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Direct analysis for the distribution of toxic glycoalkaloids in potato tuber tissue using matrix-assisted laser desorption/ionization mass spectrometric imaging

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ABSTRACT

A simple and rapid analytical method for detection and spatial distribution of glycoalkaloids in potato tubers has been developed for the first time using matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI). For effective MALDI-MSI analysis, we have developed a uniform matrix coating method using 2,5-dihydroxybenzoic acid (2,5-DHB) as the preferred matrix which results in better sensitivity than 2,4,6-trihydroxyacetophenone (2,4,6-THAP) using MALDI-TOF. The relative concentrations of two major and two minor glycoalkaloids, α-chaconine and α-solanine, dehydrochaconine and dehydrosolanine, were clearly detected and distinguished in various parts of potato tuber and their relative amounts were directly compared. We also successfully showed the relative concentrations of glycoalkaloids that were accumulated by light exposure during storage using MALDI-MSI. Therefore, MALDI-MSI has been shown to be a useful technique for screening toxic and bioactive metabolites in foods and medicinal plants.

1. Introduction

Glycoalkaloids (GAs) are commonly present in solanaceous plants such as potatoes and tomatoes. Since glycoalkaloids are toxic, their presence in potato tubers constitutes a problem in food safety. Because it is difficult to analyze for glycoalkaloids, the food industry largely relies on monitoring a greening effect for controlling glycoalkaloid levels in the food supply. The two major GAs are α-solanine and α-chaconine, which are reported to be toxic to bacteria, fungi, insects, animals and humans (Friedman, 2006; Smith, Roddick, & Jons, 1996). Although glycoalkaloids are present throughout the potato plant (*Solanum tuberosum*), the highest concentrations occur in the foliage, blossoms, periderm, cortex, and areas of high metabolic activity (e.g., eyes, green skin, stems, sprouts) followed by the peel and the tuber. α-Chaconine and α-solanine represent up to 95% of the glycoalkaloid content in potato tubers. The glycoalkaloid concentration in potato tubers varies due to environmental and genetic factors, e.g. specific species such as potato cultivar Lenape. Some factors that increase glycoalkaloid concentration include physical injury to the plant, physiological stress, immaturity (green potato), low storage temperature, and storage in bright light. Normally, potato tubers contain low concentrations of toxic glycoalkaloids unless adverse storage condition or cultivation methods increase the glycoalkaloid content (Barceloux, 2008).

Various analytical methods have been reported for the determination of GAs in potato tissues. The simplest methods, such as colorimetric, gravimetric, and titrimetric techniques, lack the required specificity and suffer from contamination by other potato components (Van Gelder, 1991). The most common methods have been chromatographic techniques such as gas chromatography (GC) or high performance liquid chromatography (HPLC). GC–MS and GC analysis require derivatization of glycoalkaloid aglycons or GAs, or hydrolysis of the GAs to obtain more volatile alkaloid (Kozukue et al., 2008; Laurila et al., 1999). Extensive sample cleanup is required for HPLC-UV methods because of UV detection in the 200–208 nm region (Friedman & Dao, 1992; Friedman, Rottman, & Kozukue, 2003; Saito, Horie, Hoshino, Nose, & Nakazawa, 1990). Immunoassays rely on the specificity of antibodies to eliminate the problems of thorough purification and extraction of samples, but in spite of that the assays developed were unable to differentiate between α-chaconine and α-solanine (Driedger, LeBlanc, LeBlanc, & Sporns, 2000; Friedman, Bautista, Stanker, & Larkin, 1998; Phlak & Sporns, 1992). Techniques such as liquid chromatography with mass spectrometry (LC-MS) can profile these analytes (Distl & Wink, 2009; Stobieckia, Matysiak-Kata, Franski, Skala, & Szopa, 2003; Väänänen et al., 2005) but provide no spatial information regarding their distribution in the potato. To demonstrate the potential of

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applying matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to food analysis, the applicability of MALDI-TOF MS for toxic potato glycoalkaloids is reported as a method of rapid analysis (Abell & Sporns, 1996). The technique has the significant advantages with high sensitivity and high throughput (Abell & Sporns, 1996), but low quantification and reproducibility by sample preparation. Although various analytical methods including chromatographic techniques coupled with mass spectrometer have been developed to determine glycoalkaloids, it was expensive and time-consuming procedure from sample preparation to detection.

MALDI imaging mass spectrometry (MALDI-IMS or MALDI-MSI) derived from MALDI-TOF MS was first developed by the Carprioli group (Caprioli, Farmer, & Gile, 1997), and has been applied recently to image the localization of plant metabolites (Cha et al., 2008; Li, Shrestha, & Vertes, 2008; Robinson, Warburton, Seymour, Clench, & Thomas-Oates, 2007). Direct analysis of plant tissues offers several intrinsic advantages over the analysis of plant extracts. The technique is particularly valuable for the analytes that may be destroyed during sample preparation. The long, tedious and complex sample preparation process often causes damage or loss of the analytes, especially those present in low levels in plant tissues. Furthermore, lots of constituents often cannot be extracted completely with a single sample preparation procedure.

