

Organic Acid Profile of "Batuan" [*Garcinia binucao* (Blco.) Choisy] Fruit

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ABSTRACT

"Batuan" fruit [*Garcinia binucao* (Blco.) Choisy], an indigenous acidulant grown in the Visayas State University, Baybay City, Leyte was analyzed for its organic acid profile at different stages of maturity for the development of potential food and non-food products. The analysis of organic acid content was done using Reverse Phase-High Performance Liquid Chromatography. Organic acids in the dried, powdered "batuan" fruit samples were extracted with the mobile phase (50mM $\text{KH}_2\text{PO}_4/\text{H}_3\text{PO}_4$, pH2.8). The sample extracts and organic acid standards (oxalic acid, tartaric acid, malic acid, citric acid, fumaric acid, lactic acid, acetic acid, and succinic acid) were injected to RP-HPLC under isocratic elution with the mobile phase at a flow rate of 1.0mL min^{-1} and using UV-vis detection at 210nm. "Batuan" fruit samples contain oxalic acid, tartaric acid, malic acid, citric acid, fumaric acid, succinic acid, acetic acid, lactic acid, and a few unidentified organic acids. Among the organic acids present, citric acid accumulated the highest in the ripe "batuan" fruit; fumaric acid, the least.

Results of this study show that "batuan" fruit could be a good natural source of acidulant for food and non-food applications.

Keywords: "batuan", citric acid, fumaric acid, organic acids, RP-HPLC

INTRODUCTION

Organic acids are the most commonly found compounds in fruits. As intermediates in the metabolic processes of fruits, organic acids are directly involved in their growth, maturation, and senescence (Gallander 1985, Moktar et al 2014). Furthermore, the nature and composition of the organic acids in fruits at different stages of maturity are of interest because of their important influence on the organoleptic properties, stability of food, beverage and the chemistry of the fruit (Igoe 2011, Moktar et al 2014). Aside from serving as a fingerprint which is distinct for each type of fruit (Shui & Leong 2002, Soyer et al 2003), the organic acid

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profile is also an important marker of fruit quality to be considered in the development of value-added products (Karadeniz 2004). However, the organic acid profile and its concentration in fruits are dependent on several factors such as species, soil, stress conditions to which the fruit was subjected and, maturity, among others (Jones 1998).

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, savory foods, and other food products (Karadeniz 2004). Aside from their influence on organoleptic properties, organic acids also play an important role in food and non-food product processing by maintaining and controlling the acidity, restricting growth of spoilage organisms, retarding the activity of enzymes involved in discoloration of fruits, as chelating agent, gelling agent and coagulating agent (Ergonul & Nergiz 2010, Igoe 2011). Among the fruits used as souring agent, the citrus family and guava (*Psidium guajava*) were found abundant in citric acid (Vera et al 2006), the tamarind (*Tamarindus indica* L.) fruit, rich in tartaric acid (Sortwell et al 1996); star fruit (*Averrhoa carambola*), in oxalic acid (Paliyath & Murr 2008).

Nowadays, fruits rich in organic acids are increasingly utilized as acidulant for beverages, food products, and other industrial applications. "Batuan" [*G. binucao* (Blco.) Choisy], an indigenous, lesser known, understudied fruit (Verheij & Coronel 1999, Florido & Cortiguerra 2003), is considered as a potential source of natural acidulant. In addition, "batuan" fruit had also been identified with commercial value and promising export potential when properly utilized (Coronel 2011). "Batuan" fruit, popular for its sour taste, has become a favorite souring agent in native dishes in lieu of tamarind for some people in Negros Occidental, Iloilo, Cebu, Leyte & Samar, among other places (Reyes 2011). The fruit can also be eaten fresh after washing with tap water and dipping in salt (Cojuanco 2012). Moreover, previous studies revealed that the "batuan" fruit is a valuable natural source of good quality protein, amino acids, lipid, fatty acids, fiber, minerals, and polyphenols (Quevedo et al 2013, 2014, 2015).

Florido & Cortiguerra (2003) reported that "batuan" trees are generally grown in the wild from Luzon to Mindanao. However, the number of trees is dwindling in some parts of the country due to natural calamities and man-made activities. Nevertheless, "batuan" trees are being cultivated nowadays as a minor tree crop in home gardens in some parts of the Visayas region and in commercial scale at the ECJ Farm, Candelaria, San Enrique, Negros Occidental for increased fruit production (Cojuanco 2012, Valencia 2013). At ECJ Farm, an affiliate company of San Miguel Corporation, "batuan" fruits are made into salted puree packed in glass bottles for the local and national market. Moreover, Cojuanco (2012) reported that the "batuan" salted puree has been transported to the US and Europe for Filipinos longing for its unique flavor.

