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RESEARCH PAPER

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Effect of processing condition on the total phenolic contents and antioxidant properties of selected tropical fruits

Ida Madiha Yusoff^{*1}, Ahmad Faris Shaharudin², Zarani Mat Taher¹, Hesham El Enshasy^{1,3,4}

¹Institute of Bioproduct Development, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia ²Politeknik Merlimau, Karung Berkunci, Pejabat Pos Merlimau, Merlimau, Melaka, Malaysia ³School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia ⁴City of Scientific Reserach and Technology Applications (SRTA), New Burg Al Arab, Alexandria, Egypt

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Abstract

Global productions and demands for tropical fruits continue to grow each year as consumers are concerned on potential health benefits. Fruits are undergoing various foods processing in order to enhance the organoleptic properties, nutrient content and extend shelf life. Therefore, the aim of this study is to determine the effect food processing parameters such as thermal pasteurization, ultrasonic treatment and high speed mixing on total phenolic contents and antioxidant properties of tropical fruits. Five tropical fruits: banana, watermelon, mango, papaya and pineapple were selected in the present study. The fruits were prepared into puree and treated with three different processing conditions i: thermal pasteurization, ii: ultrasonic treatment, iii: high speed mixing. The fruits samples were analyzed for total phenolic contents and DPPH radical scavenging activity assay. The results clearly demonstrated that ultrasonic was the best treatment for total phenolic contents and antioxidant activity. The papaya was the richest sources of antioxidant compounds. The results suggest that all selected tropical fruits have shown potential as sources of natural antioxidants.

* Corresponding Author: Ida Madiha Yusoff 🖂 idamadiha@ibd.utm.my

Introduction

The tropical fruits have drawn more attention worldwide because of sensorial properties, economic importance and also for their high antioxidant activity. Malaysia is a popular destination on the production of tropical fruits worldwide. The pineapple, banana, papaya, watermelon and mango play an important economic role. Malaysia produces tropical fruits exceed 200 metric tonnes per year. The fruits cultivation and planted area in Malaysia are approximately 33 584 ha. Johor is found to have the largest areas of fruits cultivation followed by Sarawak and Pahang. The banana is highly cultivated followed by pineapple and watermelon (Rozana *et al.*, 2017). These tropical fruits have been a valuable source of natural bioactive molecules essential for human health.

The demand on processed tropical fruits and mainly commercialized as fresh, dried fruits, juice, puree and jam have been also increased. The processing conditions are an essential procedure either to enhance the fruits quality, extend shelf life or to remove unnecessary part from the fruits. The fruits are commonly undergone many processing such as pasteurization, ultrasonic and mixing which may cause deterioration, water loss, development of offflavour and reducing the bioactive compound. Thermal pasteurization is commonly used to inactive microorganisms and enzymes to enhance the products stability and safety (Etzbach et al., 2010). The recent researches on applications of pasteurization were studied for mandarin (Cheng et al., 2020) and pineapple (Sanya et al., 2020).

Ultrasonic treatments are widely used for degassing, emulsification, extraction, cleaning and filtration. The ultrasonic treatments are one of an advanced method. These techniques are based on application of the mechanical stress along with micro-streaming stimulated by acoustic cavitation. The wave leads to faster dispersion of matrices from substrate. This action facilitates the penetration of solvent into the cells, thereby improving mass transfer and releasing bioactive compounds. The ultrasonic treatment also induces high pressure, temperature and shear force, which can causes disruption of the cell membrane (Hasan et al., 2014). One of the main differences of ultrasonic treatments instead of conventional processing technique; ultrasonic is considered of as cold and green technique. Ultrasonic treatment have been reported for extraction several fruits such as orange (Montero-calderon et al., 2019), soursoup (Aquilar-Hernandez et al., 2019) peaches, pumpkins (Altemimi et al., 2016) and blueberry (Rocha et al., 2018). The ultrasonic treatments have been used to eliminate vegetative cells of gram-positive, gramnegative bacteria and phytovirus in fruits. The high pressure and a temperature gradient create air bubbles which are causing collision with the liquids molecules. These mechanical shocks are able to disrupt the microorganism's cellular structural and functional components up the point of cell lysis (Sarkinas et al., 2018).

