CHAPTER I

INTRODUCTION

The interest of natural bioactive compounds is increased according to their economical value. Bioactive natural products have an enormous amount of economic importance as specialty chemicals. They can be used as drug, lead compounds, biological or pharmacological tools and feedstock products (raw materials for the production of drugs) [1].

Citrus fruits are rich sources of bioactive compounds such as flavonoids, limonoids and their glucosides, ascorbic acid, folic acid, carotenoids (lycopene and ß-carotene), coumarin-related compounds (auraptene), highly fermentable fiber, and potassium. These constituents may serve as chemopreventive agents [2] in addition to other beneficial effects on human health [3].

Flavonoids have a wide range of biological activities, such as inhibition of key enzymes in mitochondrial respiration, protection against coronary heart disease and anti-inflammatory, anti-tumor, and antimicrobial activities [4]. Flavonoids also possess antioxidant [5] and blood lipid lowering activities [6, 7]. Researches have shown that citrus fruits may protect against chronic diseases such as cancer [8], heart diseases [9, 10] and osteoporosis [11].

Flavonoids are polyphenolic compounds which are categorized into flavonols, flavones, flavanones, isoflavones, and catechins. Flavanones are found abundantly in citrus fruit, mainly as flavonoid glycosides [12]. Flavonoid glycosides found in citrus juice, flavedo, albedo, and leaf, display typical distribution patterns which are particular to each species [13]. The distribution of the number, kind, and amounts of the flavanone glycosides depends on the citrus cultivar [14, 15]. Using flavanone glycosides as a key, citrus cultivars can be divided into two groups: those containing the nearly tasteless hesperidin (sweet orange, mandarin, lemon) and those in which the bitter naringin is the most principal one (grapefruit, pomelo, sour orange, trifoliate orange and kumquat) [16, 17].

Citrus (*Citrus* L.) is one of the most important world fruit crops and is consumed mostly as fresh produce or juice because of its nutritional value and special flavour [18]. Citrus fruits constitute an important group of fruit crops produced all over the world. The taxonomy for *Citrus* where ten species in the subgenus *Citrus* were recognized consists of Sweet orange (*C. sinensis* Osbeck), Sour orange (*C. aurantium* L.), Pomelo or shaddock (*C. maxima* Merril; formerly *C. grandis* Osbeck), Lemon (*C. limon* (L.) Burm. f.), Grapefruit (*C. paradisi* Macf.), Mandarin or tangerine (*C. reticulata* Blanco), Lime (*C. aurantifolia* Christm.), Citron (*C. medica* L.), an Indian species that is not cultivated (*C. indica* Tan.), and a Japanese species that is not cultivated (*C. tachibana* (Mak.) Tan.) [19, 20].

Citrus is the most abundant crop with worldwide production of over 88×10^6 ton and one-third of the crop is usually being processed. Citrus fruits are processed, mainly to obtain juice, but also, in the canning industry, to produce marmalade, segment and by the chemical industry to extract flavonoids and essential oils [21]. The amount of residue obtained from the fruits accounts for 50% of the original whole fruit mass [22]. They consist of peels (albedo and flavedo), which are almost one-fourth of the whole fruit mass, seeds and fruit pulp [23]. In course of production, very large amounts of byproduct wastes, such as peel are formed every year [24, 25] (Table 1.1).

Table 1.1 Products and by-products from various tissues of citrus fruits

Whole peel or rind (pericarp)	Consists of flavedo (exterior yellow peel, epicarp) and albedo (interior whity spongy peel, mesocarp). Albedo is rich in pectin. The whole peel combined with the pulp residue (rag) and/or molasses can becomes a feed for animals. It is also used for production of human foods and food supplements
Pulp **(principal edible portion, endocarp)**	Used mainly to produce raw juice for human nutrition, after mechanical extraction and screening. The material screened from the raw juice is also called pulp and is usually combined with other residues to produce by-products used in animal nutrition
Pulp residue **(called rag in the industry)**	Consists of the fraction screened from the pulp, being cores, segment walls or membranes, juice vesicles and seeds. The pulp residue is usually combined with peel residue to manufacture by-products feeds. From the lime-treated mass peel and pulp residues, citrus processors produce such by-products as press liquor, citrus molasses, citrus pulp, citrus meal and feed yeast. It is also used for production of human foods and food supplements
Seeds	Sometimes separated from the rag to produce seed oils, seed meals and dried seed pressed cake
Waste waters (aqueous effluent emulsions from processing plants)	Have potential uses for production of such products as activated sludge and yeasts. It is also used for production of human foods and food supplements

