CHAPTER IV

RESULTS

1. Determination of Curcuminoids in *Curcuma longa*

1.1 Separation of Curcuminoids by TLC

There have been a few reports (Sasri, 1981., Stahl, 1983., Wagner, Bladt and Zgainski, 1984) on the TLC systems using silica gel plates to separate the three curcuminoid components in turmeric rhizomes. The solvents used in these works were highly different. These are,for example, n-propanol ethyl acetate-water (40:40:30) (Wagner *et al.*, 1984), benzene-methanol (90:10) (Sasri, 1981) and chloroform-benzene-95% ethanol (45:45:10) (Stahl, 1973). Attempts to repeat these TLC-systems to serve our research purpose were not fully successful owing to either their incomplete separation of the curcuminoids or their causing tailing spots of the compounds. Therefore, an improvement to obtain a better TLC system for complete separation of the curcuminoids was carried out. By careful manipulation of various solvent systems, it was found that the composition of chloroform- benzene-methanol with the ratio 80:15:5 was most suitable. Under these conditions, the silica gel plate showed maximal separation of the three curcuminoids,each of which showed a very nice

round-shape spot. The Rf values of the yellowish curcuminoids were found to be 0.65, 0.45 and 0.33. Identification of each spot was carried out as described in the next section.

1.2 Identification of Each Curcuminoid by ^{1}H NMR

Since no single pure curcumin,demethoxycurcumin and bisdemethoxycurcumin were commercially available, preparation of each curcuminoid in pure form was carried out. This was done by extraction crude curcuminoids from turmeric samples and the extracts were purified by preparative thin-layer chromatography using chloroform : benzene : methanol, 80:15:5 as a solvent system. The resulted purified yellow crystalline of each curcuminoid was then confirmed by ^{1}H NMR (200 MHz). It was found that ^{1}HNMR in CD_3OD (Fig. 15) of the three curcuminoids showed clear difference in proton NMR signals. ^{1}HNMR of curcumin showed signal for one singlet at 3.91 ppm (6H of OCH_3) (Fig.15A),demethoxycurcumin showed one singlet at 3.91 ppm (3H of OCH_3) (Fig.15B), and bisdemethoxycurcumin showed no signal at 3.91 ppm (Fig. 15C). These ^{1}HNMR data were corresponded with those described in section 7.1 of Structure and Chemical Properties of Curcuminoid. Therefore, it was clear that the curcuminoids on TLC plates with the Rf values of 0.65, 0.45 and 0.33 were corresponded to curcumin, demethoxycurcumin and bisdemethoxycurcumin, respectively.

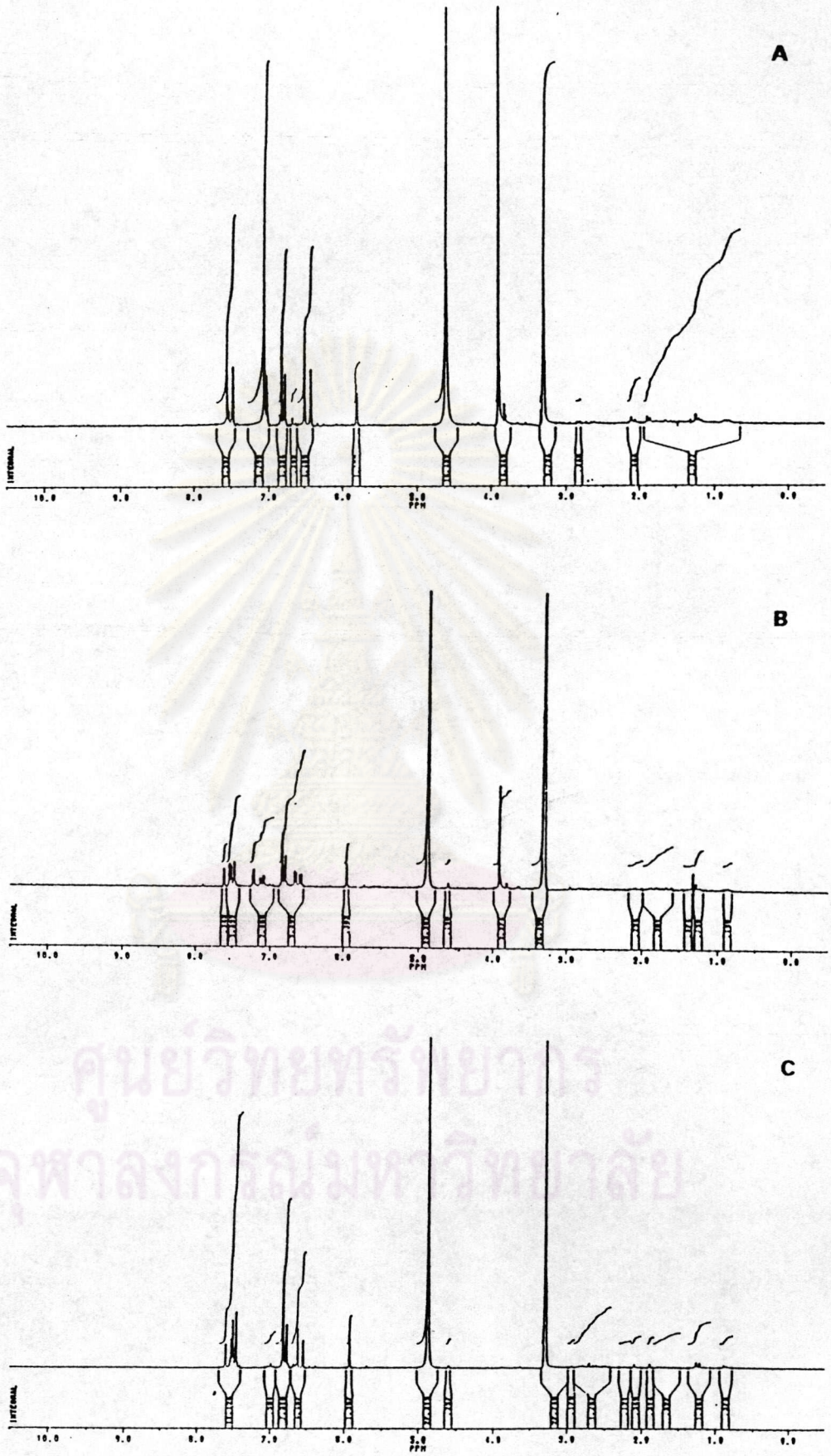


Fig. 15 ^{1}H NMR spectra (200 MHz) of curcumin (A), demethoxycurcumim (B) and bisdemethoxycurcumin (c).

1.3 TLC-Densitometric Determination of Curcuminoids

The complete separation of the three curcuminoids by TLC allowed us to quantitate each of the components by densitometry. This method is simple, rapid and allows the three curcuminoids be quantitated simultaneously. To establish this method, an appropiate wavelength to be used for scanning must be first selected. From the absorption spectrum of each curcuminoid (Fig. 16), it was found that curcumin, demethoxycurcumin and bisdemethoxycurcumin all showed very similar patterns with their λ max at around 420 nm. This wavelength was threfore chosen for performing quantitative analysis of the compounds. Figure 17 shows a typical TLC-densitometric chromatogram of a turmeric methanolic extract which was separated on a silica gel plate. It can be seen that the three symmetric peaks of curcuminoids are very well separated from one another under the established conditions. The area integrated under each peak should, therefore, be closely related to the concentration of compound.

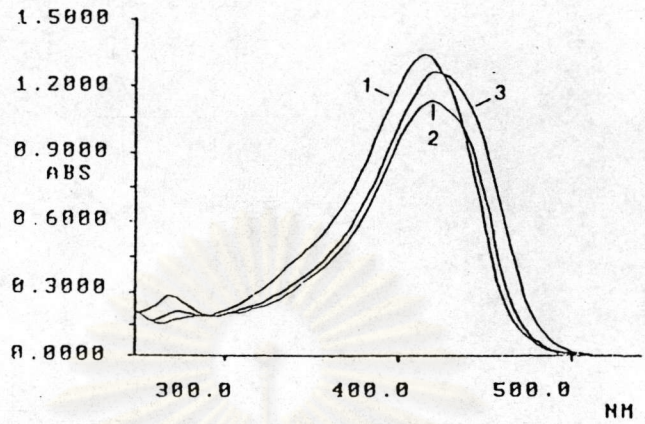


Fig. 16 Absorption spectra of the three curcuminoids (in methanol) by UV-vis spectrophotometer. 1 = bisdemethoxycurcumin, 2 = demethoxycurcumin and 3 = curcumin

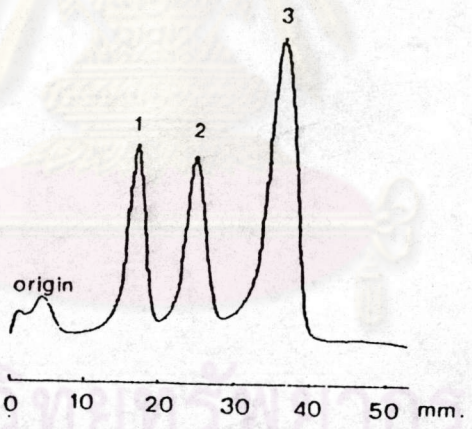


Fig. 17 TLC-chromatogram of the three curcuminoids in methanolic extract of turmeric. 1 = bisdemethoxycurcumin, 2 = demethoxycurcumin and 3 = curcumin

1.4 Calibration Curves

The standard curves of the three curcuminoids which were obtained from the TLC-densitometric method are shown in Fig.18. Each curve showed linearity of the relationship between the concentration ranges of 0.2 and 1.5 μg of each curcuminoid with the detection limit at 0.1 μg. Results of the regression analysis and the correlation coefficients (r) were found to be 0.996 for curcumin, 0.999 for demethoxycurcumin and 0.995 for bisdemethoxycurcumin.

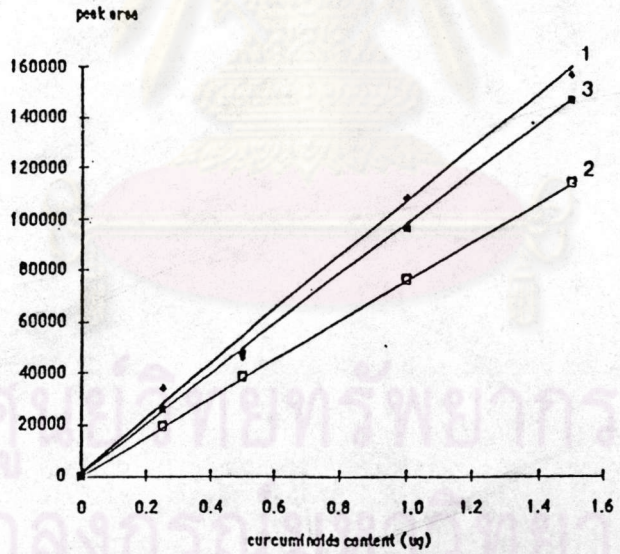


Fig. 18 Standard curves of the three curcuminoids based on TLC-densitometric method. 1 = bisdemethoxycurcumin, 2 = demethoxycurcumin and 3 = curcumin.

1.5 Sample Preparation

To ensure complete extraction of curcuminoids from the turmeric rhizome, various extraction methods, including soxhlet, reflux, maceration and sonication were investigated. In all methods, methanol was used as extracting solvent because of its high solubility for the curcuminoids. In this study, the proportion of turmeric powder and the solvent was first optimized. It was found that the ratio of 2:1 (e.g. 20 mg sample in 10 ml methanol) gave the highest curcuminoid content after one-hour reflux. When compared among various extraction methods using this sample-solvent ratio, it was found that the methods of soxhlet (6 hours), reflux (1 hour), sonication (1 hour) and maceration (overnight) all gave very similar total curcuminoid content (approx. 11% dry weight). Since the sonication is relatively convenient, rapid and allows a large number of samples to be handled, we chose this method for quantitative extraction of the curcuminoids.

