Songklanakarin J. Sci. Technol.
44 (4), 1067–1074, Jul. – Aug. 2022

http://www.sjst.psu.ac.th

Original Article

Screening for phenolic compounds and oxidative capacity of fruit peels, agricultural waste, and traditional herbal medicine for use as biodiesel fuel additive

Netnapa Chana*, Atcharaporn Muengpoon, Siriphat Pethkaew, Nattawadee Kulsin, Achariya Mahasuk, and Sirirat Srirat

Department of Chemistry, Faculty of Science,
Thaksin University, Phatthalung Campus, Pa Phayom, Phatthalung, 93210 Thailand

Received: 26 March 2022; Revised: 17 May 2022; Accepted: 23 May 2022

Abstract

One of the most efficient ways to prevent biodiesel oxidation is to use antioxidant additives. In the present study, 19 plant extracts from fruit peels, agricultural waste, and traditional herbal medicine have been evaluated for their potency as antioxidants. Ripe mango peel exhibited the highest total phenolic content (TPC) (142.99 ± 2.64 mg GAE/g). The rambutan peel had the highest radical scavenging capacities for the DPPH (IC_{50} of 5.67 ± 0.47 μg/mL), ABTS (IC_{50} of 3.94 ± 0.17 μg/mL), FRAP (109.33 ± 2.97 mg AAE/g). The performance of their extracts in improving the oxidative stability of biodiesel was assessed by using the induction period (IP). The biodiesel (B100) without the extract showed an IP of 1.47 h, while it increased to 15.00, 12.18, 12.00, and 6.38 h with the ripe mango peel, raw mango peel, rambutan peel, and longan peel extracts, respectively, while the synthetic antioxidant butylated hydroxytoluene (BHT) exhibited an IP of 6.09 h. The IP of biodiesel blends with petro-diesel (B10) with antioxidants was greater than 24.00 h in all cases. According to the results obtained, the ripe mango peel extract is a good alternative to synthetic compounds used in palm oil-derived biodiesel to improve its storage stability.

Keywords: antioxidant, biodiesel, fruit peels, agricultural waste, traditional herbal medicine

1. Introduction

Biodiesel is a renewable and non-toxic fuel produced from plant oils. The presence of poly-unsaturated chains in methyl esters leads to oxidative instability that promotes degradation during long-term storage. The addition of antioxidants to biodiesel is a cost-effective approach to increase oxidative stability. The antioxidants act to neutralize or scavenge free radicals formed during the oxidation reactions, creating more stable intermediates and, consequently, reducing the oxidation rate. Synthetic additives are widely available – including butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl gallate (PG) – and they efficaciously improve biodiesel oxidative stability due to their higher purity compared with natural additives (Yuliarita, Fathurrahman, Aisyah, Hermawan, & Anggarani, 2019). However, they are toxic and non-biodegradable.

Natural antioxidants have multiple significant advantages in health benefits over their synthetic counterparts. Natural antioxidants have received increased attention in recent years for roles in stabilizing polyunsaturated fatty acids and preventing the oxidation of vegetable and animal oils (Śpitalniak-Bajerska, Szumny, Kucharska, & Kupczyński, 2018). The properties are mainly related to phenolic compounds in the materials.

In Thailand, agriculture and fruit cultivation are major industries; fruit peels and agricultural waste are usually discarded, resulting in resource waste and environmental pollution. Extracts of peels from citrus, rambutan, sugar apple, star apple, ilama, mango, passion fruit, mangosteen, pomegranate, and lime have been widely applied in food products and serve as natural sources of antioxidants. Maize or sweet corn is an important agricultural product in Thailand.

*Corresponding author
Email address: netnapa@tsu.ac.th

The chemical compositions and antioxidant activities of silk by-products during corn processing have also been investigated (Senphan, 2019). In addition, several Thai medicinal herbs are used in primary health care for pharmacological properties including anti-inflammatory, anticancer, and antioxidant activities (Phonghanpot & Jarintanan 2021). Therefore, fruit peels, agricultural by-products, and herbs could be used as alternative natural antioxidants supplemented in fat and oil. However, very few studies have been performed on biodiesel using these natural antioxidants.

The native fruits and plants (bacuri, araçá, and rosemary) in Brazil were used to inhibit biodiesel oxidation. The ethanolic extract possessed total phenolic content (TPC) (3.67-19.60 mg GAE/g dry mater). Biodiesel (B100) mixed with these extracts showed an induction period (8.14 h) longer than the control (4.60 h) (Chendynski *et al.*, 2020). In addition, research on using natural plant extracts in biodiesel has been carried out (Fernandes *et al.*, 2015; Senthil, Pranesh, & Silambarasan, 2019). The storage stability of biodiesel fuel depends on the type of antioxidants. Antioxidant additives should have properties such as no toxicity, low volatility, effectivity at a low concentration, availability, and low cost. The antioxidant type most used in biodiesel is phenolics, due to their low cost. In recent years, the interest in phenolic derivatives has increased, in the context of plants that have them as secondary metabolites. Hence, it is necessary to find natural extracts containing the most phenolic components and with the highest antioxidant activity. In the present study, we screened the TPC and antioxidant activities of extracts from fruit peels, herbal medicines, and agricultural waste. We then selected the extract and evaluated its ability to increase the oxidative stability of biodiesel.

