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Oxidative stability of *Iresine herbstii extract*-containing coconut oil during storage

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Abstract Virgin coconut oil was used as green solvent for the extraction of total phenolics from *I. herbstii* (*I. herbstii*: coconut oil, 1:10 (w/w)). *I. herbstii* extract-containing coconut oil (ECC) had dark red in color. Acid value (AV), peroxide value (PV), thiobarbituric acid-reactive substances (TBARS), ρ-anisidine value and volatile compounds were taken as parameters for evaluation of oxidative stability of EEC sample. All parameters of EEC sample were lowered, in comparison with the control sample during storage for 7 weeks. ECC sample produced less TBARS value than the control ($p<0.05$). Abundance of volatile compounds in all samples correlated well with the TBARS value. Results revealed the use of coconut oil as green solvent for extraction of bioactive compounds and natural red pigment from *I. herbstii*, where *I. herbstii* as antioxidant to prevent lipid oxidation in vegetable oil.

Keywords: *Iresine herbstii*, Green extraction, Oxidation, Storage, Coconut oil

Introduction

Iresine herbstii, family of Amaranthaceae, commonly known as bloodleaf, is a dark red flowering plant that has a single sub-leaf, arranged opposite each other. The leaves are about 2-4 cm wide and 3-6 cm long, with visible red veins. The tip is concave red with a pink transverse stripe. Bloodleaf is native to Brazil and tropical South America (Dipankar *et al.*, 2011), meanwhile it is also found in the tropical forest in Thailand and Southeast Asia.

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It is famous for ornamental foliage. Many studies have also investigated the extract from the *I. herbstii*. Dipankar and Murugan (2012) reported that *I. herbstii* is a source of biologically active compounds necessary for the prevention of diseases due to its antiviral, antibacterial, antioxidant and cytotoxic properties. Chaudhuri and Sevanan (2012) reported the use of *I. herbstii* extracts to cure maladies. Recently, Andleeb *et al.* (2020) reported that *I. herbstii* was suggested as an important plant because it doesn't have side or toxic effects.

The interest in polyphenolic compounds with antioxidant capacity from natural materials have been increased in the food industries (Kalisz, *et al* 2020). Polyphenolic compounds can help to slow down the development of food deterioration due to lipid oxidation reactions resulting in better quality and nutritional value of food. For polyphenolic extraction, the extraction process affected the phytochemical composition and biological activities of the extract (Thouri *et al.,* 2017). Extracting organic solvents are often used for the extraction of phenolic compounds from plants such as ethanol, methanol, acetone, hexane, etc. However, some of the organic solvents are potentially toxic and unacceptable for the food and pharmaceutical industries. Currently, many researchers have tried to create extraction processes by designing environmentally friendly processes to eliminate or reduce the use of harmful chemicals. Vegetable oils are considered as green solvents for the extraction of nutritionally valuable compounds from plants and their by-products (Teramukai *et al.,* 2020). Coconut oil, the richest source of medium chain fatty acids, provides important energy and nutrients for the human body. Additionally, coconut oil is one of the common uses for cooking in Thai cuisine. Phenolic extract-containing coconut oil can be used directly in the formulation of innovative food products. However, lipid oxidation leads to the formation of a variety of decomposition products that can cause unpleasant odors and flavors in edible oil during processing and storage. Thus, oxidative stability is an important parameter for determining the quality of fat/oil products. Studies on the use of plant extracts containing phenolic components to reduce lipid oxidation rates have been investigated in many research. Phenol-rich plant extracts with good lipid oxidation inhibitory in a food product had been reported by Burri *et al.* (2020). The addition of plant extracts to coconut oil improved its oxidative stability in terms of its antioxidant properties and its characteristics (Chandran *et al*., 2017).

Therefore, the objective of this research was to study the storage stability of *I. herbstii*-containing coconut oil during accelerated storage in terms of AV, PV, TBARS, ρ-anisidine, and volatile lipid oxidation compounds.

Materials and methods

Plant materials

I. herbstii was burned and collected from a siamjannin company, Thailand. The leaves were ground using a grinder and sieved using stainless steel (sieve 80 mesh). The powdered *I. herbstii* was kept in a ziplock bag and placed at room temperature until use.

Chemicals

Folin-ciocalteau's reagent, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid and potassium persulfate were provided from Sigma-Aldrich (USA). Gallic acid was obtained from Merck. All chemical reagents used were analytical grade. Virgin coconut oil was obtained from a supermarket, Thailand.

