Analysis of Sugars and Organic Acids in Pineapple, Papaya and Star Fruit by HPLC Using an Aminex HPx-87 H Column

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บทคัดย่อ
การวิเคราะห์น้ำตาลและกรดอินทรีย์ในสับปะรด มะละกอ และมะเฟืองโดย HPLC สามารถใช้ค้อนเม็ดเพียงน้อยเกินตัว Aminex HPx - 87 column และเพล็กซ์อนที่คือ 0.01 N H₂SO₄ โดยใช้ตั้งค่าด้วย 2 ชนิดคือ RI detector สำหรับน้ำตาลและ UV detector สำหรับกรดอินทรีย์ โดยใช้ความยาวคลื่นที่ 220 nm สำหรับกรดซัลกัต และ กรดอักชิน ความยาวคลื่นที่ 245 nm สำหรับกรดออกซัลิกและกรดแอลกอฮอล์ ชนิดของน้ำตาลที่พบในสับปะรด คือ รูปโคซต์ กลูโคซ และฟูโคซีที่มีปริมาณร้อยละ 3.61 2.23 และ 2.14 ตามลำดับ ในมะละกอมี กลูโคซ และฟูโคซีในปริมาณร้อยละ 4.07 และ 4.09 ตามลำดับ ในมะเฟืองมี กลูโคซและฟูโคซีในปริมาณร้อยละ 2.25 และ 2.46 ตามลำดับ ในสับปะรดมีกรดซัลกัต และกรดอักชินในปริมาณ 182 และ 121 มิลลิกรัมต่อ 100 กรัมตามลำดับ ในมะละกอ มี กรดซัลกัต กรดอักชินและกรดแอลก็ีกในปริมาณ 91 78 และ 3 มิลลิกรัมต่อ 100 กรัมตามลำดับ ในมะเฟืองมีกรดซัลกัต กรดอักชินและกรดออกซัลิกในปริมาณ 113 และ 141 และ 4 มิลลิกรัมต่อ 100 กรัมตามลำดับ

Abstract
Analysis of sugars and organic acids in pineapple, papaya, and star fruit was carried out by HPLC using an Aminex HPx – 87 H column. Mobile phase was 0.01 N H₂SO₄. Detectors were RI detector for sugars and UV detector at wavelength 220 nm for citric and malic acids, at 245 nm for ascorbic and oxalic acid. Sugars detected in pineapple were sucrose, glucose, and fructose at 3.61, 2.23, and 2.14% respectively. Sugars in papaya were glucose and fructose at 4.07 and 4.09 % and those in star fruit were glucose and fructose at 2.25 and 2.46 %. Organic acids detected in pineapple were citric and malic acid at 182 and 121 mg/100g respectively. Acids in papaya were citric, malic, and ascorbic acid at 182, 121 and 3.3 mg/100g respectively and those in star fruit were citric, malic, and ascorbic acid at 113, 141, and 4.0 mg/100g respectively. Three kinds of tropical fruit selected for this trial were pineapple, ripe papaya and ripe star fruit.

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Introduction

Sugars and acids are major constituents in all kinds of fruit. The balance of them contribute to the acceptability and flavor of the fruit. Some of tropical fruits are very rich in sugar and acid, these include pineapple, papaya, longan, lychee, mango, mangosteen, star fruit, guava, etc. The composition of sugar and acid are tremendously varied due to the wide variety of species and condition of fruit growing. The sugar and acid are subjected to change during storage, transportation and ripening process. Determination of sugars and acids composition is a great advantage of controlling the fruit quality.

Many sugar – acid analysis methods have been developed for fruits. These started as simple as titration, thin layer chromatography, gas liquid chromatography, high pressure liquid chromatography and enzymatic method. The comparison of three sugar – acid analysis methods was reported by Reyes et al., (1982). The study revealed that there was a variability in quantity and composition of sugars and acids due to the methods used. Gas liquid chromatography gave a high sensitivity to sugar detection but a time consuming method and the method could not applied to certain organic acids. Enzymatic method gave relatively high sensitivity in detection but rather costly. High pressure liquid chromatography is somehow convenient, reproducible, and no derivatization. For good separation and quantification, both column and detector must be correctly chosen.

The sugar – acid analysis by HPLC has been carried out for fruits, vegetables and other foods. However, there are still scanty in formation on tropical fruits (Medlicott and Thompson, 1985; Wilsom, et al., 1982). Different types of column have been used for sugars and organic acids analysis. Among them, an Aminex HPX-87H (Bio Rad – Laboratories) has been used by authors (Picha, 1985; Ashoor and Knox, 1985; and Blake and Clarke, 1987). Some sugars and acids could be separated at the same time by one column.

The purpose of this study is to quantity and identity the sugars and acids content in some tropical fruits avialable in supermarket by high pressure liquid chromatography bu using with one column.

