DEVELOPMENT OF A SUPPLEMENTARY FOOD FROM SELECTED LOCAL FOOD INGREDIENTS TO OPTIMIZE NUTRITIONAL AND SENSORY QUALITIES

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A THESIS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

For Steve, Britney and Patience, the most affectionate and long-suffering persons I've ever met.

- And to all my supervisors, Kudos!

ACKNOWLEDGEMENT

This thesis took an embarrassingly long time to write; also my short-term memory is not what it was – apparently this is what happens when you are perimenopausal (not menopausal, I should stress; that's still decades away, and by the time it happens I will be splendid and back to writing more projects) – so there is a very good chance that someone may have given me invaluable help at an early stage during the research study and that I've completely forgotten. If you are that person, I am truly sorry.

Thank you to my extraordinary and visionary supervisors, Prof. J.K Imungi, Prof. M.W Okoth and Prof. H.K. Biesalski for their invaluable guidance, unstinting support and staunch supervision of my research study. We have all worked well together and it's been a blast. Special thanks and heartfelt gratitude to Prof. H.K Biesalski of the Institute for Biological Chemistry and Nutrition for his kind support in the execution and supervision of this study during the 6 months period in Germany. I wish to also thank Dr. Vadivel Vellingiri for his friendship, enthusiasm and phenomenal hard work on behalf of my manuscripts. Blessed am I, among students and authors. Several other people have acted as guinea pigs, reading the thesis and manuscripts as I wrote them, suggesting changes and improvements. Yes, many improvements. Although I may have cried at the time, I would like to stress that I am in fact very grateful for this service.

Special thanks to my husband Dr. Stephen Chege, who gave me his love, support and friendship throughout the study; and badly demanded answers to the questions I was too morto to ask. And extra-special thanks to my beautiful daughters Britney and Patience, who have given me a new direction in life through hard work and commitment. Thanks for the many funny chats and incidences during the times that I was really bogged down. In a belated attempt to give my family credit for all their love and support, this thesis is dedicated to them.

I had to do tons of research and laboratory work, which is absolutely demanding, but I had a lot of help from technical staff in the Department of Food Science, Nutrition and Technology. Thank you, Jeremiah, Rosemary and Jacinta who were incredibly generous with their time and patience.

I would like to thank the Vice Chancellor, Deputy Vice Chancellors and other relevant authorities in the University of Nairobi for giving me a study leave to carry out research in Germany for six months. I wish to sincerely express my gratitude to all the staff and students of the different institutes in the University of Hohenheim who in one way or another assisted me in the various analyses of my samples during the research period in Germany. God bless you all. Last but not the least, I wish to express my appreciation of German Academic Exchange Services (DAAD) for the scholarship award and support in my short research visit to Germany as well as the in-country DAAD scholarship which was very instrumental in the completion of this study.

ACRONYMS AND ABBREVIATIONS

AIDS – Acquired Immunodeficiency Syndrome

ART – Anti-retroviral treatment

ASAL – Arid and Semi-Arid Lands

FAO – Food and Agriculture Organization

FBF- Fortified Blended Food

FGD– Focus GROUP Discussion

GOK – Government of Kenya

HIV – Human Immunodeficiency Virus

IDP's – Internally Displaced Persons

MOA – Ministry of Agriculture

MOH – Ministry of Health

MUAC – Mid-upper arm circumference

NGO's – Non-Governmental Organizations

PEM – Protein Energy Malnutrition

PLWHA's – People Living with HIV/AIDS

RDA – Recommended Dietary Allowances

RUTF – Ready-to-use therapeutic food

UN – United Nations

UNICEF- United Nations International Children's Emergency Fund

WFP – World Food Programme

WHO – World Health Organization

TABLE OF CONTENTS

ABSTRACT

Recently there has been widespread consumption and sale of a diverse range of supplementary foods in Kenya. This situation has been prompted by increase in malnutrition, HIV/AIDS and related diseases, and consumer nutrition and health awareness. This study was therefore designed to assess the diversity and characteristics of the supplementary foods utilized by the vulnerable groups with a view to document the existing foods in Kenya. The study also sought to explore ways of harnessing local food ingredients to develop a low-cost supplementary food appropriate for vulnerable groups.

Local food ingredients such as finger millet, amaranth grain, pigeon peas, field beans, groundnuts, sweet potatoes, small dried fish (*Rastrineobola argentea*), amaranth and pumpkin were analyzed for macro- and micro-nutrients; amino acid and fatty acid profiles; bioactive compounds including phenolics, flavonoids, tannins and phytates, and their associated antioxidant and antidiabetic properties. The results of the analyses were used to identify appropriate food ingredients to be incorporated in four preliminary formulations from which the final supplement was selected as the most preffered by taste panel procedures.

The most acceptable supplement was then analyzed for nutrient contents including macro- and micro-nutrients, amino acid and fatty acid profiles, shelf life and cost of production, the latter being done to estimate the selling price. Results show that the investigated food ingredients possessed high nutritional values. The food ingredients contained 6-44% protein; 8-38% fibre; 11-43% fat; 324-497 kcal energy; and 15-57% carbohydrates. The mineral contents of the food ingredients was in the range of 25-328 mg/100 g calcium, 1.0-51 mg/100 g iron, 44-1320 mg/100g magnesium, 0.2-19 mg/100 g sodium, 60-1105 mg/100 g phosphorus, and 1.6-15 mg/100 g zinc. The indigenous vegetables exhibited 3.2-63 mg/100 g vitamin C and 0.7-5.1 mg/100 g β-carotene contents while the

grains showed 22-110 µg/100 g folic acid, 1.2-17.7 mg/100 g niacin, 0.1-1.6 mg/100 g vitamin B1 and 0.1-1.0 mg/100 g vitamin B2 contents. The total essential amino acid content ranged from 2.7 to 10.4 % in the grains and 0.9 to 12.8% for the vegetables. The levels of the fatty acids in the food ingredients were 4.8-33.6% palmitic, 1.5-9.0% stearic, 2.2-53.9% oleic, 4.5-53.7% linoleic and 0.9-60.4% α-linolenic acids. Fish powder, sunflower seeds, pumpkin seeds, amaranth grain, pumpkin and amaranth leaves exhibited the highest content of essential amino acids.

The total phenolic content of the cereals, legumes, and vegetables ranged from 0.41 to 3.00 g/100 g DM with amaranth grains (*Amaranthus cruentus*) and drumstick leaves (*Moringa oleifera*) significantly exhibiting the highest contents. The phenolic extracts showed promising levels of antioxidant activity expressed as 2,2′-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity of 81-89%, and ferric reducing antioxidant power (FRAP) of 2.33-22.30 mg/mmol Fe[II]. The antidiabetic property was demonstrated by inhibition activities of α-amylase of 10-45% and α-glucosidase of 13-80%. The vegetables exhibited higher content of flavonoids (50-703 mg/100 g) when compared to cereals, legumes and oil seeds (47-343 mg/100 g). The flavonoid extracts revealed 33-93 % of DPPH radical scavenging capacity, 0.12-2.77 µg/mM Fe[II] of Ferric reducing power, and 19-43% of α-glucosidase inhibition activity as well as 14-68% of α-amylase inhibition activity.

The tannin content of the cereals, legumes, and vegetables ranged from 1.53 to 4.35 g/100 g DM. The tannin extracts showed levels of DPPH radical scavenging activity of 77-90%, FRAP of 47.2-3.6 mg/mmol Fe[II]), α-amylase inhibition activity of 24-40% and α-glucosidase inhibition activity of 60-88%. The phytate contents of the grains ranged from 0.29 to 3.23 g/100 g DM. The phytate extracts showed levels of DPPH radical scavenging activity of 61-89%, FRAP of 37.3-3.6 mg/mmol Fe[II]), α-amylase inhibition activity of 20-72% and α-glucosidase inhibition activity of 12-91%.

The most acceptable supplement was found to be that formulated from amaranth grain, pigeon pea, sweet potato and groundnut in the ratio of 4:1:1:4. This supplement showed high nutritional levels. The consumption of only 110 g of the product could supply over 50% of the daily nutritional requirements by the vulnerable groups, as recommended by FAO/WHO. The supplement (110g DM) was found to contain 453.2 Kcal of energy, 12.7% protein, 54.3% carbohydrate, 20.8% fat and 10.2% fibre. The supplement had 93.0 mg/100 g calcium, 172.4 mg/100 g magnesium, 2.7 mg/100 g zinc and 5.7 mg/100 g iron, as well as 0.8 mg/100 g vitamin B_1, 0.2 mg/100 g vitamin B_2, 7.9 mg/100 g niacin, 100 µg/100 g folic acid, and 110 µg/100 g β-carotene. In addition, the supplement had excellent essential amino acid and fatty acid profiles. The shelf life study showed that the supplement could be stored in polythene bags, gunny bags and Kraft paper at ambient temperatures (20-27 ^{0}C), 30 ^{0}C and 35 ^{0}C for up to 4 months without significant changes in odor, moisture, peroxide value, fat acidity and reduced ascorbic acid contents. A cost analysis of the supplement was carried out to estimate the selling price of the product if commercially produced. The product could competitively be sold at KES 65.50/kg ($ 0.82/kg).

The study concluded that it is possible to formulate a low cost, acceptable and shelf stable supplementary food, affordable by the lower socio-economic vulnerable groups, using food ingredients available locally in Kenya.

CHAPTER 1

GENERAL INTRODUCTION

Daniel then said to the guard whom the chief official had appointed over Daniel, Hananiah, Mishael and Azariah, "Please test your servants for ten days: Give us nothing but vegetables or pulses to eat and water to drink. Then compare our appearance with that of the young men who eat the royal food, King's meat, and treat your servants in accordance with what you see." [15] .....At the end of the ten days they looked healthier and better nourished than any of the young men who ate the royal food.
Daniel 1:11-13 (NIV)

Cereals, legumes and vegetables have helped to fulfill the ageless need to sustain body and soul. These foods play an important role in the traditional diets of many developing countries. They are low in fat; are excellent sources of protein, carbohydrate, dietary fibre and a variety of micronutrients (Khandelwal *et al.*, 2010; Tontisirin *et al.*, 2002). Presently, increased consumption of these foods has been widely promoted because in addition supplying macro and micronutrients, they also provide many bioactive phytochemicals which are strongly associated with health maintenance and prevention of chronic diseases (De Bruyne *et al.*, 1999; Dolara *et al.*, 2005; Gin *et al.*, 1999). Currently, there is a resurgence of consumption of indigenous foods and especially vegetables in Kenya in order to curb micronutrient deficiencies by improving the nutritional quality of the predominantly starch-based staple diets. These foods constitute affordable sources of vitamins and minerals, especially vitamin A, vitamin C and iron (FAO/WHO, 2004). As a result, consumption of diverse plant food ingredients can help in addressing the double burden of micronutrient deficiencies and chronic diseases.

In many low-income countries including Kenya, large proportions of the population are nutritionally vulnerable (Tontisirin *et al.*, 2003). Generally, infants and young children, pregnant and lactating women, and the elderly are regarded as nutritionally vulnerable. In the last decade, the incidences of acute or chronic diseases have risen, which has increased the number of vulnerable groups to include also patients with Tuberculosis, People Living with HIV/AIDS (PLWHA's). The Food and Agriculture Organization (FAO) estimated that 1.02

billion people worldwide were undernourished in 2009. This represents more 'hungry people' than at any other time since 1970, and a prediction of a more worsening economic crisis resulting from reduced access to food energy by the poor people and the impact of lost dietary diversity mainly by the poor (FAO, 2009). Therefore, indigenous foods such as cereals (sorghum, millet and amaranth grain), legumes and pulses (peas and field beans), oil seeds (groundnuts, sunflower seeds and pumpkin seeds) and vegetables are playing an increasing role in alleviating nutritional insecurity of the low-income and vulnerable groups (Neumann *et al.*, 2003). In Kenya, several indigenous foods were neglected with the advent of colonization and the introduction of exotic crops but recently remarkable efforts are being made to reintroduce these nutritious foods to the diets of most communities. Subsequently, the consumption of these food ingredients would help in alleviation of hunger and malnutrition in the country.

In Kenya, the infant and the under-five mortality rates are 77 and 115 per 1000 live births respectively. Nearly, 30% of the under-five children suffer from chronic malnutrition (stunted), almost 6% are severely malnourished (wasted), while 20% are underweight (CBS/MOH/KDHS, 2010). In the Arid and Semi Arid Areas (ASAL) where food insecurity and natural disaster have affected the population, rates of acute malnutrition are between 15-20% of children under five, and sometimes substantially higher (Myatt *et al.*, 2005). This situation has been exacerbated by the rising HIV/AIDS scourge that has led to increased number of orphaned children who are at high risk of malnutrition. Nutritional deficiencies contribute to high rates of chronic diseases and death among the vulnerable groups in Kenya. The first Millennium Development Goal is to "eradicate extreme poverty and hunger by 2015". The nutrition indicator for this in Kenya is "to halve the prevalence of underweight in children less than five years of age from 35.5 per cent in 1990 to 16.25 per cent in 2015" (WHO/MPHS/UNICEF, 2010). Diet-based strategies through consumption of a broad variety

of foods are the most promising approach for a sustainable control of malnutrition and disease among the vulnerable groups (Marchione, 2002). Vulnerable groups are population groups whose immune system is compromised or depressed nutritionally, medically, or socially and require provision of extra, nutritionally high quality foods in addition to the general ration to rehabilitate or prevent deterioration in their conditions. Beside various problems being faced by the vulnerkable groups, cancer and diabetes have been increasing dramatically in the last two decades and the prevention/treatment of diabetes has received a paramount importance among the health professionals and nutritionists.

Apart from the macro- and micro- nutrients, plant foods contain many bioactive compounds which have been associated with functional properties consistent with reduced risk of several chronic diseases and other maladies (Müller & Krawinkel, 2005). Although micronutrient deficiencies mainly causing vitamin A, iron and iodine disorders remain widespread, chronic diseases, including cardiovascular disease, cancer, chronic respiratory diseases, and diabetes, are increasing globally and caused 60% of all deaths in 2005 (WHO, 2007). About 80% of the deaths from chronic diseases occur in low- and middle-income countries, and consequently a new focus in this area is imperative. Consumption of a combination of cereals, legumes, oilseeds, fruits and vegetables has been promoted due to the balance provision of micronutrients as well as a variety of bioactive phytochemicals associated with health maintenance and prevention of chronic diseases to help address this problem (Anwar *et al.*, 2007). Nutritional components in plant foods consist of at least 17 essential minerals, 13 essential vitamins, and an abundance of bioactive compounds as well as their metabolites; altogether, these are estimated to include more than 200,000 compounds (Wildman, 2001). Several studies have established positive physiological effects of some phytochemicals; they can serve as protective agents for health maintenance and promotion or possess medicinal properties (Prior & Cao, 2000; Art & Hollman, 2005). Consequently, while

accessibility of macro and micronutrient-rich foods is fundamental, comprehension about the side benefits accruing from consumption of these foods, due to the presence of phytochemicals and the fate of the nutrients and the phytochemicals during food preparation is of equal concern (Millward, 2004). Although extensive information is available on proximate composition of the indigenous food ingredients used in formulation of supplementary foods for vulnerable groups, their complete nutritional profiles, levels of bioactive compounds and effect of processing on these compounds has surprisingly not been investigated to the same extent in Kenya.

The main objective of the present study was to assess the nutritional profile and bioactive compounds as well as the health relevant functionalities of indigenous foods commonly consumed in Kenya, with the view to selecting the elite ingredients to utilize in the formulation of supplementary foods for vulnerable groups. The properties were evaluated in raw as well as traditionally processed food ingredients. The nutritional analyses carried out for minerals (iron, zinc, potassium, calcium) and vitamins (carotenes, niacin, thiamine, riboflavin and ascorbic acid) and amino acid and fatty acid profiles. The non-nutritional compounds analyzed included the phenolics, flavonoids, tannins and phytates, and their associated antioxidants and antidiabetic properties. Dietary diversification is the most important factor in ensuring intake of adequate micronutrients and phytochemicals. A further objective was to select the local food ingredients with high nutrient contents to formulate a low cost supplementary food for vulnerable groups. The currently used supplementary foods are imported and therefore not affordable by the vulnerable groups without subsidy. The study therefore focused on developing a cost-effective supplementary food to meet at least 50% of daily requirements, by drawing from energy and nutrient strengths of individual food ingredients without resulting to fortification.

Diversity, accessibility and consumption of supplementary foods in Kenya

A large diversity of supplementary foods are acquired and consumed in Kenya. Most of the foods, however, go to feed the vulnerable groups who continue to increase in number in the country. For the last two decades, supplementary feeding programmes in Kenya have been conducted by Non-Governmental Organizations (NGO's) like United Nations International Children's Emergency Fund (UNICEF) and United States Agency for International Development (USAID) (Marchione, 2002). The supplementary foods and food aid rations used in these programmes are expensive because they have always been imported. Very little consideration has been given to the use of local traditional and commonly-consumed food ingredients as alternatives in the formulations and production to bring down cost (WHO/FAO, 2003). In addition, the cost induced by transportation, storage and distribution of the fortified blended foods given by the various donors make the products beyond the reach of the common man (Navarro-Colorado, 2007). More outstandingly, rations that mainly comprise cereals grains and oil, although energy dense, do not provide a good balance of the micronutrients essential to drive the energy-giving metabolic processes that promote human nutrition and health. This increases the individual's susceptibility to malnutrition and infectious diseases during periods of prolonged hunger and poverty, and non-communicable disease, including type II diabetes and cardiovascular disease, during periods of affluence and/or nutritional adequacy (Baker, 2000; Prentice & Goldberg, 2000). The implications of poor food intake for growth and development across the life cycle are enormous.

National gaps in training, coverage and supplies of supplementary food to vulnerable groups exist. The Ministry of Health runs a National programme, on management of severe acute malnutrition, and a number of NGOs (Concern, Action Against Hunger, Merlin) support services in the North East of Kenya. An increasing number of partners are providing supplementary foods for people living with HIV and AIDS (such as the food-by-prescription

programme supported by USAID and the AMPATH project). However, these interventions are limited to selected areas, and sustained provision of supplies for therapeutic feeding (F75/100 or RUTF) remains a challenge. Kenya is developing a national advocacy and communication strategy on nutrition for people with HIV and AIDS as part of the Nutrition and HIV Strategy 2007–2010. Participants include the National AIDS Control Council and the Ministry of Health (NASCOP and the Division of Nutrition). UNICEF and WHO are named as key partners for developing, disseminating and implementing the strategy.

The Ministry of Health works closely with UNICEF, NASCOP and other partners to fulfill this role, an example of good practice that is evidenced by the number of new and revised documents that were released in 2007. Reports form the MoH show that about 80 per cent of public health facilities have adequate stocks of recommended therapeutic and supplementary foods for eligible clients. In addition, 80 per cent of district nutritionists are trained on nutritional care and support for HIV and AIDS, integrated infant and young child feeding counseling, and/or clinical nutritional care for children with HIV and AIDS. The USAID 'food by prescription' programme is serving undernourished children and adults in Kenya.

The majority of the beneficiaries in Kenya are orphaned and vulnerable children, and they receive FBFs mainly First Food and Unimix products which contain maize, millet, sorghum and soya. The nutrient density and ingredients are more similar to a supplementary food than a therapeutic food. First Food contains 435 kcal/100g, similar to Unimix at 400 kcal/100g, compared with Plumpy Nut, a therapeutic food which contains 545 kcal/100g. Insta Products are being used for both supplementary and therapeutic feeding. Therapeutic foods are ready-to-use, specialized products for use in the management of severe malnutrition typically in community and home-based settings. Insta Products also produces Advantage (for pregnant and breastfeeding mothers) and Advantage Plus (for adults). The food-by-

prescription project, which began in 2006, is still in its early stages; as yet the evidence is insufficient to determine the impact of Insta Products. As part of Kenya's *Strategy for Nutrition and HIV 2005-2010*, the country plans to standardize food and nutritional products provide to people living with HIV and AIDS and to define what is an acceptable product (MoH, 2005). This activity should be carried out using evidence based criteria and should take into account national and international product development research and programme evaluations. Most of the products identified as supplementary foods, which are largely plant-source-based diets with few animal source. The most commonly available supplementary food in Kenya is corn-soy blended flour, an inexpensive fortified cereal-legume combination that requires cooking and has been widely used in Africa for decades. Blends with higher nutrient density and lower antinutrient content, which are more appropriate for consumption by the vulnerable groups, either have high levels of animal protein or are made from high value processing technologies, which makes them more expensive than the currently recommended foods being used in Kenya.

The efficacy of supplementary feeding using fortified blended flours has raised concerns over its positive contribution in alleviation of malnutrition and hunger among the vulnerable groups (Beaton & Ghassemi, 1982; Navarro-Colorado, 2007). The nutritional composition (high protein, low fat, high dietary fibre and antinutrient contents) of the supplementary foods used by the malnourished groups are not adequate to meet the requirements of these groups (de Pee & Bloem, 2009). Small-scale food processing represents the most promising avenue that can simultaneously create both more nutritious foods for the general population and increased income for the developing societies. Interventions ranging from simply teaching people how to combine staples appropriately to setting up small-scale processing facilities can succeed in improving both nutrition and income among the vulnerable rural and urban poor groups. Local production can also help

strengthen the local economy and agricultural production. Further, the free-market competition and scaling up the production of local supplementary foods can help lower costs.

Nutritional profile of indigenous food ingredients consumed by vulnerable groups

The search for novel high quality but cheap sources of protein and energy has continued to be a major concern in many parts of the developing world including Kenya (Arinathan *et al.*, 2009). Investigations on economically viable indigenous food ingredients as alternative strategies to curb under nutrition and food insecurity are of utmost importance to broaden the essential nutrient sources for human nutrition (Barba de la Rosa *et al.*, 2009). Adaptation to adverse environmental conditions, resistance to pests, cultural acceptability and sufficient nutritional qualities are the key advantages of these indigenous foods. In addition, plants possess macronutrients, amino acids, lipids and minerals which are natural components of many cereals, legumes, oil seeds and vegetables and they play an important role in maintaining their quality and determining nutritive value in human diet (Prior & Cao, 2000).

The nutritive value of food ingredients depends primarily on their nutrient contents and presence or absence of anti-nutritional and/or toxic factors. Nutritive value is the ability of food to provide a usable form of nutrients: protein, carbohydrates, vitamins and minerals (Millward, 2004). It is well recognized that the nutritional value of proteins may differ substantially depending on their essential amino acid composition and digestibility. Among different substances that constitute grains and vegetables, amino acids are becoming more necessary in the nutrition of the vulnerable groups. Amino acids, a class of biologically active compounds present in food and beverages, are important for human nutrition and affect the quality, including taste, aroma and color (Siddhuraju & Becker, 2005). Lipids are partly responsible for the physical and chemical properties of food and those that are of major

interest are the fatty acid esters. Many lipid properties in food are explained in terms of their fatty acid composition (Applequist *et al.*, 2006).

Vitamins and minerals also play a crucial role in the proper development and good health of the human beings (Dolara *et al.*, 2005). The risk of deficiencies and attendant pathologies depends on a number of factors, such as the daily dietary intake, the chemical form of the minerals contained in the food consumed, the technological processing of the products, the presence of substance that limit or increase the bioavailability of minerals and the physiological state and overall health of the consumer (Sangronis & Machado, 2007). Data on nutrient status, amino acid and fatty acid profiles of indigenous food ingredients consumed by and used in formulation of supplementary foods for vulnerable groups in Kenya is still very limited and insufficient in spite of their expanding utilization by various organizations. The determination of the chemical composition of the indigenous grains and vegetables has become of great interest due to their extensive consumption and amplified use in the formulation of supplementary foods as well as therapeutic diets.

Bioactive compounds in food ingredients

Nutrition research and interventions are still focusing strongly on single nutrients such as protein, vitamin A, iron, iodine or zinc, which are deficient in certain population groups. Much less is known about the nutrition and health outcomes from a combination of foods and dietary patterns. However, it is becoming increasingly acknowledged that single nutrients alone are not the key to solving nutrition problems and preventing chronic diseases (Villegas *et al.*, 2008). Whole foods and nutritionally adequate diets such as a variety of vegetables and fruits as part of a mainly plant-based diet are desirable. In addition to providing several nutrients for healthy balanced diets, plant based foods exert a health-protective effect attributed mainly to antioxidants and dietary fiber (Amarowicz & Pegg, 2008). Some

phytochemicals have been reported to possess positive physiological effects on humans (Bazzano *et al.*, 2001). They can serve as protective agents for health maintainace and promotion or possess medicinal properties. Recently, a study on the nutritional value of indigenous plant foods in Kenya revealed that most of them are highly nutritious, containing high levels of both vitamins and minerals (Orech *et al.*, 2007). The authors also indicated that these foods have potential as a remedy to counter food insecurity since most are well adapted to the local environment, enabling them to resist pests, drought and diseases.

A plant-based diet with high intake of fruits, vegetables and whole grains may reduce the risk of oxidative stress-related diseases (Schlemmer *et al.*, 2009). The role of bioactive food components is important in assessing the role of dietary plants in human health and disease development. Consumption of a diversity of cereals, legumes, oil seeds and vegetable provides a combined additive or synergistic effect crucial to health benefits derived from the diet. Most bioactive food constituents are derived from plants; those so derived are collectively called phytochemicals (Halvorsen *et al.*, 2002). Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases (Lako *et al.*, 2007). The large majorities of these phytochemicals are redox active molecules and therefore defined as antioxidants. Antioxidants can eliminate free radicals and other reactive oxygen and nitrogen species, and that contribute to most chronic diseases (Prior & Cao, 2000). It is hypothesized that antioxidants originating from foods may work as antioxidants in their own right *in vivo*, as well as bring about beneficial health effects through other mechanisms, including acting as inducers of mechanisms related to antioxidant defense, longevity, cell maintenance and DNA repair (Granito *et al.*, 2008; Randhir *et al.*, 2008).

The main types of polyphenols are flavonoids, phenolic acids and tannins, which act as powerful antioxidants *in vitro* (Halvorsen *et al.*, 2002). These compounds are considered to

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carry many potential health effects, for example, in reduction of the risk of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, and osteoporosis (Fresco *et al.*, 2006; Hsu *et al.*, 2006; Islam 2006; Randhir & Shetty, 2007; Tapiero *et al.*, 2002). Therefore, the study on the importance and role of non-nutrient compounds particularly phenolics, flavonoids and high molecular tannins of plant foods as natural antioxidants have greatly increased. Food antioxidants such as α-tocopherol, ascorbic acid, carotenoids, amino acids, peptides, proteins, flavonoids and other phenolic compounds might also play a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural resistance to oxidative damage (Fresco *et al.*, 2006; Hsu *et al.*, 2006). Furthermore, there has also been interest in preserving endogenous antioxidants in food products both for stabilization and nutritional purposes (Granito *et al.*, 2008). To exploit the health-promoting functionalities of the locally available, culturally acceptable, and economically viable foods it is important to focus on evaluation of the functional properties of the bioactive compounds present in plant foods consumed by vulnerable groups.

Phenolics

Among the various bioactive substances, phenolic compounds are plants secondary metabolites, proven to exhibit many health protective effects, have received most attention (Vita, 2005). Phenolic compounds present in food ingredients such as cereals, legumes and vegetables were demonstrated to exhibit potential antioxidant (Prior & Cao, 2000), antimicrobial (Tapiero *et al.*, 2002), anti-cancer (Fresco *et al.*, 2006), anti-obesity (Hsu *et al.*, 2006), anti-diabetic and anti-hypertensive (Randhir & Shetty, 2007) as well as anti-mutagenic properties (Islam, 2006). Epidemiological studies have also suggested a positive role played by phenolics in the alleviation of oxidative stress and prevention of free-radical mediated diseases (Halvorsen *et al.*, 2002).

Flavonoids

Flavonoids are polyphenols with diphenylpropane ($C_6C_3C_6$) skeletons. They are considered to be the largest group of naturally occurring phenols and it is estimated that 2% of all the carbon photosynthesized by plants is converted into flavonoids (Alothman *et al.*, 2009). Flavonoids are widely distributed in the plant kingdom with a huge diversity of structures. They exist as six major classes: flavone, flavonone, flavonol, flavononol, isoflavone and favan-3-ol (anthocyanidins and catechins) (Iwashina, 2000). Several studies have emphasized that flavonoids from different botanical sources can act as powerful antioxidants, even more so than can the traditional vitamins. Several epidemiological studies indicate that food-derived flavonoids have been associated with decreased risk of cardiovascular diseases and cancer-prevention (Arts & Hollman, 2005; Vita, 2005) and also demonstrate antioxidant and anticarcinogenic (Ren *et al.*, 2003), anti-inflammation (Tapiero *et al.*, 2002), anti-platelet and anti-thrombotic (Vita, 2005), anti-allergic (Fresco, *et al.*, 2006) and antidiabetic properties (Arts & Hollman, 2005; Knekt *et al.*, 2002).

Tannins

Tannins are phenolic compounds divided into hydrolysable tannins which are water-soluble and condensed tannins which are water insoluble with molecular weights between 500 and 3000. They possess special properties such as ability to precipitate alkaloids, gelatin and other proteins (Amarowicz, 2007). Condensed tannins are complex polyphenols, which can be degraded into sugars and phenolic acids through either pH changes, or enzymatic or non-enzymatic hydrolysis (Fresco *et al.*, 2006). The basic units of hydrolysable tannins is either gallic or ellagic acid. Tannins do not only function solely as primary antioxidants (i.e., they donate hydrogen atom or electrons), they also function as secondary antioxidants. Tannins have the ability to chelate metal ions such as Fe (II) and interfere with one of the reaction

steps in the Fenton reaction and thereby retard oxidation. The inhibition of lipid peroxidation by tannin constituents can act via the inhibition of cyclooxygenase (Amarowicz, 2007). Numerous studies have demonstrated potentially significant biological effects of tannins such as antioxidant or radical scavenging activity as well as inhibition of lipid peroxidation and lipoxygenases *in vitro* (Amarowicz *et al.*, 2000; Gyamfi *et al.*, 2002), antimicrobial and antiviral (De Bruyne *et al.*, 1999; Dolara *et al.*, 2005), antimutagenic (Carlsen *et al.*, 2010; Dolara *et al.*, 2005), and antidiabetic properties (Anderson & Polansky, 2002; Matsui *et al.*, 2001).

Phytates

Phytate (myo-inositol 1, 2, 3, 4, 5, 6 hexakisphosphate), the salt of phytic acid, is widely distributed in the plant kingdom. It serves as a storage form of phosphorus and contains about 75% of total phosphorus of the kernels (Schlemmer *et al.*, 2009). Other parts of the plants such roots, tubers and turions, however, are very low in phytate (≈ 0.1% dm). Recently, there has been increasing interest in the phytates as phytochemicals because of their protective functions in the prevention of oxidative damage to human beings caused by reactive oxygen species (ROS) (Khattab *et al.*, 2010; Verghese *et al.*, 2006). Phytic acid has been found to play a major role in the treatment of cancer, hypercholesterolemia, hypercalcuria and kideny stones (Grases *et al.*, 2000; Plaami, 1997; Vucenik & Shamsuddin, 2003). The beneficial properties of phytate such as its antioxidant (Graf & Eaton, 1990; Khattab *et al.*, 2010), anticarcinogenic (Shamsuddin, 1995; Verghese *et al.*, 2006), anticalcification (Grases *et al.*, 2000; Schlemmer *et al.*, 2009) and hypoglycemic or hypolipidemic (Jariwalla *et al.*, 1990; Lee *et al.*, 2006; Lee *et al.*, 2007) activities are of great importance in human nutrition. Further, studies on the positive effects of dietary phytates have revived research about the

significance of phytate and other inositol phosphates in human nutrition and for human health.

Antioxidant activity of food ingredients

An antioxidant can be described in simple terms as anything that can delay or prevent oxidation of a susceptible substrate and when added to foods tend to minimize rancidity, retard the formation of toxic oxidation products, help maintain the nutritional quality and increase their shelf life (Khattab *et al.*, 2010, Lako *et al.*, 2007). Our antioxidant system is complex, however, and consists of various intracellular and extracellular, endogenous and exogenous, and aqueous and lipid-soluble components that act in concert to prevent Reactive Oxygen Species formation (preventive antioxidants), destroy or inactivate ROS that are formed (scavenging and enzymatic antioxidants), and terminate chains of ROS-initiated peroxidation of biological substrates (chain-breaking antioxidants) (Schlemmer *et al.*, 2009). In addition, metals and minerals such as selenium, copper, and zinc that are key components of antioxidant enzymes are often referred to as antioxidants. There are many biological and dietary constituents that show 'antioxidant' properties *in vitro* (Dolara *et al.*, 2005). Accordingly, regular and adequate dietary intakes of (largely) plant-based antioxidants, most notably vitamin C, vitamin E, folic acid, carotenoids, flavonoids and phenolics are neccesary. The recommendation to increase the consumption of plant-based foods and beverages is one that is widely perceived as health-promoting, and the consistent and strong epidemiological links between high dietary antioxidant intake and the greater life expectancy seen in various groups worldwide whose diet is high in plant-based foods indicate that more emphasis should be given to this particular dietary recommendation (Randhir & Shetty, 2009). Dietary strategies for health promotion in vulnerable groups should therefore be directed towards optimizing the consumption of these foods.

Given the rapidity with which traditional diets and lifestyles are changing in many developing countries, it is not surprising that chronic diseases occur in countries where under nutrition and food insecurity are endemic problems (WHO/FAO, 2003). Epidemiological studies have shown the protective effect of plant-based diets on chronic diseases and several polyphenols as well as other phytochemicals have been implicated (Amarowicz *et al.*, 2000; Carlsen *et al.*, 2010; Gyamfi *et al.*, 2002). There are many age-related disorders that, in theory at least, may be prevented or delayed by increased antioxidant defense. These disorders include arthritis, cancer, coronary heart disease, cataract, dementia, hypertension, mascular degeneration, diabetes and stroke (Dolara *et al.*, 2005; Carlsen *et al.*, 2010; Serrano *et al.*, 2007). A majority of these bioactive food constituents are redox active molecules and therefore defined as antioxidants. Antioxidants can eliminate free radicals and other reactive oxygen and nitrogen species, and these reactive species contribute to most chronic diseases (De Bruyne *et al.*, 1999; Dolara *et al.*, 2005; Gin *et al.*, 1999).

Several assays have been used to assess the antioxidant activity of foods, for example the 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity (TEAC) assay, the ferric reducing antioxidant power (FRAP) assay, DPPH radical scavenging activity (DPPH assay) and the oxygen radical absorbance capacity assay (ORAC). The current study used a combination of two assays (DPPH assay and the modified version of FRAP assay) for the analysis of the antioxidant activity of the foods. The modified FRAP assay is a simple, fast and inexpensive assay with little selectivity. The FRAP assay directly measures antioxidants with a reduction potential below the reduction potential of the Fe^{3+}/Fe^{2+} couple. The model of plant-based diet, containing whole-grain products and vegetables as primary ingredients has become one of the most important guidelines for reducing the risk of disease caused by the increased level of free radicals.

Type-II diabetes related functionality of foods ingredients

In the past two decades, numerous studies have focused on the role of nutrition and diet on health in the developing countries including Kenya. However, chronic diseases, such as cancer, cardiovascular diseases and diabetes, are also spreading and are expected to increase in the coming decennia (WHO/FAO, 2003). Consequently, the prevalence of diabetes, obesity, cardiovascular diseases and cancer is on the increase in both developing and developed countries including Kenya. Lifestyle changes and dietary habits affect diseases that include obesity, diabetes mellitus, cardiovascular disease, hypertension and stroke, and some types of cancer (Adebooye *et al.*, 2008; Adom & Liu, 2002). Epidemiological studies suggest that the consumption of whole grains, legumes, fruits and vegetables is important in the prevention of chronic diseases (Schlemmer *et al.*, 2009). Recently, there has been a keen interest in the protective biochemical function of natural bioactive compounds from dietary sources (Matsui *et al.*, 2001).

Type 2 diabetes is becoming more prevalent with increased studies on its management through dietary sources. In this context, currently there is an increasing interest in natural sources of hypoglycemic compounds since the synthetic oral hypoglycemic drugs have been found to exhibit many adverse effects (Randhir & Shetty, 2007). Small intestines α-glucosidase (EC 3.2.1.20) and pancreatic α-amylase (EC 3.2.1.1) are key enzymes of dietary carbohydrate digestion in humans. Inhibitors of these enzymes may be effective in retarding carbohydrate digestion and glucose absorption to suppress postprandial hyperglycemia. Most of the bioactive compounds such as flavonoids (Arts & Hollman, 2005), phenolics (Randhir & Shetty, 2007), tannins (Anderson & Polansky, 2002) and phytates (Schlemmer *et al.*, 2009) are known to inhibit these enzymes.

The enzyme α-amylase catalyses the hydrolysis of glycosidic linkages of starch molecules, an essential step in carbohydrate assimilation (Hosoyama *et al*, 2003). α-Amylase

inhibitors are starch blockers which affect the enzyme activity thus playing a vital role in reducing blood sugar. Inhibitory activity of the polyphenols on the amylase has been the focus of attention in the management of type II diabetes mellitus (Serrano *et al.,* 2009). Recent research has shown that the complex mixture of phytochemicals in foods provides better protective health benefits than single phytochemicals through a combination of additive and/or synergistic effects (Adom & Liu, 2002). Hence, for maximum health benefits, sufficient amounts of phytochemicals from a variety of sources such as fruits, vegetables, and whole grain-based foods are recommended.

Effects of processing on nutrients and bioactive compounds in foods

Food processing not only improves the flavour and palatability of food ingredients but also increases the bioavailability of nutrients (Miglio *et al.*, 2008). It is known that cooking induces significant changes in chemical composition, influencing the concentration and bioavailability of bioactive compounds in grains and vegetables (Turkmen *et al.*, 2005; Granito *et al.*, 2008). The reactions occurring during food processing include biochemical and chemical processes. The most important biochemical process is enzymatic oxidation, which is apparent immediately when the integrity of the cell is broken, but other types of enzymes, such as esterases, glycosidases, and decarboxylases, may also catalyze transformations and degradations of chemical compounds in foods (Cheynier, 2005). Chemical reactions take place simultaneously and gradually become prevalent as the enzymatic activities decrease. Several studies have reported both positive and negative effects of thermal treatment depending upon differences in the processing conditions, morphological and nutritional characteristics of the food ingredients (Amin *et al.*, 2006; Granito *et al.*, 2008; Siddhuraju, 2006). Thermal processing is the most extensively used method of food processing for preservation to destroy microorganisms thereby extending shelf life.

However, thermal processing has long been perceived to also increase digestibility of foods but with loss of certain heat-labile nutrients thus lowering the nutritional value (Amin *et al.*, 2006). Most indigenous foods are commonly cooked before consumption. However, both positive and negative effects of cooking have been reported depending upon differences in process conditions, and the morphological and nutritional characteristics of the food ingredients (Granito *et al.*, 2008; Miglio *et al.*, 2008).

The identification of the most elite thermal processing method that optimally preserves the bioactive compounds and their functional properties would be fundamental for the populations consuming the indigenous food ingredients with claimed medicinal effects so as to reap maximum health benefits (Khandelwal *et al.*, 2010). The effect of certain traditional processing methods, which are practiced by the ethnic groups in Kenya, on the level of bioactive compounds and their functional properties in the various indigenous food-stuffs was investigated with a view to select the viable and more suitable methods that conserve nutrients and biocative substances in the food ingredients used in the formulation of therapeutic supplementary foods for vulnerable groups. In Kenya, the most common food processing/preparation methods include the covenctional soaking, roasting, germination, fermentation, decortications, and cooking, and these are known to greatly influence the nutritive value of foods.

Formulation of the supplementary food for vulnerable groups

Cereals and legumes occupy an important place in human nutrition, especially in the dietary patterns of low-income earners of the developing countries (Oboh *et al.*, 2009). Cereals are mainly milled into flours for incorporation in formulations of supplementary foods, since they are readily available and culturally acceptable staple foods (Sadler *et al.*, 2006). Cereals have high energetic loads, based on their carbohydrate and protein contents, around 78% and

13%, respectively, and also provide minerals and vitamins, particularly thiamin. The most common cereals used to formulate supplementary foods are maize, sorghums, millets, wheat, barley, rye, oat and rice. They are used individually or mixed to obtain blended flours. Other food ingredients such as roots (tapioca, carrots, sweet potatoes etc.) or legumes and pulses (soybean, mung bean, black bean, pigeon pea, cow pea, lentils and chick pea) may also be used to formulate such foods. Legumes represent an important component of human diet in the developing countries and are considered as one of the cheapest and richest source of dietary protein (17-34%), which are used as a substitute or supplement for the relatively expensive animal protein in human diet (Huma *et al.*, 2008). Legumes are rich in proteins, complex hydrocarbons, and minerals, and exhibit lower glycemic index compared to other starchy foods (Siddhuraju *et al.*, 2006; Xu & Chang, 2008). Additionally, legumes contain a rich variety of phytochemicals, including phytosterols, natural antioxidants and bioactive carbohydrates (Amarowicz & Pegg, 2008). Epidemiological studies indicate that legume consumption is inversely associated with the risk of coronary heart disease (Bazzano *et al.*, 2001), type II diabetes mellitus, (Villegas *et al.*, 2008) and obesity (Rizkalla *et al.*, 2002). Vegetables have also been showed to exhibit protective action attributed to presence of antioxidants, especially antioxidant vitamins including ascorbic acid, α-tocopherol and β-carotene (Prior & Cao, 2000). A number of vegetables (garlic, broccoli, kales, red pepper, onion, sweet potatoes, pumpkin leaves etc.) have been reported to have high antioxidant activity among other health benefits (Kaur & Kapoor, 2002).

