KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI.

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

FACULTY OF AGRICULTURE

DEPARTMENT OF HORTICULTURE

EFFECT OF DIFFERENT PACKAGING MATERIALS ON THE QUALITY AND SHELF LIFE OF MORINGA (*Moringa oleifera*) LEAF POWDER DURING STORAGE.

A THESIS SUBMITED TO THE SCHOOL OF RESEARCH AND GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI GHANA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE (MSC.) DEGREE IN POST HARVEST PHYSIOLOGY.

MAXWELL MENSAH

JANUARY, 2011.

DECLARATION

I Maxwell Mensah, author of this dissertation titled "EFFECT OF DIFFERENT PACKAGING MATERIALS ON THE QUALITY AND SHELF LIFE OF MORINGA (*Moringa oleifera*) LEAF POWDER DURING STORAGE", do hereby declare that except for references to other peoples work which have been duly acknowledged, the work presented in this dissertation was done entirely by me and has not been presented in part, or in whole, for any degree in this university or elsewhere.

…….. ……

Maxwell Mensah Date

(STUDENT)

…… ……

Mr.Patrick Kumah Date

(SUPERVISOR)

…..…… ……..

Dr.B. K. Maaleku Date

(HEAD OF DEPARTMENT)

DEDICATIONS

I dedicate this work to my family and friends.

ABSTRACT

Transparent and opaque PET and glass bottles, transparent and opaque LDPE bags, brown paper bags, waxed paper and cardboard boxes were used to package the Moringa leaf powder. The effect of different packaging materials on the quality and shelf-life of Moringa leaf powder under ambient conditions were studied for a period of 180 days. Each treatment package was evaluated for moisture content, bacteria load, nutrients (crude protein, fat, calcium, magnesium and iron), colour and particle size. Bacterial and fungal identification was also carried out. Moisture content in the paper packages increased significantly ($P \leq 0.05$) by day 16. All the paper packages (waxed paper, paper cardboard and brown paper) were contaminated by *Aspergillus flavus* by day 16. Transparent and opaque glass packages recorded the least moisture changes followed by transparent and opaque high density polyethylene and transparent and opaque low density polyethylene packages. There was a general decline in quality of samples in terms of quantity of nutrients with storage period. Decreases in nutrients were not significantly different ($P > 0.05$) among all the packages tested. The estimated number of colony forming units of bacteria increased significantly ($P \leq 0.05$) in all the paper packages by day 16 of storage, whiles the changes in glass and plastic packages were not significantly ($P > 0.05$) different. Prominent changes in colour of Moringa samples occurred in all the paper packages by day 16 whiles there were no colour changes in the glass and plastic packages during the storage period. There was a significant effect of type of packaging on the changes of particle sizes of Moringa leaf powder. Significantly higher ($P \leq 0.05$) particle agglomeration occurred in all the paper package whiles glass packages had the least agglomeration of particle. Glass packaging was found to be the most suitable packaging material in preventing moisture absorption and growth and

activities of micro-organisms. They were also effective in preventing undesirable colour changes and particle size changes.

ACKNOWLEDGEMENTS

I would like to express my profound gratitude to all senior members of Horticulture Department, College of Agriculture and Natural Resources, K.NU.S.T for their immense support, especially to Mr. Patrick Kumah (Supervisor) for his constructive comments, ideas and help that guided me through this research work. Also to Dr. E. Moses, Mr. N. Amaglo and Mr. E. Odame for the great help and support they offered throughout the research work.

TABLE OF CONTENTS

LIST OF TABLES

TABLE **PAGE**

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

LIST OF ABBREVIATIONS

AOAC: Association of Official and Analytical Chemists.

CAC: Codex Alimentarius Commission.

CSIR: Council for Scientific and Industrial Research

FAO: Food and agricultural Organisation.

HACCP: Hazard Analysis and Critical Control Point.

IAEA: International Atomic Energy Agency.

I.C.M.S.F.: International Commission on Microbiological Specification for Foods.

LDPE: Low Density Polyethylene

PDA: Potato Dextrose Agar

PET: Polyethylene Terephthalate

1.0 INTRODUCTION

Moringa oleifera belongs to the Moringacaea family. It is indigenous in Northern India and Pakistan but has been introduced throughout the tropics and sub-tropics and has become naturalised in many African countries (Grubben and Denton, 2004). Moringa is highly patronized by people in the tropics and sub-tropics for it numerous medicinal properties (Fuglie, 2001). As a source of nutrition, Moringa leaves probably rank as the best of all tropical vegetables. They contain very strong concentrations of vitamins A and C, B-complex vitamins, iron, calcium, protein, zinc, selenium, and, unusual for a plant source, all the essential amino acids. Leaves of Moringa can be an extremely valuable source of nutrition for people of all ages. One (1) table spoonful of powdered leaves will satisfy about 14% of the protein, 40% of the calcium, and 23% of the iron and all the vitamin A needs for a child aged one to three. Six (6) table spoonfuls of powdered leaves will satisfy nearly all of a woman's daily iron and calcium needs during times of pregnancy and breast feeding (Fuglie, 2001). Ounce-for-ounce, Moringa leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas, and that the protein quality of Moringa leaves rivals that of milk and eggs (Fahey, 2005). The leaves can be dried and stored but powdering the leaves before storage is locally common (Grubben and Denton, 2004).

Quality of horticultural commodities is a combination of characteristics, attributes or properties that give the commodity value in terms of human food and enjoyment (Kader, 1993). Quality attributes of fresh produce include

appearance, texture, flavour, nutritive value and safety. The term 'quality' may also be defined in terms of end use. Some of the quality requirements under end use are market, storage, transport, eating, and processing quality (Wills *et al*., 1998). Perception of quality will increase the consumer's confidence in the product and evaluations before arriving at the final choice (Rodgers and Danley, 1994).

The quality and condition of produce sent to market are directly affected by the care taken during handling. The presence of moisture at certain levels in products enables spoilage to occur due to the activities of microorganisms and enzymes or through non-enzymatic chemical changes. Thus removing moisture from food or making it less available can lead to an extension of shelf-life of these foodstuffs (Brennan *et al.,* 1990). Consequently, many microbial problems can be minimized or eliminated by care and proper postharvest handling practices. Drying (dehydration) is, therefore, a common and important method used to reduce water content and hence extend the shelf-life of foods. Moringa leaves are usually dried in an airy place out of direct sunlight (Fuglie, 2001). If more moisture is removed the produce loses colour, flavour, nutrients and textural quality (Meshas and Rodgers, 1994). Significant loses of vitamin A can occur if leaves are exposed to sunlight during the drying process. Many food components are sensitive to light particularly in the visible blue and ultra violet part of the spectrum. Exposure of Moringa leaves to light leads to destruction of vitamins and fading of colours (Fuglie, 2001). According to Subadra *et al.*, (1997), careful drying of Moringa leaves can result in 58% of the beta carotene being retained, but after three month storage in a sealed container away from

sunlight, the leaf powder can still contain 46% of the beta-carotene of the fresh leaves. To prevent such changes, a packaging material which is opaque to light may be used (Meshas and Rodgers, 1994).

Once food is dried to the correct moisture content it should be sealed in appropriate packages. Packaging should be regarded as an integral part of food processing and preservation. The success of most preservation methods depends on appropriate packaging, for example, to prevent microbiological contamination of heat-processed product or moisture pick up by dried product (Brennan *et al*., 1990). How food is packaged is almost as important as the process used to preserve it. A faulty package can allow contamination of food by living organisms or promote the growth of organisms that are present. Dehydrated foods can re-hydrate and spoil if poor packaging lets the food come in contact with moisture. (Meshas and Rodgers, 1994). Packaging also plays an important role in maintaining the quality and extending the shelf-life of fresh produce. But the main functions of a package are to contain the product and protect it against a variety of hazards which might adversely affect its quality during handling, distribution, and storage (Brennan *et al.*, 1990). Packaging also plays the role of providing information about the produce; what it is, where it originated from, its quantity or weight or the place it is being shipped to and the owner (Brenndorfer *et al*., 1987). In planning packaging for perishable commodities, it is necessary to have information about the produce, its rate of deterioration and how it can fit into the package. The market or destination for the produce should also be known; the type of outlet, consumer requirements, as well as its shelf-life (Olympio and Kumah, 2002). Cans, glass jars, rigid plastic

containers, plastic bags and paper cardboards are commonly used to package processed food products.

2.0 LITERATURE REVIEW

2.1 ORIGIN, BOTANY AND DISTRIBUTION

Moringa oleifera is the most widely cultivated species of the Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005). It has been introduced throughout the tropics and sub-tropics and has become naturalised in many African countries (Grubben and Denton, 2004). It is already an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands (Fahey, 2005). Moringaceae comprises 13 species, of which 8 are endemic to the Horn of Africa. *Moringa oleifera* is most closely related to *Moringa concanensis* (also from India) and *Moringa perigrina* (from the region around the Red sea, the Horn of Africa, Yemen and Oman). These 3 species share the slender tree habit and the zygomorphic flowers (Grubben and Denton, 2004). *Moringa oleifera* is a perennial softwood tree with timber of low quality (Fahey, 2005).

2.2 MORPHOLOGY AND PHYSICAL CHARACTERISTICS

Moringa is a fast growing perennial tree which can reach a maximum of 7-12m and a diameter of 20-40cm at chest height (Foidl *et al*., 2001). The stem is normally straight but occasionally poorly formed and reaches a height of 1.5-2m before it begins branching but can reach up to 3m. The alternate, twice or thrice pinnate leaves grow mostly at the branch tips. They are 20-70cm long, grayish-downy when young, long petioled with 8-10 pairs of pinnae each bearing two pairs of opposite, elliptic or obovate leaflets and one at the apex, all 1-2cm long; with glands at the bases of the petioles and pinnae (Morton, 1991). The flowers

are 2.5 cm wide and are produced profusely in axillary drooping panicles 10-25cm long. They are white or cream coloured and yellow-dotted at the base. The fruits are three lobed pods which hang down from the branches and are 20-60cm in length. Each pod contains between 12 and 35 seeds (Foidl *et al*., 2001). The seeds are round with a brownish semi-permeable seed hull. The hull itself has three white wings that run from top to bottom at 120 degrees intervals. Each tree can produce between 15000 and 25000 seeds per year. The average weight per seed is 0.3g (Foidl *et al*., 2001).

2.3 PRODUCTION

In Africa local trade is mainly restricted to the leaves. Leaf production in Niger is highest during the rainy season, where a plot of 1000m^2 yields 13-14 bags per harvest which amount to about 27 bags or 600kg per month. In the dry season, monthly yields drop to 2-4 bags in the cool months and to 10-15 bags during the warmer months if irrigated. This is equivalent to an annual production of 27t/ha fresh leaves. In Kenya, some 2000 mostly small scale farmers produce Moringa green fruits for the Asian community. In Tanzania an enterprise has started with the aim of producing oil and a flocculating agent. There is considerable international trade, mostly from India, in canned and fresh fruits, oil, seeds and leaf powder, but statistics on the volumes and values are not available (Grubben and Denton, 2004).

2.3.1 Conditions for Cultivation

Moringa grows best in direct sunlight under 500 meters altitude. It tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH 6.3-

7.0), well drained sandy or loamy soil. Minimum annual rainfall requirements are estimated at 250mm with maximum at over 3000mm, but in water-logged soil the roots have the tendency to rot (Fuglie and Sreeja, 2001). It is drought tolerant and is found in locations with as little as 500mm annual rainfall (Grubben and Denton, 2004). Temperature ranges are 25-35 ºC, but the tree will tolerate up to 48ºC in the shade and it can survive a light frost (Fuglie and Sreeja, 2001).

2.3.2 Propagation and Spacing

Trees can be easily grown from seed or from cuttings. If water is available for irrigation, trees can be seeded directly and grown anytime during the year. In a large field, trees can be seeded directly at the beginning of the wet season. Hard wood cuttings are usually used. Cuttings should be 45cm to 1.5 m long and 10cm thick. Cuttings can be planted directly or planted in sacks in the nursery (Fuglie and Sreeja, 2001).

For intensive Moringa production, the trees are planted 3m in rows and 3m apart to ensure sufficient sunlight and flow. It is also recommended to plant the trees in an east-west direction. When the trees are part of an alley-cropping system, there should be 10m between the rows. The area between trees should be kept free of weeds. Moringa can be inter-cropped with maize, sunflower and other field crops. However, Moringa trees are reported to be highly competitive with eggplant *(Solanum melongena*) and sweet corn (*Zea mays)* and can reduce their yields by up to 50% (Ramachandran *et al.*, 1980).

2.3.3 Cultural Practices in Moringa Cultivation

Moringa trees do not need much watering. In very dry condition, water regularly for the first two months and afterwards only when the tree is obviously suffering. If rainfall is continuous throughout the year, Moringa trees will have a nearly continuous yield. In arid conditions, flowering can be induced through irrigation (Fuglie and Sreeja, 2001).

Moringa trees will generally grow well without adding very much fertilizer. Manure and compost can be mixed with the soil and used to fill the planting pits. Phosphorus can be added to encourage root development and nitrogen will encourage leave canopy growth (Fuglie and Sreeja, 2001). Research done in India has also shown that applications of 7.5kg farmyard manure and 0.3kg ammonium sulfate per tree can increase pod yields three folds (Ramachandran *et. al.*, 1980).

Moringa is resistant to most pests. In very water-logged conditions, *Diplodia* root rot can occur. In India, various caterpillars are reported to cause defoliation unless controlled by spraying. The budworm *Noordia morigae* and the scale insects *Diaspidotus spp*. and *Ceroplastodes cajani* are reportedly able to cause serious damage (Morton, 1991). Moringa seedlings should be protected from livestock by installing a fence or by planting a living fence around the plantation (Fuglie and Sreeja, 2001).

2.4 HARVESTING AND POSTHARVEST HANDLING OPERATIONS.

In Niger harvesting of leaves starts two and half months after sowing. Leaves are pulled from the branches, then put in bags and transported to the market. Harvesting of green fruits may start seven month after planting; harvesting of dry fruits for seed about six weeks later (Grubben and Denton, 2004). Leaves are harvested together with their branches and then stripped from the branches before drying.

2.4.1 Drying and Processing

Dehydration is the oldest method of food preservation practiced by man (Brennan, 2006). Moringa leaves are usually dried in an airy place out of direct sunlight. Significant loses of vitamin A can occur if leaves are exposed to sunlight during the drying process (Fuglie, 2001). It is estimated that only 20-40% of vitamin A content will be retained if leaves are dried under direct sunlight, but 50-70% will be retained if leaves are dried in the shade (Subadra *et al.*, 1997). Drying facilities ranges from simple sun or hot air driers to high capacity, sophisticated spray drying or freeze drying installations (Brennan, 2006). Other drying methods such as the oven and solar drying are also used commercially.

Locally, Moringa leaves can be made into powder after drying by rubbing them over a sieve or pounding the dried leaves using mortar and pestle and rubbing them over a wire screen to produce a fine powder. However, milling machines are used on large scale powder production. Moringa kernels reportedly contain up to 40% oil by weight and can, therefore, be processed to obtain high quality

edible and industrial oil. The cake remaining after the oil is extracted is rich in protein and can be used as a fertilizer in fields and gardens (Fuglie, 2001).

2.4.2 Packaging and Storage

Exposure of Moringa leaves to light leads to destruction of vitamins and fading of colours. Hence, storage and transport under dark conditions is essential. Moringa leaf powder should be packaged and stored in an opaque well sealed plastic container (Fuglie, 2001).

2.5 IMPORTANCE OF VEGETABLES

The importance of vegetables is related to their nutritional value as a major source of vitamins and minerals which are necessary in ensuring a balanced diet. As supplementary foods, some vegetables such as legumes are richer sources of proteins of higher biological value than some major staples such as rice, yam and cassava. Aromatic vegetables such as the leaves of the Fever plant (*Ocimum viridis*) and other herbs contain essential oils and flavoring substances, whiles fruits of *Capsicum spp.* contain capsaicin which imparts a hot pungent taste, which may improve the flavor of otherwise tasteless foods and also sharpen the appetite (Obeng-Ofori *et al.*, 2007). Some vegetables such as Alliums (onions and garlic), Brassicas (cabbage, cauliflower) have medicinal value. For example, garlic has proved valuable to hypertensive patients. Lycopene in tomato is known to prevent cancer (Obeng-Ofori *et al*., 2007). Moringa leaves ranks as the best of all tropical vegetables in terms of nutrients and is highly esteemed by people in the tropics and sub-tropics for its numerous medicinal properties. In

Senegal, for example, an infusion of leaf juice is commonly used to control glucose levels in case of diabetes (Fuglie, 2001).

2.5.1 Moringa as a Vegetable

As a source of nutrition, Moringa leaves probably rank as the best of all tropical vegetables (Fuglie, 2001). Moringa leaves are eaten as greens, in salads, in vegetable curries, as pickles and for seasoning (Ramachandran *et al*., 1980). Fresh leaves can be added to any dish just like spinach. When using Moringa as a vegetable, the entire seedlings or young leaves are used. It is preferable to add leaves towards the end of cooking process in order to retain the maximum vitamin and mineral content (Fuglie, 2001). The young green pods are tasty and can be boiled and eaten like green beans and the flowers can also be eaten after being blanched or raw as a tasty addition to salads (Foidl *et al*., 2001).

2.5.2 Nutritional Benefits of Moringa

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Three non-governmental organizations in particular, Trees for Life, Church World Service and Educational Concerns for Hunger Organization have advocated Moringa as "natural nutrition for the tropics." Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Moringa is especially promising as a food source in the tropics because the tree is in full leaf at the end of the dry season when other foods are typically scarce (Fahey, 2005). The leaves, flowers, roots, and immature pods of the Moringa tree are edible and they form a part of traditional diets in many countries of the tropics

and sub-tropics (Fuglie, 2001). Moringa leaves contain very strong concentrations of vitamins A and C, B-complex vitamins, iron, calcium, protein, zinc, selenium and unusual for a plant source, all of the essential amino acids. A good balance of vitamins, minerals and amino acids are very important for health. An individual needs sufficient levels of certain vitamins, minerals, proteins and other nutrients for his physical development and well being. Deficiencies in any one of these nutrients lead to health problems such as scurvy, night blindness, anemia and kwashiorkor caused by lack of vitamin C, vitamin A, Iron and Protein respectively (Fuglie, 2001).

