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Nutritional Values of *Artocarpus odoratissimus* (Terap) Fruit and its Antioxidant Capacity as Affected by Superheated-steam Treatment

Azizah Othman[1], Nurul Nadia Hassan[1], Shahrina Shah Jahan[2], Rafidah Husen[2*]
[1]Universiti Teknologi MARA, Faculty of Applied Sciences, School of Industrial Technology, 40450 Shah Alam, Selangor, Malaysia
[2]Faculty of Applied Sciences, Universiti Teknologi MARA Cawangan Sarawak, 94300 Samarahan, Sarawak, Malaysia

ABSTRACT

This study was carried out to evaluate the nutrient composition, Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and antioxidant capacity of *Artocarpus odoratissimus* by using DPPH and FRAP assays. Results showed that nutrient compositions were quite similar to other reported studies. As for the antioxidant potential of the fruit, both the flesh and seed treated with Superheated-Steam (SHS) showed significantly higher TPC, TFC, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) (except the flesh showing no significant difference) values compared to the Freeze-Dried (FD) samples. The SHS-treated seed showed the highest values in all the assays conducted, exhibiting the superior antioxidant potential of the seed over the flesh. The seed also contained a higher composition of fat, protein, ash and carbohydrate than the flesh, while the flesh, on the other hand, showed higher moisture content and crude fibre compared to the seed ($p<0.05$). This study has demonstrated that SHS has the ability to enhance the polyphenolic compounds and antioxidant capacity of terap fruit.

Keywords: antioxidant capacity, *Artocarpus odoratissumus*, nutrient composition, superheated-steam treatment, terap

INTRODUCTION

Artocarpus odoratissimus or locally known as terap/tarap, is a popular seasonal fruit in Borneo. Belonging to the Moraceae family, it is native to Southeast Asia and is related to *A. integer* (cempedak), *A. altilis* (breadfruit) and *A. heterophyllus* (jackfruit). Its common name pingan (lban), pi-ien (Bidayuh), keiran (Kelabit), terap (Malaysia), marang (Sulu), madang (Lanao), loloi (Tagalog), and khanun sampal (Thailand) varies between different places. The fruit averaged about 16 cm in length, 13 cm in diameter and weighing about 1 kg. The fruit is round to oblong, regular and thickly studded with short, brittle, greenish-yellow spines. The fruit's skin is green and thick, similar to cempedak fruit, while the flesh is white, juicy and aromatic, attached to the central core where each segment contains a seed of 8 x 15 mm (Tang *et al.* 2013).

Among the Artocarpus, terap fruit is not widely exploited, especially on its phytochemical constituents. However, various parts of the plant have been studied, such as the flesh, seed, root and stem, to identify the approximate value of each part. It has been reported that terap fruit shows high antioxidant, phenolic and flavonoid content (Bakar *et al.* 2009). Peel shows the highest total phenolic content, followed by the seed and flesh (Bakar *et al.* 2015). According to Nyokat *et al.* (2017), terap fruit is rich in phenolic compounds, including flavonoids, stilbenoids and arylbenzofurons. However, very limited studies are available on the effect of treatments on this fruit, particularly superheated-steam application in the drying process.

Superheated-Steam (SHS) is a steam at a temperature higher than its vaporisation (boiling) point at the absolute pressure where the temperature is measured (Alfy *et al.* 2016). It is a drying method with high heat transfer capability, produces condensation heat when in contact with the food, provides no oxygen environment and can be obtained under normal pressure. It also can improve product quality, such as low shrinkage, high porosity and vitamin C retention (Abdulhameed *et al.* 2014). The potential of superheated-steam application in fruit has

[*]**Corresponding Author:** email: rafidahh@uitm.edu.my
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been presented, where superheated-steam dried avocado shows a significant increase in its polyphenolic content and antioxidant capacity (Husen *et al.* 2014). It also has been reported that the radical scavenging activity in black cumin seed was improved with increased exposure to superheated-steam (Liang *et al.* 2018). To date, there is no study reported on the effect of superheated-steam treatment on the antioxidant properties of terap fruit. In this study, the effect of superheated-steam treatment on the polyphenolic compound and antioxidant capacity of terap flesh and seed was evaluated and compared with their freeze-dried counterparts, which is a drying technique that has the ability to retain nutrients and the quality of food products and is generally recommended to dry samples with heat-sensitive compounds, especially antioxidants. The nutritional composition of this fruit was also measured in this study.

