CHAPTER II

LITERATURE SURVEY

2.1 *Chlorella* spp.

Chlorella spp. are usually identified as unicellular spherical cells, less than 10 μm in diameter. Each cell contains a cup-shaped chloroplast with one parietal or more pyrenoids. Cells reproduce by production of 2, 4 or 8 autospores, released by equal or unequal fracture of cell wall. (John et al., 2003)

List of some *Chlorella* species deposited at ATCC

Chlorella ellipsoidea Gerneck

Chlorella kessleri Fott and Navakova

Chlorella luteoviridis Chodat

Chlorella miniata (Naegedi) Get manns

Chlorella protothecoides Kruger

Chlorella pyrenoidosa Chick

Chlorella saccharophila (Kruger) Migula var. *ellipsoidea* Gerneck

Chlorella saccharophila var. *saccharophila*

Chlorella sorokiniana Shihira and Krauss

Chlorella vulgaris Beijerinck

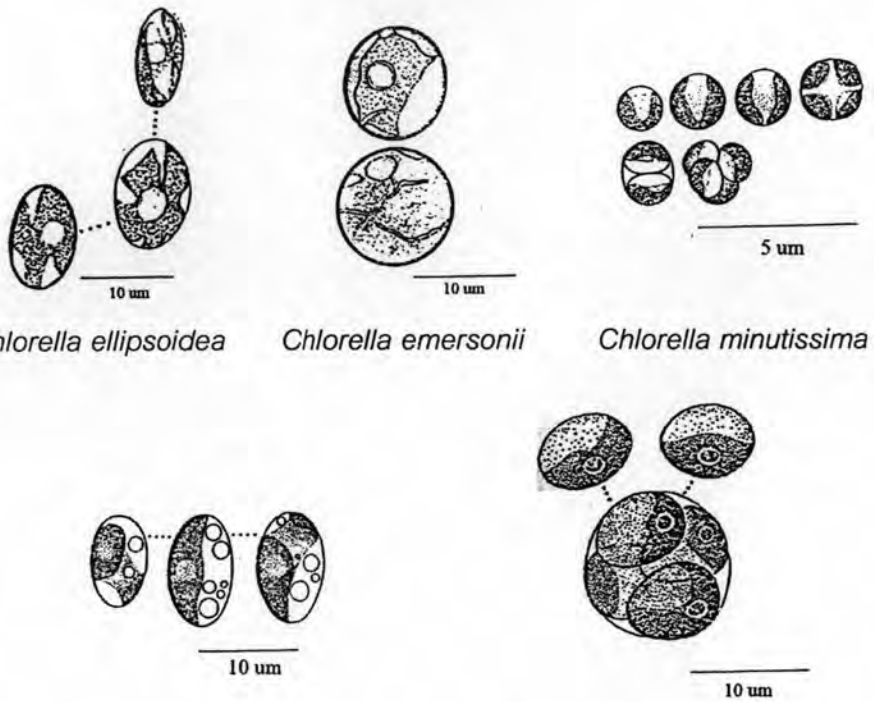
Chlorella vulgaris var. viridis Chodat

Chlorella variegata Beijerinck

Chlorella xanthella Beijerinck

Chlorella zofingiensis Donz

In 2003 John et al. listed 5 *Chlorella* spp. from the British Isles as shown in Figure 2.1. The authors reported that *Chlorella saccharophila* (Krufer) Migula var. *ellipsoidea* Gerneck was synonymous with *Chlorella saccharophila* (Kruger) var. *saccharophila*.



Chlorella ellipsoidea *Chlorella emersonii* *Chlorella minutissima*

Chlorella saccharophila *Chlorella vulgaris*

Figure 2.1 Different species of *Chlorella* spp. reported from the British Isles (John et al., 2003).

From Figure 2.1 *Chlorella* spp. could easily be identified by size and morphology. For example, the shape of cells of *C. ellipsoidea* and *C. sacchorophila* is oval. The chloroplasts are trough-like or band shaped with lobes present in mature cells of *C. ellipsoidea*. Although cells of *C. vulgaris* are oval, their chloroplasts are cup-shaped. *C. emersonii* cells are spherical with chloroplast filling most of the cells. One large pyrenoid is present in each cell. *C. minutissima* is distinctive in that the cells are relatively small with no pyrenoids in chloroplasts which are either cup-shaped or saucer-shaped.

2.2 PCR fingerprints of *Chlorella* spp.

Although some *Chlorella* spp. can easily be identified by size and morphology, in 2004 Nonticha Jamkangwan used RAPD-PCR fingerprints of 6 *Chlorella* spp. strains to show that *Chlorella* spp. strains with similar morphology could differ genetically. For example *Chlorella* sp. TISTR 8852, *Chlorella* sp. TISTR 8853, *Chlorella* sp. TISTR 8854,

Chlorella sp. TISTR 8855 and *Chlorella vulgaris* var. *vulgaris* NIES-686 had the same morphology but their PCR fingerprints with either 27f or 1492r as the primer were different.

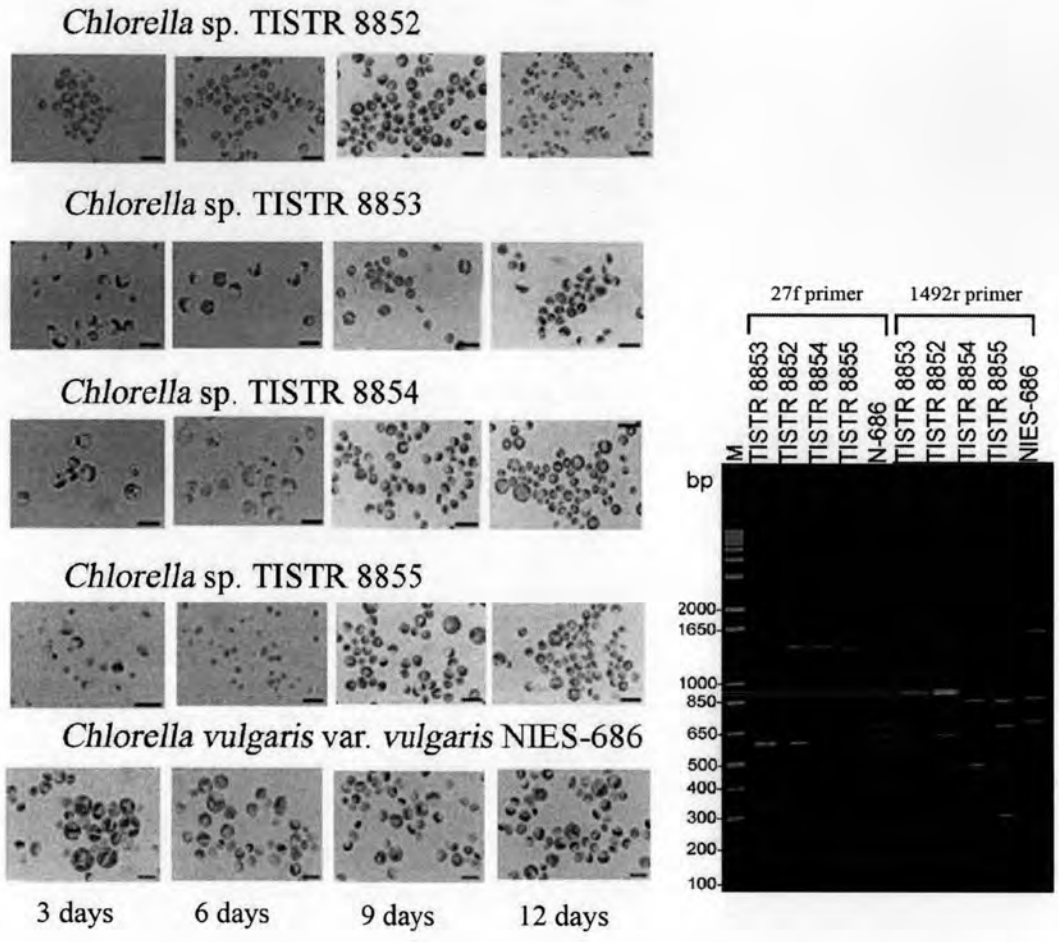


Figure 2.2 PCR fingerprints and morphology of *Chlorella* sp. TISTR 8852, *Chlorella* sp. TISTR 8853, *Chlorella* sp. TISTR 8854,*Chlorella* sp. TISTR 8855 and *Chlorella vulgaris* var. *vulgaris* NIES-686 in BG11 medium at 3, 6, 9 and 12 days (Nonticha Jamkangwan., 2004).

