The Quantitative Analysis of 3-Methylnonane-2,4-dione in Green Teas.

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Summary

3-Methylnonane-2,4-dione (3MND) has been found from green tea in 2002, it has been reported that it gives green tea leafy feeling of richness. Until now, roles of 3MND focusing as a contributor to Gyokuro tea flavor have not been studied. The detailed studies about the aging of Gyokuro are also not performed.
Therefore, we tried to quantify 3MND in various commercially available green tea and the change during storage. As a result, 3MND is contained in any types of green tea such as “gyokuro”, “sen-cha” and “houji-cha” with almost the same amount. It was observed that the amount of 3MND in green tea was increasing during preservation, and that the increasing rate depended on the condition of storage. It is known that 3MND is generated from fatty acids through the auto-oxidation in tea products, the amount of 3MND is affected by oxygen and the storage temperature. In this study, the amount of the compound in the green tea stored in air-contained package under room temperature increased faster than in the other condition, but after three-month storage, the amount of it decreased. It shows that 3MND might have degraded into other compounds by further oxidation.

Introduction

3-Methylnonane-2,4-dione (3MND) has been found from green tea by Kumazawa and Masuda (2002), it has been reported that it gives green tea leafy feeling of richness. 3MND has been also reported as one of the most important flavor compound that contribute to green tea aroma in Hattori *et al.* (2005), Minakami and Matsunaga (2013). Despite of the sensory character of 3MND with rich green, roles of 3MND focusing as a contributor to covered tea (Gyokuro, Ten-cha (Maccha) and Kabuse-cha) flavor have not been studied until now in contrast to dimethyl sulfide and β-ionone (Kawakami (2000)). During a half year aging after tea production, Gyokuro and Ten-cha lose strong green note and take change into mild flavor. But the studies about the aging of Gyokuro and Ten-cha are also not performed except Kinoshita (2005). Therefore, we tried to quantify 3MND in various commercially available tea such as Sen-cha, covered tea, Houji-cha and so on. The changes of 3NMD in Sen-cha and covered tea during storage were also investigated.

Materials and methods

Sample; Standard 3-Methylnonane-2,4-dione (3MND) was prepared in our company using the method of Kato and Yuasa (2001). Commercially available teas were purchased from tea shops in 2012 or 2013 in table 1. A covered Yabukita green tea produced in Mie prefecture was prepared and used for quantifying of 3MND after keeping in three different conditions for six months in 2012 (Table 2). The change of amount of 3MND in green tea during storage was observed using Yabukita Sen-cha produced in Shizuoka prefecture in 2012 (Table 3). At start time, after two weeks, one month, three months and six months 3MND in the Sen-cha stored in three conditions were

Table 1. Commercially available teas purchased from tea shops

Sample	Variety	Production area	Price(100g)	Purchase
A	Sen-cha	Kyoto	¥3,150	2012
B	Sen-cha	Kyoto	¥1,000	2012
C	Kabuse	Kyoto	¥1,000	2012
D	Kabuse	Kyoto	¥800	2012
E	Gyokuro	Kyoto	¥1,500	2012
F	Houji	Kyoto	¥700	2012
G	Kamairi	Japan	¥800	2012
H	Maccha	Kyoto	¥20,000	2012
I	grind Sen-cha	Sayama	¥500	2013

quantified by the following method.

Table 2. Covered green tea produced in Mie prefecture (2012 crop)

Sample name	Strage condition	Measurement piriod
Contol	4 °C, under N_2, packed and sealed in an alminium bag	6 months
Aged	4 °C, with air, packed and sealed in an alminium bag	6 months
Oxdated	25 °C ± 2 °C, with air, in a small tea strage container	6 months

Table 3. Yabukita Sen-cha produced in Shizuoka prefecture (2012 crop)

