C NMR studies have demonstrated that hydration of amylose, starch, or silk fibroin caused either substantial narrowing or displacements of ^{13}C NMR chemical shifts (Saito *et al*., 1989).

Using oat and barley β-glucan as standards, maize gum samples exhibited the following displacements, upfield chemical shifts by 5 ppm in C1, 0.38 ppm for C2, and 0.2 ppm for C6 and downfield shift by 1.27 ppm in C2, whereas the range of chemical shifts for C3 and C4 were corresponded to that of oat and barley β-glucan. Just as for oat and barley β-glucan where C3 and C4 range of chemical shifts were confounding, same observation was made for maize β-glucan (Table 4.12) (Wu *et al*., 2011; Morgan *et al*., 1999, Johansson, 2006). Curdlan, a polymer of β-1→3 glucan in its hydrated state (Saito *et al*., 1989) was compared to the hydrated maize β-glucan spectrum. Again upfield chemical shift by 4.3 ppm in C1, 0.83 ppm in C2, 15.5 ppm in C3, and 0.9 ppm in C6 were observed whereas

downfield chemical shift of 2.2 ppm and 2.82 ppm occurred at C4 and C5, respectively (Table 4.12).

Mixing of β-glucan with substances such as polyphenols is reported to create conformational stabilization leading to displacement in chemical shifts as seen in Table 4.12 Wu *et al*. (2011). Similarly, Gunnes *et al*., (2010) used ^{13}C NMR to examine bile acids and Congo red absorption by barley β-glucan and reported of displacement in chemical shifts by the bile salts. Alterations in chemical shifts between oat and maize β-glucan may be attributed to different proportions in the ratio of cellotriosyl to cellotetraosyl moieties, and (1→3)-β branching. Generally β-glucan structure varies by source with regards to the type of glycosidic linkage, degree of polymerization, as well as genotype. Section 4.10 discusses the proportion of (1→3)-β-D-glucan in maize. Hence considering spectra obtained and the assignment of chemical shifts, the resonance at 100 ppm suggests presence of starch and arabinoxylans whose anomeric carbons are in the alpha configuration. Further purification of the maize gum is required to ascertain the ^{13}C NMR spectra for maize β-glucan.

4.10 Quantification of (1→3)-β-D-glucan in maize by the aniline blue method

Figure 4.3 shows fluorescence intensities of oat and maize β-glucan. The aniline blue method was used to compare relative proportions of β-(1→3)-glucan oligomers in samples. In this method, higher intensities of fluorescence represent higher proportion of β-(1→3)-glucan branching. Fluorescence intensities of maize were 7.72 ±0.28 % and 9.41±0.20 % for 'Obatanpa GH' and 'Abeleehi' β-glucan, respectively. In contrast, oat β-glucan

exhibited much higher ($P<0.05$) fluorescence intensity of 11.29 ±0.27 % demonstrating that the degree of polymerization of β-(1→3)-glucan in oats is much higher than that in maize (Hizukuri and Takayi, 1984: Jane and Shen, 1992: Edwards *et al.*, 2002).

The disparity in fluorescence and hence degree of polymerization of β-(1→3)-glucans in the two maize samples was unexpected since both samples are maize. A plausible interpretation to the difference may be genotype dependency on degree of polymerization. Examination of morphological and the chemical composition of the two maize genotypes may explain the differences in the degree of polymerization in the two samples. 'Abeleehi' is a common maize having protein content of 10.3 %, crude fibre content of 0.33 %, and starch content of 75.02 %, whereas 'Obatanpa GH' a QPM, had protein content of 17.28 %, crude fibre content of 2.11 %, and starch content of 85.14 % (Table 4.1). Relatively higher levels of protein and fibre in 'Obatanpa GH' produced low degree of polymerization of (1→3)-glucans, while lower levels of protein and fibre produced high degree of polymerization in 'Abeleehi'. This relationship suggests inverse relation of protein and fibre with (1→3)-glucans. Because protein and fibre had positive correlation with β-glucan (Table 4.7), it means the β-glucan in 'Obatanpa GH' had lower degree of polymerization while 'Abeleehi' had higher degree of polymerization in its (1→3)-glucans moieties as displayed by the fluorescence intensities. In contrast, higher starch in 'Obatanpa GH' produced a lower degree of polymerization while lower starch in 'Abeleehi' gave higher degree of polymerization of the (1→3)-glucans. This phenomenon is corroborated by the negative correlation between starch and β-glucan. If this explanation is tenable, then 1→3)-glucans in 'Obatanpa GH' is expected to be lower than that in