METHODS

Design, location, and time

Terap fruit sample was subjected to superheated-steam drying as a pre-treatment prior to freeze-drying, while another group was directly subjected to freeze-drying. Proximate analysis (moisture, carbohydrate, protein, fat, fibre, ash and minerals) and chemical analysis (TPC, TFC, DPPH and FRAP) were then performed on both groups. Superheated-steam drying was done at the Biomass Technology Centre at Universiti Putra Malaysia, Serdang, Selangor. All experimental works were conducted in the laboratories at the Faculty of Applied Sciences, Universiti Teknologi MARA. The study started in 2016 and was completed in 2017.

Material and tools

Chemical and reagents. Aluminium chloride hexahydrate, sodium acetate trihydrate, acetic acid, petroleum ether, Trolox, gallic acid and rutin standards, and DPPH (2,2-diphenyl-2-picrylhydrazyl) were purchased from Sigma-Aldrich, Germany. Ninety percent (95%) ethanol was purchased from Merck, Germany. Hydrogen peroxide, sulphuric acid, nitric acid, boric acid, hydrochloric acid, acetone and Folin-Ciocalteu reagent were purchased from R & M Chemicals, United Kingdom. Sodium carbonate was purchased from Ajax, New Zealand. TPTZ (2,4,6-tripyridyl-s-triazine) were purchased from Fluka, Germany. Ferric chloride hexahydrate and hexane were purchased from HmbG GmbH, Denmark.

Procedures

Physical characteristics of fruit samples. Six terap fruits were obtained from Kuching, Sarawak, Malaysia, at their ripe stage. The diameter, height, width (mm) and weight (g) of all the fruits were measured.

Drying method. The fruits were cleaned and separated into flesh and seed. The flesh and seed samples were divided into two groups. The drying method was conducted according to Husen *et al.* (2014) with slight modifications. One group was subjected to SHS treatment using a SHS oven (DC Quto QF-5200C, Naomoto, Japan) at 170°C for 30 minutes, followed by lyophilisation for two weeks at -60°C by using a freeze dryer (Alpha 1-4 LD plus, Martin Christ, Germany), while the other group was freeze-dried (control). Both groups of flesh and seed were then finely ground, sieved, sealed in a normal polypropylene plastic bag and kept at -30°C until further analysis.

Extraction. Referring to the method by Shaharuddin (2021) with slight modification, 50 g and 20 g of flesh and seed samples respectively were extracted with 70% ethanol in a 1:10 wt/vol ratio. The sample was then incubated in the incubator shaker (Innova 40, Eppendorf, Germany) for 2 hours at 200 rpm at 27°C. The sample was filtered using Whatman filter paper No.1 and evaporated under vacuum at 27°C for 2–3 hours using Rotavapor (RE21, Büchi, Switzerland). The liquid extract was then lyophilised at -60°C in a freeze dryer. The dried extracts were sealed in an airtight plastic container and kept at -30°C until further analysis.

Nutritional properties. The proximate composition of terap fruit, including carbohydrate, protein, fat, moisture, ash and crude fibre, was determined according to the AOAC method (AOAC 2012). Mineral contents were determined using a method by Kumaravel and Alagusundaram (2014) with slight modification using an Atomic Absorption Spectrophotometer (Shimadzu AA-7000, Japan).

Total Phenolic Content (TPC). Total phenolic content was measured by using Folin-Ciocalteu reagent as described by Othman *et al.* (2016). Two hundred microliter (200

µL) extracts were mixed with 1,500 µL Folin-Ciocalteu reagent (diluted 10-fold with distilled water). The mixture was vortexed for 15 seconds and allowed to stand at room temperature for 5 minutes. 1,500 µL 0.56 M sodium carbonate solution was added and left to stand for another 90 minutes. The absorbance was measured using a UV-Vis Thermo Spectrophotometer (Thermo Scientific, Thermo Fisher Scientific, USA) at 725 nm. The result was expressed as mg gallic acid equivalent (GAE) per 1 g edible portion.

