



## **Changes in Phytochemical Content During Different Growth Stages in Tubers of Five Varieties of Potato (*Solanum tuberosum* L.)**

**GEOFFREY KIPKOECH KIRUI\*, SAIFUDDIN FIDAHUSSEIN DOSSAJI,  
and NELSON ONZERE AMUGUNE**

School of Biological Sciences, University of Nairobi P.O. Box 30197-00100, Nairobi, Kenya.

### **Abstract**

Potato (*Solanum tuberosum* L.) synthesizes a variety of bioactive metabolites including phenolic compounds and glycoalkaloids that protects against insects and diseases, and may influence its nutritional quality. Phenolics provide valuable health promoting antioxidants, whereas glycoalkaloid concentrations exceeding the upper safety limit of 20 mg/100 g fresh weight (Fwt) are potential neurotoxins. Therefore, efficient selection for tuber nutritional quality is dependent upon safe and reliable analytical methods. The aim of this study was to determine the changes in the concentration of glycoalkaloids and phenolic compounds during different growth stages in tubers of five selected potato varieties grown in Kenya.  $\alpha$ -chaconine and  $\alpha$ -solanine were separated and identified by HPLC. Total glycoalkaloids (TGA) and phenolics were determined by UV spectrophotometry. Recovery efficiencies for validation of analytical methods ranged from 85.9-93.5%. Significant differences in TGA and phenolic contents were detected among potato varieties. Tuber TGA content ranged from 6.80 to 10.56 mg/100g Fwt in vars. Dutch Robijn and Tigon, respectively, and were within the upper safety limit. The corresponding values for chlorogenic acid contents in the examined varieties ranged from 46.39 to 58.04 mg/100 g Fwt. Total phenolic concentration in the examined tuber extracts varied ranged from 129.24 to 192.52 mg CGA/g Fwt. Glycoalkaloid and phenolic production were significantly reduced from time of initiation to maturity at 55 and 125 days, respectively, after planting (DAP). These results demonstrate that tuber phytochemicals were strongly influenced by variety and level of maturity. For nutritional safety and quality purposes, harvesting of mature potato tubers after 125 DAP is recommended.



### **Article History**

Received: 28 September 2017

Accepted: 28 March 2018

### **Keywords**

Chlorogenic acid,  
Glycoalkaloids,  
Phenolic acids,  
Maturity stage,  
*Solanum tuberosum*.

**CONTACT** Geoffrey Kipkoech Kirui ✉ [gkirui@uonbi.ac.ke](mailto:gkirui@uonbi.ac.ke) 📍 School of Biological Sciences, University of Nairobi P.O. Box 30197-00100, Nairobi, Kenya.

© 2018 The Author(s). Published by Enviro Research Publishers

This is an  Open Access article licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (<https://creativecommons.org/licenses/by-nc-sa/4.0/>), which permits unrestricted NonCommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

To link to this article:

## Introduction

Potato (*Solanum tuberosum* L.) is a very important food crop in Kenya and many parts of the world and its expanded production may help raise livelihoods in developing countries. Besides its nutritional value, potato plants produce a variety of secondary metabolites during growth and post harvest storage. These secondary compounds include glycoalkaloids, phenolic acids, protease inhibitors and lectins<sup>1</sup>. Among these phytochemicals, glycoalkaloids and phenolic acids have been widely studied because of their toxicity to humans and plant protection against phytopathogens. The most important glycoalkaloids (GAs) are  $\alpha$ -chaconine and  $\alpha$ -solanine, because of their harmful health effects in human beings<sup>2</sup>. High levels of GAs are reported to inhibit cholinesterase and disrupt cell membranes<sup>3</sup> with clinical symptoms of poisoning that includes abdominal colic pain, diarrhea and vomiting. Given the potentially toxic nature of steroidal glycoalkaloids,  $\alpha$ -chaconine and  $\alpha$ -solanine, to human, it is of interest to assess its levels during growth to ensure that it is well below the safe level of 20 mg/100 g fresh weight (Fwt) of tubers<sup>4</sup>. Glycoalkaloid levels above this recommended safety limit have been reported in immature tubers<sup>5,6</sup> but are eventually degraded to safe levels during plant growth and maturation. There is, therefore, a need to investigate the effect of maturity stages on the level of glycoalkaloids and identify the most appropriate time of harvesting tubers for human consumption.

Previous studies have reported that the concentration and stability of phytochemicals in cultivated potato plants depends on a combination of various factors. Levels of glycoalkaloids vary significantly depending on variety, growing period, agronomic practices, maturity at harvest, storage conditions, light and mechanical injury<sup>7,8,9</sup>. Phenolic content of potatoes is influenced by genotype, conditions of cultivation including temperature and drought, day length, flooding and harvest locations<sup>10,11,12</sup>. Limited information is available regarding the variation in the levels of phytochemicals at different stages of maturity in the field. Glycoalkaloids and phenolic compounds are normal constituents in all tissues of potatoes at all stages of maturity. Potentially high glycoalkaloid concentrations are found in metabolically active tissues such as flowers, unripe

berries and young leaves and in the periderm and cortex of tubers<sup>13</sup>.

