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Waterpipe smoking: Analysis of the aroma profile of flavored waterpipe tobaccos

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ABSTRACT

In the last years the habit of smoking waterpipes has spread worldwide, especially among young people and emerged as global health issue. Although research is now under way for no less than 40 years in the field of waterpipe smoking, in comparison to cigarette smoking there is still insufficient knowledge on the real composition and the toxicity of the smoke inhaled and the resulting levels of exposure against particular hazardous ingredients. In most cases for waterpipe smoking a highly flavored tobacco called "moassel" is used. However, the number, quantity and toxicity of the added flavorings are widely unknown. In this study the static headspace gas chromatography–mass spectrometry (SHS-GC–MS) was used to identify 79 volatile flavor compounds present in waterpipe tobacco. Among these eleven compounds were analyzed quantitatively. The results show that waterpipe tobacco contains high amounts of the fragrance benzyl alcohol as well as considerable levels of limonene, linalool and eugenol, all of which are known as being allergenic in human skin. The proposed SHS-GC–MS method has been validated and found to be accurate, simple and characterized by low limits of detection (LOD) in the range of 0.016 to 4.3 μg/g tobacco for benzaldehyde and benzyl alcohol, respectively. The identification and characterization of waterpipe tobacco ingredients indeed reveals crucial for the assessment of potential health risks that may be posed by these additives in smokers.

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1. Introduction

Regardless of its form and function, waterpipes (shisha, hookah or narghile) represent a traditional aid for the consumption of tobacco. Formerly smoked mainly in Asia and Northern Africa [1], the waterpipe has spread worldwide especially among young people and emerged as global health issue [2,3]. A recent study conducted among U.S. university students revealed that waterpipe use was second after cigarettes in terms of tobacco use [4]. In the last years several studies focused on the toxicological effects of waterpipe smoke [5–9]. These studies clearly demonstrate that waterpipe smokers inhale high levels of hazardous compounds, such as carbon monoxide [5], polycyclic aromatic hydrocarbons [5,6], carbonyls [7,8] or primary aromatic amines [9], thereby pointing to considerable health risks.

For waterpipe smoking the following three kinds of tobacco are commonly used: "moassel", "jurak" and "tumbak" [10,11]. The introduction of highly flavored "moassel" in the 1990s is considered as one of the main factors for the rapid spread of this form of smoking [12]. Many waterpipe smokers explain that one of the reasons they started waterpipe smoking is the pleasant smell and the good taste of the smoke [13,14]. In addition, some smokers reported that the availability of a great variety of flavored tobaccos prompts people to start smoking [15]. An internet survey showed that today more than 250 different waterpipe tobacco flavors are commercially available. Among these "two apples" represents one of the most popular flavors. Correspondingly, in the Western hemisphere (i.e. North America and Europe) "moassel" is used almost exclusively. On the other side, the pleasant smell of the smoke may override the risk perception of smokers and therefore waterpipe smoking might be perceived less harmful.

For cigarettes it is well known that during the production process several additives are put into the tobacco. Many of these additives are used to improve taste and to decrease harshness [16]. In contrast, little is known on the composition of waterpipe tobacco. It can be assumed, however, that also to these matrix large quantities of flavoring substances are added although the toxicological effects of these flavors are largely unknown. Recently Sepetdjian et al. reported that waterpipe smoke, compared to cigarette smoke, contains 1000 times higher quantities of the artificial flavoring substance ethyl vanillin [17]. In light of this large knowledge gap it seems essential to gain much more analytical and toxicological information on the waterpipe tobacco additives used.

For the analysis of tobacco additives and volatiles mainly gas chromatography coupled to a mass selective detector (GC–MS)

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is employed. However, in most cases, prior to analysis, a preconcentration step becomes necessary [18]. Generally the following techniques are used for this: enrichment on solid sorbents [19], liquid–liquid microextraction (LLME) [20], solid-phase microextraction (SPME) [21–23], stir bar sorptive extraction (SBSE) [24,25], and static (SHS) or dynamic headspace (DHS) [26–35]. The HS-SPME-GC–MS technique for example, was used by Merckel et al. to identify 89 individual tobacco additives [21]. On the other hand, the SHS-GC–MS technique was employed for the analysis of olives and olive oil [28], green propolis [30] and lubricant oil [32]. In this study we used the SHS-GC–MS technique for the analysis of waterpipe tobacco by focusing on ethyl 2-methylbutyrate, hexanal, limonene, 1-hexanol, *cis*-3-hexen-1-ol, benzaldehyde, linalool, menthol, benzyl acetate, *trans*-anethole and benzyl alcohol. To our knowledge, data on these eleven flavored compounds have not been reported for waterpipe tobacco before. Nonetheless such data are crucial for the assessment of potential health risks associated with these additives after being inhaled by the waterpipe smoker.

2. Materials and methods

2.1. Reagents and materials

All chemicals used in this study were of analytical grade. The authentic standards of the flavoring substances listed in Table 1 were obtained from Sigma-Aldrich (Munich, Germany). Hexanal-d_{12}, 1-hexan-d_{13}-ol and benzyl-2,3,4,5,6-d_5 alcohol were used as internal standards. Hexanal-d_{12} was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), whereas 1-hexan-d_{13}-ol and benzyl-2,3,4,5,6-d_5 alcohol were purchased from Sigma-Aldrich. Ethyl acetate, sodium chloride, potassium chloride and sodium sulfate were obtained from Sigma-Aldrich. Waterpipe tobaccos were purchased from different manufacturers, namely Nakhla Tobacco (Cairo, Egypt), Middle East Tobacco (Zarqa, Jordan), Al Fakher Tobacco Trading Co. (Ajman, United Arab Emirates), Eastern Tombac & Tobacco EST. (Amman, Jordan) and Soex India Pvt. Ltd. (Mumbai, India). Some of the waterpipe tobaccos were imported to Germany and are therefore not in accordance with the German tobacco regulation. Glass beads (Ø 0.8 cm) were obtained from Omnilab (Bremen, Germany).

