THE INFLUENCE OF MICROWAVE COOKING ON THE NUTRITIONAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE UNDERUTILIZED PERAH SEED

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ABSTRACT

Background. Perah seed is one of the most underutilized oilseeds, containing high nutritional values and high percentage of α-linoleneic acid, which may have a high potential in food and pharmaceutical applications. The main objective of this study was to evaluate the influence of microwave (MW) cooking on the proximate composition and antioxidant activity of perah seeds.

Material and methods. In this study, the proximate composition and amygdalin concentration of MW irradiated perah seeds were determined. The total phenolic content (TPC), Maillard reaction products (MRPs) and antioxidant activity of methanol (PME), 70% methanol in water (PMW), ethanol (PEE), 70% ethanol in water (PEW) extracts and methanol extract of oil (PMO) were evaluated during MW cooking. The antioxidant activity was evaluated using multiple assays, namely DPPH radical scavenging activity, β-Carotene bleaching assay, and reducing power.

Results. Microwave cooking did not significantly increase crude lipid and carbohydrate content, and the amounts of other nutrients such as ash, crude protein and fibre remained almost unchanged. As evaluated by HPLC, the amygdalin concentration in the seeds was reduced by MW cooking. The TPC, MRP and antioxidant activity of the solvent extracts of perah seeds increased significantly with increasing roasting time. Of all the extracts, PMW at all MW cooking times displayed the highest antioxidant effectiveness. However, thermal treatment significantly reduced the antioxidant properties of PMO. The values for TPC, MRP and antioxidant effectiveness of the samples were ranked in the following order: PMW > PEW > PME > PEE > PMO, in both control and microwaved samples.

Conclusions. In determining the overall quality of the products, MW cooking time was found to be a critical factor. Solubilization of phenolic compounds and formation of MRPs during MW cooking could have caused the increase in antioxidant activity of the perah seeds.

Keywords: nutritional composition, antioxidant activity, microwave cooking and perah seed

INTRODUCTION

Perah (Elateriospermum tapos) seed is a type of seed that can be easily grown and is found in abundance, especially on the east coast of Malaysia and in southern Thailand. The perah fruits are about 2–2.5 in long, oblong, buff in colour and suffused pink on the exposed side, hanging singly on a stalk 1–6 in long. The perah...
seeds are about 1.75 in long and shiny and brown in
colour, with a faint ridge on each side (Corner, 1989).
Amygdalin, a type of cyanogenic glycoside, has been
detected in perah seeds. The amount of amygdalin was
much higher in fresh seeds (660 ppm) than cooked
seeds (100 ppm) and fermented seeds (25 ppm). It is
clearly shown that heat and fermentation can reduce
the amount of amygdalin in the edible seeds (Ngam-
riabsakul and Kommen, 2009). Inhabitants of this re-
gion consume perah seeds in cooked form (boiled or
roasted; Yong and Salimon, 2006). Yong and Salimon
(2006) stated that perah seed oil has the potential to
be developed for the food, pharmaceutical and oleo-
chemical industries.

The effects of various processing methods used to
prepare oilseeds for human consumption are an im-
portant subject of investigation. Roasting is a heat
treatment used to induce the development of the
typical colour, taste and flavour; it also changes the
chemical composition, modifying nutritional value
and shelf life (Ozdemir and Devres, 2000). However,
microwave cooking is a fairly recent development,
which is gaining momentum in households and es-
pecially in restaurants for its speed, convenience and
efficiency compared to conventional heating methods
(Behera et al., 2004). Tenyang et al. (2017) stated
that the proximate composition of foods was signifi-
cantly affected during roasted. Jogihalli et al. (2017)
concluded that a significant increase in total phenolic
content and antioxidant activity was observed af-
fter roasting chickpea seeds. Ali et al. (2016a) stated
that antioxidant activity increased with increasing
roasting time and extract concentration of groundnut
seeds. Baba et al. (2016) reported that microwave
cooking significantly affected the antioxidant and
anti-cancerous activities of barley. Wani et al. (2016)
found that TPC and antioxidant activity of arrowhead
was enhanced by microwave cooking. Mariod et al.
(2012) stated that microwave cooking improves the
nutritional value of safflower seeds by increasing to-
tal, and some individual, amino acids. To our knowl-
edge, there has never been a study on the effect of microwave cooking on the anti-oxidative activity and chemical composition of seeds of the perah cultivar
grown in Malaysia. The present work was therefore
carried out to investigate the effect of microwave cooking on the nutritional composition, amygdalin
concentration and antioxidant activity of the underu-
tilized perah seed.