The levels of total glycoalkaloids (TGA) in tubers of the major commercial potato varieties cultivated under Kenyan conditions ranges from 5.31 to 15.39 mg/100 g and are within the accepted safe limit of 20 mg/100 g Fwt<sup>14</sup>. Immature tubers have been shown to have elevated glycoalkaloid levels<sup>5</sup>. Thus, the safe level is significant and is used worldwide for selection and registration of novel varieties with high quality and commercial value.

Polyphenolic compounds such as chlorogenic acid (CGA), caffeic acid (CFA), gallic acid and protocatechuic acid are present in potato tubers as powerful antioxidants<sup>15</sup> and protect against pathogens such as *Phytophthora infestans* and potato-cyst nematodes *Globodera pallida* and *G. rostochiensis*<sup>16</sup>. CGA which accounts for up to 90% of total phenolic content of potato tubers contributes to after cooking blackening and browning reactions that reduce the quality of processed potatoes<sup>17</sup>. Thus, phenolic compounds have been subject of intense research as it affects the quality of processed products beside its role in plant protection. Therefore, the objective of study was to determine the changes in the concentration of  $\alpha$ -chaconine and  $\alpha$ -solanine, total glycoalkaloids (TGA), chlorogenic acid and total phenolics (TP) during different growth stages in tubers of five farmer preferred varieties of potato grown in Kenya.

## Materials and Methods

### Collection of Plant Material

Certified seeds of five commercial potato varieties selected for their varied resistance to late blight and suitability for chips, crisps and table-stock use were obtained from National Potato Research Centre (NPRC), Tigon Kenya (Table 1).

### Experimental Design

The selected potato varieties were grown under field conditions in two growing periods at the University of Nairobi, Chiromo campus in Aug-Nov, 2010 and July-Oct, 2011, respectively. The varieties were planted in five rows (75 cm  $\times$  25 cm) in a completely randomized design with three replicates.

**Table 1: Commercial potato varieties used for the study**

| Code  | Name         | Source      | Year of release | Duration to maturity (Days) | Optimal production altitude (Masl) | Late blight Resistance | Special attributes           |
|-------|--------------|-------------|-----------------|-----------------------------|------------------------------------|------------------------|------------------------------|
| 60111 | Asante       | KARI/CIP    | 1998            | 90-110                      | 1800-2600                          | Fairly tolerant        | Chipping quality             |
| 60130 | Desiree      | Netherlands | 1972            | 80-100                      | 1800-2600                          | Susceptible            | Good taste and storage       |
| 60109 | Dutch Robijn | Netherlands | 1960,s          | 90-110                      | 1600-2600                          | Moderate Susceptible   | Storage and crisping quality |
| 60110 | Kenya Karibu | KARI/CIP    | 2006            | 110-130                     | 1800-2600                          | Tolerant               | Crisping quality             |
| 60103 | Tigoni       | KARI/CIP    | 1998            | 100-120                     | 1800-2600                          | Tolerant               | Chipping quality             |

Source: Lung'aho et al.18, 19 and National Crop Variety List-Kenya maintained at Kenya Plant Health Inspectorate Service (KEPHIS).

### Tuber Sampling

Sampling was performed three times during tuber initiation, bulking and maturity at 55, 95 and 125 days, respectively, after planting (DAP). Three plants from each variety in each replicate were randomly selected and six tubers each weighing 17-22 g were collected, washed with cold water to remove extraneous materials and thoroughly dried. The unpeeled samples were cut into small pieces using a kitchen chopper. The samples were then thoroughly mixed and sub-samples of 300 g were freeze-dried. The dry samples were ground in a Wiley mill® to pass through a 40 mesh screen and stored at 4 °C until used for extraction and analysis of glycoalkaloid and phenolic components.

### Extraction of Glycoalkaloids

Extraction of glycoalkaloids was conducted employing the method adapted from Cataldi *et al.*,<sup>20</sup>. Two and a half grams of tuber sample were dissolved with 35 ml of 2% acetic acid for 2 hours. The extract was recovered by vacuum filtration, residue washed with 15 ml of 2% acetic acid and the combined filtrate centrifuged for 30 minutes at 6000 r.p.m. The supernatant was heated gently to 75 °C, allowed to cool and subsequently 15 ml of

58% aqueous NH<sub>4</sub>OH added to raise the pH to >10, alkaline condition needed to precipitate alkaloids. The alkaloids were rapidly precipitated in an ice-water bath for 1 hour. The precipitate was collected by centrifugation at 6000 r.p.m for 30 minutes at 1 °C and the pellet washed twice with 1% NH<sub>4</sub>OH prior to drying. The final pellet was placed in an oven at 60 °C overnight to evaporate the ammonia and solubilized prior to UV spectrophotometric and HPLC glycoalkaloid analysis.