'Abeleehi' as was demonstrated in the current study. This disparity in the degree of polymerization as being genotype dependent was investigated in a separate study where the fluorescence intensities of β-glucan originating from other maize genotypes were measured. The genotypes and their fluorescence intensity were 17.77% for TZm 4, 11.4 % for TZm 301 and 5.18 % for TZm 1095. These genotypes had corresponding starch contents of 67.17 % for TZm 4, 72.29 % for TZm 1095 and 69.69 % for TZm 301, hence strengthening the negative relationship between starch and β-glucan. Since cereal β-glucan is made up of (1→3)(1→4)-β-D-linkages, fewer (1→3)-β-linkages means there are more (1→4)-β-linkages. Watanabe *et al*., (2001) recorded a fluorescent intensity range of 60- 85 % for (1→3)-β-glucan in different yeast species. This observation is corroborated by various researchers who have underscored the dominance (1→3)-β-linkages in yeast (Ishihara *et al*., 2007; Sekiya-Kawasaki *et al*., 2002; Watanabe *et al*., 2001).

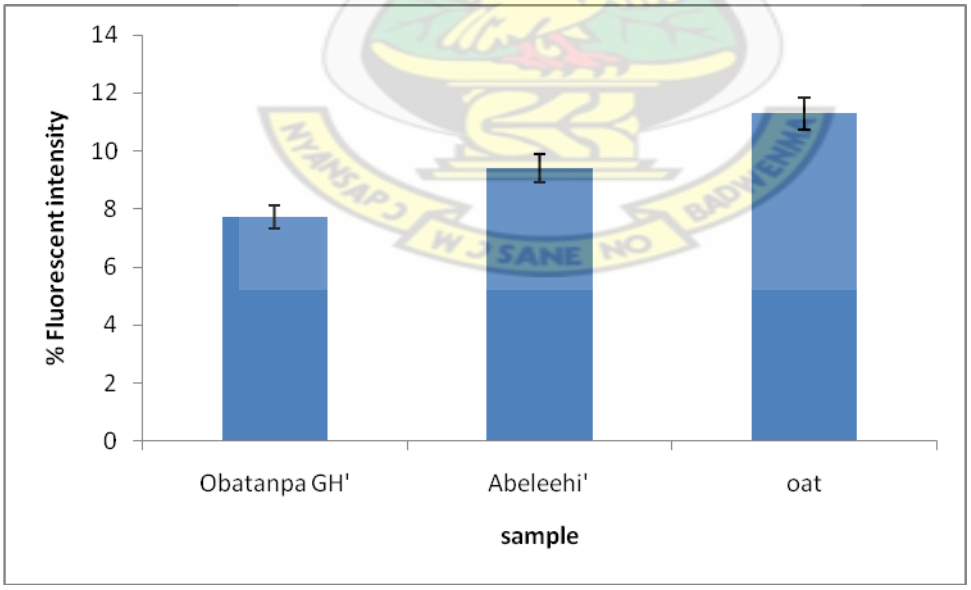


Figure 4.3: A comparism of fluorescent intensity of (1→3)-β-D-glucan in maize and oat.

4.11 Rheological characterization of maize β-glucan

The rheological properties of β-glucan were derived from correlations between stress and strain. To describe rheological properties of β-glucan, viscosities of dilute dispersions of β-glucan were measured and two graphs were generated, viz., a graph of shear stress against shear strain rate, and a graph of apparent viscosity against shear rate. Five percent maize β-glucan dispersion gave unstable viscosities of maize β-glucan hence concentration was increased to 10 % where stable viscosities were recorded and compared to 5 % oat β-glucan dispersions at 27^{o}C over shear rates of 0 to 12,800 s^{-1} were measured. Figure 4.4 shows rheograms for the three types of maize β-glucan and oat β-glucan at 27^{o} C.