MATERIAL AND METHODS

Materials
Freshly harvested and dried perah seeds (500 g, Ela-
teriospermum tapos Blume) were purchased from
the local market at Benta, Pahang, in August 2014.
Mature, healthy seeds were sorted, and stored at 4°C
in sealed plastic bags. The various chemicals and
reagents used were of analytical grade and were pur-
chased from J.T. Baker (Phillipsburg, USA), Merck
(Darmstadt, Germany) or Sigma Chemical Co. (St.
Louis, MO, USA).

Roasting protocol
A domestic-size microwave oven ((Model NN-
ST65IM, Panasonic Co. Ltd., China) at 2450 MHz,
with a power output of 900 W, was used. The perah
seeds were arranged in a single layer in a Pyrex petri
dish (12 cm diameter) and heated at a medium power
setting, for different periods (1, 2, and 3 min) after
covering the dish based on trial results. The final seed
temperatures at various MW cooking times were meas-
ured by inserting a calibrated thermocouple (Model HI
9043, Hanna Instruments Ltd., Bedfordshire, UK) into
the seeds immediately after removal from the oven.
After MW cooking, the seeds were allowed to cool to
ambient temperature. The seed coats were manually
removed from both control and roasted seeds, and the
seeds were ground to fine powder.

Proximate analysis of perah seeds
The moisture content was determined using a mois-
ture content analyzer (FD-620, Kett, CA, USA). The
crude lipid, ash and fibre contents were determined
following the standard methods of the Association of
Official Analytical Chemists (AOAC, 1990). The or-
ganic nitrogen content was quantified using the micro
Kjeldahl method, and an estimate of the crude pro-
tein content was estimated by multiplying the organic
nitrogen content by a factor of 6.25 (AOAC, 1990).
Total carbohydrate content was calculated by differ-
ence. Energy was calculated using energy conversion
factors of 4.0, 4.0 and 9.0 kcal/100 g for protein, car-
bohydrate and fat respectively.
Amygdalin concentration by HPLC

Chromatographic determination of amygdalin was carried out on a 0.2 g perah seed sample, according to the method described elsewhere (Dicenta et al., 2002; Ngamriabsakul and Kommen, 2009). The sample was extracted with 10 ml of methanol for 12 h at room temperature in the presence of 0.1 g of polyvinylpolypyrrolidone. Chromatographic separation was performed by HPLC system (PerkinElmer Series 200, PerkinElmer Life and Analytical Sciences, Connecticut, USA) furnished with a packed RP-18 column (250 × 4 mm) and a detector under UV at 218 nm using acetonitrile:water (20:80) as mobile phase at a flow rate of 0.8 ml/min. The cyanogenic glycoside was identified by comparing the retention time with that of the authentic accepted standards, amygdalin (D-mandelonitrile-β-D-gentiobioside). Quantification was based on an external standard method, where the calibration curve ranged from 2 to 15 mg/l of the reference compound, amygdalin.

Solvent extraction for antioxidant evaluation

The Soxhlet method, as described by AOAC (1990), was used to extract oil with n-hexane for 8 h. After extraction, the oil in the hexane mixture was filtered, followed by evaporation of the solvent in vacuo at 45°C. Extraction was carried out separately with methanol (PME), 70% methanol in water (PMW), ethanol (PEE), and 70% ethanol in water (PEW) from the de-fatted seeds left after extraction with n-hexane, using a shaking incubator (ST-250D, SASTEC, Selangor, Malaysia) at room temperature for 24 h. The solvent was removed from the extract by the same procedure as described earlier. The oil and other solvent extracts were weighed to calculate the yield and stored in glass containers at –16°C prior to further analysis.