2.5.3 Medicinal Benefits

Moringa is highly esteemed by people in the tropics and sub-tropics for its numerous medicinal properties. In Senegal, an infusion of leaf juice is commonly used to control glucose levels in case of diabetes. Leaf juice is sometimes used as skin antiseptic. In India, leaves are used to treat fevers, bronchitis, eye and ear infections, scurvy, and catarrh. Leaves are also considered to be anthelmintic (Fuglie, 2001). Some of these traditional uses reflect the nutritional content of the various tree parts. Some of the above traditional remedies have been supported by recent laboratory studies. Makonen *et al*., 1997 reported that Moringa leaf extract have been shown to be effective in lowering blood sugar levels within a space of 3 hours, albeit less effectively than the standard hypoglycaemic drug, glibenclamide. Effects increased with larger doses. An extract taken from dried leaves showed an impressive ability to heal ulcers in laboratory animals. Administration of daily doses by injection caused a

very significant improvement in the healing rate in induced gastric ulcers (Pal *et al*., 1995).

2.5.4 Human Consumption of Moringa

The young leaves are edible and are commonly cooked and eaten as spinach or used to make soups and salads. The leaves can be dried and made into powder to be used in place of fresh leaves to make leaf sauces. They are an exceptionally good source of pro vitamin A, vitamins B, and C, minerals and the sulphur-containing amino acids methionine and cystine. The young green pods are tasty and can be boiled and eaten like green beans. The pods are best for human consumption at the stage when they can be broken easily without leaving any visible strings of fiber (Foidl *et al.*, 2001). The dry seeds can be ground to a powder and used for seasoning sauces. The roots from young plant can also be dried and ground for use as a hot seasoning base. The flowers can be eaten after being blanched or raw as a tasty addition to salads (Foidl *et al*., 2001).

2.6 OTHER USES AND IMPORTANCE OF MORINGA

2.6.1 Socio-economic

Moringa is one of the most useful tropical trees. The relative ease with which it propagates; its low demand for soil nutrients and water after being planted makes its production and management easy. Introduction of this plant into a farm which has a bio diverse environment can be beneficial for both the farm owner and the surrounding eco-system (Foidl *et al*., 2001).

2.6.2 Water Purification

Among the many attributes of this remarkable tree are the coagulant properties of its seeds, powder from which works as a natural flocculent, which can clarify even turbid water, removing up to 99% of the bacteria in the process (Folkard *et al*., 2001). In many developing countries proprietary chemical coagulants, such as aluminum sulfate (alum) and synthetic polyelecrolytes are either not available locally or are imported, drawing on scant resources on foreign exchange. A viable alternative is the use of crushed seed of Moringa, as a natural coagulant (Folkard *et al*., 2001).

2.6.3 Animal Forage

The nutritional characteristics of the Moringa tree are excellent so it can be easily be used as a fresh forage material. The leaves are rich in protein, carotene, iron and ascorbic acid and the pod is rich in the amino acid lycine (CSIR, 1962). Leaves are readily eaten by cattle, sheep, goats, pigs and rabbits. Leaves can also be used as food for fishes. The seed cake could be used as animal feed if it is pre-treated to remove its alkanoid and saponin content (Booth and Wickens, 1988).

2.6.4 Fertilizer

Moringa can be cultivated intensely and then ploughed into the soil as green manure. Juice extracted from the leaves can be used to make a foliar nutrient capable of increasing crop yield by up to 30%. The seed cake can be used as a protein-rich plant fertilizer (Booth and Wickens, 1988).

2.6.5 Other Uses

Other uses include making ropes, paper, tannin, dye, and gum. Also used for fencing gardens and as ornamental plants. Powdered seeds can be used to clarify honey without boiling and crushed leaves are used as domestic cleaning agents (Booth and Wickens, 1988). Biogas can also be obtained from the leaves.

2.7 QUALITY AND QUALITY ATTRIBUTES

Quality can be defined as the degree of excellence or superiority and it is a highly subjective judgment related to learned criteria especially for consumers of agricultural produce. The term quality may also be defined in terms of end use. Some of the quality requirements under end use are market, storage, transport, eating, and processing quality (Wills *et al.*, 1998). To the consumers of fresh fruits and vegetables eating quality is of paramount importance where as processing quality is of paramount importance to the processor. Quality is a very important factor in the consumer decision-making process. Perception of quality will increase the consumer's confidence in the product and evaluations before arriving at the final choice (Rodgers and Danley, 1994).

Quality attributes of produce include appearance (size, shape, colour, gloss, and freedom from defects), texture (firmness, crispness, juiciness, mealiness, and toughness depending on the commodity), flavor (sweetness, sourness, astringency, aroma, and off-flavours), and nutritive value (vitamins, minerals, dietary fibre, phytonutrients). The relative importance of each quality component depends on the commodity and the individuals interest (Kader, 1992). Most postharvest researchers, producers and handlers are product

oriented in that quality is described by specific attributes of the product itself, such as sugar content, colour and firmness. In contrast consumers, marketers and economist are more likely to be consumer oriented in that consumer describes quality wants and needs (Shewfelt, 1999). Consumers are also interested in the health promoting attributes and nutritional quality of fruits and vegetables (Kader, 1988).

2.7.1 Factors Influencing Quality

Many factors contribute to quality losses in agricultural products. These include environmental conditions such as heat or drought, mechanical damage during harvesting and handling, improper postharvest sanitation, and poor cooling and environmental control (Panhwar, 2006). Quality deterioration can be caused by a variety of stresses that may be grouped into four general, but often inter-related, categories: metabolic stress, transpiration, mechanical injury stress and microbial damage (Wills *et al*., 1998).

Metabolic stress involves either 'normal' or 'abnormal' metabolism that lead to senescence or the developments of physiological disorders respectively. Transpiration and subsequent water loss can result in rapid loss of quality. Water loss mainly affects appearance, through wilting and shriveling, and texture, such as loss of crispness of lettuce. However, water loss can also affect nutritional quality. For instance, vitamin C levels fall rapidly in water-stressed leafy vegetables. Mechanical injury causes loss of visual quality characterized by unsightly abrasions, bruises, cuts and tears. Such injuries lead to an increase in general metabolic rate as the produce try to seal off the damaged tissue.

Microorganisms can be often considered a 'secondary stress', since their proliferation is generally facilitated by mechanical injury, transpiration and or metabolic changes such as senescence and physiological disorders. Consequently, many microbial problems can be minimized or eliminated by careful and proper postharvest handling practices (Wills *et al.,* 1998).

2.7.2 Handling Factors Affecting Quality

Some of the major handling factors that contribute to loss of quality of harvested produce are harvesting, transport and handling, storage, marketing and treatment residues.

2.7.2.1 Harvesting

Quality cannot be improved after harvest, only maintained; therefore it is important to harvest fruits, vegetables, and flowers at the proper stage and size and at peak quality. Immature or over mature produce may not last as long in storage as that picked at proper maturity. Mechanical damage during harvesting and associated handling operations can result in defects on produce and permit invasion by disease-causing microorganisms. The inclusion of dirt from the field can aggravate this situation. Produce can overheat and rapidly deteriorate during temporary field storage (Wills *et al*., 1998).

2.7.2.2 Transport and handling

Rough handling and transport over bumpy roads damages produce by mechanical action. At high temperatures produce will become overheated, especially if there is inadequate shading, ventilation and/or cooling. Transport

on open trucks can result in sun-scorch of the exposed produce that can lead to severe water loss. Inappropriate packaging may result in physical damage of produce due to bruising or abrasions as the commodity moves about during transport (Wills *et al*., 1998).

2.7.2.3 Storage

Delays in placing produce in cold storage after harvest often result in rapid deterioration in quality. Poor control of storage conditions, storage for too long and inappropriate storage conditions for a particular commodity will also result in a poor quality product. With mixed storage of different commodities, ethylene produced by one product can promote rapid senescence of another product. Storage at temperatures that are too low may induce physiological disorders or chilling injury. High temperature and high humidity can encourage both superficial and internal mould growth and stimulate activity of infesting insects (Wills *et al*., 1998).

2.7.2.4 Marketing

A serious reduction in quality occurs in produce displayed for lengthy periods in retail outlets because of poor organization of marketing. Major causes of quality reduction during marketing include ongoing growth, water loss leading to wilting, undesirable ripening and senescence under poor management of temperature and relative humidity, mechanical damage associated with rough handling by staff and customers, and associated disease development (Wills *et al.*, 1998).

2.7.2.5 Treatment residues

Residues of pesticides and other chemicals are another important factors impinging on postharvest quality. Insecticides are often applied pre-harvest. Fungicides may be used both pre-harvest and/or postharvest to prevent rotting. Fumigants such as methyl bromide may be used for insect disinfestations, especially in export trade or disease control. All these types of chemicals can leave residues in the commodity that, although not detectable by the consumer, is certainly considered undesirable by the consumer and must be considered in conjunction with possible health risk to the community (Wills *et al*., 1998).

2.8 FOOD PRESERVATION

One of the prime goals of food processing or preservation is to convert perishable foods such as fruits and vegetables into stabilized products that can be stored for extended periods of time to reduce their postharvest losses. Processing extends the availability of seasonal commodities, retaining their nutritive and esthetic values, and adds variety to the otherwise monotonous diet. It adds convenience to the products (Jayaraman and Das Gupta, 2006).

Several process technologies have been employed on an industrial scale to preserve fruits and vegetables; the major ones are canning, freezing, and dehydration. Among these, dehydration is especially suited for developing countries. It offers a highly effective and practical means of preservation to reduce postharvest losses and offset the shortages in supply (Jayaraman and Das Gupta, 2006).

2.8.1 Preservation by Dehydration (Drying)

The amount of water in a food is denoted by its moisture content. A food's storability is directly related to moisture content, along with temperature and oxygen availability. High amounts of available moisture lead to mold growth and microbial activity. Fruits must be dried below 30% and agricultural grains below 12% for good long-term storage (Wilhelm *et. al*., 2004). Fresh Moringa leaves contain about 75% moisture whilst the dried leaf powder contains about 7.5% moisture (Booth and Wickens, 1988). Water in fresh vegetables and fruits can account for 80 to 95% of their weight, whereas it accounts for only 12-15% of the weight of dry nuts and seeds (Dupriez and De Leener, 1989). Water in harvested produce encourages the growth of fungi that cause fermentation and rot. It is generally considered that a produce with less than 10-12% water, cannot be spoilt by microorganisms. Drying foods, therefore, involves lowering their moisture content to below these percentages (Dupriez and De Leener, 1989).

The technique of dehydration is probably the oldest method of food preservation practiced by humankind. The removal of moisture prevents the growth and reproduction of microorganisms causing decay and minimizes many of the moisture-mediated deteriorative reactions (Jayaraman and Das Gupta, 2006). The main reason for drying food is to extend its shelf life beyond that of the fresh material, without the need for refrigerated transport and storage. This goal is achieved by reducing the available moisture, or water activity to a level which inhibits the growth and development of spoilage and pathogenic microorganisms, reducing the activity of enzymes and the rate at which

undesirable chemical changes occur. Appropriate packaging is necessary to maintain the low water activity during storage and distribution (Brennan, 2006).

Drying brings about substantial reduction in weight and volume, minimizing packing, storage, and transportation costs and enables storability of the product under ambient temperature (Jayaraman and Das Gupta, 2006). These changes in weight and volume can lead to substantial savings in transport and storage costs and, in some cases, the costs of packaging. However, dehydration is an energy intensive process and the cost of supplying this energy can be relatively high, compared to other methods of preservation (Brennan, 2006).

2.8.1.1 Drying process

There are numerous methods or processes for drying food materials, whose merits can be judged by energy efficiency, time to dry, product quality achieved, etc. A balance among these factors is often required to achieve the economic aim of the manufacturing procedure while ensuring that safe and tasty food is delivered to the consumer (Dong, 2008). Dehydration is usually described as a simultaneous heat and mass transfer operation. Sensible and latent heat must be transferred to the food to cause the water to evaporate. When a wet material is placed in a current of heated air, heat is transferred to its surface, mainly by convection. The water vapour formed is carried away from the drying surface in the air stream. Placing the food in a current of heated air is the most widely used method of supplying heat. The heat is transferred by convection from the air to the surface of the food and by conduction within the food. Alternatively, the food may be placed in contact with a heated surface. The heat is transferred by

conduction to the surface of the food in contact with the heated surface and within the food (Brennan, 2006).

2.8.1.2 Sun and solar drying

For centuries, fruit, vegetables, meat and fish have been dried by direct exposure to the sun. The fruit or vegetable pieces were spread on the ground on leaves or mats while strips of meat and fish were hung on racks. While drying in this way, the foods were exposed to the vagaries of the weather and to contamination by insects, birds and animals. Drying times were long and spoilage of the food could occur before stable moisture content was attained.

But covering the food with glass or a transparent plastic material can reduce these problems. A higher temperature can be attained in such an enclosure compared to those reached by direct exposure to the sun. Most of the incident radiation from the sun will pass through such transparent materials. However, most radiation from hot surfaces within the enclosure will be of longer wavelength and so will not readily pass outwards through the transparent cover. This result in shorter drying times as compared with those attained in uncovered food exposed to sunlight (Brennan, 2006).

2.8.1.3 Mechanical drying

This drying method involves the use of mechanical devices or machines such as fans, conveyors and heaters in their operations. Mechanical drying is usually employed by large scale commercial food processing industries.

Cabinet (Tray) drying

This is a multipurpose, batch-operated hot air drier. It consists of an insulated cabinet, equipped with a fan, an air heater and a space occupied by trays of food. It can vary in size from a bench-scale unit holding one or two small trays of food to a large unit taking stacks of large trays. The air may be directed by baffles to flow across the surface of the trays of food or through perforated trays and the layers of food, or both ways (Brennan, 2006).

Tunnel drying

This type of drier consists of a long insulated tunnel. Tray loads of the wet material are assembled on trolleys which enter the tunnel at one end. The trolleys travel the length of the tunnel and exit at the other end. Heated air also flows through the tunnel, passing between the trays of food and/or through perforated trays and the layers of food. As a trolley full of fresh material is introduced into one end of the tunnel, a trolley full of dried product exits at the other end. Tunnel driers are mainly used for drying sliced or diced fruits and vegetables (Brennan, 2006).

Other mechanical drying methods are Rotary drying, Pneumatic (Flash) drier, Fluidised bed drying, Bin drying, and Conveyor (Belt) drying.

2.8.2 Quality Changes during Drying

Changes detrimental to the quality of the food may also occur during drying. In the case of solid food pieces, shrinkage can alter the size and shape of the

pieces. When the food pieces are rehydrated, their colour and texture may be significantly inferior to those of the fresh material.

2.8.2.1 Colour changes and browning

Colour and color uniformity are vital components of visual quality of fresh foods and play a major role in consumer choice. However, it may be less important in raw materials for processing (Grandison, 2004).

Colour (as an optical property) is a very important quality attribute of dried products and is subjected to appreciable changes during drying. One of the most serious colour problems is darkening that occurs during the production of dehydrated vegetable products. Enzymatic oxidation and non-enzymatic (Maillard or browning) reaction are most frequent causes for discolouration (Sablani and Mujumdar, 2006). One obstacle always encountered by the food technologists in the dehydration and long-term storage of dehydrated fruits and vegetables is the discoloration due to browning. Browning in foods is of two types: enzymatic and non-enzymatic. These reactions are sometimes desirable but in many instances are considered to be deleterious not only due to the formation of unwanted colour and flavour but also due to the loss of nutritive value. It is a major deteriorative mechanism in dry foods and is sensitive to water content (Jayaraman and Das Gupta, 2006).

The colour change during drying can be minimized by various types of pretreatments. Sulfite treatment, blanching, osmotic and microwave pretreatments have shown to reduce the colour change significantly during convectional drying of potato (Krokida and Maroulis, 2000). It has been shown

that the rate of colour change increases as temperature increases and air humidity decreases during conventional and vacuum drying (Krokida *et al*., 1998).

2.8.2.2 Loss of natural pigments

Colour is an important quality attribute in a food to most consumers. It is an index of the inherent good qualities of a food and association of colour with acceptability of food is universal. Among the natural colour compounds, carotenoids and chlorophylls are widely distributed in fruits and vegetables. The preservation of these pigments during dehydration is important to make the fruit and vegetable product attractive and acceptable. Exposure of Moringa leaves to light leads to destruction of vitamins and fading of colours (Fuglie, 2001). Carotenoids are susceptible to oxidative changes during dehydration due to the high degree of unsaturation in their chemical structure (Jayaraman and Das Gupta, 2006).

2.8.2.3 Nutritional changes

A reduction in the nutritional value of foods can result from dehydration. In particular, loss of vitamins C and A may be greater during drying than in canning or freezing (Brennan, 2006). For instance, dehydrated potato products contain the same chemical constituents as fresh potatoes, though their amounts vary. A small amount of fat in fresh potato disappears completely from dehydrated products. The protein content in dehydrated products decreases as compared to the dry matter content of fresh potato tubers. The ash content in the dehydrated products is also lower than that in raw potato. The vitamin content in

dehydrated potato products is very low in comparison with fresh potato (Sablani and Mujumdar, 2006).The effect of sun drying on the ascorbic acid content of ten Nigerian vegetables showed that there was 21–58% loss depending on the nature of the vegetables (Adenike, 1981).

2.8.2.4 Oxidative degradation and flavour loss

The acceptability of dehydrated fruit and vegetable products is highly dependent upon their flavour attributes. Loss of desirable flavour is the limiting characteristic for most dehydrated products. The natural flavour constituents are subjected to much variation and loss during pre drying operations, drying, and storage. Conditions generally responsible for the destruction of natural flavours include rough handling, delay in processing, exposure to light, high temperature, and certain chemicals. Flavour retention is especially important in products in which the principal flavour constituents are volatile oils, as in onions (Jayaraman and Das Gupta, 2006).

2.8.2.5 Texture

Texture of food materials plays a key role in consumer acceptance and market value. Texture characteristics are important factors for raw products and for processing, preparation, and consumption. Texture features are also important considerations in quality assurance and food safety issues (Wilhelm *et al*., 2004). The problem of hot air drying, which is still the most economical and widely used method for dehydrating vegetables and fruits, is the irreversible damage to the texture, leading to shrinkage, slow cooking, and incomplete rehydration. Many commercially dehydrated vegetables exhibit a dense structure

with most capillaries collapsed or greatly shrunk, which affects the textural quality of the final product. Texture of air-dried vegetables deteriorates during storage if the product is exposed to high temperature or if inadequately dehydrated (Jayaraman and Das Gupta, 2006).

