INCIDENCE OF *Aspergillus flavus* IN STORED DRIED CASSAVA PRODUCTS AND ITS ASSOCIATION WITH AFLATOXIN PRODUCTION

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ABSTRACT

Udaga is derivative of cassava products traditionally produced by farmers through peeling, fermentation and direct or indirect sun-drying of fermented cassava roots and widely consumed in Tanzania. The study was conducted in Lushoto, Rorya and Ukerewe districts in Tanzania based on diverse processing and storage methods between October and November 2012. Households (120) were interviewed on cassava processing and storage practices. Samples of dried cassava products were also obtained from interviewed households for moisture content, pH, microbial growth and aflatoxin content analysis. The factors that impact on mould and aflatoxin contamination of these products were established using regression analysis. Cassava products contained mean moisture content of 13.9%, pH 5.5 and aflatoxin ≥ 4 ppb in 8 samples. Mould incidence were *Rhizopus* spp. (59.17%), *Cladosporium* spp. (51.67%), *Penicillium* spp. (38.33%), *Fusarium* spp. (36.67%) *Aspergillus* spp. (20%) and *Mucor* spp. (4.17%). *Aspergillus flavus* was the most aflatoxin producing fungus isolated and occurred on 16.7% of all samples. Surface drying of cassava root pieces on bare ground rock surface, polypropylene sheet, rusty iron corrugated sheet roof and spread under roof (indirect sun drying) were some factors which were related to aflatoxin contamination. Other factors were fermentation of cassava products on rock surface, in polypropylene bags, heap under roof, heap on floor and cover with polypropylene bags/sheet, banana leaves, cassava peels and tree leaves. Storage practice positively significant correlated with aflatoxin contamination where cassava products were heaped under roof and stored in polypropylene bags.

DECLARATION

I, Juma Chacha do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution for a degree award.

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The above declaration is confirmed;

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DEDICATION

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LIST OF SYMBOLS AND ABBREVIATIONS

cfu/g	colony-forming unit per gram
COSCA	Collaborative Study of Cassava in Africa
DALDO	District Agricultural and Livestock Development Officer
DED	District Executive Director
DNA	Deoxyribose Nucleic Acid
E	East
FDA	Food and Drug Administration
GPS	Global Positioning System
HACCP	Hazard Analytical and Critical Control Point
IITA	International Institute of Tropical Agriculture
LD	Lethal Dose
masl	meters above sea level
ppb	part per billion
ppm	part per million
S	South
spp	species
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
TFDA	Tanzania Food and Drugs Authority
USAID	United States Agency for International Development
UV	Utra Violet
WHO	World Health Organization

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Cassava Production and Processing in Tanzania

Cassava (*Manihot esculenta* Crantz) is an important crop grown for staple food and income generation in Tanzania (Kavia *et al.,* 2007). It has stable yields especially in the semi-arid areas where cereals fail due to its drought tolerance and low soil fertility properties. Cassava is cultivated and produced in almost all regions of Tanzania. The main producing areas are Mwanza, Mtwara, Lindi, Shinyanga, Tanga, Ruvuma, Mara, Kigoma, Coast and most regions in Zanzibar. Cassava is used in fresh and dried products and flour form in most villages and alcoholic beverages in a relatively few villages (COSCA Tanzania, 1996).

Most farmers in Tanzania use indigenous technologies of processing cassava by peeling, slice the roots into products (*makopa*) or crumbs (*udaga*), fermentation (solid or wet state) and drying (Muzanila *et al*., 2000). The most common processed cassava products in Tanzania are: (i) '*Makopa'*, whereby whole or split roots are sun-dried to low moisture content after peeling. (ii) '*Udaga'*, whereby peeled roots are dried in the sun for 1 – 2 days depending on weather conditions, then heaped and covered with leaves or old sacks to allow fermentation for three days after which moulds growth is scraped off, roots pounded into small pieces and dried for two more days depending on weather condition. The whole process takes about 5 – 7 days. (iii) *Kivunde*, whereas peeled roots are soaked in water for 2 – 4 days, then shake-dry to remove excess water, broken into small pieces and sun or smoke-dried for about five days; (iv) *Chinyanya*, whereas peeled cassava roots are sliced or

crushed into smaller pieces and sun or smoke dried for about two days (Mkamilo, 2005). Generally, cassava is sun-dried on open air like on bare ground, on the shoulders of paved roads and on flat rooftops. During rainy season the cassava may not dry easily due to high humidity, inadequate sunshine and exposure to rainy that enhance mould growth and aflatoxin formation. The cassava products are stored in granaries, bare ground and floor and in the huts. The safety and quality of cassava products is usually inadequately assured because of various storage pests including moulds infection which may results in contamination with mycotoxins (FAO, 2005).

1.2 Justification

Despite its importance in the food systems, cassava production is declining due to pests, management factors and poor post-harvest handling techniques at farm level (COSCA Tanzania, 1996). A major constraint is the occurrence of aflatoxins produced by *Aspergillus* spp. caused by poor processing and storage conditions. *Aspergillus flavus* growth leads to cassava products quality deterioration, unfit for trade (marketing), household and public consumption.

It is estimated that 25% of the world food crops are affected by mycotoxins each year (FAO, 2001a). In 1967, 15-year old boy in Uganda died after eating cassava dish, which was later, found to contain 1700 $\mu g\ kg^{-1}$ aflatoxins (Kaaya and Warren, 2005). In Tanzania, aflatoxins from maize and groundnuts have been associated with stunted growth in children (Kimanya *et al.*, 2008). Current information on incidence of *Aspergillus* species and aflatoxin contamination of various crops and food items in Tanzania is limited. So there was a need to conduct study on incidence of *Aspergillus*

species and aflatoxin contamination in stored dried cassava products for developing appropriate control strategies. The study was focus to evaluate processing and storage conditions, isolation and identification of *Aspergillus* spp. infection and detection of aflatoxin in stored dried cassava products in selected regions of Tanzania.

1.3 Objectives

1.3.1 Overall objective

Effect of processing and storage conditions on incidence of *Aspergillus* spp. and aflatoxin contamination in stored dried cassava products.

1.3.2 Specific objectives

(i) To evaluate processing and storage conditions of dried cassava products in selected regions of Tanzania.

(ii) To determine the occurrence of *Aspergillus* spp. in stored dry cassava products and influence of methods of processing and storage.

(iii) To determine aflatoxin content in dried cassava products and influence of processing and storage.

CHAPTER TWO

2.0 GENERAL LITERATURE REVIEW

2.1 Aflatoxin

Aflatoxins are naturally occurring mycotoxins that are produced as secondary metabolites by many species of *Aspergillus* (*Aspergillus flavus, A. fumigatus*, *Aspergillus parasiticus* and *A. niger)* (Kaaya and Eboku, 2010. The four major naturally produced aflatoxins are known as B_1, B_2, G_1 and G_2. "B" and "G" refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively (Wild and Gong, 2010).

2.2 Ecology of Aflatoxin-Producing Fungi

Aflatoxin-producing members of *Aspergillus* are common and widespread in nature. Host crops are particularly susceptible to infection by *Aspergillus* following prolonged exposure to a high-humidity environment, or damage from stressful conditions. Stress can be caused by multiple factors; including use of ahybrid type that is unsuitable for the local geography, drought stress, high temperatures, and/or insect damage. All these factors increase the risk of the crop plant being infected by *A. flavus* and *A. parasiticus*. The main predisposing factor in postharvest aflatoxin accumulation in food is poor storage conditions; namely, excessive heat and moisture, pest-related crop damage and extensive periods of time spent in storage (Wu *et al.*, 2011). *Aspergillus* spp. is mainly saprophytes in soil, decaying vegetation, hay and grains undergoing microbiological deterioration and they can also be pathogen to plants and animals including humans.

Other factors influencing the incidence of fungal infection includes the presence of invertebrate vectors, grain damage, oxygen and carbon dioxide levels in stores, inocula load, substrate composition, fungal infection levels, prevalence of toxigenic strains and microbiological interactions (Horn, 2003). *Aspergillu*s spp. invades all types of organic substrates whenever conditions are favourable for its growth. Favourable conditions include high moisture content and high temperature. Aryee *et al*. (2006) observed that at 12% moisture cassava products had potential for long shelf life but moisture content greater than 12% allows microbial growth. Availability of water is essential for both mould growth and aflatoxin production (Klich, 2007). Optimum temperatures for aflatoxin production are between 24°C and 30°C with variation between strains and substrates (Klich, 2007).

In natural environment *Apergillus* spp. interact with other fungi species and bacteria. Kaaya and Eboku (2010), Essono *et al*. (2007) and Kaaya *et al*. (2006) observed *Rhizopus*, *Mucor*, *Penicillium*, *Aspergillus* and *Fusarium* species in cassava products and maize in Uganda and Nigeria.

2.3 Food and Feed Contamination with *Aspergillus* spp and Aflatoxins

Aspergillus spp colonize a wide variety of food commodities including roots (cassava chips, potato chips), cereals (maize, sorghum, pearl millet, rice, wheat), oilseeds (groundnut, soybean, sunflower, cotton), spices (black pepper, red pepper, coriander, turmeric, zinger), tree nuts (almonds, pistachio, walnuts, coconut), grapes and musts, tiger nuts, shelved bush mango seeds, marketed pawpaw fruit products and herbal drug plants stored for sale and milk, eggs, milk products and meat when animals are

fed with contaminated grains (Jimoh and Kolapo, 2008; Baiyewu *et al*., 2007; Makun *et al*., 2007; Adebayo-Tayo *et al*., 2006; Bankole and Mabekoje, 2004; Williams *et al*., 2004; Erdogan, 2004; Sage *et al*., 2002; Dawlatana *et al*., 2002 and Taveira and Midio, 2001).

Aspergillus spp can also produce aflatoxin in "postharvest" conditions: storage, transportation and food processing. Aflatoxin contamination is a particular problem in maize, oilseeds, spices, peanuts, tree nuts, milk (in the form of aflatoxin B1's metabolite aflatoxin M1) and dried fruit (Shephard, 2008). These fungi produce aflatoxin depends on drought stress and rainfall, suitability of crop genotype for its climate, insect damage and agricultural practices (Wu and Khlangwiset, 2010).

2.4 Effect of Food and Feed Contamination with Aflatoxin

Deterioration in grain quality due to *A. flavus* growth becomes unfit for marketing and consumption. Aflatoxins contamination in grain poses a great threat to human and livestock health as well as international trade. FAO (2001c) estimated 25% of the world food crops are affected by mycotoxins each year. And also crop loss due to aflatoxins contamination costs US producers more than $100 million per year on average including $ 26 million to peanuts ($69.34/ha) (Munkvold, 2003). Estimates of the costs of control of mycotoxins in food products in the United State reported to be $0.5 to $1.5 billion/year and $5 billion/year for Canada. Grower losses were estimated at about $2.6 million/year (Munkvold, 2003).

Bhat and Vashanthi (2003) and Otzuki *et al*. (2001), reported that export of agricultural products particularly groundnuts from developing countries have dropped considerably in recent years resulting in major economic losses to producing countries. In aflatoxin outbreak years in USA, many producers were turned away by grain elevators and other buyers since their crops exceeded the 20 ppb limit (William *et al.,* 2004).

2.5 Human and Animals Healthy Hazards Associated with Aflatoxins

Aflatoxin has been reported to be hepatic, carcinogenic, mutagens, teratogens, immune system suppressing and anti-nutritional contaminants in many food commodities (Williams *et al*., 2004; Adebayo-Tayo *et al.*, 2006). For most species, the LD_{50} value ranges from 0.5 to 10.0 mg/kg body weight.

Animal species respond differently in their susceptibility to the chronic and acute toxicity of aflatoxin. A dose of 0.25 ppm in turkey pouts and ducklings impairs growth, a dose of 1.5 ppm in broilers and 4 ppm in Japanese quail has a negative effect on growth, a dose of 0.2 mg/kg body weight can cause a decrease in weight gains in calves, a dosage of 15 mg/kg for 30 days cause strong cytotoxic effects and haemolytic anaemia in rabbits (Verma *et al.*, 1998). A study in West Africa showed a significant correlation among the aflatoxin exposure and stunted growth in children who are exposed to aflatoxin right from neonatal stages (Gong *et al.,* 2002; Gong *et al.,* 2003). Intake of these aflatoxin-contaminated foods above the level considered to be safe may be harmful to human beings and other animals.

The aflatoxin limit level of 20 μgkg^{-1} is recommended by WHO, FDA (Food and Drug Administration) of United States and Codex Alimentarius Commission for food intended for human consumption (FAO, 2004). The tolerance levels of aflatoxin M1 in dairy products for infant diets being 0.05 – 0.5 ppb milk. Small repeated doses gradually elevate the risk of liver cancer and in children's impair growth and immune system (ICRISAT, 1998). Prolonged exposure to doses of 50 ng aflatoxin B1/kg/day has clinically significant effects, no animal species has found to be resistant to the effects of aflatoxin (Murphy, 2006).

Ingestion of higher doses of aflatoxin can result in acute aflatoxicosis, which manifests as hepatotoxicity or, in severe cases, fulminant liver failure (Fung and Clark, 2004). The amount of this metabolite decides the species susceptibility as this can induce mutations by intercalating in to DNA, by forming adduct with guanine moiety in the DNA (Smela *et al.,* 2001). In 1967, aflatoxin was circumstantially associated with death of a 15 year old boy in Uganda after eating a sample of cassava, which was later found to contain 1700 μgkg^{-1} aflatoxin (Kaaya and Warren, 2005). According to Gong *et al*. (2002; 2003) and Egal *et al.* (2005), 90% of children in Benin and Togo were exposed to aflatoxin in groundnut, which led to a measurable impairment of child growth.

