GLYCOALKALOID CONTENT OF SOME POTATO (*Solanum tuberosum* L.) VARIETIES GROWN IN KENYA AS INFLUENCED BY LIGHT AND DIFFERENT STORAGE CONDITIONS

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DECLARATION

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

21/10/2002

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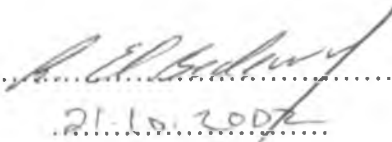

21.10.2002

Dr. Ramzy El-Bedewy

(International Potato Centre)

DEDICATION

To

My Parents, Richard and Alice

My Brothers and Sisters

Friends

"... the wonderful and secret operations of Nature are so intricate ... that it is impossible ... to pry into them, unless ... by numerous and regular series of experiments; which are the only solid foundation whence we may reasonably expect to make any advance in the real knowledge of the nature of things."

-S. Hales, 1727.

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ABBREVIATIONS

μg	micro gram
μl	micro litre
$(NH_4)_2PO_4$	Diammonium phosphate
AChe	Acetyl cholinesterase
Bwt	Body weight
CIP	International Potato Centre
cm/min	Centimetre per minute
CN	Cyanide
CV	Coefficient of variation
ELISA	Enzyme linked Immunosorbent assay
ER	Endoplasmic reticulum
FPP	Farnesyl pyrophosphate
Fwt	Fresh weight
g	grams
GC	Gas chromatography
GGPP	Geranyl geranyl pyrophosphate
GPP	Geranyl pyrophosphate
H_2SO_4	Sulphuric acid
HCL	Hydrochloric acid
HPLC	High performance liquid chromatography
HPLC-PAD	HPLC pulsed amperometric detection
ILRI	International Livestock Research Institute
IP	intraperitoneal injection
IPP	Isopentenyl pyrophosphate
IR	Infrared
IV	intravenous injection
K	Potassium
K^+	Potassium ion
KARI	Kenya Agricultural Research Institute
KEPHIS	Kenya Plant Health Inspectorate Service
kg	kilograms
KH_2P0_4	Potassium dihydrogen phosphate

LD_{50}	Lethal dose
LSD	Least significant difference
MEOH	Methanol
Mg	Magnesium
mg	milligrams
mg/100g	milligrams per 100g
ml	millilitre
Mo	molybdenum
MVA	Mevalonic acid
Na^+	Sodium ion
NaOH	Sodium hydroxide
NARP	Isocratic non-aqueous reverse phase
NH_2	Amino group
NH_4OH	Ammonium hydroxide
NMR	Nuclear magnetic resonance
NPRC	National Potato Research Centre
^oC	degree centigrade
P	Phosphorous
SD	Standard deviation
SDS	Solvent delivery system
Se	Selenium
SE	Standard error
SGT	Solanidine glucosyltransferase or solanidine galactosyltransferase
SPE	Solid phase extraction
TGA	Total glycoalkaloids
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultra violet

ABSTRACT

This study aimed at quantifying total glycoalkaloids, characterizing and quantifying α-chaconine and α-solanine of promising potato clones and varieties grown in Kenya. These compounds were studied with a view of ascertaining whether their contents varied with the potato types, changes in storage temperature and light exposure. It has been established that high levels (20mg/100g Fwt) of these compounds are toxic to animals and human and may change with storage and harvesting conditions.

Freeze-dried materials of potato clones and varieties were extracted in a mixture of THF/H_2O/acetonitrile or acetic acid for HPLC and titration with bromophenol blue, respectively. This was repeated for the tubers that were exposed to fluorescent tube (36W/54, 2500 lumens) and sunlight and those that were stored under ambient temperature and fridge conditions. The HPLC samples were ultrafiltered with a 0.45μm membrane before they were fractionated in a Nucleosil NH_2 column at a flow rate of 1mm/min and detected at 208nm and compared with pure standards of solanidine, α-chaconine, and α-solanine.

The results indicated that α-chaconine, α-solanine and solanidine were present in most varieties and CIP clones in significant quantities (5%). The elution patterns for the compounds were consistent. These patterns resulted in concentrations of total glycoalkaloids, α-chaconine and α-solanine of 3.50mg to 15.97mg/100g Fwt, 1.62mg to 4.46mg/100g Fwt, and 2.24mg to 6.41mg/100g Fwt, respectively, for the potato varieties, and 6.31mg to 17.48mg/100g Fwt, 2.24mg to 6.41mg/100g Fwt and 2.27mg to 3.80mg/100g Fwt, respectively, for the clones. The lowest and highest TGA values were recorded in Dutch Robyjn (3.50mg/100g Fwt) and Tigoni (15.97mg/100g Fwt). The results showed a significant-cultivar dependent variation in the glycoalkaloid contents.

The overall difference between the light storage and controls was significant at 5% level. The variety × light interactions for the contents were highly significant for TGA, α-chaconine, α-solanine contents. It significantly increased closer to the safety limits in some varieties that were exposed to sunlight. The results show that light can stimulate the synthesis of glycoalkaloids to levels above the safe limit (20mg/100g Fwt).

The effect of storage temperature on α-chaconine and α-solanine content was significant at the 5% level. The values of α-chaconine and α-solanine for the tubers stored under ambient room temperature ranged from 2.41mg to 5.07mg/100g Fwt and 1.85mg to 4.54mg/100g Fwt, respectively. The amount of these compounds for the potato tubers stored in the fridge varied from 2.06mg to 4.93mg/100g Fwt and 1.92mg to 4.06mg/100g Fwt, respectively. It is, therefore, suggested that storage at low temperature may greatly limit the synthesis of glycoalkaloids.

The glycoalkaloid contents in fresh tubers of the present commercial potato varieties and CIP clones are within the upper safety limit of 20mg/100g Fwt. Thus, the study demonstrates that no significant build-up in glycoalkaloids takes place under fridge and ambient temperature conditions. From these results, it is apparent that there is need to ensure that TGA, α–chaconine and α–solanine contents are maintained within the maximum limits under varying storage conditions of temperature and light. Any factors such as high light intensity and temperature that induce the contents to rise above 20mg/100g Fwt need to be controlled in order to limit the build-up of toxicity levels of TGA, α–chaconine and α–solanine.

INTRODUCTION

The solanaceae family contains many plants that are important to man. These include such diverse agricultural crop plants as potato (*Solanum tuberosum*), sweet pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), tomarillo (*Physalis ixocarpa*), tomato (*Lycopersicon esculentum*) and tobacco (*Nicotiana spp*) (Blankmeyer *et al.*, 1995). Potato is a major crop throughout the world, forming the world's most important food crops exceeded only by wheat, maize and rice in total production (Hawkes, 1990; Spoerke, 1994). Its importance is shown in providing diet for a large cross-section of the Kenyan population (Guyton *et al.*, 1994). Although it is widely used, it also contains some compounds, which tend to accumulate to toxic levels for human and animals under certain conditions.

The members of the solanaceae family and in particular *Solanum* genus, synthesise a variety of potentially toxic secondary metabolites during growth and post-harvest storage (Friedman *et al.*, 1991). These compounds include glycoalkaloids, inhibitors of digestive enzymes, lectins (haemagglutins) and mutagens (Crawford and Friedman, 1990; and Friedman and Levin, 1989). The glycoalkaloids are most frequently encountered. (Friedman *et al.*, 1997). At least 100 structurally different glycoalkaloids have been isolated and characterised in over 350 *Solanum* species (Cordell, 1981; Osman, 1983; Van Gelder, 1990), with additional ones being characterised every year (Friedman and McDonald, 1997). For most part, however, research has centred on potato glycoalkaloids (Friedman and McDonald, 1999). Different growth, storage temperature and light conditions are likely to influence the synthesis of these compounds in different parts of the crop. Kozukue *et al.* (1987) reported that the light exposure would increase the contents of α–chaconine and α–solanine in different parts of the potato plant.

Glycoalkaloids are produced in all parts of the potato plant including leaves, tubers, and sprouts (Brown *et al.*, 1999; Kozukue *et al.*, 2001). The highest levels are reported in parts with high metabolic rates such as flowers, unripe berries, young leaves, and spouts but contain much lower glycoalkaloid contents in the tubers (Van Gelder, 1990). The variation in the levels of glycoalkaloids in these parts plays an important role in the physiology of humans and animals. The two major glycoalkaloids of commercial potatoes (*Solanum tuberosum*), α–chaconine and α–solanine are reported to exert strong pharmacological and toxicological effects in human and other animals (Jadhav *et al.*, 1991(a and b); Keeler *et al.*, 1991).

Together, α–chaconine and α–solanine comprise approximately 95% of total glycoalkaloids (TGA) (Olsson, 1989). Freeze-dried potato tubers that range from 20 to 60mg/100g Fwt, typically contain TGA equivalent to 4mg to 12mg/100g Fwt (Griffiths *et al.*, 1994, Edwards and Coob, 1996). At these concentrations, glycoalkaloids are considered to enhance potato flavour. However, at concentrations in excess of 14mg/100g Fwt, they impart a bitter taste (Mondy and Gosselin, 1988; Sinden *et al.*, 1976). Maga (1980) reported that potatoes containing glycoalkaloids above 20mg/100g Fwt, are considered unfit for human consumption.

Glycoalkaloid levels of 20mg/100g Fwt in potatoes have been reported to cause poisoning in many case studies (Morris and Lee, 1984; McMillan and Thompson, 1979 and Jadhav *et al.*, 1981). The toxic dose is considered to be approximately 2 to 5mg/kg Bwt (Morris and Lee, 1984). The toxicity in animals and human include such diverse effects as anticholinesterase activity in the central nervous system (Nigg *et al.*, 1996), disruption of cell membranes, the digestive system and general body metabolism (Friedman *et al.*, 1992). The literature also reports that *Solanum* alkaloids may be associated with ancephally, *Spifina bifida* and other teratogenic changes in animals (Baker *et al.*, 1988; Renwick, 1986). The activities of these

compounds arise from the glycosylated derivatives. The nature and number of sugar residues were reported to cause biological activities of glycoalkaloids including α–chaconine and α–solanine (Rayburn *et al.* 1994). Thus, any condition that would influence the nature and number of sugar residues in the glycoalkaloids of potato varieties and clones would influence the toxicity of these compounds.

Researchers from various countries reported that glycoalkaloid contents of potato varieties can vary quite widely. For example, Norwegian varieties were found to contain TGA that vary from 4.2 to 34.5mg/100g Fwt (Baerug, 1962). Zitnak and Johnson (1970) reported that a variety used in Canada had glycoalkaloid levels consistently above 20mg/100g Fwt. The same variety was evaluated in U.S. and was found to contain high glycoalkaloid levels (Cronk *et al.*, 1974). Therefore, glycoalkaloid levels in potatoes depend on the variety and growth conditions.

The concentration of total tuber glycoalkaloids increases in response to a number of factors. These include cultivar, temperature, and exposure to light (Maga, 1980). For example, following harvest, the glycoalkaloid content can increase during marketing under the influence of light, cutting, sprouting, mechanical injury and exposure to pathogens (Friedman *et al.*, 1997). Friedman *et al.*, (1992) reported high levels in green and damaged potatoes. Other investigators have suggested that light exposure is probably the most important commercial factor influencing TGA content in potato tubers. Thus, in Kenya, where potatoes grow in varied light conditions ranging from highlands to mid altitudes and with varied storage facilities, it is expected that there is likelihood for TGA contents to increase to the toxic levels. It is, therefore, important to determine the TGA and characterise the α–chaconine and α–solanine in Kenyan potatoes.

Many methods of analysis for TGA, α–chaconine and α–solanine have been reported. These include mass spectrometry (Chen *et al.*, 1994), TLC scanning (Ferreira *et al.*, 1993), various colorimetric methods, gas chromatography (Lawson *et al.*, 1992), HPLC and immuno-assays. Each method has its relative advantages and disadvantages. Currently the most realistic methods utilised are HPLC and immuno-assays. The HPLC methods have the advantages of speed, ease and proven ability to separate and quantify potato glycoalkaloids. In Kenya where potatoes are grown in varied climatic conditions and even more varied storage and marketing conditions, there is need to use a rapid and efficient method of characterising and quantifying the glycoalkaloids.

The ware potatoes in Kenya and other countries are displayed under artificial light in most supermarkets and retail outlets. In addition, most hawkers of raw potatoes countrywide expose their wares to sunlight. Given that these conditions induce the formation of glycoalkaloids including α–chaconine and α–solanine in potato tubers, it is important to determine their concentration in potato varieties/clones grown in Kenya. It is also important to establish whether any of the conditions of storage temperature and light intensities enhance or decrease the glycoalkaloid levels. This study was, therefore, devoted to characterize and quantify α–chaconine, α–solanine and TGA in some potato varieties/clones under different light intensities and storage temperatures.

OBJECTIVES

Overall Objective

To assess the amount of TGA, α-chaconine and α-solanine in some of the Kenyan potato varieties and clones and ascertain whether storage and lighting conditions can influence their levels to toxicity limits.

Specific objectives

(1) To determine the total glycoalkaloids (TGA) in a range of promising experimental potato clones and the most widely used commercial potato varieties grown in Kenya,

(2) To characterise and quantify the α-solanine and α-chaconine of the selected potato varieties and clones, and

(3) To determine the effect(s) of variety, light and storage conditions on glycoalkaloid content of some of the selected potato varieties.

CHAPTER 1

LITERATURE REVIEW

1.1 The Origin of the Potato

The potato (*Solanum tuberosum* L.) was first domesticated in the Andes Mountains of Peru and Bolivia areas of South America over 7000 years ago (Hawkes, 1990). The Spanish introduced it to Europe in the 16th century after the conquest of Peru (Hawkes, 1990). Cultivation spread quickly throughout Europe, but the first large-scale production was in Ireland in 1807 where it became the chief food of the inhabitants (Burton, 1967). Late potato blight destroyed the Irish potato crop resulting in about 1million deaths and a massive emigration between 1845 and 1849. The British East Africa Company and Settlers started its cultivation in Kenya in the 1800's (Waithaka, 1976). Today, the potato serves as a major inexpensive food source with a worldwide production of about 350 million tons and is exceeded only by wheat, maize and rice in total production (Hawkes, 1994).

1.1.1 Botanical aspects of potato plant

The potato (*S. tuberosum* L.) is in the botanical family solanaceae, which includes such diverse agricultural crop plants as tobacco (*Nicotiana spp*), sweet pepper (*Capsicum annuum*), tomarillo (*Physalis ixocarpa*), tomato (*Lycopersicon esculentum*) and eggplant (*Solanum melongena)* (Blankmeyer *et al.*, 1995). All members of solanaceae have the ability to synthesise a variety of poisonous alkaloids (Friedman *et al.*, 1991).

The potato is an herbaceous dicotyledonous annual, 0.5 to 1M high (Horton, 1987; Burton, 1989). It has alternate, irregular, pinnate compound leaves and consists of several coloured flowers (Burton, 1967). Its roots are shallow and seldom extend deeper than 40 to 50cm. Tubers form as enlarged portions of underground stems adapted for storage of photosynthates and reproduction (Burton, 1969).

1.1.2 Potato production and consumption in Kenya

The potato, which was introduced to Kenya over 80 years ago, has become a very important food and cash crop (Ngugi, 1983). It is an excellent source of carbohydrates, vitamins (e.g. Vit. C and B_6) and minerals (e.g. P, K and Mg) (CIP, 1984; Horton and Sawyer, 1985). The crop has expanded in its cultivated area, total production and usage, and contributes a great deal to human diet in the crop growing areas and in large towns (NPCR-Tigoni, 1999).

The potato is produced mainly in the highlands and mid altitude areas in almost all provinces except coastal area due to high temperature (Kabira, 1983). The main potato growing areas are found in Central, Eastern and Rift Valley provinces. The common potatoes grown are mainly Kerr's pink and Nyayo varieties. In Kenya, the potato is the second most important food crop after maize (Guyton *et al.*, 1994).

Potatoes are mainly consumed at the commercial level as French fries (chips) and crisps served in restaurants and take-away facilities in Nairobi and other major towns in Kenya. The varieties Nyayo and Roslyn Tana are popular for French fries while Kerr's Pink and Dutch Robyjn are commonly used for crisps (Kabira, 2000). The new varieties; Asante and Furaha produce, good quality French fries while medium tubers of Tigoni could be used for crisps.

1.2 Glycoalkaloids-An overview

Glycoalkaloids are secondary plant metabolites containing nitrogen, in which the carbohydrate residues form a glycosidic linkage of one or more carbohydrate side chains at the 3-OH position of a C-27 steroid skeleton (Friedman *et al.*, 1993). They occur in members of the solanaceae family. Most research has centred on potato glycoalkaloids because of its toxicity to human and other animals (Friedman and McDonald, 1999). The two major glycoalkaloids in commercial potatoes are α-chaconine and α-solanine (Friedman *et al.*, 1991).

1.2.1 Chemical Structure and Occurrence

The chemistry of alkaloids present in solanaceous plants was reviewed extensively by Screiber (1968). Until 1954, it had been considered that the cultivated form of potato contain only one glycoalkaloid, solanine, discovered over 150 years ago. Kuhn and Löw (1954) discovered another glycoalkaloid, α-chaconine, in the leaves and shoots of the wild potato *S. chacoense.*

Besides α-chaconine and α-solanine, which represent upto 95% of the TGA contents, β and γ-forms of these glycoalkaloids were first reported in the leaves of *S. tuberosum* and *S. chacoense* (Kuhn and Löw, 1955). Leptinines and leptines (OH and acetoxy derivatives of solanines and chaconines) were reported only in *S. chacoense* (Schreiber, 1968). Because they are soluble at high pH and occur at high levels in the leaves and not in tubers in only a few lines of *S. chacoense*, they have been little studied (Sinden *et al.*, 1986b). Glycoalkaloids containing different aglycones such as tomatidenol, demissidine, and 5β-Solanidan-3α-ol have been identified in *S. tuberosum* L. (Schreiber, 1968).

Glycoalkaloids are usually classified according to the structure of their steroidal skeletons regardless of the structure of any attached sugar (Friedman and McDonald, 1999). The steroid alkaloids consist of a hydrophilic carbohydrate side chain attached to a hydrophobic steroidal skeleton (aglycone). The major *Solanum* alkaloids of pharmacological and toxicological interest possess the C-27 skeleton of cholesterol (Friedman and McDonald, 1997) (Fig. 1).

Generally, steroid alkaloids have various sugar groups attached to an aglycone at the 3-OH position (Friedman and McDonald, 1997). The side chains attached to solanidine for α–chaconine and α-solanine are β-chacotriose and α-solatriose, respectively. Solasonine and solamargine, have the same side chains as α-chaconine and α-solanine, respectively. Likewise, α-solamarine and β-solamarine are glycosides of solatriose and chacotriose. Although it is

important to study and be aware of all alkaloids that may occur in new potato varieties, at least 95% of all glycoalkaloids in commercial potatoes are α-chaconine and α-solanine. This study concentrates on these two compounds.

1.2.2 Distribution of Glycoalkaloids

In the potato plant, most of the tissues, including leaves, shoot, stems, blossoms, peels, and sprouts contain the major glycoalkaloids. The glycoalkaloid concentration is high in the shoot tips and the flowers (Kozukue *et al.*, 1987). The high level of steroid glycoalkaloids in the foliage and flowers may be due to the exposure of these parts to light (Kuć *et al.*, 1979).

The glycoalkaloids are formed in the parenchyma cells of the periderm and cortex of tubers and in areas of high metabolic activity such as eye regions (Reeve *et al.*, 1969). It is found in most tissues of the normal potato plant with only possible exception being the pith. It is generally conceded that maximum glycoalkaloid content is found in potato sprouts (Jadhav and Salunkhe, 1975). In potato sprouts, α-chaconine was the major glycoalkaloid and α-solanine was the second most predominant (Guseva *et al.*, 1960). Potato flowers are also a rich source of glycoalkaloids and are a common source of reference compounds (Lampitt *et al.*, 1943).

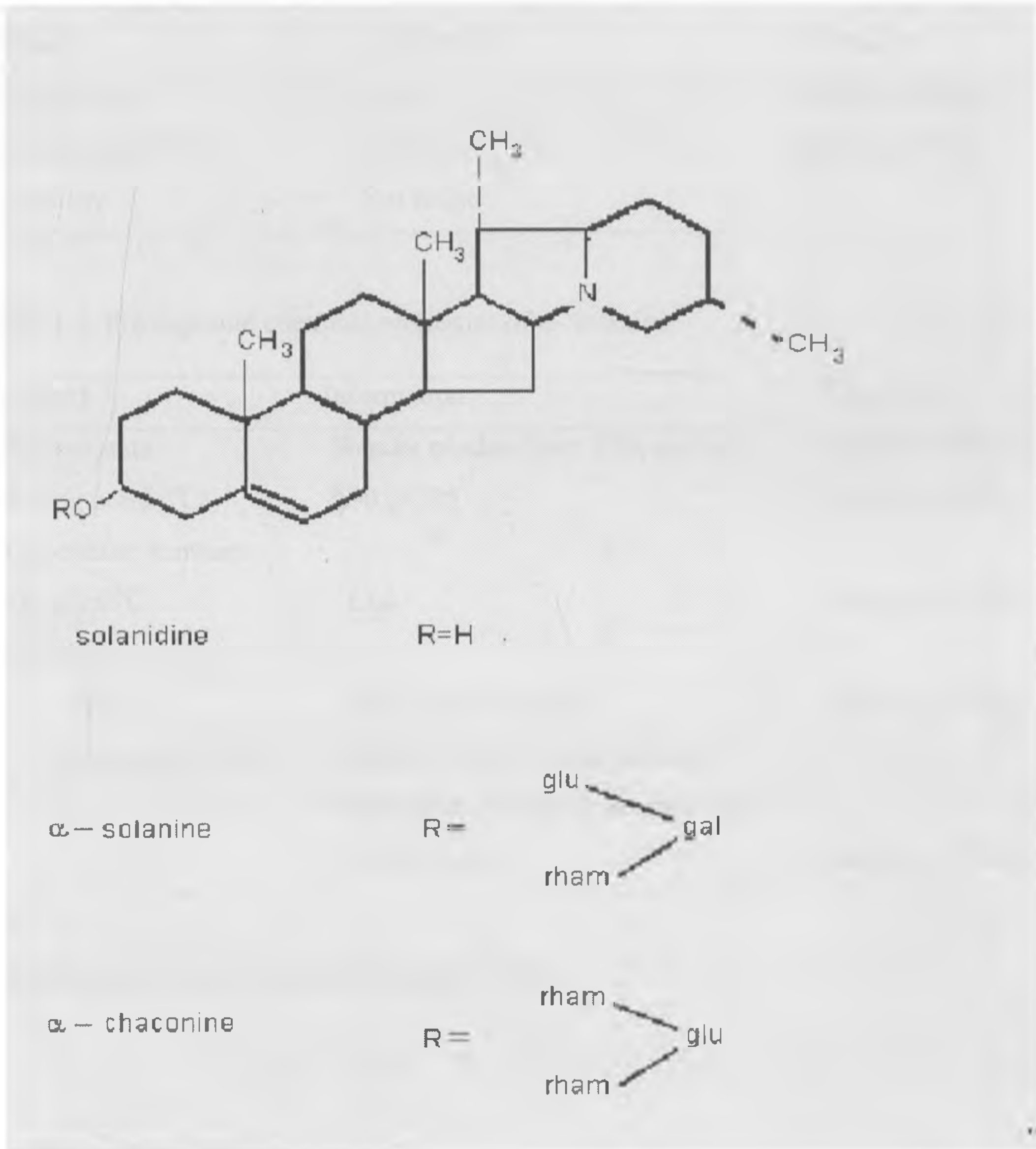

Figure 1.1. Molecular structures of α-solanine and α-chaconine. Glu, gal and rham represent glucose, galactose and rhamnose sugars, respectively. Adapted from Kozukue and Mizuno (1986).

1.2.3 Physical and chemical properties

Table 1.1. Physical and chemical properties of α-Chaconine

Property	Information	Reference
Physical state	solid	NIEHS (1997a)
Melting point (°C)	243	NIEHS (1997a)
Solubility	Not found	

Table 1.2. Physical and chemical properties of α-Solanine

Property	Information	Reference
Physical state	Slender needles from 85% alcohol	NIEHS (1997a)
Melting point (°C)	190 to 285	NIEHS (1997b)
Dissociation constant		
(Pk) at 15 °C	6.66	Budavari (1996)
Solubility:		
Water	practically insoluble	Budavari (1996)
Organic solvents	Readily soluble in hot alcohol;	
	Practically insoluble in ether and	
	Chloroform.	Budavari (1996)

Tables adapted from Zeiger and Raymond, 1998.

1.3 Physiological Effects

1.3.1 Human studies

Reviews on the toxic effects of glycoalkaloids include Baker *et al* (1991), Huxtable (1987), Morgan and Coxon (1987), Sharma and Salunkhe (1985), Van Gelder (1990), and Velisek and Hajslová (1995). The symptoms of glycoalkaloid poisoning include nausea, vomiting, diarrhoea, stomach and abdominal pain, headache, fever, rapid and weak pulse, rapid breathing, hallucination, and coma (Friedman and McDonald, 1997).

Glycoalkaloid poisoning is a case usually cited. McMillan and Thompson (1979) reported a significant case among schoolboys who became ill after eating potatoes, which contained about 330mg/kg glycoalkaloids. Ripakh and Kim (1958) and Gonmori and Yoshioka (1993) reported on poisoning in North Korea and Japan, respectively.

Hellenäs *et al.* (1992b) conducted experiments with volunteers and reported that α-solanine and α-chaconine in blood serum had biological half-lives of 10.7 and 19.1hr, respectively. Claringbold *et al.*, (1982) conducted similar experiments and reported that solanidine remained in the body longer (~34 to 68 days). They, therefore, suggested that glycoalkaloids stored in the human liver and other organs might be mobilised at times of increased metabolic stress with deleterious effects.

1.3.2 Animal studies

Besides human beings, glycoalkaloids have exhibited symptoms of glycoalkaloid poisoning in animals such as rats, mice, hamsters, rabbits and occasionally monkey, sheep, horses, pigs and dogs (Friedman and McDonald, 1997). Therefore, fresh potato haulms are poisonous and should not be used as animal feeds.

Differences have been reported on studies conducted with oral administration, intraperitoneal (IP) and intravenous (IV) injection. The α-solanine LD_{50} administered orally for all animals was 100mg/kg Bwt (Nishie *et al.* 1975). The IV LD_{50} for most animals was 10mg to 20mg/kg Bwt. The IP LD_{50} for solanine and chaconine in mice was 27mg and 30mg/kg Bwt, respectively. Friedman and McDonald (1997) reported that IP LD_{50} of various glycoalkaloids was around 30mg to 60mg/kg Bwt for most animals. Hamsters had sensitivity nearer to that of human and were recommended as the preferred animal model for research (Van Gelder, 1989).

1.3.3 Cell membrane disruption

The effects of toxic dose of glycoalkaloids are a burning sensation in the mouth, nausea, vomiting, abdominal pain and diarrhoea (Friedman and McDonald, 1997). There may also be internal haemorrhage and production of stomach lesions. These symptoms are attributed to the cell membrane disruption properties of glycoalkaloids.

Blankmeyer *et al.* (1992) established that α-solanine and α-chaconine changed the membrane potential of the embryo cells of *Xenopus laevis*. In another study, Gee *et al.* (1996) observed changes in membrane integrity following exposure of cultured cells to glycoalkaloids. The change in the membrane potential was attributed to the change in the ionic concentration, carriers and ionic pumps located near the cell membrane. A direct correlation between glycoalkaloids and inhibition of Ca^{2+}transport across the cell walls has been reported (Michalska *et al.*, 1985; and Toyoda *et al.*, 1991).

1.3.4 Anticholinesterase activity

α-solanine and α-chaconine have been established as inhibitors of the enzyme cholinesterase, a key component of the synapse. Symptoms of glycoalkaloid poisoning associated with the central nervous system such as rapid and weak pulse, rapid and shallow breathing, and coma are due to its inhibition of acetyl cholinesterase (AChe) (Friedman and McDonald, 1997).

Duan (1994) revealed that potato sprouts, α-solanine, α-chaconine, and solanidine, caused inhibition by 63.1%, 52.1%, 41.2%, and 11.4%, respectively. These studies indicate that α-solanine and α-chaconine were strong inhibitors. Harris and Whittaker (1962) reported different levels of inhibition in human population and members of the same species. Wierenga and Hollingworth (1992) found that strains of Colorado potato beetles were susceptible while some were resistant to AChe inhibition by α-chaconine. Heavy use of pesticides was suspected to have induced the observed resistance (Bushway *et al.*, 1987).

1.3.5 Damage to liver and other organs

Friedman and McDonald (1997) reported that glycoalkaloids concentrated most in the liver and were present in the kidney, heart, lungs and brain in lower concentration. Dalvi and Jones (1986) found significant increases in cholinesterase and liver enzyme activities in male rats after oral and peritoneal administration of solanine. Kusano *et al.* (1987) also reported that aglycones inhibited rat liver enzymes of cholesterol metabolism.

Azim *et al.* (1982) fed greened potatoes to rabbit and reported enlarged liver and hearts, increased blood cholesterol and glucose levels, decreased blood protein, increased plasma Ca^{2+} and decreased Na^+ and K^+ levels. Friedman *et al.* (1996) reported that consumption of aglycones resulted in increased liver weights (hepatomegaly) in mice while Caldwell *et al.* (1991) demonstrated that α-chaconine and α-solanine induced ornithine decarboxylase activities.

Bergers and Alink (1980) reported changes in heart rate and respiration in rats after IP injections of α-chaconine. Doses of 10 or 40mg/kg produced rapid heart rates (tachycardia), but intermediate doses (20mg to 30mg/kg) produced slow heart rates (bradycardia). The exact mechanism of organ damage is under investigation.

1.3.6 Teratogenicity

Renwick (1972) reported that there was a correlation between the incidence of potato blight and occurrence of spina bifida (the defective closure of the vertebral column) and anencephaly (absence of part of the brain and skull). The implications were of such importance that clinical studies were needed to test his hypothesis.

Swinyard and Chaube (1973) reported that blighted potatoes, IP injection of α-solanine, or other glycoalkaloids induced minor skeletal and kidney malformations but no neural tube defects.

Poswillo *et al.* (1972) found higher than normal cranial abnormalities in marmosets. In contrast, Bell *et al.* (1976) and Chaube and Swinyard (1976) reported no teratogenicity in pregnant mice and rats after IP injection with α-chaconine or α-solanine. Van Gelder (1989) reported that potato glycoalkaloids were not teratogenic at levels found in common commercial cultivars. These reports, however, associate glycoalkaloids with diverse effects on reproduction.

1.3.7 Relative toxicity

α-chaconine is the most toxic of all the potato alkaloids (Friedman and McDonald, 1999). It exhibits the strongest inhibition to AChe, cell disruption, organ damage, and teratogenicity in embryos (Friedman and McDonald, 1997). α-solanine on the other hand is somewhat less toxic although it is a strong inhibitor of AChe similar to α-chaconine. Solanidine is the least toxic in all the effects (Rayburn *et al.*, 1994). In a study on liver enlargement in mice, Friedman *et al.* (1996) found that the aglycones solasodine and solanidine were more active than tomatidine. It is, therefore, apparent that there is a relationship between chemical structure and biological potency and this might be used to predict the potencies of new glycoalkaloids.

Chaube and Swinyard (1976) reported that the size of the dose was more important than total amount of glycoalkaloids taken. Norred *et al.* (1976) also observed that as doses approached the toxic level, absorption seemed to increase much more rapidly. In a similar study, Morris and Lee (1984) reported that small amounts of glycoalkaloids and aglycones entering the bloodstream do not cause significant damage and are slowly detoxified in the liver. At a single dose of 1mg to 2mg/kg Bwt, cell disruption becomes noticeable and causes nausea and diarrhoea. Overtime, damaging levels of glycoalkaloids begin to collect in the liver and other organs (Friedman and McDonald, 1997). AChe inhibition as well as cell disruption occurs with a dose of 3 to 4mg/kg Bwt, cause damage to the central nervous system and major organs, although, the body may slowly recover and repair the damage.

1.4 Plant Physiology

1.4.1 Biosynthesis

All steroidal compounds such as sterols, certain sapogenins, terpenes, hormones, and alkaloids are interrelated and are synthesised via the isoprenoid pathway (Jadhav and Salunkhe, 1975; Wang *et al.*, 1999). Thus, the regular pathway starting from acetate via mevalonate, isopentyl pyrophosphate (IPP), farnesyl pyrophosphate (FPP), squalene, and cholesterol is applicable to steroidal alkaloids (Maga, 1980). This has been demonstrated in the biosynthesis of solanidine and tomatidine (Kozukue *et al.* 2001). Friedman and McDonald (1999) suggested that cholesterol is immediately and completely converted to other substances.

Guseva and Paseshnichenko (1958) initiated the first tracer work on the biogenesis of potato alkaloids that demonstrated the uptake and utilisation of radioactive acetate by potato sprouts. They suggested that acetate and mevalonate stimulate glycoalkaloids to be formed in the flowers from where they are passed into the tubers through the stems. Further improvements to the pathway have been reported (Jadhav *et al.* 1973; Jadhav and Salunkhe, 1973). The most recent progress in the study of glycoalkaloid pathway is attributed to Petersen *et al.* (1993) and Bergenstråhle (1995). They proposed that the routes to glycoalkaloids proceed from FPP, squalene, cycloartenol or lanosterol, cholesterol and solanidine.

