KWARA STATE UNIVERSITY, MALETE, NIGERIA

SCHOOL OF POSTGRADUATE STUDIES (SPGS)

PHYSICOCHEMICAL COMPOSITION AND BIOACTIVITIES OF

GLUTEN-FREE

COOKIES MADE FROM YELLOW MAIZE AND *Brachystegia eurycoma*

BLENDS

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(19/57MBC/00001)

NOVEMBER 2021

SCHOOL OF POSTGRADUATE STUDIES (SPGS)

PHYSICOCHEMICAL COMPOSITION AND BIOACTIVITIES OF GLUTEN-FREE COOKIES MADE FROM YELLOW MAIZE AND *Brachystegia eurycoma* BLENDS

A M.Sc. THESIS SUBMITTED

BY

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NIGERIA

NOVEMBER 2021

DECLARATION PAGE

I hereby declare that this thesis titled **physicochemical composition and bioactivities of gluten-free cookies made from yellow maize and *Brachystegia eurycoma* blends** is a record of my research. It has neither been presented nor accepted in any previous application for higher degree.

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APPROVAL PAGE

This is to certify that this thesis by **Khadijat Oluwashola Abdulrazaaq** has been read and approved as meeting the requirements of the Department of Medical Biochemistry and Pharmacology for the award of the degree of Master of Science (M.Sc. Hons) Degree in Biochemistry.

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DEDICATION

To Almighty ALLAH (Subhannah Watahalah) the sustainer of life and to the memory of my late father Alhaji Mohammed Nuhu Bola Issa, may his gentle soul continue to rest in peace.

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ABSTRACT

Intake of gluten-containing food products (e.g wheat cookies or bread) can cause distinct immunologically-mediated adverse reactions, including IgE-mediated food allergy in some individuals, leading to celiac disease. Presently, getting ingredients that can replace gluten in baked foods is still a major challenge to the food industries. However, recent studies have proven that hydrocolloids mimic the visco-elastic property of gluten, and therefore serve as a replacement for it. This study evaluated the physicochemical composition and bioactivities of gluten-free cookies made from yellow maize and *Brachystegia eurycoma* (a natural source of hydrocolloids) blends and their sensory attributes. *Brachystegia eurycoma* (BEF) or sodium carboxylmethyl cellulose (CMC), a reference hydrocolloid, was added to whole maize flour at 2.5 %, 5 %, 7.5 %, and 10 % proportions and cookies were baked from the blends. Wheat flour (100 %WF) cookies served as the control. The functional properties (bulk density, water absorption capacity, swelling power, pasting property, starch and free sugar content and amylose and amylopectin content), proximate composition (moisture content ,crude protein, crude fibre,

crude fat, total ash and total carbohydrates), bioactive constituents (total phenol, total flavonoids, total tannins, total saponins, and carotenoids contents) and bioactivities (DPPH scavenging ability, ABTS radical scavenging ability, reducing power, alpha-amylase and alpha-glucosidase inhibition assay) of the blends and cookies were determined using standard laboratory methods. The glycaemic index (GI) (in human subjects) and the sensory attributes of the cookies were also determined. There was a significance difference of ($p<0.05$) in functional properties of flour blends and the cookies. The water absorption capacity of the flour blends ranged from 118.26 – 174.41 % with 100 % BEF and 2.5 % BEF recorded the highest value of 174.41 % and 181.30 % respectively. 100 % wheat recorded the least value of 118.26 %. It was observed that water absorption capacity decreased with increase in addition of CMC and BEF. The bulk density results of flour blends ranged from 1.16 – 1.26 g/ml with 100 % yellow maize having the highest value of 1.26 g/ml greater than the value recorded from 100 % whole wheat (1.26 %). The results revealed a progressive increase in swelling power with increasing proportion of BEF and CMC. Peak viscosity reduced with significantly ($p < 0.05$) with an increase in BEF and CMC. The moisture content value, crude protein value, crude fiber value and crude fat value of whole yellow maize was higher than the whole wheat and whole white maize but lower in total ash and carbohydrates. The bioactive values of the cookies increased with increase in

proportion of BEF and CMC, with higher value in BEF. The antioxidant properties of the blends were decreased with increase in proportion of BEF and CMC but with higher value in BEF. BEF blends decreased the glycaemic index with increase in the proportion of the BEF. The 100 % yellow maize cookies had better sensory qualities than the 100 % whole wheat. Also the BEF blends cookies had a better sensory than the CMC blends cookies. Hence *Brachystegia eurycoma* formulation significantly improved the functional, pasting properties, bioactive, and bioactivities of the flour blends due to their higher micronutrient content than the CMC.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Celiac disease (CD) is a disease in which the small intestine is hypersensitive to gluten, leading to difficulty in digesting food (Moyo clinic,2020). Individuals with celiac disease (CD) show high levels of intestinal inflammation when exposed to gluten-containing foods (Ludvigsson *et al*., 2013; Rubio-Tapia *et al*., 2013). In western countries, the prevalence for CD is estimated at approximately 1% (Gujral *et al*., 2012; Golley *et al*., 2015). Clinically, as direct response to gluten and related prolamines in a diet, immunological processes damage intestinal mucosa and lead to villous atrophy, crypto-hyperplasia and nutrient malabsorption (Dickson *et al*., 2006; Husby *et al*., 2012). To get full remission of the symptoms, excluding gluten-containing cereals (e.g., wheat, rye, barley) in a strict lifelong gluten-free (GF) diet is indicated (El-Chammas and Danner, 2011; Green, 2009). The nutritional quality of GF products that replace cereal-based foods is pivotal for patients with CD. Previous research showed that GF food products differ in their nutrient content compared to gluten-containing counterparts (Miranda *et al*., 2014; Mazzeo *et al*., 2015). In addition, a recent evaluation of more than 600 GF foods in Australian supermarkets showed that it is unlikely that GF foods have health benefits for individuals without Celiac

disease, in particular due to the reported lower protein content in GF compared to non-GF products (Wu *et al*., 2015). However, contemporary data reporting the nutritional quality is scarce (Staudacher and Gibson, 2015), although the popularity of GF food products is increasing among consumers (Markets and markets.com, 2013).

For CD patients, adhering to a restrictive GF diet can be challenging for several reasons. First, food choices are essentially limited because cereal products are staple foods in western countries and play a predominant role in a regular diet (e.g., bread or pasta). Second, a wide range of processed foods contain gluten-based products as additional ingredients. Prior to consumption of these foods, a detailed examination of the ingredient list has to be performed to avoid being exposed to gluten. This requires fundamental nutritional knowledge and a high level of self-discipline (Mulder *et al*., 2015). Third, 20–38 % of patients with CD have some nutritional deficiencies due to their medical condition (Kinsey *et al*., 2008; Saturni *et al*., 2010); e.g., iron deficiency, deficiency in B vitamins (B6, B12) and trace minerals (e.g., zinc) (Harris *et al*., 2012; Theethira and Dennis, 2015). In a nutshell, patients with CD are needed to structure their diet in a strict manner to maintain a positive long-term health outcome. Therefore, GF products have been developed as alternatives to cereal-based formulations. A wide range of products based on teff, amaranth, buckwheat or quinoa are now

available for consumers exploring different alternatives to enhance sensory properties and shelf-life (Gallagher *et al*., 2004; Pellegrini and Agostoni, 2015).

Likewise, GF products are very popular among consumers without CD, which has led to almost exponential rise in sales for GF products over the last decade (Marketsandmarkets.com, 2013; Strom, 2014). Mardini *et al*. (2015) report updated US data from the National Health and Nutrition Examination Survey (NHANES) from 2009–2012. From all study participants (14.701 participants), 0.9 % adhered to a GF diet, even though 85 % of this group was never diagnosed with CD and 99 % had negative serological markers for CD. For the majority of consumers, GF products are perceived as healthier than conventional products (Marcason, 2011). While the evidence for this assumption is not based on solid data (Gaesser and Angadi, 2012; De Giorgio *et al*., 2015), food companies continue to market GF foods as healthier, and charge premium prices for their products (Stevens and Rashid, 2008; Singh and Whelan, 2011.). Still, there is no solid data putting GF products into proper perspective about nutritional quality and product costs.

The food industries are still faced with the challenge of getting suitable replacement for gluten. To overcome this challenge, gluten-free products (such as bread) are formulated using gluten-free flours (rice, maize, sorghum) and ingredients such as hydrocolloids, emulsifiers and shortenings, or combinations

thereof as alternatives to gluten. These ingredients improve the technological, sensory, and nutritional properties, as well as the shelf-life of the gluten-free product (Shabir *et al.,* 2016). Recent studies have shown that hydrocolloids can serve as gluten replacement in the formulation of gluten-free food products. Additives such as xanthan gum, guar gum, hydroxypropyl methylcellulose (HPMC), maize starch, or eggs are used to compensate for the lack of gluten (Lamacchia *et al*., 2014; Volta *et al*., 2015).

Generally, cookies development without gluten has involved the use of diverse ingredients and additives, with the purpose of imitating the visco-elastic properties of the gluten and consequently to obtain quality cookies products (Shabir *et al*., 2016). To overcome this challenge, gluten-free cookies formulations involve diverse approaches, such as the use of different gluten-free flours (rice, maize, sorghum) and ingredients such as hydrocolloids, emulsifiers and shortenings or combinations thereof as alternatives to gluten, to improve their technological, sensory and nutritional properties, and also the shelf-life, which leads to an increased final price (Shabir *et al*., 2016).

There are natural sources of hydrocolloids such as *B. eurycoma* and *D. microcarpum*, which have other health benefits in addition to dough formation, and are readily available. Natural sources of hydrocolloids may enhance the potential health benefits of food products, in addition to mimicking the visco-

elastic attributes of gluten. The natural sources of hydrocolloids are mainly from plant materials of which three of the commonly known plants are *Brachystegia eurycoma* (BE), *Detarium microcarpum* (DM) and *Mucuna* spp (Nwakaudu *et al*., 2017).

Maize (called corn in some countries) is *Zea mays*, a member of the grass family Poaceae. It is a cereal grain, which was first grown by people in the ancient Central America. Approximately 1 billion tons are harvested every year. However, little of this maize is eaten directly by humans. Most of it is used to make maize ethanol, animal feed and other maize products, such as maize starch and maize syrup. Maize is a healthy food for humans, which is why it is a staple food in many parts of the world. It is an excellent source of fiber and proteins, whilst being low in fat and salt. Maize contains lots of minerals, which are important for us and that too in a great quantity. These minerals include phosphorous, magnesium, manganese, zinc, copper, iron and also small amounts of calcium and potassium (Nawaz *et al*.,2013).

Maize, which is also known as corn, is a grain which was first used by people in Mexico approximately 10,000 years ago. White Maize flour, however, is different from yellow maize in terms of color (one is white and the other yellow, as the names give it away) as well as in a few qualities. Research has proven that

yellow maize has a higher nutritional value than the ordinary white maize, because of its higher levels of carotenoids (Jung *et al*., 2015).

Brachystegia eurycoma Harms (synonym *B. spiceaformis*), family *Caesalpinioideae,* is a woody plant commonly found in the forest zone in southern Nigeria and Cameroun. The tree is about 35 m tall (Nwakaudu *et al*., 2017). It is commonly called *Achi* (Igbo), *Akalado* or *Eku* (Yoruba), *Akpakpa* or *Apaupan* (Ijaw), *Okwen* (Edo), *Okung* (Efik) (Ikegwu *et al.,* 2010; Nwakaudu *et al*., 2017). It grows naturally in forest parts of Africa, flowering throughout the wet season and bearing fruits between November and January; though it may also bear in May (Ikechuku and Emmanuel, 2010). The B. *eurycoma* seeds have been shown to be composed of certain nutrients, minerals, vitamins and food like chemicals which are essential to human nutrition (Nwacaudu *et al.,*2017). Anti-nutrients such as cyanides, phytate and tannins may be toxic and result in poor palatability and bioavailability and hence, deficiency of certain nutrients e.g proteins were found to be significantly low in the seeds of *B. eurycoma* and the levels of these anti-nutrients (Bolanle *et al*;2014).There are numerous research papers and academic reviews, which have focused on the effect of additives on gluten-free cookies. However, there is paucity of scientific information on the influence of natural sources of hydrocolloids on the properties of gluten-free cookies. Hence, this study evaluated the physicochemical and bioactivities of gluten-free low-sugar cookies made from

yellow maize and *Brachystegia eurycoma* (natural source of hydrocolloids) blends.

1.1 STATEMENT OF THE PROBLEM

Dietary intake of gluten-containing food products (e.g. wheat bread or cookies) can cause distinct immunologically-mediated adverse reactions, including IgE-mediated food allergy in some individuals, leading to celiac disease. Gluten toxicity encompasses a wide spectrum of target organ pathologies, clinical disorders, and metabolic effects. Presently, getting materials that can replace gluten-containing food products is still a major challenge to the food industries.

1.2 JUSTIFICATION FOR THE STUDY

There is a need for gluten-free products produced by using alternative cereals such as maize, sorghum, rice and millet. The use of such alternative flours is restricted due to their low baking quality. Hence, this research explored the possibility of producing affordable gluten-free cookies using yellow maize and *B. eurycoma* (a natural source of hydrocolloids) blends, which may be readily available and have possible health benefits, especially for people susceptible to celiac disease.

1.3 OBJECTIVES OF THE STUDY

The broad objective of this research work was to investigate the physicochemical composition and bioactivities of gluten-free cookies made from yellow maize and *B. eurycoma* blends.

The specific objectives were to:

i. determine the nutrients and physiochemical composition of flours of yellow maize(YM), *B. eurycoma* (BE) and their composites (97.5 %YM : 2.5 % BE, 95 % YM : 5 % BE; 92.5 % YM : 7.5 % BE; 90 %YM : 10 % BE);

ii. determine the functional properties of the individual flours and their composites;

iii. evaluate the bioactivities (*in vitro* starch digestibility, pancreatic lipase, alpha-amylase, and alpha-glucosidase inhibitory activities and antioxidant activities);

iv. bake low-sugar cookies from the flours;

v. conduct a sensory evaluation of the cookies;

vi. determine the glycaemic index of the cookies in human subjects.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CELIAC DISEASE

Celiac disease is a serious auto-immune disease (Shah.S. *et al*., 2014) that occurs in genetically pre-disposed people, where the ingestion of gluten leads to damage in the small intestine. It is estimated to affect 1 in 100 people worldwide (Roy *et al*., 2016). Two and one-half million Americans are diagnosed and are at risk for long-term health complication.

Gluten is a family of storage proteins formally known as polamins that are naturally found in certain grains, such as wheat, barley and rye (Ansley *et al*., 2019). Many different polamins fall under the gluten umbrella, but they can be further classified based on the specific grains in which they're found. For instance, glutenins and gliadins are the polamins in wheat, secalins are in rye and hardeins are in barley (Ansley *et al*., 2019).

Gluten offers a variety of functional culinary benefits and is responsible for the soft, chewy texture that is characteristic of many gluten-containing, grain-based foods (Wiesee, 2007). When heated, gluten proteins form an elastic network that can stretch and trap gas, allowing for optimal leavening or rising and maintenance of moisture in cookies, breads, pasta and other similar products (Both *et al*., 2021). Because of these unique physical properties, gluten is also frequently used as an additive to improve texture and promote moisture retention in a variety of processed foods. Gluten-free diets are more common than ever, but gluten does not pose a health risk to the majority of the population. That said, people with celiac disease cannot tolerate gluten and must eliminate it from their diet to avoid harmful, adverse reactions.

Celiac disease is hereditary (meaning it runs in families). People with a first-degree relative with celiac disease (parent, child, sibling) have a 1 in 10 risk of developing celiac disease (Shah *et al*., 2014). Celiac disease can develop at any age after people start eating food or medicines that contain gluten. Left untreated, celiac disease can lead to additional serious health problems.

2.1.1 CAUSES OF CELIAC DISEASE

Celiac disease, sometimes called celiac sprue or gluten-sensitive enteropathy, is an Immune reaction to eating gluten, a protein found in wheat, barley and rye (Mayoclinic; 2019).

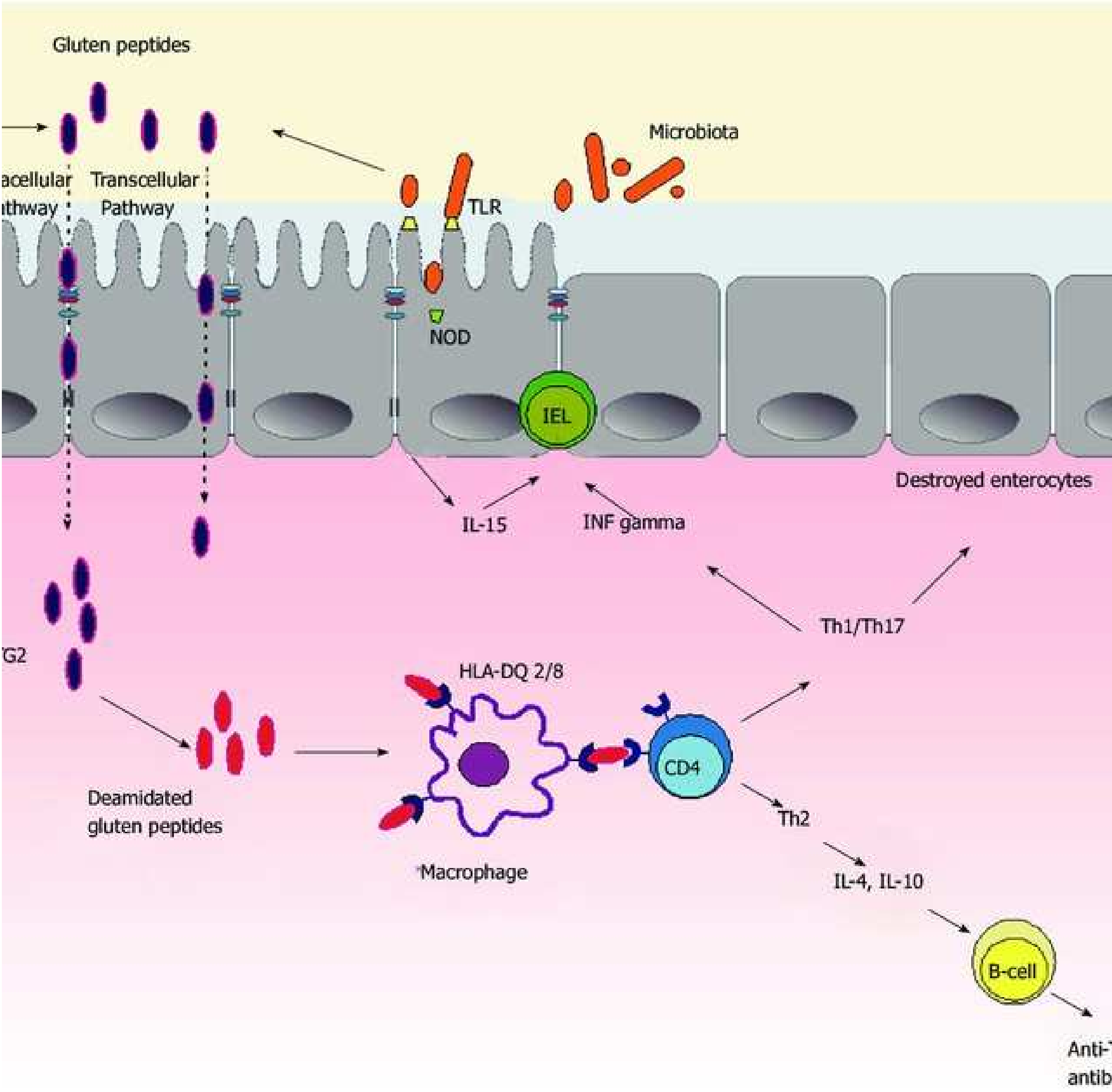
When people with celiac disease eat gluten their body mounts an Immune response that attacks the small intestine. These attacks lead to damage on the villi, small fingerlike projections that line in small intestine that promote nutrient absorption (Roy *et al.*, 2016). When the villi get damaged, nutrients cannot be absorbed properly into the body, eventually, this can lead to malnourishment as well as loss of bone density, miscarriage, infertility or even neurological diseases or certain cancer (Marrow, 2021).