2.2. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was performed on an HP 6890 gas chromatograph equipped with an Agilent MSD 5975C mass spectrometer (Agilent Technologies, Waldbronn, Germany) and a Gerstel Cold Injection System (CIS) (Gerstel, Mühlheim an der Ruhr, Germany). A Multi Purpose Sampler (MPS-2, Gerstel) was used for automated HS measurements. Chromatographic separation was achieved with a DB-WAX (30 m × 0.25 mm i.d. × 0.25 μm film) capillary column (Agilent Technologies). The GC–MS conditions were as follows: injection mode: split; split ratio: 12:1; CIS temperature program: initial temperature: 45 °C; 1. heating rate: 12 °C/s; 1. end temperature: 300 °C; 1. hold time: 2.0 min; 2. heating time: 10 °C/s; 2. end temperature: 350 °C; 2. hold time: 3.0 min; helium was used as carrier gas at a constant flow of 1.0 ml/min; oven temperature program: initial temperature: 45 °C; 1. hold time: 2.0 min; 1. heating rate: 8 °C/min; 1. end temperature: 100 °C; 2. hold time: 0.0 min; 2. heating rate: 15 °C/min; 2. end temperature: 250 °C; 3. hold time: 5.0 min; total run time: 23.88 min; detector conditions: detector: MSD; MSD acquisition mode: SCAN (*m/z* 38–380); MSD transfer line: 280 °C; MSD ion source temperature: 230 °C; MSD quadrupole temperature: 150 °C; MSD solvent delay: 4.0 min; MSD data acquisition rate: 4.13 scans/min. Chromatograms were recorded and processed with GC MSD ChemStation (E.01.00.237, Agilent Technologies) software. Data on retention times as well as quantifier and qualifier ions are summarized in Table 1. Quantification was performed by using the ion chromatograms of quantifiers upon extraction from total ion chromatograms. Additional peaks were identified by using the NIST08 (National Institute of Standards and Technology, version 8.0) and W8N05ST (Wiley, version 8.0 and NIST, version 5.0) mass spectral libraries.

2.3. Quantification of flavoring substance contents in waterpipe tobaccos

For the analysis of waterpipe tobacco 3.00 g (±0.05 g) of a well homogenized sample was added to a 20 ml headspace vial. Subsequently 4.0 g (±0.1 g) sodium chloride, 10 ml water and 35 μl of the internal standard solution (hexanal-d_{12}; 1-hexan-d_{13}-ol; benzyl-2,3,4,5,6-d_5 alcohol) were added. The headspace vials were tightly closed (18 mm magnetic screw caps; septum: butyl red/PTFE gray) and shaken vigorously for 1 min on a Vortex Genie 2 (Scientific Industries, Inc., New York, USA). The headspace vials were transferred to the autosampler tray and incubated for 15.0 min at a temperature of 90 °C (agitator speed: 250 rpm). Then 1 ml of the vial headspace (syringe temperature: 105 °C; vial penetration: 22.0 mm) was injected into the GC–MS. Each headspace vial was punctured only once and then discarded. For each waterpipe tobacco, five headspace vials were prepared and measured

Table 1
Purities, corresponding internal standards, retention times, quantifier and qualifier ions of eleven volatile flavor substances. These compounds are listed in the order of their retention times.

Substance	Purity (%)	Substance number	CAS number	Internal standard	Retention time (min)	Quantifier (m/z)	Qualifier (m/z)
Ethyl 2-methylbutyrate	99.0	4	7452–79–1	Hexanal-d_{12}	4.74	57	41
Hexanal	98.0	8	66–25–1	Hexanal-d_{12}	5.17	56	44
Limonene	97.0	16	5989–27–5	Hexanal-d_{12}	7.19	68	93
1-Hexanol	99.5	32	111–27–3	1-Hexan-d_{13}-ol	9.95	56	43
cis-3-Hexen-1-ol	98.0	34	928–96–1	1-Hexan-d_{13}-ol	10.38	67	41
Benzaldehyde	99.0	46	100–52–7	1-Hexan-d_{13}-ol	12.06	106	77
Linalool	97.0	47	78–70–6	Benzyl-2,3,4,5,6-d_5 alcohol	12.25	71	93
L-Menthol	99.0	52	2216–51–5	Benzyl-2,3,4,5,6-d_5 alcohol	13.21	71	81
Benzyl acetate	99.0	60	140–11–4	1-Hexan-d_{13}-ol	13.98	108	91
trans-Anethole	99.0	62	4180–23–8	Benzyl-2,3,4,5,6-d_5 alcohol	14.78	148	117
Benzyl alcohol	99.0	65	100–51–6	Benzyl-2,3,4,5,6-d_5 alcohol	15.13	108	79
Hexanal-d_{12}	98.8[a]	–	1219803–74–3	–	5.07	48	64
1-Hexan-d_{13}-ol	98.0[a]	–	204244–84–8	–	9.76	64	78
Benzyl-2,3,4,5,6-d_5 alcohol	98.0[a]	–	68661–10–9	–	15.12	113	84

[a] Atom % D.

Table 2
Calibration range, linear regression data, limits of detection (LOD) and limits of quantification (LOQ) of eleven volatile flavor substances.

Substance	Calibration range (μg/ml)	Determination coefficient (r^2)[a]	LOD (μg/ml)	LOQ (μg/ml)	LOQ (μg/g tobacco)	Intraday precision (%)[b]	Interday precision (%)[c]
Ethyl 2-methylbutyrate	0.10–10.0	0.997	0.071	0.176	0.585	3.0	9.9
Hexanal	0.25–5.0	0.996	0.057	0.131	0.438	2.8	8.2
Limonene	0.01–1.0	0.999	0.007	0.018	0.060	6.5	11
1-Hexanol	0.10–10.0	0.999	0.043	0.118	0.393	2.2	4.6
cis-3-Hexen-1-ol	0.10–10.0	0.999	0.059	0.157	0.525	3.4	5.7
Benzaldehyde	0.05–1.0	0.999	0.005	0.013	0.044	6.7	6.2
Linalool	0.05–1.0	0.996	0.021	0.055	0.184	7.2	5.2
L-Menthol	0.05–1.0	0.998	0.014	0.037	0.124	6.8	7.4
Benzyl acetate	0.25–5.0	0.996	0.127	0.337	1.123	5.6	8.2
trans-Anethole	0.50–10.0	0.997	0.225	0.580	1.93	6.5	4.9
Benzyl alcohol	5.0–100	0.999	1.29	2.87	9.56	3.3	9.3

[a] $n=3$.
[b] $n=6$.
[c] $n=5$.

consecutively. Standards for quantification were prepared using a slightly modified protocol. Instead of the sample (waterpipe tobacco) the required amount of the standard solution was added. To ensure that the headspaces of samples and standards were the same, seven glass beads (Ø 0.8 cm) were added.

