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Insight into the aroma profile of Bulgarian tobacco absolute oil

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ABSTRACT

The tobacco absolute usually obtained from tobacco waste, generated at various stages of post-harvest processing of the tobacco leaves and the cigarettes production, can be an important source of natural additives for tobacco compositions and cosmetics. Therefore, the chemical profile of Bulgarian tobacco absolutes derived from the three main type tobacco, grown in Bulgaria (Burley, Virginia and Oriental) was studied by means of gas chromatography-mass spectrometry (GC/MS) and gas chromatography with flame ionization detection (GC-FID). A protocol for simultaneous analysis of compounds with high structural diversity and volatility was developed involving a multistep temperature gradient and trimethylsilyl derivatives. As a result 90 compounds, mainly terpenes, but also organic acids, waxes and steroids were identified. The main constituents, representing 94.5-97.5% of the total content of the detected compounds, were quantified by means of GC-FID. Although the most important terpenoid classes, forming the heart of tobacco flavour, were found in all three samples, significant differences were observed as a reflection of the tobacco type. In Virginia and Burley absolutes only cembranoids were detected, while in Oriental compounds representative for both cembranoids and labdanoids were found. This defines a specific chromatographic fingerprint pattern that could be used for a fast identification of the tobacco absolute type.

1. Introduction

Nicotiana tabacum L., a cultivated annually-grown plant of genus *Nicotiana* L., family Solanaceae, is one of the most common non-food crops in the world with enormous social and economic importance. Probably, it is the plant with the most controversial fame in the human being history – used in sacred ceremonies and for recreational purposes, and apart from smoking, from centuries as a medicine in many countries in North and South Amerika, but also blamed for more human deaths than any other herb (Charlton, 2004; Rudgley, 2000).

Tobacco, as one of the main products of the cultivated plant is among the most extensively studied natural products, due to its commercial value and because of its influence of the human health. Up to now approximately 4 000 compounds have been identified in the leaf of various tobacco varieties and 6 000 – in the tobacco smoke (Zhu et al., 2009). In addition to the processed tobacco used for manufacturing of cigarettes and cigar, the dried tobacco leaves are important source of various aroma products such as essential oil, concrete and absolute. Recent studies rediscovered tobacco in a different light – not only as a huge risk to human health, but also as a valuable source of biologically active components with promising anti-cancer potential (El Sayed and Sylvester, 2007).

Tobacco absolute (TA) is a dense liquid with dark-brown to black-brown colour and intensive aroma of dry tobacco. It is obtained from tobacco concrete (extraction of fermented tobacco with hexane or petroleum ether) after extraction with ethanol. Tobacco absolute has been intensively used in perfumery and cosmetics, mainly in the composition of men perfumes with woody, earthy and oriental notes (Brechbill, 2009). It is an important additive in the improvement of cigarettes flavour or as an ingredient of the tobacco-flavoured liquids for electronic cigarettes (Farsalinos et al., 2015, 2013; Peng et al., 2004).

Although there are very few studies on the chemical constituents of the essential oil derived from fresh tobacco leaf (Kim et al., 1982; Rodgman and Perfetti, 2013; Stojanovic et al., 2000; Zhang et al., 2012), the chemical profile of the TA remains completely unexplored. The evaluation of its quality has been traditionally made by organoleptic experience and sensorial expertise. Therefore, the goal of this paper is to shed light on the chemical profile of the Bulgarian tobacco absolutes on the basis of the tree

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main tobacco types, grown in Bulgaria – Burley (air-cured), Virginia (flue-cured) and Oriental (sun-cured). The focus is given to the volatile and semi-volatile aroma compounds, but also organic acids, waxes and steroid fractions have been investigated by means of GC/MS. To our best knowledge no such detailed investigation has been performed up to now. Since the flavour profile as well as health influence of tobacco products are a direct result of their chemical composition, this study could give a base for more adequate quality assessment, authenticity and traceability in respect of the use of TA in the cosmetics and aromatherapy as a natural additive. The information, which allows to distinguish between the tobacco absolutes, could have strong practical effect on the development of fast and high throughput methods for analysis (Liu et al., 2007; Qin and Gong, 2016).

2. Experimental part

2.1. Materials

Tobacco absolute samples were provided by Galen-N Ltd (Sofia, Bulgaria). It is interesting to note, that the samples are produced from tobacco waste (mid ribs, leaf waste and dust), generated at various stages of post-harvest processing of the tobacco leaves and the cigarettes production.

The samples do not contain nicotine and have the following characteristics: appearance– viscous liquid; color– dark to black-brown, when in a thin layer– light brown; odor– strong, lasting, typical of dry tobacco.