1.6 Accuracy, Precision and Reproducibility

In order to evaluate its accuracy and precision, the developed TLC-densitometry was compared with the spectrophotometric method (International Organization for Standardization, 1982) which could determine the total curcuminoid content in tumeric rhizome. In doing this, a number of turmeric sample of various

sources were analysed for their total curcuminoid content by the two methods and the results obtained were compared. It can be seen in Table 4 that the values of total curcuminoid content of various turmeric samples which were determined by TLC-densitometry (the summation the three curcuminoid component) were very closed to those obtained from the spectrophotometric method. The percentage of CV was found to be 3.34. In term of precision, an at least three separate determination of each turmeric sample showed a very narrow value of standard deviation (%SD = 1.73) (Table 4). These results indicate that the accuracy and precision of the TLC-densitometric method in determining the content of individual and total curcuminoid content are reliable.

Table 4. Percentage of each curcuminoid in turmeric rhizome and the percentage of total curcuminoid content obtained from both the TLC-desitometric and UV-spectrophotometric methods.

Turmeric sample*	Origin (province)	TLC-densitometric method				UV-spectrophotometric** Total curcuminnoids (% w/w)
		curcumin (% w/w)	demethoxycurcumin (% w/w)	bisdemethoxycurcumin (% w/w)	Total curcuminoids (% w/w)	
1. Y 01128601	Phitsanulok	4.79±0.08	2.63±0.21	1.95±0.03	9.38±0.14	9.04±0.16
2. Y 01038801	Tak	7.52±0.78	4.66±0.51	4.29±0.15	16.46±1.45	16.70±0.10
3. Y 13038801	Chaiyaphum	6.26±0.19	3.66±0.02	2.64±0.07	12.56±0.25	12.10±0.05
4. Y 01038701	Chiang Rai	3.55±0.01	2.37±0.06	1.26±0.04	7.18±0.10	7.18±0.11
5. Y 12058704	Trang	3.48±0.17	1.81±0.02	1.51±0.02	6.78±0.16	7.21±0.07
6. Y 21038701	Chiang Mai	3.74±0.02	1.44±0.02	1.04±0.03	6.23±0.03	5.91±0.32
7. Y 17068801	Roi Et	4.59±0.12	3.12±0.02	3.04±0.13	10.75±0.22	10.99±0.15
8. Y 15038802	Loei	4.95±0.01	3.90±0.00	2.91±0.03	11.76±0.01	11.83±0.10
9. Y 27058703	Ratchaburi	6.85±0.01	3.72±0.07	3.88±0.15	14.46±0.21	13.77±0.09
10. Y 26038801	Chai Nat	4.38±0.08	2.41±0.04	2.80±0.16	9.59±0.28	9.98±0.03

*Samples 1-3 were obtained from the Trang Horticultural Research. Sample 4-7 were from the Phichit Horticultural Research Center and Samples 8-10 were from the Tak Horticutural Research Center.

In term of reproducibility, the developed TLC-densitometry was carried out to quantitate the individual curcuminoid in each sample with two separate determination. It was found that average value of each curcuminoid content of this duplicate determination gave very similarly and the average percentage of standard deviations showed very narrow value (%SD = 3.33) (Table 5).

Table 5 Percentage of each and total curcuminoid in turmeric sample by duplicate TLC-densitometric determination.

turmeric sample	origin	curcuminoid content (% w/w)				% SD
		curcumin	demethoxy-curcumin	bisdemethoxy-curcumin	total curcuminoid	
1	Trang	6.06±0.06 6.45±0.25	3.68±0.12 3.65±0.14	2.57±0.10 2.71±0.13	12.30±0.26 12.81±0.53	3.98
2	Phichit	5.25±0.06 5.82±0.01	3.62±0.08 3.90±0.00	2.90±0.07 2.86±0.12	12.03±0.08 12.58±0.11	4.37
3	Tak	6.76±0.21 6.33±0.08	3.92±0.11 4.11±0.03	4.15±0.12 4.83±0.04	14.83±0.12 15.28±0.16	2.95
4	Tak	6.02±0.49 6.05±0.30	3.50±0.09 3.45±0.15	3.82±0.03 3.66±0.13	13.38±0.51 13.16±0.57	1.67
5	Trang	6.98±0.25 7.50±0.20	4.02±0.15 4.30±0.11	4.03±0.16 3.50±0.01	15.04±0.57 15.30±0.32	1.70
6	Phichit	4.99±0.10 4.59±0.12	2.78±0.02 3.12±0.02	2.40±0.04 3.04±0.13	10.18±0.16 10.75±0.22	5.30

2. Curcuminoids in *Curcuma longa*

2.1 Variation of Curcuminoid Content in Turmeric Rhizomes of Different Cultivars.

Turmeric rhizomes from more than thirty cultivars which were originally obtained from various provinces in Thailand were all regrown in Phichit, Trang and Tak experimental fields in order to be exposed with the same conditions in each area. After maturation, the rhizomes of all the cultivars were collected and subjected to quantitative analysis of curcuminoid content. It was found that these rhizomes of different cultivars contained highly variable curcuminoid contents. The turmeric samples from Phichit experimental field contained curcuminoid content in the range 0.81-14.24 %w/w (Table 18 in Appendix and Fig.19) from Trang in the range 0.48-16.46 %w/w (Table 19 in Appendix and Fig. 20) and from Tak in the range 1.30-15.37 %w/w (Table 20 in Appendix and Fig 21). The average total curcuminoid content (% w/w) calculated from all turmeric cultivars at each station was as follow : 7.29% w/w for Phichit, 8.66 %w/w for Trang and 9.60% w/w for Tak. In cases of individual curcuminoid contents, it was found that the average contents of curcumin, demethoxycurcumin and bisdemethoxycurcumin were as follows : 3.48, 2.08 and 1.73% w/w for Phichit; 4.21,

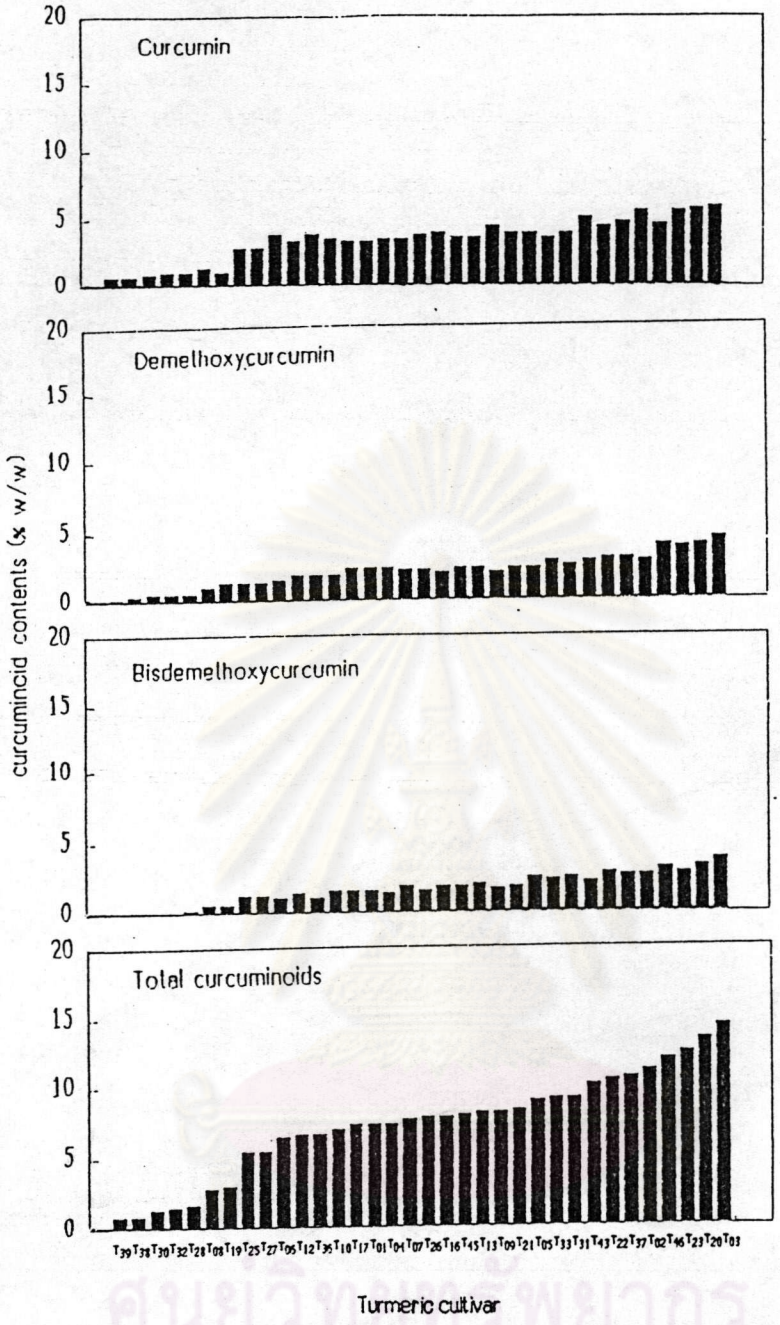


Fig.19 Variation in the contents of curcumin, demethoxycurcumin, bisdemethoxycurcumin and total curcuminoids in various turmeric rhizomes cultivated in Phichit. The rhizomes were originally obtained from different provinces in Thailand as follows :-

T_1 - Phitsanulok	T_{13} - Ratchaburi	T_{28} - Udon Thani
T_2 - Nakhon Si Thammarat	T_{16} - Indonesia	T_{30} - Sakon Nakhon
T_3 - Ranong	T_{17} - Chumphon	T_{31} - Nakhon Phanom
T_4 - Chiang Rai	T_{19} - Chiang Rai	T_{32} - Nakhon Phanom
T_5 - Chiang Rai	T_{20} - Phitsanulok	T_{33} - Chai Nat
T_6 - Chiang Mai	T_{21} - Chiang Mai	T_{36} - Malaysia
T_7 - Tak	T_{22} - Tak	T_{37} - Roi Et
T_8 - Tak	T_{23} - Chaiyaphum	T_{38} - Nong Khai
T_9 - Trang	T_{25} - Loei	T_{39} - Mukda Han
T_{10} - Trang	T_{26} - Loei	T_{43} - Bangladesh
T_{12} - Ratchaburi	T_{27} - Loei	T_{45} - Nepal
		T_{46} - Nepal

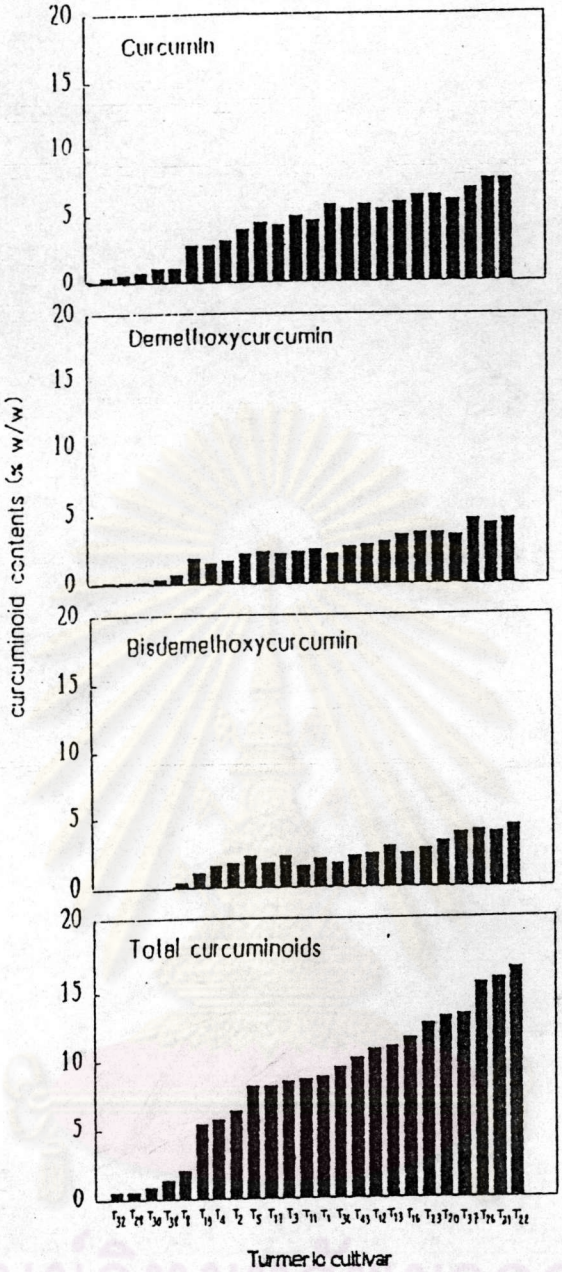


Fig. 20 Variation in the contents of curcumin, demethoxycurcumin, bisdemethoxycurcumin and total curcuminoids in various turmeric rhizomes cultivated in Trang. The rhizomes were originally obtained from different provinces in Thailand as follows :-

