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Original Article

SCREENING OF ANTIOXIDANT ACTIVITIES AND THEIR BIOAVAILABILITY OF TROPICAL FRUIT BYPRODUCTS FROM INDONESIA

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ABSTRACT

Objective: The objectives of this study were to investigate the antioxidant activities in peel and seed of six tropical fruits from Indonesia and to evaluate their bioavailability by *in vitro* digestion model.

Methods: Six tropical fruits namely kapundung, matoa, papaya, rambai salak and soursop were used. The study measured antioxidant activities through 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, thiobarbituric acid reactive substances (TBARS) assay, total phenolic and total flavonol content of the samples. Further, the bioavailability of the antioxidant was determined by *in vitro* gastrointestinal digestion

Results: Matoa peel and salak peel exhibited high DPPH radical scavenging activity with a SC_{50} values of 6.6 and 6.4 µg/ml, respectively. Soursop peel had the highest for inhibiting lipid peroxidation followed by matoa peel and salak peel. The highest value for total phenolic content had in salak peel (317.0 mg gallic acid equivalent (GAE)/g dry basis). Then, total flavonol content values in salak seed (96.7 mg quercetin equivalent (QE)/g dry basis) was the highest in this study. The antioxidant activity of matoa peel and soursop peel increased after being digested.

Conclusion: The results obtained in the present study indicate that peels from matoa (*Pometia pinnata*) and soursop (*Annona muricata* L) could serve as potential sources of antioxidant for use in food or feed supplement, and pharmaceutical industries.

Keywords: Antioxidant, Lipid peroxidation, Phenolic compounds, Fruit peel, Fruit seed, Tropical fruit

INTRODUCTION

Oxidative stress is responsible for leading to various physiological and pathological abnormalities such as hypertension, inflammation, diabetes mellitus, atherosclerosis, aging and cardiovascular [1, 2]. It was caused by imbalanced of active oxygen species (free radicals) in the human body. Excess free radicals could cause damage to cellular protein, membrane lipid, and nucleic acid, and also cell apoptosis [2]. An antioxidant is required to protect their possible damages to biological molecules and to maintain an optimum balance of free radicals in human body.

Epidemiological studies indicated that the antioxidant properties in fruits can use as protectors against certain diseases [3-5]. Fruits contained essential nutrients and micronutrients such as minerals, fibers, vitamins and secondary phytochemicals compounds [6]. Their combination seems to be responsible for reducing the level of oxidative stress and protecting cells from damage.

In addition to fruits, their non-edible part was regarded as potential resources of antioxidant that have rich of polyphenol [7-11]. The mass of byproduct of fruits has an economic and environmental impact, dietary additives, new food and pharmaceutical product, providing of nutraceuticals supplement and contributing to the recovery of agro-industrial process waste [12]. Therefore, it is important to know the phytochemicals inside of byproduct of fruits that have health benefits for human nutrition.

Tropical fruits consumption are increasing in the domestic and international market. It is because of growing recognition of its nutritional and therapeutic value. Indonesia has a large number of unexploited native and exotic fruit species that have a possible future source of income for the local population and a potential interest to the agro-industry. Indonesia is a tropical country that has the greatest variety of fruits in the world. Indonesia is third in production quantity of fresh tropical fruit worldwide, followed by India and Philippines [13]. In this present study, we used the peel and seed of six of exotic tropical fruits. These are soursop (*A. muricata*), papaya (*C. papaya* L.), matoa (*P. pinnata*), rambai (*B. motleyana*), kapundung (*B. racemosa* Reinw.) and salak (*S. zalacca*)

Many studies have determined the antioxidant capacities of fruits or their byproduct but the potential availability of antioxidants after digestion was not taken into consideration [26]. Actually, it is important to measure bioavailability of antioxidant to know whether it has effects on health or not. Being a byproduct of edible fruits that have natural antioxidant properties, their used is applicable in the pharmaceutical, cosmetic, food and feed industries, since they can be used as substitutes for synthetic antioxidant. Thus, the aim of this study was to measure antioxidant activity of peel and seed of tropical fruits from Indonesia and to evaluate the bioavailability of antioxidant activity after digestion.

MATERIALS AND METHODS

Plant materials

All samples were purchased at the local markets in Bogor, Indonesia during January-February season in 2014. Fruits without blemishes or damage were selected. The samples were peel and seed of soursop (*Annona muricata* L.), papaya (*Carica papaya* L.), matoa (*Pometia pinnata*), rambai (*Baccaurea motleyana*), kapundung (*Baccaurea racemosa* Reinw.), salak (*Salacca zalacca*) (fig. 1). All samples were dried at 50°C for 12 to 24 h and were ground to a fine powder.

