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Oxidative stability of shrimp oil-in-water emulsions as affected by antioxidant incorporation

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Abstract

Shrimp oil is one of the important sources of n-3 fatty acids, which have been known for health benefit. The incorporation of shrimp oil into food emulsion has gained increasing interest. Since shrimp oil is rich in unsaturated fatty acids, it is susceptible to oxidation, leading to development of off-odor and loss in nutritive value. Thus, this study aimed to investigate the oxidative stability shrimp oil-in-water emulsion (pH 3.6) incorporated with various antioxidants at different levels during the storage at 30°C for 12 days. With increasing storage time, progressive formation of hydroperoxide was found in all samples as evidenced by the increase in peroxide values (PV) ($p < 0.05$). The lower PV was found in those added with antioxidants; however PV values varied with different antioxidants added. Sample added with 200 ppm α-tocopherol alone had the higher PV than others ($p < 0.05$). The increase in thiobarbituric acid reactive substances (TBARS) of the control, sample added with 200 ppm *α*-tocopherol and that containing 500 ppm lecithin were noticeable when storage time increased ($p < 0.05$). Slight increases in TBARS were found in samples added with mixed antioxidants including 50 ppm EDTA + 100 ppm tannic acid, 50 ppm EDTA + 200 ppm α-tocopherol, 50 ppm EDTA + 100 ppm tannic acid + 200 ppm α-tocopherol or 50 ppm EDTA + 100 ppm tannic acid + 500 ppm lecithin + 200 ppm α-tocopherol throughout 12 days of storage ($p < 0.05$). This was concomitant with the decreased formation of volatile compounds and rancid off-odor in emulsion containing mixed antioxidants, especially 50 ppm EDTA + 100 ppm tannic acid and 50 ppm EDTA + 100 ppm tannic acid + 500 ppm lecithin + 200 ppm α-tocopherol. Thus, the use of EDTA in combination with tannic acid could retard the lipid oxidation in the shrimp oil-in-water emulsion more effectively, compared to other combinations of antioxidants tested.

Keywords: Shrimp oil; Emulsion; Antioxidant; Lipid oxidation; Rancidity

Background

Omega-3 (n-3) polyunsaturated fatty acids are nutritionally important and beneficial for individuals suffering from several diseases, e.g. coronary heart disease, diabetes, and immune response disorders (Djordjevic et al. 2004). Due to health benefits of n-3 fatty acids, the incorporation of lipids rich in those fatty acids into various food products has gained increasing attention (Let et al. 2007). Food emulsions, particularly oil-in-water emulsion such as salad dressings, beverages, etc. consist of small lipid droplets dispersed in an aqueous phase (Grigoriev and Miller 2009). In general, vegetable oils have been commonly used for most food emulsions. Thus, the use of marine

oil, which is high in n-3 fatty acid, can be a means to supplement those essential fatty acids in food emulsion. Nevertheless, the susceptibility to lipid oxidation of emulsions generally limits their shelf-life. Lipid oxidation in emulsions is expected to be initiated at the interface between oil and water (McClements and Decker 2006). Transition metals, interfacial area, processing conditions, the type of emulsifier, and droplet size etc., can affect the initiation and propagation of oxidation (McClements et al. 2006). To prevent the deteriorative reaction, the antioxidants with capacity of radical scavenging, metal chelation, and oxygen scavenging have been widely used in foods containing lipids (McClements et al. 2006). Some antioxidants can act to prevent lipid oxidation via various modes of action (Mette et al. 2007). Some proteins used as emulsifier are able to alter the properties of emulsion droplet interface in a manner that increases oxidative stability (Hu, McClements and Decker 2003). Whey protein concentrate exhibited antioxidant properties in emulsion, presumably based on their ability to bind transition metals and scavenge free radicals (Farvin et al. 2010). Furthermore, the polarity and solubility of an antioxidant determine the actual location of antioxidant in food emulsion, which directly influences antioxidative efficacy of antioxidant (Frankel 1996).

