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Original Article

Determination of volatile compounds in green tea and black tea from Turkey by using HS-SPME and GC-MS

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ABSTRACT

Background and Aims: The tea plant, *Camellia sinensis* (L.) O.Kuntze is cultivated in the temperate parts of the world because of its economical value and has been known for ages. According to the processing method, tea can be classified mainly into three types; green, oolong and black tea. The composition of tea obtained from *C.sinensis* heavily depends on geographical location, harvesting time, storage condition and manufacturing process. So the purpose of this paper is to study the volatile composition of green and black tea volatiles manufactured from *C.sinensis* cultivated in North Anatolia.
Methods: The volatiles in green and black teas were extracted by using HS-SPME and analysed by GC-MS/FID analysis. The major compounds in each tea were identified.
Results: Totally, twenty-one and sixteen compounds were separated from green tea and black tea samples, respectively. The main components of green tea aroma were cis-3-hexenyl hexanoate (11.26%), n-octanol (8.55%) and n-decanal (8.02%), while the phenylacetaldehyde (11.26%) and n-decanal (9.93%) were found abundately in black tea aroma.
Conclusion: As aroma is a strong determinant for consumer demands and decisive in tea product speciality, a suitable selection of raw material and location, modification of the manufacturing process could be a reasonable approach to enrich the target aromatic profile of tea products.

Keywords: Tea volatiles, headspace-solid phase microextraction, gas chromatography-mass spectrometer

INTRODUCTION

The tea plant, *Camellia sinensis* (L.) O.Kuntze is a member of Theaceae family and evergreen shrub native from tropical to temperate regions in Asia and also cultivated in the temperate parts of the world because of its economical value and has been known for ages. It is not grown widely in Anatolia, but in the north part it is planted for a hot drink made from its leaves.

According to the processing method, tea can be classified mainly into three types; green tea, oolong tea, and black tea. Fermentation is the cornerstone in the process of manufacturing and the degree of fermentation affects the final product. Green tea is subjected to little or no fermentation, oolong tea is a semi-fermented final product. Black tea is the final product of full fermentation (Pripdeevech & Wongpornchai, 2013; Feng et al., 2019).

Tea volatiles play a determinative role in flavour and affect the consumer preference. It is clear that the composition of tea obtained from *C.sinensis* heavily depends on geographical location, harvesting time, storage condition and manufacturing process. Besides

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phenolic compounds, the aroma is a critical parameter to define the tea quality (Das, Kim, Hong & Eun, 2019; Li et al., 2019). Aroma is also included in the defense system of *C. sinensis* against herbivore attaction (Dong et al., 2011; Zeng et al., 2017).

It has been reported that characterization of different kinds of tea depends on the balance of aroma compounds in the tea. Tea aroma can basically have a green, floral, roasted, or nutty odor depending on volatile compounds present in tea samples (Wang, You & Chen, 2002).

Volatile compounds have been studied in detail and reported to include degradation products of glycosides, carotenoids, amino acids, and carbohydrates (Chaturvedula & Prakash, 2011; Pripdeevech & Wongpornchai, 2013). Oxidation and degradation steps of these precursors have been reported to be accelerated by tea endogenous enzyme (Feng et al. 2019).

During the distillation process, a high temperature is employed which is the reason for changes in the aroma composition. Recent studies on tea volatiles have shown that the complex chemical profile of tea is due to non-volatiles such as tea pigments and lipids (Yang, Baldermann & Watanabe, 2013; Zheng, Li, Xiang & Liang, 2016). The selection of a proper extraction method is required to figure out tea volatiles. The headspace-solid phase microextraction (HS-SPME) method is a rapid, effective, and non-solvent technique, and able to link both extraction and concentration steps (Lee, Chambers, Chambers IV, Adhikari & Yoon, 2013; Lau et al., 2018 (a;b)). It is the preferable method applied in tea analysis.

So, the purpose of this paper is to study the volatile composition of green tea and black tea extracts manufactured from *C.sinensis* cultivated in North Anatolia by using HS-SPME methodology and GC/MS-FID analysis.

MATERIALS AND METHODS

Plant material

All commercial samples of green tea and black tea were supplied from a company in North Anatolia, Turkey.

Headspace-solid phase microextraction (HS-SPME)

For each extraction, 200 mg of a sample was placed in a sealed 20 mL vial with PTFE-coated silicone septum. Extraction of volatiles was carried out using a Merck Supelco SPME fibre coated with 100 µm polydimethylsiloxane. It was exposed for 30 minutes to the headspace while maintaining a temperature of 70°C. Then, the SPME fibre was immediately inserted into the injection port of GC- MS and left for three minutes for desorption of the analytes. Finally, the fibre was baked out for two minutes in a GC-MS injector after each extraction and desorption cycle to reduce the contamination.

