Evaluation of Nutritional and Microbiological Quality of biscuit fortified with malted millet flour
تقييم جودة البسكويت المدعم بدقيق الدخن المنبت

Dissertation Submitted to Sudan University of Science and Technology in Partial Fulfillment for the Requirements of the Degree of Master of Science in Food Science and Technology

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الأية

بسم الله الرحمن الرحيم

قال تعالى:

(مَثَلُ الَّذِينَ يَنْفِقُونَ أَمْوَالَهُمْ فِي سَبِيلِ اللَّهِ كَمَثَلِ حَبَّةٍ أَنْبِثَتْ سَبْعَ سَنَابِلَ
فِي كُلِّ سَنَابِلٍ مِئَةُ حَبَّةٍ وَاللَّهُ يُضَاعِفُ لِمَنْ يَشَاءُ وَاللَّهُ وَاسِعٌ عَلِيمٌ)

صدق الله العظيم

سورة البقرة الآية (261)
Dedication

To my Father and Mother
To my sisters and brothers
To all my teachers and colleagues
And finally to all my friends.

Hala.
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Abstract

This study was carried out to determine the effect of addition of different levels (5, 10, 15, and 20%) of malted millet flour on nutritional quality, microbiological safety and sensory characteristic for biscuits. Proximate analysis (moisture, protein, fat, fiber and ash) to wheat flour, millet, malted millet flour and biscuits were performed. Microbiology safety and sensory characteristic also physical properties and energy for biscuits were also done. There was significant (p<0.05) difference in moisture, protein, fat, fiber, ash and minerals between wheat flour, millet and malted millet flour. Malted millet flour had the higher protein value 16.21% as compared to wheat flour 11.24%. Energy values of biscuits were significantly (p<0.05) decreased by malted millet flour supplementation. Supplementation of biscuits with different levels of malted millet flour significantly (p<0.05) increased protein, fat, and ash content as compared to wheat flour biscuit. There was no significant (p<0.05) different in Fe, Mg, Ca and K between different types of biscuits. Pathogenic microorganisms such as coli form, staphylococcus and salmonella were not detected in any of the biscuits; therefore they are safe for human consumption. Referring to sensory characteristics including color, odor, taste, crumb texture, crumb grain and general acceptability the result did not revealed any significant (p<0.05) different between different type of biscuits. Based on the results obtained from this study, it is possible to produce a nutritious and safe biscuits supplemented with 20% malted millet flour having acceptable general characteristics as compared to control biscuits without supplementation.
ملخص الأطروحة

أجريت هذه الدراسة لتحديد أثر إضافة مستويات مختلفة (5، 10، 15 و20%) من دقيق الدخن المنبت على الجودة الغذائية والسلاسة البيولوجية والخصائص الحسية للبسكويت. تم إجراء التحليل التقربي (الرطوبة، البروتين، الدهون، الألياف والرماد) لدقيق القمح، الدخن، دقيق الدخن المنبت والبسكويت. كما تم إجراء تحليل السلامة الميكروبية والخصائص الحساسية والفيزيائية واختبارات الطاقة هناك فروق معنوية (P<0.05) في البروتين، الدخن، الألياف والرماد بالإضافة للمعادن بين دقيق القمح ودقيق الدخن ودقيق الدخن المنبت. كانت أعلى نسبة بروتين في دقيق الدخن المنبت بلغت 16.21% وفي دقيق القمح 11.24%. نسبة الطاقة انخفضت بالتدريج بدقيق الدخن المنبت في البسكويت. نتائج التحليل للبسكويت المدعوم بالدخن المنبت أظهرت زيادة في نسبة البروتين والدهون والرماد مقارنة بالبسكويت غير المدعوم والمصنوع من دقيق القمح. لا توجد فروق معنوية في الحديد، الكالسيوم، البوتاسيوم بين أنواع البسكويت المختلفة. تم تظهر النتائج وجود الميكروبات المرضية في القولون لكريوباكوات واستافيلوكوكس والسالمونيلا لذلك هو آمن لإستهلاك البشري. بالرغم للخصائص الحسية شاملة اللون، النكهة، الطعم، اللمس والقبول العام للبسكويت لا تظهر أي فروق معنوية (P<0.05) بين الأنواع المختلفة للبسكويت. بناءاً على النتائج التي توصلنا إليها في هذه الدراسة يمكن إنتاج بسكويت 20% مغذي وامن مدعم بالدخن المنبت ذو خصائص عامة مقبوله لدى المستهلك مقارنة ببسكويت دقيق القمح غير المدعم.
CHAPTER ONE

INTRODUCTION

The term cereal is a derivative from Latin word 'cerealis' meaning 'grain' which is botanically, a type of fruit called a caryopsis, composed of the endosperm, germ, and bran (Sarwar et al., 2013). The major cereal crops produced worldwide include wheat, rice, maize, barely, sorghum, millet and rye. Compositionally a cereal consists of 12 -14 % water, 65-75 % carbohydrates, 2-6 lipids and 7-12 protein. Cereal is quite similar in gross composition being low in protein and high in carbohydrates (Eliasson and Larsson, 1993).

Wheat (Triticum spp) is planted to a limited extent as a forge crop for livestock, and the nutritional value of wheat is extremely important as it takes a important place among the few crop species being extensively grown as staple food for human sources. The important of wheat is mainly due to the fact that it’s seed can be ground into fine flour (Sramkova et al., 2009). Wheat flour used for bread, biscuits, cookies and many more foods.

Millets are small-seeded cereals having excellent nutritional quality. They are comparable or superior to some commonly consumed cereals like wheat and rice (Ragaee et al., 2006). Millets play a major role in the food security and economy of many less developed countries in the world. They are commonly cultivated in India, Africa and China. Millet is thought to be one of the first grains cultivated by man (Crawford, 2006). Millet products from 100% millet flour are rarely manufactured. However in African and Asian countries, millets serve as the main ingredient for preparation of traditional foods and beverages (Saleh et al., 2013). Maximum utilization of the nutrient potential of the millet is limited by the presence of phytates, phenols, tannins...
and enzyme inhibitors but their effect can be reduced by using processing techniques like popping, roasting, malting and fermentation. Despite their beneficial nutritional properties and tolerance for adverse growing conditions, millet consumption has been less compared to major cereals such as rice, wheat and corn. Among millets, small millets have been most neglected. There is a need to increase awareness about the superior nutritional quality of millets and make them one of the important commodities in our food basket. Millets have excellent nutritional quality and are comparable to some commonly consumed cereals like wheat and rice (Ragaee et al., 2006). Millets also offer several health benefits to consumers. These crops lack gluten and hence can be consumed by people suffering from celiac disease (Gabrovska et al., 2002). Millet consumption can also lower glycemic response, which can be helpful for the treatment of type II diabetes (Choi et al., 2005).
There for the objective of this study to:-

1- Determine the chemical composition of wheat and millet flour.
2- Assess the affect of supplementation with different levels of the millet flour on nutritional value of the biscuits.
3- Evaluate the safety of the processed biscuits.
4- Assess the sensory characteristic of the processed biscuits.
CHAPTER TWO

LITERATURE REVIEW

Cereals are annual common grass members of the grass family which usually have long, thin stalks, such as wheat, rice, maize, sorghum, millet, barley and rye, whose starchy grains are used as food. Cereal grains were the first agricultural attempts by early man, and people still enjoy them today depending on where they live and what grows there well. Cereal grains are the most important calorie source in Sudanese diet (Abdelrahman, 2000). For example wheat can provide more than half of the calorie requirements in a healthy daily diet.

