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Analysis of Menthol, Menthol-Like, and Other Tobacco Flavoring Compounds in Cigarettes and in Electrically Heated Tobacco Products *

by

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SUMMARY

Although smoking is responsible for a huge variety of diseases which result in ~16% of the fatalities in the United States and Europe respectively, cigarettes are still being sold far and wide. Mentholated cigarettes were introduced in 1920, since then to today social recognition and the use of flavored tobacco products is still increasing especially within young people. The EU adopted as its measure to reduce tobacco use among adolescents the prohibition of tobacco products with a characteristic flavor by means of the directive 2014/40/EU of the European Parliament and the Council.
For this reason, we developed a method for the simultaneous determination of 14 tobacco flavors like menthol, menthol-like and other compounds via gas-chromatography coupled with mass-spectrometry (GC/MS) and analyzed 21 different tobacco products (mentholated and non-mentholated cigarettes, as well as electrically heated tobacco products (EHTPs)) of the German market regarding their flavoring compound patterns. The highest amounts of flavoring compounds were determined in menthol cigarettes (~10,000 µg/stick) whereas non-mentholated cigarettes and EHTPs featured only ~10 µg/stick. In total, seven flavoring compounds like menthol, L-menthone, L-linalool, isopulegol, geraniol, camphor and WS-3 (cooling agent) were available within the samples. Mentholated cigarettes could be clearly identified since > 99% of the measured flavoring compounds was represented by menthol. Although flavoring compounds in non-mentholated cigarettes and EHTPs were quite comparable, they could be differentiated due to different flavoring compound patterns. Brand-specific flavoring compound patterns were not recognized. [Beitr. Tabakforsch. Int. 28 (2018) 93–102]

ZUSAMMENFASSUNG

Das Rauchen verursacht eine Vielzahl an Erkrankungen, die in den USA und Europa jeweils für ca. 16% der Todesfälle verantwortlich sind. Dennoch sind Zigaretten weiterhin überall erhältlich. Mentholhaltige Zigaretten wurden im Jahr 1920 eingeführt. Seitdem nehmen insbesondere bei jungen Menschen das gesellschaftliche Ansehen und der Konsum aromatisierter Tabakprodukte zu. Als Maßnahme zur Senkung des Tabakkonsums unter Jugendlichen hat die Europäische Union mit der Richtlinie 2014/40/EU des Europäischen Parlaments und des Rates Tabakprodukte verboten, die ein charakteristisches Aroma haben.
Aus diesem Grund haben wir eine Methode zur simultanen Bestimmung von 14 Tabakaromen wie Menthol sowie mentholartigen und anderen Verbindungen mittels Gaschromatographie mit Massenspektrometrie-Kopplung (GC/MS) entwickelt und 21 verschiedene im deutschen Markt erhältliche Tabakprodukte (mentholhaltige und nicht-mentholhaltige Zigaretten sowie elektrisch erhitzte Tabakprodukte (EHTP)) hinsichtlich ihrer Aromatisierungsmuster analysiert. Die höchsten Konzentrationen an Aromaverbindungen konnten in Mentholzigaretten bestimmt werden (ca.10.000 µg/Stick) während nicht-

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mentholhaltige Zigaretten und EHTP lediglich ca. 10 µg/Stick aufwiesen. Insgesamt fanden sich sieben Aromaverbindungen wie Menthol, L-Menthon, L-Linalool, Isopulegol, Geraniol, Kampfer und WS-3 (ein "cooling agent") in den Proben. Die Mentholzigaretten waren eindeutig zu identifizieren, da Menthol über 99% der dort gemessenen Aromaverbindungen darstellte. Obwohl die Aromaverbindungen in nicht-mentholhaltigen Zigaretten und EHTP in etwa vergleichbar waren, konnte man sie aufgrund unterschiedlich Aromaverbindungsmuster unterscheiden. Markenspezifische Muster bei den Aromaverbindungsmustern wurden nicht festgestellt. [Beitr. Tabakforsch. Int. 28 (2018) 93–102]

RESUME

Bien que le tabagisme soit responsable d'un vaste éventail de pathologies à l'origine d'environ 16% des décès recensés respectivement aux Etats-Unis et en Europe, les cigarettes demeurent en vente libre dans le monde entier. Les cigarettes mentholées furent lancées en 1920 et à ce jour, la reconnaissance sociale et la consommation de produits du tabac aromatisés continuent de progresser, surtout auprès des jeunes. Dans l'espoir de réduire la consommation de tabac chez les adolescents, l'Union européenne adopta la directive 2014/40/UE du Parlement européen et du Conseil, qui interdit la vente de produits du tabac présentant un arôme caractéristique.

Par conséquent, nous développâmes une méthode permettant d'identifier simultanément, dans le tabac,14 arômes tels que le menthol, les arômes semblables au menthol et d'autres composants grâce à la chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG/SM) et nous analysâmes le comportement des composés aromatiques de 21 produits du tabac différents (cigarettes mentholées et non-mentholées ainsi que des produits du tabac chauffés par voie électrique) commercialisés en Allemagne. Les plus grandes quantités de composés aromatiques furent recensées dans les cigarettes au menthol (~10000 µg/bâtonnet) alors que les cigarettes non-mentholées et les produits chauffés par voie électrique n'indiquèrent que ~10 µg/bâtonnet. Au total, sept composés aromatiques (menthol, L-menthone, L-linalool, isopulégol, géraniol, camphre et WS-3 (agent de refroidissement)) furent relevés dans les échantillons. Les cigarettes mentholées purent être clairement identifiées puisque > 99% des composés aromatiques relevés étaient représentés par le menthol. Bien que les composés aromatiques dans les cigarettes non-mentholées et les produits chauffés par voie électrique fussent très comparables, ils purent être différenciés grâce à leurs comportements distincts. Parmi les composés aromatiques, nous ne pûmes identifier aucun comportement spécifique à une marque. [Beitr. Tabakforsch. Int. 28 (2018) 93–102]

