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The effects of pretreatment and ripening stage on nutrient content and antioxidant properties of *Lepisanthes fruticosa* whole fruit powder

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Abstract

The study on the effect of pretreatment (blanch and steam) and ripening stage (ripe and unripe) on nutrient content and antioxidant potential of *Lepisanthes fruticosa* whole fruit powder was carried out with the purpose to develop a potential functional ingredient. The results showed that blanching and steaming have significantly affected ($p<0.05$) the fat content, vitamin C, total anthocyanins content and antioxidant activity of *L. fruticosa* whole fruit powder regardless ripening stage. Both pretreatments could increase the vitamin C content, but blanching treatment alone was observed has lowered the fat content and enhanced the antioxidant activity (EC_{50}). Moreover, the ripe *L. fruticosa* whole fruit powder that has undergone blanching treatment showed higher retention of total anthocyanins content. However, higher retention of total anthocyanin content was observed in the steamed sample of the unripe stage. Upon ripening, protein and ash content were decreased, contrarily with carbohydrate and vitamin C content. Vitamin C content in the ripe sample showed an increment of more than 80% than that of the unripe sample. Meanwhile, antioxidant activity in the unripe sample showed higher activity than that of the ripe sample, although both stages showed EC_{50} values $\leq$ 1 mg/mL. These results might be important for establishing a functional ingredient made with *L. fruticosa* whole fruit.

1. Introduction

Lepisanthes fruticosa is an underutilised fruit native in Malaysia and locally known as Ceri Terengganu. The plant is commonly found in the states of Terengganu, Pahang and Johor (Lim, 2013). The plant can also be found in other regions of South-East Asia such as Indonesia, Myanmar, Thailand and the Philippines (Lim, 2013). *L. fruticosa* is a non-seasonal plant and the fruits are produced throughout the year (Mirfat and Salma, 2015). *L. fruticosa* is used in traditional medicine or consumed as food in rural areas. Previous study on *L. fruticosa f*ound that the ripe fruits showed the highest free radical scavenging activity and a great source of total phenolic contents compared with other types of underutilised fruits and commercial fruits such as guavas, oranges and apples (Mirfat and Salma, 2015). Other scientific study reported that the antioxidant activity and the naturally occurring antioxidant phytochemicals, phenols and flavanoids reached their highest levels at the unripe stage (Mirfat *et al*., 2017).

L. fruticosa fruits are perishable and have a short shelf life. Drying *L. fruticosa* fruits and processed into powder is one of the steps to preserve its nutrient and functional properties. Dried fruits showed higher antioxidant activity and polyphenolic content than fresh fruits due to their low moisture content (Vijaya *et al.,* 2010). Furthermore, fruits in dried form have longer shelf life because through drying, moisture is removed and consequently helps stop the microbial growth, reduces weight and volume (Vijaya *et al*., 2010). However, drying may not eliminate the enzymes that cause the product to be darkened (Andress *et al*., 2014). Several methods of pretreatment can be used before drying to overcome this problem. The pretreatment process is one of the important steps in the development of ingredient and food product in order to inactivate or retard bacterial and enzyme action, to prevent degenerating quality of the end product during storage. Therefore, pretreatment process before drying needs to be taken such as steaming and blanching process. Steaming and blanching methods are the most natural process of pretreatment. Furthermore, steaming and blanching are by far the most popular and commercially

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used with the advantages of simplicity and the small capital investments, because of its simple equipment and easy operation. Some studies had noted that blanching has been widely applied on pretreatment of agro-products to enhance the drying rate and improve the product quality (Doymaz and Özdemir, 2014; Doymaz, 2015; Cheng *et al.,* 2015; Ando *et al.,* 2016; Filho *et al*., 2016). However, the effectiveness of such pretreatments is dependent on the state of the fruits used which are also dependent of its cellular structure, variety, origin, state (fresh, ripe, raw) and harvesting conditions (Madaleno, 2015).

