PHYTOCHEMICAL COMPOSITION OF *IPOMOEA BATATAS* AND *MORINGA OLEIFERA* LEAVES AND CRACKERS FROM UNDERUTILISED FLOURS.

BY

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DECLARATION

I hereby declare that this thesis is the result of my own work except references cited that have been duly acknowledged. It has never been submitted for the award of any degree.

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DEDICATION

This work is dedicated to my parents Mr. E. K. Owusu and Mrs M. P. Owusu.

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PUBLICATION NOTICE

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ABSTRACT

Great emphasis is being placed on the consumption of food that will not only provide nutrients to the body but also help in the prevention of diseases such as cancer, cardiovascular diseases, and age-related macular degeneration as well as malnutrition. People are being encouraged to eat fruits and vegetables which have antioxidative and bioactive compounds that help prevent molecular damage caused by oxidation. *Ipomoea batatas* and *Moringa oleifera* are two indigenous vegetables whose nutritional potential though felt in other parts of the world are not known in Ghana. Thus the need for the analysis for the nutritional and phytochemical potential of the new varieties of these vegetables found in Ghana as well as their use in product development. Phytochemical analysis of the leaves was carried out using standard methods of analysis. The results obtained showed that *Moringa oleifera* had higher levels of crude protein, crude fibre, crude fat, iron, calcium and beta-carotene. The *Ipomoea batatas* samples, however, had higher levels of crude ash, total carbohydrate, total calorie, moisture and total phenolics. Cream cracker were developed from cassava and sweetpotato flour incorporated with these leaves using wheat flour as the control. Sensory evaluation of the cream crackers developed showed that they have good sensory properties and since the cassava and sweetpotato flour crackers do not contain gluten, they can be consumed by those who are gluten intolerant.

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CHAPTER ONE

1.0 INTRODUCTION

People of all ethnic backgrounds, the old and young, rich and poor enjoy taking snacks at various times. It is reported by Howell (2005), that now more than ever consumers' interest in healthier snacking options, resulting in significant innovation related to whole grain and nutritious crackers has increased. Consumers are slowly nibbling their way back to a more typical, if somewhat more health conscious, pattern of consumption in crackers.

As a child grows, snacks become important since he/she cannot wait many hours between the regular schedule of meals (Satter, 1987, Bobroff, 2004). Therefore parents and caregivers should provide young children with snacks that consist of nutritious foods appropriate for the child's age that make a significant contribution to the nutritional quality of the diet. Many teenagers have high calorie needs, especially during growth spurts, and snacks chosen by teenagers often are high in fat and/or sodium, and low in key nutrients such as iron, calcium, vitamin A, vitamin C, and folate (Rolfes *et al*, 1998). Teenagers need to know how to balance low nutrient-dense meals with meals and snacks rich in vitamins, minerals, and phytochemicals from whole grains, fruits, vegetables, and legumes (Position of the ADA, 2000). Gluten intolerance, a genetic condition affecting about one in 200 people in the world makes people intolerant to gluten (Gluten Intolerance and Celiac Disease, 2006). Since there is up to date no medication against this condition, the best treatment is a gluten free diet (Feighry, 1999; Sheasby, 2001; Corcoran, 2006). Since individuals on any avoidance diet are at risk of developing deficiencies of micro-nutrients

(Steinman, 2007), healthy snacks in the form of enriched crackers can be helpful in preventing nutrient deficiencies.

It is reported (Consumer Reports on Health, 1998; Palmer, 2005; Martinez and Martinez. 2007), that researchers are convinced that the nutrients in fruits and vegetables do more than just prevent deficiency diseases such as beriberi or rickets with the most publicized finding being that certain vitamins or vitamin precursors in produce, notably vitamin C and beta-carotene, and polyphenols are powerful antioxidants. Antioxidants help prevent molecular damage caused by oxidation; this protection may help fend off many diseases including cancer, cardiovascular diseases, and macular degeneration (Islam *et al*, 2002).

Ipomoea batatas leaves have been proven by Islam *et al* (2002) to be an excellent source of antioxidative polyphenolics compared to other commercial vegetables, and though consumed in Asia and some sub-Saharan countries (Duke, 1983), have generally been an underexploited green vegetable as observed by Messiaen (1994). *Ipomoea batatas* listed by Abbiw (1990) as one of the vegetables consumed by all ethnic groups in Ghana is not produced on a large scale and it must be noted that only the storage roots are consumed in large quantities. Also there is no information about the nutritive and phytochemical potential of the new varieties of *Ipomoea batatas* (Sauti, Ogyefo, Apomuden, Otoo, Hi Starch, Okumkom, and Santom Pona) to be introduced to farmers in Ghana. Yoshimoto *et al* (2002) reported that the sweetpotato leaf is considered to be tougher in terms of texture than other leafy vegetables and therefore is less popular with consumers (Woolfe, 1992). As a result, there is the need

for the development of products that will be easily accepted by the consumer and have the necessary nutrients.

Leaves of *M. oleifera* can be an extremely valuable source of nutrition for people of all ages. In some parts of the world such as Senegal and Haiti, health workers have been treating malnutrition in small children and pregnant and nursing women with Moringa leaf powder (Price, 1985). Fuglie (2005), reported that 8 g serving of dried leaf powder will satisfy a child of 1-3 years with 14 % of the protein, 40 % of the calcium, 23 % of the iron, and nearly all the vitamin A that the child needs in a day. A 100 g portion of leaves could provide a woman with over a third of her daily need of calcium and give her important quantities of iron, protein, copper, sulphur, and B-vitamins. The Moringa is a fast growing tree that does well even in arid, sandy conditions, grows to four meters, and bears fruit within the first year of growth (Fuglie, 2001).

Unfortunately, though the benefits of *M. oleifera* and *I. batatas* are being felt in some countries of the world such as Senegal and Haiti and Phillippines, that is not the case in Ghana. The leafy vegetables given *importance* in relation to consumption do not include *Ipomoea batatas* and *Moringa oleifera* leaves (Blay, 2004). Crackers, though enjoyed by all are not highly nutritious. There is therefore the need to enrich crackers to provide more nutrients for the consumer, using the underexploited vegetables.
Therefore the aims of this project were:

- To determine the phytochemical composition of *Ipomoea batatas* and *Moringa oleifera* leaves
- To develop crackers from alternative flour sources.

CHAPTER TWO

2.0 LITERATURE REVIEW

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2.1 EMPHASIS ON HEALTH

Recently, great emphasis is being placed on consumption of food that will not only provide nutrients to the body but also help in the prevention of diseases. People are being encouraged to eat food not just to satisfy their hunger but to maintain and improve their health. As a result of this, research is continually being done in the determination of the nutrient content of fruits and vegetables. Several authors (Consumer Reports on Health, 1998; Palmer, 2005; Martinez and Martinez. 2007), report of the conviction of researchers that the nutrients or bioactive compounds in vegetables and fruits do more than just prevent deficiency diseases such as beriberi or rickets. The most widely publicized finding is that certain vitamins or vitamin precursors in produce, notably vitamin C and beta-carotene, are powerful antioxidants.

Antioxidants help prevent molecular damage caused by oxidation; this protection, according to Consumer Reports on Health (1998), may help fend off not only cancer, stroke, and coronary heart disease but also arthritis, asthma, cataracts, and macular degeneration, the leading cause of blindness after age 65 years. "Bioactive compounds" are extra nutritional constituents that typically occur in small quantities in foods. They are being intensively studied to evaluate their effects on health. The impetus sparking this scientific inquiry is the result of many epidemiologic studies that have shown protective effects of plant-based diets on cardiovascular disease (CVD) and cancer (Kris-Etherton *et al*, 2002). Surh (1999) agrees with Kris-Etherton

et al (2002) and goes on to report of many bioactive compounds that have been discovered and that these compounds vary widely in chemical structure and function and are grouped accordingly. Their beneficial effects are attributed partly to the presence of numerous polyphenolic compounds, which display antioxidant and free radical scavenging properties.

Radicals are defined by Guan and Whiteman (2004) as chemical species with one or more unpaired electrons. Free radicals are radicals that have moved out of the immediate molecular environment of their generation (Guan and Whiteman, 2004). These free radicals are generated continuously in the human body as by-products of normal metabolism such as the reduction of molecular oxygen in mitochondria during cellular respiration, and degradation of fatty acids and other molecules in peroxisomes (Guan and Whiteman, 2004). Free radicals and oxidants can trigger lipid peroxidation, as well as the oxidation of proteins and DNA, causing extensive damage to body cells. They are implicated in some diseases such as Alzheimer's disease (Athar, 2002), cancer (Szweda *et al*, 2002), vascular disorders (atherosclerosis, diabetes, hypertension) (Chen *et al*, 2003) and accelerating the aging process (Szweda *et al*, 2002). Increasing the level in foods of highly active radical scavenging compounds is important for maintaining a balance between oxidants and antioxidants in the body and for eliminating oxidative stress. It is also clear from Guan and Whiteman (2004) that most of the dietary antioxidants have low or minimal toxicity, and that intake can be increased without adverse effects.

Phenolic compounds have the ability to scavenge free radicals and to contribute significantly towards antioxidant activities of vegetables and fruits. Plant phenolics

comprise a large variety of substituted phenolic compounds that give colour and astringent taste to foods and according to Fennema (1996) the glycosidic flavonoids are the largest groups of compounds among the plant phenolics. Phenolic acids such as caffeic, ferulic and gallic acids and their derivatives are abundant in many plants and they include anthocyanin pigments, yellow flavones, flavonols, chalcones and isoflavones. Phenolic compounds, including their subcategory, flavonoids, are present in all plants and have been studied extensively in cereals, legumes, nuts, olive oil, vegetables, fruits, tea, and red wine. According to several authors (Huang and Ferraro, 1991; Hagerman *et al*, 1998; Kaul and Khanduja, 1998), polyphenolics have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases.

Recently, health workers and scientist are encouraging the intake of fruits and vegetables with high polyphenolics and therefore high antioxidant content (Best, 2005) because these, as well as nutrients, are best absorbed and used by the body when they are derived from natural sources (plants and animals). These are present in naturally occurring complex compounds, and not as separate compounds as formulated in pills (Sambou, 2005). Fuglie (2005) stated that among the wide range of green leafy vegetables, Moringa is the richest source of β-carotene (pro-vitamin A), with sweetpotato leaves also having appreciable amounts of β-carotene (Yoshimoto, 2001). According to our present knowledge, Moringa (leaves, seeds, pods) contains specific plant pigments with demonstrated potent antioxidant properties such as the carotenoids – lutein, alpha-carotene and beta-carotene, xanthins and chlorophyll; contains powerful antioxidant vitamins such as vitamin C, E; and has essential micronutrients with antioxidant activity or directly linked to this

process: selenium and zinc. Moringa also contains other phytochemicals with known powerful antioxidant ability such as kaempferol, quercetin, rutin and caffeoylquinic acids (Fuglie, 2005).

Ipomoea batatas leaves, according to Islam *et al* (2002) and Komaki and Yamakawa (2007), are also an excellent source of antioxidative polyphenolics such as caffeoylquinic acid, anthocyanins, as well as beta-carotene. As reported by Marcu (2005), excess consumption of the leaves does not lead to toxicity since the polyphenols can be eliminated or deposited in the fat tissues. The plant phenols, because of their diversity and extensive distribution, are the most important group of natural antioxidants, and they contribute to the organoleptic and nutritional qualities of fruits and vegetables (Fennema, 1996; Islam *et al*, 2002).

2.2 SUMMARY OF SOME PHYTOCHEMICALS FOUND IN THE LEAVES

Beta-Carotene: Beta-carotene is strong against singlet oxygen, can stimulate DNA repair enzymes, gives better cornea protection against UV light than lycopene and can boost the activity of Natural Killer (NK) immune cells (Best, 2005).

Flavonoid Polyphenolics: Best (2005) also describes flavonoids as flavone-like substances that are usually antioxidants and sometimes anti-inflammatory. Flavonoids scavenge free radicals by forming a stable radical that can react with another flavonoid radical to produce two non-radicals.

Anthocyanins: Anthocyanins are water-soluble glycosides and acyl-glycosides of anthocyanidins. They make cherries & strawberries red, blue berries blue and sweet potato leaves red. Anthocyanins have anti-inflammatory effects and protect endothelial cells from oxidative damage (Best, 2005).

Isoflavones: Isoflavones are named as some of the phytochemicals found in sweetpotato and Moringa leaves as well as in other legumes, parsley and grains by Best (2005). They elevate HDL cholesterol (good cholesterol) and lower LDL cholesterol (bad cholesterol). Also, they are potent antioxidants against superoxide and hydrogen peroxide, have estrogenic-like qualities (phytoestrogen), may reduce menopausal symptoms, prevent bone resorption (osteoporosis) in post-menopausal women, may prevent breast cancer, inhibit prostate cancer cells by 30%, and inhibit tyrosine kinases involved in tumorigenesis.

Chlorogenic Acid: According to Best (2005) this phytochemical is very high in sweet potato tops, found in the flesh of grapes, along with ellagic acid and is most frequently an ester of caffeic acid. Caffeic acid reduces mutagenicity of polycyclic aromatic hydrocarbons, is a major contributor to the antioxidant activity of coffee and can regenerate oxidized Vitamin E.

Lycopene: Lycopene gives the red colour of tomatoes, watermelon, pink grapefruit, guava and papaya. Light and heat converts the trans form to the cis form, which is more bioavailable. Lycopene binds tightly to fibres, is freed by high heat, not soluble in water but more soluble in oil. It is classified as one of the powerful antioxidant which reduces damage to DNA and proteins and gives better skin protection against UV light than beta-carotene by Best (2005). Accounting for nearly half the total carotenoids in the blood serum, lycopene concentrates in the skin, testes, adrenal and prostate where it protects against cancer.

Alpha-Carotene: Alpha –carotene is ten times more anti-carcinogenic than beta-carotene, enhances release of immunogenic cytokines and helps in improvement of vision.

Lutein and Zeaxanthin: Lutein and zeaxanthin constitute about half of all carotenoids in the retina, give corn, avocado and egg yolk a yellow colour. Lutein and zeaxanthin are the only carotenoids in the macula of the eye (Fig 2.4). They protect the eye from macular degeneration and cataracts by absorbing damaging blue light (Best, 2005 and Sahelian, 2007). Lutein and zeaxanthin are present in nearly equal amounts in the macula.

