Optimization of an HPLC method for the simultaneous quantification of the major organic acids in different fruit extracts

Ph Baleshwor Sharma and Huidrom Sunitibala Devi

Abstract
An RP-HPLC method for the simultaneous quantification of organic acids was developed to profile different fruit extracts. An optimized extraction procedure to recover the polar fraction is presented. Aqueous phase acidified to pH 2.0 enabled optimum resolution of a mixture composed of twelve standard acids. Flow rate of 0.6 ml/min rendering column pressure of 965 psi in our specified column has served a vital parameter for precision and reproducibility. A comparable peak alignment of the mixture was set after evaluating LOD and LOQ of each standard. External calibration method was employed; retention time and spiking technique confirmed analyte identity. Repeatability expressed as percentage standard deviation was less than 1.3. Three to six organic acids could be simultaneously quantified for each fruit. The principal organic acid of *Garcinia xanthochymus*, *Garcinia pedunculata*, *Rhus semialata*, *Docynia indica*, and *Averrhoa carambola* was found as succinic, (-)-Hydroxycitric acid, malic, malonic and oxalic acid respectively.

Keywords: HPLC, standard mixture, organic acid profile, column-pressure

Introduction
Organic acid concentration and composition play important role in the flavor, organoleptic properties and consumer acceptance of a fruit. The popularity and acceptability of fruits among consumers is not only due to their high nutritive value and characteristic taste, but also due to their known health promoting properties [1]. Organic acids determine the characteristic fruit flavour and play major roles in quality assessment such as stability, colour and flavour [2, 3]. The identification and quantitative analysis of major organic acids in fruits is considered important for quality evaluation and food processing [4] and is an important indicator of the nutritional and flavour quality of fruits [5]. These organic acids content and concentration served a useful index of authenticity of fruit products, since they have a lower susceptibility to changes during processing and storage that other component of the fruit [6]. Each fruit has a unique pattern of organic acid content that in combination with sugar attributed organoleptic properties of a fruit [7]. Organic acid profile served as a chemical fingerprint for each type of fruit [8, 9]. Such profile is also an important marker of fruit quality to be considered in the development of value added products [10]. Some of the major organic acids in fruits such as citric, malic and tartaric acids are also used extensively as food additives in the manufacture of beverages, fruit and vegetable drinks, confectionery, savoury foods, and other food products [10]. Nowadays, fruits rich in organic acids are increasingly utilized as acidulant for beverages, food products and other industrial applications. Analytical methods that are routinely used for organic acids profiling are based on liquid chromatography (reverse phase or ion exchange) coupled with UV detection. Reversed-phase high performance liquid chromatography (RP-HPLC) has become more popular for analyzing certain mixtures of organic acids because of the simplicity, speed and stability of the method [11].

The present study is aimed to establish a more generalized HPLC method for simultaneous profiling and quantification of major organic acids present in different fruit species. A cost effective extraction protocol for organic acids from the different fruit sample is also designed. Recently, wild fruits have received increased interest to the consumers as well as researchers in evaluating nutritional and medicinal aspects. Five such wild fruits grown in Manipur that are widely used as a food source as well as ethnomedicine viz., *Garcinia xanthochymus*, *Garcinia pedunculata*, *Docynia indica*, *Rhus semialata* and *Averrhoa carambola* were selected for profiling their organic acid fingerprint and quantification of their major acids.
Materials and Methods

Chemicals and reagents
Organic acid kit (cat. no. 47264) and Isocratic evaluation mix (cat. no. 48270-U) were purchased from Supelco analytical, Sigma-Aldrich, USA. Conc. Sulphuric acid (HPLC grade) was purchased from Merck, Germany. Activated charcoal, phenolphthalein and sodium hydroxide were procured from Himedia, Mumbai, India. HPLC grade water was purchased from Ramkem, New, India.