INTRODUCTION

The total number of cigarette sales in the United States (US, 253.8 billion of cigarettes in 2014) (1) and in the European Union (EU, 608.8 billion sticks in 2010 (2) demonstrate that smoking still obtains social recognition. Smoking of tobacco is associated with a variety of adverse health effects, including different kinds of cancer, cardiovascular and metabolic diseases, pulmonary diseases, coronary heart diseases, diabetes mellitus, inflammation, rheumatoid arthritis, stroke and asthma (3–5). These diseases are also responsible for about 440,000 deaths (~16%) per year in the US (6–7) and for about 16% of deaths attributed to tobacco in Europe in 2004 (7).

Since the introduction of mentholated cigarettes in the 1920s, there was an increasing consumption of these flavored tobacco products (TPs) in the United States (1, 8–9) with a domestic market share of menthol cigarettes in the US of 30% in 2014 (1). Although the share of mentholated cigarettes in Europe is so far generally lower than in the US, the market share of these TPs in the EU also slightly increased from 3.4% in 2000 to 4.6% in 2010 (2). Menthol has a characteristic flavor (9) and is known to stimulate the transient receptor potential melastatin 8 receptor (TRPM8) which operates as a sensor for thermal coldness (10–11). Sensory effects of menthol are suggested to mask the harshness of tobacco smoke and the physiological properties of menthol are suspected to facilitate the inhalation of tobacco smoke (9, 12–20). The improved taste of cigarettes due to flavoring might also explain why adolescents are likely to prefer mentholated cigarettes as starter products for tobacco use (12, 16, 21–25).

The directive 2014/40/EU of the European Parliament and the Council directly prohibits tobacco products with a characteristic flavor other than tobacco that results from additives including fruit, spice, herbs, alcohol, candy, menthol or vanilla (26). The directive 2014/40/EU also prohibits the use of additives that facilitate inhalation in tobacco products for smoking. Besides menthol, there are a lot of substances such as L-menthone, L-linalool, isopulegol, geraniol, hydroxycitronellal, camphor, 2-isopropyl-*N*,2,3-trimethylbutyramide (WS-23), *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (WS-3), and L-menthyl lactate which have been shown to activate the TRPM8 receptor and therefore show menthol-like activities (27). Therefore, the application of most of these substances as TP additives is also restricted by law in Germany (28). In addition, as stipulated in directive 2014/40/EU, substances with cancerogenic, mutagenic or reprotoxic (CMR) properties, e.g., estragole or safrole-containing compounds, or substances associated with energy or vitality, e.g. α-thujone are also prohibited under German law (28).

Although there are a few studies analyzing the menthol content in mentholated and non-mentholated cigarettes, to the best of our knowledge, there is only one study by PASCHKE *et al.* dealing with tobacco flavoring compounds in cigarettes of the German market (27). Since there exists no study dealing with flavoring compounds in electrically heated tobacco products (EHTPs) of the German market, the aim of this study was to develop a method for the simultaneous determination of 14 different tobacco flavoring compounds *via* gas-chromatography coupled with mass-spectrometry (GC/MS). Moreover, these compounds were quantified in 21 TPs (including mentholated and non-mentholated cigarettes, as well as electrically heated tobacco products (EHTPs) from the German market) to assess the application of tobacco flavoring compounds in these products.

Table 1. Time segments (I-XIII), *m/z*-ratios and dwell times for the GC/EI-MS measurements of the target analytes in SIM-mode.

Analyte	Group	Time window (min)	*m/z* values	Dwell time
(−)-α-Thujone	I	0.0–13.0	81, 95, 110	100
L-Menthone	II	13.0–13.6	112, 139, 154	50
L-Linalool	II	13.0–13.6	71, 93, 121	50
Camphor	III	13.6–14.4	81, 95, 108	100
Isopulegol	IV	14.4–14.7	111, 121, 154	100
(±)-Menthol	V	14.7–15.5	71, 95, 123	100
Estragole	VI	15.5–16.0	121, 147, 148	100
Geraniol	VII	16.0–18.6	69, 93, 123	35
WS-23	VII	16.0–18.6	114, 128, 129	35
2-Methoxyphenol-3,4,5,6-d_4 [1]	VII	16.0–18.6	85, 113, 128	35
Safrole	VIII	18.6–19.8	104, 131, 162	50
Hydroxycitronellal	VIII	18.6–19.8	59, 71, 121, 139	50
L-Menthyl lactate	IX	19.8–22.3	83, 123, 139	100
1,2,4-Trimethoxybenzene [1]	X	22.3–24.5	125, 168, 153	100
WS-3	XII	26.5–28.4	100, 168, 211	100
Coumarin	XIII	28.4–30.0	89, 118, 146	100

[1] Internal standard

EXPERIMENTAL

Materials

L-Linalool (purity: 97%) and 3,7-dimethyl-7-hydroxyoctanal (hydroxycitronellal, 98%) were purchased from Acros Organics (New Jersey, NJ, USA), whereas isopulegol (> 90%) and 2-isopropyl-*N*,2,3-trimethylbutyramide (WS-23, > 98%) were obtained from TCI Chemicals (Portland, OR, USA). Camphor (95%), (−)-α-thujone (≥ 96%), (±)-menthol (99%), estragole (98%), safrole (≥ 97%), coumarin (≥ 99%), L-menthone (≥ 96%), geraniol (≥ 96%), L-menthyl lactate (≥ 97%), *N*-ethyl-2-isopropyl-5-methylcyclohexanecarboxamide (WS-3, 99%), and 1,2,4-trimethoxybenzene (97%) were purchased from Sigma Aldrich (Steinheim, Germany). 2-Methoxyphenol-3,4,5,6-d_4 was obtained from C/D/N Isotopes (Augsburg, Germany), and dichloromethane was obtained (for residue analysis) from Promochem (Wesel, Germany). Diatomaceous earth was purchased from Thermo Scientific (Waltham, MA, USA).

