CHAPTER II

HISTORICAL

1. Botanical Aspect of *Capsicum* spp.

Capsicum species are in the family of Solanaceae. This genus is comprised of two species. One is *C. frutescens* Linn. (Fig. 1). Its common names are chilli and African chilli. The other one is *C. annuum* Linn. (Fig. 2). Its common names are Tabasco pepper, Spur pepper, Long cayenne pepper etc.

Capsicum spp. originated in the Central and South America. In the fifteen century, it was introduced to Europe. By the middle of seventh century, the *Capsicum* spp. had become cultivated throughout Southern and Middle Europe to the Asian and African tropical and subtropical regions as a spice and medicinal ingredients.

Capsicum frutescens is a shrubby perennial with angled stem and branches, broadly ovate-acuminate leaves, axillary greenish-white or white flowers with peduncles, the calyx cup-shaped, embracing base of fruit multiple pods per node, the corolla rotate and often with ocherous marking in the throat. The fruit is very pungent, oblong-conical berry, up to 30 mm. in length (Youngken, 1950).

Capsicum annuum is almost alike *C. frutescens* but different in some aspects. *C. annuum* is generally one year alive plant, single pod per node, purple, white or purple white flower. The fruit is not or mild to moderate pungent, up to 150 mm in length (Jensen *et al.*, 1979, Eshbaugh *et al.*, 1983).

Fig. 1 *Capsicum frutescens* Linn.

Fig. 2 *Capsicum annuum* Linn.

2. Microscopy of Capsicum Fruit.

A transverse section of the pericarp of capsicum fruit mounted in chloral hydrate solution shows the following characteristics (Fig. 3) ; **a)** Epicarp, and outer layer of mostly rectangular to quadrangular epidermal cells up to 80 micrometer in length and up to 20 micrometer in depth, whose outer walls and greater portion of the radial walls are cutinized. The radial walls are somewhat wavy.; **b)** Mesocarp, composed of several layers of parenchyma cells, many containing yellowish-red oil globules and chromoplastids, others micro-crystals of calcium oxalate. Coursing through this region are a few bicollateral bundles with spiral and pitted tracheae.The innermost layer of cells of the mesophyll consists of very large cells (giant cells).; **c)** Endocarp, comprising a single row of cells, some of which are sclerenchymatous, others with thin, cellulose walls. The former occur directly beneath to lumina of the giant cells and have thick, lignified, porous walls.

A surface section of the epicarp exhibits rows of quadrangular or rectangular, parallel, mostly four sided, straight-walled epidermal cells, having a finely striated cuticle.

A surface section of the endocarp exhibits oval to elliptical shaped groups of stone cells separated by narrow zones of thin-walled cells containing chromoplastids. The stone cells are more or less elongated, moderately thickened, porous and slightly lignified and have beaded, wavy, vertical walls.

A transverse section of the dissepiment shows a central zone of tangentially-elongated, more or less collapsed cells, some of which contain micro-crystals. Fibrovascular tissue courses through this region. Flanking this zone on either side is an epidermis of radially elongated cells containing capsaicin (Youngken, 1950), a

pungent principle. The outer walls of the epidermal cells are cutinized. Here and there, when the cuticle has been puckered up, it is possible to see oily droplets of capsaicin.

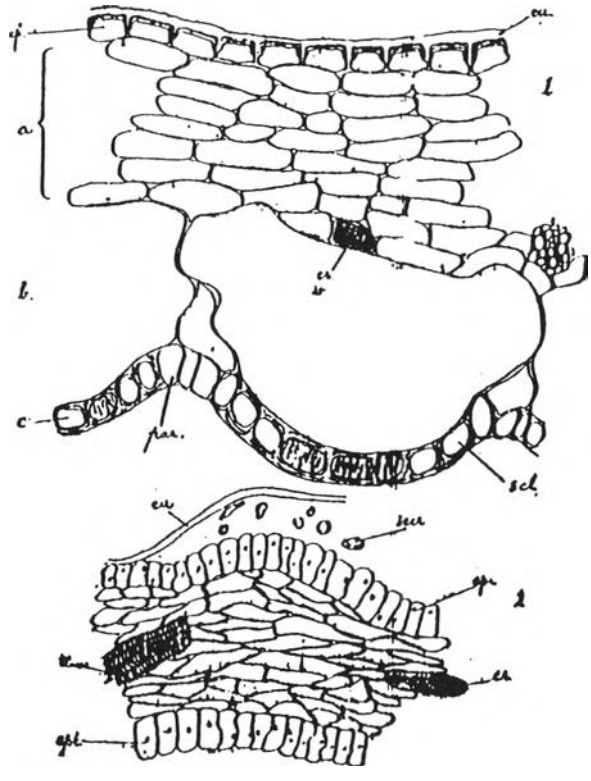

Fig. 3 Capsicum. 1. transverse section of pericarp; *a.* mesocarp parenchyma; *b.* large-celled layer of same; *c.* inner epidermis or endocarp; *cr.* microcrystals; *par*parenchymatous cells and *scl.* sclerenchymatous cells of endocarp. 2. transverse section of dissepiment; *cr.* microcrystal; *cu.* cuticle, raised by the secretion, *secr.* ; *epi.* epidermis; *v.* vascular bundle (Youngken, 1950).

A transverse section of the seeds exhibits the following peculiarities; **a)** Spermoderm, outer epidermis showing large, more or less radially elongated, stone cells with irregularly thickened radial and inner walls. The radial walls of each of these show a thickening of lignin which gradually increases toward the inner wall. The inner wall is extremely thickened in the angles, less so in the center. The epidermal stone cells along the edge of the seed possess much thicker walls than those on the flat surfaces. A narrow zone of collapsed parenchyma. Inner epidermis of row of tangentially elongated epidermal cells.; **b)** Endosperm, a broad zone of reverse parenchyma, composed of more or less thick-walled, polygonal cells containing aleurone grains.; **c)** Embryo, appearing in this kind of section as more or less circular areas of cells lined with epidermis and embedded in the endosperm. The cotyledons may be distinguished from the hypocotyl by appearing as a two plana-convex masses with plane faces opposite, in the same circular area.

