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Effects of various processing methods on hydroxycitric acid content of "*batuan*" [*Garcinia binucao* (Blanco) Choisy] fruits

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Abstract

Recent studies confirmed that *batuan* (*Garcinia binucao*), a Philippine indigenous fruit, is a potential source of hydroxycitric acid (HCA), a compound with anti-obesity property. This fruit, however, is commonly processed into different products to extend its shelf life and increase its marketability and availability all year round. This study was conducted to determine the effects of various processing methods on the HCA content of *batuan*. The fruits were steam blanched at different time intervals (10, 15, and 20 min). The effect of drying was determined by subjecting the samples to different drying temperatures (50°C, 60°C and 70°C). Whole fruits were submerged in 5% brine solution for about 3 weeks to allow fermentation, and then samples were collected every 4 days. To determine the effect of freezing, whole fruits were stored in a freezer at -18°C. Sampling was done every week for 2 months. HCA was isolated from fresh and processed fruit rinds using water extraction method. Spectrophotometric analysis was employed to quantify the isolated acid from the samples. Results revealed that fresh *batuan* fruits contain 4.81 + 0.12 g HCA/ 100 g pulp. Steam blanching was observed to reduce the HCA content of *batuan* fruit rinds by 14.55%–21.41%. On the other hand, drying can decrease the HCA content of samples by 7.28%–16.22%. Brine fermentation for 3 weeks likewise significantly reduced the HCA of *batuan* fruit by 39.08%. Freezing for 2 months decreased the HCA content of samples by 13.30%. Based on the results, freezing and drying at 60°C are the most effective methods in preserving the HCA of *batuan*.

Introduction

Obesity is more than a phenotypic manifestation of being fat. Studies revealed that it is major risk factor for several non-communicable diseases, such as cardiovascular diseases, diabetes, musculoskeletal disorders, and some cancers (Wadden, 1993; Astrup, 2001; WHO, 2017). According to the World Health Organization (2017), worldwide obesity has nearly tripled since 1975. Fact sheets show that in 2016, about 39% of adults aged 18 years and over were overweight, and 13% were obese. Also, 41 million children under the age of 5 were overweight or obese. For adolescents aged 5–19 years, over 340 million individuals were overweight or obese. Due to the alarming increase in cases of obesity around the world, many studies are focused on finding ways on preventing it.

One breakthrough was the discovery of hydroxycitric acid (HCA). It inhibits citrate cleavage enzyme, a key factor in fatty acid synthesis, thus preventing fat accumulation (Watson and Lowenstein, 1970). Today, commercially available HCA is commonly extracted from *Garcinia cambogia*. Belonging to the same genus is a Philippine indigenous fruit called *batuan* (*Garcinia binucao*). It is an under-utilized crop that is well-known in the Visayas region as a souring agent (Dela Cruz, 2012). A study conducted by Bainto *et al*. (2018) confirmed the presence of HCA in *batuan*. However, this fruit is seasonal. It is most abundant during summer, which spans from March to June. To make the fruit available all year round, increase its marketability, and extend its shelf life, *batuan* fruits are processed into high-value products such as purees, candies, powder, pickles, etc. Conversions of raw materials into these products involve combinations of different processing methods such as steam blanching, drying, brine fermentation, and freezing.

Heat treatments often affect the acid content of fruits and vegetables. According to Hagg *et al*. (1998), cooking decreases the ascorbic acid content

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of potatoes by approximately 30%. Blanching also reduces the ascorbic acid content of beans, broccoli, and cauliflower by 28%, 40% and 30%, respectively, due to the dissolution and oxidation of the vitamin during blanching (Oruna-Concha *et al*., 1998). A study conducted by Del Caro *et al*. (2004) revealed that the ascorbic acid content of prunes dried at 60°C decreased significantly compared with that of fresh samples during storage. Guava sticks dried at the same temperature to 11% moisture content has an ascorbic acid content of around 20% to 35% of the original sample (Sanjinez-Argandoña *et al*., 2005).

