Original paper

Visualization of Tocopherol Acetate Absorbed Inside Laminated Films by a Matrix-assisted Laser Desorption/Ionization-imaging Mass Spectrometry

Mitsuru TANAKA¹, Eiji TANAKA¹, Noe OBINATA² and Toshiro MATSUI^{1*}

¹Faculty of Agriculture, Graduate School of Kyushu University, Fukuoka 812-8581, Japan

²Toppan Printing Co., Ltd., 4-2-3 Takanodai-minami, Sugito-cho, Kitakatsushika-gun, Saitama 345-8508, Japan

Received April 15, 2015 ; Accepted July 21, 2015

The aim of this study was to visualize the sorption of tocopherol acetate as a penetrant into polymer films. A matrix-assisted laser desorption/ionization-imaging mass spectrometry (MALDI-IMS) technique was used to visualize the distribution of tocopherol acetate in films during the migration process. A phytic acid-aided MALDI-IMS using 2',4',6'-trihydroxyacetophenone as a matrix allowed us to successfully visualize the absorbed tocopherol acetate in both ethylene-vinyl acetate copolymer (EVA) and low-density polyethylene (LDPE) films after 1 week-storage of tocopherol acetate at 40°C. The IMS technique allowed us to also visualize the actual distribution of tocopherol acetate inside the EVA/barrier/EVA laminated films, in which tocopherol acetate sorption was blocked at the barrier layer.

Keywords: sorption, MALDI-imaging mass spectrometry, plastic films, tocopherol acetate

Introduction

The use of plastic polymer films is now in trend for drug-, food-, and cosmetic-packaging materials, because of their high processing properties and cost efficiency. However, their poor gas-barrier properties, in particular, flavor deterioration or loss of drug content by sorption, still limits their extensive use (Leufvén and Hermansson, 1994; Shimoda *et al.*, 1984). Fayoux *et al.* (Fayoux *et al.*, 1997) showed that the amount of *d*-limonene in orange juice was dramatically decreased with storage by sorption inside films. Sorption degradation was also issued in cosmetic fields, in which tocopherol acetate that is a typical cosmetic chemical was dramatically absorbed into polyethylene packaging film during 20 day-storage at 60°C (Fukahori *et al.*, 1996). In a series of studies on sorbed penetrants into films, we demonstrated that factors affecting sorption were film properties such as glass transition temperature and density as well as the film affinity for penetrant (Matsui *et al.*, 1992, 1994; Fukamachi *et al.*, 1994). In this regard, we proposed a thermodynamic sorption equation that could predict

the magnitude of sorption using a solubility parameter (SP) value (Matsui *et al.*, 1992; Fukamachi *et al.*, 1994). However, while the sorption equation was useful for homogenous films, it was restrictive when applied to laminated films. In addition, no direct information on the distribution of penetrants inside the films was available using conventional sorption experiments.

In a previous report, we visualized a fluorescent perylene distributed in cellulose acetate films using fluorescence laser spectroscopy. However, its application to visualize sorption phenomena was still restrictive to penetrants with self-fluorescence properties (Hasegawa, *et al.*, 2010a, 2010b). In contrast, a recently developed matrix-assisted laser desorption/ionization-imaging mass spectrometry (MALDI-IMS) is applicable to target compounds without any consideration of specific molecular properties. To date, in the biochemical omics field, MALDI-IMS application has increased, since it can provide hundreds of images of ionizable compounds in a single measurement. Thus, in this study, we attempted to apply the IMS technique for visualization of

*To whom correspondence should be addressed.

E-mail: tmatsui@agr.kyushu-u.ac.jp

Fig. 2. Mass spectra of tocopherol acetate (20 nmol/0.2 μ L spot) spotted on ITO-coated glass slide with various matrix reagents (CHCA, DHB, SA, and THAP, at each concentration of 5.0 mg/mL). Arrow shows the m/z value of tocopherol acetate in positive or negative mode ($[M+H]^+$ (473 m/z) or $[M-H]^-$ (471 m/z)). Visualized images of tocopherol acetate were inserted into each spectrum with a width of $\pm 0.2 m/z$ at raster spot of 100 μ m. N.D., not detected.