Powdered capsicum (Fig. 4) is dark orange, dark reddish-orange to strong yellowish brown, numerous fragments of thin-walled parenchyma containing oil globules and orange, red or yellow chromoplastids, fragments of epicarp with either striated, rectangular cells arranged in paralled rows, or with polygonal, triangular or irregular cells with or without beadded walls, endocarp stone cells with slightly wavy, beaded, lignified walls and broad lumina, numerous fragments of spermoderm composed of stone cells, showing in surface view, deeply sinnate, greatly thickened and lignified vertical walls containing numerous pore canals and, in vertical sections, beaker-shaped cells whose radiol and inner wall are more thickened than the outer walls, few fibers, fragments of small-celled parenchyma of endosperm and embryo, most of the cells of which possess fixed oil aleurone grains, numerous globules of yellowish-red oil, a few spiral and pitted tracheae, cell containing micro-crystals, occasionally tissues of the calyx and peduncle and a few more or less spheroidal starch grains from unripe fruits (Youngken, 1950).

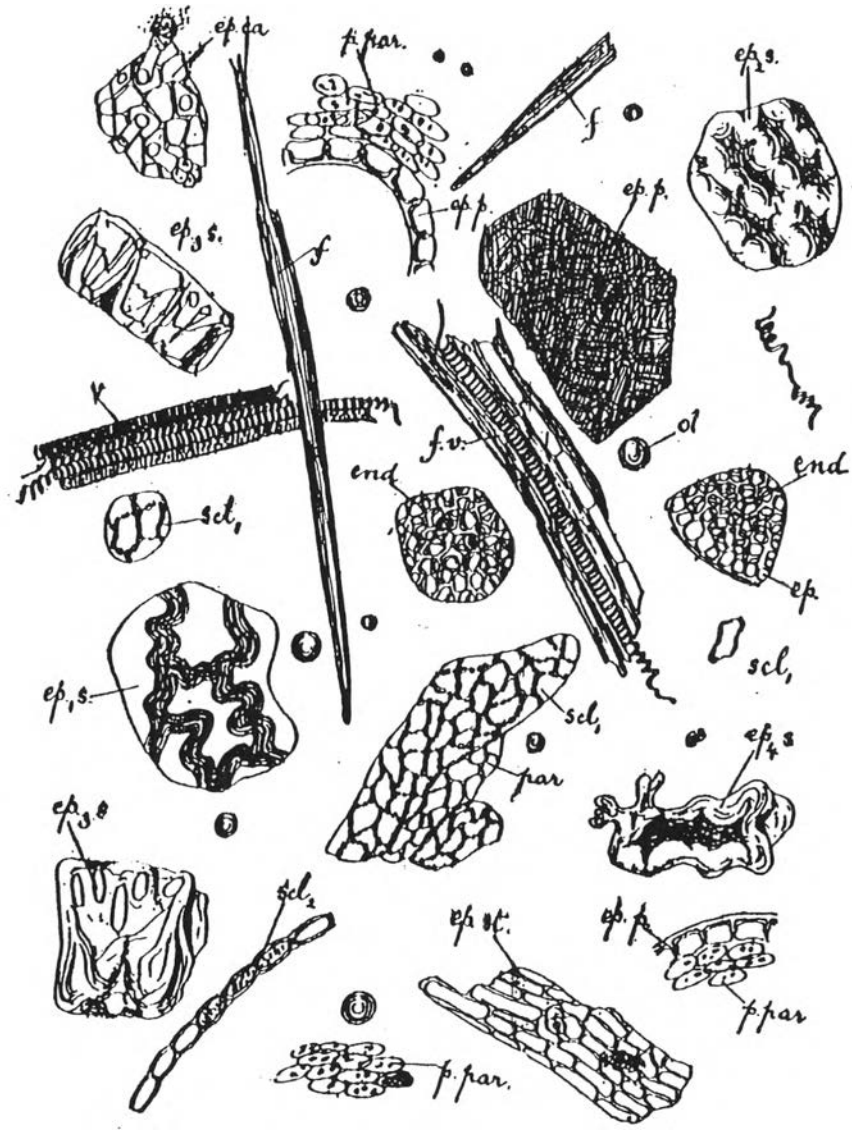

Fig. 4 Powdered Capsicum. *end.* endosperm; *ep.* epidermis of same; *ep.ca.*. upper epidermis of calyx; *ep.p.* epicarp; *ep.1s.* epidermis from flat surface of seed; *epi.2s.* epidermis from edge of seed; *ep.3s.* epidermis of seed showing one stone cell, side view; *ep.4s.* isolated epidermal cell of seed coat; *ep.st.* epidermis of stalk; *f.* sclerenchyma fibers; *f.v.* fibrovascular bundle; *ol.* oil; *par.* parenchyma of endocarp; *p.par.* parenchyma of mesocarp; *scl1.* sclerenchyma of endocarp, seen from above; *scl2.* the same, side view (Youngken, 1950).

3. The Uses of Capsicum

The domesticated capsicum is used around the world in various forms such as dried powders of different colors, paprika, tabasco sauce, pungent chilli peppers. Capsicum has high nutritional value, they are good sources of vitamins, particularly vitamin C and A (ชัยโย ชัยชาญทิพยุทธ, 2524). Although the fresh market value of capsicum is not as great as that of the processed formed use in the spice industry, consumption of the whole fruits is growing in popularity, especially amoung the mildly pungent types (Table 1).

In terms of economic importance, green peppers grown in the Southeastern portion of the United States (Texas, New Maxico and California) represent a crop value totaling in excess of $ 90 million annually (Lorenz, 1980). In South and Central America, capsicum just behind tomatoes in economic importance. A similar situation is developing in European countries such as Yugoslavia, Czecho slovakia and Bulgaria where sweet peppers are becoming increasingly popular.

Perhaps the most important use of capsicum is for spice industry. An example is the manufacture of pepper from special varieties of *C. annuum.* Types of pepper include sweet, semi-sweet, mildly pungent and pungent, depending on the varieties used. Although a large amount of the spice is manufactured in the United States, a considerable portion is exported to the United States and other parts of the world from Spain.

Table .1 Cultivars of Capsicum Important to the Food Industry.

