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Chemical Composition of the Essential Oils of *Brassica juncea* (L.) Coss. Grown in Different Regions, Hebei, Shaanxi and Shandong, of China

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ABSTRACT

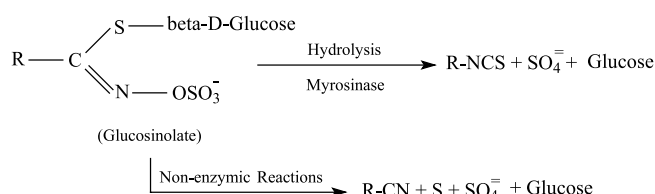
The composition of essential oils in the seeds of *Brassica juncea* (L.) Coss., grown in Hebei, Shaanxi and Shandong Province was determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). We have identified twenty-two compounds constituting 89.6% by weight of the mustard oil from Hebei Province. The main components were allyl isothiocyanate (54.8%), diallyl trisulfide (9.4%), diallyl sulfide (5.5%) and 3-butenyl isothiocyanate (4.8%). Fourteen compounds representing 94.5 % by weight of the mustard oil from Shaanxi Province were identified. They were allyl isothiocyanate (68.8%), diallyl trisulfide (7.8%) and 3-butenyl isothiocyanate (4.9%). Fifteen components were identified accounting for 94.3% by weight of the mustard oil from Shandong Province. Major constituents were allyl isothiocyanate (61.3%), diallyl trisulfide (9.7%) and 3-butenyl isothiocyanate (5.9%).

Key words: *Brassica juncea* (L.) Coss., allyl isothiocyanate, mustard oil, essential oil, gas chromatography-mass spectrometry (GC-MS)

INTRODUCTION

Brassica juncea (L.) Coss. belongs to the genus *Brassica* of the *Cruciferae* family. Seeds of this plant are widely used in America, Japan, China and other countries and regions as a traditional pungent spice, a source of edible oil and protein, and a type of medicine. The essential oil of *B. juncea*, mustard oil, has also been used in cosmetics for hair control⁽¹⁾. It is also one of the indispensable materials used for producing curry powders, pungent sauces, and yolk sauces. Its Chinese name is "Jiemo". In fact, dry seeds of *B. juncea* do not have the pungent flavor. The pungent flavor is only developed when the seeds are ground and macerated in water under specific conditions⁽²⁾. This is because there are thioglucosides (TGDs) in the seeds of *B. juncea*, which are the precursors of the essential oil of *B. juncea*. TGDs are hydrolyzed to form isothiocyanates or other sulfur-containing compounds. The hydrolytic conditions of TGDs affect the composition and yield of the essential oil⁽³⁾. The main component of mustard oil is allyl isothiocyanate^(3, 4). In addition, it was found that after the addition of 0.2% *Trichoderma reesei* cellulase (its commercial name is Celluclast TM) in mustard seeds, yield of the essential oil was approximately 50% higher than that obtained without cellulolytic pretreatment⁽⁵⁾. The hydrolytic reactions of TGDs in the seeds of mustard could be illustrated as the following⁽⁶⁾:

The pharmacological effects of mustard oil have received a great deal of interest. The essential oil of *B.*



Scheme 1. Hydrolysis of glucosinolates in *B. juncea* under different conditions⁽⁶⁾

juncea has very high application value and can be used to suppress the growth of microorganism in seafood, such as *Helicobacter pylori* and *Vibrio parahaemolyticus*. It also shows inhibitory effects on growth of bacteria that cause food poisoning and fungi⁽⁷⁻⁹⁾. The oil exhibits significant inhibitory activities against *Aspergillus niger*, *A. flavus*, *Trichoderma viride*, *Candida albicans*, *C. utilis*, *C. tropicalis*, *Cryptococcus neoformans*, *Trichosporon mucoides*, *Trichophyton tonsurans* and *Geotrichum capitatum*⁽¹⁰⁻¹³⁾. Moreover, the oil shows inhibitory effect on tumor cells and is effective in antiplatelet and anticancer⁽¹⁴⁾.