T_1 - Phitsanulok	T_{13} - Ratchaburi	T_{28} - Udon Thani
T_2 - Nakhon Si Thammarat	T_{16} - Indonesia	T_{30} - Sakon Nakhon
T_3 - Ranong	T_{17} - Chumphon	T_{31} - Nakhon Phanom
T_4 - Chiang Rai	T_{19} - Chiang Rai	T_{32} - Nakhon Phanom
T_5 - Chiang Rai	T_{20} - Phitsanulok	T_{36} - Malaysia
T_8 - Tak	T_{22} - Tak	T_{37} - Roi Et
T_{11} - Trang	T_{23} - Chaiyaphum	T_{38} - Nong Khai
T_{12} - Ratchaburi	T_{26} - Loei	T_{43} - Bangladesh

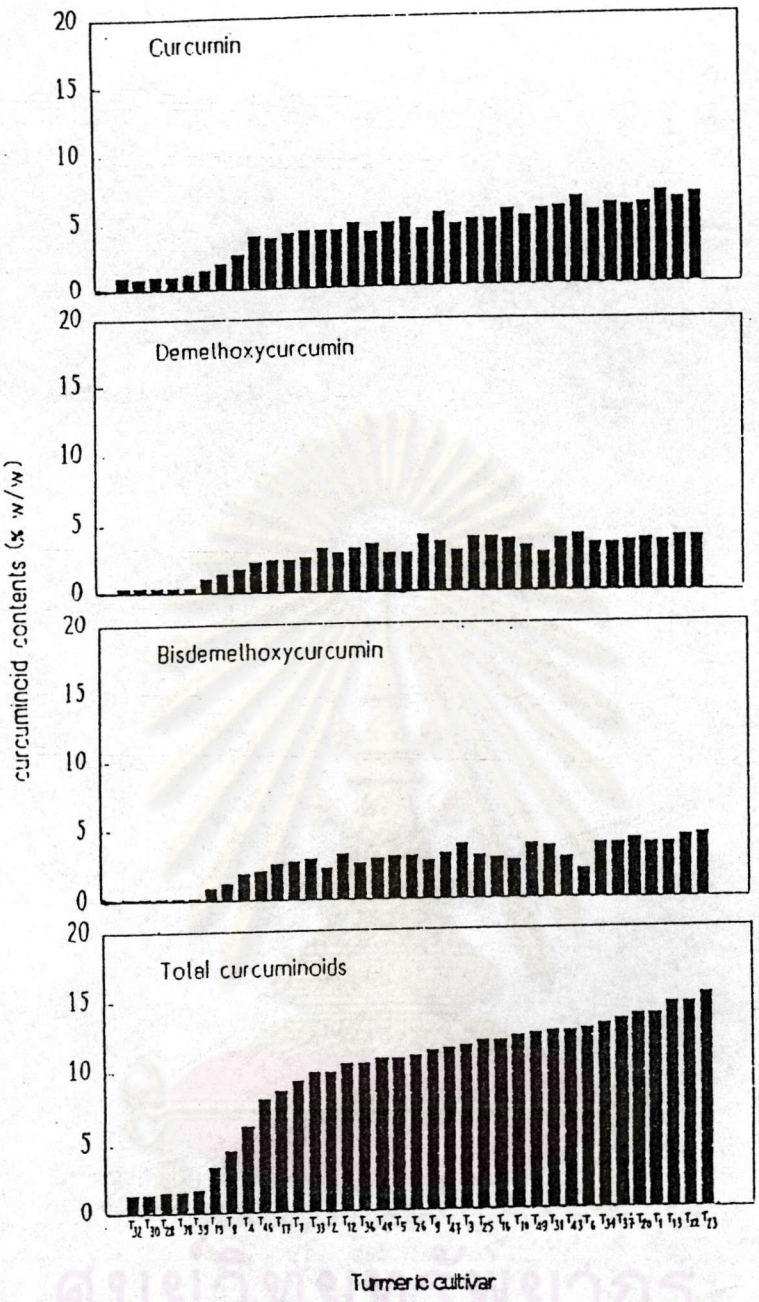
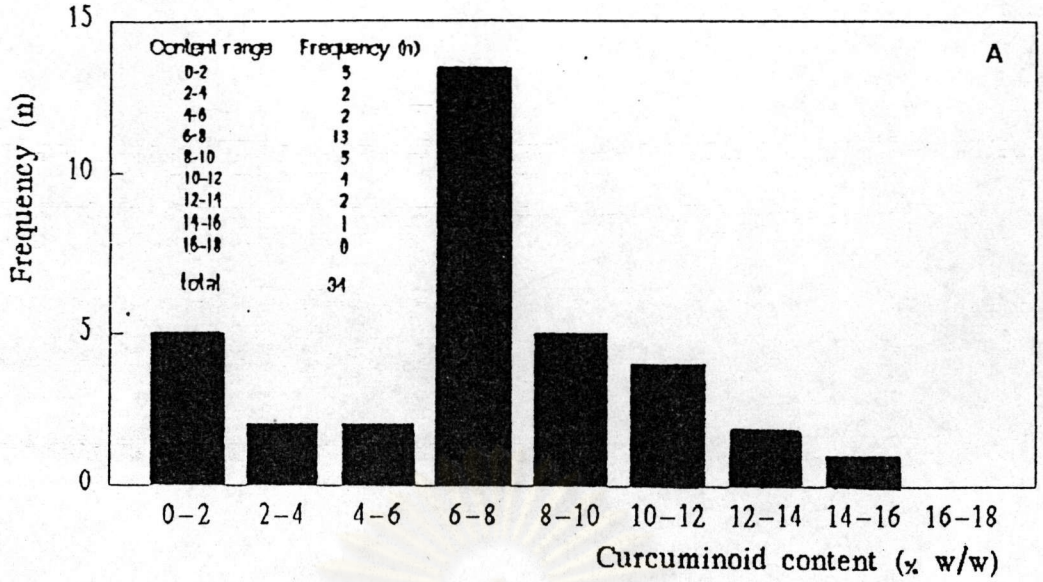
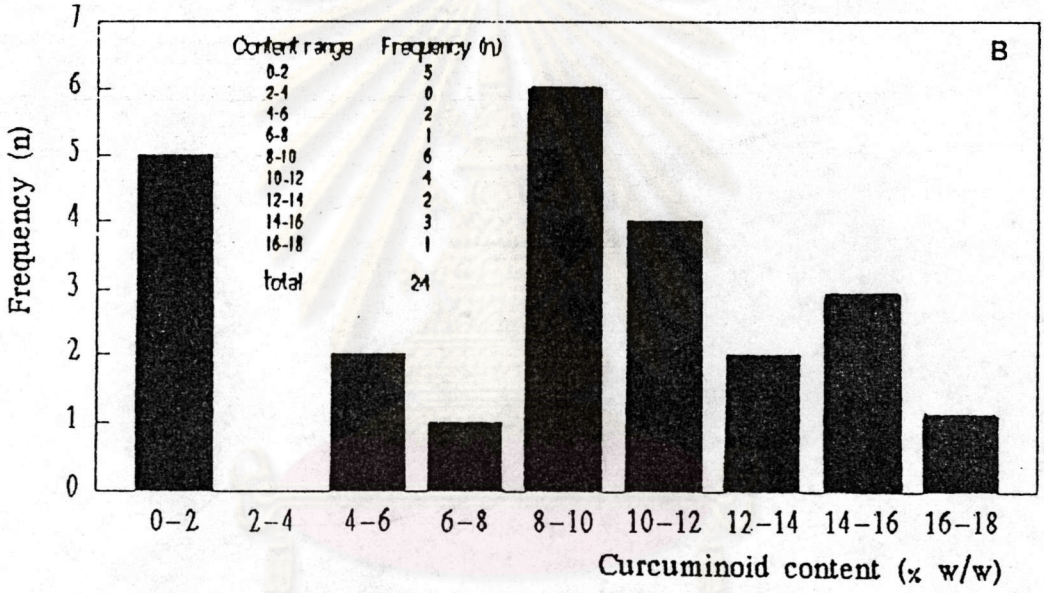


Fig.21 Variation in the contents of curcumin, demethoxycurcumin, bisdemethoxycurcumin and total curcuminoids in various turmeric rhizomes cultivated in Tak. The rhizomes were originally obtained from different provinces in Thailand as follows :-

T_1 - Phitsanulok	T_{16} - Indonesia	T_{33} - Chai Nat
T_2 - Nakhon Si Thammarat	T_{17} - Chumphon	T_{34} - Suphan Buri
T_3 - Ranong	T_{19} - Chiang Rai	T_{36} - Malaysia
T_4 - Chiang Rai	T_{20} - Phitsanulok	T_{37} - Roi Et
T_5 - Chiang Rai	T_{22} - Tak	T_{38} - Nong Khai
T_6 - Chiang Mai	T_{23} - Chaiyaphum	T_{39} - Mukda Han
T_7 - Tak	T_{25} - Loei	T_{43} - Bangladesh
T_8 - Tak	T_{26} - Loei	T_{46} - Nepal
T_9 - Trang	T_{28} - Udon Thani	T_{47} - Satun
T_{10} - Trang	T_{30} - Sakon Nakhon	T_{48} - Trang
T_{12} - Ratchaburi	T_{31} - Nakhon Phanom	T_{49} - Trang
T_{13} - Ratchaburi	T_{32} - Nakhon Phanom	





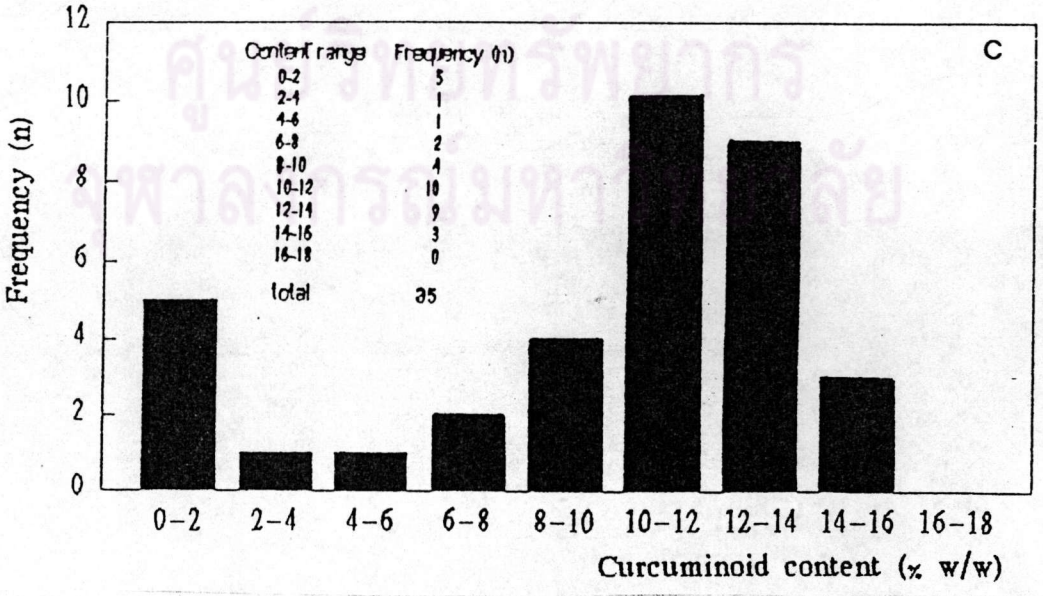


Fig. 22 Distribution of total curcuminoid contents in various turmeric rhizomes cultivated in Phichit (A), Trang (B) and Tak (C).