Sample name	Strage condition	Measurement piriod
freezing	-20 °C, under N_2, packed in an alminium bag	0 w, 2 w, 1 m, 3 m, 6 m
RT N_2	25 °C ± 2 °C, under N_2, packed in an alminium bag	0 w, 2 w, 1 m, 3 m, 6 m
RT air	25 °C ± 2 °C, with air, packed in an alminium bag	0 w, 2 w, 1 m, 3 m, 6 m

w: week, m: month

Standard addition method for quantification of 3MND using HS-SPME-GC/MS (Headspace - Solid Phase Micro Extraction – Gas Chromatography / Mass Spectrometry); 30 g of the hot water (60 °C) was added to 2 g of the tea leaves. After 10 minutes, the leaves were removed by a tea strainer. The infusion was immediately cooled to 25 ºC ± 5 ºC in an ice bath. 4 g of sodium chloride was added to 10 g infusion then stirred well. 2 g of the infusion was put into a 22 mL headspace vial and added 20 µL of standard 3NMD ethanol solution (0.05 mg/mL, 0.1 mg/mL) or blank ethanol. Volatile compounds in the vial were collected on SPME fiber (PDMS/DVB/Carboxen) for 30 minutes after 30 minutes equilibration at 60 °C. The volatile compounds were flushed in GC injector then were analyzed by GC/MS using selective ion monitoring (SIM) mode. Calibration curves were made by the peak areas of 3MND in the standard addition samples. 3MND in the infusion was quantified from the peak area of blank sample. Amount of 3MND in tea leaves were calibrated from the values of 3MND in the infusions. Three times repeat experiment was done for one sample and RSD was 8-20%. R^2 value of the calibration curves were 0.97-1.00.

Instrumental condition; 7890A GC with 5975C MSD (Agilent) equipped with BC-WAX (50 m x 0.25 mmI.D. x 0.15 µmdf, GL Sciences Inc.) was used for all experiment. The initial column head pressure was 15.6psi for constant pressure mode with splitless. The oven program started at 40 °C (3 min) to 220 °C finally at 4 °C/min. Ions of *m/z*43, 71, 72, 99 and 170 were collected with EI (70eV) for SIM detection of 3MND.

Results and discussion

1. Quantification of 3NMD in various commercially available teas; Figure 1 shows the results of the quantification of 3NMD in various commercially available teas. X axis for price (yen/100 g) is expressed in logarithmic. As a result, 3MND is contained in all samples from 0.02-0.042 µg/g in tea leaves. Sen-cha contained almost same amount of 3MND as Kabuse-cha and Maccha. Goyokuro had more 3MND than Kabuse-cha and Sen-cha, but Houji-cha had more 3MND than Gyokuro. The

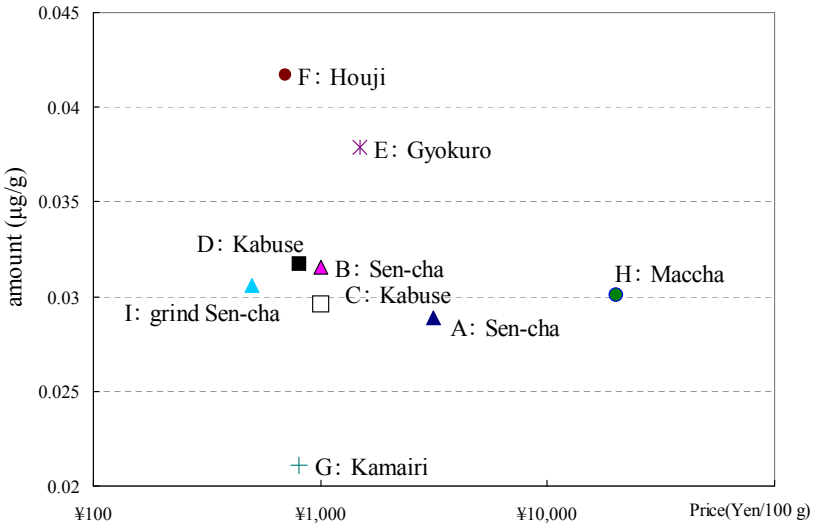

Figure 1. Amount of 3MND (µg/g tea leaves) in various comarcially available teas

correlation between green tea variety and 3MND, tea price and 3MND were not clarified in this experiment.

2. Quantification of 3MND in covered tea; Figure 2 shows the results of quantification of 3NMD in a same lot covered tea kept in three different conditions for six months. The error bars show standard deviation of three times repeat. Depending on the storage condition, the amount of 3NMD has varied. The amount of 3NMD in "Oxidized" is highest in three samples. This result suggests that 3MND increasing rate depends on existence of oxygen and temperature.

