EVALUATION OF THE PROPERTIES OF POLYSTYRENE DIVINYLBENZENE ADSORBENTS FOR DEBITTERING GRAPEFRUIT JUICE

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

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Various polystyrene divinylbenzene adsorbents were evaluated for their ability to debitter model systems and grapefruit juice in a batch process. The data provided the evidence that the solid density, the degree of crosslinkage, and the specific surface area of the adsorbents played key roles in the adsorption process of naringin and limonin.

When the solid density was greater than 1.2 g/cc (dry adsorbent), the adsorbents came in full contact with the aqueous bitter solutions. Naringin was reduced by 21.31% to 93.54% from the model systems and by 20.0% to 66.79% from grapefruit juice serum when the degree of crosslinkage and the surface area of the adsorbents increased from 16% to 65% and from 114 to 650 m²/g (dry adsorbent), respectively.
Similarly, limonin was reduced by 24.26% to 91.30% from grapefruit juice serum. The specific surface area appeared to be the major factor in the debittering process.

The rate of adsorption of naringin, limonin and recoverable oil from grapefruit juice by the 50% crosslinked PSDVB adsorbent could be approximated to two simultaneous first order reactions. Mathematical models with two exponential terms were proposed to describe the adsorption rate of these constituents. Debittering at 24°C (room temperature) was found to be more efficient than adsorption at 35°C. Moreover, the present debittering process resulted, in most instances, in negligible losses of ascorbic acid from grapefruit juice. The suitable debittering process time was found to be 20 min during which significant adsorption of naringin and limonin was achieved without significantly affecting the ascorbic acid and recoverable oil of the juice. Regeneration with 95% ethanol was more efficient than warm water for restoring the exhausted polystyrene divinylbenzene adsorbents during a 20 min regeneration in a batch system.

Sensory evaluation clearly showed that the quality of the polystyrene divinylbenzene adsorbent treated juice was significantly improved. The treated grapefruit juice was rated less bitter than the control. More than 65% of the panelists preferred the adsorbent-treated juice over the non-treated juice.
INTRODUCTION

Naringin- and limonin-induced bitterness in grapefruit imparts an objectionable taste to grapefruit juice and adversely influences consumer acceptance. Although limonin threshold is much lower than that of naringin, it has been found that the bitter taste of freshly extracted grapefruit juice is primarily due to naringin (Chandler and Nicol, 1975). Naringin is present in large amounts (generally more than 600 ppm) in juice extracted from early season fruit. Excessive bitterness of the juice constitutes an economic problem for the citrus industry because the extremely bitter juice from early season grapefruit must be mixed with the less bitter juice from late season fruits. The mixing reduces the bitter taste through dilution. However, the practice is costly to the industry since it requires refrigerated storage of early season juice concentrates and not all the bitter concentrate produced can be utilized. Other practice which is aimed at decreasing the bitter taste of grapefruit juice consists of reducing the extraction pressure of the fruits, but this results in reduced juice yield. Addition of sweeteners or substances to mask the bitter taste has not been pursued since in accordance with regulatory requirements such action would be considered
adulteration of the juice unless the label stated the presence of the additive in the product and, as a result, the juice could not be labeled as grapefruit juice. This disclosure could deter consumers from accepting the grapefruit juice and was deemed contra-indicative to the idea of expanding its market share of fruit juices.

Naringin is partially responsible for the characteristic flavor of grapefruit juice (Kesterson and Hendrickson, 1957; Buffa and Bellenot 1962). That is due to the fact that the bitter taste generally is likely to be pleasant at low concentrations but exceedingly unpleasant at high concentrations (Guyton, 1981). In Florida there are regulations to ensure the quality of grapefruit juice, which stipulate the maximal acceptable concentrations of the bitter compounds in the juice. Thus, the levels of limonin and naringin must be lower than 5 ppm and 600 ppm, respectively, for grade A grapefruit juice processed during the large part of the season (Florida Department of Citrus, 1975).

In Florida, the quality of citrus fruit juices has always been of paramount importance since a high percentage of the fruits produced are processed into juices and other products. Thus, the grapefruit juice improvement program was initiated in the 1970’s by the Florida Department of Citrus (FDOC) with the objective to boost the juice consumption. Intensive research efforts have been made to
find suitable and efficient processes for the production of more acceptable grapefruit juice and products. Biological processes involving immobilized bacterial cells and immobilized enzyme processes have been used to satisfactorily debitter citrus juices (Hasegawa et al., 1985; Olson et al., 1979; Herman et al., 1985). Unfortunately, the enzyme processes have exhibited limited industrial application owing to the unavailability of highly purified debittering enzymes in commercial quantities, the low reaction rate with immobilized enzymes, inadequate half-life of immobilized enzymes, and high optimum pH and temperature for efficient utilization of the enzymes (Chandler and Nicol, 1975; Puri, 1984). In addition, the enzymes used often required cofactors that must be supplied in order to obtain optimal activity; this practice can alter the wholesomeness of the juice (Chandler and Nicol, 1975).

Adsorbent and ion-exchange techniques have also been successfully employed to reduce the bitterness of grapefruit juice, as well as other citrus juices (Chandler et al., 1968; Chandler and Johnson, 1977; Johnson and Chandler, 1985, 1988; Mitchell et al., 1985; Onayemi and Bruemmer, 1984; Puri, 1984; Shaw et al., 1984). Furthermore, these techniques have been used to decrease the acidity of fruit juices (Johnson and Chandler, 1982; Shaw et al. 1977; Maeda et al., 1984; Huffman, 1974; Onayemi and Bruemmer, 1984). Unlike the enzymatic processes, the adsorbent and
ion-exchange methods for debittering grapefruit juice have been receiving growing interest because the resins are easier to manipulate and can be manufactured in large quantities with various physical and chemical characteristics. Thus, methods utilizing the latter materials to reduce the bitter taste of grapefruit juice are becoming a more suitable alternative for the citrus industry. Large differences have been reported between the efficiencies of the resins, and even among the resins of the same chemical type. However, the literature does not provide adequate explanation for the difference in their performance, and limited data is available concerning the specific properties of the adsorbents and exchangers which determine their ability to debitter grapefruit juice by removing naringin and limonin. Also, the contribution of the matrix structure of the resins to the debittering activities has not been extensively documented. Moreover, the ion-exchangers have not been proven to be better materials in the debittering process in comparison to the adsorbents. Therefore, one can reasonably postulate that the physical properties of the polymers used play an important role in debittering citrus juices. Thus, the objectives of this research project were

(1)- To evaluate the properties of the styrene divinylbenzene copolymer adsorbents involved in the debittering process of grapefruit juice in order to establish relationships between the
matrix structure and the efficiencies to adsorb naringin and limonin
(2)- To evaluate factors affecting the rate of adsorption of naringin and limonin and other components in order to determine the optimum conditions of utilization of some of the efficient adsorbents
(3)- To determine the organoleptic quality of the treated grapefruit juice.
REVIEW OF LITERATURE

Production of Grapefruit in the U. S. and the Problem Associated with the Bitter Taste

The United States (U.S.) is the world's leading producer of grapefruit; its production reached 57.8 million boxes of grapefruit, which represented 69.9% of the world production during the 1982-83 production year. Florida is the leading production state and has accounted for 75% of the total national crop, while Texas has averaged 14%, and California and Arizona each has averaged 11% during the five seasons between 1977 and 1982. Moreover, Florida fresh grapefruit represented 37.8% of the state grapefruit production, whereas in Texas this represented 57.9%, and in California and Arizona this was 52.4%. Florida is also the leading state in the area of processed grapefruit (Fairchild and Tuttell, 1984). From the 1983-84 season to the 1986-87 season the total production of grapefruit juice has been increasing and the amount of processed grapefruit has always exceeded fresh fruit utilization as indicated by the data in Table 1 (Behr and Brown, 1987). However, the organoleptic quality of grapefruit juice, particularly the bitterness, has constituted the major negative factor and motive for growing concern of both the citrus growers and the citrus industry; the bitter taste of the juice has been found
Table 1

Estimated Utilization of Florida Grapefruit, 1987-88 Season and Actual for Previous Seasons

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<td>Fresh</td>
<td>14.8</td>
<td>19.4</td>
<td>20.6</td>
<td>21.5</td>
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<tr>
<td>Canned</td>
<td>3.6</td>
<td>3.0</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Frozen Concentrate</td>
<td>23.0</td>
<td>21.6</td>
<td>24.1</td>
<td>24.5</td>
</tr>
<tr>
<td>Chilled Juice</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Blends</td>
<td>1.5</td>
<td>1.3</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Non-Certified</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>44.0</td>
<td>46.8</td>
<td>49.8</td>
<td>51.0a</td>
</tr>
</tbody>
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- - - - - - million boxes - - - - - -

\[ a: \text{Column does not add to total due to rounding.} \]

Adapted from Behr and Brown (1987).
objectionable by a large sector of consumers (Petrus et al., 1977). A downward trend in the retail sales of grapefruit juice for the production seasons from 1977-78 to 1982-83 has been reported (Fairchild and Gunter, 1984 and 1985). The sales declined from 105.7 million single strength gallons (or 10.5% of domestic juices sales) to 86.6 million single strength gallons (or 6.2% juices sales). In addition, the decline in the total grapefruit sales appeared to be the result of a decrease in the quantity purchased per household and in the number of buying households. This information suggested the need for more research efforts to make grapefruit juice and other related products more palatable and appealing to the consumer, and ultimately to expand the market for both the juice and products.

Because of the decrease in grapefruit juice consumption, numerous surveys have been conducted to better understand the trend and consumer attitude towards the juice. Thus, consumers rated bitter grapefruit juice unfavorably during a market survey conducted by Ting and McAllister (1977) in supermarket stores across the U. S. Rouseff et al. (1980) also observed an inverse relationship between the bitterness and flavor score of canned grapefruit juice tested during the period of 1977 to 1980. A recent field survey has shown that the bitterness of grapefruit juice had an adverse effect on its flavor and constituted the major barrier to higher consumption of the juice in the
U.S. (Fellers et al., 1987). These authors evaluated the effect of limonin concentration in grapefruit juice on consumer preference in four large U.S. cities. They found that the juice flavor was negatively affected by an increase in limonin concentration; and although the grapefruit users rated the products significantly higher than the nonusers, the preference by both groups was lowest for the highest limonin content juice (11.0 ppm limonin). They also found that the perception of bitterness and tartness increased with increasing limonin content. The sweetness perception of the juice was also diminished when limonin content increased. A significant negative correlation between overall flavor score and limonin concentrations in juice \((r = -0.970, p < 0.01)\) was obtained. These results were in agreement with those reported by Dougherty and Fisher (1977), and Ting and McAllister (1977). The threshold value of limonin in orange juice has been found to be affected by the acid and sucrose contents of the juice and by the pH (Barros et al., 1983; Dougherty and Fisher, 1977; Guadagni et al., 1973). Thus, it appears that the viability of the grapefruit industry depends on research efforts to reduce bitterness in order to improve the juice quality.

One major aspect of the taste problem of grapefruit juice is that its bitterness is due to two different compounds, namely, naringin and limonin. Consequently, any method aimed at improving the quality of grapefruit juice
must be able to remove both compounds or reduce them to levels that are not objectionable. According to regulations by the Florida Departement of Citrus (or FDOC) the limonin and naringin contents of processed grapefruit juice canned and chilled and grade A frozen concentrate grapefruit juice (FCGJ), made from juice extracted during the period of August 1 to December 1 of each season, must contain less than 5.0 ppm limonin as measured by high performance liquid chromatography (HPLC) and contain less than 600 ppm naringin as measured by the Davis test. On the other hand, grade B FCGJ must contain less than 7.0 ppm limonin and contain less than 750 ppm naringin (FDOC, 1982).

Physical and Chemical Characteristics of the Bitter Compounds, and Acidity of Grapefruit Juice

Naringin

The flavonoid naringin is one of the two compounds which greatly impart the bitter taste to grapefruit juice and its products (Kesterson and Hendrickson, 1957; Mansell, et al. 1983). In addition, Mansell and Weiler (1980) reported that naringin was one of the two naturally occurring compounds that have a major impact on the taste of grapefruit products and caused many organoleptic and economic problems. The typical bitter sensation caused by naringin has been described by Chandler and Nicol (1975) (Table 2). The summary of the response to naringin and limonin bitterness as shown in Table 3 indicates a threshold
### Table 2

**Bitterness in Citrus Fruits and their Products**

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Limonoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits vary in flavonoid type</td>
<td>Fruits vary in limonoid content</td>
</tr>
<tr>
<td>Bitter principle in tissue and juice sacs</td>
<td>Bitter principle in tissue, not in juice sacs</td>
</tr>
<tr>
<td>Bitter principle comparatively water-soluble</td>
<td>Bitter principle almost insoluble in water</td>
</tr>
<tr>
<td>Bitterness immediately apparent</td>
<td>Bitterness delayed</td>
</tr>
<tr>
<td>Bitterness in juice not avoided by instantaneous and complete pulp removal</td>
<td>Bitterness in juice avoided by instantaneous and complete pulp removal</td>
</tr>
<tr>
<td>Bitterness generally mild</td>
<td>Bitterness generally objectionable</td>
</tr>
<tr>
<td>Bitterness readily removed from palate</td>
<td>Bitterness persistent on palate</td>
</tr>
</tbody>
</table>

Adapted from Chandler and Nicol (1975)
Table 3
Organoleptic Responses to Limonin and Naringin

<table>
<thead>
<tr>
<th>Threshold</th>
<th>Limonin ppm</th>
<th>Naringin ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non bitter</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Slightly bitter</td>
<td>7-9</td>
<td>20-300</td>
</tr>
<tr>
<td>Bitter</td>
<td>10-16</td>
<td>500-1300</td>
</tr>
<tr>
<td>Very bitter</td>
<td>18</td>
<td>1500</td>
</tr>
</tbody>
</table>

Threshold ave. = 1                  ave. = 20

However, a solution of juice with 0.75 ppm limonin and 5 ppm naringin was found to be bitter (Maier et al., 1977).

Adapted from Mansell and Weiler (1980).
value of 20 ppm for naringin. Guyton (1981) stated that the perception of the bitter taste occurred on the back of the tongue where taste buds more sensitive to bitterness are located. In spite of the bitterness problems, naringin is also known to contribute to some extent to the typical flavor of grapefruit juice. Kesterson and Hendrickson (1957) reported that less than 0.05% naringin (by weight) imparted a mild and pleasant flavor to Texas grapefruit juice. Buffa and Bellenot (1962) confirmed the role of naringin in the development of the typical grapefruit juice flavor. However, the bitter taste becomes exceedingly unpleasant at high concentrations of the bitter agent (Guyton, 1981); thus, the adverse effect of the intensely bitter juice on consumer preference has been found to outweigh the slight contribution of naringin to the flavor.

Naringin is found in grapefruit (Citrus paradisi (macf.) and in the shaddock or pummelo (Citrus grandis (linn.) (Kesterson and Hendrickson, 1957). It is a member of the flavonoid compounds which are present in almost all the parts of citrus fruits, including the albedo, centre bundle and segment covers, and juice sacs (Figure 1; Chandler and Nicol, 1975).

Moreover, naringin (MW = 580.55) is a flavonoid O-glycoside with one flavonoid hydroxyl group or naringenin bound to a disaccharide moiety (glucose and rhamnose) by an acid labile hemiacetal linkage (Figure 2). Glycosylation
Albedo contains flavonoids & limonoids

Centre bundle & segment covers (rag & pulp on juicing) contain flavonoids & limonoids

Juice sacs contain flavonoids no limonoids

Figure 1: Location of flavonoid and limonoid bitter principles in the various tissues of citrus fruits
(Adapted from Chandler and Nicol, 1975)
Figure 2: Chemical structure of naringin  
(Adapted from Kefford and Chandler, 1970)
renders naringin less reactive and increases its solubility in water (Markham, 1982). Naringin is insoluble in ether, chloroform, or benzene, but soluble to some extent in water, alcohol, acetone, glacial acetic acid or pyrimidine; the solubility of naringin increases in hot water (Table 4; Kesterson and Hendrickson, 1957). Also, the solubility of naringin increases in the presence of calcium hydroxide or other alkanal. When it reacts with sodium hydroxide, a yellow coloration forms which constitutes the principle of the Davis colorimetric method (Davis, 1947). However, this coloration is not specific for the bitter compound since other non-bitter glycosides produce the same reaction (Dougherty and Fisher, 1977).

Limonin and Other Bitter Limonoids

Limonin (MW = 470.50) is the second of the two most bitter compounds of grapefruit juice. It is believed that the intensely bitter taste that develops subsequent to processing is almost entirely due to this limonoid. Maier and Beverly (1968) discovered that limonoic acid A-ring lactone (Figure 3A) localized in fruit tissues and seeds was a precursor which easily lactonized to form limonin (Figure 3B). During extraction, the non-bitter precursor diffused into the juice where the combined action of juice acids and enzyme converted it to limonin. Furthermore, the development of limonin-induced bitterness after pasteurization of citrus juices has been attributed to the heat conversion of
Table 4
Solubility of Naringin in Various Solvents at Different Temperatures

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Solvent</th>
<th>Solubility Percent by volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>35</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>45</td>
<td>Water</td>
<td>0.2</td>
</tr>
<tr>
<td>55</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>65</td>
<td></td>
<td>4.2</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td>21</td>
<td>95 % Ethyl alcohol</td>
<td>4.2</td>
</tr>
<tr>
<td>39</td>
<td></td>
<td>6.5</td>
</tr>
<tr>
<td>21</td>
<td>Glacial acetic acid</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Adapted from Kesterson and Hendrickson (1957).
Figure 3: Important limonoids in the grapefruit and conversion of the precursor to the bitter agent

(A): precursor
(B): bitter agent

(Adapted from Kefford and Chandler, 1970)
the precursor to limonin; this has been described as "delayed bitterness" in juice containing limonoids (Maeda et al., 1984). Obacunone and ichangin have also been reported as probable precursors of limonin. The character of the bitter taste due to limonin is described in Table 2. Limonin has also a lower threshold value than naringin (Table 3; Mansell and Weiler, 1980). However, Chandler and Kefford (1968) earlier observed that the threshold or the amount of limonin in citrus juices required to produce detectable bitterness depends on the sweetness and acidity of the juice as well as the sensitivity of the taster; the level of 9 ppm was found to be the threshold for most tasters.

Unlike the flavonoids, the limonoids occur in all citrus fruits, but they are localized in the albedo, central bundles and segments (Figure 1; Chandler and Nicol, 1975). Limonin is the predominant limonoid in most citrus species and hybrids (Dreyer, 1966). Limonin, a triterpenoid derivative of limonoid, is a dicarbocyclic compound with two lactone rings, a cyclic ether ring, an epoxide group, a furan ring and a ketone group (Figure 3B; Barton et al., 1961; Kefford and Chandler, 1970). It is slightly soluble in water and ether, but soluble in alcohol and glacial acetic acid. The solubility in aqueous medium increases in the presence of sugar and pectin (Chandler and Nicol, 1975). Limonin can be degraded by chemical oxidation to limononexic
acid, which is a tasteless compound. It is also readily oxidized by atmospheric oxygen in a light-catalyzed reaction at pH 7.0 (Melera et al. 1957).

Nomilin-induced bitterness has been reported by Rouseff (1982) and confirmed by Maeda et al. (1984). The latter authors have described the taste due to nomilin as a "delayed bitterness" produced by a mechanism similar to that of limonin bitterness in that there occurred a conversion of a non-bitter precursor to the bitter nomilin. However, the bitter taste caused by nomilin is less intense than that of limonin.

**Acidity of Grapefruit Juice**

The high acidity of citrus juices constitutes a factor that greatly influences the acceptance of the juices by the sector of the consumers who exhibit less tolerance to highly acidic fruit juices. Too much tartness (or acidity) / bitterness in grapefruit juice has also been found to be objectionable to consumers (Fellers et al., 1986).

Grapefruit juice contains a relatively high concentration of organic acids and its titratable acids may constitute 8% to 15% of the total soluble solids of the juice. These acids are as follows (Sinclair, 1972).

- 98.72% citric acid,
- 1.0% malic acid,
- 0.23% oxalic acid,
- 0.05% tartaric acid.
Among these, the weak acids such as citric and malic acids are the constituents that contribute to the hydrogen ion concentration. It is known that the quality of citrus juice and the palate response to acidity depend in large measure on the sugar to acid ratio or degree brix. Thus, for grade A unsweetened, single strength grapefruit juice the sugar to acid ratio has been set between 8 and 14 with a minimum brix value of 9 (USDA, 1968).

**Background Research on the Reduction of Bitterness and Acidity of Grapefruit Juice**

**Biological Methods for Debittering Grapefruit Juice**

In recent years numerous research efforts have been focused on the problem of bitterness that occurs in citrus juices. In response to the consumer dislike of excessive bitterness in grapefruit juice, an improvement program was initiated in 1973 by the Florida Department of Citrus (Dougherty and Fisher, 1977; Petrus et al., 1977). Extensive research has been performed in the development of methods and technology to produce grapefruit juice of better taste, particularly the juice extracted from early season fruits.