Study on total phenolic content (TPC) and DPPH radical scavenging activity of I. herbstii extract

Extraction of *I. herbstii*

I. herbstii was subjected to extraction using ethanol following the slightly modified method of Mohdaly *et al.* (2010). The powdered *I. herbstii* (10 g) was extracted with each 100 mL of ethanol at room temperature for 3 hr by stirring the mixture continuously at low speed using a magnetic stirrer (IKAWerke, Staufen, Germany). The extracts were filtered using filter paper No.4. The filtrates were concentrated by a vacuum evaporator (Tokyo Rikakikai, Japan) at 40 ℃ and removed all solvent by flushing with nitrogen gas. The obtained extract was weighed and calculated the yield as a percentage of the sample weight. The extract was kept in an amber bottle at -20 ℃ until analysis.

Total phenolic content (TPC)

Total phenolic in *I. herbstii* extract was detailed using Folin-ciocalteau reagent (FCR) (Kim *et al*., 2013). 0.1 mL of *I. herbstii* extract was mixed with FCR (0.75 mL, 10-fold dilution). 0.75 mL of Na_2CO_3 (6% v/v) was mixed into the mixture for 5 min. The mixed solution was placed in the dark for 60 min at 25 ℃. The absorbance was detected at the wavelength of 760 nm through a spectrophotometer (Shimadzu, Kyoto, Japan). The gallic acid concentrations in the rang of 0-100 ppm were prepared for the standard calibration. TPC was

showed in the unit of mg gallic acid equivalents (GAE) per g dry weight of the sample.

DPPH radical scavenging activity

The DPPH assay of *I. herbstii* extracts was assessed as the method by Binsan *et al*. (2008). Sample extracts (1.5 mL) were mixed with 1.5 mL of 0.15 mM 2,2-diphenyl-1-picryl hydrazyl (DPPH) in ethanol (95%). The reaction mixtures were left in the dark for 30 min at 25℃. The absorbance was measured by spectrophotometer at 517 nm. Trolox in the range of 10-50 μM was used as the standard curve. The DPPH activity of the extract was expressed as mmol Trolox equivalents (TE)/g dry *I. herbstii* extract.

Study on oxidative stability of I. herbstii extract-containing coconut oil during accelerated storage

Preparation of *I. herbstii* extract-containing coconut oil

The powdered *I. herbstii* was extracted with coconut oil at the ratios of 1:10 (w/w) at 45℃ for 30 min and filtered using a filter paper No.4. The obtained extract containing coconut oil (ECC) and virgin coconut oil (control) were subjected to analysis. ECC and control were kept in an amber bottle and capped tightly. All oil samples (30 mL/bottle) were placed at 60℃ in an incubator (Memmert, Schwabach, Germany) and were taken randomly for analyses at weeks 0, 1, 2, 3, 4, 5, 6 and 7.

Quality analyses of *I. herbstii* extract-containing coconut oil

Acid value (AV) was determined by 0.01 N alcohol potassium hydroxide (KOH) titration using the AOCS Official Method (AOCS, 1990). Peroxide value (PV) was analyzed using a standard iodometric titration method (AOCS, 1990). Para-anisidine value (ρ-AnV) was measured spectrophotometrically at 350 nm (AOCS, 1990). Thiobarbituric acid reactive substances (TBARS) were performed as described by Buege and Aust (1978).

Total volatile compounds of control and sample stored for 0 and 7 weeks at 60℃ were used for analysis. The volatile compounds of lipid oxidation in all samples were identified by using a headspace solid phase microextraction (SPME) GC-MS for the extraction, separation and analysis of the oil samples as per the method outlined by Takeungwongtrakul and Benjakul (2014) with minor modifications. The volatile compounds were compared by comparison of retention time and mass spectra with Wiley library spectra 275.L. The volatile oxidation compounds, including aldehydes, ketones, alcohols, etc., were identified in the terms of abundance of each identified volatile compound.

Statistical analysis

The data were subjected to analysis of variance (ANOVA). A comparison of means was carried out by Duncan's multiple range test. For pair comparison, t-test was used. Statistical analysis was performed using the Statistical Package for Social Science (SPSS for windows, SPSS, Inc., Chicago, IL, USA).