2.1 Importance of cereals:

All cereal grains have high energy values, mainly from the starch fraction, but, also from the fat. Apart from moisture content and inedible substances such as cellulose cereal grains contain carbohydrates mainly starches (comprising 65 to 75% total weight), as well as proteins (6 to 12%) and fat (1 to 5%) along with traces of minerals and vitamins.

The grains consist of three major parts which are (Sarwar et al., 2013)

(1) Bran: The outer layer of the grain (fiber, omega-3 fatty acids, vitamins and dietary minerals).

(2) Endosperm: The main part of the grain (mainly starch).

(3) Germ: The smallest part of the grain (vitamin E, folate, thiamine, phosphorus, magnesium).

The whole grains contain all three layers of the grain. The whole grain cereals are a rich source of many essential vitamins, minerals and
phyto-chemicals. The typical cereal food is low in saturated fat, but, is a source of poly unsaturated fats, including omega-3 linolenic acid, cholesterol free, high in both soluble and insoluble fiber and resistant starch, an excellent source of carbohydrates, a significant source of protein, a good source of B-complex vitamins, including folate, a good source of many minerals (iron, magnesium, copper, phosphorus, zinc) and a good source of antioxidants and phytochemicals that can help to lower blood cholesterol levels. The wholegrain cereals contain many different phytochemicals that have been linked to significant health benefits (Sarwar et al., 2013).

Africa with its vast land area covering 3 billion ha has 1.3 billion ha of agricultural land out of which only 252 million ha (19.36 %) is arable (2011, FAO). Africa is the center of origin and also a major producer of several cereals like sorghum, pearl millet, finger millet, teff and African rice. Another major cereal, maize, has overtaken these traditional cereals while wheat is widely cultivated in North Africa and in Sudan and Ethiopia. Agriculture is the engine for growth in Africa (Macauley, 2015).

2.2 Types of cereals:

Maize is a major staple food crop grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic backgrounds in Sub-Saharan Africa (SSA). Consumption rates being the highest in Eastern and Southern Africa (ESA) Of the 22 countries in the world where maize forms the highest percentage of calorie intake in the national diet, 16 are in Africa. Maize accounts for almost half of the calories and protein consumed in ESA, and one-fifth of the calories and protein consumed in West Africa. An estimated 208 million people in Sub-Saharan Africa (SSA) depend on maize as a source of food security and economic wellbeing (Macauley, 2015).
Sorghum is the second most important cereal after maize with 22% of total cereal area, followed by millets (pearl and finger) with 19% of the total cereal land coverage. The continuing demand for these two crops is reflected in the trend for increasing area under sorghum and millets in Africa over the last fifty years. Unfortunately however, crop productivity has not kept pace with increasing demand, due mainly to a lag in crop improvement efforts in sorghum and millets, relative to other cereals, and the extreme environmental conditions and resource constrained, low-input farming systems where these crops are grown (Macauley, 2015).

Rice has become a highly strategic and priority commodity for food security in Africa. Rice is growing faster than that of any other major staple on the continent because of high population growth, rapid urbanization and changes in eating habits. It is the single most important source of dietary energy in West Africa and the third most important for Africa as a whole (Macauley, 2015).

2.3 Wheat

Wheat is grown on around 10 million ha in Africa. It is a major staple crop for several countries and an imported commodity in all of Africa. In all African countries, wheat consumption steadily increased during the past 20 years as a result of growing population, changing food preferences and socioeconomic change associated with urbanization. African countries are the world’s biggest wheat importer with more than 45 m t in 2013 at around 15 billion US$. Wheat imports account for 60% of African’s wheat consumption and 80% of Sub-Saharan Africa (SSA) countries. North African countries have the highest per capita wheat consumption and wheat provides up to 50% of daily calories and protein. In rapidly urbanizing sub-Saharan Africa, wheat consumption is expected to grow 38% by 2023 with imports already at 23 m
tons of wheat in 2013 at a cost of $7.5 billion. Considering the growing importance wheat has for food security in Africa, African Union Heads of State endorsed their Agriculture Ministers’ endorsement in January 2013, to add wheat to the list of strategic crops for Africa (Macauley, 2015).

2.3 Wheat classification:

Kipps, (1970) reported that wheat is a group classify according to, color, texture and year seasons as follows:

2.3.1 Hard red spring wheat:

This class of wheat is noted for its high protein content and excellent bread making characteristics. It is used extensively for blends with weaker wheat throughout the world. Hard red spring and hard red winter wheat contain an average of about 11 to 15 percent protein (Kipps, 1970).

2.3.2 Hard red winter wheat:

The grain is hard and generally high in protein content. As bread wheat, it ranks second only to hard red spring wheat in quality (Kipps, 1970).

2.3.3 Soft red winter wheat:

Most of the wheat of this region has soft grain of low-protein content, which produces flour most satisfactory for pastries, such as cakes, cookies and pies. Soft wheat contains 8 to 11 percent protein and the soft red winter wheat verities were in general high in starch and low in protein (Kipps, 1970).

2.3.4 Durum wheat:

The kernels of this wheat are the hardest known and for this reason are often called "hard" wheat (Kipps, 1970).
2.3.5 White wheat:

The white wheat is mainly used for pastry purposes, but some of them go into shredded wheat and bread.

Generally bakers classify wheat by the hardness of the kernel, that is, by whether the kernel is hard or soft. Hard wheat kernels are high in protein; soft wheat kernels are low in protein. Hard wheat kernels feel harder than soft ones because protein in these kernels forms large, hard chunks. Strong flours usually have a high water-absorption value and require a longer mixing time to fully develop, but they are tolerant of over mixing. The strength of flour depends largely upon the gluten it contains, which gives to bread its elastic quality and its ability to absorb water (Blackman and Payne, 1987). Strong flours are typically used in yeast-raised products, like breads, rolls. Soft wheat flours typically form weak gluten that tears easily, and are sometimes called weak flours, and this is desirable for many cakes, cookies, and pastries.

Wheat also is a major source of protein and contributes more than 25% of the protein consumed in the human diet (Dukes et al, 1995). However, when refined by the removal of the bran and germ the remaining endosperm is mostly carbohydrate and lacks the majority of the other nutrients.

2.4. Storage:

Preferably, store in an airtight container in a cool (under 60°F), dry area. Commercial cereal should store for three years in this fashion. If stored in the cereal box, a shelf life of one year is probably maximum. If there is danger of insect infestation, store in the refrigerator. There should be a “freshness” date on the package to use as a reference for the product quality. Freezing is acceptable, but it draws moisture from the product.
2.5 Millet

Pearl millet (Pennisetum glaucum) is the most widely grown type of millet. Because of its tolerance to difficult growing conditions such as drought, low soil fertility and high temperature, it can be grown in areas where other cereal crops, such as maize (Zea mays) or wheat (Triticum aestivum), would not survive. Pearl millet production is concentrated in the developing countries which account for over 95% of the production and acreage. India continues to be the single largest producer of pearl millet in the world, although the area has been declining in the traditional growing states of Gujarat, Rajasthan and Haryana. Pearl millet is usually grown as a dry land dual purpose grain and fodder crop although it is sometimes irrigated in India, particularly the summer crop grown mainly as a forage crop (Basavaraj et al. 2010).