Biological methods which employed cells of *Corynebacterium fascians* immobilized in acrylamide gel have been used by Hasegawa et al. (1985) to metabolize limonin and nomilin of citrus juice sera. When 50 mL of citrus juice serum was eluted at a flow rate of 100 mL/hr through
a 2 cm-diameter column which contained the cells, these limonoids were significantly reduced from the juice. The procedure did not affect the other constituents such as citric, malic and ascorbic acid, fructose, glucose and sucrose. Nomilin acetyl-lyase, a bacterial enzyme, was isolated by Herman et al. (1985) for potential use in debittering citrus juices. This enzyme can convert the bitter nomilin to the non bitter obacunone by catalyzing the transelmination of acetic acid from nomilin.

Similarly, Olson et al. (1979) demonstrated a scheme of debittering unclarified grapefruit juice with immobilized naringinase prepared from Aspergillus niger in a hollow fiber reactor. Naringinase was found to be a system composed of two enzymes, namely, alpha-rhamnosidase and beta-glucosidase. Thus, naringin was converted to prunin and rhamnose after cleavage of the disaccharide bond by alpha-rhamnosidase, and this was followed by the conversion of prunin to naringenin and glucose by beta-glucosidase. The authors reported that any desired level of naringin could be achieved in grapefruit juice by recycling and properly monitoring the conditions of naringin hydrolysis. Roe and Bruemmer (1977) used naringinase to debitter the albedo and grapefruit juice. They found that the amount of enzyme required to debitter early season fruits harvested in the period of October-November was ten times higher than the amount needed to treat the late season fruit harvested in
the spring. In spite of these remarkable results, the enzyme processes were found to be limited in their industrial application because of the unavailability of purified enzymes in commercial quantities, low reaction rates and inadequate half-life of immobilized enzymes. Roe and Bruemmer (1977) noted that some juice components such as glucose and fructose completely inhibited naringinase activity whereas artificial sweetners such as saccharin and neohesperidin dihydrochalcone exhibited slight effects on the enzymes. Furthermore, the enzymes possessed a high optimum pH and/or required cofactors such that efficient utilization of these enzymes would necessitate several adjustments of the juice pH and the addition of the cofactors. Such practice would not be acceptable to the citrus industry under the Good Manufacturing Practice (G.M.P.) guidelines since the alterations for optimal enzyme activity would adversely affect the character of the juice (Chandler and Nicol, 1975; Puri, 1984).

**Adsorbent and Ion Exchanger Methods for Debittering and/or Deacidifying Grapefruit Juice**

Unlike the biological methods for debittering citrus juices, considerable work has been performed with adsorptive and ion exchange resins which, in some cases, can concurrently reduce naringin- and/or limonin-induced bitterness and acidity. Chandler and Johnson (1977) used 10 g/L of cellulose acetate to selectively remove 44 to 70% of limonin from orange juice in less than 1 hr without affecting the
hesperidin and ascorbic acid contents. The removal of limonin was attributed to a sorption mechanism rather than a surface catalyzed degradation. Johnson and Chandler (1982) reported that cellulose acetate did not have a significant effect on the acidity of grapefruit juice. They also found that polyamide resin exhibited a lack of specificity and removed up to 30% of ascorbic acid from the juice, a drawback that suggested limited industrial application for this material. Johnson and Chandler (1981) also developed mathematical models for the sorption of limonin from orange juice by cellulose acetate. Johnson (1981) reported that in commercial use, exhausted cellulose acetate beads can be readily and economically restored by washing with small volumes of warm water, which desorbed all limonin and 90% of bound hesperidin. Reactivation with methanol was found to be only partially effective, time consuming, and expensive. Successful reactivation of cellulose acetate gel bed with water washes was confirmed by Johnson and Chandler (1981).

Nisperos and Robertson (1982) reported that polyvinylpyrrolidone (PVP) significantly reduced the levels of naringin and limonin in grapefruit juice concentrate (27°B) by 78.1% and 17.5%, respectively. A loss of 23.1% ascorbic acid occurred when 5% (w/v) of PVP was contacted with the juice for 30 and 60 min in a batch experiment. However, no significant change in limonin content occurred after treating the juice with 0% to 5% (w/v) of PVP for 30 min.
Thus, PVP did not prove very useful for debittering purposes since it resulted in little reduction of limonin content, but induced high losses of ascorbic acid content from the treated juice.

Barmore et al. (1986) have reported successful reduction of bitterness and tartness in grapefruit juice with Florisil(R), an activated magnesium silicate, without affecting the ascorbic acid content. In a batch-type treatment of commercial juice, 81, 51, 57, and 50% of limonin, naringin, narirutin and total acids, respectively, were removed when 20% (w/v) Florisil(R) was used for 1 hr at 20°C. The mode of action of Florisil(R) on the bitter compounds during the debittering process was not documented by these authors. However, the chemical nature of this adsorbent would necessitate tedious preconditioning prior to contacting with grapefruit juice in order to reduce possible deleterious effects on the juice. Florisil(R) is obtained by coprecipitation of silica and magnesia. Thermally activated Florisil(R) is an adsorbent with strongly acidic sites on its surface; because of its strongly polar surface, it has been used in liquid-solid chromatography as a general-purpose adsorbent (Johnson and Stevenson, 1977). It adsorbs substances such as organic nitrogen bases and irreversibly adsorbs esters and aromatic hydrocarbons. It has been reported that acid-catalyzed reactions such as isomerization, disproportionation, polymerization of
olefins, and alcohol and ester elimination can occur when acidic adsorbents such as Florisil® are used (Snyder, 1968). The addition of 1% water causes deactivation of the polar surface of this adsorbent (Perry et al., 1972).

Shaw et al. (1984) have demonstrated that beta-cyclodextrin polymer reduced limonin, nomilin, and naringin contents in grapefruit juice, and limonin and nomilin contents in navel orange juice by about 50% when 1 g of the polymer was used for 50 mL of juice in a continuous flow fluid bed or batch processes. The soluble solids, total acids and ascorbic acid contents of the juice were not significantly affected, but the oil level was lowered by 40% along with other trace elements. It was observed that the removal of the juice components such as beta-sistoserol, nootkatone, 7-methoxy-8-(2,3 dihydroxy-isopentyl) coumarin, 5-hydroxypsoralen, and 7-hydroxypsoralen was pronounced in the first fractions from the fluid bed. These could be recovered after regeneration of the adsorbent with dilute alkali or ethanol. Nootkatone has been described as the character-impact compound of grapefruit juice (Teranish, 1966), and is considered an important component of grapefruit flavor (Shaw and Wilson, 1981). Therefore, it is essential that nootkatone be preserved after treatment of the juice to avoid alteration of the typical character of grapefruit juice.
The mechanism of removal of the bitter compounds, naringin and limonin, from aqueous solutions and citrus juices by beta-cyclodextrin was earlier elucidated by a group of Japanese researchers using the methods of solubility of these compounds in the presence of the polymer and the proton shifts within beta-cyclodextrin as measured by its H-NMR spectra in D$_2$O. The solubility of both naringin and limonin increased with the addition of beta-cyclodextrin to aqueous suspensions of the same. Furthermore, the H-NMR spectra showed that the hydrogen proton located within the cavity of the beta-cyclodextrin molecule shifted to higher field values when the molar ratio of naringin to beta-cyclodextrin increased. Hence, the reduction of bitterness was found to be the result of the formation of an inclusion complex between beta-cyclodextrin and naringin or limonin. It was also concluded, based on the proton shift, that the phenyl ring of naringin (in the naringenin moiety) was positioned within the hydrophobic cavity of beta-cyclodextrin and that the disaccharide moiety remained unaffected. Difficulties arose with limonin due to its low solubility in aqueous media (Konno et al., 1982). These authors claimed that they were the first researchers who used beta-cyclodextrins to debitter citrus juices in which naringin or limonin was the bitter constituent. In an interview by Kelly (1986), who was reporting for the USDA News, Shaw confirmed the physical mechanism of removal of
the bitter compounds from grapefruit juice. He stated that "the large molecules of the bitter compounds became physically entrapped in the hole of the doughnut-shaped molecules of cyclodextrins (P. 6)."

The important properties of cyclodextrins to encapsulate food ingredients such as vitamins and flavor compounds within their ringed structures whereby they ensure protection against loss during processing, and their role in stabilizing emulsions and foams, protecting light sensitive ingredients and facilitating the solubilization of hydrophobic materials are well documented. However, although they present potential food applications and have been used in Japan and Europe since 1978, cyclodextrins have yet to be cleared for food contact by the Food and Drug Administration (F.D.A.) in the United States. Meanwhile, the first commercial production feasibility is being investigated by the American Maize Product, Co, (Hammond, Indiana) (Pszczola, 1988; Vast range, 1987).

An important and almost similar finding by Nakabayashi about the mechanism of adsorption of hesperidin from mandarin and orange juices by methylcellulose was quoted by Misaki et al. (1981): the increase of solubility of this constituent in the presence of methylcellulose was attributed to the hydrophobic interaction between the phenolic group of hesperidin and the hydrophobic methyl groups of methyl-cellulose. Hesperidin is analogous to
naringin but is not bitter and is only present in citrus fruits other than grapefruit. These two proposed mechanisms of removal of the bitter compounds and solubilization of hesperidin in citrus juices suggested the important role of the hydrophobic sites of polymers which can bring about the interaction with the hydrophobic moiety of the bitter compounds of grapefruit juice to effect their removal as demonstrated by Konno et al. (1982).

Other polymers have found similar applications in the debittering process of citrus juices. In a review of the research on the development of syrup from grapefruit juice, Berry (1981) reported that activated carbon significantly reduced naringin content in the serum by more than 90% during a 90 min treatment in batch experiments and more than 90% in filtration column processes. It was also observed that Dowex 1 and 50 removed most of the amino acids, and Duolite S-761 adsorbed naringin. Onayemi and Bruemmer (1984) found that combinations of Dowex 1 and 50 were very effective for the removal of naringin. The anion exchange resins Dowex 1-8X, Duolite A-7 and activated carbon increased the pH of grapefruit juice sera while Dowex 50-8X lowered the pH. Amberlite XAD-7 and Duolite S-761 did not affect the pH of the juice. No off-flavor was detected in any of the treated juice samples. However, Shaw et al. (1977) found that Dowex-1 caused losses of ascorbic acid and other organic acids of the juice. Furthermore, Maeda et al.
(1984) reported that weak base anion exchange resins of styrene-divinylbenzene and acryl-divinylbenzene copolymer types effectively reduced the bitterness and acidity of Hassaku (Citrus hassaku hort. ex Tanaka) juice; about 50% of the total acidity, 60% of naringin, and 75 to 90% of limonin and nomilin were reduced from the juice by the polymeric materials in a continuous column process. Negligible losses of amino nitrogen and ascorbic acid occurred, but the losses of these valuable nutrients were relatively higher when strong base anionic resins were used.

Johnson and Chandler (1982) also reported the reduction of the bitter taste and acidity of grapefruit juice with similar resins as well as different ones. They found that Amberlite XAD-7 removed about 63% of naringin, 85% of limonin and 3% of total titratable acids, whereas Deacidite-FFID only removed 20% naringin, 8% limonin and 23% titratable acids after contacting 20 g of the dry adsorbents with 1 L of juice for 1 hr in a batch-type experiment. Also, Amberlite IRA-93 and IRA-45 each adsorbed approximately 21.5% of the titratable acids and Amberlite XAD-12, a very polar porous polymer, was found to be a very good adsorbent for titratable acids with more than 50% removal efficiency. However, the latter polymer presented two major drawbacks: it concomitantly adsorbed about 7% of the total soluble solids content and almost completely eliminated the grapefruit character from the juice. On the other hand,
Amberlite XAD-2 adsorbed very little of the titratable acids. Nylon-based adsorbents did not show a significant effect on either the bitter compounds or the acidity of grapefruit juice. The reduction of flavones by XAD-7 used in the treatment of grapefruit juice was several times higher than when grapefruit juice syrup was treated with the same polymer (Onayemi and Bruemmer, 1984).

In regards to the literature, the utilization of styrene-divinylbenzene copolymer types of resins has been growing in popularity. They have a relatively large application in the debittering process of citrus juices for a number of reasons. These resins are available in various chemical and physical forms and yield a wide range of results in debittering and/or deacidifying citrus juices; off-flavor is not generally produced and losses of desirable compounds of citrus juice are in many cases negligible. Many types of copolymers have also been approved for food contact in the United States; they have been employed in the adsorption of various organic products. Thus, a U.S. patent was granted to Puri in 1984 for a process whereby naringin and/or limonin was effectively removed from citrus juice after contact with a styrene-divinylbenzene copolymer adsorbent, Duolite S-861. Reduction by 81.23% and 90% of naringin and limonin, respectively, was achieved when 700 mL of grapefruit juice serum were passed through a 14 mL bed volume (bv) of adsorbent at a flow rate of approximately 5
to 6 bv/hr (or 1.5 mL/min) with a loss of only 6.6% ascorbic acid. In addition, the limonin content in navel orange juice was reduced by 85% when the juice was eluted through 15 bed-volumes of the adsorbent at the flow rate of 7 bv/hr (or 1.75 mL/min); this treatment represented a ratio of 1:53 (v/v) adsorbent used to juice treated. Naringin in Japanese orange juice containing 915 ppm of the same was reduced by 70% in a 15 mL bed volume column with the same flow rate. It was demonstrated that the adsorbent could effectively debitter other citrus juices: limonin in lemon, tangerine and valencia orange juices, and pulp wash solids (water extracted soluble orange solids or WESOS) was reduced by 94%, 92%, 96% and 86%, respectively, when 1 g wet Duolite S-861 was used to treat 50 mL of pulp free juice for 1 hr in a batch type-process. The exhausted adsorbent was satisfactorily regenerated with either ethanol, hot water, or alkali solution (NaOH).

Another U.S. patent allowed for the use of an ion exchange resin, Dowex 66, to remove the bitter compounds from grapefruit juice. This exchanger possessed a styrene-divinylbenzene copolymer matrix with amine functional groups. It was observed that 100, 98 and approximately 50% of the original naringin, limonin and acidity, respectively, were removed using an up-flow multistage column process without adversely affecting the desirable nutrients and flavor constituents of the juice. On the contrary, Dowex
WGR-2, a weak base anionic exchange resin having an epoxy amine matrix which carried 10% quaternary ammonium functional groups and 90% tertiary amine groups exhibited almost no effect on the naringin and limonin contents of grapefruit juice; only the juice acidity was reduced by 49% after treatment (Mitchell et al., 1985). The mechanism of adsorption of the bitter compounds by Dowex 66 was not explained by the authors. The difference in the efficiency of the two polymeric materials could be attributed to two possible factors, either their matrix type or the functional groups. The high affinity of Dowex 66 for the bitter compounds would suggest the formation of weak interaction such as hydrogen bonding, between the adsorbates and the amine groups of the resin. If this were the case, then the hypothesis would be supported by the results obtained with Dowex 1 (Shaw et al., 1977), which is a weak anionic resin with similar matrix but carries trimethyl benzyl ammonium groups. But Dowex 1 was ineffective in the debittering process. It is also not understood why Dowex WGR-2 carrying quaternary ammonium groups did not remove limonin whose structure is dominated by hydrogen accepting groups. Therefore, it is more likely that the matrix type was responsible for the difference in the debittering efficiency of these resins. Certainly, a comparison of Dowex 66 and Duolite S-861 may indicate the predominant role played by the matrix properties in the debittering process; both
resins have the same styrene-divinylbenzene copolymer matrix but differ primarily by the functional groups. Duolite S-861 is a neutral adsorbent with no functional group while Dowex 66 carries amine groups; nevertheless, they were almost equally effective in removing the bitter substances from grapefruit juice. Thus, the physical properties of the macroporous styrene-divinylbenzene resin appear to be the factor that is responsible in large part for the differences in efficiency observed between all the resins with a styrene-divinylbenzene matrix. It is known that the volume, size and diameter of the pores of the macroporous polymers can play a role in the adsorption process; these characteristics can control the diffusion of the solutes inside the polymers and the exposure of the adsorption sites in the inner part of the resins. Puri (1984) stated that the specific surface area, the chemical nature of the resin porous surface, and the physical structure of the pore could facilitate the fixation of amphoteric organic molecules; and that the hydrophobic parts of the molecules were adsorbed on the porous surface. However, no comparative investigation was conducted on Duolite S-861 and ES-865, and SYN 46 which were reported as being chemically similar but physically different with regard to their surface area, pore volume and mean pore diameter.

Segui et al. (1986) proposed a mechanism of debittering navel orange juice by ion exchange resins in batch
treatments. They found that high basicity, low degree of crosslinkage, and high porosity constituted the properties that enhanced the debittering process. On the other hand, they observed that for freshly extracted juice, a higher reduction of bitterness was obtained with highly crosslinked resins. This result may simply be attributed to the increase in the charge density that occurs with increasing the degree of crosslinkage of polystyrene divinylbenzene exchangers and subsequently the increased exchange of limonoic acid A-ring lactone, thus preventing its conversion to the bitter limonin. These observations further suggested that limonin was removed by an adsorption process. Moreover, debittering at room temperature was found to be more effective compared to the process performed under refrigeration. Based on this information, these authors concluded that apparently the physical adsorption mode was the predominant mechanism of removal of the bitter limonin from navel orange juice and that the ion exchange resins in the -OH form prevented the development of the bitter taste by removing limonoic acid A-ring lactone which contains free carboxylic acid groups. It was also stated that the adsorbing capacity of the styrenic copolymer was positively related to the degree of hydrophobicity of the adsorbate, i.e., the more hydrophobic the adsorbate, the higher the adsorbing capacity of polystyrene divinylbenzene copolymer.
The review of the literature indicates, on one hand, that the resins with a styrene-divinylbenzene copolymer matrix, particularly the neutral resins, are emerging as the most suitable materials to achieve effective debittering of grapefruit juice without affecting the nutrient content. Therefore, they constitute a good alternative for the citrus industry to improve the quality of the juice with little cost. This can result in improving consumers acceptance of grapefruit juice and in increasing cost savings by elimination of the production cost of refrigerated storage of juice concentrates from early season grapefruit. On the other hand, no extensive study has been undertaken to explain the large differences observed between the efficiencies of these adsorbents in the debittering process of grapefruit juice. Based on the observations summarized here, it can reasonably be assumed that the physical properties of the polystyrene divinylbenzene matrix play an important role and determine the ability of the adsorbents to remove the bitter compounds from grapefruit juice. It thus appears worthwhile to investigate the effects of the characteristics of the polystyrene divinylbenzene adsorbents which could play important roles in the debittering process of citrus juices.
General Properties of Styrene-Divinylbenzene Copolymer Based Adsorbents and Ion Exchangers

Construction of the Resins

The three major groups of materials used in the construction of ion exchangers and adsorbents are polystyrene or polyphenolic resins, cellulose, acrylamide and dextran. Most of the resins used in the separation of molecules are made of one type of polymer and crosslinked with another type. The polystyrene-type resins are made by reacting polystyrene with varying proportions of divinylbenzene to form a beadlike polymer. The degree of cross-linkage of the polystyrene divinylbenzene resins is determined by the concentration of divinylbenzene in the mixture during the polymerization process. The resulting solid, crosslinked macromolecular compounds are insoluble. Generally, the degree of crosslinkage of the polymers is designated by X-1, X-2, X-4, etc... with the numbers representing the percentage (wt, %) of the total polymer that is divinylbenzene (Braun et al., 1972; Cooper, 1977). Figure 4 illustrates a typical chemical structure of crosslinked polystyrene divinylbenzene resin.

The conditions of polymerization (concentrations of crosslinking agent and diluent, as well as the reaction temperature) determine the final porosity of the resulting beads. These factors can also result in various degrees of porosity in polymers with the same degree of crosslinkage.
Figure 4: Chemical structure of crosslinked polystyrene
divinylbenzene adsorbent resin
(Adapted from Skoog, 1985)
According to Seidl et al. (1967) there are basically three methods for constructing porous matrices from polystyrene divinylbenzene copolymers based on the properties of the diluent employed:

(a) Incorporation of a solvating diluent to the polymerizing mixture. Toluene and dichloroethane are usually used in the preparation; the resulting polymer has a relatively low pore volume (about 0.8 mL/g), a large internal specific surface area (50 to 500 m²/g), and a small average pore diameter.

(b) Addition of non-solvating diluent to the mixture to produce a copolymer characterized by a large pore volume (0.6 to 2.0 mL/g), with smaller internal specific surface area of 10 to 100 m²/g, and a relatively large average pore diameter. The diluent used for this purpose can be either n-heptane or n-butyl alcohol.