A study conducted by Jayabalan *et al*. (2007) demonstrated the production of organic acids at varying stages of *kombucha* tea fermentation. Results showed that acetic acid concentration slowly increased until the 15th day of fermentation. Glucoronic acid, an important key factor in the detoxifying effect of *kombucha* tea, also increased in concentration up to the 12th day of fermentation. Lactic and citric acids had the least concentrations, which were both detected on the third day of fermentation.

Low temperature preservation methods affect the ascorbic acid of vegetables. Unblanched beans and peppers lose more than 97% of their ascorbic acid content within 1 month of freezing at -22°C (Oruna-Concha *et al*., 1998). A study by Lisiewska and Kmiecik (1996) revealed that the ascorbic acid contents of broccoli and cauliflower were reduced by 15%–18% and 6%–13%, respectively, after 12 months of frozen storage at -30°C. Baked sweet potatoes were stable during 6 months of frozen-storage, with the exception of ascorbic acid, which decreased by roughly 50% during the first month (Wu *et al*., 1991).

Steam blanching, drying, brine fermentation, and freezing induce cellular damage to the raw materials. However, changes during processing may affect the availability of HCA initially present in the fresh *batuan* fruit. Hence, this study was conducted to determine the stability of HCA once the fruit is subjected to different processing conditions such as steam blanching, drying, brine fermentation, and freezing.

Materials and methods

Sample collection

Fresh and unripe *batuan* fruits were obtained from a farm located at Brgy. La Granja, La Carlota City, Negros Occidental, Philippines. The fruits used in the study have a characteristic green color, hard covering, and are about 3.7—5.5 cm in diameter. The *batuan* fruit samples were taken from the same batch to ensure uniform maturity.

Processing of batuan fruits

Blanching

The effect of heating on the HCA content of *batuan* fruits was determined by steam blanching the fruits at 100°C at pre-determined time intervals (10, 15, and 20 min). Fresh and untreated *batuan* samples served as the control. After steam blanching, the samples were cooled rapidly using an ice bath, and then the fruit rinds were collected.

Drying

The pulp from the fresh *batuan* samples was dried up to approximately 10% moisture content using a cabinet dryer. Different temperatures (50°C, 60°C and 70°C) were employed to determine the effect of drying temperatures on the HCA content of the fruit. The samples were laid on stainless steel trays layered with polyethylene to prevent the pulp from sticking onto the trays after drying. Fresh *batuan* fruits served as the control.

Brine fermentation

Approximately 500 g of *batuan* fruits were submerged into 5% brine solution for almost 3 weeks to allow fermentation. Isolation and quantification of HCA from fermented *batuan* samples were conducted every 4 days for 20 days.

Freezing

Prior to freezing, fresh *batuan* fruits were washed thoroughly and treated with chlorinated water. The samples were divided into 500 gram pack plastic bags. The fruits were stored in a freezer at -18°C for 2 months. Sampling of frozen *batuan* was done every week for 2 months. The samples were thawed thoroughly under room temperature before the isolation and quantification of HCA.

Isolation and quantification of HCA from fresh and processed batuan fruits

HCA was isolated from the samples using water extraction method based on a published protocol by Krishnamurthy *et al*. (1982). The HCA collected using this protocol was in the form of concentrated liquid.

Isolated HCA was quantified by spectrophotometric analysis using 1% ammonium monovanadate. The following procedure was based on the protocol by Antony *et al*. (1999), as cited by Bainto *et al*. (2018). Exactly 0.2 g of isolated extract was weighed. Five milliliters (5 mL) of 1N H_2SO_4 was added to dissolve the sample, and then it was diluted to 25 mL using distilled water. The solution

was filtered into a 50 mL volumetric flask. The residue was washed and diluted to volume.

