Analysis of aroma components in oral fluids of cigarette smokers

Technical Center of Shanghai Tobacco Group Co., Ltd. of CNTC

FEI Ting, P.R. China

OUTLINE

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CHINA TOBACCO

1 Introduction

2 Analytical Method

3 Sample Analysis

4 Results and Discussion

5 Conclusion

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1 Introduction

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The **sensory quality** of cigarette depends on the effect of chemical components of mainstream smoke on **the consumer's sensory organs.**

Rely on	Research mainly on	What about
Sensory quality assessment	**Mainstream cigarette smoke**	**Saliva/ Oral liquid?**

Part of the cigarette smoke ➡ **Dissolved in saliva** ➡ **Oral senses**

1 Introduction

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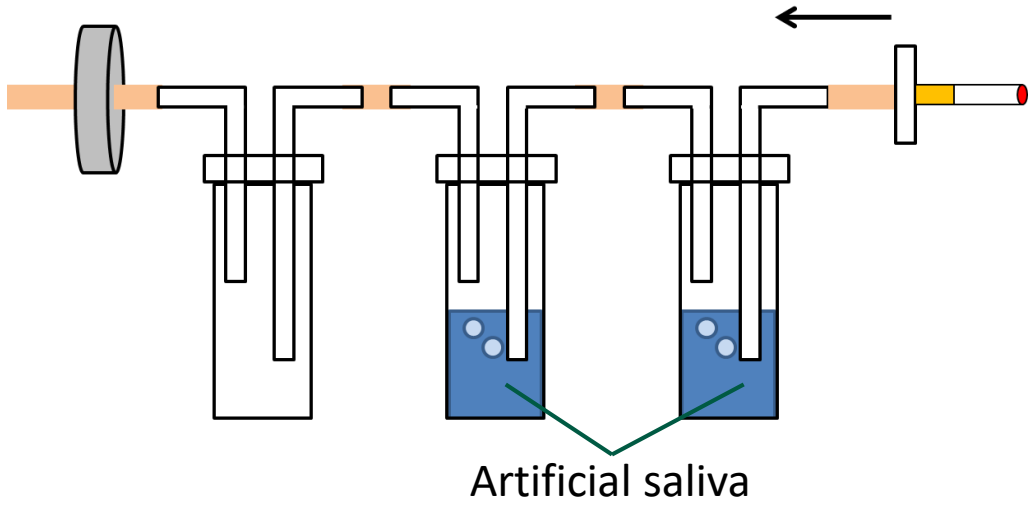
Smoking machine + Artificial saliva



CUI Yang et al. *CHINESE J ANAL CHEM*, 2010, 38(2):275-279.

Bionic absorbing device

Simulate the human sensory organs (oral and nasal) to trapping of the cigarette smoke

- Utilizing artificial saliva for oral absorption
- Utilizing water and $CHCl_3$ for nasal absorption

LI Jun. (2013). Engineering Master's Degree These, HUNAN University.

Could not represent the actual situation

1 Introduction

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Saliva collection	Oral fluid collection
Natural sampling method Physical method by chewing irritating Chemical method by taste stimulating	A small amount of purified water was used to rinse their oral cavities and then was collected.
Limited quantitative repeatability for volatile components Relative complex collection process	Good repeatability Simple, fast and convenient Avoid loss of volatile components

1 Introduction

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To provide a reference for cigarette quality assessment

1. **Analytical Method** for aroma components analysis in oral fluids
2. **Analysis of OF samples**
3. The difference of components in **OFs collected from smokers of different types of cigarettes**
4. **Comparison of OFs and mainstream cigarette smoke**

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2 Analytical Method

Pretreatment of oral fluids

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SPE procedure

Condition

6 mL dichloromethane
6 mL methanol
6 mL water,
Twice

Sample Loading

6 mL sample each time
Multiple loading

Washing

6 mL water
Vacuum pumping

Elution

4 mL eluent (95% dichloromethane, 5% methanol)

Chromabond Easy SPE column (6 mL, 500 mg)

2 Analytical Method

Heart-cutting 2DGC-MS analysis

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GC column:

1D column: DB–5MS

2D column: DB–WAX

Heart-cutting time:

1#: 5.1~10.0min

2#: 10.0~16.6min

3#: 16.6~23.5min

4#: 23.5~30.5 min

SIM+ SCAN

SIM: more than 100 compounds

MS scanning range: 33 ~ 400 amu

➡ **Semi-quantitative results of aroma components**

2 Analytical Method

Mainstream cigarette smoke analysis

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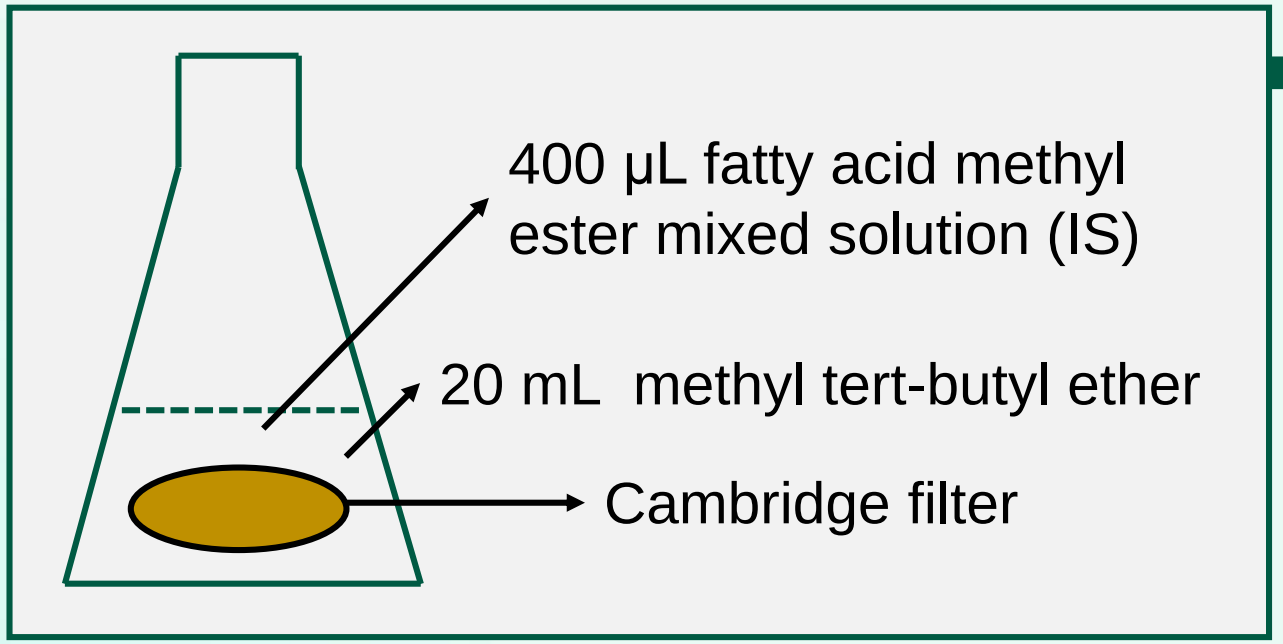
Cigarette samples equilibrated
(22 ± 2) ºC, (60 ± 2)% , 48 h

→

Cigarette smoking
ISO 3308 standard method, RM20H



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Agitated for 45 min

↓

Filtered through a 0.45 μm filter

↓

Heart-cutting 2DGC-MS analysis

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2 Analytical Method

3 Sample Analysis

4 Results and Discussion

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3 Sample Analysis

Cigarette samples

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Chinese Virginian type	British Virginian type	Blended type
Sample C1 (11 mg)	Sample V1 (11 mg)	Sample B1 (10 mg)
Sample C2 (11 mg)	Sample V2 (10 mg)	Sample B2 (10 mg)
Sample C3 (11 mg)	Sample V3 (10 mg)	Sample B3 (10 mg)
Sample C4 (11 mg)	Sample V4 (8 mg)	Sample B4 (8 mg)
Sample C5 (11 mg)		Sample B5 (8 mg)
Sample C6 (10 mg)		Sample B6 (8 mg)