### Extraction and Quantification of Phenolic Compounds

Phenolic compounds were extracted according to the modified method of Marinova *et al.*,<sup>21</sup>. The powdered samples (10 g) from potato tubers were first extracted with hexane to remove lipids. Phenolic acids were extracted from dry and defatted samples using 80% aq. methanol (1g frozen tissues per 10ml solvent), vortexed for 30 seconds, allowed to stand for 30 minutes and centrifuged at 6000 r.p.m for 10 minutes. The recovered supernatants were used for the quantification of total phenolics with Folin-ciocalteu (FC) reagent<sup>22</sup>. The absorbance of the reduced blue molybdenum-tungsten complex was measured at  $\lambda=765$  nm using UV/Vis spectrophotometer.

CGA was extracted from 200 mg of dry defatted powdered potato samples with 20 ml of 80% ethanol for 6 hours followed by centrifugation at 4000 r.p.m for 10 minutes. The supernatant was ultrafiltered using a 0.45  $\mu\text{m}$  Nylon membrane and re-adjusted to a volume of 20 ml with 80% ethanol. The absorbance of the diluted samples were then determined using UV/Vis spectrophotometer at  $\lambda=325$ .

### HPLC Analysis of Glycoalkaloids

The reversed-phase HPLC method was adapted from Friedman 23 with slight modifications on the mobile phase composition to improve peak resolution. Tuber glycoalkaloids were separated and identified using a Varian HPLC system (Varian Associates, Inc.). The HPLC system consisted of a 9050 variable wavelength UV-Vis detector, 9010 solvent delivery system, a 4400 integrator, a manually operated Rheodyne® 7125 sample injector and a 20  $\mu\text{l}$  loop. The separation was carried at room temperature on a Nucleosil 100-5  $\text{NH}_2$  column (250  $\times$  4.6 mm, 5 $\mu\text{m}$ ) (Macherey-Nagel GmbH & Co.) using a mobile phase composed of THF/0.025M  $\text{KH}_2\text{PO}_4$ /ACN (50:25:25, v/v/v) at a flow rate of 1 ml/min with UV-Vis detection at 208 nm.

The dried glycoalkaloid extracts were dissolved in 2ml of the mobile phase and ultrafiltered through 0.45  $\mu\text{m}$  microfilter prior to HPLC separation. The identities and quantities of  $\alpha$ -chaconine and  $\alpha$ -solanine in tuber extracts were calculated based on consistent retention times and HPLC peak areas of analytical grade standards ( $\alpha$ -cha and  $\alpha$ -sol) obtained from Sigma-Aldrich. Equal volumes (20  $\mu\text{l}$ ) of glycoalkaloid standards of known concentration and potato extracts were injected into the HPLC in duplicate under standard conditions and all values were averaged. The TGA content in tubers was calculated as the sum of  $\alpha$ -cha and  $\alpha$ -sol and final results were expressed as mg per 100g Fwt<sup>24</sup>.

### UV Spectrophotometric Analysis of Glycoalkaloids, Total Phenolics and CGA

Spectrophotometric measurements were carried out using a Beckman DU® 530 Life Science UV/Vis spectrophotometer (Beckman Coulter™). All potato tuber extracts were diluted appropriately before analysis to bring the sample absorbance to within the detection range of the UV-Vis spectrophotometer.

Spectrophotometric determination of glycoalkaloids was conducted on dry pellets that were reconstituted in 3 ml of a mixture of 50% ethanol and sulphuric acid (1:2; v/v). One ml of 1% formaldehyde was added dropwise to the solution while the flask was stirred vigorously in an ice-bath. The flask was then transferred to a water bath maintained at 23-25 °C for 90 mins and the absorbance of the resulting purple-red colour measured at 562 nm using the UV-Vis spectrophotometer. Equal volumes (100  $\mu\text{l}$ ) of GA standards of known concentration and potato extracts were subjected to analysis. A GA standard curve was established with commercial  $\alpha$ -solanine<sup>25</sup>. The absorbances values of TGA and phenolics were subjected to regression analysis and the resulting regression equations were used to estimate their concentrations.

The concentration of total phenolics in the tuber extracts was determined by Folin-Ciocalteu<sup>22</sup>. Fifty microlitres (50  $\mu\text{l}$ ) of each potato extract was transferred into a 15 ml glass tube, diluted with 3.95 ml of distilled water followed by addition of 250  $\mu\text{l}$  of 10% FC reagent (phosphomolybdate & phosphotungstate) and vortexed thoroughly. After 5 mins, 750  $\mu\text{l}$  of 7%  $\text{Na}_2\text{CO}_3$  was added and the solution was incubated at room temperature for 2 hrs. Upon reduction of the FC reagent, the absorbance of 100  $\mu\text{l}$  of the resulting blue complex was measured at 765 nm using a UV-Vis spectrophotometer against the reagent blank. The concentration of total phenolics was calculated from chlorogenic acid standard curve and results of total phenolic content were expressed as mg CGA equivalents per 100 g fresh weight (mg CGA equ/100 g Fwt) of tuber tissue<sup>26</sup>.

Chlorogenic acid content in the defatted potato powder was determined by UV-Vis spectrophotometry as described by Truong *et al.*,<sup>27</sup>. For electronic absorption, 100  $\mu\text{l}$  of tuber CGA extracts were diluted with 3.90 ml of ethanol, vortexed and incubated at room temperature for 5 minutes. The absorbance of the samples was measured on a UV-Vis spectrophotometer at 325 nm against corresponding distilled water blank. The standard curve was generated from commercial CGA standard (purity> 98%) (Fisher Scientific Co.).