Pacific white shrimp (*Litopenaeus vannamei*) is an important commercial species primarily cultured in Thailand (Nirmal and Benjakul 2012). In 2012, the production of 540,000 tons of Thai farmed marine shrimp was reported (FAO, 2012). Hepatopancreas, a byproduct generated from the manufacturing of whole shrimp excluding hepatopancreas, has been reported to contain high content of n-3 fatty acids (Takeungwongtrakul et al. 2012). Oil from hepatopancreas can be used for making emulsion rich in n-3 fatty acids. Due to the large surface area of oil droplet in emulsion, it is more prone to oxidation, leading to rancidity and unacceptability. Therefore, the use of appropriate antioxidants with multiple modes of actions, could prevent lipid oxidation and extend the shelf-life of emulsion rich in n-3 fatty acids effectively.

No information regarding the oxidative stability of shrimp oil-in-water emulsion and the impact of antioxidants in emulsion containing shrimp oil has been reported. Thus, this study aimed to investigate the oxidative stability and rancidity of shrimp oil-in-water emulsion incorporated without and with various antioxidants during storage of 12 days at 30°C.

Methods

Chemicals

p-anisidine, ammonium thiocyanate, α-tocopherol, L-α-phosphatidylcholine (lecithin) and tannic acid (99.5% purity) were purchased from Sigma (St. Louis. MO, USA). Trichloroacetic acid, anhydrous sodium sulphate, ferrous chloride and ethylenediamine tetraacetic acid (EDTA) were obtained from Merck (Darmstadt, Germany). 2-Thiobarbituric acid and 1,1,3,3-tetramethoxypropane were procured from Fluka (Buchs, Switzerland). Methanol, chloroform, petroleum ether, hydrochloric acid, sulphuric acid and ammonium thiocyanate were purchased from Lab-Scan (Bangkok, Thailand). Whey protein concentrate was obtained from I.P.S. International Co., Ltd. (Bangkok, Thailand).

Collection of hepatopancreas from Pacific white shrimp

Hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*) with the size of 50–60 shrimp/kg was obtained from the Sea wealth frozen food Co., Ltd., Songkhla province, Thailand during November, 2012 and January, 2013. Pooled hepatopancreas (3–5 kg) was placed in polyethylene bag. The bag was imbedded in a polystyrene box containing ice with a sample/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within approximately 2 h. The sample was stored at −18°C until use, but the storage time was not longer than 1 month. Prior to oil extraction, hepatopancreas was ground in the presence of liquid nitrogen using a blender (Phillips, Guangzhou, China) for 30 sec. The moisture content of sample was 70.5% as determined by the AOAC method (AOAC, 2000).

Extraction of oils from hepatopancreas

Oil was extracted from ground hepatopancreas by the Bligh and Dyer method (Bligh and Dyer 1959). Hepatopancreas (25 g) was homogenised with the mixture of chloroform: methanol: distilled water mixture (50:100:32.4, v/v/v) at the speed of 9500 rpm using an IKA Labortechnik homogeniser (Selangor, Malaysia) for 2 min at 4°C. The homogenate was then added with 50 mL of chloroform and homogenised at 9500 rpm for another 1 min. Thereafter, 25 mL of distilled water were added and the mixture was further homogenised at the same speed for 30 sec. The homogenate was centrifuged at 3000 × g at 4°C for 15 min and transferred into a separating funnel. The chloroform phase was transferred into the 125 mL Erlenmeyer flask containing 2–5 g of anhydrous sodium sulphate, shaken very well, and decanted into a round-bottom flask through a Whatman No.4 filter paper (Whatman International Ltd., Maidstone, England). Chloroform was then evaporated at 25°C using an EYELA rotary evaporator N-1000 (Tokyo Rikakikai, Co. Ltd, Tokyo, Japan). The residual solvent was removed by nitrogen flushing. Shrimp oil obtained was placed in a vial, flushed with nitrogen gas, sealed tightly and kept at −40°C until use for emulsion preparation.

Preparation of shrimp oil-in-water emulsion

Shrimp oil-in-water emulsion was prepared following the method of Mette et al. (2007) with a slight modification. Whey protein concentrate (2% w/w) and sodium azide (0.02% w/w) were dissolved in 10 mM sodium acetate buffer (pH 3.6). To the solution (90 mL), shrimp oil (10 mL) was gradually added within 1 min, whilst homogenisation was performed continuously using an IKA Labortechnik homogeniser at a speed of 13,500 rpm for totally 5 min.

