UNIVERSITY OF NAIROBI

DETERMINATION OF GLYCOALKALOIDS, PHENOLIC ACIDS AND PROTEASE INHIBITORS IN SELECTED CULTIVATED POTATO (*SOLANUM TUBEROSUM L.*) VARIETIES

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A thesis submitted in fulfilment of the requirements for the award of Doctor of Philosophy degree in Plant Physiology and Biochemistry in the School of Biological Sciences, University of Nairobi.

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DECLARATION

This thesis is my original work and has never been submitted elsewhere for examination or award of a degree in any other university. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

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DEDICATION

This thesis is dedicated to my wife Caroline and our children Abigael Cherono, Sandra Chemutai and Brian Kibet.

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LIST OF ABBREVIATIONS

AChe	Acetyl cholinesterase
ACB	After-cooking blackening
ANOVA	Analysis of Variance
ARS	Agricultural Research Service
BW	Bacterial Wilt
BW	Body Weight
CFA	Caffeic acid
CGA	Chlorogenic acid
CIP	International Potato Centre
CPB	Colorado potato beetle
CV	Coefficient of variation
ELISA	Enzyme Linked Immunosorbent Assay
FWT	Fresh weight
HMG	β-Hydroxy-β-methyl-glutaric acid
HPLC	High Performance Liquid Chromatography
ILRI	International Livestock Research Institute
IP	Intraperitoneal injection
JA	Jasmonic acid
KARI	Kenya Agricultural Research Institute
KEPHIS	Kenya Plant Health Inspectorate Services
LB	Late blight
LD	Lethal Dose
LSD	Least Significant Difference
MS	Mass Spectrometers
NPRC	National Potato Research Centre
PAL	Phenylalanine ammonia lyase
PIs	Protease inhibitors
TGA	Total Glycoalkaloids
THF	Tetrahydrofuran
TP	Total phenolics
UV	Ultra violet
WHO	World Health Organisation
HPLC-DAD-Ms	HPLC coupled with diode array detection and electron spray tandem mass spectrometry.

ABSTRACT

Potato (*Solanum tuberosum* L.) synthesizes a variety of potentially toxic and antinutritional compounds during growth and post harvest as a defense mechanism against pathogens, insects and other pests. As part of a program to improve its safety and nutritional quality, the concentration of glycoalkaloids (GAs), phenolic acids (PAs) and protease inhibitors (PIs) in the leaves and tubers of five field-grown potato varieties; Tigoni, Asante, Kenya Karibu, Desiree and Dutch Robijn were determined at 40, 55, 95 and 125 days after planting. Glycoalkaloid concentration was determined by means of high performance liquid chromatography (HPLC). Methanolic and ethanolic extracts of total phenolics (TP) and chlorogenic acid (CGA) were quantified using Folin-Ciocalteu's method and UV spectrophotometry, respectively. The inhibitory activities of potato Tris-HCl extracts were used to determine the concentration of PIs. The recovery values were high (85.9-93.5%) indicating the validity of the extraction methods. Foliar total glycoalkaloids (TGA) significantly ($p<0.001$) varied from 60.61 to 88.71 mg/100g fresh weight (Fwt) in vars. Dutch Robijn and Tigoni, respectively, with tuber values of 6.80 and 10.56 mg/100g Fwt, respectively which displayed a significant ($p<0.001$) reduction from the time of initiation to maturity. The foliar CGA content in the tested varieties significantly differed at $p=0.05$ and was highest (252.93 mg/100g Fwt) and lowest (244.02 mg/100g Fwt) in vars. Asante and Desiree, respectively. Tuber CGA content in the extracts was significantly ($p<0.001$) different. The vars. Ti and Dutch Robijn recorded the highest and lowest average tuber CGA concentrations of 58.04 mg/100g Fwt and 46.39 mg/100g Fwt, respectively. The highest TP contents of 603.4 mg CGA/g Fwt and 192.5 mg CGA/g Fwt were observed in the leaf and tubers of vars. Kenya Karibu and Tigoni, respectively, and the lowest amounts were detected in vars. Asante (435.9) and Desiree (127.1), respectively. Phenolic contents in the test varieties decreased significantly ($p<0.05$) during growth. The protease inhibitors (PIs) content (Units/mg) in the foliar and tuber extracts significantly ($p<0.001$) differed among the potato varieties. The chymotrypsin inhibitor (CI) and trypsin inhibitor (TI) content varied from 257.49 to 912.71 Units/mg and from 877.30 to 1646.56 Units/mg, respectively, with var. Desiree recording the highest levels. The foliar CI content was highest at 40 days after planting and declined with maturity, but peaked at maturity in tubers. These results demonstrate that the concentration of GAs, phenolic acids (PAs) and PIs was variety dependent and was strongly influenced by the stage of growth and growing season with higher levels in leaves than tubers. Light induced the synthesis of glycoalkaloids (GAs) and phenolic acids but not of protease inhibitors. The synthesis of total glycoalkaloids and total phenolics was higher in tubers that were stored under fluorescent light (FL) as compared to sunlight. The exposure to FL stimulated the synthesis of GAs to potentially toxic levels in var. Tigoni suggesting that the period between harvest and consumption of potatoes should be minimized. Routine monitoring of GAs, PAs and PIs in tubers of potato varieties at different growth stages in the field and during storage will guarantee the consumers of eating safe and good-quality diet.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum* L) is a member of the solanaceae, a large family of flowering plants with more than 3000 species (Barell *et al*., 2013). The members of this family and in particular the genus *Solanum* synthesize a variety of potentially toxic and antinutritional compounds during growth and post harvest storage. The biological active compounds present in the potato include protease inhibitors (PIs), lectins, polyphenols and glycoalkaloids (GAs) (Friedman, 2006). Available evidence supports the hypothesis that these plant secondary metabolites are synthesized as a physiological response to wounding stress including insect attack and physical injury and provide resistance against fungi, bacteria, viruses and insects (Mazid *et al*., 2011).

Until now, most of the research has centered on potato GAs because of its toxicity to human and other animals (Friedman, 2004). These neurotoxins are reported to inhibit the enzyme cholinesterase, induce teratogenesis and interrupt eukaryotic cell membrane structure (McGehee *et al*., 2000). The level of GAs of many commercial potato cultivars is generally less than the toxic limit of 200 mg/kg fresh weight (Fwt) the maximum concentration permitted by international health regulations (Carlson-Nilsson *et al*., 2012). However, their content may increase significantly when damaged or exposed to light during storage. These are largely unaltered by food cooking methods such as baking and boiling but significantly reduced during peeling, cutting, washing, blanching and deep frying at high temperatures above 210 ^{o}C (Nema *et al*., 2008).

Solanum GAs including tomatine, chaconine, demissine, commersonine and water-soluble leptines appear to be the most toxic repellants. Studies have reported that foliar GAs offer protection against Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Carlson-Nilsson *et al*., 2012), potato leafhopper, *Empoasca fabae* (Khan *et al*., 2013) and early blight, *Alternaria solani* (Esposito *et al*., 2002). Other potential defense mechanisms against CPB are glandular trichomes and PIs (Fréchette *et al*., 2010; Outchkorov, 2004).

Plant PIs are of particular attention because these contribute to the natural defense system against insect herbivores (War *et al*., 2012). They are commonly distributed within the plantae and are mainly abundant in storage organs where they may constitute 1-10% of the total protein or in exceptional cases to much higher levels (Fisher *et al*., 2015). Most of the PIs that have been isolated from plants do inhibit indigenous plant proteases but targets enzymes of animals or microbes. This led to the view that they might be involved in the protection of vulnerable plant tissues from pests and pathogens by inhibiting their digestive enzymes.

The most credible proof that PIs participates in plant protection was given by Sun *et al*. (2011) who reported that systemin and jasmonates (JA) work together as signaling molecules to induce the biosynthesis of PIs, polyphenol oxidases, lectins and other defense proteins. The induction of PIs in tomato leaves provided the best model to understand the interaction between systemin and JA in systemic defense signaling (Yan *et al*., 2013). Systematic search for defense-related genes from wound tomato foliar extracts that activate PI expression led to the isolation and characterization of systemin (an 18-amino acid peptide) and its precursor protein called prosystemin with a derived sequence of 200 amino

acids (Sun *et al*., 2011). Systemin is an active inducer for the synthesis of significant amounts of PIs such as trypsin, chymotrypsin and carboxypeptidase-A (Habib and Fazili, 2007). Evidently, different potato cultivars have different PI contents (Kim *et al*., 2009; Bauw *et al*., 2006).

Protease inhibitors have activities related to vertebrate proteases. They decrease the availability of nutrients and prevent the breakdown of proteins into their component amino acids. These interactions cause a critical shortage of essential amino acids to insects and mites (Vaseva *et al*., 2011). The potato inhibitors of carboxypeptidase remain stable during food processing. The inhibitors of trypsin and chymotrypsin are mainly denatured during food processing (Friedman and Brandon, 2001; Paiva *et al*., 2011). The presence of PIs together with GAs and phenolic acids in raw foods may reduce their safety and nutritional quality.

Potatoes also contain phenolic acids such as gallic acid (GAC), chlorogenic acid (CGA), caffeic acid (CFA), protocatechuic acids (PCA) (Azadeh *et al*., 2012). Phenolic extracts of freeze-dried peels of potato tubers have been shown to be effective as antioxidants. Chlorogenic acid is widely distributed and plays an important key role in plant protection (Plazas *et al*., 2013). This suggests that potato peel extracts might be safe to use as natural antioxidants to preserve food. Other phenolics such as flavonoids are important in pollination, seed dispersal and consumer appeal. Many phenolic acids such as CGA are involved in after-cooking blackening (ACB) and browning reactions which reduce the table and processing quality of potatoes.

Phenolic compounds that are well known for the processing quality of tubers include CGA and CFA, being the most widely distributed phenylpropanoids in essentially all plant tissues (Wang *et al*., 2015). These phenylpropanes are derivatives of cinnamic acid by the enzymatic action of phenylalanine ammonia lyase (PAL) on phenylalanine. The hydroxycinnamic acid CGA (5-o-caffeoyl quinic acid) is the major phenolic compound in potato plants that accounts for over 90% of tuber TP (Payyavula *et al*., 2015). Light stimulates large increases in CGA synthesis. In addition, its concentration increases in the cells adjacent to the wounds and in the buds during storage.

The preceding reports by Plazas *et al*. (2013), Carlson-Nilsson *et al*. (2012), War *et al*. (2012) suggest that GAs, CGA and PIs in potato cultivars and their transgenics are important components of the natural resistance complex. This natural resistance complex may aid in selection of defensive attributes that are well buffered against genetic plasticity of the target pest. Since secondary metabolites including GAs, phenolic acids and PIs are reported to interact with nutrients in a number of ways that affects the safety and quality of food, there is need to understand the complex interactions.

Recent advances in molecular biology and biotechnology have made it feasible for novel genes from unrelated sources to be transferred into potatoes. The serine PI cowpea trypsin inhibitor (CpTi), has been demonstrated to be detrimental to a number *Lepidopteran* and *Coleopteran* insects when expressed in transgenic potatoes (Bell *et al*., 2005). Transgenic potato plants expressing a gene encoding snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) have been shown to be insecticidal to peach-potato aphid (*Myzus persicae*) (Pribylova *et al*., 2006). Available literature has little information linking the induction and

expression of trypsin, chymotrypsin PI to growing season and potato stages of development which can be optimized to confer sustained pest resistance to potato plants.

Genetically modified (GM) potatoes expressing *Bacillus thuringiensis (Bt) cry3A* toxin designed for protection of plants from Colorado potato beetle (CPB) that were released commercially as varieties in the USA from 1995-2000, were banned from sale due to concerns in relation to its potential risks to the environment and human health (Grafius and Douches, 2008). It would be expected that in the process of potato transformation and selection, biochemical modifications take place. For this reason, the release and agricultural use of genetically engineered plants including potato has raised public concerns throughout the world.

The appearance of phenotypically abnormal plants at a frequency of 15-80% has been reported among populations of transgenic potatoes grown under field conditions (Barell *et al*., 2013). These off-types are attributed to somaclonal variation and exhibited shoot morphological abnormalities, resulting in stunted growth and diminished tuber yield (Davidson *et al*., 2002). Genetically modified potato tubers expressing soybean glycinin were found to contain higher GA content (Kuiper *et al*., 2001). Concerns raised in the UK on the development of transgenic herbicide-resistant crops included their negative consequences on biological biodiversity (Lövei, 2001). Thus a better understanding of secondary metabolites has coincided with an increasing environmental concern and the advent of genetic engineering.

1.2 Problem statement

Potatoes synthesize secondary compounds that include GAs, phenolic acids and PIs. Isolation, characterization and quantification of these secondary metabolites and the evaluation of their physiological roles in the potato plant and in animals have yet to be conclusively determined. In addition, there are insufficient published reports regarding the influence of stage of growth and storage on the levels of potato secondary metabolites. Given the potentially toxic nature of GAs, phenolic acids and PIs, it is important to assess the quantities present in cultivated potato varieties. In Kenya, very few studies have focused on the analysis of secondary metabolites of commercial varieties apart from the use of human taste panels to determine the quality of potatoes. Such sensory evaluation is subjective and the secondary compounds present could be hazardous to the health of the panelists. Therefore, it is necessary that more objective, safe and quantifiable measures of potato quality be developed for correct assessment to aid in the improvement of the potato. Tuber and leaf analyses of secondary metabolites could therefore be used for this purpose. This study was therefore devoted to quantify the relative amounts of GAs, phenolic acids and PIs in five commercial potato varieties during different growth stages and storage.

1.3 Justification

The analyses of secondary metabolites including GAs, phenolic acids and PIs contribute to a greater understanding of the biochemical principles, which affect the safety, quality, and suitability of potato for storage and processing for both commercial and home consumption (Friedman and McDonald, 1999; Hajšlová *et al*., 2005). It is in the consumers interest that only potatoes with low levels of anti-nutrients and toxic constituents are processed. However, to-date, researchers have mainly centered on the presence of GAs

(Friedman, 2006) in commercial varieties and transgenic potatoes and have devoted very little attention to phenolic acids and PIs for safety assessment. There has been limited potential for selecting safe and nutritive potatoes during breeding programmes until advanced clones are selected and evaluated for quality by sensory panels (Abong *et al*., 2009). This approach can subjective and risky and therefore, there is need to establish qualitative analytical methods for evaluating the quality and safety of potatoes during breeding programmes. Determination of secondary metabolites in potato varieties could provide a comprehensive understanding of the flow of carbon and guide in removal of toxic breeding lines early before they are released for commercial purposes. Therefore, development of safe and reliable chemical tests based on this study should complement organoleptic tests. Biochemical changes occur in a potato throughout storage and some of them result in significant changes in glycoalkaloids that are potentially toxic to human beings at concentrations exceeding 20 mg/100 g Fwt of tuber. Thus an understanding of various biochemical changes is required to design the optimal storage conditions and extend the quality of harvested potato crop. There has been limited research carried out to determine the variability in the accumulation potato secondary metabolites during storage. In addition, the question arises whether different conditions of storage influence the levels of GAs, phenolic acids and PIs to a similar extent. Knowledge derived from storage conditions may provide sufficient background of using biochemical tools to strengthen the approach and goals of plant breeding programs.

Existing studies clearly indicate multiple overlapping aspects of potato GAs, phenolic acids and PIs (Friedman, 2006). Since these compounds are reported to interact with

nutrients in a number of ways that affect the safety and quality of food, there is need to determine their levels in potato tubers as a component of safety evaluation. As part of an effort to contribute towards this goal, this study was designed to determine the concentration of GAs, phenolic acids and PIs of potato varieties at different stages of growth and during storage. These data are important when developing potato varieties with low levels of toxic compounds and would help in understanding the critical role of these secondary metabolites in the potato physiology during growth and storage. This study will form an important pre-requisite for the development and selection of new and improved, least toxic and most beneficial varieties of potato.

1.4 Objectives

1.4.1 Main objective

To determine the levels of glycoalkaloids, phenolic acids and protease inhibitors in selected commercial potato varieties grown in Kenya.

1.4.2 Specific objectives

1. To determine the levels of glycoalkaloids, phenolic acids and protease inhibitors in leaves and tubers of five commercial potato varieties.
2. To determine the effect of stage of growth on the conentration of glycoalkaloids, phenolic acids and protease inhibitors.
3. To investigate the postharvest changes of glycoalkaloids, phenolic acids and protease inhibitors during storage of potato tubers.

1.4.3 H_A Hypotheses

1 There are significant differences between varieties with regard to their phytonutrient content on a dry weight basis.

2 Differences in stage of growth account for the variation in the contents of phytonutrients.

3 Differences in storage conditions account for the variation in the contents of phytonutrients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The potato origin and distribution

Potato (*Solanum tuberosum* L.), is one of the world's staple food crop grown in more than 160 countries (Camire *et al*., 2009). It is the world's fourth major food crop after maize (*Zea mays* L.), rice (*Oryza sativa*) and wheat (*Triticum aestivum* L.) (Evers and Deußer, 2012). The potato was first domesticated in the Andean highlands of Peru and Bolivia, where one of its ancestral diploid wild species *S. sparsipilum* still grows (Coca-Moranthe and Castillo-Plata, 2007). The Spanish introduced it to Europe in the 16th Century from where its cultivation spread throughout the world. Today, the potato commonly recognized as the 'King of vegetables' by the natives of South America, serves as a major staple food for over one billion people worldwide (Andrews, 2015; Srivastava and Kumar, 2012).

2.2 Nutritional composition

Potato is an excellent source of carbohydrates, essential vitamins, minerals and high quality protein (Khan *et al.*, 2013). The carbohydrate constitutes about 75% of total dry matter and important sugars such as sucrose, glucose and fructose that are the main source of energy. Potatoes are rich in organic nutrients such as vitamin C, B_6 and B_{12} and also contain significant levels of potassium, copper, manganese and phosphorous (Andre *et al*., 2007; Schieber and Saldaña, 2009). Besides providing basic nutrients, potato tubers have the potential to be an important source of health promoting antioxidants (Ezekiel *et al*., 2013), pointing to their relevance in the human diet. On a dry weight basis, potatoes can have high quality protein content in the order of 10%, which is comparable to that of wheat

and higher than that of rice and maize (Friedman, 2004). These incredible benefits make the potato an important crop with potential to contribute to food security in Africa, South America and Asia.

2.3 Potato production and utilization

Potato has become an important food crop in the world. The total world potato production was estimated at 376 million tons in 2013 (FAOSTAT, 2015). According to FAO, China is at the present the World's leading potato producer, followed by India, Russia, Ukraine and the United States. In Kenya, it is ranked the second most important subsistence and commercial crop after maize (Onditi *et al*., 2012). The crop has expanded in its cultivation, total production and usage, and contributes a great deal in human diet in the production regions and in large towns. It is produced mainly in the highlands and mid altitude areas (1500-3000 m) of Central, Eastern and Rift Valley regions where the crop has higher yields than maize, rice and other food crops (Abong *et al*., 2010; Kaguongo *et al*., 2008). Many potato varieties including Nyayo, Kerr's pink, Desiree, Tigoni, Roslin Eburu, Asante, Roslin Tana, Kenya Sifa, Annet, Kenya Baraka and Dutch Robijn are currently grown and marketed in Kenya (Abong *et al*., 2009). Grown by more than 800, 000 farmers, the potato industry employ more than 2.5 million people (Onditi *et al*., 2012).

Potatoes are planted mostly in highlands and temperate areas during spring, grow quickly and tubers can be harvested during summer and autumn (Albert, 2009). In lowlands, potato farmers commonly plant sprouted tubers at the start of the cool season in areas with high soil moisture or irrigation. Cold winter is conducive for storage of ware potatoes.

In the crop growing areas, the tubers are eaten as boiled, fried, and eaten in stews or mixed with maize and beans. At the commercial level, it is mainly consumed as French Fries (chips), crisps, bhajias and other potato-based snacks (Ooko and Kabira, 2011). Potatoes are also used as animal feeds and as an industrially important source of starch and alcohol (Srivastava and Kumar, 2012). The crop generate significant returns in foreign exchange in a few regions including North Africa, Southern Europe and Middle East, but over 98% of the crop grown in developing countries is consumed by the locals (Schwartzmann, 2010). In most developing countries, potato processing into French fries, crisps and Bhajias is increasing fast in most developing countries.

The annual consumption levels of potatoes vary widely among countries and regions. Consumption exceeds 100 Kg per head per year in the UK, Poland, the Ukraine and the Russian Federation (FAO, 2008). Within developing countries, mean potato consumption levels are lowest and highest in the hot tropics and highland production zones, respectively. Potato consumption is increasing rapidly especially in those areas where the demand for food is growing fast and technology has lowered the costs of production and marketing.

As a cheap food source, the potato contributed tremendously to Europe's industrial revolution. It has also provided famine relief during periods of crop failure and war. In Ireland, however, failure of the potato crop due to late blight (LB) in the 1840s caused a great famine, one of the greatest food disasters in human history (Widmark, 2010). According to International Potato Centre (CIP), developing countries today account for over half of the world's potato production.

2.4 Constraints to potato production

Inspite of the clear potential of potato in helping meet the world's food needs, there are important constraints limiting its production. Climatic hazards like frost, hail stones, extremes in temperature and drought are major sources of production risk that reduce the absolute and marketable yield and quality of potato plants (Evers *et al*., 2007). Potato is also prone to many bacterial, fungal and viral diseases. Late blight is usually the most destructive disease worldwide where potatoes are grown and is responsible for high annual production losses of about 42% (Agu, 2004). In Kenya, incidences of LB, insect pests such as potato tuber moth (*Phthorimaeae operailella*) and aphids (*Aphis fabae, Macrosiphum euphorbia and A.gossypii*) and viral diseases [Potato virus Y (PVY) and potato leaf roll virus (PLRV)] have been reported (Were *et al*., 2013). Potato cyst nematodes (PCN), *Globodera rostochiensis* and root knot nematodes (RKN), *Meloidogyne Spp.* have been isolated in the major potato growing districts with the former presenting a significant risk to potato production in the country (Mwangi *et al*., 2015).

2.5 Potato breeding and genetic engineering

Potato breeding programs worldwide are undergoing a period of rapid change (Gorji and Polgar, 2010). As new techniques become available, breeders have adopted those which give them an advantage, particularly because a variety of biotechnological tools and techniques are available. In all respects, the aims of most potato breeding programmes have been development and release the best varieties to growers and potato industry.

Cultivated potato is tetraploid (2n=4x=48). Breeding of varieties at the tetraploid level relies primarily on phenotypic recurrent selection. Testing procedures are largely governed by practical conditions of reliability of various tests used. The biggest problem with

tetraploids is their complex patterns of tetrasomic inheritance (Muthoni *et al*., 2015). This makes genetic studies on potato and potato breeding programmes complicated. The recurrent phenotypic selection programme also requires heavy investment due to requirements of large populations and long time frame. Therefore, diploids are very useful in the study of the breeding value of parental lines, their genetics and for crossing with many wild diploid species (Watanabe, 2015). Although there is great potential for use of diploids there is also understandable reservation because of the investment required due to the fact that in the end one has to return to the tetraploid level. Because of these drawbacks, genetic transformations have been explored to improve on weaknesses in the existing varieties.

Application of recombinant DNA technology has now made it feasible to introduce desirable traits. Currently, *Agrobacterium*-mediated transformation is the dominant technology preffered for the genetic transformation of potato because of its relatively high efficiency and rapid regeneration of stable transformed plants (Chakravarty and Wang-Prunski, 2010). Transfer of genetic material from wild relatives to commercial potatoes and other genera has been successful through genetic engineering. Nowdays, a number of transgenic potato plants, particularly those with enhanced resistance to LB, PLRV, PVY, potato tuber moth (PTM), CPB and drought, are available (Barell *et al*., 2013; Cheng *et al*., 2013; Davidson *et al*., 2002; Kuhl *et al*., 2007; Missiou *et al*., 2004). To date, no transgenic potato has been developed or introduced into Kenya, but there is a possibility of introduction in future.

For transgenic crops, some researchers support the hypothesis that DNA insertion disrupts the expression of other genes including ones for toxins (Wilson *et al*., 2006). With respect to transgenic potatoes the concern is in recombination that can cause activation of the gene(s) responsible for the accumulation of GAs in tubers. Given the potentially toxic nature of GAs, it is therefore important to assess their concentration and associated biochemical imbalances in tubers of advanced potato breeding lines before their release for commercial use.

Transgenic potato lines acceptable for the market must have superior characteristics without compromising key agronomic and quality traits (Tikole *et al*., 2014). The technique allows for the transfer of a desirable gene while still retaining all the other genes derived from sexual crossing. However, genetic engineering techniques are unable to handle desirable traits controlled by polygenes e.g. yield in an organized manner. Thus sufficient knowledge of genetics and biochemical background in the potato is needed if the technique is to open possibilities for development of potato cultivars with enhanced characteristics. The biochemical data generated in this study may also guide in risk assessment of transgenic potato in future.

2.6 Biologically active components of the potato

Potatoes accumulate a variety of biologically active secondary compounds including protease inhibitors (PIs), phytoalexins, phenolic compounds and GAs that play key roles in defense against herbivores, attraction of pollinators and protection against radiation (Brown *et al*., 2005; Friedman, 2006; Zarzecka *et al*., 2013). These metabolites have a role in plant defense and varied nutritional and pharmacological properties in human beings and

animals. Therefore, there is need to develop a comprehensive understanding of the role of these metabolites in plant physiology and in animal nutrition.

2.6.1 Glycoalkaloids

Glycoalkaloids (GAs) are a group of highly toxic compounds found throughout the nightshade family. The principal GAs found in potatoes are α-solanine and α-chaconine (Figure 2.1), both of which are glycosylated derivatives of solanidine and together comprise as much as 95% of the total GAs (Ruiz de Galarreta *et al*., 2015).

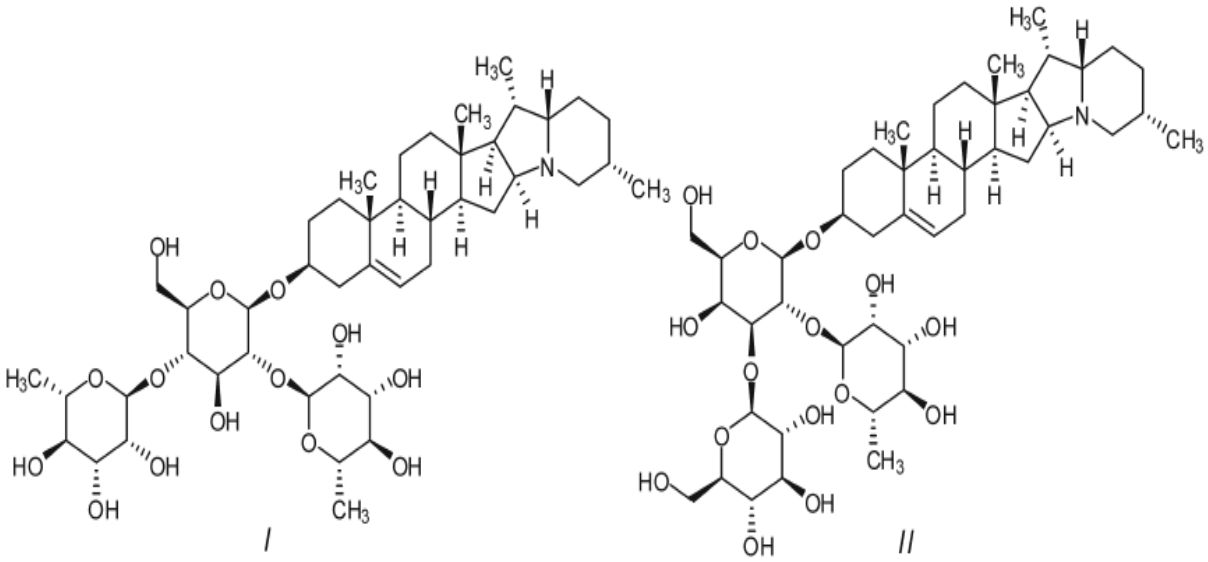


Figure 2.1: Structures of potato glycoalkaloids α-chaconine (I) and α-solanine (II). (Adapted from Liu *et al*, 2014).

Research has centred on GAs because these tend to accumulate to toxic levels for human and animal consumption (Valcarcel *et al*., 2014). A classic example is the var. Lenape, bred by the United States Department of Agriculture (USDA) during the 1970s, had desirable processing attributes derived from *Solanum chacoense* and high LB resistance derived from a wild *Solanum demissum* accession (Rommens, 2007). However, GA

content in Lenape tubers was found to be several times higher than normal during routine monitoring and was subsequently withdrawn by USDA (Sterrett *et al*., 2006).

Glycoalkaloids are normal constituents in all tissues of potatoes at all stages of growth but they accumulate to high concentration in metabolically active parts like young leaves, flowers and unripe berries (Nema *et al*., 2008; Ventrella *et al*., 2015). In tubers, higher concentrations are commonly found in the cortex and periderm, but decrease significantly towards the pith. The level of GAs is quite low in normal tubers of popular varieties e.g. Kerrs pink (12.28 mg/100g Fwt) and Desiree (7.74 mg/100g Fwt) (Kirui *et al*., 2009). The mean concentration of GAs in commercial potato varieties is usually less than the established safe level of 20 mg/100g Fwt. Although the level of GAs in commercial varieties is low, there is a danger that modern breeding techniques and the practice of introducing disease resistance characters from wild relatives may result in elevated levels in new varieties.

Potatoes with mean glycoalkaloid (GA) concentrations above 20 mg/100g Fwt are considered detrimental for human consumption because of their bitterness and toxicity (Rytel, 2012). Symptoms of severe GA poisoning in animals and human include abdominal cramps, diarrhoea and vomiting. The estimated $IPLD_{50}$ in mice for α-sol and α-cha are 27 and 30mg/kg body weight, respectively (Tek, 2006).

2.6.1.1 Glycoalkaloid synthesis and uses

Glycoalkaloids are synthesized via cholesterol, a common precursor produced by the mevalonic acid pathway (Sawai *et al*., 2014). Cholesterol does not accumulate in plants but

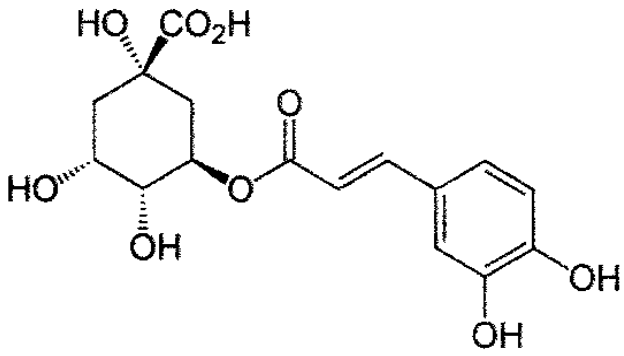
is effectively converted to steroidal glycoalkaloids including solasodine and solanidine which are eventually glycosylated to their respective GAs.

Previous studies have reported that the level of total glycoalkaloids (TGA) in cultivated potato plants depends on various factors such as genetic, growing, agrotechnical, transportation conditions and storage, exposure to pathogens, light and maturity at harvest time (Hamouz *et al.,* 2014; Khan *et al*., 2013; Valcarcel *et al*., 2014). Therefore, the level of GAs should be used for routine selection of genotypes and the most appropriate potato clones for variety registration in a breeding population.

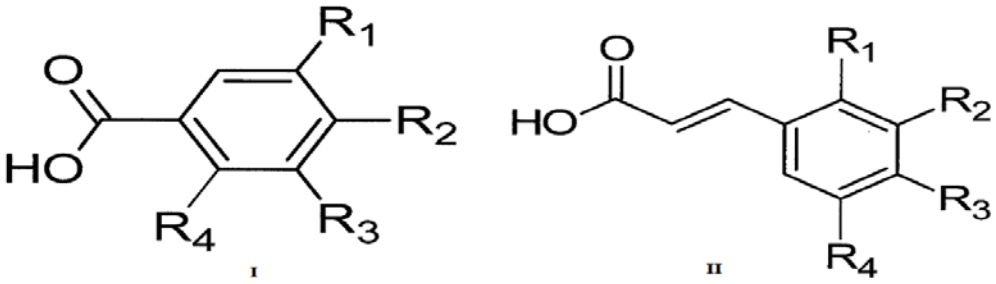
Several studies have examined the impact of GAs on the development of potato pests and diseases. High concentrations of GAs have been implicated in resistance to CPB, *Leptinotarsa decemlineata* say, leafhopper, *Empoasca fabae* and wireworm, *Agriotes obscurus* L. and as part of the natural defense against some bacterial and fungal diseases caused by *Erwinia caratovora* sub spp. *Atroseptica*, *Rhizoctonia solani*, *Phytophthora infestans* and *Fusarium coerulum* (Khan *et al*., 2013). These findings indicate an important cooperative role of steroid GAs in potato biochemical protection against important potato pests and diseases and may prove quite useful in breeding for resistant varieties. These uses suggest that though GA is important, potato breeders should check the levels do not exceed the prescribed limits in their promising varieties and transgenic potato plants before they are released.

2.6.2 Phenolic acids

Phenolic compounds of known importance, which have been identified in potato tubers, include CGA, caffeic acid (CFA), ferrulic acid (FA), gallic acid (GAC), catechin, rutin and malvidin (Camire *et al*., 2009). Total phenolic (TP) content in potato tubers has been correlated with antioxidant capacity and radical scavenging activity (Nara *et al*., 2006). The principal phenolic acid, CGA (Figure 2.2) is attributed to browning reactions and accumulates to high levels in potatoes (Lachman *et al*., 2008). Phenolic acids and other related compounds have been reported to have bacteriocidal and bacteriostatic effects. Therefore, phenolics compounds play important roles in plant and human health on one hand and cooking quality on the other.



Chlorogenic acid



Benzoic acids	**Cinnamic acids**
Protocatechuic acid (R_2=OH, R_1, R_3, R_4=H)	Caffeic acid (R_2, R_3=OH, R_1, R_4=H)
Salicyclic acid (R_4=OH, R_1, R_2, R_3=H)	*p*-coumaric acid (R_3=OH, R_1, R_2, R_4=H)
Gallic acid (R_1,R_2,R_3=OH, R_4=H)	Ferullic acid (R_2=OCH_3, R_3=OH, R_1,R_4=H)

Figure 2.2: Chemical structure of chlorogenic acid, the names and general formulas of the major potato phenolic acids derived from (I) benzoic acids: protocatechuic, salicyclic and gallic acid; and (II) cinnamic acids: caffeic, *p*-coumaric, and ferullic acid. (Adapted from Maria de Lourdes, 2013).

