CHAPTER IV

RESULTS

1. Volatile oil content of Thai pepper from various sources

Most pepper gardens at Chantaburi contain Sarawak and Sri Lankan cultivars, approximately in the ratio 70: 30, respectively. The plants of both cultivars could be differentiated from each other by their characteristics of leaves and fruit berries. The Sarawak cultivar has smaller leaves and berries than Sri Lankan cultivar (Fig. 14). This clear distinction allowed us to collect fresh berries of each cultivar without mixing with the other. However, the black pepper products obtained from the fresh berries of both cultivars appeared to have no difference in size or shape. The only clear difference between the two products was found to be their density. The density of the black pepper of Sri Lankan cultivar, 547.8 $\pm$ 28.3 g/l, was usually higher than that of the Sarawak cultivar, 517.5 $\pm$ 25.0 g/l.

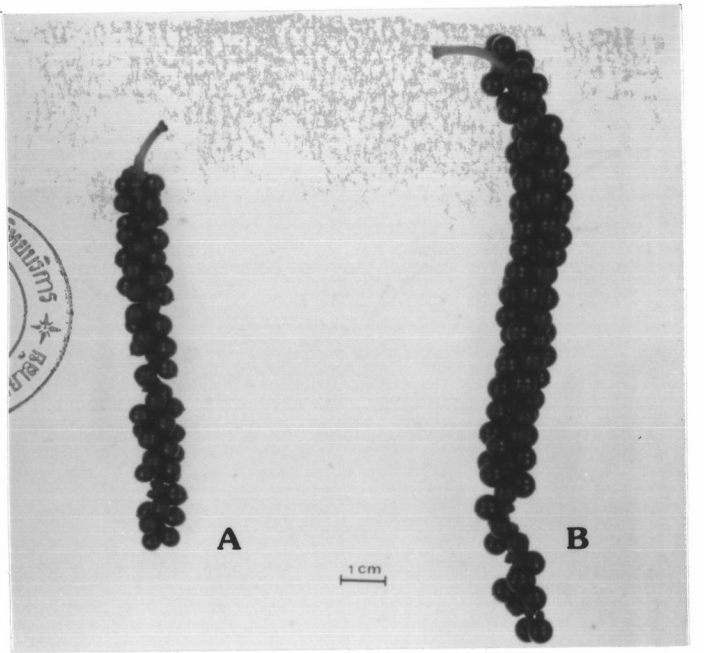


Figure 14 Fruit berries of Sarawak (A) and Sri Lankan cultivars (B).

Preliminary study on the volatile oil content of Chantaburi black pepper (produced from the mixed berries of Sarawak and Sri Lankan cultivars) showed that the product contained up to 2.3% (v/w) of volatile oil. Similary, various black pepper samples sold in Bangkok markets (mostly the products of Chantaburi) also showed their volatile oil content in the same range (Table 5). The total volatile oil content in Sri Lankan and Sarawak cultivars were the determined in for both fresh-green and dry-black pepper products. The results are shown in Table 5. It can be seen that the Sri Lankan cultivar showed significantly (α=0.05) higher level of volatile oil than the Sarawak cultivar. The Sri Lankan, either as green or black pepper, contained the volatile oil up to 2.73% while the Sarawak cultivar appeared to contain only 1.78%.

Table 5 Volatile oil content of Thai pepper from various sources

Pepper	Volatile oil content (% v/w dry weight)*
Chantaburi black pepper (mixed cultivars)	2.32 ± 0.11
Black pepper from Bangkok markets	2.12 ± 0.25
Sri Lankan cultivar	
- Fresh green pepper	2.68 ± 0.29
- Black pepper	2.73 ± 0.33
Sarawak cultivar	
- Fresh green pepper	1.57 ± 0.26
- Black pepper	1.78 ± 0.26

*Each sample was subjected to moisture content determination and the volatile oil content was calculated based on 10% moisture content of each pepper sample.

Each value represents the mean ± SD of six separate preparations.

2. Volatile oil composition of Thai black pepper from different cultivars

2.1 Gas chromatographic separation of pepper oil

In addition to the volatile oil content, composition of the pepper oils obtained from Sarawak and Sri Lankan cultivars were also compared by using gas chromatography (GC). In this study, the conditions used for the GC were developed carefully to maximize separation of all the oil components by a capillary column coated with polar polyethylene glycol stationary

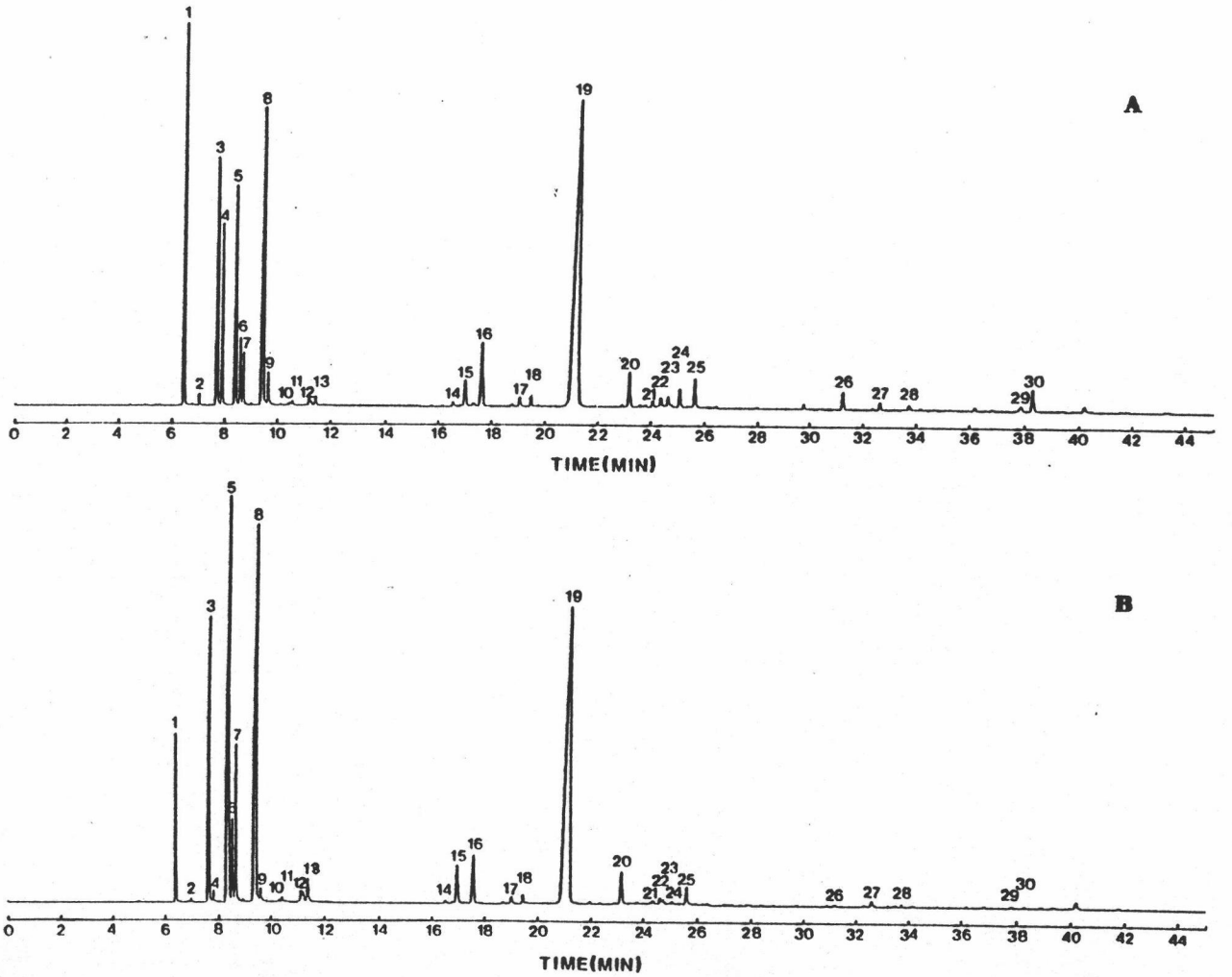


Figure 15 Typical GC-chromatograms of Thai black pepper oil obtained from Sri Lankan (A) and Sarawak (B) cultivars. Peak numbers are described in Table 6.

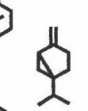
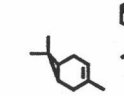
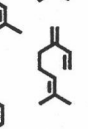
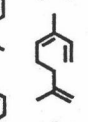
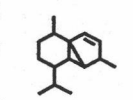
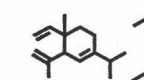
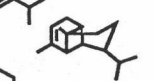
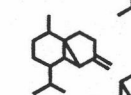
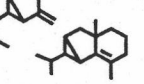
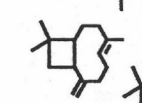
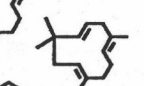
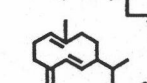
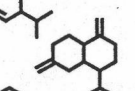
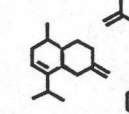
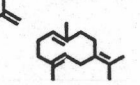
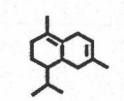
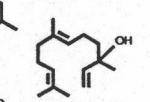
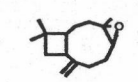
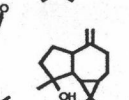
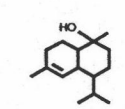
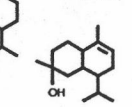
phase. The resulted GC-chromatograms for the pepper oils appeared to be similar between the two cultivars as shown in Fig. 15.

2.2 Identification of pepper oil constituents

From the chromatograms (Fig. 15), it can be seen that the pepper oils of both Sri Lankan and Sarawak cultivars are composed of some similar 30 components. Among these, peak number 1, 3, 4, 5, 8 and 19 were apparently the major components of the oil. To identify these major components as well as other minor ones, pepper oil samples were subjected to gas chromatography-mass spectroscopy (GC-MS) analysis. Under the conditions described in the Materials and Methods, all 30 peaks in the chromatograms could be identified. In practice, the mass spectrum of each component in pepper oil was compared with the mass spectra of various authentic components which were kept in the terpene library by computer (Fig. 27-56 in Appendix). Peak numbers and name of each components as well as its chemical group are shown in Table 6.

From these results, it was clear that the major components of peak numbers 1, 3, 4, 5, 8 and 19 were α-pinene, β-pinene, sabinene, Δ^3-carene, limonene and β-caryophyllene, respectively. Many other minor components of pepper oil was identified as seven monoterpene hydrocarbons, eleven sesquiterpene hydrocarbons and six oxygenated terpene compounds (Table 6).

Table 6 Thai pepper oil components separated by GC and identified by GC-MS. Peak numbers and retention times are corresponded to those shown in Fig. 15.

