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Author(s)	Taira, Shu; Shimma, Shuichi; Osaka, Issey; Kaneko, Daisaku; Ichiyanagi, Yuko; Ikeda, Ryuzo; Konishi-Kawamura, Yasuko; Zhu, Shu; Tsuneyama, Koichi; Komatsu, Katsuko
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Description	

# Mass Spectrometry Imaging of the Capsaicin Localization in the *Capsicum* Fruits

Shu Taira<sup>\*1</sup>, Shuichi Shimma<sup>2</sup>, Issey Osaka<sup>3</sup>, Daisaku Kaneko<sup>1</sup>, Yuko Ichianagi<sup>4,5</sup>, Ryuzo Ikeda<sup>1</sup>, Yasuko Konishi-Kawamura<sup>6</sup>, Shu Zhu<sup>7</sup>, Koichi Tsuneyama<sup>8</sup> and Katsuko Komatsu<sup>7</sup>

<sup>1</sup>Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan

<sup>2</sup>Osaka University, Suita, Osaka 565-0871, Japan

<sup>3</sup>Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama, Kanagawa 236-0027, Japan

<sup>4</sup>Yokohama National University, 79-5 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan

<sup>5</sup>Japan Science and Technology Agency, Precursory Research for Embryonic Science and Technology, Japan

<sup>6</sup>Ishikawa Prefectural University, 1-308 Suematsu, Nonoichi, Ishikawa 921-8836, Japan

<sup>7</sup>Institute of Natural Medicine University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

<sup>8</sup>Faculty of Medicine University of Toyama 2630 Sugitani, Toyama 930-0194, Japan

**Abstract:** We succeeded in performing mass spectrometry imaging (MSI) of the localization of capsaicin in cross-sections of the *Capsicum* fruits at a resolution of 250  $\mu\text{m}$  using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Post source decay of protonated capsaicin ion revealed structural information of the corresponding amide, vanillylamide, and C<sub>9</sub> chain fatty acid. MALDI-TOF-MSI confirmed that localization of capsaicin in the placenta is higher than that in the pericarp. In addition, it revealed no localization of capsaicin in seed and the higher localization of capsaicin at placenta surface compared with that in the internal region. A quantitative difference was detected between localizations of capsaicin at placenta, pericarp and seed in the *Capsicum* fruits. This imaging approach is a promising technique for rapid quality evaluation of general food as well as health food and identification of medicinal capsaicin in plant tissues.

**Keywords:** Capsaicin, Mass spectrometry, Imaging, *Capsicum*, Placenta.

## INTRODUCTION

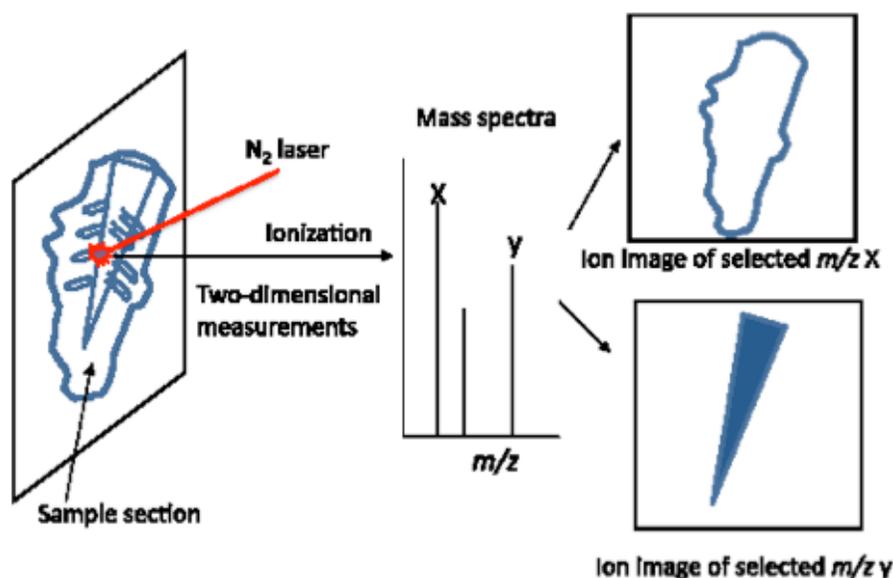
*Capsicum* fruit is an important constituent of traditional Chinese medicine as well as spice and condiment. Many pharmacological properties of the alkaloid capsaicin have been reported [1-4]. There are numerous reports on the effects of capsaicin on the nervous system [5], for antimicrobial [6], the polymorphism [7] and transient receptor potential vanilloid type 1 (TRPV1) [8].

