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EFFECT OF HEAT PRETREATMENTS ON CHEMICAL AND ANTIOXIDANT PROPERTIES OF MELON MANIS TERENGGANU (*Cucumis melo* var. Inodorus cv. Manis Terengganu 1) SEED OIL

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ABSTRACT

The main objective of this study was to determine effect of different heat pretreatments on chemical and antioxidant properties of Melon Manis Terengganu (MMT) seed oil. The seeds were treated with four different heat pretreatment which were untreated (control), roasting, steaming and microwaving. Then the MMT seed oil was extracted from each treated seed using conventional extraction method. The chemical tests determined on the MMT seed oil were free fatty acid (FFA), iodine value (IV), saponification value and fatty acid composition. The antioxidant properties for MMT seed oil evaluated was total phenolic content (TPC), 1,1- diphenyl-2-picrylhdrazyl (DPPH) scavenging capacity and Ferric reducing antioxidant power (FRAP). It was found that different heat pretreatments of MMT seed significantly ($p<0.05$) affected the oil yield (11.10–15.40%.), free fatty acid value (3.55–5.04%), DPPH radical scavenging activity (43.06–56.08%), total phenolic content (0.088–0.142 mg GAE/g) and FRAP activity (0.0723–0.110 mmole/100 mL). However, the seed heat pretreatment did not affect the fatty acid composition significantly ($p<0.05$). As for iodine value, it was different between untreated seed (107.89 g I_2/100g) and steamed seed (104.41 g I_2/100g) only ($p<0.05$).

Key words: Heat pretreatment, seed, oil, Melon Manis Terengganu, antioxidant

INTRODUCTION

Melon Manis Terengganu (*Cucumis melo* var. Inodorus cv. Manis Terengganu 1) is one of the melon species (Muhammad *et al*., 2018), with smooth yellow peel and orange in colour with orange coloured flesh. Melon has good taste and it contains carbohydrates, vitamin A, C, D, K, B and E, folic acid, carotene, minerals (potassium, magnesium, phosphorus, sodium, selenium, and calcium), and various aromatic compounds (Ivanova, 2012). Until now, only few studies have been reported on MMT including the effect of different storage temperatures on physicochemical characteristics and quality of MMT (Aishah Athirah *et al*., 2017) and effect of drying temperature and extraction solvents on antioxidant properties of immature MMT (Muhammad *et al*., 2018).

The processing of fruits has generated large quantity of by-product such as seed, seed kernel and the skin, which is usually discarded (Mallek-Ayadi *et al*., 2018). Moreover, the high costs of drying, storage and transportation of these by-products are economically unprofitable. However, in recent years, fruit seeds have received growing interest due to the important nutritional and medicinal properties of their bioactive compounds. Many researches have reported on the nutritional benefits of melon seed and seed kernel. Milind and Kulwant (2011) reported that seed kernel is commonly used in renal disorders such as kidney, bladder stone, and inflammation of the liver. According Górnaś and Rudzińska (2016), fruit seed can be extracted to produce seed oil which contains a great number of valuable bio-component and natural antioxidants. A study on three varieties of melon cultivated in Bulgaria found that the seeds were rich in oil (30–50%) and protein (12.0–35.0%) (Petkova & Antova, 2015). Honeydew melon seed oil was reported to be

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rich in biologically active substances such as tocopherols, sterols and phospholipid which give beneficial effect on humans (Zeb, 2016). Yanty *et al*. (2008) reported that honeydew melon seed possess medicinal properties and can be used as an anti-diabetic or acute eczema. Many studies have been reported on properties of seed oil such as from honeydew, bitter melon, sesame, soy, water melon, papaya, corn, egusi melon and pumpkin (*Cucurbita pepo*) (Petkova & Antova, 2015; Peiretti *et al*., 2017; Olubi *et al*., 2019).