Matrix spraying The matrix reagent (CHCA, DHB, SA, or THAP) was dissolved in acetonitrile/water (1:1, v/v) containing 0.1% trifluoroacetic acid (TFA) at a concentration of 5.0 mg/mL. Phosphoric acid, tartaric acid, citric acid, di-AC, NTMP, and phytic acid (each at 5.0 mM in matrix solution) were also used as matrix additives. To optimize the enhanced IMS visualization conditions of tocopherol acetate, an aliquot (20 nmol/0.2 μ L) of 100 mM tocopherol acetate solution was manually spotted on the ITO glass slide or on EVA film section. The matrix solution was then sprayed uniformly over the ITO glass slide with an ImagePrep automatic matrix sprayer (Bruker Daltonics). Spraying conditions were as follows: Spray power, 20%; modulation, 20%; spray time, 1.3 s; incubation time, 10 s; and dry time, 55 s. One hundred and fifty spraying cycles were performed. The amount of matrix sprayed onto the slide was controlled within 10% variation by an automated spray-generator in ImagePrep (e.g., THAP: 3.14 ± 0.26 mg/slide).

MALDI-IMS analysis MALDI-IMS measurements were performed using an Autoflex III mass spectrometer equipped with a SmartBeam III (Bruker Daltonics) in both positive and negative ion linear modes. MS data were acquired in the range of 100–1000 m/z by averaging signals from 300 consecutive laser pulses. The MS parameters were as follows: Ion source 1, 20.00 kV; ion source 2, 18.80 kV; lens voltage, 7.50 kV; gain, 2.45; laser frequency, 200 Hz; laser power, 90%. IMS analysis was performed with a spatial resolution of 20–100 μ m and the acquired MS spectra were analyzed by using Bruker FlexAnalysis software (ver. 3.3). The image data were reconstructed for

visualization with a mass filter with a width of $\pm 0.2 m/z$ using Bruker FlexImaging software (ver. 2.1).

Statistical analyses Data for signal-to-noise (S/N) ratio were expressed as mean $\pm$ SEM. The statistical difference between two groups and more than two groups were analyzed using unpaired Student's t -test and one-way analysis of variance (ANOVA), respectively, followed by Tukey-Kramer's t -test for post-hoc analysis. P values <0.05 were considered statistically significant. All analyses were performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA).

Results and Discussion

Flavor deterioration by sorption into films during storage has been a serious issue when foods with high quality are in demand. Sorption is caused by the thermodynamic condensation of flavors on the surface of the film with high affinity (Matsui *et al.*, 1992; Fukamachi *et al.*, 1993). However, sorption dynamics inside films still remained unclear. Thus, in this study, we attempted to apply MALDI-IMS for direct visualization of penetrants located inside films to get insights regarding the sorption or distribution of penetrants in films. Since, tocopherol acetate, which presents phenyl ester bonds and is non-volatile, is suitable for MALDI ionization, we used it as the penetrant compound in this study.

Optimization of MALDI-IMS measurement For enhanced MALDI-IMS detection of tocopherol acetate spotted on ITO glass slide (20 nmol/0.2 μ L spot), four matrix reagents (*i.e.*, CHCA, DHB, SA, and THAP) were examined. As shown in Fig. 2, the

highest MS detection of tocopherol acetate by IMS was observed in THAP matrix in positive mode with a high S/N ratio of 17.7 among the four common matrices. The lower tocopherol acetate detection as $[M+H]^+$ in other matrices would be due to the production of other molecular ion species such as $[M]^+$ and

$[M+Na]^+$ and/or their low ionization ability for tocopherol acetate. In addition, a 6.3-fold higher IMS detection of tocopherol acetate was observed when 5.0 mM phytic acid in THAP matrix solution was sprayed on EVA film (20 nmol/0.2 μ L spot), compared to THAP alone (Fig. 3). The phytic acid-induced high MS detection of tocopherol acetate on EVA film (Fig. 3) is most likely due to the reduction of MS-interfering complex formation with salts (e.g. Na^+) from ubiquitous impurities in film, glassware, solvent, and reagents (Chen *et al.*, 2003). As reported previously, phytic acid in matrix solution also allowed a homogenous coating (or spraying) of THAP matrix onto the target section (Hong *et al.*, 2013). In the present study, a marked MS signal of tocopherol acetate with Na^+ ($[M+Na]^+$) in THAP alone (Fig. 4A) was strongly attenuated by the presence of phytic acid in THAP (Fig. 4B), indicating the efficacy of phytic acid as a matrix additive on enhanced visualization of sorbed penetrants in films (Figs. 4C and D).

Fig. 3. Effect of matrix additives on MALDI-IMS detection of tocopherol acetate (20 nmol/0.2 μ L spot) spotted on EVA film section in positive mode. Concentration of each additive in 5.0 mg/mL THAP solution was 5.0 mM. Results are expressed as the mean of S/N ratio $\pm$ SEM. Data without common letters are significantly different ($P < 0.05$) according to Tukey-Kramer's t -test.