Fruit type	Varieties	Flavor	Uses
Bell	California Wonder	Sweet	Fresh market
large block fruit	Yolo Wonder		
thick flesh	Keystone Giant		
Pimiento	Pimiento	Sweet	Fresh market
heart shaped	Pimiento Select		
medium sized	Pimiento		
thick flesh	Perfection		
Ancho	Maxican Chili	Mildly pungent	Fresh market
long, heart shaped	Ancho		& processing
with thin flesh	Mulato		
Anahelm Chili	Sandia	Mild to	Processing
fruit elongate	Anahelm Chili	moderatly	
tapering to point,	California Chili	pungent	
medium thick	Mild California		
flesh	New Maxican Chili		
Cayenne	Cayenne Long	Pungent	Fresh market
thin flesh,	Red		& processing
Irregular shape	Cayenne Long		
	Thick		
	Cayenne Long		
	Slim		
Cuban	Cuban	Mildly	Fresh market
long, thin flesh	Cabanelle	pungent	& processing
blent ends	Pepperoncini		
	Aconcagua		
Jalapeno	Jalapeno	Pungent	Fresh market
long, cylindrical	Mild Jalapeno		& processing
Serrano	Serrano	Pungent	Fresh market
slender, cylindrical,			
taper to a point			

In addition to the production of pepper, other varieties of *C. annuum* are use to make similar types of spices such as cayenne and chilli powder that are marketed alone or as mixtures with other flavors. They are also used in the manufacture of various sauces and catsups. One special type of sauce is produced from powdered derived from a single variety of *C. frutescens*, namely tabasco sauce. Capsicum have also become increasingly important in the pickling industry as seasoning and as whole fruits.

Medicinally, capsicum is used as a stimulant, counter-irritant and stomachic. The preparation for stomachic is almost in ethanolic extraction form such as tincture of capsicum N.F. Nowadays, the stomachic drug such as flatulence^{R} uses capsicum powder as one of active ingredients. For external use as counter-irritant, the capsicum is combined in the analgesis cream such as Sloan's balm^{R}.

4. Chemical Constituents of Capsicum.

Capsicum fruits contain capsaicinoids, the group of pungent principles with the major substance of capsaicin. Capsaicin content in many varieties of capsicum varies from zero to about one percent (Kosuge, 1970). The fruit also contain color principles of carotenoids which is about 0.02 to 0.5 percent. Moreover, very little amount of volatile oil can be found in capsicum fruit (Youngken, 1950). In terms of nutrition, 100 gm of capsicum fruit has been reported to contain 10.3 calories, 2.4 gm fat, 19.9 gm carbohydrate, 6.4 gm fiber, 4.7 gm protein, 45 mg Ca, 85 mg P, 2.5 mg Fe, 11,050 units vitamin A, 0.24 mg vitamin B1, 0.29 mg vitamin B2, 2.10 mg niacin and 70 mg vitamin C (สมพร หิรัญรามเดช, 2525).

5. Capsaicin

5.1 Structure and Some Chemical Properties

Capsaicin [N-(4-hydroxy-3-methoxyphenyl)methyl)-8-methyl-6-nonenamide] : $C_{18}H_{27}NO_3$; MW 305.40 (Fig. 5).

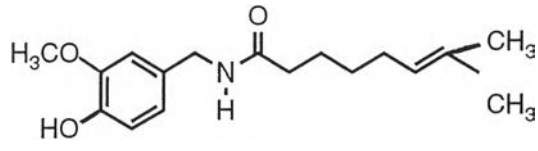

Fig. 5 The Structure of Capsaicin

Capsaicin is a major component of pungent principles that found only in *Capsicum* spp. The other pungent substances are capsaicin analogs that are similar structure. Figure. 6 and Table. 2 summerizes the structures of capsaicin and its various analogs (Cordell, 1989).

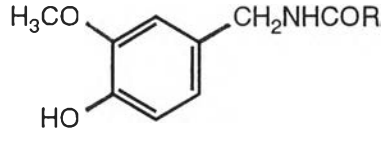

Fig. 6 The General Structure of Capsaicinoids

Table 2 Acyl Moieties of Capsaicinoids.

R	Name of Acyl Moieties	Scientific Names
$(CH_3)_2CHCH = CH(CH_2)_4$-	8-methylnon-*trans*-6-enoic	capsaicin
$(CH_3)_2CH(CH_2)_4$-	8-methylnonanoic	dihydrocapsaicin
$(CH_3)_2CH(CH_2)_5$-	7-methyloctanoic	nordihydrocapsaicin
$(CH_3)_2CHCH = CH(CH_2)_5$-	9-methyldec-*trans*-7-enoic	homocapsaicin
$CH_3CH_2(CH_3)CH(CH_2)_6$-	8-methyldecanoic	homodihydrocapsaicin

Capsaicin is odorless white needle crystal with severe burning pungency, melting point is at 64.5°C, boiling point is 210-220°C at 0.01 mm Hg and sublimate at 115°C. The ultraviolet maximum absorption was at 227, 281 nm (E = 7000, 2500). It is easily soluble in ethyl ether, ethyl alcohol, acetone, methyl alcohol and hot alkali, practically insoluble in cold water (Windholz, 1976). By alkaline hydrolysis with 25% sodium hydroxide at 180°C in an autoclave for 30 min, capsaicin gives 8-methylnon-6-*trans*-enoic acid and pyrocatechin or other decomposed phenolic products from vanillyamine with release ammonia (Saria, 1981). The data of absorption spectrophotometry and mass spectrometry have been shown in Fig. 7 and Fig. 8 (Kosuge, 1970).

5.2 Biological Activities of Capsaicin

The pungent principle in capsicum has been used as spice or as pharmaceutical aid for a long time. However, nobody knows the biological significance of capsaicin in the plant. There have been no reports elucidating the biological significance of capsaicin in the plant by showing reasonable scientific evidence. Some propose that capsaicin is nothing more than a waste product without any biological significance. Others speculate that it would have been formed to keep enemies such as birds or insects away from capsicum fruits to protect seeds and still others insist that capsaicin may have been formed to act as a phytoalexin or as an antiseptic. Although the biological significance in plant cannot identified, the biological activities in human is very attractive. There are more than 200 out of 500 papers concerning capsaicin have been related to pharmacological studies, most of which have been published over the past 15 years. All of these pappers are concluded and described in the following section..

