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Changes in Physicochemical Characteristics and Organic Acids during Ripening of Five Tropical Fruit Species in Malaysia

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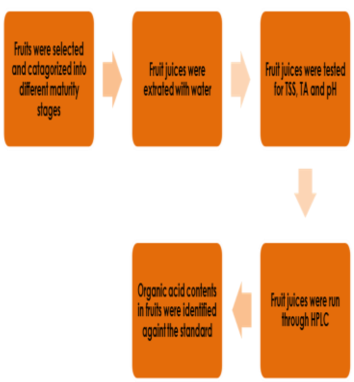
Graphical abstract



Abstract

Malaysia is a tropical country which is rich in various kinds of local fruits. Each has unique characteristics that could be explored further. Some of their compositions can play an important role in improving human life. The research aimed to evaluate the physicochemical composition and organic acid in 5 types of local fruit species in Malaysia, namely *Averrhoa bilimbi, Eleiodoxa conferta, Mangifera indica, Phyllanthus acidus* and *Bouea oppositifolia* at different maturity stages. It was observed that titratable acidity significantly decreased with the increasing maturity, while total soluble solid content and pH increased with maturity. All local fruit species at the ripe stage have higher total soluble solid content compared to young stage. Oxalic acid and ascorbic acid were present at all maturity stages of the five local fruit species. Tartaric acid was only present in *Bouea oppositifolia* while malic acid was present in all local fruit species except for *Mangifera indica*.

Keywords: Fruits, maturity stages, physicochemical, organic acid

1.0 INTRODUCTION

Among the main constituents of fruit, carbohydrate and acid, contribute a great deal to in food value of fruit [1]. The changes in the composition of sugar and organic acid compound not only determine the ripening process, but also play an important role in flavour development, chemical effect, and sensory characteristics such as microbial stability, sweetness, total acidity and pH of fruit [2]. Several sugars such as fructose, glucose and sucrose combined with a large amount of organic acids consisting of citric, malic, tartaric, oxalic and others can indicate fruit maturity [3]. The ratio of sugar to organic acids in fruit is determined by the organic acid content because it presents a wide range than the sugar contents [4]. The loss of organic acids during ripening is largely due to the utilization of these compounds as respiratory substrates and carbon skeletons for the synthesis of new compounds during the ripening process, that involve the conversion acid into sugars by physiological changes in the fruits [1]. Therefore, the present study has been conducted to find out the physicochemical composition (total soluble solid, titratable acidity and pH) and organic acids (oxalic, malic, tartaric and ascorbic acid) profiles of five local fruits species in Malaysia at three maturity stages (young, mature and ripe).

2.0 MATERIALS AND METHODS

Freshly collected fruits of *Averrhoa bilimbi, Eleidoxa conferta, Bouea oppositifolia, Mangifera indica* and *Phyllanthus acidus* were obtained from farms and market around the state of Kelantan and Pahang, Malaysia. Fruits of good condition at each maturity stage were selected. Three ripening stages of the fruits (young, mature and ripe) were determined according to the physical fruit development as stated by Federal Agriculture Marketing Authority of Malaysia.

The fruits were cut in two halves, while the seeds or skin were separated. 50 g of the sliced fruit was weighed and mixed with 100 ml sterile water before being blended with a home juice extractor. The fruit juice extract was filtered through muslin cloth. The filtered juices were immediately stored in a freezer at 0°C in the dark. The pH of the extract was measured using a digital electrode pH meter calibrated using buffer solutions [5]. The reading was taken in triplicates after a minimum period of five minutes. Total soluble solid assay (TSS) was determined by taking a direct reading of a drop of the extract in a prism glass of a refractometer (Atogo, Japan) at room temperature. The level of sugar was measured as °Brix [5]. The reading was taken in triplicates. Titratable acidity (TA) assay was conducted by titrating the fruit juice with 0.05M sodium hydroxide and the results were expressed as the concentrations of oxalic acid. It was calculated using the following formula;

$$TA\ (gml^{-1}) = \frac{V \times N \times meq.wt \times 100}{1000\ (v)}$$

Where,

V = volume of sodium hydrogen solution used for titration (ml)

N = normality of sodium hydroxide solution

Meq.wt = milliequivalent weight of oxalic acid standard, 45

v = sample volume (ml)

Fruit extract was clarified by centrifugation at 5000 RPM for 10 min and filtered through a 0.45 µm filter membrane [6]. 2 ml of sample was transferred into HPLC vial and kept at 4°C before injection. The analysis was carried out using Shimadzu HPLC equipped with a column oven C18 for organic acid separation. 1 µl injection volume was applied to an isocratic flow rate of 0.5 $mlmin^{-1}$ with UV detector set at 254 nm. Ammonium dihydrogen phosphate buffer ($NH_4H_2PO_4$, 0.5% w/v) was filtered through a 0.45 µm membrane and degassed before being used as a mobile phase [7]. Oxalic, tartaric, ascorbic and malic acids were identified by comparing their retention times with those of the corresponding standards. The quantitative analyses were carried out using calibration graphs from standard solution in the concentration range of 0.3 – 8 g. Each injection dilution was carried out in triplicate.

