

**POST HARVEST PRACTICES AND THE QUALITY OF  
FARM PROCESSED AND STORED ORANGE FLESHED  
SWEETPOTATO CHIPS IN TIRIKI WEST DIVISION,  
VIHIGA COUNTY, WESTERN KENYA**

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## DECLARATION

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

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## **DEDICATION**

**To my children Daniel, Tabitha and Steven**

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## **ABBREVIATIONS**

DAO – District Agricultural Officer

ELISA – Enzyme Linked Immunosorbent Assays

FCS – Featal calf serum

FFS – Farmers’ Field School

MoA – Ministry of Agriculture

KARI – Kenya Agricultural Research Institute

KNBS – Kenya National Bureau of Statistics

PBS – Phosphate Buffered Saline

PDA – Potato Dextrose Agar

SPSS – Statistical Package for Social Scientists

TMB – Tetra Methyl Benzilidine

## DEFINITIONS

**Post-harvest** means after separation from the medium and site of immediate growth or production. Post-harvest begins when the process of collecting or separating food of edible quality from its site of immediate production has been completed.

**Food** means weight of wholesome edible material that is consumed by humans, measured on a moisture free basis.

**Food loss** is any change in the availability, edibility, wholesomeness or quality of food that prevents it from being consumed by people.

**Food safety** is the protection of food from unintentional contamination through operational deficits or improper handling during production, storage, processing, transportation and serving. The contamination may be biological, physical or chemical and greatly leads to mild, moderate or severe illness.

**Food security** exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life.

**Food processing** refers to any changes made in any material used for food whether in the home or in a commercial establishment. Food preparation makes food ready for immediate consumption, while food preservation makes food to be available for future consumption.

**Mycotoxin** is a term used to describe the group of secondary fungal metabolites that are toxic to animals and humans.

## ABSTRACT

Post-harvest handling of sweetpotatoes (*Ipomoea batatas*) is relevant to enhance food safety and security for rural households. An observational study on post-harvest handling practices on sweetpotato farmers and determination of levels of beta-carotene, fungi and mycotoxins contamination in farm processed sun-dried orange fleshed sweetpotato chips was done. This was carried out between December 2004 and May 2005 in Tiriki West Division of Vihiga County. The purpose of the study was to promote food safety and food security through identification of practices that predispose sweetpotatoes to fungal infection with the relevant mycotoxin contamination.

Questionnaires were used to gather information on production, preparation, storage and handling practices of sweetpotato tubers. Samples of sun-dried orange fleshed sweetpotato chips of SPK 004 sweetpotato variety were then collected from the farmers for determination of beta-carotene, fungal and mycotoxin levels. Beta-carotene level was determined using the column chromatography technique. Fungi were isolated by direct plating and the fungi identified either to genera or species level. Aflatoxin and ochratoxin A levels were determined using ELISA method. Data were analyzed using SPSS Version 12 software and Excel.

Most farmers (67.8%) planted orange fleshed sweetpotato varieties. Thirty four point five percent of the farmers prepared and dried the sweetpotato roots into chips and majority (80.0%) used traditional locally available tools to do the preparation. Most of the farmers (53.3%) dried the chips by exposing them directly to the sun and majority (66.7%) of the farmers dried the chips for more than two days. Most farmers stored

sweetpotato chips anywhere (63.3%) in the house for a period of over three months (63.3%).

Beta-carotene levels were between 1.00 mg to 6.10 mg per 100g sample. The study also found that beta-carotene decreased with storage time. The major fungal genera isolated from the dry chips were *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Rhizoctonia*, *Nigrospora*, *Rhizopus*, *Alternaria* and *Microsphaera*. *Aspergillus* species isolated and identified were *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus* and *Aspergillus niger*. It was found that the levels of *Aspergillus* increased with the storage length of the dry chips, and the levels of *Fusarium* decreased with the storage length of the dry chips. The study also found that levels of *Aspergillus* and *Fusarium* were associated negatively.

Aflatoxin assessment indicated that the levels ranged from undetected level to twelve parts per billion while ochratoxin A assessment recorded levels that were undetected to fifteen parts per billion. Seventy percent of the samples recorded detectable levels of aflatoxin whereas 90% of the samples recorded presence of ochratoxin A. Aflatoxin levels and ochratoxin A levels in the farm prepared sun-dried sweetpotato chips were found to be significantly associated.

Association analysis showed the longer the storage length for the sun-dried orange fleshed sweetpotato chips the more the levels of species of *Aspergillus* and the lower the levels of beta-carotene in the samples. Association analysis also showed a negative association between species of *Aspergillus* and species of *Fusarium*, and a negative association between moisture content and aflatoxin levels.

The results of the study showed that beta-carotene level, fungal, aflatoxin and ochratoxin A contamination in the farm processed sun-dried orange fleshed sweetpotato chips could be as a result of post-harvest handling practices. All the samples were susceptible to fungal, aflatoxin and ochratoxin A contamination.

This study concludes that there is safety and health risk associated with utilization of the sun-dried sweetpotato chips and products made from the chips. This study recommends training of farmers on post-harvest handling of sweetpotatoes, further research to be done on association of beta-carotene levels and growth of fungi, aflatoxin and ochratoxin A in sun-dried sweetpotato chips under controlled study conditions of temperature, relative humidity and time.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Importance and Production of Sweetpotatoes in Kenya

Sweetpotato (*Ipomoea batatas*) is an important crop in Kenya's farming systems because it serves as a food security crop with high nutritive value and drought tolerance (MoA, 2010; Kapinga et al., 2007). It can be grown in a wide range of agro ecological zones (Kapinga et al., 2007) including low rainfall marginal lands and has low input demand. It can be produced twice per year with proper rotation. The orange fleshed sweetpotato varieties provide daily requirements of Vitamin A (Woolfe, 1992) and can be processed into juice or composite flours that can be used in making baked products and complementary foods (Hagenimana, 2001). The leaves are used as a vegetable and the vines as animal fodder (CIP, 1999). Sweetpotato has many advantages for poor households whose members depend on diverse livelihood strategies, especially those affected by HIV/AIDS (MoA, 2010). Due to health concerns by the consumers and the subsequent improvement of its food value in some varieties, its utilization as a snack and for breakfast is on the increase especially in urban areas (MoA, 2010).

Sweetpotatoes are grown mainly in Nyanza, Western, and Rift Valley and in some parts of Eastern, Central and Coast Provinces (MoA, 2010; GTZ and Ministry of Agriculture, 1998, Gruneberg et al., 2004). The total hectarage under this crop was about 61,000 Ha producing 880,000 tonnes in 2008 with marginal changes in area from year to year (MoA, 2010). However, there has been a steady increase in yields due to improved cultivars and farming practices.

Among the major constraints in sweetpotato production are biotic constraints such as insect pests (Kapinga and Carey, 2003). According to MoA (2010) the challenges faced in the sweetpotato sector include; lack of produce and product standards; lack of sorting and grading at the farm level; mechanical damage; produce is brought to the markets in inappropriate containers with no regulated weight; and there is arbitrary weight/quantity estimation. Kihurani (1994) pointed out that the main limitation to the development of sweetpotato is its low storage potential and high susceptibility to storage microorganisms.

Processing sweetpotatoes into different products enhances their usage, adds value at the farm level and reduces losses and bulkiness, thereby facilitating sale of the sweetpotato based products in the off-season and in distant markets. However, as traditionally practiced, sun-drying has various drawbacks including poor control of energy input and product quality, and frequent contamination of the food by microorganisms, dust and insects (Woolfe, 1992). The long exposure of the sweetpotato products such as chips to direct sunlight may also have negative effects on carotenoid content (Hagenimana, 1999).

Huge quantities of food are wasted every year, because they are invaded by toxic fungi and get contaminated by fungal metabolic products. Such spoilage is prominent in hotter countries, where problems of food shortages already exist. According to CAST, (2003) mycotoxins affect a quarter of the world's food crops. The aflatoxins are hepatotoxic in animals; aflatoxin B<sub>1</sub> is the most potent mutagenic and carcinogenic metabolite known and ranked as Class 1 human carcinogen (IARC, 2002). Mycotoxins



are associated with many chronic health risks, including the induction of cancer, immune suppression and blood and nerve defects (Shephard, 2006).

This study examined sweetpotato handling practices from production, preparation of chips, drying, and storage. The study involved a survey on sweetpotato farmers and laboratory analysis of samples of sun-dried sweetpotato chips collected from the farmers to determine beta-carotene levels, and assess fungal, aflatoxin and ochratoxin contamination levels.

## **1.2 Statement of the Problem**

Considering seasonal production patterns and high perishability of sweetpotatoes, their effective use as food depends on proper preservation (Hagenimana, 1999). The sweetpotato harvesting methods and tools have considerable effect on storage life (MoA, 2010) since the tubers are very easily damaged and are very susceptible to fungal rots.

The traditional methods of peeling, chipping, and drying are long and unhygienic, exposing the sweetpotato chips to possible contamination by insects, rodents and microorganisms (Kihurani, 1994). At home level, sweetpotato chips are sun-dried in the open (Woolfe, 1992). The chips are spread out in the sun on a mat or any surface and dried until they become brittle. Drying may require between one to five days depending on the weather (Marina, 1991). Most important storage problems of sweetpotato tubers in Kenya are caused by moulds such as *Botrytis cinerea* and species of *Penicillium* and rots caused by *Rhizopus stolonifer*, *Fusarium oxysporum*, species of *Mucor* and *Aspergillus* (Kihurani, 1994). Some moulds produce antibiotics and mycotoxins. It is also important

that lack of adequate post-harvest processing of the sweetpotato tubers cause losses to farmers during the glut season due to the perishable nature of the crop (MoA, 2010).

### **1.3 Objectives of the Study**

The broad objective of the study was to promote food safety and food security through identification of practices that predispose sweetpotatoes to fungal infection with the relevant mycotoxin contamination

The specific objectives were:

- i. To determine post-harvest handling practices on sweetpotatoes by farmers.
- ii. To assess levels of beta-carotene content, moisture content and water activity in samples of farmer processed sun dried orange fleshed sweetpotato chips.
- iii. To determine levels of fungal and mycotoxins contamination in farmer processed samples of sun dried orange fleshed sweetpotato chips collected from the farmers.

### **1.4 Justification of the Study**

The availability of safe food improves the health of people and is a basic human right (UNHCR, 2005). Safe food contributes to health and productivity and provides an effective platform for development and poverty alleviation (WHO, 2002). In Kenya, outbreaks of mycotoxicoses have been reported since 1978 when it was reported that a large number of dogs and poultry had died of aflatoxicoses (Manwiller, 1987). Feeds examined then showed very high aflatoxin levels ranging from 150 - 3000 ng/g compared to allowable aflatoxin levels of 20 ng/g in Kenya at that time. In 1981, twelve people were reported to have died in Eastern Province after consuming aflatoxin contaminated

grain (Ngindu, et. al., 1982 and KEPHIS, 2006). In April 2004, one of the most severe outbreaks of acute aflatoxicosis was reported in which 125 people died (Centre for Disease Control and Prevention CDC, 2004; Nyikal et al., 2004 and Lewis et. al., 2005). In 2005, another outbreak claimed 16 lives in Eastern Province (KEPHIS, 2006). Since then there has been yearly recurrent outbreaks of aflatoxicoses in Kenya which raises concern about factors responsible for these outbreaks.

Documentation of outbreaks of acute aflatoxicoses from highly contaminated food has been done in Kenya, India and Thailand (CAST, 2003 and Lewis et. al., 2005). It is now well established that mycotoxins have been responsible for major epidemics in man and animals at least during recent times (Fung and Clark, 2004). Mycotoxins have been caused by growth of specific moulds which produce one or more potent toxins, usually in one specific kind of commodity or feed. The presence of mycotoxins other than aflatoxins has also been demonstrated in breakfast cereals and infant cereals (Candlish et al., 2000; Biffi et al., 2004; Araguas et al., 2005; Molini et al., 2005). Mycotoxins are important in food security and safety considering that the fungi responsible for their contamination in foods are widely distributed in air, soil and crop residues (Diener et. al., 1987). Mycotoxins can be acutely or chronically toxic, or both, depending on the kind of toxin and the dose. In animals and humans, acute diseases include liver and kidney damage, attack on the central nervous system, skin disorders and hormonal effects (Peraica, et al., 1999). Therefore, efforts to reduce fungal contamination and toxin development to enhance food security and safety of food commodities and feedstuff need attention.