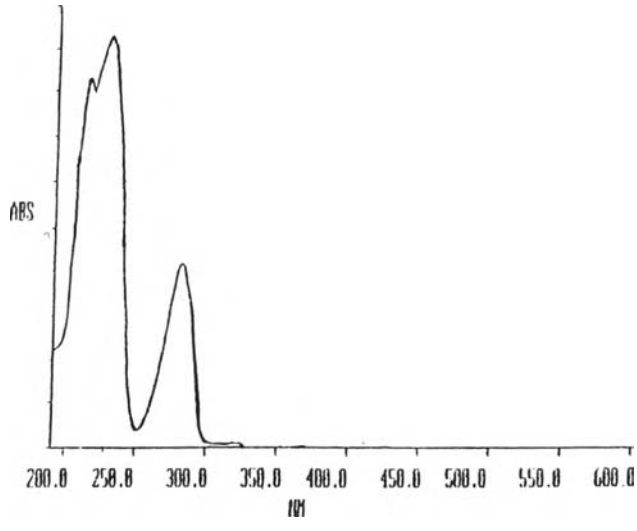

Fig. 7 The Absorption Spectrum of Capsaicin.

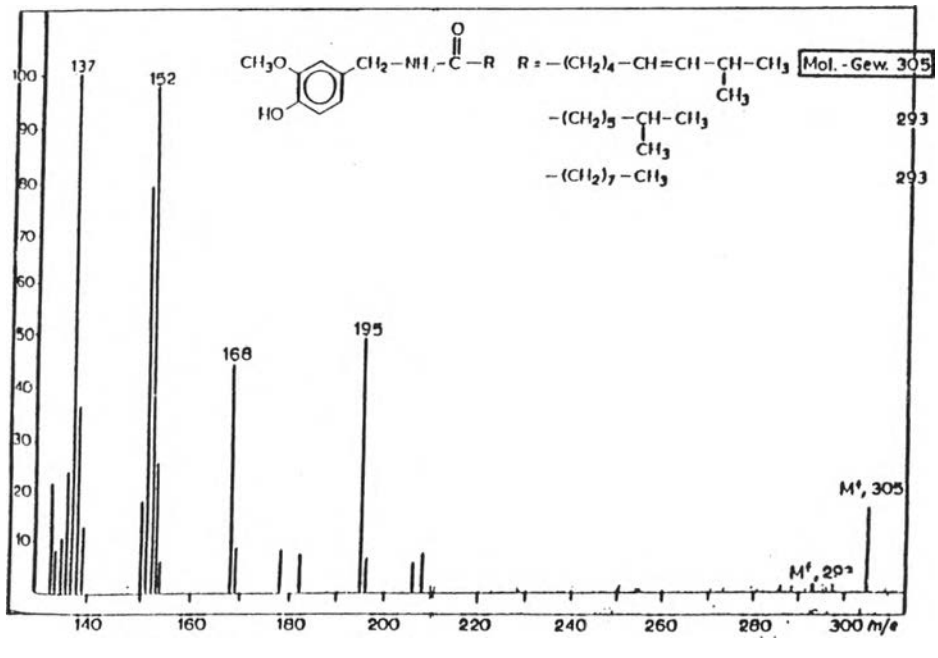

Fig. 8 Mass Spectrometry of Capsaicin (Kosuge, 1970).

5.2.1 Pharmacological Activities

Capsaicin has many effects on gastrointestinal tract both in long-term and acute effects. For the long-term effect, capsaicin stimulates the gastric juice secretion, which may occasionally cause ulcers. For the acute effect, capsaicin shows a laxative effect on the colon (Molnar, 1965; Szolcsanyi and Jancso-Gaber, 1975 ; Bartho *et al.*, 1982).

The effect of capsaicin on the cardiovascular and respiratory systems has been studied for a long time. The effect of capsaicin on blood pressure and respiration were reported as early as 1928 and 1935 (Fukuda, 1969). Extreme stimulation of respiration as well as vasoconstriction caused by injection of capsaicin to the perfusion fluid. The pulse increased in blood pressure was accounted for as a reflex increase of blood pressure similar to that produced by acetylcholine (Toda *et al.*, 1972). For the effect on respiratory system, capsaicin caused reflex bronchoconstriction that produced by pulmonary receptors sensitive to capsaicin that are accessible, *via* pulmonary circulation

A drop in body temperature induced by capsaicin administration was reportedly observed as early as in 1978. The steep drop in body temperature of mice, rats and guinea pigs caused by intraperitoneal or subcutaneous capsaicin administration seemed similar to that caused by histamine (Jancso-Gaber , 1970).

The antifungal effect of capsaicin has been reported against *Zygosaccharomyces* spp. and *Mycoplasma agalactiaee*. Moreover, the antibacterial activity of capsaicin showed bacteriostatic activity against several bacterial species, remarkably against *Bacillus cercus* and *B. subtilis*, at 1/10,000 dilution (Gal, 1965).

5.2.2 Toxicity of Capsaicin

Capsaicin not only causes strong irritation and inflammation of the skin, mucous membrane and eyes, but also show acute total toxicity when a large dose of capsaicin is given at one time (Winek *et al.*, 1982). A large amount of orally administered capsaicin causes damage to the gastrointestinal tract (Nopanitaya, 1974). Besides gastrointestinal toxicity, capsaicin induces desensitization to various stimuli especially when neonatally administered (Szolcsanyi, 1975).

5.3 Extraction and Purification

Amoung various analytical procedures, the extraction method may be the only one in which traditional procedures are still being used. Extraction is the first step in capsaicin analysis. The Joint Committee established in 1959 suggested two recommended procedures for the extraction of capsaicin from capsicum fruits. The procedures involve preparation of oleoresin by extraction capsicum powder with 96% ethyl alcohol for 48 hours followed by purification on aluminum oxide with activated charcoal and kieselguhr, and extraction and purification by ether-alkali partition extraction (The Joint Committee of Pharmaceutical Society and the Society for Analytical Chemistry on the Methods of Assay of Crude Drugs, 1959).

5.4 Determination

Organoleptic evoluation was the first method applied to identification and quantitation of capsaicin. Despite being criticized for low accuracy and poor reproducibility, organoleptic method has been proposed since the early day of capsaicin research (Jurenitsch and David, 1979).