The biosynthesis of cholesterol from acetyl-CoA and acetoacetyl-CoA involves a series of specific enzymes (Monfar *et al.*, 1990) (Fig.1.2). These precursors and enzymes synthesize FPP. The synthesis of FPP is regulated by FPP synthase. FPP is utilized for sesquiterpene production or channelled to sterol and glycoalkaloid biosynthesis via the conversion to squalene by the squalene synthase (Porter, 2000). Squalene mono oxygenase converts squalene to 2,3-Oxidosqualene. This oxidised product is subsequently converted to lanosterol and its isomer cycloartenol by lanosterol synthase and cycloartenol synthase, respectively.

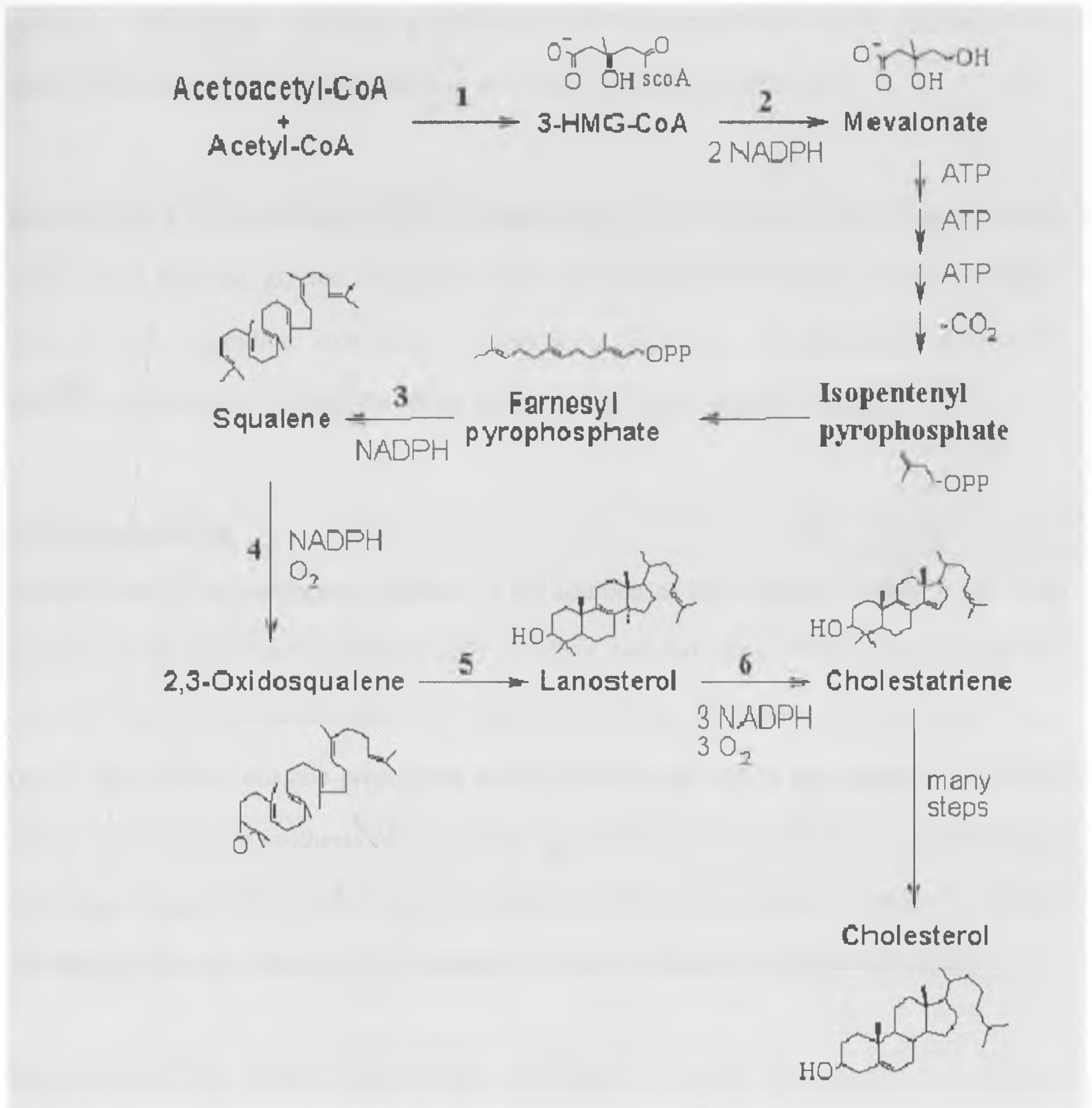

Figure 1.2. Formation of cholesterol from acetoacetyl-CoA and acetyl-CoA. The enzymes involved are: HMG-CoA synthase (**1**), HMG-CoA reductase (**2**), Squalene synthase (**3**), Squalene monooxygenase (**4**), Lanosterol synthase (**5**), and Lanosterol demethylase (**6**). Adapted from porter (2000).

Bergenstråhle (1995) and Petersen *et al.* (1993) proposed that the starting point for glycoalkaloid biosynthesis is cholesterol. In a related study, Petersen (1993) postulated that solanidine, solasodine, and tomatidine are synthesized from either cholesterol or cholesteranol (Fig.1.3). This proposal received support when cholesterol biosynthesis was suppressed by the

inhibitors tridemorph, ethylene, arachidonic and eicosapentaenoic acids decreased the glycoalkaloid formation (Bergenstråhle *et al.* (1996); Tjamos and Kuč (1982).

Kaneko *et al.* (1977b) proposed that the nitrogen atom of the F ring originates from an amino acid such as arginine, glycine, or alanine. Once incorporated, the E ring is closed in different ways to form solanidine, solasodine, or tomatidine (Friedman and McDonald, 1999). The synthesis of aglycones is complete before glycosylation begins (Ehmke, 1995).

1.4.2 Glycosylation

The final step in glycoalkaloid synthesis is the addition of the sugar side chain to the 3-OH position of the aglycone (Liljegren, 1971). Jadhav and Salunkhe (1973) demonstrated that extracts from potato sprouts and slices convert solanidine to β-glucoside. Lavintman *et al.* (1977) described an enzyme preparation isolated from potato tubers and sprouts that had the ability to catalyse the synthesis of all possible sugar derivatives (α, β, and γ) of solanine and chaconine. Maga (1980) suggested that solanidine synthesis proceeds at a rapid rate, whereas the stepwise glycosylation is slower because of limited availability of glycosidic sugars.

Bergenstråhle *et al.* (1992a) reported that solanidine was rapidly glycosylated in a stepwise manner after being formed. The formation of α-solanine and α-chaconine was accomplished by solanidine glucosyltransferase and galactosyltransferase enzymes. Studies by Paczkowski and Wojciechowski (1994) and Zimowski (1994) indicate that these enzymes occurred at very low concentrations in the plant tissue and were difficult to purify. Friedman and McDonald (1997) suggested that researchers might overcome this limitation by use of molecular biology techniques. This suggestion was adopted and a complementary DNA (cDNA) encoding solanidine glucosyltransferase was subsequently isolated from yeast (*Saccharomyces cerevisiae*) (Moehs *et al.* (1997).

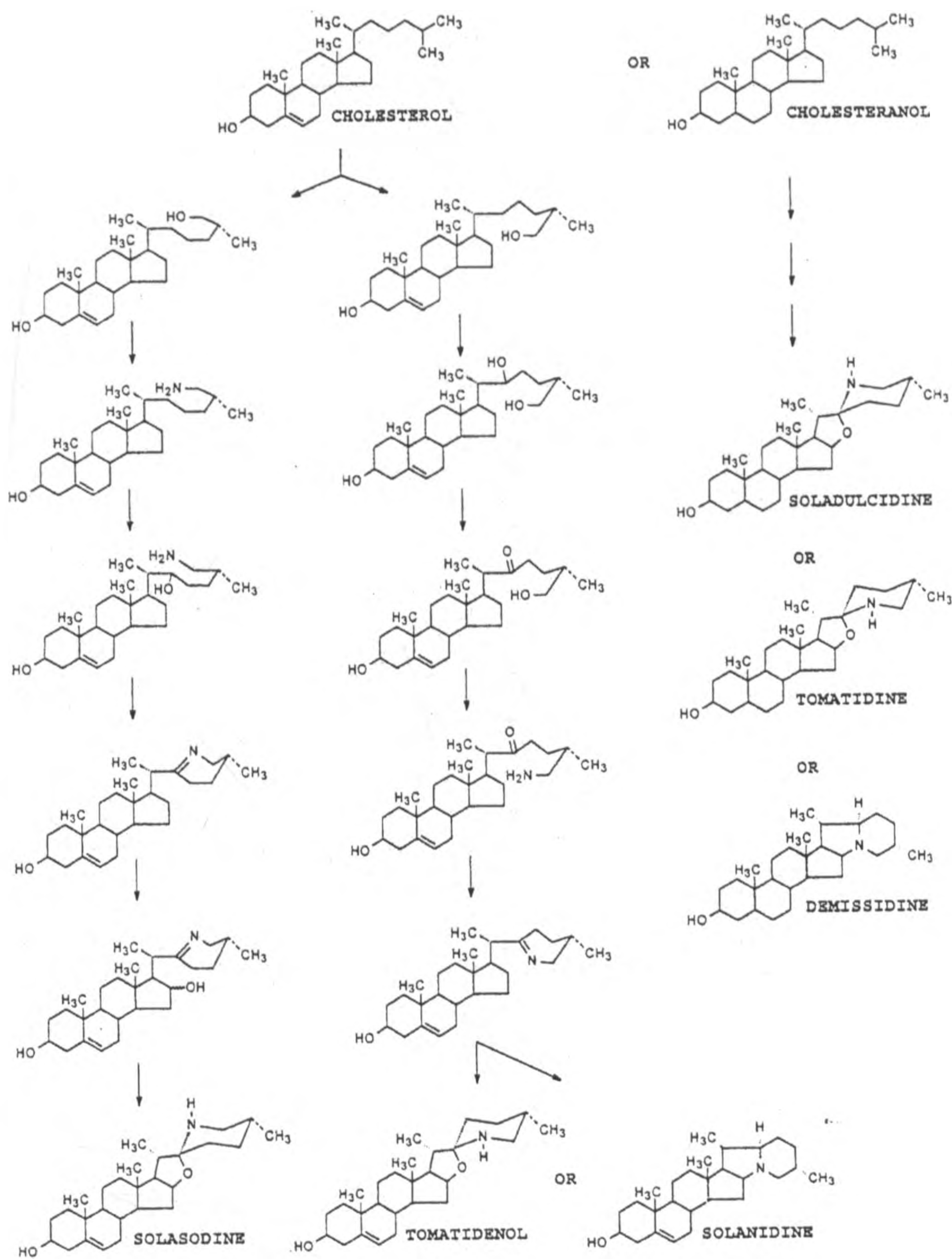

Figure 1.3. Possible synthetic pathways from cholesterol and cholesteranol to aglycones.

Adapted from Petersen (1993).

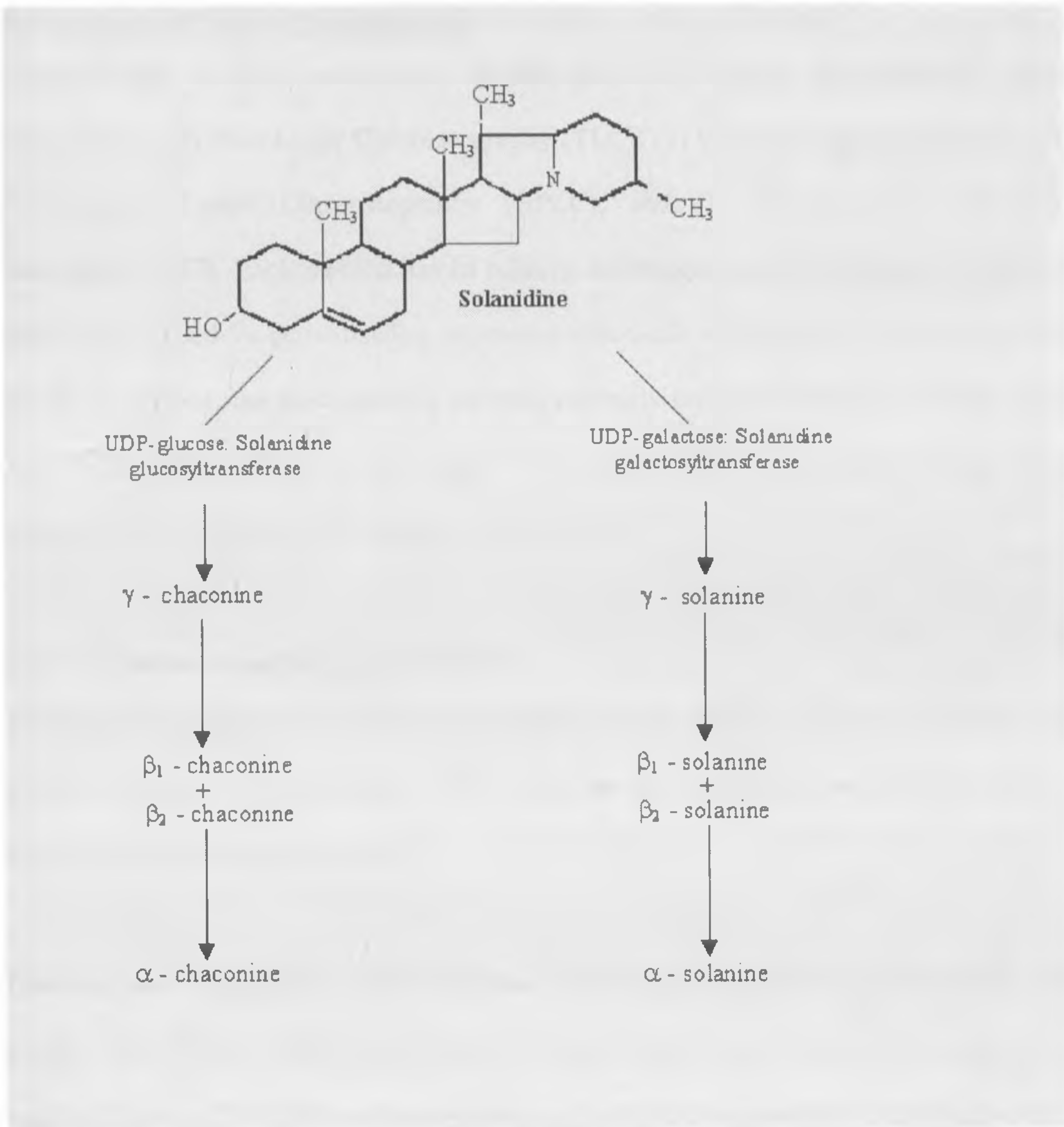

Figure 1.4. Stepwise glycosylation of solanidine.
Adapted from Bergenstråhle *et al.* (1992).

1.4.3 Hydrolysis

Glycoalkaloids are susceptible to hydrolysis by either enzymatic action or by acids. Under these conditions, the stepwise cleavage of α-solanine and α-chaconine leads to β- and γ compounds (Friedman and McDonald, 1999). The hydrolysis of α-solanine and α-chaconine is discussed in chapter four.

1.5 Analysis of Potato Glycoalkaloids

Methodologies in most current use for the analysis of potato glycoalkaloids include: (1) Colorimetry; (2) Thin Layer Chromatography (TLC), (3) Gas Chromatography (GC), (4) High Performance Liquid Chromatography (HPLC), and (5) Immunoassays (Friedman and McDonald, 1997). Each method has its relative advantages and disadvantages, such as lack of sensitivity, a need for derivatisation, expensive chemicals or excessively long preparatory steps. For these reasons, the most realistic methods currently utilised are HPLC and Immuno-assays. The HPLC methods have the advantages of speed, ease and awide variety of equipment with proven ability to separate and quantify glycoalkaloids.

1.5.1 Extraction of potato glycoalkaloids

All the glycoalkaloids except leptines are only sparingly soluble in aqueous solutions at pH 7 or above (Friedman and McDonald, 1997). Thus, for glycoalkaloids, the extraction solvents are either non aqueous, acidic or both.

Friedman and McDonald (1995) evaluated solvent systems for dried, fresh and processed tubers. Their HPLC results varied widely for α-solanine but less so for α-chaconine. They reported that the 2% acetic acid was the best solvent for dried samples and enforced the results of Speroni and Pell (1980) and Bushway *et al.* (1985). In contrast, the best solvents for fresh samples were either MeOH/chloroform or THF/H_2O/acetonitrile/acetic acid mixtures. Extraction with acetic acid, however, improves safety due to the reduced use of organic solvents and reduces the operational expenses (Friedman *et al.*, 1994).

1.5.2 Purification of potato glycoalkaloids

In traditional methods of glycoalkaloid analysis this step involved precipitation of glycoalkaloids from a solution of dilute acid (Coxon, 1984). The precipitate was usually washed with NH_2OH and dried before final quantification. The NH_2OH precipitation has a disadvantage

that while α-solanine is insoluble in basic solutions; α-chaconine seems to be partially soluble (Friedman and McDonald, 1997). Similarly, leptines are soluble at this pH and will be lost. The loss could be avoided by use of n-Butanol, which works very well with pure hydrolysed systems or by use of acetic acid for freeze-dried samples (Friedman and Dao, 1992).

The most widely used clean-up procedure in current practice for HPLC involves the use of disposable C_{18}, CN or NH_2 cartridges (Friedman and McDonald, 1997). Saito *et al.* (1990) recommended the use of C_{18} and NH_2 cartridges for clean up of low and high-lipid samples respectively. Jonker *et al.* (1992) recommended SPE-CN cartridges for clean up. The selection of clean-up method relies to a large extent on the extraction solvent and method of analysis.

1.5.3 Quantification of potato glycoalkaloids

Although there have been notable developments in extraction and clean-up procedures, most of the developments have occurred in the quantification of glycoalkaloids (Coxon, 1984). A review of methods for identification and quantification of indivitual glycoalkaloids concentrating on methodologies in current use are discussed.

1.5.3.1 Colorimetry

In early works, solanine was determined gravimetrically (Coxon, 1984), but colorimetric methods were soon developed. The colorimetric methods that involved hydrolysis were used to determine the TGA contents only and could not identify specific alkaloids. Although some of the colorimetric methods were found reliable for determination of TGA in *S. tuberosum*, their choice is limited by interference, time taken per analysis and the very unpleasant nature and toxicity of the colorimetric reagents (Friedman and McDonald, 1997).

Fitzpatrick and Osman (1974) reported the first comprehensive method of TGA analysis. The method involves extraction with MeOH/chloroform and quantification by titration with 0.067% bromothymol blue and 10% phenol in absolute methanol. Pure glycoalkaloids are titrated directly to obtain standardisation curves (Coxon, 1984). The colour change indicates when all the glycoalkaloids have been complexed with phenol (Friedman and McDonald, 1999). This method has been modified (Bushway *et al.*, 1980; Speroni and Pell, 1980).

1.5.3.2 Thin Layer Chromatography

TLC methods are extremely valuable for the quantificative identification of glycoalkaloids and their aglycones (Coxon, 1984). Early TLC work on separation of steroidal compounds was done by Screiber *et al.* (1963), Bennett and Heftman (1966), Rozumek (1969), and Hunter *et al.* (1976). Although newer methods of analysis are more powerful, TLC is still used as a simple and quick means of screening large number of samples for glycoalkaloids.

The most widely used solvent systems for separating glycoalkaloids and aglycones by TLC are combinations of chloroform/alcohol/NH_3 (Friedman and McDonald, 1997). Jellema *et al.* (1981) recommended chloroform/MeOH/2% NH_3 (70:30:5). Shih and Kuċ (1974) obtained the best results with benzene/MeOH (5:1) or cyclohexane/ethyl acetate (1:3).

Glycoalkaloids have been detected using various methods that include: antimony trichloride (Filadelfi and Zitnak, 1983), Dragendorff's reagent (Roddick and Melchers, 1985), anisaldehyde reagent (Shih and Kuċ, 1974), and iodine vapour (McCollum and Sinden, 1979). Others include Clarke reagent (Coxon and Jones, 1981), and optical brighteners such as Blankophor® BA 267% (Jellema *et al.*, 1982). The brighteners are very specific although they require fluorescence to be seen. Iodine vapour is certainly the easiest to use in preparative work (Friedman and McDonald, 1997).

1.5.3.3 Gas Chromatography

GC has been used for qualitative and quantitative analysis of glycoalkaloids and aglycones (Coxon, 1984). Herb *et al.* (1975) devised the first GC method for glycoalkaloids. The method was based on the bisolvent extraction, permethylation and chromatography on a 3% OV1 column programmed upto 330°C. It permitted qualitative identification and relative quantification of mixtures containing solanidine, β-chaconine and α-chaconine. Other GC methods were developed based on this first procedure. Siegfried (1976) reported a method for the determination of solanine: chaconine ratio by GC after trimethylation. Van Gelder (1985) and Van Gelder *et al.* (1989) developed a method of capillary GC for separation of complex mixtures of glycoalkaloids, sterols, and steroidal sapogenins using a combination of flame ionisation and N_2 source detectors.

Roddick (1980) reported a novel quantitative method for measurement of potato glycoalkaloids based on their ability to complex with stigmasterol in ethanol. The method was very specific, requires no derivatisation step, and was simple and safe to carry out. Its main disadvantages were the relatively low sensitivity and lengthy procedure (Friedman and McDonald, 1999).

GC has several disadvantages. It is relatively expensive compared with colorimetry and TLC. Due to the high temperatures involved, the columns can run ≤100 samples (Herb *et al.*, 1975). Run times are often long and do not give information on individual glycoside contents (Friedman and McDonald, 1999). Despite its disadvantages, GC present no solvent disposal problems, it gives good separation of compounds, has relatively simple detection, and is ideally suited to direct coupling with other instruments such as mass spectrometers (MS). GC-MS has proven to be of great value in structure elucidation (Evans *et al.*, 1993; Osman *et al.*, 1986).

1.5.3.4 Immunoassay methods

Immunoassays of various types provide a means of analysing many different substances of medical and biological interest (Coxon, 1984). Generally, immunochemical assays are rapid and simple in design and do not require expensive instrumentation (Friedman and McDonald, 1997). The three fundamental requirements for any immunoassay method are:

(1) An antiserum containing high affinity, highly specific antibodies to the material to be measured. A small molecule (hapten), which is non-immunogenic, is injected into an animal to produce the antiserum. A hapten is a polysaccharide responsible for the specificity of an antigen.

(2) A high specific activity 'label' that can be attached directly or indirectly to the hapten without affecting its binding to antibodies raised against the unlabelled material.

(3) A pure standard identical with the material to be measured.

In the assay labelled (known amount) and unlabelled (unknown amount) hapten are allowed to compete for a limited number of antibody binding sites in a diluted antiserum. By determining the amount of labelled hapten that is bound one can determine the amount of unlabelled material in the assay by reference to a standard curve. Methods of labelling that have been used for glycoalkaloids are radio immunoassay (RIA) (Vallejo and Ercegovich, 1978), enzyme linked immunosorbent assay (ELISA) (Morgan *et al.*, 1983), and fluorescence polarization immunoassay (Thomson and Sporns, 1995). As a labelling method, ELISA has proved to be a better option for TGA analysis (Coxon, 1984).

The first radio immunoassay (RIA) method was developed by Vallejo and Ercegovich (1978). Similar methods were reported by Matthew *et al.* (1983) and Harvey *et al.* (1985a, b). Although the methods were quite sensitive, their results were random and needs strict monitoring and control of radioactive materials (Friedman and McDonald, 1997).

Morgan *et al.* (1983) developed an ELISA method that was specific for glycoalkaloids using an antiserum from bovine serum albumin-solanine conjugates. The method has proved very useful for the determination of TGA content of potato tubers. It has greater sensitivity and a more useful selectivity than RIA method and requires no clean-up. Enzyme labelled ELISA reagents are both cheap to prepare and highly stable. In addition, the method is generally easier to use, requires less expensive equipment and do not have problems associated with the use of radioisotopes (Coxon, 1984).

Stanker *et al.* (1994, 1996a,b) developed a panel of monoclonal antibodies that bound α-solanine, α-chaconine, tomatine, solanidine or tomatidine. Some of these antibodies bind α-solanine and α-chaconine, with equal affinity. Others bind only solanidine, α-tomatine or tomatidine. None of the antibodies showed any affinity for steroids lacking ring nitrogen atom such as cholesterol, digitonin, β-sitosterol, and stigmasterol.

1.5.3.5 High-Performance Liquid Chromatography

The most popular method for determination of glycoalkaloids appears to be HPLC (Friedman and McDonald, 1997). It is a useful technique for the determination of TGA levels and for the identification of individual glycoalkaloids and their aglycones. The choice of columns and mobile phases, however, are quite critical in order to obtain the required separation (Friedman *et al.*, 1994; and Blankmeyer *et al.*, 1995).

Hunter *et al.* (1976) first used HPLC as a preparative method for collecting various aglycones. Bushway *et al.* (1979) described the first HPLC method for separating α-solanine and α-chaconine and reported that carbohydrate column eluted with THF/H_2O/acetonitrile (56:14:30) gave excellent separation with very little interference from other compounds in potato tissue extract. Crabbe and Fryer (1980) developed an HPLC method for separating partial hydrolysis

products using buffered methanol solutions with μ Bondapak C_{18} and Waters carbohydrate columns at 205nm. The method could separate combined α, β, and γ products of solanine and chaconine.

Morris and Lee (1981) investigated the separation of potato glycoalkaloids on Radial-Pak (both normal phase silica and reversed phase C_8 and C_{18}) cartridges using acetonitrile/H_2O/ethanol amine mixtures. They monitored the separations by UV absorbance at 200nm. The addition of ethanolamine to a mixture acetonitrile/H_2O greatly improved the speed of analysis and separation of hydrolysis products of solanine and chaconine.

The mobile phase is dependent on the type of HPLC column used for analysis (Friedman and McDonald, 1997). Asano *et al.* (1996) used 33% acetonitrile in 20m*M* phosphate buffer with a C_8 column at 205nm to determine glycoalkaloids in various parts of the potato plant. Kobayashi *et al.* (1989) separated α-chaconine and α-solanine using ethanol/acetonitrile/0.005M KH_2P0_4 on an NH_2 column. Friedman and Levin (1992) used 35% acetonitrile in 100m*M* $(NH_4)_2PO_4$ buffer to separate the two compounds on a C_{18} column. Houben and Brunt (1994) used 40% acetonitrile to determine α-chaconine and α-solanine on a C_{18} reverse-phase column.

The requirement to monitor glycoalkaloid separations at low wavelength (200 to 215nm) is preferable for optimum sensitivity (Coxon, 1984). This has several implications. First, the choice of eluent is limited to compounds transparent to UV, namely, water, methanol, ethanol, acetonitrile, and THF. Second, the elution should be isocratic (single solvent composition) to maintain a stable baseline (Friedman and McDonald, 1997). Finally, the clean-up procedure becomes very important, as many compounds may interfere in the UV range.

There are several advantages in using HPLC. The high molecular weight and thermal instability of glycoalkaloids, which make their analysis by GC so difficult, present no problems for analysis by HPLC (Coxon, 1984). It runs at room temperature, analyze both glycoalkaloids and aglycones without derivitization, and can give an almost complete picture of the pattern of individual glycoalkaloids present in one determination (Friedman and McDonald, 1997). Major disadvantages are the expense of the equipment, the need for extensive clean up of samples, and use of organic solvents that need to be purchased and disposed of safely. Despite these disadvantages, HPLC has certainly been shown to have a great potential for quantitative glycoalkaloid analysis.

1.6 Factors influencing glycoalkaloid production

Levels of glycoalkaloids increase during potato growth and after post-harvest depending upon environmental conditions, cultural practice and post-harvest handling procedures (Sinden and Webb, 1972). Environmental factors that can influence tuber glycoalkaloid content include soil moisture, temperature, altitude, light exposure, fertilizer level, and pesticide applications (Cronk *et al.*, 1974; Wilson and Frank, 1975). The tuber glycoalkaloids also varies significantly depending on variety, length of storage, mechanical injury, tuber size and physiological age (Zeiger and Raymond, 1998).

1.6.1 Variety

Researchers from various countries have reported that glycoalkaloid contents of potato varieties vary quite widely (Friedman and McDonald, 1997). For example, Norwegian varieties were found to vary from 4.2mg to 34.5mg/100g Fwt (Baerug, 1962). Canadian varieties ranged from 1.9mg to 7.6mg/100g Fwt (Zitnak, 1955). One Canadian variety had glycoalkaloid levels consistently above 20mg/100g Fwt (Zitnak and Johnson, 1970). The same Canadian variety was

evaluated in the U.S. and was found to contain very high levels of glycoalkaloids (Cronk *et al.*, 1974). There was a similar problem in Sweden with the Magnum Bonum variety that was in commercial production and had to be withdrawn from the market (Hellenas *et al.*, 1995b).

Morris and Petermann (1985) measured levels of individual glycoalkaloids in cultivars and breeding lines grown in Australia and reported that two of the cultivars (Lenape and Berita) had glycoalkaloid levels greater than the safety limits of 200mg/kg Fwt. The contents in several others ranged from between 100 and 200mg/kg Fwt. Similar studies have been conducted in Germany (Wünsh, 1989), Egypt (Ahmed *et al.*, 1988), India (Uppal, 1987), Korea (Hwang and Lee, 1984), Netherlands (Van Gelder and Dellaert, 1988), New Zealand (Lammerink, 1985), Pakistan (Rahim *et al.*, 1989), Poland (Mazurczyk, 1995), and U.K. (Parnell *et al.*, 1984). Sanford *et al.* (1995) demonstrated that glycoalkaloid contents of specific cultivars were genetically controlled. Sanford and Sinden (1972) observed that glycoalkaloid contents were continuous among parent and family tetraploid crosses; an indication of polygenic inheritance.

Several studies have used glycoalkaloid profiles to study the development of potato (Friedman and McDonald, 1997). Johns and Galindo Alonso (1990) reported that selection for reduced glycoalkaloid levels have occurred during the domestication of the potato. They found that some wild species that were eaten historically had potentially toxic levels of glycoalkaloids. Gregory *et al.* (1981) found that most wild species had relatively high levels of solanine, chaconine, solamarine, tomatine, demissine, and leptines. These suggest that potato breeders should check glycoalkaloid contents in their most promising new varieties.

The major potato varieties grown and consumed in Kenya are Nyayo, Kerr's pink, Desiree, Mukori, Ngure, Tigoni and Tana kamande (NPRC-Tigoni. 1999). Other important varieties by locality include Rosyln Tana (South Kinangop), Desiree Kihoro (South Kinangop), New

Desiree (South Kinangop), Asante (Laikipia), Furaha (Laikipia), Anett (Laikipia), Tiganiwe (Laikipia), Munyaka (Laikipia), Comesha (Ol kalou), Dutch Robyjn (Molo), and Romano (Central). Tigoni and Asante are new varieties that were selected from germplasm obtained from CIP in the early 1990's and released in 1998. They were well adopted by farmers due to their high yielding potential and tolerance to blight (Mussukuya *et al.*, 2000; Kabira, 2000). There are, however, no reports of glycoalkaloid contents of all these potato varieties.

The potato cultivar 'Lenape' is an instructive example of the problems glycoalkaloid biosynthesis can introduce into potato breeding programs. This cultivar was shown to have excellent chipping and storage properties (Akeley *et al.*, 1968). Lenape variety had *S. chacoense* a species with extremely high TGA levels, in its ancestry. The United States Department of Agriculture (USDA) and the Canadian Department of Agriculture (CDA) in a joint action removed Lenape from the market in 1970 and a policy was then established to test for this toxin (Jadhav and Salunkhe, 1975; Sinden *et al.*, 1984). Much time, money, and effort could have been saved if TGA analyses had been part of the breeding program.

1.6.2 Pre-harvest changes

Although the nature and relative concentrations of glycoalkaloids are genetically determined, the total concentrations are certainly influenced by environmental factors during the growing period (Friedman and McDonald, 1997). The high glycoalkaloid biosynthesis is mainly due to season and climate while cultural and soil composition had the least influence. For example, Sinden and Webb (1972) reported significant differences in tuber glycoalkaloid contents within varieties were due to factors such as length of growing season, tuber maturity, and tuber damage. These factors are important in the storage of potatoes for retail and home consumption.

Hutchinson and Hilton (1955) reported different glycoalkaloid levels in the same variety that was grown in different parts of Alberta, Canada. They reported that very bitter tuber samples

contained toxic levels of glycoalkaloids. This was attributed to frost or hail damage to the plant tops before maturity. Many investigators have concluded that increased glycoalkaloid production is the plant's reaction to stress such as prolonged cold, extreme heat, too little water, too much sunshine or too little sunshine (Yaniv *et al.*, 1984; Olsson and Carlsson, 1993).

Love *et al.* (1994) reported significantly high glycoalkaloid levels with increased nitrogen fertilization, whereas Ahmed and Müller (1979) and Van Swaaij (1992) found decreased production of glycoalkaloids. Mg and Se fertilization increased glycoalkaloid formation, whereas foliar application of Mo decreased the synthesis (Evans and Mondy, 1984; Mondy and Munshi, 1993). Ponnampalam and Mondy (1986) reported that foliar application of indoleacetic acid caused a significant decrease in tuber glycoalkaloids. The insecticide carbofuran was also found to decrease glycoalkaloid production in tubers (Wilson and Frank, 1975).