Focus on post-harvest handling practices of food commodities is justifiable especially in rural areas to reduce ingestion of aflatoxins and other toxins. In view of the growing importance for food safety standards and growing need for food security, the study is necessary to reveal the nature of post-harvest practices, levels of beta carotene, fungi, aflatoxin and ochratoxin in farm prepared sun-dried sweetpotato chips. The findings will be of use to farmers and researchers in promoting food security and safety especially as relating to sweetpotatoes.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 Importance of Sweetpotatoes as Human Food**

Sweetpotato is a promising food security crop in the face of high human population growth, modest absolute income levels for large segments of consumers, climate change, and declining farm size (MoA, 2010). Sweetpotato roots are a rich source of carbohydrates in the form of starch and sugars. They also contain vitamins and minerals. Leaves and tender shoots are used as a cooked green vegetable (Woolfe, 1992). Yellow and orange-fleshed cultivars contain much higher amount of beta-carotene than the white-fleshed cultivars (Hagenimana et al., 1999, Woolfe, 1992). Sweetpotatoes are also a good source of manganese, copper, biotin, pantothenic acid and storage proteins. Sweetpotato yields can be high in soils with low pH. It produces more edible energy, protein and dry matter on a per hectare per day basis than any other root crop (Norman et al., 1995).

Orange fleshed sweetpotato cultivars have been widely promoted for growth and utilization in Western province of Kenya, a region which experiences vitamin A deficiency (GTZ and Ministry of Agriculture, 1998). This activity offers a simple, sustainable solution to a major public health problem, as the sweetpotatoes are easy and cheap to grow. Stimulation of production through greater demands by end-users will not only increase the availability of these roots, but will also empower economically the rural women who produce most of the sweetpotatoes.

## **2.2 Sweetpotato Products and Utilization**

Products developed from sweetpotatoes include dried chips, pellets, flour, starch, bread, cookies, biscuits, chinchin, pastries, crisps, soups and composite flours (GTZ and Ministry of Agriculture, 1998). The flour can be used as a partial substitute for wheat (Martin, 1984). In addition, orange fleshed sweetpotatoes have been used for small scale processing of juice and other products (Woolfe, 1992). Exploitation of roots and tuber crops for production of these products still face challenges that require interventions (MoA, 2010).

Value added processing is one of the most practical and sustainable means of increasing commercialization of roots and tuber crops, one of them being sweetpotatoes. Value addition will increase the market worth of tubers, improve producer prices, stimulate increased farm production, increase utilization and achieve sustained rural livelihood improvements (MoA, 2010). Processing is done at various levels of the value chain and it changes form, adds value, extends shelf life and diversifies ways in which the roots and tuber crop are utilized (Woolfe, 1992).

## **2.3 Importance of Sweetpotatoes as Source of Beta-carotene**

Sweetpotato (*Ipomoea batatas*) tubers are one of the major food sources of carotenoids (Henkel, 1996; Woolfe, 1992). The color intensity of the root flesh differs from one cultivar to another, and varies from white to deep orange. The intensity of the orange color is attributed to carotenoid content (Ameny and Wilson, 1997). Carotenoids act as vitamin A precursors. When consumed, they are enzymatically transformed into retinol (vitamin A) (Simon, 1997). The orange and yellow-fleshed sweetpotato varieties

are good sources of beta-carotene (300micrograms/100 grams, fresh weight) (Woolfe, 1992). The total carotenoid content of sweetpotatoes can range from less than 100µg to more than 16,000µg per 100g (Ottaway, 1993). The vitamin A activity of beta carotene is substantially greater than that of other carotenoids (Almeida and Penteado, 1988).

The high Vitamin A content of many of the sweetpotato varieties is viewed as important by nutritionists, not only for its role in child health and development, but also because of the high incidence of households with HIV-positive members in some parts of rural Kenya (MoA, 2010). Consumption of orange-fleshed sweetpotato roots and sweetpotato based processed foods could provide sustainable, cost effective and much needed vitamin A. However, Chandler and Schwartz (1988) found that carotene content was altered by processing of sweetpotatoes, the change being dependent on the treatment employed.

Excellent food sources of beta carotene include orange-fleshed sweetpotatoes, carrots, kale, spinach, broccoli, cayenne pepper, tomatoes, and other yellow and green fruits and vegetables. Plant sources of vitamin A provide 70-90% of total vitamin A intake in the developing world (FAO/WHO, 1988). Vitamin A is essential for vision, growth, cellular differentiation and proliferation, reproduction and the integrity of the immune system and is needed on daily basis (Ottaway, 1993).

Studies have shown that diets high in carotenoids are often associated with a reduced risk of certain cancers; this protection is however seen only if the source of the beta-carotene is a food and not the supplement (Codjia, 2001; Delisle et al., 2003; FAO, 1997). Vitamin A deficiency is as a result of habitually low consumption of vitamin A in relation to requirements (Tontisirin *et al.*, 2002). A diet adequate in vitamin A reduces

risk of respiratory and gastrointestinal infections (WHO/FAO, 2004). Beta-carotene is sensitive to oxidation, which is accelerated by light and metallic catalysts particularly copper, iron and manganese. Sodium sulphide can be added to reduce the oxidative effects of metal ions during processing of foods rich in beta-carotenes (Ottaway, 1993).

## **2.4 Fungal and Mycotoxins Contamination in Foods**

Some moulds have the capacity to synthesize, during their growth period, chemical substances that are poisonous or produce toxic symptoms of various kinds when foods or feed containing them are eaten by humans or animals (CAST, 2003). These chemicals are generically referred to as mycotoxins (Lewis, et al, 2005), and the toxicity syndromes produced as mycotoxicoses. In addition to being acutely toxic, some mycotoxins are also carcinogenic in man and livestock and this has generated great global concern over food and feed safety (Fung and Clark 2004). Mycotoxins are produced by fungi growing on numerous commodities such as cereals, nuts, soybeans and other food substances. Moulds in addition to visible spoilage, can spoil foods through the formation of mycotoxins (Vedman, 2004).

The fungi that produce mycotoxins are widely distributed; are not specialized in their parasitism and become prevalent when environmental conditions favour their development (CAST, 2003). Mycotoxicoses epidemics have occurred in the populations weakened by war, stress, starvation and malnutrition (Mountney, 1988). Mycotoxins disrupt cell membranes and interfere with the synthesis of protein, RNA and DNA. Toxigenic moulds vary in their mycotoxin production depending on the substrate,



environmental factors (temperature, relative humidity, light, presence of oxygen and carbon dioxide), and seasonal and life cycle stages (CAST, 2003).

Mycotoxins remain in the food long after the mould that produced them has died, and can therefore be present in foods that are not visibly moldy (Fung and Clark 2004). Many types of mycotoxins are relatively stable substances that can survive the usual conditions of cooking or processing. Problems can also be created if livestock feed becomes contaminated by mycotoxins (Fung and Clark 2004). In addition to the losses generated by toxicity syndromes in animals, mycotoxins or their metabolic products can remain as residues in meat or be passed into milk or eggs and thus eventually be consumed by humans. Agronomic practices and insects play a major role in exacerbation of mycotoxin contamination (Bilgrami and Choudhary, 1998). Aflatoxins, fumonisins and deoxynivalenol (DON = vomitoxin) are amongst the mycotoxins that are most economically and toxicologically important worldwide (Chelkowski, 1998).

Fungal toxins have economic importance due to the effects they have on human and livestock health. In Kenya, between May and July 2004, 125 deaths were reported in Eastern Province in Makueni and Kitui districts (Nyikal et al., 2004). Long term exposures to some mycotoxins contribute to development of various cancers. Aflatoxins target the liver and result to liver cancer in both animals and humans (Toner, 1999). Epidemiological studies have reported a correlation between forms of esophageal cancer and consumption of cereals contaminated by *Fusarium moniliforme* (Landi, 1997). Another effect of mycotoxins is the impairment of the immune system in humans and livestock (Pestka and Bondy, 1994). Immunosuppression due to mycotoxins contamination is a major health issue due to the problem of HIV/AIDS in humans.

According to Prelusky (1994), mycotoxins are also a barrier to international trade as food commodities with higher levels of mycotoxins than the allowable set limits cannot be traded across national borders. Many countries have set regulatory limits on mycotoxins in order to control levels of mycotoxins in food commodities and therefore restrict human exposure to mycotoxins. In Kenya, the Kenya Bureau of Standards (KEBS) has imposed maximum tolerated levels for aflatoxin B<sub>1</sub> to 10ppb in cereal products, groundnuts and vegetable oil while tolerance levels for the other mycotoxins are yet to be established in Kenya (USAID, 2012).

Worldwide there is increasing demand for high-quality and safe food, free of chemical and physical contaminants and pathogens. Food growers and users must maintain and protect their harvested produce from insect and microbial damage (Sinha, 1995). In tropical developing countries a large proportion of the crop is harvested under humid and warm climatic conditions and most small scale farmers lack equipment for drying grains (Mendoza et al., 1982). Consequently the crop is stored while still relatively moist and warm, which results in rapid deterioration of the grains, mainly because of growth of molds. Even when the crop can be sun dried after harvest, exposure to high relative humidity during open storage may result in resumption of moisture uptake by the stored grain, with resulting enhanced deterioration (Landers and Davis, 1986). Post-harvest losses of food grains, caused by insect infestation and mold activity, have been conservatively estimated at 10–15% (Grolleaud, 2002). Molds growing on foods present a second threat, through production of mycotoxins, the secondary metabolites produced by fungi that grow on a wide range of agricultural commodities including cereals and oilseeds. Mycotoxins pose a serious health risk to both humans and animals (Vedman,

2004). Therefore, harvested grains should be dried to safe moisture levels that inhibit the activity of microorganisms (Weinberg, 2008).

The need for a meaningful term to describe the behaviour of microorganisms in environments with reduced moisture helped to establish the term water activity (Breene et al., 1988). Water activity has marked effect on the growth of microorganisms. Reducing water activity below 0.7 prevents microbial spoilage. Although, the food would not spoil from microorganisms, other deteriorative reactions can still occur. Higher water activity substances tend to support more microorganisms. For instance, bacteria usually require at least 0.91 and fungi at least 0.7 to grow (Ratkowsky, 1982). Microorganisms have different minimum levels of water activity for growth. Bacteria are generally the most sensitive and nearly all are inhibited at a water activity of less than 0.90-0.91. Molds and yeasts are more tolerant of high levels of solutes in water and therefore lower water activity. Their minima for growth are 0.70-0.80 and 0.87-0.94, respectively. A water activity of 0.60 or lower will prevent growth of all microorganisms (Ratkowsky, 1982). To successfully preserve a food product, water activity would have to be lowered to a range where the rate of deteriorative reactions is minimized.

The overall effects of contaminating factors will determine the quality of the food, its probable shelf life, and the potential public health risks. Processing of sweetpotatoes into various products adds value at the farm level and reduces perishability and bulkiness, thereby facilitating sale of root based products in the off-season and in distant markets. Processing can also help improve food security by generating employment and income for non-growers thereby enhancing their purchasing power to gain more ready access to food (MoA, 2010). All foods that undergo processing are subject to some loss in vitamin

and mineral content. Rate of vitamin A loss is a function of enzymes, water activity, storage atmosphere and temperature. Therefore, the expected loss will depend on the severity of the drying conditions and the degree of protection during storage. Losses of total carotenoids are mainly due to oxidative degradation promoted by light, heat, metals and peroxides (Woolfe, 1992).

The traditional method of sun drying is long and unhygienic and exposes the food to the possible contamination by insects, rodents and microbes. Boiling the roots reduced total carotenoid by 20%, whereas drying roots at 65% for twelve hours reduced total carotenoid content by 30%. Storing dried chips for eleven months reduced total carotenoid content an additional 11%, from 70% to 59% (Hagenimana et al., 1999). Overall losses in carotene may vary from 5 – 40% depending on temperature, time and nature of carotenoids. Drying sweetpotato pieces at temperatures below 50°C to 70°C were found to produce acceptable products; however, lower temperatures resulted in microbiological contamination (Bouwkamp, 1985).

A study carried out to assess post-harvest losses of sweetpotatoes caused by microorganisms, weevils and mechanical damages in three markets in Nairobi (Wakulima, Kangemi and Kawangware) by Kihurani et al. (1994) established that deterioration of sweetpotatoes starts soon after harvest and progresses throughout storage, transportation and at the markets. This study revealed that moldiness caused by *Botrytis cinerea* and species of *Penicillium* and rots caused by *Rhizopus stolonifer*, *Fusarium oxysporum*, species of *Mucor* and species of *Aspergillus* are the most important storage problems of fresh sweetpotato tubers in Kenya.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Materials**

The materials used in this study included orange fleshed sun dried sweetpotato chips; filter papers; aluminium foil and hand gloves.

The following reagents were among those used in this study Barium chloride; Potato Dextrose Agar; Methanol (analytical grade); Ethanol 96% volume per volume (V/V) molecular weight (M. W.) 40.07; Activated silica gel 60 – 120 Mesh made by Rankem; Sulphuric acid; Acetone GPR M. W. 58.08; Sodium chloride; Sodium hypochlorite; Aluminium oxide; Petroleum ether and Tween 20.

The equipment and tools used in the laboratory analysis were deep freezer; water activity meter, Ourotherm made in Germany; Rotary evaporator; oven Salvis model; Aluminium dishes; mortar mvortex; spectrophotometer CE 4400/ UV VIS double beam scanning; weighing balance Mentor model made in China; chromatographic tubes (30 cm x 12 mm i. d.); polystyrene microplates, Maxisorp® and ELISA reader Model Uniskan 11, type 364, Labsystems, Finland.

