Characterization of the aroma compounds in fresh and dried sapodilla (Manilkara zapota, L.) by the application of aroma extract dilution analysis

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ABSTRACT
Twenty-nine aroma-active compounds were quantified in fresh and dried sapodilla fruits grown in Malaysia. Sensory studies revealed distinct differences in the overall orthonasal aroma of the fresh and dried fruits. Whilst, the fresh fruit was characterized as typical minty, fatty/green cum woody, the dried fruit exhibited citrusy, balsamic/sweet notes. In addition, the results of the principal component analysis (PCA) showed that the fresh and dried sapodilla fruits were covaried with 13 and 19 aroma-active compounds, respectively. Calculation of the odour activity values (OAVs) of the aroma-active compounds showed that the overall aroma note of the fresh fruit was mainly caused by ethyl benzoate, E-2-hexenal, and β-caryophyllene. However, α-sinensal and to a lesser extent ethyl benzoate, E-2-hexenal, β-caryophyllene, and hexyl benzoate were responsible for the overall aroma of the dried fruit.

INTRODUCTION

Sapodilla (Manilkara zapota L.), or sapota, is also known as ‘Ciku’ in South East Asia. It belongs to the family Sapotaceae and originated from Southern Mexico, Central America and the Caribbean (Morton, 1987). Sapodilla is a medium-sized tree that produces edible fruits with characteristic delicate flavour. The fruit is very sweet and best consumed when fully ripe. In general, the fruits are between 5 and 10 cm long and weigh up to 0.15 kg. The fruit is ovoid or ellipsoid in shape and normally contains black seeds at the centre (Crane, Balerdi, & Maguire, 2016). The immature fruit has a pale green skin which turns yellow at maturity. Sapodilla is a gem among tropical fruits because it has high nutritional value. According to the National Nutrient Database for Standard References (2016) the major nutrients of sapodilla are vitamin C (39.33%), carbohydrate (37.00%), dietary fibre (33.68%), iron (24.13%) and copper (23.00%). Even though sapodilla fruit has high beneficial value, its shelf life is only around one to two weeks after plucking from the tree. To extend the shelf-life of Sapodilla and meet the consumers’ demand, the fruits are processed into juice; jam (Ahmed, Burhauddin, Haque, & Hossain, 2001), fruit bars (Rabeta, Tee, & Leila, 2016) and dried fruits (Chong & Law, 2011). Drying is one of the earliest methods used in prolonging the shelf-life of a product. However, drying also influences other characteristics of the dried products such as: palatability, flavour, aroma, viscosity, hardness and enzymatic activity (Izli, Izli, & Taskin, 2017). The effect of drying on the physicochemical properties of sapodilla fruit has been studied (Chong & Law, 2011). However, there are no reports available on the impact of drying on the volatile constituents of sapodilla fruit. For instance, drying of mango (Bonneau, Boulanger, Lebrun, Maraval, & Gunata, 2016), nectarines (Sunthonvit, Srzednicki, & Craske, 2007) and lulo fruit (Forero, Orrego, Peterson, & Osorio, 2015) resulted in significant changes in the volatile constituents of the dried fruits compared to the fresh fruits.

Consumers’ food preferences are often linked to the sensorial characteristics of a product. Flavour is considered as the first evaluation signals along with food appearance and texture encountered by consumers during eating of food (Lasekan & Otto, 2009). A major factor contributing to the

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high demand for sapodilla in South East Asia is its flavour. Surprisingly, only few studies have been conducted on sapodilla volatiles of different varieties (Laohakunjit, Kerdchoeuchuen, Matta, Silva, & Holmes, 2007; MacLeod & De Troconis, 1982). Alkyl benzoates, benzaldehyde, hexanol, methyl salicylate and trans-hex-2-enal were identified in fresh sapodilla (MacLeod & De Troconis, 1982). Laohakunjit et al. (2007) identified a total of 23 compounds in fresh sapodilla fruit. The compounds include; ethyl acetate, acetaldehyde, benzyl alcohol, and 2-butenyl benzene. The aim of this study was to identify and quantify the volatile constituents in fresh and dried sapodilla fruit.

