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Comparison of volatile and semivolatile compounds from commercial cigarette by supercritical fluid extraction and simultaneous distillation extraction*

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Abstract: Supercritical carbon dioxide fluid extraction (SFE) was studied as a rapid method for extraction of volatile and semivolatile compounds of Chinese commercial cigarettes. The method was compared with simultaneous distillation and extraction (SDE). Temperature and pressure for the SFE were optimized. The extracts obtained by the two methods showed different characters in composition and represented differently the flavor characteristics of tobacco; compared to SDE, SFE can extract compounds within a shorter time and avoid the thermal degradation and solvent contamination of samples. The extracts by the two extraction methods are complementary for investigating the flavor characteristic of tobacco products.

Key words: Volatile and semivolatile compounds, Commercial cigarette, Supercritical fluid extraction (SFE), Simultaneous distillation extraction (SDE)
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INTRODUCTION

Commercial cigarettes are blends of different tobacco types and classes selected to obtain balanced taste, smoke strength, and limited irritation (Wu *et al*., 1992), and are modified by selecting aromatic tobacco varieties and adding tobacco extracts, casings and natural and synthetic flavors (Edmond *et al*., 1985). The flavor and aroma of commercial cigarette are important qualities. The identification and quantitation of the volatile and semivolatile flavor compounds can be done using several extraction and concentration methods, including headspace (Sakaki *et al*., 1986), steam distillation (Edmond *et al*., 1985), solid phase microextraction (Švob *et al*., 1997), and other techniques (Edouard and Dominique, 1972; Ishiguro and Sugawara, 1978). The main aim of extraction is to obtain a concentrated sample containing mainly the volatile components and to improve detection limits of specific compounds.

In recent years, the use of supercritical fluid for extraction has been considered one of the most promising new methods of sample preparation. The miscibility of essential oil with carbon dioxide leads to a number of applications of SFE on the study of essential oil, as well as of flavor and fragrance compounds in the food industry and other fields. For this purpose, both offline SFE and SFE coupled with gas chromatography were used

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(Baysal and Starmans, 1999; Heikki *et al*., 1994). Extraction with supercritical carbon dioxide has several advantages such as shorter extraction time, smaller solvent consumption and lower working temperature. Simultaneous distillation extraction (SDE) is also a well-known technique used for the analysis of essential oils (Rijks and Cramers, 1983). The main disadvantage of SDE lies in its relatively high extraction temperature that might result in certain chemical changes of some temperature sensitive compounds of the extracted samples.

In the present work, SFE was used to extract the volatile aroma components from Chinese commercial cigarette. Extracts were analyzed by GC-MS. Extraction conditions were adjusted to obtain the highest yield of volatile substances, and the influence of the extraction conditions was examined. The results obtained were compared with the results obtained by SDE.

EXPERIMENTAL DETAILS

Materials and reagents

Tobacco samples were donated from a local tobacco company (Li Qun Tobacco Company, Hangzhou, China), with no further treatment before extraction. All the reagents and chemicals were of analytical reagent grade. Instrumental grade carbon dioxide was supplied in the cylinder with an SFE-CO_2 clean-up column (Isco, Lincoln, USA). The C_8-C_{29} standards (Sigma Chem. Corp., USA) were used in qualitative analysis and stored in a refrigerator.

Sampling conditions for SFE

The extractions were performed on an Isco 260 model syringe pump (Isco, Lincoln, USA) with an SFE clean-up column pressurized at different pressures. A 1.8 g tobacco sample was placed into a 10 ml extraction cell of the Isco model SFX 2–10 ml extractor. The cell was then placed into the extractor and pressurized by opening the inlet valve. After 15 min static (nonflowing) extraction, the outlet valve was opened and the extraction cell was flushed with pure CO_2 for 15 min (dynamic extraction). Then the outlet valve was opened for an additional 15 min to let the CO_2 in the cell flow out. The extraction outlet restrictor was maintained at 50 °C where the pressure was reduce to 1 atm. Extracted analytes were collected into a 7.4 ml vial containing 3 ml of dichloromethane. Then 1 μl extract was injected into the chromatographic instrument. In this study, we added 0.3 ml methanol as modifier in the sample before extraction to increase the extraction efficiencies.

Sampling conditions for SDE

Simultaneous distillation extraction was carried out in a homemade microscale simultaneous distillation extraction apparatus, as described in (Forehand *et al*., 2000). Dichloromethane was used as the solvent. For each extraction, 28 g tobacco sample and 400 ml distillation water saturated with sodium chloride were placed into a 1000 ml flask; 45 ml dichloromethane was placed into a 100 ml flask. The steam distillation process was stopped after 2.5 h, while the solvent extraction was continued for another 15 min. Then the extract was desiccated overnight. After being treated with 10% sulfuric acid and 10% sodium hydroxide solution saturated with sodium chloride, the extract was concentrated to 1.0 ml at 55 °C by distillation in a water bath.

