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Full Paper

Flavour compounds of the Japanese vegetable soybean "Chakaori" growing in Thailand

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Abstract: The vegetable soybean ("Chakaori") grown in Thailand was examined to determine its flavour components. The analyzed sample was prepared by solvent extraction and the chemical components were identified by gas chromatography–mass spectrometry (GC-MS). Twenty-seven components were detected including 2-acetylpyrrole which was identified for the first time in soybean. The most abundant flavour compounds detected were *n*–hexanal (0.91%), 1-hexanol (1.79%), 2-hexanal (0.48%), 3-hexene-1-ol (0.49%) and phenylethyl alcohol (0.40%)

Keywords: Chakaori, Soybean, Flavour Compounds, 2-acetylpyrrole

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Introduction

Vegetable soybean, called Edamame in Japan and Mao Dou in China is one of the oldest vegetables known to man. Vegetable soybeans have been grown and consumed for more than 5000 years in China and in 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), but also fiber, carbohydrates, phytoestrogens, steroids, vitamins A, C and E, minerals and flavour [1]. There are many breeds of vegetable soybeans in the world providing different flavours, the good varieties of which are mostly from Japan [2]. It is a popular food in Japan and increases in consumption of edible soybeans in other countries have been attributed to their health benefits and flavour [3]. In Thailand, many Japanese breeds of vegetable soybeans have been grown and developed. The most interesting one is "Chakaori" which provides a good flavour similar to rice [4]. A few reports concerning "Chakaori" breed showed only the maximum of the total sucrose and starch [5-6] and no report on the flavour analysis. Only one study on flavour analysis was conducted using another soybean breed from Japan, the "Dadachamame" which has a flavour similar to that of aromatic rice and found to contain 2-acetyl-1-pyrroline [4]. However, studies on the flavour composition of "Chakaori" breed have not been reported**.** Here we report on the identification of the flavour compounds in Japanese vegetable soybean "Chakaori" grown in Thailand.

Materials and Methods

1. Materials

The vegetable soybean "Chakaori" grown in Thailand was used. The fresh vegetable soybean "Chakaori" was harvested in October - November 2005 in Maejo University farm, Chiang Mai province, Thailand. Samples were packed in sealed plastic bags and kept at –20 ^{o}C for no more than 2 days before being subjected to acid-phase solvent extraction. A synthetic sample of 2-acetyl-1-pyrroline was obtained using the method outlined by Buttery [7]. All chemicals and a standard sample of 2-acetypyrrole were purchased from Fluka.

2. Acid-phase solvent extraction of aroma compounds

Fresh vegetable soybean "Chakaori" seeds (100 g) were milled, 200 mL of 0.1 M HCl was added and the mixture was stirred for 60 min before filtration. The filtrate (~200 mL) was divided into two equal parts and transferred to two 250 mL 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 then 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 ^{o}C. The concentrated extract was further concentrated under a flow of nitrogen prior to GC-MS analysis. The extract provided a similar flavour as the fresh vegetable soybean seed before extraction.

3. Capillary GC-MS conditions

GC-MS analysis was performed on an 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., and 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 ^{0}C, 3 min isothermal, then at 3°C/min to 60 °C/min (3 min isothermal), then at10 °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 ^{o}C and the GC-MS transfer line was set to 250 ^{o}C.

Results and Discussion

The results of the GC-MS analysis are shown in Figure 1. The chromatogram 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. The compounds were identified mainly by comparing their mass spectra with those 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 were 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.

