FOOD
RESEARCH

Antioxidant properties of lipote (*Syzygium polycephaloides* (C.B. Rob.) Merr.) flesh and seeds as affected by maturity and processing method

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Abstract

Lipote (*Syzygium polycephaloides* (C. B. Rob.) Merr.) is a Philippine endemic tree whose berries have a potential for functional food development. This study aimed to investigate the effect of the maturity stage (unripe, half-ripe or fully ripe) and processing (blanching at 90±5°C for 2 mins and steaming at 105±5°C for 5 mins) on the antioxidant content (total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC)) and antioxidant activity by DPPH, ABTS, and FRAP assays, of lipote flesh and skin (referred herein as flesh) and seeds. Results showed that maturity and processing significantly affect the antioxidant contents and activities of fresh lipote flesh and seeds. Blanched lipote flesh generally had higher TPC, TFC, and antioxidant activities than their unprocessed and steamed counterparts. Blanched unripe flesh had the highest TPC (33.57±0.27 mg gallic acid equivalents [GAE]/g) and antioxidant activity measured by ABTS, FRAP, and DPPH assay (92.31±1.28 mg Trolox equivalents [TE]/g, 123.83±0.86 mg TE/g, and 74.02±0.33 mg TE/g, respectively) while blanched fully ripe flesh had the highest TFC (27.98±0.08 mg quercetin equivalents [QE]/g) and TAC (18.11±0.11 mg cyanidin-3-glucoside equivalents [C3GE]/g). Lipote seeds, especially unripe ones, have appreciable antioxidant contents and antioxidant activity, even greater than that of lipote flesh. Unprocessed unripe seeds had the greatest TPC, TFC and antioxidant activity by DPPH assay (123.56±0.94 mg GAE/g, 111.90±0.14 mg QE/g and 465.89±1.86 mg TE/g, respectively). It is recommended that blanching be used as a processing step to increase the antioxidant content of lipote flesh while lipote seeds should be investigated further for nutraceutical applications due to their high antioxidant properties.

1. Introduction

Today, a rise in global interest in functional foods is evident. Functional foods are those providing health benefits other than basic nutrition. They contain physiologically active components that help reduce chronic disorders like cardiovascular diseases, hypertension, cancer, diabetes, and other diseases. Oxidative stress, the disruption of the balance between reactive oxygen species (ROS) and antioxidant defences in the body, is one condition that has been looked into as it has been associated with obesity, hyperlipidemia, neurological disorders, cancer and chronic obstructive pulmonary disease (Birben *et al.*, 2012).

Antioxidants have been known to mitigate the negative effects of oxidative stress by inactivating ROS, interrupting the radical chain reaction that generates more ROS, and chelating metal ions responsible for the production of ROS, among others (Flora, 2009). The role of dietary antioxidants in alleviating or preventing oxidative stress and related diseases have been continually studied (Duthie *et al.*, 2000; Blomhoff, 2005; Kaliora *et al.*, 2006). Natural pigments and antioxidants are commonly found in most plant materials.

Lipote (*Syzygium polycephaloides* (C.B. Rob.) Merr.) is a Philippine endemic tree. Its berry is eaten ripe and raw, or juiced and made into wine. Santiago *et al.* (2007) reported that the antioxidant activity of lipote fruits was comparable to that of Vitamin E. The study also reported that the berry contained 58.85 μg catechin

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per gram of fresh sample and exhibited a percent inhibition of linoleic acid peroxidation of 73.7%.

The antioxidant content of berries is affected by maturity and processing (Kalt, 2005; Tiwari and Cummins, 2013). Macheix *et al*. (1990) reported that the antioxidant activity of dark-coloured fruits like lipote increases with maturity. The same trend was reported by Castillo-Israel *et al*. (2020) and Sartagoda *et al*. (2020) for the 'common' and 'kalabaw' variety of bignay (*Antidesma bunius* (L). Spreng.), respectively. Processing techniques were also reported to have varying effects on the antioxidant contents of fruits and vegetables (Tiwari and Cummins, 2013). Some fruits and vegetables were reported to have increased antioxidant activity when subjected to high temperature processing techniques like boiling, microwaving, and pressure cooking (Kim *et al*., 2008; Ng and Kuppusamy, 2019). Steaming and blanching were also reported to increase the antioxidant content and activity of bignay (Castillo-Israel *et al*., 2020; Sartagoda *et al*., 2021). On the contrary, Chumyam *et al*. (2013) reported a significant decrease in the antioxidant activity of eggplant when boiled, steamed, and microwaved.

