Physicochemical Characterization and Food Application Potential of Pumpkin (*Cucurbita Sp.)* Fruit and Seed Kernel Flours

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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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To my late father, Abraham Fedha Waiti, who was called to rest by our Lord in November, 2006.

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ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
AACC	American Association of Cereal Chemists
AAS	Atomic Absorption Spectrophotometer
ACC	Administrative Committee on Co-ordination
ANOVA	Analysis of Variance
ASALs	Arid and Semi Arid Lands
AVRDC	Asian Vegetable Research and Development Centre
CAAMS	Chinese Academy of Agricultural Machinery Sciences
CIAT	International Centre for Tropical Agriculture
CPC	Corn Products Company
FAO	Food and Agriculture Organization
FEWS NET	Famine Early Warning Systems Network
GC	Gas chromatography
HPLC	High Performance Liquid Chromatography
IFPRI	International Food Policy Research Institute.
ILSI	International Life Sciences Institute
MT	Metric Tons
LSD	Least Significant Differences
MOA	Ministry of Agriculture
SCN	Standing Committee on Nutrition
VAD	Vitamin A Deficiency
OMNI	Opportunities for Micronutrient Interventions

Rpm	Revolutions per minute
RE	Retinol Equivalent
WHO	World Health Organization

ABSTRACT

The purpose of this work was to study the effect of drying on physicochemical properties and nutritional quality of fruits, seeds and seed kernels of two species of pumpkin; isolate and quantify their fruit starch; characterize the functional properties of the seed oils and proteins; carry out elemental mineral analysis and lastly formulate a food product based on pumpkin fruit flour-wheat flour blend. The investigation was done on samples of *Cucurbita maxima* and *Cucurbita moschata.* The treatment structure involved fruits with rind and those without rind (fruit pulp), whole seeds and seed kernels, and raw and dry samples (flours). Proximate composition of raw *C. moschata* fruit with rind was 87.9 % moisture (fresh weight), 1.6 g/100g crude ether extract, 4.9 g/100g crude protein, 10.9 g/100g crude fibre, 6.7 g/100g crude ash, and 76.0 g/100g nitrogen free extract (by difference) on dry weight basis. The corresponding values for *C. maxima* were 87.0 g/100g, 2.0 g/100g, and 3.9 g/100g, 9.6 g/100g, 6.9 g/100g, and 77.6 g/100g respectively. *C. moschata* fruit with rind contained significantly ($p<0.05$) higher crude protein and crude fibre than *C. maxima*. Proximate analysis of the seeds showed that *C. maxima* whole seeds had significantly ($p<0.05$) higher moisture, crude fat and crude protein content than *C. moschata* seeds. The results indicated that the pumpkin seeds are rich in lipids and proteins. Physicochemical analysis of oil extracted from *C. maxima* and *C. moschata* seeds showed that they are highly unsaturated. The lipid profile of the pumpkin seed oil indicated that the major unsaturated fatty acids were oleic and linoleic. Fruits and seeds contained varying amounts of potassium (K), phosphorus (P), magnesium (Mg), zinc (Zn), manganese (Mn), Iron (Fe), calcium

(Ca), copper (Cu) and sodium (Na). The fresh fruits with rind of *C. maxima* had significantly ($p<0.05$) higher amounts of starch (9.5 % f. w) and β-carotene (582.7 ± 3.9 µg/g d.w) than *C. moschata* fruit with rind that had corresponding values of 3.8 mg/100g and 534.5±10.2 µg/g on dry weight basis respectively. Emulsifying activity of pumpkin seed proteins ranged between 42-46 %, while foaming activity ranged between 26-43 %. The cake flavour at 5% pumpkin flour replacement level was the most acceptable. The results show that pumpkin seeds and fruits could be processed and potentially added to various food systems to improve nutrient content and as functional ingredients, hence their increased production, processing and utilization should be encouraged.

CHAPTER 1: INTRODUCTION

1.1. Background of the Study

Food and nutrition security, a situation in which all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active healthy life, is affected by a complexity of factors. These factors include unstable social and political environments that preclude sustainable economic growth, war and civil strife, macroeconomic imbalances in trade, natural resource constraints, poor human resource base, gender inequality, inadequate education, poor health, natural disasters, such as floods and locust infestation, and the absence of good governance (IFPRI, 2004). All these factors contribute to either insufficient national food availability or insufficient access to food by households and individuals.

Africa, in particular Sub-Saharan Africa, continues to lag behind in making progress in solving the food and nutrition problem due to the high prevalence of HIV/AIDS; civil war, strive and poor governance; frequent drought and famine; and agricultural dependency on the climate and environment. Food security on the continent has worsened since 1970 and the proportion of the malnourished population has remained within the 33 to 35 percent range in Sub-Saharan Africa (IFPRI, 2004). The major challenge to food security in Africa is its underdeveloped agricultural sector that is characterized by over-reliance on primary agriculture, low fertility soils, minimal use of external farm inputs, environmental degradation, significant food crop loss both pre- and post- harvest, minimal value addition and product

differentiation, and inadequate food storage and preservation that result in significant commodity price fluctuation.

Nearly half of the world's population suffers from malnutrition and an estimated two billion people are at risk of diseases resulting from deficiencies of the micronutrient iron, vitamin A, and iodine (UNICEF/MI, 2004). These deficiencies often referred to as ‘hidden hunger’; affect predominantly women, infants, and children in developing countries, impairing both physical and intellectual development. Food-based systems approaches offer sustainable solutions to problems of malnutrition, including health problems caused by micronutrient deficiencies. Food-based approaches include all activities affecting human nutrition and health, which are associated with the production, acquisition, preservation, and utilization of food (FAO, 1997).

Pumpkin (*Cucurbita sp.)* is a fruit vegetable, which belongs to the family *Cucurbitaceae* that also includes gourds, melons and squashes. The varieties of pumpkin grown widely in Kenya are of the species *C. moschata* (butternut squash) and *C. maxima* (squash pumpkin). Pumpkins are a native of Central America but have been domesticated in several tropical and sub tropical countries. In Kenya the crop is now regarded as a traditional vegetable (Chweya and Eyzaguirre, 1999) that is grown in the high potential areas as well as the arid and semi arid lands (ASALs). In Cameroon (West Africa) the species of *Cucurbitaceae* family are widely consumed and are commonly known as *egusi* (Achu *et al*., 2005).

The pumpkin has great economic potential for use both as a food and an industrial crop. It is utilized for its leaves, marrow, fruit pulp and seeds. The stem could be used as livestock feed. It has health enhancing properties and could also be used for ecological balance (FAO, 2005), since it provides good groundcover (Vodouhe and Capo-Chichi, 1998). Its cultivation and utilization in Kenya follows the rich ecological, cultural and ethnic diversity of the country (Chweya and Eyzaguirre, 1999). Exploitation of pumpkin as a food crop has been limited to a large extent by lack of sufficient information on the physicochemical, nutritional and functional properties of its fruits and seeds. Just like other traditional vegetables, the pumpkin is associated with poor rural lifestyles and low status.

Cultural change and urbanization have led to further neglect of this crop. Governments tend not to include traditional vegetable species, including pumpkins in their agricultural or food security policy strategy papers. They are seldom counted in agricultural statistics (Chweya and Eyzaguirre, 1999). Studies have shown that pumpkin fruit flesh contains β-carotene in amounts ranging between 0.06- 7.4 mg / 100g. This is important in mitigating the problem of vitamin A deficiency (VAD), which is the second most deficient micronutrient after iron that affects the vulnerable group that includes women and children (Murkovic *et al.,* 2001). The fruit also contains fibre and acetylated pectin present as 30% of the dry matter (Morris *et al.,* 1998). It contains riboflavin, vitamins C and E, plus other carotenoid pigments. Although classified as a fruit vegetable, pumpkins have been found to contain dry matter starch ranging from 3% to 60% (Corrigan *et al.,* 2001).

Pumpkin fruit could be consumed as a staple, providing substantial amounts of carbohydrates to the human diet. Studies done in Asia have shown that pumpkin seed kernel contains proteins (25-37%), lipids (33%), as well as minerals such as Fe, Zn, P, K, Mn, and Ca. (El-Adawy *et al.*, 2001). The enormous benefits notwithstanding, pumpkin remains an under exploited crop and it does not benefit widely from national or international research activities (Achu *et al.*, 2005).

Production of pumpkins has not yet reached plantation scale, unlike in the Peoples Republic of China where production and processing is about 500 tons per year and the fruit pulp is processed into flour and instant powder that is added to food products in various ways (CAAMS, 2004). The pumpkin varieties grown in Kenya are similar to those grown in other parts of the world, with minimal variation in cultivars, therefore, they have similar potential for agro-processing and utilization.

The purpose of this work, therefore, was to study the chemical, physical, nutritional and functional characteristics of raw and processed pumpkin fruit and seeds of two species grown in Kenya with a view to applying them in food systems.

1.2. Problem Statement

Processing and utilization of pumpkin fruits and seeds in Kenya is currently low. The pumpkin fruit is utilized in its primary form by boiling, adding to stews or mashing with maize and beans before consumption. The seeds are edible though they are not widely consumed. Agro-processing has concentrated on common foods such as cassava, maize and recently sweet potatoes, while neglecting others

including pumpkins. Production of this crop by farmers in the region is still minimal since it has not been promoted as an enterprise that can fetch income as well as provide family food and nutrition security. Its post harvest handling and utilization have not been given adequate attention by both research and extension organizations, despite its enormous potential. Research has been done extensively on agronomic and phenotypic characteristics of pumpkin, but this has not benefited the crop since it is still a marginalized one.

1.3. Justification

Due to the nutritional quality of pumpkins in terms of beta-carotene content and minerals, there is need to process it for preservation. The processed pumpkin products could be applied to various foods to enhance their nutritional quality for the target population.

Agro-processing helps to diversify the range of consumable farm products. There are compelling reasons therefore for encouraging agro-processing. Firstly, it improves rural incomes by adding value to produce, thereby saving on transport costs by delivering high value/ low volume products and creating opportunities for the use of by-products as inputs in other farm operations such as animal feeds, manure and fuel. Secondly, it provides an opportunity for reducing farm losses through the conversion of perishable commodities into more durable products. Thirdly, agro-processing helps create jobs in the rural areas, thereby contributing to the reduction of both poverty and rural-urban migration (MOA / GoK, 2004)

The successful characterization of the pumpkin fruit and seed kernel flours is intended to provide information that is expected to enhance production, processing and utilization of this crop. Adding value to the pumpkin will help improve its image because it is currently considered a minor crop, whose production statistics are not widely documented. Substantial amounts of starch contained in the pumpkin fruit pulp makes it suitable for agro-processing as a source of starch, alongside other crops such as cassava and sweet potatoes which are currently used. The possible outcome of this research will be more consumption of the pumpkin fruit and seeds, processing of the pumpkin fruit through drying, and utilization of pumpkin seeds at household level and at industrial level as functional ingredients in various food systems. The research findings will be published in scientific journals hence will add to the body of knowledge especially on the nutritional and physicochemical properties of the two species of pumpkin, notably *C. moschata* and *C. maxima* that would influence their utilization in food systems.

A more sustainable way to mitigate the food and nutrition problem that afflicts the population is to improve the production, processing and utilization of locally available resources, in the form of indigenous crops. This is a food-based approach recommended by the Food and Agriculture Organization (FAO) of the United Nations (FAO, 1997).

1.4. Objectives

1.4.1. Main objective

To carry out physicochemical characterization of flour from pumpkin fruit and pumpkin seed kernels and recommend their potential for application in food systems.

1.4.2. Specific objectives

a) Assess the effect of drying on the physicochemical and nutritional properties of fruit and seed kernel of *C. moschata* and *C. maxima* pumpkin species.

b) Isolate and quantify starch from the two species of pumpkin.

c) Characterize the functional properties of proteins and oils from the seed kernels of the two species of pumpkin.

d) Assess the effect of pumpkin flour on β-carotene content and sensory characteristics of cakes.

1.5. Hypothesis

The flour products of pumpkin fruit pulp and seed kernel are potentially nutritious and could be incorporated into food systems for fortification and as functional food ingredients.

CHAPTER 2: LITERATURE REVIEW

2.1. Crop description

The pumpkin is an angiosperm belonging to the *Cucurbitaceae* family that is characterized by prostrate or climbing herbaceous vines with tendrils and large, fleshy fruits containing numerous seeds (Acquaah, 2004). Pumpkin of the genus *C. maxima* is also called squash guard. The common names are Chinese pumpkin or crookneck squash and winter squash (Tindall, 1983). Its centre of origin is South America, possibly Peru, now widely distributed throughout the tropics. The areas of cultivation are tropical Asia (India, Indonesia, Malaysia, and the Philippines), tropical Africa, Central and South America, particularly Mexico and the Caribbean.

Botanically the squash guard can be described as an annual herb, rarely upright, generally trailing, and vines up to 3 m in length. Stems are slightly hairy, soft, and cylindrical in cross-section and tendrils are branched. Leaves are dark green mainly reniform, cordate, and rarely lobed, sometimes with white markings, 15-30 cm in diameter. Flowers are monoecious, female flowers are 15cm in diameter, yellow-orange; male flowers are smaller, 8.5cm in diameter with long upright thin pedicels, calyx of 5 sepals, fused at base, stamens 3, short fused, and stigmas small and yellow. The fruits are large, variable in shape, round or oblong, covered with small raised spots, weighing 2-5 kg. The rind may be soft or hard, sometimes brightly coloured. The flesh is yellow. Seeds are white or brown, ovoid, 1.3cm x 0.9cm, flattened, narrow towards point of attachment, (Rice, 1973; Tindall, 1983).

The environmental response of the crop is varied. Plants require a fairly high temperature, above 25-27^{0}C, during the growing period with fairly low humidity (Tindall, 1983). Soils with a high organic content are preferable, but in general, pumpkins are tolerant to a wide range of soil conditions. Fruits may be harvested 80-140 days from sowing or planting. The optimum harvesting stage is before the seeds ripen and when the skin of the fruit begins to harden. Maturity indices include a well corked stem, development of abscission layer and subtle changes in rind colour depending on variety (Cantwell and Suslow, 2002). Yields are 3-6 fruits per plant and individual fruits may weigh 2-5 kg.

Cucurbita moschata is closely related to *C. maxima*. Its other names are winter squash or butternut squash. Its origin is tropical South or Central America. It is the most widely grown species of the Cucurbita. It is an annual vine; stems grow up to 3 m. It is generally lacking in bristly hairs on leaves and stems, which mainly distinguishes it from *C. maxima* (Rice, 1973; Tindall, 1983). The flesh of the fruit is yellow to orange. The seeds are either white or brown in colour. The environmental response is such that *C. moschata* is more tolerant than most species of *Cucurbita* to high temperatures and is well adapted to the environment of the lowland tropics. The cultural requirements and growth period are similar to those for *C. maxima.*

2.2. Importance of pumpkin in the human diet.

2.2.1. Starch content in the fruit

Although pumpkin is botanically classified as a fruit vegetable, it is consumed by many communities as a staple, providing a substantial amount of calories on

consumption. Pumpkin fruit is a storage organ and has been found to contain starch in amounts that may go up to 60% (Corrigan *et al.*, 2001).

Starch is the major carbohydrate in plant storage organs. Starch and cellulose are the most important biopolymers on the planet. Calculated as calories, starch represents 80% of the world food supply (Guan *et al.*, 1998). Published data on the amount of starch contained in the Kenyan varieties of pumpkin is lacking yet this would serve as a basis for its inclusion in national crop statistics. Currently over 30% of children in Kenya are stunted, an indication of chronic malnutrition, and a further 6% are wasted, indicating acute malnutrition which is associated with situations of hunger and under nutrition (UNICEF, 2000).

Starch is also extensively used as animal feed and as an important industrial raw material. Starch is produced as an end product of carbon fixation during photosynthesis and is accumulated in storage organs. Most plant starches consist of a mixture of essentially linear (amylose) and highly branched (amylopectin) polymers in the ratio of approximately 1:3. The molecular weight and degree of branching of amylopectin vary widely and it is this structural variety which contributes to the differences in the chemical and physical properties of starches from diverse plant sources (Guan *et al.*, 1998).

Starches from wheat, maize, and potato continue to dominate the lucrative world markets in food and non-food industries yet alternative starch sources could be explored in order to increase their potential contribution to agricultural development

and general economic growth. Studies have shown that starch processing is more profitable when conducted alongside flour processing, which employs similar equipment. Waste from starch processing could be incorporated into flour. Since margins are small, the sales of waste materials from starch are an important factor in longer term sustainability of the process. In Kenya, a starch factory known as Kenya Corn Products Company (CPC) exports 2O, 000 MT / year of starch products to Uganda, and this implies that utilization of pumpkin starch would diversify starch sources. It is with this in mind that this research involved the isolation and quantification of starch from the pumpkin fruits of *C. maxima* and *C. moschata* species.