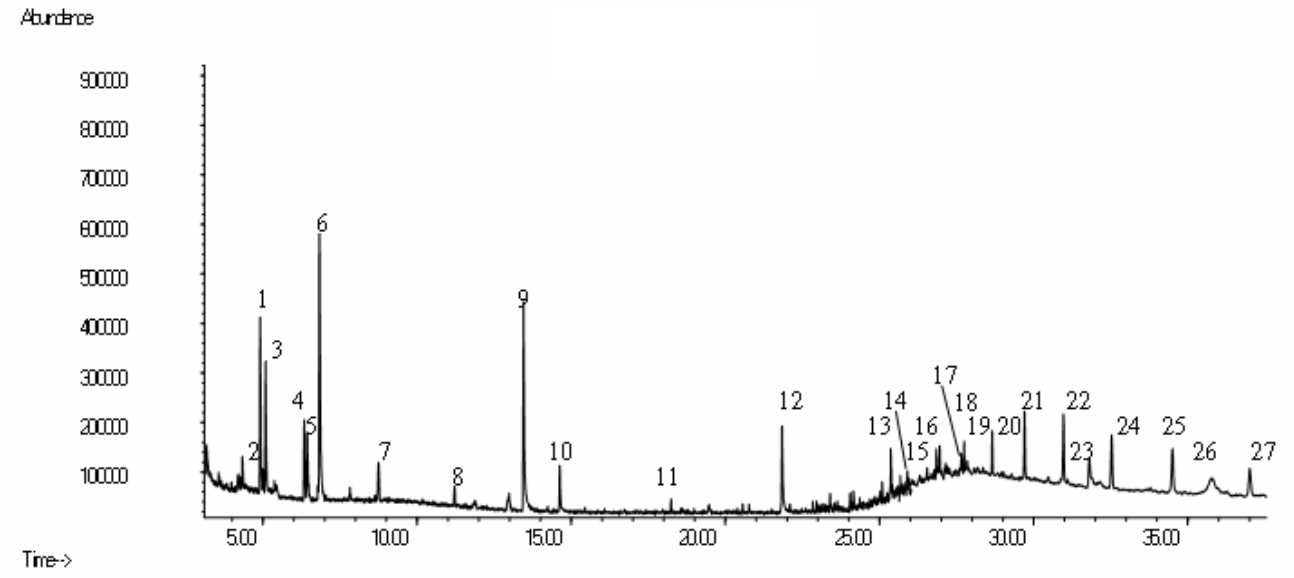


Figure 1. Reconstructed total ion chromatogram of an acid-phase solvent extract of vegetable soybean “Chakaori” obtained by GC-MS.

Table 1. Components in vegetable soybean "Chakaori".

Peak No.	Retention Time	Compounds	*m/z* (% 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)

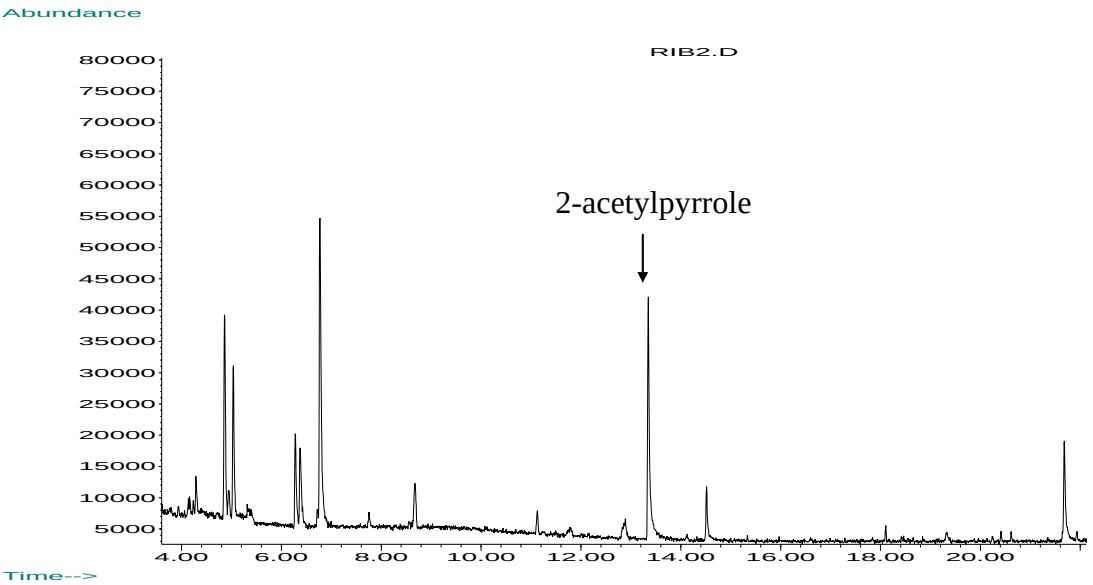
Peak No.	Retention Time	Compounds	m/z (% relative abundance)	MW[a]	Quality[b] (%)
21	29.702	*n*-Tetracosane	338, 309, 295, 281, 267, 253, 239, 225, 154, 141, 127, 113, 106, 99, 85, 71, 57(100), 43	338	98
22	30.977	*n*-Octacosane	394, 323, 309, 295, 281, 267, 253, 239, 225, 197, 183, 169, 155, 141, 127, 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

