Unilever Research and Development

Quantification of MMP in green tea infusions using MMSE comprehensive GC*GC-TOF MS

Ed Rosing, Lionel Jublot, Jeroen Klooster and Max Batenburg

Unilever R&D, Olivier van Noortlaan 120, 3133 AT Vlaardingen, the Netherlands

Corresponding e-mail: ed.rosing@unilever.com

Introduction

4-mercapto-4-methyl-2-pentanone (MMP) is reported as one of the most characteristic odour compounds in Japanese (steamed) green tea[1]. MMP has a very low odour threshold (0.8 ng/kg in water) with an odour described as black currant at low concentration, catty at higher concentration.

In order to determine the influence of manufacturing conditions on its formation, it is essential to be able to quantify this compound in green tea infusions at sub ppt level.

A combination of stable-isotope labeled internal standard with Monolithic Material Sorptive Extraction (MMSE) and Comprehensive Two-Dimensional Gas Chromatography - Time of-Flight Mass Spectrometry (GC*GC-TOF MS) was used to quantify MMP in green tea infusions.

Experimental

In this work, Monolithic Material Sorptive Extraction (MMSE) was used (a solvent free extraction technique, commercially available under the name Monotrap ™ by GL Sciences). The desorption of the Monotraps was done via a Gerstel TDU/CIS-4 thermal desorption inlet coupled to a LECO Pegasus 4D Comprehensive Two-Dimensional Gas Chromatograph–Time of-Flight Mass Spectrometer (GCxGC-TOF MS) for separation and detection. Deuterium labeled MMP (d10-MMP, supplied by AromaLab) was used as internal standard.

Infusion preparation : 1 g leaf tea /100 ml boiling mineral water (Spa Reine), 2 minutes infusion time in a closed bottle.

Monotrap extraction: 9.0 gram infusion (addition of 20 % NaCl w/w) spiked with 8 ng/kg d10-MMP in a 10 ml headspace vial, 3 hr extraction time at room temperature with Monotrap (RGC18 TD), 20 rpm shaking

TDU: solvent vent mode, initial temp. program: 35°C, ramp rate 180°C/min till 200°C (10 min).

CIS-4: solvent vent mode, initial temp -150°C, ramp rate 12°C/sec till 250°C (5 min).

GCxGC conditions: 1st dim column Rxi-5MS 30 m x 0.25 mm x 1.0 µm, 2nd dim column Rtx-Wax 1.25 m x 0.10 mm x 0.1µm, 1st dim oven temp program: 40°C (2 min)- 3 °C/min -120°C – 15°C/min – 230°C (+5 °C offset 2nd dim oven), modulation time: 3 seconds; flow: 1.0 mL/min.

MS conditions: transfer line temperature 250°C, EI (70eV), data acquisition rate 150 spectra/sec, data acquisition range m/z 33 to 250.

Results, Identification

A typical GC*GC contour plot of a Sencha green tea infusion is shown in figure 1. M/z 43, the most abundant ion in the EI spectrum of MMP, was selected to simplify the contour plot. The obtained deconvoluted MS spectrum and the library spectrum are shown in the right top corner, an excellent similarity factor (92%) was obtained. Figure 2 shows the selected ion chromatogram of m/z 132 (parent and unique ion of MMP, used for quantification). In 1D GC-TOF this ion still showed co-elution and MMP could not be detected. The separation power and the extra sensitivity of GC*GC TOF was required to achieve the level of performance desired for the quantification of MMP in green tea infusions.

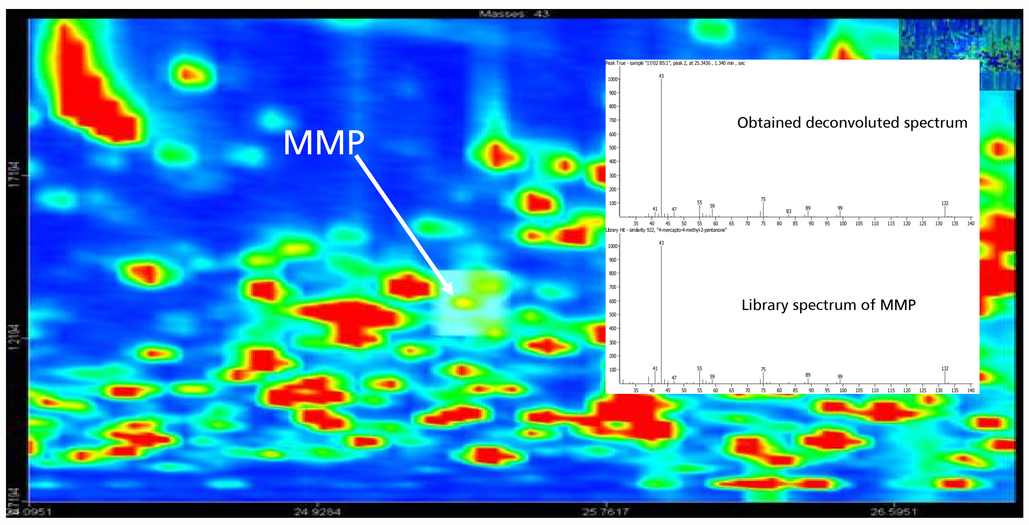

Figure 1: Detailed contour plot (m/z/ 43) of the MMP region of a Sencha green tea infusion.