#### **3.2 Methods**

##### **3.2.1 Study Design**

The design employed was observational study which involved survey on post-harvest handling practices of sweetpotato farmers in Tiriki West Division Vihiga County, and laboratory examination of samples of sun-dried orange fleshed sweetpotato chips collected from the farmers during the survey. The laboratory analysis was to assess levels

of beta carotene, fungal, and aflatoxin and ochratoxin A contamination levels, in the samples of the sun-dried orange fleshed sweetpotato chips.

### **3.2.2 The Study Area**

This study was conducted in Tiriki West Division, in the former Vihiga District, currently Vihiga County Western Province. Vihiga County borders Nandi South and Nandi North Sub-counties to the East, Siaya County to the Southwest, Kisumu County to the South and Kakamega County to the North. It lies between longitude  $34^{\circ} 30'$  East and  $35^{\circ} 0'$  East and between  $0^{\circ}$  and  $0^{\circ} 15'$  North (Jaetzold et. al., 2006). The population of the district is 554,622 people (KNBS, 2009). The County has an altitude range of 1300 metres and 1500 metres above sea level with an annual rainfall range of 1800 millimetres to 2000 millimetres, and temperature range of  $14^{\circ}\text{C}$  to  $32^{\circ}\text{C}$  (Jaetzold et. al., 2006). Over 80,000 households are engaged in small-scale farming, growing food crops such as maize, beans, pawpaws, sweetpotatoes, cassava, cash crops such as coffee, tea, and avocado, and keeping livestock such as dairy cows, goats and poultry. Sweetpotato contributes directly to the diet and income of more than 25,000 households in the district.

### **3.2.3 Sample Size Determination**

The sampling frame comprised of all sweetpotato farmers in Tiriki West Division Vihiga County. A list of the farmers growing sweetpotatoes at that time was obtained from the Ministry of Agriculture. The sample population of 138 was obtained by applying Fisher et al. (1991) formula. Where:  $n = z^2 pq / d^2$

$n$  = The desired sample size (when population is  $>10,000$ )

$z = 1.96$  (confidence level at 95%)

$p$  = Proportion in target population estimated to have a particular characteristic i. e farmers growing sweetpotatoes (0.1)

$q = 1-p$  (0.9)

$d$  = Level of precision at 5% (standard value of 0.05)

However, 87 participated in the actual survey study hence formed the sample subjects.

### **3.2.4 Sampling Procedure**

Vihiga County and Tiriki West Division of that County were chosen purposively because they were one of the highest producers of sweetpotatoes in Western Province at the time of the study. Purposive sampling technique was used to obtain the participants in the survey who were sweetpotatoes growers at the time of the interview. Out of the sample size of 138, fifty one could not be available or be reached on the day of the interview. A total of eighty seven participants were therefore interviewed in the study and were reached through purposive sampling.

### **3.2.5 Data Collection Tool and Process**

A semi structured questionnaire, see Appendix 1, was used for data collection in the survey. The questionnaire was developed, pre-tested and improved on before being administered. The method used was face to face interview. The questionnaire included the following sections: demographic profile and wealth status of the participants, farming characteristics, post-harvest features and marketing characteristics of sweetpotato tubers and products

Three research assistants who had completed secondary school and understood the local language, were identified and recruited. The assistants attended a three day training conducted by the principal investigator. The assistants were taken through the study objectives, the study sample, administration of questionnaire, interviewing technique, observance of ethics, interpersonal skills and what is expected of them during the work. The assistants were also trained on principles of ethics like maintaining confidentiality and right to privacy.

Ten sweetpotato farmers from Tiriki East Division, Vihiga County were selected and interviewed using the questionnaire for purposes of pre-testing the questionnaire. They represented the population of farmers in Tiriki West Division Vihiga County. The questionnaires used for pre-testing were analyzed before being administered in order to verify validity but were not included in the final analysis.

For the laboratory analysis work, thirty samples of sun-dried orange fleshed sweetpotato chips of the variety of SPK 004 were collected from the eighty seven farmers. The requirement was that all the interviewed farmers should provide the chips. However, only thirty of them could provide the chips at the time of the interview.

### **3.2.6 Preparation of Samples of Sun-dried Sweetpotato Chips**

In the laboratory analysis, thirty samples of sun-dried orange fleshed sweetpotato chips were collected from the farmers during the survey. Each of the samples weighed about half a kilogram. The samples were each placed and packed in brown paper bags and taken to the Food Science, Nutrition and Technology laboratory of the University of



Nairobi, Kabete Campus, within twenty four hours. In the laboratory, each sample was thoroughly mixed and the following sub samples taken from each:

- i. About one hundred grams for mycological examination which were stored at  $-15^{\circ}\text{C}$  before being plated onto suitable media 1-2 weeks after collection
- ii. About twenty grams from each sample were taken for analysis of beta-carotene.
- iii. Four grams from each sample were removed and tested in duplicate for moisture content.
- iv. Two grams from each sample were removed and tested in duplicate for water activity.
- v. The rest of the samples were put in sealed polythene bags and stored in a deep freezer at  $-15^{\circ}\text{C}$  for mycotoxin analysis.

### **3.2.7 Determination of Beta-Carotene Levels in the Sun-dried Sweetpotato Chips**

Determination of beta-carotene levels was done on each sample of the chips using the column chromatography technique as described by Imungi and Wabule (1990) and International Federation of Fruit Juice Producers – IFFJP (1985). Two grams of each sample were weighed and color extracted from the samples using a mortar and pestle with small portions of acetone until the residue became colourless. The extract was placed in a 50 ml round bottomed flask and evaporated to dryness in a rotary evaporator at about  $65^{\circ}\text{C}$  for 5 – 7 minutes. To the evaporated extract one millimeter of petroleum ether was added so as to dissolve the beta carotene. The beta-carotene was eluted through a packed chromatography column. A 15 cm column was prepared by packing slurry of activated silica gel and petroleum spirit into the column held in position by glass wool.

One gram of anhydrous sodium sulphate was placed at the top of the column in order to absorb any water that could be in the sample and also separated beta-carotene from other carotenoids that could have been in the samples. The elute was received into a 25 ml volumetric flask and the absorbance read at 450 nm on a double beam scanning spectrophotometer. Beta-carotene was calculated from the beta-carotene standard curve.

### **3.2.8 Determination of Moisture Content in the Sun-dried Sweetpotato Chips**

Determination of moisture content was done using the oven method. Two to three grams of each sample was weighed out in a covered aluminium dish and dried to constant weight at 70° C. in an oven fitted with controlled ventilation. The moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Weight fresh sample} - \text{Weight dry sample}}{\text{Weight fresh sample}} \times 100$$

### **3.2.9 Determination of Water Activity in the Sun-Dried Sweetpotato Chips**

Water activity was determined using the water activity meter model Ourotherm, Germany, which had been calibrated and standardized to work at 25<sup>0</sup> C. White filter papers were placed in the two chambers of the water activity meter. The filters were then completely wetted with saturated barium chloride. The barium chloride was used to standardize the meter. The wetted filter papers were left for three hours, afterwards they were removed and the meter wiped clean using a clean filter paper. The meter was set to read at 0.9 using the knob on the chambers. Two grams in duplicate for each sample were placed in the two chambers of the meter which was covered and left for three hours. The

water activity for each sample was recorded and converted by adding + 0.010 to each of the reading.

#### **3.2.10 Procedure for Fungal Isolation and Identification**

Fungi were isolated using the Agar Plate Method. The sun-dried sweetpotato chips were surface dried by immersing in 5% sodium hypochlorite for two to three minutes and then rinsed in two changes of sterile distilled water. Eight pieces of sun-dried sweetpotato chips were placed in each plate containing potato dextrose agar (PDA) medium for isolation of fungal species. Ten plates were used per sample. All plates were incubated at room temperature ( $23^{\circ}\text{C} \pm 2$ ) for four to five days and observed daily for fungal growth. Identification of the fungi was by colony characteristics and conidial morphology. Observations were made on colour, shape and form of each colony and number of infected chips, total number of fungal colonies and different fungal isolates recorded. Identification keys in books, manuals and other publications were used (Christensen 1981; Nelson et al., 1983; Von Arx 1987; Larone 1995). Fungal isolates were identified to species level for *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus ochraceous*. Other fungal pathogens were identified up to genus level.

#### **3.2.11 Procedure for Sample Extraction and Determination of Aflatoxin Levels**

The sun-dried orange fleshed sweetpotato chips were analyzed for aflatoxin using enzyme-linked immunosorbent assay (ELISA) techniques shown in Appendix 4 according to Gathumbi (2001) and results obtained from plotting graph of the Aflatoxin standard curve (Appendix 2). Each sample was ground finely into powder using a

laboratory blender. Five grams of each of the ground sample were weighed and put into a 50 ml tube. Twenty-five ml of methanol+H<sub>2</sub>O (55:45) were added and the mixture stirred for thirty minutes on a magnetic stirrer. Centrifugation of the mixture was done at 1500 g for 10 minutes and the supernatant recovered. A three millimeter portion of the supernatant was then defatted by homogenization with equal volume of hexane on a vortex mixer, followed by a five minute centrifugation at 1500 g. The lower methanolic layer was then recovered. The methanolic extract was diluted 1:5 in phosphate buffered saline (PBS) and again at 1:4 in methanol: PBS (1:9) before analysis by ELISA. The dilutions were obtained by diluting 200 µl of the sample extract with 800 µl of PBS and 3 ml of 10% methanol-PBS.

### **3.2.12 Procedure for Sample Extraction and Determination of Ochratoxin A Levels**

The sun-dried orange fleshed sweetpotato chips were analyzed for Ochratoxin A using enzyme-linked immunosorbent assay (ELISA) techniques shown in Appendix 4 according to Gathumbi (2001) and results obtained from plotting graph of the Ochratoxin standard curve (Appendix 3). Each sample was ground finely into powder using a laboratory blender. Five millimetres of 1 M HCl was added to two grams of each of the ground sample. The mixture was stirred for five minutes on a magnetic stirrer. This was followed by addition of ten millimetres dichloromethane and the mixture stirred for a further fifteen minutes. Centrifugation of the mixture at 1500 g for fifteen minutes followed and the lower dichloromethane layer recovered. To this recovered layer, ten millimetre of 0.13 M NaHCO<sub>3</sub> solution was added and the resultant solution mixed at high speed for fifteen minutes. Again the mixture was centrifuged at 1500 g for fifteen

minutes and the aqueous layer recovered. Two 1:3 dilutions of the aqueous extract in 0.13 M NaHCO<sub>3</sub> were prepared for ELISA analysis.

### **3.2.13 ELISA Procedure for Determining Aflatoxin and Ochratoxin Levels**

Direct competitive ELISA tests were performed as described by Gathumbi (2001). Polystyrene micro plates (Maxisorp®) were coated with specific antibodies, ELISA buffers described in Appendix 5 and micro plate titration format shown in Appendix 6. Mycotoxin standards, sample extracts and mycotoxin conjugate solutions were added simultaneously. This was followed by a two hour incubation period. The plates were then washed and an enzyme substrate solution added. The plates were further incubated for ten to fifteen minutes. The enzyme substrate reaction was stopped by adding 100 µl 1 ml/L H<sub>2</sub> SO<sub>4</sub> and the resultant absorbance read. Test results were deduced from the measured absorbance.

For analysis of aflatoxin, coating was done by adding 100 µl of antiserum Anti-AFB1 K147 at dilution 1:20,000 in bicarbonate buffer to each micro plate well and then incubating the plates for one and half hours at room temperature in a humid place. The plates were then emptied and blocking of free protein binding sites done by addition of 200 µl of 3% fetal calf serum (FCS) in PBS for twenty minutes. This was followed by washing in PBS-Tween and semi drying of the plates. Fifty microlitres of aflatoxin standard (four wells per standard dilution) and fifty microlitres of the sample extract dilution (two wells per sample) were pipetted onto the wells according to the protocol described in Appendix 6. At the same time, fifty microlitres of an AFBI – HRPO conjugate at a dilution of 1:400,000 in 1% FCS/PBS were pipetted to all wells. After two

hour incubation period, the micro plates were washed. One hundred microlitres of an enzyme substrate solution consisting of  $\text{H}_2\text{O}_2$  and tetramethylbenzidine (TMB) were added to the plates and incubated for twenty minutes. One hundred microlitres of one ml/L  $\text{H}_2\text{SO}_4$  were added to stop enzyme reaction. This was followed by reading of absorbance at  $A_{450}$  using the ELISA reader (Model Uniskan 11, type 364, Labsystems, Finland). The absorbance of the standards and samples were converted to percent inhibition by dividing the absorbance of the particular standard or sample dilution by the absorbance of the blank ( $B/B_0 \times 100$ ), where B is the absorbance of a particular standard or sample dilution and  $B_0$  is the absorbance of the blank. A standard curve was drawn using a cubic spline function with the concentration (pg / ml) of the standards on x-axis and the percent inhibition ( $B/B_0$ ) on y-axis. Appendix 2 for aflatoxin standard curve and Appendix 3 for ochratoxin A standard curve. Mycotoxin concentration in the sample extract dilution was deduced from the standard curves.