To study the effect of antioxidants on storage stability of emulsion, several antioxidants, mixed or alone, were added to obtain different final concentrations. Those included 1) control, 2) lecithin (500 ppm), 3) α-tocopherol (200 ppm), 4) EDTA (50 ppm) + tannic acid (100 ppm), 5) EDTA (50 ppm) + α-tocopherol (200 ppm), 6) EDTA (50 ppm) + tannic acid (100 ppm) + α-tocopherol (200 ppm), 7) EDTA (50 ppm) + tannic acid (100 ppm) + lecithin (500 ppm) + α-tocopherol (200 ppm).

Prior to incorporation, lecithin (500 ppm) and α-tocopherol (200 ppm) were dissolved in shrimp oil, whereas EDTA (50 ppm) and tannic acid (100 ppm) were dissolved in 10 mM sodium acetate buffer (pH 3.6).

Oxidative stability of emulsion added with different antioxidants

Different emulsions containing various antioxidants (14 mL) were transferred into the amber bottle and capped tightly. Sample without antioxidant incorporated was used as the control. The samples were stored at 30°C and were taken randomly for analyses at day 0, 2, 4, 6, 8, 10 and 12.

Peroxide value (PV)

Peroxide value (PV) was determined according to the method of Hu et al. (2003) with slight modifications. To 1 mL of emulsion sample, 2 mL of chloroform/methanol (2:1, v/v) were added and mixed using a vortex mixer for 3 s to separate the sample into two phases. The organic solvent phase (20 μL) was mixed with 2.35 mL of chloroform/methanol (2:1, v/v), followed by 50 μL of 30% ammonium thiocyanate (w/v) and 50 μL of 20 mM ferrous chloride solution in 3.5% HCl (w/v). After 20 min, the absorbance of the coloured solution was read at 500 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). Blank was prepared in the same manner, except the distilled water was used instead of ferrous chloride. PV was calculated after blank substraction and expressed as mg cumene hydroperoxide/liter (L) of emulsion. A standard curve was prepared using cumene hydroperoxide with the concentration range of 0.5–2 ppm.

Thiobarbituric acid reactive substances (TBARS)

TBARS were determined as described by Buege and Aust (1978). Emulsion sample (0.5 mL) was mixed with 2.5 mL of a solution containing 0.375% thiobarbituric acid (w/v), 15% trichloroacetic acid (w/v) and 0.25 M HCl. The mixture was heated in a boiling water (95–100°C) for 10 min to develop a pink colour, cooled with running tap water and centrifuged at 3600x*g* at 25°C for 20 min using a centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at the concentrations ranging from 0 to 6 ppm. TBARS were calculated and expressed as mg malonaldehyde/L emulsion.

Measurement of volatile compounds

The volatile compounds in the control emulsion and those containing different sets of antioxidants rendering high oxidative stability were determined after 12 days of storage using a solid-phase microextraction gas chromatography mass spectrometry (SPME GC-MS) following the method of Iglesias and Medina (2008) with a slight modification. Control emulsion at day 0 was also determined for volatile compounds.

Extraction of volatile compounds by SPME fibre

To extract volatile compounds, 1 g of emulsion sample was mixed with 4 mL of deionised water and stirred continuously to disperse the sample. The mixture was heated at 60°C in 20 mL headspace vial with equilibrium time of 10 h. The SPME fibre (50/30 lm DVB/Carboxen™/PDMS StableFlex™) (Supelco, Bellefonte, PA, USA) was conditioned at 270°C for 15 min before use and then exposed to the headspace. The 20 mL -vial (Agilent Technologies, Palo Alto, CA, USA) containing the sample extract and the volatile compounds were allowed to absorb into the SPME fibre at 60°C for 1 h. The volatile compounds were then desorbed in the GC injector port for 15 min at 270°C.