2.5.1 Millet production and importance

Millets play a major role in the food security and economy of many less developed countries in the world. They are commonly cultivated in India, Africa and China. The first recorded on the cultivation of millet dates back to about 5,500 BC in China (Crawford, 2006). Millet is extremely important crops in semi-arid regions where other crops normally do not survive. Millets ranks as the sixth most important cereal and feeds one third of the total world population (Saleh et al. 2013). They are easy to cultivate, inherently bio-diverse and can be grown together with varied crops (Rachie 1975; Dendy 1995). Another attributes of millets that make them a preferred choice in areas where they are cultivated, are their short harvest period 45-65 day (Bukhari et al. 2011).

In North American and European countries, millets are mainly used as an ingredient in composite mixes, to produce gluten-free and low glycemic index
(GI) food products. Millet products from 100% millet flour are rarely manufactured. However in African and Asian countries, millets serve as the main ingredient for preparation of traditional foods and beverages (Saleh et al., 2013). Pearl millet is the most commonly consumed millet, grown in the arid and semi-arid tropical regions of Asia, Africa and Latin America. India is the largest producer of pearl millet in Asia and is mainly grown in northwestern parts (Dendy, 1995; Obilana, 2003). It is also the major millet grown in Nepal and Bhutan (Mal et al., 2010). China however, mainly produces foxtail millet. Finger millet is cultivated in more than 25 countries in eastern and southern Africa, and across Asia, with the major producers being Uganda, India, Nepal and China. In Africa, pearl millet production is concentrated in Sahara and drier areas of northern and eastern Africa (ICRISAT, 1996; Obilana, 2003). Proso millet is mainly grown in developed countries like Australia and America (FAO, 2005).

Inclusion of millet in the human diet can also lower the risk of duodenal ulcers, anemia and constipation (Jayaraj et al., 1980; Nambiar et al., 2011). For patients suffering from allergic diseases such as atopic dermatitis, Japanese barnyard millet grains have been recommended to replace rice and wheat grains (Watanabe, 1999). Dietary fibre content in pearl and finger millet was found to be higher than that in sorghum, wheat and rice (Kamath and Belavady, 1980). Millets are also rich in phenolic acid and has high anti-oxidant activity (Chandrashekhhar and Shahidi, 2010). They are valuable sources of some essential minerals such as potassium, magnesium, calcium, iron and zinc (Ravindran, 1991).
2.6 Nutritional composition of millets

2.6.1 Sugars and starch

The free sugars found in millet are glucose, fructose, sucrose and raffinose and their contents ranges from 1-1.4% with sucrose (0.3-1.2%) being the predominant sugar (Malleshi et al., 1986). Total sugars in small millets ranged from 1.4-2% with proso having highest contents. Millets have total starch ranging from 64-79% (Krishnakumari and Thayumanavan, 1995; Geervani and Eggum, 1989). Amylose contents in millets ranges from 26-30% and amyleopectin 69-74% (Krishnakumari and Thayumanavan, 1995).

2.6.2 Protein composition

Protein in millets has three main fractions: Fraction I- albumin+globulin, Fraction II-true prolamin+prolamin like, Fraction III- true glutelin+glutelin like. The albumin and globulin fraction forms 8.5-16.26%, prolamin fraction forms 15-30%, while glutelin forms 45-55% of the total protein in small millets except foxtail which had higher prolamin (60%) than glutelin (15.23%) content. Pearl millets had prolamins from 33-49.5%, glutelins 30-45% and globulins plus albumins from 18-26% (Chanda and Matta, 1990; Parmeswaran and Thayumanavan, 1995). Differences in the amino acid composition have been shown in different millet types. Lysine is the limiting amino acid in millets similar to other cereals. Glutamic acid (16-23%) and leucine (12-22.3%) were the major amino acids in the prolamin fraction however, barnyard had higher content of alanine (18%) than leucine (Parameswaran and Thayumanavan, 1995).

2.6.3 Lipid profile

Most of the lipids in millets are present as free lipids (60-70%) followed by bound and structural lipids. Linoleic (38-40%), oleic (27-37%), palmitic
(16-22%) and linolenic (1-4%) are the major fatty acids found in millets. Unsaturated fatty acids account for more than 85% of the total fatty acid content in millets (Lai and Martson, 1980; Sridhar and Lakshminarayana, 1994). Millet pericarp and germ have considerable amount of lipids hence the total lipid content and fatty acid profile can be affected by the extent decortication of millet (Liang et al., 2010). However there is dearth information on evaluating the changes in lipid content and fatty acid profile of millets after decortication.

2.6.4 Dietary Fibre

Reports on proso millets have shown that Total Dietary Fiber (TDF) of 12–20% in whole grain varieties decreased to 3–5% after decortication (Bagdi et al. 2011). Wide variations (7–21.2%) in TDF content of ten varieties of whole grain finger millet have been reported (Premavalli et al., 2004). Therefore, millet type, variety and extent of decortication have an important effect on the IDF and SDF content; however more studies are needed in this aspect.

2.6.5 Phenolics and antioxidant capacity

Phenolic content of millet has been reported to be higher than some major cereals like barley and wheat (Chandrashekhar and Shahidi, 2010). Millets contain phenolic acids, which are located in the pericarp, testa, aleurone layer and endosperm (McDonough and Rooney, 2000). Studies have reported a high antioxidant activity in the extracts from bran rich fraction compared to refined flour (Suma and Urooj, 2012). Effects of processing namely fermentation and germination on the anti-nutrients (tannins and phytates) have been mainly studied (Khetarpaul and Chauhan, 1989; Sade, 2009). Studies on the effect of decortication on the free and bound phenolic acids have been limited. In general, major phenolic acids in millets are ferulic,
$p$-coumaric and cinnamic acids (McDonough and Rooney, 2000). Millets with dark brown pigmented testa and pericarp (kodo, finger) possess a higher phenolic content than those with white or yellow testa and pericarp (pearl, proso, foxtail, little).

2.7 Biscuits

Biscuits are the most popular bakery item consumed nearly by all levels of society (Sudha. et al., 2007). In the United States of American called cookies and crackers as reported by (Wade, 1988).

2.7.1 Biscuit ingredients

In cracker, cookie and biscuit production the characteristic texture of product depends primarily on the properties of the gluten in flour used for biscuits, the texture, flavor and color of the final product also depend on the raw materials used, such as shortening, sugar, milk solids and leavening and flavoring agents (Van et al., 2002).

2.7.2 Flour

Kernel texture in wheat has been found to be directly controlled by one or two major genes. Generally good biscuit making wheat are those with soft endosperm texture, lower protein content, more break flour and smaller particle size Study by (Labuschagne et al., 1997).

2.7.3 Sweeteners

Sweeteners provide many functional properties in addition to enhancing taste. The addition of sugar as coating, gives presweetened cereals with high sweetness levels. Topical application provides an immediate, intense flavor. Sucrose is most often used because it can be crystallized to either a white "frosted" surface or a hard, clear glaze. Sucrose, glucose and fructose are
generally used in making of cookies. Cookies with glucose were the softest and most moist, expanded vertically (Nishibori and Kawakishi, 1992).

2.6.4 Shortening

Fats and oils have been important baker ingredients for centuries. Indeed, shortening is a baker's term: fat in bakery item "shortens" (tenderizes) the texture of the finished product. Shortening plays an important part in the efficient processing of many bakery products. Shortening makes the final quality of bakery product are: tenderness, moist mouth feel, flavor and structure (Stauffer, 1998).

2.6.5 Milk and milk derivatives

Dairy milk products used to improve the Millard reaction (color and taste formation) in crackers. These products contain protein and sugars that react with each other during backing to form a variety of dark, flavored substances (Van Wakeren and Popper, 2002).