Standard stock solutions of each analyte were prepared in analytical grade ethyl acetate and stored for up to two months in a refrigerator (−20 °C). By diluting the stock solutions with ethyl acetate working standards containing all analytes of interest were prepared every two weeks and also stored in a refrigerator (−20 °C). Each working solution was set up so that no more than 10 μl per standard were added to an headspace vial (see Table 2). Internal standards were also prepared in ethyl acetate with the following concentrations: hexanal-d_{12}: 1.3 mg/ml; 1-hexan-d_{13}-ol: 2.8 mg/ml; benzyl-2,3,4,5,6-d_5 alcohol: 26 mg/ml.

2.4. Method validation scheme

The SHS-GC–MS method proposed for the quantification of eleven volatile flavor compounds in waterpipe tobacco was validated in terms of specificity, linearity, precision, recovery, limits of detection (LOD) and limits of quantification (LOQ). Identification of each substance was achieved by comparison of retention times of the respective substances to the authentic standards as well as comparing the acquired mass spectra (full-scan mode) with the library spectra. To examine the purity of each peak the peak area ratio of the qualifier and quantifier ions were determined and compared between the standards and the samples. For quantification the ion with the highest intensity was selected. Quantifier and qualifier ions are shown in Table 1. To improve the ruggedness and reliability of the method we further introduced three internal standards, namely hexanal-d_{12}, 1-hexan-d_{13}-ol and benzyl-2,3,4,5,6-d_5 alcohol, which were added during sample preparation (see Section 2.3). The assignment of the internal standards was determined by the sixfold injection of a standard solution. All analytes were evaluated with respect to all internal standards and the final assignment was made based on the lowest standard deviation (see Table 1). For determination of linearity calibration curves were established ($n=3$), whereby the calibration range for each analyte was determined in preliminary experiments and adjusted if necessary (see Table 2). The concentration of the internal standards was set as follows: hexanal-d_{12}: 4.6 μg/ml, 1-hexan-d_{13}-ol: 9.8 μg/ml and benzyl-2,3,4,5,6-d_5 alcohol: 91 μg/ml. Precision was evaluated by consecutively measuring one calibration point six times (intraday) and by measuring the same calibration point on five days within two weeks (interday) under optimized conditions [36]. For recovery experiments three analytes were selected (ethyl 2-methylbutyrate, benzaldehyde, benzyl alcohol) and added to a waterpipe tobacco sample, wherein three concentration levels were prepared (ethyl 2-methylbutyrate: 1.0, 5.0, 10 ppm; benzaldehyde: 0.1, 0.5, 1.0 ppm; benzyl alcohol: 10, 50, 100 ppm). Subsequently, the spiked waterpipe tobacco was processed as described in Section 2.3 and the recovery rates were determined. Limit of detection (LOD) and limit of quantification (LOQ) values were determined according to the German Industrial Norm (DIN) 32645 [37]. For this purpose, calibration curves were established based on which the LOD and LOQ values were calculated. In this context Brüggemann et al. [38] demonstrated that the LOD and LOQ values calculated by using the equations given in DIN 32645 correspond to those calculated in accordance with ISO 11843 and Commission Decision 2002/657/EC of the EU. The formal comparison was based on a numerical example given in DIN 32645, with all necessary equations for the calculation of the LOD and LOQ values given in the text [38].

3. Results and discussion

In this study an accurate and simple multi-analyte method for the analysis of flavoring substances was developed and applied on commercial waterpipe tobaccos. Initially various analytical techniques, including SHS-, HS-SPME- and DHS-GC–MS were tested for their suitability. The most promising results were obtained by applying the SHS-GC–MS technique. Using the HS-SPME- and DHS-GC–MS techniques revealed several disadvantages such as carryover, broad peaks and peak tailing. Subsequently, a suitable SHS-GC–MS method for the analysis of volatile flavor compounds in waterpipe tobacco has been developed and validated. In the following sections the method development and validation steps as well as the results are described.

3.1. Method development

3.1.1. Optimization of SHS parameters

Method development was undertaken in several steps. First, some of the waterpipe tobaccos were screened using a previously published SHS-GC–MS method [28]. Due to the lack of literature data the screening was necessary to gain an overview on the volatile flavor additives used in waterpipe tobaccos. Out of the compounds identified eleven were selected for further investigation and quantification (see Table 1). The final selection of the volatile flavor additives was based on their appearance in the tobacco samples, their commercial availability and from a health safety point of view.

In the second step an optimized method for the analysis of the compounds under consideration has been developed. To this end, the following parameters were considered: sample amount, addition of water and salt, incubation temperature, and incubation time. All optimization experiments were performed with real samples (waterpipe tobacco) containing the majority of the selected substances. The influence of the sample amount was determined through a weighted sample containing different amounts of waterpipe tobacco (0.10–3.00 g). The best results, with respect to a maximized peak area, were obtained with a sample amount of 3.00 g. In the literature it has been described that the addition of water to the HS vial can enhance the specificity and the precision of the method [28]. The addition of water in the range between 2.5 and 12.5 ml was evaluated and found that the best results, with respect to the lowest standard deviation, were obtained by addition of 10 ml water. In this context, the addition of salt is favorable in most cases (salting-out effect) [26,28]. A literature survey revealed that mainly sodium chloride (NaCl) is used [39,40], yet potassium chloride (KCl) [28] or sodium sulfate (Na_2SO_4) [41,42] are also occasionally used. We tested these three different salts to figure out which of them is best suited for headspace analysis. In the presence of KCl and Na_2SO_4 a distinct peak tailing occurred that can not be explained at the moment. Therefore NaCl was selected and the addition of 4.0 g (saturated solution) provided the best results. The incubation temperature was varied between 30 and 95 °C. Fig. 1 illustrates the temperature dependency of the peak areas of six selected flavoring substances. It becomes evident that the peak areas are increasing with higher incubation temperatures. The largest increases were found between 70 and 80 °C. At 90–95 °C the measured peak areas then slightly decreased again. This is probably due to a shift of the partition equilibrium of the considered compounds to lower concentrations. Therefore the incubation temperature was set to 90 °C. The syringe temperature (105 °C) was kept 15 °C above the incubation temperature to avoid condensation of the analytes. The incubation times were varied between 5 and 30 min. In Fig. 2 the time dependency of the peak areas of six selected flavoring substances is depicted. Since the highest peak areas were achieved at an incubation time of 15 min, the incubation time was set to 15 min.

