Methanolic extract of Java Plum (*Syzygium cumini* Linn.) seeds as a natural antioxidant on lipid oxidation of oil-in-water emulsions

[1,2]*Rohadi, [2]Raharjo, S., [3]Falah, I.I. and [2]Santoso, U.

[1]*Department of Processing Agricultural Product, Faculty of Agricultural Technology, Semarang University, Jl. Arteri Soekarno-Hatta, Semarang 50196, Indonesia*
[2]*Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur , Yogyakarta 55281, Indonesia*
[3]*Department of Chemistry, Faculty of Mathematics and Natural Science, Gadjah Mada University, Jl. Sekip Utara, Yogyakarta 55281 Indonesia*

Abstract

Fish oil is considered as a great nutritional importance due to a high content of essential n-3 polyunsaturated fatty acids (PUFAs) such as linolenic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). However, PUFAs are susceptible to oxidative deterioration. Methanolic extract of Java plum seed was a source of strong antioxidant polyphenols against DPPH as well as strong Ferric (Fe^{3+}) reducing agent. The aim of the research was to evaluate the effect of methanolic extract of Java Plum seed (MEJS) on lipid oxidation of fish oil from striped catfish (*Pangasius hypothalamus*) in oil-in-water emulsions as a food model at 35±2ºC during 144 h of storage. The results showed that MEJS has stronger inhibition activity than Grape Seed Extract (GSE) but lower than BHA ($p<0.05$) according to conjugated dienes (CD), peroxide values (PoV), and thiobarbituric acid reactive substances (TBARS). Inhibition activity of MEJS on lipid oxidation ranged between 28% and 38% at concentrations of 50-800 mg/L during 144 h of storage.

Introduction

Fish oil is considered as a great nutritional importance. This is due to its naturally high content of essential n-3 polyunsaturated fatty acids (PUFAs), such as α-linolenic acid (C18:3), eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6). These fatty acids have beneficial effects for heart's health (Simopoulos, 2008). However, PUFAs are susceptible to oxidation which leads to the production of short-chain chemical compounds associated with decreasing food quality attributes such as rancidity as well as textural and nutritional modification (Hsieh and Kinsella, 1989; McClements and Decker, 2000; Maqsood, 2010). The rate oxidation of PUFAs in bulk oil increases equal to their unsaturated degree. A high-PUFAs food product has more susceptible to oxidative deterioation (McClements and Decker, 2000) such as fish oil from farmed freshwater Tra catfish (*Pangasius hypothalamus*) (Ho and Paul, 2009).

Previous research showed that methanolic extract of Java Plum (*Syzygium cumini* Linn.) seed (MEJS)

has a strong antioxidant activity according to RSA-DPPH (radical scavenging activity 2,2'diphenyl-1-picrilhydrazil) and FRAP (ferric reducing antioxidant power) (Rohadi *et al*., 2016). In addition that a polar hydrophilic antioxidant is less effective in an oil-in-water emulsion compared to a non-polar lipophilic and vice versa. The polar antioxidants such as trolox, ascorbic acid, rosmarinic acid, and carnosic acid are more effective in bulk oil due to its accumulation in the air-oil interphase area while in an emulsion system, the non-polar ones such as α-tocophorol, ascorbyl palmitate, and carnosol are more effective (McClement and Decker, 2000).

Synthetic antioxidants such as BHA (Butylated Hydroxy Anisole), BHT (Butylated Hydroxy Toluene) and TBHQ (tert-butylhydroquinone) were more effective to inhibit food deterioration due to lipid oxidation during processing and storage (Frankel 1998; Maqsood, 2010). However, application of those antioxidants raised doubt in terms of toxicity-related problems of synthetic antioxidants (Madavi and Salunke, 1995; Buxiang and Fukuhara, 1997; Baydar *et al*., 2007; Vayupharp and Laksanalamal,

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*Corresponding author.
Email: *rohadij@yahoo.com*
Tel: +62 246 702757; Fax: +62 246 702272

2012). In the other hand, consumer's demand for environmentally friendly natural antioxidants has greatly increased in recent years (Perumalla *et al.*, 2011). The last decades researches have been focused on the effectiveness of natural antioxidants as a chain-breaking agent in lipid oxidation, such as tocopherol, plant extracts (rosemary, blackberries, blueberries, cherries, grapes, raspberries, strawberries, tea), and kiam wood extract (Kanner *et al.*, 1994; Frankel, 1996; Maqsood, 2010; Perumalla *et al.*, 2011).