For analysis of ochratoxin A, the above immunoassay procedure was followed with the exception that coating was done by adding one hundred microlitres of antiserum Anti-OA V pool at dilution 1:16,000 in bicarbonate buffer to each micro plate well. No blocking was done for ochratoxin.

### **3.2.14 Data Management and Analysis**

Data for the study were entered, cleaned, and analyzed using the Statistical Package for Social Sciences (SPSS) Version 12 and Excel spreadsheet. Data analysis was done by interpreting and assessing the information and results. Descriptive analysis involved statistics such as mean, frequencies, charts and tables. Total fungi counts were

expressed as percentages. Beta-carotene content was expressed in milligram per 100 g of sweetpotato chips on dry weight basis. Aflatoxin and ochratoxin A contamination was expressed in parts per billion. Correlation analysis was performed to determine the type of relationship among variables such as storage length, fungi occurrence, aflatoxin and ochratoxin A level, moisture content, water activity and beta carotene level in the sun-dried orange fleshed sweetpotato samples.

## CHAPTER FOUR

### RESULTS

#### 4.1 Demographic Characteristics of the Sweetpotato Farmers

A total of eighty seven farmers participated in the survey on post-harvest practices of sweetpotatoes in Tiriki West Division, Vihiga County. The ages of the participants ranged from twenty to sixty five years old. The demographic characteristics of the participants shown in Table 4.1 captured gender, education level and main source of income.

**Table 4.1: Demographic characteristics of sweetpotato farmers**

Characteristic	N=87	Percent
<b>Gender of interviewee</b>		
Male	26	29.9
Female	61	70.1
<b>Education level</b>		
Primary School and below	32	36.8
Secondary school	37	42.5
Secondary school	18	20.7
<b>Source of income</b>		
Farming/business	81	93.1
Formal employment	6	6.9

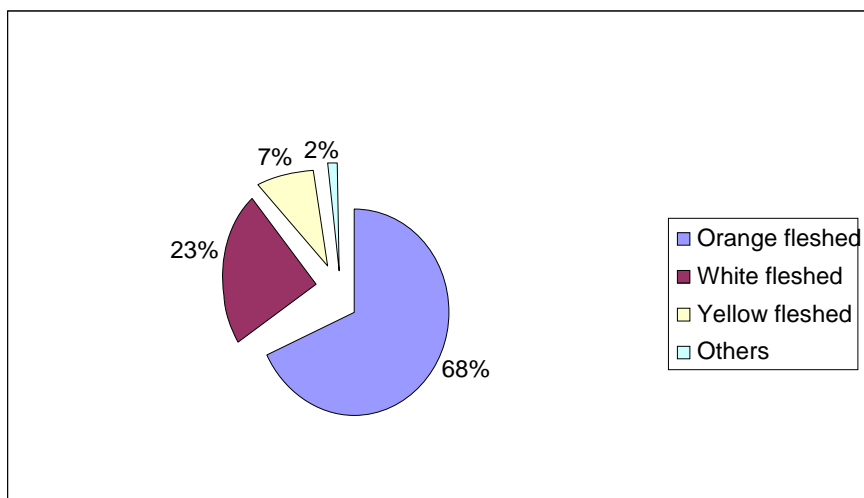
Most of the farmers interviewed were women at 70.1%. Most farmers had attained secondary school level education at 42.5%. It was also noted that most farmers interviewed depended on farming as a main source of income at 93.1% by selling farm produce. Most farmers at 20.7% farmed on one acre pieces of land in the study area. The distribution of respondents by farm size is shown in Table 4.2



**Table 4.2: Distribution of respondents by farm size**

<b>Farm size (acres)</b>	<b>N=87</b>	<b>Percentage</b>
0.25	10	11.5
0.50	17	19.5
0.75	6	6.9
1.00	18	20.7
1.25	4	4.6
1.50	10	11.5
2.00	16	18.4
2.50	6	6.9

Figure 4.1 below shows the varieties of the sweetpotatoes that were grown by the farmers in the study area. Majority of farmers, at sixty eight percent, had planted orange-fleshed sweetpotato varieties. Among the orange-fleshed sweetpotato varieties planted were SPK 004 shown in Appendix 7A at the field stage and Appendix 7B shows the tubers. The other orange fleshed varieties were *Zapalo* and *Salyboro*. Other varieties grown were yellow fleshed, white fleshed or purple fleshed. The yellow fleshed variety was KEMB10 and the white fleshed variety was *Mugande*.



**Figure 4.1: Varieties of sweetpotatoes grown by farmers**

## **4.2 Post Harvest Handling Practices on Sun-dried Orange Fleshed Sweetpotato**

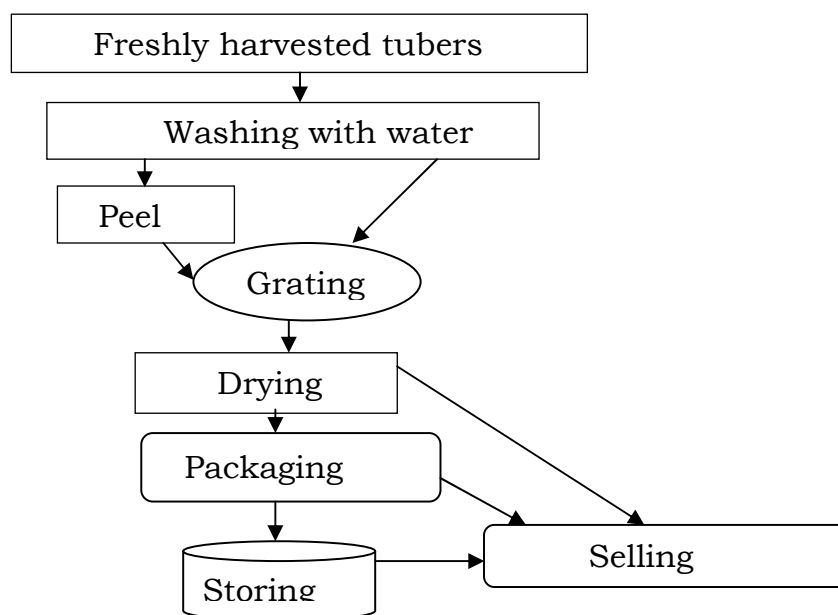
### **Chips**

The study examined the post-harvest practices of sweetpotato farmers. Some of the practices are shown in Table 4.3 and included gender of the person who harvested the sweetpotatoes, constraints in storage of tubers and method of storage of tubers. Most of those who did the harvesting of the sweetpotatoes were women at 97.7%. Rotting (81.6%) was the biggest constraint encountered by the farmers during storage of the tubers.

Some of the farmers made and dried pieces of grated sweetpotatoes known as chips which are shown in Appendix 7C. The preparation of the sweetpotato chips by farmers was done according to Figure 4.2.

**Table 4.3: Post-harvest characteristics of the sweetpotato farmers**

<b>Characteristic</b>	<b>N=87</b>	<b>Percent</b>
<b>Gender of the processor</b>		
Male	2	2.3
Female	85	97.7
<b>Method of storage</b>		
In ground	9	10.3
Process and dry	30	34.5
Does not know how to store	14	16.1
Do not store	34	39.1
<b>Constraints in storage of tubers</b>		
No storage standards	2	2.3
Rotting	71	81.6
Pest infestation	6	6.9
Others	8	9.2

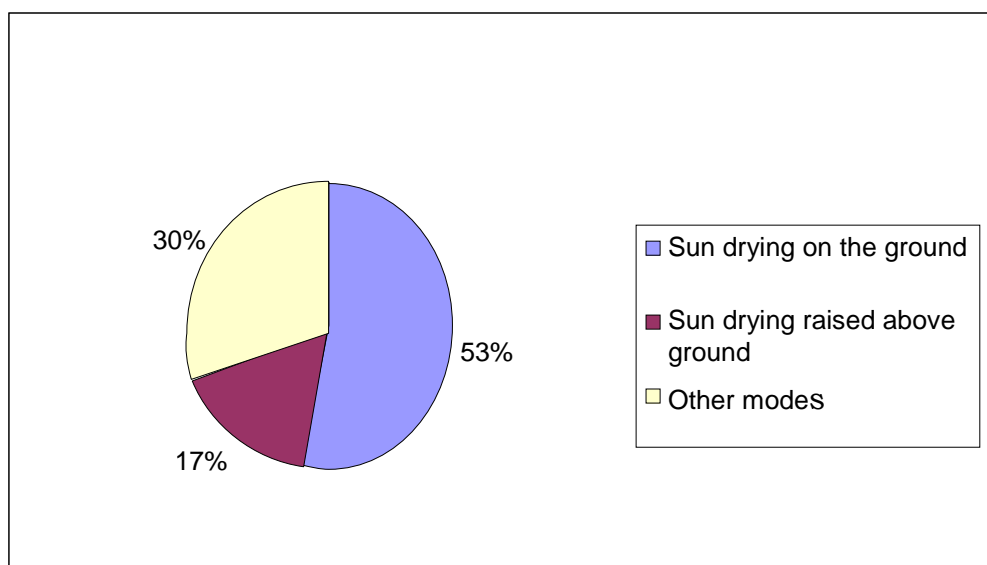


**Figure 4.2: Processing of tubers into sweetpotato chips**

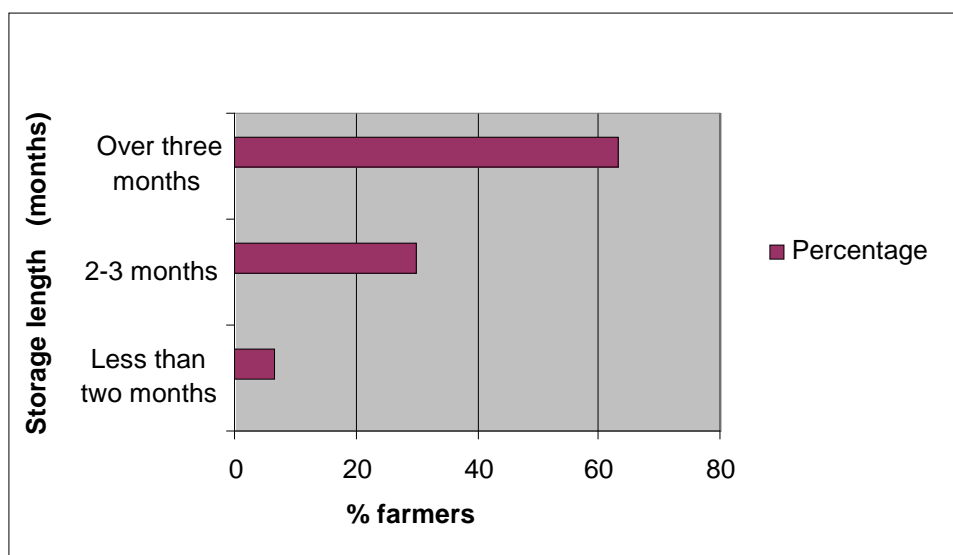
Further examination of post-harvest features was done concerning those farmers who processed the tubers into chips. This is captured in Table 4.3. Out of the eighty seven farmers interviewed, thirty of them processed the tubers into chips, this being thirty four percent of those who grew the sweetpotatoes. Majority (53.3%) of those who made chips dried them in open sunlight and spread the chips on some materials on the ground. The chips were then packaged in propylene bags as shown in Appendix 7D. Other packaging materials are indicated in Table 4.4.