The acceptable supplementary food developed in this study is based on amaranth grain, pigeon pea, sweet potatoes and groundnuts. Amaranth grain produces significant amounts of edible grain and is described as "the grain of the 21st century". It is a good source of minerals and vitamins with a protein content of 16% which is of higher nutritional quality in comparison to cereals and some legumes (Gorinstein *et al.*, 2007). Amaranth has high

contents of lysine, arginine, tryptophan and sulphur containing amino acids. Sweet potato roots are consumed in Kenya either as fresh vegetable or as boiled, roasted or baked products in the normal human diet. The roots are rich in starch, sugars and minerals and some varieties contain coloured pigments such as anthocyanin and β-carotene which are regarded as antioxidants having several physiological attributes such as anti-oxidation, anti-immunodilation, protection against cataract, ageing, muscular degeneration and liver injury (Panda *et al.*, 2009). Recently, sweet potatoes have been labeled as an 'antidiabetic' food because of some animal studies in which sweet potato helped stabilize blood sugar levels and lowered insulin resistance (Panda *et al.*, 2009).

Among food legumes, red gram or pigeon pea (*Cajanus cajan*) is a valuable source of proteins, minerals, and vitamins and occupies a very important place in human nutrition in many developing countries (Fasoyiro *et al.*, 2006). It is also observed that pigeon pea is an economically and nutritionally important legume as major source of proteins in poor communities of many tropical and subtropical regions of the world (FAO, 2003). Pigeon pea proteins are rich in lysine but are usually deficient in the sulfur amino acids, methionine and cysteine. Inspite of this, however, pigeon pea could be categorized as a high protein material to offset the amino acid deficiencies of cereal proteins (Torres *et al.*, 2006).

The development of a new supplementary food should take into account the guidelines and statement accepted at a meeting of the Technical Consultation organized by WHO in collaboration with UNICEF, WFP and UNHCR, in Geneva, 2008. The following statement was agreed upon: *When there is good reason to believe that use of a new commodity has at least equal impact compared to the currently used product (often fortified blended foods, FBF), and can be regarded as safe, it can be used at limited scale programmes for young (moderately malnourished) children with good programme*

monitoring and evaluation, while impact is also assessed under carefully controlled circumstances. Products for which there is "good reason to believe":

1. *Nutrient density in combination with family food and breast milk is consistent with adequate nutrient intake for malnourished children*
2. *Ingredients, fortificants and hygiene criteria are in accordance with Codex standards and guidelines*
3. *Production and packaging are in accordance with the Codex, with appropriate quality control and quality assurance in place*

According to WHO/UNICEF (1998), Mosha et al. (2005) and WHO (2005), formulation of low-cost, fortified supplementary foods from locally available ingredients, using appropriate small to medium-scale production technologies in community settings, can help meet the nutritional needs of vulnerable groups, hence decreasing the prevalence of undernutrition significantly. Therefore, development of supplementary foods based on locally available cereals and legumes has been suggested by Integrated Child Development Scheme (ICDS) and FAO to combat malnutrition among vulnerable groups and populations of low socio-economic groups. There is, therefore, urgent need to develop low-cost nutritious supplements through combinations of less expensive foods always available in respective localities or communities. The use of simple, inexpensive and traditional processing methods would need to be standardized to optimally conserve the nutrients during preparation of such supplementary foods.

Study objectives

Foremost, the study had been designed to generate knowledge about the diversity and characteristics of supplementary foods used for vulnerable groups in Kenya. Secondly, after displaying the nutritional profiles and health relevant functionality of the bioactive compounds present in local food ingredients, elite food ingredients to be used in formulation of supplementary foods were selected. Further, the study aimed to develop a supplementary food based on the locally available food ingredients using simple food processing methods that can be applied even at village level.

Main objective

The main objective of this study was to develop a nutritionally adequate, health promoting and low cost supplementary food for vulnerable groups by utilizing local foods as ingredients.

Sub-objectives

1. To carry out a baseline survey on the diversity, marketing and utilization of supplementary foods consumed and sold in Kenya.
2. To determine the nutritional quality (micronutrient composition, amino acid and fatty acid profiles) of the local food ingredients and products.
3. To evaluate the bioactive compounds and the in vitro health-related functionality of the common local food ingredients.
4. To develop an acceptable and high quality supplementary food based on selected locally available food ingredients.
5. To assess the sensory quality of the supplementary foods
6. To evaluate the shelf life of the final supplementary food

Thesis Layout

This study will be in nine chapters outlined as follows:

Chapter 1: A general introduction of the thesis outlining the different research areas covered in this study based on the activities carried out under different sub-objectives.

Chapter 2: Baseline survey that sought to identify and take stock of supplementary foods currently being used in the country, including market survey of the foods. The chapter details the diversity and characteristics of supplementary foods currently being used by vulnerable groups. The foods were divided into two groups: those used in rehabilitation programs in hospitals and supplementary feeding programs as well as those that are being sold in the market and targeted for the vulnerable groups. Linear programming was used to assess the percentage nutrient fulfillment of the nutritional needs of the different vulnerable groups by these foods.

Chapter 3: Laboratory analysis of selected local food ingredients to assess their nutritional quality (protein, fat, carbohydrates, energy, the micronutrients, amino acid and fatty acid profiles), to assess their suitability for use in development of nutritious supplementary foods and quality diets.

Chapter 4: Determination of the total phenolic content, antioxidant and antidiabetic properties of methanolic extracts of raw and traditionally processed food ingredients with a view to identify the elite food ingredient(s) with potential health benefits and also to select a suitable processing technology in order to utilize them in the formulation of supplementary foods for vulnerable groups living in Kenya.

Chapter 5: The flavonoid content and the associated antioxidant as well as antidiabetic activities in selected foodstuffs consumed by vulnerable groups in Kenya, with the aim to selecting the elite food ingredient(s) with the appreciable flavanoid content and favorable functional properties as well as identifying an optimal processing technology.

Chapter 6: Evaluation of the condensed tannin content of selected cereals, legumes, oil seeds and vegetables commonly consumed by vulnerable groups of Kenya as well as the associated antioxidant and antidiabetic activities. Further, the effect of certain traditional processing methods, which are particularly practiced by the ethnic group in Kenya, on the components, was evaluated with a view of selecting the foods in which the nutrients and biocative compounds are optimally conserved.

Chapter 7: Aimed at evaluating the phytate contents, and the associated antioxidant potential and antidiabetic properties of the raw and processed food ingredients with the view to identifying the most elite food ingredients, as well as suitable processing methods for preserving these functionalities.

Chapter 8: Development of a supplementary food through the effective combination of selected locally available and commonly-consumed foods, making use of their 'nutrient strength'. Blending the foods in the formulations resulted in an acceptable nutrient-enriched and relatively low cost supplement without the need for fortification. The shelf life of the final product and its selling price at the retail level were determined.

Chapter 9: This chapter gives a summary of the major conclusions of the study and also gives way forward as recommendations.

CHAPTER 2

Diversity and Characteristics of Supplementary Foods Sold and Consumed in Nairobi, Kenya

Abstract

There has been a proliferation in diversity of supplementary foods in Kenya, prompted by increase in malnutrition, HIV/AIDS and related diseases, and consumer nutrition and health awareness. This study was designed to assess the characteristics of these foods utilized in Nairobi. A previously pre-tested semi-structured questionnaire was used to interview managerial staff of the main supermarket chains, and the nutrition staff of hospitals, NGOs, rehabilitation centers and Ministry of Health. Linear programming was used to calculate the percentage fulfillment of nutrient supplementation by some foods recommended by FAO for children aged 12 – 23 months. Results showed that there were two categories of foods: Foods officially recommended for rehabilitation, but not selling in open market, and a multiplicity of commercial food supplements with varying health claims on labels and found on display in the supermarkets and sales outlets. The prices of the official foods were the same (US$ 0.92/Kg), while the prices of the commercial foods varied considerably depending on ingredients (US $ 0.71 to 2.50/Kg). The official foods satisfied the recommended nutrient supplementation better than the commercial food supplements. The study established that the official foods were imported and standardized with regard to nutrient contents unlike the locally produced commercial foods. The local commercial foods could also be optimized in terms of nutrient contents to qualify for rehabilitation.

Keywords: Supplementary foods, nutrition, health, rehabilitation, Kenyan market

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Introduction

Recently, new nutritional and health insights have emerged which have created need for development of foods and beverages with health benefits, authenticated by scientific evidence or studies (Weststrate *et al.*, 2002). The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have recognized the importance of these foods in enhancing nutrition and health (WHO, 2003). In Kenya, consumers, especially those from the middle and upper socio-economic classes, have become increasingly aware of the health benefits derived from consumption of health promoting specialized foods found in the market. Consequently, there is increasing willingness to pay for additional safety of food products, and increasing attention toward the overall safety of consumption patterns, given a more widespread knowledge of the relation between food consumption patterns and health status (FAO, 1998 and 2002). Also there has been a steady increase in malnutrition, HIV/AIDS and other diseases like diabetes (UNAC/WHO 2001). It has been reported that the percentage of malnourished people has remained at around 35%, but with absolute numbers increasing due to population growth (Rosegrant *et al.*, 2005). This has caused increase in the demand for specialized nutritious and health food supplements. Propelled by the increased demand, the diversity and volumes of the supplementary foods in the market have risen. Earlier on, most of these foods used to be imported mainly from developed countries. Recently, however, the local food industry has responded by manufacturing similar foods.

There is currently a diversity of locally manufactured foods, which sell on the 'health' display counters of many supermarkets and sales outlets in Nairobi. These foods target not just the nutritionally and health challenged individuals, but also can be used by healthy individuals and families to maintain sound nutrition and health status. The supplementary foods used for rehabilitation in Kenya can be classified as fortified blended foods (FBFs). FBFs, such as corn/soy blend and wheat/soy blend have been the choice supplementary food

commodities among many different vulnerable populations for the past 30 years or more. These FBFs consist of mixtures of 20-25% soy, 75-80% corn or wheat, and a micronutrient premix (Marchione, 2002). They have been regarded as being of reasonably good nutritional value for limited cost and are being produced in more than 20 countries across the globe (Bryce *et al.*, 2008). In Kenya, most FBF's are donated by the United States and distributed by the World Food Program (WFP). Over 50% of the supplementary feeding programs supported by WFP utilize FBFs. WFP also assists countries to enhance local manufacturing capacity, and then contract the firms to manufacture the foods for their programs. This cuts down importation costs, and because locally grown and usually cheaper food ingredients including such cereals as sorghums and millets are used in the manufacture, production cost also goes down. The local manufacturing also ensures sustainability of the programs and creates incentives for farmers to produce raw material for the industry. This in turn has the effect of improving household income and food security (Subbarao *et al.*, 1997). Approximately 50% of FBFs used by WFP are locally manufactured. Key nutritional problems are addressed during formulation and manufacture of these foods via expert consultations. Other issues taken into account include local food ingredient availability, acceptability, affordability and food patterns of the vulnerable cohorts (Allen & Gillepsie, 2001; FAO/WHO, 1998 & 2002).

Information on the supplementary foods marketed and utilized in Kenya still remains scanty. Some of the locally manufactured foods appearing on market shelves bear health claims, unauthenticated in most cases, on their labels. Health claims, refer to a health benefit from a food or food constituent and are divided into two types: claims that refer to reduction of disease risk or to improvement of children's health; and generic claims that are more straightforward and do not refer to either children's health or disease risk reduction (Aisbitt, 2007). These foods are usually either enriched with nutrient supplements or are prepared with

food ingredients known to be rich in the nutrients. This study therefore, was aimed at characterization of the various supplementary foods imported and locally manufactured, which are marketed and used in Nairobi, in terms of the ingredients used in their formulation, the target groups, nutritional values and their relative costs in the market.

Methodology

This study was carried out in Nairobi from November 2008 to February 2009. Nairobi was purposively selected because being the capital city; it has the largest number of consumers of supplementary foods and sales outlets. Nairobi has a cross-section of people from different ethnic and socio-economic backgrounds. Foods selling in Nairobi will be the same ones found selling in other metropolitan centers of the country.

Data was collected using previously pretested semi-structured questionnaires (Annex 1-3). Some qualitative data was also collected by making general observations during the interviews. Twenty-one branches of 5 major supermarkets were interviewed, including 5 branches of *Nakumatt*, 6 branches of *Uchumi*, 4 branches of *Tuskys*, 4 branches of *Ukwala* and 2 branches of *Chandarana* supermarkets. In addition nutrition staff of 5 hospitals, 13 NGOs 4 rehabilitation centers that operate rehabilitation programs and Ministry of Health were interviewed using relevant sections of the same questionnaire. Linear programming modules of Nutrisurvey (Dantzing, 2002; Briend *et al.*, 2001; Darmon *et al.*, 2002) were used to assess the nutrient adequacy of selected foods. Linear programming was used to estimate the percent fulfillment of standard and market foods specifically recommended for vulnerable children aged 12-23 months, based on FAO/WHO nutritional recommendations for malnourished children (FAO/WHO, 1998 & 2002).

Statistical analysis

The quantitative data was analyzed using Genstat statistical package, 9th Edition (Payne *et al.*, 2006). Descriptive statistics was used for the qualitative data.

Results and discussion

General market characteristics of the supplementary foods sold and utilized in Kenya

The study identified two categories of supplementary foods that are marketed and utilized in Nairobi. The first category of foods consisted of those foods that are officially recommended for use in rehabilitation and supplementary feeding programs in the country. These foods were few (5) and are shown in Table 2.1. Although the foods were targeted to the vulnerable groups, HIV/AIDS and the malnourished groups, they could also be used by healthy individuals and families to enhance health. All these foods are categorized as FBFs. These foods were imported or locally manufactured and supplied directly to rehabilitation programs. The main difference between *Foundation* and *Foundation Plus*, two of the foods in this category, is that the latter contains more energy than the former. These foods were not available for sale in the open market at the time of this study.

Table 2.1 Ingredients, target groups and cost of some supplementary foods sold in major supermarkets

Type supplementary food (Brand names)[a]	Ingredients in formulation	Target groups	Price/kg (KES)[b]
Bewa Omena mix flour **(NFBF)**	Soya, millet, sorghum, maize, fish	Adults & Children	78
Soy Afric nutria-mix recovery porridge **(NFBF)**	Maize, soya, millet, sorghum, wheat & oats	Aged, malnourished, recuperating patients, expectant & lactating mothers	64
Bewa special porridge mix **(NFBF)**	Soya, millet, beans, peanuts, green grams, maize, cassava, sorghum, peas & black beans	Adults, children & patients	86
Pure amaranth flour **(FNBF)**	Whole amaranth grain Fortified with vitamins & minerals	Malnutrition, cancer & HIV/AIDS patients, heart diseases, diabetes, gout, constipation, TB, colds etc.	190
Bewa soya-wimbi flour	Soya, millet, cassava, sorghum	Children & adults	80
Nutri-rich Proctor & Allan **(FBF)**	Maize, soya, sorghum, fortified with minerals & vitamins	Whole family	80
FAFA-D diabetic formula **(FBF)**	Special fibre preparation, toasted soya, millet, maize, sorghum, tapioca, fortified with vitamins & minerals	Diabetic patients, weight loss & people with hemorrhoids	99
Omena porridge flour **(NFBF)**	Soya, groundnuts, millet, sorghum & whole fish	General population	77
Bewa wimbi porridge flour with milk powder **(NFBF)**	Millet, maize, cassava, sorghum & milk powder	Adults & children	73
Borabora nutri mix super porridge **(NFBF)**	Soya & maize meal	General population	56
Azuri delight (BASCOT) **(NFBF)**	Beans, bananas, maize, pumpkin, carrots & amaranth	Breast milk substitute for children	186
Maspet special porridge mix **(NFBF)**	Soya, millet, beans, peanuts, green grams, maize, sorghum, peas & black beans	Adults & children	65
Maspet wimbi with milk powder **(NFBF)**	Millet, maize, sorghum & milk powder	Adults & children	65
FAFA wimbi mix sour porridge **(NFBF)**	Maize, millet, soya & souring agents	General health of population	64
FAFA porridge mix Soy afric **(NFBF)**	Soya, maize, wheat, millet & souring agents	General health of population	67
Nature's way family Health Porridge **(NFBF)**	Amaranth, millet, oats, wheat, carrots, corn, alfalfa, soya, parsley, kales & sorghum	Prevents aging, cancer & indigestion, immune boooster	140
Nature's way natural pure wimbi **(NFNBF)**	Millet flour	Diabetic patients	109
Maspet Uji mix (NFBF)	Sorghum, soya, maize & souring agents	Adults &children	60
Maspet soya wimbi flour **(NFBF)**	Soya, millet & sorghum	Adults & children	62
Pure wimbi flour Kasuku mbili **(NFBF)**	Millet flour	Growing children, aged people & diabetic patients	80
Mbogo's quality farm products **(FBF)**	Maize, sorghum, millet, cassava, soya, simsim, amaranth, black beans, and calcium	General working population, diabetic patients	92
Body builders porridge **(NFBF)**	Soya, millet, sorghum, groundnuts, black beans green grams, peas,	Health and body strength	70
Omena mix **(NFBF)**	Fish, soya, groundnuts, beans, green grams, millet & sorghum	Children & adults	76
Special porridge mix (NFBF)	Millet, beans, peanuts, green grams, maize, cassava & sorghum	Adults & children	67
Farm porridge mix **(NFBF)**	Millet, peanuts, groundnuts, soya beans	Adults, children & diabetic people	54
Pure Health uji afya **(FBF)**	Amaranth, sorghum, millet, maize, rye & oats. Fortified with vitamins & minerals	Lowers blood fat, bowel movement, protects body from pollution & protects the lungs	80
Cassava porridge mix **(NFBF)**	Cassava, millet & sorghum	Adults & children	54
Bewa strawberry flavored millet flour **(NFBF)**	Millet, cassava, sorghum, maize & strawberry flavor	Adults and children	70
Mushrooms for health **(NFBF)**	Mushrooms, sorghum & roasted ground nuts	General population	75

[a]FBF - fortified blended flour, FNBF – fortified non-blended flour, NFBF - non-fortified blended flour, NFNBF – non-fortified non-blended flour.

[b]Prices in local currency and range from US $0.71 to 2.50 per kg (1US$ = 76 KES).

The second category consisted of a group of more than 80 food supplements containing varying nutrition and health claims on their labels. These foods were found selling under the 'health' shelves in the supermarkets. The foods can be categorized as FBFs, Fortified-non-blended-foods (FNBF), Non-fortified-blended foods (NFBF) or non-fortified-non-blended foods (NFNBF), as shown in Table 2.2. About 30% of these foods could qualify for use in rehabilitation as judged by the label information. Of these foods which were not FBFs, the specific nutrients and health factors indicated on the labels were presumed to have emanated from the constitution of the ingredients used in their formulations. The results indicated that the number and consumption of this second category of foods had increased over the last few years. The increase in number was indicated as caused mainly by increased market.

Table 2.2 FBF's officially recommended for rehabilitation in Kenya showing the ingredient formulations, main target groups and product costs

Type of FBF's	Ingredients in formulation	Target group	Prices per 1 kg (KES)*
Advantage®	Precooked whole maize, soybeans, cane sugar, iodized salt, vegetable oil, whey protein concentrate 80%; fortified with vitamins & minerals	Pregnant & nursing mothers, HIV/AIDS, Malnourished	70
First food®	Precooked whole maize, millet, sorghum, soybeans, cane sugar, iodized salt, vegetable oil; fortified with vitamins & minerals	Malnourished children, HIV/AIDS	70
Foundation®	Precooked whole maize, soybeans, cane sugar and fortified with vitamins & minerals	Whole family, HIV/AIDS, malnourished	70
Foundation® +plus	Precooked whole maize, soybeans with vegetable oil, cane sugar and fortified with vitamins and minerals	People who require more concentrated energy, Malnourished, HIV/AIDS	70
UNIMIX (CBS)	Precooked corn, soya beans and fortified with vitamins and minerals	Malnourished children and adults	70

*Prices in local currency and less than one US dollar (1US$ = 76 KES)

The main reasons for the increase in consumption were indicated as presumed health benefits from the consumption of the foods by 45% (percent) of respondents and curiosity arising from the new ingredients used in their formulation and the fascinating health claims on the labels of some of them by 30% (percent) of respondents. Most of these health claims had not

been scientifically authenticated. Table 2.3 shows some of the health claims commonly featuring on the labels of these foods. The Codex Alimentarius Commission and the International Food Standards agency define health claims as "any representation that states, suggests or implies that a relationship exists between a food or nutrient or other substances contained in a food and a disease or health related condition" (Wahlquist, 2002). Health claims for products and ingredients are developed to represent both efficacy and safety. Scientific studies for substantiation of health claims need to be put in place by regulatory bodies. This requires compilation and critique of existing literature, including clinical trials on humans, epidemiological evidence, animal studies and other evidence of biological activities. The European Union PASSCLAIM Project (Process for the Assessment of Scientific Support for Claims on foods) was introduced in 2001 to provide industry, academics, consumer groups and regulators with means to evaluate the scientific basis for health claim.

Table 2.3 Major health claims labeled on packages of supplementary foods in the market

Health claims	Frequency (%)
Prevention of cancers (colon, breast and lung cancers), diabetes, heart diseases, aging and constipation	10
Control of malnutrition, marasmus and kwashiorkor.	41
Management and reduction of TB, rheumatism, bronchitis and HIV/AIDS	24
Immune boosting	13
General health improvement	9
Others	3

Advertizing also played an important role in influencing consumption of the foods. Nutrition information was not displayed on the labels of most of these products. Consumers therefore, purchased and consumed these foods regardless of their cost without being oblivious of the possibility of them being unwholesome or of lower nutritional and health quality than indicated. For example, with no shelf-life indicated on the labels, it was possible for

consumers to continue using the foods even after they had expired and were no longer able to meet their nutrition and health requirements in full as declared on the labels.

Regulatory organizations such as the Kenya Bureau of Standards (KEBS) have recently embarked on initiative to have the manufacturers mandatorily authenticate the claims on these foods and establish shelf-lives through accredited laboratory testing, before passing them to the consumer. This will obviate problems of unscrupulous manufacturers having to dump substandard goods in the market as they rush to cash on the quick money from sale of the products. No code of marketing and distribution of supplementary foods exist in Kenya so far. If organized properly, however the market for supplementary foods can contribute to poverty reduction by enhancing agricultural productivity and revenue generation of farmers who produce the raw material. The growth and successful development of supplementary food market in Kenya can also help to mainstream back into local diets the under-utilized local food crops such as sorghums, millets, cassava and sweet potatoes. As the farmers undertake to produce them as raw material for the industry, they also learn to incorporate the foods into their diets. This will improve food and nutritional security, diversify staple foods and therefore effectively curb nutritional deficiencies.

Energy dense diets from these local foods offer a low cost dietary option to the vulnerable groups (Darmon *et al.*, 2004). Fortified blended foods, however have been associated with disappointing results in supplementary feeding programmes in sub-Saharan Africa (Mahlungulu, 2007). The lack of variety and high market prices of these foods create inaccessibility to a large proportion of the vulnerable groups due to exhausted household assets with which to purchase or barter. Such people are therefore often left with no alternative but to rely on single starchy staples which also are often low in micronutrients (James *et al.*, 1997). It has also been reported that there are no FBFs that are well-adapted to meet the nutritional needs of young or moderately malnourished children (Méance et al.,

1999; Navarro-Colorado, 2007). The main concern about these blends is that they contain "anti-nutrients" that may interfere with nutrient absorption and dietary fibers that can affect appetite (Hoppe, 2008). Another concern, which is heavily publicized, is whether the lack of animal-source foods in these blends is contributing to nutritional deficiencies and growth retardation.

Ninety percent (90%) of the supermarkets and market outlets were purchasing the supplementary foods wholesale directly from the manufacturers. The foods were mainly packed in bales, each containing either 12 packets of 2kg each or 24 packets of 1kg each. Market promotion of the foods is the prerogative of the manufacturers. The sales promotion strategies employed when pitching the products to supermarkets include 1) providing a free sample of the product 2) providing free point-of-purchase displays or shelf-talkers for the products and 3) guaranteeing a minimum level of sales. All the supermarkets, however, indicated that "shelf space", "customer recommendations/requests", "price and gross margin", and "proven sales history" were the most common criteria used by supermarkets to decide whether or not to place a new product brand in their shelves. Ability to restock, product category, label information, bar code and shelf life of the product were the additional criteria used, but these were of minor importance.

Types of supplementary foods, ingredients, target groups, nutrient contents and prices in the market

The supplementary foods shown in Table 2.1 were based mainly on corn soy blend (CSB), although other cereals like wheat have also been used in place of corn. The formulations of foods for rehabilitation based on CSB have remained unchanged for almost 30 years, despite increased knowledge on alternative formulations based on other cereals and legumes that can equally meet the same nutritional requirements (Hurrel, 2002). The soybean in the blend acts

to supply cheap but quality protein from plants. The maize serves to supply carbohydrates for energy but also some protein though of lower quality than that from soybean. One food contained whey protein concentrate as source of extra protein, while another one contained sorghum and millet which contributed some protein, but acted mainly as source for additional energy. One of the foods contained vegetable oil for additional energy source. All the foods contained cane sugar as additional energy source. And all of them were fortified with vitamins and minerals. The price of all the five foods was KES 70 (approximately US$ 1.00 per Kg), because their market is controlled (Table 2.1). These foods were not found selling openly in the market.

The market supplementary foods on the other hand are shown in Table 2.2 in terms of the type, ingredient formulation, target group and price per kilogram of the foods. Most of these foods fell under the category of non-fortified blended foods (NFNBF). They were not blended with vitamins and minerals, but probably they relied on the incorporation of the ingredient constitution as source of nutrition indicated on their labels. As Table 2.2 shows, corn and soy are also the most commonly used cereal and legume in the formulation of these foods. However, in some of them, other cereals such as wheat, sorghums and millets substitute corn in the formulations. Soybean again serves as the cheap source of quality plant protein. Other legumes like the common bean and pulses such as pigeon peas, green grams and amaranth grain were also used in some of the foods as additional sources of plant protein. The source of energy in these foods was mainly maize, but also other cereals like sorghums and millets, and root crops (predominantly cassava). The foods were indicated as targeted to diverse vulnerable groups, but also to the general family for maintenance of good health and nutrition. Cereals are the main sources of most B vitamins, iron, calcium and zinc in the formulation of the foods (Posner, 2000; Harrel-Bond *et al.* 1989). Legumes and pulses on the other hand are very important sources of protein and some vitamins, especially among the

low income communities where animal proteins are unaffordable. The prices of these foods varied between about KES 54.00/Kg ($US = 0.72) to KES 190.00/Kg ($US 2.50), the prices being highest for foods meant for highly specialized vulnerable groups (Table 2.1). Amaranth flour was for example the most highly prized because it was indicated as suitable for persons suffering from malnutrition, cancer, HIV/AIDS, TB, heart diseases and gout. However, note that none of these claims have been medically or scientifically authenticated. There was indication that the prices increased if vegetables and foods of animal origin were incorporated in the formulations, sometimes the prices getting high enough to become unaffordable by some vulnerable consumers.

Blended foods based on grains, cereals and fats have been observed to be more affordable than those based on meat, fish, fruits and vegetables (Conforti & D'Amicis, 2000; Drewnowski *et al.*, 2004). Milk and milk products occupy an intermediate position as their contribution to total diet cost is equivalent to their contribution to total energy intake (Drewnowski & Darmon, 2005). However, vegetables and fruits have more favorable nutrients to energy ratio, contributing more nutrients in relation to the energy they provide (Darmon *et al.*, 2005). Cereal-based formulas low in animal products, vegetables and fruits cannot meet the nutritional recommendations for vulnerable groups and emphasizes the necessity of vegetable consumption in accompaniment with such foods in meeting the nutritional requirements of these groups for optimal nutritional status (Brown, 1991; Fawzi *et al.*, 2000).

The general nutrient composition of the official rehabilitation foods is shown in Table 2.4, while that of selected similar market foods is shown in Table 2.5 market foods. Except for Perfect Mix (with mushrooms) the other three market foods contained comparable energy, protein and the minerals iron and zinc per 100g to the official rehabilitation foods. The vitamin A and C contents of the market foods were much higher than those of the official

foods, except for UNIMIX (CBS) official food whose contents were comparable to market foods. The levels of folate were very much higher in the official foods than the market foods. Calcium levels were much higher in the market foods than in the official foods except for UNIMIX (CBS). Perfect Mix (with mushrooms) market food had generally much lower nutrient levels than both the official and market foods, except for levels of protein, which were much higher than, and folate, which were comparable to, the levels in both official and market products.

Table 2.4 Nutrient contents of the official rehabilitation supplementary foods (100 g)

Nutritional Information	Units	Foundation Plus	First Food	Advantage	UNIMIX (CSB)
Energy	Kcal	450	435	440	400
Protein	%	15	12	16	14
Fat	%	12	11	12	6
Vitamin A	IU	720	920	1050	2300
Vitamin B_1	Mg	0.39	0.25	0.29	0.28
Vitamin B_2	Mg	15.21	0.38	0.44	0.82
Niacin (B_3)	Mg	96	2.75	3.17	5.0
Vitamin B_5	Mg	1.1	1.39	1.60	2.8
Vitamin B_6	Mg	1.29	0.15	0.17	0.17
Vitamin B_{12}	Mcg	0.54	0.52	0.52	1.3
Folate	Mcg	159	108	136	0.2mg
Vitamin C	Mg	24.5	24	28.0	60
Iron	Mg	9.6	5.18	6.50	8.0
Zinc	Mg	8.79	4.80	5.50	12
Magnesium	Mg	106	112	146	105
Calcium	Mg	150	128	155	260

Table 2.5 Nutrient contents of the supplementary foods sold in the markets

Nutritional Information	Units	Soy afric Nutri-mix	Nutri-rich (P&Allan)	FAFA-D formula	Perfect mix (mushrooms)
Energy	Kcal	400	400	350	96
Protein	%	13.5	14	13.5	25
Fat	%	6.8	6.0	2.5	1.1
Vitamin A	IU	2300	2300	2300	10
Vitamin B_1	mg	0.28	0.28	0.28	0.06
Vitamin B_2	mg	0.82	0.82	0.82	0.04
Niacin (B_3)	mg	5.0	5.0	5.0	0.2
Vitamin B_5	mg	2.8	2.8	2.0	2.0
Vitamin B_6	mg	0.16	0.16	0.165	0.3
Vitamin B_{12}	mcg	1.3	1.3	1.2	5.7
Folate	mg	0.2	0.2	60	0.22
Vitamin C	mg	60	60	60	0.6
Iron	mg	8.0	8.0	8.7	8.0
Zinc	mg	12	12	12	2.4
Magnesium	mg	106	106	105	3
Calcium	mg	260	260	250	10

Table 2.6 shows the percentage fulfillment of nutrient supplementation by the supplementary foods for infants 12 -23 months old as recommended by FAO and WHO for three official foods and three commercial foods recommended for this age group. The recommended nutrient supplementation is based on the assumption that the deficits of the RDA (RDAs for the group are shown in parentheses) will be satisfied by the other foods in the diet and supplements the children receive. In Kenya, majority of the infants in this age category will be breastfeeding and all of them will be receiving vitamin A capsule supplementation every six months (Dewey *et al.*, 2004). All the foods except Perfect Mix (with mushrooms) satisfied the recommended supplementation for protein, magnesium and iron. Niacin supplementation was satisfied except by Foundation Plus and Perfect Mix (with mushrooms).

All the foods supplied a negligible proportion of the recommended supplementation for vitamin A. It was probably assumed that this nutrient will be supplied fully by other foods and breast feeding and the capsule administration. Protein is critical at this period of growth of the infant. The three official and two of the commercial foods supplied more than 100% of the recommended protein supplementation. In so doing, First food supplied 84%, Foundation Plus 93% and Unimix 92% of the RDA (for ptotein) for this group. Then of the commercial foods, Soy afric Nutrimix and Nutri-Rich (P&Allan) supplied 81% and 89% respectively of the protein RDA. Linear programming has been successfully applied in determining limiting nutrients and nutrient adequacy of the local and supplementary (food aid) in developing countries (Briend and Darmon, 2000).

Table 2.6 Percentage fulfillment of nutrient supplementation by the supplementary foods for vulnerable children aged 12-23 months*

Nutrients	Recommended Nutrient supplementation	First food	Foundation plus	UNIMIX	Soy afric Nutrimix	Nutri-rich (P&A)	Perfect Mix
Energy (kcal)	894 (30)	48	51	45	45	45	11
Protein (g)	10.9 (24)	**185**	**205**	**202**	**178**	**195**	32
Fat (g)	29.8 (-)	50	56	46	27	33	10
Retinol equiv. (ug)	400 (500)	0	0	1	1	1	0
Vitamin B1 (mg)	0.5 (50)	71	82	76	88	77	14
Vitamin B2 (mg)	0.5 (42)	79	94	90	68	78	10
Niacin equiv. (mg)	6 (46)	**107**	0	**117**	**121**	**114**	38
Pantothenic acid	2 (25)	35	26	21	48	42	23
(mg)	0.5 (25)	46	53	48	56	49	11
Vitamin B6 (mg)	160 (40)	51	55	49	45	51	6
Folic acid eq. (µg)	0.5 (17)	12	6	0	12	12	0
Vitamin B12 (µg)	30 (67)	12	9	5	11	12	1
Vitamin C (mg)	400 (50)	43	42	35	36	42	2
Calcium (mg)	60 (20)	**263**	**350**	**331**	**299**	**278**	27
Magnesium (mg)	6 (33)	**165**	**171**	**160**	**153**	**169**	20
Iron (mg)	4.1(27)	74	87	81	86	74	10
Zinc (mg)							

*Calculations are based on daily consumption of 200g of product in formulation, except Perfect mix which is based on consumption of 95g. Figures of nutrient supplementation in the parentheses represent RDAs for the group based on FAO/WHO recommendations. Figures in bold show nutrients with more than 100% adequate fulfillment of RDAs

Among the commercial foods, product cost varied from KSh 6.4 for Soy Afric NutriMix to KSh 8.0 for Nutri-rich (P&Allan). In terms of the cost of protein from the commercial products, prices varied from KSh 0.84 for First Food to KSh 1.89 for Perfect Mix (with mushrooms). Table 2.7 shows the estimated cost of the foods per 100 g product and per 100 g protein in local currency. The cost of the official foods was the same, but the protein cost was lower at KSh 0.84 for First food than for the other two which were both at KSh 1.05. Value for money in terms of protein was therefore obtained with Foundation Plus and Unimix (CBS). Nutri-rich (P&Allan) protein cost was estimated at KSh 1.12. Although the food did not satisfy the recommended protein supplementation as shown in Table 2.6, the protein from Perfect mix (with mushrooms) was the most expensive. This raises questions about the rationale of such pricing and the recommendation for the group of the product. The cost of supplementary foods is usually considered high and unaffordable by the vulnerable groups who are generally below the average income bracket (Conforti & D'Amicis, 2000).

Table 2.7 Cost of the supplementary food per 100 g protein in Kenyan shillings (KES)*

Food Products	Cost/100g product	Cost per 100g protein
Foundation Plus	7.0	1.05
First Food	7.0	0.84
UNIMIX (CBS)	7.5	1.05
Soy Afric nutrimix	6.4	0.87
Nutri-rich (P & A)	8.0	1.12
Perfect mix (with mushrooms)	7.5	1.89

*US$ 1.00 = 76 KSh as of October 2009

Packaging and labeling of market supplementary foods

The supplementary foods shown in Table 2.1 and officially recommended for rehabilitation were supplied to the institutions packed in polypropylene bag with inner polythene liner. This package has the advantage of being strong and more durable; at the same time capable of withstanding the rigors of the frequent loading and off-loading encountered in most emergency situations. The commercial supplementary foods in Table 2.2 were packaged in Kraft paper (50%), polythene bags (33%), polybags enclosed with paper cartons (11%) and polythene composite bags (6%). Kraft paper package was reported to be inappropriate in protecting the product from losses incurred through shelf display, storage and transportation, moisture permeability and microbial spoilage. Some of the supermarkets avoided these losses by storing the products on floor boards, wooden shelves or pallets, covered with polythene paper. Some supermarkets took precautions against the losses by thoroughly cleaning the food stores and shelves daily, combined with occasional store fumigation. The stores were well air conditioned, ventilated, and were maintained cool and dry as the storage conditions indicated on the product labels.

All packages contained information on the name of the product, name of manufacturer, ingredients used, target consumer group, and occasionally shelf life, storage conditions, cooking instructions and health benefits accruing from the food. Only 39% of the commercial foods studied contained some nutritional information on the packages. Claims on the health benefits from the products were found on the labels of 29% of the foods. The

standardization mark of quality by Kenya Bureau of Standards (KEBS) was found on the labels of 63% of the products. However, it was reported that the Government of Kenya had given all food manufacturers up to end of March 2009 to comply with the safety and quality standards or have their products withdrawn from the markets. Currently, there has been growing concern that the nutritional quality and safety of locally manufactured unfortified foods in the market is inexact and insufficient. Therefore, supplementary foods indicated as containing vitamin and mineral premixes are normally preferred by the consumer because they are considered as more reliable source of the nutrients indicated on their labels.

Shelf-life and deterioration of the supplementary foods

The supplementary foods in Table 2.1 indicated shelf life of 6 months, while those in Table 2.2 that did so, indicated shelf-life of 6 – 9 months. More than 50% of the foods in Table 2 had no indication of shelf life on the packages. For the shelf life to be valid, it was recommended that the FBF's be stored under cool and dry conditions, away from direct sunlight. Most of the supermarkets were purchasing only sufficient quantities which could be cleared before expiry.

Most of the supplementary foods are powders and flours, and due to their large exposed surface area therefore, they are easily amenable to spoilage through chemical reactions such as fat oxidation in the presence of air and mineral prooxidants like iron, caking due to hygroscopicity and microbial damage when suitable conditions with regard to water activity and temperature are present. Table 2.8 summarizes the different types of losses indicated as being incurred during the marketing of supplementary foods. As Table 2.8 shows, the main losses (76.2%) were caused by improper handling by store personnel and consumer during placement on the shelves and during purchase respectively, which resulted in spillage due to package tears, especially for products packaged in Kraft paper. Other

causes of product losses during marketing included microbial spoilage (52.3%), insect infestation (especially weevils) (47.6%) and shelf life expiry (66.6%).

Table 2.8 Causes of market losses of supplementary foods

Type of loss	No. of responses	Percent loss (N = 21)
Microbial spoilage	11	52.3
Insects and weevils	10	47.6
Rodents	5	23.8
Returns	2	9.5
Display losses	16	76.2
Expiry	8	66.6
Fire losses	3	14.3
Others	4	19.0

CONCLUSION

The study concluded that there is wide diversity of supplementary foods consumed in Kenya by vulnerable groups, but also by the general family. Only a very small group of these foods are, however, controlled either in importation or in manufacturing and these are officially recommended for use in rehabilitation programs. The foods are balanced in terms of nutrients through careful selection of protein and energy ingredient sources and fortification with specific vitamins and minerals. These foods are supplied directly to the rehabilitation programs and are not available in the open market. There is then diversity of supplementary foods containing nutrition and health claims on their labels, manufactured locally and sold in the open market. Most of these foods have no nutrient information and expiry periods on their labels. The market and consumption of supplementary foods is consequently rapidly expanding in Kenya because of the increased consumer awareness about the benefits accruing from the foods.

CHAPTER 3

Nutritional Composition, Amino Acid and Fatty Acid Profiles of Indigenous Food Ingredients used in Formulation of Supplementary Foods for Vulnerable Groups in Kenya

Abstract

Currently, there is an escalating demand for food to meet food and nutrition security for growing populations. Indigenous food ingredients possess high nutritive and functional properties which are associated with positive health and nutrition. The purpose of this study was to evaluate the proximate composition, amino acid and fatty acid profiles of local cereals, legumes, nuts and indigenous vegetables. The food ingredients contained 6-44% protein; 8-38% fibre; 11-43% fat; 324-497 kcal energy; and 15-57% carbohydrates. The mineral contents of the food ingredients was in the range of 25-328 mg/100 g calcium, 1.0-51 mg/100 g iron, 44-1320 mg/100g magnesium, 0.2-19 mg/100 g sodium, 60-1105 mg/100 g phosphorus, and 1.6-15 mg/100 g zinc. The indigenous vegetables exhibited 3.2-63 mg/100 g vitamin C and 0.7-5.1 mg/100 g β-carotene contents while the grains showed 22-110 µg/100 g folic acid, 1.2-17.7 mg/100 g niacin, 0.1-1.6 mg/100 g vitamin B1 and 0.1-1.0 mg/100 g vitamin B2 contents. The total essential amino acid content ranged from 2.7 to 10.4 % in the grains and 0.9 to 12.8% for the vegetables. The levels of the fatty acids in the food ingredients were 4.8-33.6% palmitic, 1.5-9.0% stearic, 2.2-53.9% oleic, 4.5-53.7% linoleic and 0.9-60.4% α-linolenic acids. The study demonstrated that the food ingredients were high in macro- and micro-nutrients, essential amino acids and fatty acids such as omega-6 and omega-9 and could potentially be used in formulation of therapeutic supplementary foods for vulnerable groups.