Therefore, it is necessary to employ combination of morphology and molecular biology techniques in strain identification of *Chlorella* spp.

2.3 *Scenedesmus* spp.

Scenedesmus spp. are phenotypically characterized as coenobia of 2, 4, 8 or more cells (rarely single) laterally attached, one daughter colony is formed in a cell; pyrenoids present. List of some *Scenedesmus* species deposited at ATCC is as follows:

Scenedesmus armatus Smith

Scenedesmus basiliensis

Scenedesmus bijugatus (Turpin)

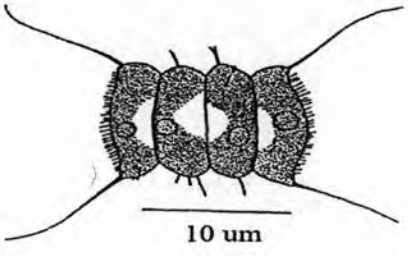
Scenedesmus communis Hegewald

Scenedesmus naegelii Chodat

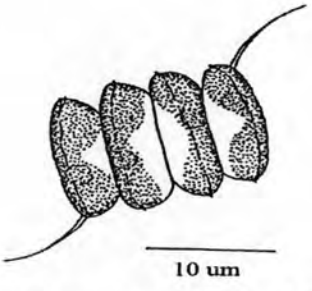
Scenedesmus obliquus (Turpin) Kutzing

Scenedesmus quadricauda (Turpin) Brebisson

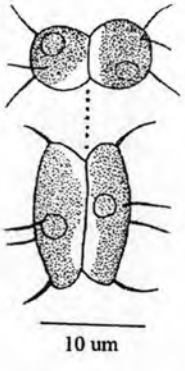
John et al. (2003) listed 46 species of *Scenedesmus* spp. from the British Isles based on the number of cells in a coenobium, wall ornamentation, and presence or absence of spines as shown in Figure 2.3



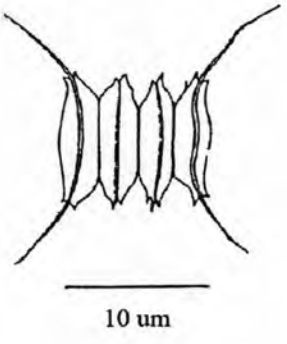
Scenedesmus armatus



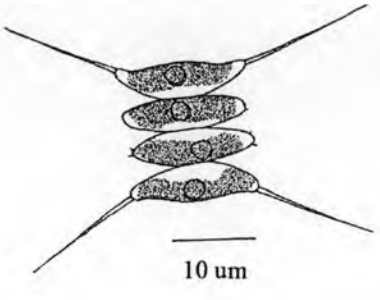
Scenedesmus armatus var. *bicaudatu*



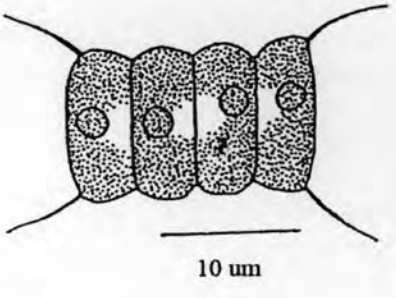
Scenedesmus flavescens



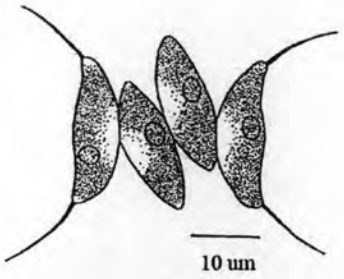
Scenedesmus opoliensis var. *carinatus*



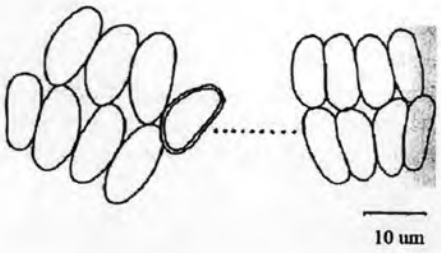
Scenedesmus opoliensis var. *mononensis*



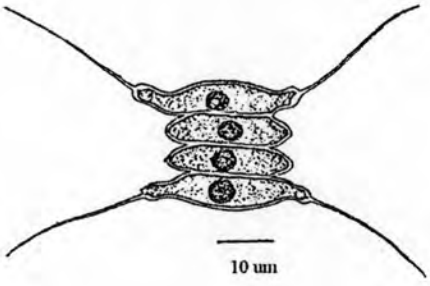
Scenedesmus communis



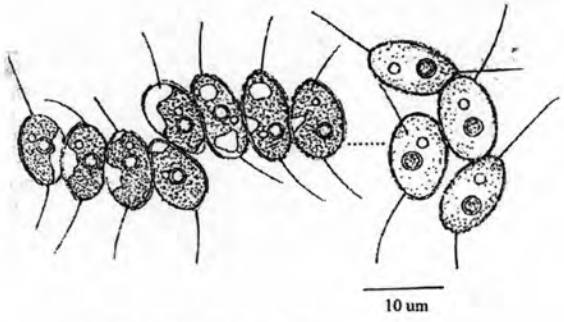
Scenedesmus opoliensis



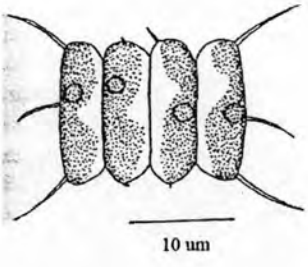
Scenedesmus arcuatus



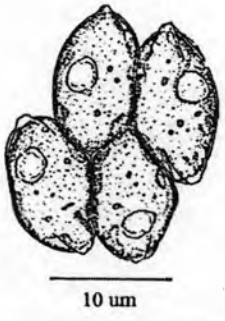
Scenedesmus protuberans



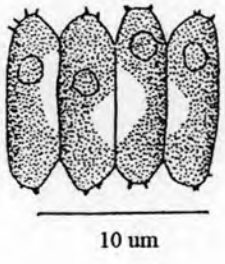
Scenedesmus kissii



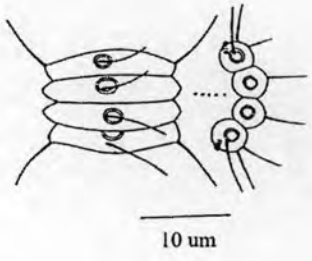
Scenedesmus abundans



Scenedesmus costatus

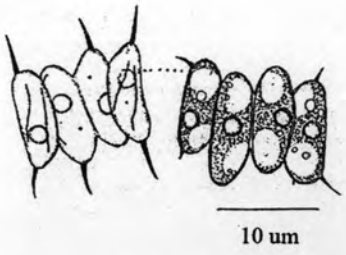


Scenedesmus aculeolatus

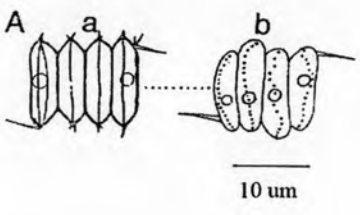


Scenedesmus asymmetricus

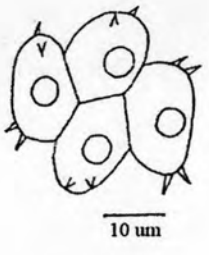
Scenedesmus intermedius var. *balatonicus*