Materials and methods

Chemicals

Reference standards of acetaldehyde, methyl acetate, ethyl acetate, 1-methoxy-2-propanol, 1-butanol, 1-penten-3-ol, 3-methyl-1-butanol, E-2-hexenal, hexan-1-ol, heptanal, 2-methylbutan-2-one, benzaldehyde, δ-3-carene, hexyl acetate, benzyl alcohol, cis-linalool-oxide, methyl benzoate, ethyl-4-hydroxyethyl-2(3H)-furanone, ethyl benzoate, decanal, methyl eugenol, pentyl benzoate, hexyl benzoate, phenyl ethyl benzoate, and β-sinensal were from Sigma-Aldrich (Steinheim, Germany). Dichloromethane, pentane and methyl eugenol, pentyl benzoate, hexyl benzoate, phenyl ethyl benzoate, and β-sinensal were from Sigma-Aldrich (St. Louis, MO, USA). β-Caryophyllene was purchased from Fluka Analytical (Steinheim, Germany). Series of n-alkanes, C₈ to C₂₀, were from Sigma-Aldrich (St. Louis, MO, USA).

Fruit materials and sample preparation

Ripe sapodilla fruits (5 kg) grown and freshly harvested in the year 2017 in Serdang, Malaysia, were purchased from a local producer. Fresh sapodilla fruits with similar maturity characteristics on the basis of size, weight, skin color and °Brix (15.80 ± 1.3) were used. The average size of the sapodilla used was 7 cm x 5 cm. The titratable acidity and pH values were 1.5% and 5.1, respectively. Fruits were manually washed with sodium hypochlorite solution (0.1 mg/mL), followed by rinsing in water. Fruits were sliced (20 mm x 50 mm x 3 mm) and divided into two batches. A batch was dried in a pilot unit (Smoke Master 250RFS, USA) at 65°C, 40% relative humidity and constant airflow for 6 h. At the end of drying, the corresponding water activity (a₀) was 0.6. The dried sapodilla fruit slices (200 g) were ground into powder and separated into fractions of 50 g and subsequently stored at −80°C until analysis. The other batch of washed fruit slices was used to analyze the volatile constituents of the fresh sapodilla fruit. The fresh fruit slices (200 g) were processed into puree with an electronic blender (Panasonic MX-798S, Malaysia). The puree was divided into fractions of 50 g. frozen under liquid nitrogen and kept at −80°C until analysis.

Sapodilla aroma profile determination

Fresh and dried sapodilla fruits (20 g) were cut into cubes (2 cm) and placed in glass containers (7 cm x 3.5 cm). Each sample was ortho-nasally evaluated by 12 trained sensory panelists (7 females and 5 males with age ranging from 18 to 30 years) using the free choice profiling in a quantitative descriptive analysis (QDA) (Munafo, Didzbalis, Schnell, Schieberle, & Steinhaus, 2014). Reference compounds dissolved in water at a concentration of 100 times above the respective thresholds of the reference compounds were used as descriptors in the QDA. The reference compounds used include hexyl acetate (fruity), ethyl-4-hydroxymethyl-3(2H)-furanone (caramel, sweet), methyl benzoate (almond, floral), 1-penten-3-ol (green, grassy), hexan-1-ol (carrot-like), β-caryophyllene (woody, spicy), and hexyl benzoate (balsamic, sweet). A seven-point scale in 0.5 increments from 0 to 3, with 0 = not detectable, 1 = weak, 2 = moderate, and 3 = strong were used to assess the orthonasal aroma of the fruits. The results were presented in a web plot.

Aroma isolates

Portions of dried fruit powder (25 g) and puree (25 g) were sequentially extracted with dichloromethane (100 mL, 50 mL) with constant shaking on an auto-shaker (28 ± 2°C, 10 min). The mixture was centrifuged (5000 rpm; 5 min) and the supernatants were subsequently combined while the residue was discarded. The organic phase was separated from the aqueous phase with the aid of a separating funnel. The organic phase was subjected to solvent-assisted flavor extraction (SAFE) distillation (Engel, Bahr, & Schieberle, 1999) at 40°C, and subsequently dried over anhydrous sodium sulphate. The distillate was concentrated first to about 2 mL using a Vigreux column (50 x 1 cm) and later to 200 µL using a small size Vigreux column (Lasekan, Buettner, & Christibuaer, 2007; Lasekan & Ng, 2015). The concentrated extract was used for the GC-MS analysis and the experiment was carried out in triplicate.