Key words: Indigenous foods; Nutrient content; fatty acids; amino acids; vulnerable groups.

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Introduction

Plant foods are important sources of energy and dietary proteins and play a significant role in human nutrition particularly in the developing countries (Oboh *et al.*, 2009). These foods are rich and inexpensive sources of protein, carbohydrates, dietary fiber, minerals and vitamins to millions of peoples in developed and developing countries, and are some of the basic foods of the indigenous populations of Africa (Luthria & Pastor-Corrales, 2006). Faced with increasing food shortages, agriculturalists and food scientists are becoming increasingly interested in previously neglected tropical grains and indigenous vegetables (Islam, 2006). The challenge for agricultural practices to increase food production to obtain food security still persists after 40 years of the green revolution (Hobbs, 2007). In addition, the first Millennium Development Goal is to reduce hunger and poverty by 2015 which can be achieved through intensified nutrition action (Dixon *et al.*, 2006).

Poverty and food insecurity seriously constrain the accessibility of nutritious diets that have high protein quality, adequate micronutrient content and bioavailability, macronutrients and essential fatty acids, and high nutrient density (Omueti, 2009) . The typical diets of vulnerable populations with high prevalence of malnutrition and undernutrition consist predominantly of starch-rich staples, such as a cereal or tuber, with limited amounts of fruits, vegetables, legumes and pulses (Solomon & Owolawashe, 2007). Such diets are bulky, have low density of energy and nutrients and a low bioavailability of minerals and will therefore result in impaired growth, development and a host of chronic diseases. Investigations on economically viable indigenous food ingredients as alternative strategies to curb undernutrition and food insecurity are of utmost importance to broaden the essential nutrient sources for human nutrition (Barba de la Rosa *et al.*, 2009). Adaptation to adverse environmental conditions, resistance to pests, cultural acceptability and sufficient nutritional qualities are the key advantages of these indigenous foods. Consequently, the search for

novel high quality but cheap sources of protein and energy has continued to be a major concern in many parts of the developing world including Kenya (Arinathan *et al.*, 2009).

In developing countries such as Kenya, because of limited access to animal products (meat, eggs and fish) that provide high intakes of minerals such as heme iron and zinc, the main dietary sources of minerals are cereals and legumes. Cereal grains and legumes constitute an important part of the human diet, providing a high portion of carbohydrates, proteins, fats, dietary fibres, B-group vitamins and minerals (Lebiedzińska & Szefer, 2006). Recent studies on traditional plant foods in Kenya have shown that most of them are highly nutritious; containing high levels of both vitamins and minerals (Orech *et al.*, 2007). In addition, grains and indigenous vegetables find increasing use in dietetic formulations for treatment and prevention of diabetes, cardiovascular diseases, cancer and lowering of blood cholesterol, which indicate their possible therapeutic value for humans (Erasto *et al.*, 2007; Gorinstein *et al.*, 2007; Oboh *et al.*, 2009). It is becoming increasingly acknowledged that single nutrients alone are not the key to solving nutrition problems and preventing chronic diseases (Villegas *et al.*, 2008). Whole foods and nutritionally-wise diets such as a variety of vegetables and fruits as part of a mainly plant-based diet are desirable (Lebiedzińska & Szefer, 2006). Therefore the traditional and novel foods should be evaluated for their nutritional contents within the context of total nutritionally balanced diets that are appropriate for *ad libitum* consumption relevant to the target populations at levels recommended in the diet to obtain maximum health benefits achievable and sustainable by the vulnerable groups.

The first Millennium Development Goal is to "eradicate extreme poverty and hunger by 2015". The nutrition indicator in Kenya is "to halve the prevalence of underweight in children less than five years old from 35.5 per cent in 1990 to 16.25 per cent in 2015" (CBS/MPHS/ORC, 2010). The incidence of micronutrient malnutrition among the vulnerable groups in developing countries and the increasing prevalence of chronic degenerative

diseases globally, necessitate the need to explore underutilized indigenous foods to overcome nutritional disorders. Data on nutrient contents including amino acid and fatty acid profiles of indigenous food ingredients used in formulation of supplementary foods for vulnerable groups in Kenya is still very limited in spite of the expanded use of the foods by relief organizations. The objective of this study was to evaluate and identify the important nutritional quality of indigenous food ingredients appropriate in the development of supplementary foods for vulnerable groups.

Materials and methods

Chemicals

Luthium dodecyl sulfate was purchased from Carl Roth, 76185, Karlsruhe, Germany; DL-Norleucine (Serva, 64271, Heidelberg, Germany); hydrochloric acid (36%, Fisher Scientific, 58239 Schwerte, Germany); Formic acid, ethylene glycol, phenol, phosphoric acid, L-amino acids,oleic acid, boron trifluride in methanol and hydrindantin dehydrate (Fluka, 82024 Taufkirchen, Germany); Linoleic acid, stearic acid, and thiamine (Sigma-Aldrich, Steinheim, Germany); and all the other analytical grade solvents and chemicals were supplied by Merck Chemicals, 64271, Darmstadt, Germany.

Materials

The samples were collected on the basis of common food ingredients used by vulnerable groups in Nairobi, Kenya. Samples included finger millet (*Eleusine coracana* L. Gaertn. P-224), amaranth grain (*Amaranthus cruentus* L.), pigeonpea (*Cajanus cajan* (L.) Millsp. Kat/Mbaazi 3), field bean (*Dolichos pupureum* L. Kat/DL-3), groundnut (*Arachis hypogea* L.), pumpkin seed (*Cucurbita maxima Duchesne ex* Lam.) and sunflower seed (*Helianthus annuus* L. PAN 7369). The vegetables selected were pumpkin (*Cucurbita maxima* L.),

butternut (*Juglans cinerea* L.), sweet potatoes *(Ipomoea batatas* [L.] Lamk. SPK 004), and leafy vegetables such as drumstick leaves (*Moringa oleifera* L.), amaranth leaves (*Amaranthus hybridus* L.) and pumpkin leaves (*Cucurbita maxima* Lam.). The food ingredients (1 kg each) were purchased from the local open-air market at Kangemi and Uchumi supermarket in Nairobi, Kenya. All the samples dried in an air oven at 60 ^{0}C for 6-10 h and milled using a laboratory mill (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland) and sieved (0.1mm) to obtain fine flour for analyzes.

Methods

Chemical analysis

The moisture, protein, fat and ash content of the food ingredients was determined according to standard methods (AOAC, 2005). Carbohydrate was determined by difference. Energy was calculated from fat, carbohydrate and protein contents using Atwater's conversion factors. For determination of minerals, the sample flours were digested with concentrated nitric acid, sulfuric acid and perchloric acid (10:0.5:2, v/v) and mineral constituents (calcium, iron, magnesium, sodium, and zinc) were determined using inductively coupled argon plasma atomic emission spectroscopy (ICP-AES, Jarrel-Ash). The mineral contents were quantified against standard solutions of known concentrations which were analyzed concurrently. The vitamins, vitamin B_1, vitamin B_2, vitamin C, β-carotene, niacin, and folic acid were analyzed using microbiological assays according to AOAC methods (AOAC, 1990).

Amino acids analysis

The amino acid profiles were determined according to standard methods of the Official Journal of the European Union (2009). The free amino acids were extracted with dilute hydrochloric acid. Co-extracted nitrogenous macromolecules were precipitated with

sulfosalicylic acid and removed by filtration then the filtered solution was adjusted to pH 2.20. The total amino acids were oxidized at 0 ^{0}C with performic acid/phenol mixture. Excess oxidation reagent was decomposed with sodium disulphite. The oxidized or unoxidised sample was hydrolyzed with hydrochloric acid (pH 3.20) for 23 hrs and the hydrolysate adjusted to pH 2.20. The amino acids were separated by ion exchange chromatography and determined by reaction with ninhydrin with photometric detection at 570 nm (440 nm for proline). For the determination of tryptophan, the sample was hydrolysed under alkaline conditions with saturated barium hydroxide solution and heated to 110 ^{0}C for 20 hrs. After hydrolysis internal standard was added in the hydrolysate. The amino acids and internal standards were measured using an amino acid analyzer (Eppendorf-Biotronic LC 3000, Laborservice Onken, 63584 Gründau, Germany).

Extraction and GC-MS analysis of fatty acid methyl esters (FAME)

The quantificantion of fatty acids of the food samples was carried out by following the method of Thurnhofer et al. (2008). Sample extraction was carried in 22 ml extraction cells filled with Isolute-HM-N using an ASE 200 system (Dionex, Idstein, Germany). Methyl esters were prepared by transmethylation using 0.5 M methanolic KOH solution at 80 ^{0}C in methanol and n-hexane determination of the fatty acids. A sample of 10 mg was mixed with 0.5 ml of 0.5 M methanolic KOH for saponification and heated to 80 ^{0}C for 5 min then cooled down on ice. 1 ml BF_3 for methylation was added with a 1 ml glass volumetric pipette then heated to 80 ^{0}C for 5 min then cooled down on ice. 2 ml n-hexane and saturated NaCl solution were added to dissolve the FAMEs and for a better phase separation. After strong shaking and phase separation, the supernatant (organic phase) was taken for measurement on the GC/FID (Hewlett Packard 5890 Series II).

The fatty acid methyl esters (FAME) were analyzed by gas chromatography in combination with electron ionization mass spectrometry (GC-EI/MS), which consists of 5890 series II gas chromatograph and a 5971 mass selective detector, MS Data analysis version C.00.07 HP 1989-1992 from Hewlett Packard, Waldbronn, Germany. For GC analysis, helium gas (99.999% purity) was used as carier with a flow arte of 1 ml/min. A fused silica capillary column (100% cyanopropylpolysiloxane, 50 m x 0.25 mm i.d. x 0.20 µm film thickness, CP-Sil 88 from Chrompack, Middelburg, The Netherlans) was installed in the GC oven. Injectionof a 1 µl volume aliquot was used at atemperature of 250 ^{0}C and analysed for 38.81 min. Under selected ion monitoring (SIM) mode, nine fragment ions were detected, and seven of them were identified during the whole run at (1) *m/z* 74 and (2) *m/z* 87 for methyl esters of saturated and monosaturated fatty acids, (3) *m/z* 81 and (4) *m/z* 79 for methyl esters of polyunsaturated fatty acids, (5) *m/z* 88 and (6) *m/z* 101 for ethyl etsers of saturated and monostaurated fatty acids. The GC experiment was replicated thrice and results were expressed in GC area % as mean values ± standard deviation.

Statistical analysis

All analyses were performed in triplicate (n = 3), and the data was presented as means standard error of deviation (± SEM) and analysis of variance was determined at 5% level of significance. GraphPad PRISM® version IV software, San Diego, CA was used for statistical analysis.

Results and discussion

Proximate composition and energy content

The results of proximate analysis and energy contents of the grains and vegetables are shown in Table 3.1. The grains were observed to contain more energy content (324-497 Kcal) when compared to the vegetables, with groundnut showing the highest content. The lipid content was highest in the groundnut (43%) and sunflower seeds (31%), while the pumpkin and amaranth leaves showed highest ash contents (11% and 16% respectively). Similarly, the lipid (42.88-66.71%), protein (7.50-21.56%), ash (1.16-3.28%) and moisture contents (1.47-9.51%) of edible nuts such as almonds, brazil nut, cashew nut, hazelnut, macadamia, pecan, pine nut, pistachio, walnut and Virginia peanut growing the U.S.A. have been reported by Venkatachalam & Sathe (2006). The oil seeds and vegetables exhibited highest fibre contents (19-38% and 8-12% respectively), while all the vegetables contained the highest moisture content (69-89%). All the legumes studied were rich in protein ranging from 18% in pigeon pea to 21% in field bean. They also contained significant amounts of crude fibre which ranged from 7.0% in pigeon pea and 19% in groundnuts.

The carbohydrate content varied from 15 % to 57%. The proximate composition of nineteen domestic legumes varieties including soybeans, black soy beans, azuki beans and mung beans had a moisture content of 8-12%, ash 4-6%, fat 0.6-18% and protein content of 19-44% growing in Taiwan (Lin & Lai, 2006). Similarly the proximate composition of the mucuna beans (*Mucuna pruriens* var. *utilis*), an underutilized legume in India was reported to have a crude protein of 30%, lipid of 4.3%, total ash of 3.5%, and crude fibre of 7.4% (Siddhuraju & Becker, 2005). The composition of the pigeon pea in the current study was comparable to those of six varieties of pigeon peas (dry matter of 87-88%, protein of 19-22%, fat of 1.2-1.3%, fiber of 9.8-13.0% and ash content of 3.9-4.3%) grown in Botswana (Amarteifio *et al.*, 2002).

Table 3.1 Proximate composition of the food ingredients [a]

Food Ingredients	Energy Kcal/100 g	Protein %	Lipid %	Fibre %	Carbohydrate %	Ash %	Moisture %
Finger Millet	335.96 ±0.01	5.68 ±0.51	1.68 ±0.62	4.38 ±0.10	75.83 ±0.02	2.21 ±0.05	11.52 ±0.08
Amaranth grain	364.96 ±0.04	13.57 ±0.40	8.20 ±0.02	4.76 ±0.05	59.22 ±0.01	2.20 ±0.01	12.05 ±0.37
Pigeon pea	325.90 ±0.05	17.95 ±0.06	2.77 ±0.57	6.98 ±0.08	57.45 ±0.04	3.58 ±0.21	11.27 ±0.04
Field bean	323.74 ±0.05	20.92 ±0.34	3.10 ±0.92	7.92 ±0.07	53.04 ±0.20	3.12 ±0.14	11.88 ±0.12
Ground nuts	496.82 ±0.08	12.59 ±0.71	42.82 ±1.17	19.00 ±0.04	15.35 ±0.01	2.81 ±0.04	7.51 ±0.04
Pumpkin seeds	288.43 ±0.04	31.60 ±0.24	2.19 ±0.03	20.64 ±0.05	35.58 ±0.09	5.29 ±0.07	4.70 ±0.08
Sunflower seeds	368.07 ± 0.07	23.37 ±0.75	30.51 ±1.13	38.10 ±0.03	36.00 ±0.09	2.93 ±0.15	7.19 ±0.29
Pumpkin	322.16 ±0.07	10.51 ±0.49	1.28 ±0.53	8.08 ±0.08	67.15 ±0.14	7.36 ±0.03	89.21 ±0.03
Butternut	335.17 ±0.23	11.56 ±0.47	1.21 ±0.55	8.22 ±0.04	69.51 ±0.05	7.25 ±0.02	86.89 ±0.20
Sweet potatoes	383.17 ±0.02	2.89 ±0.02	1.33 ±0.27	2.96 ±0.06	90.11 ±0.08	2.71 ±0.01	69.59 ±0.09
Pumpkin leaves	313.59 ±0.01	34.76 ±0.26	4.75 ±0.45	12.04 ±0.02	32.97 ±0.03	11.33 ±0.06	86.25 ±0.01
Amaranth leaves	305.06 ±0.01	29.85 ±0.41	4.26 ±0.13	8.64 ±0.04	36.83 ±0.08	16.19 ±0.17	84.78 ±0.22
Fish flour	320.00 ±0.06	44.20 ±0.71	16.00 ±0.04	2.96 ±0.02	0.00 ±0.00	2.23 ±0.03	11.10 ±0.14

[a] Values are means and ± standard error of mean (n = 3).

The grains (finger millet and amaranth) had a protein range of 5.7 – 13.6%, lipid content of 1.7-8.2%, carbohydrates of 59-75.8%, and ash contents of 2.2% with amaranth grain exhibiting the higher protein, fibre and lipid content. The proximate composition of the amaranth grain in the present study had comparable protein (14.8-15.3%), lipids (7.9-8.9%), ash (3.3-3.9%) and fiber (1.9-2.5%) contents to similar amaranth varieties grown in Mexican highlands and Southern Europe (Barba de la Rosa *et al.*, 2009). The nutritional profile of the pumpkin seeds in the current study was comparable to results reported by Younis and other (2000) for pumpkin seeds (*Cucurbita pepo*) used locally in Eritrea in terms of the oil content (35%), protein (38%), carbohydrate (37%), ash (3.3%) and moisture content (6.6%). The chemical composition of the indigenous vegetable in this study was comparable to that of underutilized green leafy vegetables such as *Cucurbita maxima, Delonix elata, Amaranthus tricolor, Digera arvensis* among others grown in India in their moisture content (73-95 g/100

g), ash (0.8-3.5 g/100 g) and ether extract (0.2-0.9 g/100 g) (Gupta *et al.*, 2005). Similarly, the chemical composition of the sweet potatoes investigated in this study was comparable to that of two sweet potatoes varieties (Koganesengan and Beniazuma) in terms of moisture (69.9-70.9 g/100 g), protein (1.28-2.13 g/100 g), lipid (0.20-0.33 g/100 g), fibre (2.30-3.42 g/100 g) and ash (1.08-1.43 g/100 g) grown in Japan (Ishida *et al.*, 2000).

Micronutrient contents

The mineral composition (calcium, iron, magnesium, sodium, phosphorus and zinc in mg/100 g) and vitamin content (β-carotene, vitamin C, vitamin B_1, vitamin B_2, niacin in mg/100 g and folic acid in µg/100 g) of the indigenous grains and vegetables are presented in Table 3.2 and 3.3 respectively. A large quantity of elements is required in trace amounts for numerous functions in the body and their essentiality has been well established (Gupta *et al.*, 2005). The mineral content of the foods ranged from 25-328 mg/100 g for Ca, 1.0-51 mg/100 g Fe, 44-1320 mg/100g Mg, 0.2-19 mg/100 g Na, 60-1105 mg/100 g P, and 1.6-15 mg/100 g Zn.

Table 3.2 Mineral composition of indigenous grains and vegetables [a]

Food Ingredients	Calcium (Ca) mg/100 g	Iron (Fe) mg/100 g	Magnesium (Mg) mg/100 g	Sodium (Na) mg/100 g	Phosphorus (P) mg/100 g	Zinc (Zn) mg/100 g
Finger Millet	319.00 ±0.01	2.70 ±0.01	124.00 ±0.08	0.98 ±0.04	245.00 ±0.01	1.95 ±0.05
Amaranth grain	105.00 ±0.06	8.70 ±1.90	253.00 ±0.24	1.15 ±0.01	522.00 ±0.50	2.85 ±4.05
Pigeon pea	80.50 ±1.22	5.60 ±1.41	108.00 ±0.02	0.33 ±0.00	334.00 ±0.00	2.70 ±0.00
Field bean	42.15 ±0.01	6.50 ±2.12	135.00 ±0.03	0.22 ±0.01	355.00 ±0.04	1.95 ±0.71
Ground nuts	25.40 ±1.22	3.80 ±7.07	140.00 ±0.07	0.20 ±0.70	268.00 ±0.10	2.30 ±0.41
Pumpkin seeds	25.00 ±1.41	7.40 ±1.40	405.00 ±0.07	0.85 ±0.02	845.00 ±0.03	8.40 ±0.01
Sunflower seeds	188.00 ±0.18	38.10 ±4.01	333.00 ±0.39	18.90 ±0.00	657.00 ±0.40	6.40 ±0.00
Fish flour	129.00 ±0.57	28.25 ±6.05	109.00 ± 0.01	10.50 ±0.00	1105.00 ±0.21	9.56 ±0.02
Pumpkin	113.00 ±0.04	6.67 ±0.05	157.00 ±0.02	4.05 ±0.70	292.00 ±0.05	4.00 ±0.00
Butternut	123.00 ±0.09	5.05 ± 0.70	141.00 ±0.11	3.40 ±0.02	139.00 ±0.12	1.55 ±0.07
Sweet potatoes	328.00 ±3.25	1.00 ±0.00	44.20 ± 2.08	3.05 ±0.00	59.50 ±5.44	4.05 ±0.00
Pumpkin leaves	167.00 ±0.28	50.80 ±2.80	941.00 ±0.23	8.85 ±0.02	525.00 ±0.13	5.10 ±0.01
Amaranth leaves	264.50 ±0.35	45.85 ±1.06	1320.00 ±0.14	17.24 ±0.14	481.00 ±0.06	15.10 ±0.14

[a] Values are mean ± standard error of means of three separate determinations ($n = 3$).

Among the vegetables, amaranth leaves were high in Ca (264 mg/100 g), Fe (46 m/100 g), Mg (1320 mg/100 g), Na (17 mg/100 g) and Zn (15 mg/100 g), while the pumpkin leaves exhibited high contents of Fe (51 mg/100 g), Mg (941 mg/100 g) and P (845 mg/100 g). Similarly, high values in Ca (41-2597 mg/100 g), Zn (1.78-9.95 mg/100 g), Fe (4.30-47.3 mg/100 g), P (16-63 mg/10 g), Na (4.7 -241 mg/100 g) and Mg (35-253 mg/100 g) were reported in commonly consumed vegetables such as amaranth leaves, sweet gourd and pumpkin leaves grown in India and Bangladeshi (Gupta *et al.*, 2005; Hels *et al.*, 2004). The Ca levels of finger millet and sweet potatoes were also high (319 and 325 mg/100 g, respectively). This value was higher than the one reported (68-73.3 mg/100 g) for two sweet potatoes varieties (Koganesengan and Beniazuma) grown in Japan and could be attributed to varietal and geographical variations (Ishida *et al.*, 2000). The mineral content of the finger millet of the current study was slightly higher in most elements as compared to reports of mineral contents (P, 288 mg/100 g; K, 280 mg/100 g; Mg, 149 mg/100 g; Ca, 51 mg/100 g; Na, 6 mg/100 g; Zn, 6.5 mg/100 g; and Fe, 20 mg/100 g) in pearl millet (*Pennisetum glaucum* L) grown in the UAE (Ragaee *et al.*, 2006). This could be attributed to varietal and climatic differences. Previous studies have also shown that finger millet (*Eleusine coracana*) has the highest calcium content (344 mg/100 g) known in foods (Hedge & Chandra, 2005).

Sunflower seeds exhibited high contents of Na (19 mg/100 g) while the pumpkin seeds exhibited high P levels (845 mg/100 g). The mineral composition of the pumpkin seeds investigated in the present study was comparable to the values reported for pumpkin *Cucurbita pepo* (Ca, 130 mg/100 g; Fe, 10.9 mg/100 g; Mg, 483 mg/100 g; Na, 38 mg/100 g; P, 1090 mg/100 g; Zn, 8.2 mg/100 g) grown in Shibin El-Kom, Egypt (El-Adawy & Taha, 2001). The mineral content of pigeon peas of the present study was comparable to the mineral contents (P 163-293, Ca 120-167, Mg 113-127, Na 11.3-12.0, Zn 7.2-8.2, Fe 2.5-4.7 mg/100 g) reported for pigeon peas grown in Botswana (Amarteifio *et al.*, 2002). However, the

calcium and phosphorus content of the legumes in the current study were higher than the range reported for similar legumes such as bambara groundnut, pigeon pea and lima bean (0.15-0.52%) growing in Nigeria (Fasoyiro *et al.*, 2006). The small dried fish showed high contents of Zn (9.6 mg/100 g) and P (1105 mg/100 g). The results of the study show that these food ingredients can be used to prevent adverse effects of micronutrient deficiencies among the vulnerable groups through regular consumption.

Table 3.3 Vitamin composition of indigenous grains and vegetables [a]

Food Ingredients	β-carotene	Vitamin C	Vitamin B_1	Vitamin B_2	Niacin	Folic acid
	mg/100 g	mg/100 g	mg/100 g	mg/100 g	mg/100 g	µg/100 g
Finger Millet	4.17 ±0.04	1.00 ±0.00	0.31 ±0.30	0.08 ±0.02	4.29 ±0.03	n.d
Amaranth grain	0.07 ±0.01	4.50 ±0.01	0.07 ±0.00	0.30 ±0.10	1.20 ±0.01	102.00 ±0.01
Pigeon pea	0.05 ±0.03	4.80 ±0.00	0.72 ±0.08	0.14 ±0.33	2.90 ±0.10	100.00 ±0.00
Field bean	0.55 ±0.01	9.05 ±0.20	0.37 ±0.16	0.12 ±0.00	2.30 ±0.04	22.00 ±0.10
Ground nuts	0.03 ±0.05	1.00 ±0.07	1.60 ±0.40	0.14 ±0.01	17.7 ±0.08	110.0 ±0.32
Pumpkin seeds	0.04 ±0.14	1.00 ±0.03	0.37 ±0.01	0.83 ±0.01	3.12 ±0.14	n.d
Sunflower seeds	0.00 ±0.00	1.00 ±0.01	1.05 ±0.20	0.27 ±0.04	3.59 ±0.11	n.d
Pumpkin	1.27 ±0.07	3.70 ±0.00	0.06 ±0.00	0.04 ±0.00	0.50 ±0.31	8.00 ±0.03
Butternut	5.08 ±0.01	3.80 ±0.04	0.05 ±0.00	0.02 ±0.20	0.50 ±0.00	n.d
Sweet potatoes	0.69 ±0.02	3.16 ±0.06	0.10 ±0.03	0.06 ±0.03	0.60 ±0.05	52.00 ±0.00
Pumpkin leaves	2.66 ±0.04	14.03 ±0.10	0.08 ±0.12	0.06 ±0.01	0.32 ±0.01	n.d
Amaranth leaves	4.29 ±0.20	62.93 ±0.03	0.42 ±0.09	0.44 ±0.03	0.70 ±0.00	85.00 ±0.02
Fish flour	3.26 ±0.01	n.d[b]	0.10 ±0.02	0.20 ±0.00	6.00 ±0.23	n.d

[a] Values are mean ± standard deviations of three separate determinations (n = 3)

[b] n.d = not detected

The vitamin content of the grains was generally higher in vitamin B_1 and B_2, niacin and folic acid when compared to the levels in vegetables, while the later contained higher β-carotene and vitamin C contents than the former. Among the grains the β-carotene content ranged from 0.0 mg/100 g in sunflower seeds to 4.1 mg/100 g in finger millet, vitamin C ranged from 1.0 mg/100 g in all the oil seeds to 9.1 mg/100 g in field bean, vitamin B_1 ranged from 0.1mg/100 g in amaranth grain to 1.6mg/100 g in groundnuts, vitamin B_2 ranged between 0.1 mg/100 g in finger millet to 1.0 mg/100 g in pumpkin seeds, niacin was ranged between 1.2 mg/100 g in amaranth grains to 17.7 mg/100 g in groundnuts, and folic acid ranged from 22.0 µg/100 g in field bean to 110.0 µg/100 g in groundnuts. Similarly, grain cereals and legumes

grown in Poland were found to be rich in thiamine (sunflower seeds, 0.55-1.05 mg/100 g; soybean, 0.91 mg/100 g; millet, 0.307 mg/100 g) and ribiflavin (sunflower seeds, 0.27 mg/100 g and pumpkin seed, 0.833 mg/100 g), pyridoxine (millet, 0.46 mg/100 g; sunflower seeds, 0.69 mg/100 g; pumpkin seed, 0.17 mg/100 g and amaranth seed, 0.56 mg/100 g) and niacin (millet, 4.29 mg/100 g; sunflower seeds, 3.59 mg/100 g; pumpkin seeds, 3.12 mg/100 g and amaranth seeds, 1.02 mg/100 g) contents (Lebiedzińska & Szefer, 2006). In addition, the ascorbic acid of 9.14 mg/100 g and 4.40 mg/100 g, and thiamine content of 0.72 mg/100 g and 0.82 mg/100 g in black beans (*P. vulgaris*) and pigeon pea (*C.Cajamus* L.) respectively, grown in Venezuela has been reported (Sangronis and Machado, 2007).

Among the vegetables, the β-carotene content ranged from 0.7 mg/100 g in sweet potatoes to 5.1 nmg/100 g in butternut, vitamin C ranged from 3.2 mg/100 g in sweet potatoes to 62.9 mg/100 g in amaranth leaves, vitamin B_1 ranged from 0.05 mg/100 g in butternut to 0.42 mg/100 g in amaranth leaves, vitamin B_2 ranged between 0.02 mg/100 g in butternut to 0.44 mg/100 g in amaranth leaves, niacin ranged between 0.3 mg/100 g in pumpkin leaves to 0.7 mg/100 g in amaranth leaves, and folic acid ranged from 8.0 µg/100 g in pumpkin to 85.0 µg/100 g in amaranth leaves. Similarly, the ascorbic acid, thiamine and β-carotene contents of the leafy vegetables in the present was within the range of values (3.0-85 mg/100 g, 0.04-0.33 mg/100 g and 1.5-10.5 mg/100 g, respectively) reported for the same vitamins in some underutilized vegetables (*Cucurbita maxima, Polygala erioptera, Amaranthus tricolor, Digera arvensis*) grown in India (Gupta *et al.*, 2005). The vitamin composition of the sweet potatoes investigated in this study was comparable to that of two Japanese sweet potatoes varieties in vitamin B_1 (0.05-0.13 mg/100 g), B_2 (0.04-0.06 mg/100 g), B_6 (0.04-0.11 mg/100 g) and niacin (0.63-0.91 mg/100 g) contents (Ishida *et al.*, 2000). The results of this study show that there are large differences in the vitamin composition between and within varieties of the grains and vegetables analyzed.

Amino acid profiles

The amino acid composition of the grains and vegetables, and FAO/WHO reference pattern EAA (FAO/WHO, 1991) are given in Table 3.4 and 3.5. The amino acid composition of the grains and fish was higher than those of the vegetables investigated in this study. Among the grains in this study, pumpkin seeds and groundnuts had the highest total essential amino acids (10.39 and 8.30% respectively) which included 0.93 % and 0.56 % of total sulfur containing amino acids respectively. Similarly, the essential amino acid content of the groundnuts in the present study was coma of edible nuts such as almonds, Brazil nut, cashew nut, hazelnut, macadamia, pecan, pine nut, pistachio, walnut and Virginia peanut growing the U.S.A. and were also shown to be limiting in threonine and the sulfur containing amino acids (Venkatachalam & Sathe, 2006). A previous study has also showed that amaranth has high contents of lysine, arginine, tryptophan and sulphur containing amino acids (Gorinstein *et al.*, 2007). The vegetables of the current study exhibited excellent amino acid composition with the highest essential amino acids being observed in pumpkin leaves (12.82 %) and amaranth leaves (11.55%) with the sulfur containing amino acids accounting for 0.83 % and 0.86 % respectively. The amino acid composition of the sweet potatoes investigated in this study was similar to the profile reported for two sweet potatoes varieties (Koganesengan and Beniazuma) in Japan (Ishida *et al.*, 2000).

Table 3.4 Amino acid compositions of the grains compared to FAO/WHO reference pattern[a]

Amino Acid (%)	Finger millet	Amaranth grain	Pigeon peas	Field bean	Groundnuts	Pumpkin seeds	Sunflower Seeds	FAO/WHO[b] (1991)
Isoleucine	0.23	0.46	0.64	0.88	0.79	0.92	0.91	2.8
Leucine	0.55	0.74	1.38	0.83	1.58	1.85	1.47	6.6
Lysine	0.21	0.76	0.25	1.41	0.92	1.21	0.69	5.8
Cystine	0.14	0.30	0.24	0.25	0.31	0.36	0.34	
Methionine	0.21	0.28	0.23	0.16	0.25	0.57	0.43	
Total sulfur amino acids	0.35	0.58	0.47	0.41	0.56	0.93	0.77	2.5[c]
Tyrosine	0.19	0.43	0.47	0.71	0.86	1.41	0.50	1.1
Phenylalanine	0.32	0.55	1.69	1.17	1.24	1.31	1.09	
Total aromatic amino acids	0.51	0.98	2.16	1.88	2.10	2.72	1.59	6.3[d]
Threonine	0.26	0.50	0.72	0.87	0.69	0.77	0.79	3.4
Tryptophan	0.09	0.20	0.15	0.21	0.25	0.41	0.31	
Valine	0.45	0.72	1.09	1.29	1.41	1.58	1.30	3.5
Total essential amino acids	2.65	4.94	6.86	7.78	8.30	10.39	7.83	
Histidine	0.15	0.33	0.66	0.65	0.57	0.72	0.52	1.9
Arginine	0.27	1.16	1.11	1.42	2.78	3.68	2.02	
Aspartic acid	0.39	1.07	1.84	2.54	2.71	2.89	2.12	
Glutamic acid	1.00	1.82	3.14	3.01	3.96	4.49	4.07	
Serine	0.33	0.82	0.99	1.31	1.23	1.49	1.04	
Proline	0.36	0.53	0.85	1.00	1.04	0.96	1.03	
Glycine	0.24	1.01	0.69	0.88	1.47	2.28	1.27	
Alanine	0.39	0.54	0.91	1.02	1.07	1.35	1.15	
Total non-essential amino acid	3.13	7.28	10.19	11.83	14.83	17.86	13.22	

[a] Average of three determinations
[b] Data from FAO/WHO (1991) reference pattern of essential amino acid requirement
[c] Cysteine + methionine
[d] Tyrosine + phenylalanine

The small whole dried fish investigated in this study had essential amino acid content of 13.26% with sulfur containing amino acids of 1.27% which was comparable to the amino acid profile of some of the grains and vegetable ingredients investigated in the current study. This is an indication of the possible role of grains and indigenous vegetables as sources of essential amino acids in the diets of the vulnerable groups. It is apparent that the indigenous vegetable protein can be used to complement the protein from cereals and legumes that are low in essential amino acids. However, the amino acid profiles of all the food ingredients studied were lower than the FAO/WHO (1991) reference pattern, but these food ingredients

can be used in combination to achieve the levels of the amino acids required in supplementary and therapeutic diets for vulnerable groups.

Table 3.5 Amino acid compositions of the indigenous vegetables and small dried fish[a]

Amino Acid	Pumpkin	Butternut	Sweet potato	Drumstick leaves	Pumpkin leaves	Amaranth leaves	Fish	FAO/WHO (1991)[b]
Isoleucine	0.45	0.18	0.09	0.68	1.37	1.17	1.24	2.8
Leucine	0.73	0.32	0.15	1.28	2.63	2.29	2.62	6.6
Lysine	0.44	0.19	0.10	0.92	1.68	1.64	2.26	5.8
Cystine	0.10	0.06	0.04	0.29	0.27	0.33	0.41	
Methionine	0.18	0.07	0.04	0.26	0.56	0.53	0.86	
Total sulfur amino acids	0.28	0.13	0.08	0.55	0.83	0.86	1.27	2.5[c]
Tyrosine	0.36	0.18	0.07	0.53	1.05	0.92	0.98	1.1
Phenylalanine	0.43	0.24	0.12	0.81	1.54	1.37	1.30	
Total aromatic amino acids	0.79	0.42	0.19	1.34	2.59	2.29	2.28	6.3[d]
Threonine	0.40	0.16	0.13	0.78	1.21	1.21	1.36	3.4
Tryptophan	0.15	0.08	0.03	0.40	0.58	0.52	0.37	
Valine	0.78	0.28	0.16	1.04	1.93	1.57	1.86	3.5
Total essential amino acids	4.02	1.76	0.93	6.99	12.82	11.55	13.26	
Histidine	0.20	0.13	0.04	0.40	0.61	0.54	0.91	1.9
Arginine	0.59	0.87	0.09	1.21	1.75	1.56	1.77	
Aspartic acid	1.48	1.37	0.28	3.14	2.84	2.51	2.80	
Glutamic acid	3.09	0.69	0.46	1.65	3.20	2.62	4.05	
Serine	0.51	0.23	0.12	0.97	1.34	1.28	1.40	
Proline	0.49	0.21	0.11	0.74	1.30	1.18	1.69	
Glycine	0.47	0.25	0.11	0.80	1.57	1.39	1.91	
Alanine	1.38	0.35	0.16	1.00	2.35	1.83	2.15	
Total non-essential amino acid	8.21	4.10	1.37	9.91	14.96	12.91	16.68	

[a] Average of three determinations
[b] Data from FAO/WHO (1991) reference pattern of essential amino acid requirement
[c] Cysteine + methionine
[d] Tyrosine + phenylalanine

Fatty acid profiles

The fatty acid composition in the grains and indigenous vegetables are listed in Table 3.6. The GC-MS analysis of the lipid extracts from the food ingredients revealed 5 major fatty acids. Palmitic acid, linoleic and α-linolenic acids were the most abundant lipids in most of the food ingredients. The essential fatty acids (EFA), α-linolenic and palmitic acids, were found in high amount (21-60%) in the leafy vegetables. This is an indication of the

significance of indigenous leafy vegetables as good supplements in human diets. α-linolenic acid (an omega-3 fatty acid), is a precursor in the biosyntheisis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DHA is one of the predominant fatty acids in the human brain and has been found to play an important role in brain development in infants (Erastos *et al.*, 2007). Linoleic acid (an omega-6 fatty acid) is the metabolic precursor of eicosanoids which are a group of biologically important lipids such as prostaglandins, thromboxanes, lipoxins and leukotrienes (Erastos *et al.*, 2007). This group of lipids plays a crucial role in immunity, inflammation and blood clotting.

The fatty acid profile of the legumes (field bean and pigeon pea) of the current study were comparable to legumes (soybean and lupin) grown in Turkey in their contents of palmitic acid (19.6-26.2 and 5.3-12.0 respectively), stearic acid (4-5.3 and 3.3-4.8 respectively), oleic acid (9.2-11.4 and 21.5-34.9 respectively), linoleic acid (52-54 and 37-54.7 respectively), and α-linolenic acid (4.7-10.3 and 4.2-9.2 respectively) (Uzun *et al.*, 2007). In addition, the fatty acid content of the oil seeds was comparable to that of other oil seeds such as peanuts and sesame in their palmitic acid (5.3-10.4), stearic acid (3.7-4.4), oleic acid (36.8-5k2.8), linoleic acid (27.1-45.3) contents, while no α-linolenic acid was detected in the peanuts as also reported in our study (Uzun *et al.*, 2007). The fatty acid profile of the pumpkin seeds of the current study is within the range of reports of other species of *Cucurbita* grown in the USA and Eriterea containing an average of 11.7-21.3% palmitic, 4.0-10.4% stearic, 13.5-37.2% oleic, and 40.3-59.0% linoleic acid (Applequist *et al.*, 2006; Younis *et al.*, 2000). All the food ingredients had high amounts of unsaturated fatty acids which consisted mainly of linoleic and oleic acids. The presence of high amounts of the essential linoleic acid suggests that these food ingredients are highly nutritious and have ability to reduce serum cholesterol.

Table 3.6 Composition of fatty acids in the indigenous food ingredients

Food ingredients	Fatty acid content (%)[a]				
	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	α-linolenic acid (18:3)
Finger millet	20.8 ±0.01	4.8 ±0.35	38.4 ±0.09	20.3 ±0.00	3.9 ±0.00
Amaranth grain	19.4 ±0.08	3.4 ±0.01	34.3 ±0.37	36.3 ±0.03	0.9 ±0.01
Pigeon pea	26.2 ±0.00	4.0 ±0.01	9.2 ±0.01	53.7 ±0.19	4.7 ±0.44
Field bean	19.6 ±0.01	5.3 ±0.02	11.4 ±0.02	51.8 ±0.24	10.3 ±0.18
Groundnut	10.4 ±0.04	3.9 ±0.02	50.5 ±0.37	28.3 ±0.02	n.d[b]
Pumpkin seed	17.7 ±0.18	9.0 ±0.40	31.4 ±0.01	41.4 ±0.33	n.d[b]
Sunflower seed	4.8 ±0.77	5.3 ±0.06	53.9 ±0.04	34.8 ±0.00	n.d[b]
Sweet potatoes	33.6 ±0.00	6.7 ±0.02	8.9 ±0.06	41.1 ±0.01	7.1 ±0.00
Pumpkin	24.4 ±0.15	3.5 ±0.02	35.7 ±0.08	21.9 ±0.06	13.9 ±0.26
Butternut	19.5 ±0.02	8.6 ±0.06	29.5 ±0.01	33.5 ±0.06	8.1 ±0.02
Drumstick leaves	20.9 ±0.00	2.1 ±0.35	2.2 ±0.01	6.8 ±0.00	60.4 ±0.04
Pumpkin leaves	23.0 ±0.01	3.3 ±0.08	3.5 ±0.00	4.5 ±0.02	55.2 ±0.04
Amaranth leaves	18.6 ±0.77	1.5 ±0.54	3.0 ±0.10	13.7 ±0.54	53.3 ±0.49
Small dried fish	22.5 ±1.54	6.7 ±0.03	20.2 ±0.13	13.9 ±0.11	2.4 ±0.24

[a] Values are mean and ± standard deviation of three separate determinations (n = 3).
[b] n.d. Not detected by GC analysis

The fatty acid profile of the small dried fish (palmitic, 22.5; stearic, 6.7; oleic, 20.2; linoleic, 13.9 and linolenic acid, 2.4) was in agreement with reports of a previous study on fatty acid profiles (palmitic, 15.5-20.5; stearic, 3.32-8.18; oleic, 6.11-20.8; linoleic, 0.93-4.03 and linolenic acid, 0.02-4.55) of eight fish species from the seas of Turkey (Özogul & Özogul, 2007). Fish are the main contributors of n3 PUFA for human diet. In addition, fish also contains other essential acids in their long-chained forms such as arachidonic acid (C20:5n-3), eicosapentaenoic acid (C20:5n-3), and docosahexaenoic acid (C22:6n-3) (Michaelsen *et al.*, 2009). Essential fatty acids are important for brain and neural tissue development. The evidence for abnormal development of children on a low intake of essential fatty acids in the Western world is becoming clear with the establishment of more sophisticated methods of

analysis (Michaelsen *et al.*, 2009). There are two types of essential fatty acids, the n-6 and the n-3 polyunsaturated fatty acids (PUFAS), which in most diets are provided by vegetable oils in the form of linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3), respectively.