Total Flavonoid Content (TFC). Total flavonoid content was measured using the colourimetric method as described by Djeridane *et al.* (2006). 1 ml of sample extract was mixed with 1 ml of 2% aluminium chloride solution and allowed to stand at room temperature for 15 mins. The absorbance was then measured using a UV-Vis Thermo Spectrophotometer at 430 nm. The result was expressed as mg rutin equivalent (RE) per 1 g edible portion.

DPPH Radical Scavenging Activity. The extract's scavenging activity was estimated using 1,1-diphenyl-2-pycrylhydrazyl (DPPH) as described by Othman *et al.* (2016). 1 ml of extract (or ascorbic acid) was mixed with 2 mL 0.15 mM DPPH and allowed to stand for 30 mins at room temperature in the dark. After the incubation period, the absorbance was measured at 517 nm using a UV-Vis Thermo Spectrophotometer. Antioxidant activity was expressed as the percentage of scavenging activity, calculated below:

Percentage of scavenging activity (%) =

$$\frac{(A_{control} - A_{sample/standard})}{A_{control}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP). FRAP was measured as described by Tang *et al.* (2013). FRAP solution was freshly prepared using 300 mM acetate buffer, 30 mM Tris(2-pyridyl)-s-Triazine (TPTZ) solution in 40 mM HCl, 30 mM ferric chloride hexahydrate with a 10:1:1 ratio. It was then incubated at 37°C for 10 minutes in an incubator shaker. 100 µL extract was mixed with 8.9 ml FRAP solution and allowed to react for 1 hour in the dark. Absorbance was measured at 593 nm using a UV-Vis Thermo Spectrophotometer. The result was expressed as mg Trolox Equivalent (TE) per 1 g edible portion.

Data analysis

All experiments were carried out in triplicates. Results were presented as mean±SD (Standard Deviation). Statistical analysis was done using analysis of variance (ANOVA), SPSS version 23 (SPSS Inc., Chicago, Illinois, USA). A significant difference between means was defined as $p<0.05$.

RESULTS AND DISCUSSION

Physical characteristics of terap fruit

Terap fruit is an underutilized fruit which is well known among the local people in the region but not widely known or consumed outside of the area. The physical measurement of the fruits can give a better idea of the fruit size, especially to those who are not familiar with the fruit. In this study, the diameter, height, width and weight were given in Table 1. Tang *et al.* (2013) also reported that the weight of terap fruit is around 0.5–1 kg depending on its size.

As given in Table 2, the moisture content of terap flesh was 72.65%, while the seed was 38.17%. These values were within the range reported by Tang *et al.* (2013), where the flesh and seed were between 67.9–73.4% and 31.0–55.0%, respectively on a wet basis. Terap flesh also showed moisture content which was within the range of the moisture content found in *A. integer* (chempedak), *A. altilis* (breadfruit), and *A. heterophyllus* (jackfruit) (67–83%) (Tang *et al.* 2013).

Fat is very important in a diet, and it helps to promote the absorption of fat-soluble vitamins. Fat content in the flesh and seed of terap was 0.36% and 3.54%, respectively. However, this value was lower compared to previously reported, which are 10.1%–28.1% (Tang *et al.* 2013) and 15.60% (Noorfarahzilah *et al.* 2017), but higher than unripe terap flour (1.89%) (Masri *et al.* 2012).

Table 1. Physical characteristics of terap fruit

Parameter	Values
Weight (g)	868.70–1402
Diameter (mm)	380–700
Height (mm)	185–250
Width (mm)	150–160

n=6

Table 2. Nutrient composition of terap fruit

	Flesh	Seed
Moisture (%)	72.65±0.43	38.17±0.47
Fat (%)	0.36±0.02	3.54±0.29
Protein (%)	0.39±0.01	7.49±0.13
Carbohydrate (%)	26.25±0.48	50.29±0.81
Ash (%)	0.34±0.03	0.50±0.00
Crude fibre (%)	1.08±0.01	0.33±0.01
Na (mg/100 g)	12.93±6.70	N/A
Ca (mg/100 g)	21.91±3.99	N/A
Mg (mg/100 g)	39.41±1.01	N/A
Zn (mg/100 g)	1.15±0.07	N/A
Fe (mg/100 g)	1.31±0.19	N/A
Cu (mg/100 g)	0.3±0.08	N/A