Among plant secondary metabolites, phenolic are natural bioactive compounds which generally distributed in all plants. The phenolic compounds are the most abundant secondary metabolite in plants compared to other compounds. The phenolic compounds are widely found in vegetable, herbal, fruits, nuts and oils. They are essential for regulating the various metabolic functions including pigmentation and resistant to different pathogens. These compounds are also crucial for protecting the plant by filter out the ultraviolet rays and fragile seeds until the plants germinate (Ding et al., 2020). The phenolic compounds are produced in shikimic acid of plants and pentose phosphate through phenylpropanoid metabolization. The basic structure of phenolic compounds comprises of an aromatic ring with one or more -OH groups. In the synthesis of phenolic compounds, the important procedure is the commitment of glucose to the pentose phosphate pathway and transforming glucose 6-phospate irreversibly to ribulose-5-phosphate (Lin et al., 2016)

The general functions of phenolic compounds are for pigmentation, growth and resistance to pathogens and predator (Noreen *et al.*, 2017). These compounds are also play important role in preventing the disease caused by oxidative stress. The oxidative stresses are induced by free radicals. The free radicals are responsible for damaging the lipid, protein and nucleic acid in cell. Attention has recently increased in phenolic compounds from fruits because of their antioxidant and free radical scavenging activities. The phenolic compounds might be responsible in preventing cell degenerative caused by reaction oxidative stress and free radicals (Gangwar *et al.*, 2014).

There are large varieties of antioxidant in fruits. Therefore, determining the antioxidant capacity of each compound separately becomes very difficult. Several methods have been developed to estimate the antioxidant capacity in the plant materials. In this present study, the method that used to determine the antioxidant was DPPH scavenging activity and expressed in methanol extract concentration in mg/mL required to inhibit 50% of radical (EC50). The DPPH assay measures the ability of the extract to donate hydrogen to the radical. DPPH molecule is considered as a very stable free radical. DPPH produces violet/purple color in methanol solution and fade shades to yellow colour in the present of antioxidant (Rahman et al., 2015). The scavenging activity of fruits had been measured using DPPH assay. Ascorbic acid was used as positive control. The unpaired electron of DPPH forms a pair with a hydrogen donated by free radical scavenging antioxidant from fruits and thus converting the purple coloured odd electron DPPH to its reduced from yellow. In DPPH assay, the lower the EC50 the better it is able to scavenge the radicals which are the propagators of the autoxidation of lipid molecules and thereby break the free radical chain reaction (Lim et al., 2007).

The instruments to identify, detect and quantify the phenolic compounds are gas chromatography (GC), high performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS) and ultraviolet-visible (UV-VIS) (Tallapally *et al.*, 2020). The phenolic compounds are reported to be useful as antioxidant. The antioxidant is any compound or substance which able to delay or prevents oxidation of oxidizable substrate (Ravimannan *et al.*, 2017).

The phenolic compounds are also described as chain breaking antioxidant through radical scavenging activities. It is relating to hydrogen and electron donating capacity and ability to delocalize or stabilizing their structure (Tallapally *et al.*, 2020). As consequences, research effort has focuses on the intake of selected tropical fruits in maintaining human health. The aim of this study is to determine the effect of thermal pasteurisation, ultrasonic treatment and juice concentration for high speed mixing on total phenolic contents and antioxidant properties of banana, watermelon, mango, papaya and pineapple.

Materials and methods

Fruits materials and processing

Five fruits were selected for this study; banana (*Musa acuminata* Colla), watermelon (*Citrullus lanatus*), mango (*Mangifera indica*), papaya (*Carica papaya*) and pineapple (*Ananas comosus*) were purchased from Pasar Awam Taman Universiti, Johor, Malaysia. The fruits were washed using tap water and dried with paper towel. The fresh fruits were manually peeled, cut and separated from their wastes. The fruits were prepared into puree and treated with three different processing conditions i: thermal pasteurization, ii: ultrasonic treatment, iii: high speed mixing.