Reports on fruit quality of *L. fruticosa* upon ripening are scarce. Changes in physiological, biochemical and morphological traits of the fruit as characterized by the process of ripening stage will determine the qualitative characteristics of fruits (Monica, 2007). The previous study reported that changes in antioxidant properties were found to be varied in different fruits such as olives, oranges and tomatoes (Zainudin *et al*., 2014). Hence, it is important to determine the ripening stage with enhanced levels of antioxidants and phytochemical compounds during maturation in targeting to increase functional properties (Fu *et al*., 2009).

Therefore, this study was undertaken to determine the nutrient content and antioxidant potential of *L. fruticosa* whole fruit powder prepared by different pretreatment and ripening stage with the intention to promote the utilisation of the fruits as a functional ingredient. This finding may provide a better understanding of the nutritional and antioxidant potential of *L. fruticosa* whole fruit powder which is important for the enhancement of the fruit species in the future.

2. Materials and methods

2.1 Chemicals and reagents

Ascorbic acid (Vitamin C), K_2HPO_4 (0.1 M), KH_2PO_4 (0.08 M), ethanol, methanol, hydrochloric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, were purchased from Sigma (St. Louis, MO, USA). Other reagents used were of analytical grade.

2.2 Instrumentations

Moisture analyzer (SARTORIUS MA 35, Metler Toledo, USA), hot air circulating drier (Model RXH-B-I, China), forced draft oven (Memmert, Germany), grinder (FRITSCH Universal Cutting Mill Pulverisette 19, Germany), HPLC (Waters system), III-1311 Milton Roy fluorimeter (Ivyland, PA), orbital shaker (Model 719, TechLab, Malaysia), benchtop centrifuge (Hettich Rotina 308, PRO Scientific Inc., USA), Lambda 25 UV/VIS spectrophotometer (Perkin Elmer, Shelton, USA), microplate reader (BIOTEK GEN5 EON Microplate Spectrophotometer, Winooski, Vermont, USA), were used in this study.

2.3 Preparation of Lepisanthes fruticosa whole fruit powder

L. fruticosa was harvested from study plot at Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. Over 5 kilograms of fruits were sampled at each stage of ripeness (ripe and unripe). Samples were washed and cleaned by removing dirt and spoilt parts, drained, separate the whole fruit (pulp with skin) from the seeds and sliced. Samples were then divided into 3 portions, which were for 2 pretreatments namely; steaming (1 min) and blanching (1 min at boiling water), and 1 portion as control (no pretreatment). Samples were dried in a hot air circulating dryer at 60°C until the moisture content was tested <5% by using moisture analyzer (SARTORIUS MA 35). Dried samples were let cooled before ground into powder using a grinder (FRITSCH Universal Cutting Mill Pulverisette 19, Germany). The powder was packed in an oriented polypropylene/ aluminium/ polyethylene (OPP/ Al/ PE) packaging (22 x 30 cm) and stored at -18°C prior to analysis.

2.4 Determination of proximate and dietary fibre

Moisture content, ash, protein, fat, soluble and insoluble dietary fibre contents of powdered samples were determined according to AOAC Official method 934.01, 930.05, 981.10, 991.36, 993.19 and 991.42, respectively (AOAC, 2000). Carbohydrate content was calculated from the sum of the percentages of crude protein, ash, fat and crude fibre and subtracted from 100. Meanwhile, the energy of the samples was calculated by multiplying the values obtained for protein, carbohydrates and fat by 4.00, 3.75 and 9.00, respectively. The results are expressed in kcal/100 g dry basis (FAO/WHO/UNU, 1985).

2.5 Determination of Vitamin C

Vitamin C of *L. fruticosa* whole fruit powder was quantified according to Courtois *et al*. (2009) by using HPLC (Waters system) using an isocratic gradient equipped with a reversed-phase C_{18} column (Waters, Spherisorb ODS 2) (5 µm packing) (250 × 4.6 mm id). Ascorbic acid was eluted under the following conditions: injected volume 20 µL; oven temperature 30°C; solvent mixture K_2HPO_4 (0.1 M), KH_2PO_4 (0.08 M), MeOH (55/25/20, v/v/v). The flow rate was 1.5 mL min^{-1}, and the total elution time was 10 min. Detection was performed by an III-1311 Milton Roy fluorimeter (Ivyland, PA) with $\lambda_{excitation}$ = 350 nm and $\lambda_{emission}$ = 430

nm. Quantification was carried out by external calibration with ascorbic acid. The calibration curve was set from 1 to 7 µg/mL ascorbic acid.

