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

*Tun Norbrillinda*, M., *Norra, I.*, *Hasri, H.* and *Helmi, M.M.A.*

*Food Science and Technology Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.*

**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* found 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

*Corresponding author.
Email: brillind@mardi.gov.my
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<sub>2</sub>HPO<sub>4</sub> (0.1 M), KH<sub>2</sub>PO<sub>4</sub> (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-1, China), forced draft oven (Memmert, Germany), grinder (FRITSCCH 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 (FRITSCCH 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<sub>18</sub> 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<sub>2</sub>HPO<sub>4</sub> (0.1 M), KH<sub>2</sub>PO<sub>4</sub> (0.08 M), MeOH (55/25/20, v/v/v). The flow rate was 1.5 mL min<sup>-1</sup>, and the total elution time was 10 min. Detection was performed by an III-1311 Milton Roy fluorimeter (Ivyland, PA) with λ<sub>excitation</sub> = 350 nm and λ<sub>emission</sub> = 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:

\[
TAC \left(\frac{mg}{100\ g}\right) = \frac{A}{L} \times MW \times D \times \frac{V}{M}
\]

(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:

\[
\text{Inhibition} \% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \tag{2}
\]

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% moisture content before cooling, grinding and processing at different pretreatment.

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^c | 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^b | 6.32±0.01^a | 6.32±0.09^b | 5.65±0.01^b | 5.67±0.08^b | 5.77±0.06^b |
| Moisture (g/100 g)   | 9.36±0.09^b | 8.95±0.54^a | 9.37±0.06^a | 7.88±0.09^a | 8.59±0.40^b | 8.03±0.10^a |
| Ash (g/100 g)        | 7.12±0.30^c | 6.20±1.12^a | 6.82±0.04^a | 2.41±0.02^b | 2.38±0.08^b | 2.55±0.04^c |
| 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^b |
| Vitamin C (mg/100 g) | 205.00±6.50^c | 314.30±9.60^c | 459.30±9.80^c | 1500.10±213^c | 2526.71±240.70^c | 2442.30±235.60^c |
| Soluble dietary fibre (g/100 g) | 9.52±1.61^c | 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^b | 35.92±1.26^b | 38.13±7.70^b | 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.
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 blanched samples before processing into powder could lower the fat content.

Steaming and blanching were also 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 Shammuga 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 Shammuga 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 (Ragaei 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; Martinez 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.

![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.](image)

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.

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
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

© 2020 The Authors. Published by Rynnye Lyan Resources
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 EC₅₀ values below 1 mg/mL. Results prove that pretreatment could enhance the antioxidant activity of L. fruticosa whole fruit powder.

Table 2. Antioxidant properties (EC₅₀) of L. fruticosa whole fruit powder

| Sample            | EC₅₀ (mg/mL) |
|-------------------|--------------|
| Control_unripe    | 0.767±0.012² |
| Steam_unripe      | 0.751±0.009⁶ |
| Blanch_unripe     | 0.431±0.008¹⁴ |
| Control_ripe      | 1.047±0.007³ |
| Steam_ripe        | 0.724±0.002¹⁴ |
| Blanch_ripe       | 0.634±0.005⁵ |
| 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 EC₅₀ 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 (EC₅₀) 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 EC₅₀. It was observed that protein, total anthocyanins content and antioxidant activity (EC₅₀) 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.

Acknowledgement

The authors were grateful for the research grant provided by Government of Malaysia, helpful staffs and facilities of Food Science and Technology Research Centre, Malaysian Agricultural and Research Development Institute.

References

Alasalvar, C., Al-Farsi, M. and Shahidi, F. (2005). Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. Journal of Food Science, 70(1), 47-52. https://doi.org/10.1111/j.1365-2621.2005.tb09064.x

Ando, Y., Maeda, Y., Mizutani, K., Wakatsuki, N., Hagiwara, S. and Nabetani, H. (2016). Impact of blanching and freeze-thaw pretreatment on drying rate of carrot roots in relation to changes in cell membrane function and cell wall structure. LWT – Food Science and Technology, 71, 40-46. https://doi.org/10.1016/j.lwt.2016.03.019

Andress, E.L. and Harrison, J.A. (2014). So easy to preserve. University of Georgia Cooperative Extension. Bulletin 989, 6, 337.

AOAC. (2000). Official methods of analysis of AOAC International, Gaithersburg, USA: AOAC.

