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Effects of tocopherol nanoemulsion addition on fish sausage properties and fatty acid oxidation

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ABSTRACT

Quality of fish sausages can be compromised due to fatty acid oxidation. The effects of tocopherol nanoemulsions (NEs) and coarse emulsions (CEs) on the physicochemical properties and lipid oxidation of fish sausages were assessed during 16-day storage at 4 °C. The addition of 250 and 500 mg/kg tocopherol NEs not only improved fish sausages' quality during cold storage but also effectively retarded lipid oxidation, as evidenced by the significantly lower peroxide value and higher polyunsaturated fatty acid content. Omega-6 fatty acids of tocopherol NEs added sausages were maintained at 31.01% for NE250 and 29.59% for NE500 on day 16. However, CEs did not exert significant antioxidant activity. The particle size of the tocopherol NEs remained below 500 nm when stored at 4 °C for 16 days while the particle size of tocopherol CEs increased from 4 to 6 μm. The smaller particle size, even distribution, and stability of tocopherol NEs might explain the better antioxidant activity of them in fish sausages. Interestingly, NEs encapsulated with 250 mg/kg tocopherol are effective to delay the lipid oxidation and improve the fish sausages quality without altering their texture properties during cold storage.

1. Introduction

Fish sausages are processed food products in which minced fish meat, along with additives, is stuffed into casings to form sausages. It is a healthier alternative to conventional meat sausages, due to its higher polyunsaturated fatty acids (PUFA). However, fish sausages can rapidly develop oxidative rancidity even under chilled or frozen conditions, subsequently resulting in quality loss (Wang, Li, Yuan, & Pavase, 2017). As fish meat contains high amount of unsaturated fatty acids, fish is highly susceptible to lipid oxidation, which results in undesirable changes in organoleptic properties, such as off-flavours.

To delay such quality losses, natural antioxidants such as phytochemicals and polyphenols from spices, herbs or other plants, have been used to reduce the oxidative effects during processing and storage of fish sausages (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007; Jongberg, Torngren, Gunvig, Skibsted, & Lund, 2013; Rysman, Van Hecke, De Smet, & Van Royen, 2016). Tocopherol is a natural antioxidant, and many studies have been done on the dietary supplementation and encapsulation of tocopherol (Raikos, 2017). However, there has been little research on the effect of tocopherol NE when added directly into meat and fish products (Channona & Trout, 2002).

Like other lipophilic compounds, tocopherol is sensitive to oxygen, light, temperature, and could be degraded during food processing or storage (Yang & McClements, 2013). In addition, with tocopherol being highly lipophilic, the dispersion of tocopherol into food products could be difficult. The encapsulation of bioactive compounds into nanoemulsions (NEs) could improve the dispersion into food products, which could increase its bioavailability in foods (Khanniri et al., 2016). Furthermore, loading tocopherol into an oil-in-water NE may improve its chemical stability, and enhance the antioxidant properties and bioavailability (Teixeira et al., 2017). However, the application of NEs in food systems to delay oxidative rancidity has not been understood yet.

As such, this project aimed to study the effect of tocopherol NEs on the physicochemical properties and oxidative stability of fish sausages during refrigerated storage. To achieve these objectives, varying amounts of tocopherol concentrations were added to fish sausages in the form of CEs and NEs, and the oxidation of fatty acid as well as physicochemical properties of fish sausages were evaluated during cold storage at 4 °C. The effects of tocopherol NEs and CEs on fish sausages were examined and compared. The particle size and rheological properties of tocopherol NEs and CEs were investigated to reveal their

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stability during storage.

2. Materials and methods

2.1. Materials

Tocopherols mixed (food grade), polyoxyethylene (20) monooleate (Tween 80, food grade) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Golden pomfret fish (*Trachinotus blochii*), canola oil (100%), and sausage casings were purchased from a local supermarket (Sheng Siong, Singapore).

2.2. Nanoemulsion preparation

Oil phase was prepared by dissolving tocopherol in canola oil with stirring until homogenous. CEs were first produced by mixing tocopherol oil phase (14%, w/w), Tween 80 (6%, w/w) and deionised water (80%, w/w) using a high shear mixer (8000 *g*, 10 min) at room temperature (Akbas, Soyler, & Oztop, 2018). The experiment was done in triplicate. The CEs were then homogenised by an ultrasound system UIP 1000 with Sonotrode BS2d34 (Hielscher, Germany) for 600 s to form NEs (Mehrad, Ravanfar, Licker, Regenstein, & Abbaspourrad, 2018). CEs and NEs prepared were stored at 4 °C and 25 °C for stability study.

2.3. Characterisation of nanoemulsion

The particle size distribution of CE and NE was determined with a Horiba laser scattering particle size distribution analyser (LA-950 V2, Horiba Ltd., Kyoto, Japan) (Dong et al., 2016).Three samples from each group were measured and averaged to get the particle size. Rheological analysis and viscosity of the NEs stored at 4 °C and 25 °C were determined using Anton Paar MCR 102 controlled-stress rheometer (Anton Paar, Graz, Austria) according to Teixeira et al. (2017). Apparent viscosity was fitted according to the Power Law model, using the following equation:

$$\tau = K \cdot \gamma^{n-1}$$

Where τ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency index (Pa·s^n) and n is the flow behaviour index.

2.4. Fish sausages preparation

Golden pomfret was purchased from a local supermarket, and transferred to the lab within 30 min after viscera removal in an ice-storage bag. The fillets were obtained and minced in a homogeniser, followed by mixing with potato starch (7.50%, w/w), salt (1.25%, w/w) and tocopherol emulsions (10%, w/w). Control sausages were prepared using water instead of emulsion. There were 6 groups of treatment sausages, which were CE0, CE250, CE500 (0, 250 and 500 mg tocopherol coarse emulsion/kg sausage) and NE0, NE250, NE500 (0, 250 and 500 mg tocopherol nanoemulsion/kg sausage) as shown in Table 1. The mixtures were then manually stuffed into 30 mm pig small intestines using a manual sausage extruder. Sausages were then heated in an oven at 150 °C for 20 min, and kept under 4 °C after cooling down to room temperature. Each group contained three samples.