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 those of 2-acetyl pyrrole (peak No. 9), *n*-hexanal (peak No. 1), 1-hexanol (peak No. 6), methyl ester of 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 the products of lipid oxidation [8], such as aldehydes, *n*-hexanal and 2-hexanal, the important products produced by oxidative cleavage of lipids [9]. 2-Acetyl-1-pyrroline, reported as an important aroma component of aroma rice [7], and found in wheat bread crust [10], popcorn [11] and vegetable soybean "Dadachamame", was expected to be found in 'Chakaori'. However, it was concluded, 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-acetylpyrrole. It was thought that 2-acetylpyrrole might result from the oxidation of 2-acetyl-1-pyrroline during the isolation process, although the method used in this study was the same as the reported extraction method for 2-acetyl-1-pyrroline [12]. The same results were found in each "Chakaori" sample analysis. The chromatograms and the mass spectra of 2-acetylpyrrole are shown in Figure 2.

A



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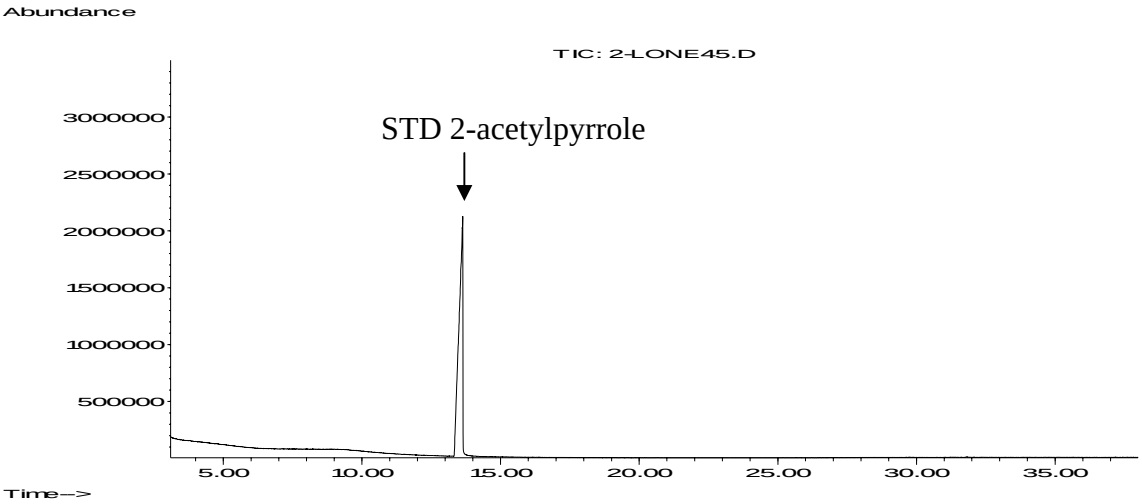
