AROMATIC COMPOUNDS FROM THE VEGETABLE SOYBEAN

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ABSTRACT

The determination of the aromatic components of the vegetable soybeans "Chakaori" have been studied. The sample was prepared by solvent extraction and the identification of the chemical components was carried out using gas chromatography–mass spectrometry (GC-MS). Twenty-seven components were detected, including 2-acetylpyrrole which identified for the first time. The most abundant flavour compounds detected were *n* – hexanal (0.907%), 1-hexanol (1.786%), 2-hexanal (0.476%), 3-hexene-1-ol (0.485%) and phenylethyl alcohol (0.399%).

KEYWORDS : Chakaori; Soybean; Aromatic Compounds; GC-MS; 2-acetylpyrrole

1. INTRODUCTION

Soybean is one of the oldest vegetables known to man. Soybeans have been grown and consumed for more than 5000 years in China and the Far East. It is not only an excellent source of vegetable protein (34 – 39%, with a balanced composition containing all the essential amino acids) and of vegetable oil (18 – 20%, containing all the essential fatty acids), it is also rich in fiber, carbohydrates, phytoestrogens, steroids, vitamins A, C and E, minerals and flavour **[1]**. There are many breeds of soybeans in the world, one of them, "Dadacha-Mame" from Japan, has a flavour similar to that of aromatic rice which containing 2-acetyl-1-pyrroline **[2]**. In Thailand, many Japanese breeds of soybean have been grown and developed. The most interesting one is "Chakaori" which provides a good flavour. However, studies on the chemical compositions of this breed have not been reported **[3]**. Here we report on the identification of the flavour compounds in vegetable soybean "Chakaori" growing in Thailand.

2. MATERIALS AND METHODS

Materials. The vegetable soybean "Chakaori" currently grown in Thailand, was used. The fresh vegetable soybean "Chakaori" was harvested in October - November 2005 in Maejo University farm, Chiang Mai province, Thailand. The samples were packed in sealed plastic bags and kept at –20 °C for no more than 2 days before being subjected to acid-phase solvent extraction. A synthetic standard of 2-acetyl-1-pyrroline was obtained using the method outlined by Buttery **[1]**. All chemicals and the standard of 2-acetypyrrole were purchased from Fluka.

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Acid-phase solvent extraction of aromatic compounds. Fresh vegetable soybean "Chakaori" seed (100 g) was milled and 200 mL of 0.1 M HCl was added and the mixture was left stirred for 60 min before filtration. The ~200 mL of filtrate was divided into two and transferred to two 250 mL pear-shaped separatory funnels. This was followed by the addition of 3 mL of 5 M NaOH to make the solution slightly basic. The aqueous phase was the extracted with dichloromethane (3 x 100 mL) After drying the combined extracts with anhydrous sodium sulfate, the solution was concentrated to ~1 mL using a rotary evaporator under reduced pressure and a temperature of 40 °C. The concentrated extract was futher concentrated under a flow of nitrogen prior to GC-MS analysis. The extract provided a similarly flavour as the fresh vegetable soybean seed before extraction.

Capillary GC-MS conditions. GC-MS analysis was performed on a Agilent 6890(GC)/HP 5975(MS). Separation was achieved using helium as the carrier gas (ca.1 mL/min) with a fused silica capillary column (HP-5MS), 30 m long, 0.25 mm i.d., 0.25 μm film thickness. The GC injector was in a splitless mode. Injector and detector temperatures were 250 °C and 260 °C, respectively. The oven temperature was programmed starting at 45 °C, 3 min isothermal, then at 5 at 3°C/min to 60 °C/min (3 min isothermal), then 10 °C/min to 250 °C (10 min isothermal).

The effluent from the capillary column went directly into the mass spectrometer. The MS instrument was operated in the full scan and electron impact ionization mode with an ionization voltage of 70 eV and an acceleration voltage of 3000 V. The ion source temperature was 230 °C, and the GC-MS transfer line was set to 250 °C.