GC–MS analysis

GC–MS analysis was performed in a HP 5890 series II gas chromatography (GC) coupled with HP 5972 mass-selective detector equipped with a splitless injector and coupled with a quadrupole mass detector (Hewlett Packard, Atlanta, GA, USA). Compounds were separated on a HP-Innowax capillary column (Hewlett Packard, Atlanta, GA, USA) (30 m ± 0.25 mm ID, with film thickness of 0.25 μm). The GC oven temperature program was: 35°C for 3 min, followed by an increase of 3°C/min to 70°C, then an increase of 10°C/min to 200°C, and finally an increase of 15°C/min to a final temperature of 250°C and holding for 10 min. Helium was employed as a carrier gas, with a constant flow of 1 mL/min. The injector was operated in the splitless mode and its temperature was set at 270°C. Transfer line temperature was maintained at 260°C. The quadrupole mass spectrometer was operated in the electron ionisation (EI) mode and source temperature was set at 250°C. Initially, full-scan-mode data was acquired to determine appropriate masses for the later acquisition in scan mode under the following conditions: mass range: 25–500 amu and scan rate: 0.220 s/scan. All analyses were performed with ionisation energy of 70 eV, filament emission current at 150 μA, and the electron multiplier voltage at 500 V.

Analyses of volatile compounds

Identification of the compounds was done by consulting ChemStation Library Search (Wiley 275.L). Identification of compounds was performed, based on the retention time and mass spectra in comparison with those of standards from ChemStation Library Search (Wiley 275.L). Quantification limits were calculated to a signal-to-noise (S/N) ratio of 10. Repeatability was evaluated by analysing 3 replicates of each sample. The identified volatile compounds, related to lipid oxidation, including aldehydes, alcohols, ketones, etc., were presented in the term of abundance of each identified compound.

Sensory evaluation

Rancidity of emulsion was conducted by 12 trained panelists with the ages of 25–35. Prior to the evaluation, the panelists were trained three times a week. Panelists were trained with standards for two sessions using a scale of 0 – 15, where 0 and 15 represent no rancidity and the strongest rancidity, respectively. The standard was prepared by incubating shrimp oil at 35°C for 0, 5, 10 and 15 days. Emulsions prepared using those oils, representing the score of 1, 5, 10 and 14, respectively, were used for training. The control emulsion and those added with different sets of antioxidants showing high oxidative stability were tested. Prior to testing, the samples were placed in the sealable plastic cups. The panelists were asked to open the sealable cup and sniff the headspace above the samples for determining rancidity (Yarnpakdee et al. 2012).

ζ-Potential measurements

The ζ-potential of the control emulsion (without antioxidants) and the emulsion added with antioxidants yielding the hightest efficacy in prevention of lipid oxidation was determined using a ζ-potential analyser model ZetaPALS (Brookhaven Instruements Co., Holtsville, NY, USA). Prior to analysis, the emulsion was diluted with deionised water to obtain droplet concentration less than 0.02% v/v. ζ -Potential of samples, adjusted to different pHs with 1.0 M nitric acid or 1.0 M KOH using an autotitrator model BI-

ZTU (Brookhaven Instruments Co., Holtsville, New York, USA), were determined. The values of the zeta potential were calculated by Smoluchowski's formula:

$$\zeta = \frac{4\pi\eta}{\varepsilon}\frac{v}{U/L}$$

where η and ε are the viscosity and dielectric constant of water, respectively, v is the mobile velocity of the oil droplets in the electric field, U is the voltage and L is the distance between the two electrodes. The **ζ**-potential of samples was calculated from the average of ten measurements.

Statistical analysis

Experiments were run in triplicate using three different lots of samples. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test. For pair comparison, *T*-test was used (Steel and Torrie 1980). Statistical analysis was performed using the Statistical Package for Social Science.