Collection and processing of fruit samples
Garcinia xanthochymus Hook. f. (ACNO 78852 BSI/ERC/2012/68), Garcinia pedunculata Roxb. (Accession number: ACNO 80728 BSI/ERC/444), Docynia indica (Wall.) Decne. (78853 BSI/ERC/2012/68), Rhus semialata Murray (ACNO 80728 BSI/ERC/444), Averrhoa carambola L. at their mature ripe stage were collected from Manipur (Figure 1). Edible portion of each of the fruit was chopped and dried in an air circulating oven until a constant weight was obtained. Dried sample was ground into powdery form by using a kitchen blender and defatted by macerating in petroleum ether for 72 h.

Extraction of organic acids
The extraction method of organic acids was adapted from Jayaprakasha et al. [12] with further modification for exhaustive extraction from smaller quantity of the sample. 0.5 g of pulverized defatted dried sample was first dissolved in 50 ml of Milli-Q water and autoclaved at 15 psi, 121 °C for 20 min. Afterward, 1 g of activated charcoal was added to the mixture and microwave assisted extraction (MAE) method was applied at 140 °C, 70 % intermittently for a total time of 10 min (Samsung-Bio) to adsorb any non-polar components. 20 ml of ethanol was then added and mixed thoroughly to dissolve any complex carbohydrate components (pectins). The mixture was filtered through a cotton plug and the residue was washed twice with less volume of ethanol. The combined filtrate was centrifuged at 3000 rpm for 10 min and the supernatant thus collected was concentrated by rotary vacuum evaporator (Buchi, Switzerland) at 60 °C to about 15 ml or less. The final volume of the extract was noted for percentage recovery. The extracts (total organic acids) were filtered through 0.22 µm syringe filter (Merck-Millipore) and kept at 4 °C for HPLC analyses.

HPLC and operating conditions
Chromatographic separations were carried out on the Waters HPLC system, equipped with 1525 separation module, Rheodyne 7725i injector (loop capacity 20 µl) and 2996 photodiode array detector controlled by Empower software. The column was fitted with Discovery® C18 columns, 25 cm × 4.6 mm, 5 µm (Supelco analytical, Sigma-Aldrich, USA). Column temperature was maintained at 25 °C (temperature control module II, Waters). The experimental condition was fixed at 25±1 °C. 271.7µl of Conc. H₂SO₄ (HPLC grade) was gently dissolved in 1 L packaged HPLC grade water and kept for 1h for dispersion. Afterward the pH was checked and adjusted to 2.00±0.01 with dil. H₂SO₄. The flow rate was set at 0.6 ml/min and detected at 210 nm by complete loop filling method for better precision.

Determination of Total Titratable acidity
Total Titratable acidity (TTA) of the polar fraction was determined by the potentiometric titration method (AOAC 2000, Method 942.15) [13] and expressed as anhydrous citric acid on weight basis.

\[ \text{TTA (g/100g) defatted dry wt} = \frac{\text{Net ml titrant} \times \text{Normality titrant}}{\text{Sample (g)} \times 6.4} \]

HPLC System calibration and validation by using standard test mixtures
An isocratic evaluation mixture procured from Supelco, Sigma Aldrich, USA was used for the system calibration and validation for the analysis of organic acids. The test mixture components, systems, parameters and conditions are given in Figure 2. The test mixture was analyzed in our specified column and HPLC system. Left panel in the Figure 2 indicates Supelco reference chromatogram and right panel of the Figure 2 showed experimental chromatogram.
HPLC method validation and quantification of organic acids