### Recovery and Reproducibility of $\alpha$ -Chaconine, $\alpha$ -Solanine and Chlorogenic Acid

The accuracy of the analytical methods was validated by recovery experiments using the previously dried potato tuber powder with known amounts of authentic internal standards. The recovery of glycoalkaloids (GAs) was performed using  $\alpha$ -chaconine as an internal standard. For this purpose, 20, 50 or 100  $\mu$ g of  $\alpha$ -chaconine was added into 2.5 g of variety Tigon potato powder sample, thoroughly mixed, extracted and analyzed for glycoalkaloids in triplicate as described previously. This procedure was repeated with another set of dry tuber powder using  $\alpha$ -solanine as an internal standard.

The applicability and reproducibility of the recovery method to chlorogenic acid (CGA) and total phenolics was determined by adding 20, 50 and 100  $\mu$ g of accurately weighed CGA to tubes containing 200 mg of var. Tigon potato powder. For CGA, the samples were mixed and extracted with 20 ml of 80% ethanol and quantified using UV-Vis spectrophotometer by reading their absorbance at 325 nm. The recovery of total phenolics was obtained with CGA spiked potato samples that were extracted with 80% aq. methanol and quantified with FC method. The percent recovery was calculated using the formula;

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

Where: RM, TC and AS are the amount of recovered metabolite, original tuber content and the amount of authentic standards added before extraction. The overall recovery values obtained for glycoalkaloids and phenolics were used to adjust their concentrations to correct for losses during extraction.

### Statistical Analysis

The results obtained for glycoalkaloid and phenolic contents were analyzed by linear regression using Genstat computer software (15<sup>th</sup> Edition). Student's t-test was used to identify the peaks of  $\alpha$ -cha and  $\alpha$ -sol in crude extracts based on the corresponding retention times of their authentic standards. One-way and two-way ANOVA were used to evaluate the differences between treatment means. Significant differences in the analytical contents of both glycoalkaloids and phenolics were compared with Fisher's statistics. The p values of  $\leq 0.05$  were considered significant. The data were expressed as mean  $\pm$  standard deviation.

### Results and Discussion

#### Recovery of $\alpha$ -Chaconine, $\alpha$ -Solanine and Chlorogenic Acid

Recovery of glycoalkaloids (GAs) ranged from 86.4 to 92.1 % and that of chlorogenic acid (CGA) from 92.5 to 94.7% as indicated in Table 2.

**Table 2: Recovery of  $\alpha$ -chaconine,  $\alpha$ -solanine and chlorogenic acid added to freeze-dried powder of potato variety Tigon determined by HPLC and UV spectrophotometry**

| Amount of added standards ( $\mu$ g) | % Recovery                   |                    |                              |
|--------------------------------------|------------------------------|--------------------|------------------------------|
|                                      | Glycoalkaloids               |                    | Chlorogenic acid             |
|                                      | $\alpha$ -chaconine          | $\alpha$ -solanine |                              |
| 20                                   | 87.7 $\pm$ 1.8 <sup>b</sup>  | 86.4 $\pm$ 1.1     | 92.5 $\pm$ 1.7 <sup>b</sup>  |
| 50                                   | 88.4 $\pm$ 2.3 <sup>ab</sup> | 89.7 $\pm$ 1.3     | 93.4 $\pm$ 1.3 <sup>ab</sup> |
| 100                                  | 89.7 $\pm$ 1.9 <sup>a</sup>  | 92.1 $\pm$ 1.6     | 94.7 $\pm$ 0.8 <sup>a</sup>  |

Values are means  $\pm$  SD of three replicates. Means in the same column followed by the same letter are not significantly different at level  $p \leq 0.05$ . Original tuber phytochemical content (mg/100g Fwt);  $\alpha$ -chaconine = 6.47 $\pm$ 0.14,  $\alpha$ -solanine = 4.13 $\pm$ 0.10 and chlorogenic acid = 63.5 $\pm$ 0.12.

The high recovery values of added glycoalkaloids and phenolics from tubers indicated the validity of the extraction methods used. Optimization of UV spectrophotometry and HPLC procedures for glycoalkaloids demonstrated that both techniques are of high accuracy and may be used to quantify total glycoalkaloids. This is in agreement with the findings of Friedman<sup>23</sup> who observed that the two methods generate comparable values. The utility of inexpensive chemicals such as ethanol and methanol in UV spectrophotometry is advantageous for large-scale surveys of total glycoalkaloids and phenolics during breeding programs. The HPLC procedure appeared more rapid and can be successfully applied for separation and analysis of  $\alpha$ -chaconine and  $\alpha$ -solanine in improved potato varieties that show potential for commercial production.

Glycoalkaloid and phenolic data were obtained from unpeeled freeze-dried tuber samples of potatoes at different stages of maturity. Since potato peels contain high levels of secondary metabolites, the reported results reflect the amount of glycoalkaloids and phenolics components present in whole tubers.