Preparation of the plant extract

We used QuEChERS method for extraction method [14] with slight modification. In a polypropylene centrifuge tube (50 ml), dried powder of the samples (each 2 or 5 g) was added. A 10 ml of distilled water and 15 ml of acetonitrile were added. The solution was homogenized for 1 min at 1000 rpm, followed by the addition of sodium chloride (1 g), trisodium citrate dehydrates (1 g), disodium

hydrogen citrate sesquihydrate (0.5 g), and anhydrous magnesium sulfate (4 g) and this mixture were shaken for 1 min. Then, the mixture was centrifuged at 3000 rpm for 5 min. The acetonitrile extract was evaporated and dried with a vacuum pump. The extracts were stored at-20 °C.

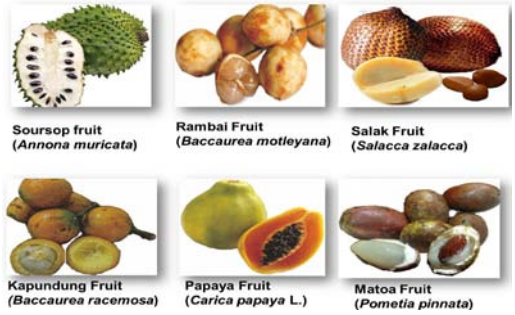

Fig. 1: All samples fruits in this experiment

Total phenolic (TP)

Total phenolic content was performed using the procedure of Folin-Ciocalteu [17]. Each sample test extracts was diluted with methanol at a concentration 1 mg/ml. Then, 20 µl of these solutions was mixed with 200 µl of 50% phenol reagent, 200 µl of 10% sodium carbonate aqueous solution, and 800 µl of distilled water. The mixture was stored for 1 h in the dark place at room temperature. The absorbance was measured at 760 nm with a spectrophotometer by 5 mm length of a quartz cell using an UV-vis spectrophotometer (JASCO V-520-SR). A calibration curve was constructed with standard gallic acid (0, 200, 400, 600, 800, and 1000 µg/ml) and the results were expressed as mg gallic acid equivalent (GAE) per g of dry basis (mg GAE/g d. b.).

Total flavonol (TF)

Total flavonol content was determined following the method of [10], using quercetin as a standard. Each sample was diluted with 95% ethanol at a concentration 5 mg/ml. A volume of 0.25 ml of the sample or standard was pipetted in a test tube, and 0.25 ml 0.1% HCl in 95% ethanol (v/v) and 4.55 ml 2% HCl (v/v) were added. The solution was mixed and allowed to stand for approximately 15 min before reading the absorbance at 360 nm with a spectrophotometer. Quercetin dissolved in 95% ethanol was used as a standard. Total flavonol content was determined from the calibration curve by extrapolating the known concentration of quercetin (1-0.0625 mg/ml) against the absorbance at 360 nm. All the determination were expressed as mg quercetin equivalent per g of dry basis (mg QE/g d. b.).

Analysis of DPPH free radical scavenging activity

The free radical scavenging activity of the extracts and standard solution (trolox) were determined using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging method [15]. Samples were diluted with methanol and acetic acid buffer (1:1). A volume of 0.25 ml of extracts or the standard in different concentrations (200, 100, 50, 10, 5, 1 µg/ml) was mixed with 0.25 ml of acetic acid buffer (0.10 M), 0.25 ml of methanol and 0.25 ml of DPPH (0.4 mM in methanol). The reaction mixtures were vigorously mixed and incubated for 30 min at room temperature in the dark. The absorbance of mixtures was measured by spectrophotometer at 517 nm. The DPPH was carried out using the following formula:

DPPH radical scavenging capacity (%) =

$$[(A_{control} - A_{sample})/A_{control}] \times 100$$

$A_{control}$ was the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} was the absorbance of the test sample. The SC_{50} value was determined by GraphPad PRISM 6 from the output of scavenging capacity values. Each sample was done in triplicate. The SC_{50} value was expressed as µg/ml.