In 2005 at least one hundred people died and several hundred became ill in Kenya after consuming mycotoxin contaminated maize (Aziz-Baumgartner *et al*., 2005). Over 98% of individuals tested in West African countries were positive for aflatoxin exposure (Gong *et al*., 2002; 2003). Diseases modulated by mycotoxins accounted

for 40% of the lost disability adjusted life years in 1993 World Bank reported on human health (Jaffee and Henson, 2004).

CHAPTER THREE

3.0 PROCESSING AND STORAGE CONDITIONS OF CASSAVA PRODUCTS IN LUSHOTO, RORYA AND UKEREWE DISTRICTS

3.1 Abstract

The study was conducted in Lushoto, Rorya and Ukerewe districts of Tanzania to evaluate diversity of cassava processing methods between October and November 2012. One hundred and twenty households were interviewed on cassava processing and storage practices. *Udaga* was the only cassava product produced from cassava roots by interviewed households in these districts. *Udaga* was a cassava products traditionally produced by many farmers through peeled of cassava roots, surface dried, solid fermented and direct and or indirect sun dried (under roof and or smoke). Cassava products were fermented by heaping under roof, on rock surface near house, on cemented floor, in *tenga* and in polypropylene bags at home. The whole fermentation process spent 1 – 9 days. During fermentation, cassava was covered either by banana leaves, or tree leaves, or cassava peels, or old cloth, or cactus leaves, or polythene sheets and sometimes left uncovered. The indirect sun drying (under roof) prolonged drying duration of cassava products for 84 – 170 h more than normal sun drying which is 12 – 24 h. The cassava products were stored mainly for less than one month (52.5%), 1 – 2 months (36.7%) and few in 3 – 4 months (7.5%) and 5 – 6 months (3.3%). The storage methods/tools mostly used for storing cassava product for more than one month were polypropylene bags (28.3%), platform-like/under roof (15.0%) and plastic containers (4.2%). The survey noted that traditional methods of cassava processing produced poor quality (unhygienic) *udaga* in the study areas. There is need to educate farmers on improved methods for quality

ugada production and develop low cost methods that are within the resource aptitude of farmers in cassava producing areas of Tanzania.

Key words: **Processing methods, cassava, storage methods, *ugada***

3.2 Introduction

Cassava (*Manihot esculenta* Crantz) is an important crop grown for staple food and income generation in Tanzania (Kavia *et al.,* 2007). It has stable yields especially in the semi-arid areas where cereals fail due to its drought and low soil fertility tolerance. The government has been emphasizing to rural households the need of cultivating cassava (Laswai, 2006). Main cassava producing areas in Tanzania include: the coastal strip along the Indian Ocean (48.8%), around Lake Victoria (23.7%) and along the shores of Lakes Nyasa (13.7%) and Tanganyika (7.9%). In these areas, cassava is regarded as the first or second staple food (Mtunda *et al.*, 2002).

The main cassava producing regions are Mwanza, Mtwara, Lindi, Shinyanga, Tanga, Ruvuma, Mara, Kigoma, Coast and most regions in Zanzibar. Cassava is used in fresh and dried products; and flour form for making various dishes in most villages including alcoholic beverages in few villages (COSCA Tanzania, 1996). Most farmers in Tanzania use indigenous technologies of processing cassava by peeling and slicing the roots into products or crumbs followed by fermentation (solid/wet state) and then drying (Muzanila *et al.*, 2000). The most common processed cassava products in these distracts is *udaga*, whereby peeled roots are dried in the sun for 1 – 2 days depending on weather conditions, then heaped and covered with leaves or old sacks to allow fermentation for three days after which moulds growth is scraped off, roots pounded into small pieces and dried for two more days depending on weather condition. The whole processes take about 5 – 7 days (Mkamilo, 2005).

Generally, cassava is sun-dried on open air like on bare ground, on the shoulders of paved roads and on flat rooftops. During rainy season the cassava may not dry easily due to high humidity, inadequate sunshine and exposure to rainy that enhance mould growth and aflatoxin formation. The cassava products are stored in granaries, bare ground or floor and in the huts. The safety and quality of cassava products is usually inadequately assured because of various storage pests including moulds infection which may results in contamination with mycotoxins (FAO, 2005).

Despite its importance in the food system, cassava production is declining due to pests, management factors and poor post-harvest handling techniques at farm level (COSCA Tanzania, 1996). Unprivileged processing and storage leads to cassava products quality deterioration, unfit for trade (marketing), household and public consumption in Tanzania. Bankole and Adebanjo (2003); pointed out that poor physical quality, chemical contamination, bacterial or mycotoxins contaminations are some of the factors that threat the food quality and safety. Likewise Manjula *et al.* (2009) reported that the low quality and safety of foods in Africa have a significant impact on human and animal health and are a major constraint to growers who need access to more remunerating markets. This study focused on assessment of processing and storage conditions of cassava products in Lushoto (Tanga regions), Rorya (Mara regions) and Ukerewe (Mwanza regions) districts.

3.3 Materials and Methods

3.3.1 Study areas

Survey was conducted in Lushoto (Tanga region), Rorya (Mara region) and Ukerewe (Mwanza region) districts between October and November, 2012 to study different

cassava processing and storage methods. Lushoto district is situated in the Northern part of Tanga region at 4^0 25’ – 4^0 55’S and 30^0 10’ – 38^0 35’ E. The district experiences bimodal rainfall pattern (October to December short rains and March to June long rains) ranging from 500 – 2000 mm per annum.

Rorya district is located in the northern-west part of Tanzania and lies between latitudes 10^0 00’ – 10^0 45’ S and longitudes $33^0$30’ – 35^0 00’ E, with an altitude range from 800 to 1 200 masl and temperatures varying from 14°C – 30°C. The annual rainfall ranges between 700 – 1 200 mm per annum with unimodal rainfall regime beginning from February to May with occasional unpredictable short rains in September to December (around Lake Victoria).

Ukerewe district is an island in the Lake Victoria situated at latitudes $1^0$45’ – $2^0$15’ S and longitude $32^0$45’– $33^0$45’ E. It receives a bimodal rainfall (October – December short rains, February – May long rains) ranging from 900 – 200 mm annually. Temperature ranges between 21^0C and 28^0C and relative humidity of 35% to 60%.

3.3.2 Cassava harvesting

Cassava roots were harvested by hands (uproot) with the aid of a hand hoe (dig up) and left at the field or transported to the rock site or homestead for processing. The tall and big roots were cut by bush knives or big knives into two or more pieces to aid peeling and transportation.

3.3.3 Cassava processing

Farmers used to process cassava at homestead for fear of theft at the field and around rock site. Cassava peeling was done by hand using knives. The peeled roots were left as a whole or further sliced to reduce the size (2 – 5 cm) of the pieces before further processing was done. The peeled and sliced cassava roots were then processed through either mechanical or traditional methods.

3.3.3.1 Mechanical processing

Cassava was being processed mechanically by peeling, washing of peeled cassava, grating by using grater machine, dewatering by pressing and sun drying for 12 h or more depending on sun shine intensity, relative humidity and drying materials. Generally, mechanical processing of cassava products spent shorter duration than traditional processing

3.3.3.2 Traditional processing

Traditional methods of cassava processing were general in Lushoto, Rorya and Ukerewe districts whereas various techniques were used to produce *udaga* through solid fermentation. However, there were differences in processing conditions, storage conditions and size of *udaga* among districts.

Cassava processing in Ukerewe was through peeling of cassava roots, sundry for 1–10 h for superficial drying then were heaped on the floor or rock surface or put in polypropylene bags and covered with tree leaves, banana leaves, old fish or mosquito nets, old clothes, fold in polyethylene and polypropylene sheets and allowed to ferment for 2 – 4 days (Fig. 3.1 and 3.2). During this period the mold grew on

cassava roots. The fermented cassava were then scraped off to reduce mold and crushed. The cassava products were then subjected to the second fermentation for 1 – 2 days, followed by sun–drying for 1 – 4 days depending on the sun intensity. Few farmers were skipping second fermentation by peeling of cassava roots, sun-dry for 6 – 8 h, ferment for 3 – 4 days then scrap off to reduce mold, crush followed by sun–drying for 1 – 2 days to obtain *udaga*. The *udaga* was smooth, small-sized boll to flour-like form.

In Lushoto district, cassava was processed traditionally by peeling, sun-dry for 1 – 4 h, fermented by heaping in polypropylene bags or *tenga*, heaped under roof or on the floor, covered with banana leaves, grasses, or old polypropylene sheets in the house for 3 – 7 days to allow mold growth. Then fermented cassava products were sun-dried on roof for 2 – 5 days or spread under roof and smoking for 3 – 7 days for drying. Generally, fermentation process took about 2 – 7 days and drying about 25 – 169 h depending on daily weather condition (Fig. 3.3). In this district the *udaga* size was in whole or halved cassava roots.

In Rorya district, cassava was initially processed as the way it was done in Ukerewe district (Fig. 3.1 and 3.2). The fermented cassava roots were either scraped off or not or mixed with large quantity of peeled unfermented chopped cassava roots (the fermented cassava roots were used as source of inocula) to underwent second fermentation for 3 – 5 days. After second fermentation the cassava product were smoothen by crushing them, before sun-dried for 1 – 2 days.

In case of shortage of food or high demand of cassava products in the market short-cut method was applied whereby cassava roots were peeled, chopped and fermented for 3 – 4 days then sun-dried for 1 – 2 days or cassava roots were peeled, fermented for 3 – 4 days, the fermented, chopped, then sundried for 1 – 2 days to obtain a *udaga* in rough large bolls to rough small size. The *udaga* has different names in this district depending on ethnic group e.g. *konzo* by Luo, *Obhotagha* by Simbiti and *Amaghoshe* by Kurya.

This study found that a complete common traditional processing of cassava took about 6 –18 days in Lushoto, 6 – 11 days in Rorya and 4 – 10 days in Ukerewe district. The cassava products were milled singly or mixed with maize, millet and or sorghum to flour. The flour was the staple food for household and was used to prepare stiff porridge (*ugali*), the dish which had different names based on ethnic groups in the location, for example, *bada* in Lushoto and *ubhukima* in Rorya.

3.3.4 Cassava storage

In Rorya, farmers were storing cassava products in polypropylene and plastic containers from 1 – 6 months. Farmers in Ukerewe were storing cassava products in polypropylene bags for 1 – 4 months and in Lushoto farmers stored cassava products by heaping under roof and in polypropylene bags for 1 – 6 months.

Generally, cassava products were being stored in farmers' houses or shelter made of bricks, mud and or wooden structures. The floor of storage structures were made of earth or cement and thatched with iron corrugated sheets or grass. Some storage

structures were not well thatched so they were found leaking and therefore caused growth of microorganisms on the stored cassava products.

3.3.5 Statistical analysis

Qualitative data of fermentation materials, storage materials, drying material, fermentation duration, drying duration and storage duration from the questionnaires were coded. After that the coded data were analysed by using Statistical Package for Social Science (SPSS 16th edition) to investigate frequency distribution (percentages), means and chi-square to compare frequencies among categorical variables.

3.4 Results

3.4.1 Cassava harvesting

It was observed that cassava products were being processed mechanically by 2.5% and traditionally by 97.5% households in Ukerewe while 100% traditional processing was taking place in Lushoto and Rorya districts (Table 3.2). This survey noticed that only one farmer was processing cassava products by grater machine in Ukerewe district and other two were possessing motorized graters machine without utilizing them, one in Mukunu village and the other one in Malegea village. Many farmers were not processing mechanically due to high cost of running the grating machines especially fuel.

3.4.2 Cassava processing to obtain *udaga*

Cassava peeling was done by hand using knives. The peeled cassava roots were left as a whole or further sliced to reduce the size of the pieces before further processing.

The peeled, sliced cassava roots were then processed through either mechanical or traditional methods.

3.4.2.1 Mechanical processing

Cassava was being processed mechanically (0.83%) by peeling, washing of peeled cassava, grating by using grater machine. Then, the grated cassava products were de-watered by pressing and sun dried for 12 h or more depending on sun shine, relative humidity and drying materials.

3.4.2 .2 Traditional processing

Traditional methods of cassava processing was common (99.17%) in Lushoto, Rorya and Ukerewe districts (Fig. 3.4) whereas various techniques were used to produce *udaga* (solid fermented cassava products) differing in form and size from one district to another depending on the end user preference. The *udaga* size in these districts are variable, in Ukerewe cassava products are processed in smooth, small sized boll to flour like form, Rorya in large bolls to small but bigger than Ukerewe and whole or halved cassava roots in Lushoto.

Traditional cassava processing in Ukerewe (97.5%) is through peeling of cassava roots and sun-dry of peeled cassava roots for 1 – 10 h for superficial drying. After surface drying the cassava root are heaped on the floor rock surface or put in polypropylene bags and covered with tree leaves or banana leaves, or old fish nets or old mosquito nets, or old clothes and or fold in polyethylene and polypropylene sheets for 2 – 4 days for fermentation. During this period the mould grow on surface

of cassava roots. The fermented cassava were then scraped to reduce mould and crushed. The cassava products are then subjected to the second fermentation for 1 – 2 days. Few farmers are skipping second fermentation by peeling of cassava roots, sun-dry of peeled cassava roots for 6 – 8 h, ferment for 3 – 4 days then scrap to reduce mould and crush followed by sun-drying for 1 – 2 days.

In Lushoto district, cassava was processed traditionally (100%) by peeling, sun-drying 1 – 4 h and ferment by heap in polypropylene bags and *tenga*, heap under roof and on floor covered with banana leaves, grasses and old polypropylene sheets in the house for 3 – 7 days to allow mould growth.

