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Vitamin C levels of selected Philippine indigenous berries as affected by fruit maturity and processing treatment

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Abstract

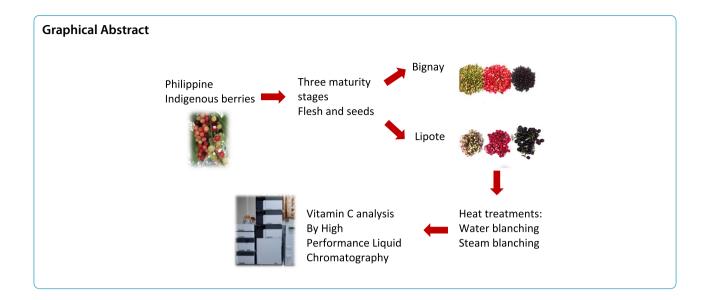
The Philippines as a tropical country is home to several indigenous berries that offer enough supply of healthpromoting bioactive compounds like vitamin C. Vitamin C is an important micronutrient in the human diet that is usually supplied by fruits and vegetables. The amount of this vitamin in different products varies depending on the species, variety, maturity, processing, and other conditions. In this study, the vitamin C contents of selected Philippine indigenous berries such as bignay and lipote were evaluated as affected by fruit maturity and processing treatment. Fruits of two bignay (Antidesma bunius (Linn.) Spreng), varieties, 'Common' and 'Kalabaw', as well as of lipote (Syzygium polycephaloides (C. B. Rob.) Merr.), at three maturity stages (unripe, half-ripe, and fully ripe) were acquired in Laguna, Philippines. Samples were subjected to two processing treatments: blanched (90 ± 5 °C, 2 minutes) and steamed $(105 \pm 5 \,^{\circ}\text{C}, 5 \,^{\circ}\text{minutes})$, while control samples did not undergo processing treatment. The flesh and seeds were separated, lyophilized, extracted, and subjected to quantification of vitamin C using reversed-phase high performance liquid chromatography. Results showed that the vitamin C levels of both fruits were significantly affected by maturity, processing, and their interaction (P < .05). In general, a concomitant increase in vitamin C content was noted as fruit maturity progressed for both flesh and seeds (0.3 to 1.7-fold increase). Lipote seeds on the other hand, had decreased vitamin C content as maturity progresses (0.6-fold decrease). Moreover, blanching the fruits resulted in the highest retention of vitamin C in the fruit samples (247% at most). The general findings of this study indicated that the utilization of these indigenous berries for future functional product development must be accompanied by the blanching - as a pretreatment process, of the fully ripe fruits to attain enhanced vitamin C contents.

Keywords Berries, Bignay, Lipote, Flesh, HPLC, Maturity, Processing treatment, Vitamin C

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Introduction

The global momentum gained by functional foods is substantially evident (Martí et al. 2009). The interest is parallel with the increasing cost of healthcare, the desire for a higher quality of life, and the overwhelming scientific evidence emphasizing the link between diet and health (Hasler 2000; Rincón-León 2003). Functional foods are natural or processed foods that impart positive health effects beyond their conventional nutritive value. These foods contain biologically active components, which, at defined levels, have clinically proven and documented benefits to health for the prevention, management, or treatment of chronic diseases. These bioactive compounds may act as antioxidants, cardioprotective, and chemo-preventive substances, which help address diseases, including cancer, obesity, and concomitant pathologies (Martirosyan & Singh 2015).

Bignay (Antidesma bunius (Linn.) Spreng), and lipote (Syzygium polycephaloides (C.B. Rob.) Merr.) are wild, fruit-bearing trees native to the Philippines. Bignay is a member of the family Phyllanthaceae (Butkhup & Samappito 2008), while lipote belongs to Myrtaceae (Janick & Paull 2008). The size of a single bignay fruit is around 1 cm, whereas a lipote fruit is about three times that size. Fruits of bignay are initially green and eventually turn dark purple or black as they ripen. On the other hand, the fruits of lipote are initially small and whitish pink and they turn dark purple upon full maturity. The ripe fruits of both plants are eaten fresh or processed. The fruits are usually used to make jam or jelly while the juice from the fully ripened berries can provide a refreshing drink and can also be made as an excellent wine. Fruit preserves and pickles may also be created from these berries. Moreover, its application can also be exploited in the baking industry and in the production of brandy and syrups (DA-BAR, Department of agriculture – bureau of agricultural research 2012; Lim 2012; Barcelo et al. 2016; ERDB, Ecosystems Research and Development Bureau 2017). Bignay and lipote have been utilized in folk medicine to treat multiple diseases (Islam et al. 2018; Micor et al. 2005). Khoo et al. (2017), among others, indicated that dark-colored fruits are naturally high in antioxidants.