Results and discussion

Oxidative stability of shrimp oil-in-water emulsion containing different antioxidants

Peroxide value (PV)

Changes in PV of shrimp oil-in-water emulsion without and with several antioxidants incorporated during 12 days of storage are presented in Figure 1A. The control sample and that added with lecithin had the increase in PV within the first 6 days of storage ($p < 0.05$). Subsequently, a slightly decrease in PV was noticeable up to day 10 ($p < 0.05$). Nevertheless, PV remained constant during 10–12 days ($p > 0.05$). For the sample added with α-tocopherol, PV increased within the first 10 days of storage ($p < 0.05$). The slight decrease in PV was found at day 12 ($p < 0.05$). PV of sample added with combined antioxidants including EDTA + tannic acid, EDTA + α- tocopherol, EDTA + tannic acid + α-tocopherol or EDTA + tannic acid + lecithin + α-tocopherol slightly increased throughout 12 days of storage ($p < 0.05$). The increase in PV of the sample indicated the increasing formation of hydroperoxide, a primary lipid oxidation product. Lipid peroxidation is a chain reaction initiated by the hydrogen abstraction or addition of an oxygen radical, resulting in the oxidative damage of polyunsaturated fatty acids (Repetto et al. 2012). When comparing PV of all samples, it was found that the sample added with α- tocopherol alone contained the higher PV than others after 2 day of storage ($p < 0.05$). Basically, antioxidant mechanism of tocopherols is hydrogen donation to lipid and/or to peroxide radicals during autoxidation. Thus, they act as chain-breaking antioxidants (Karahadian and Lindsay 1989). Tocopheroxyl radicals, formed after hydrogen donation, might abstract hydrogen from lipid hydroperoxides or fatty acids. As a consequence, reactive radicals were generated, rather than being scavenged. This result was in agreement with Kim et al. (2007) who found that α-tocopherol at the levels of 250, 500, 1000, and 1500 ppm acted as the pro-oxidant in soybean oil during 6 days of storage. Tocopherol, a lipid soluble antioxidant, was found in the hepatopancreas of shrimp (Du et al. 2006). Thus, it was more likely that the final concentration of α-tocopherol in the emulsion was higher than the designated values. Tocopherol at high concentration was reported to promote lipid oxidation (Carocho and Ferreira 2013).