Citrus byproducts represent a rich source of naturally occurring flavonoids. While flavonoids are abundant elsewhere in the plant kingdom, the peel of citrus peel is a rich source of flavanones and flavanone glycosides, which are relatively rare in other plants [26]. Surveyors have also found that peels of fruits are the major sources of natural antioxidant [27, 28] because of their abundant content of naringin, hesperidin and other flavonoids.

During the processing of citrus fruit for juice, peels are the primary byproduct. If not processed, the peels become waste and constitute a severe environmental problem [29]. In the interest of the environment, researches have been carried out on the use of this agricultural waste as a raw material in order to isolate pectin and flavonoids [30-33].

Now, more reporters are mainly focused on the beneficial effects of the grapefruit juice on human health, such as antioxidant, antiallergic, and anticarcinogenic benefits as well as protection against high blood pressure or increased cholesterol [34]. Surveyors have also found that peels of grapefruits are the major sources of natural antioxidant [28, 29] such as naringin and other flavonoids.

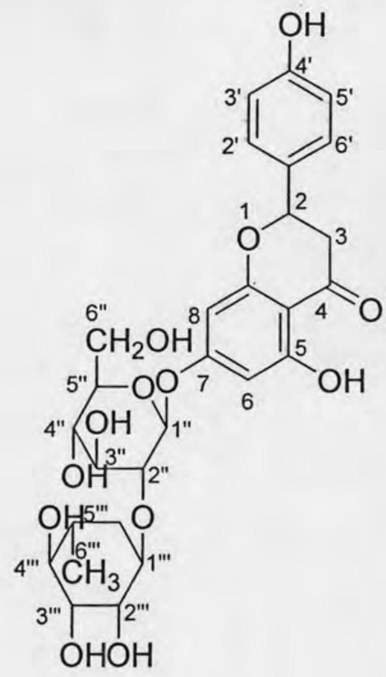


Figure 1.1 Structure of naringin

Naringin, the 7-rhamnoglucoside of the flavanone naringenin [35] is flavanone glycoside with antioxidant [36], anticancer [37] and antiviral property [38]. Researches have shown that naringin was a potential cytochrome (CYP) P450 inhibitor. The cytochrome (CYP) P450 is a family of heme containing monooxygenases that catalyzes the oxidative metabolism of many endogenous and exogenous compounds [39]. These enzymes are the principal phase I enzymes involved in the metabolism of endectocides which play a major role in the biotransformation of xenobiotics [40-42] with the far highest concentration found in the liver [43-45]. Naringin can also reduce the level of cytochrome P450 1A2 proteins [46], inhibit CYP 3A, which is the main subfamily of cytochrome P450 that is responsible for metabolizing paclitaxel, an antitumor drug [47-50]. Naringin can also inhibit lipid peroxidation in biological membranes [51] and lower blood lipid level [52]. Naringin has been reported to suppress cytotoxicity and apoptosis in mouse leukemia P388 cells exposed to a typical pro-oxidant, H_2O_2 [53] or an anticancer drug, cytosine arabinoside (1-b –D arabinofuranosylcytosine; Ara-C) [54]. Moreover naringin has demonstrated inhibition of breast cancer cell proliferation and delay of mammary tumerogenesis [55].

Naringin, the principal bitter flavonone glycoside, can be hydrolysed by naringinase, contains α-L-rhamnosidase and β-D-glucosidase expressed activities into rhamnose and prunin (one-third bitterness ratio to naringin), which can be further hydrolysed into glucose and tasteless naringenin (Figure 1.2) [56].