Two common processing steps employed in fruits and vegetables are steaming and blanching. Blanching is a mild heat treatment that primarily aims to inactivate enzymes (Vaclavik and Christian, 2014) while steaming is a cooking method that uses steam generated from boiling water (Lean, 2006).

In a similar vein, the present study aimed to investigate the effect of maturity (unripe, half-ripe, and fully ripe) and processing technique (blanching and steaming) on the antioxidant contents and *in vitro* antioxidant activity of lipote fruit. Results of this study can be used as evidence for the development of functional foods from lipote.

2. Materials and methods

2.1 Plant material preparation

Lipote (*S. polycephaloides* (C. B. Rob) Merr.) fruits were harvested in the province of Laguna, Philippines. The identity of lipote was authenticated by a curator at the Botanical Herbarium, Museum of Natural History, University of the Philippines Los Baños. The collected fruits were washed with water and divided into three maturity stages based on their size and colour (Figure 1).

Each maturity stage was subdivided into three lots. The first lot was subjected to blanching at 90±5°C for 2 mins, the second lot was steamed at 105±5°C for 5 mins, and the last lot was left unprocessed. Blanching was

carried out using a water bath while steaming was carried out using a one-layer stainless steel food steamer. All lots were then pulped to separate the seeds from the flesh. The separated flesh and seeds were freeze-dried and ground. The resulting powder was then sieved and stored at room temperature in clean, airtight containers protected from light.
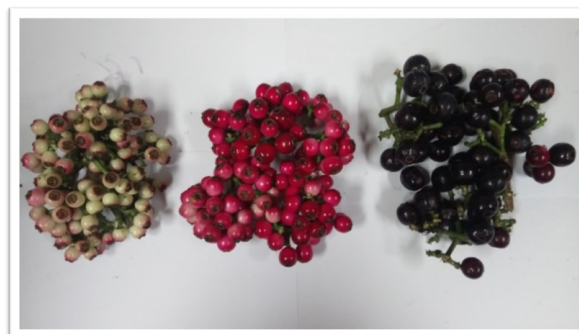

Figure 1. Ripening stages of lipote. From left to right: unripe, half-ripe and fully ripe

2.2 Sample extraction

Extraction was done according to the method described by Larrauri *et al*. (1997) with some modifications. Briefly, 0.5 g of the freeze-dried sample was extracted with 15 mL solution of methanol: water: acetic acid (50:50:1, v:v:v) in a shaker for an hour at room temperature. The mixture was then centrifuged for 15 mins. After that, the supernatant was collected and transferred to an amber glass bottle before storing it at 0-4°C. Appropriate dilutions of the extracts were done for each analysis.

2.3 Determination of total phenolic and total flavonoid content

Quantification of the total phenolic content (TPC) of the samples was done using the Folin–Ciocalteau method described by Waterhouse (2002) with some modifications. A 0.3 mL portion of the diluted extract was mixed with 1.5 mL 10% Folin-Ciocalteau reagent. The extract was allowed to stand for 5 mins at room temperature. After 5 mins, 1.2 mL of 4% Na_2CO_3 was added. The solution was vortexed and was allowed to stand for 90 mins. The absorbance readings were measured at 760 nm.

On the other hand, the total flavonoid contents (TFC) of the samples were quantified using the aluminium chloride assay of Luximon-Ramma *et al*. (2002) with some adjustments. Briefly, a 2.5 mL portion of the diluted sample was mixed with 0.5 mL of 10 % $AlCl_3$ solution. The mixture was allowed to stand for 15 mins at room temperature before its absorbance was read at 367.5 nm.

Methanolic solutions of gallic acid were used as standards for TPC analysis, while methanolic solutions of quercetin were used as standards for TFC analysis. Results were computed as milligrams standard per gram freeze-dried sample.

2.4 Determination of total anthocyanin content

The pH differential method described by Lee *et al*. (2005), was used to quantify the total anthocyanin content (TAC) of the sample. Potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.40 M, pH 4.5) were used to dilute the extracts to a minimum dilution factor of 5. Dilution factors were chosen in such a way that the absorbance of the sample diluted with the pH 1.0 buffer at 520 nm is within 0.2 – 1.2 absorbance units (AU). The absorbance of the extracts diluted with the pH 1.0 buffer and pH 4.5 buffer were then read at 520 nm and 700 nm against a blank of distilled water. After that, the total anthocyanin content (mg cyanidin-3-glucoside equivalents/L extract) was computed using Equation (1).

$$TAC \ (mg/L) = A \times MW \times DF \times (1000/(e \times L)) \qquad (1)$$

Where A is the absorbance = (A510 nm - A700 nm) pH 1.0 - (A510 nm - A700 nm) pH 4.5, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is the dilution factor, e is the extinction coefficient of cyanidin 3-glucoside (26,900 L/mol/cm) and L is the cell path length (1 cm). The computed total anthocyanin content was then converted to milligrams cyanidin-3-glucoside equivalents per grams freeze-dried sample.

