Research Article

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Solanidine Acetate – The Modified Glycoalkaloid Imparting Toxicity in Green Coloured and Sprouted Potatoes

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Abstract

Glycoalkaloids are one of the major secondary metabolites present in potatoes and also in the entire solanaceae family. Being toxic, the main function of this compound in plants is in the mechanism of disease resistance and they also act as feeding deterrents. The glycol alkaloid content was reported to be very high in the sprouted and green coloured potatoes. The glycol alkaloids extracted from the peel of potatoes during various post harvesting stages was subjected to TLC and FT/IR analysis.LC-MS showed the presence of the glycoalkaloid, α-chaconine in the purified samples of normal, sprouted and green coloured potatoes. The molecular weight of α-chaconine is 852.053kDa as represented in the MS. The specific mass fractions were also present in the spectrum. A slight change was observed in the MS of the extract from normal potato and the green coloured and sprouted potato. The mass fraction corresponding to the solanidineaglycone moiety in the normal potato was absent in both green coloured and sprouted potato instead of which there was a new mass representation of 437.71kDa indicating the presence of solanidine acetate as the modified aglycone moiety. Acetyl derivatives of glycol alkaloids generally increase toxicity and hence one of the reasons behind the increased toxicity of green coloured and sprouted potatoes might be due to the presence of this aglycone acetyl derivative.

Keywords: Glycoalkaloid, solanine, chaconine, solanidine, solanidine acetate, LC-MS Q ToF

Introduction

Solanaceae or nightshade family are an economically important family of flowering plants. They include a number of important agricultural crops, medicinal plants, weeds, and ornamentals. Many members of the family contain potent alkaloids, and some are highly toxic, but many cultures eat nightshades, in some cases as staple foods.

Glycoalkaloids, a group of nitrogen containing steroidal glycosides are biologically active secondary metabolites in plants. Glycoalkaloids are not required for plant growth and function. However they have been associated with plant resistance to pest and pathogens and also exhibit a concentration dependent toxicity to a wide range of organisms from fungi to humans [1]. One of the main reasons for this toxicity is considered as the high alkaloid content. Glycoalkaloids are the main alkaloids which contribute toxicity in potatoes, also the entire solanaceae family. More than 80 different steroidal glycoalkaloids have been identified in various potatoes. α -solanine and α-chaconine are the two important glycoalkaloids in Solanum tuberosam, which is a major caltivar. The ratio of α-solanine to α-chaconine is varying widely among tissues, genotypes and growing conditions. The amount of glycoalkaloid is also a genetic trait, but some environmental factors such as light exposure and wounding can increase the amount of glycoalkaloids in potato tubers, this may cause the tuber unsuitable for human use [2].

The toxicity of glycoalkaloids in humans is well studied, with "solanine"poisoning from green coloured and sprouted potatoes being reported as early as 1980. The mechanism of toxicity induced by glycoalkaloids is associated with their membrane disruptive properties and their inhibition of acetylcholinesterase activity. As steroidal glycoalkaloids are not destroyed during cooking, an upper limit of 20mg /100g fresh weight in potato tubers has been established for the release of commercial cultivars.The present study focuses on the various types of glycoalkaloids generated in the potatoes at different stages of post harvesting with specific reference to green and sprouted potatoes.

Materials and Methods

1. **Sample:** Three different post-harvesting stages of potato tuber (Solanum tuberosam) were taken as the sample