Peak No.	Retention time	Oil component	Structure	Chemical group
1	6.44	α-Pinene		Monoterpene hydrocarbons
2	7.02	Camphene		
3	7.69	β-Pinene		
4	7.89	Sabinene		
5	8.39	Δ^3-Carene		
6	8.57	Myrcene		
7	8.70	α-Phellandrene		
8	9.47	Limonene		
9	9.66	p-Cymene		
10	10.72	*trans* -Ocimene		
11	10.82	γ-Terpinene		
12	11.20	Terpinolene		
13	11.44	Linalool		Oxygenated monoterpene
14	16.53	α-Cubebene		Sesquiterpene hydrocarbons
15	16.98	δ-Elemene		
16	17.61	α-Copaene		
17	19.01	β-Cubebene		
18	19.43	α-Gurjunene		
19	21.20	β-Caryophyllene		
20	23.17	α-Humulene		
21	24.05	Germacrene D		
22	24.34	α-Muurolene		
23	24.62	γ-Cadinene		
24	25.04	Germacrene B		
25	25.60	δ-Cadinene		
26	32.59	*trans* -Nerolidol		Oxygenated sesquiterpenes
27	31.20	Caryophyllene oxide		
28	33.74	Spathulenol		
29	37.87	*tau* -Muurolol		
30	38.26	Torreyol		

2.3 Relative composition of Thai pepper oils from different cultivars

In term of relative quantity, however, it was found that the two cultivars were considerably different in their oil proportions of monoterpenes, sesquiterpenes and oxygenated compounds. As shown in Table 7, the Sri Lankan cultivar pepper oil was found to contain 43% monoterpenes, 50% sesquiterpenes and 3.9% oxygenated compounds whereas the Sarawak cultivar's was found to be 57%, 40% and 1.3% respectively. The higher content of the monoterpenes in the Sarawak cultivar pepper oil appeared to be contributed mainly by Δ^3-carene and limonene which were found to contain up to 20.2% and 16.3% respectively. These were much higher than the monoterpenes of the Sri Lankan cultivar which contained only 6.3% Δ^3-carene and 11.8% limonene (Table 7). For the total sesquiterpene hydrocarbons, its higher content in the Sri Lankan cultivar appeared to be contributed mainly by β-caryophyllene which contained up to 38% compared with 31% found in the Sarawak cultivar pepper oil. For the total oxygenated compounds, the higher content found in the Sri Lankan cultivar was contributed by all the oxygenated components, except for linalool (Table 7).

3. Quantitative analysis of major components in Thai pepper oil

3.1 Calibration curves

Figure 15 and Table 7 show that β-caryophyllene

Table 7 Composition of Sri Lankan and Sarawak cultivars' black pepper oil

Constituent	Pepper oil composition (% relative)*	
	Sri Lankan cutivar	Sarawak cultivar
Monoterpene hydrocarbons		
α-Pinene	8.96	3.97
Camphene	0.28	0.08
β-Pinene	6.74	8.36
Sabinene	4.44	0.14
Δ^3-Carene	6.34	20.23
Myrcene	1.69	2.46
α-Phellandrene	1.10	4.32
Limonene	11.84	16.28
p -Cymene	0.94	0.29
trans -Ocimene	0.06	0.16
γ-Terpinene	0.13	0.03
Terpinolene	0.55	0.53
Total monoterpenes	43.07	56.85
Sesquiterpene hydrocarbons		
α-Cubebene	0.20	0.10
δ-Elemene	1.12	2.05
α-Copaene	3.66	2.28
b-Cubebene	0.46	0.28
α–Gurjunene	0.46	0.28
β-Caryophyllene	38.21	30.76
α-Humulene	1.97	1.91
Germacrene D	0.38	0.04
α-Muurolene	0.48	0.24
γ-Cadinene	0.74	0.35
Germacrene B	0.87	0.24
δ-Cadinene	1.47	1.04
Total sesquiterpenes	50.02	39.57
Oxygenated compounds		
Linalool	0.30	0.91
trans -Nerolidol	0.85	0.04
Caryophyllene oxide	0.71	0.34
Spathulenol	0.33	tr**
tau -Muurolol	0.37	tr**
Torreyol	1.30	tr**
Total oxygenated compounds	3.86	1.29
Unknown fractions	3.05	2.29

*% relative=% integated area

**tr=Trace

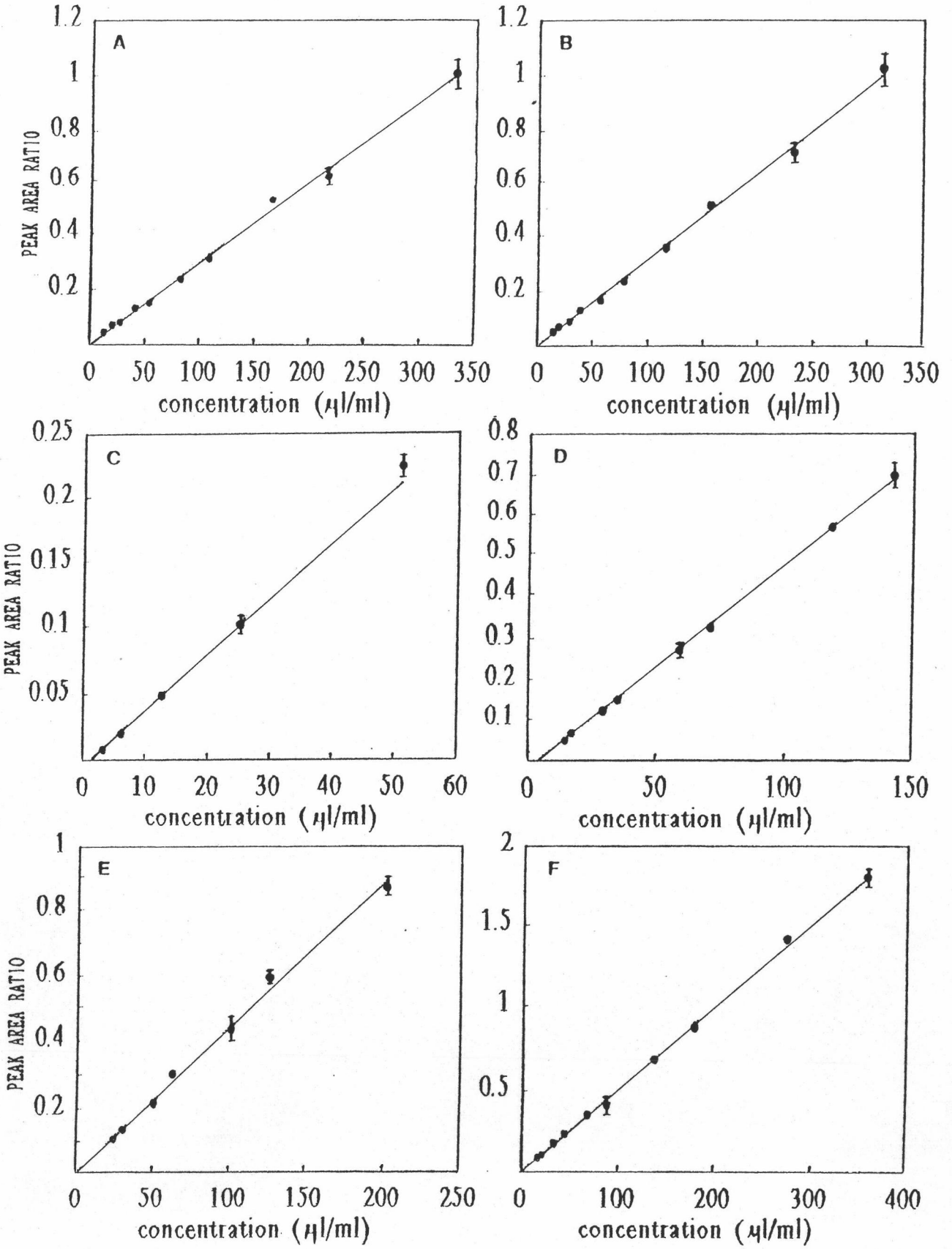


Figure 16 Calibration curves of α-pinene (A), β-pinene (B), sabinene (C), Δ^3-carene (D), limonene (E) and β-caryophyllene (F).

(sesquiterpene), α-pinene, β-pinene, sabinene, Δ^3-carene and limonene (all monoterpenes) are the major pepper oil components in Thai pepper oil. These components are important owing to their contribution to the odour quality of pepper oils. In this study, these compounds were determined for their absolute quantities in the black pepper. This quantitative analysis was carried out by GC using calibration curves of each authentic compound for calculation (Fig. 16). The calibration curves were linear over the studied range (13-332 μl/ml for α-pinene, 14-314 μl/ml for β-pinene, 3-51 μl/ml for sabinene, 14-142 μl/ml for Δ^3-carene, 25-200 μl/ml for limonene and 17-358 μl/ml for β-caryophyllene), with correlation coefficients of 0.997 or higher. Tridecane was chosen as internal standard because its peak was sharp, symmetrical and well separated from components in pepper oil. Under these conditions, the retention time of tridecane was 12.45 min.

3.2 The content of major components in Thai pepper oil

The resulted content of each major component was expressed in the unit of percent v/w of dry black pepper weight rather than percent v/v pepper oil since these values would give a more direct information on the actual level of each component in the black pepper. As shown in Table 8, it was found that the levels of α-pinene, β-pinene, sabinene and β-caryophyllene in Sri Lankan cultivar black pepper were considerably higher than those in Sarawak cultivar while the level of Δ^3-carene in Sri Lankan cultivar was slightly lower than those in Sarawak

cultivar and the level of limonene in both types of black pepper appeared to be the same. Table 8 also shows that the one percent higher of the total volatile oil of Sri Lankan cultivar (2.73%) over the Sarawak's (1.78%) is mainly the result of α-pinene, β-pinene, sabinene and β-caryophyllene. The content of these four components in the Sri Lankan cultivar is approximate 0.80% higher than their content in the Sarawak cultivar.

Table 8 The content of pepper oil major components

Major component	Content (% v/w dry weight)	
	Sri Lankan cultivar	Sarawak cultivar
Total volatile oil	**2.73±0.33**	**1.78±0.26**
α-Pinene	0.30±0.01	0.06±0.002
β-Pinene	0.24±0.01	0.14±0.01
Sabinene	0.13±0.02	0.002±0.0002
Δ^3-Carene	0.14±0.01	0.22±0.02
Limonene	0.32±0.03	0.27±0.01
β-Caryophyllene	0.87±0.03	0.54±0.03

Each value represents the mean ± SD of six separate preparations.

4. Quantitative analysis of piperine in Thai pepper

4.1 HPLC separation of piperine

The content of piperine, the major pungent alkaloid in pepper, was determined by high performance liquid chromatography (HPLC). This method could separate piperine from other derivatives of piperine such as piperettine in pepper extract. The chromatogram of standard piperine and pepper extract are shown in Fig. 17.

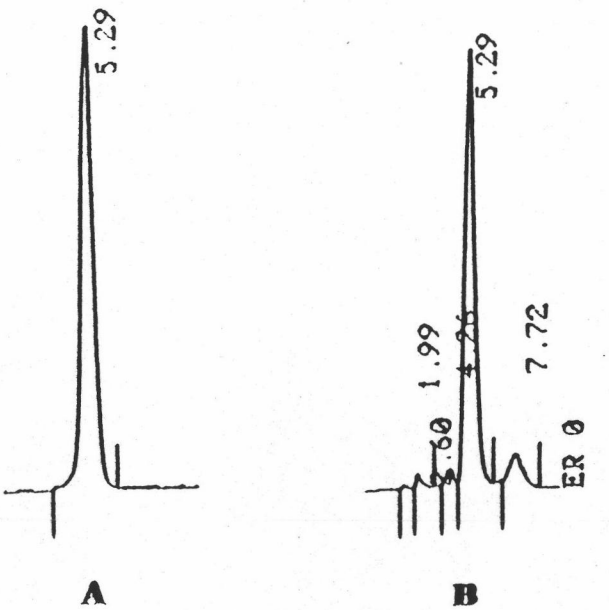


Figure 17 HPLC chromatrograms of standard piperine (A) and pepper extract (B).