2.5 DPPH radical scavenging activity assay

The DPPH radical scavenging activity assay was adapted based on the method described by Pisoschi and Negulescu (2011). A 1 mL portion of the diluted sample was mixed with 1 mL absolute methanol and 1 mL of freshly prepared DPPH solution. Methanolic solutions of Trolox were used as standards. The absorbance was then read at 517 nm against a blank of absolute methanol. Results were expressed as milligram Trolox equivalents per gram freeze-dried sample.

2.6 ABTS assay

The ABTS or TEAC assay was done according to the method described by Tomasina *et al*. (2012) with some adjustments. The ABTS radical cation was prepared by reacting a 0.25 mM ABTS stock solution with 3.45 mM potassium persulfate, allowing the mixture to stand in the dark for 12-16 hours at 4°C. After this, the ABTS radical cation solution was diluted with methanol until an absorbance of 0.70±5 at 734 nm was achieved. Then, 2.7 mL of ABTS radical cation solution was added to 0.3 mL of the diluted sample. The mixture

was allowed to stand at room temperature in the dark for 15 mins. Methanolic solutions of Trolox were used as standards. The absorbance of the solution was measured at 734 nm. Results were expressed as mg Trolox equivalents per g sample.

2.7 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay of the samples was done using a modified method described by Tomasina *et al*. (2012). Briefly, 2.7 mL of freshly prepared FRAP reagent (300 mM acetate buffer (pH 3.6):10 mM TPTZ (10 mM):20 mM $FeCl_3$ solution; (10:1:1, v:v:v) was added to 0.3 mL of the diluted sample. The reaction mixture was incubated at 37°C for 5 mins before its absorbance was read at 620 nm. Methanolic solutions of Trolox were used as standards. Results were reported as mg Trolox equivalents per g sample.

2.8 Statistical analysis

The experiments were arranged in a completely randomized design with values presented as mean±SEM computed from three values. The influence of the maturity stage and processing treatment on TFC, TPC, and TAC and antioxidant activities (ABTS, DPPH, and FRAP) of fruit seeds and flesh were analysed using a two-factor factorial ANOVA. Differences between mean values were assessed by Tukey's Honest Significant Difference (HSD) test. All the statistical tests were performed at the significance level of 0.05 using Minitab 19.0 for Windows (Minitab, 2019).

3. Results

3.1 Effect of maturity and processing method on the TPC, TFC, and TAC of lipote flesh

Maturity and processing, as well as their interaction, have significant effects on the TPC, TFC, and TAC of lipote flesh ($p \leq 0.05$). Blanched unripe lipote flesh had the highest total phenolic content (33.57±0.27 mg GAE/g) while blanched fully ripe lipote flesh had the highest total flavonoid content and total anthocyanin content (27.98±0.08 mg QE/g and 18.11±0.11 mg C3GE/g, respectively). Generally, blanched flesh had higher TPC and TFC than both their steamed and unprocessed counterparts. Moreover, steamed flesh generally had higher TPC and TFC than the unprocessed counterparts. In addition, the thermally processed seeds had greater TAC values than their unprocessed counterparts (Figure 2, Table 1).

For fully-ripe lipote flesh, blanching caused a 64.8% increase in TPC and a 38.6% increase in TFC while steaming caused a 60.2% increase in TPC and a 30.9% increase in TFC. For half-ripe lipote flesh, blanching
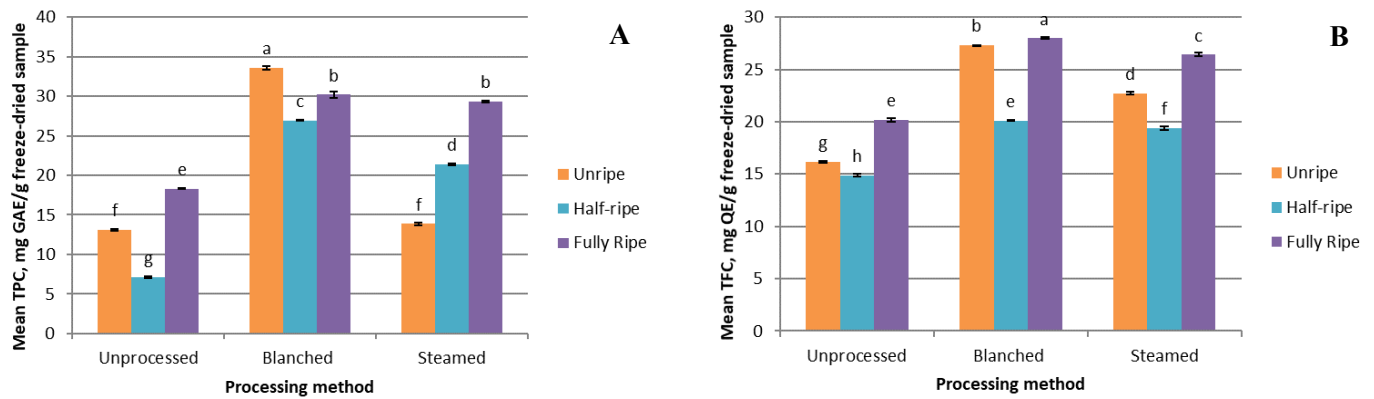
Figure 2. Total phenolic content (A) and total flavonoid content (B) of lipote flesh as affected by maturity and processing method before freeze-drying (mean±SEM). Bars with different lowercase letters denote a significant difference (p ≤ 0.05; Tukey's test)