2.9 MICROORGANISMS IN FOOD

The inner tissues of healthy plants and animals are free of microorganisms. However, the surfaces of raw vegetables and meats are contaminated with a variety of microorganisms and this depends on the microbial population of the environment from which the food was taken, the condition of the raw product, the method of handling, the time and conditions of storage (Pelczar *et al.*, 2006). A variety of microbes find their way onto foods, introduced from the soil in which they were grown, and during harvest, packaging, storage and handling. Those that are most suited for growth in the environment flourish, making end products such as acids, alcohols and gas. When present in foods in sufficiently high levels, these fungal metabolites can have toxic effects that range from acute (for example, liver or kidney deterioration), to chronic (for example, liver cancer), mutagenic, and teratogenic; and resulting symptoms range from skin irritation to immune suppression, birth defects, neurotoxicity, and death (ICMSF, 1996). Microorganisms on foods are not always undesirable. Sometimes their growth result in more pleasant taste or texture. Foods that have been intentionally altered by careful controlling activity of bacteria, yeasts, or moulds are called fermented foods. Biochemical changes in food when perceived as undesirable are called spoilage (Nesta *et al.*, 2001).

Limiting microbial growth can preserve the quality of foods and prevent food borne illness. Foods can be canned, pasteurized or irradiated to eliminate or decrease the numbers of microorganisms. Alternatively, the multiplication of microorganisms can be suppressed by storing food at cold temperatures or by adding growth inhibiting ingredients called preservatives (Nesta *et al.*, 2001).

2.9.1 Factors Influencing Microbial Growth in Foods

Fresh foods are invariably contaminated with a range of different moulds, yeasts and bacteria and whether any particular organisms will grow depends on the interaction between all the factors that influence the growth of microorganisms (Garbutt, 1997). Understanding the factors that influence microbial growth is essential to maintaining food quality, whether utilizing microorganisms to produce fermented foods or suppressing organisms to prolong the shelf life of perishable foods. The conditions, naturally present in the food, such as moisture, acidity, and nutrients are called intrinsic factors. Environmental factors affect mycotoxin presence in raw and stored commodities. Environmental conditions such as temperature and atmosphere of storage are called extrinsic factors. All of these factors combine to determine which microorganism can grow in a particular food product and the rate of that growth (Nesta *et al.*, 2001).

2.9.1.1 Intrinsic factors

The multiplication of microorganisms in a food is greatly influenced by the inherent characteristics of that food. In general, microorganisms multiply more rapidly in moist, nutritionally rich, pH neutral foods (Nesta *et al.*, 2001).

Water availability

Micro-organisms cannot grow in a water-free environment, as enzyme activity is absent, and most chemical reactions are greatly slowed down (Featherstone, 2008). Food products vary dramatically in terms of how much water is accessible to the organisms that might grow in them. Fresh meats and milk for example have ample water and will support growth of many microorganisms. Breads, nuts and dried foods, on the other hand provide a relatively arid environment. Some sugar rich foods such as jams and jellies are seemingly moist, but most the water is chemically interacting with the sugar, making it unavailable for use by microbes. Highly salted foods, for similar reasons, have little available moisture (Nesta *et al.*, 2001).

The term water activity (a_w) is used to designate the amount of water available in foods for microbial growth. By definition, pure water has an (a_w) of 1.0. Most fresh foods have an (a_w) above 0.98. Most bacteria require an (a_w) above 0.90 for growth, which explains why fresh moisture-rich foods spoil quickly than dried, sugary or salted foods. Fungi can grow at an (a_w) as low as 0.80, which explains why forgotten bread, cheese, jam, and dried foods often become mouldy (Nesta *et al.*, 2001). If the water activity of a dehydrated product is allowed to rise above a certain critical level, microbiological spoilage may occur. In such cases a packaging material with a low permeability to water vapor, effectively sealed, is required (Brennan, 2006).

Reducing the amount of water available for microbial growth is an extremely important and very ancient method of preserving foods. It influences the growth

of any food spoilage or food poisoning organisms that may be present in the raw material or introduced during processing (Garbutt, 1997).

pH

The pH of a food is also important in determining which organisms can survive and thrive on it. All microorganisms have a pH range in which they can grow and an optimum pH at which they grow best (Garbutt, 1997). Many species of bacteria, including most pathogens, are inhibited by acidic conditions and cannot grow at pH below 4.5. A commercially viable exception is the lactic acid bacteria, which can grow at a pH as low as 3.5 and are used in the production of fermented foods such as yogurt and sauerkraut. Fungi can grow at a lower pH than most spoilage bacteria, leading to some foods such as fruits becoming mouldy. For example, the pH of lemon is approximately 2.2, which inhibit the growth of bacteria, including lactic acid group, but some fungi can grow on them.

The pH of a food product may also determine whether toxins can be produced. For example, *Clostridium botulinum,* that causative agent of botulism, does not grow or produce toxin below pH 4.5, so it is not considered danger in highly acidic foods (Nesta *et al.*, 2001). Adjusting the pH of foods using organic acids is an important method of food preservation, controlling the growth of both food poisoning and food spoilage bacteria.

Nutrients

Human foods contain an abundance of nutrients that are available for the growth of a wide range of microorganisms (Garbutt, 1997). The nutrients present in a food as well as other intrinsic factors determine the kinds of organisms that can grow in it. An organism requiring a particular vitamin cannot grow in a food lacking that vitamin. A microbe capable of synthesizing that vitamin, however, can grow if other conditions are favorable. Members of the genus Pseudomonas often spoil foods because they can synthesize essential nutrients and can multiply in various environments including refrigeration (Nesta *et al.*, 2001).

Antimicrobial chemicals

Some foods contain antimicrobial chemicals that may help prevent spoilage. Egg white, for instance, is rich in lysozyme. If lysozyme susceptible bacteria breach the protective shell of an egg, they are destroyed by lysozyme before they can cause spoilage (Nesta *et al.*, 2001).

2.9.1.2 Extrinsic factors

The extent of microbial growth varies greatly depending on the conditions under which that food is stored. Microorganisms multiply rapidly in warm, oxygen-rich environments such as the surface of meat stored at room temperature (Nesta *et al.*, 2001).

Storage temperature

Microorganisms as a group are capable of active growth at temperatures well below freezing to temperatures above 100ºC (Garbutt, 1997). All micro-

organisms do, however, have an optimum temperature as well as a range in which they will grow. This preference for temperature forms the basis of dividing micro-organisms into groups. Psychrotrophs have an optimum from 20 to 30°C, but can grow at or below 7°C. Mesophiles have an optimum of 30–40°C, but can grow between 20 and 45°C. Thermophiles grow optimally between 55 and 65°C, but can grow at a temperature as low as 45°C (Featherstone, 2008). The temperature of storage affects the rate of growth of microorganisms in food. Below its freezing point, water becomes crystalline and inaccessible, effectively halting microbial growth that requires moisture. At low temperatures above freezing, many enzymatic reactions are either very slow or nonexistent, with the result that, some microorganisms are unable to grow. Those that can, do so at reduced rate (Nesta *et al.*, 2001).

Humidity of the environment

The humidity of the environment is important as it affects the water activity (a_w) of the food as well as the moisture on its surface. Food can pick up moisture from the atmosphere. Under conditions of high relative humidity storage (e.g. in a refrigerator), surface spoilage can take place, unless food is adequately protected by packaging (Featherstone, 2008).

Availability of oxygen

Although oxygen is essential for carrying out metabolic activities that support all forms of life, some micro-organisms use free atmospheric oxygen, while others metabolize the oxygen (reduced form) which is bound to other compounds such as carbohydrates. Micro-organisms can be broadly classified

into two groups; aerobic and anaerobic. Aerobes grow in the presence of atmospheric oxygen, while anaerobes grow in the absence of atmospheric oxygen (Featherstone, 2008). The presence or absence of oxygen affects the type of microbial population able to grow in food. For example, members of the genus Pseudomonas are obligate aerobes, and they cannot grow in foods stored under conditions that exclude all of their required oxygen (Nesta *et al.*, 2001).

2.9.2 Changes in Food Caused by Microorganisms

Coloured fungal hyphae are commonly red or black and spores are often green or black. The cells of some bacteria causing spoilage of foods are coloured and in large numbers, these bacterial cells cause the food to become coloured. For instance, the red pigments produced by *Halobacterium salinarum*, an organism that causes the spoilage of salted fish. Some mould fungi produce the enzyme polyphenol oxidase that causes plant tissue to go brown, example *Sclerotinia frutigena* causing brown rot of apples.

Growth of microorganisms in foods can cause textural changes associated with the breakdown of tissues. Invasion of plant tissue by microbes often involves the production of pectinases by the invading organisms. Pectinases attack the calcium pectate that welds the parenchyma cells in living tissues together causing the tissues to soften and eventually undergo maceration. Microbes are capable of producing a wide range of chemicals associated with their metabolic activities giving odours and flavours that are unacceptable or highly objectionable to the consumer (Garbutt, 1997). Growth of fungi in foods results in the production of mycotoxin. Mycotoxins are toxic products of fungal growth.

2.9.3 Control Strategies

There are many situation in which the presence and growth of micro-organisms is undesirable and even harmful to animal and plant population. Therefore the ability to control microbial population is an important concern (Atlas, 1995). Traditionally, control of mycotoxin contamination of foods has been attempted through control of water activity, pH, and quality control of incoming ingredients. Novel control avenues are emerging, including availability of genetically modified grains with increased insect resistance and, thus, lowered rates of fungal infection; improved management of grain ingredients; and inclusion of controls for mycotoxins in food manufacturing Hazard Analysis and Critical Control Point (HACCP) plans (Murphy *et al.,* 2006). Poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensils cause contamination of raw and cooked foods. Mishandling of raw and cooked foods allows bacteria to grow (Banwart, 1974).

2.9.3.1 Physical control

Several physical agents can be used to control microbial populations. High temperatures and ionizing radiations kill micro-organisms by damaging essential cell components. Enzymes such as DNA and cytoplasmic membranes are often disrupted by these physical agents. Others such as low temperature and removal of oxygen, prevents microbial growth (Atlas, 1995).

2.9.3.2 Good agricultural practices (GAPs)

The first line of defense against the introduction of mycotoxins is at the farm level and starts with implementation of good agricultural practices to prevent

infection. Preventive strategies should be implemented from pre-harvest through postharvest. Pre-harvest strategies include maintenance of proper planting/growing conditions (for example, soil testing, field conditioning, crop rotation, irrigation), antifungal chemical treatments (for example, proprionic and acetic acids), and adequate insect and weed prevention. Harvesting strategies include use of functional harvesting equipment, clean and dry collection/transportation equipment, and appropriate harvesting conditions (low moisture and full maturity). Postharvest measures include use of drying as dictated by moisture content of the harvested grain, appropriate storage conditions, and use of transport vehicles that are dry and free of visible fungal growth (CAC, 2003). While implementation of these precautions goes a long way toward reducing mycotoxin contamination of foods, they alone do not solve the problem and should be an integral part of an integrated HACCP-based management system (Lopez-Garcia *et al.*, 1999).

In the food industry, postharvest control of mycotoxins has been addressed via HACCP plans, which include use of approved supplier schemes. Implementation at pre-harvest stages of the food system needs more attention. Such action provides a critical front-line defense to prevent introduction of contaminants into the food and feed supplies. Pre-harvest HACCP programs have been documented for controlling aflatoxin in corn and coconuts in Southeast Asia, peanuts and peanut products in Africa, nuts in West Africa, and patulin in apple juice and pistachio nuts in South America (FAO/IAEA, 2001).

2.9.4 Food Borne Diseases and Health Effects

Chemicals, heavy metals, parasites, fungi, viruses and bacteria can cause food borne illness. Bacteria related food poisoning is the most common, but fewer than 20 of the many thousands of different bacteria actually are the culprits. More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus, Salmonella, Clostridium perfringens, Campylobacter, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus,* and Entero-pathogenic *Escherichia coli*. These bacteria are commonly found on many raw foods (Banwart, 1974). Fungal infections in humans are called mycoses; they include such disorders as histoplasmosis, coccidioidomycosis, and blastomycosis.

2.9.4.1 Control of food borne illness

Food borne illness is an ever-present threat that can be prevented with proper care and handling of food products. Normally a large number of food-poisoning bacteria must be present to cause illness. Therefore, illness can be prevented by controlling the initial number of bacteria present, preventing the small number from growing, destroying the bacteria by proper cooking and avoiding re-contamination (Banwart, 1974).

Biological control measures

The potential for using microorganisms to detoxify mycotoxins has shown promise. Exposure of DON to microbes contained in the contents of the large intestines of chickens completely transformed it in vitro to de-epoxy-DON (He *et al.*, 1992), which is 24 times less toxic than DON itself (Eriksen 2003).

Similar findings were demonstrated with the micro flora of cow intestines (Binder *et al.*, 1998).

Transgenic approaches

Current research efforts are focusing on methods to prevent infection at the pre-harvest stage with emphasis on mechanisms by which the affected plants may inhibit growth of moulds or destroy mycotoxins that they produce (Murphy *et al.*, 2006).

2.10 FOOD PACKAGING

It is important to look on packaging as an integral part of food processing and preservation. The success of most preservation methods depends on appropriate packaging, e.g. to prevent microbiological contamination of heat-processed foods or moisture pick up by dehydrated foods.

The main functions of a package are to contain the product and protect it against a range of hazards which might adversely affect its quality during handling, distribution and storage. The package also plays an important role in marketing and selling the product. Today most food materials are supplied to the consumer in a packaged form. Even foods which are sold unpackaged, such as some fruits and vegetables, will have been bagged, boxed or otherwise crudely packaged at some stage in their distribution (Brennan and Day, 2006).

Packaging should not give rise to any health hazard to the consumer. No harmful substances should leach from the packaging material into the food. Packaging should not lead to the growth of pathogenic microorganisms when anaerobic

conditions are created within the package. Packages should be convenient to use. They should be easy to open and re sealable, if appropriate. The contents should be readily dispensed from the container (Brennan and Day, 2006).

2.10.1 Factors Affecting the Selection of Packaging Material for a Product

2.10.1.1 Mechanical damage

Fresh, processed and manufactured foods are susceptible to mechanical damage. The bruising of soft fruits, the breakup of heat processed vegetables and the cracking of biscuits are examples. Such damage may result from sudden impacts or shocks during handling and transport, vibration during transport by road, rail and air and compression loads imposed when packages are stacked in warehouses or large transport vehicles. Appropriate packaging can reduce the incidence and extent of such mechanical damage (Brennan and Day, 2006).

Packaging alone is not the whole answer. Good handling and transport procedures and equipment are also necessary .The selection of a packaging material of sufficient strength and rigidity can reduce damage due to compression loads. Metal, glass and rigid plastic materials may be used for primary or consumer packages. Fiberboard and timber materials are used for secondary or outer packages (Brennan and Day, 2006).

2.10.1.2 Permeability characteristics

The rate of permeation of water vapor, gases (Oxygen, Carbon dioxide, Nitrogen and ethylene) and volatile odour compounds into or out of the package is an

important consideration, in the case of packaging films, laminates and coated papers. Foods with relatively high moisture contents tend to lose water to the atmosphere. This results in a loss of weight and deterioration in appearance and texture. Products with relatively low moisture contents will tend to pick up moisture, particularly when exposed to a high humidity atmosphere.

To retain the pleasant odour associated with many foods, such as coffee, it is necessary to select a packaging material that is a good barrier to the volatile compounds which contribute to that odour. Such materials may also prevent the contents from developing taints due to the absorption of foreign odours. Where some movement of vapours and/or gases is desirable, films that are semi permeable to them may be used. For products with high respiration rates the packaging material may be perforated. A range of micro perforated films is available for such applications (Brennan and Day, 2006).

2.10.1.3 Grease proofness

In the case of fatty foods, it is necessary to prevent egress of grease or oil to the outside of the package, where it would spoil its appearance and possibly interfere with the printing and decoration. Greaseproof and parchment papers may give adequate protection to dry fatty foods, such as chocolate and milk powder, while hydrophilic films or laminates are used with wet foods, such as meat or fish (Brennan and Day, 2006).

2.10.1.4 Temperature

A package must be able to withstand the changes in temperature which it is likely to encounter, without any reduction in performance or undesirable change in appearance. This is of particular importance when foods are heated or cooled in the package. Some packaging films become brittle when exposed to low temperatures and are not suitable for packaging frozen foods. The rate of change of temperature may be important. For example, glass containers have to be heated and cooled slowly to avoid breakage (Brennan and Day, 2006).

2.10.1.5 Light

Many food components are sensitive to light, particularly at the blue and ultraviolet end of the spectrum. Vitamins may be destroyed, colours may fade and fats may develop rancidity when exposed to such light waves. The use of packaging materials which are opaque to light will prevent these changes. If it is desirable that the contents be visible, for example to check the clarity of a liquid, coloured materials which filter out short wavelength light may be used. Amber glass bottles, commonly used for beer in the UK, perform this function. Pigmented plastic bottles are used for some health drinks (Brennan and Day, 2006).

2.10.1.6 Chemical compatibility of the packaging material and the contents of the package

It is essential in food packaging that no health hazard to the consumer should arise as a result of toxic substances, present in the packaging material, leaching into the contents. Materials used for food packaging may result in undesirable

chemicals migrating into foods. These include semi rigid and rigid plastic packaging materials, lacquers and sealing compounds used in metal cans, materials used in the closures for glass containers, additives and coatings applied to paper, board and regenerated cellulose films, wood, ceramics and textiles (Brennan and Day, 2006).

To establish the safety of such packaging materials two questions need to be answered: are there any toxic substances present in the packaging material and will they leach into the product? Apart from causing a health hazard to the consumer, interaction between the packaging material and the food may affect the quality and shelf-life of the food and/or the integrity of the package; and it should be avoided. Packaging materials, which are likely to react adversely with the contents, should be avoided, or another barrier substance should be interposed between the packaging material and the food (Brennan and Day, 2006).

2.10.1.7 Protection against microbial contamination

Another role of the package may be to prevent or limit the contamination of the contents by microorganisms from sources outside the package. This is most important in the case of foods that are heat-sterilized in the package, where it is essential that post process contamination does not occur.

The permeability of the packaging material to gases and the packaging procedure employed can influence the type of microorganisms that grow within the package. Packaging foods in materials that are highly permeable to gases is

not likely to bring about any significant change in the microflora, compared to unpackaged foods (Brennan and Day, 2006).

2.10.1.8 Protection against insect and rodent infestation

Dry, cool, clean storage conditions, good ventilation, adequate turnaround of warehouse stocks and the controlled use of fumigants or contact insecticides can all help to limit insect infestation. Packaging can also contribute, but an insect proof package is not normally economically feasible, with the exception of metal and glass containers. Good design of containers to eliminate as far as possible cracks, crevices and pinholes in corners and seals can limit the ingress of invading insects. The use of adhesive tape to seal any such openings can help. The application of insecticides to some packaging materials is practiced to a limited extent, e.g. to the outer layers of multiwall paper sacks. They may be incorporated into adhesives. However, this can only be done if regulations allow it (Brennan and Day, 2006). Packaging does not make a significant contribution to the prevention of infestation by rodents. Only robust metal containers offer resistance to rats and mice (Brennan and Day, 2006).