**Table 4.4: Practices in preparation of sweetpotato chips by farmers**

<b>Characteristic</b>	<b>N=30</b>	<b>Percent</b>
<b>Tools for making the chips</b>		
Traditional, locally available	24	80.0
Improved, locally available	5	16.7
Modern, fabricated	1	3.3
<b>Duration for drying the chips</b>		
Less than 2 hours	1	3.30
2-8 hours (1 day)	9	30.0
2-3 days	11	36.7
Over 3 days	9	30.0
<b>Packaging of chips</b>		
Polythene bags/paper bags	26	86.7
Jute bags	1	3.3
Crates/woven baskets	2	6.7
Others	1	3.3
<b>Challenges in storing the chips</b>		
Lack of standards	3	10.0
Rotting/moulds	5	16.7
Pest infestation	21	70.0
Others	1	3.3



**Figure 4.3: Method of drying sweetpotato chips by farmers**

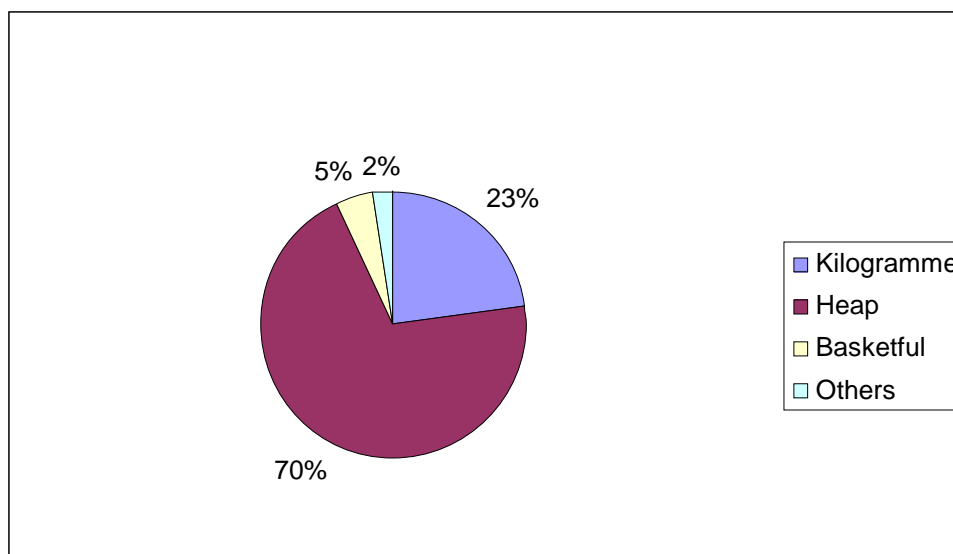


**Figure 4.4: Storage length for dry chips**

Marketing characteristics such as proximity to nearest town, mode of transport for the tubers and unit of sale were also assessed. Details are shown in Table 4.5 and Figure 4.5. Farmers sold most of their harvested tubers at the nearest town centres of Gambogi and Kiboswa.

**Table 4.5: Marketing features of the sweetpotato farmers**

<b>Characteristic</b>	<b>N=87</b>	<b>Percent</b>
<b>Proximity to nearest town</b>		
Less than 0.25km	2	2.3
0.25-0.50km	5	5.7
0.50-1.0km	30	34.5
1.0-1.5km	26	29.9
Over 1.5km	24	27.6
<b>Mode of transport for tubers</b>		
Public Service Vehicle	2	2.3
Bicycle	20	23.0
Wheelbarrow/oxcart	65	65.0
<b>Buyers of tubers/products</b>		
Locals/neighbors	5	5.7
Take to traders in nearest town centres	78	89.7
Middlepersons come to buy on farm	2	2.3
Others	2	2.3
<b>Marketing group membership</b>		
Yes	28	32.2
No	59	67.8
<b>Marketing constraints</b>		
Oversupply/seasonality	16	18.4
Price fluctuations/low prices	48	55.2
Lack of standards in quantity/quality	11	12.6



**Figure 4.5: Mode of selling the sweetpotatoes tubers**

### **4.3 Status of Beta Carotene, Moisture Content and Water Activity of Sun-dried Orange Fleshed Sweetpotato Chips**

The laboratory investigation of the sun dried orange-fleshed sweetpotato chips showed the sample with the lowest level of beta carotene had one mg per 100g on dry weight basis while the sample with the highest beta carotene content had 6.1 mg/100 g on dry weight basis. The mean level of beta carotene for the samples was 1.91 mg/100 g on dry weight basis. Details of the results on beta-carotene, moisture content and water activity for the samples are shown in Table 4.6.

The sample with the highest moisture content recorded 12.90% while the sample with the lowest moisture content had 4.82% as shown in Table 4.5. The mean moisture content of the sun dried sweetpotato chips was 10.21%.

Water activity ( $A_w$ ) of the samples was investigated. The sample with the highest water activity had 0.89 whereas the sample with the lowest water activity had 0.60. The mean water activity was 0.72. Table 4.6 provides details on water activity of the samples.



63.33% of the samples had water activity of between 0.60 and 0.70 which is the water activity critical for mould growth.

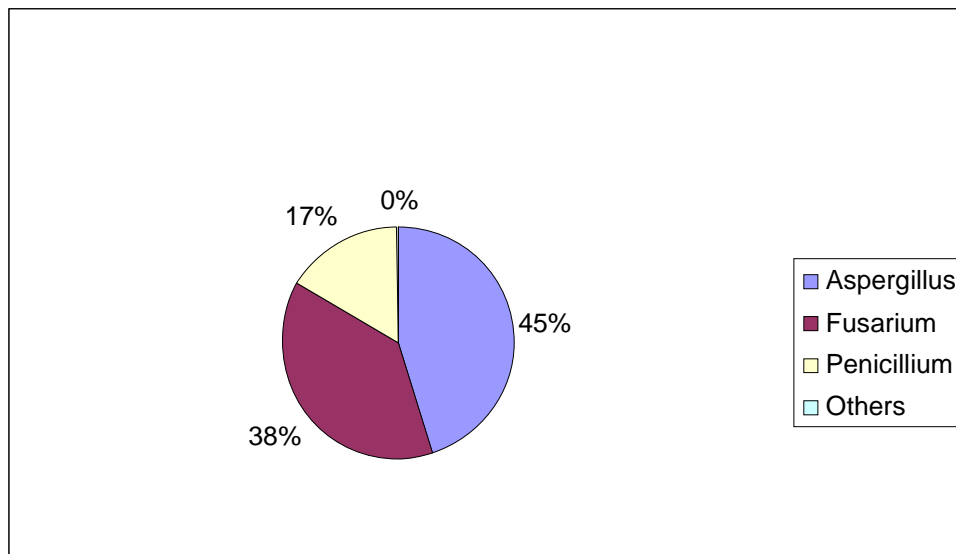
**Table 4.6: Beta-carotene levels, moisture content, water activity and length of storage of the samples**

Sample	Storage length of dried chips (in months)	Beta-carotene levels of the dried chips (mg/100g)	Water activity ( $A_w$ ) of the dried chips	M. C. (%) of the dried chips
1	5	1.58	0.68	7.00
2	5	1.52	0.68	7.71
3	7	1.55	0.68	8.06
4	7	1.52	0.70	8.82
5	5	1.17	0.70	10.00
6	5	1.02	0.74	9.23
7	5	1.09	0.68	9.00
8	5	1.46	0.70	8.57
9	5	1.59	0.66	12.26
10	7	1.40	0.66	11.54
11	5	1.57	0.67	11.00
12	2	1.49	0.71	10.51
13	2	3.35	0.68	12.29
14	3	1.09	0.67	11.00
15	2	1.00	0.74	10.43
16	4	1.21	0.69	10.98
17	4	1.60	0.75	11.62
18	7	1.55	0.69	12.90
19	5	2.23	0.68	12.38
20	2	2.40	0.73	12.02
21	2	2.76	0.77	10.68
22	7	1.07	0.77	11.37
23	4	1.17	0.81	10.00
24	7	1.52	0.80	11.37
25	3	1.48	0.74	9.71
26	2	3.03	0.88	8.78
27	2	1.88	0.89	8.72
28	4	3.29	0.69	4.83
29	1	6.10	0.62	11.56
30	1	3.71	0.60	12.08

## 4.4 Fungal and Mycotoxin Contamination in the Sun-dried Orange Fleshed Sweetpotato Chips

### 4.4.1 Fungal Contaminants of the Sun-dried Orange Fleshed Sweetpotato Chips

Nine fungal species were isolated and identified from the sun-dried orange fleshed sweetpotato chips. These are shown in Table 4.7, Appendix 7E and 7F, and included species of *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Rhizoctonia*, *Nigrospora*, *Rhizopus*, *Alternaria* and *Microsphaera*. The dominant fungal species were species of *Aspergillus*, species of *Fusarium* and species of *Penicillium* (Figure 4.7 below)



**Figure 4.6: Dominant fungal species in sun-dried orange fleshed sweetpotato chips**

*Aspergillus* species accounted for 45.19% of all total fungi isolated from the sun dried sweetpotato chips. Other fungi isolated were *Fusarium* species, which comprised 38.27% of total fungi that contaminated the sun dried sweetpotato chips. *Penicillium* species were also isolated and they comprised of 16.53% of total fungi contaminants of

the sun-dried sweetpotato chips. Other fungi isolated were *Trichoderma*, *Rhizoctonia*, *Nigrospora*, *Rhizopus*, *Alternaria* and *Microsphaera*, which together accounted for 0.01% of total fungi that contaminated the sun-dried orange fleshed sweetpotato chips.

**Table 4.7: Levels of fungal occurrence (%) in sun-dried orange fleshed sweetpotato chips**

Sample	<i>Aspergillus</i>	<i>Fusarium</i>	<i>Penicillium</i>	<i>Trichoderma</i>	<i>Rhizoctonia</i>	<i>Nigrospora</i>	<i>Rhizopus</i>	<i>Alternaria</i>	<i>Microsphaera</i>
1	39.76	8.43	21.69	3.33	2.50	0.00	20.00	4.29	0.00
2	51.52	0.00	9.09	0.00	0.00	0.00	39.39	0.00	0.00
3	60.79	11.76	1.96	2.35	0.00	9.60	0.00	5.44	8.10
4	60.35	15.52	1.72	0.00	0.00	0.00	22.41	0.00	0.00
5	3.23	6.45	6.45	0.00	0.00	0.00	83.87	0.00	0.00
6	5.26	21.05	26.32	0.00	0.00	0.00	27.22	0.00	20.15
7	0.00	21.43	0.00	0.00	0.00	0.00	78.58	0.00	0.00
8	47.06	7.84	0.00	0.00	0.00	2.00	43.10	0.00	0.00
9	61.00	0.00	0.00	0.00	0.00	0.00	39.00	0.00	0.00
10	66.67	5.56	16.67	0.00	0.00	0.00	10.00	1.10	0.00
11	36.59	7.32	17.07	0.00	0.00	0.00	39.02	0.00	0.00
12	34.10	4.55	4.55	0.00	4.20	0.00	52.60	0.00	0.00
13	25.42	35.60	1.70	0.00	0.00	0.00	37.28	0.00	0.00
14	42.11	5.26	7.89	0.00	0.00	0.00	44.74	0.00	0.00
15	14.29	28.57	0.00	0.00	0.00	0.00	46.10	11.04	0.00
16	4.92	3.28	63.93	0.00	0.00	0.00	27.87	0.00	0.00
17	23.53	19.61	0.00	0.00	0.00	0.00	41.56	15.30	0.00
18	36.00	16.00	24.00	0.00	0.00	0.00	24.00	0.00	0.00
19	26.09	60.87	0.00	0.00	0.00	0.00	13.04	0.00	0.00
20	14.89	27.66	6.38	0.00	3.30	12.20	25.52	10.35	0.00
21	13.95	29.91	0.00	0.00	0.00	0.00	56.14	0.00	0.00
22	0.00	26.09	0.00	0.00	0.00	0.00	73.01	0.00	0.00
23	42.86	14.28	0.00	0.00	0.00	0.00	42.86	0.00	0.00
24	34.78	34.78	0.00	0.00	0.00	0.00	30.44	0.00	0.00
25	44.68	6.38	4.26	0.00	0.00	0.00	34.36	0.00	10.32
26	0.00	94.29	0.00	0.00	0.00	0.00	5.71	0.00	0.00
27	15.25	10.17	54.24	0.00	0.00	0.00	20.34	0.00	0.00
28	0.00	75.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00
29	29.17	64.58	6.25	0.00	0.00	0.00	0.00	0.00	0.00
30	22.45	63.27	14.28	0.00	0.00	0.00	0.00	0.00	0.00

*Aspergillus* species isolated and identified were *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus* and *Aspergillus niger*. The mean contamination of the samples by these *Aspergilli* species altogether was 28.26%. Microscopic examination showed radiate heads with long stipes while the conidia were globose to sub globose and green in colour. *Aspergillus ochraceus* colonies were yellowish brown in colour on the obverse side while the reverse was brownish. The heads were globose while the stipes were long, thick and rough. *Aspergillus niger* colonies were black in colour with yellow mycelium on the obverse side and cream to yellow colour on the reverse side. The head was globose and stipe long, smooth and brownish in colour. The conidia were globose and black in colour.

Other fungi that were isolated included *Fusarium* species which comprised 38.27% of total fungi contaminants in the samples of the dry sweetpotato chips. *Fusarium moniliforme* was the most isolated of the *Fusarium* species with white to pinkish aerial mycelium with the colour on the reverse side of the plate ranging from white to pink to deep purple. The mycelium was cottony with powdery appearance. Microscopic examination revealed both microconidia and macroconidia. The microconidia were single-celled, oval and were formed in long chains on monophialides. The macroconidia were slightly sickle-shaped, 4-5 celled with a basal foot cell. The mean contamination by the isolated and identified species of *Fusarium* was 24.18% among the samples.

*Penicilli* species were also identified by their bluish-greenish colony. They accounted for 16.53% of total fungi isolated and identified from the dry orange fleshed sweetpotato chips. On microscopic examination the species had erect conidiophores that

were penicillatedly branched in the upper part. The mean infection by *Penicilli* among the samples was 10.45%.

