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Study on volatile isothiocyanate detected in cultivar of *Brassica* vegetable (Part 2)

Analysis of flavour of Cabbage by Gas-chromatography-Mass spectometry.

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The spectra patterns of flavour components in cabbage detected by gas chromatolgraphy were investigated in order to identify pungent components in the cabbage cultivars.

1) The flavour components in cabbage which were isolated and identified by Gas chromatography-Mass spectrometry consited of 1-cyano-2,3-epithiopropane, 3-methyl thiopropyl nitrile, menthol, *neo*-menthol, 2-H-carveol, *l*-carvone, carbitol acetate and methyl carbitol.

2) The components whose amount varied with the pH used during their extraction from cabbage were 1-pentanol, *cis*-3-hexenol, *trans*-2-hexenol and allyl isothiocyanate.

3) The inner core of the leafy balls of cabbage was found to contain higher amounts of allyl isothiocyanate than the other parts of cabbage. Unripened cabbages contained larger quantities of allyl isothiocyanate and *trans*-hexenol than ripened cabbages. (Received Apr. 30, 1985)

INTRODUCTION

The vegetables of Cruciferous contain components of pungent flavour. Hence, it is necessary to analyse these flavour components for estimation of the food taste. Because of the variety of flavours, a precise technique is necessary for isolating, identifying and quantitizing them. There are hardly any examples of analysis of such components of pungent taste for estimation of the food taste published so far. The pungent taste of cabbages of Cruciferous was studied. There are many kinds of cabbages and among them Japanese cabbages contain only little pungent taste even after a lot of improvements made on its plant breeding. The aglucon is produced as a result of hydrolyzation of glucosinolate in foreign cabbages from which Sauer-kraut is made[1]. Thiocyanate and 1-cyano-2,3-epithiopropane are reported as aglucon[2]. It is inferred that Sauer-kraut can be made from foreign cabbages because components of pungent taste reduce the number of the rotting and spoilage bacteria originated from the raw materials, but it is difficult to make Sauer-kraut from Japanese cabbages as they do not contain much pungent conponents.

We reported previously that the flavour of pickle vagetable was analyzed with the methods of headspace vapour by gas chromatography. In those reports, the volatile flavour constituents in cabbages were identified by gas chromatography-Mass spectrometry in order to study the difference of components among cultivar varieties in next report. A number of investigators, MACLEOD,[4] APPELQVIST[5], BAILEY[6], DAXENBICHER[7] and SELF[8] et al, have reported the flavours of cabbages.

MATERIALS AND METHODS

Materials

The Green ball, Top, New top and Natsuwase, cabbages were cultivated in experimental farm of Vegetable and Ornamental Crops

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Part 1 of this series is Ref 3.

Research Station and harvested on August 10 (1984). Fig 1 shows three kinds of methods of preparing flavour compounds from cabbages.

In each preparation the cabbage was first sliced except for center, added with water and salt, mixed, homogenized, filtered and distilled in vaccum (20 *mm* Hg) at 40℃. Each distillate was extracted continuously with ether: n-pentane (1: 0.5 by volume). Each extract was dried over anhydrous Na_2SO_4 for 24 hrs and the solvents were concentrated by distillation in N_2 gas. The flavour components, so collected were injected into gas chromatograph to be analyzed.

Gas-chromatography

The gas chromatography was carried out on a Shimazu GC-7 A equipped with a FID and FTD and 25 m×0.25mm fused silica W cot glass capillary column coated with Unisole 400. The column temperature was programmed linearly from 65℃ to 195℃ at 4℃/min. The injector and detector temperatures were 240℃. Carrier gas was nitrogen at the flow rate of 1.5 m*l*/min.

Gas chromatography-Mass spectrometry (GC-Mass)

GC-MASS was carried out on a Hitachi M-80 type (GC-MASS) and operation parameters were as follows: carrier gas: helium; ionization voltage: 70 eV; accelerating voltage: 3500 V; ion sauce temperature: 210℃. Relative content of each component were calculated from the percentage of the areas of peaks for 50 μg phenyl isothiocyanate as internal standard.