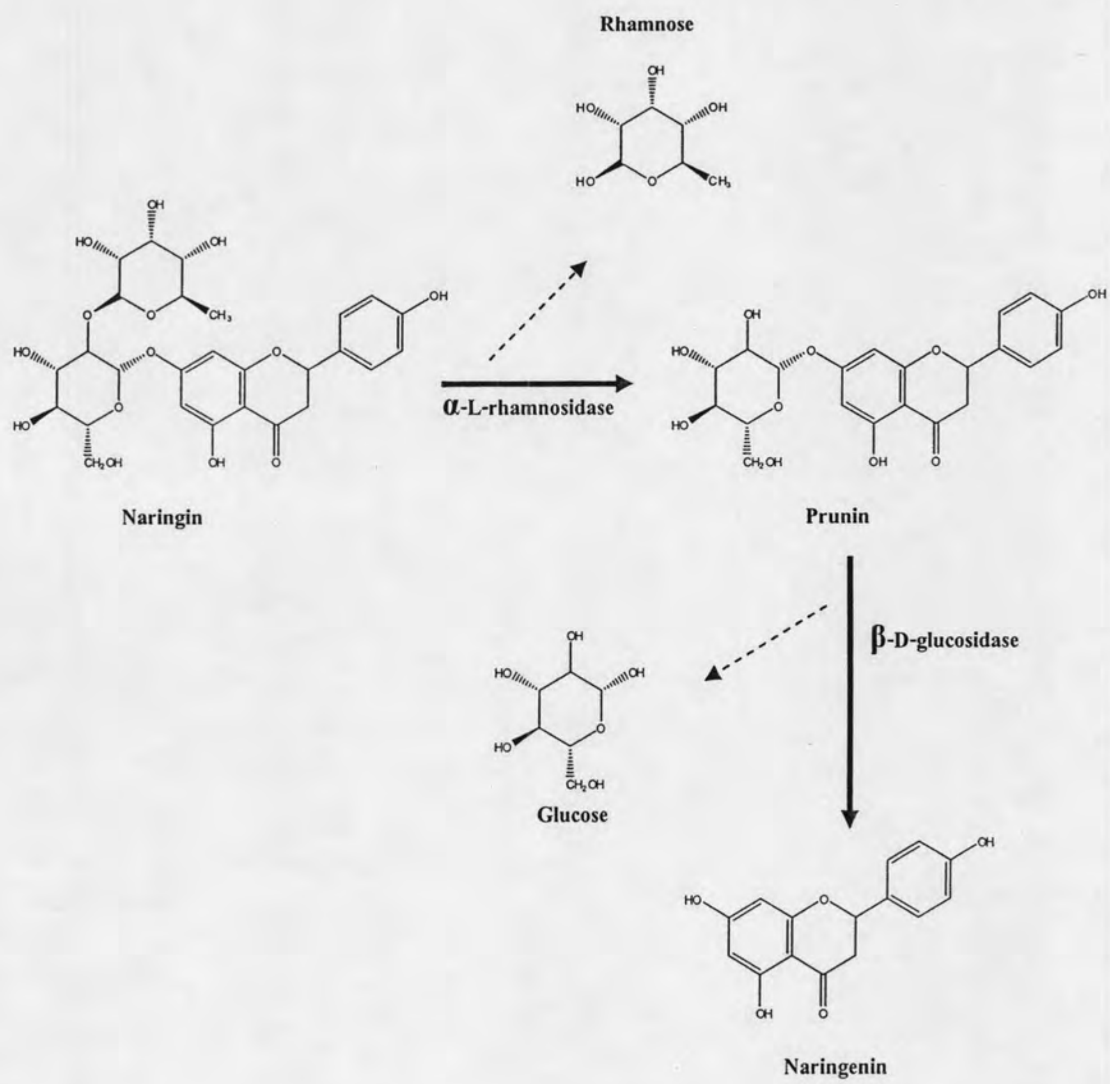


Figure 1.2 Hydrolysis of naringin into prunin, rhamnose, naringenin and glucose by naringinase expressing α-rhamnosidase and β-glucosidase activities.

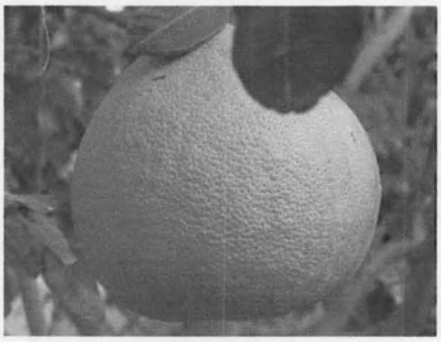
Naringin has been thought to be hydrolyzed to naringenin, presumably in the gut. It has recently been reported that after the ingestion of naringin itself, naringenin and its glucuronides were recovered from the human urine, whereas no naringin and naringin glucuronides were detected [57, 58].