3. RESULTS AND DISCUSSION

The results of the GC-MS analysis are shown in Figure 1. This, shows a great number of components that may contribute to the aroma of vegetable soybean "Chakaori". Compounds corresponding to the peaks and their major ions from mass spectra are listed in Table 1. Compounds were identified mainly by comparing their mass spectra with the mass spectra data of the standard compounds in the NIST and Wiley library together with the comparison of their GC retention times with those of standard compounds and confirmed by the standard addition technique. Tentatively identified components based only on a comparison of the mass spectra with the reference spectra of the NIST and Wiley library that yielded <90% matches were identified as unknown in Table 1.

It is clear from Table 1 that approximately 27 prominent peaks were identified. Of these, there appeared the presence of alcohols, aldehydes, ketones, hydrocarbons and others. The major peaks comprise, 2-acetyl pyrrole (peak No. 9), *n*-hexanal (peak No. 1), 1-hexanol (peak No. 6), methyl ester Hexadecanoic acid (peak No. 13), 4-methyl-6-phenyl -2-pyrimidinamine (peak No. 17), *n*-eicosane (peak No. 19), *n*-tricosane (peak No. 20), *n*-tetracosane (peak No. 21), *n*-octacosane (peak No. 22), diisooctyl 1,2-benzenedicarboxylic acid (peak No. 23), *n*-hexacosane (peak No. 24) and *n*-heptacosane (peak No. 25). Smaller peaks included 4-methyl-2-Pentanol (peak No. 3), 2-hexanal (peak No. 4), 3-hexene-1-ol (peak No. 5), phenylethyl alcohol (peak No. 10), nebirinane 6,7-dihydro (peak No. 14), lapachone (peak No. 15), *n*-eicosane (peak No. 16), and 1,7-trimethylene-2,3-dimethylindole (peak No. 18). Many of these compounds have been shown to be products of lipid oxidation **[16]** such as, aldehydes, n-hexanal and, 2-hexanal, the important products produced by oxidative cleavage of lipid of the cereals **[17]**. 2-Acetyl-1-pyrroline, reported as important aroma component of aromatic rice, **[15]** is found in wheat bread crust **[18]**, popcorn **[19]** and vegetable soybean "Dadacha-Mame" **[2]** and was expected to be found in 'Chakaori'. However, it was concluded that, after checking with library mass spectra and using the standard addition technique, that 2-acetyl-1-pyrroline was not present in the vegetable soybean "Chakaori". However, other flavour components were found including the non odourous compound, 2-acetylpyrole. It was thought that 2-acetylpyrole might result from the oxidation of 2-acetyl-1-pyrroline during the isolation process, however the method used in this study was the same as the reported extraction method for 2-acetyl-1-pyrroline **[20]**. The same results were found in each "Chakaori" samples analysis. The Chromatogram and the mass spectrum of 2-acetylpyrrole are shown in Figure 2.