3.0 RESULTS AND DISCUSSION

A significant increase of TSS was observed in all five fruit extract species as the fruits mature (Figure 1). The overall range of TSS for young fruits was between 0.47 – 3.93°Brix, mature fruits at 0.47 – 4.07°Brix and ripe fruits at 0.67 – 4.13°Brix. *Bouea oppositifolia* has the highest amount of TSS for all stages; young (3.93°Brix), mature (4.07°Brix) and ripe (4.13°Brix). Minimum TSS content was found in *Averrhoa bilimbi* at young stage (0.47°Brix). Meanwhile, a significant increase of TSS value ($p<0.05$) of *Mangifera indica* fruit was observed from mature to ripe stage. The changes in TSS from mature green stage to the yellow ripe stage is an important indicator for fruit sweetness. The increase in soluble solids content could be attributed to converting starch to sugar [8].

From young to ripe stages of the fruits, significant decrease in TA (expressed as the concentration of oxalic acid) was observed in all fruit extracts tested (Figure 2). The concentration of the TA viewed throughout the ripening process was in the range of 0.35 – 0.88 gml^{-1}. TA of *Eleiodoxa conferta* at the ripe stage (0.35gml^{-1}) showed the lowest value, meanwhile *Phyllanthus acidus* showed the highest value at the ripe stage (0.88gml^{-1}). A large reduction (with more than 0.1 gml^{-1}) during the ripening process was recorded for *Eleiodoxa conferta*, *Bouea oppositifolia* and *Phyllanthus acidus*. The acid decline during the ripening of *Pineapple* was because of the loss in the dominant organic acid, resulting in a decrease of TA [9]. Decrease in acid content may be due to change of acids into sugars by some physiological and biochemical changes in the fruits.

pH value was the lowest in young fruit and increased significantly ($p<0.05$) during the early stage of ripening as shown in Figure 3. *Bouea oppositifolia* has the highest pH value for all maturity stages compared with the other fruits. Based on the result, pH value slowly increased during maturation. Changes in pH for *Averrhoa bilimbi* were not significant from 2.68 at the young stage to 2.71 at the ripe stage. pH indicator and organic acid are important for acid-sugar balance of fruit flavour and taste. This was proven by previous study of *Mangifera indica* by [10] who showed a large decline in acidity with a pH shift from 2.31 to 4.64.

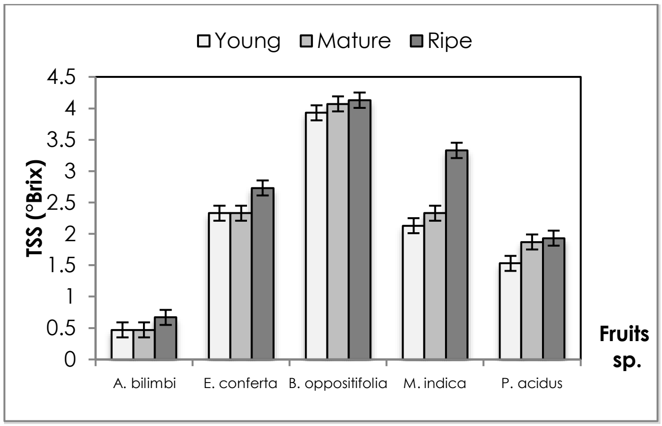


Figure 1 TSS content for five local fruits extracted at different maturity stages. Graph bar shows average mean and error bar show standard error

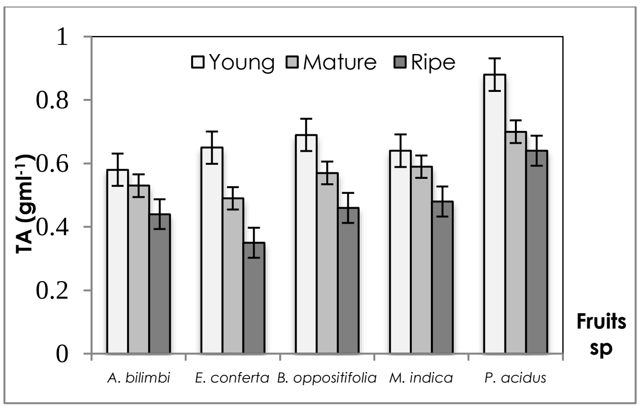


Figure 2 TA content in five local fruits extracted at different maturity stages. Graph bar shows the average mean and error bar shows the standard error

