Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

Solid-phase microextraction for flavor analysis in Harari Khat (*Catha edulis*) stimulant*

AL-FLAHI Abdulsalam#, ZOU Jian-kai (邹建凯), YIN Xue-feng (殷学锋)

(*Department of Chemistry, Zhejiang University, Hangzhou 310027, China*)

E-mail: Flahi2@yahoo.com; jklj@mail.hz.zj.cn; cheyinxf@mail.hz.zj.cn

Received Apr.16, 2003; revision accepted June 20, 2003

Abstract: This research examined the typical flavor compounds in the commonest type of Khat called Harari Khat grown in the region of Ethiopia. Twenty-eight compounds, which includes 1,2-Propanedione, 1-Phenyl, Hexanol, Hexanal compounds, Limonene, Benzaldehyde with other flavors, were extracted by polydimethylsiloxane at room temperature for 30 min from Khat samples, and identified by solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). This method needs no organic solvents and required minimal sample.

Key words: Solid-phase microextraction, Gas chromatography-mass spectrometry, Catha, Khat leaves, Khat in Yemen, Chat (Khat) in Ethiopia

Document code: A **CLC number:** O657

INTRODUCTION

Khat, a chemical extracted from a plant *Catha Edulis*, belonging to the family Celastraceae, is used as a natural stimulant in East Africa and bordering Arabic states. Other names by which Khat is known include: Catha, qat, kat, chat, kuses-salahin, mirra, tohai, tschat, quat, african tea and african salad. The plant of *Catha edulis* is an evergreen flowering tree or large shrub found in Ethiopia and spread widely in Kenya, Nyasalnd (now Malawi), Uganda, Tanganyika, Tanzania, Somalia, Djibouti, and Yemen. It attains a height ranging from 3 to 4 m (Lewis, 1931). Fresh Khat leaves are crimson-brown and glossy but become yellow-green and leathery as they age. Khat in Yemen is now considered an essential part of social life, just like coffee or tea elsewhere! In many regions of the world, catha remains acceptable at the same time with tea and cigarettes (Elmi, 1983). Khat's users consider it the focal point of night parties and social gatherings. It is consumed by chewing (without swallowing) and retaining the lump chewed inside the mouth. The most favored part of the plant are young shoots near the top of the plant. However, leaves and stems at the middle and lower sections are also used. chewing Khat leaves produces a strong aroma and generates intense thirst. Harari Khat (Fig.1) is grown in the Harar region of Ethiopia and is exported to other countries. Harar city is located in the Ethiopia's eastern part where the main livelihood of the people is agriculture.

Khat and coffee are the main stimulant crops in this region, 1300–2200 meters above sea level, with 90% of it having temperate. Climate and average annual temperature of 28 °C and annual rain-

Master's degree student in Zhejiang University, from Yemen Central Laboratories, Ministry of Oil, Sana'a Yemen

*Project supported by scholarship from China

Fig.1 Harri Khat

fall of 600 to 800 millimeters.

Khat contains cathine (*d-norisoephedrine*), cathidine (Hattab and Birgit, 2000), cathinine fluoride (Ameen and Naji, 2001), iron, calcium, and magnesium. Cathine is an alkaloid found in *Epedra-Vulgaris*. Khat is very rich in ascorbic acid,which is an excellent antidote for amphetamine type compound.

Solid-phase microextraction (SPME) had been to be a sensitive and solvent-free means of extracting chemicals directly from headspace. SPME/GC/MS is an excellent tool for detecting and identifying volatile compounds (Yang *et al*., 2002). A lot of work on SPME head-space analysis had been reported in the literature (Pawliszyn, 1999). This technique had been applied successfully for evaluation of environmental samples (Jiu, 1997; 1998), volatile drug metabolites and toxic material in human fluids at elevated temperature, volatile contaminants in food and in groundwater, and flavor in beverages and food (Jon *et al.*, 2000). It was suggested as a potentially useful technique for flavor analysis (Yang and Peppard, 1994). In this study we examined the application of SPME for analysis of fresh solid Harari Khat flavor. We propose that this method to be carried out without any organic solvents.

MATERIALS AND METHODS

The fresh Khat plant was purchased from the open market in Ethiopia, and was held in foil and stored in a refrigerator at 10 ºC until analysis.

SP-ME procedures

The SPME device was purchased from Supelco Co (Bellefonte, PA), as was the fused silica fiber coated with polydimethy-siloxane/divinylbenzene (PDMS/DVB) 65 μm.

