Effects of osmotic dehydration treatment on volatile compound (Myristicin) content and antioxidants property of nutmeg (*Myristica fragrans*) pericarp

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Abstract The effects of osmotic dehydration (OD) treatment on volatile compound (myristicin) content and the antioxidant capacity of nutmeg (*Myristica fragrans*) were studied. Fresh nutmeg pericarps were treated with varying sugar concentrations (60, 70, 80%) with different soaking periods at ambient temperature. The OD-treated nutmeg extracts were analyzed for myristicin content via Gas Chromatography Flame Ionization Detector. The phenolic content and antioxidant capacity were analyzed using Follin–Ciocalteu and a free radical scavenging activity assay. The myristicin content was highest (1.69 mg/100 mg) at 80% sugar concentration after 3 h of soaking. Total phenolic content and free radical scavenging activity were highest at 3 h of 80% sugar solution treatment with values of 76.90% and 1.75 mg GAE/g, respectively. OD treatment at varying sugar concentration levels and durations affects the production of myristicin and antioxidant composition. Treatment of nutmeg with OD at 80% sugar concentration for 3 h is preferable, resulting in an acceptable level of myristicin and high antioxidants.

Keywords Nutmeg · Myristicin · Solid phase extraction · Ultrasonic extraction · Total phenolic content

Abbreviations

OD	Osmotic dehydration
TPC	Total phenolic content
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GC-FID	Gas chromatography flame ionization detector
GAE	Gallic acid equivalent

Introduction

Nutmeg (*Myristica fragrans*) is known for its medicinal value and is one of the signature fruit of Penang. This fruit possesses strong antioxidant characteristics and finds use in antifungal, antimicrobial, anti-inflammatory, and hepatoprotective applications (Gupta et al. 2013). Despite its popularity, nutmeg is classified as an underutilized fruit, due to the production exceeds the demand. Fresh nutmeg fruit is less favored for consumption among Malaysians as it has a sour and strong taste (Hewage and Vithana 2013). The shelf-life of nutmeg is improved by processing it into a more stable and palatable product, such as pickled nutmeg. Processing pickled nutmeg involves the immersion of fruit in hypertonic solutions (salt and sugar solution), which is also known as osmotic dehydration process (OD) (Nurul and Asmah 2012).

Osmotic Dehydration or OD is one of the best methods to convert unstable and deteriorating fresh nutmeg into nutmeg with a stable shelf-life. OD is applied by the immersion of nutmeg in hypertonic medium [sugar (sucrose) and sodium chloride solution], after which the transfer of solutes within nutmeg and the medium solution takes place (Nurul and Asmah 2012). The deterioration rate can be reduced using OD, limiting the amount of water inside the fruit and consequently reducing water activity (Devic et al. 2010; Torres et al. 2007). OD involves the

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activity of partial water removal from the nutmeg. As the cell membranes are semi-permeable, they allow water to pass through the pericarp section of the nutmeg (Chavan and Amarowicz 2012).

OD has become one of the popular methods for nutmeg preservation due to the ability to retain the taste and nutritional value of nutmeg with minimal processing (Najafi et al. 2014). Changes of chemical properties especially volatile compounds (myristicin) and antioxidant capacity of nutmeg will occur after the OD process. However, according to Torres et al. (2007) and Almeida et al. (2014), the immersion of nutmeg pericarp in hypertonic solutions may trigger and enhance the production of myristicin (5-allyl-1-methoxy-2,3-methylenedioxybenzene) due to the osmotic stress that occurs within the cell tissue of the fruit while reducing the antioxidant activity. Nevertheless, in recent years, consumers have begun opting for food with high antioxidant values, which is contradict with the outcome of OD treatment.

Myristicin is a psychoactive compound and is believed to be the compound associated with the effect of hallucinogenic (Quin et al. 1998). The psychoactivity of nutmeg is due to the metabolic conversion of elemicin and myristicin into amphetamine-like compounds (Gupta et al. 2013). The doses typically vary between 5 and 30 g of nutmeg, and may cause hallucination and unconsciousness (Dawidowicz and Dybowski 2013). Such adverse effects are well reported in literature over the past century (Ehrenpreis et al. 2014; Carstairs and Cantrell 2011). Thus, it is crucial to evaluate and to reduce the amount of myristicin in nutmeg, along the food processing to produce nutmeg as a food product which is safe to eat.