One of the important parameter in edible oil production is the quality of seed oil. Pretreatment is one of the factors that can affect the yield and quality of seed oil. Roasting, microwaving, and steaming are commonly used heat pretreatments in edible oil industry. These heat pretreatments were reported to affect the quality, stability, and antioxidant properties of oil extraction from plant seed (Uquiche *et al*., 2008; Durdevic *et al*., 2017; Gumaling *et al*., 2018; Petkova & Antova, 2019). According to Uquiche *et al*. (2008), microwaving pretreatment resulting in higher oil yield and gave oil with high stability towards oxidative deterioration compared to untreated seed because microwave pretreatment can rupture the cell wall and enable the oil to move through permeable cell wall. However, Danso-Baoteng (2011) found that the steaming pretreatment yield the highest amount of sunflower seed oil. On the other hand, Potočnik *et al*. (2018) reported that iodine value decreased as roasting temperature rose. Drużyńska *et al*. (2007) reported that the values of antioxidant not only depend on the type of the solvent used but it also depends on parameters exerted by the solvent that were used for extraction. Besides that, the component that is present in seed oil, when heated can causes a decrease in antioxidant activity (Abril *et al*., 2019). Durmaz and Gökmen (2011) stated that the antioxidant activities increased with roasting pretreatment due to the formation of Mailard reaction products. Previous studies have been reported on the effect of heat pretreatment on yield and properties of seed oil including from grape seed (Oomah *et al*., 1998), African pear seed (Onyeka *et al*., 2005), cashew nut (Chandrasekara & Shahidi, 2012), honeydew seed (Zhao *et al*., 2012), sky fruit seed (Lau *et al*., 2012; Gumaling *et al*., 2018) and pomegranate seed (Đurđević *et al*., 2017). However, no study has been reported on the effect of heat pretreatments on the properties of MMT seed oil. Thus, this study aimed to determine the effects of different heat pretreatment on the oil yield as well as chemical and antioxidant properties of MMT seed oil.

MATERIALS AND METHODS

Materials

Fresh Melon Manis Terengganu (MMT) seeds were obtained from a MMT supplier in Batu Hampar, Manir, Terengganu and it was stored in a freezer until further use. Acetic acid, diethyl ether, Wjis solution, 2, 2-diphenyl-1-picrylhydrazyl reagent and, gallic acid were purchased from Sigma-Aldrich Sdn Bhd. All other chemicals and reagents used were of analytical grades.

Methods

Sample preparation

Melon Manis Terengganu (MMT) seeds were cleaned using tap water and then divided into four portions for 4 types of heat pretreatment. For untreated (control) sample, MMT seeds were dried at room temperature (30°C) under the fan for 24 hours. For roasting heat pretreatment, the seeds were roasted using a convection oven (Master 450, Garland Freeland, Pennsylvania) at 200°C for 20 min. For steaming heat pretreatment, the seeds were steamed at 100°C for 20 min. For microwaving heat pretreatment, the seeds were microwaved using a microwave (Dimension 4, The Genius National, Japan) at 2450 mHz for 15 min.

Extraction of oil

Extraction of oil from untreated (control) and treated (roasting, steamed and microwaved) samples of MMT seeds were carried out according to modified Blight and Dyer (1959) method. For each control and treated samples, 100 g of MMT seed was mixed with 200 ml methanol and 100 ml chloroform. Then, the mixture was homogenized using a Waring blender (Waring, USA). The mixture was filtered to separate the seeds and the solvent. Next, the same amount of methanol solvent (200 ml) and chloroform solvent (100 ml) were mixed with the filtered residue. After that, the mixture was transferred into a blue-capped bottle and agitated using an incubator shaker (Innova 40R, USA) at rpm 200 at 25°C for an hour. After the agitation process, the mixture was filtered to separate the seed residue and the solvent. The agitation and the filtering process were consistently conducted until a clear solvent was obtained. The solvent was then evaporated using a rotary evaporator (N-1000 Eyela, Japan) at 65 until almost all solvent was evaporated. The resulting seed oil was centrifuged in a centrifuge (Hettich Zentrifugen Universal 320, Germany) at 4000 rpm for 10 min. Finally, the supernatant was stored at -20 prior to further analysis (Yanty *et al*., 2008).

Chemical properties of MMT seed oil

Free fatty acid

Determination of free fatty acid (FFA) was conducted according to AOAC (1980) method. One gram of oil was dissolved with 25 ml diethyl ether dissolved with 25 ml of alcohol and 1 ml of 1% phenolphthalein solution. The solution was then titrated with aqueous 0.1 M of sodium hydroxide solution by shaking continuously until pink colour solution formed. The amount of aqueous 0.1 M of sodium hydroxide solution used was recorded. The percentage of FFA was calculated as follows:

$$\text{FFA (as \% oleic acid)} = \frac{\textit{volume of titration x normality of Na OH x 28.2}}{\textit{weight of oil (g)}}$$

