Localization Analysis of Natural Toxin of *Solanum tuberosum L.* via Mass Spectrometric Imaging

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Abstract: The use of mass spectrometry imaging (MSI) revealed the localization of α-solanine and α-chaconine as natural toxins for Potato (*Solanum tuberosum L.*). The content of Potato glycoalkaloids, α-solanine and α-chaconine, were quantitatively determined by high performance liquid chromatography (HPLC). Matrix assisted laser desorption/ionization-based tandem mass spectrometry (MS) could determine α-solanine and α-chaconine from raw potato extraction and section. After budbreak, α-solanine and α-chaconine were produced and localized at periderm and germ compared with that before budbreak. At germ region, these glycoalkaloids did not exist whole germ region but eccentrically localize at germ surface and central region. The amount of α-chaconine was twofold higher than α-solanine at periderm. At germ region, there was no difference between these toxins.

Keywords: Food safety, glycoalkaloid, mass spectrometry imaging, α-solanine, α-chaconine.

INTRODUCTION

Potato (*Solanum tuberosum L.*) is one of major agricultural crops and, includes vitamin B and C as well as starch as nutrition. Likewise it produces α-solanine and α-chaconine as natural toxin (Figure **1**). These are categorized as solanaceous glycoalkaloids comprised of a steroidal-like base containing aglycone to which three sugars are attached with a series of glycosidic linkages. As plant physiology, these compounds show an antifungal activity against several fungi [1-3]. Meanwhile, these induce gastro-intestinal and systemic effects in human as well as livestock due to inhibition of acetylcholine esterase [4].

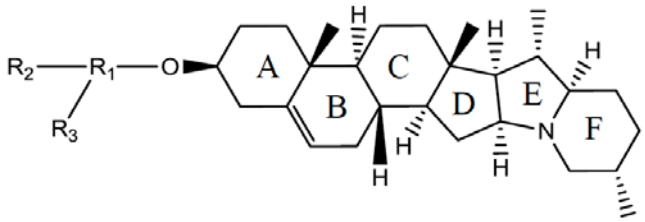

Figure 1: Structure of α-solanine: R_1 = D-galactose, R_2 = D-glucose and R_3 = L-rhamnose. Structure of α-chaconine: R_1 = D-glucose, R_2 and R_3 = L-rhamnose.

It is well known that these toxic compounds exist at germ region. In addition, previous studies have revealed and concerned the increasing of these toxins at periderm after budbreak and exposure the sunlight [5, 6]. However it is not common knowledge and involves food intoxication. Previous study has roughly described the localization of glycoalkaloids (spatial resolution of 200 μm) [7]. If detailed localization data (spatial resolution of 50 μm) indicates to public in general, visually, they will easily understand this hazard.

In order to facilitate increased direct visualization of biomolecules during biological analysis, mass spectrometry is usually used to distinguish individual molecules from each other. Mass spectrometric imaging (MSI) enables simultaneous detection of multiple analytes even in the absence of the target-specific markers such as antibodies [8].

MSI is applying to medical and pharmaceuticals fields [8-11] from the first era to the present. In the fairly recent past, MSI is received attention from other fields such as agricultural [12], chemical engineering and material fields. Recently, MSI technique was applied to agricultural and food science fields to reveal plant physiology and localization of nutrition in food body. We have also reported the localization of the nutrition in the food sample [13] and the functional compound in the body after intake [14].

Herein, we analyzed the quantity and localization of α-solanine and α-chaconine in Potato to easily understand food safety by chromatography and MSI. To quantitatively and regionally estimate these target molecules, we extracted α-solanine and α-chaconine from Potato sample. The α-solanine and α-chaconine

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were visualized at a resolution of 50 μm in the frozen potato section by MSI.

EXPERIMENTAL

Commercially potato was stored at cold dark place for 30 days to sprout it. The α-cyano-4-hydrocycinnamic acid (CHCA) as ionization assisting reagent was sprayed on frozen section of potato. The sections surface were irradiated with 1000 shots in the positive ion detection mode. To detect the laser spot area, the sections were scanned and a spot-to-spot center distance of 50 μm, respectively.

Preparation of Sample

The potato germ (1 g) was added to 10 mL of 100 % methanol and suspended with homogenizer. The suspension was centrifuged (10 min, 10,000 rpm, 4 ºC) to remove insoluble materials. The volume of the supernatant was adjusted to 10 mL with methanol. The supernatant (0.5 ml) was added to water (1 ml) in the microfuge tube. Extraction of α-solanine and α-chaconine was achived with a column (Waters Oasis HLB 1-cc). Zero point five milliliter of the sample liquid was passed through the HLB 1-cc with 40% methanol. The eluent was added to 10 ml of 40% methanol and applied on the HLB 1-cc. The α-solanine and α-chaconine were eluted from the cartridges with 100% methanol, and the volume of the sample liquid was adjust to 5 ml with the same solution.