2.6.6 Leavening agent

A chemical leavening system contains two functional components:

A leavening base and acid. Sodium bicarbonate (NaHCO3) is the most widely used leavening base and is chemically neutralized by acid according to the following reaction:

\[ HX + NaHCO3 \rightarrow NaX + H2O + CO2 \]

Where HX is the acid. Leavening and gas bubble formation occur in the stage. During mixing, air is incorporated in to dough. This provide gas cell nuclei for gas to enter during later processing steps. Some CO2 (carbon dioxide) is formed by reaction of soluble acid during mixing and holding. Heat from the oven causes less soluble acids and bicarbonate to dissolve and
react to generate more CO2. Retention of air bubbles in dough and the baked products is critical to maintain a leavened product and depend on many forces that destabilize and expand the product during baking (Cepeda et al., 2000; Dally and Navarro, 1999; LaBell, 1999).

2.6.7 Salt

Salt (sodium chloride) has marked strengthening effect on gluten, the salt level in the dough is often 1 – 1.5%.

2.6.8 Biscuit making process

2.6.8.1 Mixing

There are two basic methods of mixing dough. The first method is known as multi–stage methods. It is done in many stages using different ingredients during mixing process. Usually requires initial introduction to the mixer of shortening, sometimes the syrup, then the addition of granulated sugar and some of all other dry ingredients. Mixing is continues at a low or medium speed until the all component became homogenous and the mixture has taken up air in the form of bubbles. The second method is creaming method, the beneficial effect of the creaming mixing process as fat–coating that delays solubization and hydration of sugar and flour, and the incorporation of small air bubbles which assist in leavening and establishing the structure of the finished cookie (Matz, 1968). Mixing biscuit dough should be no more than enough to allow the dough to be gently sheeted and biscuits to be cut.

2.6.8.2 Shaping and baking

There are two ways to shape the dough, roll and cut or drop. The rolling and kneading results with flakiness biscuit, no sticky and has sufficient developed gluten. After rolling the biscuit dough is cut into shapes usually
round with a biscuit cutter, about 2 – 3 inches in diameter. The dropping method, drops the dough in an irregularly shape into grease baking sheet by your lightly floured finger tips. The dough is more sticky (Phillips, 2003). Baking is heating or cooking by hot air, and also by oven floor and trays. Moisture at the surface of the food is evaporated by the hot air, and this lead to dry crust in products such as bread and many biscuits (Kordylas, 1991; Fellows and Hampton, 1992). The cooking temperature for most ovens range between 120 and 260°C (Kordylas, 1991).
CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Millet was purchased from local market in Khartoum North. Analytical chemicals grade for analysis were purchased from local chemical supplies company in Khartoum North. Baking materials were obtained from local market as well in Khartoum North. Wheat flour (Siga) was also purchased from local market.

3.2 Methods

3.2.1 Malting of millet

The millet grains were carefully cleaned and freed from any foreign materials and broken seeds. The clean grains were steeped for 8 hrs. after that the grains were placed on aluminum foil at room temp. for 2 days. Then germinated grains were dried at room temp. for 3 days after that malted millet were cleaned by removing shoots after that milled into fine flour using roles millers. The malted millet flour was packaged in polyethylene bags and stored using covered plastic container in a freezer until used.

3.2.2 Processing of biscuits

Biscuits were prepared using the method of Vatsala and Harids Rao (1991). Formula used in biscuit processing was as follows:

- Ingredients Quantity (g)
- Biscuit flour 100 gm
- Sugar powder 30 gm
- Shortening 30 gm
- Skim milk powder 2 gm
- Sodium chloride 1 gm
- Sodium bicarbonate 0.4 gm
- Ammonium bicarbonate 1.5 gm
- Glucose 2 gm
- L-cysteine 0.02 gm
- Water (ml) 15

The ingredients were weighed for 200 g of flour, sugar powder, skim milk and glucose were creamed in Hobart N-50 mixer with a flat beaker for 3 min at 61 rpm. Salt, ammonium, bicarbonate and cysteine were dissolved separately in part of the required water and added to the cream. Mixing was done for 8 min at 125 rpm to obtain homogenous cream. Finally flour was added and mixed for 3 min at 61 rpm and then the dough was heated to a thickness of 4 mm with the help of two rulers placed at two sides of the dough. The sheeted dough was cut into round shape using 4.985 mm diameter cutter. The cut dough was transferred to an aluminum tray. The biscuits were baked in an electric oven maintained at 200°C for (11 min). The baked biscuits were cooled for about (20 min), packed in plastic bags and stored at room temperature for further analysis.

3.2.3 Analytical methods

The determination of moisture and ash were carried out on the samples according to AACC (2000) methods.

3.2.4 Determination of moisture content

Two grams of well mixed samples were weighed accurately in clean preheated moisture dish of known weight by using sensitive balance. The uncovered sample and dish were kept in an oven provided with a fan at 105°C
and left to stay overnight. The dish was covered and transferred to a desiccators, and weighed after reaching room temperature.

The loss of weight was calculated as moisture expressed as moisture.

\[
\text{Moisture content (\%)} = \frac{W_1 - W_2}{\text{Sample weight}} \times 100
\]

Where:

\( W_1 \) = Weight of sample + dish before oven drying.

\( W_2 \) = Weight of sample + dish after oven drying.

### 3.2.5 Determination of crude protein

The determination of crude protein was carried out on the samples according to AACC (2000) methods.

A 0.2 gram of sample, plus 0.4 gram catalyst mixture (potassium sulfate + cupric sulfate 10:1 by wt), and 7 ml concentrated nitrogen free sulfuric acid, were mixed in a small Kjeldahl flask (100 ml). The mixture was digested for two hours, then cooled, diluted, and placed in the distillation apparatus. Fifteen milliliters of 40\% NaOH solution were added and the mixture was heated and distilled until 50 ml were collected in a 100 ml conical flask. The ammonia evolved was received in 10 ml of 2\% boric acid solution plus 3-4 drops of universal indicators (methyl red and bromo-cresol green). The trapped ammonia was titrated against 0.02N HCL.

The percentage (g/100) of protein was calculated by using an empirical factor to convert nitrogen into protein as follows:

\[
\text{Nitrogen content \%} = \frac{\text{TV} \times N \times 14.00 \times 100}{1000 \times \text{Wt. of sample}}
\]

\[
\text{Protein content \%} = (\text{nitrogen content \%}) \times F
\]
Where:

\[ TV = \text{Actual volume of HCL used for titration (ml HCL – ml blank).} \]

\[ N = \text{Normality of HCL.} \]

\[ 14.00 = \text{Each ml of HCL is equivalent to 14 mg nitrogen.} \]

\[ 1000 = \text{to convert from mg to gm.} \]

\[ 6.25 = \text{Constant factor for other grains.} \]

\[ 5.7 = \text{constant factor for wheat flour.} \]

**3.2.6 Determination of crude fat**

Crude fat was determined according to the standard method of AACC (2000) methods. Sample of 3 g was weighed into an extraction thimble and covered with cotton; that was previously extracted with hexane (BP60-70°C), and then the sample and a pre-dried and weighed Erlenmeyer flask containing about 50 ml were attached to extraction unit for 45 minutes. At the end of distillation period, the solvent was recovered from the oil. Later, the flask with the remaining crude hexane extract was put in an oven at 105 °C for about an hour. Cooled in a desiccators, reweighed and dried extract was recorded as crude fat% (DM) according to the following formula:

\[
\text{Crude fat } \% \text{ (DM)} = \frac{\text{Dry extract w. t (g) x 100 x 100}}{\text{Wt. sample (100 – % moisture)}}
\]
3.2.7 Determination of crude fiber

Two grams of an air dried fat-free sample were transferred to a dry 600 ml beaker. The sample was digested with 200 ml of 1.25% (0.26N) H₂SO₄ for 30 minutes, and the beaker was periodically swirled. The contents were removed and filtered through Buchner funnel, and washed with boiling water. The digestion was repeated using 200 ml of 1.25% (0.23N) Na OH for 30 minutes, and treated similarly as above. After the last washing the residue was transferred to ashing dish, and dried in an oven at 105°C over night then cooled and weighed. The dried residue was ignited in a muffle furnace at 550°C to constant weight, and allowed to cool, then weighed.