caused a 277% increase in TPC and 35.4% increase in TFC while steaming caused a 199% increase in TPC and 30.6% increase in TFC. For unripe lipote flesh, blanching caused a 156% increase in TPC and 69.1% increase in TFC while steaming caused a 5.73% increase in TPC and a 40.7% increase in TFC (Figure 2).

Table 1. Total anthocyanin content of lipote flesh

Processing method	Maturity stage	Total anthocyanin content, mg cyanidin-3-glucoside equivalents/ g freeze-dried sample
Unprocessed	Unripe	ND
	Half-ripe	0.28±0.02[e]
	Fully ripe	8.02±0.43[c]
Blanched	Unripe	ND
	Half-ripe	0.89±0.00[e]
	Fully ripe	18.11±0.11[a]
Steamed	Unripe	ND
	Half-ripe	1.78±0.00[d]
	Fully ripe	13.25±0.01[b]

Values are expressed as mean±SEM, n = 3. Values with different superscript are significantly different (Tukey's test, p ≤ 0.05). ND, not detected.

TAC of lipote flesh increases as the fruit matures regardless of processing method before freeze-drying (Table 1). On the other hand, the influence of maturity in TPC is variable. For unprocessed flesh, TPC decreases from the unripe stage to the half-ripe stage and then increases markedly upon reaching the fully ripe stage. For blanched flesh, TPC decreases from the unripe stage to the half-ripe stage and decreases marginally upon reaching the fully ripe stage. For steamed flesh, TPC increases as the fruit matures. Finally, TFC is usually greatest at the fully ripe stage and the least at the half-ripe stage for all processing methods before freeze-drying (Figure 2).

3.2 Effect of maturity and processing method on the antioxidant activity (ABTS, DPPH, and FRAP) of lipote flesh

Maturity and processing, as well as their interaction, have significant effects on the antioxidant activities measured by ABTS, DPPH, and FRAP assays of lipote flesh (p<0.05). Blanched unripe lipote flesh had the highest antioxidant activity measured by ABTS, FRAP, and DPPH assay (92.31±1.28 mg TE/g, 123.83±0.86 mg TE/g, and 74.02±0.33 mg TE/g, respectively). Blanched flesh generally had higher antioxidant activities measured by ABTS, FRAP, and DPPH assays than both their steamed and unprocessed counterparts. Moreover, steamed flesh had higher antioxidant activities measured by ABTS, FRAP, and DPPH assays than their unprocessed counterparts (Figure 3).

For fully ripe lipote flesh, blanching caused an 81.4%, 85.9% and 57.4% increase in antioxidant activity measured by ABTS, FRAP and DPPH assays respectively while steaming caused a 77.7%, 107% and 60.4% increase in antioxidant activity by the aforementioned assays, respectively. For half-ripe lipote flesh, blanching caused a 192%, 365%, and 118% increase in antioxidant activity measured by ABTS, FRAP and DPPH assays respectively while steaming caused a 176%, 249%, and 74.4% increase in antioxidant activity by the aforementioned assays respectively. For unripe lipote flesh, blanching caused a 66.0%, 185% and 133% increase in antioxidant activity measured by ABTS, FRAP and DPPH assays respectively while steaming caused a 13.6%, 4.29% and 0.178% increase in antioxidant activity by the aforementioned assays respectively (Figure 3).