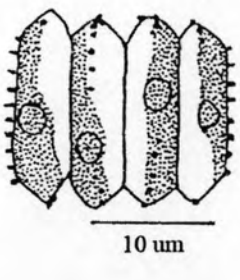
Scenedesmus pannonicus



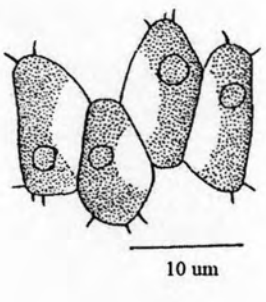
Scenedesmus semipulcher



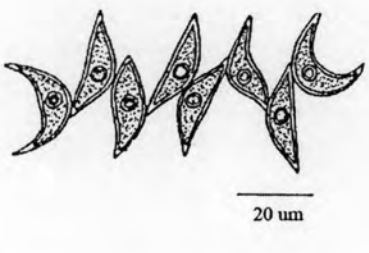
Scenedesmus denticulatus var.*disciformis*



Scenedesmus serratus



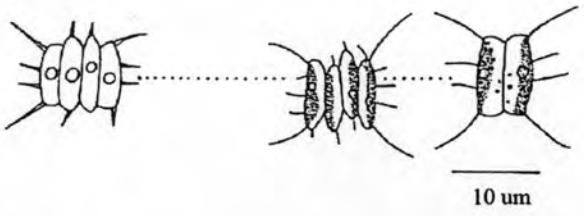
Scenedesmus denticulatus



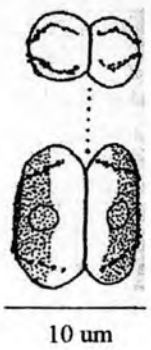
Scenedesmus bernardii

Scenedesmus dimorphus

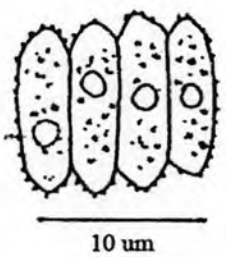
Scenedesmus acutiformis



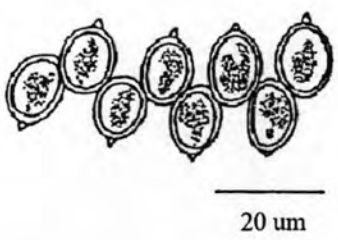
Scenedesmus subspicatus



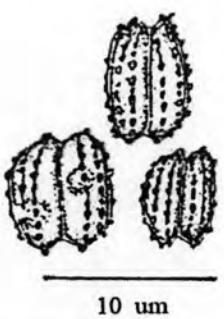
Scenedesmus grahneisii



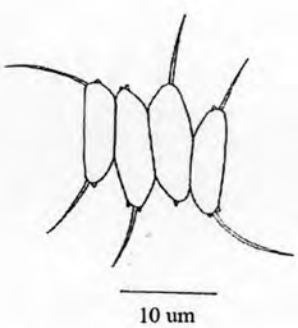
Scenedesmus granulatus



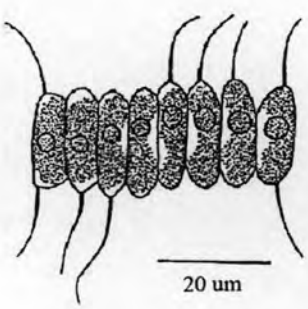
Scenedesmus apiculatus



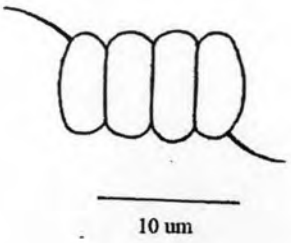
Scenedesmus costato-granulatus



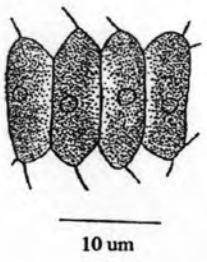
Scenedesmus caudate-aculeolatus



Scenedesmus magnus

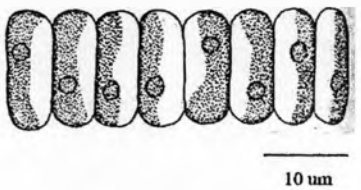


Scenedesmus bicaudatus

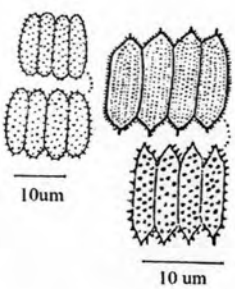


Scenedesmus dispar

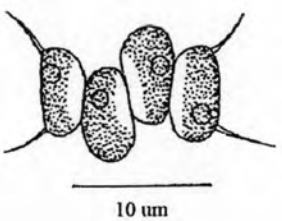
Scenedesmus acuminatus



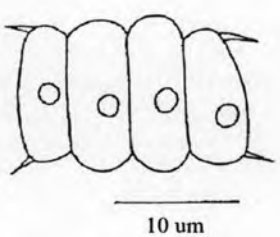
Scenedesmus ellipticus



Scenedesmus hystrix

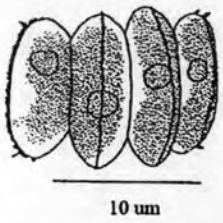


Scenedesmus intermedius

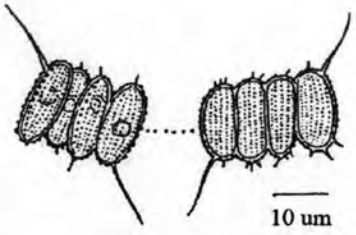


Scenedesmus microspina

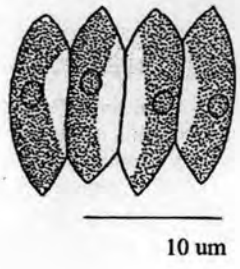
Scenedesmus falcatus



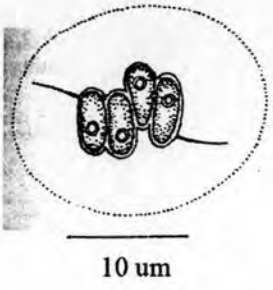
Scenedesmus circumfusus



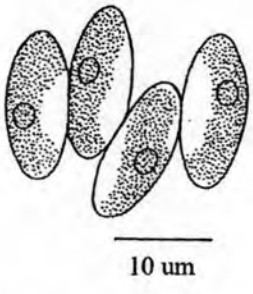
Scenedesmus insignis



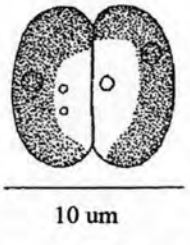
Scenedesmus obliquus



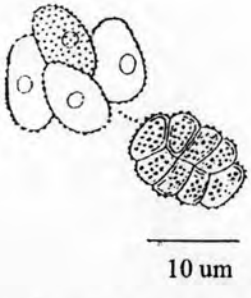
Scenedesmus intermedius var. *acutispinus*



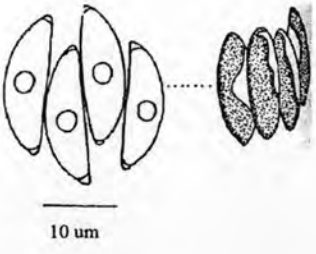
Scenedesmus obbtusus



Scenedesmus planctonicus

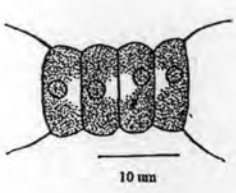


Scenedesmus verrucosus

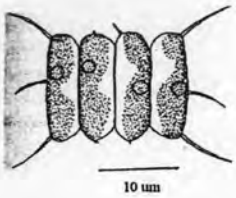


Scenedesmus raciborskii

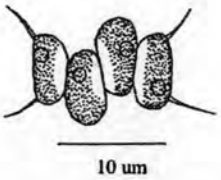
Figure 2.3 (a) Different species of *Scenedesmus* spp. reported from the British Isles (John et al., 2003).