Generally, viscosity of both maize and oat β-glucans were high at low shear rates and decreased rapidly at high shear rates. For each of the rheograms (Fig. 4.4), the shape of the curve is typical of nonNewtonian fluid, characterized by immediate deformation of the fluid under a small stress. However, because the ratio of shear stress to shear rate did not remain constant during the course of deformation and were less than unity, β-glucan in the current study were nonNewtonian in nature (Fig. 4.4). Similar studies have shown that oat β-glucan is nonNewtonian (Steff, 2006). This is the first report of nonNewtonian behavior of maize β-glucan dispersion.

In the present study, it was found that, viscosities of 10 % maize β-glucan dispersion were lower than that of oat β-glucan at 5 % dispersion. At the lowest shear rate of 6.4 s^{-1} apparent viscosity of maize β-glucan was 0.74 Pa s while that of oat was 3.6 Pa s approximately, five times higher than that of maize. Differences in viscosity of β-glucan dispersions are attributed to variations in molecular structure such as degree of

polymerization and ratio of cellotriosy/ cellotetraosyl repeats (Miller *et al*., 1993). The low viscosity of maize β-glucan could be attributed to effect of the isolation method in which extremes of pH and alcohol treatment, followed by repeated centrifugation steps may probably have had adverse impact on the molecular structure, specifically on the degree of polymerization of maize β-glucan. The low apparent viscosity of maize β-glucan compared to oat confirms the relatively low fluorescent intensities recorded in the aniline blue assay. The susceptibility of the molecular structure of β-glucan to degradation during isolation through enzymatic or chemical hydrolysis (Arnoldi, 2004) directly affects the viscosity of β-glucan (Ahmad *et al*., 2009, 2010; Åman *et al*., 2004; Tosh *et al*., 2010).

Figure 4.5 shows rheograms generated from apparent viscosity and shear strain rate for maize and oat β-glucans. The rheograms for both oat and maize exhibited a pronounced shear-thinning behaviour characterized by high apparent viscosity at low shear strain rate, with rapid decline in viscosity as shear rate increases. Thus, rheological behavior of maize β-glucan aqueous dispersion can clearly be described as non-Newtonian and shear thinning (pseudoplastic) as for oat. All three maize β-glucan types exhibited the same rheological characteristics. Rheograms of shear rate (s^{-1}) against shear stress (Pa s) for both oat and barley were reported to demonstrate the three-stage viscous response for gums where at shear rates between 0 and 6.4 s^{-1}a Newtonian behaviour was observed, followed by shear thinning range of 160 to 640 s^{-1}in which solution viscosity decreased in accordance with the Power law model, and finally an infinite shear viscosity recorded at very high shear rate (6,400 to 12,800 s^{-1}) (Rao, 2007). This observation is due to the rearrangement in the

conformation of the biopolymer molecule in the dispersion during shearing (Fig. 4.5) (Malkin and Isayev, 2006).