A total of 12 standard organic acids viz., oxalic acid, D-tartaric acid, D-malic acid, malonic acid, maleic acid, citric acid, succinic acid, fumaric acid, L-ascorbic acid, L(+)-lactic acid, acetic acid, shikimic acid, quinic acid, and (-)-hydroxycitric acid (HCA) were taken for simultaneous profiling and quantification through HPLC method (Table 1, Figure 3A). External calibration method for each standard was prepared by choosing five concentration points. Identification of each organic acid peak from the mixture was analyzed by determining the relative retention time of each acid and further confirmation by spiking technique. Limit of quantification was intrapolated from the standard calibration using the formula of ICH (2005) [14] and verifying the predicted LOD and LOQ by making 3 replicate injections. Intra-day repeatability of the method was checked by calculating the percentage relative standard deviation (% RSD) of the retention time of five replications. Inter-day repeatability was checked from three replications only. Organic acid content was intrapolated from the standard curve by correcting for the dilution factors made during sample preparation.

\[
\text{LOD (mg/L)} = \frac{3.3 \times \text{Residual Std. Deviation of y-intercept of Regression line}}{\text{Slope}}
\]

\[
\text{LOQ (mg/L)} = \frac{10 \times \text{Residual Std. Deviation of y-intercept of Regression line}}{\text{Slope}}
\]

Table 1: Linear calibration equation, range, LOD, LOQ and precision of retention time (Rt) of standard organic acids at the specified parameters: Discovery® C18 (25 cm x 4.6 mm, 5µm); mobile phase: aqueous, pH 2.0±0.01 adjusted with H₂SO₄; Operating temperature: 25 °C; Column pressure: 965 psi.

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Standard</th>
<th>Linear calibration, n = 5</th>
<th>Range (mgl⁻¹)</th>
<th>R²</th>
<th>LOD (mgl⁻¹)</th>
<th>LOQ (mgl⁻¹)</th>
<th>Rt (min)</th>
<th>% RSD of Rt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxalic acid</td>
<td>Y = 25633X + 4967.6</td>
<td>0.1-1.00</td>
<td>0.9998</td>
<td>1.349</td>
<td>4.088</td>
<td>5.691±0.013</td>
<td>0.243</td>
</tr>
<tr>
<td>2</td>
<td>D-tartaric acid</td>
<td>Y = 3473.6X + 6424.6</td>
<td>0.5-1.00</td>
<td>0.9997</td>
<td>1.437</td>
<td>4.354</td>
<td>6.111±0.014</td>
<td>0.229</td>
</tr>
<tr>
<td>3</td>
<td>D-Malic acid</td>
<td>Y = 1782.7X + 1381</td>
<td>1-10.00</td>
<td>0.9999</td>
<td>3.486</td>
<td>10.564</td>