The fibre percentage was calculated as follows:

\[
\text{Crude fibre} \% = \frac{W_1 - W_2}{\text{Dry sample weight}} \times 100
\]

Where:
W₁ = The weight of oven dry sample after treatment by H₂SO₄ and KOH
W₂ = The weight of the treated sample after ashing.

3.2.8 Determination of ash content

Crucibles weighed empty, two grams of samples were placed in a muffle furnace at 550°C for 3 hr until white grey or reddish ash was obtained. The crucible was removed from furnace and placed in a desicator to cool, then was reweighed. The process was repeated until constant weight was obtained.

\[
\text{Ash content (\%)} = \frac{(W_2 - W_1) \times 100}{W_s}
\]

Where:
W₁ = weight of empty crucible
W₂ = weight of crucible + sample after ashing
Wₛ = weight of dry sample
3.2.9 Determination of carbohydrates

The carbohydrates were calculated by difference. The sum of moisture, fat, protein and ash contents was subtracted from 100 as it was described by Wade et al. (1988).

3.2.10 Determination of minerals content

Minerals of sample were extracted according to Pearson’s (1981). The sample was burned in a muffle furnace at 550˚c, then the sample was placed in a sand bath for 10 min after addition of 10 ml of 5 N HCL, then the solution was carefully filtered in a 100 ml volumetric flask and finally distilled water was added to make up to mark. The extracts were stored in bottles for further analysis. Minerals, Ca, Mg Fe, were determined using Atomic Absorption Spectrophotometer (AA 6800) Shimadzu, Japan.

3.2.8 Potassium and sodium contents

Potassium and sodium contents of extracted sample were determined according to AACC (2000) methods using flame photometer. One milliliter of the extract was taken and diluted in a 50 ml conical flask with distilled water, The standard solutions of the KCL and NaCL were prepared by dissolving 2.54, 3.33 g of KCL and NaCL, respectively each in 1000 ml distilled water. Ten ml of this solution were taken and diluted to one liter to give a 10 ppm concentration. The flame photometer was adjusted to zero using distilled water as a blank and to 100 degree using standard solution.

Calculation:

\[ K \text{ or Na (mg/100g)} = \frac{F.R \times D.F}{103 \times S \times 10} \times 100 \]
Where:

F.R = Flame photometer reading

D.F = Dilution factor

S = Sample weight

3.2.9 Phosphorous content

The determination of phosphorous content was carried according to the method of Chapman and Pratt (1982). Two ml of the extracted samples were pipetted into a 50 ml volumetric flask. Ten ml of ammonium molybdate-ammonium vanadate reagent [(22.5 g of WH4) 6 MO7 O24 4 H2 O in 400 ml distilled water + 1.25 g ammonium vandate in 300 ml boiling water + 250 ml conc. HNO3, then diluted to 1 liter] were added. The content of the flask were mixed and diluted to volume. The density of the color was read after 30 minutes at 470 nm using using colorimeter (Lab System Analysis-9filters, J. Mitra and Bros Pvt. Ltd). A standard curve of different KH2 PO4 concentration was plotted to calculate the ion phosphorous concentration.

Calculation:

\[
\text{Phosphorous (mg/100g)} = \frac{\text{Curve reading \times ash dilution}}{103 \times \text{oven dry weight of sample}} \times 1000
\]

3.4 Microbiological methods

3.4.1 Sterilization of Glassware

Petri dishes, test tube, flask, pipettes…etc., were sterilized in hot air oven at 160 – 180˚c for 3 hours .They were washed and packed in stainless steel cans or sometimes in aluminum foil till used.
3.4.2 Sterilization of media

Culture media were prepared following manufacturing instructions then sterilization was achieved by autoclaving at 121°C for 15 minutes at 15 Pascal pressure.

3.4.3 Preparation of serial dilutions

Aseptically 10 grams of the sample were homogenized in 90 ml of sterile diluents (0.1 Peptone water). It was mixed well to give dilution \((10^{-1})\) by using sterile pipette 1ml was transferred aseptically from dilution \((10^{-1})\) to a test tube containing 1ml of sterile diluents \((10^{-2})\). In the same away the preparation of serial dilution was continued until the dilution \((10^{-6})\).

3.4.4 Total Viable Count of Bacteria

It was carried out by using the pour plate count method as described by Harrigan (1998).

One ml of each dilution was transferred into sterile petri dish, and then 15 ml of sterile melted Plate Count Agar medium were added to each plate. The inoculums was mixed medium and allowed to solidify.

The plates were incubated at 37°C for 48 hours. A colony counter was used to count the viable bacterial colonies after incubation and the results were reported as colony-forming units (CFU) per gram.

3.4.5 Determination of Coliform Bacteria

It was carried out by using the most Probable Number (MPN) technique as following:
3.4.6 Presumptive Coliform test

10, 1.0 and 0.1 ml prepared samples was inoculated in triplicates of MacConkey Borth test tube containing Durham tubes. The tubes were incubated at 37 C° for 48 hours. The production of acid together with sufficient gas to fill the concave of the Durham tube is recorded as positive presumptive test.

3.4.7 Confirmed test for Total coliforms

From every tube showing positive results using a sterile loop tubes of Brilliant Green 2% bile Broth was inoculated. The tubes were inoculated at 37 C° for 48 hours, and then the tubes showing positive and negative result were recorded. The Most Probable Number (MPN) of total colliform was found out using the Most Probable Number (MPN).

3.4.8 Confirmed *E.coli* test

Medium used was EC Broth. From every tube showing positive result in the presumptive test a small amount was used to inoculate the tube of EC in Durham tube incubated at 44.5°C for 24 hours. Tubes showing any amount of gas were considered positive. For further confirmation of *E. coli* tubes of EC Broth showing positive results at 44.5°C for 24 hours were streaked on Eosin Methylene Blue Agar (EMB) plates. The plates incubated at 37C° for 48 hours. Colonies of *E. coli* are usually small with metallic green shinng on EMB Agar.

3.4.9 *Staphylococcus aureus*

Medium used was Baird-Parker Agar; 0.1 ml from every dilution was transferred onto the surface of each well dried Baird-Parker Agar medium plates. The inoculum was spreaded all over the plate using sterile bent glass rod. The plates were incubated at 37C° for 24 hours, after incubation the
plates were examined for *Staphilococcus aureus*, which appears as black shiny convex surrounded by a zone clearing 2-5 mm in width of colony.

### 3.4.10 Yeast and Moulds

From suitable dilution of sample 0.1 ml was aseptically transferred onto solidified Potato-Dextrose Agar containing 0.1 gram chloramphenicol per one liter of medium to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod. Plates were incubated at 28°C for 72 hours. Colonies were counted using a colony counter and the result were presented as CFU/ml.

