Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
STUDIES ON THE EXTRACTION AND CHARACTERIZATION OF PECTIN AND BITTER PRINCIPLES FROM NEW ZEALAND GRAPEFRUIT AND PHILIPPINE CALAMANSI

A thesis presented in partial fulfilment of the requirements for the degree of Master of Technology in Food Technology at Massey University

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ABSTRACT

A study was conducted to determine the presence of bitter components in NZ grapefruit and Philippine calamansi; describe the effect of maturity on the bitter components and other chemical constituents of grapefruit; reduce the bitterness of grapefruit juice by adsorption on polyvinylpyrrolidone; and to extract and characterize pectin from grapefruit peel.

Naringin (995 ppm), narirutin (187 ppm), and limonoids (7.9 ppm) were detected in NZ grapefruit juice concentrate (27° Brix). Naringin was not detected in the calamansi juice, and limonin was detected at the level of 10.5 ppm in juice containing 5% crushed seeds.

Maturation of the grapefruit caused an increase in pH from 3.00 to 3.50, an increase in total soluble solids from 10.8 to 14.4 with a decline to 13.5° Brix later in the season, a steady fall in acidity from 2.50 to 1.31 g citric acid/100 mL, and a continuous rise in the Brix/acid ratio from 4.2 to 10.3. Juice yield fluctuated throughout the season. Ascorbic acid remained fairly steady in the early- and mid-season fruit but decreased in the late-season fruit. Naringin content was highest at the beginning of the season and fluctuated throughout the season. Naringin content in the grapefruit peel remained constant as the fruit matured. Narirutin was detected in the early-season fruit but disappeared later in the season. Limonoid content in both unpasteurized and pasteurized juices decreased with ripening.

The use of polyvinylpyrrolidone significantly reduced naringin in grapefruit juice by up to 78.1% and limonin by up to 17.5% depending on the amount and reaction time of the adsorbent. A loss of 23.1% in ascorbic acid occurred with 5% PVP with a reaction time of 1 h.
Pectin extraction at 85°C and the use of acidified isopropyl alcohol yielded a product with the following characteristics: 8.9% yield; 1.3% moisture content; 1.9% ash; 759 equivalent weight; 9.2% methoxyl content; 82.2% anhydrogalacturonic acid; 63.2% degree of esterification; 4.2 intrinsic viscosity; 89,362 molecular weight and setting time of 0.55 minute.
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CHAPTER 1

INTRODUCTION
Citrus fruits may fairly be regarded as one of the most important crops directly consumed as food. In New Zealand, grapefruit (C. grandis x C. reticulata) is the most important citrus fruit grown and accounts for almost all of the grapefruit acreage (Anon. 1980). The calamansi (C. mitis) is extensively grown in the Philippines and is now the leading citrus fruit being utilized primarily for its juice.

Several studies had been conducted on the composition of citrus fruits to serve the needs of the food processing and by-products industries for chemical information. However, in New Zealand and Philippines, limited information is available on the seasonal changes of bitter components, pectin extraction and characterization, and the use of adsorbents to reduce the bitter components below the organoleptically detectable level.

This study aims to present information on the bitter components of both grapefruit and calamansi, to describe the physiological and biochemical changes in grapefruit during maturation and ripening, to reduce the bitterness of the grapefruit juice by adsorption on polyvinylpyrrolidone (PVP), and to extract and characterize pectin from grapefruit peel.

1.1 BITTERNESS IN CITRUS FRUITS

Bitterness in citrus fruits and products is primarily caused by two groups of compounds - limonoids and flavonoids (Kefford and Chandler, 1970; Chandler and Nicol, 1975; Rombouts and Pilnik, 1978).
1.1.1 Limonoid Bitterness

Limonoid bitterness is associated with Navel oranges and grapefruit (Emerson, 1949; Maier and Dreyer, 1965). About 120 years after its first isolation in 1841, limonin, the major limonoid was identified as a triterpenoid derivative (I) (Arigoni et al., 1960).

Citrus seeds contain relatively large amounts of limonoids (Hasegawa et al., 1980). They have also been reported to occur in the peel and juice of oranges and grapefruit (Emerson, 1949; Maier and Dreyer, 1965). In citrus juice production, the gradual development of bitterness in the juice after extraction presents a serious problem. This phenomenon is referred to as delayed bitterness since it is not observed either in fresh fruit or in the juice immediately following extraction (Joslyn and Pílnik, 1961). Juice held at room temperature develops a definite bitterness after several hours or, if heated, within a few minutes. This delayed bitterness is characteristic of juice from early-season to mid-season Washington Navel, Australian Valencia and Israeli Shamouti oranges, as well as from Marsh and New Zealand grapefruit (Levi et al., 1974; Chandler and Kefford, 1966; Emerson, 1948; Robertson, 1980). The juice obtained from late-harvested fruit from these same varieties, however, does not develop this bitterness to the same extent.