The standard that was used in the analysis is a food grade *Garcinia cambogia* extract (80% HCA). Stock solution of the standard was prepared by dissolving exactly 0.4 g of the standard in 10 mL 1N H_2SO_4. Fifty milliliters (50 mL) of distilled water was added. The solution was then transferred into a 100 mL volumetric flask and diluted to volume using distilled water.

In the spectrophotometric analysis, 1% ammonium monovanadate (NH_4VO_3) was used. Exactly 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, and 0.6 mL from the standard stock solution were dispensed in individual 50 mL volumetric flasks and then diluted to volume. One percent of ammonium monovanadate (4.5 mL) was added to allow color development. The initial color of the solution is yellow. Through time, the yellow color slightly changed to orange red. Absorbance was noted at 467 nm after 20 min. Calibration graph was derived from the absorbance data.

The HCA concentrations of the samples were computed using the following formula:

$$\text{HCA content (g/100 g sample)} = \frac{[HCA]\ in\ solution \times Wt.\ of\ collected\ extract \times Dilution\ factor}{Weight\ of\ sample} \times 100$$

Statistical analysis

All analyses were performed in triplicates. Analysis of Variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) Test were employed to statistically analyze the gathered data using Statistical Tool for Agricultural Research (STAR) software developed by the International Rice Research Institute Laguna, Philippines.

Results and discussion

Effects of steam blanching on HCA levels

Batuan fruits were steam blanched at 100°C at different time spans. The result of spectrophotometric analysis (Figure 1) shows that the HCA content of *batuan* decreased as heating time increased up to 15 minutes. A negligible change in acid content was observed when heating continued until 20 minutes.

According to Lin and Brewer (2005), blanching may lead to the occurrence of cytoplasmic damage of cells. Consequently, the cell becomes permeable to water and solutes. An immediate effect of this is the loss of turgor pressure. Water and solutes may pass into and out of the cells; a major result is the nutrient loss from the tissue (Barrett and Theerakulkait, 1995). This explains the decrease in the acid content of *batuan* samples with steam blanching. HCA leached out from the tissues of the fruit as it was subjected to steam blanching. A continuous leaching out of acid up to 15 minutes of heat exposure was observed. On the other hand, heating beyond 15 minutes no longer decreased the HCA concentration as most of the acid near the surface of the fruit have most likely been leached out. HCA located in the inner part of the fruit was more difficult to remove by heat treatments, thus the observed HCA value at 20 min.

The result of spectrophotometric analysis using 1% ammonium monovanadate indicated the decreasing trend in the amount of HCA as heating time increased. Analysis showed that untreated sample has the highest HCA content (4.81 + 0.12 g/100 g pulp) followed by the sample heated for 10 min (4.11 + 0.49 g/100 g pulp), 15 min (3.78 + 0.31 g/100 g pulp), and 20 min (3.78 + 0.38 g/100 g pulp). Despite the observed trend, statistical analysis revealed that there were no significant differences among the treatments.

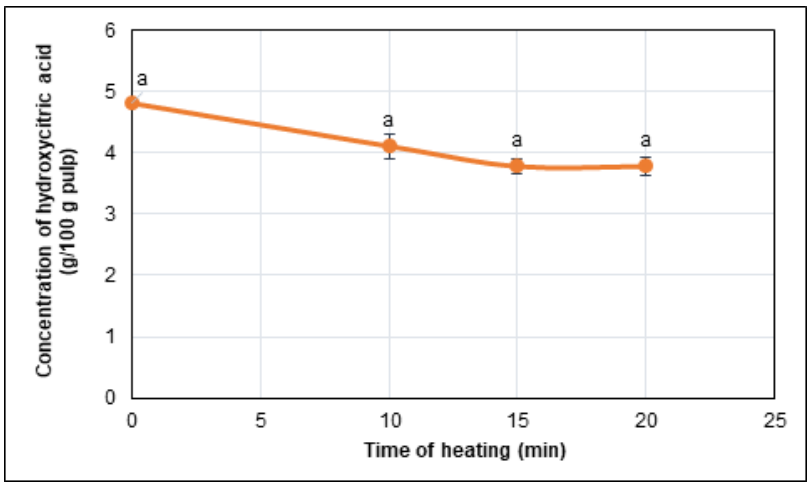

Figure 1. Effect of steam blanching on the HCA content in batuan fruit pulp. (Values with the same letters are not significantly different at P=0.05 using HSD test.)