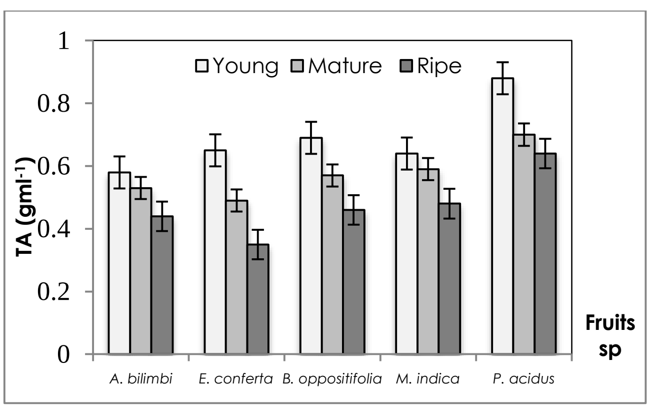


Figure 3 pH changes in five local fruits extracted at different maturity stages. Graph bar shows average mean and error bar shows standard error

Recovery of organic acids examined in five local fruits at different maturity stages (Figure 4) were analysed from chromatographic profiles. Overall, during fruit development, the amount of selected organic acid in fruit decreased from earlier to later stages of ripening. Oxalic acid and ascorbic acid were detected in all maturity stages of the fruits. Oxalic acid in *Averrhoa bilimbi* was significantly higher compared to other fruits at 2.73 gml^{-1} for young stage, 3.51 gml^{-1} for mature stage and 2.46 gml^{-1} for ripe stage. The average oxalic acid content was significantly higher than the ascorbic acid content. The content of ascorbic acid was relatively low and did not differ significantly between the five fruit extract. Meanwhile, malic acid was present in all fruits except for *Mangifera indica*. Concentration of malic acid was highest at the early stage of *Phyllanthus acidus* at 3.93 gml^{-1} and decreased to 1.58 gml^{-1} at the ripe stage followed by *Bouea oppositifolia, Averrhoa bilimbi* and *Eleiodoxa conferta*. Tartaric acid was only found in *Bouea oppositifolia* at the highest amount compared with other acids which ranges from 4.44 gml^{-1} at young stage, 5.02 gml^{-1} at mature stage to 5.10 gml^{-1} at ripe stage. Among the fruits, *Mangifera indica* was identified having the least of organic acid than others local fruit tested. In this finding, oxalic acid was determined as the major organic acid in *Averrhoa bilimbi* and *Eleiodoxa conferta*. These high levels of oxalic acid are because of its low pH value of 0.9 – 1.5[11]. Among the fruits, *Bouea oppositifolia* was found to contain all organic acids tested. Although the composition of the individual organic acid content varies between fruit species, oxalic and ascorbic acids were consistently present in all fruit species at every stage of maturity with other acids present for certain species. Most of the fruits usually contain one or two major organic acid components, whereas other organic acids are only present in small, trace quantities [12].

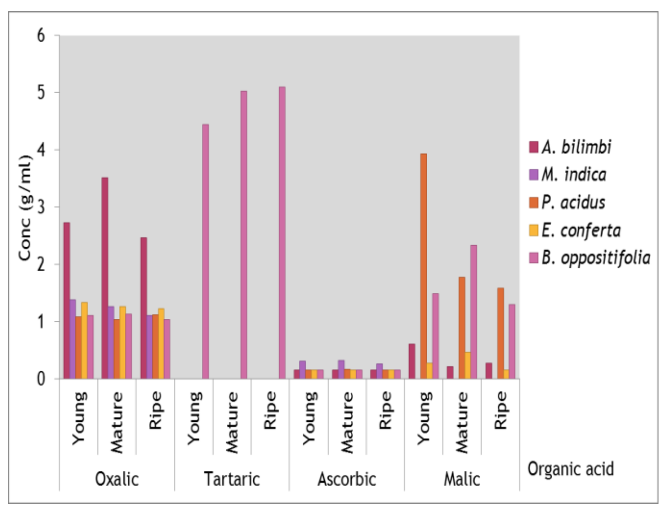


Figure 4 Comparison of the organic acid concentration determine by HPLC analysis in five local fruits extracted at young, mature and ripe stages.

4.0 CONCLUSION

The composition of organic acid decreased as fruit maturity progressed. *M. indica* L. Chok Anan and *S. zalacca* showed a decrease in the concentration of organic acids with ripening [13]. Mostly, the first two stages of fruits have a higher concentration of organic acid and lower total soluble solid content. The increase in total soluble solids provides a better palatability for the ripened fruit. The existence of oxalic acid in all fruits species gives the sour taste, depending on its strength. The content of oxalic acid in *Averrhoa bilimbi* showed significant difference than the other fruits. Thus, it shows that oxalic acid is an important for its application. With regard to the composition of total soluble solid and organic acid, the species of *Bouea oppositifolia* contains all four types of organic acid and tartaric acid. Thus, it might be as the indicators to discover their uses.

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