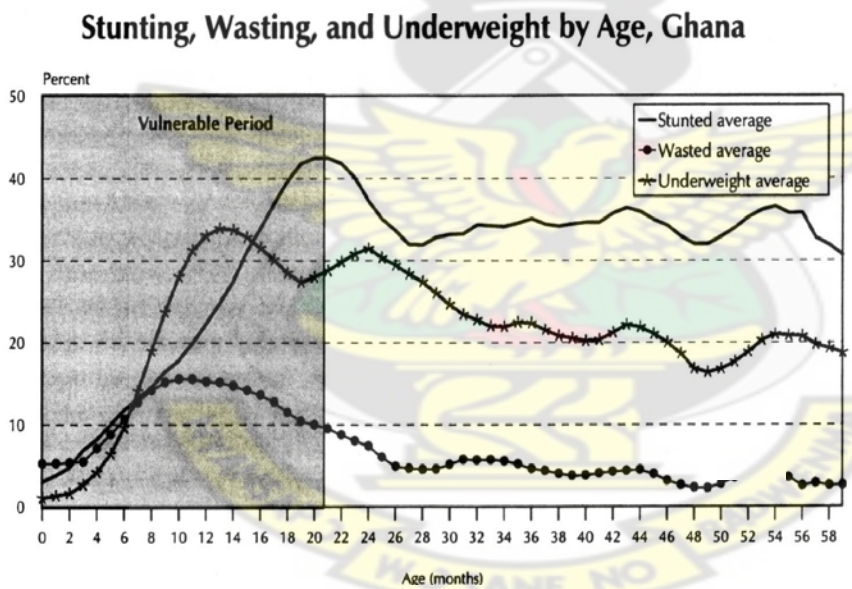
***Chlorophyll**: Chlorophyll is the most abundant pigment in plants as well as the principal light-absorbing pigment in photosynthesis (Taiz and Zeiger, 2002 and Best, 2005). It has a porphyrine ring similar to heme (of haemoglobin), but with a magnesium (not iron) central atom. It is not water soluble and forms tight molecular complexes with some carcinogens: aflatoxin-B1, polyaromatic hydrocarbons (tobacco smoke) & heterocyclic amines (cooked meat).*

2.3 BENEFITS OF THE LEAVES

2.3.1 *FIGHT AGAINST MALNUTRITION*

Malnutrition in its various forms (kwashiorkor, beriberi, anaemia, and scurvy) is reported as a major factor in the high rates of infant mortality as well as ill health in adults in the tropics (Moringa oleifera: The Miracle Tree, 2000). The former United Nations Secretary-General, Kofi Annan, lamented that Africa is the only continent where child malnutrition is getting worse rather than better (Fuglie, 2005). Black (2003) agrees that malnutrition/ micronutrient deficiencies are now recognized as an important contributor to the global burden of disease. Iron deficiency is attributed to the cause of about 800,000 deaths and 2.4 % of the global burden of disease. The Ghana Statistical Service (GSS) reported in the 2004 Ghana Demographic and Health

Survey (GDHS) that children under the age of five years and women of reproductive age are most vulnerable to malnutrition. It states that micronutrient deficiency is a major threat to maternal health as it contributes to low birth weight, lowered resistance to infection, poor cognitive development, and decreased work capacity. According to the 2004 GDHS (Fig 2.1), 30 percent of children under five are stunted and 11 percent severely stunted. Seven percent of children under five are wasted and 1 percent severely wasted. Weight-for-age results show that 22 percent of children under five are underweight, with 5 percent severely underweight.



Note: *Stunting* reflects chronic malnutrition; *wasting* reflects acute malnutrition; *underweight* reflects chronic or acute malnutrition or a combination of both. Plotted values are smoothed by a five-month moving average.

Fig 2.1 Malnourishment in children in Ghana (GDHS, 2004)

Considerable investments have been made by governments and aid agencies to promote solutions to this problem. Yet a major problem or drawback of many of the approaches used is the dependence on imported solutions and personnel from outside the country and because of these setbacks as stated in Moringa oleifera: "The Miracle Tree" (2000), progress can quickly dissipate once the programme funding finishes.

Interest is growing in the use of *Moringa oleifera* and *Ipomoea batatas* in addressing malnutrition in developing areas of the world. Church World Service (CWS), in a report by UNESCO in "Improving nutrition with Moringa 'miracle' trees in Senegal" (2003), initiated a programme in Senegal which has demonstrated that the highly nutritious leaves of the Moringa plant are very effective at helping prevent malnutrition. The nutritional value of Moringa is compared to other produce and has been shown to be richer in micronutrients. Figure 2.2 shows the relationship.

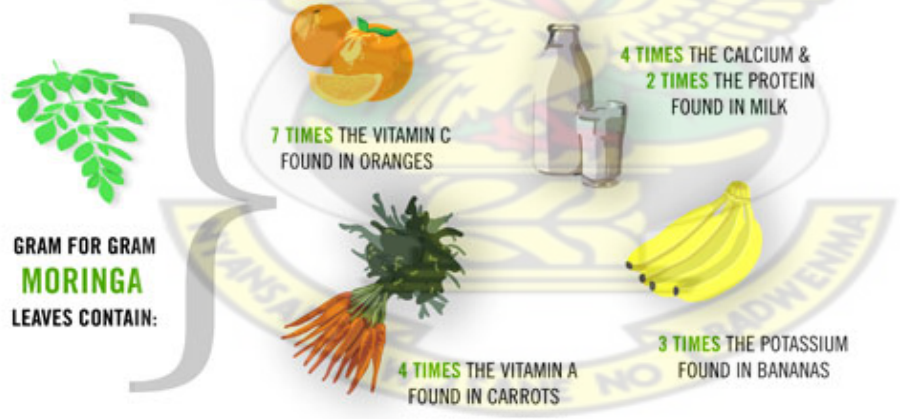


Figure 2.2 Nutritional value of Moringa leaves (Hsu *et al*, 2006).

Fuglie (2005) states that 8 g serving of dried leaf powder will satisfy a child of 1-3 years with 14 % of the protein, 40 % of the calcium, 23 % of the iron and nearly all the vitamin A that the child needs in a day. As little as 20 grams of dried leaves

would provide a child with all the vitamins A and C he needs. It is fast growing and drought resistant, remaining green when other leafy vegetables are out of season. This makes it available all year round as a supplement to the diet.

Though sweetpotato leaves are not new discoveries, and are highly nutritious, work has not been done on the new varieties found in Ghana to know their nutritional profile. Indigenous tropical vegetables including sweetpotato greens are reported by Woolfe (1992) as having low prestige in many parts of the world, being regarded as 'poor man's salad' and as such are less desirable than other vegetables. This has made the acceptability of the vegetable low in most areas of the world where they are grown, such as China, New Guinea, Sierra Leone, Tanzania and Liberia (As-Saqui, 1982). *Moringa oleifera* and *Ipomoea batatas* because of their highly nutritious leaves are a good solution to malnutrition. They provide good quantities of micronutrients and polyphenols needed for effective growth and metabolism as well as the fight against malnutrition in humans.

2.3.2 CANCER PREVENTION

Cancer is defined by Lewis *et al* (2002) as a derangement in cell cycle control since it begins with a single cell that breaks through its death and division control. These authors go on to explain that two major genes contribute to causing cancer, oncogenes and tumour suppressor genes. Oncogenes are versions of genes that normally trigger cell division, but they are over expressed and accelerate the cell cycle allowing the excess new cells to form a tumour. Tumour suppressor genes produce a protein product that normally prevent a cell from dividing or promote normal cell death. Inactivation or removal of a tumour suppressor gene can cause cancer. Heallkey,

(2006), agrees with Lewis *et al* (2002), adding that cancer is also caused by carcinogens (cancer causing substances) such as tobacco smoke; ultraviolet radiation from sunlight; industrial chemicals, viruses and genetics. Since cancer cells divide more than the cells from which they derive, when given sufficient nutrients and space, they divide uncontrollably and eternally (Lewis *et al*, 2002).

Fig 2.3 shows that most countries in the world have cancer cases of more than 151 per 100,000 people. Developing countries can learn from past mistakes by encouraging citizens to increase their intake of vegetables such as *Ipomoea batatas* and *Moringa oleifera* leaves.

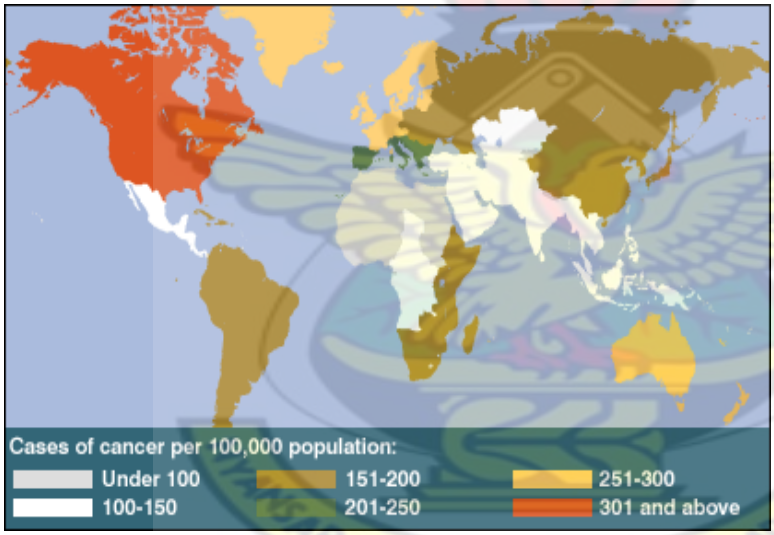


Fig. 2.3 Cancer cases worldwide (Worldwide Cancer Cases 'Double', 2005).

In the search to conquer this disease worldwide, scientists have noticed that the increased consumption of vegetables such as Moringa and sweetpotato leaves (which contain phenolic compounds) is helpful. Epidemiological studies have demonstrated the protective effects of vegetable consumption on human health. Many polyphenolic compounds have an anti-carcinogenic (anti-cancer) action by:

1. slowing cell proliferation (division) by interfering with the cell cycle
2. inducing apoptosis (cell suicide)
3. inhibiting phase 1 enzymes (enzymes that convert harmless substances into carcinogens)
4. inducing phase 2 enzymes (enzymes that can attach carcinogens to molecules that facilitate speedy excretion) (Best, 2005).

Apart from polyphenols, Null, (2004), states that beta-carotene also helps to protect the body against many types of cancer by trapping and deactivating free radical and other oxygen metabolites that damage DNA causing cell mutations that lead to cancer. A study by Watson (1991), found that beta-carotene increases the number of natural killer cells and T-helper cells. This stimulation of the immune system suggests that beta carotene has cancer-prevention potential. Many studies conducted answer the question about β-carotene and cancer prevention: Is it the beta-carotene itself that provides this protection, or is it vitamin A which beta-carotene becomes in the body? According to Gaby and Singh (1991), 17 studies distinguishing between these two nutrients found that beta-carotene offered significant protection but that preformed vitamin A (from animal sources) was not associated with the incidence of cancer. This suggests that beta-carotene has specific protective effects at the cancer sites independent of its provitamin A activity.

Ipomoea batatas and *Moringa oleifera* have been proven to be good sources of phytochemicals including polyphenols and β-carotene. Islam *et al* (2006) state that sweetpotato leaves having about fifteen different anthocyanin compounds and about six different polyphenolic compounds are excellent sources of bioactive anthocyanin

and polyphenolic constituents. Sufficient amounts of these polyphenols in the human system slow cancer proliferation, induces apoptosis, inhibits harmful enzymes and induces beneficial enzymes.

Moringa oleifera apart from being rich in the above mentioned phytochemicals, also has some anticancer compounds that are unique to the plant including, (4-(4'-*O*-acetyl-α-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (Fahey, 2005). All these phytochemicals have an anticancer effect through different actions as stated above and this goes to prove that eating a higher intake of fruit and vegetables, getting a proper diet high in antioxidants and cutting out the cigarettes are the keys not only to avoiding cancer but to living longer generally.

2.3.3 FIGHT AGAINST CARDIOVASCULAR DISEASES

The definition of cardiovascular disease (CVD) given by Medicinenet.com (Definition of Cardiovascular disease, 2007) is; a general term used to classify numerous conditions affecting the heart, heart valves, blood, and vasculature of the body. Healthy food habits can help maintain normal blood pressure, desirable blood cholesterol levels and a healthy body weight. The optimal treatment for CVD according to the Creighton University Medical Centre (Cardiovascular Disease, 2007), is prevention and modification of risk factors since the progression of these diseases beyond prevention requires surgical intervention. Studies by Granato (2003) have indicated that sufficient intake of dietary antioxidant as found in sweetpotato and Moringa leaves may help in the prevention of cardiovascular diseases. The study

shows that the goal of nutritional components and antioxidants is the elimination of cellular waste products that build up in the bloodstream. The antioxidants, found in Moringa and sweetpotato leaves, quench free radicals by donating an electron and stabilizing the compound. Beta-carotene's most powerful role is to quench singlet oxygen, the reactive oxygen species that can generate free radicals by unloading its excess energy onto other molecules. Due to the fact that β-carotene is a large molecule consisting of a long string of 11 double bonded with single bonded in between, it can absorb the singlet oxygen's energy and spread it throughout the long chain of bonds. It then releases the energy as heat and returns to its usual state (Gaby and Singh, 1991). Also as reported by Hennekens (1992), it may offer some protection against the oxidative damage associated with low density lipoproteins (LDL), which transport cholesterol through the arteries and contribute to blocked vessels. Yet another way in which polyphenols help to prevent atherosclerosis is by boosting the activity of vitamin C, which in turn increases the levels of vitamin E. This synergy increases the overall resistance to oxidative stress (Very Berry- and Grape too, 2001).

Polyphenols, carotenoids and flavones, which are found in leaves and vegetables, have been studied for their abilities to prevent lipid peroxidation, inhibiting the development of atherosclerosis and lowering blood pressure. These antioxidants found in Moringa and sweetpotato leaves even help during digestion, inhibiting some of the oxidation of fats in gastric fluid (Granato, 2003). Beta-carotene as an antioxidant is a highly effective quencher of singlet oxygen (the unstable oxygen metabolites with altered energy states) and a direct scavenger of free radicals. In addition, beta-carotene survives the process of absorbing singlet oxygen intact.

Therefore, as reported by Null (2004), a single molecule of beta-carotene can arrest up to 1,000 molecules of singlet oxygen. Some of the best-known sources of these antioxidants are sweetpotato and Moringa leaves.

2.3.4 *FIGHTING AGE-RELATED MACULAR DEGENERATION* (AMD)

Age-related macular degeneration is a disease associated with aging that gradually destroys sharp, central vision needed for seeing objects clearly and for common daily tasks such as reading and driving. It progressively destroys the macula (Fig 2.4), the central portion of the retina, impairing central vision (National Eye Institute, 2006).

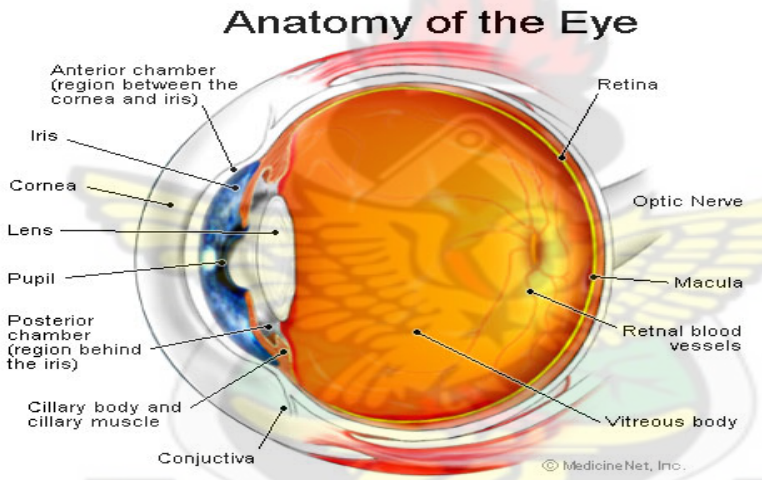


Fig. 2.4 Human Eye (MedicineNet.com, 2006)

Several authors (Henahan, 2001; National Eye Institute, 2006; Haddrill, 2006; The University of Michigan Kellogg Eye Centre, 2006; Thehealthierlife.co.uk, 2006) report that age-related macular degeneration occurs in two forms; 'wet' and 'dry'. Wet AMD occurs when abnormal blood vessels behind the retina start to grow under the macula and since these blood vessels are fragile, they leak blood and fluid which

raises the macula from its normal place at the back of the eye causing damage to the macula. Dry AMD occurs when the light-sensitive cells in the macula slowly break down, gradually blurring central vision in the affected eye. As dry AMD gets worse, you may see a blurred spot in the centre of your vision. Over time, as less of the macula functions, central vision is gradually lost in the affected eye. The retina is particularly susceptible to oxidation as its need for oxygen is large. Carotenoids, having antioxidant properties, reduce the risk of retinal cells being oxidized and damaged. Two of these carotenoids, lutein and zeaxanthin (together known as xanthophylls), are particularly important since they make up the macular pigment that is thought to limit retinal oxidative damage by filtering out blue light, quenching excited triplet state molecules or singlet molecular oxygen and scavenging further reactive oxygen species like lipid peroxides or the superoxide radical anion (Sahelian, 2007). According to the above author, supplementation with lutein has been shown to improve macular degeneration because in a study of healthy women younger than 75 years, consumption of diets rich in lutein and zeaxanthin, which are found in green leafy vegetables, such as sweetpotato and Moringa, seemed to stave off intermediate age-related macular degeneration.