Two theories have been proposed to explain the phenomenon of delayed bitterness: the precursor theory and the diffusion theory. Higby (1941) proposed that the fruit tissues contain a non-bitter, water-soluble precursor which, after disruption of the fruit tissue in juice manufacture, is extracted into the juice where it is slowly converted to limonin. Emerson (1948, 1949) studied the dihydroxy acid formed on base hydrolysis of limonin and concluded that this compound lactonized too slowly to be the precursor. He
Fig. 1. Structure of limonin (I) and limonoic acid A-ring lactone (II)
suggested that the monolactone acids or a glycoside could be the precursor, and that enzymes could be involved in its conversion to limonin.

Failure to isolate and identify the precursor led Kefferd (1959) to put forth the diffusion theory. He reported that limonin itself is present in the fruit tissues but, because of its low solubility, it takes an appreciable time to diffuse from the tissue fragments of the juice into solution and reach a concentration sufficient to impart a bitter taste. Earlier work by Samisch and Ganz (1950) had reported that the bitter principle diffused very slowly from the tissue particles.

Maier and Beverly (1968) studied the capillary membranes, albedo, juice vesicles and seeds of early season Washington Navel oranges and Marsh grapefruit and found that limonin was present only in the juice that developed bitterness. No limonin was detected in the fresh fruit to any significant degree, and the major limonoid present in the albedo and endocarp tissue was limonin monolactone. Their evidence suggested that all the limonin was in the monolactone form, contrary to the findings of the diffusion theory workers. The monolactone itself was not bitter but when it came in contact with the acidic juice, lactonization occurred, resulting in the conversion of the monolactone to the bitter compound limonin. Maier and Margileth (1969) established that A-ring monolactone (II) is the naturally occurring limonin monolactone.

Robertson (1980), on his study of solubility relationships of limonin and the phenomenon of delayed bitterness in citrus juices reported that these processes are very complicated. He presented evidence for the existence of two precursors and argued that the work on which the currently-accepted precursor theory is based lacks quantitative measurements. A need for a reassessment of previous work in
this area was therefore recommended in the light of the possible involvement of two limonin precursors and the associated enzymes and hydroxyacid forms of limonin.

Limonin (which is almost insoluble in water) can give bitter solutions of sickening intensity, especially in the presence of sugar and pectin which increases its solubility (Chandler and Nicol, 1975). Its bitterness is generally unpleasant being particularly persistent on the palate, lingering for a considerable time after swallowing the juice.

1.1.2 Flavonoid Bitterness

The peels of oranges, lemons and grapefruit contain a wide range of flavanone glycosides. Two of the best known of these compounds are hesperidin (5,7,3'-trihydroxy-4'-methoxyflavanone 7-β-rutinoside), the main flavonoid constituent of oranges and lemons, and naringin (5,7,4'-trihydroxyflavanone 7-β-neohesperidoside), the main flavonoid constituent of grapefruit (Horowitz and Gentili, 1969). Both flavanones are present as the 7-glycosides, and whereas naringin is intensely bitter, hesperidin is tasteless. The structure of hesperidin (III) consists of three parts: L-rhamnose linked α-1,6 to D-glucose, which in turn is linked to the C-7 hydroxy group of the flavanone 2(S)-hesperetin (Horowitz, 1964). The disaccharide portion 6-O-α-rhamnopyranosyl-D-glucopyranose, is referred to by its trivial name rutinose.

The structure of naringin is thought to be similar to that of hesperidin, differing only in the aglycone moiety, naringenin, in which the β-ring substitution pattern is different (IV).
Fig. 2. Structural formulae of hesperidin (III), naringin (IV), and neohesperidoses (V).
Horowitz and Gentili (1969) determined the configuration of rhamnose in naringin as well as in two other flavonoids, poncirin \((5,7\text{-dihydroxy-4\text{'-methoxyflavanone 7-\beta-neohesperidoside})}\) and neohesperidin \((5,7,3\text{-trihydroxy-4\text{'-methoxyflavanone 7-\beta-neohesperidoside})}\) in order to explain the sensory differences between naringin and hesperidin. They found that the linkage between rhamnose and glucose was \(1\rightarrow2\) in all 3 flavonoids, suggesting that they could be considered neohesperidoses \((V)\). However, the linkage between the rhamnose and glucose in hesperidin was \(1\rightarrow6\). It was therefore apparent that it was the point of attachment between the rhamnose and glucose that determined the bitterness of the flavanone-7-\beta-neohesperidosides and the tastelessness of the flavanone-7-\beta-rutinosides. Chandler and Nicol (1975) reported that the bitterness cannot be determined simply by the glycoside bonds. Not all neohesperidosides are bitter, although this disaccharide is a component of the two most bitter citrus flavonoids, naringin and poncirin. According to Horowitz and Gentili (1969), narirutin, didymin, and hesperidin (all rutinosides) are not bitter, but Kamiya et al. (1975) who synthesized narirutin described this component as bitter.