3.1.2. Optimization of GC parameters

The appropriate choice of the GC column is crucial for establishing an efficient, robust and highly specific SHS-GC method. The group of volatile flavor compounds includes a multitude of different compound classes such as alcohols, carbonyls, esters and ethers. According to recent literature for the analysis of these chemicals various mid to highly polar columns were used (e.g. DB-17MS, DB-225MS, DB-WAX; Agilent Technologies) [19,22,43,44]. For the separation of the eleven compounds listed in Table 1, two of the specified columns were tested, namely DB-17MS and DB-WAX. In principle both columns are suitable for the separation of these compounds, yet better results were obtained with the DB-WAX column. For further optimization a number of additional GC parameters were evaluated, such as the split ratio (1:1; 6:1; 12:1; 25:1; 50:1), the GC oven temperature program (temperature ramping rate (8, 15, 20, 25 °C); temperature hold time (0–5 min)), and the injection volume (250, 500, 1000, 1500, 2000 μl). The final range of parameters is listed in Section 2.2. They allow a very good separation of the analytes in less than 24 min. Fig. 3 depicts chromatograms of three commercially available waterpipe tobaccos (tobaccos "*a*" ("Two Apples"), "*h*" ("Melon"), and "*k*" ("Banana")) obtained under the optimized method parameters.

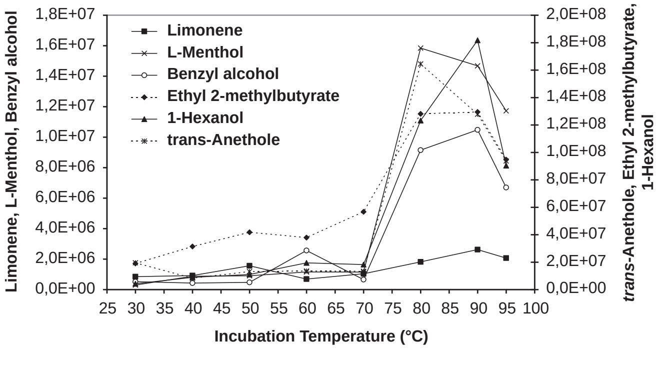

Fig. 1. Peak areas of six selected volatile flavor substances obtained by SHS-GC–MS analyses of one commercial waterpipe tobacco. The incubation temperature varied from 30 to 95 °C (sample amount: 3.00 g; amount of water: 10 ml; amount of NaCl: 4.0 g; incubation time: 20 min).

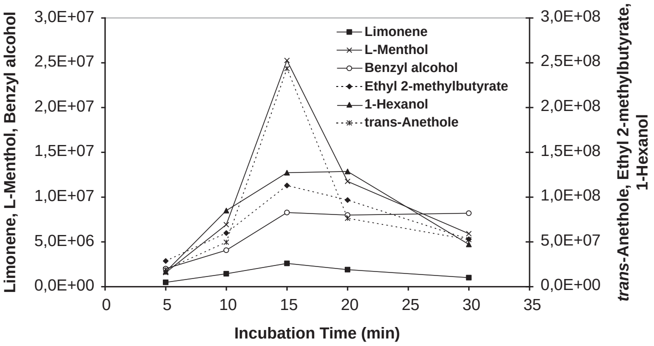

Fig. 2. Peak areas of six selected volatile flavor substances obtained by SHS-GC–MS analyses of one commercial waterpipe tobacco. The incubation time varied from 5 to 30 min (sample amount: 3.00 g; amount of water: 10 ml; amount of NaCl: 4.0 g; incubation temperature: 90 °C).

3.2. Method validation results

3.2.1. Specificity

Retention times and mass spectra of samples and standards were in accordance to each other. Furthermore the differences between the peak area ratio of the qualifier and quantifier ions (using one qualifier ion was sufficient) for standards and samples did not exceed 5.3% (tobacco "*g*", benzyl alcohol, see Table S-1) and were therefore within the range recommended by the Commission of the European Communities in the guideline "*concerning the performance of analytical methods and the interpretation of results*" [45]. The recommended value for GC–MS with electron ionization (EI) is 10%.

3.2.2. Linearity

The constructed calibration curves showed an excellent linearity for each volatile flavor substance, with typical values for the determination coefficients (r^2) between 0.996 and 0.999 (see Table 2).

3.2.3. Precision

Values for precision were found between 2.2% (for 1-hexanol) and 11% (for limonene) and thus in a good range for SHS-GC–MS. The results are presented in Table 2.