RESULTS AND DISCUSSION

1. Flavour components from cabbages were identified by GC-MASS

The retention time of flavour components in cabbages was studied by gas chromatography in order to know the kinds and composition of flavour compomemts. First of all, flavour components from cabbages were separated and identified by GC-MASS. Fig 2 shows the capillary chromatogram of the volatile flavour components by GC-MASS.

The spectra patterns of flavour components

Methods (A)
Cabbage 1*kg* 2 days stocks
├─Sliced, Suspended in 300*g* of Saslts 2 *l* of water
├─Mixer, Homogenizing
Homogenate
├─Filtrated
Filtrate
├─Extracted with Ether: n-Pentane (1 : 0.5)
├─Shaking
├─dried over anhydrous Na_2SO_4 for 24 hr
Solvent
├─Concentrated by distillation
├─Extracted with ether: 0.5 *l*
├─Shaking
├─Dried over Na_2SO_4
├─Concentrated by distillation
Aroma: Y: 9.2 *ppm*

(B)
Cabbage 1*kg* 10 days stocks
├─Sliced, Suspended Salts and water
├─Mixer, Homogenizing
Homogenate
├─Concentrated by distillation
├─Extracted with Ether: 0.5 *l*
├─Shaking
├─Dried with N_2SO_4
├─Concentrated by distillation
Aroma: Y: 3.3 *ppm*

Y: yield

(C)
Cabbage 1*kg* 14 days stocks
├─Sliced, Suspended Salts, water
├─Mixer, Homogenized
Homogenate
├─Concentrated by distillation
├─Extracted with Ether: 0.5 *l*
├─Shaking
├─Dried with Na_2SO_4
├─Concentrated by distillation
Aroma: Y; 9.0 *ppm*

Fig. 1. The preparation methodes of flavour components.

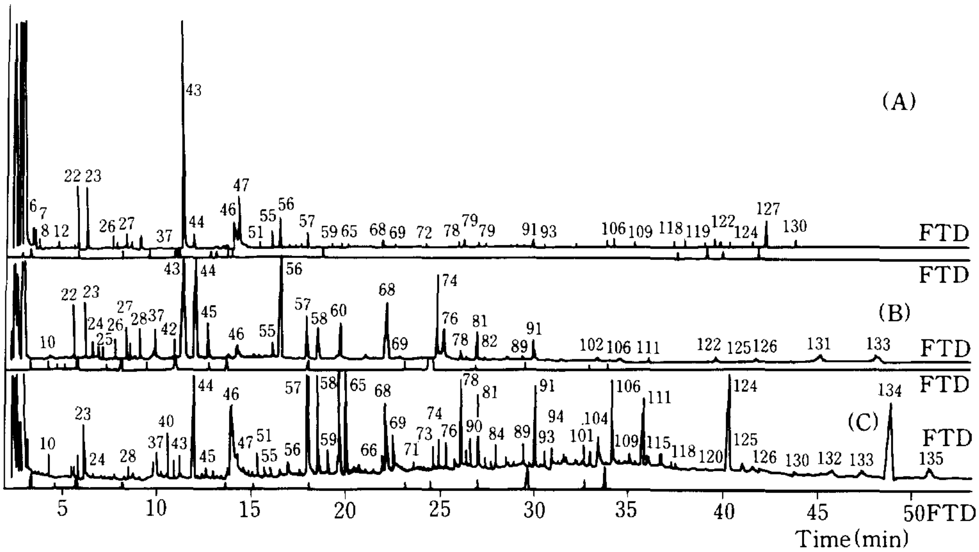

Fig. 2. Gas chromatograms of the steam volatile compounds from cabbage.
Unisole 400 (FS-Wcot) glass capillary column 25m × 0.25mm column temp 65-195℃ 4℃/min
Peak numbers refer to components listed in Table 1.

in cabbage on gas chromatogram, differed according to three kinds of extraction methods.