2.10.1.9 Other factors

There are many other factors to be considered when selecting a package for a particular duty. The package must have a size and shape which makes it easy to handle, store and display on the supermarket shelf. Equipment must be available to form, fill and seal the containers at an acceptable speed and with an adequately low failure rate. The package must be aesthetically compatible with the contents. For example, the consumer tends to associate a particular type of

package with a given food or drink. The labeling must clearly convey all the information required to the consumer and comply with relevant regulations (Brennan and Day, 2006).

2.11 FOOD PACKAGING MATERIALS

2.11.1 Rigid Containers

Rigid containers include glass and plastic bottles and jars, cans, pottery, wood, boxes, drums, tins, plastic pots and tubes. They are all to a varying degrees give physical protection to the food inside that is not provided by flexible packaging. While most rigid containers are strong, they are because of the amount of material used in their production, more expensive than flexible packaging (Fellows and Axtell, 1993).

2.11.1.1 Glass

Glass is made by heating a mixture of sand, soda ash and lime usually with a proportion (up to 30%) of broken glass or cullet to about 1500ºC until it melts into thick liquid mass. The molten glass is blown into moulds in two stages, to make bottles and jars which are then cooled under carefully controlled conditions to prevent weakness and breakage. In spite of the many developments in plastic containers, glass is still widely used for food packaging (Fellows and Axtell, 1993).

Glass is inert with respect to foods, transparent and impermeable to vapours, gases and oils. Because of the smooth internal surface of glass containers, they can be washed and sterilised and used as multi trip containers, e.g. milk and beer

bottles. However, glass containers are relatively heavy compared to their metal or plastic counterparts, susceptible to mechanical damage and cannot tolerate rapid changes in temperature (low thermal shock resistance). Broken glass in a food area is an obvious hazard (Brennan and Day, 2006). While glass is fragile to shock it is strong in terms of bearing weight so stacking on pallets is possible (Fellows and Axtell, 1993).

2.11.1.2 Pottery

Pottery is one of the most ancient forms of traditional packaging. Pottery wine and oil jars have been used for thousands of years. Although pottery containers have now been largely replaced by other materials for commercial food packaging they still are widely used in some countries for certain products, for example, cooking oil and tomato paste. They also find application when packing high value, luxury foods. In Europe, for example, very expensive marmalades, meat pastes and cheeses may be bought in glazed pots (Fellows and Axtell, 1993).

Pottery containers are made from clays either by hand or with the use of moulds. Hand-made pots vary considerable in size and shape while moulded ware is far more standard and thus more suitable for routine food packaging. Pottery is still widely used all over the world for the traditional packaging and storage of foods such as grains, pulses, wines, honey, pickles, yoghurts and dried foods. It is almost, if not totally, impossible to hermetically seal pottery containers due to the variations that occur in the neck diameter and shape. For this reason their use

is limited to products that are either very stable, such as honey, or have a short shelf-life such as yoghurts and soft cheeses (Fellows and Axtell, 1993).

2.11.1.3 Metal containers

The metal materials used in food packaging are aluminium, tinplate and electrolytic chromium-coated steel (ECCS). Aluminium is used in the form of foil or rigid metal. Metal cans are the most common metal containers used for food packaging. The traditional *three-piece can* (open or sanitary) is still very widely used for heat-processed foods (Brennan and Day, 2006). Metal containers commonly used in the food industry include steel drums, tins with push-on or screw on closures, sanitary cans (the 'tin' can), composite cans (usually a combination of paper board and steel), aerosols, aluminium cans and aluminium foil made into dishes, etc. Whilst many foods, including dried goods, can be canned the most common applications are to fruit juices, fruits in syrup, tomatoes, meats, fish and vegetables (Fellows and Axtell, 1993).

The can has distinct advantages over glass which include good heat transmission, not subject to thermal shock so rapid heating and cooling are possible, lighter in weight, not subject to breaking, and resistant to physical damage. They are also impervious to light and air. The main disadvantage of can is, of course, that the contents cannot be seen by the purchaser.

2.11.1.4 Plastic containers

Largely for cost reasons rigid plastic bottles, jars, tubes, cups and trays are increasingly replacing glass and tin cans for food packaging. Unfortunately the

widespread use of plastic is having a bad impact on the environment. However, plastic containers have great advantages of lower cost, lightness, resistance to impact damage, availability both clear and coloured, squeezability etc. Plastic containers however give less protection than coloured glass and cans against light and air. In addition they are not as strong, in terms of weight bearing and crushing, as glass or cans and are easily punctured by sharp objects.

The range of plastics and co-polymers used to make rigid food containers is wide. For most small food processors in developing countries the choice will be restricted to packaging made of polypropylene (OPP), polyethelene (high density and low density) and polyvinylchloride (PVC). Polythene tetraphthalate (PET) is however rapidly becoming more common (Fellows and Axtell, 1993).

2.11.1.5 Wooden containers

While wood is widely used for packaging fresh produce its use is limited when dealing with processed foods. The most common applications are: barrels for wines, beers, spirits, salted fish and vegetables in brine; wooden crates particularly for bottles that are returnable; tea chests; small fancy boxes for foods aimed at a tourist or gift market; to construct pallets. Wood is strong and provides better protection against crushing and impact than cardboard boxes. It is however heavier and more expensive. As a material wood is porous and so does not form a perfect barrier to moisture and air. Wood containers can be made lightproof and leakproof (Fellows and Axtell, 1993).

2.11.1.6 Paperboard

Paperboard is the general name given to a variety of different types of materials that are used to make boxes, cartons and trays to package foods. They can be used as shipping (outer) containers or as consumer packs, but only a few types of materials can be used directly in contact with foods (Fellows and Axtell, 1993). Paperboard is produced in the same way as paper but it is made thicker and often in multiple layers to protect foods from mechanical damage (crushing, puncturing, vibration).They normally are made on the cylinder machine and consist of two or more layers of different quality pulps with a total thickness in the range 300-1100 µm (Brennan and Day, 2006).Some types of paperboard used in food packaging include:

Chipboard is made from recycled paper and is used to make the outer cartons for packets of foods such as tea and cereals. It is not suitable for direct contact with foods Chipboards are seldom used in direct contact with foods (Fellows and Axtell, 1993).

Solid white board- This is the only type of paper board that is recommended for direct contact with foods (Fellows and Axtell, 1993). All plies are made from fully, bleached chemical pulp. It is used for some frozen foods, food liquids and other products requiring special protection (Brennan and Day, 2006).

Paperboards are available which are coated with wax or polymer materials such as polyethylene, polyvinylidene chloride and polyamides. These are mainly used for packaging wet or fatty foods (Fellows and Axtell, 1993).

2.11.1.7 Moulded paper

They are made from recycled waste paper. The most common moulded paper packages are egg trays and egg boxes but others such as fruit trays, small shallow dishes and protective bottle cases are available (Fellows and Axtell, 1993). They are moulded into shape either under pressure (pressure injection moulding) or vacuum (suction moulding) and the resulting containers are dried. Such containers have good cushioning properties and limit in-pack movement, thus providing good mechanical protection to the contents (Brennan and Day, 2006).

2.11.1.8 Fiberboard

This can be either solid or corrugated board. Solid fiberboard consists of a layer of paperboard, usually chipboard, lined on one or both faces with Kraft paper. Solid fiberboard is rigid and resistant to puncturing (Brennan and Day, 2006). It provides good protection against impact and compression. Corrugated fiberboard consists of one or more layers of corrugated material (medium) sandwiched between flat sheets of paperboard, held in place by adhesive. The medium may be chipboard, strawboard or board made from mixtures of chemical and mechanical pulp (Brennan and Day, 2006). Corrugated boards resist impact, abrasion and crushing damage and are therefore widely used for shipping containers for bulk foods such as dried fruit, nuts, etc., (Fellows and Axtell, 1993). Fiberboard cases are also used for goods already packaged in pouches, cartons, cans and glass containers.

2.11.2 Flexible Packaging

Flexible packaging is a major group of materials that includes plastic films, papers, foil, some types of vegetable fibers and cloths that can be used to make wrappings, sacks and sealed or unsealed bags. The wide variety of bags, wrappings and sacks that are available makes this group of packaging materials very important (Fellows and Axtell, 1993).

2.11.2.1 Papers

While paper may be manufactured from a wide range of raw materials, almost all paper used for food packaging is made from wood. Some papers are made from repulped waste paper. Such materials are not used in direct contact with foods. Treated papers are used for dry foods, fats, baked goods and confectionery. However plain paper is not heat sealable and has poorer barrier properties and therefore finds fewer applications.

Paper is produced by beating wood chips to break them down to a pulp which contains the wood fibers and then treating the fibers with alkali or acid. Papers are biodegradable in the environment because their component chemicals (mostly cellulose) are broken down by moulds, bacteria and animals (Fellows and Axtell, 1993).

Paper can be treated with wax to improve their barrier properties and make them heat sealable. These papers are used to package cereal products, bread and spices. They can also be laminated to low density polythene to make them heat sealable and improve its barrier properties to air and moisture. Other methods

include lamination to aluminium foil or to other types of plastics. Laminated papers are used to package coffee, dried soup, herbs and spices and other dried foods that require a barrier to moisture and air during a long shelf-life (Fellows and Axtell, 1993).

2.11.2.2 Films

Plastic films are becoming increasingly important in most developing countries because they have several advantages over other forms of packaging used in food processing. Several advantages of plastic films include the following: tough and durable to withstand rough handling during transport and distribution, they add very little weight to the produce, they are mostly inert and they have very good barrier properties to moisture and air.

Types of plastic films

Polythene

Polythene is the cheapest and most widespread plastic films used for food packaging in the developing countries. The thin polythene film is known as low density polyethelene (LDPE) and is transparent and glossy. LDPE have relatively poor barrier properties to moisture and air and little strength to resist puncturing, although it does not tear easily. Because they do not protect foods against mechanical damage, these packages require outer cartons or boxes for transport and distribution. Thick polythene is known as high density polyethelene (HDPE) and this is a relatively good barrier against moisture, air and odours. It is stronger, less flexible and more brittle than LDPE.

All grades of polythene have relatively poor resistance to sunlight and become less flexible and more brittle after approximately six months' exposure to light under tropical conditions. This is more noticeable with the thicker films that have less plasticizer than LDPE (Brennan and Day, 2006).

Polypropylene

It is a clear, glossy film that is fully transparent and sparkling. It is strong, heat sealable and it withstand puncturing and tearing. It does not stretch as much as polythene and has good barrier properties to moisture, air and odours which make it more suitable for foods that have a long expectant shelf-life. Polypropylene does not have the same problem of movement of plasticizers into fatty foods that is found with polythene, but it has a higher sealing temperature than temperature (Brennan and Day, 2006).

Cellulose (cellophane)

Cellulose is one of the few plastic films that are made from renewable materials instead of from petroleum products. It is made from wood pulp (mostly eucalyptus) by a complex chemical process, to produce a clear glossy transparent film that is biodegradable within approximately 100 days under tropical conditions. However, cellulose tears easily and more importantly it is not heat sealable in its plain form. It is also relatively poor barrier to moisture. As a result plain cellulose is mostly used for foods that do not require full protection against moisture (Brennan and Day, 2006).

2.11.2.3 Foil

Aluminium foil is generally expensive and thus not widely used by small and medium-scale producers. However, for some applications where very good protection of a food is needed or where local aluminium production makes foil cheaper, this can be an important material. Aluminium foil is produced from aluminium ingots by a series of rolling operations down to a thickness in the range 0.15–0.008 mm. Most foil used in packaging contains not less than 99.0% aluminium, with traces of silicon, iron, and copper and, in some cases, chromium and zinc. Foil is a bright, attractive material, tasteless, odourless and inert with respect to most food materials. For contact with acid or salty products, it is coated with nitrocellulose or some polymer material. It is mechanically weak, easily punctured, torn or abraded. Coating or laminating it with polymer materials will increase its resistance to such damage (Brennan and Day, 2006). Foil is easily damaged by handling and it should therefore be handled as little as possible.

3.0 MATERIALS AND METHODS

3.1 SOURCE OF MORINGA LEAVES

Fresh Moringa leaves were harvested from a farmer's field at Mpatasie near Trede in the Atwima Kwanwoma District of the Ashanti region. They were processed, packaged and stored at the Department of Horticulture laboratory. Samples were then sent to the Department of Biochemistry laboratory KNUST for crude protein, crude fat and fat determination, the Department of Microbiology laboratory KNUST for bacteria analysis, and the Crops Research Institute, Femusua and Soil Research Institute, Kwadaso for fungal and mineral analysis respectively.

3.2 DRYING EQUIPMENT

Solar dryer constructed with wood, transparent glass, and transparent plastic roofing sheets. Wooden trays with wire mesh base served as the drying chamber. The solar dryer had ventilation holes.

Figure 1: Solar dryer

Figure 2: Wooden trays

3.3 PACKAGING MATERIALS

All the packaging materials were purchased from the open market. The PET (polyethylene terephthalate) bottles of thickness of 0.482mm and volume of 1.2L/1200cm^3 and glass bottles of 2.921mm thickness and volumes of 1.0L/1000cm^3 and 1.1L/1200 cm^3 for opaque and transparent respectively. They were thoroughly washed and cleaned with detergents before being used as packages for the Moringa leaf powder. The LDPE (low density polyethylene) bags of thickness 0.127mm (1.2L/1200cm^3) and plain paper materials were also purchased. The Brown paper of thickness 0.127mm was designed into bags (1.2L/1200cm^3) whilst Cardboard of thickness 0.432mm and Waxed coated cardboard of 0.279mm thick were designed into boxes (1.2L/1200cm^3) as described by Fellows and Axtell, 1993. Glue was used as an adhesive to the paper packages.

The types of packaging used were as follows; transparent and opaque polyethylene terephthalate ($PET_{TRANS.}$ And PET_{OPAQUE}) bottles, transparent and opaque low density polyethylene ($LDPE_{TRANS.}$ and $LDPE_{OPAQUE}$) bags, transparent and opaque glass ($GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$) bottles, waxed coated cardboard ($PAPER_{WAXED}$) and cardboard ($PAPER_{CARDBOARD.}$) boxes, brown paper ($PAPER_{BROWN}$) bags and CONTROL (open bowl/no packaging).

Figure 3: Packaged Moringa leaf powder in the various containers.

3.4 HARVESTING AND PROCESSING

3.4.1 Harvesting, Drying and Milling

Harvesting of the Moringa was done in the morning. The leaves were harvested together with the branches with a sharp knife. Leaves were stripped from the branches and then spread thinly on the trays and kept under shed overnight to lose some moisture. The leaves were transferred to the solar dryer. The leaves were turned in the drying chamber regularly to improve air circulation which reduces drying time. The dried leaves were then transferred into dark polythene bags and tightly sealed ready for milling into powder. The dried leaves were milled with a hammer mill to a fine powder (average particle size of 0.5mm). The milled product was sealed in a dark polythene bag for subsequent packaging and storage.

3.4.2 Packaging

A weighed quantity of 350g of the powder was packaged in each of the packaging containers and the controls. Paper tape was used to seal the paper packages while the LDPE bags were heat sealed. Plastic lids were used as

closures for the PET and the Glass packages. The packaged products were well-labeled and randomly arranged in the laboratory. See Figure 3.

3.5 EXPERIMENTAL DESIGN AND ANALYSIS

The experimental design was a simple CRD (Completely Randomised Design). Data collected from the study were analysed by Analysis of variance (ANOVA) using the GENSTAT 11th Edition. The Duncan's Multiple Range Test was used to separate the means at 5%.

3.6 PARAMETERS STUDIED

The parameters studied were moisture content, nutrient content, bacteria load (numbers) and identification, colour (appearance) and particle size. Temperature and humidity of storage environment were also monitored. Moringa leaf powder samples that got deteriorated were sent to the laboratory to identify the spoilage organisms. Moisture and nutrients contents were determined by AOAC (1995) procedure. Calcium, Magnesium and Iron were estimated by using a spectrophotometer (Buck Scientific 210 VGP).

3.6.1 Moisture Content

Moisture content was determined on day 1 of storage and at 4 days interval.

A crucible dish was dried and weighed (*W1*) using an analytical balance. A weighed quantity of 2g of the wet sample was transferred to the previously dried and weighed crucible dish. The dish plus wet sample (*W2*) was placed in an electric oven (Wagtech) that was thermostatically controlled at 105°C for 5

hours. At 5 hours, the dish plus dried sample was removed from the oven and placed immediately in a desiccator to cool. It was then weighed (*W3*). The dish plus dried sample was placed again in the oven to heat, cooled in a desiccator and reweighed. This step was repeated until constant dried weight was attained. The moisture content was calculated as;

$$M.C = \frac{W2 - W3}{W2 - W1} \times 100$$

Where *W1* is weight of dish, *W2* is weight of dish and wet sample and *W3* is weight of dish and dried sample.

3.6.2 Nutrients

Nutrient contents (crude protein, crude fat, calcium, magnesium and iron) were determined on the first day of storage and then at 60 days interval until the end of storage.

3.6.2.1 Crude protein

The kjeldahl method was used in the determination of total protein. The method is based on the conversion of nitrogenous compounds in the analysed substance to ammonium sulphate by digesting the sample with concentrated H_2SO_4 in the presence of a catalyst (copper sulphate, mercury or selenium). Ammonia is liberated from the digestion mixture by making the solution alkaline and then steam distilled. The ammonia is trapped in dilute acid (Boric acid) and titrated.

A quantity of 2.00g of the sample was put into the digestion flask and half of selenium based catalyst tablet was added. A measured volume of 25ml of

concentrated H_2SO_4 was added. The flask was shaken so that the entire sample was thoroughly wet and then placed on a heating mantle, heated slowly until the resulting solution was clear. It was then cooled to room temperature (25ºC). The digested sample solution was transferred to into a 100ml volumetric flask and made up to the mark with distilled water.

The distillation apparatus was flushed out before using by boiling distilled water in the steam generator of the distillation apparatus for about 15 minutes. 25ml of 2% boric acid was pipetted into a 250ml conical flask and two drops of mixed indicator was added. Liquid was drained from the steam trap and the stopcock which drains the steam trap left open. The 250ml conical flask and its contents were placed under the condenser in such a way that the tip of the condenser was completely immersed in the solution. 10ml of the digested sample was measured and poured into the decomposition flask. An excess of 40% (about 15-20ml) was also added to the decomposition flask. To drive the liberated ammonia into the collection flask (a conical flask containing 25ml of 2% boric acid), steam was forced through the decomposition chamber by shutting the stop cock on the steam trap outlet. The boric acid changed to bluish green as soon as it came into contact with ammonia and distillation was continued for 5 minutes. The receiving flask was lowered so that the condenser tip was just above the liquid. The end of the condenser was washed with little distilled water and distillation continued for another 30 seconds. The burner was removed from the steam generator. The apparatus was flushed again before distilling another sample.