The effect of maturity on the antioxidant activities of the lipote flesh is variable. For antioxidant activity measured by the ABTS assay, antioxidant activity is greatest at the unripe stage and then markedly decreases upon reaching the half-ripe stage. For antioxidant
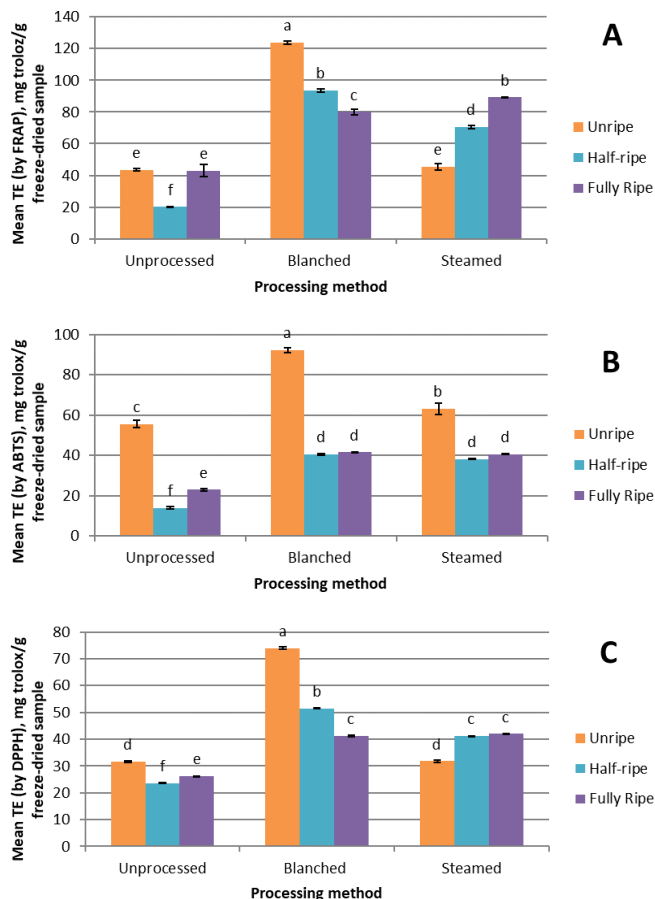
Figure 3. Antioxidant activity of lipote flesh measured by FRAP (A), ABTS (B), and DPPH (C) assays expressed as mg. Trolox equivalents per g freeze-dried sample as affected by maturity and processing method before freeze-drying (mean±SEM). Bars with different lowercase letters denote a significant difference (p≤0.05; Tukey's test).

activity measured by FRAP assay, blanched flesh decreases in antioxidant activity as the fruit matures, steamed flesh increases in antioxidant activity as the fruit matures, and finally, unprocessed flesh decreases in antioxidant activity upon reaching the half-ripe stage before returning to the same level upon reaching the fully ripe stage. For antioxidant activity measured by the DPPH assay, blanched flesh still decreases in antioxidant activity as the fruit matures. However, steamed flesh increases antioxidant activity by DPPH assay upon reaching the half-ripe stage and stays at that level upon reaching the fully ripe stage. Moreover, unprocessed flesh decreases in antioxidant activity measured by DPPH upon reaching the half-ripe stage and then increases slightly upon reaching the fully ripe stage (Figure 3).

3.3 Effect of maturity and processing method on the TPC, TFC, and TAC of lipote seeds

Maturity and processing, as well as their interaction, have significant effects on the TPC, TFC, and TAC of lipote seeds (p≤0.05). The TPC and TFC of lipote seeds generally decrease as the fruit matures. Moreover,

unprocessed unripe seeds had the greatest TPC and TFC (123.56±0.94 mg GAE/g and 111.90±0.14 mg QE/g, respectively) among all the other samples (Figure 4).

Unprocessed unripe seeds had a 42.3% decrease in TPC upon reaching the half-ripe stage and a 77.0% decrease in the same parameter upon reaching the fully ripe stage. Unprocessed unripe seeds also had a 44.9% decrease in TFC upon reaching the half-ripe stage and a 69.0% decrease in the same parameter upon reaching the fully ripe stage (Figure 4).

Blanched unripe seeds had a 17.1% decrease in TPC upon reaching the half-ripe stage and a 53.9% decrease in the same parameter upon reaching the fully ripe stage. Blanched unripe lipote seeds also had a 1.24% decrease in TFC upon reaching the half-ripe stage and a decrease in the same parameter upon reaching the fully ripe stage (Figure 4).