Naringin has long been known as the principal flavonoid of the grapefruit, *Citrus paradisi* (Chandler and Nicol, 1975). According to Hagen et al. (1966), total flavanone glycosides in grapefruit are made up of naringin \((63\% \text{ w/w})\), narirutin \((28\% \text{ w/w})\), and a small percentage of hesperidin, neohesperidin, and poncirin.

The substance now called naringin was first discovered in 1857 by de Vry who isolated it from all of the tissues (but mainly the flowers) of *Citrus decumana* in Java; he apparently did not publish his findings at that time. The structure of naringin was first established by Asahina and Inusube (1929) and then by Horowitz and Gentili (1963a). It was first synthesized by Kamiya (1967).
Naringin occurs in low concentrations in the juice of mature grapefruit, and in higher concentrations in segment membranes, core and peel (Sinclair, 1972). In citrus juice production, bitterness will rapidly increase unless the pulp and rag are removed from the juice. However, the immediate removal of all the pulp particles from the juice will not always give a non-bitter serum because the bitter principles exist in the intact juice sacs, and their concentration there may exceed the bitterness threshold (Chandler and Nicol, 1975). Only at high concentrations is the bitterness due to naringin objectionable, and at moderate levels it may be a desirable constituent. Buffa and Bellenot (1962b) suggested that a level of 0.03-0.07% naringin is required to give grapefruit juice its characteristic bitterness, juices with naringin contents below 0.03% being of poor quality.

Naringin is soluble in aqueous systems, producing a bitter taste. The bitterness of this glycoside is so pronounced that it can be detected when one part is dissolved in 50,000 parts of water (Braverman, 1949). Its bitterness is generally mild and can be readily removed from the palate by normal salivary processes.

1.2 CHANGES IN THE BITTER COMPONENTS AND OTHER CHEMICAL CONSTITUENTS OF N.Z. GRAPEFRUIT DURING MATURATION

1.2.1 Limonin

The concentration of bitter principles in all parts of citrus fruits decreases with advancing maturity (Higby, 1938). In oranges, the effect of maturity on bitterness has been studied by Keafford and Chandler (1961) and Bowden (1968); their results showed that there is a decrease in bitterness with maturity. The crude bitter principle content of the dried peel of Navel oranges on rough lemon rootstock decreased from 0.10 to 0.06% while that of Valencias on rough lemon stock decreased from 0.07 to 0.00%
during three months (Chandler, 1958).

Limonoid bitter principles generally decrease with maturity of the fruit (Emerson, 1949). Maier and Dreyer (1965) reported the presence of limonin in grapefruit juice at levels above its taste threshold. They found that limonin was present six months after the fruit had reached commercial maturity. The endocarp of the grapefruit retained limonin (in amounts up to 140 ppm on a wet weight basis) far longer after commercial maturity than did the Navel oranges where limonin decreased and disappeared as the fruit ripened beyond commercial maturity (Higby, 1938). Maier and Beverly (1968) reported that in Navel oranges, the concentration of A-ring monolactone gradually decreased in the tissue as the fruits developed beyond commercial maturity, and that the limonin content of the extracted juice showed a corresponding decrease.

1.2.2 Flavonoids

Camp et al. (1932) reported that the intensity of flavonoid bitterness is a function of fruit maturity, method of preparation and subsequent treatment of the product. Albach et al. (1969) investigated the production of naringin in grapefruit and found that naringenin rhamnoglucosides accumulated throughout the entire growing period from development of the ovary to fruit maturity. The rate of naringin formation, however, appeared to be several thousand times greater during the initial period of fruit development than in the subsequent periods. These workers also observed periodic increases in the production of naringenin glucosides which accompanied growth flushes, suggesting that it might be possible to anticipate increases in bitterness so that the processing industry could adjust its harvesting times accordingly. This would ensure the production of grapefruit products of uniform quality. The accumulation of naringin during the initial stages of fruit development appeared to coincide with the formation of new cells.
Maier (1969) also reported a rapid increase in naringin during the initial development of Marsh seedless grapefruit. The naringin accounted for 9% of the weight of the fruit (41% of the dry weight) at the end of the first month, levelling off when the fruit had reached one-quarter of its maturity. The peel thickness also reached a maximum when the amount of naringin started to level off. Kesterton and Hendrickson (1952) found that the naringin content became rather constant after the equatorial diameter had reached 50.8 mm. As the fruit grew larger, the naringin content decreased as a percentage of its overall weight.

A decrease in naringin content was observed as the season progressed (Maurer et al., 1950) and as the fruit ripened (Hagen et al., 1966). The juice at all times contained less naringin than did the peel, membrane or core.