Capsaicin is one of pungency which is composed by the amide vanillylamide and C<sub>9</sub> to C<sub>11</sub> chain fatty acid. The presence and content of capsaicin in *Capsicum* has been studied in detail. The capsaicin content in extracts of fresh *Capsicum* has been determined using liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS) [9] and transmission electron microscopy [10] which includes complicated pretreatment and special techniques. Information about

localization of nutrients is vital. If there is an easy method for optical type to understand the localization of nutrients in plant and food, we would be able to analyze plant and food sample as new quality evaluation method.

Mass spectrometry (MS) is used to distinguish individual molecules in the first generation. Especially, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS has been commonly used for analysis of metabolites [11], peptides [12], lipids [11, 13], oligonucleotides [14-18] and proteins [19] in biological samples and tissues. MALDI is a soft ionization technique used in MS in which a chemical matrix is used to assist the ionization of the analyte and to determine the structure followed by tandem mass spectrometry [20] and post source decay (PSD) [11]. As post-mass spectrographic era, in order to facilitate increased direct visualization of biomolecules during biological analysis, today, two-dimensional MS analysis of biomedical tissues by means of what is called mass spectrometry imaging (MSI) has begun to be used to analyze analyte distribution. The way to determine the existence (what is it?), localization (where is it?) of

\*Address corresponding to this author at the Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan; Tel/Fax: +81-761-51-1649; E-mail: s-taira@jaist.ac.jp



**Figure 1:** Schematic illustration of mass spectrometry imaging (MSI).

target molecules has been a dream. After two-dimensional MS measurements on sample section at regular interval, re-construction for target signals is given as an ion image (Figure 1). From one section, MSI enables simultaneous detection of multiple analytes even in the absence of the target-specific markers such as antibodies.

This is the first report on direct imaging of capsaicin compounds with *Capsicum* fruits by MALDI-TOF-MSI. The naturally occurring capsaicin in the cross-section of the *Capsicum* fruit was identified by PSD MS and visualized at a resolution of 250 $\mu$ m using the MALDI-TOF-MSI technique. Visualization of alkaloid present in plant tissues is a new and useful technique in screening of plants that have high pharmacological activities that are traditionally used in Chinese medicine as well as for evaluation of food quality.

## MATERIALS AND METHODS

### Preparation of Frozen Cross-Sections of Capsicum Fruits

*Capsicum annum* Linnaeus var. *cerasiforme* Bailey was used. The harvested fruits for MALDI-TOF-MSI were cut from whole body using a blade and the resultant frozen sample piece axially set in a cryostat (CM-3050 S; LEICA, Germany) at  $-20^{\circ}\text{C}$ . Next, they were cut into serial sections (70 $\mu$ m) and thaw-mounted on stainless target plate for MSI. The target plate for MS and MSI with the section was evacuated for 10 min. to dry the section. Saturated  $\alpha$ -cyano-4-

hydroxycinnamic acid (CHCA) as matrix (Sigma, USA) was dispersed in 1 mL of methanol. The suspension was centrifuged, and the supernatant fluid was sprayed on a target plate with the sections using an airbrush (nozzle caliber, 0.2 mm), while the CHCA matrix solution was applied to the section.