Aurore, G., Parfait, B. and Fahrasmane, L. (2009). Bananas, raw materials for making processed food products. Trends in Food Science and Technology,
Brady, C.J., Palmer, J.K., O’connell, P.B.H. and Swllie, R.M. (1970). An increase in protein synthesis during ripening of the banana fruit. *Phytochemistry*, 9(5), 1037-1047. https://doi.org/10.1016/S0031-9422(00)85224-3

Bureau, S., Renard, C.M.G.C., Reich, M., Ginies, C. and Audergon, J.M. (2009). Change in anthocyanin concentrations in red apricot fruits during ripening. *LWT - Food Science and Technology*, 42(1), 372-377. https://doi.org/10.1016/j.lwt.2008.03.010

Brindza, P., Brindza, J., Toth, D., Klimenko, S. V. and Grigorieva, O. (2009). Biological and commercial characteristics of cornelian cherry (*Cornus mas L.*) population in the Gemer region Slovakia. *ISHS Acta Horticulare*, 818, 85-94. https://doi.org/10.17660/ActaHortic.2009.818.11

Cabrita, L., Fossen, T. and Andersen, O.M. (2000). Colour and stability of the six common anthocyanin 3-glucosides in aqueous solutions. *Food Chemistry*, 68(1), 101-107. https://doi.org/10.1016/S0308-8146(99)00170-3

Castejón, A.D.R., Eichholz, I., Rohn, S., Kroh, L.W. and Huyskens-Keil, S. (2008). Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum L.*) during fruit maturation and ripening. *Food Chemistry*, 109(3), 564-572. https://doi.org/10.1016/j.foodchem.2008.01.007

Cheng, L.S., Fang, S. and Ruan, M.L. (2015). Influence of blanching pretreatment on the drying characteristics of cherry tomato and mathematical modeling. *International Journal of Food Engineering*, 11(2), 265-274. https://doi.org/10.1515/ijfe-2014-0218

Cinquanta, L., Di Matteo, M. and Esti, M. (2002). Physical pre-treatment of plums (*Prunus domestica*). Part 2. Effect on the quality characteristics of different prune cultivars. *Food Chemistry*, 79(2), 233-238. https://doi.org/10.1016/S0308-8146(02)00138-3

Courtois, F., Vedrenne, L. and George, S. (2009). Mathematical modelling of some nutrient losses during heat treatment of stewed apples. *Czech Journal of Food Science*, 27, S23-S26. https://doi.org/10.17221/966-CJFS

Doymaz, İ. (2015). Hot-air drying and rehydration characteristics of red kidney bean seeds. *Chemical Engineering Communications*, 203(5), 599-608. https://doi.org/10.1080/00986445.2015.1056299

Doymaz, İ. and Özdemir, Ö. (2014). Effect of air temperature, slice thickness and pretreatment on drying and rehydration of tomato. *International Journal of Food Science and Technology*, 49 (2), 558-564. https://doi.org/10.1111/ijfs.12337

Edem C.A. and Miranda, I.D. (2011). Chemical evaluation of proximate composition, ascorbic acid and anti-nutrients content of African star apple (*Chrysophyllum africanaum*) fruit. *International Journal of Research Reviews in Applied Sciences*, 9 (1), 146-149.

FAO/WHO/UNU. (1985). Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. WHO Technical Report Series No. 724. Geneva: WHO.

Filho, L.M., Frascareli, E.C. and Mauro, M.A. (2016). Effect of an edible pectin coating and blanching pretreatments on the air-drying kinetics of pumpkin (*Cucurbita moschata*). *Food and Bioprocess Technology*, 9(5), 859-871. https://doi.org/10.1007/s11947-016-1674-5

Fu, M., He, Z., Zhao, Y., Yang, J., and Mao, L. (2009). Antioxidant properties and involved compounds of daylily flowers in relation to maturity. *Food Chemistry*, 114(4), 1192-1197. https://doi.org/10.1016/j.foodchem.2008.10.072

Galoburda, R., Kruma, Z. and Ruse, K., (2012). Effect of pretreatment method on the content of phenolic compounds, vitamin C and antioxidant activity of dried dill. *World Academia Science Engineering and Technology*, 6, 251-255.

Garba, U. and Kaur, S. (2014). Effect of drying and pretreatment on anthocyanins, flavanoids and ascorbic acid content of black carrot (*Daucus carota l.*). *Journal of Global Biosciences*, 3(4), 772-777.