Type 1: Normal potato
Type 2: Green coloured potato
Type 3: Sprouted potato

Normal potato Green coloured potato Sprouted potato

2. **Sample collection:** The fresh potatoes were collected from Athirampuzha vegetable market, Kottayam, Kerala were used for the extraction of glycoalkaloids.
3. **Sample preparation:** The potato was peeled using sharp peeler, dried in shade and powdered.it was stored in clean and air tight bottles.
4. **Extraction of Glycoalkaloid:** 1 g of dried powder was taken from each sample and extracted with 20 ml of 5% acetic acid in water for 4 times at an interval of 30 minutes each. The extract was filtered using what Mann no.1 filter paper to separate the undissolved sample particles. The pH of acetic acid extract was found to be in a range of 3-4.The pH of the extract was adjusted to 11 with 30% ammonium hydroxide solution. This facilitates the release of free glycoalkaloids from the salt form. The alkaline extract was partitioned 4 times with 20 ml of water saturated butanol. The butanol fraction was combined together and evaporated. The residue was dissolved in 3 ml of methanol. Again the methanol was evaporated and redissolved in 1 ml of methanol to obtain a concentrated methanolic fraction of glycoalkaloid [3].
5. **Thin layer chromatography:** The methanol extract thus obtained was spotted on silica gel thin layer chromatography (TLC) plate. Silica gel plates were prepared using TLC silica gel G, 34 g of silica gel was dissolved in 100 ml of water. Thin layer plates were prepared using TLC spreader and completely dried. The prepared plates were activated at 110°C for 1 hour. The sample was chromatographed on the TLC silica gel plate using the solvents chloroform, methanol and ammonia in the ratio 10:3:0.5. The samples were allowed to run upto3/4th of the plate and the solvent front was marked. The plates were dried in hot air oven 5 minutes at 70^{o}C and visualized by spraying dragendorff's reagent. Dragendorff's reagent gives orange-red bands with nitrogen containing compounds. The bands within the similar region and Rf of the spot was scraped and dissolved in methanol [4].
6. **UV spectrum:** The methanolic extract obtained after TLC was subjected to UV spectral analysis in the range 190-1100nm using the Shimadzu UV 2600 at DBT/MSUB instrumentation centre, Mahatma Gandhi University.
7. **FT/IR:** The methanolic extract obtained after TLC was subjected to FT/IR analysis. FT/IR analysis was done using Shimadzu IR prestige 21 with ATR attachment, at DBT/MSUB instrumentation centre, School of Biosciences, Mahatma Gandhi University, with wave number ranging from 4000- 750cm^{-1}.
8. **LC-MS-Q ToF:** The sample, which is purified by TLC, was dried and redissolved in HPLC grade methanol was subjected to LC-Q-ToF analysis. Acquity H class UPLC system (Waters, USA) coupled with Xevo G2 Quadrapole Time-of-Flight (Q-ToF) high resolution mass spectrometer (Waters, USA), Quarternary solvent manager pumping system and TUV detector were used. Electron spray technique was used for ionization. The column used was Acquity UPLC BEH C_{18} carbohydrate column with 1.7µm particle size, 50 mm length and 2.1 mm diameter. A gradient elution was performed with the solvent system, water with 0.1% formic acid (solvent A): methanol (solvent B) at a flow rate of 0.3 mL/minute for 9 minutes. All the spectra were recorded in positive and negative ionization modes.LC-MS -Q ToF analysis was done at Inter University Instrumentation Centre (IUIC), School of Environmental Sciences, and Mahatma Gandhi University [5].

Results

The glycoalkaloids are successfully extracted from the potato peel through the extraction procedure mentioned in the methodology. The extracted samples were subjected to thin layer chromatography. The separation of the sample is improved by changing the solvent ratio to 10:6:0.5(chloroform: methanol: ammonia). Detection of the bands in the TLC was very easily done using dragendorff's reagent.Single and unique orange coloured bands were observed in all the three samples and the Rf value was also similar (plate 1) and (table 1).

Plate 1: Thin layer chromatography of the glycoalkaloid extracted from the green coloured potato, normal potato, and sprouted potato respectively (from left to right) using the solvent chloroform: methanol: ammonia (10:3:0.5).

1- Green coloured potato

2- Normal potato

3- Sprouted potato

Table 1: Rf value of the glycoalkaloid from potatoes in different post harvested stages.

Sample	Rf value
Normal potato	0.72
Green coloured potato	0.75
Sprouted potato	0.742

UV spectral analysis of the glycoalkaloid extracts from each sample was done. The maximum absorbance was observed in between 200nm-250nm for each sample. But there are slight differences are present in the maximum absorbance, the maximum absorbance of normal potato; green coloured potato and sprouted potato are 205nm, 208nm and 207nm respectively (figure.1). The maximum UV absorbance of α-solanine and α-chaconine was observed as 202 and 201.5nm respectively, in the literature [6].