Twenty (20ml) of the distilled sample was pippeted into a 250 ml conical flask and titrated with 0.1N HCl solution. The acid was added until the solution was colourless. The endpoint of the titration was identified as the first colour change.

Total crude protein was calculated by the formula below:

$$\%total\ nitrogen = \frac{100(VA - VB) \times NA \times 0.01401 \times 100}{W \times 10}$$

$$Crude\ protein = \%total\ nitrogen \times 6.25$$

Where *VA*, volume in ml of standard acid used in the titration, V_B, volume in ml of standard acid used in blank, N_A, normality of acid (HCl), *W*, weight in grams of the sample

3.6.2.2 Crude fat

A weighed quantity of 2.0g of the sample was transferred onto a 22x80mm thimble (filter paper). A small ball of cotton wool was placed into the thimble to prevent loss of sample. An anti bumbing granule was added to previously dried (air oven at 100°C) 250ml flask and weighed accurately (W_2). A measured volume of 150ml of petroleum spirit was added to the flask. A quickfit condenser was connected to the soxhelt extractor and refluxed for 4hours on high heating mantle. The flask was then removed and evaporated on a steam bath. The flask and fat sample was heated for 30 minutes in an oven at a temperature of 103°C. The flask and content was cooled to room temperature (25ºC) in a desiccator. The flask and its contents were then weighed (W_2) accurately to determine weight of fat collected. Weight of fat collected was determined by this formula;

$$Weight\ of\ fat = W1 - W2$$

Where *W1* is initial weight of sample plus flask and *W2*, final weight of sample plus flask.

3.6.2.3 Minerals (Calcium, Magnesium and Iron).

Samples were prepared based on the methods described by Motsara and Roy, 2008. A weighed quantity of 0.5g of the sample was put into crucible and placed in a furnace at 450°C for 3hours. After cooling the sample was wetted with a drop of distilled water. A volume 10ml of Nitric acid solution was added and placed on heating mantle until first sign of boiling appeared after which it was removed and filtered into a 50ml volumetric flask using Watman's filter paper (size 42). After filtration the filter paper was rinsed two to three times into the volumetric flask with 10mls of distilled water. It was then topped with distilled water to the 50ml mark.

Concentrations of elements were determined (read) from the prepared samples with an atomic absorption spectrophotometer (Buck Scientific 210 VGP) which had been calibrated with the elements to be determined.

3.6.3 Pathological Studies

Bacterial load (numbers) and bacterial identification were carried out on day1 and day16 and then at days 60,120 and 180 of storage. Deteriorated Moringa leaf powder samples were sent to the laboratory for examination and identification.

3.6.3.1 Fungal Analysis

PDA (Potato Dextrose Agar) media was first prepared. Fresh potatoes were thoroughly washed with running water to remove dirt. A weighed quantity of 200g of the potatoes were cut into smaller pieces of about 2g with the outer skin still intact and added to already boiling water of 0.5L and allowed to boil until the potatoes were soft. The potatoes were mashed and mixed with the boiling water and then sieved through a textile gauze material to remove larger potato particles. A weighed quantity of 20g each of agar and dextrose (glucose) were added to the mashed potato and topped with water to 1000ml and thoroughly mixed using a magnetic stirrer. The potato, dextrose and agar (PDA) mixture was then poured into storage bottles and sterilized in an autoclave for 20 minutes at a temperature of 121ºC and a pressure of 100 p.s.i.

Contaminated Moringa leaf powder samples were inoculated and incubated. The inoculating needles were first dipped in alcohol (96% ethanol) and then flamed to sterilize them. The sterilized needle was used to pick a small portion of the contaminated samples and inoculated on the surface of the PDA media in a petri dish and incubated at a normal room temperature (25ºC) for growth to occur. The needle was sterilized after every inoculation. The petri dish was labeled appropriately. After five days of incubation, grown fungi were again cultured (sub-cultured) to obtain a pure culture for examination and identification.

It is mandatory to see spore characteristics when observing under microscope to be able to make identification. Surface pigmentation (colour) and shape

characteristics of fungal spores were examined. Glass slide was rubbed with an alcohol and a drop of distilled water placed on it. Samples were taken from the fruiting bodies of the culture by streaking a sterilized needle on the surface of the culture and thoroughly mixing it with the drop of water on the glass slide. A cover slip was placed on top of the mixture and examined under microscope at a magnification of x40. The spore characteristics of shape and colour was examined and compared with reference publications by Mathur and Kongsdal, 2001 to confirm the identity of the fungi.

3.6.3.2 Bacterial Load and Identification

Serial dilutions were prepared from contaminated samples in the following procedure described by Collins and Lynes, (1989). A weighed quantity of 0.1g was thoroughly mixed with 9ml of water by inverting the sample bottle several times. An automatic pipette and a sterile 1ml pipette tip was used to take 1ml aliquot from an inch below the surface of the bottle and added to 9ml of sterile Ringers solution (diluent) in a test tube. This is the 10^{-1} dilution. The pipette was disinfected with a solution of disinfectant. A fresh sterile pipette tip was used to mix the 10^{-1} dilution by drawing the suspension up and down ten times. The 10^{-2} dilution was prepared by drawing 1ml of the 10^{-1} dilution into another test tube containing 9ml of sterile Ringers solution. Dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were further prepared by repeating the above procedure for further three times. After each dilution, the pipette was disinfected.

A measured volume of 1ml of each of the dilutions of the sample was added aseptically to universal bottles containing molten plate count agar at 40°C using

a fresh sterile pipette tip for each dilution. The sample and agar mixture were mixed by rotating the bottle between the palms taking care not to form bubbles. The mixtures were then poured aseptically into fresh sterile petri dishes. The petri dishes were well labeled and allowed to solidify and then bulked by fastening them together with cellotape. The solidified agar plates were incubated in inverted positions at 37°C for 24 hours for growth to occur. After 24 hours an electronic colony counter was used to count the number of microorganisms on the countable plates. The number of counts were reported as 'colony forming units per ml' or as 'viable count per ml'.

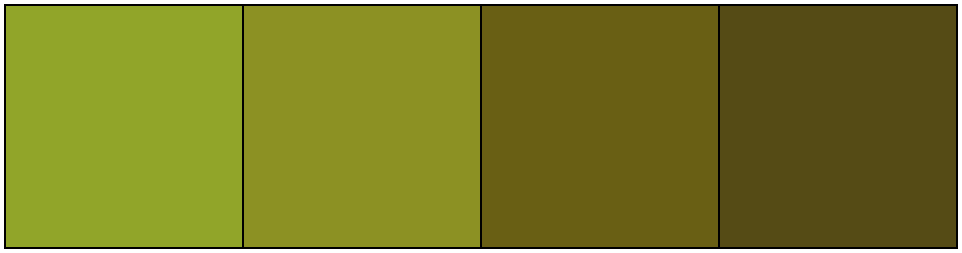
Preparation of slides and Gram staining were then carried out to identify the bacteria. The glass slides were cleaned in chromic acid, washed in water and stored in a jar of alcohol. They were removed from the jar by forceps, drained and flamed to remove the alcohol. A small drop of water was placed on the slide and a tiny portion of the bacterial colony was added to the drop of water with a flamed sterile loop and emulsified. The drop was spread and the bacteria fixed by passing the slide three times through a Bunsen flame. This coagulates bacterial protein and makes the film less likely to float off during staining (Collins and Lynes, 1989). The bacterial smear was stained with 0.5% crystal violet for two minutes, washed with water, drained and stained again with dilute iodine for two minutes (the crystal violet and iodine form a purple black complex inside the bacterial cell). Absolute alcohol was carefully dripped onto the smear and allowed to run off. This was repeated three times and then washed with water (the alcohol dissolves the lipid layer surrounding Gram negative cells and allows the crystal violet/iodine complex to wash out). The smear was again

counter stained with 1% safranin for two minutes, and the slides were washed and drained.

After staining, the slides were examined under the microscope without a cover slip. A small drop of immersion oil was placed directly onto smear and examined using an oil immersion lens of x100 magnification. Gram positive bacteria cells stain purple and Gram negative cells stain light pink (Collins and Lynes, 1989).

3.6.4 Colour (appearance)

Colour analysis were conducted at day1, day16 and then at days 60,120 and 180 of storage. Different shades of green colours were obtained from the Microsoft Office 2007 software and printed on rectangular pieces of papers. Four different shades of green were selected for identification of samples after a preliminary visual assessment. The RGB (red, green, blue) values of the four colours were; A (145:165:41), B (140:145:35), C (105:95:20) and D (85:75:21) (Kumah, 2010). The green values in the RGB were used (A=165±10, B=145±10, C=95±10 and D=75±10). The mean colour values of the sample which was equal to or closest to the green value of any of the four (4) colour shades was chosen as the colour of the sample. 30 untrained panelists were selected randomly to identify the colours of the various samples by matching the four colour shades to the samples and selecting the colour that closely matched. The different shades of green colour used are shown in the figure below.



A (165±10) B (145±10) C (95±10) D (75±10)

Figure 4: The four shades of green colour and their values in the RGB.

3.6.5 Particle size

Standard sieves of different mesh sizes were used to determine the particle sizes of the samples. The sieves consisted of a lid and a collecting bowl**.** The sieves could be stacked on each other and operated manually by shaking with both hands. The following sieve sizes were used; 422µm, 295µm, 251µm and 125 µm. The sieves were stacked on top of each other in an increasing order from the bottom to the top. 30g of the sample was weighed and poured into the first sieve on top and covered with the lid to prevent loss of sample by wind. The collecting bowl was attached to the bottom of the stacked sieves to receive the sample that passes through the last sieve. The stacked sieves and its contents were shaken for five (5) minutes. The contents of the sieves were poured out and weighed separately using an electronic scale.

3.6.6 Temperature and Humidity of Store Room

Temperature and humidity were monitored in the store room using a humidity-temperature meter (Traceable Fisher Scientific). Temperature and humidity were monitored at 4 days interval till the end of storage. Readings were taken at 9 am, 12 pm and 3 pm and then averaged.

4.0 RESULTS

Results of analysis on the effect of packaging on the shelf-life and quality of Moringa leaf powder during storage are presented on the following tables. Moringa samples in the paper packaging ($PAPER_{WAXED}$, $PAPER_{CARDBOARD.}$, and $PAPER_{BROWN}$) and control got deteriorated by day 16 of storage. They were removed from storage, discarded and ruled out as possible packaging materials for long term storage of Moringa leaf powder. That is why results for the paper packages and control are absent in the tables after day 16 of storage.

4.1 MOISTURE CONTENT

The results for the moisture content analysis during storage are shown in Table 1.

Table 1: Moisture values of Moringa leaf powder at different storage duration in different packages (%).

Treatments	Storage period(Days)							
	1	4	8	12	16	60	120	180
$PET_{TRANS.}$	5.09a	6.18b	6.02b	6.23b	6.26c	6.27b	6.12b	6.08b
PET_{OPAQUE}	5.09a	6.23b	6.04b	6.26b	6.27c	6.28b	6.14b	6.08b
$LDPE_{TRANS.}$	5.09a	6.33b	6.12b	6.39b	6.40b	6.39a	6.23a	6.20a
$LDPE_{OPAQUE}$	5.09a	6.30b	6.08b	6.36b	6.39b	6.37a	6.23a	6.18a
$GLASS_{TRANS.}$	5.09a	5.21c	5.12c	5.22c	5.24d	5.25c	5.19c	5.14c
$GLASS_{OPAQUE}$	5.09a	5.20c	5.16c	5.25c	5.27d	5.27c	5.19c	5.13c
$PAPER_{WAXED}$	5.09a	10.06a	9.84a	14.18a	15.14a	-	-	-
$PAPER_{CARD.}$	5.09a	10.08a	9.87a	14.20a	15.15a	-	-	-
$PAPER_{BROWN}$	5.09a	10.12a	9.89a	14.23a	15.20a	-	-	-
CONTROL	5.09a	10.12a	9.85a	14.24a	15.18a	-	-	-
CV(%)	-	1.4	1.4	1.0	0.6	0.6	0.7	0.8

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.
3. Higher values indicate increase in moisture content.

Table 2: Mean moisture values of Moringa leaf powder at the end of storage in different packages (%).

Treatments	Means
$PET_{TRANS.}$	6.12b
PET_{OPAQUE}	6.18b
$LDPE_{TRANS.}$	6.25b
$LDPE_{OPAQUE}$	6.22b
$GLASS_{TRANS.}$	5.19a
$GLASS_{OPAQUE}$	5.19a
CV(%)	1.7

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test
3. Higher values indicate increase in moisture content

From Table 1, the moisture content at day 1 was the same for all the treatments. There was a general increase in moisture content in all samples at day 4. $GLASS_{TRANSP.}$ and $GLASS_{OPAQUE}$ packages recorded the lowest moisture at day 4. At day 8, there was a general drop in moisture in all the samples from that of day 4. The moisture increased again at day 12 and day 16 with $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ again recording the lowest moisture whiles $PAPER_{WAXED}$, $PAPER_{CARD.}$, $PAPER_{BROWN}$ and CONTROL recorded significantly (P≤0.05) higher moisture followed by $PET_{TRANS.}$ and PET_{OPAQUE} and $LDPE_{TRANS.}$ and $LDPE_{OPAQUE}$. At day 60 $LDPE_{TRANS.}$ and $LDPE_{OPAQUE}$ packages recorded significantly (P≤0.05) highest moisture followed by the $PET_{TRANS.}$ and PET_{OPAQUE} whiles $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages maintained the lowest moisture. There was, however, a general drop in moisture at day 120 and day 180 in all the samples. $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages still

recorded the least moisture where as $LDPE_{TRANS.}$ and $LDPE_{OPAQUE}$ recorded the highest.

At the end of storage, the average moisture of samples in the $PET_{TRANS.}$, $PET_{OPAQUE,}$ $LDPE_{TRANS.}$ and $LDPE_{OPAQUE}$ packages did not differ significantly ($P>0.05$). However, the moisture of samples in the $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages were significantly different ($P\leq0.05$) from the rest of the samples. Samples in these pairs of the packaging, $PET_{TRANS.}$ and $PET_{OPAQUE,}$ $LDPE_{TRANS.}$ and $LDPE_{OPAQUE}$ and $GLASS_{TRANSPARENT}$ and $GLASS_{OPAQUE}$, showed similar moisture trends irrespective of being opaque or transparent.

4.2 NUTRIENT CONTENT

The results in Tables 3, 4, 5, 6 and 7 below show the nutrients contents of Moringa leaf powder during storage.

Table 3: Crude protein contents of Moringa leaf powder during storage (%).

Treatment	**Storage period(Days)**			
	1	**60**	**120**	**180**
$PET_{TRANS.}$	42.21a	40.37a	38.01a	35.87a
PET_{OPAQUE}	42.21a	40.44a	37.88a	35.85a
$LDPE_{TRANS.}$	42.21a	40.21a	37.94a	36.01a
$LDPE_{OPAQUE}$	42.21a	40.23a	38.03a	35.82a
$GLASS_{TRANS.}$	42.21a	40.36a	37.96a	36.03a
$GLASS_{OPAQUE}$	42.21a	40.42a	37.98a	36.00a
CV (%)	-	1.0	0.5	0.8

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same column followed by different letters differ significantly ($P\leq0.05$)
2. Mean separation were done by Duncan multiple range test.
3. Lower values indicate decrease in crude fat content.

Table 4: Crude fat contents of Moringa leaf powder during storage (%).

Treatment	Storage period(Days)			
	1	60	120	180
$PET_{TRANS.}$	5.92a	5.26a	4.18a	2.94a
PET_{OPAQUE}	5.92a	5.27a	4.24a	2.92a
$LDPE_{TRANS.}$	5.92a	5.30a	4.12a	2.97a
$LDPE_{OPAQUE}$	5.92a	5.22a	4.11a	2.95a
$GLASS_{TRANS.}$	5.92a	5.26a	4.13a	2.95a
$GLASS_{OPAQUE}$	5.92a	5.24a	4.09a	2.94a
CV (%)	-	1.8	4.4	2.6

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.
3. Lower values indicate decrease in crude fat content

Table 5: Calcium contents of Moringa leaf powder during storage (%).

Treatment	Storage period(Days)			
	1	60	120	180
$PET_{TRANS.}$	6.60a	4.04a	1.51a	0.96a
PET_{OPAQUE}	6.60a	4.01a	1.49a	0.94a
$LDPE_{TRANS.}$	6.60a	3.98a	1.48a	0.94a
$LDPE_{OPAQUE}$	6.60a	4.02a	1.51a	0.95a
$GLASS_{TRANS.}$	6.60a	3.97a	1.48a	0.96a
$GLASS_{OPAQUE}$	6.60a	3.95a	1.49a	0.95a
CV (%)	6.60a	4.3	2.1	5.1

Note: All the paper packaging and control samples got deteriorated by day 16 of storage
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.
3. Lower values indicate decrease in crude fat content

.

Table 6: Magnesium contents of Moringa leaf powder during storage (%).

Treatments	Storage period(Days)			
	1	60	120	180
$PET_{TRANS.}$	0.53a	0.49a	0.41a	0.33a
PET_{OPAQUE}	0.53a	0.48a	0.40a	0.33a
$LDPE_{TRANS.}$	0.53a	0.48a	0.40a	0.34a
$LDPE_{OPAQUE}$	0.53a	0.47a	0.40a	0.33a
$GLASS_{TRANS.}$	0.53a	0.48a	0.41a	0.33a
$GLASS_{OPAQUE}$	0.53a	0.47a	0.40a	0.33a
CV (%)	-	3.7	4.8	3.6

Note: All the paper packaging and control samples got deteriorated by day 16 of storage
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.
3. Lower values indicate decrease in crude fat content

Table 7: Iron contents of Moringa leaf powder during storage (ppm).

Treatments	Storage period(Days)			
	1	60	120	180
$PET_{TRANS.}$	31.00a	28.50a	23.00a	9.33a
PET_{OPAQUE}	31.00a	28.67a	22.67a	9.33a
$LDPE_{TRANS.}$	31.00a	28.50a	22.67a	9.50a
$LDPE_{OPAQUE}$	31.00a	29.00a	23.67a	9.33a
$GLASS_{TRANS.}$	31.00a	29.33a	22.33a	9.17a
$GLASS_{OPAQUE}$	31.00a	29.00a	22.67a	9.17a
CV (%)	-	2.9	5.0	9.5

Note: All the paper packaging and control samples got deteriorated by day 16 of storage
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.
3. Lower values indicate decrease in crude fat content

Table 8: Mean values for nutrients contents of Moringa leaf powder at the end of storage.

