Localization Analysis of Multiple Vitamins in Dried Persimmon (Diospyros kaki) Using Matrix-assisted Laser Desorption/ionization Mass Spectrometry Imaging

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Abstract: The drying process used for persimmon fruit (Diospyros kaki) can alter the composition of nutrients, and especially vitamins. We visually determined whether the amounts of vitamin A₁, vitamin B₆ and vitamin C vary after drying persimmon fruit, using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) imaging. Drying altered the amount of moisture between the fruit interior and surface. Vitamin A₁ is lipophilic and localized at the desiccated outer regions (pericarp) and not in the inner region (mesocarp and endocarp), and its concentration was increased 3.4 times in dried fruit compared with raw persimmon. Vitamin B₁ and B₆ are water-soluble and concentrated in the moist mesocarp. The vitamin C content of dried persimmon is decreased by drying in the sun. The drying process affected the localizations and amounts of all the vitamins. The observed opposite localization of vitamin A₁ compared to B₁ and B₆ was due to vitamin A₁ being lipophilic and B₁ and B₆ being water soluble. Multiple-vitamin imaging using MALDI-MSI has great potential for enhancing commodity value and for visually investigating the effects of manufacturing processes.

Key words: dry fruit, mass spectrometry imaging, matrix-assisted laser desorption/ionization, persimmon, retinol, thiamin, pyridoxal, ascorbic acid

1 Introduction

The signaling and function of a vitamin is regulated by its concentration. A physiological response can also be induced by multiple hormones in synergistic or antagonistic actions, referred to as signaling crosstalk¹ ². Vitamins are essential and functional compounds that cannot be synthesized by the human body. Vitamins are classified as either lipophilic or water-soluble. Lipophilic vitamins readily accumulate in the body whereas water-soluble vitamins are rapidly flushed from the system. Vitamins are ingested constantly in various foods and vitamin research is important in plant physiology, nutrition science, and the medical field³ ⁹. Persimmon (Diospyros kaki) is a deciduous tree belonging to the family Ebenaceae and is cultivated in many countries. The delicious fruit contains nutrients and phytochemicals such as vitamins, β-carotene, tannins and polyphenols. In Asian countries, persimmon is eaten raw or dried. Dried persimmon produced in Fukushima prefecture in Japan is watery in the center, with a desiccated outer area due to the natural cold wind in the growing region, indicating that the fruit are not mechanically dried and contain vitamins. At least four vitamins have been identified in dried persimmon fruit: vitamin A₁ (retinol), vitamin B₁ (thiamin), vitamin B₆ (pyridoxal) and vitamin C (ascorbic acid).

Information on the localization of these vitamins in dried persimmon is needed to understand the role of the vitamins in the fruit and increase the market value of the dried fruit.

Abbreviations: Mass spectrometry imaging (MSI), Matrix-assisted laser desorption/ionization (MALDI)
Current methods for determining the localization of multiple nutrients in fruit tissues are rather limited. Construction of antibodies for vitamin due to the small molecules is difficult, which limits an immunostaining method for spatial visualization of vitamins. Colorimeter analysis provides quantitative information for vitamins but no visual spatial information.

Mass spectrometry imaging (MSI), typically by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), enables the direct mapping and imaging of biomolecules present in tissue sections. Tissue cross-sections are mounted on an electric conductive glass-slide, sprayed with an organic matrix, and irradiated with laser shots. Each spot irradiated by the laser becomes a pixel in the final image. Target-specific markers such as antibodies are not required, and MSI enables the simultaneous detection of multiple analytes on a single section of animal tissue or plant tissue.

Here, we compared the localizations of lipophilic and water-soluble vitamins in raw and dried persimmon by MALDI-MSI. This is the first report of the use of MSI for the simultaneous visualization of several major vitamins.

2 Materials and Methods

2.1 Fruits and sample preparation

Raw and dried persimmon were donated by Japan Agricultural Cooperatives (JA), Fukushima. The raw sample was harvested and stored at −80°C prior to MSI analysis. Dried persimmon was prepared from fruit from the same tree as the raw sample. After removing the pericarp, the fruits were fumigated using sulfur dioxide powder on a heater for 20 min. to prevent color degradation and the oxidation of polyphenols, resulting in an amber-colored surface, then the fruit were dried under well-ventilated conditions with sunlight during the day for 40 days.