2.1.2 PATHOPHYSIOLOGY OF CELIAC DISEASE

Celiac disease appears to be multi-factorial, both in that more than one genetic factor can cause the disease, and in that more than one factor is necessary for the disease to manifest in a person (Baker and Liu, 2008). Almost all people (95%) with celiac disease have either the variant HLA-DQ2 allele or (less common) the HLA-DQ8allele. However, about 20-30% of people with celiac disease have also inherited either of these alleles (Barker and Liu, 2008). This suggests additional factors are needed for celiac disease to develop; that is, the predisposinate HLA risk allele is necessary, but not sufficient to develop celiac disease.

Furthermore, around 5% of those people who do develop celiac disease do not have typical HLA-DQ2 or HLA-DQ8 alleles. The vast majority of people with celiac disease have one or two types of the HLA-DQ protein. HLA-DQ is part of the MHC class II antigen- presenting receptor (also called the human leukocyte antigen) system and distinguishes cells between self and non-self for the purposes of the immune system (Oberhuber *et al.*, 1999). The two subunits of the HLA-DQ protein are encoded by the HLA-DQA1 and HLA-DQB1 genes located on the short arm of chromosome.

FIGURE 1:DIAGRAM OF GENETIC



2.1.3 PRIMARY MECHANISM OF CELIAC DISEASE

The primary mechanism involved in celiac disease is related to an in appropriate-adaptive immune response to gluten-derived peptides. It has been ascertained that prolamines contain critical epitopes presented by either HLA-DQ2 or HLA-DQ8 induced a CD4+ T-lymphocytes response (TronconandJaber, 2011). In celiac disease pathogenesis, the role exerted by the intestinal epithelia barrier, physiologically impermeable to macromolecules such as gliadin is actually recognized. In people with a genetic susceptibility to develop celiac disease, gliadin interacts with the intestinal cells to trigger the disassembling of the inter-enterocyte tight junctions (TJS). The impairment of the TJS determines the up-regulation of Zonulin, a peptide involved in TJ regulation and responsible for the increased gut permeability. Gliadin peptides pass through the epithelial barrier and activate T-lymphocytes located in the lamina propria. Activated CD4+ T-lymphocytes produce high levels of pro-inflammatory cytokines, inducing either a T-helper 1 dominated by IFN-Y, and a T-helper 2 pattern, which causes a clonal expansion of B-lymphocytes that subsequently differentiate in plasma – cells secreting anti-gliadin and anti-tissue-transglutaminase antibodies (Bjorck *et al.,* 2015). Some gliadin peptides that are not recognized by T-lymphocytes activate both APCs and intestinal epithelial cells; in particular, CD8+ T-lymphocytes may be stimulated by interleukin (IL)-15. An increased density of CD8+ intraepithelial cells are considered as a hall mark of celiac disease (Troncone and Jabr, 2011). Gliadin-specific T-cell responses have been found to be enhanced by the action of tissue transglutaminase, an enzyme located in the extracellular space of the sub-epithelial region or at the epithelial brush border (Marthias *et al*., 2011).

2.1.4 FEATURES OF CELIAC DISEASE

The clinical presentation of celiac disease is remarkably varied and depends on age (Van Heel and West, 2006). The classic presentation with failure to thrive, malnutrition, diarrhea, abdominal pain and distension within the first couple of years of life, represents the tip of what is commonly referred to as the celiac disease iceberg.

In contrast to the dramatic presentation noted typically in younger children, many patients with celiac disease present at a later age with subtle symptoms and the diagnosis of celiac disease may be delayed. Gastrointestinal symptoms may include abdominal pain, diarrhoea or constipation, bloating and excessive gas. Avoidance of foods containing gluten may also occur and a careful diet history is necessary to identify this symptom. Vitamin deficiencies due to fat malabsorption can also occur. With longer standing disease, patients may present with profound vitamin D deficiency resulting in rickets or hypocalcemia and tetany or coagulopathy secondary to vitamin K deficiency. Anaemia secondary to iron and/or folate deficiency is also observed (Farrell and Kelly, 2002).

Children and adolescent often present with short stature and constitutional delay of puberty. Two to 8% of children and adolescents presenting for evaluation of short stature have evidence of celiac disease (VanRyn *et al.,* 2004). Once endocrine causes of short stature have been excluded, rates of celiac disease increase two to four –fold, depending upon the population and referral base studies (VanRyn *et al.,* 2004). Access to previous growth points may be useful in the differentiation between constitutional delay of puberty and an underlying pathological cause of short stature such as celiac disease.

Children presenting with celiac disease often will experience a decline in both height and weight growth velocity, resulting in a decrease in the growth percentiles.

In contrast, children presenting with constitutional delay of puberty often have low-normal growth velocity and will have no change in their growth percentiles. In the setting of declining growth percentiles or where the data are not available, the diagnosis of celiac disease should be entertained and testing with auto-antibodies performed (Catasssi and Fasono, 2004).

Adults having diarrhoea is a major symptom of celiac disease in approximately 50% of cases (Farrell and Kelly, 2004). They may also be diagnosed in the setting of anaemia or osteoporosis. Adults may be symptomatic for years prior to other diagnosis or have short stature (suggesting long standing celiac disease). They are often initially misdiagnosed with irritable bowel syndrome and may have multiple procedures and/or hospital admissions that can ultimately be traced to their undiagnosed celiac disease (Green and Cellier, 2007).

Patients identified by screening due to genetic risk factors are often asymptomatic or mildly symptomatic for celiac disease (Hoffenberg *et al.,* 2004). This is the population of individuals with celiac disease that is rapidly growing due to increased screening effects.

2.1.5 CLINICAL DIAGNOSIS OF CELIAC DISEASE

Many people with celiac disease do not know they have it. Celiac disease can be confused with other digestive disorder. Two tests can help to diagnose it (Mayoclinic, 2018), serology testing and genetic testing for human leukocyte.

Serology testing looks for antibodies in the blood. Elevated levels of certain proteins indicated an immune reaction to gluten (Mayoclinic, 2018).

Genetic testing for human leukocyte antigens (HLA-DQ2 and HLA-DQ8) can be used to rule out celiac disease (Mayoclinic, 2019). It is important to be tested for

celiac disease before trying a gluten-free diet. Eliminating gluten from diet might make the results of blood test appear normal. If the result of the tests indicates celiac disease, the doctor will likely order one of the following tests:

- Endoscopy - This test uses along tube with a tiny camera that will be put into the mouth and passed down the throat (Upper endoscopy). The camera will enable the doctor to view the small intestine and take a small tissue sample (biospsy) to analyse for damage of villi (Mayoclinic, 2019).
- Capsule Endoscopy - This test uses a tiny wireless camera to take the pictures of the entire small intestine. The camera sits inside a vitamin-sized capsule, which travels through the digestive tract, camera takes thousands of the pictures that are transmitted to a recorder (Mayo clinic, 2019)
- Skin biopsy- In case of suspected dermatitis herpetiformis, the doctor might take a small sample of skin tissue to examine under a microscope (Mayoclinic, 2019).

2.1.6 TREATMENT OF CELIAC DISEASE

A strict lifelong gluten–free diet is the only way to manage celiac disease. Besides wheat food that contain gluten include barley, bulgur, durum, farina, graham flour, malt, rye, semolina, spelt (a form of wheat), titican, e.t.c (Mayoclinic,2019). A dietitian who works with people with celiac disease can help in planning a healthy gluten-free diet. Even trace amounts of gluten in the diet can be damaging, even if they don’t cause signs or symptoms. Gluten can be hidden in foods, medications and non-food products, including: modified food starch, preservatives and food stabilizers; prescription and over-the-counter medications; vitamin and mineral supplements; herbal and nutritional supplements; lip stick products; toothpaste and mouth wash; communion wafers; envelope and stamp glue; play dough, etc.

Removing gluten from the diet will gradually reduce inflammation in the small intestine, causing the patient to feel better and eventually heal (Mayoclinic, 2019). Children tend to heal more quickly than adults.

2.1.7 Vitamins and Minerals Supplements

If the anaemia or nutritional deficiencies are severe, the doctor or dietitian might recommend some supplements, including copper, folate, iron, vitamin B-12, vitamin D, vitamin K and zinc. Vitamins and supplements are usually taken in pill form, if the digestive tract has trouble in absorbing the vitamins, the doctor might give them by injection (Mayoclinic, 2019).

2.1.8. Follow-Up-Care

Medical follow-up at regular intervals can ensure that the symptoms have responded to a gluten-free diet. The doctor will monitor the response with blood tests. For most people with celiac disease, a gluten-free diet will allow the small intestine to heal. Children usually take three to six months and adults complete healing might take several years (Mayoclinic, 2019).

2.1.9 Medications to Control Intestinal Inflammation

When the small intestinal is severally damaged or have refractory celiac disease, the doctor might recommend steroids to control inflammation. Steroids can ease severe signs and symptoms of celiac disease, while the intestine heals. Other drugs, such as azathioprine (Azasan, Imuran) or budesonide (Entocort EC, Uceris), might be used.

2.1.9.1 Treating Dermatitis Herpetiformis

When celiac disease causes skin rash, the doctor might recommend a medication such as dapsone, taken by mouth, as well as gluten-free diet. Taking of dapsone needs regulator blood tests to check for side effects.

2.1.9.2 Refractory Celiac Disease

When the patient has refractory celiac disease, the small intestine will not heal (Mayoclinic, 2019). Then the patient will likely need to be evaluated in a specialized center. Refractory celiac disease can be quite serious, and there is currently no proven treatment.

2.1.9.3 Allowed Foods

Many basic foods are allowed in a gluten-free diet, including eggs, fresh meat, fish and poultry that are not breaded, batter-coated or marinated, fruits, lentils, most dairy products, (unless they make the symptoms worse), nuts, potatoes, vegetables, wine and distilled liquors, ciders and spirits.

Grains and starches allowed in a gluten-free diet include: amaranth, buckwheat, maize, maize meal, gluten-free flours (rice, soy, maize, potato, beans), pure maize totillas, quinoa, rice, tapioca and wild rice.

2.2 DISEASES RELATED TO CELIAC DISEASE

A set of conditions can be associated with celiac disease. The term associated conditions refer to states that are found more frequently in patients affected by celiac disease (Simnons *et al.,* 2007). These conditions include “genetic disorders” such as Down syndrome, Tuner syndrome, Williams syndrome and auto-immune or “neurological” disorders.

2.2.1 Type 1 Diabetes

One of the most recognized and widely investigated disorders associated with celiac disease is type 1-diabetes (Silverstein *et al.*, 2005). Ludvigssson *et al*. (2013) reported that type 1-diabetes constitutes a 5-to-10 fold risk increase for celiac disease in a very large cohort of children. This increasing of the risk may partly be explained by shared genetic risk represented by Human Leucocyte Antigens (HLA). A percentage approximately of 5 %-10 % of patients is affected by celiac disease-related antibodies, with up to 75 % having abnormalities on small intestinal biopsy tissue (Bondy, 2007). The prevalence of celiac disease ranges between 1 % to 19 % in patients with type 1 diabetes mellitus (Van cleve and Cohen, 2008). Prospective studies of high-risk infants for type-1 diabetes and celiac disease have been shown that early introduction of gluten is associated with an increased risk for autoimmunity (Kaur *et al.*, 2002). However, the relationship between the two conditions is still debated.

2.2.2 Auto Immune Thyroid Disorders

In a patients affected by celiac disease, an increased prevalence (nearly 2 %-5 %) of thyroid disorders (i.e, hyper-thyroidism-Grave's disease or hypo-thyroidism-Hashimoto's thyroiditis), diagnosed either before than after the diagnosis of gluten-enteropathy has been reported (Michalski *et al.*, 1996). These two conditions share genetic risk factors represented by Human leucocyte Antigens (HLA-DQ2 and DQ8). HLA-DQ2 and DQ8 haplotypes have been associated with Hashimoto's thyroiditis, while HLA-DQ2 association is less clear in Grave's disease. This difference between hyper- and hypothyroidism is reflected also by the greater risk of celiac disease in patients with Graves (Catassi, 2005). In addition to HLA, an association with gene

encoding cytotoxic T-lymphocytes- associated antigen-4 has been reported (Michalski *et al*., 1996).

Another mechanism related to the association between the two conditions is represented by abnormal absorption with consequent selenium deficiency. In the setting of autoimmune thyroid disease, it should be useful to pay attention to celiac disease marker and to monitor growth and pubertal status. In patients with celiac disease, a screening for thyroid abnormalities has also been suggested in some cases (Hadithin *et al*., 2007).

2.2.3 Auto-Immune Hepatitis and Other Forms of Liver Involvement

The involvement of liver is common among patients affected by celiac disease (Zhernakova *et al*., 2010). Hyper-transaminasemia has been reported in about 40 % of adults and in 54 % of children with a diagnosis (Green and Cellier, 2007). Conversely, celiac disease is presented in about 9 % of patients with chronic unexplained hyper-transaminesia (hammers *et al*., 2008). It has been postulated that the mechanism leading to hepatic damage is related to entry of toxins, inflammatory molecules and antigens in the portal circulation. Volta *et al*. (Shan *et al.,* 2005) pointed out the need of a serological screening for celiac disease in all patients with persistent hyper-transaminasemia of unknown cause. Moreover, exclusion of a gluten-related liver damage is necessary for all patients affected by autoimmune liver disorders or by those form of severe liver disease, whose etiology remains unknown, and for patients enrolled for liver transplant. A prolonged exposure to gluten in a patient with an overlooked celiac disease, in fact, can cause chronic hepatitis and liver cirrhosis (Shan *et al.,*2005).

2.2.4 Neurological Disorders

A potential link between celiac disease and different neurological disorders has been reported (Fleckenstein *et al*., 2002). Data concerning the association between neurological conditions and celiac disease remain poor; although ataxia, as a neurological disorder, remains indicated in some patients with celiac disease (Koning *et al*., 2005). Its clinical spectrum in association with celiac disease varies from focal to generalized, with variable outcome and response to gluten avoidance. In 1985, a more specific and rare syndrome characterized by the co-presence of celiac disease, epilepsy and occipital calcification (CEC) was described. Gobbi suggested that the HLA genotype predisposing to CEC is the same as that which predisposes to celiac disease (Mowat, 2003).

2.2.5 Complications of Celiac Disease

Untreated celiac disease can cause the following (Mowat, 2003).

i. Malnutrition: This occurs if the small intestine cannot absorb enough nutrients. Malnutrition can lead to anaemia and weight loss. In children, malnutrition can cause slow growth and short stature.

ii. Bone Weakening: Malabsorption of calcium and vitamin D can lead to a softening of the bone (osteomalcia or rickets) in children and a loss of bone density (osteopenia or osteoporosis in adult).

iii. Infertility and miscarriage: Malabsorption of calcium and vitamin D can contribute to reproductive problems of infertility and miscarriage.

iv. Lactose Intolerance: Damage to the small intestine might cause abdominal pain and diarrhea after eating or drinking dairy products that contain lactose. Once the intestine has healed then the patient might be able to tolerate dairy products again.

v. Cancer: People with celiac disease who do not maintain a gluten-free diet have a greater risk of developing several forms of cancers, including intestinal lymphoma and bowel cancer.

vi. Iron deficiency anaemia: Iron is required in red blood cells formation in the body, which carry oxygen around the body. If there is no enough iron in the body, the blood will not have enough oxygen and this can lead to tiredness and short of breath.

2.3 YELLOW MAIZE

The term yellow maize comes from the Spanish word 'maiz' and is commonly referred to as yellow maize in English-speaking countries. Contrary to its improper categorization as vegetable; it is a grain or cereal crop. It is commonly eaten as a vegetable. Maize comes in many colours, including yellow, white, blue, red, black, green, purple, tricolour and multi-colour, depending on the variety (Vaughan and Geessler, 1997).

Yellow maize grows on stalks, commonly referred to as maize stalks, with each stalk producing several ears of maize with hundreds of kernels. The kernels are commonly eaten cooked, while the dried kernels are used for animal feed or ground into flour for baking (Vaughan and Geessler, 1997). Yellow maize is commercially grown in large quantities and harvested in fresh form for human consumption. Fresh maize is sold whole with silk and husks attached, as well as cut from the cob and the kernels frozen or canned for use in recipes or as a vegetable side dish.

The fresh, canned, or frozen kernels are then boiled or otherwise cooked before being eaten.

FIGURE 2: DIAGRAM OF YELLOW MAIZE

Many commercial growers also allow the kernels to dry on the stalks before harvest. The whole or crushed dried kernels are then sold as livestock feed. In dried form, yellow maize also is crushed or ground to various consistencies for commercial and home use in cooking and baking.