Concentration of phenolics in cell walls of potato tubers are important in non-race-specific resistance to *P. infestans* and potato-cyct nematodes *Globodera pallida* and *G.rostochiensis* (Taoutaou *et al*., 2013; Ohri and Pannu, 2010). Widmark (2010) revealed that sesquiterpenoids cooperate with a multitude of other resistance factors to protect the potato tissue against *P. infestans*. All these suggest that phenolic acids play a critical role in defense response to important potato phytopathogens. Therefore, knowledge of their

genetic and biochemical background may open possibilities for their utilization in potato protection.

Nara *et al*. (2006) analyzed free and conjugated phenolic acids isolated from potato (cv. Toyoshiro) peel and flesh. Chlorogenic acid and CFA were identified as the main components of free phenolics in potato peel while FA was the predominant conjugated phenolic acid that exhibited a much stronger radical-scavenging activity. Other important phenolics present in potato peel in low amounts include GAC and protocatechuic acid (PCA) (Azadeh *et al*., 2012). Chlorogenic acid is the most important metabolic sink for phenolics in potato tubers and can comprise over 90% of TP (Payyavula *et al*., 2015. This phenolic acid has been a subject of intense study because it affects the quality of processed products.

2.6.2.1 Chlorogenic acid

Potato plants have been reported to accumulate a wider range of CGA content in various parts. The highest concentration of CGA in potato was determined in sprouts (7540 mg/Kg Fwt) followed by leaves (2235 mg), roots (263 mg) and finally tubers (174 mg) (Dao and Friedman, 1992). According to Payyavula *et al*. (2015), CGA constitutes nearly 50% of TP in flowers tubers, and up to 80% in leaves. In stored tubers, CGA levels can increase rapidly in the buds (Delgado *et al*., 2001). The values in potato varieties that range from 3-90 mg/100g Fwt have been reported (Reyes *et al*., 2005). It is also synthesized rapidly in cells adjacent to wounds (Matsuda *et al*., 2003), and shows reduction after γ-irradiation (Cisneros-Zevallos, 2003). These changes roughly parallel the GA contents and a need exists to determine and set upper levels for desirable potato quality.

2.6.2.2 Chlorogenic acid synthesis

Chlorogenic acid is oxidized by polyphenol oxidase (PPO) to O-quinone, an intermediate that normally react quickly with NH_2, SH and SCH_3 groups of lysine, cysteine and methionine, respectively and indole rings of tryptophan in the enzymatic browning reactions (Ma *et al*., 2010). These browning reactions damage essential amino acids, inhibit digestive enzymes and reduce nutritional quality of potato products. These reactions are therefore of economic and nutritional significance to growers and processors.

Phenylalanine and tyrosine serve as the initial substrates for many plant phenolics (Payyavula *et al*., 2015). The first step involving phenylalanine in CGA biosynthesis is catalyzed by PAL (Figure 2.3). Phenylalanine ammonia lyase has received most of the attention in studies of the regulation of phenolic synthesis. Although other pathways to phenolic compounds exist and other sequences for their synthesis are possible, phenylalanine and tyrosine are utilized for the synthesis of polypeptides, phenolic acids, flavonoids and lignin (Figure 2.3 and 2.4). It is therefore of interest to determine the concentration of phenolic acids alongside that of GAs.

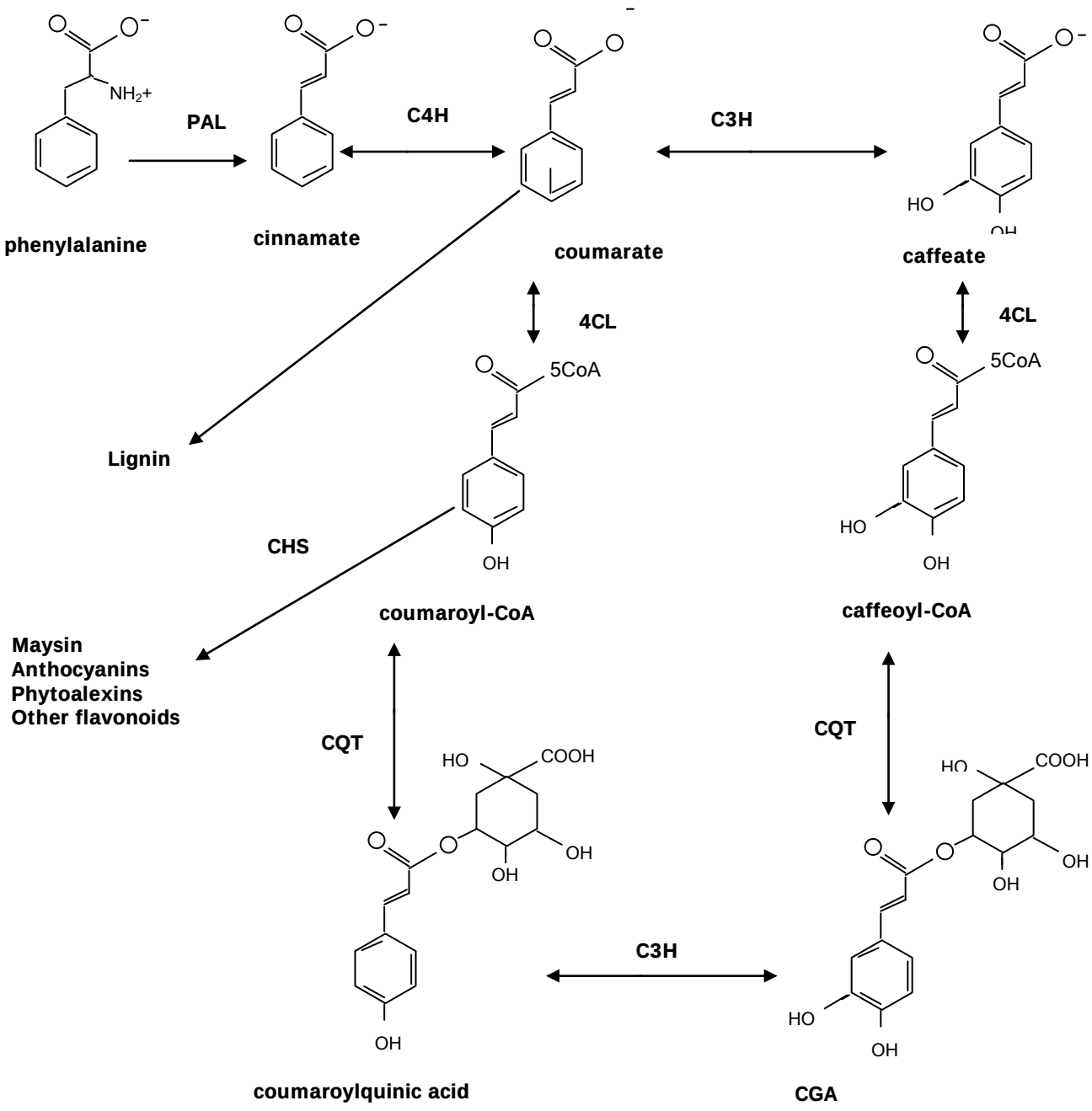


Figure 2.3: The proposed steps leading to chlorogenic acid. PAL, phenylalanine ammonia lyase, C4H, cinnamate-4-hydroxylase; C3H, coumarate-3-hydroxylase; 4CL, 4-coumaroylCoA ligase; CQT, coumaroylCoA: quinate hydroxycinnamoyl transferase; CHS, chalcone synthase. (Adapted from Bushman *et al*., 2002)

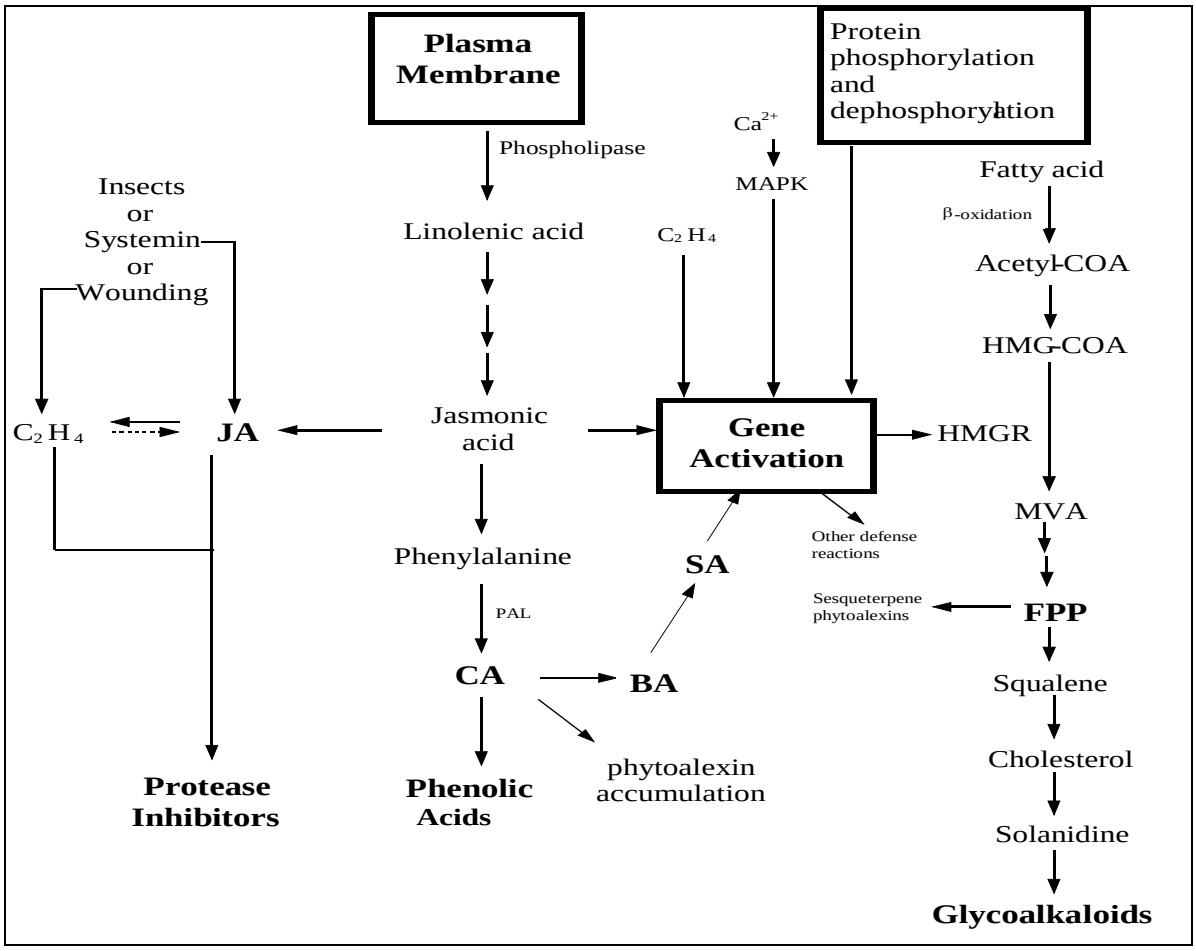


Figure 2.4 Generalized metabolic pathway showing biosynthesis of phenolic acids, glycoalkaloids and protease inhibitors. BA, Benzoic acid; SA, Salicyclic acid; JA, Jasmonic acid; CA, Cinnamic acid; MVA, Mevalonic acid; PAL, Phenylalanine ammonia lyase; FPP, Farnesyl pyrophosphate; HMGR, 3 Hydroxy 3-methyl glutaryl reductase; HMG-COA, 3 Hydroxy 3-methyl glutaryl coenzyme A; MAPK, Mitogen-activating protein kinase; BA-2H, Benzoic acid 2-Hydroxylase. (Adapted from Ginzberg *et al*., 2009; Payyavula et al., 2015; Koiwa *et al*., 1997)

2.6.3 Protease inhibitors

Protease inhibitors (PIs) are widely distributed within the plantae. They are particularly plenty in storage organs and seeds where it constitute 1-10% of the total protein or to much higher levels in exceptional cases (Fisher *et al*., 2015). PIs constitute the second major potato tuber protein after patatin (Camire *et al*., 2009) and play a key role in regulating

endogenous proteases throughout cellular development and in plant defense against pathogens and insect pests (Rashed *et al* 2008; Yang *et al*., 2008). Proteases supplies amino acids for protein synthesis during sprouting and vegetative growth as well as tuber initiation, bulking and maturation (Dwelle and Love, 2014; Weeda *et al*., 2009).

Most of the PIs that have been isolated from plants have specificities for animal or microbial enzymes especially chymotrypsin and trypsin. PIs contribute to plant resistance and tolerance to insect pests, and their value in minimizing insecticide loads in the fields has been reported (Schlüter *et al*., 2010). There is also a medical interest in the properties of PIs due to their proven ability to prevent carcinogenesis, reduce obesity and improve glucose control in diabetics (Komarnytsky *et al*., 2011; Kim *et al*., 2009).

Potato expresses diverse PIs that bind to and inhibit a variety of proteases (Beekwider *et al*., 2000). The PIs differ from each other by their amino acid sequences, substrate specificity and mechanisms of regulation (Bauw *et al*., 2006). Based on the target proteases, there are seven families of potato PIs. These inhibitors include protease inhibitor 1(PI-1) and 2(PI-2), respectively, potato cysteine PI (PCPI), potato aspartate PI (PAPI), potato Kunitz-type inhibitor (KTI), Bowman-Birk inhibitor (BBI), potato carboxypeptidase inhibitor (PCI) and other Ser- and Cys-type inhibitors (Weeda *et al*., 2009). PIs in tubers represent approximately 50% of the soluble proteins, with the most abundant being inhibitors of serine proteases from families KTI, PI-1, PI-2 and BBI (Fisher *et al*., 2015; Pouvreau, 2004). According to Pouvreau (2004), all the families (except PCI) inhibit chymotrypsin and/or trypsin.

Potatoes contain significant amount of trypsin, chymotrypsin and carboxypeptidase A inhibitors (Friedman, 2004). Inhibitors of trypsin and chymotrypsin enzymes in potato tubers are however inactivated (denatured) through food processing (Gimba *et al*., 2013). The occurrence of a number of foliar PIs are usually induced by and constitutively produced in tubers and other parts of the plant. This has led to the conclusion that they might be involved in the protection of vulnerable plant tissues from attack by pests and pathogen (Hartl *et al*., 2011).

The occurrence of PIs in tubers and the aerial parts of the plant has been widely documented (Habib and Fazili, 2007). Potato tubers are a rich source of PI (Fisher *et al*., 2015). In aerial parts, PIs are present in leaves, flowers, roots and fruits (Fan and Wu, 2005) an indication of the diverse roles of PIs in plants. According to Hartl *et al*. (2011) PIs are involved in the fine control of proteolysis by protecting specific tissues and regulating the activity of proteases. The accumulation of PIs in the potato foliage is an excellent adaptive strategy because these are the main tissues attacked by most herbivores and pathogens.

The probable role of PIs as protective agents against insect has been strongly implied. Some of the most convincing evidence for a direct role of PIs in plant protection came from studies of the wound-induced synthesis of PIs in potato and tomato plants (Ryan, 2000). Wounding of leaves by CPB or the mechanical stimulation of such wounding induced a rapid accumulation of PIs throughout the aerial tissue of the plant. The systemic response is mediated by systemin, an 18 amino acid plant peptide, which is released from

the wound site and transmitted to undamaged leaves throughout the plant where it induces the production of PIs and other defense-related proteins (Yan *et al*., 2013). These proteins can accumulate to high levels in leaves of the damaged plant and co-ordinate defensive responses by inhibiting protein digestion in the guts of herbivorous insects. According to Hartl *et al*. (2011) PIs are involved in the fine control of proteolysis by protecting specific tissues and regulating the activity of proteases.

The PIs ingested by insects have the capacity to form complexes and inhibit the hydrolytic activity of different classes of digestive proteases present in the insect mid-guts (Outchkorov *et al*, 2004).The inhibition starve the insects of essential amino acids resulting in reduced growth and survival (Parde, 2009). Serine (trypsin or chymotrypsin) and cysteine protease inhibitors have been identified to have deleterious effects including increased mortality and reduced fecundity on important insect pests mainly of the orders Coleoptera and Lepidoptera (Saguez *et al*., 2010). Therefore, it is important to integrate anti-digestive compounds such as PIs as an important component of integrated pest management.

Beyond plant protection, PIs have become good targets in pharmacology and drug development. Members of BBI and KTI families (Figure 2.5) are serine PIs that have been shown to have anti-tumor activities and to inhibit several enzymes and proteases involved in human diseases (Fisher *et al*., 2015). The therapeutic effects of KTIs in inflammation, thrombosis, acquired immune deficiency syndrome (AIDS), fungal and parasitic diseases has been documented (Oliva *et al*., 2010). Since most of the PIs are effective inhibitors of

mammalian proteases, the question of whether they might also be harmful to humans has to be addressed and their possible safe levels also need to be established.

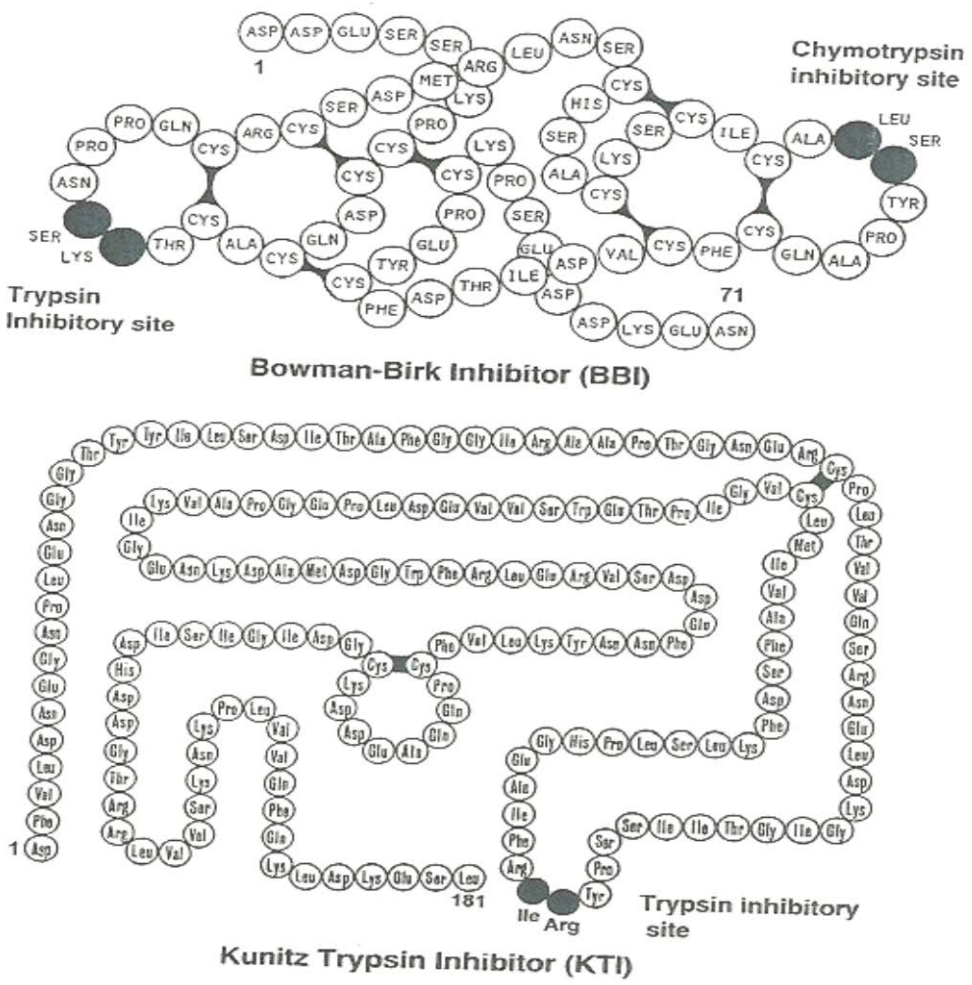


Figure 2.5: Structures of the major protease inhibitors of potatoes. Bowman-Birk protease inhibitors (BBI) has seven disulfide bonds per mole and Kunitz type trypsin inhibitor (KTI) has two. (Adapted from Friedman and Brandon, 2001)

The expression of foreign PIs in transgenic plants showing improved resistance to insect herbivores finally provided direct confirmation for the use of PIs in pest control. The first successful transfer of PI protein that encoded cowpea trypsin inhibitor (CpTi) produced transgenic tobacco which had significant insecticidal activity against tobacco hornworm

Manduca sexta L. (Parde, 2009). Subsequent bioassays established that CpTi expressing plants had significantly enhanced resistance to tobacco budworm (*Heliothis virescens)* and to a broad range of other Lepidopteran pests which are capable of eating tobacco. Transgenic plants expressing potato PIs developed increased resistance to a broad range of insect pests in rice (Bu *et al*., 2006), sugarcane (Burgess *et al*., 2002), tomato (Rashed *et al*., 2008) and Poplar (Fan and Wu, 2005). These investigations have revealed that any PI will not be effective against all pests and care must be taken over the selection of which inhibitor to transfer in any particular situation. Thus, the potential for PIs in agriculture and forestry are enormous and awaits full-scale utilization.

Earlier investigations have focused on purification and characterization of PIs of specific potato varieties (Bauw *et al*., 2006; Kim *et al*., 2005) and the roles of PIs in wound-inducible defense response against herbivores insects (Rashed *et al*., 2008). The changes in gut enzyme activities of specific insect pests (Kondrak *et al*., 2005), expression of recombinant PIs in transgenic plants (Schlüter *et al*., 2010); and infection of potato plants with known pathogens (Valueva *et al*., 2003) have also been investigated.

2.7 Influence of storage on the concentration of glycoalkaloids, phenolic acids and protease inhibitors

Proper storage of potatoes provides fresh and healthy products to the consumer for a longer duration in the market. Different factors including light, temperature, relative humidity, handling procedures and pests affect the quality, morphology and physiology of potato tubers during storage (Khanal and Uprety, 2014). According to Gachango *et al*. (2008), diffused light storage reduced sprouting and weight loss and has emerged as the best

storage method for potato seed tubers. But it remains to be determined whether the accumulation of GAs, phenolics and PIs during storage of potato seed tubers affect subsequent levels of these compounds during growth in the field.

The best table and processing quality for potato is usually determined at the time of harvest. Storage extends the shelf life and thereby helps with orderly marketing, distribution and utilization. Potato storage by farmers in Kenya is limited to a maximum of 2-3 months, while waiting for better prices. Assuming that other factors are held constant, tuber quality is prolonged at temperature of 2-4 ^{o}C and high RH levels of between 90 – 95%. High ambient temperature reduces postharvest storage life because of increased carbohydrate metabolism and light enhances the formation of chlorophyll that results in tuber greening and synthesis of secondary metabolites.

Friedman (2004) reported that GAs and CGA increases overtime during post harvest storage, while inhibitors of digestive enzymes do not. The GA and CGA content of potatoes have been reported to increase during storage (Haase, 2008; Tokuşoğlu *et al*., 2005) with the storage temperature having an appreciably greater increase. Sprouting during potato storage increase the GA content in the tuber tissue primarily within the eyes (Valcarcel *et al*., 2014). Irradiation intended to inhibit sprouting of tubers caused an increase in the total phenolic content (Ezekiel *et al*., 2013). Therefore, the presence of secondary metabolites in potato tubers is influenced by the many variables inherent during storage.

2.8 Influence of various potato processing methods on the levels of secondary metabolites and nutritional quality

Peeling of potato tubers prior to cooking removes nearly all the toxic GAs and considerably reduce the dietary fiber, minerals, phenolic compounds especially CGA, GAC, PCA and CFA (Camire *et al*., 2009). Ostry *et al*. (2010) demonstrated that peeling removes 60-96% of TGA present in whole tubers and upto 35% in varieties with high GA content. Thus, the variable partitioning of GAs and other secondary metabolites between the flesh and the peel in different potato varieties should be considered when potato flesh or peel is to be used in human and animal nutrition.

Numerous studies have revealed that baking, microwaving and boiling does little to decrease GA contents because of their heat stability and consequently, any GA present in the tubers prior to cooking will still remain afterwards. However, deep-frying is the main method that can minimize the level of potato GAs by about 77 to 94% (Valcarcel *et al*., 2014). Further accumulation of GAs is stopped as the enzymes necessary for its biosynthesis are deactivated after processing. According to Gimba *et al*. (2013), adequate heat processing inactivated the heat labile trypsin and chymotrypsin inhibitors and is effective in improving protein and starch digestibility.

Among the aspects affecting quality of processed potato are the after cooking blackening (ACB) and enzymatic browning. The occurrence of ACB is worldwide and is a key quality defect that affects the marketability of potatoes to growers (Wang-Prunski and Nowak, 2004). The development of ACB in susceptible potato cultivars is due to the oxidation of

phenolic compounds such as CGA and CFA by polyphenol oxidase to give gray, blue, purple or black end products that are perceived by several consumers as an undesirable tuber trait. The ratio of CGA to citric acid has been demonstrated as being the principal factor accounting for differences in ACB within individual tubers and is affected by a number of factors including variety, fertilizer application, soils and environmental conditions (Adams and Brown, 2007). Therefore, identification of potato varieties with acceptable tuber quality could be achieved early in the breeding program by use of effective progeny testing and subsequent clonal selection.

Enzymatic browning of peeled or cut potato tubers involving oxidation of phenolic compounds by polyphenol oxidase (PPO) has been extensively studied (Adams and Brown, 2007). The concentration of PPO, CGA, tyrosine and ascorbic acid in the tuber are accurate predictive factors with regard to the extent of enzymic discoloration. Although chemical compounds containing SH-groups such as sulphites are used as antibrowning agents that inhibit PPO activity, there is increasing concern about these additives to food and their continued use may be subject to question. The biochemical factors, which affect the freedom from discolouration after cooking, are therefore important in this respect.

Flavour is one of the most powerful attributes used by potato processors and consumers to determine the overall acceptability of new varieties of potato (Jansky, 2010). This has been made possible by professional sensory evaluation panels. Results from these organoleptic tests, is generally considered to be a combination of tuber aroma, taste and texture (Jansky, 2013). The basis of flavour development is genetic, but is influenced by growing

conditions and postharvest practices (Neibauer and Maynard, 2011). According to Dresow and Böhm (2009), flavour is produced by aromatic volatile compounds that are synthesized during plant during metabolism and modified by various cooking processes. The key flavour precursors in raw potatoes include sugars, amino acids, RNAs and lipids (Jansky, 2010). These precursors react during cooking to produce the maillard reaction compounds and other degradation products that contribute to flavour. Low levels (below 10mg/100g) of GAs and phenolic acids also influence the net organoleptic properties of potatoes (Jansky, 2013). Given that potatoes contain these GAs and phenolic compounds in different proportions depending on variety, the overall flavour could be influenced by all the active components present.

The factors influencing the potato quality traits are numerous. Quality indicators such as dry matter content, texture and browning reactions are influenced by both cultural and environmental factors that are often genotype-dependent. A comprehensive understanding of the underlying biochemical and physiological changes during growth and storage is needed to better understand these complex interactions. In addition, understanding of underlying factors affecting quality becomes of more significance to the consumer and potato industry.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was carried out at the University of Nairobi, School of Biological Sciences using potato seed tubers obtained from National Potato Research Centre (NPRC), Tigoni, a national centre mandated for potato research and development in Kenya. Laboratory assays were carried out at the Centre for Biotechnology and Bioinformatics (CEBIB), while glycoalkaloid (GA) analyses by HPLC were carried out at the Kenya Plant Health Inspectorate service (KEPHIS), Nairobi.

3.2 Potato plants

The certified seeds of the commercial potato varieties: Tigoni, Asante, Kenya Karibu, Desiree and Dutch Robijn were used in this study (Table 3.1, Plate 1 and 2). All the varieties were grown under field conditions in two growing seasons (2010 and 2011) at the Agroforestry Nursery located at the University of Nairobi, Chiromo, a high altitude location with day temperature of 20-27 ^{o}C and night temperature of 17-19 ^{o}C, relative humidity of 56-60% and red soil with a high humus content and a pH of 6.5-6.7.

Table 3.1: Source and characteristics of potato varieties used in this study

Name	Source	Year of release	Duration to maturity (Days)	Optimal production altitude (Masl)	Late blight	Special attributes
Asante	KARI/CIP	1998	90-110	1800-2600	Fairly tolerant	Chipping quality
Desiree	Netherlands	1972	80-100	1800-2600	Susceptible	Good taste and storage
Dutch Robijn	Netherlands	1960,s	90-110	1600-2600	Moderate Susceptible	Storage and crisping quality
Kenya Karibu	KARI/CIP	2006	110-130	1800-2600	Tolerant	Crisping quality
Tigoni	KARI/CIP	1998	100-120	1800-2600	Tolerant	Chipping quality

Source: Lung'aho *et al*. (2006); National Crop Variety List-Kenya maintained at KEPHIS.

3.3 Experimental design

The experiment was established as a randomized complete block design (RCBD) with three replications. The three field plots consisted of five rows of twenty seed tubers per row (Table 3.2). The planting depth was 10 cm while plant spacing was 0.75 m between rows and 0.35 m between plants. Di-ammonium-phosphate fertilizer was applied at the rate of 100 Kg/hectare. Weeding was carried out after 4 weeks of planting. Potatoes were sprayed with Duduthrin to prevent insect attacks (aphids) and with Ridomil and Dithane M 45 to protect against late blight caused by *Phytophthora infestans* every 14 days during rainy days when the relative humidity was about 70%. During dry periods, sprinkler irrigation was carried out every three days to maintain the soil moisture. The average main weather variables during the growing periods are shown in Table 3.3. The potatoes were harvested three times during the growing period at 125 days. In particular three plants from each variety were randomly selected, harvested and analyzed for GAs, phenolics and PIs.

The first leaf harvest was done after 40 days to standardize the harvesting period. Subsequent leaf samples were collected at 55 and 95 days after planting (DAP) while tubers were harvested at 55, 95 and 125 DAP.

Table 3.2: The 5 × 3 factorial combination of treatments of five levels of variety and three levels of growth stages

Replicate 1		**Replicate 2**		**Replicate 3**
Desiree		Kenya Karibu		Asante
Asante		Tigoni		Dutch Robijn
Dutch Robijn		Desiree		Tigoni
Tigoni		Asante		Kenya Karibu
Kenya Karibu		Dutch Robijn		Desiree

Table 3.3: Main weather characteristics during the potato growing seasons in year 2010 and 2011

Year		**Average temperature (^{o}C)**					**Sum of precipitation (mm)**				
		Aug	**Sept**	**Oct**	**Nov**	**Dec**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
2010	**Max**	26.00	29.00	30.00	30.00	29.30	6	4	4	67.5	2
	Min	10.00	1.00	2.00	13.00	10.00					
	Mean	18.08	18.86	20.49	20.49	20.73					
2011	**Max**	28.75*	29.75*	30.90*	30.00	28.40*	41	32.5	46.5	215	24.5
	Min	9.70*	10.60*	12.50*	15.25	12.40*					
	Mean	18.47	20.16	20.84	20.48	20.72					

*Same-day record high and low temperatures.

Source: Kenya Meteorological Department.

Plate 1: Appearance of the sprouted tubers of potato varieties evaluated in this study (Scale 1mm = 1 cm).

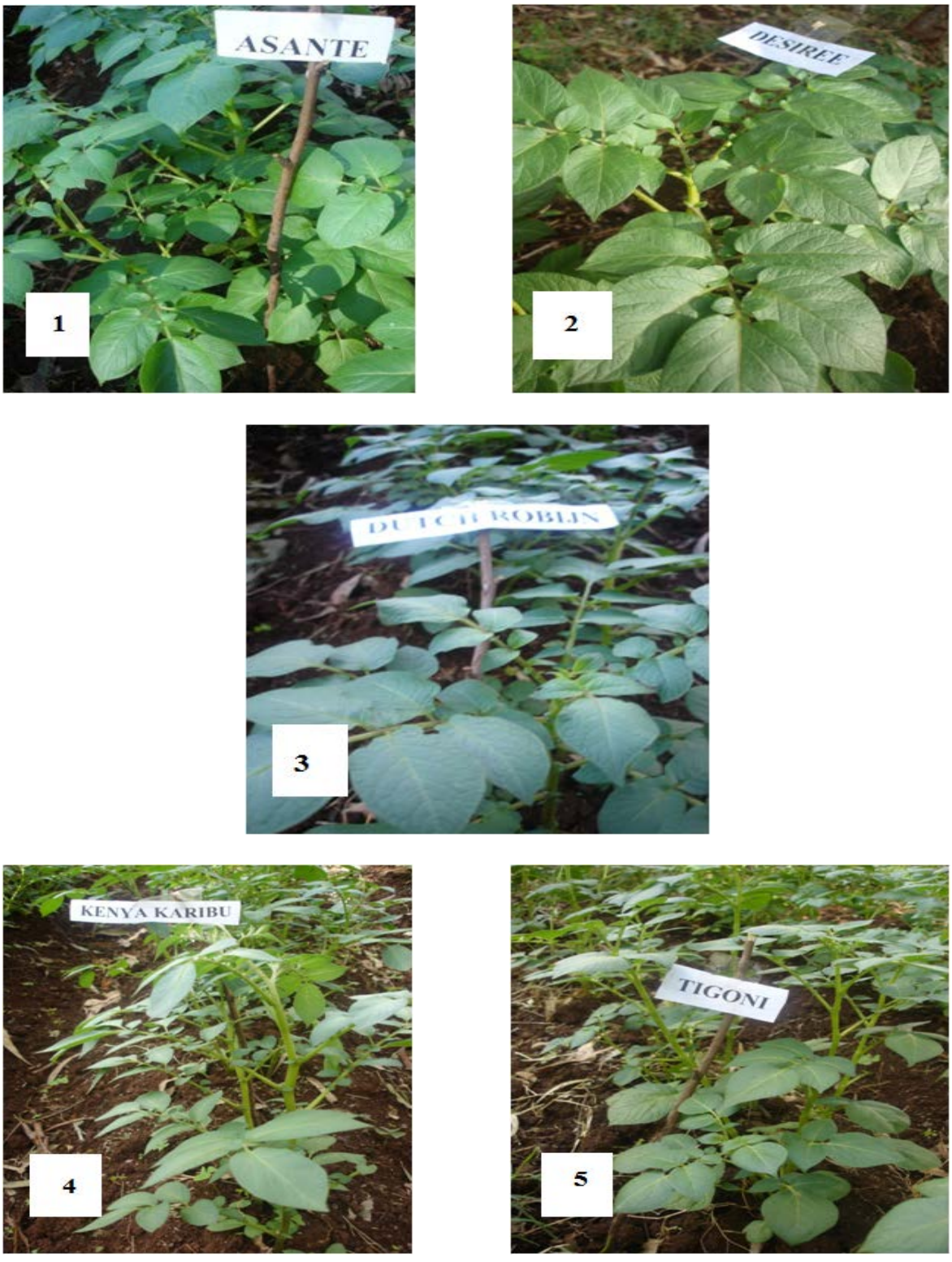


Plate 2: Appearance of the commercial potato plants after six weeks of planting at the Agroforestry Nursery, Chiromo Campus, Nairobi (Scale 1mm = 1 cm).