1.6.3 Post harvest changes

Generally, glycoalkaloids are usually present at low levels in commercially available potatoes, but because biosynthesis of glycoalkaloids in potatoes continues long after harvest, they can accumulate to higher level (Friedman and McDonald, 1997). Practical experience by plant breeders, potato growers, and distributors has resulted in numerous factors that contribute to the formation of glycoalkaloids (Maga, 1980). The suggested factors that influence glycoalkaloid formation to include light, storage conditions and mechanical injury. Light and mechanical injury were reported to be the most important environmental factors that stimulate post-harvest glycoalkaloid accumulation in potato tubers (Kuć, 1984).

1.6.3.1 Light

The effect of exposing harvested potato tubers to light, whether incandescent, fluorescent, or natural, can dramatically enhance glycoalkaloid synthesis (Jain *et al.*, 1995; Dimenstein *et al.*,

1997). Jadhav and Salunkhe (1975) found that the increase in glycoalkaloid and alkaloid levels depends on the duration of exposure, the intensity and the wavelength of light. Petermann and Morris (1985) observed that chlorophyll and glycoalkaloid syntheses were dependent on different light wavelengths.

Kozukue and Mizuno (1990) found that glycoalkaloids increased in potato tubers after exposure to fluorescent light at 10 and 15°C. Dao and Friedman (1994) reported increased glycoalkaloid (300%), chlorophyll and chlorogenic acid concentrations after exposing potatoes to fluorescent light for 20 days. Baerug (1962) reported a highly significant correlation between natural light intensity and the alkaloids accumulation. The accumulation of glycoalkaloid varied with the cultivar, temperature and tuber physiology (Burton, 1989). In connection to the last point exposure to light caused little increase in the glycoalkaloid contents of old stored tubers but considerable increases in freshly dug potatoes.

Ramaswamy *et al.* (1976), Dale *et al.* (1993) and Nair *et al.* (1981) demonstrated that chlorophyll and glycoalkaloid syntheses are intimately related. This suggestion was based on the observation that formate, glycine and pyruvate; intermediates to the mevalonic acid pathway, are precursors to chlorophyll and glycoalkaloids. In contrast, Patil *et al.* (1971) reported that light intensity had a significant effect on chlorophyll production but did not cause similar changes in glycoalkaloid levels. The question of whether increases in glycoalkaloid synthesis always parallels light induced chlorophyll synthesis remains unresolved (Kozukue and Mizuno, 1990; Dale *et al.*, 1992).

Early studies indicated that glycoalkaloid increases due to light could be significantly reduced by means of light filters. For example, Jepsen *et al.* (1974) found that red, green, or violet

filtered light reduced glycoalkaloid production. Rosenfeld *et al.* (1995) observed that tubers packaged in blue polythene bags were most susceptible to light-induced glycoalkaloids. Dale *et al.* (1993) suggest that since potato cultivars differ significantly in their ability to produce greening-related glycoalkaloids, it should be possible to find and use varieties with low rates of post-harvest glycoalkaloid synthesis.

1.6.3.2 Temperature

Many researchers have reported the importance of storage temperature in glycoalkloid formation (Salunkhe *et al.*, 1972; Kozukue and Mizuno, 1990). Hwang and Lee (1984) found that tubers stored at 1°C were lower in glycoalkaloids than tubers stored at 20°C. Linnemann *et al.* (1985) compared tubers stored for 12 weeks at 7, 16, and 28°C and reported similar results. Kaaber (1993) found that higher storage temperature caused increased glycoalkaloids in potato tubers of some varieties. In contrast, Jadhav and Salunkhe, (1975) and Bushway *et al.* (1981) found that lower temperatures produce more glycoalkaloids than higher temperatures.

Friedman and McDonald (1999) suggested that the conflicting data could be partly reconciled if the potato tuber is considered to produce glycoalkaloids in response to stress in general. Thus, stressful conditions responsible for increased tuber glycoalkaloid synthesis may include both high temperatures and lower temperature. Chungcharoen (1988) suggested that intermediate temperatures (7 to 10°C) might represent the best compromise for storage of potatoes.

1.6.3.3 Storage time and sprouting

Considerable research has demonstrated that potato storage time and sprouting can play a significant role in glycoalkaloid levels in potatoes. For example, Love *et al.* (1994) reported a

significant effect of storage time on glycoalkaloid level. Fitzpatrick *et al.* (1977), demonstrated that glycoalkaloid content increases with storage upto a maximum level, then begin to decline.

Olsson and Roslund (1994) in a storage study of potato clones kept at 4°C found that glycoalkaloid level fluctuated. In a few instances the level fell below the original levels. Wünsch and Munzert (1994) studied the effects of storage at 4°C and reported a slight reduction in glycoalkaloids overtime until sprouting occurred.

The sprouts contain the highest glycoalkaloid levels in the potato (Friedman and McDonald, 1997). They suggest that glycoalkaloid synthesis is concentrated at the growing portions of sprouts. Thus, anything that will inhibit sprouting under practical conditions of storage will reduce glycoalkaloids in stored potatoes.

1.6.3.4 Mechanical damage

Bruising, cutting and slicing of potatoes have been reported to induce the formation of glycoalkaloids (Mondy and Gosselin, 1988). Based on these reports, it appears that injury such as slicing before storage increases glycoalkaloid synthesis. Wu and Salunkhe (1976) examined the effect of damage on different potato varieties and reported that cutting produced the highest glycoalkaloid levels. Fitzpatrick *et al.* (1978) and Olsson (1986) reported that susceptibility to damage varied with cultivar. Olsson (1989) demonstrated that the increase in the level of glycoalkaloids in different genotypes after damage correlated with original contents.

1.6.3.5 Maturity

Burton, (1989) reported that when immature tubers were exposed to sunlight even for a short time, it had a significant effect on the concentration of glycoalkaloids. Cronk *et al.* (1974)

observed that tuber size could have an influence on glycoalkaloid content probably due to their larger surface area to volume ratio.

1.5.3.6 Humidity

It is a common practice in many homes that after peeling and slicing, potatoes are placed in a pot of water to reduce browning. Wu and Salunkhe (1978) and Mondy and Chandra (1979) reported that the immersion of potatoes in water reduced glycoalkaloid formation during soaking and after removal from water. These indicate that humidity may have an effect on glycoalkaloid levels in stored and sliced potatoes.

1.6.4 Food processing

Since potatoes are subject to a variety of food processing conditions including baking, boiling, frying, and microwaving, the question arises whether these conditions influence glycoalkaloid content (Friedman and McDonald, 1999). Experiments have revealed that processed potato products with high glycoalkaloid levels exceeding 140mg/kg Fwt taste bitter (Zitnak and Filadelfi; 1985). The contents in excess of 220mg/kg had mild to severe burning sensations in the mouth and throat (Friedman and McDonald, 1997). Thus, it is in the interest of the consumer that only potatoes with low glycoalkaloids are processed to improve taste and safety.

Several studies have shown that baking, boiling, and frying does little to decrease glycoalkaloid contents (Bushway *et al.*, 1983; Friedman and Levin, 1992). Boiling sprouted potatoes results in diffusion of glycoalkaloids from the sprouts into the tubers (Gonmori and Shindo, 1985). Takagi *et al.* (1990) reported that microwaving potatoes reduced the glycoalkaloid contents by about 15%. They also observed that deep-frying oil at 150°C caused little change but frying at 210°C resulted in 40% loss of glycoalkaloids. Chungcharoen (1988) showed that glycoalkaloids were stable in cooking oil at 180°C.

1.7 Pest resistance

The evidence for increased protection by glycoalkaloids from damage to insects is compelling. Several studies have reported that foliar glycoalkaloids imparted resistance to Colorado potato beetle, *Leptinotarsa decemlineata* Say (Levinson, 1976; Tingey, 1984). Sinden *et al.* (1991) reported that the nature of the glycoalkaloid was more important than the amount. Commersonine and the leptines found in *S. chacoense* were more effective against the potato beetle than α-chaconine and α-solanine. In other related studies, Tingey *et al.* (1978) and Raman *et al.* (1979) reported that the resistance to potato leafhopper, *Empoasca fabae* was highly correlated with foliar glycoalkaloid contents.

Sinden *et al.* (1973) found that the potato alkaloids inhibited the growth of *Alternaria solani* while the older leaves with lower glycoalkaloid levels were more susceptible. They concluded that glycoalkaloids impart some measure of resistance to fungal attack. Olsson (1987) observed that some genotypes with high glycoalkaloid levels were less resistant to fungal damage, suggesting that selection of low-glycoalkaloid potato cultivars may not always adversely affect the resistance of the potato to some phytopathogens.

Studies have shown that different pathogens may cause different reactions in the potato plant. Subrtová *et al.* (1993) found that infection of potato tubers with soft rot bacterium (*Erwinia carotovora*) caused increased glycoalkaloid production. Tarlakovskii (1981) observed that attack by zoospores of *Phytophthora infestans* induced glycoalkaloid accumulation in leaves. In contrast, Grassert and Lellbach (1987), and Hoogendoorn *et al.* (1992) reported that the glycoalkaloid contents had no effect on potato cyst nematodes *Globodera rostochiensis* and *G. palida*.

Plants with low levels of glycoalkaloids could produce higher levels if stressed by insect attack. For example, Hlywka *et al.* (1994) discovered that plants stressed by Colorado potato beetles produced tubers with a higher glycoalkaloid concentration than unstressed plants. In contrast, plants attacked by leafhoppers showed no increase in glycoalkaloids. These results imply that plants low in glycoalkaloids may need to be protected by other methods such as pesticides.

In conclusion, it is important to note that certain glycoalkaloids are potent against key pests of the potato, and may prove ultimately useful in breeding for resistant varieties. Therefore, scientific attempts to improve on the potato plant should not ignore the fact that glycoalkaloids are produced to protect plant against pests and that removing it entirely will benefit the pest much more than the farmer. The ideal potato plants should, therefore, have high glycoalkaloid content in the leaves to provide protection against insect and pathogens and low levels in tubers to reduce the potential hazard to human (Dao and Friedman, 1996). If this objective is to be achieved, however, considerable research is needed in areas of glycoalkaloid inheritance, routine analysis of types, levels, properties and their food quality/safety aspects.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study Area

This study was carried out at the University of Nairobi, Department of Botany using potato tubers obtained from National Potato Research Centre, Tigoni (NPRC-Tigoni). The centre has a national mandate for potato research and development in Kenya, and was chosen for potato collection because it maintains a database of all the potato varieties grown in the country. In addition, the Centre grows potatoes in replicated field trials under standard conditions (NPRC-Tigoni, 1999). This ensured that the growing conditions and handling of tubers prior to the experiment was uniform. Glycoalkaloid analysis by HPLC was carried out at the Kenya Plant Health Inspectorate Service, KEPHIS, Nairobi.

2.2 Materials

2.2.1 Chemical standards and solvents

All glycoalkaloid standards (solanidine, α-solanine and α-chaconine) were obtained from Sigma Chemical Company. These chemical standards together with the HPLC column, solvents, and Nylon filters were ordered through Ectol Limited, a chemical agent based in the UK. All quantitative determinations were based on comparison with pure standards. For HPLC, standard stock solutions (0.4μg/μl) were made up in THF/0.025M KH_2P0_4/acetonitrile (50:25:25, v/v/v). Working standard of solanidine solutions used for titration were prepared from a stock solution (1mg/ml) made up in absolute methanol.

All solvents used for HPLC were of HPLC grade and were obtained from Sigma Co. Extraction solvents were either of HPLC or Analytical grade. The analytical grade solvents that were used for extraction were obtained from ILRI.

Table 2.1. Glycoalkaloid standards used in the study

Compound	Molecular Weight	Chemical Formula	Catalogue Number	Lot. Number	Storage temperature
Solanidine (Approx. 98%)	397.6	$C_{27}H_{43}NO$	S - 7881	63H0077	2 – 8 °C
α-Solanine (Approx. 95%)	868.1	$C_{45}H_{73}NO_{15}$	S - 3757	89H7031	< 0°C
α-Chaconine (Approx. 95%)	852.1	$C_{45}H_{73}NO_{14}$	C - 3768	47H7075	< 0°C

2.2.2 Light

Fluorescent tubes from Philips (36W/54, 2500 lumens) and sunlight were used as sources of illumination.

2.2.3 Potato Tubers

The CIP clones and commercial potato varieties that were selected for this study are presented in Table 2.2 and 2.3 respectively. All the ten potato varieties and CIP clones were used to establish the glycoalkaloid contents in fresh tubers but only five selected commercial varieties were used for other phases of the research. The tubers were collected from NPRC potato stores after harvest and transported to Plant Physiology laboratory, Department of Botany, Chiromo Campus, where they were processed and analysed according to the aspect of study intended.

Table 2.2. CIP clones used in the study

CIP clones	Tuber colour	Maturity
CIP clone 382155.2	White	Medium
[a]CIP clone 382178.14	Purple	Medium - Late
CIP clone Kp91301.10	White	Late
CIP clone Kp92387.5	Red	Medium - Late
[a] CIP clone Rutuku	Pink	Medium - Late

Source-CIP
[a] CIP clones grown at the Faculty of Agriculture, University of Nairobi, Kabete.

Table 2.3. The commercial potato varieties that were used for the study

Name	Source	Strength	Disadvantage
Nyayo	Unknown	Early maturity	Late blight, BW
Kerr's Pink	UK	Premium price	Late blight, Virus
Desiree	Europe	Good taste	Low demand
Tigoni	KARI/CIP	LB tolerant	Greening, BW
Rosyln Tana	KARI/CIP	Chips, LB	Declining yield
Asante	KARI/CIP	LB	No seed
Furaha	KARI/CIP	LB	Poor cooking quality
Anett	Germany	Early maturity	Poor cooking quality
Dutch Robyjn	Holland	Good market	LB
Romano	Holland	Storability	Market

LB-late blight; BW- bacterial wilt
Source-NPRC, Tigoni, 1999.

2.3 Methods

2.3.1 The establishment of glycoalkaloid contents

Ten commercial potato varieties and five CIP clones were selected for the establishment of glycoalkaloid contents. The tubers of the potato varieties and the CIP clones used for the study were selected based on their replications at planting. Except for Asante and Tigoni, which were planted on 26th October 2000, the rest were planted on 10th November 2000 at the NPRC research farm in Tigoni. All the potato varieties, however, were harvested on 27th February 2001. The CIP clones used were planted at the same farm on 21st April 2001 and were harvested on 5th September 2001. Twelve fresh potato tubers were randomly selected from each of three replicates for uniformity from which four were randomly picked for glycoalkaloid analyses.

2.3.2 The effects of variety and light

Five commercial potato varieties were selected for the study to determine the effect of variety and light on TGA, α-solanine and α-chaconine concentration. These were Kerr's Pink, Asante, Tigoni, Nyayo and Romano. These potato varieties were planted on 25th April 2001 at the

NPRC research farm and were harvested on 28th August 2001. Twelve tubers of each variety were selected and placed in individual boxes (210 × 297mm). As controls, some of the boxes were covered. The boxes were then placed under two fluorescent tubes (36W, 2500 Lumens), left in a green house or left in the dark. There were three replications for each treatment. The potatoes were exposed to the two light sources for seven days from 30 August to 6th September 2001. The room temperature during the exposure averaged 25°C while RH during the same period was 60%. The two fluorescent tubes were 50cm from the tuber surface. After a week, four potato tubers were selected at random, cut into small cubes using a kitchen chopper and freeze-dried in a vacuum freeze-drier before they were ground into powder in a Wiley mill® (Thomas Scientific). The glycoalkaloids were extracted from the powder, and stored at 4°C in a fridge until analysis by HPLC was accomplished.

2.3.3 The Effect of Storage Conditions

Tubers of five commercial potato varieties were stored in a fridge and others were stored at ambient room conditions for two weeks. The varieties that were selected for this were Dutch Robyjn, Kerr's Pink, Nyayo, Tigoni and Asante. These potato tubers used were part of the tubers used for the light exposure experiments. There were three replications for each treatment. Twelve tubers of each variety were placed in net bags and stored under the two conditions from 30th August to 14th September 2001.

2.4 Sample Preparation

Four potato tubers of uniform size were washed with cold water to eliminate extraneous materials and dried with tissue paper. The glycoalkaloid assay was made on unpeeled tubers. The samples were cut into small cubes using a kitchen chopper and after careful mixing sub samples of 100 to 200g were placed in Kilner® jars and freeze-dried overnight in a vacuum

freeze-drier (Chemlab Instruments Inc.). The freeze-dried samples were then ground in a Wiley mill® to pass through a 40 mesh screen (Dao and Friedman, 1994). The powder was stored in a refrigerator at 4°C until they were analysed for glycoalkaloids. Exposure of glycoalkaloid powder to air and/or direct light was avoided by using amber coloured Nalgene® sample bottles. Since tuber tissues are approximately 80% water by weight, the freeze-drying of samples provides a 5-fold concentration of TGA. This allowed for powdered samples to be economically and conveniently stored for a longer period of time prior to glycoalkaloid analysis.

Freeze-drying offers numerous advantages: (1) It stops enzyme-catalysed, wound induced, and moisture dependent compositional changes that may affect the glycoalkaloid contents (Dao and Friedman, 1996); (2) It permits storage and transportation of samples for analysis at different periods; (3) It allows analysis of other potato constituents including proteins, polyphenols, and protease inhibitors (Dao and Friedman, 1994; Friedman, 1996); (4) Extracts of freeze-dried samples are much easier to clean up, as they do not undergo browning during handling as fresh tubers do; and (5) It makes it possible to relate composition to nutrition and safety. In relation to the last point, the same samples can be used for analysis of composition and incorporated into diets for animal feeding studies (Friedman *et al.*, 1996). For these reasons, only freeze-dried samples were used in the study.

2.5 Analysis of Total Glycoalkaloids

The analysis of potato glycoalkaloids has provided a challenge to chemists and biologists for many years as shown by hundreds of publications (Coxon, 1984). The purpose of initial research on glycoalkaloids included the development of methods to isolate, identify and quantify these compounds (Osman *et al.*, 1979). Initially they presented analytical problems in isolation due to their poor solubility in most solvents and in detection by spectroscopy.

The early analysts were only aware of "solanine" as the glycoalkaloid in potato and the only method for its detection involved extraction, purification and weighing (Coxon, 1984). From then until 27 years ago, the major advances in glycoalkaloid analysis were the recognition and separation of individual glycoalkaloids from *Solanum* species by TLC and the development of improved methods for quantitative extraction and colorimetric determination of TGA (Coxon, 1984). These colorimetric chemical methods, however, give the amount TGA and do not distinguish between different glycoalkaloids.

2.5.1 Methods for TGA analysis

In the early work, solanine was determined gravimetrically (Coxon, 1984), but colorimetric methods were soon developed. These colorimetric methods include: (1) Complexes with bromothymol blue (Balcar and Zalecka, 1962); (2) Complexes with methyl orange (Birner, 1969); (3) Titration with bromophenol blue and 10% phenol (Fitzpatrick and Osman, 1974); (4) Colour reaction with antimony trichloride in HCL (Wierzchowski and Wierzchowska, 1961); (5) Colour reaction with formaldehyde in H_2SO_4 (Dabbs and Hilton, 1953); and (6) Colour reaction with paraformaldehyde in 85% phosphoric acid (Clarke, 1958). Technically, method 3 is not a colorimetric method but rather is a titration method because the colour change is not proportional to the glycoalkaloids present but instead indicates when all the glycoalkaloids forms complexes with phenol (Friedman and McDonald, 1999).

The method initially used by Sanford and Sinden (1972) is a combination of the best classical procedures for glycoalkaloid analysis. It involves a lengthy Soxhlet extraction and quantification using the colorimetric reaction with antimony trichloride and HCL. The method was found reliable for the determination of TGA content in *S. tuberosum* but its choice was limited by the time taken per analysis and the very unpleasant nature and toxicity of the colorimetric reagents.

Fitzpatrick and Osman reported the first comprehensive method for TGA analysis in 1974. The method involves extraction with MeOH/chloroform. TGA was quantified by titration with a solution of 10% phenol in methanol containing bromothymol blue. Since the titration measures basic nitrogen, all aglycones are determined. Pure glycoalkaloids are also titrated directly and results used to construct standardisation curves (Coxon, 1984).

The Fitzpatrick and Osman (1974) procedure provides a relatively fast method for TGA analysis. The method is economical in the use of reagents and apparatus and can be carried out in any standard laboratory without specialised equipment (Coxon, (1984). Analysis of all the glycoalkaloids irrespective of their solubility or degree of saturation can be carried out using this method. The analysis may be completed in a few hours and therefore multiple analyses can be carried out simultaneously with minimum effort. This method has been used widely by many workers (Butcher, 1978; Bushway *et al.*, 1980; Speroni and Pell, 1980; Friedman *et al.* (1998) and Kozukue *et al.* (1994)).

2.5.2 TGA extraction

The methods of Friedman *et al.* (1998) and Kozukue *et al.* (1994) with some modifications according to Speroni and Pell (1980) were used to extract and quantify the glycoalkaloids from tuber samples of each variety. Briefly, 2.5g of unpeeled freeze-dried tuber powder were stirred with 35ml of 5% aqueous acetic acid and left to extract for two hours. The homogenised tissue was then filtered through whatman No.1 filter paper in a 9cm Buchner funnel. The extract was collected in a side-arm flask by vacuum filtration. The residue was washed with 15ml of 5% acetic acid; and the wash collected in the same side-arm flask making the total volume about 50ml. The resulting solution was centrifuged for 20 minutes at 4000 ×g to remove the insoluble materials. The resulting pellet was discarded and the supernatant was collected in a round bottom flask.

The flask containing the extract was gently heated to 75^{0}C, removed from the heat and 15ml of 58% aqueous NH_4OH was added to raise the pH to $\geq$10. The glycoalkaloids were rapidly precipitated in an ice bath. To ensure complete precipitation, the solution was refrigerated overnight or cooled in an ice bath for 1 hour prior to centrifugation. The precipitate was collected by centrifugation at 4000 ×g for 30 minutes at 1°C using a refrigerated centrifuge (Heraeus Christ® GMBH). The supernatant was decanted and the precipitate washed twice by centrifugation with 15ml of 2% NH_4OH. The final pellet was collected and placed in a 60°C oven overnight to evaporate the ammonia.

Once NH_3 vapour had dissipated, the dry pellet was dissolved in 20ml of methanol. The resulting solution was evaporated to dryness in a Buchi Rotavapor® (Buchi Laboratories, Zurich, Switzerland). The extracts were kept at reduced temperature to avoid degradation (Fitzpatrick *et al.*, 1977). These extracts were redissolved in 5ml of absolute methanol and titrated with 0.067% bromophenol blue and 10% phenol made up in absolute methanol. The TGA contents were determined by reference to a standard curve of solanidine and expressed in mg/100g Fwt.

2.5.3 Sample clean-up for TGA analysis

In methods of TGA analysis, this step involves precipitation of glycoalkaloids from a solution of dilute acid (Coxon, 1984). The precipitate is washed with NH_4OH and dried before final quantification. NH_4OH precipitation has a disadvantage that while α-solanine is insoluble in basic solutions; α-chaconine is partially soluble (Friedman and McDonald, 1997). Leptines are also soluble at this pH and will be lost. Partial loss of glycoalkaloids can be avoided by using n-Butanol which works very well with pure hydrolysed systems and acetic acid extracts for freeze-dried samples (Friedman and Dao, 1992).

2.5.4 Solanidine standard curve

The TGA content of potato varieties and CIP clones that were selected for this study were determined as solanidine equivalent (mg/100g Fwt) by reference to a standard curve of solanidine. The curve was developed using standard solutions of pure solanidine from Sigma Co. Using a stock solution of solanidine (1mg/ml) in methanol, working standards of concentrations that ranged from 0.1 to 0.6mg/ml were prepared and titrated with a solution of 0.067% bromophenol blue and 10% phenol (analytical grade) made up in absolute methanol. The volumes of titrant solutions that were recorded were plotted against the corresponding solanidine concentration. A correlation equation from the standard curve was computed and used to quantify the TGA content in the potato extracts. The final TGA values in this study were readjusted with the 98% purity value of solanidine and a recovery value of 90.25%.

2.5.5 TGA quantification

A glycoalkaloid stock solution (1mg/ml) was prepared by dissolving 10mg of solanidine (Sigma Chemical Co., UK) in 10ml of absolute methanol. Working standard solutions were prepared by diluting the stock solution with absolute methanol. The extracts were dissolved in 5ml of absolute methanol and titrated with a solution of 0.067% bromophenol blue (3', 3'', 5', 5'' Tetrabromosulphonephthalein) and 10% phenol (analytical grade) made up in absolute methanol. The colour passed from blue through blue-green to a yellow end-point. A 5-ml semi microburet graduated in 0.02ml was used in carrying out the titration. This was to achieve a high accuracy in reading the end point volume of the reaction. The titration measures the basic nitrogen group common to all the aglycones. Blank titrations of absolute methanol were subtracted from the total. The titrations were standardised against known concentration of solanidine.

2.6 Analysis of α-Solanine and α-Chaconine by HPLC

2.6.1 Extraction of α-solanine and α-chaconine

Isolation and quantitative determination of α-solanine and α-chaconine were carried out according to the methods of Friedman *et al* (1998) and Kozukue *et al* (1994) with some modifications based on the available equipment. Briefly, 2.5g of freeze-dried sample powder were stirred with 40ml of THF/H_2O/ acetonitrile (5:3:2, v/v/v) in a Sorvall Omni mixer (Newton, CT) followed by vacuum filtration using a 9cm Buchner funnel fitted with Whatman No.1 filter paper. This mixture was then centrifuged for 30 minutes at 4000 ×g for 20 minutes using a refrigerated centrifuge operating at 1°C. This was aimed at removing the insoluble material. The residue was resuspended in 20ml of extracting solvent and treated as above. The combined filtrates were evaporated to about 4ml on a Buchi Rotavapor®.

0.2N HCL was then added to the concentrate while stirring. The flask was rinsed twice with 10ml of 0.2N HCL and centrifuged at 4000 ×g at 1°C for 20 minutes using a refrigerated centrifuge. The supernatant was then transferred to a 250ml Erlenmeyer flask followed by the addition of 30ml of concentrated NH_4OH to precipitate the glycoalkaloids. The solution was refrigerated overnight or cooled in an ice bath for 1 hour prior to centrifugation to ensure complete precipitation. The precipitate was collected by centrifugation at 4000 ×g at 1°C for 30 minutes and washed twice with 2% NH_4OH.

The pellet was collected and placed in a 60°C oven overnight to evaporate the ammonia. The dry pellet was dissolved in 2ml of a mixture of THF/0.025M KH_2P0_4/acetonitrile (50:25:25, v/v/v). 20μl of each sample was subjected to HPLC for analysis. The samples were reconstituted just shortly prior to HPLC runs and the remains were kept in a fridge in 20ml eppedorf® tubes.

2.6.2 Sample clean-up for HPLC

Impurities were removed from glycoalkaloid samples before they were subjected to HPLC for glycoalkaloid analysis. This was important because potato tissue extract contains impurities and other compounds that may interfere with the separation in the UV range by causing baseline noise and drifts. It could also reduce the life of the column and the entire HPLC system.

The glycoalkaloid extracts from 2.5g of potato varieties and CIP clones used were reconstituted in 2ml of the mobile phase, typically THF/0.025M KH_2P0_4/acetonitrile (50/25/25, v/v/v). The samples were ultra-filtered through a 0.20μm Sartorius Minisart® microfilters by using Plastipak® sterile syringes before they were loaded into the HPLC system.

2.6.3 HPLC solvent requirements

All liquids passing through the HPLC system were clean and of high quality in order to protect the HPLC system and to ensure that the desired results were obtained. This was important because impure or dirty solvents not only cause baseline noise and drift, it also reduce the life of the gradient mixing valves and cause damage to the pump head assemblies.

HPLC solvents used were ultrafiltered using a 250ml Millipore® filter fitted with a Sigma-Aldrich® Nylon-66 membrane filter (Lot No. 10b146771). The Nylon membranes had a diameter of 0.47mm and pore size of 0.22μm. During the run, solvents were further filtered through a solvent filter fitted to the end of each delivery tube from the solvent reservoirs. All solvents were degassed to minimize the risk of air bubbles being trapped in the detection flow cell or in the pump head. Degassing of the mobile phase by priming and equilibration by purging was done before daily runs.

2.6.4 Instrumentation

The HPLC analysis of glycoalkaloids was carried out at the Kenya Plant Health Inspectorate Service, KEPHIS, Nairobi. The analysis was carried out with the aid of a Varian HPLC system (Varian Associates, Inc.) equipped with a Varian 9050 variable wavelength UV-visible detector, Varian 9010 solvent delivery system and a Varian 4400 integrator. The HPLC system was fitted with a manually operated Rheodyne® 7125 sample injector and a 20µl loop. The type of column used was Nucleosil NH_2. Operator inputs were generated by front panel key selections on the UV-visible detector, solvent delivery system and the integrator.

2.6.4.1 The wavelength detector

The Varian 9050 variable wavelength UV-visible detector used in this study was a programmable detector controlled entirely from the local front panel keypad. All entries were displayed in fields. The detector has a deuterium lamp, an automatically controlled second order filter and operates in the spectral range 190-700nm with a wavelength resolution of 1nm. It is operated as a stand-alone HPLC detector or integrated into an HPLC system controlled remotely from a workstation. For this study, the instrument was used in the stand-alone mode. The detector measured the sample absorbance at the selected wavelength. The absorbance was output as an analog signal to an external integrator. The value of peak threshold (PT) was 200, while the attenuation and chartspeed were set at 16 and 1cm/min, respectively.

The appropriate flow cell was fitted prior to the initial runs. The deuterium lamp, which is part of the detector optics, was replaced. A calibration procedure was initiated automatically when the detector was switched on initially and when it was reset. After an initialization period of 30 to 35 seconds, the lamp goes through a calibration and monitor period for approx. 1.5 minutes. The 'Ready' indicator would go on and the detector was usable at full sensitivity after 1 hour.

2.6.4.2 The solvent delivery system

The Varian 9010 solvent delivery system (SDS) used had a reciprocating single piston pump that delivers the solvent. The piston's design and electronically controlled stroke provided a nearly pulseless flow in the system. A mixing chamber assured adequate mixing of solvent prior to injection and a transducer converted the pressure in the solvent system into an electrical output that was digitally displayed on the front panel. All controls for setting pump operating conditions and building methods were located on the front panel.

The elution solvents were stored in external vessels and were drawn into the system by the pump. Checks were always made to ensure that there was sufficient solvent in the reservoirs before daily runs. Proportioning valve positions were labelled, A, B, and C. The solvent reservoirs were also labelled accordingly and connected to the matching proportional valve. The reservoir ends of these solvent lines are terminated with 10μ porous metal filters. When starting up, it was necessary to prime the pump then purge the hydraulics of the pumping system.

The SDS pump required three solvent inlet lines connected to the proportioning valves. In the flow line between the proportioning valves and the pump, the solvents were mixed in a mixing chamber. It was essential that all liquids passing through the pump were clean and of high quality. During preliminary runs, it was noted that KH_2PO_4 was precipitated out within the mixing chamber. For this reason, a mobile phase mixture was prepared before being pumped into the machine and a sufficient supply of distilled water was made available for rinsing the entire system.

2.6.4.3 The sample injector

The HPLC system used in this investigation was fitted with a manually operated Rheodyne[R] 7125 sample injector (Cotati, California, U.S.A) and a 20µl sample loop. The model was a rotary valve designed for HPLC. It is an important component because a precise, quantitative result of HPLC required injection of well-defined sample volumes in a reproducible manner.

Glycoalkaloid samples were always introduced into the rheodyne needle port in the load position by means of a 25µl Rheodyne[R] 702-SNR microsyringe (Alltech Europe, Begoniasraat 5, 9731 EKE, Belgium). By rotating the knob through 60°C switches the valve from load to inject. Upon injection, the solvent flows from the pump, through the loop into the column. Care was taken to ensure that the syringe was pulled out straight to avoid bending or breaking it during sample loading. The rheodyne syringe and the rheodyne sample injector were rinsed thoroughly with distilled water after each sample change and after daily use. To clean the rheodyne syringes, the plunger was removed from the barrel, gently wiped with a lint- free tissue before rinsing with deionized water.

2.6.4.4 The column

The type of HPLC column used was Nucleosil NH_2 (25cm × 4.6mm i.d, Particle diameter, 5µm) (Catalogue No. 5CM 125, Capital HPLC Ltd, UK). The column was packed with Nucleosil NH_2. The column was protected from particulate material by use of on-line filters and pre-column filtration as already mentioned. In addition, the column was never allowed to go dry, as dry stationary phase will shrink, leading to voids. The column was washed thoroughly with water at the end of each day and was securely capped at both ends and stored when it was not in use. The same direction of flow was maintained.

2.6.5 HPLC System, Conditions and Procedure

Isocratic non-aqueous reverse phase (NARP) HPLC was used. Glycoalkaloids were eluted with THF/0.025M KH_2PO_4/acetonitrile (50:25:25, v/v/v) at the flow rate of 1ml/min. The HPLC mobile phase for glycoalkaloid analysis was prepared by combining the required volume of THF with 0.025M KH_2PO_4 solutions, followed by acetonitrile. This sequence was followed to avoid precipitating the KH_2PO_4 salt.