In Rorya, cassava is processed traditionally (100%) by peeling small quantity of cassava roots, sun-dry of peeled cassava roots for 0 – 4 h then fermented by heap in polypropylene bags, on rock surface near the house, on floor covered with cuctus leaves, old mosquito net, cassava peels, cassava and tree leaves (Fig. 3.2) for 2 – 4 days to allow mould growth and become soft (*mbute*).

The fermented cassava roots were scraped or not scraped and mixed (as fermentation catalyst) with large quantity of peeled unfermented cassava roots and crushed or chopped together by bush knife or piece of wood known as *ehori* in Simbiti or Kurya languages. The crushed or chopped cassava roots were then fermented (second fermentation) for 3 – 5 days. After second fermentation the cassava product would be smoothen by crushing then sun-dried for 1 – 2 days.

In case of shortage of food or high demand of cassava products in the market short cut method was applied (*Konzo*) whereby cassava roots were peeled, crushed or chopped and fermented for 3 – 4 days then sun-dried for 1 – 2 days (Luo) or cassava roots were peeled, fermented for 3 – 4 days; the fermented cassava roots would be crushed then sun-dried for 1 – 2 days (to form *mbute* in Kurya or Simbiti languages). Fermentation methods, duration and phases were statistically significant different ($p \leq 0.05$) among the districts.

3.4.3 Drying of cassava products

The methods used to dry cassava products reported by framers in all districts are presented in Table 3.1. Farmers in Rorya and Ukerewe (100%) dried cassava by direct sun drying. In Lushoto, farmers dried cassava products by direct sun drying (37.5%), spread under roof and smoke (20%), under roof only and direct sun drying, under roof and smoke (17.5%) and direct sun drying, under roof (7.5%). The cassava products were spread to dry on platform like under roof only (45%), rusty roof constructed by iron corrugated sheet (32.5%), under roof (17.5%) or on polypropylene sheet and (2.5%) on rock surface (Table 3.1).

Overall, Majority farmers dried cassava products on rock surface (50%) followed by on platform placed under roof (15%) and on roof made of rusty iron corrugated sheet (10.5%). Minority farmers in these districts dried the cassava products on rock surface + polypropylene or polythene sheet and on roof made of rusty iron corrugated sheet + platform placed under roof (5.8%), on cemented floor (5%), on polypropylene or polythene sheet (4.2%), on rock surface + cloth + polypropylene or polythene sheet (2.5%) and iron vessel (0.8%).

The results from this study showed that farmers in all districts (77.5%), dry cassava between 12 to 48 h. Few farmers 17.5%, 4.2% and 0.8% dry cassava products between 49 to 85 h, 86 to 122 h and 160 to 169 h respectively. Farmers dry cassava between 12 to 48 h in Rorya (100%), Ukerewe (95%) and Lushoto (37.5%). In Lushoto 47.5% farmers and 5% farmers in Ukerewe dry cassava between 49 to 85 h. Drying duration , methods and tools were statistically significant ($p \leq 0.05$) differ among the districts.

The interviwed farmers in Lushoto and Ukerewe districts reported that the drying durations of cassava products were protracted during rain season. In Ukerewe district, the rain interfered the drying duration of cassava products. It caused the cassava products to be shifted from the rain to the shelter for a certain period of time and then returned back on the sun for drying after the rain or the following day depending on weather condition. The under roof drying was the other cause of extended drying period in Lushoto district because the cassava products were not received direct sunshine. These conditions promoted mould growth on the cassava products.

3.4.4 Cassava storage

The results of the survey on storage methods and storage materials used by farmers for cassava products are presented in Table 3.3. It was observed that 47.5% of farmers stored their cassava products for more than one month. It was found that 52.5% of farmers stored cassava products for very short period of time. Some farmers (59.6%) stored cassava products in polypropylene bags (28.3%) heap them

under roof (16.7%) and in plastic containers (4.2%). Farmers stored cassava products by heaping under roof in Lushoto (45%), Ukerewe (45%) and Rorya (20%). Storage containers used were polypropylene bags (20%) in Lushoto and plastic containers in Ukerewe (10%) and Rorya (2.5%).

Generally, cassava products were stored in farmers' houses or shelter made of bricks, mud and or wooden structure with earth or cemented floor thatched with iron corrugated sheets and or grasses. The reason for storing cassava products in the houses or shelters was to avoid theft. Some houses or shelters were not well made to resist leaking when it rains thus allowed wetness of stored cassava products which provided a favourable conditions for the growth of mold.

More than fifty percent (52.5%) of cassava products of samples collected from farmers in these districts were stored less than 1 month. Cassava products were stored for 1 to 2 months (36.7%), for 3 to 4 months (7.5%) and for 5 to 6 months (3.3%). Numerous cassava samples were stored for less than one months in Rorya (75%) and for 1 to 2 months in Ukerewe (52.5%) and Lushoto (42.5%) and for fewer months in Rorya (15%). Cassava products (12.5%) in Lushoto, (7.5%) in Rorya and (2.5%), in Ukerewe were stored for 3 to 4 months. Only some cassava samples (7.5%) in Lushoto and (2.5%) in Rorya were stored for 5 to 6 months. Storage duration and storage methods were statistically significant ($p \leq 0.05$) different among the districts.

Reason given by farmers for not storing cassava products were shortage of food (high demand of food than supply). Cassava could not be stored for longer period of time without being destroyed by insects and other storage organisms. Decline of cassava production was due to weather changes, edaphic factors, diseases and insects pests.

3.5 Discussion

The study found that farmers harvest, process and store cassava products for future consumption and other uses. This implies that cassava products are vital source of food and income for majority of people in these locations. The main processing period in Lushoto and Rorya districts was during dry season when there was adequate sunshine and less rain for easy drying of cassava products.

In Ukerewe district farmers are used to process cassava products during rainy season for easy harvesting, intensive use of labour and time saving (integrating of harvesting and land preparation) while they were preparing their fields for planting next season crops like cassava and other crops depending on farmers preference. This alternation in time of cassava processing and drying implies that cassava was dried in all seasons, the process that exposes cassava products to various weather conditions. During rainy seasons the cassava products may not dry easily due to high humidity, inadequate sunshine and exposure to rain.

The survey showed that farmers practiced both traditional and mechanical methods of cassava product processing whereby solid state heap fermentation under roof

(indirect sun-drying) and direct sun-drying of cassava products were performed. Likewise, Manjula *et al.* (2009) and Westby *et al*. (2002) reported that cassava chips and flour were processed by the traditional method whereby cassava roots were sometimes fermented in Mtwara, Mkongi, Zanzibar and Ugunja Island regions of Tanzania.

Traditional methods of cassava processing was general in Lushoto, Rorya and Ukerewe districts whereby *udaga* was the only cassava products produced in various forms or size. In Ukerewe cassava products were processed in smooth, small-sized bolls to flour-like form, while in Rorya they were processed in small to large bolls but bigger than Ukerewe and whole or halved cassava roots in Lushoto. Mkamilo (2005) reported that in Tanzania, traditional cassava processing is common whereas various techniques are used to produce different processed products from one place to another depending on the intended use of the end product.

The survey in these districts noticed that in traditional methods, the harvested cassava roots were peeled and might be cut into varying sizes, dried directly on the sun on bare ground or other drying materials or heaped to ferment before the drying process. During fermentation fungi grew on cassava root surface with white, black, green or orange colouration. Likewise, Manjula *et al.* (2009) and Westby *et al*. (2002) reported that cassava chips and flour were processed by the traditional methods by manually peeling the roots, chopped them into small pieces, and sometimes fermented and finally manually pounded, sun-dried and stored in Mtwara, Mkongi, Zanzibar and Ugunja Island regions of Tanzania. During fermentation

cassava products were covered by banana leaves, tree leaves, cassava peels, old cloth, cactus leaves and polythene sheets and sometimes left uncovered.

In mechanical methods, cassava roots were grated by machine, de-watered by hand pressing and then direct sun-dried. Mechanical method of cassava processing is not guaranteed to be free from contamination because during peeling contaminants might get into cassava.

Generally, in Lushoto district cassava products were dried while retaining the fungal growth until the time of preparation for milling when they were scraped off. Sometimes in Rorya district, fermented cassava roots were dried and milled for food without scrapping off the fungi which grew during fermentation. Similarly, the same observation in Ghana by Wereing *et al.* (2001) reported mold growth on dried fermented cassava product (*Kokonte*) in Ghana. Likewise Kaaya and Eboku (2010) reported mold growth on fermented cassava roots in Kumi districts, Eastern Uganda. The processing tools might be the source of inocula transmission, the scraping knives are not sterilized after each scrapped root and sometimes the processing tools are being exchanged from one household to another.

This survey recognized that cassava products form and size were the results of harvesting season and end user preferences. The cassavas harvested at dry season were in large bolls, some pieces to whole roots and those harvested during rainy season were in small bolls to flour-like. For example, majority of cassava products were crushed into small bolls to flour-like especially in Ukerewe district as aid of fast drying, small to large bolls in Rorya and some pieces or whole roots in Lushoto,

although the fermented cassava were likely to be crushed. Crushing is experienced when the drying is to be achieved fast (Kaaya and Eboku, 2010). Out of farmers preference cassava products would be in flour form due to shifting of crushed cassava product from rain and return back to the sun which enhanced breaking from bolls into flour-form. Similarly heaping of cassava products that are not thoroughly dried leads to heat emission the situation that boost breakage of cassava bolls into flour-form.

The survey further revealed that cassava roots were dried on bare ground by many farmers and 50% of the farmers dry cassava products on rock surface. Other farmers dried cassava products on rusty iron sheet roof and platform-like (under roof only). Very few farmers dry their cassava products on polypropylene sheets and cemented floor. Other materials found to be used for drying cassava products were iron vessels, dry grass or grass thatched roof (pieces or whole cassava roots), cattle hides, cloths like bed sheets and *khanga*. The result in this survey is reliable with Kaaya and Eboku (2010) who reported that cassava had been dried on rock surface by many farmers and very few on polyethylene sheets or concrete paved surfaces in Kumi district-Eastern Uganda. Similarly, FAO (2005) showed that cassava in Uganda is dried on any open surface including bare ground, bare rock and on shoulders of roads. Poor drying material may be caused by financial crisis of the farmers in Tanzania which hold up the failure of purchasing improved drying materials like polyethylene sheets and or cemented floors. Kaaya and Eboku (2010) reported that drying cassava on bare ground, rock surface and shoulder of roads was attributed to the low income status of the farmers in Uganda too.

Drying of cassava roots on the bare ground or any other materials rested on ground like polypropylene, bed sheets and *khanga* exposes the cassava to contamination with soil, dust, fungal spores and other foreign materials. Exchanging of drying materials like polyethylene sheet among farmers may be the source of inocula dissemination especially when one farmer among the chain has contaminated cassava products. Generally, rock surface and cemented floor are neither cleaned nor sterilized for life in many areas; they may be also the sources of inocula.

This study noticed that cassava products had been dried for 12 to 170 h depending on weather condition and methods of drying. Rainy, cloudy, misty and high atmospheric relative humidity delayed drying of cassava products in Ukerewe district. Likewise indirect sun-drying (under roof) prolonged drying duration of cassava products in Lushoto district, hence chance for microbial growth. Delay in drying period may promote microbes and insect infestation and can lead to discolouration and changes in flavour (Knoth, 1993).

Generally, in Lushoto, Rorya and Ukerewe districts cassava products were being stored in farmers' houses or shelter (used as house and kitchen) made of bricks, mud and or wooden structure with earth or cemented floor thatched by iron sheets and or grasses. The reason of storing cassava in houses or shelters was to avoid theft of their food. Similarly, Kaaya and Eboku, (2010) showed that most farmers in Kumi stored dried cassava in the huts which double as housing and kitchen in order to protect the produce against theft. Fandohan *et al*. (2005) reported that such structures are built using mud and wood and may have little or no ventilation. Mestres *et al.* (2004)

observed that heaps of yam chips in a poorly ventilated room was not favourable for moisture loss and favoured mold growth and insect infestation. In these structures cassava products were being stored in polypropylene bag, heaped under roof and in plastic containers in Lushoto, Rorya and Ukerewe districts. Likewise Kaaya and Eboku (2010) mentioned that cassava was either heaped or stored in various containers like Jerri cans, clay pots or polypropylene bags in Kumi district-Eastern Uganda.

Majority of farmers in Lushoto Rorya and Ukerewe districts stored cassava products for less than one month. This may be due to scarcity of food that all cassava products processed were used as food or sold within a short time, avoiding storage insects and microbes. Majority farmers among those who storing cassava products for more than one month, have been stored cassava products for 1 – 2 months. Contrary, Wereing *et al*. (2001) reported similar storage period averaging 8 –12 weeks among majority of farmers in Ghana who stored *kokonte*, a Ghanaian dried fermented cassava product. The study reveals that most cassava products processed traditionally in these districts are poor in quality and low safety. Currently, poor quality and safety of cassava products is attributable to poor processing techniques, which are a draw back in the exploitation of market (Mkamilo, 2005).

3.6 Conclusion

Cassava were processed traditionally by many farmers as a result poor quality *udaga* was produced that might cause dangerous conditions to consumers. The situation was due to illiterate in high quality processing and storage of cassava products.

Education on high quality udaga processing and storage is very important. The government should bear in mind the way it can help in knowledge dissemination to farmers and processors so that good quality udaga can be produced. The processing machines provided to farmers should be of low running cost that farmers can afford rather than those provided before that farmers couldn't use due to high running cost.

