LabWorks APPNOTE

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Determination of Flavor Compounds in Cut Tobacco by TD-SBSE-GCMS

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Keywords

Cigarette, Cut tobacco, SBSE, TDU, GCMS

Abstract

To enhance the fragrance and taste of cigarettes smoke, flavor additives are often incorporated into cut tobacco. Analyzing the aroma components of cut tobacco holds significant importance. Therefore, it is crucial to select an efficient method for extracting the aroma and odor components from cut tobacco. In this study, a GERSTEL LabWorks Platform using Thermal Desorption TDU/ Stir Bar Sorptive Extraction (SBSE) combined with gas chromatography-mass spectrometry is employed for the analysis and identification of volatile flavor and aroma components in cut tobacco. The Automatic Mass Spectral Deconvolution and Identification System (AMDIS, NIST) software are utilized to identify co-eluted chromatographic peaks. Additionally, the retention index is employed to facilitate the identification of flavor components in cut tobacco. This whole approach allows for the identification the composition of flavor compounds in cut tobacco.

Introduction

To enhance the aroma, refine the smoking experience, mask undesirable odors, and improve the overall sensory profile of tobacco, cigarettes often incorporate flavorings. These flavorings, derived from hundreds of synthetic and natural sources, encompass spice powders, extracts, tinctures, oleoresins, essential oils, and individual flavor chemicals. Their addition aims to impart a mellower, sweeter, and fresher taste to the tobacco, thereby enhancing the pleasure and satisfaction of smoking.

Moreover, these flavorings play a crucial role in masking the inherent bitterness and impurity taste of tobacco, contributing to an overall more enjoyable smoking experience. As an integral component of tobacco products, flavor additives are instrumental in defining a product's distinctive taste and appeal.

The analysis of flavor compounds in cut tobacco holds immense significance for studying flavor additive levels, discerning market trends, and ensuring the judicious addition of flavorings to achieve desired sensory outcomes.

The extraction of flavor compounds from tobacco traditionally involves methods such as solvent extraction, simultaneous extraction and distillation (SDE), or solid-phase extraction (SPE). However, these approaches often demand substantial amounts of solvent, a large sample volume, and subsequent concentration steps to eliminate residual solvents. Moreover, they may introduce side reactions or artifacts, making the process time-consuming, laborious, and operationally complex.

Solid-phase microextraction (SPME) is an alternative, but its quantification capabilities are sometimes unsatisfactory, and its sensitivity is limited. Accelerated solvent extraction (ASE) is another option, but it necessitates higher temperatures and pressures, potentially triggering undesired reactions. Furthermore, concentration steps are still required to remove solvents.

In contrast, Stir Bar Sorptive Extraction (SBSE) represents a modern, solvent-free, and environmentally friendly extraction technique. SBSE is characterized by its efficiency, high sensitivity, and ease of operation when extracting aroma and fragrance. In this study, SBSE is employed to extract volatile flavor and aroma components from cut tobacco. The analysis involves the use of a Cooled Inlet System (CIS 4), thermal desorption TDU 2, and gas chromatography-mass spectrometry. The Automatic Mass

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Spectral Deconvolution and Identification System (AMDIS, NIST) software assists in identifying co-eluted chromatographic peaks, while retention index values aid in identifying specific flavor components in cut tobacco. This method not only offers a greener alternative but also enhances efficiency and sensitivity in the analysis of tobacco flavor compounds.

Experimental

Samples

Cigarettes (from local market).

Instrumentation

GERSTEL LabWorks Platform with Cryostatic Cooling Device CCD 2 option combined with Agilent 7890/5975 GC/MSD.

Analysis Conditions LabWorks Platform

SBSE	PDMS twister stir bar
	10 mm length, 1 mm thickness
TDU	Splitless
	25 °C (0.2 min); 100 °C/min; 250 °C (8 min)
CIS	Tenax liner
	Solvent Vent (50 mL/min), split 11:1
	-30 °C (0.5 min); 12 °C/sec; 250 °C (10 min)
	(note: MSD/ODP split ratio is with 1:1)

Analysis Conditions Agilent GC 7890

Column	60 m HP-Innowax column (Agilent, USA)
	d_i=0.25 mm d_f=0.25 µm
Pneumatics	He (> 99.999%)
	P_i=208.18 kPa, Constant Flow 1.8 mL/min
Oven	40°C (2 min); 5°C/min; 250°C (20 min)