Standard solutions

The stock mix solution (2,500 mg/L per compound) was prepared by dissolving 25 mg of the flavor standards in 10 mL dichloromethane. The internal standard (ISTD) stock solution (2,500 mg/L per compound) consisting of 1,2,4-trimethoxybenzene and 2-methoxyphenol-3,4,5,6-d_4 was prepared the same way. The ISTD standard solution was obtained by diluting the ISTD stock solution in dichloromethane to a final concentration of 25 mg/L. 2-Methoxyphenol-3,4,5,6-d_4 was used for (−)-α-thujone, L-menthone, L-linalool, camphor, isopulegol, (±)-menthol, estragole, geraniol, and WS-23. 1,2,4-Trimethoxybenzene was used as internal standard for safrole, hydroxycitronellal, L-menthyl lactate, WS-3, and coumarin.

Gas chromatography coupled with electron ionization mass spectrometry (GC/EI-MS)

The Agilent 6890N/5973 GC/MS system (Waldbronn, Germany) was equipped with a DB-WAX UI column (60 m × 0.25 mm internal diameter × 0.25 µm film thickness, Agilent, Waldbronn, Germany). Injection of the sample solutions (1 µL) was performed by a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) into a split/splitless injector operated in splitless mode. Helium gas (purity: 99.999%) obtained from Air Liquide (Düsseldorf, Germany) was used as carrier gas at a constant flow rate of 1.0 mL/min. The GC oven was initially set for 5 min to 50 °C, and then the temperature was ramped up at 20 °C/min to 160 °C, then at 2.5 °C/min to 190 °C (2 min), and finally at 20 °C/min to 250 °C (8 min). The transfer line, quadrupole, and ion source temperatures were set at 250 °C, 180 °C, and 230 °C, respectively. The mass spectrometer (MS) was operated in the selective ion monitoring (SIM) mode by means of several time segments (Table 1). All analytes were identified by comparison of their mass spectra and retention times with those of authentic standards (Figure 1). Data was evaluated using MassHunter Workstation Software Quantitative Analysis (for GCMS), version B.07.01 (Agilent, Waldbronn, Germany).

Samples and sample preparation

Cigarettes and electrically heated tobacco products (EHTPs) of different manufacturers were obtained from different retailers in Germany. Tobacco filler from 18 different unopened packs of cigarettes (ten non-mentholated and eight mentholated) as well as from three unopened packs of EHTPs, each stored at room temperature, were analyzed. For a comparison of tobacco flavoring compounds used in mentholated and non-mentholated cigarettes, cigarette samples (n = 18) were obtained from 11 different cigarette brands. In five cases, both mentholated

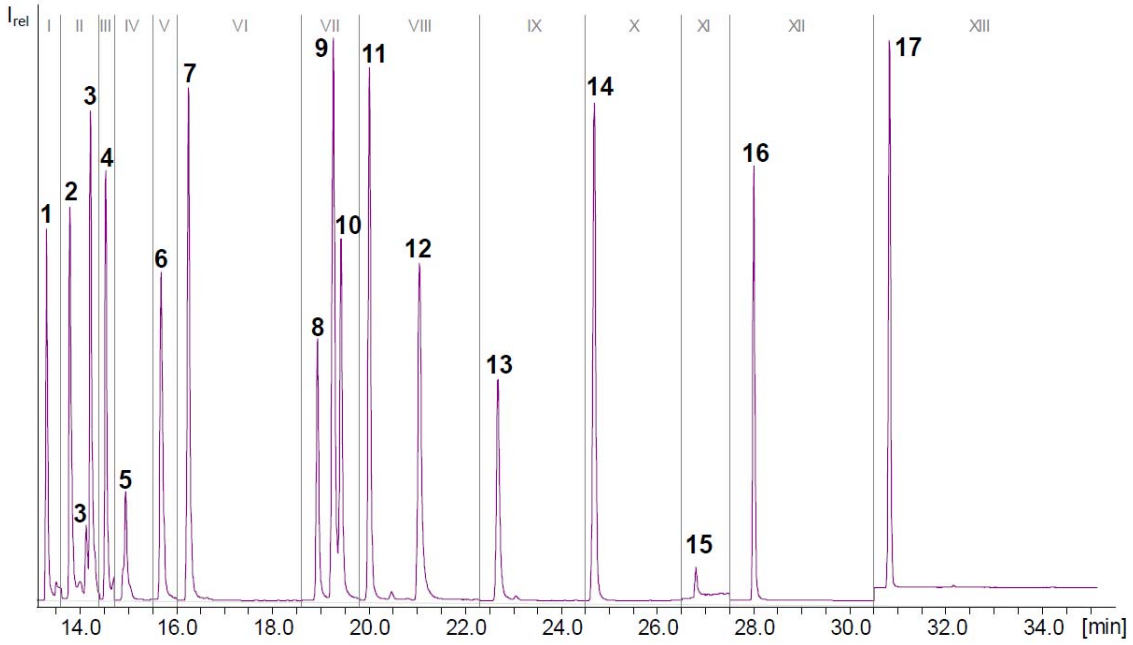

Figure 1. GC/EI-MS SIM chromatogram with time segments (I–XIII) of the standard solution spiked with internal standards (10: 2-methoxyphenol-3,4,5,6-d_4, 14: 1,2,4-trimethoxybenzene). 1: (−)-α-thujone, 2: L-menthone, 3: L-linalool, 4: camphor, 5: isopulegol, 6: (±)-menthol, 7: estragole, 8: geraniol, 9: WS-23, 11: safrole, 12: hydroxycitronellal, 13: L-menthyl lactate, 15: PMD 38 (not relevant for this study), 16: WS-3, 17: coumarin.