Vitamin C, also known as ascorbic acid, is one of the essential bioactive compounds in the human diet. Fruits such as currants (black, red, and white), grapefruits, honeyberries or haskap berries, kiwis, lemons, mandarins, papayas, oranges, and strawberries have exceptionally high levels of this water-soluble vitamin (Giannakourou & Taoukis 2021; Grobelna et al. 2020; Sapei & Hwa 2014). According to the Commission Regulation (EU) 432/2012 (2012), there are several permitted health claims concerning vitamin C. One of which is its fundamental role in the biosynthesis of collagen for the normal function of blood vessels, bones, gums, cartilage, teeth, and skin. Other contributions of vitamin C to human health include enhanced iron absorption, preservation of vitamin E, and its participation in the normal functioning of the immune and nervous systems. Different studies have also shown that vitamin C preserves the levels of highdensity lipoproteins and reduces the content of total cholesterol, low-density lipoproteins, platelet aggregation, and fibrinogen in the plasma. Research also provides scientific proof that vitamin C enhances the bioactivity of nitric oxide, a known potent vasodilator, that helps widen the blood vessels, thereby increasing blood

flow and decreasing blood pressure (Dludla et al. 2022; Domitrović 2016; Hillstrom et al. 2003; Juraschek et al. 2012). Moreover, vitamin C is an excellent antioxidant that helps ameliorate the negative impact of oxidative stress, a condition resulting from an imbalance of reactive oxygen species (ROS; e.g. superoxides, peroxides, radicals, etc.) levels relative to intrinsic antioxidant defense systems (Savini et al. 2013). It acts by donating or receiving a supplementary electron that terminates the chain of oxidative reaction (Halliwell, 2007). Based on those mentioned properties, vitamin C has been recognized as an effective agent for the prevention of cardiovascular and neurodegenerative diseases, certain types of cancer, atherosclerosis, and diabetes (Chambial et al. 2013; Moser & Chun 2016). Furthermore, ascorbic acid is considered the primary indicator of quality during the processing and storage stages. Generally, the retention of ascorbic acid translates to the retention of other bioactive compounds. Nevertheless, owing to the reducing and antioxidative properties of ascorbic acid, it is commonly used in the food industry for preventing the enzymatic browning of horticultural crops and for the curing of meats (Giannakourou & Taoukis 2021).

As food innovators and manufacturers continue to invest in adding a health criterion in their products, there have been numerous efforts to boost ascorbic acid retention in functional foods even after processing and storage. According to Lee and Kader (2000), the content of vitamin C in fruits and other horticultural crops can be influenced by various factors like species, variety, maturity, and processing methods. Ascorbic acid may be higher or lower during the peak of maturity, depending on the fruit type (Brandão et al. 2011; Sapei & Hwa 2014). Common processing methods, such as blanching and steaming also affect vitamin C content (Rickman et al., 2007). Blanching is a type of mild heat treatment popularly used to inactivate enzymes in fruits before storage or further processing (Huang et al. 2016). Steaming is a cooking method that uses steam by continuous boiling of water until it vaporizes (Lafarga et al. 2018).