Results

Total phenolic content (TPC) and DPPH radical scavenging activity of I. herbstii extract

The extraction yield of the ethanolic extract was 2.59%. Total phenolic content and DPPH radical scavenging activity in ethanolic extract were noted as 1972.87 µg GAE/g extract and 1261.88 mmol TE/g extract, respectively (data not shown).

Oxidative stability of I. Herbstii extract-containing coconut oil during accelerated storage

Acid and peroxide values

AV and PV of *I. Herbstii* extract-containing coconut oil were investigated during the accelerated storage (Figure 1). The AV in coconut oil with/without the *I. herbstii* extract was developed from 0.44 to 0.64 mg KOH/g oil for 7 weeks ($p>0.05$). For PV, a sharp increase in PV within the 2nd week was observed, followed by remaining value on the 4th week of storage. After that, the PV was increased with increasing storage time. However, PV of ECC remained low at 13.97 meq O_2/kg oil for 6 weeks, whereas pure coconut oil showed higher PV ($p<0.05$).

TBARS and ρ-anisidine values

TBARS and ρ-anisidine values of *I. Herbstii* extract-containing coconut oil stored for 7 weeks at 60 ℃ are present in Figure 2. TBARS values were 1.26 mg MDA/kg and 1.59 mg MDA/kg for ECC and control samples after 7 weeks of storage, respectively. In addition, ρ-anisidine value of the control sample was constant throughout the storage ($p>0.05$) (Figure 2B). The ρ-anisidine value in the ECC sample before 2 weeks of storage did not differ from the ρ-anisidine value in the initial sample ($p>0.05$). However, an increase in ρ-anisidine value was found in the ECC sample during 4 weeks of storage and decreased at 6th week.

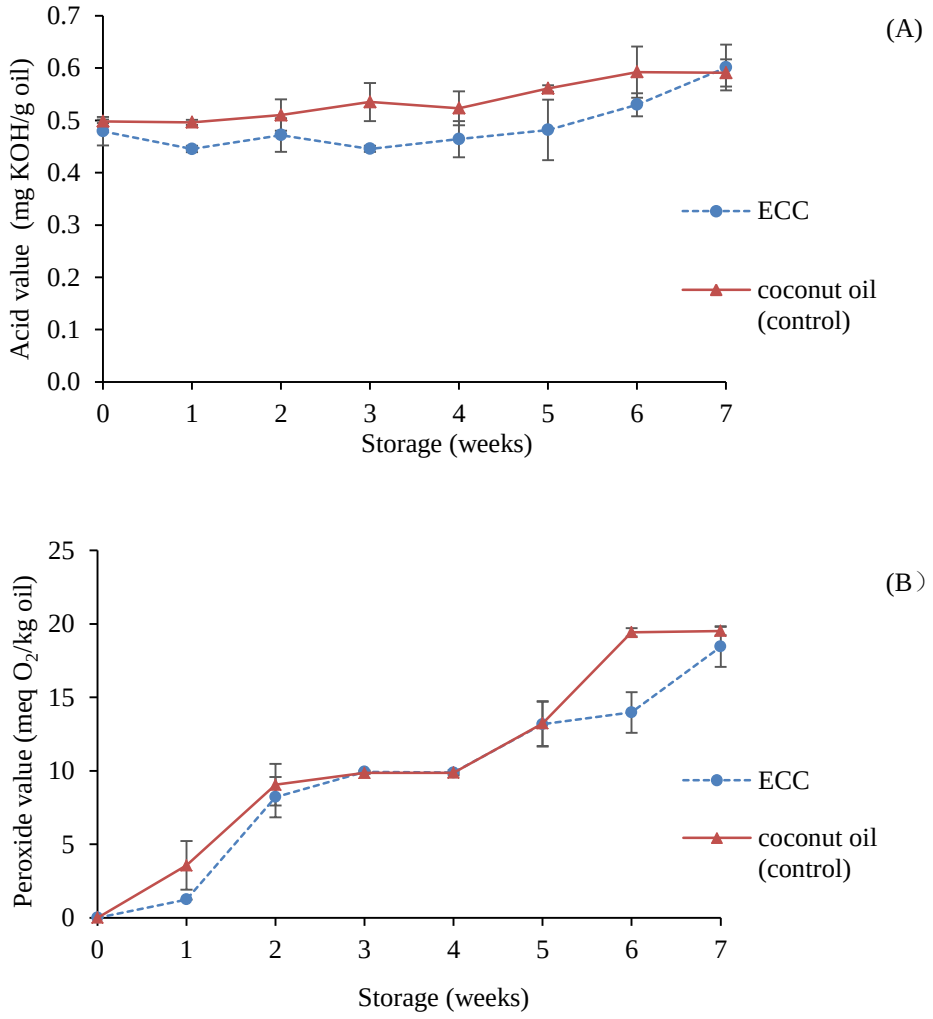

Figure 1. Acid and peroxide values in coconut oil with the *I. herbstii* extract addition during storage for 7 weeks