Table 1
Composition of tocopherol emulsions.

	NE0 & CE0 (%, w/w)	NE250 & CE250 (%, w/w)	NE500 & CE500 (%, w/w)
Tocopherol	0	0.25	0.5
Canola oil	14	13.75	13.5
Tween 80	6	6	6
Deionised water	80	80	80

2.5. pH, texture and colour analyses

Each sample (2 g) was homogenised with distilled water (8 mL), and the pH was measured. Fish sausage samples were cut from the center into cubes of 15 mm for texture profile analysis by TA-XT2i texture analyser (Stable Micro Systems Co. Ltd., UK) (Mohtar, Perera, Quek, & Hemar, 2013). Colour of fish sausage samples was determined using a Minolta Colorimeter CM-3500d (Konica Minolta Inc., Japan) (Kittiphattanabawon, Benjakul, Sinthusamran, & Kishimura, 2016). Colour difference (ΔE^*) was calculated according to the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

ΔL^*, Δa^* and Δb^* are the differences between the colour parameter of the sample and that of a white standard ($L^* = 93.63$, $a^* = -0.92$ and $b^* = 0.42$).

2.6. Lipid extraction and POV determination

After removing and discarding the outer casing, the fish sausages were minced in a food blender. Each sample (5 g) was mixed with a chloroform/methanol/deionised water mixture (1:2:1 v/v, 40 mL) and homogenised. The homogenate was further treated with chloroform (10 mL) and water (5 mL), and centrifuged at 5000 *g* for 15 min at 4 °C. The supernatant was transferred to a flask and the organic phase was decanted into a conical flask containing 0.4 g anhydrous sodium sulfate. After vigorous shaking, the solution was filtered into a round-bottom flask, and subjected to rotary evaporation at 40 °C to obtain the fish oil extract. The POV of extracted fish oil (0.3 g) from each fish sausage sample was assessed and expressed as milliequivalents of peroxide/kg oil (meq O_2/kg oil) (Balzan et al., 2017).

2.7. Determination of fatty acid composition

Fatty acids were derivatised according to O'Fallon, Busboom, Nelson, and Gaskins (2007). Fatty acid methyl esters (FAMEs) were stored at −20 °C until GC analysis. The resultant FAME samples were injected into a Shimadzu GC-MSQP2010 Ultra Gas Chromatography-Mass Spectrometer coupled with a Shimadzu AOC-5000 Autosampler and a Shimadzu Flame Ionization Detector-2010 (Shimadzu Corporation, Kyoto, Japan) (Intarasirisawat, Benjakul, Visessanguan, & Wu, 2014). The solutions containing FAMEs (1 μL) were injected and analysed on a BPX70 (70% cyanopropyl polysilphenylene-siloxane) capillary column (SGE Analytical Science Pte. Ltd., USA). Injection port temperature was set at 250 °C while detector temperature was set at 270 °C. The initial temperature of the column was set at 170 °C and increased to 225 °C at a rate of 1 °C/min, and then held at 225 °C for an additional 20 min. Helium was used as the carrier gas with a split ratio of 10:1 (v/v) (Intarasirisawat et al., 2014). Fatty acids were identified and quantified based on chromatographic retention times using reference standard Supelco 37 component FAME mix (Sigma Aldrich Chemical Co., St. Louis, MO, USA). Results obtained were presented as percentage of total fatty acids.

2.8. Statistical analysis

All experiments were carried out in triplicate and the results were reported as mean ± standard deviation. The differences among different groups were determined by analysis of variance (ANOVA) and Duncan's multiple range test using SPSS software, with $P < 0.05$ being statistically significant.