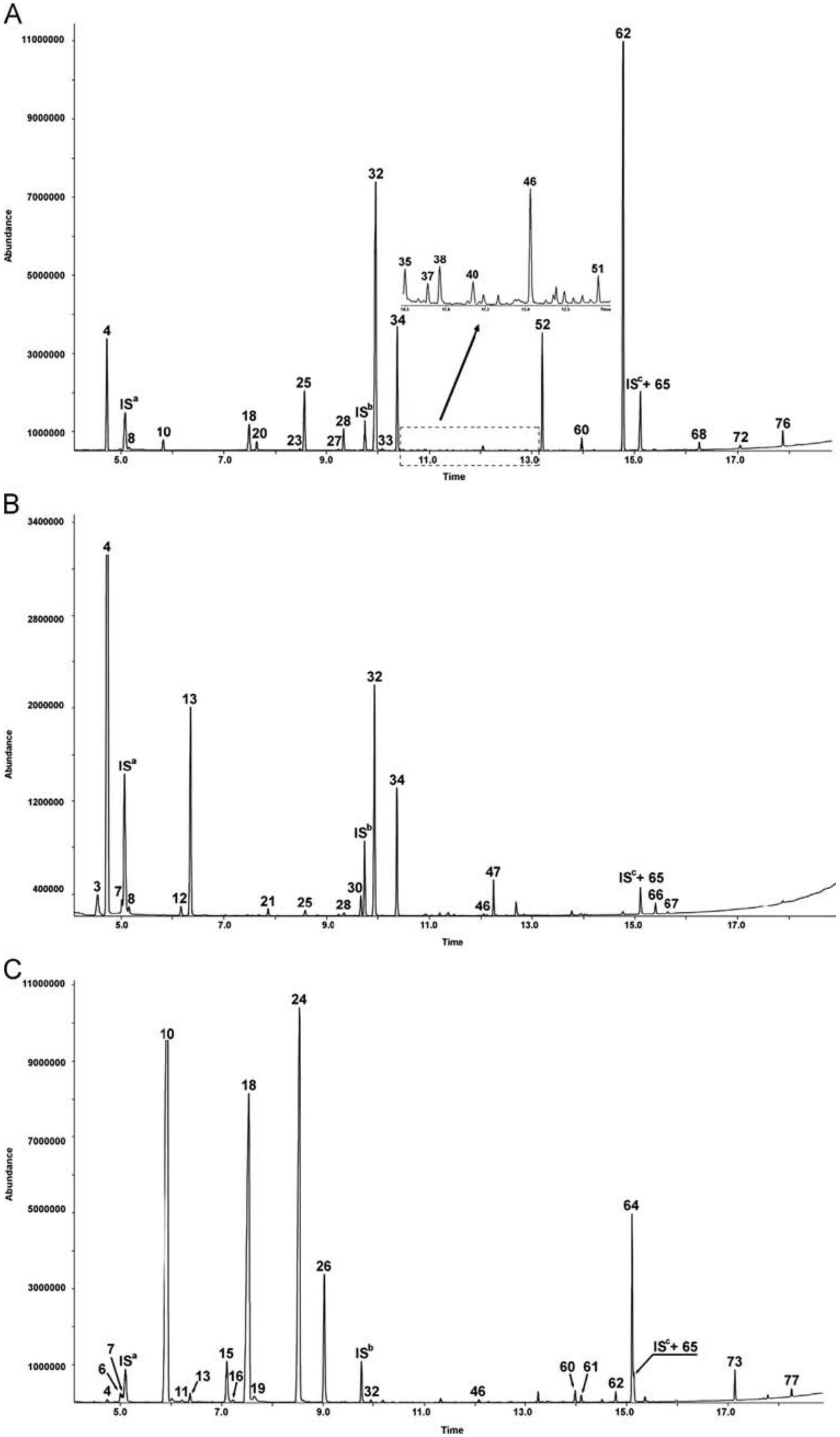

Fig. 3. SHS-GC–MS chromatograms (SCAN mode) of three commercially available waterpipe tobaccos (A, tobacco "*a*" ("Two Apples"); B, tobacco "*h*" ("Melon"); C, tobacco "*k*" ("Banana")); IS, internal standard (a, hexanal-d$_{12}$; b, 1-hexan-d$_{13}$-ol; c, benzyl-2,3,4,5,6-d$_5$ alcohol); for peak assignment see Table 4.

3.2.4. Recovery

The recovery values based on three independent measurements were found in the range between 80 and 120%, in particular as follows: 81.3% (1.0 ppm), 112% (5.0 ppm) and 118% (10 ppm) for ethyl 2-methylbutyrate, 117% (0.1 ppm), 116% (0.5 ppm) and 113% (1.0 ppm) for benzaldehyde and 114% (10 ppm), 96% (50 ppm) and

Table 3

Results for the determination of eleven volatile flavor substances in twelve commercially available waterpipe tobaccos and its comparison to literature data of cigarette tobacco (concentration in μg/g tobacco).

Substances	Tobbaco												
	a “Two Apples” (RSD)[b]	*b* “Two Apples” (RSD)[b]	*c* “Two Apples” (RSD)[b]	*d* “Apple”[a] (RSD)[b]	*e* “Apple”[a] (RSD)[b]	*f* “Apple” (RSD)[b]	*g* “3 Apples Flavour” (RSD)[b]	*h* “Melon” (RSD)[b]	*i* “Raspberry”[a] (RSD)[b]	j “Five Stars” (RSD)[b]	*k* “Banana” (RSD)[b]	*l* “Fruits Molasses” (RSD)[b]	Cigarette tobacco
Ethyl 2-methylbutyrate	27.6 (8.6)	20.3 (22)	47.9 (14)	n.d.	n.d.	133 (4.3)	55.9 (11)	51.4 (8.9)	n.d.	BLQ	BLQ	71.8 (4.5)	2.5[c]
Hexanal	1.48 (9.7)	2.15 (16)	1.14 (24)	BLQ	BLQ	2.84 (5.2)	5.29 (5.3)	BLQ	BLQ	n.d.	n.d.	BLQ	3[c]
Limonene	n.d.	BLQ	0.29 (20)	n.d.	n.d.	0.71 (14)	1.40 (11)	n.d.	n.d.	0.35 (34)	BLQ	BLQ	–
1-Hexanol	336 (11)	214 (6.8)	196 (12)	14.6 (3.4)	BLQ	201 (3.1)	286 (8.3)	89.2 (1.6)	BLQ	n.d.	BLQ	60.5 (6.3)	15[c]
cis-3-Hexen-1-ol	293 (15)	135 (13)	122 (16)	11.7 (3.5)	BLQ	117 (4.2)	232 (3.8)	118 (7.3)	35.3 (6.1)	6.32 (14)	BLQ	137 (8.5)	2.5[c]
Benzaldehyde	7.57 (13)	2.10 (6.2)	3.02 (13)	1.84 (3.2)	0.83 (13)	3.73 (9.6)	7.43 (4.6)	0.59 (12)	6.77 (4.4)	1.35 (9.0)	2.15 (15)	1.87 (6.3)	28–56[d]
Linalool	n.d.	n.d.	BLQ	n.d.	n.d.	0.22 (18)	0.31 (11)	1.70 (56)	n.d.	0.22 (17)	BLQ	BLQ	10[c]
L-Menthol	9.86 (17)	1.26 (28)	3.51 (18)	0.60 (11)	1.55 (47)	5.03 (19)	14.3 (14)	n.d.	BLQ	BLQ	BLQ	5.62 (30)	0.019–13.3[d]
Benzyl acetate	13.0 (24)	BLQ	1.69 (20)	BLQ	BLQ	1.29 (10)	10.9 (17)	n.d.	BLQ	n.d.	2.23 (33)	1.81 (23)	–
trans-Anethole	31.3 (15)	6.69 (22)	40.2 (17)	BLQ	BLQ	50.2 (13)	51.3 (20)	n.d.	n.d.	n.d.	BLQ	16.3 (26)	0.0046–0.23[e]
Benzyl alcohol	830 (7.5)	140 (5.4)	84.3 (7.9)	371 (2.6)	155 (6.1)	79.8 (8.1)	1020 (8.8)	53.6 (2.3)	484 (3.0)	10.6 (14)	424 (13)	280 (4.6)	4.7–41[d]
Sum [μg/g tobacco]	1550	522	500	400	157	595	1680	314	526	18.8	428	575	–

RSD, relative standard deviation; BLQ, below limit of quantification; n.d., not detected.

