



Antioxidants Effect of Two Aminomethyl Derivatives of 2-Methoxyphenol on Thermal and Storage Oxidative Stability of Coconut Oil

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Abstract

The antioxidants effect of two aminomethyl derivatives of 2-methoxyphenol: 4,6-di[(morpholin-4-yl)methyl]-2-methoxyphenol (DMMMP) and 5-[(pyrrolidin-1-yl)methyl]vanillic acid (PMVA) on enhancing thermal and oxidative storage stability of coconut oil was performed using tert-butylhydroxyquinone (TBHQ) as a comparative standard. The efficacy on thermal stability test was carried out by heating at 180°C for 1, 3, and 6 hours, while the efficacy on the storage stability test was performed using an accelerated method by heating at 60°C for 5 weeks. The concentrations for DMMMP was 200, 350, and 500 ppm; for PMVA was 200, 275, and 350 ppm; and for TBHQ was 200 ppm. Free fatty acid (FFA) level, peroxide value (PV), and p-anisidine value (p-AV) were used as parameters to assess the level of oxidative stability of coconut oil. The results showed that the addition of DMMMP 200, 350, and 500 ppm, and PMVA 200 and 275 ppm did not inhibit FFA, peroxide, and aldehyde formation. In thermal stability study, PMVA (350 ppm) could inhibit the free fatty acid release, and formation of secondary oxidation products compounds on thermal stability comparable to TBHQ (200 ppm) addition, but only TBHQ that could inhibit peroxide formation for 6 h. In storage stability, DMMMP (all concentration) could not delay the fatty acid release, while PMVA (all concentration) and TBHQ (200 ppm) delayed that for 2 and 5 weeks, respectively. DMMMP (all concentration) delayed peroxide formation for 2 weeks, while PMVA (all concentration) and TBHQ (200 ppm) delayed peroxide formation for 5 weeks. All compounds only delay the formation of secondary oxidation products for 1 week. In conclusion, the efficacy of PMVA as an antioxidant against thermal and storage oxidative stability of coconut oil is higher than DMMMP but lower than TBHQ.



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Introduction

Oxidative deterioration and rancidity is a major issue limiting the utilization of fatty oil. The rancidity leads to bad taste, discoloration, and nutritional degradation quality of the oil.¹⁻² Coconut oil is vegetable oil widely used for cooking in Southeast Asian countries, Sri Lanka, Brazil, and many countries in West Africa, North America, and Europe.³⁻⁴ Coconut oil relatively slow to oxidize and resistant to rancidification.⁵ However, during processing, such as frying and other heating, it may increase the oxidation and rancidification of fatty oil. Formation of several primary and secondary oxidation products such as lipid hydroperoxides, aldehydes, ketones, hydrocarbons, and alcohol found and may be harmful to human health.⁶⁻⁹

The addition of antioxidants is one of the most common ways to maintain the oxidative stability of fatty oil.¹⁰ Some synthetic antioxidants such as butyl-hydroxyanisole (BHA), butyl-hydroxytoluene (BHT), propyl gallate, and *tert*-butyl-hydroxyquinone (TBHQ) was frequently used.¹¹ The synthetic antioxidants were used for a several reasons, including inexpensive, high purity, and effectiveness.¹² TBHQ (Figure 1) is the most commonly applied for fats and oils, especially vegetable oils,¹³ with a maximum level of use allowed is 0.02%.¹⁴ However, several

studies indicated that chronic exposure of TBHQ might induce carcinogenicity. The semiquinone anion radicals derived from oxidized forms of TBHQ and reactive oxygen species (ROS) generation suggested contributing to that carcinogenic effects.¹⁵ Considering their potential toxicity of synthetic antioxidants, there is a growing demand to replace synthetic antioxidants with natural ones. However, the application still faces numerous challenges. The most important barrier is the economic cost.¹⁶⁻¹⁷ The introduction of new synthetic antioxidants produced cheaply and safer is another alternative that can be studied.¹²

Two aminomethyl derivatives of 2-methoxyphenol: 4,6-di[(morpholin-4-yl)methyl]-2-methoxyphenol (DMMMP) and 5-[(pyrrolidin-1-yl)methyl]vanillic acid (PMVA) (Figure 1) are new antioxidants successfully synthesized cheaply. This compound showed a free radical scavenger activity 300-437 folded than of vanillic acid used as starting material.¹⁸ However, their efficacy on enhancing thermal and oxidative storage stability of fatty oil and their biological safety has not been elucidated. Hence, this study aimed to evaluate the efficacy of DMMMP and PMVA as antioxidants on enhancing the stability of coconut oil during frying and storage.