2.34 and 2.11 %w/w for Trang and 4.32, 2.73 and 2.55 %w/w for Tak, respectively.

Based on the total curcuminoid content, it was found that most of the turmeric cultivars contain curcuminoid content in the range 6-8% for those cultivated in Phichit, 8-10% for those in Trang and 10-12 % w/w for those in Tak (Fig. 22 A, B and C). Out of 34 samples of turmeric cultivated in Phichit, it was found that up to 13 samples contained 6-8 % w/w of the total curcuminoid content equivalent to 38.23% of the population (Fig.22A). In Trang experimental field, it was found that 6 out of 24 collected samples contained 8-10 %w/w of total curcuminoid content (25%) (Fig. 22B). For Tak experimental field, from which 35 samples of turmeric samples were collected, 10 samples were found to contain 10-12 % w/w (28.57%) and 9 samples contained 12-14 % w/w (25.71%) (see Fig 22C). Among these three experimental field turmeric sample from Tak appeared to contain significantly higher curcuminoid content than those from the other two sources.

When considering high curcuminoid-producing cultivars among the *C. longa* population grown in the three experimental fields, it was found that the cultivars T_{20}, T_{22}, T_{23}, T_{31} and T_{37} could consistently produce turmerics with high curcuminoid content. (Table 6). These

cultivars were originally obtained from Phitsanulok, Tak, Chaiyaphum, Nakhon Phanom and Roi Et, respectively, and were found to contain more than 10 % w/w curcuminoid in their dry roots when regrown in all the three experimental fields.

Table 6 Turmeric samples containing high curcuminoid contents.

Turmeric cultivar	Origin	Total curcuminoid content (% w/w)		
		Experimental field		
		Phichit	Trang	Tak
T_{20}	Phitsanulok	13.20±1.31	13.06±0.76	13.61±0.05
T_{22}	Tak	10.21±0.10	16.46±1.45	14.52±0.85
T_{23}	Chaiyaphum	12.31±0.27	12.56±0.25	15.06±0.22
T_{31}	Nakhon Phanom	9.04±1.04	15.71±1.54	12.38±0.64
T_{37}	Roi Et	10.44±0.31	15.17±0.13	13.27±0.11

When considering low curcuminoid-producing cultivars, it was found that T_{38} (Nong Khai), T_{32} (Nakhon Phanom), T_{30} (Sakon Nakhon), T_{28} (Udon Thani) and T_8 (Tak) were the cases(see Table 7). These cultivars produced low curcuminoid-containing turmeric (less than 4.5% w/w) in all the three experimental fields.

Table 7 Turmeric samples containing low curcuminoid contents

Turmeric cultivar	Origin	Total curcuminoid content (% w/w)		
		Experimental field		
		Phichit	Trang	Tak
T_{38}	Nong Khai	0.82±0.04	1.33±0.13	1.55±0.05
T_{32}	Nakhon Phanom	1.50±0.09	0.48±0.09	1.30±0.12
T_{30}	Sakon Nakhon	1.30±0.13	0.86±0.09	1.36±0.13
T_{28}	Udon Thani	1.58±0.07	0.55±0.02	1.49±0.02
T_8	Tak	2.85±0.15	1.98±0.15	4.48±0.005

In addition, the turmeric cultivars which produced highly variable curcuminoid content among the three experimental fields were also considered. It was found that T_1 (Phitsanulok), T_{12} (Ratchaburi), T_{16} (Indonesia), T_{22} (Tak), T_{26} (Loei) and T_{31} (Nakhon Phanom) cultivars belonged to this group. These cultivars when were regrown in Tak and Trang contained higher curcuminoid content than regrown in Phichit for approximately 40-60% (Table 8).

Table 8 Turmeric samples containing highly variable curcuminoid contents

Turmeric cultivar	Origin	Total curcuminoid content (% w/w)		
		Experimental field		
		Phichit	Trang	Tak
T1	Phitsanulok	7.16±0.58	8.80±0.58	13.61±0.69
T12	Ratchaburi	6.44±0.17	10.74±0.43	10.32±0.14
T16	Indonesia	7.66±0.13	11.68±0.09	11.79±0.76
T22	Tak	10.21±0.10	16.46±1.45	14.52±0.85
T26	Loei	7.60±0.15	15.50±0.30	10.73±0.61
T31	Nakhon Phanom	9.04±1.04	15.71±1.54	12.38±0.64

Finally, the group with very low variable curcuminoid contents were found to be T_{17} (Chumphon), T_{20} (Phitsanulok), T_{23} (Chaiyaphum) and T_{43} (Bangladesh) cultivars. These cultivars had a variation in curcuminoid content only in the range 0-20% (Table 9).

Table 9 Turmeric samples containing low variable of curcuminoid contents

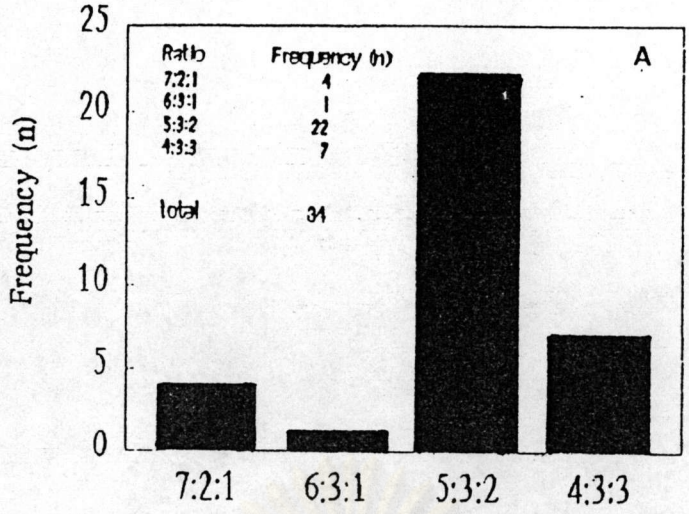
Turmeric cultivar	Origin	Total curcuminoid content (% w/w)		
		Experimental field		
		Phichit	Trang	Tak
T_{17}	Chumphon	7.14±0.02	8.20±0.05	8.52±0.24
T_{20}	Phitsanulok	13.20±1.31	13.06±0.76	13.61±0.55
T_{23}	Chaiyaphum	12.31±0.27	12.56±0.25	15.06±0.22
T_{43}	Bangladesh	9.96±0.27	10.04±0.33	12.53±0.21

2.2 Ratio of Curcumin : Demethoxycurcumin : Bisdemethoxycurcumin

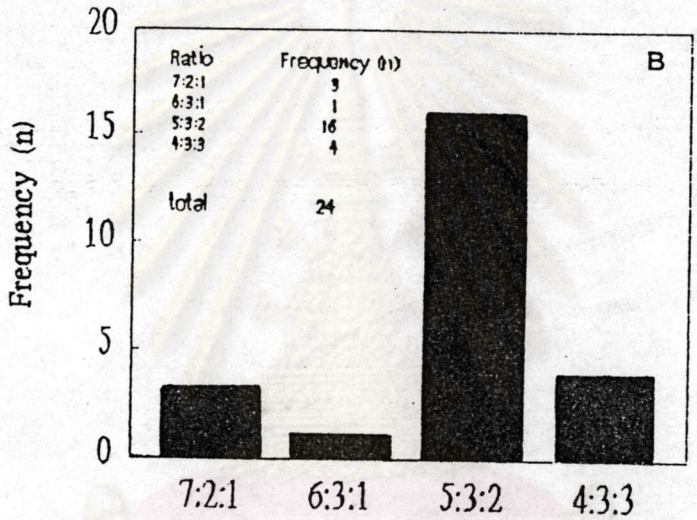
From the results on the content of individual curcuminoids, the ratio of the three components in each cultivar was evaluated. It was found that the ratio curcumin : demethoxycurcumin : bisdemethoxycurcumin was mostly 5:3:2 for turmeric rhizomes regrown in Phichit experimental field (Fig 23A). From 34 turmeric samples, up to 22 samples (64.71%) were found in this ratio. This was followed by the ratio 4:3:3 (20.59%), 7:2:1 (11.76%) and 6:3:1 (2.94%) respectively. Similarly,turmeric samples

regrown in Trang experimental field (Fig 23B) were also mostly found to contain curcuminoids in the ratio 5:3:2 (66.67%), followed by 4:3:3 (16.67%), 7:2:1 (12.50%) and 6:3:1 (4.17%) respectively. On the other hand, turmeric samples regrown in Tak experimental field were mostly found in the ratio of 4:3:3 (48.57%) followed by 5:3:2 (37.15%), 7:2:1 (11.43%) and 6:3:1 (2.86%) respectively (see Fig 23C).

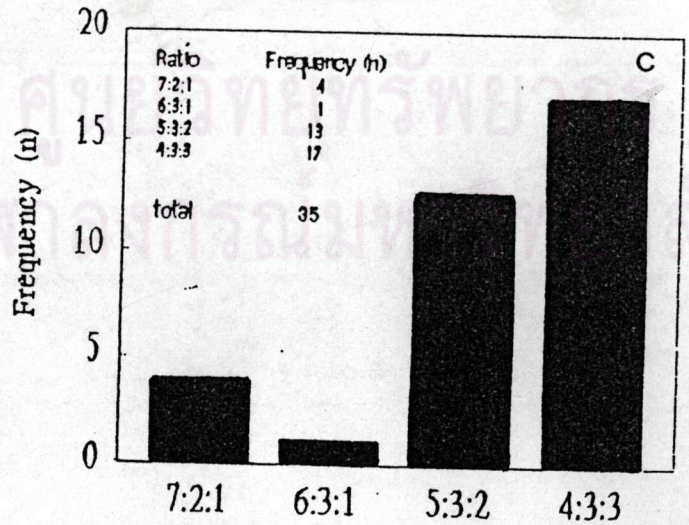
It should be noted that most turmeric samples with 4:3:3 ratio contained total curcuminoid content $\geq$ 8 %w/w while those with 5:3:2 ratio was found to contain $\geq$ 5 %w/w (Table 18, 19 and 20 in Appendix). For turmerics with 6:3:1 and 7:2:1 ratios, they both found to contain total curcuminoid content $\leq$ 5% w/w when regrown in the three experimental fields.



Ratio of curcumin : demethoxycurcumin : bisdemethoxycurcumin



Ratio of curcumin : demethoxycurcumin : bisdemethoxycurcumin



Ratio of curcumin : demethoxycurcumin : bisdemethoxycurcumin

Fig. 23 Ratio of the three curcuminoids in various turmeric rhizomes cultivated in Phichit (A), Trang (B) and Tak (C).

3. Volatile Oil in *Curcuma longa*

3.1 Variation of Volatile Oil Content in Various *Curcuma longa* Rhizomes

The dried rhizomes of *C. longa* obtained from various cultivars which were grown in Phichit, Trang and Tak experimental fields were analyzed for their volatile oil contents. It was found that various turmeric samples obtained from Phichit, Trang and Tak contained volatile oil in the range of 4.30-10.12, 4.16-10.86 and 5.34-16.74 %v/w respectively (Table 21 in Appendix and Fig. 24). In term of frequency, almost 50% the turmeric samples from Phichit (16 out of 34) and Trang (11 out of 24) were found to be in the range of 6-8% volatile oil content (Figs 24 A and B). From Tak, on the other hand, about half of the samples (17 out of 35) were in the higher content ranging from 10 to 14% (Fig. 24C). Therefore, it appeared that turmeric samples which regrown in Tak contain more volatile oil content than the samples regrown in Phichit and Trang. The frequency of distribution of the total oil content in various turmeric rhizomes are shown in Fig. 25.