As a source of human food, yellow maize has a high starch content and is in fresh form a source of fibre and Vitamin C (Nawaz *et al*., 2015). In canned form, yellow maize loses its vitamin–A property, as well as some fibre content, but increases in vitamin C. When yellow maize is dried and coarsely ground; it is sold as maize grits. Maize grits are a fat-free food that contains iron, fibre, and protein (Nuss and Tanumchardjo, 2010). Grits are often used to make polenta, masa and porridge, or added to baked goods for added texture.

Yellow maize that is finely ground is often referred to as maize meal or maize flour. This is often used to make pancakes, cookies, waffles, maize bread, muffins, and other baked foods (Nuss and Tanumchardjo, 2010). Yellow maize is a versatile crop with many other uses including in the production of maize syrup, grains alcohol such as bourbon, maize oil and biofuel production. Many maize farmers set up labyrinths and mazes through their maize fields during the autumn months and provide tours and other harvest activities.

Maize seeds are used as a nutritional source for humans, while the stems and leaves are utilized as folders for cattle throughout the world. Maize silks and maize cobs are usually discarded as waste.

All parts of maize plant are good sources of varieties of bioactive phytochemical compounds, which possess antioxidant activity (Nuss and Tanumchardjo, 2010).

The principal phytochemicals of biological importance present in maize seed and silk include polyphenols, phenolic acids, flavonoids, anthocyanins, glycosides, carotenoids, and polysaccharides (reducing compounds) and some water-soluble vitamins (Nawaz *et al*., 2015). The presence of these phytochemicals makes maize a medicinal plant, which shows various biological activities, particularly the antioxidant, antimicrobial, antidiabetic, anti-obesity, anti-proliferative, hepato-protective, cardio-protective and renal-protective activities. On the account of its high antioxidant activity, all parts of maize plant can be used for the management of oxidative stress and the treatment of various diseases.

2.3.1 NUTRITIONAL COMPOSITION OF MAIZE

Due to its high nutritional quality, maize is a permanent global crop used to fulfil the nutritional requirements of human and cattle (Nuss and Tanumchardjo, 2010). Maize is rich in nutritional compounds such as carbohydrates, proteins, vitamins, and minerals including calcium, magnesium, potassium, and sodium salts (Nuss and Tanumchardjo, 2010). Maize seed contain sugars (16.39-21.20 g/100gdw), protein (11.46-12.70 g/100gdw) and crude oil (5.73-6.21 g/100gdw) (Nawaz *et al*, 2013). Maize silk contains moisture (9.65-10.4 %), protein (9.42-17.6 %), fat (0.29-4.74 %), ash (1.2-3.91 %), dietary fibre (7.34 %) and carbohydrates (65.5-74.3%) and good composition of vitamins and minerals as sodium, potassium (28, 1360 mg/100gdw) respectively. Calcium, magnesium, iron, zinc, manganese, and copper (0.1869, 0.1939, 0.005, 0.0165, 0.0109 and 0.0073 mg/g respectively (Emmanuel *et al*; 2016).

The processed maize silk contains significant amounts of crude fiber (13 %), crude protein (13 %) and carbohydrates (69 %). Being low in crude fat content; maize silk can be preferably used in the preparation of fat-free food formulation (Cabrera *et al.,* 2015)

2.3.2 PHYTOCHEMICAL COMPOSITION OF YELLOW MAIZE

Phytochemicals are the non-nutritional bioactive compounds found in various parts of plants. In plants, these compounds perform vital functions particularly in protection from predators and harsh environmental conditions. These compounds are also important in pharmaceutical and medicinal field due to their antioxidant, antimicrobial and other biological properties. Flavonoids are the bioactive phytochemical compounds which make the plant resistant to the attack of microbes and insects. It also protects the animals against various diseases (Silver *et al.,* 2002). The anthocyanins have been found to protect against ischemic reperfusion injury in rats (Toufeksian *et al.,* 2008). These have been also found to show antioxidant and antiradical activities which are further associated with certain health-promoting activities such as anticancer, anti-inflammatory, anti-obesity, anti-diabetic, cardio-protective and hepato-protective activities (Wang and Stoner; 2008). Tannins are polyphenolic compounds which show several biological activities such as anti-inflammatory, antioxidant, free-radicals-scavenging and anti-mutagenic activities (Kaur *et al.,* 1998).

Various parts of maize plant such as silk, seed, stem, leaves and roots are good sources of bioactive phytochemical compounds such as phenolic acids, flavonoids, steroids, alkaloids, carotenoids, tannins, saponins, anthocyanins and other phenolic compounds (Ebrahimzadeh *et al.,* 2008). Maize seeds contain polyphenols, phenolic acids, flavonoids, anthocyanins, carotenoids, vitamins, sugars, polysaccharides, and other phytochemicals of medicinal importance (Yang and Zhai; 2010). Maize silk contains a number bioactive phytochemicals compounds including phenols, polyphenols, phenolic acids, flavonoids, flavone glycosides, anthocyanins, carotenoids, terpenoids, alkaloids, steroids, lutein, tannins, saponins, volatile oils, vitamins, some sugars and polysaccharides (Table1) (Hasanudin *et al.,* 2012). The maize silk flavonoids have been also reported to reduce the oxidative stress and show anti-fatigue activity in mice (HUG-L *et al.,* 2010). The content of the major phytochemical compounds found in various parts of the maize are summarized in Table below:

TABLE 1: BIOACTIVE PHYTOCHEMICAL COMPONENTS IN VARIOUS PARTS OF MAIZE

MAIZE PART	CLASS OF PHYTOCHEMICALS	PHYTOCHMEICAL COMPONENTS	REFERENCES
Maize SILK	Polyphenols	Tannins, saponins, Flavonoids, Alkaloids, Steroids, Cardiac glycosides, Allantoins, Anthocyanins, Hesperdin and resins.	Emmanuel *et al.,* 2016.
	Phenolic acids	Para-aminobenzoicacid (PABA), Vanillic acid, P-coumaric acid, chlorogenic acid, Protocatechuric acid, caffeic acid, Ferulic acid, Maizenicacid, Hydroxycennamicacid, Ester and 3-0 Caffeoyl-quinic acid.	Jana *et al.,* 2016
MAIZE SILK	Flavonoids	Catechin, Protocatechin, Quercetin, Rutin, Flavones, 3-Hydroxyl, 4-Hydroxyl, 5-Hydroxyl, and 7-Hydroxyl flavones and Isoflavones-2-0-α-L-rhamnosyl-6-c-3-deoxyglucosyle-3-methoxylxuteolin and 6,4-dihydroxy-3-methoxy-flavone-7-0-glucoside. Isoorientin-2-2-0-α-L-rhamnoside, cardic glycosides, Luteolins, 2-0-α-L-rhamnosyl-6-c-quinovosy-luteolin, 2-0-α-L-rhamnosyl-6-C-fucosylluteolin, and 2-0-α-L-rhmanosyl-6-C-fucosyl-3-methoxylluteolin-2-0-α-L-rhamsnosyl-6-C-3-deoxyglucosyl-3-methylether, Kaempferol, tetrahydroxyl-flavone, ax-5-methane-3-methoxyflavone-7-0-glucosides, 7-4-dehydroxy-3-	Yang and Zhai, 2010 Jana *et al.,2016*

		methoxyflavone-2-0-α-L-rhamnosyl-6-C-fucoside.	
		β-Carotene, Zeaxanthin	Liu *et al.,*2011
		Phytosterols like stigmasterol, beta-sitosterol.	
	Carotenoids	Gallotannins Phlobatannins	
	Sterols		
	Tannins	Methol, Carvacrol, Thymol, eugenol, neolso-3-thujanol, cis-sabinene hydrate, 6,11-oxidoacor-4-ene-citronelle, trans-pinocamphone, cis-sabinene hydrate, cis-R-terpineol and neo-Iso-3-thu-janol	
	Volatile compounds		
		Vitamin C, Vitamin K, Vitamin E.	El-Ghorab *et al.,* 2007
		Dextrose, Xylose	
		Polysaccharides, geronul, Apigenin, Pelargo limonene, terpenoids, nedin, anthra-alpha-terpinol, citronellol, quinones, Trans-pinocamphone, Xanthoproteins	Rahman and Rosli, 2014
	Vitamins		
	Sugar		
	Miscellaneous		
		Tannis, Saponins, rutin, allantoins, Querceting,	Zhao *et al.,*2012

		Isoquercertin, morin, naringenin, kaemferol.	
MAIZE SEED	Polyphenols	Gallic acid, chlorogenic acid, syringicacid, hydroxycinnamicacid derivatives, Ferulic acis, 7-Hydroxy-2-Indolinone-3-acxetic acid, Caffeic acid.	Zhao *et al.,* 2012
	Phenolic Acid	Anthocyanins, Quercetin and Catechin	
	Flavonoids	Carotenes including Lutein. Cyclosadol, B-CRYPTOXANTHIN, Zeaxanthin, &- and B-Carotene, & and B-Cryptoxanthin.	Lewer and Banduriski,1987 Jung *et al.,*2015
	Carotenoids	Cyanogenic glycosides including Pelargonidin-3-glucoside, Cyanidin-3-glucoside, and Peonidin-glucoside.	Jung *et al.,* 2015
	Anthocyanins	Vitamin E (Tocopherols), Vitamin B (Biotin, Riboflavin, Pantothenic acid, Folic acid, niacin, Pyridoxine, Thiamine) and Vitamin C	
	Vitamins	Polysaccharides, Sugars, Proteins, Inositols, Resins, Hexaphosphoric and Maizenic acid, Esters of Indole-3-acetic acid, d-glucose hydroxyl-2-Indolinone-3acetic acid, N-Coumanyl tryptamine 6-methoxylbenzoazoine oxalic	Yang and Zhai; 2010

		acid, essential fatty acids, and Choline.	
	Miscellaneous compound		
			Gueveral *et al.,* 2000
		Methyl (E)-P-Cumarate, Methyl (Z)-P-Cumarate, Methyl Ferulate and 1, 3-O-deferuloyl glycerol	
		Tetrahydro-4, 6-bis (4-hydroxyl-3-methoxylphenyl)-1H, 3H-furo (3-4-C) furan-1-one.	Jung *et al.,* 2014
CORN STEM	Phenolic compounds	Tricin, Salcolin A and Salcolin B.	Jung *et al.,* 2015
	Ligan	Cyanidin-3-glucoside, Pelargonidin-3-glucoside, and peonidin-3-glycoside.	
			Jung *et al.,* 2015
	Flavonoids	Flavonoids, Terpenoids, Alkaloids, Tannins, Phlobatanins, Saponins	Toufektsian *et al.,* 2008
	Anthocyanins		

MAIZE ROOT AND SHOOT	Polyphenols, Flavonoids and Others		Nurhanan; 2012

2.3.3 ANTIOXIDANT PROPERTIES OF MAIZE

The pharmaceutical and medicinal significance of medicinal plants is usually based on their antioxidant phytochemical composition. Antioxidants are the substances which can prevent the oxidation reaction in living and non-living systems. They possess hydrogen donating ability due to which they reduce other species and are themselves oxidized. These substances have the ability to terminate the free radical chain reactions in the living system. Owing to their antiradical and reducing properties; the antioxidant phytochemicals play a key role in the preparation of pharmaceutical formulations against various diseases (Add a reference to support this paragraph).

The diversity in the phytochemical quality and high content of bioactive antioxidant phytochemicals make maize a valuable candidate for pharmaceutical applications. Among various parts of maize, the maize silk is a rich source of antioxidant activity, ferric reducing iron chelating, copper-reducing properties and free radical-scavenging capacities are presented in table 2-4.

The maize extracts have been also reported to improve the antioxidant status of various organs by affecting the activity of antioxidant enzyme (Huql and Deng, 2011)

Table 2: Total antioxidant activity and β – Carotene-bleach capacity of extracts from various parts of maize

MAIZE PART	EXTRACTING SOLVENT	TAOA	β-CABC	REFERENCE
MAIZE SILK	Water	73-44.19%		Nurhanan and WR; 2012
	Methanol		66.05%	
	Ethanol	5061-9.98mgFeSO4 gdw	52.92%	Nurhanan and WR; 2012
	Ethyl acetate	2.15-2.735mgGAE/gdw	38.65% 26.33%	Nurhanan and WR; 2012
MAIZE SEED	Methanol acidified with citric acid	1827.5-2429.3µmol TE/100gdw, 3.8µmol TE/g dw, 17.9-32.19%		Khampas *et al.,* 2013
		18-100µmol AAE/100g		
	Methanol acidified with HCl	22.95% TE		Kukm *et al.,* 2014
	Ethanol acidified with citric acid	0.3-10.2µmol/gdw		Herrera *et al.,* 2017
MAIZE COB	Methanol	31.10%		Khampas *et al.,* 2015

MAIZE HUSK	Methanol acidified with citric acid Methanol acidified with citric acid	11.85%		Simla *et al.,* 2015 Simla *et al.,* 2016

KEY:

TAOA: Total antioxidant activity fw: Fresh weight

β-CABC: β-Carotene-bleaching capacity dw: Dry weight

GAE: Gallic acid equivalent TE: Trolox equivalent

AAE: Ascobic acid equivalent

2.3.4 BIOLOGICAL ACTIVITIES OF YELLOW MAIZE

Epidemiological studies have demonstrated a relationship between the consumption of food with high quantities of phenolic compounds and a reduction in the risk of chronic and degenerative diseases. Maize seed possesses antidiabetic, antioxidant, anti-proliferative and anti-cataractogenic activities. (Nawaz and Shad., 2013).

Table 3: The biological activities of various extracts of different parts of maize.

MAIZE PART	EXTRACTING SOLVENT	ACTIVITY	REFERENCE
MAIZE SILK	Water	Diuretic and Kaliuretic activity with reduced glomerular function, anti-hepatocarcinomic, antiadipogenic, antiobesitic, antihyperglycaemic, antidiabetic, lipid lowering, hematinic, anti-	Chen *et al.,* 2014

		inflammatory, and analgesic activity.	
	Hot Water	Antioxidant activity and inhibition of IgE antibody formation in mice.	Namba *et al.,* 1993
	Methanol	Antioxidant, antimicrobial, anti-hyperthyroidism, Inhibition of lipid-peroxidation, Immunomodulation activity by enhancing the innate immunity, Lipid, lowering and cardioprotective activity.	Emmanuel *et al.,* 2016
	Ethanol	Inhibition of tumour necrosis factor-α and adhension of leukocytes to cell surface, activation of human peroxisome proliferator activator receptors, Induction of antioxidant enzymes and reduction of oxidative stress, antioxidant and free radical-scavenging, urease inhibitory, anti-hyperlipidemic and diuretic activity.	Karami *et al.,* 2014
	Aqueous alcohol	Anti-fatigue, hepatoprotective and renal protective activity in terms of inhibition of lipid peroxidation.	Jana *et al.,* 2016
	Aqueous acetone	Antioxidant activity	Nessa *et al.,* 2012

	Various Polarity solvents	Antioxidant activity in terms of free radical-scavenging, metal-reducing and beta-carotene-bleaching capacities and antimicrobial activity.	Catap *et al.,* 2015
	maize silk powder	Antioxidant and Immuno-stimulating activity in fish.	Karami *et al.,* 2014
MAIZE SEED	Methanol	Antioxidant activity in terms of free radical scavenging and metal reducing capacity.	Thiraphathanavong *et al.,* 2014
	Aqueous alcohol	Antioxidant and anti-cataractogenic activity against diabetic cataract.	Jung *et al.,* 2015
MAIZE STEM	Methanol	Anti-Inflammatory, neuroprotective, hepato-protective and antioxidant	Okokon *et al.,* 2017
MAIZE HUSK	Ethanol	Nephro-protective activity by dose-dependent increase antioxidant enzymes in diabetic rat.	

2.3.5 MEDICINAL IMPORTANCE OF YELLOW MAIZE

Maize seed kernel is commonly used as nutritional purpose but owing to its good phytochemical composition and biological properties; it has great medicinal value. The toxicological assessment of maize seed at various doses against various clinical parameters has proven it clinically nontoxic and can be used for nutritional and medicinal purposes (Hassanudin *et al*., 2012). Anthocyannins in purple waxy maize have been reported to be effective against diabetic cataract (Thiraphathanavong *et al.,* 2014). Maize silk is usually discarded as waste and not used for nutritional purpose. However, it has a great medicinal importance due to the presence of valuable bioactive phytochemical compounds. It has been traditionally used as an effective herbal remedy for the treatment of hyperglycemia, diabetes, obesity, *hypercholesterodemia*, hyperthyroidism, rheumatism, arthritis, gout, tumours, hepatitis, heart problems, jaundice, malaria, inflammation, asthma, prostatitis, cystitis, nephritis, kidney stones, bed wetting, renal conditions and other kidney-related diseases. Maize silk is also known to be urine laxative, antihypertensive and immune enhancer. Maize silk tea has been used as diuretic for the treatment of urinal irritation. In combination with other herbs, maize silk has been found to be effective against mumps or inflammation of the bladder. It has been also reported to be useful in gonorrhoea, acute and chronic cystitis and bladder irritation due to uric acid and phosphate gravel (Hassanudin *et al.,* 2012).

Recently, maize silk polysaccharides have been suggested to be a good choice as functional food or medicine for the treatment of type 2 diabetes mellitus due to its hypoglycaemic activity (Pan *et al.,* 2017).

2.3.6 FACTORS AFFECTING THE PHYTOCHEMICAL PROFILE AND ANTIOXIDANT POTENTIAL OF YELLOW MAIZE

There are several factors which have been reported to affect the phytochemical quality and antioxidant potential of various parts of maize. The phytochemical

composition and antioxidant profile of maize have been observed to be different in different varieties and at various stages of maturity (Nawaz *et al.,* 2013). The phytochemical content of silk has been found to be enhanced by treatment with red algae (Al-saman *et al.,* 2015). The location, climatic, water, stress, irrigation method and plant density significantly affect the growth, metabolism and physiological characteristics of maize plant (Bahadori *et al.,* 2015). The spraying of salicylic acid and collection period have been found to increase the growth rate and phytochemical content of maize silk (MHA-M; 2017).

The fermentation of maize samples has been found to result in an increase in carotenoid and ascorbic acid content with a slight decrease in antioxidant activity (Oladeji *et al.,* 2017). The germination conditions between light and dark periods have been also found to affect the morphological structures, biochemical and phytochemical composition and antioxidant activity of maize sprout (Xiang *et al.,* 2017).