Iodine value

Iodine value of the MMT oils was measured according to AOAC (2000) method. Oil sample (0.2 g) was weighed in a 250 ml conical flask. Then, 20 ml of cyclohexane was added and swirled until the oil was completely dissolved. Next, 25 ml of Wijs solution was added into the mixture. The mixture was kept in the dark for one hour. Then, 20 ml of 15% potassium iodide was added into the flask followed by 100 ml distilled water. The starch indicator was added and the mixture was titrated with 0.1 N sodium thiosulphate till colourless end point. The blank sample was conducted without the sample. The iodine value was calculated as follows:

$$\text{Iodine value (IV)} = \frac{(B–S)xNx12.69}{\textit{weight of oil (g)}}$$

Saponification value

Saponification value of the MMT oils was measured according to AOAC (2000) method. The oil sample (2 g) was weighed in a conical flask. Next, 25 ml alcoholic potassium hydroxide was added. The mixture was then heated in boiling water using a water bath (TE-10D Temp., Techne, UK) at 100°C for 1 hour. Then, 1 ml of phenolphthalein solution was added and the solution, and was titrated with hot excess alkali of 0.5 M hydrochloric acid. The saponification value was calculated as follows:

Saponification value = mg of KOH consumed by 1g of oil

Weight of KOH = (28.05 x volume of KOH) / sample weight (g)

Volume of KOH consumed by 1 g oil = [Blank – test] ml

Peroxide value

Analysis of peroxide value was conducted according to AOAC (2000). The oil sample (1 g) was placed in a 250 ml conical flask and then dissolved with 10 ml of chloroform and 15 ml acetic acid at a ratio of 2:3. Then, 1 ml of concentrated potassium iodide was added and kept in the dark for 5 min. After 5 min, 75 ml of distilled water was added. Next, 1 ml of 2% starch indicator was added and the mixture was titrated with 0.01 N sodium thiosulphate until the colourless end point was attained. The blank sample was conducted without the sample. The peroxide value (PV) was calculated as follows:

$$\text{PV} = \frac{\text{volume of titration in ml (sample-blank)} \times (\text{normality of } Na_2S_2O_3 \times 1000)}{\text{weight of oil (g)}}$$

Fatty acid composition

Esterification of fatty acid methyl ester (FAME) was carried out according to Timms (1978). Oil sample (0.5 g) was weighed in a 5 ml vial and mixed with 1 ml hexane and 0.2 ml of sodium methoxide using a vortex (MS1 Minishaker, IKA Works, Guangzhou) for 1 min. The mixture was allowed to separate into two layers and the upper layer was pipetted into 2 ml vial.

Analysis of fatty acids was conducted using gas chromatography (Shidmadzu GC-2010, Japan) equipped with flame ionization detector (FID) and BPX-70 column (30 m length x 0.25 mm internal diameter x 0.2m film thickness). The flow rate of the carrier gas (helium) was 1 ml/min and the split ratio was 1:10. The injector and detector temperatures were 230°C and 250°C, respectively. The oven temperature was programmed initially at 70°C, holding for 5 min, and increased to 140°C at 10°C/min, then held isothermal for 5 min. The temperature was increased from 140°C to 240°C at 4°C/min and held for 1 min. A 37 components of fatty acid methyl esters mix (Sigma, St. Louis, Missouri, USA) was used as external standard for peak identification.

Antioxidant properties of MMT seed oil

Total phenolic content

Oil sample (0.5 ml) was mixed with 2 ml of 10% of diluted Folin–Ciocalteau reagent. The solution was kept for 5 min and 1.6 ml of 7.5% of sodium carbonate was added. The solution was vortexed and was kept in room temperature (25°C) for 60 min. The absorbances were measured at 765 nm using a UV-spectophotometer against methanol blank (Gutfinger, 1981).

DPPH radical scavenging activity

Oil sample (3 ml) in methanol (300 mg/ml) was mixed with 3 ml of methanolic solution that contained DPPH radical (0.2 mM). The mixture was vortexed (IKA Works, Guangzhou) for 1 min at 2000 rpm and allowed to stand for 30 min in the dark. Next, the absorbance was read against blank using a spectrophotometer (GENEYS 20 Thermo-spectronic, Thermo Electron Corp., USA) at 517 nm. Percentage of DPPH inhibition activity (%) was calculated as described by Shimada *et al*. (1992):

$$\text{Percentage inhibition activity (\%)} = \frac{\textit{Absorbance of control} - \textit{absorbance of sample}}{\textit{absorbance of control}} \times 100$$

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) reagent was prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mM HCl and 20 mM ferric chloride solution in a proportion of 10:1:1 (v/v/v). An aliquot of 400 L of the diluted sample was mixed with 3.6 ml of FRAP reagent. Then, the mixture was kept in the water bath (TE-10D Temp., Techne, UK) at 37 for 10 min. The absorbance was measured at 593 nm using a UV-VIS spectrophotometer. The standard curve was elaborated at 10 concentration levels of iron sulphate according to Muhammad *et al*. (2018).