<td>7.495±0.061</td>
<td>0.821</td>
</tr>
<tr>
<td>4</td>
<td>Malonic acid</td>
<td>Y = 1914.5X + 191.36</td>
<td>10-1000</td>
<td>0.9999</td>
<td>3.165</td>
<td>5.052</td>
<td>7.712±0.069</td>
<td>0.870</td>
</tr>
<tr>
<td>5</td>
<td>Shikimic acid</td>
<td>Y = 9409.5X + 15637</td>
<td>0.05-10</td>
<td>0.9975</td>
<td>0.431</td>
<td>1.306</td>
<td>7.928±0.056</td>
<td>0.712</td>
</tr>
<tr>
<td>6</td>
<td>L-Ascorbic acid</td>
<td>Y = 19445X + 5900</td>
<td>1-100</td>
<td>0.9999</td>
<td>0.345</td>
<td>1.055</td>
<td>8.303±0.082</td>
<td>0.987</td>
</tr>
<tr>
<td>7</td>
<td>L(+)-Lactic acid</td>
<td>Y = 1509.3X + 4341.6</td>
<td>5-1000</td>
<td>0.9999</td>
<td>4.306</td>
<td>13.05</td>
<td>8.566±0.0357</td>
<td>0.417</td>
</tr>
<tr>
<td>8</td>
<td>Acetic acid</td>
<td>Y = 113757X + 16716</td>
<td>4.5-900</td>
<td>0.9996</td>
<td>16.326</td>
<td>49.474</td>
<td>8.885±0.042</td>
<td>0.475</td>
</tr>
<tr>
<td>9</td>
<td>Maleic acid</td>
<td>Y = 224199X + 43701</td>
<td>0.1-100</td>
<td>0.9999</td>
<td>0.4905</td>
<td>1.486</td>
<td>12.357±0.112</td>
<td>0.91</td>
</tr>
<tr>
<td>10</td>
<td>Citric acid</td>
<td>Y = 2393.9X + 14078</td>
<td>10-1000</td>
<td>0.9997</td>
<td>15.783</td>
<td>47.828</td>
<td>14.53±0.078</td>
<td>0.543</td>
</tr>
<tr>
<td>11</td>
<td>Succinic acid</td>
<td>Y = 11094X + 2241.6</td>
<td>10-2000</td>
<td>0.9996</td>
<td>38.51</td>
<td>116.7</td>
<td>14.871±0.117</td>
<td>0.788</td>
</tr>
<tr>
<td>12</td>
<td>Fumaric acid</td>
<td>Y = 242579X + 18447</td>
<td>0.05-10</td>
<td>0.9998</td>
<td>0.2404</td>
<td>0.728</td>
<td>15.463±0.150</td>
<td>0.97</td>
</tr>
<tr>
<td>13</td>
<td>Quinic acid</td>
<td>Y = 1026.3X + 1899.2</td>
<td>1-1000</td>
<td>0.9998</td>
<td>13.202</td>
<td>40.000</td>
<td>6.201±0.037</td>
<td>0.60</td>
</tr>
<tr>
<td>14</td>
<td>l(-)-HCA</td>
<td>Y = 2050X + 165.46</td>
<td>5-100</td>
<td>0.9998</td>
<td>1.73</td>
<td>5.24</td>
<td>7.51±0.01</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Rt: Retention time given in mean ± standard deviation; % RSD: percentage relative standard deviation of Rt. NB: SN 13(aQuinic acid) & 14(b-) -HCA were not included in the standard mixture due to coelution problem.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three independent determinations. Calculation of linear regression, Internal response factor (IRF), separation factor, and other calculations required in quantification were performed in MS excel 2007.