Instrumental analysis

An HP 6890 Gas Chromatograph, equipped with an HP-Innwax (30 m×0.25 mm id), film thickness (0.25 μm) capillary column, linked to an HP 5973 mass spectrometer system. The ionization energy was 70 eV; the temperature of the injection block was 250 ºC; the oven temperature program was 40 ºC, rising at 5 ºC/min to a final temperature of 220 ºC. Peak areas and retention times were calculated using Hewlett-Packard software. Helium was used as carrier gas at flow rate of 0.87 ml/min. The sample was crushed into powder in a mortar and then placed into a 20 ml serum bottle and clamped with a PTFE-lined cap for headspace analysis. SPME fiber was inserted into a sample vial containing 1.0 g sample at room temperature for 30 min, where it equilibrated; for thermal adsorption, the SPME was allowed to remain in the injector for 3 min, splitless injection mode was used, the split valve being opened after 2 min.

RESULTS AND DISCUSSION

Twenty-eight flavor compounds in Khat found and identified by SPME/GC/MS, are shown in Table 1, and the total ion chromatography is shown in Fig.2. This methodology is excellent for accounting for the strong aroma of Harari Khat.

The most prominent analyte peaks and high abundance were observed at 12.64 min 1-hexanol (peak 11), 13.497 min 3-hexen-1ol (z)-(peak 12), 14.073 min 2-hexen-1ol (e)-(peak 13), 16.936 min benzaldehyde (peak 17), and 23.552 min 1,2-propandione, 1-phenyl-(peak 25).

Cathinone is considered Khat leaves' main CNS-active component (Ripani, 1996).One Cathinone derivative (1,2-Propandion, 1-phenyl-) (Fig.3)

Table 1 Analytical result of the flavor compounds in *Catha*

No	Compounds	Retention time (min)	Relative content (%)	Molecular weight (MW)	Quality (%)
1	Hexanl	5.522	2.104	100	85
2	1-Penten-3ol	7.543	3.137	86	86
3	Limonene	8.344	3.137	136	94
4	1-Butanol, 3-methyl-	8.801	2.827	88	90
5	2-Hexenal, (E)-	9.010	6.324	98	91
6	1-Pentanol	9.938	1.245	88	80
7	Acetic acid, hexyl ester	10.526	1.311	144	86
8	Benzene, 1-methyl-4- (1-methylethl)-	10.324	0.419	134	94
9	2-Hexen-l-ol, acetate, (Z)	11.742	4.702	142	78
10	2-Penten-1-ol, (Z)-	11.884	4.702	86	80
11	1-Hexanol	12.640	23.403	102	90
12	3-Hexen-1-ol, (Z)-	13.497	18.646	100	91
13	2-Hexen-1-ol, (E)-	14.073	6.733	100	91
14	1-Octen-3-ol	15.226	0.198	128	38
15	1-Heptanol	15.334	0.205	116	85
16	Cedrene	15.978	0.141	204	90
17	Benzaldehyde	16.936	7.542	106	96
18	1,6-Octadien-3-ol, 3,7-dimethyl-	17.639	0.180	154	83
19	Caryophllene	18.508	0.427	204	95
20	Benzene acetaldehyde	19.836	0.471	120	72
21	Nonanol	20.259	0.207	144	87
22	Butanoic acid, 2-methyl- and butanoic acid, 3-methyl-butanoic acid, 3-methyl-	20.491	0.469	102	85
23	Methyl salicylate	22.718	0.210	120	95
24	Lzeraniol	23.350	0.131	154	90
25	1,2-Propanedione, 1-phenyl-	23.552	4.296	148	87
26	Hexanoic acid	24.310	0.102	116	80
27	Benzyl Alcohol	24.907	1.210	108	94
28	Phenylethyl alcohol	25.606	0.481	122	94

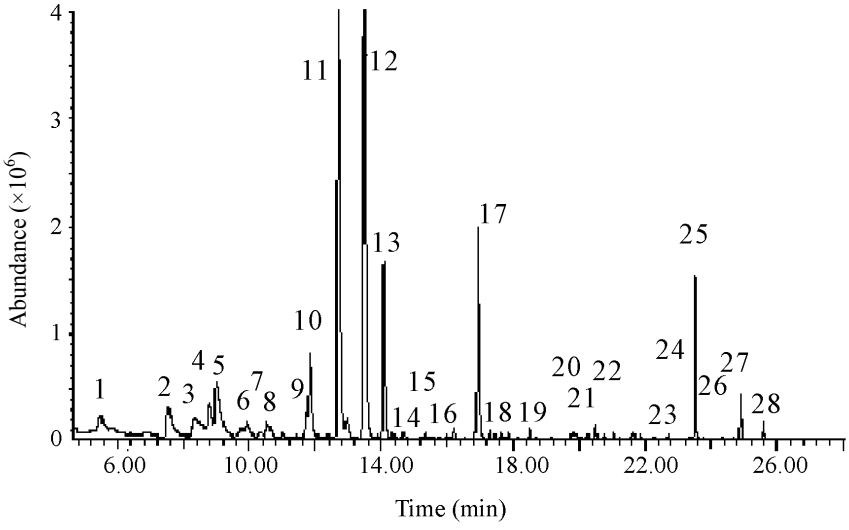

Fig.2 Total ion chromatography from headspace SPME GC/MS analysis of KHAT flavor sample