4.2 Calibration curve

The calibration curve of piperine is shown in Fig. 18. It showed linearity of the relationship in the range 48-274 μg/ml and the correlation coefficients was found to be 0.999 for piperine.

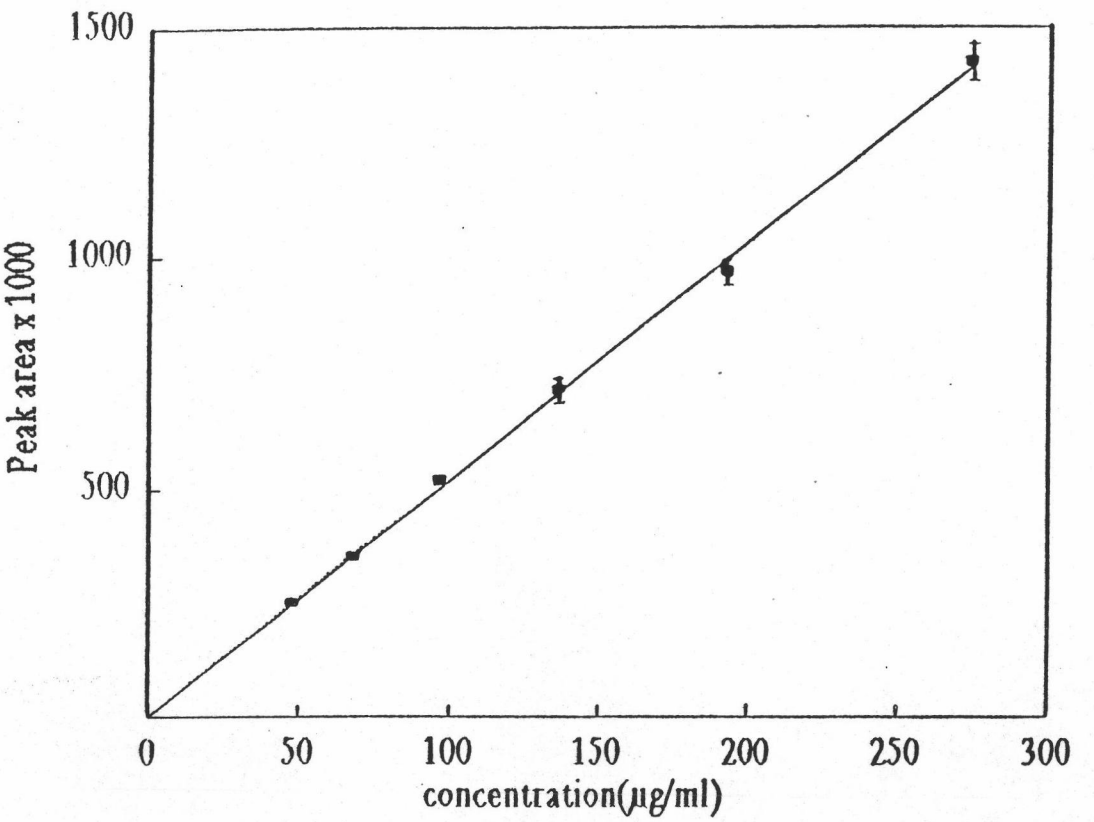


Figure 18 Calibration curve of piperine.

4.3 Piperine content in the pepper cultivated in Thailand

In addition to the pepper oil content, the amount of piperine accumulated in Thai black pepper of various sources was also examined. Using the HPLC method, it was found that piperine in pepper from Sri Lankan cultivar (4.96% w/w) contained significantly (α=0.05) higher level than those from Sarawak cultivar (3.82%) (Table 9). The piperine content of Chantaburi black pepper which is produced from the mixed berries of Sarawak and Sri Lankan cultivars showed an intermediate value of 3.94% of piperine. Similarly, various black pepper samples sold in Bangkok markets (mostly the product of Chantaburi) also showed the same range of piperine content of 3.46%.

Table 9 Piperine content in black pepper from various sources

Black pepper	Piperine (% w/w)
Chantaburi black pepper (mixed cultivars)	3.94±0.10
Black pepper from Bangkok markets	3.46±0.11
Sri Lankan cultivar	4.96±0.19
Sarawak cultivar	3.82±0.32

Each value represnts the mean ± SD of six separate preparations.

5. Comparison of volatile constituent and piperine content among black pepper products of Thailand and other countries

5.1 Volatile oil and piperine contents

Table 10 shows the contents of volatile oil (determination by distillation) and piperine (by HPLC) in the black pepper obtained from India, Brasil, Malaysia and in the local products of Thailand. The black pepper from Malaysia (3.20% v/w) and India (3.19%) was found to have highest levels of volatile oil, followed closely by the Brasil pepper (3.10%). The Thai black pepper, however, showed lower levels of the volatile oil content, with 2.73% in the Sri Lankan and 1.78% in the Sarawak cultivars.

For the piperine content, on the other hand, the black pepper from Thailand's Sri Lankan cultivar was found to contain the highest level with 4.96% w/w. This was more than one percent higher than the piperine level found in the black pepper from Malaysia, India and Brasil (3.78, 3.71 and 3.58%, respectively). For Thailand's Sarawak cultivar, piperine content (3.82%) was also found to be significantly (α=0.05) lower than the Sri Lankan cultivar but slightly higher the foreign black pepper.

Table 10 Volatile oil and piperine contents of black pepper obtained from Brasil, India, Malaysia and Thailand.

Pepper	Volatile oil (% v/w)	Piperine (% w/w)
Brasil	3.10±0.16	3.58±0.10
India	3.19±0.14	3.71±0.14
Malaysia	3.20±0.14	3.78±0.14
Thailand		
- Sri Lankan cultivar	2.73±0.33	4.96±0.19
- Sarawak cultivar	1.78±0.26	3.82±0.12

Each value represents the mean ± SD of six separate preparations.

5.2 Volatile oil composition

Volatile oil composition in pepper oil distilled from the black pepper of various countries were compared by using gas chromatography (GC). GC chromatograms of the pepper oils from Thailand, Brasil, India and Malaysia are shown in Fig. 19. Among these, the black pepper oils from Thailand's Sri Lankan cultivar and Brasil showed significantly higher levels of α-pinene than those from other countries. The proportion of sabinene in the pepper oils from Thailand's Sri Lankan, Brasil and India showed high level whereas the sabinene contents in Thailand's Sarawak cultivar and in Malaysian pepper oil

were very low. In addition, the chromatograms show that black pepper oils from Thailand's Sarawak cultivar, India and Malaysia contained high levels of Δ^3-carene whereas the proportion of α-phellandrene in the pepper oil from Thailand's Sri Lankan cultivar and β-caryophyllene in pepper oil from India showed the lowest levels.

From the chromatograms, the relative level of each component in the black pepper oil of different sources was determined. As shown in Table 11, the proportion of total monoterpene hydrocarbons in the pepper oil from India (61.54%) appeared to be highest and the level of pepper oil from Brasil (57.02%) was similar to that of Thailand's Sarawak cultivar (56.85%). The lowest level of total monoterpene hydrocarbons was found in the pepper oil from Thailand's Sri Lankan cultivar (43.07%). In term of the proportion of total sesquiterpene hydrocarbons, the pepper oil from Thailand's Sri Lankan cultivar (50.02%) showed the highest level and the pepper oils from Brasil (38.52%) and from Thailand's Sarawak cultivar (39.57%) were found to have similar moderate contents. The pepper oil from India, on the other hand, showed the lowest level of total sesquiterpene hydrocarbons (31.52%). For the proportion of total oxygenated compounds, the Indian pepper oil showed the highest level (4.17%) whereas the Malaysian pepper oil (1.22%) showed the lowest level.

The pepper oil composition from Brasil was devoid of γ-terpinene, α-cubebene and *tau*-muurolol, the pepper

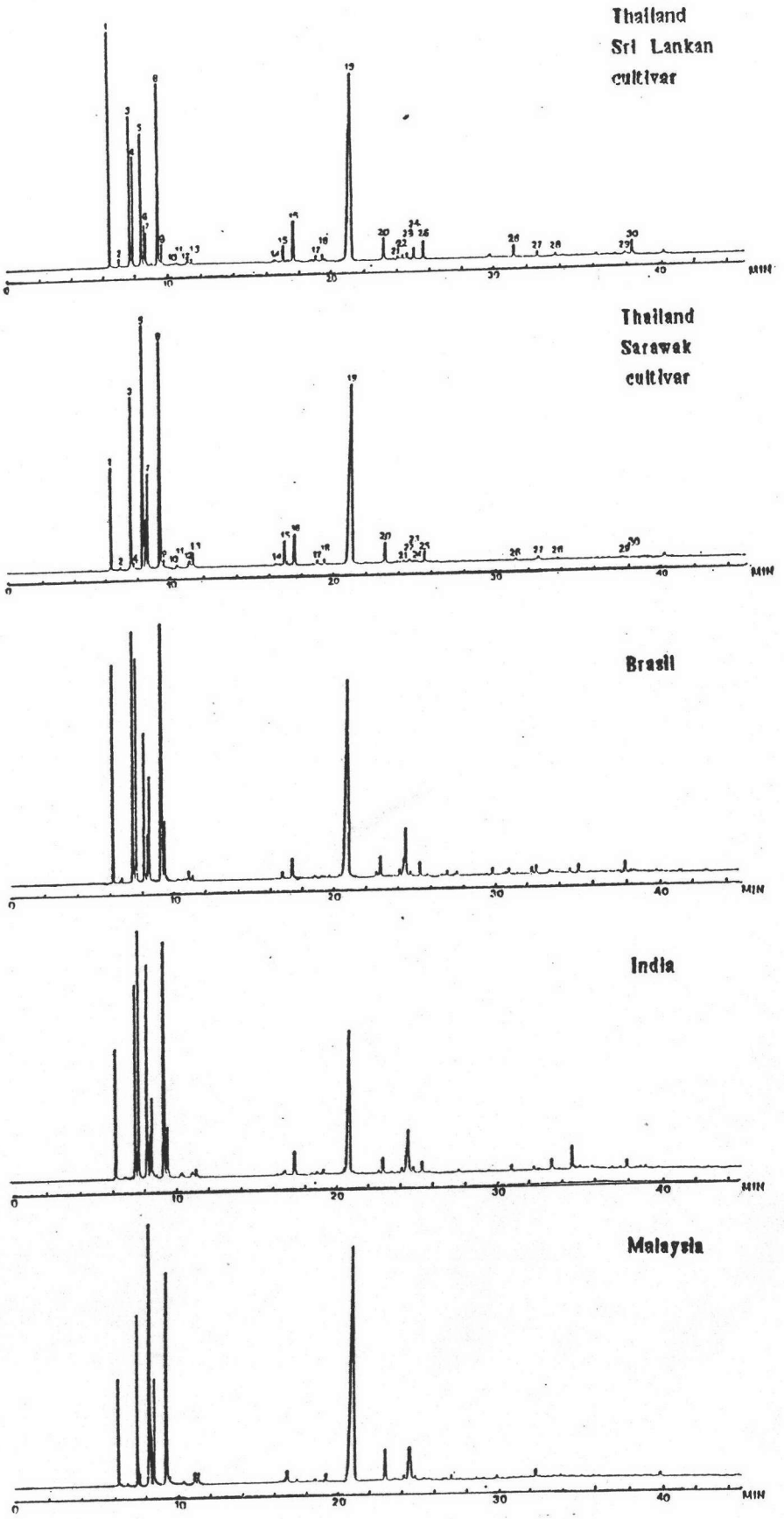


Figure 19 Typical GC-chromatograms of black pepper oil from Thailand , Brasil , India and Malaysia. Peak numbers are described in Table 6.