Results and Discussions

Extraction of organic acids

Defatting treatment prior to the extraction of organic acids proved to be useful, especially for those fruits with high lipid, grease and wax content viz., R. semialata and G. xanthochymus. It also removes coloured pigments and other undesirable lipophilic substances, thereby increasing surface contact with the aqueous system for exhaustive recovery of polar fraction. In hot extraction, the extractability of organic acids was reported maximum in water from amongst acetone and methanol Jayaprakasha et al. [13] due to high polarity of the water. Complex hetero-polysaccharides particularly pectin extracted during MAE was allowed to precipitate by ethanol. Their removal minimized thickening of the extract as well as improved baseline and resolution in HPLC analyses. Polar fraction obtained from each 0.5 g defatted dried fruit sample of the G. xanthochymus, G. pedunculata, R. semialata, D. indica and A. carambola was concentrated to 11.0, 16.0, 15.0, 10.5 and 8.5 ml respectively. Extraction procedure for the organic acid from the defatted dried sample was found reproducible and cost effective with minimal matrix interferences during HPLC analyses.

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### Table 2: Organic acid composition of the five wild fruits as determined by HPLC and titration method.

<table>
<thead>
<tr>
<th>Fruit sample</th>
<th>Oxalate (mg/L)</th>
<th>Tartarate (mg/L)</th>
<th>Malate (mg/L)</th>
<th>(-)-HCA (mg/L)</th>
<th>Maleate (mg/L)</th>
<th>Shikimate (mg/L)</th>
<th>Ascorbate (mg/L)</th>
<th>Lactate (mg/L)</th>
<th>Acetate (mg/L)</th>
<th>Maleate (mg/L)</th>
<th>Succinate (mg/L)</th>
<th>Total TTA (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. xanthochymus</td>
<td>0.08±0.01</td>
<td>0.03±0.00</td>
<td>0.05±0.01</td>
<td>0.23±0.03</td>
<td>-</td>
<td>-</td>
<td>trace</td>
<td>-</td>
<td>-</td>
<td>trace</td>
<td>-</td>
<td>8.24±0.81</td>
</tr>
<tr>
<td>G. pedunculata</td>
<td>0.13±0.03</td>
<td>0.05±0.00</td>
<td>14.86±0.75</td>
<td>1.93±0.52</td>
<td>-</td>
<td>trace</td>
<td>-</td>
<td>1.87±0.42</td>
<td>-</td>
<td>0.46±0.03</td>
<td>0.46±0.03</td>
<td>17.37±1.25</td>
</tr>
<tr>
<td>R. semialata</td>
<td>0.17±0.02</td>
<td>0.05±0.01</td>
<td>4.33±0.12</td>
<td>1.93±0.52</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.06±0.27</td>
<td>8.65±0.94</td>
<td>8.7±1.2</td>
<td>2.06±0.27</td>
<td>19.84±0.94</td>
</tr>
<tr>
<td>D. indica</td>
<td>0.15±0.04</td>
<td>0.58±0.06</td>
<td>-</td>
<td>4.23±0.09</td>
<td>0.01±0.00</td>
<td>trace</td>
<td>0.29±0.04</td>
<td>Trace</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.26±0.23</td>
</tr>
<tr>
<td>A. carambola</td>
<td>3.14±0.09</td>
<td>0.51±0.04</td>
<td>-</td>
<td>1.06±0.08</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.67±0.21</td>
</tr>
</tbody>
</table>

Values are given in mean ± standard deviation of five to six independent determinations. ddw: defatted dry weight. TTA: Total Titratable acidity.

### HPLC method optimization and validation for simultaneous profiling

The standard test mixture was separated in an approximately same pattern as given in Supelco analytical standard chromatogram (cf. Lt. and Rt. Panel of Figure 2). Minor differences observed between two comparative chromatograms were due to different HPLC systems, column length and operating temperature. Minor fluctuation in the temperature affects the pressure in the column, and consequently the flow rate. Column pressure, which precisely observed in the experimental conditions (25°C), was 965 psi. Minor changes in the column pressure were monitored and adjusted accordingly in order to keep constant at 965 psi. Reversed phase-HPLC coupled with UV-detection is one of the common techniques for organic acid analyses. The separation of organic acids with liquid chromatography and their quantitative determinations is extremely difficult because there is no difference between their structural and spectral characteristics besides; pKa values of most of the organic acids are rather similar thus limiting the usage of pH for chromatographic separation. Acidic eluent (pH 1.5-2.5) is generally required to ensure that organic acids remain protonated, allowing the best interaction between organic acids and stationary phase for an optimum resolution. Generally, pH range for most of the C18 columns is 3-8 limiting separation at pH lower than 3.0. In our study, Supelco C18 whose allowable pH range is 2-8 was used. Resolution performance of twelve standard organic acids’ mixture was checked in mobile phases of pH ranging 2-3. Several species of organic acids tended to co-elute with mobile phase having pH more than 3.0. Co-elution of some organic acids, particularly oxalic and tartaric acid was observed in several points, pH 2.3, 2.5, 2.7 and 3.0. Finally, mobile phase having pH 2.0 provided optimum resolution of a standard mixture comprising twelve organic acids. Low acidic mobile phase, pH ≤ 2.00 may ensure absolute protonation and increased resolution, however; it was limited by column efficiency. LOD and LOQ for each organic acid were quite different, thereby hindering the simultaneous profiling process. However, in order to achieve a comparable peak height alignment; mixture constituting different concentration of each standard was used (Figure 3A). Calibration range, LOD, LOQ, retention time (tR) and % RSD of tR were presented in Table 1. The linearity of response of each compound in their quantification limits was good with correlation coefficient, R2 > 0.999. % RSD of intra-day retention time (tR) was less than 1.0 while that of inter-day was less than 1.3.