The spectrum patterns showed each peak and its figure indicating respectively a particular components and its quantity.

The mass spectra of peak No 73 nad 74 on gas chromatogram are shown in Fig 3. The mass spectrum of epithionitriles gives intense molecular ion, regular losses of masses 27 (HCN) and 40 (CH_2CN). Fragments of m/c 72 (M^+-HCN), 67 (M^+-S), 59 (M^+-CH_2CN), 54 (M^+-CHS), 45 (CHS), 27 (HCN) and 40 (CH_2CN) indicated the molecular weight 99.

The mass spectra of No 73 and 74 were isomers, and were estimated to be 1-cyano-2, 3-epithiopropane, were similar to those of epithionitrile except that they differed at m/c 27, 28, 41 and 59.

The epithionitriles were identified as hydrolysis products from the mustard seed (Sinigrin)[4] but not from cabbages.

The mass spectrum of No 71 is shown in Fig 3. The methylthio propyl compound is unique in that M^+-41 (CH_3CN) is prominent, The base peak at m/c 61 represents $CH_3SCH_2^+$ and a series of M^+47 (loss of CH_3) is clearly seen. The compound of No 71 indicating the molecular weigh 115, was estimated to be 3-methyl thionitrile. 3-methyl thionitrile was obtained from glucosinolate (Glucoibervirin)[4] in cauliflower by autolysis. Phenyl propionitrile of No 91 was contained in many Brassica vegetables. The mass spectra of No 58 and 64 are shown in Fig 4. Prominent features of No 58 and 64 in the Mass were: 138 (M^+-H_2O), 123, 109, 95, 81, 55, 43, 28 and 156 (molecula ion). The compound peak No 58 and 64 were isomers and were estimated to be *neo*-menthol and menthol which were also present in peppermint but not in Cruciferous vegetables.

The mass spectra of No 57, 63, 67 and 68 are shown in Fig 5.

2-H-carveol (No 67) and *l*-carvone (No 68), which had been detected as the aromatic flavour in spearmint oil, were identified in cabbages. Methyl carbitol (No 57) and carbitol acetate (No

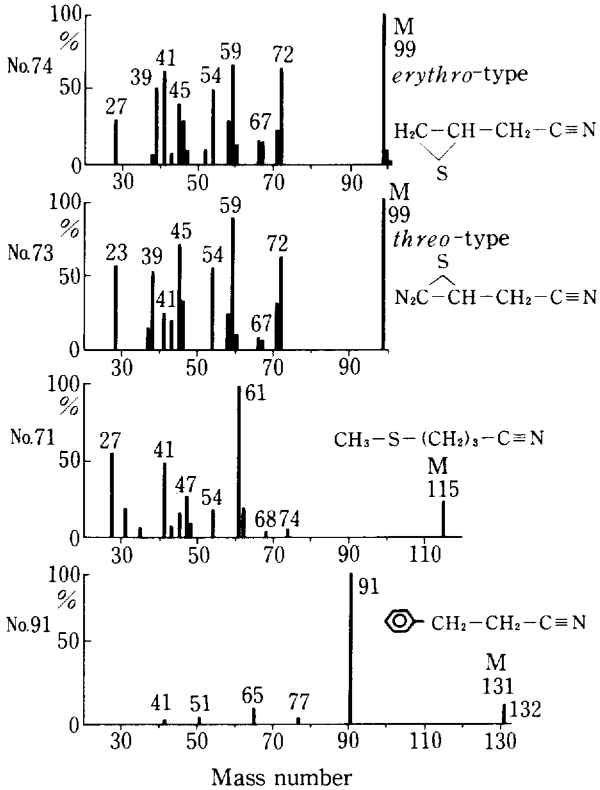

Fig. 3. Mass spectra of *threo*-1-cyano-2, 3-epithiopropane (No.74), *erythro*-1-cyano-2, 3-epithiopropane (No.73), 3-methyl thio propylnitrile (No.71) and phenyl propionitrile (No.91).