The storage conditions, processing techniques and cooking methods have been also found to affect the phytochemical content and free radical-scavenging activity of maize (Cabrera *et al.,* 2015). Recently, studies in our laboratory have shown that high-dose gamma irradiation result in a decrease in antioxidant properties of maize flour (Nawaz *et al.,* 2016).

*2.4 Brychystegia eurycoma (*BE)

Bryschytegia eurycoma is an economic tree that belongs to the family caesalpiniaceae. It is a dicotyledinous legume that grows in the swaps or rain forest and well-drained soil of Southern-Eastern Nigeria and Western Cameroun. It is a huge tree which has twisted and spreading branches with a back that often exudes a buttery gum (Giami and Wachuku, 1997). Its flowers spring path between April and May and the fruits ripen between September and January. The fruits occur as broad leathery dark purplish brown pods containing four to six brown shiny flat disk like brown seeds with a hard dull (Igwe and Okwu, 2013).

B. *eurycoma* is called “Achi” in Igbo, “Ekalodo” or “Eku” in yoruba, “Okweri” in Edo, “Akpakpa” or “Taura” in Hausa, “Apaupan” in Ijaw and “Odukpa” in Ibibio (Bafor *et al.,*2017).

The seed flour, which is a good source of carbohydrate and fiber is used as flavour and thickening agents for soups in Eastern Nigeria (Okoli *et al.,*2015). The seeds are used in folkloric medicine to maintain body temperature, soften stool and protect against colon and rectal cancer (Ndukwu,2009). A range of proximate, phytochemical and pharmacological screening has been carried out in different parts of the plant following anecidotal account of its nutritious and medicinal value by local residents and traditional medicinal practitioners, respectively, in the localities where the plant predominantly grows. Phytochemical screening has shown that B. *eurycoma* contains diverse bioactive compounds alkaloids, saponins and tannins. Nutritious compound present in the plant include carbohydrate, proteins, lipids and minerals (Okwu and Okoro, 2006). Other than its nutritional value, different parts of the plants have been demonstrated to possess biological/pharmacologic activities, namely, analgesic, anti-inflammatory, anti-microbial, wound healing, anti-oxidant, anti-cancer and blood glucose lowering activities as well as lipid profile and liver enzyme modulation activities (Bhat and Krim,2009).

2.4.1 NUTRITIONAL VALUE OF *B. eurycoma*

The B. *eurycoma* seeds have been shown to be composed of certain nutrients, minerals, vitamins and food like chemicals which are essential to human nutrition (Nwacaudu *et al.,* 2017). Anti-nutrients such as cyanides, phytate and tannins may be toxic and result in poor palatability and bioavailability and hence, deficiency of certain nutrients e.g proteins were found to be significantly low in the seeds of *B. eurycoma* and the levels of these anti-nutrients detected were below the lethal dosage approved by regulatory body like National Agency for food drug and control in Nigeria (Bolanle *et al.,* 2014).Additionally, it has been proven that different processing methods of the seeds are subjected to the process of using it as food or medicine or for analysis could successfully reduce, remove or deactivate these anti-nutrients(Bhat and Krim, 2009).This evidence indicates that overall, the seeds

of *B. eurycoma* are composed of nutrients, essential minerals and vitamins and the presence of anti-nutrients is not an obstacle to its use as food or medicine.

Table 4: Anti-nutrient values of B. *eurycoma* harm seeds (Bolanle *et al.,* 2014).

Anti-nutrients	Value%
Cyanide	0.84
Phytate	0.296
Tannins	0.039

The plant, B. *eurycoma* is an affordable source of protein, carbohydrates and calories, while these nutrients are all essential to human nutrition, their composition in the plant differs (Nwakaudu *et al.,* 2017).Proximate analysis of the seeds has revealed a value of 7.2 % protein,14.0 % fat,59 % carbohydrate,<3 % crude fiber and>5%ash (Whitegbu *et al.,* 2009).In another report, the proximate analysis of the seeds showed the values of proteins, fat, carbohydrate, crude fibers and ash to be 8.75 %,4.49 %,53.51 %,17.2 % and 5 % respectively(Bolanle *et al.,* 2014).Yet another report revealed protein, carbohydrate, lipid and fiber contents of the seeds to be 7 %,71.74 %,4.2 % and 3.76 % respectively(Okwu and Okoro, 2006).The seeds have also been demonstrated to yield a total oil content of 5.87 g and analysis of this oil revealed the presence of fatty acids such as linoleic ,palmitic, oleic and stearic acids. The composition was similar to that of sunflower and groundnut seed oil, thus indicating the seeds as an alternative source of edible oil (Ajayi *et al.,* 2006).Similarly, fatty-acids in addition to phospholipids such as phosphatidyethanolmine have also been found to be present in the seed flour(Ajayi *et al.,* 2006).On the other hand, Ikegwu *et al.,* 2010 reported the crude protein, crude fat, crude fiber, moisture, total ash and starch content of the seed flour to be 12.77 %,10.52 %,2.2 %,10.25 %,1.48 % and 58.77 % respectively. The differences in these nutrients composition of the seeds highlighted in these report may be several factors such as environmental factors, the age of the plant, time of collection as well as difference in the method of processing the seeds for proximate analysis. This is more so as variations in proximate and nutritive compositions of the seeds of B. *eurycoma*

have been demonstrated to be due to different processing method (Aremu *et al.,* 2014).

2.4.2 MINERAL COMPOSITION

Analysis has revealed the presence of essential minerals (macro and micro elements) in the seeds of B. *eurycoma*. The macro and microelements that have been shown to occur in the B. *eurycoma* seeds are sodium, calcium, magnesium, potassium, phosphorous, iron, copper and zinc respectively. Harmful heavy metals or microelements such as lead, cobalt, chromium, arsenic and cadmium were not found following analysis of the seeds (Okwu and Okoro, 2006). The percentage occurrence of these minerals was different in these reports. This was probably due to the different methods used in preparing or processing the seeds for analysis as shown by Aremu and Ohale, 2014.

2.4.3 VITAMIN COMPOSITION

Nutritionally valuable water soluble vitamins necessary for different processes and functions in the human body have also been detected in the seeds of B. *eurycoma*. These include vitamins C (ascorbic acid), niacin (nicotinic acid), riboflavin (vitamin-B2) and thiamine (vitaminB1) (Okwu and Okoro, 2006). The realization of the vitamin content, as well as the mineral and nutrients content have continued to make the seeds attractive for culinary applications in the preparation of food, food products and nutraceuticals.

2.4.4 PHYTOCHEMICAL CONSTITUENTS

Phytochemical screening has revealed the presence of diverse secondary plant metabolites like flavonoids, alkaloids, phenolic compounds, saponin and tannins in stem bark and seeds of the plant. The presence of flavonoids ,alkaloids, saponin and tannin has been detected in the seed flour of the plant(Okwu and Okoro, 2006). Steroids,in addition to alkaloids, saponin and tannins have been also detected in the cold and hot aqueous extract and the ethanol extract of the stem bark(Okoli *et al.,* 2015). Ethanol extracts of the

seed flour and stem bark have also shown to contain alkaloids, tannins, flavonoid and phenol (Igwe and Okwu, 2013).Reports on the isolation and characterization of compounds from the plants are scarce. A new compound isolated from the seeds by column and thin layer chromatography methods has been characterized and name 2-(-4-ethylphenyl)-5-hydoxy-3-methyl-6,7-hyddro furo-chromen-4-0ne (Igwe and Echeme, 2013). Another furo-chromen-4-one, isolated by column and thin layer chromatographic techniques from the seeds are characterized as 5-hydroxyl-3-methyl-2-phenyl-6,7-dihydrofuro-chrome-4-one, has also been reported (Igwe, 2013).

Furthermore, another new polyphenol tertiary butyl compound name bis-6,6-methylenebis(4-ter-butyl2-methylphenol) has been isolated from the stem exudates of the plant (Igwe and Okwu, 2013). Several compounds have been isolated from the volatile oil extracted from the leaves of the plant. These compounds include oxygenated monoterpenoids (35.9 %), sesquiterponoid hydrocarbons (30.7 %);1,8-cinede (23.7 %) acorenone (10 %), β-caryophyllene (5.6 %), and geranyl acetone (4.5 %) (Ogunbinu and Ogunwande, 2006).

Other compounds that have been isolated include starch carbon and hydrocarbon (Ikegwu *et al*, 2010). The detection of several bioactive secondary plant metabolites and compounds on the plant has party prompted, researchers to screen extracts and isolate from parts of the plant for different pharmacological activity.

2.4.5 CULINARY APPLICATION

The seed flour of B. *eurycoma* is used in local culinary practices as food additives in soup making in Southern Eastern Nigeria. It serves as condiment flavouring agent, and thickening agent in soups (Ndukwu, 2009). It also serves the purpose of stabilization and emulsification in soups commonly consumed in south eastern Nigeria (Uhuegbu *et al.,* 2009). The soups in which it is normally used as food additive include egusi (melon), *ofe onugbu* (bitter leaf), oha (made from oha leaves) and ofe nsala. Additionally, it has been used in bakery products and meat-based products as a functional agent due to its absorption (Ikegwu *et al.,* 2010). Other than the flavor it imparts in soups, it also imparts a gummy texture when used in soups and this is a desirable

characteristic for the eating fufu, garri, pounded yam and other staple food normally eaten with soups (Nwosu *et al.,* 2011).

The observation that hydrocolloids-starch extracted from the seed flour has good pasting, temperature characteristic and swelling power properties (Nwosu *et al.,* 2011) prompted its trial as a stabilizing agent in watermelon fruit juice, and it was found to favourably compete with established hydrocolloids or food gums such as gum Arabic and guargum (Nwakaudu *et al.,* 2017). Additionally, it application as a stabilizer in Yoghurt has also been investigated. The sensory scores of yoghurt made with B. *eurycoma* seeds were generally accepted by a 20—man judging panellista and the youghurt contained higher protein, fat, ash and carbohydrate levels and lower moisture level versus control. The microbial count from the yoghurt was within acceptable range and no mould growth was observed. Overall, the evidence from this investigation showed that stabilizer from B.eurycoma improved the proximate ,organoleptic and physiochemical properties of stirred yoghurt (Mbaeyi-Nwaoha *et al.,* 2017). Besides its use as food based on its nutritional value, (nutraceutical value) has also been explored. The dietary inclusion of the seeds has been evaluated in the prevention of colon carcinogenesis in rat (Atawodis and Illiemene, 2017). Reports in this regard are very rare and the only one motioned in this review is still inconclusive. However, it is worthy of mention given that with time research may establish its eligibility as a useful neutraceutical while the evidence reviewed so far highlights its consumption as food due to its nutritional and medicinal values, other evidence which is highlighted in next paragraph shows that it is also consumed solely as medicine because of its medicinal value.

2.4.6 HEALTH BENEFITS OF *B. eurycoma*

Achi has a high medicinal value in its own right and also an excellent source of anti-oxidant and rich in vitamins (Irondi *et al.,* 2014). Studies have shown that BE has the following health benefits;

2.4.6.1 Analgesic and anti-inflammatory properties

BE extract has been reported to have analgesic, and anti-inflammatory properties. BE achieves this by blocking pain signals to the brain or interfering with the brain's interpretation of those signals. Anti-inflammatory agents block certain substances in the body that cause inflammation. They are used to treat many different conditions. Some anti-inflammatory agents are being studied in the prevention and treatment of cancer (Igwe and Okwu, 2013).

2.4.6.2 Anti-microbial and wound healing properties

Extracts and purely isolated compounds from the plant have been reported to have anti-microbial and wound healing abilities (Adekunle, 2000).

A 2013 study reported that the stem exudate has effective antibacterial activity and used traditionally in the treatment of wound and infections (Adekunle, 2000).

2.4.6.3 Anti-oxidant and anti-cancer properties

BE is listed as a good antioxidant. Antioxidant protect cells from damage caused by an unstable molecules known as free radicals. Laboratory and animal research has shown antioxidants help prevent the free radical damage that is associated with cancer (Atawodi and Iheme, 2017).

2.4.6.4 Anti-diabetic activity

The extracts and purely isolated compounds from the plant have been reported to have blood glucose lowering activities (Onyeso *et al.,* 2016). High blood sugar (hyperglycemia) affects people who have diabetes. Several factors can contribute to hyperglycemia in people with diabetes, including food and physical activities choices, illness, non-diabetes medications or skipping or not taking enough glucose-lowering medication.

2.4.6.5 Promotes heart health

This plant has been reported to have lipid lowering activities (Agukwe and Muazu, 2014). The fact is elevated low-density lipoprotein (LDL), the bad cholesterol, is a major cause of heart disease. LDL causes the build-up of fatty deposits within the arteries, reducing or blocking the flow of blood and oxygen the heart needs. This can lead to chest pain and heart attack.

2.4.6.6 Liver enzyme and gastrointestinal motility modulation activities

A new study suggests that the stem bark extract of *B. eurycoma* possesses anti-diarrheal property through antimotility, antisecretory effects possibly mediated by activity on muscarinic receptors and/or interaction with calcium channels (Okwu *et al.,* 2010).

2.5 SODIUM CARBOXYMETHYL CELLULOSE (CMC)

Sodium Carboxy-methyl cellulose (CMC) is a sodium salt derivative of cellulose. Unlike cellulose, it is water soluble and can function as a suspending agent stabilizer, film former or thickening agent (Hoefler, 2019). CMC funds use in gluten –free baking by providing dough with viscosity and cookies with volume much like gluten proteins do. It also functions well in filling as a thickner and in glazes as an agent to slow down sugar crystallization.

CMC can provide different functionality depending on its degree and uniformity of substitution by sodium ions, chain length and cellulose back bone. For example, CMC with uniform substitution is known for smooth flow properties and works well in frostings. CMC with non-uniform substitution is known to be thixotropic, forms a stable gel that becomes more fluid when agitated and reforms to a gel over time. Non –uniform substituted CMC works well in filling or sauces (Xin *et al.,* 2018).

CMC is derived from cellulose, the linear glucose based polymer found in plant material. Producing CMC is a two-step process. In the first step, cellulose is suspended in an alkaline solution which opens the cellulose chains and allows water to enter. When this happens, the cellulose can react with sodium monochloroacetate and yield sodium carboxymethyl cellulose (Hoefler, 2019).

Some baked good applications where CMC finds use include:

Frozen dough: Asa 0.5 % replacement for wheat flour and with a D.S of 1.1, CMC weakens the influence of frozen treatment on the gluten starch structure of the dough (Xin *et al.,* 2018).

Tortillas: CMC is added to tortillas for shelf life extension and to maintain a pliable texture.

Gluten free bread and cookies: Improves the internal structure like gluten proteins and helps with moisture retention and mouth free.

Fried dougs: At the level of 0.35 %, CMC can reduce oil absorption and improve the texture of fried products (Zecher and Gerrish, 1997).

Cookies: CMC functions as a release aid and spread controller (Zecher and Gerrish, 1997).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Chemicals and Reagents

Folin-Ciocalteu reagent, Na_2CO_3, gallic acid, Folin-Denis reagent, tannic acid, aluminum chloride, potassium acetate, methanol, distilled water, quercetin, acetic acid, NaOH, ethanol, iodine solution, amylose standard, $ABTS^{*+}$, $K_2S_2O_8$, ethanol, phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, DPPH. All other chemicals and reagents were of analytical grade.

3.1.1 Materials

Refined wheat *(Triticum aestivum*) flour (RWF), yellow maize grains (*YM*), and B. *eurycoma* (BE), were obtained from a local market in Ilorin, Kwara State.

3.1.2 Preparation of Samples

The samples (yellow maize grains and *B. eurycoma* seeds) were milled separately to obtain their individual flours. Composite flours were formulated by incorporating *B. eurycoma* flour (BEF) to whole meal yellow maize at four different

proportions (2.5, 5, 7.5 and 10%). Carboxyl methylcellulose (CMC) and refined wheat flour (RWF) flour served as reference hydrocolloid and flour, respectively. These were then used in cookies production, in which the cookies made from CMC composites and RWF served as the control cookies.

3.2. Determination of Functional Properties

3.2.1 Determination of Bulk Density (BD)

The method described by Nwakaudu *et al*. (2017) and Onwuka (2005) was adopted to determine the Bulk Density (BD) of flour samples. Graduated cylinder 100 ml was weighed dry, and gently filled with 50 g of flour sample. The bottom of the cylinder was then tapped gently on a laboratory bench several times. This was continued until no further diminution of the test flour in the cylinder after filling to mark was observed. Weight of cylinder plus flour was measured and recorded. The bulk density was calculated as follow:

$$\textbf{Bulk density (g/ml)} = \frac{\textbf{weight of sample (g)}}{\textbf{volume (ml)}}$$

3.2.2 Determination of Water Absorption Capacity (WAC)

The procedure described by Nwakaudu *et al*. (2017) and Onwuka (2005) was followed to determine the WAC of flour samples. Sample (1g) was mixed with 10ml of distilled water in a graduated centrifuged tube for 30 seconds. The suspension was left for 30 minutes at room temperature, and then centrifuged at

400 rpm for 15 min. Water absorption capacity was expressed as percent water bound per gram of the sample.

3.2.3. Determination of Swelling Power and Solubility

The method by Leach *et al.* (1959) was adopted for swelling power and solubility determination. Briefly, 1 g of the flour was mixed with 15 ml of distilled water in a 100 ml conical flask. The suspension was shaken for 5 min at low speed. It was then transferred into a water bath and heated for 40 minutes at 80-85 ^{o}C with constant stirring. Thereafter, 7.5 ml of distilled water was added and centrifuged at 2,200 rpm for 20 min. The supernatant was carefully decanted into a pre-weighed can and dried at 100 ^{o}C to constant weight. The can was allowed to cool to room temperature and then weighed. The sediment with the centrifuge tube was also weighed.

$$\textbf{Swelling power} = \frac{\textbf{Weight of sediment (g)}}{\textbf{Sample weight (g)} - \textbf{Weight of soluble (g)}}$$

3.2.4 Determination of Pasting Properties

The pasting properties of the flours were determined by the method of Deffenbaugh and Walker (1989), as reported by Irondi *et al.* (2019a), using a Rapid

Visco Analyzer (RVA) (model: RVA-4, Perten Scientific, Springfield, IL). The RVA was equipped with a personal computer (PC) containing the Thermocline software. The pasting characteristics of a suspension obtained from 3 g flour in 25 ml of distilled water, including peak viscosity, trough, breakdown, final viscosity, set back, peak time, and pasting temperature, was read on the PC, with the help of the Thermocline software. The results were expressed in Rapid Visco Analyzer units (RVU).