Zhang and Lin (2009) reported that Java plum (*Syzygium cumini* Linn.) extract has high tannin content and antioxidant activity toward DPPH and FRAP test, therefore, it has a high potential as a natural antioxidant. Among part of the plant, the seed has the highest polyphenols content (Vasi and Austin, 2009; Rydlewsky, 2013; Saha *et al.*, 2013). Rohadi *et al.* (2016) reported that yield of Java Plum seed extract using 50% methanol (v/v) was 16.29% (db) and contain 45.99±0.25% total phenolic compound expressed as g gallic acid equivalents (GAE/100 g dry extract), 2.28±0.07% total flavonoid expressed as g quercetin equivalents (QE/100 g dry extract), and 26.9±0.07% total tannin as expressed as g tannic acid equivalents (TAE/100 g dry extract). This result showed that extract of Java plum seed has a high potential as a natural antioxidant. Several studies have reported the natural polyphenol of plant extracts were more effective to inhibit lipid oxidation in an oil-in-water emulsion (Roedig-Penman and Gordon, 1997; Maqsood and Benjakul, 2010; Skowyra *et al.*, 2014; Azman *et al.*, 2016). However, the scientific information of Java plum seed as a natural antioxidant on lipid oxidation of fish oil in an oil-in-water emulsion is still limited. Therefore, the aim of this research was to evaluate the effect of methanolic extract of Java Plum seed (MEJS) on lipid oxidation of fish oil from striped catfish (*Pangasius hypothalamus*) in an oil-in-water emulsion as a food model during storage.

Materials and Methods

Materials

Live striped catfish (*Pangasius hypothalamus*) were obtained from "Mina Jaya" farmer group in Margo Mulyo Village, Seyegan, Sleman, Yogyakarta. Java plum (*Syzygium cumini* Linn. var. *Genthong*) were collected from Semarang, Central Java, Indonesia during December 2014. Grape seed extract (GSE) contained 95% oligoprotoanthocianidin (OPC) (Bulk powder, UK), 2,2'-diphenyl-1-picrilhydrazil (DPPH) and 1,1,3,3-tetra methoxypropane (TMP) (Aldrich Chemical Co.), and butylatedhydroxyanisole (BHA)

(Sigma Chemical Co.) were an analytical grade.

Preparation of methanolic extract of Java plum seed

The seed was separated from the fruit pulp and then was sliced with stainless steel knife, dried using cabinet dryer at 47±3° C for 24 h. The dried seed was ground into powder using a grinder then sieved through 80 mesh. Twenty five grams of Java Plum seed powder were exhaustively three times extracted with aqueous-methanol 50% (v/v) at a ratio of 1:10 (w/v) using the maceration method for 6 h at ambient temperature according to (Vasi and Austin, 2009) with slight modification. The extracts were collected and concentrated using a rotary vacuum evaporator (IKA-RV 10 Basic Germany) then the solid crude extract was freeze-dried for solvent removal and kept in the refrigerator for further analysis. This extraction procedure was repeated three times.