Mass Spectrometry and Imaging Mass Spectrometry

Standard α-solanine or α-chaconine dissolved in 70% methanol (Wako, Japan). Standard α-solanine or α-chaconine (10 pmol) was dropped on a CHCA coated target MS plate. MSI was achieved by a time of flight (TOF) MS (Ultra-flex, Bruker, Germany). A CHCA (10 mg/mL) suspension was dispersed in 70% methanolic solution and was sprayed on Indium Tin Oxide-coated glass slides with an airbrush on some sections (Linear Compressor L5, nozzle caliber, 0.2 mm, Mr. Hobby Co., Japan). The section surface was irradiated with 1,000 laser shots in the positive ion detection mode of the mass spectrometer. To detect the laser spot area, the sections were scanned and a spot-to-spot center distance of 50 μm in each direction at the germ, the periderm and tuber of the potato were measured. For the germ, periderm and tuber regions, the related intensities were processed by discarding background peaks. The remaining intensities formed the set of variables that were used for imaging and semiquantitative analysis.

RESULTS

Quantitative Analysis

HPLC was used for quantitative analysis of the germ, periderm and tuber. The LC showed that the signal at 20 and 21 min. corresponded to α-solanine and α-chaconine. We monitored α-solanine and α-chaconine peaks and prepared these calibration curves, respectively. For the regional comparison of α-solanine and α-chaconine, total amount was almost same at germ region. The α-chaconine inhered two times higher than α-solanine at periderm. We could not determine the amount of α-solanine and α-chaconine due to no peaks from tuber. For the comparison between a regions, the germ region showed fifteen and seven times higher peaks of α-solanine and α-chaconine than that of periderm, respectively (Table **1**). As a control, the potato before germination showed no peaks corresponded to α-solanine and α-chaconine from every regions.

Table 1: Quantitative Analysis of Potato Extraction by HPLC

	α-solanine	α-chaconine
Germ	123	125
Periderm	8	17
Tuber	N.D.	$N.D._{(mg/g)}$

Mass Spectrometry

We investigated ionization of these target molecules from the extraction of potato sample. Fractionated germ extract by HPLC showed MS signals at *m/z* 868.8 (elution time: 20 minutes) and 852.7 (elution time: 21 minutes), respectively. To prove whether these target mass were α-solanine and α-chaconine, structural analysis was achieved on potato section by tandem MS. We could detect *m/z* 868.8 and 852.7 on germ region of potato section. Both molecules were glycoside that consist of solanidene and trisaccharide. A α-solanine possesses D-galactose (D-Gal), L-rhamnose (L-Rha) and D-glucose (D-Glc). A α-chaconine possesses D-Glc and two L-Rha. The tandem mass spectrum of α-solanine showed a specific pattern of the precursor ion at *m/z* 868.8 and four derivative ions at *m/z* 722.8, 706.6, 398.6, 380.3,