3 Sample Analysis

Oral fluids collection

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Rinse the mouth

↓

Smoke two cigarettes

↓

Rinse the mouth with 15-20 mL purified water

↓

Collect the oral fluid

↓

Combine six oral fluid samples into one, internal standard added

↓

SPE pretreatment

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1 Introduction

2 Analytical Method

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4 Results & Discussion

Method Evaluation

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SPE procedure optimized

Good repeatability achieved (RSDs: 1.51%-6.98%)

Compounds	RSD (%)	Compounds	RSD (%)
3-Penten-2-one	2.05	Furfuryl alcohol	5.35
2-Methylfuran	1.51	3-Methyl-1,2-cyclopentanedione	2.72
2-Cyclopentenone	5.66	Acetaldehyde cyanohydrin	5.24
2-Methyl-2-cyclopenten-1-one	5.26	2-Methoxyphenol	3.80
3-(Methylthio)propionaldehyde	2.64	Benzyl alcohol	3.44
2-Furaldehyde	5.01	Phenol	4.78
3-Vinylpyridine	4.44	Triacetin	4.04
3-Methyl-2-cyclopenten-1-one	6.06	p-Cresol	2.10
2,3-Dimethyl-2-cyclopenten-1-one	6.14	4-Ethylphenol	2.34
5-Methyl furfural	5.78	2,3-Dihydrobenzofuran	3.48
4-Cyclopentene-1,3-dione	6.98	5-Hydroxymethylfurfural	3.99

4 Results & Discussion

OFs Analysis

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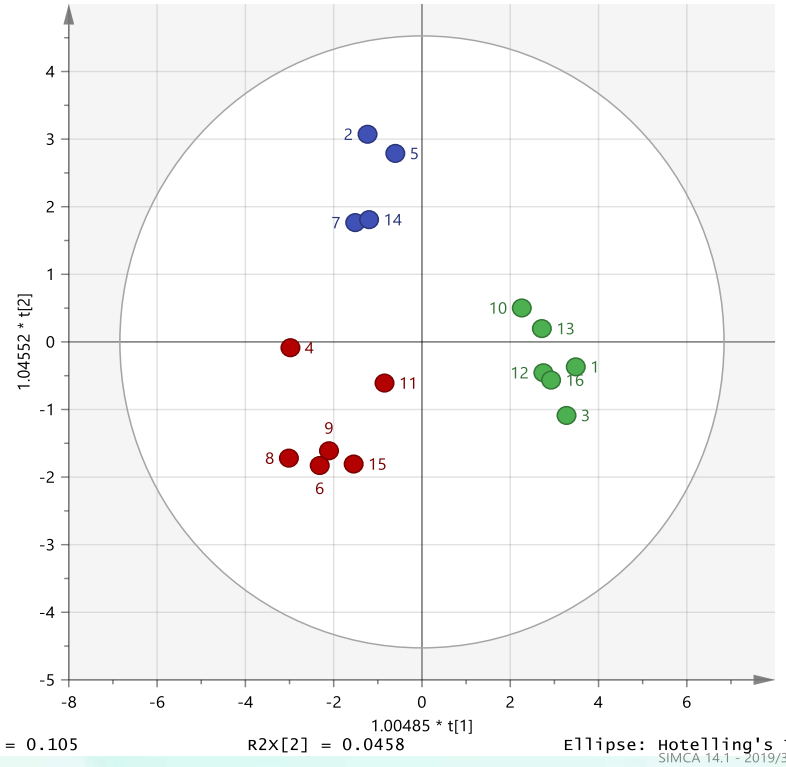
Chinese Virginian type
British Virginian type
Blended type

