Investigation of odour-active compounds in grapefruit (*Citrus paradisi*)

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Abstract

Odour-active components of grapefruit essence oil obtained by distillation of grapefruit juice were investigated by aroma extract dilution analysis (AEDA). The results showed a total of 15 components exhibited high flavour dilution (FD) factors in the range of 64-1024. By application of a GC omission test, it was clarified that a woody odorant contributed to the juicy aroma of grapefruit. Subsequently, a multi-dimensional GCMS-Olfactometry (MDGC/MS-O) analysis revealed the woody odorant was mustakone. Enantiomeric distribution of mustakone in grapefruit showed (−)-mustakone was predominant over (+)-mustakone. A GC omission test and a reconstitution test with a mixture of both synthesized enantiomers indicated that mustakone enriched the natural juicy aroma note like original essence oil.

Introduction

Grapefruit (*Citrus paradisi*) has a characteristic odour and its volatile components have been investigated for several decades. So far, over 320 volatile compounds have been identified including intense odorants such as 1-*p*-menthene-8-thiol and 4-mercapto-4-methylpentan-2-one [1]. However, there still remain some unknown odour-active components because the preparation of high quality reconstituted grapefruit flavour like original natural grapefruit aroma is extremely difficult even by blending reported compounds.

Essence oils, obtained by distillation during the concentrated fruit juice production process, are highly concentrated juice aroma. Therefore, they are used as flavour ingredients to enrich the juicy aroma note of foodstuffs and/or beverages. Among them, grapefruit essence oil is one of the most expensive ingredients because the production for processing gradually decreases year by year. Hence, clarification of the remaining odour-active components in grapefruit is very important for us to cope with the essence oil supply shortage and the increase in price.

Omission experiments are enormously effective to evaluate the actual contribution of certain compounds to the overall aroma of the original sample. Recently, we have developed a novel sample preparation method for omission tests using preparative capillary GC [2]. The procedure of our method is: 1) determination of target odorants by GC-O, 2) preparation of original and omitted samples by preparative GC, 3) evaluation of differences of the aroma between the samples by sensory analysis. More details regarding sample preparation, the original sample is a collection of all components of the aroma eluted from preparative GC. The omitted samples are the same collections expect they do not include each target odorant. The preparation is accomplished by preparative GC based only on the retention time range of target odour-active regions. Therefore, this method requires no qualification and quantification. Furthermore, there is little change in the ratio of all components during the experiment.

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The aim of this study is to reveal novel odour contributors in grapefruit essence oil and their effect through omission experiments without complex processes.

Experimental

Materials

Grapefruit essence oil was purchased from Peace River Citrus Products Inc. (Florida, US). Essence oils are obtained by distillation during concentrated juice production processing.

GC-Olfactometry (GC-O) and Aroma Extract Dilution Analysis (AEDA)

GC-O was conducted on a 7890A GC (Agilent, Santa Clara, USA) equipped with a sniffing port (GL Sciences, Inc., Tokyo, Japan) and a BC-Wax column (50 m × 0.25 mm, 0.15 µm, GL Sciences, Inc.). At the end of the separation column, the effluent was split between an FID and a sniffing port (1:10). For AEDA, the sample was diluted stepwise (1:4) with ethanol and each dilution was investigated by GC-O. All analyses were repeated in triplicate by three trained panellists.

Preparative-gas chromatography

Preparative-GC was carried out on a GC-2010 plus equipped with an FID (Shimadzu Corp., Kyoto, Japan) and a BC-Wax column (30 m × 0.53 mm, 1.0 µm, GL Sciences Inc.) as a separation column. The end of column was connected to both a VPS2800 GC fraction collector (GL Sciences, Inc.) and the FID via a splitter. GC conditions: Helium was used as carrier gas at a head pressure of 103 kPa (flow rate, 6.8 mL /min). The splitter make-up pressure set at 80 kPa. The oven temperature was set at 70 °C, ramped at 4 °C/min to 230 °C and held for 20 min. The temperature of the injector and detector were set at 230 °C. The target compounds were collected using the GC fraction collector equipped with traps cooled at −30 °C.