(c) Addition of a linear polymer like polystyrene to the mixture to produce a polymer with smaller pore volume (less than 0.5 mL/g), and internal specific surface area (less than 10 m²/g), and a large average pore diameter. These three types of polymers have been referred to as porous by solvent, porous by precipitation and porous by macromolecular material, respectively. Addition of a combination of two diluents to the reaction mixture can give various types of resins with characteristics different from the forms cited above (Sederel and de Jong, 1973). Also,
the mechanism of formation of the pore srtucture has been described by these authors: during the polymerization reaction, the agglomeration of polymer chains forms nuclei with diameters estimated between 50 and 200 angstroms. As the polymerization reaction proceeds, the nuclei grow further to form the microspheres with diameters ranging from 600 to 5,000 Angstroms, a size which can then be observed using electron microscopy. A final agglomeration of microspheres constitutes the actual beads. The size of the beads ranges between $10^6$ and $10^7$ Angstroms which is measurable with a micrometer. By convention, the term "gel porosity" is used to designate the porosity that occurs in the swollen state of the macromolecular network, i.e., an ion exchanger swollen in water or a crosslinked matrix swollen in an organic solvent. "Macroporosity" refers to the void volume between the microspheres and their agglomerations whose size may exceed 250 angstrom diameter. "Microporosity" refers to the pore space within the microspheres, among the nuclei and their agglomerations; their diameter average from 10 to 100 Angstroms. These latter pores constitute the internal surface of the resin bead. A typical structure of a polystyrene divinylbenzene adsorbent bead is shown in Figure 5A. The bead has a continuous gel phase and a continuous pore phase as illustrated by Figure 5B (Rohm and Haas, 1982).
Figure 5: Structure of a single bead of crosslinked polystyrene divinylbenzene adsorbent
For an amphoteric sorbate molecule:
- o: hydrophilic section
- -: hydrophobic section
(Adapted from Rohm and Haas, 1982)
Effect of the Characteristics of the Matrix of the Resins

Generally, the nature of the supporting matrix determines the flow properties, ion accessibility in the inner portion, and chemical and mechanical stability of the resins. Rothbart (1973) and Cooper (1977) reported that when the degree of crosslinkage in polystyrene or phenolic resins increased, the permeability of the resins decreased because they would swell less. Consequently, the accessibility of the interior region of the resin by the relatively large molecules is limited and equilibration time increases. Also, increasing the degree of crosslinkage results in increasing the exchange capacity of the ion exchangers since the number of exchangeable sites per unit volume and the density of ionic sites on the resins increase. In effect, highly crosslinked resins swell only slightly resulting in more exchangeable sites per unit volume compared to a resin with less crosslinkage. Furthermore, the size of the beads (referred to as diameter or mesh size) was found to influence the flow rate, equilibration time, and capacity of the exchanger (capacity refers to equivalents of ions bound per gram or volume of exchanger, or milliequivalents per milliliter of column bed volume of exchanger). When the mesh size of the resins increased, the capacity and the equilibration time increased but the flow rate decreased (Cooper, 1977). In addition, the author reported that the charge of weakly basic and
acidic ion exchangers depended on the pH of the environment. It is known that weaker exchangers of lower charge density are recommended for the separation of large molecules with high charge density; this procedure limits irreversible binding between the ionic molecules and the exchangers.

Weber (1981) discussed the parameters that contributed to the adsorption of organic compounds by activated carbon, an adsorbent used in waste-water treatment applications. It was noted that the characteristics of the organic substances that determine the extent of adsorption include concentration, molecular weight, molecular size, molecular structure, molecular polarity, steric form or configuration, and the presence of competitive organic substances. The temperature and pH of the solution also influence adsorption since they can produce changes in the aforementioned properties of organic molecules. The pH of the solution can affect the strength of binding of an ionizable solute; the pH can either make the solute predominantly positive and then allows stronger binding to a cationic exchanger (negatively charged resin) or make it more negative and favors a stronger binding to an anionic exchanger (positively charged resins). These observations indicate the possible effects of some of the juice components on the adsorption in terms of modification of the resin surface properties, the competitive adsorption between limonin and
naringin for the reaction sites on the resins, and the solubility of these bitter compounds in aqueous medium.

**Principle of Adsorption and Ion Exchange**

**Adsorbents and Principle of Adsorption**

The adsorbent resins (or neutral resins) are polymers with well-defined characteristics but having no ionizable functional groups. Generally, the separation of the solutes by these resins is based on the adsorption process. The adsorption phenomenon involves the concentration of solute and quasi-soluble materials (solute) from a solution at an interface or surface. The material that is concentrated at the polymer surface is called the adsorbate (Figure 5B). There exists a quantitative equilibrium distribution between the solution and the adsorbent surface phase. The character of this distribution is affected by various factors related to the characteristics of the adsorbate, the adsorbent, and the solution in which adsorption takes place (Weber, 1981).

Weber (1981) attributed the adsorption of organic compounds from water by activated carbon to various types of binding which involve electromagnetic interactions. The types of adsorption can be physical or chemical and are defined as follows.

(a) Physical adsorption involves the action of van der Waals forces which in turn include both London dispersion forces and classical electrostatic forces. These are weak bonds between the solute and the adsorbent.
(b) Chemical adsorption results from the reaction of an adsorbate with an adsorbent which generally results in the transformation of the adsorbate. The binding is localized at the active centers on the adsorbent and is stronger than the physical attraction through van der Waals forces.

The forces involved in the adsorbent-adsorbate interaction (London dispersion, electrostatic, chemisorptive) determine the affinity of an adsorbent for a given solute. It has also been reported that the extent of adsorption was greatly influenced by the degree of insolubility or hydrophobicity (which refers to the degree of 'dislike') for water, of an organic compound. Moreover, solubility can be interpreted as a bond between an organic compound and water that must be broken before adsorption takes place. That is why increasing hydrophobicity generally favors the adsorption of hydrophobic solutes by neutral adsorbents (Rohm and Haas, 1983).

**Ion Exchangers and Principle of Operation**

Ion exchangers can be defined as porous materials carrying ionogenic groups called functional groups (Rothbart, 1973). Positively- and negatively-charged functional groups are covalently bound to the matrix in the case of anion and cation exchangers, respectively. These charged groups determine the type and strength of binding of the solutes to be separated out of mixtures by the exchangers. The counterion is the ion that is
electrostatically bound to the exchanger; the net charge must be opposite to that of the exchanger for the binding to occur (Cooper, 1977).

The ion exchange process is defined as the reversible exchange of a given ion in solution with a counterion bound to an insoluble supporting matrix. The like charged and uncharged molecules in the solution cannot bind to the exchanger and are easily washed away (Rothbart, 1973; Cooper, 1977). Cooper (1977) discussed the ion exchange mechanism for the case of a cation exchanger having $X^+$ as counterion (or Exch-$X^+$); he described that, as a general principle, when a mixture of cation species such as $YH^+$ and $Z^+$ are eluted through the exchanger they progressively replace the counterion $X^+$ by forming electrostatic binding with the cation exchanger Exch- which results in displacing an equivalent amount of counterions. These cations can be in turn removed from the exchanger in two ways, i.e., by introducing increased concentrations of $X^+$ in the medium or increasing the pH of the eluent. Since the strength of binding depends on the quantity of charge possessed by the ionic species $YH^+$ and $Z^+$, a higher concentration of $X^+$ will be needed to elute them (if they are highly charged). The latter procedure causes the conversion of $YH^+$ and $Z^+$ to $Y^0$ and $ZOH$ (uncharged species) and consequently weakens their attractive forces with the exchanger. The resulting reactions can be written as follows (Cooper, 1977).
\[ \text{YH}^+ + \text{OH}^- \rightarrow \text{YO} + \text{H}_2\text{O} \]
\[ \text{Z}^+ + \text{OH}^- \rightarrow \text{ZOH}. \]

Also, the pH of the system affects the strength of binding by changing the \( pK_a \) of the ionic molecule and therefore its ionic state. The higher the \( pK_a \) of the ionic molecule, the higher the pH required to elute it (Cooper, 1977; Rieman and Walton, 1970).

The removal of acids from citrus juices, which takes advantage of a weakly basic ion exchange resin, constitutes an illustration of the ion exchange process. Removal of the citrate ion occurs by displacing the hydroxyl ion on the exchanger with subsequent formation of water. The reaction is outlined in the following equation (Varsel, 1980):
\[ 3\text{R}^+.\text{OH}^- + (\text{C}_6\text{H}_5\text{O}_7)^{3-}.3\text{H}^+ \rightarrow (3\text{R}^+).\text{C}_6\text{H}_5\text{O}_7^- + 3\text{H}_2\text{O} \]

where \( \text{R}^+ \) represents an anion exchange resin unit and \( \text{C}_6\text{H}_5\text{O}_7^- \) the citrate ion. The weakly basic anion exchanger exhibits a stronger retention of the strong acids, but ascorbic and folic acids are less strongly retained. The affinity of this type of resin for citric acid is also influenced by the mass action of other components of the juice such as limonoic acid A-ring lactone.

When using the polystyrene crosslinked divinylbenzene resins, the selection of the type of exchanger (functional group and charge density) is based on the strength of binding desired. Strongly acidic or basic exchangers of high charge density are generally employed for ionic species.
that are resistant to drastic changes of pH and ionic strength. Conversely, ionic species that are sensitive to these variations require weaker exchangers of low charge density since they can only withstand elution under gentle conditions of pH and ionic strength. Weaker exchangers of lower charge density are also used to prevent irreversible binding that often occurs during separation of large molecules with high charge density. Generally, polystyrene and polyphenolic ion exchange resins possess a higher charge density because substitution is nearly complete during manufacturing. The resins also exhibit a lower permeability towards large molecules.

**Transports Involved in the Adsorption and Ion Exchange Processes**

In the case of the adsorption of organic compounds by activated carbon, the rate of adsorption has been found to be dependent on three consecutive mass transports: bulk transport, film transport, and intraparticle transport of the adsorbates (Weber, 1981). The bulk transport occurs in the void volume between the bulk of the solution and the surface film. This form of transport is favored by continuous mixing and rapid flow of the solution. However, the rate-limiting step in the adsorption process is the transport of organic compounds through the surface film to the exterior surface of the active carbon. This film transport barrier is caused by a relatively stagnant film around the adsorbent; extensive mixing can significantly
reduce that barrier whereas a stagnant surface film drastically impedes the passage of the compounds through the film. The intraparticular transport is formed by the diffusion of the compounds within the pores of the carbon and/or along the pore wall surfaces, and it is influenced by the geometry of the pore in relation to that of the moving compounds (adsorbates). Thus, this transport constitutes another rate-limiting step in the adsorption by activated carbon. Similar transport occurs with other adsorbents and ion exchangers having supporting matrices such as polystyrene divinylbenzene, cellulose, etc.... In addition to the above transports, the kinetic process of ion exchange includes another process which is the exchange of ions at the fixed groups within the resin; the film and particle diffusion were again reported as the slowest processes in ion exchange (Rothbart, 1973).

Method of Determination of the Surface Area of Resins

The following discussion is adapted from Lowel (1979). The author stated that the kinetic theory for the measurement of the surface area of porous material is based on the prediction of the number of adsorbate molecules required to uniformly cover the solid with a single molecular layer. The cross-sectional area of the adsorbate molecule (or the effective surface covered by each molecule on the surface of the porous material) constitutes an important factor. Thus, by definition, "the surface area of
an adsorbent is the mathematical product of the number of molecules in a completed monolayer and the effective cross-sectional area of the adsorbate (P. 16)." So, based on the theory of adsorption of gas on an adsorbent surface and assuming a monolayer coverage of the surface, Langmuir (1918) established that the adsorption of a gas follows a type I isotherm. That is, the number of molecules \( N_M \) colliding with each square centimeter of surface per second is given by the following equation adapted from Lowel (1979)

\[
N_M = \frac{N \cdot P}{(2 \pi R T)} \tag{1}
\]

where \( N \) is Avogadro's number, \( P \) is adsorbate pressure, \( R \) is the universal gas constant and \( T \) is absolute temperature.

After balancing the adsorption process of the gas with the rates of adsorption and desorption, the equation for the type I isotherm was established as follows.

\[
\frac{W}{W_M} = \frac{K \cdot P}{1 + K \cdot P} \tag{2}
\]

where the ratio of \( W \) to \( W_M \) is the weight of adsorbate molecule relative to the weight of molecule adsorbed in a completed monolayer, and \( K \) is the Langmuir constant which is equal to the ratio of \( N \) to \((2 \pi R T)\). Rearrangement of the above equation gives

\[
\frac{P}{W} = \frac{P}{W_M} + \frac{1}{K \cdot W_M} \tag{3}
\]

and the plot of \( P/W \) versus \( P \) will give a straight line of slope \( 1/W_M \) and intercept \( 1/K \cdot W_M \) from which \( K \) and \( W_M \) can be
obtained. Knowing $W_M$, the adsorbent surface area $S_t$ can be calculated from the equation

$$ S_t = N_M A = \frac{W_M N A}{M} $$

where $A$ is the cross-sectional area, $M$ is the molecular weight of the adsorbate molecule, and $N$ is Avogadro's number. Although the Langmuir equation provides information for the calculation of the adsorbent surface area, it lacks precision and presents the following disadvantages (Lowel, 1979):

(a) It does not adequately treat physical adsorption and the other types of adsorption isotherms, namely, types II, III, IV and V.

(b) The surface area measurement made from type I isotherms are subject to uncertainties regardless of whether chemisorption or physical adsorption takes place.

Using the approach of the Langmuir theory of type I isotherm adsorption of gas onto an adsorbent, Brunauer, Emmett and Teller (or BET; 1938) expanded the adsorption to the formation of multiple layers of adsorbate on the adsorbent surface from which the monolayer can be determined since exactly a single monomolecular layer is never actually formed. "The BET method allows for a quick experimental determination of the number of molecules required to form a monolayer. This latter theory assumes that the uppermost molecules in the adsorbed layers are in dynamic equilibrium with the vapor, and so the number of molecules in each layer
remains constant (P. 20)." Thus, after extrapolation and rearrangement, the final form of the BET equation becomes:

\[ \frac{1}{W[(P_o/P)-1]} = \frac{1}{W_M} C + \frac{C-1}{W_M} C \times \frac{P}{P_o} \]  \hspace{1cm} (5)

where \( C \) is the BET constant, \( W_M \) is the weight of a monolayer of adsorbate molecules, \( W \) is the weight of adsorbed molecules of adsorbates, \( P_o \) is the initial pressure of the adsorbate gas and \( P \) the pressure at equilibrium. The determination of the surface area of the adsorbent from the BET theory is a direct application of equation (5); thus, a plot of \( 1/W[(P_o/P)-1] \) versus \( P/P_o \) gives a straight line generally in the range \( 0.05 \leq P/P_o \leq 0.35 \) (Figure 6). The slope \( s \) of the line is equal to \( (C-1)/W_M \) and the intercept \( i \) is equivalent to \( 1/(W_M C) \). The solutions of this double equation give \( W_M = 1/(s+1) \) and \( C = s/i + 1 \). Having established \( W_M \), the total surface of the adsorbent material can be calculated from the earlier described Langmuir equation (4) afromentioned. The specific surface area (S) is obtained by dividing the surface area \( S_i \) by the sample (adsorbent) weight (Lowel, 1979). \( S \) is expressed in \( m^2 \) when \( W_M \) is in grams, \( M \) the adsorbate molecular weight, \( N \) the Avogadro's number \( (6.02 \times 10^{23} \text{ molecules per mole}) \), \( A \) is in square Angstrom per molecule and the ratio is multiplied by \( 10^{-20} \). The adsorbate cross-sectional area is obtained from the following equation which takes into account the molecular packing on the surface:

\[ A = 1.09 (V/N)^{2/3} 10^{16} \text{ Å}^2 \]  \hspace{1cm} (6)
Figure 6: Typical BET plot
(Adapted from Lowel, 1979)
where \( V \) is the liquid molar volume and 1.09 represents the packing factor.

Lowel (1979) stated that "the BET theory is popular and widely used to determine the surface of porous materials because of its ease of application, its definitiveness, and its ability to accommodate all five types of isotherms. Most importantly, in the region of relative pressure near the completed monolayer, \((0.05 \leq P/P_0 \leq 0.35)\) the BET theory and experimental isotherms agree very well. Therefore, the BET method is both powerful and extremely useful for surface area determinations of porous materials (P. 28-29)."

Nitrogen has been accepted as the standard and universal adsorbate for surface area determination because of its inherent characteristics that make it an ideal gas. That is, nitrogen gas exhibits no localized adsorption since it has a small BET \( C \) value, but it is large enough to prevent the adsorbed layer from behaving as a two-dimensional gas.

**Determination of Porosity and Pore Size by the Mercury Porosimetry Method**

The mercury porosimetry method basically consists of measuring the intrusion volume of mercury required to fill the pore of a test sample in a high-pressure mercury porosimeter under vacuum (Lowel, 1979). A typical apparatus for measuring the intrusion volume of mercury is shown in Figure 7. A summary of the operation of the apparatus described by Lowel (1979) is adapted as follows. A high vacuum applied to the system first removes entrapped air
Figure 7: Capacitive probe; high pressure cavity and seals for continuous intrusion measurements (Adapted from Lowel, 1979)
from the pores of the sample. Then, mercury is applied to the sample and the observed change in the mercury level in the stem of the dilatometer can be monitored with a capacitance probe that translates the resistance of a wire in the stem into voltage that increases linearly with decreasing level of mercury in the dilatometer stem. The mercury level in the stem and the sheath surrounding the dilatometer constitute the two plates of the capacitor whose capacitance changes when the mercury level varies due to the change in the effective plates area.

Another mercury porosimeter that is able to provide simultaneously the volume of intrusion and the volume of extrusion of mercury, hence data about the pore volume and the pore size, respectively, of porous materials is also adapted from Lowel (1979) and is illustrated in Figure 8. This latter apparatus consists of two sides:

(a) A pressure generator side which can generate up to 60,000 psi, where the extrusion volume is measured. A motor drives a rod into a narrow cavity filled with hydraulic oil whereby the penetration of the rod into the cavity causes compression of the oil in the entire porosimeter. A strain gauge transducer located on top of the pressure generator produces an electrical signal that is proportional to the pressure which is in turn translated into pore size.

(b) A pressure vessel side containing the sample cell and where the intrusion volume is monitored. The principle
Figure 8: Continuous recording mercury porosimeter
(Adapted from Lowel, 1979)
of measurement of the pore volume is similar to that of the apparatus described in Figure 7.
The two sides are connected through a high pressure tubing. The signals from the pressure tranducer and from the capacitance probe can be plotted on a X-Y recorder as pore size and intrusion volume, respectively, where X represents the pressure axis (the pressure applied in increments) and Y is the volume axis.

The pore size is determined according to the following principle. The signal coming from the pressure tranducer can either be recorded linearly or logarithmically. For the linear mode, the X axis (or pressure axis) is inversely proportional to the pore radius as shown in this equation

\[ Pr = 2 \gamma \cos \theta \]  \hspace{1cm} (7)

where \( \gamma \) is the surface tension of mercury and \( \theta \) represents the contact angle of mercury on the test sample surface. By taking the logarithm of both sides, a linear equation is obtained to calculate the pore radius.

\[ \log P = \log A - \log r \]  \hspace{1cm} (8)

where \( A \) represents \( (2 \gamma \cos \theta) \), \( r \) is the pore radius and \( P \) is the applied pressure. Since the electrical signal \( E \) is proportional to \( \log P \), then

\[ \log P = KE \]  \hspace{1cm} (9)

Using equations (8) and (9), suggest that differential rate equation applies

\[ K \, dE = - \frac{dr}{r} \]  \hspace{1cm} (10)
which describes a first order chemical reaction; integration of the equation (10) gives

\[ r = K e^{-KE} \]

(11)

and indicates that the pore radius decreases exponentially with decreasing signals from the transducer. Using semi-log paper in the X-Y recorder, the pore radius can be plotted directly from the larger pores to the small ones to produce a profile of the pores in the sample (called pore distribution).

This latter apparatus has several advantages as it provides a continuous plot of both intrusion and extrusion curves, simultaneously, in a short period of time (about 15 min) and no relaxation of pressure occurs during the experiment. This also indicates that no extrusion takes place after intrusion. A blank run with mercury alone in the sample cell is not necessary since the effect of mercury compression and the resulting compression heating of the oil are electronically compensated, and mercury alone shows less than 1% of a full scale signal over the operation range from 0 to 60,000 psi.
EXPERIMENTAL

Sources and Preparation of Materials

Adsorbent Resins

Commercial polystyrene divinylbenzene (PSDVB) copolymer adsorbent resins in the dry or wet forms (6, 8, 16, 20 and 50% crosslinked PSDVB adsorbents) and Kastel 111 and 112 resins (65% crosslinked PSDVB resins) in the wet form were obtained from Dow Chemical Company (Dow Chemical U.S.A., Midland, MI). They were all food grade neutral adsorbent resins and approved for food contact use. The 8 (or 8a), 16, 20 and 50% crosslinked PSDVB adsorbents were white-opaque while the 6% and a separate 8% (or 8b%) crosslinked PSDVB adsorbents were translucent. The 65a% crosslinked PSDVB adsorbent resin (Kastel 111) was white while a separate 65b% crosslinked PSDVB adsorbent (Kastel 112) was amber in color. Other physical properties of the adsorbents are listed in Table 5.

