Identification of a key odorant contributing to Muscat aroma of Darjeeling black tea

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Abstract

The aroma of Darjeeling black tea was investigated. An unknown odorant reminiscent of Muscat grape was detected with multidimensional gas chromatography–mass spectrometry/olfactometry, and the chemical structure of the unknown component was successfully identified as 4-methylene-2-(2-methylprop-1-enyl)oxane (i.e. dehydrorose oxide or DHRO), which has Muscat-like and floral notes on its own. The present study is the first to detect DHRO as an odorant in Darjeeling black tea. The odour threshold of DHRO was calculated to be 0.20 µg/kg in water, and a stable isotope dilution assay showed that the amount of DHRO in a Darjeeling black tea infusion was 0.028 µg/kg. The odour activity value (OAV) of DHRO in the Darjeeling black tea infusion was 0.14. A sensory evaluation was conducted to obtain the orthonasal aroma profiles of black tea aroma model solutions and revealed the effect of DHRO in Darjeeling black tea. DHRO was found to contribute to the characteristic attributes such as Muscat-like, floral, fruity and green aroma of Darjeeling black tea, although the OAV was less than 1.

Keywords: Darjeeling black tea, 4-methylene-2-(2-methylprop-1-enyl)oxane, stable isotope dilution assay, Muscat aroma, sensory evaluation

Introduction

Darjeeling black tea is known for a unique aroma reminiscent of the Muscat grape. Several studies have focused on the aroma of black tea [1-4], however, it remains difficult to recombinate this characteristic aroma with known odorants. Therefore, there may be unknown odorants that contribute to this aroma. This work aimed to determine and evaluate the key odorants that contribute to the Muscat-like aroma of Darjeeling black tea.

Experimental

Darjeeling black tea sample

Darjeeling black tea (Goomtee Muscatel Valley, FTGFOP1, 2016-DJ19) was purchased from a local shop (Lupicia, Tokyo, Japan).

Preparation of the volatile concentrate of Darjeeling black tea

Boiling hot water (500 mL) was added to the tea leaves (10 g), and the mixture was allowed to stand still for 3 min. After extraction, the leaves were separated with a strainer, and the infusion was immediately cooled to approximately 25 °C in an ice bath. The obtained infusion (420 g) was distilled at 40 °C using the solvent assisted flavour evaporation (SAFE) method [5]. Sodium chloride (63 g) was added to the distillate, which was then extracted with diethyl ether (1 × 60 mL, 2 × 30 mL) followed by dichloromethane (1 × 60 mL, 2 × 30 mL). The organic phases were individually dried over anhydrous sodium sulphate. The dichloromethane solution was first concentrated to approximately 1 mL by distilling it off the solvent at 48 °C; then, it was added to the diethyl ether solution. The combined organic phase was concentrated at 43 °C to yield 2.3 mg of the volatile concentrate.

Identification of an unknown odorant in Darjeeling black tea

Gas chromatography–mass spectrometry/olfactometry (GC-MS/O) and multidimensional (MD) GC-MS/O were applied to identify the volatile compounds in the concentrate. MDGC-MS/O was also used to analyse DHRO, which was synthesised in our laboratory.

Quantification of DHRO in the Darjeeling black tea infusion

A stable isotope dilution assay (SIDA) was conducted by preparing 0.5 mL of a diluted solution of [d_6]-DHRO in dichloromethane and adding it to 200 g of the Darjeeling black tea infusion. The tea infusion was stirred for 10 min. Then, the volatile concentrate was prepared with the SAFE method and solvent extraction as described

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above. The calibration curve obtained for the standard solution with MDGC-MS/O was used to quantify DHRO in the Darjeeling black tea infusion.

Sensory evaluation

Sensory evaluation was conducted using a descriptive analysis to elucidate the effect of DHRO in aroma recombinant of Darjeeling black tea. A recombinant model solution was prepared by mixing the key odorants of Darjeeling black tea at quantified values according to published data [3]. The solution was orthonasally compared with another solution to which was added the model solution with DHRO at a concentration quantified via SIDA.

Results and discussion

Detection of the Muscat-like aroma from a volatile concentrate of Darjeeling black tea

A volatile concentrate of Darjeeling black tea infusion was prepared by the SAFE method and solvent extraction using diethyl ether and dichloromethane. GC-MS/O was applied to the concentrate. A characteristic Muscat-like aroma was successfully detected at a retention index (RI) of 1390 on a polar column. However, the mass spectrum of the target odorant was not observed.

Elucidation of the Muscat-like aroma component

MDGC-MS/O was used to elucidate the chemical structure of the unknown component with the Muscat-like aroma. At the retention time when the Muscat-like aroma was detected in the first polar column, the effluent was introduced to the second non-polar column and sniffed. The mass spectrum of the unknown component was obtained successfully in the second column. However, this mass spectrum did not match the data from the libraries. Hence, the chemical structure of the unknown component needed to be estimated from analytical data. The RI, odour quality and mass spectrum of *trans*-rose oxide (*t*RO) were conducive to revealing the chemical structure of the unknown component. The RIs of both columns and the odour quality of the unknown component were quite similar to those of *t*RO (Table 1). Thus, the chemical structure of the unknown component was concluded to resemble that of *t*RO. Additionally, when the mass spectra of the two components were compared, the molecular mass (*m/z* 152) of the unknown component was less than that of *t*RO by 2 (Figure 1). The same applied to *m/z* 137 of the unknown component. Thus, the chemical structure of the unknown component was proposed to be dehydrogenated *t*RO.