Degassing of the mobile phase by priming for 10 minutes was done before daily runs. Degassing minimised baseline noise by reducing solvent out-gassing at the flow cell and also minimised the risk of air bubbles being trapped in the pump head. Flow rate of the mobile phase was set at 1.0ml/min. and a backpressure of 14.1± 0.2mpa was maintained during the HPLC runs. Chromatography was done at room temperature.

The glycoalkaloid samples were introduced into the rheodyne by means of a 25μl Rheodyne® microsyringe. The size of each injection sample was 20μl. The samples were injected at a uniform time. This was important because the analysis greatly depends on the retention time. The rheodyne syringe was held in place until the run had completed 1 min to ensure that all the injected volume was taken up. After which, the syringe was pulled out straight to avoid bending or breaking it. The solvent flow rate was 1ml/min, and the UV detector was set at 208nm. Glycoalkaloids (solanidine, α-solanine and α-chaconine) were quantified from HPLC chromatographic peak areas by reference to the standard curves that were constructed for use to quantify the compounds.

2.6.6 Standard curves and HPLC runs

Standard curves were run each day by injecting 2µl, 4µl, 6µl, 8µl and 10µl of a mixture containing 1mg each of α-chaconine and α-solanine dissolved in 2.5ml of THF/0.025M KH_2PO_4/acetonitrile (50:25:25, v/v/v). Peak areas were used to determine the amount of each glycoalkaloid since peak area is directly proportional to concentration under the chromatographic conditions of the assay.

After preparing the curves, the dry glycoalkaloid extracts were dissolved in 2ml of a mixture of THF/0.025M KH_2PO_4/acetonitrile (50:25:25, v/v/v), and were ultra-filtered through a 0.20µm Sartorius Minisart® microfilters by using Plastipak® sterile syringes before they were loaded into the HPLC system. 20µl of the filtrate were subjected to HPLC. Each sample was injected two times and all values were averaged.

2.6.7 HPLC glycoalkaloid identification and quantification

The glycoalkaloid standards were used to identify the different HPLC chromatographic peaks. Analytical grade solanidine, α-solanine and α-chaconine used were obtained from Sigma Chemical Co. Equal volumes (20µl) of glycoalkaloid standards of known concentration and potato extracts were injected into the HPLC and run under standard conditions. Identification of these glycoalkaloids in the samples was based on consistent retention times. During HPLC, the retention times of the glycoalkaloid standards and potato extracts were compared. Their similarity and identity were statistically evaluated by t-test.

HPLC quantification of glycoalkaloids was based on chromatographic peak areas. Absolute quantities were derived from peak area-concentration correlation computed from HPLC standard curves of the respective glycoalkaloids.

2.6.8 Recovery experiments

A series of recovery experiments were carried out to establish the extent of recovery of added glycoalkaloids from potato tubers. Specifically, various amounts of solanidine, α-solanine and α-chaconine were added to freeze-dried Tigoni potato powder available from earlier study. The samples were thoroughly mixed, extracted as above, and analysed at the end of each analytical procedure by both titration and HPLC methods. The Recoveries were expressed as a percentage. Final glycoalkaloid content values in the study were readjusted with the overall recovery to compensate for experimental loses.

2.7 Statistical Analysis

Data was analysed using General Statistics (GENSTAT) software (Lawes Agricultural Trust, Rothamsted Experimental Station, 1995). One way and two-way ANOVA, t-test, correlation and regression analyses were used in this investigation. Means were compared with LSD when there was a significant ($P \leq 0.05$) F value in the ANOVA. Transformations were not necessary because all glycoalkaloid determinations were carried out using the freeze-dried potato tubers (Dao and Friedman, 1996).

CHAPTER 3

RESULTS

3.1 Standardization

3.1.1 Recovery experiments

Recovery experiments revealed that the titration method affords an 88 to 94 % recovery of added solanidine (Table 3.1). When solanidine was added in the range 0.5 to 4.0mg to freeze-dried Tigoni potato powder, the extent of recovery increased with the amount added.

Table 3.1. Recovery of solanidine added to freeze-dried Tigoni potato powder[a]

	Added solanidine	Expected total (mg/100g)[b]	Experimental total (mg/100g)	% Recovery[c]
1	0.50	16.19	14.25 ± 1.0	87.63 ± 2.0
2	1.00	16.69	15.03 ± 0.4	89.42 ± 1.2
3	1.50	17.19	15.65 ± 2.4	90.18 ± 0.4
4	4.00	19.69	18.71 ± 1.6	93.75 ± 0.8

---[a] The indicated amount of solanidine was added to 2.5g of Tigoni powder. The samples were mixed, extracted and analyzed by the 5% acetic acid extraction procedure.

---[b] The original TGA content (mg/100g Fwt) of the Tigoni tubers was 15.69 ± 0.18. All values are means of three separate determinations ± standard deviation.

---[c] $\% \text{ recovery} = \frac{\text{Glycoalkaloid recovered}}{\text{Tuber TGA + solanidine}} \times 100\%$

Table 3.2 shows the % recovery of α-chaconine and α-solanine added to freeze-dried potato powder before extraction. Recoveries for α-chaconine and α-solanine ranged from 89 to 95% of the added glycoalkaloids.

Table 3.2. Recovery of α-chaconine and α-solanine added to freeze-dried Tigoni potato powder[a]

Amount of glycoalkaloids added (μg)	% Recovery	
	α-chaconine	α-solanine
20	91.7 ± 3.3	8 9.5 ± 0.8
50	90.8 ± 2.4	93.6 ± 2.7
100	92.8 ± 1.8	94.8 ± 0.9
Mean ± SD	91.8 ± 1.0	92.6 ± 2.8

[a] The original glycoalkaloid content of 2.5g of Tigoni potato powder in mg/100g were 4.46 ± 0.10 and 4.51 ± 0.11 for α-chaconine and α-solanine, respectively. All values are means of three separate determination ± standard deviation.

3.1.2 HPLC standardization

The optimum HPLC operation conditions were developed from trial runs using the glycoalkaloid standards. The most appropriate conditions for best retention and run time included a mobile phase of THF/0.025M KH_2PO_4/acetonitrile (50:25:25, v/v/v) with a flow rate of 1.0ml/min at ambient temperature. Attempts to use a mobile phase composition with 50:20:30 for THF/0.025M KH_2PO_4/acetonitrile, respectively, resulted in the precipitation of the KH_2PO_4 salt both in the reservoir and in the HPLC mixing chamber.

During standardization, it was noted that variation in the retention time of samples analyzed on the same day varied from run to run. However, the variations stabilized in subsequent runs. HPLC runs were avoided during the afternoons due to excessively high column temperatures.

3.1.3 Glycoalkaloid standard curves

Figure 3.1 is a linear plot of the titration of solanidine in the range of 0.1 to 0.7mg by bromophenol. Solanidine, α-chaconine and α-solanine peak area responses were plotted from serial concentrations of 0.02 to 0.18mg/ml, 0.1 to 0.8mg/ml and 0.05 to 0.3mg/ml, respectively. Figures 3.2, 3.3 and 3.4 shows linear relationships for the concentration of solanidine, α-chaconine and α-solanine.

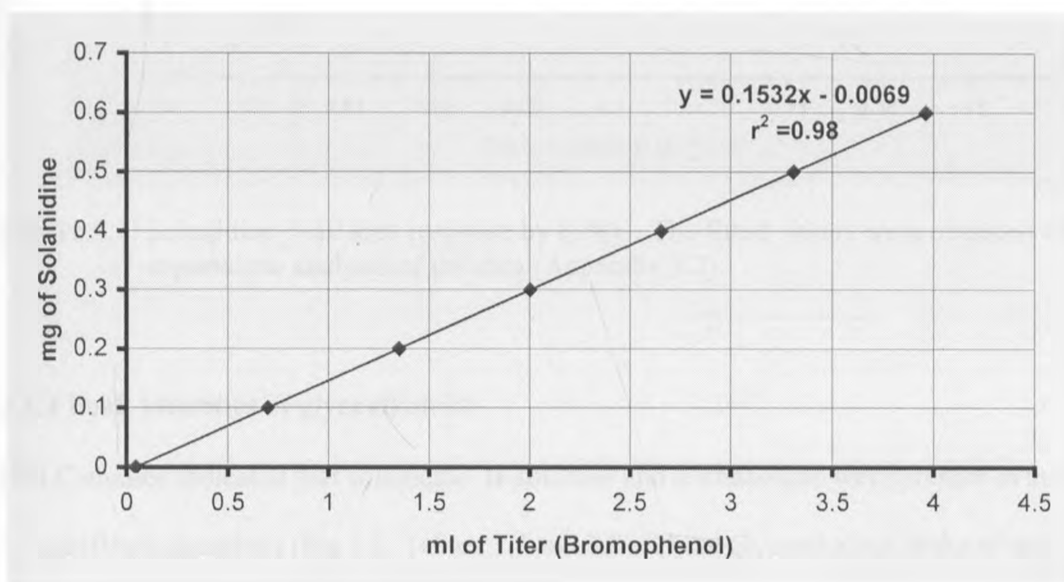

Figure 3.1. Titration of solanidine with bromophenol blue. The fitted values were obtained after regressions analysis of the data (Appendix 3.1).

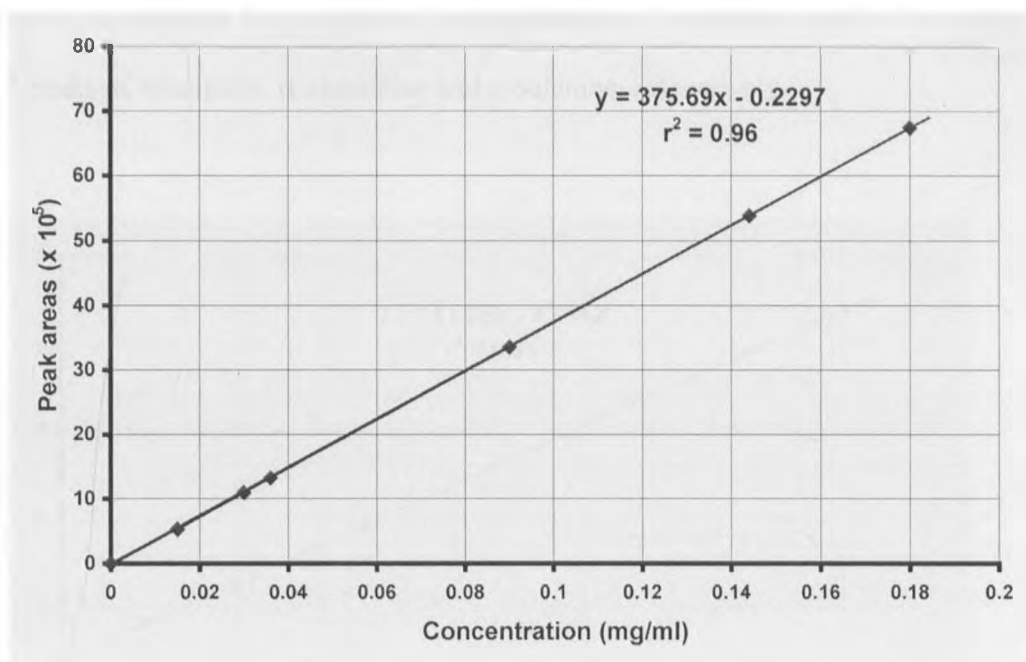

Figure 3.2. Solanidine peak area response by HPLC. The fitted values were obtained after regressions analysis of the data (Appendix 3.2).

3.1.4 Peak identities of glycoalkaloids

HPLC studies indicated that solanidine, α-solanine and α-chaconine were present in most tubers in significant quantities (Fig 3.6, Tables 3.7 and 3.8). These glycoalkaloid peaks of interest were designated as P1, P2 and P3. The mean retention times of these glycoalkaloids were 3.26, 4.57 and 6.04 minutes, respectively (Table 3.2). The three compounds were positively identified as solanidine, α-chaconine and α-solanine, respectively. Beside these three compounds, peaks of some unknown compounds appeared in most of the runs including the standards (Fig 3.5).

3.1.5 Identification of glycoalkaloids

Identification of solanidine, α-chaconine and α-solanine in potato tuber extracts were based on consistent retention times. The retention times of the three compounds from the tubers and the pure standards were compared for similarity. Statistical comparisons showed that the retention times of P1, P2 and P3 were similar to those of solanidine, α-chaconine and α-solanine,

respectively (Appendix 3.2 a, b and c). It was, therefore, concluded that P1, P2 and P3 were HPLC peaks of solanidine, α-chaconine and α-solanine, respectively.

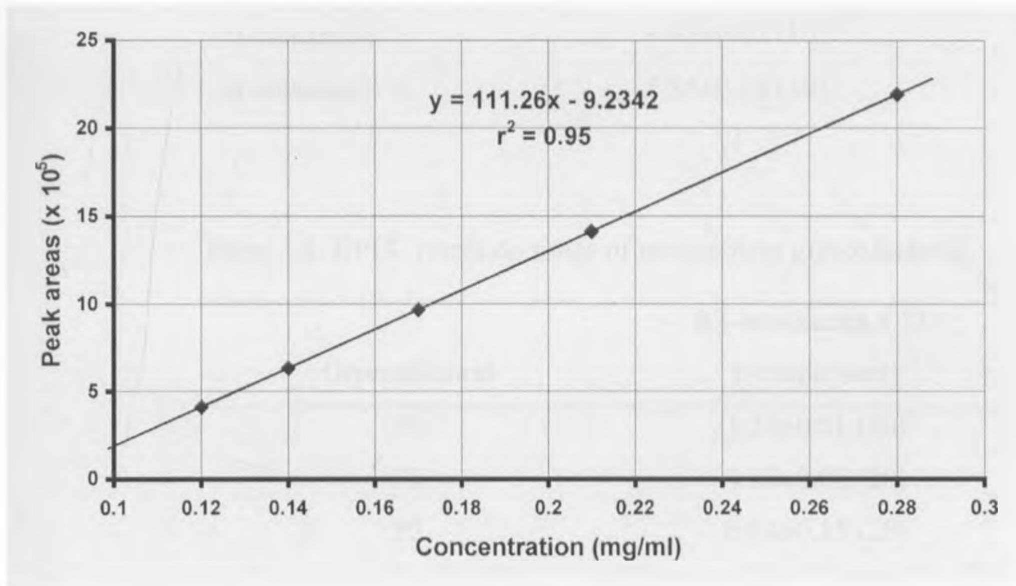

Figure 3.3. α-chaconine peak area response by HPLC. The fitted values were obtained after regressions analysis of the data (Appendix 3.2).

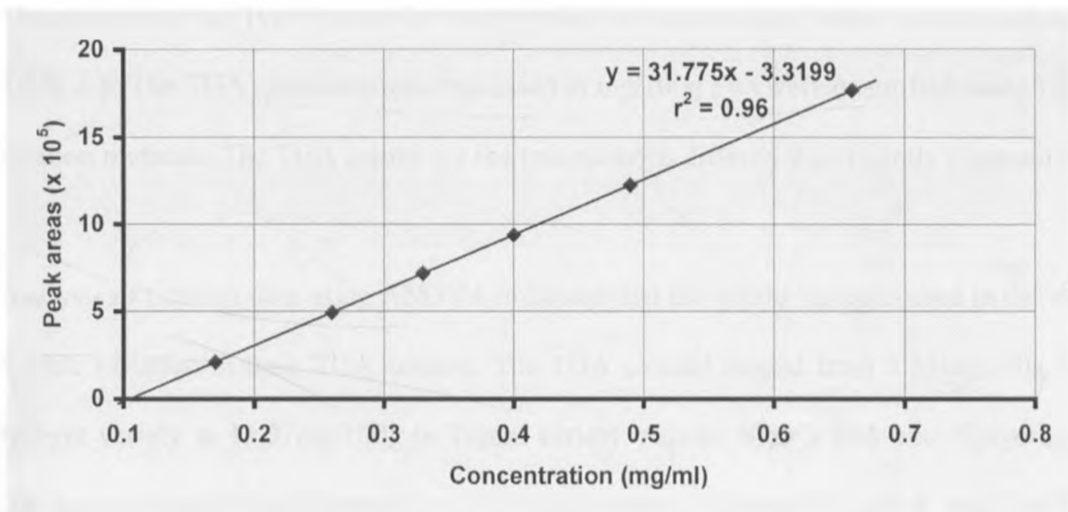

Figure 3.4. α-solanine peak area response by HPLC. The fitted values were obtained after regressions analysis of the data (Appendix 3.2).

Table 3.3. HPLC retention times of glycoalkaloid standards

Glycoalkaloid standard	RT in minutes ± SD (Sample size)
Solanidine	3.34±0.02 (10)
α-chaconine	4.43±0.03 (10)
α-solanine	5.85±0.16 (10)

Table 3.4. HPLC retention times of main potato glycoalkaloids

Glycoalkaloid	RT in minutes ± SD (Sample size)
P1	3.26±0.01 (20)
P2	4.57±0.06 (20)
P3	6.04±0.15 (20)

3.2 Experimental Results

3.2.1 TGA content of potato varieties

The results for the TGA content of tubers from ten commercial potato varieties are shown in Table 3.5. The TGA concentrations expressed in mg/100g Fwt were quantified using HPLC and titration methods. The TGA results for the two methods differed significantly (Appendix 3.1).

Analysis of titration data using ANOVA indicated that the potato varieties used in the study had a wide variation in their TGA content. The TGA content ranged from 3.51mg/100g in Dutch Robyjn variety to 15.97mg/100g in Tigoni variety. Asante, Kerr's pink and Nyayo had larger but approximately equal amount of TGA. In contrast, Romano, Desiree and Roslyn Tana contained lower TGA contents, but that were not significantly (P>0.05) different.

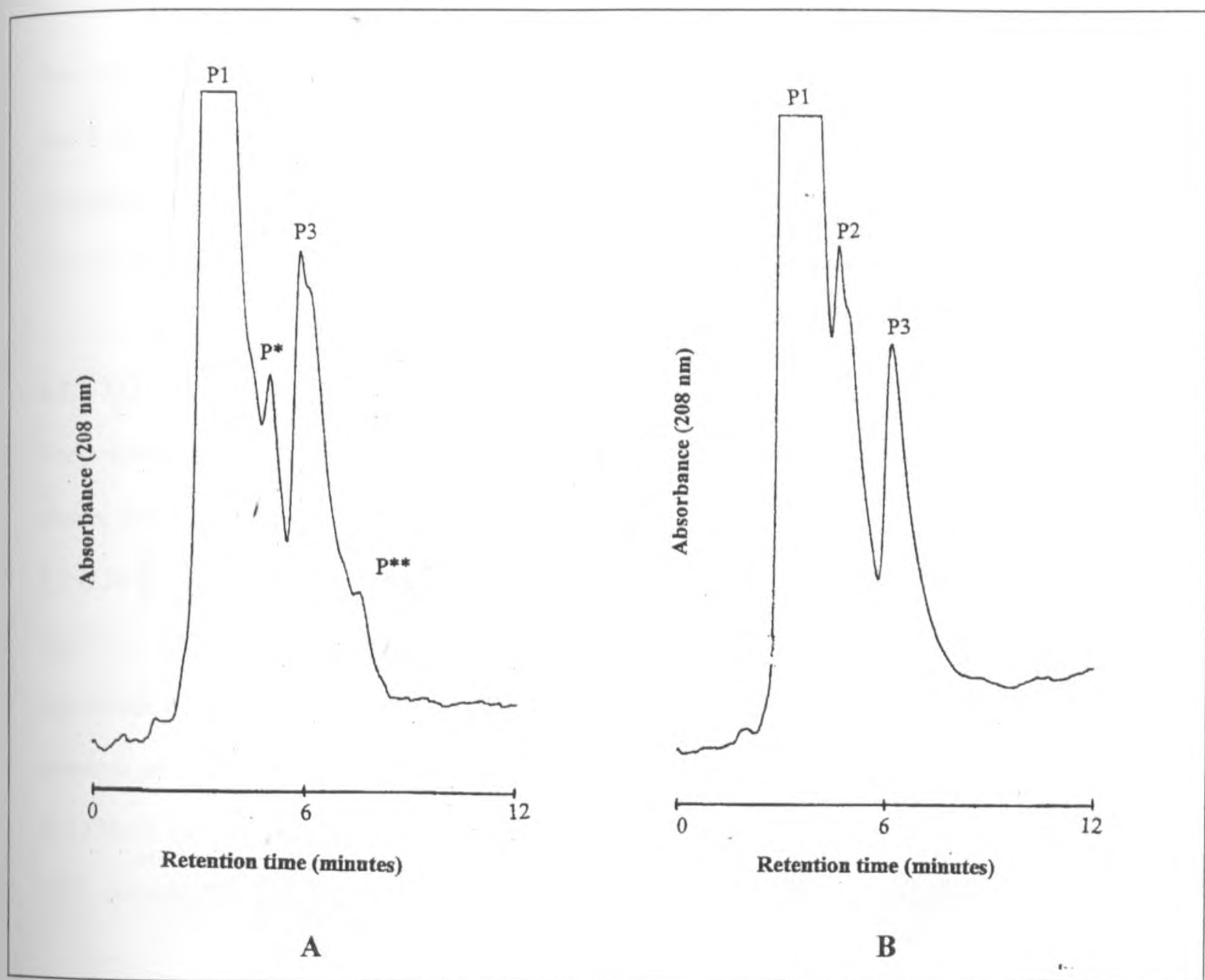

Figure 3.5. HPLC chromatograms of glycoalkaloid standards of (A) 3.2μg of α-solanine.

Peaks: P1=solanidine, P3=α-solanine, P*=unknown peak and P**= unknown tailing shoulder. (B) α-chaconine and α-solanine of 2μg each. Peaks: P1=solanidine, P2=α-chaconine and P3=α-solanine. (Mobile phase: THF/0.025M KH_2P0_4/acetonitrile. Flow rate: 1.0ml/min. Detection: 208nm).

The titration results indicated that Tigoni potato variety had a significantly (P<0.05) higher TGA content compared to the other test varieties. Dutch Robyjn had the least TGA content. No significant (P>0.05) difference was observed between TGA content of Desiree and Romano.

From the HPLC results there is evidence that the mean TGA content in Desiree, Romano and Rosyln Tana were not significantly (P>0.05) different. Similarly, the TGA content of Furaha, Anett and Rosyln Tana were not significantly different. Asante, Kerr's Pink and Nyayo contained higher but approximately equal amount of TGA. In general, TGA was found to range from 5.31mg to 15.38mg/100g Fwt. 60% of the test varieties had below 9mg/100g Fwt.

3.2.2 TGA content of CIP clones

The amount of TGA as determined by the titration method significantly differed among the CIP clones evaluated in the study (Table 3.6). The TGA contents of the CIP clones KP91301.10 and KP92387.5 were significantly higher compared to the other clones. Rutuku and CIP clone 382155.2 contained lower TGA contents of about the same amount. Similarly, there was no significant difference in TGA content between the CIP clones KP91301.10 and KP92387.5. The contents of CIP clones 382155.2, 382178.14 and Rutuku were all below 7mg/100g. CIP clone 382178.14 had the least TGA content of 3.49 whereas CIP clone KP92387.5 had the highest TGA content.

The TGA contents of the CIP clones obtained from HPLC method showed that the clonal effect on the TGA content was highly significant. The TGA content of CIP clone KP91301.10 was not significantly different from that of CIP clone KP92387.5 The TGA content of CIP clone 382178.14 was significantly lower than that of the other test clones. The coefficient of variation was 7.4%. The greatest difference in the TGA content of the samples determined from the two methods was 44.5 % in CIP clone 382178.14 with most other clones varying at below 25%.

Table 3.5. TGA contents of the commercial potato varieties

	TGA (mg/100g Fwt)	
Variety	HPLC method	Titration method
Tigoni	15.39	15.97
Asante	12.11	13.49
Rosyln Tana	8.48	5.04
Furaha	9.18	11.51
Kerr's Pink	12.27	13.69
Desiree	7.73	5.70
Romano	7.73	5.75
Anett	8.88	6.22
Dutch Robyjn	5.31	3.51
Nyayo	12.33	14.15
LSD (0.05)	0.81	
SE (m)±	0.40	
CV (%)	5.00	

Table 3.6. TGA contents of the CIP clones

	TGA (mg/100g Fwt)	
CIP clone	HPLC method	Titration method
CIPclone 382178.14	6.29	3.49
CIPclone 382155.2	8.47	6.31
CIPclone KP91301.10	11.99	14.81
CIPclone KP92387.5	12.14	17.48
Rutuku	7.63	6.90
LSD (0.05)	1.21	
SE (m)±	0.58	
CV (%)	7.4	

3.2.3 α-chaconine and α-solanine contents of potato varieties

The results for the α-chaconine contents in tubers ranged from 1.62mg to 4.46mg/100g Fwt (Table 3.7). The α-chaconine contents in tubers of Kerr's Pink, Nyayo, Asante and Tigoni were not significantly ($P>0.05$) different. The α-chaconine content of Dutch Robyjn was the lowest. The differences in the α-chaconine among Dutch Robyjn, Desiree, Romano, Furaha, Anett and Rosyln Tana, the difference was not significant.

The α-solanine content of the potato varieties ranged from 1.45mg to 4.51mg/100g (Table 3.7). The α-solanine content in the tubers of Desiree, Romano, and Rosyln Tana were not significantly ($P>0.05$) different. Similarly, Romano, Rosyln Tana and Anett contained α-solanine of about the same amount. Asante and Kerr's Pink varieties contained approximately equal amount of α-solanine, but that was significantly higher than that of Nyayo and Anett.

3.2.4 α-Chaconine and α-solanine contents of CIP clones

The α-chaconine contents of the CIP clones studied are presented in Table 3.8. The data indicate that Rutuku contained the least α-chaconine content of 2.39mg/100g. There was no significant difference in the α-chaconine content among the CIP clones 382178.14, 382155.2 and Rutuku. Similarly, no significant difference in α-chaconine content between CIP clones KP91301.10 and KP92387.5 was evident. However, the mean α-chaconine content of the latter was significantly ($P<0.05$) higher compared to that of the other test clones.

The α-solanine contents ranged from 1.58mg to 3.62mg/100g Fwt. No significant ($P>0.05$) difference in the α-solanine content was observed in CIP clone 382178.14 and Rutuku, and between CIP clone 382155.2 and Rutuku. The α-solanine content ranged from 1.58mg to

3.62mg/100g Fwt. Overall, the α-solanine content of the CIP clones was highest in KP92387.5 followed by KP91301.10 and was lowest in 382178.14.

Table 3.7. The glycoalkaloid contents of the commercial potato varieties as determined by HPLC

Variety	α-chaconine mg/100g Fwt	α-solanine mg/100g Fwt	Solanidine mg/100g Fwt	TGA mg/100g Fwt
Tigoni	4.46	4.51	6.41	15.38
Asante	3.92	3.51	4.68	12.11
Rosyln Tana	2.64	2.25	3.59	8.48
Furaha	2.51	1.87	4.80	9.18
Kerr's Pink	3.67	3.47	5.13	12.27
Desiree	2.47	1.93	3.33	7.73
Romano	2.57	2.11	3.05	7.77
Anett	2.47	2.44	3.97	8.88
Dutch Robyjn	1.62	1.45	2.24	5.31
Nyayo	4.31	2.81	5.21	12.33
LSD (0.05)	0.98	0.43	0.46	1.00
SE (m)±	0.47	0.20	0.22	0.48
CV (%)	18.6	9.4	6.3	5.9

3.2.5 Solanidine contents

The TGA contents of potato varieties and clones evaluated were determined by calculating the sum of solanidine, α-solanine and α-chaconine. Due to long storage period, α-solanine and α-chaconine had undergone slow enzymatic hydrolysis to yield solanidine that was detectable at levels as low as 0.020mg/ml. At this concentration range, the UV response was linear from 0.02mg to 0.18mg/ml. At higher levels, however, the peak became too large to integrate accurately because it migrates with the solvent front.

The results for solanidine contents in potato tubers followed similar trends to those of α-solanine and α-chaconine, with some shifts across the distribution classes by some individual varieties. For example, Furaha, Romano and Asante had shifts towards lower solanidine content while Furaha had a shift towards higher solanidine content. Similar shifts were observed in the CIP clones studied. Since solanidine determination was not a major concern, their concentrations were used only for TGA determination.

Table 3.8. The glycoalkaloid contents of CIP clones as determined by HPLC

CIP clone	α-chaconine mg/100g Fwt	α-solanine mg/100g Fwt	Solanidine mg/100g Fwt	TGA mg/100g Fwt
CIP clone382178.14	2.39	1.58	2.32	6.29
CIP clone 382155.2	2.62	2.20	3.65	8.47
CIP clone KP91301.10	3.51	3.34	5.14	11.99
CIP clone KP92387.5	3.80	3.62	4.72	12.14
Rutuku	2.27	1.61	3.75	7.63
LSD (0.05)	0.43	0.61	0.96	1.39
SE (m)±	0.18	0.26	0.42	0.60
CV (%)	7.8	13.1	13.0	7.9

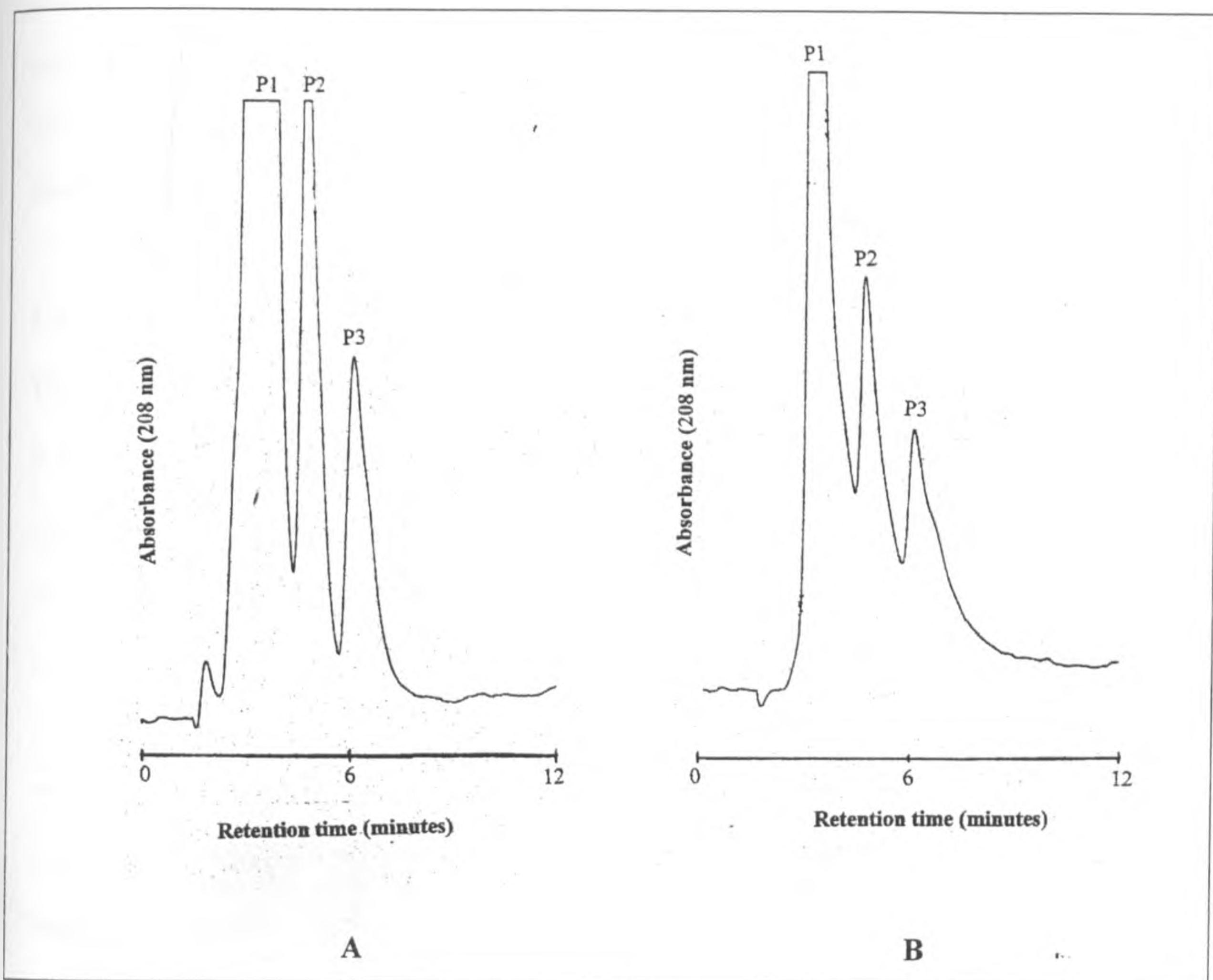

Figure 3.6. HPLC chromatograms of glycoalkaloids from fresh potato tubers of (A) Nyayo variety; (B) CIP clone KP91301.10. Peaks: P1=solanidine, P2=α-chaconine and P3=α-solanine (Mobile phase: THF/0.025M KH_2PO_4/acetonitrile. Flow rate. 1.0 ml/min. Detection. 208nm).

3.3 The effect of light

Table 3.9 shows the glycoalkaloid content of potato tubers that were exposed to light during this study. The concentration of α-chaconine, α-solanine and solanidine were present in all tubers in significant quantities. The effect of variety × light × cover was significant at the 5% level and, therefore, the means presented in the table include all the interactions (Appendix 3.4). There was a general difference in the concentration of α-chaconine, α-solanine and solanidine among the different light treatments indicative of changing glycoalkaloid concentrations and a high dilution factor was used where necessary in order to achieve good peak separation.