2.2.2. Micronutrient content in pumpkin fruit and seed kernels

2.2.2.1. Vitamins

Pumpkin fruit pulp contains beta-carotene, a pro-vitamin A, which plays a major role in human nutrition. Beta-carotene has been used for many years as a food colouring agent, pro-vitamin A in food and animal feed, an additive to cosmetics, multivitamin preparations, and in the last decades as a health food product under the claim 'anti-oxidant' (Ben Amotz and Fishler, 1997). Murkovic *et al.,* (2001) reported that β-carotene content in pumpkins ranged between 0.06 and 7.4 mg per 100 g. This study aimed at analysing and quantifying the amounts of β-carotene in the two species of pumpkins.

Vitamin A occurs only in animal tissues such as fish liver oil, livers of animals, milk fat and in egg yolk (Belitz and Grosch, 1985). Plants are devoid of vitamin A.

Vitamin A in nature originates from carotenes, which are the yellow and red pigments responsible for colour of many vegetables and fruits. Of the 500 or so naturally occurring carotenoids, about 60 posses the vitamin A activity in varying degrees but only 5 or 6 of these are commonly found in food. The major carotenoids are β- carotene, α-carotene, cryptoxanthin, lutein, zeaxanthin, and lycopene. The most common and active of the pro-vitamins is β-carotene, found in fruits and vegetables. It exists naturally as the all–trans isomer (Frigg, 1999). One of the most important features of carotenoids is that they are organic compounds with long unsaturated chains. These chains are responsible for their bright colour. The unsaturated property is easily destroyed by oxidation in air or by hydrogenation (Ihekororonye and Ngoddy, 1985).

The carotenoids yield vitamin A by cleavage of the centrally located double bond (Belitz and Grosch, 1986). The carotenoids which have the unsubstituted β-ionone ring can be cleaved oxidatively to yield retinaldehyde which is reduced to retinol (Bender, 1992). Once ingested, β-carotene and other pro-vitamin A carotenoids are cleaved in the intestinal mucosa by carotene dioxygenase yielding retinaldehyde (Combs, 1992). Central oxidative cleavage of β-carotene gives rise to two molecules of retinaldehyde. The retinaldehyde is reduced to retinol, which is esterified and enters the circulation in chylomicrons. Animals are unable to biosynthesize carotenoids, but assimilate them through their diet in the form of vitamin A (Woolfe, 1992).

Pro-vitamin A accounts for 60-90% of vitamin A intake. Populations depending on it as dietary source of vitamin A are in South East Asia, Africa and West Pacific, where animal sources of vitamin A are out of reach. The biological value of dietary carotene varies widely depending on the efficiency of absorption but it is taken as an average of one-sixth that of all trans-retinols (Woolfe, 1992). This disparity in biological activity is primarily due to inefficiency of carotene absorption and subsequent bioconversion to retinol. The bioavailability is greatly influenced by the nature of the embedding matrix (fibre) and the composition of the accompanying meal (Bender, 1992).

2.2.2.1.1. Stability of carotenoids

In general carotenoids are destroyed or altered by acids and free halogens, particularly in the presence of light and high temperatures (Latham, 1997). The carotenes are easily oxidized in the presence of oxygen or oxidizing agents, in conjunction with co-oxidation of unsaturated fatty acids. In foods the carotenoids are mostly dissolved in the fat matrix where they are protected from the oxidizing action of atmospheric oxygen by vitamin E and other anti-oxidants (Latham, 1997).

Carotenoids are susceptible to isomerization and oxidation during processing and storage (Rodriguez-Amaya, 2001). The practical consequences are loss of colour and biological activity and the formation of volatile compounds that impart undesirable flavour in some foods, especially in fruits. The occurrence of oxidation depends on the presence of oxygen, metals, unsaturated lipids, enzymes, oxidants; type and physical state of the carotenoid present and the severity of treatment during

food preparation (Bender, 1992). Oxidation of the pro-vitamin A carotenoids may occur when the ultra structure that protects the carotenoid is destroyed, when the surface area is increased and during heat treatment. Heating promotes trans-*cis* isomerization and therefore the duration and temperature used in food preparation should be controlled (Rodriguez-Amaya, 2001).

2.2.2.1.2. Role of carotenes in the diet

Some of the most severe nutritional problems the world over have been caused by the so called 'hidden hunger'. This is malnutrition caused by micronutrient deficiencies. One such micronutrient is vitamin A. Most of the vitamin A requirements of man, which are less than a milligram per day, are met in large part by green and yellow vegetables, such as lettuce, spinach, sweet potatoes, pumpkins and carrots, which are rich in carotenes (Leninger, 1992). On consumption, the carotenoids are converted to vitamin A. This vitamin is an essential micronutrient for the normal functioning of the visual system, growth and development, maintenance of the epithelial cellular integrity, immune function and reproduction (ACC/SCN, 2000). Food-based approaches used to mitigate this problem include diet diversification and food fortification. One important advantage of food-based strategies is that foods provide several essential micronutrients, simultaneously addressing a combination of deficiency problems. In addition, physiological interactions between vitamins and minerals can enhance the body's ability to absorb essential micronutrients.

Promoting consumption of micronutrient- rich foods fosters better overall health for all members of the society. Most importantly, food-based strategies promote sustainable improvements by encouraging market solutions and long term behaviour change in food preference among high-risk groups. This is more so because some foods, like the pumpkin, have a sweetish taste that may not be liked by many people. Processing it into flour for addition to various food products is one way of increasing their utilization. In addition, food based strategies are often linked to income generating activities (FAO, 1997).

2.2.2.1.3. Beta-carotene as a functional food ingredient in wheat products

Wheat is a major component of most diets of the world. Throughout the centuries, wheat has been used in variety of human foods such as breads, cakes, crackers, pasta and noodles. (Cho-Ho Lee *et al*., 2002). In Kenya, wheat flour is consumed mainly as bread and cakes. Wheat and other grains contain a limited amount of naturally occurring β-carotene. In Asian countries, synthetic β-carotene has been used for providing colour and vitamin A in a variety of bakery products including sweet rolls, Danish pastries, frozen waffles, bagels, soft cookies and snack foods (Gordon *et al.*, 1985 and Heinonen *et al*., 1989). Bakery products in Kenya are coloured using permitted food colourings to enhance acceptability without adding any nutritional or functional value. Although pumpkin is a good source of β-carotene and is served on its own, it has not yet been added to cakes. This study will assess the effect that pumpkin flour added to cake mixtures will have on β-carotene content and sensory properties of cakes.

2.2.2.2. Minerals

The pumpkin seed and seed kernels contain substantial amounts of minerals such as phosphorus, magnesium, potassium and calcium. Other minerals that are available in small amounts are zinc, iron and sodium (El-Adawy, 2001). Minerals are important for enzymatic activity and normal physiological function in the human body.

Calcium is a macro- mineral that is required in amounts of more than 1mg per day. The recommended daily allowance (RDA) is 1000-1200 mg for adults and 1300mg for those aged between 9-18 years. Calcium is important in the formation and maintenance of strong bones and teeth throughout the life cycle. It is also involved in blood clotting and aids in nerve impulse transmission, muscle contractions and contributes to cell permeability (Fawzi and Hunter, 1998). Calcium deficiency can cause osteoporosis. Vitamin D is needed for the body to absorb Ca from the diet. People most at risk of deficiencies are women. Good sources of dietary calcium are dairy products, fish, leafy vegetables and nuts.

Phosphorus is a micro-mineral that participates in the energy cycle that turns the food we eat into energy in form of Adenosine Triphosphate (ATP), for use by the body. Like calcium, phosphorus is also involved in muscle contraction and nerve transmission, and it is needed in protein synthesis. Phosphorus is part of deoxyribonucleic acid (DNA) which controls heredity. Phosphorus is required for bone and tooth strength. As part of cell membranes, phosphorus plays a protective role by regulating what comes in and goes out. The RDA for phosphorus is 700 mg for those aged above 18 and 1250 mg for those aged between 9-18 years.

Phosphorus is found in nearly all foods and dietary deficiency is unknown, though too much phosphorus can cause calcium deficiency, leading to osteoporosis. Besides carbonated drinks, phosphorus is found in milk and milk products, meat, fish, nuts and legumes (Wardlaw and Kessel, 2002)

Magnesium provides bone strength and is a component of enzymes involved in energy production. Magnesium also helps in nerve and heart function. Women and patients on thiazide diuretics are at risk of magnesium deficiency. The RDA is 450 mg for men and 350 mg for women. Dietary sources of magnesium are whole grains, legumes, leafy green vegetables, nuts and chocolate. Deficiencies can cause muscle spasms, tremor, convulsions and mental derangement. The mineral is not toxic, and unlike calcium it is not given up by bones for all the functions it has to perform. If it is not supplied by food or supplements there is risk of a deficiency state (Fawzi and Hunter, 1998).

Sodium and potassium are also macro minerals that are required by the human body in amounts of more than 1mg per day. They both function as major cations of intracellular fluid and aid in nerve impulse transmission. They are important in maintaining the water balance in the body and controlling the composition of blood and other body fluids (Gibson, 2003). Sodium chloride is present in processed foods and in small amounts in vegetables, fruits and grains, condiments, soups, sauces and chips. The RDA of sodium is 500 mg. Deficiency leads to muscle cramps. The RDA of potassium is 2000 mg and deficiency leads to irregular heartbeat, loss of appetite and muscle cramps. Foods rich in potassium include spinach, pumpkin, bananas,

orange juice, other vegetables and fruits, milk, meat, legumes and whole grains (Gibson, 2003).

The key micro- minerals also referred to as trace elements that were studied include zinc, iron, copper and manganese. Zinc is significant in the human diet since it is required in more than 100 enzymes involved in growth, immunity, alcohol metabolism, sexual development and reproduction. The human body contains 2-3 g of zinc (Gibson, 2003). There are no specific storage sites for zinc hence a regular supply in the diet is required. Zinc is found in all parts of the body, 60% is found in muscle, 30% in bone and about 5% in our skin (Gibson, 2003). Particularly high concentrations are in the prostate gland and semen. The RDA for zinc is 11 mg for men and 8mg for women (Wardlaw and Kessel, 2002).

The first signs of zinc deficiency are impairment of taste, a poor immune response and skin problems. Other symptoms of zinc deficiency include hair loss, diarrhoea, fatigue, delayed wound healing, and decreased growth rate and mental development in infants (Wardlaw and Kessel, 2002). Zinc supplementation can help skin conditions such as acne and eczema, prostate problems, anorexia nervosa, alcoholics and those suffering from trauma or post surgery. Zinc is present in a variety of foods, particularly in association with protein foods. A vegetarian diet contains less zinc than a meat based diet and so it is important for vegetarians to eat plenty of foods that are rich in this vital mineral (Gibson, 2003). Good sources of zinc for vegetarians include dairy products, beans, sesame, lentils, whole grain cereals and green vegetables. Pumpkin seeds provide one of the most concentrated vegetarian

food sources of zinc. All meats and sea foods are rich sources of zinc (Wardlaw and Kessel, 2002).

Iron is a functional component of haemoglobin and other key compounds used in respiration, immune function and cognitive development. Iron plays an important role in changes in some neurotransmitters in the brain and brain development (Latunde-Dada, 2000). People most at risk are infants, preschool children, adolescents and women in child bearing age (Dallman, 1992). The RDA for men is 8 mg and 18 mg for women Nutrient dense dietary sources include meats, sea food, broccoli, peas, pumpkin seeds, bran and enriched breads. Vitamin C helps in iron absorption. Deficiency symptoms include fatigue, small pale, red blood cells and low blood haemoglobin values (Levin, 1996).

The trace element copper is important in iron metabolism, works with many antioxidant enzymes and those involved in protein metabolism and hormone synthesis. Deficiency symptoms are anaemia, low white blood cell count and poor growth. The RDA for humans is 900 µg. Dietary sources are liver, cocoa, beans, whole grains and dried fruits.

Manganese is a micro- mineral that is a co-factor of some enzymes such as those involved in carbohydrate metabolism. There are no known deficiency symptoms in man. The RDA or adequate intake is 1.8-2.3 mg. Rich dietary sources are nuts, oats, beans and tea. The mineral content of the fruits, seeds and seed kernels of the two of pumpkin species were analysed in this study. Comparison was made between the

elemental mineral content of fruit and seed flours for each of the pumpkin species (Wardlaw and Kessel, 2002).

2.2.3. Proteins and oils in pumpkin seed kernels and their role in human diet

Pumpkin seeds are utilized directly for human consumption as snacks after salting and roasting in Arabian countries (Al-Khalifa, 1996). The seeds have been found to contain high levels of protein, in amounts of 35-37% and oil (50-51%) on dry weight basis (El- Adawy and Taha, 2001). A review of literature on functional properties of the pumpkin seed kernel proteins which include protein solubility in distilled water, water and oil absorption capacities, foam capacity and stability found them to be 24.3%, 2.55 g H_2O/g flour, 3.85 ml oil/ g flour, 18.2% volume increase, 15.9 ml, respectively, and these values were excellent when compared to those of watermelon seed flour (El- Adawy and Taha, 2001), which were found to be 23.9%, 2.51g H_2O, 3.89 ml oil/ g flour, 18.2% volume increase 15.2 ml, and paprika seed flour which had values of 24.9%, 2.10 g H_2O/g flour,3.10 ml oil/ g flour, 12.8% volume increase, and 10.1 ml respectively (El- Adawy and Taha, 2001).

Flour samples of the seeds and seed kernels could be potentially added to food systems such as bakery products and ground meat formulations, not only as nutrient supplements, but also as functional ingredients in these formulations (El-Adawy and Taha, 2001). Pumpkin seed oil has been produced in the southern parts of Austria, Slovenia and Hungary (Murkovic *et al.*, 1996). These oils are used in cooking in some countries of West Africa and the Middle East. The physicochemical properties

of these oils have been found to be comparable to the conventional oils frequently consumed in the human diet. Several reports exist on the nutritive values of proteins and oils of pumpkin seeds (Al-Khalifa, 1996 and Lazos, 1986).

However, more information needs to be reported on the physicochemical and functional properties of pumpkin oils and seed proteins because the varieties within the pumpkin species are many and research on them is not yet exhaustive. Furthermore, no work has been reported on the physicochemical properties of the two species of the pumpkin studied. Characterization of pumpkin proteins arises from the need to develop other sources of concentrated plant proteins other than those which are extracted from soybean, pigeon pea and cowpeas.

Properties of the proteins from these cereals have been reported by Mwasaru *et al*., (1999). Proteins are an important component of the human diet. They are macromolecules made up of small units called amino acids. They are essential for growth, tissue repair and replacement. Besides their nutritional importance, proteins can be applied in food systems as functional ingredients that serve as soup thickeners or meat replacers (El-Adawy and Taha, 2001).

2.3. Technology for processing fruit vegetable into powder

Drying of fruits and vegetables is important in that it reduces bulkiness, adds value, and diversifies their utilization. The technology for processing vegetable into powder has been developed in recent years with applications mainly for potatoes (flour, flakes, granulated); carrots (powder) and red tomatoes (powder) (FAO,

1995). The vegetables are either dried to a final water content of below 4% followed by grinding, sieving and packing of products or they are transformed by boiling and sieving into purées which are then dried on heated surfaces (under vacuum preferably) or by spraying in hot air. The pumpkin fruit has been found to be suitably dried to a moisture content of 10% while the seeds are stable at final moisture of between 5% and 6%.

2.4. Drying pre-treatments

2.4.1. Heat blanching

The pumpkin fruit is blanched before drying. Blanching is an important step which involves exposing the vegetable very quickly to heat (around 100^0C) to inactivate the naturally occurring enzymes. Exposure for a particular vegetable should not be too short to be ineffective or too long to soften the tissue due to excessive cooking (AVRDC, 1992). It is recommended that before drying pumpkins, the fruits should be cut into stripes and peeled. The peeled stripes are cut into slices of about 0.6cm thick after which they are immersed in 2% common salt solution for about 10 minutes, blanched in 2% boiling common salt solution for 3 to 4 minutes and dried (AVRDC, 1992).

Two of the more heat resistant enzymes important in vegetables are catalase and peroxidase. If these are destroyed then the other significant enzymes in vegetables will also have been inactivated. The heat treatment to destroy catalase and peroxidase in different vegetables are known, and sensitive chemical tests have been

developed to detect the amounts of these enzymes that might survive a blanching treatment (AVRDC, 1992).