Analysis Conditions Agilent 5975 MSD

EI	70 eV
Interface	250 °C
Ion source	230 °C
Quadrupole	150 °C
Full scan	33 – 400 amu

Extraction

The volatile flavoring compounds in cut tobacco were extracted using the Stir Bar Sorptive Extraction (SBSE) technique employing a PDMS twister stir bar. The cut tobacco was carefully prepared into small pieces. Approximately 0.2 grams of these small tobacco pieces (the exact amount depending on the flavorings content) were combined with 8 grams of a saturated NaCl water solution within a 20 mL headspace vial. The PDMS twister was carefully immersed into the solution, and the extraction of volatile compounds took place for 60 minutes at room temperature on a stirrer, with a stirring rate set at 1000 rpm.

Following the extraction period, the PDMS twister was delicately removed using forceps or a mounting tool for Thermal Desorption Unit (TDU). Subsequently, the twister was briefly rinsed in distilled water, dried with a clean, lint-free tissue, and then transferred to a thermal desorption tube for subsequent Gas Chromatography-Mass Spectrometry (GC-MS) analysis. This method ensures a thorough and efficient extraction of volatile flavor compounds for precise analysis.

Thermal Desorption

For the subsequent GC–MS analysis, the PDMS twister was introduced into the Thermal Desorption Unit (TDU 2), adhering to the parameters outlined in the analysis conditions above. Please refer to the specified TDU 2 and CIS 4 parameters for further details.

Data Process

MS ChemStation Data Analysis version E.02.01.1177 (Agilent Technologies).

The Automatic Mass Spectral Deconvolution and Identification System (AMDIS), Version 2.66, NIST, National Institute of Standards and Technology.

Results and Discussion

Sample Preparation Method

When employing the classical solvent extraction method to extract flavorings from cigarette tobacco, challenges such as solvent consumption, interference, reduced sensitivity, and operational complexity may arise. The matrix of cut tobacco is intricate, necessitating a simple, rapid, and preferably solvent-free or low-solvent technique for determining its aroma components.

In contrast to conventional extraction methods like Liquid-Liquid Extraction (LLC), Simultaneous Distillation and Extraction (SDE), Solid-Phase Extraction (SPE), Solvent Assisted Fluid Extraction (SAFE), and Accelerated Solvent Extraction (ASE), which often involve complex steps, solvent consumption, and the need for subsequent concentration, Stir Bar Adsorption Extraction (SBSE) emerges as a solvent-free technique for extracting and concentrating trace organic compounds. Its notable attributes include high sensitivity, good reproducibility, minimal sample dosage, straightforward and speedy operation, surpassing the sensitivity of ordinary Solid-Phase Microextraction (SPME). SBSE is particu-

larly well-suited for determining aroma and flavor compounds in cut tobacco.

Given that the flavorings added to cut tobacco frequently employ polar solvents like propylene glycol and glycerol, these substances can pose challenges by interfering with the determination of flavoring components. Additionally, propylene glycol and glycerol may co-elute with other flavoring components. To address this issue, the addition of a saturated sodium chloride water solution proves effective in mitigating the influence of polar solvents, such as propylene glycol and glycerol. The salting-out effect further enhances the extraction efficiency of other flavoring components.

Analysis of Aroma Volatile Compounds in Cut Tobacco

The Total Ion Chromatography (TIC) of volatile aroma and flavor compounds in a specific cut tobacco, extracted using Stir Bar