Materials and methods

1. Preparation of fruit extract

Tropical fruits; pineapple, papaya and star fruit, were purchased from local supermarket. The fruits were in ripen stage and had been kept in a cold room before analysis. A 50 grams of fruit
pulp was homogenized with 50 ml deionized water for 3-5 minutes, then samples were centrifuged at 7000 rpm for 20 minutes at room temperature. The supernatant was stored in refrigerator for further use.

2. Sample preparation for organic acids determination

A 10 ml of fruit extract was passed through a glass column containing 8 grams of anion exchange resin (Dowex – WGR-2, Cl form) (Shaw and Wilson, 1983). The column was washed with 20 ml deionized water to elute out sugars and other substances. Then, 2 x 10 ml 4 N formic acid was passed through the column to remove organic acids and followed by 2 x 10 ml deionized water. The collected solution was concentrated to dryness under vacuum at 50°C by using rotary evaporator. The residue was redissolved in 10 ml deionized water and 0.1 mM NaI0₄ was added at ratio 1 500, this was to prevent a co-elution of malic acid and ascobic acid. Prior to HPLC analysis, the sample was filtered through 0.45 μm Millipore filter (water associates).

3. Sample preparation for ascorbic acid and oxalic acid determination

The fruit extract was passed through Sep-pak C18 (water asociaties) with out passing through anion resin exchange column (Wilson, Shaw and Campbell, 1982.). Before HPLC analysis, the sample was filtered through 0.45 μm Millipore filter.

4. Sample preparation for sugar determination

The fruit extract was passed through Sep-pak C18 and then filtered through 0.45 μm Millipore filter prior to HPLC analysis.

5. HPLC system

The Bio-Rad HPLC pump Model 1330 was used. A 20 μl Rheodyne injector was connected to the pump. A guard column 125-0131 (Bio Rad laboratories) was placed between the injector and the column. A 300 mm x 7.8 mm i.d Aminex HPX-87H column (Bio Rad laboratories) was used for both sugar and acid analysis. Two types of detector were connected to the system at each analysis, a differential refractometer detector (Water R 401) for sugar analysis, and a U.V detector (Bio Rad Model 1305) at 220nm for citric and malic acid, and at 245 nm for ascorbic and oxalic acid. Mobile phase
was 0.01 N \( \text{H}_2\text{SO}_4 \), flow rates were 0.5 ml/min for sugars and 0.6 ml/min for acids. Quantification of sugars and acids was achieved by data processor (Shimadzu C-R3A chromatopac).

6. Standards

Individual sugars and acids were identified by comparison with reference standards. Calibration curves were made from purified sugars and acids.

Results and discussion

The major sugars found in three tropical fruits are shown in Table 1. Pineapple contained a larger amount of sucrose than glucose and fructose. Sucrose was not detected in papaya and star fruit. Glucose and fructose were found in both papaya and star fruit.

Table 1  Sugars content (g/100g fresh fruit) in pineapple, papaya and star fruit.

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Total sugar</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pineapple</td>
<td>7.98</td>
<td>3.61 ± 0.016</td>
<td>2.23 ± 0.009</td>
<td>2.14 ± 0.004</td>
</tr>
<tr>
<td>Papaya</td>
<td>8.16</td>
<td>-</td>
<td>4.07 ± 0.01</td>
<td>4.09 ± 0.54</td>
</tr>
<tr>
<td>Star fruit</td>
<td>4.71</td>
<td>-</td>
<td>2.25 ± 0.009</td>
<td>2.46 ± 0.009</td>
</tr>
</tbody>
</table>

The sugars content in pineapple were 3.61% for sucrose, 2.23% for glucose, and 2.14% for fructose. Dull, (1971) reported that the amount of sucrose, glucose, and fructose were in the range of 5.9-12.0%, 1.0-3.2%, and 0.6-2.3% respectively. It was obvious that sucrose content in the sample was some how lower than the reported value but not with fructose and glucose.

The total sugar content in papaya was 8.16%, which comprised of 4.07% of glucose and 4.06% of fructose. It was previously reported as 9% (de Arriola et al., 1975) and 5.21% - 12.0% (Jagtiani et al., 1988). Unfortunately, no individual sugar was reported. However, the reducing sugar in papaya was ranged from 0.08 to 6.0% (Jagtiani et al., 1988). No detection of sucrose in ripe papaya might due to the invertase. Inactivating of invertase by heating, the proportion of sugars in papaya were reported as 48.3% sucrose, 29.8% glucose, and 21.9% fructose (Chan and Kwok, 1975).