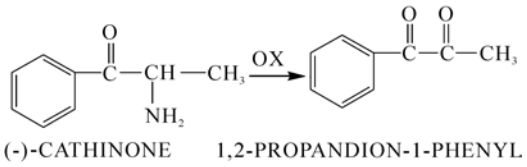

Fig.3 Pathway from cathinone to 1,2-Propandion, 1-phenyl-

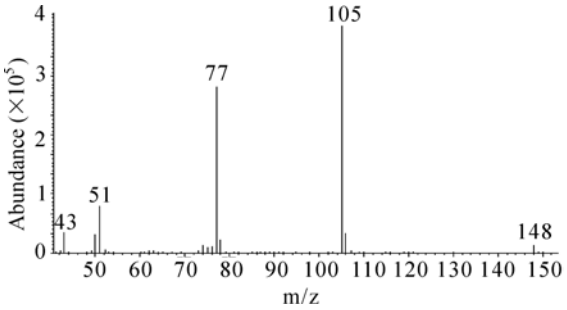

Fig.4 Mass spectrum of 1,2-Propandion, 1-phenyl peak in Fig.2

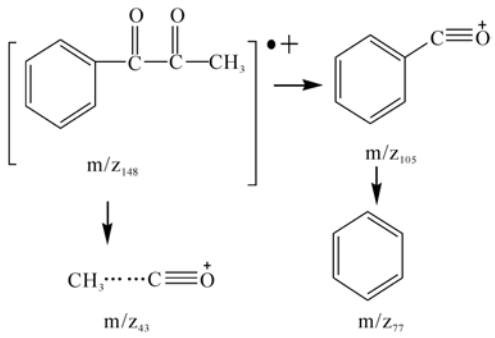

Fig. 5 1,2 Propandion, 1-Phenyl fragmentation

was extracted by SPME at room temperature as shown by peaks (m/z 148, m/z 105, m/e 77 and m/z 43) in the mass spectrum (Fig.4), which is indicative of 1,2-Propandion, 1-phenyl fragmentation as we see in Fig.5.

CONCLUSION

The strong flavor in Harari Khat was identified by SPME GC/MS, which is very simple, rapid and does not require any organic solvent.

References

Ameen, J.R.M., Naji, J.A., 2001. Causal models for road accident fatalities in Yemen. *Accident Analysis Prevention*, **33**:547-561.

Elmi, A.S., 1983. The chewing of Khat in Somalia. *J. Ethnopharmac,* **l8**:163-176.

Hattab, F.N., Birgit, A.M., 2000. Fluoride content in Khat (*Catha edulis*) chewing leaves. *Archives of Oral biology,* **45**:253-255.

Jiu, A., 1997. Headspace solid-phase microextraction dynamics and quantitative analysis before reaching a partition equilibrium. *Anal. Chem*, **69**:3260-3266

Jiu, A., 1998. Solid-phase microextraction in headspace analysis. dynamics in non-steady-state mass transfer. *Analytical Chemistry*, **70**:4822-4826.

Jon, G.W., Eric. D.C., Yongkyoung, K., Manuel, H., John, B.S., Dwight, W.M., 2000. Sample preparation for the analysis of flavors and off-flavor in foods. *Journal of Chromatography*:*A*, **880**:3-33.articls

Lewis, L., 1931. Phantastica, Narcotic and Stimulating Drugs (Translation of 1924, German edition). Routle and Kegan Paul, London.

Pawliszyn, J., 1999. Application of Solid Phase Microextraction (Book), Chapters 1 & 2 on Quantitation. *Royal Society of Chemistry*, **85**:404-525.

Ripani, L., Schiavone, S., Garofano, L., 1996. GC/MS identification of *catha edutis* stimulant-active. *Forensic Science International*, **78**:39-46.

Yang, X., Peppard, T., 1994. Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem.*, **42**:1925-1930.

Yang, S.S., Huang, C.B., Smetena, I., 2002. Microextraction for volatile compounds in Tobacco. *Journal of Chromatography*:*A*, **942**:33-39.