Isomerization of Feruloylputrescine in Orange Juice by Light Exposure

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Exposure to light may adversely affect the quality of foods. This investigation of how light exposure affects citrus (orange and mandarin blend) juice in polyethylene terephthalate (PET) bottles demonstrated that the isomeric form of a compound in the juice changed during storage. This compound was identified as feruloylputrescine (FP; CAS: 501-13-3; $C_{14}H_{20}N_2O_3$) using LC/MS (Q-TOF). LC/MS and NMR measurements showed that the content of the original form, trans-FP, decreased as it isomerized to cis-FP during storage. This phenomenon could be observed in citrus fruit juices containing FP, such orange and grapefruit juices. Therefore, determining the content of these two isomers of FP could be used to indicate the level of light exposure experienced by citrus fruit products.

Key words: feruloylputrescine, orange juice, light exposure, mass spectrometry, principal component analysis

Introduction

Exposure of foods to light is increasing. The increasing number of round-the-clock grocery stores is one cause. Clear packages are desired for food safety, which also contributes to light exposure. According to the Japan Soft Drink Association, in Japan, the production of polyethylene terephthalate (PET)-bottled beverages increased from nine million kL in 2003 to fifteen million kL in 2015, while the production of canned beverages and paper-container beverages was three million kL and two million kL respectively in 2015 [1]. It is reported that light exposure affects the quality of various PET-bottled beverages and causes color-change or fading [2,3]. Coincidence of color-change and fading makes it difficult to evaluate the degradation of products by measurement of color. Therefore, it is desirable to find marker-substances to evaluate the effect of light exposure. In this report, the effect of light exposure of PET-bottled citrus (orange and mandarin blend) juices was investigated and isomerization of feruloylputrescine (FP, CAS: 501-13-3) was found on light exposure. The structure of FP is shown in Fig. 1. Some plants, such as tobacco [4], potato [5], eggplant [6] and bamboo [7], are reported to produce this substance, but we have not found any relevant reports about orange. In this paper, we report finding FP in orange juice, its isomerization under light, and the possibilities of using these properties.

Materials and methods

Materials. Bottled 100% citrus juices (orange and mandarin blend) in 350-mL PET bottles were purchased directly from the manufacturer in lot units (Ehime Beverage Inc., Ehime, Japan).

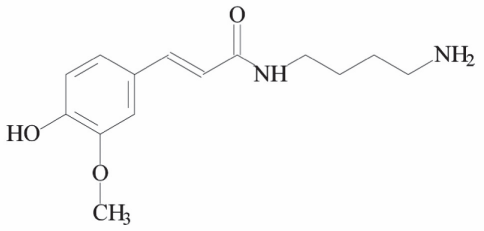

Fig. 1 Structure of feruloylputrescine

These bottles were stored in a dark place (4°C) until use. Other beverages and fruits were purchased from supermarkets in Japan. Feruloylputrescine trifluoroacetic acid (purity 96%) was obtained from Santa Cruz Biotechnology Inc. (Dallas, USA). Other chemicals were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). In this paper, FP stands for feruloylputrescine, and FP standard stands for feruloylputrescine trifluoroacetic acid.

Light exposure of bottled citrus juices. For the preparation of artificially degraded samples, bottled juices were stored in three conditions: a dark place at 4°C (D4); a dark place at 25°C (D25); or a light place at 25°C under 4000 lx fluorescent light (L25). The storage periods were 1, 2 and 4 weeks. After each period, three bottles were picked up and stored in the dark at 4°C until analysis.

In addition to the conditions mentioned above, bottled citrus juices were also stored at 25°C under 800 lx fluorescent light for 2, 4, 7 and 10 weeks, on the assumption that these juices are sold in stores and therefore exposed to low-intensity light for long periods. After each period, a bottle was picked up and stored in the dark at 4°C until analysis. In order to reveal the effect of temperature, bottled citrus juice was stored at 4°C under 1000 lx

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fluorescent light for 11 days.