GC-MS and GC-FID analyses

The GC-MS and GC-FID analysis were performed using a Shimadzu (Kyoto, Japan) QP-5050A GC-MS equipped with a GC-17A Ver.3, a flame ionization detector (FID) and fitted with a DB-5 column (30 m x 0.32 mm i.d., film thickness 0.25 µm; Scientific, Inc., Ringoes, NJ) (Lasekan, 2017). The gas chromatographic and mass spectrometric conditions were the same as described previously by Lasekan and Ng (2015).

GC-O analysis

GC-O was conducted with a Trace Ultra 1300 gas chromatograph (Thermos Scientific, Waltham, MA, USA) fitted with a DB-5 column (30 m x 0.32 mm i.d., film thickness, 0.25 µm, Scientific Instrument Services, Inc., Ringoes, NJ) and an ODP 3 olfactory Detector Port (Gerstel, Mulheim, Germany), with additional supply of humidified purge air (12 mL min⁻¹). The humidified air in the sniffing cone maintained olfactory sensitivity and help to reduce dehydration of mucous membranes in the nasal cavity. The sniffing port was held at 250°C and operated as earlier reported by Lasekan, Khatib, Juhari, Patiram, and Lasekan (2013). The split ratio between the sniffing port and the FID detector was 1:1. Two replicate samples were sniffed by three trained panelists who presented normalized responses, with strong agreement with one another.

Identification and quantification

The linear retention indices were calculated according to Kovats method using a mixture of normal paraffin C₈-C₂₀ as external references (Lasekan, 2017). The identification of compounds was as described by Lasekan and Ng (2015).
Semi-quantitative data were obtained by relating the peak area of each compound to that of the corresponding reference standard and were expressed as µg kg⁻¹.

Aroma extracts dilution analysis (AEDA)

Aroma extract dilution analysis (AEDA) was adopted to obtain the aroma profile of the fruit. Three experienced sniffers performed the AEDA experiments. Two days before the analysis, the sniffers conducted preliminary sniffing tests on some aroma-active reference compounds and they repeatedly confirmed the retention time and odour quality of the compounds. The extract obtained by SAFE was diluted stepwise with dichloromethane (1:1, v/v) and sniffing of dilution continued until no odour could be detected by the sniffers (Lasekan, 2017). Each of the obtained dilution was injected into the GC-O. The highest dilution in which an aroma compound was observed is referred to as the flavour dilution (FD) factor of that compound (Schieberle, 1995).

Statistical analysis

SPSS version 16.0 Windows (SPSS INC., Chicago, IL) was employed for the statistical analyses. The significance of difference between means was tested by one-way analysis of variance (ANOVA). Results were expressed as mean ± SD (Standard deviation of triplicate analyses). The mean concentrations of the 29 aroma-active compounds (Table 1) were the data statistically analysed by Principal Components Analysis (PCA). The multivariate statistical analyses were performed with SIMCA-P software (V. 10.0, Umetrics, Umea, Sweden) using mean centred scaling method.

Results and discussion

The results of sensory evaluation of freshly cut pieces of fresh fruits and the dried pieces revealed distinct differences (Figure 1). The aroma of the fresh sapodilla fruit was characterized as typical minty, fatty/green, woody and spicy. While the dried fruit exhibited citrusy, balsamic/sweet, and fatty/green notes. In order to elucidate the reasons behind this observation, the fruits (fresh and dried) were subjected to aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry (GC-O).

The application of AEDA and GC-O, on the SAFE extracts of the fresh and dried fruits, revealed a total of 26 and 29 aroma-active compounds in the fresh and dried fruits, respectively. In addition, the aroma-active compounds were in the flavour dilution (FD) factor range of 4 to 64 (Table 1). The aroma-active compounds exhibited an array of odour qualities such as pungent/fruity, nail-polish-like, sweet/fruity, green/grass-like, malty, almond-like, minty, woody/spicy and sweet/balsamic. Sniffing of serial-dilution of the fruit extracts revealed that the minty (ethyl benzoate), almond/floral-like (Methyl benzoate), floral-like (pentyl benzoate), and malty (3-methyl-1-butanol) possessed the highest FD factors (32–64) (Table 1).