Therefore, the assessment of myristicin content and antioxidant capacity in nutmeg before and after OD treatment is crucial for the future development of processing nutmeg, as there is limited literature related to the OD processing of nutmeg compared to other fruits. Thus, in this study, the myristicin content and antioxidant capacity of nutmeg related to the treatment of osmotic dehydration was evaluated. The effects of different concentrations and soaking time on the antioxidant capacity of nutmeg were also examined.

Materials and methods

Chemicals

The analytical standard of myristicin was supplied by Fluka (Buchs, Switzerland). Sucrose, sodium chloride, hexane, methanol, Follin–Ciocalteu's, sodium carbonate, gallic acid, and 1,1-diphenyl-2-picryl-hydrazyl (DPPH)

was purchased from Sigma Chemical Co. (St., Louis, USA). All chemicals used were analytical grade.

Sample preparation

Fresh nutmeg fruits (immature nutmeg) were purchased from the local Chowrasta Market, Penang. The whole nutmeg fruits were washed and the skins were manually peeled with a knife, while the pericarp and seed were separated. The skin and the seed were removed, while the pericarp was sliced into small pieces ($1 \times 1 \times 1.5 \text{ cm}^3$) prior to further processing. Three different concentrations of aqueous sugar solution (60, 70, 80%) were used as the osmotic dehydration media. These concentrations were selected based on the preliminary analysis of sensory test (results not shown). The sugar solution was prepared by dissolving 60, 70, and 80 g of sugar in water and made up to 100 mL, respectively, at 70 °C to ensure complete dissolution. The OD-treated nutmeg was prepared by soaking the nutmeg pericarp in sugar solutions at varying levels of sugar (60, 70, 80%) and times (3, 6, 9, 12, 15 h) prior to further analysis.

Myristicin content

Sample extraction: ultrasonic extraction (USE)

The ultrasonic solvent extraction procedure was performed according to the procedure of Dawidowicz and Dybowski (2012) with slight modification. An amount of 200 mg/kg of nutmeg pericarp was added to 1250 μL methanol in a glass vial and sonicated in an ultrasonic bath (Elma D-78224 Singen/HTW, Germany), at 50 °C for 30 min. Methanol was used as an extracting solvent. Then, the glass vials were centrifuged at 3000 rpm for 10 min using a table-top centrifuge (Kubota Model 4000, Japan). A volume of 500 μL obtained supernatant was diluted with deionized water up to 2500 μL and subjected to SPE procedure.

Sample purification: solid phase extraction (SPE)

The SPE procedure was performed according to the procedure of Dawidowicz and Dybowski (2012) using an SPE cartridge packed with 0.5 g of SeptraC18-E and a combination of Agilent 10-port Vacuum Magnifold from the US. SPE cartridges were washed with 5 mL of n-hexane and vacuum-dried for 5 min. The n-hexane functioned as the extracting solvent in this method. Then, 2 mL of the sample extracts were passed through the preconditioned cartridge. The cartridges were washed again with 1 mL of the 20% methanol/water mixture. The retained analytes

were eluted into a calibrated flask with 5 mL n-hexane and analyzed by GC. The eluting solvents were passed through the sorbent bed at a flow rate of one drop per second (s^{-1}).

Quantitative determination of myristicin by gas-chromatographic (GC) analysis

The amount of myristicin component in the samples was determined according to Dawidowicz and Dybowski (2012) using GC with flame ionization detection (FID) (GC-2010 Plus, Shimadzu, Japan). A ZB5-MS fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ L film thickness; Phenomenex, USA) was used. Helium (grade 5.0) was used as a carrier gas. One micro liter of the sample was injected by an AOC-500 Plus type auto-sampler. The injector temperature was 310 °C. The following temperature increase up program was applied: 1 min at 50 °C followed by a linear temperature increase up to 250 °C at the rate of 6 °C/min. Qualitative analysis was carried out comparing the retention indices with the retention data from myristicin standard. The quantitative results of compounds examined were calculated using calibration curves. The applied GC-FID equipment was calibrated using myristicin standard solutions. The working solutions were obtained via serial dilutions of the stock solution with hexane to obtain the following myristicin concentrations: 0.05, 0.2, 0.4, 0.6 and 1 mg/100 mL.