Preparation of striped catfish fillet and its lipid extraction

Six live striped catfish (*Pangasius hypothalamus*) is weighed of approximately 1 kg per head. They were cleaned and filleted in 2 x 5 cm^2 size using stainless steel knife, stored in polyethylene (PE) bag, and freezed prior to oil extraction. The lipid content of striped catfish fillet was extracted according to Bligh and Dyer (1959). Twenty five grams of thawed fillet cuts were ground using a meat grinder and added with 200 mL of a mixture of chloroform: methanol:distilled water (1:2:1, v/v) then centrifuged at 3000 rpm and 4°C for 5 min. A-50 mL of chloroform and 25 mL of distilled water were added into homogenate and furthermore mixed for 2 min at a similar speed. The homogenate was centrifuged at 4°C with speed 3000 rpm for 45 min using J-6B Centrifuge (Beckman, Fullerton, CA, USA). Thereafter, the supernatant was put into a separating funnel, which chloroform fraction was drained off into Erlenmeyer glass previously filled with 25 g of activated anhydrous sodium sulphate with gentle shaken. The mixture was filtered using Whatman filter paper No.4. The solvent was evaporated from filtrate using a vacuum rotary evaporator at 25°C, and the remaining solvent residue was removed using nitrogen gas.

Determination of fatty acid profile

Fatty acid composition of striped catfish oil was determined as Fatty Acid Methyl Ester (FAME) according to Latimer (2000). Into 0.5 mL of fish oil, 1.5 mL of methanolic sodium was added then heated at 70°C for 5-10 min while vigorously shaken. The mixture was cooled and added with 2 mL of boron trifluoride methanoate then reheated at 70°C for

5-10 min and cooled again. Thereafter, the mixture was extracted using 1 mL of heptane and 1 mL of saturated NaCl. The top layer was taken and put into Eppendorf tubes. One µL of sample was injected into GC-2010 Shimadzu (Shimadzu, Kyoto Japan) equipped with flame ionization detector (FID) at 260ºC. Capillary column (CP-Sil8-CB 30 m x 0.25 mm) was used. The standard retention time of FAME was used to identify chromatogram peak of each sample. Fatty acid content was quantified based on peak area ratio as g fatty acid /100 g oil (%).

Preparation of fish oil emulsion

Fish oil emulsion was prepared based on Maqsood, (2010) with slight modification. Twenty five mL of striped catfish oil were mixed with 225 mL of 0.1 M buffer acetate (pH 5.4) and 2.5 mL of Tween 40 then homogenized in 400 mL plastic jars for making 250 mL of oil-in-water emulsion. The mixture was kept in an ice-bath (10±2ºC) during homogenization at 6500 rpm (Ultra Turrax T50, IKA Werke, Germany) for 5 min. The methanolic extract of Java plum seed (MEJS) powder was mixed into fish oil emulsion at various concentration (25, 50, 100, 200, 400 and 800 mg/L) using vortex (Velp Scientifica Europe) at 3000 rpm for 3 min. The mixture (10 mL) was then kept at 35±2ºC in the dark room. The control of oil-in-water emulsion was prepared in the same procedure but distilled water was added instead of the extract. BHA and grape seed extract (GSE) were used as the reference antioxidant. Samples were taken at every 24 h for the determination of conjugated diene (CD) value, peroxide value (PoV) and the thiobarbituric acid reactive substances (TBARS).

Determination of lipid oxidative deterioration

The lipid oxidation was determined using evaluation of conjugated diene (CD), peroxide value (PoV), and thiobarbituric acid reactive substances (TBARS) value.

Analysis of conjugated diene (CD)

Determination of conjugated diene was carried out according to Frankel et al. (1996) in (Maqsood, 2010). Fish oil emulsion (0.1 mL) was dissolved in 5 mL of methanol and the absorbance was measured at λ=234 nm using spectrophotometer UV-1601 (Shimadzu, Kyoto Japan). The absorbance of λ 234 nm indicated CD value.

Analysis of peroxide value (PoV)

Determination of peroxide value was done according to Sakanaka et al. (2004). Fish oil emulsion (50 µL), 2.35 mL of 75% ethanol, 50 µL

of 30% ammonium thiocyanate, 50 µL of 20 mM ferrous chloride ($FeCl_2$) in 3.5% HCl were mixed and shaked using a vortex. After 3 min the absorbance of the sample was measured using spectrophotometer UV-1601 (Shimadzu, Kyoto Japan) at λ = 500 nm. An increase absorbance at λ = 500 nm indicated the formation of peroxide. The percent inhibition of peroxidation of fish oil-in water emulsion of MEJS was calculated as follow:

$$\% \text{ inhibition} = 100 - \left(\frac{\text{Absorbance increase of sample}}{\text{Absorbance increase of control}} \times 100 \right)$$

