Monitoring on Some Organic Acids in Fresh and Processed Rural Plant Leaves in Thailand

Pornpimol Muangthai[1], Piyatip Nookaew[2]

Department of Chemistry, Srinakharinwirot University, Bangkok, THAILAND

[1] pornpi@g.swu.ac.th

ABSTRACT

The aim of this work was to monitor three organic acids such as tartaric acid, malic acid and citric acid in 9 types of rural plant leaves found in Thailand such as Cowa leaf, Moringa leaf, Neem tree leaf, Noni leaf, Tamarind leaf, Soap pod leaf, Indian gooseberry leaf, Ceylon Spinach leaf and Gac leaf. The work was studied acid content in fresh rural plant leaves and effect on treatment leaves samples by boiling and freezing. The result showed that three organic acids could be detected in all fresh leaves of rural plants in different quantity. Tartaric acid, malic acid and citric acid contents were found in the range of 0.55 ± 0.01 - 685.28 ± 12.78 mg/100g, 0.50± 0.01 – 1496.64 ± 10.33 mg/100g and 0.20 ± 0.01 - 76.66 ± 9.54 mg/100g of plant leaves samples, respectively. Tartaric acid was found highest content in Neem tree leaf and malic acid, citric acid were found highest quantity in Cowa leaf. The quantity of organic acid not depends on the taste of leaves. The organic acid content in plant leaves depend on heating time of boiling process, the cooling process as freezing could preserved organic acids in all plant leaves. Those rural plants may be used as AHA source for future acid preparation and could increase value added of rural plant leaf as a one ingredient in cosmetic industry for the future economy.

Keywords: Citric acid, Malic acid, Organic acid, Thai Rural Plant, Tartaric acid

INTRODUCTION

An organic acids are weak acid organic compounds that contain carboxylic acid group (-COOH) in theirs formula and completely dissociate in water[1]. In general organic acids were found in fruits and some vegetables and their content depends on many factors such as species, soil and stress conditions[2]. Many organic acids were very useful for healthy and were applied as food additives in beverages industry[3], as an antioxidant in cosmetic industry[4].The main oraganic acids used in the industries are citric acid, malic acid and tartaric acid and ascorbic acid. The organic acids content was the quality evaluation index for some food and beverage. Those organic acids often influence on flavor, stability, and storage quality of food[5]. Thus some organic acid was proposed to be an index of maturity, ripeness, spoilage in some foods.In this work will scope the three types of organic acids such as tartaric acid, malic acid and citric acid.

Tartaric acid is a white crystalline diprotic aldaric acid and naturally found in many plants, especially in fruit such as grapes, bananas, and tamarinds. It is a food additive to other foods that give a sour taste, and is also used as an antioxidant[6].

Malic acid is a dicarboxylic acid with the general formula as $HO_2CCH_2CHOHCO_2H$, it contributes to the pleasantly sour taste of fruits as apple[7-8] It is used as a food additive. Malic acid plays a vital role[6] in improving overall muscle performance, reversing muscle fatigue

following exercise, reducing tiredness and poor energy levels, as well as improving mental clarity. These actions can make it a beneficial treatment for sufferers of fibromyalgia and CFS (both these conditions involve muscle pain, joint tenderness and low energy levels

Citric acid is a weak tricarboxylic acid, name as 2-hydroxy-1,2,3-propanetricar-boxylic acid($C_6H_8O_7$). This acid is naturally concentrated in citrus fruits. Citric acid is frequently used as a food additive to provide acidity and sour taste to foods and beverages. Among fruits, citric acid is most concentrated in lemons and limes[9]. It is widely used as a pH adjusting agent in creams and gels of all kinds. In this role, it is classified in most jurisdictions as a processing aid and so does not need to be listed on ingredient lists

In general many fruits always are a souce of all organic acid, but in leaves of some plant have not detail too much. However, some Thai rural plants were interesting, thus the aim of this work was to monitor tartaric acid,malic acid and citric acid in their leaves for evaluation on the potency source of AHA. The effect from cooking was also studied to evaluate the stabilities of organic acids.

MATERIALS AND METHODS

Standards and Reagents

Tartaric acid .malic acid and citric acid standards (Analar grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from *Carlo Erba*. Potassium dihydrogenphosphate (Analar grade) was purchased from Merck. Sulfuric acid (laboratory grade) was purchased from Duksan. All the water used in the study was ultrapure, obtained from a Direct-Qsystem (Millipore Corporation, France).

