CHAPTER V

DISCUSSION

1. Determination of Curcuminoids in *Curcuma longa*

We have shown in this study that the turmeric curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) can be determined simultaneously and effectively by using a TLC-densitometric method (Fig. 17). This method was developed carefully to ensure accuracy precision (Table 4) and reproducibility (Table 5) in the quantitative analysis of the three curcuminoids. The method is also simple and rapid since the analysis can be performed directly from the crude extracts without prior purification.

The crucial part of the method development is the step of searching a solvent system that allows complete separation of the three curcuminoids. It appears that the composition of chloroform-benzene-methanol with the ratio 80:15:5 is the best solvent system for the separation. The identity of each curcuminoid as curcumin, demethoxycurcumin and bisdemethoxycurcumin (Fig. 17) was clearly demonstrated by ^{1}H NMR (Fig. 15).

In addition, other factors that may affect the reliability and sensitivity of the method were also examined. For example, the step of sample preparation by sonication (section 1.5 of the "RESULTS") was optimized to allow exhaustive extraction of the accumulated curcuminoids in the turmeric rhizome. Also, the choosing of the wavelength 420 nm for the analysis was supported by the absorption spectrum of each curcuminoid (Fig. 16).

2. Variation of Curcuminoid and Volatile Oil Contents in Turmeric Rhizomes from Different Cultivars

Curcuma longa can be found in almost every province of Thailand. Many of them have been grown locally for a long period of time without being evaluated for their quality based on curcuminoid and volatile oil content. This study was therefore designed for the evaluation of various turmeric cultivars collected from different part of Thailand. However, in order to minimize many variable factors caused by different geographic conditions of different provinces, various turmeric cultivars (more than 30) were all regrown in three different experimental fields in Phichit, Trang and Tak provinces.

Using the developed TLC-densitometric method for curcuminoid determination, the rhizomes of different cultivars show highly variable curcuminoid contents in all the three experimental fields (Figs. 19, 20 and 21). By average, all the cultivars from Tak experimental field produce the highest total curcuminoid content (9.58% w/w), followed by those from Trang (8.49% w/w) and from Phichit (7.42 % w/w). Considering, each of the curcuminoid (curcumin, demethoxycurcumin and bisdemethoxycurcumin), it also appears that the cultivars from Tak (4.32, 2.73 and 2.55%) contain the content of each curcuminoid higher than those from Trang (4.21, 2.34 and 2.11%) and Phichit (3.48, 2.08 and 1.73% w/w) respectively. These results indicate that the geographical and climatic conditions have an effect on the curcuminoid production in turmeric rhizomes. Those cultivars regrown under the conditions of Tak (850 m. from sea level) seem to be induced for curcuminoid accumulation more than those regrown under the condition of Phichit (50 m. from sea level) an Trang (60 m. from sea level) Furthermore, the enhancement of the curcuminoids is due to the increase of all the individual components.

Detailed study on the ratio of curcumin : demethoxycurcumin : bisdemethoxycurcumin in each cultivar (Fig. 23) shows that most of the turmeric rhizomes regrown in Phichit and Trang experimental fields (64.71 % and 66.67 %) have such a ratio of 5:3:2, respectively. On the other hand, those from Tak mostly have the ratio of 4:3:3 (48.57 %) followed by 5:3:2 (37.14 %) (Fig. 23). This suggests that the environmental factors in Tak tend to induce more accumulation of bisdemethoxycurcumin in turmeric rhizomes and causing the shift of the regular 5:3:2 ratio to 4:3:3. For other turmeric samples which contain high proportion of curcumin (7:2:1 and 6:3:1 ratios), These always contain low absolute curcuminoid content. For example, the samples with the ratio of 4:3:3 usually contain the total curcuminoid content more than 8 % w/w, those with the ratio 5:3:2 contain more than 5 % w/w and those with 6:3:1 or 7:2:1 were usually contain the total curcuminoid content less than 5% w/w. These data indicated that *C.longa* rhizomes usually contained curcumin as the major component followed by demethoxycurcumin and bisdemethoxycurcumin, respectively.

It should be noted that among the *C. longa* population grown in the three experimental fields, the high curcuminoid-producing cultivars (more than 10 % w/w) appears to be the cultivars of T_{20} (Phitsanulok), T_{22} (Tak), T_{23} (Chaiyaphum), T_{31} (Nakorn Phanom) and T_{37} (Roi-Et) (Table 6). These cultivars should be promoted for cultivation in any area of Thailand. For the volatile oil content, the results show that the geographical and climatic conditions have an effect on volatile oil accumulation in turmeric rhizomes. In this case, the conditions of Tak seem to enhance more volatile oil accumulation in *C. longa* rhizomes than the conditions of Trang and Phichit (Fig. 25). Therefore, the Tak experimental field is apparently superior to the other to obtain high-curcuminoid and high volatile oil-containing turmeric rhizomes. The samples from the cultivars of T_1 (Phisanulok), T_5 (Chiang Rai), T_{17} (Chumphon), T_{22} (Tak) and T_{31} (Nakorn Phanom) consistently show high volatile oil content ($\geq$ 8 % v/w) when grown in the three experimental fields. From these results, the common cultivars which give both high curcuminoid and volatile oil contents appear to be T_{22} (Tak) and T_{31} (Nakhon Phanom). Both cultivars should be promoted for cultivation. However, the production yield (kg/rai) of the turmeric cultivars should be evaluated first.