2.6 Determination of total anthocyanins content (TAC)

Total anthocyanins content (TAC) was measured according to the method employed by Cinquanta *et al*. (2002). A hundred milligram of powder sample was extracted with 10 mL of 1.5 N hydrochloric acid/ water/95% ethanol solution (ratio 1:29:70). The mixture was shaken with orbital shaker for 30 mins, centrifuged at 3000 x *g* (5175 rpm) at 25°C for 5 mins and the supernatant was filtered with Whatman filter paper no. 1. The extraction was performed two times and supernatants collected were combined. The absorbance of the supernatant was measured using a Lambda 25 UV/ VIS spectrophotometer (Perkin Elmer, Shelton, USA) at λ_{max} of 533 nm and the pigment content was calculated as cyanidin-3-glucoside equivalent. TAC was calculated by the following formula:

$$TAC\left(\frac{mg}{100\ g}\right) = \frac{\mathrm{A}}{\mathrm{eL}} \times MW \times D \times \frac{V}{M} \quad (1)$$

Where, A = absorbance, e = molar absorbance for cyanidin-3-glucoside (26900), L = cell path length (1 cm), MW = molecular weight of cyanidin-3-glucoside (449.2 Da), D = dilution factor, V = final volume (mL) and M = the dry weight of powder sample (mg).

2.7 Determination of free radical scavenging activity-DDPH test for EC_{50}

Free radical scavenging activity was determined using spectrophotometric assay which uses stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent, according to a slightly modified method of Lu and Foo (2000). About 100 µL of the extracts at concentrations ranging from 0.0781 – 5.0 mg/mL was added to 200 µL of a 0.007% methanol solution of DPPH. Trolox and ascorbic acid (Vitamin C), i.e. the well-known standards with strong antioxidant activities, were used as positive controls. After 40 mins incubation period in a dark at room temperature, the absorbance was read against a blank at 517 nm using microplate reader (BIOTEK GEN5 EON Microplate Spectrophotometer, Winooski, Vermont, USA). The percentage of inhibition of free radical DPPH by the extracts was calculated as follows:

$$\text{Inhibition (\%)} = (A_{blank} - A_{sample}/\ A_{blank}) \times 100 \quad (2)$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Inhibition (%) was plotted against concentration and the EC_{50} was calculated graphically.

2.8 Statistical analysis

All measurements were carried out in triplicate for each sample and data were expressed as mean±standard deviation. All statistical analyses were carried out using MINITAB version 16 Sub100 for the analysis of variance (ANOVA) with Tukey's test at a significance level of $p<0.05$.

3. Results and discussion

3.1 Effect on proximate and dietary fibre

Nutrient compositions of whole fruit powder from unripe and ripe *L. fruticosa* processed at different pretreatment are presented in Table 1. Powder moisture contents ranging from 7.88 to 9.37% and were not significantly different ($p>0.05$) for all samples since the drying process has been monitored until it gets 4 to 5% of moisture content before cooling, grinding and

Table 1. Nutrient composition of whole fruit powder from unripe and ripe *L fruticosa* processed at different pretreatment

