Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(7):2456-2459

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Analysis of the volatile components of brown coal distillate using the headspace extraction-GC-MS

Hui Zhu[1], Yi Qin[1], Lingdi Ma[1], Mi Zhang[1], Yang Zhao[1], Baocai Li[1*] and Huifen Zhang[2*]

[1]Faculty of Life Science and Technology, Kunming University of Science and Technology. Kunming, China
[2]Food Security Research Institute of Yunnan Province, Kunming University of Science and Technology, Kunming, China

---

ABSTRACT

The headspace extraction-GC-MS was used to separate and identify the volatile components of brown coal distillate(BCD)in this paper, while the retention index (RI)was verified using the C_8-C_{40} n-alkane mix standard. There were 15 kinds of volatile components of BCD which had been identified; the main ingredients included acids, alcohols, esters, ketones, etc. And the quantity of each component was measured by the method of peak area normalization, the following several components content were higher: N-hydroxy ethyl amidine (5.396%), acetic acid (22.567%), urea (11.536%), methyl formate (8.475%), 2, 5-adipic ketone (5.521%), acrylic acid (12.470%), ethyl phthalate (8.446%), etc. The results indicate that the GC-MS in combination with RI can significantly improve the accuracy of the unknown compounds qualitative test, and increase the reliability of the test results. The method of measuring volatile components of BCD established in this paper provide technical reference for exploring the anti-salt stress active substance andits mechanism, as well as further development and utilization.

Key words: BCD; volatile components; headspace extraction; GC-MS; RI

INTRODUCTION

Brown coal is a kind of low calorific value coal in the primary stage, and belongs to the inferior coal, but it occupies an important position in the reserves of coal resources of the world. The characteristic of brown coal is low degree of coalification, low calorific value, high moisture, and containing a large amount of humic substances. It has been traditionally used as the fuel directly, such a heating and power generation. However, direct combustion not only give rise to wasting of resources, but also cause serious environmental pollution[1-3].

Extraction of humic substances is an efficient utilization method of brown coal, which has been earlier researched. Because of its biological activities, humic substances can be used in agriculture production, such a regulating crop growth (short growing period and improve production), increasing crop resistance(protect crop from drought, cold, dry-hot wind, lodging, diseases and insect pests), improving the quality of crops[4-5], respectively. And it can be made into fertilizer yet which has been used widely. Study shows that the plant physiological activity of humic substances is closely related to its small molecular compounds. It has a significantly performance especially in promoting growth, improving the resistance and the quality of crops[6-8]. The effective way of humic acid extracting is oxidative degradation, of which brown coal with nitric acid or hydrogen peroxide as the oxidant. Therefore, it increases the small molecular components and finds the bioactive compounds, then made into high-grade fertilizer.

The research group got a distillate in the process of enrichment degradation liquid of brown coal(using the hydrogen peroxide as the oxidant). The distillate has a stronger activity of easing the wheat seed to salt stress than humic acid substances, at the same time it can improve the germination rate significantly and promote the growth of embryo and radicle under the absence of salt stress[9].The distilled fluid which we named brown coal distillate (BCD) is colorless and transparent, pH is 2~3, density is 1.02~1.05g/cm^3, the largest molecular weight of the components is less than 500 measured by LC-MS. The enrichment, isolation and structure identification of BCD is very difficult, due to its chemical composition is volatile, strong polarity. On account of the comparison of various methods, we choose the headspace extraction-GC-MS[10-15] as the method of separation and analysis of the chemical constituents from BCD, and combine the retention index (RI) for validation. The main volatile components of BCD are identified successfully for the first time. The material basis of BCD activity for the resistance to salt stress has been clarified in this study, which could be benefit to the active mechanism research, and also provide a scientific basis for further development and utilization of BCD in agriculture.

EXPERIMENTAL SECTION

Materials

Agilent GC/MS 6890N/5975C spectrometer and Agilent 7694E automatic headspace sampler: Agilent, USA; N-alkanes mixed standard Standard: C_8-C_{40}, U.S. Accu Standard company; Yunnan Eshan brown coal: Shed rent coal mine; Rotary evaporator: R1002, Shanghai Shenshun Biotechnology Co.ltd.; 30% hydrogen peroxide: AR, Tianjin East Tengen Reagent Factory.