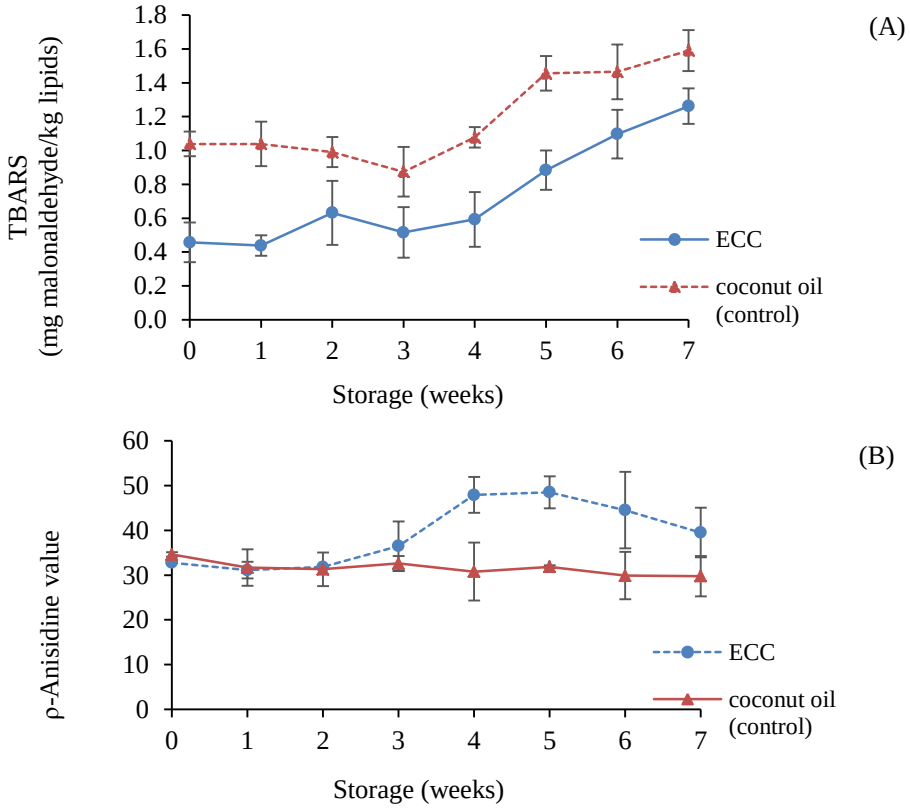

Figure 2. TBARS and ρ-anisidine values in coconut oil with the *I. herbstii* extract addition during storage for 7 days

Volatile lipid oxidation compounds of I. herbstii-containing coconut oil during accelerated storage

Result showed the list of volatile lipid oxidation compounds extracted from the control and ECC samples at 0 and 7 weeks of storage (Table 1). These volatile compounds included aldehydes, ketones and alcohols. There were 33 volatile compounds detected from all samples. All compounds presented in coconut oil with/without *I. herbstii* extract at day 0 were lower in abundance than those found after 7 weeks of storage. Nevertheless, 2-heptanol, 2-furanmethanol, isobutyraldehyde, 2-butanone, 2,3-pentanedione, 2-heptanone, 3(2H)-furanone, dihydro-2-methyl- and 2H-pyran-2-one, tetrahydro-6-pentyl, and in the control sample were also lower in abundance after 7 weeks of storage. For ECC sample, 1-hexanol, undec-3-en-2-ol, 2-furanmethanol, 2-furan-carboxaldehyde, 2-butanone, 2-heptanone, 3(2H)-furanone, dihydro-2-methyl- and 2(3H)-furanone, dihydro decreased after storage. After the storage,