Ghai, K., Gupta, P.K. and Gupta, A.K. (2016). Physiochemical behaviour changes during ripening in fruits of *Trewia nudiflora Linn.* *Perspectives in Science*, 8, 596-598. https://doi.org/10.1016/j.pisc.2016.06.031

Hwang, H., Kim, Y.J. and Shin, Y. (2020). Assessment of physicochemical quality, antioxidant content and activity, and inhibition of cholinesterase between unripe and ripe blueberry fruit. *Foods*, 9(6), 690. https://doi.org/10.3390/foods9060690

Hwang, H., Shin, Y. and Kim, Y.J. (2019). Influence of ripening stage and cultivar on physicochemical properties, sugar and organic acid profiles, and antioxidant compositions of strawberries. *Food Science Biotechnology*, 28, 1659-1667. https://doi.org/10.1007/s10068-019-00610-y

Laleh, G.H., Frydoonfar, H., Heidary, R., Jameci, R. and Zare, S. (2006). The effect of light, temperature, pH
and species on stability of anthocyanin pigments in four Berberis species. *Pakistan Journal of Nutrition*, 5(1), 90-92. https://doi.org/10.3923/pjn.2006.90.92

Lee, Y.L., Tsung, Y.M. and Mau, J.L. (2007). Antioxidant properties of various extracts from *Hypsigizus marmoreus*. *Food Chemistry*, 104(1), 1-9. https://doi.org/10.1016/j.foodchem.2006.10.063

Leong, S.Y. and Oey, I. (2012). Effect of processing on anthocyanins, carotenoids and vitamin C in summer fruits and vegetables. *Food Chemistry*, 133(4), 1577-1587. https://doi.org/10.1016/j.foodchem.2012.02.052

Li, B.W., Andrews, K.W. and Pehrsson, P.R. (2002). Individual sugars, soluble, and insoluble dietary fiber contents of 70 high consumption foods. *Journal of Food Composition and Analysis*, 15(6), 725-733. https://doi.org/10.1006/jfca.2002.1096

Lim, T.K. (Ed.) (2013). Edible Medicinal and Non-Medicinal Plants. Volume 5, Fruits. Netherlands: Springer. https://doi.org/10.1007/978-94-005-653-3

Lu, Y. and Foo, Y.L. (2000). Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chemistry*, 68(1), 81-85. https://doi.org/10.1016/S0308-8146(99)00167-3

Mahmood, T., Anwar, F., Bhatti, I.A. and Iqbal, T. (2013). Effect of maturity of proximate composition, phenolics and antioxidant attributes of cherry fruit. *Journal of Botanical Pakistan*, 45(3), 909-914.

Martínez, S., López, M., González de Lez-Raurich, M. and Alvarez, A.B. (2005). The effects of ripening stage and processing systems on vitamin C content in sweet peppers (*Capsicum annuum* L.). *International Journal of Food Sciences and Nutrition*, 56(1), 45-51. https://doi.org/10.1080/09637480500081936

Madaleno, R.O. (2015). Effect of Pre-treatments and Post-treatments on Drying Processes. Portugal: ISEC - Coimbra Institute of Engineering, MSc. Thesis.

Mercado-Silva, E., Benito-Bautista, P. and García-Velasco, M.A. (1998). Fruit development, harvest index and ripening changes of guavas produced in central Mexico. *Postharvest Biology and Technology*, 13(2), 143-150. https://doi.org/10.1016/S0925-5214(98)00003-9

Ministry of Health. (2014). Food Act 1983 (Act 281) and Regulations. Malaysia.

Miraf, A.H.S., and Salma, I. (2015). Ceri Terengganu: The future antioxidant superstar, p. 6 Malaysia: MARDI Scientia 6.

Miraf, A.H.S., Zaulia, O., Joanna, C.L.Y., Erny, S.M.N. and Salma, I. (2017). Antioxidant activity and phytochemical content of fresh and freeze-dried *Lepisanthes fruticosa* fruits at different maturity stages. *Journal of Agricultural Science*, 9(2), 147-153. https://doi.org/10.5539/jas.v9n2p147

Monica, F. (2007). Diurnal and seasonal course of the rate of the photosynthesis in the apple tree case in the conditions from the fruit growing region *Pitesti Maracineni*. *Buletin USAMV-CN*, 64, 223-228.

Mozafar, A. (1994). Plant vitamins: agronomic, physiological and nutritional aspects. Boca Raton, FL: CRC Press.

Mphahlele, R.M., Stander, M.A., Fawole, O.A. and Opara, U.L. (2014). Effect of fruit maturity and growing location on the postharvest contents of flavonoids, phenolic acids, vitamin C and antioxidant activity of pomegranate juice (cv. Wonderful). *Scientia Horticulturae*, 179, 36-45. https://doi.org/10.1016/j.scienta.2014.09.007

Ndawula, J., Kabasa, J.D. and Byaruhanga, Y.B. (2004). Alterations in fruit and vegetable β-carotene and vitamin C content caused by open-sun drying, visqueen-covered and polyethylene-covered solar-dryers. *African Health Service*, 4(2), 125-130.

Nithiya Shannmuga Rajan, Rajeev Bhat and Karim, A.A. (2014). Preliminary studies on the evaluation of nutritional composition of unripe and ripe ‘Kundang’ fruits (Bouea macrophylla Griffith). *International Food Research Journal*, 21(3), 985-990.