Table 1: Comparison between the RIs and odour qualities of the two components.

	RI		odour quality
	polar column	non-polar column	
unknown component	1390	1096	Muscat-like, floral
trans-rose oxide (*t*RO)	1372	1117	Muscat-like, floral

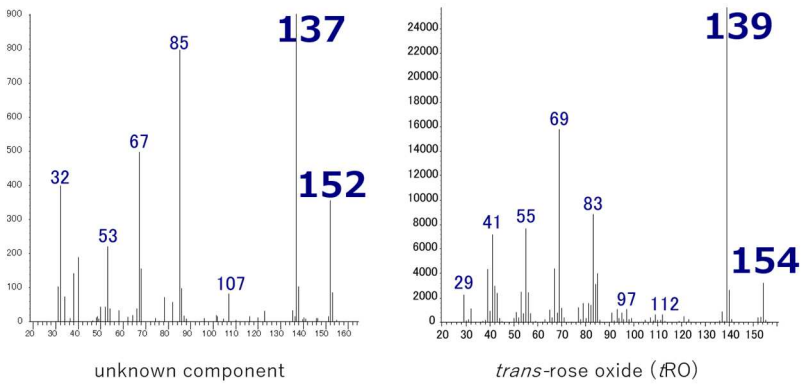
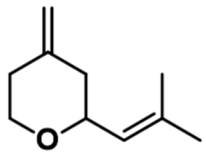


Figure 1*: Comparison between mass spectra of the two components.*

The candidate compounds were synthesised and analysed. Among the compounds, the RI, mass spectrum and odour quality of DHRO (Figure 2) showed good agreement with those of the unknown component detected from the volatile concentrate of the Darjeeling black tea infusion.

DHRO

Brunke *et al.* detected DHRO as one of the volatile components in *Achillea wilhelmsii*, although they did not refer to its aroma [6]. Königsmann *et al.* filed a patent regarding the utilisation of DHRO as a synthetic intermediate of rose oxide [7]. Ohloff *et al.* described the odour qualities of its enantiomers as floral notes [8]. However, the detection of DHRO in Darjeeling black tea is novel. When orthonasal recognition test was conducted, the odour threshold of DHRO was 0.20 µg/kg in water, and the panellists described the aroma as Muscat-like, floral, lychee-like, rosy and green.

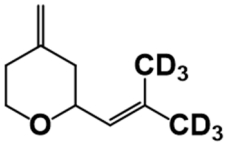


DHRO

Figure 2*: Chemical structure of the Muscat-like odorant newly identified in the Darjeeling black tea infusion.*

Quantification of DHRO by SIDA

SIDA was conducted to quantify the amount of DHRO in the Darjeeling black tea infusion. Figure 3 shows 4-methylene-2-(2-(methyl-d_3)-prop-1-enyl-*3,3,3-*d_3)oxane ([d_6]-DHRO), which was synthesised as the stable isotope internal standard. The concentration of DHRO in the tea infusion was found to be 0.028 µg/kg. The odour activity value (OAV) was calculated as 0.14 from the concentration and odour threshold.



[d_6]-DHRO

Figure 3*: Chemical structure of [*d_6*]-DHRO.*

Sensory evaluation

Although the OAV suggests that DHRO does not contribute to the aroma of Darjeeling black tea, the aroma characteristics of DHRO were extremely interesting because they were similar to the unique Muscat-like aroma of Darjeeling black tea. Additionally, the effect of DHRO in Darjeeling black tea was unknown because it was the odorant identified in the Darjeeling black tea infusion for the first time. Hence, a sensory evaluation was conducted to obtain the orthonasal aroma profiles of two black tea aroma model solutions. Twelve panellists were asked to examine the effect of DHRO in Darjeeling black tea via descriptive analysis. Figure 4 shows the aroma profiles of the two model solutions. DHRO was confirmed to contribute to the aroma of Darjeeling black tea because it enhanced the Muscat-like, floral, fruity and green aroma of Darjeeling black tea, although the amount of DHRO in the infusion was less than the odour threshold.

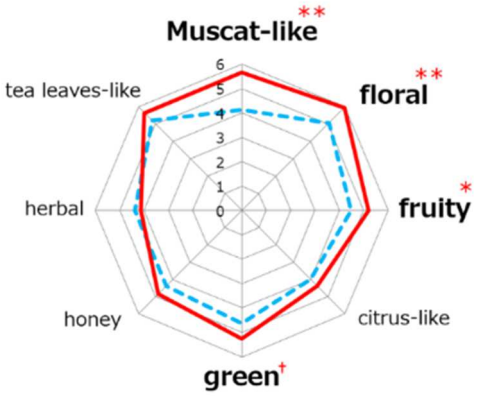


Figure 4: *Orthonasal aroma profiles of the recombinant model solution (dotted line) and the solution with added DHRO at the quantified amount (solid line); n = 12, t-test, **p < 0.01, *p < 0.05, †p < 0.1.*

Conclusion

To elucidate the unknown odorant contributing to the Muscat-like aroma in Darjeeling black tea, a volatile concentrate was prepared and analysed with MDGC-MS/O. DHRO, which has a Muscat-like aroma on its own, was identified in the Darjeeling black tea infusion. This is the first study to detect DHRO in Darjeeling black tea. A sensory evaluation showed that DHRO contributed to the characteristic attributes of Darjeeling black tea, although the OAV of DHRO in the tea infusion was less than 1. Our further examination detected DHRO in other foods such as coffee, lychees and hops (data not shown). It is thought that DHRO widely exists in natural products and may affect their aroma.

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