The different spectrophotometric was recommended by the Joint Committee of Pharmaceutical Society and the Society for Analytical Chemistry and the TLC or column chromatography were applied for this purpose (Kraus, 1969; Todd, 1975). By

TLC separation, capsaicin is located as yellow spot on the browish background by spraying with a solution of potassium permanganate in a 2% sodium carbonate solution (Benett , 1968).

Nowadays, a new technique of separation have been offered. Gas chromatrography (GC) and High performance liquid chromatrography (HPLC) are of interest because of its high sensitivity, separatility and speed. GC analysis is very complicate because of many steps of purification and derivatization (DeCecco, 1976; Lee *et al.*, 1976; Pokharkar and Dethe, 1981).

High performance liquid chromatrography (HPLC) is a powerful analytical technique that has been applied to capsaicin determination. The adventage of capsaicin analysis by HPLC is that it does not require modification of capsaicin prior to injection (Iwai et al., 1979; Jurenitsch and Kampelniihler, 1980).

5.5 The Proposed Biosynthetic Pathways of Capsaicin

The biosynthetic pathway of capsaicin has been proposed to proceed by the usual pathway, known as the phenylpropanoid pathway. Its originates by enzymatic changes of phenylalanine to many intermediates to the end of vanillylamine part of capsaicin (Leete, 1968; Fujiwake *et al.*, 1980; Rangoonwala, 1969). The fatty acid side chain of capsaicin originates from valine that is converted to α-ketoisovalerate, then to isobutyryl CoA after decarboxylation, followed by conversion to even-numbered acyl moieties after chain elongation (Kawada *et al.*, 1985). The acyl-CoAs and vanillylamine are condensed with capsaicinoid-synthetase which yield capsaicin and its analogs (Neumann, 1966) (Fig.9).

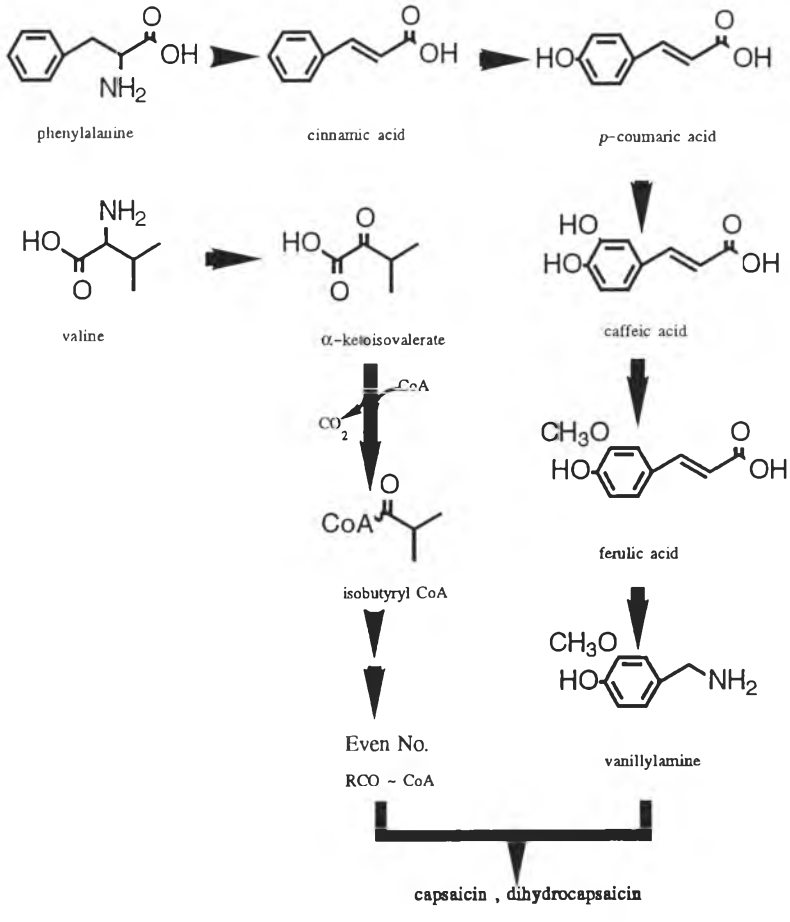

Fig. 9 Biosynthesis Pathways of Capsaicin and Analogs.

6. Carotenoids of Capsicum

6.1 Composition of Capsicum Carotenoids.

The carotenoids of capsicum are obtained by extraction of dried riped capsicum with many kinds of organic solvents. The carotenoid extract has been used commercially in form of oleoresin. The total carotenoid content in capsicum extract is approximately 0.1-0.5 percent (Christopher, 1981). Capsanthin and capsorubin are generally considered to be the main carotenoids in capsicum whereas other minors are composed of at least 30 kinds of carotenoid species (Barber et al., 1961).

The composition of capsicum carotenoid has been studied intensively (Lord and Tirimanna, 1976). The individual carotenoids isolated from red riped fruit of capsicum are summerized in Table 3 and some of their structures are shown in Fig. 10.

6.2 Biological Activities of Carotenoids.

The function of carotenoids in plant is believed to be a protective agents againt photosensitization (Krinsky, 1971). Carotenoids are used by human as a source of vitamin A (Goodman and Ltuang, 1966). This group of compounds is also used for coloration of human skin. This property, of accumulating in human skin, has led to another medical use of carotenoids in man, as light-protective agents in patient with photosensitivity dieseases (Mathews , 1964; Frossberg et al., 1959).

In pharmaceutical products, carotenoids act as coloring agents for many medical preparations, such as β-carotene and canthaxanthin in sugar-coated tablet colors, coloring of suppositories (Munzel and Fuller, 1961), soft and hard gelatin capsules.

Table. 3 The Carotenoid Composition in Red Riped Fruit of Capsicum.