the control sample contained new volatile compounds included ethanol, 2-butoxy-, pentanal and 2-heptanone, 3-methyl-, whereas ECC sample showed 1-hexanol, 2-ethyl-, hexanal, 3-buten-2-one, 3-buten-2-one, 3-methyl-, 2-butanone, 3-hydroxy-, 2-undecanone and 2(3H)-furanone, 5-ethyl dihydro-, as new volatile compounds. The highest amount of lipid oxidation products were 2-propanone, 2H-pyran-2-one, tetrahydro-6-methyl-, 2,3-butanediol and 2-heptanone which were found in coconut oil without *I. herbstii* extract after 7 weeks. 2H-Pyran-2-one, tetrahydro-6-methyl was the most numerous in ECC sample at 7 weeks, followed by 2H-pyran-2-one, tetrahydro-6-pentyl-, 3-buten-2-ol, 2-methyl- and 2-propanone.

Amongst all the volatile alcohols, 2,3-butanediol was identified as a major alcohol in control sample, followed by 2-furanmethanol and ethanol, 2-butoxy-, respectively. 3-Buten-2-ol, 2-methyl- and 1-hexanol, 2-ethyl- were found to be the volatile alcohol compounds in the ECC sample. Additionally, five aldehyde compounds (butanal, 3-methyl-, butanal, 2-methyl-, 2-furan-carboxaldehyde, pentanal and hexanal) and eleven volatile ketones (2-propanone, 2H-pyran-2-one, tetrahydro-6-methyl-, 2-heptanone, 2H-pyran-2-one, tetrahydro-6-pentyl-, 2-nonanone, 2-pentanone, 2-heptanone, 3-methyl-, 2-butanone, 2(3H)-furanone, dihydro-, 2-propanone, 1-hydroxy- and 3(2H)-furanone, dihydro-2-methyl-) were also found in control sample. Butanal, 2-methyl-, butanal, 3-methyl-, 2-butenal, 2-furan-carboxaldehyde and hexanal were aldehyde volatile compounds in the ECC sample. The oxidized volatile ketones from ECC sample were 2H-pyran-2-one, tetrahydro-6-methyl-, 2H-pyran-2-one, tetrahydro-6-pentyl-, 2-propanone, 2(5H)-furanone, 5,5-dimethyl-, 3-buten-2-one, 2-heptanone, 2-butanone, 3-hydroxy-, 2-nonanone, 3-buten-2-one, 3-methyl-, 2-butanone, 2(3H)-furanone, 5-ethyl dihydro-, 3(2H)-furanone, dihydro-2-methyl- and 2-undecanone.

Table 1. Volatile compounds in coconut oil with and without the *I. herbstii* extract during storage for 7 weeks

Volatile compounds	**Peak area (Abandance)**			
	Control		**ECC**	
	week 0	**week 7**	**week 0**	**week 7**
Alcohols				
1-Hexanol	ND	ND	18×10^{5}	ND
3-Buten-2-ol, 2-methyl-	ND	ND	19×10^{5}	27×10^{6}
2-Heptanol	71×10^{4}	58×10^{4}	ND	ND
Undec-3-en-2-ol	ND	ND	17×10^{5}	ND
Ethanol, 2-butoxy-	ND	70×10^{4}	ND	ND
1-Hexanol, 2-ethyl-	ND	ND	ND	45×10^{4}
2,3-Butanediol	60×10^{6}	73×10^{6}	ND	ND