Despite its potential commercial importance, "batuan" fruit has not received much attention among business entrepreneurs and the scientific community. Its utilization is limited probably due to the very little to no information available on its chemical composition and nutritional properties. Suni et al (2000) pointed out that certain acids have a lowering effect of the postprandial blood glucose and insulin responses. Moreover, Soumya and Nair (2014) also reported that malic acid maintains the liver in a healthy condition and helps in the digestion process. Literature search showed that no studies have been done on the organic acid

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profile of "batuan" fruit which would provide insights into its processing and nutritional properties. For this reason, the organic acid composition of "batuan" fruit at different stages of maturity was analyzed to provide new significant information on a potential natural source of acidulant. The result of this study is not only useful for the agriculturists, food experts, researchers, industrialists, nutritionists, but to local, national and the international community. In this paper, the identity and quantity of the organic acids present in "batuan" at different stages of fruit maturity using RP-HPLC method were reported.

MATERIALS AND METHODS

Materials

"Batuan" fruit samples at different stages of maturity were obtained from trees growing at the Pomology Project and the nearby hills of the Visayas State University (VSU), Visca, Baybay City, Leyte on June 2012.

Pure standards of citric acid, DL-malic acid were purchased from SUPELCO; L(+)-fumaric acid from FLUKA; L(+)-tartaric acid, L(+)-oxalic acid dehydrate and acetic acid from Sigma Chemical Co. All other solid and liquid reagents used were of analytical/HPLC grade.

Methods

Sample preparation. "Batuan" fruits were authenticated at the University of the Philippines Los Baños (UPLB) Museum of Natural History, College, Laguna, Philippines.

Immediately after collection, disease-free "batuan" fruits were washed with tap water and air-dried. Composite samples representing the immature, fully mature and ripe fruits were prepared. The fruits were classified into three stages of maturity according to their firmness, skin/pulp color, aroma and comparison on cross-section of the fruits (Table 1).

All samples were dried at 70°C for 16 h using a fabricated food cabinet dryer at the Department of Food Science and Technology (DFST), VSU. The dried samples were ground into powder using homogenizer/blender (Osterizer), passed through

Table 1. State of maturity of "Batuan" [*G. binucao* (Blco.) Choisy] fruit based on fruit color, skin/pulp color, firmness, aroma and cross-sectional appearance

State of Maturity	Fruit Color	Skin/Pulp Color	Firmness	Aroma	Cross-sectional Appearance
Immature	Light green	Thin skin; white pulp	Firm	None	watery pulp and less than half-endosperm filled seeds
Mature	green	Thin skin; creamy white pulp	Firm	None	Less watery pulp; more than half-endosperm filled to fully developed seeds; thick, woody-hard pericarp
Ripe	Light yellow	Soft, thin skin; creamy yellow pulp	Soft	Fruity	thick, woody-hard pericarp; fully developed endosperm

a 60-mesh sieve, stored in an air tight plastic container (Ziploc) and kept at room temperature.

Organic acid analysis. Dried, powdered samples were sent for organic acid content analysis to the Jefcor Analytical Service Laboratory, Dasmariñas, Cavite, Philippines and analyzed following the Scherer et al (2012) method with slight modification. Organic acids in the dried, powdered "batuan" fruit samples were extracted with the mobile phase (50mM $\text{KH}_2\text{PO}_4/\text{H}_3\text{PO}_4$, pH2.8), and centrifuged. All sample extracts and organic acid standard solutions were filtered through a 0.45 μm membrane (Millipore, USA) before injecting into the instrument.

Determination of the organic acids was carried out on Shimadzu liquid chromatograph (Shimadzu, Tokyo, Japan) system equipped with Teknokroma Mediterranean Sea₁₈ (5 μm particle size, 250mm x 4.6mm I.D kept at 25°C) column, and a SPD-10AVVP UV-Vis detector set at 214nm. The elution with 50mM $\text{KH}_2\text{PO}_4/\text{H}_3\text{PO}_4$ (pH2.8) was carried out at a flow-rate of 1.0mL min⁻¹ under isocratic condition. Identification of organic acids present in the sample was done by comparing the retention time of sample peaks with that of authentic standards. The compounds were quantified using a Shimadzu CR8A Chromatopac data processor.

Statistical analyses. All analyses were done in triplicate and results were expressed as means. Results of the quantity of the organic acids in "batuan" fruit samples were statistically analyzed and compared using one-way Analysis of Variance (ANOVA) with statistical significance for difference set at $p < 0.05$, followed by Tukeys (HSD) at $\alpha = 0.05$ to locate significant differences for treatment effects.