Gas chromatography-mass spectrometer/ flame ionisation detector (GC-MS/FID) analysis

The GC analysis of volatiles was carried out using the Shimadzu GC-17A/QP5000 system equipped with a flame ionisation detector (FID) and mass selective detector (MSD). The column used was a Supelcowax-10 capillary column (30 m × 0.32 mm i.d., 0.25 film thickness), and the gas carrier was helium at a rate of 1.8 mL/min. The oven temperature program consisted of a two minute hold at 40°C, followed by a 2°C/minute ascent to 220°C, and a 30 minute hold at 220°C. The injector and MS transfer line temperature were maintained at 200°C and 250°C, respectively. The FID temperature was 300°C. The analytes were detected after electron impact ionization (70 eV) in the SCAN-mode from m/z 50 to 550. Identification of volatile compounds was performed by comparing spectra and retention times of the standards. The components were identified based on the comparison of their relative retention time to a C8–C32 n-alkanes mixture and mass spectra with those of NBS75K, Wiley 7, NIST MS search 2.0 library data of the GC-MS system, literature data, and standards of the main components. The results were also confirmed by comparison of the compounds elution order with their relative retention indices on WAX columns. All analysis were performed in triplicate.

RESULT AND DISCUSSION

Unlike distillation, HS-SPME is able to quantify the high volatiles of tea samples that are more important for aroma perception (Du et al., 2014; Yang et al., 2013). So the purpose of this study is to determine the volatile profile of green and black tea by using HS-SPME coupled with GC-MS. The aroma profile of both samples are shown in Table 1.

Totally, twenty-one and sixteen compounds were separated from green tea and black tea samples, respectively and their structures were determined by MS and retention index data. We detected a total of six aldehydes, four ketones, eight alcohol, two esters, and one other compound representing 75.76±1.19% of the green tea volatile oil. The major identified constituents of green tea aroma were cis-3-hexenyl hexanoate (11.26±0.44%), n-octanol (8.55±0.38%), and n-decanal (8.02±0.31%). Four aldehydes, five ketones, one ester, and six alcohols representing 65.11±1.24% totally were found in black tea volatile oil. Phenylacetaldehyde, that has a honey-like odour, was determined as a major compound in black tea aroma with a value of 11.26±0.52%. Furfural, 1-penten-3-ol, nonanol, n-hexanal, cis-3-hexenal, and cis-3-hexenyl hexanoate were found in only green tea aroma and 1-penten-3-one was found to be included in black tea aroma with a value of 0.34±0.05%. Similar to green tea, n-decanal (9.93±0.32%) which is possibly one of the lipid degradation products and 2,6,6-trimethyl-2- hydroxy cyclohexanone (8.10±0.42%) originated from carotenoid degradation were found abundantly in black tea samples.

According to previous reports, the major compounds in the aroma are formed from carotenoids, lipids, glycosides, and amino acids/carbohydrate (Ho, Zheng &, Li, 2015; Feng et al., 2019; Tan et al., 2019). In general, the linalool and hexanal contents play a key role in the quality of green teas (Kato & Shibamoto, 2001; Pripdeevech & Wongpornchai, 2013).

The flavor and volatile composition of Kangra orthodox black tea extracted by simultaneous distillation extraction (SDE) and hydrodistillation were compared. The major volatiles identified were E-2-hexenal, pentene-3-ol, Z-3-hexenol, linalool, linalool oxides, geraniol, methyl salicylate, and 3,7-dimethyl-1,5,7-oc-

Table 1. Chemical composition of volatile oils of green tea and black tea.

