Aroma-active compounds of Georgian black tea infusions as determined by gas chromatography-olfactometry

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Summary

Aroma belongs to the most important quality characteristics of black tea infusions. Volatile aroma-active compounds were analysed in infusions of black tea produced in Georgia in two major production locations. For this purpose, solid phase microextraction was used to isolate the volatile fractions, which were subsequently analysed by gas chromatography-olfactometry and gas chromatography-mass spectrometry. The method used involves a combination of instrumental separation and sensory analysis by the human nose, which provide quantitative data to characterize the organoleptic quality and allow the detection of volatile compounds with strong aroma perception even if present at low concentration levels. Overall, 59 odouric zones were identified in the lowland black tea and 57 odouric zones were identified in the highland black tea. The aroma-active compounds comprised alcohols, aldehydes, terpenes, ketones, phenolics lactones, furanes, furanones and long chain fatty acids. Odorously most pronounced compounds in both Georgian black tea infusions were benzaldehyde, β-ionone, δ-decalactone, (*E*)-linalool-3,7-oxide, furaneol, hexanal, indole and 2-phenylethanol. The results demonstrated that Georgian black tea is of good quality, being compatible with international requirements, while indicating specific components that might be interesting for its use in premium blends.

Keywords

black tea; volatiles; aroma; gas chromatography-olfactometry

Tea is the most widely consumed beverage in the world. It is a freshly prepared hot water infusion of processed leaves and buds of *Camellia sinensis*. The beverage is characterized by stimulating physiological effects, which are caused by the contained caffeine, and by a quite complex, generally pleasant taste and aroma [1, 2].

One of the most popular types of tea is black tea. In this case, the plant material is processed by a technology that involves biochemical changes and oxidation of catechins to quinones to a high degree. The formed theaflavins and thearubigins are responsible for the main characteristics of black tea infusions but further important contributors to quality of black tea are various aroma-active compounds, contents of which may vary between different origins, gardens, seasons and processing technologies. The compounds perceived to be deleterious to quality of black tea, imparting green grassy aroma, form a group of Volatile Flavour Compounds I (VFC I group). The compounds perceived to be beneficious to quality of black tea, imparting sweet flowery aroma, form VFC II group. Absence of VFC I group compounds and high contents of aroma-active compounds from VFC II group characterize premium quality black teas [3–5].

Georgia (Sakartvelo) is a country with subtropi-

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Fig. 1. Geographical location of tea garden regions in Georgia.

1 – Martvili lowland tea gardens, 2 – Tkibuli highland tea gardens.

cal climate and specific fertile soil that are favourable for cultivation of tea, the major tea gardens being located in the lowland region of Martvili and the highland region of Tkibuli (Fig. 1). *C. sinensis* varieties Georgian selection N1, Georgian selection N2 and Kolkheti are mainly cultivated. The orthodox technology with dedicated equipment is most commonly used for tea processing, which involves withering at 36–42 °C with residual moisture reduced to 62–64 %, three-fold twisting of tea leaves, fermentation at 20–22 °C and 98% relative humidity, drying in stages at 90–120 °C to reduce the residual moisture to 3–4%, sorting and packing. At the end of fermentation, the oxidation depth of the tannin-catechin complex reaches 45 %.

Georgian tea has been facing difficulties in sales due to the loss of traditional markets in former Soviet Union and due to the strong competition [6]. However, opportunities for this original product may be derived from the fact that black tea produced in various countries is known to have, to a certain extent, unique chemical composition and may have interesting organoleptic properties, which could help to succeed in the market [7].

In this study, we revealed and characterized the aroma-active compounds in infusions of two types of Georgian black tea with an intention to evaluate their qualitative composition and odouric quality. For this purpose, we used gas chromatography-olfactometry, which is a highly effective analytical technique that combines instrumental and sensory approach, allowing to detect volatile compounds with strong aroma perception even if present at low concentration levels. Thanks to the use of a sophisticated analytical method, original, detailed and reliable data were obtained, which will be useful for quality management of black tea production in Georgia.