Scenedesmus communis

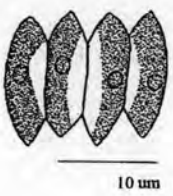


Scenedesmus abandons



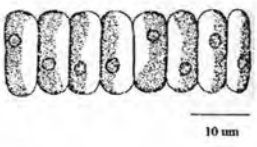
Scenedesmus intermedius

Scenedesmus circumfusus

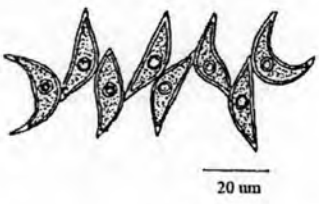


Scenedesmus obliquus

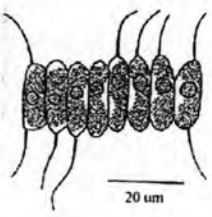
Scenedesmus dimorphus



Scenedesmus ellipticus



Scenedesmus bernardii



Scenedesmus magnus

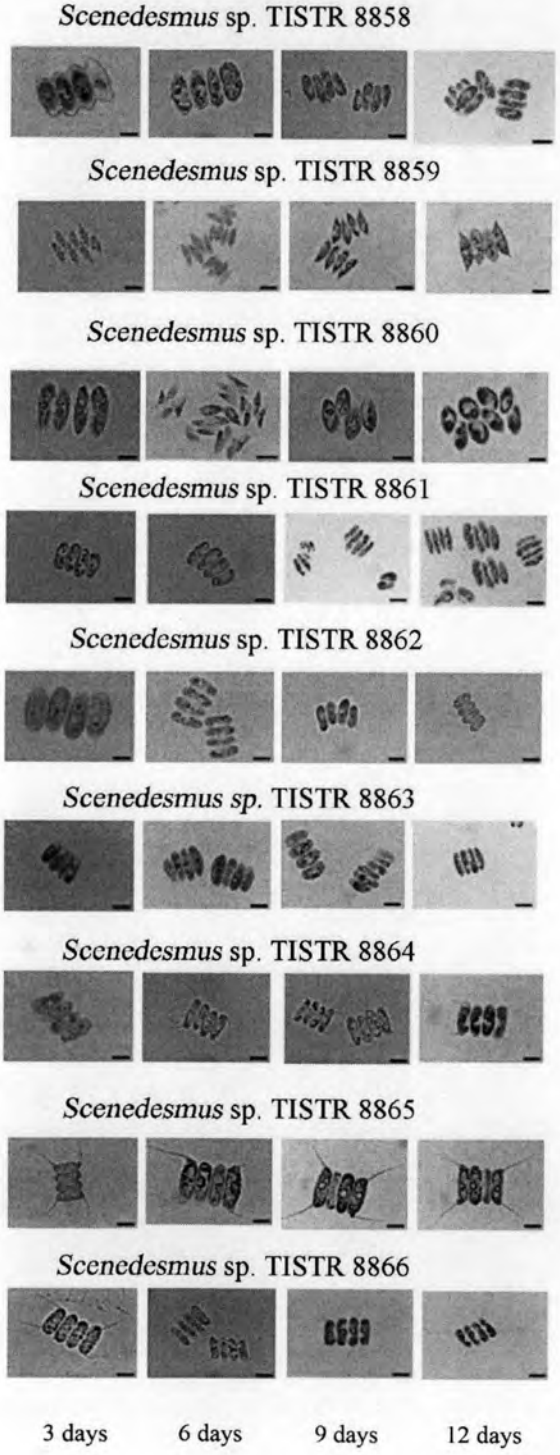
Figure 2.3 (b) Examples of *Scenedesmus* spp. with 4-8 cells with and without spines (John et al., 2003)

From Figure 2.3 (b) *Scenedesmus* spp. could easily be identified by morphology, different types of spines could be observed in *Scenedesmus communis* and *Scenedesmus abandons*. The spines in middle part of outer convex wall of *Scenedesmus abandons* range from 1-3. *Scenedesmus intermedius* has smaller spines.

2.4 PCR fingerprints of *Scenedesmus* spp.

Although some *Scenedesmus* spp. can easily be identified by morphology, in 2004 Jamkangwan used RAPD-PCR fingerprints of 9 *Scenedesmus* spp. strains to show that *Scenedesmus* spp. strains with similar morphology could differ genetically. For example, 6 day cultured *Scenedesmus* sp. TISTR 8860 could be mistakenly identified as

Scenedesmus sp. TISTR 8859. RAPD-PCR fingerprints revealed the two strains differed at the genotypic level. Similarly, based on morphology, 12 day cultured *Scenedesmus* spp. TISTR 8858 could be mistakenly identified as *Scenedesmus* spp. TISTR 8861. *Scenedesmus quadricauda* TISTR 8864 could be mistakenly identified as *Scenedesmus quadricauda* TISTR 8866.



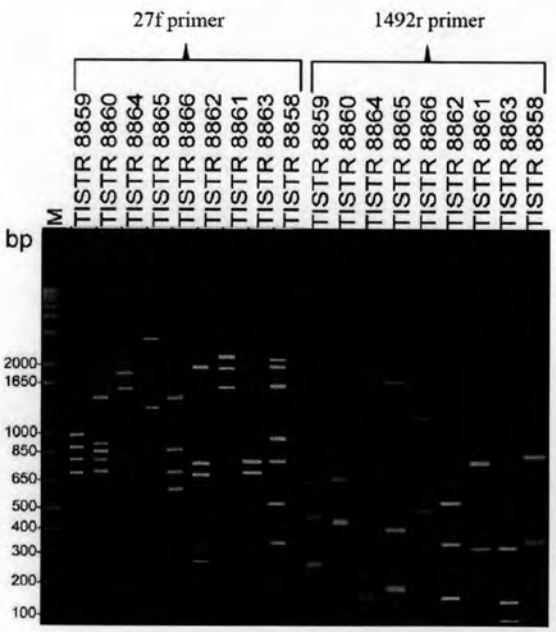


Figure 2.4 PCR fingerprints and morphology of *Scenedesmus* sp. TISTR 8858, *Scenedesmus* sp. TISTR 8859, *Scenedesmus* sp. TISTR 8860, *Scenedesmus* sp. TISTR 8861, *Scenedesmus* sp. TISTR 8862, *Scenedesmus* sp. TISTR 8863, *Scenedesmus* sp. TISTR 8864, *Scenedesmus* sp. TISTR 8865 and *Scenedesmus* sp. TISTR 8866 in BG11 medium at 3, 6, 9 and 12 days (Jamkangwan., 2004).

2.5 Research on taxonomy of *Chlorella* spp. and *Scenedesmus* spp. in Thailand

There is a limited amount of research on taxonomy of *Chlorella* spp. and *Scenedesmus* spp. in Thailand Yuwadee Peerapornpisal (2005) reported one *Chlorella* sp. and 6 *Scenedesmus* spp. from the Northern part of Thailand. The *Scenedesmus* spp. consisted of *S. perforatus* Lemmermann var. *perforatus*, *S. bicaudatus* Dedusenko, *S. apiculatus* var. *indicus* Hortobagyi, *S. acuminatus* (Lagerheim) Chodat, *S. acuminatus* var. *tetradesmoides* Smith, *S. pectinatus* Meyen. The micro-algae were identified by their morphology. Rungnapa Phitaktansakul et al. (2004) obtained nucleotide sequences of approximately 600 bp fragments of 18S rDNA from two isolates each of *Chlorella* spp. and *Scenedesmus* spp. The authors compared percent homology of the 18S rDNAs with those deposited at NCBI. The partial 18S rDNA of the first *Chlorella* sp. isolate was found to have 96.9% homology with *Chlorella kessleri* while that of the second *Chlorella* sp. isolate was found to have 90% homology with *C. mirabilis*. Partial 18S rDNA sequences of the two *Scenedesmus* spp. were found to have 96.1% and 90.8% homology with *Scenedesmus ovalternus* and *Scenedesmus quadricauda* respectively. Thus, the authors identified the two *Chlorella* spp. isolates and the two *Scenedesmus* spp. isolates as *Chlorella kessleri*, *Chlorella mirabilis*, *Scenedesmus ovalternus*, and *Scenedesmus quadricauda*. Sequencing data as well as construction of a dendrogram based on the partial 18S rDNA sequence indicated that the two *Chlorella* spp. isolates belonged to the same cluster. The two *Scenedesmus* spp. isolates were also found in the same cluster. However, the authors should not have identified the *Chlorella* spp. and *Scenedesmus* spp. up to the species level because only partial 18S rDNA sequences were used. Normally, the whole 18S rDNA sequences should be obtained and they should have at least 99.9% homology to conclude that the strains belonged to the same species. At present, there has been no research on molecular taxonomy of *Chlorella* spp. and *Scenedesmus* spp. in Thailand (Abstract