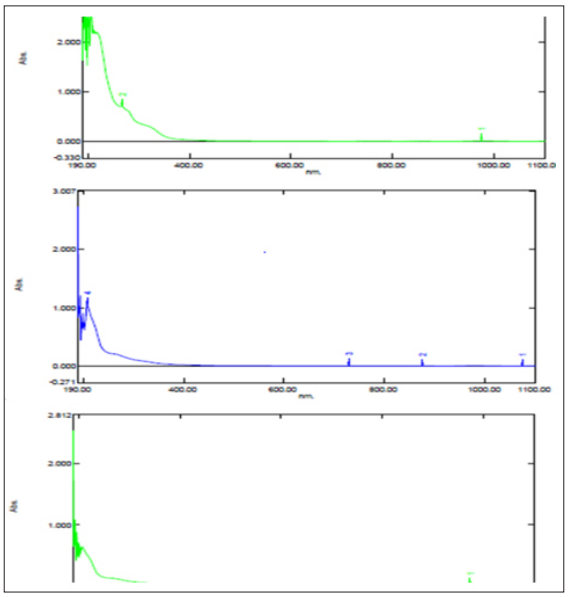
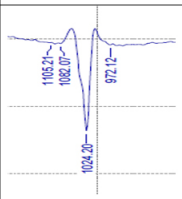
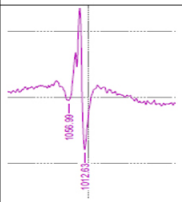
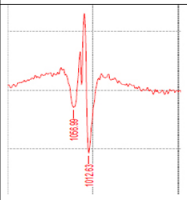


Figure 1: UV spectral analysis of the glycol alkaloid extracted from potatoes in three different post harvesting stages, normal potato, green coloured potato and sprouted potato respectively from top to bottom.

FT/IR analysis of the glycoalkaloid from different post harvesting stages of potato was done. The peaks in the spectrum were observed almost at the same regions, but there are slight differences in the banding pattern also present. One of the principle peak was obtained in between 1000 and 1100nm. FT/IR spectrum of normal potato shows the peak at 1020.24cm^{-1} and that in green coloured potato and sprouted potato is 1012.63cm^{-1}.This peak has the maximum peak area than the other peaks in all the three samples, normal potato, green coloured potato and sprouted potato. The similar regions in FT/IR spectrum shows in the Table 2.

Table 2: Similar regions in FT/IR spectrum of potatoes belong to various post harvesting stages.

Sample	Similar regions in FT/IR
Normal potato	
Green coloured Potato	
Sprouted potato	

The samples obtained after thin layer chromatography containing the purified glycoalkaloid was subjected to LC-MS Q ToFanalysis for the molecular level analysis of the glycoalkaloids in different post harvested stages of potatoes (figure 2 and 3).