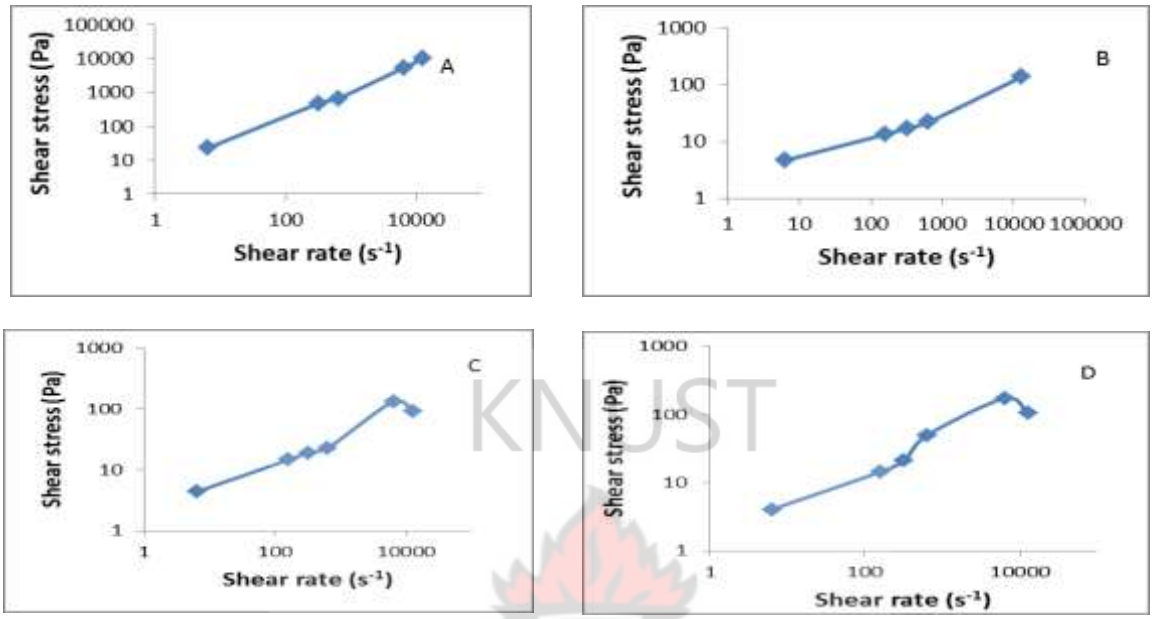


Fig. 4.4. Rheograms of β-glucan dispersions. (A) 5 % oat, (B) 10% 'GH9', (C) 10 % 'Obatanpa GH', (D) 10 % 'Abeleehi'.

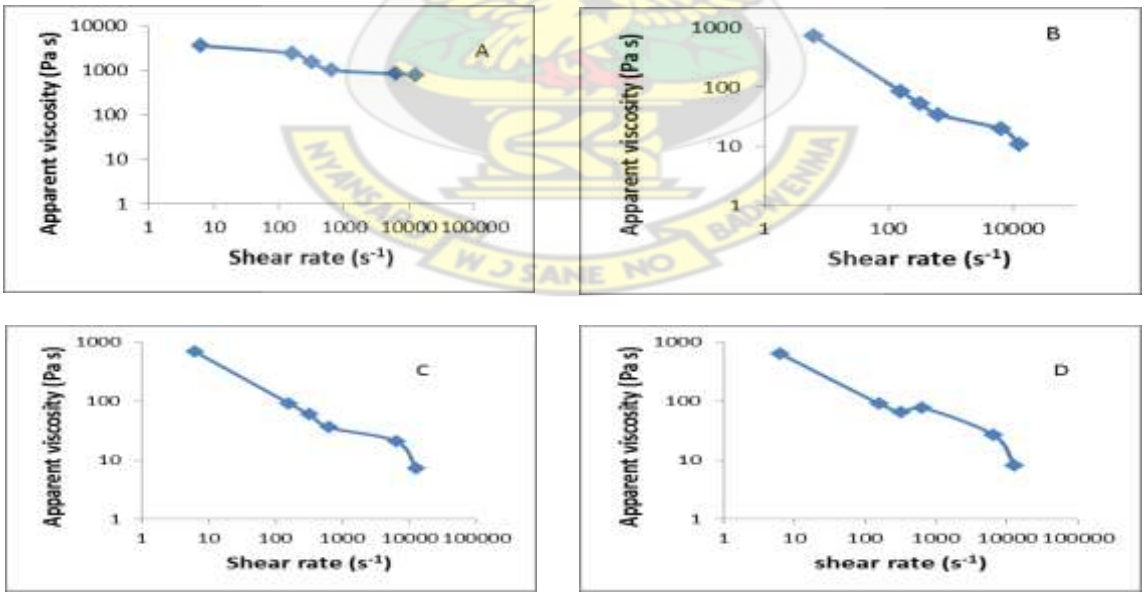


Figure 4.5: Apparent viscosity as a function of shear rate. (A) 5 % oat, (B) 10% 'GH9', (C) 10 % 'Obatanpa GH', (D) 10 % 'Abeleehi'.