[a] Waterpipe tobacco made from non-tobacco herbal basic materials (sugarcane).

[b] $n=5$.

[c] See Ref. [54].

[d] See Ref. [21].

[e] See Ref. [55].

Table 4
Volatile flavor substances detected by SHS-GC–MS in twelve commercially available waterpipe tobaccos, their linear retention index (LRI), molecular weight (MW), boiling point (BP), frequency and abundance in the tobaccos analyzed and occurrence in other tobacco products.

Substance		CAS number	LRI[a] (DB-WAX)	MW (g/mol)	BP[b,c] (°C)	Frequency in 12 waterpipe tobaccos	Abundance in 12 waterpipe tobaccos	Reported in other tobacco products[d]
No.	Name							
1	α-Pinene[e]	80-56-8	–	136	155–156	1	0.2%	Yes
2	Ethyl butyrate	105-54-4	1015	116	121	5	0.1–18%	Yes
3	1-Propanol	71-23-8	1019	60	97	1	1.6%	Yes
4	Ethyl 2-methylbutyrate	7452-79-1	1033	130	133	9	0.1–51%	Yes
5	Ethyl isovalerate	108-64-5	1049	130	135	2	0.5–13%	Yes
6	Isopentyl formate	110-45-2	1050	116	124	1	0.2%	Yes
7	Butyl acetate	123-86-4	1053	116	127	2	0.2–0.4%	Yes
8	Hexanal	66-25-1	1063	100	129	9	0.2–0.9%	Yes
9	β-Pinene	127-91-3	1084	136	165–166	1	0.1%	Yes
10	Isoamyl acetate	123-92-2	1107	130	142	9	0.4–22%	Yes
11	Butyl propionate	590-01-2	1128	130	146	1	0.1%	Yes
12	Propyl 2-methylbutyrate	37064-20-3	1128	144	155–156	1	0.4%	No
13	1-Butanol	71-36-3	1137	74	118	2	0.6–9.7%	Yes
14	Methyl hexanoate	106-70-7	1178	130	151	1	0.2%	Yes
15	Isoamyl propionate	105-68-0	1181	144	161	1	2.9%	Yes
16	Limonene	5989-27-5	1188	136	175	7	0.2–0.8%	Yes
17	1,8-Cineole	470-82-6	1205	154	176–177	2	0.2–2.8%	Yes
18	3-Methyl-1-butanol	123-51-3	1207	88	131	10	0.5–41%	Yes
19	Butyl butyrate	109-21-7	1212	144	166	1	0.4%	Yes
20	Hexyl formate	629-33-4	1216	130	155–156	5	0.1–0.6%	Yes
21	Butyl 2-methylbutyrate	15706-73-7	1228	158	179	1	0.3%	No
22	Ethyl hexanoate	123-66-0	1229	144	168	1	2.3%	Yes
23	*cis*-3-Hexenyl formate	33467-73-1	1263	128	154–156	1	0.2%	Yes
24	Isoamyl butyrate	106-27-4	1263	158	178	3	1.2–13%	Yes
25	Hexyl acetate	142-92-7	1268	144	172	8	0.2–3.8%	Yes
26	Isoamyl isovalerate	659-70-1	1293	172	195	2	2.5–2.8%	Yes
27	2-Ethyl-1-butanol	97-95-0	1309	102	149	2	0.1%	Yes
28	*cis*-3-Hexenyl acetate	3681-71-8	1316	142	165–167	7	0.1–1.2%	Yes
29	4-Methyl-2-pentyl-1,3-dioxolane	1599-49-1	1325	158	182–184	1	0.1%	Yes
30	6-Methyl-5-hepten-2-one	110-93-0	1339	126	173	1	0.8%	Yes
31	Rose oxide	16409-43-1	1358	154	86[f]	1	0.4%	Yes
32	1-Hexanol	111-27-3	1359	102	157	11	0.1–20%	Yes
33	*trans*-3-Hexen-1-ol	928-97-2	1367	100	155–156	4	0.1%	Yes
34	*cis*-3-Hexen-1-ol	928-96-1	1387	100	157	10	0.9–7.9%	Yes
35	3-Octanol	589-98-0	1395	130	173	2	0.1–0.2%	Yes
36	Fenchone	1195-79-5	1404	152	193–194	1	0.2%	Yes
37	Hexyl butyrate	2639-63-6	1418	172	204–206	6	0.1–0.7%	No
38	Hexyl 2-methylbutanoate	10032-15-2	1430	186	203–204	3	0.1–0.2%	Yes
39	Hexyl valerate	1117-59-5	1431	186	226–227	1	0.1%	No
40	Furfural	98-01-1	1465	96	162	1	0.1%	Yes
41	*cis*-3-Hexenyl butyrate	16491-36-4	1466	170	191–192	3	0.1–1.2%	No
42	Menthone	10458-14-7	1474	154	207	1	3.6%	Yes
43	2-Ethyl-1-hexanol	104-76-7	1493	130	182	1	0.2%	Yes
44	Acetic acid	64-19-7	1497	60	118	3	0.1–1.5%	Yes
45	Ethyl sorbate	2396-84-1	1513	140	195–196	1	0.1%	No
46	Benzaldehyde	100-52-7	1530	106	179	12	0.1–2.2%	Yes
47	Linalool	78-70-6	1551	154	199	7	0.1–1.1%	Yes
48	Menthyl acetate	89-48-5	1573	198	228–229	1	0.5%	Yes
49	Hydroxyacetone	116-09-6	1578	74	147[g]	2	0.1%	Yes
50	(−)-Isopulegol	89-79-2	1585	154	216–218	1	0.2%	Yes
51	Hexyl hexanoate	6378-65-0	1616	200	245–246	5	0.1–0.4%	No
52	L-Menthol	2216-51-5	1651	156	212	11	0.3–9.1%	Yes
53	*cis*-3-Hexenyl hexanoate	31501-11-8	1664	198	222–224	2	0.1%	Yes
54	Acetophenone	98-86-2	1667	120	202	1	0.1%	Yes
55	(+)-Pulegone	89-82-7	1667	152	220–222	1	0.2%	Yes
56	Butyric acid	107-92-6	1672	88	163	1	0.1%	Yes
57	Ethyl benzoate	93-89-0	1682	150	213	1	0.1%	Yes
58	Estragole	140-67-0	1682	148	215–216	1	0.4%	Yes
59	α-Terpineol	98-55-5	1709	154	214–218	2	0.2–0.6%	Yes
60	Benzyl acetate	140-11-4	1740	150	214	4	0.2–0.8%	Yes
61	Carvone	99-49-0	1757	150	231	2	0.2–1.1%	Yes
62	*trans*-Anethole	4180-23-8	1843	148	234–237	8	0.5–43%	Yes
63	α-Ionone	6901-97-9	1872	192	257–258	3	0.6–1.4%	Yes
64	Benzyl butyrate	103-37-7	1887	178	238–240	3	0.4–2.1%	Yes
65	Benzyl alcohol	100-51-6	1891	108	206	12	0.2–12%	Yes
66	2-Phenylethanol	60-12-8	1928	122	220	1	0.6%	Yes
67	β-Ionone	14901-07-6	1966	192	126-128[h]	2	0.3%	Yes
68	*p*-Anisaldehyde	123-11-5	2052	136	247–249	7	0.2–1.3%	Yes
69	Methyl cinnamate	103-26-4	2103	162	260–262	1	1.9%	Yes
70	Ethyl 3-methyl-3-phenylglycidate	77–83-8	2109	206	272–275	1	1.8%	Yes
71	Ethyl cinnamate	103-36-6	2158	176	271	1	0.4%	Yes
72	γ-Decalactone	706-14-9	2176	170	153[i]	7	0.1–0.6%	Yes