Most of the studies on the vitamin C content of bignay are limited to using fresh, ripe fruits. For instance, Khomdra et al. (2017) found that the ascorbic acid content of an Indian bignay cultivar was 7.80 mg/100g fresh weight. Castillo-Israel et al. (2020) assessed the effects of maturity and processing on the antioxidant properties of bignay 'Common', but excluding its vitamin C content. The blanched bignay seeds and flesh generally had higher antioxidant activity than steamed and unprocessed samples. The seeds and flesh of fully ripe fruits had generally higher antioxidant levels and antioxidant activities relative to unripe and half-ripe samples. Studies on lipote are even more scant and limited to plants under the same genus. For example, Nunes et al. (2016) reported that lyophilized samples of Malay Apple (Syzygium malaccense) had $171.14 \pm 1.61 \text{ mg AA}/100 \text{ g}$, while Ayyanar and Subash-Babu (2012) found that Jambolan (Syzygium cumini (L.) Skeels) had 5.70-18.00 mg AA/100 g. The vitamin C content of bignay and lipote as affected by maturity and processing has not been quantified yet. Therefore, this study aimed to determine the influence of maturity stages and processing techniques (specifically blanching and steaming) on the vitamin C content of the selected Philippine indigenous berries. The industrial use of bignay and lipote can be expanded as this study laid down some basis for these fruits to be used as functional food ingredients. For example, different products may be derived from these berries like spray-dried and freeze-dried juice extracts for instant beverages and freeze-dried pulp powder for immediate healthy snacks. It can also be incorporated into a known commercial product (e.g. juices and purees) for possible enhanced bioactive content (Grobelna et al. 2019b). Information on the degradation of vitamin C brought about by processing is relevant in development of processing methods for functional foods which will be developed out of these fruits in the future. The potency of the final product is the most important attribute of a functional food, and this study has shown trends in vitamin C levels when subjected to various heat treatments.

Materials and methods

Chemicals

All chemicals used in this study were HPLC grade or analytical grade. HPLC water and acetonitrile were supplied by Duksan Pure Chemicals Co., Ltd. (South Korea). Phosphoric acid (H_3PO_4) and sodium hydroxide (NaOH) were purchased from RCI Labscan (Bangkok, Thailand). L-ascorbic acid was procured from Sigma-Aldrich Corporation (Singapore), ethylenediaminetetraacetic acid (EDTA) was obtained from J.T. Baker[®] (New Jersey, USA), sulfuric acid (H_2SO_4) was acquired from Macron Fine ChemicalsTM (New Jersey, USA), and metaphosphoric acid (MPA) was purchased from Merck (Darmstadt, Germany).

Sample preparation

Bignay (*Antidesma bunius* (Linn.) Spreng), varieties, 'Common' and 'Kalabaw', and lipote (*Syzygium polycephaloides* (C. B. Rob.) Merr) fruits were harvested in the province of Laguna, Philippines. The identity of the plant materials was validated by a curator from the Botanical Herbarium, Museum of Natural History, University of the Philippines, Los Baños. The collected fruits were washed and cleaned with tap water and divided into three maturity stages according to their size and color. Each maturity stage was subdivided into three groups. The first group was blanched in a water bath at 90 ± 5 °C for 2 minutes, the second group was steamed through a one-layer stainless-steel food steamer at 105 ± 5 °C for 5 minutes, and the last group was left unprocessed. The seeds and the flesh with the skin were manually separated and were lyophilized using a freeze-dryer. The lyophilized samples were pulverized using a DV Tech[®] grinder Model 525 (Madrid, Spain), and the resulting fine powder was sieved through 10-mm mesh sieves, then stored in a clean, dry, and airtight container away from direct light at room temperature until further use.

Vitamin C extraction

The extracting solution used was based on the method described by Franke et al. (2004) with some modifications. About 167 mg of the powdered freeze-dried samples were extracted with 5 mL of the extracted solution containing 3% MPA, 0.15 M $\rm H_2SO_4$, and 1 mM EDTA for 60 minutes at 4°C, in the dark. The supernatants were collected and transferred to an amber glass bottle and stored at 4°C before subjecting to chromatographic analysis.

Vitamin C analysis via HPLC

All standard solutions and sample extracts were filtered using a 0.22-µm PVDF syringe filter. Exactly 500 uL of the filtered standards and samples were placed in 1.5 mL HPLC vials, and sample injection volume was set at 10 uL. Analysis of vitamin C was performed using Shimadzu Prominence HPLC (Tokyo, Japan) equipped with an LC-20AD pump with DGU-20A5R degasser, Sil-20AHT UFLC autosampler, and SPD-M20A diode array detector. Separation was carried out using an Inertsil ODS-3 $(250 \text{ mm} \times 4.5 \text{ mm} \times 5 \mu \text{m})$ reverse-phase C18 column protected with an Inertsil ODS-3 $(4.0\,mm \times 10\,mm \times 5\,\mu m)$ guard column. The entire chromatographic analysis was maintained at 25°C using a CTO-10ASVP column oven and isocratic elution was conducted using 88:12 (v/v) acetonitrile:water (at pH2.50, acidified with H_3PO_4) at a flow rate of 1 mL/min. Vitamin C detection was carried out at 254 nm using an M20A diode array detector. Chromatograms were obtained and analyte peaks were analyzed using the LabSolution software and all analyses were done in triplicate.