The antioxidants (xanthophylls) that protect the retina by either delaying the progression of macular degeneration if one already has the eye condition (meaning that vision won't get worse as quickly) or by preventing the development of this serious eye disorder are listed as some of the phytochemicals found in *I. batatas* and *M. oleifera* (McLaren and Frigg, 2001; Best, 2005; Liu *et al*, 2006; Zija:Drink life in, 2007).

2.3.5 DEFENCE AGAINST AGING

In Mader (2006), aging is defined as a slow process during which the body undergoes changes that contribute to an increased risk of infirmity, disease, and death. According to him, there is great interest in gerontology, the study of aging, because in the next half-century, the number of people over the age of 75 years is expected to rise from the present 8 million to 14.5 million, and the number over age 80 years is expected to rise from 5 to 12 million (Mader, 2006). The present goal of gerontology is not necessarily to increase the life span, but to increase the health span, the number of years an individual enjoys the full function of body parts and processes.

Boelsma *et al*, (2001) describes the various effects aging has on the body. The skin becomes thinner and less elastic. There is less adipose tissue in the subcutaneous layer; therefore, older people are more likely to feel cold. Homeostatic adjustment to heat is also limited because there are fewer sweat glands for sweating to occur. The heart shrinks because of a reduction in cardiac muscle cell size. Growing inelasticity of lung tissue means that ventilation is reduced. Blood supply to the kidneys is also reduced. The kidneys become smaller and less efficient at filtering wastes. Salt and water balance are difficult to maintain, and the elderly dehydrate faster than young people. After age 50 years, the ability to hear tones at higher frequencies decreases gradually. The lens of the eye does not accommodate as well and also may develop a cataract. Glaucoma is more likely to develop because of a reduction in the size of the anterior cavity of the eye. Aging is accompanied by a decline in bone density.

Osteoporosis, characterized by a loss of calcium and mineral from bone, according to Perricone (2002) and Mader (2006) is not uncommon.

Clearly, aging and age-associated diseases are promoted by free radicals and inflammation. The process can be dealt with by increasing dietary antioxidants, which can boost endogenous defences together with the body's antioxidants such as superoxide dismutases (SOD) and glutathione (Borek, 1997) in decreasing the incidence of age-associated disease in all organ systems.

As has been stated earlier, *Moringa oleifera* and *Ipomoea batatas* leaves are rich sources of dietary antioxidants such as: anthocyanins that have an anti-aging effect making those who consume it healthier (Anti-aging Supplement/Anthocyanins, 2007); vitamin E a defence against lipid oxidation, heart diseases (Borek 1997), cataracts (Robertson, 1991) and macular degeneration (Snodderly, 1995), protecting cells against the cancerous effects of x-rays, chemicals, air pollutants and ultraviolet light (Borek, 1997); carotenoids especially β-carotene which can disarm reactive oxygen molecules generated by sunlight and air pollution, prevent free radical damage to skin, eyes and lungs (Krinsky, 1989), prevent cancer risk in many sites as well as heart diseases; flavonoids such as isoflavones, resveratrol and catechins which inhibits the body's production of chemicals that increase inflammation and protects against cancer and heart diseases (Borek, 1997). Due to the fact that free radical damage accumulates with age, people should consume vegetables and fruits which contain antioxidants from an early age to achieve long-term benefits.

2.4 CRACKERS

2.4.1. *HISTORY OF CRACKERS*

A cracker is defined by the American Heritage Dictionary of English Language as a thin crisp wafer made from flour and water with or without leavening and shortening; unsweetened or semisweet. History is not clear when people first began to make hardtack (crackers), but it's quite probable that its history began in prehistory. Prehistoric people boiled grains; they cooked grains and added vegetables and herbs to the mixture; and sometimes they ground it into a powder, mixed it with water, and dried it on a hot stone. Six thousand year-old unleavened biscuits have been found in Switzerland (Stradley, 2004).

The development of the cracker is attributed to Sylvester Graham, an American clergyman who in 1829 concocted the recipe for a cracker whose main ingredient was un-sifted, coarsely ground whole wheat flour. Touting his product as a health food, he produced and sold it locally. Over time, it became known the Graham cracker. According to the history given in Graham crackers, definition (2006), due to its popularity and innovation, other bakeries copied his recipe and eventually developed methods for its mass production. It was touted as a health food in the early 1800's, when Americans believed good eating meant as much meat and fowl as one could consume, the fatter the better (Barnhart and Metcalf, 1997). The different types of crackers depending on the ingredients used and nutritional value described in Crackers (2000) are: *Saltine*- a cracker sprinkled with salt before baking; *Soda cracker*- unsweetened cracker leavened slightly with soda and cream of tartar; *Oyster cracker*- a small dry usually round cracker; *Graham cracker*- semisweet whole-wheat cracker; *Wheat crackers*- crackers with more white than wheat flour; *Cream crackers*- crackers made with cream.

2.4.2 *SALIENT CHARACTERISTICS OF CRACKERS*

i. Ingredients:

The crackers shall consist of flour, water, leavening ingredients, and vegetable shortening or oil. The crackers may consist of salt, emulsifiers or other stabilizers, flavours, and other ingredients appropriate for the type of crackers

ii. Appearance and colour:

The crackers shall have a uniformly brown crust characteristic of the product. The crackers shall have typical volume, characteristic grain, and be evenly baked with colour highlights without evidence of scorching or burning. There shall be no foreign colour to the product. The delivered crackers shall not be crushed or damaged.

iii. Odor and flavor:

The crackers shall have a flavor and aroma characteristic of cream crackers. There shall be no foreign odors or flavors such as, but not limited to, scorched, stale, rancid, or mouldy.

iv. Texture:

The texture of the crackers shall have a characteristic texture for crackers. The crackers shall possess a firm, crisp crust.

v. Enrichment:

When enriched, the crackers shall have the enrichment ingredients evenly distributed in the finished product.

vi. Foreign material:

All ingredients shall be clean, sound, wholesome, and free from evidence of rodent or insect infestation.

vii. Fat content:

The fat content for crackers shall not be less than 5.0 percent or more than 15.0 percent.

viii. Moisture content:

The moisture content shall not exceed 6.0 percent (The U.S. Department of Agriculture, 1998).

2.4.3 *CURRENT CONDITIONS*

The U.S. Chamber of Commerce noted that cookie and cracker manufacturing was the fastest growing segment of the bakery industry in 1992. Shipments of all bakery products rose an average of 1.3 percent per year from the years 1987 to 1992. Sales of crackers for the same years, however, increased by rates of 2.3 percent (American Industries Information about Cookies and Crackers, 2005). Well into the late 1980s, bakery goods showed a consistent increase in sales, but consumption of sweet baked goods began to decline around 1992 (Howell, 2005).

It is reported by the American Industries Information about Cookies and Crackers (2005) that in the late 1990s crackers became the second largest segment of the dry grocery category in supermarkets, with carbonated beverages coming in first. Almost 98 percent of all households purchase cookies and crackers. Due to this market saturation, manufacturers of cookies and crackers have had to work harder at

expanding their sources of sales. These sales points now include various countries in the world, Ghana inclusive.

2.5 GLUTEN INTOLERANCE

The definition of gluten given by Sheasby (2001) is a protein that occurs naturally in wheat and rye and is related to similar proteins in oats and barley. This means that gluten is present in all foods that are made from these grains. Gluten intolerance also known as celiac disease is a genetic condition that makes people intolerant to gluten. This life-long condition affects about one in 200 people in the world (Gluten Intolerance and Celiac Disease, 2006). In fact in celiacs, this protein actually *attacks* the lining of the small intestine causing damage that flattens out the tiny villi. When a celiac eats food containing gluten, the intestine responds to the food as if it were a foreign body. The lining of the intestine becomes inflamed and this causes the villi to become flattened, reducing the surface area of the gut which is then no longer able to absorb nutrients efficiently. Over time, according to Sheasby (2001), weight loss and wasting can occur, leading to malnutrition. Severe symptoms reported by Corcoran, (2006), include chronic abdominal bloating and pain, diarrhea, constipation, weight loss and sometimes a blistery rash. Other symptoms include bone or joint pain, fatigue, depression, osteoporosis and anemia. Dermatitis herpetiformis (DH) is another form of gluten intolerance that affects the skin by forming lesions that are watery and *itchy* blisters. DH is only present when the patient has inherited the gene. In this case they always also have the intestinal symptoms as described above (Gluten Intolerance and Celiac Disease, 2006).

Up to date, no medication has been formulated against gluten intolerance and the best method of treatment is a gluten-free diet (Feighry, 1999; Sheasby, 2001; Corcoran, 2006). This means the avoidance of all foods made from grains containing gluten. Although avoidance of products containing gluten is straightforward, the complex manufacture of modern processed food means that the ongoing advice of a trained dietician is required. Individuals on any avoidance diet are at risk of developing deficiencies of micro-nutrients (e.g., thiamine, riboflavin, niacin, iron, selenium, chromium, magnesium, folacin, phosphorus and molybdenum) (Steinman, 2007). It is therefore essential that patients consume lots of fruits and vegetables such as *Moringa oleifera* and *Ipomoea batatas*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCE OF RAW MATERIALS

Leaves of seven (7) new varieties of *I. batatas* (Sauti, Ogyefo, Apomuden, Otoo, Hi Starch, Okumkom, and Santom Pona) were harvested from the Crops Research Institute (CRI) at Fumesua, Kumasi. One specie from the Moringaceae family common in Ghana *M. oleifera* leaves used for this project was obtained from the Horticulture Department of the Faculty of Agriculture, KNUST, Kumasi. Both species of leaves were grown in the wet semi-equatorial climate of Ghana with temperatures between 26 - 30°C and relative humidity between 70 - 80%. Soil in the area is of the forest ochrosol type which is less leaching and contains greater nutrients (Dickson and Benneh, 1980).

~~Sweetpotato tops of the seven varieties used for this work were obtained from the Crops Research Institute (C.R.I) at Fumesua. The varieties used were Sauti, Ogyefo, Apomuden, Otoo, Hi Starch, Okumkom, and Santom Pona. The *Moringa*~~

~~*oleifera* leaves used for this project were obtained from the KNUST Horticulture Department of the Faculty of Agriculture.~~

3.2 SAMPLE HARVESTING

The *I. batatas* leaves were harvested during the rainy season at the phase of rapid growth of vines and hence large increase in leaf area. Leaves for *M.* oleifera were harvested during the same period as the *I. batatas* leaves, when new branches and pinnae were being formed. The *I. batatas* tops obtained were harvested after 15 cm apical tip of the vine, including the stem, petioles and tender leaves of the plant. The *M.* oleifera leaves were also harvested at 15 cm from the tip of the pinnae. The young tips were harvested because they are tender and not as tough as the older leaves (Woolfe, 1992; Fuglie, 2001).

~~Seven (7) varieties of Sweetpotato tops were harvested for the project. The tops obtained were harvested at about 3 cm from the tip of the vine and this part included some of the leaves as well as the vines of the plant. For the Moringa leaves, the fresh leaves on the Moringa tree were harvested and used for the production.~~

PREPARATION OF LEAVES (Blanched).

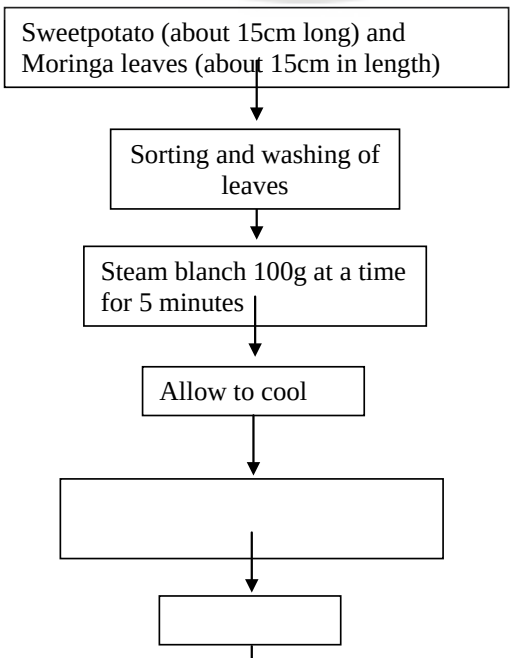


Fig 3.1 Preparation of Leaves

3.3 PREPARATION OF THE LEAVES

The *Ipomoea batatas* tops (about 15 cm in length from the tip of the plant) and *Moringa* leaflets obtained after harvesting were sorted to eliminate damaged ones and washed. The leaves were steam blanched hundred grams (100 g) at a time for five minutes to ensure same treatment for all the tops (for both Moringa and Sweetpotato). This was done to inactivate the polyphenol oxidase enzyme which causes browning by oxidizing the phenolics present when the leaves are bruised as well as to preserve the nutritional value of the leaves. The samples after the steam blanching were allowed to cool and shade dried until constant weight. They were then milled and packaged into polypropylene films to avoid moisture absorption. The packaged leaves were placed in black polyethylene to avoid photo-oxidation. All the samples were

stored in a deep freezer prior to analysis. In all, eight (8) different samples were obtained from the *Moringa* leaves as well as the sweetpotato tops. Pictures in Appendix A.

3.4 CHEMICAL ANALYSIS

3.4.1 DETERMINATION OF MOISTURE

Two grams (2 g) of sample was weighed into a previously dried and weighed glass crucible and dried in a thermostatically controlled oven at 105 ^{o}C for 6 hours. The glass crucible containing the dried sample was removed and placed in a desiccator to cool, and then weighed. This was then placed in the oven, heated, cooled and weighed again, repeating until a constant weight was obtained. The moisture content was then determined by difference and expressed as a percentage of the initial weight of the sample (AOAC, 1990). This was done in duplicates.

3.4.2 TOTAL ASH

Two grams (2 g) ~~of the sample~~ was weighed from each variety of the samples in duplicates into a previously washed, dried, ignited and weighed Gouch porcelain crucible and then the dish with its contents placed in a Muffle furnace (Gallenkamp, England) preheated to 600^{o}C for two hours (2 hrs). The crucibles and their contents were removed and cooled in a desiccator after which they were weighed. The ash content was then calculated by difference and expressed as a percentage of the initial weight of the sample.

3.4.3 DETERMINATION OF MINERAL MATTER

Mineral composition was determined by acid digestion of the ash after it had been weighed. 5 ml of 5 N HCl was added to the ash, allowed to cool, transferred into a 50 ml volumetric flask and made to the mark with distilled water. The mineral contents were then measured using the Atomic Absorption Spectrophotometric technique (AAS) (UNICAM model 929).

3.4.4 *CRUDE FAT DETERMINATION*

Two grams (2 g) of the moisture free sample was put in a paper thimble and plugged with cotton wool. The thimble was placed in a soxhlet extraction apparatus and extracted with petroleum ether (b.p 40-60 ^{o}C) and methanol mixed properly in the ratio 1:1, at low heat for 6 hours in a continuous extraction manner. The extract was collected in a flask and dried at 100 ^{o}C, cooled and weighed. The difference in weight between the empty flask and the flask and its dry contents was recorded as crude fat (Antia et al, 2006).

3.4.5 CRUDE FIBRE DETERMINATION

Two grams (2 g) of the de-fatted sample was transferred into a 750 ml Erlenmeyer flask and ½ g of asbestos was added. 200 ml of hot and boiling 1.25 % H_2SO_4 solution was added and heated to boil under reflux until the sample was thoroughly wetted for 30 minutes on a hot plate. The sample was then filtered through linen cloth in a funnel and washed thoroughly with boiling water until the washings were no longer acidic (did not change blue litmus paper red). The sample and asbestos were washed back into the flask with 200 ml boiling 1.25 % NaOH solution. The flask with its contents were refluxed for another 30 minutes. The sample with asbestos were again filtered and washed with boiling water until the washings were no longer

alkaline (did not turn red litmus paper blue). They were then washed with 15 ml alcohol and the residue left was transferred into a Gouch porcelain crucible. The crucible and its contents was dried for 1hour at 105 ^{o}C in a drying oven and cooled in a desiccator, and weighed. The crucible and its content were then ignited in a muffle furnace for 30 minutes at 600 ^{o}C, cooled in a desiccator and reweighed. The loss of weight was reported as percentage crude fibre, (AOAC, 1990).