3.2.5 Determination of Starch and Free Sugar Content

The sample (0.02 g) was mixed with 10 ml of 80 % hot ethanol to extract sugar and starch. The suspension will be centrifuged for 10 min at 2000 rpm, and the supernatant (S1) was obtained and used for free sugar analysis (Onitilo *et al.,* 2007). To determine free sugar content, an aliquot of the supernatant (0.2 ml) was mixed with 0.5% phenol solution (0.5 ml) and conc. H_2SO_4 (2.5 ml). After cooling to room temperature, the absorbance of the reaction mixture was read at 490 nm. For starch determination, the residue was hydrolyzed with perchloric acid (7.5 ml) for 1h, and the solution will be diluted with distilled water to 25ml. The mixture was then filtered through filter paper (Whatman No. 2). The filtrate (0.05 ml) obtained was mixed with 0.5 ml of phenol solution (5 %) and 2.5 ml conc. H_2SO_4. The absorbance of the solution was read after it was cooled to room

temperature. The total free sugar and starch contents were obtained by calculation from a glucose standard curve.

3.2.6. Determination of Amylose and Amylopectin Contents

Amylose content of flour samples was determined using the method of Juliano *et al*. (1981). Briefly, 100 mg of sample was mixed with 1 ml 100 % (v/v) ethanol in a 50 ml centrifuged tube and 9 ml of sodium hydroxide (NaOH) was been carefully added, and the centrifuge tube was covered. The sample was heated for 10 min in a boiling water bath to gelatinize the starch, after which it was removed from the water bath and allowed to cool to room temperature. It was then filled up to the mark with distilled water, and well shaken thoroughly. 5 ml of the mixture was pipetted into another 50 ml volumetric flask. 1ml acetic acid and 2 ml of iodine solution was added, and the reaction mixture was made up to mark with distilled water. Absorbance was read using spectrophotometer at 620 nm against reagent blank. Subsequently, amylose content of the samples was calculated using amylose as a standard.

Amylopectin content of the samples was calculated by difference using the formula previously reported by Juan *et al.* (2006) as follows:

Amylopectin (%) = 100 − amylose (%).

3.3. Preparation of Methanolic Extract

Methanolic extract of each flour sample was prepared following the method of Chan *et al.* (2007) with a slight modification, 10 ml of methanol was added to 1 g of sample contained in a covered 50 ml centrifuge tube and was shaking continuously for few minutes at room temperature. The mixture was kept overnight, after which it was filtered to obtain the supernatant (subsequently referred to as methanolic extract), which was collected and stored at -4^oC until analysis

3.4. Quantitative Determination of Bioactive Constituents

The bioactive constituents (phytochemicals) in the samples were quantitatively determined according to the following standard procedures.

3.4.1 Determination of Total Phenol Content

The total phenolics content of samples methanolic extracts was determined according to the Folin–Ciocalteu method reported by Chan *et al.* (2007). Briefly, 300 µL of extract was dispensed into test tube. To this was added 1.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 ml of Na_2CO_3 solution (7.5 % w/v). The reaction mixture was shaken, and then incubated for 30 min at room temperature, before the absorbance was read at 765 nm against a blank, prepared by dispensing 300 µL of distilled instead of sample extract. Total phenolics content was expressed as gallic acid equivalent (GAE) in mg/g material.

3.4.2 Determination of Total Flavonoids Content

Total flavonoids content of sample extract was determined using aluminum chloride method as reported by Kale *et al*. (2010). Methanolic extract (0.5 ml) was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10 %), 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The reaction mixture was shaken, and then incubated at room temperature for 30 minutes, before absorbance was read at 514 nm. Total flavonoids content was expressed as quercetin equivalent (QE) in mg/g material.

3.4.3 Determination of Total Tannins Content

Tannins content of the sample extract was determined following the method reported by Amorim *et al*. (2008). Briefly, 0.1 ml of the supernatant was mixed with 7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, 1 ml of 35 % sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 mins and absorbance was read in a UV/Visible spectrophotometer at 760 nm. Blank was prepared with distilled water instead of the sample. Tannins content was expressed as tannic acid equivalent in mg/g material

3.4.4 Determination of Total Saponins

Total saponins content of the extract was determined by the method described by Makkar *et al*. (2007). In a test tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8 % vanillin in ethanol) and 2.5 ml of 72 % aqueous H_2SO_4 was added. The reaction mixture in the tubes was heated in a water bath at 60°C for 10 min. Then tubes were cooled in ice for 4 min and then allowed to acclimatize to room temperature. Subsequently, the absorbance was read in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard, and the results obtained was expressed as mg diosgenin equivalent per gram.

3.4.5 Quantification of Carotenoid Contents.

The carotenoid content of the flours and cookies were determined by adopting the method described by Howe and Tanumihardjo (2006). Carotenoids were extracted from the flours by mixing 0.6 g of the sample with 6 mL of ethanol (containing 0.1 % butylated hydroxyl toluene). The mixture was placed in a water bath at 85 ^oC for 5 minutes. Next, the interfering oil in the mixture was saponified with potassium hydroxide (80 % w/v) at 85 ^oC in a water bath for 5 min. The suspension was then mixed using a vortex machine and returned to the water bath for another 5 minutes. It was immediately transferred into a bath of ice, and 3 mL of cold deionized water was added. The carotenoid contents from the mixture were separated three consecutive times with 3 mL of n-hexane by centrifuging at 1000 rpm for 10 s. The upper layer of the mixture was dispensed into a 50 mL concentrator tube. The combined hexane fraction was washed thrice with deionized water, vortexed, and centrifuged for 10 s at 1000 rpm. The n-hexane fraction was dried down using a Turbo Vap (LIV) Concentrator under nitrogen gas for 25 min. The dried extract was reconstituted with

methanol/dichloromethane (1 mL, 50:50 v/v), and a 100 _L aliquot was injected into the HPLC system to quantify the carotenoids. The HPLC system (Water Corporation, Milford, MA, USA) comprised a guard column, C30 YMC carotenoid column (4.6 _ 250 mm, 3 _M), binary HPLC pump (Waters 626), auto-sampler (Waters 717), and a photodiode array detector (Waters 2996). The system operated with Empower 1 software (Waters Corporation). The mobile phase consisted of solvent A, containing methanol water (92:8 v/v) with 10 mmol/L ammonium acetate, and solvent B, containing 100 % methyl tertiary-butyl ether. Gradient elution was performed at a flow rate of 1 mL/min under the following conditions: 29 min of linear gradient from 83 % to 59 % A; 6 min of linear gradient from 59 % to 30 % A; 1 min of hold at 30 % A; 4 min of linear gradient from 30 % to 83 % A and a 4-min hold at 83 %. Chromatograms of the carotenoids were generated at 450 nm, and the specific carotenoids were identified and quantified using the external standards method based on the calibration curve from pure standards and comparison of the absorption spectrum and co-elution with standard carotenoids.

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3.5. Formulation and Baking of Cookies

Cookies were formulated and baked by the method reported by Okaka and Isiah (1990). Flour (200 g) from each sample of different flour blends was creamed with margarine (100 g) until light and fluffy constituency was obtained using Kenwood Chef with initial minimum speed and the speed was increased step wise until the mark of 6 on the chef indicator was obtained. Whole egg (60 g) was added, then flour (200 g), powdered milk (20 g), baking powder (0.1 g) and salt (1 g) was added

and mixed until a stiff paste (batter) was obtained. The batter was being rolled on a flour board using rolling pin to a thickness of 0.2 to 0.3 cm. The rolled batter was cut into shapes and arranged on a greased tray and baked at 150°C for 20 min. The cookies were brought out, cooked and packaged in cellophane bag until used.

3.6 *In Vitro* Antioxidant Activity

3.6.1. Determination of 1,1-Diphenyl-2-Picrylhydrazyl Free-Radical ($DPPH^*$) - Scavenging Ability

The free radical-scavenging ability of the methanolic extracts of flours and cookies samples against $DPPH^*$ was determined as described by Cervato *et al.* (2000), with slight modification. Briefly, appropriate dilution of the extracts (1 ml) was mixed with 3 ml of 60 µM methanolic solution of DPPH radicals; the mixture was left in the dark for 30 minutes before the absorbance was read at 517 nm. The decrease in absorbance of $DPPH^*$ on addition of test samples in relation to the control was used to calculate the percentage inhibition (% inhibition) following the equation:

$$\%\ \text{inhibition} = \frac{(\text{A517control} - \text{A517sample})}{\text{A517control}} \times 100$$

The SC_{50}, which stands for the concentration of extract required for 50 % scavenging activity, was calculated from the dose-inhibition linear regression equation of each extract.

3.6.2 Determination of 2,2-Azinobis (3-Ethyl-Benzothiazoline-6-Sulfonic Acid) Radical Cation ($ABTS^{*+}$) Radical-Scavenging Ability

The ABTS radical-scavenging ability of the extracts of flours and cookies samples was determined according to the method described by Sellappan and Akoh (2002). The ABTS radical was generated by incubating equal volume of a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 16 h at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95 % ethanol. Then 0.2 ml appropriate dilution of the extract was added to 2.0 ml $ABTS^{*+}$ solution and the absorbance was read at 734 nm after 15 min. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

3.6.3 Determination of Reducing Power

The reducing power of the methanolic extracts of flours and cookies samples was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5 ml aliquot of the extract was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was added. This mixture was centrifuged at 650 rpm for 10 minutes. Then 5 ml supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was read at 700 nm. The ferric reducing power was subsequently calculated.

3.7 *In Vitro* Enzyme Inhibition Assay

3.7.1 *In Vitro* Alpha-Glucosidase Inhibition Assay

Alpha-glucosidase inhibitory activity was conducted following the method reported by Kim *et al*. (2005) using α-glucosidase (EC 3.2.1.20) and para-nitrophenyl glucopyranoside (PNPG) as the substrate. Briefly, five units, aliquot of α-glucosidase was incubated with 20 µg/ml of the different flours and cookies methanol extracts for 15 min. Next, 3 mM PNPG dissolved in 20 mM phosphate buffer, pH 6.9 was added as a substrate to initiate the hydrolytic reaction. The hydrolytic reaction was allowed to proceed for 20 minutes at 37 °C, after which it was terminated by adding 2 ml of 0.1 M Na_2CO_3. The absorbance of the yellow *p*-nitrophenol released from PNPG hydrolysis was read at 400 nm. The percentage (%) inhibition of α-glucosidase was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{Absorbance400}_{\text{Reference}} - \text{Absorbance400}_{\text{Sample}}}{\text{Absorbance400}_{\text{Reference}}} \times 100$$

3.7.2. *In Vitro* Alpha-Amylase Inhibition Assay

Alpha-amylase inhibition assay was conducted using the method described by Kwon *et al*. (2008). Porcine pancreas α-amylase (EC 3.2.1.1) and soluble starch (substrate) was used in this assay. Different dilutions of methanol extract of flours and cookies samples totaling 500 µl, and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/ml α-amylase solution was incubated

at 37 °C for 10 minutes. Afterward, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer was added. Next, the reaction mixture was incubated at 37 °C for 15 min, after which 1.0 ml of DNSA color reagent (1 % 3, 5-dinitrosalicylic acid and 12 % sodium potassium tartrate in 0.4 M NaOH) was added to terminate the reaction. Next, the reaction mixture was incubated for 5 minutes in a boiling water bath, cooled to room temperature, and diluted with 10 ml of distilled water. The absorbance was read at 540 nm. The percentage (%) inhibition of α-amylase will be calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{Absorbance540}_{\text{Reference}} - \text{Absorbance540}_{\text{Sample}}}{\text{Absorbance540}_{\text{Reference}}} \times 100$$

%Inhibition= [(Absorbance540$_{\text{Reference}}$ − Absorbance540$_{\text{Sample}}$)/ Absorbance540$_{\text{Reference}}$] ×100

3.8 GLYCEAMIC INDEX EVALUATION OF THE COOKIES

Ethical approval: The study on the GI was approved by the Kwara state ministry of health with approval number MOH/KS/EU/777/493.

Sixty apparently healthy, non-diabetes human subjects (30 males and 30 females) between 28 to 50 years were recruited for the clinical trials upon obtaining their information consent. Measurement and calculation of GI were based on the method described by FAO/WHO (1998). Subjects used were with no known diseases and were not on any medications which could influence the results of the

study. They were strictly informed to abstain from cigarette smoking or alcohol and caffeine-containing drinks within the period of the study. All subjects fasted overnight (7.30 pm to 7.30 am) before the morning of testing and were instructed not to engage in strenuous physical activities prior to testing days. Data on height, weight, age, were obtained for each subject before the test.

3.9 Sensory Evaluation of cookies

Freshly baked cookies of the composite flours and a control cookie of 100 % WF were used for the sensory evaluation. Sensory evaluation was conducted according to the method described by Ranasalva and Visvanathan (2014) with a slight modification. Ethical approval was obtained from Kwara State Ministry of Health, Ilorin. The attributes of the cookies evaluated were: color, flavor, taste, chewiness and overall acceptability. Twenty panelists, out of which 12 were male and 8 were female genders of age ranging from 25 – 40, were given a hedonic scale questionnaire to evaluate the attributes using a 9 points scale (1- extremely dislike, 2- dislike very much, 3- dislike moderately, 4- dislike slightly, 5- neither like nor dislike, 6- like slightly, 7- like moderately, 8- like very much, and 9 - extremely like).

3.9.1 Data Analysis

Results of triplicate experiments were expressed as mean ± standard error of mean (SD). Analysis of variance (ANOVA) was carried out on the result data using SPSS software (21st version). Also, Duncan's Multiple Range Test was performed to

compare the means at 95 % confidence level. Graph pad prism 8 was used in plotting the graphs.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 PHYSICOCHEMICAL PROPERTIES OF THE FLOUR SAMPLES

TABLE 5: Function Properties of Samples

SAMPLES	Bulk density (g/ml)	% Water absorption Capacity	SOLUBLE SUGAR (g/100g)	% Amylopectin	% Amylose
100% yellow maize	1.26±0.00 [m]	170.53± 0.00 [c]	13.68 ± 0.02 [j]	81.69±0.00 [f]	18.31±0.00 [c]
100 % wheat flour	1.17±0.00 [d]	118.26±15.28 [a]	10.25 ± 0.03 [c]	76.19±1.70 [g]	20.8.1±1.70 [b]
100 % white maize	1.24±0.00 [k]	164.90± 0.20 [c]	10.80 ± 0.01 [d]	82.07±0.00 [i]	17.93±0.00 [a]
100 % BEF	1.23±0.00 [i]	174.41±0.06 [cd]	7.94 ± 0.12 [b]	98.04±0.00 [e]	1.96±0.00 [d]
2.5 % BEF	1.22±0.00 [g]	151.26±0.00 [b]	12.85 ± 0.00 [h]	91.94±0.24 [c]	8.06±0.24 [f]
5 % BEF	1.25±0.00 [l]	181.30±0.26 [d]	12.13 ± 0.14 [f]	89.11.10±0.97 [a]	10.89±0.97 [h]
7.5 % BEF	1.17±0.00 [c]	144.14±0.05 [b]	12.00 ± 0.03 [f]	87.63±0.00 [f]	12.37±0.00 [c]
10 % BEF	1.22±0.00 [f]	121.66±0.03 [a]	11.07 ± 0.03 [e]	86.97±1.95 [g]	13.03±1.95 [b]

2.5 % CMC	1.16±0.00 ^{b}	164.59±0.10 ^{c}	13.00 ± 0.02 ^{i}	71.31±0.24^{d}	28.68±0.24 ^{e}
5 % CMC	1.22±0.00 ^{h}	172.84±0.04 ^{cd}	13.87 ± 0.00 ^{k}	78.72±1.46 ^{e}	21.27±1.46 ^{d}
7.5 % CMC	1.16±0.00 ^{a}	165.78±0.05 ^{c}	12.79 ± 0.10 ^{h}	62..87±0.00 ^{h}	37.13±0.00 ^{i}
10 % CMC	1.23±0.00 ^{j}	126.58±0.08 ^{a}	12.44 ± 0.01 ^{g}	59.65±0.00 ^{g}	40.65±0.00 ^{b}
100 % CMC	1.19±0.00 ^{e}	150.14±0.02 ^{b}	5.82 ± 0.04 ^{a}	55.62±3.90 ^{b}	44.37±3.90 ^{g}

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

Table 5 presents the functional properties of the flours with their composites, and the mean values for water absorption capacity (WAC), bulk density (BD), water solubility (WS), and swelling power of the flour. Water absorption capacity is the amount of water taken up by the flour to achieve the desired consistency. Usually, it is defined based on the flour weight. WAC is important in bulking and consistency of products as well as baking application (Niba *et al.,* 2001). Ojinaka *et al*. (2009) reported that high WAC and retention suggests better performance in texture of baked product. Also, Amandikwu (2012) reported that water absorption capacity is a useful indication of whether flour can be incorporated into aqueous food formulation especially those involving dough formulations. Water absorption capacity is important in the development of ready to eat foods and a high absorption capacity may assure product cohensiveness (Houson and Ayenor, 2002).

In this study, the water absorption capacity of the flour samples ranges from 118.26 to 174.41 % with 100 % Achi and 2.5 % BEF recording the highest value of 174.41 % and 181.30 % respectively.100 % wheat recorded the least value of 118.26 %. It was observed that WAC decreased with increase in addition of CMC and BEF. This suggests that if the composite flours are incorporated into the food formulation like dough, it will improve its handling characteristic and

also freshness as reported by Ojinaka *et al*. (2009). Increase in WAC increases the tendency for increase digestibility (Ayele and Nip, 1994).

Bulk density (BD) is the weight of a unit volume of a loose material to the same volume of water. It is important in relation to its packaging (Okpala *et al.,* 2013). Increase in BD is desirable, in that it offers greater packaging advantages as greater quantities may be packed within constant volume (Molina *et al.,* 1983). Udensi and Eke (2000) reported that higher BD is desirable for greater ease of dispersibility and reduction of paste thickness. Also, Lewis (1990) reported that high BD is good physical attribute when determining mixing quality of particulate matter. Onimago and Egbekun (1998) buttressed the fact that a low BD is advantageous for the infants as both calorie and nutritional density is enhanced per feed of the child. The results of the bulk density ranged 1.16 – 1.26 g/ml. 100 % wheat had the lowest BD of 1.17 g/ml while 100 % yellow maize had the highest value of 1.26 g/ml. There was a significant difference ($p < 0.05$) between the samples. The variation in bulk density could be as a result of variation in starch content. Iwe and Onadipe (2110) reported that starch content increases bulk density.