Method validation

Magnusson and Örnemark (2014) mentioned various parameters that need to be established to validate an analytical method for its intended purpose. These include linearity, accuracy, precision, LOD, LOQ, and robustness. Linearity was defined by identifying the coefficient of determination (R2) from the established calibration graph (plot of concentration versus peak area) of the series of ascorbic acid standard solutions (25-800 µg/mL). The accuracy was assessed through the standard addition method wherein samples were spiked with 50 µg/mL and 100 µg/mL ascorbic acid standard and calculating the percent recovery (% recovery=recovered amount*100/ injected amount) of the standard in the mixture. Precision was evaluated by determining the percent relative standard deviation (%RSD) of the analytical readings (retention time and peak area) of six ascorbic acid standard solutions from the same batch. The limit of detection (LOD) and limit of quantitation (LOQ) were obtained by multiplying the ratio of the standard deviation of the y-intercepts and the slope of the established calibration graph by 3.3 and 10, respectively. Robustness was assessed by computing the percent RSD of the experimental values upon varying the pH of the mobile phase (pH 2.5 ± 0.5), and the temperature $(25^{\circ}C \pm 5^{\circ}C)$ of the chromatographic analysis.

Statistical analysis

The experiment was laid out in a completely randomized design (CRD) with results presented as mean \pm SD of triplicate determinations. Two-way ANOVA was used to evaluate the influence of the maturity stages and processing treatments on the vitamin C content of the flesh and seed of the berry samples, followed by Tukey's Honest Significant Difference (HSD) test for mean comparisons. Data analysis was carried out using Minitab 18.0 for Windows at 95% significance level.

Results and discussion

Extraction and HPLC analysis of vitamin C in Philippine indigenous berries

Minimal degradation and maximum recovery of vitamin C from bignay and lipote fruits were attained using a high sample-to-solvent ratio (1:30 w/v), protecting the extraction medium from light, working at low temperature (~4°C), and utilizing vitamin C-preserving chemicals such as MPA, EDTA, and sulfuric acid. Increasing the sample-to-solvent ratio creates a higher concentration gradient and induces mass transfer between the analyte and solvent while conducting the process devoid of light and at a low temperature reduces the possible oxidation of vitamin C (Durdun et al. 2016; Lee & Kader 2000). Similar observations were also noted by Kalisz and Kieliszek (2021) in their study wherein the vitamin C content of their berry sample was found most stable in a refrigerated condition (4°C) and away from direct light contact. Moreover, the vitamin C extraction process in the presence of MPA, EDTA, and sulfuric acid provides

additional stability and protection of the analyte against various degrading agents (Nelis et al. 1997).

The chosen HPLC parameters offered appreciable results in terms of the overall chromatographic characteristics (peak's sensitivity, shape, and area) of vitamin C which eluted at around 2.477 minutes. A typical chromatogram of the standard ascorbic acid measured at 254 nm is shown in Fig. 1, along with the representative chromatograms of bignay and lipote sample extracts. Based on the extracted chromatograms, the detected peak of the samples corresponds to the peak of the standard ascorbic acid, thereby confirming the identity of vitamin C in the samples.

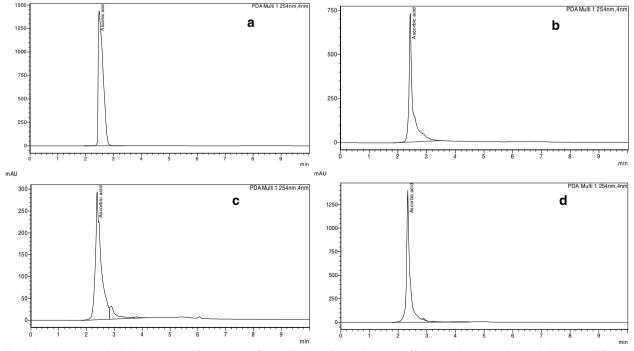
Method validation

The HPLC method used here for the quantification of vitamin C in Philippine indigenous berries was validated by determining accuracy (recovery), precision, recovery, linearity, limit of detection (LOD), limit of quantitation (LOQ), and robustness. A good linearity (R^2 value = 0.9995) was achieved within the concentration range (25–800 µg/mL). A high average recovery (103.81%) was noted and low percent RSD values (below 1%) were obtained from the repeatability test of the retention time and peak area of the standard ascorbic acid indicating high precision. LOD and LOQ