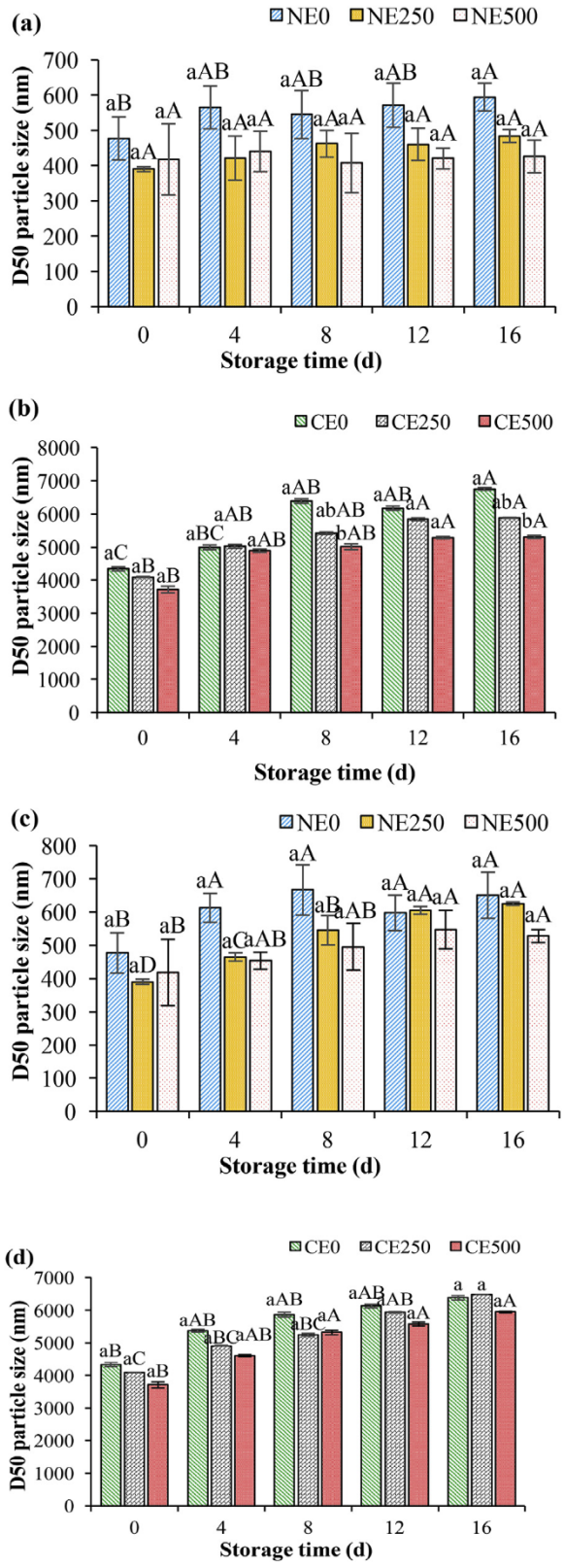

Fig. 1. Average particle size (D50) of tocopherol NE (a) and CE (b) when stored at 4 °C, and of NE (c) and CE (d) when stored at 25 °C during storage period of 16 days.

*Values with different lower case letters at the same day and capital letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively. NE0, NE250, and NE500 represent nanoemulsions with 0, 250, and 500 mg/kg tocopherol, respectively; CE0, CE250, and CE500 represent coarse emulsions with 0, 250, and 500 mg/kg tocopherol, respectively.

3. Results and discussion

3.1. Stability of tocopherol CEs and NEs during storage

3.1.1. Changes of particle size

Fig. 1 shows the particle size of CEs and NEs stored under 4 and 25 °C. In Fig. 1, no significant increase ($P < 0.05$) was observed in particle sizes for NE250 and NE500, whereas CE0, CE250, CE500 were found to show an appreciable increase under 4 °C. This shows that NEs were more stable against droplet aggregation during cold storage. It has been widely reported that NEs exhibit increased stability to aggregation and subsequently gravitational separation due to their relatively smaller particle size (An, Yan, Li, & Li, 2014; Zha, Dong, Rao, & Chen, 2019). Comparatively, CEs are more susceptible to droplet growth due to either coalescence or Ostwald ripening. It was observed that particle size for NE0 showed a significant increase from 476.7 nm on day 0–594.3 nm on day 16 under 4 °C; however, there was no significant difference between different groups. The particle size of tocopherol NEs increased slightly under 25 °C; however, it still remained in nano ranges during the 16-day storage.

3.1.2. Changes in rheological properties

The stability of NEs is also associated with its rheological properties. The flow behaviour index n and consistency index K of various emulsions are presented in Table 2 while the viscosity of tocopherol NEs and CEs is shown in Fig. 2. Table 2 showed that all emulsions followed a shear thinning behaviour, as all n values were below 1, at both 4 and 25 °C. This result was consistent with that reported by Lu, Zheng, and Miao (2018), in which ß-carotene enriched NEs with 10% oil phase were also found to exhibit shear-thinning behaviour. The consistency index K gives an indication of the viscosity. K values for all emulsions were within the range of 0.00218–0.00282 Pa·s^n, which were found to be similar to α-tocopherol NEs prepared by Teixeira et al. (2017). As seen from Fig. 2a, viscosity for emulsions stored at 4 °C was observed to be similar among all treatment groups during 16-day storage. NEs were observed to be more stable than CEs at 4 °C, in which CE0, CE250 and CE500 showed slight decreases in viscosity after 8 days ($P < 0.05$). It was found that viscosity of the treated emulsions, when stored at 25 °C (Fig. 2b), generally showed an appreciable increase after 8 days of storage, before decreasing again at day 16.

3.2. Physical changes of fish sausages during storage

3.2.1. Changes in pH

Changes in the pH of fish sausages treated with tocopherol NEs or CEs are shown in Fig. 3. From Fig. 3, initial pH values of all fish sausage samples at day 0 were around pH 6.5, with no significant differences among the groups ($P < 0.05$). It was observed that pH generally

Table 2
Rheological properties of nanoemulsions and coarse emulsions stored at 4 and 25 °C. *

	Flow behaviour index n		Consistency index K ($\times 10^{-3}$ Pa·s^n)	
	4 °C	25 °C	4 °C	25 °C
NE0	0.85 ± 0.21^a	0.98 ± 0.01^{ab}	2.45 ± 0.14^{ab}	2.33 ± 0.14^a
NE250	0.96 ± 0.01^a	0.97 ± 0.02^{ab}	2.72 ± 0.22^a	2.39 ± 0.21^a
NE500	0.86 ± 0.17^a	0.88 ± 0.14^{ab}	2.82 ± 0.79^a	2.48 ± 0.21^a
CE0	0.99 ± 0.01^a	0.85 ± 0.14^b	2.25 ± 0.13^b	2.4 ± 0.47^a
CE250	0.99 ± 0.01^a	0.99 ± 0.02^{ab}	2.21 ± 0.13^b	2.18 ± 0.24^a
CE500	0.98 ± 0.01^a	0.99 ± 0.01^a	2.36 ± 0.16^b	2.28 ± 0.11^a

*Within each column, groups with different small case letters are significantly different ($P < 0.05$). NE0, NE250, and NE500 represent 0, 0.25, and 0.5 g tocopherol addition, respectively, in 100 g nanoemulsions; CE0, CE250, and CE500 represent 0, 0.25, and 0.5 g tocopherol addition, respectively, in 100 g coarse emulsions.