3.4 Sampling of leaves and tubers at different growth stages

3.4.1 Leaf tissue sampling at 40, 55 and 95 days after planting

At forty days of growth, the first harvest was carried out and during that time (three terminal leaflets and the next two opposing leaflets) were cut as a unit from the plant to minimize the variability (Brown *et al*., 1999). These five leaflets cut as a unit comprised the leaf tissue sample from one plant. Five plants were randomly selected for harvest at 40, 55 and 95 days after planting (DAP). The leaflets were immediately placed in a Marina coolbox (with ice) after harvest. After sampling of various varieties from each replicate, the leaf samples were immediately transferred to the lab, freeze-dried and within the following week, ground in a Wiley mill® and stored at 4 ^{o}C in a refrigerator prior to extraction and analysis of GAs, phenolic acids (PAs) and protease inhibitors (PIs).

3.4.2 Tuber sampling at 55, 95 and 125 days after planting

Six tubers of average weight (each weighing 17-22 g) freshly harvested from each replicate were randomly selected, washed with cold water to remove extraneous materials and dried with tissue paper. The chemical assays involved use of unpeeled tubers. The unpeeled samples were cut into small pieces with a kitchen chopper and following careful mixing homogenous sub-samples of 300 g were freeze-dried immediately in a vacuum freeze-drier (Chemlab Instruments Inc.). The freeze-dried samples were ground in a Wiley mill® to pass through a 40 mesh screen and the powders were refrigerated at 4 ^{o}C for subsequent extraction and analysis of GAs, PAs and PIs.

3.5 Extraction and characterization of potato glycoalkaloids

3.5.1 Extraction of foliar glycoalkaloids

Glycoalkaloid extraction and analyses of potato leaves and tuber tissue was carried out according to the HPLC protocol described by Cataldi *et al*. (2005) with some modifications on the mobile phase composition and detection scheme. Accurately weighed 1 g sample of ground leaf tissue were extracted with 100 ml of 2% acetic acid for 2 hours. The crude extract was recovered by vacuum filtration and concentrated to 10 to 15 ml on a rotary evaporator at 50 ^{o}C. The flask containing the sample was placed in a water bath heated to 75 ^{o}C for 30 minutes, removed from the heat and the pH was adjusted to 11 using 15 ml of 58% NH_4OH. The GAs were rapidly precipitated in ice bath for 1hour prior to centrifugation.

The GA precipitate was recovered by centrifugation at 6 000 rotation per minute (r.p.m) for 30 minutes at 1 ^{o}C using a refrigerated centrifuge (Heraeus Christ® GMBH). The pellet was washed twice with 1% NH_4OH prior to drying. The final pellet was placed in an oven at 60 ^{o}C overnight to evaporate the ammonia before it was subjected to HPLC and UV spectrophotometry for GA analysis.

3.5.2 Extraction of tuber glycoalkaloids

The HPLC method of Cataldi *et al*. (2005) with slight modifications was used to extract GAs from tubers of each variety. Approximately 2.5 g tuber powder was weighed and dissolved in 35 ml of 2% acetic acid for 2 hours. The crude extract was recovered by vacuum filtration, washed with 15 ml of 2% acetic acid and the resulting solution centrifuged for 30 minutes at 6000 r.p.m. The crude extract was heated gently to 75 ^{o}C,

removed from the heat and 15 ml of 58% aqueous NH_4OH added to increase the pH to 10. GAs were quickly precipitated in an ice-water bath for 1 hour or refrigerated overnight.

The precipitated GA was collected by centrifugation at 6000 r.p.m for 30 minutes at 1 ^oC. The pellet was then washed twice with 1% NH_4OH prior to drying. The final pellet was placed in an oven at 60 ^oC overnight to evaporate the ammonia before the GAs were analyzed using the HPLC and UV spectrophotometric methods with solvents of HPLC spectroquality grade.

3.5.3 HPLC analysis of glycoalkaloids

The HPLC analyses of GAs were carried out at KEPHIS analytical chemistry laboratory in Nairobi. Analysis was carried out by means of a Varian HPLC system (Varian Associates, Inc.) with a 9050 variable wavelength UV-visible detector, 9010 solvent delivery system and a 4400 integrator. The HPLC system was equipped with a manually operated Rheodyne® 7125 sample injector, a 20 µl loop and a Nucleosil NH_2 column (250 mm × 4.6 mm i.d). Flow rate of 1 ml/min with isocratic elution mixture of THF/0.025M KH_2PO_4/ACN (50:25:25, v/v/v) and detection was made at 208 nm at room temperature.

The GA extracts were dissolved in THF/0.025M KH_2PO_4/ACN (50:25:25, v/v/v), ultrafiltered through 0.45 µm microfilter prior to separation by HPLC. The identification and concentration of α-chaconine (α-cha) and α-solanine (α-sol) in the extracts was calculated by comparison of HPLC peak areas and retention times of known amounts of standard compounds. Equal volumes (20 µl) of GA standards (Analytical grade α-cha and α-sol, obtained from Sigma-Aldrich) of known concentration and potato extracts were

injected into the HPLC and runs conducted under standard conditions. Each sample was injected twice at a uniform time and all values were averaged. The total glycoalkaloid (TGA) content was calculated as the addition of the individual values obtained for α-cha and α-sol and expressed as mg TGA per 100g Fwt (Birch *et al.,* 2002).

3.5.4 UV Spectrophotometry

UV-spectrophotometric analysis was carried out at CEBIB laboratory, University of Nairobi. The instrument used was a Beckman DU® 530 Life Science UV/vis spectrophotometer (Beckman Coulter™) equipped with various built-in programs. This UV spectrophotometer has a spectral range of 190-1100 nm and uses a single cell module that supports a wide variety of cell holders. The cells used for the test potato extract and the blank were always kept clean and the UV spectrophotometer was always calibrated before carrying out the measurements. The absorbances found in the assays were interpolated on the standard curves.

For UV spectrophotometric analysis of GAs, the dry pellet was reconstituted in 3 ml of a mixture of 50% ethanol and sulphuric acid (1:2; v/v). One ml of 1% formaldehyde was added dropwise to the solution while the flask was spinned in an ice-bath. The resulting solution was allowed to stand at 23-25 ^{o}C for 90 minutes and the purple-red colour measured at 562 nm using the UV spectrophotometer. Equal volumes (100 µl) of GA standards of known concentration and potato extracts were subjected to analysis. A standard curve of GA was established with commercial α-solanine (Zarzecka *et al*., 2013).

3.6 Extraction and characterization of phenolic acids

Potato powder from tuber or leaf tissues were mixed with 80% aqueous methanol (1g frozen tissues per 10 ml solvent), vortexed for 30 seconds, allowed to stand for 30 minutes and centrifuged at 6000 r.p.m for 10 minutes. The recovered supernatants were used for the quantification of PAs with Folin-ciocalteau reagent (Azadeh *et al*., 2012). The absorbance readings of extracts were recorded at 765 nm. Chlorogenic acid (CGA) was used as a standard and TP content was expressed as milligrams of CGA equivalents per 100 grams of potato tissue fresh weight (mg CGA equ/100 g Fwt) (Burgos *et al*., 2013).

Chlorogenic acid was determined by UV spectrophotometry as described by Truong *et al*. (2007). Freeze-dried potato powder was defatted by extracting with hexane in a soxhlet extractor for 16 hours. A sample of defatted powder (200 mg) was then extracted with 20 ml of 80% ethanol for 6 hours before it was ultrafiltered using a 0.45 μm Nylon membrane. The filtrate was adjusted to a volume of 20 ml with 80% ethanol. To establish the extent of recovery of CGA, the entire analysis was performed on 200 mg of tuber powder and 100 mg sample of leaf powder spiked with known amounts of CGA standards.

UV spectrum 250-400 nm determined from the ethyl alcohol extract after a dilution with 80% ethanol was used. The concentration of CGA was calculated from the main peak at 325-328 nm from a standard curve generated from CGA standard purchased from Fisher scientific and Sigma-Aldrich chemical Co.

3.7 Protease inhibitor assays

PI assays were carried out using potato leaves and tubers. The assays for enzyme inhibition of trypsin and chymotrypsin were carried out following the protocol of Xu *et al*. (2004) with slight modifications on concentration of reagents. All the spectrophotometric assays were performed with foliar and tuber extracts and the final concentration of trypsin inhibitors (TI) and chymotrypsin inhibitors (CI) expressed in units/mg (U/mg). Trypsin activity is defined by the equation:

Units/mg = ($\Delta A_{247/min}$ x 1000 x 3)/(540 x mg of trypsin used). A trypsin unit (TU) is defined as the quantity of trypsin that can catalyze the hydrolysis of 1μmol of N-α-tosyl-arginine methyl ester (TAME)/min. A trypsin inhibitor unit (TIU) is equivalent to the decrease in trypsin activity by 1 TU. The activity of chymotrypsin is defined by the following equation: units/mg = (ΔA_{256}/min x 100 x 3)/(964 x mg of chymotrypsin used). One chymotrypsin unit (CU) is defined as the quantity of chymotrypsin that can catalyze the hydrolysis of 1μmol of N-benzoyl-L-tyrosine ethylester (BTEE)/min. A chymotrypsin inhibitor unit (CIU) is the reduction in chymotrypsin activity by 1 CU. Authentic Bowman-Birk inhibitor (BBI) and soybean Kunitz trypsin inhibitor (KTI) were used as standards for chymotrypsin and trypsin inhibitor assays, respectively.

3.7.1 Trypsin inhibitor assay

The dry powder (100 mg) of potato was suspended in 10 ml of Tris-HCl buffer (pH 8.1), vortexed for 30 seconds and allowed to extract for one hour at room temperature. The resulting suspension was again vortexed for 30 seconds, allowed to settle for 5 minutes and then centrifuged at 10 000 r.p.m for 10 minutes. The supernatant was diluted 1:2 with Tris-

HCl buffer. Equal volumes (20 µl) of the resulting solution was used for trypsin inhibition assay under the following controlled conditions: temperature 25 ^{o}C; buffer 0.046 M Tris-HCl containing 0.0115 M $CaCl_2$, pH 8.1, 10 mM N-α-tosyl-arginine methyl ester (TAME) (37.9 mg/10 ml of H_2O); enzyme 1 mg/ml (10 mg dissolved in 10 ml of 1 mM HCl solution). The trypsin enzyme solution was diluted to 10-20 µg/ml with 1 mM HCl solution (Xu *et al*., 2004).

Activity towards TAME in samples with inhibitors was determined as follows: 2.6 ml of buffer was added to a cuvette along with 0.1 ml of trypsin solution and 20 µl of inhibitor solution prepared to give 50% inhibition and the mixture was incubated at room temperature for 6 minutes. The reaction was started by adding 0.3 ml of substrate (TAME) and absorbance readings were taken at intervals of 30 seconds for 3 minutes at 247 nm (A_{247}) on a UV spectrophotometer. The activity in control samples without inhibitor was determined as follows: 2.6 ml of assay buffer (Tris-HCl) and 0.3 ml of TAME was added to a 3 ml cuvette followed by 0.3 ml of diluted trypsin solution. The increase in absorbance at 247 nm was determined from the first stage of the reaction with precisely known substrate concentration from the initial linear part of the curve. The calculated values were based on extracts diluted to produce trypsin inhibition of 40-60% at 25 ^{o}C.

3.7.2 Chymotrypsin inhibitor assay

The dry potato powder (200 g) was dispersed in 10 ml of Tris-HCl (pH7.8) buffer, vortexed for 30 seconds and allowed to extract for 1 hour at 25 ^{o}C. The resulting suspension was again vortexed for 30 seconds, allowed to settle for 5 minutes and then centrifuged at 10 000 r.p.m for 10 minutes. Equal volumes (20 µl) of the supernatant were

used for the inhibitor assay under the following standardized conditions: Buffer 0.08 M Tris-HCl containing 0.1 M $CaCl_2$ (pH7.8); substrate, 1.07 mM BTEE (8.4 mg/25 ml of 50% methanol); enzyme, 1 mg/ml (10 mg dissolved in 10 ml of 1 mM HCl solution). The chymotrypsin stock solution was diluted to a concentration of 10-20 μg/ml with 1 mM HCl solution (Xu *et al*., 2004).

In the absence of any inhibitor, 1.5 ml of buffer, 1.4 ml BTEE and 0.1 ml of chymotrypsin solution were added and the increase in absorbance at 256 nm (A_{256}) recorded for 3 minutes. The reaction rate (ΔA_{256}/min) was extrapolated from the rate on the initial linear part of the curve when the substrate concentration was known. In the presence of a chymotrypsin inhibitor, 1.5 ml of buffer, 0.1 ml of chymotrypsin solution and 20 μl of inhibitor was incubated for six minutes before 1.4 ml of BTEE was added and the increase in absorbance recorded as above. The calculated values were based on sample dilutions prepared to inhibit chymotrypsin trypsin activity to 40-60% at 25 ^{o}C.

3.8 Determination of glycoalkaloids, phenolic acids and protease inhibitors at different stages of storage

Sixty freshly harvested (after 125 DAP) tubers of Tigoni, Asante, Kenya Karibu, Desiree and Dutch Robijn potato varieties were cleaned with a slightly damp paper towel and divided into three groups/batches. A third of the potatoes were stored under fluorescent lights in a laboratory that was moderately opened to excess air and the other third were stored in the green house open to sunlight light. The remaining one third was stored at room temperature (20-25 ^{o}C) in a dark and dry place (as controls). The tubers were stored for three weeks (dormancy period before sprouting) and were rotated every week in all the

three set ups. The average main weather variables during the storage periods are shown in Table 3.4. Six tubers average weight (each weighing 17-22 g) from each of the set up were selected after every 7 days. The selected unpeeled potatoes were cut up into small cubes, placed in jars and freeze-dried immediately in a vacuum freeze-drier. The freeze-dried samples were ground to pass through a 40-mesh screen on the laboratory Wiley mill. The resulting powder from stored potatoes was refrigerated at 4 ^{o}C until analyzed for GAs, PAs and PIs described in sections 3.5 to 3.7.

Table 3.4: Main weather characteristics during the potato storage seasons in the year 2010 and 2011

Year		**Average weekly temperature (^{o}C)**			**Average weekly Relative Humidity (%)**		
	Storage time	**Week 1 (Day 1-7)**	**Week 2 (Day 8-14)**	**Week 3 Day (15-21)**	**Week 1 (Day 1-7)**	**Week 2 (Day 8-14)**	**Week 3 Day (15-21)**
2010	**Max**	28.10	29.00*	28.75*	50.80	60.10	60.00
	Min	13.50	13.00*	13.50*	50.60	50.00	50.00
	Mean	21.04	21.58	20.94	50.70	53.10	51.74
2011	**Max**	30.50*	28.70*	29.50	70.40	70.40	60.00
	Min	9.50*	9.50*	11.20	50.40	50.70	50.10
	Mean	20.92	19.38	20.48	58.48	59.14	50.41

*Same-day record high and low temperatures.
Source: Kenya Meteorological Department.

3.8.1 Recovery experiments

After the extraction and quantification of glycoalkaloids, phenolic acids and protease inhibitors, a series of tests were conducted to ascertain the extent of recovery of the various metabolites from potato leaves and tubers. Specifically, the recovery was tested by running

recovery experiments with spiked samples of freeze-dried potato powder. At the end of each analytical procedure, the recoveries were expressed as a percentage. Final values were adjusted with the overall recovery to compensate for the experimental losses.

To test the recovery of glycoalkaloids (GAs), 2.5 g of Tigoni potato powder was spiked with α-chaconine (α-cha) to obtain four different concentrations between 0 and 100 µg of pure α-cha standard and extracted in duplicate according to the procedure given in section 3.5.2. The experiment was repeated with potato powder spiked with α-solanine standard. The dry powder of var.Tigoni had the highest amount of TGA. The recovery of chlorogenic acid (CGA) and total phenolics was determined by adding 20 µg, 50 µg and 100 µg of accurately weighed CGA to tubes containing 200 mg of var. Tigoni potato powder. All samples were mixed thoroughly and extracted using 95% ethanol and quantified by reading their absorbance at 325 nm.

The applicability and reproducibility of the recovery method to protease inhibitors (PIs) was also determined. In particular, 20 µg, 50 µg and 100 µg of KTI and BBI were weighed and added to tubes containing 100 mg and 200 mg of freeze-dried potato powder. The samples were mixed and extracted using Tris-HCl and quantified using UV spectrophotometer by reading their absorbance at 247 nm and 256 nm, for trypsin and chymotrypsin inhibitors, respectively.

The recovery (%) in all experiments was calculated using the formula;

$$\text{Recovery (\%)} = [RM/TC+AS] \times 100$$

Where: RM is the amount of recovered metabolite, TC is the original tuber content and AS is the amount of authentic standards added before extraction. The mean recovery values were used to adjust the final values so as to compensate for losses during the extraction of GAs, phenolics and PIs in all experiments.

3.9 Statistical analyses

All experiments were carried out in three replications from two years of investigation and the present results show the means of all data combined. Quantitative data were analyzed using analysis of variance in Genstat 15th edition statistical software. One-way and two-way ANOVA were used to analyze GAs, phenolic acids and PIs concentration data from all treatments. Student's t-tests were used to conclusively identify the peaks of α-cha and α-sol in crude extracts based on their corresponding retention time values in the standards. The concentration of potato metabolites in the extracts were calculated from standard curves prepared from pure standards of known concentration and regression analyses. The differences between the means for significant treatments were compared by Fishers's protected least significant differences (LSD) at 5% ($p \leq 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Standardization

4.1.1 Recovery of α-chaconine, α-solanine, chlorogenic acid, Bowman-Birk inhibitor and Kunitz-type inhibitor

The recovery of glycoalkaloids (GAs), chlorogenic acid (CGA) and protease inhibitors (PIs) added to freeze-dried potato powder prior to extraction ranged from 86.4 to 92.1 %, 92.5 to 94.7%, and 85.3 to 88.9%, respectively (Table 4.1.1). The recovery values varied depending on the nature of the added metabolite.

Table 4.1.1: Recovery of glycoalkaloids (GAs) α-chaconine and α-solanine, chlorogenic acid (CGA), Bowman-Birk inhibitor (BBI) and Kunitz-type inhibitor (KTI) added to freeze-dried potato var. Tigoni powder.

Amount of added standards (μg)	% Recovery				
	GAs		CGA	BBI	KTI
	α-cha	α-sol			
20	87.7±1.8^{b}	86.4±1.1	85.3±1.9^{b}	86.7±1.5^{a}	85.3±1.9^{b}
50	88.4±2.3^{ab}	89.7±1.3	85.9±1.7^{ab}	87.5±1.3^{a}	85.9±1.7^{ab}
100	89.7±1.9^{a}	92.1±1.6	86.4±1.3^{a}	88.9±1.1	86.4±1.3^{a}

Values are means ± SD of three replicates. Means with the same letter within the same column are not significantly different at level $p \leq 0.05$. The original tuber contents (mg/100g Fwt) of α-cha, α-sol and CGA in potato var. Tigoni were 6.47±0.14, 4.13±0.10 and 63.5±0.12, respectively, while its trypsin and chymotrypsin inhibitor contents (U/g) were 1050.6 ± 9 and 268.5±9, respectively.

4.1.2 Standard curves of α-solanine, α-chaconine, chlorogenic acid and inhibitors of trypsin and chymotrypsin

Figure 4.1.1 is a linear plot of the absorbance of α-solanine concentrations in the range of 0.1 to 0.3 mg/ml.

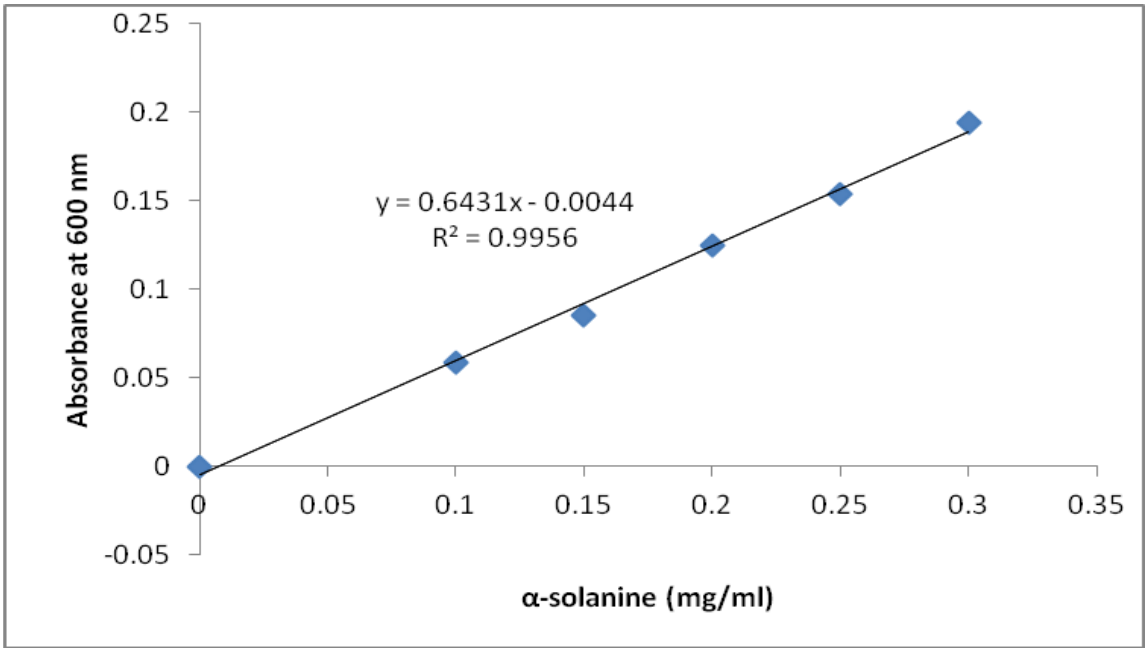


Figure 4.1.1: Regression correlations between α-solanine content (mg/ml) and absorbance at 600 nm.

Peak areas were used to compute the concentration of α-cha and α-sol in the sample extracts, since the area under each peak is directly proportional to the amount of that analyte which has passed through the detector under identical chromatographic conditions. The calibration curves showed strong positive linear relationships (R^2=0.96) for α-cha and α-sol for the concentrations ranging from 0.1 to 0.8 mg/ml and 0.05 to 0.3 mg/ml, respectively, as indicated in Figure 4.1.2 and 4.1.3.

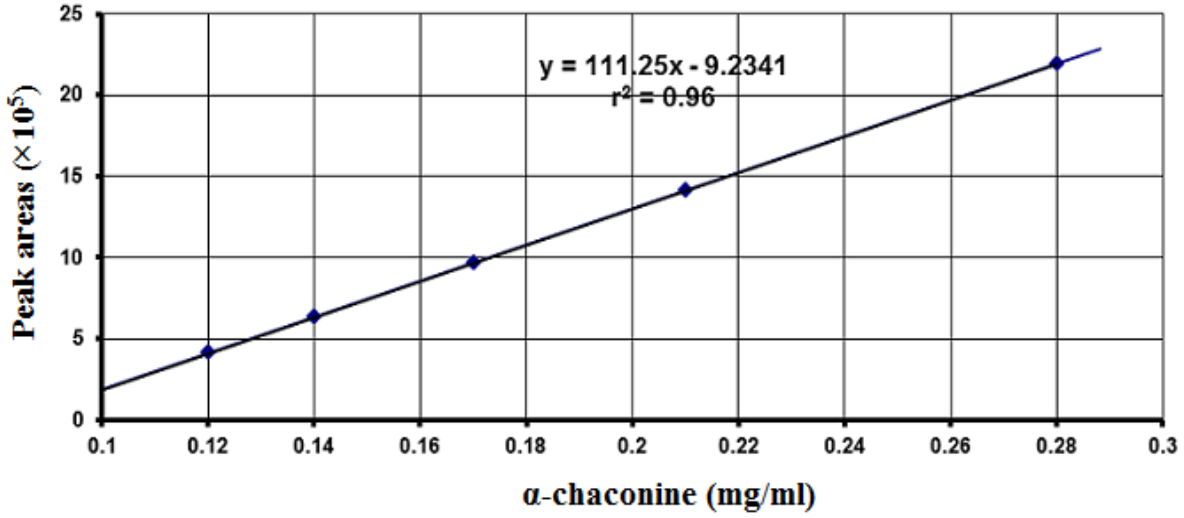


Figure 4.1.2: HPLC calibration plot for α-chaconine in the concentration range of 0.1 - 0.8 mg/ml.

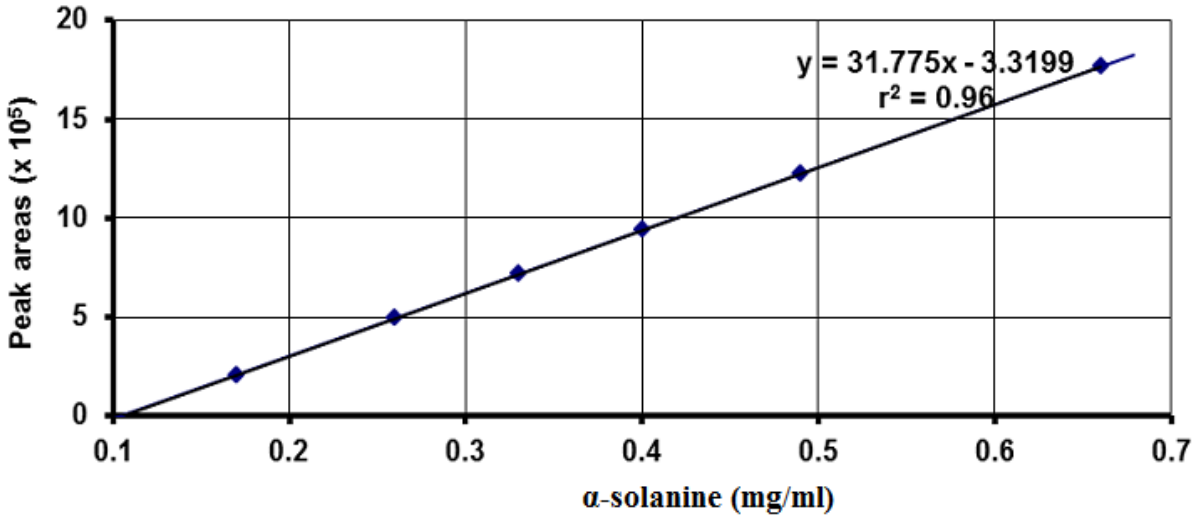


Figure 4.1.3: HPLC calibration plot for α-solanine in the concentration range of 0.05 - 0.3 mg/ml.

The absorbance readings of chlorogenic acid (CGA) were plotted from serial concentrations that ranged from 0.25 to 1.5 mg/l and 20 to 100 mg/l, respectively. Figure 4.1.4 show positive linear relationships for the concentration of CGA and absorbance values at 325 nm and 765 nm. CGA and total phenolics were estimated based on the

calibration curves of chlorogenic acid (CGA) for absorbance values measured at 325 nm and 765 nm, respectively.

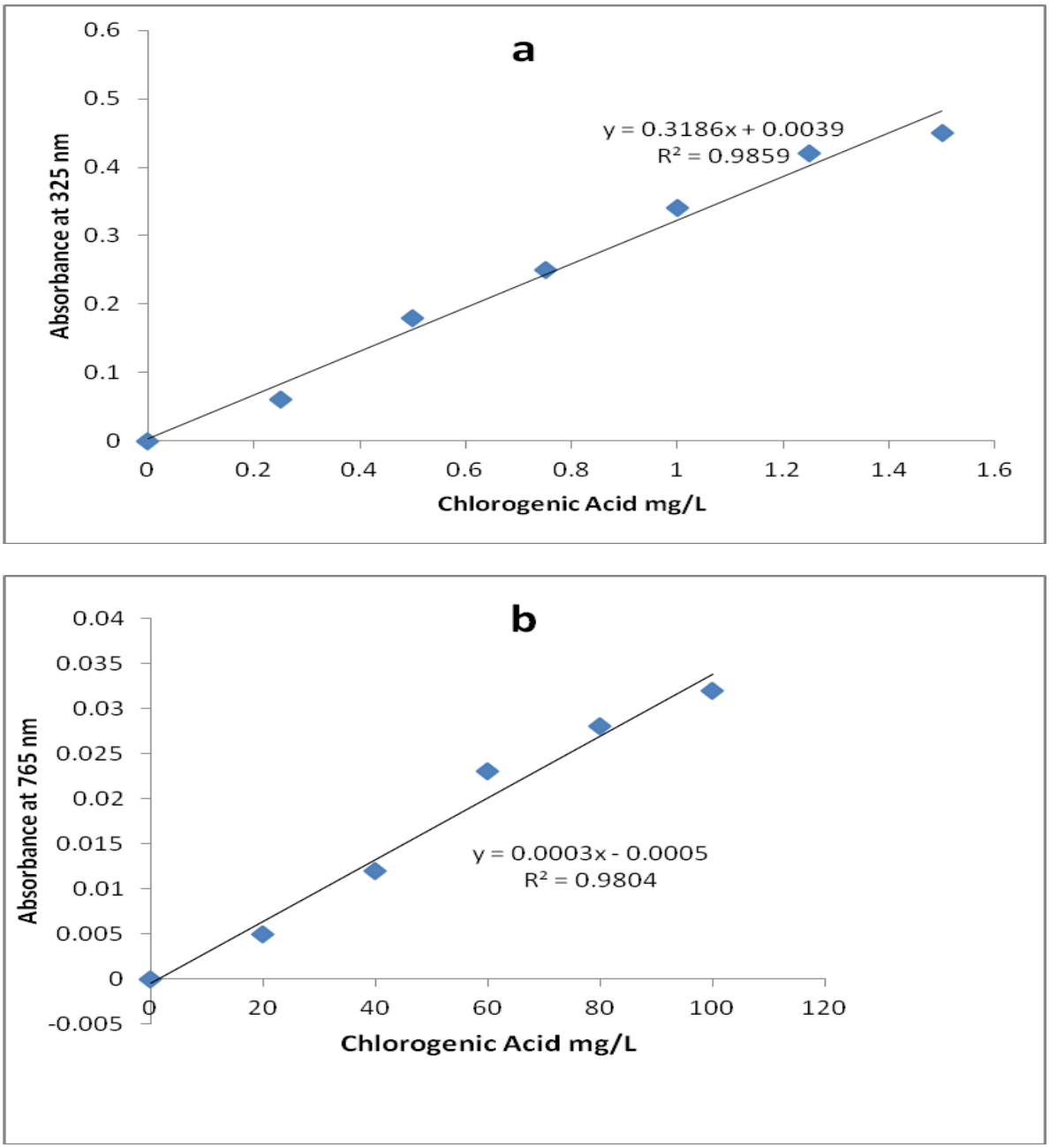
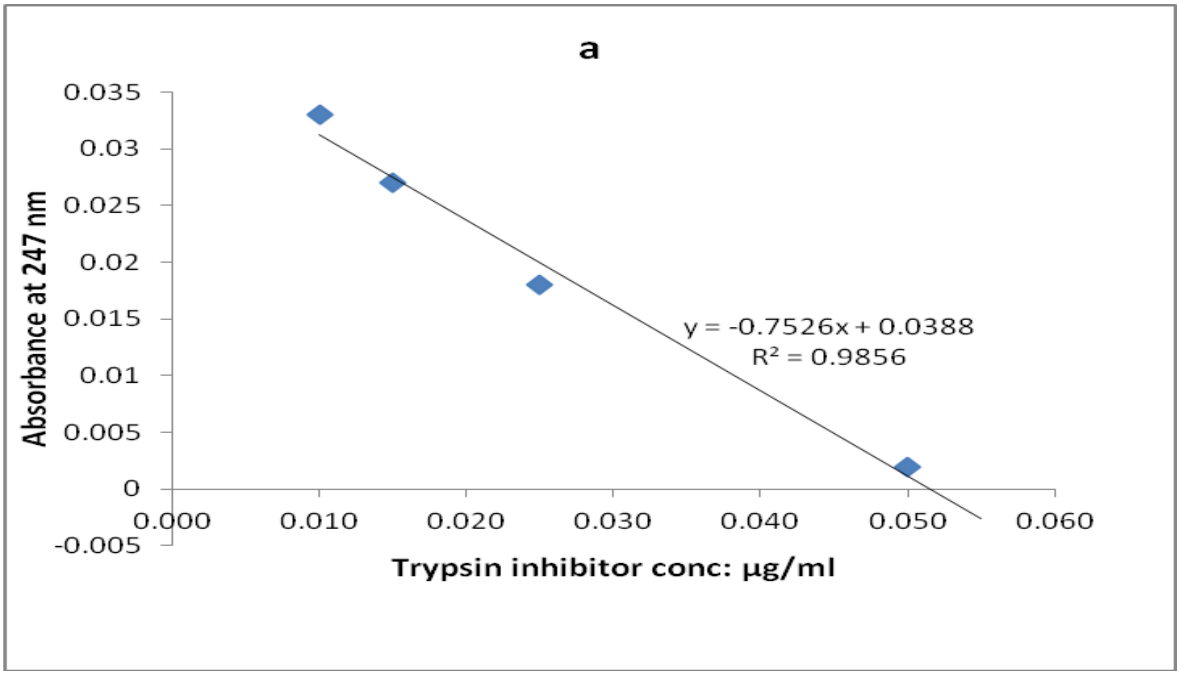


Figure 4.1.4: Regression correlations between CGA content (mg/ml) and absorbances at 325 nm and 765 nm. The absorbance readings and the concentration of CGA showed strong positive linear relationships (R^2>0.98).

Figure 4.1.5 show negative linear relationships for the loss of tryptic and chymotryptic activities upon addition of increasing quantities of protease inhibitors (PIs). At constant enzyme concentrations, adding known quantities of potato PI extracts resulted in loss of absorbances. Since the quantities of trypsin and chymotrypsin used in incubation were known, the amount of each of the enzyme that was inactivated by 20 µl of the extract was calculated based on the equations of the reactions.