Table 1. Aroma-active constituents detected in SAFE extracts from fresh and dried sapodilla fruits.

| No | Compound* | Odour quality | RI on DB-5 | FD Factor | Conc. µg kg⁻¹ DM |
|----|-----------|---------------|------------|-----------|-----------------|
| 1  | 2-Butenyl benzene | - | 186 | nd | nd | 15.9 ± 0.1 | 9.2 ± 0.0 |
| 2  | Benzaldehyde | - | 207 | nd | nd | 52.6 ± 0.1 | 16.9 ± 0.1 |
| 3  | Acetaldehyde | Pungent, fruity | 500 | 4 | 4 | 18.6 ± 0.0 | 41.4 ± 0.2 |
| 4  | 3-Methyl acetate | Nail polish | 522 | 8 | 8 | 62.3 ± 1.0 | 12.4 ± 0.1 |
| 5  | Ethyl acetate | Sweet fruity | 615 | 16 | 16 | 11.6 ± 0.0 | 25.6 ± 1.5 |
| 6  | 1-Methoxy-2-propanol | Sweet, ether | 673 | nd | 8 | nd | 112.4 ± 3.5 |
| 7  | 1-Butanol | Alcoholic | 719 | nd | 4 | nd | 75.5 ± 2.0 |
| 8  | 1-Penten-3-ol | Green, grass | 736 | 32 | 32 | 12.8 ± 0.0 | 210.1 ± 6.3 |
| 9  | 3-Methyl-1-butanol | Malty | 853 | 16 | 16 | 76.5 ± 2.5 | 97.6 ± 1.7 |
| 10 | 2,2-Dimethylpropane | Fatty, green | 871 | 8 | 8 | 12.1 ± 0.0 | 31.3 ± 1.2 |
| 11 | 2-Heptanone | Carrot-like | 903 | nd | 4 | t | 26.8 ± 1.0 |
| 12 | 2-Methyl butanoate | Sweet, fruity | 927 | 16 | 16 | 12.7 ± 0.1 | 35.9 ± 1.0 |
| 13 | Benzaldehyde | Almond-like | 963 | 16 | 16 | t | 20.8 ± 1.0 |
| 14 | 2,3-Dimethylbutanal | Pungent | 1010 | 4 | 4 | 112.2 ± 4.0 | 17.6 ± 0.1 |
| 15 | Hexanal | Fruity | 1014 | nd | 4 | nd | 78.4 ± 2.0 |
| 16 | Benzaldehyde | Aromatic | 1039 | 16 | 16 | 15.1 ± 0.1 | 31.5 ± 1.2 |
| 17 | 2-Cis-Linalool oxide | Floral, sweet | 1074 | 4 | 4 | 113.3 ± 0.0 | 9.3 ± 0.0 |
| 18 | Methyl benzoate | Floral | 1091 | 32 | 32 | 375.5 ± 9.0 | 87.3 ± 2.0 |
| 19 | Ethyl-4-hydroxymethyl-3(2H)-furanone | Caramel | 1138 | nd | 8 | t | 26.6 ± 0.1 |
| 20 | Ethyl benzoate | Minty | 1170 | 64 | 64 | 7540 ± 21.0 | 137.4 ± 8.7 |
| 21 | Decanal | Tallow | 1207 | nd | 4 | nd | 57.6 ± 1.6 |
| 22 | Benzyl isothiocyanate | Watercress-like, medicinal | 1361 | 4 | 4 | 46.7 ± 2.7 | 18.6 ± 0.1 |
| 23 | Methyl eugenol | Spicy | 1401 | 4 | 4 | 26.1 ± 0.1 | 15.2 ± 0.0 |
| 24 | β-Caryophyllene | Woody | 1418 | 16 | 16 | 175.4 ± 3.5 | 76.8 ± 1.2 |
| 25 | Pentyl benzoate | Floral | 1462 | 32 | 32 | 30.6 ± 1.0 | 10.5 ± 0.1 |
| 26 | Hexyl benzoate | Balsamic | 1595 | 16 | 16 | 1903 ± 7.2 | 300.0 ± 11 |
| 27 | α-Sinensal | Citrus | 1707 | nd | 8 | t | 26.7 ± 0.1 |
| 28 | Phenyl ethyl benzoate | Honey | 1840 | nd | 4 | nd | 113.4 ± 6.0 |

*Compounds were identified by comparing their retention indices on DB-5 column, their mass spectra, and their odour notes with that of respective reference compounds.