Visualization of tocopherol acetate in EVA and LDPE films by MALDI-IMS Under the optimal MALDI-MS conditions, sections of EVA and LDPE films immersed in tocopherol acetate (10 mg/mL) at 40°C for 1 week were subjected to an IMS analysis. As shown in Fig. 5, tocopherol acetate was sorbed into the inner EVA and LDPE films with storage. Although the MS detection intensity by MALDI-MS measurement was semi-quantitative (Albrechtsen *et*

Fig. 4. Mass spectra of tocopherol acetate (20 nmol/0.2 μ L spot) spotted on EVA film in positive mode in the absence (A) and presence (B) of 5.0 mM phytic acid in THAP matrix solution. Observed product ions regarding matrix and tocopherol acetate (TocAc) were $[THAP+Na]^+$ (191 m/z), $[TocAc+Na]^+$ (495 m/z), together with $[THAP+H]^+$ (169 m/z) and $[TocAc+H]^+$ (473 m/z). S/N ratios of each target product ion for THAP (C) and tocopherol acetate (D) in the presence (■) and absence (□) of 5.0 mM phytic acid are expressed as mean $\pm$ SEM. Significant difference between groups was analyzed by Student's t -test.

et al., 2011), a ca. 8.0-fold higher intensity of tocopherol acetate in EVA film, compared with that in LDPE film (Fig. 5), suggested that tocopherol acetate was favorably sorbed into EVA film rather than in LDPE film. The favorable sorption in EVA film may be explained by the high affinity or small distance in SP values (δ_c) between tocopherol acetate and EVA ($\delta_{c, \text{EVA}} = 4.4 \text{ MPa}^{1/2}$, $\delta_{c, \text{LDPE}} = 9.0 \text{ MPa}^{1/2}$) (Matsui *et al.*, 1992). So far, few investigators attempted to use MALDI-IMS to evaluate polymer characteristics such as cross-linking (Crecelius *et al.*, 2011) and surface analysis (Krueger *et al.*, 2013). However, to the best of our knowledge, this is the first application of the technique for dynamic evaluation of sorption behavior in films.

Evaluation of sorption barrier property of laminated EVA/

barrier/EVA film by MALDI-IMS Since no direct observation of sorption in films permitted to clarify the distribution of the penetrant inside the sorbed films, barrier characteristics of laminated films remained in question so far. Thus, we first attempted to visualize the absorbed tocopherol acetate in laminated EVA film with isocyanate-cured acrylic layer. As shown in Fig. 6, the inside of the laminated EVA film at 1 and 4 week-storage was successfully visualized by MALDI-IMS. At 1 week-storage, tocopherol acetate absorption was only observed in the upstream side of the laminated EVA film, indicating that tocopherol acetate sorption was completely blocked at the barrier layer. The IMS technique also provided useful information on the evaluation of the barrier property in laminated films, since after a longer (4 week-)

Fig. 5. Distribution of tocopherol acetate sorbed into EVA and LDPE films for 1 week-storage at 40°C by MALDI-IMS. The visualized image of tocopherol acetate ($[M+H]^+$, $m/z = 473$) was obtained by MALDI-IMS with a width of $0.2 m/z$ at raster spot of $20 \mu\text{m}$. White dotted lines in pictures represent the edges of cross sectional film segment on ITO-coated slide glass. Scale bar: $100 \mu\text{m}$.

Fig. 6. Distribution of tocopherol acetate sorbed into laminated EVA film for 1 and 4 week-storage at 40°C. Laminated EVA/barrier/EVA ($250/2/250 \mu\text{m}$) films composed of isocyanate-cured acrylic polymer as the barrier layer were used for sorption experiments. The image of tocopherol acetate ($[M+H]^+$, $m/z = 473$) was obtained by MALDI-IMS with a width of $0.2 m/z$ at raster spot of $20 \mu\text{m}$. White and red dotted lines in pictures represent the edges of the cross-sectional film segment and barrier layer, respectively. Scale bar: $100 \mu\text{m}$.

storage period, tocopherol acetate sorption was observed beyond the barrier layer (Fig. 6).

Conclusion

The present study demonstrated for the first time that a phytic-acid-aided MALDI-IMS technique was applicable for the visualization of sorption dynamics in laminated films. As reported by Palmgrén *et al.* (2006), the reduction of packed drugs with polymer films by sorption was also issued in the pharmaceutical field. Thus, the advantage of direct IMS visualization of penetrants distributed across cross-sectional directions in films will provide new insights regarding evaluation and protection on sorption in any packaging fields.