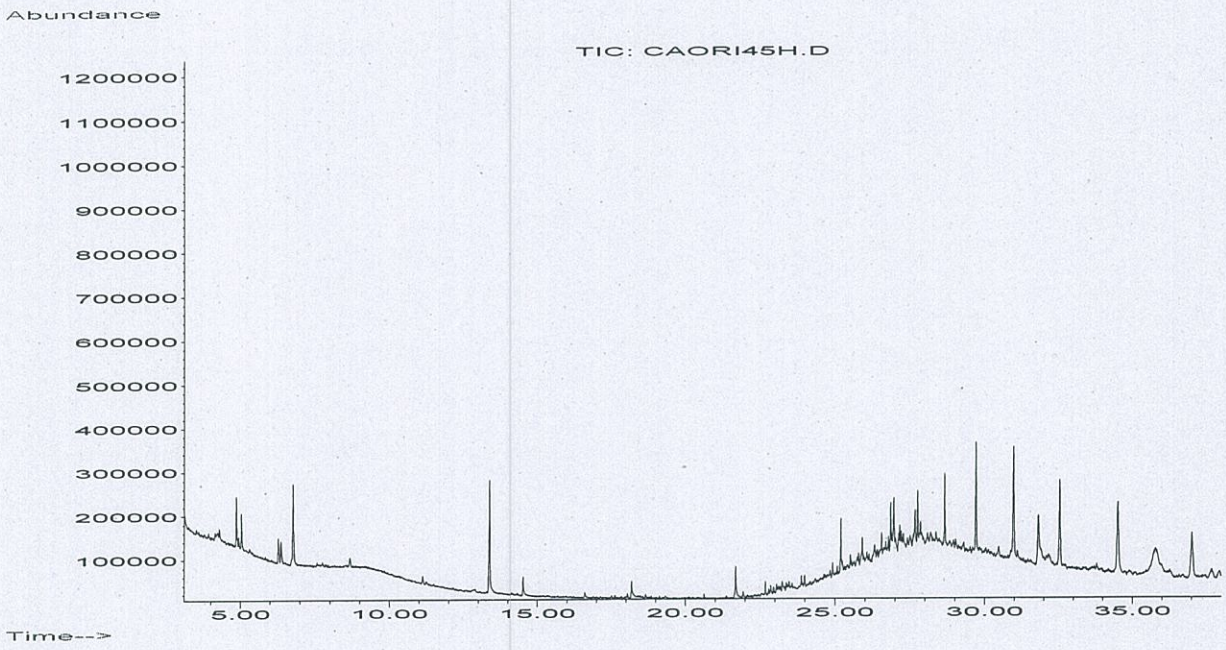
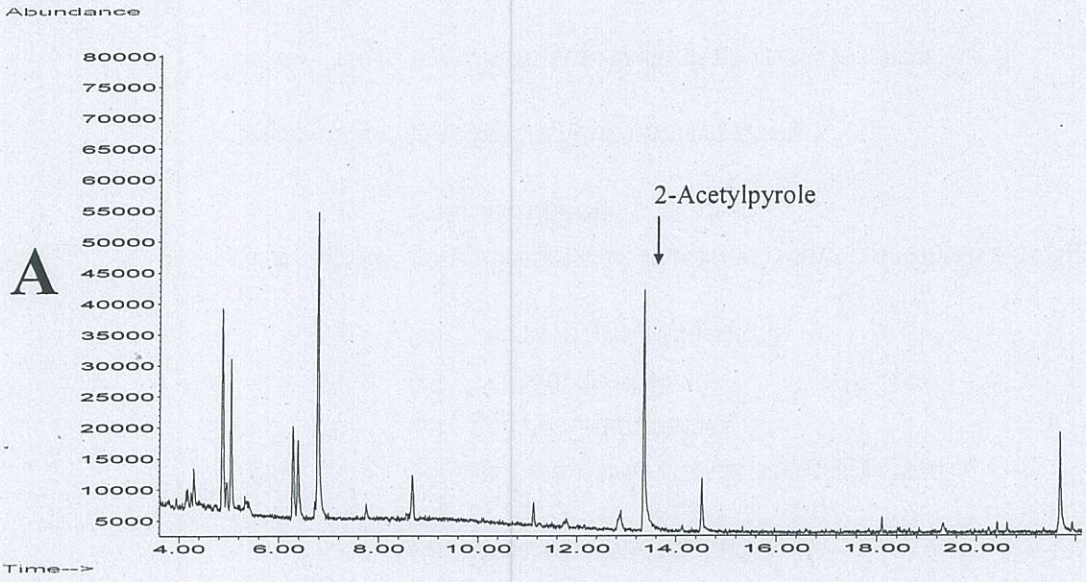
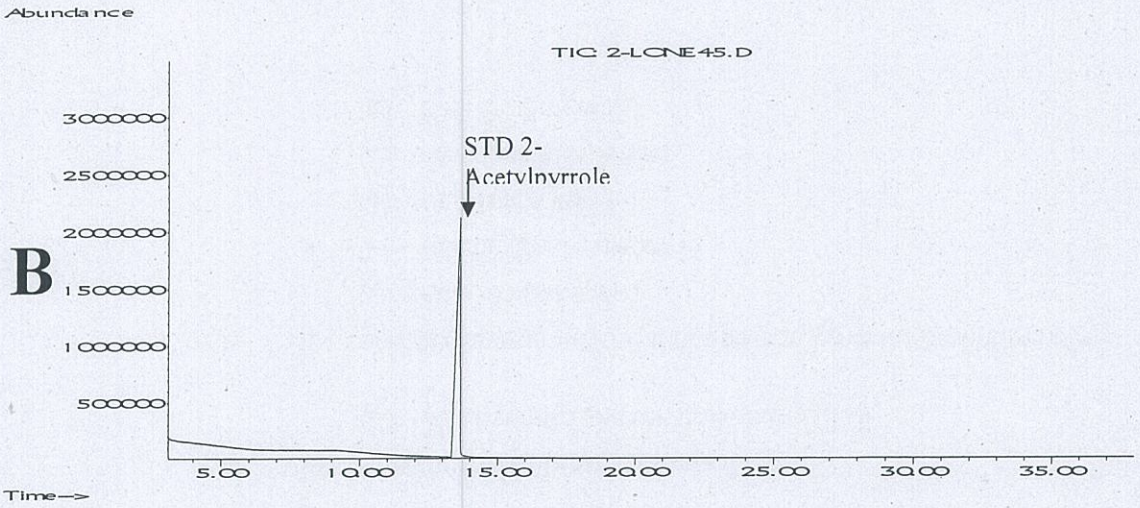