Table 11 Composition of pepper oil obtained from Brasil,India,Malaysia and Thailand

Constituent	Pepper oil composition (% relative)*				
	Brasil	India	Malaysia	Thailand Sri Lankan cutivar	Thailand Sarawak cultivar
Monoterpene hydrocarbons					
α-Pinene	7.04	5.45	3.14	8.96	3.97
Camphene	0.15	0.09	0.06	0.28	0.08
β-Pinene	9.61	8.74	6.15	6.74	8.36
Sabinene	7.64	11.04	0.28	4.44	0.14
Δ^3-Carene	5.77	11.18	20.12	6.34	20.23
Myrcene	1.79	1.99	1.94	1.69	2.46
α-Phellandrene	3.95	3.48	3.79	1.10	4.32
Limonene	17.92	16.07	11.71	11.84	16.28
p -Cymene	2.34	2.61	0.25	0.94	0.29
trans -Ocimene	0.08	0.17	0.10	0.06	0.16
γ-Terpinene	tr**	0.18	tr**	0.13	0.03
Terpinolene	0.73	0.54	1.04	0.55	0.53
Total monoterpenes	**57.02**	**61.54**	**48.78**	**43.07**	**56.85**
Sesquiterpene hydrocarbons					
α-Cubebene	tr**	0.12	0.09	0.20	0.10
δ-Elemene	0.50	0.35	0.73	1.12	2.05
α-Copaene	1.47	1.93	0.18	3.66	2.28
b-Cubebene	0.18	0.20	tr**	0.46	0.28
α–Gurjunene	0.09	0.24	0.24	0.46	0.28
β-Caryophyllene	28.54	19.86	39.20	38.21	30.76
α-Humulene	1.47	1.23	2.22	1.97	1.91
Germacrene D	0.10	0.10	tr**	0.38	0.04
α-Muurolene	0.43	0.51	0.30	0.48	0.24
γ-Cadinene	4.47	5.47	4.60	0.74	0.354
Germacrene B	0.40	0.58	0.32	0.87	0.24
δ-Cadinene	0.87	0.93	0.11	1.47	1.04
Total sesquiterpenes	**38.52**	**31.52**	**47.99**	**50.02**	**39.57**
Oxygenated compounds					
Linalool	0.37	0.55	0.73	0.30	0.91
trans -Nerolidol	0.31	0.34	tr**	0.85	0.04
Caryophyllene oxide	0.45	0.26	0.49	0.71	0.34
Spathulenol	0.20	0.85	tr**	0.33	tr**
tau -Muurolol	tr**	1.59	tr**	0.37	tr**
Torreyol	0.68	0.58	tr**	1.30	tr**
Total oxygenated compounds	**2.02**	**4.17**	**1.22**	**3.86**	**1.29**
Unknown fractions	2.45	2.77	2.01	3.05	2.29

*% relative=% integated area

**tr=Trace

oil from Malaysia was devoid of β-cubebene, germacrene D, *trans*-nerolidol, spathulenol, *tau*-muurolol and torreyol, the pepper oil from Thailand's Sarawak cultivar was devoid of spathulenol, *tau*-muurolol and torreyol while the pepper oil from India and Thailand's Sri Lankan cultivar contained all volatile components possibly identified by GC-MS.

The major pepper oil components from Brasil, India, Malaysia and Thailand are shown in Table 12. It was found that α-pinene level in the pepper oil from Brasil was similar to that in Thailand's Sri Lankan cultivar (0.28% and 0.30%, respectively) while Thailand's Sarawak cultivar showed the lowest level of α-pinene (0.06%). For β-pinene, the pepper oils from Brasil and India contained high levels of β-pinene (0.36% and 0.35%, respectively) and the proportion of β-pinene in the pepper oil from Malaysia was similar to the level in Thailand's Sri Lankan cultivar (0.23% and 0.24%, respectively) while Thailand's Sarawak cultivar showed the lowest level of β-pinene (0.14%). The content of sabinene was found to be highest and lowest in the pepper oil from India (0.34%) and Thailand's Sarawak cultivar (0.002%), respectively. For the level of Δ^3-carene, the pepper oil from Malaysia showed the highest content (0.47%) while those from Thailand's Sri Lankan cultivar and Brasil were found to be low (0.14% and 0.15%, respectively). The pepper oils from Brasil and India contained the same highest levels of limonene (0.50%) and those from Malaysia was similar to the level of limonene

in Thailand's Sri Lankan cultivar (0.34% and 0.32%, respectively) while Thailand's Sarawak cultivar contained the lowest level of limonene (0.27%). In addition, the proportion of β-caryophyllene showed the highest level in pepper oil from Malaysia (0.95%) and contained relatively high level in the pepper oil from Thailand's Sri Lankan cultivar (0.87%) while those from India contained the lowest level of β-caryophyllene (0.47%). The total major pepper oil components from these five sources contained in the range 67-73% of their own pepper oil content.

Table 12 The content of volatile oil major components obtained from the black pepper of Brasil, India, Malaysia and Thailand

Major component	Content (% v/w dry weight)				
	Brasil	India	Malaysia	Thailand Sri Lankan cultivar	Thailand Sarawak cultivar
Total volatile oil	**3.10±0.16**	**3.19±0.14**	**3.20±0.14**	**2.73±0.33**	**1.78±0.26**
α-Pinene	0.28±0.02	0.23±0.02	0.13±0.01	0.30±0.01	0.06±0.002
β-Pinene	0.36±0.02	0.35±0.02	0.23±0.01	0.24±0.01	0.14±0.01
Sabinene	0.22±0.02	0.34±0.02	0.01±0.004	0.13±0.02	0.002±0.0002
Δ^3-Carene	0.15±0.03	0.28±0.03	0.47±0.03	0.14±0.01	0.22±0.02
Limonene	0.50±0.01	0.50±0.02	0.34±0.01	0.32±0.03	0.27±0.01
β-Caryophyllene	0.66±0.01	0.47±0.01	0.95±0.04	0.87±0.03	0.54±0.03

Each value represents the mean ± SD of six separate preparations.

6. Changes in volatile oil and piperine contents during the maturation of pepper berries

6.1 Changes in volatile oil and piperine contents

During the maturation of pepper berries, the contents of volatile oil and piperine were found to fluctuate during a period of 6 months (Table 13). For Sri Lankan cultivar, the black pepper showed a rise in both volatile oil content, from 2.87% in the first month to 7.13% in the third month, and piperine content, from 0.51% from the first month to 8.29% in the third month. Thereafter, the level of both components decreased continuously until maturation of the berries which contained 2.79% volatile oil and 4.06% piperine. The Sarawak cultivar, pepper berries showed similar patterns of volatile oil and piperine accumulation except the levels of both components were lower than the Sri Lankan cultivar at all stage of development. Again, the maximum volatile oil and piperine contents were found in the third month with 5.30 and 6.42% respectively. The mature berries (6 months old) finally contained 2.35% volatile oil and 2.99% piperine. In term of density, the black pepper berries of both cultivars reached their maximum values at the fifth month (647.0 and 613.8 g/l in Sri Lankan and Sarawak cultivars, respectively) and declined after that. At the third month of pepper berries, although the volatile oil and piperine contents of both cultivars appeared to be maximal, their density were significantly lower than the fifth month (Table 13). Consequently, the pepper berries

Table 13 Volatile oil content, piperine content and density of pepper berries.

Pepper berries (months)	Volatile oil (% v/w)	Piperine (% w/w)	Density (g/l of black pepper)
Sri Lankan cultivar			
1	2.87±0.07	0.51±0.02	212.8±7.9
2	5.80±0.05	3.17±0.15	216.2±2.9
3	7.13±0.11	8.29±0.24	422.3±6.2
4	5.22±0.24	8.16±0.18	533.2±9.2
5	3.74±0.01	4.98±0.25	647.0±5.3
6	2.79±0.03	4.06±0.19	631.5±6.6
Sarawak cultivar			
1	3.10±0.05	0.83±0.04	220.4±3.6
2	5.18±0.13	5.30±0.23	228.8±5.9
3	5.30±0.15	6.42±0.20	347.4±3.4
4	4.38±0.08	3.40±0.15	342.0±2.3
5	3.00±0.12	3.24±0.07	613.8±5.9
6	2.35±0.08	2.99±0.18	595.8±5.8

Each value represents the mean ± SD of six separate preparations.

at the third month were considered being immature and too early for harvesting. The 5 months old of pepper berries were, therefore, likely to be suitable for harvesting although it contained volatile oil and piperine lower than the maximum.

6.2 Changes in volatile oil composition

The volatile composition of the pepper oil obtained during the maturation of pepper berries of Sri Lankan and Sarawak cultivars are shown in Tables 14 and 15, respectively. It can be seen that the pepper berries from both cultivars with one month old showed very low level of total monoterpene hydrocarbons (19.22% for Sri Lankan and 20.78% for Sarawak cultivars) but fairly high level of total sesquiterpene hydrocarbons (72.39% for Sri Lankan and 75.66% for Sarawak cultivars). It should be noted that the one month old pepper berries contained undetectable *p*-cymene in both cultivars and undetectable sabinene in the pepper oil from Sarawak cultivar.

At the second month, the proportion of total monoterpene hydrocarbons and each component of monoterpene hydrocarbon were found to increase up to 43.85 and 53.37% in the pepper oil of Sri Lankan and Sarawak cultivars, respectively. On the other hand, the proportion of total sesquiterpene hydrocarbons and each component of sesquiterpene hydrocarbon were decreased to 52.08% for Sri Lankan and 46.35% for Sarawak cultivars. For subsequent stages of maturity (from 2 to 6 months), the components in pepper oil from both cultivars showed no significant variation.

Table 14 Composition of pepper oil from Sri Lankan cultivar pepper berries during the development (% relative*).

Constituent	Age of berries (months)					
	1	2	3	4	5	6
Monoterpene hydrocarbons						
α-Pinene	6.75	12.20	13.02	12.31	12.00	11.05
Camphene	0.21	0.36	0.35	0.34	0.32	0.32
β-Pinene	3.52	8.95	9.28	9.10	9.23	8.88
Sabinene	0.56	4.22	4.40	4.99	4.87	4.31
Δ^3-Carene	1.87	6.54	7.00	6.90	6.68	6.30
Myrcene	0.64	1.81	2.00	2.02	1.97	1.74
α-Phellandrene	0.65	1.84	2.02	2.04	1.92	1.95
Limonene	4.74	14.03	14.26	15.54	15.20	14.69
p -Cymene	tr**	0.47	0.50	0.54	0 45	0.40
trans -Ocimene	0.28	0.45	0.50	0.48	0.45	0.43
Total monoterpenes	19.22	50.87	53.33	54.26	53.09	50.07
Sesquiterpene hydrocarbons						
α-Cubebene	0.60	0.25	0.21	0.12	0.13	0.16
δ-Elemene	2.91	1.39	1.20	0.94	0.90	0.89
α-Copaene	10.33	3.82	3.60	3.20	3.14	3.53
b-Cubebene	0.98	0.45	0.42	0.37	0.40	0.41
α–Gurjunene	0.31	0.19	0.16	0.12	0.10	0.12
β-Caryophyllene	41.32	34.28	33.52	34.05	34.68	35.74
α-Humulene	2.61	1.68	1.54	1.58	1.56	1.75
Germacrene D	0.52	0.28	0.23	0.23	0.24	0.25
α-Muurolene	2.20	0.67	0.60	0.52	0.44	0.51
γ-Cadinene	1.28	0.58	0.50	0.46	0.48	0.59
Germacrene B	3.60	1.18	1.02	0.90	0.66	0.76
δ-Cadinene	5.73	1.65	1.54	1.42	1.22	1.61
Total sesquiterpenes	72.39	46.42	44.54	43.91	43.95	46.32
Oxygenated compounds						
Linalool	0.12	0.31	0.28	0.21	0.30	0.26
trans -Nerolidol	0.44	0.30	0.44	0.54	0.68	0.40
Caryophyllene oxide	0.14	0.07	0.10	0.10	0.10	0.12
Spathulenol	0.19	0.14	0.14	0.12	0.29	0.18
tau -Muurolol	0.50	0.30	0.32	0.34	0.34	0.36
Torreyol	1.73	1.17	0.85	0.34	1.21	1.18
Total oxygenated compounds	3.12	2.29	2.13	1.65	2.92	2.50
Unknown fractions	5.27	0.42	0	0.18	0.04	1.11

*% relative = % integated area
**tr=Trace

Table 15 Composition of pepper oil from Sarawak cultivar pepper berries during the development (% relative*).