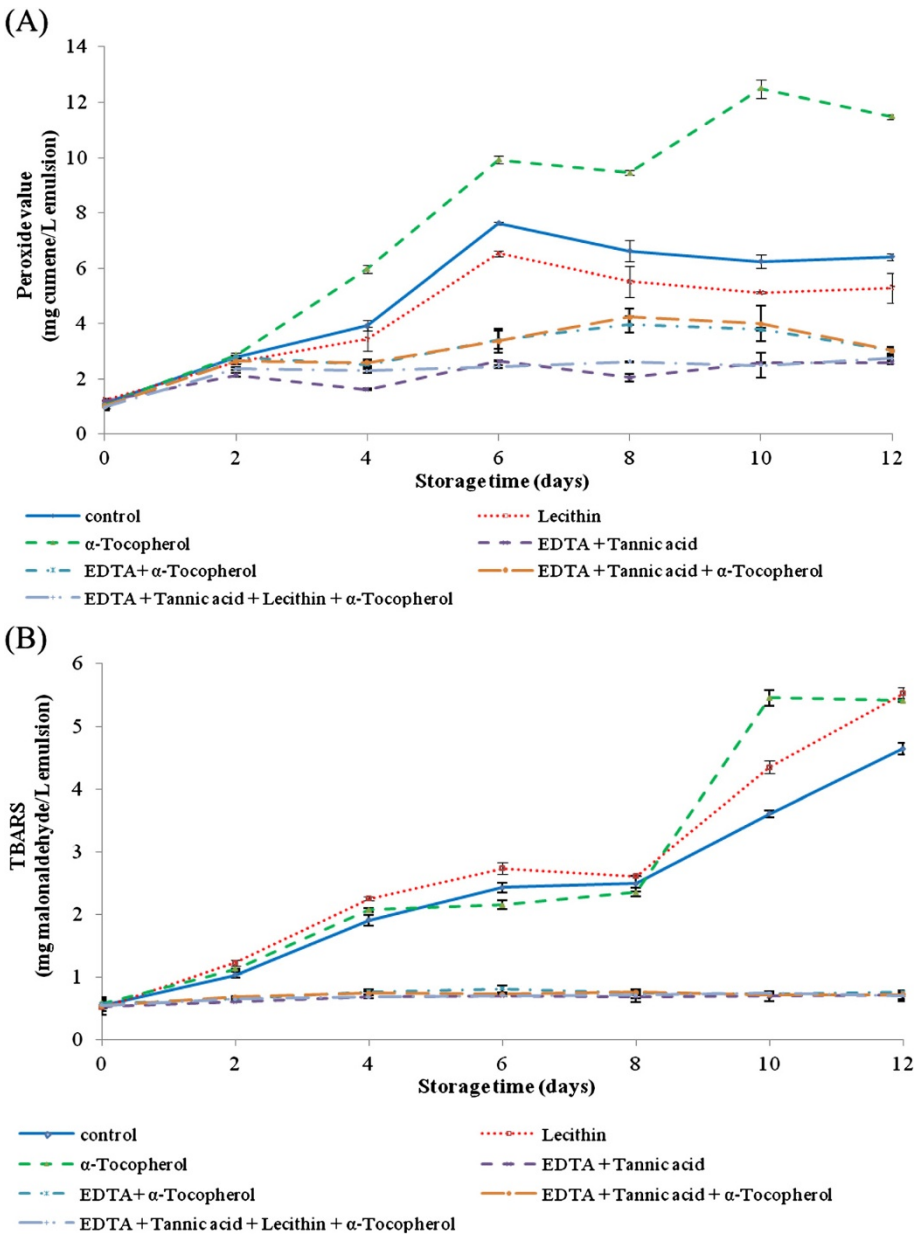

Figure 1 Peroxide values (A) and TBARS values (B) of shrimp oil-in-water emulsion during storage at 30°C of 12 days. Bars represent standard deviations (n = 3).

For other antioxidants used in the present study, they were able to lower the increase in PV in shrimp oil-in-water emulsion, compared with the control ($P < 0.05$). Sample added with lecithin alone showed slightly lower PV than the control after 2 days of storage, indicating its role as antioxidant in the emulsion. Lecithin was found as antioxidant in vegetable oil and fish oil (Chen et al. 2011; Judde et al. 2003). Phosphate residue might act as the chelator of metal, a pro-oxidant in the system. Lecithin possesses the unique chemical structure containing both lipophilic and hydrophilic groups. The antioxidant activity of lecithin was speculated to be due to their ability to form structures within the lipid phase of the emulsions droplets or to chelate metals (Chen et al. 2011). When comparing PV of sample containing EDTA + α-tocopherol and

sample added with α-tocopherol alone, it was found that the sample containing EDTA + α-tocopherol had the lower PV than those of sample added with α-tocopherol alone throughout 12 days of storage ($p < 0.05$). The result suggested that EDTA alone might have antioxidative activity or exhibited the synergistic effect with α-tocopherol. PV in the sample added with EDTA + α-tocopherol was not different from those found in the sample containing EDTA + tannic acid + α-tocopherol throughout 12 days of storage ($p > 0.05$). EDTA has been known as the potential metal chelator (Djordjevic et al. 2004; Wang and Regenstein 2009). In emulsion with acidic pH, transition metal ions become soluble (Mette et al. 2007). In the present study, whey protein concentrate was used as an emulsifier. At pH 3.6 used for emulsion preparation, proteins surrounding oil droplets became positively charged (+ 14 to + 16 mV) as determined by zeta potential analysis. Emulsion had the positive charge throughout storage of 12 days (data not shown). Under such a condition, Fe^{2+} or Fe^{3+} was repelled from oil droplet, thereby preventing the oxidation of polyunsaturated fatty acid in the droplets (Surh et al. 2006). The electrical characteristics of emulsions are important because they determine the droplet stability towards aggregation, as well as their interactions with pro-oxidant cationic transition metal ions (Charoen et al. 2011). Thus, whey protein concentrate partially contributed to the oxidative stability of emulsion in conjunction with antioxidants added. It was noted that EDTA scavenged the metal ions in emulsions, thereby lowering lipid oxidation in emulsion. EDTA has been shown to protect lipid oxidation of foods during storage (Djordjevic et al. 2004). In the present study, EDTA in combination with tannic acid and the mixture of EDTA, tannic acid, lecithin and *α*-tocopherol showed the highest efficacy in retardation of lipid oxidation in emulsion during the storage. It was reported that activity of *α*-tocopherol could be increased in oil when it was conjugated to the polar head group of lecithin. Lecithin could increase partitioning of the reactive portion of *α*-tocopherol into the water phase, which could make tocopherol a more efficient free radical scavenger (Laranjinha and Cadenas 1999). Tannic acid contained a large number of hydrophobic portions, which could align themselves at the oil–water interface and functioned as a hydrogen donor or radical scavenger (Maqsood and Benjakul 2010). In the present study, tannic acid in conjunction with EDTA was therefore considered as the most potential antioxidants for prevention of lipid oxidation in shrimp oil-in-water emulsion.