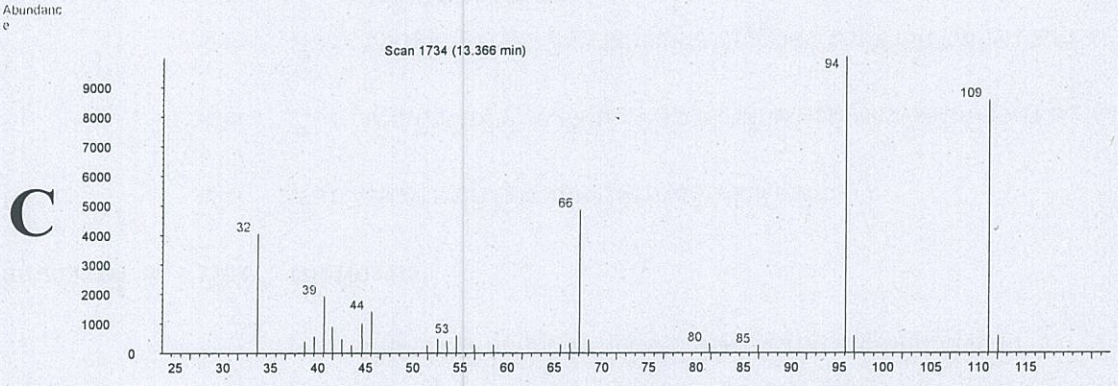
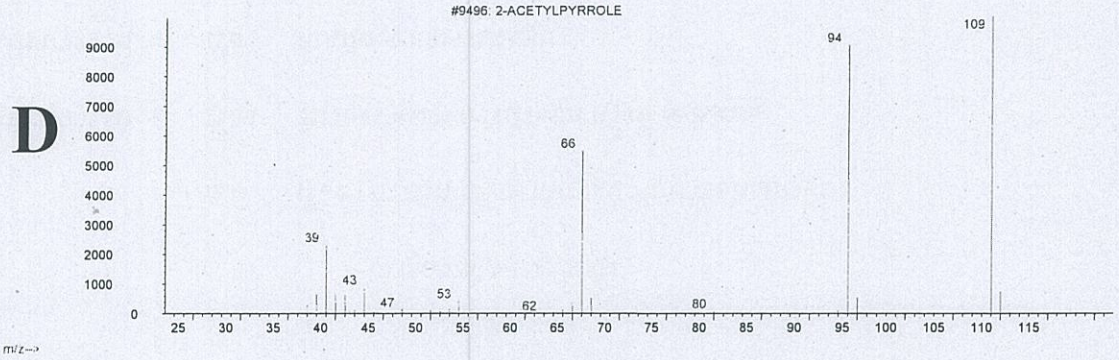