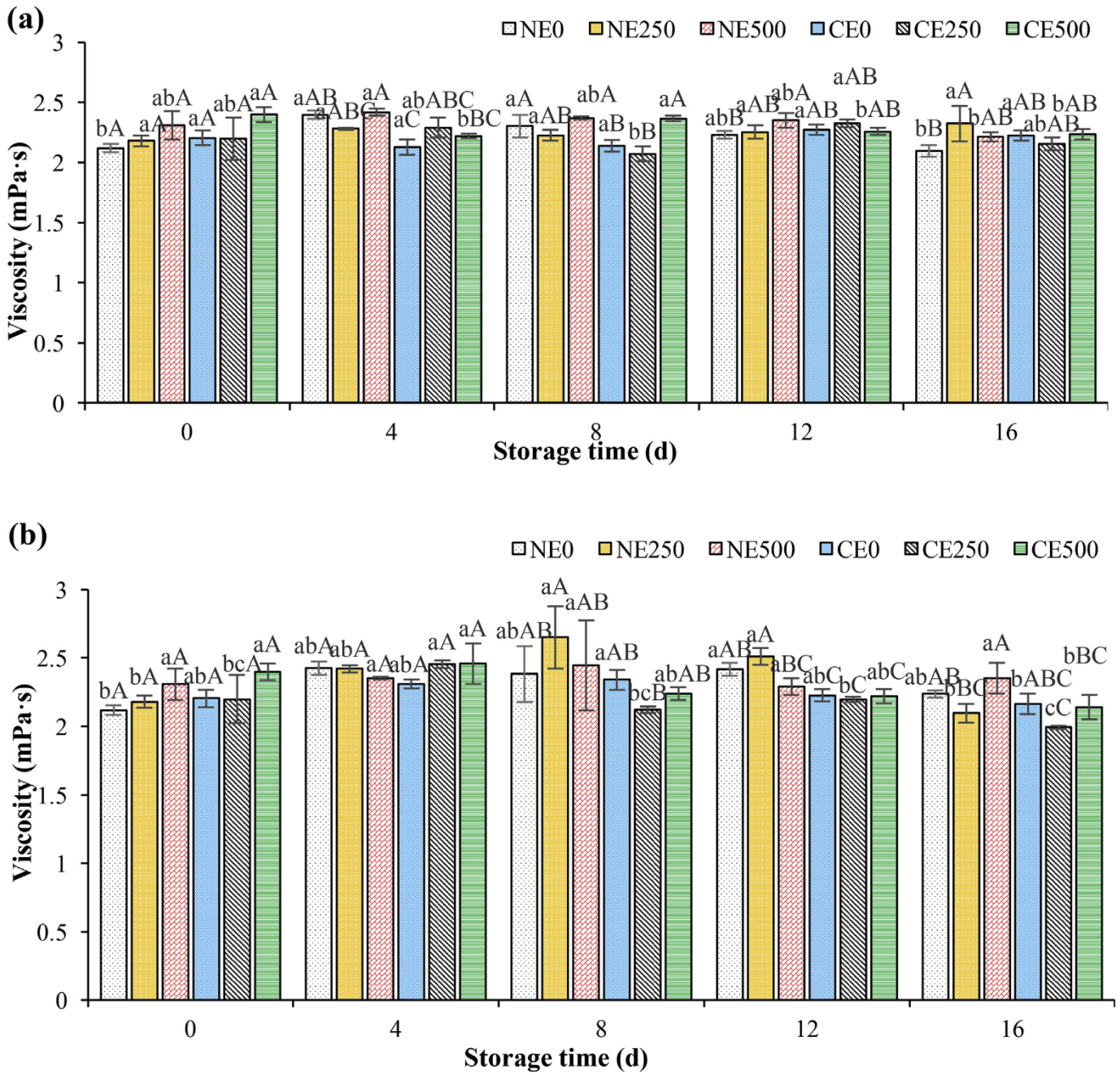

Fig. 2. Viscosity of tocopherol nanoemulsions and coarse emulsions over 16-day storage at 4 °C (a) and 25 °C (b).
*Values with different lower case letters at the same day and capital letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively. NE0, NE250, NE500 represent nanoemulsions with 0, 250, 500 mg/kg tocopherol, respectively; CE0, CE250, CE500 represent coarse emulsions with 0, 250, 500 mg/kg tocopherol, respectively.

maintained constant from day 0 to day 8, before showing appreciable increases from day 12 to day 16. On day 16, control samples exhibited the highest pH of 7.57, which was not significantly different from the pH of NE0. The increase of pH could be attributed to the accumulation of alkaline compounds such as ammonia or trimethylamine from possible microbial spoilage or from the degradation of protein and amino acids within the fish sausages (Georgantelis et al., 2007). It was also found that a fermented and rancid taste of frankfurters was detected after 18 days of 4 °C storage, indicating significant changes of sensory properties and spoilage (Ranucci, Miraglia, & Branciari, 2018). Comparatively, on the 16th day, it was observed that pH values of NE250 (pH 7.10) and NE500 (pH 7.13) were significantly lower than that of control (pH 7.57). This suggests that the spoilage deterioration within the sausages treated with NEs containing 250 or 500 mg/kg tocopherol was significantly retarded.

3.2.2. Changes in textural properties

The effect of tocopherol treatment on textural properties of fish sausages is shown in Fig. 4. At day 0, textural parameters showed no observable differences among all samples, indicating that addition of tocopherol emulsions did not affect the intrinsic structure and texture of fish sausages. In general, as observed from Fig. 4, no significant changes in textural properties were observed in fish sausages with different treatments over the 16-day storage period.