3.3.1 Controls

The α-chaconine contents of the controls ranged from 1.62 to 4.46mg/100g Fwt and 1.46 to 4.39mg/100g Fwt for the exposed and the covered samples, respectively (Table 3.9). There was no significant ($P>0.05$) difference in α-chaconine contents among Asante, Nyayo and Tigoni, and between Kerr's Pink and Asante. The effect of cover on α-chaconine contents in the controls was not significant.

The range for α-solanine contents was 1.45 to 4.51mg/100g Fwt for the exposed samples. The corresponding range for the covered samples was 1.38 to 4.09mg/100g Fwt. There was no significant difference between the α-solanine contents of Kerr's Pink and Asante. There was a marked decrease in the TGA content in all the covered controls. The TGA contents ranged from 5.31 to 15.39mg/100g Fwt and 5.10 to 15.07mg/100g Fwt for the exposed and the covered samples, respectively. These glycoalkaloids were significantly lower than the upper safety limit of 20mg/100g Fwt.

In general, there was no significant difference in the glycoalkaloid contents between the covered and the exposed tubers. Tigoni and Dutch Robyjn contained the highest and lowest glycoalkaloid contents, respectively.

3.3.2 Tube light

The data for TGA, α-chaconine and α-solanine contents of potato varieties that were exposed to tube light for seven days are presented in table 3.9. Except for Kerr's pink, there were significantly higher α-chaconine contents in the exposed than in the covered samples. The difference due to cover was highest in Tigoni, followed by Nyayo and lowest in Kerr's pink.

The effect of exposure to tube light on α-solanine content was similar to that for α-chaconine. The α-solanine contents in Kerr's pink, Asante and Nyayo were not significantly different. The contents ranged from 3.61 to 5.54mg/100g Fwt and 3.57 to 5.07mg/100g Fwt for the exposed and the covered samples, respectively.

Under tube light condition, the TGA content for the exposed tubers ranged from 9.43 to 19.01 mg/100g Fwt. The range for the covered samples was from 10.20 to 16.38mg/100g Fwt. The tubers that were exposed to light accumulated significantly higher TGA contents compared to the covered samples and the controls. And as observed in α-solanine determination above, there was no significant difference in the TGA contents of Kerr's pink, Asante and Nyayo.

3.3.3 Sunlight

The glycoalkaloid contents for the potato tubers that were exposed to sunlight were significantly higher compared to the controls and those kept under tube light conditions (Table 3.9).

The α-chaconine contents in the tubers that were exposed to sunlight ranged from 3.67mg to 9.61mg/100g Fwt. The corresponding range for the covered samples was 3.02 to 9.61mg/100g Fwt. There was no significant difference in α-chaconine content between Asante and Nyayo, and between Nyayo and Kerr's Pink. The effect of cover on α-chaconine was significant for Tigoni and Dutch Robyjn, which in addition contained the highest and lowest concentrations, respectively. The chromatograms for the two varieties are presented in Fig.3.7

The α-solanine content for the tubers exposed to the sun ranged from 3.62 to 5.58mg/100g while the range in the covered samples was 3.25 to 4.96mg/100g Fwt (Table 3.9). These results were not consistent with the tube light data in that Asante, Nyayo and Kerr's pink contained higher concentration of α-solanine in the covered samples. The reason for this inconsistency could not be immediately established.

The effect of sunlight on TGA contents was similar to that of α-solanine. The range for the TGA content was 10.80 to 20.10mg/100g Fwt and 9.37 to 15.35mg/100g Fwt for the exposed and the covered samples, respectively. The TGA contents for Asante, Kerr's Pink and Nyayo were not significantly different. In addition, the TGA contents in these three varieties were higher in the covered as compared to the exposed samples. A similar observation was made for the α-solanine contents.

In general, all tubers exhibited an increase in α-chaconine, α-solanine and TGA contents over the 7 days of exposure to light. The glycoalkaloid concentration was relatively higher in potato tubers that were exposed to light compared to the covered samples. Tubers that were exposed to sunlight recorded significantly higher glycoalkaloid contents compared to the controls and the tubers that were exposed to tube light. The effect of light × cover × variety was highly significant. This shows that light is important in the biosynthesis of glycoalkaloids.

Table 3.9. The effect of light on glycoalkaloid contents (mg/100g Fwt)

Light source	Variety	α-chaconine		α-solanine		TGA[1]	
		NC	Cov	NC	Cov	NC	Cov
Controls[2]	Tigoni	4.46	4.39	4.51	4.09	15.39	15.07
	Asante	3.92	3.80	3.51	3.39	12.10	12.00
	K.Pink	3.67	3.61	3.47	3.67	12.28	12.06
	Nyayo	4.31	4.20	2.81	2.71	12.33	12.19
	D.Robyjn	1.62	1.46	1.45	1.38	5.31	5.10
Tube light	Tigoni	8.51	6.49	5.54	5.07	19.01	16.38
	Asante	5.70	5.08	4.98	5.22	15.35	14.28
	K.Pink	5.21	4.90	4.77	4.97	14.36	13.31
	Nyayo	5.87	5.15	5.35	4.23	15.89	12.92
	D.Robyjn	3.49	4.12	3.61	3.57	9.43	10.20
Sunlight	Tigoni	9.61	5.93	5.58	4.96	20.19	15.35
	Asante	4.94	5.16	4.77	4.93	13.93	14.63
	K.Pink	5.75	5.15	4.50	4.83	14.54	14.90
	Nyayo	5.11	5.07	4.88	5.09	14.46	14.78
	D.Robyjn	3.67	3.02	3.62	3.25	10.80	9.37
	LSD (0.05)	0.60		0.49		0.83	
	SE (m)±	0.30		0.24		0.41	
	CV (%)	7.7		7.2		3.8	

NC-Not covered; Cov-Covered

[1]TGA values: calculated as the sum of α-chaconine, α-solanine and solanidine.

[2]Controls: tubers stored in the dark.

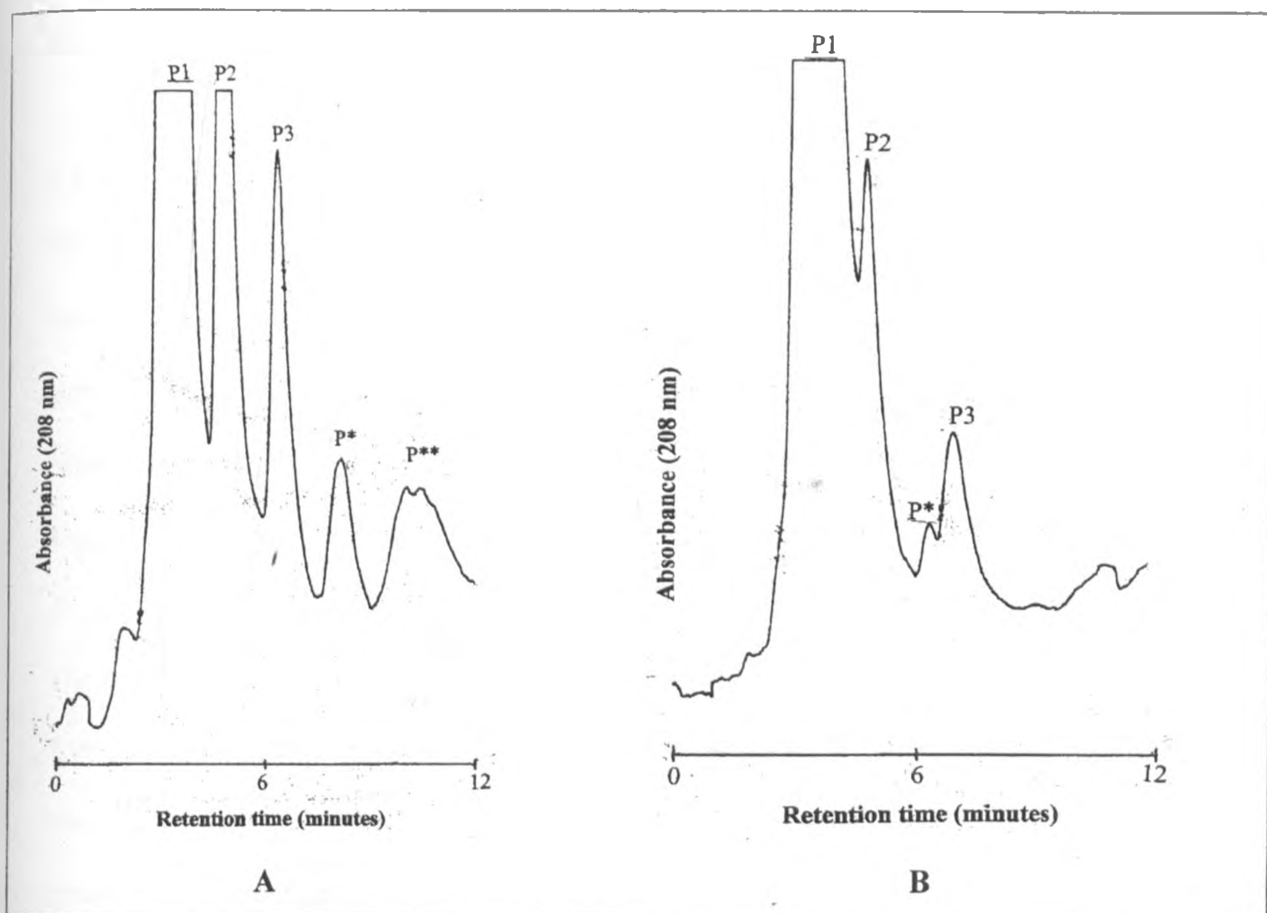

Figure 3.7. HPLC profiles of glycoalkaloids extracted from tubers that were exposed to sunlight for a week. (A) Tigoni. (B) Dutch Robyjn. Peaks: P1=solanidine, P2=α-chaconine, P3=α-solanine, P* and P** unknown peaks (Mobile phase: THF/0.025M KH_2PO_4/acetonitrile. Flow rate: 1.0ml/min. Detection: 208nm).

3.4 The effect of different storage temperature

The HPLC analyses of glycoalkaloid contents in the potato tubers indicated that they were little affected by the storage conditions used in the study. Except for α-solanine, storage × variety interaction was not significant (Appendix 3.5). Analysis of variance was carried out on the glycoalkaloid contents of the potato varieties and the results are presented in table 3.10.

3.4.1 Controls

The control tubers had lower glycoalkaloid contents as compared to the other storage conditions. The α-chaconine contents ranged from 2.60 to 4.40mg/100g Fwt. There was no significant difference in the α-chaconine contents between Furaha and Dutch Robyjn and between Asante and Tigoni. The highest and lowest α-chaconine contents were recorded in Tigoni and Dutch Robyjn, respectively.

The α-solanine and TGA contents in the controls ranged from 1.42 to 4.50mg/100g Fwt and 7.06 to 14.56mg/100g Fwt, respectively. The TGA contents for Dutch Robyjn and Furaha were not significantly different. And as for α-chaconine, α-solanine and TGA contents were consistently higher and lower in Tigoni and Dutch Robyjn, respectively.

3.4.2 Refrigerated conditions

Some of the potato varieties that were stored in the fridge accumulated low TGA, α-chaconine, and α-solanine contents (Table 3.10). The results provide some evidence that fridge condition could afford lower glycoalkaloid contents. The HPLC profiles for Tigoni and Dutch Robyjn, however, indicated that the glycoalkaloids that were present in the tubers before storage were retained and could be detected by HPLC after two weeks of storage (Fig. 3.8).

The concentration range for α-chaconine content was 2.06 to 4.93mg/100g Fwt in Dutch Robyjn and Tigoni, respectively. The two varieties showed increased and reduced α-chaconine contents, respectively as compared to the controls. There was no significant difference in α-chaconine content between Asante and Tigoni.

The values for α-solanine were much lower as compared to the corresponding α-chaconine values. The concentration range was 1.92 to 4.06mg/100g Fwt. The difference in α-solanine contents between Furaha and Asante and between Asante and Tigoni were not significant. Except for Tigoni that recorded reduced α-solanine content, the remaining varieties recorded increased concentrations as compared to the controls.

TGA analyses ranged from 7.98 to 12.00mg/100g Fwt. Dutch Robyjn variety recorded a marginal increase and a significant increase in TGA was recorded in Furaha. In contrast, a marginal and a significant increase were recorded in Asante and Tigoni, respectively. The results seem to suggest that the potato varieties selected for this study had varying concentration of the enzymes required for glycoalkaloid biosynthesis, hence inactivated to varying extents by the low temperature in the fridge.

3.4.2 Ambient temperature conditions

After two weeks of storage at ambient temperature, the α-chaconine contents in the potato tubers ranged from 2.41 to 5.07 mg/100g Fwt. All the test varieties except Dutch Robyjn contained higher α-chaconine contents as compared to the controls. The small decrease in the α-chaconine content in Dutch Robyjn could be attributed to the effect of hydrolytic enzymes after extraction.

The concentration range for α-solanine was 1.85 to 4.54mg/100g Fwt. There was a general increase in α-solanine contents in all the test varieties as compared to the controls. The increase in α-solanine was significant only in Furaha. On the other hand, the HPLC analyses on TGA contents showed a significant increase in Furaha and Dutch Robyjn varieties as compared to the controls. There was a marginal increase and decrease in Tigoni and Asante varieties, respectively.

Some general trends in the glycoalkaloid contents were noted during this investigation. The amount of glycoalkaloids in the tubers of potato varieties was consistently lower in Dutch Robyjn and consistently higher in Tigoni under all experimental conditions used for this study. The occurrence of glycoalkaloids did not exhibit any relations with flesh colour. The tubers of Asante and Kerr's pink, both with red flesh contained approximately equal amount of glycoalkaloids as Nyayo, a variety with white flesh. It should be interesting to investigate the relationship between the glycoalkaloid content and flesh colour. It is important, however, that stress factors such as high light intensity and temperature, which may be capable of inducing glycoalkaloids to rise above 20mg/100g Fwt, should be given more consideration.

Table 3.10. The effect of storage conditions on glycoalkaloid contents (mg/100g Fwt)

Storage condition	Variety	α-Chaconine	α-Solanine	TGA[1]
	Tigoni	4.40	4.50	14.56
	Asante	3.97	3.46	12.40
Controls[2]	Furaha	2.51	1.87	7.38
	D.Robyjn	2.60	1.42	7.06
	Tigoni	4.93	4.06	12.00
	Asante	4.10	3.58	11.76
Fridge	Furaha	3.28	3.09	9.37
Temp. 4 °C	D.Robyjn	2.06	1.92	7.98
	Tigoni	5.07	4.54	14.61
Ambient	Asante	5.02	3.92	11.94
temperature	Furaha	3.50	2.69	9.18
(25°C, RH 60%)	D.Robyjn	2.41	1.85	8.26
	LSD (0.05)	0.86	0.47	1.06
	SE (m)±	0.41	0.23	0.51
	CV (%)	14.2	9.0	6.8

[1]TGA values: calculated as the sum of α-chaconine, α-solanine and solanidine.

[2]Controls: Fresh tubers

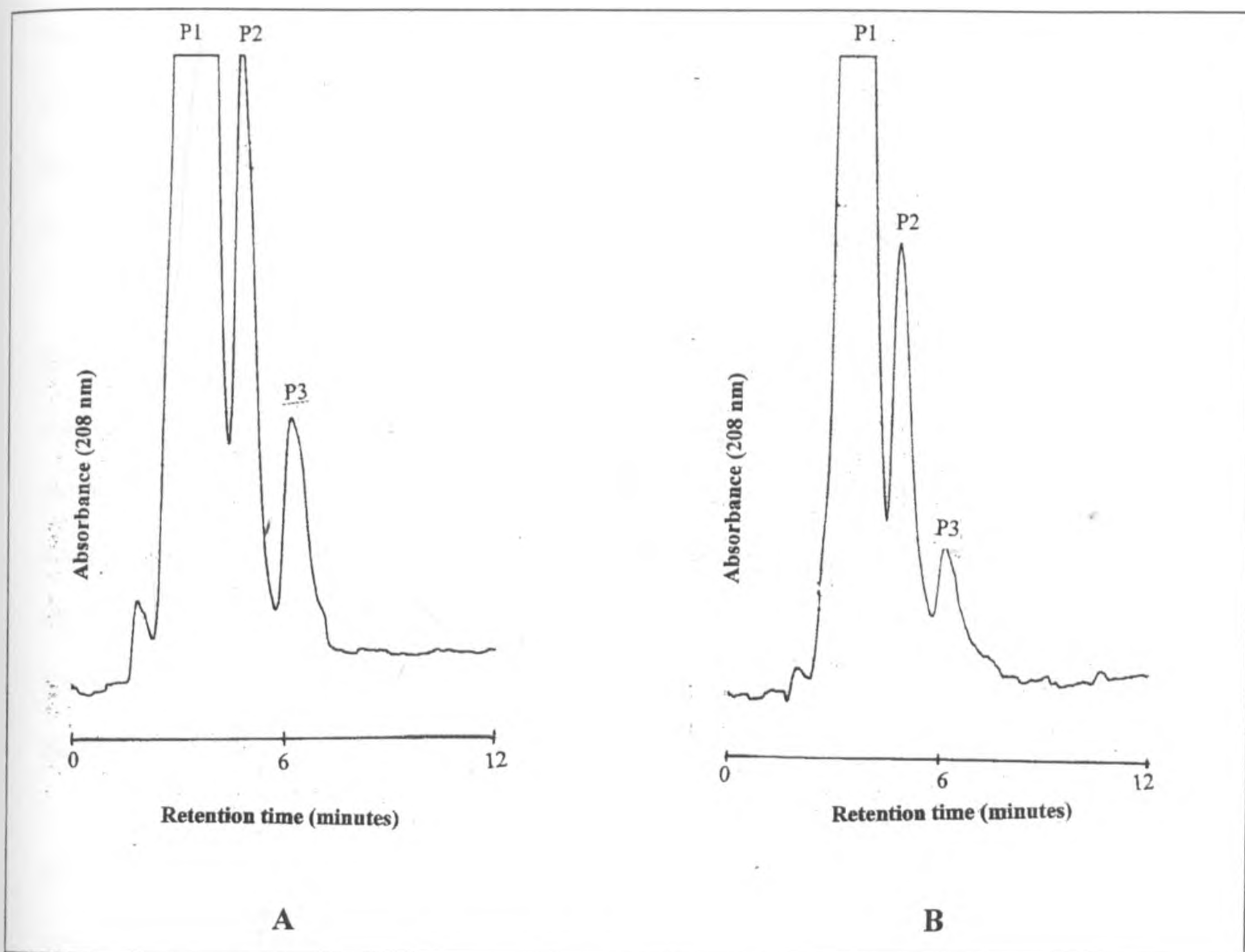

Figure 3.8. HPLC profiles of glycoalkaloids extracted from tubers that were stored in a fridge. (A) Tigoni. (B) Dutch Robyjn. Peaks: P1=solanidine, P2=α-chaconine and P3=α-solanine (Mobile phase: THF/0.025M KH_2PO_4/acetonitrile. Flow rate: 1.0 mL/min. Detection: 208nm).

CHAPTER 4

DISCUSSION

4.1 Techniques

The described extraction, titration and HPLC procedure used for glycoalkaloid analyses in this study demonstrated that it could accurately measure α-chaconine, α-solanine, and TGA contents of potato tubers. The experiments were conducted using tubers harvested from fully mature crops. Freeze-dried samples were used in all experiments because of their smaller variation compared to fresh tubers (Dao and Friedman, 1996).

Since majority of the glycoalkaloids are present near the potato skin, the samples used in the study were not peeled. Therefore, the results reported reflect the amount that would be eaten assuming that the potatoes were oven cooked and consumed together with the 'jackets' (Davies and Blincow, 1984). The 5% acetic acid extraction procedure was used for extracting TGA for titration whereas a mixture of THF/H_2O/acetonitrile (5:3:2, v/v/v) was used for HPLC. Extraction with acetic acid provided a more refined technique for extracting glycoalkaloids. Safety was also improved because of reduced use of organic solvent (Friedman *et al.*, 1994).

During titration, the colour changes from blue to blue-green, to yellow-green, and finally to a yellow-orange end point. The method measures all glycoalkaloids irrespective of their solubility or their degree of saturation and does not include non-nitrogenous steroids (Fitzpatrick and Osman, 1974). In addition, this method utilizes common chemicals as well as inexpensive and simple equipment. Within a day, multiple analyses were carried out simultaneously with minimum effort. The simplicity and the safety of this titration method made it a universally available analytical procedure for potato glycoalkaloids (Fitzpatrick and Osman, 1974). Because of these advantages, bromophenol blue procedure was adapted for tuber TGA analyses for this study.

During HPLC standardization, it was noted that there was variation in the retention times of samples from run to run. Reports of HPLC studies in other compounds have indicated that the variations in retention time are attributed to the mechanical variation in the HPLC system, the mobile phase composition and ambient temperature differences (K'osambo, 1998).

Mechanical variation in the performance of an HPLC system may arise from several sources. It could be in the performance of the solvent delivery system, flow cell, or the variable wavelength monitor (K'osambo, 1998). The performance of these components could be affected by heating from continuous use, oxidative residues clogging the pump heads and leaks in the flow cell. In this study, there was evidence of precipitation of KH_2PO_4 salt in the mixing chamber and so the precipitation of the same salt in other parts of the system could not be ruled out. These could have affected the flow rate and consequently the retention times of the eluting glycoalkaloid species. To minimize this effect, all extracts and solvents were ultrafiltered before they were pumped into the machine and a sufficient supply of distilled water was made available for rinsing the entire HPLC system before and after daily runs.

It has been reported that ambient temperature affects the performance of HPLC components and the interaction of analytes with the stationary and mobile phases (Craft, 1992). In the present study, the Varian HPLC system used was designed to operate within specifications (10 to 35°C) at column pressures. The reason for this was to provide relatively mild conditions that keep side reactions to a minimum (Friedman and McDonald, 1995). HPLC runs were therefore avoided during sunny afternoons to minimize the possible interference by high room temperature.

The effect of the above factors was more pronounced in glycoalkaloid extracts from potato samples than in glycoalkaloid standards. This was because (a) potato sample runs were carried

out over long periods while standard runs were usually done in a single day and (b) larger samples were involved in potato samples than in glycoalkaloid standard analyses. These explained the difference in the variance of the retention times of glycoalkaloids in the potato and glycoalkaloid standards.

HPLC runs of supposedly pure α-chaconine and α-solanine standards were not composed of single compounds. It was noticed that prominent peaks and 'shoulders' of some unestablished compounds appeared in most runs including the standards (Fig.3.5). It is possible that hydrolysis products of α-solanine and α-chaconine could have been present. It is possible that the tailing shoulders that were observed in some runs could have been *cis* isomers (Craft, 1992, Khachik *et al.*, 1992). The *trans-cis* isomerization could have occurred during transport, storage and during analysis.

In spite of the variations discussed above, inferences made from retention time data on the identities of solanidine, α-chaconine and α-solanine were conclusive. The mean retention times of these glycoalkaloids in potato extracts and pure standards were consistent (Tables 3.3 and 3.4). This could be achieved by ensuring that the set experimental conditions were always the same. This is because, under ideal situations, the retention time of the solvent front should always be constant. However, since this is not usually the case, adjustments were made to compensate for the shift in the position of the solvent front. More conclusive proof could be given by further analyses of HPLC glycoalkaloid fractions. The components giving rise to the various peaks are trapped as they emerge from the column and identified separately by techniques such as IR, NMR and Mass Spectroscopy.

4.2 The Established Glycoalkaloid contents

HPLC studies revealed that potato varieties and the CIP clones that were selected contained α-chaconine, α-solanine, and solanidine in significant quantities (Tables 3.7 and 3.8). The results also show that there was a significant cultivar-dependent variation in the glycoalkaloid contents.

The recovery figures were used to adjust the values of glycoalkaloid contents as reported by Blincow *et al.* (1982). Each laboratory should determine the recovery factor so that individual working practices are taken into consideration. In view of this, it is not likely that the recoveries developed for this study underestimates the glycoalkaloid content; nor does available evidence suggests that they are greatly in excess of the expected recovery levels. However, recovery experiments of this sort are only good for ensuring that there is little loss of glycoalkaloids in the clean up and analysis after extraction.

The use of a large recovery factor might have been expected to give rise to considerable higher values of glycoalkaloids. However, for potato varieties, α-chaconine and α-solanine content varied from 1.62mg to 4.46mg/100g Fwt and 1.45mg to 4.51mg/100g Fwt, respectively (Table 3.7). The contents ranged from 2.27mg to 3.80mg/100g Fwt and 1.58mg to 3.62mg/100g Fwt for α-chaconine and α-solanine, respectively, among the CIP clones (Table 3.8). The TGA contents ranged from 5.31mg/100g Fwt in Dutch Robyjn to 15.39mg/100g Fwt in the variety Tigoni. The corresponding values for the CIP clones ranged from 6.29mg to 12.14mg/100g Fwt. This was attributed to the genetic materials that were used by CIP for breeding, which were imported mainly from Lima, Peru. These materials were possibly selected for reduced beneficial agricultural features including low glycoalkaloids before they were imported.

The potato varieties studied represent a large portion of potatoes that are used for table stock and processed products throughout the country. In these varieties, the TGA contents measured

by the titration method ranged from 3.51mg/100g Fwt in Dutch Robyjn to 15.97mg/100g Fwt in the variety Tigoni. These varieties also recorded the lowest and the highest glycoalkaloid contents, respectively. Since Desiree (Urgenta × Depesche), Dutch Robyjn (Rodestar × Preferent) and Romano (Desiree × Draga) were bred in Netherlands (NIVA, 2000), it is possible that they have been improved for reduced glycoalkaloids.

The α-chaconine contents ranged from 1.62mg to 4.40mg/100g Fwt in the potato varieties whereas, the CIP clones had α-chaconine contents that ranged from 2.27mg to 3.80mg/100g Fwt. This observation is in agreement with other reports (Friedman and Dao, 1992; Rodriguez-Saona *et al.*, 1999). Friedman and Dao (1992) reported that the potato varieties that were obtained from Aberdeen had α-chaconine contents that ranged from 1.17mg to 13.5mg/100g Fwt. The α-chaconine levels determined in different potato clones at USDA Agriculture Research Service ranged from 0.5mg to 21.2mg/100g Fwt (Rodriguez-Saona *et al.*, 1999).

The accumulation of α-chaconine in potato tubers could be attributed to cultivar differences due to germplasm, developmental stages or environmental/stress factors (Rodriguez-Saona *et al.*, 1999). Tingey (1984) and Roddick *et al.* (1990) suggested that α-chaconine accumulates in the potato plant in order to provide resistance against several insect pests and herbivores. It was hypothesized that the production of a mixture of α-chaconine and α-solanine by potato tubers results in synergistic effects leading to increased plant protection (Roddick *et al.*, 1990; Friedman and McDonald, 1997).

The assay values for α-solanine contents in potato varieties and CIP clones are presented in tables 3.7 and 3.8. α-solanine contents ranged from 1.45mg to 4.50mg/100g Fwt in Dutch Robyjn and Tigoni, respectively. The corresponding values for α-solanine in CIP clones ranged from 1.58mg to 3.62mg/100g Fwt in 382178.14 and KP9238.5, respectively. These CIP clones

also recorded the lowest and the highest α-solanine contents, respectively. These results are within the limits reported in other countries. Several potato varieties that were grown at Aberdeen contained α-solanine that ranged from 0.58mg to 5.9mg/100g Fwt (Friedman and Dao, 1992). Christen (1997) reported that the α-solanine concentration of Australian varieties ranged from 0.0mg to 5.1mg/100g Fwt.

As mentioned earlier, it is possible that hydrolysis products of α-solanine and α-chaconine, were present in the HPLC runs of the potato extracts and standards. Guseva and paseshnichenko (1958) reported that potato contain hydrolytic enzymes rhamnosidase, glucosidase and galactosidase, which cleave rhamnose, glucose and galactose residues, respectively. In a related study, swain *et al.* (1978) confirmed that an enzyme preparation from potato sprouts cleaved rhamnose side chain at the 2-position of α-chaconine and further transformed the resulting β_2-chaconine to solanidine, apparently without producing γ-chaconine. The same enzyme mixture produced β-solanine by removal of rhamnose, then γ-solanine by the removal of galactose.

Bushway *et al.* (1988, 1990) reported that the hydrolytic enzymes were present and work under laboratory conditions and they seem to have little activity in intact mature tubers. Generally, only traces of β- and γ compounds were found in tubers although Morris and Peterman (1985) found significant amount of β-chaconine in some Australian varieties that have been proved to contain high glycoalkaloid content. Friedman and Dao (1992) also found β_2-chaconine in potato roots. Friedman and McDonald (1995a) noted that after prolonged storage of sprouted potatoes at 3 to 4°C, the α-chaconine in the sprouts was converted to β_2-chaconine whereas α-solanine was largely unaffected.

In general, the results from this study indicated that no fresh potato variety or CIP clone sampled had glycoalkaloid content exceeding 20mg/100g Fwt. All the commercial potato

varieties evaluated had glycoalkaloid contents that fall within 1mg to 15g/100g Fwt. This distribution of glycoalkaloids implies that they are not likely to pose any public health and safety concern. Therefore, there is no immediate problem in using CIP clones evaluated for the development of new potato varieties. Care must, however, be taken in producing new potato varieties so that glycoalkaloids does not rise to a high level. This will help ensure the safety of future varieties.

4.3 The Effect of Light

This study used two fluorescent tubes (36W/54, 2500 Lumens) and sunlight to investigate the effect of light on glycoalkaloid contents of some commercial potato varieties grown in Kenya. The two light sources were chosen because they are conditions that tubers are exposed to during storage or marketing. For example, ware potatoes are displayed under white light (2000~8000 lux) conditions in most supermarkets and retail outlets (Kozukue *et al.*, 1993). Most hawkers of raw potatoes countrywide and in major towns in Kenya including Nairobi expose their wares to sunlight. It is estimated that the intensity (visible region) of sunlight at the earth surface at noon on a cloudless day is ~100,000 lux (Nobel, 1983). Since these light conditions induce tuber glycoalkaloid production to a higher level appropriate controls are needed.

The results from this study indicated that there was a significant effect of light on glycoalkaloid concentration in potato varieties. All the samples that were exposed to fluorescent tube light (~1500 lux) and sunlight (~100,000 lux) showed a significant increase in glycoalkaloid contents (Table 3.9 and Appendix 3.4). From the results, it was apparent that glycoalkaloid contents had risen in all the samples by the end of the light exposure period. The increase was much greater in the tubers that were exposed to light as compared to the controls and the tubers that were covered. Sunlight increased glycoalkaloid contents to a higher level than tube light (Table 3.9).

The TGA, α-chaconine and α-solanine contents in potato tubers after the exposure period ranged from 10.80mg to 20.19mg/100g Fwt, 3.67mg to 9.61mg/100g Fwt and 3.62mg to 5.58mg/100g Fwt, respectively. The corresponding values for the tubers kept covered under sunlight over the same period of time were 9.37mg to 15.35mg/100g Fwt, 3.02mg to 5.93mg/100g Fwt and 3.25 to 4.96mg/100g Fwt, respectively. The glycoalkaloid contents were lowest and highest in Dutch Robyjn and Tigoni varieties, respectively.

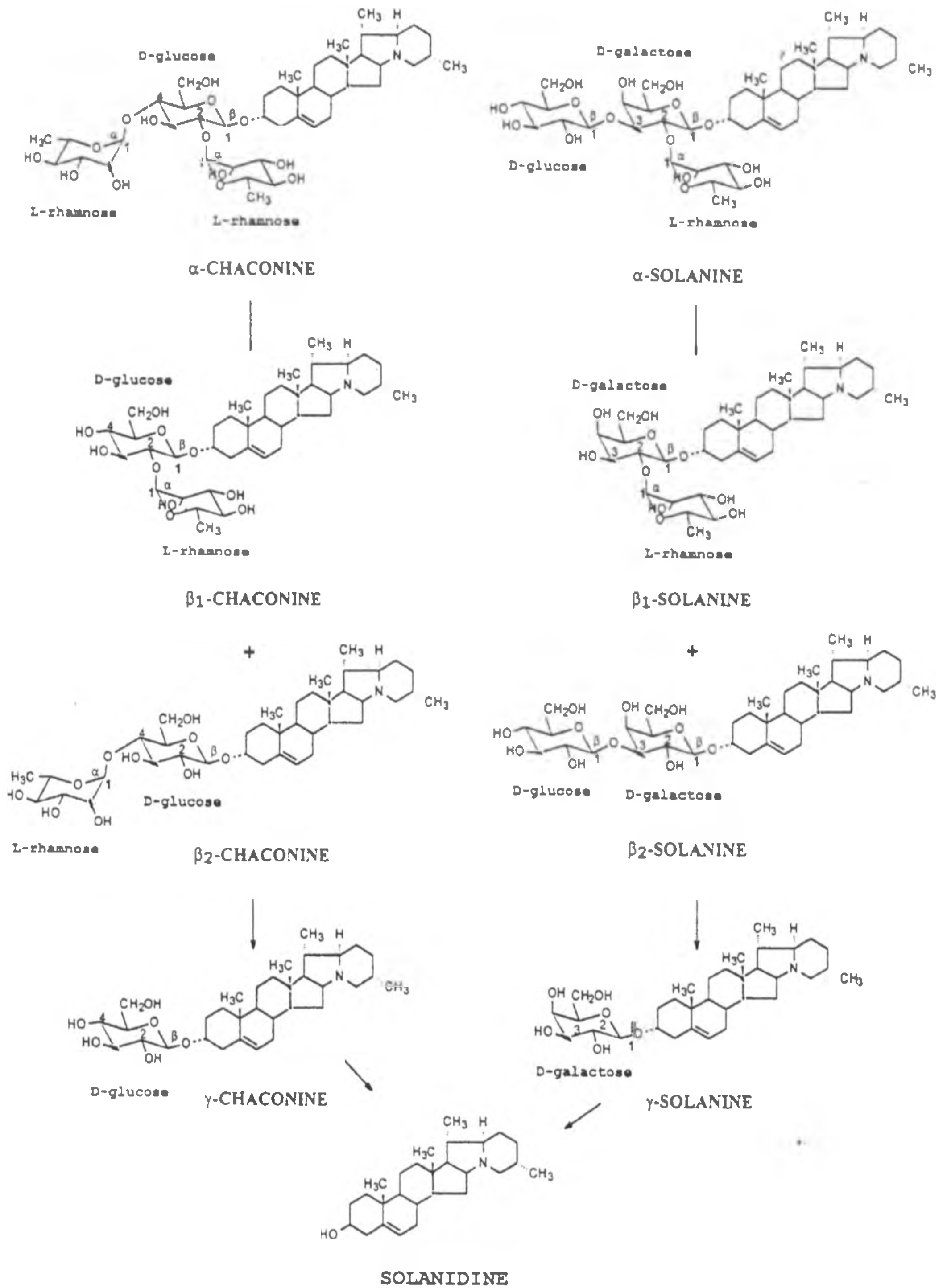

Figure 4.1: Structures of the hydrolysis products of α-solanine and α-chaconine.