2. Materials and Methods

2.1 Raw materials

The plants examined are presented in Table 1. Nine selected fruit peels based on the local fruits in the southern part of Thailand and four agricultural wastes were purchased from the Papayom market in Phatthalung province. Six samples of traditional herbal medicines were obtained from Trang province, Thailand.

2.2 Ethanol extracts

Nineteen plant samples were washed, dried and ground. The powder was extracted with 95% ethanol by conventional maceration method. An extraction process consists of immersing plant materials in a solvent inside a container. The phenolic contents are well soluble in ethanol as solvent with maceration procedure (Alara, Abdurahman, & Ukaegbu, 2021; Ghomari *et al.*, 2019). Fifty grams of the sample powder was soaked in 500 mL of solvent for 7 days before being filtered through Whatman No.1 filter paper. The process was repeated two times. The solvent was removed by rotary evaporator at 40 °C. The extract yields were calculated using the formula (1):

$$\% \text{ Yield} = (\text{mass of the extract/mass of the plant sample}) \times 100 \quad (1)$$

2.3 Determination of the TPC in extracts

This analysis was performed by using Folin–Ciocalteu reagent (Kim, Jeong, & Lee, 2003) with some modifications. The extract solution (30 µL, 1 mg/mL) was mixed with diluted Folin–Ciocalteu reagent (110 µL), and then the samples were shaken vigorously. After 3 min, Na_2CO_3 solution (110 µL, 7.5%) was added, and the mixture was incubated for 15 min at 45 °C. The absorbance was determined at 765 nm by a spectrophotometer plate reader. Gallic acid was processed similarly to provide a standard curve. The result of TPC is expressed as mg of gallic acid equivalents per g of dry extract (mg GAE/g extract).

2.4 Antioxidant assays

2.4.1 2,2-Diphenyl-2-picrylhydrazyl (DPPH) test

The antioxidant activity was determined through the DPPH method with some modifications (Zhu, Lian, Guo, Peng, & Zhou, 2011). Briefly, several concentrations of each sample were added to 96-well plates (100 µL). 150 µL of 0.2 mM DPPH was mixed with the extract and the plate was shaken vigorously. The 96-well plate was then incubated in the dark for 30 min. The absorbance of the resulting mixture was recorded at 517 nm. The control was prepared as described above without the extract. Ascorbic acid was used as a reference standard. The percentage of radical scavenging activity was calculated using the equation (2):

$$\text{Radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (2)$$

where $Abs_{control}$ is the absorbance of the control and Abs_{sample} is the absorbance in the presence of the extract. The results are reported as the half-maximal inhibitory concentration (IC_{50}). All tests were performed in triplicate.

2.4.2 ABTS radical scavenging test

The ABTS radical scavenging test was adapted from the method reported by Re *et al.* (Re *et al.*, 1999). Briefly, the ABTS radical cation solution was generated by reacting the following solution; 7 mM ABTS reagent and 2.45 mM $K_2S_2O_8$ solution in a 2:1 volumetric ratio. Afterward, this solution was diluted with ethanol to obtain the ABTS working solution with absorbance of 0.70 units at 743 nm. A volume of 270 µL of diluted $ABTS^{\bullet+}$ solution was transferred to 96-well plates containing 30 µL of the ethanolic extract at different concentrations. After 6 min in the dark, the absorbance was measured at 734 nm in a UV-Visible spectrophotometer. Ascorbic acid was used as a positive control. Percentage ABTS inhibition was calculated by using equation (3):

$$\text{ABTS inhibition (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (3)$$

The IC_{50} was calculated from the plot of ABTS inhibition percentages against sample concentration. All tests were performed in triplicate.

Table 1. Ethnobotanical information and extraction yields of the tested plant species

Plant	Common name	Scientific Name	Part of plant used	% Yield
Fruit peels	Tamarind	*Tamarindus indica* L.	Peel	7.13
	Longan	*Dimocarpus longan*	Peel	11.02
	Raw mango (Nam Dok Mai)	*Mangifera indica* Linn.	Peel	15.12
	Lime	*Citrus aurantifolia (Christm.) Swingle*	Peel	16.07
	Mangosteen	*Garcinia mangostana* Linn.	Peel	23.95
	Ripe mango (Nam Dok Mai)	*M. India*	Peel	28.04
	Mandarin orange	*Citrus reticulata Blanco*	Peel	29.58
	Rambutan	*Nephelium lappaceum* Linn.	Peel	36.91
	Pomegranate	*Punica granatum* Linn.	Peel	42.33
Agricultural waste	Sweet corn	*Zea mays* L. var. *saccharata*	Silk	4.83
	Waxy Corn	*Zea mays* L.	Silk	5.92
	Waxy Corn	*Z. mays*	Husks	10.01
	Sweet corn	*Z. mays* var. *saccharata*	Husks	11.71
Traditional herbal medicine	Garlic	*Allium sativum* L.	Whole bulb	1.25
	Laurel clockvine	*Thunbergia laurifolia* L.	Leaves	2.22
	Baiyanang	*Tiliacora triandra* (Colebr.) Diels	Leaves	5.84
	Great morinda	*Morinda citrifolia* L.	Leaves	5.97
	Betel Piper	*Piper betle* L.	Leaves	6.57
	Cha muang	*Garcinia cowa* Roxb.	Leaves	37.11