Table 3.1: Drying systems and period for sampled dried cassava products in Lushoto, Ukerewe and Rorya districts

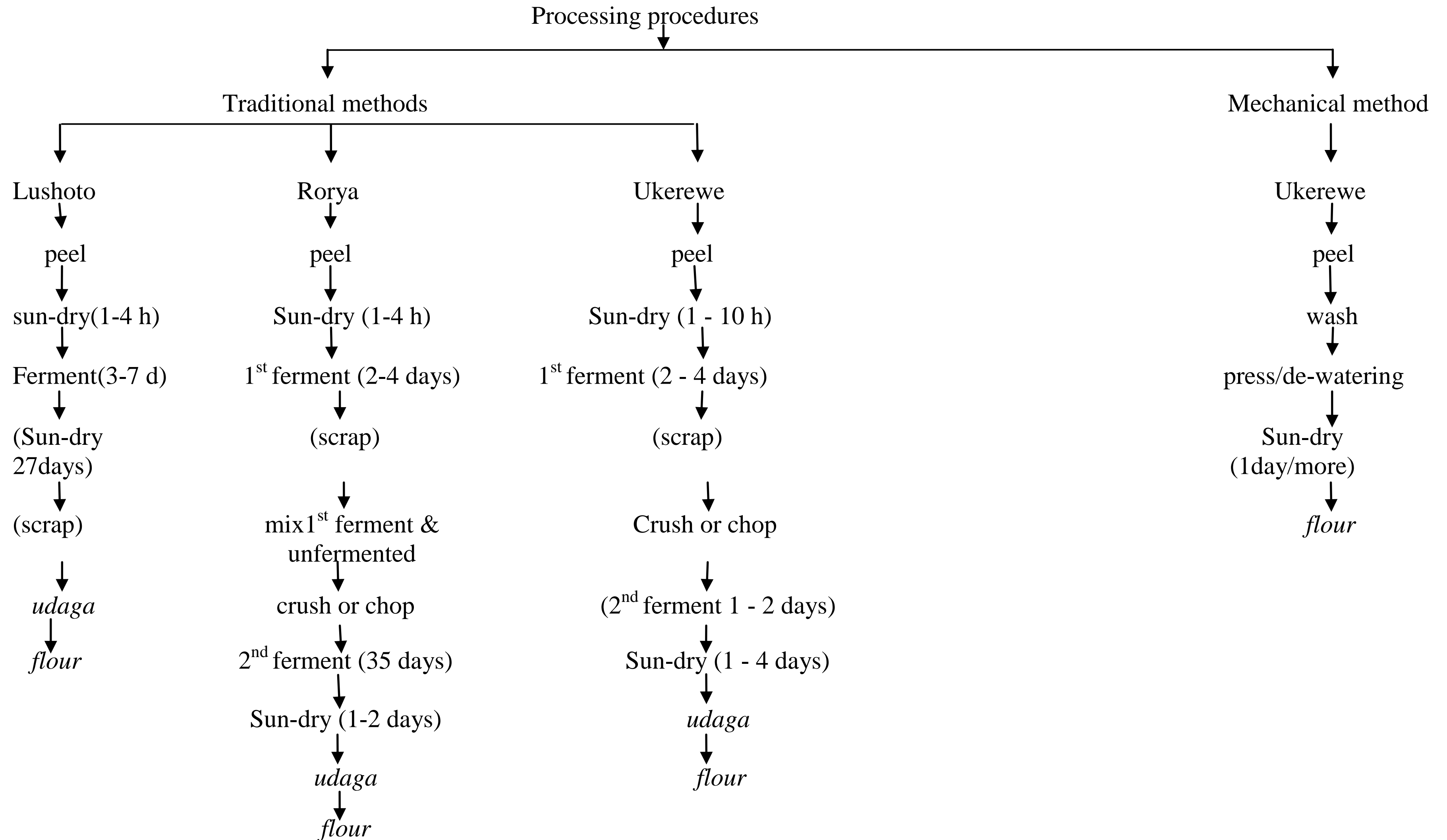
Parameter	Category	Lushoto (N = 40)		Ukerewe (N = 40)		Rorya (N = 40)		Mean	χ2	Asymptotic significant
		n	%	n	%	n	%	%		
Drying methods	direct sundry	15	37.5	40	100.0	40	100.0	79.2	263.17[a]	0.000
	under roof	7	17.5	0	0.0	0	0.0	5.8		
	under roof and smoke	8	20.0	0	0.0	0	0.0	6.7		
	direct sundry and under roof	3	7.5	0	0.0	0	0.0	2.5		
	direct sundry, under roof and smoke	7	17.5	0	0.0	0	0.0	5.8		
Drying materials	(a)Polypropylene sheet	1	2.5	3	7.5	1	2.5	4.2	199.65[b]	0.000
	(b) Rock surface	29	72.5	30	75.0	1	2.5	50.0		
	(c) Roof made of rusty iron corrugated sheet	0	0.0	0	0.0	13	32.5	10.8		
	(d) Platform placed under roof	0	0.0	0	0.0	18	45.0	1.0		
	(e) As (c) and (d)	0	0.0	0	0.0	7	17.5	5.8		
	(f) As (a) and (b)	5	12.5	2	0.5	0	0.0	5.8		
	(g) Iron vessel	1	2.5	0	0.0	0	0.0	0.8		
	(h) Rock surface, cloth and polypropylene sheet	3	7.5	0	0.0	0	0.0	2.5		
	(i) Cemented floor	1	2.5	5	12.5	0	0.0	5.0	183.87[b]	0.000
Drying duration	12 - 48 hours	15	37.5	40	100.0	38	95.0	77.5		
	49 - 85 hours	19	47.5	0	0.0	2	5.0	17.5		
	86 - 122 hours	5	12.5	0	0.0	0	0.0	4.2		
	160 - 196 hours	1	2.5	0	0.0	0	0.0	0.8		
	12 - 48 hours	15	37.5	40	100.0	38	95.0	77.5		
	49 - 85 hours	19	47.5	0	0.0	2	5.0	17.5		
	86 - 122 hours	5	12.5	0	0.0	0	0.0	4.2		
	160 - 196 hours	1	2.5	0	0.0	0	0.0	0.8		

Table 3.2: Fermentation systems and period for cassava products sampled from farmers in Lushoto, Ukerewe and Rorya districts

Parameter	Category	Rorya (N = 40)		Ukerewe (N = 40)		Lushoto (N = 40)		Mean	χ2	Asymptotic significant
		n	%	n	%	n	%	%		
Fermentation phase	Single fermentation phase	40	100.0	4	10.0	10	25.0	45.0	58.55[d]	0.000
	Double fermentation phases	0	0.0	36	90.0	29	72.5	54.2		
	No fermentation	0	0.0	0	0.0	1	2.5	0.8		
Fermentation method	In tenga	1	2.5	0	0.0	1	2.5	1.7	120.27[c]	0.000
	Heap on rock surface	0	00..0	27	67.5	12	30.0	32.5		
	In plastic container	0	0.0	1	2.5	0	0.0	0.8		
	In polypropylene bag/sheet	2	5.0	12	30.0	16	40.0	25.0		
	Heap on cemented floor	5	12.5	0	0.0	7	17.5	10.0		
	Fold in old mosquito/fish net	0	0.0	0	0.0	3	7.5	2.5		
	Heap under roof	32	80.0	0	0.0	0	0.0	26.7		
	None	0	0.0	0	0.0	1	2.5	0.8		
Fermentation duration	No ferment	0	0.0	0	0.0	1	2.5	0.8	122.47[c]	0.000
	1- 3 days	17	42.5	3	7.5	7	17.5	22.5		
	4 - 6 days	19	47.5	31	77.5	30	75.0	66.7		
	7 - 9 days	4	10.0	6	15.0	2	5.0	10.0		

Table 3.3: Percentage of farmers reporting the use of different storage systems for cassava products

Parameter	Category	Rorya (N = 40)		Ukerewe (N = 40)		Lushoto (N = 40)		Mean	χ2	Asymptotic significant
		n	%	n	%	n	%	%		
Storage method	Fresh consumption (No storage)	31	77.5	18	45.0	12	30.0	50.8	62.5^{b}	0.000
	Under roof	0	0.0	0	0.0	20	50.0	16.7		
	Polypropylene bags	8	20.0	18	45.0	8	20.0	28.3		
	Plastic containers	1	2.5	4	10.0	0	0.0	4.2		
Storage duration	Less than 1 month	30	7.0	18	45.0	15	37.5	52.5	80.7^{b}	0.000
	1-2 months	6	15.0	21	52.5	17	42.5	36.7		
	3-4 months	3	7.5	1	2.5	5	12.5	7.5		
	5-6 months	1	2.5	0	0	3	7.5	3.3		



NB. Processes stages in brackets are optional

Figure 3.1: Steps in cassava processing in Lushoto, Rorya and Ukerewe District in Tanzania

A: Cassava products were covered with cactus leaves, cassava peels and blue old mosquito net.

B: Cassava products were placed in the polypropylene bag, covered with cassava leaves, cassava peels and tree leaves.

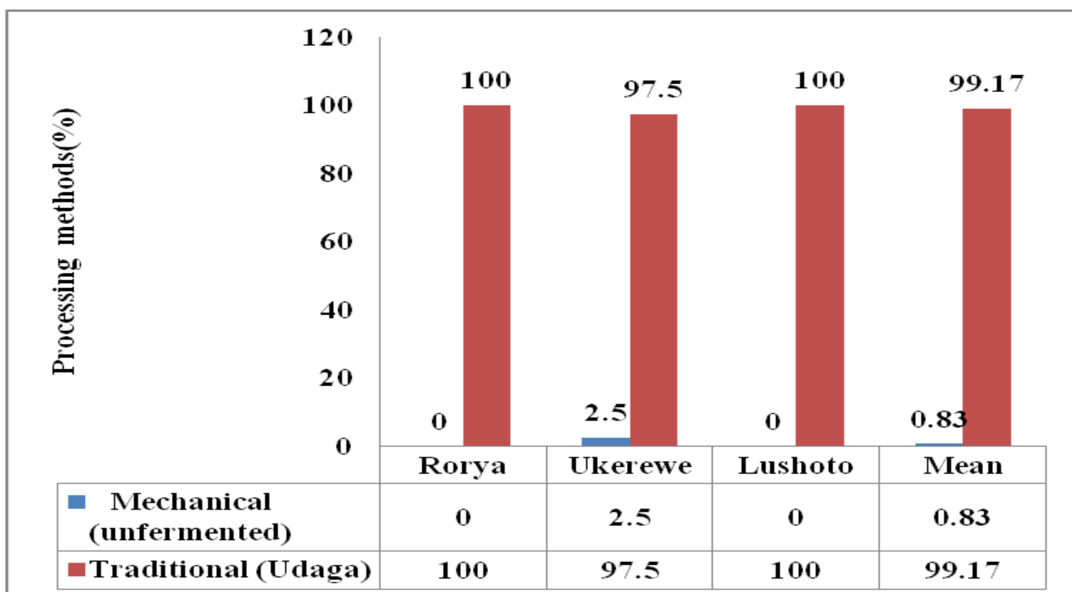
Figure 3.2: Heap fermentation on rock surface

A: Drying of cassava products on cattle hide and polypopylene sheet placed on ground.

B: Drying of cassava products on rock surface.

C: Drying of cassava products on rock surface and fermentation of small amount at the middle.

Figure 3.3: Drying of *udaga* in Rorya district



	Rorya	Ukerewe	Lushoto	Mean
Mechanical (unfermented)	0	2.5	0	0.83
Traditional (Udaga)	100	97.5	100	99.17

Figure 3.4: Cassava products processing methods

CHAPTER FOUR

4.0 ISOLATION AND IDENTIFICATION OF *Aspergillus* spp. AND DETECTION OF AFLATOXIN IN STORED DRIED CASSAVA PRODUCTS OBTAINED FROM LUSHOTO, RORYA AND UKEREWE DISTRICTS

4.1 Abstract

The study was conducted in Lushoto, Rorya and Ukerewe districts in Tanzania to evaluate diversity of cassava processing and storage methods of cassava products in 120 households. Samples of dried cassava products were collected for moisture, pH and microbial growth analysis. The factors that impact on microbial and aflatoxin contamination of these products were established using regression analysis. Cassava products had moisture content 13.87%, pH 5.507% and microbial counts whereby *Rhizopus* spp. was the most prevalent (59.17%) followed by *Cladosporium* spp. (51.67%), *Penicillium* spp. (38.33%), *Fusarium* spp. (36.67%), *Aspergillus* spp. (20%), *Muco*r spp. (4.17%) and *A. flavus* was the most contaminant mycotoxigenic fungus isolated and occurred on 16.67% of the samples. Effort should be made to improve the quality of cassava by educating farmers better-quality processing and storage practices for management of *Aspergillus flavus* and other toxin producing microbes.

Key words: ***A. flavus, microbe count, cassava, incidence, udaga,***

4.2 Introduction

Cassava (*Manihot esculenta* Crantz) is an important food crop in Tanzania, especially in drier areas (United Republic of Tanzania, 2008), grown for staple food and income generation in Tanzania (Kavia *et al*., 2007). Cassava is a liable substrate to fungal flora development and to mycotoxin formation (Essono *et al.,* 2007) if unhygienic processed and stored. The contaminated cassava when consumed as a staple food, become a major dietary source of mycotoxins of importance to both public and animal health such as aflatoxins. Fungi that produce toxins in food are classified into field fungi and storage fungi based on their ecological requirements for growth (Owolade *et al.,* 2005). The first group requires grain moisture above 20% in cereals and often causes ear rot diseases and toxin production before harvest, when the crop is still in the field. The important genera of field fungi include *Fusarium, Cladosporium* and *Alternaria* (Bankole and Adebanjo, 2003). The storage fungi usually grow in grain with moisture content in equilibrium with 70 – 90% relative humidity, which corresponds to less than 18% moisture content in cereals and the most important genera are *Aspergillus* and *Penicillium*. They are infrequently associated with crops in the field, but are also associated with plant debris, plant surfaces, atmosphere and other surfaces where the water activity is relatively low. The risk posed by these mycotoxins in developing countries is compounded by the fact that the financial and technological resources to tackle these problems are very limited in these regions.

