Evaluation and optimization of sample preparation techniques towards the regional differentiation of Chinese green teas

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Abstract

Aroma profiles of 18 green tea samples from Guizhou province were investigated and compared to teas from other Chinese regions.

Several preparation techniques like Static (SHS) and Dynamic Headspace (DHS), Solid-Phase Microextraction (SPME), Stir-Bar Sorptive Extraction (SBSE) and Solvent Assisted Flavour Evaporation (SAFE) were performed to compare their efficiency and applicability. All methods were measured with gas chromatography coupled to mass spectrometry (GC-MS).

HS-SPME proved to be the most efficient method regarding enrichment of the analytes, robustness, flexibility, ease of handling, and in economic regards. The optimized method identified 43 volatile organic compounds (VOC). Subsequently, all green teas were classified on the basis of these VOC into their provinces. Here, Partial Least Squares Discriminant Analysis (PLS-DA) achieved a separation of teas of the provinces Anhui and Guizhou, whereas the VOC profiles of teas from the remaining provinces overlap with others.

Introduction

Tea contains substantial amounts of polyphenols, caffeine, volatile oils, vitamins, aroma-forming substances and other compounds that have unique biological activities and health benefits [1].

The flavour of green tea is characterized by up to 300 volatile compounds, of which only eight are responsible for the formation of the distinct green tea aroma [2].

Green tea is mainly consumed in Asia. The overall market in 2016 for Chinese tea was about 37.5 billion Euro. Especially high-quality green teas from special regions like the Guizhou province are of interest. The price per kilogram tea can vary from ten to several thousand euros. The increasing demand for those teas, however, misleads certain producers to sell their low-quality products as high-quality ones from specific regions. As a result, a large number of adulterations have already been registered on the markets. In order to protect consumers from adulteration and food fraud, instrumental analysis is necessarily required to clearly indicate distinctions between the tea aroma profiles of specialty goods and inferior ones. Due to its popularity, the characterization of green tea aroma compounds is widely spread, but does not address the situation mentioned above. Essentially, a method investigating the regional origin may also consider VOC other than key food odorants.

Experimental

All green teas were high quality teas from special Chinese regions. 18 green teas derive from Guizhou, six from Anhui, two of each from Henan and Zhejiang and one from Jiangsu.

GC settings: Column: Rxi-5ms and Rtx-624 (Restek GmbH, Bad Homburg, Germany) 60 m x 250 µm x 0,25 µm; oven: initial 40°C, hold 4 min; 5°C/min to 100°C; 2°C/min to 138°C; 5°C/min to 210°C, hold 2 min; 15°C/min to 230°C, hold 16 min; carrier gas: Helium, constant flow = 1 mL/min; aquisition mode: Scan mode, 35-500 amu; GC system: Agilent 7890 (Waldbronn, Germany); MS/MS system: MS Triple Quad 7000 C Agilent.

For the following analyses, an amount of 0.3 g green tea was ground with liquid nitrogen and poured with 8 mL distilled water at 80°C. For direct SPME and SBSE a filtration step was included. At SHS analysis the sample was incubated for one minute at 80°C in the incubator oven of the autosampler (MultiPurposeSampler MPS2, Gerstel, Mülheim a.d. Ruhr, Germany). After that an aliquot of 600 µL was injected into the GC injector.

In the course of DHS (Automated Dynamic Headspace, Gerstel), the sample was incubated for one minute at 45°C. Soon afterwards the headspace was flushed with 100 mL nitrogen (flow of 40 mL/min) and subsequently injected onto a cryogenic trap cooled with nitrogen at –120°C (KAS 4, Gerstel), heated and injected onto a Rtx-624 in an Agilent 6890 GC.

In SBSE (coupled to a Thermal Desorption Unit TDU, Gerstel) and SPME (automated SPME using the Gerstel MPS2) analysis the analytes were incubated for 1 min at 80°C and enriched on a PDMS-based Twister® SBSE respectively a 50/30 µm DVB/Carboxen/PDMS fibre and analysed on a Rtx-624 as well.