Compound	LRI*	RI[lit]	Green tea (%)	Black tea (%)
1-penten-3-one[b]	1024	1024	-	0.34±0.05
trans-2-hexenal[b]	1061	1061	3.09±0.15	0.32±0.04
n-hexanal[a]	1097	1095	0.31±0.07	-
cis-3-hexenal[b]	1135	1135	0.95±0.12	-
1-penten-3-ol[b]	1175	1173	0.49±0.02	-
Pentanol[a,b]	1252	1256	1.19±0.23	0.31±0.06
n-hexanol[a]	1262	1271	2.45±0.14	0.95±0.05
2,6,6-trymethyl-2- Hydroxycyclohexanone[a,b]	1282	1288	7.06±0.55	8.10±0.42
cis-3-hexenol[a]	1386	1386	3.37±0.27	2.12±0.35
n-nonanal[a,b]	1390	1396	6.66±0.39	8.09±0.65
trans-2-hexenol[b]	1409	1419	4.63±0.22	4.92±0.31
β-ionone[a]	1463	1462	2.45±0.11	3.33±0.31
Furfural[a]	1473	1474	1.15±0.05	-
n-decanal[a,b]	1502	1504	8.02±0.31	9.93±0.32
n-octanol[b]	1565	1569	8.55±0.38	3.15±0.28
cis-3-hexenyl hexanoate[b]	1642	1642	11.26±0.44	-
Phenylacetaldehyde[b]	1648	1648	5.40±0.38	11.26±0.52
Nonanol[a]	1663	1663	0.62±0.10	-
Benzylacetate[a,b]	1682	1686	1.32±0.11	2.92±0.09
α-ionone[b]	1863	1860	3.72±0.23	5.20±0.32
Geraniol[b]	1865	1864	0.62±0.05	2.27±0.11
Geranyl acetone[a]	1867	1867	2.45±0.23	1.90±0.21
		Alcohols	*21.92±0.59*	*13.72±0.56*
		Aldehydes	*24.43±0.66*	*29.60±0.89*
		Esters	*12.58±0.45*	*2.92±0.09*
		Ketones	*15.68±0.65*	*18.87±0.65*
		Others	*1.15±0.05*	-
		TOTAL	**75.76±1.19**	**65.11±1.24**

*LRI: Linear retention indices (WAX column) calculated against n-alkanes. % calculated from FID data with standart. [a]Compounds listed in order of elution from a WAX column. [b]Identification of components based on standard compounds; All values are mean ± standart deviation of triplicates; RI[literature]: https://pubchem.ncbi.nlm.nih.gov

tatrien-3-ol and the SDE technique was reported as more efficient than hydrodistillation (Rawat et al., 2007; Pripdeevech & Wongpornchai, 2013).

Chen et al. studied key aroma compounds of Hanzhong black tea infusion and compared it with other black tea samples. They mentioned that the growing altitude area of *C. sinensis* affects the aroma profile of black tea samples (Chen et al., 2019).

In addition to climatic and geographical conditions, the manufacturing process deeply affects aromatic precursors and glycosidase enzyme content and leads to a great variation of the aromatic profile of tea (Lee et al., 2013; Zheng et al., 2016; Ravichandran & Parthiban, 1998). The volatile compounds of both green tea and black tea were reported to be varied within storage duration (Wang et al., 2002; Choi, Jung & Yun, 2016; Zheng et al., 2016). The off-flavour of green tea was mentioned as the most important problem during preservation, because of light and storage conditions like packaging material, moisture, and temperature (Horita, 1987; Katsuno et al., 2014). Tontul et al. have studied the impact of the shooting period and shading degree on two different tea clones in Turkey and reported that the volatile aroma of green tea samples could be different from each other by a few identical compounds but with considering the shading treatment in general, there were no significant differences in aroma profiles (Tontul et al., 2013).

Many studies have been interested in the volatiles of tea. Common volatile alcohols were mentioned such as hexanols, benzyl alcohols, linalool, and terpineol. Aldehydes commonly found were heptenal, (E)-2-hexanal, pentanal, and nonanal (Das et al, 2019). Le et al. (2013) studied the volatile profile of 24 green tea samples. Linalool and hexanal were found in almost all green tea samples but, the green teas from Africa had 1-penten-3-ol, 2-penten-1-ol, and benzaldehyde. Nonanal was reported to be present generally in green teas from Southeast Asia and India. Additionally, Northeast Asia samples were found to contain nonanal, benzene ethanol, and jasmone (Lee et al., 2013). Both sensory factors and aromatic compounds of black tea and instant teas manufactured by using freeze-dried, spray-dried, and decaffeinated methods were determined to show variations on the basis of processing method (Kraujalyte, Pelvan & Alasalvar, 2016).

CONCLUSION

It is very clear that tea aroma formation depends on many factors especially; raw material, harvesting location, manufacturing process, and storage condition. Tea is consumed all over the world using different preparation methods. So aroma is a strong determinant for consumer demands and decisive in tea product speciality. The HS-SPME extraction method is known as solvent-free, low cost, and rapid extraction procedure, and it is particularly used to quantify the high volatile profile of tea samples that are believed to be decisive in consumer preference. In overall evaluation, a suitable selection of raw material and location, modification of the manufacturing process could be a reasonable approach to enrich the target aromatic profile of tea products.

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