Hardness of fish sausages increased slightly in NE250 and NE500 sample after 12 days of storage, with NE250 increasing from 16.70 to

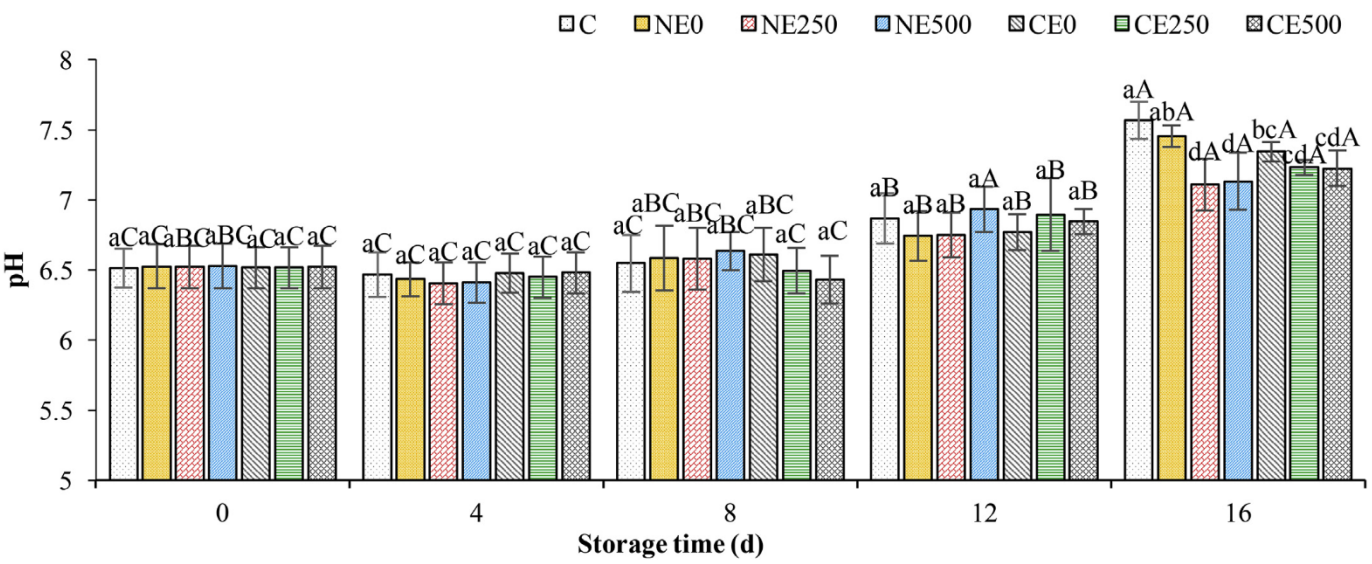

Fig. 3. Effects of tocopherol emulsion on the pH of fish sausages during 16-day cold storage.
*Values with different lower case letters at the same day and capital letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively. C, control with canola oil emulsion; NE0, NE250, NE500 represent nanoemulsions with 0, 250, 500 mg/kg tocopherol, respectively; CE0, CE250, CE500 represent coarse emulsions with 0, 250, 500 mg/kg tocopherol, respectively.

20.57 N, and NE500 increasing from 17.58 to 19.95 N (Fig. 4). This hardening of fish sausages could be due to the decrease in moisture content of sausages, which was also observed by Sriket, Sriket, and Nalinanon (2015) through the use of Ya-nang leaves in tilapia fish sausages.

As observed from Fig. 4, no significant changes in springiness, cohesiveness and chewiness were observed in fish sausages. The lack of significant changes in textural attributes could be due to small extent of protein microstructural changes, which is associated with changes in the inter- and intra-molecular interactions of peptide chains. Hence,