Effects of drying on HCA levels

Figure 2 shows the appearance of *batuan* fruit pulp after drying at different drying temperatures. The samples dried at 50°C had a notably darker color compared to the other two samples.

Results show that drying reduces the amount of HCA from the *batuan* fruits (Figure 3). Among the three drying temperatures analyzed, the samples dried at 60°C yielded the highest concentration of HCA (4.46 + 0.22 g/100 g *batuan* pulp), followed by 70°C (4.22 + 0.34 g/100 g *batuan* pulp), and the least was 50°C with 4.03 + 0.24 g/100 g *batuan* pulp. However, the untreated sample still had the highest HCA content, which was equal to 4.81 + 0.12 g/100 g *batuan* pulp.

The drying process reduces the nutritional quality of food due to the degradation and evaporation of volatile compounds (Brennan, 2006). The result of the experiment revealed that this principle also applies to HCA. The moisture content of fresh *batuan* fruit pulp is around 85%. Drying at 50°C entailed a longer time to reduce the moisture content to the desirable level. The samples were dried for 42 hours at 50°C. Exposing the samples to a high temperature for a prolonged period decreases the acid content due to several deteriorative reactions that may include oxidation. According to Mizobutsi *et al*. (2010), polyphenol oxidase, the enzyme responsible for enzymatic browning, can still be active at 50°C. This reaction is also responsible for the darker color of samples dried at 50°C. Meanwhile, employing 70°C for drying resulted to a considerable decrease in acid content due to a relatively higher temperature that could have introduced a more deteriorating effect, although it required a shorter time of exposure (Wang and Wang, 2009). Only 18 hours of drying at this temperature is sufficient to reduce the moisture to approximately 10%. Based on the results, the optimum drying temperature is 60°C. This temperature requires 24 hours only to reduce the moisture content to 10%, and 60°C is also not too high to cause drastic undesirable changes to the samples. Essentially, it has the least decrease in acid content among the drying temperatures.

Statistical analysis showed no significant differences among the treatments and control. Thus, drying as a food processing method can preserve the HCA content of *batuan* fruit.

(a) (b) (c)

Figure 2. Appearance of batuan fruit pulps after drying at (a) 50°C, (b) 60°C, and (c) 70°C.

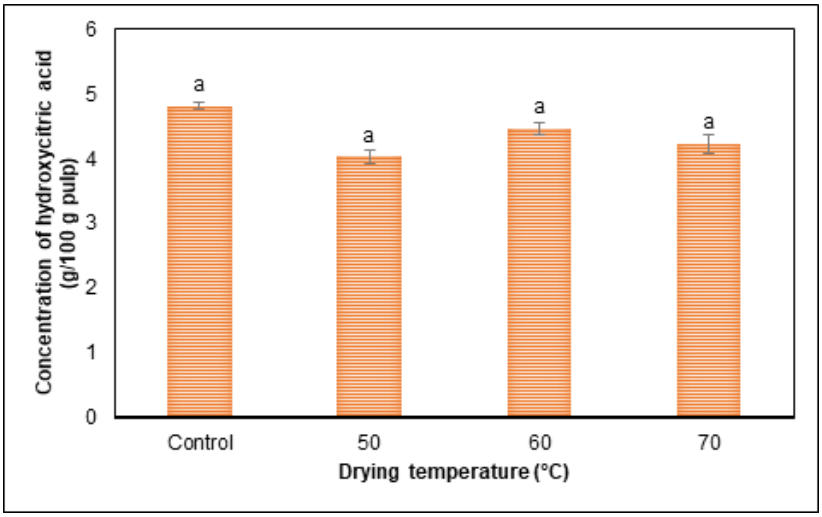

Figure 3. Effect of different drying temperatures on the HCA content of batuan fruit pulp. (Values with the same letters are not significantly different at P=0.05 using HSD test.)