1.2.3 Ascorbic Acid

Cattoni and Gonzales (1932) stated that grapefruit juice had a scurvy-preventing potency equal to that of lemon juice, and that its curative value was superior. The grapefruit peel was subsequently found to be especially rich in ascorbic acid (Atkins et al., 1945). They found that the concentration of ascorbic acid in the juice was only one-seventh and one-fifth of the vitamin C concentration in the flavedo and albedo respectively. On a whole fruit basis, the juice contained about 17% of the total ascorbic acid of the fruit.

New Zealand grapefruit juice contains from 21 to 49 mg/100 mL ascorbic acid (Hyatt, 1936; Strachen, 1967; Anon., 1966; Dawes, 1970; Robertson, 1975). As fruit ripens and increases in size, the concentration generally decreases (Harding and Fisher, 1945). Metcalfe et al. (1940), in the Rio Grande Valley of Texas, concluded that there is a very definite decrease in the ascorbic acid content of the fruit (6 varieties) by the end of the season.
Ugon and Bertullo (1944) and Krezdorn and Cain (1952) stated that the amount of ascorbic acid in grapefruit juice decreased as the season advanced. Previous work, however, had reported that the more mature fruits from a given tree sometimes contained more ascorbic acid than the less mature ones (Melas-Joannides, 1939). A later paper by Ugon and Bertullo (1945) reported that the ascorbic acid concentration in the grapefruit juice increased as the season advanced.

The seasonal decrease in concentration of ascorbic acid in grapefruit should be considered in relation to the increase in volume of the juice and in the size and weight per fruit during growth and maturation. Harding and Fisher (1945) showed that when expressed as mg per mL of juice, the ascorbic acid decreased significantly during the season. Since growth of the fruit is accompanied by a large increase in juice volume and fruit weight, the concentration of ascorbic acid would decrease, resulting in less ascorbic acid per mL of the juice. On the other hand, if the ascorbic acid values are reported on the basis of mg per fruit, an increase occurs during the growth and development.

1.2.4 Acidity

pH and titratable acidity are the two measures of acidity used in physiological studies on grapefruit. Grapefruit contains a relatively high concentration of organic acids. The titratable acidity may constitute 8 to 15 percent of the total soluble solids of the juice from the edible portion (Sinclair, 1972). Wolf (1958) determined the organic acids in grapefruit juice and reported that the total acidity was composed of 87.2% citric acid, 2.17% malic acid, 1.7% quinic and phosphoric acid, and 7.0% unidentified acid. Traces of oxalic and tartaric acids in grapefruit were reported in earlier work (Braverman, 1949).
Many investigators have shown that the concentration of titratable acids in grapefruit gradually decreases as the fruit develops and matures (e.g. Sinclair and Bartholomew 1944; Harding and Fisher 1945). Ting and Vines (1966) on their study of Marsh grapefruit found that the citric acid content increased throughout the growth cycle, declined up to about 5 months from fruit set and then remained roughly constant. Karaoulanis and Margaris (1975) in their study of the seasonal changes in grapefruit reported that as the fruit ripened, there was a decrease in acidity. Sinclair and Ramsey (1944) reported that the decrease in titratable acidity was considered to be due to dilution as the fruit increased in size and juice content.

Decrease in the concentration of acid with the gradual increase in total sugars during development results in an increase in the ratio of total soluble solids to acidity, which is the basis for determining the legal maturity of the fruit as well as its palatability. In view of this, titratable acidity of the juice is highly associated with quality of the fruit (Sinclair, 1972).

Harding and Fisher (1945) observed a gradual increase in the pH of grapefruit as the fruit matured. The change in pH was slight, indicating the strong buffer capacity of grapefruit juice. Karaoulanis and Margaris (1975) observed the same trend, with a marked increase in the pH of the juice during ripening.

A linear relationship between the pH of grapefruit and the log of the free acid concentration expressed as milliequivalents per gram of soluble solids was demonstrated by Kilburn (1958) and Kilburn and Davis (1959) who were able to draw a common regression line through the points. This means that at a uniform soluble solids content (°Brix), the pH of citrus juices is proportional to the log of the titratable acidity.
1.2.5 Total Soluble Solids

There is a fairly close correlation between total soluble solids and sugar content because a high proportion of the soluble solids are sugars. Grapefruit juice contains 6-13% soluble solids, of which more than half are sugars (Kefford, 1959; Sinclair, 1972). The major sugars are reported to be sucrose, glucose and fructose with trace quantities of acid-hydrolyzable glycosides (flavanones) containing galactose and rhamnose (McReady et al., 1950). Ting and Deszyck (1961) reported that in the peel, the total sugar content of grapefruit was about 80% of the total soluble solids and the sugars present were glucose, fructose and sucrose with traces of xylose and rhamnose.