There have been a few studies on the composition of the oil. Vaughan et al. reported on the composition of North American mustard oil⁽¹⁵⁾, while Kojima et al. determined the low mass volatile constituents of Japanese mustard oil⁽¹⁶⁾. Kharchenko et al. found that the major components of Indian mustard oil were allyl, butyl, phenylethyl, and pentenyl isothiocyanates⁽⁴⁾. Osik et al. described the chemical components of seed and oil of leaf mustard. Special attention was given to fatty acids, glucosinolates, and essential oils⁽¹⁷⁾. Inahata et al. determined the quantity of 3-butenyl isothiocyanate in mustard oil and found that it

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was an important component in wasabi flavor (Japanese)⁽¹⁸⁾.

Mustard oil has often been labeled and sold as (natural) mustard oil or adulterate mustard oil with synthetic chemicals like allyl isothiocyanate. It was well known that allyl isothiocyanate is a major component of the mustard oil. It can be synthesized at a much lower price than its cost when extracted from mustard seeds. Therefore, the adulteration of mustard oil by adding synthetic allyl isothiocyanate is very profitable. In order to detect such a fraud, the detailed analysis of mustard oils obtained from three different regions, Hebei (N39°, E117°), Shaanxi (N34°, E108°) and Shandong (N37°, E122°), in China are performed and this study also makes it possible to propose an available method for adulterate analysis of mustard oils.

MATERIALS AND METHODS

I. Plant Materials

The seeds of *B. juncea* (L.) Coss. were collected in November, 2000 from Hebei, Shaanxi and Shandong Province, China. The seeds were dried at 60°C for 8 hrs, ground to powders (40 mesh) and placed in a desiccator prior to use.

Samples of the essential oils were extracted by the following procedure^(16, 17): 50 g of mustard powders, 150 mL of distilled water, 5 mL of buffer solution (pH = 4.5, the buffer was prepared by mixing 0.1 mol/L sodium hydroxide with 0.2 mol/L potassium biphthalate in a volumetric proportion of 2 to 5) and 1 mL of 1 mg/mL ascorbic acid were added to a 250-mL stopper round flask. After thorough mixing, the mixture sample was extracted at 70°C for 2 hrs. The essential oil samples were separated by steam-distilled and dried using anhydrous sodium sulfate and filtered through the 0.22 μ m film (Millipore Company, Bedford, Massachusetts, USA) before analysis. The oil obtained was a pale yellow pungent liquid. The yields lied in the range of 0.4-0.7% (V/W).

II. Gas Chromatography (GC) Conditions

Gas chromatography (GC) analyses of the oils were performed on a Hewlett-Packard chromatograph (Hewlett Packard Company, Palo Alto, CA, USA), model 6890 Series II, equipped with a capillary column (HP-5MS, crosslined 5% PH ME siloxiane, 30 m \times 0.25 mm i. d., 0.25 μ m film thickness, HP part No. 19091s-433) and a FID detector. The flow rate for the helium carrier gas was 1.0 mL/min. The injector temperature was 250°C. A 1.0 μ L sample was injected in the split mode with a split ratio of 12:1. The temperature program was: 35°C for 3 mins, 35 to 150°C at a rate of 5°C/min and subsequently held isothermally for 2 mins, 150 to 250°C at a rate of 4°C/min, and finally held isothermally for 6 mins.

III. Gas Chromatography-Mass Spectrometry (GC-MS) Conditions

Gas chromatography-mass spectrometry (GC-MS) analyses of the oils were performed on a Hewlett-Packard chromatograph, model 6890 Series II, equipped with a Hewlett-Packard 6890 Series injector and a mass spectrometer selective detector 5973 (MS) (Hewlett Packard Company, Palo Alto, CA, USA). GC conditions: The column was coupled directly to the MS, and the flow rate for the helium carrier gas was 1.0 mL/min. The injector temperature was 250°C. MS conditions: Ionization voltage (EI) was 70 eV. Ion source temperature was 280°C. Scan mass range was 25-400 m/z. Solvent delay time was 1 min. The column used and other operating conditions were the same as those of GC.