MATERIALS AND METHODS

Samples

Georgian lowland Martvili black tea (loose, broken, 100 g packing, harvest 2019) and Georgian highland Tkibuli black tea (loose, medium leaves, 60 g packing, harvest 2019) in food-grade packings were obtained from a specialized tea shop in Kutaisi, Georgia. For infusion, 2.5 g portions were weighed in dedicated paper filter bags (Finum Extra slim; Riensch und Held, Hamburg, Germany) declared to be flavour-neutral, made from abaca pulp, cellulose and sealing fibre. As a reference, Ceylon black tea blend (bagged à 2.5 g, individually wrapped, broken; Taylors of Harrogate, Harrogate, United Kingdom) was used. Infusions were prepared with 125 ml of freshly boiling medium-hardness tap water (pH 7.6, less than 0.05 $mg \cdot l^{-1}$ free chlorine) by immersing the bag for 4 min.

Reference standards

Chemicals used as reference standards to support identification of volatiles (listed in Tab. 1) were gifts donated by Bedoukian Research (Danbury, Connecticut, USA), Graz University of Technology (Graz, Austria) or French National Institute for Agricultural Research (INRA) laboratories (Dijon, France).

Solid phase microextraction

Individual samples of tea infusions (5 ml) were incubated statically in a 40 ml glass vial in a metallic block thermostat (Liebisch, Bielefeld, Germany) at 80 °C for 30 min and the volatile fraction was separated a solid phase microextraction (SPME) fibre placed in the headspace of the sample. The SPME fibre DVB/Carboxen/PDMS, film thickness 50/30 μm, film length 2 cm, Stable Flex, "For odours" (cat. no. 57328-U; Supelco, Bellefonte, Pennsylvania, USA) was used. The fibre was initially conditioned by heating in the injector block of the gas chromatograph at 250 °C for 1 h. SPME samples were desorbed at 250 °C in the gas chromatography (GC) injector block during the entire analytical run.

Gas chromatography-mass spectrometry

Volatiles separated by SPME were analysed by gas chromatography-mass spectrometry (GC-MS) using a gas chromatograph Agilent 6890N (Agilent

Technologies, Palo Alto, California, USA) coupled to a mass spectrometric detector 5973 inert (Agilent Technologies) equipped with a HP-INNOWax column (30 m × 0.25 mm × 0.50 μm; Agilent Technologies) operating with a temperature programme 40 °C (1 min), 5 °C·min^{-1}, 250 °C (1 min). The linear velocity of carrier gas helium was 45 cm·s^{-1} (measured at 143 °C). Pulse splitless injection (80 kPa for 1 min) was used at an injector temperature of 250 °C. Ionization energy (EI) was 70 eV.

Gas chromatography-olfactometry

In order to reveal key aroma-active compounds of black tea infusions, in parallel with GC-MS, volatiles separated by SPME were analysed by a combined technique of GC with flame ionization detection and olfactometry (GC/FID-O), using the concept of detection frequency of posterior assessment [8]. The sniffing procedure panel was formed of 5 judges (2 men, 3 women, aged 29, 47, 50, 57 and 61) who were chosen from 11 assessors trained in sensory evaluation. Results of GC/FID-O analyses were expressed as average values of aroma intensities in a scale from 0 to 3 with increments of 0.5, obtained from five independent measurements. However, it was essential to comply with the requirement of at least 4 citations within each sensory perception. The gas chromatograph Agilent 7890A (Agilent Technologies) was coupled to a flame ionization detector and to an olfactory detector port ODP3 (Gerstel, Mülheim an der Ruhr, Germany). The effluent of the GC column was split to FID and the olfactory detector port at a ratio of 1 : 1. The column was DB-WAX (30 m × 0.32 mm × 0.25 μm; Agilent Technologies) with a polar stationary phase, operated with a temperature programme 40 °C (1 min), 5 °C·min^{-1}, 230 °C (1 min). Hydrogen was used as a carrier gas at a linear velocity of 45 cm·s^{-1} (measured at 143 °C). Pulse splitless injection (80 kPa for 1 min) was used at an injector temperature of 250 °C. The olfactory detector port (ODP) operated at a temperature of 180 °C, interface temperature was 230 °C and the flow of the added nitrogen in the olfactory detector port's humidifier was 12 ml·min^{-1}. The sniffing time of individual judges did not exceed 40 min.