RESULTS AND DISCUSSION

Table 2 shows that the "batuan" fruit at different stages of fruit maturity exhibited a common organic acid profile. Results revealed that the "batuan" fruits contained various organic acids such as oxalic acid, tartaric acid, malic acid, lactic acid, acetic acid, citric acid, fumaric acid, succinic acid and other unidentified acids (Table 2 & Figure 1). Furthermore, the concentrations of the citric acid, acetic acid and lactic acid varied significantly with the stages of fruit maturity while those of oxalic acid, tartaric acid, malic acid, fumaric acid, and succinic acid did not. Among the organic acids present, citric acid had significantly accumulated the highest (3.12mg g⁻¹ dry wt).

Table 2. Organic acids (mg g⁻¹ dry weight basis) in "batuan" [*G. binucao* (Blco.) Choisy] fruits at different stages of maturity

Sample	Organic Acid (mg g ⁻¹ dry weight basis) ^{1/}							
	Oxalic acid ^{ns}	Tartaric acid ^{ns}	Malic acid ^{ns}	Citric Acid	Fumaric acid ^{ns}	Succinic acid ^{ns}	Acetic Acid	Lactic Acid
Immature Fruit	0.30	0.47	1.33	0.93 ^b	0.01	1.57	1.62 ^b	0.25 ^b
Mature Fruit	0.41	0.63	0.74	1.94 ^b	0.04	1.75	1.51 ^b	0.26 ^b
Ripe Fruit	0.41	0.96	0.88	3.12 ^a	0.06	1.54	2.64 ^a	0.79 ^a

^{1/}Means with different letters differ significantly from each other at 5% level, Tukey's HSD. ns – not significant

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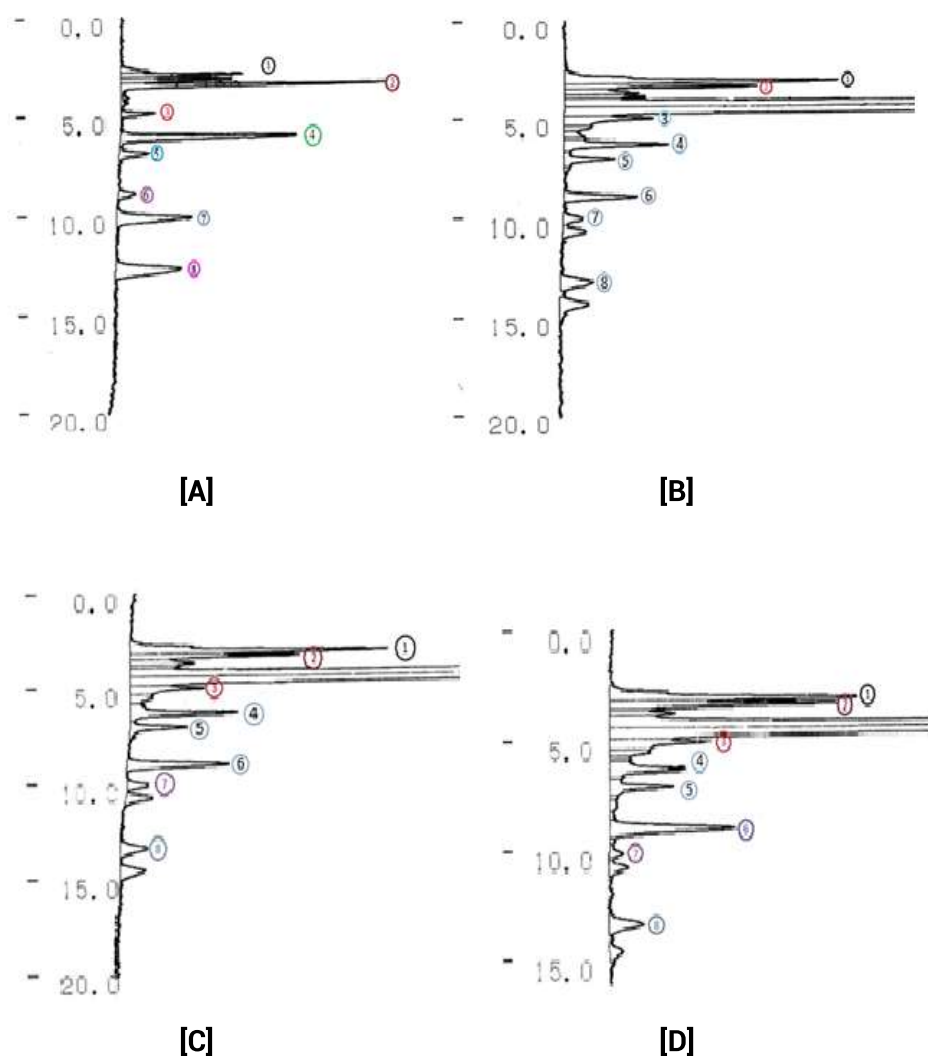


Figure 1. High performance liquid chromatogram. A) mixture of standards: 1) oxalic acid; 2) tartaric acid; 3) malic acid; 4) lactic acid; 5) acetic acid; 6) citric acid; 7) fumaric acid and 8) succinic acid; B) immature "batuan" fruit. C) mature fruit D) ripe fruit. Detection at 215nm.