Swelling power (SP) is the ability of flour to increase in volume when mixed with water. The extent of swelling however depends on the presence of water, heat, species of starch, extent of starch damage due to thermal and mechanical processes (Ojukwu *et al.,*2012). Table 5.3, presented the value for swelling power as one of the functional properties. There was a significant difference ($p < 0.05$) among the flour samples. The values ranged from 30.30 - 52.94 ml/ml in 100 % yellow maize and 100 % BEF, respectively. The results revealed a progressive increase in SP with increasing proportion of BEF and CMC. The control wheat flour had SP of 31.10 ml/ml.

The amylose content ranged from 1.96 -49.89 %, which differed significantly ($p<0.05$) among all the samples. Amylose content is an important

starch property as low amylose content leads to increased relative crystallinity of starch due to the reduced amorphous region within the starch granule (Olapade and Adeyemo, 2014). The amylose and amylopectin content of flours were reported by Irondi *et al*. (2017) to influence their functional properties and their food and industrial applications. In addition, amylose and amylopectin content of the flours impacts their glycaemic index (Shanita *et al.,* 2011). Generally, amylopectin content of the flours was higher than the amylose content, in line with the reports of previous studies that indicated that amylopectin contents is the predominant component of most starches (Yotsawimon *et al.,* 2008). The amylose and amylopectin content of flours were reported to influence their functional properties and consequently their food and industrial applications (Irondi *et al.,* 2017). In addition, amylose and amylopectin content of the flours or starches impacts their glycaemic index; with a lower amylose and a higher glycaemic index (Shanita *et al.,* 2011), due to their relative ease of digestion by alpha-amylase in human duodenum (Birt *et al.,* 2013). Thus, the low amylose contents of 2.5, 5, 7.5, and 10 % BEF blends relative to those of CMC, is an indication that they will have higher digestibility than the CMC formulations and consequently, higher glycaemic index.

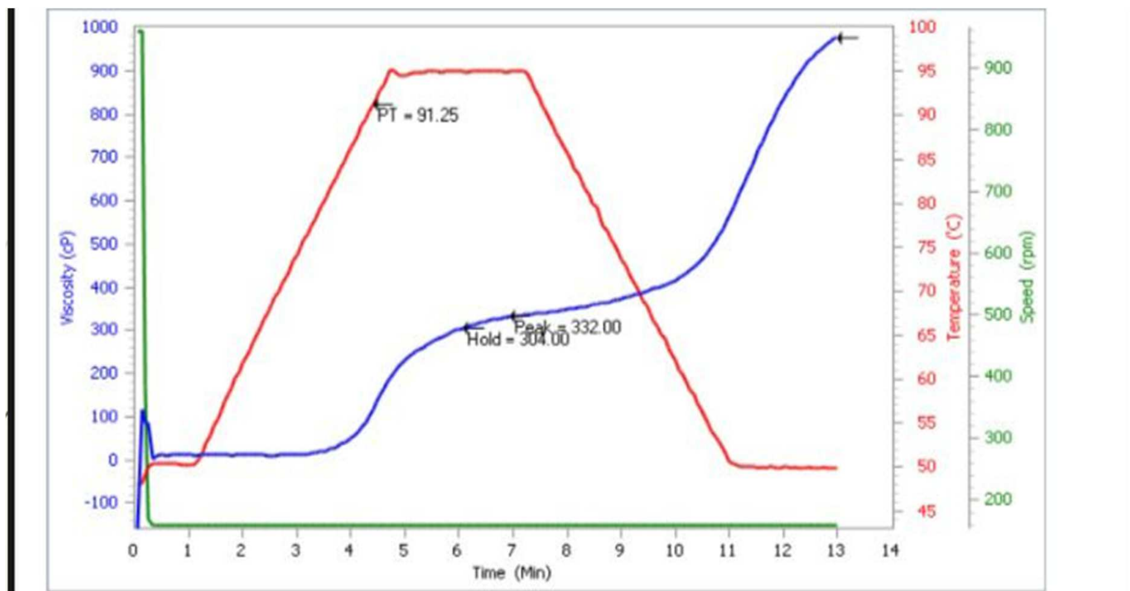
TABLE 6: PASTING PROPERTIES OF FLOUR SAMPLES

SAMPLES	PEAK (RVU)	TROUGH(RVU)	BREAKDOWN(RVU)	FINAL VISCOSITY(RVU)	PEAKTIME (MIN)	PASTING TEMP(^{O}C)	SET BACK(RVU)
100 %yellow maize	332[b]	304[c]	28[a]	976[b]	6.93[d]	91.25[c]	672[d]
100 % wheat	1007[c]	662[d]	345[c]	1451[c]	5.67[b]	87.3[c]	789[d]
100 %white maize	608[c]	495[c]	113[b]	1427[c]	4.93[a]	82.3[b]	932[d]
100 % BEF	5204[d]	2794[e]	2410[d]	8896[e]	7.0[d]	88.7[c]	6102[e]
2.5 % BEF	529[b]	473[c]	56[a]	1520[c]	7.0[d]	87.2[b]	1047[d]

5 % BEF	393^{b}	334^{c}	59^{a}	1221^{c}	7.0^{d}	92.05^{c}	887^{d}
7.5 % BEF	555^{b}	446^{c}	109^{b}	1800^{c}	7.0^{d}	89.6^{c}	1354^{e}
10 % BEF	831^{c}	652^{d}	179^{b}	2556^{d}	7.0^{d}	86.4^{c}	1904^{e}
2.5 % CMC	165^{a}	152^{a}	13^{a}	246^{a}	7.0^{d}	88.1c	94^{b}
5 % CMC	91^{a}	81^{a}	10^{a}	113^{a}	6.4^{c}	88.1^{c}	32^{a}
7.5 % CMC	505^{b}	463^{b}	42^{a}	780^{b}	-	79.15^{b}	317^{c}
10 % CMC	281^{a}	262^{b}	19^{a}	386^{a}	-	87.28^{c}	124^{b}
100 % CMC	14708^{e}	12772^{f}	1936^{d}	17673^{f}	6.0^{c}	50.05^{a}	4901^{e}

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

FIGURE 3: REPRESENTATIVE AMYLOGRAM OF PASTING PROPERTY



Pasting properties of a food refers to the changes that occur in the food as a result of application of heat in the presence of water. The changes affect texture, digestibility and end use of the food product. The results of the pasting properties of the flours are presented in the Table 4. There were significant differences ($p < 0.05$) in the pasting profile of the normal yellow maize flour, wheat flour, BEF, CMC flour and their composite. Several changes may occur upon heating a starch-water system, including enormous swelling, increased viscosity, translucency and solubility and loss of anisotropy (birefringence).

These changes are defined as gelatinization. The RVA results indicated that wheat flour, yellow maize flour, and flour from *B. eurycoma* seeds had distinct pasting properties compared to white maize flour and CMC flour. The pasting temperature of yellow maize, wheat flour, BEF and CMC flour were 91.25, 87.3, 88.7, and 50.0 ^{o}C, respectively.

The peak viscosity is the maximum viscosity attainable during heating cycle and it ranged from 91 RVU to 5204 RVU for the samples. Peak viscosity reduced with significantly ($p < 0.05$) with an increase in BEF and CMC. Reduction in peak viscosity could be due to lowering of starch as well as interaction between the starch fat and protein contents of the blends. Peak viscosity has been reported to be correlated with water binding capacity of starch which takes place at equilibrium point between swelling which causes an increase in viscosity while rupturing and realignment causes its reduction (Sanni *et al.*,2001). The trough viscosities for 100 % yellow maize and wheat flour were 304 and 662 RVU, respectively. The break down viscosity ranged from 13 to 2410 RVU in (2.5 % CMC and 100 % BEF, respectively) and differ significantly ($p<0.05$) among the flour samples. Adebowale *et al*. (2011) reported that the higher the break down viscosity the lower the ability of the flour sample to with stand heating and shear stress during cooking. 100 % Yellow maize flour had 672 RVU while whole wheat flour had 789 RVU respectively for set-back viscosity. The 100 % yellow maize recorded the highest peak time value of 6.93 minutes while wheat flour recorded an average peak time value of 5.67 minutes.

4.2 PROXIMATE COMPOSITION

TABLE 7: PROXIMATE COMPOSITION OF FLOUR SAMPLE

SAMPLE	Moisture content (%)		Crude protein (%)	Total ash (%)	Crude fibre (%)	Crude fat (%)	Total carbohydrate (%)	FOOD ENERGY (CAL/100g)
100%yellowmaiz	6.25±.006^{def}		12.78 ± 0.08 ^{l}	1.05 ± 0.04 ^{b}	2.94 ± 0.12 ^{h}	2.21 ± 0.08 ^{g}	74.76 ± 0.14 ^{a}	370.05 ± 2.09 ^{a}
100 %wheat	5.54 ± 0.02 ^{b}		11.01 ± 0.04 ^{g}	1.85 ± 0.15 ^{e}	1.06 ± 0.02 ^{c}	1.79 ± 0.09 ^{d}	78.73 ± 0.24 ^{g}	375.15 ± 3.11 ^{c}
100 %white maize	5.91 ± 0.12 ^{c}		9.61 ± 0.01 ^{f}	1.06 ± 0.01 ^{b}	1.14 ± 0.02 ^{c}	1.69 ± 0.00 ^{c}	80.58 ± 0.18 ^{i}	376.01 ± 1.04 ^{c}
100 % BEF	5.24 ± 0.02 ^{b}		12.02 ± 0.00 ^{j}	1.95 ± 0.12 ^{e}	0.58 ± 0.01 ^{b}	0.72 ± 0.01 ^{b}	79.48 ± 0.08 ^{h}	372.50 ± 0.43 ^{b}
2.5 % BEF	6.39 ± .021 ^{efg}		11.99 ± 0.02 ^{i}	1.69 ± 0.01 ^{d}	2.35 ± 0.01 ^{g}	2.54 ± 0.02 ^{h}	75.04 ± 0.01 ^{b}	370.98 ± 0.24 ^{a}
5 % BEF	6.67 ± 0.31 ^{g}		12.59 ± 0.09 ^{k}	1.52 ± 0.00 ^{c}	2.16 ± 0.04 ^{f}	2.11 ± 0.02 ^{f}	74.94 ± 0.15 ^{ab}	369.17 ± 1.63 ^{a}
7.5 % BEF	6.13 ± 0.21^{cde}		11.64 ± 0.01 ^{h}	1.58 ± 0.02 ^{cd}	2.06 ± 0.02 ^{de}	1.95 ± 0.02 ^{e}	76.62 ± 0.02 ^{de}	370.61 ± 2.33 ^{a}
10 % BEF	6.00 ± 0.63 ^{cd}		12.48 ± 0.02 ^{j}	1.48 ± 0.04 $_{c}$	2.02 ± 0.00 ^{d}	1.62 ± 0.02 ^{c}	76.38 ± 0.02 ^{d}	370.04 ± 2.94 ^{a}

2.5 % CMC	6.27 ± 0.03^{def}		7.66 ± 0.01 ^{b}	2.99 ± 0.02 ^{f}	2.05 ± 0.14 ^{d}	3.52 ± 0.04 ^{i}	77.50 ± 0.03 ^{f}	372.34 ± 4.31 ^{b}
5 % CMC	6.49 ± 0.01 ^{fg}		7.99 ± 0.04 ^{c}	3.04 ± 0.00 ^{f}	2.21 ± 0.02 ^{f}	3.64 ± 0.00 ^{j}	76.62 ± 0.04 ^{de}	371.24 ± 5.02 ^{a}
7.5 % CMC	6.07 ± 0.32 ^{cd}		8.54 ± 0.02 ^{e}	3.38 ± 0.09 ^{g}	2.36 ± 0.01 ^{g}	4.11 ± 0.01 ^{l}	75.58 ± 0.09 ^{c}	373.49 ± 2.71 ^{b}
10 % CMC	5.54 ± 0.02 ^{b}		8.11 ± 0.05 ^{d}	3.32 ± 0.00 ^{g}	2.14 ± 0.01 ^{ef}	3.88 ± 0.01 ^{k}	76.78 ± 0.02 ^{e}	374.50 ± 1.32 ^{c}
100 % CMC	3.97 ± 0.01 ^{a}		1.09 ± 0.00 ^{a}	0.86 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}	0.01 ± 0.0 ^{a}	94.04 ± 0.02 ^{j}	380.65 ± 2.91 ^{d}

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

Table 7 presents the proximate composition of the flour samples. The moisture content ranged from 3.97 - 6.67 %, with 100 % CMC having the least value of 3.97 % and 5 % BEF having the highest. The 100 % yellow maize had 6.25 % moisture content value and the refined wheat (control) flour had 5.91 %. The observed moisture contents were within the acceptable limit prescribed by the standard organization of Nigeria as reported by Sanni *et a*l, (2005). Fat content of the flour samples ranged from 0.01 - 4.11 %. The lowest fat content was recorded in 100 % CMC, followed by 100 % BEF, which had 0.72 % fat content. 100 % yellow maize had the higher fat content of 2.21 % than the control wheat flour which recorded 1.79 %. There was a significant difference among the samples as fat content reduced with increase in the proportion of BEF and increase with increase in the proportion of CMC. The 100 % yellow maize flour had the highest protein content of 12.78 % while the control wheat flour had 11.01 %. The 100 % BEF was higher in protein content than the 100 % CMC, with values of 12.02 % and 1.09 %, respectively. There were significant differences ($p < 0.05$) among the samples for protein. The percentage of crude fiber decreased with an increase in the percentage of BEF in the composite flour and increased with an increase in proportion of CMC. The crude fiber

value ranged from 0.03-2.94 %. 100 % CMC recorded the highest carbohydrate value of 94.04 %, follow by white maize which had 80.58 %. 100 % yellow maize and 100 % wheat flour had a very close range of total carbohydrates with 74.76 % and 78.73 % respectively. There were significant differences ($p<0.05$) among the samples for total ash content, which was an indication of the total mineral content of the flour samples. The value ranged from 0.86 - 3.38 %, with 7.5 % CMC flour recorded the highest value.

4.3 BIOACTIVE CONSTITUENTS

TABLE 8: Bioactive Constituents of Flour Samples

SAMPLES	TANNIN (mg/kg)	TOTAL PHENOLIC (mg/kg)	SAPONIN (mg/kg)	FLAVONOIDS (mg/kg)
100 % Yellow maize	1.42 ± 0.03 [a]	4.18 ± 0.07 [a]	0.38 ± 0.01 [a]	2.13 ± 0.04 [a]
100 %Wheat flour	4.80 ± 0.04 [f]	7.35 ± 0.03 [f]	1.99 ± 0.05 [g]	4.32 ± 0.06 [d]
100 % White maize	4.47 ± 0.01 [e]	6.87 ± 0.19 [e]	1.84 ± 0.04 [f]	4.08 ± 0.09 [c]
100 % BEF	10.32 ± 0.04 [g]	13.51 ± 0.01 [g]	5.13 ± 0.02 [h]	6.49 ± 0.36 [e]
2.5 % BEF	1.99 ± 0.04 [b]	5.17 ± 0.03 [b]	0.52 ± 0.04 [b]	1.95 ± 0.01 [a]
5 % BEF	2.09 ± 0.01 [b]	5.26 ± 0.01 [b]	0.97 ± 0.08 [c]	2.01 ± 0.04 [a]
7.5 % BEF	2.52 ± 0.04 [c]	5.58 ± 0.06 [c]	1.40 ± 0.03 [d]	2.20 ± 0.04 [ab]
10 % BEF	2.99 ± 0.05 [d]	6.39 ± 0.01 [d]	1.73 ± 0.03 [e]	2.41 ± 0.03 [b]
2.5 % CMC	1.42 ± 0.03 [a]	4.18 ± 0.07 [a]	0.38 ± 0.01 [a]	2.13 ± 0.04 [a]
5 % CMC	1.99 ± 0.04 [b]	5.17 ± 0.03 [b]	0.52 ± 0.04 [b]	1.95 ± 0.01 [a]
7.5 % CMC	2.09 ± 0.01 [b]	5.26 ± 0.01 [b]	0.97 ± 0.08 [c]	2.01 ± 0.04 [a]

10 % CMC	2.52 ± 0.04^{c}	5.58 ± 0.06^{c}	1.40 ± 0.03^{d}	2.20 ± 0.04^{ab}
100 % CMC	2.99 ± 0.05^{d}	6.39 ± 0.01^{d}	1.73 ± 0.03^{e}	2.41 ± 0.03^{b}

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

Phytochemicals are naturally found in plants and are responsible for providing colour, flavor and aroma to fruits and vegetables. They are biologically active and function to protect plants against invasion, disease and infection. Phenolic compounds were reported to have antioxidant, anti-inflammatory and anti-microbial properties (Yadav and Agarwala, 2011). Flavonoids are polyphenolic compounds that have been reported to have, anti-allergic, antioxidant, anticancer, vasodilatory, anti- inflammatory, anti- microbial and immune-stimulating activities (Igwe and Okwu, 2013a). Tannins are generally regarded as anti-nutrients because they interact with proteins and form insoluble complexes, thereby reducing their bioavailability. Their potential health benefits cannot be overlooked as it is widely accepted and known that tannis confer anti-tumors as well as biocidal activities and are utilized in ulcer management, wound healing and control of bleeding and burns.

Tannin content of the flour sample ranged from 1.99 mg/kg to 10.32 mg/kg. 100 % yellow maize and 2.5 % CMC composite had the least value of 1.99 mg/kg while 100 % BEF recorded the highest value of 10.32 %, followed by the control wheat flour which had (4.80 mg/kg). 100 % BEF recorded the highest value for total phenolic content (13.51 mg/kg), the control wheat recorded (7.35 mg/kg) and 100 % yellow maize, had the least value of (4.18 mg/kg). Saponin content ranged from 0.38 mg/kg to 5.13 mg/kg with 100 % yellow maize and 2.5 % CMC recorded the least value of 0.38 mg/kg with 100 % BEF recorded the highest value of 5.13 mg/kg. The control wheat flour had saponin value of 1.99 mg/kg.

Flavonoids ranged from 1.95 % to 6.49 mg/kg. 100 % BEF had the highest value of 6.49 mg/kg, followed by 100 % wheat which had 4.32 mg/kg. 100 % yellow maize recorded 2.13 mg/kg value of flavonoid.