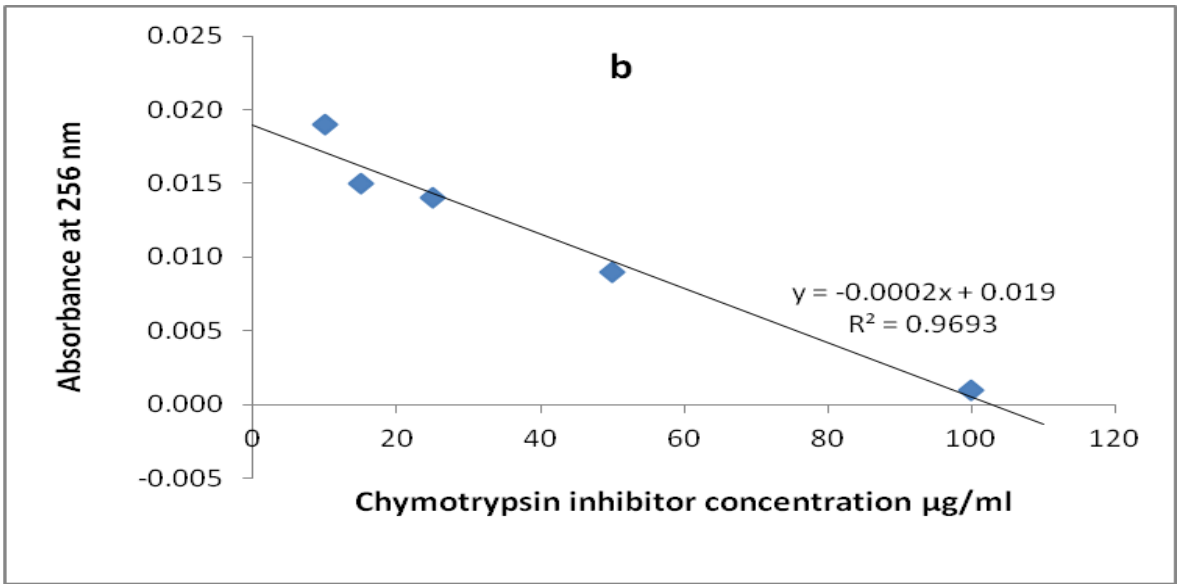


Figure 4.1.5: Reduction of tryptic and chymotryptic activity upon addition of increasing quantities of protease inhibitors. Calibration curves, with correlation coefficients ≥0.96, were established using concentration ranges from 0.01 to 0.05µg/ml and 10 to 100 µg/ml for trypsin and chymotrypsin inhibitors, respectively.

4.1.3 HPLC standardization

The optimum HPLC operation conditions were developed from trial runs using the glycoalkaloid standards (Sigma-Aldrich Co., UK). The following conditions gave optimum separation of α-chaconine and α-solanine: Column; Nucleosil NH_2 (5 µm, 4.6×250 mm); mobile phase of THF/0.025M KH_2PO_4/acetonitrile (50:25:25, v/v/v); flow rate 1.0ml/min; column temperature, 25 ^oC; UV detector, 208 nm and sample volume of 20 µl.

4.1.4 Peak identities of glycoalkaloids

The HPLC glycoalkaloid peaks of interest were designated as G1 and G2. The mean retention times of the two main potato glycoalkaloids (GAs) in the extracts were 4.53±0.01 and 6.05±0.03 minutes, respectively (Table 4.1.2, Figure 4.1.6). When the comparison was done it was found that the retention times of peaks G1 and G2 in the extracts were similar to those of known standards of α-cha and α-sol, respectively (Appendix 1). It was, therefore, concluded that G1 and G2 were HPLC peaks of α-cha and α-sol, respectively. Alongside these two main GAs, peaks of some unidentified compounds also appeared in most of the HPLC runs (Figure 4.1.7 and 4.1.8).

Table 4.1.2: HPLC retention times of glycoalkaloid standards and the main potato glycoalkaloids.

Glycoalkaloid standards		Main potato glycoalkaloids	
Standard	RT in minutes ± SD	Glycoalkaloid	RT in minutes ± SD
α-cha	4.54±0.01 (10)	1	4.53±0.01 (20)
α-sol	6.04±0.03 (10)	2	6.05±0.03 (20)

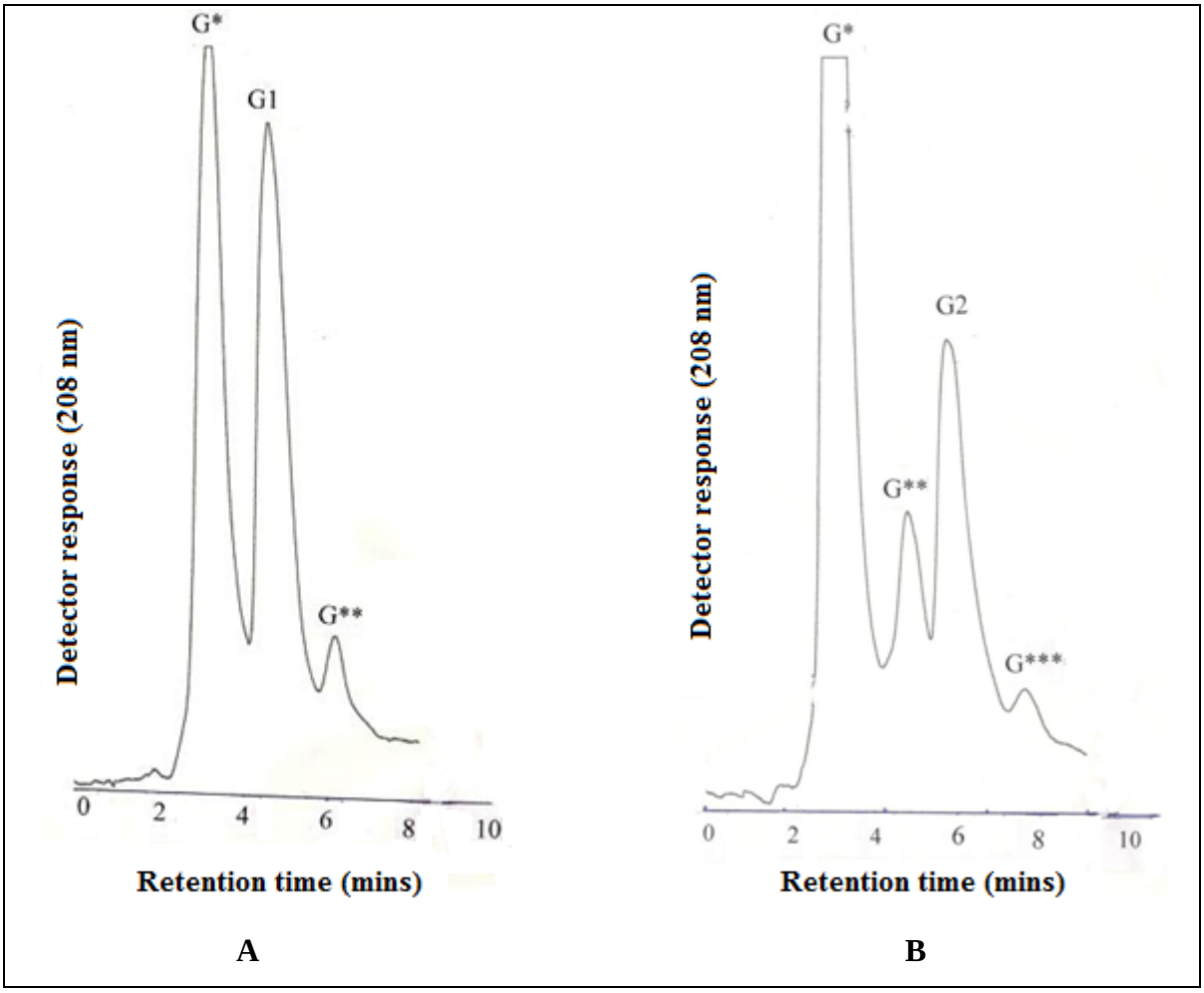


Figure 4.1.6: HPLC profiles of glycoalkaloid standards. Absorbance peaks G1 and G2 corresponds to α-chaconine and α-solanine, respectively, while G*, G**, and G***were not identified. Chromatographic conditions: mobile phase; THF/0.025M KH_2PO_4/acetonitrile; column temperature, 25 ^{o}C; flow rate 1.0ml/min; injection volume, 20 µl; UV detector, 208 nm.

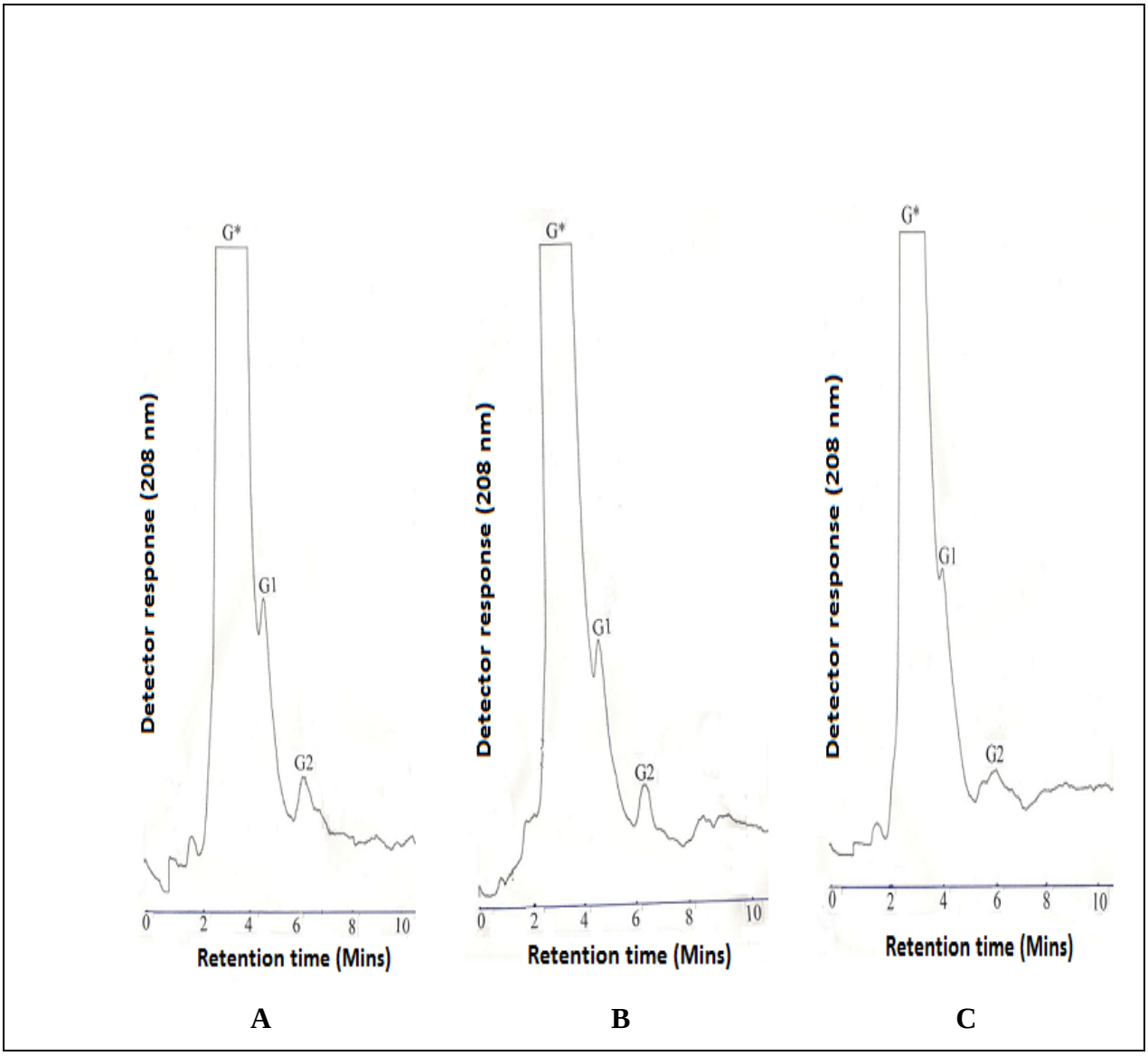


Figure 4.1.7: HPLC profiles of glycoalkaloids in tuber extracts of freeze-dried potato var. Dutch Robijn at (A) 55, (B) 95 and (C) 125 days after planting. The peaks G1and G2 corresponds to α-chaconine and α-solanine, respectively, while G* was not identified.

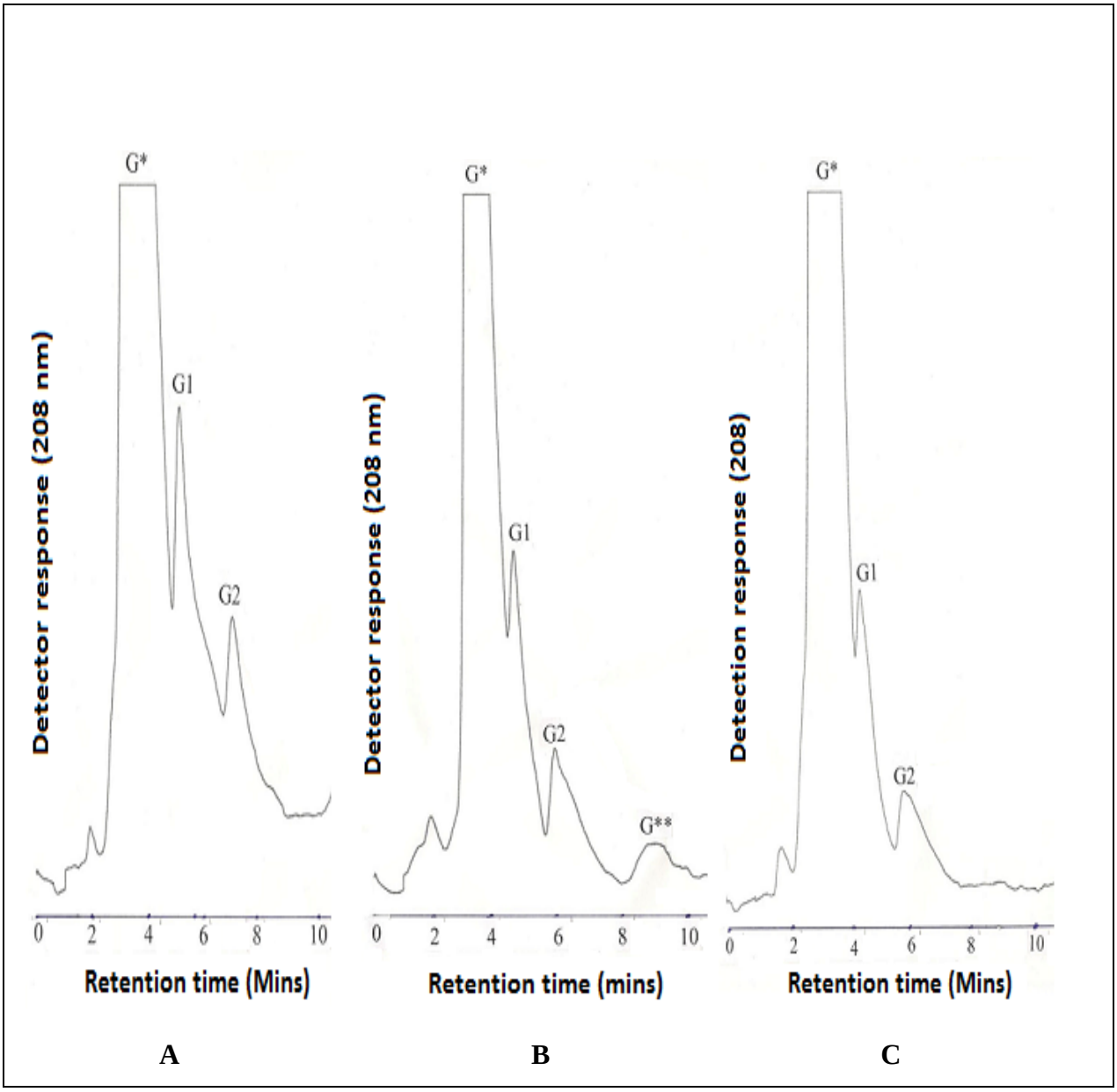


Figure 4.1.8: HPLC chromatograms of tuber glycoalkaloids extracted from the potato var. Tigoni at (A) 55, (B) 95 and (C) 125 days after planting. Absorbance peaks G1and G2 corresponds to α-chaconine and α-solanine, respectively. G* and G** were not identified.

4.2 Glycoalkaloid content of potato varieties at different growth stages

4.2.1 Total glycoalkaloid content of potato varieties at different growth stages

The total glycoalkaloid (TGA) content of leaves from five commercial potato varieties for the two seasons as determined by HPLC and UVspectrophotometry are shown in Table 4.2.1. The foliar TGA concentrations were expressed as mg/100g Fwt. Except for var. Desiree harvested at 95 days after planting (DAP), the foliar TGA values for the two methods differed significantly (Appendix 2A).

Table 4.2.1: Foliar total glycoalkaloid (TGA) content (mg/100g) at different growth stages of potato as determined by HPLC and UVspectrophotometry

	Total glycoalkaloid content (mg/100g)					
Variety	**HPLC Analysis**			**UV spectrophotometry**		
	Days after planting			**Days after planting**		
	40	**55**	**95**	**40**	**55**	**95**
Asante	77.73	69.43	94.42	63.31^{A}	49.26^{A}	69.70^{A}
Desiree	70.04	63.28	74.13^{a}	45.59	40.17	76.11^{a}
Dutch Robijn	60.58	54.38	66.86	29.38	25.87	63.28
Kenya Karibu	82.68	72.71	97.72^{A}	62.27^{A}	56.39	70.45^{A}
Tigoni	88.89	79.39	97.87^{A}	63.30^{A}	47.36^{A}	83.82
LSD (0.05)	SG	V	SG×V	SG	V	SG×V
(n=3)	0.17	0.13	0.30	2.83	2.19	4.91

Values are mean of three replicates and two growing seasons for year 2010 and 2011. Means along each row with the same lower case letters are not significantly different (LSD test, $p \leq 0.05$). Means with the same upper case letters within each column are not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction. LSD (0.05) for method = 1.15.

Analysis of HPLC data using ANOVA indicated that the commercial potato varieties used in the study had a wide variation in their foliar TGA contents (Table 4.2.2). The influence of variety and stage of growth on potato TGA concentration were significant ($p<0.001$). At 40 DAP the mean foliar TGA content ranged from 60.61 to 88.71 mg / 100g in vars. Dutch Robijn (DR) and Tigoni, respectively. The lowest levels of mean foliar TGA in all the potato varieties were recorded 55 DAP and ranged from 54.38 to 79.39 mg /100g. The highest mean foliar TGA was recorded at 95 DAP and ranged from 66.86 mg to 97.87 mg/100g. The TGA content in vars. Tigoni (97.87 mg/100g) and Kenya Karibu (KK) (99.72 mg/100g) was not significantly ($p>0.05$) different (Table 4.2.2).

The foliar TGA values determined by UV spectrophotometry at 40 DAP ranged from 39.51 to 64.83 mg / 100g. At this stage of growth, the TGA contents among vars. Asante, KK and Tigoni were not significantly ($p>0.05$) different. The foliar TGA content reduced progressively to the lowest levels at 55 DAP. During this stage, the foliar TGA content between vars. Asante and Tigoni was not significantly ($p>0.05$) different. The foliar TGA then gradually increased with growth in all varieties to the highest levels at 95 DAP. At this stage of growth, the TGA values for vars. Asante and Tigoni were not significant. The results from HPLC and UVspectrophotometry indicate that the stage of growth had a significant ($p<0.001$) effect on the concentration of TGA in all the potato varieties.

The foliar TGA content of different potato varieties at different stages of growth during 2010 and 2011 growing seasons obtained by UVspectrophotometry are presented in Table 4.2.2. Except for a few variety and stage of growth combinations in vars. Desiree, KK and Tigoni, the foliar TGA values for the two growing seasons differed significantly.

Table 4.2.2: Foliar total glycoalkaloid (TGA) content (mg/100g) of potato plants at different growth stages as determined during year 2010 and 2011 seasons

Variety	**Stage of growth (Days after planting)**	**Total glycoalkaloid content (mg/100g)**	
		2010 season	**2011 season**
Asante	40	74.06	52.55
	55	52.07	46.50
	95	73.38	65.98
Desiree	40	46.55[a]	44.74[a]
	55	41.22[a]	39.20[a]
	95	86.72	65.40
Dutch Robijn	40	74.75	49.78
	55	69.11	43.67
	95	74.56	66.43
Kenya Karibu	40	33.62	25.27
	55	25.60[a]	26.23[a]
	95	61.70[a]	64.93[a]
Tigoni	40	73.99	52.52
	55	47.01[a]	47.87[a]
	95	94.23	73.40
LSD (0.05) (n=3)	SG	2.33	2.34
	V	3.01	3.04
	SG×V	5.21	5.22

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

Analysis of data using ANOVA indicated that the influence of growing season on the concentration of potato foliar TGA was significant ($p<0.001$). There were significant differences due to season irrespective of variety and stage of growth. The mean foliar TGA content was higher in samples collected during 2010 growth period than that of 2011 (Figure 4.2.1).

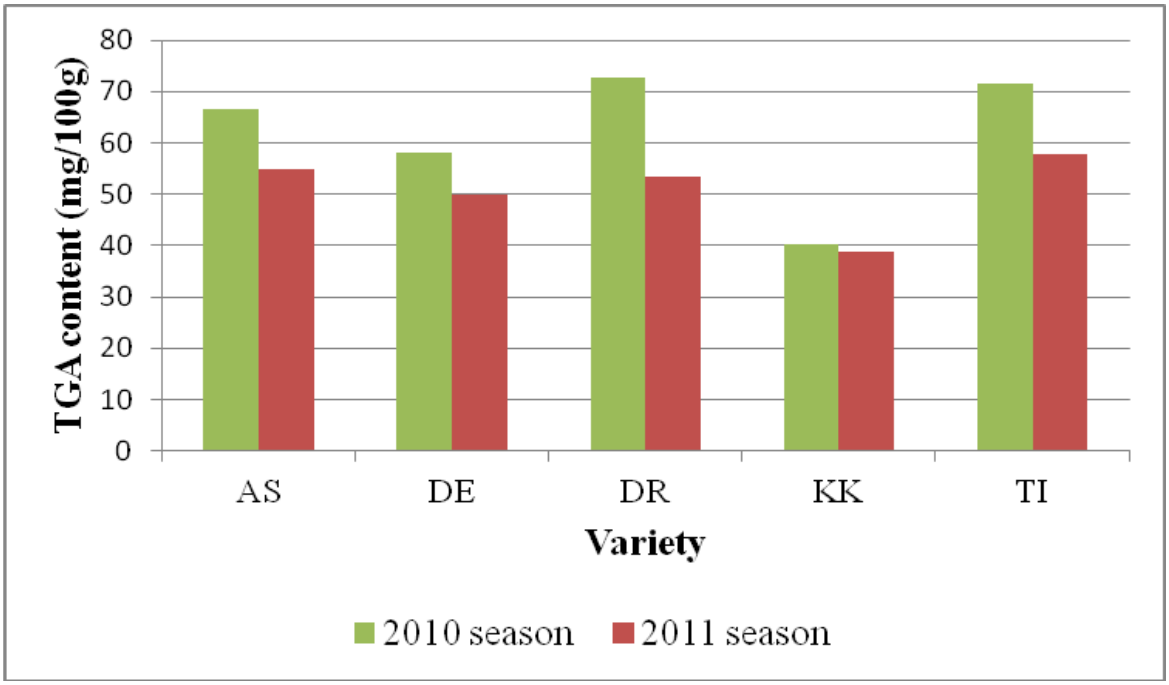


Figure 4.2.1: Influence of growing season on the concentration of foliar total glycoalkaloids (TGA) in different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni. $LSD_{0.05}$ for TGA = 3.01 (2010) and 3.04 (2011).

The tuber TGA contents from five commercial potato varieties for the two seasons as determined by HPLC and UV spectrophotometry are shown in Table 4.2.3. The concentration of tuber TGA was significantly ($p<0.001$) influenced by the variety of the potato plant. No significant ($p>0.05$) difference in tuber TGA was observed between the two methods.

Table 4.2.3: Tuber total glycoalkaloid (TGA) content (mg/100g) of potato plants at different growth stages as determined by HPLC and UV spectrophotometry

	Total glycoalkaloid content (mg/100g)					
Variety	**HPLC Analysis**			**UV spectrophotometry**		
	Days after planting			**Days after planting**		
	55	**95**	**125**	**55**	**95**	**125**
Asante	9.40	8.67^{a}	8.17^{A}	10.07^{B}	8.86^{aB}	9.14^{A}
Desiree	9.02^{a}	7.75^{b}	7.03^{c}	9.12^{a}	7.79^{b}	7.03^{c}
Dutch Robijn	7.84^{a}	6.73^{b}	5.82^{c}	7.88^{a}	6.78^{b}	6.17^{c}
Kenya Karibu	10.48^{a}	9.69^{b}	8.38^{cA}	10.85^{aA}	9.76^{bA}	8.02^{c}
Tigoni	12.22	10.21	9.24^{a}	10.57^{AB}	9.49^{AB}	9.69^{aA}
LSD (0.05)	SG	V	SG×V	SG	V	SG×V
(n=3)	0.21	0.28	0.48	0.59	0.76	1.32

Values are mean of three replicates and two growing seasons for year 2010 and 2011. Means with the same lowercase letters along each row are not significantly different (LSD test, $p \leq 0.05$). Means within each column with the same uppercase letters are not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction. LSD (0.05) for method = 0.27.

The HPLC method gave tuber TGA contents that ranged from 6.80 mg to 10.56 mg / 100 Fwt. in vars. DR and Tigoni, respectively. The stage of tuber growth had a significant ($p<0.001$) effect on TGA level. The mean tuber TGA values varied from 7.84 mg to 12.22 mg/100g, 6.73 mg to 10.21 mg/100g and 5.82 mg to 9.24 mg/100g at 55, 95 and 125 days after planting (DAP), respectively (Table 4.2.4). The tuber TGA levels in the tested varieties were highest at 55 DAP but decreased significantly ($p<0.001$) during consecutive stages of growth. Tuber TGA level decreased by 10.7 and 21.7% in vars. Tigoni and DR, respectively.

The tuber TGA values of the same cultivars measured by UV spectrophotometry ranged from 6.94 mg to 9.92 mg / 100 Fwt. The tubers from vars. DR and Tigoni contained the lowest and highest concentration of mean TGA, respectively. Analysis of data using ANOVA indicated that the influence of variety and stage of growth on tuber TGA content were significant ($p<0.001$). The levels of TGA in tubers varied from 7.88 mg to 10.85 mg/100g, 6.78 mg to 9.76 mg/100g and 6.17 mg to 9.69 mg/100g at 55, 95 and 125 days after planting (DAP), respectively (Table 4.2.4). These data showed a significant ($p<0.001$) reduction in tuber TGA content from the time of tuber initiation up to maturity.

The tuber TGA content of different potato varieties at different stages of growth during 2010 and 2011 growing seasons obtained by UV spectrophotometry are presented in Table 4.2.4. Except for vars. Desiree and Tigoni, the tuber TGA values for the two growing seasons differed significantly.

Table 4.2.4: Tuber total glycoalkaloid (TGA) content (mg/100g) of potato plants at different growth stages as determined during year 2010 and 2011 seasons

Variety	**Stage of growth (Days after planting)**	**Total glycoalkaloid content (mg/100g)**	
		2010 season	**2011 season**
Asante	55	8.52	11.64
	95	7.88	9.84
	125	8.05	10.23
Desiree	55	8.84[a]	9.40[a]
	95	7.48[a]	8.10[a]
	125	6.90[a]	7.16[a]
Dutch Robijn	55	7.19	8.58
	95	5.83	7.74
	125	5.45	6.89
Kenya Karibu	55	9.80	11.90
	95	8.49	11.03
	125	6.91	9.13
Tigoni	55	9.74	11.40
	95	8.75	10.23
	125	9.46[a]	9.92[a]
LSD (0.05) (n=3)	SG	1.01	1.19
	V	0.78	0.92
	SG×V	1.74	2.07

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The ANOVA for TGA concentration obtained by UV Vis spectrophotometry indicated that the influence growing season on tuber TGA content was significant ($p<0.05$). A significant variety × season interaction was also observed for tuber TGA. The TGA concentration was significantly higher in tuber samples of vars. Asante, Desiree and DR collected during 2011 growth period than that of 2010 (Figure 4.2.2).

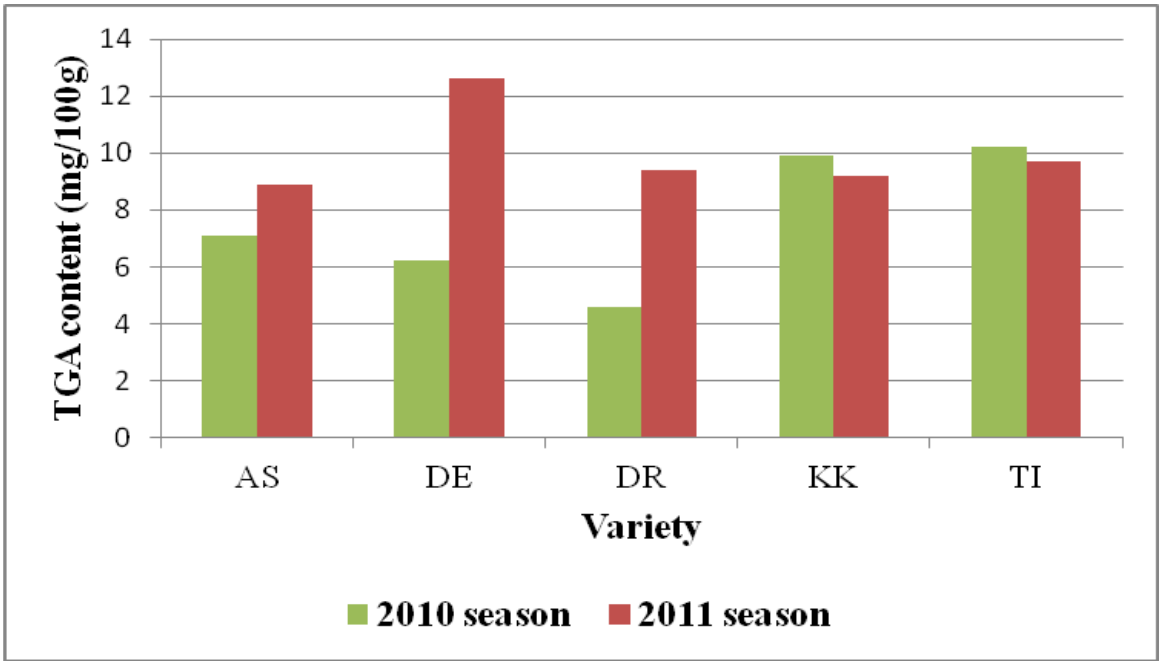


Figure 4.2.2: Influence of growing season on the concentration of tuber total glycoalkaloids (TGA) in different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni. $LSD_{0.05}$ for TGA = 0.78 (2010) and 0.92 (2011).

4.2.2 α-chaconine and α-solanine content of potato varieties at different growth stages

The results for α-chaconine (α-cha) and α-solanine (α-sol) content of leaves and tubers from five commercial potato varieties for the two growing seasons are shown in Tables 4.2.5 and 4.2.6. The concentration of α-cha and α-sol were quantified using HPLC and expressed in mg/100g Fwt.

Table 4.2.5: α-chaconine and α-solanine content (mg/100g) of potato leaves at different growth stages

Variety	**α-chaconine content (mg/100g)**			**α-solanine content (mg/100g)**		
	Days after planting			**Days after planting**		
	40	**55**	**95**	**55**	**95**	**125**
Asante	42.24	39.11	53.26	35.49	30.32	41.16
Desiree	39.72	37.59	46.08	30.32	25.69	28.05[b]
Dutch Robijn	37.52	33.14	38.80	23.06	21.24	28.06[b]
Kenya Karibu	44.76	40.18	53.97[a]	37.92	32.53	43.75[a]
Tigoni	50.19	46.23	54.10[a]	38.70	33.16	43.77[a]
LSD (0.05)	SG	V	SG×V	SG	V	SG×V
(n=3)	0.23	0.30	0.52	0.13	0.17	0.29

Values are means of three replications and two growing seasons for year 2010 and 2011. Means along each column with the same lowercase letters are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The data clearly indicate that the effects of variety and stage of growth on the concentration of α-cha in potato leaves were significant ($p<0.001$) (Appendix 2B). The mean foliar α-cha contents ranged from 36.49 to 50.17 mg/100g. The varieties DR and Tigoni contained the least and the highest concentration of α-cha over the three stages of growth, respectively. The lowest concentration of foliar α-cha in all the tested varieties was observed at 55 DAP, while the highest values were obtained at 95 DAP. The foliar α-cha contents of vars. KK and Tigoni at 95 DAP were not significantly ($p>0.05$) different.

The α-solanine (α-sol) content in the examined foliar extracts varied significantly among the five potato varieties (Table 4.2.6). The effect of stage of growth on the concentration of

foliar α-sol content was significant ($p<0.001$). The α-sol content in potato leaves at 40 DAP ranged from 23.06 mg in var. DR to 38.70 mg/100g in Tigoni. In all the varieties, the foliar α-sol content at different stages of growth followed a pattern similar to that of α-cha. The lowest α-sol levels were detected from potato leaf samples harvested at 55 DAP. From this stage, the concentration of foliar α-sol increased significantly ($p<0.05$) to the highest levels at 95 DAP that ranged from 28.05 mg to 43.77 mg/100g in vars. Desiree and Tigoni, respectively. No significant ($p>0.05$) difference in foliar α-sol content was observed between vars. DR and Desiree and between Tigoni and KK at 95 DAP.

The α-cha and α-sol content of tubers from five commercial potato varieties evaluated in this study are shown in Table 4.2.7. The vars. DR and Tigoni contained the lowest and highest levels of α-cha when tubers were harvested at 55, 95 and 125 DAP. The results indicated that the effect of variety and stage of growth on the concentration of α-cha in potato tubers were significant ($p<0.001$).

Table 4.2.6: α-chaconine and α-solanine content (mg/100g) of potato tubers at different growth stages

Variety	Stage of growth (Days after planting)	Glycoalkaloid content (mg/100g)	
		α-chaconine	**α-solanine**
Asante	55	5.19[a]	4.21
	95	5.13[a]	3.54[b]
	125	4.97	3.20
Desiree	55	5.12[a]	3.90
	95	4.41	3.34
	125	3.93	3.10
Dutch Robijn	55	4.29	3.55[b]
	95	4.17	2.56
	125	3.53	2.29
Kenya Karibu	55	6.11	4.37[a]
	95	5.57	4.12
	125	4.86	3.52[b]
Tigoni	55	7.33	4.89
	95	5.91	4.30[a]
	125	5.46	3.78
LSD (0.05) (n=3)	SG	0.03	0.02
	V	0.04	0.03
	SG×V	0.07	0.05

Values are means of three replications and two growing seasons for year 2010 and 2011. Means along each column with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The mean tuber α-cha contents ranged from 4.29 mg to 7.33 mg/100g, 4.17 mg to 5.91 mg/100g and 3.53 mg to 5.46 mg/100g at 55, 95 and 125 days after planting (DAP), respectively (Table 4.2.7). No significant ($p>0.05$) difference in α-cha content between vars. Asante and Desiree when tubers were harvested at 55 DAP. Tuber α-cha contents decreased significantly ($p<0.001$) with progressing growth stages. One exception to this trend was observed with Asante variety in which tuber α-cha values at 55 and 95 DAP were not significantly ($p>0.05$) different.