LC/MS measurement (triple-quadrupole, QQQ) of degraded samples. Each sample (i.e., D4, D25 and L25 stored for 1, 2, and 4 weeks) was filtered with a three-layered filter (Whatman syringe filter, φ25 mm, 0.45 μm) and injected into a LC/MS system comprising an Agilent 1260 Infinity binary LC and an Agilent 6430 triple-quadrupole LC/MS (Agilent Technologies Inc., California, USA). Analysis was carried out according to the method described by Kammerer et al. [8], with small modifications. Parameters were: column: Synergi Hydro-RP 100A (100 mm × 3 mm, φ2.5 μm; Phenomenex Inc., California, USA); column oven temperature: 40°C; mobile phase: (A) 2% acetic acid, (B): 0.5% acetic acid/acetonitrile (1:1 v/v); flow rate: 0.4 mL/min; injection volume: 5 μL; ion source: ESI (positive and negative ion modes); dry gas: nitrogen (350°C, 12 L/min); nebulizing gas: nitrogen (60 psi); capillary voltage: ±2500 V; fragmentor voltage: 100 V; and mass range: *m/z* 100–1000. Throughout this paper, the solvent changes were applied as a linear gradient, not a stepwise gradient. Solvent B was increased from 10% to 24% at 8 min, to 30% at 16 min, to 55% at 24 min, to 100% at 30 min until 33.2 min, then decreased to 10% from 34 min until 36 min.

Principal component analysis of degraded samples. Principal component analysis (PCA) was performed using the statistical software Pirouette (Infometrix Inc., Washington, USA). Base ion chromatograms of samples were transformed into ANDI format using the analysis software Qualitative Analysis (Agilent Technologies Inc., California, USA) and aligned using the alignment software LineUp (Infometrix Inc.). A data matrix of retention time and peak intensity was generated using PiroTran ver. 1.35 (GL Science Inc., Tokyo, Japan). Peak intensity per 0.01 min was averaged and normalized.

LC/MS (Q-TOF) measurement of degraded samples. Sample L25 (4 weeks) was filtered with a three-layer filter (Whatman syringe filter φ25 mm, 0.45 μm) and injected into a LC/MS (Q-TOF) system using sodium acetate as an inner calibrant. The LC/MS system was comprising a LC20A (Shimadzu Corp., Kyoto, Japan) and a micrOTOF-QII (Bruker Daltonics Inc., Billerica, MA, USA). LC parameters were the same as mentioned above. MS parameters were: detection mode: AutoMSMS; mass range: *m/z* 50–1000; dry gas: nitrogen (200°C, 8 L/min); nebulizing gas: nitrogen (1.6 bar); capillary voltage: −4500 V for positive ion; hexapole RF: 200 V; quadrupole ion energy: 5 eV; collision gas: nitrogen (1.6 bar); collision energy: 10 eV; collision RF: 200 V; end plate offset: 500 V. Analysis was performed using the software Data Analysis (Bruker Daltonics Inc.).

Product ion scan of degraded samples and FP standard using LC/MS (QQQ). L25 sample (4 weeks) was diluted 10 times with pure water and filtered with a membrane filter (0.45-μm). FP standard solution (2 ppm) and L25 sample were injected into LC/MS (QQQ). The measurement was performed in product ion scan mode, using parameters precursor ion: *m/z* 265 and collision energy: 5 eV. The LC/MS system and other parameters were the same as described above.