were 3.840 and $11.635 \,\mu$ g/mL, respectively, implying a high sensitivity of the method. The low variability (RSD below 5%) among the data points obtained upon varying the pH of the mobile phase (3.177% RSD) and the temperature of the chromatographic analysis (0.932% RSD) demonstrated the capacity of the present analytical procedure to remain unaffected by small changes in the method parameters indicating an appreciable robustness. Overall, the obtained R², % RSD, % recovery, LOD, and LOQ values indicated a linear, precise, accurate, sensitive, and robust HPLC method for the quantitative determination of vitamin C in bignay and lipote berries.

Effect of fruit maturity and processing treatment on vitamin C levels

An increase in vitamin C levels of the berry samples was observed as fruit maturity progressed (Fig. 2). The vitamin C content of fully ripe bignay 'common' and 'kalabaw', and lipote flesh increased by 1.7-, 1.0-, and 0.3-fold, respectively, compared to their unripe counterparts. Moreover, processing the samples generally enhanced the retention of vitamin C. Blanching and steaming of bignay (cv. 'Common' and 'Kalabaw') fruit flesh slightly increased the vitamin C content by 1–38%, while pretreatment of lipote fruit flesh greatly enhanced the



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Fig. 1 Representative HPLC chromatograms ($\lambda = 254$ nm) of **a** standard ascorbic acid, extracts of **b** bignay 'Common', **c** bignay 'kalabaw', and **d** lipote

yield of vitamin C by 247 and 121% for blanching and steaming, respectively. Vitamin C levels of bignay and lipote seeds were significantly affected by fruit maturity and processing treatment (Fig. 3). Apart from bignay 'Kalabaw' seeds, the interaction between the two variables was also found to be significant (P < 0.05). It was observed that as the fruit matures, the vitamin C content in bignay ('Common' and 'Kalabaw') seeds increased while a decline in lipote seed vitamin C levels was noted (Fig. 3). Fully ripe bignay 'Common' and 'Kalabaw' seeds increased by 0.9- and 0.3-fold, respectively, compared to their unripe equivalents. In contrast, about 0.6-fold decrease in vitamin C levels of lipote seeds were noted in relation to maturity progression. In addition, except for the unprocessed lipote seeds, the blanching treatment generally enhanced the retention of vitamin C in the berry samples. A general increase of about 38, 5, and 108% in the vitamin C levels of blanched bignay 'Common', bignay, 'Kalabaw', and lipote seeds was observed, respectively. On the other hand, the steaming procedure slightly decreased the vitamin C level in bignay ('Common' and 'Kalabaw') seeds and moderately enhanced it in the half-ripe and fully ripe lipote seeds. A decrease of about 4 and 0.3% and an increase of around 104% were observed, respectively, for the steamed berry samples.

Fruit maturity is one of the major factors that affect the chemical composition of fruits (Lee & Kader 2000). In the present study, it was observed that the amount of vitamin C in the Philippine indigenous berries mainly increased with maturity. Also, apart from the lipote seeds, the rest of the fully ripe berry samples generally exhibited the highest vitamin C content compared to other maturity stages. The study of Valšíková-Frey et al. (2017) reported similar findings wherein an increase of about 134% in the vitamin C levels of tomato fruits were observed upon full maturation. Badejo et al. (2012) also reported that a 2-fold increase in ascorbic acid content was noted for fully ripe micro-tom fruits. Other similar findings by Barata-Soares et al. (2004) in papayas, Martínez et al. (2005) in red peppers, Cruz-Rus et al. (2010) on grapes, and Padmanabhan (2016) on strawberries revealed a positive correlation between vitamin C content and maturity of the fruits. In the case of lipote seeds, a similar trend was also reported in the study of Liang et al. (2017) on sweet cherries with decreasing ascorbic acid content in relation to maturity progression. Despite the observed decrease in ascorbic acid content per unit fresh weight, the ascorbic acid content per fruit was reported to generally increase upon maturity. Since the ascorbic acid content per fruit was obtained by the product of the average fruit weight