Treatments	Means				
	Pro.(%)	Fat.(%)	Ca.(%)	Mg.(%)	Fe.(ppm)
$PET_{TRANS.}$	37.90a	4.14a	2.15a	0.41a	20.7a
PET_{OPAQUE}	38.02a	4.13a	2.13a	0.40a	20.3a
$LDPE_{TRANSP}$	37.92a	4.11a	2.11a	0.41a	20.2a
$LDPE_{OPAQUE}$	38.33a	4.14a	2.12a	0.41a	19.8a
$GLASS_{TRANS.}$	38.23a	4.14a	2.13a	0.41a	20.3a
$GLASS_{OPAQUE}$	38.31a	4.15a	2.12a	0.41a	20.3a
CV (%)	5.6	27.8	74.9	18.1	49.6

Note: All the paper packaging and control samples got deteriorated by day 16 of storage
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.
3. Lower values indicate decrease in crude fat content

From Table 3, the protein content at day 1 was the same for all samples. There was a general decrease in quantity of crude protein in all the samples tested at day 60, day 120 and day 180. There were, however, no significant differences (P>0.05) in the quantity of crude protein among all the samples. Tables 4, 5, 6 and 7 also show a general decrease in quantity of crude fat, calcium, magnesium and iron respectively during storage. There were no significant differences (P>0.05) in the quantities crude fat, calcium, magnesium and iron among all the samples tested during storage.

From Table 8, the average contents of nutrients at the end of storage did not differ significantly (P>0.05) among the packages. By day 180 of storage the amount of crude protein, crude fat, calcium, magnesium and iron reduced by

about 15%, 50%, 86%, 38% and 70% respectively in the remaining packages. See appendix Table 6.

4.3 PATHOLOGICAL STUDIES

4.3.1 Fungal Analysis

Figures 5-8 are showing deteriorated Moringa leaf powder samples by day 16 of storage in the various paper packages and control.

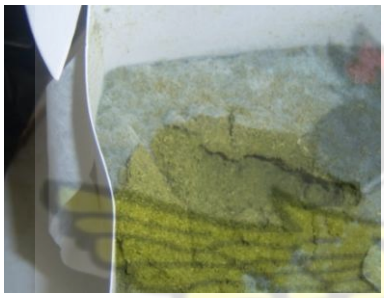


Figure 5: Deteriorated Moringa leaf powder in wax coated cardboard

Figure 6: Deteriorated Moringa leaf powder in cardboard box

Figure 7: Deteriorated Moringa leaf powder in brown paper bag.

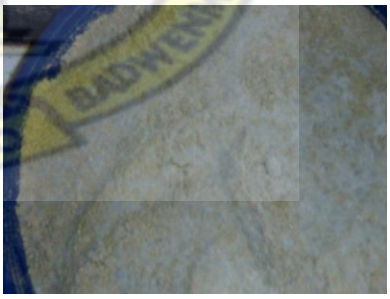


Figure 8: Deteriorated Moringa Leaf powder in control.

The cultural and microscopic characteristics of fungi examined in the laboratory were the same for all the deteriorated samples. The cultural and spore characteristics (Figures 9 and 10) were compared with reference publication by Mathur and Kongsdal, 2001 for identification and confirmation.

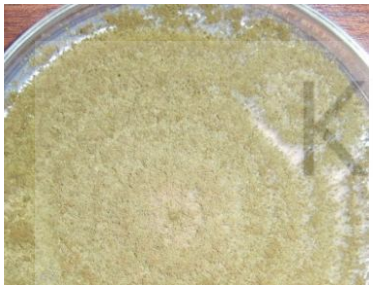


Fig 9: Mycellium colour of fungi *Aspergillus flavus*

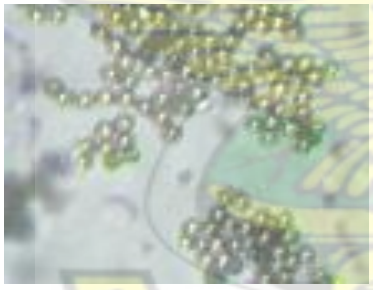


Fig 10: *Aspergillus flavus* spores under microscope (x40).

4.3.2 Bacterial Analysis

The results of the estimation of bacterial numbers during storage are presented in Table 9 below.

Table 9: Estimation of colony forming units (cfu) of bacteria ($x10^7$ colonies/g)

Treatments	Storage period(Days)				
	1	16	60	120	180
$PET_{TRANS.}$	2.36	2.36b	2.37a	2.40a	2.38a
PET_{OPAQUE}	2.36	2.35b	2.36a	2.38a	2.38a
$LDPE_{TRANSP}$	2.36	2.39b	2.35a	2.38a	2.39a
$LDPE_{OPAQUE}$	2.36	2.38b	2.36a	2.36a	2.37a
$GLASS_{TRANS.}$	2.36	2.36b	2.34a	2.35a	2.35a
$GLASS_{OPAQUE}$	2.36	2.36b	2.35a	2.39a	2.37a
$PAPER_{WAXED}$	2.36	7.35a	-	-	-
$PAPER_{CARD.}$	2.36	7.33a	-	-	-
$PAPER_{BROWN}$	2.36	7.37a	-	-	-
CONTROL	2.36	7.36a	-	-	-
CV(%)	-	1.4	2.7	1.4	1.4

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test
3. Higher values indicate increase in number of colony forming units of bacteria

Table 10: Mean values for colony forming units (cfu) of bacteria ($x10^7$ colonies/g) at the end of storage period.

Treatments	Means
PET_{TRANSP}	2.38a
PET_{OPAQUE}	2.38a
$LDPE_{TRANS.}$	2.39a
$LDPE_{OPAQUE}$	2.37a
$GLASS_{TRANS.}$	2.38a
$GLASS_{OPAQUE}$	2.39a
CV (%)	1.54

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test

From Table 9, the numbers of colony forming units of bacteria estimated on day 1 were the same for all samples. However at day 16, the numbers of colony forming units greatly increased significantly ($P \leq 0.05$) in $PAPER_{WAXED}$, $PAPER_{CARD.}$, $PAPER_{BROWN}$, and CONTROL packages whiles there were no significant changes ($P > 0.05$) in the numbers estimated in $PET_{TRANS.}$, PET_{OPAQUE}, $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$,$GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages even though $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ recorded the least values. There were slight changes in the number of colony forming units of bacteria estimated at day 60 in the $PET_{TRANS.}$, PET_{OPAQUE}, $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages but were not significantly ($P > 0.05$) different from each other and that of day 16. Again, there were no significant changes ($P > 0.05$) in the numbers estimated at day 120 and 180 of storage for all the treatments, a trend similar to that of day 60 of storage. The average estimated numbers of colony forming units of bacteria during the storage period in the remaining samples were significantly ($P > 0.05$) not different from each other.

The cultural, microscopic and Gram reactions of Bacteria at all the days of experiment during storage were similar in all the samples examined. The microscopic characteristics were compared with reference publications by Garbutt, 1997 for identification and confirmation. The gram reactions (i.e. purple) of the bacteria were also used in identifying the bacteria. Figures 11 and 12 are showing the microscopic and gram reaction of the bacteria examined.

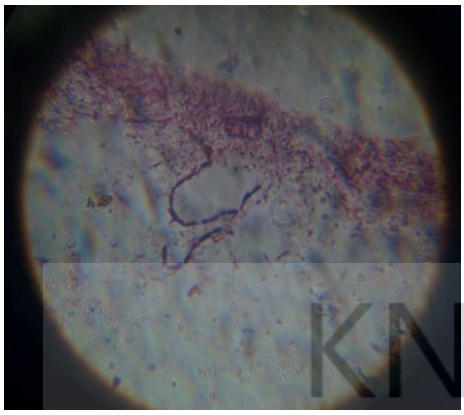


Figure 11: Gram positive *Bacillus spp*.bacteria.under microscope (x100)

Figure 12: Gram positive *Bacillus spp*.bacteria under microscope (x100)

4.4 COLOUR (APPEARANCE)

The results for the mean colour of samples are shown on the table below.

Table 11: Mean colour of samples during storage.

Treatments	Storage period (Days)									
	1		16		60		120		180	
	value	colour	value	colour	value	colour	value	colour	value	colour
$PET_{TRANS.}$	157.7	A	156.6	A	155.7	A	156.1	A	157.2	A
PET_{OPAQUE}	157.7	A	157.7	A	158.1	A	155.2	A	158.3	A
$LDPE_{TRANS.}$	157.7	A	156.1	A	156.8	A	158.7	A	156.1	A
$LDPE_{OPAQUE}$	157.7	A	158.3	A	156.8	A	157.0	A	155.9	A
$GLASS_{TRANS.}$	157.7	A	158.1	A	155.3	A	156.6	A	159.0	A
$GLASS_{OPAQUE}$	157.7	A	158.3	A	159.8	A	155.6	A	156.3	A
$PAPER_{WAXED}$	157.7	A	82.1	C	-	-	-	-	-	-
$PAPER_{CARD.}$	157.7	A	82.6	C	-	-	-	-	-	-
$PAPER_{BROWN}$	157.7	A	84.6	C	-	-	-	-	-	-
CONTROL	157.7	A	82.6	C	-	-	-	-	-	-

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.

Mean values that were closer to the values of green in the RGB was chosen as the colour of the sample. Value of green in RGB (A=165±10, B=145±10, C=95±10, D=75±10)

Table 12: Mean values for colour of Moringa leaf powder at the end of storage period.

Treatments	Means	
	Value	Colour
PET_{TRANSP}	156.33a	A
PET_{OPAQUE}	157.20a	A
$LDPE_{TRANSP}$	157.20a	A
$LDPE_{OPAQUE}$	156.57a	A
$GLASS_{TRANSP}$	156.97a	A
$GLASS_{OPAQUE}$	157.23a	A
CV (%)	0.99	

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test

At day 1, a mean green value of 157.7 which is closer to the green value of 165, hence colour A was chosen for all the samples. At day 16, Colour A was maintained in $PET_{TRANS.}$, PET_{OPAQUE}, $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages with mean values closer to that of the green values in the RGB. This indicates that, there were no colour changes in those packages. There was however a change in colour from A to colour C in the $PAPER_{WAXED}$, $PAPER_{CARD.}$, $PAPER_{BROWN}$ and CONTROL with mean values closer to green value of 95. At day 60, day 120 and day180, colour A was again maintained in the $PET_{TRANSP.}$, PET_{OPAQUE}, $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ And $GLASS_{OPAQUE}$ packages with mean values closer to green value of 165 in the RGB. From Table 12 above, average colour score during the storage period in the remaining samples were not significantly (P>0.05) different from each other.

It was also observed in all the transparent packages that the Moringa leaf powder by the surface of the packages exposed to light faded in colour. The fading, however, only occurred at the surface of the packages that were exposed to light and did not have any effect on the colour of the samples stored in them.

Figure 13: Faded Moringa leaf powder in glass bottle.

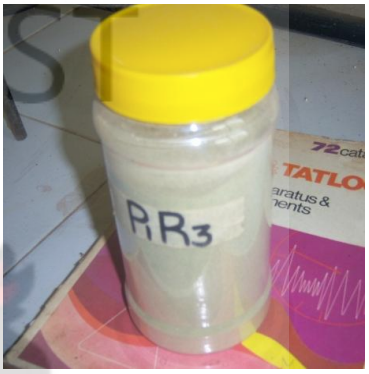


Figure 14: Faded Moringa leaf powder in PET bottle.

Figure 15: Moringa leaf powder in glass bottle after shaking.

Figure 16: Moringa leaf powder PET bottle after shaking.

4.5 PARTICLE SIZE

Changes in particle size during storage (in percentages) are presented in Table 13 and 14.

Table 13: Changes in particle size during storage (%).

Storage days	Sieve size(µm)	Treatments										CV (%)
		$PET_{TRANS.}$	$PET_{OPAQ.}$	$LDPE_{TRANS.}$	$LDPE_{OPAQ}$	$GLASS_{T.}$	$GLASS_{O}$	$PAPER_{WAX.}$	$PAPER_{CD.}$	$PAPER_{BRW.}$	CTRL	
1	422	8.4a	8.4a	8.4a	8.4a	8.4a	8.4a	8.4a	8.4a	8.4a	8.4a	-
	295	14.37a	14.37a	14.37a	14.37a	14.37a	14.37a	14.37a	14.37a	14.37a	14.37a	-
	251	21.23a	21.23a	21.23a	21.23a	21.23a	21.23a	21.23a	21.23a	21.23a	21.23a	-
	125	27.97a	27.97a	27.97a	27.97a	27.97a	27.97a	27.97a	27.97a	27.97a	27.97a	-
	>125	28.03a	28.03a	28.03a	28.03a	28.03a	28.03a	28.03a	28.03a	28.03a	28.03a	-
	Total %	100	100	100	100	100	100	100	100	100	100	
16	422	8.69d	8.71cd	8.75bc	8.77b	8.49e	8.48e	19.47a	19.51a	19.48a	19.48a	0.2
	295	14.78b	14.79b	14.80b	14.78b	14.48c	14.51c	15.71a	15.73a	15.75a	15.73a	0.2
	251	21.45b	21.45b	21.44b	21.46b	21.47b	21.45b	22.23a	22.23a	22.22a	22.21a	0.2
	125	27.57b	27.55bc	27.52bc	27.51c	27.88a	27.89a	24.12d	24.07d	24.08d	24.12d	0.1
	>125	27.51b	27.50b	27.49b	27.48b	27.69a	27.68a	18.46c	18.47c	18.48c	18.45c	0.1
	Total %	100	100	100	100	100	100	100	100	100	100	

1. Any two values in the same row followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.
3. Higher values in the bigger sieves indicate accumulation of particles.

Table 14: Changes in particle size during storage up to day 180.

Storage days	Sieve size(µm)	Treatments						CV(%)
		$PET_{TRANS.}$	PET_{OPQ}	$LDPE_{TRANS.}$	$LDPE_{OPQ}$	$GLASS_{TRANS}$	$GLASS_{OPQ}$	
60	422	8.68b	8.66b	8.73a	8.75a	8.46c	8.46c	0.2
	295	14.77a	14.78a	14.78a	14.78a	14.53b	14.50b	0.1
	251	21.45ab	21.47a	21.48a	21.49a	21.41b	21.43b	0.1
	125	27.58b	27.58b	27.57b	27.55b	27.78a	27.81a	0.1
	>125	27.52b	27.51b	27.43c	27.44c	27.81a	27.81a	0.1
	Total %	100	100	100	100	100	100	
120	422	8.53b	8.55b	8.65a	8.67a	8.46c	8.44c	0.2
	295	14.72a	14.73a	14.71a	14.71a	14.46b	14.47b	0.2
	251	21.34bc	21.35abc	21.39ab	21.40a	21.33c	21.31c	0.1
	125	27.71b	27.70b	27.63c	27.61c	27.89a	27.89a	0.1
	>125	27.70b	27.68b	27.62c	27.61c	27.87a	27.89a	0.1
	Total %	100	100	100	100	100	100	
180	422	8.55b	8.57b	8.70a	8.73a	8.45c	8.46c	0.3
	295	14.64b	14.66ab	14.70a	14.67ab	14.49c	14.50c	0.2
	251	21.31b	21.32b	21.39a	21.39a	21.30bc	21.28c	0.1
	125	27.77b	27.74b	27.64c	27.63c	27.90a	27.88a	0.1
	>125	27.72b	27.71b	27.57c	27.58c	27.86a	27.88a	0.1
	Total %	100	100	100	100	100	100	

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same row followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.
3. Higher values in the bigger sieves indicate accumulation of particles.

Table 15: Mean values for changes in particle size at the end of storage period.

Treatments	Means				
	422 µm	295 µm	251 µm	125 µm	Less125µm
$PET_{TRANS.}$	8.58b	14.71a	21.37a	27.70b	27.64b
PET_{OPAQUE}	8.59b	14.72a	21.36a	27.69b	27.64b
$LDPE_{TRANS.}$	8.69a	14.72a	21.42a	27.63b	27.54b
$LDPE_{OPAQUE}$	8.73a	14.71a	21.41a	27.60b	27.54b
$GLASS_{TRANS.}$	8.47c	14.47b	21.34a	27.87a	27.86a
$GLASS_{OPAQUE}$	8.46c	14.48b	21.34a	27.86a	27.86a
CV (%)	0.6	0.4	0.3	0.2	0.3

Note: All the paper packaging and control samples got deteriorated by day 16 of storage.
1. Any two values in the same column followed by different letters differ significantly (P≤0.05)
2. Mean separation were done by Duncan multiple range test.

From Tables 13 and 14, the quantity of Moringa powder in the various sieves after sieving were the same for all samples at day1. Less Moringa remained in the 422µm sieve and 295µm whiles greater quantities were collected from the 125µm and less125µm sieves at day 1. At day 16, there was a slight increase in the quantity of Moringa in the 422µm, 295µm and 251µm sieves with a corresponding decrease in quantity in the 125µm and less125µm for $PET_{TRANS.}$, PET_{OPAQUE}, $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages. However, a significantly ($P\leq0.05$) greater increase in quantity occurred in the 422µm, 295µm and 251µm sieves with a corresponding significant decrease ($P\leq0.05$) in the 125µm and less125µm sieves for $PAPER_{WAXED}$, $PAPER_{CARD.}$, $PAPER_{BROWN}$ and CONTROL samples. There were also significant differences ($P\leq0.05$) in changes that occurred in glass, polyethylene terephthalate and low density polyethylene (both transparent and opaque) with the glass samples having the smallest changes in particle sizes

followed by polyethylene terephthalate and low density polyethylene at day 16. At day 60, there was a slight increase in quantity in all packages in the 422µm, 295µm and 251µm sieves with a corresponding decrease in the 125µm and less125µm sieves in all samples. There were, however, significant differences ($P \leq 0.05$) in the changes in particle sizes that occurred in the glass, high polyethylene terephthalate, and low density polyethylene (transparent and opaque) samples with the glass (transparent and opaque) recording the lowest and highest quantity in the bigger and smaller sieves respectively. Similar trends were also observed at day 120 and day180 of storage showing significant differences ($P \leq 0.05$) in particle sizes in the glass, polyethylene terephthalate, and low density polyethylene (transparent and opaque) samples.

From Table 16, the average quantity of Moringa leaf powder during storage in the 422µm sieve for the GLASS, PET, and LDPE packages were significantly ($P \leq 0.05$) different from each other with GLASS having the lowest quantity of Moringa but, however, recorded the highest quantity in the 125µm and less125µm sieves which were again significantly ($P \leq 0.05$) different from that of PET and LDPE.