RESULTS AND DISCUSSION

The essential oils in seeds of *B. juncea* grown in Hebei, Shaanxi and Shangdong Province were identified by comparison of mass spectra with those of authentic standards of a mass spectra library (Wiley275.L Database) and explanation and certification of spectra. Twenty-five components were identified, which represented 89.6-94.5% by mass of the mustard oils. The total ionic chromatograms (TIC) of the oils are shown in Figure 1. The chemical compositions of the oils are compiled in Table 1.

Although the mustards were obtained from different regions of China, the oils are essentially similar in chemical composition. Isothiocyanates were shown to be the main group of constituents in the essential oils of *B. juncea*. In this fraction, allyl isothiocyanate (54.8 – 68.8%), 3-butenyl isothiocyanate (4.8 – 5.9%) and phenethyl isothiocyanate (2.4 – 3.4%), which were present in all samples, represented more than 62.9% of the total essential oil. The above result was different from that of synthetic mustard oil. Only allyl isothiocyanate existed in synthetic mustard oil and its quantity often was over 90%.

The sulfides were present in relatively small amounts (14.8 – 23.4%). Diallyl trisulfide (7.8 – 9.7%), diallyl sulfide (3.2 – 5.5%) and diallyl disulfide (2.7 – 4.1%) were the main component of this fraction.

Comparing chemical compositions of Chinese mustard oil with those of other countries' mustard oils, we found that there were certainly differences in the components of the oils from different countries. Twenty-two new components with relatively low concentrations were first identified in Chinese mustard oil, but these compounds did not exist in other countries' mustard oils^(4, 7, 9, 16), including diallyl sulfide, methyl allyl disulfide, 2,3-dimethyl 2-butenic acid, diallyl disulfide, methyl allyl trisulfide, benzenepropanenitrile, 2-propen-1-thiol, diallyl trisulfide, 6-undecanol, diallyl tetrasulphide 4-(4,5-dihydro-4,4-dimethyl-2-oxazolyl)-isoxazole, *N, N'*-diallyl thiourea, *anti*-9-methyl-1,6-methano-fluorene and four advanced alkanes. Moreover, allyl-, butyl-, 3-butenyl-, 3-butyl-, pentyl- and phenethyl isothiocyanate in Chinese mustard oil have been previously found in essential oil from other countries^(4, 7, 15).