TBARS

TBARS values of shrimp oil-in-water emulsion without and with added antioxidants incorporated during 12 days of storage are presented in Figure 1B. TBARS levels of the control, sample added with *α*-tocopherol and that containing lecithin increased continuously ($p < 0.05$), and reached the plateau at day 6. After day 8, the marked increases in TBARS were observed up to 12 days ($p < 0.05$), expect for that containing tocopherol, which had the constant TBARS during 10–12 days of storage. The increase in TBARS value indicated the formation of the secondary lipid oxidation products (Chaijan et al. 2006). TBARS have been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes (Nawar 1996). The increases in TBARS were coincidental with the decrease in PV, especially with increasing storage time. This was probably due to the destruction of hydroperoxides into the secondary

oxidation products, including aldehydes, in the later stages of lipid oxidation (Chaijan et al. 2006). Nevertheless, there were no changes in TBARS of sample added with EDTA + tannic acid, EDTA + α-tocopherol, EDTA + tannic acid + α-tocopherol and EDTA + tannic acid + lecithin + α-tocopherol throughout 12 days of storage ($p < 0.05$). The result indicated that EDTA was essential to prevent the oxidation of shrimp oil-in-water emulsion. Nevertheless, radical scavenger, especially tannic acid, was required to terminate the propagation as evidenced by the negligible change in both PV and TBARS throughout the storage of 12 days.

Volatile compounds

Volatile compounds in shrimp oil-in-water emulsion without and with EDTA + tannic acid or EDTA + tannic acid + lecithin + α-tocopherol after 12 days of storage are displayed in Table 1. Volatile compounds in initial emulsion (days 0) was also determined. In general, all compounds detected in emulsion at day 0 were lower in abundance than those found after storage, expect for nonanal, which was obtained only in the initial emulsion. The volatile compounds derived from the oxidation of lipids, due to their low perception thresholds, are amongst those chiefly responsible of sensorial properties of products (Montel et al. 1996). Shrimp lipids from hepatopancreas contained high content of unsaturated fatty acids and linoleic acid (C18:2(n-6)) was the dominant fatty acid, followed by oleic acid (C18:1(n-9)) (Takeungwongtrakul et al. 2012). After storage, the control sample contained several new volatile compounds including 4-ethylcyclohexanol, myrtenol and 4H-pyran-4-one. The higher amount of lipid oxidation products such as benzaldehyde, 3-methoxy-1-(4-methoxyphenyl)-2(E)-propen-1-one, hexadecanal, 2,4-ditert-butylphenol and octadecanal were found in the control sample after 12 days. Several derivatives of aldehyde, ketone and alcohol can be formed by the oxidation of unsaturated fatty acids (Varlet et al. 2006). Aldehydes are the most prominent volatiles produced during lipid oxidation and have been used successfully to follow lipid oxidation in a number of foods. Amongst all the aldehydic compounds, hexadecanal was found to be the major aldehyde in control samples, followed by

Table 1 Volatile compounds in shrimp oil-in-water emulsion in the absence and presence of mixed antioxidants after storage for 12 days at 30°C