3. Volatile Oil Components in Turmeric Rhizomes

In order to examine whether the volatile oils obtained from different cultivars would have similar or different composition, a technique of gas chromatography (GC) was used to facilitate separation of the volatile oil components. The GC conditions developed for this purpose shows a good separation of the oil components. The GC conditions developed for this purpose show a good separation of the oil components within 45 minutes. The results of GC analysis of all turmeric samples from all the three experimental fields show that all have very similar GC chromatogram (Fig. 26) although their proportion of the oil components may be different in certain degree.

To identify chemical structure of each peak in the chromatogram, GC-MS was used and the resulted fragmentation pattern of each peak was compared with the pattern of authentic compounds in the terpene library. The results (Table 12) show that the major components of turmeric oil are α-curcumene, α-zingiberene, β-sesquiphellandrene, ar-turmerone, α-turmerone and β-turmerone. Ar-tumerone appears to be present in highest amount (approximately 30% relative) in all turmeric rhizomes. It is likely that this component has a major contribution to the characteristic odor of turmeric.

The obtained GC chromatograms also allow us to determine the relative quantity of volatile oil components in turmeric samples. The results show that the turmeric samples obtained from Phichit and Trang experimental fields are essentially similar in their oil proportions of monoterpenes, sesquiterpenes and sesquiterpene-ketones

(3.08, 21.00 and 75.92% for Phichit; 2.61, 18.03 and 79.36% for Trang) whereas those from Tak appear to have relative low monoterpene proportion respectively (Fig. 27 and Table 13).

This suggested that the climatic and geographical conditions of Tak are different from those of Phichit and Trang in influencing *C. longa* plants to produce monoterpene group of compounds.

4. Curcuminoid and Volatile Oil Contents in the Rhizomes of Some Selected Zingiberaceous plants

Determination of curcuminoids in some 14 selected zingiberaceous plants clearly shows that some species in zingiberaceae family contain curcuminoids and some do not (Figs.28 and 29). Also, the results show a high variation of the curcuminoid contents and components among the selected plants in the family. For example, *G.malaccensis* contains only two curcuminoids in its rhizome (curcumin and demethoxycurcumin) and *C. mangga* contains only demethoxycurcumin while other contain all the three curcuminoids (Table 14 and Fig. 30).

For their volatile oil content, it also appears to have a high variation of volatile oil content among the plants in this family (Table 15). Usually, the plants which contain high curcuminoid contents are also contain high volatile oil contents (Fig. 32). The reason for such a relationship is still unknown.

In addition, GC was also used to study the volatile oil composition of these selected zingiberaceous plants. Again, many different types of chromatograms are observed among their volatile oils (Table 16), particularly their oil proportions of monoterpenes, sesquiterpenes and sesquiterpene-ketones. Therefore, the volatile oil of each zingiberaceous plant seems to have its own characteristic finger print and odor. This information may be important for the study of the relationship and evolution of various zingiberaceous plants.

CONCLUSION

From this research topic of "Curcuminoid and Volatile Oil Determination in Turmerics from Various Locations in Thailand", the following conclusions can be drawn :-

1. The established TLC-densitometry for curcuminoid determination is an efficient and reliable technique for simultaneous determination of the three curcuminoids in *C. longa* or other zingiberaceous plants potentially contain the curcuminoids.

2. Turmeric samples which are derived from different provinces in Thailand inherently have different potential in their production of curcuminoids and volatile oils.

3. The geographical and climatic conditions have an effect on curcuminoid and volatile oil production in turmeric. The more high level from the sea , the more curcuminoids and volatile production in turmerics as well.

4. Turmeric samples which were regrown in Tak (Doi Musor) experimental field contain higher curcuminoid and volatil oil content than those from Phichit and Trang.

5. The ratio of curcumin : demethoxycurcumin : bisdemethoxycurcumin found in various turmeric rhizome is mostly 5:3:2.

6. The five turmeric samples that contain high curcuminoid content (10-16 % w/w) are T_{20}, T_{22}, T_{23}, T_{31} and T_{37} cultivars. They were derived from Phitsanulok, Tak, Chaiyaphum, Nakhon Phanom and Roi Et cultivars, respectively.

7. The five turmeric samples that contain high volatile oil contents (8-16 % v/w) are T_1 (Phitsanulok), T_5 (Chiang Rai), T_{17} (Chumphon), T_{22} (Tak) and T_{31} (Nakhon Phanom).

8. Turmeric samples that contain high curcuminoid content are usually contain high volatile oil content as well.

9. Ar-turmerone, β-turmerone, α-turmerone, α-curcumene, α-zingiberene and β-sesquiphellandrene are the major components in turmeric oil.

10. Turmeric oil proportion of monoterpenes, sesquiterpenes and sesquiterpene-ketones found in most rhizomes is as follow : 0-5% monoterpenes, 20-25% sesquiterpenes and 70-80% sesquiterpene-ketones.

11. Curcuminoid contents and components in the selected zingiberaceous plants are considerably different. Some contain all the three curcuminoids while other such as *C. mangga* and *G. malaccensis* contain only one and two components respectively.

12. Among the zingberaceous plants selected for this study, turmeric is the species that contains the highest curcuminoid and volatile oil contents.

13. The zingiberaceous plants which contain no curcuminoids are *Z.officinale, C. comosa* and *C.aeruginosa.*