2.2. Methods

2.2.1. Derivatization

Trimethylsilyl derivatives were prepared by using a mixture of BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) and TMCS (trimethylchlorosilane), 99:1 (Supelco, Bellefonte, PA) as derivatization reagent. 50 μl dry pyridine and 75 μl BSTFA/TMCS were added to 5 mg of each sample, dried under a stream of nitrogen and stored over the night in exicator; the mixture was then heated for 30 min at 80 °C, cooled to the ambient temperature and analyzed by GC/MS.

2.2.2. Analysis

2.2.2.1. Gas chromatography–mass spectrometry (GC/MS). The GC/MS analysis was performed on a Shimadzu GCMS-QP2010 Plus gas chromatograph coupled with a mass selective detector (Shimadzu Corporation, Kyoto, Japan). The ultra-inert fused silica capillary column DB–5ms UI (J&W Scientific, Folsom, CA) with 30 m column length, 0.25 mm i.d., 0.25 μm film thickness was used. The oven temperature was programmed from 60 (2.5 min held) to 175 °C at a rate of 15 °C/min, from 175 to 200 °C at a rate of 1 °C/min, and from 200 to 300 °C at a rate of 10 °C, 30 min held at the final temperature was applied. Helium (99.999%) was used as a carrier gas at a constant flow rate of 0.8 ml/min. The split ratio was 1:50, the inlet temperature was set to 300 °C and the transfer line temperature was 320 °C. Mass selective detector operated in electron impact ionization (EI) mode at 70 eV electron energy, the ion source temperature was set to 230 °C, and the quadrupole temperature was 150 °C. The mass scan range was 30–950 amu. Instrument control and data collection were carried out using Shimadzu LabSolutions GC/MS solution software (Rev.2.1, Shimadzu Corporation).

2.2.2.2. Gas chromatography with flame-ionization detector (GC-FID). On Thermo GC Ultra gas chromatograph equipped with flame ionization detector and Thermo TriPlus autosampler (Thermo Scientific, Bremen, Germany) under the same temperature gradient as above described. Instrument control and data collection were carried out using Thermo Xcalibur software (Rev.2.0, SR1, Thermo Scientific).

2.2.2.3. Identification and quantitative analysis. The identification of the compounds was performed using commercial mass spectral libraries (NIST 05, Wiley 7th Mass spectra register) and retention times (Kovats retention indices, RI). In the cases of lack of the corresponding reference data, the structures were proposed on the basis of their general fragmentation and/or using reference literature mass spectra. The quantification of the main compounds was carried out by internal normalisation method with response factor 1 for all of the sample constituents.

3. Results and discussion

3.1. GC/MS and GC –FID analysis

Tobacco absolute represents complex mixture containing compounds with high structural diversity which leads to significant variations in their polarity, respectively volatility. This makes comprehensive characterization of its chemical profile a challenging, not straightforward, task. DB-5ms UI fused silica capillary column (polydimethylsiloxane with 5% phenyl siloxane), which is characterized with low polarity and high thermal stability and extended high temperature limits (up to 350 °C), was preferred for the gas chromatography separation, regardless of some loss of selectivity in comparison with the columns with polar stationary phase on the base of polyethylene glycol (Innowax, etc.), because it allows simultaneous separation of constituents very different in their volatility. The temperature gradient was carefully designed in a way to provide effectively separation of the analytes, with very few co-eluting components. An extra separation power was achieved by using trimethylsylil derivatives–which, increasing volatility and thermal stability, improves chromatographic behavior of highly polar compounds (peak width and symmetry).

The total ion current (TIC) chromatograms of TA samples are shown in Fig. 1. Although the chromatograms contain more than 250 individual peaks, distinct areas of the aromatics/heterocyclic compounds, followed by abundant terpenes/terpenoids fraction and sterol fraction have been noticed.

As a result of the GC/MS analysis over 150 compounds with concentrations higher than 0.05% were detected and about 90 of them containing C_9-C_{30} carbon atoms were identified (Table 1). The quantitative analysis was performed by GC-FID and the results for 33 individual compounds, representing 94.5–97.5% of the total content of the detected components, are collected in Table 2.

3.2. Macrocomponents

The macrocomponents (components with concentration, as determined by GC-FID, >0.5%) are representatives of the terpenoid classes of diterpenenoids and triterpenoids (predominantly phytosterols). Fatty acids, higher alcohols and waxes were found as well.

3.2.1. Acyclic tepenoids

The most abundant component, aliphatic diterpene neophytadiene (7,11,15-trimethyl-3-methylidenehexadec-1-ene), belonging to the group of key tobacco flavour compounds, is responsible for the unique tobacco organoleptic properties and forming of tobacco smoke aroma. Neophytadiene is often used as electronic cigarettes additive, giving “real” cigarette burning aroma. Generally, its content being low in the green tobacco leaf, increases significantly during the yellow phase.