Isolation of a component changing according to light exposure. FP standard (50 mg) was dissolved in 30 mL of 70% EtOH. The solution was poured into a glass test tube with a screw cap and stored under 4000 lx fluorescent light at 25°C for 1 week. After light exposure, the solution was evaporated at 50 °C using a miVac Duo (Genevac Ltd., Suffolk, UK). The dried residue was dissolved in 0.5 mL pure water and injected into a preparative LC system comprising a Delta600 HPLC and Fraction Collector III (Waters Corp., Milford, MA, USA). The isolation program parameters were: column: Discovery HS F5-5 (25 cm × 10 mm, Φ5 μm; Sigma-Aldrich Co. LLC., Missouri, USA); column oven temperature: 40°C; mobile phase (A): 0.1% formic acid, (B): acetonitrile; flow rate 8 mL/min; and injection volume: 250 μL. The solvent B profile was as follows: 5% for 5 min, then increase from 5% to 25% over 5 min, to 35% over 10 min, to 95% over 15 min, hold at 95% for 5 min, then decrease to 5% for the next injection. Samples were injected twice.
Each fraction was diluted 5 times with pure water and injected into LC/MS (QQQ). Fractions with a component change on light exposure were collected. After removal of acetonitrile using an evaporator, the collected solution was freeze-dried. Finally, 20 mg of the isolate was obtained from 50 mg FP standard.

NMR measurements. The whole isolate (20 mg) was dissolved in 1.2 mL D_2O. FP standard was also dissolved in D_2O (1 mg/mL) for comparison. D_2O included 0.1% 3-(trimethylsilyl)-propionic acid sodium salt as an internal standard. ^{1}H, ^{13}C, H-H Correlated Spectroscopy (COSY), Heteronuclear Multiple Quantum Coherence (HMQC), and Nuclear Overhauser Spectroscopy (NOESY) of the isolate and FP standard were recorded with a ECA400 (JEOL Ltd., Tokyo, Japan). Other parameters are shown in Table 1. The number of scans was decided depending on the methods used and the sample. All data were analyzed using Delta NMR Software ver. 5.02 (JEOL Ltd.).

Quantification of FP. Degraded juices, other beverage products and fresh juices squeezed from raw fruits were diluted 10 times with pure water and filtered with 0.45-μm membrane filters. LC/MS (QQQ) parameters followed the previous description with small modifications: ion source: ESI (positive ion mode) and capillary voltage: −3500 V. The solvent

Table 1 NMR Parameters

			Data points	Relaxation delay (s)	Offset (ppm)	Sweep (kHz)	Remarks
1D NMR	^{1}H		32000	5	5	7.50	temperature: 25℃ presaturation mode
	^{13}C		32000	5	100	31.40	temperature: 25℃
2D NMR	COSY	f1	2560	1.5	4	4.49	temperature: 4℃ for component A 25℃ for FP standard
		f2	256	1.5	4	3.60	
	HMQC	f1	2560	1.5	4	4.49	temperature: 4℃ for component A 25℃ for FP standard
		f2	256	1.5	97.5	16.70	
	NOESY	f1	1024	1.5	4.22	4.58	temperature: 4℃ for component A 25℃ for FP standard
		f2	256	1.5	4.22	3.66	

conditions were as follows: increase solvent B from 10% to 24% over 8 min, then to 100% at 8.01 min until 10 min, decrease to 10% at 10.01 min until 12 min, and then to 5% for the next injection.

Quantification was performed using a standard curve (FP standard concentrations: 0.1, 1, 3 and 5 ppm). FP concentration was calculated considering the ratio of FP (molecular weight: 264.3) and FP standard (FP trifluoroacetic acid, molecular weight: 378.3). The detection limit of FP was 0.03 ppm.

Heating experiment of FP to assess reversibility of isomerization reaction. Isolated cis-form FP was resolved in water (5 ppm). The solution was dispensed into 19 microtubes (1 mL/tube). The tubes containing FP solution were heated using a dry bath incubator (FastGene FG-01N; Nippon Genetics Co. Ltd., Tokyo, Japan) for 1, 2 and 4 h at 30, 40, 50, 60, 70 and 80°C. After each period, a tube was picked up and applied to measurement. Samples were injected into LC/MS (QQQ) and ratio of trans-form FP to whole FP was determined for each sample.