An inspection of the graphs shows that the intercept on the Y-axis, which is the yield stress, is zero. Therefore, flow behavior of maize β-glucan can be described by the Power Law model,

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

A regression analysis on the logarithmic data points permitted the estimation of model parameters. Table 4.13 shows the flow behavior index and consistency coefficient of oat and maize β-glucan dispersions. Maize β-glucan in the study was non-Newtonian in nature because its flow behavior index was less than 1.00. Though shear stress increases with shear rate, the relationship is not directly proportional and so the flow behavior, *n*, lies between 0 and 1 (Marcotte *et al*., 2001).

Table 4.13: Flow behavior (n) and consistency coefficient (K) of 5% oat and 10% maize gum dispersions determined at shear rates of 0 to 12,800 s^{-1} at 27°C

Sample	Power Law parameter	Estimate
10% "Obatanpa GH'	Yield stress	0
	Flow behavior index (*n*)[1]	0.50
	Consistency coefficient (K)	1.15
	Mean square	6.71
	Standard error	0.01
	R^2	0.75
10% 'GH9'	Yield stress	0
	Flow behavior index (n)	0.52
	Consistency coefficient (k)	1.17
	Mean square	7.21
	R^2	0.76
	Standard error	0.17
10% 'Abeleehi'	Yield stress	0
	Flow behavior index (n)	0.48
	Consistency coefficient (k)	1.23
	Mean square	6.24
	Standard error	0.01
	R^2	0.74
5% Oat	Yield stress	0
	Flow behavior index (n)	0.91
	Consistency coefficient (k)	2.13
	Mean square	22.28
	Standard error	0.04
	R^2	0.81

[1]The lower and upper limits of 95 % confidence intervals of the flow behavior index of beta-glucan dispersions are 0.45 to 0.55 for 'Obatanpa GH', 0.46 to 0.57 for 'GH 9', 0.42 to 0.54 for 'Abeleehi' and 0.82 to 1.00 for oat.

Many polymer melts and solutions exhibit flow behaviour index in the region 0.3 to 0.7 depending upon the concentration and molecular weight (Chhabra and Richardson, 2008; Barnes, 1997). The flow behaviour index obtained for 10 % maize β-glucan showed 0.48 for 'Abeleehi', 0.50 for 'Obatanpa GH' and 0.52 for 'GH9'while that of 5% oat was 0.91. The proximity of the R^2 values to unity attests to the precision of the rheological data. Bhatty (1995) and Burkus (1996) reported a flow behaviour index 0.7 for oat and barley β-glucan gums. In the current study, both flow behavior index and consistency coefficient of maize β-glucan suspensions were about one-half that of oat.

The viscosity and/or flow behavior of maize gum at 10 % dispersion demonstrates its utilizability as thickening agents to modify texture, consistency, and appearance of food formulations, and may also qualify as fat substitutes in the development of calorie-reduced diets (Lathia, 2011). Beta-glucan-rich fractions from cereals may be incorporated into products such as breakfast cereals, pasta, noodles and baked goods (bread, muffins), as well as dairy and meat products (Lazaridou and Biliaderis, 2007).

4.12 Water Binding Capacity

The water binding capacity (WBC) measures the amount of water retained by a material after centrifugation (Ahmad *et al*., 2009). These hydration properties of maize β-glucan are important in many food applications and have an impact on shelf life of food product. In the current research, the WBC ranged from highest ($P<0.05$) value of 232.6 ±8.4 % for 'GH 9' β-glucan to 131 ± 28.5 % for 'Obatanpa GH' which was not significantly different from that of 'Abeleehi' (126±1.3 %). The water binding capacity for alcohol-enzyme

extracted oat beta-glucan was 395 % (Ahmad *et al*., 2009) and for barley, 291 % (Ahmad *et al*., 2010). The relatively high value of WBC for the gums makes them suitable for incorporation into sauces, dips, yoghurt, tomato ketchup and spreads where syneresis must be prevented (Ahmad *et al*., 2009, 2010).