2.4.2. Sulphur dioxide treatment

Sulphur dioxide may function in several ways (FAO, 1995):

(a). Sulphur dioxide is an enzyme poison against common oxidising enzymes;

(b). It also has antioxidant properties; i.e., it is an oxygen acceptor (as is ascorbic acid);

(c). SO_2 minimises non enzymatic maillard type browning by reacting with aldehyde groups of sugars so that they are no longer free to combine with amino acids;

(d). Sulphur dioxide inhibits microbial growth

In many processing pre-treatments two factors must be considered:

Sulphur dioxide must be given time to penetrate the fruit or vegetable tissues; SO_2 must not be used in excess because it has a characteristic unpleasant taste, odour and causes allergenic reactions. International food laws limit the SO_2 content of fruit products, especially of those which are consumer oriented (except semi-processed products oriented to further industrial utilisation). Commonly a 0.25% solution (except for semi-processed fruit products which are industry oriented and use a 6% solution) of SO_2 or its SO_2 equivalent in the form of solutions of sodium sulphite, sodium bisulphite or sodium/potassium metabisulphite are used (FAO, 1995).

CHAPTER 3: MATERIALS AND METHODS

3.1. Research design

The research involved dehydrating two species of pumpkin (*C. moschata* and *C. maxima*) and grinding them into flour for physicochemical analysis. The treatment structure involved fresh and dry samples, fruit with rind and without rind (pulp), whole seeds and seed kernels. For each treatment, three pumpkins were analyzed each one in duplicate. A completely randomised design (CRD) was employed in the study, where treatments were allocated at random to experimental units.

3.2. Materials

1. Chemicals were purchased from Kobian Chemicals Suppliers Limited, Nairobi.
2. Pumpkins were sourced from farmers in Machakos District Matungulu Division. Selection of the pumpkin fruit samples was based on maturity. Fully ripe pumpkins were used in the study. They were stored at temperatures of 10^0C before analysis.

Plate 1: *Cucurbita moschata* fruits

Plate 2: *Cucurbita maxima* fruits

3.3. Methods

3.3.1. Proximate composition of fresh and dried pumpkin fruit.

Moisture content was determined by AOAC method 930.04, (AOAC, 1995) total ash by AOAC method 930.05, crude fibre by AACC method 32-06, crude fat by AACC method 30-25 and crude protein (N% x 6.25) by AOAC method 978.04 (AACC, 1995). The carbohydrate was calculated by the difference.

3.3.2. Starch isolation and quantification in raw pumpkin fruits

Starch was isolated from the pumpkin fruits using the method reported by Badenhuizen (1964) with slight modification (Kasemsuwan *et al.,* 1995). Two fruits per species were used for starch isolation. Squash pulp was ground in distilled water and filtered through 106µm mesh. The starch residue was washed three times with distilled water, twice with ethanol then recovered by filtration using Whatman No.1 filter paper. Purified starch cake was dried in an air oven at 35^0C for 24 hours then quantified using an analytical weighing balance.

3.3.3. Flour preparation from pumpkin fruits and seed kernels

3.3.3.1. Flour from fruits

Mature pumpkins of *C. moschata* and *C. maxima* species grown in Machakos District were used. The fruits were sourced from the farmer's fields. Processing of the fruits was done at Kenya Industrial Research and Development Institute (KIRDI), Nairobi. The fresh pumpkins were cleaned, peeled (some fruits were not peeled) seeds removed, and chipped into pieces of 2.5cm length and 0.31cm thick

using a motorized electric chipper (Model Skymsen PA-7C, Brazil). Different known weights of slices were subjected to four treatments: (blanching and treating with 0.5% sodium metabisulfite, blanching only, sulfiting only, and no treatment). Drying was done using an electrical drier (KIRDI- EDSC Division, Nairobi) to a moisture content of 10%. This was achieved by drying at a temperature of 49^0C for 4 hours. The dried fruits were then ground into flour using a fine mill (Model Bauermeister, Hamburg-Altona, Germany) after which the flour samples were subjected to physicochemical analysis.

3.3.3.2. Flour from seed kernels

This was done according to a method described by El-Adawy and Taha (2001). Pumpkins were cut by a sharp knife and the seeds hand collected washed with water, then oven dried at 60^0C for 12 hours using an Isuzu hot air rapid drying oven (Soyokaze type ASF-113S). The dried seeds were shelled to remove the kernels, which were ground to pass through a 60 mesh (British standard screen). The fine flour of the whole seeds and seed kernels were put in an air tight jar and kept in a refrigerator (10^0C) until analysis.

3.3.4. Physicochemical analysis of fresh and processed pumpkin fruits

3.3.4.1. Material yield

The material (dry matter) yield was calculated by finding the weight of flour relative to the original weight of the fruit for samples not subjected to any drying pre-treatment; this was expressed as a percentage.

3.3.4.2. β-Carotene content in fresh and dried pumpkin fruits

This was determined by AOAC Official Methods (Method 941.15), (AOAC, 1995) and procedures similar to those described by Murkovic *et al.*, (2002) using HPLC and modified by (Rodriguez-Amaya and Kimura, 2004) as follows:

In the case of fresh fruits the material was finely cut with a knife and ground in a food chopper (waring blender) to achieve a homogenous sample. If analysis could not be performed immediately the samples were blanched in boiling water for 5 minutes and stored in frozen condition (-4^0C) . About 5g of the homogenous representative sample was weighed in a beaker. It was then transferred to a mortar and 3g of hyflosupercel (celite) added. The mixture was ground with 50 ml of cold acetone (acetone refrigerated for about 2 h). Filtration was done with suction through a Buchner funnel with 2 µm filter membranes.

The second step after extraction is partitioning to petroleum ether (PE). Twenty five millilitres PE was put in a 500 ml separatory funnel with Teflon stop-cock and the acetone extract added in amounts of 20 ml. Distilled water (200 ml each time) was slowly added, letting it flow along the walls of the funnel. The two phases were

allowed to separate and the lower aqueous phase discarded. The washings were done 3 times to remove residual acetone.

The third step is saponification. Saponification is an effective means of removing chlorophylls and unwanted lipids, which may interfere with the chromatographic separation and shorten the column's life. The PE phase was collected in a volumetric flask and an equal volume of 10% methanolic potassium hydroxide added. Also added was a few drops of 0.1% butylated hydroxytoluene (BHT) and the mixture let to stand overnight in the dark at room temperature. After overnight saponification, the solution was again washed with distilled water to remove the alkali. It was dried with anhydrous sodium sulphate and concentrated. It was made up to 100 ml volume then it was ready for HPLC analysis. For dry samples, 2 g of the material was weighed and soaked in water for 30 minutes, after which 20 ml of cold acetone added and let to stand for 15 minutes. The same steps were then followed as for fresh samples before HPLC analysis.

The samples in volumes of 10 µL were injected onto a reversed phase column (HPLC mode: Shimadzu, column packing size: 150 cm x 4.6 mm). The columns were eluted with a mobile phase of acetonitrile/methanol/dichloromethane (70/10/20). The flow rate was 1.0 ml per minute and the absorption of the effluent was monitored at a wavelength of 450 nm.

Identification of the peaks for beta-carotene was done using pure beta-carotene standard that was injected into the HPLC. The retention time for the sample was

similar to that of the pure standard. A solution of a pure standard of beta-carotene with concentrations ranging from 0 ppm to 2.5 ppm were injected into the HPLC and from the peak areas a calibration curve was drawn and the regression equation calculated (Figure 1). Concentration of beta-carotene (x) in ppm in the samples was calculated by dividing the peak area (y) by the regression constant, 41944.

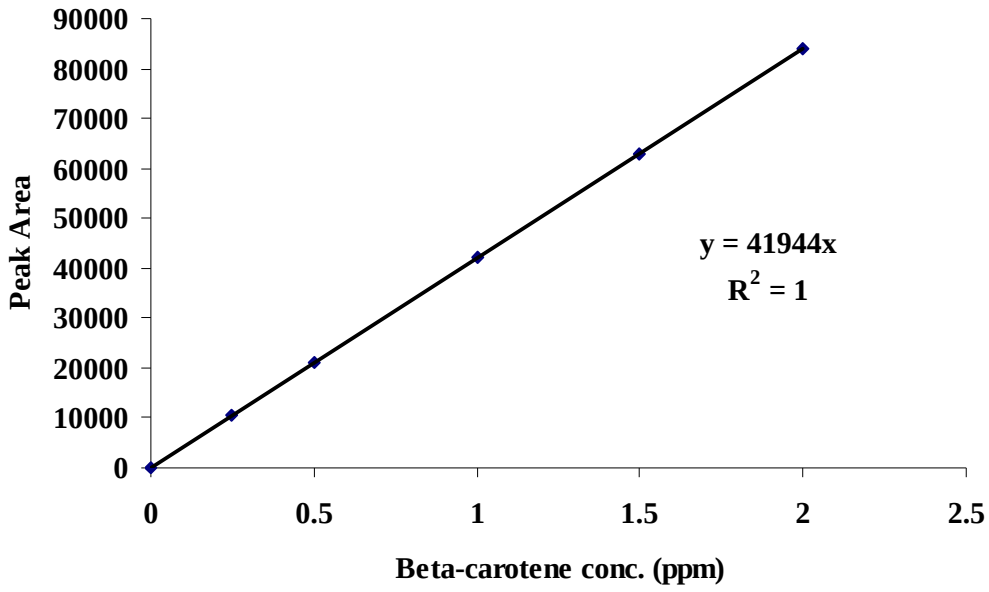

Figure 1: Standard calibration curve for β-carotene determination in pumpkin fruit samples

3.3.4.3. Analysis of specific minerals

The pumpkin fruits, seeds and seed kernels were analysed for elemental mineral content of Ca, Zn, and Fe Mg, Mn, Na, K, and Cu using an Atomic Absorption Flame Emission Spectrophotometer (Shimadzu Corp., Kyoto Japan, Model AA-6200) using the respective cathode lamps (AOAC, 1995) while P was determined by the Ascorbic acid method using UV-Visible spectrophotometer. Two grams of each of the samples were dry-ashed in a muffle furnace at 550^0C for about ten hours. The ash was dissolved in 1% HCl acid in a conical flask and made up to 100 ml mark using a standard volumetric flask. The individual mineral element composition was calculated from the AAS or U-Visible spectrometer readings obtained for both the blank and the test solution. Analyses were done in triplicate.

3.3.5. Extraction and characterization of kernel oils

3.3.5.1. Lipid extraction

Crude oil extraction was done using petroleum ether (boiling point 60-80^0C) in a soxhlet extractor for 16 h (Pearson, 1976). The preparation of the sample for solvent extraction involved drying followed by grinding. It is often necessary to dry samples prior to solvent extraction, because many organic solvents cannot easily penetrate into foods containing water, and therefore extraction would be inefficient. Dried samples were then finely ground prior to solvent extraction to produce a more homogeneous sample and to increase the surface area of lipid exposed to the solvent.

3.3.5.2. Specific gravity and refractive index

The specific gravity of the oil was determined using a 25 ml pycnometer at 15.5^0C while refractive index was determined using Abbe refractometer at 25^0C. The specific gravity was calculated by dividing the weight of the sample by the weight of the equal volume of water.

3.3.5.3. Peroxide value

One of the most commonly used methods to determine peroxide value utilizes the ability of peroxides to liberate iodine from potassium iodide (Pearson, 1976). The lipids were dissolved in a solvent mixture of acetic acid and carbon tetrachloride, warmed with saturated potassium iodide (13 g KI dissolved in 7 ml of hot water):

$$ROOH + KI_{excess} \rightarrow ROH + KOH + I_2$$

Once the reaction had gone to completion, the amount of ROOH that had reacted was determined by measuring the amount of iodine that was formed. This was done by titration with 0.01 N sodium thiosulphate and a starch indicator (1% starch solution):

$$I_2 + \text{starch} + 2Na_2S_2O_3 \text{ (blue)} \rightarrow 2NaI + \text{starch} + Na_2S_4O_6 \text{ (colourless)}$$

The amount of sodium thiosulphate required to titrate the reaction is related to the concentration of peroxides in the original sample.

3.3.5.4. Iodine value

The iodine value is expressed as the grams of iodine absorbed per 100g of lipid. One of the most commonly used methods for determining the iodine value of lipids is "Wij's method" (Pearson, 1976). The lipid to be analyzed was weighed and dissolved in carbon tetrachloride, to which iodine chloride was added. Some of the ICl reacted with the double bonds in the unsaturated lipids, while the rest remained:

$$R\text{-}CH{=}CH\text{-}R + ICl_{excess} \rightarrow R\text{-}CHI\text{-}CHCl\text{-}R + ICl_{remaining}$$

The amount of ICl that reacted was determined by measuring the amount of ICl that remained after the reaction had gone to completion ($ICl_{reacted} = ICl_{excess} - ICl_{remaining}$). The amount of ICl that remained was then determined by adding excess potassium iodide to the solution to liberate iodine, and then titrating with a 0.1N sodium thiosulfate ($Na_2S_2O_3$) solution in the presence of starch to determine the concentration of iodine released:

$$ICl_{remaining} + 2KI \rightarrow KCl + KI + I_2$$

$$I_2 + \text{starch} + 2Na_2S_2O_3 \text{ (blue)} \rightarrow 2NaI + \text{starch} + Na_2S_4O_6 \text{ (colourless)}$$

Iodine itself has a reddish brown colour, but this is often not intense enough to be used as a good indication of the end-point of the reaction. For this reason, starch is usually used as an indicator because it forms a molecular complex with the iodine that has a deep blue colour. Initially, starch was added to the solution that contained the iodine and the solution turned dark blue. Then, the solution was titrated 0.1N sodium thiosulphate solution.

While there is any I_2 remaining in the solution it stays blue, but once all of the I_2 has been converted to I^- it turns colourless. Thus, a change in solution appearance from blue to colourless was used as the end-point of the titration.

The concentration of C=C in the original sample was therefore calculated by measuring the amount of sodium thiosulphate that was needed to complete the titration. The higher the degree of un-saturation, the more iodine absorbed, and the higher the iodine value (Pearson, 1976).

3.3.5.4. Acid value

The acid value is a measure of the amount of free acids present in a given amount of fat. The lipids were extracted from the food sample and then dissolved in an ethanol solution containing a phenolphthalein indicator. This solution was then titrated with alkali (0.5 N KOH) until a pinkish colour appeared. The acid value is defined as the mg of KOH necessary to neutralize the fatty acids present in 1g of lipid (Pearson, 1976).

3.3.5.6. Saponification value

The lipid was first extracted and then dissolved in an ethanol solution which contained a known excess of KOH. This solution was then heated so that the reaction could go to completion. The un-reacted KOH was then determined by adding an indicator and titrating the sample with HCl. The saponification number was then calculated from knowledge of the weight of sample and the amount of KOH which reacted (Pearson, 1976). The smaller the saponification number the larger the average molecular weight of the triacyl-glycerols present. The

saponification value content of the oil was obtained by refluxing the alcoholic potassium hydroxide solution of the oil and then titrated with 0.5 M HCL using Phenolphthalein indicator (Pearson, 1976).

3.3.5.7. Fatty acid profile

The fatty acid profile of pumpkin seed oil was determined using the Gas Chromatograph (Shimadzu GC 9A, Kyoto, Japan). The lipid sample of 3 g was pipetted into a flask. Five percent of methanolic- HCl was added and the mixture heated under reflux for 1 hour for methylation of fatty acids. After cooling the sample, it was transferred to a glass stoppered tube and methyl esters extracted with 3 portions (1 ml) of hexane. The hexane layer was washed with 1 portion (3-4 ml) of distilled water.

The hexane layer was transferred to a flask and the solvent evaporated with evaporator. The residue was dissolved using a drop of hexane then it was ready for injection into the GC. A Shimadzu gas chromatograph with a flame ionization detector was used in the presence of nitrogen as a carrier gas. The stationery phase was diethylene glycosuccinate (DEGS 15%). Standard fatty acid methyl esters were used for identification. The area under each peak was expressed as a percentage in regard to the total area as explained by El-Adawy and Taha (1999).

3.3.6. Extraction and Characterization of seed kernel proteins

3.3.6.1. Extraction of proteins

The protein extraction technique described by Mwasaru *et al.*, (1999) and adopted from methods described by Lopez and Ordorica- Falomir (1986) were applied. The protein isolates were obtained by extracting pumpkin seed kernel meal with 0.1 M NaOH (1:10: meal: solvent, w/v) adjusted to pH value of 8.5. The suspension was homogenized (Ultra Turrax T 25 Janke and Kunkel & Co., Stauffen, Germany) at 8500 rpm for 30 min followed by centrifugation at 1500 rpm for 15 min. The supernatant was filtered and protein precipitated by adjusting the pH to 4.5 using 0.5 M HCl. The precipitated protein was recovered by centrifuging at 1500 rpm for 10 min followed by washing three times in excess water. The protein isolate was dried at 50 ^{0}C for 48 h and was kept in air tight jars and refrigerated until analysis.