Conclusion

The nutrient composition of the selected cereals, legumes, oil seeds and indigenous vegetables indicated that they are good sources of many nutrients. Therefore, they could make substantial contributions to intakes of carbohydrates, protein, fat and fibre as well as vitamins and minerals. The fatty acid and amino acid profiles were also comparable to the patterns recommended by FAO. Consequently, these food ingredients could help in overcoming malnutrition and hunger among the vulnerable groups in Kenya. These food ingredients can be successfully combined to formulate therapeutic supplementary foods with adequate nutrition for use by vulnerable groups in Kenya.

CHAPTER 4

Total Phenolic Content, Antioxidant and Antidiabetic Properties of Methanolic Extract of Raw and Traditionally Processed Kenyan Indigenous Food Ingredients

Abstract

Bioactive compounds present in foods are reported to exhibit potential health benefits and preventive effects against certain chronic diseases. Certain indigenous plant foods recommended as supplemental foods for vulnerable groups in Kenya are not yet evaluated for phenolic content and functional properties. The present study was therefore designed to analyze the phenolic content, antioxidant and antidiabetic properties of the methanolic extract of raw and traditionally processed food ingredients with a view to identifying the elite food ingredient(s) with potential therapeutic properties. Total phenolic content of the cereals, legumes, oil seeds and vegetables ranged from 0.41 to 3.00 g/100 g DM with amaranth grains (*Amaranthus cruentus*) and drumstick leaves (*Moringa oleifera*) exhibiting the highest contents. The methanolic extract of the investigated samples showed promising levels of DPPH radical scavenging activity (81-89%); ferric reducing/antioxidant power (FRAP, 44-744 mmol Fe[II]/g); α-amylase (10-45%) and α-glucosidase (13-80%) inhibition activities. The food ingredients with high phenolic content exhibited relatively higher antioxidant and antidiabetic activities. Among the traditional processing methods attempted in this study, roasting and blanching caused significant losses of antioxidant and antidiabetic activities of most grains and vegetables, respectively. On the other hand, soaking + cooking for grains and cooking for vegetables were found to be more suitable mild treatments for preserving phenolic compound and the functional properties.

Keywords: Indigenous food ingredient, total phenolic, methanolic extract, antioxidant activity, antidiabetic activity, vulnerable groups.

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Introduction

In recent years, there has been increasing interest in consumption of plant-based foods including traditional staple foods which are widely perceived to possess health promoting benefits. These foods have a strong epidemiological link to reduce the risk of cardiovascular diseases, neuro-degenerative diseases, and certain types of cancer (Anwar *et al.*, 2007). Currently, there is also a resurgence of consumption of indigenous vegetables to curb micronutrient deficiencies in Kenya. The consumption of such vegetables improves the nutrient quality of cereal-based diets through provision of affordable vitamins and minerals, especially provitamin A, vitamin C, and iron (FAO/WHO, 2004). Therefore, indigenous cereals (finger millet and amaranth grains) and legumes (pigeon peas and field beans), oil seeds (groundnuts, sunflower and pumpkin seeds) and vegetables (Pumpkin, butternut, sweet potatoes) and leafy vegetables (drumstick, pumpkin and amaranth leaves) are playing a major role in attaining nutritional security of the low-income and vulnerable groups in Kenya (Neumann *et al.*, 2003).

Plant foods are rich in macro- and micro-nutrients as well as bioactive compounds, and have been recognized as major source of dietary antioxidants with potential therapeutic benefits (Prior & Cao, 2000). The non-nutritive health-promoting bioactive components present in foodstuffs have the potential to exert beneficial effects against certain chronic diseases such as diabetes, obesity, cardiovascular diseases and cancer (Art & Hollman, 2005). At present, the natural bioactive compounds such as phenolics, flavonoids, tannins and phytic acid have been reported to exhibit many health benefits including excellent antioxidant property (Halvorsen *et al.*, 2002). Among the various bioactive substances, phenolic compounds which are plants secondary metabolites and have been proven to exhibit many health protective effects, have received most attention (Vita, 2005). Phenolic compounds present in food ingredients such as cereals, legumes and vegetables were demonstrated to

exhibit potential antioxidant (Prior & Cao, 2000), antimicrobial (Tapiero *et al.*, 2002), anti-cancer (Fresco *et al.*, 2006), anti-obesity (Hsu *et al.*, 2006), anti-diabetic and anti-hypertensive (Randhir & Shetty, 2007) as well as anti-mutagenic properties (Islam, 2006). Epidemiological studies have also suggested a positive role played by phenolics in the alleviation of oxidative stress and prevention of free-radical mediated diseases (Halvorsen *et al.*, 2002).

For the last two decades, supplementary feeding programmes have been conducted by Non-Governmental Organizations (NGO's) like United Nations International Children's Emergency Fund (UNICEF) and United States Agency for International Development (USAID) to nourish the vulnerable groups in Kenya (Marchione, 2002). To formulate the supplementary foods, different food ingredients have been used on the basis of their nutritional profiles. However, due to the increasing trend of many oxidative-stress related diseases, particularly type-2 diabetes, it has been considered essential to include the bioactive compounds such as phenolics in the formulation of the supplementary foods. Hence, the quest for natural food products having substantial antioxidant and antidiabetic characteristics has been the main focus of recent research efforts (Matsui *et al.*, 2004; Islam, 2006). The outcome of such research on indigenous foods as in the present study could be vital to nutritionists, food manufacturers as well as consumers in formulating supplementary foods for vulnerable groups.

In Kenya, cereals and legumes are usually soaked + cooked or roasted, whereas the vegetables are cooked or blanched before use. During such thermal processing operations, the phenolic content and its functional properties may be altered among different food ingredients and even within the same food and can result in either decrease (Randhir *et al.*, 2008) or increase of antioxidant activity of plant foods (Granito *et al.*, 2008; Randhir *et al.*, 2008). Hence, appropriate processing method should be established for each food ingredient

in order to increase the functional properties of phenolic compounds in Kenyan indigenous food ingredients.

Even though many studies have reported the nutritional value of various food ingredients consumed by vulnerable groups in Kenya (Neumann *et al.*, 2003), only limited information is available regarding the phenolic content and its functional properties in indigenous foodstuffs of Kenya. Hence, in the present study, the methanolic extract of raw and traditionally processed food ingredients was subjected to analysis of total phenolic content, antioxidant and antidiabetic properties with a view to identify the elite food ingredient(s) with potential health benefits and also to select a suitable processing technology in order to utilize them in the formulation of supplementary foods for vulnerable groups living in Kenya.

Materials and methods

Chemicals

The chemicals, (+) catechin (Ref. No. 22-402-2), 2,2′-Diphenyl-1-picryl hydrazyl (DPPH, Ref. No.: 217-591-8,); 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ, Ref. No. 3682-35-7), 4-Nitrophenyl-α-D-glucopyranoside (PGPP, Ref. No. 3767-28-0), Butylated Hydroxytoluene (Ref. No. 204-881-4), poly-vinyl-polypyrolidone (PVPP, CAS 25249-54-1, P6 155-259, Batch no. 019K1145), starch (Ref. No. 232-679-6), α-amylase (Ref. No. 9001-19-8), and α-glucosidase (Ref. No. 9001-42-7) were obtained from Sigma chemical, USA, and all other chemicals were purchased from Merck, Darmstadt, Germany.

Sample collection

The samples were collected on the basis of common food ingredients used by vulnerable groups in Nairobi, Kenya. Samples included cereals such as finger millet (*Eleusine coracana*

L. Gaertn. P-224) and amaranth grain (*Amaranthus cruentus* L.); legumes such as pigeonpea (*Cajanus cajan* (L.) Millsp. Kat/Mbaazi 3) and field bean (*Dolichos pupureum* L. Kat/DL-3); oil seeds such as groundnut (*Arachis hypogea* L.), pumpkin seed (*Cucurbita maxima* Duchesne ex Lam.) and sunflower seed (*Helianthus annuus* L. PAN 7369). The vegetables selected were pumpkin (*Cucurbita maxima* L.), butternut (*Juglans cinerea* L.), sweet potato (*Ipomoea batatas* [L.] Lamk. SPK 004), and leafy vegetables such as drumstick leaves (*Moringa oleifera* L.), amaranth leaves (*Amaranthus hybridus* L.) and pumpkin leaves (*Cucurbita maxima* Lam.). The food ingredients (1 kg each) were purchased from the local open-air market at Kangemi and Uchumi supermarket in Nairobi, Kenya.

Processing of cereals, legumes and oil seeds

The grains (cereals, legumes, and oil seeds) were each randomly divided into three batches of 100 g each. The first batch constituted the control sample and was stored as such without any treatment. The second batch was washed with tap water and then soaked in 200 ml distilled water for 8 h while in the dark at 25 $\pm 1^0$C, then cooked at 90-95^{0}C for 120 min in fresh distilled water in the ratio of 1:4. The third batch was roasted in an open-pan iron container to a golden brown color for 30 min using traditional charcoal burner at 150^{0}C with continuous stirring to avoid burning of the seed coat. After cooking and roasting, the samples were cooled to room temperature.

Processing of vegetables

The fresh vegetables were randomly divided into three batches of 100 g each and the first batch was used as control. The second batch was cut into small pieces or cubes and washed under running tap water, then cooked in 200 ml of distilled water at 90-95^{0}C for 5 min for all samples, except sweet potatoes, pumpkin and butternut which are cooked for 15 min. The

vegetables in the third batch were cut into pieces (4 mm cubes or strips) then blanched by immersing in boiling water for 5 min

Preparation of methanolic extract

All the raw, cooked and blanched samples were dried in hot-air oven at 50^0C for 6 h and then milled using a laboratory hammer mill (Type DFH48, No. 282521/UPM 6000, Switzerland) and sieved through 100 micron sieve before analyses. One gram of defatted flour was extracted with 10 ml of 100%, 80% & 50% methanol acidified with 1% conc. HCl in an ultra-sonic bath (Bandelin Sonorex, RK – 514 H, Berlin, Germany) for 30 min. After centrifugation at 13,000 rpm for 5 min, all the supernatants were pooled and made up to a known volume (50 ml). The extract was purified by treating with 1 g of PVPP at 0°C for 30 min and then the contents were purified by using a Solid Phase Catridge (SPC) (Strata-x-33 um polymeric sorbent, 8B-S100-FCH-S, from Phenomenex, USA).

The phenolics were eluted with 10 ml of 50% and 100% methanol. The solvents were evaporated by using rotary vacuum evaporator (Büchi Rotavapor, CH-9230, Switzerland) at 40°C and dried in lyophilier (Virtis Freezemobile 25 EL, New York) for 1 h and finally the residue was weighed and the total dry yield of extract was calculated. Then the extract was re-dissolved in water: methanol: formic acid (47.5:47.5:5%, v/v/v) solution the ratio of one milligram of extract per millilitre of solvent and used for the analysis of antioxidant and antidiabetic properties.

Analysis of total phenolics

Total free phenolics were estimated by using Folin-Ciocalteu reagent (Singleton et al., 1999). The absorbance was measured at 765nm with UV-Vis Spectrometer (Perkin-Elmer, Lambda 35, USA). Based on the standard curve prepared with (+)-catechin hydrate (20-100 µg), the

amount of total phenolics in the extract was calculated and expressed in gram per 100 gram sample on dry matter basis.

Antioxidant activity

DPPH Radical scavenging activity

The DPPH radical scavenging activity of methanolic extract was analyzed by following the method of Sanchez-Moreno et al. (1998). A methanol solution (0.1 ml) of the sample extract was added to 3.9 ml (0.025 g/L) of DPPH solution. BHT solution, (10 mg/10 ml) was used as a positive control. The solutions were incubated at room temperature (25°C) for 30 min and the decrease in absorbance at the end of incubation period was determined at 515 nm with the same Spectrometer. From the absorbance value, the free-radical scavenging activity of methanolic extract was calculated and expressed as percentage basis.

FRAP Assay

The Ferric-reducing/antioxidant power (FRAP) of methanolic extract was estimated according to the procedure described by Pulido et al. (2000). FRAP reagent (900 µl), prepared fresh and incubated at 37°C for 30 min, was mixed with 90 µl of distilled water and 30 µl of test sample or methanol (for the reagent blank) or BHT (10 mg/10 ml as positive control). Then test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The FRAP reagent contained 2.5 ml of 20 mM TPTZ solution (2,4,6-Tris (2-pyridyl)-s-triazine) in 40 mM HCl and 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 0.3 M acetate buffer, (pH 3.6). At the end of incubation the absorbance readings were taken immediately at 593 nm using the Spectrometer. Methanolic solutions of known Fe(II) concentration ranging from 100 to 2000 µM ($FeSO_4.7H_2O$) were used for the preparation of calibration curve. The antioxidant activity of methanolic extracts was expressed in mmol Fe(II) per gram.

Antidiabetic effect

α-Amylase inhibition activity

One hundred microlitre of buffer (0.02 M sodium phosphate buffer, pH 6.9) was added to 100 µl of methanolic extract, 100 µl of α-amylase enzyme (1 ml liberates 1.9 µg of maltose), and 100 µl of 1% starch. All the reagents were prepared with phosphate buffer (pH 6.9). After the ncubation at 25°C for 30 min, the reaction was stopped with 1.0 ml of dinitrosalicylic acid eagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to oom temperature. The reaction mixture was then diluted with the addition of 5.4 ml of listilled water and the absorbance was measured at 540 nm. The readings were compared with the control, which contained buffer instead of sample extract. Based on the absorbance value, the percent inhibition activity was calculated for the samples.

α-Glucosidase inhibition activity

One hundred microlitre of methanolic extract and 100 µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 unit/ml) were taken in tubes and pre-incubated at 25°C for 5 min. After the pre-incubation, 100 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution prepared in 0.1 M phosphate buffer (pH 6.9) was added to each tube and the reaction mixture was incubated at 25°C for 5 min. Then the aliquots were diluted 10-fold with distilled water, and the absorbency was recorded at 405 nm and compared to a control that had 100 µl of buffer solution in place of the extract. The results were calculated and expressed as percentage of α-glucosidase inhibition

Statistical analysis

All analyses were performed in triplicate (n = 3), and the data was presented as means ± standard error of means. The results obtained were analyzed by using two-way ANOVA to

determine the significant differences between the experimental batches by taking the raw samples as control. GraphPad PRISM® version IV software, San Diego, CA was used for statistical analysis.

Results and discussion

Total phenolic content

The total phenolic content of methanolic extract of grains is shown in Fig. 4.1. The cereals including finger millet and amaranth grains exhibited total phenolic content of 1.05 and 1.07 g/100 g DM and these values were found to be comparable to those of buckwheat (0.91 g/100g) and rice (0.92 g/100g) reported by Gorinstein et al. (2007). The total phenolic content of finger millet (1.05 g/100 g) observed in the present study was higher than that of finger millet (*Eleusine coracana* var. GPU-26 and CO 13) and kodo millet (*Paspalum scrobiculatum*) (0.053-0.10 g/100 g) reported by Hedge and Chandra (2005) and Sripriya et al (1996). Such higher levels of total phenolics noticed in finger millet of the present study might be due to repeated extraction of the compound with acidified methanol and also the varietal differences between the different finger millets used as well as different growing agro-climatic locations.

The total phenolic contents of legumes of the current study ranged from 0.68 to 1.00 g/100 g and were higher than the values reported for some under-utilized legumes in Korea such as pigeonpea (0.024-0.058 g/100 g), groundnut (0.03-0.06 g/100 g) and kidney bean (0.025-0.032 g/100 g) (Oboh *et al.*, 2009) but comparable to those reported for kidney bean (0.7 g/100 g), pinto bean (0.75 g/100 g) and soybean (0.65 g/100 g) (Boateng *et al.*, 2008). The total phenolic content of raw groundnut (0.85 g/100 g) of the present study was slightly higher than the values reported in the same species (0.10 to 0.65 g/100 g) by Craft et al (2010) and Yang et al (2009). The variation in the total phenolic content was attributed to

many factors including genotype, agronomic practices, maturity at harvest, postharvest storage, climatic and geographical locations.

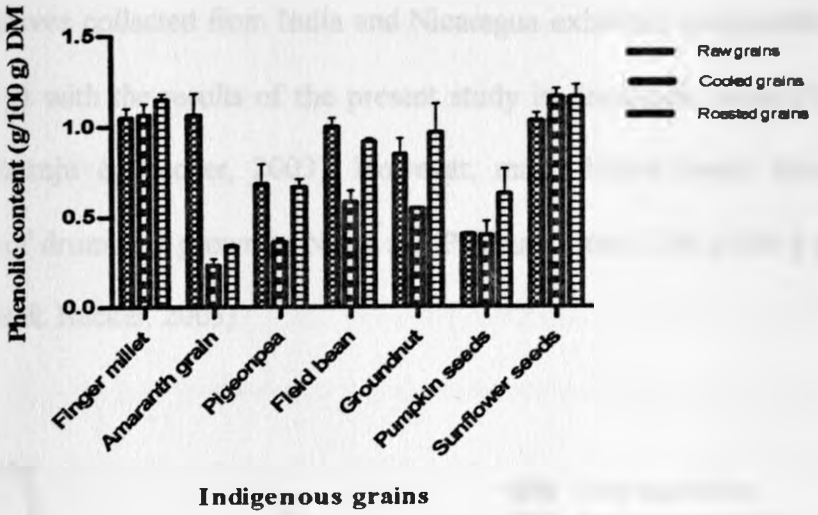

Fig 4.1 Effects of processing on the phenolic content of selected indigenous grains

The common vegetables exhibited phenolic content of 0.42-1.27 g/100g. The total phenolic content in the presently analyzed vegetables was comparable to the ranges of most commonly consumed vegetables of the world including tomato (1.42-1.56 g/100 g), carrot (1.40-1.58 g/100 g), onion (1.22-1.48 g/100 g) and broccoli (1.61-2.36 g/100 g) reported by Cieślik et al (2006). The phenolic content of sweet potato of the present study falls within the range of values (0.19-1.16 g /100 g) reported for different varieties of sweet potato (*Dakol, Emelda, Haponita*, PSBSP and Violet) growing in The Philippines (Rumbaoa *et al.*, 2009) but lower than the values reported for 'murasakimasari' cultivar in Japan (3.12 g /100 g) (Ishiguro et al., 2007). The total phenolic content of pumpkin (1.27 g/100 g) reported in this study was higher than the values revealed for the same species (0.09-0.10 g/100 g) (Azizah *et al.*, 2009).

The total phenolic contents of the leafy vegetables ranged from 1.50 to 3.00 g/100 g DM (Fig. 4.2). Drumstick leaves and amaranth leaves contained significantly higher levels of

total phenolics as compared to other vegetables. The leaves of other species of amaranthus (*Amaranthus paniculatus, A. blitum and A. viridis*) also exhibited higher levels of total phenolics (6.94-10.7 g/100 g) according to reports by Amin et al (2006). The total phenolic content of drumstick leaves collected from India and Nicaragua exhibited comparable levels (2.94 and 4.25 g/100 g) with the results of the present study in drumstick leaves (Iqbal & Bhanger, 2006; Siddhuraju & Becker, 2003). However, much higher levels have been reported in the leaves of drumstick grown in Niger and Pakistan 3.66-11.94 g/100 g (Verma *et al.*, 2009; Siddhuraju & Becker, 2003).

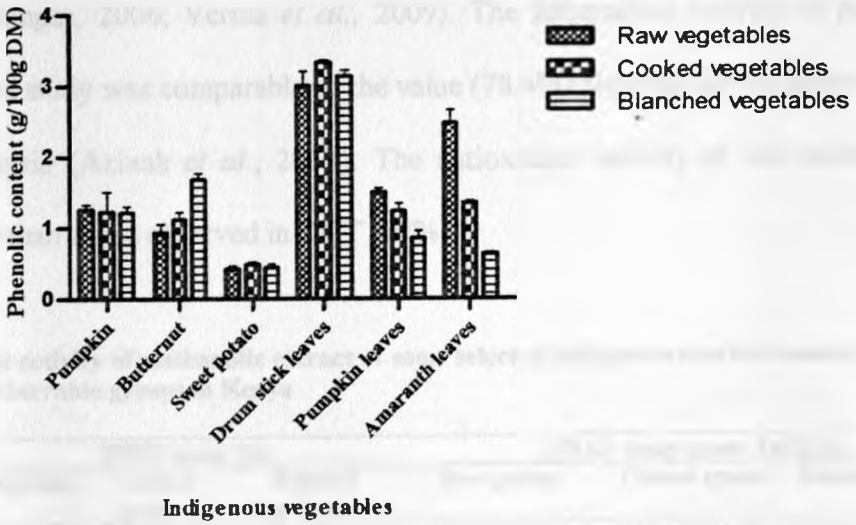

Fig 4.2 Effects of processing on the phenolic content of selected indigenous vegetables

Antioxidant activity

DPPH Assay

The DPPH free-radical scavenging activity (82-87%) of the methanolic extract of grains is presented in Table 4.1. The DPPH free-radical scavenging activity in raw cereals ranged from 79 to 86%, which is comparable to the values reported for finger millet, rice, wheat, sorghum and amaranth (70- 94%) (Sripriya *et al.*, 1996; Hedge & Chandra, 2005; Choi *et al.*, 2007;

Nsimba, 2008). The percent DPPH radical scavenging ability of legumes of the present study (86-87%) falls within the range reported for some under-utilized legumes such as pigeonpea, groundnut and kidney bean (40-90%) growing in Korea (Oboh *et al.*, 2009).

The DPPH radical scavenging capacity of methanolic extract in raw vegetables was found to fall between 75 and 89% with the highest values observed in sweet potatoes (89%) and drum stick leaves (87%). Similar levels of DPPH radical scavenging activity were reported for sweet potato (87%) growing in the USA and Japan (Cevallos-Casals & Cisneros-Zrvallos, 2003; Oki *et al.*, 2002). The antioxidant activity of drumstick leaves of this study falls within the range reported for the drumstick leaves collected from India and Pakistan (86-96%) (Iqbal & Bhanger, 2006; Verma *et al.*, 2009). The antioxidant activity of pumpkin (81%) of the current study was comparable to the value (78.4%) reported for the same sample cultivated in Malaysia (Azizah *et al.*, 2009). The antioxidant activity of the investigated samples was lower than those observed in BHT (97%).

Table 4.1 Antioxidant activity of methanolic extract of some selected indigenous cereals, legumes and oil seeds consumed by vulnerable groups in Kenya

Food samples	DPPH Assay (%)			FRAP Assay (mmol Fe[II]/g)		
	Raw grains	Cooked grains	Roasted grains	Raw grains	Cooked grains	Roasted grains
Finger Millet	81.67^{a} ±1.33	86.67^{b} ±0.33	84.67^{c} ±0.33	190.63^{a} ±12.85	215.41^{a} ±11.99	459.64^{c} ±14.10
Amaranth	84.67^{a} ±0.67	87.33^{b} ±0.88	81.00^{c} ±0.01	44.94^{a} ±1.35	233.78^{b} ±34.37	417.56^{c} ±15.24
Pigeon pea	86.67^{a} ±0.33	87.33^{a} ±0.88	84.00^{c} ±0.01	445.18^{a} ±48.99	308.39^{b} ±11.21	423.06^{a} ±10.75
Field beans	86.00^{a} ±0.58	88.33^{a} ±0.67	84.00^{a} ±0.01	393.51^{a} ±24.12	278.21^{b} ±22.71	265.43^{c} ±3.27
Groundnuts	86.67^{a} ±0.33	87.67^{a} ±0.67	84.00^{c} ±0.01	304.14^{a} ±46.07	317.17^{a} ±1.01	309.38^{a} ±41.89
Pumpkin seeds	82.67^{a} ±0.88	76.33^{b} ±0.88	83.67^{a} ±0.33	148.05^{a} ±5.41	514.91^{b} ±67.83	180.66^{a} ±30.08
Sunflower seeds	79.67^{a} ±0.67	78.33^{a} ±0.33	84.67^{c} ±0.67	394.42^{a} ±16.73	356.11^{a} ±15.90	672.89^{c} ±60.74
BHT		97%			2370	

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

FRAP Assay

The FRAP values of the raw grains ranged from 44 to 445 mmol Fe[II]/g with the highest reducing power being observed in pigeon pea and sunflower seeds (445 and 394 mmol Fe[II]/g) (Table 4.1). The FRAP values of legumes of the current study (304-445 mmol

Fe[II]/g) were comparable to the levels reported in legumes such as yellow pea (54-159 mmol Fe[II]/g), green pea (62-116 mmol Fe[II]/g), black bean (113-1103 mmol Fe[II]/g), chick pea (73-113 mmol Fe[II]/g), soy bean (127-993 mmol Fe[II]/g) and red kidney beans (285-922 mmol Fe[II]/g) collected in USA (Xu & Chang, 2007).

The FRAP value of raw vegetables were observed to vary from 104 to 744 mmol Fe[II]/g with the highest level observed in pumpkin leaves and butternut (Table 4.2) which falls within the range of values reported for carrots, courgettes and broccoli (50 to 10200 mmol Fe[II]/g) (Miglio *et al.*, 2008). Certain vegetables such as potato, kale, cabbage, cucumber, brussel sprouts and spinach contained concentrations ranging from 0.24 to 2.65 mmol Fe[II] based on the Frap values and showed higher antioxidant power than the presently studied vegetables (Halvorsen *et al.*, 2002). The ferric reducing/antioxidant power of the investigated samples was lower than those observed for BHT (1730-2320 mmol Fe[II]/g).

Table 4.2 Antioxidant activity of methanolic extract of some selected indigenous vegetables

Food samples	DPPH Assay (%)			FRAP Assay (mmol Fe[II]/g)		
	Raw vegetables	Cooked vegetables	Blanched vegetables	Raw vegetables	Cooked vegetables	Blanched vegetables
Pumpkin	81.00^{a} ±0.00	83.00^{a} ±0.01	78.00^{c} ±1.00	286.97^{a} ±22.25	310.26^{a} ±25.90	380.06^{a} ±22.01
Butternut	85.33^{a} ±0.67	80.33^{b} ±0.67	81.67^{c} ±0.67	475.25^{a} ±46.31	239.05^{b} ±29.56	343.41^{c} ±18.62
Sweet potatoes	89.00^{a} ±0.01	84.67^{b} ±0.67	86.33^{c} ±0.33	249.75^{a} ±13.38	327.01^{a} ±13.85	336.75^{a} ±31.64
Drumstick leaves	87.00^{a} ±0.01	89.00^{a} ±1.00	87.67^{a} ±0.33	186.11^{a} ±7.35	72.92^{b} ±4.65	80.94^{a} ±2.36
Pumpkin leaves	75.00^{a} ±0.01	83.67^{b} ±0.33	64.00^{c} ±1.00	744.44^{a} ±29.40	265.06^{b} ±31.61	301.95^{c} ±10.71
Amaranth leaves	85.33^{a} ±0.33	85.67^{a} ±0.33	78.33^{c} ±0.33	103.80^{a} ±6.11	181.59^{a} ±2.80	292.02^{c} ±2.72
BHT		97%			2370	

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

Antidiabetic activity

α-Amylase inhibition activity

In recent years, natural sources of α-amylase inhibitors have received a lot of interest due to search for alternatives for synthetic enzyme inhibitors such as acarbose, metformin and orlistat, which have been found to have adverse effects, mild efficacy and can cause

gastrointestinal distress as a side effect (Randhir *et al.*, 2008). Certain plant phenolics have the ability to partially inhibit the activity of α-amylase enzyme hence exhibiting therapeutic benefits such as hypoglycemic effects and are therefore useful in dietary management of type 2 diabetes (Chethan *et al.*, 2008). Phenolics are able to bind to the reactive sites of α-amylase and alter its catalytic effects.

The α-amylase inhibition activity of methanolic extract of grains ranged between 21 and 45% with sunflower seeds exhibiting the highest level (Table 4.3). These values are comparable to the range of values reported for certain related foods such as wheat, buckwheat, oats, corn mung beans, pumpkin, and butternut (20-90%) (Kwon *et al.*, 2007; Randhir *et al.*, 2008). Inhibitory activity of the polyphenols on the amylase has been the focus of attention in the management of type II diabetes mellitus. Similarly, Chethan et al (2008) and Randhir et al. (2008) reported the non-competitive inhibition of amylase by polyphenolics extracted from millets and mung bean respectively.

Table 4.3 Antidiabetic effect of methanolic extract of some selected indigenous cereals, legumes and oil seeds

Food samples	α-Amylase inhibition (%)			α-Glucosidase inhibition (%)		
	Raw grains	Cooked grains	Roasted grains	Raw grains	Cooked grains	Roasted grains
Finger Millet	$21.00^{a} \pm 5.00$	$25.67^{a} \pm 9.17$	$26.33^{a} \pm 3.18$	$62.67^{a} \pm 4.26$	$81.33^{b} \pm 2.67$	$91.00^{c} \pm 1.53$
Amaranth	$35.33^{a} \pm 1.67$	$28.67^{a} \pm 12.17$	$21.00^{a} \pm 0.01$	$40.33^{a} \pm 4.41$	$34.67^{a} \pm 5.89$	$14.00^{c} \pm 2.52$
Pigeonpea	$33.33^{a} \pm 4.67$	$33.33^{a} \pm 6.33$	$37.33^{a} \pm 10.68$	$32.00^{a} \pm 5.00$	$16.67^{a} \pm 2.67$	$34.00^{a} \pm 3.79$
Field bean	$22.67^{a} \pm 1.67$	$38.33^{a} \pm 6.33$	$15.67^{a} \pm 5.33$	$31.33^{a} \pm 4.37$	$18.00^{a} \pm 3.79$	$37.00^{a} \pm 6.43$
Ground nuts	$24.33^{a} \pm 1.67$	$43.33^{a} \pm 3.18$	$26.00^{a} \pm 0.01$	$30.00^{a} \pm 2.52$	$44.33^{a} \pm 4.33$	$39.67^{a} \pm 1.45$
Pumpkin seeds	$31.67^{a} \pm 14.11$	$48.00^{a} \pm 2.31$	$44.33^{a} \pm 3.71$	$14.00^{a} \pm 3.06$	$27.67^{a} \pm 0.33$	$27.67^{a} \pm 1.20$
Sunflower seeds	$45.00^{a} \pm 4.00$	$40.67^{a} \pm 3.33$	$35.33^{a} \pm 1.67$	$14.67^{a} \pm 1.45$	$40.67^{b} \pm 3.67$	$39.33^{c} \pm 1.41$

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

The α-amylase inhibition activity of the vegetables ranged from 10 to 29% with sweet potatoes, and pumpkin exhibiting the highest activity (Table 4.4). Kaushik et al. (2010) reported varying degree of hypoglycemic and anti-hyperglycemic activity of commonly consumed vegetables in India. The authors also suggest that pigeonpea, pumpkin and sweet

potato were having high antidiabetic activity and recommended for the management of diabetes mellitus.

Table 4.4 Antidiabetic effect of methanolic extract of some selected indigenous vegetables

Food samples	α-Amylase inhibition (%)			α-Glucosidase inhibition (%)		
	Raw vegetables	Cooked vegetables	Blanched vegetables	Raw vegetables	Cooked vegetables	Blanched vegetables
Pumpkin	28.33^{a} ±3.67	26.00^{a} ±0.01	5.00^{a} ±0.01	13.33^{a} ±2.96	17.67^{a} ±3.33	22.67^{a} ±3.71
Butternut	10.67^{a} ±0.33	0.00	0.00	40.67^{a} ±1.86	34.33^{a} ±4.09	8.00^{c} ±2.10
Sweet potatoes	29.00^{a} ±8.00	0.00	0.00	23.67^{a} ±2.40	12.00^{a} ±3.00	5.67^{c} ±1.45
Drumstick leaves	17.00^{a} ±0.01	36.33^{a} ±2.85	35.67^{a} ±1.33	80.67^{a} ±0.33	92.33^{a} ±0.33	91.67^{a} ±0.33
Pumpkin leaves	26.00^{a} ±0.01	41.67^{a} ±2.60	50.00^{a} ±3.00	49.33^{a} ±3.18	21.33^{b} ±2.91	63.67^{a} ±1.33
Amaranth leaves	41.00^{a} ±0.33	40.67^{a} ±10.04	21.00^{a} ±2.89	28.00^{a} ±3.06	22.67^{a} ±4.91	31.00^{a} ±0.58

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

α-Glucosidase inhibition activity

The effective management of diabetes mellitus, especially the non-insulin-dependent type, involves the prevention of excessive rise of the blood glucose level. α-Glucosidase, which is a membrane-bound enzyme located in the epithelium of the small intestines, catalyzes the cleavage of glucose from disaccharides for subsequent absorption (Chethan *et al.*, 2008). Earlier studies have reported that the retardation α-glucosidase enzyme by inhibitors would be one of the most effective ways to control non-insulin-dependent diabetes (Chethan *et al.*, 2008; Islam, 2006).

The α-glucosidase inhibition activity of methanolic extract of the grains ranged from 14 to 62% with finger millet demonstrating the highest activity (Table 4.3). The legumes (pigeonpea, field bean and groundnut) exhibited lower levels of α-glucosidase inhibition activity (30 to 32%) when compared to cereals, but higher levels than those of oil seeds. Among the various grains, finger millet recorded the highest level of α-glucosidase inhibition activity. Shobana et al. (2009) reported strong inhibition of finger millet phenolics towards α-glucosidase. Epidemiological studies have also reported lower incidences of diabetes in

populations consuming millets in their regular diets (Shobana *et al.*, 2009), which might be due to the potential α-glucosidase inhibition activity as revealed by the present study. α-Glucosidase inhibition activities demonstrated by the vegetables of the present investigation falls between 13 to 81% with the drum stick leaves showing the highest level. To our knowledge, this is the first study reporting the α-glucosidase inhibition activity of these vegetables.

Effects of processing

Soaking + cooking of grains

Fig. 4.1 shows the effects of soaking + cooking on the total phenolic content of the grains. Soaking + cooking caused significant ($p \leq 0.05$) reduction (35-79%) of phenolic content in most of the raw cereals and legumes. Similarly, a 55% decrease of total phenolics in *Phaseolus vulgaris* and 61-79% loss in black and pinto beans were reported during cooking (Granito *et al.*, 2008; Xu & Chang, 2009). The decreases could be attributed to the leaching out of water soluble phenolics into the soaking medium as well as heat degradation of phenolic compounds during cooking.

However, soaking and cooking did not cause any significant loss of DPPH-radical scavenging capacity, FRAP, α-amylase and α-glucosidase inhibition activities of the grain samples except losses in the reducing power of pigeonpea and field bean (Table 4.1 & 4.3). Cooking of foods does not necessarily cause the loss of antioxidant and antidiabetic properties and may in fact assist in the release of bound antioxidant compounds from the food matrix and result in apparent increase of antioxidant activity of the cooked samples when compared to the raw food ingredients. Furthermore, it is also likely that matrix softening and increased extractability upon cooking were accompanied by the conversion of methanolic extracts into active chemical species, which were not yet identified and acted synergistically

to elicit the high antioxidant capacity. Significant increase in DPPH-radical scavenging capacity and FRAP were observed in the current study for finger millet, amaranth grain and pumpkin seeds. Similarly, Nsimba et al. (2008) and Randhir et al. (2008) also reported a substantial increase in antioxidant activity of food ingredients such as amaranth grains, wheat, buckwheat, corn and oat during cooking treatment. These increases were attributed to release of bound bioactive compounds from breakdown of cellular constituents and cell walls and also polymerization, aglycosylation and/or oxidation of the compounds.

Roasting of grains

Roasting of the grains did not show any significant reduction in the total phenolic contents (Fig. 4.1). Slight increase in total phenolic content (12%) of groundnuts after roasting was observed in the current study which is in agreement with report by Craft et al. (2010). Such increases could be due to some other phenolics which are not endogenous in the grains that may be formed as by-products of thermal degradation, maillard reactions, caramelization, chemical oxidation of phenols and maderization.

No significant changes were noticed on FRAP, α-amylase and α-glucosidase inhibition activities in most of the grains (Table 4.1 & 4.2). Further, roasting of finger millet, sunflower seeds and amaranth grain resulted to significant increases on the FRAP values of phenolics. Such increases could be due to formation of maillard products such as hydroxymethylfurfuraldehyde from the methanolic extracts during roasting, which may yield high antioxidant activity. However, significant loss of DPPH radical scavenging activity was recorded in all the samples, except finger millet and sunflower seeds during roasting. Such decreases could be due to the effect of high roasting temperature which leads to modification of chemical properties of bioactive compounds. Roasting of most of the grains did not result in significant changes on α-amylase and α-glucosidase inhibition activities. Randhir et al.

(2008) suggested that the α-amylase inhibitory activity depended on the spectrum of phenolics that are mobilized by thermal processing and not on the total phenolic contents.

Cooking of vegetables

Cooking did not cause any significant changes on the phenolic content of all the vegetables, except amaranth leaves (45% reduction) (Fig 4.2). In contrast, slight increase was observed in the total phenolic content of cooked sweet potatoes (14%), which might be attributed to the inactivation of polyphenoloxidase enzyme. Similar increases have been reported for different sweet potato cultivars (Beauregard and Hernandez) in the United States (Truong *et al.*, 2007). Nonetheless, significant decreases in total phenolic contents (40-50%) of different vegetables such as squash, peas and leek have also been reported by Turkmen et al. (2005) and Xu & Chang (2008).

Cooking demonstrated no significant loss of DPPH radical scavenging, FRAP, α-amylase and α-glucosidase inhibition activities in most of the vegetables analyzed in the present study (Table 4.2 & 4.4). However, losses were observed in the radical scavenging activity and reducing power of butternut, drumstick and sweetpotato. Similarly, significant decrease in the DPPH radical scavenging in cooked vegetables such as broccoli, pepper, spinach, green beans, peas, leek, chick pea, lentil and squash have been reported (Turkmen *et al.*, 2005). This loss of the DPPH radical scavenging activity and reducing power could be attributed to counteractions of several types of chemical reactions and leaching of water soluble antioxidant compositions.

Blanching of vegetables

Blanching showed no significant loss of phenolics in the presently studied vegetables, except in amaranth and pumpkin leaves (74 and 43%, respectively). Similarly, blanching in boiling

water was reported to cause a loss of up to 80% of phenolics in brassica vegetables (Ninfali *et al.*, 2005). Amin et al. (2006) observed that the total phenolic contents of different amaranth species tend to decrease after blanching with losses of 31-51% after 5 minutes and 71% after 15 minutes of blanching. Blanching is mainly carried out to inactivate the peroxidase, which is necessary to prevent undesirable changes in the taste and odor of vegetables. The heat treatment partially denatures the enzymes and prevents enzymatic degradation of phenolics but also exposes them to thermal modifications. Substantial loss of phenolics in vegetables during blanching is likely to be a as result of enzymatic browning and maillard reactions.

No significant reduction on FRAP values was noticed in most of the vegetables, whereas significant loss of DPPH radical scavenging activity was recorded in all the vegetables except drumstick leaves. Significant increases in FRAP values were noticed in the amaranth leaves. Similarly, earlier report indicated that the antioxidant activity significantly increased or remained the same in vegetables such as spinach, pepper, green beans and broccoli during steaming and cooking procedures (Turkmen *et al.*, 2005). Blanching did not affect the α-amylase and α-glucosidase inhibition activities of vegetables except, in butternut and sweet potatoes (which showed no inhibition activity). Such absence of inhibition activity observed for butternut and sweet potato might be due to denaturation of phenolics during blanching.

Conclusion

Among the investigated raw food ingredients, amaranth grain, sunflower seed, drumstick and amaranth leaves with high phenolic content exhibited relatively higher antioxidant as well as antidiabetic activities. When considering the effect of processing methods, roasting of grains and blanching of vegetables modified the chemical nature of the phenolic compound and thus adversely affected the antioxidant and antidiabetic activities. On

the other hand, soaking + cooking of grains and cooking of vegetables were observed as the most suitable methods of processing to preserve the chemical and functional properties of phenolic compounds. Hence, such viable and mild treatments could be recommended for formulation of antioxidant-rich therapeutic diets and supplementary foods with antidiabetic properties for the vulnerable groups in Kenya. Further, the synergistic or antagonistic association of phenolics from different indigenous food ingredients of the present study warrants further investigation, before using their combinations in supplementary food formulations.