#### Glycoalkaloid Content of Potato Varieties at Different Stages of Maturity

The total glycoalkaloid (mg/100g Fwt) content of tubers from five commercial potato varieties at different stages of maturity as determined by HPLC and UV spectrophotometry are shown in Table 3. The results indicate that the influence of variety and stage of maturation on the concentration of TGA in potato tubers were highly significant ( $p < 0.001$ ).

**Table 3: Total glycoalkaloid (TGA) (mg/100g) content of potato tubers at different stages of maturity as determined by HPLC and UV spectrophotometry**

| Variety             | Stage of maturity<br>(Days after planting) | Total glycoalkaloid (mg/100g) content |                         |
|---------------------|--|---------------------------------------|-------------------------|
|                     |  | HPLC<br>Analysis                      | UV<br>spectrophotometry |
| Asante              | 55   | 9.40 <sup>a</sup>                     | 10.07 <sup>a</sup>      |
|                     | 95   | 8.67 <sup>a</sup>                     | 8.86 <sup>a</sup>       |
|                     | 125  | 8.17                                  | 9.14                    |
| Desiree             | 55   | 9.02 <sup>a</sup>                     | 9.12 <sup>a</sup>       |
|                     | 95   | 7.75 <sup>a</sup>                     | 7.79 <sup>a</sup>       |
|                     | 125  | 7.03 <sup>a</sup>                     | 7.03 <sup>a</sup>       |
| Dutch Robijn        | 55   | 7.84 <sup>a</sup>                     | 7.88 <sup>a</sup>       |
|                     | 95   | 6.73 <sup>a</sup>                     | 6.78 <sup>a</sup>       |
|                     | 125  | 5.82 <sup>a</sup>                     | 6.17 <sup>a</sup>       |
| Kenya Karibu        | 55   | 10.48 <sup>a</sup>                    | 10.85 <sup>a</sup>      |
|                     | 95   | 9.69 <sup>a</sup>                     | 9.76 <sup>a</sup>       |
|                     | 125  | 8.38 <sup>a</sup>                     | 8.02 <sup>a</sup>       |
| Tigoni              | 55   | 12.22                                 | 10.57                   |
|                     | 95   | 10.21 <sup>a</sup>                    | 9.49 <sup>a</sup>       |
|                     | 125  | 9.24 <sup>a</sup>                     | 9.69 <sup>a</sup>       |
| LSD (0.05)<br>(n=3) | SM   | 0.05                                  | 1.22                    |
|                     | V  | 0.06                                  | 1.58                    |
|                     | SM×V                                       | 0.11                                  | 2.74                    |

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

The tuber total glycoalkaloid (TGA) contents determined by HPLC method ranged from 7.84 mg to 12.22 mg/100g, 6.73 mg to 10.21 mg/100g and 5.82 mg to 9.24 mg/100g at 55, 95 and 125 days after planting (DAP), respectively. The tubers from Dutch Robijn and Tigoni varieties had the lowest (6.80 mg) and highest (10.56 mg/100g) concentration of mean TGA, respectively. The mean tuber TGA concentration obtained by spectrophotometry ranged from 6.94 mg to 9.92 mg/100g Fwt, in var. Dutch Robijn and Tigoni, respectively. The data clearly shows that there was a significant ( $p < 0.001$ ) TGA reduction from the period between tuber initiation and maturity.

#### $\alpha$ -Chaconine and $\alpha$ -Solanine Content of Potato Varieties at Different Stages of Maturity

The HPLC results for  $\alpha$ -chaconine ( $\alpha$ -cha) and  $\alpha$ -solanine ( $\alpha$ -sol) content (mg/100g Fwt) of tubers from five commercial potato varieties are shown in Table 4. The varieties Dutch Robijn (DR) and Tigoni contained the least and the highest concentration of  $\alpha$ -cha during tuber initiation, bulking and at harvest at 55, 95 and 125 days after planting (DAP), respectively. The results show that the influence of variety and stage of maturity on the concentrations of  $\alpha$ -cha and  $\alpha$ -sol in potato tubers were highly significantly ( $p < 0.001$ ).

**Table 4:  $\alpha$ -chaconine and  $\alpha$ -solanine content (mg/100g) of potato tubers at different stages of maturity**

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

Values are mean of three replicates. Means followed by the same letter along each column are not significantly different at level  $p \leq 0.05$ . LSD = least significant differences, SM = stage of maturity, V= variety, SMxV= stage of maturity and variety interaction.

The mean tuber concentration of  $\alpha$ -chaconine ( $\alpha$ -cha) ranged from 4.29 mg/100g to 7.33 mg/100g, 4.17 mg to 5.91 mg/100g and 3.53 mg to 5.46 mg/100g at 55, 95 and 125 DAP, respectively

(Table 3). There was a gradual decrease in  $\alpha$ -cha content in tubers between the time tuberization at 55 DAP and at maturity 125 DAP. The tuber  $\alpha$ -solanine ( $\alpha$ -sol) content in all the tested varieties

followed a pattern similar to that of  $\alpha$ -cha. The  $\alpha$ -sol concentration in tubers at 55, 95 and 125 DAP ranged from 3.55 mg to 4.89 mg/100g, 2.56 mg to 4.30 mg/100g and 2.29 mg to 3.78 mg/100g, respectively. Overall, the average  $\alpha$ -solanine ( $\alpha$ -sol) content of tubers was highest (4.32 mg/100g) and lowest (2.80 mg/100g) in the vars. Tigoni and Dutch Robijn, respectively.