Identification of aroma-active volatiles

Aroma-active volatiles were identified on the basis of comparison of their linear retention indices, mass spectra, analysis of standard compounds and data on occurrence as well as aroma description in the literature [9] and in our in-house database. Linear retention indices (*LRI*) were calculated using the equation of Van den Dool and Kratz [10] with *n*-alkanes C9–C26 as reference compounds. *LRI* values were compared and confirmed with *LRI* data obtained by measurement of relevant standard compounds. For this purpose, our in-house database of *LRI* values was used. Identification of compounds by comparison of their mass spectra was done using Mass Spectral Library NIST 20 (National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

RESULTS AND DISCUSSION

In order to provide chemically objective data on components of the aroma complex of selected Georgian tea infusions, analysis of volatiles separated by SPME was carried out using GC/FID-O with support of GC-MS. For this purpose, black tea infusions were prepared at 2 g per 100 ml, which is a double strength as usually consumed. This setting was chosen based on the results of our preliminary experiments, which showed that a majority of peaks separated by GC-MS provided sufficient mass spectra at this concentration. We believe that analytical results obtained for infusions of this concentration are comparable with those that would be obtained with „normal strength“ infusions, albeit they are richer. However, we found that certain principal aroma-active compounds with strong odouric perception in GC/FID-O analyses did not provide satisfactory mass spectra at these standard analytical conditions. In order to obtain sufficient mass spectra, additional black tea infusions at 2 g per 10 ml were also prepared and analysed.

Results are summarized in Tab. 1 showing that 59 and 57 aroma zones were detected in Martvili lowland black tea and Tkibuli highland black tea infusions, respectively. This means that the aroma of both of the analysed Georgian teas was rich and complex, comparable in this parameter with Ceylon reference tea, in which 56 aroma zones were detected. The aroma-active compounds comprised alcohols, aldehydes, terpenes, ketones, phenolics lactones, furanes, furanones and long chain fatty acids. Most of the identified compounds were previously detected in Darjeeling black tea leaves, although that study used the solvent assisted flavour evaporation (SAFE) technique for extraction of volatiles and aroma extract dilution analysis (AEDA) for GC-O [11]. Thirty-seven aroma-active compounds were common to all three tested black tea infusions. For both of the Georgian teas, VFC II compounds were determined at high levels and VFC I compounds were determined at low

Tab. 1. Key aroma-active compounds in headspace SPME extracts of Georgian and reference Ceylon black tea infusions.