Data expressed as mean±SD
N/A: Data not available

Protein plays a part in food's organoleptic properties and is a source of amino acids. The protein content of terap fruit was found to be high in the seed (7.49%) and low in the flesh (0.39%). This was in agreement with Tang *et al.* (2013), where lower protein content in the flesh is 1.2–1.5% and higher in the seed at 5.1–6.6%. The protein content in the seed measured in this study was slightly lower than previously reported by Noorfarahzilah *et al.* (2017) on terap seed flour, with a value of 8.78%. The stage of maturity and growing environment commonly affect the protein content in the fruit (Noorfarahzilah *et al.* 2017). In pineapple (*Ananas comosis)* for example, total protein content increases with the increase in the days of maturity stage, while in sweet pepper (*Capsicum annuum* L.) seeds show an increase in albumin, globulin and prolamins content as a function of the fruit maturation stage (Sabahelkhier *et al.* 2010; Colombari *et al.* 2022).

Ash represents the total mineral content in food. In this study, the ash content in the seed (0.50%) was slightly higher than in the flesh (0.34%). This result was lower compared with Tang *et al.* (2013), which shows that ash content for seed and flesh are in the range of 1.0–1.5% and 0.6–0.8%, respectively. The ash content in the seed is higher than in the flesh could be due to the seeds having higher concentrations of minerals than flesh because they are the reproductive organs and therefore need more nutrients for germination and growth. Terap has less ash content compared to other Artocarpus, where *A. heterophyllus* (jackfruit) and *A. integer* (chempedak) showed 2.2% and 1.2% of ash content respectively in their flesh (Morton 1987). Nevertheless, terap flour possessed higher ash content with a value of 2.84% (Masri *et al.* 2012) and 1.17% for terap seed flour (Noorfarahzilah *et al.* 2017).

Total carbohydrate content includes fibre and other components such as organic acids. Energy from fruits comes mainly from carbohydrates. In this study, total carbohydrates in the flesh and seed were 26.25% and 50.29% respectively. The carbohydrate content in the flesh reported by Tang *et al.* (2013) is lower than this study (12.0–25.2%,) while in the seed, the carbohydrate content was similar with terap seed flour (49.65%) (Noorfarahzilah *et al.* 2017).

Crude fibre helps maintain normal peristaltic movement of the intestinal tract. A diet containing high fibre is important to prevent constipation, which in turn could be helpful in order to prevent other chronic diseases. As reported by Tang *et al.* (2013), the fibre content in the flesh and seed of terap is 2.8–4.2% and 5.5–10.0% respectively. The seed flour contains 12.30% crude fibre (Noorfarahzilah *et al.* 2017), and terap flour contains 2.65% crude fibre (Masri *et al.* 2012). In this study however, the crude fibre content in terap fruit was found to be lower, which was 1.08% and 0.33% in the flesh and seed, respectively. Generally, seeds have higher fibre content than flesh due to the higher content of complex carbohydrates that are resistant to digestion, such as cellulose, hemicellulose, and lignin. These substances provide structural support and protection for the seeds, as well as a source of energy and nutrients for germination and seedling growth (Oso & Ashafa 2021).

Potassium was found to be the most abundant mineral in terap flesh (Masri *et al.* 2012; Tang *et al.* 2013; Noorfarahzilah *et al.* 2017), where Tang *et al.* (2013) shows 176–298 mg/100 g potassium content. The mineral content of terap flesh in this study is given in Table 2. In this study, magnesium gave the highest value (39.41 mg/100 g), followed by calcium (21.91 mg/100 g) and sodium (12.93 mg/100 g). Tang *et al.* (2013) reported lower magnesium, calcium and sodium content (15–31 mg/100 g, 0.5–1.4

mg/100 g and 1.1–1.7 mg/100 g respectively). Iron (1.31 mg/100 g) and zinc (1.15 mg/100 g) measured in this study were also higher than the reported values (0.3–0.5 mg/100 g and 0.17–0.45 mg/100 g for iron and zinc respectively) (Tang *et al.* 2013). Among all the minerals analysed, copper content was the lowest (0.3 mg/100 g), which gave a similar value as previously reported (Tang *et al.* 2013). No lead was found in both the flesh and seed of the fruit. Hence, it is safe for consumption (Tang *et al.* 2013).