3.3.6.2. Water and oil absorption capacity

This was determined by the centrifugation method of Lin *et al.*, (1974), as explained by Mwasaru *et al.*, (1999). One gram of flour was mixed with 10ml of distilled water or sunflower oil and centrifuged at 6600 rpm for 30 min. The volume of the supernatant was measured. The water holding capacity was expressed as the number of grams of water held by 1 g of flour. The oil-holding capacity was expressed as the number of ml of oil held by 1 g of flour.

3.3.6.3. Emulsifying activity and emulsion stability

Emulsifying properties were determined according to the method of Sathe *et al.*, (1983). One hundred millilitres of 7% (w/v) flour suspension at pH 7 was

homogenized at 11000 rpm for 30s using a warring blender. One hundred millilitres of sunflower oil was then added, and homogenized for a further one minute. The emulsions were centrifuged in 50 ml graduated centrifuge tubes at 1200 x g for 5 minutes and the volume of the remaining emulsion was measured. Emulsifying activity (EA) was calculated as follows:

EA% = (volume of emulsified layer / volume of whole layer in centrifuge tube) x 100. To determine emulsion stability (ES), emulsions prepared by the above procedures were heated at 80^0C for 30 min, cooled to room temperature and centrifuged at 1200 x g for 5 min. ES was calculated as follows: ES% = (volume of remaining emulsified layer / original volume of emulsion) x 100.

3.3.6.4. Foaming capacity and foam stability

Whipping properties (foam expansion and stability) were determined according to the method described by Kabirulla and Willis (1982). One hundred ml of 2.5% (w/v) flour suspension was whipped at 'low' speed in a 250 ml waring blender for 5 min, and foam volumes were recorded after 30 seconds. Foam capacity (FC) was expressed as percent increase in foam volume measured after 30 seconds, and foam stability (FS) was determined by measuring the FC after standing for 60 min.

3.3.6.5. Gelation properties

Gelation properties were determined according to the method described by Carcea and Bencini (1986) with slight modifications. Flour suspensions of 2-20% (w/v) were prepared in 100 ml distilled water by mixing in a 250 ml waring blender

(Model: HPB 300 U, Waring, UK) at the 'Hi'speed for 2 min. The suspensions were boiled in test tubes in a water bath for 1 hour, followed by rapid cooling under running cold tap water. The lowest concentration at which the gel formed was regarded as the Least Gelation Concentration (LGC).

3.3.6.6. Nitrogen solubility

Nitrogen solubility of the flours and protein isolates in distilled water at 5% (w/v) was determined over a pH range of 2-12 according to the method described by Narayana and Narasinga Rao, (1982): 1 g of flour was used with a flour or isolate: water ratio of 1: 60 and shaking for 2 h at room temperature. The pH of the suspension was adjusted by the addition of 2 M HCl or 2 M NaOH. After extraction, the suspension was centrifuged (Sorvall WX 80 Ultra Series Centrifuge, USA) for 20 min at 400 rpm at room temperature, and Nitrogen in the supernatant was estimated by the micro-Kjeldahl method (Pearson, 1976). Nitrogen extracted was expressed as percentage of the flour or isolate nitrogen content. Analyses were done in triplicate.

3.3.6.7. The pH of the pumpkin seed flours

The pH of the flours and protein isolates was measured using a pH meter (conductivity meter and pH meter, Sartorious Mechatronics, UK) after dispersing the solids in water at 10% w/v.

3.3.8. Food application potential of pumpkin flour

3.3.8.1. Cake preparation

Cakes were prepared by using the modified AACC method 10-90 (AACC, 1995). Pumpkin flour was added to wheat flour at 0, 5, 10, and 15% replacement levels of wheat flour, with the control having no pumpkin flour added (0%). Initially, 50 ml of water was added in the first mixing stage, with subsequent additions being adjusted commensurate with the percentage of pumpkin flour added. The batters weighing between 496-606 g were scaled into each of the 20 cm diameter cake pans and baked at 180 ^{O}C for 45 minutes in an electric oven. Baking trials were replicated two times. Cakes were cooled for 60 minutes, stored in plastic bags overnight and evaluated the next day.

Table 1: Cake formulation

Ingredient	Pumpkin flour replacement level				
	0%	5%	10%	15%	20%
Wheat Flour	200g	190g	180g	170g	160g
Pumpkin flour	0g	10g	20g	30g	40g
Sugar	100g	100g	100g	100g	100g
Shortening(margarine)	100g	100g	100g	100g	100g
Baking powder	4g	4g	4g	4g	4g
Whole eggs	2	2	2	2	2
Water	90 ml	90 ml	90 ml	90 ml	90 ml

3.3.8.2. Beta-carotene content, colour and sensory evaluation of cake

Beta-carotene retained in the cake was analysed by HPLC method 941-15 (AOAC, 1995), as detailed in section 3.3.4.2. Crumb and crust colour measurement was done using a Minolta Colour Difference Meter (Model C-R -200, Osaka, Japan) to obtain L, a, b values. Hue angle, Ɵ, ($\tan^{-1}$ b/a) was calculated to define the actual colour for the cakes (Lee *et al*., 1991)

Laboratory consumer testing using a sensory panel was done using nine untrained participants who included staff and graduate students from the Department of Food Science and Postharvest Technology at Jomo Kenyatta University of Agriculture and Technology. The panellists were presented with coded samples of the cakes and asked to check the category on a hedonic scale that indicated how much they liked the samples in terms of colour, flavour, texture, appearance and general acceptability as described by Meiselman (1984) (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like or dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely**).** Immediately before sensory tests, cakes were cut into 25 mm cubes. Cake samples were served on white plastic plates labelled with three-digit codes from a random number table.

3.3.10. Statistical analysis

Analysis of variance (ANOVA) was done using the GenStat Discovery 5th edition (Roger, *et al.*, 2001). The level of significance was 5%.

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Proximate composition of fresh and processed pumpkin fruits and seeds

4.1.1. Moisture content of fruits and seeds under different treatments

The moisture content of the fruits and seeds under study are presented in Table 2. The results show that both species have high moisture content. One way analysis of variance showed that there were significant ($P<0.05$) differences between moisture levels of fruits with rind and those without rind for both species. The latter were found to contain less moisture

Table 2 also presents the moisture content of seeds and seed kernels of pumpkins. A one-way analysis of variance showed that there were no significant ($P<0.05$) differences between moisture levels of sun dried seeds and seed kernels for the *C. moschata* species and the inverse was true for the *C. maxima* species.

These values for dry seeds are similar to those obtained by Kershaw and Hackett (1987) for other edible oilseeds such as cottonseeds (6.46%), peanuts (4.58%), palm kernel (5.31%), sesame (4.60%), and sunflower seeds (6.58%). They are lower than those of soybean (11.07%) and coconut seeds, 14.3% (FAO, 1982). The low moisture levels of pumpkin seeds enable them to be preserved for long periods.

Table 2: Moisture content of fruit vegetables and seeds of two pumpkin species (% d.w)

Treatment	*C. moschata*	*C. maxima*
Fruit with rind	87.9±0.0[d]	87.1±0.2[e]
Fruit without rind	89.5± 0.3[e]	88.2±0.4[f]
Whole seed (fresh)	33.2±0.4[c]	34.1±0.1[d]
Seed kernel (fresh)	28.5±0.9[b]	30.9±0.1[c]
Whole seed (sun dried)	5.7±0.4[a]	6.1±0.1[b]
Seed kernel (sun dried)	5.6±0.0[a]	5.5±0.2[a]
L.S.D. (P<0.05)	0.9	0.4

Values are mean ± S.D, n = 3

[a-f] Values in the same column with different superscripts are significantly different at 5% level.

Moisture levels of food products have a bearing on their dry matter content. The higher the moisture content the lower the dry matter yield on drying. Therefore information on moisture content would help the food processors make decisions on the economics of thermal processing of the foods.

4.1.2. Comparison of moisture content in fruits of two pumpkin species

Relative amounts of moisture in the fruits and seeds of the two pumpkin species are presented in Figure 2. It was observed that the moisture levels of fruits and seeds of the two pumpkin species are similar. We therefore inferred that differences in the

physicochemical properties of the samples analyzed were not dependent on the moisture levels.

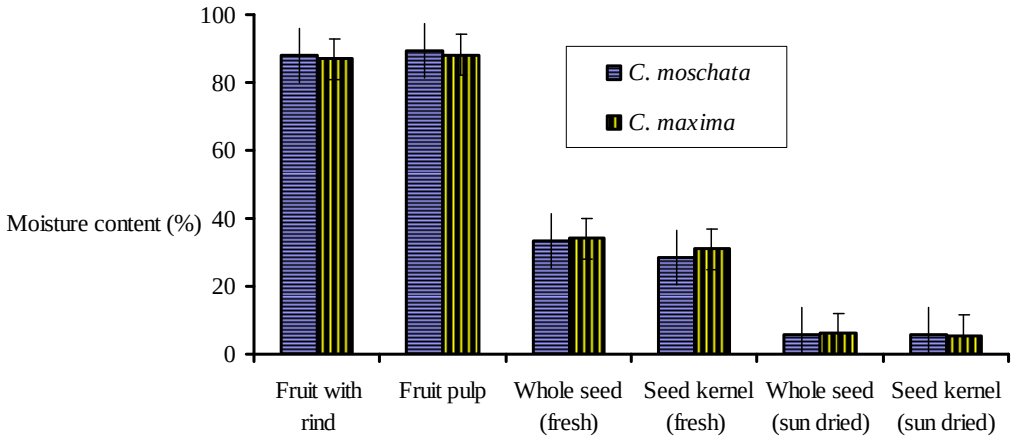

Figure 2: Moisture content of fruits and seeds of two pumpkin species. Vertical bars are Mean ±S.D

4.1.3. Crude protein content of raw and dried pumpkin fruits and seeds

The protein content of fruits and seeds of pumpkins studied are presented in Table 3. One way ANOVA showed that no significant ($P<0.05$) differences exist between protein contents of raw and dry samples for fruit with rind of the *C. moschata* species. For the other treatments it was found that drying and grinding either reduced or increased the protein content of the samples. Seed kernels contained significantly ($p<0.05$) higher protein than seeds. *C. maxima* seed kernels contained higher protein than *C. moschata* kernels. The values obtained for whole seeds are similar to those of Achu *et al.,* (2005), who found them to contain 29-35%. Martin (1998) got 35%, which was higher than cashew nuts (22.8%) and sesame (18.8%). The fluted pumpkin (*Telfairia occidentalis*) seed was found to contain 30.1g/100g protein (Asiegbu, 1987).

Table 3: Crude protein content of raw and processed samples of fruits and seeds of two species of pumpkins (g/100g d.w)

Treatment	Raw samples		Dry samples		L.S.D.
	C. moschata	*C. maxima*	C. *moschata*	*C. maxima*	P<0.05
Fruit with rind	4.9±0.2[c]	3.9±0.1[a]	4.9±0.0[c]	4.0±0.0[b]	0.1
Fruit without rind	4.0±0.1[c]	3.6±0.0[a]	4.3±0.0[d]	3.7±0.0[b]	0.1
Whole seed	36.2±0.3[c]	35.8±0.1[b]	35.4±0.3[a]	36.3±0.2[c]	0.3
Seed kernel	37.7±0.3[a]	39.5±0.2[c]	37.4±0.2[a]	40.3±0.3[b]	0.4

Values are mean ± S.D, n = 3

[a-d] Values in the same row with different superscripts are significantly different at 5% level.

An adult male of about 70 kg body weight requires 35 g of protein daily, therefore, only 98.93 g of *C. moschata* seed would be required to provide the minimum daily protein need. However, 122 g should be consumed to meet the requirement; if an allowance of 25% is made to take care of digestibility and the limiting sulphur amino acid (Fagbemi and Oshodi, 1991).

The proteins are of the globulin type and are deficient in lysine and sulphur bearing amino acids (Vodouhe and Capo-Chichi, 1998). The results show that these Kenyan species of pumpkins are rich in proteins hence good for children, lactating mothers and old people who need more proteins for growth, maintenance and repair of worn out tissues. These substantial amounts of proteins led us further to extract them and subject them to analysis of their functional properties which are important in their utilization in food systems.

4.1.4. Comparison of crude protein content in different parts of pumpkin fruit

The levels of crude protein contained in the different parts of pumpkin fruit of the two varieties is shown in Figure 3 below. Analysis of variance showed that significant differences existed between the different fruit parts. Highly significant differences ($P<0.05$) were observed between fruits and seeds, with the latter containing higher amounts of crude protein. Both raw and dry samples showed a similar trend in the amounts of the crude protein contained in the different parts of the pumpkin fruit.

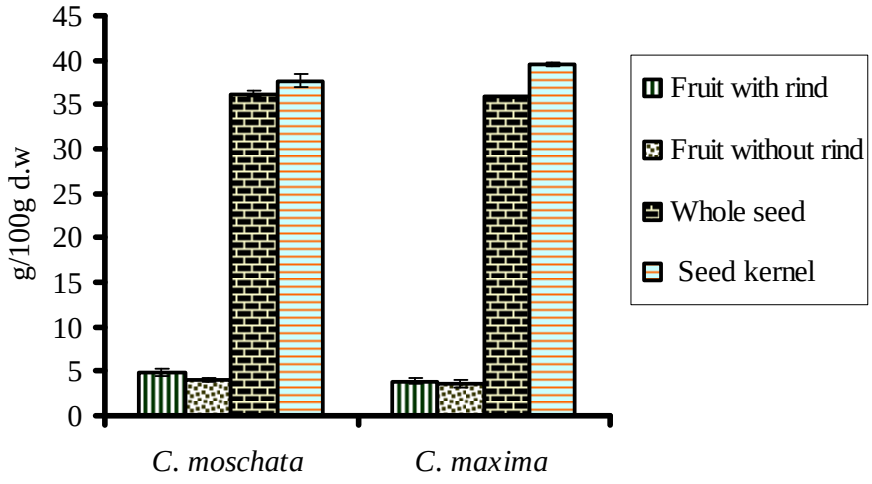

Figure 3: Crude protein content in different parts of pumpkin fruit

These results show that pumpkin fruits contain less amounts of crude protein than the seeds and seed kernels. A review of existing literature does not show any work that has involved comparison of nutritional content between different plant parts.

4.1.5. Crude fat content of raw and processed pumpkin fruits and seeds

The crude fat content of the pumpkins under study is presented in Table 4. One way ANOVA showed that there were no significant ($P<0.05$) differences between crude fat levels of fruits of the two species and between the fresh and dry samples. Both species contain low levels of crude fat. Generally fruits and vegetables contain low levels of lipids except avocado that contains 20% oil at ripe stage.

Table 4: Crude fat content of raw and processed pumpkin fruits and seeds (g/100g d.w)

Treatment	Raw Samples		Dry samples		L.S.D.
	C. moschata	*C. maxima*	*C. moschata*	*C. maxima*	(P<0.05)
Fruit with rind	1.6 ± 0.0^{b}	2.0 ± 0.0^{d}	1.4 ± 0.1^{a}	1.7 ± 0.0^{c}	0.1
Fruit without rind	1.5 ± 0.0^{a}	2.3 ± 0.1^{c}	1.5 ± 0.0^{a}	1.9 ± 0.0^{b}	0.1
Whole seed	34.7 ± 0.0^{a}	36.6 ± 0.2^{b}	35.1 ± 0.4^{a}	36.9 ± 0.5^{b}	0.6
Seed kernel	44.4 ± 0.1^{a}	48.3 ± 0.2^{b}	44.4 ± 0.2^{a}	48.4 ± 0.1^{b}	0.4

Values are mean ± S.D, n = 3

[a-d]Values in the same row with different superscripts are significantly different at 5% level.

The results in Table 4 also show the crude fat values for seeds and seed kernels. From analysis of variance, no significant differences were observed between raw and dry seeds and seed kernels ($P<0.05$). For both species the seed kernels contain significantly ($p<0.05$) higher crude fat than the whole seeds. *C. maxima* seeds and

seed kernels contain significantly higher ($p<0.05$) crude fat than those of *C. moschata.* On average, the seed kernels contain high lipid levels (46.3%).