Books, the1st and 2nd Conferences on "National Symposium on Algae and Planktons, 2003, 2005). In 2004 Nonticha Jamkangwan reported on the use of 16S rDNA sequencing primers to obtain 16S rDNA sequences and PCR fingerprints of 6 *Chlorella* spp. strains and 9 *Scenedesmus* spp. strains. However, the micro-algae were not identified up to the species level. Therefore, a lot of research is needed to be conducted in Thailand on the molecular taxonomy of micro-algae such as *Chlorella* spp. and *Scenedesmus* spp. especially *Scenedesmus* spp. because some members this genus have been proposed to be transferred to genus *Desmodesmus* based on variations in ITS2 rDNA sequences. Of the 42 species recorded from the British Isles the following 21 have been transferred to the genus *Desmodesmus*: *S. abundans*, *S.armatus*, *S.armatus* var.*bicaudatus*, *S.asymmetricus*, *S.brasiliensis*, *S.communis*, *S. costato-granulatus*, *S. dispar*, *S. flavescens*, *S. grahneisii*, *S. hystrix*, *S. insignis*, *S. intermedius*, *S. kissii*, *S. maximus*, *S. opoliensis*, *S. opoliensis* var. *carinatus* and *mononensis*, *S. pannonicus*, *S. protuberans*, *S. subspicatus*, *S. arthrodesmus*, *S. denticulatus*, *S. serratus*. Five British species of *Scenedesmus* have been transferred to the new genus *Acutodesmus*: *S. acuminatus*, *S. dimorphus*, *S. incrassatulus*, *S. obliquus*, *S. pectinatus* (John et al., 2003).

2.6 β-carotene

In spinach leaves, two molecules of β-carotene have been reported in PSII with 6 molecules of chlorophyll a while in green halotolerant algae, *Dunaliella bardawil*, under high light intensity, β-carotene was reported to be in oil droplets at the inner periphery of cells (Kobayashi et al., 1990). β-carotene has been shown to be a precursor of vitamin A in human as show in Figure 2.5

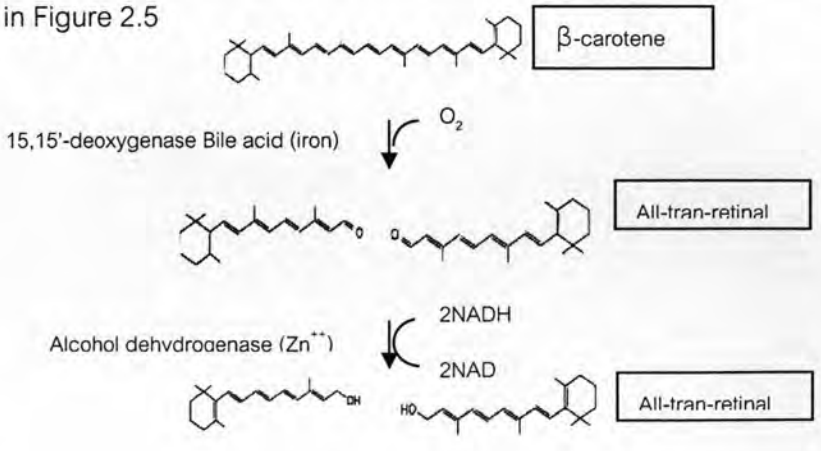
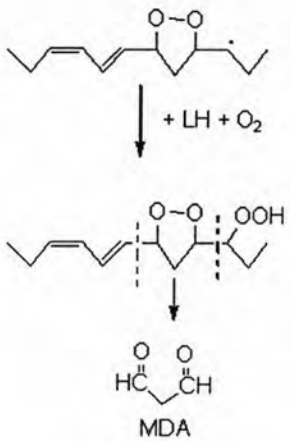


Figure 2.5 Structure of β-carotene as precursor of vitamin A (Voet and Voet, 1995)

Antioxidant functions of β-carotene (http://www.cyberlipid.org/perox/)

In *Chlorella* spp. and *Scenedesmus* spp. as well as in other chlorophyll-containing organisms, β-carotene acts as quenchers of singlet oxygen ($^1O_2$) (Krinsky, 1989). However, in humans, β-carotene acts as an antioxidant against lipid peroxidation which leads to the production of malondialdehyde (MDA) which is a mutagen. MDA reacts with DNA to form adducts (new structures) to deoxyguanosine and deoxyadenosine. The formation of MDA which is a secondary peroxidation product is as follows:

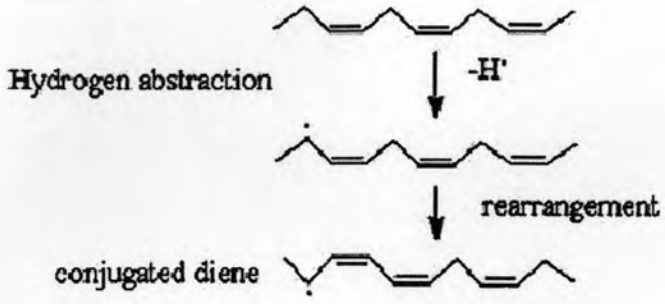


Lipid peroxidation is a naturally-occurring process. A great deal of knowledge on lipid peroxidation has come from studies of oxidation of simple model fatty acid molecules with various degrees of unsaturation including the membrane lipid phosphocholine. Primary products of lipid peroxidation such as peroxyl radicals or hydroperoxides are obtained by either a non-enzymatic auto-oxidation or photo-oxidation process or an enzymatic process. As far as antioxidation by β-carotene (and Quercetin) is concerned the two antioxidants prevent auto-or photo-oxidation. Auto-oxidation occurs via free radical reaction as follows:

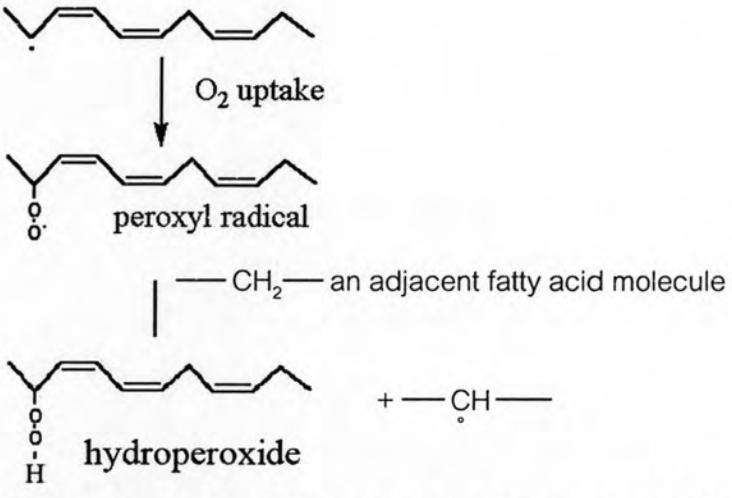
$$—CH_2— + {}^{\bullet}OH \longrightarrow — {}^{\bullet}CH — + H_2O$$