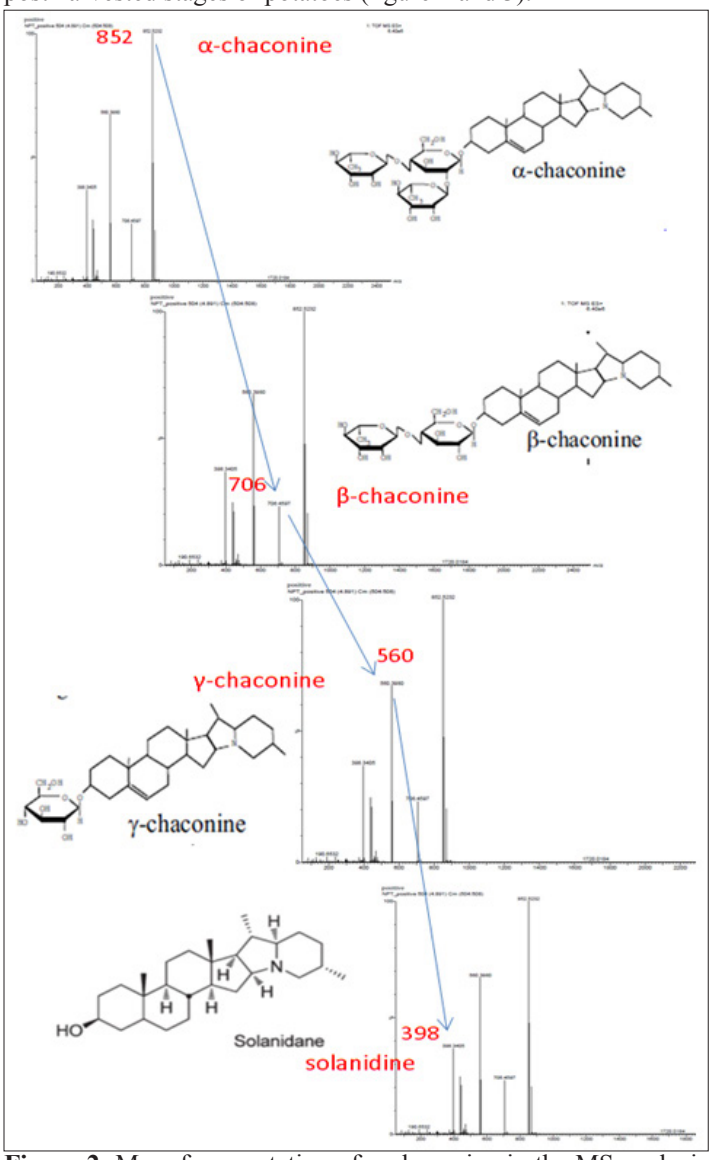


Figure 2: Mass fragmentation of α-chaconine in the MS analysis of the normal potato.

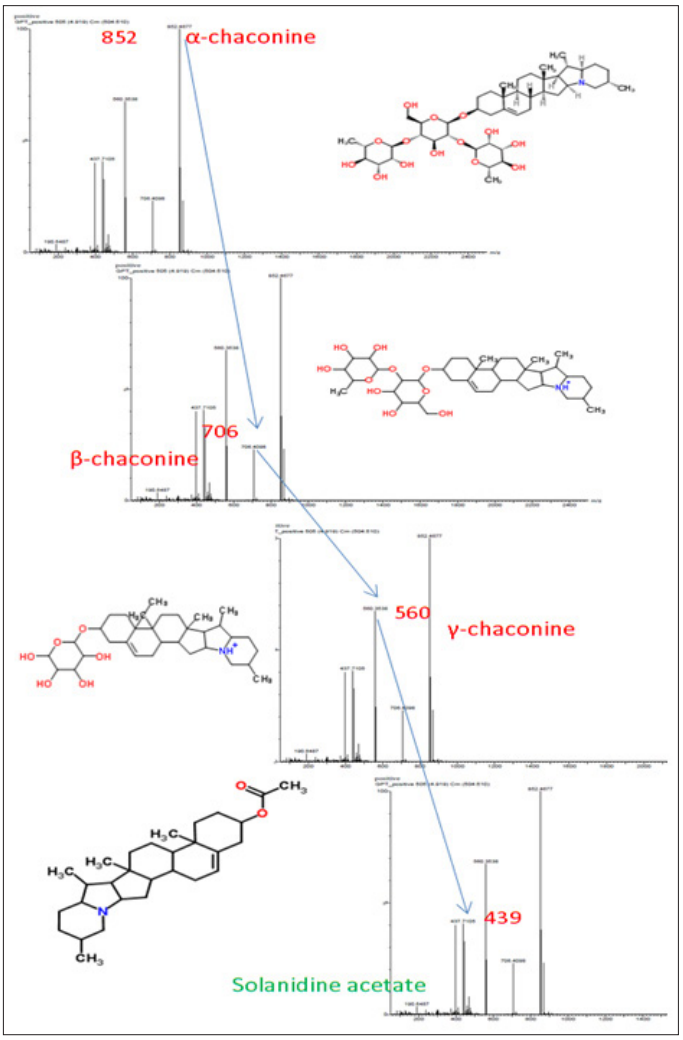


Figure 3: Mass fragmentation of α-chaconine in the MS analysis of green coloured and sprouted potatoes.