These values are close to those obtained by El- Adawy *et al.,* (2001) for *C. pepo* seeds with oil content of 51.01%. Martin (1998) found cucurbit seeds to contain 50% lipids while Achu *et al.*, (2005) found the seeds to contain 41-54 % lipids. These lipid values are similar to those of sunflower (45.6%), sesame (53.5%), and peanuts (47.5%) (FAO, 1982), but lower than those for soybean 19.1% (Oyenuga,1968).These seeds can be considered good sources of vegetable oils.

Fats provide more energy than proteins and carbohydrates; they make a meal more satisfying, enrich its flavor and delay onset of hunger, and more importantly, are a medium of fat soluble vitamins (A, D, E, K) and are a source of antioxidants and bioactive compounds. Fats are also incorporated as structural components of the brain and cell membranes (Wardlaw and Kessel, 2002).

4.1.6. Comparison of crude fat content in different parts of pumpkin fruit

The crude fat content in fruits and seeds of pumpkin is represented in Figure 4 below. The trend is similar to what was observed for crude protein. The fruits contained significantly lower ($p<0.05$) amounts of crude fat than the seeds. The raw and dry samples both exhibited the same trend in amounts of the crude fat in the different treatments of the pumpkin fruit parts. Fruits and vegetables have been found to contain less fat than nutty seeds (Wardlaw and Kessel, 2002).

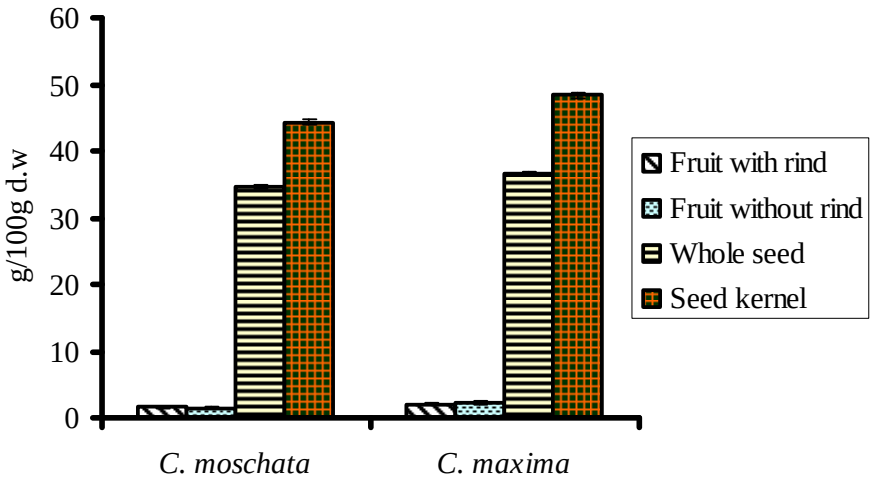

Figure 4: Comparison between crude fat content in different pumpkin fruit parts

4.1.7. Crude ash content of raw and processed pumpkin fruits and seeds

The total ash content of the fruits and seeds under study are presented in Table 5. No significant differences were observed between species and between raw and dry samples of whole seeds and seed kernels. The differences observed between fresh and dry samples of fruits could be attributed to analytical error. Fruits were found to have significantly ($p<0.05$) higher crude ash content than the seeds and seed kernels. Fruit with rind had significantly higher crude ash content than the fruit pulp.

Values for seeds and kernels are similar to those obtained by Achu *et al*., (2005) which ranged from 3.5-5.3%. These values are similar to those of soybean (5.06 %), cottonseed (4%), sesame (3.8%), and sunflower seed (4.1%) (FAO, 1982).

Table 5: Crude ash content of raw and processed fruits and seeds of pumpkin (g/100g d.w)

Treatment	Raw Samples		Flour Samples		L.S.D.
	C. moschata	*C. maxima*	*C. moschata*	*C. maxima*	(P<0.05)
Fruit with rind	6.7±0.1[a]	6.9±0.3[ab]	7.1±0.2[bc]	6.8±0.1[ac]	0.3
Fruit pulp	5.9±0.1[b]	6.5±0.1[c]	5.6±0.4[a]	5.9±0.1[b]	0.2
Whole seed	4.0±0.1[bc]	3.7±0.3[a]	4.1±0.3[c]	3.7±0.4[a]	0.2
Seed kernel	4.4±0.4[bc]	4.1±0.1[a]	4.4 ±0.2[c]	4.3±0.1[ab]	0.2

Values are mean ± S.D, n = 3

[a-c]Values in the same row with different superscripts are significantly different at 5% level.

4.1.8. Comparison of crude ash content in different fruit parts

The crude ash content in the different parts of pumpkin fruit is presented in figure 5 below. The fruit with rind had the highest amount of crude ash while whole seed contained the lowest amounts. The fruit with rind contained significantly higher ($p<0.05$) amounts of crude ash than the fruit pulp. Conversely, the seed kernels contained higher amounts of crude ash than the whole seed. This trend implies that the rind of pumpkin fruit contains mineral elements while the seed hull does not contain significant levels of minerals. A review of literature does not find any work done to compare amounts of ash in the pumpkin rind as compared to the respective levels in the fruit pulp. The seed kernels of pumpkin have been found to contain more crude ash levels than the whole seed (Achu *et al.*, 2005)

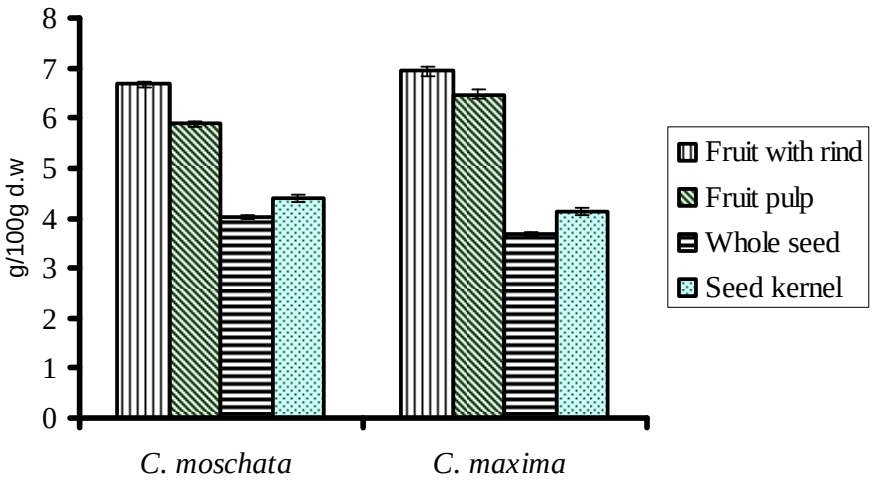

Figure 5: Crude ash content in different parts of pumpkin fruit

4.1.9. Crude fibre content of fruits and seeds of raw and processed pumpkins

The results of values of total fibre content of fresh (raw) and dry pumpkin fruits and seeds are presented in Table 6. One way analysis of variance showed that there were no significant ($P<0.05$) differences between fresh and dry samples for the fruits with rind for both species. Fruit with rind had significantly ($p<0.05$) higher crude fibre content than fruit pulp. Whole seeds had significantly ($p<0.05$) higher crude fibre content than seed kernels. Values for seed kernels were similar to those obtained by El-Adawy *et al.*, (2001). Crude fibre made up of cellulose, pectin, and hemicelluloses form bulk in the intestines and stomach, which stimulates peristalsis and thus preventing constipation. Dietary fibre refers to cell wall components that are not digestible by human or any other mammalian digestive enzymes. According to Dietary Reference Intake tables, the daily recommended intakes of dietary fibre

for individuals range between 25-38 g per day depending on age or sex with males and pregnant females requiring more (Institute of Medicine, 2005).

Table 6: Crude fibre content of raw and processed pumpkin fruits and seeds (g/100g d.w)

	Raw Samples		Dry samples		L.S.D.
Treatment	*C. moschata*	*C. maxima*	*C. moschata*	*C. maxima*	(P<0.05)
Fruit with rind	10.9±0.1[a]	9.6±0.2[a]	10.3±0.1[a]	9.1±0.1[a]	1.8
Fruit without rind	9.4±0.3[b]	8.2±0.1[a]	9.4±0.1[b]	8.0±0.1[a]	0.2
Whole seed	12.6±0.1[b]	12.0±0.3[a]	12.6±0.1[b]	11.9±0.1[a]	0.2
Seed kernel	4.2±0.1[b]	3.9±0.2[a]	4.2±0.4[b]	3.9±0.1[a]	0.1

Values are mean ± S.D, n = 3

[a-b]Values in the same row with different superscripts are significantly different at 5% level.

The Dietary Reference Intakes have been continuously developed since 1996 by the Food and Nutrition Board, Commission on Life Sciences, National Research Council, to replace the Recommended Dietary Allowances. According to institute of Medicine, (2005), the total fibre content in g/100g on fresh weight basis of selected fruits and vegetables is as follows: watermelon, 0.5; mango, 1.8; fresh pineapple, 1.2; banana, 2.4; apple with skin, 2.7; French beans, 3.1; potato, 3.2; carrot, 5.7; and bitter gourd, 16.6. Fibre content of vegetables varies owing to many factors which include growth condition (climate, soil), time of harvest or species (Ozcan and Haydar (2004).

Fibre is determined as material insoluble in dilute acid and dilute alkali. Crude fibre is a useful parameter in food and feed analysis. It is commonly used as an index of the feeding value of poultry and stock feeds; seeds high in crude fibre content are low in nutritional value. A determination of crude fibre is used in evaluating the efficiency of milling and separating bran from the starchy endosperm. It is a more direct index of flour purity than colour or ash (Yeshajahu, 1994). Crude fibre is also useful in the chemical determination of succulence of fresh fruits and vegetables. Over mature products have increased levels of fibre.

4.1.10. Crude fibre content in different fruit and seed parts

Different parts of the pumpkin species were found to contain different levels of crude fibre as shown in Figure 6. Of all the fruit parts, that is, fruit with rind, fruit pulp, whole seed and seed kernel, the whole seed was found to contain the highest amount of crude fiber, followed by fruit with rind, then fruit pulp, with the seed kernel having the least amount.

Both pumpkin species exhibited a similar trend in the amounts of crude fibre in these fruit parts. Fibre rich foods are usually recommended to diabetics since they are supposed to reduce glycaemic response to the food and consequently reduce the need for insulin (Guillon and Champ, 2000). Diverse sources of fibre are recommended for the young population to avoid insulin resistance syndrome and to decrease the incidence of other metabolic diseases such as obesity and cardiovascular disease (Guillon and Champ, 2000). Therefore the whole pumpkin seed could serve as a good source of dietary fibre.

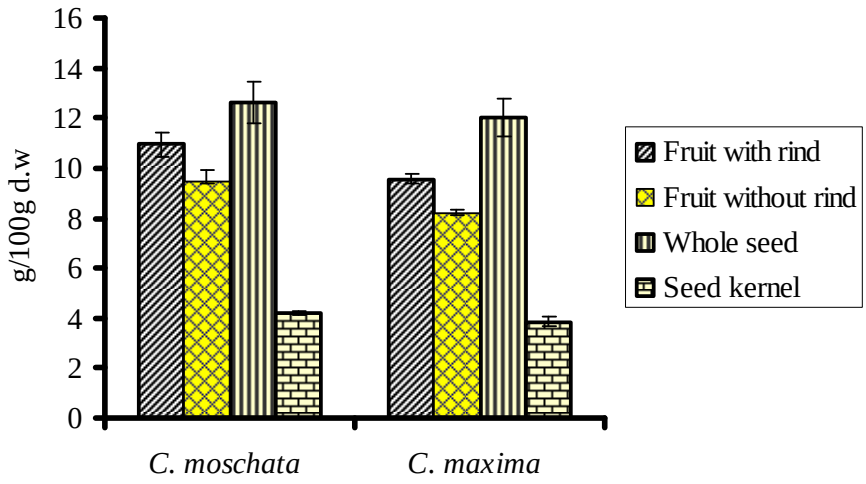

Figure 6: Crude fibre in different fruit and seed parts of pumpkin

4.1.11. Carbohydrate content of raw and dry pumpkin fruits and seeds

The results for carbohydrate content of fruits and seeds of raw and dry pumpkins are presented in Table 7. To obtain these values, the amounts of crude protein, fat fiber and ash were subtracted from one hundred for each treatment. Analysis of variance showed that on dry weight basis the pumpkin fruit pulp contained significantly ($p<0.05$) higher carbohydrate than fruit with rind. Whole seed contained significantly ($p<0.05$) higher carbohydrate than seed kernels for both species.

The values for whole seeds are similar to those reported by Platt (1962), 10%, and lower than for peanuts (18.6 %), sesame (20.2 %) (FAO, 1982). The carbohydrate is made up of monosaccharide (glucose and fructose), oligosaccharides and polysaccharides (starch, glycogen). The carbohydrate is divided into structural polysaccharides, which are mechanical structures in plants, and these include

cellulose, hemicellulose and lignin. The nutrient polysaccharides include starch and glycogen that are metabolic reserves in plants and animals (Southgate, 1991).

Table 7: Carbohydrate content of raw and processed pumpkin fruits and seeds (g/100g)

	Raw samples		Dry Samples		L.S.D.
Treatment	*C. moschata*	*C. maxima*	*C. moschata*	*C. maxima*	(P<0.05)
Fruit with rind	76.0±0.9[a]	77.6±0.7[b]	76.3±1.4[a]	78.3±0.8[c]	0.5
Fruit pulp	79.1±2.3[a]	79.5±0.7[a]	79.2±0.7[a]	80.4±0.9[b]	0.4
Whole seed	12.6±0.9[b]	11.8±0.5[ab]	12.9±0.9[b]	11.3±0.8[a]	1.0
Seed kernel	9.4±0.7[a]	4.2±0.7[c]	9.6±0.9[a]	3.1±0.5[b]	0.7

Values are mean ± S.D. n = 3

[a-b]Values in the same row with different superscripts are significantly different at 5% level.

The results show that pumpkins can potentially be good sources of carbohydrates in the human nutrition continuum.

4.1.12. Carbohydrate content in different parts of pumpkin fruit

The carbohydrate content in the different fruit parts is represented in Figure 7. The fruit pulp of both pumpkin species contained significantly higher amounts of carbohydrates than either the whole seed or seed kernel. This could be attributed to the fact that the whole seeds and seed kernels do contain higher amounts of crude fat than the either the fruit pulp or fruit with rind.

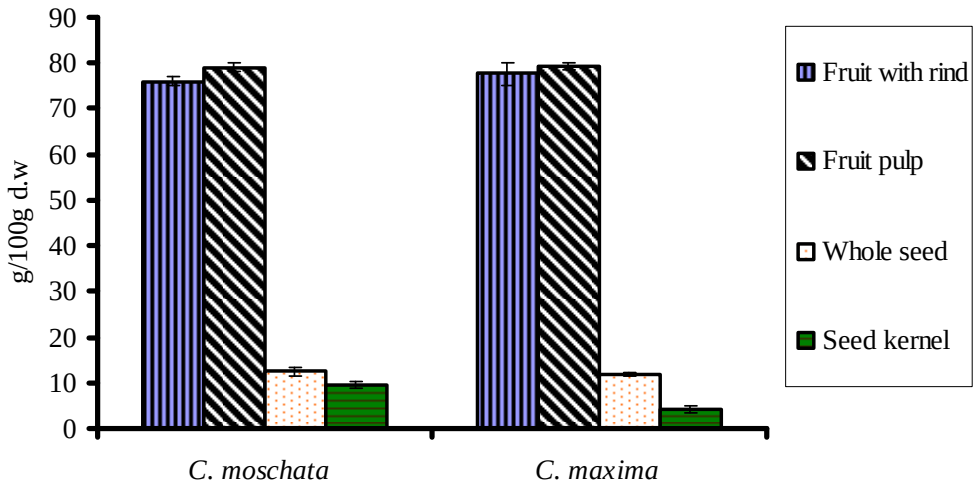

Figure 7: Carbohydrate content in different parts of pumpkin fruits. Vertical bars are mean ± S.D.

4.2. Hunter (L^*, a^*, b^*) colour and hue angle values for pumpkin fruit flours

The Hunter (L^*, a^*, b^*) colour values for pumpkin fruit pulp that was dehydrated and ground into flour are presented in Table 8. Drying pre-treatments had varying effects on the final colour of the pumpkin flour. The treatments of blanching resulted in significantly ($p<0.05$) lower chromatic L^* values than the control. The flour samples from fruit pulp that was not subjected to any drying pre-treatment had significantly

($p<0.05$) high chromatic L^* values for both species. This implies that the samples lost their pigment to become lighter. Blanching is a heat treatment commonly applied to tissue systems prior to freezing, drying or canning. Blanching prior to dehydration is done primarily to inactivate enzymes.