Constituent	Age of berries (months)					
	1	2	3	4	5	6
Monoterpene hydrocarbons						
α-Pinene	1.72	3.94	4.02	4.35	4.26	3.72
β-Pinene	3.72	7.99	7.91	9.03	8.88	8.27
Sabinene	tr**	0.13	0.56	0.38	0.14	0.20
Δ^3-Carene	5.56	17.16	16.06	15.98	15.72	15.70
Myrcene	0.78	2.27	2.18	2.40	2.30	2.18
α-Phellandrene	1.45	4.17	3.90	4.20	4.00	4.39
Limonene	7.55	15.66	15.64	17.23	17.01	17.26
p -Cymene	tr**	0.08	0.10	0.08	0.06	0.09
Total monoterpenes	20.78	51.37	50.37	53.65	52.37	51.81
Sesquiterpene hydrocarbons						
α-Cubebene	0.44	0.16	0.10	0.10	0.10	0.12
δ-Elemene	5.32	2.06	2.14	2.18	1.39	1.15
α-Copaene	10.06	3.52	3.25	3.40	2.64	2.43
b-Cubebene	0.88	0.34	0.30	0.36	0.32	0.28
α–Gurjunene	0.48	0.20	0.22	0.20	0.11	0.23
β-Caryophyllene	46.32	35.30	35.67	35.09	38.94	39.52
α-Humulene	2.78	1.80	1.73	1.90	1.83	1.85
α-Muurolene	0.58	0.24	0.20	0.26	0.21	0.22
γ-Cadinene	3.91	1.05	0.88	0.58	0.30	0.40
Germacrene B	0.66	0.28	0.20	0.30	0.18	0.21
δ-Cadinene	4.23	1.40	1.20	1.02	0.02	0.98
Total sesquiterpenes	75.66	46.35	45.89	45.39	46.04	47.39
Oxygenated compounds						
Linalool	0.23	0.86	0.64	0.64	0.56	0.52
Caryophyllene oxide	0.23	0.17	0.28	0.24	0.19	0.22
Total oxygenated compounds	0.46	1.03	0.92	0.88	0.75	0.74
Unknown fractions	3.10	1.05	2.82	0.08	0.84	0.06

*% relative = % integated area

**tr=Trace

6.3 Changes in the content of major oil components

In term of major oil components in the pepper oil obtained during the maturation of the pepper berries from both cultivars (Fig 20), it was found that both Sri Lankan and Sarawak cultivars showed low levels of α-pinene, β-pinene, sabinene, Δ^3-carene and limonene. Especially, sabinene was devoid in Sarawak cultivar while β-caryophyllene showed high level in both cultivars at one month old of the pepper berries.

For subsequent stages of maturity, the proportion of α-pinene, β-pinene, sabinene, Δ^3-carene and limonene in pepper oil from both cultivars were markedly increased at the second month of pepper berries to the relatively constant level throughout, the course of 6-month period of maturity. On the other hand, the proportion of β-caryophyllene in pepper oil from both cultivars were decreased at the second month of pepper berries and slightly decreased to a minimum at the forth month, and then increased slowly until the sixth month of maturity. Therefore, it appeared that the patterns of accumulation of major oil components during the maturation of the pepper berries from both Sri Lankan and Sarawak cultivars were essentially the same.

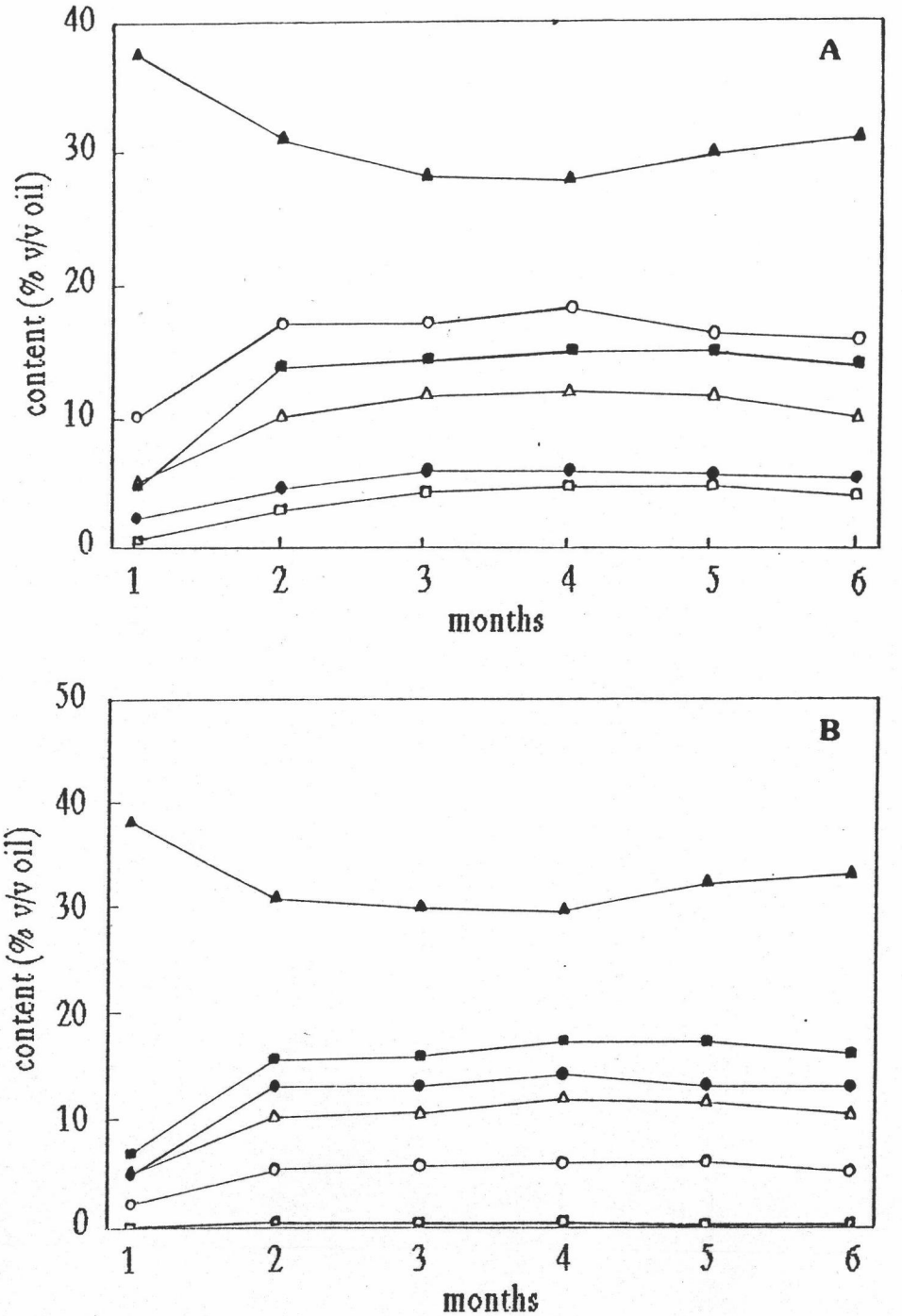


Figure 20 Changes in the contents of major components in pepper oil obtained from Sri Lankan (A) and Sarawak cultivars (B) pepper berries. α-Pinene (○——○), β-pinene (△——△), sabinene (□——□), Δ^3-carene (●——●), limonene (■——■) and β-caryophyllene (▲——▲).

7. Stability of volatile constituents in black pepper and pepper oil during storage

7.1 Volatile oil in stored black pepper

7.1.1 Changes in volatile oil content

Changes in the volatile oil content in black pepper during storage for a period of eight months at room temperature in tight container was carried out. It was found that the volatile oil level in black pepper had no significant (α=0.05) change during such a storage (Fig. 21). These results indicate that the black pepper from fully developed berries can be stored at least eight months without affecting the volatile oil constant.

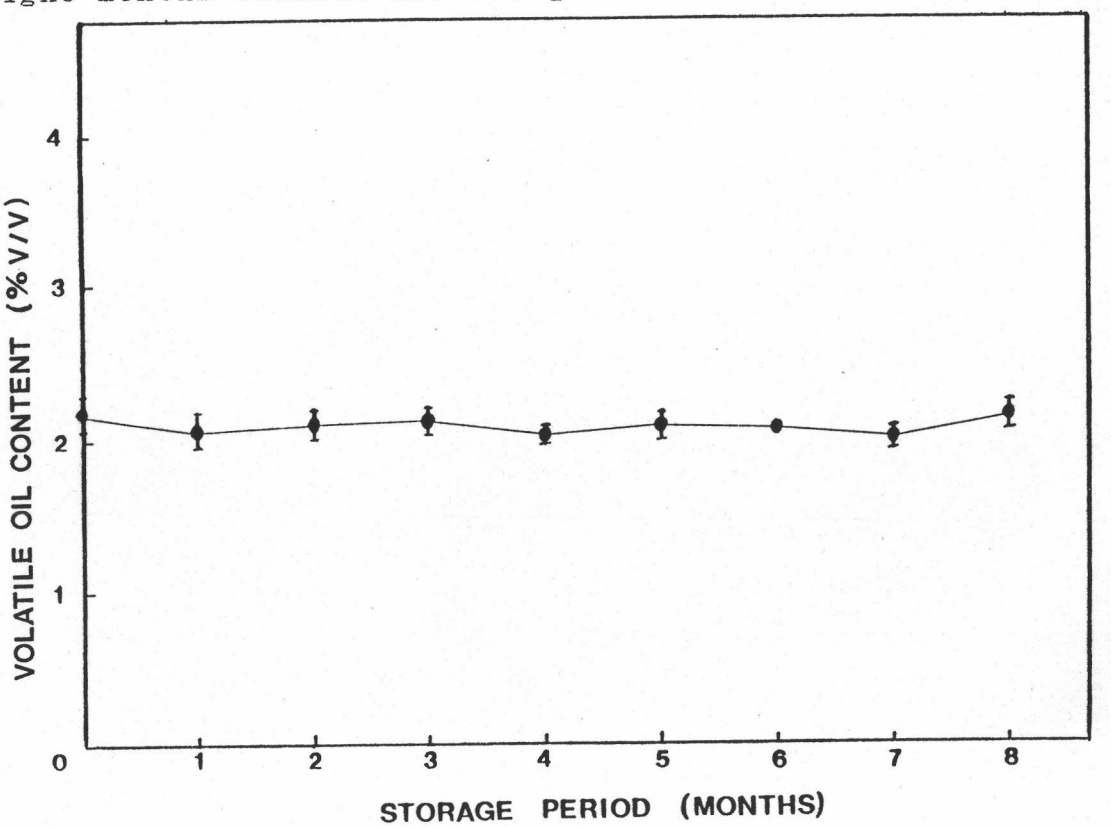


Figure 21 Changes in the volatile oil content in black pepper during a period of 8-month storage at room temterature.