Table 4 (*continued*)

Substance No.	Name	CAS number	LRI[a] (DB-WAX)	MW (g/mol)	BP[b,c] (°C)	Frequency in 12 waterpipe tobaccos	Abundance in 12 waterpipe tobaccos	Reported in other tobacco products[d]
73	Eugenol	97-53-0	2188	164	252	1	0.7%	Yes
74	Methyl anthranilate	134-20-3	2271	151	260	1	0.2%	Yes
75	γ-Undecalactone	104-67-6	2293	184	297	1	0.5%	Yes
76	Methyl dihydrojasmonate	24851-98-7	2311	226	109–112	9	0.1–2.1%	Yes
77	Isoeugenol	97-54-1	2373	164	266–268	1	0.2%	Yes
78	Ethyl vanillin	121-32-4	2553	166	285–294	5	0.2–0.5%	Yes
79	Vanillin	121-33-5	2607	152	285–286	6	0.1–0.2%	Yes

The substance number is identical with the peak number in Fig. 2.
LRI, Linear retention index; MW, Molecular weight; BP, Boiling point.

[a] Determined according to [56].
[b] See Ref. [57].
[c] See Ref. [58].
[d] See Ref. [59].
[e] Determination of an LRI for α-Pinene was not possible.
[f] 27 hPa.
[g] Decomposition temperature.
[h] 16 hPa.
[i] 20 hPa.

97% (100 ppm) for benzyl alcohol. We are aware that the selected approach is based on the assumption that the added analyte behaves similar to the native analyte and that a high recovery of the added analyte is necessary but not necessarily a sufficient condition. The three analytes (ethyl 2-methylbutyrate, benzaldehyde, and benzyl alcohol) were selected because they encompass the entire chromatographic range in terms of volatility and boiling point (i), as well as the selected concentration ranges (ii) and represent each internal standard (iii).

3.2.5. *Limit of detection (LOD) and limit of quantification (LOQ)*

The results obtained are summarized in Table 2. Furthermore, the LOQ values based on the amount of tobacco were also determined. The LOD values were found between 0.005 μg/ml (benzaldehyde) and 1.29 μg/ml (benzyl alcohol). This range is also reflected by the corresponding calibration curves. Switching to single ion monitoring rather than a full spectral scan should improve the LOD values. However, the use of the full-scan mode (m/z 38–380) enables the recording of spectral information for a qualitative analysis of additional waterpipe tobacco constituents.

3.3. *Waterpipe tobacco*

The proposed method was applied for the analysis of 12 commercially available waterpipe tobaccos. The following flavors were analyzed: “Two Apples” (3 tobaccos), “Apple” (3 tobaccos); “3 Apples Flavour” (1 tobacco), “Melon” (1 tobacco), “Raspberry” (1 tobacco), “Five Stars” (1 tobacco), “Banana” (1 tobacco) and “Fruits Molasses” (1 tobacco) (see Table 3). Among these samples there were also three tobaccos made from non-tobacco herbal basic materials (sugarcane; see Table 3). The samples were analyzed fivefold, and the mean concentration values have been reported (Table 3). Among the selected tobaccos, “Apple” flavor tobaccos (“Apple”, “Two Apples”, and “3 Apples Flavour”) are dominating (7 tobaccos) because they belong to the most popular brands. The obtained results are summarized in Table 3. The results for each analyte are presented as per-gram tobacco which simplifies data analysis and enables direct tobacco-to-tobacco comparisons. Example chromatograms for three waterpipe tobaccos are depicted in Fig. 3, wherein each of the main components is designated. The chromatograms shown (A, B, C) represent tobaccos “*a*” (“Two Apples”), “*h*” (“Melon”) and “*k*” (“Banana”). By comparing these three representative chromatograms significant differences between the individual waterpipe tobacco brands become obvious. For example, tobacco “*a*” contains high amounts of *trans*-anethole (peak 62), whereas tobaccos “*h*” and “*k*” contain only low concentrations of this compound (see Table 3). The same applies for tobacco “*h*” and 1-butanol and for tobacco “*k*” and isoamyl acetate. On the other hand, the comparison of chromatograms obtained with one particular flavor (e.g. “Two Apples”) reveals comparable results (data not shown). Exceptions are the tobaccos made from non-tobacco herbal basic materials (sugarcane). They contain lower amounts of the detected analytes when compared to the tobaccos made from tobacco leaves.