and non-mentholated products (#4–13) were available from the same brands (B–F), and there was one brand (A) which provided two non-mentholated and one mentholated cigarette (#1–3) (Figure 2). The remaining samples consisted of three non-mentholated and two mentholated cigarettes (#14–18) of individual brands (G–K) (Figure 2). The three EHTP samples (#19–21) were supplied by the same brand (L) but with three different tastes (Figure 2).

For sample preparation, the tobacco filler was separated from the cigarette paper and filter and was homogenized for 10 sec with a blender (Grindomix 200, Retsch, Haan, Germany) operating at 0.35 × 1000 rpm. Extraction of the tobacco filler was performed by means of Accelerated Solvent Extraction (ASE). For this purpose 11 mL ASE extraction cells were packed as follows: a first cellulose filter covered with superfine glass wool, a second cellulose filter, 2 g of the homogenized tobacco mixed with 2 g diatomaceous earth and spiked with 50 µL ISTD, a third cellulose filter, glass pearls, and finally a fourth cellulose filter. ASE extraction was then performed on an ASE 200 system (Dionex, Idstein, Germany). Each cell was extracted twice with 15 mL of dichloromethane (100 °C, 10 MPa, heating time of 10 min). ASE extracts were evaporated by a gentle stream of nitrogen at room temperature to a volume of 5 mL in a calibrated flask. An aliquot of the concentrated sample solution was measured by GC/EI-MS.

Validation

For validation, analyte-free, fine-cut tobacco was homogenized with a blender (10 sec, 0.35 × 1000 rpm, Grindomix 200, Retsch, Haan, Germany) and was used as a blank sample. For calculation of the limits of detection (LOD) and limits of quantification (LOQ), a standard mix was prepared by diluting the stock solution with dichloromethane to a final concentration of 100 mg/L. In quintuplicate, 2 g of the fine-cut tobacco was spiked with 50 µL of the standard mix and 50 µL ISTD standard solution. Sample preparation was performed as described above (section samples and sample preparation) and sample solutions were measured by GC/EI-MS in SIM-mode (Table 1).

For calibration curves within the recovery experiments, a set of seven standard mix solutions was prepared in the range of 0.1 and 50 mg/L, and 50 µL ISTD standard solution was added to each standard mix solution before measuring. On two different days, 2 g of the fine-cut tobacco was spiked in quintuplicate with 10 µL and 50 µL of the stock mix solution, respectively. Sample preparation was performed as described above before measuring by GC/EI-MS in SIM-mode (Table 1).

RESULTS AND DISCUSSION

Validation

The limits of detection (LOD) and limits of quantification (LOQ) were determined by means of signal-to-noise (S/N) ratios using fine-cut tobacco spiked in quintuplicate with standard mix at a low level (1 mg/L, section validation). The calculated LODs ranged from 0.047–1.8 mg/kg tobacco (LOQs: 0.16–6.1 mg/kg tobacco) (Table 2). The

Table 2. Validation results. LOD and LOQ were determined as mg/kg tobacco. Recovery rates were averaged over both spiking levels.

Analyte	LOD (mg/kg)	LOQ (mg/kg)	R^2	Recovery ± RSD (%)
(−)-α-Thujone	0.16	0.54	0.9985	133 ± 9.1
L-Menthone	0.065	0.22	0.9981	112 ± 12
L-Linalool	0.22	0.72	0.9967	107 ± 12
Camphor	0.13	0.42	0.9994	119 ± 12
Isopulegol	1.2	3.8	0.9998	97 ± 8.6
(±)-Menthol	1.5	5.0	0.9985	102 ± 6.2
Estragole	0.047	0.16	0.9951	93 ± 5.1
Geraniol	0.90	3.0	0.9989	135 ± 7.5
WS-23	0.11	0.35	0.9995	110 ± 12
Safrole	0.32	1.1	0.9981	77 ± 11
Hydroxycitronellal	0.14	0.47	0.9996	92 ± 12
L-Menthyl lactate	0.36	1.2	0.9993	107 ± 7.8
WS-3	0.53	1.8	0.9994	124 ± 8.2
Coumarin	1.8	6.1	0.9968	111 ± 7.4

calibration curves were linear over two orders of magnitude (0.1–50 mg/L) and showed high coefficients of determination (R^2) between 0.9951 and 0.9998 (Table 2). Acceptable recovery rates from 77–135% and satisfying precisions from 5.1–12% were achieved within both spiking levels and no significant differences between the lower and higher spiking levels were observed (Table 2). The fluctuation of recovery rates might have been induced by matrix components interacting with active sites in the GC inlet and column and by loss of analytes during sample preparation (29).

Percentages of flavoring compounds in cigarettes and EHTPs

In general, only seven out of 14 different tested flavoring compounds were present within the samples (WS-3, geraniol, menthol, isopulegol, camphor, L-linalool, and L-menthone). α-Thujone, estragole, WS-23, safrole, hydroxy-citronellal, L-menthyl lactate, and coumarin were not detected within the samples. More than half of the tobacco products (12 samples) contained four or five different flavoring compounds. Two samples contained only two compounds (#12: isopulegol + WS-3 and #16: isopulegol + menthol, respectively) whereas there was only one cigarette sample solely containing menthol.