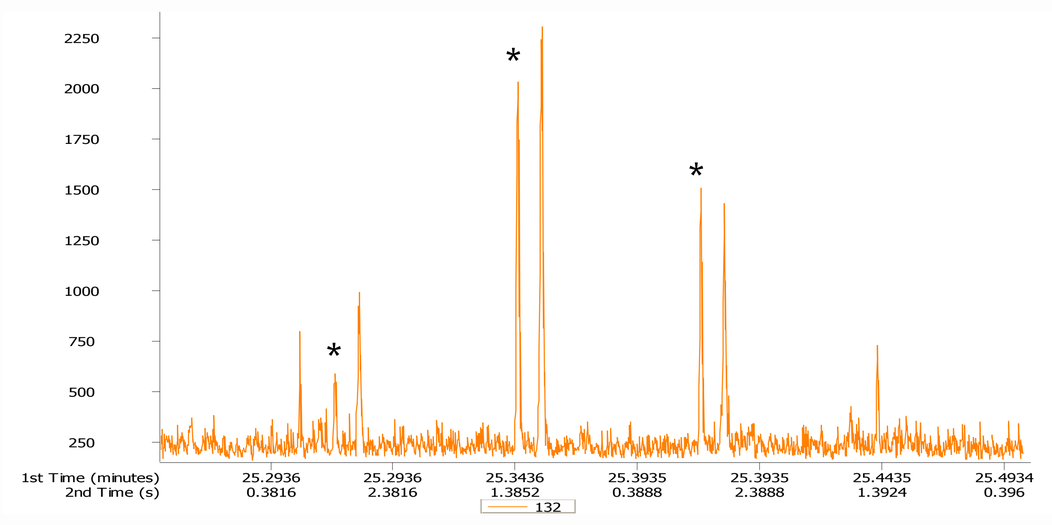

Figure 2: Selected ion chromatogram of MMP (m/z 132) in a Sencha green tea infusion, three modulations are shown and MMP is indicated with an *. The concentration of MMP in this sample was 0.6 ng/kg.

Results, Quantification

Quantification was carried out by adding d10-MMP to the sample as an internal standard. The linearity of the method was evaluated by adding known amounts of MMP to a green tea sample. The obtained curve (after subtraction of the base concentration) is shown in figure 3. From the calibration line the Limit Of Quantification (LOQ) was estimated to be 0.2 ng/kg (ppt), which is below its reported odour threshold.

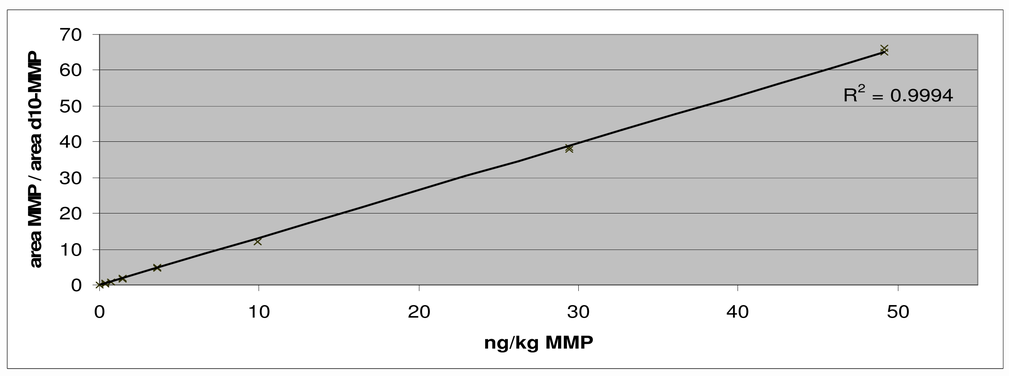

Figure 3: Calibration line for MMP with the use of 8 ng/kg d10-MMP.

The combination of stable-isotope labeled internal standard with Monotrap – GC*GC-TOF MS was applied to quantify MMP in green tea infusions from various origin. Results are presented in figure 4.

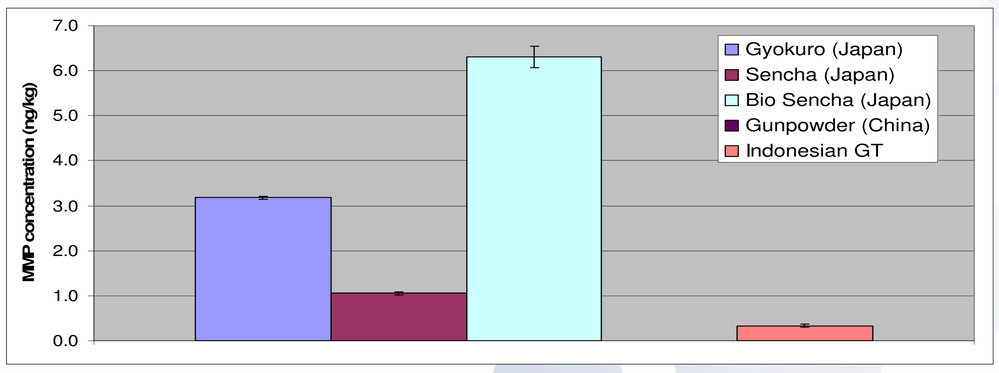

Figure 4 : MMP concentration in various green tea infusions analysed using the proposed method.

Conclusion

The combination of Monotrap with GC*GC-TOF MS enabled to quantify MMP at sub-ppt level in green tea infusions. Such analytical could then be applied to study impact of manufacturing condition on MMP formation.

[1]K. Kumazawa, Flavor Chemistry of Tea and Coffee Drinks, Food Sci. Technol. Res., 12 (2), 71-84, 2006