The results demonstrate that the concentration of glycoalkaloids at different stages of maturity was variety-dependent and are within the limits that have reported by other authors<sup>23,25</sup>. The glycoalkaloid values in this study are also in agreement with findings in potato varieties grown commercially in North America, Germany and UK<sup>28</sup>. Potato varieties with genetically high TGA have higher capability to synthesize glycoalkaloids to potentially fatal levels

when grown under extremely stressful conditions such as high salt, drought and flooding<sup>29</sup>. Exceptionally high levels GA led to a ban on commercial cultivation of Vars. 'Lenape' and Magnum Bonum in USA and Sweden, respectively<sup>30,31</sup>. Therefore, breeders should identify and subject suitable parental lines to different environmental conditions before selection and registration of varieties with low glycoalkaloid (GA) content and other additional traits.

#### Chlorogenic Acid and Total Phenolic Content of Potato Tubers at Different Stages of Maturity

The phenolic content of tubers from five commercial potato varieties determined by UV-spectrophotometry is shown in Table 5. The mean CGA and TP values for potato tubers ranged from 46.39 to 58.04 mg/100 g and 129.24 to 192.52 mg CGA/g, respectively.

**Table 5: Chlorogenic acid (CGA) and total phenolic (TP) content of potato tubers at different stages of maturity**

| Variety             | Stage of maturity<br>(Days after planting) | Phenolic content     |                      |
|---------------------|--|----------------------|----------------------|
|                     |  | CGA (mg/100g)        | TP (mg CGA/g)        |
| Asante              | 55   | 60.78 <sup>ab</sup>  | 129.80 <sup>de</sup> |
|                     | 95   | 54.93 <sup>de</sup>  | 122.82 <sup>de</sup> |
|                     | 125  | 51.39 <sup>g</sup>   | 135.10 <sup>de</sup> |
| Desiree             | 55   | 55.08 <sup>cd</sup>  | 132.32 <sup>de</sup> |
|                     | 95   | 50.11 <sup>gh</sup>  | 123.51 <sup>de</sup> |
|                     | 125  | 44.23 <sup>i</sup>   | 125.49 <sup>de</sup> |
| Dutch Robijn        | 55   | 51.77 <sup>fg</sup>  | 153.30 <sup>c</sup>  |
|                     | 95   | 46.81 <sup>hi</sup>  | 135.33 <sup>d</sup>  |
|                     | 125  | 40.59                | 120.32 <sup>de</sup> |
| Kenya Karibu        | 55   | 63.81 <sup>a</sup>   | 231.53 <sup>a</sup>  |
|                     | 95   | 56.79 <sup>c</sup>   | 195.20 <sup>b</sup>  |
|                     | 125  | 53.04 <sup>ef</sup>  | 132.41 <sup>de</sup> |
| Tigoni              | 55   | 63.94 <sup>a</sup>   | 234.61 <sup>a</sup>  |
|                     | 95   | 58.30 <sup>b</sup>   | 182.71 <sup>b</sup>  |
|                     | 125  | 51.89 <sup>efg</sup> | 160.23 <sup>c</sup>  |
| LSD (0.05)<br>(n=3) | SM   | 2.55                 | 11.70                |
|                     | V  | 3.30                 | 15.00                |
|                     | SM×V                                       | 5.71                 | 26.10                |

Values are mean of three replicates. Means followed by the same letter along each column are not significantly different at level  $p \leq 0.05$ . LSD = least significant differences, SM = stage of maturity, V= variety, SM×V= stage of maturity and variety interaction.

The results indicate that variety and stage of maturity affect the concentration of tuber CGA and TP were highly significant ( $p < 0.001$ ). The CGA content among potato varieties ranged 46.39 to 58.04 mg/100g with vars. Dutch Robijn and Tigonni recording the lowest and highest concentrations, respectively. The corresponding total phenolic (TP) content varied from 127.1 to 192.5 CGA/100g Fwt with the highest and lowest concentration measured in vars. Tigonni and Desiree, respectively. The observed variations of TP and CGA among potato varieties evaluated are within the acceptable limits reported in previous investigations<sup>32,33</sup> in which tuber TP and CGA contents ranged from 1.0-181 mg CGA/100g Fwt and 3.0-90 mg/100g Fwt, respectively. This study has also established that vars. Tigonni and Kenya Karibu (KK) have great potentials as a source of TP which can be positive in terms of the antioxidant intake but at the same time could speed potato browning reactions that lower their quality. The var. Asante exhibited higher levels of TP and a lower level of CGA. Therefore, adoption within Kenyan farming communities of potato vars. Tigonni, Asante and Kenya Karibu with enhanced phenolic content could increase the antioxidants in the diet.