Results and Discussion

Analysis of degraded citrus juices

Comparison among base peak ion chromatograms of samples D4, L25 and D25 (4 weeks exposure for each) showed that the intensity of a peak with a retention time of 5.0 min increased on light exposure (Fig. 2). PCA of these chromatograms (positive ion mode) yielded clusters of samples stored in dark and light places (Fig. 3); samples stored in light places clustered according to the storage period. Considering the score plot, peaks around 5.1 min were observed at the positive side of the first principal component axis (PC1), and these around 6.8 min at the negative side, which indicates peaks around 5.1 min contributed to clusters of light-exposed sample and these around 6.8 min to the cluster of samples stored in the dark place (Fig. 4). Both peaks contained a molecular ion ($[M+H]^+$) with *m/z* 265.1. Hereafter, the component at 5.1 min is named A, and that at 6.8 min is named B. There was no component A found in samples D4 and D25, and no changes in the amount of component B were observed in any of the samples stored in the dark. However, depending on the period of light exposure, the amount of component B decreased and that of component A increased (Fig. 5).

In previous studies, PCAs of GC/MS data, which have higher completeness and resolution than LC/MS data, have been reported to be useful in searching for fluctuating components [9,10]. Since results of LC/MS are highly dependent on parameters such as solvent and columns, it is often considered difficult to use LC/MS data for PCA. However, in this case, we succeeded in finding a fluctuating component in citrus juices using LC/MS data, which indicates LC/MS data can also be used in PCA.

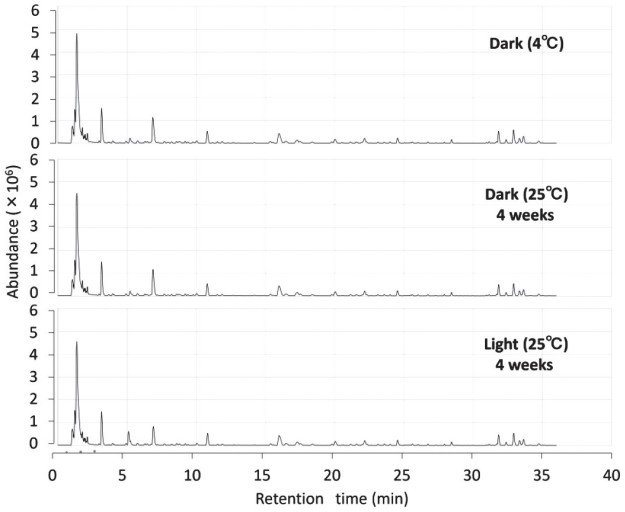

Fig. 2 LC/MS (QQQ) base peak ion chromatograms of stored samples (positive ion).Chromatograms of samples stored in the at 4°C (D4), in the dark at 25°C (D25), or in the light at 25°C (L25) from above.

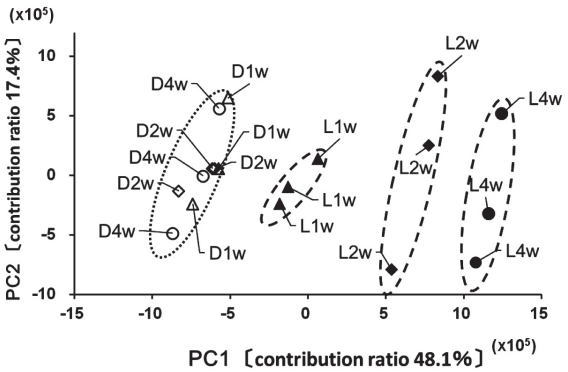

Fig. 3 Principal component (PC) analysis score plot for citrus juices stored in the light or in the dark.Sample labels (n = 3): △ , Dark 1 week (D1w); ◇, Dark 2 weeks (D2w); ○, Dark 4 weeks (D4w); ▲, Light 1 week (L1w); ◆, Light 2 weeks (L2w); ●, Light 4 weeks (L4w).