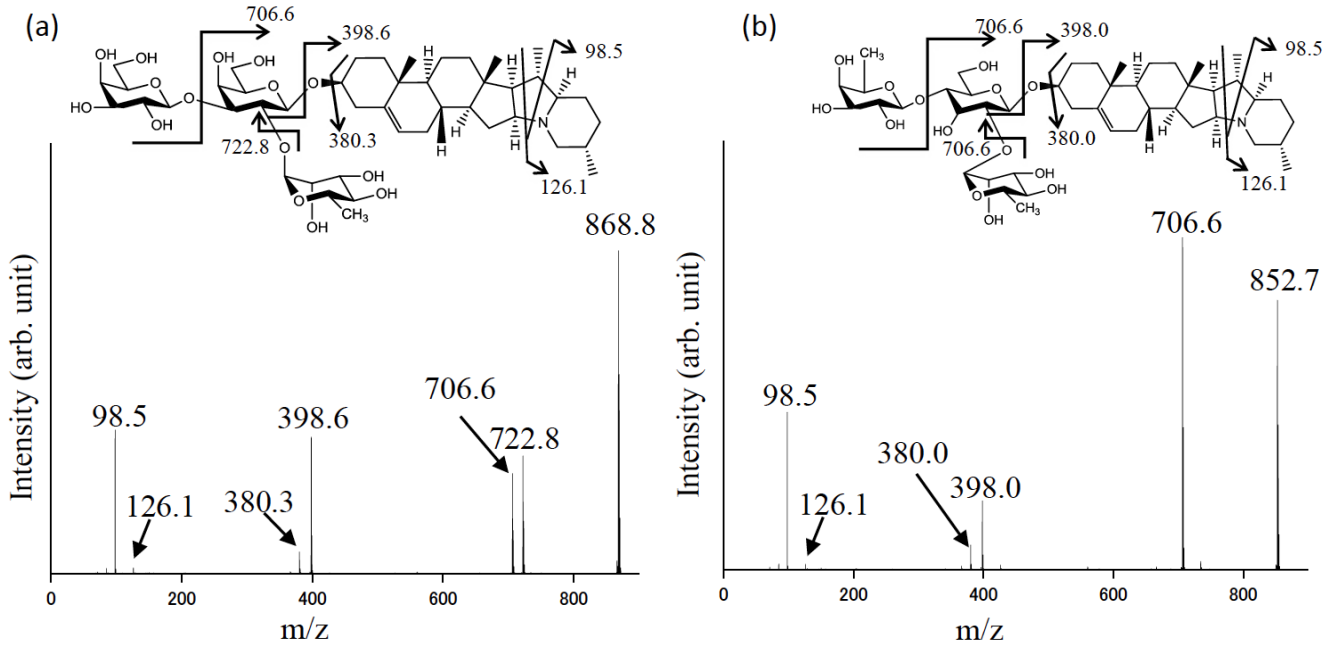

Figure 2: Tandem MS spectra of (**a**) α-solanine and (**b**) α-chaconine, generated by trapping the protonated ions at *m/z* 868.8 and 852.7 on potato section.

126.1 and 98.5 as fragment ion (Figure **2a**). The *m/z* 722.8, 706.6 could assigned to the cleavage of glycosidic bond by D-Rha and D-Glc. The *m/z* 398.6 showed solanidine portion. The *m/z* 380.3 attributed to anhydrated-solanidine ion. The *m/z* 126.1 and 98.5 ascribed fragmentation of E-ring. The tandem mass spectrum of standard α-chaconine showed the precursor ion at *m/z* 852.7 and fragment ion at *m/z* 706.6, 398.0, 380.0, 126.1 and 98.5 (Figure **2b**). The fragment ion of *m/z* 722.8 was not seen in α-chaconine but specific ion of α-solanine. Due to this, we could distinguish α-solanine and α-chaconine using *m/z* 722.8. We could also confirm same precursor ions and tandem MS spectra from standard α-solanine and α-chaconine sample (data not shown).

Mass Spectrometry Imaging

Figure **3** showed the result of mass spectrometry imaging (MSI) on the potato sections. Optical image could define each regions of potato before and after budbreak. We could clearly find germ region after budbreak (Figure **3a-i** and **d-i**). Figure **3a-ii** and **d-ii** indicated MSI performed-area, which scanned the sections and determined the laser spot area with spot-to-spot center distance of 50 μm.

Both protonated-α-solanine (*m/z* 868.8) and α-chaconine (*m/z* 852.7) obviously localized the periphery and center of the germ, and periderm but didn't exist in the tuber and near the cambium (Figure **3b-i** and **ii**, and **3c-i** and **ii**). At the periderm, α-chaconine was more abundant than that of α-solanine. Generally, it is well known that these glycoalkaloids were produced at sprout after a budbreak and periderm when potato are exposed to illumination such as sunlight and room tight. In this time, for the periderm, we stored potato sample at cold dark place until potato sprouted. The result indicated that illumination was not only trigger to produce alkaloid-based toxins but a long-term conservation and budding. From before budbreaked potato as control, we managed to image α-solanine and α-chaconine where budbreak would occur (Figure **3e-i** and **ii**, and **3f-i** and **ii**). We hypothesis that both glycoalkaloids are produced at this point before budbreak as defensive capability of plant.

Semiquantitative regional analysis for α-solanine and α-chaconine was performed by matching the MS intensities with the pericarp, outer and inner regions of germ. The values were converted by the basis of intensity of α-solanine at pericarp. The total intensity ratios for samples after budbreak were 1:3:0.5 for α-solanine and 1.7:2.5:0.4 for α-chaconine in the pericarp, outer germ and inner germ regions, respectively. For both glycoalkaloids, the intensity of pericarp was lower than that of germ region. In the pericarp, the intensity of α-chaconine was approximately twofold higher than that for α-solanine. No difference was observed at other region. MSI data