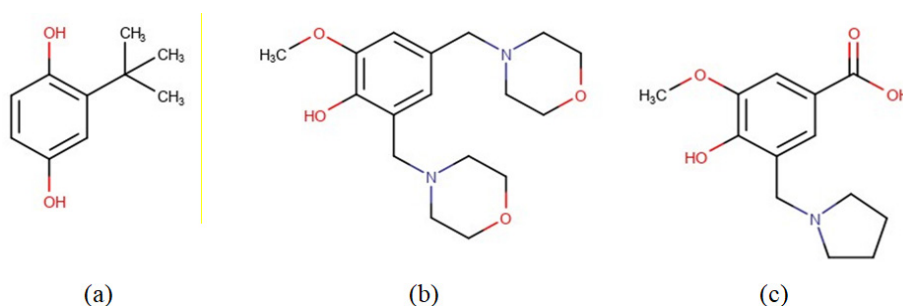


Fig.1: Chemical structure of *tert*-butyl-hydroxyquinone (TBHQ) (a), 4,6-di[(morpholin-4-yl)methyl]-2-methoxyphenol (DMMMP) (b), and 5-[(pyrrolidin-1-yl)methyl]vanillic acid (PMVA) (c)^{12,18}

Material And Methods

Materials

Fresh coconut oil was prepared traditionally by heating coconut flesh (*Cocos nucifera*) according to reported method.¹⁹ The coconut is picked from coconut tree garden in Bandar sub-district, Batang Regency, Central Java, Indonesia. The vegetable

oil was analyzed to determine its suitability against Indonesian National Standard (ISN) 2902 (2011)²⁰ concerning the characteristic standard of crude coconut oil. The organoleptic, water content, iodine value, free fatty acids level, peroxides value, and saponification value was assessed according to ISN 01-3555 (1998)²¹ method concerning evaluation

oils and fats. 4,6-Di[(morpholin-4-yl)methyl]-2-methoxyphenol (DMMMP) and 5-[(pyrrolidin-1-yl)methyl]vanillic acid (PMVA) were obtained from earlier researcher.¹⁸ Tertiary butyl hydroxyquinone (TBHQ), chemical reagents, and solvents were analytical grade and purchased from Sigma Aldrich, USA, or Merck, Germany.

Efficacy on Thermal Frying Stability

The efficacy of DMMMP and PMVA on thermal frying stability of coconut oil was carried out according to the frying stability method reported previously with a little modifications.^{3, 22-24} TBHQ is used as a comparative standard, and coconut oil without antioxidants was used as a negative control. Coconut oil is heated at a temperature of 180°C for 1, 3, and 6 hours. The concentration of DMMMP was 200, 350, and 500 ppm, PMVA was 200, 275, and 350 ppm, and TBHQ was 200 ppm. The following chemical parameters: free fatty acid (FFA) level (%), peroxide value (PV) (mEq peroxide/kg), and p-anisidine value (p-AV) were analyzed using reported procedure.^{8,22} FFA levels were determined by a volumetric titration technique using sodium hydroxide solution as a titer.⁸ The peroxide values (PV) were determined spectrophotometrically by measuring the absorption value of iron (III) chloride resulted from oxidation of iron (II) chloride by peroxide formed.²⁵ While the p-anisidine value (p-AV) was determined by measuring spectrophotometrically, the amount of p-anisidine that reacts with the aldehyde compound formed.²⁵

Efficacy on Storage Accelerated Stability.

The efficacy of DMMMP and PMVA on the storage stability of coconut oil was carried out according to the accelerated stability method reported previously with a little modification.^{3,22-26} TBHQ is used as a comparative standard, and coconut oil without antioxidants is used as a negative control. Coconut oil with and without the addition of the antioxidants is stored at 60°C in the oven for nine weeks. The concentration of DMMMP was 200, 350, and 500 ppm, PMVA was 200, 275, and 350 ppm, and TBHQ was 200 ppm. Samples were taken at the week of 0, 2, 5, and 9 to be analyzed for their chemical parameters [free fatty acid (FFA) levels, peroxide value (PV), and p-anisidine value (p-AV)] using same procedures used in the thermal frying stability study mentioned above.^{8,25}

Statistical analysis

All determination were triplicated, and the reported data were the mean \pm standard deviation (SD). The data were analyzed by one way ANOVA and Kruskal-Wallis methods with their statistical significance ($p \leq 0.05$) using SPSS package (IBM, SPSS Statistics 21.0)

Result and Discussion

The result of the initial analysis indicated that the characteristic of coconut oil used for the study meets the Indonesian National Standard 2902 (2011).²⁰ The crude oil presented as a specific smell and taste for coconut oil with a clear yellow colour. Water content, iodum value (IV), free fatty acid (FFA) level, peroxide value (PV), and saponification value (SV) of the oil were 0.2%, 7.0 g/100 g, 0.14%, 0.01 mEq peroxide/kg, and 269.5 mg KOH/g, respectively.