The predominance of citric acid was also observed in other fruit acidulants such as lemon, lime, and guava (Morton 1987, Sortwell et al 1996). On the other hand, fumaric acid content in "batuan" was found the lowest (0.01 to 0.06mg g^{-1} dry wt. basis) regardless of the state of maturity. Meanwhile, malic acid content had the highest value of 1.33mg g^{-1} (dry wt. basis) in the immature fruit which did not differ significantly with those of the mature and ripe fruits. A similar

observation of high malic acid content in the immature fruit of *A. bilimbi* was also obtained by Soumya and Nair (2014). FAIA (2012) also pointed out that malic acid is often found in the unripe fruit. These observations suggest that the fruit accumulates the simple organic acid, malic acid, in the early stage of fruit development. Furthermore, no literature data on the organic acid profile of "batuan" fruit are available with which to compare the results of this study.

The presence of various organic acids in "batuan" at different stages of fruit maturity suggests that the fruit would be a very good natural acidulant in the formulation of food and beverage with more complex and authentic flavour profile (Sortwell et al 1996). Moreover, the additive effect and blend of the unique properties of each organic acid in "batuan" fruit could also be exploited in the development of food and non-food products. According to Univar (2013), acetic acid functions as a flavour enhancer, pH control agent and preservative. The presence of acetic acid in "batuan" fruit makes it a very suitable acidulant in the making of savory foods such as fish salads, processed meat, sauces and salad dressings. Meanwhile, the high citric acid content in "batuan" also makes the fruit a suitable acidulant for all types of food and beverage products. Univar (2013) revealed that lactic acid has a mild taste, and is generally used as preservative, flavor enhancer, antimicrobial agent, pH control agent, and is dairy compatible as well. This would also make "batuan" fruit a good choice of acidulant for product formulation involving meats, snacks, confections, soups, dairy, bakery, salad dressings, nutritional supplements and oral hygiene (FAIA 2012, Univar 2013). Fumaric acid which is present in the least amount, was reported to provide the greatest degree of sourness, and function in leavening, pH reduction, flavour enhancement, heat resistance, and low solubility (Univar 2013). This implies that a "batuan"-based product such as dry mixes of beverage, dessert, bakery, and confectionery will be less sour (a less fumaric-flavour impact) and a lower acidulant cost as well as (Sortwell et al 1996). On the other hand, tartaric acid which is used as a leavening agent, dough conditioner, flavour enhancer, and pH control agent (Brenntag Food & Nutrition Europe, n.d.) had also been used as moisturizer against dry skin as well as in inhibiting the precipitation of melanin (Mahmood et al 2012). As "batuan" fruit contains tartaric acid, this could also be explored as a valuable moisturizing agent for cosmetic applications. In addition, "batuan" fruit which contains modest amount of succinic acid that also functions as flavour enhancer, pH control agent, microbial control agent, would find wide application in the formulation of instant beverages, dairy and desserts products, bouillons and stocks, and flavourings (Brenntag Food & Nutrition Europe, nd). Moreover, malic acid had been used as preservative, pH control agent and enhancer of fruit flavors and aspartame sweetness in beverages, cocktail mixes and confectionery products (Sortwell et al 1996, Univar 2013). For this reason, the malic in "batuan" fruit would make it a strong flavour enhancer and flavour blender.

CONCLUSIONS

The organic acid profile of "batuan" fruits at different stages of maturity was determined using RP-HPLC to evaluate its potential as natural acidulant for food, beverage and other industrial applications.

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"Batuan" fruit samples were found to contain oxalic acid, tartaric acid, malic acid, citric acid, fumaric acid, succinic acid, acetic acid, lactic acid and a few unidentified organic acids. The concentrations of the citric acid, acetic acid and lactic acid varied significantly with stages of fruit maturity while those of oxalic acid, tartaric acid, malic acid, fumaric acid and succinic acid did not. Among the organic acids present, citric acid had significantly accumulated the highest (3.12 mg/g dry wt basis) in ripe fruit samples. On the other hand, fumaric acid content was the lowest (0.01 to 0.06 mg/g dry wt basis) regardless of state of maturity. Furthermore, malic acid was found highest (1.33 mg/g dry wt basis) in the immature fruit which did not differ significantly as maturity progressed.

The significantly high amount of citric acid, acetic acid and lactic acid in ripe fruit than the immature and mature ones together with the other organic acids present make this stage of the fruit very suitable for utilization as natural acidulant for different purposes.

"Batuan" fruit has a very promising potential as a natural acidulant and could be an important source of valuable organic acids for food and non-food applications.

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