Many investigators have reported that sugars increase as the season advances. According to Harding and Fisher (1945) the reducing, non-reducing and total sugars increased as grapefruit continued to ripen on the tree. Usually before or at maturity, the sucrose decreases and the reducing sugars increase due to the breaking down of sucrose into glucose and fructose. On the other hand, Grebinskii (1940) reported that the sucrose content of Rumanian grapefruit juice reached a maximum and then began to decrease long before the fruit was fully mature. Hilgeman and Smith (1940), in studies of Marsh grapefruit from September to May reported that total sugars increased until January and then decreased. Results of a 2-year study in the Naples area showed that the fruits reached maximum soluble solids during the month of February and then decreased (Gioffe, 1976). A marked increase in the soluble solids and total sugars was observed in grapefruit harvested from three different regions of Greece (Karaoulanis and Margaris, 1975). Such a pattern of increasing to a maximum and then levelling off or declining with advancing maturity has also been reported in other areas, e.g. in California.
and Arizona (Rygg and Getty, 1955); Texas (Krezdorn and Cain, 1952; Burdick, 1961; Lime et al. 1954, 1956); Florida (Stenstron and Westbrook, 1956) and New Zealand (Robertson, 1975).

1.3 METHODS OF DEBITTERING CITRUS JUICES

Because bitterness continues to be an important economic problem in the citrus industry, several methods have been developed and research is still being conducted to reduce (or preferably totally eliminate) bitterness. Maier et al. (1977) reported that there are several approaches to controlling juice bitterness among which are pre-harvest factors, post-harvest fruit treatment, processing conditions and treatment of the juice. Processors try to minimize limonoid and flavonoid bitterness in grapefruit products by carefully selecting the fruit, by controlling the pressure used in juice extraction and by blending bitter and non-bitter juices where possible.

1.3.1 Removal of Limonoid Bitterness

1.3.1.1 Enzymic Methods

As early as 1950, McColloch reported that citrus juices could be debittered by enzymic degradation of the pectic substances which caused a change in the physical state of limonin. Pectin-destroying enzymes from fruits or fungi were found to debitter orange juice after several hours of treatment at 4-10°C, but the process was associated with the development of off-flavour and loss of cloud. Recently Robertson (1980) attempted to reduce limonoid bitterness by degrading the pectin in Navel orange juice. However, he was unable to demonstrate any reduction in limonin concentration, even after all the pectin has been degraded.
Several studies have been directed towards the use of enzymes of bacterial origin to degrade limonin. An enzyme, limonoate dehydrogenase was isolated from Arthrobacter globiformis (Hasegawa et al., 1972) and Pseudomonas sp. (Hasegawa et al., 1974b) which catalyzed the dehydrogenation of the free 17-hydroxyl group of limonoate A-ring lactone to produce 17-dehydrolimonoate A-ring lactone which is not bitter.

In addition to limonoate dehydrogenase, Hasegawa et al. (1974c) isolated another limonoid metabolic system from Pseudomonas sp. 321-18. The addition of limonin to this system produced exocellular traces of deoxylimonin together with considerable quantities of deoxylimonoate as metabolites.

Vaks and Lifshitz (1976) identified an enzyme from Acinetobacter sp. capable of attacking limonin close to the natural pH of the juice. This enzyme was distinct from those discussed previously and was inactive at pH 8.5, where limonin is in the open-ring limonoate form. However, the mechanism of the reaction catalyzed and the nature of the products formed were not discussed.

Limonin-degrading enzymes are also present in orange albedo (Chandler, 1971). Hasegawa et al., (1974a) found limonoate dehydrogenase activity in the albedo tissues of Navel oranges. This confirmed the ability of citrus fruits to form 17-dehydrolimonoate A-ring lactone from limonin.

The interruption of limonoid metabolism in postharvest fruit in the absence of oxygen supported the limonoate dehydrogenase pathway as the one operating in citrus fruits (Eskin, 1979). Nicol and Chandler (1978) in a paper detailing the optimum conditions for the extraction of this enzyme, referred to it simply as the limonin precursor degrading (LPD) enzyme without specifically identifying its substrate. The substrate used for their assays was orange albedo.
Enzymic methods for reducing limonoid bitterness pose several problems. The enzymes of bacterial origin are expensive and have very limited activity at the natural pH of citrus juices. The high limonin-degrading activity of citrus albedo is encountered only over a narrow period during maturation of the fruit, and the isolation of extracts of high activity is difficult (Chandler and Nicol, 1975). Although enzymic methods pose a number of problems, the search for more suitable enzymes is still in progress (Brewster et al., 1976).

1.3.1.2 Adsorption Methods

The problem of bitterness appears to have been solved through the recent use of chemical sorbents. An early attempt to reduce limonin was reported by McColloch (1950) using activated carbon. However, this material was too selective to be of any practical value.

More recent interest in chemical sorbents led to an examination of polyamide powders by Chandler et al. (1968). They found that much of the limonin from pasteurized Navel orange juice could be adsorbed by polyamides. The most efficient adsorption involved a two-step treatment bringing the limonin concentration below the organoleptically detectable level. The powder was, however, found to be more effective in removing flavonoids as well as some ascorbic acid and this lack of specificity was a major drawback in the industrial utilization of these powders.