4.13 Sensory evaluation of pie crusts

Maize beta-glucan was incorporated into pastry at levels of 15 and 20 % for the purpose of evaluating their functionality as fat replacers in pie crust. In baked goods the function of fat is to assist in aerating and maximizing the viscosity of batter (Lee *et al*., 2005; O'brien, 2009). Fat also imparts crispiness, tenderness, as well as prevent starch retrogradation in baked products (Fennema, 2008). Table 4.14 shows sensory evaluation of full fat (control), 15 and 20 % fat-replaced pie crust and. Plate 1 shows images of the pie crusts. In a typical short crust pastry such as pie crust, reducing shortening content by 20 % resulted in a pie crust that was tougher and required much effort to either bite or break. As expected, the replacement of the shortening with gums affected the handling properties of the batter formulation during kneading and thus had effect on the physical and sensory parameters of the product.

Plate 1: Samples of pie crust used for sensory evaluation.RTZ= control, BYA= 15% fat replacement, XMH= 20% fat replacement

At both levels of replacement panelists rated colour as identical to that of control (Plate 1). Notwithstanding the apparent differences in sensory scores, fat replacement up to 20 % did not show significant difference (P>0.05) from that of full fat pie crust (Table 4.14).

Table 4.14: Descriptive statistics for brittleness and acceptance of β-glucan full fat and fat-replaced pie crust.

Statistic	Brittleness			Acceptance		
	Full fat	15 % replacement	20 % replacement	Full fat	15 % replacement	20 % replacement
N	54	54	54	54	54	54
Mean	5.30[a]	5.11[a]	4.78[a]	5.70[a]	5.60[a]	5.30[a]
Median	5	5	5	6	6	5
SD	1.06	1.28	1.50	1.19	1.04	1.44
[1]SEM	0.14	0.17	0.20	0.16	0.14	0.20
CV (%)	20.2	25.67	30.01	19.86	17.35	26.10
Min.	3	2	1	1	2	2
Max.	7	7	7	7	7	7

Numbers in rows followed by same superscripts are not significantly different (P>0.05).[1]Standard error of mean. Brittleness $LSD_{.05}$= 2.56; Acceptance $LSD_{.05}$= 2.44

For full fat pie crust, approximately 50 % of the sensory scores were in the interval 3 to 5 and the next 50 % located in the interval 5 to 7. For 15 % fat-replaced pie crust, 50 % of the brittleness scores were in the range of 2 and 5 and the next 50 % were located in the interval 5 and 7. While brittleness scores of full fat and 15 % fat-replaced pie crust had 50 % of scores between 3 and 5, in the 20 % fat-replaced pie crusts, 50 % of scores were between 1 and 5, indicating greater dislike for this product. A low coefficient of variation ranging between 20 and 30 % indicated high precision and demonstrated consistency in the scores.

Acceptance scores for the three products did not indicate significant difference (Table 4.14). The relatively lower coefficient of variation again demonstrates the precision and consistency in the sensory scores. Generally 20 % fat replaced pie crust samples received relatively lower brittleness and acceptance scores for the 20 % product could be attributed insufficient amounts of fat needed to inhibit the development of tough gluten networks (Fennema, 2008). This observation is corroborated by Chysirichote (2010) who stated that, fat has the ability to coat the surface of flour particles thus disrupting the development of tough gluten proteins in order to obtain softer and tender dough in baked products. Fifteen percent fat replacement was selected due to its similarity to the full fat pie crust. The performance of individual beta-glucan as fat replacers at 15 % replacement in pie crust is shown in Table 4.15.

Table 4.15: Descriptive statistics for15% fat-replaced β-glucan pie crust

Statistic	Brittleness			Acceptance		
	'Obatanpa GH'	'GH9'	'Abeleehi'	'Obatanpa GH'	'GH 9'	'Abeleehi'
N	18	18	18	18	18	18
Mean	5.39[a]	5.28[a]	4.67[a]	5.83[a]	5.71[a]	5.00[a]
Median	6	6	5	6	6	5
SD	1.58	0.97	1.18	0.99	1.09	0.91
SEM	0.29	0.22	0.21	0.17	0.19	0.18
CV %	37.2	27.8	22.9	23.2	25.8	21.4
Min.	2	2	2	3	2	4
Max.	7	7	6	7	7	6