7.1.2 Changes in volatile oil composition

In addition to the volatile oil content, the volatile oil composition of the stored black pepper was also examined. As shown in Table 16 and Fig. 22, the proportion of total monoterpene hydrocarbons showed no significant variation (α=0.05) from 56.96% at the first month to 57.39% at the eighth month of storage. The proportion of total sesquiterpene hydrocarbons showed no significant variation (α=0.05) from 42.39% to 39.10%. For the oxygenated compounds, the amount of caryophyllene oxide could only be detected in pepper oil after 4 months of storage. It was possible that the decrease in β-caryophyllene from 34.11% at the first month to 32.28% at the eighth month resulted from its conversion to caryophyllene oxide.

7.1.3 Changes in the content of major oil components

For quantitative analysis of major oil components in the stored black pepper, it was found that the levels of α-pinene, β-pinene, Δ^3-carene, limonene and β-caryophyllene were slightly decreased during such an 8-month storage (Fig. 23). This suggested that the major oil components were relatively stable during being stored in the black pepper. Therefore, the black pepper can be stored at least eight months at room temperature in a tight container.

Table 16 Composition of pepper oil from black pepper during storage at room temperature(% relative*)

Constituent	Storage period (months)								
	0	1	2	3	4	5	6	7	8
Monoterpene hydrocarbons									
α-Pinene	3.44	3.39	3.36	3.56	3.44	3.92	3.61	3.44	3.69
Camphene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
β-Pinene	8.07	7.92	7.87	8.30	8.11	8.58	8.08	7.80	8.08
Sabinene	0.16	0.15	0.12	0.12	0.14	0.11	0.08	0.12	0.12
Δ^3-Carene	20.16	20.04	19.99	20.45	20.44	20.20	19.78	19.74	20.02
Myrcene	2.20	2.14	2.46	2.43	2.47	2.56	2.32	2.37	2.52
α-Phellandrene	4.33	4.38	4.35	4.63	4.50	4.59	4.00	4.26	4.64
Limonene	17.47	17.36	17.33	17.48	17.53	17.70	16.95	16.71	17.28
p -Cymene	0.22	0.21	0.26	0.14	0.18	0.17	0.14	0.10	0.12
trans -Ocimene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
γ-Terpinene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
Terpinolene	0.91	0.92	0.90	0.96	0.97	0.92	0.88	0.90	0.92
Total monoterpenes	**56.96**	**56.51**	**56.64**	**58.13**	**57.78**	**58.75**	**55.84**	**55.44**	**57.39**
Sesquiterpene hydrocarbons									
α-Cubebene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
δ-Elemene	1.80	1.81	1.75	1.51	1.66	1.57	1.60	1.97	1.54
α-Copaene	2.64	2.62	2.51	2.30	2.28	2.23	2.53	2.39	2.25
β-Cubebene	0.33	0.32	0.30	0.28	0.27	0.26	0.28	0.26	0.25
α-Gurjunene	0.28	0.29	0.26	0.20	0.15	0.14	0.16	0.12	0.07
β-Caryophyllene	34.11	34.71	34.70	33.36	33.26	32.37	34.41	34.34	32.28
α-Humulene	1.74	1.68	1.64	1.56	1.56	1.53	1.62	1.61	1.50
Germacrene D	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
α-Muurolene	0.10	0.12	0.11	0.14	0.12	0.15	0.12	0.14	0.11
γ-Cadinene	0.47	0.43	0.46	0.44	0.42	0.45	0.50	0.46	0.26
Germacrene B	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
δ-Cadinene	0.92	0.86	0.85	0.87	0.78	0.78	0.89	0.84	0.84
Total sesquiterpenes	**42.39**	**42.84**	**42.58**	**40.60**	**40.50**	**39.48**	**42.11**	**42.13**	**39.10**
Oxygenated compounds									
Linalool	0.64	0.61	0.70	0.76	0.82	0.90	0.88	0.91	1.00
trans -Nerolidol	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
Caryophyllene oxide	tr**	tr**	tr**	tr**	0.34	0.43	0.48	0.50	0.48
Spathulenol	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
tau -Muurolol	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
Torreyol	tr*	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
Total oxygenated compounds	**0.64**	**0.61**	**0.70**	**0.76**	**1.16**	**1.33**	**1.36**	**1.41**	**1.48**
Unknown fractions	0.1	0.04	0.08	0.51	0.56	0.44	0.69	1.02	2.03

*% relative=% integated area

**tr=Trace

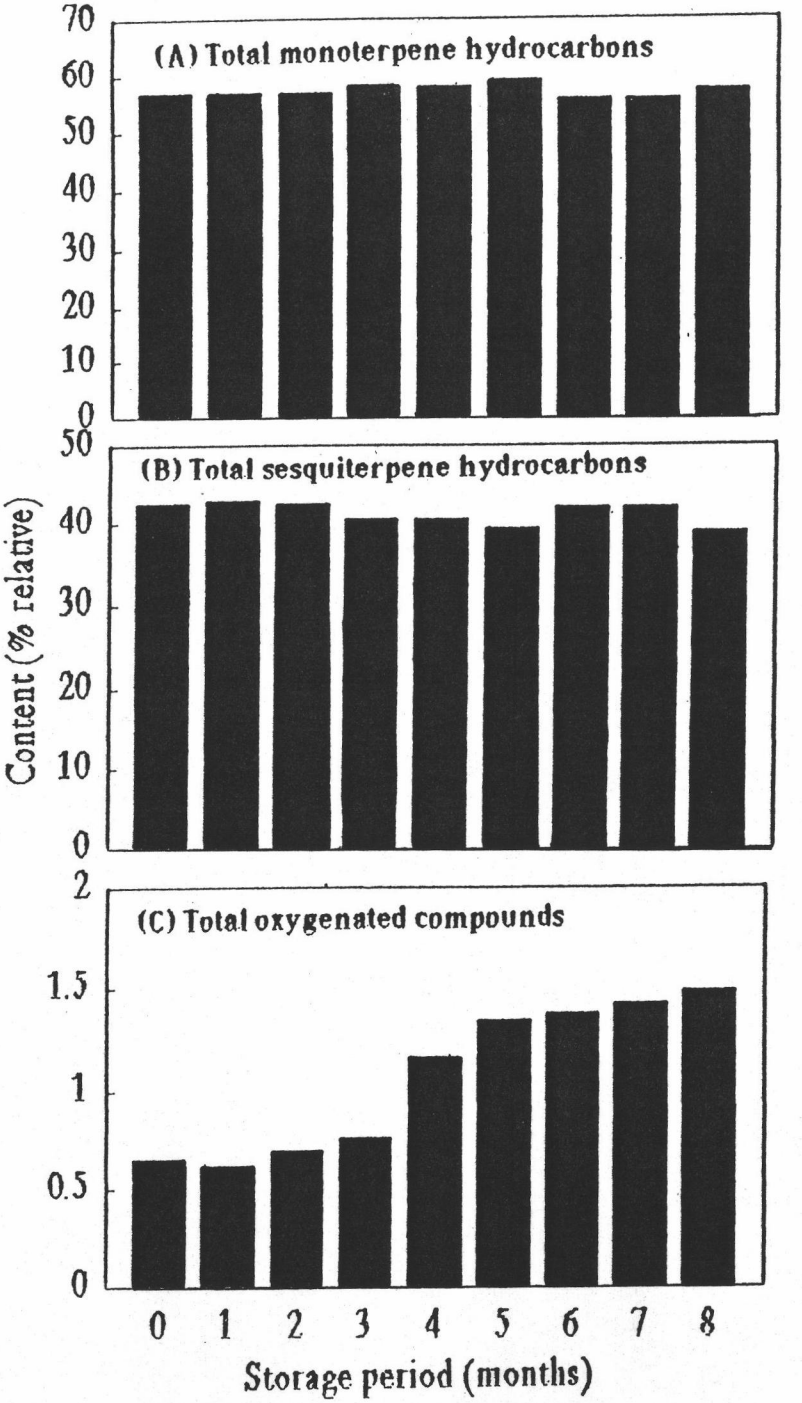


Figure 22 Relative changes in the contents of total monoterpenes (A), total sesquiterpenes (B) and total oxygenated compounds (C) in the pepper oil that were extracted from the stored black pepper at room temperature for an 8-month period.

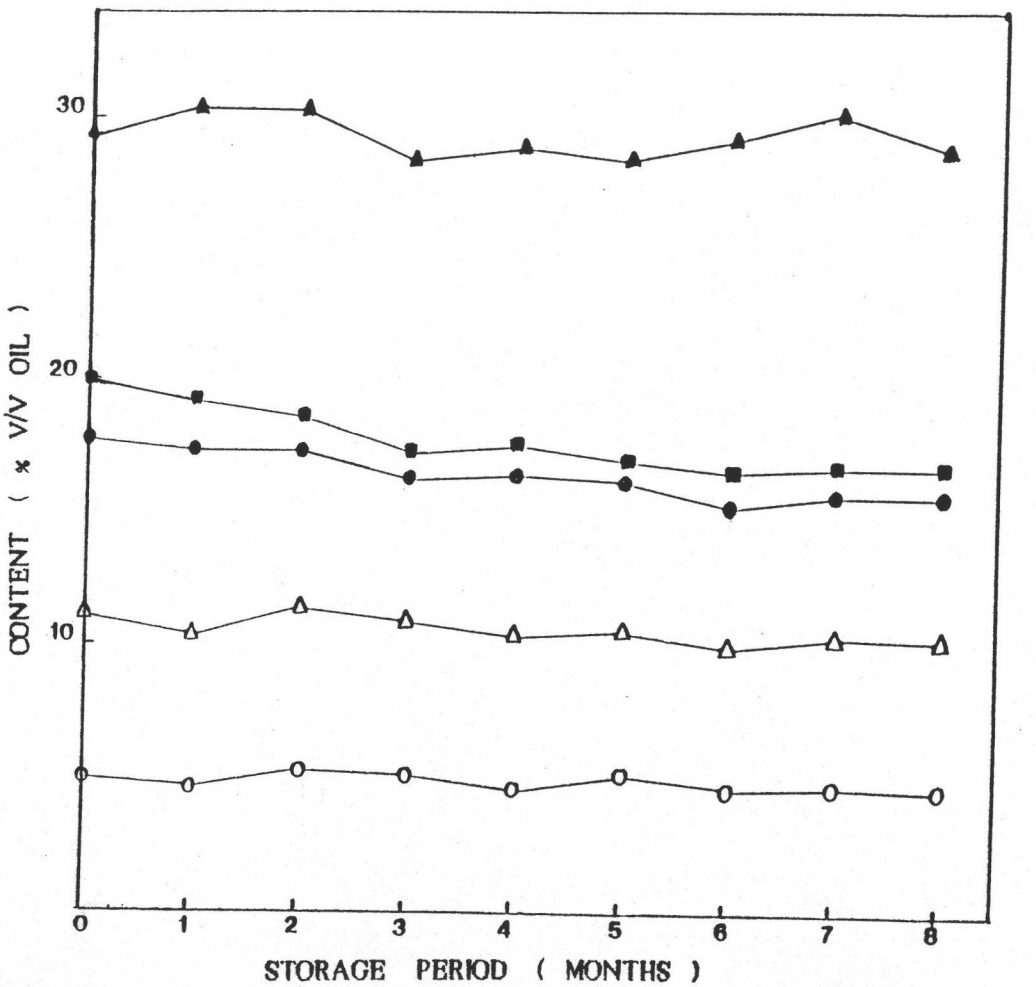


Figure 23 Changes in the contents of major components in pepper oils that were extracted from the stored black pepper at room temperature for a 8-month period. α-Pinene (O——O), β-pinene (Δ——Δ), Δ^3-carene (●——●), limonene (■——■) and β-caryophyllene (▲——▲).