3.4.6 DETERMINATION OF CRUDE PROTEIN

The Kjeldahl method was used for the determination of the total nitrogen. Two grams (2 g) of sample was digested with 25 ml of concentrated sulphuric acid (H_2SO_4) in Kjeldahl digestion flask in the presence of a catalyst (Selenium tablet (½ tablets)) and anti bumping agents, in a fume chamber, until the solution was clear. The clear digested solution was transferred into a 100 ml volumetric flask and made to the mark with distilled water after cooling at room temperature. Distillation was carried out using the steam distillation apparatus.

25 ml of 2 % Boric acid was poured into a 250 ml conical flask and 2 drops of mixed indicator added. It was placed under the condenser outlet with the tip of the condenser completely immersed in the solution (Boric acid), 10 ml of the digested sample solution and about 20 ml of 40 % NaOH solution were transferred into the decomposition flask and well closed. Ammonia (NH_3) liberated during the distillation was collected by the Boric acid solution turning it bluish green. The distillation was

continued until about 5 minutes after the solution in the conical flask has changed to bluish green. The distillate was titrated with 0.1N Hydrochloric Acid (HCl) solution and the end point or titre, recorded. The titre values obtained were used to calculate the total Nitrogen. This was then converted into percentage crude protein by multiplying this percentage by an appropriate conversion factor (6.25). A blank was carried out using distilled water (AOAC, 1990).

3.4.7 TOTAL CARBOHYDRATE AND CALORIC VALUE

Total carbohydrate content of the samples was determined by subtracting the values of crude protein, crude fat, crude fibre and crude ash content from one hundred. The caloric value was determined by multiplying the protein, fat, and carbohydrate content by their Atwater factor (FAO, 2006a).

3.4.8 TOTAL PHENOLICS EXTRACTION

Total phenolics were extracted by a modification of the procedure described by Makkar *et al* (1993). Four hundred milligrams of plant samples (dried and finely ground) was weighed into centrifuge tubes. 20 ml of 70% aqueous acetone (with pH adjusted to 3 with acetic acid) was added to the samples and allowed to stand at room temperature for 20 min with intermittent gentle vortexing. The tubes were subjected to centrifugation for 10 min at approximately 3000 rpm at 4 °C. The supernatant (containing polyphenols) was collected and kept on ice.

3.4.9 SPECTROPHOTOMETRIC ASSAY ON FILTERATE

This assay is based on the principle that phenols or phenolic compounds react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium, to produce a blue coloured complex (molybdenum blue), which absorbs in the UV-Visible region. The total phenolic content was determined by a modification of the procedure described by Makkar *et al* (1993). 0.1 milliliters of aliquot of the polyphenol-containing extract was put into test tubes and the volume made up to 2 ml with distilled water. 1 ml of the Folin-Ciocalteau reagent (1N) and 5 ml of 20 % sodium carbonate solution was added. The tubes were vortexed and absorbance read at 725 nm after 40 minutes. The amount of total phenols was determined as tannic acid equivalent from a calibration curve prepared using standard tannic acid solution (0.1mg/ml). Total phenolic content was expressed on a dry matter basis ~~(x%)~~.

3.4.10 BETA-CAROTENE DETERMINATION

0.05 g of the sample was weighed and ground smoothly with celite using mortar and pistil. 50 ml acetone was added while grinding to extract carotene. The extracts were filtered using the hand aspirator and the filtrate added to 20 ml of pet ether in a separating funnel. Water was gently added at the side of the funnel with each addition being allowed to separate with the removal of residual acetone, dried over anhydrous sodium sulphate. 100 ml of the concentration was evaporated to dryness with nitrogen gas and re-dissolved (reconstituted) in varying volumes of the mobile phase depending on anticipated concentration. The absorbance was then determined by spectrophotometer and HPLC (Detector-shimadzu SPD – 6AY; recorder-shimadzu C-R6A; Injector – Model 17125; Pump-shimadzu LC-^A; column-Zorbox ODS columns) (Rodriguez-Amaya and Kimura, 2004).

Formulae for calculations shown in Appendix B.

3.5 PREPARATION OF THE CRACKER

3.5.1 ~~*3.5.1*~~ *CRACKER PREPARATION WITH BUTTER*.

The oven was preheated to 218.3 °C~~425 ºF~~. Four hundred and fifty grams (450 g) of flour, 28.35 g of sugar, 4.77 g of salt, and 2 g of each sample were sifted together into a large bowl. 56.25 g of butter was cut with 2 knives until it looked like cornmeal. 237 ml of milk was stirred in until the dough formed a stiff ball. On a lightly floured board, with a lightly floured rolling pin, the dough was rolled out until it was about 1/8 of an inch thick. With a 2-inch round cookie cutter dipped in flour, round crackers was cut out. These were placed on an ungreased cookie sheet and pricked on the top in several places with a fork. The top of each cracker was brushed with milk. The crackers were baked for 15-20 minutes, or until they were light gold, cooled on a rack and stored airtight at room temperature in a glass jar (Hodgman, 1995).

3.5.2 *CRACKER PREPARATION WITH CREAM*

The oven was preheated to 176.7°C~~350º F~~. ~~two~~ Two hundred and twenty five grams (225 g) flour, 4.77 g salt, 7.16 g sugar, 4.77 g baking powder and 2 g each of the samples were combined in a bowl. 158.79 ml of heavy cream was slowly added

whiles stirring until the dough held together in a ball. The dough was rolled out to a thickness of about 1/8 inch and cut with a 3 inch cookie cutter. It was baked on one side for eight minutes, turned over and baked for a further 6-8 minutes. The crackers were then removed and placed on a rack to cool (Cream Crackers, 2005). Figure 3.2 shows the outline of the preparation. Pictures in Appendix C.

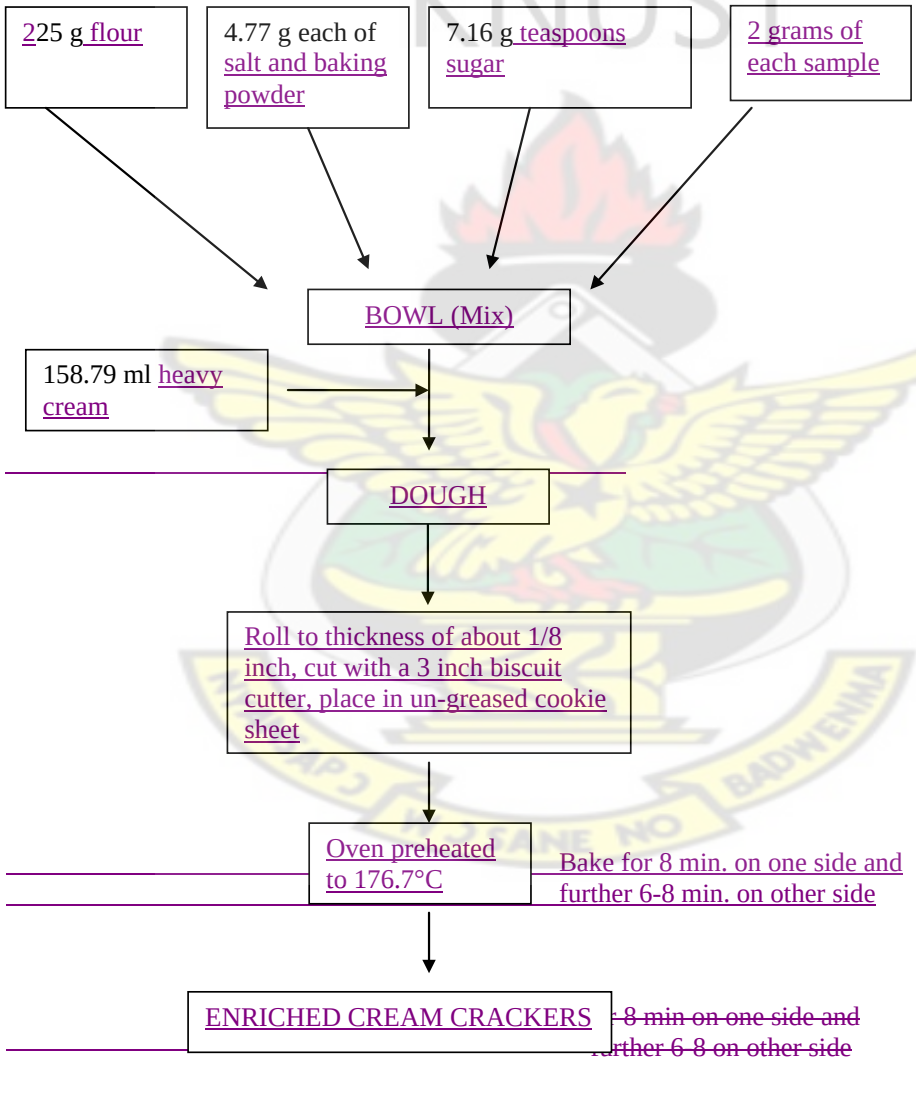


Fig. 3.2 Outline of preparation of cream crackers (Cream Crackers, 2005)

3.6 ~~3.6~~ SENSORY ANALYSIS

3.6.1 SENSORY ANALYSIS WITH UNTRAINED PANELISTS

Sensory analysis was conducted on the cracker samples that were developed using cassava and ~~and~~ sweetpotato flour and ~~flour with~~ wheat flour ~~crackers (~~as the control~~)~~ to find the degree of acceptability. Thirty ~~U~~untrained panelist who constitute the consumers of the end product were used to ~~The trained panelists were to~~ assess the colour, aroma, mouth-feel, texture, taste, and the overall acceptability of the crackers using the two preparation methods. ~~There were untrained panelists who constitute the consumers of the end product.~~ This was to enable the selection of the best preparation method. Each panelist was given the crackers in a random order; they did not specifically follow the serving orders but were randomly given each sample to assess. They evaluated samples using a hedonic scale of 1 – 5 with one representing like extremely and 5 dislike extremely. The sensory evaluation form is shown in Appendix ~~2~~D. The data generated were put together and analyzed using the SPSS 11 analysis software and Microsoft Excel. The means were calculated and significant differences among the crackers were also determined and used in the discussion.

3.6.2 SENSORY ANALYSIS WITH TRAINED PANELISTS

From the preliminary sensory~~this~~ analysis, the best preparation method, which was crackers made with cream, was selected ~~which was crackers made with cream~~. This was then assessed by fifteen trained panelist. The panelists were re-trained for the sensory attributes and salient characteristics of cream crackers. They were then given

the products and control and asked to mark the intensity of the stated qualities on a line scale of 10 centimeters. A ruler was then used to measure the length from the beginning of the line to the point marked by the panelists and the length was noted. The line scale was used because it gives the intensity of each attribute being assessed (Stone and Sidel, 1996). The data generated were put together and analyzed using the SPSS 11 analysis software and Microsoft Excel. The means were calculated and significant differences among the crackers were also determined. The sensory evaluation form in shown in Appendix D.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

~~4.0~~

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4.1 MOISTURE CONTENT

The moisture content of fresh samples showed Otoo as having the highest moisture content of 88.20 % with *Moringa oleifera* having the lowest moisture content of 76.53 % (Table 4.1) meaning that Otoo is more prone to deterioration. Statistical analysis of the data showed significant differences ($p<0.05$) between the samples except Okumkom and Ogyefo between which there was no significant difference ($p>0.05$). The significant differences are attributed to varietal differences. Values of moisture for both sweet potato leaves and *M. oleifera* leaves corroborated with standard references for sweetpotato: 82.21-87.48% (Woolfe, 1992; Antia et al., 2006; FAO, 2006[b]) and for *M. Oleifera*: 75.00% (Fuglie, 2001; Nutritional Value of Malunggay Pods/Leaves, 2006). The moisture content of the fresh samples was close to the literature value of 84.7 % and 84.1 % for leaves and tips respectively (FFTC, 2001).

It has long been recognized that there is a relationship between water content of food and its perishability (Fennema and Tannenbaum, 1996). Concentration and drying processes are conducted primarily for the purpose of decreasing the water content of a food, simultaneously increasing the concentration of solutes, and thereby decreasing perishability.

Table 4.1. Moisture content of Fresh, Blanched and Dried Leaf Samples*

Sample	Fresh Leaves (%)	Blanched Leaves (%)	Dried Leaves (%)
Apomuden	83.17±0.03[a]	89.17±0.08[a]	17.26±0.08[a]
Hi Starch	80.16±0.08[b]	87.73±0.06[b]	21.21±0.10[bc]
Moringa oleifera	76.53±0.02[c]	76.43±0.06[c]	18.48±0.06[ad]
Ogyefo	85.15±0.06[d]	89.51±0.07[d]	17.07±0.04[a]
Okumkom	85.15±0.06[d]	89.55±0.03[d]	19.14±0.08[cd]
Otoo	88.20±0.09[e]	87.65±0.04[b]	15.43±0.03[e]
Santom Pona	83.39±0.09[f]	89.05±0.01[a]	20.71±0.05[bcd]
Sauti	84.33±0.07[g]	88.38±0.08[e]	16.90±0.09[a]

* Mean values ± Standard Deviation Values
[a-h] Means in same column but with different superscripts differ significantly ($p<0.05$)

During the blanching process, a general trend was observed in which all the varieties except Otoo picked up moisture (Table 4.1). Of all the samples, Okumkom picked up the most water having a moisture content of 89.55 % followed by Ogyefo with a moisture content of 89.51 %. The Moringa leaves had the lowest moisture content of 76.43 %. No significant difference upon statistical analysis was observed between Apomuden and Santom Pona; Ogyefo and Okumkom; and Otoo and Hi Starch. There were however significant differences between Sauti and all the other samples as well as *Moringa oleifera* and all the other samples. The reason why the samples picked up water according to Taiz and Zeiger (2002) is because the water potential in the cell

was lower than that in the environment. As water moves into the cell, the hydrostatic pressure, or turgor pressure of the cell increases. Consequently, the cell water potential increases, and the difference between the inside and outside water potentials is reduced eventually reaching equilibrium. Otoo lost water because as explained above, the water pressure in the cell was higher than that in the environment so it lost water to the environment. This deviation from the trend observed is attributed to varietal difference in which the cell membrane of Otoo is able to keep in water better than the other varieties (Taiz and Zeiger, 2002). *Moringa oleifera* neither lost nor gained moisture during the blanching and this could be attributed to the water potential at equilibrium (Taiz and Zeiger, 2002).

Upon air drying under shade until constant weight, all the samples lost moisture (Table 4.1). Otoo had the lowest moisture content of 15.43 % followed by Sauti with a moisture content of 16.90 %. Hi Starch had the highest moisture content of 21.21 %. Statistical analysis of the data showed no significant differences between Apomuden, *Moringa oleifera*, Ogyefo and Sauti. Also there were no significant differences between Hi Starch, Okumkom and Santom Pona; *Moringa oleifera*, Okumkom and Santom Pona; Hi Starch and Santom Pona and this could be attributed to varietal differences. The low moisture content of the samples means that there is a concentration of solutes and decreased ability to perishability (Fennema and Tannenbaum, 1996). This makes the leaves ideal in usage since they are less likely to grow mouldy or help in the proliferation of bacteria. Low moisture content and water activity according to Fennema and Tannenbaum (1996) inhibit micro organisms. Therefore the dried leaves are more stable than the fresh leaves.

