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RESEARCH PAPER

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Use of Cashew (*Anacardium occidentale* L.) Leaf Extract for Prevention of Lipidoxidation in Mayonnaise Enriched with Fish Oil

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Abstract

Effects of cashew leaf extract (CE) or BHT at levels of 100 and 200 mg/kg on the oxidative stability of mayonnaise enriched with fish oil during storage of 30 days at 30°C were investigated. Samples containing either CE or BHT had the lower peroxide value (PV), thiobarbituricacidreactive substances (TBARS) and *p*-anisidine (AnV) values throughout the storage, compared to the control (P<0.05). Among all samples, that containing 200 mg/kg BHT exhibited the lowest PV, TBARS and AnV values (P<0.05). At day 30, a lower abundance of selected volatile compounds was found in mayonnaise added with 200 mg/kg CE as compared to that of the control. Addition of CE (at 200 mg/kg) decreased L* value but increaseda*, b* and ΔE* values (P<0.05). However, CE at both levels were effective in retard the formation of rancid odor and fishy odor in the mayonnaise. Total viable count of all samples was below 10^3 CFU/g throughout the storage. Thus, the use of CE at 200 mg/kg could retard lipid oxidation and the formation of fishy odor and rancid odor of mayonnaise enriched with fish oil during the storage of 30 days at 30°C.

Introduction

Fish oil, an excellent food ingredient rich in long-chain polyunsaturated fatty acids (PUFA), has been fortified in food products such as bread, yoghurt drinks and milk as well as mayonnaise (Ganesan, Brothersen, &Mcmahon,2014).These fatty acids are known to have several benefits for human health, e.g. reducing the risks of cardiovascular diseases, carcinogenesis and allergies (Bakry *et al*., 2016). However, PUFAs are prone to oxidation, which is associated with the changes in taste, odor, texture, appearance and shortened shelf-life. Moreover, it also causes some diseases in human beings such as cardiovascular disease, cancer and neurological disorders and aging process (Essick & Sam, 2010).

Mayonnaise is an oil-in-water (o/w) emulsion having high fat content where egg yolk is used as an emulsifier (Li, Wang, Jin, Zhou, & Li, 2014). Lipid oxidation of mayonnaise is generally initiated at the interface between the oil and water and progresses in the oil phase (Sørensen, Nielsen, Hyldig, & Jacobsen, 2010). There are many factors that can potentially influence the rate of lipid oxidation in oil-in-water emulsions. Those include fatty acid composition, aqueous phase pH and ionic composition, type and concentrations of antioxidants, prooxidants, and oxygen (Waraho, McClements, & Decker, 2011).

To prevent lipid oxidation in emulsion, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ethylenediaminetetraacetic acid (EDTA) are commonly used. Nevertheless, the use of synthetic antioxidants is limited in many countries. Natural antioxidants from plants have become the alternative antioxidants in the food industry. They are able to retard the oxidative degradation of lipids, improve the quality and nutritional value of food as well as prevent the occurrence of diseases (Shahidi & Ambigaipalan, 2015).

Cashew (*Anacardiumoccidentale*L..) is a member of the family *Anacardiaceae*(Razali, Razab, Junit, & Aziz, 2008), which are abundant in the southern part of Thailand. Cashew leaves has been consumed as fresh in some regions of Asian and American countries. It has been reported that it could be used as folk medicine to treat gastrointestinal disorders (acute gastritis, diarrhea), mouth ulcers as well as throat problems (Kudi, Umoh, Eduvie, & Gefu, 1999). Several parts of cashew tree, especially its leaves, contain phenolics with bioactivities, especially antioxidative activity. Chotphruethipong, Benjakul, and Kijroongrojana (2017)reported that ethanolic cashew leaf extract showed antioxidative activity in a dose dependent manner. Additionally, it has been reported that cashew leaf extracts had antimicrobial activity against several microorganisms (Omojasola & Awe, 2004). Since cashew leaf extract had high antioxidative activity, it could be used as a natural antioxidant to retard lipid oxidation, especially in food emulsion enriched with PUFA, such as mayonnaise. However, no information regarding the use of cashew leaf extract in prevention of lipid oxidation in mayonnaise enriched with fish oil exists. Thus, the present study aimed to examine the effect of cashew leaf extract on the retardation of lipid oxidation of mayonnaise enriched with fish oil during storage of 30 days at 30°C.

Materials and Methods

Chemicals

Butylated hydroxytolulene (BHT) was obtained from (Guangzhou Huangpu Chemical Co., Ltd., Guangzhou, China). 1,1,3,3-tetramethoxypropane and 2-thiobarbituric acid were purchased from Fluka (Buchs, Switzerland). Ammonium thiocyanate, *p*-anisidine, and pyridine were procured from Sigma (St. Louis. MO, USA). Trichloroacetic acid, isooctane and ferrous chloride were obtained from Merck (Darmstadt, Germany). Chloroform, methanol and hydrochloric acid were purchased from Lab-Scan (Bangkok, Thailand).