Numbers in rows followed by same superscripts are not significantly different (P>0.05).Brittleness $LSD_{.05}$=2.59; Acceptance $LSD_{.05}$= 2.11

At 15 % fat replacement, pie crust made from the three beta-glucan fat replacers were not different (P>0.05) in brittleness nor acceptance. The lower coefficient of variation again

demonstrates precision and consistency in the sensory scores. The closeness of sensory scores of products made with 'Obatanpa GH' β-glucan to 7 (like extremely) showed that it was most liked by panelists. It can be inferred that the quality characteristics of pie crust will be maintained when the fat content is replaced with "Obatanpa GH' and 'GH9' β-glucan at levels of 15 % replacement. This study has demonstrated that maize beta-glucan can be used as a fat replacer in pie crust without compromising sensory qualities.

4.14 Chemical composition of pie crust

Crude fat and moisture content of the pie crusts were determined (Table 4.16). As expected, low levels of fat replacement led to pie crust with high fat content. In contrast, low levels of replacement led to high moisture content of pie crust. The full fat pie crust recorded the highest crude fat content followed by 15 and 20% fat replaced pie crust.

Table 4.16: Crude fat and moisture composition of pie crust containing 15 and 20 % β-glucan and full fat formula (control).

Chemical constituent		**'Obatanpa GH'**		**'GH9'**		**'Abeleehi'**	
	Control	15 %	20 %	15 %	20 %	15 %	20 %
Crude fat	32.25^{a} ±1.56	26.51^{b} ±2.36	21.23^{c} ±1.11	27.68^{b} ± 2.34	20.06^{c} ± 2.56	27.86^{b} ±3.58	24.33^{d} ±1.15
Moisture	9.43^{a} $\pm 1.01^{a}$	7.36^{b} ±0.89	4.16^{c} ±0.94	6.99^{b} ± 1.22	4.03^{c} ±0.20	7.01^{b} ±1.78	5.82^{d} ±0.69

Numbers in rows followed by different superscripts are significantly different ($P<0.05$).

The mean moisture content of the pie crust treatments ranged between 4.03 and 9.43% with the full fat pie crust recording the highest followed by 15 and 20% fat replaced pie crust (Table 4.16). There was a significant decrease ($P<0.05$) in moisture content with

increasing fat replacement. The results conflict with the work of Paintsil (2008) who observed an increase in moisture content with increasing fat replacement. This observation could be attributed to the varying volume of water incorporated in the pastry to achieve the desired consistency prior to baking. One of the functions of shortening in baked products is to enhance lubrication and consistency. As such pastry with less fat requires more water to achieve the desired consistency.

4.15 Implications of the β-glucan content of maize

This work has demonstrated that maize genotypes differ in their β-glucan content. Genotypes with high βglucan may be used as commercial sources in tropical regions where barley and oat do not thrive. Genotypes with low β-glucan will find uses in brewing industry where influence of β-glucan on saccharification process is detrimental. Though slightly lower values of β-glucan were found in maize compared to oat and barley, they can be utilized as base germplasm for β-glucan enhancement in breeding programmes.

Chapter Five

Conclusions and Recommendation

5.1Conclusion

The acid and alcohol-based isolation methods gave the highest yield of gum while hot water and alkaline isolation methods demonstrated lower extractability. The genotypes 'Obatanpa GH' gave the highest gum yield by the enzymic method while 'Abeleehi' produced the highest gum yield with the acid isolation method. The gum yield was found to be genotype-dependent. Hybrid varieties had the highest gum content while the landraces had the lowest. Beta-glucan content of kernels was found to correlate with protein, fibre (positively) and nitrogen free extract (negatively). A striking feature was the negative correlation of β-glucan with starch content of the kernels. This observation suggests that maize genotypes having relatively low starch content are likely to have high β-glucan contents.

With regards to actual β-glucan content of gums, 'GH 9' had the highest β-glucan of 2.56 %. This value, though lower than that of barley and oat, is still higher than β-glucan content of wheat, sorghum and rye. Maize with this concentration of β-glucan is enough to supply the World Health Organization recommended level of 0.75 g/ serving of β-glucan in the diet in order to maintain good health status with regards to blood sugar and cholesterol levels.