![Fig. 3](image-url) Chromatograms of (A) standard mixture of 12 organic acids with peak assignment: (1) Oxalic acid 27.9 mgL⁻¹, (2) D-tartarate 185 mgL⁻¹, (3) D-Malic acid 416.75 mgL⁻¹, (4) Malonic acids 416.75 mgL⁻¹, (5) Shikimate 8.33 mgL⁻¹, (6) L-Ascorbic acid 41.67 mgL⁻¹, (7) L(+)-Lactic acid 595.2 mgL⁻¹, (8) Acetic acid 3030 mgL⁻¹, (9) Maleic acid 8.32 mgL⁻¹, (10) Citric acid 520.82 mgL⁻¹, (11) Succinic acid 1389.57 mgL⁻¹, and (12) Fumaric acid 5.2 mgL⁻¹. (A) HCA (B) Garcinia xanthochymus, (C) Garcinia pedunculata, (D) Docynia indica, (E) Rhus semialata and (F) Averrhoa carambola.
Simultaneous profiling and quantification of organic acids for each fruit extract

The order of acidity (citric acid equivalent) based on titration method amongst five fruits was found as G. pedunculata (19.84 g/100 g ddw) > G. xanthochymus (9.29 g/100 g ddw) > R. semialata (19.84 g/100 g ddw) > D. indica (5.26 g/100 g ddw) > A. carambola (4.57 g/100 g ddw) (Table 2). TTA determined by the titration method was slightly higher (ca. 7%) than HPLC method in the case of G. xanthochymus, D. indica and R. semialata. In the case of G. pedunculata, ca. 12% was found higher in TTA than HPLC method. Results show an acceptable figure because TTA determined by titration method is based on the available free protons irrespective of their parent molecules. However, in A. carambola, TTA (4.67±0.21) by HPLC method was slightly higher than the titration method (4.57±0.46). An Organic acid mixture comprising 12 standards viz., oxalic acid, D-tartaric acid, D-malic acid, malonic acid, maleic acid, citric acid, succinic acid, fumaric acid, L-ascorbic acid, L (+)-lactic acid, acetic acid, and shikimic acid were simultaneous profiled within 16 min run time (Figure 3A). Quinic acid tends to co-elute with oxalic acid while (-) - HCA with malic acid and hence found difficult to profile together with 12 others at the defined parameters. Hence, these two organic acids were separately quantified. Quinic acid (Peak no. 13) and (-) -HCA (peak no. 14) were solely detected in D. indica and G. pedunculata respectively (Fig. 3 E, C respectively). Ripe fruit of G. xanthochymus contained oxalic (80 mg/100 g ddw), tartaric (30 mg/100 g ddw), malonic (50 mg/100 g ddw), malonic (240 mg/100 g ddw) and succinic acid (8.24 g/100 g ddw) of which succinic acid (88.69 % of the TTA) was found as the principal acid (Table 2). Lactic and acetic acid were detected in trace amounts (Figure 3B). G. pedunculata contained oxalic (130 mg/100 g ddw), tartaric (50 mg/100 g ddw), (-)-HCA (14.86 g/100 g ddw), ascorbic (1.87 g/100 g ddw) and succinic acid (460 mg/100 g ddw) of which (-) -HCA was found as the principal acid contributing ca. 74% of the TTA of the fruit (Table 2). Jayaprakasha et al. [12] reported ca. 70% of the TTA, contributed by (-) -HCA, which is close to our determined value. This fruit is well known for anti-obesity properties furnished by (-) -HCA. Additionally, they quantified oxalic, citric acids and hydroxycitric acid lactone in the fruit rind.