Many people in the developing countries are not even aware of the effect of consuming mouldy products. Due to the poor education levels and other

socio-economic factors, even if steps are taken to make food products safe the consumers will be unwilling to pay the extra costs and will still prefer to buy the cheap commodities (Bankole and Adebanjo, 2003). The sub-region also has poorly developed infrastructures such as processing facilities, storage, transportation and skilled human resources. Predictive mycology, by providing tools allowing for the prediction of fungal growth and mycotoxin production, could play a very important role in improving the quality and safety of food (Dantigny *et al.,* 2005).

In addition, although the omnipresent and broad-based nature of most fungi result in these problems occurring worldwide, these become more prominent in tropical developing countries most common when crops are exposed during harvesting and storage, provided that hot and humid weather conditions, improper and unsanitary storage exist for a prolonged period (Hamid *et al.,* 2013; Mardani *et al*., 2011; Obuseh *et al.,* 2011).

The East African countries have tropical climate with an all year round high ambient temperature and relative humidity that provide optimal condition for the growth of toxigenic moulds.

Current information on incidence of *Aspergillus* species in cassava products in Tanzania is limited. So there was a need to conduct study on incidence of *Aspergillus* species and other fungi in stored dried cassava products. This study focused on isolation and identification of *Aspergillus* spp. and other fungi in stored dried cassava products collected from Lushoto, Rorya and Ukerewe districts of Tanzania.

4.3 Materials and Methods

4.3.1 Study area

Survey was conducted in Lushoto (Tanga region), Rorya (Mara region) and Ukerewe (Mwanza region) districts between October and November, 2012 to study different cassava processing and storage methods. Lushoto district is situated in the Northern part of Tanga region at 4^0 25’ – 4^0 55’S and 30^0 10’ – 38^0 35’ E. The district experiences bimodal rainfall pattern (October to December short rains and March to June long rains) ranging from 500 – 2 000 mm per annum.

Rorya district is located in the northern-west part of Tanzania and lies between latitudes 10^0 00’ – 10^0 45’ S and longitudes $33^0$30’ – 35^0 00’ E, with an altitude range from 800 to 1 200 masl and temperatures varying from 14°C – 30°C. The annual rainfall ranges between 700 – 1 200 mm per annum with unimodal rainfall regime beginning from February to May with occasional unpredictable short rains in September to December (around Lake Victoria).

Ukerewe district is an island in the Lake Victoria situated at latitudes $1^0$45’ – 2015’ S and longitude $32^0$45’– $33^0$45’ E. It receives a bimodal rainfall (October – December short rains, February – May long rains) ranging from 900 – 200 mm annually. Temperature ranges between 21^0C and 28^0C and relative humidity of 35% to 60%.

4.3.2 Cassava products sample collection

The totals of 120 samples were collected from 17 villages in study areas whereby 40 samples were obtained from each district of Lushoto, Rorya and Ukerewe. The

respondents (households) were selected randomly in the villages (at least 10 households/village), relative to number of cassava growers in the hamlets. The households were interviewed on cassava products processing and storage methods by using structured questionnaires. The numbers of households interviewed were equivalent to the number of samples collected.

Sampling was performed according to (Kaaya and Eboku, 2010) and 1 kg cassava processed products were obtained from each household. In the situation where many containers were found having processed cassava products in the same store, the representative composite sample was obtained by drawing samples from each container and mixed them thoroughly. Samples were placed in plastic bags (well labeled and sealed) to maintain their conditions.

Global position system (GPS) was used to specify coordinates. The samples were apportioned and transported to Sokoine University of Agriculture (SUA), Morogoro and Institute of International Tropical Agriculture (IITA), Kibaha laboratories where they were stored at $4^{o}C$ for further analyses. The determination of total moisture content, pH and aflatoxin content were conducted at Kibaha IITA laboratory and microbial growth analysis at SUA.

4.3.3 Preparation of cassava samples for laboratory analysis

The processed cassava products were divided into four quarter (sub-samples) each approximately 250 g. The four quarters were milled by small milling machine. The 100 g out of 250 g of each milled sample (flour) was taken as representative

sub-sample, making total of four sub-samples. The left milled sample was kept as back up. Sub-sample 1 was used for moisture content analysis; sub-sample 2 for pH analysis, sub-sample 3 for total aflatoxin content analysis and sub-sample 4 for microbial growth analysis.

4.3.3.1 Analysis of moisture content

Moisture content of collected samples was analyzed using the air oven method (AOAC, 1999). The empty Petri dishes (a) were weighed. Then the Petri dishes with approximately two grams cassava flour (b) of each sample from sub-sample 1 were weighed in duplicate and dried to constant weight at 105°C for 24 h in an oven. Finally the Petri dishes with samples(c) were weighed after being cooled in the desiccators for 30 min. The mean moisture content of duplicate samples were calculated and expressed as percentage on weight loss (Kaaya and Eboku, 2010).

$$\text{Formula: mc \%} = \frac{(b - c) \times 100}{(b - a)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Whereby:

% mc = percentage moisture content,

a = weight of empty Petri dishes,

b = weight of Petri dish + sample before oven drying and

c = weight of Petri dish + sample after oven dry.

4.3.3.2 Analysis of pH

Ten (10) grams flour of each cassava sample from sub-sample 2 was weighed into a 250 ml beaker in triplicates. Then 25 ml of sterile distilled water (sdH_2O) adjusted to pH 7.0 with 0.1 M KOH or 0.1 M HCl was added to each beaker containing samples

and mixed well by rocking for 25 min to allow cassava flour to dissolve thorough before pH determination. The pH was determined by using single electrode *HANNA* pH meter (Zhaoyuan DAMING Instrument Co., Ltd.). The pH meter was calibrated daily prior to start reading with standard buffers.

4.3.3.3 Isolation and identification of fungi

The standard blotter method (Anonymous, 1976) was used to detect a wide range of fungi present in the cassava samples. Cassava flour sample approximately 1 g from each sample was placed on moisten sterile blotter papers (5 ml H_2O) in 100 mm diameter Petri dishes in triplicates. The Petri dishes with samples were incubated for 7 – 14 days at 25^0C in the dark to provide ideal conditions for fungal growth and sporulation. *Aspergillus* spp. and other fungi were identified by macro and microscopic characteristics using keys described by Pitt and Hocking (1999) after observation under compound microscope.

Furthermore, single spore of presumptive *Aspergillus* spp. was sub-cultured on acidified Czapek Dox Agar medium adjusted to pH 5.5 with 0.1M H_2SO_4 to suppress bacteria. The plates were incubated for 7 – 14 days at 25^0C in the dark to provide ideal conditions for fungal growth and sporulation. *Aspergillus* spp. were identified by macro and microscopic characteristics using keys described by Pitt and Hocking (1999) after observation under compound microscope. The percent incidence of fungal species was determined by dividing the number of positive samples for the species by the total number of sample of cassava products analysed (Essono *et al*., 2007).

4.3.3.4 Enumerations of fungi

For determination of *Aspergillus* spp. content, 5 g of ground cassava product was suspended in 500 ml of distilled water (dH_2O). The determination of fungal content was carried out by three-fold serial dilutions. The dilution was made by transferred 1 ml sample solution in each subsequent test tubes contained 9 ml agar solution. Each dilution (1 ml) was added to plate containing melted acidified Czapek Dox Agar medium in three replicates, spread by using glass-rod and allowed to settle. The plates were incubated at 25^0C in the dark for 7 days (Essono *et al.,* 2009). The counts of triplicate plates made from one dilution were averaged together. The colonies that developed in the media were calculated using the formula: cfu/g = (No. of colonies × dilution factor. (cfu/g = No. of colonies × 10^1(dilution factor) + No. of colonies × 10^2 (dilution factor) + No. of colonies × 10^3 (dilution factor))/3. The appropriate dilution factor was selected by considering the plates that contained 10 – 140 colonies. This range of colonies was preferred because more than 40 colonies per plate tended to grow together, making colony counts and isolation difficult. The results were expressed as colony forming units per gram (cfu/g) of sample. The cfu/g was then transformed to coefficient base exponent then $\log_{10}$ (N+1) before analysis (Kaaya and Eboku, 2010).

4.3.4 Statistical analysis

The moisture content, pH and fungal growth were subjected to analysis of variance (ANOVA) after Completely Randomized Design (CRD) experimental lay out by using GenStat Software 14th version. In CRD experimental lay out in three replicates where by the districts were standing for replication, cassava samples for

experimental units and mc, pH and cfu/g for treatments. The differences were separated by using DMRT ($p \leq 0.05$). Multiple Linear Regression analysis was used to compare relationship between variables (cfu/g (*A. flavus*) versus moisture content, pH, storage duration, fermentation duration and drying duration. Regression equation was expressed as:

$Y = 1/n\ (a + b_1X_1 + b_2X_2 + \ldots + l_nX_n + E)$ ………….................................... (2)

Where:-

X1, X_n = independent variable,

a = estimate of the intercept,

b_1, b_n = estimates relate to independent variables (pH, mc, storage duration, fermentation duration and drying duration),

n = estimate number of independent variables,

Y = dependent variable (cfu/g).

4.4 Results

4.4.1 Moisture content and pH of cassava products

The result showed that mean moisture content of cassava products were high in Lushoto followed by Ukerewe and Rorya and were significantly different ($p \leq 0.05$) (Table 4.1). The pH content in cassava samples from Lushoto, Rorya and Ukerewe were 6.9, 4.9 and 4.8 respectively. The pH in Rorya and Ukerewe samples were not significantly different ($p \geq 0.05$) but both differ to those from Lushoto (Table 4.1).

4.4.2 Identification of aflatoxin- producing fungi and other fungi

Results of the incidence of different fungal species isolated and identified from cassava products are indicated in (Table 4.2). The results showed that *Rhizopus* spp.

were the most prevalent (59.17%) fungi followed by *Cladosporium* spp. (51.67%), then *Penicillium* spp. (38.33%), *Fusarium* spp. (36.67%), *Aspergillus* spp. (20.0%), yeast (10.83%) and 4.17% for both *Mucor* spp. and *Curvularia* spp respectively. These fungi were isolated from cassava products in all districts except *A. niger* which was not isolated in casssava products from Lushoto. Also *Mucor* spp. was not isolated from Rorya samples and *Curvularia* spp. was not isolated in Ukerewe samples.

Aspergillus flavus was the most prevalent aflatoxigenic fungus species identfied and occurred in 16.67% of the samples. Gross cultural characteristics, colour of conidial heads and shift in colour with age were used as diagnostic criteria on Czapek Dox Agar medium (CDA). Ultimate identification was based mainly on microscopic observations of micro-conidiogenesis from single spore cultures.

After plating on CDA, mold growth was observed in all samples and counts ranged from 2.0 x 10^1 to 1.874 x 10^6. The *Cladosporium* spp. was the dominant fungus followed by *Rhizopus* spp., *Fusarium* spp. and *Penicillium* spp., *A. niger*, *Mucor* spp., yeast and *Curvularia* spp. (Table 4.3). Many colony counts (45.99%) were observed on cassava product from Lushoto, followed by Rorya (31.13%) and Ukerewe (22.88%).

4.4.3 Correlation coefficient of the relationship between aflatoxin producing fungi and processing and storage conditions

Aflatoxin producing fungi levels were further determined in cassava samples in relation to the moisture content, pH, storage facilities, storage duration, processing

methods and drying duration. The results of the correlation between aflatoxin producing fungi contamination and moisture content, pH, drying duration, fermentation duration and storage duration are presented in (Table 4.4). *A. flavus* (cfu/g) was positive non significant ($p \geq 0.05$) correlated (0.084) to drying duration, that the increase in storage duration have miniature positive influence in the growth of *A. flavus* (cfu/g). *A. flavus* (cfu/g) was negative non significant ($p \geq 0.05$) correlation to fermentation duration (-0.612), storage duration (-0.024), moisture content (-0.482) and pH (-0.561). The association between pH and fermentation duration was positive while the association between pH and drying duration and storage duration were negative. The associations were not significant ($p \geq 0.05$)

4.5 Discussion

The results established that cassava products are vital source of food and income for majority people in these locations but they had been contaminated by number of fungal species, of which most of them are dangerous to human being. *Rhizopus* spp., *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Fusarium* spp., *Mucor* spp., *Curvularia* spp. and yeast have been isolated from 120 samples of cassava products collected from Lushoto, Rorya and Ukerewe districts. Likewise, Kaaya and Eboku (2010) observed *Rhizopus, Mucor, Penicillium, Aspergillus* and *Fusarium* species in cassava products from Kumi district, Uganda. Essono *et al.* (2007) recovered 13 species of *Aspergillus* from 72 samples of dried cassava chips from Cameroon. Wareing *et al.* (2001) isolated predominantly *Fusarium* spp. on Ghanaian cassava chips and to a lesser extent *Aspergillus* and *Penicillium* spp.

The most species of *Aspergillus* isolated in this study was *A. flavus* (16.67%) from samples of heap-fermented cassava in Lushoto (20%), in Rorya (7.5%) and Ukerewe (22.2%), the other was *A. niger* (3.37%). The variation in methods of processing of *udaga*, storage and geographical conditions might be the cause of variation of incidence of fungi in these districts. Similarly, Essono *et al.* (2007) recovered 13 species of *Aspergillus* from 72 samples of dried cassava chips from Cameroon whereby *Aspergillus flavus* was the most predominant of isolated aflatoxin producing species; the others were *A. nomius* and *A. parasiticus.* The majority of these molds are known to produce mycotoxins. *Aspergillus flavus* is known to produce aflatoxin (Klich, 2007). *Aspergillus niger* produces oxalic acid and malformin (Moake *et al*., 2005).