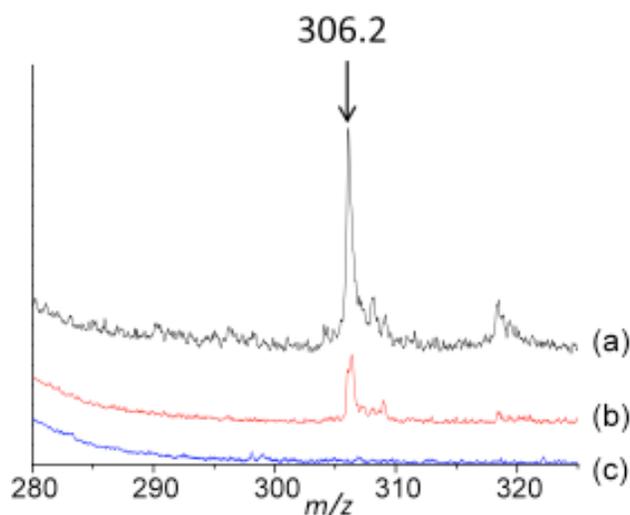
### MALDI-MSI of Capsaicin Localization in Capsicum Fruits

The efficacy of MSI was confirmed by a MALDI-TOF instrument (Voyager-DE-RP; Applied Biosystems, Germany) using  $\text{N}_2$  laser emitting at 337 nm. In order to detect the laser spot area, the sections were scanned and laser spot areas (40 $\times$ 120 spots) were detected with a spot-to-spot center distance of 250 $\mu$ m in each direction of the whole *Capsicum* fruit. The tissue surface was irradiated with 20 laser shots in the positive ion detection mode. For each placenta, pericarp and seed areas related intensities were processed by discarding peaks with back ground. The remaining intensities constituted the set of variables that was used for statistical analysis.

## RESULTS AND DISCUSSION

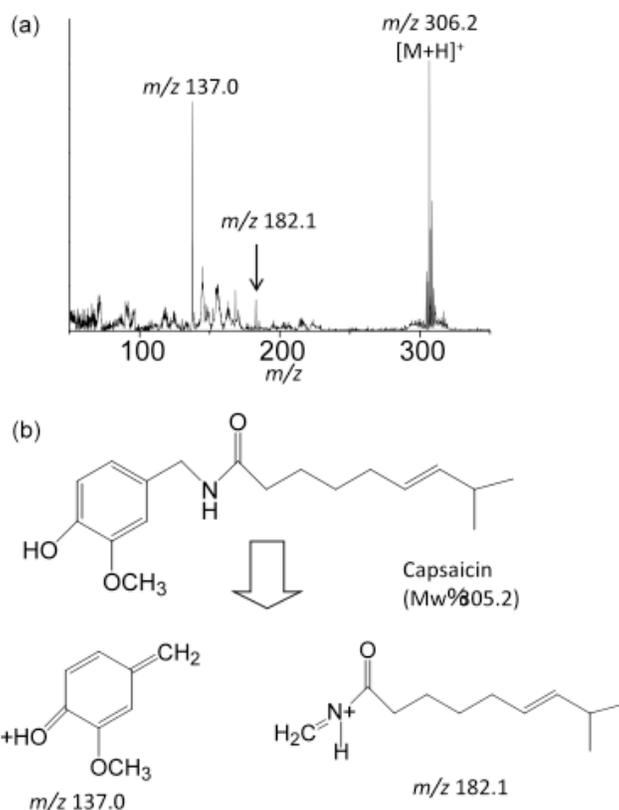
MS measurement was achieved to detect capsaicin from the *Capsicum* fruit before MS imaging. A number of signals were detected in the mass spectrum obtained from the *Capsicum* section at placenta, pericarp and seed regions, respectively, seeped with CHCA (Figure 2a-c). We focused on one signal for  $m/z$  306.2 which is the theoretical value of protonated