Carotenoids	Spectral Absorption Maxima (nm)
Phytoene	298,286,276
Phytofluene	366,348,332
α-Carotene	436,456,431,339
β-Carotene	447,449,424,338
ξ-Carotene	423,397,377
Mutatochrome-like	451,428,402
Hydroxy-α-carotene	473,444,420,332
Cryptoxanthin	476,447,422,338
Hydroxy-α-carotene-like	473,445,423,333
Cryptoflavin-like	451,426,402
Cryptocapsin	497,470,445,353
Capsolutein	486,455,430,339
Zeaxanthin	493,463,437,346
P-482, diol	482,451,424,336,320
Capsolutein 5,6-epoxide	483,451,425,336,322
Antheraxanthin	487,456,431,338
Capsolutein 5,8-epoxide	459,430,406,318,303
Mutatoxanthin	464,437,412,320
Violaxanthin	484,452,425,336,321
Luteoxanthins	460,431,406,318,303
Capsanthin	510,483,363
P-441, tetrahydrocapsorubin	441,414,392
Hydroxycapsolutein	486,456,430,338
Capsanthin 5,6-epoxide	509,478,455,357,344
Capsochrome	483,456,431,345
Capsorubin	522,487,460
Hydroxycapsolutein 5,6-epoxide	482,449,421,336,321
Neoxanthin	478,447,421,335,321
Trolliflor-like	482,449,423
Carbonyl	458
Hydroxycapsanthin-like	478,358

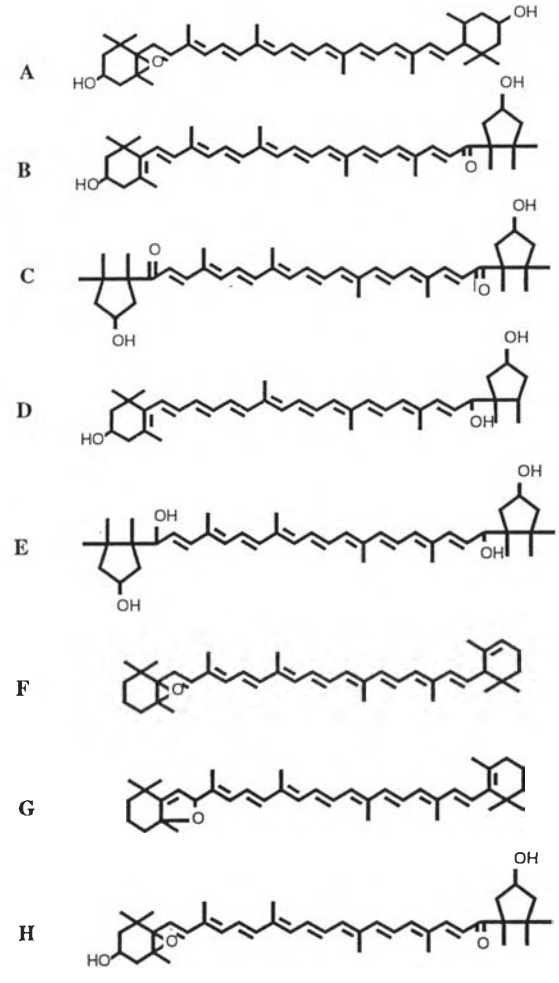

Fig. 10 The structures of some carotenoid pigments in capsicum : A, antheraxanthin; B, capsaxanthin; C, capsorubin; D, capsanthol; E, capsorubinol; F, a-carotene 5,6-epoxide; G, mutatochrome; H, capsanthin 5,6-epoxide

6.3 Extraction and Purification of Capsicum Carotenoids

A simple method of extracting carotenoids from capsicum tissue involves blending or grinding the tissue in sequential portion of acetone, each portion of acetone being removed by decantation of filtration before a new portion is added. Relatively large amounts of acetone are required, and the extracted pigments must be transferred to a hydrophobic solvent before subsequent steps may be taken. To purify carotenoids from fats and oils may be simple or difficult, depending on the amount of carotenoids and other material. Carotenoids in fats and oils are in solution and may be measured by direct spectral measurement in the absence of interfering material. This is possible to

apply oil samples to chromatographic column and isolate carotenoids (Simpson et al., 1964). Better separation can be obtained by removing the neutral fat fraction by saponification

6.4 Determination of Capsicum Carotenoids

Quantitation of carotenoids is based on spectral absorption of the compounds. The visible spectrophotometry method has been used for this purpose since it is rapid and easy (Christopher, 1981). High performance liquid chromatrography (HPLC) have been used to separate individual carotenoids both in normal and reverse phase (Stewart and Wheaton, 1971). This method however is complicated and time-consuming.

6.5 The Proposed Biosynthetic Pathway of Carotenoids

The earliest investigations on the biosynthesis of carotenoids were carried out before 1950. The carotenoids that constitute a large group of naturally occuring pigments are formed by the condensation of eight isoprenoid units called "phytoene". The formation of phytoene can be devided into three stages; **(a)** the formation of isopentanyl pyrophosphate (Fig. 11), **(b)** the conversion of isopentanyl pyrophosphate to geranylgeranyl pyrophosphate (Fig. 12), **(c)** the condensation of two molecules of geranylgeranyl pyrophosphate to form phytoene. Phytoene can be converted to lycopene (Fig. 13), which is a precursors of many kinds of carotenoids in capsicum (Fig. 14) (Camera, 1980, Camera and Monegor,1981).