Collection of Cashew Leaves and Preparation of Cashew Leaf Extract

Collection of Cashew Leaves

Cashew (*Anacardiumoccidentale* L.) leaves were collected from an orchard in Songkhla province, Thailand during November and December 2016. The trees were approximately 15-20 years old. Leaves from III (apical) to V (basal) of twig were used. Collected cashew leaves were washed with tap water, dried, blended and sieved as described by Chotphruethipong *et al*. (2017). Thereafter, the obtained powder was subjected to chlorophyll removal using chloroform at the powder/solvent ratio of 1:20 (w/v) (Chotphruethipong *et al*., 2017), followed by drying at 105°C for 1 h. Prepared cashew leaf powder was subjected to extraction.

Preparation of Cashew Leaf Extract

To cashew leaf powder (10 g), 200 mL of 80% ethanol were added. Ultrasound-assisted extraction was performed using an ultrasonic equipment (Sonics, Model VC750, Sonica & Materials, Inc., Newtown, CI, USA) at the amplitude of 77% for 31 min with the frequency of 20 kHz ± 50 Hz and high intensity power of 750 W. The temperature was controlled at 35±5°C using the ice bath. After extraction, the mixtures were centrifuged at 5000xg for 30 min at room temperature using a RC-5B plus centrifuge (Beckman, JE-AVANTI, Fullerton, CA, USA). The supernatants was then filtered through a Whatman filter paper No.1 (Chotphruethipong *et al*., 2017). The filtrate was evaporated at 40°C using an EYELA rotary evaporator (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan). The extract was lyophilized using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). Dried extract was packed in a ziplock bag and stored in a desiccator until used.

Collection and Extraction of Fish Oil from Visceral Depot Fat of Seabass

Collection of Fish Oil from Visceral Depot Fat of Seabass

Depot fat from viscera of seabass, acquired from a market in Hat Yai, Songkhla province, Thailand, was dissected manually. The sample was placed in polyethylene bag and then embedded in ice with a fish/ice ratio of 1:2 (w/w), and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within30 min. The sample was immediately washed with tap water, drained and used for lipid extraction.

Extraction of Fish Oil from Visceral Depot Fat of Seabass

Lipid was extracted according to the method of Sae-Leaw and Benjakul (2017). Visceral depot fat (100 g) was placed in a bottom flask equipped with a rotary evaporator. The sample was heated at 70 ºC for 20 min under vacuum. To the obtained oil, 2-5g of anhydrous sodium sulfate were added. The mixture was shaken well and decanted into a centrifuge tube through a Whatman No.4 filter paper. The mixture was centrifuged at 10,000xg for 20 min at 4°C using a refrigerated centrifuge (CR22N, Hitachi, Hitachi Koki Co., Ltd., Tokyo, Japan). The oil was collected using a Pasteur pipette. The oil sample was placed in the amber bottle and purged with nitrogen gas. The vials were capped tightly and kept at -40°C until used.

Preparation of Mayonnaise

Mayonnaise (800 g/batch) were prepared using the following ingredients: soy bean oil (60.25%), fish oil (7.80%), egg yolk (6.78%), salt (0.50%), sugar (14.27%), lemon juice (5.00%), vinegar (3.80%) and water (1.60%). All the mixtures except soy bean oil and vinegar were blended using a blender (Panasonic, Model MK-GB1, New Taipei City, Taiwan) at a speed of 800 rpm for 3 min. Thereafter, half of amount of soy bean oil was gradually added and the mixture was blended for 5 min. Subsequently, vinegar was added and blending was performed for 3 min. Then the additives or distilled water (control) were added while blending. Finally, the rest of soy bean oil was added slowly and the mixture was blended for another 5 min. Two additives were used in the present study. BHT at two different levels (100 and 200 mg/kg) was used as the positive control. BHT was previously dissolved in oil before addition into mayonnaise. For cashew leaf extract, it was dissolved in distilled water before being added into mayonnaise. The final concentrations of 100 and 200 mg/kg were used.

The resulting mayonnaises were transferred into glass jar, and the lid was closed. The samples were kept at room temperature (30 ± 2°C). At designated times (0, 5, 10, 15, 20, 25 and 30 days), the samples were taken for analyses.

Analyses

Measurement of Peroxide Value (PV)

PV was measured as per the method of Sae-Leaw and Benjakul (2014). PV was expressed as mg cumenehydroperoxide/kg sample.

Measurement of Thiobarbituric Acid-reactive Substances (TBARS)

TBARS was determined according to the method of Egan *et al*. (1981). TBARS was calculated and expressed as mg malonaldehyde/kg sample.