SAFE was performed at 10^{-5} mbar. Before analysis, the apparatus was set thermostatically at 40°C. 2 g with liquid nitrogen grounded tea were poured with 50 mL of 80°C hot distilled water and stirred for 15 min. 40 mL of the sample was then added to the SAFE apparatus by decantation. After 30 min distillation time the distillate was extracted twice with 15 mL diethyl ether and the ether phases were combined. 1 µL of the ether extract was injected directly for GC-MS analysis.

0.4 g cryo-ground green tea were analysed without infusion. The volatiles were enriched in 20 mL SPME vials by HS-SPME after which GC-MS was performed. For SPME analysis a 50/30 µm DVB/Carboxene/PDMS fibre (2 cm) was used and the analysis was performed with the GERSTEL-Multi Purpose Sampler MPS2. The incubation of the samples took place at 70°C for 40 min. After that the SPME fibre was exposed into the sample's headspace for 40 min extraction at 70°C. A temperature programmed transfer of the analytes was performed in the GC-injector. To include very volatile compounds as well, the initial GC oven temperature was set on 40°C.

Results and discussion

HS-SPME analysis was preferred over the tested methods direct SPME, direct SBSE, DHS, SHS and SAFE. In a direct comparison, higher signal intensities and a wider range of analytes have been achieved by HS-SPME [5]. SHS merely turns out as a screening method for the analysis of very volatile compounds (e. g. 2-methybutanal or 3-methylbutanal). Later eluting compounds as well as typical terpenoid components of green tea like linalool or geraniol were not covered by this method. DHS, on the other hand, should be able to analyse a wider range of substances and be able to achieve higher

signal intensities as it includes concentration steps like purging and trapping. Due to this the equilibrium between liquid and gas phase is constantly resetting. This ensures that even substances with low volatility and low concentrations can be detected. This method, however, turns out as not suitable for the characterisation of green tea under the given sample preparation conditions. Aqueous samples like tea infusions were rather problematic for DHS analysis. The difficulty is that condensed water may block the GC injector by freezing out. To minimize this effect, DHS analysis was performed at a low incubation temperature of 45°C, which results in a small spectrum of VOC extracted from green tea. According to the results, DHS performance was limited in the case of aqueous samples and was thus not suitable for tea analysis. Additionally, DHS was not preferred over HS-SPME due to economic aspects.

When comparing SPME and SBSE, HS-SPME proves to be preferred over direct SPME and direct SBSE. Compared to the first, HS-SPME is the more robust method, because in direct analysis, the tea infusion had to be filtered before starting the analysis in order to protect the fibre from contamination. In consequence, very volatile compounds escaped during the filtration process. On the contrary, HS-SPME analysis allowed a direct investigation in the vial immediately after tea infusion, so that these losses did not occur. Similar to direct SPME, the deficit of very volatile substances appeared in direct SBSE as well.

Another advantage of HS-SPME is the triple coated fibre that guarantees a wider range of aroma compounds of different polarities. PDMS-Twister® are selective for non-polar compounds, while polar compounds are less effectively analysed. In total, only 8 green tea components were determined by direct SBSE analysis, while 43 substances were analysed by the HS-SPME method [5]. Polar compounds such as volatile esters and alcohols can be determined more efficient by magnetic stirrers coated with ethylene glycol and polyacrylate. On the other hand, extraction deficits would result due to polar materials regarding non-polar substances, so that there should be no advantage over the triple-coated SPME fibre. Additionally, the analysis of several sorption phases with different polarities are connected with extra efforts and a longer analysis time which lead to economic disadvantages.

SAFE was also carried out in comparison with the automated methods. This application, however, was not suitable due to the low analyte concentrations in those green teas.