Adapted from Friedman and McDonald (1997).

A significant variety × light interaction was established for TGA and α-solanine. This was consistent with a study on glycoalkaloid biosynthesis in response to light in New Zealand varieties (Patchett, 1976). The results were also in agreement with other reports on light-induced glycoalkaloid synthesis. For example, Baerug (1962) reported that TGA contents of harvested tubers of a Norwegian variety increased from 5mg to 20mg/100g Fwt after 6 hours exposure to direct sunlight. Zitnak, (1953) found as high as 45mg/100g Fwt in 'Netted Gem' tubers exposed for 72 hours to intense sunlight under freezing temperature conditions.

The glycoalkaloid contents of tubers that were exposed to tube light were lower compared to those of sunlight (Table 3.9). The tubers that were exposed to tube light also accumulated more glycoalkaloids compared to the controls. In addition, Tigoni and Dutch Robyjn contained the highest and lowest glycoalkaloid contents, respectively. In all the varieties evaluated, the variety × tube light interaction was significant for TGA and α-solanine contents at the 5% level. This observation suggests that both light conditions may be utilized in the production of NADPH and ATP required for glycoalkaloid synthesis. Potato tubers are rich in starch and the need for the synthesis of carbohydrates in general is limited. Therefore, the amount of CO_2 fixed during light is comparatively much less than that for normal plant leaves and most of it is directly reduced to formate and channelled to the synthesis of acetate and mevalonate and eventually to glycoalkaloid biosynthesis. This could be one way to explain why glycoalkaloid synthesis appeared to be coupled to the photosynthesis.

The results from this study indicate that fluorescent tube lights of intensity used (~1500 lux) could enhance glycoalkaloid synthesis in tubers during storage or marketing. This is supported by reports from similar studies (Baerug, 1962; Gull and Isenberg, 1960; Haard, 1977; Jadhav and Salunkhe, 1975). Haard (1977) reported that fewer reports of whole-tuber TGA in excess of 20mg/100g Fwt arising from exposure of potato tubers to artificial light, and suggested that this

could be due to both the wavelength and the intensity. It is also reported that the UV portion of the light spectrum may be more effective than the higher wavelengths in inducing glycoalkaloid synthesis (Conner, 1937). Greening of potatoes due to low levels of artificial light does not necessarily indicate that tubers have excessive TGA contents (Sinden *et al.*, 1984).

During this study, it was observed that α-chaconine was significantly lower in covered treatments. Under field conditions, diffuse sunlight can filter through an adequate layer of soil and induce some glycoalkaloid biosynthesis even if tubers are covered with soil (Sinden *et al.*, 1984). Hutchinson and Hilton (1955) reported that potatoes planted with only minimal soil cover and not hilled up during the growing season developed a higher glycoalkaloid level than those planted deeply and kept well covered. Potato farmers in Kenya and other countries should, therefore, protect growing potato tubers from sunlight by providing sufficient soil cover.

The light enhanced glycoalkaloid formation could also be explained by the fact that the potato is a C_3 plant (Vayda, 1994). High light, high O_2 and low CO_2 coupled with high temperature favours photorespiration. The process involves formation of phospho glycolate in the chloroplast, oxidation to glycolate and amination to glycine in the peroxisomes. The presence of glycine in particular accelerates the incorporation of CO_2 via formate and is essential for the uninterrupted pathway, which guarantees the supply of acetyl-CoA (Ramaswamy *et al.*, 1976). The low rates of glycoalkaloid accumulation in some varieties that were exposed to light could, therefore, be attributed to their low glycine and other enzymes of glycoalkaloid synthesis.

Although, little studies have been done to locate the enzymes and precursors of glycoalkaloids in the plant cells, the evidence from the MVA pathway suggests that it could involve many gene products in sub cellular locations that includes the plastids, mitochondria, peroxisomes, endoplasmic reticulum (ER) and cytosol (Thomas *et al.*, 1990; Monfar *et al.*, 1990). The well coordinated synthesis of glycoalkaloids would ensure a constant supply of ATP, NADH and

acetate for the formation of IPP and FPP. FPP provides a branching point for sesquiterpene phytoalexins, squalene and GGPP for the synthesis of carotenoids, chlorophyll, and ent-kaurenes (Figure 4.2). FPP transferase, which was located in the peroxisomes, converts FPP into squalene used for the subsequent glycoalkaloid production.

Squalene synthase located in endoplasmic reticulum catalyses the condensation of FPP to yield squalene, which rearranged to form cholesterol (David Nes, 1990). The HMG-CoA reductase, which controls the synthesis of cholesterol in the cytosol, was located in the smooth ER between the outer envelope of the mitochondria, at the tonoplast and at the membranes (Thomas *et al.*, 1990). These locations are the proposed sites for the synthesis of chlorophylls, ubiquinones and glycoalkaloids, respectively. IPP isomerase and FPP transferase were located in the peroxisomes (Keller *et al.*, 1985, 1986). GGPP metabolism was located in the plastids. It is also possible that the synthesis of UDP-glucose, UDP-galactose and UDP-rhamnose in the cytosol induces the glycosylation to take place in a favourable manner. The absence of the sugars could induce a solanidine feedback regulation in the biosynthesis of glycoalkaloids. The cytosol location ensures that the intermediates do not diffuse towards a competing pathway as represented in Figure 4.2.

The experimental evidence presented in this work indicates that light stimulates glycoalkaloid biosynthesis in potato tubers. The glycoalkaloid contents of tubers that were exposed to sunlight were significantly higher than that for tubers exposed to tube light and the controls. These suggest it is not likely that exposure of potatoes to tube light for seven days would be long enough to cause significant glycoalkaloid accumulation. Therefore, storage of potatoes in the dark is a very effective means of avoiding high concentration of glycoalkaloids in potato tubers.

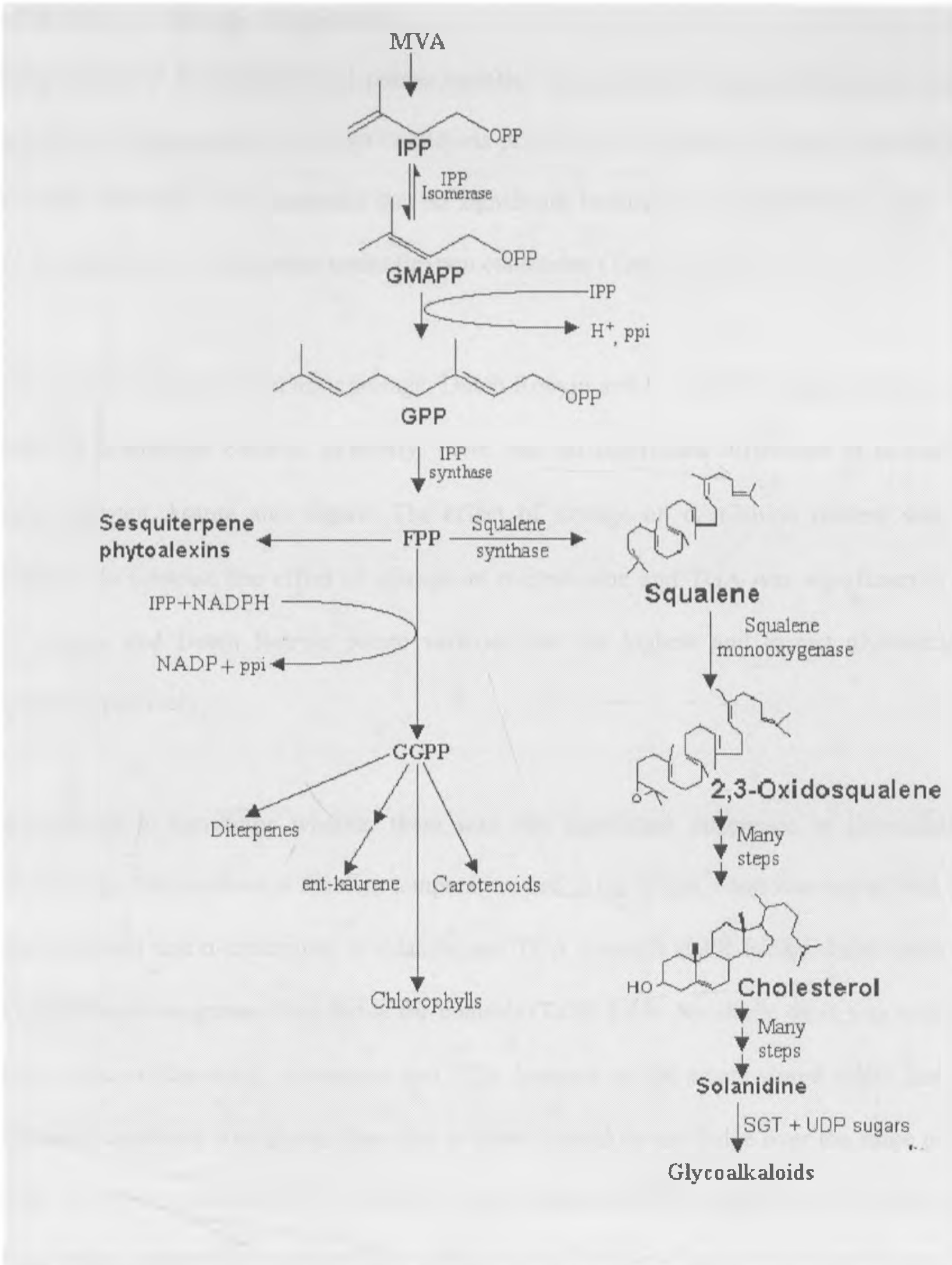

Figure 4.2. Regulation of glycoalkaloid biosynthesis.
MVA – Mevalonic acid,
IPP – Isopentenyl pyrophosphate, FPP – Farnesyl pyrophosphate,
GPP- Geranyl pyrophosphate, GGPP - Geranyl geranyl pyrophosphate,
SGT- Solanidine glucosyltransferase and Solanidine galactosyltransferase.
Adapted from Lyon (2002).

4.4 The Effect of Storage Temperature

Healthy tubers of four commercial potato varieties were selected and stored for two weeks under ambient temperature and fridge conditions prior to glycoalkaloid extraction and analysis. The results from this study indicated that no significant build-up of glycoalkaloids would take place in potato tubers maintained under the two conditions (Table 3.10).

HPLC analyses indicated that after storage, Dutch Robyjn and Furaha had approximately equal amount of α-solanine content. Similarly, there was no significant difference in α-solanine content between Asante and Tigoni. The effect of storage on α-solanine content was not significant. In contrast, the effect of storage on α-chaconine and TGA was significant at 5% level. Tigoni and Dutch Robyjn potato varieties had the highest and lowest glycoalkaloid contents, respectively.

In an attempt to determine whether there was any significant difference in glycoalkaloid contents of the tubers stored at ambient temperature and in the fridge, t-test was carried out. The results indicated that α-chaconine, α-solanine and TGA contents in the tubers stored under the two conditions were greater than that of the controls (Table 3.10). Similarly, there was evidence that the mean α-chaconine, α-solanine and TGA contents in the tubers stored under ambient temperature condition was greater than that of tubers stored in the fridge over the same period of time. However, refrigeration of potatoes is not recommended because they are sensitive to chilling which converts the starches they contain to high levels of sugar that cause potatoes to turn brown very quickly when fried (Friedman and McDonald, 1997).

There is little research that has been done to determine the precise cellular location of glycoalkaloid storage in potato cells. Evidence from varied sources, however, suggests that the

possible deposition site is the vacuole. This proposal is consistent with a suggestion by Hughes and Genet (1973) that all alkaloids in the living systems are stored in the vacuoles. This ensures that the harmful glycoalkaloids are safely removed and do not freely return to the cytoplasm where they can destroy the enzymes of other important pathways. The storage of glycoalkaloids in the vacuoles is also consistent with one report that glycoalkaloids accumulate in the parenchyma cells of the periderm and cortex (Reeve *et al.* 1969). These reports suggest that vacuoles provide a storage compartment that is important in regulation of the glycoalkaloid pathway. They also indicate that the biosynthesis of glycoalkaloids is under interplay of many regulatory controls operating at the organ, cellular, sub cellular and enzyme level. The simplified model presented in Figure 4.3 is consistent with this proposal.

The sub cellular compartments of glycoalkaloid biosynthesis described in this report indicate that there is a physical separation of the reaction sequences leading to the synthesis of glycoalkaloids. This shows that the overall rate of synthesis might be controlled by the transport of intermediates rather than by the rate of individual metabolic conversion. Temperature changes become important in influencing the membrane permeability and stability of the intermediates.

Research on storage temperature and time has demonstrated that these factors play a significant role in glycoalkaloid biosynthesis in potato tubers. However, the conclusions reached by various groups are not always in agreement. For example, Hilton (1951) concluded that low-temperature storage resulted in more bitter tasting potatoes, with bitterness resulting from glycoalkaloid accumulation, than higher temperatures. In contrast, Zitnak (1953) reported that storage at higher temperatures resulted in lower glycoalkaloids than lower temperatures. Potato glycoalkaloids were reported to increase with storage due to sprouting (Kozukue and Mizuno,

1989). The sprouting effect was minimised during this study by the relatively short storage period. All these factors have been shown to increase glycoalkaloid contents during storage.

Several interesting results dealing with glycoalkaloid formation rates in potato slices deserve mention because of the potential application to potato processors. Research strongly indicated that the practice where potatoes were sliced and held for long hours in processing plants that were exposed to high light intensities might increase the glycoalkaloid levels. Salunkhe *et al* (1972) demonstrated that dependent upon storage temperature, the glycoalkaloid contents of potato slices stored in the dark could increase significantly. A similar but expanded study clearly demonstrated that glycoalkaloid content in potato varieties that were maintained at $44^{o}F$ and 85% RH increased with storage (Fitzpatrick *et al*., 1977). TGA contents for most varieties of the potato slices increased dramatically to reach a maximum early in storage then decreased gradually over the storage period. Thus, wound-stimulated glycoalkaloid biosynthesis can best be explained by its effect on respiration.

The respiration process provides ATP and NADH, which are required in the acetate-mevalonate pathway (Perez, 2002). This suggests that the processes such as mechanical damage and sprouting that promote respiration concomitantly increase the FPP pools in the potato cells and consequently increase glycoalkaloid biosynthesis. Charles *et al*. (1990) observed that although infection by pests stimulates glycoalkaloid synthesis, more FPP is diverted to the synthesis of phytoalexins. This could explain the observed higher glycoalkaloid contents in varieties that are resistant to late blight such as Tigoni, Asante and Furaha.

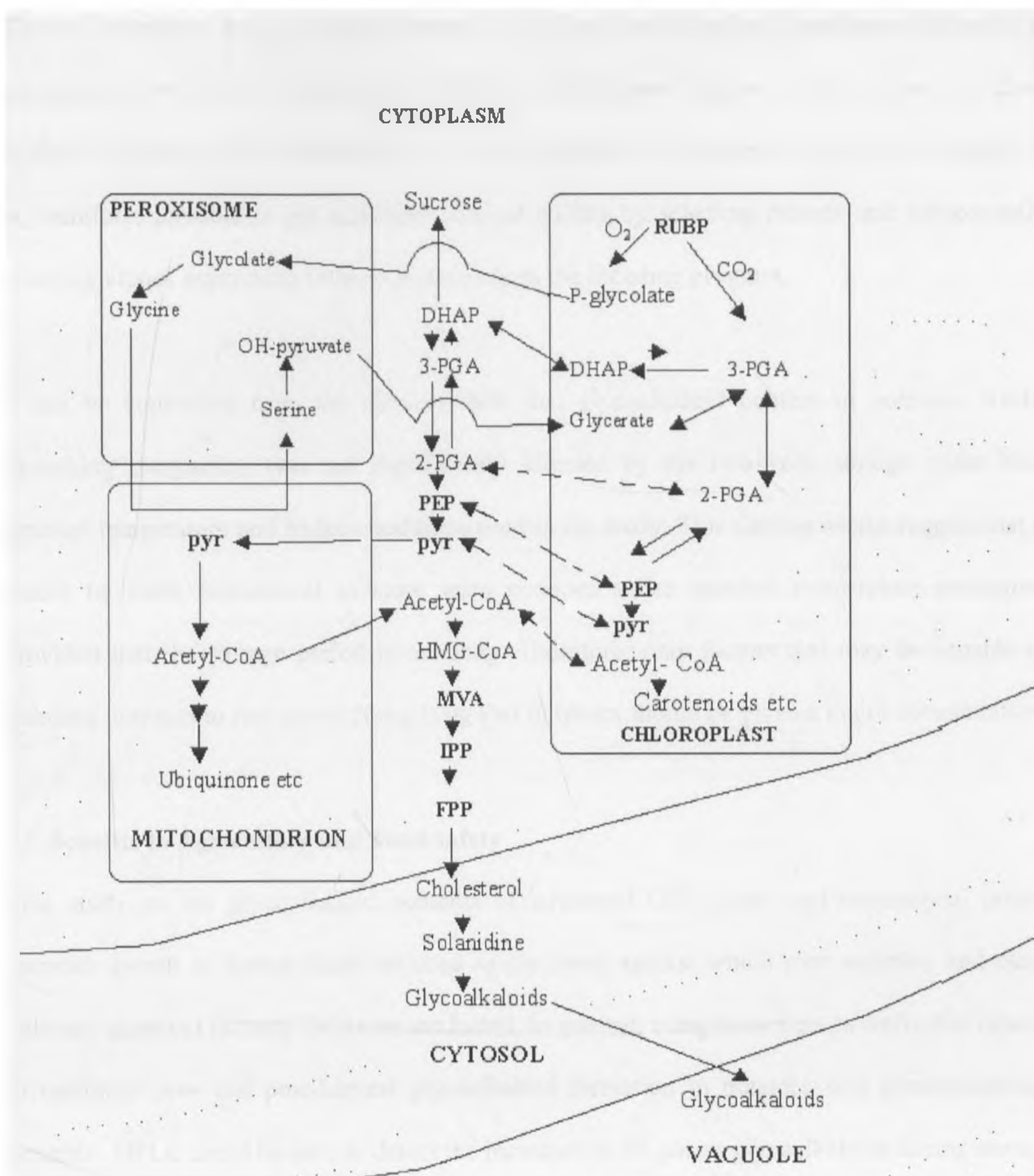

Figure 4.3. A simplified model illustrating the compartmentalization of glycoalkaloid biosynthesis. The abbreviations:

DHAP - Dihydroxy acetone phosphate,
PEP - Phosphoenol pyruvate,
FPP – Farnesyl pyrophosphate,
PGA – Phosphoglycerate, MVA – Mevalonic acid,
IPP – Isopentenyl pyrophosphate,
Pyr – Pyruvate, OH-Pyr – Hydroxy pyruvate,
HMG–CoA– 3-Hydroxy-3-methyl glutaryl coenzyme A.

There is, therefore, enough evidence that glycoalkaloids are complex characters with heritable components, which are influenced by various environmental factors. Little is known of their detailed inheritance. The evidence there is indicates that it is inherited in a polygenic manner. It is, therefore, possible to get materials of good quality by selecting parents and subsequently selecting clones expressing little contents early in the breeding program.

It can be concluded from the above results that glycoalkaloid content of potatoes, whilst increasing marginally, was not significantly affected by the two-week storage under both ambient temperature and fridge conditions used in the study. This finding would suggest that it would be more economical to store ware potatoes under ambient temperature conditions provided that the storage period is not long. Therefore, only factors that may be capable of inducing contents to rise above 20mg/100g Fwt in tubers should be given a major consideration.

4.5 Benefits to Agriculture and Food safety

This study on the glycoalkaloid contents of advanced CIP clones and commercial potato varieties grown in Kenya could be used as the basis against which new varieties and other cultivars grown at farmers' fields are evaluated. In general, using these data as well other reports to minimize pre- and post-harvest glycoalkaloid formation in potatoes will provide several benefits. HPLC could be used to detect the increases in the potato glycoalkaloids during storage and shipping to ensure that the product that reaches the market place is completely safe. Secondly, the breeding programs aimed at improving the agronomic characteristics of potatoes could incorporate glycoalkaloid tests to ensure that new cultivars do not have more than allowable levels of known glycoalkaloids.

4.6 Control of Glycoalkaloid Formation

Because of the toxicological effects of glycoalkaloids discussed exhaustively in chapter one, glycoalkaloid formation in potatoes can be a serious health concern. Adequate and varied methods should, therefore, be available to retard or minimize glycoalkaloid formation.

4.6.1 Genetics

The glycoalkaloid contents of potato varieties can vary significantly (Friedman and McDonald, 1997). Furthermore, Sinden *et al.* (1984), reported that although significant interactions between variety and environment have been demonstrated, a cultivar that synthesizes relatively high levels under one set of environmental conditions will tend to have high levels wherever it is grown (Sinden and Webb, 1972, 1974). when subjected to stresses or improper handling, cultivars with high average contents were more likely to synthesize levels in excess of the 20mg/100g limit.

The potato cultivar 'Lenape' is an instructive example of some of the problems glycoalkaloid biosynthesis can introduce into potato breeding programs. This cultivar was shown to have a high solid content and a low level of reducing sugars which resulted in excellent chipping and storage properties (Akeley *et al.*, 1968). However, because of its high glycoalkaloid content, 'Lenape' had to be withdrawn from the market (Zitnak and Johnston, 1970). This cultivar remains such a superior chipper that it is apparently still widely used in potato breeding programs. The potato variety 'Atlantic', a cross between 'Lenape' and Wauseon, has low TGA contents, and is the standard for chip quality in the USA and Canada. This indicates that Tigoni variety and CIP clone KP92387. 5 could be improved further for agronomic characteristics and reduced glycoalkaloid contents.

It has been reported that glycoalkaloid levels are highly heritable in tetraploid *S. tuberosum* (Sinden *et al.* 1984; and Ross *et al.*, 1978). They observed that once very high levels of TGA levels were introduced from wild species gene sources into parental lines in a breeding program, higher than normal levels would persist amongst some of the offspring. Therefore, wild species with very high glycoalkaloid levels should be used with caution in potato breeding programs. Glycoalkaloid analyses of selected offspring are also necessary to maintain normal levels of TGA among potential varieties.

4.6.2 Packaging

Retail packaging can also contribute to increased glycoalkaloid accumulation (Wayne, 1995). Since many fresh potatoes are marketed in clear polyethylene bags or in bulk displays under grocery and fluorescent lighting, it is important to devise ways to eliminate or greatly reduce the glycoalkaloid contents.

Certain coloured filters (red, violet, green) were shown statistically to result in lower glycoalkaloid levels (Maga, 1980). However, packaging and marketing potatoes in these materials was not practical because of consumer acceptance. It is, therefore, advisable to protect potato tubers from light, breed for resistant varieties and control the storage conditions.

4.6.3 Chemical treatment

Extensive research has been conducted using a wide variety of chemicals and methods of applications (dips, foliar sprays and vapour exposure) in an attempt to control glycoalkaloid formation. Parups and Hoffman (1967) demonstrated that vacuum injection of nicotinic acid could inhibit glycoalkaloid biosynthesis. Patil *et al.* (1971) used this technique to demonstrate the effectiveness of 2-chloroethyl phosphoric acid and succinic acid –2, 2- dimethylhydrazide.

The later compound was found to depress the incorporation of mevalonic acid into isoprenoids (Ryugo and Sachs, 1969).

Other chemicals that have been evaluated and found to be effective in reducing glycoalkaloid biosynthesis includes 2 or 3% common household detergents (Sinden, 1971), various types of oils (Wu and Salunkhe, 1972), post harvest application of 2, -(*p*-chlorophenylthio) and 1,3-dichloropropene and levels of glycine ranging from 10 to 30% (Jadhav and Salunkhe, 1974). Increased health concern over the use of agricultural chemicals, however, restricts the widespread use of most of the chemicals.

In addition to the need to minimize post harvest glycoalkaloid production, studies are needed to reduce pre-harvest glycoalkaloid formation by suppressing the enzymes and genes governing their biosynthesis (Friedman and McDonald, 1997). Such an approach provides a variety of benefits which includes the introduction of some selected varieties that cannot currently be released due to their higher than acceptable levels of glycoalkaloids.

4.7 Molecular Genetics

Friedman and McDonald (1999) reviewed the metabolism of glycoalkaloids in the plant with the goal of reducing the levels of the most toxic compounds. In one study, characterisation of individual steps was carried out and the single step requiring the action of SGT was identified as an appropriate target for genetic manipulation (Moehs *et al.* 1997). The use of brewers' yeast allowed for the successful isolation of the SGT gene, which was used to construct an artificial gene that functioned to inactivate the SGT step without altering the existing genes. This technology was used in Peru, Bolivia, and Equador.

Initial results suggested that this strategy could decrease the activity of solanidine glycoalkaloids by 40% (Stapleton *et al.*, 1991,1992; Moehs *et al.*, 1997; Zimowski, 1997). The presence of the antisense gene copies in the potato genome results in degradation of the message conveyed by the natural gene and as a result the plants made fewer glycoalkaloids. It is hoped that this technology has a tremendous potential to down regulate the glycoalkaloid biosynthetic pathway so as to reduce glycoalkaloids to acceptable level. This will help improve the health of the communities who grow and consume a lot of potatoes.

It has been suggested that other approaches might succeed in achieving the same objectives (Bergenstråhle *et al.*, 1992a; Bushway *et al.*, 1988, 1990; Larini *et al.*, 1996; Osbourn *et al.*, 1996; Sandrock *et al.*, 1996). The first possible approach is to clone and suppress potato genes encoding aminotransferase enzymes that are postulated to catalyse the introduction of nitrogen during the biosynthesis of glycoalkaloids from cholesterol (Fig. 1.3). The second possibility is to clone and suppress potato genes encoding enzymes that catalyse the introduction of galactose and rhamnose moieties into glycoalkaloids. The third option is to introduce genes into potatoes that encode glycoalkaloid-degrading enzymes. Studies are, therefore, required to characterize all the genes, which are responsible for glycoalkaloids. The studies should focus on their promoters, repressors, suitable restriction enzymes, ligases and vectors.

The above discussion of dietary and safety aspects of glycoalkaloids suggests the need to minimize the synthesis of UDP-glucosyltransferase and related enzymes both in the plant and in the tuber after harvest. This could permit the development of new potato varieties that produce glycoalkaloids that are non-toxic or minimally toxic to humans, but would still protect plants from insects and other pathogens. The molecular genetic studies, however, need continuing guidance from safety evaluations in order to develop safe transgenic potatoes.

4.8 Protective Effects of Nutrients

In order to devise new effective ways that prevent the accumulation of the more toxic glycoalkaloids and to manipulate the diet to minimize their adverse effects, an understanding of multiple overlapping aspects of glycoalkaloids are required. This aspect has the most relevance to food safety because people do not eat individual glycoalkaloids but a combination of two or more along with other foods.

Rayburn *et al.* (1995a) reported that glucose–6–phosphate (G_6P) and nicotinamide adenine dinucleotide phosphate (NADP) protected frog embryos against adverse effects of α-chaconine. The authors suggested that G_6P could exert protective effect by competing with carbohydrate groups for acceptor sites of cell membranes in frog embryos. Although, the mechanisms of reducing toxicity were not established fully, it was certain that G_6P and NADP reduce the toxicity of α-chaconine. This suggests that type 2 diabetics who have less G_6P are more vulnerable to glycoalkaloid poisoning.

Type 2 diabetes is the most common form of diabetes (ADA, 2002). The statistics indicate that almost 16 million Americans are diabetics while some 5.4 million do not know that they have the disease (Vern, 2001). 600, 000 new cases are diagnosed every year and nearly 200,000 people die from it. The serious nature of this disease and the fact that the patients have low level of G_6P suggest that complications related to glycoalkaloid intake may arise even for potatoes containing TGA within the upper safety limit of 20mg/100g Fwt. Further risk assessments studies are, therefore, needed to adequately establish the acceptable levels.

Friedman *et al.* (1997b) reported that folic acid protected against α-chaconine-induced disruption of frog embryo cell membranes and developmental toxicity. They proposed that the protective effect might be due to a competition between α-chaconine and folic acid for receptor

sites or channels of the membranes. These observations suggest that ingredients present in complex diets may protect against adverse effects of glycoalkaloids.

In conclusion, efforts should continue to measure the glycoalkaloids individually and in combinations found in different potato varieties and clones. The results will provide information on the mechanism of action of glycoalkaloids, which may facilitate the development of improved potato cultivars. Further study is also needed to determine the ingredients present in the diet that protect against glycoalkaloid toxicity.

CHAPTER FIVE

RECOMMENDATIONS AND CONCLUSION

5.1 Recommendations

The present research as well as other reports warrant making the following recommendations:

(1) Potato growers should maintain sufficient soil cover above the potato seeds and keep enough hills. They should control soil erosion, which may expose the tubers to light. Harvest mature tubers while avoiding bruising, and exposure to light after harvesting.

(2) Select unblemished potatoes and store them in a cool, dark place. Where necessary, low-wattage incandescent light may be used for short periods.

(3) As in the field and storage, light should be avoided during marketing. Potato displays should be located in sections with low light intensity. Incandescent light bulbs may be used, but should be switched off at night to reduce the time potatoes are exposed to light.

(4) Peeling is effective in removing most of the affected tissue. Potatoes with pronounced greening or damage should not be eaten.

(5) Potato breeders should assess the glycoalkaloid contents particularly in the initial parental materials and advanced lines used within a breeding program. Such tests allows for unacceptable lines to be identified and discarded. Backcrossing or crossing with parents of low contents may be used to improve the advanced breeding materials that contain glycoalkaloids in excess of 20 mg/100 g. New potato varieties should be checked for low glycoalkaloid levels (below 20 mg/100g Fwt) before they are released commercially.

5.2 Future research needs

To facilitate progress in enhancing the value of the potato as a safe and nutritious food, we are also challenged to respond to additional research needs as outlined below:

(1) Develop improved sampling, extraction, and analytical methods for glycoalkaloids and metabolites. In this respect more informative methods like gradient HPLC and HPLC-PDA analysis should be used.

(2) Determine the relative susceptibilities to greening, mechanical damage, and increases in glycoalkaloid levels of the newly developed and all the present major commercial potato varieties. Recommend adoption and use of cultivars showing the greatest resistance to greening, bruising, and post-harvest glycoalkaloid production.

(3) Evaluate the changes in TGA throughout the storage life of potatoes with the estimate of amounts removed through peeling as well as the proportion of individual glycoalkaloids.

(4) Evaluate films made from safe agricultural products with built-in-chromophores that absorb light to protect potatoes against greening, browning and spoilage.

(5) Study the responses of different cultivars to environmental factors such as rainfall, temperature, soil types and altitudes.

(6) Evaluate food-compatible enzyme inhibitors such as citric acid and sulphur amino acids and substrate inhibitors for their ability to inactivate SGT and other enzymes catalyzing glycoalkaloid biosynthesis.

5.3 Conclusion

This study provides information on the glycoalkaloid content of some of the advanced CIP clones and commercial potato varieties grown in Kenya. All the varieties and clones evaluated had glycoalkaloid contents that were much less than the upper safety limit of 20mg/100g Fwt. These results are useful in the development of new and more improved potato varieties with proven benefit to health, particularly in terms of glycoalkaloids.

The overall difference between the exposure to light and controls was significant at 5% level. The variety × light interactions for the contents were highly significant for TGA, α-chaconine, and α-solanine contents. It significantly increased closer to the safety limits in some varieties that were exposed to sunlight. The results show that light can stimulate the synthesis of glycoalkaloids to levels above the safe limit (20mg/100g Fwt). The extent to which tubers exposed to different light quantities varies. The period between harvest and consumption should therefore be short to reduce the time potatoes are exposed to light and other conditions. Special care should be taken when handling and marketing potatoes.