Analysis of thiobarbituric acid reactive substances (TBARS)

Determination of TBARS was done according to (Buege and Aust, 1978) in (Maqsood, 2010). Fish oil emulsion (0.5 mL), 2.5 mL of TBA (0,375% thiobarbituric acid, 15% trichloroacetic acid-TCA and 0.25 N HCl) was mixed and shaked using a vortex. The mixture was heated in boiling water for 10 min until pink color appeared then cooled in water flow and mixed using a vortex at 3000 rpm (25ºC) for 10 min and the absorbance was measured at λ 532 nm. A standard curve was conducted using 1.1.3.3-tetramethoxypropane (TMP) at various concentrations ranged 0.61-20.9 µM/L. TBARs value was expressed as µM–MDA equivalent/L.

Statistical analysis

All data was presented as a mean value±standard deviation (SD). Analysis of the variance was determined and the mean comparison was examined by the Duncan's Multiple Range Test (DMRT) at the significance level of 0.05 using SPSS statistic software series 22 for Windows (SPSS Inc. Chicago, USA). Regression analysis was applied to determine correlation among variables.

Results and Discussion

Lipid content and fatty acid composition of striped catfish oil

The lipid content of striped catfish fillet (*Pangasius hypothalamus*) was 9.45%, similar to previous research reported by (Maqsood, 2010) which showed lipid content of striped catfish from Thailand was 9.2%. Meanwhile, (Ho and Paul, 2009) reported lipid content of Tra catfish from Mekong River delta in Vietnam was only 2.55%.

In the present study, striped catfish contains 43.57% saturated fatty acid (SFA), 40.01% monounsaturated fatty acid (MUFA), and 16.42% polyunsaturated fatty acid (PUFA) (Table 1). Unsaturated fatty acid content

Table 1. Fatty acid profile of lipid extracted from striped catfish
(*Pangasius hyphotalamus*)

Fatty acid	Formula	Fatty acid content (g/100 g lipid)	Fatty acid content (g/100 g lipid)*
Lauric acid	C12:0	4.3	n.d
Myristic acid	C14:0	6.28	4,97±0,25
Pentadecanoic acid	C15:0	0.55	n.d
Palmitic acid	C16:0	28.31	29,33±1,70
Heptadecanoic acid	C17:0	1.11	n.d
Stearic acid	C18:0	0.7	7,58±0,47
Arachidic acid	C20:0	0.39	0,47±0,08
Heneicosanoid acid	C21:0	0.56	n.d
Tricosanoic acid	C23:0	1.61	n.d
Lignoceric acid	C24:0	0.17	0,47±0,18
Σ Saturated fatty acid		43.98	42,63±0,95
Palmitoleic acid	C16:1 n-7	2.54	1,53±0,32
Trans-9-Elaidic acid	C18:1 n-9	0.43	n.d
Cis-9-Oleic acid	C18:1 n-9	36.71	30,93±1,05
Cis-11 Eicosaenoic acid	C20:1 n-9	n.d	1,66±0,43
Erucic acid	C22:1 n-9	0.18	n.d
Nervonic acid	C24:1 n-9	0.15	n.d
Σ Monounsaturated fatty acid		40.01	34,69±1,57
Linoleic acid	C18:2 n-6	8.28	8,43±0,72
Linolenic acid	C18:3 n-3	2.01	1,07±0,19
Cis-11,14-Eicosadienoic acid	C20:2 n-6	1.34	n.d
Cis-8,11,14-Eicosatrienoic acid	C20:3 n-6	2.42	n.d
Cis-5,8,11,14-Eicosatetraenoid acid	C20:4 n-6	1.51	1,03±0,03
Cis-5,8,11,14,17-Eicosapentanoid acid	C20:5 n-3	0.4	0,31±0,08
Cis-13,16-Docosadienoic acid	C22:2 n-6	0.46	n.d
Docosotetraenoic acid	C22:4 n-6	n.d	0,82±0,42
Docosopentaenoic acid	C22:5 n-3	n.d	1,29±0,04
Cis-4,7,10,13,6,19-Docosahexaenoic acid	C22:6 n-3	n.d	4,74±0,93
Σ Polyunsaturated fatty acid		16.42	17,69±1,26
Source = *Ho and Paul, (2009)	n.d. = no data		