**Not detectable, Trace (concentration < 10 µg kg⁻¹). Mean ± SD.

*Los compuestos fueron identificados mediante la comparación de sus índices de retención en la columna DB-5, sus espectros de masas y sus notas aromáticas con los de los compuestos de referencia respectivos.

**No detectado, Trazas (concentración < 10 µg kg⁻¹). Media ± DE.
High FD factors ≥ 8 and < 32 were determined for 3-methyl acetate, ethyl acetate, E-2-hexenal, hexan-1-ol, 2-methyl butanoate, benzaldehyde, benzyl alcohol, β-caryophyllene and hexyl benzoate. In addition, high FD factor was determined for α-sinensal in the dried fruit. Results further revealed significant (p < 0.05) changes in the concentrations of aroma-active compounds in the fresh fruit compared to the dried fruit. On the other hand, the dried fruit produced higher concentration for 1-methyl-2-propanol, 1-penten-3-ol, 3-methyl-1-butanol, α-sinensal and phenyl ethyl benzoate.

In order to gain a better understanding of the distribution of aroma compounds in the fresh and dried sapodilla fruits, principal component analysis (PCA) was used. PCA provides a pictorial relationship between the fruits and their aroma-active compounds. This method was used to establish the relationship between the fruits and their aroma-active compounds. Based on the samples grouping from PCA, a partial least square discriminant analysis (PLS-DA) was established (Figure 2A). The scatter plot of scores of the first two components (in the PLS-DA which explained 95.0% of the total variance in the data) revealed distinct differences between the fresh and dried sapodilla fruits. In addition, the PLS-weight plot (Figure 2B) showed how the aroma-active compounds covaried with the fresh and dried fruits.

The fresh sapodilla fruits covaried with 13 aroma-active compounds with most of them exhibiting a floral-like
Table 2. The orthonasal thresholds and odour activity values (OAVs) of compounds with FD ≥ 8 in fresh and dried sapodilla fruits.

| No | Compound                              | Odour quality       | Threshold (μg kg⁻¹ of water) | OAVs of fresh fruit | OAVs of dried fruit |
|----|---------------------------------------|---------------------|------------------------------|---------------------|---------------------|
| 1  | 3-Methyl acetate                      | Nail polish         | 88⁰                          | < 1                 | < 1                 |
| 2  | Ethyl acetate                         | Sweet, fruity       | 5x10⁻⁶                      | < 1                 | < 1                 |
| 3  | 1-Methoxy-2-propanol                  | Ether-like          | NA                          | -                   | -                   |
| 4  | 3-Methyl-1-butanol                    | Malty               | 1x10⁻⁴                      | < 1                 | < 1                 |
| 5  | E-2-Hexenal                           | Fatty, green        | 17⁰                         | 5                   | 6                   |
| 6  | Hexan-1-ol                            | Carrot-like         | 2.5 x10⁻⁶                   | < 1                 | < 1                 |
| 7  | 2-Methyl butanoate                    | Sweet, fruity       | 60⁰                         | < 1                 | < 1                 |
| 8  | Benzaldehyde                          | Almond-like         | 350⁰                        | < 1                 | < 1                 |
| 9  | Benzyl alcohol                        | Aromatic            | 1x10⁻⁴                      | < 1                 | < 1                 |
| 10 | Methyl benzoate                       | Almond, floral      | NA                          | -                   | -                   |
| 11 | 2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone | Caramel, sweet | 43⁰                         | < 1                 | < 1                 |
| 12 | Ethyl benzoate                        | Minty               | 60⁰                         | 13                  | 2                   |
| 13 | β-Caryophyllene                       | Woody               | 64⁰                         | 3                   | 1                   |
| 14 | Pentyl benzoate                       | Floral              | NA                          | -                   | -                   |
| 15 | Hexyl benzoate                        | Sweet, balsamic     | 250⁰                        | < 1                 | 1                   |
| 16 | α-Sinensal                            | Citrusy             | 0.05⁰                       | nd                  | 5340                |

NA: Not available.

OAV: Odour activity values were calculated by dividing the concentration of compounds by their thresholds in water.