Acknowledgements The cost of publication was supported in part by the Research Grant for Young Investigators of Faculty of Agriculture, Kyushu University. The authors thank Ms. Kaori Miyazaki for her technical assistance.

References

- Albrethsen, J. (2011). The first decade of MALDI protein profiling: a lesson in translational biomarker research. *J. Proteomics*, **74**, 765-773.
- Chen, H., He, M., Pei, J., and He, H. (2003). Quantitative analysis of synthetic polymers using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Anal. Chem.*, **75**, 6531-6535.
- Crecelius, A. C., Alexandrov, T., and Schubert, U. S. (2011). Application of matrix-assisted laser desorption/ionization mass spectrometric imaging to monitor surface changes of UV-irradiated poly(styrene) films. *Rapid Commun. Mass Spectrom.*, **25**, 2809-2814.
- Fayoux, S. C., Seuvre, A. M., and Voilley, A. J. (1997). Aroma transfers in and through plastic packagings: orange juice and *d*-limonene. A review. Part I: Orange juice aroma sorption. *Packag. Technol. Sci.*, **10**, 69-82.
- Fukahori, M., Kakinuma, K., Seki, M., Takahashi, H., Uchino, Y., Sato, H., and Yamamoto, K. (1996). Effects of added lower alcohols on solubilized tocopherol acetate sorption to polyethylene film in aqueous solution. *Chem. Pharm. Bull.*, **44**, 196-200.
- Fukamachi, M., Matsui, T., Shimoda, M., Nakashima, M., and Osajima, Y. (1993). Sorption depression of flavors into modified EVA film by lowering film-flavor affinity. *J. Agric. Food Chem.*, **41**, 929-932.
- Fukamachi, M., Matsui, T., Shimoda, M., and Osajima, Y. (1994). Derivatization of thermodynamic sorption equation of flavors with packaging films (2). *J. Agric. Food Chem.*, **42**, 2893-2895.
- Hasegawa, T., Matsui, T., and Matsumoto, K. (2010a). Application of confocal laser scanning microscope technique to visualize dynamic sorption behavior of perylene in cellulose acetate film. *J. Appl. Polym. Sci.*, **116**, 1552-1557.
- Hasegawa, T., Matsui, T., and Matsumoto, K. (2010b). Comparative study of additives on visualized dynamic sorption behavior of perylene in cellulose acetate film by confocal laser scanning microscope technique. *J. Appl. Polym. Sci.*, **116**, 1710-1717.
- Hong, S.M., Tanaka, M., Yoshii, S., Mine, Y., and Matsui, T. (2013). Enhanced visualization of small peptides absorbed in rat small intestine by phytic-acid-aided matrix-assisted laser desorption/ionization-imaging mass spectrometry. *Anal. Chem.*, **85**, 10033-10039.
- Krueger, K., Terne, C., Werner, C., Freudenberg, U., Jankowski, V., Zidek, W., and Jankowski, J. (2013). Characterization of polymer membranes by MALDI mass-spectrometric imaging techniques. *Anal. Chem.*, **85**, 4998-5004.
- Leufvén, A. and Hermansson, C. (1994). The sorption of aroma components from tomato juice by food-contact polymers. *J. Sci. Food Agric.*, **64**, 101-105.
- Matsui, T., Nagashima, K., Fukamachi, M., Shimoda, M., and Osajima, Y. (1992). Application of the solubility parameter in estimating the sorption behavior of flavor into packaging film. *J. Agric. Food Chem.*, **40**, 1902-1905.
- Matsui, T., Fukamachi, M., Shimoda, M., and Osajima, Y. (1994). Derivatization of thermodynamic sorption equation of flavors with packaging films (1). *J. Agric. Food Chem.*, **42**, 2889-2892.
- Palmgrén, J.J., Mönkkönen, J., Korjamo, T., Hassinen, A., and Auriola, S. (2006). Drug adsorption to plastic containers and retention of drugs in cultured cells under in vitro conditions. *Eur. J. Pharm. Biopharm.*, **64**, 369-378.
- Shimoda, M., Nitanda, T., Kadota, N., Ohta, H., Suetsuna, K., and Osajima, Y. (1984). Adsorption of Satsuma mandarin juice aroma on plastic films. *NIPPON SHOKUHIN KOGYO GAKKAISHI*, **31**, 697-703.