Onwuka, G.I. and Onwuka, N.D. (2005). The effects of ripening on the functional properties of plantain and plantain based cake. *Journal of Food Properties*, 8(2), 347-353. https://doi.org/10.1016/j.jfp.20059489

Ping, C.S., Hock, E.K. and Azlan, A. (2013). Comparison of nutrient composition of ripe and unripe fruits of *Nypa fruticans*. *Fruits*, 68, 491-498. https://doi.org/10.1051/fruits/2013089

Ragae, S., Abdel-Aal, E.M. and Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, 98(1), 32-38. https://doi.org/10.1016/j.foodchem.2005.04.039

Rahmadi, A., Puspita, Y., Nursayekti, D., Sinaga, I.S., Oktalina, R., Setiawan, H. and Murdianto, W. (2016). Analisis proskimat, senyawa fenolik, sifat antioksidan dan antibakteri kulit buah *Lepisanthes alata*. *Jurnal Teknologi dan Industri Pangan*, 27(2), 115-122. https://doi.org/10.6066/jtip.2016.27.2.115

Sharaf, A., Ahmed, F.A. and EI-Saadany, S.S. (1989). Biochemical changes in some fruits at different ripening stages. *Food Chemistry*, 31(1), 19-28. https://doi.org/10.1016/0308-8146(89)90147-7

Shin, Y., Ryu, J.A., Liu, R.H., Nock, J.F. and Watkins, C.B. (2008). Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant...
contents and activity, and inhibition of cell proliferation of strawberry fruit. *Postharvest Biology Technology*, 49(2), 201-209. https://doi.org/10.1016/j.postharvbio.2008.02.008

Siriwoharn, T., Wrolstad, R.E., Finn, C.E. and Pereira, C.B. (2004). Influence of cultivar, maturity, and sampling on blackberry (*Rubus L. Hybrids*) anthocyanins, polyphenolics, and antioxidant properties. *Journal of Agricultural Food Chemistry*, 52, 8021-8030. https://doi.org/10.1021/jf044861y

Umi Kalsom, H.Z. and Mirfat, A.H.S. (2014). Proximate composition of Malaysian underutilised fruits. *Journal of Tropical Agriculture and Food Science*, 42(1), 63-72.

Vijaya, K.R.C., Sreeramulu, D. and Raghunath, M. (2010). Antioxidant activity of fresh and dry fruits commonly consumed in India. *Food Research International*, 43(1), 285-288. https://doi.org/10.1016/j.foodres.2009.10.006

Wahyuningsih, D. (2008). The effect of method and blanching time on anthocyanin and ascorbic acid content of red *Sesbania grandiflora L.* (Pers) flower. Yogyakarta, Indonesia: Mercu Buana University, PhD Thesis.

Wall, M.M. and Biles, C.L. (1993). *Alternaria* fruit rot of ripening chile peppers. *Phytopathology*, 83, 324-328. https://doi.org/10.1094/Phyto-83-324

Wallace, T.C. and Giusti, M.M. (2015). Anthocyanins. *American Society for Nutrition. Advanced Nutrition*, 6(5), 620-622. https://doi.org/10.3945/an.115.009233

Wang, S.Y. and Lin, H.S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural Food Chemistry*, 48, 140-146. https://doi.org/10.1021/jf9908345

Yang, H., Kim, Y.J. and Shin, Y. (2019). Influence of ripening stage and cultivar on physicochemical properties and antioxidant compositions of aronia grown in South Korea. *Foods*, 8(12), 598. https://doi.org/10.3390/foods8120598

Zainudin, M.M. A., Abdul Hamid, A., Anwar, F., Osman, A. and Saari, N. (2014). Variation of bioactive compounds and antioxidant activities of carambola (*Averrhoa carambola L.*) at different ripening stages. *Scientia Horticulturae*, 172, 325-331. https://doi.org/10.1016/j.scienta.2014.04.007

Zielinski, A.A.F., Goltz, C., Yamato, M.A.C., Ávila, S., Hirooka, E.Y., Wosiacki, G., Nogueira, A. and Demiate, I.M. (2015). Blackberry (*Rubus spp.*): influence of ripening and processing on levels of phenolic compounds and antioxidant activity of the ‘Brazos’ and ‘Tupy’ varieties grown in Brazil. *Ciência Rural*, 45(4), 744-749. https://doi.org/10.1590/0103-8478cr20120715

Zhang, Y., Chen, S., Huo, J. and Huang, D. (2019). Deciphering the nutritive and antioxidant properties of Malay cherry (*Lepisanthes alata*) fruit dominated by ripening effects. *RSC Advances*, 9, 38065-38076. https://doi.org/10.1039/C9RA05312C