### 3.4.11 Detection of *Salmonella*

Ten gram of the sample were added to a conical flask containing 90 ml of sterile Nutrient Broth and incubated at 37°C for 24 hours. A loopfull of 24 hours incubated Nutrient Broth was transferred aseptically to sterilized Selenite Cysteine Broth and incubated at 37°C for 24 hours. A loopfull of 24 hours inoculums of Selenite Cysteine Broth was streak on Bismuth Sulphite Agar surface and incubated at 37°C for 24-72 hours. A black metallic sheen discrete colony indicates the presence of *Salmonella*.

### 3.4.12 Statistical analysis

The analysis of variance was performed to examine the significant effect in all parameters measured. Duncan Multiple Range Test was used to separate the means.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate composition of flour samples

Table (1) showed the chemical composition of wheat flour, millet and malted millet flour.

4.1.1 Moisture content

The moisture content of wheat flour, millet and malted millet flour were 5.43, 7.37, and 6.34 respectively (Table 1). Bashir (2006) has reported higher value of moisture content for wheat flour (6.37%). On the other hand Abdelrahman (2005) reported that the moisture content of millet ranged from 7.7% to 8.9%. Elyas et. al (1999) gave lower value of 6.4% for millet. However Eltayeb (2006) stated value range between 5.4% - 6.48% for millet flour. The variation in moisture might be due to variety difference.

4.1.2 Protein content

As presented in (Table 1) protein content of wheat flour, millet and malted millet were 11.24, 15.34 and 16.21%, respectively. The level of protein was higher in millet flour than that value of wheat flour. Finger millet has nearly 7% protein but large variations in protein content from 5.6 to 12.70% have been reported by various studies. The average protein content of millet is reported to be from 7.7-11.8% (Hulse et al. 1980). Moreover, Bashir (2006) found that protein content of three local wheat cultivars (Debira, WadiElneel and Elneelain) varied recording value of 13.57, 11.97 and 10.77% respectively. Rao (1994) has reported lower protein value of 8.2 – 11.3% for brown and white varieties of malted finger millet respectively as compared to
our result in (Table 1). The variation in protein of wheat might be variety dependent.

### 4.1.3 Fat content

In (Table 1) the fat content for wheat, millet and malted millet flour were 3.40, 5.94, and 3.39%, respectively. Unlike other millet types, finger millet has almost equal proportions of free and bound lipids (Sridhar and Laxminarayana, 1994). Bashir (2006) reported lower levels of fat content for three local wheat cultivars (Debira, WadiElneel and Elneelain) which were 1.22, 1.13 and 1.04%, respectively. Referring to fat of millet Hadimani et al. (1995) stated fat value ranged between 1.5 to 5% for millet. The free lipid content for kodo, finger, barnyard, little, proso, foxtail millet have been reported to be 3.4%, 5.2%, 5.7%, 5.4%, 5.6% and 5% respectively (Sridhar and Laxminarayana 1992, 1994). These values for fat were similar to our finding for millet (Table 1). Pearl millet has free lipid content from 6-8% while bound and structural lipids of small millets were reported to be 1.3-5% and 0.4-0.9%, respectively (Lai and Martson, 1980).

### 4.1.4 Fiber content

Fiber content of wheat, millet and malted millet are presented in (Table 1). Ali (2008) showed that the fiber content of seven imported wheat and local commercial wheat cultivars (72% extraction rate) ranged from 1.08 to 1.24%. While Joshi and Katoch, (1990) had reported 3.7% crude fiber in finger millet which was higher than the value presented in (Table 1).

### 4.1.5 Ash content

Ash content is showed in (Table 1). Wheat, millet and malted millet ash were 2.05, 1.41 and 1.31% respectively. Duke (1981) and Huisman an Vander Poel (1994) had reported that wheat contain 2-4.8% ash. For decorticated
millet flour lower values of 1.58% and 1.75% were reported by Eltayeb (2006). Ash content had been found to be nearly 1.7 to 4.13% in finger millet (Singh and Srivastava, 2006).

4.1.6 Carbohydrate

Carbohydrate content of wheat, millet and malted millet flour is showed in (Table 1) were 76.21, 68.79 and 70.51% respectively. Wheat flour has the higher carbohydrate content as compared to millet and malted millet flour. The variation in carbohydrate is due to difference in level of other specific components. Chemical composition of finger millet revealed that the total carbohydrate content is ranged of 72 to 79.5% (Singh and Srivastava, 2006).
Table (1): Chemical composition of raw materials.

<table>
<thead>
<tr>
<th>Raw materials</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Fiber</th>
<th>Ash</th>
<th>Carbo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>5.43±0.15ᵇ</td>
<td>11.24±0.82ᵇ</td>
<td>3.40±0.20ᵇ</td>
<td>1.55±0.26ᵇ</td>
<td>2.05±0.69ᵃ</td>
<td>76.21±1.20ᵃ</td>
</tr>
<tr>
<td>Millet</td>
<td>7.37±0.08ᵃ</td>
<td>15.34±0.05ᵃ</td>
<td>5.94±0.55ᵇ</td>
<td>1.13±0.17ᵇ</td>
<td>1.41±0.26ᵃ</td>
<td>68.79±0.74ᵇ</td>
</tr>
<tr>
<td>Malted millet</td>
<td>6.34±1.00ᵇᵇ</td>
<td>16.21±0.46ᵃ</td>
<td>3.39±0.28ᵇᵇ</td>
<td>2.21±0.16ᵃ</td>
<td>1.31±0.09ᵃ</td>
<td>70.51±1.23ᵇ</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicate independent analysis.

Values in the same column carrying different superscribe litter are significant different at (P<0.05).
4.2 Minerals content

Minerals contents of raw materials revealed in the experiment were display in (Table 2).

Iron (Fe) in millet decreased by malting as showed in (Table 2). The Fe in wheat, millet and malted millet were 1.82, 2.44 and 2.28 mg/100g respectively. (Singh and Srivastava, 2006) reported the Fe content of finger millet varieties ranged from 3.61 mg/100g to 5.42 mg%. In general Millet has the higher content of Fe as compared to its level in wheat. Fe content of different finger millet fraction was reported from 2 to 3 mg/100g (Viswanath et al., 2009). Our result in (Table 2) is within the range by Viswanath, (2009).

Magnesium (Mg) content of millet and malted millet flour were almost six time higher than its level in wheat flour. Araujo et al., (2008) stated that the Mg content of 54 wheat cultivars varied from 19 – 51 mg/100g. However Abdelrahman et al., (2005a) gave higher rate 75 and 93 mg/100g for Mg for two pearl millet cultivars which were similar to our result in (Table 2).

Wheat, millet and malted millet are a very good source of Ca reporting values of 230.2, 240.78 and 225.36mg/100g. Araujo et al., (2008) determined that the Ca content in wheat cultivars ranged from 11 – 196 mg/ 100g based on variety. Viswanath et al., (2009) also found that millet flour has the highest percentage of Ca content as compared to its level in wheat and malted millet flour.Ca content of various finger millet fraction content ranges from 140 to 340 mg/100g. Ca content of 36 genotypes of finger millet ranged from 162 to 487 mg %. (Singh and Srivastava, 2006).

Phosphor (P) Millet and malted millet are good source of P (Table 2). Levels of P in wheat, millet and malted millet were 115.00, 263.69 and
244.11mg/100g. Millet flour has the higher value of P as compared with its level in wheat flour. Arujo et al., (2008) reported P value range from 89 – 715 mg/100g for wheat flour. Moreover Shobana and Malleshi (2007) stated that, the phosphorus content for native and decorticated finger millet were 211mg/100g and 109 mg/100g respectively.