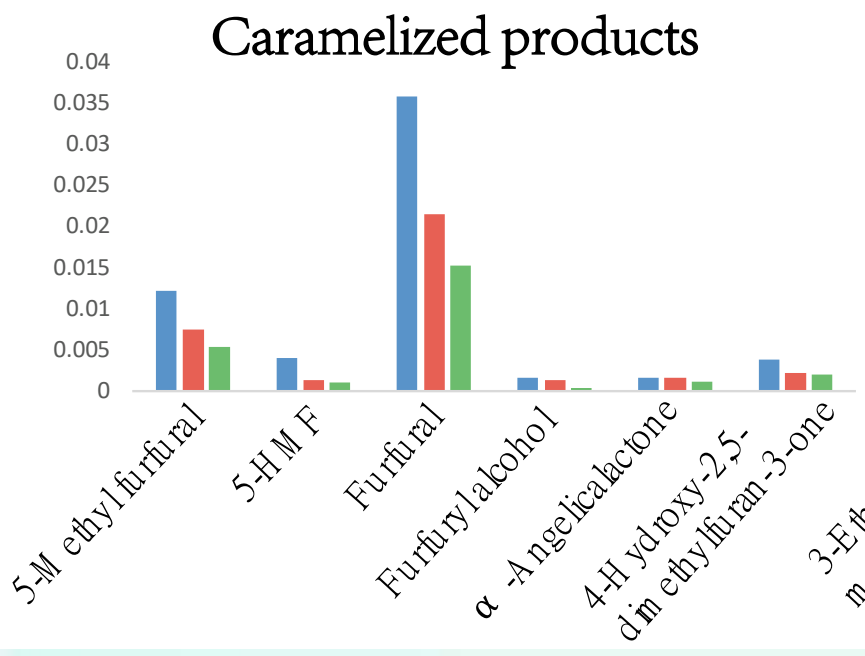


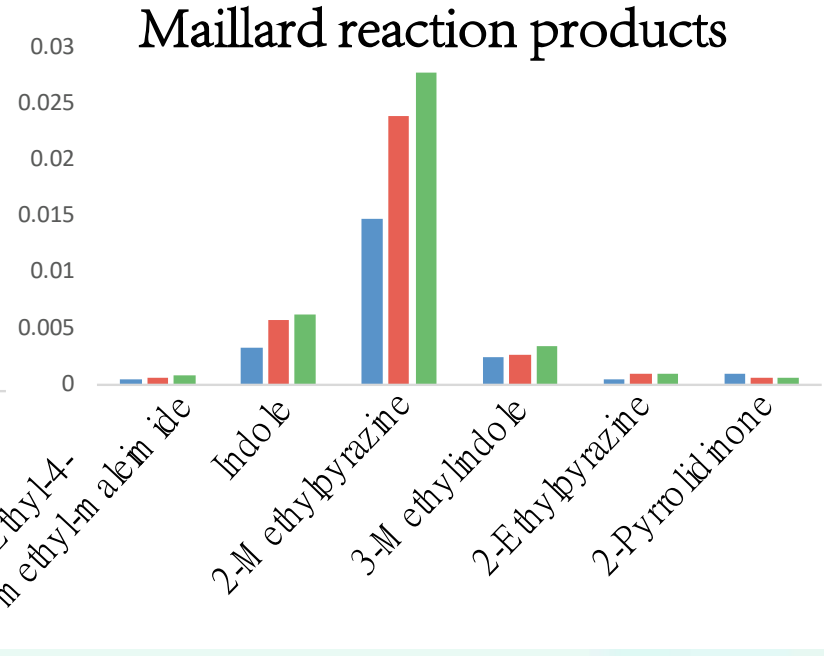
	Differential components (VIP value >1)
Phenols	2-Methoxyphenol, 4-Vinylguaiacol, Eugenol, 2,6-Dimethylphenol, o-Cresol, Isoeugenol, 2,6-Dimethoxyphenol
Caramelized products	5-Methyl furfural, 5-HMF, 2-Furaldehyde, Furfuryl alcohol , α-Angelica lactone, Furaneol
Cyclopentenones	2-Cyclopentenone, 3-Ethyl-2-cyclopentenone
Maillard reaction products	3-Ethyl-4-methyl-maleimide, Indole, 2-Methylpyrazine, 3-Methylindole, 2-Ethylpyrazine , 2-Pyrrolidinone
Else	2,3'-Bipyridine, Solanone, Cinene