These fungal species are mostly related with processing and storage practices in these districts. The main processing period is during dry season when there is adequate sunshine and less rain for Lushoto (land preparation is done during dry season) and Rorya districts and during the rainy season in Ukerewe for easy harvesting while they are preparing their farms (land preparation done during rainy season). In traditional methods, the survey showed that farmers practice both solid state heap fermentation and direct sun-drying (in mechanical methods) of cassava products. In traditional methods, the harvested cassava roots were peeled and cut into varying sizes, dried by direct sun on bare ground or other drying materials or heaped to ferment before the drying process. Then the cassava roots were fermented for average of 4 – 6 days (majority). During fermentation fungi grow on cassava products with white, black, green, yellow or orange colouration. The fungi may be

removed by scrapping with small knives or piece of sticks before crushing and or before or after drying the cassava roots in order to improve the quality of the final products.

Generally, in Lushoto cassava products were dried with fungi till the time of preparation for milling when they were scraped off. Sometimes fermented cassava roots (*mbute*) were dried and milled for food mixed with other cereals or without mixing and retention of fungal growth in Rorya. During rainy seasons the cassava products would not dry easily due to high humidity, inadequate sunshine and exposure to rain. Wereing *et al.* (2001) reported mold growth on dried fermented cassava products *(kokonte*) in Ghana. Likewise, Kaaya and Eboku (2010) reported mold growth on fermented cassava roots in Kumi districts, Eastern Uganda. In mechanical method, the peeled cassava roots may be grated by machine, de-watered by pressing then direct sun drying.

Peeling of cassava roots removes the natural protective tissues, the process that expose the inner part to contaminants. Liu *et al*. (2006) and Udoh *et al*. (2000) reported that natural protection such as grain husks has been reported to protect maize and rice from weevils and mold infestation and aflatoxin contamination. This kind of defense is not available in cassava after peeling and processing into cassava products making them susceptible to attack by fungi. According to Hell *et al*. (2003) and Kaaya *et al*. (2006), aflatoxin has been reported to increase when maize cobs were dried on ground. Drying of cassava roots on the ground or any other materials rested on ground-like bed sheets and *khanga* exposes the cassava to contamination

with soil, dust and fungi spores. Cassells (1990) proved that *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. were exogenously found in soils, water and plant surfaces. Similarly, Suryanarayanan *et al*. (2000) reported that *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. are also endophytes in some plant species. This study noticed that cassava products are being dried for 12 – 170 h depending on weather condition and methods of drying. Rainy, cloudy and high atmospheric relative humidity delayed drying of cassava products in Ukerewe likewise indirect sun-drying (under roof) plus smoke prolonged drying duration of cassava products in Lushoto, hence chance for microbial growth. Likewise, Knoth (1993) showed that delay in drying period may promote microbes and insect infestation and can lead to discolouration and changes in flavour.

The moisture content, total incidence and colony count of fungi species were higher in Lushoto than Rorya and Ukerewe though in Lushoto drying of cassava products were accompanied with smokes. The results were contrary to Manjula *et al*. (2009) who reported that smoking of cassava chips probably reduced insect infestation, fungal infection and aflatoxin accumulation by lowering the moisture content and Udoh *et al.* (2000) who confirmed the effectiveness of smoke-drying in reducing insect and fungal infection.

The variation in moisture content, total incidence and colony count of fungi species among the districts may be caused by geographical conditions since most surveyed areas in Lushoto 726 masl and 1646 masl were hotter and cooler respectively than Rorya (1152 – 1290 masl) and Ukerewe (1141 – 1216 masl). Also great variation in

altitude in Lushoto (203%) may have created various favourable conditions for fungi contamination in cassava products.

The cassava samples moisture content was ranging from 12.12% to 28%. The average moisture content of 13.87% was higher than 12% recommended by Tanzania Bureau of Standards for cassava. The high moisture content under optimal temperature may favour fungal growth. This was also observed by Aryee *et al*. (2006) that at 12% moisture cassava products had potential for long shelf life but moisture content greater than 12% allowed microbial growth. In the same way Klich (2007) reported that availability of water was essential for both mold growth and aflatoxin production.

During fermentation cassava were covered by banana leaves, tree leaves, cassava peels, old cloth, cactus leaves and polythene sheets and sometimes left uncovered whereby they came into contact with dust, infested by insects, birds and animals. These materials (especially plant tissues) used for covering cassava product might be the source of variable microbes (contaminants) of food. Msogoya *et al*. (2012) observed that *Aspergillus, Fusarium, Penicillium* and *Candida* were the main fungal contaminants of banana in vitro cultures. Similarly, *Fusarium* has been reported as an endophytic fungus in banana and pumpkin plants while *Penicillium* spp. and *Aspergillus* spp. were found in internal tissues of mallow plants (Suryanarayanan *et al*., 2000; Odutayo *et al*., 2007).

Fandohan *et al*. (2005) reported that structures built using mud and wood had little or no ventilation. Mestres *et al.* (2004) observed that heaps of yam chips in a poorly ventilated room was not favourable for moisture loss and favoured mold growth and insect infestation. In these structures cassava products were being stored in polypropylene bag, heaped under roof and in plastic containers in Lushoto, Rorya and Ukerewe districts.

Storage areas and materials for cassava products and cereals were used for long time without being cleaned, the condition that possibly might be the source of fungi inocula to newly processed cassava products. Likewise, Kaaya and Eboku (2010) mentioned that cassava was either heaped or stored in various containers like Jerri cans, clay pots or polypropylene bags in Kumi district-Eastern Uganda.

4.6 Conclusion

This research has shown that cassava products in these districts were infected with many fungi some of them are mycotoxins producers. Poor processing and storage condition were the core sources of cassava product contamination by these fungi. Processing and storage tools should be put in hygienic conditions to avoid fungi inocula and inoculation to newly processed products.

Education on processing high quality *udaga* is not well known to cassava growers and processers in these districts, still among interviewed farmers nobody was aware of *Aspergillus flavus* and mycotoxins. Education on production of high quality udaga and management of aflatoxin producing fungi is very crucal to cassava growers and

processors that can enable them to handle the produce effectively to reduce the chances of contamination. Since cassava products from these districts are transported to many areas in Tanzania, there is possibility of many people to consume contaminated food.

Table 4.1: Status of mould growth, moisture content and mould count of cassava products sampled from farmers in Lushoto, Rorya and Ukerewe districts

	Mean moisture content (%)	Mean pH content	Mean *A. flavus* (cfu/g)
Lushoto	15.77a	6.86a	0.55a
Rorya	12.12b	4.84b	0.24a
Ukerewe	13.73c	4.83b	0.56a
Grand mean	13.90	5.50	0.56
se	3.10	17.00	1.10
cv%	22.60	17.30	234.00
LSD	1.40	0.40	0.50
Fpr	<.001 Sd	<.001 Sd	0.294 NS

Sd = Significant deference ($P \leq 0.05$), NS = no Significant deference ($p \geq 0.05$)

Values followed by the same letters within column are not significantly different. *A. flavus* (cfu/g) means transformed to the $\log_{10}$, i.e., $y = \log (N+ 1)$ previous to analysis.

Table 4.2: Number of samples tested positive and % incidence of mould species in cassava products (N = 40)

Fungus species	Lushoto		Rorya		Ukerewe			
	Number of samples tested positive	Incidence (%)	Number of samples tested positive	Incidence (%)	Number of samples tested positive	Incidence (%)	Total Number of samples tested positive	(%)Total incidence
Rhizopus sp.	28	70.0	24	60.0	19	47.5	71	59.17
A. flavus	8	20.0	3	7.5	9	22.5	20	16.67
A. niger	0	0.0	2	5.0	2	5.0	4	3.33
Fusarium sp.	14	35.0	17	42.5	13	32.5	44	36.67
Mucor sp.	3	7.5	0	0.0	2	5.0	5	4.17
Curvularia sp.	3	7.5	2	5.0	0	0.0	5	4.17
Cladosporium sp.	26	65.0	16	40.0	20	50.0	62	51.67
Penicillium sp.	20	50.0	12	30.0	14	35.0	46	38.33
Yeast	1	2.5	8	20.0	4	10.0	13	10.83

Incidence was calculated as the number of samples contaminated (n) by the fungal species divided by the number of samples analysed (N) times 100. Total add to more than 100% because mould species occurred in more than one sample and at least once in each sample. A total of 40 samples were collected from each district

Table 4.3: Quantity of fungal species contamination in cassava products

	Colonies (cfu/g)			
Fungal species	**Lushoto**	**Rorya**	**Ukerewe**	**Mean**
Rhizopus sp.	157 220.0	276 450.0	272 960.0	235 543.0
A. flavus	990.0	13 000.0	18 700.0	12 896.7
A. niger	0.0	10 430.0	2 100.0	4 176.7
Fusarium sp.	200 360.0	99 760.0	224 350.0	174 823.0
Mucor sp.	20 000.0	3 420.0	3 420.0	8 946.7
Curvularia sp.	0.0	20.0	0.0	6.7
Cladosporium sp.	1 223 730.0	515 210.0	134 630.0	624 523.0
Yeast	7 890.0	186 530.0	13 770.0	69 396.7
Penicillium sp.	173 800.0	102 920.0	217 420.0	164 713.0
% colony	45.9	31.1	22.9	33.3

0^* counts mean no colony was observed.

Table 4.4: Correlation coefficient for relationships between occurrences of aflatoxin producing fungi (*Aspergillus flavus*), processing and storage condition

	A. Flavus (cfu/g)	Drying duration	Fermentation duration	Storage duration	Moisture content	pH
Cfu/g	1					
Drying duration	0.084	1				
Fermentation duration	-0.612	0.003	1			
Storage duration	-0.024	-0.024	0.071	1		
Moisture content	-0.482	-0.237	0.063	0.033	1	
pH	-0.561	-0.329	0.208	-0.173	-0.18	1

Non significant difference ($p \geq 0.05$)

CHAPTER FIVE

5.0 DETECTION OF AFLATOXIN IN STORED DRIED CASSAVA CHIPS IN LUSHOTO, RORYA AND UKEREWE DISTRICTS OF TANZANIA

5.1 Abstract

The survey was conducted in Lushoto, Rorya and Ukerewe districts in Tanzania where 120 samples collected and analyzed for aflatoxin content. A lateral flow immunochromatographic assay with a cut-off level of 4 ppb aflatoxin (Agra Strip® Total AflatoxinTest-COKAS1100) based on an inhibition immunoassay format was implemented to enumerate the qualitative level for total aflatoxin content. Eight of the samples were contaminated by aflatoxin whereas 112 were clean. The levels of aflatoxin ranged between greater than 4 ppb as positive sample with mean 0.49 and less than 4 ppb as negative sample. The relationship on mold, storage duration, processing method, drying duration, moisture content and pH and aflatoxin contamination of these products was established using regression analysis. Storage duration was positively significant ($p \leq 0.05$) correlated to aflatoxin content ($\geq$ 4 ppb). Effort should be made to educate farmers on sources of aflatoxin in cassava products, their side effects to human health and control measures.

Key words: Aflatoxin, dried cassava products

5.2 Introduction

Cassava (*Manihot esculenta* Crantz) is an important staple food crop grown for staple food and income generation in Tanzania (Kavia *et al*., 2007). The crop is grown in 39 African countries, of which Nigeria, Democratic Republic of Congo, Ghana, Tanzania and Mozambique are among the top ten producers in the world (FAO, 2001c). Tanzania is the fourth producer of cassava in Africa and annual root production is estimated at 5 500 000 tons from 761 100 hectares (Mkamilo, 2005). In Tanzania, cassava production is mainly dominated by small scale farming with an average farm size ranges from 0.5 – 2.0 ha per household (Manyama *et al*., 2002; Temu and Nyange, 2001; Saleh, 2001). This is about one-third of the total household farm size and it accounts 70 percent of the total land under root and tuber crops (Temu and Nyange, 2001).

In Tanzania, cassava is one of the key staple foods that are increasingly in its contribution to the household regular income to meet farmers' immediate obligations such as paying school fees, day-to-day household expenditures, festivals and funeral ceremonies (Temu and Nyange, 2001; Mlingi *et al*., 2000). Cassava is highly rich in carbohydrates, being the third source of calories in the tropics after rice and maize (FAO, 2002). In Tanzania according to FAOSTAT (2007), cassava produces about 409 calories per person per day. Cassava and similar crops could represent the future of food security in the poorest regions and contribute to achieving the first of the eight Millennium Development Goals that aims to halve worldwide hunger and poverty by the year 2015 (FAO, 2002).

Underprivileged agronomic practices of cassava, processing and storage condition of cassava products may possibly result contamination by mould and mycotoxins.
Derived from the *Aspergillus flavus* fungus, the toxigenic strains of aflatoxins are among the most harmful mycotoxins producers. Aflatoxin induces hepatocellular carcinoma in humans and other animals (Groopman *et al.* 2008). Aflatoxins are found in the soil as well as in grains, nuts, dairy products, tea, spices and cocoa, as well as animal and fish feeds (Waliyar *et al*., 2008). Aflatoxins are especially problematic in hot, dry climates (+/- 30 to 40 degrees latitude) and their prevalence is exacerbated by drought, insect pests' prevalence, delayed harvest, insufficient drying and poor post-harvest handling.

Aflatoxin has been reported to be hepatic, carcinogenic, mutagens, teratogens, immune system suppressing and anti-nutritional contaminants in many food commodities (Williams *et al*., 2004; Adebayo-Tayo *et al.*, 2006). There are six forms of aflatoxin: B1, B2, G1 and G2 are found in plant-based food, while M1 (metabolite of B1) and M2 are found in foods of animal origin. The form of aflatoxin B1 is the most harmful due to its direct link to human liver cancer (Leslie *et al*., 2008; USAID, 2012). Much of Sub-Saharan Africa is at risk of unsafe levels of aflatoxin exposure that can negatively affect human health, food security and economic trade (Williams *et al*., 2004).