(56.43%) was 1.3 times higher than saturated fatty acid content (43.57%), which indicated that lipid content of the fish was vulnerable to oxidation. The high percentage of SFA (43.57%) was similar to the previous study reported by (Ho and Paul, 2009). The MUFAs content was 36.71% *cis*9 oleic acid (C18:1 n-9), 2.54% palmitoleat acid, and 0.43% oleic acid isomer (trans-9 oleic acid). These values showed that oleic acid was abundant in striped catfish oil.

The PUFAs content of striped fish oil consisted of 8.28% linoleic acid (C18:2 n-6), 2.42% eicosatrienoic acid (C20:3 n-6), 2.01% linolenic acid (C18:3 n-3), 1.51% eicosatetraenoic acid (C20:4 n-6), and 1.34% eicosadienoic acid (C20:2 n-6). Eicosapentaenoic acid (C20:5 n-3) or EPA content was 0.4%, higher than reported by Ho and Paul, (2009) and Maqsood, (2010), i.e. 0.31% and 0.13%, respectively. However, the striped catfish oil did not contain docosahexaenoic acid (C22:6 n-3), which was similar to the previous studies (Ho and Paul, 2009; Maqsood, 2010). The difference of lipid content and fatty acid composition of striped catfish could be contributed to a different method of oil extraction, kind of feed, and type of fish farmed.

Table 2. The phenolic compounds of *Syzygium cumini* seed were extracted using three different solvents. Different subscript letters within the same kind of phenolic compound mean significant differences (p < 0.05) (n=3).

Extractant	Phenolic compounds Total phenolic (mg GAE/ kg-seed)	Phenolic compounds Total Flavonoid (mg QE/ kg seed)	Phenolic compounds Total Tannin (mg TAE/ kg seed)
EtO-Ac 85	277.50±14.24[a]	8.37±0.05[a]	162.86±0.70[a]
Et-OH 50	1092.23±8.76[b]	58.84±1.32[b]	572.41±2.6[b]
Met-OH 50	1179.64±6.32[c]	67.50±17.5[b]	693.13±1.6[c]

Effect of MEJS on lipid oxidation of fish oil emulsion

Methanolic Java plum seed extract (MJES) was prepared from dry Java plum seed powder which extrated using methanol 50% (v/v), rather than ethanol 50% (v/v) and ethyl acetate 85% (v/v). Among the extracts, methanolic extract of Java
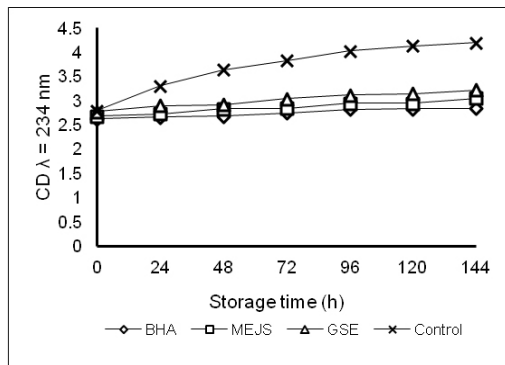
Figure 1(a). Effect of methanolic extract of Java Plum (*Syzygium cumini* Linn.) seed (MEJS) on lipid oxidation products formation, conjugated diene (CD) in striped catfish (*Pangasius hyphotalamus*) oil-in-water emulsion at 35±2ºC during 144 h of storage, compared to BHA and grape seed extract (GSE). For all treatments, a final concentration of 50 mg/L was used in the system (n=3).
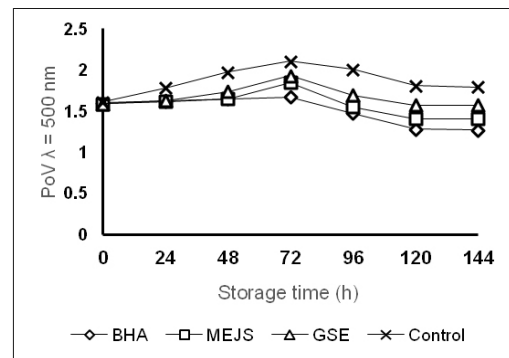