A series of sorbents were examined by Chandler and Johnson (1977), who showed that cellulose acetate was far more specific than polyamide powders. Treatment of orange juice serum with the powder removed 44-70% of the limonin content in less than an hour, at the same time removing relatively negligible amounts of hesperidin and ascorbic acid. Other materials, including related carbohydrate derivatives and polymers were tested but only cellulose acetate butyrate shared the unusual sorptive properties of cellulose acetate.
Chandler (1977), in reviewing this bitterness problem discussed the production of cellulose acetate gel beads that were successfully used in a "gel debittering process". This process provided a simple and inexpensive method for the removal of limonin, thus permitting the commercial use of citrus juices affected by the delayed bitterness phenomenon. The cellulose ester adsorbents may be used in gel bead form packed in columns, or as a powder. A drawback is the regeneration of the adsorbent, which requires rather large volumes of water (Rombouts and Pilnik, 1978).

The use of other chemical sorbents has been investigated by Maeda et al. (1979) who used PVP, Nylon-66 and High Porous Polymer in the adsorption of naringin but not limonin from Natsudaidai juice.

1.3.2 Removal of Flavonoid Bitterness
1.3.2.1 Enzymic Methods

The bitter flavour of naringin is associated with the 1→2 linkage between rhamnose and glucose as discussed in Section 1.1.2. The susceptibility of this glycosidic bond to hydrolytic enzymes suggested the possibility of hydrolyzing naringin to rhamnose and prunin (5,7,4'-trihydroxyflavanone 7-β-D-glucoside). Prunin can be further hydrolyzed to glucose and naringenin, neither of which is bitter. Naringenin is very poorly water soluble and has no bitter taste. Prunin seems to retain at least part of the bitterness (Horowitz and Gentili, 1969) but is certainly less soluble than naringin. Ting (1958) found in certain commercial pectic enzyme preparations (Pectinol 10-M and Pectinol 100-D), an enzyme that would hydrolyze naringin to rhamnose, glucose and naringenin. An enzyme was isolated from the preparation, naringinase, which was active over the pH range 3-5. This enzyme was partially purified by Thomas et al. (1958) who found that it was composed of two glycosidases. Dunlap et al. (1962) separated these two enzymes and identified
them as rhamnosidase and glucosidase.

Versteeg et al. (1977) studied the mode of action of naringinase using a thin-layer chromatographic-fluorodensitometric method. Two enzyme preparations were used which both hydrolyzed naringin and narirutin to prunin (rhamnosidase activity) and prunin to naringenin (glucosidase activity). In grapefruit juice, hydrolysis of both naringin and narirutin to prunin went at approximately the same rate. The prunin accumulated in the intermediary stage of the reaction, but was finally converted to naringenin. The glucosidase was inhibited by glucose but other inhibitors which may affect the activities of both enzymes may also be present in the juice.

The enzymatic approach has since been the subject of many patents and the three most recent patents are by Ito and Takiguchi (1970), and Fukumoto and Okada (1972, 1973). The enzyme naringinase has been isolated from a wide range of moulds grown on media containing naringin or rhamnose and in tests of 132 strains from 39 genera, Aspergillus niger and Coniella diplodiella have been reported to yield the most active preparations, hydrolyzing naringin within a few hours at room temperature and at the natural pH of most citrus juices (Chandler and Nicol, 1975). The first commercial preparations of naringinase were so heavily contaminated with pectinase that undesirable loss of cloud resulted from their use. This problem has led to attempts to compensate for the added costs of naringinase purification by immobilizing the enzyme onto solid supports, thereby allowing it to be re-used. Goldstein and co-workers (1971) claimed that an immobilized naringinase system could be used in a continuously generated bath or column process for removing the bitterness from clarified juices. Further work has been done in this area (Dinelli and Morisi, 1972; Krasnobaev, 1973, 1974) but attempts to obtain stable immobilized naringinase preparations have not been successful so far (Chandler and Nicol, 1975).
1.3.2.2 Adsorption Methods

The use of chemical sorbents to remove flavonoid bitterness was first reported by Horhammer et al. (1957) who found that polyamides have a pronounced affinity towards flavonoids due to phenolic groups on the flavonoids. Chandler et al. (1968) in their attempt to remove limonin from bitter orange juice, found that in a double batch treatment, most of the polyphenolics were removed in the first treatment while limonin was removed only during the second treatment. Polyvinylpyrrolidone (PVP) was recently used to remove naringin from Natsudaidai juice (Maeda et al., 1979). Other chemical sorbents were also used: Nylon-66 which adsorbed more than 74% of the naringin present in the juice and High Porous Polymer (HPP) which adsorbed about 59%.