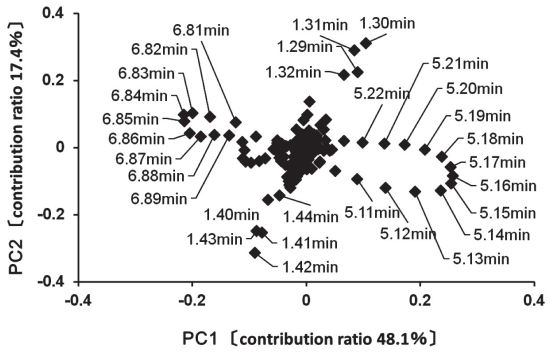

Fig. 4 PC analysis loading plot. Retention times (min) are marked for each spot.

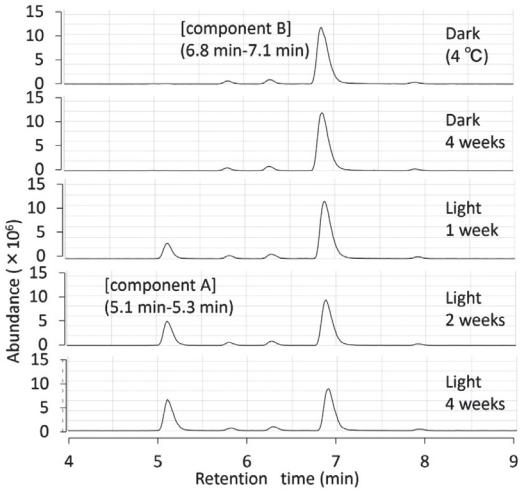

Fig. 5 LC/MS (QQQ) extracted ion chromatograms of samples (positive ion, m/z = 265.1). Chromatograms of D4, D25 (4 weeks) or L25 (1, 2 and 4 weeks) from above. Peak with retention time of 5 min is [component A], and that with retention time of 7 min is [component B].

Structural identification of component B

Accurate mass analysis of components A and B (n = 2) showed that they had almost the same $[M+H]^+$ ions, A: 265.15495 and 265.15459; B: 265.15520 and 265.15464. Structures of both $[M+H]^+$ were estimated to be $C_{14}H_{21}N_2O_3$ (monoisotopic weight: 265.15522). Thus, components A and B were possibly structural isomers.

Based on structural information from ChemSpider, feruloylputrescine ($C_{14}H_{20}N_2O_3$) was a candidate molecule. To compare FP and components A and B, citrus juice (D4, diluted 10 times), FP standard (feruloylputrescine trifluoroacetic acid; 2 ppm), and a mixture of them (1:1 v/v), were injected into LC/MS (QQQ). Component B and FP standard showed the same retention time and MS spectrum (data not shown). A product ion scan of component A and FP standard (precursor ion: m/z 265) indicated that component A produced the same fragment ion peak as FP standard (m/z 177; Fig. 6). From these results, component B was identified as FP, and component A had the same partial structure as component B and FP standard.

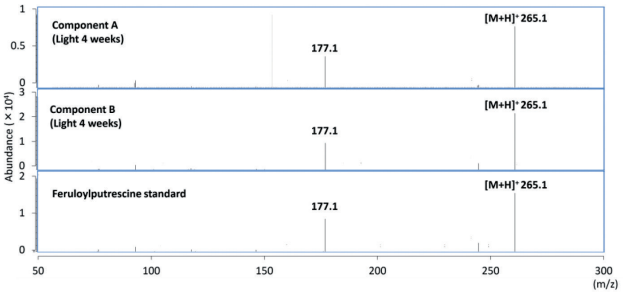

Fig. 6 Product ion spectra of component A (5.1 min retention time), component B (6.8 min) and feruloylputrescine (FP) standard from above. Precursor ion m/z = 265, CE 5 eV. Each peak represents fragment.