Figure 1(b). Effect of methanolic extract of Java Plum (*Syzygium cumini* Linn.) seed (MEJS) on lipid oxidation products formation, peroxide value (PoV) in striped catfish (*Pangasius hyphotalamus*) oil-in-water emulsion at 35±2ºC during 144 h of storage, compared to BHA and grape seed extract (GSE). For all treatments, a final concentration of 50 mg/L was used in the system (n=3).

Plum seed (MEJS) has stronger antioxidant activity according to DPPH and Feric (Fe^{3+}) reducing capacity and a moderate activity for linoleic acid oxidation inhibition (Rohadi *et al*., 2016). The high antioxidant activity of Java Plum seed extract could be associated with the high content of phenolic compounds (Table 2). The phenolic compound acts both as primary antioxidants donate hydrogen to scavenge alkoxy and peroxy radicals to form unreactive antioxidant radical (chain breaking antioxidants), and also as secondary antioxidants, such as metal chelating agents, singlet oxygen quenchers, and oxygen scavengers (Frankel, 1998; Brewer, 2011; Saha *et al*., 2013).

A model emulsion was used to assess the deterioration of lipids at two stages of oxidation, i.e. primary oxidation products (CD and PoV) and secondary oxidation products (TBARS). Maqsood and Benjakul, (2010) reported that the effectiveness of four phenolic compounds to inhibit lipid oxidation in Menheden fish oil emulsion system during 168 h of storage. Meanwhile, Azman *et al*. (2016) investigated that the potential of bearberry leaf extract as a natural antioxidant to inhibit lipid oxidation of sunflower oil emulsion during 40 days of storage.

Effect of MEJS on conjugated diene (CD) formation

The effect of MEJS (50 ppm) on lipid oxidation of striped catfish oil emulsion during storage based on conjugated diene (CD) and peroxide value (PoV) is presented in Figure 1(a) and 1(b). The CD values of samples gradually increased until 144 h ($p<0.05$), whereas PoV increased up to 96 h ($p<0.05$) then decreased slightly until the end of the storage period (except control). A similar pattern was also found in
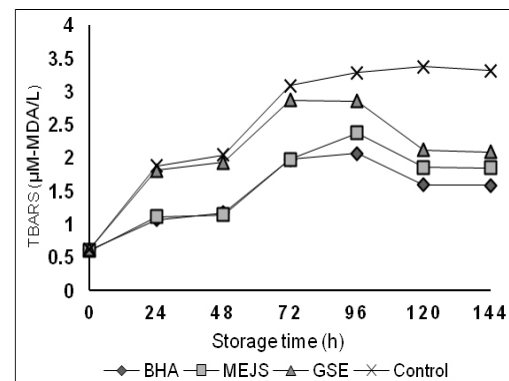

Figure 1(c). Effect of methanolic extract of Java Plum (*Syzygium cumini* Linn.) seed (MEJS) on lipid oxidation products formation, thiobarbituric acid reactive substances (TBARS) in striped catfish (*Pangasius hyphotalamus*) oil-in-water emulsion at 35±2ºC during 144 h of storage, compared to BHA and grape seed extract (GSE). For all treatments, a final concentration of 50 mg/L was used in the system (n=3).

TBARS value (Figure 1c). TBARS value reflects the concentration of lipid oxidation secondary products such as aldehyde (malondialdehyde, 4-hydroxy nonenal) equivalent to malonaldehyde (µM-MDA eq/L).