The superscript alphabets are odour thresholds as reported in the literature: *Takeoka, Flath, Mon, Teranishi, and Guentert (1990); Belitz et al. (2009); Takeoka et al. (1989); Rychlik, Schieberle, and Grosch (1998).

NA: No disponible.

OAV: Se calcularon los valores de actividad odorífica dividiendo la concentración de los compuestos por sus umbrales en agua.

Las letras en superíndice representan los umbrales aromáticos que se han reportado en la literatura: *Takeoka, Steinhaus, Buettner, and Schieberle (2005); Takeoka, Flath, Mon, Teranishi, and Guentert (1990); Belitz et al. (2009); Takeoka et al. (1989); Rychlik, Schieberle, and Grosch (1998).

Nou. The compounds were; hexyl acetate, cis-linalool, methyl benzoate and pentyl ethyl benzoate. Other compounds, identified were; spicy methyl eugenol, β-caryophyllene, benzyl isothiocyanate, δ-3-carene, 3-methyl acetate, benzenedioli and 2-butenyl benzene. On the other hand, the dried sapodilla fruits covaried mostly with alcohols and aldehydes such as; 1-methoxy-2-propanol, 1-penten-3-ol, 3-methyl-1-butanol, hexan-1-ol, benzyl alcohol, acetaldehyde, E-2-hexenal, heptanal, benzaldehyde and decanal. In addition, other compounds such as ethyl acetate, 2-methyl butanoate, ethyl-4-hydroxymethyl-3(2H)-furanone (EHMF), hexyl benzoate, phenyl ethyl benzoate and α-sinensal were strongly correlated with the dried sapodilla fruits.

Finally, to gain an insight into how these aroma-active compounds impart on the overall aroma quality of the fresh and dried sapodilla fruit, sixteen aroma-active compounds with FD factors ≥ 8 were further investigated (Table 2). Results of the aroma potencies showed that the fresh sapodilla fruit exhibited higher potency for ethyl benzoate (minty), E-2-hexenal (fatty, green), and β-caryophyllene (wood, spicy) as revealed by their high odour activity values (Table 2). However, the dried fruit produced higher potency for α-sinensal (sweet, citrusy), and to a lesser extent E-2-hexenal (fatty, green), ethyl benzoate (minty), β-caryophyllene (woody, spicy), and hexyl benzoate (sweet, balsamic). Interestingly, aroma-active compounds that exhibited high concentrations in the fresh and dried fruit such as methyl benzoate, δ-3-carene and 1-butanol, gave relatively low orthonasal OAVs and their contributions to the overall orthonasal aroma impression of sapodilla fruit can be assumed to be low. Again, with the exception of α-sinensal, both fresh and dried fruits showed more potency for the green note E-2-hexenal and ethyl benzoate. E-2-Hexenal is normally formed as a result of enzymatic activity (lipoxigenase and hydroperoxide lyase) on linoleic and linolenic acids (Bartley & Schwede, 1989). Sapodilla fruit is known to contain an average of 0.5% triglyceride materials (Brito & Narain, 2002), and this probably accounted for the source of E-2-hexenal. Alpha-sinensal a sesquiterpene which was obtained in high amount in the dried sapodilla fruit is assumed to play a major role in the overall aroma of the dried fruit. Alpha-and beta-sinensal have been reported in most citrus fruits (Sharon-Asa et al., 2003) and are known to contribute particularly to the specific sweet orange aroma.

Conclusion

This study has revealed the key aroma-active compounds responsible for the overall orthonasal aroma of fresh and dried sapodilla fruits grown in Malaysia. The results of the sensory studies and OAVs showed distinct differences in the aroma notes of the fresh and dried fruits. Whilst, the fresh fruit exhibited characteristic minty, fatty/green, cum woody notes, the dried fruit produced citrusy, balsamic/sweet nuances. In addition, while the results of the PCA showed that the fresh sapodilla fruits covaried mostly with 13 aroma-active compounds, the dried fruits showed a strong correlation with 19 compounds, respectively. Furthermore, fresh fruit showed higher potency for ethyl benzoate, E-2-hexenal, and β-caryophyllene. However, the dried fruit exhibited very high potency for α-sinensal, and to a lesser extent, E-2-hexenal, ethyl benzoate, β-caryophyllene and hexyl benzoate.

Disclosure statement

No potential conflict of interest was reported by the authors.

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