Structural identification of component A

Component A was isolated from light-exposed FP standard solution using a preparative LC/MS system. Purity was shown to be 98% by LC/MS (QQQ).

The structure of FP is presented in Fig. 1. ^{1}H NMR spectra and ^{13}C NMR spectra of FP standard and component A are shown in Figs. 7 and 8. The assignment of each spectrum is presented in Table 2. Comparing ^{1}H NMR and ^{13}C NMR results for component A and FP standard, signals related to double-bond such as H10, 11 and C10, 11, 12 were shifted more than other signals. Furthermore, coupling constants between H10–H11 (see Fig. 1) were 15.7 Hz and 12.3 Hz for FP standard and component A respectively. It is reported that when two protons are in cis-form, the coupling constant between them is smaller than for the trans form (around 8–12 Hz for cis and 14–17 Hz for trans) [11]. In NOESY spectra, cross peaks are observed between protons located in a spatially close distance. Comparison of NOESY spectra of FP standard and component A indicated that a cross peak of H10–H11 was observed only in component A (Figs. 9 and 10). These results strongly suggest that FP standard and component A are cis-trans isomers, where FP standard is the trans-form, and component A is the cis-form.

There are some reports on substances generated by light-exposure of fruit juices, but FP has not been mentioned [12, 13]. Generally, trans-form compounds are said to be more stable than cis-form, due to the smaller effect of steric hindrance. Therefore, the structural change from trans to cis is an interesting phenomenon. As one example, some trans-form carotenoids in vegetables and fruits were reported to isomerize into cis-forms by the influence of heat or light [14]. However, we can find no report of light exposure of FP changing its structure from trans to cis.

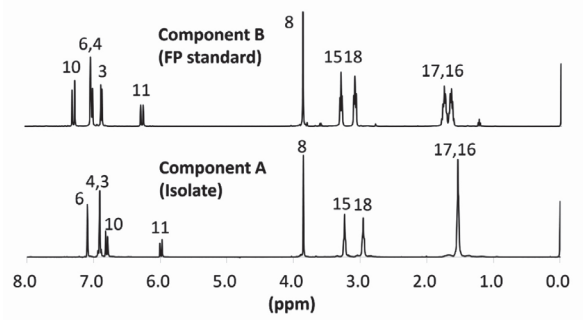

Fig. 7 ^{1}H NMR spectra of FP standard and isolated component A. Numbers on peaks refer to positions of protons.

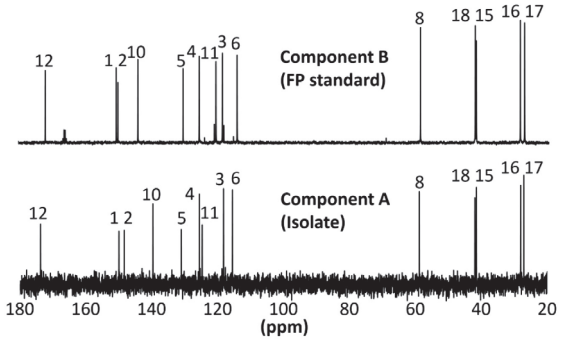

Fig. 8 ^{13}C NMR spectra of feruloylputrescine standard and isolated component A. Numbers on peaks refer to positions of carbons.

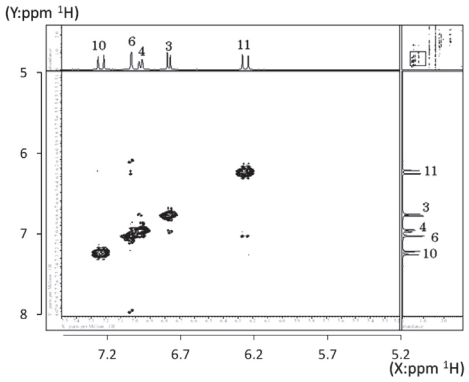

Fig. 9 NOESY spectrum of FP standard (extended). ^{1}H NMR spectra of feruloylputrescine standard is shown on X-Y axis.