Heat treatment of oil samples

The cooked or control oil samples (80 g) were weighed into 100 ml glass beakers and placed in an electric oven at 170°C, in order to accelerate the lipid oxidation and thermal degradation. Oil samples were extracted at intervals of 0, 4, 8, and 12 h and flushed with nitrogen, covered with parafilm and kept at –16°C for further analysis.

Methanolic extract of oil sample

Methanolic extracts of the oil (PMO) sample were obtained after heat treatment, and prepared according to the method of Durmaz and Gökmén (2011). One gram of oil sample and 1 ml 70% methanol were mixed in an Eppendorf tube and agitated with a vortex mixer for 1 min. Samples were centrifuged at 3000 rpm for 5 min and the upper methanolic phase was extracted. One millilitre of fresh 70% methanol was added to the extraction. This was repeated three times, upper phases were combined and washed with three portions of 2 ml of n-hexane to remove oil residues. The final methanolic solution was diluted to achieve the desired concentration for antioxidant evaluation.

Antioxidant activity assay

Total phenolic content (TPC). The TPC of the sample was determined using the method of Gutfinger (1981). The sample (1 mL, 1 mg/ml) was mixed with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na₂CO₃, and centrifuged at 13 400 × g for 5 min. The absorbance of the upper phase was measured using a spectrophotometer (Jenway 7305, Spectrophotometer, Scientific Instrument Services, NJ, USA) at 750 nm after 30 min incubation at room temperature. Tannic acid was used as the reference standard, and the results were expressed as mg tannic acid equivalents TAE/g defatted extract or mg TAE/g oil.

DPPH (1-diphenyl-2-picrylhydrazyl) radical scavenging activity. The radical scavenging activity was determined following the method of Negro et al. (2003). One millilitre of sample solution was thoroughly mixed with 4 mL of DPPH (0.1 mmol/l) solution, and allowed to stand for 30 min in the dark. The control solution contained equivalent 70% methanol, instead of the sample solution. The absorbance was computed at 517 nm against a blank control without the sample. DPPH free radical-scavenging activity was calculated according to the following equation:

\[
\text{DPPH radical-scavenging activity, } \% = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100
\]

β-Carotene bleaching assay. The β-carotene bleaching assay was performed as described by Durmaz and Alpaslan (2007). In brief, a 2 ml solution of β-carotene was...
in chloroform (0.2 mg/ml) was added to 40 mg of linoleic acid and 400 mg of Tween-20. The chloroform was evaporated in vacuo at 45°C for 4 min, and 100 ml distilled water was added with vigorous agitation to form an emulsion. 3 ml of this emulsion was added to each tube containing 50 µl samples of oil. The tubes were incubated in a water bath at 50°C for 120 min. Absorbance was measured at 470 nm, immediately, against a blank consisting of the emulsion without β-carotene. Antioxidant activity (AA), which was expressed as the delay in the bleaching of β-carotene, was determined using the following formula: \( AA = 100 \left(1 - \frac{(A_t - A_o) \times A_o}{(A_o - A_t)}\right) \); where \(A_o\) and \(A_t\) represent the absorbance values measured at zero time of incubation for the test sample and the control respectively. The \(A_t\) and \(A_o\) represent the absorbance measured in the test sample and the control respectively, after incubation for 120 min.

Reducing power. The reducing power of the extracts was determined according to the method of Atmani et al. (2009). One millilitre of sample (1 mg/ml), 2 ml phosphate buffer (0.2 M, pH 6.6), and 2.5 ml potassium ferricyanide (1%, w/v) were mixed and incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture. A portion of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and then the absorbance was measured at 700 nm at a reaction time of 30 min.