1.4 PRODUCTION OF DIHYDROCHALCONEs AND PECTIN FROM CITRUS PEEL

1.4.1 Types of Dihydrochalcones

Flavonoids found in grapefruit are classified as 7-8 neohesperidosides and 7-8 rutinosides. These bitter and non-bitter flavonoids are available in large quantities and can be readily reduced to extremely sweet dihydrochalcones. These findings have considerable commercial potential. Because of the comparatively high quantities which can be isolated, the flavonoids have often attracted attention as a means of increasing the profitability of citrus processing (Vincent, 1962; Hendrickson and Kesterton, 1965). In Florida, about 7,000 tonnes of hesperidin can be recovered annually from the residues of the processors and although their processed grapefruit contains several thousand tonnes of naringin, little is recovered.

Presently accepted artificial sweeteners such as saccharin and cyclamate have enjoyed steadily increasing demand during the last decade. With waste materials from the
citrus processing industry as starting material, dihydrochalcones which are low-calorie sweeteners could be produced to replace sucrose in the diet.

Currently, the three most promising dihydrochalcone sweeteners are neohesperidin dihydrochalcone, naringin dihydrochalcone and hesperidin dihydrochalcone.

Neohesperidin dihydrochalcone. Neohesperidin is the predominant bitter principle of the Seville orange peel and can be readily converted to the intensely sweet dihydrochalcone derivative upon alkaline hydrogenation. Neohesperidin dihydrochalcone is 1,000-1,900 times sweeter than sucrose or 20 times sweeter than saccharin (Horowitz and Gentili, 1969). However, because the starting material neohesperidin occurs in the sparsely cultivated Seville or bitter orange (Citrus aurantium), it is not a commercial product. Much work has therefore been done in the production of neohesperidin using naringin as starting material.

Naringin dihydrochalcone. The large scale preparation of naringin dihydrochalcone from commercially available naringin can be accomplished easily and in high yield by catalytic hydrogenation in alkaline solution. Naringin dihydrochalcone is reported to have the same sweetness as saccharin or 20 times sweeter than sucrose (Horowitz and Gentili, 1969).

Hesperetin dihydrochalcone. Hesperetin dihydrochalcone is derived from hesperidin which is the most ubiquitous of the citrus flavonoids (Horowitz and Gentili, 1969b; 1971b). The hesperidin is reduced in alkal to hesperidin dihydrochalcone followed by a partial acid or enzymic hydrolysis to remove L-rhamnose (Horowitz and Gentili, 1969b; 1971b). This compound can also be made by partial hydrolysis of neohesperidin dihydrochalcone.
This type of dihydrochalcone exhibits a sweetness about equal to that of saccharin, on a molar basis. Moreover, hesperetin dihydrochalcone glucoside imparts a more agreeable sweetness, i.e., less clinging and absence of bitter or other taste effects, than some of the dihydrochalcone sweeteners such as naringin dihydrochalcone or prunin dihydrochalcone (Horowitz and Gentili, 1971a).

Studies at Dynapol, Palo Alto, California have now conclusively established that the structural elements of the dihydrochalcones responsible for inducing the sweet-taste response reside entirely on the aromatic nucleus (Crosby, 1976).

1.4.2 Extraction and Characterization of Pectin

Pectic substances are found in fruits and vegetables, being most abundant in limes, lemons, grapefruit and oranges. They are the major constituents of the middle lamella as well as structural elements in the primary cell wall (McClandon, 1964; Talmadge et al., 1973). Three distinct classes of pectic substances have been recognized: protopectin, the water-insoluble parent pectic material; pectinic acids or high methoxyl pectins and pectinates; and pectic acids or low methoxyl pectins and pectates (Pilnik and Voragen, 1970). Pectin is composed of anhydrogalacturonic acid units that exist in a chain-like combination with each unit connected through the 1,4 glycosidic linkages forming a polygalacturonic acid (Rouse, 1977; Doesburg, 1965). Some of the carboxyl groups are esterified with methanol, some are neutralized with cations and some are free acids (Fig. 3). Secondary acetyl groups occur in many pectins. Schultz (1965a) reported values from 0.2% in apple, citrus and cherry pectins to 3-4% for peach, pear and sugar beet pectins. Pectin acetyl are important because they affect the gelation of pectin.
Fig. 3. Part of polygalacturonic acid molecule esterified with methanol, also showing cross linkage through a polyvalent ion. (From Baker and Goodwin, 1941).
Generally, extraction temperatures range from 80 to 100°C, time of extraction from 20 to 60 minutes and pH from 1.4 to 2.6. Crandall and Kesterton (1976) used a hot water leach (88-90°C) for the recovery of pectin, with naringin being recovered as a specialty product from the hot water leach.