Steamed unripe seeds had a 27.9% decrease in TPC stage and a 43.8% decrease in the same parameter upon reaching the fully ripe stage. Steamed unripe seeds also had a 28.7% decrease in TFC upon reaching the half-ripe stage and a 47.4% decrease in the same parameter upon reaching the fully ripe stage (Figure 4).
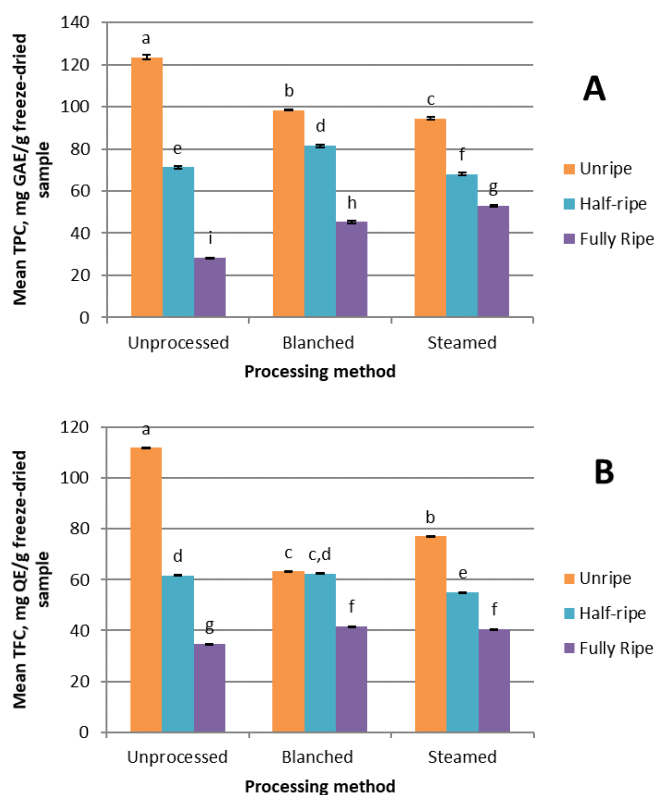

Figure 4. Total phenolic content (A) and total flavonoid content (B) of lipote seeds as affected by maturity and processing method before freeze-drying (mean±SEM). Bars with different lowercase letters denote a significant difference (p≤0.05; Tukey's test)

The TAC of lipote seeds ranged from 0.22±0.01 mg cyanidin-3-glucoside/g to 1.13±0.00 mg cyaniding/g. Processing generally increases anthocyanin content of the seeds. Both steamed and blanched seeds have greater TAC than their unprocessed counterparts (Table 2).

Table 2. Total anthocyanin content of lipote seeds

Processing method	Maturity stage	Total anthocyanin content, mg cyanidin-3-glucoside equivalents/ g freeze-dried sample
Unprocessed	Unripe	ND
	Half-ripe	ND
	Fully ripe	0.08±0.00[e]
Blanched	Unripe	ND
	Half-ripe	0.22±0.01[c]
	Fully ripe	0.40±0.00[b]
Steamed	Unripe	ND
	Half-ripe	0.13±0.00[d]
	Fully ripe	0.78±0.00[a]

Values are expressed as mean±SEM, n = 3. Values with different superscript are significantly different (Tukey's test, p ≤ 0.05). ND, not detected.

3.4. Effect of maturity and processing method on the antioxidant activity (ABTS, DPPH, and FRAP) of lipote seeds

Maturity and processing, as well as their interaction, have significant effects on the antioxidant activities measured by ABTS, DPPH, and FRAP assays of lipote flesh (p≤0.05).

The antioxidant activity of unripe seeds is generally greater than that of their half-ripe and unripe counterparts. For unprocessed seeds, antioxidant activities measured by ABTS, FRAP and DPPH assays decreased by 51.9%, 34.3%, and 50.2% upon reaching the half-ripe stage and 68.1%, 78.8% and 80.5% upon reaching the fully ripe stage. On the other hand, for blanched seeds, antioxidant activities measured by ABTS, FRAP and DPPH assays decreased by 15.1%, 23.1% and 27.8% upon reaching the half-ripe stage and 61.2%, 55.7% and 57.5% upon reaching the fully ripe stage. Finally, for steamed seeds, antioxidant activities measured by ABTS, FRAP and DPPH assays decreased by 56.4%, 36.5%, and 41.3% upon reaching the half-ripe stage and 58.1%, 48.0% and 35.2% upon reaching the fully ripe stage.