Table 8: Hunter (L^*, a^*, b^*) colour and hue angle values of pumpkin fruit flours

Species/pre-treatment	L^*	a^*	b^*	Hue Angle Θ
C. moschata no treatment	74.0 ± 0.6^{e}	13.8 ± 0.6^{c}	64.3 ± 0.3^{g}	77.9^{c}
C. moschata blanched	48.9 ± 0.1^{c}	15.1 ± 0.4^{d}	29.1 ± 0.6^{c}	62.5^{a}
C. maxima no treatment	77.1 ± 0.2^{f}	5.4 ± 0.5^{a}	41.1 ± 0.4^{d}	82.5^{d}
C. maxima blanched	39.7 ± 0.4^{a}	9.9 ± 0.7^{b}	24.4 ± 0.6^{a}	67.7^{b}
L.S.D ($P<0.05$)	1.2	0.8	0.7	3.5

Values are Mean± S.D, n = 3

$^{a-g}$ Values in the same column with different superscripts are significantly different ($p< 0.05$)

Un-blanched dried foods exhibit rapid changes in such properties as colour, flavour and nutritive value as a result of enzyme activity. In the present study it can be seen from Table 8 that un-blanched samples lost their colour upon drying, as indicated by the high L^* values and high hue angle values. There was a direct correlation between the chromatic L^* values and the hue angle values. Where the hue values were high, the corresponding L^* values were also high and vice versa.

The chromatic L^* value is an indication of brightness, the higher the L^* value, the brighter the samples. The samples that were un-blanched depicted high hue angle values, implying that they lost some of their orange colour to become more brightly yellow and less orange. Hue is the characteristic associated with the conventionally perceived colour. An angle of 90^0 represents yellow hue.

Objects with a higher hue angle are greener while those with a lower hue angle are more red -orange (Francis, 1980). The chromatic a^* values represent redness. The higher the a^* values the more red the samples are. The chromatic b^* value represents yellowness. The higher the b^* value, the more yellow the sample is. The results showed that the blanched samples had significantly ($p<0.05$) higher a^* values than the un-blanched samples. This shows that the blanched samples retained their colour. The un-blanched samples had significantly ($p<0.05$) lower b^* values than the blanched ones, which means that the latter were less yellow (more orange) since the blanching helped them retain their colour.

Blanching may provoke some loses of carotenoids, but the inactivation of oxidative enzymes that occurs in this type of heat treatment prevents further and greater losses of carotenoids during holding before thermal processing, slow processing and storage (Rodriguez-Amaya and Kimura, 2004).

Two of the more heat-resistant and widely distributed enzymes in plant tissues are peroxidase and catalase. Activity of these enzymes, therefore, can be used to evaluate the effectiveness of a blanching treatment. If both are inactivated then it can

be assumed that other significant enzymes also are inactivated. In this study the peroxidase test was done and between the blanching times of 0, 2, 4, 5, 6, 8; and 10 minutes, the peroxidase test was negative at 5 minutes, at which time there was no colour change in the solution. Below 5 minutes, there was a deep pink–brown colour in the resulting solution. Therefore 5 minutes is the most efficient blanching time at a temperature of 96^0C for pumpkin slices of 12 cm length and 1.5 cm thickness.

4.3. Beta-carotene content in raw and dried pumpkin fruits

4.3.1. Beta-carotene content in raw pumpkins

The β-carotene content in the two species of pumpkin studied is presented in Table 9. The results show that fresh fruits with rind of *C. maxima* contain significantly ($p<0.05$) higher beta-carotene than *C. moschata*. For both species generally the fruit with rind contains more beta-carotene than the fruit pulp.

Table 9: Beta-carotene content in raw pumpkin fruits (µg/g d.w)

Variety/treatment	B-carotene content
C. maxima with rind	582.7 ± 3.9^c
C. maxima pulp	538.0 ± 1.7^b
C. moschata with rind	534.5 ± 10.2^b
C. moschata pulp	518.7 ± 6.9^a
L.S.D. ($P<0.05$)	18.5

Values are mean ± S.D, n = 3

[a-c] Values in the same column with different superscripts are significantly different at 5% level

A review of literature does not find any values of beta-carotene for fruits with rind. Pumpkins are potentially good sources of β-carotene, especially if their worldwide availability, ease of production, and long shelf life are considered. In some countries, including Kenya, the flowers and leaves of these fruit vegetables are also consumed (Pepping *et al*., 1998).

Data from Tee and Lim (1991) show that β-carotene (from 0.5 to 15 µg/g) contents of squashes and pumpkins to be very low to low, while some studies show moderate to high β-carotene levels in these fruit vegetables. Studies carried out by Arima and Rodriquez-Amaya (1988) on four commercial Brazilian cucurbits by open column chromatography found that the native *C. moschata* presented the highest mean levels of α-carotene (23 µg/g) and β-carotene (39 µg/g) at the mature stage.

In the United States, some samples of pumpkin analysed by HPLC contained 24 to 84 µg/g of β-carotene (Bushway 1986; Quackenbush 1987; Khachik and Beecher 1987).The varied differences in the β-carotene amounts in pumpkins may be attributed to the long period during which these fruit vegetables can be harvested, and to their extended shelf life. Some of the low levels reported may be due to analyses of immature pumpkins Rodriguez-Amaya (1997). The values in this study were obtained from mature pumpkin fruits. Since the fruits with rind contain more beta-carotene than the fruit pulp, people could be encouraged to eat whole pumpkins without peeling after boiling. The fruit slices could also be dried without peeling in order to maximize on the content of β-carotene.

4.3.2. Beta carotene content of flours from blanched and un-blanched dried pumpkin fruits

The values for β-carotene content in dried pumpkin fruit pulp subjected to blanching and those not blanched are presented in Table 10. The blanched samples depicted a significantly ($p<0.05$) higher content of β-carotene than the un-blanched ones. Blanching is an important thermal treatment that inactivates oxidative enzymes that would degrade carotenoids (Rodriguez-Amaya and Kimura, 2004). This drying pre-treatment also preserves colour of the tissues. This was observed in the results of colour presented earlier in Table 8. If processing of pumpkins by drying is to be adopted both at cottage and industrial level then it would be important to incorporate this thermal procedure.

The results in Table 10 also show that without any prior treatment, flours from fruits with rind had significantly ($p< 0.05$) higher beta-carotene levels than the respective pulp. This trend was also observed earlier for fresh fruits. Some investigations have shown that carotenoids, including the provitamin A, are more concentrated in the peel than in the pulp of some fruits (Gross, 1987). Thus, the peeling of fruits and vegetables can reduce the beta-carotene content considerably. In paired samples of immature *C. pepo* and *C. moschata*, the whole fruits had β-carotene content five times greater than the peeled samples (1.5 compared to 0.3 and 1.0 compared to 0.2 µg/g, respectively (Arima and Rodriguez-Amaya, 1988).

The peel of the cucurbit hybrid Tetsukabuto has been found to contain 101 µg/g β-carotene while the pulp had only 16µg/g β-carotene (Rodriguez-Amaya and Kimura, 2004). In this study, the rind (peel) was not analyzed separately from the pulp but it

was never the less observed that the fruits with rind contained more β-carotene than the pulp. The flour from *C. maxima* contained significantly more β-carotene than the flour from *C. moschata* fruits. This difference could be due to the species differences since the period of analysis after harvest was the same.

Comparison of the β-carotene levels of raw fruits (Table 9) and that of dried fruit (flours) in Table10, shows that thermal processing of the pumpkin fruit vegetables caused substantial loss of the β-carotene (61.0% for *C. moschata* and 44.3% for *C. maxima*). This notwithstanding, the retention levels of the β –carotene in the flours would still be sufficient to make the products good sources of pro-vitamin because the raw material used was mature enough to provide high amounts of β-carotene.

Table 10: Beta-carotene content in pumpkin fruit flours (µg/g d.w)

Treatment	Variety	
	C. moschata	*C. maxima*
Fruit pulp no treatment	244.1±3.8[a]	252.6±2.6[a]
Fruit with rind no treat'nt	289.2±7.2[c]	362.7±3.5[c]
Fruit pulp blanched	262.2±4.5[b]	299.7±0.1[b]
Fruit with rind blanched	315.8±7.2[d]	492.8±6.8[d]
L.S.D. (P<0.05)	8.7	5.8

Values are mean ± S.D, n = 3

[a-d] Values in the same column with different superscripts are significantly different at 5% level.

The major cause of carotenoid destruction during processing and storage of foods is enzymatic or non-enzymatic oxidation. Isomerization of *trans*-carotenoids to the *cis*-isomers, particularly during heat treatment, alters their biological activity and discolors foods, but not to the same extent as oxidation. Enzymatic degradation of carotenoids may be a more serious problem than thermal decomposition in many foods (Rodriguez-Amaya, 2001).

In a study by Arima *et al*., (1988) canned sweetened *C. moschata* pumpkin, which was subjected to drastic processing conditions (10-minute blanching, disintegrating, thermal processing in an open steam jacketed kettle for 40 minutes, and immersion of filled and sealed cans in boiling water for 10 minutes), the β-carotene content decreased by 35%. Therefore, thermal processing could be used to add value and diversify the utilization of pumpkins.

4.4. Starch content in pumpkin fruits

Starch was isolated from the two species of pumpkin and quantified using an analytical weighing balance. The results obtained showed that *C. maxima* yielded significantly ($p<0.05$) higher starch content (9.6 ± 2.2 %) than *C. moschata* (3.8 ± 1.4 %). The results agree with those reported by Corrigan *et al.,* (2001) and Hurst *et al*., (1995) who reported that the fruit dry matter starch content may range from < 3% to > 60 % respectively. Studies have shown that other plant food materials like partly ripe bananas contain 8.8% starch while fresh corn and white sweet potatoes contain about 15% starch (Yeshajahu *et al*., 1994). The *C. maxima* species can be a potential source of starch especially in consideration of its high yields of

approximately 22 tons per acre. Starch is an important biopolymer that has found various applications in the food and non-food industrial sector as a raw material. As mentioned earlier, starch, calculated as calories, represents 80 % of the world's food supply (Guan *et al*., 1998).

4.5. Elemental mineral content of pumpkin fruit and seed flours

4.5.1. Mineral content of pumpkin fruit and seed flours of *C. moschata*

The results for mineral composition of fruits and seeds under various treatments for dry samples are presented in Table 11. The fruits and seeds of *C. moschata* contained significantly ($p<0.05$) higher levels of P, K, Mg and Na than Fe, Zn, Cu and Mn. The fruits and seeds can be good sources of all the essential minerals required by human beings for a healthy and active life. Generally, it can be observed from the results that seeds flours contain significantly ($p< 0.05$) higher amounts of minerals than the fruit flours.

The seed kernels contained significantly ($p<0.05$) higher amounts of minerals than the seeds. This could imply that the hull contains less minerals and this is similar to the trend observed for crude ash values. This trend is consistent with studies done on *C. maxima* by Alfawaz (2004) who also found that the seeds contained less crude ash than the seed kernels (4.59% as compared to 5.15%). Of all the nine elements studied, copper was in the smallest amounts, except for the *C. moschata* fruit pulp in which manganese was in the smallest amount.

Table 11: Mineral composition of fruit and seed flours of *C. moschata* (mg/100g d.w)

	Value				
	C. moschata fruit flours		*C. moschata* seed flours		L.S.D.
Element	Fruit pulp	Fruit with rind	whole seed	seed kernel	P<0.05
Copper	0.9 0.2[a]	0.9±0.1[a]	1.5±0.2[b]	1.6±0.1[b]	0.3
Zinc	3.6±0.4[a]	5.3±0.3[b]	8.1±0.4[c]	11.1±0.3[d]	0.6
Manganese	0.7±0.1[a]	2.1±0.1[b]	3.1±0.1[c]	3.9±0.1[d]	0.1
Magnesium	68.4 ±1.1[a]	159.3±2.1[b]	318.4±2.9[c]	537.8±2.7[d]	4.7
Sodium	40.9±0.8[c]	61.2±0.8[b]	24.2±0.4[a]	22.8±1.1[a]	1.8
Potassium	1040.9±13.6[c]	1253.8±11.9[d]	417.3±6.9[a]	493.6±17.1[b]	17.5
Phosphorus	562.6±2.8[a]	714.9±4.8[b]	928.9±1.9[c]	980.8±4.4[d]	8.1
Iron	5.4±0.1[a]	9.3±0.4[c]	7.4±0.1[b]	13.6±0.9[d]	1.2
Calcium	6.3±0.2[c]	9.3±0.4[d]	5.0±0.2[b]	2.4±0.1[a]	0.5

Values are Mean ± S.D, n = 3

[a-d]Values in the same row with different superscripts are significantly different at 5% level.

The major minerals required in the diet are sodium, calcium, magnesium, potassium, phosphorus, chlorine, and sulphur. The latter two were not analyzed in this study. Other minerals that are required in only micro quantities (less than 100 mg/day) are called trace elements and they include iron, zinc, copper, selenium, iodide, fluoride, chromium, manganese and molybdenum. Out of these those analyzed in this study were zinc, copper, manganese and iron.

4.5.2. Mineral composition of pumpkin fruit and seed flours of *C. maxima*

The composition of minerals in the fruit and seed flours of the *C. maxima* species of pumpkin is presented in Table 12. In the seed and fruit flours, levels of P, K, and Mg were significantly ($p<0.05$) higher than the other elements. For most elements the seed kernels contain higher amounts than whole seeds. These results are consistent with those reported by El-Adawy *et al*., (2001). This can be explained by the fact that the hull of the seeds does contain low levels of mineral elements if any, thus lowering the overall content of the minerals in the whole seed. Pumpkin seeds are rich plant source of the mineral zinc which has been reported to be good for the male prostate gland and for body immune system. Zinc has a range of functions. It plays a crucial role in growth and cell division where it is required for protein and DNA synthesis, in insulin activity, in the metabolism of the ovaries and testes and in liver function. It is a component of many enzymes that are involved in the metabolism of proteins, carbohydrates, lipids and energy. This vital role of zinc makes infants, children, adolescents and pregnant women especially at risk for an inadequate zinc intake (Docrep, 2003).Consumption of pumpkin seeds by all age groups should therefore be encouraged through nutrition programs.

Table 12: Mineral composition of fruit and seed flours of *C. maxima* (mg/100g d. w)

	Value				
	C. maxima fruit flours		*C. maxima* seed flours		L.S.D.
Element	Fruit pulp	Fruit with rind	whole seed	seed kernel	P<0.05
Copper	0.47±0.1[a]	0.5±0.3[b]	2.4±0.1[d]	1.9±0.1[c]	0.1
Zinc	2.4±0.2[a]	4.0±0.2[b]	8.7±0.3[d]	7.6±0.4[c]	0.6
Manganese	0.3±0.1[a]	1.3±0.2[b]	3.1±0.2[c]	3.1±0.2[c]	0.3
Magnesium	35.9±1.7[b]	15.2±0.3[a]	286.0±4.4[c]	399.4±2.7[d]	6.3
Sodium	41.3±0.6[c]	64.4±0.4[d]	17.8±0.2[a]	20.6±0.2[b]	0.8
Potassium	981.8±4.3[c]	1148.7±3.1[d]	351.6±7.2[a]	399.6±9.3[b]	13.9
Phosphorus	239.6±7.2[a]	440.5±7.2[b]	824.8±5.2[d]	776.9±2.3[c]	8.3
Iron	1.1 ±0.2[a]	5.1±0.1[b]	6.1±0.9[c]	12.8±0.4[d]	0.9
Calcium	12.3±0.4[c]	15.2±0.1[d]	3.3±0.2[b]	2.1±0.2[a]	0.5

Values are mean ± S.D, n = 3

[a-d]Values in the same row with different superscripts are significantly different at 5% level.

There is still lack of quantitative estimates on prevalence of zinc deficiencies globally, hence policy makers are unable to address the problem (Brown *et al*., 2001).

4.5.3. Relative mineral content in fruit flours of *C. moschata* and *C. maxima*

4.5.3.1. Macro-minerals in fruit flours

The macro-minerals, namely magnesium, sodium, potassium, phosphorus and calcium contained in the fruits of the two pumpkin species are illustrated in Figure 8. Comparatively, for magnesium the *C. maxima* fruit pulp contained the highest

levels. In the case of potassium and phosphorus *C. moschata* fruit with rind contained higher amounts than *C. maxima* fruit with rind. Both species are good sources of the macro minerals.