CHAPTER 5

Flavonoid content in ethanolic extracts of selected raw and traditionally processed indigenous foods consumed by vulnerable groups of Kenya: Antioxidant and Type II diabetes-related functional properties

Abstract

The present study evaluated the flavonoid content, antioxidant as well as type II diabetes related enzyme inhibition activities of ethanolic extract of certain raw and traditionally processed indigenous food ingredients including cereals, legumes, oil seeds, tubers, vegetables and leafy vegetables, which are commonly consumed by vulnerable groups in Kenya. The vegetables exhibited higher flavonoid content (50-703 mg/100 g) when compared to the grains (47-343 mg/100 g). The ethanolic extract of presently studied food ingredients revealed 33-93% of DPPH radical scavenging capacity, 486-6389 mmol Fe(II)/g of reducing power, 19-43% of α-amylase inhibition activity and 14-68% of α-glucosidase inhibition activity. Among the different food-stuffs, the drumstick and amaranth leajves exhibited significantly higher flavonoid content with excellent functional properties. Roasting of grains and cooking of vegetables were found to be suitable processing methods in preserving the functional properties. Hence, such viable processing techniques for respective food samples will be considered in the formulation of functional supplementary foods for vulnerable groups in Kenya.

Keywords: Kenyan indigenous foods, flavonoids, antioxidant activity, functional property, processing methods, vulnerable groups.

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Introduction

Recent advancements in nutritional research give speculative health insights that allow for development of foods and beverages with claimed health benefits, the so called functional foods (Weststrate *et al.* 2002). Dietary diversification and diet-based strategies through consumption of broad variety of foods is the most promising approach for a sustainable control of malnutrition and diseases among the vulnerable and socio-economically weaker sections of populations in Kenya. Studies on the food intake and dietary patterns in Kenya show that the diets are mainly cereal-based, with tubers and a variety of vegetables and fruits (Bwibo & Neumann, 2003; Marchione, 2002). The consumption of such foods is crucial to the nutritional security of these groups as it provides affordable nutrients such as vitamins and minerals, especially provitamin A, vitamin C, and iron (FAO/WHO, 2004).

Apart from the macro- and micro- nutrients, plant foods contain many bioactive compounds which have been associated with functional properties consistent with reduced risk of several chronic diseases and other maladies (Müller & Krawinkel, 2005). To exploit the health-promoting functionalities of the locally available, culturally acceptable, and economically viable indigenous foods it is important to focus on their functional properties. Among the various bioactive substances, the phenolic compounds such as flavonoids, phenolic acids, and polyphenols are the most abundant antioxidants in commonly consumed foods of plant origin.

Flavonoids are the most common and widely distributed group of plant phenolics and are an integral part of human diet (Aherne & ÓBrien, 2002). They are a large family of plant secondary metabolites consisting of >9000 individual molecules found in all plant tissues and are principally recognized for their health-promoting properties in human nutrition. Most flavonoids have strong antioxidant capacity as compared to ascorbate and α-tocopherol because of their strong ability to donate hydrogen atoms (Cieślik *et al.*, 2006). Several

epidemiological studies indicate that food-derived flavonoids have been associated with decreased risk for cardiovascular diseases and cancer-prevention (Art & Hollman, 2005; Vita, 2005) and also demonstrated to possess potential antioxidant and anticarcinogenic (Ren *et al.*, 2003), anti-inflammation (Tapiero *et al.*, 2002), anti-platelet and anti-thrombotic (Vita, 2005), anti-allergic (Fresco et al. 2006) and anti-hyperglycemic effects (Art and Hollman, 2005; Knekt *et al.*, 2002).

Flavonoids in foods are relatively resistant to heat, dryness and moderate degrees of acidity, but can be modified by light and also certain traditional food processing methods may affect the flavonoids (Aherne & ÓBrien, 2002). The flavonoid content of foods and its functional properties may be altered among different food ingredients and even within the same food depending upon the thermal processing conditions and can result in either decrease (Randhir *et al.*, 2008) or increase of antioxidant activity of plant foods (Granito *et al.*, 2007; Randhir *et al.*, 2008). Hence, appropriate processing method should be addressed for each food ingredient in order to increase or preserve the functional properties of flavonoid compounds present in Kenyan indigenous food ingredients.

Vulnerable groups are population groups whose immune system is compromised or depressed nutritionally, medically, or socially and require provision of extra, nutritionally high quality foods in addition to the general ration to rehabilitate or prevent deterioration in their conditions. They include: the malnourished; children under 5 years of age; Pregnant and lactating women; Medical referrals (malnourished adults in cases of vitamin or mineral deficiencies, TB, diabetes, cancer, AIDS); people living with HIV/AIDS (PLWHA's) refugees; internally displaced persons (IDP's) and socially vulnerable groups (orphans or unaccompanied children; the elderly, the disabled and any individuals separated from family and unable to fend for themselves). Beside various problems being faced by the vulnerable groups, cancer and diabetes have been increasing dramatically in the last two decades and the

prevention/treatment of diabetes has received a paramount importance among the health professionals and nutritionists.

To our knowledge, very little information exists concerning the flavonoid content and functional properties of certain indigenous food ingredients consumed by vulnerable groups in Kenya, in addition to lack of knowledge on the impact of various traditional processing methods. Hence, the purpose of the present study was to determine the flavonoid content, antioxidant and type II diabetes related enzyme inhibition activities of ethanolic extract of certain selected raw and traditionally processed foodstuffs consumed by vulnerable groups in Kenya, with the aim to identify the elite food ingredient(s) with appreciable flavonoid content and favorable functional properties as well as optimal processing method(s).

Materials and methods

Sample collection

Samples included cereals such as finger millet (*Eleusine coracana* L. Gaertn. P-224) and amaranth grain (*Amaranthus cruentus* L.); legumes such as pigeonpea (*Cajanus cajan* (L.) Millsp. Kat/Mbaazi 3) and field bean (*Dolichos pupureum* L. Kat/DL-3); oil seeds such as groundnut (*Arachis hypogea* L.), pumpkin seed (*Cucurbita maxima* Duchesne ex Lam.) and sunflower seed (*Helianthus annuus* L. PAN 7369). The vegetables selected were pumpkin (*Cucurbita maxima* L.), butternut (*Juglans cinerea* L.), sweet potatoes (*Ipomoea batatas* [L.] Lamk. SPK 004), and leafy vegetables such as drumstick leaves (*Moringa oleifera* L.), amaranth leaves (*Amaranthus hybridus* L.) and pumpkin leaves (*Cucurbita maxima* Lam.). The cereals, legumes, oil seeds and vegetable samples (1 kg each) were randomly obtained from Kenya Agricultural Research Institute (KARI), Kenya as well as different parts of Kenya from the agricultural fields. Then they were mixed to obtain a representative sample which was then subdivided into three portions for different treatments with three replicates.

Chemicals

The chemicals used include quercetin dehydrate (Ref. No. 83370), 2,2′-Diphenyl-1-picryl hydrazyl (DPPH, Ref. No.: 217-591-8,); 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ, Ref. No. 3682-35-7), Butylated Hydroxytoluene (BHT, Ref. No. 204-881-4), 4-Nitrophenyl-α-D-glucopyranoside (PGPP, Ref. No. 3767-28-0), starch (Ref. No. 232-679-6), α-amylase (Ref. No. 9001-19-8), and α-glucosidase (Ref. No. 9001-42-7) were obtained from Sigma chemical, USA, and all other chemicals were purchased from Merck, Darmstadt, Germany.

Processing of cereals, legumes and oil seeds

The grains (cereals, legumes, and oil seeds) were collected and randomly divided into three batches. Each batch was treated under different condition depending on the processing method. The first batch was stored as such without any treatment at ambient temperature (25 ± 1 ^{0}C) for 2 days and regarded as raw samples. The second batch of samples (100 g each) was washed with tap water and then soaked in distilled water (200 ml) for 8 h in the dark at 25 ± 1 ^{0}C. The soaking water was discarded and the grains cooked at 90-95 ^{0}C for 120 min in fresh distilled water in the ratio of 1:4. The third batch of samples was roasted in an open-pan iron container to a golden brown color for 30 min using traditional charcoal burner at 150 ^{0}C with continuous stirring to avoid burning of the seed coat. After roasting, the samples were cooled to room temperature.

Processing of vegetables and leafy vegetables

The fresh vegetables were collected and randomly divided into three batches for treatment using different processing methods. The first batch was stored as such without any treatment at ambient temperature (25 ± 1 ^{0}C) for 2 days and regarded as raw samples. The second batch of samples (100 g of edible portion) were cut into small pieces or cubes and washed under

running tap water, then cooked in 200 ml of distilled water at 90-95 ^{0}C for 5 min for all samples, except sweet potatoes, pumpkin and butternut which are cooked for 15 min. The third batch of vegetables was blanched by immersing in boiling water for 5 min after cutting into small pieces or cubes.

Extract preparation

All the raw, cooked and blanched samples were dried in hot-air oven at 50 ^{0}C for 6 h (As per the traditional method practiced by the vulnerable group in Kenya for the preparation of food) and then milled using a laboratory mill (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland) and sieved (0.1mm) to obtain fine flour. The samples were defatted by adding petroleum ether in 1:10 ratio (W/V) and kept in an ultrasonic bath for 30 mins. After centrifugation at 13,000 rpm for 5 min, the supernatant was discarded and the residue was air-dried. The defatted sample flour (1 g) was extracted by using 10 ml of 50% aqueous ethanol for 30 min repeatedly in an ultrasonic bath (Bandelin Sonorex, RK – 514 H, Berlin, Germany) and centrifuged at 13,000 rpm for 5 min after each extraction. The supernatants were pooled together and made up to a known volume with ethanol. The solvent was evaporated by using rotary vacuum evaporator (Büchi Rotavapor, CH-9230, Switzerland) at 40°C, freezed overnight at -80 °C and dried in lyophilier (Virtis Freezemobile 25 EL, New York) for 10 h and finally the residue was weighed and the total dry yield of extract was calculated, which ranged from 73 – 95 mg/g sample for the presently analyzed samples. Then the extract was re-dissolved in ethanol:formic acid (97.5:2.5, v/v) solution in the ratio of one mg/ml and used for further analysis.

Flavonoid content

The analysis was performed according to the spectrophotometric method adapted from Jia et al. (1999). Sodium nitrite (50%, 150 µl) and 2 ml of distilled water were added to 500 µl of the extract and allowed to stand for 5 min in the dark. Then 150 µl of aluminium chloride was added to the aliquots and allowed to stand for another 5 min in the dark. Finally, 1 ml of 1 M sodium hydroxide and 1.5 ml of distilled water were added and the absorbance was read at 510 nm with UV-Vis Spectrophotometer (Perkin-Elmer, Lambda 35, USA). Based on the standard curve prepared with (+) quercetin (20-120 µg), the amount of flavonoids in the extract was calculated and expressed in milligrams per 100 g sample extract on dry matter basis.

DPPH Radical scavenging activity

The DPPH radical scavenging activity of ethanolic extract was analyzed by following the method of Sanchez-Moreno et al. (1998). A methanol solution (0.1 ml) of the sample extracts at various concentrations was added to 3.9 ml (0.025 g/L) of DPPH solution. BHT (10 mg/10 ml) was used as a positive control and pure DPPH solution alone was used as negative control. The solutions were incubated at room temperature (25 ^{0}C) for 30 min and the decrease in absorbance was determined at 515 nm at the end of incubation period with a Spectrophotometer. The radical scavenging activity of the tested samples was measured as a decrease in the absorbance of DPPH radical and was calculated by using the equation, DPPH radical scavenging activity (%) = $(1 - A_{\text{samples}}/A_{\text{negative control}}) \times 100$.

Ferric reducing antioxidant power (FRAP)

The reducing property of the ethanolic extract was estimated according to the procedure described by Pulido et al. (2000). FRAP reagent (900 µl), prepared freshly and incubated at

37 ^{0}C, was mixed with 90 µl of distilled water and 30 µl of test sample or methanol (for the reagent blank) or BHT (10 mg/10 ml) as positive control. The test samples and reagent blank were incubated at 37 ^{0}C for 30 min in a water bath. The FRAP reagent contained 2.5 ml of 20 mM TPTZ solution (2,4,6-Tris (2-pyridyl)-s-triazine) in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 0.3 M acetate buffer, pH 3.6 (16.8 g of glacial acetic acid, MW 60.05 and 2.69 g of sodium acetate trihydrate, MW 136.1 dissolved in 1 L of distilled water). At the end of incubation the absorbance readings were taken immediately at 593 nm using a Spectrophotometer. Methanolic solutions of known Fe(II) concentration ranging from 100 to 2000 µM ($FeSO_4.7H_2O$) were used for the preparation of the calibration curve.

α-Amylase inhibition activity

The α-amylase inhibition activity was measured by following the method of Worthington (1993). One hundred microlitre of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to 100 µl of each extract in 100 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (1 unit liberates 1.9 µl of maltose from starch in 1 min at pH 6.9 and temperature 25 ^{0}C), and was incubated at 25 ^{0}C for 30 min. After the incubation, the reaction was stopped with 1.0 ml of dinitrosalicylic acid reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted to 10-folds with distilled water and the absorbance was measured at 540 nm. The readings were compared with the control, which contained buffer instead of sample extract. Based on the absorbance value, the percent inhibition activity was calculated for all the samples.

α-Glucosidase inhibition activity

The α-glucosidase inhibition activity was determined according to the method described by Worthington (1993). One hundred microlitre of ethanolic extract and 100 µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 unit/ml) were taken in tubes and incubated at 25 ^{0}C for 5 min. After the pre-incubation, 100 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each tube and the reaction mixture was incubated at 25 ^{0}C for 5 min. After the incubation period the aliquots were diluted with 10-fold distilled water, and the absorbance readings recorded at 405 nm and compared to a control that had 100 µl of buffer solution in place of the extract. The results were calculated and expressed as percentage of α-glucosidase inhibition.

Statistical analysis

All analyses were performed in triplicate (n = 3), and the data was presented as means standard deviation (± SD). The results obtained were analyzed by using two-way ANOVA to determine the significant differences between the experimental batches by taking the raw samples as control. GraphPad PRISM® version IV software, San Diego, CA was used for statistical analysis.

Results and discussion

Flavonoid content

The flavonoid contents of some western foods have been reported in the USDA flavonoid database (USDA, 2009). However, little is known about the flavonoid content and other bioactive compounds of foods from developing countries like Kenya. The flavonoid content of grains (cereal, legumes and oil seeds) varied from 47 to 343 mg/100 g DW (Figure 5.1). The flavonoid content of the cereals of the present study (47-167 mg/100 g) was comparable

to the values reported for cereals and pseudocereals such as buckwheat (146 mg/100 g), amaranth grains (72-75 mg /100 g), Jasmine rice (38 mg/100 g) and quinoa (102 mg/100 g) (Gorinstein *et al.*, 2007). Groundnuts and sunflower seeds had the highest flavonoid content. Groundnuts have already been reported to have several flavonoids and are rich in resveratrol with cardio-protective and anticancer effects (Yang *et al.*, 2009). The flavonoid content of groundnuts (343 mg/100 g) of the present study is higher than those reported for shelled peanuts (190 mg/100 g) in the USA by Yang et al. (2009).

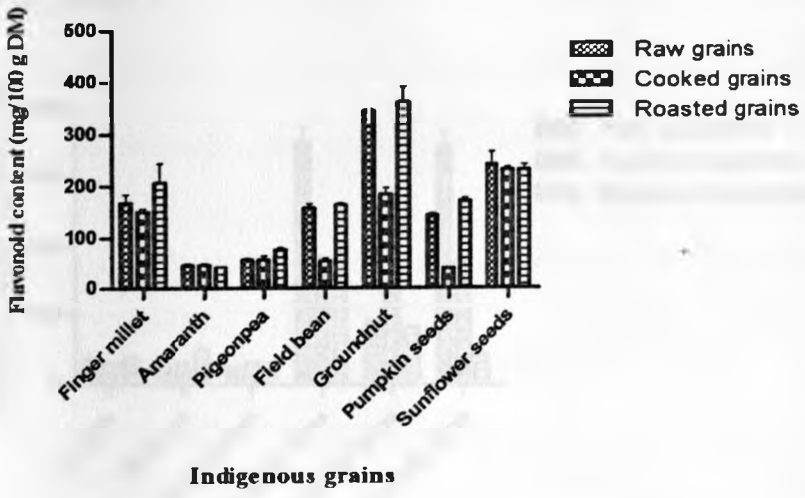

Fig 5.1 Effects of processing on flavonoid content of selected indigenous grains

The flavonoid content of the legumes (57-343 mg/100 g) of the current study was comparable to the levels reported in legumes such as yellow pea (18-32 mg /100 g), green pea (8-39 mg/100 g), black bean (98-321 mg/100 g), chickpea (18-316 mg/100 g), lentil (72-221 mg/100 g), soy bean (25-257 mg/100 g), and red kidney beans (85-293 mg/100 g) (Xu and Chang 2007) as well as broad bean (154.6 mg/100 g) (Santos-Buelga *et al.*, 2000), but slightly lower to that of pinto and black bean (177.90 and 677.36 mg/100g, respectively) as reported by Xu and Chang (2009). The difference could be attributed to various reasons but not only limited to genotype, agronomic practices, maturity at harvest, post-harvest storage, climatic conditions, growing and storage conditions.

The flavonoid content of the vegetables was significantly higher in drumstick leaves (703 mg/100 g) and amaranth leaves (687 mg/100 g) as compared to the other vegetables (50-177 mg/100 g) (Figure 2). These values are comparable to the flavonoid content of edible tropical vegetables reported in Malaysia such as semambu leaves (204 mg/100 g), onion leaves (272 mg/100 g) and cekur manis (78 mg/100 g) (Miean and Mohamed, 2001) as well as indigenous vegetables growing in Indonesia such as *Sauropus androgynus* (L) *Merr* (143 mg/100 g), *Cosmos caudatus* H.B.K. (52.2mg/100 g) and *Polyscias pinnata* (52.2 mg/100 g) (Andarwulan *et al.*, 2010).

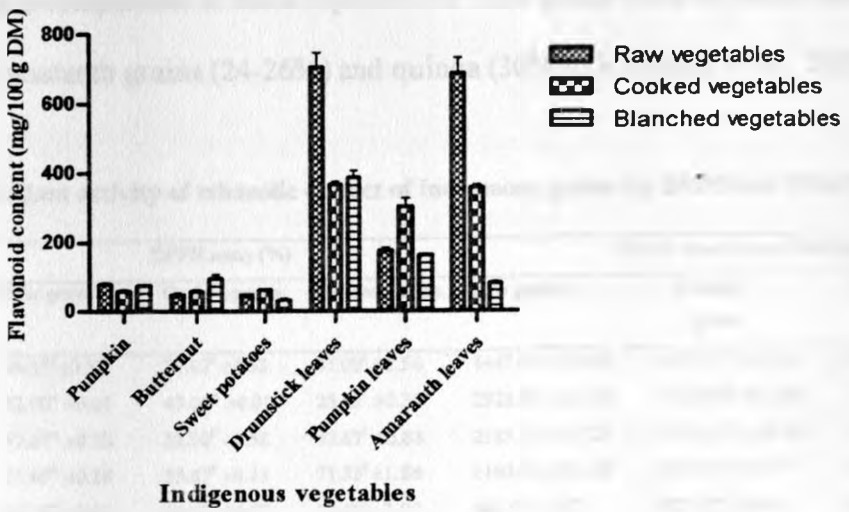

Fig 5.2 Effects of processing on flavonoid content of indigenous vegetables

The flavonoid content of drumstick leaves of the present study was higher than that reported for similar species growing in Malaysia (23 mg/100 g), but lower than those reported for the same species growing in Pakistan (6930-11900 mg/100 g) (Miean & Mohamed, 2001; Iqbal & Bhanger, 2006). Among the common vegetables of the current study (50-80 mg/100 g), sweet potato showed comparable flavonoid content to similar species growing in Taiwan (22.0-35.5 mg/100 g) (Huang *et al.*, 2006), and pumpkin had higher values than those growing in Malaysia (37 mg/100 g) (Miean & Mohamed, 2001). These differences may be attributed to the differences in methods used (HPLC vs

spectrophotometric methods), pre-harvest factors such as climate, geography, agronomic practices and varietal differences.

DPPH Radical scavenging activity

Flavonoids in foods have been reported to possess strong antioxidant and free radical scavenging activities (Miean & Mohamed, 2001). The DPPH free-radical scavenging activity of ethanolic extract of the presently studied grains was found to range from 42 to 74% with the field bean (72%) and sunflower seeds (74%) exhibiting the highest activity (Table 5.1). These values are comparable to those reported for other grains such as buckwheat (80%), soy beans (33%), amaranth grains (24-26%) and quinoa (30%) (Gorinstein *et al.*, 2007).

Table 5.1 Antioxidant activity of ethanolic extract of indigenous grains by DPPH and FRAP assay

Food samples	DPPH assay (%)			FRAP Assay (mmol Fe[II]/g)		
	Raw grains	Cooked grains	Roasted grains	Raw grains	Cooked grains	Roasted grains
Finger millet	69.33^{a} ±0.33	77.67^{b} ±0.33	65.00^{c} ±1.56	1447.00 ±119.68	1470.33^{a} ±31.34	1692.33^{a} ±173.32
Amaranth	42.00^{d} ±0.01	43.00^{d} ±0.01	25.33^{c} ±0.33	2928.00 ±661.00	1128.00^{b} ±61.00	1986.00^{c} ±14.00
Pigeonpea	57.67^{a} ±0.33	52.00^{b} ±0.58	47.67^{c} ±0.88	2183.33 ±107.20	2031.67^{a} ±143.19	2862.67^{a} ±8.67
Field bean	71.67^{a} ±0.88	53.67^{b} ±0.33	71.33^{a} ±1.86	1169.67 ±101.98	1953.67^{a} ±60.87	1963.00^{a} ±268.12
Groundnut	64.33^{a} ±0.33	16.67^{b} ±0.67	55.00^{c} ±2.00	486.67 ±1.67	827.00^{a} ±18.00	615.67^{a} ±44.63
Pumpkin seed	52.33^{a} ±0.33	11.00^{b} ±1.00	32.00^{c} ±0.01	903.67 ±18.68	3111.33^{b} ±91.14	734.67^{a} ±12.73
Sunflower seed	73.67^{a} ±2.40	71.67^{a} ±2.85	72.33^{a} ±1.76	1689.33 ±220.11	1693.00^{a} ±48.14	1482.33^{a} ±46.74
BHT		97%			2370	

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

The DPPH free-radical scavenging activity of ethanolic extract of the vegetables of the present study ranged from 34 to 93% with the drumstick leaves (93%) and amaranth leaves (92%) showing the highest activity among all the foods samples (Tables 5.2). Similarly, the DPPH free-radical scavenging activity of different drumstick leaves varieties was reported to range from 88 to 96% depending on production location in Pakistan (Iqbal & Bhanger, 2006). Kaur and Kapoor (2002) have also reported excellent antioxidant activity of

vegetables such as white cabbage, cauliflower, kale, spinach, brussel sprouts, alfalfa sprouts, broccoli, beets, red-bell pepper, onion, corn, egg plant and cucumber. The radical scavenging activity of the drumstick and amaranth leaves of the current study was comparable to that observed for synthetic antioxidant BHT (97%), which confirms that these two leafy vegetables could be used as a dietary source with potential free radical scavenging property in the formulation of supplementary foods for vulnerable groups in Kenya.

FRAP Assay

The reducing power of the grains ranged from 486 to 2928 mmol Fe[II]/g with amaranth grain and pigeonpea exhibiting the highest activity (2928 and 2183 mmol Fe[II]/g, respectively) (Table 5.1). The reducing power of the legumes of the current study (486 to 2183 mmol Fe[II]/g) were comparable to the levels reported in legumes such as yellow pea (54-159 mmol Fe[II]/g), green pea (62-116 mmol Fe[II]/g), black bean (113-1103 mmol Fe[II]/g), chick pea (73-113 mmol Fe[II]/g), soy bean (127-993 mmol Fe[II]/g) and red kidney beans (285-922 mmol Fe[II]/g) collected from USA (Xu & Chang, 2007).

Table 5.2 Antioxidant activities of ethanolic extracts in indigenous vegetables by DPPH and FRAP assay

Food samples	DPPH assay (%)			FRAP Assay (mmol Fe[II]/g)		
	Raw vegetables	Cooked vegetables	Blanched vegetables	Raw vegetables	Cooked vegetables	Blanched vegetables
Pumpkin	76.00^{a} ±1.00	80.67^{a} ±0.33	65.67^{c} ±0.33	6389.00 ±200.62	8723.00^{b} ±514.45	6190.33^{a} ±360.08
Butternut	62.67^{a} ±0.33	54.33^{b} ±1.67	77.67^{c} ±0.67	7603.33 ±80.27	5350.33^{b} ±390.50	3562.67^{c} ±190.56
Sweetpotato	33.67^{a} ±0.67	37.33^{a} ±0.67	36.00^{a} ±0.01	2288.67 ±144.33	2007.67^{a} ±75.33	3111.33^{c} ±91.14
Drumstick leaves	93.00^{a} ±0.01	90.33^{a} ±0.33	89.67^{a} ±1.33	724.33 ±3.18	437.33^{a} ±45.39	365.33^{a} ±19.94
Pumpkin leaves	51.67^{a} ±0.88	75.00^{b} ±0.58	33.67^{c} ±0.33	2357.33 ±171.49	1604.67^{a} ±146.81	965.00^{c} ±61.07
Amaranth leaves	92.00^{a} ±0.01	91.00^{a} ±1.53	27.00^{c} ±0.01	734.33 ±60.80	1400.67^{a} ±42.55	1101.00^{a} ±97.18
BHT		97%			2370	

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

The FRAP values of vegetables in the current study ranged from 724 to 6389 mmol Fe[II]/g with butternut and pumpkin exhibiting the highest content of 7603 and 6389 mmol Fe[II]/g,

respectively (Table 5.2). These values are higher than the reducing properties of cashew shoot (3.59 mmol Fe[II]/g), a vegetable consumed in Malaysia (Razali *et al.*, 2008). The FRAP value of most of the investigated samples was comparable to that of BHT control (1730-2320 mmol Fe[II]/g), which is an indication of high antioxidant activity of the food ingredients. Thus, the results could be useful in implementing these food ingredients in the formulation of supplementary foods for vulnerable groups in Kenya.

α-Amylase inhibition activity

Type II diabetes is becoming more prevalent with increased studies on its management through dietary sources. In this context, currently there is an increasing interest in natural dietary source of hypoglycemic compounds, since the synthetic oral hypoglycemic drugs have been found to exhibit many adverse effects (Randhir & Shetty, 2007). α-Amylase catalyses the hydrolysis of glycosidic linkages in starch which is essential in carbohydrate assimilation. α-Amylase inhibitors are starch blockers, which affect the enzyme activity and thus playing a vital role in reducing blood sugar. Inhibitory activity of the polyphenols on the amylase has been the recent focus of attention in the management of type II diabetes mellitus. Synthetic flavonoids were reported to have protective effects against the development of diabetes as well as a mitigation effect of diabetes consequences (Li *et al.*, 2009). Chethan et al. (2008) also reported the non-competitive inhibition of amylase by polyphenolics extracted from millets.

The α-amylase inhibition activity of the grains was observed to range from 23 to 41% with the legumes such as pigeonpea and field bean (41%) exhibiting the highest activity (Table 5.3). Randhir et al. (2008) reported a high α-amylase inhibition activity in oats, buckwheat, wheat and corn (20-90%). The α-amylase inhibition activity of the vegetables ranged from 19 to 43% with the sweet potato and pumpkin exhibiting the highest activity of

43 and 42%, respectively (Table 5.4). A review published by Kaushik et al. (2010) shows varying degree of hypoglycemic and anti-hyperglycemic activity of commonly consumed plant foods in India, which also indicated high anti-hyperglycemic activity of pumpkin and sweet potato. It should be noted that these results are based on biochemical tests are *in vitro* and indicative of anti-glycemic effects in the prevention/management of type II diabetes and have limited implications to what happens *in vivo*.

Table 5.3 Antidiabetic effects of ethanolic extract of cereal, legume and oil seeds consumed by vulnerable groups in Kenya

Food samples	α-Amylase inhibition			α-Glucosidase inhibition		
	Raw grains	Cooked grains	Roasted grains	Raw grains	Cooked grains	Roasted grains
Finger Millet	22.67^{a} ±7.45	35.33^{a} ±4.67	43.67^{c} ±6.74	23.00^{a} ±1.00	34.67^{a} ±11.47	31.67^{a} ±1.67
Amaranth	38.67^{a}±13.20	44.67^{a} ±2.67	42.00^{a} ±5.69	20.33^{a} ±7.69	19.50^{a} ±6.00	13.33^{a} ±4.33
Pigeonpea	41.00^{a} ±5.51	40.67^{a} ±0.67	46.00^{a} ±2.31	18.67^{a} ±6.12	20.00^{a} ±0.33	41.67^{c} ±1.33
Field beans	40.67^{a} ±4.84	46.00^{a} ±4.00	49.00^{a} ±3.22	27.33^{a} ±2.67	17.50^{a} ±0.33	43.00^{a} ±0.01
Groundnut	28.33^{a} ±2.67	22.00^{a} ±0.58	36.33^{a} ±4.70	14.33^{a} ±6.39	10.00^{a} ±3.00	30.00^{a} ±0.01
Pumpkin seeds	35.33^{a} ±4.67	30.00^{a} ±0.01	47.33^{a} ±1.33	17.33^{a} ±4.91	20.33^{a} ±1.67	27.33^{a} ±1.33
Sunflower seeds	35.33^{a} ±4.81	28.33^{a} ±0.67	43.33^{a} ±3.53	21.67^{a} ±6.44	23.33^{a} ±3.18	28.67^{a} ±1.33

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

α-Glucosidase inhibition activity

Certain plant flavonoids exhibit the ability to partially inhibit the activity of α-glucosidase enzyme and hence show therapeutic benefits such as hypoglycemic effects and are useful in the dietary management of type II diabetes (Chethan *et al.*, 2008). The α-glucosidase inhibition activity of grains was observed to range from 14 to 27% with field bean and finger millet exhibiting the highest activity of 27 and 23%, respectively (Table 5.3). Epidemiological studies have also reported lower incidences of diabetes in population consuming millets in their regular diet (Shobana *et al.*, 2009), which might be due to the potential α-glucosidase inhibition activity as revealed by the present study. The α-glucosidase inhibition activity of the vegetables ranged from 22 to 68% with the drumstick and pumpkin

leaves exhibiting the highest activity of 68 and 43%, respectively (Table 5.4). Recent studies also reported that certain indigenous foods such as pumpkin, corn, beans, sweetpotato, cabbage, radish and pea exhibit α-glucosidase inhibition activity and have the potential to reduce hyperglycemia-induced pathogenesis (Matsui *et al.*, 2001; Kwon *et al.*, 2007).

Table 5.4 Antidiabetic effects of ethanolic extract of vegetables consumed by vulnerable groups in Kenya

Food samples	α-Amylase inhibition			α-Glucosidase inhibition		
	Raw vegetables	Cooked vegetables	Blanched vegetables	Raw vegetables	Cooked vegetables	Blanched vegetables
Pumpkin	42.33^{a} ±0.33	34.67^{a} ±2.03	14.67^{c} ±4.81	24.67^{a} ±1.33	23.00^{a} ±3.79	14.33^{a} ±2.67
Butternut	29.33^{a} ±1.67	28.00^{a} ±1.53	0.00^{c}	22.00^{a} ±0.01	20.00^{a} ±3.00	5.67^{a} ±1.67
Sweetpotato	43.00^{a} ±1.73	25.67^{a} ±3.53	8.00^{c} ±0.33	23.00^{a} ±1.00	14.33^{a} ±1.33	5.67^{a} ±1.67
Drumstick leaves	19.00^{a} ±2.31	22.00^{a} ±0.33	26.00^{a} ±0.01	68.33^{a} ±3.84	80.00^{a} ±3.00	61.00^{a} ±6.66
Pumpkin leaves	26.00^{a} ±2.00	25.67^{a} ±2.67	29.67^{a} ±4.41	43.00^{a} ±0.01	35.00^{a} ±0.01	42.00^{a} ±3.00
Amaranth leaves	22.67^{a} ±3.67	26.33^{a} ±5.46	30.67^{a} ±0.33	33.33^{a} ±1.67	28.67^{a} ±1.33	26.00^{a} ±0.01

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

Effect of processing of grains

Food processing not only improves the flavour and palatability of food ingredients but also increases the bioavailability of nutrients. Traditional processing of food ingredients can cause significant changes on the flavonoid content as well as its functional properties. Soaking and cooking caused no significant decrease of flavonoid content in all the presently analyzed food samples, except groundnut, field bean and pumpkin seed (Figure 5.1). But in contrast, soaking + cooking were reported to cause significant decrease in total flavonoid content of pinto and black beans (*Phaseolus vulgaris* L.) (Xu & Chang, 2009) and total phenolic content of Brazilian bean cultivars (*Phaseolus vulgaris* L.) (Ranilla *et al.*, 2009). Loss of significant level of flavonoids noticed in the groundnut, field bean and pumpkin seeds could be attributed to water soluble flavonoids that were leached into soaking and cooking water as well as degradation of flavonoid compounds at elevated temperature during cooking. Interestingly, on the other hand roasting did not cause any significant loss on the

content of all the cereals, legumes and oil seeds (Figure 5.1). Generally, both soaking + cooking and roasting resulted in significant losses of the DPPH free-radical scavenging activity of all the grains, except finger millet and amaranth grain. Similarly, soaking was observed to cause significant reduction in the antioxidant capacity of Brazilian bean cultivars (*Phaseolus vulgaris* L.) as opposed to treatments without soaking and where cooking water was not discarded (Ranilla *et al.*, 2009). Such a drop in radical scavenging property of the ethanolic extract of processed samples can be attributed to synergistic combinations of different classes of bioactive compounds or counteractions of several types of chemical reactions, leaching out of the water-soluble compounds during cooking, and the formation or breakdown of phytochemical substances during roasting. Nonetheless, cooking and roasting did not result in reduction of the FRAP values of grains (Table 5.1).

Both soaking + cooking and roasting did not cause any significant losses of the α-amylase and α-glucosidase inhibition activities of the grains. Surprisingly, significant increases were observed in the finger millet and pigeonpea samples (Table 5.3). Cooking of foods does not necessarily cause the loss of functional properties but could promote the release of bioactive compounds from the food matrix and result in the formation of new compounds. Furthermore, it is also likely that matrix softening and increased extractability upon cooking were accompanied by the conversion of bioactive compounds into very active chemical species, which concurred synergistically to determine the high functional property. Such increases could imply that α-amylase and α-glucosidase inhibition activities may depend on spectrum of the bioactive compounds of ethanolic extract that are mobilized by thermal processing.

Effect of processing of vegetables

Food processing and preparation, particularly the mild heat treatment can improves the organoleptic properties of food ingredients, increase the content of absorption enhancers, and thus improves the bioavailability of bioactive compounds located in plant foods in addition to enhancing their functional properties. Most of the vegetables did not show any significant loss of flavonoid content after blanching and cooking, except in drumstick and amaranth leaves (Figure 5.2). Such losses observed in drumstick and amaranth leaves are probably due to the leaching out of flavonoids into the cooking water and/or chemical or thermal degradation (Aherne & ÓBrien, 2002). Steaming has been reported to decrease the contents of flavonoid from 34.23 to 21.01 mg in sweet potatoes from Taiwan (Huang *et al.*, 2006). However, pumpkin leaves showed a significant increase in total flavonoid content (42%) after cooking. Processing increases the flavonoid level in foods as a consequence of enzymatic hydrolysis of flavonoid conjugates during thermal treatments (Aherne & ÓBrien, 2002).

Cooking did not cause any loss in the DPPH free-radical scavenging activity and reducing power of all the vegetables, except in butternut (Table 5.2). Also, significant increase in the radical scavenging activity of pumpkin and pumpkin leaves as well as reducing power of pumpkin were observed in this study. This increase was attributed to the break down and release of bound bioactive compounds into simple classes of compounds as a result of heat treatments.

Blanching caused significant decrease in the DPPH free-radical scavenging activity of most of the vegetables, except an increase that was observed in butternut. Blanching is mainly carried out to inactivate the peroxidase, which is necessary to prevent undesirable taste and smell of vegetables. The thermal heat treatment partially denatures the enzymes and prevents enzymatic degradation of bioactive compounds, but exposes them to thermal

modifications. Such substantial loss of free radical scavenging activity of ethanolic extract of vegetables during blanching is likely to be a as result of enzymatic browning and maillard reactions attributed to the enzyme polyphenols oxidase, which can be activated during blanching, resulting in degradation and consequent loss of antioxidant activity of the flavonoids. However, blanching did not cause any significant loss in the reducing power of vegetables analyzed in this study except in butternut and pumpkin leaves. Sweet potatoes exhibited an increase in the FRAP values during blanching. Such improved reducing power could be attributed to the additive and synergistic effects of different classes of bioactive compounds.

The α-amylase and α-glucosidase inhibition activities of all the vegetables were not affected by cooking (Table 5.4). Blanching did not cause any significant loss in α-glucosidase inhibition activity of the vegetables of the current study but caused significant loss in the α-amylase inhibition activity of sweet potato and pumpkin as well as complete loss in butternut. Such decreases observed in sweet potato and pumpkin, and absence of inhibition activity noticed in butternut might be due to degradation of heat sensitive compounds during blanching.

Conclusion

Among the various indigenous food ingredients tested, amaranth leaves (*Amaranthus hypondricus*) and drumstick leaves (*Moringa oleifera*) exhibited the highest flavonoid content, antioxidant as well as type II diabetes related enzyme inhibition properties. When considering the effect of processing methods, soaking + cooking of grains and blanching of vegetables degrades the chemical property of flavonoids and thus adversely affect the antioxidant and functional properties of the food ingredients. On the other hand, roasting of grains and cooking of vegetables were observed as the most suitable mild treatments to

preserve the flavonoids level and its functional properties. Hence, such viable and mild treatments for the respective food samples can be recommended for the formulation of antioxidant-rich supplementary foods for the vulnerable groups in Kenya. Further, the synergistic or antagonistic association of flavonoids from different indigenous food ingredients of the present study warrants further investigation, before using them together in supplementary food formulations.

CHAPTER 6

Antioxidant and Antidiabetic Properties of Condensed Tannins in Acetonic Extract of Selected Raw and Processed Indigenous Food Ingredients from Kenya

Abstract

Recently, tannins have received considerable attention as health-promoting component in various plant foods and several studies have reported on its nutraceutical properties. However, no study has established the role of condensed tannins in indigenous foods of Kenya. Therefore, this study was designed to evaluate the antioxidant activity (DPPH and FRAP) and antidiabetic effects (α-amylase and α-glucosidase inhibition activities) of condensed tannins in some selected raw and traditionally processed indigenous cereals, legumes, oil seeds and vegetables. The condensed tannin content of the grains and vegetables ranged between 2.55 and 4.35 g/100 g DM and 1.53 and 5.73 g/100 g DM, respectively. The scavenging effect of acetonic extract on DPPH radical ranged from 77 to 90% while the reducing power was found to be 31-574 mmol Fe(II)/g DM in all the investigated food ingredients. The condensed tannin extracts of the analyzed samples showed promising antidiabetic effects with potential α-amylase and α-glucosidase inhibition activities of 23-44% and 58-88% respectively. Condensed tannins extracted from the amaranth grain, finger millet, field bean, sunflower seeds, drumstick and amaranth leaves exerted significantly higher antioxidant and antidiabetic activities than other food ingredients. Among the traditional processing methods, roasting of grains and cooking of vegetables were found to be more suitable mild treatments for preserving the tannin compound and its functional properties as opposed to soaking + cooking and blanching treatments. The identified elite sources of optimally processed indigenous food ingredients with promising results could be used as health-promoting ingredients through formulation of therapeutic diets.

Keywords: Condensed tannins; indigenous food ingredients; antioxidant activity; antidiabetic effect; traditional processing methods.

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Introduction

Tannins are widespread in the foods of plant origin, particularly in legume seeds, cereal grains, fruits, vegetables and different beverages such as wine, tea, cocoa as well as cider. They are an integral part of human diets and have positive implications for health and nutrition (Serrano *et al.*, 2009). Currently there is an increasing interest in tannins as bioactive component of foods as well as biological antioxidants. Tannins are a unique group of water-soluble phenolic metabolites of relatively high molecular weight and having the ability to complex strongly with carbohydrates and proteins (Chavan *et al.*, 2001). In the past, tannins have been viewed as one of the antinutrients of plant origin because of their ability to precipitate proteins, inhibit the digestive enzymes and decrease the absorption of vitamins and minerals (Khattab *et al.*, 2010). However, recently several health benefits have been attributed to intake of tannins and some epidemiological correlations with the decreased incidence of chronic diseases have been established (Serrano *et al.*, 2009). Numerous studies have demonstrated potentially significant biological effects of tannins such as antioxidant or radical scavenging activity as well as inhibition of lipid peroxidation and lipoxygenases *in vitro* (Amarowicz *et al.*, 2000; Gyamfi *et al.*, 2002), antimicrobial and antiviral (Dolara *et al.*, 2005; De Bruyne *et al.*, 1999), antimutagenic (Dolara *et al.*, 2005; Carlsen *et al.*, 2010), and antidiabetic properties (Matsui *et al.*, 2001; Anderson & Polansky 2002). The antioxidant activity of tannins results from their free radical- and reactive oxygen species-scavenging properties, as well as the chelation of transition metal ions that initialize the oxidation process (Serrano *et al.*, 2009). Antioxidants have also been reported to provide synergistic benefits for the treatment of diabetes because of their insulin enhancing potential (Madhujith *et al.*, 2004).