Except one sample (#12), menthol was detected in all tobacco products with a relative amount of 5.79–100% in relation to the total flavor compound concentration (Figure 2). Thereby tobacco products which were non-mentholated showed smaller amounts of menthol (5.79–23.0%) compared to mentholated cigarettes (generally > 99% menthol) (Figure 2). Surprisingly, one non-mentholated cigarette (sample #16) also contained

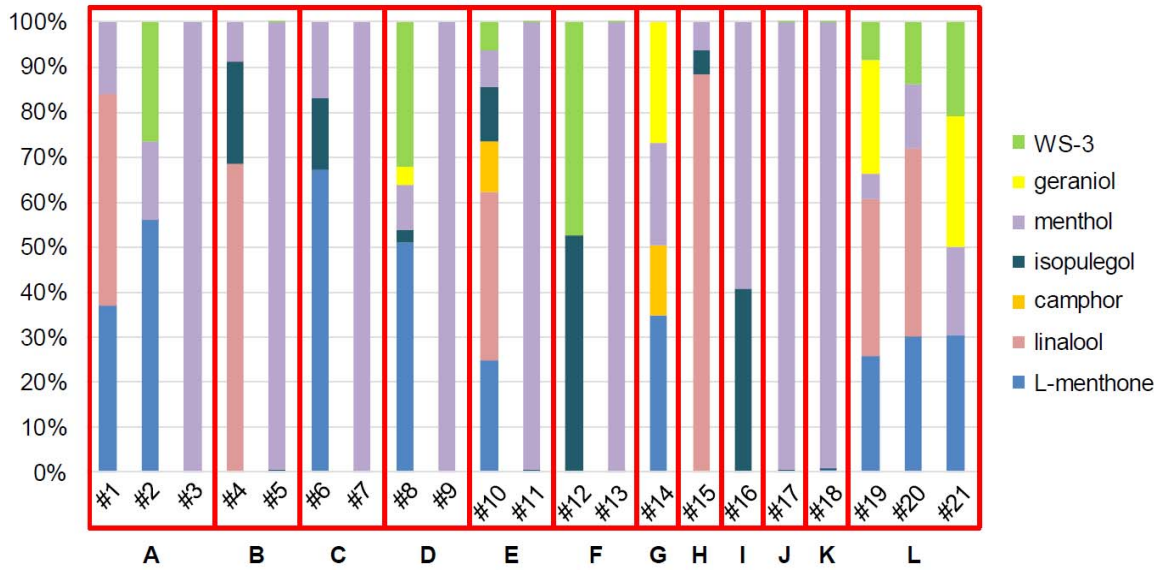

Figure 2. Percentage of tobacco flavoring compounds within the analyzed cigarette samples (#1–18) and EHTP samples (#19–21). Tobacco products of the same brand (A–L) are bordered red. Mentholated cigarettes: #3, #5, #7, #9, #11, #13, #17 and #18; non-mentholated cigarettes: #1, #2, #4, #6, #8, #10, #12, #14, #15 and #16. EHTP samples were supplied by the same brand but with three different tastes.

Table 3. Total amount of flavoring compounds in the samples (#1–21) sub-divided into three TP categories (menthol cigarettes, non-menthol cigarettes, and EHTPs).

Tobacco product	Sample	L-Menthone (ng/stick)	L-Linalool (ng/stick)	Camphor (ng/stick)	Isopulegol (ng/stick)	(±)-Menthol (ng/stick)	Geraniol (ng/stick)	WS-3 (ng/stick)	Total (ng/stick)
Menthol cigarettes	#3	4620	n.d.	n.d.	385	12.6×10^6	n.d.	n.d.	12.6×10^6
	#5	5320	n.d.	1480	4290	12.3×10^6	n.d.	263	12.3×10^6
	#7	8100	n.d.	n.d.	4270	18.6×10^6	n.d.	n.d.	18.6×10^6
	#11	5430	4550	n.d.	5500	15.3×10^6	n.d.	329	15.3×10^6
	#13	5450	4580	1490	269	15.1×10^6	n.d.	949	15.1×10^6
	#17	5860	n.d.	n.d.	2150	6.93×10^6	n.d.	1230	6.94×10^6
	#18	9790	4470	1470	2690	11.4×10^6	n.d.	905	11.4×10^6
	#9	n.d.	n.d.	n.d.	n.d.	275	n.d.	n.d.	275
Non-menthol cigarettes	#1	3580	4540	n.d.	n.d.	1540	n.d.	n.d.	9660
	#2	3350	n.d.	n.d.	6.74	1040	n.d.	1580	5970
	#4	n.d.	5280	n.d.	1770	679	n.d.	n.d.	7730
	#6	3350	n.d.	n.d.	798	841	n.d.	n.d.	4990
	#8	3350	n.d.	n.d.	173	657	268	2100	6540
	#10	3350	5030	1500	1660	1060	n.d.	847	13,500
	#12	n.d.	n.d.	n.d.	1310	n.d.	n.d.	1180	2480
	#14	3350	n.d.	1500	n.d.	2220	2580	n.d.	9640
	#15	n.d.	5070	n.d.	303	354	n.d.	n.d.	5730
	#16	n.d.	n.d.	n.d.	73.9	107	n.d.	n.d.	181
EHTPs	#19	3350	4480	n.d.	n.d.	748	3260	1090	12,900
	#20	3510	4830	n.d.	n.d.	1650	n.d.	1580	11,600
	#21	3350	n.d.	n.d.	n.d.	2180	3210	2300	11,000