The sugars content in star fruit were 2.25% of glucose and 2.46% of fructose. In general, sucrose is found in almost all kinds of fruit. The absence of sucrose need further investigations. The
composition of sugars in fruit is inevitably changing during the storage period especially at the ripening stage. Most of the changes resulted from the action of all kinds of enzymes. In order to get comparable results, inactivation of certain enzymes like invertase might be considerably necessary. Besides that the stage of fruit maturity, variety, growing condition must be indicated.

The analysis of sugars by high pressure liquid chromatography with the aid of Aminex HPX-87H column and RI detector gave a beautifully peak separation. The chromatograms of sugars in pineapple, papaya and star fruit are presented in Fig 1, 2, and 3, respectively.

![Fig 1 Sugars in pineapple](image1)
![Fig 2 Sugars in papaya](image2)
![Fig 3 Sugars in star fruit](image3)

1 = sucrose  2 = glucose  3 = fructose

Organic acid in fruit is another major constituent that contributes to the fruit quality. Analysis of organic acid is somehow more difficult than that of sugar, since many interferences can be detected by U.V detector. The sample clarification through ion exchange resin is probably a must for acid analysis. In pineapple juice the detectable amount of citric acid and malic acid were 182.7 and 121.76 mg/g of fresh fruit respectively. The same acids were previously reported as 0.32 – 1.22% and 0.1 – 0.47% (Dull, 1971). The amount of citric acid detected was a bit lower but the amount of malic acid was appreciably comparable. The small amount of oxalic acid, 0.005%, was also found in
pineapple as reported by Dull (1971). The citric acid and malic acid in papaya were 91.3 and 78.64 mg/100 g fresh fruit respectively. Chan et al., (1971) reported that organic acids in papaya were citric acid, malic acid, alfa- ketoglutaric acid and small amount of ascorbic acid. No quantity of each acid was reported. The citric and malic acid in star fruit were 113 mg/g and 141 mg/g respectively.

Citric acid was the major acid found in pineapple and papaya. Malic acid was the major one found in star fruit. Oxalic acid was not detected in papaya only in star fruit. The major organic acids found in pineapple, papaya and star fruit are presented in Table 2.

Table 2 Organic acids content (mg/100g fresh fruit) in pineapple, papaya and star fruit

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Citric acid</th>
<th>Malic acid</th>
<th>Oxalic acid</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pineapple</td>
<td>182.7 ± 0.37</td>
<td>121.76 ± 3.96</td>
<td>Trace</td>
<td>-</td>
</tr>
<tr>
<td>Papaya</td>
<td>91.3 ± 16.96</td>
<td>78.64 ± 9.43</td>
<td>-</td>
<td>3.30 ± 0.045</td>
</tr>
<tr>
<td>Star fruit</td>
<td>113.85 ± 13.07</td>
<td>141.04 ± 9.14</td>
<td>4.25 ± 0.95</td>
<td>0.249 ± 0.009</td>
</tr>
</tbody>
</table>

The chromatograms of citric, malic acid in pineapple, papaya and star fruit are shown in Fig 4, 5 and 6. Organic acid analysis might be interfered by some other water extractable substances, such as nucleotides, small peptides. If a proper clearification is not applied to the sample, several peaks might appear and can fool the interpretation.

Fig 4 Organic acids in pineapple  Fig 5 Organic acids in papaya  Fig 6 Organic acids in star fruit
1 = periodate  2 = citric acid  3 = malic acid  4 = formic acid
Detection of oxalic and ascorbic acid was carried out at 245 nm. Star fruit was rather a special tropical fruit which contains a large amount of oxalic acid. The detectable amount at 245 nm was 4.25 mg/100 g. Oxalic acid was not successfully recovered from the anion resin, therefore the detection of oxalic acid at 220 nm was not carried out.

The amount of ascorbic acid detected in papaya and star fruit was relatively low. De Arriola et al., (1975) reported that the ascorbic acid content in papaya increased gradually during ripening process, the maximum value at the ripen stage was 55 mg/100 g. The ascorbic acid in pineapple was not detectable, this might due to the fruit was over ripe. The chromatograms of oxalic acid and ascorbic acid are presented in Fig 7 and 8.

![Fig 7](image7.png)  Organic acids in papaya
1 = periodate  2=citric acid  3 = malicacid
4 = formic acid  5 = ascorbic acid

![Fig 8](image8.png)  Organic acids in star fruit
1 = oxalic acid  2 = ascorbic acid

**Conclusion**

The analysis of sugars and acids in tropical fruits by HPLC was satisfactorily achieved with an Aminex HPX-87H column. The results showed that sucrose, glucose and fructose were major sugars found in pineapple. Glucose and fructose were found in papaya and star fruit. The quantity of sugars
in the sample was comparable to the previous report. Malic acid and citric acid were found in all samples. Oxalic acid was quantitatively detected only in star fruit. Ascorbic acid presented in a detectable amount in papaya and could not detected in pineapple.

References