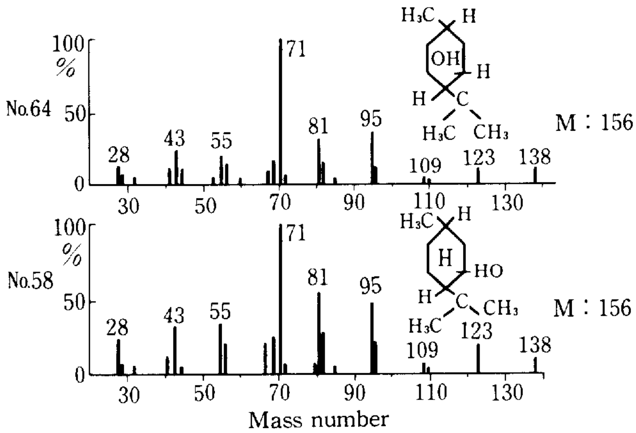

Fig. 4. Mass spectra of menthol (No.64) and *neo*-menthol (No.58).

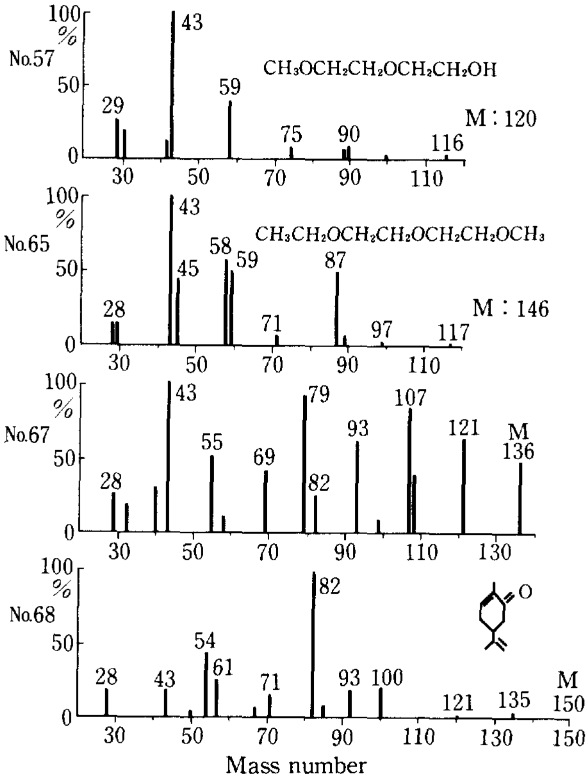

Fig. 5. Mass spectra of 2-H-carveol (No.67), l-carvone (No.68) methyl carbitol (No.57) and carbitol acetate (No.65).

65), which had been detected as contents of the packing materials, were identified as hydrocarbons.

Table 1 shown the approximate relative percentage of flavour components of cabbages in Fig. 2 which were isolated and identified. 15 isothiocyanates, 9 nitriles, 19 alcohols, 8 esters, 10 hydrocarbons, aromatic compounds and 10 other compounds were identified as volatile components in cabbages. Among them, allyl isothiocyanate (No 43), allyl cyanide (No 23) and *cis*-3-hexenol (No 44) were in high proportion. Allyl isothiocyanate, 3-butenyl isothiocyanate (No 51), phenethyl isothiocyanate (No 106), 2-butenyl isothiocyanate (No 33), methyl isothiocyanate (No 14) and isoamyl isothiocyanate (No 47) were identified as isothiocyanate of pungent odour and taste. Dibutyl phthalate and diethylene glycol monoethyl ether were identified as contents of packing materials. However, the rest of the mass spectra could not be identified.