n.d.: not detected

about 60% menthol (Figure 2). L-menthone was present in 16 of 21 tobacco products, but in different percentages: Mentholated cigarettes generally contained < 1% L-menthone, whereas the nine non-mentholated L-menthone-positive TPs contained generally > 20% (Figure 2). More than half of the cigarettes (15 samples) featured isopulegol, but in lower amounts compared to menthol and L-menthone: Most of the menthol cigarettes contained < 1% isopulegol, whereas the non-mentholated cigarettes typically featured > 10% isopulegol (Figure 2). Five mentholated cigarettes contained < 1% WS-3. In contrast, there were up to 50% WS-3 (6%–47%) within non-mentholated tobacco products (Figure 2). Three menthol cigarette samples contained < 1% of the flavoring compound L-linalool whereas six non-mentholated tobacco products showed higher percentages of L-linalool (35–89%) (Figure 2). Camphor (0.05–15%) was detected in five cigarette samples whereas geraniol was exclusively detected in two non-menthol cigarette samples (4% and 26%) and two EHTP samples above the LOQ (25% and 29%) (Figure 2).

Flavoring compound patterns within TPs of the same brand

Furthermore, we wanted to investigate whether tobacco products of the same brand provided a brand specific flavoring compound pattern. Although for cigarettes labelled as mentholated, this flavouring comprised > 99% of the total content of flavoring compounds and most of the non-mentholated samples showed higher amounts of L-menthone and L-linalool, in addition to comparatively small amounts of menthol. It is thus not possible to state that there are brand-specific flavoring compound patterns (Figure 2). Flavoring compounds used in EHTPs were quite comparable to those used in non-mentholated cigarettes since EHTPs contained lower amounts of menthol but different substitute compounds (L-linalool, L-menthone, WS-3 and geraniol) in a balanced share (Figure 2). Noteworthy: in comparison to non-mentholated cigarettes geraniol was used almost exclusively within EHTPs but no isopulegol could be detected (Figure 2).

Although all three cigarettes of brand A contained L-menthone and menthol, there were marked differences, since the mentholated cigarettes (sample #3) contained > 99% menthol and small amounts of L-menthone and isopulegol whereas the non-mentholated sample #1 contained L-menthone, menthol, and L-linalool (Figure 2). In addition to L-menthone and menthol, WS-3 and small amounts of isopulegol were detected within the non-mentholated sample #2 (Figure 2). Both samples of brand B contained isopulegol and menthol (Figure 2). In addition to > 99% menthol, sample #5 contained further L-menthone, camphor and WS-3 while the non-mentholated sample #4 contained only ~9% menthol, 23% isopulegol but a high share of L-linalool (68%) (Figure 2). Samples of brand C were the only cigarettes of the same brand with comparable qualitative applications of flavoring compounds since both contained L-menthone, isopulegol and menthol (Figure 2). However, their shares differed considerably: The non-mentholated sample #6 contained more than 50% of L-menthone and a balanced share of isopulegol and menthol (both ~15%) whereas sample #7 contained > 99% menthol and < 1% L-menthone and isopulegol (Figure 2). Whereas sample #9 of brand D was

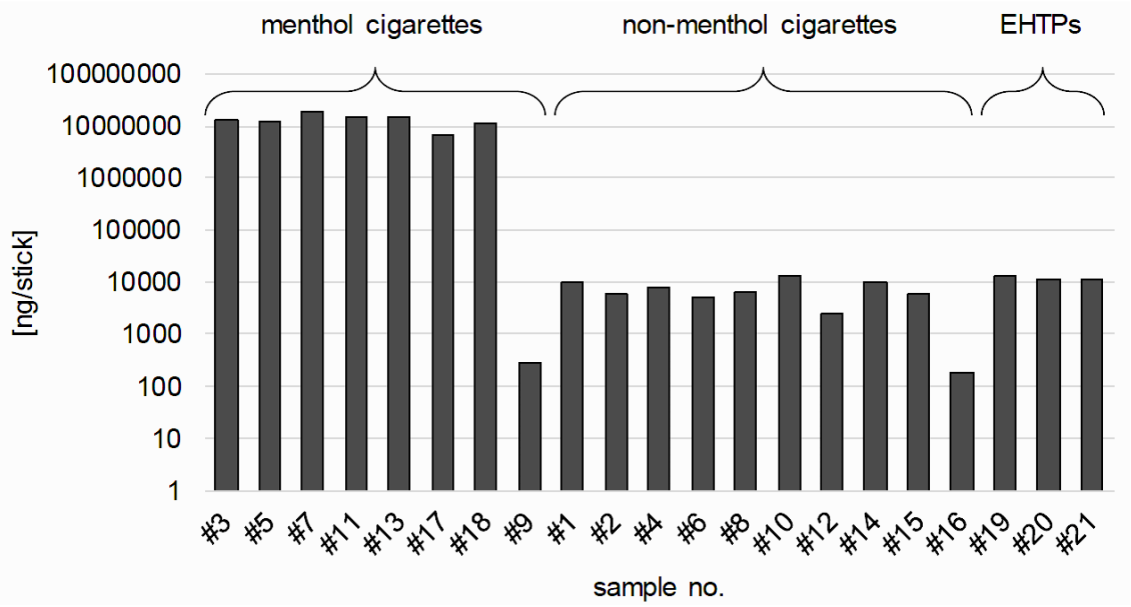

Figure 3. Total amount of flavoring compounds detected in the samples (#1–21) sub-divided into three TP categories (menthol cigarettes, non-menthol cigarettes, EHTPs).