In this study, the efficacy of DMMMP and PMVA compared to TBHQ on thermal frying stability and storage oxidative stability of coconut oil was evaluated. The concentration for compound tested was applied based on the ratio between the IC_{50} value of radical DPPH scavenger activity of the compound tested, and TBHQ reported earlier.¹⁸ The concentration of TBHQ refers to a maximum level of use allowed.¹⁴

Efficacy on Thermal Frying Stability.

Frying in high-temperature oil (around 180°C) is one of the most frequently used food processing methods. At high temperatures, the oil will underwent significant changes due to many chemical reactions occurred, such as hydrolysis, oxidation, isomerization, cyclization polymerization, and cyclization.⁷ The hydrolysis occurred was measured by free fatty acids (FFA) levels measurement. While the presence of oxidation was determined by the peroxide value (PV) measurement. Peroxide is unstable and will decompose to form aldehydes, ketones, alcohols, acids, and others. This secondary product leads to rancidity can be measured by the p-anisidine value (p-AV) method.^{8,25}

Free Fatty Acid (FFA) Levels

An increase in free fatty acid (FFA) levels was detected at the 1st-hour observation in the control sample and samples that were added with DMMMP at concentrations of 200, 350, and 500 ppm. The

samples with the addition of PMVA at concentrations of 200 and 275 ppm, no increasing of FFA levels observed until the 3rd hour, and the samples added with PMVA (350 ppm) or TBHQ (200 ppm) were not observed a significant increase in free fatty acids levels (Figure 2). These indicated that PMVA

(350 ppm) dan TBHQ (200 ppm) could inhibit the hydrolysis of triglyceride to fatty acid on coconut oil. The results on the addition of TBHQ in this study are in line with the antioxidative effect of TBHQ on the thermal stability of sesame oil reported earlier.⁸

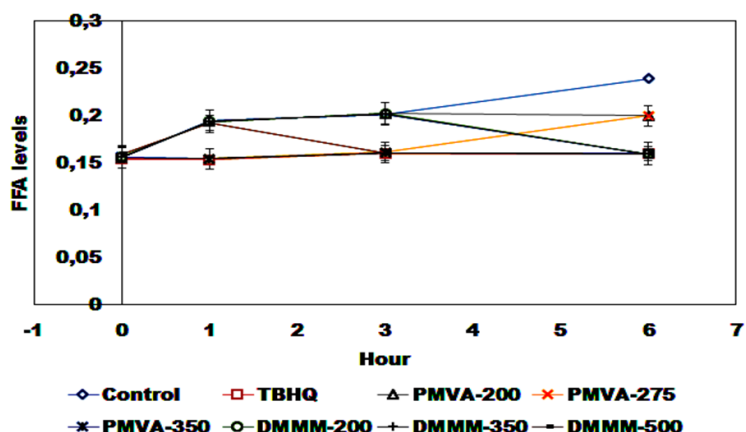


Fig.2: Free fatty acid (FFA) levels (%) of coconut oil on elevated heating at 180°C without and with antioxidant addition (Results are expressed as mean ± SD of three measurements)

Peroxide Value (PV)

The elevated heating at 180°C of the control sample and samples added with DMMMP or PMVA was observed a significant increase in the peroxide value (PV). While in the sample added with TBHQ, the increasing of PV was smaller than other samples (Figure 3). Based on the data above,

it indicated that the DMMMP and PMVA could not delay peroxide formation in a frying temperature of 180°C of coconut oil. According to previous reports, the antioxidative effect of TBHQ decreased significantly at temperatures ≥ 135°C,¹³ but our study indicated that it didn't show a decrease.