Nutrient composition	Unripe *L. fruticosa* whole fruit powder			Ripe *L. fruticosa* whole fruit powder		
	Control	Steaming	Blanching	Control	Steaming	Blanching
Energy (kcal/100 g)	418.67±57.33^{a}	377.24±6.40^{a}	374.03±0.00^{a}	394.57±0.77^{a}	393.81±1.55^{a}	408.83±26.14^{a}
Fat (g/100 g)	0.72±0.06^{a}	0.43±0.04^{b}	0.22±0.00^{c}	0.80±0.08^{a}	0.62±0.02^{b}	0.26±0.10^{c}
Protein (g/100 g)	6.34±0.02^{a}	6.32±0.01^{a}	6.32±0.09^{a}	5.65±0.01^{b}	5.67±0.08^{b}	5.77±0.06^{b}
Moisture (g/100 g)	9.36±0.09^{a}	8.95±0.54^{a}	9.37±0.06^{a}	7.88±0.09^{a}	8.59±0.40^{a}	8.03±0.10^{a}
Ash (g/100 g)	7.12±0.30^{a}	6.20±1.12^{a}	6.82±0.04^{a}	2.41±0.02^{b}	2.38±0.08^{b}	2.55±0.04^{b}
Carbohydrate (g/100 g)	85.82±0.16^{b}	87.60±0.92^{b}	86.64±0.20^{b}	91.14±0.06^{a}	91.34±0.46^{a}	91.43±0.04^{a}
Vitamin C (mg/100 g)	205.00±6.50^{c}	314.30±9.60^{c}	459.30±9.80^{bc}	1500.10±213^{ab}	2526.71±240.70^{a}	2442.30±235.60^{a}
Soluble dietary fibre (g/100 g)	9.52±1.61^{a}	9.55±2.79^{a}	9.16±0.83^{a}	7.33±0.44^{a}	6.50±0.77^{a}	7.50±0.18^{a}
Insoluble dietary fibre (g/100 g)	36.24±0.23^{a}	35.92±1.26^{a}	38.13±7.70^{a}	38.95±0.05^{a}	38.19±0.35^{a}	39.93±0.32^{a}
Total dietary fibre (g/100 g)	42.76±1.84^{a}	45.47±4.05^{a}	47.29±8.53^{a}	46.28±0.48^{a}	44.70±0.42^{a}	47.43±3.50^{a}

Values are expressed as mean±standard deviation, n=3. Means that do not share a letter are significantly different based on Tukey 95 % Simultaneous Confidence Intervals.

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packing. The moisture content was slightly increased when dried samples were ground into powder because the surface area of samples increased when the particle size was smaller. The moisture content of food is an index of water activity, which indicates the stability and susceptibility to microbial contamination (Edem and Miranda, 2011). Low moisture content (<10%) in both ripe and unripe *L. fruticosa* whole fruit powder can be beneficial to improve the shelf-life and can play an important role on the storage stability of functional ingredient developed using this fruit.

Results of energy showed no significant difference ($p>0.05$) between samples of both pretreatments and ripening stages with control sample. This showed that pretreatments (steaming and blanching) and ripening stage did not affect the energy of the samples. The powder also can be categorized as low fat as the content were between 0.22 to 0.80%, which was lower than that reported in other studies of dried samples such as *L. alata* (2.25 - 2.80%), *Nypa fruticans* (0.52 – 4.33%) and plantain (1.40 - 2.52%) (Onwuka and Onwuka, 2005; Ping *et al.,* 2013; Zhang *et al.,* 2019). Fat content was observed to decrease when samples were steamed and blanched with a loss percentage of 20 – 40% in steamed and 67 – 69% in blanched samples. Since blanched samples had the lowest fat content than the steamed sample, it is suggested that blanching samples before processing into powder could lower the fat content.

Steaming and blanching were also had not significantly affected ($p>0.05$) protein and ash content, but were decreased upon ripening in all samples. The ash content of the unripe *L. fruticosa* whole fruit powder was significantly higher ($p<0.05$) than that of the ripe sample (Table 1), hence showed that the unripe fruit might be rich in minerals content than that of ripe fruits. The previous study also reported that protein content was decreased upon ripening in sweet banana pulp (Aurore *et al*., 2009) and freeze-dried Kundang fruits (Nithiya Shanmuga Rajan *et al*., 2014). However, protein content in *Trewia nudiflora Linn*., cherry (*Prunus avium* L.), plantain and banana were reported to increase upon ripening (Brady *et al*., 1970; Onwuka and Onwuka, 2005; Mahmood *et al*., 2013; Ghai *et al.,* 2016). The protein content of *L. fruticosa* whole fruit powder ranged between 5.65 to 6.34% and the values were in the range reported by Umi Kalsom and Mirfat (2014) (2.19 - 7.30%), but higher than that in *L. alata*, *Cornus mas* L. and *Prunus avium* L. (Brindza *et al*., 2009; Mahmood et *al.,* 2013; Rahmadi *et al.,* 2016; Zhang *et al*., 2019).

