DISCUSSION

1. Development of a Solid-Phase Extraction and HPLC for Capsaicin Determination.

The major difficulty in the determination of capsaicin in Capsicum fruits is the chemical complexity of the crude extracts. The methanolic extracts of capsicum contain various pungent principles of capsaicinoids and various color principles of carotenoids. It is therefore, unlikely to determine capsaicin content directly in the capsicum crude extracts using HPLC, GC or spectrophotometric methods. This is due to the possible interference of other constituents in the extracts. Recently, the method of solid-phase extraction has been used successfully in the prepurification of an extract prior to the analysis (Van Horne, 1990). Attempts were therefore made in this study to optimize the conditions of solid-phase extraction to clean the capsicum extracts.

The results obtained from the study indicate that the method of solid-phase extraction can be applied to our case for capsaicin determination in capsicum fruits. The method involves the use of octadecylsilane reverse-phase (RP-18) as a sorbent bed. Preconditionine of the column with 50% methanol allows capsaicin in the crude extract be absorbed to the sorbent bed whereas other constituents are either passed through or washed out the column. Finally, the absorbed capsaicin can be eluted quantitatively by using the solvent of 70% methanol. The resulted eluate contains maninly capsaicin as analyzed by HPLC (Fig. 22). The chromatogram of the eluate is very similar to the chromatogram of the commercially available capsaicin (Sigma No.

M-3403, 60% purity) which has only two major peak of capsaicin and dihydrocapsaicin (Fig. 22). Therefore, the determination of capsaicin by the solid-phase extraction followed by HPLC very effective.

The developed solid-phase extraction technique gives not only high purity of capsaicin but also high recovery (approx. 90%) of the compound. This was demonstrated by HPLC analysis of the capsicum extracts both before and after passing the step of solid-phase extraction (Table 6) and Fig. 24. Although this experiment shows that capsaicin can be detected directly HPLC without sample cleaning, it is not suitable because the crude extracts have many impurities. These impurities would decrease the efficiency of the HPLC column and reduce the column-life. Therefore, the step of sample cleaning is very important and should be carried out before the HPLC analysis.

2. Capsaicin Content in Various Thai Capsicum Fruits.

Based on the developed method of capsaicin determination by solid-phase extraction and HPLC, we can obtain information on the capsaicin content in various Thai capsicum fruits. From more than 40 capsicum samples of various sources, the results shows that Thai capsicum fruits contain capsaicin from zero to 0.8% on the dry weight basis on from zero to 3.9 mg capsaicin on the basis of total content per fruit (Table 7). Study on the distribution of the population shows that the capsaicin content in most *C. annuum* is in the range of 0.1-0.4% whereas *C. frutescens* is in the range of 0.4 to 0.8% (Fig. 28). As expected, there is a direct correlation between the weight of the fruit and the percentage of capsaicin content (Fig. 27).

3. Carotenoid Content in Various Thai Capsicum Fruits.

The carotenoid content in Thai capsicum fruits was determined by a spectrophotometric method as described in AOAC. This method is simple and convenient for detection of total carotenoids based on β-carotene equivalent. Although HPLC method is accepted to be a reliable method but it not suitable for total carotenoid determination. This is because the capsicum crude extract is composed of at least thirty-five different kind of carotenoids (Section. 6, Chapter. II). It is difficult for using HPLC to separate them from each other and determine its content separately. Although it may be possible with gradient elution systems, it would be very time-consuming and not practical for working on a large number of samples as caried out in this study.

Based on the spectrophotometric method, we can obtain information on the carotenoid content in various capsicum fruits. Using the same samples for capsicum fruits coutain total carotenoids in the range from 0.075% to 0.56% (dry weight) or from 0.2 to 9.4 mg on the basis of total content per fruit (Table 12). Study on the distribution of the sample population shows that most *C. annuum* fruits have carotenoid content in the range of 0.1-0.3% whereas most *C. frutescens* fruits are in the range 0.3 to 0.4%. Unlike capsaicin, no correlation between the fruit weight and the carotenoid content was observed.

4. Application of the Information on Capsaicin and Carotenoid Content in Thai Capsicum Fruits.

4.1 Capsicum Fruits as Food Additives

The fresh capsicum fruits which are traded in the markets have many physical appearance. It is difficult to specify what cultivars are the most important for

the fresh market trading because it depends on the satisfaction of each consumer. For dried capsicum fuits, they are not only traded locally but also in the whole market.

The information obtained from this study is not only useful for knowing the quality of Thai capsicum fruits but only for the production of ground chilli with different degrees of pungency. Presently, the consumption of hot chilli has been more and more popular in Japan and European countries. The Thai chilli products specified with capsaicin content would be satisfied the requirements of foreign importers.