The concentration of chlorogenic acid and total phenolic content decreased significantly from the period between tuber bulking at 55 days after planting (DAP) and the period when mature tubers were ready for harvest at 125 DAP. This demonstrates that the stage of maturity influences the level of phenolic compounds in potato tubers, an

observation which is consistent with results of Reyes *et al.*,<sup>11</sup> who reported decreased anthocyanins and total phenolics during tuber growth and development. The observed changes suggest that phenolic profiles can be useful to potato breeders and growers in selecting the optimum time for harvesting to increase antioxidant properties in food.

The research findings have demonstrated the influence of variety and stage of maturity on the levels of phytochemicals in potato tubers. The glycoalkaloid content in fresh mature tubers of all potato varieties studied were well below the upper safety limit of 20 mg/100g Fwt which implies that they are unlikely to pose any public health and safety concern. This study has also revealed large variations in total phenolic and chlorogenic acid contents among the potato varieties at different stages of maturity. The glycoalkaloid and phenolic levels decreased with increasing maturity of potato tubers. Therefore, harvesting at full maturity is essential for consumer safety as well as improved nutritional and commercial value.

#### Acknowledgments

This study was supported by research funds from Deutscher Akademischer Austauschdienst (DAAD) and University of Nairobi. We thank the National Potato Research Center for supplying the tubers of potato varieties used in this study. We are grateful to KEPHIS for the use of HPLC facility and Rosemary Nganga, formerly Principal analytical Chemist for her excellent technical assistance.

#### References

1. Friedman, M. Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. *Journal of Agricultural and Food Chemistry*; **54**:8655-8681: (2006).
2. Rytel, E. Changes in the levels of glycoalkaloids and nitrates after the dehydration of cooked potatoes. *American Journal of Potato Research*; **89**:501-507: (2012).
3. McGehee, D.S., Krasowski, M.D., Fung, D.L., Wison, B., Gronert, G.A. and Moss, J. (2000). Cholinesterase inhibition by potato glycoalkaloids slows mivacurium metabolism. *Anesthesiology*; **93** (2): 510-9.
4. Carlson-Nilsson, U., Zoteyeva, N. and Reslow, F. Glycoalkaloid content in potato tubers with different levels of resistance to *Phytophthora infestans*. *PPO-Special Report no.*; **15**: 195-200: (2012).
5. Zolnowski, A.C., Ciecko, Z. and Wyszowski, M. Glycoalkaloids content in potato tubers as affected by fertilization during vegetation and storage. Mat. I conference of the Interfood Network, 12-14.09.2002 Olsztyn, Poland: 27-30: (2002).
6. Senguel, M., Keles, F., Keles, M.S. The effect of storage conditions (temperature, light,

- time) and variety on the glycoalkaloid content of potato tubers and sprouts. *Food Control*, **15**:281-286: (2004).
7. Hamouz, K., Pazderui, K., Lachman, J., Orsák, M., Pivec, V., Hejtmánková, K., Tomášek, J. and Cížek, M. Effect of cultivar, flesh colour, location and year of cultivation on glycoalkaloid content in potato tubers. *Plant, Soil and Environment*; **60**(11): 512-517: (2014).
  8. Skrabule, I., Grauda, D., Mikelsone, A. and Vasariete, A. Adaptation of glycoalkaloids detection method for evaluation of Latvian potato genetic resources. *Agronomy Research*, **8**(3):705-710: (2010).
  9. Valcarcel, J., Reilly, K., Gaffney, M. and O'Brien, N. Effect of Genotype and environment on the glycoalkaloid content of rare, heritage and commercial potato varieties. *Journal of Food Science*; **79**(5): 42-48: (2014).
  10. Islam, M.S., Yoshimoto, M., Ishiguro, K., Okuno, S. and Yamakawa, O. Effects of artificial shading and temperature on radical scavenging activity and polyphenolic composition in sweetpotato (*Ipomoea batatas* L.) leaves. *Journal of the American Society for Horticultural Science*; **128**:182-187: (2003).
  11. Reyes, L.F., Miller Jr. J.C. and Cisneros-Zevallos, L. Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. *American Journal of Potato Research*; **81**(3):187-193: (2004).
  12. Lin, K.-H., Chao, P.-Y., Yang, C.-M., Cheng, W.-C., Lo, H.-F. and Chang, T.-R. The effects of flooding and drought stresses on the antioxidant constituents in sweet potato leaves. *Botanica Studies*; **47**: 417-426: (2006).
  13. Ventrella, E., Marciniak, P., Adamski, Z., Rosínski, G., Chowánski, S., Falabella, P., Scranò, L. and Bufo, S.A. Cardioactive properties of *Solanaceae* plant extract glycoalkaloids and pure on *Zophobas atratus*. *Insect Science*; **22**: 251-262: (2015).
  14. Kirui, G.K., Misra, A. K., Olanya, O.M., Friedman, M., El-Bedewy, R. and Ewell, P.T. Glycoalkaloid content of some superior potato (*solanum tuberosum* L.) clones and commercial cultivars. *Archives of Phytopathology and Plant Protection*; **42**(5): 453-463: (2009).
  15. Azadeh, M.S., Hashem, P., Nima, N. and Amirhosein, E. Phenolics in potato peels: Extraction and utilization as natural antioxidants. *World Applied Sciences Journal*; **18**(2): 191-195: (2012).
  16. Taoutaou, A., Socaciu, C., Pamfil, D., Balazs, E. and Botez, C. Role of some phenolic compounds in a resistant gene pyramided potato genotype to late blight. *Bulgarian Journal of Agricultural Science*; **19**(1):126-132: (2013).
  17. Payyavula, R.S., Shakya, R., Sengoda, V.G., Munyaneza, J.E., Swamy, P. and Navarre, D.A. Synthesis and regulation of chlorogenic acid in potato: Rerouting phenylpropanoids flux in HQT-silenced lines. *Plant Biotechnology Journal*; **13**:551-564: (2015).
  18. Lung'aho C., Nderitu S.K.N., Kabira J.N., El-Bedewy R., Olanya O.M. and Walingo A. Yield performance and release of four late blight tolerant potato cultivars in Kenya. *Journal of Agronomy*; **5**:57-61: (2006).
  19. Lung'aho C., Chemining'wa G.N., Fu Y.-B., Shibairo, S.I., Hutchinson, M.J. and Paniagua, H.G. Genetic diversity of Kenyan potato germplasm revealed by simple sequence repeat markers. *American Journal of Potato Research*; **88**:424-434: (2011).
  20. Cataldi, T.R.I., Ielario, F. and Bufo, S.A. (2005). Analysis of tomato glycoalkaloids by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, **19**: 3103-3110.
  21. Marinova, D., Ribarova, F. and Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. University of Chemical Technology and metallurgy*, **40**(3): 255-260.
  22. Chirinos, R., Campos, D., Arbizu, C., Rogez, H., Rees, J-F. O., Larondelle, Y., Noratt, G. and Cisneros-Zevallos, L. Effect of genotype, maturity stage and post-harvest storage on phenolic compounds, carotenoid content and antioxidant capacity, of Andean mashua tubers (*Tropaeolum tuberosum* Ruiz & Pavón). *Journal of the Science of Food and Agriculture*, **87**:437-446: (2007).