4.2 TOTAL ASH CONTENT

~~4.2~~

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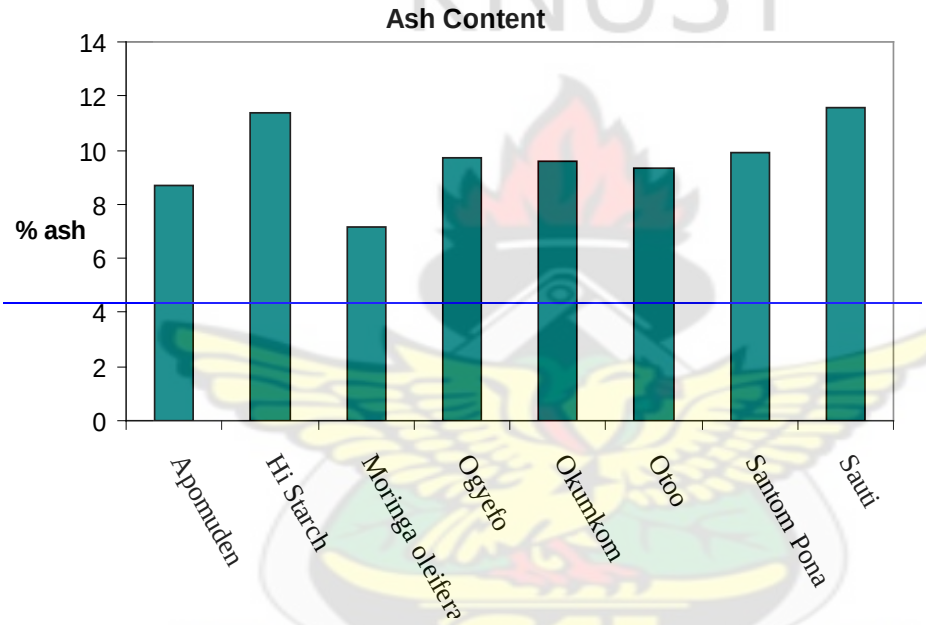
Of all the eight samples, Sauti had the highest ash content (11.60 %) followed by Hi Starch with a value of 11.37 %. It was noticed that all the samples had higher ash content than *Moringa oleifera* (7.13 %) (Table 4.2) which means that the sweetpotato samples have higher total mineral content than the Moringa leaves. Of the sweetpotato leaf samples, Apomuden had the lowest ash content of 8.71 %. The results obtained for the crude ash contents of these indigenous vegetables are in agreement with those in literature (Woolfe, 1992; Antia *et al*, 2006) Statistical analysis of the data showed significant differences ($p<0.05$) among all the samples and this is attributed to difference in variety or specie (Haard, 1996; Woolfe, 1998). Ash is composed of various minerals in different proportions in different plants and is used to express the total mineral content of plant tissue and is a useful index of mineral matter (dirt or sand) (Pomeranz and Meloan, 1987; Haard, 1996). A high ash content means that the mineral content of the food is also high. According to Haard (1996) the most abundant mineral elements in plants are potassium, calcium, magnesium, iron, phosphorus, sulphur, and nitrogen. This means that samples with high ash content (high total mineral content of the plant) are good in treating or preventing malnourishment.

Table 4.2 Proximate and energy composition of the leaf samples*

Sample	**Ash Content (%)**	**Crude Fat (%)**	**Crude Protein (%)**	**Crude Fibre (%)**	**Carbohydrate Content (%)**	**Caloric Value (cal/g)**
Apomuden	8.71±0.03[a]	1.91±0.09[a]	23.39±0.05[a]	11.62±0.09[a]	54.38±0.03[a]	328.23±0.07[a]
Hi Starch	11.37±0.06[b]	0.83±0.02[b]	21.89±0.00[b]	10.10±0.06[b]	55.81±0.01[b]	318.28±0.09[b]
Moringa oleifera	7.13±0.03[c]	2.23±0.03[c]	27.51±0.00[c]	19.25±0.07[c]	43.88±0.01[c]	305.62±0.32[c]
Ogyefo	9.75±0.03[d]	0.38±0.01[d]	22.69±0.00[d]	9.39±0.04[d]	57.79±0.00[d]	325.38±0.07[d]

Okumkom	9.56±0.07^{e}	1.46±0.03^{e}	19.41±0.00^{e}	12.14±0.00^{e}	57.42±0.11^{e}	320.49±0.11^{e}
Otoo	9.31±0.09^{f}	1.61±0.02^{f}	21.85±0.05^{b}	10.66±0.18f	56.57±0.19^{f}	328.21±1.19^{a}
Samtom Pona	9.90±0.09^{g}	1.67±0.02^{f}	25.39±0.00^{f}	9.75±0.04g	53.29±0.03^{g}	329.76±0.27^{f}
Sauti	11.60±0.03^{h}	1.50±0.02^{g}	16.78±0.00^{g}	11.11±0.00^{h}	59.01±0.05^{h}	316.67±0.06^{g}

* Mean values ± Standard Deviation Values
$^{a-h}$ Means in same column but with different superscripts differ significantly ($p<0.05$).



~~4.8~~4.3 MINERAL CONTENT

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4.3.1 IRON CONTENT

Of all the samples, *Moringa oleifera* had the highest iron content (28.29 mg/100 g) (Table 4.3). Among the *Ipomoea batatas* samples, Hi Starch had the highest iron content of 23.02 mg/100 g followed by Okumkom (20.73 mg/100 g) with Otoo having the lowest iron content (9.62 mg/100 g) (Table 4.3). Statistical analysis of the iron content of the samples showed significant differences among the samples and this may be attributed to the different types of varieties. The iron content of all the

leaf samples was higher than that of amaranth, cassava, cowpea, okra, pumpkin and taro leaves (FAO, 2006[b]).

The Ghana Demographic and Health Survey (GDHS, 2004) states that children, women of reproductive age and pregnant women are most vulnerable to micronutrient deficiency and anaemia. As a result they need to take in food with high iron content. Since iron deficiency affects about two billion people and recent estimates by Black (2003) shows that iron deficiency anemia is responsible for a fifth of early neonatal mortality and a tenth of maternal mortality, as well as reducing cognitive development and work performance, these leaves can be used to combat this problem. The high iron content of these leaves makes them a good source of iron for the invalid, anaemic, women, children and convalescent. They can also be used in the fight against iron deficiency.

Table 4.3 Iron and Calcium Composition of Leaf Samples*

Sample	Iron (mg/100g)	Calcium (mg/100g)
Apomuden	16.57±0.00[a]	1402.27±0.03[a]
Hi Starch	23.02±0.00[b]	1334.78±0.05[b]
Moringa oleifera	28.29±0.05[c]	2009.79±0.02[c]
Ogyefo	12.27±0.02[d]	1310.52±0.00[d]
Okumkom	20.73±0.04[e]	1326.19±0.06[e]
Otoo	9.62±0.07[f]	13.52.27±0.03[f]
Santom Pona	13.52±0.00[g]	1316.45±0.02[g]
Sauti	12.39±0.01[h]	1315.70±0.03[h]

* Mean values ± Standard Deviation Values
[a-h] Means in same column but with different superscripts differ significantly ($p<0.05$).

4.3.2 CALCIUM CONTENT

For calcium, Moringa had the highest calcium content of 2009.79 mg/100 g (Table 4.3). This was followed by Apomuden (1402.27 mg/100 g) and Otoo (1352.27 mg/100 g) with Ogyefo having the lowest value of 1310.52 mg/100 g. There were significant differences among all the samples at the 0.05 level of significance. The high level of calcium in *Moringa oleifera* means that it is a better source of calcium than the sweetpotato samples. However, in places where there is no Moringa tree, sweetpotato can equally be used to supplement the diet. According to FAO (2006[b]), the sweetpotato and Moringa leaf samples have higher calcium content than taro, pumpkin, amaranth, cassava and okra leaves.

It has long been known that calcium helps in good skeletal growth (Whitney and Hamilton, 1984) and it is very important especially in the diet of pregnant and lactating women, older women and children in the growing years. Whitney and Hamilton (1984) made it known that a developing calcium deficiency does not show any symptoms like other diseases or deficiencies and becomes apparent only when a hip or pelvic bone suddenly shatters into fragments. Calcium plays other roles in the body apart from skeletal development (cell membrane integrity, regulation of ion transport, muscle action, transmission of nerve impulses, blood clotting, cofactor for several enzymes) and as a result, foods that are high in calcium are needed by the body. However it must be noted that approximately 85 % of kidney stones are composed predominantly of calcium compounds. The most common cause of calcium stone production is excess calcium in the urine (hypercalciuria). Excess calcium is

normally removed from the blood by the kidneys and excreted in the urine. In hypercalciuria, excess calcium builds up in the kidneys and urine, where it combines with other waste products to form stones. Low levels of citrate, high levels of oxalate and uric acid, and inadequate urinary volume may also cause calcium stone formation (Kidney Stones; Overview, 2008).

Common sense has long held that consumption of too much calcium could promote the development of calcium kidney stones. However, current evidence suggests that the consumption of low-calcium diets is also associated with a higher overall risk for the development of kidney stones. This is perhaps related to the role of calcium in binding ingested oxalate in the gastrointestinal tract. As the amount of calcium intake decreases, the amount of oxalate available for absorption into the bloodstream increases; this oxalate is then excreted in greater amounts into the urine by the kidneys. In the urine, oxalate is a very strong promoter of calcium oxalate (CaO_x) precipitation, about 15 times stronger than calcium (Kidney Stone, 2008).

4.4 CRUDE FAT CONTENT

For fat analysis, Ogyefo had the lowest crude fat content of 0.38 % with Apomuden having a higher fat content of 1.91 % for the sweetpotato leaves. Moringa leaves had the highest fat content (2.23 %) among all the samples. Statistical analysis showed significant differences between Apomuden (1.91 %) and all other samples; Hi Starch (0.83 %) and all other samples; *Moringa oleifera* (2.23 %) and all samples and; Ogyefo (0.38 %) and all samples. These differences are attributed to differences in variety. There was however no significant difference between Otoo (1.61 %) and

Santom Pona (1.67%) as well as Sauti (1.50 %) and Okumkom (1.46 %). Table 4.2 shows the crude fat contents.

A diet including *Moringa oleifera* should be more palatable than that with sweetpotato leaves because according to Lindsay (1996[a]) and Pomeranz and Meloan, (1987), dietary fats function in the increase of palatability of food by absorbing and retaining flavours. A diet providing 1 – 2 % of its caloric energy as fat is said to be sufficient to human beings, as excess fat consumption is implicated in certain cardiovascular disorders such as artherosclerosis, cancer and aging (Davidson *et al*, 1975; Kris-Etherton *et al*, 2002).

4.5 CRUDE FIBRE CONTENT

~~4.4~~

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The crude fibre content of the sweetpotato samples was lower than that of Moringa leaves (19.25 %) (Table 4.2). Of the sweetpotato samples, Okumkom had the highest fibre value of 12.14 % followed by Apomuden (11.61 %) with Ogyefo having the lowest value (9.39 %). There were significant differences ($p<0.05$) amongst all the samples which may be attributed to varietal differences. Non-starchy vegetables are the richest sources of dietary fibre (Agostoni *et al*., 1995) and are according to Saldanha (1995) employed in the treatment of diseases such as obesity, diabetes and gastrointestinal disorders. This makes *Moringa oleifera* a more favourable vegetable since high fibre content of foods help in digestion, prevention of constipation, and the prevention of colon cancer (IWES, 1971; Saldanha, 1995; UICC/WHO, 2005).

4.6 TOTAL PROTEIN CONTENT

~~4.5~~

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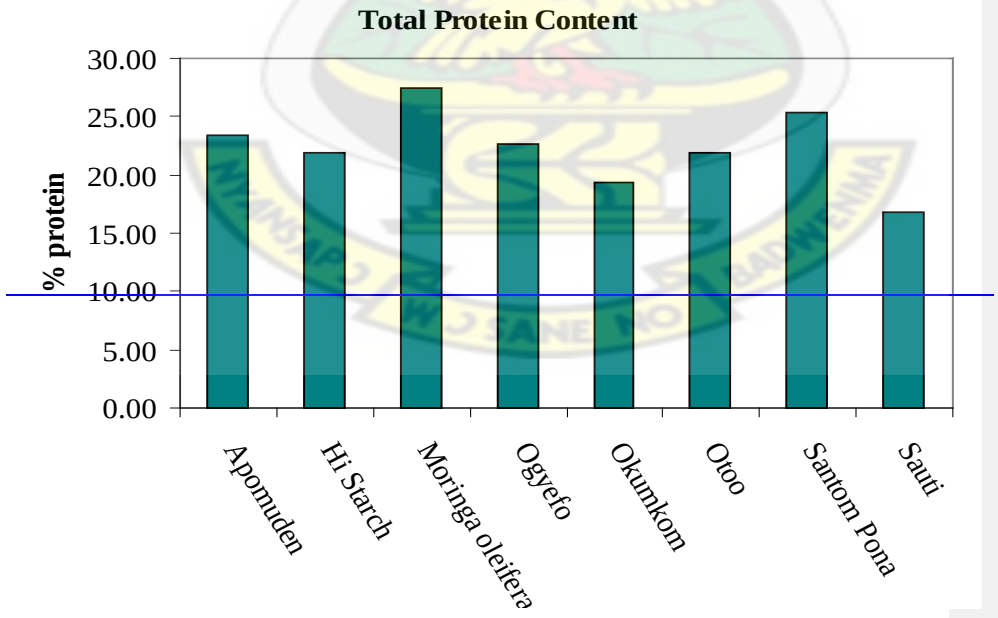
Crude protein levels were between 16.78 % and 27.51 %. With the exception of Sauti (lowest value of 16.78 %) and Okumkom (19.41 %), all the leaf samples had protein levels above 20 % (Table 4.2). These levels are comparable with crude protein levels of lentil, cowpea and pigeon pea (Kay, 1979) which are highly recommended as substitute for animal protein. Comparatively Moringa had the highest protein value of 27.51 %. *Ipomoea batatas* leaves contain crude protein comparable with that of other leafy vegetables (cassava 24.88 %) (Akindahunsi and Salawu, 2005). Statistical analysis of the data obtained showed significant differences amongst all the samples except between Otoo and Hi Starch. According to Woolfe (1992), the major source of variation in leaf protein content is cultivar. It must be noted that the protein content for all the samples followed literature value (Price, 1985; Woolfe, 1992; Abbey *et al*, 2006) for sweetpotato and Moringa leaf proteins and is better when compared to other vegetables such as amaranth, taro leaves, pumpkin leaves and okra leaves (FAO, 2006[b]).



Lewis *et al* (2002) and Nelson and Cox (2000) stated that proteins consist of monomers of amino acids linked together to form polypeptide chains. Some amino acids listed as being present in Moringa leaves are, arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine and valine (Nutritional value, 2006). Apart from their nutritional significance, proteins play a large part in the organoleptic properties of foods (Pomeranz and Meloan, 1987), this means that the leaves with high protein content will generally be more palatable than others with low protein content. From the protein values obtained, it can be deduced that these leaves can be used to supplement diets low in protein.

4.7 TOTAL CARBOHYDRATE CONTENT

~~4.6~~

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Total carbohydrate content for the samples as shown in Table 4.2 indicate low levels ranging from 43.88 % (Moringa) to 59.01 % (Sauti). All the samples had total carbohydrate content below 60 %. Statistical analysis of the data showed significant differences ($p<0.05$) among all the samples. These differences are attributed to differences in cultivar. The low carbohydrate content of *Moringa oleifera* means that it is a more suitable vegetable for those who want to cut down on carbohydrate intake and for the obese who need less carbohydrate in their diet. This is because excess glucose, which is the subunit of carbohydrate (Nelson and Cox, 2000), in the body is converted to fat which in the end leads to obesity (Whitney and Hamilton, 1984). The leaves will also be good for diabetics who need less sugar or glucose in their diet.

4.8 CALORIC CONTENT

~~4.7~~

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The caloric value obtained in Table 4.2 showed *Moringa oleifera* as having the lowest value (305.62 cal/g or 1296.09 kJ/g). Among the sweetpotato specie, Sauti had the lowest value (316.67 cal/g or 1343.96 kJ/g) whiles Santom Pona had the highest value (329.76 cal/g or 1399.41 kJ/g). The caloric value of these leaves make them a good source of energy for people and a good addition to the diet of the obese and those who do not want to gain too much weight. Moringa having the lowest caloric content means that it is a better vegetable when compared to sweetpotato leaves in terms of lower calories for the obese and diabetic. There were significant differences between the samples except between Apomuden and Otoo which showed no significant difference ($p>0.05$).