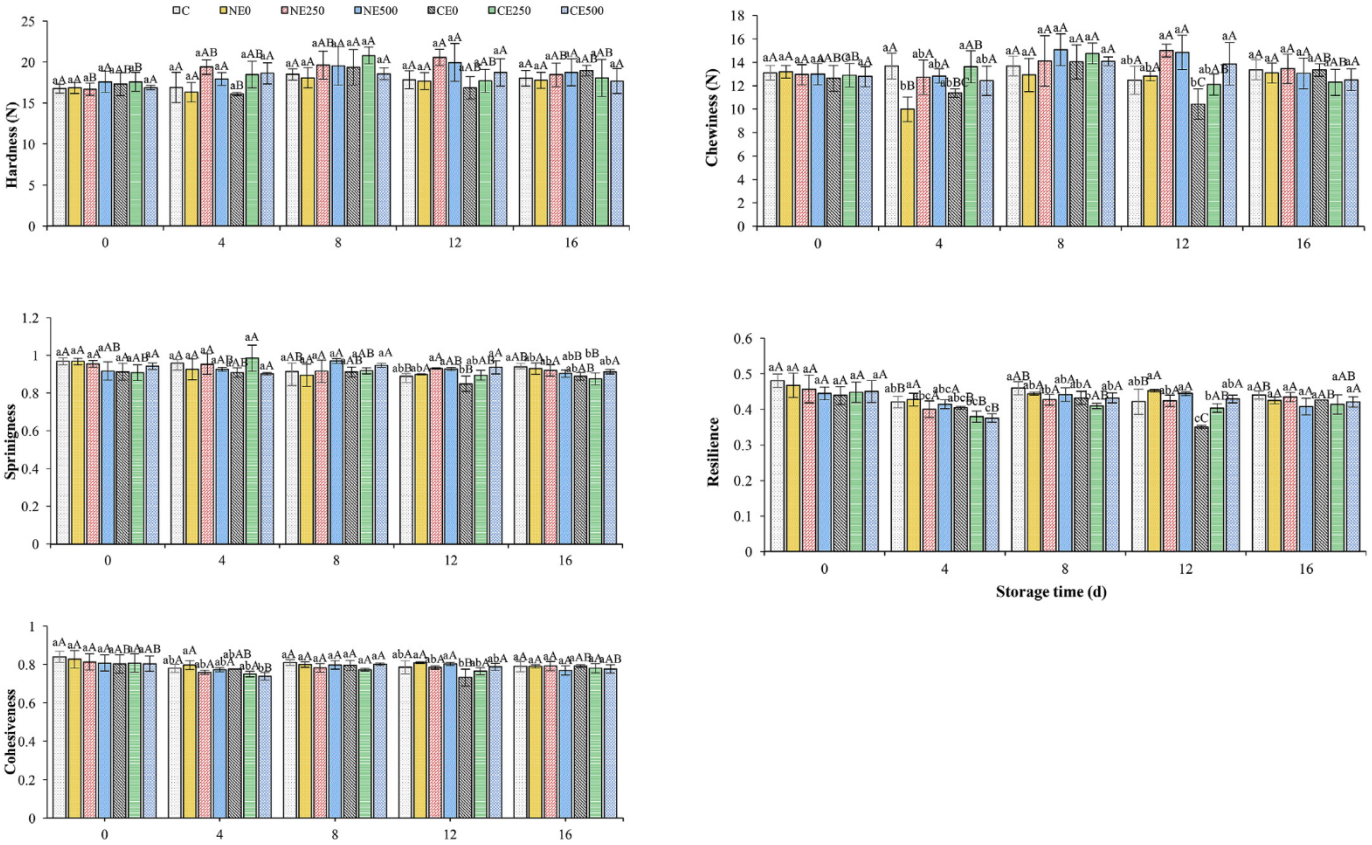

Fig. 4. Effects of tocopherol emulsion on the texture properties of fish sausages during 16-day cold storage.
*Values with different lower case letters at the same day and capital letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively. C, control with canola oil emulsion; NE0, NE250, NE500 represent nanoemulsions with 0, 250, 500 mg/kg tocopherol, respectively; CE0, CE250, CE500 represent coarse emulsions with 0, 250, 500 mg/kg tocopherol, respectively.

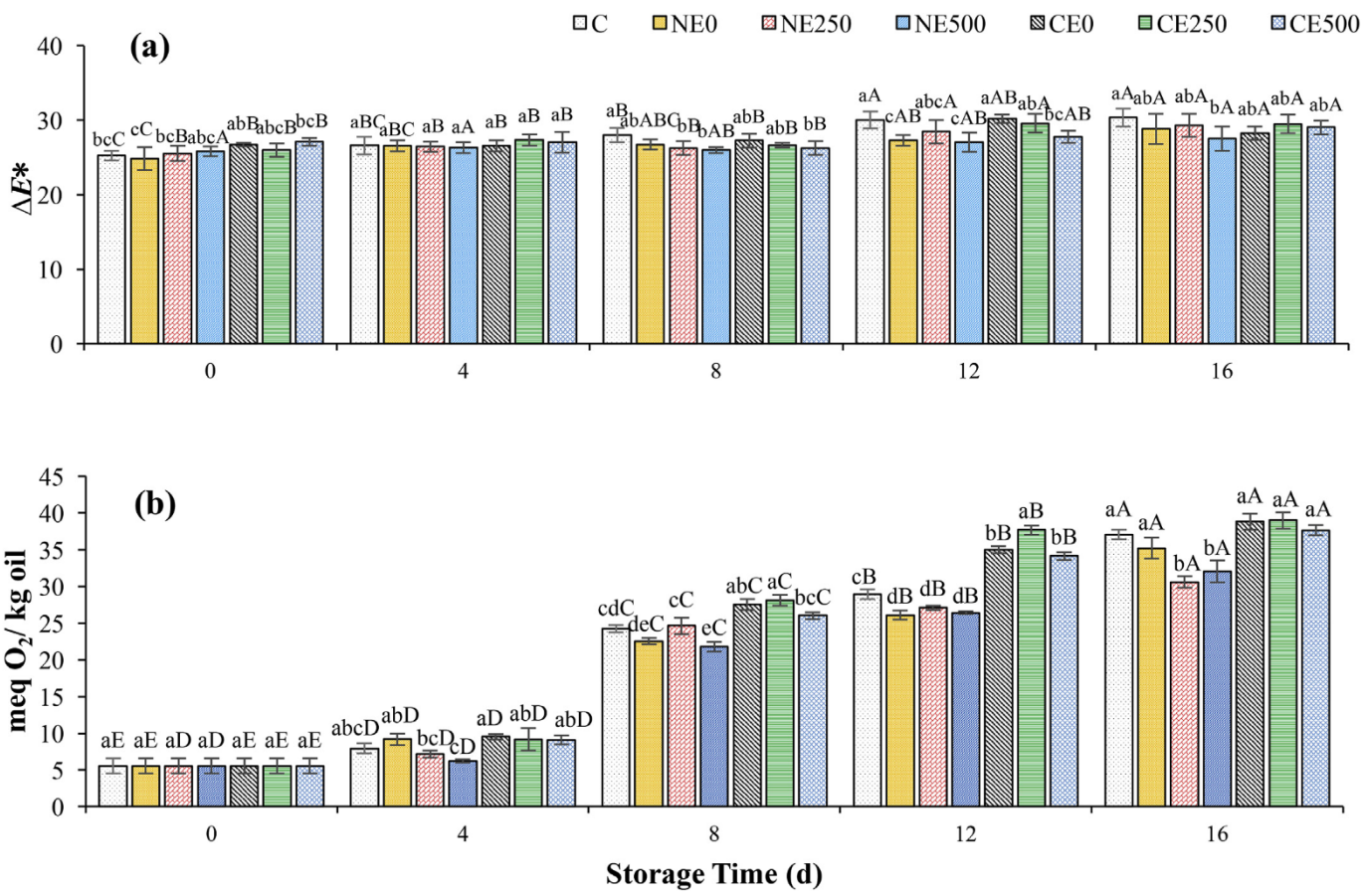

Fig. 5. Effects of emulsion addition on the (a) colour changes and (b) peroxide value (POV) of fish sausages during 16-day cold storage.
*Values with different lower case letters at the same day and capital letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively. C, control with canola oil emulsion; NE0, NE250, NE500 represent nanoemulsions with 0, 250, 500 mg/kg tocopherol, respectively; CE0, CE250, CE500 represent coarse emulsions with 0, 250, 500 mg/kg tocopherol, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

conformational changes within the protein matrix were not sufficient to cause observable changes of texture properties (Feng, Fu, & Yang, 2017). This is also observed by Wang, Li, Yuan, Lin, and Pavase (2017) on the textural parameters in fish mince gel added with polyphenols, alpha-tocopherol, and ascorbic acid, in which fish mince showed a marked decrease in breaking force only after 30 days of frozen storage.