Indigenous cereals, legumes and vegetables play an important role in the traditional diets of many developing countries and they are low in fat; excellent sources of protein,

dietary fibre, as well as a variety of micronutrients (Tontisirin *et al.*, 2002). Recent experimental and epidemiological studies have also shown that grains, vegetables, and fruits have a large variety of bioactive constituents called "plant chemicals" or "phytochemicals" (Adebooye *et al.*, 2008; Adom & Liu 2002). Phytochemicals refer to every naturally occurring chemical substance present in plants, especially those that are biologically active such as tannins, phenolics, flavonoids, phytates and others. Now-a-days, increased consumption of these foods has been widely promoted because in addition to provision of micronutrients they also provide in diets these bioactive phytochemicals which are strongly associated with health maintenance and prevention of chronic diseases (De Bruyne *et al.*, 1999; Dolara *et al.*, 2005; Gin *et al.*, 1999). As a result, consumption of diverse plant food ingredients can help in addressing the double burden of micronutrient deficiencies and chronic diseases. Current research efforts have indicated that the complex mixture of bioactive compounds in foods provides better protective health benefits than single phytochemicals through a combination of additive and/or synergistic effects (Adom & Liu, 2002).

Most of the plant food ingredients undergo thermal processing, which has been demonstrated to significantly alter their chemical profiles. In most communities, it is a common practice for most of the food ingredients to undergo thermal treatment such as soaking, blanching and cooking, or dry heating before being consumed. Several studies have reported both positive and negative effects of thermal treatment depending upon differences in the processing conditions, morphological and nutritional characteristics of the food ingredients (Amin *et al.*, 2006; Granito *et al.*, 2008; Siddhuraju, 2006). The quantity of tannins in foods was severely modified during various food processing techniques due to their highly reactive nature, which ultimately affects their antioxidant activity and other functional properties and thus decrease the nutraceutical property of plant foods (Khandelwal

et al., 2010). As a result, identification of the most elite thermal processing method that preserves the condensed tannins and their functional properties would be fundamental for the populations consuming these indigenous food ingredients with claimed medicinal effects so as to reap maximum health benefits.

A review by Chung Kim-Thom et al. (1998) has raised the curiosity on potential role of the tannins as health-promoting components in plant food-stuffs. Nonetheless, the antioxidant and antidiabetic properties of condensed tannins present in indigenous cereals, legumes, oil seeds and vegetables growing in Kenya have not been reported so far. Therefore, the aim of this study was to evaluate the condensed tannin content in the acetonic extract of selected cereals, legumes, oil seeds and vegetables commonly consumed by vulnerable groups of Kenya as well as to screen for their antioxidant and antidiabetic activities that could be of the essence in human nutrition and health. Further, the effect of certain traditional processing methods, which are particularly practiced by the ethnic group in Kenya, on the level of condensed tannins and functional properties of various indigenous food-stuffs was investigated with a view to select the viable and more suitable processing techniques in order to incorporate the optimally processed elite food ingredients in the formulation of therapeutic supplementary foods for vulnerable groups of Kenya.

Materials and methods

Chemicals

The chemicals used include 2,2′-Diphenyl-1-picryl hydraxyl (DPPH, Ref. No.: 217-591-8,); 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ, Ref. No. 3682-35-7), Butylated Hydroxytoluene (Ref. No. 204-881-4), o-vanillin, 99% (2-Hydroxy-3-methoxybenzaldehyd, Ref. No. 05614BJ030), α-amylase (Ref. No. 9001-19-8), and α-glucosidase (Ref. No. 9001-42-7) were obtained from Sigma chemicals, USA, and all other chemicals were purchased from Merck, Darmstadt, Germany.

Sample collection

Samples included cereals such as finger millet (*Eleusine coracana* L. Gaertn. P-224) and amaranth grain (*Amaranthus cruentus* L.); legumes such as pigeonpea (*Cajanus cajan* (L.) Millsp. Kat/Mbaazi 3) and field bean (*Dolichos pupureum* L. Kat/DL-3); oil seeds such as groundnut (*Arachis hypogea* L.), pumpkin seed (*Cucurbita maxima* Duchesne ex Lam.) and sunflower seed (*Helianthus annuus* L. PAN 7369). The vegetables selected were pumpkin (*Cucurbita maxima* L.), butternut (*Juglans cinerea* L.), sweet potatoes (*Ipomoea batatas* [L.] Lamk. SPK 004), and leafy vegetables such as drumstick leaves (*Moringa oleifera* L.), amaranth leaves (*Amaranthus hybridus* L.) and pumpkin leaves (*Cucurbita maxima* Lam.). All the food ingredients (1 kg each) were obtained from the Kenya Agricultural Research Institute (KARI), Nairobi, Kenya.

Processing of cereals, legumes and oil seeds

The grains (cereals, legumes, and oil seeds) were randomly divided into three batches for each. The first batch was stored without any treatment and regarded as raw samples. The second batch of samples (100 g each) was washed with tap water and then soaked in distilled water (200 ml) for 8 h in the dark at 25 ± 1^0 C and cooked at 90-95^0 C for 120 min in fresh distilled water in the ratio of 1:4. The third batch of samples was roasted in an open-pan iron container to a golden brown color for 30 min using traditional charcoal burner at 150^0 C with continuous stirring to avoid burning of the seed coat. After roasting, the samples were cooled to room temperature.

Processing of vegetables and leafy vegetables

The fresh vegetables were randomly divided into three batches and the first batch was stored as such without any treatment and regarded as raw samples. The second batch of samples

(100 g of edible portion) were cut into small pieces or cubes and washed under running tap water, then cooked in 200 ml of distilled water at 90-95^0 C for 5 min for all samples, except sweet potatoes, pumpkin and butternut which are cooked for 15 min. The third batch of vegetables was blanched by immersing in boiling water for 5 min after cutting into small pieces or cubes.

Preparation of acetonic extract

All the raw, cooked and blanched samples were dried in hot-air oven at 50^0 C for 6 h and then milled using a laboratory mill (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland) and sieved (0.1mm) to obtain fine flour for further analyzes. The condensed tannins were extracted from the grains and vegetable samples, by taking 1 g of defatted flour sequentially with 10 ml of 100%, 90%, 80% and 70% acetonic acidified with 1% conc. HCl. After centrifugation at 16,000 rpm for 10 min, all the supernatants were pooled together and made up to a known volume (50 ml) with acetonic. Then the extract was purified by using Sephadex LH-20 column chromatography (96 x 1.6 cm) with acetonic:water (50:50, v/v) as a solvent. After collecting 20 fractions (5 ml each), the active fractions were identified and pooled together in a round-bottom flask. The solvent was evaporated by using rotary vacuum evaporator (Büchi Rotavapor, CH-9230, Switzerland) at 40° C, freezed overnight at -80° C and dried in lyophilier (Virtis Freezemobile 25 EL, New York) for 10 h and finally the residue was weighed and the total dry yield of extract was calculated. Then the extract was re-dissolved in distilled water:formic acid (97.5:2.5, v/v) solution in the ratio of one mg/ml and used for further analysis.

Analysis of condensed tannins

The partially purified acetonic extract of different indigenous food ingredients were used for the quantification of condensed tannins by using modified vanillin-HCl reagent method (Price *et al.*, 1978). The aliquot (100 µl) was taken in a test tube and made up to 1 ml with distilled water and treated with 3 ml of 4% vanillin dissolved in methanol (W/V) and 5 ml of 70% HCl-methanol (V/V). After standing for 12 min at room temperature, the contents were mixed well and the absorbance was measured at 500 nm in a UV-Visible Spectrophotometer (Perkin-Elmer, Lambda 35). The standard curve was prepared by taking different concentrations of catechin and the level of tannins was calculated and expressed on g/100 g DM basis.

Antioxidant activity

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of acetonic extract was analyzed by following the method of Sanchez-Moreno et al. (1998). The acetonic extract (0.1 ml) of each sample was added to 3.9 ml (0.025 g/L) of DPPH solution. BHT, (1 mg/ml) was used as a positive control. The solutions were incubated at room temperature (25 ^{0}C) for 30 min and at the end of incubation period the decrease in absorbance was determined at 515 nm with a Spectrophotometer. The absorbency of pure DPPH solution incubated at 25° C for 30 min was regarded as control and the radical scavenging activity of acetonic extract of each sample was calculated and the results were expressed as percent basis.

Ferric reducing antioxidant power (FRAP)

The reducing property of the acetonic extract was estimated according to the procedure described by Pulido et al. (2000). FRAP reagent (900 µl), prepared freshly and incubated at

37 °C, was mixed with 90 µl of distilled water and 30 µl of test sample or methanol (for the reagent blank) or BHT (10 mg/10 ml) as positive control. The test samples and reagent blank were incubated at 37 °C for 30 min in a water bath. The FRAP reagent contained 2.5 ml of 20 mM TPTZ solution (2,4,6-Tris (2-pyridyl)-s-triazine) in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 0.3 M acetate buffer, pH 3.6 (16.8 g of glacial acetic acid, MW 60.05 and 2.69 g of sodium acetate trihydrate, MW 136.1 dissolved in 1 L of distilled water). At the end of incubation the absorbance readings were taken immediately at 593 nm using a Spectrophotometer. Acetonic solutions of known Fe (II) concentration ranging from 100 to 2000 µM ($FeSO_4.7H_2O$) were used for the preparation of the calibration curve.

Antidiabetic activity

α-Amylase inhibition activity

The α-amylase inhibition activity was measured by following the method of Worthington (1993). One hundred microlitre of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to 100 µl of acetonic extract of each sample in 100 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (1 unit liberates 1.9 µl of maltose from starch in 1 min at pH 6.9 and temperature 25 °C), and was incubated at 25 °C for 30 min. After the incubation, the reaction was stopped with 1.0 ml of dinitrosalicylic acid reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted to 10-folds with distilled water and the absorbance was measured at 540 nm. The readings were compared with the control, which contained buffer instead of sample extract. Based on the absorbance value, the percent inhibition activity was calculated for all the samples.

α-Glucosidase inhibition activity

The α-glucosidase inhibition activity was determined according to the method described by Worthington (1993). One hundred microlitre of acetonic extract and 100 µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 unit/ml) were taken in tubes and incubated at 25 ^{0}C for 5 min. After the pre-incubation, 100 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each tube and the reaction mixture was incubated at 25 ^{0}C for 5 min. After the incubation period the aliquots were diluted with 10-fold distilled water, and the absorbance readings recorded at 405 nm and compared to a control that had 100 µl of buffer solution in place of the extract. The results were calculated and expressed as percentage of α-glucosidase inhibition.

Statistical analysis

All analyses were performed in triplicate (n = 3), and the data was presented as means standard error of deviation (± SEM). The results obtained were analyzed by using two-way ANOVA to determine the significant differences between the experimental batches by taking the raw samples as control. GraphPad PRISM® version IV software, San Diego, CA was used for statistical analysis.

Results and discussion

Tannin content

The condensed tannin content of acetonic extract of the raw indigenous cereals, legumes and oil seeds are shown in Fig. 6.1. Condensed tannin content of the grains of the present study ranged from 2.55 to 4.35 g/100 g DM with the finger millet and field bean exhibiting the highest levels. These values falls within the range reported for grains and pulses such as beach pea, Indian grass pea, Canadian grass pea, red gram, Bengal gram, green gram, lentil

and canola seeds (0.11 - 11.6 g/100 g) (Chavan *et al.*, 2001; Khandelwal *et al.*, 2010; Khattab *et al.*, 2010). On the other hand, the condensed tannin content of legumes (3.1 - 3.7 g/100 g) of the current study are comparable to the levels reported for two varieties of horse gram seeds (2.7 - 3.9 g/100 g) consumed in India (Siddhuraju & Manian 2007) but higher than those observed in moth bean (1.91 g/100 g) (Siddhuraju, 2006).

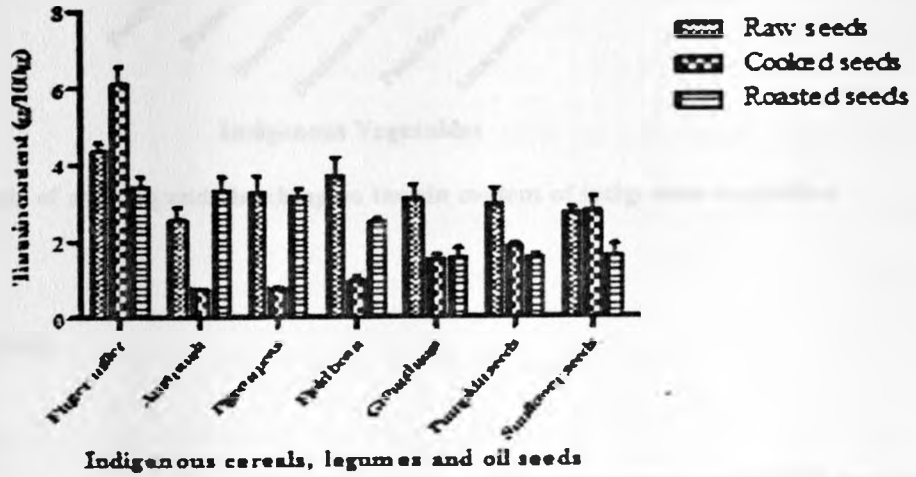

Fig 6.1 Effects of cooking and roasting on tannin content of the grains

The vegetables investigated in the current study were observed to contain condensed tannins between 1.53 and 5.73 g/100 g DM with the drumstick leaves and sweetpotatoes exhibiting the highest levels (Fig. 6.2). The tannin content of the indigenous leafy vegetables (3.15-5.73 g/100g) of the current study are within the levels (0.15-13.30 g/100g) reported in underutilized green leafy vegetables such as *Coleus aromaticus* and *Delonix elata* in India (Gupta *et al.*, 2005). The difference was attributed to the variation in the type of vegetables analyzed and agronomic practices in the two regions of studies. No recent work has been published on the condensed tannin content of Kenyan indigenous vegetables and to our knowledge this is the first report.

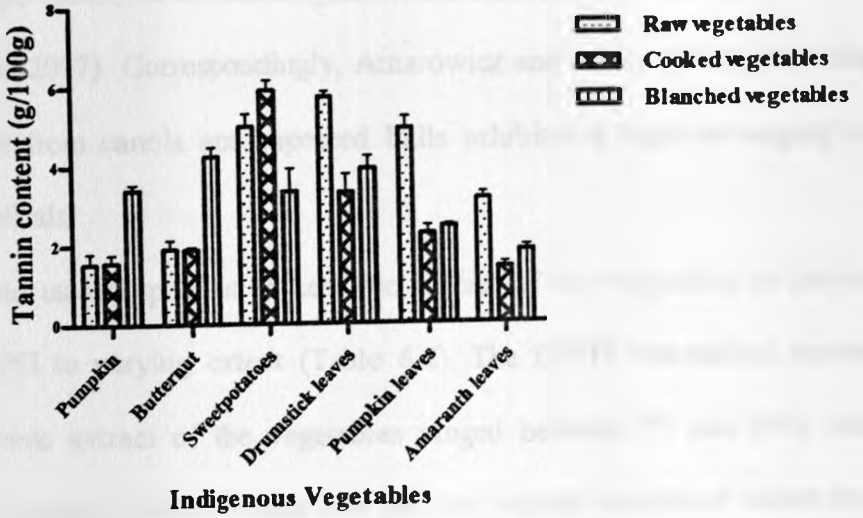

Fig 6.2 Effects of cooking and blanching on tannin content of indigenous vegetables

Antioxidant activity

DPPH Assay

In general, acetonic extracts from the raw grains exhibited promising DPPH free-radical scavenging activity (86 - 89%), comparable with that of synthetic antioxidant BHT (94%) (Table 6.1). BHT is a synthetic antioxidant with phenolic structure and it has been used in various food systems because of its high stability, low cost, and other practical advantages. However, its use in foods has been questioned due to possible toxic or carcinogenic compounds formed during its degradation. Finger millet of the current study had the highest tannin content (4.35 g/100 g) among the investigated grains and also exhibited a high level of scavenging activity (89%) against the DPPH free radical. Sripriya et al. (1996) demonstrated that the brown and red variety of finger millet, which is commonly available, had higher activity (94%) than the white variety (4%) using the DPPH method. DPPH method has also shown that the finger millet is a more potent radical scavenger than rice, wheat or sorghum. Similarly, the DPPH radical scavenging potential of oil and legume grains of the present study was higher when compared to the earlier report on tannins extracted from canola oil

seeds (45%) and two varieties of horse gram seeds (56-68%) (Khattab *et al.*, 2010; Siddhuraju & Manian 2007). Correspondingly, Amarowicz and others (2000) observed that the tannins extracted from canola and rapeseed hulls exhibited a high scavenging ability against the DPPH radicals.

The condensed tannins present in acetonic extract of the vegetables in the current study quenched DPPH to varying extent (Table 6.2). The DPPH free-radical scavenging activity of the acetonic extract of the vegetables ranged between 77 and 90% with the sweetpotatoes and drumstick leaves, which also had the highest condensed tannin content, exhibiting the highest activity. The DPPH scavenging activity of acetonic extract in majority of the food ingredients was comparable to that of BHT (90-94%) which shows the potential use of these foods as natural antioxidants. Recently, there has been scientific evidence demonstrating that high concentration of tannins extracted from stem bark of *Cassia fistula* had an elevated DPPH radical quenching capacity (Siddhuraju *et al.*, 2002). Tannins have been considered superior antioxidants as their eventual oxidation may lead to oligomerization via phenolic coupling and enlargement of the number of reactive sites, a reaction which has never been observed with the other phenol compounds such as flavonoids (Siddhuraju & Becker 2007).

Table 6.1 Antioxidant activity of tannin in acetonic extract of raw and processed indigenous grains by DPPH and FRAP assay

Food samples	DPPH assay (%)			FRAP Assay (mMol Fe(II)/g)		
	Raw seeds	Cooked seeds	Roasted seeds	Raw seeds	Cooked seeds	Roasted seeds
Finger Millet	89.00^{a} ±0.01	89.67^{a} ±0.33	89.00^{a} ±0.01	120.72^{a} ±1.60	79.67^{a} ±2.47	156.49^{a} ±8.33
Amaranth	89.67^{a} ±0.33	87.00^{b} ±0.01	89.33^{a} ±0.33	31.01^{a} ±3.91	97.22^{b} ±15.50	26.59^{a} ±1.35
Pigeon pea	89.00^{a} ±0.01	89.67^{a} ±0.33	89.00^{a} ±0.01	83.09^{a} ±6.33	203.17^{b} ±3.98	105.17^{a} ±8.15
Field bean	86.67^{a} ±0.33	89.00^{b} ±0.01	87.00^{a} ±0.01	98.33^{a} ±1.18	260.77^{b} ±20.83	178.38^{c} ±2.93
Groundnuts	89.00^{a} ±0.01	89.67^{a} ±0.33	88.33^{a} ±0.67	71.02^{a} ±11.39	75.56^{a} ±1.02	185.50^{c} ±4.65
Pumpkin seeds	88.33^{a} ±0.67	85.33^{b} ±0.33	86.67^{a} ±0.33	46.66^{a} ±4.48	71.57^{a} ±3.67	80.24^{a} ±3.67
Sunflower seeds	89.67^{a} 0.33±	86.67^{b} ±0.33	88.33^{a} ±0.67	180.71^{a} ±1.87	154.43^{a} ±18.52	340.29^{c} ±48.18

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

FRAP Activity

The FRAP activity of acetonic extract of the raw grains ranged from 31.01 to 180.71 mmol Fe[II]/g with the highest reducing power observed in sunflower seeds and finger millet (Table 6.1). The FRAP values of legumes of the current study (76 - 261 mmol Fe[II]/g) were comparable to the levels reported in acetonic extract of legumes such as yellow pea (128 - 134 mmol Fe[II]/g), green pea (103 - 108 mmol Fe[II]/g), black bean (931 - 1103 mmol Fe[II]/g), chick pea (73 - 108 mmol Fe[II]/g), soy bean (27 - 993 mmol Fe[II]/g) and red kidney beans (382 - 922 mmol Fe[II]/g) collected in USA (Xu & Chang 2007), and Desi chickpea (73 - 90 mmol Fe[II]/g) indigenous to Pakistan (Zia-Ul-Haq *et al.*, 2008). Such potential reducing power could be attributed to the presence of dihydroxy type of benzene derivatives, (+) catechin and (-) epicatechin, which are the integral part of condensed tannins, present in the grains' seed coats. The reducing power of bioactive compounds including the high molecular tannins extracted from peanut hulls and stem bark of Indian laburnum have been strongly linked to high antioxidant activity, specifically scavenging of free radicals (Siddhuraju *et al.*, 2002).

Table 6.2 Antioxidant activities of tannin in acetonic extracts of raw and processed indigenous vegetables

Food samples	DPPH assay (%)			FRAP Assay (mMol Fe(II)/g)		
	Raw vegetables	Cooked vegetables	Blanched vegetables	Raw vegetables	Cooked vegetables	Blanched vegetables
Pumpkin	87.00^{a} ±0.01	88.33^{a} ±1.33	83.67^{c} ±0.33	286.09^{a} ±33.86	338.52^{a} ±34.92	114.37^{c} ±4.22
Butternut	89.33^{a} ±0.33	88.67^{a} ±0.88	87.67^{a} ±0.67	238.58^{a} ±29.13	217.50^{a} ±5.88	158.89^{c} ±5.88
Sweetpotatoes	90.00^{a} ±0.01	90.33^{a} ±0.33	89.67^{a} ±0.33	46.45^{a} ±1.83	41.08^{a} ±1.08	37.86^{a} ±6.85
Drumstick leaves	89.00^{a} ±0.01	91.00^{a} ±0.01	90.67^{a} ±0.33	339.15^{a} ±8.47	83.72^{b} ±10.62	71.38^{c} ±4.71
Pumpkin leaves	76.67^{a} ±0.33	84.67^{b} ±1.20	80.67^{c} ±0.33	85.90^{a} ±4.63	246.88^{b} ±18.00	122.02^{a} ±6.11
Amaranth leaves	88.33^{a} ±0.67	87.33^{a} ±0.88	78.33^{c} ±1.20	573.53^{a} ±22.62	366.91^{b} ±18.14	147.08^{c} ±5.89

[1]Values are mean and ± standard error of means of three separate determinations (n = 3).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

The FRAP value of acetonic extract of raw vegetables were observed to vary from 46.45 to 573.53 mmol Fe[II]/g with the highest level observed in amaranth and drumstick leaves (Table 6.2). The reducing ability of the vegetables of the current study is comparable

to the values reported for a variety of vegetables (100 - 4810 mmol Fe[II]/g) consumed worldwide such as curly kales, celery, leaves of African baobab tree, artichoke and broccoli (Carlsen *et al.*, 2010). However, when compared to the synthetic antioxidant such as BHT (2370 mmol Fe[II]/g), all the tested food ingredients showed significantly ($p < 0.05$) lower reducing power.

Antidiabetic activity

α-Amylase inhibition activity

The α-amylase inhibition activity of acetonic extract of grains ranged between 29 and 40% with the field bean and amaranth grains exhibiting the highest level (Table 6.3). Correspondingly, tannins extracted from the black bean (*Phaseolus vulgaris var. Cubagua*) grown in Venezuela were observed to inhibit α-amylase by 50% (Carmona *et al.*, 1996). Tannins extracted from different plants have been shown to exhibit inhibition of α-amylase enzyme activity (Gin *et al.*, 1999; Hosoyama *et al.*, 2003). Tannins can delay intestinal glucose absorption and the onset of insulin-dependent diabetes mellitus by producing an insulin-like effect on insulin-sensitive tissues which lowers glucose levels as well as regulating the antioxidant environment of pancreatic β cells (Serrano *et al.*, 2009). Consequently, it is not astounding that the acetonic extract of the presently investigated samples that contained high level of condensed tannins (field bean, sweet potato and amaranth leaves) were exhibiting more effective α-amylase inhibition activity.

The α-amylase inhibition activity of acetonic extract of the presently studied vegetables ranged from 24 to 44% with amaranth leaves and sweetpotatoes exhibiting the highest activity (Table 6.4). Similarly, the tannins and ellagic acid derivatives from Banaba (*Lagerstroemia speciosa* L.) leaves have been reported to be potent inhibitors of α-amylase (Hosoyama *et al.*, 2003). Zhang and Kashket (1997) also identified tannins as the active

constituents in green and black teas responsible for inhibition of α-amylase. The levels of the α-amylase inhibition activity exhibited by the food ingredients in this study were at an appreciable level and would represent adequate synergistic potential with insulin in controlling post-meal blood glucose levels.

Table 6.3 Antidiabetic effects of tannin in acetonic extract of cereal, legume and oil seeds

Food samples	α-amylase inhibition			α-glucosidase inhibition		
	Raw seeds	Cooked seeds	Roasted seeds	Raw seeds	Cooked seeds	Roasted seeds
Pearl Millet	32.33^{a} ±2.33	32.67^{a} ±2.96	39.00^{a} ±0.58	85.00^{a} ±5.00	95.00^{a} ±0.01	91.00^{a} ±1.00
Amaranth	39.00^{a} ±0.58	40.33^{a} ±0.33	46.33^{a} ±1.67	78.33^{a} ±4.41	68.33^{a} ±6.67	68.33^{a} ±3.33
Pigeon pea	35.67^{a} ±2.19	35.67^{a} ±1.33	43.00^{a} ±3.00	88.33^{a} ±1.67	63.33^{b} ±7.27	90.00^{a} ±1.16
Field bean	39.67^{a} ±1.76	38.33^{a} ±0.88	39.33^{a} ±3.53	83.33^{a} ±4.41	56.67^{b} ±4.41	91.00^{a} ±2.00
Groundnuts	29.67^{a} ±5.49	47.33^{b} ±0.88	27.67^{a} ±2.03	73.33^{a} ±1.67	87.67^{a} ±1.33	87.33^{a} ±0.33
Pumpkin seed	35.00^{a} ±2.52	42.67^{a} ±6.12	38.00^{a} ±1.00	65.00^{a} ±7.64	87.00^{b} ±2.08	74.00^{a} ±7.00
Sunflower seed	33.67^{a} ±4.70	38.33^{a} ±6.49	40.00^{a} ±0.58	81.67^{a} ±4.41	85.33^{a} ±1.86	86.00^{a} ±1.00

[1]Values are mean and ± standard error of means of three separate determinations (n = 3).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

α-Glucosidase inhibition activity

The condensed tannins present in acetonic extract of the presently investigated food ingredients caused significant inhibition of α-glucosidase (59-88%). The extent of inhibition of α-glucosidase was related to their condensed tannin content. For example, finger millet, field bean, sweet potato and drumstick leaves, which had the highest tannin content, were the most effective inhibitors of α-glucosidase. Tannins have been reported to effectively inhibit intestinal α-glucosidase activity similar to synthetic inhibitors such as acarbose and voglibose, which are already being used therapeutically to control non-insulin-dependent diabetes mellitus (Matsui *et al.*, 2001). A study of patients with non-insulin dependent diabetes mellitus revealed that tannic acid and tannin-rich nonalcoholic compounds of red wine can reduce the serum glucose levels after starch-rich meals (Gin, 1999). The α-glucosidase inhibition activity of acetonic extract of the grains ranged from 65 to 88% with pigeon pea and finger millet demonstrating the highest activity (Table 6.3). Similarly, Matsui

et al. (2001) reported α-glucosidase inhibition activity of 10-25% in corn. α-Glucosidase inhibition activity of the presently investigated vegetables falls between 59 and 83% with the sweetpotatoes and drumstick leaves showing the highest level. These values are higher than the α-glucosidase inhibition activity reported for vegetables in Japan such as sweet potato, cabbage and radish (20-45%) (Matsui *et al.*, 2001). This disparity could be attributed to varietal differences, climate and geographical location. Tannins from tea extract have been reported to possess insulin-enhancing properties and are known to regulate hepatic glucose output (Anderson & Polansky, 2002), which also could help in the management of diabetic patients.

Table 6.4 Antidiabetic effects of tannin in acetonic extract of raw and processed indigenous vegetables

Food samples	α-amylase inhibition			α-glucosidase inhibition		
	Raw vegetables	Cooked vegetables	Blanched vegetables	Raw vegetables	Cooked vegetables	Blanched vegetables
Pumpkin	32.00^{a} ±1.00	14.00^{b} ±3.06	38.33^{a} ±5.24	70.67^{a} ±7.45	65.33^{a} ±6.36	82.33^{a} ±3.84
Butternut	0.00^{a} ±0.00	3.00^{a} ±0.01	3.33^{a} ±1.45	74.00^{a} ±3.79	59.00^{a} ±1.53	69.33^{a} ±5.78
Sweetpotatoes	40.67^{a} ±1.20	33.67^{a} ±3.33	31.00^{a} ±1.00	83.00^{a} ±4.36	94.33^{a} ±3.67	92.00^{a} ±2.08
Drumstick leaves	32.67^{a} ±1.33	8.00^{b} ± 0.88	13.33^{c} ±6.33	75.67^{a} ±1.33	73.00^{a} ±2.08	73.67^{a} ±1.67
Pumpkin leaves	23.67^{a} ±0.33	54.33^{b} ±4.26	30.00^{a} ±4.73	60.00^{a} ±2.52	57.67^{a} ±0.33	55.00^{a} ±2.52
Amaranth leaves	44.33^{a} ±1.33	50.00^{a} ±1.00	30.00^{c} ±4.04	58.67^{a} ±0.67	54.33^{a} ±1.33	53.67^{a} ±4.33

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

Effects of processing

Soaking + cooking of grains

Soaking + cooking caused significant level of reduction of tannin content, DPPH radical scavenging ability and α-glucosidase inhibition activity of some of the grains such as amaranth grain and the legumes of the present study. This was attributed to leaching due to the solubility of tannins. Other causes of the loss would be the possible complexing of tannins with proteins present in high content in the amaranth grain and legumes, with the eventual effect of rendering the tannins unavailable for detection by the analytical methods used. Such complexing would also lower the antioxidant and antidiabetic properties of tannins. However,

increases were observed in the tannin content of finger millet, DPPH scavenging ability of field bean and the α-glucosidase inhibition activity of pumpkin seeds. Regarding the tannins, soaking + cooking caused significant decrease ($p \leq 0.05$) of 52-76% with the highest losses being recorded for the pigeon pea, field bean and amaranth grain (Fig 6.1). Similarly, Granito et al. (2008) when studying the effects of thermal processing on the tannin content of *Phaseolus vulgaris* beans found that cooking produced a significant decrease of 55.5%. The same authors observed that cooking exerted a great negative impact on the DPPH scavenging activity of the Phaseolus beans, which is in agreement with the results of the present study.

The tannin contents of Faba beans (*Vicia faba*) and four Indian pulses was reported to be significantly reduced by domestic processes such as soaking for 12 h (22.1-51%) and cooking (33.3-81%) (Serrano *et al.*, 2009; Khandelwal *et al.*, 2010). From the present study, it is observed that the cooked samples had a low concentration of tannins and functional properties possibly due to (1) the degradation or polymerization of tannins into more highly-polymerized compounds, which could result in incomplete extraction or quantification, and (2) tannins are not completely extracted by solvent, due to the formation of insoluble tannin-protein and tannin-carbohydrate as well as cell wall polysaccharide complexes. The reductions observed in the α-glucosidase inhibition activity of pigeon pea and field bean of the present investigation could be due to the complexing of tannins with proteins during soaking and cooking of the seeds, which may render them unable to interact with the digestive enzyme.

The FRAP values and α-amylase inhibition activity of the grains in the current study were not affected by soaking + cooking. However, it caused significant increases in the FRAP activity of amaranth, pigeon pea and field bean, while high α-amylase inhibition activity was noticed in groundnuts. Similarly, Randhir and others (2008) noticed substantial increases in antioxidant and α-amylase inhibition activity in selected grain sprouts and

seedlings due to thermal processing. Processing can promote the oxidation of polyphenols to an intermediate oxidation state, which can exhibit a higher radical scavenging efficiency than the non-oxidized ones. Likewise, the formation of either insoluble or soluble tannin-protein complexes, which are formed as a result of food/grain processing, has been reported as a potential free radical scavenger and radical sinks (Siddhuraju & Manian, 2007).

Roasting of grains

Roasting did not cause any significant losses in the tannin content of most of the grains of the presently investigated samples. Roasting is a dry heating method which would not adequately degrade the tannins in most of the food ingredients and any losses observed could be attributed to oxidation of the tannin compound. However, significant reductions were noticed in field bean, groundnuts and pumpkin seeds. The losses observed during roasting of groundnuts in the current study were similar to the small but significant reduction in tannins (14% loss at 204 ^{0}C in 5 min) reported during roasting of walnuts (Sze-Tao *et al.*, 2001). Siddhuraju and Becker (2007) also reported the presence of significantly higher level of tannins in raw legumes such as cowpea seeds than in dry heated samples, which are comparable to the results observed for field bean of the present study. The higher levels of tannins observed in raw grains than processed grains could be attributed to increased activity of tannin degradation enzymes such as polyphenol oxidase and other catabolic enzymes during the thermal treatments.

No significant losses were observed in the DPPH radical scavenging activity, FRAP values, α-amylase and α-glucosidase inhibition activities of all the grains investigated in the current study. However, significant increases were noticed in the FRAP values of groundnuts, field bean, and sunflower seeds as well as α-amylase and α-glucosidase inhibition activity of groundnuts and pumpkin seeds respectively. Similarly, Siddhuraju and Becker (2007) noticed

high radical scavenging activity of 83.6% and 68.3%, in dry heated samples of light brown and dark brown cow pea seed extracts. Randhir et al. (2008) have also demonstrated that thermal processing increase the α-amylase inhibition activity in buck wheat and oats as well as α-glucosidase inhibition activity of wheat, buckwheat and oats. An increase in antioxidant activity was observed in the presently analyzed roasted samples was probably because of the matrix softening and increased extractability of compounds, which could be partially converted into more antioxidant chemical species.

Cooking of vegetables

The tannin content of the vegetables of the present study was not significantly affected by cooking, except in some leafy vegetables. The losses could be attributed to the leaching of tannins into the cooking water. Adebooye et al. (2008) have also observed losses in tannin contents (66.1 - 73.5%) of two leafy vegetables (*Solanum nigrum* L. and *Amaranthus cruentus* L.) consumed in India after cooking regardless of the pretreatment methods used. Losses may also be attributed to decreases in extractability, as polymerization of lower molecular weight compounds, thus becoming insoluble in water.

Cooking of vegetables did not cause any significant decreases in the DPPH radical scavenging activity, FRAP values, α- glucosidase inhibition activity of all the presently investigated samples. However, significant increases were observed in the above-mentioned parameters for pumpkin leaves. The increases observed in the cooked vegetables might be due to the Maillard reaction products, in addition to tannin constituents, which could also be effectively participating as radical scavengers. Processing may enhance a foods' potential as a good antioxidant source by increasing the amount of antioxidants released from the food matrix which otherwise would be less or not at all available for absorption (Carlsen *et al.*, 2010). The α-amylase inhibition activity of the vegetables in the current study was not

affected by cooking except significant losses noticed in pumpkin and drumstick leaves. Correspondingly, thermal processing was reported to cause a significant decrease in the α-amylase inhibitory activity of wheat and corn sprouts and seedlings (Randhir *et al.*, 2008).

Blanching of vegetables

Blanching caused significant reductions in the tannin content, DPPH radical scavenging activity, FRAP values and α-amylase inhibition activity of some of the vegetables in the current study (Table 6.2 & 6.4). These decreases could be attributed to the enzyme polyphenols oxidase, which can be activated during blanching, resulting in degradation and consequent loss of tannins and other polyphenolic compounds. Similarly, blanching has been reported to be very effective in decreasing assayable tannins (98% reduction in 2 min of blanching) (Sze-Tao *et al.*, 2001). Amin et al. (2006) observed that the antioxidant activity in all types of raw spinach was higher than that of the blanched counterparts indicating that blanching may greatly influence the loss of antioxidant components in leafy vegetables. Further, these authors also noticed that the DPPH radical scavenging activity of the spinach (*Amaranthus* sp.) decreased tremendously after blanching for up to 15 minutes.

However, significant increases were noticed in the tannin content of pumpkin and butternut, DPPH activity of pumpkin leaves as well as FRAP values of amaranth leaves. Recently, even mild thermal processing techniques such as blanching have raised concern regarding the impact on functional components even though it is an important method of preserving vegetables (Amin *et al.*, 2006). Blanching did not affect the α-glucosidase inhibition activity in all the presently investigated vegetables. Heating of the tannins during blanching of vegetables did not affect its inhibitory properties, confirming the thermostable character of these compounds when exposing to a high temperature for a short time.

Therefore, condensed tannins are likely to remain active even after thermal processing of foods under similar conditions of temperature and time of blanching.

Conclusion

In recent years, the tannin compounds receive more attention as a nutraceutical compound and have been reported to associate with many potential health benefits such as antioxidant, antidiabetic, anti-carcinogenic and antimicrobial activities, even though some previous literature indicates their adverse nutritional effects. Hence, the beneficial properties of dietary tannins need to be investigated extensively against their anti-nutritional properties. In this juncture, the condensed tannins in the acetonic extract of various indigenous food ingredients of Kenya investigated in the present study exhibited potential antioxidant and antidiabetic properties. Among the food-stuffs analyzed, finger millet, field bean, sweetpotatoes and drumstick leaves were found to possess a high level of condensed tannins, which correlated well with high DPPH radical scavenging activity, ferric reducing power, α-amylase and α-glucosidase inhibition activities observed for these foods. Generally, processing did not adversely affect the functional properties of acetonic extract of most food ingredients investigated in this study except reductions observed for some food ingredients after cooking of grains and blanching of vegetables. Thus, the soaking + cooking treatment for grains and blanching for vegetables were considered as aggressive practices, while the roasting and cooking were appears to be the more suitable mild treatments for the processing of grains and vegetables, respectively, to preserve the level of condensed tannins and their functional properties. Therefore, such viable processing techniques will be taken into account for the formulation of therapeutic supplementary foods for the vulnerable groups in Kenya in future.

CHAPTER 7

Antioxidant and Type II Diabetes-related Functional Properties of Phytic Acid Extract from Kenyan Indigenous Food Ingredients: Effects of Traditional Processing Methods

Abstract

Emerging scientific evidences revealed that phytic acid has several positive effects on human health. The antioxidant and type II diabetes-related enzyme inhibition properties of phytic acid extract prepared from raw and traditionally processed indigenous grains and vegetables collected from Kenya were evaluated. Phytic acid content of raw grains and vegetables ranged between 2.81-3.01 and 0.29-3.23 g'/100 g DM, respectively. The phytic acid extract from raw samples revealed 59-89% of DPPH radical scavenging capacity, 27-3526 mmol Fe(II)/g of reducing power, 20-72% of α-amylase inhibition activity and 8-91% of α-glucosidase inhibition activity. Cooking and roasting have improved the antioxidant and functional properties of phytic acid extract obtained from Kenyan indigenous vegetables and grains, respectively.

Keywords: Indigenous food ingredients, phytic acid, antioxidant activity, functional property, traditional processing methods.

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Introduction

Phytic acid (myo-inositol 1,2,3,4,5,6, hexakis-dihydrogen phosphate; IP_6) is the major storage form of phosphorus in plants comprising 60-90% of total phophorus. It is an ubiquitous seed constituent, composing 1.0-5.0 % of edible portion of all nuts, cereals, legumes and oil seeds (Champ, 2002). Recently, there has been an increasing interest on the protective biochemical functions of phytic acid and its application in the prevention of oxidative stress related diseases in human beings (Khattab *et al.*, 2010; Verghese *et al.*, 2006). There is growing scientific and epidemiological evidences associating phytic acid intake with reduced incidences of cardiovascular disease, cancer and age-related degenerative processes (Jariwalla *et al.*, 1990).

Phytic acid was found to play a major role in the treatment of cancer, hypercholesterolemia, hypercalcuria and kidney stones (Grases *et al.*, 2000; Plaami, 1997; Vucenik & Shamsuddin, 2003). Subsequently, the beneficial properties of phytic acid such as antioxidant (Graf & Eaton, 1990; Khattab *et al.*, 2010), anticarcinogenic (Shamsuddin, 1995; Verghese *et al.*, 2006), anticalcification (Grases *et al.*, 2000; Schlemmer *et al.*, 2009) and hypoglycemic or hypolipidemic (Jariwalla *et al.*, 1990; Lee *et al.*, 2006, 2007) were evaluated, which have great importance in human nutrition. Therefore, studies on the positive effects of dietary phytic acid have revived research about its significance in human nutrition and health.

Phytic acid is predominantly present in unprocessed foods, but can be degraded during thermal processing and resulting in a broad range of inositol phosphates, which are being consumed in the diet (Khattab & Arntfield, 2009). The profiles and quantities of phytic acid in foods are affected by processing due to their highly reactive nature, which may also affect their functional properties (Egounlety & Aworh, 2003; Schlemmer *et al.*, 2009). Hydrolysis of phytic acid during soaking, cooking and fermentation is reported mainly due to

phytic acid-degrading activity of phytase enzyme that naturally present in plants. Consequently, food processing is crucial in affecting the phytic acid content by dephosphorylation and regulation of composition of partially phosphorylated myo-inositol phosphate ester with health benefits.