Other fungi that were isolated include *Trichoderma*, *Rhizoctonia*, *Nigrospora*, *Alternaria* and *Microsphaera*. *Trichoderma* colonies covered the whole petri dish showed irregular outline with isolated tufts. The colonies had white to yellow mycelium, with bright to dull yellow green conidia developing over the whole surface or in patches. The reverse side of the plate was yellowish. The conidiophores consisted of highly branched structures, with a stipe bearing branches and the branches re-branching, appropriately at right angles to form a pyramidal shape, with each bearing phialides irregularly. The phialides bore conidia singly.

Species of *Alternaria* were off-white to grayish dark green in colour on the obverse side and brown to nearly black on the reverse side. The species produced large brown club shaped conidia with both longitudinal and transverse septa, and a distinct conical narrowing at the apical end. *Rhizopus* species were characterized by very fast growth filling the petri dish within a very short period. The mycelium was aerial and coarse brownish in colour. Examination under the microscope revealed numerous rhizoids and sporangiophores in groups. The sporangia were globose and black in colour.

*Nigrospora* species colonies were gray to deep bluish gray on the reverse side of the petri dish and grayish to black colour on the obverse side. The colonies produced large, solitary, jet black, smooth walled and oblate conidia. The conidiophores were short bearing very dark, globose one-celled conidium from aerial mycelium.

#### **4.4.2 Levels of Aflatoxin and Ochratoxin A in the Sun-dried Orange Fleshed Sweetpotato Chips**

The thirty samples of sun dried orange-fleshed sweetpotato chips were analyzed for aflatoxin and ochratoxin A. The results are shown in Table 4.8. The levels of aflatoxin and Ochratoxin A were obtained from the standard curves of the two mycotoxins whose figures are shown in Appendices 2 and 3. The overall mean level of aflatoxin in the sun dried orange fleshed sweetpotato chips was 1.45 ppb. The aflatoxin levels ranged from undetected to 12.00 ppb. The maximum acceptable level for aflatoxin is 10 ppb. Most of the dry orange fleshed sweetpotato chips tested in this study had levels below maximum acceptable level for aflatoxin.

The overall mean level for ochratoxin A was 3.51 ppb. The sample with the lowest level had 0.00 ppb while the sample with highest ochratoxin level had 15.00 ppb. One sample had the highest recorded levels of both aflatoxin and ochratoxin at 12 ppb and 15 ppb respectively. However, this sample had only *Aspergillus parasiticus* at 5% contamination level. The same sample had the lowest moisture content at 4.83% and water activity at 0.69. The fungi isolated from this sample were species of *Fusarium* and species of *Penicilli*. Seventy percent of the samples reported detectable levels of aflatoxin whereas ninety percent of the samples reported detectable levels of ochratoxin A.

**Table 4.8: Aflatoxins and Ochratoxin A levels in the sun-dried samples of sweetpotato chips**

Sample	Aflatoxins level (ppb)	Ochratoxin A level (ppb)
1	0.5	0.00
2	0.0	0.01
3	0.8	0.04
4	0.0	0.02
5	0.5	0.025
6	0.0	0.001
7	0.8	0.00
8	1.5	0.03
9	1.8	0.06
10	0.8	0.07
11	0.8	0.03
12	2.0	3.00
13	0.0	0.00
14	1.8	0.10
15	1.0	0.50
16	1.5	2.30
17	0.0	0.10
18	0.8	2.00
19	0.5	5.00
20	0.5	4.50
21	0.0	6.00
22	1.8	7.50
23	2.0	10.05
24	0.8	9.00
25	1.0	9.00
26	1.5	4.00
27	0.0	6.00
28	12.0	15.00
29	0.0	4.50
30	0.0	6.00



#### **4.5 Association Analysis on Storage Length and Levels of Beta-Carotene, Moisture Content, Water Activity, Fungi, Aflatoxin and Ochratoxin of the Sun-dried Sweetpotato Chips**

Association analysis was done and the results of the analysis are shown in Table 4.9. The results shows that the longer the storage period for the sun dried orange fleshed sweetpotato chips the more the levels of species of *Aspergillus* in the samples with a correlation coefficient of 0.367 and p-value of 0.046 which is significant at 0.01 level of significance. However, the correlation coefficient was -0.376 and p-value of 0.041 for storage length and species of *Fusarium*. Association analysis also showed a negative association between species of *Aspergillus* and species of *Fusarium* at correlation coefficient of – 0.475 and significance of  $p = 0.008$ .

The association analysis revealed that the longer the storage length of the sun dried sweetpotato chips, the lower the beta-carotene at correlation coefficient of – 0.558 and p-value of 0.001. The analysis from the study showed a negative correlation between moisture content and aflatoxin levels at a correlation coefficient of – 0.506 and p-value of 0.004. The analysis also showed a positive relationship between species of *Fusarium* and beta-carotene levels at  $p > 0.05$ .

Further, the association revealed a positive relationship between species of *Fusarium* and Ochratoxin A at  $p > 0.05$  at 0.01 level of significance. There was also a positive relationship between aflatoxin and Ochratoxin A at  $p > 0.05$  at 0.01 level of significance.

**Table 4.9: Results of associations analysis on storage length and laboratory analysis work**

		Storage Length	W.A.	M.C. (%)	Species of <i>Aspergillus</i> (%)	Species of <i>Fusarium</i> (%)	Species of <i>Penicillium</i> (%)	Aflatoxin (ppb)	Ochratoxin (ppb)	Betacarotene (mg/100g)
Storage Length	Pearson Correlation	1	-.096	-.098	.367*	-.376*	-.052	.023	-.194	-.558**
	Sig. (2-tailed)		.614	.607	.046	.041	.786	.904	.304	.001
	N	30	30	30	30	30	30	30	30	30
W.A.	Pearson Correlation		1	-.175	-.333	.109	.060	-.024	.350	-.225
	Sig. (2-tailed)			.354	.072	.567	.754	.901	.058	.232
	N		30	30	30	30	30	30	30	30
M.C. (%)	Pearson Correlation			1	.069	-.040	-.162	-.506**	-.168	.095
	Sig. (2-tailed)				.717	.832	.391	.004	.374	.618
	N			30	30	30	30	30	30	30
Species of <i>Aspergillus</i> (%)	Pearson Correlation				1	-.475**	-.232	-.221	-.301	-.153
	Sig. (2-tailed)					.008	.218	.240	.106	.418
	N				30	30	30	30	30	30
Species of <i>Fusarium</i> (%)	Pearson Correlation					1	-.170	.314	.478**	.709**
	Sig. (2-tailed)						.370	.091	.007	.000
	N					30	30	30	30	30
Species of <i>Penicillium</i> (%)	Pearson Correlation						1	.128	.056	-.058
	Sig. (2-tailed)							.502	.767	.760
	N						30	30	30	30
Aflatoxin (ppb)	Pearson Correlation							1	.578**	.100
	Sig. (2-tailed)								.001	.598
	N							30	30	30
Ochratoxin (ppb)	Pearson Correlation								1	.304
	Sig. (2-tailed)									.103
	N								30	30
Betacarotene (mg/100g)	Pearson Correlation									1
	Sig. (2-tailed)									
	N									30

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

## **CHAPTER FIVE**

### **DISCUSSION**

#### **5.1 Post Harvest Handling Practices of Sweetpotatoes by Farmers**

Postharvest storage of sweet potatoes is an important practice that enhances household food security in rural communities. The tubers are bulky and once harvested have short life. Post-harvest storage of the roots occurs in two forms, in the fresh form and in the dry form as seen from the study. Fresh storage in the soil consists of intermittent harvesting of the larger tubers while leaving the others in the soil to develop further (Degras, 2003). However, in fresh storage the tubers are prone to attack by insect pests, disease and vertebrate pests as is found in this study, this is in agreement with Stathers et al., (2005) and findings by Kihurani et al. (1995) and Gathumbi et al. (1992). From the study findings, fresh storage of harvested roots was prone to rotting at 81.6% and this is supported by the studies done by Karuri and Hagenimana (1995), Kapinga et al., (1997), and Kihurani (2004).

According to the findings of this study, thirty four percent of the farmers process the roots into dry chips which were used as a form of dry storage of sweetpotatoes. The processed chips were dried by spreading on any material direct on the ground by 53.3% of the farmers thereby exposing the chips to contamination by dust, soil, birds and micro-organisms. The current study matches the study done by Silayo et al., (2003) on effect of sun drying on some quality characteristics of sweetpotato chips. However, household level processing of sweetpotato chips was shown to be done during the dry season when the tubers are ready for harvesting. This finding is in agreement with the study done by Peters and Wheatley (1997). From this study, most farmers used locally available tools to

process the chips and 58.6% of the farmers process unpeeled roots. This is in agreement with the studies done by Owori (2000), Silayo et al., (2000) and Owori (2003). In West Africa, smallholder farmers use dried slices of sweetpotatoes since these can be stored for periods of up to four months (Hall, 1998). This concurs with the current study findings in which some farmers (9.2%) stored the dry sweetpotato chips for over three months.

From this study, 93.1% of the farmers package the roots and chips in polythene bags before storage, transportation or sale. This is in line with the study done by Antarlina and Bedjo (1997) who concluded that sweetpotato products could be stored using sealed plastic bags and jars to keep their quality.

Marketing of sweetpotato roots and chips was done mainly in the nearest towns of Gambogi and Kiboswa. Most of the tubers were sold soon after harvesting and the selling was done in heaps (70.1%). Each heap was sold at Kshs. 10. The biggest constraint faced by the producers at the time of marketing the tubers was price fluctuation of the fresh tubers. The other challenges that sweetpotato farmers faced as recorded in the current study, included seasonality of supply of the fresh tubers, and lack of organized marketing groups. Concerning marketing of the chips, pests (71.3%) and moulds (17.2%) were the main challenges the farmers faced. There was also concern about lack of standards for sweetpotato dry chips (9.2%) as is the case with maize. In this study, the farmers were producers as well as traders of the sweetpotato roots and dry chips. This is in agreement with studies done by Damardjati et al., (1990), and Alumira and Obara (1997). Most of the farmers (93.1%) interviewed indicated that they had short supply of sweetpotato roots for sale and all the selling was done individually thus reducing the bargaining ability. Farmers also indicated they had ready markets for the tubers and

products. In this study, women (97.7%) dominated the processing and marketing of the sweetpotato tubers. This is in agreement with the study done by Rono et al., (2005).

## **5.2 Levels of Beta-Carotene Content, Moisture Content and Water Activity in the Samples**

From the study, the mean beta carotene level of the samples was 1.91 mg/100 g of the sample on dry weight basis. The dry orange fleshed sweetpotato chips sample with the lowest level of beta carotene had 1.00 mg/100 g of sample on dry weight basis while the sample with the highest beta carotene level had 6.1 mg/100 g of sample on dry weight basis. The findings of the current study are in agreement with studies done by Hagenimana et al., (1999), Bengtsson et al., (2008), Nascimento et al., (2006), Almeida et al., (1988), Simonne et al., (1993), Ameny and Wilson (1997), Hagenimana et al., (1998), Picha (2006), Van Jaarsveld et al., (2006), Nascimento et al., (2006) and Takahata et al., (1993). Indeed this study is in line with the study done by Hagenimana et al., (1999) in which SPK 004 dried chips variety lost beta-carotene over months of storage. Boiling the roots reduced total carotenoid by 20%, whereas drying roots at 65% for 12 hours reduced total carotenoid content by 30%. Storing dried chips for 11 months reduced total carotenoid content an additional 11%, from 70% to 59% (Hagenimana et al., 1999). Overall losses in carotene may vary from 5 – 40% depending on temperature, time and nature of carotenoids. Drying sweetpotato pieces at temperatures between 50°C to 70°C were found to produce acceptable products; however, lower temperatures resulted in microbiological contamination (Bouwkamp, 1985). Other related studies to evaluate

carotenoid content of various foods have been done by Edwards and Lee (1986), Imungi et al., (1990), Sian and Ishak (1991) and Simon (1997).

The sample with the highest moisture content recorded 12.90% whereas the sample with the least moisture content had 4.82%. The mean moisture content of the samples was 10.21%. According to Woolfe (1992), sweetpotato dry chips should be stored at moisture content of less than 17%, 12.5% and 11% if they are to be stored in temperatures of 6 - 10° C, 15-20° C and 27- 29 °C respectively. According to study done by Okungbowa and Osagie (2009) on mycoflora of sun-dried sweetpotato, the high moisture content of sweet potato samples (71.8-72.6% before sun-drying; and 9.0-9.1% after drying) as well as the humidity and high temperatures encouraged the germination of fungi and colonization by fungi such as *Aspergillus*, *Rhizopus*, and *Penicillium*. Some or all of the fungi isolated were previously reported to cause spoilage of food and have been isolated from various food items such as cotton seeds (Okungbowa, 2005; Okungbowa and Dede, 2005), cassava (Jimoh and Kolapo, 2008), grapes (Valero et al., 2007), gari (Ogiehor et al., 2004), yam chips (Ekundayo, 1986), plantain pudding (Ohenhen et al., 2006) and cocoyam chips (Uguanyi, 2007). They are fungi that occur in the air and easily contaminate exposed foods.