Instrument

The HPLC system consisted of a binary pump (Agilent Hewlett-Packard HP 1100), a vacuum degasser, 20μL injector, RP-C18 column (SphereClone 5 μm ODS, 250 x 4.60 mm, Phenomenex) and photodiode array detector. All samples were filtered through 0.45 μm PTFE (polytetrafluoroethylene) from International scientific.

Thai Rural Plant Samples

The rural plant leaves samples such as Cowa leaf, Moringa leaf, Neem tree leaf, Noni leaf, Tamarind leaf, Soap pod leaf; Indian gooseberry leaf, Ceylon Spinach leaf and Gac leaf were brought from rural market in Thailand and also examined their family by botanist.

Sample Preparation

The preparation sample modified from Shiraishi et al.,(2010).[11] Each of fresh plant samples were weighed 10 g before homogenized by the electrical blender. The accurately weight 1 g of the homogenized sample was transferred to a test tube and sulfuric acid (0.001 $molL^{-1}$) 5 mL was added and mixed with vortex mixer for 5 minutes. The suspension was centrifuged at 3000 rpm for 10 min. The supernatant was then collected and filtered through a 0.45 μm PTFE membrane filter before analysed by HPLC using optimum condition of HPLC analysis.

The leaves samples were heated at 100^{o}C for 5, 10, 20 and 30 minutes. Those heated leaves were also treated as the fresh leaves samples before analyzed by HPLC. However, the leaves samples were freezed at -20 ^{o}C in frozen cabin and analyzed same as the fresh leave too.

Study the Optimum Condition for the Analysis of Organic Acids

Mobile phase systems. The two mobile phase systems were investigated as the following

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a. System 1: 0.001 mol/L sulfuric acid: methanol as 90: 10, 95 : 5 and 98 : 2 (v/v)[12]
b. System 2: potassium dihydrogen phosphate 0.001 mol/L[13]

Flow rate of mobile phase. Three flow rates were studied including 0.3, 0.5 and 1.0 mL/min

The mobile phase solvent was controlled by isocratic grediant system.

Statistical Analyses

All determinations were carried out at least in five replicates and values were averaged. For all statistics, ANOVA and Microsoft Excel were used for calculate and graph presentation in this work.

RESULTS AND DISCUSSION

Optimization Method

In this study, HPLC analysis of tartaric acid and ascorbic acid was performed on a Vertisep^{TM} UPS C_{18} (150 x 4.6 mm id) equipped with a guard column (4.6 x10 mm) and detected by Diode array detector at 210 nm. The optimum condition for the analysis of malic acid, tartaric acid and citric acid by HPLC showed the was the mobile phase of 0.001 molL^{-1} sulfuric acid and methanol (98:2 v/v) at a flow rate of 1.0 mLmin^{-1}. The retention times of tartaric acid, malic acid and citric acid are 2.3, 3.0 and 5.6 mins, respectively as showed the typical chromatogram in Figure1.

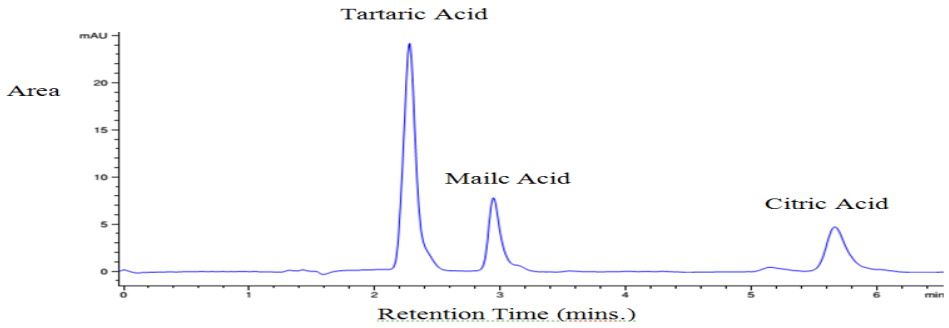

Figure 1. Chromatogram of tartaric acid, malic acid and citric acid standard solutions

The method showed good analytical features. The linearity was in the range of 5.00 to 1000 mgL^{-1} for above three organic acids as the obtained calibration curves are shown in Figure 2.