Generally, an increase in quantity of Moringa leaf powder in the 422µm, 295µm and 251µm led to a decrease in the 125µm and less125µm sieves in all the samples, but the changes were significantly ($P \leq 0.05$) greater in the $PAPER_{WAX}$, $PAPER_{CARDBOARD}$, $PAPER_{BROWN}$ and CONTROL than all the other treatments analysed.

4.6 TEMPERATURE AND HUMIDITY OF STORE ROOM

The highest temperature recorded was 30.0°C with a humidity of 84.16% which was also the lowest humidity recorded. The lowest temperature recorded was 25.5°C with 89.0% humidity being the highest during the storage duration. The average temperature and humidity of the storage room were 27.20°C and 86.56% respectively. As the temperature of the storage environment increased humidity decreased and vice versa. Warm air absorbs more moisture than cool air, therefore, the amount of moisture that can be held by warm air is greater than that held by the same volume of air at a lower temperature, so for the same amount of moisture in a known volume of air, a higher relative humidity will be recorded at a lower temperature while a low relative humidity will be recorded at a higher temperature Obeng-Ofori and Boateng (2008).

5.0 DISCUSSION

5.1 MOISTURE CONTENT

Increase in moisture by the Moringa leaf powder could be attributed to the increase in relative humidity of the storage environment from 84.2% at day1 to 87.9% at day 4. When food commodities having low moisture activity are stored in atmosphere of high relative humidity water will transfer from the gas phase to the food (Adams and Moss, 1995). The general decrease in moisture in all the samples at day 8 could be due to a decrease in the humidity of the storage environment from 87.9% at day 4 to 86.9% at day 8. Moisture content of hygroscopic material such as dry food is in direct relation to the humidity of the surrounding air (Wilhelm *et al.*, 2004). Generally, changes in moisture content in all the samples during the storage period were due to changes in humidity of the storage atmosphere. $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages had the least moisture values because they were better moisture barriers than $PET_{TRANS.}$ and PET_{OPAQUE} and $LDPE_{TRANS.}$ and $LDPE_{OPAQUE}$ packages which allowed in relatively more moisture. Even though GLASS materials are impermeable to moisture, the changes in their moisture could be attributed to ingress of moisture through the lids which were not hermetically sealed. The higher moisture content in $PAPER_{WAXED}$, $PAPER_{CARD.}$, $PAPER_{BROWN}$ and CONTROL packages could be attributed to their poor moisture resistance ability. Papers are, therefore, not good packages for foods that require protection against air and moisture pickup over a long storage period (Fellows and Axtell, 1993). The higher moisture in the paper materials and control could also be due to metabolic activities of micro-organisms. According, to Adams and Moss (1995),

once micro-organisms have started to grow and become physiologically active, they usually produce water as an end product of respiration.

Even though there was relatively lower moisture pick up by the $GLASS_{TRANS.}$, $GLASS_{OPAQUE}$, $PET_{TRANS.}$, PET_{OPAQUE} , $LDPE_{TRANSP.}$ and $LDPE_{OPAQUE}$ packages, it was not enough to have caused spoilage by micro-organism. Thickness of the packaging materials could also affect moisture absorption and moisture loss in a package. This could explain why samples in $PAPER_{BROWN}$ absorbed more moisture than $PAPER_{CARD.}$ packages and also LDPE packages absorbed more moisture than PET packages. The opaqueness or transparency of the packages did not have a significant effect on the moisture absorption because both the transparent and opaque packages were of the same thickness and thus exhibited similar moisture exchange capabilities.

5.2 NUTRIENT CONTENT.

The general decrease in the amount of nutrients in all the treatments with storage period could be attributed to storage duration and temperature. Nutritional quality of vegetables decreases with increasing storage time and temperature (Yamaguchi, 1983). Bender (1974) stated that reduction in protein quality can take place at room temperature during storage. The average storage room temperature recorded over the storage duration was 27.38°C. Different types of packaging used did not have any significant effect on the rate of nutrients loss analysed because there were no significant differences ($P>0.05$) in the amount of nutrients lost among all the packages over the storage period. Nonetheless, other nutrients such as Vitamins A and C could be affected by light and heat

respectively, therefore, an appropriate packaging that is opaque to light may be used (Meshas and Rodgers, 1994). Ascobic content remained constant when canned French beans were stored at 0°C but at 30°C an increase in degradation was observed (Charalambous, 1986). Lee *et. al.*, 1979, indicated that storage of canned vegetables at lower temperatures improved the retention of thiamine. Fuglie (2001) reported that exposure of Moringa leaves to light leads to destruction of vitamin A.

5.3 PATHOLOGICAL STUDIES

5.3.1 Fungal analysis

By comparing the cultural and microscopic characteristics of the fungus studied to the reference publication by Mathur and Kongsdal, 2001, *Aspergillus flavus* was identified as the organism responsible for the early spoilage. The growth of fungi in the Moringa leaf powder samples in all paper packages and control could be due their water activity (a_w). Fungi can grow at a water activity (a_w) as low as 0.80, which explains why dried foods often become mouldy (Nesta *et al.*, 2001). If the water activity of a dehydrated product is allowed to rise above a certain critical level, microbiological spoilage may occur. In such cases a packaging material with a low permeability to water vapour and effectively sealed, is required (Brennan, 2006).

Hoseney, in 1994, worked on wheat flour and reported that at lower moisture, fungi will not grow but at about 14% moisture content or slightly above, fungal growth takes place. The early spoilage in $PAPER_{WAX}$, $PAPER_{CARDBOARD}$, $PAPER_{BROWN}$ and CONTROL packages was as result of higher moisture

availability in these packages. According to Wilhelm *et al.* (2004), high available moisture leads to mould growth and microbial activities. The source of contamination could be from the field during harvesting and processing operations such as drying and milling. The storage environment could also have been a source of contamination.

5.3.2 Bacterial Analysis

The results from Table 10, clearly indicates a significant increase ($P \leq 0.05$) in the number of colony forming units of bacteria in the $PAPER_{WAX}$, $PAPER_{CARDBOARD}$, $PAPER_{BROWN}$ and CONTROL samples by day16 of storage. This could be attributed to the availability of moisture suitable for microbial activities because there was as much as 15% moisture (three times the initial moisture content) in those samples by day16 of storage. According to Jay (1992), bacteria require relatively high levels of moisture for their growth. The higher numbers estimated could also be due to the inability of those packages to serve as a physical barrier to oxygen, which is essential for carrying out metabolic activities by micro-organisms. The lower bacterial numbers recorded in $PET_{TRANS.}$, $PET_{OPAQUE,}$ $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages could be attributed to their good moisture barrier properties and also because the packaging containers could have acted as effective physical barrier against bacteria.

The number of colony forming units of bacteria estimated at day 1 and at the end of storage period in the $PET_{TRANS.}$, $PET_{OPAQUE,}$ $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages could be due to the presence of

bacteria spores from the field in which they were grown or contamination during harvesting and processing operations. A variety of microbes find their way onto foods, introduced from the soil in which they were grown, and during harvest, packaging, storage and handling (ICMSF, 1996).

The bacteria were identified as a Gram positive *Bacillus spp*. because of their microscopic characteristics of shape and spore form and purple Gram reaction (Garbutt, 1997).

5.4 COLOUR (APPEARANCE)

From Table 12, colour A was chosen as the colour of all the samples at day1 of storage. Colour C was selected for $PAPER_{WAXED}$, $PAPER_{CARD.}$, $PAPER_{BROWN}$ and CONTROL treatments at day16. This is an indication of a prominent colour change from colour A to colour C. This colour change could be attributed to chemical changes in the food as a result of microbial activities because these packages recorded the highest number of micro-organisms estimated at day16. Some micro-organisms give rise to chemical changes that alter the colour of food (Garbutt, 1997). The colour changes could also be due to the presence of oxygen in those packages which led to oxidation of certain substances in the plant tissue. Grandison (2006) stated that oxidation of phenolic substances in plant tissue by phenolase leads to browning. The colour changes could also be due to non-enzymatic browning (Maillard reaction). Chemical reaction between amino acids in proteins and reducing sugars in food materials causes browning (Dobraszczyk *et al.,* 2004).The colour of samples in $PET_{TRANS.}$, $PET_{OPAQUE,}$ $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages were

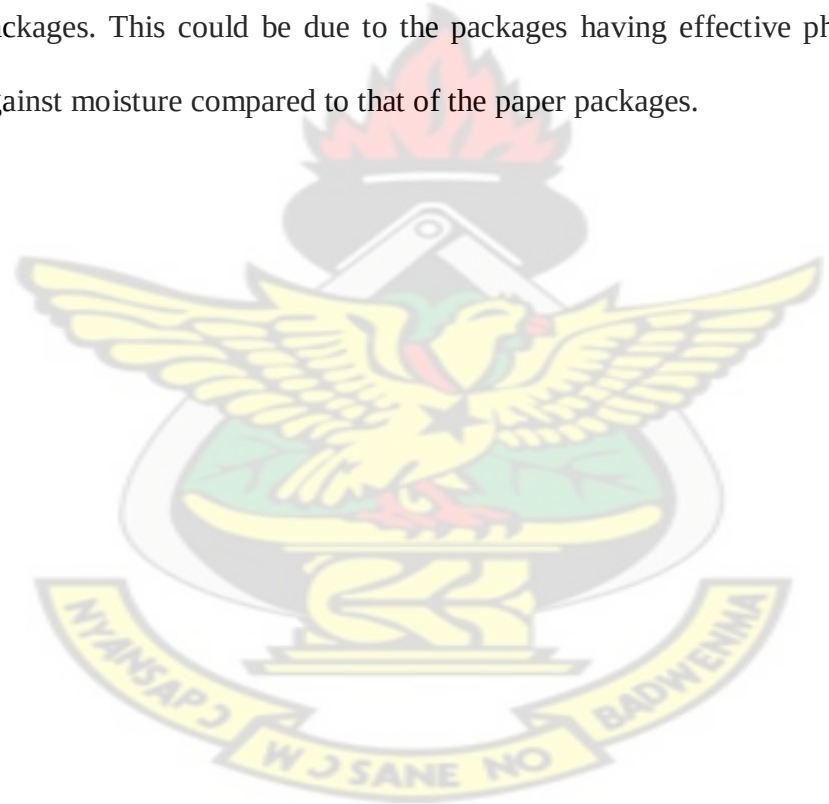
retained at day 16. This is an indication of no colour changes in these packages. These packages also recorded the least numbers of micro-organisms estimated and lower moisture than the rest of the packages. Again, at day 60, day 120 and day 180, the colour of samples in $PET_{TRANS.}$, $PET_{OPAQUE,}$ $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages did not change. The colour maintenance could be attributed to their good barrier properties against moisture, oxygen and micro-organisms.

It was observed, however, that the fading that occurred at the surface in all the transparent packages could be attributed to exposure of Moriga to light. Fuglie, 2001, reported that, exposure of Moringa leaves to light lead to destruction of vitamins and fading of colours. Since the fading only occurred at the surface of the transparent packages exposed to the light, it could not significantly change the colour of the entire content of samples in them. Fading, however, has an unattractive appearance and could detract consumers from buying because colour and colour uniformity are vital components of visual quality of foods and play a major role in consumer choice (Grandison, 2006).

5.5 PARTICLE SIZE

The increase in the amount of the Moringa leaf powder in the bigger sieves (422µm, 295µm and 251µm) and a corresponding decrease in the smaller sieves (125µm and less125µm) during the entire storage duration is an indication of agglomeration of the particles. This could be attributed to absorption of moisture by the Moringa leaf powder which binds smaller particles together to form larger ones. There was a direct relationship between moisture absorption and

increase in quantity of samples in the bigger sieves. The significant ($P \leq 0.05$) increase in quantities of samples in the 442µm, 295µm and 251µm sieves and a significant decrease ($P \leq 0.05$) in the 125µm and less 125µm sieves in the $PAPER_{WAXED}$, $PAPER_{CARD.}$, $PAPER_{BROWN}$ and CONTROL samples at day 16 is an indication of agglomeration of Moringa leaf powder particles as a result of increased moisture in these packages. Changes in particle sizes in $PET_{TRANS.}$, $PET_{OPAQUE,}$ $LDPE_{TRANS.}$, $LDPE_{OPAQUE}$, $GLASS_{TRANS.}$ and $GLASS_{OPAQUE}$ packages were significantly ($P \leq 0.05$) far smaller compared to that of the paper packages. This could be due to the packages having effective physical barrier against moisture compared to that of the paper packages.



6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Moringa leaf powder stored in the waxed paper, paper cardboard, and brown paper packages all had relatively shorter shelf-lives of 16 days in a storage environment of about 86.85% humidity and a temperature of 27.20ºC. Both transparent and opaque glass, polyethylene terephthalate and low density polyethylene packages were found to prolong the shelf-life of Moringa leaf powder. They were also were found to be effective against moisture absorption from the storage environment and against the growth and activities of micro-organisms such as fungi and bacteria. *Aspergillus flavus* and *Bacillus spp.* were the main postharvest fungi and bacteria, respectively, found to be causing spoilage in Moringa leaf powder stored in the paper packages. High moisture content and increased microbial activities also altered the colour of the Moringa leaf powder in the paper packages.

There was also a direct effect of different packaging on the agglomeration of particles due to moisture. The paper packages which absorbed more moisture had the highest agglomeration of particles where as both transparent and opaque glass, polyethylene terephthalate and low density polyethylene packages with less moisture absorption recorded low agglomeration of particles. There was an influence of storage duration on nutrients content. Stored Moringa leaf powder lost most of their nutrients when stored at an average temperature of 27.20°C for a period of six months. The research however revealed that the different types of packaging used did not have any significant effect on the rate of nutrients loss because there were no significant differences ($P>0.05$) in the amount of nutrients

lost among all the packages over the storage period. Although, there were no significant differences (P>0.05) among the colour of samples in the LDPE, PET and the glass packages, the surfaces of samples in the transparent packages exposed to light got faded (lost the green colour). Both transparent and opaque glass packaging containers were found to be the most effective type of packaging for Moringa leaf powder during the storage period.

6.2 RECOMMENDATIONS

Glass packaging proved to be the most effective packaging for Moringa leaf powder especially in terms of barrier against moisture since moisture absorption poses a great postharvest threat to highly hygroscopic food commodities. Glass packaging, however, have relatively high cost, extra weight and are fragile. Due to these reasons, a more lighter and durable and less expensive packaging material such opaque polyethylene terephthalate (PET) may be recommended for packaging highly hygroscopic and light sensitive foods such as Moringa leaf powder.

Storage of Moringa leaf powder for a long period in high temperature and high humidity environments (27.20°C and 86.85%, respectively) should be avoided because it loses most its nutrients. By the end of 6 months storage of Moringa leaf powder, the amount of crude protein, crude fat, calcium, magnesium and iron reduced by about 15%, 50%, 86%, 38% and 70% respectively. Packaging of Moringa powder in all forms of paper packaging could not be an acceptable practice especially in high temperature and high humidity environments (27.20°C and 86.85%, respectively).

REFERENCES

Adams, M.R. and Moss, M.O. (1995) Food Microbiology**.** The Royal Society of Chemistry, Cambridge. Pp398.

Adenike, A.A. (1981). Ascorbic acid retention of stored dehydrated Nigerian vegetables, Nutrition Reports Int., 24(4):769.

AOAC (1995). Association of Official Analytical Chemists. Official Methods of Analysis. Washington D.C. U.S.A.

Atlas, R.M. (1995). Principles of Microbiology. Mosby-Year Book, Inc. Pp 888

Banwart, G.J. (1974). Basic Food Microbiology, CBS Publishers and Distributers. Pp 781

Bender, A.E(1974). Food Processing and Nutrition. Academic Press, London. Pp 243.

Binder, E.M., Binder, J., Ellend, N., Schaffer, E., Kriska, R. and Braun, R. (1998). Microbiological degradation of deoynivalenol and 3-acetyl-deoxynivalenol. In: Mycotoxins and phycotoxins—developments in chemistry, toxicology and food safety. (Eds. Miraglia, M., VanEgmond, H., Brera, C. and Gilbert, J.). Fort Collins, Colo.: Alaken, Inc. p 279–285.

Dobraszczyk, B.J., Ainsworth, P., Ibanoglu, S. and Bouchon, P. (2004). Baking, Extrution and Frying. Pp 237-290.In: Food Processing Handbook. (Ed.Brennan, J.G.). WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Booth, F.E.M. and Wickens, G.E. (1988). Non-timber uses of selected arid zone trees and shrubs in Africa. F.A.O Conservation Guide, Rome. Pp. 92-101.

Brennan, J.G., Butters, J.R., Cowell, N.D., and Lilley, .E.V. (1990). Food Engineering Operations. 3rd Edition, Elsevier Science Publishers Limited. Pp 700

Brennan, J.G. and Day, B.P.F. (2006). Packaging. In: Food Processing Handbook. (Ed. Brennan, J.G). WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Pp 291-350.

Brennan, J.G. (2006). Food Processing Handbook. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Pp 602

Brenndorfer, B., Kennedy, L., Oswin, Bateman, C.O., Mrema, G.C., Trim D.S. and Wereko-Brobbey, C. (1987). Solar Dryers- their role in postharvest processing. Commonwealth Science Council, Pall Mall. Pp 286

CAC. (2003). Code of practice for the prevention and reduction of mycotoxin contamination in cereals, including annexes on ochratoxin A, zearalenone, fumonisins and tricothecenes. Codex Alimentarius Commission. CAC/RCP 51–2003.

Charalambous, G. (Ed.).(1986). The Shelf-life of Foods and Beverages: Procedings of the 4th International Flavour Conference. Elsevier, Amsterdam.

Collins, C.H and Lynes, P.M. (1989). Microbiological Methods. 6th Edition. Butterworth and Co. (Publishers) Ltd. Pp 407

Council of Scientific and Industrial Research. (1962). *The Wealth of India. A dictionary of Indian raw materials and industrial products. Raw materials, volume 6*: L-M, New Delhi, CSIR, India.

Dong C. X. (2008). Drying as a Means of Controlling Food Biodeterioration. In: Food Biodeterioration and Preservation. (Ed. Tucker, G.S.). Pp 137-163.

Dupriez, H. and De Leener, P. (1989). African Gardens and Orchards; Growing vegetables and fruits. McMillan Publishers. Pp 333

Eriksen, G.S. (2003). Metabolism and toxicity of trichothecenes. Swedish Univ. of Agricultural Sciences, Uppsala, Sweden. Pp 38

Fahey, J.W. (2005). *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. Trees for Life Journal 2005, 1:5

FAO/IAEA. (2001). Manual on the application of the HACCP system in mycotoxin prevention and Control. F.A.O. Food and Nutrition Paper 73, Rome: Food and Agriculture Organization/International Atomic Energy Agency.