The above reaction shows a hydroxyl radical extracts a hydrogen atom from a methylene group of a fatty acid. The presence of a double bond in a fatty acid molecule weakens the C-H bonds on the adjacent carbon atom allowing the hydrogen atom to be

removed more readily. The carbon radical is stabilized by the formation of a conjugated diene as follows:



Under aerobic condition, oxygen uptake leads to the formation of a peroxyl radical which, is turn, extracts a hydrogen atom from the methylene group of an adjacent lipid molecule in the propagation step



In photo-oxidation, hydroperoxides are produced by the reaction of singlet oxygens ($^1O_2$), formed by photo oxidation of oxygen by light. Photo-oxidation of lipids is faster than the free radical auto-oxidation reactions.

$$2(CH_3\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}CH_2\text{-}(CH_2)_6\text{-}COOH) \xrightarrow{^1O_2}$$

$$CH_3\text{-}(CH_2)_7\text{-}C(OO)\text{-}CH_2\text{-}CH_2\text{-}(CH_2)_6\text{-}COOH \text{ or } CH_3\text{-}(CH_2)_7\text{-}CH_2\text{-}C(OO)\text{-}CH_2\text{-}(CH_2)_6\text{-}COOH$$

Peroxyl radicals

The peroxyl radicals formed by photo-oxidation propagate themselves in the same way as described for the propagation of peroxyl radicals formed by photo-oxidation reactions.

The genes encoding enzymes involved in the biosynthetic pathway of β-carotene in micro-algae are shown in Figure 2.6

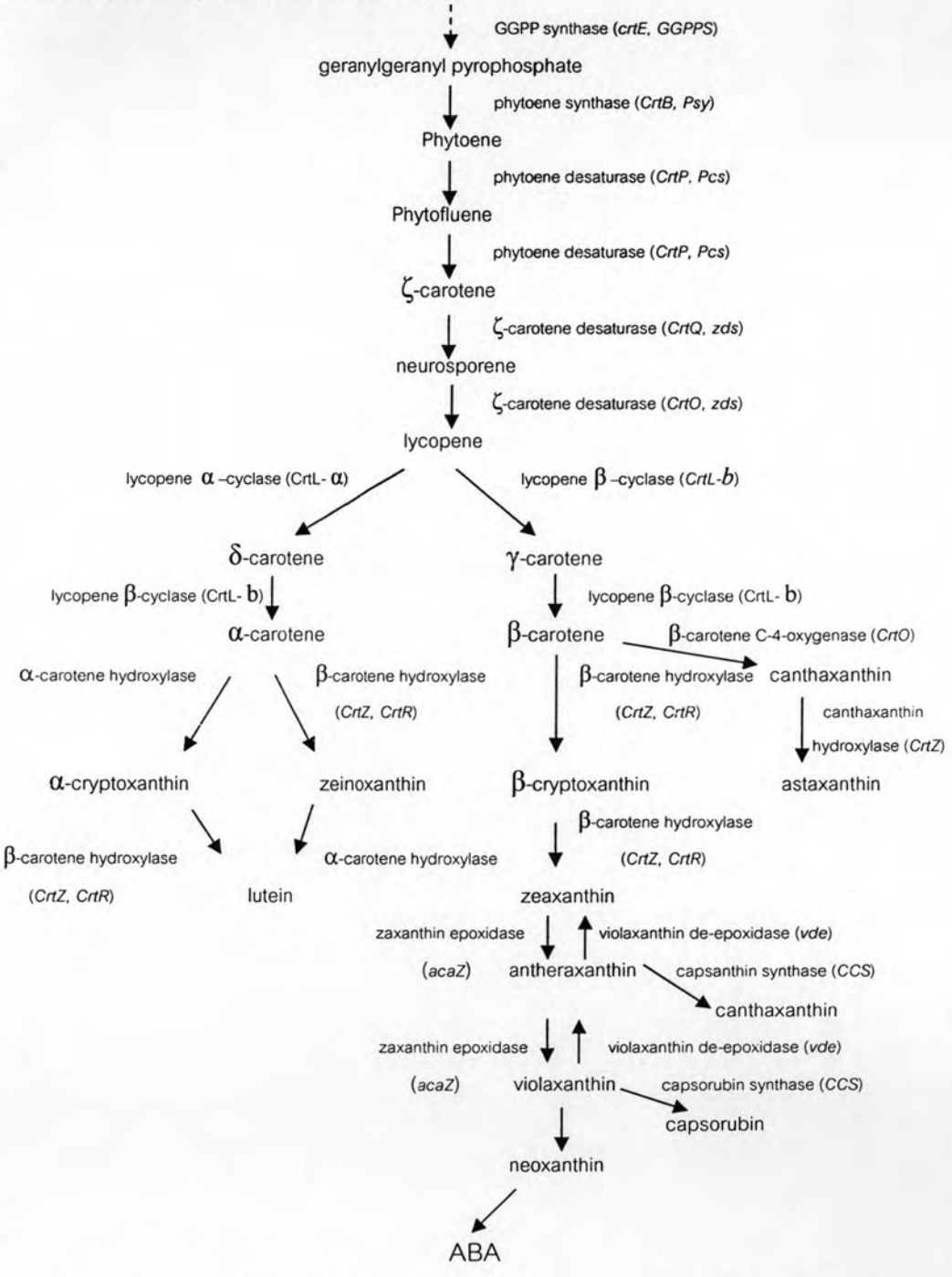


Figure 2.6 The biosynthetic pathway of β-carotene in micro-algae (Hirschberg et al., 1997)

Inbaraj et al. (2006) reported that green micro-algae producted high amounts of lutein, zeaxanthin, β-carotene, α-carotene, violaxanthin and neoxanthin during their normal growth phase. Bour et al. (1995) found that *Chlorella zofingiensis* grow under high light intensity (300 mμmole.m^{-2}.s^{-1}) in a nitrogen-free medium increased primary carotenoids: lutein and β-carotene during the first 8 hours of stress after which, levels of the primary carotenoids decreased and those of secondary carotenoids (astaxanthin and canthaxanthin) increased.

2.7 β-carotene contents in *Chlorella* spp. and *Scenedesmus* spp.

In 2006, Inbaraj et al. photoisomerized 100 μg.ml^{-1} all *trans*- β-carotene standard (Sigma) dissolved in 10 ml methylene chloride at 25 °C under four fluorescent tubes (20 W each) at a distance of 30 cm for 24 h. The illuminated standard was evaporated to dryness, reconstituted in 1 ml methanol-methylene chloride (50:50 v/v) and filtered through a 0.2 μm membrane filter for reversed-phase HPLC analysis with conditions indicated in Table 2.1. Figure 2.7 showed reversed-phase HPLC chromatogram of illuminated β-carotene standard.

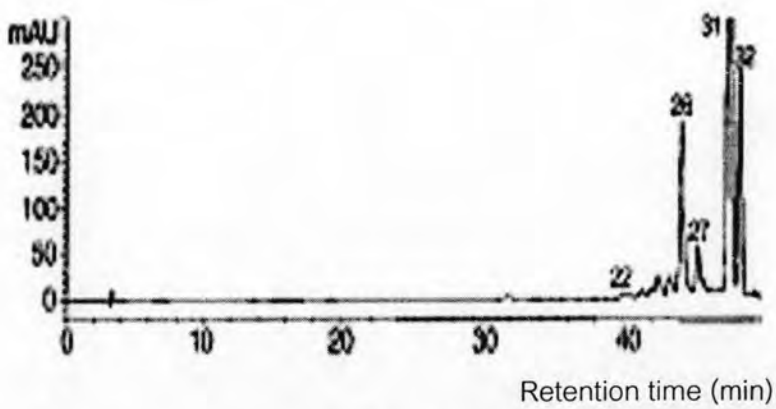


Figure 2.7 Reversed-phase HPLC chromatogram of illuminated β-carotene standard. Peak (26) 13-or 13' *cis* β-carotene: (27) 9-or 9' *cis* β-carotene: (31) all-*trans*- β -carotene; (32) 9-or 9'- *cis* β-carotene (Inbaraj et al., 2006)

β-carotene exists in various isomeric forms: 9-or 9'- *cis* β-carotene, 13-or 13' *cis* β-carotene and all *trans* β-carotene.