Aflatoxins from maize and groundnuts have been associated with stunted growth in children in Tanzania (Kimanya *et al*., 2008). According to Gong *et al*. (2002; 2003) and Egal *et al.* (2005), 90% of children in Benin and Togo were exposed to aflatoxin

in groundnut, which led to a measurable impairment of child growth. In 1967, aflatoxin was circumstantially associated with death of a 15 year old boy in Uganda after eating a sample of cassava, which was later found to contain 1700 $\mu g\ kg^{-1}$ aflatoxin (Kaaya and Warren, 2005). According to FAO (2001b) estimates, 25% of the world food crops are affected by mycotoxins each year. Crop loss due to aflatoxins contamination costs United States producers more than $100 million per year on average including $ 26 million to peanuts ($69.34/ha) (Munkvold, 2003)

Few studies have reported on mycotoxin contamination in cassava products (Gnonlonfin *et al*., 2008) when compared with the number of studies conducted on cereals, peanuts, dairy products, wheat and dried chilies (Bankole and Adebanjo, 2003). Current information on aflatoxin contamination of cassava products in Tanzania was limited. So there was a need to conduct study on aflatoxin contamination in stored dried cassava products for strategic control measures. This study focused on detection of aflatoxin in stored dried cassava products collected from Lushoto, Rorya and Ukerewe districts of Tanzania.

5.3 Materials and Methods

5.3.1 Total aflatoxin content analysis

The qualitative level for the presence of total aflatoxin was determined by lateral flow immunochromatographic assay with a cut-off level of 4 ppb aflatoxin (Agra Strip® Total AflatoxinTest-COKAS1100 product of Romer Labs Singapore Pte Ltd) based on an inhibition immunoassay format. Ten grams of thoroughly composite sample was placed into a clean jar and sealed. Twenty milliliters (20 ml) of 50% ethanol extraction solution (i.e. 50/50 (v/v) ethanol/water) was added in jar and

tightly sealed. The mixture was vigorously shaken for 1 min and the extracts filtered through a Whatman number one filter paper and the filtrate collected, adjusted to a pH of 6 – 8. Using a single channel pipette, 50 ml of assay diluents was added to each micro-well. To dissolve the coating conjugate in the micro-well the content was pipetted up and down 5 times. To each micro-well 50 ml of sample extracts were added then well mixed by pipetting it up and down for 3 times. One test strip was put in one well and allowed to develop colour for 5 min and test results interpreted immediately whereby 2 lines were visible for the sample contained total aflatoxin less than 4 ppb (negative sample), 1 line was visible for the sample contained total aflatoxin $\geq$ 4 ppb (positive sample) and where no line in control zone observed (invalid test), the sample was re-tested by using a valid test strip.

5.3.2 Statistical analysis

The moisture content, pH, *A.flavus* (cfu/g) and aflatoxin content were subjected to analysis of variance (ANOVA) in Completely Randomized Design (CRD) by using GenStat Software 14th version, whereby the districts were stand for replication, cassava samples for plots/experimental units and aflatoxin ($\geq$ 4 ppb), mc, pH and cfu/g for treatments. The aflatoxin content was transformed into natural logarithm before analysis and relationship between aflatoxin level of the products and other variables was explored. The aflatoxin levels in cassava samples (y), were transformed such that amount of aflatoxin in cassava sample (Y_1 cassava) = log (y+3) (Kaaya and Eboku, 2010). The mean differences were separated by using DMRT ($p \leq 0.05$). Multi- linear regression analysis was used to compare relationship between variables *Aspergillus flavus* (cfu/g) versus moisture content, processing methods, fermentation duration and drying duration).

Regression equation was expressed as: -

$Y = 1/n\ (a + b_1X_1 + b_2X_2 + \ldots + l_nX_n + E)$ ……………………………………….. (3)

Where:-

X1, X_n = independent variable,

b_1, b_n= estimates relate to independent variable (pH, mc, drying duration, fermentation duration and storage duration),

a = estimate of the intercept,

n = estimate number of independent variables,

Y = dependent variable (cfu/g).

5.4 Results

5.4.1 Aflatoxin content in cassava products

Eight (8) samples possessed some aflatoxins $\geq$ 4 ppb (Table 5.1). Aflatoxins were detected in four cassava samples from Lushoto, two cassava samples from Rorya and two cassava samples from Ukerewe. Although there was a variation in the number of samples contaminated with aflatoxin between Lushoto, Rorya and Ukerewe districts the means were not significant ($p \geq 0.05$) among the districts.

5.4.2 Relationship between samples of cassava products infected with *A. flavus* and total aflatoxin content

Among 20 tested samples, 8 contained aflatoxins greater than 4 ppb and had *A. flavus* (Table 5.2). Only 50%, 66.67% and 22.22% cassava samples were contaminated with *A. flavus* from Lushoto, Ukerewe and Rorya respectively and were found to contain aflatoxins (Fig. 5.1).

5.4.3 The correlation between aflatoxin content and aflatoxin producing fungi, processing and storage conditions in *udaga* from Lushoto, Rorya and Ukerewe districts

The results of the correlation between aflatoxin contamination and aflatoxin producing fungi contamination, moisture content, pH, drying duration, fermentation duration and storage duration are presented in Table 5.3. Aflatoxin content (≥ 4 ppb) was high positive non significant ($p \geq 0.05$) correlated (0.71) to pH and positive significant ($p \leq 0.05$) correlated (0.05***) to storage duration. Aflatoxin contamination was negative non significant ($p \geq 0.05$) correlated with moisture content, *A. flavus* (cfu/g), fermentation and drying duration. The association between pH and storage duration was positive while the association between pH, moisture content, drying duration and fermentation duration were negative. The association between moisture content and fermentation duration, drying duration and storage duration was positive. The association between *A. flavus* (cfu/g) and fermentation duration, drying duration, storage duration was also positive. The association between storage duration and fermentation duration and drying duration was negative. The association between fermentation duration and drying duration was positive.

5.5 Discussion

The survey established implies that cassava products are vital source of food and income for majority people in Lushoto, Rorya and Ukerewe districts. Generally, in Lushoto districts cassava products which contained fungal growth retained them till the time of preparation for milling when are scraped. This condition may contribute

to high contamination of aflatoxins when compared to Rorya and Ukerewe districts. Sometimes fermented cassava roots (*mbute*) are dried and milled for food mixed with other cereals or lonely without scrapped in Rorya district. Mycotoxins may have already been produced between the time of fungi developing and scraping hence making the product perilous to consumers.

Peeling of cassava roots removes the natural protective tissues, the process that expose the inner part to contaminants by aflatoxin. Liu *et al*. (2006) and Udoh *et al*. (2000) reported that natural protection such as grain husks has been reported to protect maize and rice from weevils and mould infestation and aflatoxin contamination. This kind of defence is not available in cassava after peeling and processing into cassava products making them susceptible to attack by fungi hence mycotoxins.

Aflatoxin contaminated samples were dried for 24 – 97 h with mean of 44.13 h by direct sun drying on rock surfaces, rusty ironed roof and on polypropylene sheet and indirect sun drying (under roof plus smoke). This situation enhances fungi growth and aflatoxin formation in and could explain the existence of aflatoxin in cassava products. Though the geographical condition for Lushoto was conducive for *A. flavus* contamination and aflatoxin production only 10% samples were contaminated with aflatoxins greater than 4 ppb. The low contamination may be contributed by the usage of smoke in drying of cassava products, the situation that was proved by Manjula *et al.* (2009) and Udoh *et al*. (2000). Manjula *et al*. (2009) reported that smoking of cassava chips could probably reduce insect infestation,

fungal infection and aflatoxin accumulation by lowering the moisture content and the incidence of aflatoxin was indeed quite low (1.27 ppb) on smoked cassava chips. Similarly the effectiveness of smoke drying in reducing insect and fungal infection was confirmed by Udoh *et al*. (2000).

The moisture content of aflatoxin contaminated samples with greater than 4ppb was ranging from 9.38% to 19% with mean of 13.37%. This high moisture content under optimal temperature may favour fungi growth and aflatoxin production. Aryee *et al*. (2006) observed that at 12% moisture cassava products had potential for long shelf life but moisture content greater than 12% allows microbial growth. Klich (2007) reported that availability of water was essential for both mould growth and aflatoxin production. FAO (2001a) reported that safe moisture content is one of the prerequisites in preventing mycotoxins using the Hazard Analytical and Critical Control Point (HACCP) approach.

Generally during fermentation, the moisture availability, cassava as substrate and adequate temperature may facilitate fungal growth and aflatoxin production. *Aspergillus flavus* has been observed to produce the highest aflatoxin level at water activity of 0.996 and temperature of 30°C between 5 – 15 days of storage (Gqalen *et al*., 1997). Optimum temperatures for aflatoxin production are between 24°C and 30°C with variation between strains and substrates (Klich, 2007).

Majority of aflatoxin contaminated cassava products had been stored in polypropylene bags and few heaped under roof in farmers’ shelters used as house and

kitchen in the study areas. Storage areas and materials are being used for storing cassava products and cereals for long time without being cleaned, the condition that may be the source of fungi inocula to newly processed cassava products.

Aflatoxin contaminated samples were stored for variable period of time ranging from less than a month to one month and showed positive correlation between aflatoxin content and storage duration that the increase of storage duration may support aflatoxin contamination in cassava products. Similarly Mestres *et al.* (2004) reported an increase of aflatoxin contamination with storage time in yams. Manjula *et al.* (2009) reported that probably storing cassava products for long time is one of the factors predisposing the products to aflatoxin contamination.

The results of aflatoxin examination in cassava products explain fairly low (6.67%) incidence of aflatoxin contaminated cassava samples. The reputed aflatoxin range of ≤ 4 ppb and ≥ 4 ppb during cassava samples examination showed that many cassava products had not been contaminated or contaminated with low level of aflatoxin compared to limits for B1 of 5 μg/kg in rice (TBS, 2004), cashews, barley, sorghum flour and pearl millet grains, as well as total aflatoxin limits set at 10 μg/kg and 15 μg/kg for cashews and groundnuts established by TFDA in Tanzania (TBS, 2003a; 2003b; 2003c; 2003d). This study reported the presence of aflatoxin in cassava but with low incidence. Muzanila *et al.* (2000) reported no aflatoxin in 18 samples of cassava processed by smallholder farmers using sun drying and solid fermentation in Tanzania. Manjula *et al*. (2009) reported that the cassava samples from markets in Tanzania showed AFB1 levels in the range of 0.86 to 33.8 ppb. Tanzania's estimated

aflatoxin exposure among humans (and its HCC prevalence) is much lower than Nigeria's, but it is still elevated. The same is true for much of Sub-Saharan Africa (Liu and Wu, 2010). Butalao-Jayme *et al*. (1982) showed that increased consumption of aflatoxin-loaded foods was associated with cases of cancer in the Philipines even when the level of contamination was moderately low. Kimanya *et al*. (2008) reported that Tanzanians are at risk of exposure to fumonisins and aflatoxins in maize.

The occurrence of aflatoxins in cassava indicates that Tanzanians can be at risk of aflatoxicosis because cassava products are staple food not only in Lushoto, Rorya and Ukerewe districts but also in other range of districts which this study could not reach because of financial resource limitation. Cassava products from Ukerewe are often sold in Lake Victoria islands and Mwanza city (very populated city) and cassava products from Lushoto are transported to Dar es Salaam and Arusha cities.

5.6 Conclusion

This research has shown that cassava products in these districts are infected with fungi (*Aspergillus* spp) and contained aflatoxins. Cassava products from these districts are transported to many areas; there is possibility of many people to consume contaminated foods in Tanzania. Hence, people who feed on these cassava products are at danger of aflatoxicosis. Aflatoxicosis may be occurring regularly around the Tanzania and may be mistaken for other conditions. Aflatoxicosis, associated with extremely high doses of aflatoxin, is characterized by hemorrhage, acute liver damage, edema and death in humans. Conditions increasing the likelihood of aflatoxicosis in humans include limited availability of food, environmental

conditions that favor fungal development in crops and commodities and lack of regulatory systems for aflatoxin monitoring and control. The government should enforce standards and provide crucial support to benefit the rural poor, such as improving their level of education about aflatoxin exposure. Education will enable Stakeholders along the value chain to be aware of the potential impacts of aflatoxin and they may be more interested in implementing measures to alter their families' exposure to aflatoxin since among interviewed farmers nobody was aware of *Aspergillus flavus* and aflatoxins. Information about the level of fungi and aflatoxin contamination of cassava and other foodstuffs should be disseminated at all levels of society in Tanzania

Table 5.1: Aflatoxin content (≥ 4 ppb) in cassava products

Districts	Samples analysed	No. positive samples	% positive samples	Mean aflatoxin content (ppb)	s.e.	cv%	LSD	Fpr
Lushoto	40	4	10	0.4896a	0.0314	6.5	0.014	0.592NS
Rorya	40	2	5	0.4834a				
Ukerewe	40	2	5	0.4834a				

NS = no significant difference

Values (means) followed by the same letters along the column are not significantly different

Means transformed to the $\log_{10}$, i.e., $y = \log(x + 3)$ prior to analysis

Table 5.2: Relationship between aflatoxin (≥ 4 ppb) and *A. flavus* contamination in cassava products (N = 20)

Districts	Nsc *A. flavus*	%Nsc *A. flavus*	Nsc aflatoxin	%Nsc aflatoxin	%Nsc aflatoxin and *A. flavus*
Lushoto	8	40	4	20	50
Rorya	3	15	2	10	66.67
Ukerewe	9	45	2	10	22.22

Nsc = number of samples contaminated

$$\%\text{Nsc aflatoxin and } A.\ flavus \text{ obtained} = \frac{\text{Nsc aflatoxin} \times 100}{\text{Nsc } A.\ flavus}$$

Table 5.3: Relationships between aflatoxin content and factors associated with aflatoxin production: aflatoxin producing fungi contamination (cfu/g), moisture content (%), pH, drying duration (h), fermentation duration (days) and storage duration (months)

	Total aflatoxin content (≥ 4 ppb)	Drying duration (h)	Fermentation duration (days)	Storage duration (months)	*A. flavus* content (cfu/g)	Moisture content (%)	pH
Total aflatoxin content (≥ 4 ppb)	1						
Drying duration (h)	-0.73	1					
Fermentation duration	-0.81	0.77	1				
Storage duration (months)	0.05***	-0.27	-0.55	1			
A. flavus content (Cfu/g)	-0.78	0.65	0.43	0.2	1		
Moisture content	-0.83	0.69	0.56	0.17	0.69	1	
pH	0.71	-0.95	-0.68	0.13	-0.68	-0.82	1

***Highly significant ($p \leq 0.05$)

Parameters used in correlation analysis are shown in Appendix 5.1.