Antioxidant properties

Total Phenolic Content (TPC). Table 3 shows the result of the TPC of terap flesh and seed dried with superheated steam treatment and freeze-drying. SHS-treated terap seed gave the highest TPC at 4.69 mgGAE/g, which was significantly higher than the FD seed (4.10 mgGAE/g). For the flesh, TPC values were significantly lower than the seed, with the SHS-treated sample being significantly higher (0.72 mgGAE/g) than the FD counterpart (0.65 mgGAE/g).

This increase in TPC was possibly due to the matrix liberation of phenolic compounds and/or inactivation of polyphenol oxidase during the superheated steam treatment process. According to Tomás-Barberán and Espín (2001), fruit subjected to stress conditions may increase the level of phenolic compounds. Stress conditions include UV radiation, infection by pathogens and parasites, wounding, air pollution, and exposure to extreme temperatures. Therefore, SHS might cause structure damage to fruit cells, resulting in increased liberation of phenolic compounds from the flesh and seed of the terap samples. A study by Bakar *et al.* (2009) reported that the seed contained higher TPC than the flesh, with values of 14.67 mgGAE/g and 4.39 mgGAE/g respectively. Another part of terap fruit, the peel, shows even higher phenolic content (42.38 mgGAE/g) (Bakar *et al.* 2015). TPC of terap fruit seed is lower when compared to A. heterophyllus (jackfruit) at 27.7 mgGAE/g (Soong & Barlow 2004). According to Soong and Barlow (2004), gallic acid, ellagic acid, coumarin, cinnamic acid, ferulic acid and caffeic acid might contribute to the total phenolic acid in the seed. Phenolic compounds found in the seed are responsible during seed germination to act as antioxidants to avoid internal damage.

Total Flavonoid Content (TFC). The total flavonoid content in terap seed was also higher compared to the flesh (Table 3). Similar to the TPC result, TFC in SHS-treated seed and flesh were significantly higher (2.12 mgRE/g and 0.43 mgRE/g respectively) compared to their FD counterparts (1.10 mgRE/ g and 0.28 mgRE/g respectively). Bakar *et al.* (2009) also showed higher TFC in terap seed (3.65 mgRE/g) compared to the flesh (1.08 mgRE/g) ($p<0.05$). Flavonoids are found at high levels in most plant seeds as they offer vital roles in defence against pathogens and predators and contribute to physiological functions such as seed maturation and dormancy (Shirley 1998).

Flavonoids are the most common and extensively distributed group of plant phenolic compounds. They are ubiquitously present in fruits and vegetables. Flavonoids have high pharmacological activities as radical scavengers, making them essential for human health to avoid oxidation (Baliga *et al.* 2011). A. odoratissimus consists of artosimmin, a phytochemical compound derived from the flavonoid group. It displays cytotoxicity activity against breast cancer and promyelocytic leukaemia (CL kEe *et al.* 2010).

Table 3. Total phenolic content, total flavonoid content and antioxidant activity of terap fruit

Sample	TPC (mgRE/ g)	TFC (mgGAE/ g)	Antioxidant activity	
			DPPH (%)	FRAP (mgTE/ g)
Flesh Superheated-Steam (SHS)	0.72±0.00^{a}	0.43±0.01^{a}	11.24±0.37^{a}	1.55±0.01^{a}
Flesh Freeze-Dried (FD)	0.65±0.00^{b}	0.28±0.00^{b}	8.46±0.28^{b}	1.60±0.08^{a}
Seed Superheated-Steam (SHS)	4.69±0.00^{a}	2.12±0.01^{a}	90.52±0.15^{a}	129.81±1.95^{a}
Seed Freeze-Dried (FD)	4.10±0.00^{b}	1.10±0.00^{b}	89.19±0.16^{b}	59.75±1.27^{b}

DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power

Results expressed as mean±S.D; (n=3); Different letters in the same group indicate a significant difference ($p<0.05$)

Polyphenols and flavonoids are powerful free radical scavengers. Phenolic compounds generally occur as soluble conjugated and insoluble forms, while flavonoids present as glycosides with single or multiple sugar molecules linked through an OH group (O-glycoside) or Carbon-Carbon Bond (C-glycoside) (Acosta-Estrada *et al.* 2014). Phenolic phytochemicals are a group of compounds that play an important role in determining antioxidant properties in plants (Minatel *et al.* 2017).