Table 9A: CAROTENOIDS OF THE FLOUR SAMPLES

SAMPLES	TC (µg/g)	LUT (µg/g)	ZEA (µg/g)	β-Cryp (µg/g)	α-Caro (µg/g)	13-Cis-β (µg/g)	trans-β (µg/g)	9 Cis-β (µg/g)
2.5% BEF	16.06^{b}	3.52^{e}	9.51^{d}	1.92^{c}	0.75^{c}	0.49^{c}	0.99^{c}	0.47^{b}
5% BEF	2.81^{a}	3.46^{e}	6.89^{c}	1.79^{b}	0.72^{c}	0.45^{c}	0.90^{c}	0.45^{b}
7.5% BEF	0.84^{a}	2.70^{d}	7.20^{c}	1.43^{b}	0.55^{b}	0.35^{b}	0.76^{b}	0.39^{b}
10% BEF	0.71^{a}	2.98^{d}	8.01^{d}	1.67^{b}	0.62^{c}	0.37^{b}	0.85^{b}	0.42^{b}
100%Yellow M	15.34^{b}	3.52^{d}	9.88^{d}	2.14^{c}	0.78^{c}	0.52^{c}	1.07^{c}	0.56^{c}
100% Wheat	13.82^{b}	1.01^{b}	2.09^{b}	-	-	-	-	-
100% White M	12.50^{b}	1.67^{b}	-	-	-	-	-	-
100% BEF	13.76^{b}	0.47^{a}	-	-	-	-	-	-
2.5% CMC	15.20^{b}	3.24^{e}	8.79^{d}	1.93^{c}	0.71^{c}	0.46^{c}	0.98^{c}	0.46^{b}
5% CMC	15.01^{b}	3.28^{e}	9.06^{d}	1.90^{c}	0.72^{c}	0.47^{c}	0.97^{c}	0.46^{b}

7.5% CMC	14.69^{b}	3.02^{d}	8.32^{d}	1.82^{b}	0.70^{c}	0.39^{b}	0.93c	0.45^{b}
10% CMC	14.66^{b}	3.08^{d}	8.50^{d}	1.78^{b}	0.68^{c}	0.45^{c}	0.91^{c}	0.45^{b}
100% CMC	1.06^{a}	2.08^{c}	0.19^{a}	0.19^{a}	0.19^{a}	0.19^{a}	0.19^{a}	0.19^{a}

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

Table 9B: CAROTENOIDS OF THE FLOUR SAMPLES

SAMPLES	Total Xanthophyl (μg/g)	Total βC (μg/g)	PVA (μg/g)	Retinol activity (μg/g)
2.5 % BEF	13.03^{d}	1.94^{b}	3.28^{c}	0.55^{c}
5 % BEF	10.35^{c}	1.80^{b}	3.06^{c}	0.51^{b}
7.5 % BEF	9.90^{c}	1.50^{b}	2.49^{b}	0.42^{b}
10 % BEF	11.00^{c}	1.64^{b}	2.79^{b}	0.46^{b}
100 % yellow maize	13.40^{d}	2.14^{c}	3.60^{d}	0.60^{d}
100 % wheat flour	3.10^{b}	-	-	-
100 % white maize	1.67^{b}	-	-	-
100 % BEF	0.47^{a}	-	-	-
2.5 % CMC	12.03^{c}	1.91^{b}	3.23^{c}	0.54^{c}
5 % CMC	12.34^{d}	1.91^{b}	3.22^{c}	0.54^{c}
7.5 % CMC	11.34^{c}	1.77^{b}	3.03^{c}	0.50^{b}
10 % CMC	11.58^{c}	1.80^{b}	3.04^{c}	0.51^{b}

100 % CMC	2.28^{b}	0.58^{a}	0.78^{a}	0.13a

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

Carotenoids are class of phytochemicals with more than 600 naturally occurring pigments, synthesized by plants, algae and photosynthetic bacterial. Carotenoids are found in the most richly coloured yellow, orange and red fruits and vegetables like pumpkin, tomatoes, tangerines, carrots, and water melons. Carotenoids are well-known for their health benefits including vitamin A activity (Elemosho *et al.,* 2020), beta–carotene can be converted to retinol, which is a pre-cursor to vitamin A and it is important for maintaining healthy vision. Dietary intake of carotenoids has been shown to lower risk of chronic disease (Bertram, 1999; Johnson, 2002), Protection against chronic disease for example cardiovascular disease, Prostate cancer (Elemosho *et al.,* 2017) and antioxidant activity which has been identified as the mechanism under pinning their health benefits (Seifried *et al.,* 2007). In addition, Suguira *et al*. (2015) reported that consumption of carotenoids rich diets may confer protection against the development of type-2 diabetes.

Tables 9A and 9B presents the carotenoids profile of wheat, white maize, yellow maize, BEF, CMC and their composite flours. Carotenoids were lack in wheat flour, white maize flour and *B. eurycoma* flour but abundant in yellow maize and its composite flours. Total β-carotene (9-cis-β-carotene + 13-cis-β-carotene + all trans-β-carotene) ranged from 0.58 μg/g -1.94 μg/g; total xanthophylls (lutein + zeaxanthin) ranged from 2.28 μg/g- 13.40 μg/g and total provitamin A- carotenoids (β- cryptoxanthin +β- carotene + α-carotene) ranged from 0.78 μg/g – 3.60 μg/g. There was a significance difference in the carotenoids content of the composites flours. The ranges of the carotenoids

obtained in this study were similar to those previously reported by (Alamu *et al.,* Elemosho *et al*.,2021) for provitamin-A biofortified yellow maize hybrids.

4.4 ANTIOXIDANT COMPONENTS OF THE FLOUR SAMPLES

TABLE 10: ANTIOXIDANT COMPONENTS OF THE FLOUR SAMPLES

SAMPLES	DPPH* SC_{50} (mg/mL)	ABTS*+ TEAC (µM/g)	Reducing power (GAE mg/g)
100 % yellow maize	14.09 ± .005 ^{e}	63.20 ± 0.00 ^{b}	5.31 ± 0.05 ^{cd}
100% wheat flour	28.94 ± .004 ^{g}	59.97 ± 0.02 ^{bc}	4.04 ± 0.03 ^{b}
100 % white maize	12.71 ± .002 ^{de}	70.99 ± 0.00 ^{a}	6.20 ± 0.02 ^{d}
100 % BEF	8.19 ± .002 ^{a}	55.84 ± 0.04 ^{c}	5.84 ± 0.04 ^{cd}
2.5 % BEF	24.22 ± .033 ^{g}	51.99 ± 0.03 ^{def}	5.15 ± 0.04 ^{bcd}
5 % BEF	17.50 ± .013 ^{f}	48.24 ± 0.01 ^{fg}	4.75 ± 0.01 ^{bc}
7.5 % BEF	11.84 ± .002 ^{cd}	53.04 ± 0.01 ^{def}	5.43 ± 0.02 ^{cd}
10 % BEF	12.85 ± .002 ^{de}	52.99 ± 0.01 ^{def}	5.46 ± 0.03 ^{cd}
2.5 % CMC	10.31 ± .016 ^{b}	53.80 ± 0.01 ^{de}	4.67 ± 0.10 ^{bc}

5 % CMC	9.77 ± .014 [b]	47.86 ± 0.00 [fg]	2.48 ± 0.05 [a]
7.5 % CMC	10.56 ± .029 [bc]	48.96 ± .01485 [efg]	5.05 ± 0.09 [bcd]
10 % CMC	10.02 ± .032 [b]	44.59 ± .04596 [e]	6.28 ± 0.00 [d]

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

DPPH free radical scavenging method offers the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources. Free radicals are inevitably produced in biological systems and also encountered exogenously, and are known to cause various degenerative disorders such as mutagenesis, carcinogenesis, cardiovascular disturbances and ageing (singh and singh, 2008). These antioxidants are also produced by the biological system, and occur naturally in many foods and the balance between oxidants and antioxidants decides the health and vigor of individual (Halliwell, 1996). Thus it is important to know the antioxidant content and their efficacy in food for preservation or protection against oxidative damage to avoid deleterious changes and loss of commercial and nutritional value (Halliwell, 1997). The antioxidant activities of the flours are presented in the table 10. The DPPH and $ABTS^+$ scavenging, and Fe^{2+} chelating abilities of the whole wheat, whole white maize, BEF, CMC with their composite flours were presented. The DPPH scavenging ability ranged from 8.19 to 41.17 mg/ml. 100 % BEF had a low value of SC_{50}, 8.19 mg/ml. The DPPH SC_{50} of the flours differs significantly ($p<0.05$). Compared to the DPPH* SC_{50} range (15.4 – 40.2 mg/ml) in orange maize hybrids recently reported by Alamu *et al*. (2021), the yellow maize used in this study had a lower DPPH* SC_{50} of 14.09 mg/ml, indicating a stronger DPPH* scavenging activity. The control wheat flour had a higher value of SC_{50} (28.94 mg/ml). It well-known that a lower DPPH* SC_{50} value represents a stronger capacity of a sample to scavenge DPPH* (Irondi *et al.,* 2019c).

The results of $ABTS^+$ radical assays were presented as Trolox equivalent antioxidant capacity (TEAC) using Trolox as reference standard. The TEAC values ranged from 38.49 to 70.99 TEAC (µmolTrolox/g). 100 % BEF had the highest ABTS scavenging ability (55.84). The wheat flour had ABTS* scavenging value of 59.97 µmg/g while yellow maize had 63.20 µmg/g. Similarly, Fe^{2+} chelation SC_{50} of the flours differs significantly ($p<0.05$) from 2.48 to 6.30 GAE mg/g. 100 % yellow maize had Fe^{2+} chelating SC_{50} of 5.31 GAE mg/g while the control wheat flour had 4.04 GAEmg/g. The increased trend of the antioxidant activity of the flour blends observed in this study is similar to that of wheat flour blended with flour of some other staples, as reported by some previous studies (Ahmed, 2014; Irondi *et al.,* 2017). This increase may be attributed to the increase of the levels of various phenolic compounds due to BEF flour substitution. The increase of the free radicals scavenging ability of the flour blends suggests that the substitution of BEF may boost the ability of the baked food products to protect the body from harmful effects of free radicals such as oxidative damage to cellular biomolecules (protein, nucleic acids, carbohydrate and lipids (Takemoto *et al.,* 2009) and oxidative stress –related chronic diseases (Ward *et al.,* 2008).

4.5 STARCH-DIGESTING ENZYMES INHIBITORY ACTIVITIES OF FLOUR SAMPLES

TABLE 11: ALPHA-AMYLASE OF THE FLOUR SAMPLES

SAMPLES	IC_{50} VALUE (µg/ml)
2.5 % BEF	2.95
5 % BEF	3.28
7.5 % BEF	4.62
10 % BEF	11.03
100% Yellow maize	6.36
100% Wheat	11.22
100 % White maize	50.00

100 % BEF	47.44
2.5 % CMC	9.86
5 % CMC	6.81
7.5 % CMC	7.61
10 % CMC	4.43

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

TABLE 12: ALPHA-GLUCOSIDASE OF THE FLOUR SAMPLES

SAMPLES	IC_{50} VALUE (µg/ml)
2.5 % BEF	11.34
5 % BEF	11.2
7.5 % BEF	10.62
10 % BEF	11.03
100% Yellow maize	9.65
100 % wheat	12.2
100% White maize	11.4
100% BEF	7.15
2.5 % CMC	3.29
5 % CMC	14.9
7.5 % CMC	5.24
10 % CMC	3.81

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

Both alpha-amylase and alpha-glucosidase are involved in the digestion of dietary carbohydrates. Alpha-amylase in the small intestine hydrolyses starch α-

1,4 bonds to release oligosaccharides and dissacharides, α-glucosidase in the brush border of the small intestine complete the digestion by further hydrolyzing the oligosaccharides and disaccharides to yield absorbable monosaccharides including glucose and fructose (Tucci *et al.,*2010). Hence, the inhibition of these two digestive enzymes is well- established therapeutic approach for alleviating postprandial hyperglycemia in type-2 diabetes management and a key mechanism of action of many anti-diabetes agent (Irondi *et al.,*2019a) including drugs, natural product and functional foods. The lowest IC_{50} values of both α-amylase and α-glucosidase, displayed the strongest ($p<0.05$) inhibitory activity of these two enzymes.

4.6 COOKIES RESULTS

TABLE 13: Proximate Composition of Cookies

SAMPLES	% ASH	% PROTEIN	% FAT	% MOISTURE	% CHO	FOOD ENERGY (cal/100g)
100 %Yellow Maize	1.65 ± 0.28^{bc}	7.71 ± 0.27 ^{bc}	21.23 ± 0.19 ^{b}	10.93 ± 0.03 ^{f}	59.22 ± 0.21 ^{d}	458.84 ± 2.04 ^{b}
100 % Wheat Flour	1.32 ± 1.52^{ab}	7.42 ± 0.07 ^{b}	24.69 ± 1.27 ^{c}	9.52 ± 0.14 ^{de}	56.04 ± 1.08 ^{c}	476.07 ± 7.45 ^{c}
2.5 % BEF	2.10 ±0.09^{bcd}	6.65 ± 0.00 ^{a}	29.82 ± 0.29 ^{e}	8.38 ± 0.24 ^{bc}	53.03 ± 0.45 ^{b}	507.12 ± 0.89 ^{f}
5 % BEF	2.48 ±0.16^{bcd}	7.53 ± 0.10 ^{bc}	24.38 ± 0.55 ^{c}	8.62 ± 0.47 ^{bc}	56.97 ± 0.19 ^{c}	477.45 ± 5.31 ^{cd}
7.5 % BEF	2.69 ± 0.16^{cd}	8.56 ± 0.03 ^{d}	18.91 ± 0.38 ^{a}	9.61 ± 0.50 ^{e}	60.21 ± 1.08 ^{d}	445.31 ± 0.77 ^{a}
10 % BEF	0.28 ± 0.21 ^{a}	7.65 ± 0.18 ^{bc}	23.79 ± 0.69 ^{c}	8.01 ± 0.12 ^{ab}	60.26 ± 0.77 ^{d}	485.74 ± 3.90 ^{de}

2.5 % CMC	2.58 ± 0.19^{cd}	7.84 ± 0.00^{c}	23.49 ± 0.61^{c}	8.92 ± 0.08^{cd}	57.15 ± 0.89^{c}	471.47 ± 1.97^{c}
5 %CMC	2.73 ± 0.02^{cd}	7.77 ± 0.19^{bc}	28.72 ± 0.50^{e}	10.06 ± 0.04^{e}	50.85 ± 0.55^{a}	493.05 ± 1.54^{e}
7.5 % CMC	2.86 ± 0.18^{cd}	7.42 ± 0.13^{b}	20.55 ± 0.42^{b}	9.62 ± 0.27^{e}	59.53 ± 1.02^{d}	452.32 ± 0.42^{ab}
10 % CMC	3.16 ± 0.01^{d}	7.33 ± 0.18^{e}	26.16 ± 0.72^{d}	7.65 ± 0.41^{a}	55.69 ± 0.46^{ab}	487.54 ± 5.34^{e}

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

The proximate composition of the cookies produced from the composites of yellow maize and BEF, and yellow maize and CMC is presented in Table 13. The moisture content value ranged from 7.65 % to 10.93 %. 10 % CMC had the lowest moisture content of 7.65 % while 100 % yellow maize recorded the highest value of 10.93 %. Cookies made from 100 % wheat flour had a value of 9.52 %. These values are within the range of value obtained by Okpala (2011) using pigeon pea and cocoyam flours in their native state to produced cookies. The moisture content obtained in this study falls within 10 % limit recommended for safe storage pf cookies (FAO/WHO, 1994).

The protein content of the cookies ranged from 6.65 to 8.56 % in 2.5 % BEF 7.5 % BEF cookies, respectively. The control cookies made from 100 % wheat had protein content of 7.42 %. An increase in the proportion of BEF showed a progressive increase in the protein content of the cookies samples. The fat content of the cookies ranged between 18.91 – 29.82 % in 7.5 % BEF and 2.5 % BEF, respectively. Cookies made from 100 % wheat flour had 24.69 % fat. The pronounced increase in the fat contents in these cookies compared to the raw flours, therefore may be as a result of the butter fat used in the preparation of the cookies sample. The ash content of cookies increased with increase in proportion of CMC and BEF. The results recorded for ash ranged

between 0.28 - 3.16 % for 10 % BEF and 10 % CMC, respectively. The results obtained in this study for ash and crude fiber therefore suggested that incorporation of BEF could enhance the mineral intake of many people as ash is the indicative of the amount of minerals contained in a food sample (Atinuke, 2014). Also fiber content of all the cookies were with the recommended range of not more than 5 g dietary fiber per 100 g dry matter (FAO/WHO, 1994). The carbohydrate content of the cookies ranged between 50.85 % and 60.26 % for 5 % CMC and 10 % BEF. Cookies made from 100 % wheat flour had a value of 56.04 %. These values slightly different from those obtained in a study by Okpala (2011) in which the values ranged between 69.03 % and 72.13 % for cookies made with a composite of pigeon pea and cocoyam starch in their native forms. The cookies made from this composite may serve as a functional food product for many health managements especially in management of protein-energy malnutrition in children because of higher content of energy value/ cal. Incorporation of BEF into yellow maize flour for cookies production made it possible, since BEF is high in protein and mineral content

TABLE 14: BIOACTIVE CONSTITUENTS OF THE COOKIES

SAMPLES	**Total flavonoids (mg/kg)**	**Total phenolics (mg/kg)**	**Tannins (mg/Kg)**	**Total saponins (mg/Kg)**
2.5 % BEF	121.15 ± 0.18 ^{g}	118.05 ± 0.00 ^{c}	4.87 ± 0.01 ^{j}	1.05 ± 0.00 ^{g}
5 % BEF	80.00 ± 0.36 ^{c}	118.05 ± 0.00 ^{c}	4.50 ± 0.01 ^{i}	1.03 ± 0.00 ^{d}
7.5 %BEF	106.53 ± 0.18 ^{f}	118.05 ± 0.00 ^{c}	4.04 ± 0.01 ^{f}	1.04 ± 0.00 ^{f}
10 % BEF	59.35 ± 0.54 ^{a}	118.05 ± 0.00 ^{c}	4.41 ± 0.00 ^{h}	1.04 ± 0.00 ^{e}
100 % Yellow maize	144.48 ± 0.18 ^{h}	118.05 ± 0.00 ^{c}	3.70 ± 0.02 ^{e}	1.04 ± 0.00 ^{f}
100 % Wheat flour	93.84 ± 0.36 ^{d}	90.40 ± 39.10 ^{bc}	3.12 ± 0.00 ^{a}	1.02 ± 0.00 ^{a}
2.5 % CMC	59.48 ± 0.36 ^{a}	52.57 ± 0.20 ^{a}	3.36 ± 0.00 ^{b}	1.03 ± 0.00 ^{d}

5 % CMC	95.76 ± 0.18 [e]	55.44 ± 0.20 [a]	3.41 ± 0.00 [c]	1.02 ± 0.00 [b]
7.5 % CMC	58.71 ± 0.36 [a]	58.02 ± 0.20 [a]	3.44 ± 0.00 [d]	1.03 ± 0.00 [c]
10 % CMC	71.92 ± 0.54 [b]	76.79 ± 0.40 [ab]	4.08 ± 0.00 [g]	1.03 ± 0.00 [c]

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

Table 14 presents the bioactive content of the cookies. Total tannis of the cookies ranged from 3.12-4.87 mg/kg for (100 % wheat and 2.5 % BEF). 100 % wheat had 3.12 mg/kg of tannins and yellow maize recorded 3.7 0mg/kg, a value higher than the control wheat. Total phenolics ranged from52.57 mg/kg-118.05 mg/kg for 2.5 % CMC and 2.5 5 BEF respectively. 100 % maize had value higher than 100 % than control whole wheat (118.05 and 90.40 mg/kg respectively). Yellow maize (1.04mg/kg) and BEF composites (1.05 mg/kg) recorded the values higher than the whole wheat flour (1.02 mg/kg) nd CMC composites (1.03 mg/kg) respectively. Yellow maize had higher value of total flavonoids (144.48 mg/kg) than the control wheat of (93.84 mg/kg) and its ranged from 58.71-144.48 mg/kg (7.5 % CMC and whole yellow maize).