4.9 TOTAL PHENOLIC CONTENT

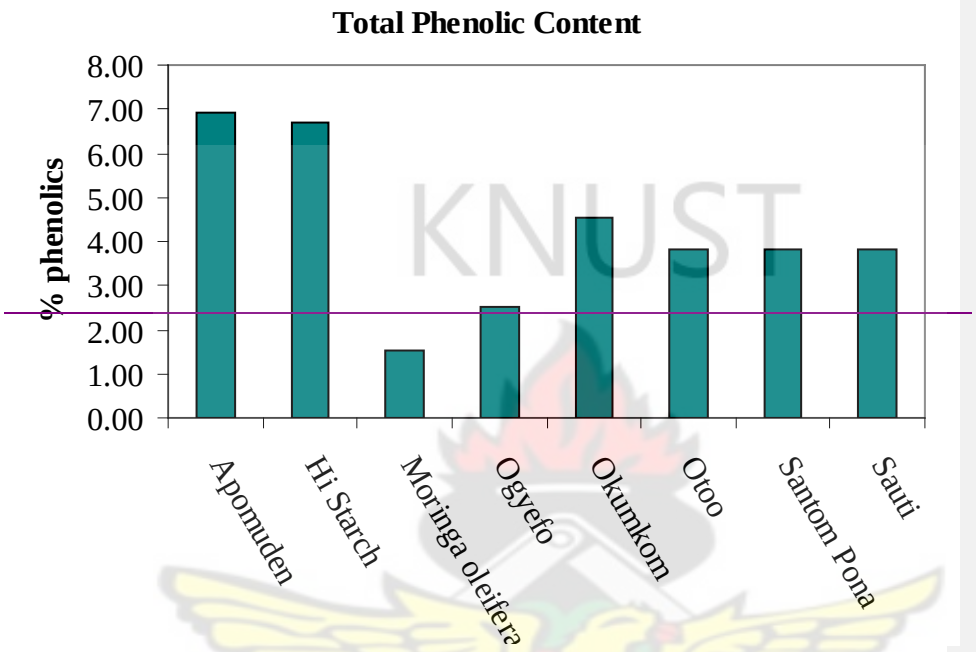
Apomuden had the highest level of total phenolics (6.92 %) followed by Hi Starch (6.68 %) and Okumkom (4.55 %) as shown in Table 4.4. Of all the samples, *Moringa oleifera* had the lowest phenolic level of 1.51 %. Statistical analysis showed no significant difference between Otoo and Santom Pona. There were however significant differences between all the other samples at the 0.05 significance level attributed to varietal differences. Phenolic acids, a subset of polyphenols, protect the body's tissues against oxidative stress. Phenolics include anthocyanins, flavonoids, proanthocyanidins and tannins (Lindsay, 1996[b]). Islam *et al* (2006) stated that sufficient amounts of these phenolics in the human system slow cancer proliferation, induces apoptosis, inhibits harmful enzymes and induces beneficial enzymes.

Table 4.4 Total Phenolics and Beta-carotene content of Samples*

Sample	Phenolic content (%)	Beta-carotene content (mg/100g)
Apomuden	6.92±0.01[a]	9.89±0.00[a]
Hi Starch	6.68±0.00[b]	4.76±0.00[b]
Moringa oleifera	1.51±0.00[c]	23.43±0.00[c]
Ogyefo	2.51±0.00[d]	8.76±0.01[d]
Okumkom	4.55±0.00[e]	9.89±0.00[e]
Otoo	3.83±0.02[f]	10.02±0.00[f]
Santom Pona	3.81±0.00[f]	11.54±0.01[g]
Sauti	3.16±0.00[g]	5.14±0.00[h]

* Mean values ± Standard Deviation Values

[a-h] Means in same column but with different superscripts differ significantly ($p<0.05$)



Phenolics and carotenoids, which are found in leaves and vegetables, have been studied for their abilities to prevent lipid peroxidation, inhibiting the development of atherosclerosis and lowering blood pressure. These plant antioxidants even help during digestion, inhibiting some of the oxidation of fats in gastric fluid (Granato, 2003). Yet another way in which phenolics help to prevent atherosclerosis is by boosting the activity of vitamin C, which in turn increases the levels of vitamin E. This synergy increases the overall resistance to oxidative stress (Very Berry- and Grape too, 2001).

4.10 BETA-CAROTENE CONTENT

The beta-carotene levels of the leaves (Table 4.4) ranged from 4.76 mg/100g to 11.54 mg/100g for the sweetpotato leaves. Santom Pona had the highest β-carotene level of 11.54 mg/100g followed by Otoo with 10.02 mg/100g and Apomuden with 9.89 mg/100g. Hi Starch had the lowest level of 4.76 mg/100g. In all, *Moringa oleifera* had the highest β-carotene content of 23.43 mg/100g. Statistically all the leaf samples were significantly different at the 0.05 % significance level. The variation is attributed to varietal differences.

The level of beta-carotene in the samples exceed the recommended daily allowance for children ages 1-3 (1.5 mg/100g) (Fuglie, 2001). The high level of beta-carotene in the leaf samples make them good for human consumption since it works strongly against singlet oxygen, gives better cornea protection against UV light than lycopene and can boost the activity of Natural Killer (NK) immune cells (Best, 2005). ~~Beta-carotene~~

Null (2004), states that beta-carotene also helps to protect the body against many types of cancer by trapping and deactivating free radical and other oxygen metabolites that damage DNA causing cell mutations that lead to cancer.

Upon analysis for the various nutritional components, Apomuden from the sweetpotato varieties was chosen. This is because it had higher total phenolics, calcium, and fibre. It also had appreciable levels of protein, iron and beta-carotene making it the overall best among the sweetpotato leaf samples. The Apomuden was added to the *Moringa oleifera* leaves in a one to one ratio and added to each cracker preparation.

4.11 -SENSORY EVALUATION OF PRODUCTS BY UNTRAINED PANELISTS.

4.11.1 COLOUR ACCEPTABILITY

Amongst all the samples, the colour of cream crackers made with wheat was the one most liked with a mean score of 2.53 (Graph in Appendix E). Butter crackers made with cassava (3.23) and cream crackers made with sweetpotato (3.00) and cassava flour (3.23) were neither liked nor disliked. Statistical analysis of the data showed significant differences between butter crackers made from cassava flour and butter crackers made from wheat and sweetpotato flour as well as cream crackers made from wheat flour; butter crackers made from wheat flour and cream crackers made from cassava flour; butter crackers made from sweetpotato flour and cream crackers made from cassava flour; cream crackers made from cassava flour and cream crackers made from wheat flour; cream crackers made from wheat flour and that made from sweetpotato flour.

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4.11.2 AROMA

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The aroma of cream crackers made from the three different types of flours was liked moderately with crackers made from cassava flour being the most liked with a mean score of 2.07 (see Appendix E). The aroma of the butter crackers were neither liked nor disliked with butter crackers made from wheat having the highest mean score of 3.40. There were significant differences ($p < 0.05$) between all the butter cracker samples and cream cracker samples but no significant differences between samples made with butter crackers as well as between samples made with cream crackers. This result could be as a result of the type of fat found in the heavy cream used in the cream cracker preparations not found in butter. According to Pomeranz and Meloan, (1987) and Lindsay (1996[a]), dietary fats function in the increase of palatability of food by absorbing and retaining aromas.

4.11.3 MOUTHFEEL

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The mouthfeel of wheat and sweetpotato flour cream crackers were the most liked with mean scores of 2.40 and 2.70 respectfully. All the other samples were neither liked nor disliked. Cassava and sweetpotato flour butter crackers had the highest scores of 3.10 each meaning that they were the least preferred. Statistical analysis at the 0.05 significance level showed significant differences between butter crackers made from cassava flour and wheat and sweetpotato flour cream crackers. There were also significant differences between cream crackers made from wheat flour and all samples except cream crackers made from sweetpotato flour as well as between

cream crackers made from cassava flour and cream crackers made from wheat and sweetpotato flour. Cream crackers made from wheat flour were the most preferred and this could be as a result of gluten in the wheat flour on which the leavening agent, baking powder, worked. This made the crackers more flaky and crisp (Leavening, 1996) and therefore more easy to chew.

4.11.4 TEXTURE

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The mean scores for texture of the samples fell in a range from 2.57 (cream crackers made from wheat flour) to 3.20 (butter crackers made from wheat flour). There were significant differences ($p<0.05$) between butter crackers made from wheat flour and all the samples but no significant difference amongst all the other samples. This result was expected due to the fact that the baking powder acts on gluten found in wheat flour releasing carbon dioxide and therefore making the crackers more crisp and firm. The texture of butter crackers made from wheat flour was the least like in texture and this could also be attributed to the lack of baking powder in the preparation.

4.11.5 TASTE

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The taste of cream crackers made from wheat flour was the most preferred having a mean score of 2.37. This means that the crackers were liked moderately. This was followed by sweetpotato flour cream crackers with a mean score of 2.63. There were significant differences ($p<0.05$) between all the cream cracker samples and all the butter cracker samples except that made from sweetpotato flour. These significant differences could be attributed to the heavy cream used in the preparation of the cream crackers because as stated earlier, dietary fat retain and release flavour (taste and aroma) of foods (Lindsay, 1996[a]).

4.11.6 OVERALL ACCEPTABILITY

~~4.11~~For overall acceptability, all the cream cracker samples were liked moderately with mean scores of 2.03 (wheat flour), 2.07 (sweetpotato flour) and 2.40 (cassava flour). Butter crackers made from cassava, wheat and sweetpotato flours were neither liked nor disliked. Statistical analysis of the data showed significant differences between all the cream cracker samples and the butter cracker samples at the 5% significance level. There were however no significant differences between the butter cracker samples. This means that on the whole cream cracker samples were the most preferred (Appendix E).

4.12 RODUCT DEVELOPMENT AND CHEMICAL COMPOSITION

4.12.1 PRODUCT DEVELOPMENT

From preliminary sensory evaluation~~analysis~~ with untrained panellists, crackers made with cream, which was preferred was analysed for nutritional composition and also used ~~developed~~ for further sensory evaluation~~analysis~~ by ~~the~~ trained panellists. ~~Upon analysis for the various nutritional components, Apomuden from the sweetpotato varieties was chosen. This is because it had higher total phenolics, calcium, and fibre. It also had appreciable levels of protein, iron and beta-carotene making it the overall best among the sweetpotato leaf samples. The Apomuden was added to the *Moringa oleifera* leaves in a one to one ratio and added to each cracker preparation.~~

~~*4* ENRICHED CREAM CRACKERS~~

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~~7 Fig. 4.14 Outline of preparation of Cream crackers~~

~~8 Source: Cream Crackers, 2005~~

~~4.11.2~~4.12.2 *PROXIMATE COMPOSITION OF PRODUCTS*

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Proximate analysis of the product and control (Table 4.5), showed crackers made from wheat flour (control) as having the highest moisture content of 5.08 % and that made from cassava flour as having the lowest (3.14 %). Statistically, there were differences in the moisture content of all the product types. As stated earlier, the higher moisture content of the wheat flour means that it is more prone to deterioration and mould growth (Fennema and Tannenbaum, 1996). The moisture content did not exceed the 6.0 % level recommended by the U. S. Department of Agriculture (1998).

Ash content ranged from 2.75 % (wheat flour crackers) to 3.31 % (sweetpotato flour crackers). The ash content of the products were significantly different ($p<0.05$). The high ash content of the products is attributed to the incorporation of the Moringa and sweetpotato leaves. This is inferred because the ash content of the cassava and sweetpotato flours were lower than the ash content of their respective crackers. Also the ash content of wheat flour crackers was higher than that of commercial crackers on the market. Ash content is reflective of the total mineral content of the products (Pomeranz and Meloan, 1987; Haard, 1996) and this means that crackers made from the sweetpotato flour had higher total mineral content.

Cassava flour crackers had higher fibre content (1.92 %) than all the products. Wheat flour crackers had the lowest (0.55 %). Statistical analysis showed significant differences amongst the crackers. Since fibre content of foods help in digestion, prevention of constipation, and the prevention of colon cancer (IWES, 1971;

Saldanha, 1995; UICC/WHO, 2005), cassava flour crackers is better than the others in terms of fibre content and the prevention of these diseases. It was noticed that the fibre content of the sweetpotato and cassava flour crackers was higher than the fibre content of the flours. This is attributed to the incorporation of the Moringa and sweetpotato leaf samples.

Crude protein analysis showed crackers made from wheat flour as having the highest level (15.69 %) with cassava flour crackers having the lowest (3.29 %) amongst the developed products. This is expected because of the presence of gluten, a form of protein not found in cassava and sweetpotato flours (Sheasby, 2001). It can be said that the leaf samples added to the protein content of all the developed crackers since their protein content were higher than that of the flours and commercial crackers. This result shows that those who need more protein can add crackers made from wheat flour to their diets.

Crude fat content ranged from 10.31 % (cassava) to 16.47 % (wheat). The fat content of all the products except wheat flour crackers (16.47 %) fell in the range (5.00 %-15.00 %) given by the U.S. Department of Agriculture (1998). There were significant differences ($p<0.05$) among the products. Since fat contributes to total calories of a diet, those who do not want to gain too much weight can pick crackers with lower fat content (cassava flour crackers) for consumption. The increase in fat content of the products could be attributed to the heavy cream used in the preparation. However, it must be noted that according to Pomeranz and Meloan, (1987) and Lindsay (1996[a]), dietary fats function in the increase of palatability of food by absorbing and retaining

flavours. Therefore, it can be said that crackers made with wheat flour was more palatable than the others.

Total carbohydrate content of the products was high ranging from 65.08 % to 81.28 %. Significant differences ($p<0.05$) were detected among the products. The high carbohydrate content of the products makes them a good source of energy for all people. However, the obese and those who do not want to gain weight should consider the caloric value of the products when consuming them.

Caloric value showed wheat flour as having the highest calorie per gram. This ranged from 431.02 cal/g for crackers made with cassava flour to 471.33 cal/g for wheat flour crackers (control). There were statistical differences among the products at the 0.05 % significance level. These caloric values means that the control is better for the obese followed by cassava flour crackers.

Table 4.5 Proximate composition of crackers and flour*

Sample	Moisture (%)	Ash (%)	Fibre (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Calorie (%)
Commercial crackers	3.21	1.41	2.80	9.86	13.51	67.6	431.0
Wheat flour crackers	5.08^{a}	2.75^{a}	0.55^{a}	15.69^{a}	16.47^{a}	65.08^{a}	471.33^{a}
Sweetpotato flour	3.06	4.5	1.24	1.03	1.14	90.6	376.78
Sweetpotato flour crackers	4.87^{b}	3.31^{b}	1.79^{b}	4.23^{b}	13.29^{b}	77.38^{b}	446.09^{b}
Cassava flour	3.53	1.68	1.75	1.56	0.70	85.32	353.82
Cassava flour crackers	3.14^{c}	3.20^{c}	1.92^{c}	3.29^{c}	10.31^{c}	81.28^{c}	431.02^{c}

* Mean values ± Standard Deviation Values

$^{a-c}$ Means in same column but with different superscripts differ significantly ($p<0.05$)

4.12SENSORY EVALUATION OF PRODUCTS

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*4.12.1*SENSORY EVALUATION BY UNTRAINED PANELISTS.

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4.12.1.1COLOUR ACCEPTABILITY

Amongst all the samples, the colour of cream crackers made with wheat was the one most liked with a mean score of 2.53 (Graph in Appendix E). Butter crackers made with cassava (3.23) and cream crackers made with sweetpotato (3.00) and cassava flour (3.23) were neither liked nor disliked. Statistical analysis of the data showed significant differences between butter crackers made from cassava flour and butter crackers made from wheat and sweetpotato flour as well as cream crackers made from wheat flour; butter crackers made from wheat flour and cream crackers made from cassava flour; butter crackers made from sweetpotato flour and cream crackers made from cassava flour; cream crackers made from cassava flour and cream crackers made from wheat flour; cream crackers made from wheat flour and that made from sweetpotato flour.