Naringin is found in few varieties of citrus, mainly grapefruit (*Citrus paradisi* Macfadyen) and pomelo or shaddock (*Citrus grandis* Osbeck) [59, 60]. Grapefruit and pummelo are major *Citrus* species in terms of cultivation and utilization in the world [61]. Pomelo, the largest of citrus fruits with a thick, spongy peel belongs to the family Rutaceae [61]. Pomelo is a giant citrus and parents of grapefruits and tangelos. It is the largest of all Citrus fruits [61]. The pomelo is a native citrus species of southeastern Asia. The main areas of production in the Orient are southern China, southern Japan, Thailand, Vietnam, Malaysia and Indonesia. Pomelo is also cultivated in the (California and Florida), the Caribbean islands, and Africa [61]. There are many caltivars of the pomelo in Thailand, such as Kao Taeng-gwa, Kao Yai, Kao Nam Pheung, Tong Dee, etc.

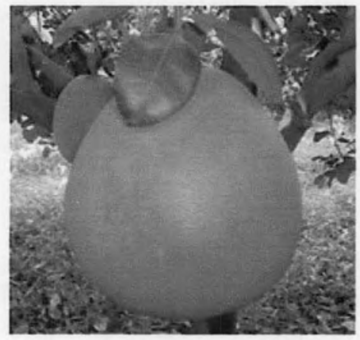
Taxonomic classification of pomelo [62].

Kingdom:	Plantae
Division:	Mognoliophyta
Class:	Magnoliopsida
Subclass:	Rosidae
Order:	Sanpindales
Family:	Rutaceae
Genus:	*Citrus*
Species:	*C. grandis* (L.) Osbeck*; C. maxima* Merril

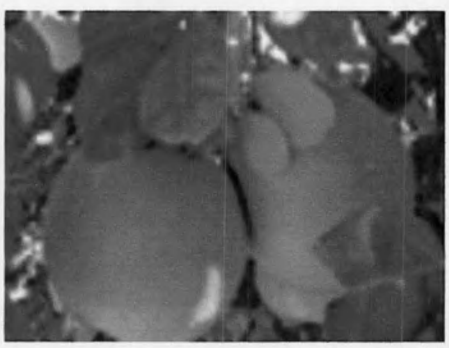
In this work the four popular cultivars, Khao Taeng-gwa, Khao Yai, Khao Nam Pheung and Tong Dee (Figure 1.3) were studied. Their characteristics are summarized in table 1.2.



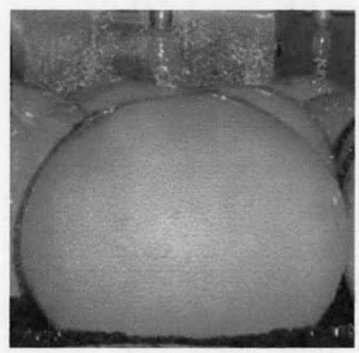
Khao Taeng-gwa



Khao Nam Pheung



Khao Yai



Tong Dee

Figure 1.3 Four Pomelo cultivars [63]

Table 1.2 Characteristics of four pomelo ciltivars [64]

Characteristics	Four Pomelo Cutivars			
	Khao Taeng-gwa	Khao Yai	Khao Nam Pheung	Tong Dee
Area of cultivation	Chainat, Nakhonsawan and Uthaithani	Samutsongkhram, Nakhonpathom and Samutsakhon	Nakhonpathom Samutsakhon and Ratchaburi	Nakhonpathom Samutsakhon and Ratchaburi
Havesting period	September	August-September	August-September	August-September
Fruit	Medium round with a flattened bottom	Medium altitude round product	Medium altitude round product	Medium round with a flattened bottom with pucker head product
Diameter	≈14-16 cm.	≈14-18 cm.	≈17 cm.	≈14-16 cm.
Pulp colour	White petal film	White petal film	White petal film	Pink petal film
Fruit colour	Shrimp-coloured, white-yellowish	Dry shrimp-coloured, white-yellowish	Shrimp-coloured, honey	Juicy shrimp-coloured, light pink
Flavor	Acid-sweet taste	Acid-sweet taste	Acid-sweet taste	Sweet taste

The aim of this research was to assess the potential of pomelo peel as a source of naringin. Efficient process of isolation of the naringin was explored and the isolated compound was also evaluated for its purity. Comparision of naringin content in pomelo peels from four cutivars, Khao Taeng-gwa, Khao Yai, Khao Nam Pheung and Tong Dee were also reported.