2. The extracting condition on analyzed flavour.

It is reported[10] that different nitriles are produced according to the difference of pH at autolysis, because some flavour compounds were produced from glucosinolates that were hydrolysed by myrosinase after cells had been broken.

The effect of extracts at different pH and the enzymatic reaction time on analysis of flavour components were studied by the head space

Table 1. Relative percentages of flavour components of cabbage.

Peak No.*	Identification Compounds	Sample peak: relation retention time	Sample A	Sample B	Sample C	Peak No.*	Identification Compounds	Sample peak: relation retention time	Sample A	Sample B	Sample C
1	Diethyl ether	3.1				72		21.9		0.1	φ
2	Ethyi formate	3.2	11.0	1.85	10.6	73	1-Cyano-2, 3-epithio propane (*threo*)	22.1	φ	0.1	0.63
3	Methyl acetate	3.4	11.0	2.41	3.4	74	1-Cyano-2, 3-epithio propane (*erythro*)	22.2		3.34	0.77
4	Ethyl acetate	3.5	11.0	7.05	1.2	75		22.4		1.19	φ
5	Ethyl alcohol	3.6	11.0	18.6	10.1	76	2-Methoxy phenol (Guaiacol)	22.9			0.72
6	Diacetyl	3.8	0.5	0.3	0.9	77		23.2			0.33
7	3-Metyl-3-buten-2-one	3.9	0.3	0.1	0.14	78	Benzyl alcohol	23.4	0.26	0.3	2.4
8		4.0	0.7	0.1	0.1	79		24.0	0.35	0.1	0.5
9		4.1	0.4		0.1	80					φ
10	Methyl butenol	4.2	0.3	0.1	0.58	81	Phenethyl alcohol	24.3	φ	1.0	1.9
11	1,3,5-cycloheptatriene	4.4		0.1	0.05	82	n-Propyl-4-octyl keton	25.3	0.17	0.1	0.3
12		4.5	0.2	0.1	0.05	83	3-Methylthio propylisothiocyanate				0.1
13	*Cis*-isopropenyl-1-methyl-2-cyclohexane	4.6			0.02	84					0.5
14	Methyl isothiocyanate	4.8			φ	85			φ		
15	2-Propoxyethanol (Pentan-2-ol	4.9			φ	86					0.3
16		5.0			φ	87					0.2
17	3-Methy 1-3-buten 2-ol	5.1			φ	88					φ
18	1-Pentanol	5.2			φ	89				0.14	0.48
19	1-Penten-3-ol	5.4			0.3	90					0.1
20	2-Methyl-3-buten-2-ol	5.5			φ	91	Phenyl propionitrile	27.7	0.3	0.81	2.12
21	Ethylene glycolmtnomethylether	5.7	0.2		0.1	92		28.0	0.2		0.1
22			2.9	1.9		93		29.4	φ		0.3
23	Allyl cyanide	5.8	2.9	2.2	1.34	94	6-methylthio hexanenitrile	30.2		0.1	0.48
24	2-Isopropoxyethanol	5.9	0.4	0.7	0.14	95					φ
25		6.0		0.2	φ	96					0.29
26		6.6	0.6	0.89	φ	97	2-Methyl-3-hept-isopropyl keton	30.6			φ
27		6.7	0.7	1.11	φ	98					φ
28	3-Methyl-butanol	6.8	0.3	0.7	0.3	99			0.2		φ