the only sample made of 100% menthol, the non-mentholated sample #8 contained > 50% L-menthone, > 30% WS-3, > 10% menthol and a small amount of isopulegol (Figure 2). Furthermore, the non-mentholated sample #8 was one of two non-mentholated cigarettes which contained geraniol (Figure 2). Both samples of brand E were comparable in their qualitative application of flavoring compounds since both contained L-menthone, L-linalool, isopulegol, menthol and WS-3 (Figure 2). Compounds in the non-mentholated sample #10 were used in a very balanced manner and sample #11 contained next to > 99% menthol < 1% of the other flavoring compounds each (Figure 2). The non-mentholated sample #12 of brand F is the only cigarette exclusively containing two different compounds in the same proportion (50% isopulegol, 50% WS-3) (Figure 2). Since > 99% of menthol was within sample #13, it also contained marginal percentages of L-menthone, L-linalool, camphor, isopulegol and WS-3 (Figure 2). Each of the three EHTPs contained L-menthone, menthol and WS-3 (Figure 2). Differences in taste might be achieved by means of a slight variation in flavoring compound application. Whereas sample #19 contained L-linalool and geraniol both, sample #20 only contained L-linalool as additive compound and sample #21 contained geraniol as the only compound besides menthole and L-menthone (Figure 2). Nothewor-thy: none of the EHTPs contained isopulegol (Figure 2).

Flavoring compound patterns within the three different TP categories

In general, the total amount of flavoring compounds detected in TPs was, with two exceptions, in the same order of magnitude within one TP category: Levels in menthol cigarettes ranged from 7,000 µg/stick to 20,000 µg/stick. However, sample #9 only featured 0.275 µg/stick (Figure 3, Table 3). The content of flavouring compounds other than menthol in non-menthol cigarettes ranged from 2.00–13.0 µg/stick, hence they were about three orders of magnitude lower in concentration than in menthol cigarettes (Figure 3, Table 3). The only exception among the non-mentholated cigarettes was sample #16 which contained only 0.181 µg of flavorings/stick (Figure 3, Table 3). The total flavoring content in EHTPs (~10 µg/stick) was in the same order of magnitude like the one detected in non-menthol cigarettes (Figure 3).

While seven flavoring compounds (L-menthone, L-linalool, camphor, isopulegol, menthol, geraniol and WS-3) were traceable in the non-menthol cigarettes, no geraniol could be detected in concentrations above the LOD in mentholated ones. Neither isopulegol nor camphor were present in EHTPs (Figure 4–5). Instead, EHTPs showed relatively higher amounts of L-menthone, L-linalool and WS-3 as menthol substitute compounds (Figure 4–5). Furthermore, α-thujone, estragole, WS-23, safrole, hydroxycitronellal, L-menthyl lactate and coumarin were generally below the LOD.

Flavoring compounds present in all three TP categories

Comparison of menthol cigarettes, non-menthol cigarettes and EHTPs indicated that the highest amounts of flavoring compounds for menthol were determined in mentholated cigarettes (mean: 11.5 mg/cig stick) which was well comparable to levels described in the literature (0.516–19.6 mg/cig) (9, 27, 30–31). Menthol levels in non-mentholated cigarettes and EHTPs were about four orders of magnitude lower (mean: 0.944 µg/cig stick and 1.524 µg/EHTP stick, respectively) (Figure 4) and were in the same range reported by Ai *et al.*, Paschke *et al.*, and Merckel *et al.* for non-mentholated cigarettes (0.019–

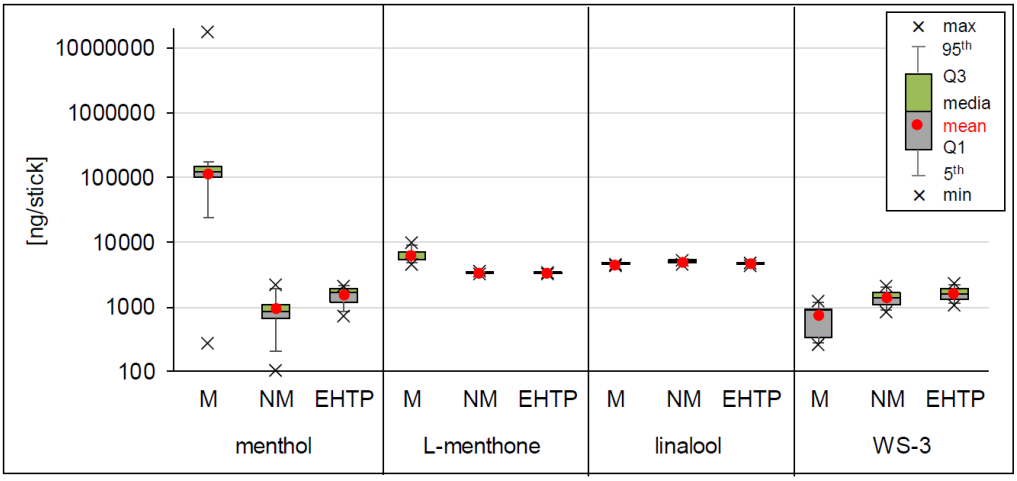

Figure 4. Boxplot of the concentrations of flavoring compounds (menthol, L-menthone, linalool, and WS-3) determined in menthol cigarettes (M), non-menthol cigarettes (NM), and EHTPs in ng/stick.