Puree production

For puree, the fruits were crushed to a paste-like state for approximately 1 min by the pestle and mortar method. Then, the fruits were blended (Waring commercial two speed laboratory blender, Torrington, USA) and strained through a sieve (Etzbach *et al.*, 2019). The puree was kept in -20°C for further analyses.

Thermal pasteurization

For thermal pasteurization, 55g puree was heated at 75°C in water bath system (Etzbach *et al.*, 2019). The water bath systems were prepared earlier by heating the tap water into boiling condition. The beakers with fruits puree were immersed into the boiling water. The beakers were covered with aluminium foil to reduce heat loss. The puree temperature was monitored and maintained at 75°C. The thermal pasteurizations were conducted for 10 minutes.

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Ultrasonic treatments

The fruits puree were placed in beakers and treated using ultrasonic water bath (Elmasonic S 30 H, Elma-Hans Schmidbauer GmbH, Germany) with operating frequency; 50/60 Hz and power; 280 W for 45 minutes (Madiha *et al.*, 2017).

High speed mixing

The fruits juice was produced by diluting fruits puree with distilled water and added into the concentration of 30, 50 and 70% w/v. The calculation as below:

 $\frac{\text{Weight of puree (g)}}{\text{Volume of distilled water (ml)}} = \text{Concentration of juice (% w/v)}$

The fruits juices were mixed using vortex with high speed of 2500 rpm ((DLAB MX-B, China) for 2 minutes.

Sample preparation

The treated fruits purees were transferred into 100mL volumetric flask and 50% v/v ethanol were added up to the mark. The mixture was shaken with a vortex mixer for 10 minutes. The mixtures were used further for analysis (Lim *et al.*, 2007).

Determination of total phenolic contents

The total phenolic contents were determined by Folin-Ciocalteu (Fisher Scientific Inc., Waltham, MA, USA). The 0.3mL samples were introduced into the test tube followed by 1.5mL of Folin-Ciocalteu's reagent and 1.2mL of sodium carbonate (7.5% w/v). The test tubes were vortexed, covered with parafilm and allowed to stand for 30 minutes. Absorptions were measured at 765 nm. Gallic acid (0 to 1000 mg/mL) was used for calibration of standard curve. The calibration curve was y = 0.0111x + 0.0127 where y refers to absorbance reading and x is gallic acid equivalents (GAE) in microgram of GAE per miligram of fruits. The results were expressed as gallic acid equivalents (GAE) in micrograms of GAE per miligram of fruits puree.

DPPH radical scavenging activity assay

The antioxidant activity was analysed through the method of 1,1-diphenyl-2picryhydrazyl (DPPH) free radical-scavenging (Chua *et al.*, 2013).

The DPPH solution (20mg/L) was prepared by dissolving 2mg of DPPH in 100mL of methanol. A 0.75mL of sample solution at different concentration was added to 1.5mL of DPPH solution. The absorbance was measured at 517nm after 15 minutes of incubation at 25°C. Ascorbic acid was used as positive control. The concentration of sample required to scavenge 50% of DPPH (EC50) was determined based on the ascorbic acid calibration (o to 200µg/mL). The experiment was performed in triplicate. The rapid colour changing from purple to yellow indicates strong antioxidant. The solution were shaken and left in dark condition for 30 minutes. The samples were measures using UV-Vis spectrophotometer at 517nm.

Statistical analysis

All experiments were performed triplicate. The application of MINITAB 16 software was used to analyse the data for analysis of variance (ANOVA). The turkey's was used to identify the significance difference (p<0.05) between all the treatments \pm standard deviation (SD) of triplicate analysis.