Objective of research

1. Screening for free-radical scavenging, tyrosinase-inhibition and UV absorption activities in organic extracts of pomelo peels.
2. Finding a process to obtain naringin.
3. Comparing naringin contents among Khao Taeng-gwa, Khao Yai, Khao Nam Pheung and Tong Dee.

Literature reviews.

In 1987, Rouseff and coworker determined the citrus flavanone glycosides, narirutin, naringin, hesperidin, and neohesperidin concentrations in juices from 52 citrus cultivars using HPLC. Neither naringin nor neohesperidin was detected in sweet orange *(Citrus sinensis),* tangerine *(Citrus reticulata),* tangerine-like hybrids, or most tangelos. Grapefruit *(Citrus paradisi),* sour orange *(Citrus aurantium),* and K-Early (a grapefruit-like tangelo) juices all contain similar amounts of naringin and widely varying amounts of neohesperidin [15].

In 1995, Ortuño and coworker studied the effects on production of secondary metabolite including flavanones narirutin, naringin, and neohesperidin and of the sesquiterpene nootkatone of variety, rootstock, and geographical location in grapefruit and pummelo. The work attempts to identify the most productive varieties and to ascertain the effect of variety, rootstock, and cultivation area on the levels of some secondary metabolites. Flavonones including narirutin, naringin, and nehoesperidin were determined by HPLC analysis. The highest flavanone levels was found in immature fruit while the sesquiterpene nootkatone, was highest in mature grapefruit and pummelo. Moreover, variety of these fruit was influence on the expression on secondary metabolites. The highest levels of naringin are produced by Marsh and Red Blush grapefruit on Cleopatra, Davis Seedless on sour orange, Thompson and Reed varieties on Citrange Troyer [65].

In 2001, Gorinstein and coworker evaluated the antioxidant properties of some citrus fruits. The contents of dietary fibre, total polyphenols, essential phenolics, ascorbic acid and some trace elements in lemons, oranges and grapefruits were determined and compared with their total radical-trapping antioxidative potentials (TRAP). The results showed that the content of total polyphenols in peeled lemons and their peels ware significantly higher than in peeled oranges, peeled grapefruits and their peels. The content of total polyphenols and TRAP in the peels were significantly higher than in peeled fruits. Lemons possess the highest antioxidant potential among the studied citrus fruits. The peels of all citrus fruits are rich in dietary fibres and phenolic compounds and suitable for industrial processing [66].

In 2005, Li and coworker evaluated the total phenolic contents of five citrus peels (Yen Ben lemon, Meyer lemon, grapefruit, mandarin and orange). Ethanol extracts and aqueous extracts were quantified for phenolic contents and the antioxidant activities using the Folin-Ciocalteu assay (FRAP assay). Ethanol was found to be the best solvent for the extraction that gave highest extraction yield (about 74%). Among 5 citrus peels, grapefruit was the greatest source of polyphenolics and highest total antioxidant activity, followed by mandarin, Yen Ben lemon, orange and Meyer lemon peel [67].

In 2006, Anagnostopoulou evaluated radical scavenging activity of Navel sweet orange (*Citrus sinensis*) peel extracts by the DPPH$^{\bullet}$ and luminol induced chemiluminescence methods. They also determined the total phenolic content by the Folin–Ciocalteu method within seven different extracts, fractions and residues of Navel sweet orange *(Citrus sinensis)* peel. Ethyl acetate exhibited the high total phenolic content and the best radical scavenging activities. The active compounds constituent flavonoids (flavanone glycosides, flavones and flavonols) [68].

In 2006, Peterson and coworker reported that flavanones (hesperidin, naringin, narirutin, neohesperidin, didymin, neoericitrin and poncirin) (Figure 3) constitute the majority of flavonoids in citrus fruits such as sweet *(Citrus sinensis)*, sour oranges *(C. aurantium)*, tangerines/mandarins *(C. reticulata)*, tangors (*C. reticulate x C. sinensis*) and tangelos (C. *reticulata x C. paradisi*). Sour oranges had a distinct flavanone profile dominated by naringin and neohesperidin [69].