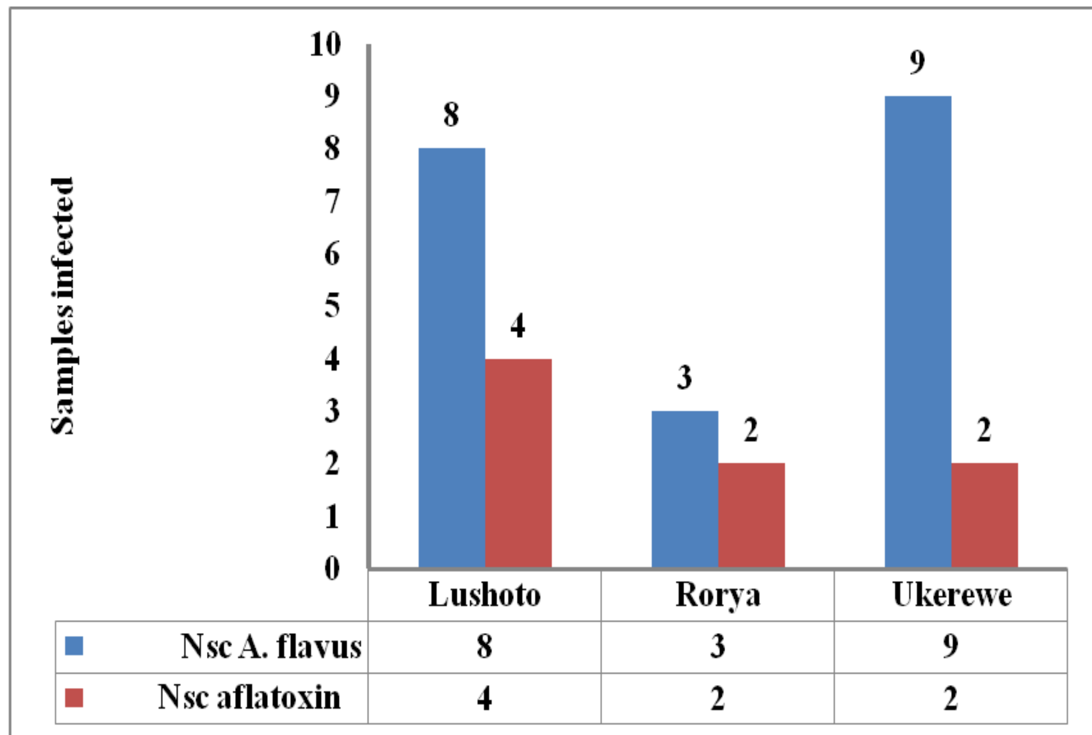


Figure 5:1 Relationship between samples of cassava products infected with *A. flavus* and total aflatoxin content

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APPENDICES

Appendix 1: Characteristics of samples contaminated by aflatoxin (≥ 4 ppb) in Lushoto, Rorya and Ukerewe districts in Tanzania

Location	Moisture content	pH	*A. flavus* (cfu/g)	Fermentation duration (days)	Drying duration (h)	Storage duration (months)
Lushoto	13.5	9.71	2.00	4.0	25.0	1.0
Lushoto	13.4	7.58	2.70	4.0	85.0	1.0
Lushoto	17.7	5.84	2.69	3.0	37.0	0.0
Lushoto	19.0	8.56	2.78	3.0	97.0	0.0
Rorya	11.6	4.86	2.00	6.0	24.0	1.0
Rorya	12.0	5.09	4.00	5.0	24.0	1.0
Ukerewe	10.3	4.98	4.16	4.0	30.0	0.0
Ukerewe	9.4	4.04	2.08	4.0	31.0	1.0
Means	**13.4**	**6.33**	**2.80**	**4.1**	**44.1**	**0.6**

Appendix 2: Levels of moisture content (%), pH, total aflatoxin content (≥ 4 ppb) and aflatoxin producing species concentration (cfu/g), drying duration (h), fermentation duration (days) and storage duration (months) associated with stored cassava chips in Lushoto district.

Sample number	Moisture content (%)	pH	*Aspergillus* spp (cfu)	Total aflatoxin content (≥ 4 ppb)	Storage duration (months)	Fermentation duration (days)	drying duration (h)
1	11.57	5.86	0.0	0.48	0	3	49
2	13.59	9.17	0.0	0.48	3	3	49
3	18.50	6.63	0.0	0.48	3	4	38
4	10.84	7.73	0.0	0.48	0	4	48
5	13.34	6.31	3.3	0.48	1	4	61
6	11.69	5.94	0.0	0.48	3	5	62
7	23.09	8.16	0.0	0.48	0	3	50
8	13.38	6.26	0.0	0.48	1	4	52
9	11.74	6.28	0.0	0.48	6	4	37
10	15.72	9.73	0.0	0.48	3	4	49
11	16.57	7.72	0.0	0.48	0	4	85
12	17.55	7.96	0.0	0.48	6	3	37
13	25.57	5.04	3.3	0.48	0	7	169
14	11.19	5.46	0.0	0.48	2	4	85
15	14.46	6.89	0.0	0.48	1	4	36
16	15.42	5.57	3.0	0.48	1	4	36
17	15.77	7.26	0.0	0.48	2	3	87
18	13.57	5.69	0.0	0.48	1	4	50
19	13.49	9.71	2.0	0.60	1	4	25
20	13.04	7.19	0.0	0.48	1	4	50
21	15.59	7.43	0.0	0.48	2	4	49

22	13.40	7.58	2.7	0.60	1	4	85
23	28.06	5.4	0.0	0.48	0	3	26
24	15.57	5.94	0.0	0.48	1	3	85
25	14.29	5.44	0.0	0.48	1	4	62
26	20.22	9.02	0.0	0.48	0	3	60
27	17.73	5.84	2.7	0.60	0	3	37
28	20.68	9.55	0.0	0.48	1	6	38
29	18.55	5.37	0.0	0.48	1	7	49
30	10.72	5.95	0.0	0.48	0	5	39
31	14.31	6.45	0.0	0.48	0	3	37
32	13.19	6.2	0.0	0.48	1	7	25
33	14.71	5.85	0.0	0.48	4	3	87
34	19.00	8.56	2.8	0.6	0	3	97
35	15.01	8.25	0.0	0.48	0	3	102
36	16.00	5.85	0.0	0.48	6	3	70
37	12.59	5.55	0.0	0.48	0	7	86
38	19.54	6.29	2.3	0.48	0	3	37
39	11.99	6.04	0.0	0.48	1	3	49

a' Data for Total aflatoxin content (≥ 4 ppb) (y) were log (x + 3) transformed before analysis and a’ Data for cfu/g (y) were $\log_{10}$ (x + 1) transformed before analysis

Appendix 3: Levels of moisture content (%), pH, total aflatoxin content (≥ 4 ppb) and aflatoxin producing species concentration (cfu/g), drying duration (h), fermentation duration (days) and storage duration (months) associated with stored cassava chips in Rorya district.

Sample number	**Moisture content** (%)	**pH**	***Aspergillus* spp** (cfu)	**Total aflatoxin content** (≥ 4 ppb)	**Storage duration** (months)	**Fermentation duration** (days)	**drying duration** (h)
41	12.99	4.66	0.0	0.48	0	2	12
42	11.13	4.26	0.0	0.48	0	6	16
43	11.16	4.70	0.0	0.48	0	5	12
44	11.05	4.35	0.0	0.48	0	5	12
45	8.89	4.45	0.0	0.48	0	6	12
46	12.63	4.71	0.0	0.48	3	8	13
47	14.42	4.55	0.0	0.48	0	8	12
48	11.50	4.60	0.0	0.48	0	9	12
49	10.62	4.34	0.0	0.48	0	5	12
50	15.46	4.38	0.0	0.48	0	8	12
51	11.61	4.86	2.0	0.60	1	6	24
52	23.92	4.62	0.0	0.48	1	6	26
53	10.37	4.16	0.0	0.48	0	6	24
54	10.94	4.91	0.0	0.48	0	6	24
55	12.01	5.09	4.0	0.60	1	5	24
56	12.22	4.27	0.0	0.48	0	4	24
57	12.65	5.67	0.0	0.48	0	6	24
58	10.30	4.96	0.0	0.48	4	6	24
59	10.53	7.27	0.0	0.48	0	6	24
60	13.70	4.87	0.0	0.48	0	4	24

61	9.85	6.77	0.0	0.48	0	5	24
62	11.91	6.03	0.0	0.48	0	4	24
63	10.98	5.91	0.0	0.48	0	4	24
64	12.87	4.63	0.0	0.48	0	4	24
65	11.24	4.44	0.0	0.48	0	4	24
66	10.36	5.74	3.5	0.48	5	3	12
67	10.25	4.11	0.0	0.48	2	7	24
68	10.04	4.03	0.0	0.48	0	8	24
69	14.39	4.54	0.0	0.48	1	6	12
70	12.49	4.74	0.0	0.48	1	6	12
71	16.84	5.80	0.0	0.48	0	6	24
72	12.89	4.27	0.0	0.48	0	6	12
73	10.19	4.09	0.0	0.48	0	6	24
74	12.00	5.25	0.0	0.48	0	3	12
75	12.67	5.64	0.0	0.48	0	4	12
76	11.95	4.09	0.0	0.48	0	6	12
77	9.41	4.67	0.0	0.48	0	5	24
78	11.93	4.29	0.0	0.48	0	6	12
79	10.57	4.34	0.0	0.48	0	6	12
80	13.97	4.33	0.0	0.48	3	6	28

a' Data for Total aflatoxin content (≥ 4 ppb) (y) were log (x + 3) transformed before analysis and a’ Data for cfu(y) were log_{10} (x + 1) transformed before analysis

Appendix 4: Levels of moisture content (%), pH, total aflatoxin content (≥ 4 ppb) and aflatoxin producing species concentration (cfu/g), drying duration (h), fermentation duration (days) and storage duration (months) associated with stored cassava chips in Ukerewe district.

Sample number	Moisture content (%)	pH	*Aspergillus* spp (cfu)	Total aflatoxin content (≥ 4 ppb)	Storage duration (months)	Fermentation duration (days)	drying duration (h)
81	13.53	4.21	2.00	0.48	1	5	40
82	10.89	4.31	0.00	0.48	1	3	29
83	16.21	4.26	2.88	0.48	1	3	24
84	15.80	4.27	0.00	0.48	0	3	30
85	10.69	4.39	0.00	0.48	1	3	18
86	14.80	4.40	0.00	0.48	0	4	30
87	12.82	5.06	3.34	0.48	0	3	30
88	14.78	4.12	0.00	0.48	0	4	54
89	9.59	4.67	0.00	0.48	1	4	18
90	10.48	5.16	0.00	0.48	0	7	36
91	13.15	5.10	0.00	0.48	1	5	33
92	16.39	5.10	0.00	0.48	1	9	26
93	11.93	4.96	0.00	0.48	0	5	42
94	18.85	4.31	0.00	0.48	0	4	40
95	12.51	4.81	0.00	0.48	1	4	21
96	9.74	4.88	0.00	0.48	0	3	48
97	14.56	4.81	0.00	0.48	1	5	41
98	12.39	3.97	2.00	0.48	0	4	28
99	10.33	4.98	4.16	0.60	0	4	30
100	11.40	5.02	0.00	0.48	1	4	15

101	12.87	5.01	0.00	0.48	0	4	15
102	9.38	4.04	2.08	0.60	1	4	31
103	15.00	4.34	2.00	0.48	2	4	19
104	15.21	4.18	0.00	0.48	0	4	18
105	14.57	6.34	3.00	0.48	1	4	20
106	18.15	6.11	0.00	0.48	2	3	30
107	12.30	4.65	0.00	0.48	0	5	51
108	19.33	4.54	0.00	0.48	3	4	18
109	15.64	4.70	0.00	0.48	1	0	12
110	8.45	4.28	0.00	0.48	1	4	28
111	15.01	4.38	0.00	0.48	1	4	28
112	15.02	5.46	0.00	0.48	0	4	38
113	14.50	5.31	0.00	0.48	1	4	28
114	18.28	5.34	0.00	0.48	1	5	26
115	14.04	4.42	1.04	0.48	0	4	25
116	12.63	5.65	0.00	0.48	1	4	35
117	13.79	6.55	0.00	0.48	1	4	31
118	11.42	4.76	0.00	0.48	0	4	35
119	18.54	4.60	0.00	0.48	0	4	35
120	14.12	5.56	0.00	0.48	0	4	36

a' Data for Total aflatoxin content (≥ 4 ppb) (y) were log (x + 3) transformed before analysis and a' Data for cfu(y) were $\log_{10}$ (x + 1) transformed before analysis.