Conditions of GC-MS

The analysis was performed by a trace 2000 GC/MS (Finnagan, USA), operated in the EI+ mode. The ionization energy was 70 eV, and the scan range was 35–540 m/z. The injector temperature was kept at 250 °C with a helium carrier flow of 10 ml/min in a split mode of 1:10. Extract (1.0 μl) was injected into a 30 m long×0.25 mm id (0.25 μl film thickness) DB-5 capillary column (J&W Scientific, USA). In the SFE analysis, the oven temperature was kept at 45 °C for 2 min, ramped at 6 °C/min to 260 °C and held for 10 min. In the SDE analysis, the oven temperature was kept at 35 °C for 2 min, ramped at 8 °C/min to 150 °C, and 3 °C/min to 260 °C, then kept for 10 min. Volatile and semivolatile compounds were tentatively identified using comparison of GC retention time to those of standard compounds and mass spectral library searches comparison of their mass spectra with mass spectra of standard compounds. For mass spectra, the NIST98 (National Institute of Science and Technology, USA) library was used. Duplicate analyses were performed on each SFE and SDE extraction.

RESULTS AND DISCUSSIONS

Optimization of SFE conditions

The first step of SFE was to optimize the working temperature and pressure in order to maximize the recoveries of volatile substances, avoiding coextraction of unwanted substances. The equipment was operated at low pressure and temperature, under which the selectivity of the supercritical fluid was higher but its solvent power was lower. To investigate the effect of changing pressure and temperature on the extraction efficiency, a series of extraction experiments were carried out on the samples over a temperature range of 20–65 °C and a pressure range of 8–15 MPa. Temperature higher than 60 °C and pressure higher than 15 MPa were not tested in order to avoid the coextraction of higher molecular weight compounds (Díaz-Maroto *et al*., 2002). For the extraction with subcritical liquid carbon dioxide at 20 °C, the yields of most compounds were relatively low and the density of the liquid carbon dioxide was low. By increasing the temperature to 30 °C and 45 °C, the yields of all the compounds increased dramatically. When the temperature reached 60 °C, the extraction efficiency decreased. It was concluded that the reduced extraction strength of carbon dioxide was not compensated for by the increased volatility of these compounds with the increase of temperature and thus resulted in the extraction ability being decreased, especially the compounds of low molecular weight compounds, while the yield of nonvolatile substance such as high molecular weight lipids were greatly increased with the increase of temperature. The effect of pressure was studied at a constant temperature of 45 °C. The increase in the pressure up to 15 MPa resulted in little increasing peak areas of polar substances such as acetic acid butyl ester, terpineol, but a significant increase of the yield of lipids such as *n*-heptacosane was noticed. There were no significant changes in the chromatogram obtained in the narrow pressure range from 8 MPa to 15 MPa at 45 °C in this experiment, but it seemed that relatively high pressure could increase the extraction efficiency of high molecular weight compounds that would cause difficulties during gas chromatographic analysis.

Comparative analysis of the extracted components using SFE and SDE

The oils extracted by SFE were almost colorless to pale yellow when the 0.3 ml methanol was added as modifier. A chromatogram of a typical SFE extraction with 0.3 ml methanol as modifier is shown in Fig.1. The extract was dominated by neophytadiene, nicotine, 9,12,15-octadecatrienoic acid, and hexadecanoic acid (which together account for 43% of the extract). Other important components in the chromatogram were compounds such as 1,2,3-propanetriol diacetate, terpineol and its isomers, 9,12-octadecadienioc acid, *n*-heptacosane and cuticular diterpeneols (which together account for the difference between 43% and 70% of the extract). This results accorded with the results of Matsukura *et al.* who also reported that neophytadiene and nicotine represented the largest amount of semivolatiles in roasted tobacco (Matsukura *et al*., 1983). A relatively high level of terpineol and its isomers were extracted by SFE, while only a small amount of these compounds were extracted by SDE. Furthermore, high proportion of duvanes (cembranes) such as α&β-4,8,13-duvatriene-1,3-diols and α&β-4,8,13-duvatriene-1-ols were examined, but it was not possible to confirm the stericstructure of these compounds by mass spectra, which was the only identifying method in this study. Duvanes are major cuticular chemicals of roast and burley tobacco and had been postulated to be the precursor of numerous identified ketone and aldehyde in the flavor of different tobacco types (Severson *et al.*, 1984). Compared with SFE, SDE can extract large amount of oxygenated compounds such as aldehydes and alcohols, which have important impact on flavor (Fig.2). Both methods can extract compounds such as butyl ester acetic acid, megastigmatrienane, geranylacetore, hexadecanoic acid methyl ester and soalnone, which can be found in volatile compounds of tobacco or tobacco additive. In conclusion, SDE yielded higher amounts of polar compounds, whereas the SFE extracts contained higher amounts of alcohol terpenes, nicotine and paraffins. Each method has its own advantages and disadvantages, but SFE extraction has no apparent hydrolysis or pyrolysis. On the whole, the extracts by the two extraction methods are complementary for investigating the flavor characteristic of natural products.