7.2 Stored pepper oil

7.2.1 Changes in volatile oil composition

Samples of pepper oil were kept at both room temperature and 4°C in tight containers in order to study changes in volatile components in pepper oil during storage. As shown in Table 17 and Fig. 24, the proportion of total monoterpene hydrocarbons in pepper oil at 4°C was decreased from 52.31% at the first month to 47.37% at the eighth month of storage. The decrease was contributed by every component of the monoterpene hydrocarbons, especially terpinolene which decreased to an undetectable amount by 5 months of storage. Also, the proportion of total sesquiterpene hydrocarbons also decreased from 45.90% at the first month to 42.72% at the eighth month of storage which resulted from the decrease of each component of sesquiterpene hydrocarbons. On the other hand, the proportion of total oxygenated compounds was found to increase continuously from 1.56% at the first month to 6.09% at the eighth month of storage. The increase could be observed clearly at the third month of storage in which a number of oxygenated compounds were increased (Table 17). Among the oxygenated compounds, linalool, *trans*-nerolidol and torreyol were found to increase first followed by caryophyllene oxide, spathulenol and *tau*-muurolol which were found to increase after three months of storage. Therefore, at 4°C, it appeared that there was a relative decrease in the components of monoterpene and sesquiterpene hydrocarbons but increase in the proportion of oxygenated compounds.

At room temperature (Table 18 and Fig. 25), it was found that the proportion of total monoterpene hydrocarbons decreased more than 50% from 49.24% at the first month to 23.97% at the eighth month of storage. Particularly, camphene, *p*-cymene and terpinolene were found to decrease to an undetectable amount at 5 months of storage and α-phellandrene was undectectable at 6 months of storage. Similarly, The proportion of total sesquiterpene hydrocarbons also decreased from 48.34% at the first month to 31.89% at the eighth month of storage. This was due to the decrease of every sesquiterpenes, especiaally α-muurolene which decreased to an undetectable amount at 5 months of storage. On the other hand, the level of total oxygenated compounds was found to dramatically increase from 1.64% at the first month to 25.96% at the eighth month of storage. This increase was resulted from the rise of every oxygenated component, particularly, caryophyllene oxide, spathulenol and *tau*-muurolol which were undetectable in the fresh pepper oil. The sharp increase of this group was observed after 3 months of storage, especially caryophyllene oxide which is present up to 22.04% at 8 months of storage. This result indicate that monoterpene and sesquiterpene hydrocarbons in pepper oil are much more unstable at room temperature (Fig. 25) than at 4°C (Fig. 24), although it seens that no significant difference in the oil compositions during the first two months of storage under the two different temperatures.

Table 17 Composition of pepper oil during storage at 4°C (% relative*)

Constituent	Storage period (months)								
	0	1	2	3	4	5	6	7	8
Monoterpene hydrocarbons									
α-Pinene	6.51	6.45	6.36	6.26	6.48	6.40	6.38	6.69	6.35
Camphene	0.17	0.16	0.17	0.166	0.17	0.17	0.18	0.17	0.17
β-Pinene	7.96	7.95	7.92	7.89	7.95	7.96	7.90	8.19	7.68
Sabinene	2.50	2.57	2.44	2.47	2.46	2.46	2.46	2.60	2.46
Δ^3-Carene	13.13	13.04	13.02	12.96	13.03	12.82	12.65	12.63	11.85
Myrcene	2.01	2.08	2.06	2.00	1.96	1.93	1.90	1.89	1.75
α-Phellandrene	2.35	2.34	2.30	2.26	2.23	1.73	1.40	1.27	0.75
Limonene	16.71	16.70	16.67	16.65	16.38	16.70	16.40	16.73	16.16
p -Cymene	0.40	0.37	0.35	0.35	0.37	0.34	0.30	0.13	0.20
trans -Ocimene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
γ-Terpinene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
Terpinolene	0.54	0.54	0.54	0.52	0.52	tr**	tr**	tr**	tr**
Total monoterpenes	**52.31**	**52.20**	**51.83**	**51.52**	**51.55**	**50.55**	**49.57**	**50.30**	**47.37**
Sesquiterpene hydrocarbons									
α-Cubebene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
δ-Elemene	1.10	1.04	1.03	0.93	0.91	0.82	0.73	0.61	0.09
α-Copaene	3.20	3.16	3.13	3.02	3.17	3.33	3.38	3.21	3.38
β-Cubebene	0.39	0.37	0.38	0.40	0.38	0.39	0.38	0.35	0.37
α-Gurjunene	0.18	0.16	0.13	0.14	0.16	0.19	0.18	0.18	0.18
β-Caryophyllene	36.92	36.50	35.95	36.66	35.98	37.06	36.72	34.67	35.06
α-Humulene	1.75	1.74	1.64	1.70	1.66	1.72	1.72	1.60	1.63
Germacrene D	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
α-Muurolene	0.24	0.21	0.24	0.23	0.26	0.22	0.20	0.20	0.16
γ-Cadinene	0.62	0.60	0.61	0.62	0.58	0.60	0.60	0.58	0.41
Germacrene B	0.38	0.36	0.35	0.36	0.34	0.28	0.24	0.20	0.22
δ-Cadinene	1.12	1.10	1.05	1.08	1.07	1.06	1.01	0.86	0.62
Total sesquiterpenes	**45.90**	**45.24**	**44.51**	**45.14**	**44.51**	**45.67**	**45.16**	**42.46**	**42.72**
Oxygenated compounds									
Linalool	0.48	0.54	0.72	0.75	0.75	0.83	0.93	1.11	1.22
trans -Nerolidol	0.55	0.54	0.53	0.50	0.49	0.52	0.54	0.53	0.56
Caryophyllene oxide	tr**	tr**	tr**	0.60	0.56	1.04	1.46	2.39	3.06
Spathulenol	tr**	tr**	tr**	0.17	0.14	0.17	0.20	0.22	0.26
tau -Muurolol	tr**	tr**	tr**	0.20	0.20	0.19	0.18	0.14	0.22
Torreyol	0.53	0.57	0.61	0.68	0.66	0.72	0.73	0.71	0.77
Total oxygenated compounds	**1.56**	**1.65**	**1.86**	**2.90**	**2.80**	**3.47**	**4.03**	**5.10**	**6.09**
Unknown fractions	0.23	0.91	1.80	0.44	1.14	0.31	1.24	2.14	3.82

*% reletive=% integated area

**tr=Trace

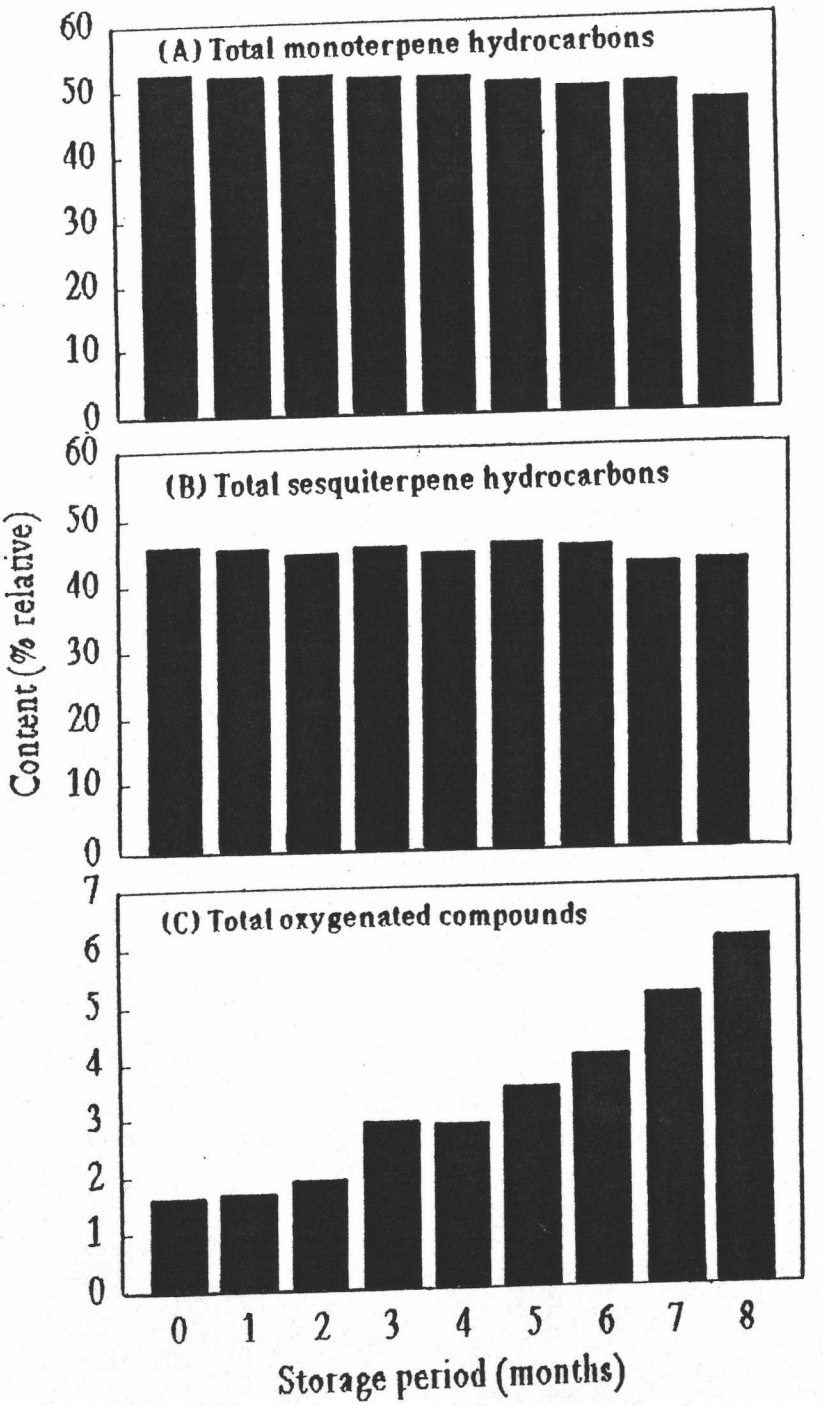


Figure 24 Relative changes in the contents of total monoterpenes (A), total sesquiterpenes (B) and total oxygenated compounds (C) in the stored pepper oil at 4 °C for an 8-month period.