The mean daily intake of phytic acid for infants (~1½ - 2½ years) and pupils (7 - 9 years) is 1066 and 2390 mg, respectively in Kenya (Schlemmer *et al.*, 2009). Even though reports of studies on the nutritional value of indigenous Kenyan foods are abounding (Neumann *et al.*, 2003), the levels of phytic acid and its functional properties in these foods remain unexplored. Moreover, the effects of commonly applied domestic processing techniques on the concentration of phytic acid and its functional properties have been studied even less. Therefore, the present study was aimed to evaluate the antioxidant potential and type II diabetes related enzyme inhibition characteristics of phytic acid extract obtained from raw and processed Kenyan indigenous food samples with a view to identify the most elite food sample(s) as well as suitable processing method(s) in preserving the functionalities.

Materials and Methods

Chemicals

The chemicals used include sodium phytate (Ref: 238-242-6); 2,2′-Diphenyl-1-picryl hydrazyl (DPPH, Ref. No.: 217-591-8,); 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ, Ref. No. 3682-35-7), Butylated Hydroxytoluene (Ref. No. 204-881-4), α-amylase (Ref. No. 9001-19-8), and α-glucosidase (Ref. No. 9001-42-7) were obtained from Sigma Chemical, USA, and all other chemicals were purchased from Merck, Darmstadt, Germany.

Sample collection and preparation

The samples were collected on the basis of common food samples used by vulnerable groups in Nairobi, Kenya. Samples included cereals such as finger millet (*Eleusine coracana* L. Gaertn. P-224) and amaranth grain (*Amaranthus cruentus* L.); legumes such as pigeonpea (*Cajanus cajan* (L.) Millsp. Kat/Mbaazi 3) and field bean (*Dolichos pupureum* L. Kat/DL-3); oil seeds such as groundnut (*Arachis hypogea* L.), pumpkin seed (*Cucurbita maxima* Duchesne ex Lam.) and sunflower seed (*Helianthus annuus* L. PAN 7369). The vegetables selected were pumpkin (*Cucurbita maxima* L.), butternut (*Juglans cinerea* L.), sweetpotatoes (*Ipomoea batatas* [L.] Lamk. SPK 004), and leafy vegetables such as drumstick leaves (*Moringa oleifera* L.), amaranth leaves (*Amaranthus hybridus* L.) and pumpkin leaves (*Cucurbita maxima* Lam.). The cereals, legumes, oil seeds and vegetable samples (1 kg each) were randomly obtained from Kenya Agricultural Research Institute (KARI), Kenya as well as different parts of Kenya from the agricultural fields. Then they were mixed to obtain a representative sample, which was then subdivided into three portions for different treatments with three replicates.

Processing of cereals, legumes and oil seeds

The grains (cereals, legumes, and oil seeds) were randomly divided into three batches for each. The first batch was stored as such without any treatment and regarded as raw samples. The second batch of samples (100 g each) was washed with tap water and then soaked in distilled water (200 ml) for 8 h in the dark at 25 ±1 ^{0}C and cooked at 90-95 ^{0}C for 120 min in fresh distilled water in the ratio of 1:4. The third batch of samples was roasted in an open-pan iron container to a golden brown color for 30 min using traditional charcoal burner at 150 ^{0}C with continuous stirring to ensure even roasting. After roasting, the samples were cooled to room temperature.

Processing of vegetables and leafy vegetables

The fresh vegetables were randomly divided into three batches and the first batch was stored as such without any treatment and regarded as raw samples. The second batch of samples (100 g of edible portion) were cut into small pieces or cubes (approximately 1cm in size) and washed under running tap water, then cooked in 200 ml of distilled water at 90-95 ^{0}C for 5 min for all samples, except sweet potatoes, pumpkin and butternut which are cooked for 15 min. The third batch of vegetables were each cut as above and blanched by immersing in boiling water for 5 min.

All the raw, cooked and blanched samples were dried in hot-air oven at 50 ^{0}C for 6 h (As per the traditional method practiced by the vulnerable group in Kenya for the preparation of food) and then milled using a laboratory mill (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland) and sieved (0.1mm) to obtain fine flour for further analyzes.

Analysis of phytic acid

The phytic acid was extracted from raw and differentially processed samples by taking 1 g of defatted flour with 10 ml of 2.4% HCl and incubated for 10 min in ultra-sonic bath followed by magnetic stirrer for 30 min. Then the contents were centrifuged at 13,000 x g for 5 min and the supernatant was collected. Similarly, the residue was re-extracted twice and all the supernatants were pooled together and made up to a known volume with distilled water. The extract was purified by using an anionic-exchange column chromatography (0.7 cm x 15 cm) containing 0.5 g of anion-exchange resin (100–200 mesh, chloride form; AG1-X4, Bio-Rad Co., CA, USA) and phytic acid was eluted with 2 M HCl.

Phytic acid was quantified according to Latta and Eskin method (1980). The partially purified extract (500 µl) was diluted to 2.5 ml with distilled water and 1 ml of Wade reagent

(0.03% $FeCl_3.6H_2O$ and 0.3% sulfosalicylic acid) was added. The contents were vortexed and centrifuged at 3500 x g for 5 min and the absorbance of the supernatant was measured at 500 nm using a UV-Vis Spectrometer (Perkin-Elmer, Lambda 35, USA). The phytic acid content was calculated by using the standard curve prepared with synthetic sodium phytate and expressed as g/100 g on dry weight basis. The extract was frozen at – 80 °C for overnight and dried in lyophilier (Virtis Freeze mobile 25 EL, New York) for 8 h and finally the residue was weighed and the total dry yield of extract was calculated. The extract was re-dissolved in distilled water the ratio of one milligram of extract per millilitre of solvent and used for further analysis.

DPPH radical scavenging activity

The DPPH radical scavenging activity of phytic acid extract was analyzed by following the method of Sanchez-Moreno et al. (1998). The aqueous extract (0.1 ml) of each sample was added to 3.9 ml (0.025 g/L) of DPPH solution. BHT (1 mg/ml) was used as a positive control and pure DPPH solution alone was used as negative control. The solutions were incubated at room temperature (25 ^{0}C) for 30 min and at the end of incubation period the decrease in absorbance was determined at 515 nm with a Spectrophotometer. The radical scavenging activity of the tested samples was measured as a decrease in the absorbance of DPPH radical and was calculated by using the equation,

DPPH radical scavenging activity (%) = $(1 - A_{\text{samples}}/A_{\text{negative control}}) \times 100$.

Ferric reducing antioxidant power (FRAP)

The reducing property of the aqueous extract was estimated according to the procedure described by Pulido et al. (2000). FRAP reagent (900 µl), prepared freshly and incubated at 37 ^{0}C, was mixed with 90 µl of distilled water and 30 µl of test sample or distilled water (for

the reagent blank) or BHT as positive control. The test samples and reagent blank were incubated at 37 ^{0}C for 30 min in a water bath. The FRAP reagent contained 2.5 ml of 20 mM TPTZ solution (2,4,6-Tris (2-pyridyl)-s-triazine) in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 0.3 M acetate buffer, pH 3.6 (16.8 g of glacial acetic acid, MW 60.05 and 2.69 g of sodium acetate trihydrate, MW 136.1 dissolved in 1 L of distilled water). At the end of incubation the absorbance readings were taken immediately at 593 nm using a Spectrophotometer. Methanolic solutions Different concentrations of Fe(II) ranging from 100 to 2000 µM ($FeSO_4.7H_2O$) were used for the preparation of the calibration curve.

α-Amylase inhibition activity

The α-amylase inhibition activity was measured following the method of Worthington (1993). One-hundred microlitre of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to 100 µl of aqueous extract of each sample in 100 µl of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (1 unit liberates 1.9 µl of maltose from starch in 1 min at pH 6.9 and temperature 25 ^{0}C), and was incubated at 25 ^{0}C for 30 min. After the incubation, the reaction was stopped with 1.0 ml of dinitrosalicylic acid reagent. The test tubes were incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted to 10-folds with distilled water and the absorbance was measured at 540 nm. The readings were compared with the control, which contained buffer instead of sample extract. Based on the absorbance value, the percent inhibition activity was calculated for all the samples.

α-Glucosidase inhibition activity

The α-glucosidase inhibition activity was determined according to the method described by Worthington (1993). One-hundred microlitre of aqueous extract and 100 µl of 0.1 M

phosphate buffer (pH 6.9) containing α-glucosidase solution (1 unit/ml) were taken in tubes and incubated at 25 ^{0}C for 5 min. After the pre-incubation, 100 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each tube and the reaction mixture was incubated at 25 ^{0}C for 5 min. After the incubation period the aliquots were diluted with 10-fold distilled water, and the absorbance readings recorded at 405 nm and compared to a control that had 100 µl of buffer solution in place of the extract. The results were calculated and expressed as percentage of α-glucosidase inhibition.

Statistical analysis

All analyses were performed in triplicate (n = 3), and the data was presented as means standard error of deviation (± SEM). The results obtained were analyzed by using two-way ANOVA to determine the significant differences between the experimental batches by taking the raw samples as control. GraphPad PRISM® version IV software, San Diego, CA was used for statistical analysis.

RESULTS AND DISCUSSION

Phytic acid content

Phytic acid contents for the grain samples analyzed in the present study ranged from 2.57 to 3.01 g/100 g DM with the finger millet and pumpkin seeds exhibiting the highest contents (Fig. 7.1). The phytic acid content of the oil seeds (2.81-3.01 g/100 g DM) in the present study was comparable to the previous values reported for phytic acid in canola oil seed (2.16-3.75 g /100 g) (Khattab *et al.*, 2010). Earlier report indicated that phytic acid occur in various foods at different concentrations ranging from 0.1 to 6.0 g/100 g (Verghese *et al.*, 2006). Data obtained from this study for phytic acid was comparable with that of cereals such as maize, wheat, rice, millet, sorghum and barley (0.06-3.35 g/100 g) as well as legumes such as kidney

beans, pinto beans, black eye beans, broad beans, cowpeas and peas (0.22-2.90 g/100 g) reported in a review on phytic acid in foods by Schlemmer et al. (2009). The variation in range of phytic acid contents in different grains reflect the great number of botanical; varieties of seeds; various growing environmental or climatic conditions and different stages of seed maturation.

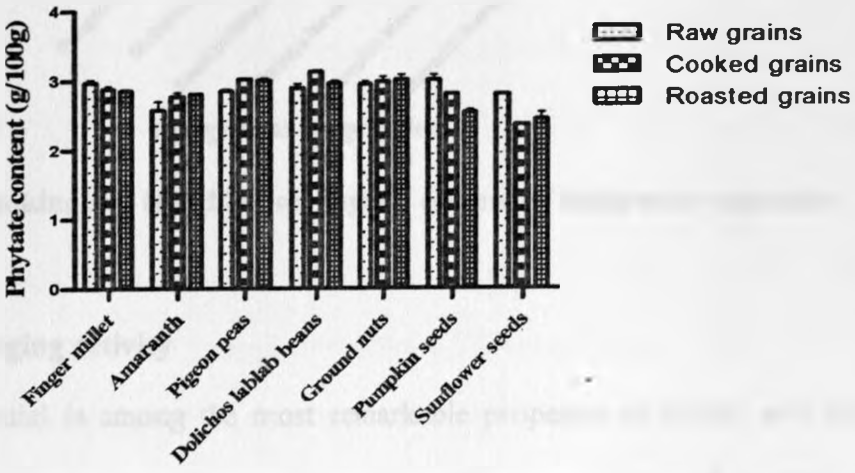

Fig 7.1 Effects of cooking and roasting on the phytate content of grains

Among the vegetables investigated in this study, the phytic acid content was found to range from 0.29 to 3.23 g/100 g (Fig. 7.2). Sweetpotato and butternut were found to possess highest levels of phytic acid among the vegetables, while the leafy vegetables had significantly low phytic acid content. The phytic acid content of the leafy vegetables (0.29-2.95 g/100 g) evaluated in this study were comparable to those reported for green leafy vegetable such as amaranth (*Amaranthus tricolor*), bathua (*Chenopodium album*), fenugreek (*Trigonella foenumgrecum*) and spinach (*Spinacia oleracia*) (129.67-234.50 mg/100 g) grown in India (Yadav & Sehgal, 2003). The variation in phytic acid content of vegetables could be due to varietal differences and growing locations.

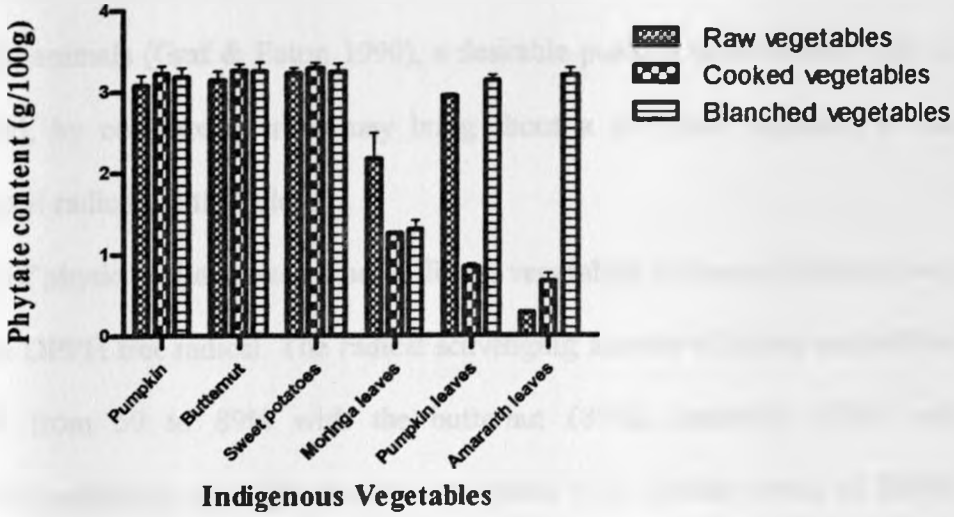

Fig 7.2 Effects of cooking and blanching on phytate content of indigenous vegetables

DPPH radical scavenging activity

The antioxidant potential is among the most remarkable properties of phytic acid and is mainly based on complexing iron between three phosphate groups through inhibition of hydroxyl radical formation from H_2O_2 via Fenton reaction by the Fe^{2+}-phytic acid complex (Schlemmer *et al.*, 2009). The ability of phytic acid extracted from raw and processed grains to scavenge the stable synthetic DPPH free radical is shown in Table 7.1. The phytic acid extract of raw grains exhibited promising levels of the DPPH free-radical scavenging activity (78 - 85%), but lower than the synthetic antioxidant BHT (94%).

Among the studied grains, the phytic acid extract of finger millet and pumpkin seeds, which had high levels of phytic acid were observed to also exhibit significantly high radical scavenging activity. The phytic acid extracted from oil seeds in the current study exhibited excellent radical scavenging activity (83-85%) which was comparable to the earlier values reported for canola oil seed (63%) (Khattab *et al.*, 2010). Phytic acid occurring in grains acts as an antioxidant through the formation of chelates with pro-oxidant transition metals. In the past, due to this property phytic acid was considered as an antinutrient due to the mineral

binding activity. However, recently phytic acid has been reported to reduce the risk for colon and breast cancer in animals (Graf & Eaton 1990), a desirable positive health effect. This is because phytic acid, by complexing iron, may bring about a favorable reduction in the formation of hydroxyl radicals in the colon.

The ability of phytic acid extracted from different vegetables to donate hydrogen was estimated using the DPPH free radical. The radical scavenging activity of phytic acid of the vegetables ranged from 59 to 89% with the butternut (89%), pumpkin (87%) and sweetpotatoes (87%) exhibiting the highest activities (Table 7.2). Similar levels of DPPH radical scavenging activity were reported for sweetpotato (87%) grown in Japan (Oki *et al.*, 2002). The antioxidant activity of pumpkin (87%) of the current study was comparable to the value (78.4%) reported for the same sample cultivated in Malaysia (Azizah *et al.*, 2009). The butternut, pumpkin and sweetpotatoes were also observed to possess higher phytic acid content among the presently studied vegetables and thus exhibiting high antioxidant property.

Table 7.1 Antioxidant activity of phytic acid extract of raw and processed indigenous grains

	DPPH assay (%)			FRAP Assay (mmol Fe[II]/g)		
Food samples	**Raw seeds**	**Cooked seeds**	**Roasted seeds**	**Raw seeds**	**Cooked seeds**	**Roasted seeds**
Finger Millet	$84.67^{a} \pm 2.85$	$74.33^{a} \pm 0.88$	$77.67^{a} \pm 2.33$	$52.52^{a} \pm 5.47$	$46.88^{a} \pm 0.86$	$93.57^{b} \pm 1.84$
Amaranth grain	$81.33^{a} \pm 0.33$	$72.33^{a} \pm 0.33$	$71.67^{a} \pm 1.20$	$26.86^{a} \pm 0.96$	$103.80^{b} \pm 2.51$	$51.72^{a} \pm 2.20$
Pigeonpea	$81.33^{a} \pm 0.33$	$82.67^{a} \pm 0.33$	$72.67^{a} \pm 2.40$	$45.11^{a} \pm 3.50$	$75.84^{b} \pm 2.83$	$77.64^{c} \pm 1.45$
Field bean	$78.00^{a} \pm 0.58$	$75.67^{a} \pm 3.18$	$83.00^{a} \pm 1.00$	$77.94^{a} \pm 10.06$	$59.41^{a} \pm 2.55$	$118.53^{b} \pm 6.75$
Ground nuts	$83.33^{a} \pm 0.33$	$83.33^{a} \pm 5.69$	$84.67^{a} \pm 2.85$	$44.50^{a} \pm 0.58$	$56.86^{a} \pm 0.60$	$75.93^{b} \pm 0.69$
Pumpkin seeds	$85.33^{a} \pm 0.88$	$65.00^{b} \pm 2.00$	$81.67^{a} \pm 1.76$	$109.31^{a} \pm 6.84$	$73.69^{b} \pm 2.50$	$171.57^{c} \pm 6.90$
Sunflower seeds	$85.33^{a} \pm 1.67$	$66.33^{b} \pm 1.76$	$86.00^{a} \pm 3.51$	$106.59^{a} \pm 4.21$	$207.20^{b} \pm 24.45$	$86.50^{a} \pm 1.14$

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

FRAP activity

Phytic acid forms an iron chelate, which accelerates Fe^{2+}-mediated oxygen reduction yet blocks iron-driven hydroxyl radical generation and suppresses lipid peroxidation (Khattab *et al.*, 2010). The FRAP activity of phytic acid extract of the raw grains ranged from 25.86 to

109.31 mmol Fe[II]/g with the highest reducing power observed in pumpkin seeds and sunflower seeds, which also exhibited high DPPH radical scavenging activity (Table 7.1). The FRAP values of legumes of the current study (78-119 mmol Fe[II]/g) were comparable to the levels reported in other legumes such as yellow pea (54-159 mmol Fe[II]/g), green pea (62-116 mmol Fe[II]/g), black bean (113-1103 mmol Fe[II]/g), chick pea (73-113 mmol Fe[II]/g), soy bean (127-993 mmol Fe[II]/g) and red kidney beans (285-922 mmol Fe[II]/g) collected from USA (Xu & Chang, 2007).

The FRAP activity of phytic acid extract of the presently studied vegetables ranged from 42.59 to 3526.46 mmol Fe[II]/g with the high reducing power recorded in the leafy vegetables, particularly in the amaranth and pumpkin leaves. Certain vegetables notably potato, kale, cabbage, cucumber, brussel sprouts and spinach have been reported to have slightly lower antioxidant power (ranging from 24 to 265 mmol Fe[II]/g) (Halvorsen *et al.*, 2002) than the presently studied vegetables. Phytic acid may supply an important antioxidant function by its metal binding characteristics.

Table 7.2 Antioxidant activities of phytic acid extracts of raw and processed indigenous vegetables

Food Samples	DPPH assay (%)			FRAP Assay (mmol Fe[II]/g)		
	Raw vegetables	Cooked vegetables	Blanched vegetables	Raw vegetables	Cooked vegetables	Blanched vegetables
Pumpkin	86.67^{a} ±4.33	84.00^{a} ±3.22	55.00^{b} ±2.65	72.42^{a} ± 1.39	154.74^{a} ± 2.12	203.89^{b} ± 4.78
Butternut	88.67^{a} ±2.60	75.67^{a} ±3.71	66.00^{b} ±1.00	92.28^{a} ± 5.62	118.70^{a} ± 1.31	183.23^{a} ± 2.97
Sweet potatoes	87.33^{a} ±2.33	79.33^{a} ±4.09	72.00^{b} ±1.24	42.59^{a} ± 2.79	71.30^{a} ± 1.26	39.88^{a} ± 4.37
Drumstick leaves	61.33^{a} ±5.33	59.67^{a} ±0.88	69.00^{a} ±2.00	228.15^{a} ± 9.23	390.63^{b} ± 8.66	481.57^{c} ± 6.96
Pumpkin leaves	59.33^{a} ±1.20	65.67^{a} ±1.76	71.00^{a} ±6.00	238.41^{a} ± 16.17	528.60^{b} ± 3.15	181.18^{a} ± 1.71
Amaranth leaves	81.67^{a} ±1.45	74.00^{a} ±1.16	72.00^{a} ±8.88	3526.46^{a} ± 68.78	1148.15^{b} ± 93.97	119.89^{c} ± 2.70

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

α-Amylase inhibition activity

Foods that result in low blood glucose response have been shown to have immense nutritional significance in the prevention and/or management of type II diabetes mellitus. Inhibition of

α-amylase by phytic acid lowers the blood glucose response and may prove to be useful in the clinical management of hyperglycemia and diabetes (Jariwalla *et al.*, 1990; Lee *et al.*, 2006). The *in vitro* reduction of starch digestion was positively correlated with the *myo*-inositol phosphate concentration and negatively with the number of phosphate groups on the *myo*-inositol ring (Lee *et al.*, 2006). The α-amylase inhibition activity of phytic acid of presently analyzed grains was ranged between 55 and 72% with the pumpkin seeds and finger millet exhibiting the highest level (Table 7.3).

The α-amylase inhibition activity of phytic acid extract of the presently studied vegetables ranged from 24 to 67% with sweetpotatoes and drumstick leaves exhibiting the highest activity (Table 7.4). Kaushik et al. (2010) reported varying degree of hypoglycemic and anti-hyperglycemic activity of commonly consumed vegetables in India. The authors revealed that sweetpotato also exhibited high antidiabetic activity and were recommended for the management of type II diabetes mellitus, in addition to other food samples. All the presently analyzed phytic acid extracts inhibited α-amylase action, indicating that phytic acid would have a potential function to suppress the elevation of postprandial glucose level from starch. It should be noted that till to date there are no studies to reveal the inhibition of α-amylase enzyme activity and in turn reducing the starch digestion by phytic acid from Kenyan indigenous food samples and so, to our knowledge this is the first report.

Table 7.3 Type II diabetes related properties of phytic acid extracted from cereals, legumes and oil seeds

Food samples	α-Amylase inhibition (%)			α-Glucosidase inhibition (%)		
	Raw grains	Cooked grains	Roasted grains	Raw grains	Cooked grains	Roasted grains
Finger millet	65.67^{a} ±3.53	60.33^{a} ±3.84	65.33^{a} ±1.67	91.33^{a} ±0.88	44.00^{b} ±1.35	73.00^{c} ±3.79
Amaranth	65.00^{a} ±3.22	65.00^{a} ±3.06	63.67^{a} ±1.76	14.00^{a} ±2.08	35.00^{b} ±4.04	25.67^{a} ±5.93
Pigeon pea	55.33^{a} ±2.33	55.67^{a} ±2.60	66.00^{a} ±5.13	60.67^{a} ±1.33	33.33^{b} ±8.21	79.00^{c} ±3.51
Field beans	56.67^{a} ±5.24	48.00^{a} ±4.16	71.67^{b} ±2.96	49.33^{a} ±0.67	7.00^{b} ±0.01	27.67^{c} ±3.18
Groundnuts	57.00^{a} ±3.00	63.67^{a} ±2.19	65.67^{a} ±5.84	16.33^{a} ±0.67	28.33^{a} ±6.39	54.67^{b} ±4.33
Pumpkin seeds	71.67^{a} ±2.60	46.00^{b} ±2.52	64.33^{c} ±3.84	27.33^{a} ±1.45	26.67^{a} ±0.88	69.33^{b} ±0.67
Sunflower seeds	56.00^{a} ±2.52	53.67^{a} ±2.60	78.00^{b} ±1.16	40.67^{a} ±1.86	27.33^{a} ±2.67	13.67^{b} ±0.67

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).

[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

α-Glucosidase inhibition activity

The phytic acid interaction with starch and divalent metals has been shown to result in low glycemic index and also reduces the participation of iron in oxidation metal mediated effects related with low diabetes (Schlemmer *et al.*, 2009). Phytic acid extract of the presently investigated food ingredients caused significant inhibition of α-glucosidase enzyme activity (8 - 91%). The extent of inhibition of α-glucosidase of food samples was directly related to their phytic acid content which could prove to be synergistic to their potential therapeutic effect on post-meal blood glucose level. For example, finger millet and butternut, which had the highest phytic acid content in the presently investigated food ingredients, were the most effective inhibitors of α-glucosidase. Generally, the grains were observed to exhibit greater α-glucosidase inhibition activity when compared to the vegetables. The α-glucosidase inhibition activity of phytic acid extract of the grains ranged from 14 to 91% with pigeon pea and finger millet demonstrating the highest activity (Table 7.3). Epidemiological studies have reported the low incidence of diabetes in populations consuming millets in their regular diets (Shobana *et al.*, 2009), which might be due to their potential α-glucosidase inhibition activity as revealed by the present study.

α-Glucosidase inhibition activity of the presently investigated vegetables falls between 8 and 66% with the butternut and amaranth leaves showing the highest level (Table 7.4). Recent studies have reported that certain indigenous foods such as pumpkin, corn, beans and sweet potato exhibit α-glucosidase inhibition activity and have the potential to reduce hyperglycemia-induced pathogenesis (Kwon *et al.*, 2007). To our knowledge, this is the first study reporting the α-glucosidase inhibition activity of these indigenous vegetables.

Table 7.4 Type II diabetes related properties of phytic acid extracted from raw and processed indigenous vegetables

Food samples	α-Amylase inhibition (%)			α-Glucosidase inhibition (%)		
	Raw vegetables	Cooked vegetables	Blanched vegetables	Raw vegetables	Cooked vegetables	Blanched vegetables
Pumpkin	62.33^{a} ±2.02	55.00^{a} ±4.16	45.67^{b} ±5.04	11.67^{a} ±3.28	8.33^{a} ±0.88	5.00^{a} ±0.01
Butternut	20.33^{a} ±4.33	38.67^{b} ±2.33	38.00^{c} ±4.16	66.00^{a} ±1.53	31.67^{b} ±2.03	5.00^{c} ±0.01
Sweet potatoes	68.00^{a} ±1.53	56.67^{a} ±2.03	63.00^{a} ±3.51	8.00^{a} ±0.01	4.33^{a} ±0.06	62.00^{b} ±6.00
Drumstick leaves	67.33^{a} ±2.19	36.00^{b} ±2.51	49.33^{c} ±1.67	36.33^{a} ±0.67	30.67^{a} ±3.18	29.67^{a} ±1.45
Pumpkin leaves	62.00^{a} ±0.58	69.00^{a} ±0.58	65.00^{a} ±1.53	40.67^{a} ±0.67	29.33^{a} ±0.67	17.00^{b} ±1.00
Amaranth leaves	64.00^{a} ±2.08	64.33^{a} ±2.67	64.00^{a} ±5.86	48.00^{a} ±2.08	35.33^{a} ±7.33	19.33^{b} ±1.33

[1]Values are mean and ± standard error of means of three separate determinations ($n = 3$).
[2]Values in the same row with different alphabet superscripts are significantly different ($p < 0.05$).

Effects of processing

Soaking + cooking of grains

Phytic acid is quite stable up to 100 ^{0}C and it cannot be easily denatured by heat treatment such as that applied in house-hold cooking, roasting, pressure cooking and fermentation. However, enzymatic degradation of phytic acid by phytase occurs naturally during food processing and may result in strong phytic acid hydrolysis (Schlemmer *et al.*, 2009). Phytases are naturally present in plant parts, which involved in enzymatic dephosphorylation of phytic acid. In the present study, soaking and cooking caused no significant losses in the phytic acid content, FRAP activity, DPPH radical scavenging activity and α-amylase inhibition effect of some of the grains (Fig. 7.1, Tables 7.1 & 7.3). However, significant losses were observed in the phytic acid content of sunflower seeds (16%); DPPH radical scavenging activity of pumpkin and sunflower seeds; FRAP values and α-amylase inhibition activity of pumpkin seeds; and α-glucosidase inhibition activity of finger millet, pigeon pea and field bean. Similarly, the phytic acid content of cowpea, pea and kidney pea seeds were significantly reduced during soaking (43-49%) and cooking (31-69%) (Khattab & Arntfield, 2009).

Soaking of cereals and legumes can promote diffusion of phytic acid into soaking water, which was attributed to the fact that phytic acid in dried grains exist wholly as a water soluble salt such as sodium/potassium phytate. A significant phytic acid reduction can be

realized by discarding the soaked water since phytic acid is water soluble, in addition to the action of endogenous phytases. Xu and Chang (2008) have also reported a decrease in DPPH activity of boiled green pea, yellow pea and chick pea due to leaching of soluble antioxidant components into boiling water.

In addition, soaking and cooking of grains resulted in significant increases in the phytic acid content of field bean, FRAP activity of the amaranth grain, pigeon pea and groundnuts, as well as α-glucosidase inhibition activity of amaranth grain. Similarly, increased phytic acid content after soaking of soybean, ground bean and cowpea for 12-14 h has been observed by Egounlety and Aworh (2003). During food processing, phytic acids can be dephosphorylated to produce degradation products such as myo-inositol pentakis (IP_5), tetrakis (IP_4), tris- (IP_3), bis- (IP_2) and monophosphate (IP_1).

Roasting of grains

Roasting did not cause significant reduction in the phytic acid content in all the grains, except the pumpkin (15%) and sunflower seeds (13%) (Fig 7.1). Similarly, Khattab and Arntfield (2009) reported significant reduction (35-40%) of phytic acid content during roasting of cowpea, pea and kidney pea seeds which were attributed partly due to the formation of insoluble complexes between phytic acid and other components. Roasting also did not cause any significant losses in the DPPH radical scavenging activity, FRAP values and α-amylase inhibition activity of all the grains investigated in the present study (Tables 7.1 & 7.3). However, significant losses were noticed in the α-glucosidase inhibition activity of finger millet, field bean and sunflower seeds.

Significant increases were observed in the phytic acid content of amaranth grain; FRAP activity of finger millet, amaranth grain, pigeon pea and groundnuts; α-amylase inhibition activity of field bean and sunflower seeds; as well as α-glucosidase inhibition

activity of pigeon pea, ground nuts and pumpkin seeds during roasting. Reports from other studies indicate that roasting caused no significant losses in the phytic acid content of small red kidney beans as well as α-amylase inhibition activity of both red peanuts and kidney beans, but losses were observed after roasting of red peanuts (Ejigui *et al.*, 2005).

Cooking of vegetables

Cooking degrades phytic acid to myo-inositols with a lower number of phosphate groups with similar beneficial effects like those of IP6, as it has been found that myo-inositols are involved in cell signaling in mammalian cells (Vucenik & Shamsuddin, 2003). Cooking caused no significant reduction in the phytic acid content of most of the vegetables, except losses observed in the drumstick leaves (42%) and pumpkin leaves (71%) (Fig 7.2). Similarly, it has been reported that cooking of some green leafy vegetable such as amaranth (*Amaranthus tricolor*), bathua (*Chenopodium album*), fenugreek (*Trigonella foenum grecum*) and spinach (*Spinacia oleracia*) did not cause significant changes their phytic acid content (Yadav & Sehgal, 2003).

Cooking did not cause any significant losses in the DPPH radical scavenging activity, FRAP activity, α-amylase and α-glucosidase inhibition activities of all the vegetables (Tables 7.2 & 7.4). During cooking endogenous phytases are inactivated by heat and made ineffective to breakdown the phytic acid, which can then only be degraded by high temperature (Yadav & Sehgal, 2003). However, slight losses were noticed in α-amylase inhibition activity of drumstick leaves as well as α-glucosidase inhibition activity of butternut while significant increases were observed in FRAP activity of all the leafy vegetables and α-amylase inhibition activity of butternut. Phytic acid is heat stable and significant heat destruction of phytic acids during cooking is not expected to occur. Therefore, considerable phytic acid dephosphorylation during cooking only takes place either by discarding the cooking water or

by enzymatic phytic acid hydrolysis due to the action of the intrinsic plant phytases during the early part of the cooking phase.

Blanching of vegetables

Blanching caused no significant loss of phytic acid content in most of the vegetables investigated in this study, except reductions noticed in the drumstick leaves (Fig. 7.2). However, significant losses were observed in DPPH radical scavenging activity of pumpkin, butternut and sweetpotatoes; FRAP activity of pumpkin and amaranth leaves; α-amylase inhibition activity pumpkin and drumstick leaves; as well as α-glucosidase inhibition activity of butternut, pumpkin and amaranth leaves (Tables 7.2 & 7.4). Blanching of some selected vegetables such as *bathua*, fenugreek and spinach leaves from India was observed to reduce the phytic acid level due to the rupture of cell walls that causes soluble phytic acid to leach out in the blanching medium (Yadav & Sehgal, 2003). Significant increases were observed in the phytic acid content and FRAP activity of amaranth leaves and pumpkin, as well as α-amylase inhibition activity of butternut and α-glucosidase inhibition activity of sweetpotatoes.

Recent studies have shown conflicting findings with relation to effects of blanching on the antioxidant activity of vegetables. Reports by several authors have demonstrated that blanching significantly influence the antioxidant activity of vegetables and the effects were not consistent in different foods with some showing increases in antioxidant activity while others noticing decreases (Amin *et al.*, 2006; Turkmen *et al.*, 2005; Xu & Chang 2008). The increased antioxidant potential caused by processing could be due to improvement of naturally occurring compounds or formation of novel compounds, possessing high antioxidant activity. On the other hand, leaching of antioxidant compounds into water during

boiling and degradation or formation of complexes can contribute to lower antioxidant activity.

CONCLUSION

The present study revealed that certain selected Kenyan indigenous grains and vegetables contain high levels of phytic acid, associated with high antioxidant and type II diabetes related enzyme inhibition properties. A good relationship has been observed between phytic acid content and antioxidant as well as functional properties. Finger millet and amaranth leaves exhibited excellent type II diabetes-related enzyme inhibition properties while pumpkin and sunflower seeds showed promising antioxidant activity. Cooking of vegetables and roasting of grains have improved the antioxidant and functional properties of phytic acid extract. The presently studied indigenous grains and vegetables could contribute to the significant supply of dietary antioxidants to prevent oxidative stress related chronic diseases including type II diabetes, which is a growing problem among vulnerable groups in Kenya. The elite food sources and potential processing methods identified from the present investigation could be considered further for the formulation of supplementary foods with functional properties for the vulnerable group of Kenya.

CHAPTER 8

Nutritional Characteristics, Acceptability, Shelf-stability and Cost of a Supplementary Food for Vulnerable Groups Developed from Local Food Ingredients

Abstract

Food based approaches have been advocated as the best strategies to curb hunger and malnutrition in developing countries. In the present study, a low-cost food supplement was developed using different processed local foods to meet the nutrient requirements for vulnerable groups. Initially four formulations consisting of cereals, legumes, nuts, fish and vegetables were developed based on their superiority of nutritional profiles. The most acceptable supplement among the four formulations was selected by taste panel procedures. This supplement was formulated from amaranth grain, pigeon pea, sweet potato and groundnut. Nutritional evaluation showed that the food supplement could meet satisfactorily over 50% RDA's of the fundamental nutrients requirements for vulnerable groups. This supplement contained 453.2 Kcal of energy per 100 g, 12.7% crude protein, 54.3% soluble carbohydrate, 20.8% crude fat and 10.1% crude fibre. The supplement also had 93.0 mg/100g calcium, 172.4 mg/100g magnesium, 2.7 mg/100g zinc and 5.7 mg/100g iron, as well as 0.8 mg/100g vitamin B_1, 0.2 mg/100g vitamin B_2, 7.9 mg/100g niacin, 100 µg/100g folic acid, and 140 µg/100g retinol equivalent. The supplement also contained 21% total essential amino acid Palmitic, stearic, oleic, linoleic and α-linolenic fatty acids were detected in the fat extracted from the supplement. The shelf life study showed that the supplement could be stored in each of three types of packaging; polythene bags, gunny bags and Kraft paper at ambient temperature (20 -26 ^{0}C), 30 ^{0}C and 35 ^{0}C for up to 4 months without significant changes in odor, moisture, peroxide value, fat acidity and reduced ascorbic acid contents. A cost analysis of the supplement was carried out to assess the potential selling price of the product, if commercially produced. The product could be competitively sold at KES 65.50/kg ($ 0.82).

Keywords: Supplementary food; development; nutrient content; acceptability; shelf life; cost analysis

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Introduction

Food insecurity, chronic hunger, starvation and malnutrition continue to plague millions of people throughout the developing world, especially the Sub-Saharan Africa (Omueti, 2009). Malnutrition and under-nutrition are the major causes of morbidity and mortality among the vulnerable groups in most of the developing countries (Ejigui *et al.*, 2007). In Kenya, malnutrition is exacerbated by recurrent drought conditions and emergency situations that may result from adverse environmental factors such as floods or political conflict. High fertility rates, increased poverty levels and inadequate household food production are also key contributing factors (CBS/MOH/KDHS, 2010). In addition, limited availability, accessibility and inadequate intake of a diversity of cereals, legumes and vegetables at the household level and lack of knowledge about their nutritional contribution to the diet contribute to poor diet quality (Leenstra *et al.*, 2005). Formulation of supplementary foods using available/affordable staple food commodities is a possible approach that has been recommended to reduce malnutrition (Solomon & Owolawashe, 2007). State-sponsored nutrition intervention programs have been therefore, designed to supply low-cost supplementary foods to the vulnerable and minimize the adversities of malnutrition (Milán-Carrillo *et al.*, 2007).

The joint recommendations from World Health Organization (WHO) and Food and Agriculture Organization (FAO) on diet, nutrition and prevention of chronic diseases have suggested the promotion of dietary diversity based on locally available nutrient-rich foods as a vital strategy against food insecurity, malnutrition and disease (Muller & Krawinkel, 2005; WHO, 2003). In the face of persistent food emergencies and the scale of global hunger, addressing nutrient deficiencies remains an immediate priority (Duvenage & Schönfeldt, 2007). Food deficit and low dietary diversity in both animal and plant foods simultaneously result in inadequate intake of nutrients among the vulnerable groups. The current low cost of

staples relative to non-staple foods means that diets can be adequate in energy but deficient in micronutrients (John, 2006). Diversification of diet with locally available traditional whole grains, legumes and vegetables through optimization of nutrients in formulation of supplementary foods improves the diet quality. Therefore, foods based on local processed cereal and legume provides models of nutritionally rational products for use in supplementation and other public health measures (Patel *et al.*, 2005; Ejigui *et al.*, 2007).

According to WHO (2005), WHO/UNICEF (1998) and Mosha et al. (2005), formulation of low-cost, fortified supplementary foods from locally available ingredients, using appropriate small to medium-scale production technologies in community settings, can help meet the nutritional needs of vulnerable groups populations of low socio-economic groups. Consequently, there is urgent need to develop low cost nutritious supplements through combinations of less expensive foods available in respective localities or communities (Omueti, 2009; Nnam, 2000). Therefore, the main objective of this study was to develop a supplementary food from traditional and commonly-consumed local food ingredients, making use of their 'nutrient strength' to provide a nutrient-enriched end product with little or no fortification. The supplement was developed within a cultural context and utilized local food preparation and processing methods to formulate a product with acceptable sensory characteristics for a wider population.

Material and methods

Food ingredients

The local food ingredients included the finger millet (*Eleusine coracana* L. Gaertn. P-224), amaranth grain (*Amaranthus cruentus* L.), pigeonpea (*Cajanus cajan* L. Millsp. Kat/Mbaazi 3), field bean (*Dolichos pupureum* L. Kat/DL-3) and groundnut (*Arachis hypogea* L.). The vegetables included pumpkin (*Cucurbita maxima* L.), sweet potatoes (*Ipomoea batatas* L.

Lamk. SPK 004), and leafy vegetables such as amaranth leaves (*Amaranthus hybridus* L.) and pumpkin leaves (*Cucurbita maxima* Lam.). Small dried fish (*Rastrineobola argentea*) was also included. These food ingredients (1 kg each) were purchased mainly from the local open-air markets and supermarkets in Nairobi.

Chemicals

All the chemicals were of analytical grade and were purchased from either Sigma Chemical Co. (St. Louis, Mo., U.S.A.) or Aldrich (Milwaukee, Wis., U.S.A.) through local chemical stores.