BCD sample preparation

Thedried Eshan brown coal was pulverized and sieved through 80 mesh sieve, so that its physicochemical property such as moisture, ash, total acidic group content can be analyzed. The results can be shown in Tab.1.

Tab.1 The main physicochemical properties of the feedstock coal

Sample	The water content	The ash content	The total HA content	The free HA content
Eshan brown coal	18.30	13.16	51.29	53.25

1L30%(v/v)H_2O_2wasadded into the reactor with 1kg sieved brown coal, continuously stirring for 3h at40℃.When the oxidation reaction is finished, the product is centrifuged 15 min (speed 3500 r/min) and the filtered supernatant fluid is the degradation liquid. The BCD is collected by vacuum-rotary evaporation procedure at 50℃, which is colorless and transparent and pH value is 2.5, density is 1.04g/cm^3.

Experimental conditions

Headspace conditions Bottle temperature:70℃; ambienttemperature:80℃; transmission line temperature: 90℃; bottle equilibrium time: 10 min; carrier gas: purity He; Sample pressure: 103.42 kPa; pressing time: 10 s; injection time: 1.0 min .

Chromatographic conditions Column: HP-FFAP fused silica capillary column (30.0 m × 0.25 mm × 0.25 μm); carrier gas: He, flow rate: 2.0mL/min, constant current mode; injector temperature:180 ℃; split injection, split ratio of 10:1; the initial temperature is maintained at 60℃ for 7 minutes, programmed to 180℃at a rate of 2℃/min, hold for 10 minutes. transmission line temperature: 280℃.

MS conditions Using electron bombard iron source under the condition of electronic energy 70 eV; electron multiplier voltage:980V;Solvent delay time: 7min; the temperature of ionization source and quadrupole are set: 250℃,150℃;scanning mode: full scan, mass range of m/z 50 to 550 atomic mass units and the scan rate is 0.5 scan/s.

Operating steps

Sealing the 20 mL headspace bottle with a rubber gasket and aluminum cap after adding 5 mL BCD in it, and then put it into headspace injector. Using GC/MS analysis the sample under the above conditions.

Determination of RI According to the conditions mentioned above, each n-alkane standard peak retention time values is measured, using linear heating program of retention index formula: $RI = 100Z + 100 [TR(X) - TR(Z)] / [TR(Z+1) - TR(Z)]$ to calculate the volatile components of the RI values, wherein: TR (X), TR (Z), TR (Z+1) is the peak retention time value of the sample and n-alkanes standards substance which carbon atoms are Z, Z+1 respectively, moreover $TR(Z) < TR(X) < TR(Z+1)$. The RI of analyzed components is obtained through calculate the retention time of components getting by GC, verify the structure of component further[16-17].

RESULTS AND DISCUSSION

BCD is analyzed by GC/MS under the above conditions, and the total ion current chromatogram of volatile components of BCD which is shown inFig.1.