Results and discussion

Consumptions of tropical fruits have been related to reduce the possibility of cancer and chronic disease. The previous research suggests that these health benefits are associated with antioxidant as phytochemical compounds such phenolic compounds. The contents of phenolic compounds are based on the reduction of molybdenum/ tungsten salts by phenol groups in Folin-Ciocalteu. These methodologies evaluate antioxidant reducing capacity by the redox reaction. The high reducing capacity of chemical species may indicate a high antioxidant capacity. The polyphenols are believed to be potent scavenger of peroxyl radicals might be due to the presence of high mobility of hydrogen in their molecular structure (Chua et al., 2013).

The results for phenolic content in this study was varied from $51.138 \pm 0.084\mu$ g GAE/ mg to $16.18 \pm 1.58\mu$ g GAE/ mg. The highest content of total phenolic contents obtained from papaya using ultrasonic treatment whereas watermelon with 30% w/v juice concentration was the lowest. The results for total phenolic and DPPH scavenging activity are shown as in the Table 1, 2 and 3.

	Juices	Phytochemical			
Fruits	concentration for High Speed Mixing (% w/v)	Total phenolic contents (μg GAE/ mg)	EC ₅₀ (DPPH mg/mL)		
Banana	30 50 70	$\begin{array}{l} 30.46 \pm 0.16^{a} \\ 33.76 \pm 0.83^{b} \\ 35.27 \pm 0.16^{b} \end{array}$	32.052 ± 0.559^{a} 36.024 ± 2.474^{a} 29.752 ± 0.951^{a}		
Mango	30 50 70	35.02 ± 1.79^{a} 41.27 ± 0.23^{a} 43.89 ± 0.47^{c}	$\begin{array}{l} 29.850 \pm 1.811^{a} \\ 29.006 \pm 1.305^{b} \\ 27.440 \pm 1.330^{b} \end{array}$		
Watermelon	30 50 70	$\begin{array}{c} 16.18 \pm 1.58^{a} \\ 18.24 \pm 0.43^{b} \\ 23.52 \pm 0.54^{c} \end{array}$	31.375 ± 1.226^{a} 30.247 ± 0.494^{b} 29.042 ± 1.543^{b}		
Pineapple	30 50 70	32.19 ± 1.77^{a} 36.18 ± 1.22^{b} 39.83 ± 0.16^{c}	32.256 ± 0.725^{a} 29.784 ± 2.354 ^a 26.618 ±2.689 ^a		
Papaya	30 50 70	38.40 ± 0.07^{a} 47.43 ± 2.66 ^b 49.21 ± 3.29 ^b	$\begin{array}{l} 32.257 \pm 1.007^{a} \\ 30.359 \pm 2.140^{a} \\ 26.095 \pm 1.710^{a} \end{array}$		

Table 1. The effect of juice concentration on total phenolic contents and DPPH a
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Value are expressed as mean \pm standard deviation (n=3). Means within row followed by different small letters (a, b, c) are significantly different at the level P < 0.05.

Table 2. The effect of processing condition on total phenolic contents of selected tropical fruits.

Analysis	Processing	Banana	Mango	Watermelon	Pineapple	Papaya
	Condition					
	Thermal	28.979 ± 1.778^{a}	38.680 ± 0.516 ^a	17.801 ± 0.558^{a}	36.815± 1.351ª	36.105 ± 1.150^{a}
Total phenolic	pasteurization (TP)					
contents	Ultrasonic treatment	$39.477 \pm 0.789^{\mathrm{b}}$	50.312 ± 0.512 b	$28.432\pm0.468^{\mathrm{b}}$	41.134 ± 0.155^{b}	51.138 ± 0.084^{b}
(µg GAE /mg)	(UT)					
	High Speed Mixing	35.272 ± 0.155^{b}	43.887 ± 0.472 °	$23.523 \pm 0.541^{\circ}$	39.831 ± 0.155^{b}	49.209 ± 3.285^{b}
	(HSM) with 70% w/v					

Value are expressed as mean \pm standard deviation (n=3). Means within row followed by different small letters are significantly different at the level P < 0.05.