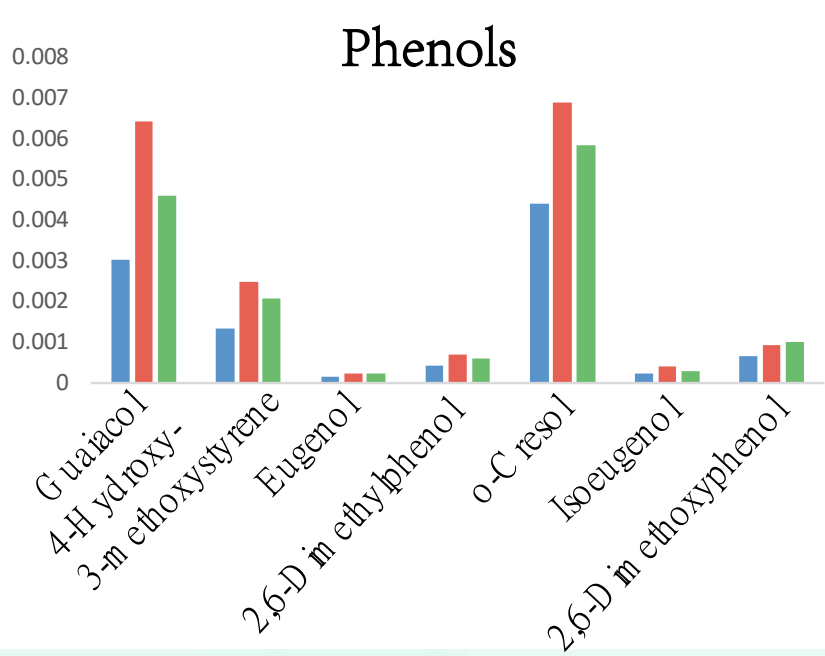
4 Results & Discussion

OFs Analysis

Chinese Virginian type British Virginian type Blended type







Chinese Virginian type:
The relatively high sugar content of flue-cured tobacco

Blended type:
Maillard reaction products produced by the burley tobacco casing and baking

British Virginian type:
The higher content of lignin (important precursor of simple phenol) in stems and flakes

4 Results & Discussion

Mainstream cigarette smoke vs OF

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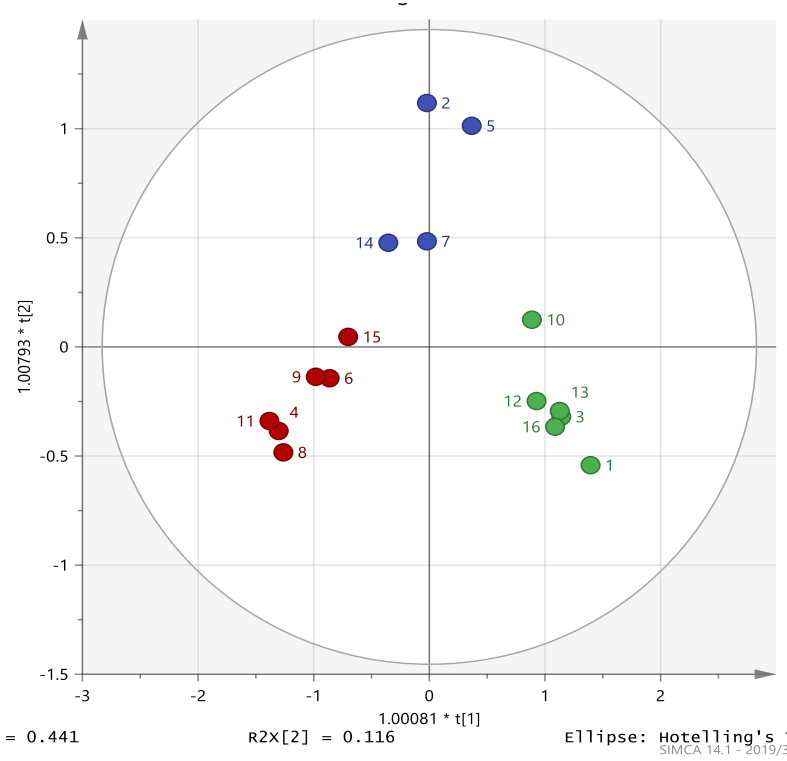
The kinds and contents of aroma components differed significantly between mainstream cigarette smoke and oral fluids of cigarette smokers.