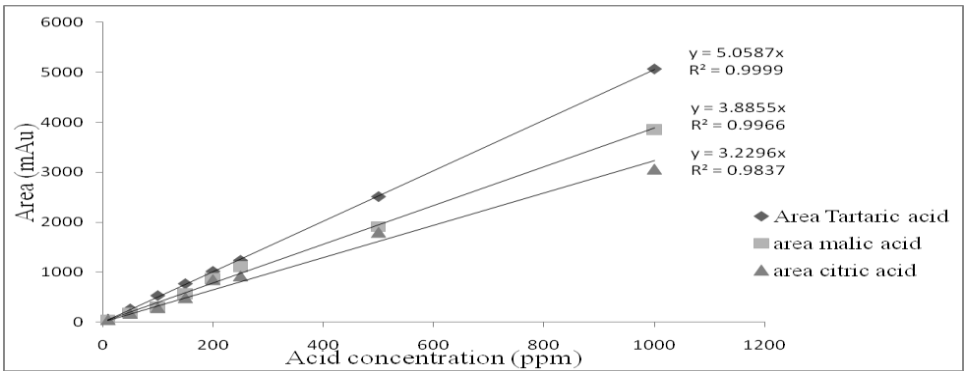

Figure 2. Standard calibration curve of standard organic acids

Limit of detection (LOD) and limit of quantitation (LOQ) were evaluated based on S/N = 3 and S/N = 10 respectively. The obtained LOD for tartaric acid, malic acid and citric acid were 1.33 ± 0.54, 2.27± 0.76 and 2.20 ± 0.57 mgL^{-1}, and LOQ value were 4.44 ±0.24, 7.54 ± 0.06

and 7.34 ± 0.20 mg/L respectively. The percentage recovery was in the range of 90.25 ± 4.46 – 101.58 ±3.18 %.

Analysis of Samples

The plant leaves were extracted by acid as referred and filtered through a 0.45 µm cellulose membrane filter before injected to C18 column of HPLC. The chromatogram was recorded and compared the area of peak at the retention time of standard each acid. The example of the filtrated leaves sample chromatogram as in figure 3.

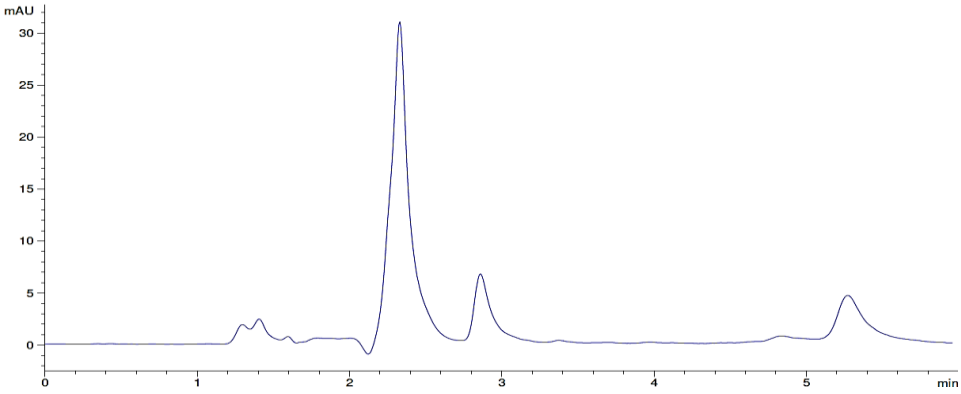

Figure 3. Chromatogram of Neem tree leaves

The quantity of acids was calculated by regression equation from Figure 2 as in figure 4.