23. Friedman, M. Analysis of biologically active compounds in potatoes (*Solanum tuberosum* L), tomatoes (*Lycopersicon esculentum*), and jimson weed (*Datura stramonium*) seeds. *Journal of Chromatography A*; **1054**:143-155: (2004).
24. Birch A.N.E., Geoghegan I.E., Griffiths D.W. and McNicol J.W. The effect of genetic transformations for pest resistance on foliar solanidine-based glycoalkaloids of potato (*Solanum tuberosum*). *Annals of Applied Biology*; **140**:143-149: (2002).
25. Zarzecka, K., Gugala, M. and Mystkowska, I. Glycoalkaloid contents in potato leaves and tubers as influenced by insecticide application. *Plant, Soil and Environment*; **59**:183-188: (2013).
26. Burgos, G., Amoros, W., Mu M.L., Sosa P., Cayhualla E., Sanchez C. and Bonierbale M. Total phenolic, total anthocyanins and phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes affected by boiling. *Journal of Food Composition and Analysis*; **30**: 6-12: (2013).
27. Truong V.-D., Mcfeeters R.F., Thompson R.T., Dean L.L. and Shofran B. Phenolic acid content and composition in leaves and roots of common commercial sweetpotato (*Ipomea batatas* L.) cultivars in the United States. *Journal of Food Science*; **72**(6):343-349: (2007).
28. Dale, M.F.B. and Mackay, G.R. Inheritance of table and processing quality. In: Bradshaw, J.E. & Mackay, G.R. (Eds.). *Potato genetics*. CAB International, Wallingford; 285-315: (2007).
29. Friedman, M.L. and McDonald, G.M. Potato glycoalkaloids: Chemistry, analysis, safety, and plant physiology. *Critical Reviews in Plant Sciences*; **16**(1): 55-132: (1997).
30. Hellenäs, K.-E., Branzell, C., Johnson, H. and Slanina, P. High levels of glycoalkaloids in the established Swedish potato variety Magnum bonum. *Journal of Science, Food and Agriculture*; **68**: 249-255: (1995).
31. Nahar, N. Regulation of sterol and glycoalkaloid biosynthesis in potato (*Solanum tuberosum* L.) – Identification of key genes and enzymatic steps. PhD Thesis. Swedish University of agricultural Sciences, Uppsala; (2011).
32. Im, H.W, Suh, B-S., Lee, S-U., Kozukue, N., Ohnisi-Kameyama, M., Levin, C. and Friedman, M. Analysis of phenolic compounds by HPLC and LC/MS in potato plant flowers, leaves, stems, and tubers and in home processed potatoes. *Journal of Agricultural and Food Chemistry*; **56**: 3341-3349: (2008).
33. Reyes, I.F., Miller Jr. J.C. and Cisneros-Zevallos, L. Antioxidant capacity, Anthocyanins and total phenolics in purple- and red-fleshed potato (*Solanum tuberosum* L.) genotypes. *American Journal of Potato Research*; **82**:271-277: (2005).