4.12.1.2 AROMA

The aroma of cream crackers made from the three different types of flours was liked moderately with crackers made from cassava flour being the most liked with a mean score of 2.07 (see Appendix E). The aroma of the butter crackers were neither liked nor disliked with butter crackers made from wheat having the highest mean score of 3.40. There were significant differences ($p < 0.05$) between all the butter cracker

samples and cream cracker samples but no significant differences between samples made with butter crackers as well as between samples made with cream crackers. This result could be as a result of the type of fat found in the heavy cream used in the cream cracker preparations not found in butter. According to Pomeranz and Meloan, (1987) and Lindsay (1996[a]), dietary fats function in the increase of palatability of food by absorbing and retaining aromas.

4.12.1.3 MOUTHFEEL

The mouthfeel of wheat and sweetpotato flour cream crackers were the most liked with mean scores of 2.40 and 2.70 respectfully. All the other samples were neither liked nor disliked. Cassava and sweetpotato flour butter crackers had the highest scores of 3.10 each meaning that they were the least preferred. Statistical analysis at the 0.05 significance level showed significant differences between butter crackers made from cassava flour and wheat and sweetpotato flour cream crackers. There were also significant differences between cream crackers made from wheat flour and all samples except cream crackers made from sweetpotato flour as well as between cream crackers made from cassava flour and cream crackers made from wheat and sweetpotato flour. Cream crackers made from wheat flour were the most preferred and this could be as a result of gluten in the wheat flour on which the leavening agent, baking powder, worked. This made the crackers more flaky and crisp (Leavening, 1996) and therefore more easy to chew.

4.12.1.4TEXTURE

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The mean scores for texture of the samples fell in a range from 2.57 (cream crackers made from wheat flour) to 3.20 (butter crackers made from wheat flour). There were

~~significant differences ($p<0.05$) between butter crackers made from wheat flour and all the samples but no significant difference amongst all the other samples. This result was expected due to the fact that the baking powder acts on gluten found in wheat flour releasing carbon dioxide and therefore making the crackers more crisp and firm. The texture of butter crackers made from wheat flour was the least like in texture and this could also be attributed to the lack of baking powder in the preparation.~~

~~*4.12.1.5TASTE*~~

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~~The taste of cream crackers made from wheat flour was the most preferred having a mean score of 2.37. This means that the crackers were liked moderately. This was followed by sweetpotato flour cream crackers with a mean score of 2.63. There were significant differences ($p<0.05$) between all the cream cracker samples and all the butter cracker samples except that made from sweetpotato flour. These significant differences could be attributed to the heavy cream used in the preparation of the cream crackers because as stated earlier, dietary fat retain and release flavour (taste and aroma) of foods (Lindsay, 1996[a]).~~

~~*4.12.1.6OVERALL ACCEPTABILITY*~~

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~~For overall acceptability, all the cream cracker samples were liked moderately with mean scores of 2.03 (wheat flour), 2.07 (sweetpotato flour) and 2.40 (cassava flour). Butter crackers made from cassava, wheat and sweetpotato flours were neither liked nor disliked. Statistical analysis of the data showed significant differences between all the cream cracker samples and the butter cracker samples at the 5% significance level. There were however no significant differences between the butter cracker~~

~~samples. This means that on the whole cream cracker samples were the most preferred (Appendix E).~~

4.13

~~4.12.2~~SENSORY EVALUATION BY TRAINED PANELISTS.

4.13.1 COLOUR ACCEPTABILITY

The colour of the control, wheat flour crackers (mean score: 2.97), was picked as being the closest to the typical colour of crackers. This was reflected in the result given by the untrained panellists because they chose the colour of cream crackers made from wheat as the best. The colour of the cassava flour crackers was further away from the typical colour of crackers (mean score: 2.28). Statistical analysis showed no significant differences ($p > 0.05$) existed among the developed products. These scores were expected because of the addition of the Moringa and sweetpotato leaves, which give a faint greenish colour to the crackers due to the presence of chlorophyll. The results are shown in Fig 4.4.

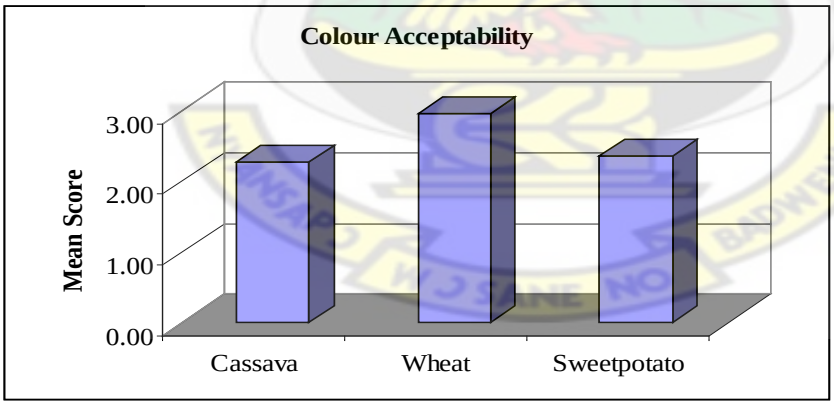

Fig 4.1 Mean Scores for Colour Acceptability of Products

4.13.2 APPEARANCE

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Cream crackers made with cassava flour were less bumpy than all the other samples with a mean of 5.51 followed by that of sweetpotato flour with a mean score of 5.35. Of all the samples, the wheat flour crackers were the most bumpy with a mean score of 3.87 (Fig. 4.5). Statistical analysis of the samples showed no significant differences ($p>0.05$). The appearance of the products conformed with specifications, not being burnt or scorched (U. S. Department of Agriculture, 1998). Cream crackers are supposed to have a bumpy or rough surface due to the release of carbon dioxide from the leavening agent used (Leavening, 1996). The release of carbon dioxide expands the dough thereby increasing the volume of the cracker and also making the cracker flaky. The dough must be suitable for holding the expanded shape, before, during, and after cooking. Crackers made from cassava and sweetpotato flour were closer to being smooth because of the lack of gluten which help hold the volume even after the crackers cool and the leavening gases contract.

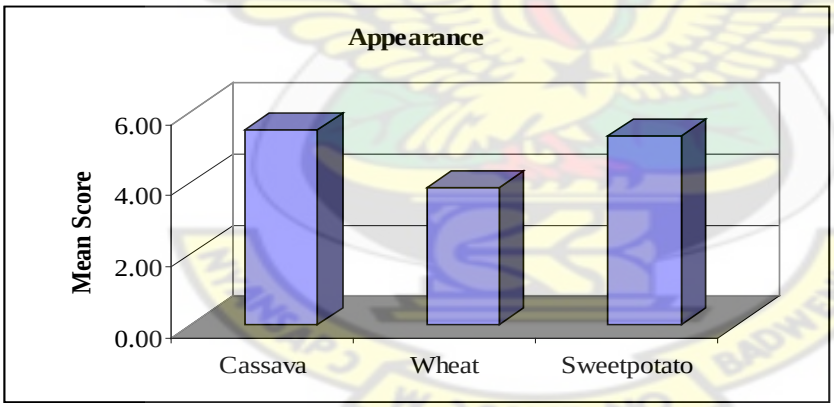

Fig. 4.2 Mean Scores for Appearance acceptability of products.

4.13.3 AROMA

The mean score for aroma of all the samples, shown in Fig. 4.6, passed the halfway mark and this means that the aroma of all the samples was closer to that of cream

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crackers. The aroma of the control was the closest to that of cream crackers with a mean score of 5.79, followed by sweetpotato flour crackers (5.48), and cassava flour crackers (5.33). Significant differences were not observed between the products. These results were expected because of the addition of heavy cream in the preparation. The creamy aroma was enhanced also due to the fat in the cream. As stated earlier, dietary fats function in the increase of palatability of food by absorbing and retaining flavours (Lindsay,1996[a]; Pomeranz and Meloan, 1987). In terms of aroma, any of the products would be liked by consumers.

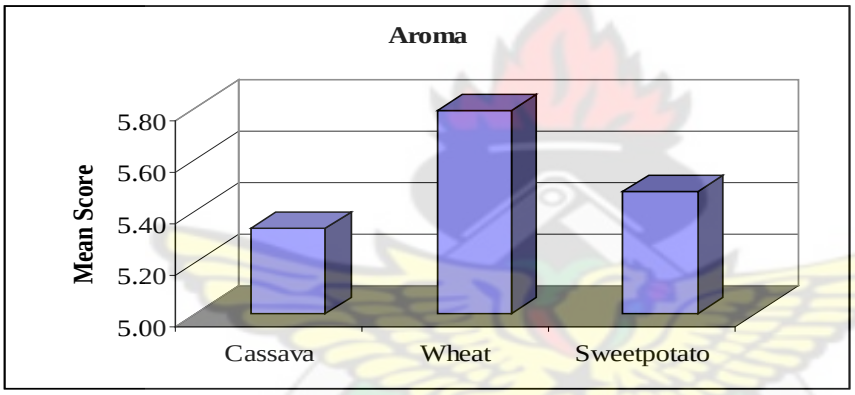

Fig. 4.3 Mean Scores of Aroma

4.13.4 TASTE

The taste of wheat flour crackers which was preferred by the untrained panellists was described by the trained panellists as being the closest to the typical taste of cream crackers (5.89). There was a slight bitter aftertaste tasted by the panellists and this is attributed to the presence of the leaf samples in the crackers. This is because some people claim to have a slight bitter aftertaste in their mouths after consumption of moringa leaves though this has not been proven scientifically. Crackers made from the wheat flour had a less bitter after-taste among the three products. The taste of

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crackers made from cassava flour was the least typical of cream crackers (5.48) and incidentally the one with the highest bitter after-taste (4.18). The means score for bitter after-taste did not exceed the halfway mark for all products. Statistical analysis showed no significant differences ($p>0.05$) between the products for both taste and bitter aftertaste. These results are shown in Fig 4.4 and 4.5.

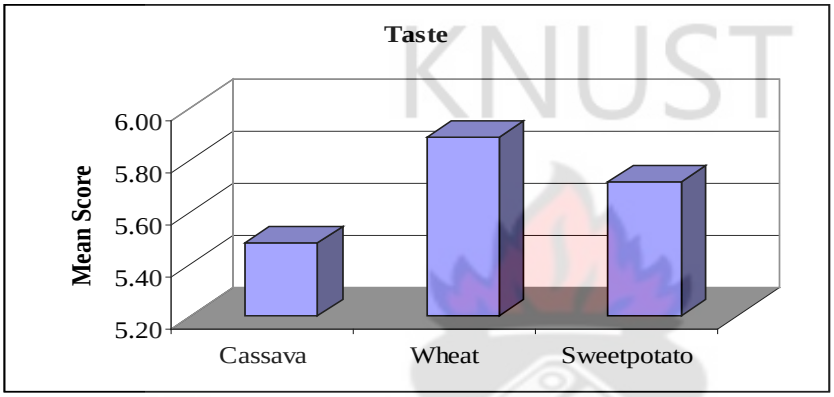

Fig 4.4 Mean Scores for Taste of Samples

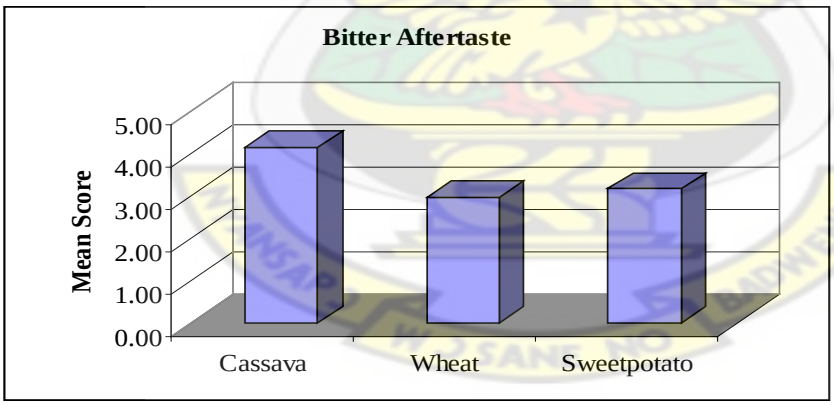

Fig. 4.5 Mean Scores for Bitter Aftertaste of Samples

4.13.5 TEXTURE

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For texture (Fig. 4.6 and 4.7), all samples were crisp and firm. The control wheat flour crackers was crispiest (6.58), followed by cassava flour crackers (6.21) and sweetpotato flour crackers (5.36). When it came to firmness, the products were very firm. Crackers made from cassava flour were more firm (7.55) than all the others (sweetpotato flour crackers: 7.21, wheat flour crackers: 6.42). The lower score of the control meant it was more prone to breakage which was observed when the packs were opened. This was also reflected in the results given by the untrained panellists who preferred the texture of cream crackers made from wheat which was crispier. There were no significant differences between the products statistically. -According to the U. S. Department of Agriculture (1998), the crackers shall possess a firm, crisp crust. From this, it can be seen that the products conform to specifications.

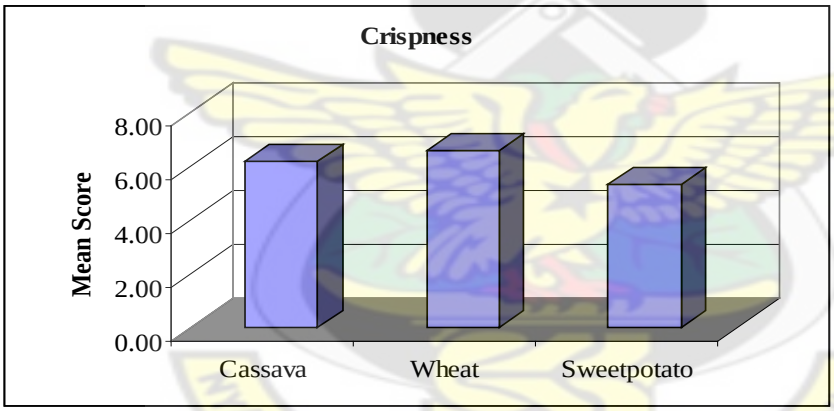

Fig. 4.6 Mean Scores for Crispness of Samples

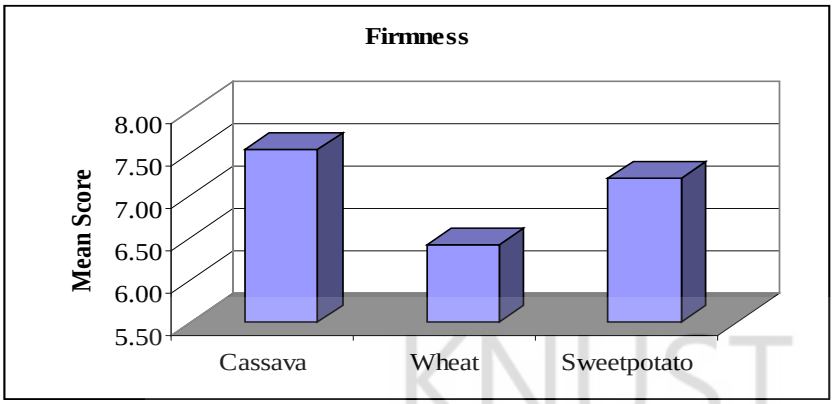

Fig. 4.7 Mean Scores for Firmness of Samples.

4.13.6 MOUTHFEEL

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The chewiness of crackers made from cassava flour (5.24) was higher than all the samples as shown in Fig 4.8. The ~~crackers made from wheat flour was~~crackers made from wheat flour were the least chewy. There was however no significant differences among the products. The chewiness is described as how long one has to chew the crackers before swallowing it. Crackers should not be chewed for long because of their flaky nature due to the presence of a leavening agent. Crackers made from wheat flour were less chewy and this correlated with the texture profile of the products. Due to the fact that crackers made from wheat flour were more crisp and less firm, they should also be less chewy. It must be noted that the result from sensory analysis by untrained panellists reflected in these results. This is because the mouthfeel of cream crackers made from wheat flour were the most liked. This was because the cracker was not chewed for long before swallowing. The same principle applied to crackers made from cassava and sweetpotato flours.