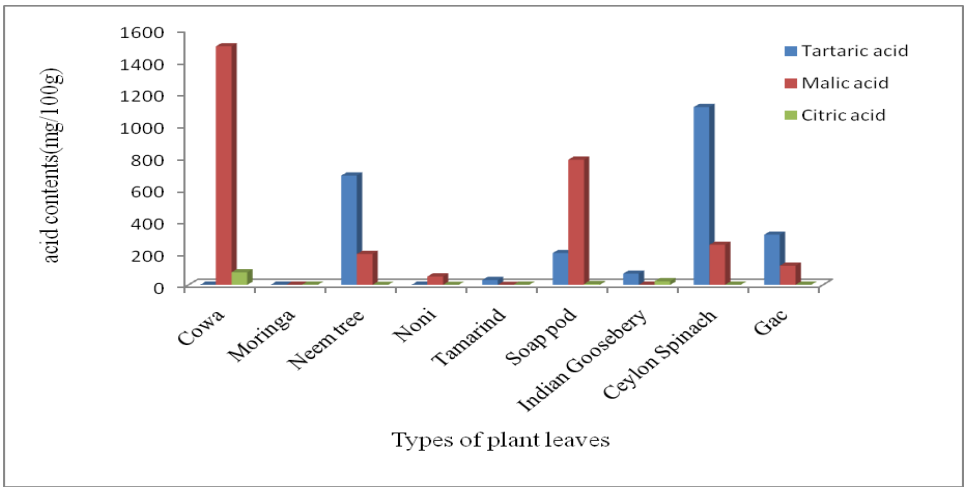

Figure 4. Organic acid contents in fresh plant leaves

From figure 4, the Cowa leaf contained the highest malic acid content at the level of 1500 ± 120.36 mg/100 g and citric acid content at the level of 78.60 ± 5.22 mg/100g .Ceylon Spinach showed the highest tartaric acid content at the level of 1200 ± 11.56 mg/100g.The moringa leaf showed the lowest three organic acids content at the level of 0.45 ± 0.02 mg/100 g of tartaric acid, 0.52 ± 0.12 mg/100 g of malic acid and 1.56 ± 0.45 mg/100 g of citric acid. The organic acid in rural plants showed the usefulness of plant to be a good organic acid source for further application from plant leaves. An interesting knowledge of the work also showed that the taste of some plant leaves could not evaluate to organic acid content such as the, Ceylon Spinach leaf, Moringa leaf and Gac leaf, because the leaves of all three plants did not show a sour taste as Cowa leaf. There was no report about the organic acids of above rural plant; this was the first work that searches the details about organic acids in Thai rural plant. Cowa plant is an important source of bioactive compounds[14]. Among the parts of this tree, the fruit, twig and stem are the best source of metabolites, thirty substances of which have

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been isolated, i.e. onedepsidone, one α,β-unsaturated cyclohexenone, three flavonoids, six phloroglucinols and nineteen xanthones. Some of these compounds show interesting pharmacological activities. α-Mangostin, cowanol and cowanin[15-16] are commonly found in all parts of Cowa and they can be used as chemotaxonomic markers of this species. The details about its leaf were not referred about the organic acid.

However, all plant leaves were heated 100°C for 5, 10, 20 and 30 minutes and analysis of tartaric acid in each plant leaf as showed in figure 5.

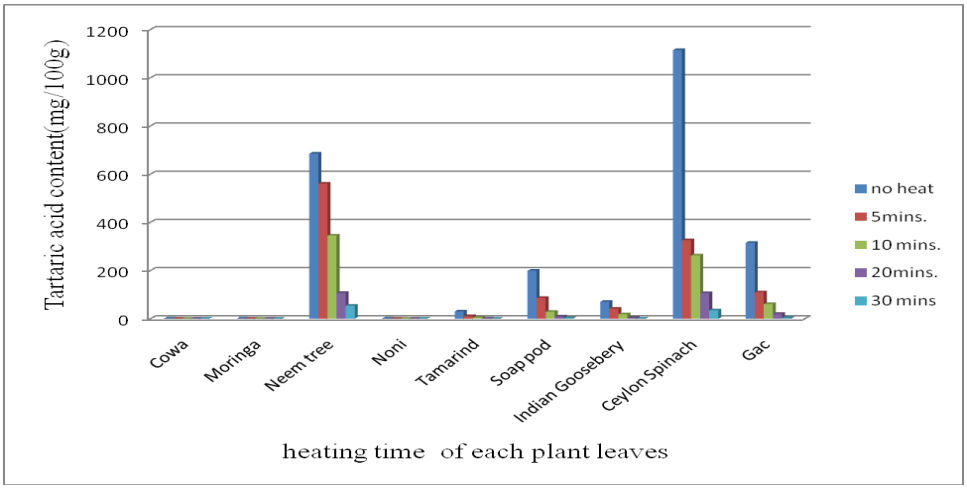

Figure 5. Tartaric acid contents in plant leaves after heating for 5 – 30 minutes

From figure 5, all plant leaves showed the same trend since tartaric acid trend to reduce after heating for 30 minutes. However, some plant as Neem tree and Ceylon Spinach still contained some tartaric acid, so this may from the high content of tartaric acid at initial and nature of plant. For malic acid study, Cowa leaf which contained the highest malic acid content was heated for the same period and shown in figure 6.