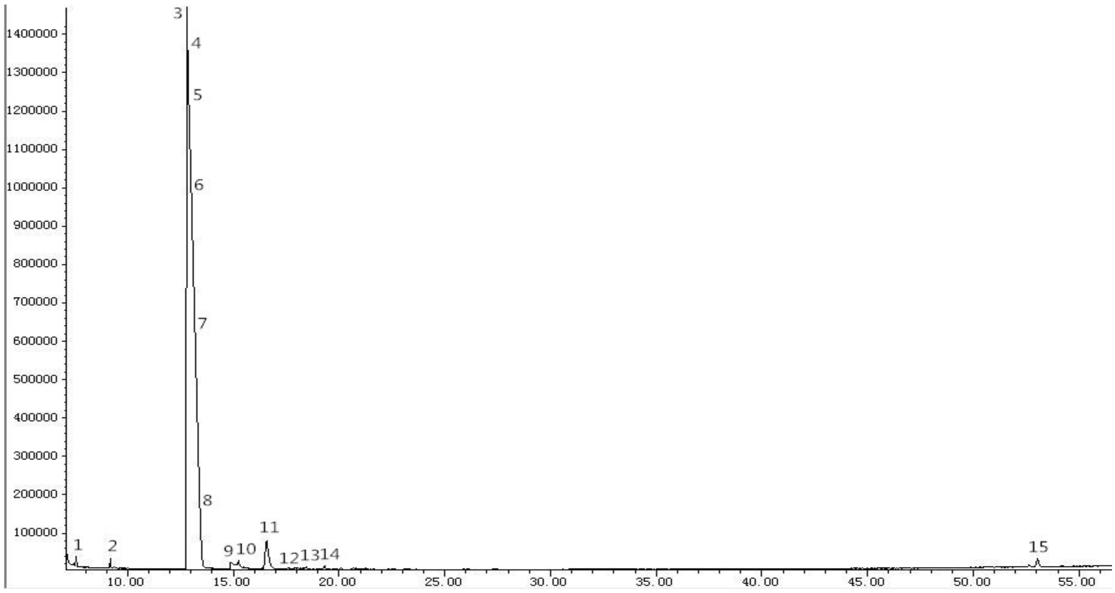

Fig.1The total ion current chromatogram of volatile components of BCD

Fig.1 indicates that the separation degree and quantitative accuracy of spectrogram are better under the chromatographic conditions. The relative percentage of the volatile components is obtained through artificial analyzing of spectrogram and the peak area normalization calculating. Comparing the results of RI values, 15 kinds of main volatile components of BCD is obtained which have the big similarity. The results are shown in Tab.2.

Tab.2 The identification results of volatile components of BCD

NO	Compound	Retention time/min	Molecular formula	Similarity /%	RI	Relative content /%	The main ion peak of mass spectrum
1	Pyridinium,dinitrometh-ylide	7.567	$C_6H_5N_3O_4$	86.2	972	4.227	51、52、59、78、79
2	Acetamideorime	9.175	$C_2H_6N_2O$	95.4	1018	5.396	53、55、56、58、74
3	Acetic acid	12.791	$C_2H_4O_2$	97.8	1097	22.567	15、43、45、60
4	Isopropanol	12.983	C_3H_8O	92.2	1102	4.837	15、19、27、43、
5	Urea	13.106	CH_4N_2O	93.5	1105	11.536	16、17、28、44、60
6	Methyl formate	13.312	$C_2H_4O_2$	89.3	1110	8.475	15、29、31、32、60
7	Methoxyacetlc acid	13.581	$C_3H_6O_3$	93.7	1116	3.174	14、29、31 42、45
8	Diethyl oxalate	14.385	$C_6H_{10}O_4$	92.4	1136	1.353	27、28、29、45、74
9	Acetonylacetone	15.255	$C_6H_{10}O_2$	89.6	1157	5.521	15、43、57、99、114
10	Hydrazine,1,2-dimethyl	15.916	$C_2H_8N_2$	85.5	1173	0.756	28、30、45、59、60
11	Propionic acid	16.587	$C_3H_6O_2$	94.7	1190	12.470	28、45、57、73、74
12	Phenylacetic acid	17.244	$C_8H_8O_2$	96.4	1205	0.582	39、51、63、91、136
13	2(3H)-Furanone,dihydro-5,5-dimethyl	18.445	$C_6H_{10}O_2$	91.1	1229	0.328	55、59、70、91、99
14	Gamma-Valerolactone	19.338	$C_5H_8O_2$	92.6	1246	1.154	55、56、57、85、100
15	Diethyl phthalate	53.050	$C_{12}H_{14}O_4$	88.7	2154	8.446	89、105、149、150、176、177

The molecular of all the volatile components are small, and the special are less, but the difference of component relative percentage is big. The compounds of acid, alcohol, ester, and ketone are the main ingredients. The identified components account for 90.8% of the total volatile. Acetic acid, Urea, Methyl formate, Propionic acid, Diethyl phthalate, etc., is the major component of BCD, accounting for 63.494%. The content Acetic acid and Propionic acid is higher and have a great contribution to the acidity of solution. Urea is an indispensable source of nitrogen for plants [18-19].In addition, Methoxyacetlc acid, Diethyl oxalate, Hydrazine, 1, 2-dimethyl, Phenylacetic acid, Gamma-Valerolactone, etc. is the components of BCD also. These components have a low content, but the activity is very strong. Phenylacetic acid is a natural plant growth regulator, which could improve the rate anther callus differentiation and plant regeneration rate significantly, and also shorten the seedling period[20-22]. According to Yamada P[23], Gamma-Valerolactone structure analogue is one of the main active structural unit of pharmacological activities of humic acid.