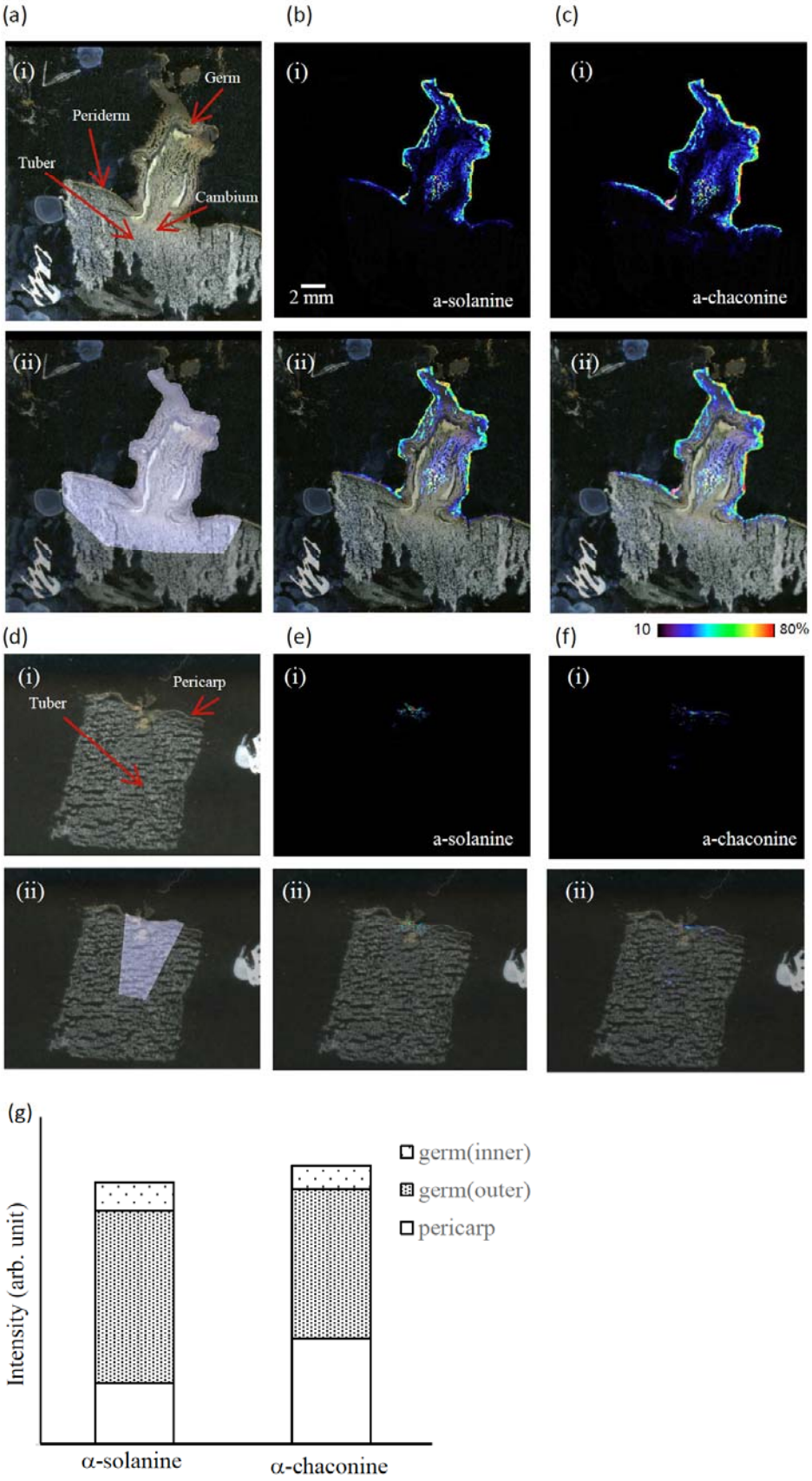

Figure 3: Imaging MS of α-solanine and α-chaconine: optical image of potato sections (**a** and **d**); MSI of α-solanine (**b** and **e**); and of α-chaconine (**c** and **f**); stacked bar chart of α-solanine and α-chaconine in the samples, reconstructed from the MSI data by binarization (**g**).

only shows relative comparison although it was in good agreement with quantitative HPLC data (Table **1**).

CONCLUSION

MALDI MSI revealed that α-solanine and α-chaconine localized at germ surface and periderm after budbreak. At germ, both glycoalkaloids showed no difference, meanwhile α-chaconine was produced twice compared with α-solanine at periderm region. On the other hand, no these toxins were detected from potato sample before budbreak. MSI data was coincident with HPLC data, semiquantitatively. In this research, we could detail localization of glycoalkaloids at germ region. Glycoalkaloids localized germ surface and central region not whole region. These data are becoming increasingly important not only in the areas of fundamental plant science but also in food sanitation areas. Glycoalkaloids are not destroyed during food-processing treatments such as baking, frying or boiling even at high temperatures. Thus visualization of natural toxins easily gives us scientific food safety information, which indicate edible or non-edible part. MSI is a powerful tool for fundamental science, agricultural chemistry, food hygiene as well as food safety and quality.

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