Figure 1. Reconstructed total ion chromatogram of a acid-phase solvent extraction of Vegetable soybean "Chakaori" by capillary column GC-MS.




Abundance
TIC: 2-LCNE45.D
STD 2-
Acetylpyrrole
B
3000000
2500000
2000000
1500000
1000000
500000
5.00
10.00
15.00
20.00
25.00
30.00
35.00
Time-->


Abundance
Scan 1734 (13.366 min)
C
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
32
39
44
53
66
80
85
94
109
25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115
m/z-->


Abundance
#9496: 2-ACETYLPYRROLE
D
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
39
43
47
53
62
66
80
94
109
25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115
m/z-->

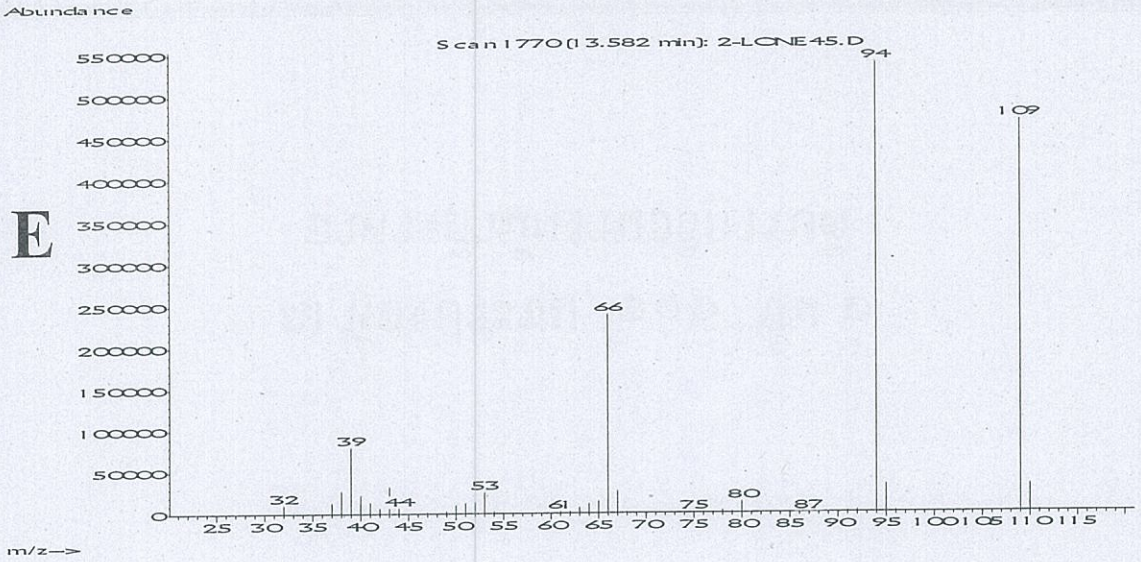


Figure 2. (A) Reconstructed total ion chromatogram of the extract of vegetable soybean "Chakaori" obtained by GC-MS using a HP-5MS column. (B) Reconstructed total ion chromatogram of the standard 2-acetylpyrrole. (C) EI mass spectrum of 2-acetylpyrrole present in the sample. (D) EI mass spectrum of 2-acetylpyrrole from the MS library. (E) EI mass spectrum of standard 2-acetylpyrrole.

4. CONCLUSIONS

Twenty-seven components were detected in an acid-phase solvent extract of vegetable soybean "Chakaori", including 2-acetylpyrrole, which was found for the first time in the vegetable soybean 'Chakaori' from Thailand. The chemical components could conveniently be classified into the following groups, alcohols, aldehydes, ketones, hydrocarbons and others. Those compound identified as potential contributors to the flavour of vegetable soybean "Chakaori" were *n* – hexanal (0.907%), 1-hexanol (1.786%), 2-hexanal (0.476%), 3-hexene-1-ol (0.485%) and phenylethyl alcohol (0.399%)

5. ACKNOWLEDGEMENTS

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Table 1. Components in Vegetable Soybean "Chakaori".