2.4.3 FRAP test

The ferric ion-reducing antioxidant power (FRAP) test was conducted according to the method described by Benzie and Strain (Benzie & Strain, 1999). Briefly, the FRAP reagent was prepared by mixing three solutions (300 mM sodium acetate buffer, pH = 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$) in a 10:1:1 volumetric ratio. The reaction was performed in 96-well plates containing 10 μL of different concentrations of samples and 290 μL of FRAP reagent and mixed. The plate was then kept in the dark at room temperature for 5 min to perform the reducing reaction. The absorbance of each solution was recorded at 593 nm in a spectrophotometer. The reducing power is expressed as mg of ascorbic acid equivalents per g of extract (mg AAE/g extract). All tested were performed in triplicate.

2.5 Oxidative stability of biodiesel

Biodiesel (B100) was bought from the Prince of Songkla University; it was produced from palm oil. Commercial diesel with 10% content of biodiesel (B10) was purchased from PTT station in Phattalung province, Thailand. Dilutions of the ripe mango peel, raw mango peel, rambutan peel, and longan peel extracts in biodiesel were prepared at final concentrations of 500 mg/L. A biodiesel sample without extract (B100 and B10) was set as the blank. The samples were then stored for 28 days in a storage room. Oxidative stability testing was performed by determining the induction period (IP) from the WI-RES-EC Meter-001 and with an in-house method based on EN 15751 (Intek Inc., Korea). The assay was carried out at 110 °C.

2.6 Acidity value and water content of biodiesel during storage

The monitoring of the acidity value of biodiesel during the 28 days of storage was performed according to the standard specified in EN 14104. Water content determination in biodiesel stored for 7 days was measured according to ISO 12937 with coulometric Karl Fischer titrators, Metrohm, Switzerland.

2.7 Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple-range test were performed. Significant differences among the extracts were called when $p < 0.05$. SPSS Statistics version 17.0 was used for analysis.

3. Results and Discussion

3.1 TPC of the plant extracts

To screen the antioxidant compounds in plant materials, crude extracts were prepared from fruit peels, herbal medicines, and agricultural waste (Table 1). The yields of the 19 tested extracts ranged from 1.25% to 42.33%, with the pomegranate peel extract having the highest yield. Cha muang leaves and rambutan peel showed yields of 37.11% and 36.91%, respectively. The yield depends on the solubility of the substance in 95% ethanol (Chairerk, Pongyeela, Chungsiriporn, & Rakmak, 2021). However, the application of non-conventional methods and fractionation of crude extracts should be further evaluated to provide bioactive ingredients-rich extract from the plant with the most phenolics or highest antioxidative potential.

Because phenolic compounds are considered the major contributors to the antioxidant capacity of many plants, the TPC of the 19 extracts was determined by using the Folin–Ciocalteu assay (Figure 1). The TPC in the extracts ranged from 18.72 ± 0.06 mg GAE/g extract (great morinda leaves) to 142.99 ± 2.64 mg GAE/g extract (ripe mango peel extract). There was a relatively high TPC for rambutan peel (131.05 ± 0.61 mg GAE/g extract), raw mango peel (109.67 ± 2.08 mg GAE/g extract), and waxy corn silk (99.67 ± 1.04 mg GAE/g extract), whereas several herbal plants like cha muang leaves, Baiyanang leaves, and great morinda leaves contained <30 mg GAE/g extract. Fruit peels from orange and lime also has <30 mg GAE/g extract.

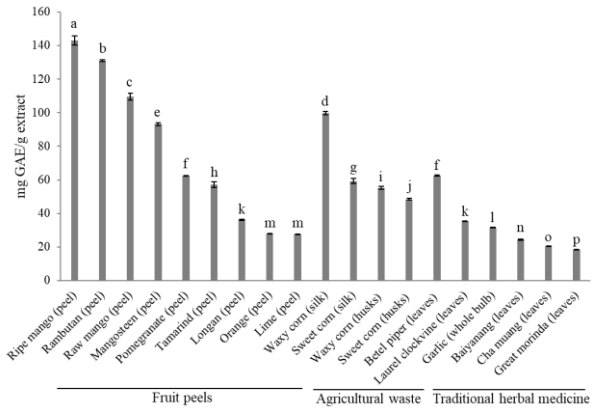

Figure 1. The total phenolic contents of the ethanol extracts of fruit peels, agricultural waste, and traditional herbal medicine. Abbreviations: GAE, gallic acid equivalents. Different letters in the bar graph indicate statistically significant differences ($p < 0.05$) using Duncan's multiple-range test.