2.2 Preparation of persimmon cross-sections for mass spectrometry imaging (MSI)

The raw and dried samples were cut into quarters. The cut samples included the epidermis (raw sample) and pericarp, mesocarp and endocarp, and were embedded in super cryo embedding medium (SCEM, Leica Biosystems, Wetzlar, Germany) and frozen in liquid nitrogen. The specimen block was cut into 10 µm sections using a cryostat (NX70, Thermo Fisher Scientific, USA) set at −25°C for the chamber and at −23°C for the object holder. The sections were gently mounted on slides coated with indium tin oxide (ITO) (Bruker Daltonics, Billerica, MA, USA). Optical images of the sections were obtained using a scanner (GT-X830, Epson, Tokyo, Japan) before analysis by MALDI-MSI.

2.3 MALDI-MSI

A 10 mg/ml solution of the matrix α-cyano-4-hydroxycinnamic acid (CHCA, Nacalai Tesque, Japan) was suspended in 6 ml of acetonitrile/water/trifluoro acetic acid: 50/49/1 v/v and sprayed on persimmon tissue sections on ITO-coated glass slides (Bruker Daltonics) using an automated pneumatic sprayer (TM-Sprayer, HTX Tech., Chapel Hill, NC). Ten passes were sprayed using the following conditions: flow rate 120 µl/min, air flow 10 psi, nozzle speed 1100 mm/min.

Ionization and imaging of the vitamins were confirmed with a MALDI-TOF-MS (rapifleX, Bruker Daltonics, Billerica, MA, USA). Tandem MS spectra on section were obtained using a collision-induced dissociation method. Precursor ion (obtained using 1000X shots) and fragment ion (obtained using 4000X shots) signals were integrated using flexControl 3.4 (Bruker Daltonics, Billerica, MA, USA). Spectra were recorded in positive linear tandem MS mode (ion source 1 voltage, 20 kV; lens voltage, 18 kV). The collision energy was about 1.3 eV, estimated using MALDI TOF/TOF (PSD) mode and CID via Ar (Manufacturer default settings).

For MSI, the laser spot areas were detected by scanning the sections. The laser spot areas (200 shots) were detected with a spot-to-spot center distance of 120 µm in each direction of the persimmon fruit. Signals between m/z 100-800 were corrected. The section surface was irradiated with YAG laser shots in the positive or negative ion detection mode. The laser power was optimized to minimize in-source decay of the targets. The obtained MS spectra were reconstructed to MS images with a mass bin width of m/z ± 0.05 from the exact mass using FlexImaging 4.0 software (Bruker Daltonics, Billerica, MA, USA). The peak intensity values of the spectra were normalized by dividing by the total ion current (TIC) to achieve semi-quantitative analysis between raw and dried persimmon.

3 Results and Discussion

3.1 Detection of vitamins using MALDI-MS and tandem MS of persimmon sections

Applying MALDI-MS analysis to sample sections, we could detect deprotonated vitamin A$_1$ ion at m/z 285.4 and vitamin C ion at m/z 175.1 in negative ion mode, and protonated vitamin B$_3$ ion at m/z 265.1 and vitamin B$_1$ ion at m/z 168.1 in positive ion mode. We confirmed that the detected ions were target vitamins by tandem MS using the above-detected ion as precursor ions (Fig. 1).

The precursor ions of the four compounds were cleaved and detected as product ions. In negative ion mode, the m/z values of the precursor ions of vitamin A$_1$ and vitamin C were at m/z 285.4 and m/z 175.1, respectively. These ions were cleaved at the hydroxy group and the lactone ring to
provide additional ions. As expected from their chemical structures, the fragment ions were observed at \( m/z \) 269.0 and \( m/z \) 115.0 (Fig. 1(a) and (d)).

In positive ion mode, the \( m/z \) values of the precursor ions of vitamin B1 and vitamin B6 were at \( m/z \) 265.1 and \( m/z \) 168.1, respectively. These ions were cleaved at the pyrimidine moiety and the hydroxy group to provide additional ions. As expected from their chemical structures, the fragment ions were observed at \( m/z \) 122.0 and 144.4, and \( m/z \) 150.0 (Fig. 1(b) and (c)). This suggests that the peaks of the precursor ions detected by MALDI-MSI matched the structures of the target analytes.