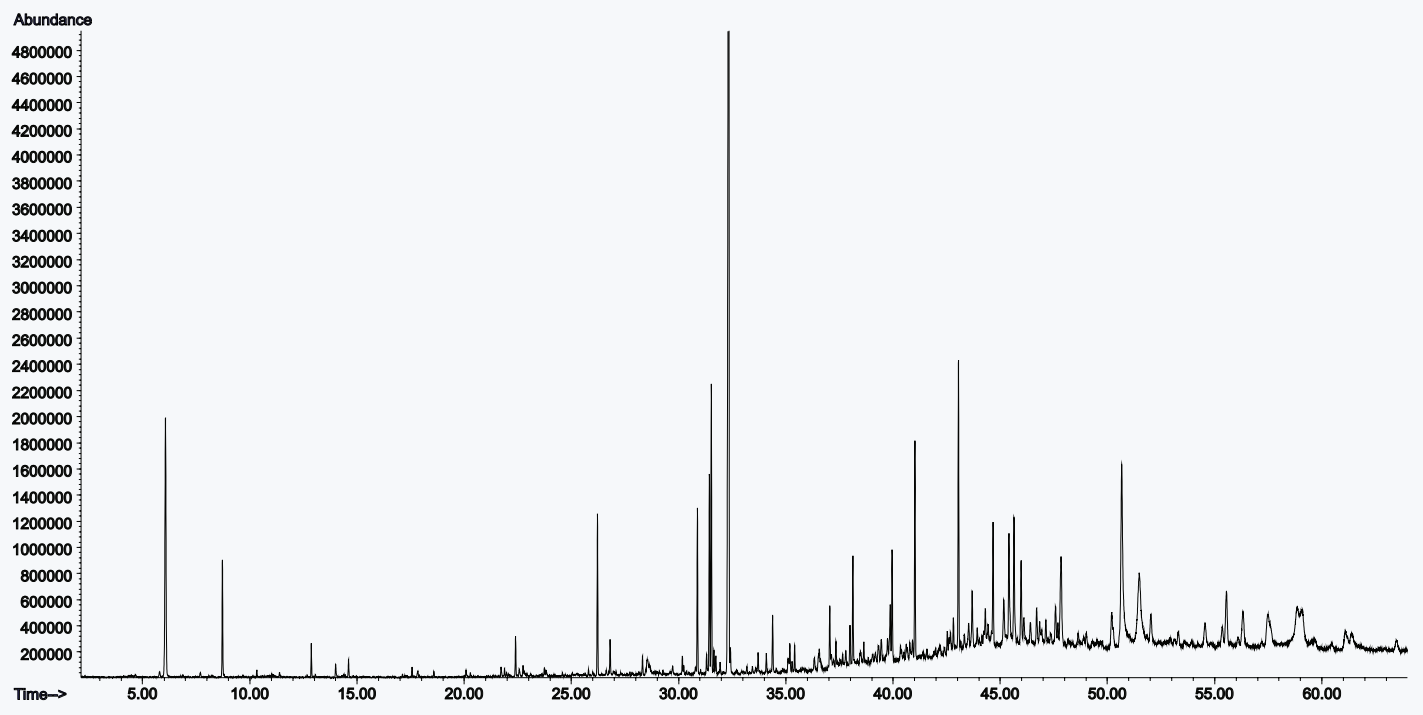

Figure 1: Total ion chromatogram (TIC) of volatile aroma and flavor compounds in certain cut tobacco extracted by SBSE.

Sorptive Extraction (SBSE), is presented below.

Approximately 68 volatile flavor compounds were identified through the extraction of volatile compounds from cut tobacco using Stir Bar Sorptive Extraction (SBSE). Neophytadiene (No. 28) emerged as the predominant compound, constituting over 41% of the total, establishing itself as the principal aroma component in tobacco. Other noteworthy compounds with substantial content include menthol, cooling agent WS23 (No. 21), and nicotine (No. 25), all of which are pivotal odor components in cigarettes.

The spectrum of identified compounds encompasses various terpenes, aldehydes, alcohols, ketones, esters, phenols, acids, pyrrole, and several aromatic compounds released from cut tobacco.

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Table 1: components of cut tobacco extracted by SBSE.