3.2 Oxidative capacity of the ethanolic extract

The antioxidant capability of the 19 extracts was evaluated by employing the DPPH and ABTS radical scavenging assays and the FRAP assay. The DPPH assay demonstrated a high antioxidant potential of rambutan peel extract (IC_{50} = 5.67 ± 0.47 µg/mL), which was close to the IC_{50} of ascorbic acid (4.88 ± 0.29 µg/mL) (Table 2). The rambutan peel extract also showed an IC_{50} of 1.97 ± 0.06 µg/mL with the ABTS method and 109.27 ± 2.97 mg AAE/g extract with the FRAP method. Considering all samples, the DPPH radical scavenging activity IC_{50} ranged from 5.67 to >1500 µg/mL. The DPPH radical scavenging activity of the rambutan peel extract was higher than that of BHT; these results are consistent with previously published research (Sukatta *et al.*, 2021). Among the plants screened, the extracts of fruit peels including rambutan peel, ripe mango peel, raw mango peel, longan peel are the most active and their IC_{50} values are 5.67 ± 0.47, 8.09 ± 0.12, 9.81 ± 0.61, and 26.07 ± 0.18 µg/mL, respectively. Other plant extracts of mangosteen peel, pomegranate peel, tamarind peel, waxy corn silk, and betel piper leaves also possessed significant activity and their IC_{50} values were between 36.11 and 65.36 µg/mL. Little antioxidant activity (IC_{50} >200 µg/mL) was observed for fruit peel including lime peel and orange peel, agricultural waste including waxy corn husks, sweet corn silk, and sweet corn husks, traditional herbal medicine including great morinda leaves, garlic bulb, and cha muang leaves. The ABTS activities of the rambutan peel extract, ripe mango peel, and raw mango peel were higher than that of ascorbic acid. The high antioxidant activity (low IC_{50}) with ABTS assay from the extracts had the rank order of rambutan peel extract (IC_{50} 1.97 ± 0.06) > ripe mango peel (IC_{50} 2.41 ± 0.08) > raw mango peel (IC_{50} 2.78 ± 0.05) > longan peel (IC_{50} 5.73 ± 0.02) > betel piper leaves (IC_{50} 10.40 ± 0.49) > mangosteen peel (IC_{50} 21.79 ± 0.44) > pomegranate peel (IC_{50} 29.28 ± 0.75). The FRAP activity showed the rank order rambutan peel extract > ripe mango peel > raw mango peel > longan peel > pomegranate peel > mangosteen peel and betel piper leaves. These results are concordant with the DPPH radical scavenging assay.

The ripe mango peel, raw mango peel, and rambutan peel possessed a high TPC and showed a good oxidative capacity when compared to the agricultural waste or to traditional herbal medicine. This might be because of the larger number of OH groups and phenolic rings in their peels providing these compounds greater antioxidant capacity and reducing power, when compared with the other tested plant extracts (Pereira, Valentão, Pereira, & Andrade, 2009).

The antioxidant properties of plant extracts are attributed to their active components. The rambutan peel extract showed the highest antioxidant activity among the tested samples, a reflection of its high TPC. The main components of rambutan peel extract are corilagin, ellagic acid, geranin, and gallic acid, all phenolic compounds with antioxidant activity (Sukatta *et al.*, 2021). Moreover, rambutan peel extract also contains non-phenolic antioxidant compounds such as ascorbic acid and β-carotene (Fregolente, Fregolente, & Wolf Maciel, 2012). The main bioactive compounds in mango peel (varieties 'Haden' and 'Tommy Atkins') are flavanols, flavonols, and phenolic acids (Coelho *et al.*, 2019). The majority in longan peel extract are phenolic compounds including ellagic acid conjugates, flavone glycosides, and other phenols (Jaitrong, Rattanapanone, & Manthey, 2006).

3.3 Oxidative stability of biodiesel (B100) and B10

The oxidative stability of biodiesel is related to the chemical composition of unsaturated esters, which can react with oxygen at double bonds to form peroxide. Antioxidants prevent this process by scavenging the free radicals that attack oxygen reactive sites of the fatty acid ester chain (Agarwal, Singhal, Singh, Arora, & Tanwer, 2018). The mango, rambutan, and longan peel extracts exhibited excellent antioxidative activity, which encouraged us to explore their ability to improve the oxidative stability of biodiesel. This ability was assessed by using the induction period (IP) with oxidative stability testing at 110 ºC based on EN 15751.

The IP values obtained are shown in Table 3. The B100 commercial palm oil biodiesel without antioxidants showed an IP of 1.47 h, while it increased to 15.00, 12.18, 12.00, and 6.38 h with the ripe mango peel, raw mango peel, rambutan peel, and longan peel extracts, respectively. The greatest stabilization was obtained with the use of ripe mango peel extract (10.20 fold) when compared to biodiesel without an antioxidant additive. For raw mango peel, rambutan peel, and longan peel extract, the stabilizing factors were 8.29, 8.16, and 4.34 fold, respectively.