Inbaraj et al. (2006) reported that chlorella tablets in *Chlorella pyrenoidosa* contained only 2.6% of all-*trans*- β-carotene and its *cis* isomer. Carotenoids contents in

Chlorella pyrenoidosa tablets followed the order : all *trans*-lutein and its *cis* isomer (93.1%)>> all-*trans*- β-carotene and its *cis* isomer (2.6%)> zeaxanthin (1.3%)> epoxy-containing compounds (0.2%) ~ β-cryptoxanthin (0.2%). *cis* isomers of β-carotene (2159.3 μg/g) were reported to be present in approximately the same concentration as all *trans*- β-carotene (2155.0 μg/g) in *C. pyrenoidosa*. 9-*cis*- β-carotene was reported to possess higher antioxidant property when compared to its all-*trans*-isomer

Analysis of carotenoids in different *Chlorella* spp. has been carried out with C_{18} column as shown in Table 2.1.

Table 2.1 Reversed phase HPLC separation of carotenoids in *Chlorella* spp.

Green micro-algae	Reversed phase HPLC condition	β-carotene content	Reference
Chlorella sorokiniana (2000)	-YMC Carotenoid S5 250 x Id. 4.6 mm. -Mobile phase: MeOH: *t*-butylmethylether = 92:8 containing 0.1% ammonium acetate with 1mlmin^{-1} flow rate and detection at 450 nm.	600 ug/g	Matsukawa et al.
Chlorella pyrenodoisa	-YMC C_{30} Column (250 mmx4.6mm ID., 5 μm) Water (Milford, USA) -Mobile phase: methanol: acetonitrile: water (84:14:2 v/v/v) (A) and Methylene Chloride (100%) (B) with Gradient elution with 1mlmin^{-1} flow rate and detection at 450 nm.	*cis* isomers 2159.3 ug/g all *trans* isomer 2155.0 ug/g	Inbaraj et al. (2006)

The results of β-carotene contents in two *Chlorella* spp. and three strains of *Scenedesmus* spp. obtained in this research will form baseline data for further research into mechanisms for massive accumulation of β-carotene in freshwater *Chlorella* spp. and *Scenedesmus* spp. In 1990s, a lot of research was conducted on massive accumulation of β-carotene in marine unicellular green algae *Dunaliella bardawil* (Lers et al., 1990) and *Dunaliella salina* (Borowitzka et al., 1990). Cultivation of *Dunaliella* sp. in outdoor ponds for commercial production of high beta-carotene dry algae meal used

as supplementary food was well-documented (BenAmortz et al., 1989). In *D. bardawil* β -carotene is present as all-*trans*- β-carotene and its 9-*cis* isomer. The carotenoid was reported to act as a protective screen to absorb blue light region of the spectrum when irradiated at high light intensity (BenAmotz et al., 1989).

2.8 Quercetin in *Chlorella* spp. and *Scenedesmus* spp.

Quercetin is a type of flavonoids which was found as the major potentially anticarcinogenic flavonoid in vegetables and fruits (Hertog et al.,1992). According to Goodwin and Mercer (1983), plant flavonoids are phenolic derivatives which are brightly colored (red, crimson, purple or yellow). They are found in vacuoles of chromogents or chromoplasts. Flavonoid molecules are glycosides and the structures of their aglycone units are based on the flavan structure (Figure 2.8) which consists of two aromatic rings joined in a chroman structure by a three-carbon unit (C_6-C_3-C_6)

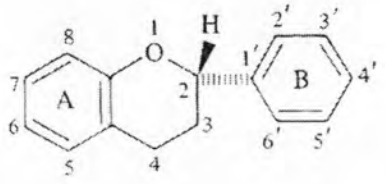


Figure 2.8 Common flavan (C_6-C_3-C_6) structure of flavonoids (Goodwin and Mercer, 1983)

The aglycones are classified according to the state of oxidation of the C3 unit (C-2, 3, 4) in the molecule (Figure 2.9).

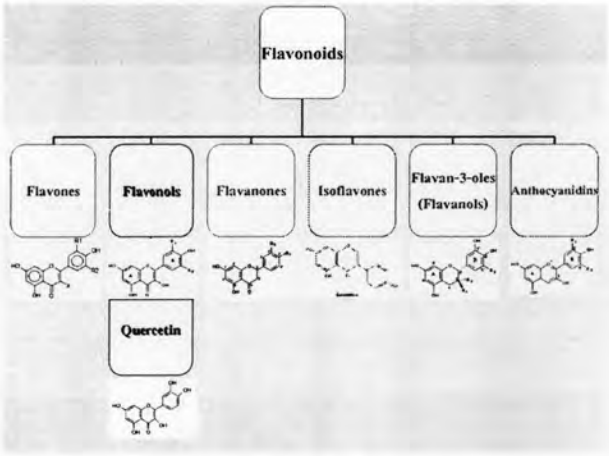


Figure 2.9 Classification of flavonoids. (Crozier et al., 2000).

The chemical structure of Quercetin is 3, 3', 4', 5, 7 pentahydroxyflavone as shown in Figure 2.10

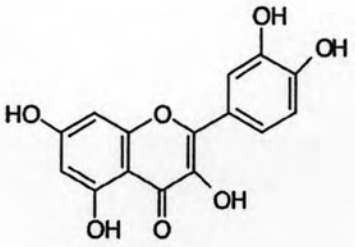
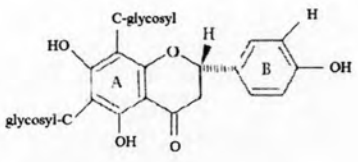
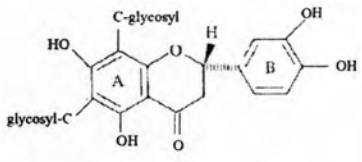


Figure 2.10 Chemical structure of Quercetin (Tsimogiannis and Oreopoulou, 2004)

Markham and Porter (1969) reported the first identification of flavonoids in green algae. The authors reported that the relatively advanced green algae, *Nitella Hookeri* which is a solf, branched filamentous green algae (Family Characeae, Class Charaphyceae, Division Chlorophyta), contained at least 10 flavonoid glycosides, two of which were identified by UV spectra of hydrolyzed flavonoids as 6,8-di-C apigenin glycosides (Vicenin) and 6,8-di-C luteolin glycosides (Lucenin). The authors did not report the flavonoids contents. Structures of some flavonoid glycosides are given in Figure 2.11.





(A) 6, 8-di-C apigenin glycosides (Vicenin) (B) 6, 8-di-C luteolin glycosides (Lucenin)

Figure 2.11 Structures of some flavonoid glycosides; (A) 6, 8-di-C apigenin glycosides (Vicenin) and (B) 6, 8-di-C luteolin glycosides (Lucenin) (Markham and Porter, 1969).

