CHAPTER V

DISCUSSION

1. Qualitative and quantitative determination of volatile oil and piperine in Thai black pepper

As mentioned in the "Introduction", the constituents of pepper responsible for its value as food additive are the volatile oils for aroma and the alkaloid piperine for the characteristic pungency. Generally, black pepper contains approximately 2-4% of the volatile oil and 5-9% of total alkaloids including piperine (Youngken, 1950). The content of both components in pepper products has been reported to depend on the cultivars of the pepper plants (Buckle *et al.*, 1985; Rathnawathie and Buckle, 1984). The most well-known pepper cultivars include Sri Lankan, Sarawak (Kuching) and Indian (Panniyur).

Thai pepper products are mostly obtained from a mixture of Sarawak and Sri Lankan cultivars which are grown mainly at Amphor Tamai, Chantaburi province. In order to evaluate the quality of Thai pepper products, particularly the black pepper, the pepper berries obtained from the Sarawak and Sri Lankan cultivars were studied separately with respect to their volatile oil and piperine components. For this purpose of quantitative

analysis, the conditions and systems of GC and HPLC were developed in this study for the analysis of pepper oil components and piperine, respectively.

The resulted GC chromatogram (Fig. 15) showed the presence of more than thirty volatile components and each of which was identified by a powerful GC-MS analysis (Table 6). This complete separation and identification of the volatile oil composition allows each constituent be quantitated. Particularly, those major oil components (α-pinene, β-pinene, sabinene, Δ^3-carene, limonene and β-caryophyllene) which contribute to the over all odour of the pepper oil. When compared with other pepper oil's GC chromatograms reported previously (Buckle *et al.*, 1985; Jansz *et al.*, 1984), our chromatogram shows much better separation of the oil components. This is accomplished by using a high resolution capillary column (coated with polar polyethylene glycol stationary phase) rather than a simple packed column (10% Carbowax) used in those studies. For the piperine analysis by HPLC, the chromatogram (Fig. 17) shows the presence of mainly piperine (ca. 88%) in the methylene dichloride extract of black pepper. This indicates the relative specificity of this organic solvent in extracting piperine from pepper samples as detected at the wavelength of 336 nm. The complete separation of piperine as show in its chromatogram also indicates the reliability of HPLC method in the quantitative analysis of piperine.

After establishing these appropiate analytical methods, pepper samples were then collected. This was done by collecting fresh pepper berries at Amphor Tamai in order to avoid any possible mixing of the two different cultivars' berries (Sri Lankan and Sarawak). At least 6 different gardens at various areas of Tamai were chosen so that the results can be the representative of this area of plantation. The collected pepper samples were subjected to volatile oil content determination using the standard AOAC method (method 962.17, AOAC, 1990). Both the fresh and dried black pepper were determined for the oil content in order to observe the effect of drying on the original pepper oil content.

The results (Table 5) clearly shows that Sri Lankan cultivar's pepper, either as green or black pepper, contains significantly (α=0.05) higher level of volatile oil than the Sarawak cultivar's pepper (2.73% and 1.78%, respectively). Therefore, the volatile oil content of 2.1-2.3% detected in the commercially available black pepper from Chantaburi must be contributed mainly from the Sri Lankan cultivar. Furthermore, the results also indicate that the process of drying to obtain black pepper has no significant (α=0.05) effect on the total volatile oil content accumulated in the pepper berries. The higher level of the pepper oil from Sri Lankan cultivar also suggests that it has more aroma than the Sarawak cultivar. It should be noted that the pepper oil content in Sri Lankan reaches the standard level of the

Ministry in Industry of Thailand (มาตรฐานผลิตภัณฑ์อุตสาหกรรม, สำนักงาน, 2526) while that in Sarawak cultivar does not.

In term of pepper oil composition, by overall, it is concluded the chemical components of the pepper oils from both Sri Lankan and Sarawak cultivars are essentially the same (Fig. 15). The relative quantities of the pepper oils from the two cultivars are, however, considerably different in their oil proportions of monoterpenes, sesquiterpenes and oxygenated compounds (Table 7). According to the literatures, pepper oil from various sources have been reported to contain 50-80% monoterpene hydrocarbons, 20-40% sesquiterpene hydrocarbons and less than 4% of oxygenated terpene compounds (Hasselstrom *et al.*, 1957; Ikeda *et al.*, 1962; Jennings and Wrolstad, 1961; Nigam and Handa, 1964; Richard *et al.*, 1977; Wrolstad and Jennings, 1965). Based on this information, it can be concluded that while the Sarawak cultivar pepper oil has similar oil composition to the other pepper oils, the Sri Lankan has its own unique composition. It can be described to have low monoterpene content but high sesquiterpene and oxygenated terpene level.