The effect of storage temperature on α-chaconine and α-solanine content was significant at the 5% level. However, these glycoalkaloid contents were much less than the upper safety limit of 20mg/100g Fwt. The study demonstrates that no significant build-up of glycoalkaloids takes place under fridge and ambient temperature conditions and any factors such as high light intensity and temperature that induce the contents to rise above 20mg/100g Fwt need to be controlled. Thus, post-harvest handling of potatoes during storage, shipping, and processing should be improved so as to control their glycoalkaloid contents. A better understanding of the fate of the glycoalkaloids from harvest to consumption is also needed to better assess the impact of glycoalkaloids on the quality, safety, and nutritional value of potatoes.

In order to devise new effective ways that prevent the accumulation of the more toxic glycoalkaloids and to manipulate the diet to minimize their adverse effects, an understanding of multiple overlapping aspects of glycoalkaloids are required. Efforts should continue to measure the safety of glycoalkaloids individually and in combinations found in different potato varieties. These studies are relevant to the scientists in the laboratory and field, and would enhance the quality of potatoes.

REFERENCES

Ahmed, S.S. and Müller, K. (1979). Seasonal changes and the effect of Nitrogen and Potash Fertilization on the solanine and chaconine content of various parts of the potato plant. *Z. Pflanzenernähr*, 142: 275-279.

Ahmed, S.S.; Abd-El Goward, A.; Omer, E.A. and El-Balal, A.S. (1988). Variety-Environment interaction in solanine and α-chaconine content of potatoes grown in Egypt. *Egypt J. Hort.*, 15:20-212.

Akeley, R.V.; Mills, W.R.; Cunningham, C.E. and Watts, J. (1968). Lenape: a new potato variety high in solids and chipping quality. *Am. Potato J.*, 45:142-145.

Asano, M.; Goto, N and Isshiki, K. (1996). Simple and Rapid analysis of Potato glycoalkaloids. *J. JPn.Soc. Fd. Sci.Technol.*,43:593-597.

Azim, A.; Shaikh, H.A. and Ahmad, R. (1982).Effect of feeding greened potatoes on different visceral organs and blood plasma of rabbits. *J. Sci. Food Agric.*, 33:1275-1279.

Baerug, R. (1962). Influence of different rates and intensities of light on solanine content and cooing quality of potato tubers. *Eur. Potato J.* 5, 242-251.

Baker, D.C.; Keeler, R.F. and Gaffield, W. (1988). Mechanisms of death in Syrian hamsters' gavaged potato sprout material. *Toxicologic Pathol.*, 16: 333-339.

Baker, D.C.; Keeler, R.F. and Gaffield, W. (1991). Toxicosis from steroidal alkaloids of *Solanum* species. In: *Handbook of Natural Toxins.* Keeler, R.F. (ed.). Marcel Dekker, New Yolk, Vol.6, 71-80.

Balcar, E. and Zalecka, M. (1962). Determination of the solasodine content in the leaves of *Solanum aviculare* and *Solanum laciniatum*. *Biul. Inst. Roslin Leczniczych*, 8:90, 1962.

Bell, D.P.; Gibson, J.G.; McCarroll, A.M. and McLean, G.A. (1976). Embryotoxicity of solanine and aspirin in mice. *J. Reprod. Fert.*, 46:257-259.

Bennett, R.D. and Heftman, E. (1966). Separation of closely related steroids by an improved technique for continuous development of Thin-Layer Chromatograms. *J. Chromatogr.*, 21; 488-490.

Bergenstråhle, A. Glycoalkaloid synthesis in potato tubers. (1995). Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Bergenstråhle, A.; Tillberg, E. and Jonsson, L. (1992a). Characterization of UDP glucose: solanidine glucotransferase from potato tubers. *Plant Sci.*, 84:35-44.

Bergenstråhle, A.; Tillberg, E. and Jonsson, L. (1992b). Regulation of glycoalkaloid accumulation in potato tuber discs. *J. Plant Physiol.*, 140:269-275.

Bergenstråhle, A.; Borga, P. and Jonsson, L. M. (1996). Regulation of glycoalkaloid accumulation in tuber discs in relation to glycoalkaloid synthesis. *Phytochemisty* 41:155-161.

Bergers, W.W.A. and Alink, G.M. (1980). Toxic effects of the glycoalkaloids solanine and tomatine on cultured neonatal rat heart cells. *Toxicol. Lett.*, 6:29-32.

Birner, J. Determination of total steroid bases in *Solanum* species. (1969). *J. Pharm. Sci.*, 58:258-259.

Blankmeyer, J.T.; Atherton, R. and Friedman, M. (1995). Effect of potato glycoalkaloids α-chaconine and α-Solanine on sodium active transport in frog skin. *J. Agric. Food Chem.* 43:636–639.

Blankmeyer, J.T.; Stringer, B. K.; Rayburn, J. R. and Friedman, M. (1992). Effect of potato glycoalkaloids α-chaconine and α-solanine on the membrane potential of frog embryos. *J. Agric. Food Chem.*, 40:2022-2026.

Blincow, P.J.; Davies, A.M.; Bintcliffe, E.J.; Clydesdale, A.; Drapper, S.R. and Allison, M.J. (1982). A screening method for the determination of glycoalkaloids in tubers of potato varieties. *J. Natl. Inst. Agric. Bot.*, 16:92-97.

Brown, M.S.; McDonald, G.M. and Friedman, M. (1999). Sampling leaves of young Potato (*Solanum tuberosum*) Plants for Glycoalkaloid Analysis. *J. Agric. Fd. Chem.*, 47,2331-2334.

Budavari, S. Ed. (1996). *Solanine*. In: Merck Index. 12th ed., Merck & Co. Inc., Whitehall, NJ.

Burton, W.G. (1967). The Potato: A survey of its History and of factors influencing its Yield, Nutritive Value, Quality and Storage. Second Edition. H. Veenman & Zonen N. V. Wageningen, Holland.

Burton, W.G. (1969). The sugar balance in some British Potato Varieties during storage. II. The

effects of tuber age, previous storage temperature, and intermittent refrigeration upon low-temperature sweetening, *Eur. Potato J.*, 12, 81-95.

Burton, W.G. (1989). *The potato*. Longman Scientific, Harlow, England, 421-596.

Bushway, A.A.; Bushway, R.J. and Kim, C.H. (1988). Isolation, Partial purification and Characterization of a potato peel glycoalkaloid glycosidase. *Am. Potato J.*, 65:621-631.

Bushway, A.A.; Bushway, R.J. and Kim, C.H. (1990). Isolation, Partial purification and Characterization of a potato peel α-solanine cleaving glycosidase. *Am. Potato J.*, 67:233-238.

Bushway, R.J. and Ponnampalam, R. (1981). α-chaconine and α-solanine content of potato products and their stability during several modes of cooking. *J. Agric. Food Chem.*, 29:814-817.

Bushway, R.J. (1983). Sources of Alkaloid and Glycoalkaloid Standards for Potato Breeding Programs and other Research. *Am. Potato J.*, 60:793-797.

Bushway, R.J.; Barden, E.S.; Wilson, A.M. and Bushway, A.A. (1980). Analysis of potato glycoalkaloids by HPLC. *J. Food Sci.*, 45:1088-1089.

Bushway, R.J.; Savage, S.A. and Ferguson, B.S. (1987). Inhibition of acetylcholinesterase by solanaceous glycoalkaloids and alkaloids. *Am. Potato J.*, 64:409-413.

Butcher, H. (1978). Total Glycoalkaloids and Chlorophyll in potato cultivars bred in New Zealand. *N. Z. J. Exp. Agric.*, 127-130.

Caldwell, K.A.; Grosjean, O.K.; Henika, P.R. and Friedman, M. (1991). Hepatic Ornithine Decarboxylase Induction by Potato Glycoalkaloids in rats. *J. Food Toxicol.*, 29, 531-535.

Candle, L.S.; Stelzig, D.A.; Harper, K.L. and Young, R.J.(1978). Thin-Layer Chromatographic system for identification and quantification of potato tuber glycoalkaloids. *J. Agric. Food Chem.*, 26: 1453-1454.

Charles, A.W.; Augusto, F.L.; Karen, A.W. and Yue-Ying, R. (1990). Diterpenoid phytoalexins: Biosynthesis and Regulation. In: *Biochemistry of the Mevalonic Acid pathway to Terpenoids*. (eds. Neil Tower, G.H. and Stafford, H.A.). Plenum, New York. Pp.219-248.

Chaube, S.J. and Swinyard, C.A. (1976). Teratological and toxicological studies of alkaloidal and phenolic compounds from *Solanum tuberosum* L. *Toxicol. Appl. Pharmacol.*, 36:227-237.

Chen, S.; Derrick, P.J.; Mellon, F.A. and Price, K.R. (1994). Analysis of Potato shoots and tomatoes by four sector tandem mass spectrometry with scanning- array detection. Comparison of ion positive and negative ion methods. *Anal. Biochem.*, 218:157-169.

Christen, S. (1997). Glycoalkaloid levels in Potato Products. Health protection Service-Food Survey Report.

Chungcharoen, A. (1988). Glycoalkaloid content of Potatoes grown under controlled Environments and stability of glycoalkaloids during Processing. Ph.D. Thesis, University of Wisconsin.

CIP. (1984). Potato for the developing World: A collaborative Experience. International potato Centre, Lima, Peru. P. 150.

Claringbold, W.D.B.; Few, J.D. and Renwick, J.H. (1982). Kinetics of retention of solanine in man. *Xenobiotica*, 12: 293-303.

Clarke, E.G.C. (1958). Identification of solanine. *Nature* (London), 181:1152-1153.

Clement, E. and Verbist, J.F. (1980). Determination of solanine in *Solanum tuberosum* L. tubers: Comparative study of 9 colorimetric methods. *Lebensm.-Wissen. U. Technol.*, 13:202-206 [French].

Conner, H.W. (1937). Effect of light on solanine synthesis in the potato. *Plant Physio*l. 12:79-98.

Cordell, G.A. (1981). *Introduction to Alkaloids-A Biogenetic Approach*, Wiley Interscience, NY.

Coxon, D.T. and Jones, P.G. (1981). A rapid screening method for the estimation of total glycoalkaloids in potato tubers. *J. Sci. Food Agric.*, 32:366-370.

Coxon, D.T. (1984). Methodology for glycoalkaloid analysis. *Am. Potato J.*, 61:169-183.

Coxon, D. T.; Price, K. R. and Jones, P. G. (1979). A simplified method for the Determination of Total Glycoalkaloids in potato tubers. *J. Sci. Food Agric.*, 30: 1043-1049.

Crabbe, P.J. and Fryer, C. (1980). Rapid quantitative analysis of Solasodine, Solasodine Glycosides and Solasodiene by HPLC. *J. Chromatogr.*,187:87-100.

Craft, N.E. (1992). Carotenoid Reverse-phase HPLC methods: Reference compendium. *Methods in Enzymology* 213:185-205.

Crawford, L. and Friedman, M. (1990). The effects of low levels of dietary toxic weed seeds, Jimson weed (*Datura stramonium*) and Sicklepod (*Cassia obtusifolia*) on the relative size of rat liver levels and function of Cytochrome P-450. *Toxicology Letters* 54, 175-185.

Cronk, T.C.; Kuhn, G.D. and McArdle, F.J. (1974). The influence of maturity, Level of Nitrogen fertilization , and storage on the concentration of Solanine in tubers of three Potato cultivars. *Bull. Environ. Contamin. Toxical.*, 11:163-168.

Dabbs, D.H. and Hilton, R.J. (1953). Methods of analysis for solanine in tubers of *Solanum tuberosum*. *Can. J. Technol.* 31:213-220.

Dale, M.F.B.; Griffiths, D.W. and Bain, H. (1992) Glycoalkaloids in Potatoes-Shedding light on an important problem. *Aspects of Applied Biology.*, 33, 221-227.

Dale, M.F.B.; Griffiths, D.W.; Bain, H. and Todd, D. (1993). Glycoalkaloid increases in *Solanum tuberosum* on exposure to light. *Ann. Appl. Biol.*, 123:411-418.

Dalvi, R.R. and Jones, R.H. (1986). Role of microsomal cytochrome P-450 containing monooxygenases in the metabolism and toxicity of certain alkaloids. *Curr. Sci.*, 55:558-561.

Dao, L. and Friedman, M. (1994). Chlorophyll, Chlorogenic acid, glycoalkaloids and protease inhibitor content of fresh and green potatoes. *J. Agric. Food Chem.*, 42:633-639.

Dao, L. and Friedman, M. (1996). Comparison of glycoalkaloid content of fresh and freeze-dried Potato Leaves determined by HPLC and Colorimetry. *J. Agric. Food Chem.*, 44: 2287-2291.

David Nes, W. (1990). Control of Sterol Biosynthesis and its importance to Developmental Regulation and Evolution. In: *Biochemistry of the Mevalonic Acid pathway to Terpenoids*. (eds. Neil Tower, G.H. and Stafford, H.A.). Plenum, New York. Pp.283-327.

Davies, A. M. C. and Blincow, P. J. (1984). Glycoalkaloid content of potatoes and potato products sold in U.K. *J. Sci. Food. Agric.* 35: 533-557.

Dimenstein, L.; Lisker, N.; Kedar, N. and Levy, D. (1997). Changes in the content of steroidal glycoalkaloids in potato tubers grown in the field and in the greenhouse under conditions of light, temperature and daylength. *Physiol. Mol. Plant Pathol.*, 50, 391-402.

Duan, G.M. (1994). Inhibition of potato glycoalkaloids to human blood cholinsterase. *Chinese Biochem. J.*, 10:575-578. [Chinese].

Edwards, E.J. and Coob, A. H. (. 1996). Improved HPLC method for the analysis of potato (*Solanum tuberosum* L.) Glycoalkaloids. *J. Agric. Food Chem.*

Ehmke, A. (1995). Steroidal glycoalkaloids in cell and shoot cultures of *Solanum dulcamara. Plant Tiss. Cult.,* 43: 191-197.

Evans, D. and Mondy, N.I. (1984). Effect of Magnesium on glycoalkaloid formation in potato tubers. *J. Agric. Food Chem.*, 32:465-466.

Evans, S.; Buchanan, R.; Hoffman, A.; Mellon, F.A.; Price, R.K.; Hall, S.; Walls, F.C.; Burlingame, A.L.; Chen, S. and Derrick, P.J. (1993). Structural Characterization of a glycoalkaloid at the femtomole level by means of four-sector tandem mass spectrometry and scanning- array detection. *Org. Mass Spectr.*, 28:289-290.

Ferreira, F.; Moyna, P.; Soule, S. and Vazquiez, A. (1993). Rapid determination of solanum glycoalkaloids by TLC Scanning. *Journal of Chromatogr.*, 653:380-384.

Filadelfi, M.A. and Zitnak, A. (1983). A simple TLC Standard for Identification of Potato Glycoalkaloids. *Can. Inst. Fd. Sci. Technol J.*, 16:151-153.

Fitzpatrick, T.J. and Osman, S.F. (1974). Comprehensive method for determination of total glycoalkaloids. *Am. Potato J.*, 51:318-323.

Fitzpatrick, T.J.; Herb, S.F.; Osman, S.F. and McDermott, J.A. (1977). Potato Glycoalkaloids: Increases and variations of ratios in aged slices over prolonged storage. *Am. Potato J.*, 54:539-544.

Fitzpatrick, T.J.; McDermott, J.A. and Osman, S.F. (1978). Evaluation of injured Commercial Potato samples for Total Glycoalkaloid content. *J. Fd. Sci.*, 43:1617-1618.

Friedman, M. and Dao, L. (1992). Distribution of glycoalkaloids in potato plants and commercial

potato products. *J. Agric. Fd. Chem.*, 40:419-423.

Friedman, M. and Levin, C. E. (1989). Composition of Jimson weed (*Datura stramonium*) seeds. *J. Agric. Fd. Chem.*, 31, 998-1005.

Friedman, M. and Levin, C.E. (1992). Reversed-Phase HPLC separation of potato glycoalkaloids and hydrolysis products in acidic columns. *J. Agric. Fd. Chem.*, 40: 2157-2163.

Friedman, M. and McDonald, G. M. (1995). α-Tomatine content of tomato and tomato products determined by HPLC with Pulsed amperometric detection. *J. Agric. Fd. Chem.*, 43:1507-1511.

Friedman, M. and McDonald, G.M. (1995a). Acid catalyzed partial hydrolysis of carbohydrate groups of the potato glycoalkaloid α-chaconine in alcoholic solutions. *J.Agric.Fd. Chem.*,43:1501-1506.

Friedman, M. and McDonald, G.M. (1997). Potato Glycoalkaloids: Chemistry, Analysis, and Plant Physiology. *Critical Reviews in Plant Sciences*, 16(1):55-133.

Friedman, M. and McDonald, G.M. (1999). Steroidal Glycoalkaloids. In: *Naturally Occurring Glycosides*. (ed. Raphael, I.). John Wiley & Sons Ltd. Pp. 311-343.

Friedman, M. (1994). Improvement in the Safety of Foods by SH-containing Amino Acids and Peptides. *J. Agric. Fd. Chem.*, 42:3-20.

Friedman, M.; Baustita, F. F.; Stanker, L. H. and Larkin, K. (1998). Analysis of potato glycoalkaloids by a new ELISA kit. *J. Agric. Fd. Chem.* 46:5097–5102.

Friedman, M.; Henika, P.R. and Mackey, B.E. (1996). Feeding of potato, tomato and eggplant alkaloids affect consumption and body and liver weights in mice. *J. Nutr.* , 126:989-999.

Friedman, M.; Kozukue, N. and Harden, L. A. (1997). Structure of tomato glycoalkaloid tomatidenol – 3 - β - lycotetraose (dehydrotomatidine). *J. Agric. Fd. Chem.* 45, 154–1547.

Friedman, M.; Levin, C.E. and McDonald, G.M. (1994). α-Tomatine Determination in tomatoes by HPLC Using Pulsed amperometric detection. . *J. Agric. Fd. Chem.*, 42:1959-1964.

Friedman, M.; McDonald, G.M. and Haddan, W.F. (1993). Kinetics of Acid-catalyzed hydrolysis of

Potato Glycoalkaloids α-Chaconine and α-Solanine. *J. Agric. Fd. Chem.* 41, 1397-1406.

Friedman, M.; Rayburn, J.R. and Bantle, J.A. (1991). Developmental toxicology of potato alkaloids in the frog embryo teratogenesis assay–Xenopus (FETAX). *Fd. chem. Toxicol.*, 29:537–547.

Friedman, M.; Rayburn, J.R. and Bantle, J.A. (1992). Structural relationships and Developmental toxicity of *solanum* alkaloids in the frog embryo teratogenesis assay – Xenopus. *J. Agric. Fd. Chem.* 40:1617–1624.

Gee, J.M.; Wortley, G.M.; Johnson, I.T.; Prince, K.R.; Rutten, A.A.; Houben, G.F. and Penninks, A. (1996). Effect of Saponins and glycoalkaloids on the permeability and viability of mammalian intestinal cells and the integrity of tissue proportions invitro. *Toxicol. Invitro*, 10:117-128.

General Statistics (GENSTAT) Software. Lawes Agricultural Trust (Rothamsted Experimental Station, 1995).

Gonmori, K. and Shindo, S. (1985). Effect of cooking on the concentration of solanine in potato. *Res. Pract. Forens. Med.*, 28:91-93.

Gonmori, K. and Yoshioka. N. (1993). The risk of solanine poisoning in a folk remedy. In: *Contributions to Forenzic Toxicology, proceedings of the 31st International meeting of the International Association of Forenzic Toxicologists*, Mueller, R.K., Molina Press, Leipzig, 247-251.

Grassert, V. and Lellbach, H. (1987). Investigation of Glycoalkaloid content of Potato hybrids resistant to potato cyst eelworms, *Globodera rostochiensis* and *Globodera Pallida*. *Biochem. Biophys. Pflanzen*, 182:473-479.

Gregory, P.; Sinden, S. L.; Osman, S. F.; Tingey, W. M. and Chassin, D. A. (1981). Glycoalkaloids of wild tuber-bearing *Solanum* Species. *J. Agric. Fd. Chem.*, 29:1212-1215.

Griffiths, D.W.; Dale, M.F.B. and Bain, H. (1994). The effect of Cultivar, maturity and Storage on Photoinduced changes in total glycoalkaloid and chlorophyll content of Potatoes (*Solanum tuberosum* L.). *Plant Sci.*, 98:103-109.

Gull, D.D. and Isenberg, F.H. (1960). Chlorophyll and solanine content and distribution in four Varieties of potato tubers. *Pro. Am. Soc. Hort. Sci.*, 75:545-556.

Guseva, A.R. and Paseshnichenko, V.A. (1958). A study of the biogenesis of potato glycoalkaloids by the method of labelled atoms. *Biokhimiya*, 23:385 [Russian].

Guseva, A.R.; Borikhima, M.G. and Paseshnichenko, V. A. (1960). Utilization of acetate for the biosynthesis of chaconine and solanine in potato sprouts. *Biokhimiya*, 25:213-214 [Russian].

Guyton, B.; Sogo, F.; Mogire, J. and Njuguna, R. (1994). Kenya's Irish Potato subsector characteristics, performance and participant's information needs. Government of Kenya Market Information System Report No. 94-01, Nairobi.

Haard, N. (1977). Glycoalkaloid content of Newfoundland grown potato tubers and potato wart tissue. *J. Fd. Biochem.*, 1:57:65.

Harris, H. and Whittaker, M. (1962). Differential inhibition of serum cholinesterase phenotypes by solanine and solanidine. *Ann. Hum. Genet.*, 26:73-76.

Harvey, M.H.; McMillan, M.; Morgan, M.R.A. and Chan, H.W.S. (1985a). Solanidine is present in serum of healthy individuals and in amounts dependent on their dietary potato consumption. *Human Toxicol.*, 4:187-194.

Harvey, M.H.; Morris, B.A.; McMillan, M. and Marks, V. (1985b). Measurement of potato steroidal alkaloids in Human serum and saliva by radioimmunoassay. *Hum. Toxicol.*, 4:503-512.

Hawkes, J.G. (1994). Origins of Cultivated Potatoes and Species Relationships. In: *Potato Genetics*. (eds. Bradshaw, J.E, and Mackay, G.R.). Colset Pte Ltd, Singapore, University Press Cambridge.

Hawkes, J. G. (1990). *The Potato Evolution , Biodiversity and Genetic Resource,* Smithsonian Institution Press, Washington , D.C.

Hellenäs, K.E.; Branzell, C.; Johnson, H. and Slanina, P. (1995). High levels of glycoalkaloids in the established Swedish potato Variety Magnum Bonum. *J. Sci. Fd. Agric.*, 68:249-255.

Hellenäs, K.E.; Nyman, A.; Slanina, P.; Lööf, L. and Gabrielsson, J. (1992). Determination of potato glycoalkaloids and their aglycone in blood serum by HPLC. Applications to Pharmacokinetic studies in Humans. *J. Chromatogr.*, 573: 69-78.

Herb, S.F.; Fitzpatrick, T.J. and Osman, S.F. (1975). Separation of potato glycoalkaloids by gas

chromatography. *J. Agric. Fd. Chem.*, 23:250-253.

Hilton, R.J. (1951). Factors in relation to tuber quality in potatoes. II. Preliminary trials on bitterness in Netted gem potatoes. *Sci. Agr.* 31, 61-70.

Hlywka, J.J.; Stephenson, G. R.; Sears, M. K. and Yada, R. Y. (1994). Effects of insect damage on glycoalkaloid content of potatoes (*Solanum tuberosum*). *J. Agric. Fd. Chem.*, 42:2546-2550.

Hoogendoorn, J.; Dellaert, L.M.W.; Van Gelder, W.M.M.; Van Eeuwijk, F.A.; Jonker, H.H.; Keizer, L.C.P. and Vinke, J. H. (1992). Glycoalkaloids and resistance to potato cyst nematodes (*Globodera* spp.). Proc. Joint Conference EAPR-Breeding and EUCARPIA- Potato, 208-210.

Horton, D.E. (1987). Potatoes: Production Marketing and Programs for Developing Countries. West View Press (Boulder) It Publications, London pp 1-50.

Horton, D. and Sawyer, R.L. (1985). The Potato as a World Food Crop, with special reference to developing areas. In: *Potato Physiology.* (ed. Paul, H.L). Academic press Inc. London. Pp.1-32.

Houben, R.J. and Brunt, K. (1994). Determination of glycoalkaloids in potato tubers by Reversed-Phase HPLC. *J. Chromatogr.,* 661:169: 169-174.

Hughes, D.W. and Genest, K. (1973). "Alkaloids". In: *Phytochemistry*, Vol. II. (ed. Miller, L.P). Van Nostrand Reinhold Corp., New Yolk, P. 118.

Hunter, I.R.; Walden, M.K.; Wagner, J. R. and Heftman, E. (1976). HPLC of steroidal alkaloids. *J. Chromatogr.*, 119:223-226.

Hutchinson, A. and Hilton, R.J. (1955). The influence of certain cultural practices on solanine content and tuber yields in Netted Gem Potatoes. *Can. J. Agric. Sci.*, 35:485.

Huxtable, R.R. (1987). The toxicology of alkaloids in foods and herbs. In: *Food Poisoning -Handbook of Natural Toxins*. Tu, A.T., Ed., Marcel Dekker, New Yolk, Vol. 7, 237-262.

Hwang, C.S. and Lee, S.W. (1984). Glycoalkaloid content as influenced by varieties, parts and weight of potatoes. *Korean J. Food Sci. Technol.*, 16:383-388.

Jadhav, S. J. and Salunkhe, D. K. (1973). Enzymatic glycosylation of solanidine. *J. Food Sci.*, 38:

1099- 1100.

Jadhav, S.J. and Salunkhe, D.K. (1974). Effects of certain chemicals on Photoinduction of chlorophyll and glycoalkaloid synthesis and sprouting of potato tubers. *Can. Inst. Fd. Sci. Technol. J.*, 7:178-182.

Jadhav, S.J. and Salunkhe, D.K. (1975). Formation and Control of chlorophyll and glycoalkaloids in tubers of *Solanum tuberosum* L. and evaluation of glycoalkaloid toxicity. *Adv. Fd. Res.*, 21:307-354.

Jadhav, S.J. Kumar, A and Chavan, J K. (1991b). Glycoalkaloids. In: Potato: *Production, Processing, Products*. Salunkhe, D.K, Kadam, S.S, and Jadhav, S.J, Eds., CRC Press, Boca Raton, FL, 203-245

Jadhav, S.J.; Mazza, G. and Desai, U.T. (1991a). Post harvest handling and storage. In: *Potato Production, Processing, and Products*. (eds. Salunkhe, D.K, Kadam, S.S, and Jadhav, S.J.) CRC Press, Boca Raton, FL, 69-109.

Jadhav, S.J.; Salunkhe, D.K.; Wyse, R.E. and Dalvi, R.R. (1973). *Solanum* alkaloids: biosynthesis and inhibition by chemicals. *J. Fd. Sci.*, 38:433- 455.

Jadhav, S.J.; Sharma, R.P. and Salunkhe, D.K. (1981). *Naturally occurring toxic alkaloids in foods. CRC Crit. Rev. Toxicol.* 9:21-104.

Jain, S.; Shoo, S.L. and Vijavergia, R. (1995). Influence of light on growth and production of steroids and glycoalkaloids in Solanum species in vivo and in vitro. *Indian J. Pharm. Sci.*, 57:100-101.

Jellema, R.; Elema, E.T. and Malingre, T.M. (1982). A rapid quantitative determination of individual glycoalkaloids in tubers of *Solanum tuberosum* L. *Potato Res.*, 25:247-255.

Jellema, R.; Elema, E.T. and Malingre, T.M. (1981). Fluorodensitometric determination of potato glycoalkaloids on thin-layer chromatograms. *J. Chromatogr.*, 210: 121-129.

Jeppsen, R.B.; Wu, M.T.and Salunkhe, D.K. (1974). Some observations on the occurrence of chlorophyll and solanine in potato tubers and their control by N^6-benzyladenine, ethephon and filtered lights. *J. Food Sci.*, 39:1059-1061.

Johns, T. and Galindo Alonso, J. (1990). Glycoalkaloid change during domestication of the potato

Solanum section petota. *Euphytica*, 50:203-210.

Jonker, H.H.; Koops, A.J. and Hoogendoorn, J.C. (1992). A rapid method for the determination of steroidal glycoalkaloids by Reversed-Phase HPLC. *Potato Res.*, 35:451-455.

Kaaber, L. (1993). Glycoalkaloids, green discoloration and taste development during storage of some potato varieties (*Solanum tuberosum* L.). *Norw. J. Agric. Sci.*, 7:221-229.

Kabira, J.N. (2000). French fries and crisps processing characteristics of selected potato varieties in Kenya. In: African Potato Association Conference proceedings, vol.5.pp.507-511.

Kabira, J.N. (1983). Storage and Processing of three Kenyan Potato Varieties. Msc. Thesis, University of Nairobi.

Kaneko, K.; Tanaka, M.W.; Takahashi, E. and Mitsuhashi, H. (1977). Teinemine and iso- teinemine, two new alkaloids from *Veratrum grandiflorum*. *Phytochemistry*, 16: 1620-1622.

Keeler, R.F.; Baker, D.C. and Gaffield, W. (1991). Teratogenic *Solanum* species and the responsible teratogens. In: *Handbook of Natural Toxins*. Keeler, R.F. and Wu, A.T., Marcel Dekker, New York, Vol. 6, 83-99.

Keller, G.A.; Barton, M.C.; Shapiro, D.J. and Singer, S.J. (1985). 3-hydroxy-3-methylglutaryl-Coenzyme-A reductase is present in peroxisomes in normal rat liver cells. *Proc. Natl. acad. Sci.* USA 82: 770-4.

Keller, G.A.; Pazirander, M. and Krisans, S. (1986). 3-hydroxy-3-methylglutaryl-Coenzyme-A reductase localization in rat liver peroxisomes and microsomes of control and cholestyramine – treated animals: quantitative biochemical and immunoelectron microscopical analysis. *J. Cell Biol.* 103:875-86.

Khachik, F.; Beecher, G.R.; Goli, M.B. and Lusby, W.R. (1992). Separation and Quantification of Carotenoids in Foods. *Methods in Enzymology* 213: 347-357.

Kobayashi, K.; Powel, A.D.; Toyoda, M. and Saito, Y. (1989). HPLC method for simultaneous analysis of α-Solanine and α-chaconine in plants cultures in vitro. *J. Chromatogr.*,462:357-364.

K'osambo, M.D.O. (1998). Carotenoids content and vitamin a value of sweet potato (*Ipomea batatas* L.) cultivars as influenced by Root age, Farming site and Cooking. Msc. Thesis, University of Nairobi.

Kozukue, N.; Kozukue, E. and Mizuno, S. (1987). Glycoalkaloids in potato plants and tubers. *Hortscience,* 22:294-296.

Kozukue, N.; Kozukue, E.; Yamashita, H. and Satoshi, F. (1994). *J.Food. Sci.* 59(6): 1211-1212.

Kozukue, N. and Mizuno, S. (1986). Determination of α-chaconine and α-solanine in May Queen potatoes by HPLC. *Nippon Shokuhin Kogyo Gakkaishi* Vol.33, No.4, pp.232-237.

Kozukue, N. and Mizuno, S. (1990). Effect of light exposure and storage temperature on greening and glycoalkaloid content in potato tubers. *J. Jpn. Soc. Hort. Sci.*, 59:673-677. [Japanese].

Kozukue, N.; Tsuchida, H. and Friedman, M. (2001). Tracer Studies on the incorporation of ($2^{-14}C$) – Mevalonate into Chlorophylls a and b, α-Chaconine and α-Solanine of Potato Sprouts. *J. Agric. Fd. Chem.*, 49, 1, 92-97.

Kuć, J.J.; Henfling, J.; Garas, N. and Doke, N. (1979). Control of terpenoid metabolism in Potato-Phytophthora infestans interaction. *J. Food Protect.* 42:508-511.

Kuć, J. (1984). Steroidal glycoalkaloids and related compounds as potato quality factors. *Am. Potato J.*, 61: 123-139.

Kuhn, R. and Löw, I. (1955). Chaconine. *Ann. Acad. Sci. Fen. Ser. A chem.*, 60: 488-495 [German].

Kuhn, R. and Löw, I. (1954). The constitution of solanines. *Angew Chem.*, 66:639-640 [German].

Kusano, G.; Takahashi, A.; Sugiyama, K. and Nzoe, S. (1987). Antifungal properties of *Solanum* alkaloids. *Chem. Pharm. Bull.*, 35:4862-4867.

Lammerink, P. (1985). Total glycoalkaloid content of new potato cultivars. *N. Zealand J. Exp. Agric.*, 13: 413-414.

Lampitt, L.H.; Bushill, J.H.; Rooke, H. and Jackson, E. M. (1943). Solanine Glycoside of the potato. II. Distribution in potato plant. *J. Soc. Chem. Ind.*, 62:48-51.

Larini, K.; Perez-Espinosa, A.; Pineda, M. and Ruiz-Rubio, M. (1996). Purification and

Characterization of tomatidinase from *Fusarium oxysporium f.sp. lycopersici*. *Physiol. Plant Pathol.*, 2:17-25.

Lavintman, N.; Tandecarz, J. and Cardini, C.E. (1977). Enzymatic glucosylation of steroid alkaloids in potato tuber. *Plant sci. Lett.*, 8:65-69.