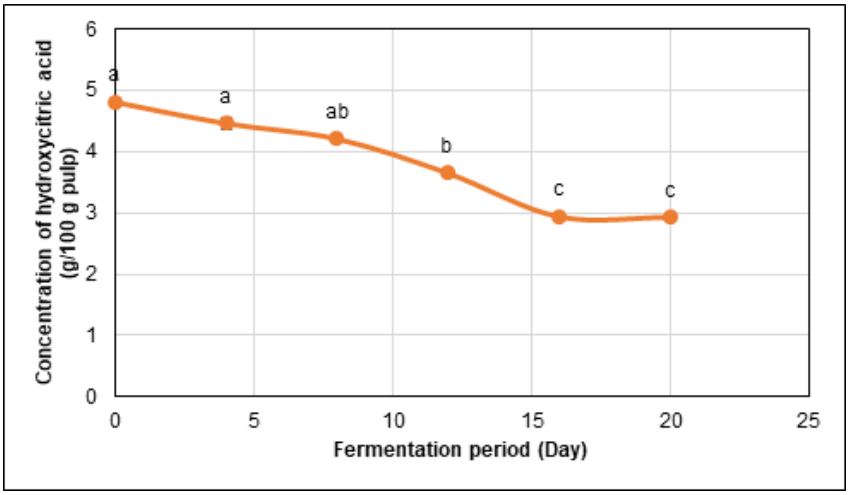

Figure 4. HCA content of batuan fruit pulp during brine fermentation. (Values with the same letters are not significantly different at P=0.05 using HSD test.)

Effects of brine fermentation on HCA levels

Traditionally, fermented fruits are produced to extend their shelf life. Brine fermentation by lactic acid bacteria may be considered a simple technique for maintaining and/or improving the safety, nutritional, sensory, and shelf life properties of fruits (Rodriguez *et al*., 2009). Aside from lactic acid bacteria, other microorganisms such as yeasts may be involved, depending on salt concentration and other factors. Sodium chloride is a major flavor and modifying ingredient in food. It also prevents the spoilage of fermented fruits by inhibiting the growth of undesirable microorganisms.

A continuous decrease in HCA content of *batuan* samples was observed until day 20 (Figure 4). A decreasing trend was observed from day 0–16, whereas the HCA level remained constant from day 16 until day 20. The addition of sodium chloride to the fermentation solution is the key factor for the observed decreasing trend. Sodium chloride creates an osmotic gradient between the *batuan* fruit and the brine solution. Osmotic exchange leads to the leaching of substrates such as carbohydrates (glucose mainly, as well as fructose, mannitol, and sucrose) and organic acids, including HCA. Consequently, the brine solution becomes a good medium for the growth of desired microorganisms (Piga and Aggabio, 2003). The decrease in HCA concentration may be attributed to the migration of acid from the fruit tissues to the brine solution where it is submerged. Moreover, oxidation, one possible deteriorative reaction of HCA, could have occurred. The high solubility of HCA to water also contributed to this decrease in acid content.

In the brine fermentation of *batuan* fruits, it is recommended to immerse the fruit in 5% brine solution for a maximum of 8 days only to maintain high levels of HCA.

Effects of freezing on HCA levels

Freezing preservation retains the quality of agricultural products over long storage periods. As a method of long-term preservation for fruits and vegetables, freezing is generally regarded as superior to canning and dehydration with respect to retention in sensory attributes and nutritive properties (Fennema, 1977).