Unprocessed unripe seeds and steamed unripe seeds had the greatest antioxidant activity measured by ABTS assay (227.93±12.21 mg TE/g and 270.17±2.24 mg TE/ g). On the other hand, unprocessed unripe seeds and blanched unripe seeds had the greatest antioxidant activity as measured by FRAP assay (479.10±0.82 mg TE/g and 480.80±1.91 mg TE/g, respectively). Finally, only unprocessed unripe seeds had the greatest

antioxidant activity as measured by the DPPH assay (465.89±1.86 mg TE/g) (Figure 5).
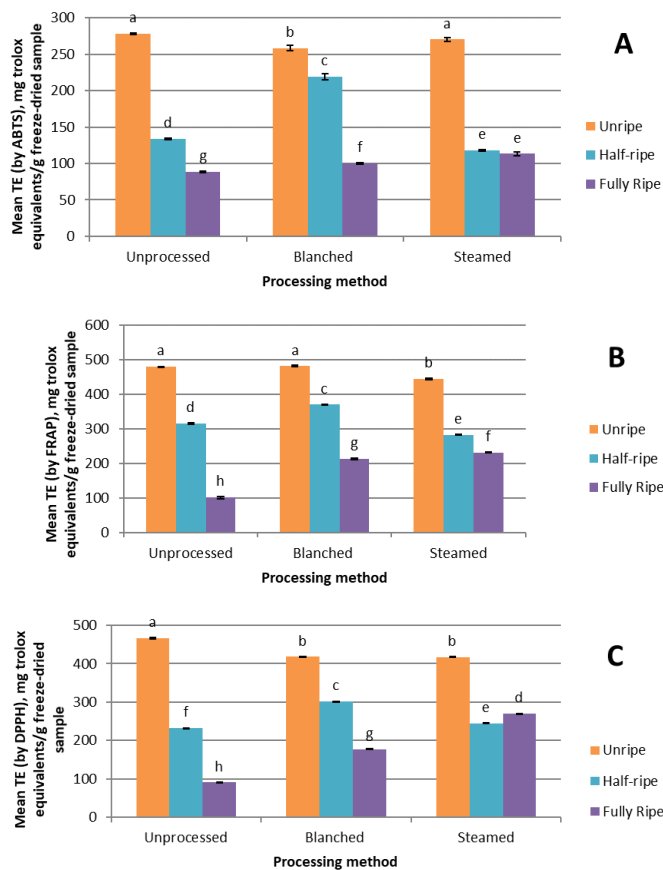

Figure 5. Antioxidant activity of lipote seeds measured by ABTS (A), FRAP (B), and DPPH (C) assays (top to bottom) expressed as mg Trolox equivalents per g freeze-dried sample as affected by maturity and processing method before freeze-drying (mean±SEM). Bars with different lowercase letters denote a significant difference (p ≤ 0.05; Tukey's test)

4. Discussion

This study was undertaken to investigate the effect of maturity stage and processing (specifically blanching and steaming) on the antioxidant contents and antioxidant activities of lipote flesh and seeds for the purpose of developing functional foods.

The total phenolics, total flavonoids, and total anthocyanin content of lipote flesh as well as their antioxidant activities measured by ABTS, DPPH, and FRAP were all significantly associated with both maturity stage and processing method (p≤0.05). Moreover, on all these parameters, the interaction between the maturity stage and processing method was significant. This was also observed in the study of Sartagoda *et al*. (2021) on bignay 'kalabaw' and Castillo -Israel *et al*. (2020) on bignay 'common.'

HPLC analysis by Chirinos *et al*. (2010) on Peruvian *camu-camu* and by Hammouda *et al*. (2013) on Tunisian dates showed that the concentration of phenolic

compounds changes during ripening. These changes may account for the effect of maturity in the antioxidant content and antioxidant activity of the samples. These may also account for the interaction between maturity and processing, as the compounds that changed in concentration may have different sensitivity to heat treatment. Still, this needs further investigation.

The total phenolic content and total antioxidant activities of blanched flesh are generally greater than that of the unprocessed flesh. This was also observed in the study of Sartagoda *et al*. (2021) on bignay 'kalabaw' and Castillo-Israel *et al*. (2020) on bignay 'common.' Increases in the total phenolic content and antiradical potential by DPPH assay due to blanching pre-treatment prior to freeze-drying was also observed in the study of Franceschinis *et al*. (2015) on cherry discs. Results of this study showed that blanching prior to air-drying and freeze-drying produced a product that has a greater TPC and ARP by DPPH assay than those that were not subjected to treatment and those that were subjected to sugar infusion prior to the two aforementioned drying techniques. The authors of this study speculated that this could be due to the inactivation of polyphenol oxidase during blanching, preventing the breakdown of some polyphenols in the fruit. In a similar study by Sablani *et al*. (2011), steam blanching prior to air drying of all but one of the four types of red raspberries and four types of blueberries studied resulted in a greater phenolic content of the product as compared to those air-dried without blanching as a pre-treatment. However, results on the effect of steam blanching on antioxidant activity are more variable, with some types having greater antioxidant activity when blanching was used a pre-treatment prior to air-drying, some types having lesser antioxidant activity and some types having no significant differences in antioxidant activities. Blanching was also found to increase the ellagatannin content of blackberries for juice processing (Hager *et al*., 2010). In addition to the inactivation of enzymes, blanching can also break down the cell matrix, which can subsequently increase the extraction of phenolics (Nayak *et al*., 2015). Blanched unripe lipote flesh had the highest antioxidant activity measured by DPPH, ABTS, and FRAP. This finding coincides with it having the greatest phenolic content out of all the other samples.