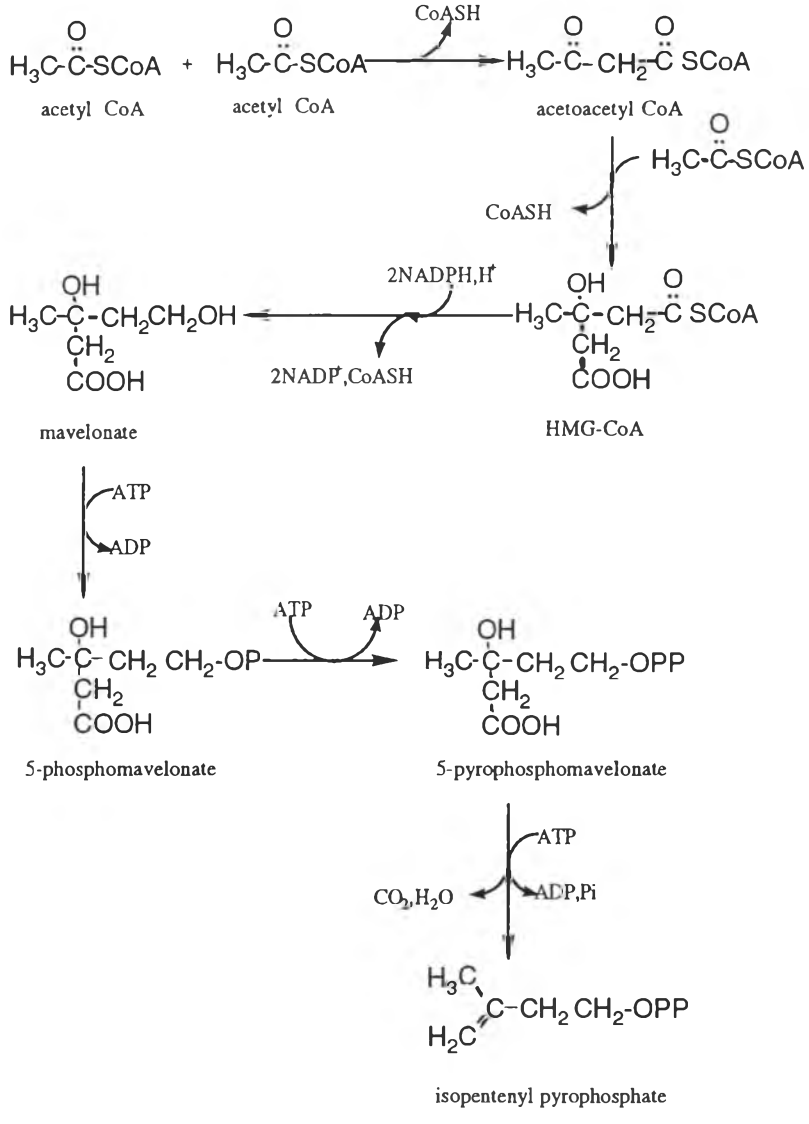

Fig.11 Pathway of Formation of Isopentenyl Pyrophosphate from Acetyl CoA.

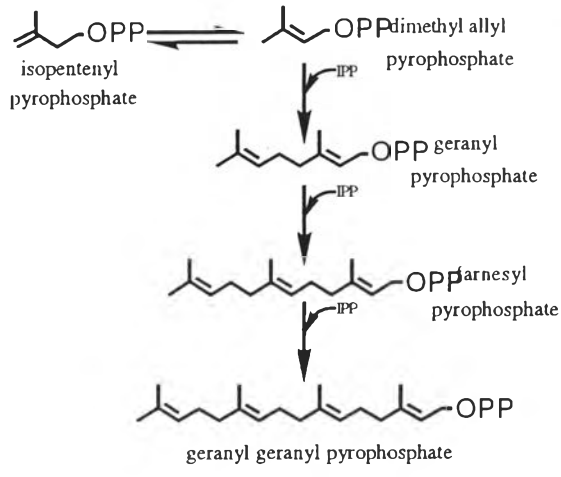

Fig. 12 Pathway of Conversion of Isopentenyl Pyrophosphate to Geranylgeranyl Pyrophosphate

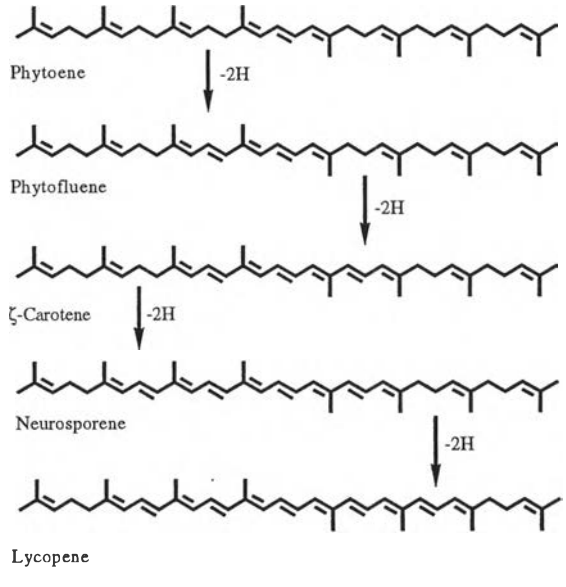

Fig. 13 Pathway of Conversion of Phytoene into Lycoene.