TABLE 15A: CAROTENOIDS PROFILE OF THE COOKIES

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

SAMPLE CODES	TC (µg/g)	LUT (µg/g)	ZEA (µg/g)	β-Cryp (µg/g)	α-Caro (µg/g)	13-Cis-β (µg/g)	trans-β (µg/g)	9 Cis-β (µg/g)
2.5 %BEF	7.73^{c}	0.62^{a}	1.40^{b}	1.02^{b}	0.47^{b}	0.83^{a}	2.44^{a}	0.54^{a}
5%BEF	3.74^{a}	0.88^{b}	2.10^{c}	1.63^{c}	0.61^{c}	1.06^{a}	3.15^{b}	0.75^{c}
7.5%BEF	6.68^{b}	0.96^{b}	2.23^{c}	1.61^{c}	0.58^{c}	1.30^{b}	3.15^{b}	0.72^{c}
10%BEF	9.38^{e}	0.93^{b}	2.31^{c}	1.54^{c}	0.59^{c}	1.33^{b}	3.16^{b}	0.68^{b}
100% Wheat	8.91^{d}	0.76^{a}	1.51^{b}	1.16^{b}	0.52^{b}	1.10^{a}	2.70^{a}	0.50^{a}
100%Yellow M	9.13^{e}	1.63^{c}	0.83^{a}	0.19^{a}	0.19^{a}	1.41^{b}	3.99^{c}	0.65^{b}
2.4%CMC	9.32^{e}	0.72^{a}	2.23^{c}	1.56^{c}	0.58^{c}	1.23^{b}	3.19^{b}	0.73^{c}
5% CMC	9.20^{e}	1.07^{b}	2.48^{c}	1.75^{c}	0.61^{c}	1.37^{b}	3.50^{c}	0.79^{c}
7.5%CMC	9.04^{d}	0.98^{b}	2.24^{c}	1.65^{c}	0.62^{c}	1.39^{b}	3.25^{b}	0.78^{c}
10%CMC	8.73^{d}	0.87^{b}	2.07^{c}	1.49^{c}	0.62^{c}	1.34^{b}	3.03^{a}	0.74^{c}

TABLE 15B: CAROTENOIDS PROFILE OF THE COOKIES

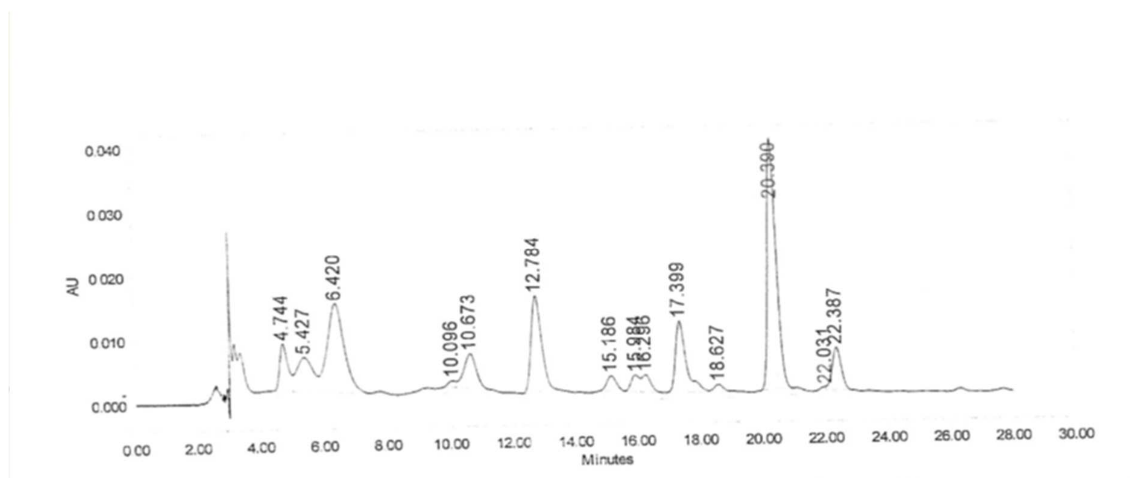
SAMPLES	Total Xanthophyl (µg/g)	Total βC (µg/g)	PVA (µg/g)	Retinol activity (µg/g)
2.5 %BEF	2.02^{a}	3.81a	4.55a	0.76a
5 % BEF	2.97^{b}	4.96b	6.08b	1.01
7.5 % BEF	3.18b	5.18c	6.27b	1.04b
10 % BEF	3.24b	5.17c	6.24b	1.04b
100% Wheat	2.27a	4.30b	5.14a	0.86a
100% Yellow M	2.46a	6.06d	6.25b	1.04b
2.5 % CMC	2.95b	5.14c	6.22b	1.04b

5 % CMC	3.55c	5.65c	6.83c	1.14b
7.5 % CMC	3.22b	5.42c	6.55c	1.09b
10% CMC	2.93b	5.11c	6.17b	1.03b

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

Table 15A and 15B contain the carotenoids content of the cookies. The high level of total xanthophyll, total β- carotene, PVA, and retinol activities of the cookies relatives to their corresponding flours may be attributed to the addition of egg and butter in the production of the cookies. The co-consumption of whole egg with carotenoids-rich foods may increase overall carotenoids absorption via lipid-rich egg yolk (Kim *et al.,* 2016).

FIGURE 4: REPRESENTATIVE AMYLOGRAM OF CAROTENOIDS



4.7 STARCH-DIGESTING ENZYMES INHIBITORY ACTIVITIES OF THE COOKIES

TABLE 16: ALPHA-AMYLASE OF THE COOKIES

SAMPLES	IC_{50} VALUE (µg/ml)
2.5 % BEF	2.97
5 % BEF	2.23
7.5 % BEF	3.52
10 % BEF	1.94
100% BEF	1.42
100 % Wheat	2.63
2.5 % CMC	1.96
5 % CMC	2.17
7.5 % CMC	1.77
10 % CMC	1.91

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

TABLE 17: ALPHA-GLUCOSIDASE OF THE COOKIES

SAMPLES	IC_{50} VALUE (µg/ml)
2.5 % BEF	1.34
5 % BEF	2.51
7.5 % BEF	6.72
10 % BEF	1.94
100% Yellow maize	1.42
100% Wheat	2.29
2.5 % CMC	1.12
5 % CMC	2.15
7.5 % CMC	1.33
10 % CMC	2.51

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

The results of enzymes (α-amylase and α-glucosidase) inhibitory activities of the wheat, yellow maize, CMC, BEF with their composite flours and cookies are presented in Figure 3 in-terms of their IC_{50} values. Among the flours, 100 % yellow maize had the lowest IC_{50} values indicating the strongest inhibitory activities towards the tested enzymes, while 100 % wheat

had higher values of IC_{50}, compared to yellow maize (the weaker inhibitory effect). Relatives to the IC_{50} values reported for red sorghum variety against α-amylase, and α-glucosidase 16.93 ± 1.08 and 10.78 ± 0.63 µg/ml, respectively (Irondi *et al.,* 2019a). The IC_{50} values of yellow maize used in this study against the same enzymes (6.36 and 9.65 µg/ml respectively) are higher than the reported sorgum. The variations in the IC_{50} values obtained in this study against the tested enzymes and those previously reported, may be attributed to differences in the sample extraction methods, genotype and environmental factors (Mpofu *et al.,* 2006). Furthermore, the IC_{50} values of the cookies in this research(towards the tested enzymes decreased relatively to their corresponding flours, indicating an increase in the level of bioactive constituents in the cookies, in comparison to their corresponding flours. The bioactive constituents analysed in the flours and the cookies (phenolics, saponins, tannins, and total flavonoids), have been shown to inhibit digestive enzymes, including α-amylase and α-glucosidase (Irondi *et al.,* 2019a; Liu and Xu, 2015). Phenolic compounds, for instance have a high affinity for proteins through hydrogen and hydrophobic interaction, a property that enables them to inhibit digestive enzymes by protein denaturation (Villiger *et al.,* 2015).

4.8 TABLE 18: SENSORY ATTRIBUTES OF THE COOKIES

Samples	Taste	Appearance	Colour	Crispiness	Aroma	Overall aceptability	Texture

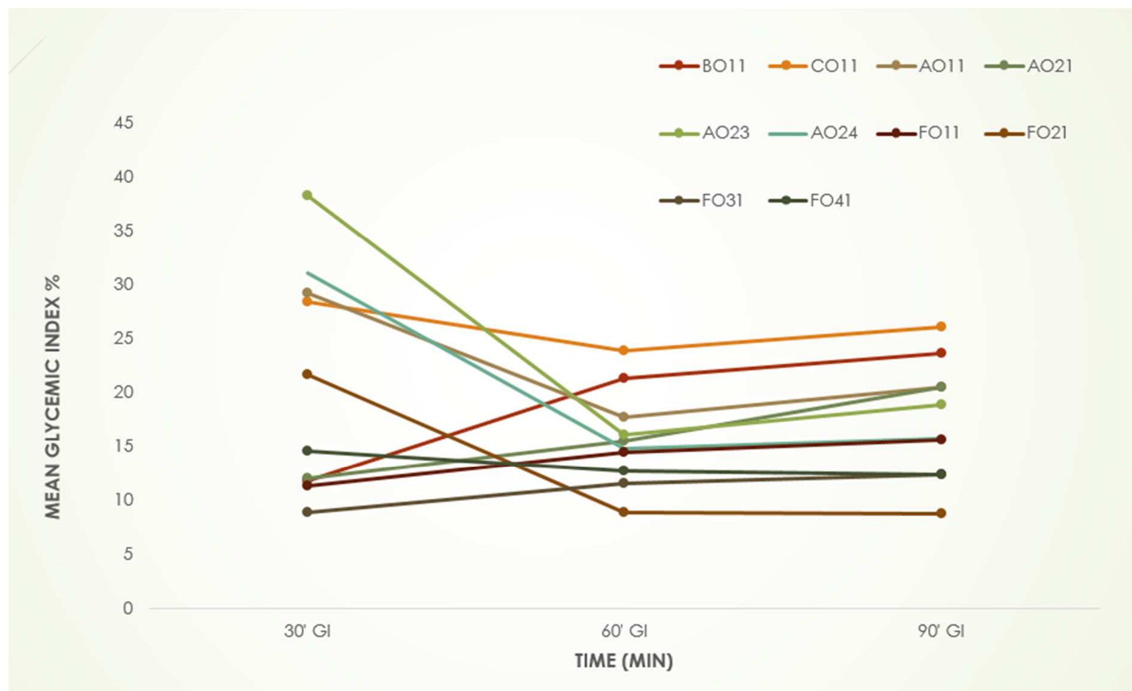
100% Yellow Maize	7.70 ± 1.65 ^{c}	8.29 ± 1.45^{b}	8.11 ± 1.23 ^{c}	7.35 ± 1.15 ^{a}	7.82 ± 0.85 ^{c}	7.88 ± 1.15 ^{b}	7.23 ± 1.15 ^{a}
100% Wheat	7.1 ± 1.23 ^{a}	7.2 ± 1.02^{a}	7.2 ± 0.75 ^{a}	7.1 ± 2.05 ^{b}	7.65 ± 1.08 ^{a}	7.90 ± 1.05 ^{b}	7.70 ± 0.8 ^{c}
2.5% BEF	7.9 ± 1.08 ^{c}	8.0 ± 0.00^{b}	8.1 ± 1.11 ^{c}	7.35 ± 1.20 ^{b}	7.45 ± 1.25 ^{a}	8.40 ± 1.20 ^{c}	7.9 ± 1.25 ^{d}
5% BEF	7.2 ± 2.28 ^{a}	7.25 ± 1.25^{b}	7.5 ± 1.25 ^{a}	7.25 ± 0.75 ^{b}	7.40 ± 1.50 ^{a}	7.6 ± 1.50 ^{a}	7.2 ± 1.25 ^{a}
7.5% BEF	7.0 ± 0.00 ^{a}	8.0 ± 0.00^{b}	7.75 ± 0.75 ^{b}	7.2 ± 0.84 ^{b}	7.50 ± 0.95 ^{b}	8.1 ± 2.10 ^{c}	7.45 ± 1.05 ^{b}
10% BEF	7.15± 1.89 ^{a}	7.95 ± 0.75 ^{b}	8.1 ± 1.25 ^{c}	7.65 ± 1.25 ^{b,c}	7.55 ± 1.65 ^{b}	7.9 ± 1.25 ^{b}	7.65 ± 1.08 ^{a}
2.5% CMC	7.4 ± 2.04 ^{b}	8 ± 15.28 ^{b}	8.15 ± 1.05 ^{c}	6.65 ± 1.97 ^{a}	7.6 ± 0.75 ^{b}	7.95 ± 1.26 ^{b}	7.6 ± 1.05 ^{c}
5% CMC	7.8 ± 2.34 ^{c}	7.95 ± 0.75^{b}	8.1 ± 1.05 ^{c}	7.25 ± 0.75 ^{b}	7.85 ± 1.05 ^{c}	7.95 ± 0.65 ^{b}	7.55 ± 1.05 ^{b}
7.5% CMC	7.8 ± 1.24 ^{c}	8.05 ± 1.28 ^{b}	7.75 ± 0.50 ^{b}	7.1 ± 1.62 ^{a}	7.65 ± 1.15 ^{a}	7.9 ± 0.21 ^{b}	7.2 ± 0.85 ^{a}
10% CMC	7.25 ± 2.18 ^{a}	7.95 ± 1.35 ^{b}	8.1 ± 1.24 ^{c}	7.95 ± 1.22 ^{c}	7.60 ± 0.75 ^{a}	7.75 ± 1.05 ^{a}	7.6 ± 1.10 ^{c}

Results are mean ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$).

The sensory attributes (colour, flavor, taste, crispness, aroma, texture and overall acceptability) are presented in table 4.16. Relative to the 100% wheat flour cookies (reference cookies), the cookies of 100 % whole maize flours and the blends (2.5 %BEF, 5 % BEF, 7.5 % BEF, 10 % BEF, 2.5 % CMC, 5 % CMC, 7.5 % CMC, 10 % CMC) were rated significantly higher ($p<0.05$) in all the sensory attributes. The cookies sample that had the lowest sensory ratings, especially in colour, aroma and overall acceptability was 100 % wheat flour, this may be attributed to the colour of the wheat flour.

4.9 GLYCEAMIC INDEX OF THE COOKIES

Figure 5: GLYCEAMIC INDEX OF THE COOKIES



KEYS: AO11=2.5 % BEF, AO21=5 % BEF, AO23=7.5 % BEF, AO25=10 % BEF, BO11=100 % YELLOW MAIZE, CO11=100% WHEAT FLOUR, DO11=100 % WHITE FLOUR, EO11=100 % BEF, FO11=2.5 % CMC, FO21=5 % CMC, FO31=7.5 % CMC, FO41=10 % CMC

Figure 5 showed the glycaemic index of all the cookies at 0 minute, 30minutes, 60minutes and 90minutes intervals. The mean GI of all the cookies were between 10 to 45, which apparently fall to the value of low GI (Jenkin *et al*,1981) with significance difference of ($P<0.05$).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The results of this study showed that addition of *Brachystegia eurycoma*) and CMC (sodium methylcellulose) at four different proportions (2.5, 5,7.5 and 10 %) had increase effects on the functional properties, phytochemical composition, antioxidant and enzyme Inhibitory activities of yellow maize and its blends. Beta-carotene was very high in the yellow maize, but lacking in wheat flour. Hence, the presence of four major carotenoids (alpha-carotene, beta-carotene, lutein and zeaxanthin) available in yellow maize made it a good substitute for gluten- free cookies. Whole yellow maize flour: *brachystegia eurycoma* (BEF) composite flours cookies produced gradual rise in blood sugar level, which lower the glycaemic index. BEF displayed highest nutritional contents, phytochemical contents and antioxidant activities than the CMC, the cookies produced from the composite flours may serve as functional food products for protein-energy malnutrition in children, type-2 diabetes and most importantly celiac disease. Therefore, the addition of BEF and CMC into whole yellow maize flour may be a viable option for developing gluten-free cookies with enhanced health benefits for people susceptible to gluten allergy and the resultant celiac disease.

5.2 RECOMMENDATION

Based on the findings of this study, further research is recommended to establish the bioactivities of the gluten-free cookies formulated from the blend of whole yellow maize flour and natural hydrocolloids (BEF) in a clinical trial. When the efficacy of the gluten-free cookies is established in a clinical trial, they can be produced in a large-scale, as functional cookies for people that are susceptible to celiac disease and type-2 diabetes.

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