3.2 MALDI-MSI of persimmon sections

Figure 2 shows the MALDI-MSI data for four vitamins in raw and dried persimmon. Optical images provide information on persimmon morphology. The epidermis, pericarp, mesocarp and endocarp could be observed in raw persimmon (Fig. 2(a)) whereas only the pericarp, mesocarp and endocarp were confirmed in dried persimmon (Fig. 2(b)) due to cutting the epidermis before drying.

Vitamin A1 (VA1) was marginally imaged in the epidermis zone of raw persimmon (Fig. 2(c)) but was clearly localized at the pericarp in dried persimmon and was present throughout the inner region, including the mesocarp (Fig. 2(d)).

We semi-quantitatively analyzed the trends in vitamin A1 distribution by correlating the MS intensities in each area of the image. The total intensity per unit area of dried persimmon was about 3.4 times higher than that in raw persimmon.

Vitamin B1 (VB1) broadly localized throughout the region, especially to the outer region (pericarp and upper part of mesocarp) in raw persimmon (Fig. 2(e)) but was broadly localized to the inner region (mesocarp and endocarp) of dried persimmon and was 2.5-fold higher than in raw persimmon.

Specific localization of vitamin B6 (VB6) was not observed and rather it was imaged throughout the raw sample (Fig. 2(g)). After drying, VB6 localized to the inner zone, similar to VB1, and was 2.3-fold higher in dried persimmon compared to raw persimmon.
Fig. 2 MALDI-MS imaging of vitamins in persimmon sections. Optical images of (a) raw and (b) dried persimmon sections. MS image of vitamin A in (c) raw and (d) dried, vitamin B in (e) raw and (f) dried, vitamin B in (g) raw and (h) dried, and vitamin C in (i) raw and (j) dried. Cross sections of the persimmon were placed on ITO-coated glass and vitamin-related signals from the cross sections were normalized to the total ion count. Their relative intensities are shown as a heat map (warm color indicating high intensity and cold color indicating low intensity; intensities are expressed as a percent of the highest intensity of each ion in the image) and normalized versus total ion count (right bar chart).
pared with raw persimmon.

Vitamin C (VC) mainly localized at the epidermis and the pericarp close to the epidermis in raw persimmon (Fig. 2d) but was absent in dried persimmon. VC was marginally detected around the seed (Fig. 2g).

Our imaging results for VA



1 and VB


6 were different in raw persimmon but similar after drying. The dried persimmon was moist at the inner zone and dry at the outer zone. These textures are desirable in persimmon as a commodity product and also affect the localizations of vitamins. VA


1 is lipophilic and VB


1 and VB


6 are water-soluble. Thus, VA


1 localized at the outer dried zone and VB


1 and VB


6 localized to and were concentrated in the moist inner zone. Due to drying process, the kind of VB did not increase or decrease due to that the kind of VB originally existed in the raw persimmon fruit. Shrink of cubic volume of persimmon due to drying process affected more concentration of VB


1 and VB


6.

VC mainly localized at the pericarp and fruit surface due to its antioxidant function. VC is photo-degraded by sunlight during the drying process and thus we did not detect or image VC from dried persimmon.

VA

1, also called retinol, is an essential nutrient for vision, reproduction, growth and maintenance of epithelial and bone structures. In addition, VA

1 is noted in cosmetic anti-wrinkle and medical dermatological treatments. VB

1 (thiamin) plays a critical role in energy metabolism and prevents beriberi. Interestingly, VB

6, a pyridoxal, plays a role in amino acid metabolism and neurotransmission and is a precursor molecule of pyridoxal phosphate, which helps in recovery from hangovers and is available as an ethical drug. Our results show that multiple vitamins can be ingested by eating dried persimmon, making dried persimmon a healthy and delicious food.

To our knowledge, this is the first study to simultaneously visualize several major vitamins in raw and dried persimmon tissues. Our vitamin images are only semi-quantitative but make it possible to compare the relative concentrations of a single analyte in a single image. Quantification by MALDI-MSI is difficult because the signal intensity depends on many factors (e.g., ionization efficiency, extraction efficiency from the tissue, and sample preparation). Mass spectrometry imaging (MSI) is a powerful tool for the direct visualization of fruit compounds and biomolecules in biological tissues, and may have applications in agriculture relevant to commercial food products.

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