Total anthocyanin content markedly increases during the ripening of the flesh. This is consistent with the study of Yang *et al*. (2016) on mulberries and Seraglio *et al*. (2018) on *Myrciaria cauliflora, Myrcianthes pungens* and *Syzygium cumuni*. This also coincides with the study of Sartagoda *et al*. (2021) on bignay 'kalabaw' and Castillo-Israel *et al*. (2020) on bignay 'common.' This correlates with the colour development of the fruit.

Moreover, in the study of Sablani *et al*. (2011), steam blanching prior to air drying decreases the degradation of anthocyanins. This may coincide with the greater TAC content observed in the thermally processed samples as compared to the unprocessed samples.

The total phenolics, total flavonoids, and total anthocyanin content of lipote seeds as well as their antioxidant activities measured by ABTS, DPPH, and FRAP were also all significantly associated with both maturity stage and processing method (p≤0.05). The interaction between the maturity stage and processing method in all these parameters was also significant. This was also observed in the study of Sartagoda *et al*. (2021) on bignay 'kalabaw' and Castillo-Israel *et al*. (2020) on bignay 'common.'

Unripe lipote seeds generally had greater TPC, TFC, and antioxidant activities than their half-ripe and fully ripe counterparts. This is in contrast to the study of Sartagoda *et al*. (2020) on bignay 'kalabaw' and Castillo-Israel *et al*. (2020) on bignay 'common' which reported that fully ripe seeds of both berries generally had the greatest TPC, TFC and antioxidant activities by ABTS, FRAP and DPPH than their half-ripe and fully ripe counterparts. In the study of Moulehi *et al*. (2012), the TPC, TFC, and antioxidant activity measured by DPPH of mandarin orange and bitter orange were also affected by maturity. The effects of maturity on TPC varied in the two types of seeds. Like those of the lipote seeds, the TPC of the mandarin orange seeds in this study also decreased during ripening. However, in both the mandarin orange and bitter orange seeds, the antioxidant activity measured by DPPH increased during ripening. Differences in the antioxidant activities exhibited by the orange seeds and lipote seeds may be due to the nature of the fruit themselves. Total phenolic contents of seeds of *Syzygium cumini* was also reported to decrease as the fruit matures (Kheaw-on *et al*., 2008).

Little to no anthocyanins was detected in the seeds of lipote. This coincides with the study of Aqil *et al*. (2012) and Kheaw-on *et al*. (2008) which reported that anthocyanins were not detected in the seeds of *Syzygium cumini*. Moreover, greater TAC of the seeds of the thermally processed fruits may be due to the migration of anthocyanins from the flesh to the seeds during processing.

The TPC, TFC, and antioxidant activities measured by ABTS, DPPH, and FRAP of lipote seeds were considerably higher than that of the lipote flesh. Seeds of *Syzygium cumini*, were also reported to contain higher phenolic content and reducing capacity by FRAP assay than its flesh (Kheaw-on *et al*., 2008). Seeds also contained higher phenolic content than the skin and pulp

of Muscadine grapes (*Vitis rotundifolia*), according to the study of Sandhu and Gu (2010).

Possible implications of these findings for functional food development are as follows. First, blanching can be used to increase the phenolic content and antioxidant activity of lipote flesh. Second, lipote seeds, especially unripe ones, have good potential for nutraceutical applications due to their appreciable antioxidant contents.

4. Conclusion

The maturity stage and processing method both significantly affect the total phenolic content, total flavonoid content, total anthocyanin content, and antioxidant activities of lipote seeds and flesh. Moreover, the interaction of maturity and processing on all these parameters was statistically significant.

Generally, blanched flesh was found to have greater total flavonoid content, total anthocyanin content, and antioxidant activities than their unprocessed and steamed counterparts. Therefore, for functional food development, blanching can be used as a processing step to increase the antioxidant content of lipote flesh.

Seeds have appreciable total flavonoid content, total anthocyanin content, and antioxidant activities, even greater than that of lipote flesh. Moreover, unripe seeds generally have greater total flavonoid content, total anthocyanin content, and antioxidant activities than their half-ripe and fully ripe counterparts. For future functional food development, seeds can be investigated further for nutraceutical applications.

Acknowledgement

References

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