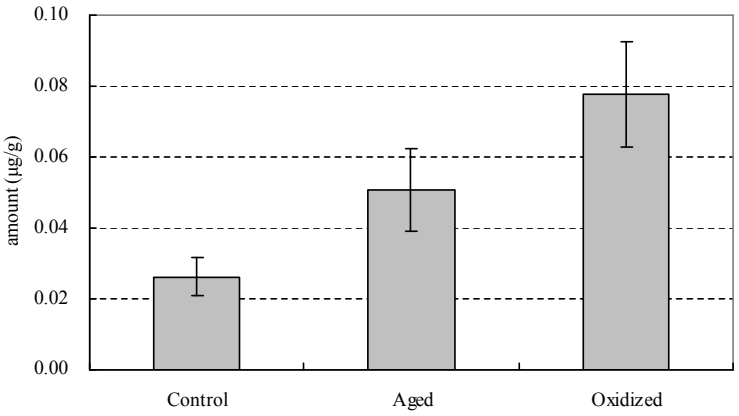

Figure 2. Quantification of 3MND in covered tea after 6 months(μg/g)

In a sensory test, 20 g of hot water (90 °C) was put on 2 g of tea leaves in a cup, and then two panelists smelled and tasted the infusion of the tea. "Control" showed a strong green note of C6 aldehydes and dimethyl sulfide like fresh green tea. "Aged" had a less fresh green smell and had a mild green note. "Oxidized" gave an oxidatively degraded tea smell like autumn leaves. It is shown that 3MND has a possibility to measure aging level of covered tea.

3. The change of amount of 3MND; The change of 3MND during storage in Yabukita Sen-cha is shown in figure 3. The error bars show standard deviation of three times repeat. It was observed that the amount of 3MND in "RT air" was significantly increasing during preservation. 3MND in the other teas did not increase or slightly increased. The amount of 3MND in "RT air" increased faster than in the other condition, but after three-month storage, the amount of it decreased. It is known that 3MND is produced from oxidation of fatty acid (Guth and Grosch (1989)). These results show that 3MND might have degraded into other compounds by further oxidation.

In sensory test, the same samples using for experiment of 3MND quantity were used and one panelist smelled and tasted the infusions. After one month "RT N_2" and "RT air" showed mild green note. After three month, "RT air" had autumn leave note. After six month, "freezing" showed fresh green and "RT N_2" had a mild green note, in contrast to "RT air" indicated smell of deteriorated tea.

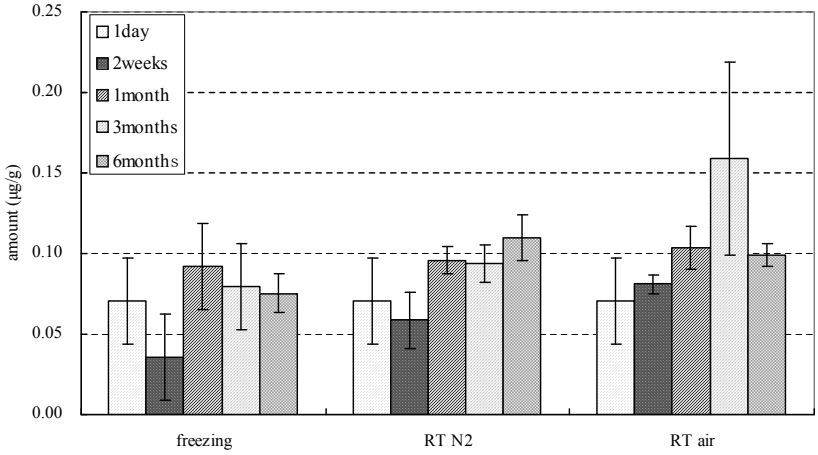

Figure 3.The change of 3MND during storage inYabukita Sen-cha(μg/g)

The amounts of 3MND in Sen-cha after preservation were more than the amount of it in covered green teas in experimental 1 and 2. According to this result, 3MND is a common compound for all types of green teas and the amount of it depends on existence of oxygen and temperature.

Conclusion

3MND is one of the contributor to green tea note, but in terms of quantities of it in many types of green teas,

3MND is NOT a specific compound in covered tea such as Gyokuro, Maccha and Kabuse-cha. 3MND in covered tea and Sen-cha increase during preservation and the speed of increasing might depend on condition of preservation. In this study, it is shown that 3MND has a possibility to measure aging level of green tea. Especially as covered teas such as Gyokuro and Ten-cha are usually take six months for aging, if using 3MND as an index of aging of covered tea, it might be possible to investigate best condition and time for aging. The studies about the aging of covered tea are not enough for clarifying mysterious change of their aroma character. Further experiments are required for changing green tea aroma during aging.

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