Using HS-SPME-GC-MS analysis and PLS-DA, it can be stated that round 83% investigated green teas are well classified. Especially samples from Guizhou and Anhui 100% separated from each other. The two samples from Zhejjang have similar aroma profiles like samples from Anhui and Guizhou. In the same way, the aroma profile of green teas from Jiangsu and Henan overlap with these from Guizhou (cp. figure 1).

In general, this data analysis shows that a classification of the green teas by means of their volatiles profile into their provinces is possible. General conclusions for specific provinces can hardly be stated. A broader analysis with equal sample numbers of each province are required to draw general and valid statements. The statistical power of this study is limited, since only 29 teas with varying shares of regions were analysed. For example, Jiangsu province was only represented by one green tea sample (T19).
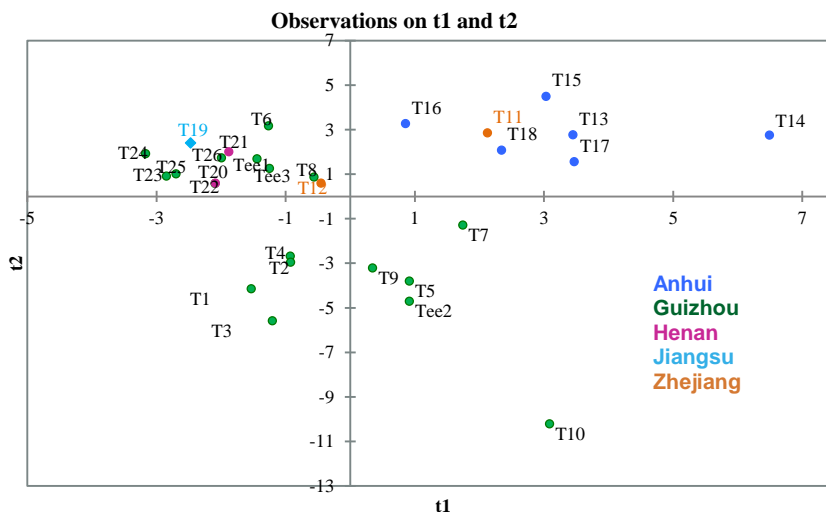
Observations on t1 and t2

Figure 1: PLS-DA plot of 29 green teas of five different provinces; based on 43 identified VOC

Future studies, should therefore contain a variety of green teas from different regions. It would be advantageous if the same numbers of green tea were examined in each province, to assure even weighting of the samples. In parallel, a trained sensory panel should accompany the study and establish relations to influencing factors.

Further work should be carried out in the context of organic teas. By statistical analysis the organic tea of this study (Tea 10) was significantly distinguished from all other 28 teas (s. figure 1). To confirm whether such teas and why are different from conventional teas, a larger number of organic teas should be included in the statistics.

Finally, the analysis of low-volatility components can as well be performed by liquid chromatography coupled to mass spectrometry, since e.g. flavonoids are important constituents with varying and potentially fingerprint concentrations in green tea.

References

1. Sereshti H., Samadi S., and Jalali-Heravi M. (2013) J. of Chromatogr. A, 1280 pp. 1-8
2. Dunkel A., Steinhaus M., Kotthoff M., Nowak B., Krautwurst D., Schieberle P., and Hofmann T. (2014) Angewandte Chemie. International edition 53, pp. 7124-7143
3. Huo D., Wu Y., Yang M., Fa H., Luo X., and Hou C. (2014) Food Chem 145, pp. 639-45
4. Ye N., Zhang L., and Gu X. (2011) Anal Sci. 27, pp. 765-769
5. Ho T. T. (2017) Master Thesis: Evaluierung von Probenvorbereitungsmethoden und Optimierung der Aromaanalytik mit Hilfe von GC-MS zur Herkunftsbestimmung von chinesischem Grüntee, Lebensmittelchemie, University Wuppertal, Germany (in German).