The addition of MEJS can inhibit CD formation of an oil-in-water emulsion as well as BHA, moreover, significantly higher than grape seed extract (GSE) (Figure 1a). This result could be associated with the stronger free radical scavenging activity of BHA and MEJS during initiation or propagation stage, therefore, it can inhibit free radical formation in further stages. CD values of the sample were higher than earlier study (Maqsood and Benjakul, 2010) (ranged of 0.8-2.0 at λ 234 nm). The difference in CD value could

be contributed to a different fatty acid composition of fish oil and phenolic compounds. Maqsood and Benjakul, (2010) investigated Menheden fish oil and single phenolic compund (not plant extract).

A strong correlation between RSA-DPPH activity of MEJS or GSE and CD formation during initial 72 h was 0.98 and 0.84, respectively. Moreover, a strong correlation was also found between MEJS or GSE and CD formation using FRAP method, i.e. 0.96 and 0.95, respectively.

Effect of MEJS on peroxide value (PoV) formation

Peroxide value (PoV) reflects the development of primary oxidation products in emulsion food model. The PoV of emulsion with or without 50 ppm MEJS during storage was presented in Figure 1(b). PoV of the samples gradually increased during 72 h then decreased until the end of storage period. The PoV of the samples with MEJS, BHA, and GSE were lower than control ($p<0.05$). The effectiveness of MEJS and BHA to inhibit PoV formation during initial 72 h of storage were similar, even higher than GSE ($p<0.05$). Previous studies of the effectiveness of emulsion containing plant extract antioxidant have a similar pattern. Roedig-Penman and Gordon, (1997) reported that emulsions containing green tea extract required 8 days to reach the end of the induction time. Meanwhile, Maqsood and Benjakul, (2010) found that an emulsion containing 100 mg/L of the tannic acid took 96 h to reach more than 4.5 (λ=500 nm). An emulsion containing 48 μg/mL of Tara extract took 13 days to reach more than 10 meq hydroperoxide/kg (Skowyra *et al.*, 2014). Furthermore, Azman *et al.* (2016) noted that the sample containing bearberry leaf extract (BL) 1 g/kg reached the end of the induction time after 20 days, while BHT samples after 36 days of storage.

Inhibition of lipid oxidation among three antioxidant agents (i.e. MEJS, GSE, BHA) at all concentration (50-800 ppm) differed significantly ($p<0.05$). BHA has the highest of oxidation inhibition (45-55%), followed by MEJS (38-40%), and the lowest GSE (18-22%) (Figure 2). Phenolic composition of MEJS which consisted of 45.99±0.25% total phenolic (g GAE/100g), 2.28±0.07% flavonoid (g QE/100g), and 26.9±0.07% total tannin (g TAE/100g) may be strongly contributed on inhibition of lipid oxidation in fish oil emulsion system. Meanwhile, GSE contained 95% of oligoproto-anthocianidin (OPC) family (Bulkpowders, 2015).

Maqsood, (2010) reported that tannic acid was very effective to inhibit CD and PoV in menheden fish oil emulsion system. Tannic acid can be strongly inhibited the formation of hydroperoxide and a
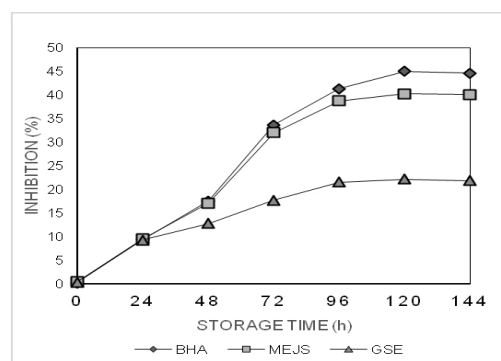

Figure 2. Effect of methanolic extract of Java Plum (*Syzygium cumini* Linn.) seed on inhibition of lipid oxidation in striped catfish (*Pangasius hyphotalamus*) oil-in-water emulsion stored at 35±2ºC for 144 h, compared to BHA and grape seed extract (GSE). For all of the treatment, a final concentration of 200 mg/L was used in the system, (n=3).