Table 18 Composition of pepper oil during storage at room temperature (% relative*)

Constituent	Storage period (months)								
	0	1	2	3	4	5	6	7	8
Monoterpene hydrocarbons									
α-Pinene	6.01	5.94	5.90	5.86	5.92	5.67	4.97	3.34	2.85
Camphene	0.18	0.18	0.17	0.16	0.16	tr**	tr**	tr**	tr**
β-Pinene	7.75	7.74	7.70	7.64	7.64	7.59	6.98	5.36	4.02
Sabinene	2.32	2.31	2.32	2.30	2.27	2.22	1.98	1.53	0.98
Δ^3-Carene	12.82	12.79	12.77	12.54	12.06	11.83	10.03	7.53	6.01
Myrcene	1.54	1.53	1.50	1.48	1.29	0.87	0.44	0.16	0.09
α-Phellandrene	1.77	1.74	1.71	1.66	1.08	0.42	tr**	tr**	tr**
Limonene	16.15	16.10	16.02	16.16	15.49	14.18	12.91	11.46	10.02
p -Cymene	0.23	0.21	0.21	0.20	0.20	tr**	tr**	tr**	tr**
trans -Ocimene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
γ-Terpinene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
Terpinolene	0.47	0.44	0.41	0.38	0.36	tr**	tr**	tr**	tr*
Total monoterpenes	**49.24**	**48.98**	**48.71**	**48.38**	**46.47**	**42.78**	**37.31**	**29.38**	**23.97**
Sesquiterpene hydrocarbons									
α-Cubebene	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
δ-Elemene	1.13	1.10	1.04	0.94	0.88	0.94	0.98	0.99	0.92
α-Copaene	3.48	3.51	3.43	3.46	3.53	3.96	4.22	4.59	4.19
β-Cubebene	0.46	0.46	0.42	0.41	0.42	0.45	0.47	0.46	0.46
α-Gurjunene	0.25	0.23	0.20	0.15	0.22	0.25	0.26	0.19	0.15
β-Caryophyllene	38.67	38.48	38.04	37.82	36.82	37.34	36.43	30.33	24.24
α-Humulene	1.84	1.81	1.78	1.79	1.75	1.86	1.85	1.69	1.04
Germacrene D	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**	tr**
α-Muurolene	0.28	0.26	0.25	0.23	0.18	tr*	tr**	tr**	tr**
γ-Cadinene	0.68	0.68	0.62	0.60	0.66	0.62	0.71	0.58	0.42
Germacrene B	0.35	0.38	0.30	0.28	0.21	0.18	0.20	0.19	0.10
δ-Cadinene	1.20	1.18	1.15	1.13	1.08	1.03	0.87	0.53	0.37
Total sesquiterpenes	**48.34**	**48.09**	**47.23**	**46.81**	**45.75**	**46.63**	**45.99**	**39.75**	**31.89**
Oxygenated compounds									
Linalool	0.58	0.61	0.72	0.85	1.09	1.09	1.12	1.04	0.96
trans -Nerolidol	0.50	0.51	0.53	0.53	0.56	0.64	0.70	0.75	0.84
Caryophyllene oxide	tr**	tr**	tr**	1.48	2.36	4.72	8.12	16.44	22.04
Spathulenol	tr**	tr**	tr**	0.20	0.20	0.23	0.25	0.28	0.19
tau -Muurolol	tr**	tr**	tr**	0.21	0.22	0.20	0.21	0.21	0.25
Torreyol	0.56	0.64	0.70	0.74	0.76	0.98	1.19	1.19	1.68
Total oxygenated compounds	**1.64**	**1.76**	**1.95**	**4.01**	**5.19**	**7.86**	**11.59**	**19.91**	**25.96**
Unknown fractions	0.78	1.17	2.11	0.80	2.59	2.73	5.11	10.96	18.18

*% relative= % integated area

**tr=Trace

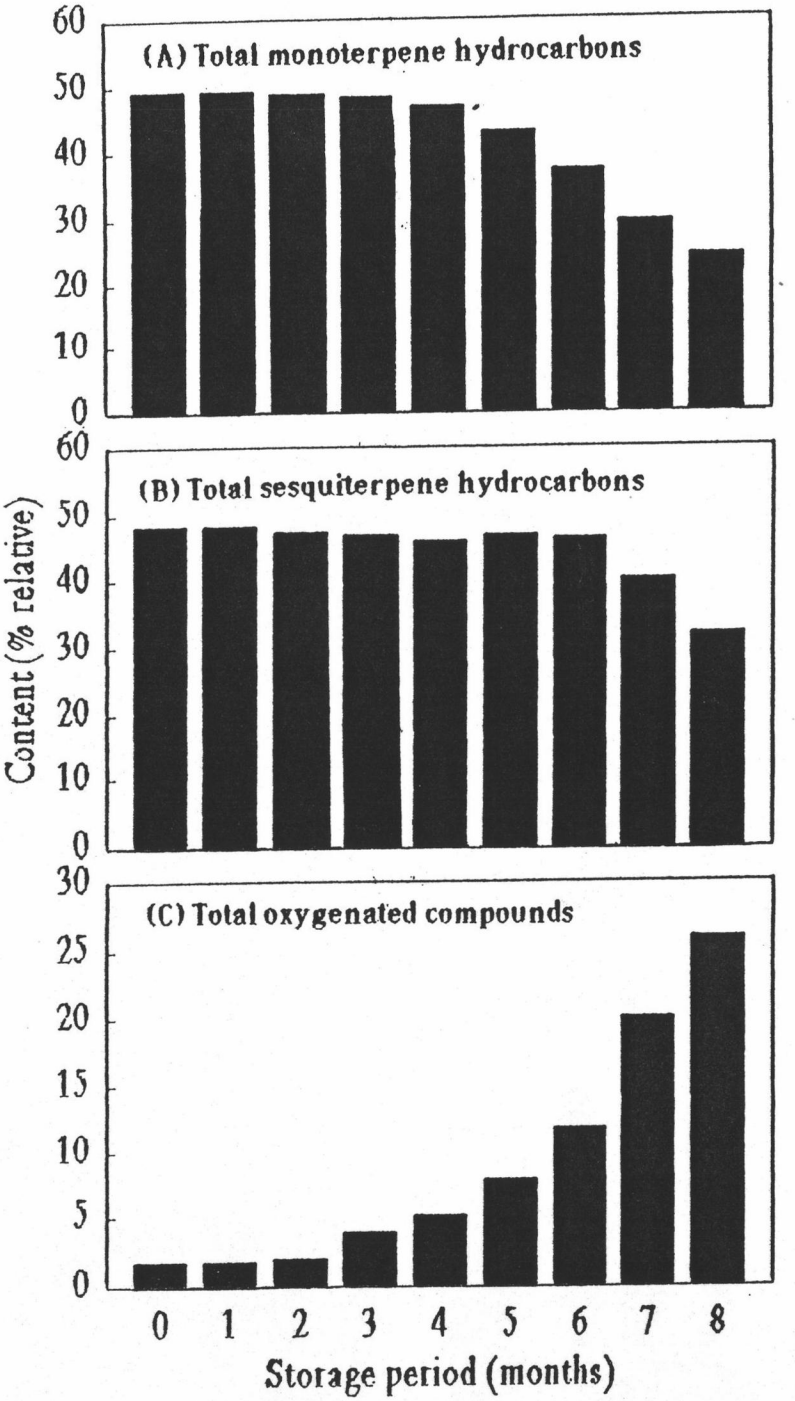


Figure 25 Relative changes in the contents of total monoterpenes (A), total sesquiterpenes (B) and total oxygenated compounds (C) in the stored pepper oil at room temperature for an 8-month period.

7.2.2 Changes in the content of major oil components

In term of major oil components, it was found that their content also disappear with faster rate at room temperature than at 4°C (Fig. 26). The levels of α-pinene, β-pinene, limonene, Δ^3-carene, sabinene and β-caryophyllene during the storage at 4°C were found to slightly decrease while those at room temperature decreased markedly. At 4°C, the major oil components that were found to be decreased in the range 6-15% included sabinene changed 6% and Δ^3-carene changed 15% at 8 months of storage. At room temperature, the major oil components that were found to be decreased in range 49.74% included β-caryophyllene changed 49% and α-pinene changed up to 74% at 8 months of storage. These results also showed that the content of the major oil components changed at room temperature markedly after 3 months of storage.

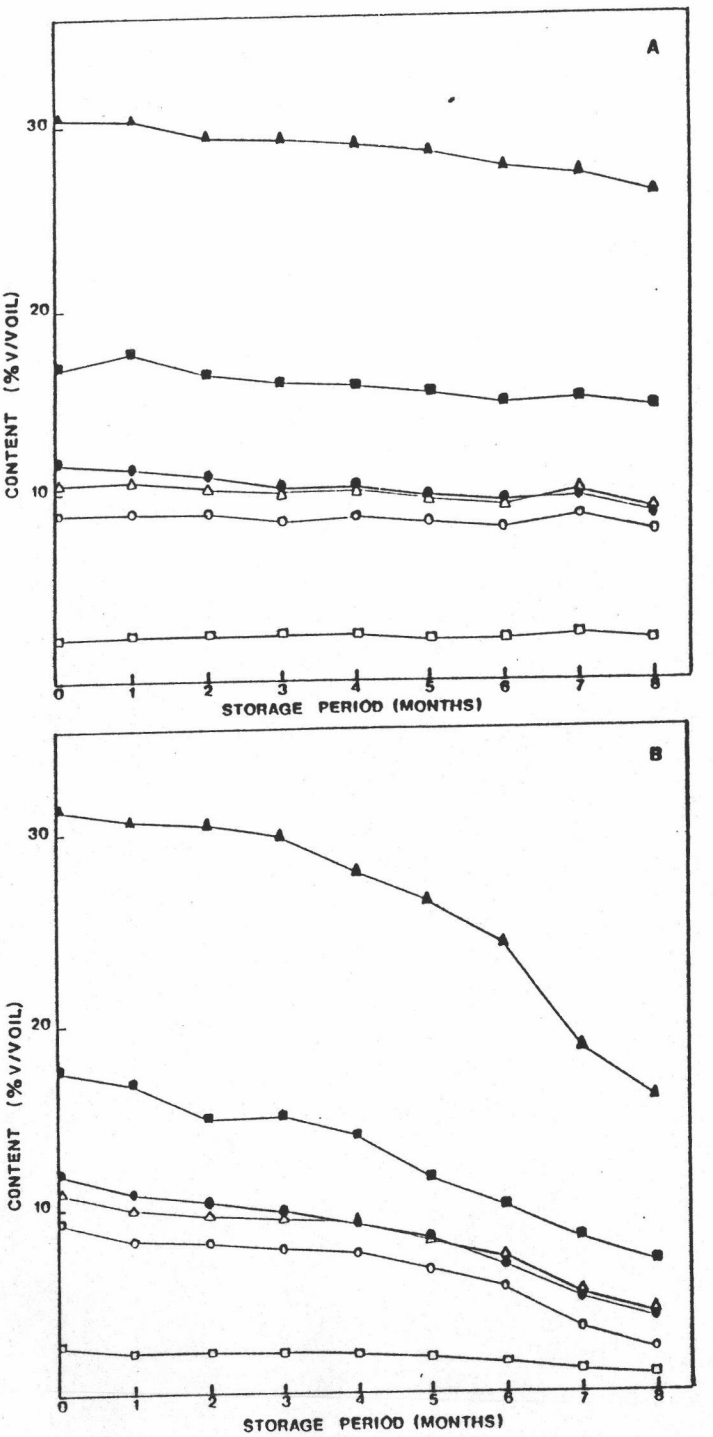


Figure 26 Changes in the contents of major components in pepper oils stored at 4 °C (A) and room temperature (B) for an 8-month period. α-Pinene (o——o), β-pinene (△——△), sabinene (□——□), Δ^3-carene (●——●), limonene (■——■) and β-caryophyllene (▲——▲).