Statistical analysis

All analyses were carried out in triplicate (n=3). Data were presented as mean standard deviation. The significance difference between means were determined by a Tukey's Multiple Comparison test following a one-way analysis of variance (ANOVA) at a significance level of $p<0.05$ using a Minitab software Version 16 (Minitab Inc., USA).

RESULTS AND DISCUSSION

Effect of heat pretreatment on yield of extracted oil from MMT seed

Table 1 shows the oil yield, free fatty acid content, iodine value, saponification value and peroxide value MMT seed oil obtained from different heat pretreatments. The oil yield ranged from 11.10 to 15.40%. There was a significant difference in the oil yield of MMT seeds with different heat pretreatment (p0.05), except for microwaving and roasting heat pretreatment. The highest oil yield was obtained from microwaving, followed by roasting and steaming, and finally untreated MMT seed.

The oil yields in the present study were similar to that of grape seed (13.9–14.8%; Oomah *et al*., 1998) and African pear seed (2.74–13.21%; Onyeka *et al*., 2005), but higher than that of corn (3.1–5.7%; O' Brien, 2004). However, the yield of MMT seed oils in the present study were lower compared to honeydew and hybrid 1 (41.6%; Petkova & Antova, 2015), Dessert 5 (44.5%; Petkova & Antova, 2015), agrestis melon (23.3%; Mariod *et al*., 2009), Kalahari melon (30.5%; Nyam *et al*., 2009a), bitter melon (19.3%; Mariod *et al*., 2009), watermelon (50%; Baboli & Kordi, 2010), pumpkin (31.9–39.44%; Yanty *et al*., 2008), soy (18–22%; O' Brien, 2004), rapeseed (41%; Yang *et al*., 2014), corn (18–20%; Petkova & Antova, 2015) and sunflower (40%; Petkova & Antova, 2015).

In the present study, the oil yield of roasted MMT was higher than the untreated MMT seed. A similar finding was reported for African pear seed (Onyeka *et al*., 2005), cashew nut (Chandrasekara & Shahidi, 2012) and honeydew seed (Zhao *et al*., 2012). Meanwhile, Oomah *et al*. (1998) reported that the oil yield of microwaved grape seed was higher than the untreated grape seed. A different finding was reported by Lau *et al*. (2012) on sky fruit seed where the oil yields obtained from microwave

Table 1. Chemical properties of Melon Manis Terengganu seed oil as affected by different heat pretreatments of seeds

Heat pretreatment	Oil Yield (%)	Free fatty acid (% oleic acid)	Iodine value (g I_2/100g)	Saponification value (mg KOH g^{-1})	Peroxide value ($meqO_2$/kg)
Untreated	11.10 ± 0.63^{c}	4.48 ± 0.12^{c}	107.89 ± 1.73^{a}	171.32 ± 1.16^{b}	12.79 ± 0.75^{c}
Microwaving	15.40 ± 0.34^{a}	3.55 ± 0.19^{d}	105.60 ± 1.77^{ab}	163.83 ± 1.72^{c}	13.70 ± 0.55^{b}
Roasting	14.84 ± 0.86^{a}	4.83 ± 0.097^{b}	106.62 ± 0.6^{ab}	185.19 ± 1.87^{a}	14.57 ± 0.26^{a}
Steaming	12.13 ± 0.98^{b}	5.04 ± 0.057^{a}	1074.41 ± 0.56^{b}	165.71 ± 1.17^{c}	11.52 ± 0.47^{d}

Values are mean standard deviation from three replications (n=3).
Values with different superscript letter in the same column are significantly different at p 0.05

and untreated seeds were higher (28.46–29.5%) compared to roasting and steaming (23.29–24.82%) samples. The variations in oil yields in these studies may be contributed to differences in plant cultivar, cultivation climate, ripening/maturity stage and the oil extraction method used (Nyam *et al*., 2009b; Petkova & Antova, 2015).