Processing of the ingredients

The grains (cereals and legumes) were sorted to remove extraneous matter, washed with tap water and then soaked in distilled water (1 kg grain: 2 litres) for 8 h in the dark at 25 $\pm 1^0$C. They were drained and cooked at 90-95^{0}C for 120 min in fresh distilled water in the ratio of grain: water of 1:4. The groundnuts were roasted in an open-pan iron container to a golden brown color for 30 min using traditional charcoal burner at 150^{0}C with continuous stirring to avoid burning of the seed coat. After roasting to a characteristic roast flavor, they were cooled to room temperature.

The fresh vegetables were cut into approximately 1 cm pieces, then washed under running tap water and blanched in boiling water for 5 min. They were then dried in an air oven at 60 ^{0}C for 6 h and milled into fine powder to pass through 0.1 mm sieve using a hammer mill (DFH48, No. 282521/UPM 6000; Glen Creston Ltd, London, England).

The small dried fish (*Rastrineobola argentea*) was sorted in tap water to remove sand, dirt, and other extraneous matter, and soaked in a solution containing 0.5% $NaHCO_3$ and 1% NaCl for 30 Min. The soak liquor was decanted and the soak-decant process repeated again. The

fish sample was then rinsed three times with distilled water, dried at 60 ^{0}C in an air oven for 6 h and milled into a fine powder to pass through 0.1 mm sieve using a hammer mill (DFH48, No. 282521/UPM 6000; Glen Creston Ltd, London, England). The soaking of the fish was intended to reduce levels of the water-soluble nitrogenous compounds, namely trimethylamine oxide, trimethylamine and the fish oil, which cause the characteristic fishy and rancid odors.

Supplement development

The supplementary food development was carried out in two stages. In the first stage, four formulations were optimized in terms of their nutritional contents using selected local food combinations. The concept of food multi-mixes and four food square systems were used for selecting the staple food ingredients for inclusion in the formulation. A number of permutations and combinations of low-cost, locally grown and commonly consumed food ingredients were theoretically calculated to satisfy specific macro- and micro-nutrient contributions to the RDA's of the vulnerable groups. Sugar was added to the formulations to increase the energy content. The ratio of blending the different local cereals, legumes and vegetables was 4:1:1:1:4. The composition, cost and processing methods for the different food supplements are given in Table 8.1.

In the second stage, the four formulations were subjected to sensory testing to identify the most preferred supplement (Annex 4). The four supplements were assessed for acceptability using taste panel procedures. Sensory attributes of the supplements were evaluated using a five-point Hedonic rating scale (1= dislike extremely and 5 = like extremely). Fifteen trained panelists comprising of staff members from the Department of Food Science, Nutrition and Technology in the University of Nairobi. They were asked to

assess the products for six sensory attributes namely, colour, appearance, flavour, texture, taste and overall acceptability (Meilgaard et al., 1999).

Table 8.1 Composition, cost and processing methods of developed food supplements.

Ingredients	Amount (g)	Cost (KES)*	Processing methods
Supplement I			
Finger millet	40	2.80	Washing & roasting
Pigeon pea	10	0.25	Soaking & cooking
Pumpkin leaves	10	0.10	Washing & blanching
Brown sugar	10	0.60	
Groundnuts	40	1.50	Roasting
Supplement II			
Amaranth grains	40	3.00	Washing & roasting
Field bean	10	1.00	Soaking & cooking
Amaranth leaves	10	0.10	Washing & blanching
Brown sugar	10	0.60	
Groundnuts	40	1.50	Roasting
Supplement III			
Amaranth grains	40	3.00	Washing & roasting
Pigeon peas	10	0.25	Soaking & cooking
Groundnuts	10	1.50	Roasting
Brown sugar	10	0.60	
Sweet potatoes	40	0.40	Blanching & air drying
Supplement IV			
Finger Millet	40	2.80	Washing & roasting
Small dried fish	10	0.80	Soaking & sun drying
Sweet potatoes	10	0.40	Blanching & air drying
Brown sugar	10	0.60	
Groundnuts	40	1.50	Roasting

*1 KES = 0.013 (1 $ is equivalent to KES 80)

For presentation to the panelists, the products were prepared as porridges as follows: The supplements were prepared by mixing 10 g of the supplement flour with 40 ml of water to form slurry (flour: water ratio of 1:4) and then heating slowly to boiling with constant stirring to form a smooth porridge. The heat was then reduced and the porridge left to simmer at 50 °C for 7 – 10 min since the ingredients had been pre-cooked then cooled to room temperature under running tap water. The porridges were served in polypropylene cups which had been coded with symbols. The sensory evaluation data were calculated as means of the panelists' scores for each attribute and used to select the most acceptable supplement. The selected supplement was then analyzed for nutrient and chemical composition. Shelf life and cost analysis of this supplement was also carried out.

Analytical methods

All the analyses were performed in triplicate. The moisture, protein, fat and total ash contents were analyzed according to standard AOAC methods (AOAC, 2005). Energy was calculated by multiplying the fat, carbohydrate and protein contents with the Atwater's conversion factors. The sample flours were digested with concentrated nitric acid, sulfuric acid and perchloric acid (10:0.5:2, v/v) for analysis of the mineral constituents (calcium, iron, magnesium, sodium, and zinc) using inductively coupled argon plasma atomic emission spectroscopy (ICP-AES, Jarrel-Ash). The vitamins; vitamin B_1, vitamin B_2, niacin, vitamin B_6, and folic acid were analyzed by microbiological AOAC methods (AOAC, 1990).

The amino acid profiles of the supplement were determined according to standard methods of the Official Journal of the European Union (2009) using automated amino acid analyzer (Eppendorf-Biotronic LC 3000, Laborservice Onken, 63584 Gründau, Germany). The quantificantion of fatty acids of the food samples was carried out by following the method of Thurnhofer et al. (2008). Sample extraction was carried in 22 ml extraction cells filled with Isolute-HM-N using an ASE 200 system (Dionex, Idstein, Germany). The fatty acid methyl esters (FAME) were analyzed by gas chromatography in combination with electron ionization mass spectrometry (GC-EI/MS), which consists of 5890 series II gas chromatograph and a 5971 mass selective detector, MS Data analysis version C.00.07 HP 1989-1992 from Hewlett Packard, Waldbronn, Germany. For GC analysis, helium gas (99.999% purity) was used as carier with a flow arte of 1 ml/min. A fused silica capillary column (100% cyanopropylpolysiloxane, 50 m x 0.25 mm i.d. x 0.20 µm film thickness, CP-Sil 88 from Chrompack, Middelburg, The Netherlands) was installed in the GC oven. Injectionof a 1 µl volume aliquot was used at atemperature of 250 ^{0}C and analysed for 38.81 min. Under selected ion monitoring (SIM) mode, nine fragment ions were detected, and seven of them were identified during the whole run at (1) *m/z* 74 and (2) *m/z* 87 for methyl

esters of saturated and monosaturated fatty acids, (3) *m/z* 81 and (4) *m/z* 79 for methyl esters of polyunsaturated fatty acids, (5) *m/z* 88 and (6) *m/z* 101 for ethyl etsers of saturated and monostaurated fatty acids. The GC experiment was replicated thrice and results were expressed in GC area % as mean values ± standard deviation.

Shelf life evaluation

Shelf life was assessed by storage in three packages (polythene bags, kraft paper and gunny bags) under three temperature regimes (ambient temperature 20-26 ^{0}C, 30 ^{0}C and 35 ^{0}C to simulate the temperature ranges of different regions of Kenya). Each package contained 500 g of product and was stored for 4 months consecutively. The samples were analyzed pre-storage and monitored at the end of every month of storage for moisture, peroxide value, fat acidity and reduced ascorbic acid contents using AOAC methods (AOAC, 2005). Odor test was carried out on each packaged product every month using modified method of Amonsou et al. (2009).

Product selling price

The selling price was estimated by carrying out a cost analysis as shown in Table 8.7. Farm gate prices of the food ingredients as well as prevailing costs of labor, rentals for the processing premises and utilities were incorporated in the calculations. A margin marker of 20% of the production cost was added to account for the profit margin of the producer and vendor.

Statistical analyses

All the analyses were performed in triplicate (n = 3) except for sensory analysis (n = 15), and the data was presented as means and standard of deviation (± SD). The results obtained were

analyzed using two-way ANOVA to determine the significant differences ($p < 0.05$) between the experimental batches. GraphPad PRISM® version IV software, San Diego, CA was used for statistical analysis.

Results and discussion

Supplement formulation

The composition, cost and processing methods for the four supplements I –IV formulated in the preliminary study are presented in Table 8.1. These supplements were subjected to sensory evaluation to identify the most acceptable supplement in terms of color, texture, appearance, taste, aroma and overall acceptability. On a five-point hedonic scale, the acceptable level is ≥ 3.0. Results showed that there was no statistically significant differences between the four supplements in terms of color, appearance and texture ($p<0.05$) (Table 8.2). Supplement I was not acceptable in regard to its color and appearance attributes while supplement II was unacceptable in color. Supplement III and IV scored significantly higher color and appearance when compared to supplement I and II, which was attributed to the presence of the greenish color of the pumpkin and amaranth leaves.

Supplement IV was significantly different from all others in taste and aroma, which was attributed to the repulsive odor of the added small dried fish flour. In addition, supplement IV had a significantly low overall acceptability. All the panelists indicated that supplement IV revealed a repulsive and fishy odor which was undesirable in porridge. Supplement III, had overall highest and acceptable mean scores for all the sensory attributes, and thus was most acceptable supplement. Further analyses and evaluation were therefore, based on this supplement.

Table 8.2 **Sensory characteristics of food supplements** [b,c]

Formulations	Sensory attributes and characteristics						
	Color	Appearance	Taste	Aroma	Texture	Overall acceptance	Mean score
Supplement I	2.73 ±1.34[b]	2.94 ±1.53[b]	4.13 ±0.83[a]	3.60 ±0.99[a]	4.53 ±0.52[a]	4.20 ±0.68[a]	3.69
Supplement II	2.73 ±1.22[b]	3.13 ±1.36[a]	3.07 ±1.28[a]	3.00 ±1.41[a]	3.53 ±1.46[a]	3.13 ±1.25[a]	3.10[b]
Supplement III	4.20 ±0.41[a]	4.33 ±0.72[a]	4.20 ±0.68[a]	4.00 ±0.66[a]	4.27 ±0.96[a]	4.07 ±0.60[a]	4.18[c]
Supplement IV	4.67 ±0.62[a]	4.53 ±0.64[a]	3.07 ±1.28[a]	2.53 ±1.30[b]	4.20 ±0.68[a]	2.93 ±1.49[b]	3.66

[b] Values are mean and ± standard deviation of 15 panelists (n =15).
[c] Values in the same column with different alphabet superscripts are significantly different ($p < 0.05$).

Nutritional composition

The nutrient composition of the most acceptable food supplement is presented in Table 8.3. The FAO/WHO (2002) nutrient intake recommendations were used to calculate the per cent nutrient fulfilment of the supplement for the vulnerable group of children of 12-23 months. The results show that consumption of 110 g of the supplement could meet over 100% of the nutritional requirements for protein, magnesium, vitamin C, niacin and vitamin B_1 and over 50% of the requirements for energy, fat, iron, zinc and folic acid. However, the supplement fulfilled less than 50% of the requirements for vitamin B_2, calcium and vitamin A. Similarly, reports on complementary foods developed from maize, soybeans and cray fish in Nigeria give comparable levels of protein (14.16-17.66%), fat (18.6-12.6%), ash (2.98-4.02%) and fiber (2-2.5%) to the present study (Folake & Bolanle, 2006; Nnam, 2000). A supplementary food developed from a blend of rice, field pea, guar gum, locust bean gum and fenugreek gum in New Zealand contained higher levels of protein (20.6%) but comparable levels of fat (1.97 g) and total ash (2.19 g) when compared to the supplement of the present study (Ravindran *et al.*, 2011).

Table 8.3 Nutritional composition of the food supplement [a]

Nutrient	Food supplement (110 g DM)[b]	RDA (FAO/WHO, 2002)[c]	% of Fulfillment
Energy (kcal)	453.22	894	51
Crude Protein (g)	12.66	10.9	116
Crude Fat (g)	20.83	29.8	70
Retinol equivalent (ug)	139.97	400	40
Vitamin B_1 (mg)	0.75	0.5	150
Vitamin B_2 (mg)	0.19	0.5	38
Niacin equiv. (mg)	7.91	6	132
Folic acid eq. (µg)	100	160	63
Reduced ascorbic acid (mg)	35.22	30	117
Calcium (mg)	93.01	400	23
Magnesium (mg)	172.42	60	287
Iron (mg)	5.66	6	94
Zinc (mg)	2.74	4.1	67

[a] Ingredients: Amaranth grain, sweet potatoes, ground nut and sugar.
[b] Based on a daily ration of 110 g; Daily energy supplied: 453 Kcal. Cost per ration: KES. 6.01 per 110g or $ 0.08 per 110 g.
[c] FAO and WHO (2002) recommended nutrient intakes for a selected vulnerable group (12-23 months).

The mineral contents of the developed supplementary food were higher than the contents of calcium (0.13 mg/100 g), magnesium (0.14 mg/100 g), iron (0.01 mg/100 g) and zinc (0.004 mg/100 g) reported for a legume-cereal based complementary food blend used in Nigeria (Solomon & Owolawashe, 2007). Similarly, the calcium (93 mg/100 g) and iron (5.66 mg/100 g) contents of the developed supplementary food were higher than the levels (32-82 mg/100 g and 2.7-3.7 mg/100 g respectively) reported for a cowpea/maize/sweetpotato complementary food developed in Nigeria (Nnam, 2000).

The essential amino acids and fatty acid contents of the supplementary food are shown in Table 8.4. The total essential amino acid was 21 % and the fatty acid content was 96.7 %. The results showed that the levels of the essential amino acids, isoleucine (1.98%), leucine (3.85%), lysine (2.03%), tyrosine (1.8%), phenylalanine (3.6%), threonine (2.04), valine (3.38%), cysteine (0.89%), methionine (0.8%), and tryptiphan (0.63%) were comparable to the FAO/WHO reference pattern for human milk in Africa (FAO/WHO, 1991). Similar levels of the amino acids glutamic acid (17.2%), aspartic acid (10.8%), leucine (8.90%), arginine (7.09%), cysteine (1.43%) and methionine (1.15%) were reported in a legume-cereal based

mplementary food blend used in Nigeria (Solomon & Owolawashe, 2007). The addition of gh protein legumes to cereal-based supplementary foods improves the quality of protein by mplementary amino acids (Ejigui *et al.*, 2007; Nnam, 2000). This is in agreement with the sults observed in the present study.

ble 8.4 Amino acid and fatty acid profiles of the supplementary food*

sential amino acid	Amino acid content (%)	Essential fatty acid Profile	Fatty acid content (%)
leucine	1.98	Palmitic acid (16:0)	22.40
ucine	3.85	Stearic acid (18:0)	4.50
sine	2.03	Oleic acid (18:1)	25.73
stine	0.89	Linoleic acid (18:2)	39.85
ethionine	0.8	α-linolenic acid (18:3)	4.23
rosine	1.8		
nenylalanine	3.6		
hreonine	2.04		
ptophan	0.63		
aline	3.38		

Average values of two independent determinations of the amino acid and fatty acid profiles

The levels of the fatty acids found in the developed supplementary food are presented in Table 8.4. Among the unsaturated fatty acids detected, linoleic acid was the most abundant (39.85%), followed by oleic acid (25.73%). For the saturated fatty acids, palmitic acid was the most abundant (22.40% of total), followed by stearic acid (4.50%) and α-linolenic acid (4.23%). The percent fatty acid content was well above the recommendations by FAO/WHO (1995) joint consultation on fats and oils in human nutrition. Total saturated (31.13%) and total unsaturated (65.60%) as well as ratio of unsaturated to saturated (2:1) well exceeded the FAO/WHO recommendations of 3.5, 44.8-52.9, 1.16-0.84 respectively (FAO/WHO, 2003). The ratio of linoleic to α-linolenic acid was 10:1 as compared to 5:1-10:1 recommended by WHO/FAO (2003). Similar reports have been made on a soyabean-based weaning food studied in Nigeria (Solomon & Owolawashe, 2007).

The results of the present study demonstrated that the nutritive values of individual components of the diet can be effectively combined to improve the nutritional quality without necessarily resulting to fortifying the products or supplementing them with synthetic nutrients. Similarly, local foods have been used to successfully formulate supplementary foods in many developing countries of Africa including Nigeria (Folake & Bolanle, 2006; Omueti, 2009; Ejigui *et al.*, 2007; Nnam, 2000). A nutritionally enhanced and affordable food formulation from local ingredients for identified inadequancies in nutrient intakes would help alleviate nutrient deficits of low-income households (Duvenage & Schönfeldt, 2007). The methods employed in the production of the supplements are simple and applicable at the village level and the ingredients used were low-cost, locally available and with acceptable tastes (Ejigui *et al.*, 2007; Egounlety, 2002). The possibility of production of an acceptable supplementary food would present a business opportunity for establishment of small-scale and cottage industries for generation of income by small-scale entrepreneurs.

The acceptable supplementary food developed in this study was based on amaranth grain, pigeon pea, sweet potatoes and groundnuts. Amaranth grain produces significant amounts of edible grain and is described as "the grain of the 21st century". It is a good source of minerals and vitamins with a protein content of up to 16%, which is of higher nutritional quality in comparison to cereals and some legumes (Gorinstein *et al.*, 2007). Sweet potato roots are consumed in Kenya prepared in different forms. The roots are rich in starch, sugars and minerals and some varieties contain coloured pigments such as anthocyanins and β-carotenes which are both regarded as antioxidants possessing several physiological attributes including anti-immunodilation, protection against cataract, ageing, muscular degeneration and liver injury (Panda *et al.*, 2009). The carotenes also serve as vitamin A precursors. Recently, sweet potatoes have been labeled as an 'antidiabetic' food because some animal studies have showed that they can help stabilize blood sugar levels and lower insulin

esistance (Panda *et al.*, 2009). Pigeon pea (*Cajanus cajan*) is a valuable source of proteins, minerals and vitamins, and occupies a very important place in human nutrition in many developing countries (Fasoyiro *et al.*, 2006). Pigeon pea is also an economically and nutritionally important legume as major source of proteins in poor communities of many tropical and subtropical regions of the world (FAO, 2003). Pigeon pea protein is a rich source of lysine, but are usually limiting in the sulfur-containing amino acids, methionine and cysteine. Although low in some essential amino acids, pigeon pea could be considered a good protein source to offset the amino acid deficiencies of cereal proteins (Torres *et al.*, 2006).

Shelf life evaluation

Most of the supplementary foods are powders and flours, and due to their large surface area exposed therefore, are easily amenable to spoilage through chemical reactions such as fat oxidation especially in the presence of air and mineral prooxidants like iron. They are also amenable to caking due to hygroscopicity and to microbial damage when suitable conditions with regard to water activity and temperature are present (Fasoyiro *et al.*, 2009).

Table 8.5 shows the mean reduced ascorbic acid and moisture contents of the supplement stored in kraft paper, polythene and gunny bags at different temperature of storage (20-26 ^{0}C, 30 ^{0}C and 35 ^{0}C) for four months. Results show that the reduced ascorbic acid content of the fresh pre-storage supplement was 310.3 mg/100 g. Upon storage for four months, significant losses in the reduced ascorbic acid content of the stored samples were observed depending upon the type of packages, duration of storage and temperature. The supplements stored in all the three packages showed significant reductions in the reduced ascorbic acid content at all temperatures from the second month of storage indicating a 59-81% loss in four months. However, no significant differences were observed between different packing and temperature conditions.

Table 5 Mean reduced ascorbic acid ... and moisture content ...

Packing's	Temperature	Reduced ascorbic acid (mg/100 g) 0 Month	1 Month	2 Months	3 Months	4 Months	Packing's	Temperature	Moisture content (%) 0 Month	1 Month	2 Months	3 Months	4 Months
Kraft paper	26 °C	310.3[a]	245.0[a] (21)	124.0[b] (60)	105.1[b] (66)	83.78[b] (73)	Kraft paper	26 °C	6.09[a]	7.25[a] (19)	7.43[a] (22)	7.69[a] (26)	7.78[a] (28)
	30 °C	310.3[a]	201.0[b] (35)	109.3[b] (65)	96.12[b] (69)	78.03[b] (75)		30 °C	6.09[a]	6.09[a] (0)	6.14[a] (0.8)	6.16[a] (1)	6.16[a] (1)
	35 °C	310.3[a]	283.0[a] (9)	122.9[b] (60)	101.0[b] (67)	81.19[b] (74)		35 °C	6.09[a]	6.11[a] (0.3)	6.17[a] (1)	6.19[a] (2)	6.24[a] (2)
Polythene	26 °C	310.3[a]	260.1[a] (16)	66.99[b] (78)	69.21[b] (78)	58.39[b] (81)	Polythene	26 °C	6.09[a]	6.91[a] (13)	7.80[a] (28)	7.93[b] (30)	7.99[b] (31)
	30 °C	310.3[a]	279.0[a] (10)	70.07[b] (77)	66.00[b] (79)	62.96[b] (80)		30 °C	6.09[a]	6.36[a] (4)	7.13[a] (17)	7.18[a] (18)	7.24[a] (19)
	35 °C	310.3[a]	245.0[a] (21)	63.91[b] (79)	60.30[b] (81)	59.85[b] (81)		35 °C	6.09[a]	6.02[a] (1)	6.00[a] (1)	5.27[b] (13)	5.31[b] (13)
Gunny bags	26 °C	310.3[a]	291.0[a] (6)	126.0[b] (59)	106.3[b] (66)	78.46[b] (75)	Gunny bags	26 °C	6.09[a]	7.40[a] (22)	7.63[a] (25)	7.72[a] (27)	8.01[b] (32)
	30 °C	310.3[a]	162.2[b] (48)	78.54[b] (75)	78.00[b] (75)	76.23[b] (75)		30 °C	6.09[a]	6.13[a] (0.7)	6.14[a] (0.8)	6.22[a] (2)	6.49[a] (7)
	35 °C	310.3[a]	205.6[a] (34)	84.70[b] (73)	80.03[b] (74)	61.37[b] (80)		35 °C	6.09[a]	5.69[a] (7)	4.62[a] (24)	4.80[a] (21)	4.99[a] (18)

[a] The values in the 0 month column represent the mean of the prestorage sample. Values in the same row with different lowercase superscript letters are significantly different ($p<0.05$).
* Figures in parentheses indicate percent decrease in reduced ascorbic acid content and per cent increase/decrease in moisture content over 0 months values.

Table 6 Mean fat acidity and peroxide values of supplement III stored in different packing's at different temperatures.

Packing's	Temperature	Fat Acidity (mg/100 g) 0 Month	1 Month	2 Months	3 Months	4 Months	Packing's	Temperature	Peroxide value (mg/100 g) 0 Month	1 Month	2 Months	3 Months	4 Months
Kraft paper	26 °C	19.00[a]	26.02[b] (37)	30.25[b] (59)	49.21[b] (159)	68.50[b] (261)	Kraft paper	26 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)
	30 °C	19.00[a]	21.06[a] (11)	26.00[b] (37)	56.10[b] (195)	73.80[b] (288)		30 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)
	35 °C	19.00[a]	29.36[b] (55)	47.00[b] (147)	51.11[b] (169)	57.50[b] (203)		35 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)
Polythene	26 °C	19.00[a]	25.16[a] (32)	34.00[b] (79)	60.02[b] (216)	67.80[a] (257)	Polythene	26 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)
	30 °C	19.00[a]	21.91[a] (15)	31.75[b] (67)	66.42[b] (250)	93.00[b] (389)		30 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)
	35 °C	19.00[a]	74.69[b] (293)	80.00[b] (321)	88.66[b] (367)	93.80[b] (394)		35 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)
Gunny bags	26 °C	19.00[a]	19.41[a] (2)	19.75[a] (4)	35.21[b] (85)	61.80[b] (225)	Gunny bags	26 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)
	30 °C	19.00[a]	20.36[a] (7)	27.25[b] (43)	48.95[b] (158)	69.50[b] (266)		30 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)
	35 °C	19.00[a]	33.51[b] (76)	46.75[b] (146)	51.03[b] (169)	58.00[b] (205)		35 °C	0.00[a]	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)	0.00[a] (0)

[a] The values in the 0 month column represent the mean of the prestorage sample. Values in the same row with different lowercase superscript letters are significantly different ($p<0.05$).
* Figures in parentheses indicate percent increase or decrease over 0 months values.

The effects of storage on the moisture content of the supplement in different packages at 20-26 ^{0}C, 30 ^{0}C and 35 ^{0}C are shown in Table 8.5. The moisture content of the pre-storage supplement was found to be 6.09%. There were no significant differences in the moisture contents of the supplements when stored in kraft paper and gunny bags at all the temperatures of storage, but significant increases were observed in the fourth month of storage in the gunny bag. The supplement stored in the polythene bags showed no significant changes in moisture content at 30 ^{0}C and 35 ^{0}C but significant increase were noticed at 26 ^{0}C in the third and fourth month of storage. The observed results indicated that the supplement could be stored in kraft paper and gunny bags at 20-26 ^{0}C to 35 ^{0}C as well as polythene bag at 30 ^{0}C and 35 ^{0}C without significant changes in the moisture content. Water plays a crucial role in food quality characteristics and moisture content higher than 14% for such products will affect storage quality by promoting mold growth, insect infestation, and agglomeration of the food particles (Atwell, 2001).

The means of fat acidity and peroxide value of the supplement stored in kraft paper, polythene and gunny bags at different temperature of storage (20-26 ^{0}C, 30 ^{0}C and 35 ^{0}C) for four months are shown in Table 8.6. The fat acidity of the fresh pre-storage sample was 19.00 mg KOH/100 g. Results revealed that the supplement stored in the kraft paper and polythene bags showed significant increases ($p< 0.05$) in the fat acidity content from 0-4th month at all temperatures. The supplement stored in gunny bags exhibited significant increases in fat acidity from the 3rd to 4th month at 26 ^{0}C but these changes were only observed in the 2nd and 1st month of storage at 30 ^{0}C and 35 ^{0}C respectively. The increases in fat acidity were attributed to the possible lipolysis of the fat in the supplement due to higher storage temperatures (30 ^{0}C and 35 ^{0}C).

The effect of packaging and period of storage interaction on the peroxide value of the stored supplement was not significant ($p< 0.05$) at all the temperatures of storage (Table 8.6).

The possible fat lipolysis likely to occur in the supplement during storage was monitored by determining fat acidity values (mg/100 g) in food supplement. The peroxide values for all the samples stored at different temperatures were not detectable. The primary intermediate products of lipid oxidation are generally peroxides. Therefore, this analysis uses the concentration of peroxide in the supplement samples as a measure of the extent of oxidation and to produce off-flavour or rancidity. The absence of peroxides in the samples is also consistent with the lack of increases in the moisture content.

An odor test was carried out on all the stored samples in comparisons to a fresh sample. All the supplements stored in all the packages had non-significant variations in the odor up to 4 months of storage. Thus, it was concluded that the supplements can be stored in all the three types of packages at the three temperatures for up to 4 months of storage without significant changes in the organoleptic characteristics.

Results from the shelf life study showed that all the packaging materials (polythene bags, kraft paper and gunny bags) didn't show any significant changes in the chemical composition and sensory attributes of the supplement during storage for four months at different temperature regimes. Proper packaging of products is fundamental in prolonging the shelf-life and safety of supplementary foods, the later one of which is of major concern in consumption of these foods (Avermaete *et al.*, 2004). Generally the package used has to be strong and durable with low gas and moisture permeability, and at the same time be capable of withstanding the rigors of the frequent loading and off-loading encountered in most emergency situations.

Cost analysis of the supplement

The cost analysis of the supplementary food is shown in Table 8.7. The production cost was shown to be KES 60.10 per kg. A margin mark-up of 20% was incorporated to calculate the

selling price of the supplement by the retail vendor which was estimated to be KES 65.50 per kg. The selling price for the developed supplement is lower than most of the prevailing prices (KES 54.00-190.00/kg) for supplementary foods sold in Kenya. The present supplement was precooked, which is not the case with similar products found in the market. Therefore, addidtional saving in cost is realized during cooking. This study used low-cost and locally available food ingredients to reduce production cost and lower the selling price of the supplement. Recent studies have shown that about $US 8 billion is necessary per year to assist 100 million families to protect their children from hunger and malnutrition (UNICEF, 2006) and yet current donor spending on programmes to reduce under nutrition is only about $US 250 –300 million annually (Macdonald, 2008). The cost of commercially available supplementary foods is usually considered to be high and unaffordable by the vulnerable groups who are generally in the low income bracket (Conforti and D'Amicis, 2000). The results of this study will therefore be useful in helping with local production of affordable low-cost supplementary foods to spread the available donor funding further maong the vulnerable goups than the current coverage.

Table 8.7 **Production cost analysis of the supplement***

ITEMS	Cost/110 g Product (KES)
Raw materials	
Amaranth grain (40 g @ KES 75/kg)	3.00
Pigeon pea (10 g @ KES 25/kg)	0.25
Sweet potato (40 g @ KES 10/kg)	0.40
Brown sugar (10 g @ KES 60/kg)	0.60
Groundnuts (10 g @ KES 150/kg)	1.50
Milling cost of 110 g product	0.10
Labor to produce 110 g product	0.03
Packaging cost (100/= per 100pcs polythene bag)	0.01
Energy costs (for 110 g product)	0.01
Processing room (10tons of product/month)	0.01
Transport costs (3 tons of raw materials /trip)	0.10
Total cost/110 g product	**6.01**

* The cost per 100 g = 5.46 (KES 54.60 per kg). Using a margin marker of 20%, the selling price of the supplement will be KES 7.20 per 110 g (KES 65.50 per kg) or $ 0.82/kg (1 $ = 80 KES)

Conclusion

The results of the study show that a supplementary food that possess adequate nutrition and acceptable shelf life for vulnerable groups could be formulated from the local food ingredients; amaranth grains, pigeon pea, sweet potatoes, brown sugar and groundnuts in the ratio of 4:1:1:1:4. The product conformed to the specifications of the FAO/WHO with respect to protein, energy and micronutrient contents and could provide between 50-100% of the requirements of the major macro- and micronutrients. The proposed selling price of the supplement was lower than the selling price range of similar products currently found in the Kenyan market.

CHAPTER 9

CONCLUSIONS AND RECOMMENDATIONS

The present study documented the supplementary foods that are currently being sold and consumed by the vulnerable groups in Kenya. All the supplementary foods documented by the study are plant-based foods. Generally, cereals, legumes, oil seeds and indigenous vegetables remain a staple component of diets in Kenya. They make substantial contributions to intakes of carbohydrates, protein, fat and fibre as well as vitamins and minerals. In addition to this, their role in promoting good health goes beyond merely the provision of nutrients; there is much evidence to suggest that regular consumption of these food ingredients may have a role in prevention of chronic diseases such as cardiovascular diseases, diabetes and cancers. Furthermore, the whole grains promote feelings of satiety and the regular consumption of cereal-based diets at mealtimes appears to be key drivers of healthier dietary patterns.

The nutrient composition of the cereals, legumes, oil seeds and indigenous vegetables investigated in the present study indicated that they are good sources of many nutrients. Therefore, they could make substantial contributions to intakes of carbohydrates, protein, fat and fibre as well as vitamins and minerals. The fatty acid and amino acid profiles were also comparable to the patterns recommended by FAO. Consequently, these food ingredients could help in overcoming malnutrition and hunger among the vulnerable groups in Kenya. These food ingredients can be successfully combined to formulate therapeutic supplementary foods with adequate nutrition for use by vulnerable groups in Kenya.

The study also revealed that certain selected Kenyan indigenous grains and vegetables contain high levels of bioactive compounds (phenolics, flavonoids, condensed tannins and phytic acid), associated with high antioxidant and type II diabetes related enzyme inhibition properties. A good relationship has been observed between these substances and antioxidant

is well as functional properties. Cooking of vegetables and roasting of grains were observed to improve the antioxidant and functional properties of the bioactive compounds. The presently studied indigenous grains and vegetables could contribute to the significant supply of dietary antioxidants to prevent oxidative stress related chronic diseases including type II diabetes, which is a growing problem among vulnerable groups in Kenya.

The elite food sources and potential processing methods identified from the present investigation could be considered further for the formulation of supplementary foods with functional properties for the vulnerable group of Kenya. Given the importance of dietary habits and food components to health, the provision of phytochemical and antioxidant information of a diversity of foods locally available and consumed by vulnerable groups in Kenya is vital to support the future studies in assessing the protective status of vulnerable people from chronic degenerative disorders. In this regard, food-based approaches would be essential for sustainable solutions to combat the alarming prevalence of malnutrition, nutritional deficiencies, and chronic diseases such as cancer, coronary heart diseases and diabetes. The identified health benefits of these foods may prompt research into the assessment and determination of potential rich sources of antioxidant compounds in agricultural produce that could improve cultivar development, production practices, postharvest storage and food processing. However, further study is needed before firm conclusions can be drawn regarding long-term health benefits of increasing antioxidant defense per se, whether through food or supplements. The challenge in nutritional and biomedical science remains to develop tools that will allow the measurement of biomarkers of functional and nutritional strategies to promote health and functional longevity.

The results of the study show that a supplementary food possessing adequate nutrition and acceptable shelf life for vulnerable groups could be formulated from the local food ingredients, amaranth grains, pigeon pea, sweet potatoes, brown sugar and groundnuts in the

atio of 4:1:1:1:4. The product conformed to the specifications of the FAO/WHO with respect o protein, energy and micronutrient contents and could provide between 50-100% of the equirements of the major macro- and micronutrients. The proposed selling price of the supplement was lower than the selling price range of similar products currently found in the market.

Apart from the formulation a nutrient and phytochemical-rich supplementary food for the vulnerable groups in Kenya, the study also provides valuable information that is a useful tool for health professionals and consumers in choosing antioxidant and phytochemical rich foods. Plant breeders can also use this information in developing programs designed to increase antioxidant components in foods for human consumption. Therefore, nutrition education and counseling is important and it's advisable that educational campaigns are initiated to encourage the vulnerable groups to understand the health benefits of foods that are nutrient-rich sources of antioxidants and phytochemicals to avoid or prevent the development of diseases. The significant antioxidant activity observed in the studied food ingredients demonstrated that they may alleviate oxidative damage induced by oxygen radicals, thereby, being beneficial to human health and effectively employed as ingredients in food applications. On the basis of a conmprehensive chemical analysis, this study has demonstrated that it is possible to use traditional food processing technologies in the processing of supplementary foods without significant changes in the nutrients and bioactive compounds present in the raw materials. When properly optimized and used in urban and rural communities, these technologies can help to reduce the risk of micronutrient deficiencies as well as chronic diseases among the vulnerable groups in Kenya.

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APPENDICES

ANNEX 1

Baseline and market survey

Information on supplementary foods that are commonly utilized and sold in major supermarkets and other retail outlets in the city of Nairobi, Kenya

QUESTIONNAIRE FOR SUPERMARKETS

Name of Supermarket/ Outlet: _____

Location: _____

Name of Enumerator: _____

Q1. What types of supplementary foods do you sell in this supermarket? Please list them.

_____

_____

Q2. Which type of supplementary food is frequently bought by consumers?

Q3. Of the supplementary foods that you sell, what approximate percentage represents the imported products? _____

Q4. Please, give me the price list of the supplementary foods sold in this supermarket. List of products, unit cost and quantity sold.

Q5. What size of the population do you target for the marketing and consumption of supplementary foods? _____

Q6. What groups of consumers generally buy/purchase supplementary foods from this outlet?

_____

Q7. Which supplementary foods are most preferred by your customers? List them in order of preference 1. _____

2. _____

3. _____

Q8. Have you ever observed any altitudes and beliefs among the consumers of supplementary foods? List them, if any.

1) _____

2) _____

3) _____

Q9 How are the supplementary food products stored and transported from the producers/manufacturers to the market?

Q10. For transportation to the market, how are the supplementary foods packaged? How are these foods protected from spoilage during storage?

Q11. Do experience any duration or periods of seasonal shortages of the supplementary foods in the market?

Q12. How frequent do you experience changes in brands of supplementary foods in the market?

Q13. Do you sell any specialized formulations of supplementary foods for specific groups of consumers?

Q14. In your opinion, what is the trend of prices and consumption of supplementary foods in Kenya?

_____

Q15. What are the reasons for these trends?

_____

Q16. Do you incur any losses after storage and transportation?
If yes, state the types of losses incurred a) _____
b) _____ c) _____ d) _____

Q17. What are the losses due to? Tick the appropriate
a) Expiry
b) Microbial spoilage (fungal/bacterial)
c) Insects/weevils
d) Rodents
e) Returns
f) Other Specify_____

Q18. Which other problems do you encounter in marketing of supplementary foods in Kenya?

Q19. Are there any differences in prices are between locally produced and imported supplementary foods?
If yes, what are the reasons for these differences? _____

_____

Q20. Do you supply supplementary foods to any rehabilitation centres or institutions?
If yes, list the names of the rehabilitation centres /institutions.
1) _____
2) _____
3) _____

Q21. Do you receive any request from suppliers of supplementary foods to place these foods under categories of health products/ health enhancing products?

_____

If yes, list the products below.
1) _____
2) _____
3) _____

NNEX 2

UESTIONAIRRE FOR REHABILITATION CENTRES (Baseline Study)

ame of the interviewer: _____

ate of the interview: —— /—— /2009

ame of the interviewee: _____

ofession & Affiliation: _____

1) How many vulnerable groups are living in this institution or rehabilitation centre?
2) Please, give me the names of all the vulnerable groups in these institution or rehabilitation centre.
3) What services do you provide for the vulnerable groups in this centre/institution?
4) Do the vulnerable groups in this centre consume any supplementary foods?
5) Please, give me a list of all the supplementary foods that are used to feed these groups?
6) What are the sources of supplementary foods consumed by the vulnerable groups? (Purchase, NGO's, donors, UN agencies, international agencies, MoH, GoK). Rank those in order of frequency and quantity supplied.
7) Is there a positive change in the nutritional status of vulnerable groups consuming supplementary foods?
8) Is the change in the nutritional status dependent upon the type and protocol of supplementary feeding?
9) What problems are encountered in the administration and consumption of supplementary foods b the vulnerable groups?
10) What are the management and evaluation protocols used by caregivers and health workers in use of supplementary foods?
11) How do you prepare, distribute and feed the supplementary foods to the vulnerable groups?
12) How frequent do the vulnerable groups consume these foods?
13) Have you observed any health related benefits or improvement in health of the vulnerable groups consuming these foods?
14) How is the acceptability and palatability of the supplementary foods as observed in the vulnerable groups?
15) Have ever had any problems with the safety and quality of the supplementary foods consumed by the vulnerable groups? Please indicate any cases of vomiting, diarrhea and other complications and symptoms after consumption.
16) How do you prepare the supplementary foods for use by the vulnerable groups? Please indicate the handling practices, hygiene, and preservation, cooking methods and cooking time.
17) Do you have any therapeutic feeding in this institution?
18) At what level of malnourishment are the supplementary foods used for the vulnerable groups?

ANNEX 3

QUESTIONNAIRE FOR MANUFACTURERS

Name of the interviewee: _____

Date of the interview: —— /—— /2009

Profession & Affiliation: _____

1. What types of supplementary foods do produce in this industry? Please list them down.
   i.
   ii.
   iii
2. Do you import any supplementary foods from other countries? Please, provide me with general information on these imported foods.
3. What ingredients are used in the formulation of the supplementary foods?
4. Where do you obtain the ingredients used in production of these foods?
5. How do you process the supplementary food? Provide me with a detailed flow diagram of the unit processes.
6. What quality control and safety measures are implemented in the production?
7. What is the production capacity of the supplementary foods?
8. How much is supplied to government, UN agencies and NGO's?
9. Do you supply any rehabilitation centres, hospitals and food aid programmmes? Please, provide me with a list of you suppliers.
10. What vulnerable groups do target in formulation and production of these foods?
11. What is the shelf-life of these foods? Please give me general information on duration of storage in the factory and at the outlets you supply.
12. What health related benefits do you incorporate in production of these foods?
13. What type of package is used for these foods? State the type, quantity and unit price per pack.
14. How are these foods transported to the suppliers?
15. Are there any problems and challenges encountered during production, transport and storage of these products?

ANNEX 4

SENSORY EVALUATION OF SUPPLEMENTARY PORRIDGES

Name (Optional) ---------- Date----------

COLOR AND APPEARANCE

You have been given four uji samples. Without tasting them, score your liking of the color and appearance of each on the basis of the evaluation chart shown below:

ATTRIBUTE	SAMPLE CODE			
	□	⦿	◇	*
Colour				
Appearance				

TASTE, AROMA (ODOUR), TEXTURE (MOUTHFEEL) AND OVERALL ACCEPTANCE

Now taste the products one at a time in any order and for each product score your liking of the taste, aroma (odour), texture (mouthfeel) and overall acceptance of each on the basis of the evaluation chart shown below:

ATTRIBUTE	SAMPLE CODE			
	□	⦿	◇	*
Taste				
Aroma (odour)				
Texture (Mouthfeel)				
Overall acceptance				

EVALUATION CHART

5 = Like very much
4 = Like slightly
3 = neither like nor dislike
2 = Dislike slightly
1= Dislike very much

Any other comment:

_____

_____

_____

THANK YOU