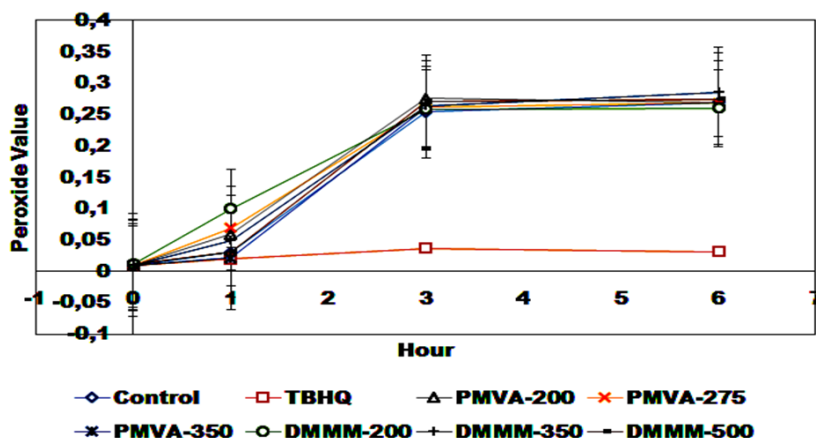


Fig.3: Peroxide value (PV) (mEq peroxide/kg) of coconut oil on elevated heating at 180°C without and with antioxidant addition (Results are expressed as mean ± SD of three measurements. Statistically significant ($p \leq 0.05$) of sample added with TBHQ (200 ppm) from the control and other samples)

p-Anisidine Value (p-AV)

At the 3rd and 6th hours after high-heating at 180°C, the control sample, the sample added with DMMMP with a concentration of 200, 350, and 500 ppm, and sample added with PMVA with the concentration of 200 ppm and 275 ppm were observed the increasing of p-AV significantly. Whereas the sample added

with TBHQ (200 ppm) or PMVA (350 ppm) showed p-AV relatively stable (Figure 4). Based on this result indicated that DMMMP at all concentrations and PMVA at a concentration of 200 ppm and 275 ppm, did not have a better effect compared to PMVA (350 ppm) and TBHQ (200 ppm) in the secondary oxidation product formation of coconut oil.

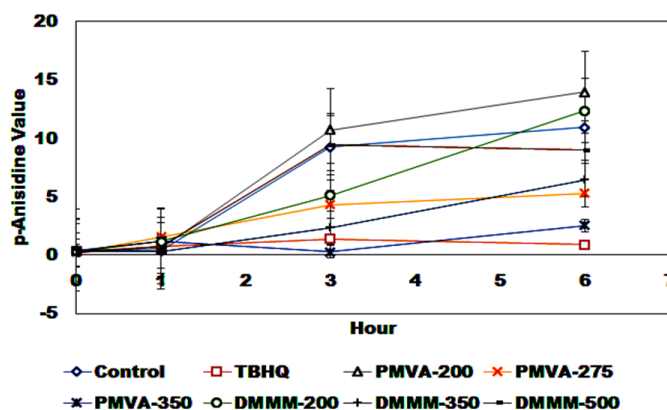


Fig.4: p-Anisidine value (p-AV) of coconut oil on elevated heating at 180°C without and with antioxidant addition (Results are expressed as mean ± SD of three measurements. Statistically significant value ($p \leq 0.05$) of samples added PMVA (350 ppm) or TBHQ (200 ppm) from the control and other samples)

Efficacy on Storage Accelerated Stability.

Oxidative stability is a test to determine the storage duration until rancidity is formed. Tests carried out under normal conditions can determine realistic stability. However, testing in this condition requires

a very long time, so in this study, the accelerated storage test method was performed using the Schaal oven test method.²⁵ An increase in temperature was increasing an oxidation reaction that can be evaluated both organoleptically and chemically.

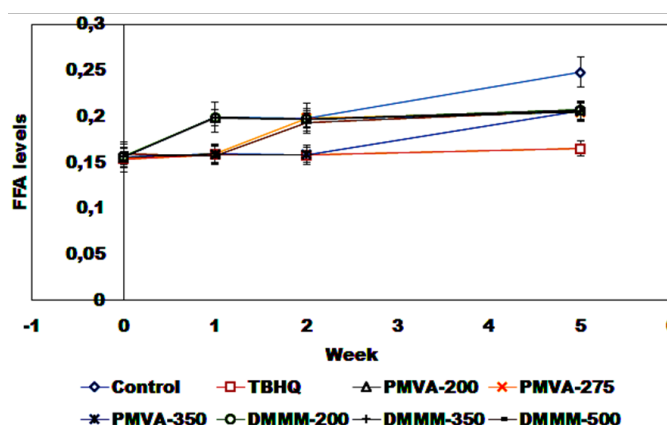


Fig.5: Free fatty acid (FFA) levels (%) of coconut oil on storage accelerated stability without and with antioxidant addition. (Results are expressed as mean ± SD of three measurements. Statistically significant ($p \leq 0.05$) of sample added with TBHQ (200 ppm) from the control and other samples)

Free fatty acid (FFA) levels

The FFA levels in the control sample, sample added with DMMMP (200 ppm), DMMP (350 ppm), PMVA (200 ppm), and PMVA (275 ppm) increased from week 1 to week 5. Whereas the sample added with DMMMP (500 ppm), the increasing began to occur after the 1st week, and in sample added with PMVA (350 ppm), an increase in FFA levels occurred after the 2nd week. Samples with the addition of TBHQ (200 ppm) showed the FFA levels that were stable until the 5th week (Figure 5). The above data indicate that the effectiveness of TBHQ in inhibiting triglyceride hydrolysis was higher than DMMMP and PMAV

Peroxide value (PV).