Lipid peroxidation inhibition (TBARS assay)

Lipid peroxidation inhibition was measured by using a thiobarbituric acid (TBA) method [16]. The capacity of each sample solution (10 mg/ml) to inhibit lipid peroxidation was evaluated using the modified assay of thiobarbituric acid reactive substances (TBARS). Linoleic acid (5 mg) was mixed with 4.8 ml of 0.2% SDS Tris-HCl buffer and 100 µl of the sample solution. Then, 100 µl of 20 mM ferrous sulfate aqueous solution was added to oxidize the induction of lipid peroxidation, and the mixture was incubated for 16 h at 37 °C.

The production of TBARS, mainly malondialdehyde, was measured in the following way. One ml of the reaction solution above was mixed with 3 ml of 0.05 N HCl and 1 ml of 0.05 M TBA-50% acetic acid and then incubated for 30 min at 100°C. After cooling to room temperature, 4 ml of *n*-butanol was added and the mixtures were shaken vigorously and adding 200 µl of EtOH to remove air bubbles. The mixtures were centrifuged (10 min, 2500 rpm) and the absorbance of the *n*-butanol layer (upper layer) was measured at 535 nm. To make a standard curve, 1, 1, 3, 3-tetraethoxypropane standard solution (0, 2.5, 5, 10, 20, 30, 40, 50, 60, 70, 80 nmol/ml) were measured. Lipid peroxidation was expressed in nmol of MDA per 1 mg of linoleic acid (nmol MDA/mg linoleic acid).

The Bioavailability of antioxidant in *in vitro* digestion

An *in vitro* gastric and intestinal digestion was performed as described [27] with a little modification. Samples dried powders used in this *in vitro* digestion are matoa peel, salak peel, soursop peel with three difference concentration that equal with extract concentration (10, 50, and 1000 µg/ml). Briefly, samples were mixed with 5 ml of pepsin buffer solution (1600 unit/ml pepsin, 3.6 mM CaCl2, 12 mM KCl, 1.5 mM MgCl2.6H2O, 49 mM NaCl, 6.4 mM KH2PO4) in a 25 ml conical flask. The mixture was acidified with 2 M HCl until it reached to pH 2.5 and the mixture was placed in a shaker bath at 37 °C for 1 hour. Thereafter, intestinal digestion was performed with the addition of 5 ml of pancreatin-bile solution (0.682 g of pancreatin and 0.062 g bile extract in 155 ml of 0.1 M NaHCO3) and the pH of the solution was adjusted to 6.8 with 2 M HCl. The mixture was incubated in a shaker bath at 37 °C for 2 hour. The digesta was stored at freezer temperature until further analysis.

The analysis for the bioavailability of antioxidant was DPPH radical scavenging activity in the concentration of 50 µg/ml, total phenolic content in a concentration of 1000 µg/ml and lipid peroxidation concentration of in 1000 µg/ml. The units of DPPH radical scavenging activity, total phenolic content, and lipid peroxidation were %, mg GAE/g sample, and µmol/mg linoleic acid respectively.

Statistical analysis

All data are expressed as mean±standard deviation (SD) in triplicate at least. For comparisons among samples, data were analyzed by ANOVA and Duncan test (SPSS, version 16.0). A probability of 5% or less was accepted as statistically significant.

RESULTS AND DISCUSSION

Total phenolic (TP)

Phenolic compounds are secondary metabolites in fruits or plants. It is formed by derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants [18]. It can be used as natural antioxidant properties. The results of the TP in the peels and seeds of tropical fruit were presented in table 1. The peels have a higher phenolic content than the seeds. Tomas-Barberan *et al.*[31] found that peel tissues usually contained a larger amount of phenolics. Phenolic compounds tend to accumulate in the dermal tissues of the plant body because of that they have an important role in protecting against ultraviolet radiations, acting as attractants in fruit dispersal, and as defence chemicals against pathogens and predators [8]. The TP in all samples ranged between 47-317 mg GAE/g d. b. In the peels, salak was found to have the highest value of TP, followed by matoa, papaya, soursop, kapundung and rambai. In the seeds,

soursop had a higher value of TP than others. Based on the data of TP in this study, salak peel contained the highest phenolic content (317 mg GAE/g d. b).

The phenolics concentration of fruits evaluated in this work was higher than that found by other authors. Studied of [19] established that total phenolics on soursop peel was 79% lower than our result (134.9 vs 75.04 mg GAE/g sample). Then, Ribeiro *et al.* [6] found the TP value of by-product of soursop and papaya was 14.4 and 7.8 mg GAE/g d. b, respectively. The TP value in soursop peel was similar to Surinam cherry byproduct from Brazil [6]. These differences might be because of several factors such as the type of cultivation, climate, fruit variety, geographic origin, ripeness and extraction method [20, 25]. All the peel and seed of the fruits, except rambai seed, could be considered as a good source of phenolic compounds and antioxidants.