Precipitation of the filtered, clarified and concentrated pectin is accomplished using acetone (Hinton, 1940), alcohol (ethyl or isopropyl) or aluminium hydroxide (Rouse, 1977). Sinclair and Joliffe (1958) reported that recovery of acetone-insoluble solids is much more difficult than recovery of alcohol-insoluble solids, owing to the poor filtration and sticky adhesion of the former to the filter paper and sides of the container, possibly because of the precipitation of more colloidal materials. Thus alcohol is preferable to acetone for the recovery of pectic substances from citrus because of the greater ease of recovery of the insoluble solids. Aluminium hydroxide precipitation proved to be superior, but the method was more lengthy due to pH adjustments and a series of washings and rinsings to remove excess aluminum and acid.

The alcohol-insoluble solids resulting from the citrus peel extraction are composed chiefly of the structural constituents of the cell, i.e., cellulose, hemicellulose, pectin, protein, etc. (Sinclair and Crandall, 1949a). Pectin is composed of long chains of galacturonic acid residues with varying degrees of esterification of the carboxyl groups. The chemical nature of the pectin molecule can be ascertained by taking advantage of certain chemical reactions of polygalacturonic acid which can be determined quantitatively. Chemical and physical properties of pectin are affected by the extent of acetylation of the hydroxyl groups, of methyl esterification of carboxyl groups, and by the degree of polymerization.
In citrus fruits, pectin is the naturally occurring colloidal stabilizer that gives juice its viscosity or "body" (Rouse, 1977). Pectin is produced from the white spongy albedo or from the whole peel of citrus fruits where primary walls are said to contain a large proportion of protopectin (Kertesz, 1951). Royo et al. (1980) in a study of the preparation of dried peel for pectin production from different citrus fruits reported that dried lemon peel yields the highest proportion of crude pectin, followed by orange and then by grapefruit. Rouse and Crandall (1976) reported a similar finding.

Pectin content generally increases with maturity (Rygg and Harvey, 1938; Ting and Deszyck, 1961). Sinclair and Joliffe (1958, 1961) observed a steep initial rise in the pectin substances in the peel and pulp followed by a gradual decrease throughout the season. Gaddum (1934) reported that as Valencia oranges ripened, the water-soluble pectin in both the albedo and the pulp increased to a peak and then declined. Pectin in the peel, membrane, juice and juice sacs was found to be generally higher in the fruits from young trees.

The commercial extraction of pectin is usually accomplished with mineral acids such as sulphurous (Wilson, 1925; Joseph and Havighorst, 1952; Snyder, 1970; Wiles and Smit, 1971), sulphuric (Myers and Baker, 1929, 1931, 1932), combination of hydrochloric with ion-exchange resins (Doesburg, 1973; Huang, 1973a, 1973b), or nitric (Rouse and Crandall, 1976). Many organic acids and their salts such as oxalic acid and ammonium oxalate (Manabe and Tarutani, 1966), tartaric acid (Myers and Baker, 1929), polyphosphates (McReady et al., 1947) and many others (Joslyn and Deuel, 1963) have also been used. Meyers and Rouse (1943) reported the use of a H-Zeolite ion exchange resin for the extraction of pectin. A very low yield of pectin obtained from dried orange peel was reported (Bailly, 1956) using Zeocarb as extractant at 85-90°C.
Generally, extraction temperatures range from 80 to 100°C, time of extraction from 20 to 60 minutes and pH from 1.4 to 2.6. Crandall and Kesterton (1976) used a hot water leach (88-90°C) for the recovery of pectin, with naringin being recovered as a specialty product from the hot water leach.

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In foods, regular pectin is used in the manufacture of fruit jellies, jams, preserves, confectionery jelly pieces, thickeners for low-calorie fruit syrup and beverages, flavour emulsions and salad dressings (Glicksman, 1969). Low methoxyl pectin is used in the manufacture of salad and dressing gels with imitation flavour and colour, salad and dressing gels of fruit and vegetables juices, milk gels and puddings, low-calorie jam-like fruit gels for dietetic use and, fruit and berry gels for ice cream use. Non-food uses include pharmaceutical and medicinal. Ahmadsiddiqui et al. (1971) demonstrated the beneficial medicinal effect of pectin in disorders of the alimentary canal. Works on the effect of pectin on blood had been conducted by Keys et al. (1961) and Fisher et al. (1964). Rouse (1977) suggested further research on the application of metallic pectinates to relieve physical suffering.

New Zealand imports pectin mainly from Denmark, United Kingdom and United States. Other sources are Italy, Switzerland, Fed. Rep. of Germany, Australia, Israel and Sweden. For the year 1979-80, New Zealand imported 20,805 kg pectin substances from Denmark, 15,375 kg from U.K. and 1,376 from U.S.A. with the cost (including insurance and freight) being NZ $150,964, $69,990 and $14,882 respectively (New Zealand Department of Statistics, 1981). Ten years earlier the comparable figures were 17,954 kg from Denmark, 17,219 kg from U.K. and 7,864 kg from U.S.A. with the cost (including insurance and freight) being NZ $4,962, $11,377 and $20,337, respectively.