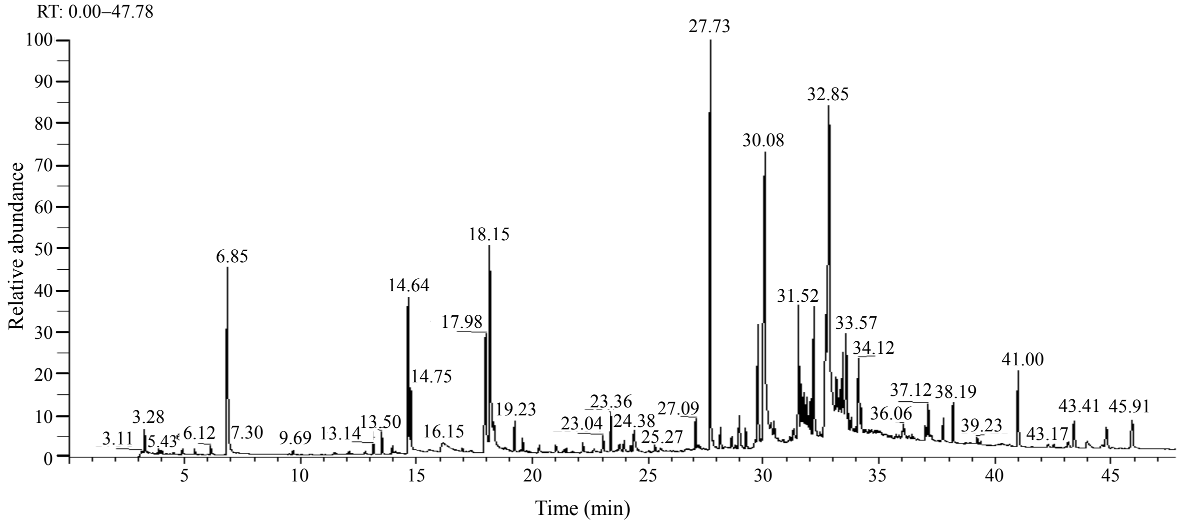

Fig.1 The total ion chromatogram of the commercial cigarette extract obtained by SFE

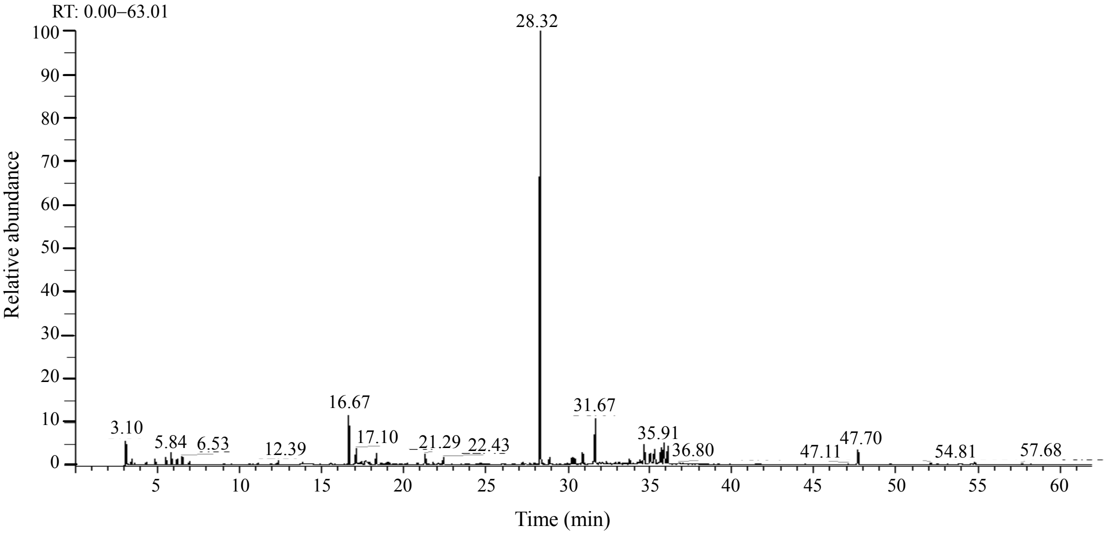

Fig.2 The total ion chromatogram of the commercial cigarette extract obtained by SDE

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