capsaicin. To confirm whether the obtained signal for  $m/z$  306.2 is capsaicin or not, the *Capsicum* fruit section was subjected to post source decay (PSD) for identification of a capsaicin based on structural analysis. The PSD spectrum of capsaicin showed a specific pattern of the precursor ion at  $m/z$  306.2 and two derivative ions at  $m/z$  182.1 and 137.0 as fragment ion that corresponds to that of the 8-methyl-6-nonenamide and 2-methoxy-4-methylphenol moieties, respectively (Figure 3a, b). The signal at  $m/z$  306.2 was detected from placenta, pericarp and seed regions, respectively, although the difference of signal intensity was observed. Especially, the signal at  $m/z$  306.2 of placenta region was the highest. The ratio of signal intensity of the placenta, the pericarp and the seed was 33:10:1. From these MS spectra, it was concluded that capsaicin was most existing at placenta region, semi-quantitatively.



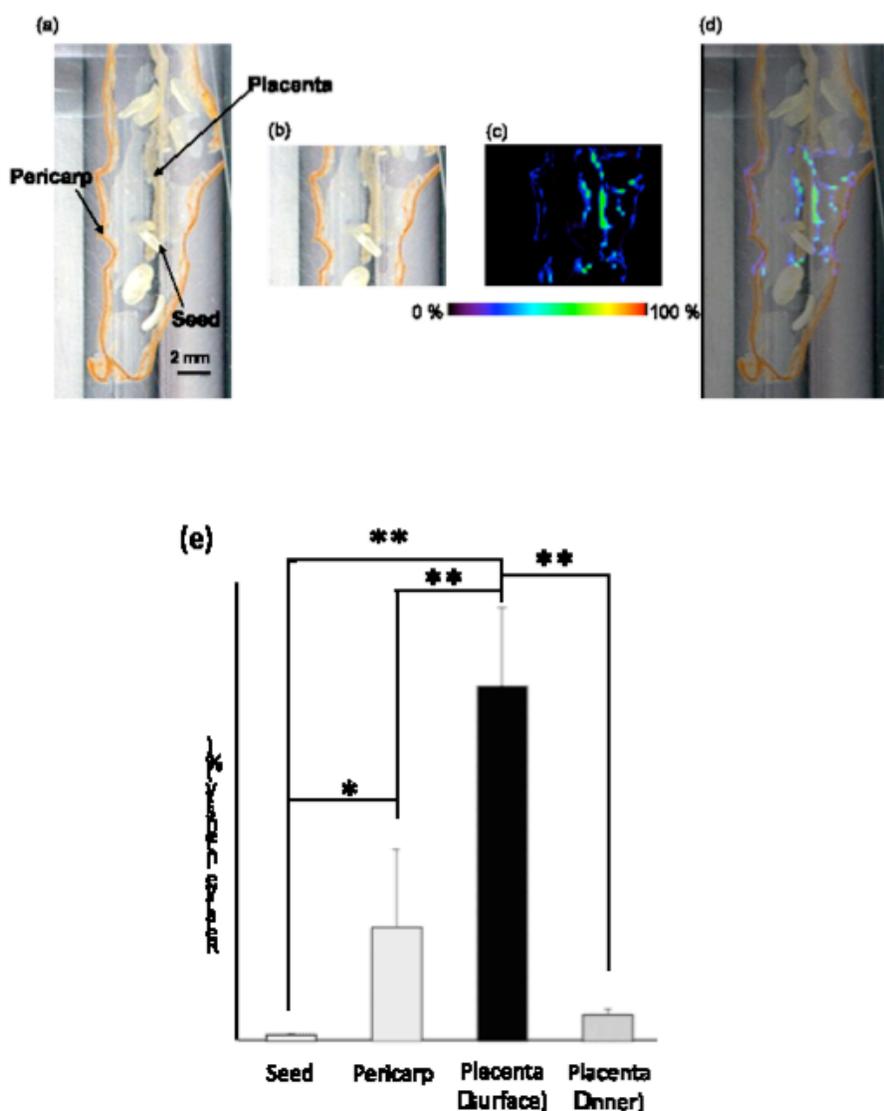
**Figure 2:** MS spectrum of *Capsicum* fruit at placenta (a), pericarp (b) and seed (c).