In general, higher oil yields were obtained from microwaved or roasted seeds. This probably was due to damage of cell membrane at high temperature during heat pretreatment, which increased the yield of seed oil extraction (Chandrasekara & Shahidi, 2012). According to Zhong *et al*. (2018), microwave is an efficient means for accelerating solvent extraction from plant matrices and it requires less solvent. Microwave penetrates to the seed oil due to the convection from electromagnetic energy to thermal energy. The cell membrane was ruptured by microwave radiation and created permanent pores and allowed easier oil extraction (Mazaheri *et al*., 2019). Ramanadhan (2005) stated that microwave heat for oil extraction has many advantages compared to conventional methods. For example, improvement of extracted oil yield and quality, direct extraction capability, lower energy consumption, faster processing time and reduced solvent contents. On the other hand, roasting heat pretreatment can produce condiment oils and extensive processing method that can increase the availability of nutrients and inactive enzymes. According Mazaheri *et al*. (2019), desired flavours, colours and promoting palatability of oil can be achieved from roasting heat pretreatment.

Effect of heat pretreatments on chemical properties of MMT seed oil

Free fatty acid content

Free fatty acid (FFA) is the one of the parameters to monitor the quality of edible oil. The FFA content of MMT seed oils extracted from different heat pretreatments in the present study ranged from 3.55% to 5.04%. There was a significant difference in FFA content of MMT seed oils between all different heat pretreatments ($p<0.05$). The highest FFA content was found in the MMT seed oil extracted from steamed (5.04%), followed by roasted (4.83%), untreated (4.48%) and microwaved (3.55%) seeds.

The FFA content of MMT seed oils in the present study were higher than *Cucumis melo* Maazoun cultivar (0.31%), honeydew melon (2.5%), watermelon (1.41%), pumpkin (1.44%) (Mallek-Ayyadi *et al*., 2019), oriental melon (2.54%; Chen *et al*., 2014) and honeydew melon (2.5%; Yanty *et al*., 2008) seed oils. This finding indicated that MMT seed oils in the present study were more susceptible to lipolysis. Lipolysis process is caused by water content and lipase enzymes in the seed. Lipase enzyme in the oil will accelerate the hydrolysis process (Lau *et al*., 2012). According to Japir *et al*. (2017), an increase in the FFA content in crude palm oil was due to the lipolysis by lipase enzyme. They also stated that FFA content is very important to determine quality index for the length of the storage, marketing and production of palm oil products. The breakdown of triglyceride and the release of FFA are usually due to evolving climate, torrential rainfall, humidity and unsuitable storage condition (Japir, 2017).

Onyeka *et al*. (2005) reported that the FFA contents in the roasted and steamed African black pear seeds were higher than untreated seed due to greater liberation of FFA. However, for sky fruit seed oil, no significance difference was reported between untreated sky fruit seed with other heat pretreatments (1.48–1.85%) (Lau *et al*., 2012).

Iodine content

Iodine value (IV) is a measure of degree of unsaturation of oil, solidification temperature and oxidation stability. The higher the unsaturation, the higher the iodine value and the higher the susceptibility to oxidation. The IV of extracted MMT seed oils in the present study ranged from 104.41 to 107.89 g I_2/100 g. This study showed that the IV of the MMT seed oils were lower than Kalahari melon (125.0 g I_2/100 g; Nyam *et al*., 2009a), *Cucumis melo* Maazoun cultivar (139.5 g I_2/100 g; Mallek-Ayadi *et al*., 2019), honey dew (153.4 g I_2/100 g), water melon (156 g I_2/100 g; Yanty *et al*., 2008), sunflower (137 I_2/100 g; Yanty *et al*., 2008) and soy (131 I_2/100 g; Yanty *et al*., 2008) seed oils.

A lower IV of MMT seed oils indicated a lower degree of unsaturation and lower melting point than the above-mentioned seed oils. A higher IV was found in the untreated MMT seed oil compared to steamed seed oil ($p<0.05$). Similar finding was reported by Potočnik *et al*. (2018) where IV gradually decreased with increasing heating power setting and time. Meanwhile, the IV of the roasted and microwaved MMT seed oils were not significantly different from other heat pretreatments ($p>0.05$). However, Yanty *et al*. (2008) reported a contrasting finding whereby the microwaved and roasted honeydew seed oils gave significant reduction in IV due to formation of polar compound when heating. The reduction in IV during heating were often taken as a measure of lipid oxidation because high temperature accelerates lipid oxidation the number of unsaturation sites reduced as a result of oxidation, polymerization, or breakage of the long-chain fatty acid (Anjum *et al*., 2006).