Table 3	. The effect	of processing	condition on DPPH	scavenging a	ctivity of	selected tropical fruits.
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Analysis	Processing Condition	Banana	Mango	Watermelon	Pineapple	Papaya
DPPH	Thermal pasteurization(TP)	32.572 ± 3.014^{a}	30.990 ± 3.242 ^a	36.508 ± 1.180ª	33.849 ± 2.368ª	34.839 ± 3.062ª
(mg/mL)	Ultrasonic treatment (UT)	27.719 ± 0.696ª	24.943 ± 0.834^{b}	28.262 ± 1.204 ^b	27.154 ± 2.784ª	23.998 ± 1.163 ^b
	High Speed Mixing (HSM) with 70% w/v	29.752 ± 0.951^{b}	27.440 ± 1.330^{b}	29.042 ± 1.543^{b}	26.618 ± 2.689ª	26.095 ± 1.710 ^b

Value are expressed as mean \pm standard deviation (n=3). Means within row followed by different small letters are significantly different at the level P < 0.05.

Effect of different processing condition on total phenolic contents and DPPH scavenging activity

Food processing techniques involve a sequence of operation bringing the desired changes to raw materials. The quality of tropical fruits deteriorated after exposed to processing condition. These problems have driven the emergence of various new processing conditions with main purpose to enhance product quality. In this study, there were three processing condition, i: thermal pasteurization, ii:

ultrasonic treatment, iii: high speed mixing. Ultrasonic treatment has been applied to extract bioactive compound from various types of plant. The results showed that the total phenolic content and DPPH scavenging activities were higher for all selected tropical fruits compared to thermal pasteurization and high speed mixing. According to statistical results of phenolic contents between thermal pasteurization, ultrasonic treatments and high speed mixing, the significance difference (p<0.05) was observed for all fruits using ultrasonic treatment and thermal pasteurization. The ranged of total phenolic contents for thermal pasteurization were from 28.432 ± 0.468 to $51.138 \pm 0.084 \mu g$ GAE/mg. The ranged of DPPH scavenging activities for ultrasonic treatment were from 23.998 ± 1.163 to 28.262 ± 1.204 mg/mL. Ultrasonic treatment is effective processing methods might be due to mechanical stress by acoustic cavitation (Fig. 1). The acoustic cavitation enhanced the mass transferred which are leading to matrices dispersion from substrate into solvent. This phenomenon might be the reason that the ultrasonic treatment showed the highest total phenolic contents and DPPH scavenging activity among other processing technique.



Fig. 1. The fruits in water A) 70% w/v, B) 50% w/v, C) 30% w/v.

There is no significant difference (p<0.05) between Ultrasonic treatment and high speed mixing for banana, pineapple and papaya. The high speed mixing might be one of other alternative instead of Ultrasonic treatment to obtain the high phenolic content and DPPH scavenging activity. Moreover, high speed mixing are common technique used in juices industry as this method is low in cost and easier to handle.

Effect of fruits on total phenolic contents and DPPH scavenging activity

The papaya exhibited the highest total phenolic content compared to mango, watermelon, pineapple and banana and similiar to Morais, *et al.* (2015). Morais *et al.* (2015) studied on tropical fruits such as avocado, pineapple, banana, papaya, watermelon and melon (Morais *et al.*, 2015). The papaya flesh contains high antioxidant might be due to contain high levels of carotenoids such as lycopene, β -carotenes and β -cryptoxanthin. β -carotenes and β -cryptoxanthin. β -carotenes and β -cryptoxanthin β -carotenes and β -

mature stages of papaya fruits (Sangsoy *et al.*, 2017). The mechanisms of β -carotenes and β -cryptoxanthin as antioxidant is still unclear. However, β -carotenes and β -cryptoxanthin are well known and efficient in physical or chemical quenchers of single oxygen as well as potent scavenger to reactive oxygen species (Fiedor *et al.*, 2014).

The papaya also contains chitinases (Lucas-bautista et al., 2020). Chitinases are essential glycosyl hydrolases for the biotransformation of chitin to chit oligosaccharides. Chitinase activities are also contributing in antioxidant activity by controlling free radical oxidation because of their amino and acetamido and hydroxyl functional group can react with these radicals (Kidibule et al., 2020). Chitinases are reported as can be synthesized in responds to the attack of phytopathogens. The papayas actually are susceptible to the attack of the fungus Colletotrichum gloeosporioides. The function of antioxidant in plant is to protect the plant. It is similar to chitinases in protecting the papayas from bacterial and fungal attack (Lucas-bautista et al., 2020).