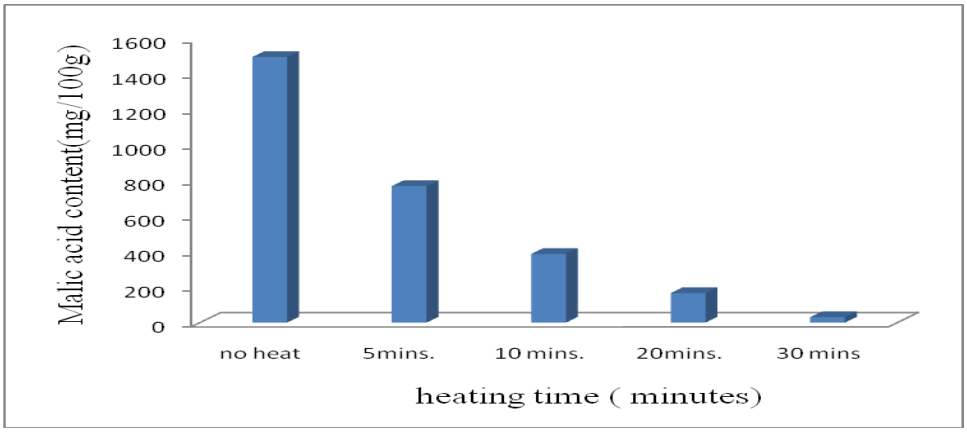

Figure 6. Malic acid contents in Cowa leaves after heating for 5 – 30 minutes

The malic acid in Cowa leaf also change in the same trend as tartaric acid by acid content depend on heating time. This also mean that the cooking process at boiling temperature on plant leaves destroyed some part of organic acid same as other important nutrient such as vitamin[17].

The leaves samples were freezed at -20 °C in frozen cabin for 3 months and analyzed the organic acid in all samples and showed in fiqure 7-9.