3.2.3. Changes in colour

The effects of tocopherol treatment on the colour changes of fish sausages are shown in Fig. 5. On day 0, ΔE^* values for NE or CE treated samples were not significantly different from control samples, indicating that addition of tocopherol emulsion did not change the colour of sausages. The high value of ΔE* on day 0 for all sausages could be due to the haem pigments from golden pomfret (Joseph, Nair, & Suman, 2015).

In Fig. 5, control sausage samples became darker over the storage period, with ΔE^* value increasing significantly from 25.3 at day 0–30.4 at day 16. Within NE groups, ΔE^* of NE0 and NE250 also increased. In contrast, NE500 sausage samples, which had the highest amount of tocopherol, showed no significant changes in ΔE^* values at the end of storage compared with day 0 samples. This suggests that 500 mg/kg tocopherol NE was able to retard the colour changes.

Myoglobin, accounts for around 80% of the colour of the fish, is prone to oxidation and can be oxidised to form brown metmyoglobin. Lipid oxidation products or free radicals can also accelerate the oxidation of myoglobin, causing darkening of the meat colour (Joseph et al., 2015). Therefore, 500 mg/kg tocopherol NE could have exerted significant antioxidant effects and retarded myoglobin oxidation to maintain the colour of fish sausages during cold storage. Although CE500 contained the same amount of tocopherol as NE500, an increase in ΔE* values was observed at the end of storage, with significant differences from samples at day 0. Hence, it can be deduced that the instability of CEs, as shown by the increase of particle sizes in Fig. 1, could have affected the antioxidant activity of tocopherols.

3.3. Effects on lipid oxidation of fish sausages

3.3.1. Fatty acid composition

The impact of tocopherol emulsions on lipid oxidation in fish sausage samples is shown in Supplementary Table S1. Fish sausages were found to contain a wide variety of fatty acids, with higher proportion of unsaturated fatty acids (UFA) compared with saturated fatty acids (SFA). For samples at day 0, PUFA were 33.7%, which were the most abundant during the storage, followed by monounsaturated fatty acids (MUFA) of 29.9%. Linoleic acid (C18:2 n-6) was found to be the dominant PUFA within the sausages at 30.2%, while the major MUFA was oleic acid (C18:1 n-9) of 19.87% on day 0. It was reported that linoleic acid and oleic acid in sea-cage cultured golden pomfret was 20.73% and 25.70%, respectively (He et al., 2019), which was comparable with our results. Palmitic acid (C16:0) was found to be the dominant SFA at 21.7% on day 0, which was consistent with previous finding that palmitic acid was 24.96% in golden pomfret muscle (He et al., 2019).

It was observed that refrigerated storage had a significant effect on

the fatty acid composition of fish sausages ($P < 0.05$). From Supplementary Table S1, PUFA were found to decrease significantly ($P < 0.05$) in control samples from 33.70% at day 0–22.51% at day 16, which indicated the susceptibility of PUFA to oxidation due to oxidative and hydrolytic reactions (Channona & Trout, 2002). It was observed that NE250 and NE500 samples contained a significantly higher proportion of PUFA after 16 days of refrigerated storage, which were 32.80% and 31.37% respectively compared to control (22.51%) and NE0 (22.14%), indicating the protective effects of tocopherol NE towards lipid oxidation during storage. It was also noted that no significant difference was observed between NE250 and NE500 samples, indicating that it is not necessary to increase the tocopherol from 250 mg/kg to 500 mg/kg for better antioxidant effect.

However, the maintaining of PUFA was not observed in the CEs added groups. It was observed that CE250 and CE500 did not show any differences in PUFA content as compared to control group on day 16. This could be attributed to the different stability between tocopherol NEs and CEs as shown by the increase in particle size of CEs when stored at 4 °C over 16 days (Fig. 1b). Possible coalescence of the CEs could have occurred within the fish sausages, which damaged the uniform distribution of tocopherol in the food system, leading to the loss of antioxidant effects (Salminen, Herrmann, & Weiss, 2013). The aggregation of oil particles and formation of oil pockets within fish sausages would inhibit tocopherol from exerting its antioxidant effect (Rahimabadi, Jasour, Ehsani, Rahnama, & Arshadi, 2012). The schematic diagram of the effects of tocopherol emulsion on fish sausage properties and fatty acids oxidation is shown in Fig. 6.

In Supplementary Table S1, it was observed that linoleic acid (C18:2 n-6) in control, NE0 and CE treated samples decreased significantly. However, linoleic acid in NE250 and NE500 samples were well preserved, with a proportion of 29.94% and 28.67% respectively on day 16 ($P < 0.05$). A similar trend was observed in oleic acid (C18:1 n-9) as well, with a higher proportion of oleic acid observed in NE250 (28.27%) and NE500 (28.8%) samples, as compared to control of 22.91%. It was noticeable that the amount of EPA was only 0.1%, while DHA was not detected on day 0. This result was different from previous findings that EPA and DHA was 2.76% and 6.17% in broadhead catfish sausages, which may be due to the mild thermal processing of pre-incubation at 55 °C for 40 min and cooking at 80 °C for 15 min (Intarasirisawat et al., 2014). It is worthy to mention that the EPA and DHA in raw golden pomfret was 2.61% and 2.88%, respectively, indicating the degradation of EPA and DHA after heating in oven at 150 °C for 20 min. In addition, on day 16, NE250 and NE500 samples had the highest proportion of n-3 and n-6 fatty acids while there were no significant differences between NE250 and NE500 groups. The tocopherol NE maintained the n-3 and n-6 fatty acids in fish sausages during cold storage, and further addition to 500 mg/kg was not essential to further improve the antioxidant effects.