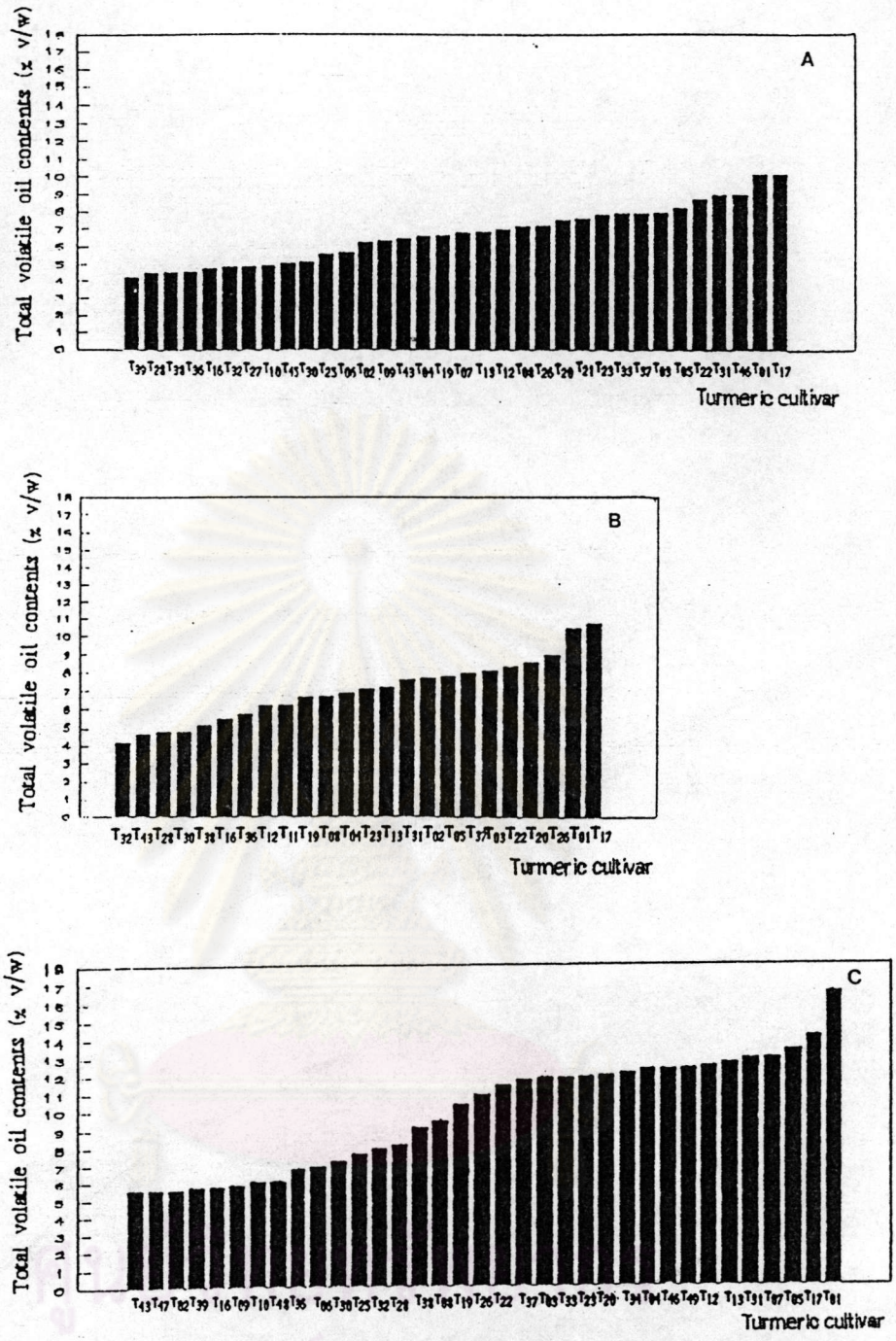


Fig.24 Variation of total volatile oil content in various turmeric rhizomes cultivated in Phichit (A), Trang (B) and Tak (C). The rhizomes were originally obtained from different provinces in Thailand as follows :-

T_1 - Phitsanulok	T_{16} - Indonesia	T_{33} - Chai Nat
T_2 - Nakhon Si Thammarat	T_{17} - Chumphon	T_{34} - Suphan Buri
T_3 - Ranong	T_{19} - Chiang Rai	T_{36} - Malaysia
T_4 - Chiang Rai	T_{20} - Phitsanulok	T_{37} - Roi Et
T_5 - Chiang Rai	T_{22} - Tak	T_{38} - Nong Khai
T_6 - Chiang Mai	T_{23} - Chaiyaphum	T_{39} - Mukda Han
T_7 - Tak	T_{25} - Loei	T_{43} - Bangladesh
T_8 - Tak	T_{26} - Loei	T_{46} - Nepal
T_9 - Trang	T_{28} - Udon Thani	T_{47} - Salun
T_{10} - Trang	T_{30} - Sakon Nakhon	T_{48} - Trang
T_{12} - Ratchaburi	T_{31} - Nakhon Phanom	T_{49} - Trang
T_{13} - Ratchaburi	T_{32} - Nakhon Phanom	

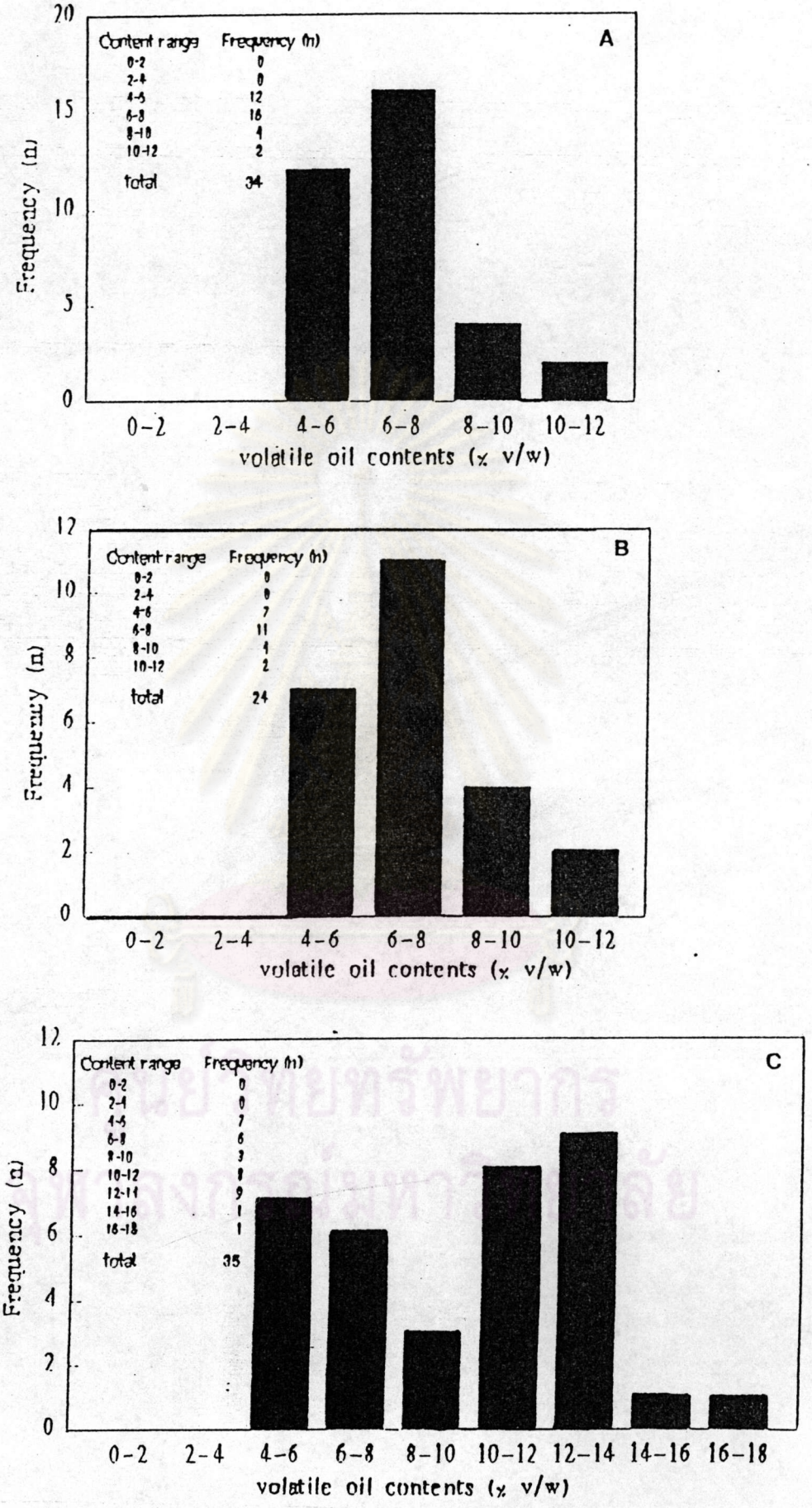


Fig. 25 Distribution of total volatile oil contents in various turmeric rhizomes cultivated in Phichit (A), Trang (B) and Tak (C).

3.2 High and Low Volatile Oil-Containing Turmeric Cultivars

Based on the variation of total volatile oil content in turmeric samples which were obtained from various cultivars grown in Phichit, Trang and Tak (Fig.24). It was found that there were at least five cultivars that always gave high volatile oil content. As shown in Table 10, the cultivars T_1, T_5, T_{17}, T_{22}, T_{31} gave good yield of turmeric oil either grown in Phichit, Trang or Tak. These cultivars were originally obtained from Phitsanulok, Chiang Rai, Chumphon, Tak and Nakhon Phanom, respectively. According to Table 10, it can be seen that the samples from Tak usually contain the highest volatile oil contents. Among these cultivars, T_1 (from Phitsanulok) appeared to be the best.

Table 10 Turmeric samples containing high volatile oil contents

Turmeric cultivar	Origin	Total volatile oil content (% w/w)		
		Experimental field		
		Phichit	Trang	Tak
T1	Phitsanulok	10.10±0.03	10.46±0.13	16.47±0.06
T5	Chiang Rai	8.20±0.03	7.76±0.16	13.35±0.10
T17	Chumphon	10.11±0.01	10.72±0.14	14.08±0.12
T22	Tak	8.71±0.10	8.29±0.04	11.40±0.20
T31	Nakhon Phanom	8.88±0.08	7.60±0.04	12.82±0.10

For low volatile oil-containing turmeric cultivars, it was found that T_{16}, T_{30}, T_{32}, T_{36} and T_{43} were found to be the cases. As shown in Table 11, the turmerics of these cultivars contain essentially less than 6% of volatile oil (or less than 8% in Tak). These cultivars were originally obtained from Indonesia, Sakon Nakhon, Nakhon Phanom, Malaysia and Bangladesh, respectively. Among these cultivars, T_{16} (from Indonesia) appeared to be the lowest.