The papayas are also composed of pectins, polygalacturonans, celluloses and hemicelluloses. All these compounds are important in ripening process (Jiang *et al.*, 2019). The study by Smirnov, *et al.* (2017) found that plant pectin fractions exhibited as potential antioxidant agent. All these reasons might be indicated that papaya is a good source of antioxidant (Smirnov *et al.*, 2017).

The fruits with the highest total phenolic contents with decreasing order, were papaya > mango > pineapple > banana > watermelon. The pineapple exhibited high total phenolic content (41.134 \pm 0.155µg GAE / mg) and compared to banana (39.477 \pm 0.789µg GAE / mg) as similiar in the study by Alothman, *et al.* (2009). The findings were in contradiction with the previous study conducted by Morais, *et al.* (2015) they reported that the banana exhibited high total phenolic content compared with banana (Morais *et al.*, 2015). However, there is no significance difference between total phenolic content of banana and pineapple in their study. The results

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also exhibited the watermelon as the lowest total phenolic contents and DPPH scavenging activity compared to banana, mango, pineaaple and papaya.

Effect of juice concentration on total phenolic contents and DPPH scavenging activity

The tropical fruits are largely used in food industry for production of beverages and juices. In this study, we suggested to use the concentration of 70% w/v fruits into fruits juice as at 70% w/v of fruit juices, the tropical fruits exhibited the best for total phenolic contents (49.21 ± 3.29µg GAE / mg) and DPPH scavenging activity (26.095 ± 1.710 DPPH mg/mL). The tropical fruits may protect themselves from oxidative stress caused by strong sun exposure by producing large amount of antioxidant compounds (Parvez et al., 2020). The tropical fruits exhibited in increasing total phenolic contents and radical scavenging with increasing concentration of fruit juices. It might be due to the bioactive compounds were high in high concentration compared to low concentration as in Fig. 2.



Fig. 2. A) Cavitation bubbles in ultrasonic water bath, B) Fruits plant cell before ultrasonic treatment, C) Plant cell raptured and compound released after ultrasonic treatment, D) Phenolic compounds from fruits.

Correlation between total phenolic contents and DPPH scavenging activity

In this study, to understand the correlation between total phenolic content and DPPH assay, the Pearson Correlation was used. It was observed that the significant correlation (P<0.05) between total phenolic content and DPPH. The results suggest that total phenolic content may be the important contributor to the antioxidant in this study. These results agreed with previous finding by Almeida, *et al.*, (2011) in their research on pineapple, soursop, sugar apple, jackfruits, murici, papaya, mangaba, sapodilla, ciruela, umbu and tamarind (Almeida *et al.*, 2011). The similarity in present research with Almeida, *et al.* (2011) can be found in the method which the fruits were peeled first and fresh pulps were used for the experiment. Previous study also confirmed that phenolic compounds are the main micro constituents in contributing to the antioxidant activities the fruits (Alothman *et al.*, 2009). It might be due to they found the correlation between total phenolic contents from banana, guava and pineapple with DPPH values. The higher phenol in the fruits, the lower DPPH values. The DPPH values were reflected the antioxidant capacity of the fruits.

Conclusion

This study showed that ultrasonic treatment was the best treatment for total phenolic contents and antioxidant properties followed by high speed mixing. High speed mixing might be another alternative for ultrasonic treatments. Among the selected tropical fruits, papayas were the best sources of total phenolic content and antioxidant properties compared to banana, watermelon, mango and pineapple. This study also recommended that the best concentration to produces juices was 70% w/v. The results suggest that the selected tropical fruits have shown potential as sources of natural antioxidants. Exploitation of these abundant and low-cost renewable antioxidant resources from tropical fruits in developing ingredient for functional food and pharmaceutical products may enhance the food industry

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