General Procedure for Preconditioning the Polystyrene Divinylbenzene Adsorbent Resins

Three mg of neutral PSDVB adsorbent resins were preconditioned by soaking for 1 hr in 50 mL of 95% ethanol in 125 mL Erlenmeyer flasks, followed by 5 rinses with distilled deionized water (DDW, 100 mL water per rinsing) over 1 hr period. The resins were occasionally agitated
Table 5

Physical Properties of the Polystyrene Divinylbenzene (PSDVB) Adsorbents Used

<table>
<thead>
<tr>
<th>Adsorbents (% Crosslinkage)</th>
<th>Solid Density (g/cc, dry)</th>
<th>Specific Surface Area (m²/g, dry)</th>
<th>Bulk Density (g/cc, dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1.08</td>
<td>123</td>
<td>0.639</td>
</tr>
<tr>
<td>8ₐ</td>
<td>1.108</td>
<td>130</td>
<td>0.588</td>
</tr>
<tr>
<td>8ₐ</td>
<td>1.073</td>
<td>110</td>
<td>0.633</td>
</tr>
<tr>
<td>16</td>
<td>1.266</td>
<td>114</td>
<td>0.511</td>
</tr>
<tr>
<td>20</td>
<td>1.315</td>
<td>138</td>
<td>0.400</td>
</tr>
<tr>
<td>50</td>
<td>1.522</td>
<td>450</td>
<td>0.417</td>
</tr>
<tr>
<td>65ₐ</td>
<td>1.330</td>
<td>550</td>
<td>0.597</td>
</tr>
<tr>
<td>65ₐ</td>
<td>1.227</td>
<td>650</td>
<td>0.601</td>
</tr>
</tbody>
</table>

Solid density was determined by the Air comparison pychnometer and bulk density by the graduated cylinder measurement.

Specific surface area was measured by the BET method (Dow Chemical Co., Midland, MI).
Table 5 -- continued

<table>
<thead>
<tr>
<th>Adsorbents (% Crosslinkage)</th>
<th>Pore Volume (cc/g, dry)</th>
<th>Mean Pore Diameter (Angstrom)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.950</td>
<td>310</td>
</tr>
<tr>
<td>8_2</td>
<td>1.400</td>
<td>240</td>
</tr>
<tr>
<td>8_b</td>
<td>0.660</td>
<td>430</td>
</tr>
<tr>
<td>16</td>
<td>0.430</td>
<td>152</td>
</tr>
<tr>
<td>20</td>
<td>0.860</td>
<td>250</td>
</tr>
<tr>
<td>50</td>
<td>0.350</td>
<td>100</td>
</tr>
<tr>
<td>65_2</td>
<td>0.340</td>
<td>25</td>
</tr>
<tr>
<td>65_b</td>
<td>0.810</td>
<td>50</td>
</tr>
</tbody>
</table>

All data were obtained from Dow Chemical Co. (Dow Chemical Co, Midland, MI).
during soaking and rinsing; the water was decanted with much of the fines. They were then filtered through a 30 mesh screen (0.013 inch wire diameter, 0.0203 inch opening) with distilled deionized water. The resin surface water was removed by quickly transferring them onto Whatman filter paper no 541. Care was taken to avoid air drying of the adsorbents; the amount of resins needed was immediately weighed into 125 mL Erlenmeyer flasks prior to treatment.

Preparation of the Model Systems

Two model systems containing 100 ppm (5 mg/50 mL solution) and 600 ppm (30 mg/50 mL solution) naringin (Sigma Chem. Co, St Louis, MO) were prepared by dissolving the bitter compound in methanol (6.7% v/v). They were then mixed with an aqueous solution containing 4.5, 3.7, and 2.3% (w/v, final concentration), of sucrose, glucose and fructose, respectively. The pH of the solution was adjusted to 3.2 by adding solid citric acid. The final soluble solids content was 10.5°Brix.

Preparation of Single Strength Grapefruit Juice

Commercial grapefruit juice concentrate (65°Brix) was obtained from Ocean Spray (Vero Beach, FL) and kept frozen until needed. Single strength grapefruit juice was prepared by diluting the concentrate to 10°Brix with DDW. Brix measurement was taken using a refractometer maintained at room temperature and corrected for acid content and temperature of the sample. The juice was then centrifuged
at 2200 rpm (800 x g) for 10 min in a RC-5 Superspeed Refrigerated Centrifuge (E.I. du Pont de Nemours & Co. Doraville, GA). The pulp was separated and discarded and the supernatant or juice serum containing approximately 640 ppm naringin and 17 to 19 ppm limonin was frozen for later use. Juice containing approximately 400 ppm naringin and 11.5 ppm limonin was also used for some experiments.

**Methodology**

**General Procedure of the Batch Operation and Determination of the Properties of the PSDVB Adsorbents Involved in the Debittering Process of Model Systems and Grapefruit Juice**

**Procedure of the batch operation**

The experiments in this study were all conducted in a batch system. The juice serum was thawed and brought to the desired temperature prior to the actual experiment. The amount of conditioned resin needed for the experiment was transferred to a 125 mL Erlenmeyer flask and mixed with the desired volume of bitter solution. The mixtures were shaken at 150 rpm for a predetermined treatment time in a Giratory(R) water bath shaker Model G76 (New Brunswick Scientific Co., Inc., Edison, N.J.). Monitoring the length of the treatment started immediately after the adsorbent and the solution were mixed. The treated solutions were separated from the resins through Whatman filter paper no 541 for subsequent analyses. Control samples were similarly treated except that no adsorbent was added. The samples for
limonin assay were immediately frozen and stored until analysis.

**Determination of the properties of the adsorbents important in the debittering process of model systems and grapefruit juice**

The determination of the properties of the PSDVB adsorbents involved in the debittering process was conducted by contacting 2 g of the preconditioned adsorbents with 50 mL of either the model systems or grapefruit juice serum. Treatment of each solution was done with all the adsorbents on the same day. The mixtures in 125 mL Erlenmeyer flasks were agitated for 20 min followed by separation through Whatman filter paper no 541.

The treated solutions were divided into two samples for naringin and limonin assays. The amount of the bitter compounds, $B$, adsorbed from the solutions was expressed as percent reduction which was calculated as follows.

$$\% \ Reduction = \left( \frac{B_i - B_t}{B_i} \right) \times 100$$

where $B_i$ and $B_t$ refer to the initial and final concentrations of a bitter compounds in the control and treated samples, respectively.

**Sonication of the 6% and 8% crosslinked PSDVB adsorbents**

The adsorbents with 6% and 8% ($8_a$ and $8_b\%$) crosslinkage which showed a strong hydrophobicity during the normal batch treatment were sonicated in a Branson sonicator model B-220 (Shelton, Con) to examine the effects of the mode of treatment on the performance of these lower crosslinked
PSDVB adsorbents. The preconditioned adsorbents were combined with 50 mL of the 100 ppm naringin containing model system in 125 mL Erlenmeyer flasks and the mixtures were sonicated intermittently during a 20 min treatment period to avoid heating them. The percent reduction was calculated as above. The data were compared to those obtained from the treatment by agitation.

**Determination of the density of the adsorbents**

The polymers used in the experiments are hydrophobic. Therefore, they would exhibit poor contact with the bitter aqueous solutions employed for contact. The densities of the polymers were measured to evaluate their effects on the behavior of the adsorbents in the aqueous solutions.

The solid density of the PSDVB adsorbents was determined with the Air comparison pychnometer (Figure 9). This instrument is used to measure the true volume of porous materials (Mohsenin, 1980) based upon the equilibration pressure between two chambers, A and B, after a sample is introduced into the measuring chamber B.

In the first step, the pistons in the chambers were brought to an initial position to read 1059 with the connecting (coupling) valve closed. The sample cup was filled with dry polystyrene divinylbenzene adsorbent beads up to the rim and gently vibrated with a Vortex-Genie (Scientific Industries, Inc. Bohemia, N.Y.) at low speed for 10 sec to ensure good packing prior to the weighing step.
Figure 9: Diagram of the Air comparison pycnometer
A: reference chamber
B: measuring chamber
(Adapted from Mohsenin, 1980)
The next step consisted of introducing the cup filled with the sample in the measuring chamber thereby creating a pressure differential between the reference chamber A and the measuring chamber B monitored with a pressure differential indicator. The difference in pressure that caused a deflection of the indicator was brought to zero by withdrawing the piston in the measuring chamber B while the reference piston in chamber A was being advanced.

The distance, \( d_x \), that the measuring piston B travelled from its initial position was previously calibrated to provide a direct reading of the true sample volume (in cc). Thus, the solid density of a given polymer was calculated from the following formula:

\[
\frac{\text{weight of beads and cup (g)} - \text{weight of cup (g)}}{\text{true volume of solid material (cc)}}
\]

The bulk density of the dry beads was obtained by determining the volume of a known weight of beads in a graduated glass cylinder. The sample was transferred in a graduated cylinder and gently vibrated as described above. The bulk density was calculated from the ratio:

\[
\frac{\text{weight of dry beads (g)}}{\text{volume of dry beads (cc)}}
\]

Regeneration of the adsorbent resin

After being separated from the model system at the end of the treatment period, the 20% crosslinked PSDVB adsorbent was quickly rinsed with distilled deionized water at room
temperature to remove any solution remaining on the resin bead surface. Fifty mL of the regenerant water at room temperature, 35°, 45° and 55°C and 95% ethanol were subsequently employed to study the efficiency of different types of regenerants. The resin-regenerant mixtures were shaken for 20 min as in the batch process, followed by the separation of the resin and the regenerant through Whatman filter paper no 541. Naringin content of the regenerant was then determined by the HPLC procedure.

In preliminary experiments, the 16, 20, 65a and 65b% crosslinked PSDVB adsorbents employed in the treatment of grapefruit juice were regenerated with 95% ethanol and water at room temperature and at 35°C. Also, 95% ethanol was used to restore all the adsorbents following treatment of the model systems. The regeneration procedure was identical to that described above.

Determination of the Rate of Adsorption of the Bitter Compounds and other Constituents of Grapefruit Juice

The rates of adsorption of naringin, limonin, recoverable oil and total acids were measured in a batch system as described previously. Preliminary experiments indicated that a rapid reduction in the levels of the above constituents occurred at the onset of the debittering process. Thus, the rate of adsorption was carried out at relatively short time intervals up to 60 min, and also at longer time intervals up to 300 min.
Individual samples of 1 g (dry) of the 50% crosslinked PSDVB adsorbent were preconditioned as described above and mixed with 100 mL of grapefruit juice serum. Series of mixtures were agitated for 5, 10, 15, 20, 40, 60, 100, 140, 180, 220 and 300 min at room temperature. After each treatment, the juice serum was separated from the adsorbent through Whatman filter paper no 541 and analyzed for naringin, limonin, recoverable oil, and total acid. The control juice was also passed through the filter paper.

Study of the Effect of the Juice Temperature on the Adsorption of the Bitter Compounds and Ascorbic Acid

In order to obtain further information about the mechanism of adsorption of naringin and limonin from grapefruit juice, and to assess the effects of some of the potentially useful resins on ascorbic acid content (a valuable nutrient of the juice), the effect of the juice temperature on the debittering process was examined. The adsorption was carried out both at room temperature (24°C) and in a water bath at 35°C.

The frozen juice serum was thawed and brought to room temperature. Fifty mL aliquots of the juice were transferred into Erlenmeyer flasks. In the experiment performed in a water bath, the juice temperature was equilibrated at 35°C prior to the addition of the adsorbent. Two g of the preconditioned adsorbent resin, either 50% or 65b% (Kastell 112) crosslinked PSDVB adsorbent, was mixed with the juice serum, and agitated for 20, 40, 100 and 140
min as described earlier, before separation on Whatman filter paper no 541. The juice samples were rapidly transferred to a refrigerator for cooling. Samples for limonin assay were frozen until needed. The effect of the 35°C water bath on juice controls (without adsorbent contact) was observed by running several controls for the various treatment periods employed in the experiment.

**Sensory Evaluation of Single Strength Grapefruit Juice Screening of the panelists**

**Preparation of the bitter solution for the screening test.** A stock solution containing 200 ppm naringin was prepared by dissolving naringin in distilled deionized water. The suspension was heated in boiling water bath for 30 min with frequent shaking, and allowed to cool after complete dissolution of the naringin. A 100 ppm naringin aqueous solution was prepared by diluting an adequate amount of the stock with distilled water (1:1 dilution). This diluted sample was paired with distilled water (control) for the screening experiment. The samples were chilled prior to the screening test.

**Screening test.** In this preliminary step, a pool of 35 informal panelists were screened to assess their ability to detect bitterness. A pair of coded samples of bitter and non-bitter (distilled water) solutions were randomly presented to the panelists under red light in separate booths in the sensory evaluation facility of the department.
Each booth was equipped with an extra empty cup and running water for rinsing between tastings. The panelists were asked to determine whether there was a difference between the pair of samples and to indicate the degree of bitterness, if any, as presented in the evaluation form in Table A11. The panelists who could differentiate between the bitter and the non-bitter samples were selected to participate in the sensory evaluation of the pairs of adsorbent-treated and untreated grapefruit juice samples.

**Evaluation of bitterness and preference of the juice**

**Preparation of grapefruit juice.** Grapefruit juice was treated with 65.6% crosslinked PSDVB adsorbent, Kastel 112, at a ratio of 2 g preconditionned adsorbent per 50 mL of juice. Juice-adsorbent mixtures were agitated in a batch-like process for 20 and 40 min after which the adsorbent was separated from the juice. The untreated and adsorbent-treated juices were chilled prior to the sensory evaluation.

**Sensory evaluation of grapefruit juice.** The bitterness of untreated and polystyrene divinylbenzene adsorbent-treated grapefruit juice samples was evaluated using analytical-descriptive and affective tests (IFT, 1981 and Larmond, 1970). Paired-comparison tests were employed to determine differences in bitterness between the two samples; when a difference between the samples was detected by panelists, a preference test was used to determine the least preferred juice sample. The juice samples were also rated
for bitterness according to the procedure used by Barmore et al. (1986), but on a 5-point scale where 1 was trace and 5 represented extreme bitterness (Table A12). A pool of 20 panelists who were screened in the preliminary test for their ability to detect bitterness, were used in the evaluation of the juice samples.

The pair of coded samples of juice (treated and non treated) were randomly presented to the panelists in separate booths in the taste panel facility of the department, under red light in order to mask any effect of the juice color on the panelists evaluation. The evaluation was conducted in 2 replications in mid-morning for two days with the same panelists. The data were analyzed using a two tailed Students' t-test.

Methods of Analysis

Analysis of naringin

Sample preparation for naringin assay. The resin-treated juice and the control juice serum samples were cleaned with Sep-pak C\textsubscript{18} cartridges (Waters Associates, Milford, Mass) prior to analysis. The cartridges were preconditioned with 2 mL HPLC grade methanol and rinsed with 5 mL distilled deionized water before introducing 2.5 mL of the juice serum. The water soluble components were removed with 2.5 mL distilled deionized water followed by 2.5 mL HPLC grade methanol for a quantitative extraction of naringin. The methanol extracts were diluted with the
mobile phase before analysis by HPLC. All the solutions were injected through the catridges using Luer tip syringes.

**Analysis of naringin by the HPLC procedure.** The HPLC procedure for naringin analysis (Fisher and Wheaton, 1976) was modified to shorten the retention time to less than 10 min. This was accomplished by increasing the acetonitrile (Fisher Scientific, Fairlawn, N.J.) concentration to 21% and decreasing the water content to 79% in the solvent mixture used as mobile phase.

The column system employed for the separation of naringin consisted of a 25 cm x 4.6 mm precolumn packed with Synchrosorb\textsuperscript{(R)} RP-P (SynChrom, Inc., Linden, IN), a 5 cm x 4.6 mm guard column packed with Zorbax octadecyl silane RP 433 5μ (Dupont Company, Wilmington, DE) and a 25 cm x 4.6 mm Lichrosorb 5 C18 column (Phenomenex, Rancho Palos Verdes, CA) in series. An aqueous mobile phase containing 21% acetonitrile in HPLC grade water was delivered to the system using a Waters chromatography pump (Model M 6000, Waters Associates, Inc. Milford, Mass) at a flow rate of 1.8 mL/min.

The solvent delivery system was later in the study replaced with an Eldex pump (Model AA-100-S) equipped with a digital pump monitor (Eldex Laboratories, Inc. San Carlos, CA). Furthermore, the analytical column was replaced by a 25 cm X 4.0 mm Hibar LiChrospher 100 CH 18/2 5 μ C18 column (E. Merk Darmstadt, F.R. Germany) and the flow rate was
reduced to 1.35 mL/min; this later change was made to reduce the head pressure in the HPLC system under 2500 psi while still maintaining a retention time below 10 min for naringin with a good resolution.

A Rheodyne injector (Model 7125, Rheodyne, Coati, CA) equipped with a 5 µL loop was used for sample injection. Naringin was detected with a Spectra Physics Sp 8440 UV/VIS detector (Spectra Physics, Santa Clara, CA) set at 280 nm wavelength and at a sensitivity of 0.32 absorbance units (A.U.) and a time constant of 0.5 sec. The peaks were recorded on a Soltec recorder (Model 261) (Soltec, Encino, CA) at a chart speed of 8 in/hr in line with an HP integrator, Model 3390A (Hewlett Packard, Avondale, PA). A typical chromatogram is presented in Figure 10.

Naringin concentrations in the samples were determined by reference to an analytical curve (standard curve). The analytical curve was prepared for the concentrations of naringin between 2 ppm and 100 ppm. A linear regression procedure was applied to derive a mathematical equation relating naringin concentration in the 6 serial dilutions to the peak area. A typical regression curve is shown in Figure 11. Values of $r^2$ between 0.995 and 0.999 were obtained. Naringin concentrations of the juice samples and the model systems were calculated using the equation obtained from the linear regression analysis.
Figure 10: Separation of naringenin rutinoside and naringin in grapefruit juice by the method of Fisher and Wheaton (1976). Refer to text for more details.
Figure 11: Analytical curve for naringin from the HPLC procedure
Analysis of limonin by the Bitterdetek™ procedure

Limonin concentrations in the PSDVB adsorbent-treated and control juice serum samples were determined using the Bitterdetek™ limonin immunoassay (Bitterdetek, Inc., San Bruno, CA). The assay is a competitive enzyme-linked immunoassay which is based on the immunological principle of antigen-antibody complex formation (Idetek, 1987; Poy, 1987) as illustrated in Figure 12. Aliquots of a given juice sample and the alkaline phosphatase conjugate, also called limonin oxime or tracer (which is limonin tagged with the enzyme), mixture are transferred to the reaction wells and incubated at 35°C for 30 min. Then a competitive reaction takes place between the limonin in the juice sample and the tracer for the antibody made to limonin which is precoated onto the reaction well walls. In the second step, the unbound tracer and limonin are rinsed out and aliquots of p-nitrophenyl phosphate (pnpp), the substrate, are added to the wells followed by a second incubation as indicated above. The enzyme of the tracer bound to the antibody then reacts with the substrate to form p-nitrophenol, a yellow colored product. The optical density of the colored solution is measured at 405 nm with a Titertek Miniskan spectrophotometer (Idetek, San Bruno, CA). The intensity of the color is inversely related to the limonin concentration in the juice sample. A representative analytical curve, which is shown in Figure 13, was obtained from the 4
Limonin antibody coated Reaction Well

Add mixture

Incubate for 30 minutes at 37°C

Wash

Add substrate(s), p-nitrophenyl phosphate

Read absorbance at 405 nm

Incubate for 30 minutes at 37°C to yield yellow color of product (p), p-nitrophenol

Figure 12: Enzyme immunoassay protocol for Bitterdetek™ limonin test (Adapted from Idetek, Inc., 1987)
Optical Density at 405 nm

D.D. = -0.427 \log(\text{limonin}) + 2.239

r^2 = 0.983

Figure 13: Analytical curve for limonin from the immunoassay test.
standard orange juice samples included in the limonin test kit received from Bitterdetek Inc., which contained 2, 4, 8 and 16 ppm limonin. A $r^2$ value of 0.983 was found for the linear regression between the exponential transformation of the absorbance of substrate-limonin oxime reaction product and limonin concentration of the standards supplied. Limonin concentrations in the PSDVB adsorbent treated and control grapefruit juice sera were calculated using the following mathematical equation derived from the linear regression procedure.

$\text{Limonin concentration (ppm)} = b \times \exp(a \times \text{absorbance})$

where the constants $a$ and $b$ were obtained from the regression analysis between limonin concentrations in the standards and the corresponding absorbance values.