Figure 5 shows the amount of HCA isolated from *batuan* fruits frozen at different time intervals. The obtained data indicate that frozen samples have a lower HCA content compared to fresh samples (Week 0). However, the trend was not definite as fluctuations in the computed amounts of acid were observed.

The abrupt decrease in the concentration of HCA after the first week can be attributed mainly to the cellular damage of *batuan* fruits due to ice formation during freezing. The water and dissolved solutes inside the rigid plant cell walls provide support to the plant structure and texture to the fruit or vegetable tissue. In the process of freezing, when water in the cells freezes, an expansion occurs, and ice crystals cause the cell walls to rupture (Desrosier and Desrosier).

Fast and slow freezing are two general types of freezing which can influence the extent of cellular damage. When heat is removed rapidly, ice forms rapidly, thus, the ice crystals tend to be small due to multiple nucleation sites that can be formed. The concentration of the external unfrozen matrix rises rapidly because of fast ice formation. Osmotic transfer of water is limited; thus, the cells freeze internally and a small amount of water translocates. In slow freezing, the ice forms slowly outside the cell. As a result, the time is sufficient for a large amount of osmotic transfer of water from the cells. This phenomenon results in cell shrinkage that can damage the membranes (Meryman, 1971; Steponkus, 1984).

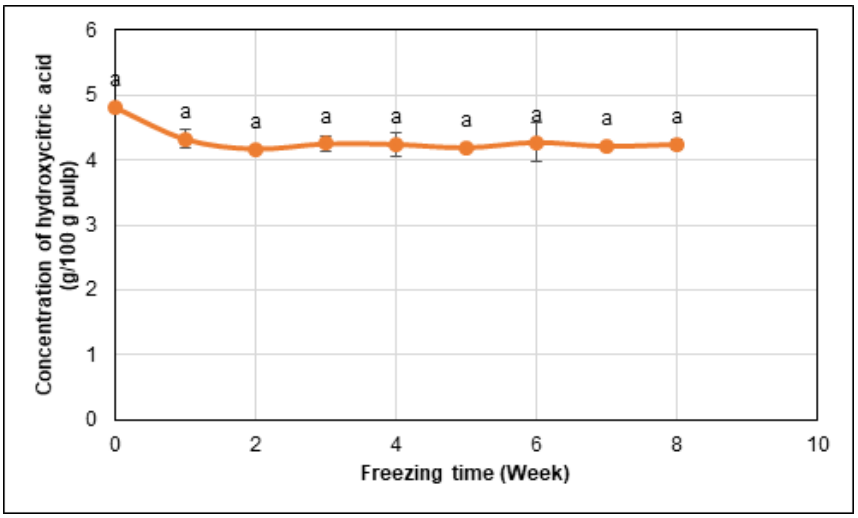

Figure 5. HCA content of batuan fruit pulp during frozen storage at -18°C. (Values with the same letters are not significantly different at P=0.05 using HSD test.)

In the experiment, drip loss was observed after thawing. It is important to note that the water that translocates from the cell contains nutrients and other compounds including HCA. Due to cell wall damage as a result of the freezing process, this water does not return to the cells on thawing and instead becomes drip loss.

Statistical analysis revealed that the control (Week 0) was not significantly different from other treatments despite the observed decrease in the amount of acid after subjecting the samples to freezing and thawing. This result indicates that HCA content was stable while in the frozen state. Therefore, it can be concluded that freezing up to 2 months does not significantly reduce the HCA present in *batuan* fruits.

Conclusion

Steam blanching at 100°C for 10 minutes, drying at 60°C, and brine fermentation for 8 days are the processing conditions that maintained the HCA content of *batuan* fruits. Freezing up to 2 months can be considered acceptable in terms of HCA content. Of all the processing methods used in this study, freezing can be considered the best method for preserving HCA.

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