The tuber α-sol concentration in the potato varieties evaluated followed similar trend as α-cha (Table 4.2.6). Significant ($p<0.001$) differences in tuber α-sol contents were found among the test potato varieties for each growth stage. The influence of stage of growth on α-sol concentration was significant ($p<0.001$) and varied from 3.55 mg to 4.89 mg/100g, 2.56 mg to 4.30 mg/100g and 2.29 mg to 3.78 mg/100g, in tubers harvested at 55, 95 and 125 DAP, respectively. Overall, the average tuber α-sol content was highest (4.32 mg/100g) and lowest (2.80 mg/100g) in the vars. Tigoni and DR, respectively.

4.3 Phenolic content of potato varieties at different growth stages

4.3.1 Chlorogenic acid content of potato plants at different growth stages

The chlorogenic acid (CGA) content of leaves and tubers from five commercial potato varieties determined by UV spectrophotometry are summarized in Table 4.3.1. The mean CGA values for potato leaves ranged from 246.85 mg/100 g to 252.93 mg/100 g Fwt. Results derived from three technical replicates for each variety gave consistent results.

Table 4.3.1: Foliar chlorogenic acid (CGA) content (mg/100g) of potato plants at different stages of growth

Variety	Leaf CGA content (mg/100g)			Tuber CGA content (mg/100g)		
	Days after planting			Days after planting		
	40	55	95	55	95	125
Asante	260.48	250.12^{aA}	248.19^{abA}	60.78^{a}	54.93^{b}	51.39^{a}
Desiree	250.34^{c}	246.19^{bcA}	244.02^{cdA}	55.08	50.11^{c}	44.23
Dutch Robijn	251.69^{bcA}	249.86^{aA}	242.27^{d}	51.77	46.81^{c}	40.59
Kenya Karibu	255.87^{a}	245.71^{c}	251.59^{a}	63.81^{a}	56.79^{ab}	53.04^{a}
Tigoni	255.09^{ab}	248.08^{abA}	245.87^{bcA}	63.94^{a}	58.30^{a}	51.89^{a}
LSD (0.05) (n=3)	SG 3.01	V 3.88	SG×V 6.72	SG 2.55	V 3.30	SG×V 5.71

Values are means of three replications and two growing seasons for year 2010 and 2011. Means along each column with the same lowercase letters are not significantly different (LSD test, $p \leq 0.05$). Means with the same uppercase letter within each row indicate that the effect of stage of growth for each potato variety is not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The results indicate that the effects of variety and stage of growth on the concentration of foliar CGA among examined potato varieties were significant ($p<0.05$) (Appendix 3A). The vars. Asante and Desiree had the highest (252.93 mg/100g) and lowest (244.02 mg/100g) concentration of CGA, respectively. At 40 DAP, the foliar CGA values between vars. KK and Tigoni, Tigoni and DR and DR and Desiree were not significantly ($p>0.05$) different. Foliar CGA contents between vars. Tigoni and Desiree, KK and Desiree, and among Asante, DR and Tigoni, were not significantly different at 55 DAP. At the last foliar growth stage evaluated (95 DAP) the CGA contents between vars. KK and Asante, Asante and Tigoni, Tigoni and Desiree, Desiree and DR were not significantly ($p>0.05$) different. The mean foliar CGA contents decreased significantly ($p<0.05$) during growth between 40 and 95 DAP. The differences in foliar CGA contents observed with var. DR

between 40 and 55 DAP, and with leaves of vars. Asante, Desiree and Tigoni when harvested between 55 and 95 DAP were not significantly different ($p>0.05$). The interaction between variety and growing season on foliar CGA content was significant ($p<0.001$), with a higher mean during 2011 (250.72 mg/100g) than 2010 (248.82 mg/100g) (Table 4.3.2, Figure 4.3.1 and Appendix 3B).

Table 4.3.2: Foliar chlorogenic acid (CGA) concentration (mg/100g) of potato plants at different growth stages as determined during year 2010 and 2011 seasons

Variety	**Stage of growth (Days after planting)**	**Chlorogenic acid content (mg/100g)**	
		2010 season	**2011 season**
Asante	40	261.8[a]	259.2[a]
	55	251.4[a]	248.8[a]
	95	249.4[a]	246.9[a]
Desiree	40	235.6	265.1
	55	231.1	263.4
	95	227.7	260.4
Dutch Robijn	40	245.9	258.6
	55	244.1	257.6
	95	225.9	255.7
Kenya Karibu	40	263.4	248.3
	55	262.2	241.0
	95	261.9	229.5
Tigoni	40	262.5	247.7
	55	260.0	246.8
	95	249.4	231.7
LSD (0.05) (n=3)	SG	6.94	5.83
	V	8.96	7.53
	SG×V	15.52	13.04

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p\leq0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

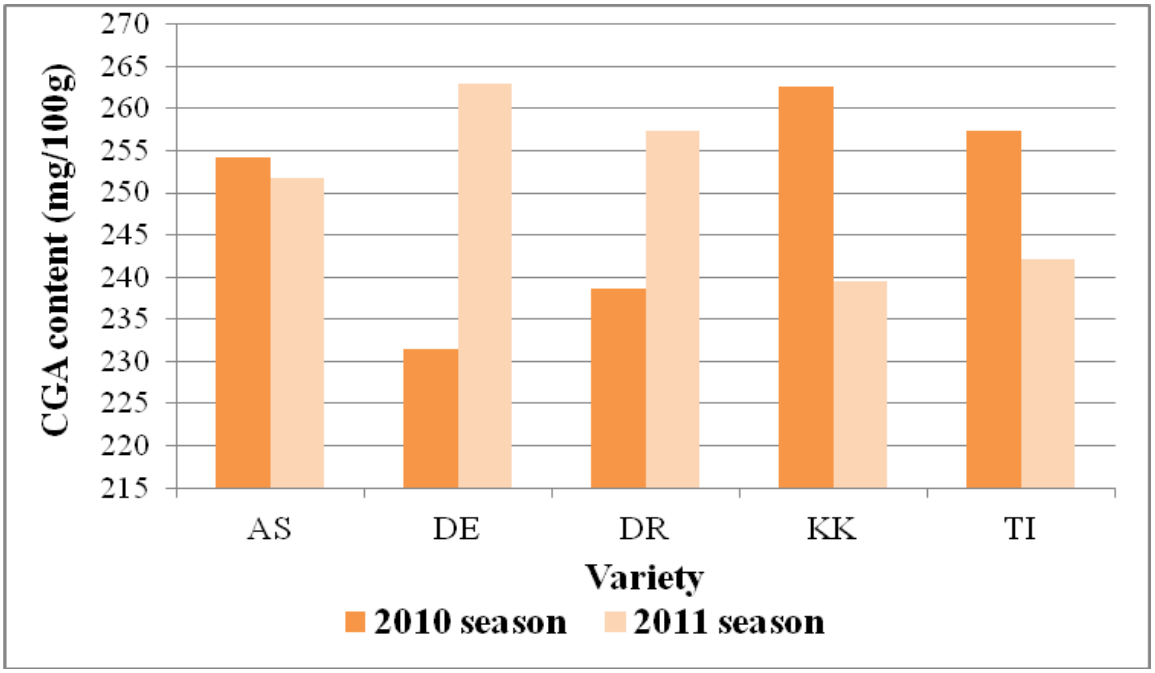


Figure 4.3.1: Seasonal variation in the concentration of foliar chlorogenic acid in different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

The chlorogenic acid (CGA) content in tubers from the five potato varieties examined show significant varietal differences ($p<0.001$). The mean CGA content among potato varieties ranged 46.39 to 58.04 mg/100g with vars. DR and Tigoni recording the lowest and highest concentrations, respectively. At 55 and 125 DAP the mean tuber CGA among Asante, KK and Tigoni were not significantly ($p>0.05$) different. The mean CGA between vars. KK and Tigoni, KK and Asante and Desiree and DR were not significantly ($p>0.05$) different when tubers were harvested at 95 DAP. The concentration of CGA in tubers decreased significantly from the period between tuber bulking and tuber growth at 55 and 125 days after planting (DAP), respectively (Table 4.3.3).

Table 4.3.3: Tuber chlorogenic acid (CGA) concentration (mg/100g) of potato plants at different growth stages as determined during year 2010 and 2011 seasons

Variety	Stage of growth (Days after planting)	Chlorogenic acid content (mg/100g)	
		2010 season	2011 season
Asante	55	56.17	65.47
	95	48.47	61.37
	125	45.93	56.83
Desiree	55	55.33[a]	54.75[a]
	95	51.92	48.37
	125	43.71[a]	44.73[a]
Dutch Robijn	55	57.63	46.02
	95	49.48	44.17
	125	39.65[a]	41.60[a]
Kenya Karibu	55	65.37	62.19
	95	53.23	60.37
	125	48.47	57.43
Tigoni	55	62.83[a]	65.01[a]
	95	54.77	61.87
	125	45.57	58.33
LSD (0.05) (n=3)	SG	2.49	1.64
	V	3.22	2.12
	SG×V	5.58	3.67

Values are mean of three replications. Means with the same letter along each row are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The effect of growing season on tuber CGA concentration was significant (p=0.007) (Figure 4.3.2 and Appendix 3B). Tuber samples collected in 2011 had significantly higher mean CGA content than that found in samples collected in 2010.

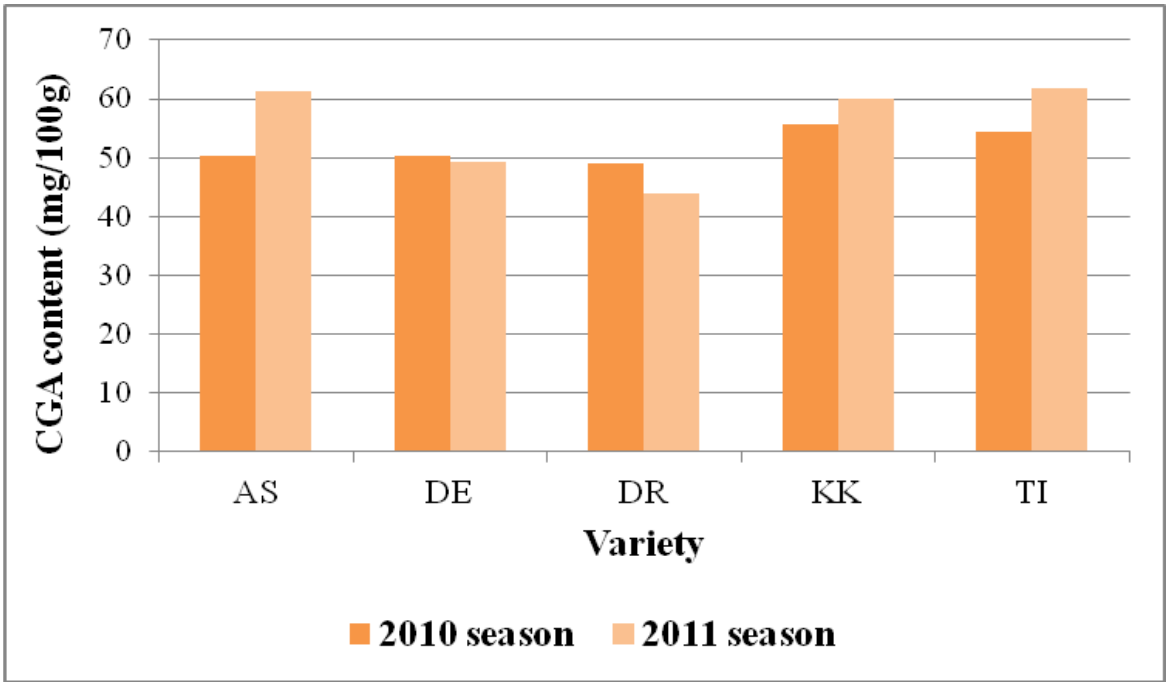


Figure 4.3.2: Seasonal variation in the concentration of tuber chlorogenic acid in different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

4.3.2 Total phenolic content of potato leaves and tubers

Total phenolic (TP) content of potato leaf extracts was determined using the Folin-Ciocalteu's reagent and expressed in terms of chlorogenic acid equivalent (the standard curve equation: $y = 0.0003x - 0.0005$, $r^2=0.9804$). The results for TP content (mg CGA/g) in the leaves and tubers of the five potato varieties used in this study are presented in Table 4.3.4.

Table 4.3.4: Total phenolic (TP) content (mg CGA/g) of potato leaves and tubers at different stages of growth

Variety	**Leaf TP content (**mg CGA/g**)**			**Tuber TP content (**mg CGA/g**)**		
	Days after planting			**Days after planting**		
	40	**55**	**95**	**55**	**95**	**125**
Asante	737.80	514.91^{aA}	560.23^{A}	129.80^{bAB}	122.82^{bB}	135.10^{aA}
Desiree	672.73	469.42^{ab}	400.61^{a}	132.32^{bA}	123.51^{bA}	125.49^{abA}
Dutch Robijn	554.91^{ab}	470.03^{ab}	370.60^{a}	153.30	135.33^{b}	120.32^{b}
Kenya Karibu	554.40^{b}	394.41^{bA}	358.89^{aA}	231.53^{a}	195.20^{a}	132.41^{ab}
Tigoni	608.22^{a}	488.89^{a}	392.20^{a}	234.61^{a}	182.71^{a}	160.23
LSD (0.05) (n=3)	SG 59.40	V 76.40	SG×V 132.80	SG 11.7	V 15.0	SG×V 26.0

Values are means of three replications and two growing seasons for year 2010 and 2011. Means within each column with the same lowercase letters are not significantly different (LSD test, $p \leq 0.05$). Means along each row with the same uppercase letter indicate that the effect of stage of growth for each potato variety is not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The results demonstrate an effect of variety on the concentration of total phenolics (TP) in potato leaves was significant ($p<0.001$) (Appendix 3C). Foliar TP content ranged from 435.9 to 603.4 mg CGA/g. The var. Asante had the highest mean concentration and var. Kenya Karibu had the lowest mean concentration. The effect of stage of growth on the concentration of TP was also significant ($p<0.001$) (Appendix 3C). Generally, the concentration of mean TP decreased from the period between vegetative growth and senescence at 40 and 125 days after planting (DAP), respectively.

The effect of growing season on foliar TP content was significant at $p<0.05$. The concentration of TP in leaf samples collected during the 2010 growing season was

significantly higher (p=0.021) than that found in samples collected in 2011 (Table 4.3.5, Figure 4.3.3 and Appendix 3D).

Table 4.3.5: Total phenolic concentration (mg CGA/g) of potato leaves at different growth stages as determined during year 2010 and 2011 seasons

Variety	**Stage of growth (Days after planting)**	**Total phenolic content (mg CGA/g)**	
		2010 season	**2011 season**
Asante	40	877.22	598.33
	55	585.00	444.89
	95	692.06	428.33
Desiree	40	781.67	563.74
	55	352.78	587.22
	95	351.67	448.33
Dutch Robijn	40	607.22	502.67
	55	483.89[a]	456.11[a]
	95	372.78[a]	368.33[a]
Kenya Karibu	40	632.76	476.11
	55	372.78[a]	416.11[a]
	95	399.44[a]	318.33[a]
Tigoni	40	758.33	458.11
	55	437.22	540.56
	95	353.89[a]	430.56[a]
LSD (0.05) (n=3)	SG	73.62	34.00
	V	95.04	43.90
	SG×V	164.62	73.03

Values are mean of three replications. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

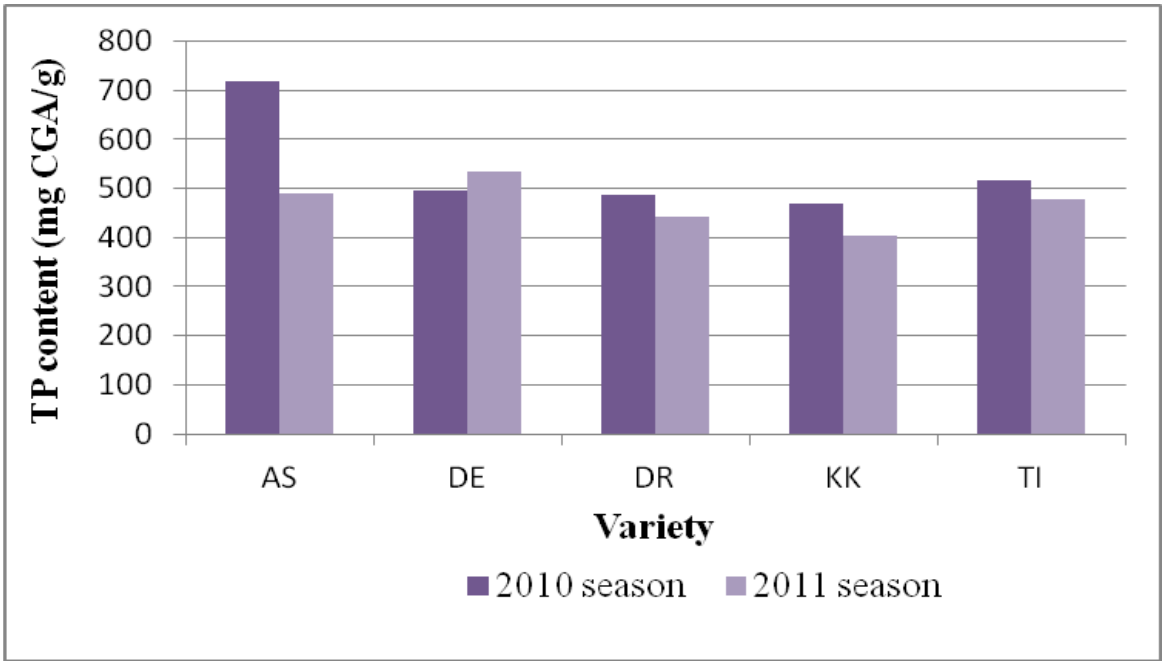


Figure 4.3.3: Seasonal variation in the concentration of foliar total phenolics in different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

The total phenolic (TP) content in the examined tuber extracts varied significantly ($p<.001$) among different commercial potato varieties (Appendix 3C). The TP content in tubers varied from 127.1 to 192.5 CGA/100g Fwt with the highest and lowest concentration measured in vars. Tigoni and Desiree, respectively (Table 4.3.4). The effect of stage of growth on the concentration of tuber TP was significant ($p<0.001$). In most of the varieties the concentration of TP generally decreased from the period between tuber bulking at 55 DAP and the period when mature tubers were ready for harvest at 125 DAP.

The influence of growing season on TP content of potato tubers at different stages of growth are indicated in Table 4.3.6.

Table 4.3.6: Total phenolic content (mg CGA/g) content of potato tubers at different growth stages as determined during year 2010 and 2011 seasons

Variety	Stage of growth (Days after planting)	Total phenolic content (mg/100g)	
		2010 season	2011 season
Asante	55	109.20	150.43
	95	104.10	141.42
	125	116.10	155.10
Desiree	55	113.20	151.43
	95	103.90	143.17
	125	106.50	144.63
Dutch Robijn	55	139.30	167.30
	95	121.00	149.70
	125	106.70	133.88
Kenya Karibu	55	252.80	210.17
	95	216.10	174.17
	125	129.50[a]	135.33[a]
Tigoni	55	245.00	224.20
	95	186.10[a]	179.33[a]
	125	125.00	195.44
LSD (0.05) (n=3)	SG	11.82	2.53
	V	15.26	3.26
	SG×V	26.43	5.65

Values are mean of three replications. Means with the same letter along each row are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The mean TP concentration in tuber samples from the 2011 growing season was significantly ($p=0.003$) higher than that found in samples collected in 2010 (Figure 4.3.4, Appendix 3D).

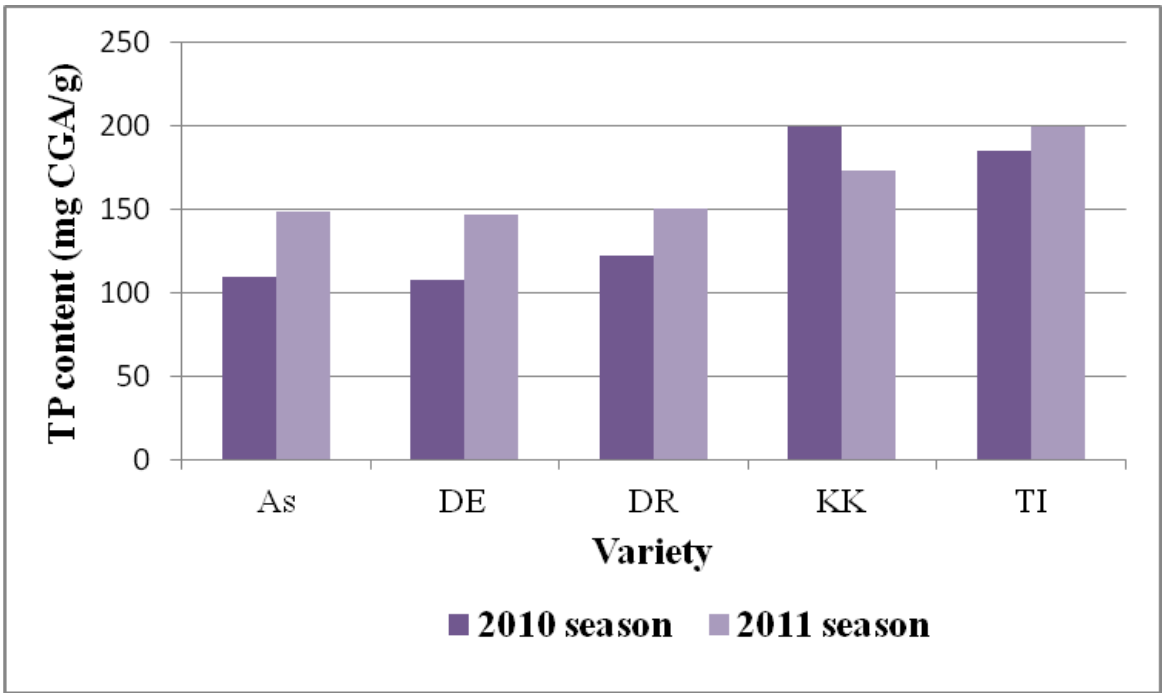


Figure 4.3.4: Seasonal variation in the concentration of tuber total phenolics in different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

4.4 Protease inhibitor content of potato plants

4.4.1 Chymotrypsin inhibitor content of potato leaves and tubers

Table 4.4.1 shows the mean chymotrypsin inhibitor (CI) content in potato leaves and tubers at different stages of growth during two growing seasons of year 2010 and 2011, respectively.

Table 4.4.1: Chymotrypsin inhibitor content (U/mg) of potato leaves and tubers at different growth stages

Variety	Leaf chymotrypsin inhibitor content (U/mg)			Tuber chymotrypsin inhibitor content (U/mg)		
	Days after planting			Days after planting		
	40	55	95	55	95	125
Asante	674.84^{bc}	932.32^{aA}	901.80^{aA}	250.33^{A}	252.24^{A}	269.90^{c}
Desiree	775.10^{a}	1099.60	863.44^{ab}	264.22^{b}	292.70^{a}	313.89
Dutch Robijn	748.32^{ab}	818.24^{bA}	808.03^{A}	283.31^{aA}	$282.04^{a\ A}$	279.23^{bcA}
Kenya Karibu	639.80^{c}	840.53^{bA}	879.09^{abA}	278.90^{aB}	284.53^{aAB}	292.88^{aA}
Tigoni	670.44^{bc}	958.70^{a}	864.82^{ab}	271.44^{ab}	286.90^{aA}	289.84^{abA}
LSD (0.05) (n=3)	SG 67.20	V 86.70	SG×V 150.20	SG 9.76	V 12.60	SG×V 21.83

Values are means of three replications and two growing seasons (2010 and 2011). Means within each column with the same lowercase letters are not significantly different (LSD test, $p \leq 0.05$). Means with the same uppercase letters within each row indicate that effect of growth for each potato variety is not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

In the leaves, the average chymotrypsin inhibitor (CI) content was highest at 55 days after planting then reduced by 7% at 95 DAP. The effects of variety and growing season on the amount of CI in potato leaves were significant at $p<0.05$. The mean CI content for the different varieties ranged from 786.47 to 912.71 U/mg with vars. Kenya Karibu and Desiree recording the lowest and highest concentrations, respectively.

The influence of growing period on CI content of potato leaves at different stages of growth is indicated in Table 4.4.2.

Table 4.4.2: Chymotrypsin inhibitor content (U/mg) of potato leaves at different growth stages as determined during year 2010 and 2011 seasons

Variety	Stage of growth (Days after planting)	Chymotrypsin inhibitor content (U/mg)	
		2010 season	2011 season
Asante	40	676.80[a]	672.80[a]
	55	889.45	975.14
	95	932.65[a]	870.94[a]
Desiree	40	909.95	640.31
	55	1163.44	1035.77
	95	812.64	914.14
Dutch Robijn	40	834.48	662.13
	55	779.96	856.48
	95	792.96[a]	822.95[a]
Kenya Karibu	40	640.95[a]	638.64[a]
	55	905.12	775.96
	95	1026.94	731.30
Tigoni	40	694.97[a]	645.79[a]
	55	1042.27	875.14
	95	924.62	804.94
LSD (0.05) (n=3)	SG	118.26	38.91
	V	152.68	50.24
	SG×V	264.44	87.01

Values are mean of three replications. Means with the same letter along each row are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The mean CI content in leaf samples collected during the 2010 growing season was significantly ($p=0.037$) higher than that of 2011 (Figure 4.4.1).

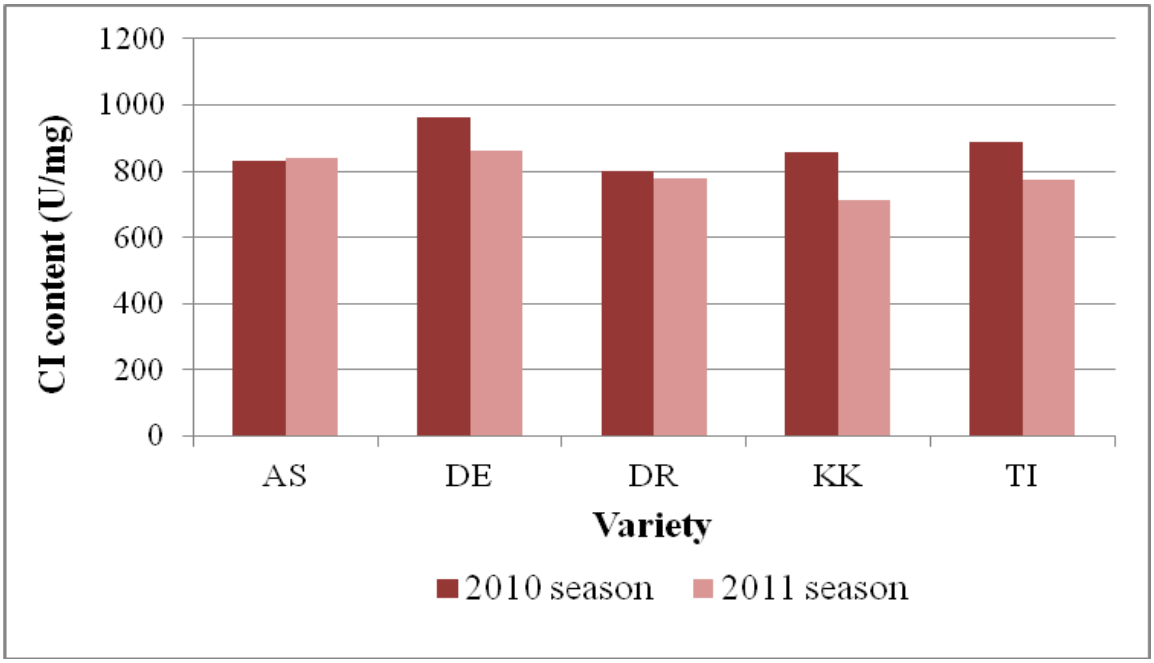


Figure 4.4.1: Seasonal variation in foliar chymotrypsin inhibitor content of different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

The ANOVA data obtained for tubers clearly show that the effects of variety and stage of growth on chymotrypsin inhibitor (CI) concentration were significant ($p<0.001$). The tuber CI content ranged from 257.49 U/mg in var. Asante to 290.27 U/mg in var. Desiree. The concentration of CI in tubers increased significantly during growth and attained the highest concentration at 125 days after planting when the tubers were fully developed and ready for harvest (Tables 4.4.1 and 4.4.3).

Table 4.4.3: Chymotrypsin inhibitor content (U/mg) of potato tubers at different growth stages as determined during year 2010 and 2011 seasons

Variety	Stage of growth (Days after planting)	Chymotrypsin inhibitor content (U/mg)	
		2010 season	2011 season
Asante	55	253.49[a]	247.03[a]
	95	236.65	267.67
	125	255.16	284.65
Desiree	55	288.65	239.76
	95	299.99	285.43
	125	282.49	345.32
Dutch Robijn	55	305.48	261.13
	95	287.65	276.40
	125	278.16[a]	280.32[a]
Kenya Karibu	55	292.48	265.40
	95	285.32[a]	283.70[a]
	125	293.66[a]	292.15[a]
Tigoni	55	283.83	259.00
	95	289.82[a]	283.93[a]
	125	292.16[a]	287.48[a]
LSD (0.05) (n=3)	SG	11.43	6.17
	V	14.76	7.97
	SG×V	25.57	13.81

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The influence of growing season on potato tuber CI content was not significant (p=0.315)

(Figure 4.4.2).

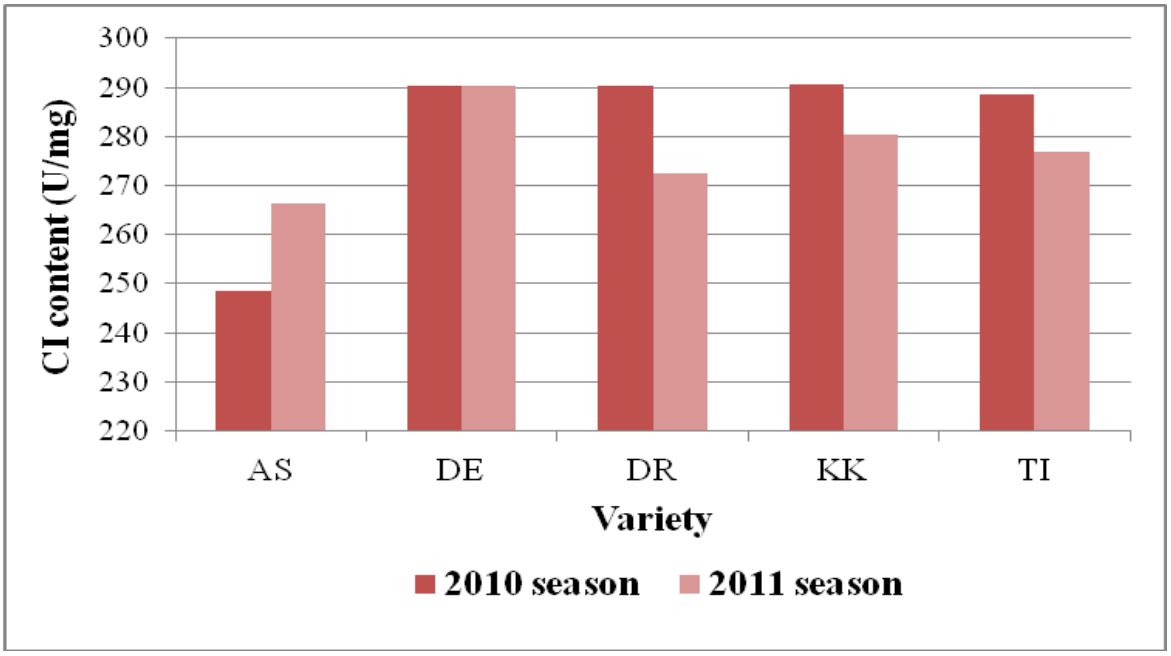


Figure 4.4.2: Seasonal variation in tuber chymotrypsin inhibitor content of different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

4.4.2 Trypsin inhibitor content of potato leaves and tubers

Table 4.4.4 shows the trypsin inhibitor (TI) content in leaves and tubers of the five potato varieties at different stages of growth.

Table 4.4.4: Trypsin inhibitor content (U/mg) of potato leaves and tubers at different growth stages

Variety	Leaf trypsin inhibitor content (U/mg)			Tuber trypsin inhibitor content (U/mg)		
	Days after planting			Days after planting		
	40	55	95	55	95	125
Asante	1385.94[a]	1744.41[a]	1687.27[a]	781.28[a]	1016.35[A]	1056.88[A]
Desiree	1265.88[b]	1681.40	1566.40[b]	728.16[b]	935.13[aA]	973.35[aA]
Dutch Robijn	1229.95[b]	1600.02	1547.58[b]	743.62[ab]	926.75[aA]	961.47[aA]
Kenya Karibu	1415.77[a]	1768.93[a]	1683.67[a]	776.55[a]	938.88[aA]	972.87[aA]
Tigoni	1520.40	1742.55[a]	1676.59[a]	775.35[a]	926.87[a]	977.83[a]
LSD (0.05) (n=3)	SG 33.67	V 43.47	SG×V 75.29	SG 46.38	V 59.88	SG×V 103.72

Values are means of three replications and two growing seasons for years 2010 and 2011. Means within each column with the same lowercase letters are not significantly different (LSD test, $p \leq 0.05$). Means with the same uppercase letter within each row indicate that effect of growth for each potato variety is not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The results indicate that there was significant variation among varieties at level $p<0.05$. The average concentration of trypsin inhibitor (TI) in the leaves ranged from 1459.18 to 1646.51 U/mg with vars. Tigoni and Dutch Robijn recording the highest and lowest concentrations, respectively. The accumulation of trypsin inhibitors and chymotrypsin inhibitors in potato leaves generally followed similar patterns. The effect of stage of growth on the concentration of TI was significant ($p<0.001$) (Tables 4.4.4 and 4.4.5, Appendix 4C). The foliar TI concentration was highest at 55 days after planting at vegetative stage when leaves, stem and root systems were fully developed. This was followed by a slight decline as the plants developed to full maturity.