GC omission test

Grapefruit essence oil was divided into 3 fractions using preparative-GC. The odour-active region of the target odorant by GC-O was 32.9 to 33.0 min, therefore, each fraction was collected as below. Fr.1: 0 min to 32.9 min, Fr.2: 32.9 to 33.0 min, Fr.3: 33.0 to 60 min. The recombination with these fractions provided the original sample A (Fr.1 + Fr.2 + Fr.3) and the omitted sample B (Fr.1 + Fr.3). Subsequently, sensory evaluation was applied in order to compare the overall aroma of these samples [2].

Multi-Dimensional GC/MS-O (MDGC/MS-O)

MDGC/MS-O analysis was performed on a MDGC/GCMS-2010 (Shimadzu Corp.). The first GC column was a BC-Wax (30 m × 0.25 mm, 0.15 µm, GL Sciences, Inc.) and the second GC column was an Rxi-5ms (30 m × 0.25 mm, 0.25 µm, Restek Corp., Bellefonte, USA). For chiral analysis, the second GC column was a CP-Chirasil-DEX CB (25 m × 0.25 mm, 0.25 µm, Agilent).

Synthesis of chiral mustakone

(+)- and (−)-mustakone were obtained by oxidation of (+)- and (−)-alpha-copaene, respectively [3].

Preparation of reconstituted sample

The original grapefruit essence oil was subjected to preparative-GC to give Fr.1, Fr.2 and Fr.3. A mixture of Fr.1 and Fr.3 was used as the omitted sample B. The mustakone solution D was a mixture of both authentic enantiomers in the resulted enantiomeric ratio.

A reconstituted sample C was prepared by the addition of a mustakone solution D to an omitted sample B in a concentration of 100 ppm. All samples were diluted to 1% (w/w) with ethanol and then the solutions were diluted to 0.1% (w/w) with water. The aqueous solutions were subjected to sensory evaluation.

Sample A: Fr.1 + Fr.2 + Fr.3 (recombined original sample)

Sample B: Fr.1 + Fr.3 (without the target woody odorant)

Sample C: sample B + sample D (replaced Fr.2 with mustakone)

Sample D: synthetic mustakone solution

Sensory evaluation

All samples were evaluated by trained panellists. The omitted sample B and the reconstituted sample C were evaluated by 10 trained panellists. The panellists were asked to rate given descriptors for each sample on a scale from 0 to 5 in 1 increments, where 0 = very weak and 5 = very strong.

Results and discussion

Investigation of a potent odorant contributing to grapefruit juicy aroma

Odour-active components of grapefruit essence oil were investigated by AEDA. The results showed a total of 14 components exhibited high FD factors in the range of 64-1024 (Table 1). Among them, a woody odorant **13** was focused because the structure could not be identified by conventional GCMS analysis. The GC omission test was carried out to confirm the effect of **13** on overall aroma of grapefruit. By application of the GC omission test, the original sample A and the omitted sample B were prepared using preparative-GC and then a sensory evaluation of overall aroma was performed. The result showed that the typical grapefruit juicy aroma note of the omitted sample B was clearly less intense than that of the original sample A. Thus, the woody odorant **13** should contribute to grapefruit juiciness.