LRI	Aroma compound	Aroma description	Odour intensity		
			A	B	C
914.8	2-Methyl butanal [a]	Black tea, dried leaves, cocoa powder-like	0.5	0	1
918.6	3-Methyl butanal [a]	Unpleasant, sweaty, malty	0	0	1
NC	Ethanol [b]	Weak alcoholic waft, ethereal	0.5	0.5	0.5
954.8	2-Ethyl furan [a]	Sweetish, ethereal, bready	0	0	0.5–1
981.6	Pentanal [a]	Sweetish, slightly caramel-like	0.5	0.5	0.5
1 085.2	Hexanal [a]	Green, grass-like, fresh	2	2	2
1 129.4	(*E*)-2-Pentenal [a]	Green, fresh, plant-like	0.5–1	0	0.5–1
1 167.3	Myrcene [a]	Herbal, green, plastic, balsamic-resinous	1.5	0.5–1	0.5
1 186.0	2-Heptanone [a]	Pleasant, sweetish, herbal	0	1	0
1 188.7	Heptanal [a]	Pleasant, fresh, herbal	1	0	1
1 200.0	Limonene [a]	Pleasant, fresh, terpenic-soapy, citrus-like	0.5–1	0	0.5–1
1 219.4	(*E*)-2-Hexenal [a]	Green, leafy, vegetable, fresh	0.5–1	0.5–1	0.5–1
1 236.7	2-Pentyl furan [a]	Green bean-like, metallic, vegetable	1.5	1.5	0
1 245.2	(*Z*)-4-Heptenal [a]	Herbaceous, oily	0.5–1	0.5–1	1
1 261.8	Pentanol [a]	Fusel, oily, balsamic, bready	0.5	0.5	1
1 306.5	1-Octen-3-one [c]	Mushroom-like, earthy	2	2	0
NC	1-Hydroxy-2-propanone [d]	Slightly burnt caramel-like, sweet	0.5–1	0	0
NC	2,2,6-Trimethylcyclohexanone [d]	Camphoraceous tobacco notes, dried herbs-like, thujonic note	0.5–1	1.5	0.5–1
NC	(*Z*)-2-Hexenol [d]	Fresh green, vegetable	1.5	2	0.5
1 325.2	(*E*)-2-Heptenal [a]	Green, vegetable, nettle-like	1	1	1
NC	Unknown	Mushroom-like, plant-like, mixed odour	1	0	0
1 342.1	6-Methyl-5-hepten-2-one [a]	Herbaceous, fresh, green, citrus-like	1	1.5	1.5
1 366.0	Hexanol [a]	Green, herbal, fresh, grassy	1.5	2	1.5
NC	L-Rose oxide [d]	Heavy scent with sweetish nuance, rose-like	1	1	1
NC	(*Z*)-3-Hexenol [d]	Green, plant-like	1.5	1.5	0.5
1 397.7	Nonanal [a]	Fresh, citrus, fatty, waxy	0.5	2.5	0.5–1
1 431.2	(*E*)-2-Octenal [a]	Green, leafy, fatty, nutty	1	2	0.5
1 494.5	(*E*,*E*)-2,4-Heptadienal [a]	Fatty, slightly hazelnut-like	1.5	0	0.5–1
1 520.9	Benzaldehyde [a]	Sharp, bitter, vegetable	2	2.5	1
1 559.9	Linalool [a]	Pleasant, flowery, gentle, fresh scent	2	1	2
1 571.7	Octanol [a]	Green, fatty, waxy, green paprika-like	1.5	2	0
NC	(*E*,*E*)-3,5-Octadien-2-one [d]	Herbs-like, bitterish, green	2 [f]	1.5	1.5
NC	6-Methyl-3,5-heptadien-2-one [d]	Balsamic, green, plant-like, fatty, spicy	1.5	1.5	0
1 619.7	β-Cyclocitral [a]	Fresh, minty, green	1	1	1
1 640.2	Phenylacetaldehyde [a]	Harsh, honey-like, green	2.5	1.5	2
1 674.3	Nonanol [a]	Pleasant, sweetish, fresh, orange, rose	0	1.5	0
1 683.2	Neral [(*Z*)-citral)] [a]	Citrus-like, gently fresh	0	0	1.5
1 702.5	(*E*,*E*)-2,4-Nonadienal [a]	Fatty, green, waxy, plant-like	0	1	0
1 735.1	Geranial [(*E*)-citral] [a]	Pleasant, fresh, green plant-like	1	1.5	1
NC	(*E*)-Linalool-3,7-oxide [d]	Fresh, herbal, cooling, mentol-like nuance	2	2	1.5
NC	Unknown	Bitterish, tea-like	0	1.5	0
NC	Unknown	Straw-like	0	0	1.5