29		7.0			φ	100					0.2
30	Butyl cyanide	7.4	0.6	0.2	0.1	101	2, 4-Decandienal	31.0			0.48
31	Isobutyicyanide + sec butylcyanide	7.5			0.1	102	4-Methyl thiobutyl isothiocyanate				0.34
32		7.6				103	2-Ethyl hexyl benzoate	31.4	0.2	0.1	φ
33	2-Butenyl isothiocyanate	7.7		1.11		104			0.35		0.7
34	Amy vinyl ketone	7.8		0.14		105					0.67
35		8.0			φ	106	Phenethyl isothiocyanate	31.6		0.1	2.17
36	2-Methyl-1-pentanol	8.2		φ	0.4	107	P-Isopropyl benzene	32.4			φ
37	*Cis*-hexenyl acetate	8.4	0.2	1.18	0.4	108	Ethyl palmitrate	33.9			φ
38	2,5-Dimethy-1,3-dioxolan-4-one	8.6		0.22		109					0.24
39	Ethy lactate	8.8			0.19	110					φ
40	1-Hexanol	9.0			1.16	111	Tri ethyl citrate	38.1		0.1	1.69
41	*Cis*-hexanol	9.3	0.2	0.7	0.43	112					0.24
42	2-Methoxy ethanol (ethylene glycol monomethy ether)	9.4			φ	113					0.24
43	Allyl isothiocyanate	9.7	18.4	6.17	0.5	114					0.38
44	*Cis*-hex-3-en-ol (cis-3-hexenol	9.8	0.6	11.9	4.9	115					0.2
45	*trans*-2-Hexenol			1.4	0.3	116			0.61		
46	4-Pentenenitrile		0.2	φ	0.1	117			0.35		0.2
47	Isoamyl isothiocyanate		0.2	0.2	0.2	118	1-Cyano-4-methylsulfinyl butane		φ		φ
48	Acetic acid	11.4	1.3	0.5	1.5	119					φ
49	5-Hexenenitrile		2.5	φ	0.5	120					φ
50			0.3	φ	0.1	121					φ
51	3-Butenyl isothiocyanate				0.5	122	3-Mehylsulfinylpropyl isothiocyanate		0.35	0.22	φ
52		12.1			0.2	123					φ
53	2-Ethyl-1-hexanol	12.6		0.14		124	Dibutyl phthalate	45.4	φ	φ	2.6
54	4-Pentenyl isothiocyanate	13.2		φ		125			φ	φ	0.2
55	Benualdehye	13.6	0.8	0.6	0.6	126	4-Methylsulfinyl butylisothiocyanate			0.1	0.2
56			1.5	18.6	0.3	127		60.0	1.49		
57	Methyl carbitol	15.3	0.8	1.63	10.6	128					φ
58	*Neo*-Menthol	15.7	0.1	1.19	3.18	129					φ
59	Hexyl isothiocyanate			φ	0.53	130					φ
60	Menthol	16.1	0.3	1.41	4.91	131				0.2	φ
61	Diethylene qlycol monoethyl ether	16.4	0.2	0.1	2.41	132					0.1
62		16.5				133					0.2
63	2,2-Dimethyl butane	16.6				134			φ	0.2	3.8
64	Iso-Menthol	16.9		0.1		135					0.3
65	carbitol acetate	17.1	0.3	φ	2.75	136					0.2
66	Heptadecane	18.9		φ	0.24	137					φ
67	2-H-Carveol	19.3		0.1	0.49	138			φ		
68	ℓ-Carvone	19.6	0.4	2.2	1.88	139					0.2
69	Methy allyl keton	19.9	0.2	0.1	0.9		Total components (sq.mms)		113	134	207
70		20.6		φ	φ						
71	3-Methyl thiopropylnitrile	21.0		φ	0.3						