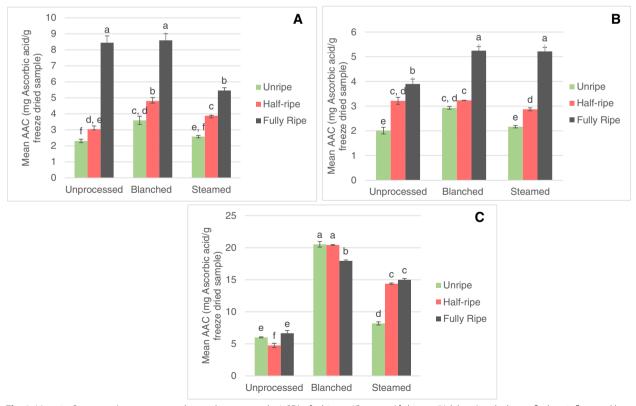


Fig. 2 Vitamin C content (mean mg ascorbic acid per g sample \pm SD) of **a** bignay 'Common', **b** bignay 'Kalabaw', and **c** lipote flesh as influenced by fruit maturity and processing treatment. Bars with different lowercase letters denote significant difference (P < 0.05, Tukey's HSD test)

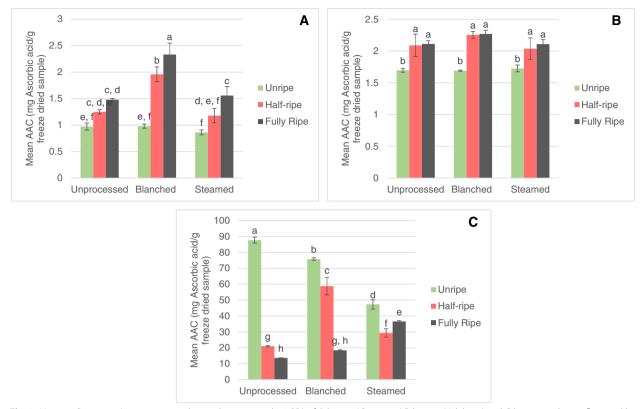


Fig. 3 Vitamin C content (mean mg ascorbic acid per g sample \pm SD) of **A** bignay 'Common', **B** bignay 'Kalabaw', and **C** lipote seeds as influenced by fruit maturity and processing treatment. Bars with different lowercase letters denote significant difference (P < 0.05, Tukey's HSD test)

and the concentration of ascorbic acid based on fresh weight, the increase in size and weight of sweet cherries during maturity may be the reason for the overall increase in the ascorbic acid content per fruit (Liang et al. 2017). The lipote fruit also exhibits the same characteristics upon maturity as sweet cherries, thus it is possible that the ascorbic acid contents of the half-ripe and fully ripe fruits may be greater than the ascorbic acid content of the unripe fruit when measured on a per fruit basis.

According to Atkinson et al. (2015), the vitamin C content of most fruits is generally low during the early stages of fruit maturity because the fruit is still in the process of cell development. As the fruit proceeds towards physiological maturity, the ascorbic acid content increases due to the rise of the concentration of free sugars (Mellidou et al., 2012). Monosaccharides such as mannose and galactose are important precursors in the biosynthesis of ascorbic acid in plants. These sugars are also derived from glucose through various isomerization and activation steps (Cruz-Rus et al. 2010). Another physiological manifestation associated with fruit maturity is cell wall degradation (Waldron et al. 2003). Fenech et al. (2019) reported that the degradation of cell wall pectin can provide an abundant substrate for the biosynthesis of ascorbic acid. The common biosynthetic pathway of ascorbic acid in plants under L-galactose and D-galacturonate pathways. The latter includes degradation of cell wall pectin to galacturonate, conversion of galacturonate to galactonate, and oxidation to galactono-1,4-lactone, towards ascorbic acid. On the other hand, L-galactose pathway involves the conversion of mannose to galactose, oxidation of galactose to galactono-1,4-lactone, and further oxidation to ascorbic acid. The last step of ascorbic acid biosynthesis is the oxidation of L-galactono-1,4-lactone to L-ascorbic acid catalyzed by L-galactono-1,4-lactone dehydrogenase (GalLDH). Enzymatic studies indicate that it is an important regulatory step for L-ascorbic acid biosynthesis (Valpuesta & Botella 2004). The study of Pateraki (2004) revealed that the ripeningassociated increases in GalLDH mRNA levels were accompanied by subsequent increases in ascorbic acid content in melons. This suggests a positive correlation between ripening and ascorbic acid content in fruits. In addition, fruit ripening is a developmental process wherein several oxidative reactions occur, and various reactive oxygen species (ROS) accumulate (Jimenez et al., 2002). To balance the excess ROS, the plant's mechanism is to increase the levels of antioxidants such as ascorbic

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acid (Meitha et al. 2020). Fruit ripening is also highly associated with ethylene, the "ripening hormone". Ascorbic acid is necessary as a cofactor for 1-aminocyclopropane-1-carboxylic acid oxidase (ACCO) in the final step of ethylene biosynthesis. This condition may explain the increased ascorbic acid content in mature fruits.