Here we propose the novel application of the MALDI-MSI technique to monitoring toxic or bioactive metabolites in foods and medicinal plants. For effective MALDI-MSI analysis, we developed a uniform matrix coating method with 2,5-dihydroxybenzoic acid (2,5-DHB) in this study. We could analyze the distribution of two major and two minor GAs, and directly compare the ratio of glycoalkaloid contents in the various parts of potato tubers by MALDI-MSI without traditional sample preparations. The study clearly demonstrates some advantages of direct analysis of plant tissues over the traditional analysis of sample extracts, while yielding comparable analytical results. Therefore, MALDI-MSI can serve as a valuable technique to improve the precision and reliability of ingredient analysis in foods and herbal medicines for quality control and safety.

2. Materials and methods

2.1. Materials

The common matrices, 2,4,6-trihydroxyacetophenone (2,4,6-THAP) and 2,5-Dihydroxybenzoic acid (2,5-DHB) and peptide calibration standard II were purchased from Bruker Daltonics (Bremen, Germany). HPLC-grade acetonitrile and methanol were purchased from Merck. Trifluoroacetic acid, acetic acid, and α-solanine were purchased from Sigma (St Louis, MO). All chemicals used in this study were of the highest purity available. Metal MALDI imaging templates were developed by chemical etching and photolithography. The plate was 300 μm thick and of microscopy slide glass dimensions (75 mm × 25 mm). Also, the conductive ITO glass with teaching points was used, and it has a thin indium-tin-oxide (ITO) layer on glass. The glass was 0.7 mm thick, and its conductivity and transparency were 15 Ω and 88% (λ = 550 nm), respectively.

2.2. Matrix deposition method for MALDI-MSI

The concentration of 2,5-DHB matrix was 40 mg/ml in 70% MeOH with 0.1% TFA. MALDI matrix solution was deposited onto MALDI imaging templates with sprayer (ImagePrep, Bruker Daltonics) or a prototype of pico-liter emitting device (μMatrix Spotter, Hudson Surface Technology) developed in our laboratory (Fig. 1).

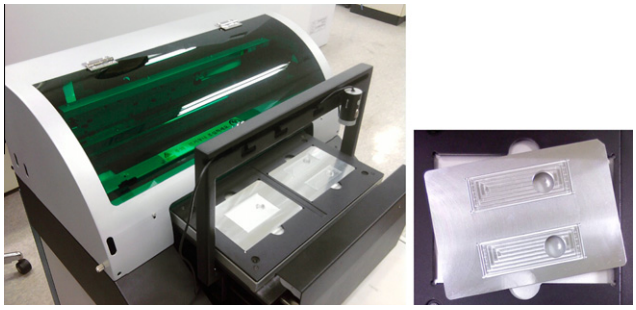

Fig. 1. Photographic images of a novel pico-liter emitting device.

A pico-liter emitting device had an inherent resolution of 5760 × 1440 dpi and a six channel piezoelectric head that dispensed 3 pL of matrix solution per drop onto MALDI imaging template. MALDI imaging template in 5-mm-thick aluminum holder (Fig. 1) was mounted on the device. Coreldraw software was used to control the matrix deposition pattern of specimen on the MALDI imaging template automatically.

2.3. Preparation of potato tissue sample

Potatoes were obtained from a farm (Gwangju, Korea), and potato tubers were harvested after three months storage at room temperature in the dark. Commercial potatoes were purchased from a market (E-mart, Suwon, Korea). The half parts of each potato tuber were wrapped with aluminum foil, and stored under fluorescent light for ten days. A part of potato tuber that contained sprouts was cut and frozen in liquid nitrogen. Before starting sectioning, MALDI imaging templates and the brush kept inside the cryostat chamber (Cryotome FE/FSE, Thermo scientific) at −18 °C for 30 min to thermally equilibrate. The frozen potato piece was cut into 6 μm sections. The sections were attached onto frozen MALDI imaging templates. The cold slide was kept inside the cryostat with the section in place until the section appeared to be completely dry. The potato tissue sections were stored at −80 °C until matrix deposition. Matrix solution (40 mg/ml 2,5-DHB in 70% MeOH with 0.1% TFA) was dispensed 80 passes with 1 min drying time between each coating by pico-liter emitting devices.

2.4. Mass spectrometry

All mass spectra were obtained on a MALDI-TOF/TOF system (Ultraflex III, Bruker Daltonics, Germany) equipped with a 200 Hz Smartbeam laser. The extraction voltage was 25 kV in reflector mode using delayed extraction, and gated matrix suppression up to 300 *m/z* was applied to prevent the saturation of the detector by matrix ions. MS/MS spectra were measured in TOF/TOF Mode and summed 1000 laser shots in 10 different positions. The tissue sections were scanned with a spatial resolution of 200 μm spots and 150 laser shots in each pixel at the optimized parameters. Data acquisitions and reconstructions from the spectra were processed by using FlexImaging 2.0 software (Bruker Daltonics). Data preparation such as average peak list calculation, peak calculation, recalibration, and peak statistic results was accomplished by ClinproTool 2.1 software (Bruker Daltonics).

3. Results and discussion

3.1. Matrix selection: 2,5-DHB is better than 2,4,6-THAP for the mass sensitivity

The selection of the matrix, the matrix/analyte ratio and the laser power each play an important role in obtaining high quality