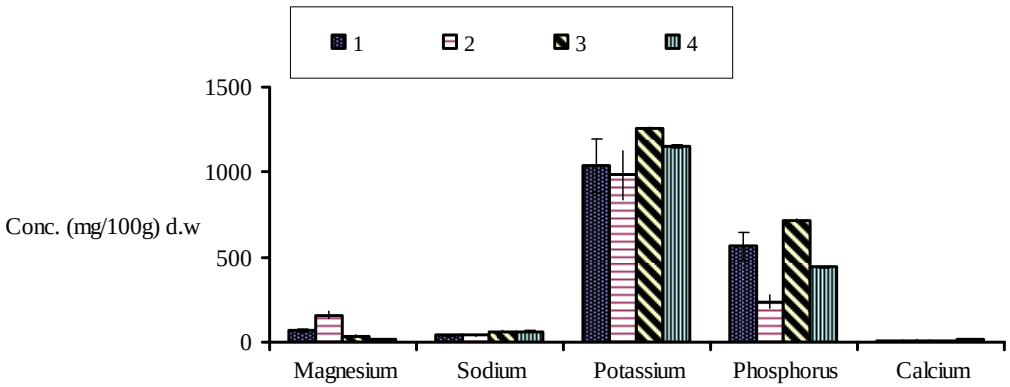

Figure 8: Macro-minerals in *C. moschata* and *C maxima* flours

Legend: 1 - C. *moschata* fruit pulp, 2 - *C. maxima* fruit pulp, 3 - *C. moschata* fruit with rind, 4 - *C. maxima* fruit with rind

4.5.3.2. Micro-minerals in fruit flours

Figure 9 is an illustration of the comparative content of micro-minerals copper, zinc, Iron and manganese in the fruit flours of the two species of pumpkin. It is evident that both species contain appreciable amounts of Iron and zinc. *C. moschata* with rind contains more of each of the micro-minerals than the corresponding *C. maxima***.**

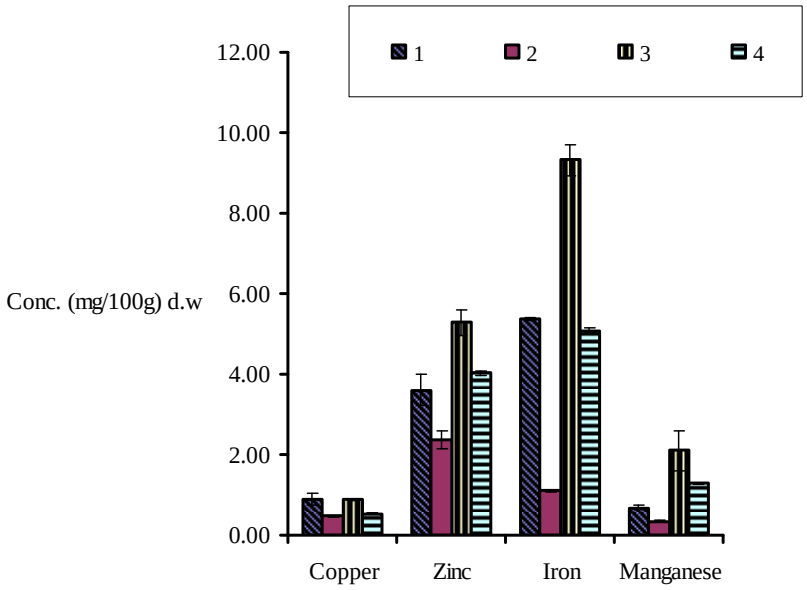

Figure 9: Micro-minerals in *C. moschata* and *C. maxima* fruit flours

Legend: 1 - *C. moschata* fruit pulp, 2 - *C. maxima* fruit pulp, 3 - *C. moschata* fruit with rind, 4 - *C. maxima* fruit with rind.

4.5.4. Mineral content in seed flours of *C. moschata* and *C. maxima*

4.5.4.1. Macro-minerals in seed flours

The macro-mineral content in seed flours of both pumpkin species are illustrated in Figure 10. Both species contained significantly higher amounts of magnesium, potassium and phosphorus than calcium and sodium. *C. moschata* seed kernel was found to contain higher amounts of each of the macro-minerals than its corresponding whole seeds and the seed kernels and whole seeds of *C. maxima.* A review of literature has not found any work that has compared the amounts of minerals in whole seeds and seed kernels of pumpkin or any other nuts.

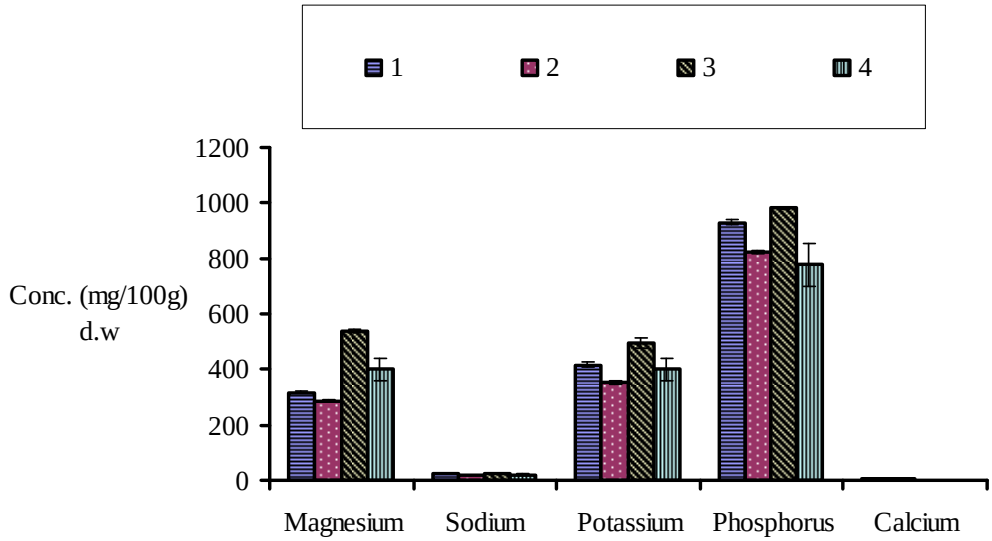

Figure 10: Macro-minerals in *C. moschata* and *C. maxima* seed flours.

Legend: 1 - *C. moschata* whole seed, 2 - *C. maxima* whole seed, 3 - *C. moschata* seed kernel, 4 - *C. maxima* seed kernel.

4.5.4.2. Micro-minerals in seed flours

A comparison between mineral content in whole seeds and seed kernels of the two species of pumpkin are illustrated in Figure 11. It was found that both seeds and seed kernels contained almost equal amounts of zinc, copper and manganese. In the case of Iron *C. maxima* whole seed contained the lowest amounts while *C. moschata* seed kernel contained the highest amounts of the micro-mineral. From these results it can be seen that *C. moschata* is superior in terms of micro-mineral content.

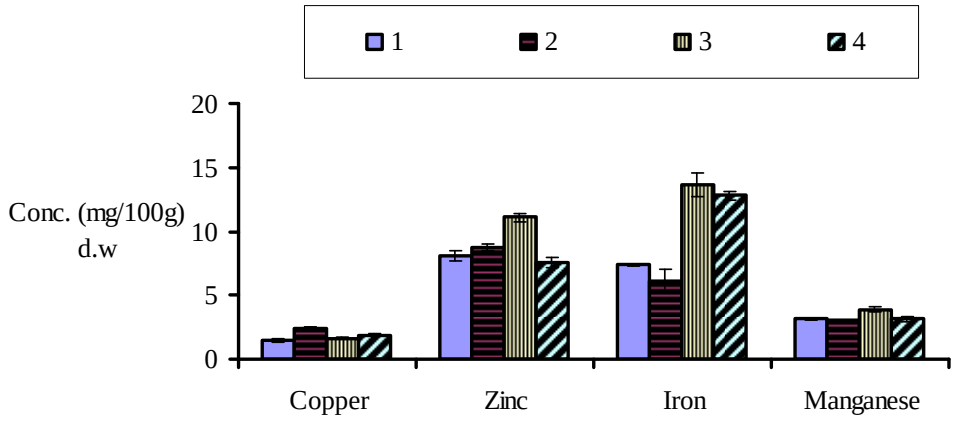

Figure 11: Micro-minerals in *C. moschata* and *C. maxima* seed flours

Legend: 1 - *C. moschata* fruit pulp, 2 - *C. maxima* fruit pulp, 3 - *C. moschata* fruit with rind, 4 - *C. maxima* fruit with rind

4.6. Characteristics of pumpkin seed oils

4.6.1. Physicochemical properties of pumpkin seed oil

The results of values for the physicochemical properties of pumpkin seed oils of the two species of pumpkin under study are presented in Table 13. A one way analysis of variance showed that there were no significant ($P<0.05$) differences in specific values of the oils of the two species, except for acid value in which *C. maxima* oil had a significantly ($p<0.05$) higher amount. Saponification value gives an indication of molecular weight. The smaller the saponification number the larger the average molecular weight of the triacylglycerols present. The high iodine values indicate that the oil contains unsaturated fatty acids. Kinkela (1990) reported that *Cucurbitaceae* seed oils contain 68.5% linoleic acid. The oils could be used as drying oil for manufacture of cosmetics, oil paints and varnishes (Odoemelam, 2005).

Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali:

Triacylglycerol + 3 KOH → Glycerol + 3 Fatty acid salts of potassium

The saponification number is defined as the mg of KOH required to saponify one gram of fat.

Table 13: Physicochemical characteristics of pumpkin seed oil

Property	*C. moschata*	*C. maxima*	L.S.D. (P<0.05)
Refractive index (25^0C)	1.5±0.0[a]	1.5±0.0[a]	0.0
Specific gravity (15.5^0C)	0.9±0.0[a]	0.9±0.0[a]	0.0
Saponification value	201.9±4.9[a]	205.4±3.9[a]	10.2
Wij's Iodine value	109.7±1.1[a]	112.2±1.6[a]	3.2
Acid value	1.2±0.0[b]	2.7±0.1[a]	0.1
Peroxide value	3.5±0.2[a]	3.8±0.3[a]	0.5

Values are mean ± S.D, n = 3

[a-b]Values in the same row with different superscripts are significantly different at 5% level.

4.6.2. Fatty acid profile of pumpkin seed oil

The fatty acid composition of lipids from the seeds of *C. moschata* and *C. maxima* are presented in Figure 12. There were wide variations in the contents of palmitic, Stearic, oleic and linoleic acids of the seed oils. The *C. maxima* seed oil contained higher percentages linoleic acid than *C. moschata* oil.

The major unsaturated fatty acids in both oils were linoleic (9, 12-octadecadienoic acid) acid followed by oleic acid (9-octadecenoic acid). The presence of high amounts of the essential linoleic acid suggests that these oils are highly nutritious, due to their ability to reduce the serum cholesterol. Both oils could be utilized as edible cooking or salad oils or for margarine manufacture owing to their rich content of both oleic and linoleic acids. The major saturated fatty acids in both oils were palmitic acid (hexadecanoic acid) and stearic acid (octadecanoic acid). These results are consistent with the findings of Al-Khalifa (1996) and Tarek *et al*. (2001).

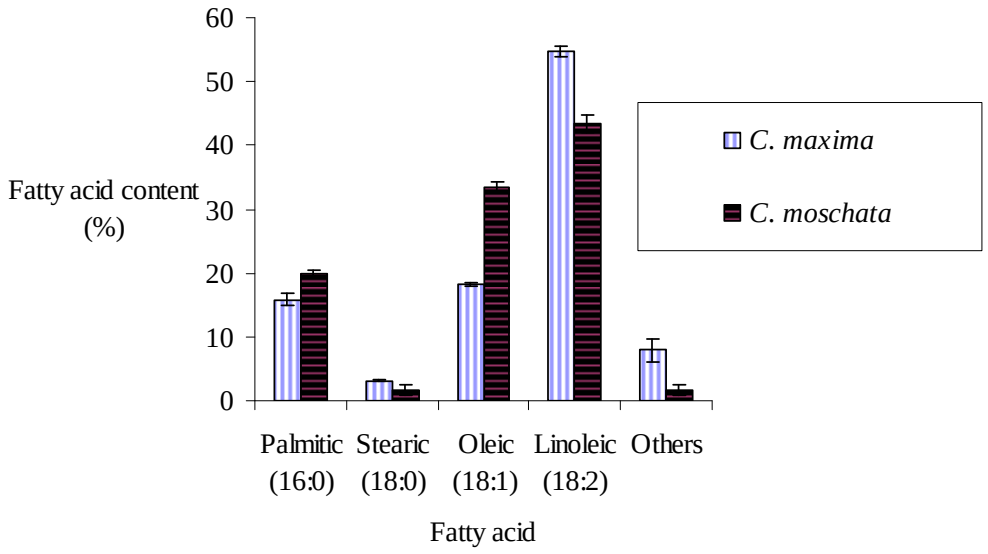

Figure 12: Fatty acid content in *C. maxima* and *C. moschata* seed oil

The comparative content of different fatty acids in the two species of pumpkin analyzed showed that *C. maxima* seed oil contained more stearic and linoleic fatty

acids than *C. moschata* seed oil, while palmitic and oleic acids were in higher amounts in *C. moschata* than *C. maxima.*

4.7. Functional properties of pumpkin seed flours and protein isolates

4.7.1. Protein solubility profile

Protein solubility characteristics are influenced by factors such as origin, processing conditions, pH, ionic strength and the presence of other ingredients (Kinsella, 1981). The protein solubility profiles at various pH values of protein isolates and defatted flours of two species of pumpkin are illustrated in Figure 13.

From the results it was found that nitrogen solubility was pH –dependent. Minimum solubilities were observed at pH 4 which is called the isoelectric point (pI). At this point there was a sharp increase in nitrogen solubility on either side. The pI of a protein is the pH at which the protein has an equal number of positive and negative charges; it is the pH at which the protein carries no net charge. The pI is of significance in protein purification because it is the pH at which solubility is often minimal and at which mobility in an electro- focusing system is zero (and therefore the point at which the protein will accumulate) and less water interacts with the protein (Vojdani, 1996).

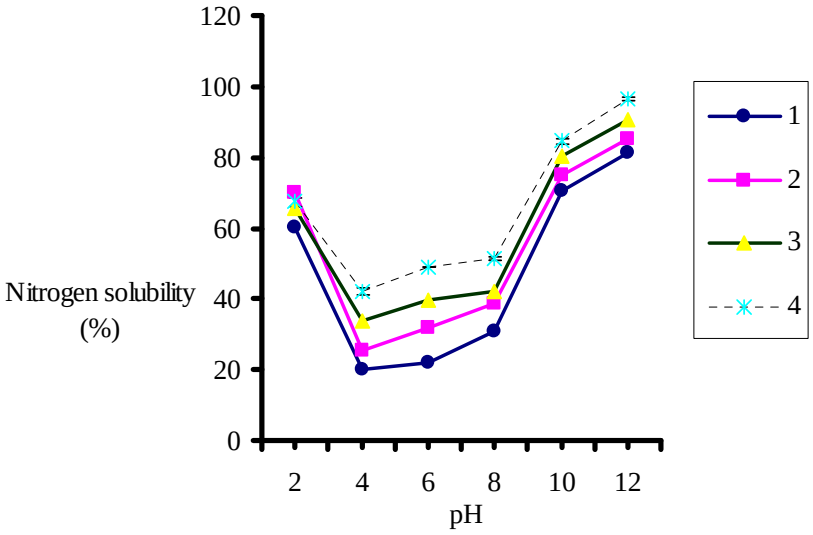

Figure 13: Protein solubility profiles of protein isolates and defatted flours of pumpkin seeds.

Legend: 1 - *C. moschata* flour, 2 - *C. moschata* protein isolate, 3 - *C. maxima* flour, 4 - *C. maxima* protein isolate.

The protein isolates were more soluble than the seed flours due to the differences in the total protein content. In general, proteins used for functionality are required to have high solubility, in order to provide good emulsion, foam, gelation and whipping properties. The pumpkin seed flours and isolates depicted high solubility of over 80% at pH values above 9. This shows that they could find useful application in various food systems.

4.7.2. pH values of pumpkin seed kernel flours and protein isolates

The pH values of the flours and protein are presented in Table 14. The protein isolates were found to be more acidic than the flour samples due to their higher concentration of protein. The acidity of these samples was in general agreement with pH values of many legume flours, including soybean flour (Sosulski *et al*., 1976).

4.7.3. Water- and oil-holding capacity

The water- and oil –holding capacities of the flours and protein isolates are shown in Table 14. For both species the protein isolates had higher water holding capacity than the respective flour samples. This could be attributed to the amount of protein available in the samples.