Lawson, D.R.; Erb, W.A. and Miller, A.R. (1992). Analysis of solanum alkaloids using Internal Standardization and Capillary Gas Chromatography. *J. Agric. Fd. Chem.*, 40: 2186-2191.

Levinson, H.Z. (1976). The defence role of Alkaloids in Insects and Plants. *Experentia*, 32:408-411.

Liljergren, D.R. (1971). Glucosylation of solasodine by extracts from *Solanum laciniatum*. *Phytochemistry*, 10:3061-3064.

Linnemann, A.R., Van Es, A. and Hartmans, K. J. (1985). Changes in the content of L-ascorbic acid, glucose, fructose, sucrose and total glycoalkaloids in potatoes (Cv Bintje) stored at 7, 16, and 28°C. *Potato Res.*, 28:271-278.

Love, S.L.; Herman, T.J.; Thompson-Johns, A. and Baker, T.P. (1994). Effect of Interaction of crop management factors on glycoalkaloid concentration of potato tubers. *Potato Res.*, 37:77-85.

Lyon, G.D. (2002). Metabolic pathways of diseased potato. http://www.scri.sari.ac.uk/TiPP/pps/charttxt.htm. Accessed on 13/05/2002.

Maga, J. (1980). Potato Glycoalkaloids. *Crit. Rev. Fd.Sci. Nutr.*, 12; 371-405.

Matthew, J.A.; Morgan, M.R.A.; McNerner, R.; Chan, H.W. and Coxon, D.T. (1983). Determination of Solanidine in human plasma by RIA. *Fd. Chem. Toxicol.*, 21:637-641.

Mazurczyk, W. (1995). Chemical composition of 30 potato cultivars. *Biul. Inst. Ziemniaka*, 44: 55-63.

McCollum, G.D. and Sinden, S. L. (1979). Inheritance study of potato tuber glycoalkaloids in a wild potato, *Solanum chacoense* Bitter. *Am. Potato J.*, 56:95-113.

McMillan, M. and Thompson, J.C. (1979). *An outbreak of suspected solanine-poisoning boys. An examination of criteria of solanine poisoning. Quart. J.Med.N.S* 48, 227-43.

Michalska, L.; Nagel, G.; Swiniarski, E. and Zydowo, M. M. (1985). The effect of α-solanine on the active calcium transport in rat intestine. *Gen. Pharmacol.*, 16: 69-70.

Moehs, C.P.; Allen, P.V.; Friedman, M. and Belknap, W.R. (1997). Cloning and expression of solanidine UDP-glucosyltransferase (SGT) from potato. *Plant J.*, 11: 101-110.

Mondy, N.I. and Chandra, S. (1979). Reduction of Glycoalkaloid Synthesis in Potato slices by water soaking. *Hortscience*, 14:173- 174.

Mondy, N.I. and Gosselin, B. (1988). Effects of peeling on total phenols, total glycoalkaloids, discoloration and flavor of cooked potatoes. *J. Fd. Sci.,* 53; 756-759.

Mondy, N.I. and Munshi, C.B. (1993). Effect of soil and foliar application of molybdenum on the glycoalkaloid and nitrate concentration of potatoes. *J. Agric. Fd. Chem.*, 41:256-258.

Monfar, M.; Caelles, C.; Bacelles, L.; Ferrer, A.; Hegardt, F.G. and Boronat, A. (1990). Molecular Cloning and Characterization of plant HMG-CoA Reductase. In: *Biochemistry of the Mevalonic acid Pathway to Terpenoids*. (eds. Neil Tower, G.H. and Stafford, H.A.). Plenum, New York. Pp. 33-97.

Morgan, M.R.A. and Coxon, D.T. 1987. Tolerances: glycoalkaloids in potatoes. In: *Natural toxicants in Foods: Progress and Prospects*. Watson, D.H., Ellis Horwood Ltd., Chichester, England, 221-230.

Morgan, M.R.A.; McNey, R.; Matthew, J.; Coxon, D.T. and Chan, H.W.S. 1983. An Enzyme Linked Immunosorbent Assay for Total Glycoalkaloids in Potato tubers. *J. Agric. Fd. Chem.* 34:593-598.

Morris, S.C. and Lee, T.H. (1981). Analysis of Potato glycoalkaloids with Radially Compressed HPLC Cartridges and ethanolamine in the Mobile Phase. *J. Chromatogr.*,219:403-410.

Morris, S.C. and Lee, T.H. (1984). The toxicity and teratogenicity of solanaceae glycoalkaloids, particularly those of the potato (*Solanum tuberosum* L.). *Fd. Tcchnol. Australia*, 36:118-124.

Morris, S.C. and Petermann, J. B. (1985). Genetic and Environmental effects on levels of glycoalkaloids in cultivars of Potato (*Solanum tuberosum* L.). *Food Chem.*, 18:271-282.

Mussukuya, E.; Walingo, A.M., Kinyae, P.M., Lungaho, C.; Kabira, J.N., and Nganga, N. (2000). An assessment of the impact of semi-informal seed system for Asante and Tigoni potato varieties.

In: *Impact of selected research programes and projects in KARI. Impact Assessment Report* 3103, KARI HQ, Nairobi.

Nair, P. M.; Behere, A. G. and Ramaswamy, N. K. (1981). Glycoalkaloids of *Solanum tuberosum* Linn. *J. Sci. Ind. Res.*, 40: 529-535.

Ngugi, D. N. (1983). Potato Production in Kenya: Potentials and limitations. In: ''*Proceedings of the International Congress Research for the potato in the year 2000*''. (ed. Hooker, W.J.). CIP, Lima, Peru PP. 140-142.

NIEHS (National Institute of Environmental Health Science). (1997a). Information profile for NIEHS nominations on chemicals to the National Toxicology Program for α-chaconine.

NIEHS (National Institute of Environmental Health Science). (1997b). Information profile for NIEHS nominations on chemicals to the National Toxicology Program for α-solanine.

Nigg, H.N.; Ramos, L.E.; Graham, E.M.; Sterling, J.; Brown, S. and Cornell, J.A. (1996). Inhibition of Human plasma and serum butyrl cholinsterase by α-chaconine and α-solanine. *Fund. Appl. Toxicol.,* 33, 272-281.

Nishie, K.; Norred, W.P. and Swain, A.P. (1975). Pharmacology and Toxicology of Chaconine and tomatine. *Res. Commun. Chem. Pathol. Pharmacol.*, 12:657-668.

NIVAA, Netherlands catalogue of Potato varieties. (2000). The Netherlands Consultative Potato Institute.

Nobel, P.S. (1983). Biophysical Plant physiology and Ecology. W.H. Freeman and company, San Francisco. Pp. 195.

Nobuyuki, K.; Hironobu, T. and Susumu, M. (1993). Effect of Light Intensity, Duration and Photoperiod on Chlorophyll and Glycoalkaloid production by potato tubers. *J. Japanese Soc. Hort. Sci.* Vol. 62, 3:669-673.

Norred, W.P.; Nishie, K. and Osman, S.F. (1976). Excretion, distribution and metabolic rate of ^{3}H α-chaconine. *Res. Commun. Chem. Pathol. Pharmacol.*, 13:161-167.

NPRC. (1999). Annual Report. Kenya Agricultural Research Institute, National Potato Research

Centre -Tigoni.

Olsson, K. (1986). The influence of genotype on the effects of impact damage on the accumulation of glycoalkaloids in potatoes potato tubers. *Potato Res.*, 29: 1-12.

Olsson, K. and Carlson, C. (1993). Do wet field conditions influence glycoalkaloids in potatoes? In: *Abstracts from the 12th Triennial Conference of the European Association of Potato Research,* Paris, 192-193.

Olsson, K. and Roslund, C-A. (1994). Changes in glycoalkaloid content of potato during long term storage. In: *Abstracts from Joint meeting of the Agronomy and Utilization sections of the European association of Potato Research*, Vila real, Portugal, June 24-30, 41.

Olsson, K. (1989). Impact Damage, Gangrene and Dry rot in Potato: Important Biochemical factors in Screening for Resistance and Quality in breeding material. Ph.D. Thesis, The Swedish University of Agricultural sciences, Svalov.

Olsson, K. (1987). The influence of glycoalkaloids and impact damage on the resistance to *Fusarium Solani* Var. *coerruleum* and *Phoma exigua* Var. *foveata* in potato tubers. J. *Phytopathol.*, 118:347-357.

Osbourn, A.; Bowyer, P.; Lunness, P.; Clark, B. and Daniels, M. (1996). Fungal pathogens of oat roots and tomato leaves employ closely regulated enzymes to detoxify different host plant saponins. *Mol. Plant-Microbe Interact.*, 9:971-978.

Osman, S.F. and Sinden, S.L. (1977). Analysis of mixtures of solanidine and demissidine glycoalkaloids containing identical carbohydrate units. *J. Agric. Fd. Chem.*, 25: 955-957.

Osman, S.F. (1983). Glycoalkaloids in potatoes. *Fd. Chem.*, 11: 235-247.

Osman, S.F.; Johns, T.A. and Price, K.R. (1986). Sisunine, a glycoalkaloid found in hybrids between *Solanum acaule* and *Solanum ajanhuiri. Phytochemistry*, 25:967-968.

Osman, S.F.; Zacharius, R.M.; Kalan, E.B.; Fitzpatrick, T.J. and Klulick, S. (1979). Stress metabolites of the potato and other solanaceous plants. *J. Food Protect.*, 42:502-507.

Paczkowski, C. and Wojciechowski, Z.A. (1994). Glucosylation and galactosylation of diosgenin and

solasodine by soluble glucosyltransferase(s) from Solanum melongena leaves. *Phytochemistry*, 35:1429-1434.

Parnell, A.; Bhuva, V.S. and Bintcliffe, E.J.B. (1984). The glycoalkaloid content of Potato Varieties. *J. Natl. Inst. Agric. Bot.*, 16:535-541.

Parups, E.V. and Hoffman, I. (1967). Induced alkaloid levels in potato tubers. *Am. Potato J.*, 44:277-280.

Patchett, B.J.; Cunningham, P.S. and Lill, R.E. (1976). Glycoalkaloid levels in New Zealand potatoes. *N. Z. J. Exp. Agric.*, 5:55-57.

Patil, B.C.; Salunkhe, D.K. and Singh, B. (1971). Metabolism of solanine and chlorophyll in potato tuber as affected by light and specific chemicals. *J. Food Sci.*, 36:474-476.

Petermann, J.B. and Morris, S.C. (1985). The spectral responses of chlorophyll and glycoalkaloid synthesis in tubers (*Solanum tuberosum*). *Plant Sci.*, 39:105-110.

Petersen, H.W. (1993). Steroidal Glycoalkaloids in Tuber-bearing *Solanum* species. Ph.D. Thesis. Royal Danish School of Pharmacy, Copenhagen, Denmark.

Ponnampalam, R. and Mondy, N.I. (1986). Effect of foliar application of Indoleacetic acid on the total glycoalkaloids and Nitrate nitrogen content of potatoes. *J. Agric. Fd. Chem.*, 34:686-688.

Porter, T. D. (2000). Squalene monooxygenases. University of Kentucky Medical Centre. http://www.uky.edu/. Accessed on 30/5/2002.

Poswillo, D.E.; Sopher, D. and Mitchell, S.J. (1972). Experimental induction of foetal malformation with ''blighted'' potatoes: a preliminary report. *Nature*, 239: 462-464.

Rahim, J.; Hussain, M. and Khan, F.K. (1989). Effect of Potato Variety and growing season on the total glycoalkaloids (TGA) content in tubers and its portions, grown in N.W. Pakistan. *Sarhad. J. Agric.*, 5:449-452.

Raman, K.V.; Tingey, W.M. and Gregory, P. (1979). Potato glycoalkaloids: Effects on Survival and feeding behaviour of the Potato Leafhopper. *J. Econ. Entomol.*, 72:337-341.

Ramaswamy, N.K.; Behere, A.G. and Nair, P.M. (1976). A novel pathway for the synthesis of solanidine in the isolated chloroplasts from greening potatoes. *Eur. J. Biochem.*, 67: 275-282.

Rayburn, J.R.; Bantle, J.A. and Friedman, M. (1994). Role of Carbohydrate side chains in developmental toxicity. *J. Agric. Fd. Chem.*, 42: 1511-1515.

Rayburn, J.R.; Bantle, J.A.; Qualls, C.W., Jr. and Friedman, M. (1995a). Glucose-6-phosphate and NADP protect frog embryos against developmental toxicity induced by α-chaconine. *Fd. Chem. Toxicol.*, 33:1021-1025.

Reeve, R.M.; Hautala, E. and Wever, M. L. (1969). Anatomy and compositional variation within potatoes. II. Phenolics, enzymes and other minor components. *Am. Potato J.*, 46:257.

Renwick, J.H. (1972). Hypothesis. Ancephally and spina bifida are usually preventable by avoidance of a specific but unidentified substance present in certain potato tubers. *Br. J. Prev. Soc.Med.*, 26: 67-88.

Renwick, J.H. (1986). Our ascorbate defence against solanaceae. In: *Solanaceae: Biology and Systematics*. (ed. D'arcy, G.) Columbia University press, New York, 569-576.

Ripakh, L.A. and Kim, A. (1958). Case of mass poisoning by solanine. *Sovetskaia Meditsina* 22:129-131. [Russian].

Roddick, J.G. (1980). A Sterol-binding assay for potato glycoalkaloids. *Phytochemistry.*, 19:2455-2457.

Roddick, J.G.; Rijnenberg, A.L. and Weissenberg, M. (1990). Membrane-disrupting properties of Steroidal Glycoalkaloids Solasonine and Solamargine. *Phytochemistry*, 29:1513-1518,

Roddick, J.G. and Melchers, G. (1985). Steroidal glycoalkaloid content of potato, tomato, and their somatic hybrids. *Theor. Appl. Genet.*, 70:655-660.

Rodriquez-Saona, L.E.; Wrolstad, R.E. and Pereira, C. (1999). Glycoalkaloid Content and Anthocyanin Stability to Alkaline treatment of Red-Fleshed potato extracts. *J.Food. Sci.*, vol. 64, No.3, Pp.445-450.

Rosenfeld, H.J.; Sundell, H.A.; Lea, P. and Ringstad, M. (1995). Influence of packaging materials and temperature on the glycoalkaloid content of potato tubers. *Fd. Res. Int.*, 28: 481-4844.

Ross, H.; Pasemann, P. and Nitzche, W. (1978). The dependence of the glycoalkaloid content of

potatoes on location and year of production and its relation to taste. *Z. Pflanzenzüchtg.*, 80:64-79 [German].

Rozumek, K.E. (1969). Thin- Layer Chromatographic separation of tomatidenol, solasodine and soladulcidine and yamogenin, diosgenin and tigogenin. *J. Chromatogr.*, 40:97-102 [German].

Ryugo, K. and Sachs, R.M. (1969). In vitro and In vivo studies of Alar and related substances. *Am. Potato J.*, 94:529.

Saito, K.; Horie.; Hoshino, Y.; Nose, N. and Nakazawa, H. (1990). HPLC determination of Glycoalkaloids in Potato Products. *J. Chromatogr.*, 508:141-147.

Salunkhe, D.K.; Wu, M.T. and Jadhav, S.J. (1972). Effects of light and temperature on the formation of solanine in potato slices. *J. Food Sci.*, 37:969-970.

Sandrock, R. W.; Dellapenna, D. and Van Etten, H. D. (1996). Purification and Characterization of β_2-tomatidinase, an enzyme involved in the degradation of α-tomatine and isolation of genes encoding β_2-tomatidinase from *Septoria lycopersici. Mol. Plant-Microbe Interact.*, 8:960-970.

Sanford, L. L.; Deahl, K. L.; Sinden, S. L. and Kobayashi, R. S. (1995). Glycoalkaloid content in tubers of hybrid and backcross populations from *Solanum tuberosum* X *chacoense* cross. *Am. Potato J.*, 72: 261-271.

Sanford, L.L. and Sinden, S.L. (1972). Inheritance of potato glycoalkaloids. *Am. Potato J.*, 49:209-217.

Screiber, K. (1968). On the glycoalkaloids of tuber-bearing Solanum species, *Kulturpflanze*, 11:422-450 [German].

Screiber, K.; Aurich, O. and Osske, G. (1963). Solanum alkaloids. XVII. Thin layer chromatography of *Solanum* steroid alkaloids. *J.Chromatogr.*, 12:63-69 [German].

Sharma, R.P. and Salunkhe, D. K. (1985). Solanum Glycoalkaloids. In: Toxicants of Plant Origin. (ed. Morgan, S.). John Wiley and Sons, New Yolk, 180-236.

Shih, M.J. and Kuć, J. (1974). α- and β-Solamarine in Kennebec *Solanum tuberosum* leave and aged tuber slices. *Phytochemistry*, 13: 997-1000.

Siegfried, R. (1976). Determination of the solanine-chaconine ratio in potato sprouts and tubers. *Z. Lebensm. Unters. Forsch.*, 162:253-258.

Sinden, S.L. (1971). Control of potato greening with household detergents. *Am. Potato J.*, 48:53-56.

Sinden, S.L. and Webb, R.E. (1972). Effect of Variety and Location on the Glycoalkaloid content of potatoes. *Am. Potato J.*, 49:334-338.

Sinden, S.L.; Cantello, W.W.; Sanford, L.L. and Deahl, K.L. (1991). Allelochemically mediated Host resistance to the Colorado potato beetle, leptinotarsa decemlineata (Say) (Coleoptera: Chrysomelidae). *Mem. Ent. Soc. Can.*, 159:19.

Sinden, S.L.; Deahl, K.L. and Aulenbach, B.B. (1976). Effect of glycoalkaloids and phenolics on potato flavor. *J. Fd. Sci.,* 41:520-523.

Sinden, S.L.; Goth, R.W. and ÕBrien, M.J. (1973). Effect of Potato alkaloids on the growth of *Alternaria solani* and their possible role as resistance factors in potatoes. *Phytophatology*, 63:303-307.

Sinden, S.L.; Sanford, L.L. and Webb, R.E. (1984). Genetic and environmental control of potato glycoalkaloids. *Am. Potato J.*, 61:141-156.

Sinden, S.L.; Sanford, L.L. and Deahl, K.L. (1986b). Segregation of leptine glycoalkaloids in *Solanum chacoense* Bitter. *J. Agric. Fd. Chem.*, 34:372-377.

Smittle, D.A. (1971). A comparison and modification of methods of total glycoalkaloid analysis. *Am. Potato J.*, 48:410-413.

Speroni, J.J. and Pell, E.J. (1980). Modified Method for tuber glycoalkaloid and leaf glycoalkaloid analysis. *Am. Potato J.*, 57:537-542.

Spoerke, D. (1994). The mysterious Potato. *Vet. Human Toxicol.*, 36:324-326.

Stanker, L.H.; Kamps-Holtyapple, C. and Friedman, M. (1994). Development and Characterization of monoclonal antibodies that differentiate between potato and tomato glycoalkaloids and aglycones. *J. Agric. Food Chem.*, 42:2360-2366.

Stanker, L.H.; Holtzapple, C.K. and Friedman, M. (1996a). Monoclonal Antibodies to Potato, Tomato, and Eggplant Glycoalkaloids and Assays for the same. U.S. Patent application P.C. 0195.94.

Stanker, L.H.; Kamps- Holtzapple,C.; Beier, R.C.; Levin, C.E . and Friedman , M. (1996b). Comparison of ELISA and HPLC Methods for detection and Quantification of Potato and tomato glycoalkaloids. ACS Symposium Series, 621:243-255.

Stapleton, A.; Allen, P.V.; Belknap, W.R.; and Friedman, M. (1992). Partial amino acid sequence of potato solanidine-UDP glucose glucosyltransferase purified by new ion exchange and size exclusion media. *Protein Expression Purif.*, 3, 85-92.

Stapleton, A.; Allen, P.V.; Friedman, M. and Belknap, W.R. (1991). Isolation and Characterization of solanidine glucosyltransferase from potato sprouts. *J. Agric. Fd. Chem.*, 39, 1187-1202.

Subrtová, D.; Hejtmánková, A.; Vanková, Z. and Hubácek, J. (1993). Changes in the content of Volatile substances, solanine and starch caused by soft rot infection. *Potravin Vedy*, 11:31-41[Chech.].

Swain, A.P.; Fitzpatrick, T.J.; Talley, E.A.; Herb, S.F. and Osman, S.F. (1978). Enzymatic Hydrolysis of α-chaconine and α-solanine. *Phytochemistry*, 17:800-801, 1978.

Swinyard, C.A. and Chaube, S. (1973). Are potatoes teratogenic to experimental animals? *Teratology*, 8:349-357.

Takagi, K.; Toyoda, M.; Fujiyama, Y. and Saito, Y. (1990). Effect of cooking on the contents of α-chaconine and α-solanine in potatoes. *J. Food Hygienic Soc. Jpn.*, 31:67-73.

Tarlakovskii, S.A. (1981). Studies on sterol and sterol glycoalkaloids in potato leaves in relation to the problem of their field resistance to *Phytophthora infestans*. Vsesoiuznyi Nauchno- Issledovatel' Zashchity Rastenii, Trudy, Leningrad, 59-60, [Russian].

Thomas, J.B.; Thomas, W. and Anja, M. (1990). Some properties of enzymes involved in the biosynthesis and metabolism of mevalonic acid to terpenoids. In: *Biochemistry of the Mevalonic acid Pathway to Terpenoids*. (eds. Neil Tower, G.H. and Stafford, H.A.). Plenum, New York. Pp. 1-59.

Thomson, C.A. and Sporns, P. (1995). Fluorescence Polarization immunoassays for potato glycoalkaloids. *J.Agric.Fd. Chem.*, 43:254-260.

Tingey, W.M. (1984). Glycoalkaloids as pest resistance factors. *Am. Potato J.*, 61:157-167.

Tingey, W.M.; MacKenzie, J. D. and Gregory, P. (1978). Total glycoalkaloids and resistance of wild potato species to *Empoasca fabae* (Haris). *Am. Potato J.*, 55:577-585.

Tjamos, E. and Kuč, J. (1982). Inhibition of steroid glycoalkaloid accumulation by arachidonic and eicosapentanoic acids in potato. *Science* 217:542-544.

Toyoda, M.; Rausch, W.D.; Inoue, K.; Ohno, Y.; Fujiyama, Y.; Takagi, K. and Saito, Y. (1991). Comparison of solanaceous glycoalkaloid-invoked Ca^{2+} influx in different types of cultured cells. *Toxicol. Invitro*, 5:347-351.

Uppal, D.S. (1987). Varietal and environmental effect on the glycoalkaloid content of potato *Solanum tuberosum* L.). *Plant Fds. Hum. Nutri.*, 37:333–340.

Vallejo, R.P. and Ercegovich, C.D. (1978). Analysis of Potato for glycoalkaloid contents by Radioimmunoassay (RIA). In: *Methods and Standards for Environmental Measurements,* Publication 519 National Bureau of standards, Washington, D.C., 333-340.

Van Gelder, W.M.J. and Dellaert, L.M.W. (1988). Alkaloids in potatoes. *Prophyta*, 425:236-238. . [Dutch].

Van Gelder, W.M.J. (1990). Chemistry, Toxicology, and Occurrence of Steroidal glycoalkaloids; Potential contaminants of the Potato (*Solanum tuberosum* L.). In: *Poisonous Plants Contaminating Edible Plants*. (ed. Rizk, A-F.M.). CRC press, Boca Raton, Florida, 117-156.

Van Gelder, W.M.J. (1985). Determination of the total C_{27}-Steroidal alkaloid composition of *Solanum* species by high-resolution gas chromatography. *J. Chromatogr.*, 331: 285-293.

Van Gelder, W.M.J. (1989). Steroidal Glycoalkaloids in Solanum species: Consequences for potato Breeding and Food safety. Ph.D. Thesis, University of Wageningen, Holland.

Van Gelder, W.M.J.; Tuinistra, L.G.M. Th.; Van der Greef, J. and Scheffer, J.J.C. (1989). Characterization of novel steroidal alkaloids from tubers of *Solanum* species used by combined gas-mass spectrometry. Implications for potato breeding. *J. Chromatogr.*, 482:13-22.

Van Swaaij, A.C. (1992). Effect of growth conditions on glycoalkaloid accumulation in potato tubers. *Potato Res.*, 35:68-69.

Vayda, M.E. (1994). Environmental Stress and its impact on potato yield. In: Potato Genetics (eds. Bradshaw, J.E. and MacKay, G.R). Pp. 239-261.

Velísek, J. and Hajslová, J. (1995). Alkaloids. In: *Natural Toxic Compounds of Foods- Formation and changes during Processing and Storage*. (ed. Davidek, J.). CRC Press, Boca Raton, Florida, 15-44.

Vern, S. C. (2001). New Hope for America's 16 million Diabetics. http://www.diabetes-help.com/. Accessed on 6/07/2002.

Waithaka, J.G. (1976). Potato Cultivation in Kenya. In: *Regional workshop on potato seed production and marketing.*

Wang, S.L.; Bedford, C.L. and Thompson, N.R. (1972). Determination of Glycoalkaloids in potatoes (*Solanum tuberosum* L.) with a bisolvent extraction method. *Am. Potato J.*, 49:302-308.

Wang, C.W.; Oh, M.K. and Liao, J.C. (1999). Engineered Isoprenoid Pathway Enhances Astaxanthin production. *Biotechnol. Bioeng.* 62:235-241.

Wierenga, J.M. and Hollingworth, R.M. (1992). Inhibition of insect acetylcholinesterase by the potato glycoalkaloid α-chaconine. *Natural Toxins*, 1:96-99.

Wierzchowski, P. and Wierzchowska, Z. (1961). Colorimetric Determination of solanidine and solanine with $SbCl_3$. *Chem. Anal.* (Warsaw), 6:579-585.

Wilson, J.M. and Frank, J.S. (1975). The effect of systematic pesticides on total glycoalkaloid content of potato tubers at harvest. *Am. Potato J.*, 52:179-184.

Wu, M. and Salunkhe, D.K. (1972a). Control of Chlorophyll and solanine syntheses and sprouting of tubers by hot paraffin wax. *J. Fd. Sci.*, 37:629-630.

Wu, M.T. and Salunkhe, D.K. (1972b). Inhibition of chlorophyll and solanine formation and sprouting of potato tubers by oil dipping. *J. Amer. Soc. Hort. Sci.*, 97:614-616.

Wu, M.T. and Salunkhe, D. K. (1978). After effect of anoxia water treatment on greening and glycoalkaloid formation of potato tubers. *J. Food Sci.*, 43: 1330-1331.

Wu, M.T. and Salunkhe, D.K. (1976). Changes in glycoalkaloids content following mechanical injuries to potato tubers. *J. Am. Soc. Hort. Sci.*, 101:329-331.

Wünsch, A. and Munzert, M. (1994). Effect of storage and Cultivar on the distribution of glycoalkaloids in potato tuber. *Potato Res.*, 37:3-10.

Wünsch, A. (1989). Spartial distribution of glycoalkaloids of different potato varieties throughout the tuber. *Chem. Mikrobiol. Technol. Lebensm* 12:69-74.

Yaniv, Z.; Palevitch, D. and Weissenberg, M. (1984). The effect of water stress on growth and solasodine content in Solanum khasianum. *Planta Medica* 50:60-65.

Zeiger, E. and Raymond T. (1998). α-chaconine (20562-03-2) and α-solanine (20562-02-1). Review of Toxicological Literature. Institute of Environmental Health Science.

Zimowski, J. (1994). Characterization of UDP-galactose: tomatidine galactosyltransferase from tomato (*Lycopersicon esculentum*) leaves. *Acta Biochim. Polonica*, 41:202-204.

Zimowski, J. (1997). Synthesis of γ-chaconine and γ-solanine are catalyzed in potato by two separate glycosyltransferases: UDP-glucose: solanidine glucosyltransferase and UDP-galactose: solanidine galactosyltransferase. *Acta Biochem. Polonica*, 44:209-214.

Zitnak, A. (1955). Factors influencing the initiation and rate of solanine synthesis in tubers of *Solanum tuberosum* L. PhD thesis, Univ. Alberta.

Zitnak, A. and Filadelfi, M.A. (1985). Estimation of taste thresholds of three potato glycoalkaloids. *J. Can. Inst. Food Sci. Technol.*, 18:337-339.

Zitnak, A. and Johnson, G. R. (1970). Glycoalkaloid content of B5141-6 potatoes. *Am. Potato J.*, 47: 256-260.

Zitnak, A. (1953). The influence of certain treatments upon solanine synthesis in potatoes. M.S. Thesis, University of Alberta, Edmonton, Canada.

APPENDICES

Appendix 3.1. Regression analysis

Source	df	Bromophenol ms	Solanidine ms	α-chaconine ms	α-chaconine ms
Regression	1	10.74815**	3235.32**	199.539*	153.217**
Error	5	0.04073	71.27	7.853	1.473
Total	6	1.82530	661.32	55.775	31.822

*-Significant at 5% level
**-Significant at 1% level

df -degrees of freedom
ms -mean square

Appendix 3.2a. Comparison of retention time of potato glycoalkaloid peak P1 and solanidine

	P1 (1)	Solanidine (2)
Mean RT (min)	3.24	3.34
Variance	0.001	0.001
SD	0.030	0.023
F-test for the hypothesis "variance 1=variance 2"		
F-value	1.74	
Numerator df	19	
Denominator df	19	
Probability	0.24	
Result: Significant F, reject the null hypothesis of equal variances		
T-test for the hypothesis "mean 1≠mean 2"		
t' value	11.89	
Effective df	38	
Probability	0.001	
Result: Not significant t, accept the null hypothesis.		
95% confidence interval for difference in means (0.08215, 0.1159)		

Appendix 3.2b. Comparison of retention time of potato glycoalkaloid peak P2 and α-chaconine

	P2 (1)	α-chaconine (2)
Mean RT (min)	4.55	4.43
Variance	0.008	0.001
SD	0.088	0.026
F-test for the hypothesis "variance 1=variance 2"		
F-value	11.39	
Numerator df	19	
Denominator df	19	
Probability	0.001	
Result: Not significant F, accept the null hypothesis of equal variances		
T-test for the hypothesis "mean 1≠mean 2"		
t' value	-6.03	
Effective df	22	
Probability	0.001	
Result: Significant t, reject the null hypothesis and accept the alternate hypothesis. 95% confidence interval for difference in means (-0.1660, -0.08104)		

Appendix 3.2 c. Comparison of retention time of potato glycoalkaloid peak P3 and α-solanine

	P3 (1)	α-chaconine (2)
Mean RT (min)	6.027	5.882
Variance	0.004	0.003
SD	0.059	0.058
F-test for the hypothesis "variance 1=variance 2"		
F-value	1.05	
Numerator df	19	
Denominator df	19	
Probability	0.92	
Result: Significant F, reject the null hypothesis of equal variances		
T-test for the hypothesis "mean 1≠mean 2"		
t' value	-7.80	
Effective df	38	
Probability	0.001	
Result: Significant t, reject the null hypothesis and accept the alternate hypothesis. 95% confidence interval for difference in means (-0.1826, -0.1074)		

Appendix 3.3a. ANOVA table of the effect method of analysis on the TGA contents of potato varieties

Source	df	ss	ms
Replication	2	0.5064	0.2532^{ns}
Method (M)	1	2.9084	2.9084**
Variety (V)	9	767.3199	85.2578**
M × V	9	61.9914	6.8879**
Error	38	9.0904	02392

Appendix 3.3b. ANOVA table of the effect of method of analysis on the TGA contents of CIP clones

Source	df	ss	ms
Replication	2	1.7170	0.8585 ^{ns}
Method (M)	1	1.8007	1.8007 ^{ns}
Clone (C)	4	444.8432	111.2108**
M × C	4	72.3313	18.0828**
Error	18	9.0157	0.5009

*-Significant at 5% level
**-Significant at 1% level
ns-not significant

df -degrees of freedom
ss-sum of squares
ms -mean squares

Appendix 3.4. ANOVA table of the effects of variety and light on glycoalkaloid contents

		α-chaconine	α-solanine	TGA
Source	df	ms	ms	ms
Replication	2	1.1983^{ns}	0.22607^{ns}	0.9165*
Cover (C)	1	7.0953**	0.51984*	16.4267**
Light (L)	2	37.5893**	26.1834**	79.7573**
Variety (V)	4	30.1888**	11.5811**	169.7030**
C × L	2	1.4172**	0.0614^{ns}	2.7541**
C × V	4	2.6553**	0.3399**	4.6672**
L × V	8	1.6582**	0.5301**	2.7528**
C×L ×V	8	1.1882**	0.2139*	3.3592**
Error	58	0.1334	0.0897	0.2553

*-Significant at 5% level
**-Significant at 1% level
ms -mean square

ns-not significant
df -degrees of freedom

Appendix 3.5. ANOVA table of the effects of variety and storage condition on glycoalkaloid contents

		α-chaconine	α-solanine	TGA
Source	df	ms	ms	ms
Replication	2	1.0794*	0.74679**	3.0785**
Storage (S)	2	2.2828**	0.57180**	5.0486**
Variety (V)	3	14.2768**	12.28886**	11.9812**
S × L	6	0.1155^{ns}	0.37885**	0.7000^{ns}
Error	22	0.2572	0.07646	0.3927

*-Significant at 5% level
**-Significant at 1% level
ms -mean square

ns-not significant
df -degrees of freedom

Of making many books there is no end ...
- Ecclesiastes 12:12