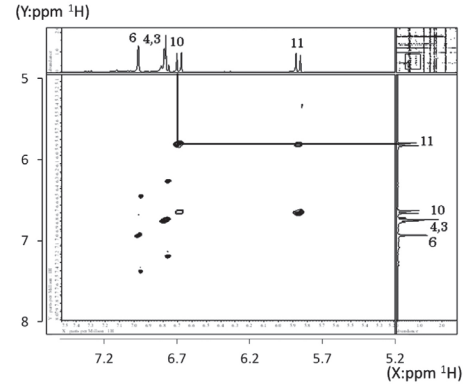

Fig.10 NOESY spectrum of component A (extended). 1H NMR spectra of component A is shown on X-Y axis. Characteristic cross-peak of H10-H11 is indicated by black lines.

Table 2 Assignments of 13C and 1H NMR

Position	^{13}C Chemical shift (ppm)			^{1}H Chemical shift (ppm)		
	FP standard (Comoponent B)	Component A	⊿δC	FP standard (Comoponent B)	Component A	⊿δH
1	150.40	149.92	0.48			
2	149.89	148.30	1.59			
3	118.38	118.20	0.18	6.92 (m, −)	6.91 (m, −)	0.01
4	125.36	125.51	−0.15	7.11 (m, −)	6.91 (m, −)	0.20
5	130.23	131.03	−0.80			
6	113.94	115.51	−1.57	7.18 (s, 0 Hz)	7.09 (s, 0 Hz)	0.09
8	58.68	58.80	−0.12	3.89 (s, 0 Hz)	3.85 (s, 0 Hz)	0.04
10	143.86	139.62	4.24	7.38 (d, 15.9 Hz)	6.80 (d, 12.4 Hz)	0.58
11	120.33	124.68	−4.35	6.40 (d, 15.9 Hz)	5.99 (d, 12.4 Hz)	0.41
12	171.79	173.70	−1.91			
15	41.82	41.49	0.33	3.32 (t, 6.7Hz)	3.23 (t, 6.2 Hz)	0.09
16	28.57	28.05	0.52	1.64 (m, −)	1.52 (m, −)	0.12
17	27.28	27.11	0.17	1.72 (m, −)	1.52 (m, −)	0.20
18	42.15	41.96	0.19	3.05 (t, 7.6 Hz)	2.94 (t, 6.8 Hz)	0.11

Time-course change and distribution of FP in retail products

In most grocery stores, the light intensity is around 700 to 1000 lx. To reproduce this circumstance, bottles of citrus juice were exposed to 800 lx fluorescent light at 25°C. Quantification of FP in the juices showed that component B decreased and component A increased depending the period of light exposure (Fig. 11). The sum of component A and B at each time point showed a 5.6% variation coefficient, which indicated that isomerization proceeded stoichiometrically. These findings indicate that juices containing FP can be used as an indicator of light exposure. In addition to that, in citrus juice exposed to 1000 lx fluorescent light at 4°C for 11 days, the concentration of trans-form and cis-form FP were 20.5 and 1.4 ppm each. The concentrations of trans-form and cis-form FP in citrus juices exposed to 800 lx fluorescent light at 25°C for 14 days were 18.1 and 1.1 ppm each (Fig.11). Even though there were little differences in the intensity of light (1000 or 800 lx) and exposure-periods (11 or 14 days), it could be concluded that the difference of storage temperature under 25°C didn't have big effect on isomerization reaction of FP.