It is generally accepted that the level of monoterpene hydrocarbons in related to pepper oil's peppery notes (Govindarajan *et al.*, 1973; Lewis *et al.*, 1969b) while the level of sesquiterpene hydrocarbons contributes to pepper odours (Lewis *et al.*, 1969b) and the level of oxygenated compounds makes an important

contribution to the spicy notes, or overall odour quality of pepper oils (Lewis *et al.*, 1969a; 1969b). In these aspects, the Sarawak cultivar black pepper oil seems to be rich in peppery notes, moderate pepper odour and low odour quality of spicy notes. On the other hand, the Sri Lankan cultivar has low peppery notes but is rich in pepper odours and highly aroma of spicy notes.

It has been proposed that the proportion of pinenes is related to either of two top-notes which are described as refreshing/piney and terpentine/eucalyptus. The high level of pinenes is related to the "terpentine-like" off-odour of pepper oil (Govindarajan *et al.*, 1973; Lewis *et al.*, 1969b). The Sarawak cultivar is, therefore, rich in the top peppery notes since it has lower content of pinenes than the Sri Lankan cultivar (Table 8). For β-caryophyllene, this is one of the major sesquiterpenes which smells sweet and flowery and is desirable in products for perfumery applications such as useful adjunct in the preparation of soap perfumes (Pocher, 1974). The Sri Lankan cultivar which is rich in β-caryophyllene (Table 8) appears to be suitable for the perfumetry industry.

For piperine content, the higher level of piperine fron the Sri Lankan cultivar (Table 9) also suggests that it has more pungency than the Sarawak cultivar. The piperine content in pepper from Sri Lankan cultivar (4.96%) reaches the standard level of Ministry in Industry of Thailand (มาตรฐานผลิตภัณฑ์อุตสาหกรรม, สำนักงาน,

2526) while that in Sarawak cultivar (3.82%) does not. This study clearly indicates that the Sri Lankan cultivar grown in Thailand produces better black pepper quality than the Sarawak cultivar, with respect to the pepper oil and piperine contents.

2. Comparison of volatile constituents and piperine content among black pepper products of Thailand and other countries

In order to know the quality standard of Thai black pepper, a comparative study was carried out. This was done by comparing the compositions and contents of volatile oil and piperine of various world's famous black pepper products. These included the black pepper from India, Brasil and Malaysia which we asked these samples from a Germany Spices Company, Gewurzmuller Dr.E.Rendlen Gmbh & Co. in Stuttgart.

Various black pepper samples to be analyzed were prepared simultaneously under the same procedure. The results indicate that the black pepper of Thailand, both Sri Lankan and Sarawak cultivars, contains lower level of volatile oil but higher level of piperine than those from India, Brasil and Malaysia (Table 10). This suggests that Thai black pepper has more pungency but less aroma than those from other countries. However, the Sri Lankan cultivar's black pepper seems to be only slightly inferior than the foreign pepper with respect to the aroma, as compared to the Sarawak cultivar which is low in its volatile oil content (Table 10).

In term of pepper oil composition, the results show relatively high variation of the pepper oil composition obtained from different sources of pepper (Fig 19 and Table 11). In general, however, it can be said that Thailand's Sri Lankan pepper has its volatile oil pattern similar to the Brasil and India's pepper products while Thailand's Sarawak pepper has the pattern similar to the Malaysia's pepper. Nevertheless, their differences in the relative constituents may give each of them to have its own characteristic odour.

The variation in the composition of pepper oils from different sources is important when pepper is used as a component of food flavorings (Buckle and Rathnawathie, 1985). The pepper oil from India is rich in the top peppery notes as Thailand's Sarawak cultivar since it has the high monoterpene concentration with a consequential lower content of pinenes. Product of black pepper from Malaysia which is rich in ß- caryophyllene appears to be suitable for the perfumery industry like Thailand's Sri Lankan cultivar.