Total flavonol (TF)

In this experiment, the standard chemical that was used to measure total flavonol was quecertin. Quecertin is a great antioxidant activity, and it is abundant in fruit and vegetables. The meant value of flavonol contained in the peel and seeds of tropical fruit were 28.0-96.7 mg QE/g d. b. (table 1). Moo-Huchi *et. al* [21] reported that total flavonoid in purple star apple was higher than other tropical fruit from Mexico (844 mg QE/100 g dry weight). All samples tested showed a significant difference to the total flavonol values. Salak seed had the highest flavonol content, followed by soursop peel, papaya peel, salak peel and soursop peel. Rambai peel had the lowest flavonol content in the study. Several types of flavonols are found in the fruit byproduct are quecertin, rutin, kaempferol, catechin and tangeritin [4].

DPPH radical scavenging activity

The method of DPPH radical scavenging activity was very popular to evaluate the antioxidant activity. The result of DPPH radical scavenging capacity is interpreted with SC_{50} values. All samples had an activity to scavenge free radicals (table 1). The SC_{50} values of DPPH of peel and seed in six fruits were significant differences and ranged between 6.4 and 281 µg/ml. Among all peels and seeds tested, salak peel and matoa peel had the highest DPPH radical scavenging activity with an SC_{50} value at 6.4 and 6.6 µg/ml, respectively. This SC_{50} value was equal to the SC_{50} value of trolox standard (4.4 µg/ml).

The highest of DPPH radical scavenging activity in salak and matoa peel was possibly caused by secondary phytochemicals compounds in them such as phenolic acid and flavonoid compounds [7, 8, 12]. Strong correlations (r = 0.58, the equation: y =-0.6444x+170.48) between the DPPH assay and the values of TP in this study were found, which prove that phenolic compounds contribute to the inhibiting ability of tropical fruit byproducts.

Table 1: The DPPH radical scavenging activity, lipid peroxidation, total phenolic and total flavonol of tropical fruits byproduct from Indonesia

Sample name	Scavenging capacity 50 value of DPPH* (µg/ml)	Lipid peroxidation** (nmol MDA/mg Linoleic acid)	Total phenolic*** (mg GAE/g d. b.)	Total flavonol*** (mg QE/g d. b.)
Kapundung seed	57.9±5.8^{d}	9.4±1.7^{f}	60.0±4.1^{ab}	69.0±3.3^{de}
Matoa seed	60.8±9.9^{d}	5.5±2.4^{bcdef}	80.5±3.4^{bc}	67.9±1.3^{de}
Papaya seed	78.3±11.3^{e}	7.1±1.1^{cdef}	94.8±3.4^{cd}	41.0±5.0^{b}
Rambai seed	>100	24.7±4.6^{g}	47.6±2.3^{a}	63.9±2.0^{d}
Salak seed	28.9±4.7^{b}	5.3±1.5^{abcde}	120.7±15.0^{de}	96.7±0.0^{f}
Soursop seed	33.7±5.3^{bc}	7.5±2.0^{def}	134.9±4.7^{fg}	74.5±7.6^{e}
Kapundung peel	40.2±3.9^{c}	9.2±5.0^{ef}	96.9±3.8^{cd}	50.8±1.7^{c}
Matoa peel	6.6±0.3^{a}	1.9±0.8^{ab}	265.5±27.2^{g}	37.5±1.5^{b}
Papaya peel	30.9±4.0^{bc}	4.5±1.2^{abcd}	155.6±49.7^{f}	71.5±1.2^{e}
Rambai peel	>100	21.4±3.3^{g}	53.1±3.2^{ab}	28.0±0.3^{a}
Salak peel	6.4±1.8^{a}	3.2±0.8^{abc}	317.0±29.2^{h}	71.7±11.7^{e}
Soursop peel	24.6±5.4^{b}	1.2±0.4^{a}	126.8±11.2^{ef}	74.1±1.5^{e}
Trolox	4.4±0.3^{a}			
Control		34.4±7.9^{g}		

*Each value represents mean±SD (n=3), **Each value represents mean±SD (n=6), ***Each value represents mean±SD (n=4), Means with different small letters within a column were significantly different ($p<0.05$).