PV is the general method for determining the oxidative stability of oils. The amount of peroxide formed indicates the degree of primary oxidation associated with rancidity.⁶⁻⁹ An increase in PV indicated the formation of hydroperoxide during the lipid oxidation process. In the study, the control sample underwent an increase in the PV during storage at week 0 to week 5. The PV of the DMMMP sample has increased at the 2nd week of storage, while the sample with PMAV and TBHQ can withstand the PV until the 5th week (Figure 6). It indicated that PMAV and TBHQ could delay peroxide formation in the storage.

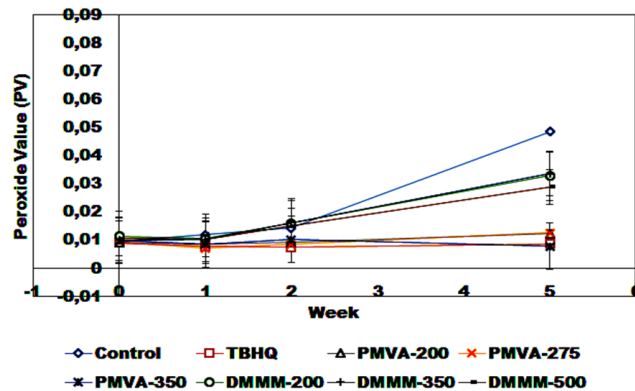


Fig.6: Peroxide value (PV) (mEq peroxide/kg) of coconut oil on storage accelerated stability without and with antioxidant addition. (Results are expressed as mean ± SD of three measurements. Statistically significant ($p \leq 0.05$) of sample added with TBHQ (200 ppm) and PMVA (all concentration) from the control and sample added DMMMP (all concentration)

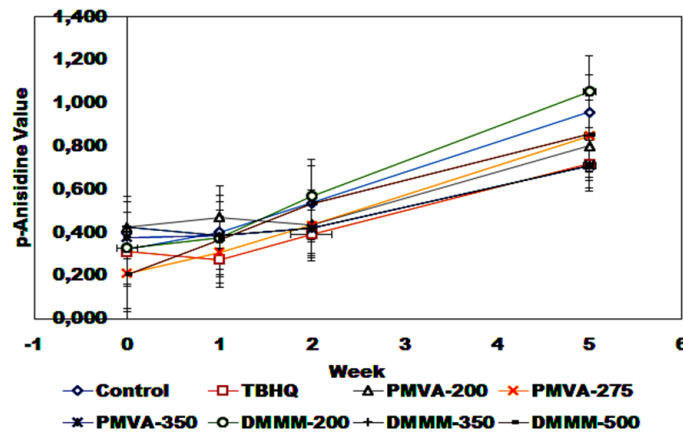


Fig.7: p-Anisidine value (p-AV) of coconut oil on storage accelerated stability without and with antioxidant addition. (Results are expressed as mean ± SD of three measurements. Statistically, nonsignificant ($p \leq 0.05$) between the samples

p-Anisidine value (p-AV)

In all samples, there was an increase in p-AV in the second-week storage. The increase in p-AV in the sample with the addition of TBHQ was not as high as in the other samples (Figure 7). However, this difference was not significant.

Conclusion

Evaluation of the efficacy of antioxidants of two aminomethyl derivatives of 2-methoxyphenol: 2-methoxy-4,6-di[(morpholin-4-yl)methyl]phenol (DMMMP) and 5-[(pyrrolidin-1-yl)methyl]vanillic acid (PMVA) compared to *tert*-butyl hydroxy quinone (TBHQ) against oxidative stability during frying and storage of coconut oil has been done successfully. Free fatty acid level, peroxide value, and p-anisidine value were used as parameters to assess the level of oxidative stability of the oil with and without the addition of antioxidants. The results indicated that the

efficacy of PMVA as an antioxidant against thermal and storage oxidative stability of coconut oil is higher than DMMMP but lower than TBHQ.

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Conflict of interest

The authors declare that there is no conflict of interest.

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