Meanwhile, carbohydrate of the powders also showed no significant difference ($p>0.05$) between pretreatments and control, showing that pretreatment did not affect the carbohydrate content of the samples. However, the carbohydrate value showed a slight increment upon ripening. Ping *et al.* (2013), Mahmood et *al.* (2013), Nithiya Shanmuga Rajan *et al.* (2014) and Sharaf *et al*. (1989) were also reported an increment of carbohydrate content upon ripening in *Nypa fruticans*, *Prunus avium* L., kundang, apricot and mango, respectively. Result of carbohydrate content of *L. fruticosa* whole fruit powder (85.82 – 91.43%) was also observed to be higher than that in *Nypa fruticans*, cherry laurel, pekmez and *Prunus avium* L. (Alasalvar *et al.,* 2005; Ping *et al*., 2013; Mahmood *et al*., 2013).

Soluble and insoluble dietary fibre showed no significant difference ($p>0.05$) in all samples with the percentage of insoluble dietary fibre in all samples were higher than that in soluble dietary fibre. The total dietary fibre of *L. fruticosa* whole fruit powder ranged from 42.76 to 47.29% (Table 1). The values were lower than that reported by Zhang *et al*. (2019) in *L. alata* samples which ranged between 58 to 59%. However, the total dietary fibre of the samples are still higher than that in ripe apples (Li *et al*., 2002) and even with wheat flours and whole grain cereals (Ragaee *et al*., 2006). According to Malaysia's Food Act 1983, food or ingredients in the solid state which contains total dietary fibre more than 6% can be considered as high dietary fibre (Ministry of Health, 2014). Hence, *L. fruticosa* whole fruit powder can appear to be a good potential functional food ingredient with high dietary fibre content.

3.2 Effect on vitamin C

Vitamin C is a potent water-soluble antioxidant in humans and was mostly stated to be heat sensitive and prone to degradation under the influence of many factors including enzymes, temperature and leaching (Garba and Kaur, 2014). The values of vitamin C in *L. fruticosa* whole fruit powder were between 1500 to 2526 mg/100 g and 205 to 459 mg/100 g in ripe and unripe samples, respectively. These values were much higher compared with dried *L. alata*, black carrot, peppers and mango (Ndawula *et al*, 2004; Martínez *et al.,* 2005; Garba and Kaur, 2014; Rahmadi *et al.,* 2016). Results showed that ripe *L. fruticosa* whole fruit powder can be a natural source of vitamin C.

The vitamin C in the blanched unripe sample was found to be slightly higher than the steamed sample, whereas in the ripe sample, both pretreatments did not show any significant different ($p>0.05$) in vitamin C content. In this study, vitamin C in both pretreatments observed to have higher values than the control sample, hence we may conclude that pretreatment might enhanced vitamin C content in *L. fruticosa* whole fruit powder. Previous studies on black carrot (Garba and

Kaur, 2014), sweet pepper (Martinez *et al.,* 2005) and dried dill (Galoburda *et al*., 2012) confirmed the effect of blanching on vitamin C content of many fruit and vegetables, where vitamin C was found to increase when submitted to blanching before drying.

It was observed that vitamin C content in *L. fruticosa* whole fruit powder was increased dramatically upon ripening, showing more than 80% higher than that in the unripe samples. The result was in trend with reports by Mercado-Silva *et al. (*1998) in guava and Martinez *et al*. (2009) in pepper, where the ascorbic acid was increased during ripening. According to Mozafar (1994), the concentration of ascorbic acid in fruits is probably associated with carbohydrate metabolism. The concentration of ascorbic acid in fruits also related to sugar accumulations which are at maximum levels in ripe fruits (Wall and Biles, 1993). Since the increment of carbohydrate content upon ripening showed a similar trend, it may conclude that there is a positive correlation between the vitamin C content and carbohydrate content in *L. fruticosa* whole fruit powder.