Maillard reaction products (MRPs). The amount of MRPs was evaluated according to the non-enzymatic browning measurement (Kim et al., 2011). The sample was diluted to 1:100 with distilled water to give absorbance signals on a scale. The samples were allowed to stand for 1 h and filtered using a 0.45 µm filter, prior to measuring optical density using a spectrophotometer (Jenway 7305, Scientific Instrument Services, NJ, USA) at 420 nm.

Statistical analysis
All data were expressed as the mean and standard deviation (SD) and were subjected to one way analysis of variance (ANOVA). Mean values were compared at \(p < 0.05\) significant level by Duncan’s multiple range test using IBM SPSS 22 statistics.

RESULTS AND DISCUSSION

Changes in proximate composition
Microwave cooking had a remarkable effect on the levels of moisture in all the samples. As shown in Table 1, with increasing MW cooking time, the moisture content of perah seeds was found to decrease. The results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Microwave cooking, min</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>18.16 ±0.46c</td>
</tr>
<tr>
<td>Crude lipid, %</td>
<td>33.71 ±0.47a</td>
</tr>
<tr>
<td>Ash, %</td>
<td>3.39 ±0.10a</td>
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<tr>
<td>Crude protein, % N</td>
<td>29.33 ±0.17a</td>
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<tr>
<td>Crude fibre, %</td>
<td>9.39 ±0.02a</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>6.03 ±1.14a</td>
</tr>
<tr>
<td>Energy, kcal/100 g</td>
<td>444.80 ±0.24a</td>
</tr>
<tr>
<td>Amygdalin concentration, ppm</td>
<td>139.66 ±0.43a</td>
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</table>

Each value in the table represents the mean of three replicates ±SD. Means followed by different letters in the same row of individual parameter present significant difference (\(p < 0.05\)).
are in agreement with the findings reported by Ali et al. (2016b) for pumpkin seeds. However, the amount of moisture in the control sample significantly reduced from 18.16% to 12.16% after 3 min MW cooking. The crude lipid content increased insignificantly for the first 2 min of MW cooking, then increased significantly with further increasing cooking time (Table 1). The sample which was microwaved for 3 min contained the highest amount (35.62%) of crude lipid compared to the raw sample (33.71%). The obtained results concur with previously published results by some authors (Ali et al., 2016b; Jau-Tien et al., 2016) who reported that the lipid content increased as a result of roasting the seeds. Exposure to microwave radiation decreases seed moisture content, which directly influences the amount of lipid extracted from the seeds. Moreover, MW cooking ruptures cell membranes, which enables oil to move through the permeable cell walls more easily (Takagi et al., 1999). The amounts of ash, crude protein, and fibre in MW roasted perah seed samples were found to be almost the same to those of the control sample (Table 1). The present results are in agreement with the results reported by Anjum et al. (2006) for sunflower seeds. However, the ash, crude protein and fibre content were in the range of 3.33–3.39%, 29.18–29.33%, and 9.37–9.48% respectively. The minor reduction in protein content may be linked to the denaturation of nitrogenous compounds during MW cooking (Jain et al., 2016). The carbohydrate and energy contents of perah seeds are reported in Table 1. Fresh perah seeds contained 6.03% carbohydrate, which increased to 10.33% after 3 min MW cooking. In fact, this value was highly dependent on the moisture, ash, protein, fat and fibre contents of the seed, since carbohydrate content was measured by difference of dry weight of seeds to them. The amount of energy in microwaved perah seed samples was higher than control samples. The energy value of unroasted perah seeds was 444.80 kcal/100 g, which rose to 445.84, 455.28 and 478.66 kcal/100 g respectively for 1-, 2- and 3-min microwaved samples. The higher energy content is a result of the higher fat content after MW cooking.