Peak No.	Time	Compounds	*m/z* c% relative abundance	MW[a]	Quality[b] (%)
1	4.859	*n*-Hexanal	100, 82, 72, 67, 56, 51, 49(100), 39	100	90
2	4.936	Unknown	59(100), 44, 40(100)	87	59
3	5.031	4-Methyl-2-Pentanol	87, 69, 57, 51, 45(100), 39	102	40
4	6.264	2-Hexanal	98, 83, 69(100), 63, 55(100), 50, 40(100)	98	96
5	6.365	3-Hexene-1-ol	100, 97, 82, 72, 67(100), 55, 50, 41	100	94
6	6.762	1-Hexanol	84, 69, 56(100), 51, 43, 38	102	91
7	8.694	Unknown	123, 107, 91, 81, 69, 55, 40(100)	182	45
8	11.125	Unknown	85, 82, 81, 73, 72, 71, 70, 69, 68, 67, 60, 58, 57(100), 56, 55, 54, 53, 51, 44(100), 43, 42, 41, 40, 39	100	43
9	13.379	2 – Acetyl Pyrrole	109(100), 94(100), 80, 66, 53, 44, 39	109	94
10	14.515	Phenylethyl Alcohol	122, 91(100), 86, 77, 65, 51	122	95
11	18.185	Unknown	100,(100)58,44(100),39	100	25
12	21.682	Plasticizer	222, 177, 149(100), 132, 121, 105, 93, 81, 76, 65 55, 50, 39	222	97
13	25.185	Methyl ester Hexadecanoic acid	270, 277, 239, 213, 199, 185, 171, 157, 143, 135, 129, 123, 115, 109, 101, 95, 87(100), 74(100), 69, 55, 43	270	98
14	25.909	Nebirinane 6,7-dihydro	282, 258, 240, 215, 193, 179, 165, 141, 119, 85, 71(100), 57(100), 43	288	97
15	26.537	Lapachone	242, 227(100), 214, 199, 171, 157, 128, 105, 76, 55	242	90
16	26.845	*n*-Eicosane	252, 238, 183, 169, 155, 141, 127, 111, 97, 91, 85, 79 71, 57(100), 43	282	98
17	26.746	4-Methyl-6-phenyl- 2-Pyrimidinamine	185(100), 128, 105, 77, 44	185	93
18	27.651	1,7-Trimethylene-2,3-dimethylindole	185(100), 179, 171, 157, 128, 89	185	92
19	27.746	*n*-Eicosane	253, 239, 195, 155, 141, 127, 111, 97, 91, 85, 71, 57(100), 43	282	97
20	28.647	*n*-Tricosane	324, 281, 252, 239, 183, 169, 155, 141, 125, 111, 97, 85, 71, 57(100), 43	324	97

(continue)

Peak No.	Time	Compound	m/e c%relative abundance	MW[a]	Quality[b] (%)
21	29.702	*n*-Tetracosane	338, 309, 295, 281, 267, 253, 239, 225, 154, 141, 127, 113, 106, 99, 85, 71, 57(100), 43	338	98
22	30.977	*n*-Octacosane	394, 323, 309, 295, 281, 267, 253, 239, 225, 197, 183, 169, 155, 141, 127, 113, 97, 85, 71(100), 57(100), 43	394	98
23	31.819	Diisooctyl 1,2-Benzenedicarboxylic acid	279, 167, 149(100), 121, 112, 104, 83, 70, 55, 41	390	90
24	32.524	*n*-Hexacosane	366, 309, 295, 281, 267, 253, 239, 225, 197, 183, 169, 155, 141, 127, 111, 99, 85, 71, 57(100), 43	366	96
25	34.504	*n*-Heptacosane	380, 351, 323, 309, 295, 281, 267, 253, 239, 225, 207, 183, 169, 155, 141, 127, 113, 97, 85, 71(100), 57(100), 43	380	98
26	35.749	Unknown	396(100), 159, 145, 135, 121, 105, 95, 81(100), 69, 55(100)	396	53
27	37.023	Unknown	397, 313, 309, 295, 281, 267, 253, 225, 183, 169, 155, 141, 121, 113, 97, 85, 71(100), 57(100), 43	397	58

[a] molecular weight from GC-MS (EI) data
[b] MS quality comparison with database