Antioxidants capacity

Preparation of nutmeg extract

The sample extract was prepared by mixing 3 g of the fruit pericarp with 50 mL 80% methanol (v/v) in a conical flask which is wrapped with an aluminium foil and kept at room temperature overnight, shaken on an orbital shaker at 150 rpm according to the procedure of Tan (2013), with slight modification. The extract was filtered through Whatman filter paper no. 42 to obtain a clear solution.

Total phenolic content (TPC)

Total phenolic levels were determined by using Follin–Ciocalteu (FC) reagent according to the procedure of Tan (2013), with slight modification. A volume of 200 μ L samples extracts was introduced into test tubes followed by 1.5 mL of Follin–Ciocalteu's reagent (10 times dilution). The mixture was allowed to be at room temperature for 5 min. Next, 1.5 mL of sodium carbonate (7.5%, w/v) was added. The tubes were allowed to stand at room temperature for 30 min before measured using a UV–Vis spectrophotometer at an absorbance of 725 nm. A calibration standard curve was prepared using 25, 50, 100, 150, 200,

250, 300 and 500 mg/L solutions of gallic acid. The equation of standard curve was $y = 0.0062x$ ($R^2 = 0.99$), where y is the absorbance and x is the gallic acid concentration in mg/L (figure not shown). All tests were conducted in triplicate. The result was expressed as gallic acid equivalent in mg/g.

Free radical scavenging activity assay

The free radical-scavenging activity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Lima et al. 2011). Volumes of 300 μ L of the samples extract were added to 2 mL of DPPH (5.9 mg per 100 mL methanol). After 30 min at room temperature, absorbance was measured using a UV–VIS spectrophotometer at 515 nm. The decreased absorbance of the DPPH solution at $\lambda = 515$ nm indicates an increase in free radical scavenging activity (Ahmed et al. 2015). Triplicate measurements were carried out and their activity was calculated based on the percentage of DPPH discoloration.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences Software (SPSS, version 22.0, IBM). Two-way ANOVA was carried out and Duncan's new multiple range tests were used to determine the significant differences. Pearson correlation (r) was used to determine the correlation between various dependent variables. Significant differences were set at $p < 0.05$. All results were presented as mean $\pm$ standard deviation of triplicates.

Results and discussion

Prior to further analysis, the myristicin chromatographic peak was identified at a retention time of 22.70 min (Fig. 1). The identity of myristicin peak in the samples was confirmed by the chromatographic peak of the analytical myristicin standard and the calibration curve ($y = 7463x$) shows high linearity ($R^2 = 0.9948$) (Fig. 2).

The myristicin concentrations of osmotic dehydrated nutmeg pericarps at different soaking times were analyzed (Table 1). Fresh nutmeg was used as a control. The myristicin content of fresh nutmeg pericarp was 0.38 ± 0.01 mg/g prior to OD treatment. This increased after being treated with OD treatment based on the increased time. After 3 h of immersion time, the myristicin content was increased to 1.02 ± 0.01 , 1.54 ± 0.02 and 1.69 ± 0.03 for 60, 70 and 80% (g/v) of sugar solution,

Fig. 1 Myristicin standard retention time (min)

Fig. 2 The calibration plot of the myristicin standard

Table 1 The concentration of myristicin compound in nutmeg pericarp before and after undergoes osmotic dehydration (OD) treatment at different sugar concentration and different immersion time