3.3.2. Peroxide value (POV)

Peroxide values of fish sausage samples with different tocopherol NEs and CEs are shown in Fig. 5. Peroxide values for all treatment groups generally increased over the storage period. A slight increase of POV was observed during the first 4 days of storage for all groups, and the POV of NE500 sausages on day 4 was the lowest among all groups. It can be observed that there was a sharp increase for all sausage samples from day 8 onwards (Fig. 5). An increase in POV indicates the presence of primary lipid oxidation, mainly due to hydroperoxide formation (Intarasirisawat et al., 2014). It was interesting to find out that the POV of NE0 group was lower than that of CE0, CE250, CE500 on day 8 and day 12. It could be due to that the unsaturated fatty acids such as oleic acid and linoleic acid of canola oil in nanoemulsions could exert antioxidant activity towards lipid oxidation in fish sausages (Quiroga, Nepote, & Baumgartner, 2018). It was noticeable in Fig. 1 that the particle size of coarse emulsions was around 10 folds of the particle size of nanoemulsions, and the nanoemulsions with higher specific surface area might be easier to be oxidised so as to exert antioxidant activity and maintain the POV values of fish sausages. The antioxidant activity of NE0 could be further investigated and verified in the next work.

As for the control group, water was added to replace emulsions, which decreased the total lipid content in the sausages. Therefore, the decreased lipid content in control sausages may contribute to the lower POV value compared with CE added sausages on day 12. Longer shelf-life study is needed for better clarification. On day 16, it was observed that NE250 and NE500 fish sausages had the lowest POV values of 30.5 and 32.0 meq O_2/kg oil, respectively, which were significantly lower than the control of 40.3 meq O_2/kg oil ($P < 0.05$). On the contrary, from day 8 to the end of storage, all CE treated samples exhibited significantly higher POV than NE250 and NE500 samples, indicating that antioxidant property of tocopherol was not effective when added as CEs.

4. Conclusions

In this study, tocopherol NEs with concentrations of 250 and 500 mg/kg were able to delay lipid oxidation of fish sausages during refrigerated storage. Samples treated with tocopherol NEs had lower peroxide values and higher polyunsaturated fatty acids at the end of storage than the control. However, CEs neither exerted antioxidant activity nor prevented the PUFA from decreasing in fish sausages, which could be due to the lability and uneven distribution of tocopherol

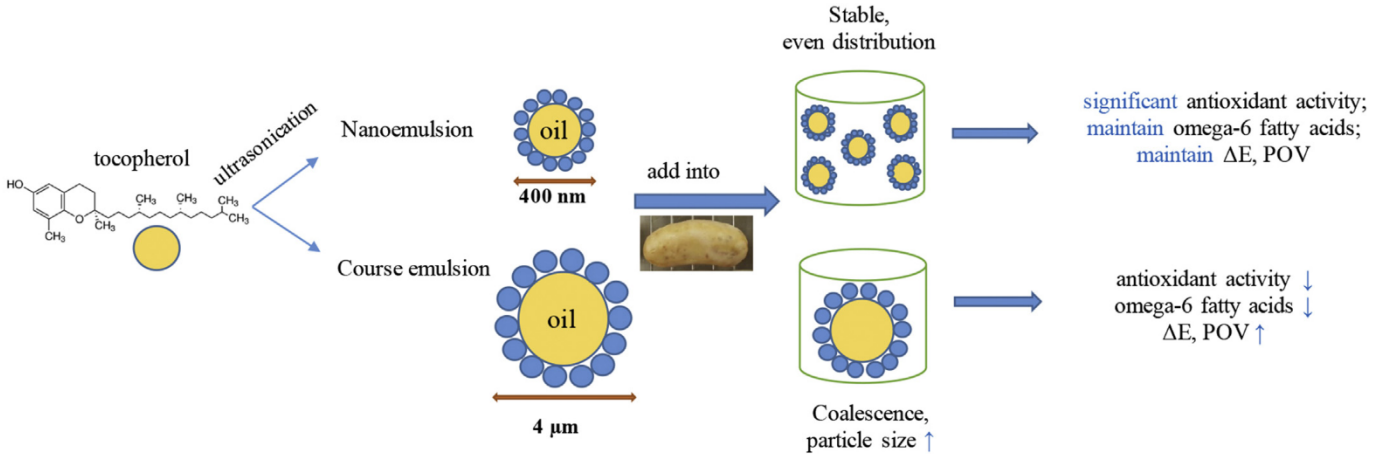

Fig. 6. Effects of tocopherol emulsion on fish sausage properties and fatty acid oxidation.

CEs in fish sausages during cold storage. Treatment of 500 mg/kg tocopherol NEs/kg sausage was effective in preventing colour changes after 16-day refrigerated storage, but there were no significant effects in texture and pH. Tocopherol NEs were more stable than CEs when stored at both 4 and 25 °C, and exerted better effects on delaying lipid oxidation than CEs. Moreover, the antioxidant effect was not dose-dependent. Hence, treatment of 250 mg/kg tocopherol NEs/kg sausage is effective in delaying the lipid oxidation of fish sausages and improving their quality during cold storage. Thus, they can be applied in fish sausages' manufacturing and production.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.108737.

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