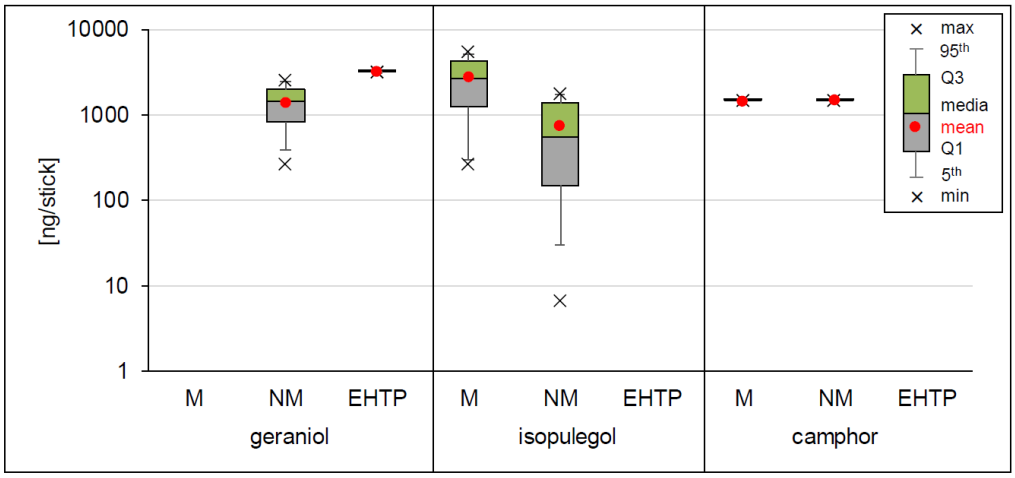

Figure 5. Boxplot of the concentrations of flavoring compounds (geraniol, isopulegol, and camphor) determined in menthol cigarettes (M), non-menthol cigarettes (NM), and EHTPs in ng/stick.

73.5 µg/cig) (9, 27, 32). The second highest concentrated flavoring compound was L-menthone in mentholated cigarettes with a mean value of 6.37 µg/cig stick (Figure 4) which was equal to values provided by PASCHKE *et al.* (1.38–37.8 µg/cig) (27). The mean level of L-menthone in non-mentholated cigarettes and EHTPs was about a factor of two lower than those determined in menthol cigarettes, and was quite comparable in both tobacco products with mean values of 3.39 µg/cig stick and 3.40 µg/EHTP stick, respectively (Figure 4). PASCHKE *et al.* reported a L-menthone content of 0.0359–0.0379 µg/cig and 0.0226–0.103 µg/cig respectively in American Blend cigarettes and additive-free cigarettes, which is one to two orders of magnitude lower than the data presented herein (27). L-Linalool was the only compound which showed comparable mean values for all three tobacco products (4.53 µg/cig stick in mentholated cigarettes, 4.98 µg/cig stick in non-mentholated cigarettes and 4.65 µg/stick in EHTPs) (Figure 4). These values were in the range of those determined by PASCHKE *et al.* for menthol cigarettes (0.67–4.05 µg/cig) (27). WS-3 was detected in each TP category with an increasing share in the following order: menthol cigarettes (0.736 µg/cig stick) < non-mentholated cigarettes (1.42 µg/cig stick) < EHTPs (1.66 µg/stick) (Figure 4).

Flavoring compounds present only in two TP categories

The mean level of geraniol in EHTPs (3.23 µg/stick) was comparable to the level of L-menthone (3.40 µg/stick) in EHTPs, whereas the geraniol content in non-mentholated cigarettes (1.42 µg/cig stick) was half the mean level of L-menthone (3.39 µg/cig stick) (Figure 5). Geraniol could not be detected in menthol cigarettes, which was in agreement with observations reported by PASCHKE *et al.* (27) (Figure 5). Compared to non-mentholated cigarettes,

mentholated ones contained three times more isopulegol with a mean value of 2.79 µg/stick compared to 0.761 µg/stick (Figure 5). PASCHKE *et al.* could not determine isopulegol within American blend cigarettes or additive-free cigarettes but the isopulegol content in mentholated cigarettes was similar to the one presented in this study (0.73–6.00 µg/cig) (27). In contrast to the cigarette samples, no isopulegol could be detected within EHTPs (Figure 5). Camphor content was nearly identical within mentholated cigarettes and non-mentholated ones (1.48 µg/stick and 1.50 µg/stick) but this flavoring compound was not detected in EHTPs (Figure 5).

CONCLUSIONS

Although ten of the 18 analyzed cigarette samples were non-mentholated, each of them (except for one sample) contained significant amounts of menthol. Higher menthol content was detected within mentholated cigarettes where menthol represents > 99% of the measured flavouring compounds. Since the menthol content was generally lower within non-mentholated cigarettes, they contained higher percentages of L-menthone and L-linalool which were most likely used as menthol surrogate flavoring compounds. Flavoring compounds within EHTPs were quite comparable to those added to non-mentholated cigarettes, though they contained an increased share of geraniol.

Due to specific flavoring compound patterns within the three tobacco product categories, mentholated cigarettes and non-mentholated ones could be clearly distinguished. Furthermore, initial assessments could be made, whether the TP could be assigned to a non-mentholated cigarette or EHTP. However, no brand-specific flavoring compound patterns were identified within the analyzed samples. Menthol, L-menthone, L-linalool, and WS-3 were detected within each TP category. Thereby, the highest amounts of flavoring compounds were determined for menthol and L-menthone in mentholated cigarettes, whereas L-linalool was the only compound detectable at comparable levels in all three TP categories. Geraniol, isopulegol and camphor were also detected in low amounts in the samples but not in each TP category. It is worth mentioning that the well-known and often used flavoring compound isopulegol was not detected in EHTPs but higher amounts of L-menthone were determined within these samples. The content of L-menthone, L-linalool, isopulegol, menthol and geraniol determined within the TPs was comparable to values described in the literature.

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