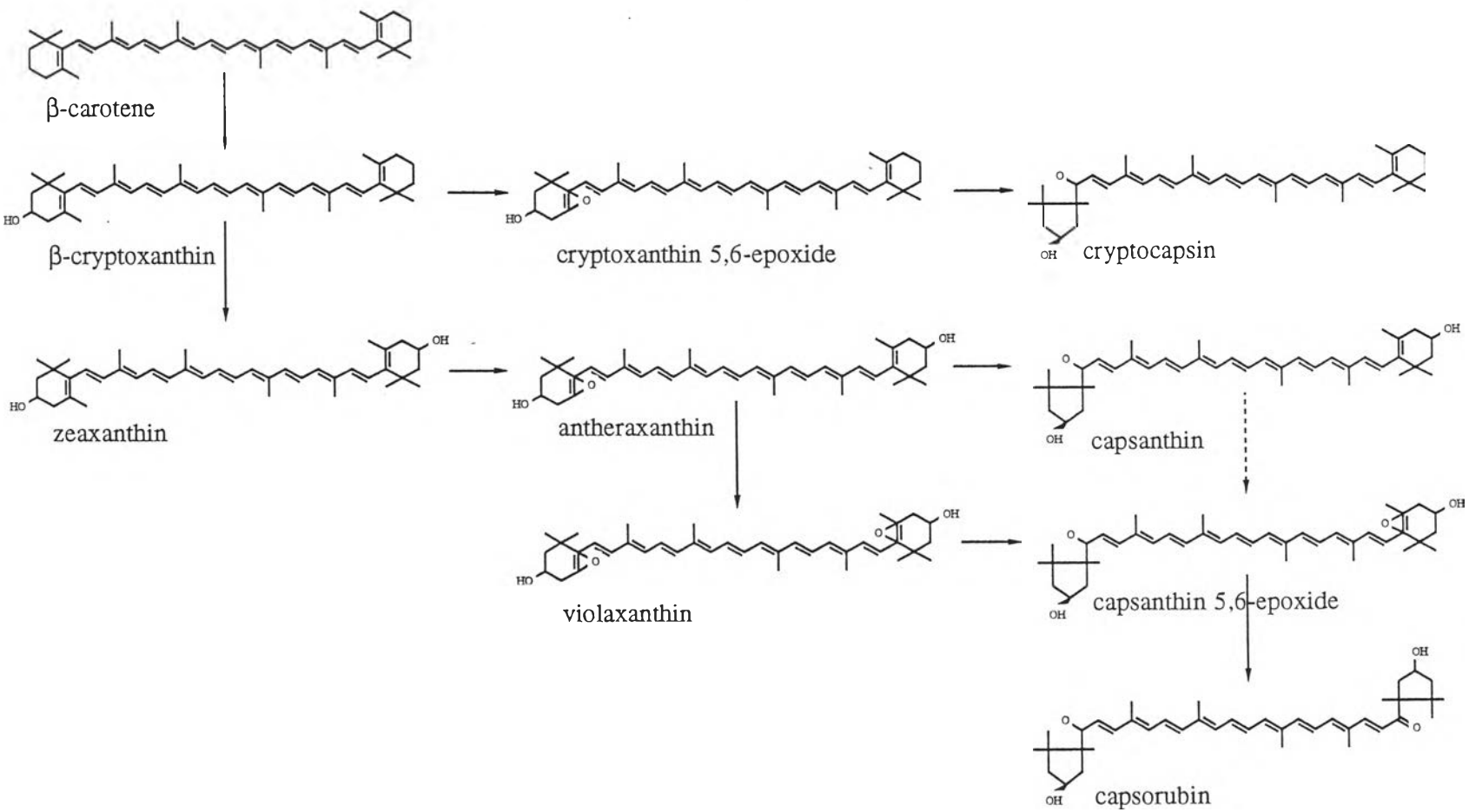

Fig. 14 Possible Pathway of the Biosynthesis of Cryptocapsin, Capsanthin, Capsanthin 5,6-epoxide, and Capsorubin.

7. Introduction to Solid-phase Extraction Technique.

The solid-phase technique is the method which is use to purify the crude extract to get rid of some impurities. This technique is composed of two major components. The first is the absorbent that usually packed in the minicolumn called "sorbent bed". The second is the suitable solvent for washing or cleaning the sample which concentrate on the sorbent bed. This technique is widely use because of many kind of sorbent beds and solvents could be selected to use for highly purify samples.

The solid-phase extraction is not a really new technique but it base on the classical method, "column chromatrography". The different between column chromatrography and solid-phase extraction are the column chromatrography almost use a long time for large scale purification of as " quanlitative analysis" but the solid-phase extraction almost use a short time for mini-scale purification or as "quantitative analysis".

The crude sample extract can be classified in two major parts. The first is the component of interest which we want to purity from the crude extract called "isolate". The second is the other components which we want to eradicate called "impurities". When the crude extract solution passes through the sorbent bed, the isolate will be trapped and concentrated on the sorbent surface, while the other components pass through the bed. Very seclective extraction resulting in highly purified and concentrated isolate can be achieved by choosing a kind of sorbent with an attraction for the isolated but not for the other sample components and by choosing solvents which can eluting only isolate but let the other components trap in sorbent bed (Fig. 15).

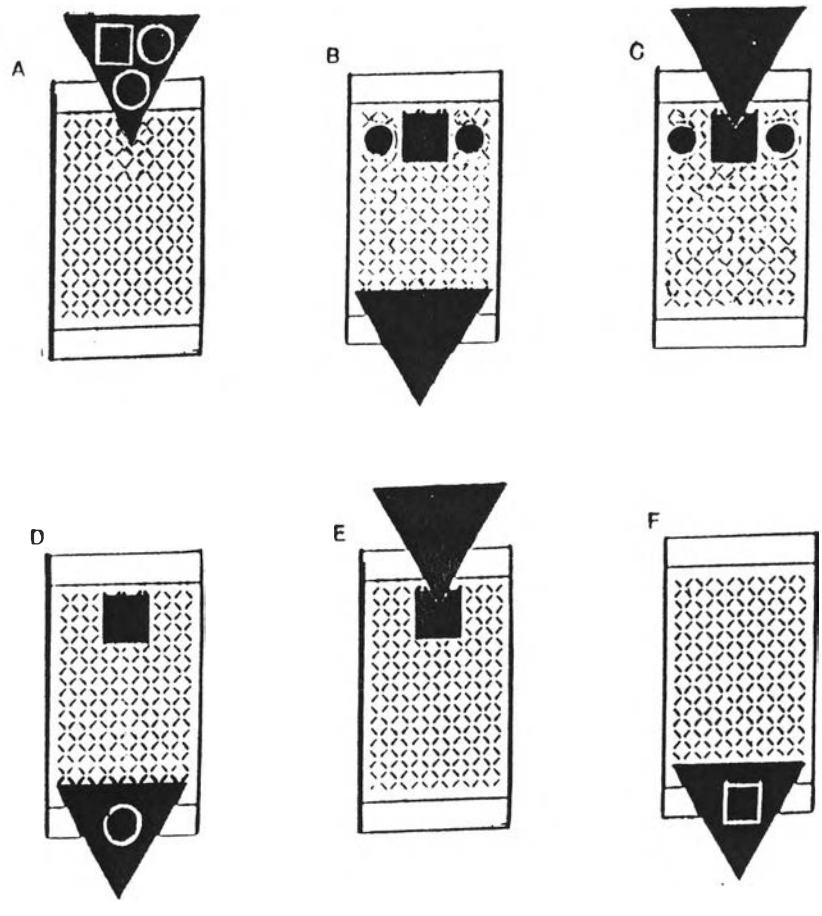

Fig. 15 The Basis of Solid-phase Extraction Technique.

A. A crude extract is loaded into the sorbent bed of solid-phase extraction column

B. The isolate (■) and impurities (●) are trapped and concentrated in the sorbent bed

C. The suitable (washing) solvent is loaded into the bed to get rid off the impurities.

D. The impurities are get rid off the bed by suitable solvent and left the isolate trapped in the bed.

E. The selected (eluting) solvent is loaded into the bed to eluted the isolate.

F. The highly pure isolate is eluted from the bed which is ready for analysis.