**Changes in amygdalin concentration**

Under chromatographic conditions, a symmetrical chromatographic peak for obtained was standard amygdaline eluted at a retention time of 1.55 min. Changes of amygdalin concentration with MW cooking times in perah seeds are presented in Table 1. During MW cooking, perah seed samples showed a clear decrease in amygdalin concentration. This might be explained by the effect of heating and drying during the MW cooking process, which reduced the amount of amygdalin in the seeds. Amygdalin and other cyanogenic glycosides easily release the cyanide group upon hydrolysis (Dicenta et al., 2002). The amount of amygdalin significantly reduced from 139.66 ppm in uncooked perah seeds to 83.72 ppm in those MW cooked for 3 mins. Ngamriabsakul and Kommen (2009) also reported that heating and fermentation reduced the amount of amygdalin in perah seeds.

**Changes in antioxidant activity**

Microwave cooking had a significant effect on the level of TPC in all extracts; the amounts of TPC were increased by the MW cooking process (Fig. 1a and 1b). The levels of TPC in control sample were lower, ranging from 3.46 mg TAE/g (PMO) to 6.34 mg TAE/g (PMW), compared with MW roasted (at 3 min) sample values that ranged from 4.52 mg TAE/g (PMO) to 8.94 mg TAE/g (PMW). After 3 min MW cooking, the samples showed a TPC of 4.52 mg TAE/g or more: PMW (8.94 mg TAE/g) > PEW (7.54 mg TAE/g) > PME (6.42 mg TAE/g) > PEE (5.70 mg TAE/g) > PMO (4.52 mg TAE/g). A similar trend was found by Ali et al. (2016a) for microwaved groundnut, and by Win et al. (2011) for oven-roasted peanut. The roasting process most likely increased the TPC through the following mechanisms: roasting might partially destroy the cell structure, resulting in the release of some bound phenolic compounds, which could then become more extractable in aqueous ethanol and acetone (Zou et al., 2015). In case of PMO, TPC contents significantly (p < 0.05) decreased with increasing heating time and reached to 3.69 mg TAE/g after 12 h heating of the 3 min MW cooked sample (Fig. 1b). Herchi et al. (2016) also confirmed that the heating process caused a loss of phenolic acid content in flaxseed hull oil. This might confirm that thermal treatment causes the oxidation and polymerization of phenolic compounds present in the oil. Figure 1c and 1d illustrate a significant (p < 0.05) increase in DPPH radical-scavenging effect as the MW cooking time of seeds increased. The extracts
PMO, PEE, PME, PEW and PMW from control seeds showed 50.48, 52.89, 55.26, 57.03 and 66.34% DPPH scavenging activity respectively, whereas the same solvent extracts from the samples MW roasted for 3 min showed 60.61, 67.06, 69.64, 72.78, and 78.04% activity respectively. Similar results were also reported for arrowhead by Wani et al. (2016). In this work, PMW exhibited higher DPPH scavenging activities than the other solvent extracts. In general, the methanol extract exhibited higher DPPH scavenging activity than the other solvent extracts, which might be due to a greater release of antioxidants (Baba et al., 2016). During thermal treatment, the scavenging activity of PMO decreased significantly (p < 0.05) from 60.61 to 41.29% in the 3-min MW cooked PMO sample. Hertchi et al. (2016) concluded that heated flaxseed hull oil contained fewer antioxidant compounds, which decreased the DPPH radical scavenging capacity. The scavenging action of plant constituents has been found to relate to polyphenolic compounds (Siger et al., 2008).

The assay of β-carotene bleaching is dependent on the loss of the yellow colour of β-carotene, due to its interaction with radicals created by linoleic acid oxidation in an emulsion (Nanasombat and Wimittoesol, 2011). In the β-carotene/linoleic acid assay, the oxidation of linoleic acid generates peroxyl free radicals, which will then oxidize the highly unsaturated fatty acids, which means the presence of antioxidants will minimize the oxidation of β-carotene compounds. The β-carotene bleaching assay values of the control and MW cooked perah seeds were determined and shown in Figure 2a and 2b. Inhibition of β-carotene and linoleic acid oxidation ranged from 0% to 80%.