DPPH radical scavenging activity. DPPH radical scavenging activity is a method to measure antioxidant activity in food and has a broad absorption band in the visible region at 517 nm. It is protonated by an antiradical compound by losing this property (Scalzo 2008). In this study, the highest percentage of scavenging activity was observed in the SHS-treated seed, followed by the FD seed, SHS-treated flesh and FD flesh, with values of 90.52%, 89.19%, 11.24% and 8.46% respectively (Table 3). Similar to the previously described results on TPC and TFC, higher radical scavenging activity was observed in the seed than in the flesh. Bakar *et al.* (2009) also reported that the seed of terap fruit contains higher scavenging activity (13.69 mgAEAC/g) compared to the flesh (2.44 mgAEAC/g). The radical scavenging activity was also significantly higher when SHS treatment was applied in both seed and flesh. The SHS may have caused the inactivation of oxidative enzymes and contributed to better preservation of phenolic compounds which can increase antioxidant activity (Samoticha *et al.* 2016).

Ferric Reducing Antioxidant Power (FRAP). FRAP assay was also performed to determine the antioxidant capacity of the samples. It measures the ability of phytochemicals to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) through electron donation (Benzie & Strain 1996). The result also showed that terap seed gave significantly higher FRAP values than flesh (Table 3). When treated with SHS, the antioxidant activity doubled in the treated seed, at 129.81 mgTE/g, significantly higher than the FD sample (59.75 mgTE/g). The flesh, however, showed no significant difference when treated with superheated steam.

Fruits and vegetables have higher biological activities due to chemical changes during heat treatment (Dewanto *et al.* 2002). The effect of the high temperature of SHS might be one cause for the significant increase in antioxidant activity (Husen *et al.* 2014). Citrus pomace extract treated with SHS at 300˚C exhibited higher scavenging activity with IC_{50} values ranging from 0.13 to 0.20 mg/mL (Wang *et al.* 2018), while black cumin seed treated with SHS for 30 min resulted in higher scavenging activity compared with conventional hot air drying (Liang *et al.* 2018). It was thought that any materials treated with high temperatures would most likely lose their antioxidant activity. However, this is not necessarily the case, as shown by the application of SHS in food processing. SHS process has the advantage of heating at high temperatures within a short period of time. The steam temperature of SHS is over its boiling point at an absolute pressure in a closed system that may cause strong penetrability and solvency, which effectively enrich the antioxidant without destroying relevant compounds in a short period of time (Wang *et al.* 2018). Moreover, SHS can enhance the amount of phenolic and flavonoid compounds compared to FD. SHS works in the absence of oxygen, where steam is generated and is given additional heat to raise its temperature above the saturation temperature under normal pressure. Therefore, because of the absence of oxygen in the SHS system, there is less oxidation and, in turn, higher antioxidant properties in both samples. Research has shown that SHS-heated food retains antioxidants, vitamins, and other essential nutrients due to the absence of oxygen (Pronyk *et al.* 2004).

Relationship between antioxidant compounds and antioxidant activities in terap fruit. The correlation between antioxidant compounds and antioxidant activities of terap fruit was also evaluated. TPC gave a strong positive correlation ($p<0.01$) with both DPPH and FRAP antioxidant activities, with r2 values at 0.953 and 0.960, respectively. TFC on the other hand, only showed a strong positive correlation with FRAP with r^2 at 0.970. These strong positive correlations indicated that antioxidant activities in terap fruit were attributed to the presence of both phenolic and flavonoid compounds in the fruit.

CONCLUSION

Terap is a nutritious fruit which can provide a certain amount of both macro and

micronutrients. Terap fruit dried using SHS exhibited significantly higher TPC, TFC, DPPH and FRAP values than that of freeze dried. Generally, food materials processed at high temperatures are likely to lose their nutrients and antioxidant properties. However, the application of SHS in this study has demonstrated its advantage over freeze-drying as a method which can be applied in food processing due to its ability to enhance the antioxidant capacity. Furthermore, a significant increase especially shown in the seed of terap fruit treated with SHS shows the potential of this fruit to be developed into functional food products such as seed flour and powders. For future studies, it is recommended that terap fruit seed be used to develop food or functional food products.

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DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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