Next, we performed MALDI-TOF-MSI with the *Capsicum* fruits to visually prove localization of capsaicin. Successive lateral *Capsicum* fruit sections were prepared for identifying the regions where MSI was performed; further, the placenta, pericarp, and seed regions were defined (Figure 4a, b). In the reconstituted MS image, distinct capsaicin localizations was observed at  $m/z$  306.2 ( $[M+H]^+$ ) (Figure 4c). This image revealed the concentration of the capsaicin in the placenta region. In contrast, lower MS intensities were observed in the pericarp. For the seed region, we could marginally image from seed surface. Interestingly, we detected that capsaicin was unevenly distributed in the inner portion and was more abundant in the adverse side of placenta (Figure 4d).



**Figure 3:** Structural analysis of  $m/z$  306.2 from *Capsicum* fruits by PSD MS (a). Fragmentation form of capsaicin (b).

A semi-quantitative analysis was performed by correlating the MS intensities and whole placenta pericarp and seed regions of imaging area. For the total MS intensity per unit area, we detected that MS intensity at the pericarp and seed were lower than that at the placenta. Significant difference between the MS intensities at the placenta and the pericarp or seed was detected at the  $m/z$  value 306.2. Due to negligible MS intensity at seed region, a significant difference between MS intensities in the pericarp and seed region was observed. The capsaicin locally existed at placenta surface compared with that of inner region. We evaluated the localization between adverse side and inward of capsaicin. A significant difference was detected between MS intensities in the adverse side and the inward at the placenta region (Figure 4e). It is well known that the capsaicin is a second metabolite of *Capsicum* fruits and works as antibacterial agent or fungicide to protect the seed from foreign foe such as bacteria and fungi. *Capsicum* seeds take hold on placenta region and receive capsaicin from placenta surface. Therefore, the seeds are protected from foreign foe in spite of no production of capsaicin by itself. Our resultant image visually revealed botanic



**Figure 4:** MALDI-TOF-MSI of capsaicin. Optical images of *Capsicum* sections (a). The MSI region of *Capsicum* (b). MS spectra reconstructed as ion images for  $m/z$  306.2 (c). Merged image of optical *Capsicum* and MSI for  $m/z$  306.2 (d). Total MS intensity at pericarp, seed and placenta of *Capsicum* fruits (e). These areas were measured to quantify the MS intensity, respectively. Obtained values were commuted to per unit area. The values are expressed in terms of mean  $\pm$  SEM. \* $P < 0.05$  and \*\* $P < 0.01$  with Student's  $t$ -test.  $n=3$ .

instinct in the nature. In addition, the intake of placenta region of *Capsicum* fruit is reasonable as the gastrointestinal drug of Chinese medicine as well as the condiment for our wellness.

## CONCLUSION

MALDI-based MS is a useful technique for the identification and visualization of capsaicin in the *Capsicum* fruits. The capsaicin was found to be more dominantly distributed in placenta than pericarp and seed: capsaicin preferably existed to ward the outer portion of placenta compared with that of inner region.

MALDI-TOF-MS imaging is constructed by MS spectra of sample section and, therefore, it becomes a direct and easy-to-use technique for the determination of target molecule localization without any marker such as antibody and fluorescent reagent. This method can be applied as a new screening method of raw plants that possess a high pharmacological activity, to serve as a new quality evaluation system of food. In future, we intend to obtain an effective spatial resolution image by nanoparticle-assisted laser desorption/ionization (nano-PALDI) MS imaging to reveal a more accurate distribution of molecules such as their cellular resolution(12) and the selection and culture of high

pharmaceutical activity regions of herbal medicine as well as normal food for human wellness.

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