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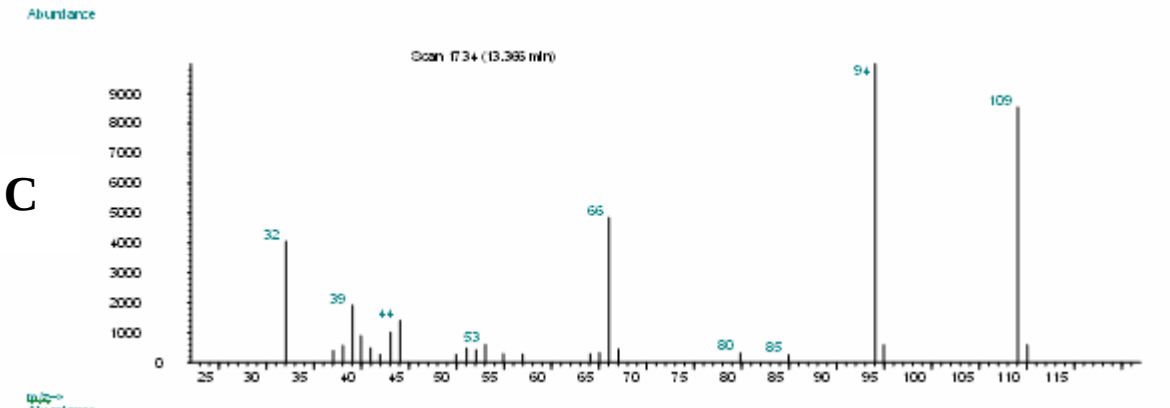
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85
94
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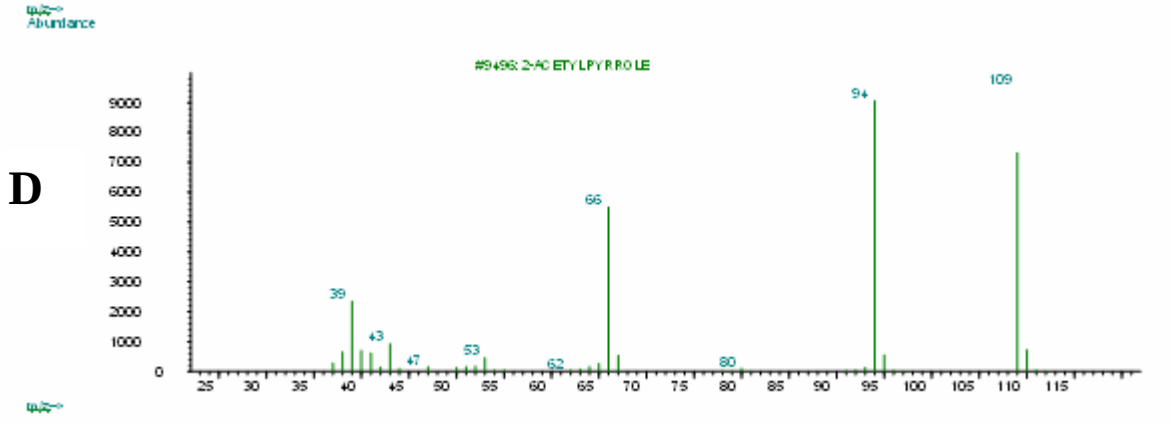
D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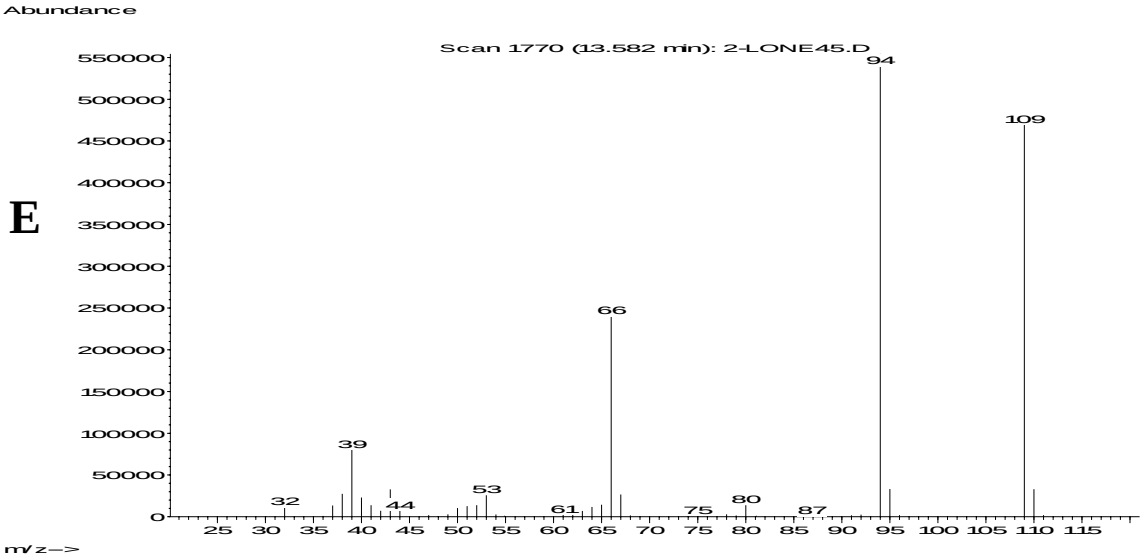

Figure 2. (A) Reconstructed total ion chromatogram of the extract of vegetable soybean "Chakaori" obtained by GC-MS using a DB-5 column. (B) Reconstructed total ion chromatogram of the standard 2-acetylpyrrole. (C) EI mass spectrum of 2-acetylpyrrole present in the sample. (D) EI mass spectrum of 2-acetylpyrrole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

Conclusion

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. Those compounds identified as potential contributors to the flavour of vegetable soybean "Chakaori" were *n*–hexanal (0.91%), 1-hexanol (1.79%), 2-hexanal (0.48%), 3-hexene-1-ol (0.49%) and phenylethyl alcohol (0.40%)

Acknowledgements

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