Water activity ( $A_w$ ) of the samples was investigated. The sample with the highest water activity had 0.89 whereas the sample with the lowest water activity had 0.60. The mean water activity of the samples was 0.72. Water activity is a critical factor that determines shelf life of foods. Most bacteria, for example, do not grow at water activities below 0.91, including pathogens such as *Clostridium botulinum*. Below 0.80 most molds cannot be grown and below 0.60 no microbiological growth is possible. However, there

remain a number of food spoilage microbes that can grow within the range 0.6 - 0.8. By measuring water activity, it is possible to predict which microorganisms will and will not be potential sources of contamination of food. In addition to influencing microbial spoilage, water activity can play a significant role in determining the activity of enzymes and vitamins in foods and can have a major impact on their color, taste, and aroma.

A study done by Ayub et al., (2003) to make a comparison for better food quality with respect to microbial load, moisture content and water activity, so that consumers may get a nutritive, more hygienic and shelf stable product at their homes, established that the effect of treatments and storage period was significant ( $p < 0.05$ ) on moisture content,  $A_w$  and microbial growth on the samples of bread. Water activity is related to moisture content in a non-linear relationship known as a moisture sorption isotherm curve. These isotherms are substance and temperature specific. Isotherms can be used to help predict product stability over time in different storage conditions according to a study done by Roos (1993). Another study carried out by Abbas et al., (2009) indicated that the water activity ( $A_w$ ) played an important factor in fish spoilage and that the growth of different microorganisms depends on its level.

### **5.3 Levels of Fungi in the Sun-dried Orange Fleshed Sweetpotato Chips**

The results from this study indicated a high level of contamination in the sun dried orange fleshed sweetpotato chips by *Aspergillus* spp. (45.19%). Other fungi that had contaminated the chips were *Fusarium* spp., *Penicillium* spp., *Trichoderma*, *Rhizoctonia*, *Nigrospora*, *Rhizopus*, *Alternaria* and *Microsphaera*. *Aspergillus* spp. are serious fungal infecting sweetpotato chips and flour. This is in agreement with studies

done by Okungbowa and Osagie (2009), Antarlina and Bedjo (1997) and Silayo et al., (2003). Some or all of the fungi isolated were previously reported to cause spoilage of food and have been isolated from various food and feed items such as maize (Tanaka et al., 2001), cotton seeds (Okungbowa, 2005; Okungbowa and Dede, 2005), cassava (Jimoh and Kolapo, 2008 and Wareing et al., 2001), grapes (Valero et al., 2007), garri (Ogiehor et al., 2004), yam chips (Ekundayo, 1986), feed (Carlson et al., 2003 and Gathumbi, 1993), plantain pudding (Ohenhen et al., 2006) and cocoyam chips (Uguanyi, 2007). They are fungi that occur in the air and easily contaminate exposed foods and feeds.

In the current study, nine fungal species were isolated and identified from the sun dried orange fleshed sweetpotato chips. These are *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Rhizoctonia*, *Nigrospora*, *Rhizopus*, *Alternaria* and *Microsphaera*. The dominant fungal species were *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. In a similar study done by Okungbowa and Osagie (2009), a total of twelve fungal species were isolated from sun dried sweet potato slices. *Aspergillus* was the single genus that recorded most different species. This is in agreement with the current study.

However, more studies have been done on fungi infecting sweetpotato tubers than studies on fungi contaminating sweetpotato dry chips. The studies included those done by Kihurani, (2004), Kapinga, et al., (1997), Muhanna, (2001) and Kihurani et al. (1994). These include studies done on micro-organisms that contaminate sweetpotato roots and flour, Owori, (2000), Rees et al., (1997) and Rees et al. (2003).



#### **5.4 Levels of Aflatoxin and Ochratoxin A in the Samples of Sun-dried Sweetpotato Chips**

According to findings of this study, the overall mean level of aflatoxin in the sun dried orange fleshed sweetpotato chips was 1.45 ppb. The sample with the lowest aflatoxin level had undetected level of aflatoxin whereas the sample with the highest level had 12.00 ppb. The maximum acceptable level or safe level for aflatoxin is 10 ppb. Most of the dry orange fleshed sweetpotato chips tested in this study had levels below maximum acceptable level for aflatoxin.

From this study, the overall mean level for ochratoxin A in the dry orange fleshed sweetpotato chips was 3.51 ppb. The sample with the lowest level of ochratoxin A had undetected level of ochratoxin A whereas the sample with highest ochratoxin level had 15.00 ppb. The maximum permitted level of ochratoxin A is 10 ppb for coffee. Of the sun dried orange fleshed sweetpotato chips tested in this study, 3.3% had levels above maximum acceptable level for ochratoxin A.

Co-occurrence of mycotoxins occurred in the sun-dried orange fleshed sweetpotato chips examined in this study where aflatoxins and ochratoxin A were reported in the samples. Seventy percent of the samples reported detectable levels of aflatoxin whereas 90% of the samples reported detectable levels of ochratoxin A. This finding is in agreement with those of other researchers (Medina and Marinez, 2000; Camargos et al., 2001; Li-FengQin et al., 2001; Ono et al., 2001; Valente et al., 2001 and Vargas et al., 2001) who also reported the co-occurrence of mycotoxins in foods. Consumers are therefore predisposed to simultaneous exposure to more than one mycotoxin every time they consume contaminated foods or food products.

## **CHAPTER SIX**

### **CONCLUSION AND RECOMMENDATIONS**

#### **6.1 CONCLUSIONS**

The conclusion from this study is that farmers in Tiriki West Division of Vihiga County carried out post-harvest handling practices on sweetpotatoes that exposed them to fungal, aflatoxin and ochratoxin contamination, and influenced beta carotene, moisture content and water activity levels of the sun dried orange flesh sweetpotato chips. These practices included using any tool for cutting up the tubers into chips, spreading the chipped tubers on any material such as old sacks and pieces of polythene bags and drying the chipped tubers on the ground and for more than two days. Also storage of the chipped tubers was done in any available container or material and the chips were kept anywhere in the farmers' houses and some farmers kept the chipped tubers for more than three months.

The findings of this study conclude that the methods of processing at farm level by farmers could contaminate the sweetpotato chips and put at risk the health of the consumers of the products made from the chips. There was no consistency in practice and standards by the farmers as each farmer handled the sweetpotatoes differently from another farmer. Therefore processing varied from one farmer to another and the samples were not handled and stored under standardized conditions and period.

This study also concludes that sun drying of chipped sweetpotatoes at ordinary farm level conditions does not reduce moisture content and water activity sufficiently low enough to eliminate fungal contamination of the chips.

From this study it was also concluded that several fungal species are associated with farmer processed sun dried orange fleshed sweetpotato chips, some of them potential mycotoxin producers. The fungal species included species of *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Rhizoctonia*, *Nigrospora*, *Rhizopus*, *Alternaria* and *Microsphaera*. The most dominant fungi in the sun dried orange fleshed sweetpotato chips were *Aspergillus* species, *Fusarium* species and *Penicillium* species.

The study reported presence of aflatoxin at 70% and ochratoxin A at 90% in the farm processed sun-dried orange fleshed sweetpotato chips. This does suggest that there is safety and health risk associated with utilization of the sweetpotato chips and products made from the chips, thereby compromising food security. There was concurrent contamination of the samples by aflatoxin and ochratoxin A deduced from the positive relationship between aflatoxin and ochratoxin following the correlation analysis between the two mycotoxins.

There was a possibility that contamination of some sun-dried orange fleshed sweetpotato chips could have happened at some point during handling, but on analysis there were no *Aspergillus* species isolated yet the same had detectable levels of aflatoxin and ochratoxin A.

Finally, it is concluded that the results from this study are important in providing baseline information for a more systematic, better organized experimental design on sweetpotatoes post-harvest study where programmed processing is carried out and storage times known in which all treatments are specified across the samples.

## **6.2 RECOMMENDATIONS**

- From this study it is recommended that the issue of post-harvest handling of sweetpotatoes by farmers be addressed. One way is to train farmers to adopt better post-harvest handling practices by use of standardized tools, drying the chipped tubers on raised surface, drying the sweetpotato chips for the shortest time possible and storing the dried chips for short length of period.
- Further investigation is recommended aimed at determining fungal and toxin levels and beta carotene depletion in sun-dried orange fleshed sweetpotato chips. This may include studies on the association of fungi, mycotoxins and beta-carotene in sweetpotatoes both in fresh form and in the processed form.
- This study also recommends more research be done to assess effects of specific post-harvest handling practices on development of mycotoxins in processed sweetpotatoes under experimental conditions, and further under farm conditions.

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## APPENDICES

### Appendix 1: Survey Questionnaire

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#### SWEETPOTATO POST HARVEST PRACTICES SURVEY TIRIKI WEST DIVISION, VIHIGA DISTRICT

##### **Section A: Background Information**

- |  |                             |                      |                     |
|--|-----------------------------|----------------------|---------------------|
| 1. a. Date of interview:                         | b. Name of interviewer:     |                      |                     |
| c. Location:                                     | d. Sub location:            |                      |                     |
| e. Village:                                      | f. Household No.            |                      |                     |
| 2. Name of household head:                       | Age: Sex:                   |                      |                     |
| 3. Name of Respondent:                           | Age: Sex:                   |                      |                     |
| 1=male   | 2=female                    |                      |                     |
| 4. Relationship of respondent to household head: |                             |                      |                     |
| 1=head   | 5=daughter                  |                      |                     |
| 2=husband  | 6=employee                  |                      |                     |
| 3=wife   |                             |                      |                     |
| 4=son  | 7=other (specify)           |                      |                     |
| Code for head                                    |                             |                      |                     |
| 1=head   | 5=daughter                  |                      |                     |
| 2=husband  | 6=employee                  |                      |                     |
| 3=wife   | 7=other (specify)           |                      |                     |
| 4=son  |                             |                      |                     |
| 5. Marital status of household head:             |                             |                      |                     |
| 1=married  | 2=single                    | 3=separated/divorced | 4=widowed           |
| 6. Religion of household head:                   |                             |                      |                     |
| 1=catholic                                       | 2=Protestant                | 3=Muslim             | 4= others (specify) |
| 7. Proximity to nearest town:                    |                             |                      |                     |
| 1=0-10km   | 3=21-30km                   |                      |                     |
| 2=11-20km  | 4= over 30km                |                      |                     |
| 8. State of the road near village/farm:          |                             |                      |                     |
| 1=barely passable all year                       | 3=easily motorable all year |                      |                     |
| 2= barely motorable all year                     | 4= tarmac                   |                      |                     |

##### **Section B: Wealth Status (interviewer):**

9. Farm size:  
10. What is the gender of the household head?



1=male

2=female

11. Level of education:

0=no formal education

3=post-secondary

1=primary school

4=adult literacy education

2=secondary school (O-level)

5=other (specify)

12. What are your sources of income?

1=farming/business

3=children

2=formal employment

4=no income

13. How many people are living/working on farm? Enter in table

Age	Males	Females	Totals
<8-16yrs			
17-24yrs			
25-45yrs			
46-65yrs			
>65yrs			

### **Section C: Role of sweetpotatoes in sample area**

14. Proportion of people who engage in growing sweetpotatoes:

1= none

3= some

2= few

4= many

15. What are the most common sweetpotato varieties grown?

1=orange fleshed

3=yellow fleshed

2=white fleshed

4=others (specify)

16. Sweetpotato planting seasons in sample area:

1= long rains

2=short rains

3=from time to time

17. Sources of planting material:

1=own farm

4=government program or FTC

2=neighbors

5=KARI

3=market

6=other (specify)

18. What is the purpose of cultivating the sweetpotatoes?

1=Home consumption only

4=Livestock feed + home consumption

2=Income source + home consumption

5=Home consumption + soil conservation

3=Income source only

6=others (specify)

19. What are the constraints in production of sweetpotatoes?

### **Section D: Mode of transport for harvested sweetpotatoes and products**

20. What mode of transport do you use for transporting the tubers/products?

1=saloon car

6=wheelbarrow

2=pick up

7=donkey cart

3=lorry

8=hand cart

4=tractor

9=matatu

5=bicycle

10=any other (specify)

**Section E: Harvesting, Processing and Packaging of sweetpotato tubers in sample area**

21. Who harvests the tubers?

1=self

3=children

2=spouse

4=hired labour/others

22. Who owns the harvested tubers?

1=Wife

2= Husband

3=Children

4=Others

23. How do you process the tubers?

1=wash- cook- with peel

5=wash-peel-grate-dry-mill

2=wash- peel- boil

6=wash-grate-dry-mill

3=wash-peel-grate-fry

7=wash-peel-blanch-grate-dry-mill

4=roast

8=others (specify)

24. Who does the processing?

1=self

2=spouse

3=Children

4=Hired laborers

5=others (specify)

25. How long does it take to process the sweetpotatoes?

1=less than 2 hours

3=over 2 days

2=2-8 hours

4=do not process

26. When is the processing done (time of the day)?

1=anytime

3=afternoon hours

2=morning hours

4=do not process

27. Why do you process the tubers?

1=home consumption

3=home consumption and income

2=income source

4=do not process

28. Of the harvested tubers, how much is processed?

1=100%

2=75%

3=less than 50%

4=do not process

29. If drying of product is done, how long does it take to dry?

1=less than 2 hours

3=2 days and over

2=2-8 hours

4=do not dry

30. How do you dry the product?

1=open sun drying, on ground

3=solar drier

2=open sun drying, raised above ground

4=others (specify)

31. What is the quality of the product?

1=retained color

3=big change in color

2=slight change in color

4=not apply

32. Are there more raw materials available than can be processed locally?

1=Yes

2=No

33. Are there seasonal shortages of the raw material?

1=Yes

2=No

34. If the sales of processed sweetpotato products are an income source, what proportion of the income do

a. Women earn?