Determination of the recoverable oil content of the juice

The recoverable oil of grapefruit juice serum (d-limonene) was analysed by the bromate titration method, also known as the Scott method (Praschan, 1977). Twenty five mL of juice was mixed with 25 mL of 2-propanol and 50 mL of distilled deionized water in a 500 mL round bottom distillation flask. After boiling chips and 2 drops of antifoam were added, the mixture was distillated at full heat, but the rheostat was monitored to a point to obtain an adequate rate of distillation without over boiling the mixture. Antifreeze liquid at 0°C was circulated in the condensor by an Ultra Kryomat TK 30 (Messgerat-werlaura, W. Germany)
pump-freezer system. Thirty (30) mL of condensate was collected in a 100 mL graduated cylinder and mixed with 10 mL dilute HCL (1 + 2) in water; The mixture was titrated with 0.0247N potassium bromide-bromate titrant containing 0.1% methyl orange indicator until disappearance of the red color. The oil content of the juice sample was calculated using the following formula:

\[
\% \text{ recoverable oil (by volume)} = 0.004 \times \text{mL titrant}
\]

where the titrant volume was corrected with a blank.

**Determination of the titratable acidity of the juice**

The titratable acidity of grapefruit juice samples was determined by the alkali titration method in the presence of phenolphthalein indicator (FMC, 1977): 25 mL of single strength grapefruit juice was mixed with 75 mL of distilled deionized water and 5 drops of phenolphthalein. The mixture was titrated with 0.3125N NaOH solution to the first pink endpoint. The acid content of the juice was expressed as percent by weight of total acidity and determined as anhydrous citric acid with the following formula:

\[
\% \text{ citric acid (w/w)} = \frac{\text{mL NaOH} \times 6.4}{\text{grams juice}}
\]

**Determination of the ascorbic acid content of the juice**

The ascorbic acid content of grapefruit juice sample was analyzed by the indophenol titration method for single strength juice (Praschan, 1977). Ten mL of well-mixed juice was mixed with 10 mL metaphosphoric acid-glacial acetic acid
solution and titrated immediately with a sodium 2,6 dichlorobenzenoneindophenol solution (standard dye solution) to a persistent pink endpoint. Ascorbic acid concentration was calculated from the following formula:

\[
\text{mg AA} / \text{100 mL juice} = \text{Dye titer} \times \text{mL indophenol} \times \frac{100}{10}
\]

where the dye titer was obtained by standardization of the dye solution with standard ascorbic acid solution. The titer was calculated as follow.

\[
\text{dye titer (or factor)} = \frac{\text{mg ascorbic acid used}}{\text{mL dye solution used}} = \frac{\text{(mg)}}{\text{(mL)}}
\]
EXPERIMENTAL DESIGN

Determination of the Solid Density and Bulk Density

An experiment with 7 different PSDVB adsorbent resins was conducted in two replications with duplicate analyses to determine the solid density and the bulk density of the adsorbents. For each replication, all 8 adsorbents were measured on the same day. The means for the solid and bulk densities for each adsorbent were computed with their standard deviations.

Regeneration of the Adsorbent

A regeneration procedure was undertaken to compare the effectiveness of different regenerants, 95% ethanol and water at 24°C, 35°C, 45°C and 55°C, in regenerating one adsorbent (20% crosslinked PSDVB adsorbent) after contacting with a model system. Each procedure was conducted in two replications with duplicate analyses. The mean for the calculated efficiency of each regenerant was computed with the corresponding standard deviation.

Evaluation of the Properties of the Adsorbents Involved in the Debittering Process

Debittering experiments were undertaken with 8 different adsorbents, namely, the 6, 8a, 8b, 16, 20, 50, 65a, and 65b% crosslinked PSDVB adsorbent resins to evaluate the properties of the adsorbents which were the most
involved in the debittering process. Grapefruit juice serum and 2 naringin-containing model systems (100 ppm and 600 ppm) constituted the media to debitter. The tests were conducted in 3 replications with duplicate analyses for each medium. Each replication consisted of utilizing all the adsorbents to be compared on the same day. Thus, a series of 2 g of preconditioned adsorbents were contacted with 50 mL of a desired solution and the mixtures were shaken for 20 min. Percent reduction of the bitter constituents was calculated as described earlier.

Correlation coefficients were computed to explore the relationship between the percent removal of the bitter compounds and the quantitative properties of the adsorbents such as the degree of crosslinkage, specific surface area, pore volume and pore diameter. From the literature search, these properties appeared most likely to affect the adsorption of the bitter compounds by these adsorbents. The mean for the properties such as the specific surface area and degree of crosslinkage which were highly correlated with the ability of the adsorbents to adsorb the bitter compounds were computed. The two adsorbents with 65 percent crosslinkage were also analysed for the contribution due to their specific surface area. Regression models were fitted to the experimental data using a linear regression package that produced various regression models based on the independent variables, X values, represented by the specific
surface area or degree of crosslinkage of the adsorbents and the response, Y values, represented by the percent reduction (Tecktronix, 1980). The selection of the best model was based on the $r^2$ value and the minimum maximum residual of the models. When the best regression model was determined, the predicted response values were computed for the independent variables within the range of the experimental values, i.e., from 16 to 65% crosslinkage and from 114 to 650 m$^2$/g for the degree of crosslinkage and the specific surface area of the adsorbents, respectively.

Determination of the Rate of Adsorption of Limonin, Naringin and Recoverable Oil of Grapefruit Juice

Experiments were carried out with the 50% crosslinked PSDVB adsorbent in order to study the rate of adsorption of limonin, naringin and the recoverable oil of grapefruit juice. A series of 1 g of preconditioned adsorbent resin were employed to treat 100 mL grapefruit juice in a batch system for various lengths of time (from 5 to 300 min as mentioned earlier) in 2 replications. Each replication consisted of performing the series of treatments of the juice on the same day. Analysis of these components of the control and adsorbent-treated juice samples was done in duplicate.

The rate of depletion was determined by calculating the quantities of limonin, naringin and oil remaining in the treated juice and plotting fractional concentration versus time. These same data were then plotted on semi-log plots
to test for applicability of first order reaction kinetics as suitable mathematical model. Since these semi-log plots showed an initial deviation from linearity, it was clear that more than one term would be needed in the mathematical model. The method of successive residuals (Mohsenin, 1980) was employed to determine the number of terms in the model and the coefficients and exponents needed in each term. The procedure for determining the number of terms and constants that make up the mathematical rate expression was follows: in the first step, linear regression analysis was used on the linear portion of the original semi-log plot of the fractional concentration versus time. The intercept, $i_1$, of this straight line portion of the original curve gave the coefficient, $a$, of the first exponential term while the slope, $s_1$, of this line gave the rate constant, $k_1$, for this same first term.

The second step of this method consisted of plotting the first residual. This was done by plotting the difference between the extended straight line and the original curved portion of the curve against time on the same semi-log graph. Linear regression analysis was also applied to this new straight line. The intercept, $i_2$, and the slope, $s_2$, of this residual plot were taken as the coefficient, $b$, and the exponent, $k_2$, of the second term, respectively. This first residual was also examined for evidence of any further deviation from linearity to see if additional terms
were required. The true values of the adsorption rate constants were calculated by multiplying the slopes by 2.303, which was the conversion from log base 10 to natural log. The rate of adsorption expression could then be represented by the general expression as follows.

$$C_t / C_0 = a \cdot \text{Exp}(-k_1 \cdot t) + b \cdot \text{Exp}(-k_2 \cdot t)$$

where $C_t / C_0$ represented the fractional concentration of a component of the juice.

**Effect of the Juice Temperature on the Rate of Adsorption of Limonin, Naringin and Ascorbic Acid from Grapefruit Juice**

The juice temperature was changed in order to study the effect of grapefruit juice serum held at different temperatures (24°C or room temperature, and 35°C water bath) on the adsorption of naringin, limonin and ascorbic acid. A series of 1 g of the 50% or 65% crosslinked PSDVB adsorbents were employed to treat 50 mL grapefruit juice serum for 20, 40, 100 and 140 min in 2 replications with duplicate analyses. For each adsorbent, a replication consisted of the treatments at 24°C and 35°C on the same day.

Preliminary experiments showed that raising the temperature of the juice and maintaining it at 35°C did not affect its naringin and limonin contents in reference to the control that was maintained at room temperature. Therefore the untreated juice (at room temperature) was used as the control and the amounts of the constituents remaining in the juice were calculated based on the initial concentrations of
these constituents in the controls. The Students’ t-test was employed to compare the overall effect of juice treated at 24°C and 35°C on naringin, limonin and ascorbic acid contents, with some of the potentially useful PSDVB adsorbents.

Raising the juice temperature to 35°C without adding any adsorbent resulted in reducing the ascorbic acid content, especially when treatment proceeded for 100 and 140 min. Because the overall effect of the treatment conditions (juice temperature and adsorbent combined) on ascorbic acid levels were to be evaluated, the means of percent reduction of ascorbic acid were computed without correction to compare the 2 modes of treatments (i.e. the two temperatures).

**Sensory Evaluation of the PSDVB Adsorbent Treated and Non-treated Grapefruit Juice**

The organoleptic quality of grapefruit juice samples was evaluated in two replications with a pool of 20 panelists screened in a preliminary test. The juice samples were treated with 2 g of the 65% crosslinked PSDVB adsorbent (Kastel 112) per 50 mL of juice for 20 min or 40 min. This adsorbent was found to be the most potentially useful adsorbent.

A paired-comparison test procedure was used to detect the difference in bitterness between the adsorbent treated and non-treated juices. In addition a preference test was employed to determine the most preferred juice sample for a given treatment time. The samples were also rated for their
degree of bitterness. A treated juice and the control juice constituted the pair of samples which were coded and randomly presented to the panelists for simultaneous evaluation. The tasting of each pair of juice samples was conducted in 2 sessions, each in the mid-morning with the same panelists under the conditions outlined earlier.

The data collected for rating the juice bitterness was analyzed using the two tailed Students’ t-test. The mean rating score of the treated and control juice samples were compared. The results for the difference and preference tests were expressed as percentage relative to the total number of panelists who participated in the tasting session.
RESULTS AND DISCUSSION

Determination of the Properties of the Polystyrene Divinylbenzene Adsorbents Involved in the Adsorption of the Bitter Compounds

Behavior of the Polystyrene Divinylbenzene Adsorbents in the Solutions

The behavior of the preconditioned polystyrene divinylbenzene adsorbents in the solutions during the treatment is shown in Figure 14. In both the model systems and grapefruit juice serum, the 6 and 8% crosslinked PSDVB adsorbents floated on the solutions and formed clusters at the surface. The 16, 20, 50, and 65% crosslinked PSDVB adsorbents remained submerged in the solutions and had complete contact with the solutions during the treatment period. After the 20 min treatment time the latter adsorbents became deposited at the bottom of the solutions as shown in Figure 14. It was also observed that the 6 and 8% crosslinked PSDVB adsorbent resins formed clusters on the surface of the distilled water used for rinsing during the resin preconditioning step.

Even after agitation, the 6 and 8% crosslinked PSDVB adsorbents did not fully contact the solutions. That could suggest that entrapped air was in the adsorbent matrix. Therefore, mixtures of the resins in a model system (100 ppm
Figure 14: Behavior of various polystyrene divinylbenzene adsorbents in different aqueous solutions.
naringin) were sonicated to release air bubbles that were possibly entrapped in the open structure of the 6 and 8% crosslinked PSDVB adsorbents. The data were compared to those obtained from the treatment by agitation (Table 6). They showed that the treatment in a sonicator was not satisfactory since it did not improve the performance of the adsorbents of low crosslinkage. Entrapped air could thus be ruled out as the cause of the poor contact of these resins with the aqueous solutions. It was expected that the hydrophobic nature of the polystyrene divinylbenzene adsorbent resins was the factor responsible for the very poor dispersion within aqueous solutions employed in the experiment. Rieman and Walton (1970) reported that a styrene divinylbenzene copolymer without ionogenic groups (neutral resin) was swollen by hydrocarbons but not by water. In contrast, ion exchangers with the same matrix, but carrying ionogenic groups, could be swollen by water. However, their swelling decreased regularly as the crosslinkage was increased and the hydrated radius of the exchanger ion decreased. Since there was no attractive force (from hydrophilic, polar groups) to draw water into the neutral resins used in the experiment, identical behavior for all the resins would be expected when they were placed in contact with the aqueous bitter solutions and water during preconditioning. But, as was observed, the neutral resins with crosslinkage above 16% did not float in the
Table 6

Comparison of the Modes of Treatment of the Model System Containing 100 PPM Naringin by the 6% and 8% Crosslinked PSDVB Adsorbents

<table>
<thead>
<tr>
<th>Crosslinkage</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sonication</td>
</tr>
<tr>
<td>6</td>
<td>2.01</td>
</tr>
<tr>
<td>8_a</td>
<td>2.17</td>
</tr>
<tr>
<td>8_b</td>
<td>--</td>
</tr>
</tbody>
</table>

Data are means of duplicate analyses of three replications.
aqueous solutions and water. Instead they were well mixed and immersed into the solutions. This observation suggested that clearly there were other physical properties, such as the solid density, which allowed the resins with 16% crosslinkage and above to remain in full contact with the aqueous solutions.

Effect of the Solid Density of Polystyrene Divinylbenzene Adsorbents on their Behavior in Aqueous Solutions

The physical properties of the adsorbents used are shown in Table 5. The solid density was determined with the Air comparison pychnometer and calculated as the ratio of the weight of the solid material to the true volume of the same. It appeared that the density of the adsorbents increased with percent crosslinkage. This result was in agreement with a report by Rieman and Walton (1970) who stated that "the density of a swollen cation-exchanger resin is chiefly dependent on the degree of crosslinking; so the higher the crosslinking of a particle, the greater its density". It appeared in the present case that the 6 and 8% crosslinked PSDVB adsorbents had solid density values of approximately 1 g/cc (dry adsorbent) whereas the adsorbents with 16% crosslinkage and above had values well above 1.2 g/cc (dry adsorbent; greater than the density of water). Based on these data, the adsorbents were classified into two groups; the first group encompassed the adsorbents with a solid density lower than 1.2 g/cc (dry adsorbent) and the second group included the adsorbents with a solid density
greater than 1.2 g/cc (dry adsorbent) (the 16, 20, 50 and 65% crosslinked PSDVB adsorbents). Although they were all known to be hydrophobic adsorbents, the latter group of adsorbents which had solid densities greater than 1.2 g/cc (dry adsorbent) were fully in contact with the solutions during rinsing with water in the resin preconditioning phase and during the treatment of the model systems and grapefruit juice serum. The solid densities of the 6 and 8% cross-linked PSDVB adsorbents were too low to allow submersion in the aqueous solutions. The results indicated that the solid density of the adsorbents was a major factor that determined the behavior of hydrophobic polystyrene divinylbenzene adsorbents in the model systems and with grapefruit juice serum. However, a trend was not observed between the extent of debittering and the solid density above 1.2 g/cc (dry adsorbent). It was thus concluded that the solid density favored only adequate contact of the adsorbents when aqueous solutions were used. Solid density must be sufficiently greater than that of water to assure total submersion of neutral adsorbents in aqueous solutions. These observations also suggested that after full contact and immersion in the aqueous solutions were ensured, other properties of the adsorbents played important and essential roles in the debittering process.

In addition, the excessively large coefficients of variation obtained for the 6 and 8% crosslinked PSDVB
adsorbents were attributed to their poor and uneven contact with the solutions. The adsorbents which had solid densities greater than 1.2 g/cc (dry adsorbent) and immersed in the solutions during the treatments exhibited uniform contact with the solutions and showed relatively lower coefficients of variation (Table A1).

The behavior of the adsorbents with a solid density lower than 1.2 g/cc (dry adsorbent) showed that they did not have full contact with the solutions. Thus, it was not possible for these adsorbents to fully utilize their physical characteristics (e.g. surface area, etc...) which may be involved in the debittering process of the model systems and grapefruit juice. Therefore, the 6 and 8% crosslinked PSDVB resins were excluded from the statistical analysis of the data employed to establish the correlation between the physical properties of the adsorbents and their ability to debitter the solutions. This analysis enabled identification of the essential properties that governed the ability of the polystyrene divinylbenzene adsorbents to adsorb the bitter compounds.

**Correlations Between the Adsorbents Physical Properties and the Adsorption of Naringin and Limonin**

Correlation procedures were performed to examine how the physical properties of the adsorbents with a solid density greater than 1.2 g/cc (dry adsorbent) were related to the ability to adsorb naringin from the model systems and naringin and limonin from grapefruit juice serum as well as
to explore the relationship and explain the difference between the performances of the different adsorbents. The relationship could also be used to construct mathematical models which may be useful in predicting and optimizing the debittering process. The computed correlation coefficients are presented in Tables 7 and 8.

The percent reduction of naringin from the model system containing 100 ppm naringin was highly correlated with both the specific surface area \((r = 0.90)\) and the degree of crosslinkage \((r = 0.89)\) of the adsorbents. Also, high correlation coefficient values were obtained between the percent reduction of naringin from the model system containing 600 ppm naringin and both the surface area \((r = 0.97)\) and the degree of crosslinkage \((r = 0.98)\) of the adsorbents. The pore diameter, in contrast, did not correlate as well with the percent reduction of naringin from either the 100 ppm \((r = -0.63)\) or the 600 ppm \((r = -0.83)\) naringin-containing model system (Table 7).

Likewise, relatively high correlation coefficients were observed between the percent reduction of naringin from grapefruit juice and both the specific surface area \((r = 0.88)\) and the degree of crosslinkage \((r = 0.84)\); the percent reduction of limonin from grapefruit juice serum was also highly correlated with both the surface area \((r = 0.85)\) and the degree of crosslinkage \((r = 0.82)\) of the adsorbents. Furthermore, relatively lower coefficients were observed
Table 7
Correlation Coefficients Between the Physical Properties of the PSDVB Adsorbents and the Removal of the Bitter Compounds from the Model Systems

<table>
<thead>
<tr>
<th>Properties</th>
<th>100 PPM M.S.</th>
<th>600 PPM M.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosslinkage</td>
<td>0.90</td>
<td>0.98</td>
</tr>
<tr>
<td>Surface Area</td>
<td>0.89</td>
<td>0.97</td>
</tr>
<tr>
<td>Pore Diameter</td>
<td>-0.63</td>
<td>-0.83</td>
</tr>
<tr>
<td>Pore Volume</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

M.S.: represents model system.
Table 8

Correlation Coefficients Between the Physical Properties of the PSDVB Adsorbents and the Removal of the Bitter Compounds from Grapefruit Juice Serum

<table>
<thead>
<tr>
<th>Properties</th>
<th>Naringin</th>
<th>Limonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosslinkage</td>
<td>0.84</td>
<td>0.82</td>
</tr>
<tr>
<td>Surface Area</td>
<td>0.88</td>
<td>0.85</td>
</tr>
<tr>
<td>Pore Diameter</td>
<td>-0.71</td>
<td>-0.70</td>
</tr>
<tr>
<td>Pore Volume</td>
<td>0.284</td>
<td>0.273</td>
</tr>
</tbody>
</table>
between the pore diameter and the percent reduction of naringin \((r = -0.71)\) and limonin \((r = -0.70)\) from grapefruit juice serum (Table 8).

Negligible correlation was found between the pore volume of the adsorbents and the percent reduction of naringin from the model systems (Table 7) and naringin and limonin from grapefruit juice serum (Table 8). This result was expected since the pore volume, as measured by the mercury intrusion method (Figure 8), encompassed both the pore space inside and between the beads; the pore space between the beads could not influence the adsorption in the batch process. Attempts to determine the true pore volume inside the bead particles by using the Air comparison pychnometer with an impervious coating applied to the beads were unsuccessful because the diluted water base paint and varnish used to coat the beads reacted with the polystyrene divinylbenzene and resulted in shrinking of the beads and development of off-odors. The polystyrene divinylbenzene bead has a porous "sponge-like" or elastic structure (Samuelson, 1963) and exhibited compressibility (Skoog, 1985), which caused the resins to retract when the coating material dried and hardened, as observed with a microscope.

In view of these results, the specific surface area and the degree of crosslinkage appeared to be the primary physical properties that influenced the performance of the polystyrene divinylbenzene adsorbents with solid densities
greater than 1.2 g/cc (dry adsorbent) in adsorbing naringin and limonin in the batch treatment. The negative relationship observed between the pore diameter and the ability of the resins to adsorb the bitter compounds indicated the effectiveness of the resins with smaller average pore diameters.

**Effect of the Mean Pore Diameter of the Adsorbents on the Adsorption of Naringin and Limonin**

The mean pore diameter values of the polystyrene divinylbenzene adsorbents used are presented in Table 5. The values were inversely related to the degree of crosslinkage, i.e., when the degree of crosslinkage increased the mean pore diameter decreased. This is in agreement with numerous reports which indicated that increasing the crosslinkage of the copolymer resulted in making the structure of the bead more compact and reducing the pore dimension (Collins et al., 1982; Rieman and Walton, 1970 and Skoog, 1985). This type of structure can impede the accessibility of larger molecules to the inner parts of the bead or even prevent entry inside the resin bead by a steric hindrance, depending upon the size of the solid in relation to the geometry of the solute (Webber, 1981).