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

The stage of harvesting pepper berries is usually dependent on the product to be prepared. In Sri Lankan, berries are harvested at 5-6 months, 4.5-5 months and 6 months for the preparation of black, green and white

pepper products,respectively (Rathnawathie and Buckle, 1984). In Thailand, fully-matured pepper berries are also obtained 6 months after fruit setting. The berries at this stage are usually harvested for black pepper preparation. However, no information has been established whether the harvesting of 6-month-old pepper berries at Chantaburi is the most suitable timing with respect to the accumulated contents of volatile oil and piperine. This study, therefore, aims to investigate the changes in both chemical constituents during the maturation of pepper berries of both the Sri Lankan and Sarawak cultivars grown under the environmental conditions of Chantaburi.

The results from the study (Table 13) indicate that the percent contents (v/w) of volatile oil and piperine in the berries of both Sri Lankan and Sarawak cultivars are highest at the third month of maturation. Thereafter, these values of percent content (v/w) are decreased. However, the weight of this 3-month-old berries is much lower than the fully-mature berries (Table 13) and therefore, the berries are too young for harvesting. On the other hand, pepper berries at 5 months appear to be relatively mature for harvesting because they have the highest weight with moderate levels of both piperine and volatile oil. Furthermore, the volatile oil composition of the 5-month-old berries appear to be similar to that of the fully-mature berries (Tables 14 and 15). This confirms that Thai pepper berries at 5 month olds are optimum for harvesting

in all aspects (eg. their chemical content and composition and the pepper yield). However, between the Sri Lankan and Sarawak cultivars, it is clear that former is superior to the latter with respect to the volatile oil and piperine contents as well as the yield of the pepper berries (Table 13). There is no question that the Sri Lankan cultivar grown in Thailand give good quality of pepper products. Our study on the maturity of the chemical constituents of Thai pepper is similar to the Indian data (Dwarakanth *et al.*, 1963) but relatively different from the results obtained in Sri Lanka which showed the highest levels of chemical constituents around 5 to 5.5 months (Jansz *et al.*, 1984). This different maturity patterns may due to the effect of geographic conditions of each country.

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

The storage of black pepper is necessary in almost every step, starting from keeping the product for selling at appropriate timing, transportation to the importing countries and finally storing in the warehouse of manufacturers. Question arises as to whether the stored black pepper has the same quality as the original harvested pepper particularly the volatile constituents. This study, therefore, aims to monitor the level of the volatile oil and its composition in black pepper during a period of eight months. The stability of pure volatile oil was also monitored in order to observe

their differences in the stability. The normal room temperature and at 4°C were chosen for the study.

The results indicate that black pepper can be stored at room temperature in a sealed plastic bag at least eight months. During this period, the volatile oil content and composition show relatively constant. In the case of stored pepper oil at room temperature and 4°C, the proportion of oxygenated compounds were increased markedly especially caryophyllene oxide. These results possibly indicate that the proportion of β-caryophyllene changes into caryophyllene oxide. It can be seen that pepper oil stored at 4°C is more stable than at room temperature. This may be partly due to changes in the pepper oil composition into the oxygenated compounds of monoterpenes and sesquiterpenes.

CONCLUSION

From this research work of "Studies on Volatile Oil and Piperine Contents in Pepper Cultivated in Thailand", the conclusions can be drawn as follows. The Sri Lankan cultivar grown in Thailand produces better black pepper quality than the Sarawak cultivar, with respect to its volatile oil and piperine contents. The pepper oil composition analysis by GC and GC-MS indicate that both Sri Lankan and Sarawak cultivars contain at least 30 similar components. Among these, α-pinene, β-pinene, sabinene, Δ^3-carene, limonene and β-caryophyllene appear to be the major constituents. The better quality of the Sri Lankan cultivar black pepper over the Sarawak's suggests that the plantation of the former should be promoted in Thailand. The black pepper cultivated in Thailand (both Sri Lankan and Sarawak cultivars) has more pungency but less aroma than those from India, Brasil and Malaysia. The accumulation of volatile oil and piperine appears to be maximum at 3 months old of pepper berries and thereafter it decreased. However, the optimum age of the berries to be harvested for black pepper product is 5 months old. The pepper oil composition has no significant variation during 2 to 6 months maturity of pepper berries. Finally,black pepper can be stored at room temperature for at least eight months without affecting its chemical constituents.