1=100%                      2=75%                      3=less than 50%                      4=none

b. Youth earn?

1=100%                      2=75%                      3=less than 50%                      4=none

35. How do you package the tubers/products?

1=polythene sacks/paper bags

3=crates/woven baskets

2=jute bags

4=do not package/not apply

### **Section F: Processing Technology and Labor**

36. What are the tool(s) you use for preparing sweetpotato products?

1=traditional, locally available

3=modern, imported/fabricated

2=improved, locally available

4=not apply

37. How much does the tool cost?

1=less than ksh. 100/-

3=over ksh. 500/-

2=between ksh. 100/- and ksh. 500/-

4=not apply

38. Where did you buy it? (Source of tool)

1=local market

3=KIRDI/KARI/Government

2=nearest big town

4=not apply

39. How many people assist you in processing?

1=1person

3=over 5 people

2=less than 5 people

4=not apply

40. What is the gender of those who assist in processing the tubers?

1=male

2=female

3=both male and female

41. How much do you pay for the labor per day?

1=less than ksh. 50/-

3=over ksh.100/-

2=ksh.50/- to ksh.100/-

4=not apply

### **Section G: utilization**

42. How do you consume the sweetpotato tubers?

1= boil with/out peel

3=make flour for ugali/porridge

2= roast with/out peel

4=make snacks/doughnuts

43. Frequency of consumption:

1=eat once per week

3=eat when no other food

2=eat only during famine

4=like to eat more than once per week

### **Section H: Storage**

44. How do you store sweetpotato tubers after harvesting?

1=in ground

3=process and dry

2=do not know

4=do not store

45. How do you store the product(s)? (Storage conditions)

- |                                   |                |
|-----------------------------------|----------------|
| 1=special room, well ventilated   | 3=anywhere     |
| 2=special room, poorly ventilated | 4=do not store |

46. How do you package the products before storage?

- |                  |                        |
|------------------|------------------------|
| 1=polythene bags | 3=crates/woven baskets |
| 2=jute bags      | 4=not apply            |

47. For long do you keep the products before selling/consuming?

- |                       |                 |
|-----------------------|-----------------|
| 1=less than one month | 3=2-3 months    |
| 2=1-2 months          | 4=over 3 months |

48. What is the quality of the products after storage period?

- |                          |                       |
|--------------------------|-----------------------|
| 1=retained color         | 3=big change in color |
| 2=slight change in color | 4=not apply           |

49. What constraints do you encounter when storing sweetpotato tubers/products?

### **Section I: Marketing**

50. Who are the buyers of the tubers/chips/flour?

- |                     |                     |
|---------------------|---------------------|
| 1=locals            | 3=to middle persons |
| 2=at market centers | 4=flour millers     |
| 5=hotel s           |                     |

51. In what quantities do you sell the tubers/products?

- |            |        |             |             |
|------------|--------|-------------|-------------|
| 1=kilogram | 2=heap | 3=basketful | 4=not apply |
|------------|--------|-------------|-------------|

52. What is the selling price per heap of the tubers?

- |                       |                         |
|-----------------------|-------------------------|
| 1=do not sell         | 3=ksh. 10/- to ksh.20/- |
| 2=ksh.5/- to ksh.10/- | 4=over ksh.20/-         |

53. Do you belong to any cooperative society or group?

- |       |       |
|-------|-------|
| 1=yes | 2= no |
|-------|-------|

54. What constraints do you encounter in marketing the tubers/products?

## Appendix 2: Aflatoxin Standard Curve

standard	Absorbance					
	Rep 1	Rep 2	Rep 3	Rep 4	Average	% Inhibition
S1 (100ppb)	0.206	0.193	0.199	0.227	0.206	17
S2 (33ppb)	0.298	0.300	0.309	0.300	0.301	25
S3 (11 ppb)	0.517	*	0.517	0.518	0.517	42
S4 (3.7 ppb)	0.911	0.978	0.946	0.966	0.950	78
S5 (1.2ppb)	1.207	1.152	1.133	1.137	1.157	94
S6 (0 ppb)	1.196	1.200	1.236	1.269	1.225	100

\*outlier

## Appendix 3: Ochratoxin A Standard Curve

Standard	Absorbance					
	Rep 1	Rep 2	Rep 3	Rep 4	Av.	% Inhibition
S1 (40ppb)	0.139	0.144	0.152	0.141	0.144	12
S2 (13.3ppb)	0.265	0.251	0.264	0.257	0.259	21
S3 (4.4ppb)	0.402	0.433	0.456	0.464	0.439	36
S4 (1.4 ppb)	0.742	0.730	0.641	0.653	0.692	57
S5 (0.47ppb)	0.949	0.955	0.944	0.977	0.956	76
S6 (0 ppb)	1.221	1.163	1.224	1.259	1.217	100

## Appendix 4: Procedure for direct enzyme-linked immunosorbent assays (ELISA)

for quantification of aflatoxins and ochratoxin A in foods and feeds

### Sample Extraction

#### a) Aflatoxin (AF)

1. Grind the sample into a fine powder (to pass through 1mm aperture) using a blender.
2. Weigh 5g of the sample and add 25 ml methanol+H<sub>2</sub>O (distilled).
3. Stir for 30 minutes on a magnetic stirrer.

4. Centrifuge at 1500g for 10 minutes and recover the supernatant. Alternatively, filter through a filter paper.
5. Defat at 3 ml supernatant /filtrate by homogenization with an equal volume of hexane on a vortex mixer, followed by a 5 minute centrifugation at 1500g and the recovery of the lower methanolic layer (Optional step).
6. Dilute the methanol content of the extract to 10% with Phosphate Buffered Saline (PBS).
7. Prepare further two 1:3 dilution of the extract in 10% methanol-PBS. Save the three dilutions for ELISA.

**b) Ochratoxin A (AO)**

1. Grind the sample into a fine powder (to pass through 1mm aperture) using a blender.
2. To 2g sample, add 5ml 1M HCl and mix at full speed for 5 minutes on a magnetic stirrer.
3. Add 10ml dichloromethane and stir at full speed for a further 15 minutes.
4. Centrifuge the mixture at 1500g for 15 minutes and recover the lower dichloromethane layer.
5. Add 10ml 0.13M NaHCO<sub>3</sub> solution to the dichloromethane layer and mix at high speed for 15 minutes.
6. Centrifuge as in step 4 and recover the aqueous layer.
7. Prepare two 1:3 dilutions of the aqueous extract in 0.13M NaHCO<sub>3</sub> and save for ELISA analysis.

**c) Procedure for direct competitive ELISA**

1. Coat each well of an immunoplate using 100µl of a solution of the specific antiserum in bicarbonate buffer at the following dilutions:

<b>Toxin</b>	<b>Antiserum</b>	<b>Dilution</b>
Aflatoxins (AF)	Anti-AFB1 K147	1:20,000
Ochratoxin A	Anti-OA Vpool	1:16,000

2. Incubate overnight at room temperature or 3 days at 4° C.
3. Empty the microplate wells, semi-dry. To block free protein sites, add 200µl of 3% fetal calf serum (FCS) in PBS for aflatoxin. No blocking is done for ochratoxin A.
4. Wash the plates three times with PBS-Tween and semi-dry.
5. Add 50µl of mycotoxin standards dissolved in the solvents and at the concentration ranges shown in the table below. Pipet 4 wells per each standard dilution.

<b>Toxin</b>	<b>Diluent</b>	<b>Standard dilution range</b>
Aflatoxins (AF)	Methanol+PBS (10+90)	Initial dilution of 1ng/ml. Serial diluted to obtain a total of 6 dilutions. Include a diluent blank.
Ochratoxin A	0.13M NaHCO <sub>3</sub>	Initial dilution of 4ng/ml. Serial diluted to obtain a total of 6 dilutions. Include a diluent blank.

6. Add 50µl sample extract dilution. For each sample extract dilution, pipet 2 wells.
7. Immediately after steps 5 and 6, add mycotoxin-enzyme conjugates as detailed in the table below:

<b>Toxin</b>	<b>Mycotoxin conjugate</b>	<b>Diluent and dilution factor</b>
Aflatoxins (AF)	AFBI-oxim-HRPO	1:400,000 in 1% FCS/PBS
Ochratoxin A	Ochratoxin A-AE-HRPO	1:300,000 in 1% Tween 20/0.13M NaHCO <sub>3</sub> solution.

8. Incubate in the dark for 2 hours at room temperature.
9. Wash as in step 4.
10. Add 100µl of substrate solution into each well
11. After enough colour develops, usually in about 10 – 20 minutes, stop the reaction by adding 100µl of 1mol/L H<sub>2</sub>SO<sub>4</sub>.

12. Read absorbance at 450nm using the ELISA reading machine.

#### **Appendix 5: Buffers and substrates for ELISA**

1. Coating buffer – Bicarbonate buffer pH 9.6 (9.4-9.8)

$\text{Na}_2\text{CO}_3$  – 1.59g

$\text{NaHCO}_3$  – 2.93g

Distilled  $\text{H}_2\text{O}$  – 1000ml

2. Washing solution – saline Tween (PBS-T)

$\text{NaCl}$  – 8.55g

Tween 20 – 0.25ml

Distilled water 1000ml

3. Phosphate buffered saline (PBS) pH 7.0-7.5

$\text{NaCl}$  – 6.79g

$\text{Na}_2\text{HPO}_4$  – 1.47g

$\text{KH}_2\text{PO}_4$  – 0.43g

Distilled water 1000ml

4. The substrate is prepared by mixing two components;  $\text{H}_2\text{O}_2$  buffer and TMB solution

$\text{H}_2\text{O}_2$  buffer: pH 3.9 – 4.0

Mix 8.81g citric acid monohydrate, 40ml 1M KOH and 160ml distilled water and adjust the pH to 3.9-4.0. Add 65 $\mu\text{l}$   $\text{H}_2\text{O}_2$ .

TMB solution

Mix 50.4mg tetramethylbenzidine, 1ml acetone and 9ml methanol.

Just before use, mix 20 parts of the  $\text{H}_2\text{O}_2$  buffer and 1 part of TMB solution.

5. Stopping reagent – 1 mol/L sulphuric acid



### Appendix 6: ELISA Microplate Titration Format

	1	2	3	4	5	6	7	8	9	10	11	12
A	E	S1	S2	S3	S4	S5	S6	S7rb	P1	P2	P3	E
B	E	S1	S2	S3	S4	S5	S6	S7rb	P1	P2	P3	E
C	E	S1	S2	S3	S4	S5	S6	S7rb	P1	P2	P3	E
D	E	S1	S2	S3	S4	S5	S6	S7rb	P1	P2	P3	E
E	E	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	E
F	E	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	E
G	E	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	E
H	E	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	E

KEY: E – Empty wells

P – Samples

S – Standards

S7rb – Reagent blank

## Appendix 7: Photo Plates



7A: Sweetpotato field



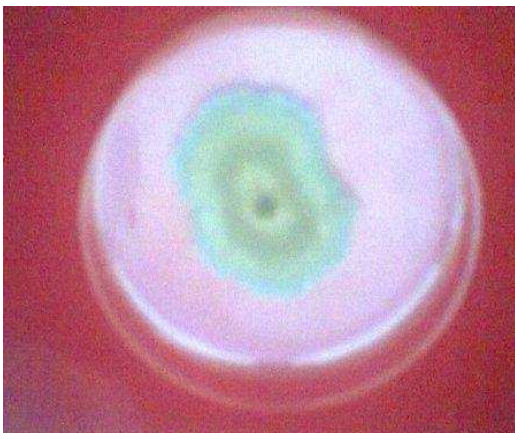
7B: Orange flesh (SPK 004) sweetpotatoes



7C: Sweetpotato chipped tubers being sun-dried



7D: Storing dried Sweetpotato Chips



7E: Two weeks old *Aspergillus parasiticus* plate