It was observed that the greatest reductions in naringin and limonin levels in grapefruit juice and naringin from the model systems were achieved with adsorbents which possessed lower mean pore diameters. The percentage reduction of the bitter compounds decreased when the mean pore diameter
increased from 50 to 250 Angstroms (Figure 15 and 16). However, the overall performance of the adsorbent with 50 Angstroms mean pore diameter (and 650 m²/g, dry adsorbent, specific surface area) was relatively superior to that of the adsorbent having 25 Angstroms size (and 550 m²/g, dry adsorbent, specific surface area). Furthermore, the adsorbent with 250 Angstroms mean pore diameter (the 20% crosslinked PSDVB adsorbent with specific surface area equal to 138 m²/g dry) performed relatively better in removing naringin and/or limonin from the test solutions as compared to the adsorbent with 152 Angstroms mean pore diameter (the 16% crosslinked adsorbent with specific surface area equal to 114 m²/g, dry adsorbent). From the results of the present study, it appeared that an average pore size of 25 Angstroms did not prevent the bitter compounds from penetrating inside the bead of the polymers. The highly crosslinked adsorbents (with 65% crosslinkage) which possessed average pore sizes of either 25 or 50 Angstroms removed greater amounts of naringin from the model systems (Figure 15) and naringin and limonin from grapefruit juice serum (Figure 16). However, from comparison of the performance of the resins that possess 16% and 20% crosslinkage and 152 and 250 Angstroms mean pore diameter, respectively, there appeared that an indirect relationship existed between the mean pore diameter of the adsorbents used and their efficiency, so the lower the mean pore diameter the higher
Figure 15: Effect of the pore diameter of polystyrene divinylbenzene adsorbents on the adsorption of naringin.
Figure 16: Effect of the pore diameter of polystyrene divinylbenzene adsorbents on the adsorption of naringin and limonin from grapefruit juice
the adsorption efficiency. Further examination of the two groups of resins (the two 65% crosslinked PSDVB adsorbents, and the 16 and 20% crosslinked PSDVB adsorbents) suggested that penetration of the bitter solutes inside the adsorbent bead was not totally hindered and that another factor, such as the specific surface area of the adsorbents, superceded the effect of the smaller pore size in the highly cross-linked PSDVB adsorbent resins.

It has been shown that limonin is less bulky than naringin and its molecular dimensions were found to be approximately 7 x 10 x 14 Angstroms (Arnott et al., 1960). Limonin would not encounter difficulties diffusing through the mean pore diameter of the resins studied. Resistance to its diffusion might occur only along the narrower pores in the interior of the beads. It also appeared that the diffusion of both limonin and naringin to the interior of the resins was not impeded and that most of the surface area of the resins was accessible to them. Physical inclusion of naringin through its most hydrophobic moiety, naringenin, and inclusion of limonin by beta-cyclodextrin (with a cavity diameter of 7 Angstroms; Bender and Komiyama, 1978) has been demonstrated by Konno et al. (1982).

**Effect of the Degree of Crosslinkage of the adsorbents on the Adsorption of Naringin and Limonin**

For statistical purposes and convenience of interpretations, the data collected for the two adsorbents with 65% crosslinkage (65a% and 65b%) were averaged to yield a single
value to use in the discussion of the effect of the degree of crosslinkage (Tables A2 and A4).

The effect of the degree of crosslinkage of the adsorbents on the removal of naringin from the model systems and grapefruit juice serum is presented in Figure 17. Reduction of naringin from the 100 ppm naringin model system increased rapidly from 36% to 89% when the degree of crosslinkage of the adsorbents increased from 16% to 50%, but leveled off as the degree of crosslinkage exceeded 50%.

When the concentration of naringin was increased to 600 ppm in the model system, the percentage reduction increased steadily from 21% to 86% as the degree of crosslinkage increased. In addition, the trend was quite similar to that of the reduction of naringin from grapefruit juice containing 640 ppm naringin. In the latter case, the reduction of naringin increased from 20% to 58% when the degree of crosslinkage increased. But, for both solutions the curves did not level off when the degree of crosslinkage was increased to 65%. That was perhaps due to the fact that as sufficient naringin was available in the external solution, the removal by the highly crosslinked PSDVB adsorbents was proceeding to a point where all of the available sites of the matrix were exhausted. Whereas in the solution with a relatively low concentration of naringin, the concentration of the adsorbate acted as a limiting factor. Agitation during treatment made the
Figure 17: Effect of the degree of crosslinkage of polystyrene divinylbenzene adsorbents on the adsorption of naringin
diffusion of the adsorbate through the bulk of the solution up to the resin particle surface area negligible (Lederer and Lederer, 1957).

From a comparison of the graphs in Figure 17 corresponding to the model systems and grapefruit juice serum, one could observe a gradual change in the pattern of removal of naringin. This change may be attributed to the presence of competing compounds in the grapefruit juice complex (i.e. limonin) for the adsorption sites. Grapefruit juice contains various potential adsorbates that can concentrate onto the adsorbent matrix; primarily, naringin and limonin could behave as competitors with one another for the adsorption sites on the matrix.

The percentage reductions of naringin and limonin from grapefruit juice are presented in Figure 18. As shown by the curve, the removal of limonin increased with the degree of crosslinkage. The percentage reduction of limonin increased from 25% to 79%, as opposed to that of naringin which increased from 20% to 58% with the degree of crosslinkage (Table A4). Limonin was initially present in the juice at a concentration of 19 ppm and the concentration of naringin was 640 ppm. Thus, it appeared that the two bitter compounds may be removed from grapefruit juice by similar processes and that, most importantly, the polystyrene divinylbenzene adsorbents were relatively more selective for limonin. It has been reported that limonin
Figure 18: Effect of the degree of crosslinkage of polystyrene divinylbenzene adsorbents on the adsorption of naringin and limonin from grapefruit juice
is relatively more hydrophobic (Kefford and Chandler, 1970; Konno et al., 1982) and relatively less bulky (MW = 470.50) than naringin (MW = 580.55, Johnson and Chandler, 1988). These characteristics could contribute to the slightly higher adsorption of limonin.

Although both compounds are relatively small molecules, one would not expect such an increase in their adsorption when the degree of crosslinkage of the adsorbents increased. Polystyrene divinylbenzene adsorbents are network matrices whose structures get compact (tight) as the degree of crosslinkage increases (Rieman and Walton, 1970; Collins et al., 1973). Extensive crosslinking in the resins results in creating very small pores in the inner portion of the bead structure. This phenomenon was illustrated by their average pore size as indicated in Table 5. The present results have indicated that even the narrow pores of the highly cross-linked adsorbents (i.e. the 65% crosslinked PSDVB adsorbents) did not impede the removal of the bitter compounds. This suggested that limonin and naringin were not totally excluded from the inner structure of the highly crosslinked adsorbents. Rather, the narrow structure appeared to promote the adsorption of the bitter compounds. The extent of adsorption increased with the degree of crosslinkage in the following order: 16% < 20% < 50% < 65% crosslinkage. This result suggests that the removal of limonin and naringin occurred through forces known to be
active in the adsorption process. In addition, unlike the work reported by Segui et al., (1986), the present investigation has shown that in both the model systems and the processed grapefruit juice, the efficiency of polystyrene divinylbenzene adsorbents increased directly with the degree of crosslinkage (from 16% to 65%), when the solid density of the adsorbents was greater than 1.2 g/cc (dry adsorbent). The removal of both bitter agents followed the same pattern, but the limonin removal curve was consistently higher than that of naringin.

**Effect of the Surface Area of the Adsorbents on the Adsorption of Naringin and Limonin**

Figures 19 and 20 show the effect of the adsorbent specific surface area on the removal of naringin from the model systems and the juice, and naringin and limonin from grapefruit juice, respectively. The data for the effect of the surface area are presented in Tables A3 and A5.

The reduction of naringin from the 100 ppm and 600 ppm naringin-containing model systems increased rapidly from 36% to 94% and from 21% to 87%, respectively, when the specific surface area of the adsorbents increased from 114 to 650 m²/g (dry adsorbent) (Figure 19, Table A3). With the 100 ppm naringin-containing model system the earlier discussed plateau was reached as the surface area approached 450 m²/g. In contrast, in the 600 ppm naringin-containing model system the plateau was reached at a higher specific surface area value (after 550 m²/g). Therefore the concentration of the
Figure 19: Effect of the specific surface area of polystyrene divinylbenzene adsorbents on the adsorption of naringin
Figure 20: Effect of the specific surface area of polystyrene divinylbenzene adsorbents on the adsorption of naringin and limonin from grapefruit juice.
adsorbate might have acted as a limiting factor even before
the adsorption sites of the adsorbents were totally
exhausted. The 600 ppm naringin model system provided
larger amounts of naringin in the internal solution of the
adsorbent beads which tended to create a force that drove
more adsorbate onto the adsorbent matrix. Many authors
discussed the phenomenon of the concentration difference
which is responsible, to a large extent, for the creation of
the force that drives the sorbate inside the resin phase

Furthermore, concerning the adsorption of naringin from
grapefruit juice containing approximately 640 ppm naringin,
the pattern of removal of naringin was quite different from
the ones described for the model systems. In the latter
case, the percentage reduction of naringin was lower for all
the adsorbents as opposed to its uptake when the 600 ppm
model system was employed (Figures 17 and 19). This
discrepancy in the adsorption curve patterns suggested that
there were competitors in the juice that impedes the removal
of naringin by either slowing down the transport through the
pore or occupying the same adsorption sites. Grapefruit
juice is a complex system. The presence of many potential
adsorbates in the juice system can also generate a
competitive attraction on the resin matrix between the
bitter compounds and other constituents. The pulp remaining
in the serum even after centrifugation could also block, to
some extent, some of the pores in the beads and hinder the inner diffusion of the adsorbates.

However, the removal of naringin did not show a plateau within the range of the surface area investigated; the uptake of naringin increased with increasing the specific surface area of the adsorbents for the same reason given to explain the difference between the 100 ppm and 600 ppm naringin-containing model systems.

Figure 20 shows the effect of the surface area on the reduction of the levels of both naringin and limonin from grapefruit juice. The similarity of the patterns of the two curves may suggest that naringin and limonin were competitors in the adsorption processes, i.e., they were being removed by identical process and perhaps on the same sites of the adsorbents. The percent reduction of limonin was greater than that of naringin. It appeared that the adsorbents had a relatively higher affinity for limonin than naringin.

Polystyrene divinylbenzene adsorbent resins possess a continuous pore structure (Dow Chemical, Co, 1971; Rohm and Haas, 1983) and utilization of the inner surface is important in terms of determining the extent of adsorption. The results of this study indicated that the adsorption increased with the specific surface area in the following order: 116 m²/g < 138 m²/g < 450 m²/g < 550 m²/g < 650 m²/g (dry adsorbent).
It was observed that the general trends in the removal of naringin and limonin were similar when the degree of crosslinkage and the surface area of the resins were examined. That is, the curves in Figures 17 and 19, and those in Figures 18 and 20 clearly indicate that the effect of the surface area increased in much the same manner as that of the degree of crosslinkage. This similitude could be attributed to the linear relationship that was found between these two physical properties of the polystyrene divinylbenzene adsorbent resins investigated (Figure 21). It appeared that crosslinking is simply part of means by which higher surface area is achieved.

Furthermore, it was observed that the resins with the same degree of crosslinkage (65% crosslinked PSDVB adsorbents) but having different surface areas exhibited different efficiencies in adsorbing both naringin and limonin. The resin having a larger specific surface area (650 m²/g, dry adsorbent) adsorbed higher amounts of the bitter compounds as compared to the amounts adsorbed by the one with relatively less specific surface area (550 m²/g, dry adsorbent). The difference in the efficiency of sorption of the bitter compounds by these two adsorbent resins was very pronounced in the treatment of grapefruit juice as illustrated in Figure 22. Therefore, the surface area appeared to be the major factor that determined the extent of adsorption of naringin and limonin. This finding
Figure 21: Relationship between the specific surface area and the degree of crosslinkage of polystyrene divinylbenzene adsorbents
Figure 22: Effect of the specific surface area of the adsorbents on the adsorption process. Comparison of the adsorption efficiency of the adsorbents with 65% crosslinkage and different surface areas.
was in agreement with numerous reports that attributed the adsorption to a surface phenomenon; the efficiency of adsorbents in the adsorption of various compounds has been reported to be directly related to the surface area of the adsorbent material available for the adsorption process (Johnson and Chandler, 1988; Puri, 1984; Weber 1981). Johnson and Chandler (1988) reported that when other adsorbent materials such as nylon and cellulose acetate, were made with high surface area, they become effective adsorbents of the bitter agents of citrus juice. They also attributed the effectiveness of an adsorbent in removing the bitter compounds to two factors, namely, the affinity of the adsorbent to the adsorbate and a large surface area which is accessible to the solute. In the case of polystyrene divinylbenzene adsorbents, the surface area combines with the macroreticular structure of the adsorbents (Dow Chemical Co, 1971; Rohm and Haas, 1982) to render the resin an ideal polymer for debittering grapefruit juice.

Unlike other adsorbents, macroreticular resins possess a true macroporous structure which facilitates the diffusion of solutes inside the resin particles (Rohm and Haas, 1982). This type of resin is obtained by suspension copolymerization of styrene and divinylbenzene in the presence of a substance that is a good solvent for the monomers but a poor swelling agent for the resulting polymer (Samuelson, 1963). It has also been reported that the adsorptive property of
polystyrene divinylbenzene adsorbents was derived from their continuous pore structure, broad range of pore sizes, high surface area and aromatic nature of their surface (Dow Chemical, 1971; Rohm and Haas, 1982). The positive effect of the surface area on physical adsorption of gases and vapors has also been reported (Brunauer, 1945).

Mathematical expressions were fitted to the experimental data to describe the relationship between the specific surface area and the degree of crosslinkage of the adsorbents, and their ability to adsorb naringin and limonin (Figures 23 through 27). These expressions have been defined for the values within the range of specific surface area and degree of crosslinkage of the PSDVB adsorbents used in the present study, i.e., from 114 to 650 m²/g (dry adsorbent) and 16% to 65% crosslinkage, respectively. They are very useful for prediction of the debittering efficiency by PSDVB adsorbents.

Regeneration of the Adsorbent

Previous research has shown that cellulose acetate could be readily and economically regenerated by washing with small volumes of warm water as opposed to methanol which was only partially effective (Johnson, 1981, Johnson and Chandler, 1981). In preliminary tests, 95% ethanol was used to recover naringin from the adsorbents after regeneration with warm water failed to restore the used adsorbents. Also, 95% ethanol was employed to regenerate
Figure 23: Comparison of calculated and experimental effects of crosslinkage of the adsorbents on the adsorption of naringin from the 100 ppm model system

\[
\% \text{reduction} = 110.999 - \left( \frac{1095.492}{\% \text{crosslinkage}} \right)
\]

\[r^2 = 0.937\]
Figure 24: Comparison of calculated and experimental effects of specific surface area of the adsorbents on the adsorption of naringin from the 100 ppm model system.
Figure 25: Comparison of calculated and experimental effects of specific surface area of the adsorbents on the adsorption of naringin from the 600 ppm model system
Figure 26: Comparison of calculated and experimental effects of specific surface area of the adsorbents on the adsorption of naringin from grapefruit juice serum
Comparison of calculated and experimental effects of specific surface area of the adsorbents on the adsorption of limonin from grapefruit juice serum

Figure 27:

\[
\% \text{Reduction} = \frac{1}{(0.0430 - 0.00005 \times \text{surface area})} \\
R^2 = 0.873
\]

Percent Reduction

Specific Surface Area (m²/g, dry resin)
the 16, 20, 65\textsubscript{a}, and 65\textsubscript{b} % crosslinked PSDVB adsorbents after contacting with grapefruit juice. The data indicate that a recovery from 79\% to 91\% was achieved in restoring the PSDVB adsorbents used to contact grapefruit juice (Table A6). In addition, recoveries from 89\% to 100\% were achieved when these adsorbent resins were regenerated with 95\% ethanol following the treatment of either the 100 ppm or 600 ppm naringin-containing model system.

In order to test the regeneration power of various solutions, water at room temperature (24°C), 35°C, 45°C and 55°C and 95\% ethanol were employed to restore the 20\% crosslinked PSDVB adsorbent used in the treatment of the 600 ppm naringin-containing model system. The results are presented in Table 9. The data indicated that the recovery of naringin adsorbed onto the adsorbent increased from 1.5 to 14\% when the water temperature increased from 24°C to 55°C whereas regeneration with 95\% ethanol allowed for recovery of 82\% of adsorbed naringin.

Perhaps, the increased recovery of naringin with warm water can be attributed to its relative solubility in hot aqueous solutions (Kesterson and Hendrickson, 1957; Poore, 1934). Poore (1934) reported that naringin was soluble in alcohol, but soluble only to the extent of about 1 part in 2000 in water at 20°C. It can thus be inferred that 95\% ethanol was a very efficient regenerant for naringin adsorbed onto the 20\% crosslinked PSDVB adsorbents, but warm
Table 9
Regeneration of Polystyrene Divinylbenzene Adsorbent with Different Regenerants

<table>
<thead>
<tr>
<th>Regenerants</th>
<th>% Recovery of Naringin</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% Ethanol</td>
<td>81.725 ± 4.394</td>
</tr>
<tr>
<td>Water (°C)</td>
<td></td>
</tr>
<tr>
<td>24 (room temp.)</td>
<td>1.500 ± 0.453</td>
</tr>
<tr>
<td>35</td>
<td>5.474 ± 0.319</td>
</tr>
<tr>
<td>45</td>
<td>9.795 ± 0.519</td>
</tr>
<tr>
<td>55</td>
<td>14.256 ± 0.703</td>
</tr>
</tbody>
</table>

The adsorbent used was the 20% crosslinked PSDVB adsorbent following treatment of the 600 ppm naringin-containing model system. Data are the means of duplicate analyses of 2 replications.
water was inefficient for restoring the adsorbents in one regeneration for 20 min in a batch process.

Discussion of the Probable Mode of Adsorption by the Polystyrene Divinylbenzene Absorbent Resins

The polystyrene divinylbenzene adsorbent resins are network polymers (Collins et al., 1973; Samuelson, 1963). They possess a non-polar (neutral) matrix (Dow Chemical, 1971; Rohm and Haas, 1983; Skoog, 1975) with no ionogenic groups as represented in Figure 4. Therefore, they are hydrophobic and preferentially adsorb non-polar hydrophobic and amphoteric substances from aqueous solutions (Rohm and Haas, 1983). On the other hand, naringin has very limited solubility in water (Poore, 1934; Pullley, 1936; Kesterson and Hendrickson, 1957) while limonin is insoluble in aqueous medium (Chandler et al. 1968). The relative solubility of naringin in water has been attributed to glycosylation that binds the dissacharide portion, formed by glucose and rhamnose, to the naringenin moiety (Markham, 1982; Figure 2). Limonin and naringin are both hydrophobic compounds, but their solubility in citrus juice is due to the presence of the complex hydrocolloid formed by pectin and sugars (Chandler and Nicol, 1975; Kefford and Chandler, 1970). So, when the adsorbents were added to the aqueous solutions (model systems and grapefruit juice), there occurred an accumulation of naringin and limonin onto the hydrophobic matrix surface of the PSDVB. A similar phenomenon was previously demonstrated by Burnham et al. (1972) who found
that non-ionic, neutral organic substances were retained from dilute aqueous solution on the matrix of PSDVB adsorbent while the ionic substances were unretained and passed through a column containing the polymer. Furthermore, they found that the adsorption of weakly ionic organic compounds, such as carboxylic acids, phenols and amines, depended on the pH of the solution (the pH influences the ionic charge of the ionizable solutes); they also found that the adsorbents had a higher efficiency of sorption for aromatic compounds.

Also, the relationship between the degree of hydrophobicity of a solute and the extent of adsorption on PSDVB adsorbents has been reported (Rohm and Haas, 1983). It was shown that the extent and the selectivity of adsorption of a solute by the PSDVB adsorbents increased with the degree of hydrophobicity. This phenomenon was substantiated by the adsorption of various phenols with different degrees of chlorine substitution. It is known that as the level of chlorine substitution increased, the phenolic compounds became less soluble in water. These authors found that the adsorption capacity of PSDVB absorbent for phenols increased when the chlorine substitution increased. Since limonin is more insoluble in water as compared to naringin, one can expect the adsorbents to show a relatively higher affinity for the former compound. The present results appeared to
support this theory of adsorption of hydrophobic solute from the aqueous solutions.

It has also been reported that the adsorption of an amphoteric solute onto the matrix of PSDVB adsorbent occurred through the hydrophobic portion of the adsorbate molecule (Rohm and Haas, 1982). Konno et al. (1982) demonstrated that inclusion of naringin by the beta-cyclodextrin polymer occurred via the naringenin moiety. Also, Rieman and Walton (1970) stated that the phenol was much more strongly attracted by van der Waals’ forces to uncharged benzene ring than the charged ring. These ideas are, in principle, applicable to the adsorption of naringin by the polystyrene divinylbenzene adsorbents, i.e., naringin molecule would likely interact with the adsorbent matrix through its naringenin moiety.