3.3 Effect on total anthocyanins content (TAC)

Pigments of anthocyanins are so much special and can be related to the colour of fruits or plant. These pigments are polyphenolic that belong to flavanoid group which gives the colour ranging from red-orange to blue-violet colours in plant organs (Wallace and Giusti, 2015). The stability of anthocyanins influenced by temperature, light, pH and structure (Laleh *et al*., 2006). Results of total anthocyanins content on the powders produced were shown in Figure 1. The percentage loss of anthocyanins in unripe samples was 30% in the steamed sample and 63% in the blanched sample. Higher reduction in anthocyanin content was observed in the blanched unripe sample than that in the steamed unripe sample. The lower value recorded for the blanched unripe sample was probably due to the high leaching of the pigment observed during blanching. Wahyuningsih (2008) recorded a decreased in anthocyanin content of red turi (*Sesbania grandiflora L.*) flower which was ascribed to the leaching of anthocyanin in the blanching media. Heating was also reported to encourage cellular fluids, containing phytochemicals to diffuse from the plant cell to the water media. Thus, the phytochemical content after blanching is the combined result of increased in extraction, degradation and leaching (Leong and Oey, 2012).

However, in the ripe sample, the loss percentage of anthocyanins in the blanched ripe sample was lower than that in the steamed ripe sample. The percentage loss of anthocyanins in the steamed and blanched sample was 46% and 34%, respectively. Therefore, the ripe *L. fruticosa* whole fruit powder submitted to blanching treatment presented the highest retention of TAC. This scenario might be explained by non-uniform blanching effects because of the sample surface area was smaller than the unripe sample because the sample was sliced slightly bigger than the unripe sample since the texture of ripe fruit was soft and mushy.

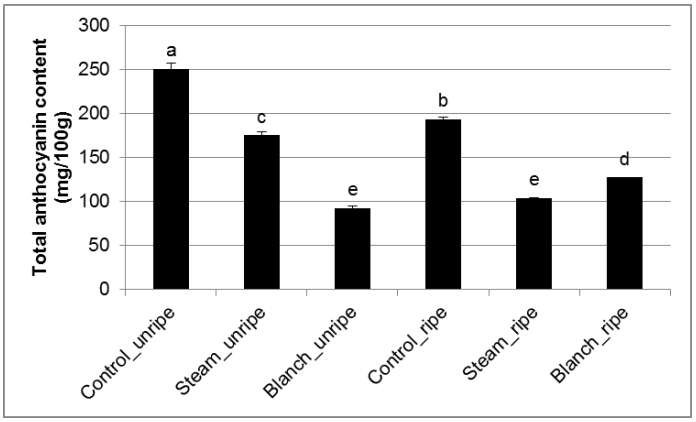

Figure 1. Total anthocyanins content of *L. fruticosa* whole fruit powder. Values are expressed as mean±standard deviation, n=3. Means that do not share a letter are significantly different based on Tukey 95 % Simultaneous Confidence Intervals.

In this study, TAC in ripe samples was lower than that of the unripe sample. Results also in agreement with TAC in apricots, where the pigment content decreased toward the end of the ripening stage (Bureau *et al*., 2009). In most species, fruit anthocyanin concentrations increase with ripening, as their biosynthesis proceeds faster than fruit expansion (Bureau *et al*., 2009). This is consistent with the findings reported for blueberry, aronia, blackberry, raspberry and strawberry fruits tended to increase upon ripening (Wang and Lin, 2000; Siriwoharn, 2004; Castrejón *et al*. 2008; Yang *et al*, 2019; Hwang *et al*., 2020). Meanwhile, Zhang *et al*. (2019) and Zielinski *et al*. (2015) reported that the pigments found only in the ripe fruit of *L. alata* and blackberry, respectively. Castrejón *et al*. (2008) concluded that anthocyanin biosynthesis is highly related to the developmental stages of the fruit, and enzyme activities are controlled in response to different developmental and environmental cues. Based on these results, total anthocyanins content varies according to cultivar and maturity. The possible explanations for the anthocyanin decrease might be because of the degradation of the anthocyanins, molecules which are known to be unstable in weakly acidic conditions (Cabrita *et al*., 2000).