Water holding capacity as an index of the amount of water retained within the protein matrix shows the functional capacity of the seed protein in thickening and food formulations.

Mwasaru *et al*., (1999) studied the effect of extraction pH on the water and oil holding capacity, but in this study the proteins were all extracted at pH 8.5. The values for oil absorption indicate that proteins isolates had significantly ($p<0.05$) higher oil holding capacity than the flour samples. Oil absorption is attributed to physical entrapment of oil and is important in flavour retention and mouth feel of foods (Mwasaru *et al*., 1999).

Table 14: Functional properties of seed flours and protein isolates of pumpkin seeds

Functional properties	*C. maxima* flour	*C. maxima* protein	*C. moschata* Flour	*C. moschata* Protein	L.S.D. P<0.05
Ph	6.6±0.1[d]	5.9± 0.1[b]	6.3±0.1[c]	5.6±0.1[a]	0.1
Water holding capacity (mg/g)	2.2±0.2[b]	2.7 ±0.1[c]	1.9 ±0.1[a]	2.4 ± 0.2[b]	0.2
Oil holding capacity (ml/g)	1.7± 0.1[b]	2.2 ± 0.1[d]	1.5 ± 0.1[a]	2.0 ± 0.1[c]	0.2
Emulsifying activity (%)	44.1±0.2[b]	47.0±2.0[c]	42.1±0.2[a]	46.2±0.6[c]	1.9
Emulsifying stability (%)	55.2±0.6[b]	60.6±0.9[d]	52.0 ± 0.5[a]	57.2±0.6[c]	1.3
Foaming activity (%)	26.2±0.2[a]	31.3±0.3[b]	38.4± 0.6[c]	43.0 ±0.8[d]	0.9
Foaming stability (%)	60.0±1.0[c]	65.3±1.5[d]	39.2± 0.3[a]	44.6±0.6[b]	1.8

Values are Mean ± S.D, n=3

[a-d]Values in the same row with different superscripts are significantly different at 5% level.

4.7.4. Emulsifying activity and emulsion stability

The values of emulsifying activity and stability for seed flours and protein isolates for the two pumpkin species are presented in Table 14. The analyses were done without modifying the pH of the solution. There were significant differences between the emulsifying capacities and emulsion stabilities of the flours and protein isolates, with flours having lower values of each property tested ($p<0.05$).

The ability of proteins to aid in the formation and stabilization of emulsions is important in applications in food systems such as chopped, comminuted meats, cake batters, coffee creamers, mayonnaise, salad dressings and frozen desserts (Kinsella, 1981). It has also been reported that emulsifying properties can be manipulated by enhancing their solubility properties since the two properties are inter related.

4.7.5. Whipping properties

The foam expansion and foam stability profiles of flours and protein isolates of pumpkin seeds are presented in Table 14. The results show that there were significant differences ($p<0.05$) between whipping properties of the flours and isolates, with the latter depicting higher values. The foam stability values were higher than the respective values for foam expansion. The foaming power of proteins can be enhanced by partial hydrolysis which, however, results in decreased foam stability, thus a compromise has to be made between foamability and foam stability depending on the application (Mwasaru *et al*., 1999).

The foaming property is important in the manufacture of foods such as whipped toppings, fudges, chiffon desserts and angel cakes. Proteins stabilize foams by forming a cohesive but deformable film which ideally resists excessive localized thinning (Kinsella, 1981). It can be seen that the pumpkin proteins can be ideal for these food applications.

4.7.6. Gelation Properties

The least gelation concentration (LGC) of seed flours and protein isolates of the two pumpkin species are given in Table 15. The flour samples of both seeds had an LGC of 8% while the protein isolates had LGC of 6%. The value for the flours is in agreement with the value of 8% obtained for defatted pumpkin flours for *C. maxima* (Lazos, 1992).

Table 15: Gelation properties of seed flours and protein isolates of pumpkin seeds

Flour and protein isolate concentration % (w/v)	*C. maxima* Flour	*C. maxima* Protein isolate	*C. moschata* Flour isolate	*C. moschata* Protein isolate
2	–	–	–	–
4	–	±	–	±
6	±	+	±	+
8	+	+	+	+
10	+	+	+	+
12	+	+	+	+
14	+	+	+	+
16	+	+	+	+
18	+	+	+	+
20	+	+	+	+

–,Not gelled; ±, gelled slightly; +, gelled

A commercial soy protein isolate required a minimum concentration of 12% to form a stable gel (Mwasaru *et al*., 1999). Previous workers have reported LGC of 8% for soy isolate (Kinsella, 1981), 10% for mung bean isoelectric protein isolate (Coffman and Garcia, 1977).

Proteins are composed of three dimensional matrices or networks of intertwined, partially associated polypeptides in which water is entrapped. Proteins are able to form gels and provide a structural matrix for holding water, flavours, sugars and other ingredients and based on this they play an important role in many food applications in particular comminuted sausage products and tofu (Fleming *et al*.,1975).

4.8. Food application potential of pumpkin fruit flour

4.8.1. Colour of pumpkin flour-wheat flour cakes

The ingredients of the cakes made with addition of pumpkin powder are presented in Table 1 in chapter 3. The amounts of sugar, shortening, baking powder water and whole eggs were constant for all samples.

The mean values for crumb and crust cake colours are given in Table 16. The results show significant differences ($p<0.05$) between the L^*, a^*, and b^* values at all levels of pumpkin powder replacement. The crust and crumb of the cakes became significantly darker (more orange) than the control with subsequent replacements of the wheat flour with the pumpkin flour.

Table 16: Mean values for crust and crumb colours of cakes prepared with various levels of pumpkin flour replacing wheat flour

	% pumpkin powder replacement					L.S.D.
Colour values	0	5	10	15	20	P<0.05
Crust colour						
L^*	58.1± 0.3^{e}	55.3±0.3^{d}	46.2±0.4^{c}	43.3± 0.4^{b}	42.9±0.3^{a}	0.6
a^*	10.5± 0.2^{a}	11.2±0.6^{a}	14.3±0.6^{b}	15.4 ± 0.2^{c}	16.8±0.4^{d}	0.9
b^*	31.8±0.2^{b}	37.2±0.2^{e}	36.4±0.1^{d}	35.3±0.5^{c}	29.4±0.5^{a}	0.6
Θ	71.8	73.3	68.6	66.5	60.3	0.9
Crumb colour						
L^*	66.3±0.3^{d}	65.1±0.3^{c}	60.1±0.1^{b}	60.2± 0.3^{b}	58.4±0.6^{a}	0.7
a^*	14.1±0.1^{d}	17.5±0.1^{c}	22.0±0.0^{a}	32.0±0.03^{b}	38.0 ±0.0^{ab}	0.1
b^*	42.2± 0.3^{a}	45.3±0.4^{b}	52.3±0.4^{c}	56.4± 0.7^{d}	57.9±0.2^{e}	0.6
Θ	74.0	68.9	67.2	60.4	56.7	1.5

Values are Mean ± S.D

$^{a-d}$ Values in the same row with different superscripts are significantly different at 5% level. Θ: Hue angle = $\tan^{-1} b^*/a^*$

This is evident from the values of the hue angle values, which became subsequently lower with increasing amount of pumpkin powder added. Visually, this can be seen from pictures of the cakes (plates 3 and 4). These results are similar with the findings by Lee *et al*., (1993) who found that on substituting egg with bovine plasma product the cake crust and crumb colours became significantly darker and tanner than those for the control when 25% or more of the egg was replaced by plasma.

In the present study colour changes were observed to occur from the addition level of 5%. Colour as a physical property of food is important in consumer acceptability of food products. The yellow colour is appealing to both adults and children and would influence the purchasing choice (Lee *et al*., 1999).

Plate 3: Effect of pumpkin flour addition on cake colour from left to right: control, 5%, 10%, 15%, and 20%.

Plate 4: A cross section of cakes with added pumpkin flour from left to right: control, 5%, 10%.15%, and 20%.

4.8.2. Beta-carotene content in cakes with blending of pumpkin flour with wheat flour

The effect of pumpkin flour replacements on cakes is shown in figure 14. It was observed that the beta carotene content increased with subsequent increase in the percentage of pumpkin flour added.

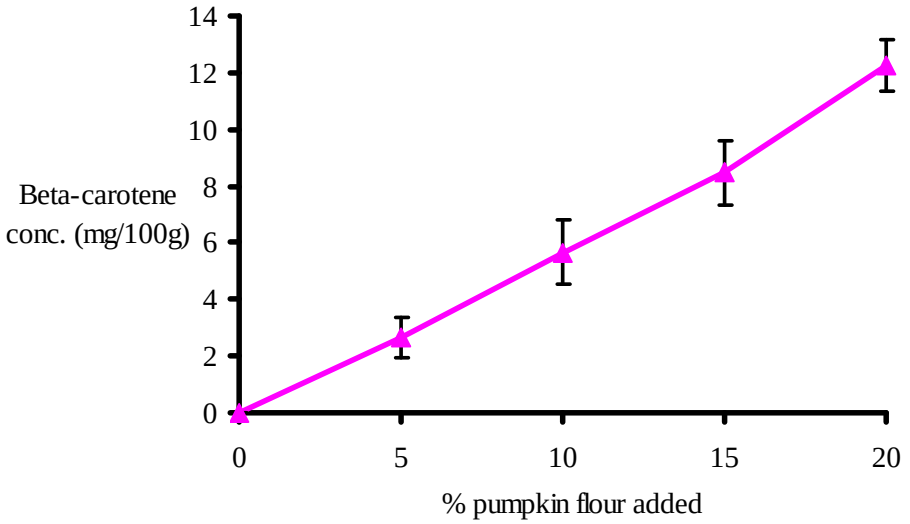

Figure 14: Beta-carotene concentration in cakes with various additions of pumpkin powder

Beta-carotene, alpha carotene and beta-cryptoxanthin are carotenes that are converted to vitamin A in the body. About 300 carotenoids have been isolated from natural sources (Ong and Tee, 1992). The most important sources for carotenoids are plants, where often the brilliant colours of the carotenoids are covered by the green chlorophillic pigments. Most yellow and orange coloured fruits and vegetables contain carotenoids.

In human beings, carotenoids can serve several important functions. The most widely studied and well understood nutritional role of carotenoids is their provitamin A activity. Deficiency of vitamin A is a major cause of premature death in developing nations, particularly among children. The vitamin A can be produced within the body from beta-carotene (Britton *et al*., 1995; Patton *et al*., 1990). Dietary beta-carotene is obtained from a number of fruits and vegetables, such as carrots, pumpkins, spinach, sweet potatoes (Mangels *et al*., 1993).

Fortification of foods with products rich in beta-carotene would go a long way in reducing health problems like low body immunity and night blindness that are associated with deficiency of vitamin A. In a study conducted by Abebe *et al.,* (2006) in Ethiopia it was found that the use of pumpkin improved the vitamin A density of corn: kidney bean: pumpkin (CBP) diet and kocho: kidney bean: pumpkin (KBP) diet. Compared with the un-supplemented corn and kocho diet, the improvement in the vitamin A value was more than 25-fold in CBP and more than 180-fold in KBP. The amounts of vitamin A required to prevent night blindness were evaluated by the Food and Nutrition Board (2001) and an Estimated Average Requirement (EAR) of 112 µg/day.

4.8.3. Sensory Evaluation of cakes

Sensory rating of cakes is presented in Table 18. Colour rating was lowest (5.44) for the control (0% pumpkin flour addition) as compared to the colour rating of 7.78 at 5% pumpkin flour addition to the wheat flour. The control cake was white in colour while addition of pumpkin flour gave the cakes yellow colouration which appealed

to the panellists. The lowest score (5.78) for general acceptability was observed at 20% pumpkin flour addition compared to 7.89 score for 5% pumpkin flour replacement level.

Table17: Sensory evaluation scores of cakes made from pumpkin-wheat flour blends

Property	% Pumpkin powder added					
	0	5	10	15	20	LSD
Colour	5.4±1.0[a]	7.8±0.7[b]	7.1±1.5[b]	7.4±0.9[b]	7.0±1.0[b]	0.9
Flavour	6.8±1.3[a]	8.1±0.9[b]	7.7±0.9[bc]	7.1±1.6[ac]	6.8±1.4[a]	0.9
Texture	7.2± 1.5[b]	7.7±1.2[b]	7.1±1.6[b]	7.3 ±1.2[b]	5.6±1.42[a]	1.1
Appearance	6.2±1.8[a]	7.8±1.5[a]	6.9±1.76[a]	7.2 ±1.7[a]	6.6 ±1.8[a]	1.3
General acceptability	6.6± 0.7[ac]	7.9±0.9[b]	7.3±1.3[b]	7.2 ±1.5[bc]	5.8±2.0[a]	1

Values are mean ±S.D, n= 9

[a-c]Values in the same column with different superscripts are significantly different at 5% level.

The cake flavour at 5% replacement level attracted the highest score (8.1), while 0% (control) and 20% attracted the lowest rating (6.8). Generally, replacing the wheat flour in cake formulations improved their sensory rating. The most acceptable flour combination was 10g pumpkin flour (5% of mixture) and 190g wheat flour.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

From this study, it can be concluded that both species of pumpkins contain high moisture levels, with fruits yielding a dry matter of approximately 12% on dehydration. Conversely, the pumpkin seeds contain low moisture levels (5.7%-6.1%). Therefore the seeds can be stored over long periods of time, owing to their low moisture levels.

The pumpkin seeds are better sources of protein than the pumpkin fruits, since pumpkin fruits were found to contain relatively low amounts of proteins (4-4.9%), as compared to the seeds (35-40%). The pumpkin fruits contained negligible amounts of crude fat (1-2 %) as compared to seeds and seed kernels which contained relatively high amounts of crude fat (34-48%). Therefore pumpkin seeds can contribute substantially to the dietary fat in human nutrition.

The crude ash content of pumpkin fruits was found to be higher than that for the seeds, with fruits ranging between 5-7% while seeds 3.6-4.4%, therefore mineral intake in the diet can be enhanced by increased consumption of pumpkin fruits. The crude fiber content of pumpkin fruits ranged between 8-10.4%. Fruits with rind contained more crude fiber than fruit pulp. Whole seeds contained crude fiber ranging between 11-12.6%, while seed kernels had lesser amounts of 3.8-4.2%. The whole seeds contain half the amount of fiber as sesame seeds per 100g. These results show that consumption of whole pumpkin fruits without peeling can enhance the

amounts of fibre consumed, also the seeds could be consumed without de-hulling in order to ensure adequate intake of dietary fibre.

From this study it can be seen that both raw and processed pumpkin fruits contained substantial amounts of Beta-carotene which ranged between 518.7-582.7µg/g for raw fruits and 244-492.8 µg/g for processed fruits on dry weight basis. The pumpkin fruits and seeds contained substantial amounts of all minerals analyzed. Zinc and Iron were more in the seeds than in the respective fruits. Therefore processing of the fruits by drying should be encouraged in order to reduce bulkiness since this does not affect their nutritive value.

This study also showed that the oils are nutritious due to their ability to reduce serum cholesterol and could be utilized as edible cooking or salad oils or for margarine manufacture The physicochemical properties of seed lipids showed them to be desirable since the iodine value was 109-112 g I/100g oil, acid value 1.2-2.7mg/KOH /g oil and peroxide value 3.5-3.7 $Meq._{thio}$ /kg sample. The high iodine value gave an indication of highly unsaturated fatty acids. This was confirmed by the analysis of fatty acid profile that found the lipids to contain 43.4-54.9 % linoleic acid which is a poly- unsaturated fatty acid. Therefore consumption of pumpkin oil in the diet as cooking oil, margarine or salad dressing would improve the nutritional status of the most vulnerable in the population.

Owing to their high protein content, the seed proteins could be used in various food systems such as sausages and comminuted foods since they have desirable

functional properties. The pumpkin fruit flour would be used in flour fortification in order to enhance their beta-carotene content, flavour and colour of resulting food products. From this study, it can be concluded that *C. maxima* species is a better species than *C. moschata* owing to its nutritional composition and physicochemical properties.

This study proves to be true the hypothesis that the four products of pumpkin fruits and seeds are potentially nutritious and could be incorporated into food systems for fortification and as functional food ingredients. Pumpkins therefore have good potential for increased production, processing and utilization.

5.2. Recommendations

It is recommended that further studies be conducted to optimize blanching methods to ensure minimal losses of beta-carotene. Studies should also be done with more possible types of foods that can be made with incorporation of pumpkin seed and fruit flours.

Future work should involve the study of influence of storage conditions on beta carotene content and colour of the processed pumpkin fruits. It is also recommended that the drying kinetics of the pumpkin fruits be studied in order to come up with information on the optimal drying conditions.

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