Compound	Peak area (Abundance) × 10^7		
	Without antioxidant	EDTA + Tannic acid	EDTA + Tannic acid + Lecithin + α-Tocopherol
Nonanal	ND (300)*	ND	ND
4-Ethylcyclohexanol	270 (ND)	ND	ND
Benzaldehyde	270 (80)	170	100
Myrtenol	310 (ND)	ND	ND
3-Methoxy-1-(4-methoxyphenyl)-2(E)-propen-1-one	500 (300)	480	410
4H-pyran-4-one	44 (ND)	ND	ND
Hexadecanal	1000 (420)	730	770
2,4-Ditert-butylphenol	190 (120)	200	170
Octadecanal	350 (170)	320	240

ND non-detectable.
* Value in the parenthesis represent the abundance of compound in emulsion at day 0.

octadecanal. After 12 days of storage, it was noted that nonanal was not detected, plausibly due to its decomposition or interaction with other compounds in emulsion. It was postulated that higher lipid oxidation and greater decomposition of hydroperoxide occurred during preparation and storage.

However, the formation of volatiles was reduced as the mixed antioxidants were incorporated in shrimp-oil-in-water emulsion. No 4-ethylcyclohexanol, myrtenol and 4H-pyran-4-one were found in emulsion added with mixed antioxidants. Other volatile compounds found in the control were also lower in abundance, compared with those present in the control. The result confirmed that both of EDTA + tannic acid and EDTA + tannic acid + lecithin + α-tocopherol were effective in retarding the lipid oxidation, thereby preventing the formation of volatile lipid oxidation compounds, which contributed to rancidity in shrimp oil-in-water emulsion.

Sensory property

Changes in rancidity of shrimp oil-in-water emulsion without and with EDTA + tannic acid or EDTA + tannic acid + lecithin + α-tocopherol are shown in Table 2. There were no differences in rancidity between all samples at day 0 of storage ($p > 0.05$). At day 12, the control sample showed the higher rancidity than other samples ($p < 0.05$). Rancid odour of the sample added with EDTA + tannic acid was not different from that of the sample incorporated with EDTA + tannic acid + lecithin + α-tocopherol ($p > 0.05$). The result was in agreement with the TBARS values (Figure 1B), in which there was no difference between these two samples. The result suggested that the addition of EDTA + tannic acid or EDTA + tannic acid + lecithin + α-tocopherol could prevent off-odour, mainly rancidity in shrimp oil-in-water emulsion effectively. Also, those antioxidants contributed to prevention of nutritional loss and lowering the risk of health problem associated with lipid oxidation (Hayes et al. 2011).

Conclusion

Shrimp oil-in-water emulsion was susceptible to oxidation during 12 days of storage at 30°C. Antioxidants used had the impact on oxidative stability differently. Tocopherol alone exhibited prooxidative effect in emulsion during the extended storage. EDTA was a potential antioxidant in the emulsion. The use of EDTA in combination with tannic acid effectively inhibited lipid oxidation of shrimp oil-in-water emulsion.

Table 2 Rancid odour of shrimp oil-in water emulsion in the absence and presence of mixed antioxidants before and after storage for 12 days at 30°C

Samples	Day	
	0	12
Control	5.41 ± 1.42[‡] B[†]a[*]	10.78 ± 1.58Aa
EDTA + Tannic acid	5.15 ± 1.05Aa	4.30 ± 1.35Ab
EDTA + Tannic acid + Lecithin + α-Tocopherol	5.18 ± 1.74Aa	4.14 ± 1.63Ab

[‡] Values are given as means ± SD (n = 3).
[†] Different uppercase letters in the same row indicate significant differences ($P < 0.05$).
[*] Different lowercase letters in the same column indicate significant differences ($P < 0.05$).

Abbreviations
n-3: Omega-3; *p*-anisidine: Para-anisidine; α-tocopherol: Alpha-tocopherol; EDTA: Ethylenediamine tetraacetic acid; PV: Peroxide value; TBARS: Thiobarbituric acid reactive substances; SPME GC-MS: Solid-phase microextraction gas chromatography mass spectrometry; EI: Electron ionization; S/N: A signal-to-noise; ζ-Potential: Zeta-potential.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
SB developed the initial idea and designed the study. ST was responsible for conducting experiments and analysis of data. SB and ST carried out the analytical work. ST wrote the manuscript with assistance from SB. SB read and approved the final manuscript.

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