Saponification value

Saponification was defined as the number of mg of potassium hydroxide (KOH) necessary to saponify 1 g of lipid sample. The saponification value of MMT seed oils in the present study ranged from 163.83 to 185.19 mg KOH g^{-1}. There was a significant difference in saponification value of MMT seed oils for all heat pretreatments ($p<0.05$) except between microwaved seeds and steamed seeds. The highest saponification value found in the roasted MMT seeds, followed by untreated, steaming and microwaved seeds.

The saponification values of MMT seed oils were in similar range with Kalahari melon (173.2 mg KOH g^{-1}), kenaf (171.0 mg KOH g^{-1}), pumpkin (185.3mg KOH g^{-1}), roselle (172.3 mg KOH g^{-1}) and *Suaeda salsa L* (174.24 mg KOH g^{-1}) seed oils (Nyam *et al*., 2009a; Baogu *et al*., 2013). A lower saponification value was reported for *Cucumis melo* var Maazoun cultivar (139.8 mg KOH g^{-1}; Mallek-Ayadi *et al*., 2019). On the other hand, a higher saponification values were reported for bitter melon (190.7 mg KOH g^{-1}), *Cucumis melo* hybrid AF-522 (210.62 mg KOH g^{-1}), honeydew melon (210.2 mg KOH g^{-1}) and *Cucumis melo var saccharinus* (191.4 mg KOH g^{-1}) seed oils (Yanty *et al*., 2008; Nyam *et al*., 2009b; Baogu *et al*., 2013).

A higher saponification value indicates the presence of low content of long chain fatty acid, making it suitable as edible oil. A higher saponification value of oil was useful in the production of liquid soap and shampoos because it contains more of the unsaturated fatty acids and indicates greater liquidity (Lau *et al*., 2012). The saponification values of MMT seed oils with different heat pretreatment were within the range for certain crude vegetables oil like corn oil (187–197 mg KOH g^{-1}) and mustard oil (170–184 mg KOH g^{-1}) (Codex Alimentarius Commission, 2001).

Peroxide value

Peroxide value (PV) is defined as the milliequivalent (mEq) of peroxide per kilogram of fat or oil and it is an indicator of the initial stage of lipid oxidation. The PVs of MMT seed oils in the present study ranged from 11.52 to 14.57 $mEqO_2$/kg. The PV of MMT seed oils were higher than bitter melon, Kalahari melon, kenaf, pumpkin and roselle (1.5–6.5 mEq O_2/kg; Nyam *et al*., 2009a), watermelon (3.24 mEq O_2/kg oil; Baboli & Kordi, 2010) and sky fruit (1.98–3.25 mEq O_2/kg; Lau *et al*., 2012) seed oils. The PVs of MMT seed oil in the present study were lower than 15 $mEqO_2$/kg, which nearly reached the Codex limit for virgin oil (Codex Alimentarius Commission, 2001). These findings indicated that the MMT seed oil were unstable and susceptible to rancidity. Moussata and Akoh (1998) also reported that the stability of melon seed was low compared to other seeds because it was rich in linoleic acid (64.5%).

The PVs of MMT seed oils were significantly different for all heat pretreatments ($p<0.05$). The highest PV was found in roasted followed by microwaved, untreated and finally steamed MMT seed oils. This finding shows that the oils extracted from heat treated MMT seeds contained higher peroxide values than untreated seed, except for steamed seed. Similar findings were reported by Lau *et al*. (2012) whereby the roasted sky fruit seed had highest value of peroxide (3.25 $mEqO_2$/kg) and followed by microwaved (2.96 $mEqO_2$/kg), untreated (2.61 $mEqO_2$/kg) and steamed sky fruit seed oil (1.98 $mEqO_2$/kg). Similar finding also has been reported in roasted pumpkin oil (Potočnik *et al*., 2018). A high PV in roasted seed is due to the damaged of the structure of lipid storage cell (Lau *et al*., 2012). However, Lee *et al*. (2004) reported a decrease in PV of roasting safflower oil due to the non-enzymatic reaction and caramelization product formed during roasting.