The structure of maize β-glucan was found to be similar to that of oat and barley as demonstrated by ^{13}C NMR in that each had chemical shift between 60 and 105 ppm. With

regards to maize β-glucan, slight displacements of upfield and downfield chemical shift were observed at the individual carbon atoms. Application of the aniline blue fluorescence fluoremetry revealed lesser β-(1→3) linkages in maize β-glucan compared to oat. 'Abeleehi' β-glucan demonstrated higher β-1→3 branching than 'Obatanpa GH' β-glucan demonstrating genotypes dependency. In a structure-function relationship, the occurrence of less β-1→3 branching means relatively lower values of viscosity, molecular weight and water binding capacity. At the lowest shear rate of 6.4 s^{-1}, apparent viscosity of 10 % maize β-glucan dispersion was 0.74 Pa s while that of oat was 3.6 Pa s approximately, five times higher than that of maize. Similarly, though the water binding capacity of 232 % maize β-glucan was high, it was about half that of oat. A study of rheological properties of maize β-glucan revealed that aqueous dispersions had flow behavior index of less than 1 and consistency coefficient greater than unity. These characteristics make maize β-glucan a pseudoplastic nonNewtonian fluid.

A study of the functionality of maize β-glucan as a fat replacer in pie crust demonstrated that at 15 % replacement, brittleness, colour, and acceptability were not significantly different from that of full fat pie crust. This may be explained by the high water binding capacity of maize β-glucan which was able to mimic the role of fat in the pie crust.

5.2 Recommendation

The study has demonstrated the potential use of maize as good source of β-glucan in tropical climates where oat and barley are not grown. An extension of the screening to cover more maize genotypes may reveal even higher contents with better quality with regards to degree of polymerization, and functionality. It is recommended that genotypes with low β-glucan content be investigated as raw material for brewing. Further work should concentrate on the elucidation of the molecular weight of maize β-glucan. Functionality studies of β-glucan in other food products should be carried out. Regarding its health benefits, maize β-glucan must be investigated for its cholesterol reducing, blood glucose reducing and its anti-cancer activity. The gum samples need further purification and analysis to ascertain the ^{13}C NMR spectra for maize β-glucan. Also, it is recommended that further work on the intrinsic viscosity and flow behaviour at varying gum concentrations be done using a rheometer.

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APPENDIX

Appendix 1: Calculation of moisture content

$$\% \text{ Moisture} = \frac{(\text{weight of wet sample} - \text{weight of dry sample}) \times 100\ \%}{\text{weight of wet sample}}$$

Appendix 2: Calculation of ash content

$$\% \text{ Ash} = \frac{(\text{weight of crucible and ash residue} - \text{weight of empty crucible}) \times 100\ \%}{\text{weight of sample}}$$

Appendix 3: Calculation of crude fat content

$$\% \text{ Crude fat} = \frac{(\text{weight of flask and fat residue} - \text{weight of dry empty flask}) \times 100\ \%}{\text{weight of sample}}$$

Appendix 4: Calculation of total nitrogen content

$$\% \text{ Total nitrogen} = \frac{100\ (S_T - S_b) \times 0.1 \times 0.01401 \times 100\ \%}{\text{weight of sample} \times 10}$$

Appendix 5: Calculation of crude fibre content

$$\% \text{ Crude fibre} = \frac{(\text{weight of crucible and sample before ignition} - \text{weight of crucible and ash}) \times 100\ \%}{\text{weight of sample}}$$

Appendix 6: Ballot score sheet for sensory evaluation

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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

SAMPLE: PIE CRUST

NAME…… SEX……..

You have been provided with pie crust, you are expected to make a fair assessment based on a seven point hedonic scale

7 points hedonic scale
1= like extremely
2= like very much
3= like moderately
4= neither like nor dislike
5= dislike moderately
6= dislike very much
7= dislike extremely

CODE	COLOUR	TASTE	BRITTLENESS	CRISPINESS	OVERALL ACCEPTANCE
……	…….	……..	……	…….	……
……	……	……	……	…….	……
…….	……	……	……	……..	.....

Any other comments
……
……

Thanks very much for your co-operation.