The adsorption process onto the PSDVB adsorbents has been attributed to a surface phenomenon which involves Van der Waals’ interactions (Mantell, 1951; Rieman and Walton, 1970; Rohm and Haas, 1982; Samuelson, 1963). Also, Rieman and Walton (1970) and Samuelson (1963) attributed the absorption of non-electrolytes and less polar solutes by ion-exchange resins to London (or Van der Waals’) forces of attraction between the hydrocarbon part of the resin matrix and the hydrocarbon part of the solute. Based on the chemical characteristics of naringin and limonin one can reasonably postulate that similar interactions can occur
between the bitter compounds and the matrix of polystyrene
divinylbenzene adsorbents during the debittering process of
the model systems and grapefruit juice.

**Rate of Adsorption of the Bitter Compounds and Certain Constituents of Grapefruit Juice by the 50% Crosslinked PSDVB Adsorbent**

**Rate of Adsorption of the Bitter Agents of Grapefruit Juice**

The depletion of naringin and limonin from 100 mL of
grapefruit juice serum by 1 g of 50% crosslinked PSDVB
adsorbent, one of the potentially useful resins, was
measured. This experiment was conducted to follow the time-
dependent adsorption process of the bitter agents and to
derive mathematical expressions which could be used to
determine contact times required for the desired reduction
of the bitter compounds.

The rate of depletion of naringin from grapefruit juice
serum is presented in Figure 28. The depletion of this
bitter agent was very rapid in the first 20 min post-mixing,
followed by a relatively slow rate over the remaining 280
min. The rate of depletion of limonin from grapefruit juice
conducted under identical conditions is shown in Figure 29.
The removal of this other bitter principle of grapefruit was
also very rapid during the first 40 min and slowed down
thereafter, for the remainder of the 300 min treatment
period. Based on the assumption that static equilibrium was
approached after the extended period of adsorption, a first
order reaction approximation was applied to the data. The
Figure 28: Time-dependent adsorption of naringin from grapefruit juice. 50% crosslinked polystyrene divinylbenzene adsorbent
Figure 29: Time-dependent adsorption of limonin from grapefruit juice. 50% crosslinked polystyrene divinylbenzene adsorbent.
following mathematical expression of a first order reaction described by Hill and Grieger-Block (1980) was applied between the limits of the bitter compound concentrations \( C_0 \) at initial time, \( t_0 \), and \( C \) at any given reaction time, \( t \):

\[-\frac{dC}{dt} = KC \]  

(1). After rearrangement, equation (1) takes the following form

\[
\frac{dC}{C} = -kdt 
\]  

(2) and integration of equation (2) between the limit of the bitter compounds concentrations \( C_0 \) and \( C \), and between the initial and any given times of adsorption, \( t_0 \) (with \( t_0 = 0 \) min) and \( t \), respectively, yields the next equation of the form:

\[
\ln\left(\frac{C}{C_0}\right) = -Kt 
\]  

(3) which, when converted to the logarithm base 10, gives the following equation:

\[
2.3 \times \log\left(\frac{C}{C_0}\right) = -kt 
\]  

(4) This expression theoretically describes a straight-line relationship between \( \log\left(\frac{C}{C_0}\right) \) and \( t \). The adsorption rate constant, \( k \), can be derived from the slope of the semi-logarithmic plot of \( C/C_0 \) versus \( t \). The slope of this plot is equal to \(-k/2.303 \). Thus, the rate constant is obtained by multiplying the slope by 2.303.

This analysis was applied to the present data. From the semi-log plots of the fractional concentrations \( N_t/N_0 \) and \( L_t/L_0 \) versus time for naringin and limonin, respectively, (with \( N_0 \) and \( L_0 \) corresponding to the initial concen-
trations of naringin and limonin, respectively, and \( N_t \) and \( L_t \) the concentrations at a given time of the same compounds), it would appear that the adsorption process did not follow a first order reaction rate because the initial portion of the curves deviated from the straight line pattern as shown in curve (A) in Figures 30 and 31. Curve (A) in Figures 30 and 31 may be characteristic of two or more simultaneous and independent first order reactions. The method of successive residuals previously described (Mohsenin, 1980) was used to determine the number of additional exponential terms required in the mathematical model of the rate of adsorption of the bitter agents. The results are illustrated by the plot of the first residual (curve (B)) in Figures 30 and 31. Since the plot of the first residual had no deviation from linearity, no further terms were needed and the rate equation could be described as the sum of two exponential terms. The parameters of the first exponential term were obtained from the linear regression analysis of the straight line portion of the original semi-log plot (curve (A) in Figures 30 and 31). Those of the second exponential term were calculated from the linear regression analysis of the first residual (curve (B) in Figures 30 and 31). Thus, the general form of the mathematical expression of the rate of adsorption would be:

\[
\frac{C_t}{C_0} = a\exp(-k_1 t) + b\exp(-k_2 t)
\]  

(5).

The second exponential term characterized a very rapid
Figure 30: Semi-log plot of fractional concentration versus time showing the rate of adsorption of naringin from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent
Figure 31: Semi-log plot of fractional concentration versus time showing the rate of adsorption of limonin from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent
adsorption of the bitter compounds that may likely occur because of the readily available outer surface area of the beads at the beginning of the adsorption process. At the same time another first order reaction is likely taking place characterizing the relatively slower adsorption rate caused by the diffusion of the bitter compounds inside the pore of the beads. This reaction was described by the first exponential term. At any given time the amount of a given bitter compound adsorbed would be the sum of the two simultaneous processes.

From the above approach the parameters in the expressions for the rate of adsorption of naringin and limonin from grapefruit juice were computed and the mathematical models were found to be as follows.

For naringin:
\[ \frac{N_t}{N_0} = 0.789 \exp(-8.751 \times 10^{-4} t) + 0.184 \exp(-3.960 \times 10^{-2} t) \]
and for limonin:
\[ \frac{L_t}{L_0} = 0.718 \exp(-7.139 \times 10^{-3} t) + 0.256 \exp(-8.823 \times 10^{-2} t). \]

Figures 32 and 34 represent the plots of the calculated and experimental fractional concentrations versus time for naringin and limonin, respectively. For each compound the curves were very similar. Moreover, the scatter diagrams suggested that the mathematical models proposed above provided adequate description of the rate of adsorption of the bitter compounds (Figures 33 and 35). Therefore, these
Figure 32: Experimental and calculated fractional concentrations versus time for adsorption of naringin from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent
Figure 33: Scatter diagram of calculated versus experimental adsorption of naringin from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent
Figure 34: Experimental and calculated fractional concentrations versus time for adsorption of limonin from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent.
Figure 35: Scatter diagram of calculated versus experimental adsorption of limonin from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent
expressions could be adopted as true mathematical models of the adsorption kinetics of the bitter compounds.

**Rate of Adsorption of the Recoverable Oil and Acid from Grapefruit Juice**

Figure 36 shows the time-dependent adsorption of the recoverable oil from the juice by the 50% crosslinked PSDVB adsorbent. The initial concentration of the recoverable oil in the juice sample was 0.0037% (v/v solution). Initially, there occurred a very rapid adsorption of the recoverable oil during the first 20 min of contact between the juice and the adsorbent. This was followed by a slow removal of oil as indicated by the plateau that appeared after 140 min of contact time.

When the first order procedure was applied to the data, a straight line was obtained for a portion of the semi-log plot of the fractional concentration, \( \frac{O_t}{O_0} \), versus the contact time, \( t \) (Figure 37). Because of the curved portion on the original curve in Figure 36 (curve A), further determination of the relationship between the removal of recoverable oil and contact time was conducted using the method of the successive residuals as in the case of the bitter compounds. Two exponential terms were found necessary to describe the rate equation. The following mathematical model was obtained.

\[
\frac{L_t}{L_0} = 0.69 \times \exp(-1.313 \times 10^{-2} t) + 0.154 \times \exp(-1.417 \times 10^{-1} t).
\]

The plot of experimental and calculated fractional concentrations versus contact time is shown in Figure 38.
Figure 36: Time-dependent adsorption of recoverable oil from grapefruit juice. 50% crosslinked polystyrene divinylbenzene adsorbent.
Figure 37: Semi-log plot of fractional concentration versus time showing the rate of adsorption of recoverable oil from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent
Figure 38: Experimental and calculated fractional concentrations versus time for adsorption recoverable oil from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent.
There was a good match of the curves. Also, the scatter
diagram of calculated versus experimental fractional
concentration showed a straight line relationship, which
suggested that the above model gave an adequate description
of the rate of adsorption of the recoverable oil from
grapefruit juice (Figure 39). This model could be adopted
as true mathematical model of the adsorption kinetics of the
recoverable oil.

The loss of oil that occurred after 20 min contact time
was approximately 40% of the initial concentration. The
present study showed that the recoverable oil could be
totally stripped from the juice if adsorption was allowed to
proceed for 300 min. The loss of oil could be detrimental,
to some extent, to the flavor of grapefruit juice if the
debittering process was carried out for an extended period
of time. In practice, the flavor and aroma compounds which
evaporate during preparation of the juice concentrate, are
added back to the single strength juice preparation prior to
commercialization. Therefore, it would be beneficial to
treat grapefruit juice before restoring the single strength
juice flavor.

Figure 40 shows the adsorption rate of the juice acid
(expressed as citric acid) by the 50% crosslinked PSDVB
adsorbent. The data indicated that the loss of the juice
acid was not as rapid as that of the other components
(naringin, limonin and recoverable oil). A plateau was very
Figure 39: Scatter diagram of calculated versus experimental adsorption of recoverable oil from grapefruit juice onto 50% crosslinked polystyrene divinylbenzene adsorbent
Figure 40: Time-dependent adsorption of titratable acids from grapefruit juice. 50% crosslinked polystyrene divinylbenzene adsorbent.
quickly established at approximately 15 min of contact between the juice and the adsorbent. Furthermore, only 2% of the total acid was removed after 300 min of treatment. From these results one can infer that the acid of the juice was barely affected by the 50% crosslinked PSDVB adsorbent.

**Effect of the Juice Temperature on the Adsorption of the Bitter Compounds and Ascorbic Acid by the 50% and 65% Crosslinked PSDVB Adsorbents**

**Comparison of the Effect of the Juice Temperature on the Adsorption of Naringin and Limonin from Grapefruit Juice**

Figures 41 and 42 compare the effect of the juice temperature, at 24°C and 35°C, on the adsorption rate of naringin from grapefruit juice by the 50 and 65% crosslinked PSDVB adsorbents, respectively. There was no difference in the adsorption of naringin by the 50% crosslinked PSDVB adsorbent as a function of temperature. However, after 100 min of contact time, removal of the bitter agent at room temperature was increased (Figure 41). But, there was no significant difference in the overall effects of the two temperatures of the juice on the adsorption of naringin.

On the contrary, when the 65% crosslinked PSDVB adsorbent was employed, the adsorption of naringin from the juice samples maintained at 24°C remained consistently greater than the removal of the bitter agent from the juice held at 35°C (Figure 41). Furthermore, the overall effects of the juice temperatures were significantly different ($\alpha = 0.025$) as indicated by the one tailed Students’ $t$-test of the data (Table 10).
Figure 41: Effect of juice temperature on the adsorption of naringin onto 50% crosslinked polystyrene divinylbenzene adsorbent.
Figure 42: Effect of juice temperature on the adsorption of naringin onto 65% crosslinked polystyrene divinylbenzene adsorbent
Table 10
Comparison of the Effect of the Juice Temperature on the Adsorption of Naringin and Limonin by the 50 and 65% Crosslinked PSDVB Adsorbents

<table>
<thead>
<tr>
<th>Juice Temperature (°C)</th>
<th>Naringin</th>
<th>Limonin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Crosslinked PSDVB Adsorbents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>65</td>
</tr>
<tr>
<td>24</td>
<td>12.590a</td>
<td>7.516a</td>
</tr>
<tr>
<td>35</td>
<td>12.296a</td>
<td>10.185b</td>
</tr>
</tbody>
</table>

Data are overall means of mg remaining of the bitter compounds in 50 mL of grapefruit juice treated with 1 adsorbent. Experiment was conducted in 2 replications duplicate analyses. Means with different letters are significantly differ α = 0.025, t-test.
The effects of the juice temperature on the adsorption of limonin by the 50% and 65% crosslinked PSDVB adsorbents are shown in Figures 43 and 44, respectively. In both cases, the rate of adsorption of limonin at 35°C was consistently lower than that at 24°C. But, the overall effect of the juice held at 24°C was significantly different from that of the juice held at 35°C for only the 65% crosslinked PSDVB adsorbent ($\alpha = 0.025$, Table 10).

The kinetics of the adsorption reaction of naringin and limonin by these two PSDVB adsorbents when the juice was held at either temperature was not explored because enough experimental points were not available between the initial and 20 min adsorption periods.

**Effect of the Adsorbent Treatment and the Temperature of the Juice on the Ascorbic Acid Content of Grapefruit Juice**

The ascorbic acid content of the grapefruit juice serum treated with the 50% and 65% crosslinked PSDVB adsorbents when the juice were held at 24°C and 35°C was analyzed to study the effect of the PSDVB adsorbent treatment on this valuable grapefruit juice component. Preliminary experiments indicated that losses of ascorbic acid from the control juice occurred when the samples were maintained at 35°C for extended periods of time (40 to 140 min). For the present study one was interested in assessing the combined effects of the temperature and PSDVB adsorbent treatment on the ascorbic acid of grapefruit. Therefore, no correction was made for the data collected. Thus, the losses of
Figure 43: Effect of juice temperature on the adsorption of limonin onto 50% crosslinked polystyrene divinylbenzene adsorbent
Figure 44: Effect of juice temperature on the adsorption of limonin onto 65% crosslinked polystyrene divinylbenzene adsorbent
ascorbic acid were calculated based on the initial concentration of ascorbic acid of the juice held at room temperature.

The data in Tables 11 and 12 indicate that there occurred an increasing loss of ascorbic acid when the contact time of the juice with both adsorbents was increased from 20 min to 140 min. In the case of the juice samples held at 24°C and 35°C and treated with the 50% crosslinked adsorbent, the losses varied from approximately 6% to 17% of the initial ascorbic acid content (Table 11) when the contact time was increased from 20 min to 140 min. Treatment with the 65% crosslinked adsorbent resulted in losses from 11% to 16% and 4% to 20% when the juice temperature was held at 24°C and at 35°C, respectively, and the contact time was increased from 20 min to 140 min. But, for each adsorbent, the overall effect of the treatment at 24°C (room temperature) was not significantly different from the effect of the treatment at 35°C ( = 0.025, Table 13).

Comparison of the data in Tables 11 and 12 shows that the overall effect of the treatment with the 65% crosslinked PSDVB adsorbent was slightly greater than that of the 50% crosslinked PSDVB adsorbent. However, the reduction of the ascorbic acid from grapefruit juice treated with both resins for 20 min appeared to be negligible. This treatment time period was found to be a suitable debittering condition in most cases since significant amounts of naringin and
Table 11
Effect of Grapefruit Juice Temperature on the Ascorbic Acid Content of Juice treated with the 50% Crosslinked PSDVB Adsorbent

<table>
<thead>
<tr>
<th>Contact Time (Min)</th>
<th>Room Temperature (24°C)</th>
<th>Water Bath (35°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>6.44 ± 1.51</td>
<td>5.71 ± 2.74</td>
</tr>
<tr>
<td>40</td>
<td>7.08 ± 1.71</td>
<td>8.09 ± 0.03</td>
</tr>
<tr>
<td>100</td>
<td>12.83 ± 1.95</td>
<td>13.76 ± 4.06</td>
</tr>
<tr>
<td>140</td>
<td>17.05 ± 0.30</td>
<td>16.54 ± 3.46</td>
</tr>
</tbody>
</table>

Data are means of duplicate analyses of 2 replications.
Table 12
Effect of Grapefruit Juice Temperature on the Ascorbic Acid Content of Juice Treated with the 65% Crosslinked PSDVB Adsorbent

<table>
<thead>
<tr>
<th>Contact Time (Min)</th>
<th>Room Temperature (24°C)</th>
<th>Water Bath (35°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>10.70 ± 0.75</td>
<td>4.33 ± 3.14</td>
</tr>
<tr>
<td>40</td>
<td>12.06 ± 1.11</td>
<td>9.16 ± 1.72</td>
</tr>
<tr>
<td>100</td>
<td>15.14 ± 0.29</td>
<td>14.29 ± 2.38</td>
</tr>
<tr>
<td>140</td>
<td>16.32 ± 1.89</td>
<td>19.93 ± 1.80</td>
</tr>
</tbody>
</table>

Data are means of duplicate analyses of 2 replications.
Table 13
Comparison of the Effect of the Juice Temperature on the Reduction of Ascorbic Acid Content by the 50\% and 65\% Crosslinked PSDVB Adsorbents

<table>
<thead>
<tr>
<th>Juice Temperature °C</th>
<th>% Crosslinked PSDVB Adsorbents 50</th>
<th>% Crosslinked PSDVB Adsorbents 65</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>10.850&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.555&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>35</td>
<td>11.025&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.928&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are overall means of percent reduction of ascorbic acid from 50 mL juice treated with 1 g of adsorbent. Means with different letters are significantly different at $\alpha = 0.025$. The experiment was conducted in 2 replications with duplicate analyses.
limonin were adsorbed during the treatment with small losses of the juice recoverable oil and ascorbic acid.

The data of this study shows that increasing the juice temperature to 35°C resulted in decreasing the amounts of naringin and limonin removed by the adsorbents and in decreasing the losses of ascorbic acid content of grapefruit juice. These results may be explained in part by the solubility of these juice components which increased as the juice temperature was raised. Numerous authors have reported the increased solubility of naringin and limonin with increasing temperature of aqueous solutions (Kefferd and Chandler, 1970; Kesterson and Hendrickson, 1957; Poore, 1934; Pulley, 1936). It has also been pointed out that there is an indirect relationship between the solubility of a solute in aqueous solution and its adsorption by neutral polystyrene divinylbenzene adsorbent from the medium (Rohm and Haas, 1983). Also, the relative solubility of the bitter agents in warm water might have favored the motion of the molecules of naringin and limonin away from the adsorbent surface. This, in turn, might have caused a reduction of the number of molecules adsorbed. Brunauer (1945) stated that in physical adsorption, the adsorbate is not rigidly held to a position on the adsorbent surface and that thermal energy contributes to the free movement over the surface. Therefore, the results of the present study
appeared to support the principle of removal of the bitter compound by weak physical adsorption forces.

Sensory Evaluation of the Treated and Non-treated Grapefruit Juice

Adsorption of limonin and naringin from grapefruit juice is of interest from the standpoint of improving the organoleptic quality of the juice. Also, sensory quality is essential for consumer acceptance of grapefruit juice. The method employed to achieve significant improvement of the organoleptic quality of the juice in terms of significantly reducing the bitterness without adversely affecting the flavor and desirable components of the juice, are of paramount commercial interest to the citrus industry.

The data of the sensory evaluation of the grapefruit juice before and after treatment for 20 min with the most potentially useful resin, the 65\% crosslinked PSDVB adsorbent with 650 m\(^2\)/g (dry adsorbent) specific surface area (Kastel 112), are presented in Table 14. The data show that all the panelists detected a difference between the pair of non-treated (control) juice samples containing 693 ppm and 19 ppm naringin and limonin, respectively, and the adsorbent-treated juice which contained 261 ppm naringin and less than 2 ppm limonin. Furthermore, 65\% of the panelists preferred the treated juice as compared to the non-treated form. As for the degree of bitterness, the treated juice bitterness mean score was significantly lower than that of the non-treated juice (\(\alpha = 0.05\)). The former juice sample
Table 14

Sensory Evaluation of PSDVB Adsorbent-Treated and Non-Treated (Control) Grapefruit Juice

<table>
<thead>
<tr>
<th>Items</th>
<th>Treatment Period</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
<td>40 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference Test (%)</td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preference Test (%)</td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
<td>Treated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bitterness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Score</td>
<td>3.45*</td>
<td>2.1*</td>
<td>3.5**</td>
<td>2.03***</td>
<td></td>
</tr>
</tbody>
</table>

(a): 20 Screened Panellists participated in the sensory evaluation test that was conducted in 2 replications. The juice was treated with 65% crosslinked PSDVB adsorbent.

* & **: Means are significantly different (α = 0.05, t-test). Based on a 5-Point rating scale, 1 was trace and 5 was extreme.
scored on the average 2.1 on a 5-point rating scale, which translated to a slightly bitter sample as opposed to the latter sample that had an average score of 3.5 and translated to moderately to very bitter juice.