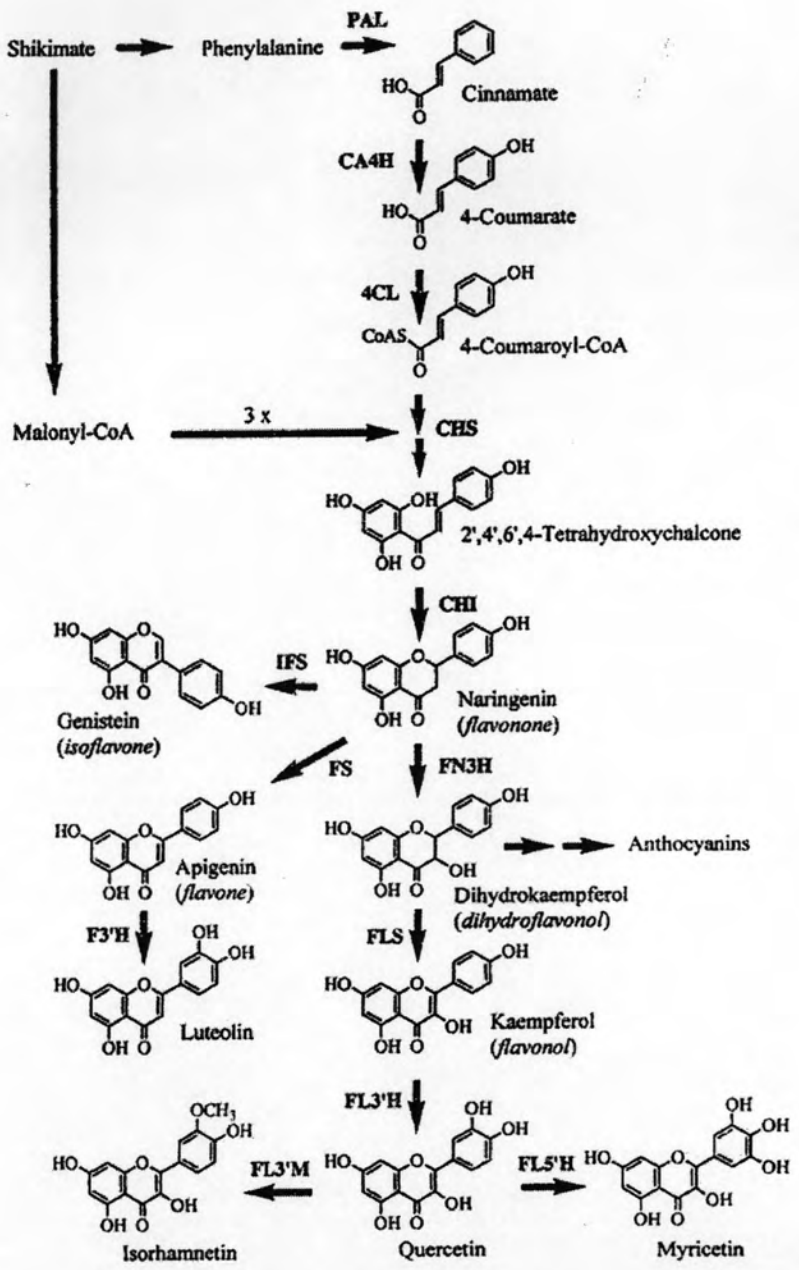


Figure 2.12 The phenylpropanoid pathway by which plants synthesize a wide range of secondary metabolites. Chalcone synthase (CHS) is the first step in the branch of the pathway that produces the flavonoids including isoflavones, flavones, flavonols and anthocyanins. (Crozier et al., 2000).

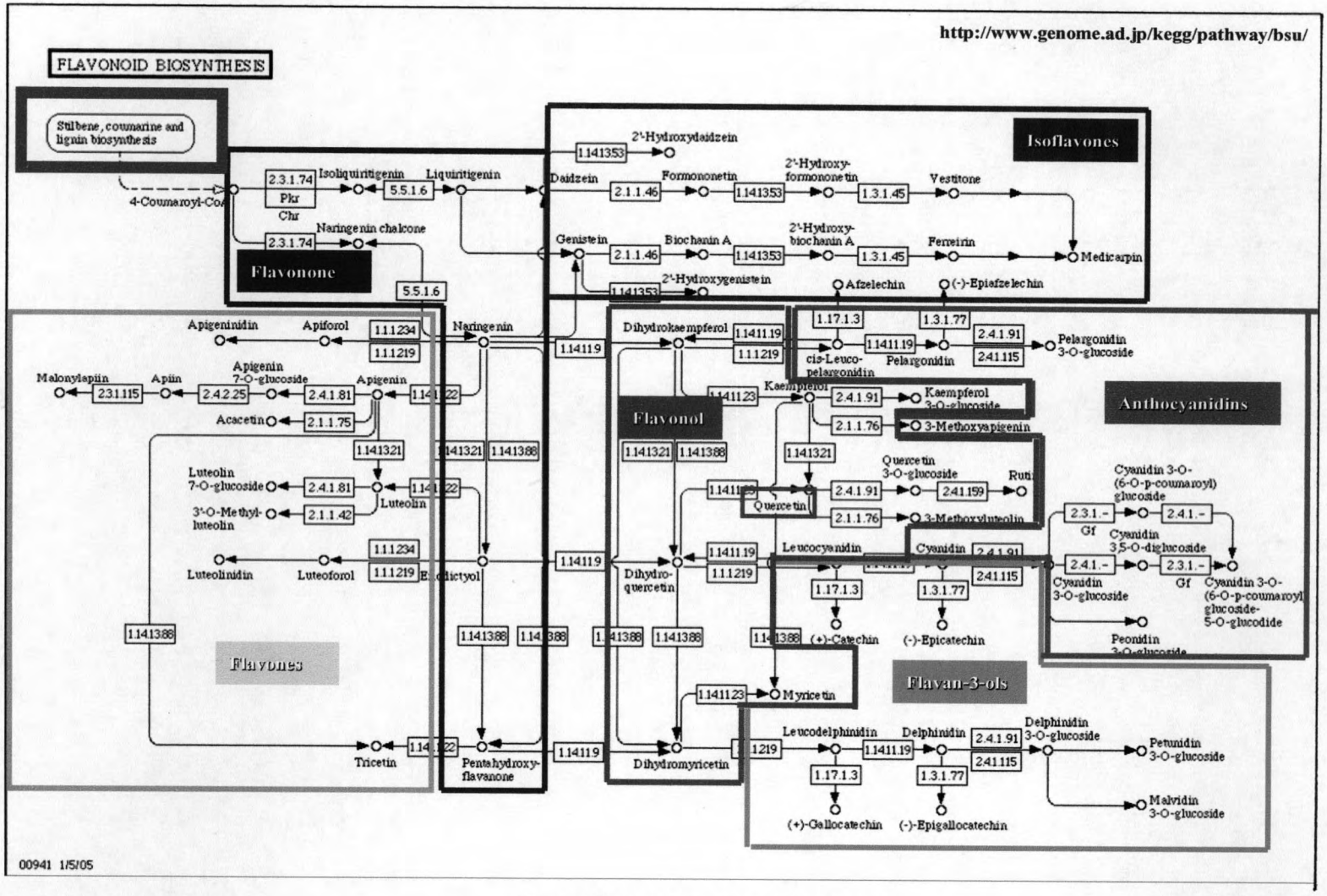


Figure 2.13 Flavonoid biosynthesis.

Figure 2.12 shows the biosynthetic pathway of Quercetin in plants. It is observed that the precursor of Quercetin is 4-Coumaroyl-CoA which is also a precursor of lignin (Figure 2.13). Since green algae do not contain lignin, Markham and Porter (1969) reported a considerable excitement when flavonoids whose precursor was also the precursor of lignin were detected for the first time in green algae with no lignin. In the following study, Quercetin contents in non-lignoferous green algae *Chlorella* spp. and *Scenedesmus* spp. were determined since Quercetin was reported as the major potentially anticarcinogenic flavonoid in plants and vegetables (Hertog et al., 1992) and it would be interesting if this flavonoid molecule is detected in non-lignoferous green micro-algae.

In the following experiments, two strains of *Chlorella* spp. and three strains of *Scenedesmus* spp. were isolated and their 27f, 1492r, CRL-7 primer directed PCR fingerprints obtained. In addition, β-carotene and Quercetin contents in the isolated green micro-algae were determined.