Mainstream cigarette smoke



= 0.441 R2X[2] = 0.116 Ellipse: Hotelling's T2 (95%) = 0.105

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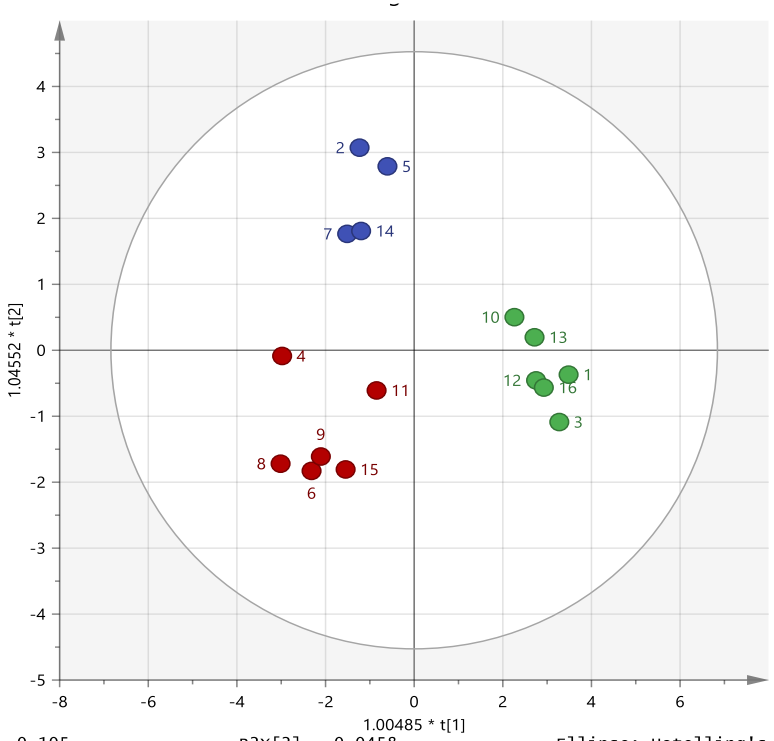
Oral fluid



R2X[2] = 0.0458 Ellipse: Hotelling's T2 (95%)

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- Chinese Virginian type
- British Virginian type
- Blended type

4 Results & Discussion

Mainstream cigarette smoke vs OF

VIP Value Ranking	Differential components in mainstream cigarette smoke (Ranking in OF)	Differential components in OF (Ranking in mainstream cigarette smoke)
1	2-Furaldehyde (5)	Guaiacol (23)
2	3-Furaldehyde (N*)	5-Methyl furfural (86)
3	2-Cyclopentenone (16)	5-HMF (8)
4	2-Acetylfuran (N*)	3-Ethyl-4-methyl-maleimide (36)
5	α-Angelica lactone (14)	Furfural (1)
6	2-Methyl-2-cyclopentenone (N*)	4-Hydroxy-3-methoxystyrene (35)
7	Cyclopentanone (N*)	Furfuryl alcohol (19)
8	5-HMF (3)	Indole (13)
9	Myosmine (N*)	Eugenol (67)
10	Methyl 2-furoate (N*)	Solanone (84)

The evaluation of sensory quality using only mainstream cigarette smoke may neglect some components which are more important for the oral senses.

4 Results & Discussion

Compound contribution

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- The distribution ratio of each compound was different in mainstream cigarette smoke and OFs
- The nicotine contents in OFs were less than 1/10 of the nicotine content in the mainstream cigarette smoke
- The content of some compounds such as limonene and neophytadiene is very low in OFs

- **The contributions of different compounds to the oral senses of consumers were different**
- **Compounds like pyridines, pyrrolidines, pyrazines, cyclopentenones, furfurals and phenols were more likely to retain in oral fluids.**

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5 Conclusion

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- **SPE-Heart cutting-2DGC/MS method** developed for the analysis of OFs collected from cigarette smokers
- The difference of components in **oral fluids of smokers of different type cigarettes** might related to the differences of cut tobacco structure
- The **kinds and contents of aroma components** differed significantly between mainstream cigarette smoke and oral fluids of cigarette smokers
- The **contributions of different compounds to the oral senses** of consumers were different

The aroma components in oral fluids of smokers could reflect their oral feelings more intuitively.

Thank you for your attention!

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