In 2006, Rehman use of citrus peel extract as antioxidant in edible oil. They evaluated free fatty acid content (FFA), peroxide value (POV) and iodine value (IV) of refined corn oil that had been added with citrus peel extracts. The study revealed that citrus peel extract gave 8–10 times higher inhibition of rancidity in corn oil, than BHA and BHT. Therefore, the use of citrus peel extract is recommended as a natural antioxidant to suppress development of rancidity in oils and fats [70].

In 2006, Wu and coworker determined flavonoids and ascorbic acid from grapefruit *(Citrus paradisi Mact.* (Rutaceae)*)* peel and juice using capillary electrophoresis with electrochemistry detection (CE-ED). The result showed five flavonoids (hesperidin, naringin, hesperedin, narigenin and rutin) and ascorbic acid (Figure 4) in grapefruit juice and two flavonoids (hesperidin, naringin) and ascorbic acid in extract of grapefruit peel [71].

In 2007, Abeysinghe and coworker determined the contents of bioactive compounds and the total antioxidant capacity (TAC) in different edible tissues, namely juice sacs (JS), segment membrane (SM), and segment (Seg), of fruit of four citrus species using ferric reducing antioxidant power (FRAP) assay. The results showed that SM contained significantly higher amounts of total phenolics, total flavonoids, two flavanones (naringin and hesperidin) (Figure 1.4), and higher antioxidant activity than Seg and JS of four citrus species. Vitamin C contributed significantly to the TAC in JS and Seg of all citrus species. Hesperidin is the major contributor in SM of *C. unshiu, C. reticulata and C. sinensis species*. The results indicated that SM of citrus fruit contained higher in contents of bioactive compounds and TAC than Seg and JS [72].

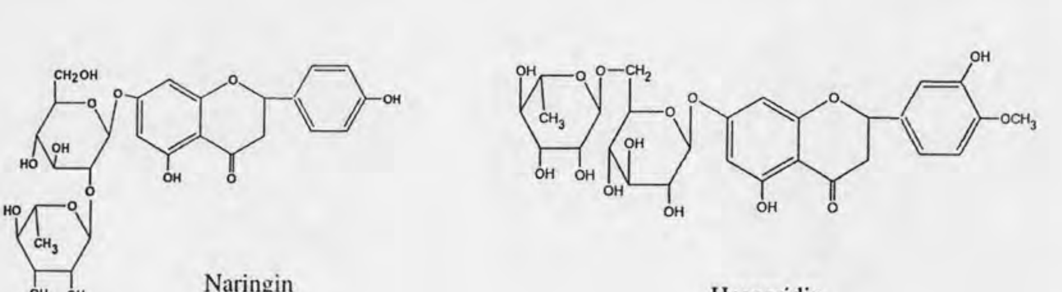


Figure 1.4 Structure of naringin and hesperidin

In 2007, Jayaprakasha and coworker extracted pummelo and navel oranges using five different solvents including hexane, ethyl acetate, acetone, methanol and methanol:water (8:2). The antioxidant capacities was measured using three different methods i.e., a,a-diphenyl-b-picrylhydrazyl (DPPH), 2,20-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical assay and ORAC methods. The total phenolic content of the extracts was determined by Folin–Ciocalteu method and expressed as catechin equivalents. Ethyl acetate extract from navel orange and pummelo gave highest phenolics and exhibited significantly higher free radical scavenging activity, whereas hexane extract from pummelo and methanol extract from navel orange did not show any phenolics [73].

In 2007, Martín and coworker described the chemical composition of citrus by-products from three of the most important fibre sources: wastes from chemical companies, wastes from canning companies and wastes from citrus juice production. The chemical changes produced from the processing of citrus wastes into fibre were evaluated in terms of total antioxidant activity, and physical properties, such as LHC (lipid-holding capacity) and WHC (water-holding capacity). The results showed that final composition was more dependent on the industrial process than on the type of citrus. Citrus fibre showed losses of functional values during the process; i.e. soluble dietary fibre and ascorbic acid content decreased when waste products were transformed into fibres. The water-holding and lipid-holding capacities of analyzed citrus fibres were also affected [74].