Table 4.4.5: Trypsin inhibitor content (U/mg) of potato leaves at different growth stages as determined during year 2010 and 2011 seasons

Variety	Stage of growth (Days after planting)	Trypsin inhibitor content (U/mg)	
		2010 season	2011 season
Asante	40	1389.37[a]	1382.50[a]
	55	1729.63[a]	1759.20[a]
	95	1704.75[a]	1669.79[a]
Desiree	40	1269.04[a]	1262.72[a]
	55	1679.07[a]	1683.73[a]
	95	1570.57[a]	1562.23[a]
Dutch Robijn	40	1224.39[a]	1235.51[a]
	55	1547.37	1652.67
	95	1566.66[a]	1528.50[a]
Kenya Karibu	40	1451.50	1380.05
	55	1762.72[a]	1775.13[a]
	95	1691.37[a]	1675.96[a]
Tigoni	40	1678.87	1361.93
	55	1747.37[a]	1737.73[a]
	95	1667.71[a]	1685.46[a]
LSD (0.05) (n=3)	SG	62.82	18.99
	V	48.66	14.71
	SG×V	108.81	32.90

Values are mean of three replicates. Means with the same letter along each row are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The effect of growing season on the concentration of trypsin inhibitors (TI) in the leaves was not significant ($p=0.423$) (Figure 4.4.3, Appendix 4D).

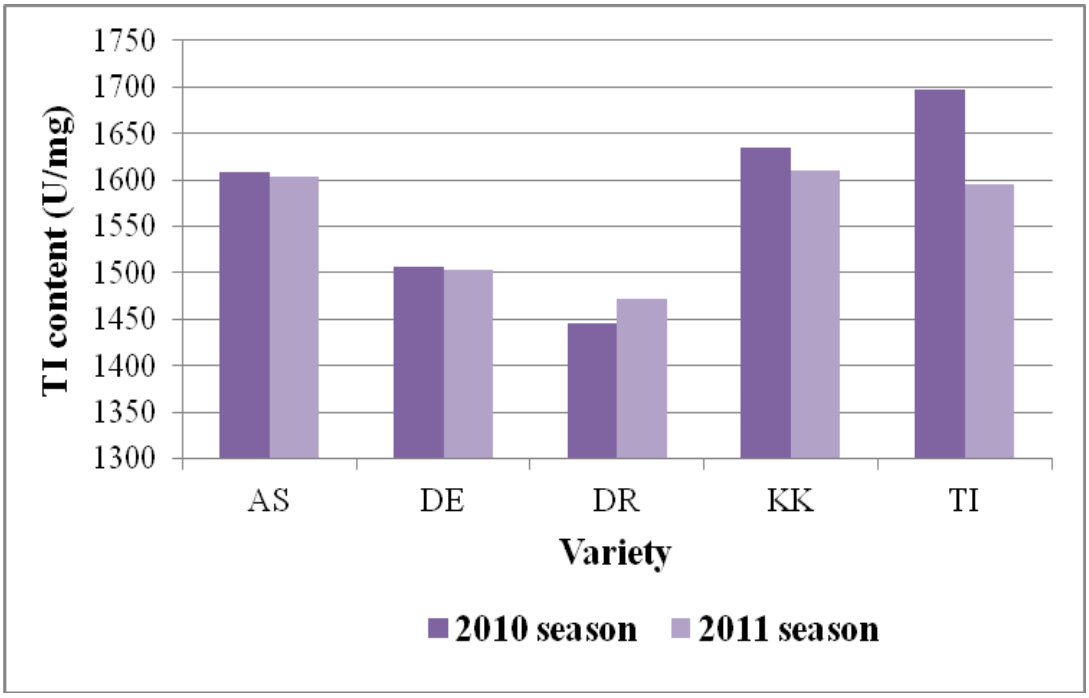


Figure 4.4.3: Seasonal variation in foliar trypsin inhibitor content of different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

Table 4.4.4 shows the TI contents found in tubers from the extracts of potato varieties studied. The mean TI content ranged from 877.28 to 951.51 U/mg in the vars. Dutch Robijn and Asante, respectively. The difference in the concentration of TI in tubers of the five potato varieties was not significant ($p=0.098$) (Appendix 4C). The data indicates that the amount of TI significantly ($p<0.001$) increased with growth and was highest at 125 days after planting when tubers were mature and senescence of plant canopy had started (Tables 4.4.4 and 4.4.6).

Table 4.4.6: Trypsin inhibitor content (U/mg) of potato tubers at different growth stages as determined during year 2010 and 2011 seasons

Variety	**Stage of growth (Days after planting)**	**Trypsin inhibitor content (U/mg)**	
		2010 season	**2011 season**
Asante	55	705.20	857.37
	95	934.63	1098.07
	125	969.30	1144.47
Desiree	55	653.43	802.90
	95	854.17	1016.10
	125	885.77	1060.93
Dutch Robijn	55	670.43	816.80
	95	845.80	1007.70
	125	873.97	1048.97
Kenya Karibu	55	699.87	853.23
	95	857.90	1019.87
	125	885.37	1060.37
Tigoni	55	702.17	848.53
	95	845.83	1007.90
	125	890.30	1065.37
LSD (0.05) (n=3)	SG	11.72	11.68
	V	15.13	15.08
	SG×V	26.21	26.12

Values are mean of three replicates. LSD = least significant differences, SG = stage of growth, V= variety, SG×V= stage of growth and variety interaction.

The mean tuber TI content in samples collected during the year 2011 was significantly higher than that of the year 2010 in most of the test varieties (Figure 4.4.4).

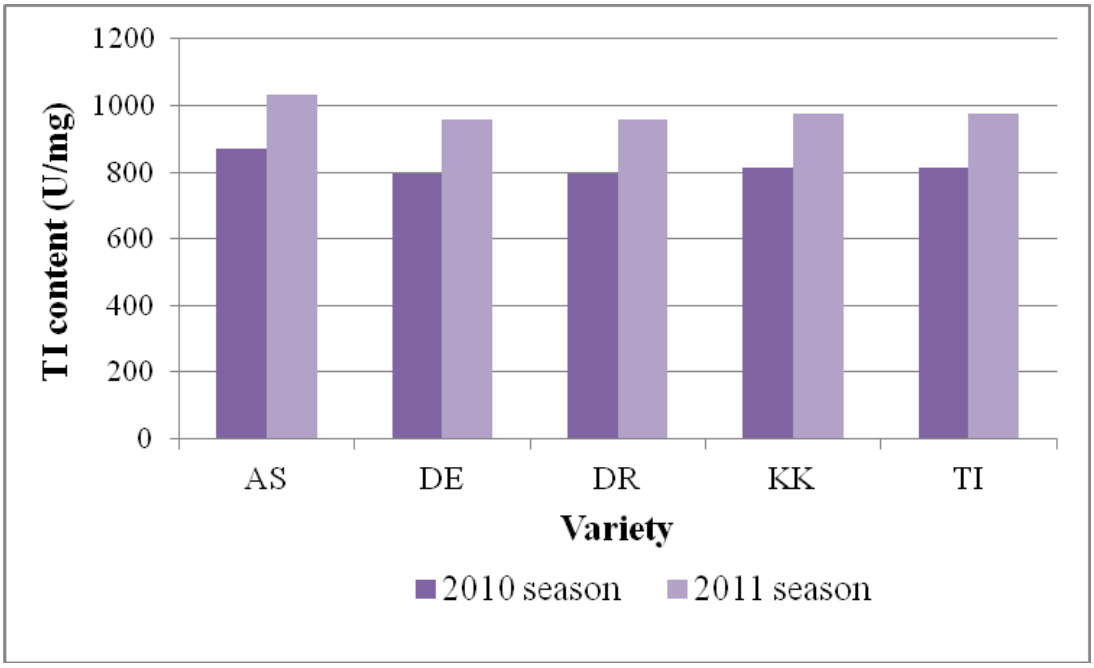


Figure 4.4.4: Seasonal variation in tuber trypsin inhibitor content of different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

4.5 Effect of storage on phytonutrient levels in different potato varieties

4.5.1 Effect of storage on the level of glycoalkaloids in different potato varieties

The concentration of tuber total glycoalkaloid (TGA) from five commercial potato varieties at different storage times during year 2010 and 2011 seasons are shown in Table 4.5.1.

Table 4.5.1: Effect of storage time on tuber glycoalkaloid content of different potato varieties

Variety	**Storage time (Days)**		
	7	**14**	**21**
Asante	9.40^{B}	9.91^{aAB}	10.88^{aA}
Desiree	5.64^{ab}	7.40^{bA}	8.41^{bA}
Dutch Robijn	5.31^{bA}	6.23^{bA}	8.20^{b}
Kenya Karibu	7.03^{a}	9.41^{a}	11.81^{a}
Tigoni	15.80	19.30	23.02
LSD (0.05) (n=3)	ST 1.28	V 1.65	ST×V 2.85

Values are means of three replications. Means within each column with the same lowercase letters are not significantly different (LSD test, $p \leq 0.05$). Means with the same uppercase letter along each row indicate that the effect of storage time for each potato variety is not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, ST = storage time, V= variety, ST×V= storage time and variety interaction.

The results indicate that the effects of variety, storage time and storage season on the concentration of TGA in potato tubers were significant ($p<0.001$). The mean TGA values ranged from 5.27mg to 15.79 mg/100g, 6.24 mg to 19.25 mg/100g and 8.23 mg to 23.04 mg/100g Fwt in tubers stored for 7, 14 and 21 days, respectively. The TGA levels increased to the highest levels in tubers stored for 21 days, with the highest being for variety Tigoni (Table 4.5.2).

Table 4.5.2: Tuber total glycoalkaloid concentration (mg/100g) of potato varieties at different storage times as determined during year 2010 and 2011 seasons

Variety	**Storage time (Days)**	**Storage season**	
		2010	**2011**
Asante	7	8.18	10.55
	14	8.04	11.68
	21	9.52	12.22
Desiree	7	5.61[a]	5.65[a]
	14	6.52	8.34
	21	6.95	9.94
Dutch Robijn	7	5.16[a]	5.37[a]
	14	5.85[a]	6.63[a]
	21	7.10	9.36
Kenya Karibu	7	6.62[a]	7.32[a]
	14	8.04	10.73
	21	10.09	13.52
Tigoni	7	14.35	17.23
	14	16.84	21.67
	21	19.76	26.31
LSD (0.05)	ST	1.46	1.98
(n=3)	V	1.89	2.56
	ST×V	3.27	4.44

Values are mean of three replicates. Means with the same letter along each row are not significantly different at level $p \leq 0.05$. LSD = least significant differences, ST = storage time, V= variety, ST×V= storage time and variety interaction.

The TGA in potato tubers after three weeks of storage ranged from 8.24 mg to 25.87 mg/100g Fwt and 5.79 mg to 19.37 mg/100g Fwt, for tubers kept under fluorescent light and sunlight conditions, respectively (Table 4.5.3). The corresponding values for tubers kept in the dark room over the same period ranged from 5.71 mg to 12.84 mg/100g Fwt.

The total glycoalkaloid (TGA) contents were highest and lowest in vars. Tigoni and Dutch Robijn, respectively.

Table 4.5.3: Tuber total glycoalkaloid concentration (mg/100g) of potato varieties at different storage conditions as determined during year 2010 and 2011 seasons

Variety	**Storage condition**	**Storage season**	
		2010	**2011**
Asante	D	8.75	15.68
	FL	12.22	13.77
	SL	8.78[a]	9.00[a]
Desiree	D	5.87[a]	6.20[a]
	FL	6.80	11.13
	SL	6.41[a]	6.60[a]
Dutch Robijn	D	5.58[a]	5.84[a]
	FL	6.86	9.62
	SL	5.68[a]	5.90[a]
Kenya Karibu	D	7.34[a]	7.58[a]
	FL	7.96	14.28
	SL	9.44[a]	9.72[a]
Tigoni	D	12.70[a]	12.98[a]
	FL	24.72	27.01
	SL	13.51	25.23
LSD (0.05) (n=3)	SC	0.94	1.41
	V	1.21	1.82
	SC×V	2.10	3.15

Values are mean of three replicates. Means with the same letter along each row are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SC = storage condition, V= variety, SC×V= storage condition and variety interaction.

The results show that the effects of variety, storage conditions and duration on the concentration of TGA in all potato varieties were significant ($p<0.001$) (Appendix5A). From the experimental results, it was apparent that TGA content had increased significantly after three weeks of storage. The increase was much greater in tubers that were exposed to fluorescent light compared to tubers that were exposed to sunlight and the

controls stored in the dark. Exposure to fluorescent light induced TGA accumulation to a higher level than sun light (Figure 4.5.1).

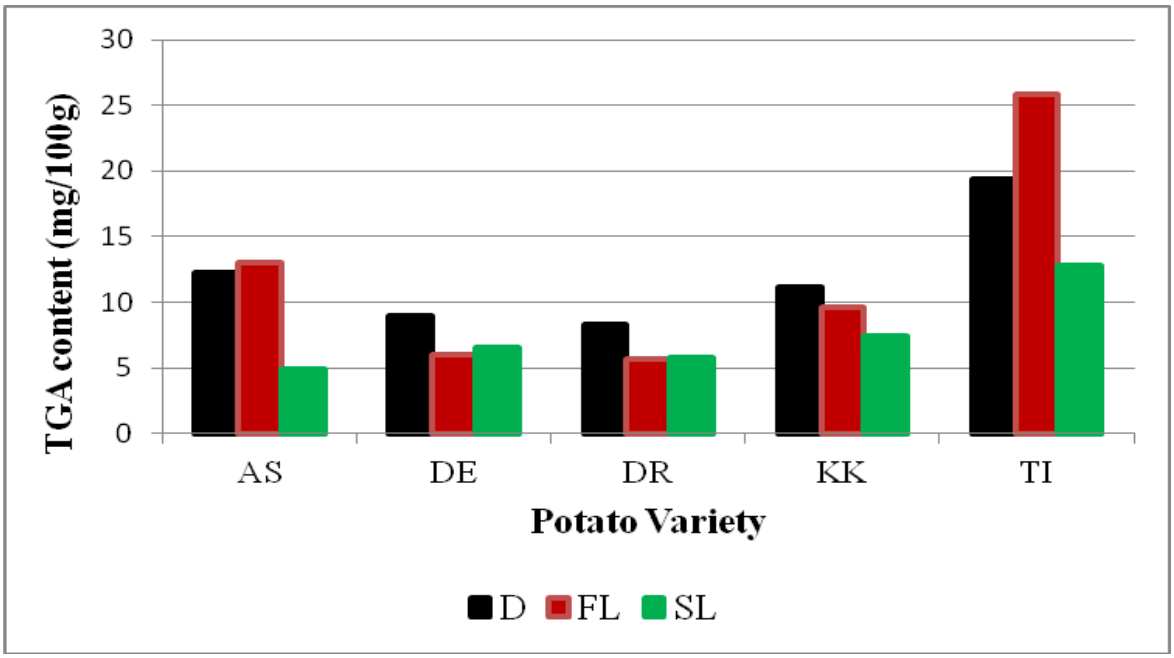


Figure 4.5.1: Influence of light on potato tuber TGA content during storage. Varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni. Storage conditions: SL; sunlight, FL; fluorescent light, and D; dark room.

The tuber samples stored during 2011 season exhibited significantly higher ($p<0.05$) mean TGA content (12.04 mg/100g) compared to that of 2010 (9.52 mg/100g) (Figure 4.5.2).

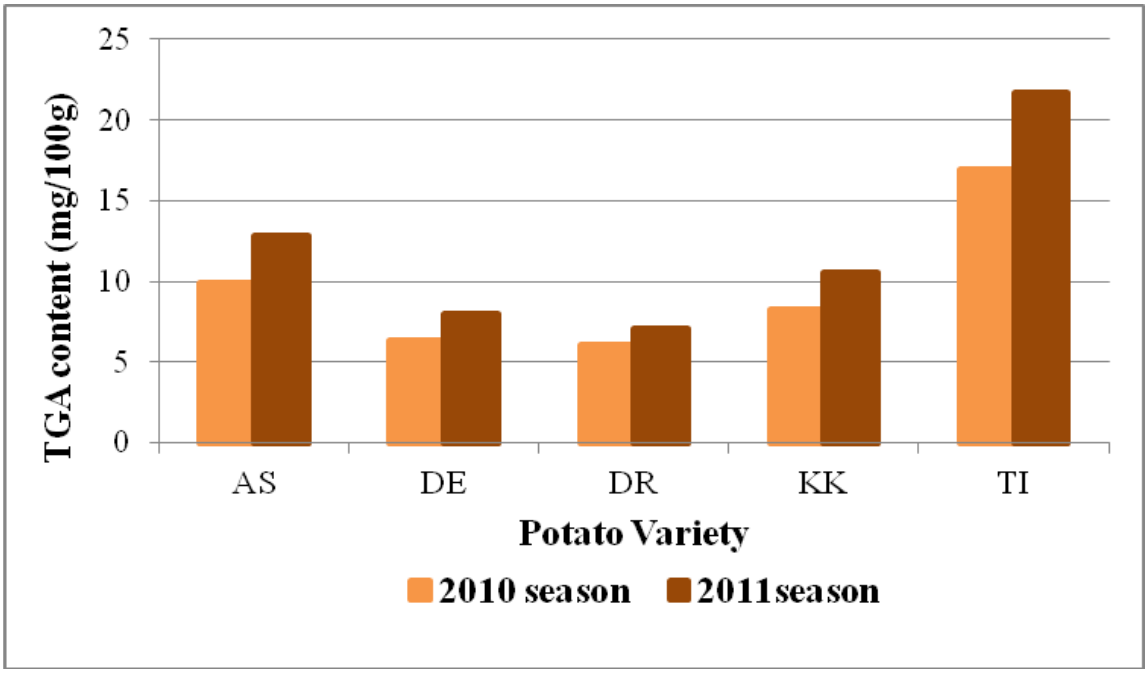


Figure 4.5.2: Influence of storage season on potato tuber TGA content. Varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

The storage season and variety interaction effects were not significant (p=0.24) suggesting that the varieties behaved the same for TGA across different storage seasons.

Absorbance peaks of unidentified compounds were also observed in the HPLC chromatograms of stored tubers (Figure 4.5.3 and 4.5.4).

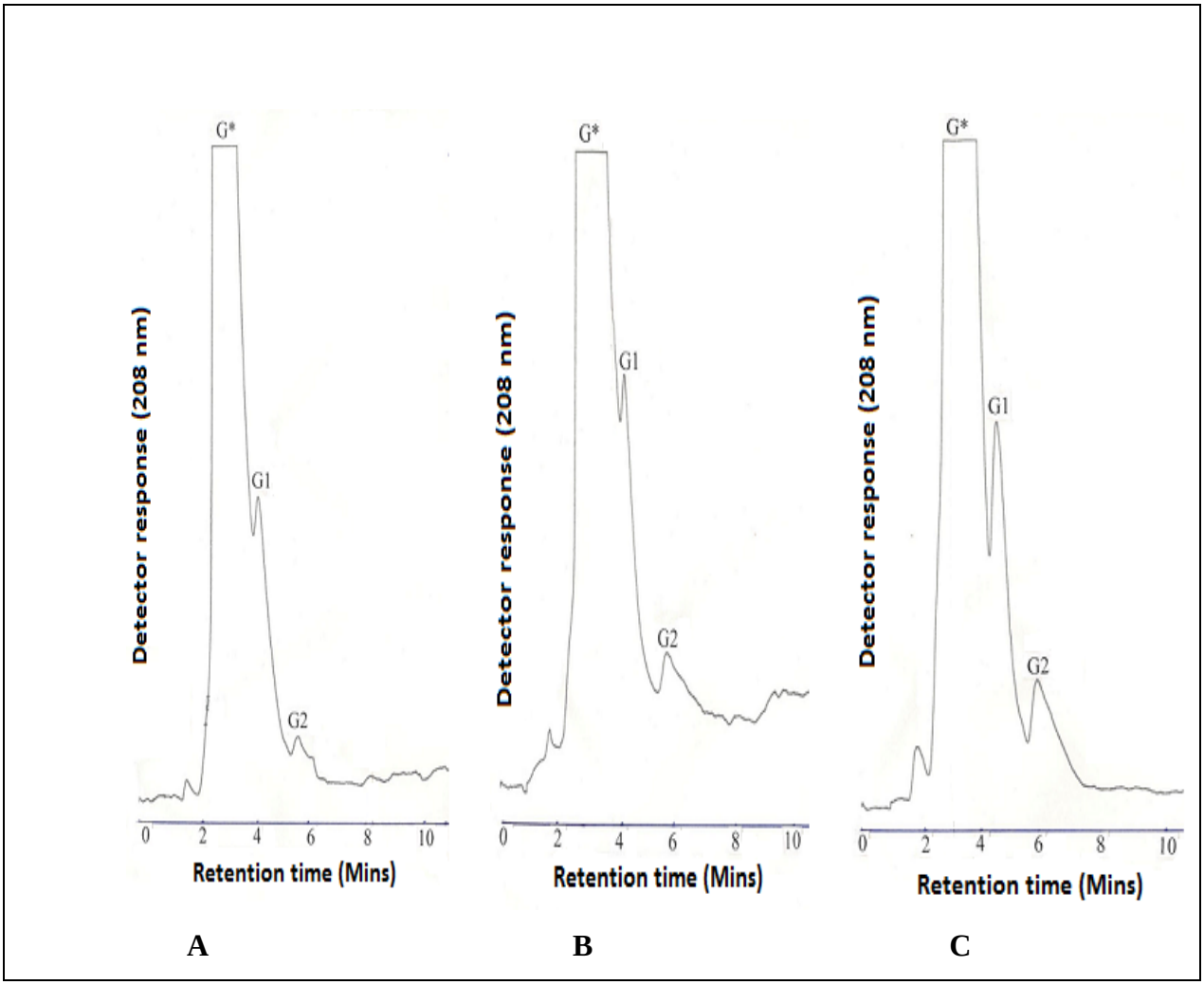


Figure 4.5.3: HPLC profiles of glycoalkaloids in tuber extracts of potato var. Dutch Robijn stored for 21 days under the following conditions (A) dark room, (B) sunlight and (C) fluorescent light. Peaks G1and G2 corresponds to α-chaconine and α-solanine, respectively, while G* was not identified.

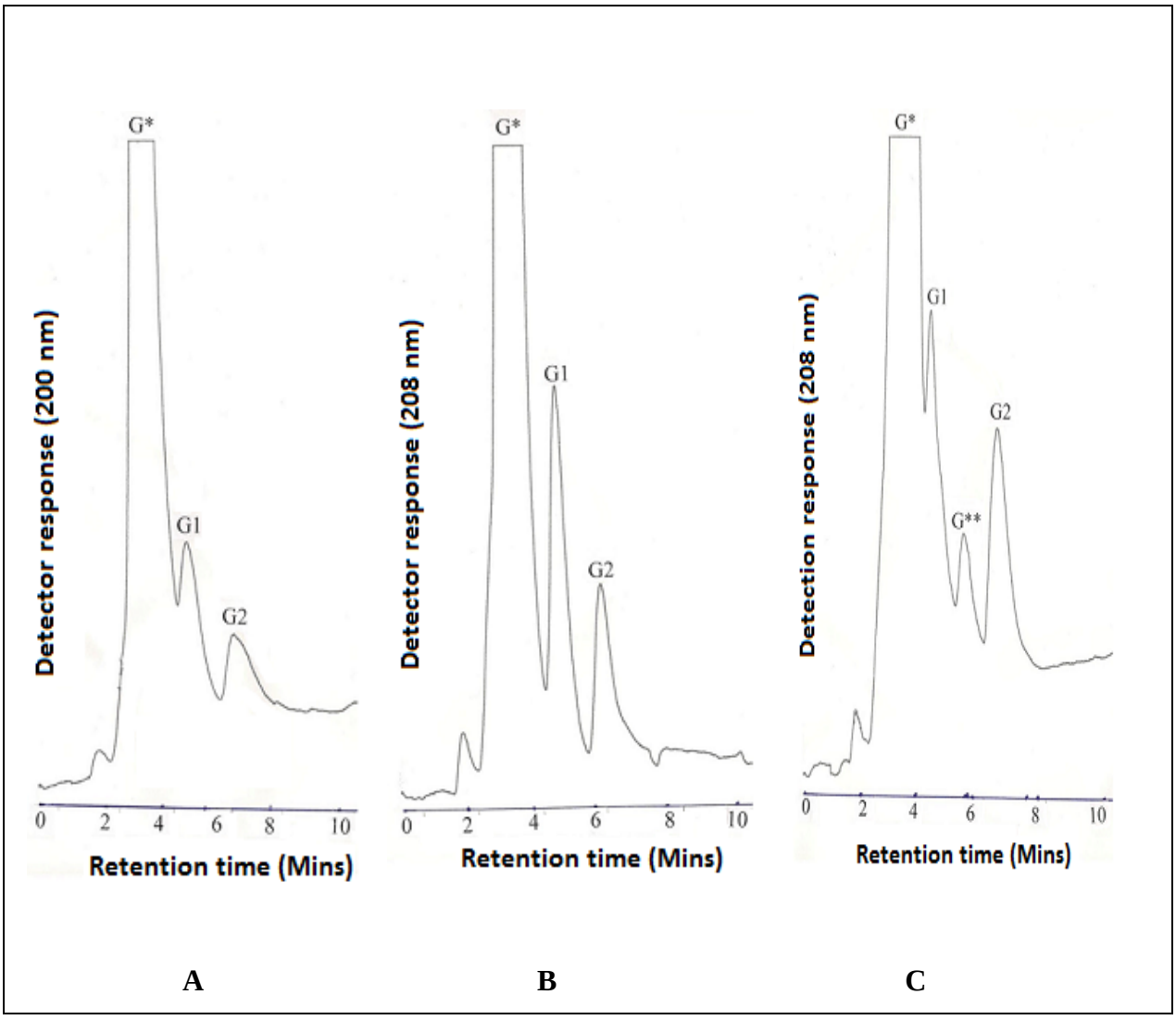


Figure 4.5.4 HPLC chromatograms of tuber glycoalkaloids in potato var. Tigoni stored for 21 days at (A) dark room, (B) sunlight and (C) fluorescent light. Absorbance peaks G1and G2 corresponds to α-chaconine and α-solanine, respectively, while G* and G** were unidentified.

4.5.2 Effect of storage on the levels of chlorogenic acid and total phenolics in different potato varieties

The effect of storage for three consecutive weeks on the concentration of chlorogenic acid (CGA) and total phenolics (TP) in different potato varieties are presented in Table 4.5.2.

Table 4.5.4: Chlorogenic acid and total phenolic concentration in potato tubers at different storage times

Variety	**CGA content (mg/100g)**			**TP content (mg CGA/g)**		
	Storage time (days)			**Storage time (days)**		
	7	**14**	**21**	**7**	**14**	**21**
Asante	46.87^{a}	54.13^{a}	63.80^{a}	140.06^{ab}	156.04^{ab}	169.20^{b}
Desiree	39.09^{b}	41.77	52.23	143.27	161.26^{a}	176.51^{b}
Dutch Robijn	37.84^{b}	45.72	57.05	129.57^{b}	150.32^{b}	193.38^{a}
Kenya Karibu	46.44^{a}	51.90^{a}	62.96^{a}	140.50^{aA}	149.86^{bA}	190.90^{a}
Tigoni	48.04^{a}	54.31^{a}	61.33^{a}	161.39^{A}	164.87^{aA}	166.53^{bA}
LSD (0.05)	ST	V	ST×V	ST	V	ST×V
(n=3)	2.11	2.72	4.72	9.86	12.72	22.04

Values are means of three replicates. Means within each column with the same lowercase letters are not significantly different (LSD test, $p \leq 0.05$). Means with the same uppercase letters along each row indicate that the effect of storage time for each potato variety is not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, ST = storage time, V= variety, ST×V= storage time and variety interaction.

The results indicate that the influence of variety, storage time and storage season on the concentration of potato tuber chlorogenic acid (CGA) were significant ($p<0.001$) (Table 4.5.5). The concentration of CGA increased significantly with time of exposure to light. The increases were greatest in all the examined varieties after storage for between 14 and 21 days.

Table 4.5.5: Chlorogenic acid (CGA) content (mg/100g) of potato tubers at different storage times as determined during year 2010 and 2011 seasons

Variety	Storage time (Days)	Storage season	
		2010	**2011**
Asante	7	50.77	42.97
	14	55.81	52.46
	21	63.08[a]	64.50[a]
Desiree	7	43.27	34.91
	14	42.48[a]	41.07[a]
	21	53.71	50.76
Dutch Robijn	7	41.07	34.61
	14	46.86[a]	44.57[a]
	21	57.95[a]	56.16[a]
Kenya Karibu	7	45.94[a]	46.94[a]
	14	52.56[a]	51.23[a]
	21	64.03[a]	61.90[a]
Tigoni	7	49.78	46.30
	14	54.96[a]	53.67[a]
	21	62.00[a]	60.66[a]
LSD (0.05) (n=3)	ST	2.589	3.305
	V	3.342	4.267
	ST×V	5.788	7.390

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, ST = storage time, V= variety, ST×V= storage time and variety interaction.

In the study the concentration of CGA in potato tubers exposed to fluorescent light and sunlight were higher as compared with the negative controls that were kept in the dark room (Table 4.5.6). The highest increase was observed on tubers exposed to fluorescent light (Figure 4.5.4).

Table 4.5.6: Chlorogenic acid (CGA) content (mg/100g) of potato tubers at different storage conditions as determined during year 2010 and 2011 seasons

Variety	Storage condition	Storage season	
		2010	2011
Asante	D	51.88	48.31
	FL	59.11[a]	59.17[a]
	SL	58.66	52.45
Desiree	D	42.92	39.03
	FL	48.68[a]	47.60[a]
	SL	47.91	40.10
Dutch Robijn	D	42.81	38.94
	FL	55.88	48.87
	SL	47.19[a]	47.52[a]
Kenya Karibu	D	47.62	43.76
	FL	57.82	61.20
	SL	57.08[a]	55.12[a]
Tigoni	D	45.73	41.84
	FL	61.09[a]	61.86[a]
	SL	59.90[a]	56.92[a]
LSD (0.05) (n=3)	SC	1.085	3.817
	V	1.401	4.928
	SC×V	2.426	8.535

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SC = storage condition, V= variety, SC×V= storage condition and variety interaction.

The mean CGA concentration in the potato tubers ranged from 48.14 to 61.51 mg and 44.20 to 58.40 mg/100g, for tubers kept under fluorescent light and sunlight conditions, respectively. Statistically significant ($p=0.012$) interaction was found for the storage condition and variety on CGA content (Appendix 5B).

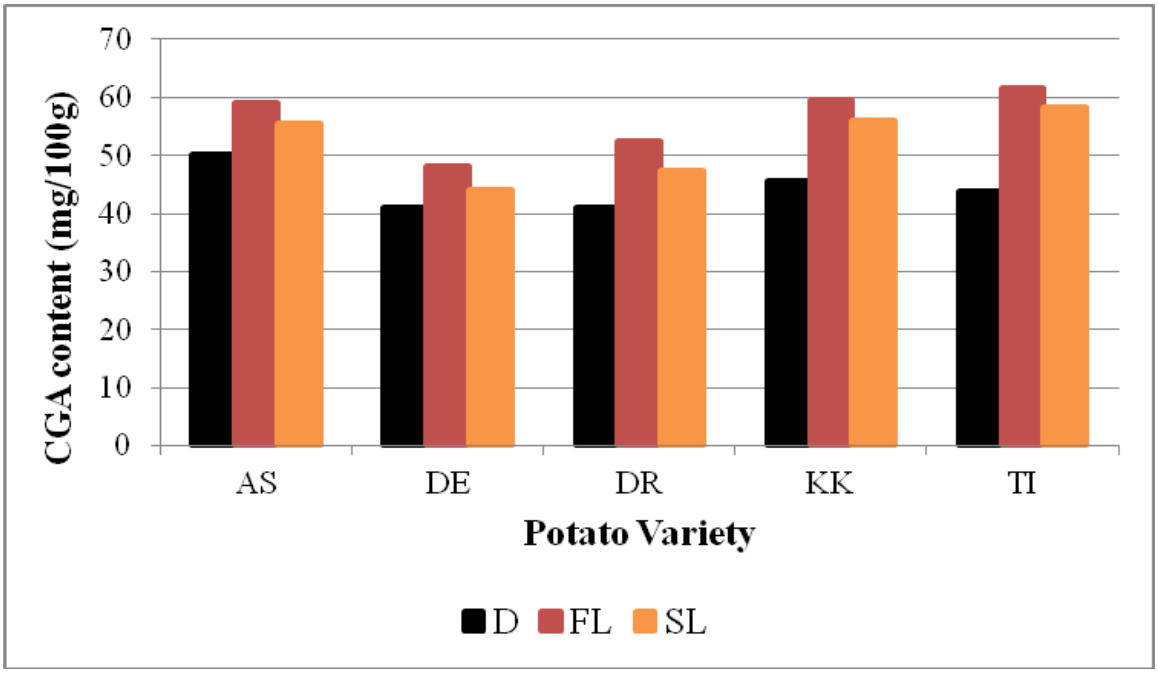


Figure 4.5.5: Influence of light on potato tuber CGA content. Varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni, were stored during 2010 and 2011 seasons. Storage conditions: SL; sunlight, FL; fluorescent light, and D; dark room.

Higher tuber CGA content was observed during 2010 (52.28 mg/100g) storage season as compared to that of 2011 (49.51 mg/100g). Analysis of variance indicated no statistically significant ($p=0.90$) difference on mean CGA contents between the two seasons (Figure 4.5.6).

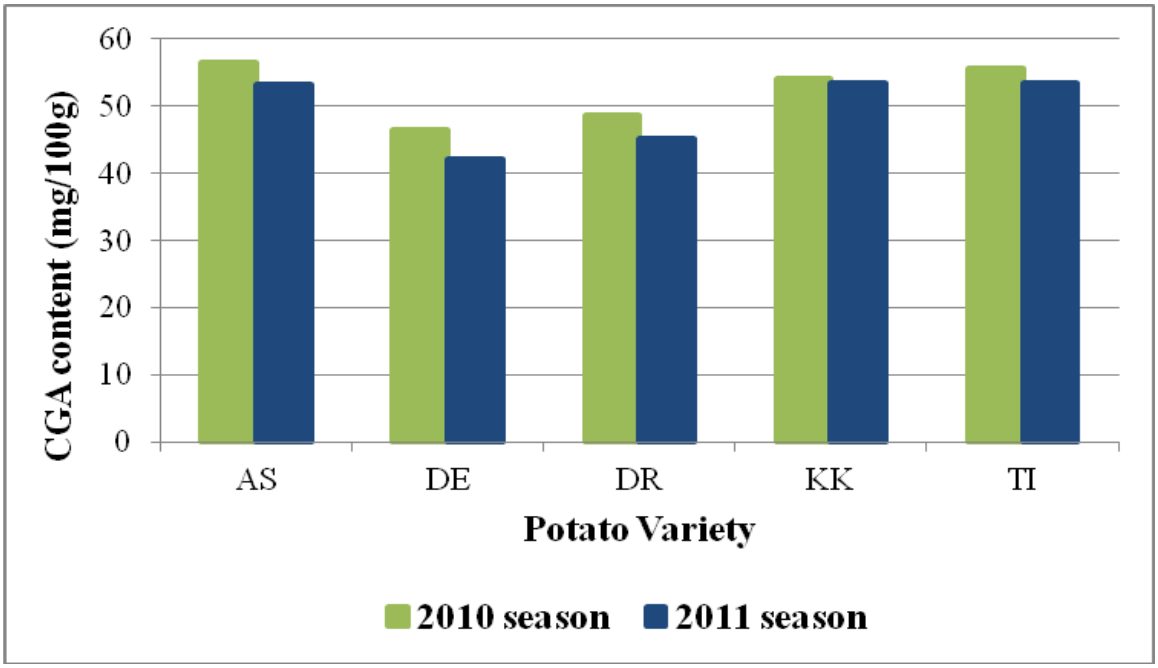


Figure 4.5.6: Influence of storage season on potato tuber CGA content. Varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

The ANOVA results show that the influence of storage time on total phenolic (TP) in potato tubers was significant ($p<0.001$) (Table 4.5.7). The concentration of TP increased significantly with storage time and the increases were greatest after storage for 21 days in all the examined varieties.