3.4 Effect on radical scavenging activity

All samples which include control (no pretreatment) showed the value of EC_{50} below 1 mg/mL (Table 2). According to Lee *et al*. (2007), EC_{50} values lower than

10 mg/mL are indicative of the effective antioxidant activity. Antioxidant activity in *L. fruticosa* whole fruit powder was found to have a lower value than that of *Hypsizigus marmoreus* mushroom extract with a value of 24.6 mg dried sample/mL (Lee *et al*., 2007), but slightly higher than *L. alata* peels with a value of 0.25 mg dried sample/mL (Rahmadi *et al*., 2016). Sample with pretreatments (steam and blanch) were found to be significantly ($p<0.05$) stronger radical scavenger than control with all EC_{50} values below 1 mg/mL. Results prove that pretreatment could enhance the antioxidant activity of *L. fruticosa* whole fruit powder.

Table 2. Antioxidant properties (EC_{50}) of *L. fruticosa* whole fruit powder

Sample	EC_{50} (mg/mL)
Control_unripe	0.767 ± 0.012^{b}
Steam_unripe	0.751 ± 0.009^{c}
Blanch_unripe	0.431 ± 0.008^{f}
Control_ripe	1.047 ± 0.007^{a}
Steam_ripe	0.724 ± 0.002^{d}
Blanch_ripe	0.634 ± 0.005^{e}
Vitamin $C_{Positive\ control}$	0.0012±0.0001
$Trolox_{Positive\ control}$	0.0130±0.0010

Values are expressed as mean±standard deviation, n=3. Means that do not share a letter are significantly different based on Tukey 95 % Simultaneous Confidence Intervals.

Unripe fruits have been reported to have the highest level of bioactivities but decreased upon ripening (Mphahlele *et al*., 2014). Our result is in accordance to study by Mirfat *et al*. (2017) on the extracts of fresh and freeze-dried whole fruits of *L. fruticosa,* which showed that antioxidant and phytochemical contents decreased with fruit maturation and suggested that the lower the maturity, the higher the antioxidant activity. A similar trend had been reported for antioxidant compounds and activities of Jewel strawberries, aronia and Janghee fruits, shown significant higher values for the unripe stage than the ripe stage (Shin *et al*., 2008; Yang *et al*., 2019; Hwang *et al*., 2019). However, Hwang *et al*. (2019) reported that the total antioxidant activity of ripe and unripe Seolhyang strawberry fruit showed no differences. Based on these results, antioxidant activity varies according to cultivar and maturity. In this study, the changes in EC_{50} according to maturity showed a pattern similar to the changes in the total anthocyanins content. This shows a positive correlation between the antioxidant activity and TAC in *L. fruticosa* whole fruit powder.

4. Conclusion

In conclusion, the study showed that the fat content, vitamin C, total anthocyanins content and radical scavenging effect (EC_{50}) were significantly affected by pretreatments. Results showed that blanching might decrease the fat content of the sample. Meanwhile, the antioxidant activity of *L. fruticosa* whole fruit powder showed that the fruit is an excellent source of antioxidant with the blanching sample was identified having the most remarkable antioxidant with a lower value of EC_{50}. It was observed that protein, total anthocyanins content and antioxidant activity (EC_{50}) are at their highest levels at the unripe stage, whereas vitamin C value was significantly increased upon ripening. Therefore, ripe *L. fruticosa* whole fruit powder could have the potential as a natural source of vitamin C, while the unripe *L. fruticosa* whole fruit powder could have the potential as antioxidant and anthocyanins rich ingredient. Although the total dietary fibre content of *L. fruticosa* whole fruit powder was not affected by pretreatments and ripening stage, the high content of the sample (>40%) showed that *L. fruticosa* whole fruit powder has excellent potential as a functional ingredient with high dietary fibre. Hence, both stages of the fruits could be used as a promising ingredient for developing a functional ingredient.

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