The synthetic antioxidant BHT exhibited an IP of 6.09 h (4.14 fold) when added to the biodiesel. The biodiesel (B100) without the extracts had comparatively poor stability indicated by the low induction time. It does not meet the quality requirement of 6 h according to EN 14112 (Domingos, Saad, Vechiatto, Wilhelm, & Ramos, 2007). Palm oil contains almost 50% PUFA, which makes it quite susceptible to oxidation (Helwani *et al.*, 2021). After the addition of ripe mango peel, raw mango peel, rambutan peel, and longan peel, the biodiesel remained stable when exposed to heat at 110 ºC. The mixture of biodiesel with these extracts showed more stability than with the synthetic BHT. BHT is efficient for low-level biodiesel blends but is an ineffective antioxidant in

Table 2. Antioxidant capacities of 19 plant extracts from fruit peels, agricultural waste, and traditional herbal medicine measured by the DPPH (IC_{50}), ABTS (IC_{50}), and FRAP assays.

Plant	Common name	DPPH assay IC_{50} (µg/mL)	ABTS assay IC_{50} (µg/mL)	FRAP value (mg AAE/g extract)
Antioxidant standards	Ascorbic acid	4.88 ± 0.29^{a}	3.86 ± 0.04^{ab}	ND
	Butylated hydroxytoluene (BHT)	9.54 ± 0.12^{a}	ND	ND
Fruit peels	Rambutan (peel)	5.67 ± 0.47^{a}	1.97 ± 0.06^{a}	109.27 ± 2.97^{a}
	Ripe mango (peel)	8.09 ± 0.12^{a}	2.41 ± 0.08^{a}	99.17 ± 1.89^{b}
	Raw mango (peel)	9.81 ± 0.61^{a}	2.78 ± 0.05^{a}	89.92 ± 1.87^{c}
	Longan (peel)	26.07 ± 0.18^{ab}	5.73 ± 0.02^{ab}	86.31 ± 1.15^{d}
	Mangosteen (peel)	36.11 ± 0.78^{bc}	21.79 ± 0.44^{d}	20.69 ± 1.59^{f}
	Pomegranate (peel)	$40.04 \pm 1.25b^{bc}$	29.28 ± 0.75^{e}	23.95 ± 4.50^{e}
	Tamarind (peel)	65.36 ± 5.20^{c}	15.09 ± 8.90^{c}	13.16 ± 0.23^{g}
	Lime (peel)	538.69 ± 11.96^{h}	81.46 ± 8.22^{g}	4.42 ± 0.56^{i}
	Orange (peel)	990.09 ± 55.15^{k}	>150	1.19 ± 0.09^{j}
Agricultural waste	Waxy corn (silk)	54.10 ± 1.13^{c}	ND	ND
	Waxy corn (husks)	715.13 ± 8.41^{j}	ND	ND
	Sweet corn (silk)	$1{,}405.78 \pm 13.97^{k}$	ND	ND
	Sweet corn (husks)	>1,500.00	ND	ND
Traditional herbal medicine	Betel piper (leaves)	43.69 ± 4.80^{bc}	10.40 ± 0.49^{bc}	21.05 ± 0.83^{f}
	Baiyanang (leaves)	108.374 ± 25.27^{d}	87.36 ± 5.32^{h}	3.76 ± 0.17^{ij}
	Laurel clockvine (leaves)	152.28 ± 16.97^{e}	54.96 ± 1.92^{f}	8.29 ± 0.44^{h}
	Great morinda (leaves)	324.51 ± 11.20^{f}	112.71 ± 3.86^{i}	4.65 ± 0.16^{i}
	Garlic (bulb)	419.06 ± 7.12^{g}	74.84 ± 6.80^{g}	3.55 ± 0.45^{ij}
	Cha muang (leaves)	625.498 ± 25.31^{i}	>150	1.76 ± 0.24^{ij}

Table 3. Induction period (IP) of biodiesel during the oxidative stability test at 110 °C with the ripe mango peel, raw mango peel, rambutan peel, and longan peel extracts or BHT additive

Type of fuel	Case	Concentration of antioxidant (mg/L)	IP (h)	Stabilizing factor
Commercial palm oil-derived biodiesel (B100)	B100 without additive	-	1.47	1
	B100 + BHT	500	6.09	4.14
	B100 + Ripe mango peel extract	500	15.00	10.2
	B100 + Raw mango peel extract	500	12.18	8.29
	B100 + Rambutan peel extract	500	12.00	8.16
	B100 + Longan peel extract	500	6.38	4.34
Commercial diesel with 10% blend with biodiesel (B10)	B10 without additive	-	12.26	1
	B10 + BHT	500	>24	>1.96
	B10 + Ripe mango peel extract	500	>24	>1.96
	B10 + Raw mango peel extract	500	>24	>1.96
	B10 + Rambutan peel extract	500	>24	>1.96
	B10 + Longan peel extract	500	>24	>1.96

high-level biodiesel blends. In addition, natural antioxidants contain more OH groups than the monohydric BHT (Varatharajan & Pushparani, 2018). Hence, the high TPC and antioxidant properties of the extract can improve the stability of B100 better than BHT antioxidant. For B10, all antioxidants boost the oxidation stability compared to that without antioxidant (IP > 24 h) (Table 3). Diesel has a better oxidative stability than biodiesel.