Studied of Kanlayavattanakul *et al.* [3] reported that the SC_{50} values of DPPH scavenging radical activity on ethyl acetate fraction of the salak peel (2.93 µg/ml) had high antioxidant activity compared with of 70% ethanol fraction (8.87 µg/ml), and aqueous (4.12 µg/ml). In this study, the SC_{50} value of salak peel had in between the SC_{50} value of 70% ethanol fraction and ethyl acetate fraction of [3] studied. The differences of SC_{50} values of DPPH scavenging radical activity might be caused by differences solvent used, cultivation, extraction method and varieties of fruit. Despite some samples in this study were similar to the samples in other studies, nevertheless there were some data of fruits still undiscovered for their antioxidant activities, total phenolics, and total flavonols.

The DPPH radical scavenging activity is a good way to determine of antioxidant capacities in the sample. However, only one method to determine antioxidant activity is not enough. Likewise, the biggest limitation of DPPH assay is that is not related to specific free radicals that have physiological relevance [22]. Based on this, we also studied the antioxidant activity through the lipid peroxidation inhibition.

Lipid peroxidation

Lipid peroxidation is one of the markers of oxidative stress. Lipid peroxidation always occurred in polyunsaturated fatty acids such as linoleic acid, linolenic acid, and arachidonic acid (cell membrane component) which are oxidized in various pathological conditions [1]. Malondialdehyde (MDA) and 4-hydroxy-2-hexenal (HHE) are one of the end products from the lipid peroxidation process. Those were ascribed cytotoxic, mutagenic, and neurotoxic properties and can promote cancer development in the gastrointestinal (GI) tract and liver [23, 24].

The effect of peel and seed of tropical fruits on lipid peroxidation is summarized in table 1. In this study, there was a significant variation in the effect of peel and seed of tropical fruits on lipid peroxidation (1.2 to 24.7 nmol MDA/mg of linoleic acid). In lipid peroxidation, the lower result of MDA product, the stronger activity of inhibited lipid peroxidation. The soursop peel (1.2 nmol MDA/mg of linoleic acid) had the strongest activity in inhibiting of lipid peroxidation, followed by salak peel and matoa peel.

All fruit peels and seeds tested possessed a good activity of inhibited lipid peroxidation except in peel and seed of rambai fruit. It is likely that aromatic compound with vicinal methoxy hydroxy or dihidroxy groups is present in these samples tested [16]. In addition, phenolic compounds that have in these samples also had strong correlations (r = 0.62) to the inhibiting lipid peroxidation.

Three groups were clearly distinguishable for the lipid peroxidation inhibition; the first group including soursop peel (96.4%), matoa peel (94.4%), salak peel (90.8%), papaya peel (87.9%), salak seed (84.7%) and matoa seed (84.1%); the second group including sample having 50-80%, i.e., papaya seed, soursop seed, kapundung peel, kapundung seed, and the third group including samples with "low" activity<50%, rambai peel and rambai seed.

The Bioavailability of antioxidant in *in vitro* digestion

The result from the screening of antioxidant activity, lipid peroxidation and total phenolic in six tropical fruit byproducts exhibited that matoa peel, salak peel, and soursop peel had a high antioxidant activity. Thus, these three samples were evaluated in the bioavailability of antioxidants after *in vitro* digestion (fig. 2).