Sample Immersion time (h)	Control Myristicin concentration (mg/100 g)	N60	N70	N80
0	0.38 ± 0.01^a	–	–	–
3	–	1.02 ± 0.01^{bA}	1.54 ± 0.02^{bB}	1.69 ± 0.03^{bB}
6	–	1.56 ± 0.01^{cA}	1.62 ± 0.01^{bcAB}	1.85 ± 0.04^{bcB}
9	–	1.68 ± 0.01^{dA}	1.77 ± 0.02^{cdA}	1.97 ± 0.01^{cdB}
12	–	1.83 ± 0.01^{eA}	1.86 ± 0.03^{dA}	2.09 ± 0.04^{cdA}
15	–	1.95 ± 0.01^{fA}	2.02 ± 0.02^{eAB}	2.11 ± 0.01^{eB}

* Values are given as mean with standard deviation (n = 3)

* ^{a–e} Values with a different superscript letter in the column are significantly different ($p < 0.05$) at each sugar concentration with different immersion time

* ^{A–B} Values with different superscript letter in the column are significantly different ($p < 0.05$) at each immersion time with different sugar concentrations

* Control indicates fresh nutmeg without osmotic dehydration treatment

* N60 indicates fresh nutmeg after osmotic dehydration treatment at 60% sugar solution

* N70 indicates fresh nutmeg after osmotic dehydration treatment at 70% sugar solution

* N80 indicates fresh nutmeg after osmotic dehydration treatment at 80% sugar solution

* Information about the treatment

respectively. However, at 15 h immersion time, the myristicin content of the nutmeg pericarp was 1.95 ± 0.01 , 2.02 ± 0.02 , and 2.11 ± 0.01 mg/100 g for 60, 70 and 80% of osmotic sugar solution, respectively. In addition, higher content of myristicin were obtained from nutmeg pericarp (1.7–2.1 mg/100 g) at 80% sugar concentration with different immersion times. Two-way ANOVA revealed that osmotic sugar concentration ($p < 0.05$) and time ($p < 0.05$) have a significant difference in the nutmeg pericarp myristicin content. There was also a significant difference ($p < 0.05$) in the interaction of osmotic sugar concentration and time with the myristicin content in nutmeg pericarp.

The increasing myristicin content in nutmeg pericarp at high sugar concentration was in agreement with the previous studies that have examined the effect of OD treatment on the chemical properties, such as the volatile profile of fruits sample. A number of studies have investigated the influences of osmotic process on volatile profile of fruits (Zhao et al. 2016; Chiralt and Talens 2005; Escriche et al. 2000; Scalzo et al. 2001). The findings of Zhao et al. (2016), Chiralt and Talens (2005) and Scalzo et al. (2001) showed that osmotic treatment causes an increase in ester compounds, which is an important contributor to the sense of fresh kiwi flavor and muskmelon. This leads to an increase in the enzyme system and the promotion of enzyme activity of the cells, due to the osmotic stress triggered the chemical changes in osmotic dehydration mechanism. The increased enzyme activity enhances the aroma development process (Kucner et al. 2013; Escriche et al. 2000). Myristicin, an aromatic compound in nutmeg,

is developed due to the promoted activity of alcohol acyl transferase enzymes (AAT). The AAT enzyme is well known as a key enzyme that played an important role in the formation of myristicin in nutmeg through esterification process and also responsible in the synthesizing of other secondary metabolites volatile esters (Chedgy et al. 2015). According to Gethins et al. (2015) and Engelberth et al. (2003), the AAT enzyme has been reported to have stress-related gene which will react and act as a defense response when plants are under stress, during which the formation of volatile compounds will be increased.

The effect of OD treatment was further evaluated for changes of antioxidant compound contents (Table 2). Generally, the yield and antioxidant activities of extracts depend on the different chemical characteristics and polarities of the extracting solvent. The selection of an appropriate solvent system is important in optimizing the recovery of antioxidant compounds from a given sample (Ahmed et al. 2015).

The solubility of phenolic compounds in the solvent has an impact on the recovery of polyphenols from extracts (Allothman et al. 2009). According to Sultana et al. (2009),

80% (v/v) of methanol may be used as an extracting solvent for recovery of polyphenol compounds from nutmeg matrix. Benmeziane et al. (2014) reported that the extraction of polyphenols in nutmeg was optimized by using 80% (v/v) of methanol compared to another solvent (acetone and ethanol) at the same concentration. Thus, 80% (v/v) methanol was preferable for use in solvent extraction for the antioxidants extracted in this study.