Tab. 1. *continued*

LRI	Aroma compound	Aroma description	Odour intensity		
			A	B	C
NC	α-Damascone [d]	Woody, herbal, black tea-like	1	0	1
NC	Unknown	Slightly caramel-like	0	0	1
1 774.2	Methylsalicylate [a]	Herbal, camphoreous, woody, tobacco note, spicy	0	0.5–1	1.5
1 812.0	(*E,E*)-2,4-Decadienal [a]	Fatty, fried potatoes-like, mastic-like	1.5	1.5	0.5–1
1 823.9	β-Damascenone [a]	Dried plums, sweet	1.5	2	1.5
1 860.5	Geraniol [a]	Floral, rose-like	1.5	2	1.5
NC	2-Methoxyphenol [guaiacol] [d]	Smoky, bitterish	2	2	1.5
NC	Unknown	Fatty, mastic-like	0	1.5	0
NC	γ-Ionone [d]	Pleasant, floral, sweetish	0	2	0.5–1
NC	Benzylalcohol [d]	Pleasant, slightly floral	0.5–1	0.5–1	0
1 917.9	2-Phenylethanol [a]	Floral, rose-like, honey	2	2	1.5
NC	2-Phenyl-2-butenal [d]	Pleasant, sweetish, slightly floral	0	0	1.5
1 941.0	β-Ionone [a]	Strong floral, sweetish, viola, freesia	2	3	2.5
NC	Cyclodecane [d]	Musty	1.5	0	0
1 981.1	Dodecanol [a]	Pleasant, sweetish, gently soapy	1	2	0.5–1
NC	β-Ionon-5,6-epoxide (unknown isomer) [d]	Pleasant, sweetish, fruit caramel-like	1	1	0.5
NC	Isopropyl myristate [d]	Oily, fatty	2 [g]	1.5	1.5 [h]
2 028.3	γ-Nonalactone [a]	Sweetish, coconut-like	0	2.5	
2 043.3	Furaneol [c]	Sweet, caramel-like, candy cotton-like	2	3	1
2 090.0	*p*-Cresol [c]	Specific heavy phenolic mixed odour, cresylic, medicinal, plastic-like	2	2	1.5
NC	Unknown	Fruity, sweetish	1	1	0
NC	*p*-Cymen-7-ol [d]	Fresh plant-like, slightly cooling, caraway-like	1	0	0
2 143.9	γ-Decalactone [c]	Fruity, sweet, peach-like	0	1.5	0
2 186.6	Tetradecanol [a]	Waxy, fruity, orris, slightly coconut-like	2	1.5	1.5
2 194.6	δ-Decalactone [c]	Coconut-like, creamy, sweet milky, peach-like	3	3	2
NC	Sotolon [e]	Spicy, seasoning curry-like, maggi-like	1	2	1
2 225.7	Methyl palmitate [a]	Waxy, pleasant balsamic odour, orris	1	1	0.5–1
2 291.9	Methyl dihydrojasmonate [hedione] [a]	Gentle jasmine note with a citrus freshness, magnolia blossom-like	0.5–1	1.5	0.5–1
NC + 2 392.6	Dihydroactinidiolide [d] + hexadecanol [a]	Pleasant, fragrant, apricot, berry, woody + waxy, floral	0.5–1	0.5–1	0.5–1
2 448.8	Indole [a]	Unpleasant, putrid plants-like	3	3	1.5
NC	Dodecanoic acid [d]	Faint bay leaf-like, soapy	1	0	0
NC	Tetradecanoic acid [d]	Waxy, soapy, slightly sweetish, citrus peel-like	1	2	0

Odour intensities are presented as mean values of 5 or 4 analyses.
LRI – linear retention index (determined on DB-WAX column), A – lowland Martvili tea, B – highland Tkibuli tea, C – reference Ceylon tea, NC – not calculated.

Aroma compounds were identified on the basis of the following criteria:
a – identification based on mass spectrum, linear retention index, analysis of an authentic standard, aroma description and literature data;
b – identification based on mass spectrum and aroma description;
c – identification based on linear retention index, analysis of an authentic standard, aroma description and literature data;
d – identification based on mass spectrum, aroma description and literature data;
e – tentative identification based on aroma description and literature data;
f – mixed with tentatively identified (*Z*)-3-hexenyl propanoate with a fresh, green plant, leafy, waxy aroma;
g – mixed with an unknown compound with a balsamic, plant essential oil-like, bay leaf aroma.
h – isopropyl myristate was overlapped with γ-nonalactone.

levels, corresponding to characteristics of premium quality black teas, although the composition of these fractions differed to a certain extent. The ratio between linalool, a representative of VFC II, and (*E*)-2-hexenal, a representative of VFC II [12], was good for Martvili lowland tea and moderate for Tkibuli highland tea.