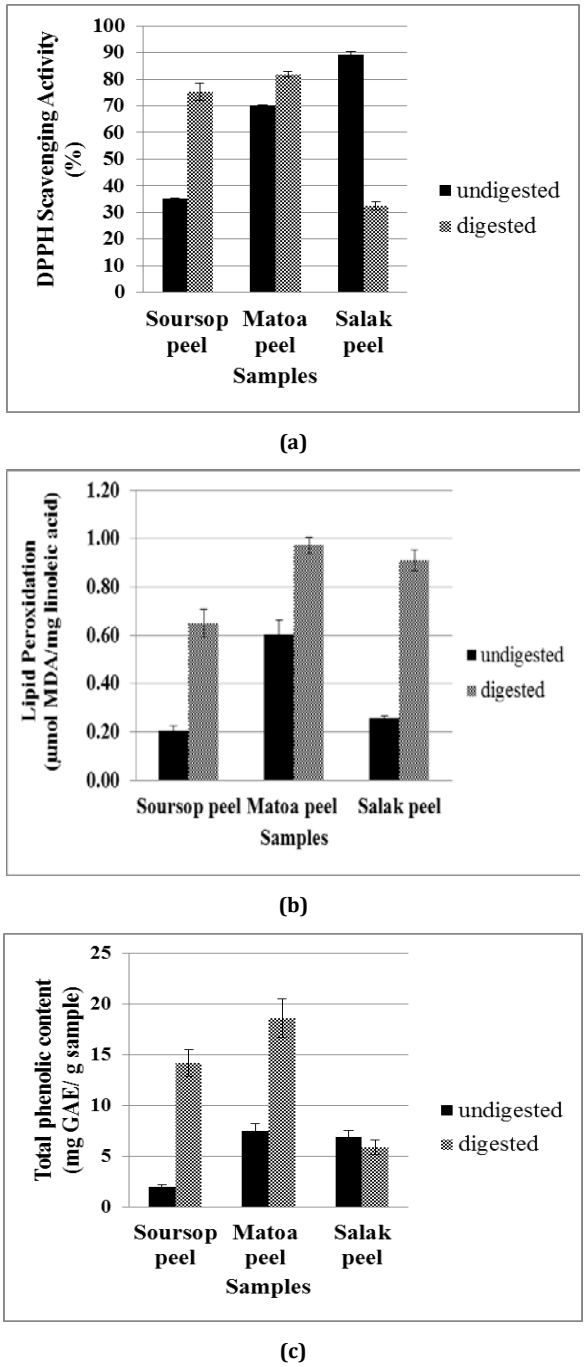

Fig. 2: The DPPH radical scavenging activity (a), lipid peroxidation (b) and total phenolic (c) on undigested and digested of soursop peel, matoa peel and salak peel

Fig. 2 presented that there was significant ($p<0.05$) difference between the undigested values and digested of tropical fruit peels in the DPPH radical scavenging activity, lipid peroxidation, total phenolic content. The soursop peel (35.0 to 75.3%) and matoa peel (70.2 to 81.8%) after *in vitro* digestion showed significant increased ($p<0.05$) in the DPPH radical scavenging activity, which was similar to what reported by [26] in the analysis of jackfruit. The pH and enzymatic interactions during digestion affected the result of antioxidant activity [26].

In the other hand, lipid peroxidation values after *in vitro* digestion showed significantly increased in all samples tested (fig. 2). It had same resulted as [29] in their studied that MDA content by lipid peroxidation after digestion was increased compared to before digestion. The enzymes in the gastrointestinal tract such as lipase [29] might be causing linolieic acid breakdown quickly before the samples protected it completely. The soursop peel (0.65 µmol MDA/mg linoleic acid) resulted in the lowest concentration for lipid peroxidation compared matoa peel (0.97 µmol MDA/mg linoleic acid) and salak peel (0.91 µmol MDA/mg linoleic acid). The MDA content in this present study is still in the range of 0.356 and 1.14 µmol MDA/mg linoleic acid as [28] and [29] had reported before.

Total phenolic (TP) content in salak peel exhibited a significant decrease ($p<0.05$) after *in vitro* digestion with a value of 6.9 to 5.9 mg GAE/g sample (fig. 2). A similar resulted was found in papaya and araticum extracts that TP value in these samples was decreased after digested [26]. After *in vitro* digestion, matoa peel exhibited the highest value of TP (18.6 mg GAE/g sample) followed by soursop peel (2.0 mg GAE/g sample) and salak peel (6.9 mg GAE/g sample). The increasing of total phenolic after *in vitro* digestion could be caused by releasing of polyphenols and changing their structural form which has affected their chemical and functional properties [30]. We suspected that the matrix obtained from samples contained other substances that were not analyzed in this study, including non-phenolic substances involved in changed of antioxidant activity value after the *in vitro* digestion process. These compounds, such as amino acids, peptides, were released during digestion or were changed [26].

CONCLUSION

Overall the peel and seed of tropical fruit have an antioxidant activity and lipid peroxidation inhibition activity, as well as phenolic compounds and flavonol content. The bioavailability of the antioxidant after the screening was also evaluated. Matoa peel and soursop peel indicate the most promising antioxidant sources of these tropical fruit byproducts. This study performed that these tropical fruit by-products are good sources of the antioxidant compound and could be used in the pharmaceutical, food, and feed industries. Their antioxidant activities and total phenolic contents are enhanced after the digestion process. It could provide good advantages for health. Regarding the result, *in vivo* experiments are warranted for further studies.

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CONFLICT OF INTERESTS

Declared none

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