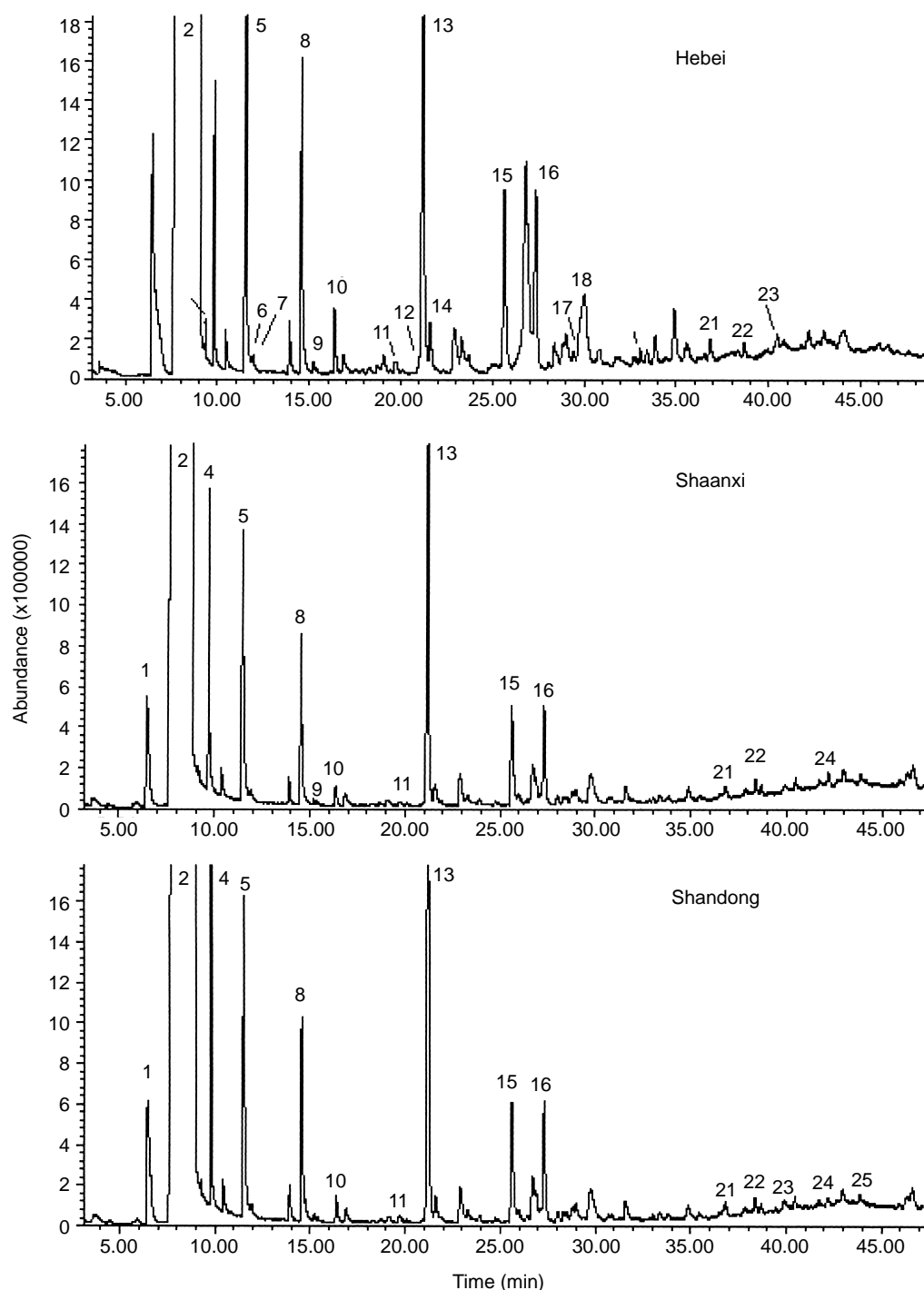


Figure 1. Total ionic chromatograms of the essential oil of *B. juncea* (L.) Coss. obtained from Hebei, Shaanxi and Shandong, China

¹⁶). In addition, dimethyl trisulfide, 3-methylthiopropyl isothiocyanate, phenylacetonitrile, phenylpropionitrile and phenylpropionamide were not found in Chinese mustard oil, but they existed in Japanese oil⁽¹⁹⁻²¹⁾. 5-methylisothiazole existed in Korean mustard oil⁽⁹⁾, but it was not found in Chinese mustard oil. The high concentrations of allyl isothiocyanate (54.8 – 68.6%) are present in Chinese mustard oils. It was well reported that this compound showed strong antimicrobial activity against a wide spectrum of bacteria (22-24).

The composition of the essential oil of *B. juncea* differs, depending on where it is grown. The differences in chemical composition, which can affect the biological activities and pharmaceutical applications, are probably due to the differences in the climatic and geographical conditions (temperature, rainfall, altitude, hours of sunshine, etc.). The results suggest that different chemo-types of *B. juncea* exist in China. These results are useful for future studies on the health benefits of Chinese mustard oil and for adulterate analysis of the mustard oil.

Table 1. Composition of the essential oils of *Brassica juncea* (L.) Coss. collected from Hebei, Shaanxi and Shandong, China