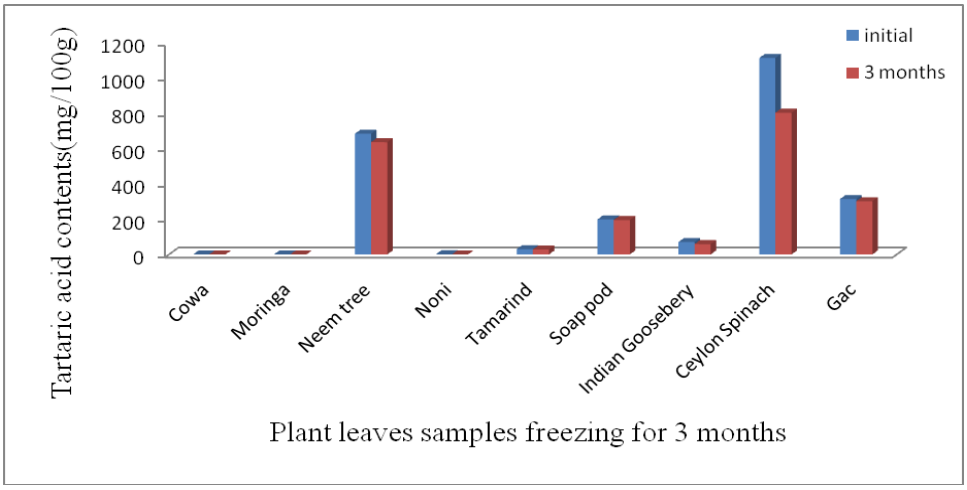

Figure 7. Tartaric acid contents in leaves samples after freezing for 3 months

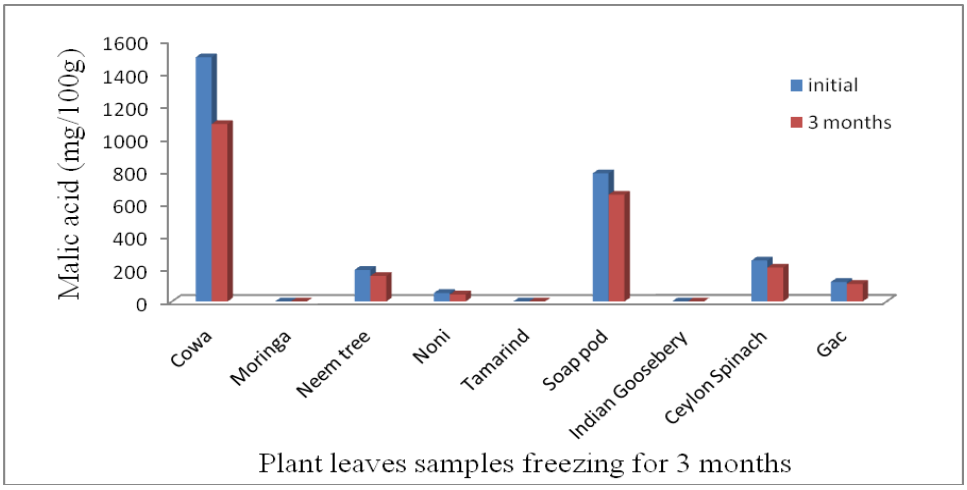

Figure 8. Malic acid contents in leaves samples after freezing for 3 months

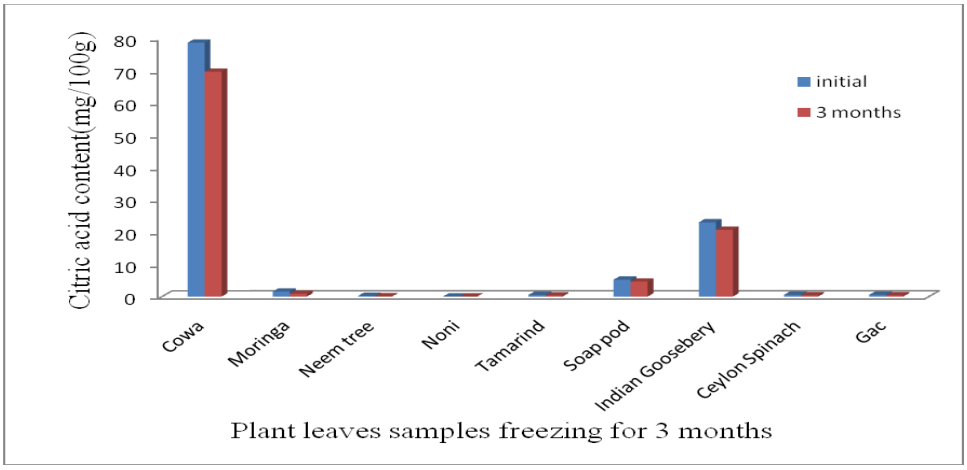

Figure 9. Citric acid contents in leaves samples after freezing for 3 months

After storaging for three months in freezing cabin at – 20^{o}C, it presented that storage in cool temperature for three months could save the organic acid because all organic acids showed a few change not more than 15 percent. The cooling process is good for preserve acid content in cellulose fiber of plant leaf[18]. However, cooling step as known is important to stop many reaction in food product which opposite on heating process could destroy an important

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nutrients as referred by Bolin and Stafford (1974)[19]. This work also confirms that organic acid which was one important nutrient from plant could be preserve under freezing condition.

CONCLUSIONS

The analysis of all organic acids in 9 types of rural plant leaves of Thailand such as Cowa leaf, Moringa leaf, Neem tree leaf, Noni leaf, Tamarind leaf, Soap pod leaf, Indian gooseberry leaf,Ceylon Spinach leaf and Gac leaf by high performance liquid chromatography. The result showed that organic acids could be detected in all leaves of rural plants in different quantity which depend on nature of plant. Tartaric acid, malic acid and citric acid contents that found in rural plants were in the range of 0.55 ± 0.01 - 685.28 ± 12.78 mg/100g, 0.50± 0.01 – 1496.64 ± 10.33 mg/100g and 0.20 ± 0.01 - 76.66 ± 9.54 mg/100g, respectively. Neem tree leaf showed the highest content of tartaric acid. Malic acid and citric acid were found highest quantity in Cowa leaf. The quantity of organic acid not depends on the taste of leaves. The organic acid content in plant leaves depend on heating time of boiling process, the cooling process as freezing could preserved organic acids in all plant leaves. This study gave the important data about tartaric acid, malic acid and citric acid in rural plant leaves of Thailand concerning on heating and freezing process. Those rural plants may be used as AHA source for future acid preparation and could increase value added of rural plant leafs as an one ingredient in cosmetic industry for the future economy.

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