The fruit of R. semialata contained oxalic (170 mg/100 g ddw), tartaric (50 mg/100 g ddw), malonic (4.43 g/100 g ddw), malonic (1.93 g/100 g ddw) and succinic acid (2.06 g/100 g ddw) of which malonic acid (50.91% of the TTA) was found as major component; acetic acid was found in trace amounts. Kossah et al. [16] have reported malic acid as the principal acid from amongst four organic acids (malic, citric, tartaric and fumaric acid) determined respectively in Rhus coriaria L. and Rhus typhina L. Mavllyanov et al. [17] detected malic, citric acid, succinic acid, fumaric acid, maleic and tartaric acid in R. glabra; malic, citric, fumaric and tartaric acid in R. coriaria; malic, succinic, fumaric and ascorbic acid in R. typhina. They reported the presence of a considerable amount of organic acids (5–7 %) of which malic acid as a major organic acid constituting up to 25% of the total amount of the acids in those three species. Abundance of malic acid in R. coriaria and in fact for other Rhus species was further confirmed by the presence of malic acid isomers and various malate derivatives as determined by HPLC–DAD–ESI-MS/MS in the investigation of Abu-Reidah et al. [18] D. indica contained oxalic (150 mg/100 g ddw), quinic (trace), tartaric (580 mg/100 g ddw), malonic (4.23 g/100 g ddw), shikimic (10 mg/100 g ddw) and lactic acid 290 mg/100 g ddw); of which malonic acid (75% of the TTA) was found as a major component. Acetic and maleic acid was found in trace amounts. Oxalic (3.1 g/100 g ddw), malonic (1.06 g/100 g ddw) and tartaric acid (510 mg/100 g ddw) were quantified in the mature ripe fruit of A. carambola. Oxalic acid (67.83% of TTA) was found as the principal organic acid of A. carambola (Table 2). Vines and Grierson [19] reported oxalic acid and tartaric acid as the main organic acids in green-mature stage and subsequently as the fruit reached yellow-mature stage (edible stage), tartaric acid decreases, while oxalic acid and α-ketoglutaric acid increased. They reported 74 % oxalic acid and 17 % α-ketoglutaric content of the total acids, whereas citric, malic, and succinic acid were not detected in the mature ripe stage. Similarly, in our analysis, citric, malic and succinic acids were not detected in the mature ripe fruit and α-ketoglutaric acid and certain other acids could be identified due lack of standard. Oxalic acid as the principal acid is supported by different reports [19, 20, 1]. Unlike other reports, malonic acid was found second most abundant acid in the mature ripe fruit stage and our result is partially supported by the malonic acid content in the A. bilimbi [21]. Lastly, it would be worthwhile to mention that several studies on the variation of organic acids and sugars in fruits have been reported with regard to cultivars and maturation stages [12, 23, 24, 25]. All five fruits contained oxalic and tartaric acid as common acids, though in different quantity. Basic organic acid profile of such underutilised wild fruits shall be helpful to the consumers as well as food enterprises.

Conclusions

Extraction of total organic acids from defatted sample and presented RP-HPLC method for the simultaneous determination of organic acids present in a fruit sample is simple and cost effective. Twelve organic acids were simultaneously profiled within 16 min. Constant column pressure was maintained to render a more precise and reproducible chromatogram. % RSD of retention time of all the twelve organic acids was below 1.0 indicating the precision of the method. Reproducibility, accuracy and sensitivity of the method were satisfactory. Total titratable acidity determined by the titration method was found higher than the HPLC method which is true because titration method determines all available protons. The relative content of organic acids found in the fruits shall be useful and suitable to be used as food additives. The fruit of G. pedunculata has appreciable amounts of (-) - HCA, ca. 74 % of the total organic acids and therefore could be commercialized as an anti-obesity herbal formula. Value addition of such fruit products is also encouraged to create more market opportunities and accrued benefits.

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Conflict of interest

The authors have declared no conflicts of interest.

References