Volatile compounds	Peak area (Abandance)			
	Control		ECC	
	week 0	week 7	week 0	week 7
2-Furanmethanol	77×10^5	59×10^5	10×10^5	ND
Aldehydes				
Isobutyraldehyde	39×10^5	ND	ND	ND
2-Butenal	ND	ND	11×10^5	42×10^5
Butanal, 2-methyl-	14×10^6	16×10^6	19×10^5	91×10^5
Butanal, 3-methyl-	11×10^6	17×10^6	16×10^5	57×10^5
Pentanal	ND	67×10^5	ND	ND
Hexanal	78×10^4	63×10^5	ND	13×10^5
2-Furan-carboxaldehyde	20×10^5	69×10^5	26×10^5	15×10^5
Ketones				
2-Heptanone, 3-methyl-	ND	52×10^5	ND	ND
2-Propanone	10×10^7	18×10^7	35×10^5	10×10^6
2-Butanone	39×10^5	27×10^5	21×10^5	12×10^5
3-Buten-2-one	ND	ND	ND	47×10^5
2-Pentanone	37×10^4	90×10^5	ND	ND
3-Buten-2-one, 3-methyl-	ND	ND	ND	13×10^5
2,3-Pentanedione	96×10^4	ND	ND	ND
2-Heptanone	80×10^6	58×10^6	51×10^6	24×10^5
3(2H)-Furanone, dihydro-2-methyl-	19×10^5	13×10^5	84×10^4	78×10^4
2-Butanone, 3-hydroxy-	ND	ND	ND	21×10^5
2-Propanone, 1-hydroxy-	19×10^5	20×10^5	ND	ND
2-Nonanone	14×10^5	10×10^6	64×10^4	21×10^5
2-Undecanone	ND	ND	ND	66 ×104
2(5H)-Furanone, 5,5-dimethyl-	ND	ND	41×10^5	78×10^5
2(3H)-Furanone, dihydro-	20×10^5	26×10^5	75 ×104	ND
2(3H)-Furanone, 5-ethyl dihydro-	ND	ND	ND	10×10^5
2H-Pyran-2-one, tetrahydro-6-methyl-	53×10^6	78×10^6	59×10^6	65×10^6
2H-Pyran-2-one, tetrahydro-6-pentyl-	20×10^6	18×10^6	20×10^6	45×10^6

ND = non-detectable
Control = pure coconut oil
EEC = *I. herbstii* extract containing coconut oil
Volatile compounds of the extract were determined based on more similarity than 80% with Wiley library spectra 275.L.

Discussion

The *I. herbstii* extract was prepared using maceration extraction method with ethanol as solvent. The use of ethanol as extraction solvent can recover the phytocompounds from *I. herbstii* leaves (Ijioma *et al.,* 2017). The ethanol solvent can attract both polar and nonpolar molecules, resulting in a high amount of extraction yield. Andleeb *et al*. (2020) explained that the main

phytochemical compound found in *I. herbstii* extracts using acetone or ethanol were alkaloids, anthraquinones, disaccharide compounds, flavonoids, glycosides, saponins, organosulfur compound, phenols, protein, and terpenoids. From the result, total phenolics in ethanolic extract showed the high value. Total phenolics of *I. herbstii* extract have beneficial effects including antioxidant activity in DPPH assay. The correlation between total phenolic contents and DPPH antioxidant activity coefficients were a positive relationship. This proved that phenolic compounds were responsible for the antioxidant properties of plant extracts. Moverover, these data might be supporting the potential of *I. herbstii* extracts in reducing lipid oxidation in lipid-rich system. However, some researcher suggested that the higher scavenging DPPH radical activity of an antioxidant could not be predicted that it has greater inhibitory capacity for lipid oxidation (Zhang *et al*., 2015).

Vegetable oils have been used instead of organic solvents, which are toxic, volatile, and flammable. Bioactive compounds from natural sources were extracted by vegetable oils that can be used directly in food products (Portillo-López *et al.*, 2021) Virgin coconut oil was used as green solvent for the extraction of total phenolics from *I. herbstii*. *I. herbstii*-containing coconut oil (ECC) had dark red in color. The result indicated that coconut oil could extract the red pigments from the *I. herbstii*. The bioactive compounds from plant materials could be diffused into the edible oil (Teramukai *et al.*, 2020). Several reports have studied the extraction of antioxidants and natural pigments with vegetable oil (Li *et al.*, 2013; Roy *et al.*, 2010; Teramukai *et al.*, 2020). The EEC could be used as natural bioactive pigments for food products, especially edible oil, or oil-in-water food emulsions.

Changes in AV, PV, TBARS and ρ-anisidine values of ECC and control samples during the accelerated storage are shown in Figure 1 and 2. AV is used to monitor the quality of the oil. The presence of AV in the oil might be caused by hydrolytic activities from lipase enzymes, autoxidation and poor processing. Both EEC and control samples had low AV throughout the storage of 7 weeks (Figure 1A). AV of control sample was significantly higher than that of EEC sample throughout the storage ($p<0.05$). Similar trends of AV were also reported by Drinić *et al.* (2020). For PV, PV has been used as the primary indicator for measuring lipid oxidation in the initial phases. Primary lipid oxidation products such as hydrogen peroxide are generated early during storage. PV had increased continuously in all oil samples through the storage time (Figure 1B). However, PV of ECC remained low for 6 weeks, whereas virgin coconut oil showed higher PV. Additionally, hydroperoxide is an unstable compound that readily decomposes, leading to the formation of aldehydes, epoxides, and ketones, reducing peroxide values and accumulating