φ = trace

* Refers to peak number of both curves in Fig 2.

vapour methods[3]. The amount of flavour components increased along with reaction time during which glucosinolate were hydrolyzed by myrosinase. It is discussed that flavour components differed according to extraction methods or parts of cabbage. At pH of cabbage decreased even under cooling after harvest. Table 2 shows the relation between extracts at different pH and the amount of flavours. The relation between extracts at pH and flavour was studied using Top cabbage with a little pungent taste and Natsu-Wase cabbage with strong pungent taste. 1-pentanol and *cis*-3-hexenol were found to be produced best at low pH (pH5.6-5.9). Allyl isothiocyanate and *trans*-2-hexenol were found to be produced best at high pH (7.0-7.5) in phoshate buffer.

3. The flavour relation among the parts of cabbages and among cabbages of different ripening stages.

The flavour components with respect to the different parts of surface, middle and center of a ripened cabbage were analyzed.

The flavour components with respect to middle parts of a cabbage of different ripening

Table 2. The relation between extract pH and the amount of flavour.

Flavour	Cabbage Top				Natsu-Wase			
components	pH 5.6	5.9	7.0	7.5	5.6	5.9	7.0	7.5
Allyl cyanide	0.20	0.18	0.46	0.31	0.29	0.52	0.75	0.85
Allyl isothiocyanate	trace	trace	0.29	0.48	0.81	0.91	2.59	4.02
1-Penten-3-ol	0.86	0.36	-	1.47	1.41	0.79	0.84	0.84
1-Pentanol	6.76	2.33	0.91	0.86	8.42	4.47	1.35	1.21
Cis-3-hexenol	0.92	0.91	0.12	trace	1.20	0.92	0.80	trace
Trans-2-hexenol	0.11	0.26	1.16	0.98	0.15	0.20	2.39	2.63

The percentages indicating the ratio of peak area for 50 μg phenyl isothiocyanate as internal standard.
Top; alittle pungent, Natsu wase cabbage; strong pungent taste.

Table 3. The amount of flavour according to parts of cabbage.

Flavour components / Parts of cabbage	Allyl cyanide	Allyl iso thiocyanate	1-Penten-3-ol	1-Pentanol	*Cis*-3-Hexenol	*Trans*-2-hexenol
Surface-5	0.40	2.63	0.92	1.42	trace	3.92
6-15	0.86	4.92	0.66	1.22	trace	2.82
6-center	0.62	4.82	0.70	2.11	trace	4.22

Natsu-wase cabbage

Table 4. The amount of flavour in terms of ripening of cabbage.

Flavour components / ripening time	Allyl cyanide	Allyl iso thiocyanate	1-Penten-3-ol	1-Pentanol	*Cis*-3-hexen-ol	*Trans*-2-Hexenol
unripen(I)	3.20	6.21	2.21	1.22	trace	5.91
unripen(II)	2.27	4.87	2.09	1.43	trace	4.21
ripen	2.17	4.12	1.05	1.26	trace	3.49

(New top cabbage)

stages were analyzed. Table 3 shows the relation between parts of ball cabbage and flavour components. Table 4 shows the relation between ripening stages and flavour components. The difference between flavour produced in different parts of cabbage and defferent ripening time was small. The center of balled and ripened cabbages were found to contain higher amount of allyl isothiocyanate in comparison with the other parts of cabbage. Unripened cabbages had more allyl isothiocyanate and *trans*-2-hexenol than the ripened cabbages.

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つけな類の揮発性辛味成分の研究（第２報）
キャベツの香味成分のGC-Massによる分析

伊藤寛・矢野昌充・岡田憲幸・新国佐幸

キャベツの品種間の辛味成分の研究をするため、ガスクロマトグラフィーを用いて、キャベツ香気成分のスペクトル　パターンを調べた。

１．GC-Mass分析計によって、キャベツの香気成分の分離同定を行った。１－シアノ－２，３－エピチオ　プロパン，３－メチル　チオプロピルニトリル，メントール，ネオメントール，2H－カルボーオール，ℓ－カルボン，カルビトールアセテートとメチル　カルビトオールを検出した。

２．キャベツから香気成分を抽出する時のpHにより、量的に１－ペンタノール，シス－３－ヘキセノール，トランス－２－ヘキセノール，アリル　イソチオシアネートに差を認めた。

３．成熟し、結球したキャベツの中心部は他の部分よりアリルイソチオシアネートが多く、未熟キャベツは成熟したキャベツより，アリルイソチオシアネートやトランス－２－ヘキセノールが多く含まれていた。