Discussion

Three different samples considered for this study are normal potato, green coloured potato and sprouted potato. Both green coloured potato and sprouted potato represented the potatoes at various post harvesting stages. The former is formed on longer exposure to increased light intensity during storage and the latter is formed on storage under increased moisture content. Both these types are generally not recommended for human consumption. There are many references, which proved that the amount of glycoalkaloid is increased by greening and sprouting of the potato tuber during post harvesting period. But there is no complete evidence for the reason of this increase. The present project was initiated to explore the types of glycoalkaloids in normal potato and in the green coloured and sprouted ones. The potato peel was taken for the extraction of glycoalkaloids, it is because of the peel contain more glycoalkaloid than the flesh. Extraction from the peel gave more concentrated sample for the analysis than from the flesh or whole tuber.

The extracted samples were subjected to thin layer chromatography. Solvent system was changed to 10:3:0.5 (chloroform: methanol: ammonia). Increase in the amount of chloroform retarded the movement of the sample due to change in polarity of the solvent mixture. Detection of the bands was very easy with the dragendorff's reagent, which specifically reacted with the tertiary amine of the glycoalkaloid.

Glycoalkaloids has a maximum UV absorbance in the 200nm range. The literature shows, pure solanine has a maximum absorbance at 202nm and chaconine has a maximum absorbance at 201.5nm (figure.1). The extracted samples also showed their maximum absorbance at this range, confirming the presence of glycoalkaloid in the extracted sample (Table 3).

Table 3: UV absorbance maxima of glycoalkaloids extracted and purified from potato in different post harvesting stages.

	Normal potato	Green coloured potato	Sprouted potato
Wavelength	205nm	208nm	207nm
Absorbance	3.484	1.061	0.652

When the FT/IR analysis of the scraped spot of TLC was done, a principle peak was observed at 1020.24cm^{-1}in normal potato and 1012.63cm^{-1} in green coloured and sprouted potato (Table.2). The 1020.24cm^{-1} represents C-O-C asymmetric stretching and that of 1012.63cm^{-1} represented C-O-C asymmetric stretching. The spectrum also contained a peak at the range of 3400cm^{-1} in all the three samples, possibility indicating the presence of O-H stretching in the extracted samples.

The three FT/IR spectrums carry a similar region(1000cm^{-1}-1200cm^{-1}) that are shown in the table.2, these peaks are exactly similar in green coloured and sprouted potatoes but slightly different from normal potato. This indicated that even though the basic structural identity of glycoalkaloids in the three samples remained same, there have been specific structural changes in the glycol alkaloids of green coloured and sprouted ones possibly indicating the slight structural changes that have occurred to the glycoalkaloids at the time of post harvesting stages.

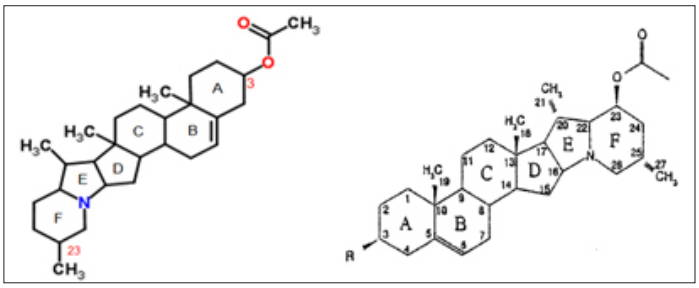
LC-MS Q-ToF analysis of all three samples was done. The retention time of pure glycoalkaloid in a total ion chromatogram is 4.8min. The total ion chromatogram of the three samples in the present study also contained major peaks in the range of 4.8min. The retention time of the major peak of normal potato was at 4.89min and green coloured and sprouted potatoes were at 4.92min. All the peaks obtained in the total ion chromatogram were subjected to the MS, the MS of only the major peak in the LC is being considered for further analysis.

The MS of the major peak contained four mass fragments. In normal potato the molecular ion is 852.5232m/z, representing the molecular weight of α-chaconine, the other three is 706.4597,560.3980 and398.3405m/z.These fractions represented the molecular weight of β-chaconine, γ-chaconine and aglycone solanidine respectively. The MS of green coloured potato and sprouted potato also contained four major mass fragments. Three of them are similar to the peaks in the MS of normal potato but the fourth one was found to be different. It corresponded to 437.9435m/z, representing the molecular weight of solanidine acetate, its original molecular weight being 439.673kDa. It followed that Solanidine in the normal potato has been derivatised to solanidine acetate both in the green coloured and sprouted potatoes. The acetylated products of alkaloids are present in the natural condition and also acetylation reaction is present in plants. This possibly indicated that, acetylation of the aglycone might haveoccurred

at the time of greening and sprouting during post harvesting stage.