In the process of researching of BCD activity and its chemical composition, using a polar fused silica capillary column HP-FFAP combining with headspace extraction technology is a key to analyzing the volatile components.BCD not only has activity of alleviating the salt stress, but also has the potential of drought resistance, activity of keeping fresh. Although it is a single or a variety of ingredients synergistic effect is unclear now, but the research about the components and activity of BCD has a great theoretical and practical value in the area of promoting and enhancing the growth and the yield of crops under the environment of salt inverse. Research of BCD activity of resistant to salt stress, a problem which need solved urgently is that ascertain the active intergradient and the action mechanism.

CONCLUSION

It is a very good analysis method to the volatile components of BCD that use the headspace extraction-GC-MS in combination with RI. As the low content of BCD volatile component, it is very difficult to enrichment, separation and structural identification. The use of headspace extraction GC-MS analysis can be a very good method, and the use of RI as auxiliary means to verify the results of mass spectrometry after parsing make the results more accurate and credible. Meanwhile this method don't need any reagents, the process of analysis is convenient, fast and sensitive.BCD is a classification of molecular weight in the process of lignite oxidation, which has smaller molecular weight and more prominent activity. The breakthrough of this paper provides technical reference for exploring the anti-salt stress active substance and its mechanism, as well as further development and utilization.

Acknowledgements
This work was supported by the national natural science foundation of China (NO.21166013) and applied basic research project of Yunnan, China (NO.2013FZ025).

REFERENCES

[1] XX Ma; HB Zhao,*China Coal*, **2004**, 30(9),47-49.
[2] Tree DR; Clark AW,*Fuel*,**2009**,79(13),1687-1695.
[3] Dennis JS; Scott SA; Hayhurst AN,*Journal of the Energy Institute*,**2006**, 97(3),181-190.
[4] LQ Hu; M Zeng; WA Zeng; et al.,*Journal of chemical and Pharmaceutical Research*, **2014**, 6(3),447-451.
[5] RMAtiyeh; S Lee; CA Edwards,*BioresourceTechnology*, **2002**, (84), 7-14.
[6] D Cheng; YM Peng; XYCui; et al.,*Humic Acid*,**1996**(3), 33-34.
[7] YL Chen; M Cao; YY Li,*Plant Physiology Communications*, **2000**,36(4), 311-314.
[8] TG Gao; JS Yang; HL Yuan,*Humic Acid*, **2009**(1),14-18.
[9] LD Ma; YQin; H Zhu;et al., *Life Science Research*,**2014**,18(6).
[10] Safarova VI; Sapelnikova SV; Djazhenko EV; et al.,*Journal of chromatography B*,**2004**,800(1/2),325-330.
[11] Ines M; Lotfi S; Bassem J; et al.,*Journal of chromatography Science*, **2009**,47(4), 257-262.
[12] Shinya H; Toshiyuki T,*Journal of Separation Science*,**2001**,24(2), 97-103.
[13] Drosos JC; ViolaRhenals M; VivasReyes R,*Journal of chromatography A*, **2010**, 1217(26), 4411-4421.
[14] RehanaBanuH; NagarajanN, *Journal of chemical and Pharmaceutical Research*,**2013**,5(4), 279-285.
[15]Anbuselvi S; Jeyanthi Rebecca L; Sathish Kumar M; et al., *Journal of chemical and Pharmaceutical Research*, **2012**, 4(2), 1246-1250.
[16]YH Wang; PK Wong,*Chemosphere*, **2003**, 50(4),499-505.
[17] RJ Hu; HX Liu; RS Zhang; et al.,*Talanta*, **2005**, 68(1),31-39.
[18] YL Chen,*Chinese Bulletin Botany*,**2001**,17(1),11-16.
[19] C Li; KH Liu; JK Lan; et al.,*Journal of Guangxi Academy of sciences*,**2001**, 17(3), 129-132.
[20] Marassi MA;Bovo A;Lavia GL,*Plant Physiol*, **1993**(141),610-614.
[21] Leuba V; Tourneau DL, *Plant Growth Regul*, **1990**, 9,71-76.
[22] Ziauddin A;Marsolai A;Simion E; et al.,*Plant Cell Reports*,**1992**(11),489-498.
[23] Yamada P;Isoda H; Han JK,*Bioscience Biotechnology and Biochemistry*,**2007**,71(5), 1294-1305.