The ripe peels were found to have more carotenoid content compared to the raw peels (Raspuri and Badami varieties) (Ajila, Naidu, Bhat, & Rao, 2007). They also have the ability to scavenge free radicals (Young & Lowe, 2018). Hence, the ripe mango peel extract gave a greater increase in IP compared with raw peel and rambutan peel extracts. Rambutan peel extract also had a high radical scavenging activity but some phytochemical compounds might inhibit the biodiesel oxidative stability. With the standard procedure described in EN 15751, the requirement of oxidative stability according to the Rancimat test is 6 h, so the mango peel and rambutan peel additives provided an excellent improvement in the IP.

The increase in IP we found in this study is higher than that reported by Senthil *et al.* (Senthil *et al.*, 2019) (Table 4). In this study, biodiesel contains a low level of natural antioxidants (500 mg/L) and had a stabilizing factor exceeding 8 fold. This is a cost-effective way to use a small quantity of antioxidants for preventing the oxidation of biodiesel.

3.4 Acidity value and water content during storage

The oxidation reaction products degrade biodiesel quality by increasing acidity, viscosity, and insoluble molecules (Fu, Turn, Takushi, & Kawamata, 2016). Thus, the acidity index was monitored during the 28 days of storage, and the results are shown in Figure 2. All samples with added extracts had minimal acid values (0.24-0.33 mg KOH/g) within the standards set for oil according to EN 14104 (0.5 mg KOH/g), during 28 days of storage. Thus, the quality of

Table 4. Comparison between different antioxidants for biodiesel

Material	Antioxidants	Concentration (mg/L)	Blank IP (h)	IP with antioxidants (h)	Stabilizing factor	Reference
Jamun oil methyl ester (JOME)	-Albizia Lebbeck (AL)	500	8	11.5	1.44	(Senthil *et al.*, 2019)
	-Melia Azedarach (MA)	500		10.4	1.3	
	-Psidium Guajava (PG)	500		9.5	1.19	
Commercial biodiesel	-*Moringa oleifera* Lam leaf	1000	5.51	7.75	1.41	(Fernandes *et al.*, 2015)
	-BHT	1000		8.01	1.45	
Commercial palm oil-derived biodiesel (B100)	-Rambutan peel	500	1.47	12.00	8.16	This study
	-Ripe mango peel	500		15.00	10.20	
	-Raw mango peel	500		12.18	8.29	
	-Longan peel	500		6.38	4.34	
	-BHT	500		6.09	4.41	