secondary oxidation products. A similar result was also observed for TBARS (Figure 2A). In all samples of coconut oil, there was an increase in the amount of secondary oxidation products. After storage for 7 weeks, the ECC sample showed a significantly lower TBARS value than the untreated coconut oil. Higher value of TBARS is considered rancid and unacceptable for consumption. In addition, no change in ρ-anisidine value of the control sample was monitored through the storage time ($p>0.05$) (Figure 2B). Normally, the ρ-anisidine value is an important method to determine the second oxidation product of vegetable oils, namely non-volatile aldehydes (Zuo *et al.*, 2017). The result indicated that the reaction of ρ-anisidine with some compounds of coconut oil which were not a non-volatile secondary oxidation product. A benefit of *I. herbstii* extract in the coconut oil resulted in a low level of lipid oxidation, preventing the rancid flavor of the oil. The results were consistent with those reported by Chandran *et al.* (2017) who examined incorporating black pepper and ginger extract in coconut oil to improve the product stability and in flavor characteristics. The *I. herbstii* extract was rich in phenolic compounds that exhibited potential antioxidant activity and reduced secondary oxidation products monitored by TBARS value. Thus, *I. herbstii* extract could delay or prevent lipid oxidation that caused off-odor in coconut oil during storage.

Effects of I. herbstii extract on the generation of volatile lipid oxidation compounds during storage

These volatile compounds, including aldehydes, ketones and alcohols, are markers of lipid oxidation in all samples. *I. herbstii* extract had affected quantitatively and qualitatively on the volatile compounds of control and ECC samples. There were 33 volatile compounds detected from all samples. All compounds present in coconut oil with/without *I. herbstii* extract at day 0 were lower in abundance than those found after 7 weeks of storage. Those volatile compounds decreased due to the decomposition or volatilization.

After the storage, the control sample contained new volatile compounds. The highest amount of lipid oxidation products was found in control sample after 7 weeks especially methyl-ketones such as 2-propanone and 2-heptanone. Methyl-ketones could result from beta oxidation of the carbon chain followed by decarboxylation (Selke *et al.*, 1975). 2H-Pyran-2-one, tetrahydro-6-methyl- was the most numerous in ECC sample at 7 weeks. Takahashi *et al.* (2014) reported that 2H-pyran-2-one, tetrahydro-6-methyl- showed strong correlation between hexanoic acid concentrations and fatty odor. Amongst all the volatile alcohols, 2,3-butanediol was identified as a major alcohol in control sample. 3-

Buten-2-ol, 2-methyl- and 1-hexanol, 2-ethyl- were found to be the volatile alcohol compounds in the ECC sample. Additionally, five aldehyde compounds and eleven volatile ketones were also found in control sample. Butanal, 2-methyl-, butanal, 3-methyl-, 2-butenal, 2-furan-carboxaldehyde and hexanal were aldehyde volatile compounds in the ECC sample. Some cyclic compounds such as furan were derived from unsaturated fatty acids (Ghorbani Gorji *et al.*, 2019). These compounds indicated the off-flavour of soybean oil (Kochhar, 1996). Moreover, there were some volatile compounds showed in the control sample but were not detected in the ECC sample. Abundance of volatile compounds in all samples correlated well with the TBARS value as presented in Figure 2A, in which ECC sample had the lowest TBARS value after storage. Thus, the use of *I. herbstii* was effective in reducing oxidative changes in coconut oil.

The extraction solvent used had a significant effect on the polyphenol content and antioxidant activity of the *I. herbstii* extract. Ethanol is the choicest solvent for extracting phytochemical compounds from *I. herbstii*. Additionally, *I. herbstii* are soluble in edible oil. The coconut oil treated with *I. herbstii* showed significant reductions in lipid oxidation and maintained the quality of the lipid during storage for 7 weeks. Based on the research results obtained, the *I. herbstii* extract could be used as additive by preventing rancidity in food to retard lipid oxidation and extend shelf life of oil and fats products. Additionally, the ECC with bioactive phytochemicals could be applied as natural colorants for food and cosmtic products.

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