Several VFC II compounds were more odorously pronounced in both Georgian teas than in the reference Ceylon tea, such as 2-phenylethanol, (*E*)-linalool-3,7-oxide or benzaldehyde. Other strong aroma-active compounds were also more odorously pronounced in both Georgian teas than in the reference Ceylon tea, such as δ-decalactone, furaneol or guaiacol. Most of the important VFC II compounds (e.g. β-damascenone, geraniol or β-ionone) were at a similar odour intensity level in Georgian teas as in the reference Ceylon tea, with the exception of methylsalicylate and 2-phenyl-2-butenal, which were at low odour intensity levels in Georgian teas. γ-Ionone and γ-decalactone were strongly pronounced only in Tkibuli highland tea.

On the other hand, several VFC I compounds were more odorously pronounced in both Georgian teas than in the reference Ceylon tea, such as 2-pentyl furan, (*Z*)-3-hexenol or (*E*)-2-octenal. Important VFC I compounds hexanal and hexanol were present in all analysed teas at similar odour intensity levels. Several other aroma-active compounds that may decrease the quality of black tea were sensory more pronounced in both Georgian teas than in the reference Ceylon tea, such as 1-octen-3-one, 6-methyl-3,5-heptadien-2-one or (*E*,*E*)-2,4-decadienal. Regarding compounds that may have detrimental effect on the tea aroma, Georgian teas were characterized by lower odour intensity levels of 3-methyl butanal and pentanol in comparison with Ceylon tea.

A specific case was indole. It was detected by GC/FID-O as a strong off-flavour (intensity 3) in a late-eluting aroma zone of both Georgian teas and, at a lower odour intensity of 1.5, also in the reference Ceylon tea. In our earlier analyses, its concentration was found to be too low to record a sufficient, full mass spectrum. Later, it was recorded in a specially prepared concentrated infusion. In odouric terms, depending on its concentration, indole can be very repulsive or immensely seductive. This compound is known to have an intense putrid off-odour, but at very low, trace or ultra-trace concentrations, its odour is flowery [13]. At high dilutions, i.e. in concentrations lower than 0.1 % or in compositions, the odour of indole is reminiscent of jasmine or orange blossoms. Presence of indole in black tea may suggest that it should be used in blends rather than pure, in order to dilute the off-flavour.

A part of the presented results that regards Ceylon black tea can be compared with data reported in a recent study [14]. Of course, a direct quantitative or odour-intensity comparison is impossible due to the obvious differences between gardens, seasons and batches of the Ceylon black tea samples, as well as the different extraction techniques used, along with certain other differences in analytical techniques. However, that study revealed the same dominant aroma-active compound as here in this study, β-ionone, several same key aroma-active compounds were detected at prominent odour levels (e. g. 2-methyl butanal, 3-methyl butanal, hexanal, linalool, phenylacetaldehyde, guaiacol), together with a number of aroma-active compounds at various odour levels, which were detected and identified in both studies. Indole was not sensorially detected in that study but its derivative 3-methylindole was detected.

CONCLUSION

This study provided objective information on aroma-active compoounds of Georgian black tea infusions as obtained by GC-O. The analytical method used involves sensory description of individual compounds and is therefore much more suitable for characterizing organoleptic quality than the widely used GC-MS approach alone. Of course, further investigation of more samples would be necessary to encompass the intra- and inter-season variability as well as differences between gardens and producers. However, the presented results demonstrate that Georgian black tea is generally of good quality, being compatible with international requirements, while containing specific aroma-active compounds that might be interesting for its use in premium blends.

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