Table 11 Turmeric samples containing low volatile oil contents

Turmeric cultivar	Origin	Total volatile oil content (% w/w)		
		Experimental field		
		Phichit	Trang	Tak
T16	Indonesia	4.74±0.07	5.42±0.17	5.67±0.02
T30	Sakon Nakhon	5.11±0.14	4.72±0.01	7.18±0.12
T32	Nakhon Phanom	4.83±0.02	4.15±0.06	7.88±0.10
T36	Malaysia	4.59±0.07	5.69±0.02	6.70±0.04
T43	Bangladesh	6.43±0.04	4.60±0.04	5.34±0.05

3.3 Gas Chromatographic Chromatogram of Turmeric Oil and Component Identification

In addition to the volatile oil content, composition of the turmeric oil prepared from *Curcuma longa* rhizome was examined by using gas chromatography (GC). In this study, the conditions used

for GC was developed carefully to maximize separation of the oil components by a capillary column coated with polar polyethylene glycol stationary phase. As shown in the chromatogram of Fig. 26, some 25 components were detected in turmeric oil. Among these, peaks number 9, 11, 12, 13, 14 apparently the major components of the oil. To identified these major and other minor components, turmeric oil was subjected to gas chromatography-mass spectroscopy (GC-MS) analysis. Under the conditions described in the Material and Method 2.6, 14 peaks of the chromatogram could be identified. Peak number and name of each component are shown in Table 12 and GC-MS were shown in Fig. 33 in Appendix. From these results, it was clear that the major components of peak number 9, 11, 12, 13, and 14 were α-curcumene + α-zingiberene, β-sesquiphellandrene, ar-turmerone, β-turmerone and α-turmerone respectively.

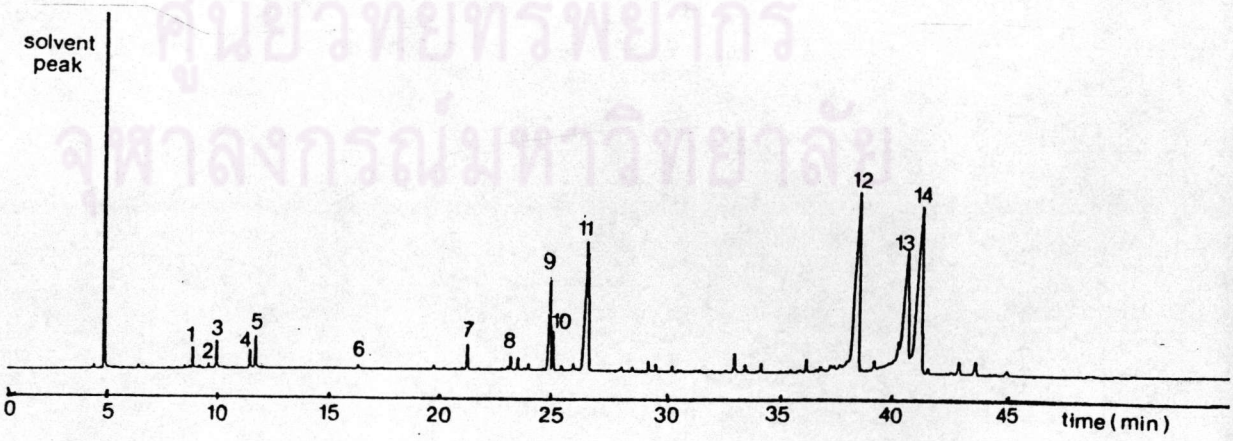
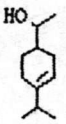
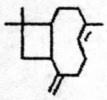
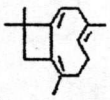
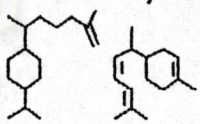
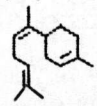
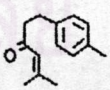
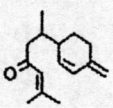
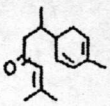


Fig. 26 A typical GC chromatogram of turmeric oil

Table 12 Turmeric oil components separated by GC and identified by GC-MS. Peak numbers and Retention time are correspondence to those shown in Fig. 26

Peak No.	Retention time (min)	Oil component	Structure	Chemical group
1	8.93	α-phellandrene		monoterpenes
2	9.66	p-cymene		
3	10.04	1,8-cineol		
4	11.53	p-cymenene		
5	11.77	terpinolene		
6	16.53	α-terpineol		
7	21.39	β-caryophyllene		sesquiterpenes
8	23.31	α-caryophyllene		
9	25.11	α-curcumene+a-zingiberene		
10	25.24	bisabolene		
11	26.72	β-sesqniphellandrene		
12	38.91	ar-turmerone		sesquiterpene ketones
13	41.07	β-turmerone		
14	41.67	α-turmerone		

In term of relative quantity, it was found that turmeric samples obtained from Phichit and Trang experimental fields were relatively similar in their oil proportions of monoterpenes, sesquiterpenes and sesquiterpene-ketones (Fig 27A and B) where as those from Tak experimental field had different relatives volatile oil composition from Phichit and Trang especially the presence of very low amount of monoterpenes (Fig 27C). As shown in Table 13, turmeric oils from Phichit experimental field were found to contain 3.08% monoterpenes, 21.00% sesquiterpenes and 75.92% sesquiterpene-ketones, from Trang were found to contain 2.61% monoterpenes, 18.03% sesquiterpenes and 79.35% sesquiterpene-ketones whereas those from Tak experimental field were found to be 0.35%, 19.59% and 80.06% respectively. The relative low content of monoterpenes in the Tak's turmeric oils appeared to resulted from the low content of all the individual monoterpenes (especially terpinolene) as compared with the oils from Phichit and Trang. For the sesquiterpenes and sesquiterpene-ketones, no significant different in the amount of the individual components were observed among the three experimental fields.

Therefore, based on the % relative of volatile oil components (Table 13), it was suggested that the

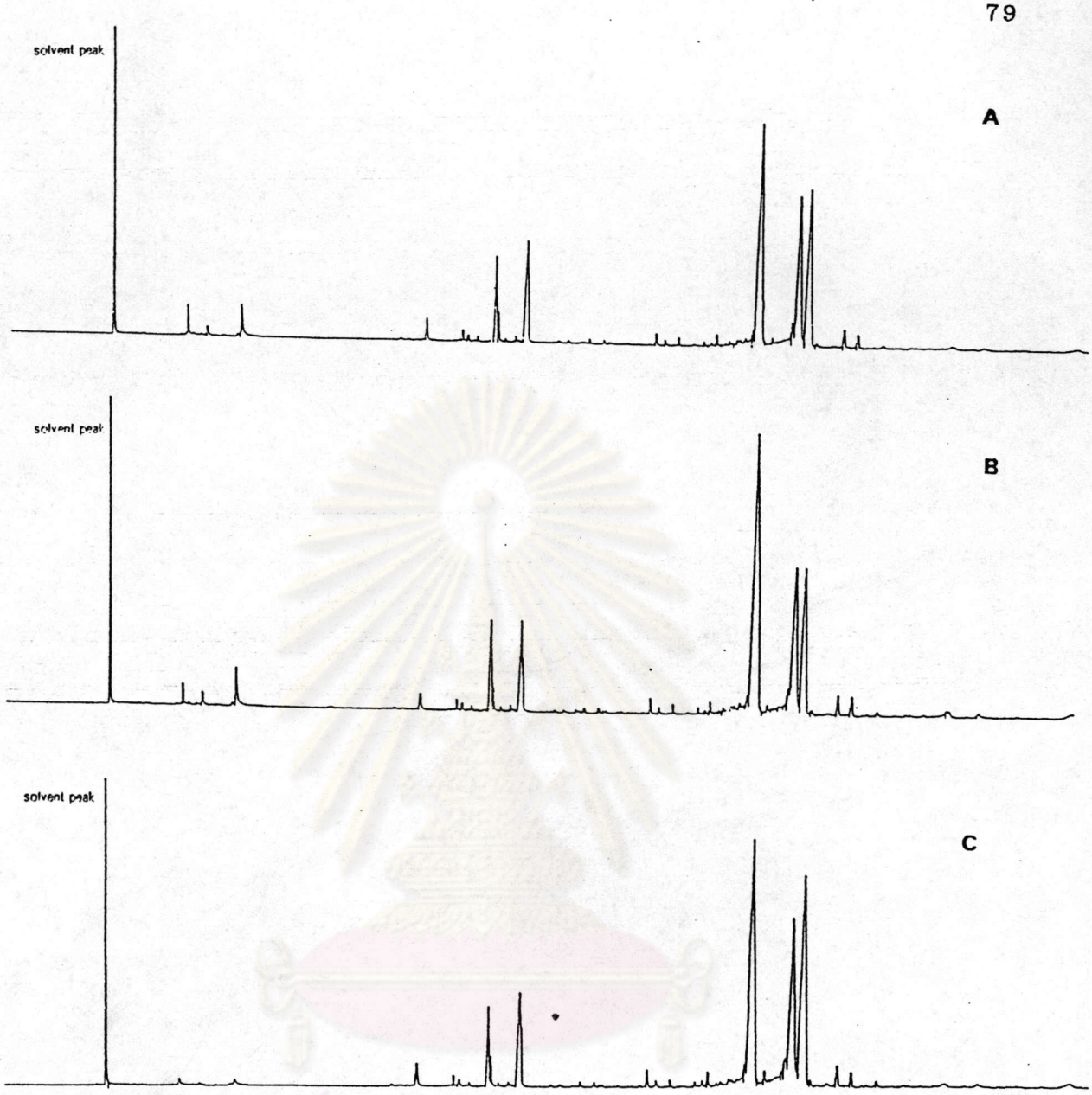


Fig. 27 GC chromatogram of turmeric oil obtained from turmeric samples regrown in Phichit (A), Trang (B) and Tak (C).

Table 13 Composition of turmeric oil from Phichit, Trang and Tak experimental fields

Component	% relative* of volatile oil component		
	Experimental field		
	Phichit	Trang	Tak
Monoterpenes			
α-phellandrene	0.82	0.57	0.22
1,8-cineol	0.57	0.57	0.06
p-cymenene	0.07	0.12	0.03
terpinolene	1.54	1.31	0.01
minor components	0.08	0.04	0.03
total monoterpenes	**3.08**	**2.61**	**0.35**
Sesquiterpenes			
β-caryophyllene	1.23	1.01	1.49
α-caryophyllene	0.32	0.34	0.38
α-curcumene + α-zingiberene	6.87	5.20	5.60
β-bisabolene	1.24	0.98	1.04
β-sesquiphellandrene	8.56	6.82	8.47
minor components	2.78	3.68	2.61
total sesquiterpenes	**21.00**	**18.03**	**19.59**
Sesquiterpene Ketones			
ar-turmerone	32.54	32.72	32.51
β-turmerone	17.43	18.03	18.54
α-turmerone	17.70	17.80	22.33
minor components	8.25	10.81	6.68
total sesquiterpene ketones	**75.92**	**79.36**	**80.06**

* % relative = calculated from the average value of each component obtained from the turmeric samples regrown in Phichit, Trang and Tak experimental fields.

turmeric samples obtained from Phichit and Trang experimental fields were similar in their volatile oil composition but were different from the sample from Tak experimental field with respect to the monoterpene's relative composition.