Potassium (K) content of wheat is lower as compared to its level in millet, millet and malted millet (Table 2). Level of K in wheat, millet and malted millet were 215.70, 273.7 and 267.5mg/100g respectively. Study on K content of 54 wheat cultivars in Brazilian found value ranged from 76 to 319 mg /100g (Araujo et al, 2008). Another study by Abdelrahman et al., (2005b) reported 370.47 mg/100g K for different millet cultivars.

On the other hand zinc (Zn) contents of wheat, millet and malted millet as showed in (Table 2) were 0.86, 2.74 and 2.28 mg/100g. Millet flour is almost three times high Zn than that of wheat flour.
Table (2): Minerals content of raw materials

<table>
<thead>
<tr>
<th>Raw materials</th>
<th>Fe</th>
<th>Mg</th>
<th>Ca</th>
<th>P</th>
<th>K</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>1.82±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.67±3.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>230.2±27.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.00±11.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>215.70±6.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Millet</td>
<td>2.44±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.07±2.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>240.78±9.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>263.69±10.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>273.7±20.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.74±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Malted millet</td>
<td>2.28±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.55±1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>225.36±4.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>244.11±11.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>267.5±19.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.28±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicate independent analysis.

Values in the same column carrying different superscribe litter are significant different at (P<0.05).
4.3 Physical characteristics of biscuits supplemented with different levels of malted millet

Physical characteristics were showed in (Table 3). There was no significant deference (p≤0.05) between control biscuits and biscuits made with different levels of malted flour in thickness and width. Generally the analysis of variance showed no significant difference (p ≤ 0.05) among biscuits sample in term of width. The low width value of biscuit made from 100% wheat flour as compared to malted millet biscuits.

4.3.1 Biscuits spread ratio

The spread ratio of biscuits prepared from wheat and malted millet flour mixtures are shown in (Table 3). There was no significant deference (p≤0.05) between biscuits made from wheat flour and biscuits containing different levels of malted millet flour in terms of spread ratio. The spread ratio of 100% wheat flour were 4.37 decreased to 3.55 in B (95%wheat flour +5% millet flour) and increased again to 4.16 in C (90% wheat flour + 10% malted millet flour). These results are contrary to the Lorenz et al., (1980) who found that cookie spread ratio increased with increasing amounts of millet flour. Badi et al., (1976) reported decrease in cookies spread ratio with increased levels of millet flour.
Table (3): Physical characteristics of Biscuits samples

<table>
<thead>
<tr>
<th>Types of biscuits</th>
<th>Thickness</th>
<th>Width</th>
<th>Spread ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>53.29 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.20 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.37 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>52.17 ± 1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.68 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.55 ± 0.081&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>52.89 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.70 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.16 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>53.44 ± 1.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.70 ± 0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.97 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>52.54±1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.34±1.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.96±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicate independent analysis.
Values in the same column carrying different superscribe litter are significant different at (P<0.05).
Where:
A: 100% wheat flour.
B: 95% wheat flour + 5% malted millet flour.
C: 90% wheat flour + 10% malted millet flour.
D: 85% wheat flour + 15% malted millet flour.
E: 80% wheat flour + 20% malted millet flour.
4.4 Chemical composition of biscuits supplemented with different levels of malted millet:

Chemical composition including moisture, protein, fat, fiber, ash and carbohydrate of biscuits supplemented with different levels of malted millet is showed in (Table 4).

4.4.1 Moisture content

Moisture contents of biscuits supplemented with different levels of malted millet was significantly (p˂ 0.05) decrease than the control biscuit made from wheat flour. Malted millet has the lower value of moisture content than the control biscuits. Bolarinwa et. al., (2016) reported that biscuits produced from malted sorghum and soybeans composite flour had moisture content ranged from 2.93% to 4.12%.

4.4.2 Fat content

Supplementation of biscuit with different levels of malted millet significantly (p< 0.05) improves its fat levels. Bolarinwa et.al., (2016) showed that in biscuits made with malted sorghum-soy biscuits the fat content increase from 25.08% to 30.79%. This is because supplementation with legume soy which has high level of fat improves its level in soy biscuits.

4.4.3 Protein content

Protein content of biscuits made with different levels of malted millet is showed in (Table 4). Biscuits made with malted millet flour have higher content of protein than the control. Bolarinwa et. al., (2016) reported that the protein content of malted sorghum-soy biscuits samples ranged from 7.28% to 11.74% which was similar to malted millet biscuits as presented in (Table 4).
4.4.4 Fiber content

Fiber contents of biscuits supplemented with different levels of malted millet significantly (p< 0.05) improved 4 to 7 times higher than in the control biscuit (Table 4). Olusegun et al., (2016) reported that the crude fiber of biscuits made with malted sorghum-soy flour ranged between 2.56% to 3.46%, similar to our finding in malted millet biscuits.

4.4.5 Ash content

In (Table 4) levels of ash were 1.16, 3.16, 3.34, 2.92 and 3.34% in control biscuit and biscuits supplemented with different levels of malted millet flour. Bolarinwa et al.,(2016) reported that ash of biscuits supported with malted sorghum-soy flour ranged between 2.20% to 2.81%. These values of ash in malted sorghum-soy biscuits is lower than that in (Table 4) for biscuits supplemented with malted millet flour.

4.4.6 Carbohydrate content

Carbohydrate content of biscuit and biscuits supplemented with different levels of millet flour ranged from 75.00 to 71.39% as showed in (Table 4) Bolarinwa et al., (2016) stated lower carbohydrate content of 47.08-59.95% in malted sorghum-soy biscuits that maybe due to high fat and protein content of soy biscuits.
Table (4) Chemical composition of biscuits supplemented with different levels of malted millet

<table>
<thead>
<tr>
<th>Types of biscuits</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fat</th>
<th>Fiber</th>
<th>Ash</th>
<th>Carbohydrate</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.16±0.42a</td>
<td>11.21±0.94b</td>
<td>0.89±0.07b</td>
<td>0.55±0.04c</td>
<td>1.16±0.16b</td>
<td>74.34±7.16a</td>
<td>380.47±3.64b</td>
</tr>
<tr>
<td>B</td>
<td>4.79±0.81b</td>
<td>11.46±0.27ab</td>
<td>3.52±0.30a</td>
<td>2.05±0.03d</td>
<td>3.16±0.32a</td>
<td>75.00±0.63a</td>
<td>377.56±6.16bc</td>
</tr>
<tr>
<td>C</td>
<td>5.12±0.23b</td>
<td>11.64±0.34ah</td>
<td>3.49±0.11a</td>
<td>2.41±0.19c</td>
<td>3.34±0.11a</td>
<td>73.98±0.24a</td>
<td>373.96±0.68bcd</td>
</tr>
<tr>
<td>D</td>
<td>5.26±0.83b</td>
<td>12.43±0.23ab</td>
<td>3.49±0.16a</td>
<td>3.36±0.12ba</td>
<td>2.92±0.51a</td>
<td>72.53±0.53a</td>
<td>373.96±0.68cde</td>
</tr>
<tr>
<td>E</td>
<td>5.35±0.64b</td>
<td>12.64±0.15a</td>
<td>3.44±0.22a</td>
<td>3.82±0.14o</td>
<td>3.34±0.23a</td>
<td>71.39±0.31a</td>
<td>367.14±0.24de</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicate independent analysis.