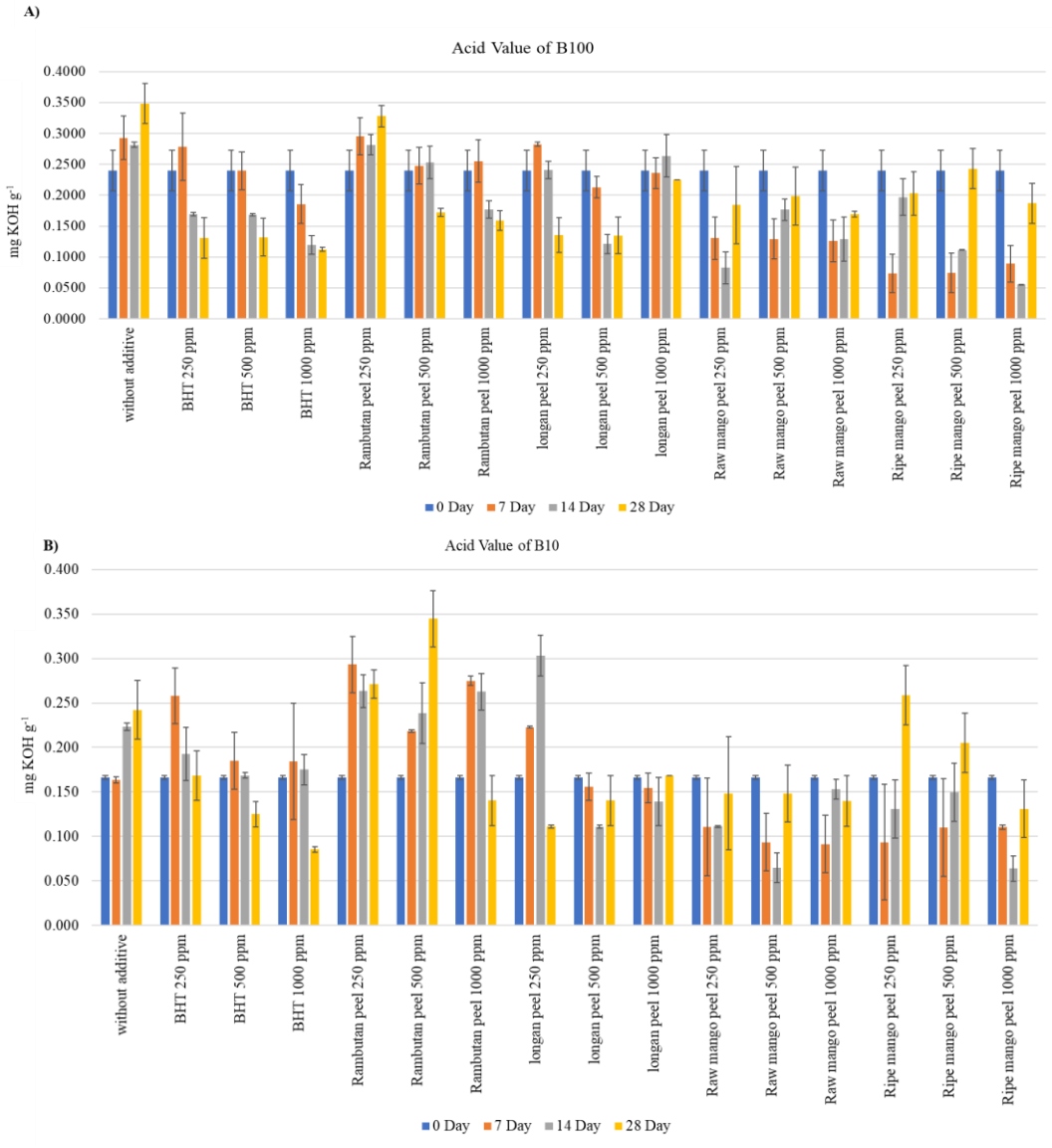

Figure 2. Acidity values measuring during 28 days of biodiesel (B100) (A), and B10 (B) storage

biodiesel as a fuel was preserved concerning this parameter. It can be seen that acid value for B10 (Figure 2B) was lower than for B100 (Figure 2A). It is proven that palm oil-derived biodiesel is more degraded than mineral oil diesel. The water content in B100 ranged from 992 to 1331 mg/kg (Figure 3). Water content in fuels has also been a problem, promoting

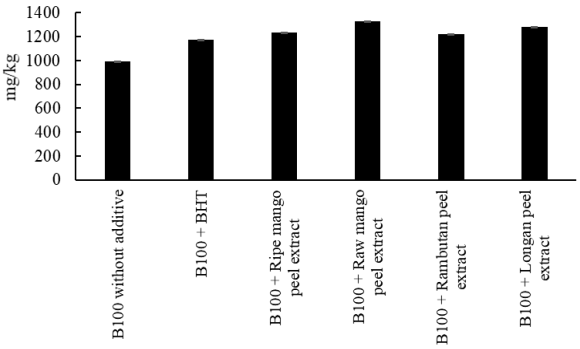

Figure 3. Water content in biodiesel (B100) stored for 7 days

microbial growth in storage tanks (Sekar, 2020). However, water removal and assessing other biodiesel quality parameters should be subjects of a further analysis. The results demonstrate that mango peel and rambutan peel could provide economically and environmentally interesting alternative antioxidants to improve the oxidative stability of biodiesel.

4. Conclusions

The ripe mango peel, raw mango peel, and rambutan peel extracts had high TPC levels and antioxidant activities and were thus tested for their ability to improve the oxidative stability of biodiesel. The IP of biodiesel (B100) samples mixed with 500 mg/L of the ripe mango peel extract had increased 10.20 fold. This exceeds the improvement by the synthetic antioxidant BHT (4.14 fold). All antioxidant extracts can greatly improve the oxidation stability of B10. These results indicate that the ripe mango peel extract was the most effective biodiesel fuel additive among the extracts tested.

Acknowledgements

This work was supported by the Thailand Science Research and Inno Office of National Higher Education Science Research and Innovation Policy Council, Thaksin University (research project grant) Fiscal Year 2021. Our thanks go to the Department of Chemistry, Faculty of Science, Thaksin University, for allowing us to use laboratory facilities.

References

Agarwal, S., Singhal, S., Singh, M., Arora, S., & Tanwer, M. (2018). Role of antioxidants in enhancing oxidation stability of biodiesels. *ACS Sustainable Chemistry and Engineering, 6*(8), 11036-11049.

Ajila, C., Naidu, K., Bhat, S., & Rao, U. P. (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry, 105*(3), 982-988.

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current Research in Food Science, 4*, 200-214.

Benzie, I. F., & Strain, J. (1999). [2] Ferric reducing/ antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in enzymology, 299*, 15-27.

Chairerk, N., Pongyeela, P., Chungsiriporn, J., & Rakmak, N. (2021). Ethanol extraction of active ingredients and antioxidant from germinated sangyod rice. *Applied Science and Engineering Progress, 14*(1), 52-59.