The presence of solanidine acetate in the green coloured and sprouted potato is being reported for the first time. This might have been formed as a result of long storage during the post harvesting stage. It is to be noted that the type of alkaloids i.e. chaconine is same in all the normal, green coloured and sprouted potato. The structural change has been observed on the aglycone moiety alone.

Leptinine and leptine are glycoalkaloids present in a wild variety of potato (Solanumchacoense Bitter) which have more pest resistance property. These glycoalkaloids are natural antifeedants to Colorado potato beetle. The aglycone part of leptinine and leptine are leptinidine and acetylleptinidine respectively [7]. This is a solid evidence for the existence of acetylated glycoalkaloids in plants. Leptine is more toxic than the chaconine. Acetylleptinidine is the acetylated form of leptinidine and also solanidine acetate is the acetylated form of the solanidine. This solanidine acetate may also the reason for high toxicity of green coloured and sprouted potatoes than the normal potato.



Solanidine acetate Acetylleptinidine

The α-Chaconine is one of the major glycoalkaloid in the potato and β-chaconine and γ-chaconine are the hydrolytic products of α-chaconine. Solanidine is the aglycone of chaconine, which is the last hydrolytic product of chaconine. This indicated that the extracted sample contain pure glycoalkaloids. Solanine is another major glycoalkaloid in potatoes, but the corresponding mass fragments are absent in the MS spectrum of the samples.

Figure 2 Represented explanations for the mass distribution in the MS of the glycoalkaloid extracted from normal potato and figure 3, Represented explanation for the mass distribution in the MS of the glycoalkaloids extracted from the green coloured and sprouted potato. It clearly indicated fragmentation pattern of α-chaconine in normal and also in sprouted/green coloured potatoes strongly justifying the formation of acetylated solanidine as an aglycone derivative in both green coloured and sprouted potatoes.

Table 4: Major Peaks obtained in the LC- MS and the suspected molecular mass of that peaks and their corresponding compounds.

	Normal potato	Green coloured potato	Sprouted potato
Peak in LC	4.77,**4.89**,5.82,6.07, 6.54,6.72,6.86,6.93, 7.36,7.66,7.98,8.24	**4.92**,6.61,6.87,7.65, 7.83,8.07	4.18,4.78,**4.92**,5.81, 6.07,6.72,6.87,6.93, 7.32,7.66,7.83
Analysed peak	4.89	4.92	4.92
Molecular mass	852.5332 706.4597 560.3980 398.3405	852.4677 706.4093 560.3538 **437.7105**	852.5305 706.4626 560.3959 **437.7435**
Suspected mass and compound	852.059(α-chaconine) 706.9256(β-chaconine) 560.7844(γ-chaconine) 397.636 (solanidine)	852.059 (α-chaconine) 706.9256(β-chaconine) 560.7844(γ-chaconine) 439.673(solanidine acetate)	852.059(α-chaconine) 706.9256(β-chaconine) 560.7844(γ-chaconine) 439.673(solanidine acetate)

Conclusion

The amount of glycoalkaloids increase when the tubers become green coloured and sprouted during post- harvest period. The present study evaluated the effect of different post harvesting stages on potato glycoalkaloids. Glycoalkaloids were extracted from normal potato, green coloured potato and sprouted potato successfullyusingstandardised procedure and the extracted sampleswere subjected to TLC analysis, UV analysis, FT/IR analysis and then to LC/MS spectroscopy. All these analysis indicated the presence of glycoalkaloids in the extracted samples. The LC-MS showed the presence of the α-chaconine in the extracted sample. The molecular weight of α-chaconine was 852.053kDa as represented in the MS. The mass of its fractions are also present in the spectrum. The slight change observed in the MS between the extract of normal potato and the green coloured and sprouted potato. The mass fragment of the solanidine aglycone is absent in the green coloured and sprouted potatoes, but a mass fragment which represents the solanidine acetate in the samples. This may be because of the change in the glycoalkaloid during greening and sprouting process in the post harvesting stages.

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