Values in the same column carrying different superscribe litter are significant different at (P<0.05).

Where:
A: 100% wheat flour.
B: 95% wheat flour + 5% malted millet flour.
C: 90% wheat flour + 10% malted millet flour.
D: 85% wheat flour + 15% malted millet flour.
E: 80% wheat flour + 20% malted millet flour.
4.5 Minerals content

The minerals content of biscuits supplemented with malted millet is showed in (Table 5).

Supplementation of biscuits with malted millet increased levels of Fe, Mg, Ca, P, K and Zn due to the higher level of millet as compared to wheat. Bolarinwa et al., (2016) reported that content of malted sorghum-soy biscuits ranged between 1.28 to 1.98 mg/100g. Mineral content of biscuits supplemented with millet flour show increasing as compared to wheat flour biscuits.
Table (4): Minerals content of biscuits supplemented with different level of malted millet

<table>
<thead>
<tr>
<th>Types of biscuit</th>
<th>Fe</th>
<th>Mg</th>
<th>Ca</th>
<th>P</th>
<th>K</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.49±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.72±0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>227.73±7.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.2±55.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>277.45±11.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16±0.44&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>8.06±10.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.34±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>224.77±15.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124.39±1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>248.9±36.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.07±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>2.06±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.58±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>220.50±2.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>131.48±0.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>243.7±24.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.72±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>2.28±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.36±2.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>236.2±27.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.22±1.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>251.3±18.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>2.22±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.06±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>232.81±4.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.28±1.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>241.16±9.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicate independent analysis.

Values in the same column carrying different superscribe litter are significant different at (P<0.05).

Where:
A: 100% wheat flour.
B: 95% wheat flour + 5% malted millet flour.
C: 90% wheat flour + 10% malted millet flour.
D: 85% wheat flour + 15% malted millet flour.
E: 80% wheat flour + 20% malted millet flour.
4.6 Safety of biscuits supplemented with different levels of malted millet

Food is considered safe when it free from pathogenic microorganism (staphylococcus, E. coli and Salmonella) biscuits supplemented with different levels of malted millet flour were safe. Pathogenic microorganism did not exist in all types of biscuits therefore its safe for human consumption as it showed in (Table 6).
Table (5): Safety of biscuits supplemented with different level of malted millet.

<table>
<thead>
<tr>
<th>Types of biscuit</th>
<th>Total count bacteria</th>
<th>Yeast &amp; moulds</th>
<th>Coli form</th>
<th>Stafilococcus</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>Nill</td>
<td>Nill</td>
</tr>
<tr>
<td>C</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
</tr>
<tr>
<td>D</td>
<td>Nill</td>
<td>&lt; 10</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
</tr>
<tr>
<td>E</td>
<td>&lt; 10</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
<td>Nill</td>
</tr>
</tbody>
</table>

Where:
A: 100% wheat flour.
B: 95% wheat flour + 5% malted millet flour.
C: 90% wheat flour + 10% malted millet flour.
D: 85% wheat flour + 15% malted millet flour.
E: 80% wheat flour + 20% malted millet flour.
4.7 Sensory evaluation of biscuits

There were no significant difference (P<0.05) in color, odor, taste, crumb texture grain and general acceptability between the control biscuit and other prepared by supplementation with different levels of malted millet flour (Table 7). Referring to these results, it is possible to produce biscuits supplemented with 20% malted millet flour having acceptable general characteristics as compared to control biscuits without supplementation.
Table (6): Sensory characteristic of biscuits supplemented with different level of malted millet.

<table>
<thead>
<tr>
<th>Type of biscuits</th>
<th>Color</th>
<th>Odor</th>
<th>Taste</th>
<th>Crumb texture</th>
<th>Crumb grain</th>
<th>General acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.13±1.35a</td>
<td>1.86±0.99a</td>
<td>2.40±1.45a</td>
<td>2.26±1.22a</td>
<td>2.20±0.94a</td>
<td>2.40±1.24a</td>
</tr>
<tr>
<td>B</td>
<td>2.53±1.12a</td>
<td>2.46±1.24a</td>
<td>2.26±0.88a</td>
<td>2.33±0.90a</td>
<td>2.46±0.83a</td>
<td>2.26±1.22a</td>
</tr>
<tr>
<td>C</td>
<td>2.26±1.22a</td>
<td>2.53±0.83a</td>
<td>2.93±1.03a</td>
<td>2.60±0.91a</td>
<td>2.73±1.03a</td>
<td>2.73±1.03a</td>
</tr>
<tr>
<td>D</td>
<td>2.13±1.12a</td>
<td>2.53±0.99a</td>
<td>2.53±1.24a</td>
<td>2.33±0.97a</td>
<td>2.46±0.91a</td>
<td>2.73±1.16a</td>
</tr>
<tr>
<td>E</td>
<td>2.06±0.88a</td>
<td>2.40±0.91a</td>
<td>2.40±1.29a</td>
<td>2.26±0.96a</td>
<td>2.33±0.97a</td>
<td>2.73±1.10a</td>
</tr>
</tbody>
</table>

Values are mean ±SD of triplicate independent analysis.

Values in the same column carrying different superscribe litter are significant different at (P<0.05).

Where:
A: 100% wheat flour.
B: 95% wheat flour + 5% malted millet flour.
C: 90% wheat flour + 10% malted millet flour.
D: 85% wheat flour + 15% malted millet flour.
E: 80% wheat flour + 20% malted millet flour.
CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results obtained in this study shown that millet flour has different level of protein, fat, and ash contents compared to wheat flour. Biscuits supplemented with different levels of millet flour showed increasing in protein, fat, fiber and ash content, also has increasing value of Fe, P, Ca, and Zn % as compared to control biscuits. But also has lower content in Mg and K as compared to control biscuits. On other hand biscuits were safe for human consumption since no pathogenic microorganisms detected in all biscuits. Therefore malted millet flour is a good choice for development of quality and nutritious convenience biscuit products.

5.2 Recommendation

1. Malted millet and millet based convenience biscuit food products can perhaps be popularized through proper marketing strategies to ensure consumer acceptability.
2. Use of composite flour will reduce over dependence on imported wheat flour so as to save foreign exchange.
3. More studies should done on biscuits supplemental with millet flour.
References


adiponectin, HDL-cholesterol, and insulin levels in genetically type 2 diabetic mice. Bioscience, Biotechnology and Biochemistry 69: 31-37.


Appendices

Plate 1.

A: Biscuit supplemented with 100% wheat flour.
Plate 2.

B: Biscuit supplemented with 95% wheat flour + 5% malted millet flour.
Plate 3.

C: Biscuits supplemented with 90% wheat flour + 10% malted millet flour.
Plate 4.

D: Biscuit supplemented with 85% wheat flour + 15% malted millet flour.
Plate 5.

E: Biscuit supplemented with 80% wheat flour + 20% malted millet flour.
Plate 6.
Panel test.
Sensory Evaluation of Biscuit Samples

Please evaluate the following samples according to their color, odor, taste, crumb texture, crumb grain, general acceptability. The ranking scores are given below:

1= Excellent  2= Very good  3= Good  4= Acceptable  5= unacceptable

<table>
<thead>
<tr>
<th>Samples</th>
<th>Colour</th>
<th>Odour</th>
<th>Taste</th>
<th>Crumb texture</th>
<th>Crumb grain</th>
<th>General acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
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<td>D</td>
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<tr>
<td>E</td>
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</tbody>
</table>