4. Curcuminoids in Selected Zingiberaceous Plants.

The established TLC-densitometric method for the determination of the three curcuminoids in turmeric rhizomes (described in Material and Methods, section 1.5) was also found to be applicable for the quantitative analysis of the curcuminoids in some selected zingiberaceous plants. The plants of interest which were contain curcuminoids expected to included some species in the genus of *Curcuma*, *Globba* and *Zingiber*. The name of the selected plants are shown in Table 14. Table 14 also shows the characteristics of TLC-densitometric chromatograms of curcuminoid extracts obtained from various zingiberaceous species. The chromatograms were produced from the well separated TLC-pattern of the corresponded samples as shown in Fig. 28. It was found that most of the selected zingiberaceous plants contained curcuminoids either all three or only one or two components. As shown in Table 14, the species of *C. mangga* contains only demethoxycurcumin,

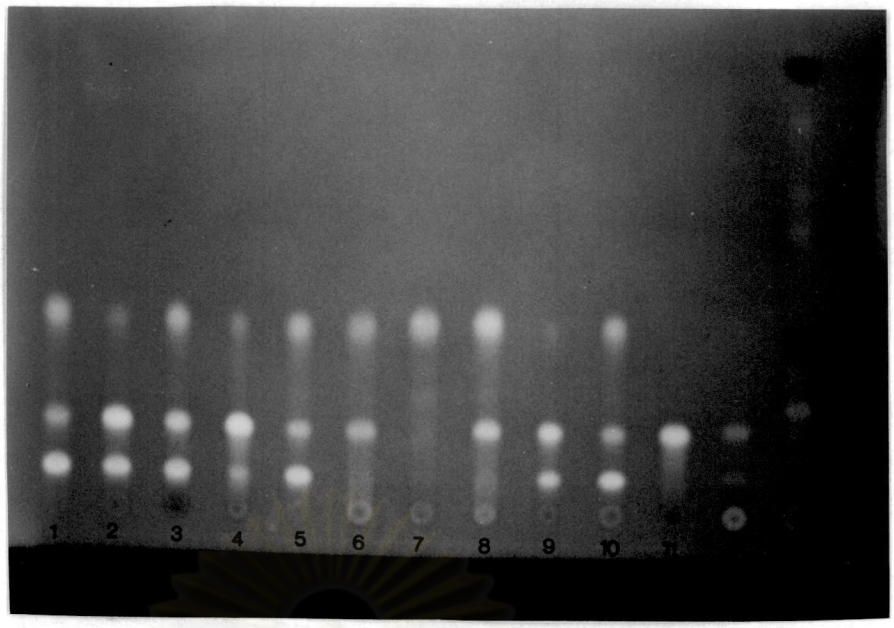


Fig.28 TLC chromatogram of crude extract of selected zingiberaceous plants (under UV-vis 365 nm) coresponded to the species in Table 14.

Table 14. TLC-densitometric chromatogram of the three curcuminoids in selected zingiberraceous plants. 1= bisdemethoxycurcumin, 2= demethoxycurcumin and 3= curcumin.

Species	TLC-densitometric chromatogram	B:D:C* ratio
1 *C. longa*	origin 1 2 3	2 : 3 : 5
2 *C. zedoaria*		1.5 : 8 : 0.5
3 *C. sp* (Thaya Waan)		3 : 4 : 3
4 *C. aromatica*		1 : 7.5 : 1.5
5 *C. caesia*		1.5 : 3.5 : 5
6 *C. sp* (Waan Ma Luoang)		1 : 4.5 : 4.5
7 *Z. cassumunar*		1 : 1 : 8
8 *C. malaccensis*		0 : 4.5 : 5.5
9 *Z. zerumbet*		1 : 8 : 3
10 *C. sp* (Waan En Luoang)		2.5 : 2.5 : 5
11 *C. mangga*		0 : 10 : 0
12. *Z. officinale*		-
13 *C. comosa*		-
14 *C. aeruginosa*		-

*B:D:C=bisdemethoycurcumin:demethoxycurcumin:bisdemethoxycurcumin

Z. cassumunar contains essentially curcumin and *G. malaccensis* obviously contains these two components. On the other hand, none of the three curcuminoids were detected in the species of *Z. officinale*, *C. comosa* and *C. aerugionosa*. In addition to the characteristic densitometric chromatogram, the ratio of curcumin : demethoxycurcumin : bisdemethoxycurcumin could also be another criteria for chemical differentiation of the different zingiberaceous plants. This ratio for each plant that was examined is shown in Table 14.

Quantitatively, the contents of total curcuminoid and of each component were found to be highly variable among the selected zingiberaceous plants. *C. longa* was found to be highest for the total content (almost 10%) followed by *C. zedoaria* (3.8%), Phaya Waan (unknown species of *Curcuma*, 1.3%), *C. aromatica* (0.9%) and so on (see Table 22 in Appendix). In term of individual curcuminoid content, *C. longa* also showed high content of all the three components when compared with the other species (Table 22 in Appendix). However, *C. zedoaria* was found to contain relatively high content of demethoxycurcumin. For other species the content of each component was normally lower than 0.5% w/w (Table 22 in Appendix, Fig. 29 and Fig. 30).

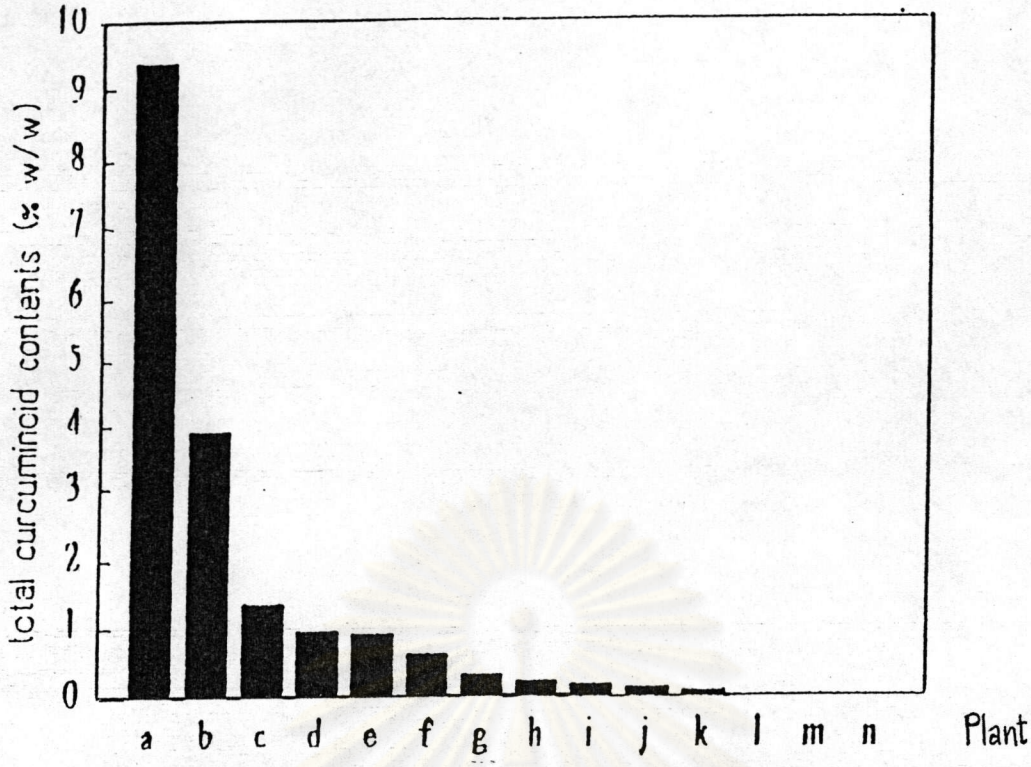


Fig. 29 Total curcuminoid content (%w/w) obtained from selected zingiberaceous plants.

ous plants

a. *Curcuma longa*
b. *C.* species (Phaya Waan)
c. *C. zedoaria*
d. *C. caesia*
e. *Zingiber zerumbet*
f. *C. aromatica*
g. *Z. cassumunar*
h. *Z. officinale*
i. *C.* species (Waan Ma Lueang)
j. *C.* species (Waan En Lueang)
k. *Globba malaccensis*
l. *C. aeruginosa*
m. *C. mangga*
n. *C. comosa*

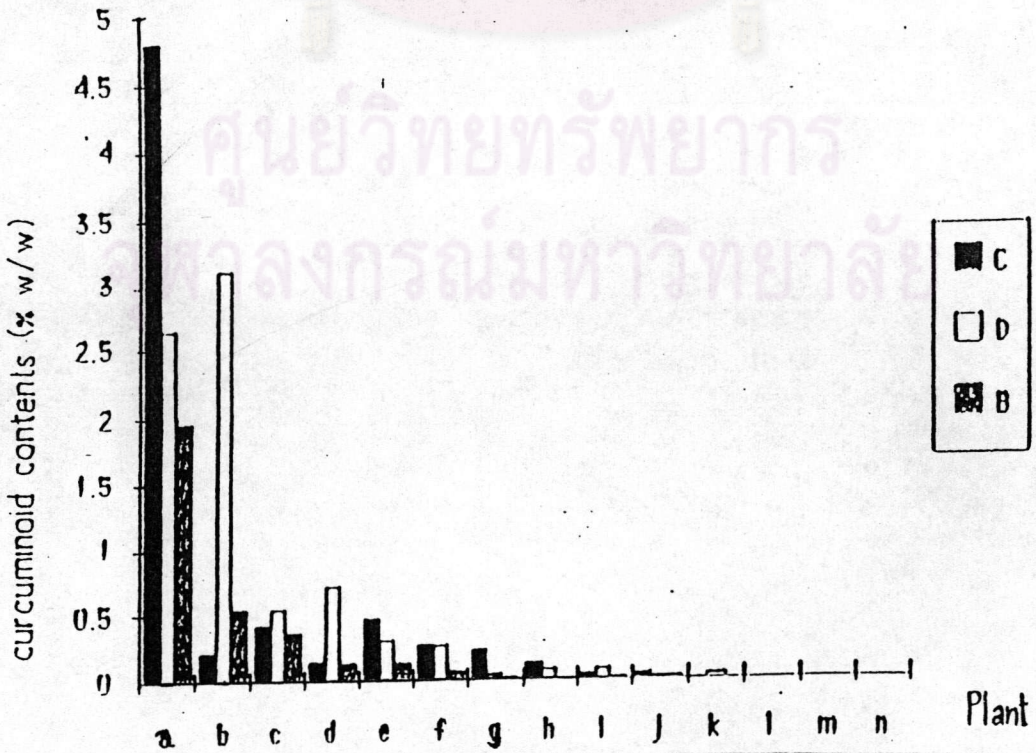


Fig. 30 Each curcuminoid content (%w/w) obtained from selected zingiberaceous plants.

5. Volatile Oil Content and Composition in the Rhizomes of Some Selected Zingiberaceous Plants

5.1 Volatile Oil Content

As shown in Table 15,the volatile oil content of some selected zingiberaceous plants were found to contain highly variable amount of extractable volatile oils (from 0.1 to 10 %v/w). Among the selected plants, *Curcuma longa* was found to contain up to 10.86% which was the highest volatile oil content. This was followed by an unknown *Curcuma* species (known in Thai as Phaya Waan) which contained 6.67% and *C. zedoaria* (6.13%), *C. caesia* (4.61%), *C. aromatica* (3.40%) and *Zingiber zerumbet* (4.19%) were also found to have moderately high volatile oil level. *C. comosa* appeared to contain the lowest volatile oil content (0.14%). The oil in other species were varied from 0.14 to 2.3% (Table 15). These results are also easily shown in a form of bar graph (Fig. 31).

Table 15 Volatile oil content (% v/w) of some selected Zingiberaceous plants.