Processing treatment is another factor that also affects the composition and quality of fruits (Lee & Kader 2000). It is imperative to select suitable processing techniques to preserve the bioactive compounds and to produce products that have high nutritional content (Grobelna et al. 2019a). In the current study, the results obtained were variable, especially in the seeds of the berry samples. The amount of vitamin C retained in the berry samples generally increased upon subjecting to thermal processing treatment, especially blanching. These were mainly noted on the fruit flesh of the berry samples. Except for the unprocessed lipote seeds, the blanched berries showed the highest retention of vitamin C. Moreover, apart from the bignay ('Common' and 'Kalabaw') seeds and the unprocessed lipote seeds, steamed berry samples also had enhanced retention of the said bioactive compound. The increase in vitamin C retained upon blanching of the fruits of Philippine berries studied here was consistent with the findings of Jeevitha et al. (2014), wherein they reported that blanched peppers had greater vitamin C retention than their unblanched counterparts. High retention of vitamin C was also found in dried mango after the blanching treatment (Guiamba et al., 2018). In the case of steaming, Popova (2019) reported that about 70% of the ascorbic acid in potato and cauliflower was retained after the processing treatment, and he further suggested that steaming is a suitable pretreatment for vitamin C retention.

Heat treatments like blanching and steaming are said to improve the levels of bioactive compounds in plant foods by enhancing the membrane permeability of the cell matrix and through thermal inactivation or reduction of enzymes associated with the degradation of these components (Castillo-Israel et al. 2020). In the case of vitamin C, heat treatment inhibits the activity of ascorbic acid oxidase (AAO), the main enzyme responsible for the oxidation of ascorbic acid (Xanthakis et al. 2018). Lee et al. (2017) further pointed out that to prevent more degradation and to retain much higher vitamin C during the heat treatment procedure, the plant material must be thermally processed at a high temperature over a short time (HTST). This was highlighted because Guiamba et al. (2018) mentioned that heat treatment at a low temperature over a longer time (LTST) resulted in a slower inactivation of the AAO enzyme, thus leaving a higher chance of ascorbic acid oxidation in the samples. The study of Wawire et al. (2011) revealed that heating at temperatures above 90 °C for short times (HTST) resulted in complete inactivation of AAO, thereby providing a protective effect for ascorbic acid against enzyme-catalyzed oxidation. These findings coincide with the current processing treatments wherein the blanching procedure (90 ± 5 °C for 2 minutes) generally provided greater vitamin C retention in the sample than the steaming process (105 ± 5 °C for 5 minutes).

Conclusion

Berries are rich in health-promoting compounds such as vitamin C. In order for these plant materials to be incorporated into various products in the future, it is important to evaluate the factors (e.g. maturity and processing) that provide the highest vitamin C retention in the samples. Based on the present study, the vitamin C content of bignay 'Common', bignay 'Kalabaw', and lipote fruits are significantly affected by fruit maturity and processing treatments (blanching and steaming). In general, the fully ripe bignay and lipote fruits studied here yielded the highest amount of vitamin C. Moreover, subsequent thermal processing treatments, especially blanching $(90\pm5^{\circ}C \text{ for})$ 2 minutes), generally enhanced the retention and amount of vitamin C in the said berry samples. Future functional food development initiatives (e.g. spray-dried and freezedried juice extracts for instant beverages and freeze-dried pulp powder for immediate healthy snacks) involving these Philippine indigenous berries may take advantage of blanching the fully ripe fruits in order for the products to have improved amounts of vitamin C that may be beneficial to consumers.

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Authors' contributions

KATCI conceptualized the research, obtained project funding, supervised the experiments, edited the manuscript. LELF optimized the protocol, analyzed the samples and data, and was the major contributor in writing the manuscript. APPT supervised the experiments, edited the manuscript. KJDS conducted experiments and contributed to manuscript writing. MCMC conducted experiments and contributed to manuscript writing. All authors read and approved the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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