Data for the evaluation of the juice treated for 40 min with the same adsorbent are also presented in Table 14. The final concentrations of the bitter compounds in the treated juice were less than 1 ppm and 174 ppm for limonin and naringin, respectively. The results show that 98% of the panelists detected the difference between the pair of treated and control juices. The 40 min-treated juice was rated slightly bitter with an average score of 2.03 as opposed to the control which received an average score of 3.5 which represented a moderately to very bitter juice on the rating scale. The mean scores for bitterness of the treated and non-treated juice samples were found to be significantly different ($\alpha = 0.05$) and more than 72% of the panelists preferred the treated grapefruit juice.

The comments from the panelists revealed that the control juice exhibited an objectionable bitter taste which was significantly improved after treatment with the adsorbent resin. Thus, the results indicate that high bitterness had an adverse effect on grapefruit juice preference by consumers. This confirmed the results of the survey reported by de Jager (1983), that is, as the degree of preference for grapefruit juice decreased when the bitter
compound levels in the juice increased, the bitterness scores increased. The study reported here show also that when the most potentially useful adsorbent with 65\% cross-linkage was used to treat grapefruit juice (2 g adsorbent per 50 mL juice) for only 20 min there was a significant improvement in one of the major sensory attribute of grapefruit juice, namely, the bitterness of grapefruit juice.
CONCLUSION

The results of the present study provided the evidence that certain physical properties of polystyrene divinylbenzene adsorbents play an important role in the process of debittering grapefruit juice. Solid densities greater than 1.2 g/cc (dry adsorbent) allowed total contact of the adsorbents with the naringin-containing model systems and grapefruit juice serum in order to adsorb the bitter compounds naringin and limonin.

The specific surface area and the degree of cross-linkage of the PSDVB adsorbents were found to be the properties which determined the extent of adsorption of the bitter compounds from aqueous solutions. The adsorption of naringin and limonin from grapefruit juice increased rapidly when the degree of crosslinkage and the specific surface area of the adsorbents increased from 16% to 65% and from 114 to 650 m²/g (dry adsorbent), respectively. Although these two properties have been treated equally in terms of their effects on the adsorption of the bitter compounds from grapefruit juice, the specific surface area of the adsorbents appeared to play a key role in the adsorption process. However, sufficient number of adsorbents with the same degree of crosslinkage was not available to conclu-
vely substantiate this finding. The adsorbents appeared to be relatively more selective for limonin. Mathematical models which included either the degree of crosslinkage or the specific surface area of the PSDVB adsorbents were fitted to the experimental data.

Naringin could be recovered from the exhausted adsorbents by 95% ethanol washes with recovery efficiency of 86% to 100%. Although recovery of naringin slightly increased with increasing water temperature, water was found to be an inefficient regenerant for the polystyrene divinylbenzene adsorbents. Naringin was not degraded on the surface of the adsorbents. It can be recovered from the PSDVB adsorbents for use as bittering agent in various preparations.

The adsorption processes of naringin, limonin and recoverable oil by the 50% crosslinked PSDVB adsorbent were found to be time-dependent reactions. The adsorption kinetics of each constituent appeared to be a first order reaction that could be characterized by a mathematical model with two simultaneous first order terms. The term with the highest adsorption rate constant described the rapid adsorption that predominated at the onset of the treatment and the other term represented the relatively slower adsorption which occurred as the treatment proceeded. But, at any given time, the amount of each constituent removed was the sum of these two simultaneous reactions.
Adsorption of the recoverable oil of grapefruit juice was substantial after an extended treatment period. Because of the loss of a large amount of recoverable oil during prolonged treatment with the 50% crosslinked PSDVB adsorbent, it will be beneficial to debitter grapefruit juice before restoring the peel oil of the single strength juice. Titratable acidity was unaffected by the adsorbent treatment.

Increasing the juice temperature to 35°C did not improve the adsorption of either naringin or limonin by the 50% and 65% crosslinked PSDVB adsorbents. On the contrary, debittering at room temperature was found to be more efficient. The losses of ascorbic acid caused by both the 50 and 65% crosslinked PSDVB adsorbents were negligible after a 20 min treatment period, while naringin and limonin were greatly reduced from the juice.

Sensory evaluation of 65% crosslinked PSDVB adsorbent-treated grapefruit juice showed a significant reduction of the bitterness. This, in turn, resulted in a significant increase in the panelists' preference for the juice as compared to the control. It is the authors' opinion that no off-flavor was produced after debittering the juice. Furthermore, more than 65% of the panelists preferred the treated juice. Therefore, the adsorbent treatment could significantly increase the acceptability of grapefruit juice by the consumer.
Table A1

Adsorption of Naringin from 100 PPM Model System by the 6% and 8% Crosslinked PSDVB Adsorbents

<table>
<thead>
<tr>
<th>Adsorbents (%Crosslinkage)</th>
<th>%Reduction of Naringin C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.288 ± 0.29 103.083</td>
</tr>
<tr>
<td>8_a</td>
<td>0.374 ± 0.51 137.344</td>
</tr>
<tr>
<td>8_b</td>
<td>2.218 ± 1.90 85.799</td>
</tr>
</tbody>
</table>

C.V.: represents the coefficient of variation.
Table A2

Effect of the Percent Crosslinkage of the PSDVB Adsorbents on the Reduction of Naringin in the Model Systems Containing 100 PPM and 600 PPM Naringin

<table>
<thead>
<tr>
<th>Percent Crosslinkage</th>
<th>Percent Reduction 100 PPM M.S.</th>
<th>Percent Reduction 600 PPM M.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>36.01 ± 2.00</td>
<td>21.31 ± 1.71</td>
</tr>
<tr>
<td>20</td>
<td>65.06 ± 4.45</td>
<td>35.83 ± 2.94</td>
</tr>
<tr>
<td>50</td>
<td>89.39 ± 2.56</td>
<td>56.99 ± 1.48</td>
</tr>
<tr>
<td>65</td>
<td>89.55 ± 3.15</td>
<td>86.03 ± 1.46</td>
</tr>
</tbody>
</table>

M.S.: represents the model system.
Table A3

Effect of the Surface Areas of the PSDVB Adsorbents on the Reduction of Naringin in the Model Systems Containing 100 PPM and 600 PPM Naringin

<table>
<thead>
<tr>
<th>Surface Area (M²/G, dry)</th>
<th>Percent Reduction</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 PPM M.S.</td>
<td>600 PPM M.S.</td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>36.01 ± 2.00</td>
<td>21.31 ± 1.71</td>
<td></td>
</tr>
<tr>
<td>138</td>
<td>65.06 ± 4.45</td>
<td>35.83 ± 2.94</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>89.39 ± 2.56</td>
<td>56.99 ± 1.48</td>
<td></td>
</tr>
<tr>
<td>550</td>
<td>89.55 ± 3.15</td>
<td>85.29 ± 2.08</td>
<td></td>
</tr>
<tr>
<td>650</td>
<td>93.54 ± 2.66</td>
<td>86.78 ± 0.83</td>
<td></td>
</tr>
</tbody>
</table>

M.S.: represents the model system.
Table A4

Effect of the Percent Crosslinkage of the PSDVB Adsorbents on the Reduction of Naringin and Limonin in Grapefruit Juice Serum

<table>
<thead>
<tr>
<th>Percent Crosslinkage</th>
<th>Percent Reduction</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 PPM M.S.</td>
<td>600 PPM M.S.</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>20.00 ± 0.82</td>
<td>24.63 ± 5.86</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>26.72 ± 1.39</td>
<td>31.89 ± 10.86</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>28.79 ± 0.37</td>
<td>34.03 ± 4.51</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>57.96 ± 2.08</td>
<td>78.63 ± 2.56</td>
<td></td>
</tr>
</tbody>
</table>

M.S.: represents the model system.
Table A5

Effect of the Surface Areas of the PSDVB Adsorbents on the Reduction of Naringin and Limonin in Grapefruit Juice Serum

<table>
<thead>
<tr>
<th>Specific Surface Area (M²/G, dry)</th>
<th>Percent Reduction</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Naringin</td>
<td>Limonin</td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>20.00 ± 0.82</td>
<td>24.26 ± 5.86</td>
<td></td>
</tr>
<tr>
<td>138</td>
<td>26.72 ± 4.45</td>
<td>31.89 ± 10.86</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>28.79 ± 3.05</td>
<td>34.03 ± 5.15</td>
<td></td>
</tr>
<tr>
<td>550</td>
<td>49.14 ± 2.06</td>
<td>65.95 ± 1.92</td>
<td></td>
</tr>
<tr>
<td>650</td>
<td>66.79 ± 3.53</td>
<td>91.30 ± 3.19</td>
<td></td>
</tr>
</tbody>
</table>
Table A6

Regeneration of the Adsorbents after Contacting with Grapefruit Juice Serum - Naringin Analysis

<table>
<thead>
<tr>
<th>Adsorbents (%Crosslinkage)</th>
<th>95% ethanol</th>
<th>Water</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25°C</td>
<td>35°C</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>79.38</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>87.61</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>65&lt;sub&gt;a&lt;/sub&gt;</td>
<td>90.92</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>65&lt;sub&gt;b&lt;/sub&gt;</td>
<td>90.51</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

The data are from the preliminary experiment conducted in one replication with duplicate analyses.

** : Negligible.
Table A7

Time-Dependent Adsorption of Naringin and Limonin of Grapefruit Juice onto the 50% Crosslinked PSDVB Adsorbent

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Naringin (mg remaining)</th>
<th>( \frac{N_t}{N_0} )</th>
<th>Limonin (mg remaining)</th>
<th>( \frac{L_t}{L_0} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40.969 ± 1.392</td>
<td>1</td>
<td>0.894 ± 0.013</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>39.237 ± 1.788</td>
<td>0.958</td>
<td>0.749 ± 0.005</td>
<td>0.838</td>
</tr>
<tr>
<td>10</td>
<td>37.957 ± 2.181</td>
<td>0.926</td>
<td>0.670 ± 0.005</td>
<td>0.749</td>
</tr>
<tr>
<td>15</td>
<td>36.576 ± 2.011</td>
<td>0.893</td>
<td>0.651 ± 0.033</td>
<td>0.728</td>
</tr>
<tr>
<td>20</td>
<td>36.199 ± 2.915</td>
<td>0.884</td>
<td>0.618 ± 0.034</td>
<td>0.691</td>
</tr>
<tr>
<td>40</td>
<td>34.843 ± 1.921</td>
<td>0.850</td>
<td>0.485 ± 0.041</td>
<td>0.543</td>
</tr>
<tr>
<td>60</td>
<td>33.399 ± 2.167</td>
<td>0.815</td>
<td>0.407 ± 0.063</td>
<td>0.455</td>
</tr>
<tr>
<td>100</td>
<td>29.902 ± 0.026</td>
<td>0.730</td>
<td>0.320 ± 0.060</td>
<td>0.358</td>
</tr>
<tr>
<td>140</td>
<td>28.836 ± 1.122</td>
<td>0.704</td>
<td>0.239 ± 0.045</td>
<td>0.267</td>
</tr>
<tr>
<td>180</td>
<td>26.974 ± 1.307</td>
<td>0.658</td>
<td>0.174 ± 0.038</td>
<td>0.195</td>
</tr>
<tr>
<td>220</td>
<td>26.239 ± 0.284</td>
<td>0.640</td>
<td>0.203 ± 0.007</td>
<td>0.227</td>
</tr>
<tr>
<td>300</td>
<td>25.162 ± 0.561</td>
<td>0.614</td>
<td>0.168 ± 0.005</td>
<td>0.188</td>
</tr>
</tbody>
</table>

The ratios \( \frac{N_t}{N_0} \) and \( \frac{L_t}{L_0} \) correspond to the fractional concentrations where \( N_0 \) and \( L_0 \) are the initial concentrations of naringin and limonin, respectively, in the juice; \( N_t \) and \( L_t \) are the concentrations of naringin and limonin in the juice, respectively, at any given time of treatment.
Table A8

Time-Dependent Adsorption of Recoverable Oil and Titratable Acidity of Grapefruit Juice onto the 50% Crosslinked PSDVB Adsorbent

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Recoverable Oil (mL remaining)</th>
<th>$O_t/O_0$</th>
<th>Titratable Acidity (mg remaining)</th>
<th>%Reduc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.370 ± 0.006</td>
<td>1</td>
<td>1.222 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.291 ± 0.003</td>
<td>0.786</td>
<td>1.204 ± 0.005</td>
<td>1.489</td>
</tr>
<tr>
<td>10</td>
<td>0.259 ± 0.015</td>
<td>0.700</td>
<td>1.204 ± 0.009</td>
<td>1.489</td>
</tr>
<tr>
<td>15</td>
<td>0.240 ± 0.012</td>
<td>0.649</td>
<td>1.197 ± 0.011</td>
<td>2.043</td>
</tr>
<tr>
<td>20</td>
<td>0.234 ± 0.014</td>
<td>0.632</td>
<td>1.201 ± 0.012</td>
<td>1.745</td>
</tr>
<tr>
<td>40</td>
<td>0.162 ± 0.018</td>
<td>0.438</td>
<td>1.199 ± 0.012</td>
<td>1.830</td>
</tr>
<tr>
<td>60</td>
<td>0.111 ± 0.029</td>
<td>0.300</td>
<td>1.199 ± 0.013</td>
<td>1.915</td>
</tr>
<tr>
<td>100</td>
<td>0.067 ± 0.012</td>
<td>0.181</td>
<td>1.201 ± 0.011</td>
<td>1.745</td>
</tr>
<tr>
<td>140</td>
<td>0.038 ± 0.009</td>
<td>0.103</td>
<td>1.199 ± 0.012</td>
<td>1.830</td>
</tr>
<tr>
<td>180</td>
<td>0.025 ± 0.005</td>
<td>0.0676</td>
<td>1.201 ± 0.015</td>
<td>1.745</td>
</tr>
<tr>
<td>220</td>
<td>0.020 ± 0.002</td>
<td>0.0541</td>
<td>1.199 ± 0.013</td>
<td>1.915</td>
</tr>
<tr>
<td>300</td>
<td>0.005 ± 0.004</td>
<td>0.0135</td>
<td>1.197 ± 0.011</td>
<td>2.043</td>
</tr>
</tbody>
</table>

The ratios $O_t/O_0$ correspond to the fractional concentrations where $O_0$ is the initial concentration of oil and $O_t$ is the concentration at any given time in the juice.

%Reduc. represents the % reduction, which is calculated as the ratio of the concentration of acid removed at any given time to the initial concentration of acid times 100.
Table A9

Effect of the Juice Temperature on the Adsorption of Naringin and Limonin by the 65\% Crosslinked PSDVB Adsorbent

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Naringin (mg remaining)</th>
<th>N_t/N_o</th>
<th>Limonin (mg remaining)</th>
<th>L_t/L_o</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19.255 ± 0.178</td>
<td>1</td>
<td>0.534 ± 0.042</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>11.704 ± 0.077</td>
<td>0.608</td>
<td>0.143 ± 0.005</td>
<td>0.268</td>
</tr>
<tr>
<td>40</td>
<td>8.455 ± 0.019</td>
<td>0.439</td>
<td>0.107 ± 0.001</td>
<td>0.200</td>
</tr>
<tr>
<td>100</td>
<td>5.670 ± 0.397</td>
<td>0.294</td>
<td>0.097 ± 0.001</td>
<td>0.182</td>
</tr>
<tr>
<td>140</td>
<td>4.233 ± 0.280</td>
<td>0.220</td>
<td>0.081 ± 0.026</td>
<td>0.152</td>
</tr>
</tbody>
</table>

24°C

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Naringin (mg remaining)</th>
<th>N_t/N_o</th>
<th>Limonin (mg remaining)</th>
<th>L_t/L_o</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19.255 ± 0.178</td>
<td>1</td>
<td>0.534 ± 0.042</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>13.158 ± 0.382</td>
<td>0.683</td>
<td>0.226 ± 0.012</td>
<td>0.423</td>
</tr>
<tr>
<td>40</td>
<td>11.498 ± 0.043</td>
<td>0.597</td>
<td>0.225 ± 0.034</td>
<td>0.421</td>
</tr>
<tr>
<td>100</td>
<td>7.257 ± 1.808</td>
<td>0.377</td>
<td>0.187 ± 0.052</td>
<td>0.350</td>
</tr>
<tr>
<td>140</td>
<td>8.825 ± 0.656</td>
<td>0.458</td>
<td>0.193 ± 0.058</td>
<td>0.361</td>
</tr>
</tbody>
</table>

35°C
### Table A10

**Effect of the Juice Temperature on the Adsorption of Naringin andLimoin by the 50% Crosslinked PSDVB Adsorbent**

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Naringin (mg remaining)</th>
<th>Limonin (mg remaining)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N&lt;sub&gt;t&lt;/sub&gt;/N&lt;sub&gt;o&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24°C</td>
</tr>
<tr>
<td>0</td>
<td>19.209 ± 0.021</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>15.252 ± 0.731</td>
<td>0.794</td>
</tr>
<tr>
<td>40</td>
<td>13.980 ± 1.011</td>
<td>0.728</td>
</tr>
<tr>
<td>100</td>
<td>10.921 ± 1.335</td>
<td>0.569</td>
</tr>
<tr>
<td>140</td>
<td>9.031 ± 1.043</td>
<td>0.470</td>
</tr>
<tr>
<td>35°C</td>
<td>19.209 ± 0.021</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>15.156 ± 0.499</td>
<td>0.789</td>
</tr>
<tr>
<td>40</td>
<td>13.751 ± 0.233</td>
<td>0.716</td>
</tr>
<tr>
<td>100</td>
<td>10.801 ± 1.751</td>
<td>0.562</td>
</tr>
<tr>
<td>140</td>
<td>10.651 ± 0.329</td>
<td>0.554</td>
</tr>
</tbody>
</table>
Table A11
Sensory Evaluation of Model Systems of Grapefruit Juice

Difference test:
Paired Comparison Test

Taster name:  

Date:  

Please taste these two samples and answer the following questions. Also, indicate the level of bitterness in the two samples on the appropriate rating scale. Thank you!

(1) Are you a grapefruit user?  Yes _____  No _____

(2) Is there a difference in bitterness between the two samples?

Yes ___________  No ___________

If your answer is 'Yes', which sample tastes the most bitter?

Indicate the code ___________

<table>
<thead>
<tr>
<th>Bitterness</th>
<th>555</th>
<th>260</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely bitter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very bitter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately bitter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slightly bitter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not bitter</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table A12

Sensory Evaluation of Single Strength Grapefruit Juice

Difference tests:
Paired Comparison & Preference Tests

Taster name: _________________________________

Date: _________________________________

Please taste these two grapefruit juice samples and answer the following questions. Also, indicate the degree of bitterness in the two samples on the appropriate rating scale. For better evaluation, use the cup provided to rinse your mouth after tasting each sample. Thank you!

(1) Are you a grapefruit user?  Yes ____  No ____

(2) Is there a difference in bitterness between the two samples?
   Yes _______________  No _______________

If your answer is 'Yes', which sample do you prefer?
Indicate the code ____________

| Bitterness Score |
|-----------------|-----|-----|
| 475             | 121 |

Extremely bitter .......  | 475 | 121 |
Very bitter ........... |     |     |
Moderately bitter ...... |     |     |
Slightly bitter ....... |     |     |
Not bitter (trace) ..... |     |     |
Comments............... |     |     |
REFERENCES


Johnson, R.L. 1981. The reactivation of 'exhausted' cellulose acetate gel beads used commercially for debittering orange juice. J. Food Agric. 32: 602-612.


Maier, V.P. and Beverly, G.D. 1968. Limonin monolactone, the non bitter precursor responsible for delayed bitterness in citrus juice. J. Food Sci. 33: 488-490.


USDA 1968. US standards for grades of grapefruit juice. USDA, Washington D.C


BIOGRAPHICAL SKETCH

The author, Michel Manlan, was born on June 27, 1956, in Ayame, Ivory Coast. He started primary school in 1962 in his hometown Ayame and graduated in 1969. Then he successfully completed the following two sections of high school studies: the first in 1973 with the diploma B.E.P.C. and the second section in 1976 with the diploma Baccalaureat D. Michel entered the National University of the Ivory Coast in 1976 to start an agricultural science program where he graduated in 1978. He was then moved to ENSA (National School of Agronomy) from where he graduated in June, 1980 with the degree of Agricultural Engineering.

The author came to the U.S.A. in August, 1980 and enrolled in a 6-month intensive English program at the University of Illinois. He joined the Department of Food Science and Human Nutrition at the University of Florida in January, 1981 to obtain his Master of Science in August, 1983. He came back in the summer of 1984 to pursue a Ph.D. program in the same field. Michel expects to complete the program during the summer of 1988.
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

R. F. Matthews, Chairman
Professor of Food Science and Human Nutrition

R. L. Rouseff, Cochairman
Professor of Food Science and Human Nutrition

R. C. Littel
Professor of Statistics

M. R. Marshall
Associate Professor of Food Science and Human Nutrition
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H. A. Moye  
Professor of Food Science and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

A. A. Teixeira  
Associate Professor of Agricultural Engineering

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1988  
Dean, College of Agriculture

Dean, Graduate School