The microwaved MMT seed oil also had higher PV compared to the untreated MMT seed oil. This finding is in agreement Lau *et al*. (2012) where the PV of microwaved sky fruit seed oil was also higher (2.96 mEq O_2/kg) compared to the untreated seed oil (2.61 mEq O_2/kg). Microwaved grape seed oil (Oomah *et al*., 1998) also gave similar trend. This is because of the reactive radicals formed during exposure of the seed to microwave energy (Uquiche *et al*., 2008).

Fatty acid composition

Table 2 shows no significant difference between all MMT seed oils in the present study ($p>0.05$). Fatty acid composition of MMT seed oils comprised of linoleic acid (60.85–60.89%), oleic acid (20.85–20.89%), palmitic acid (17.72–17.83%), cis-11-eicosanoic acid or gondoic acid (0.430–0.439%) and myristic acid (0.06–0.069%). Similar findings were reported by Silva *et al*. (2014), for melon seed oil where linoleic acid was the main fatty acid (52–69%), followed by oleic acid (12–32%) and palmitic acid (9–24%). Mallek-Ayadi *et al*. (2019) also reported the dominant fatty acids in *Cucumis melo*, Maazoun cultivar seed oil were linoleic acid (68.98%), oleic acid (15.84%) and palmitic acid (8.76%). A high content of linoleic acid may have favourable nutritional implications and beneficial physiological effect in the prevention of coronary heart disease and cancer (Oomah *et al*., 2000).

The MMT seed oils in the present study contained 17.78–17.89% total saturated fatty acid, 21.28–21.325% monounsaturated fatty acid and 60.85–60.89% polyunsaturated fatty acid. The ratio of unsaturated fatty acid to saturated fatty acid for MMT seed oils ranged from 3.75 to 4.06. A high

Table 2. Fatty acid composition of Melon Manis Terengganu seed oil as affected by different heat pretreatments of seeds (%)

Heat pretreatment	Untreated	Microwaving	Roasting	Steaming
Myristic acid C14:0	0.06 ± 0.02	0.069 ± 0.01	0.064 ± 0.05	0.060 ± 0.01
Palmitic acid C16:0	17.74 ± 0.09	17.78 ± 0.04	17.83 ± 0.021	17.72 ± 0.05
Oleic acid C18:1	20.87 ± 0.01	20.85 ± 0.06	20.85 ± 0.02	20.89 ± 0.05
Linoleic acid C18:2	60.89 ± 0.021	60.87 ± 0.02	60.85 ± 0.04	60.89 ± 0.07
Gondoic acid C20:1	0.439 ± 0.02	0.434 ± 0.05	0.430 ± 0.02	0.435 ± 0.09
Total saturated fatty acid	17.8	17.85	17.89	17.78
MUFA	21.31	21.28	21.28	21.325
Total unsaturated fatty acid	72.26	69.87	67.14	71.56
Unsaturated FA / saturated FA	4.06	3.91	3.75	4.02

Table 3. Antioxidant activities of Melon Manis Terengganu seed oil as affected by different heat pretreatments of seeds

Heat pretreatment	DPPH free radical scavenging activity (%)	Total Phenolic content (mg GAE/g)	Ferric reducing antioxidant power (mmol Fe^{2+} /100 ml)
Untreated	56.08 ± 0.87^{a}	0.109 ± 0.002^{c}	0.110 ± 0.0047^{a}
Microwaving	45.95 ± 0.76^{b}	0.135 ± 0.003^{b}	0.0723 ± 0.0047^{d}
Roasting	0.55^{c}	0.142 ± 0.002^{a}	0.0803 ± 0.0015^{c}
Steaming	54.64 ± 0.49^{a}	0.088 ± 0.002^{d}	0.0930 ± 0.0036^{b}

Values are mean ± standard deviation from three replications (n=3).
Values with different superscript letter in the same column are significantly different at $p<0.05$.

content of unsaturated fatty acid may lead to more lipid oxidation during storage as compared to saturated fatty acid. The ratio of unsaturated fatty acid to saturated fatty acid for *Cucumis melo* (Mazoun cultivar) seed oil (5.79) was higher than MMT seed oil (Mallek-Ayadi *et al*., 2019). Meanwhile, honeydew melon seed oil contained a lower PUFA (59.20%) and total saturated fatty acid (15.70%) and higher MUFA (25%) (Górnaś & Rudzińska, 2016) compared to the MMT seed oils.