Table 1: Flavour Dilution factors (FD factors ≥ 64) of potent odorants in grapefruit essence oil

			LRI [1]		
No.	odorant	FD factor	exp.	ref [2]	odour description
1	alpha-pinene	256	1045	1043	piney
2	*cis*-3-hexenal	256	1138	1128	green, leafy
3	limonene	256	1218	1207	citrus
4	octanal	1024	1284	1282	peely, citrus
5	nonanal	64	1381	1383	peely, waxy
6	linalool	64	1529	1531	floral, citrus
7	(*E*, *E*)-2,4-decadienal	64	1785	1787	oily, fried
8	*cis*-calamenene	64	1809	1813	spicy, phenolic
9	geraniol	64	1824	1828	floral, citrus
10	*trans*-4,5-epoxy-(*E*)-2-decenal	256	1977	1977	metallic, green
11	*trans*-4,5-epoxy-(E, Z)-2,7-decadienal	256	2032	2034	metallic, green
12	eugenol	64	2140	2146	spicy, clove-like
13	unknown	64	2231	-	woody, peppery, powdery
14	rotundone	256	2244	2248	woody, peppery

[1] Linear Retention indices on BC-Wax calibrated by *n*-alkanes

[2] According to internal database

Identification of the potent odorant

MDGC/MS-O was conducted to determine the structure of **13**. As a result of mass spectrum analysis, the woody odorant **13** was estimated as mustakone. Its mass spectrum, retention time and odour characteristics were in good agreement with those of an authentic mustakone standard. Therefore, the woody odorant **13** was determined to be mustakone. To the best of our knowledge, mustakone was found for the first time in grapefruit. Mustakone has a pair of enantiomers as shown in Figure 1. As a result of chiral analysis by MDGC/MS, the enantiomeric ratio (+) : (−)-mustakone in the grapefruit essence oil was 8.5 : 91.5 (Fig. 1).

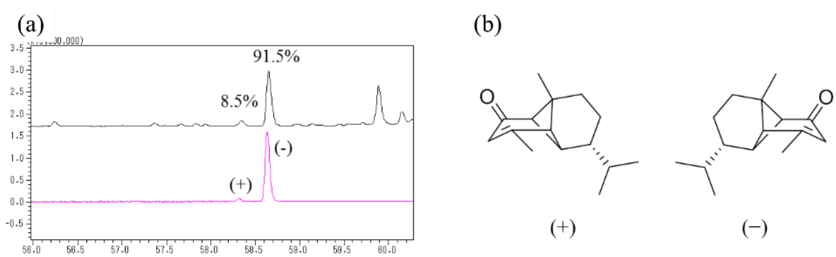

Figure 1: (a) MDGC second dimension chromatograms of grapefruit essence oil (top) and mixture of synthesized mustakone ((+) : (−) = 1 : 9) (bottom). (b) Structures of two enantiomers of mustakone.

Evaluation of the effect of Mustakone on overall aroma

To confirm the effect of mustakone on grapefruit odour, the omitted sample B and the reconstituted sample C were prepared and then evaluated by sensory analysis. The result indicated that “impact”, “voluminous”, “woody” and “sweet” were rated slightly more intense in reconstituted sample C (Fig. 2). For the overall aroma, all sensory panellists judged that mustakone enriched natural juicy aroma note like original essence oil. It was concluded that mustakone was responsible for the juicy aroma of grapefruit.

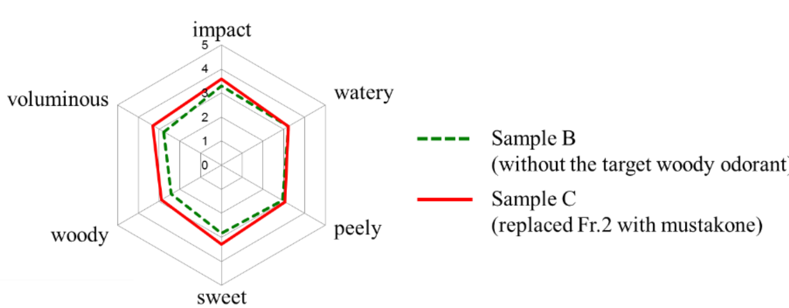

Figure 2: Aroma profiles of grapefruit essence oils omitted the woody odorant and added mustakone to omitted sample.

References

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