Chendynski, L., Cordeiro, T., Messias, G., Mantovani, A., Spacino, K., Zeraik, M., & Borsato, D. (2020). Evaluation and application of extracts of rosemary leaves, araçá pulp and peel of bacuri in the inhibition of the oxidation reaction of biodiesel. *Fuel, 261*, 116379.

Coelho, E. M., de Souza, M. E. A. O., Corrêa, L. C., Viana, A. C., de Azevêdo, L. C., & dos Santos Lima, M. (2019). Bioactive compounds and antioxidant activity of mango peel liqueurs (Mangifera indica L.) produced by different methods of maceration. *Antioxidants, 8*(4), 102.

Domingos, A. K., Saad, E. B., Vechiatto, W. W., Wilhelm, H. M., & Ramos, L. P. (2007). The influence of BHA, BHT and TBHQ on the oxidation stability of soybean oil ethyl esters (biodiesel). *Journal of the Brazilian Chemical Society, 18*, 416-423.

Fernandes, D. M., Sousa, R. M., de Oliveira, A., Morais, S. A., Richter, E. M., & Muñoz, R. A. (2015). Moringa oleifera: A potential source for production of biodiesel and antioxidant additives. *Fuel, 146*, 75-80.

Fregolente, P. B. L., Fregolente, L. V., & Wolf Maciel, M. R. (2012). Water content in biodiesel, diesel, and biodiesel–diesel blends. *Journal of Chemical and Engineering Data, 57*(6), 1817-1821.

Fu, J., Turn, S. Q., Takushi, B. M., & Kawamata, C. L. (2016). Storage and oxidation stabilities of biodiesel derived from waste cooking oil. *Fuel, 167*, 89-97.

Ghomari, O., Sounni, F., Massaoudi, Y., Ghanam, J., Kaitouni, L. B. D., Merzouki, M., & Benlemlih, M. (2019). Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. *Biotechnology Reports, 23*, e00347.

Helwani, Z., Zahrina, I., Tanius, N., Fitri, D. A., Tantino, P., Muslem, M., . . . Idroes, R. (2021). Polyunsaturated fatty acid fractionation from crude palm oil (CPO). *Processes, 9*(12), 2183.

Jaitrong, S., Rattanapanone, N., & Manthey, J. A. (2006). *Analysis of the phenolic compounds in longan (Dimocarpus longan Lour.) peel.* Paper presented at the Proceedings of the Florida State Horticultural Society.

Kim, D.-O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry, 81*(3), 321-326.

Pereira, D. M., Valentão, P., Pereira, J. A., & Andrade, P. B. (2009). *Phenolics: From chemistry to biology* (Volume 14, pp. 2202-2211). Basel, Switzerland: Molecular Diversity Preservation International.

Phonghanpot, S., & Jarintanan, F. (2021). Antiproliferative, antibacterial, and antioxidant activities of Bauhinia strychnifolia Craib aqueous extracts in gut and liver perspective. *BMC complementary medicine and therapies, 21*(1), 1-12.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237.

Sekar, M. (2020). Rambutan fruits extract in aging skin. In V. R. Preedy & V. B. Patel (Eds.), *Aging* (2nd ed. pp. 303-307), London, England: Academic Press.

Senphan, T. (2019). Comparative studies on chemical composition and antioxidant activity of corn silk from two varieties of sweet corn and purple waxy corn as influenced by drying methods. *Food and Applied Bioscience Journal, 7*(3), 64-80.

Senthil, R., Pranesh, G., & Silambarasan, R. (2019). Leaf extract additives: A solution for reduction of NOx emission in a biodiesel operated compression ignition engine. *Energy, 175*, 862-878.

Śpitalniak-Bajerska, K., Szumny, A., Kucharska, A. Z., & Kupczyński, R. (2018). Effect of natural antioxidants on the stability of linseed oil and fish stored under anaerobic conditions. *Journal of Chemistry, 2018*.

Sukatta, U., Rugthaworn, P., Khanoonkon, N., Anongjanya, P., Kongsin, K., Sukyai, P., . . . Chollakup, R. (2021). Rambutan (Nephelium lappaceum) peel extract: Antimicrobial and antioxidant activities and its application as a bioactive compound in whey protein isolate film. *Songklanakarin Journal of Science and Technology, 43*(1).

Varatharajan, K., & Pushparani, D. (2018). Screening of antioxidant additives for biodiesel fuels. *Renewable and Sustainable Energy Reviews, 82*, 2017-2028.

Young, A. J., & Lowe, G. L. (2018). *Carotenoids—antioxidant properties* (Volume 7, pp. 28). Basel, Switzerland: Molecular Diversity Preservation International.

Yuliarita, E., Fathurrahman, N., Aisyah, L., Hermawan, N., & Anggarani, R. (2019). *Comparison of synthetic and plant extract antioxidant additives on biodiesel stability.* Paper presented at the IOP Conference Series: Materials Science and Engineering.

Zhu, K.-X., Lian, C.-X., Guo, X.-N., Peng, W., & Zhou, H.-M. (2011). Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. *Food Chemistry, 126*(3), 1122-1126.