In marking such ground chilli products with different degrees of pungency, different kinds of chilli can be blended to obtain final product characteristics which are required by the market. In this case, the information on the capsaicin content of diferent capsicum will be very useful in the blending. In addition, the solid-phase extraction and HPLC can be always used for the determination of the capsaicin content of the final products.

4.2 Capsicum Fruits for Pharmaceutical Products.

4.2.1 Colorant and Skin Protective Agents.

Some pharmaceutical products such as suppository contain natural pigments such as carotenoids from plant as a colorant. This topical carotenoid cream can be used as skin protectant (Section. 6.2, Chapter II). The capsicum cultivars which are suitable for this aim should have specific properties. These include no capsaicin but high carotenoid content, so that the skin protection cream or as colorant in pharmaceutical products such as suppository or in cosmetic products will not cause irritation to the skin. The suitable capsicum cultivars are shown in Table 17

Table. 17 The Suitable Capsicum Cultivars for Colerants and Skin Protectants.

Capsicum cultivars	Capsaicin contents (mg/fruit)	Carotenoids contents (mg/fruit)
1. Supan Buri (Far Kew) (C38) (สุพรรณบุรี พันธุ์ฟ้าเขียว)	0.000	2.790
2. Supan Buri (Bang Laung) (C36) (สุพรรณบุรี พันธุ์บางหลวง)	0.000	1.586

The two cultivars are very suitable because of no capsaicin was detected. Both of these cultivars were grown in Supan Buri but different gardens. The local name of two cultivars are "Far Keaw" and "Bang Laung" cultivar.

4.2.2 Analgesic, Counterirritant

One of the most important uses of capsicum fruits is its pungency taste. The pungency taste of capsicum is the result of capsaicin presents in the fruits. The effect of capsaicin on the skin are the analgesis and counter-irritant (Section. 5.2, Chapter II). The capsicum fruits which are suitable for this purpose should have a specific property of high a capsaicin but very low carotenoid. This raw material will be useful for the production of a highly effective drug that contains very little unwanted color when applied on the skin.

Table. 18 The Suitable CapsicumCultivars for Analgeisc and Counterirritant.

Capsicum cultivars	Capsaicin contents (mg/fruit)	Carotenoids contents (mg/fruit)	Ratio of capsaicin / caroteniods
1. Chaing Rai (C24) (เชียงราย)	2.243	0.349	6.43
2. Kanjanaburi (C25) (กาญจนบุรี)	1.213	0.293	4.14
3. Songkla (C17) (สงขลา)	1.002	0.280	3.58

Table 18 shows the capsicum cultures obtained from this study which are suitable for this purpose. These include Chiangrai cultivar because the ratio of capsicin/carotenoids is 6.43 which is the highest among the sample tested. The second is Kanjanaburi and Songkla cultivars which this ratio are 4.14 and 3.58 respectively.

CONCLUSION

From this research work of "Study on Capsaicin and Carotenoid Contents in *Capsicum* Cultivars in Thailand.", some conclusions can be drawn. First, the fruits of *Capsicum* species obtained from various parts of Thailand have many variation of capsaicin content, ranging from zero to 0.793% dry weight or from zero to 3.856 mg in one fruit. Generally, *C. frutescens* showed more percentage of capsaicin (0.478% to 0.793%) than *C. annuum* (zero to 0.534%). When expressed using the unit of total capsaicin in one fruit, *C. anuum* showed more content of capsaicin (zero to 3.856 mg) than *C. frutescens* (0.228 mg to 0.537 mg) owing to the difference in their dry fruit weight of the two species.

Second, the capsicum fruits also contain variable content carotenoids as determined by visible spectrophotometric method. Since the capsicum fruits are composed of many kinds of carotenoids, the spectrophotometry was preferred to detect the total carotenoid content in unit of β-carotene equivalent. The results showed that the carotenoid in capsicum fruits had variation, ranging from 0.065% to 0.505% dry weight or from 0.195 mg to 9.364 mg in one fruit. In terms of carotenoid content, it seems that no different in variation between the percentage of carotenoids in *C. frutescens* (ranging from 0.237% to 0.484% dry weight) and *C. annuum* (ranging from 0.065% to 0.505% dry weight). The siginificant difference between the two species was observed by means of total carotenoid in one fruits. The range of total carotenoid of *C. frutescens* were from 0.195 mg to 0.348 mg in one fruit while *C. annuum* were from 0.23 to 9.364 mg in one fruit.

Finally, in this research, we have developed the method of simple capsaicin determination by using solid-phase extraction techique to pre-purify the sample folowed by HPLC analysis of capsaicin content in various capsicum cultivars found in Thailand.

The solid-phase extraction technique for capsaicin purification was proved to be highly effective, good recovery and gave highly purity of capsaicin which showed by the similariy of HPLC chromatogram and absorption spectrum between sample after solid-phase extraction process and standard capsaicin.