Peak No.	R.T. ^a (min)	Compound	Hebei		Shaanxi		Shandong	
			mg/100g ^b in seeds	%, w/w ^c in oil	mg/100g in seeds	%, w/w in oil	mg/100g in seeds	%, w/w in oil
1	6.45	Diallyl sulfide	22.4 ± 0.4 ^d	5.5 ± 0.1	20.5 ± 1.9	3.2 ± 0.0	25.5 ± 0.7	3.7 ± 0.1
2	9.06	Allyl isothiocyanate	224.8 ± 2.1	54.8 ± 0.5	440.4 ± 7.5	68.8 ± 1.2	424.3 ± 2.8	61.5 ± 0.4
3	9.35	Methyl allyl disulfide	0.7 ± 0.2	0.2 ± 0.0	— ^e	—	—	—
4	9.71	Butyl isothiocyanate	—	—	19.1 ± 0.6	3.0 ± 0.1	25.1 ± 0.7	3.6 ± 0.1
5	11.55	3-Butenyl isothiocyanate	19.5 ± 0.4	4.8 ± 0.1	31.0 ± 0.7	4.9 ± 0.1	40.4 ± 2.1	5.9 ± 0.3
6	11.85	2,3-dimethyl 2-butenic acid	0.3 ± 0.1	0.1 ± 0.0	—	—	—	—
7	11.94	3-Butyl isothiocyanate	0.7 ± 0.1	0.2 ± 0.0	—	—	—	—
8	14.58	Diallyl disulfide	16.8 ± 0.8	4.1 ± 0.2	17.3 ± 1.3	2.7 ± 0.2	22.7 ± 1.4	3.3 ± 0.2
9	15.22	Pentyl isothiocyanate	0.5 ± 0.0	0.1 ± 0.0	0.6 ± 0.2	0.1 ± 0.0	—	—
10	16.37	Methyl allyl trisulfide	3.7 ± 0.2	0.9 ± 0.0	2.5 ± 0.2	0.4 ± 0.0	3.3 ± 0.5	0.5 ± 0.0
11	19.69	Benzenepropanenitrile	1.1 ± 0.1	0.3 ± 0.0	0.9 ± 0.1	0.1 ± 0.0	1.2 ± 0.3	0.2 ± 0.0
12	21.00	2-Propen-1-thiol	0.6 ± 0.1	0.2 ± 0.0	—	—	—	—
13	21.25	Diallyl trisulfide	38.6 ± 0.9	9.4 ± 0.2	49.9 ± 0.9	7.8 ± 0.1	66.8 ± 4.8	9.7 ± 0.7
14	21.58	6-Undecanol	3.2 ± 0.2	0.8 ± 0.0	—	—	—	—
15	25.68	Phenethyl isothiocyanate	13.7 ± 0.5	3.4 ± 0.1	15.6 ± 0.2	2.4 ± 0.0	20.6 ± 0.7	3.0 ± 0.1
16	27.37	Diallyl tetrasulphide	13.5 ± 0.4	3.3 ± 0.1	4.5 ± 0.2	0.7 ± 0.0	16.8 ± 0.6	2.4 ± 0.1
17	29.44	4-(4,5-dihydro-4,4-dimethyl-2-oxazolyl)-isoxazole	0.3 ± 0.1	0.1 ± 0.0	—	—	—	—
18	29.83	N, N'-diallyl thiourea	3.6 ± 0.1	0.9 ± 0.1	—	—	—	—
19	30.82	anti-9-Methyl-1,6-methano-fluorene	0.7 ± 0.2	0.2 ± 0.0	—	—	—	—
20	33.04	Tetradecane	0.9 ± 0.1	0.2 ± 0.0	—	—	—	—
21	36.82	Eicosane	0.8 ± 0.3	0.2 ± 0.1	1.0 ± 0.3	0.2 ± 0.0	0.8 ± 0.3	0.1 ± 0.1
22	38.68	Heneicosane	0.6 ± 0.1	0.2 ± 0.0	1.3 ± 0.3	0.2 ± 0.0	0.9 ± 0.2	0.1 ± 0.0
23	40.45	Docosane	0.2 ± 0.0	0.1 ± 0.0	—	—	0.5 ± 0.1	0.1 ± 0.0
24	42.16	Tricosane	—	—	0.6 ± 0.1	0.1 ± 0.0	0.9 ± 0.0	0.1 ± 0.0
25	43.88	Eicosane	—	—	—	—	0.7 ± 0.2	0.1 ± 0.0

a: Retention time.

b: Content per component in mg/100g of dried mustard seeds measured by external standard of allyl isothiocyanate.

c: Content per component in mustard oil measured by external standard of allyl isothiocyanate.

d: Mean ± standard deviation based on three measurements.

e: No existence in the mustard seeds and oil.

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