According Silva *et al*. (2018), the fatty acid profile of melon seeds oil is very similar to soybean and sunflower oils, two of the most commonly used vegetable oils. It is also very analogous to grape seeds oil and paprika seeds oil, although grape seeds oil has a lower content of saturated fatty acids. Silva *et al*. (2018) also stated that the content of linoleic acid in melon seeds oil is very similar to blackcurrant and watermelon seeds oils.

Effect of heat pretreatment on antioxidant properties of MMT seed oil

Total phenolic content

Table 3 shows the antioxidant activities of Melon Manis Terengganu seed oil as affected by different heat pretreatments of seeds. Table 3 shows that there was a significant difference in total phenolic content (TPC) in the MMT seed oils for all heat pretreatments ($p<0.05$). Roasted MMT seed oil contained the highest TPC (0.142 mg GAE/g). This finding is consistent with Mazaheri *et al*. (2019) where the roasted black seed oil contained a higher amount of caffeic acid (4812.77 mg caffeic acid/kg of oil) compared to the unroasted seed (4527.90 mg caffeic acid /kg of oil). The decreasing in caffeic content due to thermal decarboxylation occur during black seed heat pretreatment (Yang *et al*., 2014). The reduction of moisture content occurred when the seeds were roasted and the increase of total phenolic content (Azhari *et al*., 2014). This is because, denaturation of protein is linked to the phenolic compound which resulted in easier extraction (Yang *et al*., 2014).

The microwaved MMT seed oil also contained higher TPC (0.135 mg GAE/g) compared to untreated and steamed MMT seed oils. Similar findings were reported by Mazaheri *et al*. (2014), where the TPC of black seed oil increased from 3546.58 to 6889.02 (mg caffeic acid /kg of oil) when the sample was heated by microwave. Mazaheri *et al*. (2014) stated that the increasing amount of microwave irradiation had a positive effect on the extraction of phenolic compound.

The total phenolic content for steamed MMT seed oil were the lowest which was only 0.088 mg GAE/g. High temperature with long drying time could destroy the phenols and decreasing water presents. The antioxidant values were reduced because it associated with thermal degradation of phytochemicals, enzymatic degradation of phenolic compounds and loss of antioxidant enzymes

activities (Nyam *et al*., 2009b). Total phenolic content of bitter melon (0.32 mg GAE/g), kalahari melon (0.23 mg GAE/g) and kenaf (0.24 mg GAE/g) seed oils were higher compared to that of MMT in the present study.

DPPH radical scavenging activity

Table 3 shows that the untreated MMT seed oil (56.08%) had the highest DPPH free radical scavenging activity, followed by steamed (54.64%), microwaved (45.95%) and roasted (43.06%) MMT seed oils. This shows that the free radical scavenging capacities of microwaved and roasted MMT seed oil were significantly reduced due to heating process. Heating has resulted in release of phenolic compound from the cell wall that affects the bioaccessibility of total phenolics. Muhammad *et al*. (2018) reported a radical scavenging activity of melon seed oils extracted using different solvents extraction (methanol, ethanol and distilled water) ranging from 23.38% to 50.36%.

Ferric reducing antioxidant power (FRAP)

Table 3 shows that MMT seed oil gave low FRAP activity (0.0723 - 0.110 mmol Fe^{2+}/100 ml). This study found that the highest FRAP activity was given by MMT seed oil from untreated > steaming > roasting > microwaving. According to Abril *et al*. (2019), heating can cause a decreasing in antioxidant activity in the seed oil. However, Muhammad *et al*. (2018) reported that MMT sample that was dried at 60 exhibited the highest value of FRAP value (49.30%) and similar with bitter guard melon. This is because polyphenol oxidase activity was fully activated at higher temperature (60 and thus the oxidation product from polyphenol oxidase contributed to the increased antioxidant capacities.

CONCLUSION

It was found that different heat pretreatments of MMT seeds significantly affected the oil yield, free fatty acid value, DPPH radical scavenging activity, total phenolic content and ferric reducing antioxidant power (FRAP) ($p<0.05$). However, the seed heat pretreatment did not affect the fatty acid composition significantly ($p>0.05$). Among the four pretreatments, the preferable treatments were microwave and roasting. Microwave gave the highest yield and the lowest free fatty acid content, while roasting also gave the highest yield and the highest total phenolic content.

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