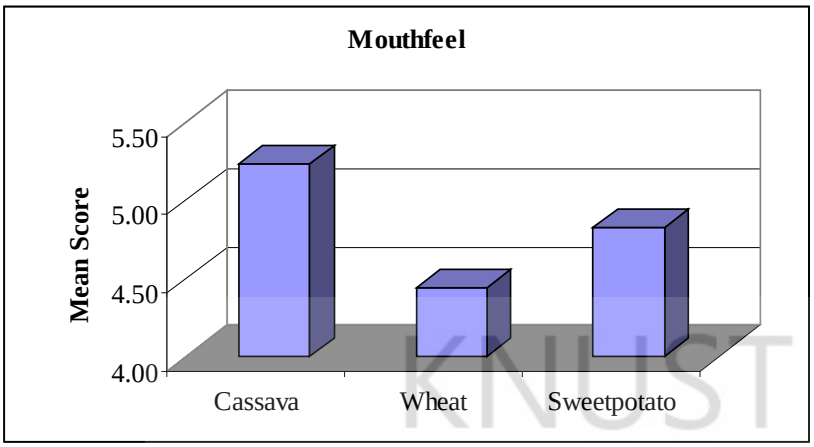

Fig. 4.8 Mean Scores for Mouth feel (chewiness) of Crackers

4.13.7 OVERALL ACCEPTABILITY

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Of all the products, crackers made from cassava flour were the most preferred with a mean score of 5.81, whiles crackers made from sweetpotato flour had the least mean score (5.29). It must be noted that the mean score of all the samples passed the halfway mark (Fig. 4.9), which means that the samples were acceptable. There were no significant differences between the developed products. This means that those with gluten intolerance can opt for crackers made from cassava or sweetpotato flour and enriched with *Ipomoea batatas* and *Moringa oleifera* leaves without fear of celiac disease.

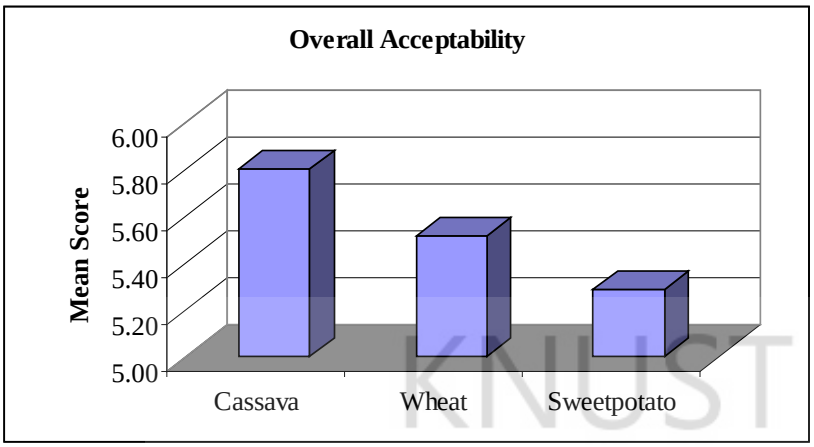

Fig 4.9 Mean Scores for Over-all acceptability of Crackers.

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Moringa oleifera and *Ipomoea batatas* leaves are very nutritious when compared to vegetables such as cassava leaves, amaranth, mushrooms, taro, and pumpkin leaves. Relatively, Moringa leaves contain higher levels of calcium, iron and proteins making it a very rich source of dietary nutrients compared to the *I.batatas* leaves. However both leaves can contribute significantly to the nutrient requirements of humans.

Two species of leaves were used in the development of cream crackers using wheat, cassava and sweetpotato flour. Sensory evaluation of the products showed that crackers made from cassava and sweetpotato flour were comparable to crackers made from wheat flour and as a result can be consumed by those who are gluten intolerant. The crackers can also be consumed by children, teenagers and the elder as a source of additional nutrient and good snacking habit.

5.2 RECOMMENDATION

From the study conducted, the following recommendations are given:

- Public education about the high nutritional value of the leaves by organizing seminars, printing of flyers, and through the media.
- Animal studies to determine the bioavailability of the nutrients found in the leaves.
- Further research into the nutritional potential of other species of both Moringa and *Ipomoea batatas* leaves.
- Research into product development using sweetpotato and cassava flour.

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APPENDIX A

PLATE OF LEAVES

Blanched and unblanched Apomuden

Dried Apomuden

Blanched and unblanched Otoo

Dried Otoo

Blanched and unblanched Santom Pona

Dried Santom Pona

Fresh *Moringa oleifera*

Blanched *Moringa oleifera*

Dried *Moringa oleifera*

APPENDIX B

FORMULAE FOR CALCULATIONS.

1. % Moisture content = $\frac{\text{loss in weight of sample}}{\text{Original weight of sample}} \times 100$

2. % Ash = $\frac{\text{weight of ash}}{\text{dry weight of sample}} \times 100$

3. % Nitrogen = $\frac{100 \times (VA - VB) \times NA \times 0.01401}{W \times 100}$

   % Crude Protein = % total nitrogen × 6.25

4. % Crude Fat = $\frac{\text{Weight of fat}}{\text{dry weight of sample}} \times 100$

5. % Crude Fibre = $\frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$

6. β-carotene content µg/µl = $\frac{\text{Absorbance} \times 10{,}000}{2592 \times \text{sample weight}}$

7. Iron (mg/100g) = $\frac{50 \times \text{Concentration}}{\text{Sample weight} \times 10}$

8. Calcium (mg/100g) = $\frac{50 \times \text{Concentration} \times 100}{\text{Sample weight} \times 10}$

9. % Phenolics = $\frac{\text{Tannic acid} \times 20 \times 100}{0.1 \times \text{sample weight}}$

APPENDIX C

PLATE OF PRODUCTS

Cassava flour crackers

Wheat flour crackers

Sweetpotato flour crackers

Package of Crackers

Cream crackers

APPENDIX D

Kwame Nkrumah University of Science and Technology
Department of Biochemistry and Biotechnology
Sensory Evaluation Form

Name: _____ Sample Code: _____

Please tick the box which indicates your preference for the attribute of the sample.
Please rinse your mouth with the water provided after tasting each sample.

Colour: 1 Like extremely [ ]
2 Like moderately [ ]
3 Neither like nor dislike [ ]
4 Dislike moderately [ ]
5 Dislike extremely [ ]

Aroma:1 Like extremely [ ]
2 Like moderately [ ]
3 Neither like nor dislike [ ]
4 Dislike moderately [ ]
5 Dislike extremely [ ]

Mouth-feel: 1 Like extremely [ ]
2 Like moderately [ ]
3 Neither like nor dislike [ ]
4 Dislike moderately [ ]
5 Dislike extremely [ ]

Texture:1 Like extremely [ ]
2 Like moderately [ ]
3 Neither like nor dislike [ ]
4 Dislike moderately [ ]
5 Dislike extremely [ ]

Taste: 1 Like extremely [ ]
2 Like moderately [ ]
3 Neither like nor dislike [ ]
4 Dislike moderately [ ]
5 Dislike extremely [ ]

Over-all acceptability: 1 Like extremely [ ]
2 Like moderately [ ]
3 Neither like nor dislike [ ]
4 Dislike moderately [ ]
5 Dislike extremely [ ]

Comments:

Kwame Nkrumah University of Science and Technology
Department of Biochemistry and Biotechnology
Sensory Evaluation Form

Name: _____

Sample Code: _____

Please mark the intensity of the attributes indicated below in reference to the sample.

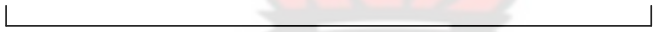
Colour:

1 10
Not typical of crackers Typical of crackers

Appearance:

1 10
Bumpy Smooth

Aroma:



1 10
Not typical of crackers Typical of crackers

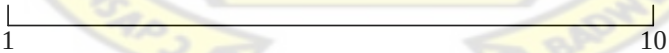
Taste:

1 10
Not typical of crackers Typical of crackers

1 10
No bitter after-taste Bitter after-taste

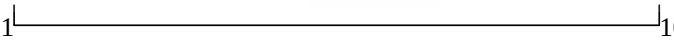
Texture:

1 10
Not crisp Crisp



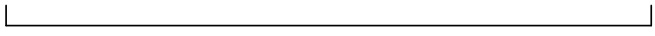
1 10
Not firm Firm

Mouth-feel



1 10
Not chewy Very chewy

Over-all acceptability

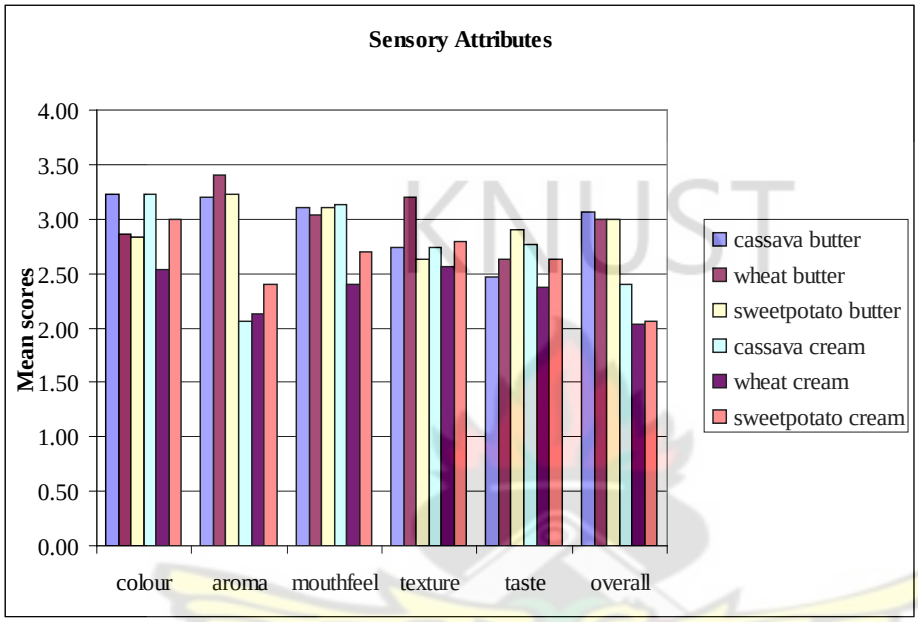


1 10
Dislike extremely Like extremely

Comments:

APPENDIX E

SENSORY ATTRIBUTE SCORES FOR UNTRAINED PANELLISTS



Sensory attributes scores for untrained panellist.

APPENDIX F

ANOVA Tables

ANOVA

FRESMOIS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	175.125	7	25.018	5277.493	.000
Within Groups	3.792E-02	8	4.740E-03		
Total	175.163	15			

ANOVA

MOISBLAN

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	272.178	7	38.883	11310.004	.000
Within Groups	.028	8	.003		
Total	272.206	15			

ANOVA

DRYMIOS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.385	7	10.912	20.280	.000
Within Groups	4.305	8	.538		
Total	80.689	15			

ANOVA

ASH

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.297	7	4.042	1165.986	.000
Within Groups	2.774E-02	8	3.467E-03		
Total	28.325	15			

ANOVA

FAT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.842	7	.692	483.913	.000
Within Groups	1.143E-02	8	1.429E-03		
Total	4.853	15			

ANOVA

FIBRE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	140.565	7	20.081	2669.948	.000
Within Groups	6.017E-02	8	7.521E-03		
Total	140.625	15			

ANOVA

PROTEIN

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	154.355	7	22.051	36001.230	.000
Within Groups	4.900E-03	8	6.125E-04		
Total	154.360	15			

ANOVA

CARBO

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	318.936	7	45.562	6664.353	.000
Within Groups	.055	8	.007		
Total	318.991	15			

ANOVA

CALORIE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	921.145	7	131.592	501.171	.000
Within Groups	2.101	8	.263		
Total	923.245	15			

ANOVA

IRON

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	575.699	7	82.243	65911.568	.000
Within Groups	9.982E-03	8	1.248E-03		
Total	575.709	15			

ANOVA

CALCIUM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	804791.8	7	114970.251	8.4E+07	.000
Within Groups	1.089E-02	8	1.361E-03		
Total	804791.8	15			

ANOVA

PHENOLIC

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	32.900	7	4.700	80687.005	.000
Within Groups	4.660E-04	8	5.825E-05		
Total	32.901	15			

ANOVA

BETA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	469.373	7	67.053	5364268	.000
Within Groups	.000	8	.000		
Total	469.374	15			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MOISPRDT	Between Groups	4.509	2	2.254	735.094	.000
	Within Groups	.009	3	.003		
	Total	4.518	5			
ASHPRDT	Between Groups	1.479	2	.739	1249.297	.000
	Within Groups	.002	3	.001		
	Total	1.480	5			
FIBREPRD	Between Groups	2.283	2	1.142	6567.172	.000
	Within Groups	.001	3	.000		
	Total	2.284	5			
CARBPRDT	Between Groups	31.127	2	15.564	9248.914	.000
	Within Groups	.005	3	.002		
	Total	31.132	5			
CALPRDT	Between Groups	365.048	2	182.524	1354.545	.000
	Within Groups	.404	3	.135		
	Total	365.452	5			
PROTPRDT	Between Groups	190.549	2	95.274	2684071	.000
	Within Groups	.000	3	.000		
	Total	190.549	5			
FATPRDT	Between Groups	38.019	2	19.009	11886.768	.000
	Within Groups	.005	3	.002		
	Total	38.023	5			

ANOVA TABLE FOR UNTRAINED PANELIST SENSORY ATTRIBUTES

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
COLOUR	Between Groups	10.717	5	2.143	4.345	.001
	Within Groups	85.833	174	.493		
	Total	96.550	179			
AROMA	Between Groups	54.828	5	10.966	19.489	.000
	Within Groups	97.900	174	.563		
	Total	152.728	179			
MOUTHFEE	Between Groups	13.244	5	2.649	5.401	.000
	Within Groups	85.333	174	.490		
	Total	98.578	179			
TEXTURE	Between Groups	7.444	5	1.489	3.024	.012
	Within Groups	85.667	174	.492		
	Total	93.111	179			
TASTE	Between Groups	5.628	5	1.126	3.040	.012
	Within Groups	64.433	174	.370		
	Total	70.061	179			
OVERALL	Between Groups	35.494	5	7.099	18.744	.000
	Within Groups	65.900	174	.379		
	Total	101.394	179			

ANOVA TABLE FOR TRAINED PANELISTS SENSORY ATTRIBUTES

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
COLOUR	Between Groups	4.161	2	2.081	.334	.718
	Within Groups	261.847	42	6.234		
	Total	266.008	44			
APPEARAN	Between Groups	24.320	2	12.160	1.715	.192
	Within Groups	297.716	42	7.088		
	Total	322.036	44			
AROMA	Between Groups	1.656	2	.828	.330	.721
	Within Groups	105.527	42	2.513		
	Total	107.183	44			
TASTE	Between Groups	1.254	2	.627	.480	.622
	Within Groups	54.865	42	1.306		
	Total	56.119	44			
BITTERAF	Between Groups	12.400	2	6.200	.717	.494
	Within Groups	362.972	42	8.642		
	Total	375.372	44			
CRISP	Between Groups	11.723	2	5.862	.758	.475
	Within Groups	324.669	42	7.730		
	Total	336.392	44			
FIRMNESS	Between Groups	9.922	2	4.961	1.902	.162
	Within Groups	109.524	42	2.608		
	Total	119.446	44			
CHEWYNES	Between Groups	4.721	2	2.361	.375	.690
	Within Groups	264.727	42	6.303		
	Total	269.448	44			
OVERALL	Between Groups	6.963	2	3.482	3.322	.046
	Within Groups	44.021	42	1.048		
	Total	50.984	44			

APPENDIX G

HPLC Readings for Beta-carotene