Among the analyzed waterpipe tobacco brands tobaccos “*g*” and “*a*” contain the highest amounts of the selected volatile flavor compounds with 1680 and 1550 μg/g tobacco, respectively, followed by tobacco “*f*” that contains 595 μg/g tobacco (see Table 3). The high quantities in tobaccos “*g*” and “*a*” are mainly caused by benzyl alcohol (61 and 54%, respectively). Furthermore both tobaccos contain high amounts of 1-hexanol (286 and 336 μg/g tobacco, respectively) and *cis*-3-hexen-1-ol (232 and 293 μg/g tobacco, respectively). Tobacco “*f*” contains mainly 1-hexanol (201 μg/g tobacco), ethyl 2-methylbutyrate (133 μg/g tobacco) and *cis*-3-hexen-1-ol (117 μg/g tobacco). The lowest amounts of the eleven selected volatile flavor compounds were found in tobacco “*j*” (18.8 μg/g tobacco). Furthermore, benzyl alcohol and benzaldehyde were detected in each of the waterpipe tobaccos analyzed. Compared to cigarette tobacco most of the analytes were found at higher values in waterpipe tobacco. Based on their values per gram tobacco the contents of *trans*-anethole and 3-hexen-1-ol were 223 and 117 times higher in waterpipe tobacco. In the case of cigarettes *trans*-anethole is added to neutralize or reduce the lingering smoke odor and it has a characteristic smell of anise [16]. On the other hand, 3-hexen-1-ol is added to increase the organoleptic characteristics of cigarette tobacco and it has a characteristic smell of newly mown grass [16]. By contrast, the contents of benzaldehyde and linalool revealed as exception. Their amounts were found lower in waterpipe tobacco when compared to cigarette tobacco.

Performing the GC–MS measurements in a full spectral scan mode enabled the identification of 68 additional volatile flavor compounds, besides the established eleven compounds listed in Table 1. The entire range of all 79 identified compounds is provided in Table 4 along with the corresponding CAS numbers and linear retention indices (LRI). In addition, the frequency each compound was detected in the analyzed waterpipe tobaccos as well as the corresponding abundance was also specified. The abundance was calculated by dividing the individual peak areas for each compound by the total peak area. Furthermore we found that 72 out of the 79 listed compounds have already been identified in other tobacco products, e.g. cigarette tobacco. The list comprises aliphatic and aromatic alcohols (e.g. 1-butanol (#13), benzyl alcohol (#65)), carbonyles (e.g. hexanal (#8)) and esters (e.g. butyl butyrate (#19)), as well as some terpenes (e.g. α- (#1) and β-pinene (#9), limonene (#16)) and lactones (e.g. γ-decalactone (#72)). The large number of volatile flavor substances in the waterpipe tobaccos raises the question of whether they represent only additives, or not. To answer this question a literature survey was conducted and the emission profiles of the waterpipe tobaccos (e.g. "Banana", respectively, "Melon" flavor) were compared to their corresponding natural flavors. For example, tobacco "*k*" contains the natural banana flavors isoamyl acetate (peak 10), 1-butanol (peak 13), isoamyl alcohol (peak 18; main component), isoamyl butyrate (peak 24), isoamyl isovalerate (peak 26) and eugenol (peak 73) [44,46]. However, hexanal and ethyl butyrate which are also characteristic for banana flavor were not detected in tobacco "*k*" whereas benzyl butyrate (peak 64) is not a constituent of banana flavor (see Fig. 3) [44,46]. Another example is tobacco "*h*", containing the following melon flavor components: ethyl 2-methylbutyrate (peak 4), butyl acetate (peak 7), 1-butanol (peak 13), 6-methyl-5-hepten-2-one (peak 30), *cis*-3-hexen-1-ol (peak 34) and benzyl alcohol (peak 65) [20]. However, ethyl butyrate and acetoin, which are also characteristic for melon flavor, were not detectable in tobacco "*h* "whereas linalool (peak 47) is not a constituent of melon flavor (see Fig. 3) [20]. These results suggest that the analyzed waterpipe tobaccos were produced by adding synthetic mixtures of volatile flavor substances to imitate the respective natural flavor. This assumption is further supported by the finding that ethyl vanillin, an artificial flavoring substance, was also detected in some of the waterpipe tobaccos analyzed (5 tobaccos). Furthermore, Sepetdjian et al. reported that waterpipe smoke, in comparison to cigarette smoke, contains 1000 times higher quantities of ethyl vanillin [17]. On the other hand, some of the compounds listed in Table 4 are naturally occurring tobacco ingredients, e.g. benzyl alcohol, benzaldehyde or menthol [47].

Among the 79 identified flavoring compounds five allergenic fragrances were found (eugenol, isoeugenol, benzyl alcohol, limonene, linalool). The same compounds are restricted, e.g. in toys or cosmetic products [48,49]. Four out of the 79 flavoring compounds (eugenol, limonene, furfural, benzyl acetate) were evaluated and classified by the International Agency for Research on Cancer (IARC) and classified among IARC class 3 ("not classifiable as to its carcinogenicity to humans") [50–53]. By contrast, data on the health risks associated with the inhalation of flavoring compounds are scarce. Moreover, some of the flavoring compounds may represent precursors for toxic compounds that occur when being heated. Due to the high contents of the flavoring compounds in the waterpipe tobacco, the smoke generated confers a sweet and pleasant smell which is not perceived as nuisance. This may obscure the risk perception of the smokers and therefore waterpipe smoking might be considered less harmful. However, it seems likely that the main health risks associated to the flavoring additives would not result from an increase in toxicity of the smoke but rather from a wider acceptance among the human population [47].

4. Conclusion

The aim of this study was to identify volatile flavor compounds which occur in waterpipe tobacco. This may then contribute to the assessment of the potential health risks posed to the smokers. The SHS-GC–MS method worked out in this study was found suitable to detect a large range of volatile flavor compounds in this complex matrix. Altogether 79 volatile flavor compounds were identified, eleven of which were quantified. Out of these eleven substances benzyl alcohol, an allergenic fragrance, showed the highest concentrations in waterpipe tobacco. It is also possible to integrate additional analytes into the method. Overall, the occurrence of a variety of volatile flavor compounds in waterpipe tobacco, including allergenic fragrances, may pose a potential health risk to the smoker. To reveal this, further studies are urgently required.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.06.022.

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