Table 4.5.7: Total phenolic content (mg CGA/g) content of potato tubers at different storage times as determined during year 2010 and 2011 seasons

Variety	Storage time (Days)	Storage season	
		2010	2011
Asante	7	143.90[a]	136.23[a]
	14	148.88	163.21
	21	160.21	178.19
Desiree	7	148.63	137.91
	14	156.21	166.31
	21	157.79	195.24
Dutch Robijn	7	143.31	115.84
	14	146.51[a]	154.14[a]
	21	158.87	227.89
Kenya Karibu	7	145.75	135.27
	14	149.74[a]	149.97[a]
	21	175.68	206.13
Tigoni	7	146.43	176.35
	14	152.36	177.39
	21	155.23	177.82
LSD (0.05) (n=3)	ST	12.54	13.61
	V	16.19	17.57
	ST×V	28.04	30.43

Values are mean of three replicates. Means with the same letter along each row are not significantly different at level $p \leq 0.05$. LSD = least significant differences, ST = storage time, V= variety, ST×V= storage time and variety interaction.

The concentration of TP in tubers exposed to light as compared with the negative controls that were stored in the dark room (Table 4.5.8). The highest mean tuber TP content was recorded in tubers stored under fluorescent light (Figure 4.5.7).

Table 4.5.8: Total phenolic content (mg CGA/g) content of potato tubers at different storage conditions as determined during year 2010 and 2011 seasons

Variety	Storage condition	Storage season	
		2010	2011
Asante	D	147.68	134.93
	FL	154.08	175.56
	SL	151.23	167.14
Desiree	D	134.25	148.03
	FL	171.79	199.12
	SL	156.59[a]	152.31[a]
Dutch Robijn	D	126.85[a]	134.85[a]
	FL	167.07	194.31
	SL	151.46	163.28
Kenya Karibu	D	130.19	140.28
	FL	185.08[a]	190.28[a]
	SL	159.24[a]	166.23[a]
Tigoni	D	134.80	168.81
	FL	178.78	188.10
	SL	175.50[a]	174.65[a]
LSD (0.05) (n=3)	SC	10.64	16.27
	V	13.73	21.01
	SC×V	23.79	36.39

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SC= storage condition, V= variety, SC×V= storage condition and variety interaction.

The mean total phenolic (TP) content ranged from164.82 to 187.68 mg CGA/g and 154.45 to 175.07 mg CGA/g, for tubers stored under fluorescent light and sun light conditions, respectively. Interaction effects of variety and light condition on tuber TP content was significant (p=0.026).

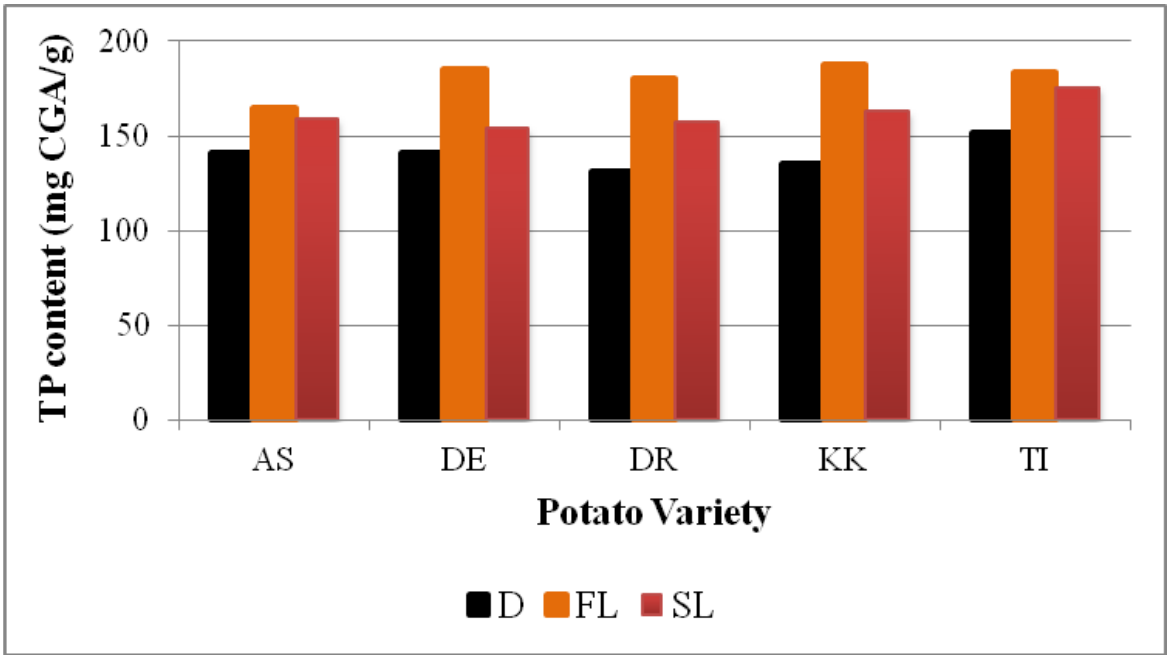


Figure 4.5.7: Influence of light on potato tuber TP content. Varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni, were stored during 2010 and 2011 seasons. Storage conditions: SL; sunlight, FL; fluorescent light, and D; dark room.

Potato tubers kept in the dark accumulated the lowest levels of TP.

Higher tuber TP content was recorded during 2011 (166.53 mg CGA/g) storage season as compared to 2010 (155.00 mg CGA/g). The difference in mean TP values between the two seasons was significant ($p=0.002$) (Figure 4.5.8).

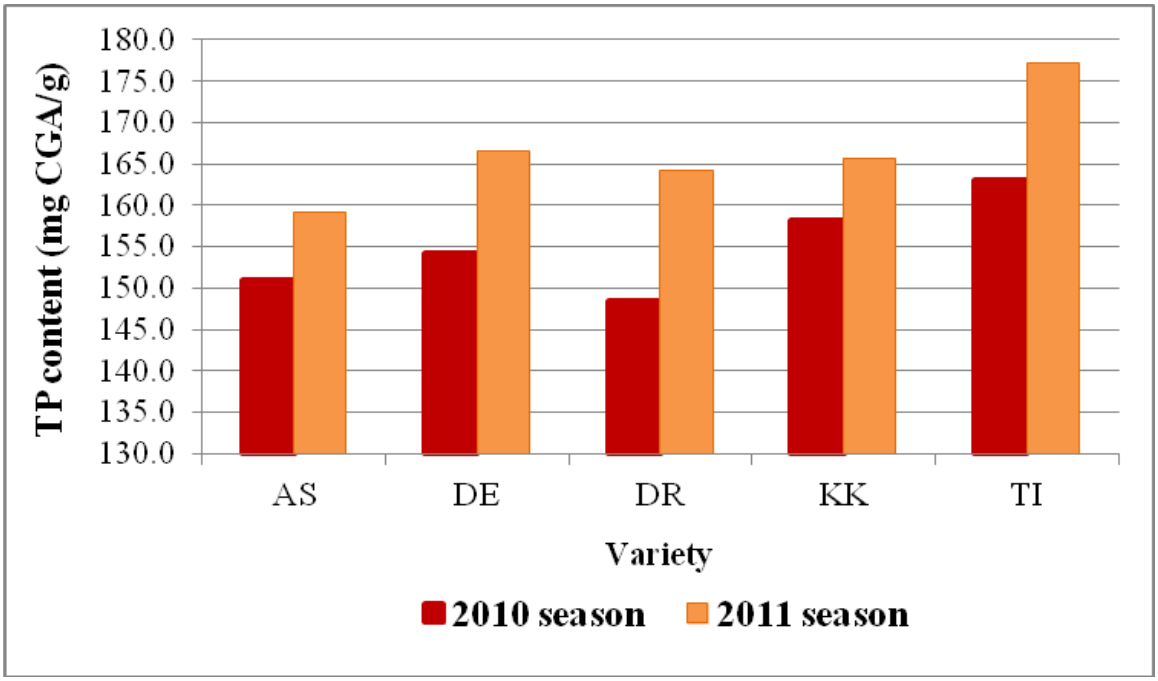


Figure 4.5.8: Influence of storage season on potato tuber TP content. Varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

4.5.3 Effect of storage on the level of protease inhibitors in different potato varieties

Trypsin and chymotrypsin inhibitor concentration in stored potato tubers were determined by UV spectrophotometry as described in chapter three. The data summarized in Table 4.5.9 show that the three weeks storage time used in this study had no significant influence on the concentration of these inhibitors.

Table 4.5.9: Influence of storage time on chymotrypsin inhibitor and trypsin inhibitor concentration in tubers of different potato varieties

Variety	**Chymotrypsin inhibitor (U/mg)**			**Trypsin inhibitor (U/mg)**		
	Storage time (days)			**Storage time (days)**		
	7	**14**	**21**	**7**	**14**	**21**
Asante	274.14^{aB}	275.32^{aA}	274.91^{bAB}	1122.10	1123.18	1123.23
Desiree	285.18	286.36^{A}	286.52^{A}	1107.94	1109.46	1109.16
Dutch Robijn	286.83	288.07^{A}	287.99^{aA}	1039.44	1041.34	1045.45
Kenya Karibu	274.94^{aA}	275.02^{aA}	274.93^{bA}	1138.37	1138.76	1138.58
Tigoni	290.54^{A}	289.69^{A}	289.04^{a}	1140.57	1142.03	1142.18
LSD (0.05)	ST	V	ST×V	ST	V	ST×V
(n=3)	0.88	1.13	1.96	3.37	4.35	7.54

Values are means of three replicates. Means within each column with the same lowercase letters are not significantly different (LSD test, $p \leq 0.05$). Means with the same uppercase letters along each row indicate that the effect of storage time for each potato variety is not significantly different (LSD test, $p \leq 0.05$). LSD = least significant differences, ST = storage time, V= variety, ST×V= storage time and variety interaction.

The chymotrypsin inhibitor (CI) content in tuber extracts after one week of storage ranged from 274.14 to 290.54 U/mg in the vars. Asante and Tigoni, respectively. After three weeks of storage, the CI content in different potato varieties varied from 274.91 to 289.04 U/mg. The effect of variety on tuber CI contents was statistically significant ($p<0.001$) (Table 4.5.10).

Table 4.5.10: Chymotrypsin inhibitor (CI) content (U/mg) of potato tubers at different storage times as determined during year 2010 and 2011 seasons

Variety	Storage time (Days)	Storage season	
		2010	2011
Asante	7	273.61	274.67
	14	274.86	275.79
	21	274.77[a]	275.05[a]
Desiree	7	280.52	289.84
	14	282.33	290.39
	21	282.38	290.66
Dutch Robijn	7	286.56	287.11
	14	287.98[a]	288.17[a]
	21	286.31	289.67
Kenya Karibu	7	271.28	278.60
	14	272.01	278.39
	21	271.48	276.39
Tigoni	7	286.87	294.21
	14	288.24	291.13
	21	287.84	290.24
LSD (0.05) (n=3)	ST	0.63	0.11
	V	0.82	0.14
	ST×V	1.42	1.25

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, ST = storage time, V= variety, ST×V= storage time and variety interaction.

The results indicate that the influence of storage time on tuber CI content was not significant ($p=0.087$).

Potato tubers stored under fluorescent light accumulated higher mean CI levels compared to tuber samples that were kept in the dark room and sunlight conditions (Table 4.5.11).

Table 4.5.11: Chymotrypsin inhibitor content (U/mg) of potato tubers at different storage conditions as determined during year 2010 and 2011 seasons

Variety	Storage condition	Storage season	
		2010	2011
Asante	D	273.81	274.67
	FL	275.54 [a]	275.79[a]
	SL	273.89	275.05
Desiree	D	280.69	289.84
	FL	283.82	290.66
	SL	280.72	290.39
Dutch Robijn	D	286.09	287.11
	FL	287.90	289.67
	SL	286.86	288.17
Kenya Karibu	D	270.69	276.39
	FL	272.32	278.60
	SL	271.77	278.78
Tigoni	D	286.89	290.24
	FL	288.43	294.21
	SL	287.63	291.13
LSD (0.05) (n=3)	SC	0.56	0.31
	V	0.73	0.40
	SC×V	1.26	0.70

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, SC = storage condition, V= variety, SC×V= storage condition and variety interaction.

The mean CI content for tubers stored under fluorescent light and sun light conditions varied from 275.46 to 291.32 U/mg and 274.47 to 289.38 U/mg, respectively (Figure 4.5.9). Interaction effects of variety and storage condition on tuber CI content was not significant (p=0.21).

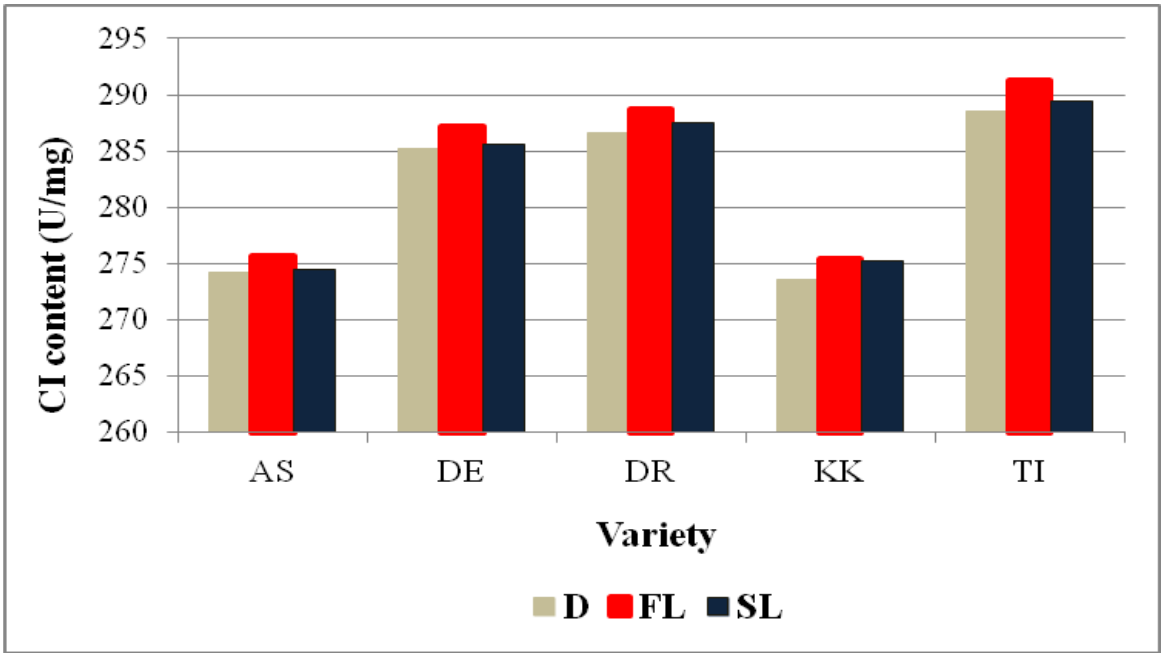


Figure 4.5.9: Influence of light on potato tuber chymotrypsin inhibitor content. Varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni. Storage conditions: SL; sunlight, FL; fluorescent light, and D; dark room.

The ANOVA for potato tuber CI content indicate that the influence of storage season was significant ($p<0.001$) (Figure 4.5.10).

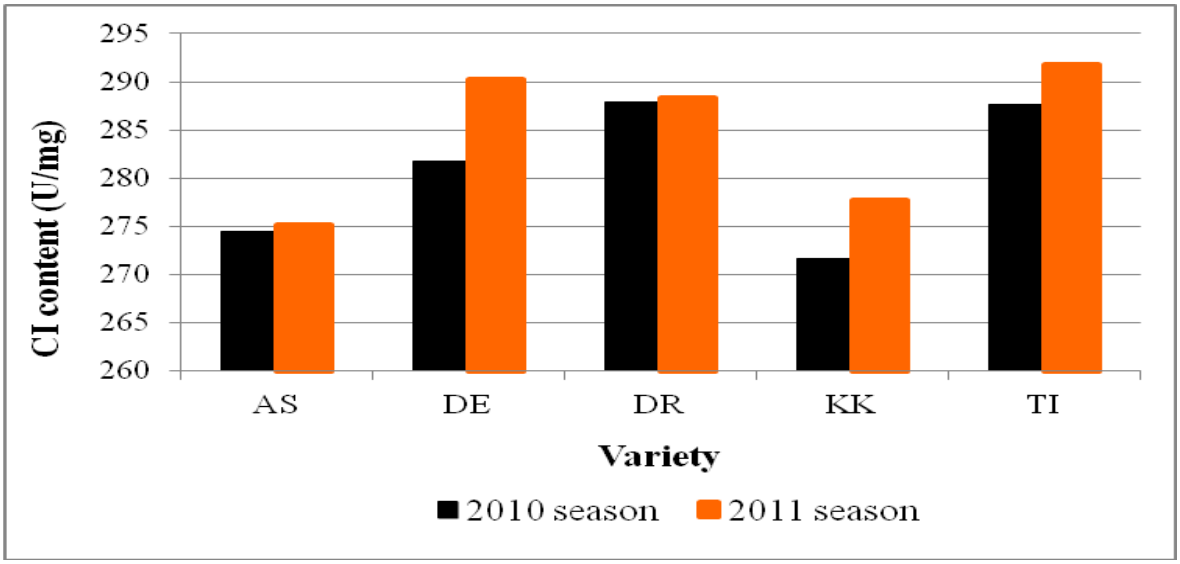


Figure 4.5.10: Seasonal variation in tuber chymotrypsin inhibitor content of different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

The mean trypsin inhibitor (TI) content of tubers from five commercial potato varieties at different storage times during 2010 and 2011 seasons are shown in Table 4.5.12.

Table 4.5.12: Trypsin inhibitor (TI) content (U/mg) of potato tubers at different storage times as determined during year 2010 and 2011 seasons

Variety	**Storage time (Days)**	**Storage season**	
		2010	**2011**
Asante	7	1121.77[a]	1122.44[a]
	14	1122.57[a]	1123.78[a]
	21	1123.01[a]	1123.45[a]
Desiree	7	1104.40	1111.49
	14	1106.29	1112.64
	21	1106.10	1112.21
Dutch Robijn	7	1032.01	1046.87
	14	1033.28	1049.39
	21	1042.16	1048.73
Kenya Karibu	7	1135.81[a]	1140.92[a]
	14	1136.13[a]	1141.40[a]
	21	1136.76[a]	1140.39[a]
Tigoni	7	1140.10[a]	1141.04[a]
	14	1139.01	1145.05
	21	1123.75	1160.61
LSD (0.05) (n=3)	ST	12.15	12.61
	V	9.41	9.77
	ST×V	21.05	21.84

Values are mean of three replicates. Means along each row with the same letter are not significantly different at level $p \leq 0.05$. LSD = least significant differences, ST = storage time, V= variety, ST×V= storage time and variety interaction.

The results show that after seven and twenty one days of storage in dark room and sun light conditions, the trypsin inhibitor content in tuber extracts ranged from 1039.44 to 1140.57 U/mg and from 1045.45 to 1142.18 U/mg in the vars. Dutch Robijn and Tigoni, respectively. The effect of storage time on concentration of tuber trypsin inhibitor (TI) in the tested potato varieties was no significant (p=0.831) (Appendix 5E).

The effects of storage condition and variety on TI content were statistically significant ($p<0.001$) (Table 4.5.13).

Table 4.5.13 Trypsin inhibitor content (U/mg) of potato tubers at different storage conditions as determined during year 2010 and 2011 seasons

Variety	**Storage condition**	**Storage season**	
		2010	**2011**
Asante	D	1116.15	1107.20
	FL	1128.74	1137.48
	SL	1122.46[a]	1124.97[a]
Desiree	D	1085.12[a]	1086.06[a]
	FL	1117.58	1126.06
	SL	1114.09	1124.24
Dutch Robijn	D	1023.26	1058.80
	FL	1048.44	1059.71
	SL	1035.74	1026.49
Kenya Karibu	D	1120.77	1126.51
	FL	1152.22	1158.38
	SL	1135.71[a]	1135.82[a]
Tigoni	D	1053.07	1061.09
	FL	1142.77	1156.17
	SL	1120.77[a]	1116.06[a]
LSD (0.05) (n=3)	SC	3.55	0.58
	V	4.59	0.75
	SC×V	7.45	1.30

Values are mean of three replicates. Means with the same letter along each row are not significantly different at level $p\leq0.05$. LSD = least significant differences, SC = storage condition, V= variety, SC×V= storage condition and variety interaction.

Potato tubers stored in the dark accumulated the lowest levels of mean TI that varied from 1041.03 to 1111.67 U/mg in the vars. DR and Asante, respectively. The highest mean TI was recorded under fluorescent light conditions and ranged from 1051.07 to 1155.30 U/mg in the vars. DR and Kenya Karibu, respectively. There were significant ($p<0.001$) interactions between variety and storage conditions on TI content (Figure 4.5.11).

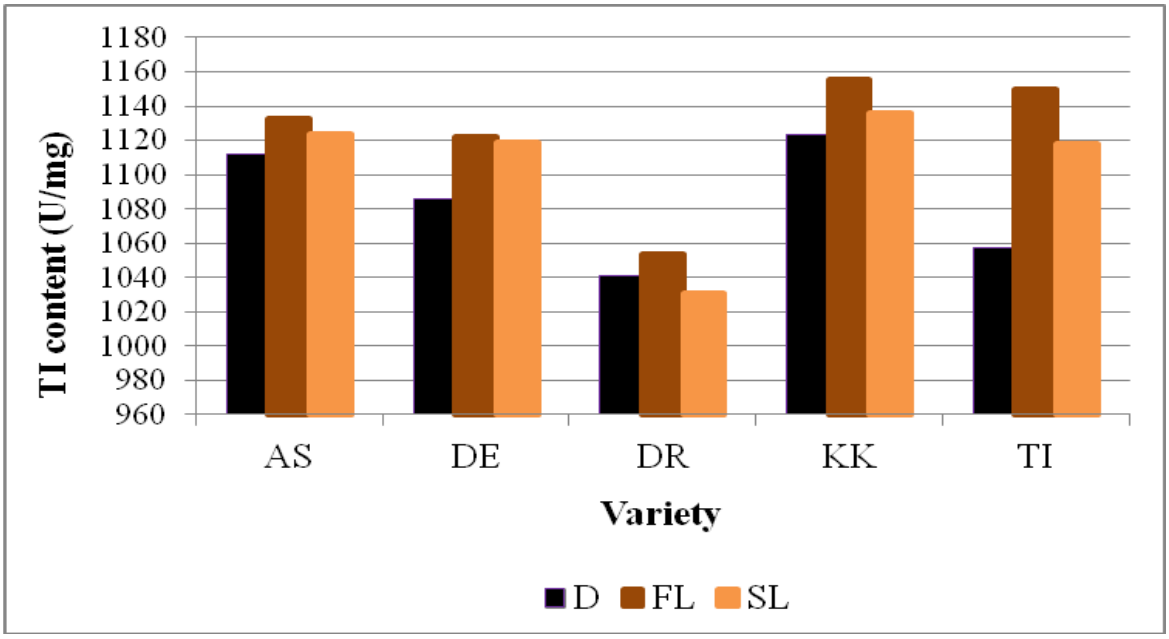


Figure 4.5.11: Influence of light and variety on trypsin inhibitor content of potato varieties: AS; Asante, DE; Desiree, DR; Desiree, KK; Kenya Karibu and TI; Tigoni. Storage conditions: SL; sunlight, FL; fluorescent light, and D; dark room.

The highest mean TI content of stored tubers was recorded under florescent light conditions and varied from 1054.07 to 1155.30 U/mg in DR and KK, respectively. The corresponding values for the tubers kept under sunlight ranged from 1031.11 to 1135.76 U/mg.

The ANOVA results indicate that the effect of storage season on the level of TI in potato tubers was significant (P=0.026) (Figure 4.5.12).

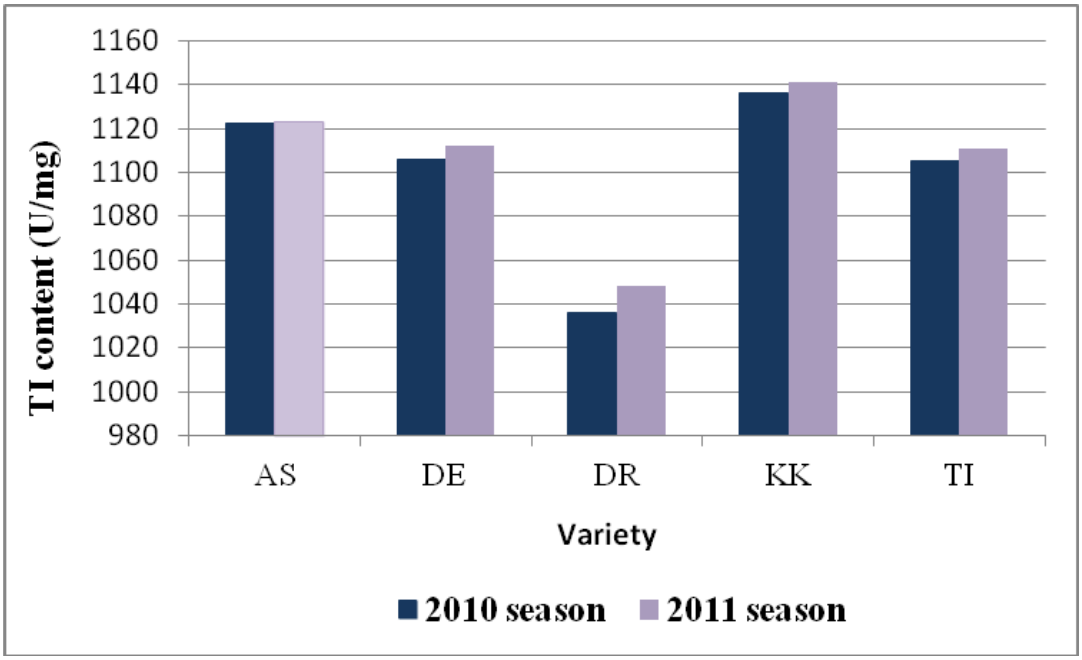


Figure 4.5.12: Seasonal variation in tuber trypsin inhibitor content of different potato varieties: AS; Asante, DE; Desiree, DR; Dutch Robijn; KK; Kenya Karibu and TI; Tigoni.

The mean tuber TI content was higher in samples collected during 2011 (1107.13 U/mg) growth period than that of 2010 (1101.10 U/mg). No significant ($p>0.05$) difference in mean TI values was observed between the two years in vars. Asante and KK. ANOVA also indicated that interaction effects of variety and storage season on tuber TI content was not significant ($p=0.74$).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Analytical techniques

The described extraction methods, HPLC and UV/Vis spectrophotometry used for glycoalkaloid analyses in this study could accurately determine the α-chaconine (α-cha), α-solanine (α-sol) and total glycoalkaloid (TGA) contents of potato tissues. The percentage recovery of phenolic acids and protease inhibitors (PIs) from potato tissues was quite high (85.9-93.5%), indicating the validity of spectrophotometry. The use of freeze-dried potato powdered samples harvested at different stages of growth provided a large surface area for rapid reconstitution and extended shelf life.

Total glycoalkaloid contents analyzed using HPLC and UV/Vis spectrophotometry and expressed in mg/100g Fwt showed that both techniques are of high accuracy. This result is in conformity with findings of Friedman (2004) who observed that these methods generally generate comparable values. The advantages of spectrophotometry over HPLC included utility of common inexpensive chemicals such as ethanol and methanol, simple equipment and its rapid nature that ensured multiple analyses within a short time. However, although spectrophotometry is a useful technique for the determination of TGA, it does not quantify individual glycoalkaloids.

The observed retention time (RT) variability in the analyses could be attributed to mechanical variation of HPLC system, mobile phase composition and ambient temperature. Similar observations were reported by K'osambo (1998) who attributed

retention shifts to heating from continuous use, residue clogs in the pump and leaks in the flow cell. In this study, shifts in retention times (RTs) were minimized by ultrafiltration of all extracts and solvents before HPLC analysis and rinsing the entire system with distilled water after daily runs. HPLC determinations of glycoalkaloid standards were not composed of single compounds and peaks and 'shoulders' that appeared in most runs could possibly be hydrolysis products. The conclusive identities of α-chaconine and α-solanine were determined based on the means of their consistent RTs in potato extracts and standards.

The presence of a large variety of flavonoids and other compounds in plant extracts interfere with peak purity when estimating the chlorogenic acid (CGA) content of potato tissues using HPLC (Nakatani *et al*., 2000; Olszewska, 2007). Olszewska (2007) observed both CGA and flavonoids in the chromatograms obtained from methanolic extracts. In this study, the use of UV spectrophotometry in the analyses of CGA, total phenolics (TP) and PIs in potato tissues did not detect any interfering substances. Thus, UV-spectrophotometry is recommended for quantitative assay for profiling important phenolic compounds in cultivated crops that are rich in flavonoids (Abugri and McElhenney, 2013; Chandra *et al*., 201;, Kale *et al*., 2010; Zhu *et al*., 2009).

5.2 Influence of variety, stage of growth and growing season on glycoalkaloid content

The levels of foliar and tuber glycoalkaloids in this study are within the limits that have been reported by other studies (Friedman, 2004; Zarzecka *et al*., 2013). The glycoalkaloid content in the major potato varieties grown in North America, Germany and UK ranged

from 2-13 mg/100g Fwt, 2-22 mg/100g Fwt and 3.6-14.2 mg/100g Fwt, respectively (Dale and Mackay, 2007). Friedman and McDonald (1997) suggested that varieties expressing elevated levels are more likely to have high rate of GA synthesis. Excessive GA production above the 20 mg per 100 g Fwt safe limit led to the removal of cvs.'Lenape', Magnum Bonum and Ulster Chieftain from the USA and Swedish markets (Hellenäs *et al*., 1995; Nahar, 2011). These findings clearly demonstrate that the GA content in potato is variety-dependent and their changes become more evident at different stages of growth under fluctuating environmental conditions that occur during the growing season. Therefore, breeders should identify suitable parents for selection of varieties with low glycoalkaloid (GA) content in addition to other desirable traits.

This study revealed a variety dependent variation in foliar total glycoalkaloids (TGA) content. The late blight (LB) tolerant vars. Tigoni and Karibu Kenya contained higher levels of foliar α-chaconine and α-solanine as compared to the relatively susceptible vars. Desiree and Dutch Robijn. This variation could be attributed to the effect of breeding programmes used for development of LB resistance. The observed high foliar total glycoalkaloid concentration (above 50 mg/100 g Fwt) in all varieties suggests a protective role of these compounds against key pests and pathogens as reported by Ventrella *et al*. (2015). This suggestion is supported by previous reports (Coombs *et al*., 2003; Andreu et al., 2001) that indicated that high foliar glycoalkaloids (GAs) contributes to host resistance mechanisms for controlling CPB and fungal pathogens. These findings imply that GAs are important for protection against key potato pests and pathogens including aphids and

Phytophthora infestans detected during the experiments and may prove ultimately useful in breeding for TGA-based resistant varieties with commercial potential.

The observed high GA level in potato foliage is in agreement with previous reports (Dinkins *et al*; 2008; Zarzecka *et al*, 2013; Żołnowski, 2001). Żołnowski (2001) found that the GA content in potato leaves tested during the growing period was about 50 times higher than the level determined in tubers. The potato alkaloid α-cha is considered to be the more toxic and present in relatively higher amount than α-sol (Friedman, 2006; Jensen, 2008). Lachman *et al*. (2001) established that the level of glycoalkaloids (GAs) in potatoes is strongly affected by light. This might explain why these compounds are normally present at elevated levels in the aerial parts of the plant.

In this study, the level of foliar GAs increased with plant growth in all varieties during both growing seasons. The observed changes in the levels of foliar GAs with maturation in potato plants followed a pattern similar to that reported by Zarzecka *et al*. (2013) who related the high level of foliar GAs to application of insecticides. During the growing period, the experimental plants were sprayed with Duduthrin 5EC at 3.3ml L^{-1} to control insect pests and a mixture of Ridomil Gold® and Dithane M 45 at 2.5 kg/ha and 1.7 kg/ha, respectively, to protect them from late blight. All chemicals were applied as foliar on potato plants every 14 days upto 80 DAP. Therefore, only leaves of the same physiological age and subjected to similar agrochemicals during growth may be compared for their GA levels.

The glycoalkaloid (GA) level in the five varieties were higher in the immature than in mature tubers. The high GA content in immature tubers may be attributed to high metabolic activity which generally decreased as the tubers mature. This observation is supported by documented field reports (Senguel *et al*., 2004; Zolnowski *et al.*, 2002; Papathanassiou *et al*., 1999; Nitithamyong *et al*., 1999). In contrast, Petersen (1993) identified three Danish potato cultivars that did not have lower glycoalkaloid (GA) content at maturity and linked the cultivar responses to growing conditions. Therefore, any environmental factor that retards maturation contributes to increased GA concentration in potato tubers.

In addition to variety and stage of growth, GA levels recorded in potato plants during the two year period of study revealed a seasonal variability. The seasonal variation of GA contents of potatoes is in agreement with reports from previous studies (Skrabule *et al* 2010; Zarzecka *et al* 2013; Hamouz *et al* 2014). In these reports, it was observed that light, temperature and rainfall cause significant variations in GA content in potato plants growing in the field. Zarzecka *et al.* (2013) determined the highest foliar GA under the coolest and humid weather and the highest tuber GA content during the wet and warm year. Hamouz *et al.* (2014) observed the highest tuber GA content during the year with high rainfall, low temperature and global radiation. Therefore, awareness of prevailing environmental conditions during the growing season may be important when screening potato varieties for GA content and those with low GA content such as Dutch Robijn and Desiree may be promoted for adoption and production in warmer areas of Kenya.