Among beverage products in various packages (PET bottles, cans and papers), trans-FP was found in orange juice (9–21 ppm) and grapefruit juice (>30 ppm), but no trans-FP was detected in mandarin orange juice, apple juice, grape juice or tomato juice. Among fresh fruit juices squeezed from raw fruits, trans-FP was found in orange juice (9 ppm), grapefruit juice (50 ppm) and lime juice (1.5 ppm), but no FP was detected in mandarin orange juice or lemon juice. Cis-form FP was only found in a fresh grapefruit juice (0.5 ppm).

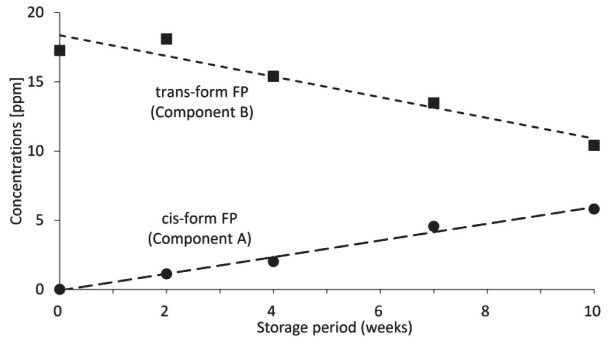

Fig. 11 Light-exposure assay of feruloylputrescine (FP) standard under 800 lx. Concentration of trans-form or cis-form FP (ppm) is plotted as a function of sample storage period (weeks). ■, concentration of trans-form FP; ●, concentration of cis-form FP.

Reversibility and possible utility of FP isomerization

Reversibility of the isomerization reaction of FP was confirmed by heating cis-form FP (30–80°C)—heating at >60°C was needed to isomerize FP from cis to trans (Fig. 12).

These days, commercial product delivery processes and product packages vary widely, and quality control techniques are well-developed. Even so, defective products are sometimes found, and, in many cases, a quick response is desired. FP could be used in researching the cause of defects. For example, when defects are found in products containing FP, quantification of cis and trans-form FP could be used to estimate the amount of light that products or crude materials were exposed to. For food products, other industrial products, and packaging development, the amount of each FP isomer could give information about light-blocking effects. As, cis-form FP was found to isomerize into the trans-form by heating at >60°C, it could be used as an indicator of the thermal history of a product. Industrial use of FP thus has great potential.

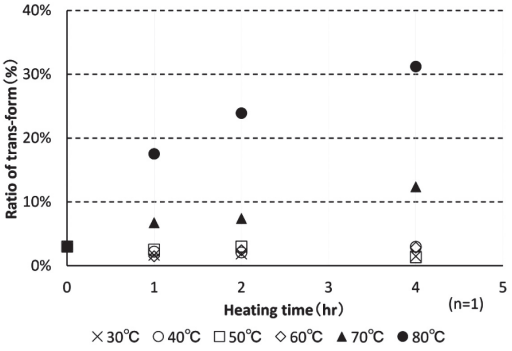

Fig. 12 Heating assay of cis-form feruloylputrescine (FP) (aqueous solution of component A). Ratio of trans-form FP (%) is plotted as a function of heating time (h).

Summary

Because of increasing concerns about food safety, clear packages are often used, which results in increasing light exposure of foods. In this report, we found two components in citrus juices that changed according to the light exposure. LC/MS (Q-TOF) measurement showed that both components had the formula $C_{14}H_{20}N_2O_3$, and during the storage period one component increased while the other decreased. Further analysis using LC/MS and NMR showed that trans-form FP isomerized into the cis-form on exposure to light. FP could be used as indicator of light exposure of citrus juices.

Author contribution

Conceived and designed the experiments: Hidenobu Sumitani. Performed the experiments: Hidenobu Sumitani, Kayako Ogi, Takako Otsuka. Performed the PCA: Hidenobu Sumitani and Takako Otsuka. Analyzed NMR datas: Kayako Ogi. Contributed to the writing of the manuscript: Kayako Ogi and Hidenobu Sumitani. All the authors approved the final

version of the manuscript.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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