28.48, 50.43, 54.98, 53.59 and 55.58% in the control to 39.97, 61.49, 65.76, 66.49 and 70.23% in the 3-min MW cooked samples of the PMO, PEE, PME, PEW and PMW extracts respectively. Durmaz et al. (2010) stated that, in the β-carotene linoleate assay, the increase in protection of β-carotene from bleaching was attenuated after 20 min of roasting but slightly decreased for the 30-min roasted sample from apricot kernel oil. Moreover, the values decreased steadily as heating time of the PMO sample increased and, after 12 h of heating, it reached to 30.94 for the 3-min MW cooked sample (Fig. 2b). As shown in Figure 2c and 2d, the MW cooking of perah seeds significantly (**p < 0.05**) increased reducing power. This increase in reducing power may be attributed to the production of Maillard reaction products, which contributed to antioxidant properties after cooking (Woffenden et al., 2002). During MW cooking, the absorbance at 700 nm increased from 0.37 to 0.58 for PMO, 0.52 to 0.57 for PEE, 0.55 to 0.59 for PME, 0.70 to 0.81 for PEW, and 0.76 to 0.89 for PMW. Microwave cooking enhanced the reducing power of arrowhead (Wani et al., 2016). Data also revealed that reducing power was roasting- or heating time-dependent, and increasing the heating time at 170°C led to a significant decrease in reducing power, in the control and roasted sample of PMO. The PMW sample roasted for 3 min displayed the highest value, whereas the lowest value was detected in the control samples of PMO. The MRPs, which form as a consequence of intense heat treatment, are found in the molecular weight range of <30 kDa, and they generally have strong antioxidant properties (Manzocco et al., 2000; Summa et al., 2006). In this work, non-enzymatic browning measurements were performed at 420 nm to evaluate the degree of roasting and MRPs in perah seeds. According to the other antioxidative
assays, as stated, the MW cooking process enhanced the antioxidant activity of perah seed extracts. Non-enzymatic browning showed the same pattern under the same conditions. The values of optical density at 420 nm significantly (p < 0.05) increased from 0.98, 1.19, 1.27, 1.29, and 1.33 in the uncooked control to 1.12, 1.86, 1.89, 1.94, and 2.00 in the 3-min MW cooked samples of PMO, PEE, PME, PEW and PMW respectively. Kim et al. (2011) reported that heat treatment was effective in increasing the degree of browning in small black soybean. In addition, the optical density of PMO was significantly (p < 0.05) reduced by thermal treatment, and this decrease gradually and significantly increased with increasing heating time at 170°C. At the end of 12 h of thermal treatment, the optical density in PMO reduced to 0.55 for the 3-min MW cooked sample of PMO.

CONCLUSIONS

The MW cooking process was not as effective in increasing the nutritional quality of perah seeds as was suggested by determining ash, crude protein and fibre. MW cooking substantially enhanced the TPC and antioxidant activity of the perah seed flour and in this regard, MRPs formed during the cooking process are able to contribute to antioxidant activity. Moreover, MRPs might lead to an increase in the amount of total phenolics or phenolic-like complexes that further contribute to higher absorbance readings measured by the Folin assay. The duration of microwave cooking also affected the total phenolic levels in the extracts, which led to changes in their antioxidant activities. The thermal treatment of oil at 170°C significantly reduced antioxidative properties. In the present study, MW cooking caused a significant reduction of the amygdalin concentration in perah seeds, which may result in the seeds being more suitable for human consumption, as uncooked seeds taste bad and can cause dizziness. The aqueous methanol extract of microwaved perah seeds are a potent source of antioxidant agents compared to other solvent extracts. Therefore, the perah seeds can be considered a good source of important bioactive constituents which are beneficial to human health.

REFERENCES


Fig. 3. Changes in Maillard reaction products of PEE, PME, PEW, PMW extracts (a) and PMO (b) extracts from control (MW-0) and microwave cooked (MW-1, microwave cooked for 1 min; MW-2, microwave cooked for 2 min and MW-3, microwave cooked for 3 min) perah seeds. Each value is the mean ± standard deviation of triplicate determinations. Values in each solvent extract or heating time grouped with different letters on the bar are significantly different (p < 0.05)