The distribution of glycoalkaloids in this work implies that they are not likely to pose any public health and safety concern. All the tested varieties had tuber glycoalkaloid contents that were below the upper safety limit of 20 mg/100 g Fwt, and may be used for the development of new and improved commercial potato varieties with beneficial agronomic features including low glycoalkaloids.

5.3 Influence of variety, stage of growth and growing season on phenolic acid content

The phenolic acids are among the diverse phytochemicals that occur in potato plants, which contribute to variation in their total phenolic content. The differences in phenolic content observed in the present study suggest that potato varieties have different phenolic profiles. The observed variation of TP and CGA among potato varieties used in this study is within the acceptable limits reported in previous investigations (Im *et al*., 2008; Reyes *et al*., 2005). Based on these two reports, the TP and CGA contents ranged from 1.0-181 mg CGA/100g Fwt and 3.0-90 mg/100g Fwt, respectively in different potato genotypes. Purple- fleshed potatoes generally contain higher levels of phenolic compounds including CGA and anthocyanins. The results from the present study demonstrate that TP and CGA contents in potatoes are variety-dependent and indicate the presence of a large diversity of phenolic compounds.

The five selected potato varieties showed higher phenolic content in the leaves as compared with their corresponding tubers. This distribution suggests the functional diversity of phenolics in plant development. These observations are consistent with results of Griffiths *et al*. (1995) who reported that leaves of potato plants growing in the field are

exposed to light which stimulates the biosynthesis of CGA, an important polyphenol with antioxidant and antibacterial properties, and intermediate in lignin biosynthesis.

Phenolics contents in potato plants varied depending on their stage of growth. The observed reduction in foliar and tuber phenolics during growth is consistent with previous published reports that indicate that potato phenolics decreased with maturity (Reyes *et al*., 2004; Marinova *et al*., 2005). The observed changes suggest that variety and stage of growth are the most important variables when evaluating potato phenolic profiles.

Total phenolics and CGA content in the potato plants were also influenced by the growing season. This can be supported by the observed low TP levels in potato plants that were harvested in 2011 and the high tuber chlorogenic acid (CGA) contents during the same growing season. This can be attributed to high rainfall and diurnal temperature fluctuations during the year. These observations clearly demonstrate that tissues of potato plants respond differently to prevailing environmental conditions by synthesizing phenolic acids and other compounds. Although, the results obtained do not provide any direct evidence, it is possible that the higher response indicated by higher TP and a drop in CGA contents in leaf tissues could be ascribed to synthesis of other specific phenolic compounds.

The experimental evidence presented in this work has shown a great variability in TP and CGA contents in the potato varieties. The vars. Tigoni and Kenya Karibu (KK) have great potentials as a source of antioxidants. The var. Asante exhibited higher levels of TP and a lower level of CGA. Therefore, it is possible to develop potato varieties of good quality for

human consumption by selecting parents expressing high levels of TP and low level of CGA. Given the importance of phenolics, the high antioxidant varieties, should be adopted by farming communities to enhance potato quality.

The results from the present study confirms that environmental conditions such as temperature and rainfall determine to a great extent the total phenolic content and the potato varieties that may be cultivated. Previous studies on potatoes have shown that phenolic compounds are influenced by various factors including temperature, day length, drought and flooding (Islam *et al*., 2003; Reyes *et al*., 2004; Lin *et al*., 2006). According to Reyes *et al*. (2005) harvest locations with varied environmental conditions could enhance the synthesis of different types and quantities of phenolic compounds in plants. Total phenolics (TP), anthocyanins and flavonoids are closely associated with strong antioxidant activity (Lachman *et al*., 2008; Leo *et al*., 2008; Reyes *et al*., 2005). Therefore, adoption within Kenyan farming communities of potato vars. Tigoni, Asante and Kenya Karibu with enhanced phenolic content could increase the antioxidants in the diet.

.5.4 Influence of variety, stage of growth and growing season on protease inhibitor content

The present study revealed significant differences in protease inhibitor content among the potato varieties tested. The observed high levels of chymotrypsin inhibitors in the leaves and tubers of var. Desiree and the high trypsin inhibitor content in leaves and tubers of vars. Tigoni and Asante, respectively, suggest a differential expression of the two protease inhibitors (PIs). The tissue specific expression patterns of protease inhibitors were strongly

dependent on variety. The influence of variety on protease inhibitor contents has been reported in previous studies (Bauw *et al*., 2006; Feldman *et al*., 2000, Kim *et al*., 2006). The presence of PIs in potatoes is related to high level of field resistance and increased protein nutritional quality (Dijkistra *et al*., 2003; Weeda *et al*., 2009). For potato production, the enhanced resistance to insect pests could lead to improved yield and nutritional quality of the harvested tubers.

This study has shown that the accumulation of foliar PIs during plant establishment increased with growth and followed a sigmoid pattern. Early in development at 40 days after planting (DAP), leaves accumulated low levels of PIs. The concentration of foliar PIs then increased rapidly to a maximum at 55 DAP when the plant systems were fully developed. The slight decline in PI levels towards full maturity coincided with the decreased metabolic activity during this growth phase. A similar observation reported by Etienne *et al*. (2007) demonstrates that PIs function in nitrogen remobilization during leaf development. These findings therefore indicate that proteases dominate early stages of vegetative growth to allow for the synthesis and translocation of proteins to the different parts involved in plant growth and development.

The protease inhibitor (PI) values obtained in developing tubers as a function of maturation displayed some similarities with the leaves at the initial stages but the different accumulation patterns observed for trypsin inhibitor (TI) and chymotrypsin inhibitor (CI) suggest that these might be distinct proteins. The increase in TI content was most rapid between 55 and 95 days after planting (DAP) suggesting high TI specific activities at this

stage. The concentration of CI increased steadily during tuber bulking and attained a maximum level that averaged 289.15 U/mg at full maturity. The average low PI level of 269.64 U/mg at 55 DAP as compared to other stages of growth suggests that the early stages of tuberization are dominated by proteases. The increase in the levels of PI between 55 and 125 DAP during tuber bulking indicates that attenuation of protease activity could be necessary to facilitate accumulation of food reserves. Similar findings have been reported by several authors (Hartl *et al*., 2011; Lehesranta *et al*., 2006; Weeda *et al*., 2011). Weeda *et al*. (2009) revealed that proteins of fully developed potato tubers are dominated by patatin and protease inhibitors (PIs). Hartl *et al*. (2011) have attributed the functional diversity of serine PIs in *Solanum nigrum* to evolutionary dynamics. Hence, the occurrence of stage and tissue specific expression of PIs may be optimized to devise a sustainable strategy for enhancing potato resistance to pests.

This study has demonstrated different patterns of seasonal variation of PI in potato tissues. The accumulation of high foliar CI during the 2010 growing season may be attributed to low rainfall amounts over the entire period of plant establishment. High rainfall and temperature fluctuations during tuberization may be responsible for the high tuber CI levels in potato plants that were harvested during the 2011 growing season. The observed different accumulation patterns of protease inhibitors (PIs) in plants grown at different growing periods could be attributed to varied responses to changing environmental conditions. This is consistent with previous published reports that show that many PIs are produced in response to stresses including pathogens, insects, wounding, salt, heat and water (Benedict, 2003; Fan and Wu, 2005; Haq *et al*., 2004). According to Dwelle and

Love (2014), any interruption of optimal growth can result in reduced tuber yield and quality. Therefore, alleviating of stressful conditions caused by water and temperature during growth could ensure improved tuber yield and quality.

The application of PIs in potato pest management requires a better understanding of how insects respond and adapt to PIs. In this study, aphids, which limits potato production in Kenya, were detected early during the experiments and were controlled exclusively with Duduthrin. Although, the metabolic adaptation of aphids to PIs is beyond the scope of this research, it is important to better understand the interaction of aphids with potato PIs. Some insects such as *Spodoptera littoralis* (Lepidopteran) and Colorado potato beetle (CPB) can overcome the deleterious effects of PIs by synthesizing different PI-insensitive proteases (Jaber *et al*., 2010; Dunse *et al*., 2010) and have emerged as a major threat to global agriculture. These findings suggest that a better understanding of the multiple physiological functions of potato protease inhibitors may open possibilities for their utilization in protection of potato plants against pests and diseases.

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5.5 Influence of storage conditions on the level of total glycoalkaloids, phenolic acids and protease inhibitors

The present study has shown that controlled light conditions helps in maintaining the quality and enhancing shelf life of the potato tubers in storage facilities. This can be explained by the fact that biochemical activities of potato tubers are closely connected with the physiological status and with the post-harvest environmental conditions such as temperature and relative humidity (Weeda *et al*., 2011). The storage conditions influence

the post-harvest biosynthesis of glycoalkaloids, phenolic acids and protease inhibitors (PIs) and affect the quality and overall acceptability of tubers at the end of storage.

Storage duration play a critical role in determining the magnitude of phytochemical accumulation in potato tubers. Tubers that were stored for 21 days under fluorescent light (FL, ~1500 lux) accumulated the highest levels of glycoalkaloids, phenolic acids and protease inhibitors than tubers that were subjected to 7 and 14 day storage treatments. The observed changes in the levels of glycoalkaloids and phenolic compounds in tubers stored for 21 days were significant (1% level), whereas the concentration of protease inhibitors did not change significantly over the same period. These observations indicate the importance of the duration of storage of tubers when profiling phytochemicals.

The exposure of tubers to FL induced the synthesis of GAs to a higher level as compared to direct sunlight (SL, ~100,000 lux). Eltayeb *et al*. (2003) attributed the high glycoalkaloid concentration for FL to less than 300 nm UV light considered being active elicitor of glycoalkaloid synthesis. Consequently, var. Tigoni accumulated potentially toxic TGA concentration (23.02 mg/100g Fwt) after exposure to FL for 21 days. Tubers exposed to SL also accumulated more glycoalkaloids compared to tuber samples that were stored in the dark room for the same duration. These light induced changes in TGA content are in harmony with studies by Grunnenfelder *et al*. (2006) who observed variable light-induced TGA levels in fresh market potatoes and suggested that levels below 20 mg/100 g Fwt are not of health concern. Hossain and Rai (2014) observed that the accumulation of glycoalkaloids (GAs) peaked at 30 days of storage.

The present study shows that the 21 day storage under FL and sunlight (SL) conditions increased the total phenolic (TP) and chlorogenic (CGA) contents to higher levels as compared with the potato tubers stored in the dark. Phenolic content of tubers varied significantly according to variety, storage time and light in a pattern similar to that of TGA. According to Dicko *et al*. (2006), phenolic compounds and related enzymes are quality grade markers for several plant derived food products.

Current investigations established that potato tuber TP content was significantly ($p<0.001$) influenced by storage time and storage condition. The TP contents increased by an average of between 3-47% during the three weeks of storage and the highest mean content of 170.6 mg CGA/g was observed in tubers exposed to fluorescent light. The possible reason for the lower average total phenolic (TP) value of 162.7 mg CGA/g for tubers exposed to sunlight may be attributed to destruction of some phenolic components. Similar observations were made by Laleh *et al*. (2006) who reported that light affects TP contents by accelerating the destruction of anthocyanins. In general, the rate of TP accumulation was greatest after 14-21 days of storage. The highest TP content was determined in var. Tigoni followed by var. Kenya Karibu and the lowest content was found in var. Dutch Robijn with mean values of 164.3, 160.4 and 153 mg CGA/g, respectively. This observation is in general agreement with recent studies that have shown that total phenolic (TP) content is a genotype-dependent parameter that is significantly influenced by storage conditions (Blessington *et al*., 2010; Murniece *et al*., 2013). Grace *et al*. (2014) also observed a decline in TP content in a sweet potato variety that was stored for 8 months. According to Dale *et al*. (2003), storage would be the major effect impacting on contents of antioxidants. Therefore, results

of the present study indicate that storage of tubers in a dark room for 14 days could enhance the health beneficial components of ware potatoes.

The 21-day storage period significantly ($p<0.001$) increased the accumulation of chlorogenic acid (CGA) in tubers exposed to light with higher values that ranged from 53.4-55.0 mg/100g compared to the controls stored in the dark that had a mean 44.3 mg/100g. The CGA contents in controls after 21 days was apparently higher as compared with those stored for one week. The rates of accumulation of chlorogenic acid (CGA) in stored tubers was cultivar–dependent with the highest and lowest contents determined in vars. Tigoni and Desiree, respectively. The variations in light-induced CGA contents in potato varieties may indicate the presence of different phenolic synthesizing enzymes. This is consistent with previous studies in which, the magnitude of light-induced CGA synthesis was found to be cultivar-dependent. Chlorogenic acid content increased more than twice in the light-stored potatoes (Dao and Friedman, 1994). Griffiths and Bain (1997) demonstrated that the rapid increase in total CGA content during light exposure was primarily due to an increase in 5-caffeoyl quinic acid. Therefore, storage of tubers in a dark for 7-14 days may minimize CGA content and after cooking blackening in processed potato products. The present findings suggest that the potato tubers are rich in phenolic compounds and light is a key factor to consider when designing storage facilities necessary to preserve useful antioxidants.

The analysis of protease inhibitors (PIs) established no significant changes in the levels of chymotrypsin inhibitor (CI) and trypsin inhibitor (TI) in potato tubers stored for 21 days.

Statistically significant ($p<0.001$) effect of variety on tuber protease inhibitor (PI) contents was observed. Insignificant differences in PI content ($p>0.05$) were found in analyzed samples within the same period of storage. Potato tubers stored under fluorescent light accumulated higher PI levels as compared to tuber samples that were kept in the dark and sunlight conditions. Potato tubers stored in the dark accumulated the lowest levels of protease inhibitors. According to the results obtained, storage condition significantly ($p<0.001$) affected the tuber TI content but had insignificant ($p=0.154$) effect on the concentration of CI. This observation suggests that light plays an important role in regulating tuber trypsin inhibitor (TI) content after harvest and the low protease inhibitor capacity in the dark can preserve the nutritional value of ware potatoes.

The results of protease inhibitor content reported in this study are in agreement with data published by Dao and Friedman (1994) which was attributed to pre-sprouting activities. According to Weeda *et al*. (2011), PIs play a major role in maintaining tuber integrity and regulating protein content during prolonged storage. The authors suggested that the inactivation of PIs at the start of sprouting may be crucial for the protease-mediated degradation of storage proteins for mobilization of nitrogen. Thus, by modulating the activities of proteases, PIs may have a significant role in regulating the protein content and nutritive value of potato tuber.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

(1) The concentration of glycoalkaloids (GAs), phenolic acids (PAs) and protease inhibitors (PIs) in potato plants were variety-dependent. The var. Tigoni recorded the highest levels of GAs compared to all the other test varieties. The highest foliar and tuber TP contents were determined in vars. Asante and Tigoni, respectively. All potato varieties accumulated higher levels of GAs, PAs and PIs in their leaves as compared to tubers.

(2) Foliar GAs increased during growth in all varieties while tuber GAs decreased. All commercial potato varieties evaluated had GA contents that were lower than the upper safety limit of 20mg/100g Fwt.

(3) Total phenolics (TP) and CGA contents in potato leaves and tubers generally decreased during growth.

(4) The concentration of PIs in the leaves and tubers increased with growth and the different accumulation patterns for TI and CI revealed that these are distinct proteins. The highest levels of TI in leaves and tubers were recorded in vars. Tigoni and Asante, respectively.

(5) The changes in levels of GAs, phenolics and PIs of field grown potato plants are dynamic and appear to be interdependent.

(6) The duration and conditions of storage significantly increased the levels of GAs and PAs in potato tubers. The concentration of CGA in stored tubers was highest

and lowest in vars. Tigoni and Desiree, respectively. No significant changes in the levels of PIs in stored potato tubers were observed.

(7) Exposure to light increased the biosynthesis of total glycoalkaloids (TGA) and phenolic acids in potato tubers during the 21 day storage period. The exposure of tubers to fluorescent light induced the synthesis of GAs and PAs to a higher level as compared with potato tubers stored under sunlight or dark room. Fluorescent light stimulated the synthesis of GAs to potentially toxic levels (above 20mg/100g Fwt) in var. Tigoni. Therefore, light is an important factor to be closely monitored in stores, supermarkets and other retail outlets to prevent accumulation of TGA to toxic levels.

6.2 Recommendations

The present research investigation warrants making the following recommendations:

(1) Potato breeders should evaluate the changes in levels of GAs, phenolic acids and PIs in all varieties used within a breeding program.

(2) Glycoalkaloid determination should be used for routine control of new varieties to ensure that total glycoalkaloid content remains below the recommended food safety limit (20mg/100g Fwt).

(3) Given the importance of phenolics, breeders while selecting novel high antioxidant varieties, should maintain other traits such as low glycoalkaloids to enhance the safety of the potato.

(4) The observed variations of potato phenolics at different stages of growth should guide in designing appropriate sampling procedures.

(5) The characterization of specific potato phenolic compounds including gallic, caffeic, protocatechuic and *p*-coumaric acid might be useful in understanding their metabolism in plants.

(6) Potato displays during marketing should be located in sections with low light intensity for short durations. Vars. Tigoni and Kenya Karibu with capacity to accumulate high TGA content should be subjected to shorter duration of light exposure during storage.

(7) A better understanding of the coordinated changes and overlapping functions of potato phytochemicals during development in the field and storage are relevant for designing proper handling practices and conditions necessary to preserve useful antioxidants and proteins while minimizing the levels of glycoalkaloids (GAs) and chlorogenic acid (CGA).

(8) The present study provides valuable information which may facilitate postharvest retention of protein. This information is important to breeders and researchers and may prove ultimately useful in selection of novel varieties with high yields, quality, nutritional and commercial value.

6.3 Future research needs

To facilitate further progress in enhancing the safety and nutritional quality of the potato, breeders/ researchers are also challenged to undertake analytical research in the priority areas outlined below:

(1) Research scientists should develop simplified and improved HPLC methods for phenolic acids and PIs.

(2) Characterize the GAs, phenolic acids and protease inhibitor contents of other commercial varieties grown in Kenya.

(3) Recommend adoption and use of varieties with low pre- and post-harvest glycoalkaloid production.

(4) Determine the distribution of important phenolic compounds (chlorogenic acid (CGA), caffeic acid and ferullic acid) in commercial potatoes in relations to their antioxidant activities.

(5) Additional knowledge on the mechanism of CGA accumulation in potato tubers is important in minimizing after-cooking blackening (ACB) in processed products.

(6) Further research is needed to establish the tissue specific induction, developmentally-linked expression, exact subcellular location and physiological functions of PIs in cultivated potato plants.

(7) Since most of the PIs are effective inhibitors of mammalian proteases, the question of whether raw potato tubers might also be harmful to humans as to the pests has to be addressed.

(8) Develop a mathematical index that may be used to predict the relative beneficial effects of new potato varieties based on their glycoalkaloids, phenolic acids and protease inhibitors.

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APPENDICES

Appendix 1A. Comparison of retention time of α-chaconine (α-cha) standard and GA peak G1

	α-cha (1)	G1 (2)
Mean RT(min)	4.545	4.543
Variance	0.00009737	0.00007395
SD	0.009868	0.008599
F-test for the hypothesis ‘‘σ1 = σ2’’		
F-value	1.32	
Numerator df	19	
Denominator df	19	
Probability	0.55	
Result: F-value close to 1, p > α, accept null hypothesis of equal variances		
T-test for the hypothesis ‘‘μ1 = μ2’’		
t’ value	0.51	
Effective df	38	
Probability	0.611	
Result: Not significant t, p > α, accept the null hypothesis that the two means are equal		

σ1 and σ2-variances 1 and 2, α- alpha (0.05), μ1 and μ2- means 1 and 2.

Appendix 1B. Comparison of retention time of α-solanine (α-sol) standard and GA peak G2

	α-sol (1)	G2 (2)
Mean RT(min)	6.051	6.048
Variance	0.0003292	0.0002379
SD	0.01814	0.01542
F-test for the hypothesis ‘‘σ1 = σ 2’’		
F-value	1.38	
Numerator df	19	
Denominator df	19	
Probability	0.49	
Result: F-value close to 1, p > α, accept null hypothesis of equal variances		
T-test for the hypothesis ‘‘μ1 = μ2’’		
t’ value	0.66	
Effective df	38	
Probability	0.515	
Result: Not significant t, p > α, accept the null hypothesis that the two means are equal		

σ1 and σ2-variances 1 and 2, α- alpha (0.05), μ1 and μ2- means 1 and 2.

Appendix 2A. ANOVA table on the effect of method of analysis on potato TGA content

TGA	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf TGA	Method (M)	1	5396.9	5396.9**	52.35	<.001
	Variety (V)	4	2078.7	519.7*	5.04	0.001
	M × V	8	2396.6	599.2**	5.81	<.001
	Residual	80	8247.2	103.1		
	Cv % - 14.7			SE – 10.5		
Tuber TGA	Method (M)	1	0.0896	0.0896^{ns}	0.10	0.758
	Variety (V)	4	116.7478	29.1970**	31.26	<.001
	M × V	4	0.1035	0.0259^{ns}	0.03	0.998
	Residual	80	74.7055	0.9338		
	Cv % - 11.0			SE – 0.966		

Appendix 2B. ANOVA table showing significance of variety, stage of growth and interaction effects for foliar TGA, α-cha and α-sol content

Source of variation	df	Mean squares			
		TGA (UV)	TGA(HPLC)	α-cha	α-sol
Stage of growth (SG)	2	6534.57**	1376.7772**	436.77663**	263.19857**
Variety (V)	4	1907.97**	1024.2136**	195.18792**	368.51796**
SG × V	8	304.30 **	14.7180**	26.69865**	18.00422**
Residual	75	74.28	0.1390	0.09620	0.03119
CV%		15.3	5.0	7.0	2.0
SE		8.62	0.3729	0.3102	0.1766

Appendix 2C. ANOVA table showing significance of variety, growing season and interaction effects for potato TGA content

TGA	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf TGA	Growing season (GS)	1	2689.4	2689.4**	12.24	<.001
	Variety (V)	4	7631.9	1908.0**	8.68	<.001
	GS × V	4	800.8	200.2^{ns}	0.91	0.462
	Residual	80	17584.7	219.8		
	Cv % - 26.3			SE – 14.83		
Tuber TGA	Growing season (GS)	1	125.024	125.024**	57.95	<.001
	Variety (V)	4	112.766	28.191**	13.07	<.001
	GS × V	4	183.976	45.994**	21.32	<.001
	Residual	80	172.593	2.157		
	Cv % - 16.7			SE – 1.47		

Appendix 2D: ANOVA table showing significance of variety, stage of growth and interaction effects for tuber TGA, α-cha and α-sol content

		Mean squares			
Source of variation	df	TGA (UV)	TGA(HPLC)	α-cha	α-sol
Stage of growth (SG)	2	22.794ns	15.117094**	3.697749**	2.102176**
Variety (V)	4	28.191*	11.363902**	2.736242**	5.383892**
SG × V	8	1.353ns	0.742908**	0.570982**	0.280776**
Residual	75	5.669	0.004009	0.001767	0.001002
Cv %		17.1	7	8	8
SE		2.38	0.6332	0.4203	0.3166

Appendix 3A. ANOVA table showing significance of variety, stage of growth and interaction effects for potato chlorogenic acid (CGA) content

CGA	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf CGA	Stage of growth (SG)	2	541.77	270.88*	4.43	0.021
	Variety (V)	4	1948.76	884.63**	14.46	<.001
	SG × V	8	788.03	98.50^{ns}	1.61	0.164
	Residual	30	1834.72	61.16		
	Cv % - 11.8			SE – 5.84		
Tuber CGA	Stage of growth (SG)	2	1645.78	822.89**	73.55	<.001
	Variety (V)	4	313.63	78.41**	7.01	<.001
	SG × V	8	128.77	16.10^{ns}	1.44	0.221
	Residual	30	335.63	11.19		
	Cv % - 9.3			SE – 4.97		

Appendix 3B. ANOVA table showing significance of variety, growing season and interaction effects for potato CGA content

CGA	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf CGA	Growing season (GS)	1	23.25	23.25^{ns}	0.63	0.428
	Variety (V)	4	418.93	104.73*	2.85	0.029
	GS × V	4	1136.56	284.14**	7.74	<.001
	Residual	80	2935.34	36.69		
	Cv % - 12.2			SE – 6.06		
Tuber CGA	Growing season (GS)	1	249.94	249.94*	7.54	0.007
	Variety (V)	4	1948.76	487.19**	14.69	<.001
	GS × V	4	741.12	185.28**	5.59	<.001
	Residual	80	2653.13	33.16		
	Cv % - 10.8			SE – 5.76		

Appendix 3C. ANOVA table showing significance of variety, stage of growth and interaction effects for potato total phenolic (TP) content

TP	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf TP	Stage of growth (SG)	2	713308.	356654.**	26.74	<.001
	Variety (V)	4	294526.	73632.**	5.52	<.001
	SG × V	8	66307.	8288.^{ns}	0.62	0.757
	Residual	75	1000443.	13339.		
	Cv % - 23.0			SE – 115.50		
Tuber TP	Stage of growth (SG)	2	26203.1	13101.5**	25.53	<.001
	Variety (V)	4	75189.6	18797.4**	36.63	<.001
	SG × V	8	25389.6	3173.7**	6.18	<.001
	Residual	75	38485.2	513.1		
	Cv % - 14.7			SE – 22.65		

Appendix 3D. ANOVA table showing significance of variety, growing season and interaction effects for potato TP content

TP	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf TP	Growing season (GS)	1	104235.	104235.*	5.54	0.021
	Variety (V)	4	294526.	73632.*	3.91	0.006
	GS × V	4	170706.	42677.^{ns}	2.27	0.069
	Residual	80	1505117.	18814.		
	Cv % - 27.3			SE – 137.16		
Tuber TP	Growing season (GS)	1	7860.5	7860.5*	9.11	0.003
	Variety (V)	4	75189.6	18797.4**	21.78	<.001
	GS × V	4	13164.0	3291.0*	3.81	0.007
	Residual	80	69053.3	863.2		
	Cv % - 19.0			SE – 29.38		

Appendix 4A. ANOVA table showing significance of variety, stage of growth and interaction effects for potato chymotrypsin inhibitor (CI) content

CI	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf CI	Stage of growth (SG)	2	826429.	413214.**	24.22	<.001
	Variety (V)	4	184395.	46099.*	2.70	0.037
	SG × V	8	223550.	27944.^{ns}	1.64	0.128
	Residual	75	1279694.	17063.		
	Cv % - 15.7			SE – 130.62		
Tuber CI	Stage of growth (SG)	2	5721.8	2860.9**	7.94	<.001
	Variety (V)	4	11745.2	2936.3**	8.15	<.001
	SG × V	8	4968.2	621.0^{ns}	1.72	0.106
	Residual	75	27006.6	360.1		
	Cv % - 6.8			SE – 18.98		

Appendix 4B. ANOVA table showing significance of variety, growing season and interaction effects for potato CI content

CI	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf CI	Growing season (GS)	1	122053.	122053.*	4.57	0.036
	Variety (V)	4	184395.	46099.^{ns}	1.73	0.152
	GS × V	4	71731.	17933.^{ns}	0.67	0.614
	Residual	80	2135889.	26699.		
	Cv % - 19.6			SE – 163.40		
Tuber CI	Growing season (GS)	1	430.3	430.3^{ns}	1.02	0.315
	Variety (V)	4	11745.2	2936.3**	6.97	<.001
	GS × V	4	3541.5	885.4^{ns}	2.10	0.088
	Residual	80	33724.7	421.6		
	Cv % - 7.3			SE – 20.53		

Appendix 4C. ANOVA table showing significance of variety, stage of growth and interaction effects for potato trypsin inhibitor (TI) content

TI	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf TI	Stage of growth (SG)	2	2076357.	1038178.**	152.58	<.001
	Variety (V)	4	513974.	128493.**	18.88	<.001
	SG × V	8	94674.	11834.^{ns}	1.74	0.103
	Residual	75	510307.	6804.		
	Cv % - 5.2			SE – 82.49		
Tuber TI	Stage of growth (SG)	2	885956.	442978.**	54.47	<.001
	Variety (V)	4	66111.	16528.^{ns}	2.03	0.098
	SG × V	8	18187.	2273.^{ns}	0.28	0.971
	Residual	75	609930.	8132.		
	Cv % - 10.0			SE – 90.18		

Appendix 4D. ANOVA table showing significance of variety, growing season and interaction effects for potato TI content

TI	Source of variation	df	ss	ms	v.r.	Fpr.
Leaf TI	Growing season (GS)	1	21217.	21217.^{ns}	0.65	0.423
	Variety (V)	4	513974.	128493.*	3.93	0.006
	GS × V	4	42952.	10738.^{ns}	0.33	0.858
	Residual	80	2617169.	32715.		
	Cv % - 11.5			SE – 180.87		
Tuber TI	Growing season (GS)	1	592650.	592650.**	51.46	<.001
	Variety (V)	4	66111.	16528.^{ns}	1.44	0.230
	GS × V	4	26.	6.0^{ns}	0.00	1.000
	Residual	80	921397.	11517.		
	Cv % - 11.9			SE – 107.32		

Appendix 5A. ANOVA table showing significance of variety, storage time, storage condition, season and interaction effects for tuber TGA content

Storage parameters	Source of variation	df	ss	ms	v.r.	Fpr.
Storage time (ST)	ST	2	676.08	338.04**	17.92	<.001
	Variety (V)	4	5746.32	1436.58**	76.16	<.001
	ST × V	8	183.80	22.97^{ns}	1.22	0.289
	Residual	255	4810.19	18.86		
	Cv % - 14.3			SE – 4.34		
Storage condition (SC)	SC	2	1220.29	610.14**	49.55	<.001
	Variety (V)	4	5746.32	1436.58**	116.67	<.001
	SC × V	8	1309.88	163.73**	13.30	<.001
	Residual	255	3139.90	12.31		
	Cv % - 13.4			SE – 3.51		
Storage season (SS)	SS	1	430.82	430.82**	21.83	<.001
	Variety (V)	4	5746.32	1436.58**	72.81	<.001
	SS × V	4	109.12	27.28^{ns}	1.38	0.240
	Residual	260	5130.11	19.73		
	Cv % - 12.4			SE – 4.44		

Appendix 5B. ANOVA table showing significance of variety, storage time, storage condition, season and interaction effects for tuber CGA content

Storage parameters	Source of variation	df	ss	ms	v.r.	Fpr.
Storage time (ST)	ST	2	11499.64	5749.82**	111.39	<.001
	Variety (V)	4	5228.81	1307.20**	25.32	<.001
	ST × V	8	332.30	41.54^{ns}	0.80	0.599
	Residual	255	13162.83	51.62		
	Cv % - 14.1			SE – 7.18		
Storage condition (SC)	SC	2	6010.57	3005.28**	43.55	<.001
	Variety (V)	4	5228.81	1307.20**	18.94	<.001
	SC × V	8	1388.34	173.54*	2.51	0.012
	Residual	255	17595.86	69.00		
	Cv % - 16.3			SE – 8.31		
Storage season (SS)	SS	1	518.10	518.10*	5.53	0.019
	Variety (V)	4	5228.81	1307.20**	13.94	<.001
	SS × V	4	98.50	24.62^{ns}	0.26	0.902
	Residual	260	24378.17	93.76		
	Cv % - 19.0			SE – 9.68		

Appendix 5C. ANOVA table showing significance of variety, storage time, storage condition, season and interaction effects for tuber TP content

Storage parameters	Source of variation	df	ss	ms	v.r.	Fpr.
Storage time (ST)	ST	2	44766.	22383. **	19.86	<.001
	Variety (V)	4	2515.	629. ^{ns}	0.56	0.693
	ST × V	8	34389.	4299. **	3.81	<.001
	Residual	255	287449.	1127.		
	Cv % - 21.0			SE – 33.57		
Storage condition (SC)	SC	2	29390.	14695. **	11.89	<.001
	Variety (V)	4	2515.	629. ^{ns}	0.51	0.729
	SC × V	8	21984.	2748. *	2.22	0.026
	Residual	255	315230.	1236.		
	Cv % - 22.0			SE – 35.16		
Storage season (SS)	SS	1	13026.	13026. *	9.67	0.002
	Variety (V)	4	2515.	629. ^{ns}	0.47	0.760
	SS × V	4	3177	794. ^{ns}	0.59	0.671
	Residual	260	350400.	1348.		
	Cv % - 23.0			SE – 36.71		

Appendix 5D. ANOVA table showing significance of variety, storage time, storage condition, season and interaction effects for tuber CI content

Storage parameters	Source of variation	df	ss	ms	v.r.	Fpr.
Storage time (ST)	ST	2	43.75	21.88 ^{ns}	2.46	0.087
	Variety (V)	4	11432.24	2858.06**	321.61	<.001
	ST × V	8	12.317	1.540 ^{ns}	0.17	0.994
	Residual	255	2266.107	8.887		
	Cv % - 1.1			SE – 2.98		
Storage condition (SC)	SC	2	32.423	16.211 ^{ns}	1.88	0.154
	Variety (V)	4	11432.234	2858.059**	331.91	<.001
	SC × V	8	93.989	11.749 ^{ns}	1.36	0.213
	Residual	255	2195.763	8.611		
	Cv % - 1.0			SE – 2.93		
Storage season (SS)	SS	1	1201.582	1201.582**	576.26	<.001
	Variety (V)	4	11432.236	2858.059**	1370.67	<.001
	SS × V	4	578.454	144.613**	69.35	<.001
	Residual	260	542.138	2.085		
	Cv % - 0.5			SE – 1.44		

Appendix 5E. ANOVA table showing significance of variety, storage time, storage condition, season and interaction effects for tuber TI content

Storage parameters	Source of variation	df	ss	ms	v.r.	Fpr.
Storage time (ST)	ST	2	189.9	94.9	0.19	0.831
	Variety (V)	4	293008.0	73252.0**	143.20	<.001
	ST × V	8	217.3	27.2 ^{ns}	0.05	1.000
	Residual	255	130438.4	511.5		
	Cv % - 2.0			SE – 22.62		
Storage condition (SC)	SC	2	24952.43	12476.21**	170.84	<.001
	Variety (V)	4	293007.96	73251.99**	1003.07	<.001
	SC × V	8	87270.94	10908.87**	149.38	<.001
	Residual	255	18622.16	73.03		
	Cv % - 0.8			SE – 8.55		
Storage season (SS)	SS	1	2454.8	2454.8*	5.01	0.026
	Variety (V)	4	293008.0	73252.0**	149.47	<.001
	SS × V	4	971.3	242.8 ^{ns}	0.50	0.739
	Residual	260	127419.5	490.1		
	Cv % - 2.0			SE – 22.14		