Plant	Volatile oil content (% v/w)	Volatile oil characteristic
Curcuma longa	10.86	yellow
C. zedoaria	6.13	pale yellow
C. sp (Phaya Waan)	6.67	transparent
C. aromatica	3.40	pale yellow
C. caesia	4.61	pale yellow
C. sp (Waan Ma Lueang)	2.01	brown - green
Zingiber cassumunar	2.30	transparent
Globba malaccensis	1.20	brown - green
Z. zerumbet	4.19	pale yellow
C. sp. (Waan En Lueang)	1.71	yellow
C. mangga	0.50	transparent
Z. officinale	2.21	pale yellow
C. comosa	0.14	yellow
C. aeruginosa	0.59	brown - green

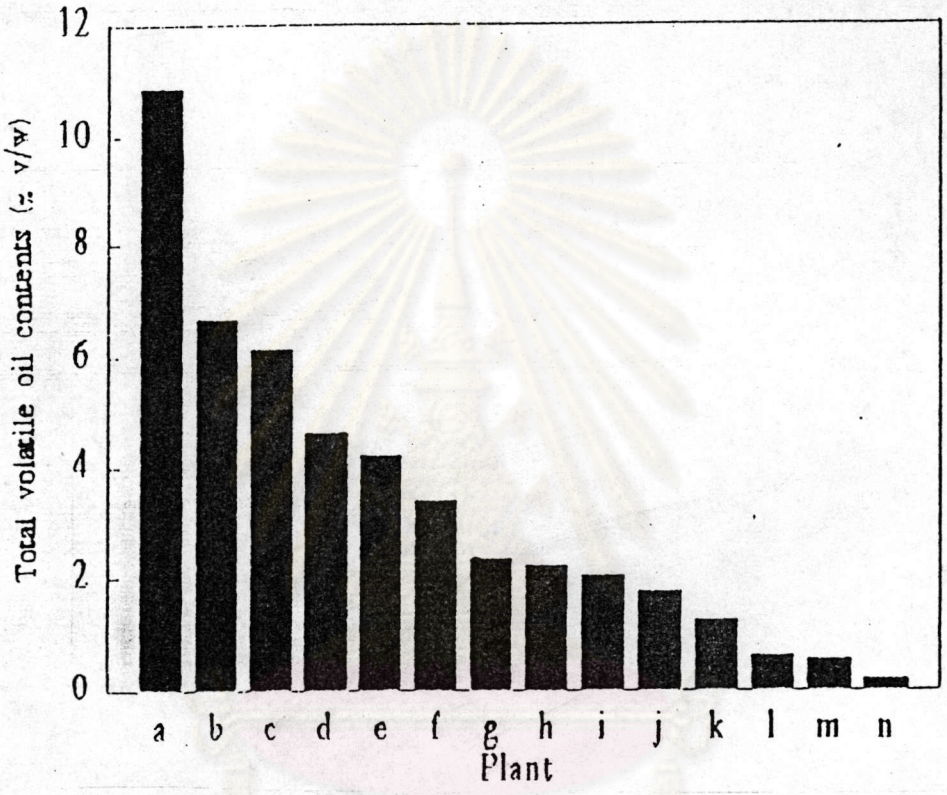


Fig. 31 Volatile oil content (%v/W) obtained from selected zingiberaceous plants.

a. *Curcuma longa*
b. *C.* species (Phaya Waan)
c. *C. zedoaria*
d. *C. caesia*
e. *Zingiber zerumbet*
f. *C. aromatica*
g. *Z. cassumunar*
h. *Z. officinale*
i. *C.* species (Waan Ma Lueang)
j. *C.* species (Waan En Lueang)
k. *Globba malaccensis*
l. *C. aeruginosa*
m. *C. mangga*
n. *C. comosa*

When the content of both curcuminoids and volatile oils in each species were considered simultaneously, it was found that the species containing high curcuminoid content also usually contained high volatile oil content (Fig. 32).

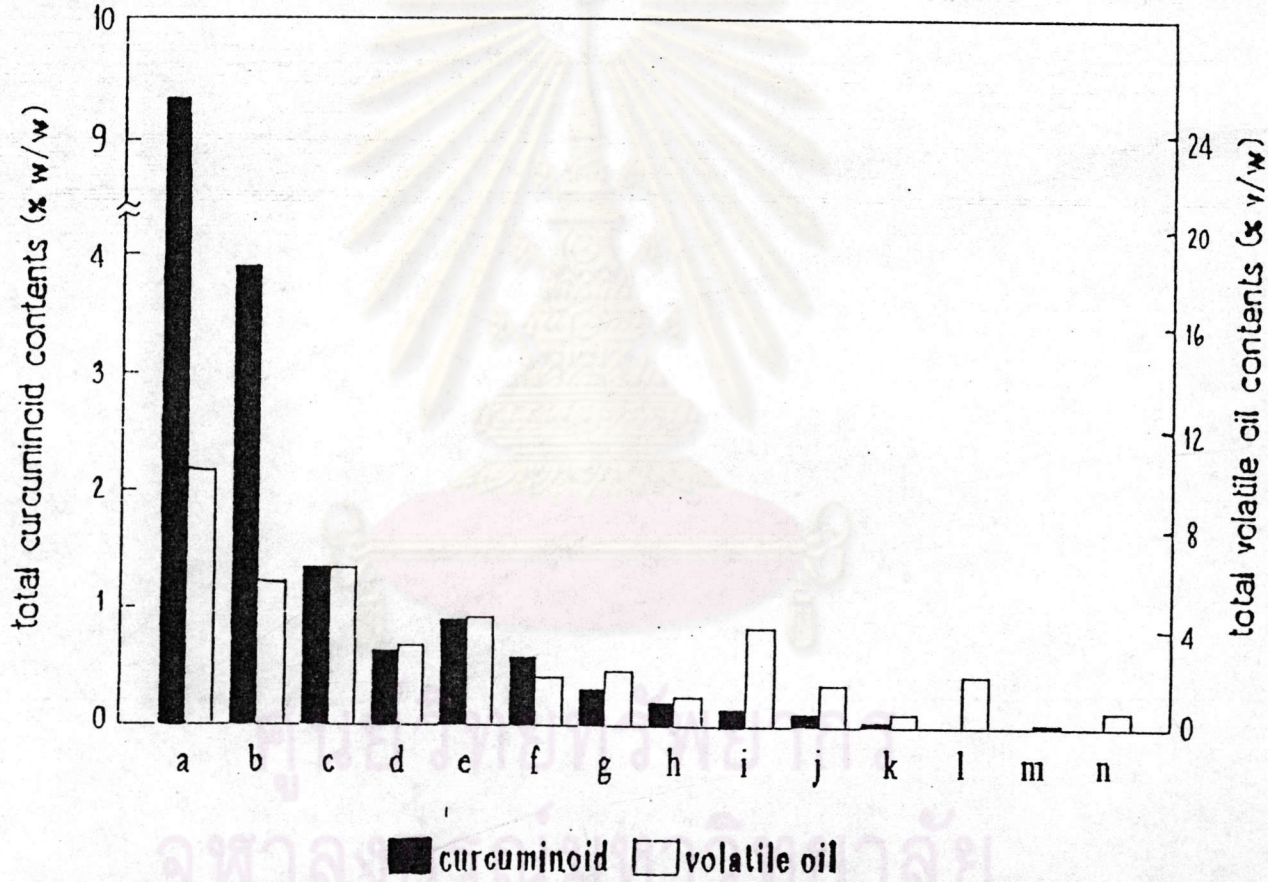


Fig. 32 Curcuminoid and volatile oil contents obtained from selected zingiberaceous plants.

a. *Curcuma longa*
b. *C.* species (Phaya Waan)
c. *C. zedoaria*
d. *C. caesia*
e. *Zingiber zerumbet*
f. *C. aromatica*
g. *Z. cassumunar*
h. *Z. officinale*
i. *C.* species (Waan Ma Lueang)
j. *C.* species (Waan En Lueang)
k. *Globba malaccensis*
l. *C. aeruginosa*
m. *C. mangga*
n. *C. comosa*

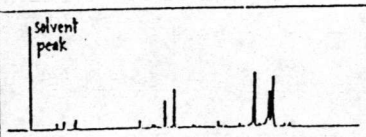
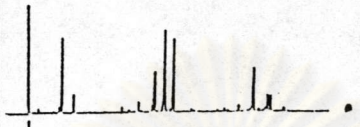
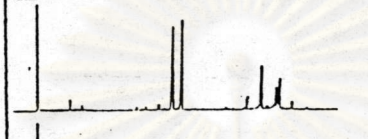
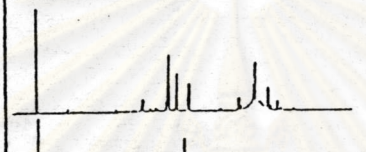
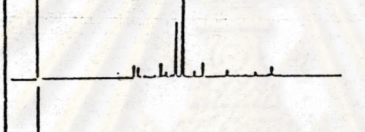
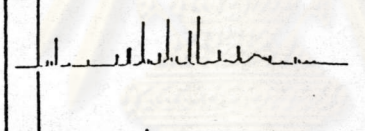
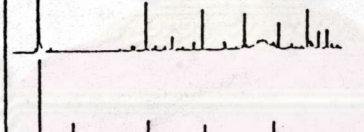
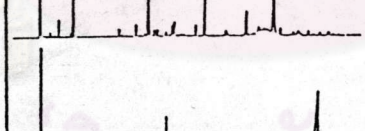
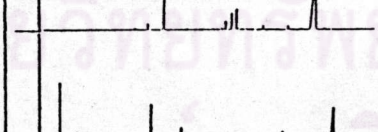
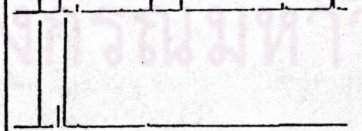
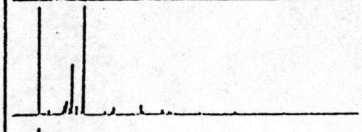
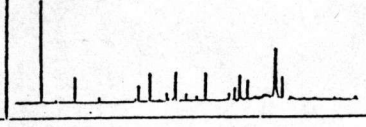
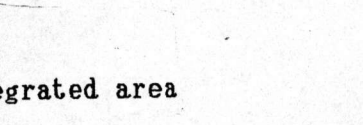
5.2 GC Chromatograms of Volatile Oils

In addition to the volatile oil content, composition of the volatile oil in the selected zingiberaceous plants were also compared by using gas chromatography. The typical GC chromatograms of the volatile oil from various plants are shown in Table 16. Among these plants, it was found that *C. zedoaria, C. caesia, C. aromatica* and *C.* species (known in Thai as Phaya Waan) had their GC chromatograms similar to that of *C. longa*. The second group included *G. malaccensis*, *C. aeruginosa* and two unknown species of *Curcuma* which known in Thai as Waan Ma Lueang and Waan En Lueang. This group was found to have a number of volatile oil components of monoterpenes, sesquiterpenes and sesquiterpene-ketones distributed over the chromatograms and no obvious major components. The third group belongs to *C. mangga* and *C. comasa* which were found to contain mainly monoterpene components and minor of sesquiterpenes and sesquiterpene-ketones components. Finally, the fourth group which included *Z. zerumbet* and *Z. cassumunar*, they found to contain the major components of sesquiterpene-ketones.

In term of the relative quantity, it was found that various volatile oils from the selected plants were considerably different in their oil proportions of monoterpenes, sesquiterpenes and sesquiterpene-ketones

(Table 16). With this aspect, the plant samples could be divided into 3 groups. The first group was found to contain high % relative of sesquiterpene-ketones, such as *C. longa* (73%), *G. malaccensis* (66%), *Z. zerumbet* (86%), *Z. cassumunar* (54%) and *C. aeruginosa* (64%). The second group contained high % relative of sesquiterpenes. These included *C. zedoaria* (62%), *C. caesia* (63%), *C. species* (Phaya Waan) (63%),*C. aromatica* (94%) and *C. species* (Waan Ma Lueang) (62%). For the last group which contained high % relative of monoterpenes, it was found to be *C. mangga* (98%) and *C. comosa* (89%).

Table 16. Typical GC chromatogram of selected zingiberaceous plants and percent relative of their volatile oil components

Plant	Typical GC chromatogram	*%relative		
		monoter-penes	seequiter-penes	seequiter-pene Ketones
1 *C. longa*		3.0	24.0	73.0
2 *C. zedoaria*		13.0	62.0	25.0
3 *C. caesia*		2.0	63.0	35.0
4 *C. sp* (Phaya Waan)		1.0	63.0	36.0
5 *C. aromatica*		tr*	94.0	6.0
6 *C. sp* (Waan Ma Liwang)		19.0	62.0	19.0
7 *G. malaccensis*		tr*	34.0	66.0
8 *C. sp* (Waan En Liwang)		13.0	41.0	46.0
9 *Z. zerumbet*		0.5	13.5	86.0
10 *Z. cassumunar*		23.0	23.0	54.0
11 *C. mangga*		98.0	2.0	tr*
12 *C. comosa*		89.0	9.0	2.0
13 *C. aeruginosa*		6.0	30.0	64.0

*tr=trace

*%relative= integrated area