TPC and free radical scavenging activity assays were conducted to measure the total antioxidant activity, as the presence of other food components may affect the results (Patthamakanokporn et al. 2008). Table 2 shows the amount of antioxidant capacity of osmotic dehydrated nutmeg pericarp after osmotic treatment.

TPC of fresh and osmotic dehydrated nutmeg pericarp was analyzed due to their strong correlation with antioxidant activity (Yao et al. 2009). Total phenol levels of the extracts in terms of Gallic acid equivalent (GAE) (standard curve equation: $y = 0.0062x$, $R^2 = 0.99$ (figure not shown)) are shown in Table 2. TPC obtained from different concentrations of osmotic sugar solution varied in nutmeg pericarp with varying immersion times. Phenolic content in

Table 2 The amount of antioxidant capacity of nutmeg pericarp after undergoes osmotic dehydration (OD) treatment

Sample	Immersion time (h)	Analysis	
		TPC (mg GAE/g)	Free radical scavenging activities (%)
Control	–	1.87 ± 0.11^a	80.29 ± 0.45^a
N60	3	1.55 ± 0.01^{bA}	68.31 ± 1.46^{bA}
	6	1.30 ± 0.06^{bA}	66.92 ± 1.06^{bA}
	9	1.14 ± 0.07^{cA}	58.13 ± 1.82^{cA}
	12	1.10 ± 0.04^{cA}	56.10 ± 2.08^{cA}
	15	1.03 ± 0.08^{cA}	51.92 ± 2.86^{dA}
N70	3	1.73 ± 0.01^{bB}	68.40 ± 0.94^{bA}
	6	1.34 ± 0.07^{cB}	61.74 ± 0.83^{cB}
	9	1.32 ± 0.03^{cB}	59.46 ± 2.60^{cdA}
	12	1.29 ± 0.07^{cB}	58.10 ± 1.54^{dA}
	15	1.18 ± 0.07^{dB}	51.66 ± 1.62^{eA}
N80	3	1.75 ± 0.10^{bC}	76.90 ± 1.73^{bB}
	6	1.39 ± 0.12^{cC}	73.80 ± 2.98^{bC}
	9	1.27 ± 0.11^{dC}	64.36 ± 0.77^{cB}
	12	1.23 ± 0.11^{dC}	59.60 ± 2.50^{dA}
	15	1.13 ± 0.13^{dC}	54.71 ± 1.96^{eA}

* Values are given as mean with its standard deviation ($n = 3$)

* ^{a–c} Values with a different superscript letter in the column are significantly different ($p < 0.05$) at each sugar concentration with different immersion time

* ^{A–B} Values with a different superscript letter in the column are significantly different ($p < 0.05$) at each immersion time with different sugar concentration

* Control indicates the fresh nutmeg without undergoes osmotic dehydration treatment

* N60 indicates the fresh nutmeg undergoes osmotic dehydration treatment at 60% of sugar solutions

* N70 indicates the fresh nutmeg undergoes osmotic dehydration treatment at 70% of sugar solutions

* N80 indicates the fresh nutmeg undergoes osmotic dehydration treatment at 80% of sugar solutions

* Information about the treatment

fresh nutmeg pericarp decreased from 1.87 ± 0.11 to 1.55 ± 0.01 , 1.73 ± 0.01 and 1.77 ± 0.06 mg GAE/g after 3 h of immersion time for 60, 70 and 80% sugar solution, respectively. The phenolic content in nutmeg pericarp was concurrently reduced by osmotic dehydrated treatment. After 15 h of immersion, the phenolic content in nutmeg pericarp decreased to 1.03 ± 0.08 , 1.08 ± 0.07 and 1.28 ± 0.05 mg GAE/g for 60, 70, and 80% sugar solution, respectively. Results show a significant differences in TPC of nutmeg pericarp with concentration of sugar solution ($p < 0.05$) and immersion time has significant influence ($p < 0.05$) in the phenolic content of osmotic dehydrated nutmeg pericarp. Gupta et al. (2013) reported phenolic content in nutmeg seed as 0.61 mg GAE/g. The amount of phenolic content obtained from this study was higher than the previous studies. This indicates that nutmeg pericarp has higher levels of phenolic content that is 1.87 mg GAE/g compared to nutmeg seed. In the future it would be of research interest to further identify phenolic content from different parts of the nutmeg fruit.