Featherstone, S. (2008). Control of Biodeterioration in Food. Pp 1-35. In: Food Biodeterioration and Preservation. (Ed. Tucker, G.S.). Blackwell Publishing Ltd.

Fellows, P. and Axtell, B. (1993). Appropriate Food Packaging. International Labour Organisation /TOOL Publications, Amsterdam. Pp 136

Folkard, G.K., Sutherland, J.P. and Al-Khalili, R.S. (2001). Water clarification using *Mornga oleifera* seed coagulant. Pp 47-51. In: The Miracle tree. *Moringa oleifera*: Natural nutrition for the tropics. (Ed. Fuglie, L.J.). CWS, Darkar, Senegal.

Foidl, N., Makkar, H.P.S. and Becker, K. (2001). The potential of Moringa oleifera for agricultural and industrial uses. Pp 45-76. In: The Miracle Tree: The Multiple Attributes of Moringa. (Ed. Fuglie, L.J.). CTA, Wageningen, The Netherlands.

Fuglie, L.J., and Sreeja, K.V. (2001). Cultivation of Moringa. Pp 123-128. In: The Miracle tree. *Moringa oleifera*: Natural nutrition for the tropics. (Ed. Fuglie, L.J.). CWS, Darkar, Senegal.

Fuglie,L.J. (2001). The Miracle Tree. *Moringa oleifera* : Natural nutrition for the tropics. CWS, Dakar , Senegal. Pp 164

Garbutt, J. (1997). Essentials of Food Microbiology. Hodder Headline Group, London. Pp 251

Grandison, A.S. (2004). Postharvest Handling and Preparation of Foods for Processing. Pp 1-32.In: Food Processing Handbook. (Ed.Brennan, J.G.). WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Grubben, G.J.H. and Denton, O.A. (2004). Plant Resources of Tropical Africa 2. Vegetables. PROTA Foundation, Wageningen , Netherlands /Backhuys Publishers, Leiden , Netherlands /CTA, Wageningen , Netherlands . Pp 688

He, P., Young, L.G. and Forsberg, C. (1992). Microbial transformation of deoxynivalenol (vomitoxin). Applied Environmental Microbiology. 58:38 57-63.

Hoseney, R.C. (1994). Principles of Cereal Science and Technology. 2nd ed. Am. Assoc. of Cereal Chem. Inc., St. Paul, Minnesota, USA.

I.C.M.S.F. (1996). International Commission on Microbiological Specification for Foods. Microorganisms in foods. Volume 5, Characteristics of microbial pathogens. London: Blackie Academic & Professional. P 513.

Jay, J.M. (1992). Modern Food Microbiology, 4th Edition. Chapman Hall, New York. Pp 701

Jayaraman, K.S. and Das Gupta, D.K. (2006). Drying of fruits and vegetables. In: Hand Book of Industrial Drying, 3rd ed. (Ed. Mujumdar, A.S.). Taylor & Francis Group, LLC. Pp 606-631

Kader, A.A. (1988). Influence of pre-harvest and postharvest environment on nutritional composition of fruits and vegetables. In: Horticulture and human health, contributions of fruits and vegetables. (Eds. Quebedeaux, B. and Bliss, F.A.). Prentice-Hall. Englewood Cliffs, NJ, Pp. 18-32.

Kader, A.A. (1992). Postharvest Technology of Horticultural crops. 2ND Edition, University of California, Division of Agric. And Nat. Resources, Publ.3311. Pp 296

Kader, A.A. (1993). Post harvest handling: In: The Biology of Horticulture an Introductory Textbook. (Eds. Preece, J.E. and Reed, P.E.). John Willey and Sons Inc. Pp 353-375.

Krokida, M.K., Karathanos, V.T. and Maroulis, Z.B. (1998). J Food Eng 35: 369–380.

Krokida, M. and Maroulis, Z. (2000). In: Drying Technology on Agriculture and Food Science. (Ed. Mujumdar, AS.). Science Publishing, Inc., Enfield, NH, pp. 61–105.

Kumah, P. (2010). Personal communication. Lecturer. Department of Horticulture, Faculty of Agriculture, KNUST, Kumasi, Ghana.

Lee, W.N., Kramer, .A and Onishi, Y. (1979). Journal of Food Quality (2). Pp 257-268.

Lopez-Garcia, R., Park, D.L. and Phillips, T.D. (1999). Integrated mycotoxin management systems. Tunis,Tunisia: Third Joint FAO/WHO/UNEP International Conference on Mycotoxins. Document No. MYC-CONF/99/6a.

Makonnen, E., Hunde, A. and Damecha, G. (1997). Hypoglycaemic effect of *Moringa stenopetala* aqueous extracts in rabbits. *Phytoterapy Research.* 11:147-148.

Mathur, S. B. and Kongsdal, O. (2001). *Common Laboratory Seed Health Testing Methods for Detecting Fungi.* Kandrup Bogtrykkeri, Arhusgade 88, DK – 2100 Copenhagen, Denmark.

Meshas, K.Y. and Rodgers, S.L. (1994). Food Science and You, 2nd editon. Glencoe, McGraw-Hill. Pp 399

Morton, J.F. (1991). The Horseradish Tree, *Moringa Ptergosperma* (Moringaceae)-A Boon to Arid Lands? Economic Botany. 45:318-333.

Motsara, M.R. and Roy, R.N. (2008). Guide to Laboratory Establishment for plant nutrient analysis. F.A.O Fertilizer and Plant Nutrition Bulletin 19. F.A.O, Rome. Pp 204

Murphy, P.A., Hendrich, S., Landgren, C. and Bryant, C.M. (2006). Food Mycotoxins: An Update. Journal of Food Science Vol. 71, Nr. 5. Pp 1-15.

Nesta, E.W., Anderson, D.G., Roberts, C.E. and Nesta, M.T. (2001). Microbiology, A Human Perspective, 3rd edition. McGraw-Hill. Pp 820

Obeng-Ofori, D. and Boateng B.A. (2008). The Stored Product environment: In Postharvest Science and Technology. (Eds. Cornelius, E.W and Obeng-Ofori, D). Smartline Publishing Ltd. for College of Agriculture and Consumer Sciences University of Ghana, Legon.

Obeng-Ofori, D., Danquah, E.Y. and Ofosu-Anim, J. (2007). Vegetable and Spice Crop Production in West Africa. The City Publishers Ltd. Pp 1-7.

Olympio, N.S. and Kumah, P. (2002). Packaging for Marketing of Fruits, Vegetables and Floricultural Produce in Ghana . Ghana Journal of Horticulture. Volume 1, 2002. Pp 109-116.

Pal, S.K., Mukherjee, P.K. and Saha, B.P. (1995). Studies on the Antiulcer Activity of *Moringa oleifera* Leaf Extract on Gastric Ulcer Models in Rats. *Phytoterapy Research.* 9:463-465

Panhwar, F. (2006). Postharvest technology of fruits and vegetables. http://www.farzanapanhwar.blogspot.com (27th July, 2010).

Pelczar, M.J., Chan, E.C.S, Krieg, N.R. (2006). Microbiology, 5th edition. Tata McGraw-Hill Publishing Company Limited, New Delhi.

Rodgers, E.S. and Danley, K.S. (1994). *"The residential needs and preference of persons with serious mental illness"*: A comparison of consumers and family members. The Journal of Mental Health Administration. Vol.21 issue 1, Pp. 42-51.

Sablani, S.S., and Mujumdar, A.S. (2006). Drying of Potato, Sweet Potato, and Other Roots. In: Hand Book of Industrial Drying, 3rd edition. (Ed. Mujumdar, A.S.). Taylor & Francis Group, LLC. Pp 647-662

Shewfelt, R.L. (1999). What is quality? Postharvest Biol. Technol., 15:197-200.

Subadra, S., Monica, J. and Dhabhai, D. (1997). Retention and storage stability of beta-carotene in a dehydrated drumstick leaves (*Moringa oleifera)*. *International Journal of Food and Nutrition.* 48: 373 – 379.

Wilhelm, L.R., Suter, D.A. and Bruzwitz, G.H. (2004). Physical Properties of Food Materials. In: Food and Process Engineering Technology, 23-62. St Joseph, Michigan: ASAE. American Society of Agriculture Engineers. Pp 29 – 34.

Wills, R., McGlasson, B., Graham, D. and Joyce, D. (1998). Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables, 4th Edition. UNSW Press, South Australia. Pp 159-168.

Yamaguchi, M. (1983). World Vegetables: Principles, Production and Nutritive Values. The AVI Publishing Company, Inc., Westport, Connecticut, USA. Pp 32-46.

APPENDICES

ANOVA TABLES FOR THE PARAMETERS STUDIED.

Table 1a: Moisture content.

Storage period	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
4	Trt Residual Total	9 20 29	130.63156 0.23213 130.86370	14.51462 0.01161	1250.54	<.001
8	Trt Residual Total	9 20 29	124.68512 0.22627 124.91139	13.85390 0.01131	1224.56	<.001
12	Trt Residual Total	9 20 29	496.322163 0.188667 496.510830	55.146907 0.009433	5845.96	<.001
16	Trt Residual Total	9 20 29	613.494853 0.063333 613.558187	68.166095 0.003167	5845.96	<.001
60	Trt Residual Total	5 12 17	4.627044 0.015933 4.642978	0.925409 0.001328	696.96	<.001
120	Trt Residual Total	5 12 17	3.958628 0.021733 3.980361	0.791726 0.001811	437.15	<.001
180	Trt Residual Total	5 12 17	4.024178 0.026533 4.050711	0.804836 0.002211	364.00	<.001

Table 1b: Mean values for moisture content at the end of storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt Residual Total	5 12 17	4.070867 0.113533	0.814173 0.009461 4.184400	86.05	<.001

Table 2a: Crude protein

Storage period	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
60	Trt Residual Total	5 12 17	0.1463 2.1265 2.2728	0.0293 0.1772	0.17	0.971
120	Trt Residual Total	5 12 17	0.04433 0.40907 0.45340	0.00887 0.03409	0.26	0.926
180	Trt Residual Total	5 12 17	0.12293 0.92867 1.05160	0.02459 0.07739	0.32	0.893

Table 2b: Crude fat

Storage period	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
60	Trt	5	0.012178	0.002436	0.28	0.916
	Residual	12	0.104800	0.008733		
	Total	17	0.116978			
120	Trt	5	0.04787	0.00957	0.29	0.909
	Residual	12	0.39393	0.03283		
	Total	17	0.44180			
180	Trt	5	0.003561	0.000712	0.12	0.986
	Residual	12	0.072467	0.006039		
	Total	17	0.076028			

Table 2c: Calcium

Storage period	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
60	Trt	5	0.01791	0.00358	0.12	0.984
	Residual	12	0.34673	0.02889		
	Total	17	0.36464			
120	Trt	5	0.0024278	0.0004856	0.50	0.769
	Residual	12	0.0116000	0.006039		
	Total	17	0.0140278			
180	Trt	5	0.001133	0.000227	0.10	0.991
	Residual	12	0.028467	0.002372		
	Total	17	0.029600			

Table 2d: Magnessium

Storage period	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
60	Trt	5	0.0007111	0.0001422	0.45	0.806
	Residual	12	0.0038000	0.0003167		
	Total	17	0.0045111			
120	Trt	5	0.0003167	0.0000633	0.17	0.970
	Residual	12	0.0045333	0.0003778		
	Total	17	0.0048500			
180	Trt	5	0.0001833	0.0000367	0.26	0.924
	Residual	12	0.0016667	0.0001389		
	Total	17	0.0018500			

Table 2e: Iron

Storage period	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
60	Trt Residual Total	5 12 17	1.6667 8.3333 10.0000	0.3333 0.6944	0.48	0.785
120	Trt Residual Total	5 12 17	3.167 15.333 18.500	0.633 1.278	0.50	0.774
180	Trt Residual Total	5 12 17	0.2361 9.3333 9.5694	0.0472 0.7778	0.06	0.997

Table 2f: Mean values for nutrients contents of Moringa leaf powder at the end storage

Nutrients	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Crude pro	Trt Residual Total	5 12 17	0.572 53.882 54.454	0.114 4.490	0.03	1.000
Crude fat	Trt Residual Total	5 12 17	0.002 15.837 15.839	0.000 1.320	0.00	1.000
Calcium	Trt Residual Total	5 12 17	0.003 30.504 30.506	0.001 2.542	0.00	1.000
Manessium	Trt Residual Total	5 12 17	0.000383 0.065867 0.066250	0.000077 0.005489 1216.6	0.01	1.000
Iron	Trt Residual Total	5 12 17	1.1 1215.5 1216.6	0.2 101.3	0.00	1.000

Table 3a: Estimation of colony forming units (cfu) of bacteria

Storage day	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
16	Trt Residual Total	9 20 29	178.967480 0.069400 179.036880	19.885276 0.003470	5730.63	<.001
60	Trt Residual Total	5 12 17	0.001578 0.047867 0.049444	0.000316 0.003989	0.08	0.994
120	Trt Residual Total	5 12 17	0.004511 0.014133 0.018644	0.000902 0.001178	0.77	0.592
180	Trt Residual Total	5 12 17	0.002628 0.013800 0.016428	0.000526 0.001150	0.46	0.801

Table 3b: Mean values for estimation of colony forming units (cfu) of bacteria during storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	5	0.000894	0.000179	0.13	0.982
Residual	12	0.016200	0.001350		
Total	17	0.017094			

Table 4: Mean values for colour of samples during storage.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Trt	5	2.178	0.436	0.18	0.964
Residual	12	28.707	2.392		
Total	17	30.885			

Table 5a: Changes in particle sizes during storage(422µm)

Storage day	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
16	Trt	9	8.457E+02	9.397E+01	1.620E+05	<.001
	Residual	20	1.160E-02	5.800E-04		
	Total	29	8.458E+02			
60	Trt	5	0.2601167	0.0520233	199.24	<.001
	Residual	12	0.0031333	0.0002611		
	Total	17	0.2632500			
120	Trt	5	0.1393111	0.0278622	135.55	<.001
	Residual	12	0.0024667	0.0002056		
	Total	17	0.1417778			
180	Trt	5	0.2123611	0.0424722	79.64	<.001
	Residual	12	0.0064000	0.0005333		
	Total	17	0.2187611			

Table 5b: Changes in particle sizes during storage(295µm)

Storage day	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
16	Trt	9	8.1076300	0.9008478	916.12	<.001
	Residual	20	0.0196667	0.0009833		
	Total	29	8.1272967			
60	Trt	5	0.2828500	0.0565700	130.55	<.001
	Residual	12	0.0052000	0.0004333		
	Total	17	0.2880500			
120	Trt	5	0.2609167	0.0521833	56.25	<.001
	Residual	12	0.0111333	0.0009278		
	Total	17	0.2720500			
180	Trt	5	0.1248000	0.0249600	27.73	<.001
	Residual	12	0.0108000	0.0009000		
	Total	17	0.1356000			

Table 5c: Changes in particle sizes during storage(251µm)

Storage day	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
16	Trt Residual Total	9 20 29	4.295733 0.028067 4.323800	0.477304 0.001403	340.12	<.001
60	Trt Residual Total	5 12 17	0.0124667 0.0051333 0.0176000	0.0024933 0.0004278	5.83	0.006
120	Trt Residual Total	5 12 17	0.0189111 0.0098667 0.0287778	0.0037822 0.0008222	4.60	0.014
180	Trt Residual Total	5 12 17	0.0318278 0.0027333 0.0345611	0.0063656 0.0002278	27.95	<.001

Table 5d:Changes in particle sizes during storage(125µm)

Storage day	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
16	Trt Residual Total	9 20 29	91.459737 0.027733 91.487470	10.162193 0.001387	7328.50	<.001
60	Trt Residual Total	5 12 17	0.2116944 0.0093333 0.2210278	0.0423389 0.0007778	54.44	<.001
120	Trt Residual Total	5 12 17	0.2385833 0.0046667 0.2432500	0.0477167 0.0003889	122.70	<.001
180	Trt Residual Total	5 12 17	0.1976278 0.0072667 0.2048944	0.0395256 0.0006056	65.27	<.001

Table 5e:Changes in particle sizes during storage(less251µm)

Storage day	Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
16	Trt Residual Total	9 20 29	5.954E+02 1.933E-02 5.954E+02	6.615E+01 9.667E-04	68431.58	<.001
60	Trt Residual Total	5 12 17	0.4668278 0.0053333 0.4721611	0.0933656 0.0004444	210.07	<.001
120	Trt Residual Total	5 12 17	0.2220667 0.0027333 0.2248000	0.0444133 0.0002278	194.99	<.001
180	Trt Residual Total	5 12 17	0.2650278 0.0068667 0.2718944	0.0530056 0.0005722	92.63	<.001

Table 5f: Means values for changes in particle sizes during storage

Sieve size	Source of variation	d.f.	s.s.	m.s.	v.r	F pr.
422	Trt Residual Total	5 12 17	0.185161 0.033667 0.218828	0.037032 0.002806	13.20	<.001
295	Trt Residual Total	5 12 17	0.235600 0.041200 0.276800	0.047120 0.003433	13.72	<.001
251	Trt Residual Total	5 12 17	0.017600 0.049000 0.066600	0.003520 0.004083	0.86	0.534
125	Trt Residual Total	5 12 17	0.195517 0.036933 0.232450	0.039103 0.003078	12.71	<.001
Less125	Trt Residual Total	5 12 17	0.314444 0.096733 0.411178	0.062889 0.008061	7.80	0.002

Table 6: Percentage decrease in nutrients at the end of storage.

Treatment	**Crude pro.**	**Crude fat**	**Calcium**	**Magnesium**	**Iron**
$PET_{TRANS.}$	15.02	50.33	85.45	37.74	69.90
PET_{OPAQUE}	15.07	50.66	85.76	37.74	69.90
$LDPE_{TRANS.}$	14.67	49.83	85.76	35.85	69.35
$LDPE_{OPAQUE}$	15.14	50.17	85.61	37.74	69.90
$GLASS_{TRANS.}$	14.64	50.17	85.45	37.74	70.42
$GLASS_{OPAQUE}$	14.71	50.33	85.61	37.74	70.42

Table 7: Temperature and humidity of the store room during the storage period

Storage period(Days)	Temperature (°C)	Humidity (%)
1	30.00	84.16
4	26.50	87.94
8	27.00	86.94
12	26.00	88.11
16	25.50	89.00
30	26.00	88.33
60	25.67	88.67
90	27.00	87.00
120	29.00	84.11
150	29.34	84.21