No	Name	RI test	RT [min]	Area [rel.%]
1	PROPANONE	812	5.792	0.169
2	TOLUENE	1049	10.324	0.138
3	ALDEHYDE C 6	1092	11.383	0.100
4	XYLENE, M-	1150	13.056	0.000
5	LIMONENE	1202	14.604	0.391
6	BENZENE, 1,2,4-TRIMETHYL-	1292	17.124	0.050
7	METHYL HEPTENONE, 6,5,2-	1346	18.585	0.119
8	ALDEHYDE C 9	1401	20.084	0.157
9	ETHLENE GLYCOL MONOBUTYL ETHER	1409	20.287	0.065
10	ACETIC ACID	1465	21.728	0.506
11	ISOOCTANOL	1491	22.396	1.812
12	BENZALDEHYDE	1545	23.741	0.364
13	2-HYDROXYPROPYL ACETATE	1579	24.582	0.054
14	HEXADECANE	1598	25.042	0.034
15	MENTHOL	1648	26.222	3.882
16	ACETOPHENONE	1673	26.812	0.851
17	TERPINEOL, ALPHA-	1708	27.6	0.015
18	SOLANONE E	1740	28.326	0.697
19	DOWANOL DB	1804	29.733	0.100
20	BUTANONE, 1-PHENYL-2-	1826	30.188	0.406
21	COOLING AGENT WS23	1859	30.889	4.016
22	GERANYLPROPANONE	1866	31.039	0.038
23	BUTYRIC ACID-3-HYDROXY-2,2,4-TRIMETHYL-PENTYLESTER, ISO-	1879	31.315	0.475
24	2,2,4-TRIMETHYL-1,3-PENTANEDIOL DIISOBUTYRATE	1885	31.45	5.035
25	NICOTINE	1889	31.537	6.219
26	BENZYLALCOHOL	1895	31.658	0.747
27	BUTYRIC ACID-1-HYDROXY-2,2,4-TRIMETHYL-3-PENTYLESTER, ISO-	1899	31.75	0.333
28	NEOPHYTADIENE	1928	32.33	41.656
29	PHENYLETHYL ALCOHOL, 2-	1932	32.403	0.441
30	ALCOHOL C 12	1970	33.172	0.092
31	EDULANE, CIS-7-OXO-	1982	33.428	0.061
32	ACETYLPYRROLE-2	1996	33.699	0.383
33	IONONE, 5,6-EPOXY-	2015	34.081	0.360
34	EDULANE, TRANS-7-OXO-	2030	34.371	2.238
35	PYRROLALDEHYDE, 2-	2055	34.841	0.019

Table 1 (cont.): components of cut tobacco extracted by SBSE.

No	Name	RI test	RT [min]	Area [rel.%]
36	TRIACETIN	2084	35.402	0.498
37	HEXADECANAL	2145	36.553	0.169
38	NORSOLADIONE	2171	37.041	1.115
39	PELARGONIC ACID	2175	37.119	0.123
40	TABANONE P.3	2187	37.327	0.766
41	Nicotine oxide compounds isomer	2203	37.636	0.073
42	DIHYDRO BOVOLIDE	2212	37.791	0.165
43	METHYL HEXADECANOATE	2224	37.999	0.494
44	TABANONE P.4	2231	38.125	2.280
45	DECANOL, 2-HEXYL-	2249	38.454	0.184
46	ETHYL PALMITATE	2260	38.637	0.590
47	HELIOTROPIN	2272	38.85	0.046
48	FRESCOLAT MGA isomer	2298	39.315	0.103
49	TABANONE P.5	2306	39.45	0.054
50	FRESCOLAT MGA	2323	39.74	0.287
51	TABANONE P.1	2335	39.953	2.161
52	OCTADECANAL	2358	40.35	0.130
53	DIHYDRO ACTINIDIOLIDE	2398	41.022	4.648
54	METHYL STEARATE	2431	41.578	0.027
55	METHYL OLEATE	2456	41.984	0.031
56	2,3'-Dipyridyl	2522	43.053	0.000
57	ETHYL LINOLEATE	2539	43.329	0.031
58	DAMASCONE, 3-HYDROXY-BETA-	2562	43.692	0.786
59	METHYL LINOLEATE	2577	43.929	0.299
60	MALZOXAZINE	2601	44.311	0.533
61	IONOL, 3-OXO-ALPHA- 1	2666	45.409	4.637
62	HEPTACOSANE	2700	45.979	1.935
63	MYRISTIC ACID	2706	46.1	0.230
64	DIBUTYL PHTHALATE	2723	46.42	0.467
65	3-HYDROXYSOLAVETIVONE	2797	47.842	0.743
66	NONACOSANE	2901	50.211	0.429
67	PALMITIC ACID	2918	50.676	3.548
68	HENTRIACONTANE	3101	56.325	0.149
	Sum			99.75

For a comprehensive breakdown, please refer to Table 1, detailing the components of cut tobacco obtained through SBSE.

Conclusion

Stir Bar Sorptive Extraction (SBSE) stands out as a solvent-free method for extracting and concentrating trace organic compounds. Its distinguishing features encompass high sensitivity, excellent reproducibility, minimal sample requirement, and a straightforward, rapid operation, outperforming the sensitivity of conventional Solid-Phase Microextraction (SPME). SBSE proves especially effective for discerning aroma and flavor compounds in cut tobacco. Through SBSE-TD-GCMS, a total of approximately 68 volatile flavor compounds were successfully identified, showcasing the method's robust capabilities in comprehensive compound analysis.

References

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