conjugated diene, which have a strong correlation with DPPH and ABTS analysis. MEJS has also rich in tannin acid and exhibited a strong inhibition of lipid oxidation according to RSA-DPPH and FRAP methods (Rohadi *et al.*, 2016). A strong correlation between RSA-DPPH activity of MEJS or GSE (25-800 mg/L) and PoV formation during initial 72 h was found, i.e. $y = 5.67x +1.002$, $R^2 = 0.87$ for MEJS and $y = 0.296x + 1.89$, $R^2 = 0.966$ for GSE. A similar pattern was also obtained using FRAP methods, i.e. $y = -10.95x + 20.26$, $R^2 = 0.82$ for MEJS and $y = -18.52x + 36.75$, $R^2 = 0.875$ for GSE. Mielnik *et al.* (2006) reported that commercial GSE (Grapines-High Potency) at 0.4-1.6 g/kg concentration was relatively effective to prevent lipid oxidation in cooked meat.

The difference in PoV inhibition among samples could be influenced by the difference of polarity. McClement and Decker, (2000) reported that hydrophilic antioxidant was less effective in oil-in-water (o/w) emulsion system than the lipophilic and vice versa.

Effect MEJS on TBARS formation

TBARS (thiobarbituric acid reactive substances) method is widely used to measure malondialdehyde (MDA), a secondary product of lipid oxidation, which is reacted with thiobarbituric acid (TBA), to give a red color at λ 532 nm. Malondialdehyde (MDA) are responsible for the formation off flavor, rancid odor and the undesirable taste in the foods. The effect of MEJS on TBARS formation in the emulsion system is presented in Figure 1(c). TBARS of the control sample was greater than 3.0 μM-MDA/L ($p<0.05$) in 72 h of storage, whereas, the samples treated with antioxidant were less than 2.5 μM-MDA/L emulsions. Over 144 h of storage, TBARS of all of the samples were less than 4.0 μM-MDA/L (approximately 0.25

mg MDA/L), and may not change the physical and visual properties of the emulsion. The addition of BHA in the emulsion could inhibit lipid oxidation and exhibited the lowest TBARS value throughout the storage period. Meanwhile, the addition of MEJS in the emulsion exhibited the lower of TBARS than the sample with GSE ($p<0.05$). In addition, the product was quantified using standard curve equation of $y = 0.1509x + 0.029$, $R^2 = 0.9971$ (x-axis = concentration μM-MDA/L; y-axis = absorbance). Nollet and Toldra, (2011) reported that the acceptable limits of TBARS value in fat products was 1.0 mg MDA/kg (1 mg-MDA/kg≈ 13.7055 μM-MDA/kg).

During the initial stage of oxidation, TBARS concentration was 0.6 μM-MDA/L, then increased up to the peak at 72 h ranged between 3.1 μM-MDA/L (GSE and control) and 1.97 μM-MDA/L (BHA and MEJS). After the peak, TBARS decreased in all of the samples, except the control. The decreasing of TBARS could be associated with the inhibition of secondary product of lipid oxidation, particularly the volatile short-chain carbon compounds which easily evaporate. The interaction of thiobarbituric acid (TBA) with many other carbonyl-containing compounds such as carbohydrates, pigment, and amino acid could be also contributing to TBARS values. Malondialdehyde and the short-chain compound as a lipid oxidation product were also labile and easily decomposed into alcohol and organic acids thus, undetectable using TBA analysis (Borneo *et al*., 2009; Maqsood, 2010). Azman *et al*. (2016) reported that the addition of lyophilized bearberry leaf extract (1 g/kg) as an antioxidant in the emulsion food model significantly inhibited lipid oxidation during 20 days of storage.

Conclusion

Methanolic extract of Java Plum seeds (MEJS) had a great potential as the natural antioxidant to inhibit the oxidative deterioration of oil-in-water (o/w) emulsion system. Inhibition activity of lipid peroxidation of MEJS was 28-38%, lower than BHA (40-45%) but stronger than grape seed extract (GSE) 18-23%. MEJS could be significantly able to suppress the TBARS generation due to lipid oxidation.

Acknowledgement

References

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