The reduction of TPC indicated that the phenolic compounds may have transfer from fruit to osmotic solution during the OD process under the influence of concentration gradient, leading to a lower phenolic content (Kebe et al. 2014). According to Yadav and Singh (2012), the pigments, flavor precursors, and volatile compounds are transferred from fruit to osmotic solution during the process. Changes in the composition of phenolics in an array may interfere with the antioxidant activity in extracts. So, the reduction of TPC with increasing of immersion time indicated that the decreasing of antioxidant activity in extracts. However, the retention of antioxidant activity is greater at higher sugar levels. These results agreed with those of Almeida et al. (2014). They mentioned that a high concentration of sucrose promotes a protective effect on the surface of the fruit by preventing the outflow of antioxidant compounds.

A free radical scavenging activity assay was conducted to complement the study. The antioxidant activity was evaluated in terms of hydrogen donating or radical scavenging ability by using the free radical scavenging activity assay (Ali et al. 2010; Xie and Schaich 2014). The percentage of free radical scavenging activity of methanol extract of fresh nutmeg pericarp from Table 2 was $80.29 \pm 0.45\%$, which decreased to $68.31 \pm 1.46\%$, $68.40 \pm 0.94\%$, and $77.90 \pm 1.73\%$ after 3 h of immersion time for 60, 70 and 80% sugar solutions, respectively. The free radical scavenging activity of nutmeg pericarp decreased with an increase in immersion time. At 15 h immersion, the percentage of free radical scavenging activity is reduced to $51.92 \pm 2.86\%$, $51.66 \pm 1.62\%$, and $54.71 \pm 1.96\%$ for 60, 70, and 80% sugar solution, respectively. The high scavenging

Fig. 3 Correlation of TPC (mg GAE/g) and free radical scavenging activity (%) for osmotic dehydrated nutmeg pericarp

property of nutmeg may be due to the hydroxyl groups in the chemical structure of the phenolic compound. The presence of tannin, flavonoids and terpenoids compounds in nutmeg leads to high antioxidant capacity in free radical scavenging activity measurement (Assa et al. 2014). These compounds serve as electron donors and contribute to extraction of antioxidants. Statistical tests show that significant differences in concentration of sugar solution ($p < 0.05$) and immersion time ($p < 0.05$) significantly influenced the free radical scavenging activity of nutmeg pericarp after OD treatment.

Overall, strong linear correlations were observed in osmotic dehydrated nutmeg pericarp between extract total phenolic content and the antioxidant activity with a correlation coefficient of $R^2 = 0.97$ (Fig. 3). Increased levels of phenolic compounds in the extracts also increased free radical scavenging activity. Therefore, the content of phenolic compounds may be an important indicator of antioxidant capacity.

Conclusion

It was revealed that processing nutmeg using OD treatment, as practiced by the nutmeg industry in Malaysia, has been proven to increase the concentration of myristicin content and lower antioxidant content. The higher concentration of the osmotic solution resulted in higher values of the myristicin in nutmeg pericarp, while the antioxidant properties of TPC and free radical scavenging activity were found to decrease with an increase in immersion time. The study has found that nutmeg treated with OD treatment of 80% sugar concentration at 3 h is the most suitable treatment providing acceptable levels of myristicin and high antioxidants. The results also suggest that the effects of OD treatment and time variation on myristicin content and antioxidant capacity should be studied independently. It is also crucial to evaluate effects of other treatments, such as

heat treatment, on the myristicin content and antioxidant properties of nutmeg pericarp. A combination of thermal pre-treatment and the OD process is expected to give higher levels of myristicin production.

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