Measurement of *P*-anisidine Value

p-Anisidine value of the sample was determined according to the method of AOCS (AOCS, 1990). Sample (2 g) was added in 25 mL of isooctane. After mixing thoroughly, the sample was centrifuged at 5000xg for 10 min. The supernatant (5 mL) was mixed with 1 mL of 0.5% (w/v) *p*-anisidine in acetic acid for 10 min. The absorbance was read at 350 nm. The *p*-anisidine value was calculated using following equation:

$$p\text{-anisidine value} = 25 \times [(1.2 \times A_2 - A_1)/W]$$

where A_1= A_{350} before adding *p*-anisidine, A_2 = A_{350} after adding *p*-anisidine and W= weight of sample (g).

Microbiological Determination

Total viable count (TVC) was measured. Mayonnaise samples (25 g) were added with 225 mL of 0.85% sterile saline solution, and mixed using a Stomacher blender (Stomacher M400, Seward Ltd., Worthington, England) for 1 min. A series of 10-fold dilutions were made by the same diluent. TVC was determined by plate count agar (PCA) after the incubation at 35 ± 2°C for 2 days (Fan, Chi, & Zhang, 2008).

Color

Mayonnaise samples were subjected to color measurement using a CIE colorimeter (Hunter associates laboratory, Inc., VA, USA) with the port size of 0.75 inch. The color of the mayonnaise was expressed as L*-value (lightness), a*-value (redness/greenness), b*-value (yellowness/blueness) and total difference of color (*ΔE**) were calculated as follows (Ghanbarzadeh, Almasi, & Entezami, 2010):

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

where ΔL*, Δa*and Δb* are the differences between the corresponding color parameter of the sample and that of white standard (L* = 92.25, a* = -0.99 and b* = 1.78).

Sensory Evaluation

Sensory evaluation of mayonnaise samples was conducted using multisample difference test (Meilgaard, Civille, & Carr, 2007). Twelve trained panelists (4 females and 8 males) with the ages of 20-35 years were used. Prior to the evaluation, the panelists were trained three times a week for 1 month with standards using a 15 cm line scale. Two sessions were performed each time. Four attributes evaluated included acetic acid odor, fishy odor, rancid odor and color. The score ranking was as follows: 0 is the absence and 15 is the strongest intensity. Mayonnaise enriched with fish oil stored at 35°C for 30 days was used for preparing the fishy odor and rancidity standards. The standards were prepared by dissolving the prepared mayonnaise in water to obtain the concentrations of 0%, 25%, 50% and 100% (w/v), representing scores of 0, 3, 5 and 8, respectively. For acetic odor, the mayonnaise containing only soy bean oil (no fish oil) was prepared as standard. The mayonnaise was dissolved in water to obtain the concentrations of 0%, 25%, 50% and 100% (w/v), representing scores of 0, 6, 11 and 15, respectively. For color training, mayonnaise added with the cashew leaf extract at levels of 0, 200, 400, 600 and 800 mg/kg were used, representing the score of 0, 4, 7, 11 and 15, respectively. Evaluation was carried out at day 0, 10, 20 and 30 of storage. The panelists were asked to open the sealable cup and sniff the headspace above the samples for odor evaluation (Yarnpakdee, Benjakul, Nalinanon, & Kristinsson, 2012).

Determination of Volatile Compounds

Sample at day 0, the sample without the extract or BHT (control), and that added with the cashew leaf extract at the concentration of 200 mg/kg at day 30 were collected for analysis of volatiles. Volatile compounds were extracted and adsorbed by the SPME fiber (75 µm CarboxenTM/PDMS StableFlexTM; Supelco, Bellefonte, PA, USA). The volatile compounds were then desorbed and subjected to GC-MS analysis as per the method of Sae-leaw and Benjakul (2014).

Statistical Analysis

All experiments were carried out in triplicate. Data were subjected to analysis of variance (ANOVA) and mean comparisons were run using the Duncan's multiple range test. Statistical analysis was performed using the statistical package for social sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

Results and Discussion

Peroxide Value (PV)

Changes in PV of mayonnaise enriched with fish oil without and with the addition of cashew leaf extract or BHT at levels of 100 and 200 mg/kg during storage of 30 days at 30°C are depicted in Figure 1A. The control sample (without antioxidants) had the higher PV than those containing cashew leaf extract or BHT throughout 30 days of storage ($P<0.05$). All samples had markedly increases in PV during the first 15 days of storage ($P<0.05$). Nevertheless, no difference in PV was observed between the sample added with cashew leaf extracts at 200 mg/kg and the sample added with BHT at day 5 and day 15 of storage ($P>0.05$). Moreover, there were no differences in PV between the samples containing BHT at both concentrations at day 10 of storage ($P>0.05$). After 15 days of storage, a continuous decrease in PV was found in the control with increasing storage time ($P<0.05$). On the other hand, other samples had the increasing PV up to 25 days ($P<0.05$), followed by the slight decrease till the end of storage time ($P<0.05$). The decrease in PV was due to the decomposition of hydroperoxide to the secondary oxidation products (Chaijan, Benjakul, Visessanguan, & Faustman, 2006). In general, the mayonnaise is susceptible to lipid oxidation due to their large surface area that facilities the interactions between the oil and water-soluble prooxidants(Gorji, Smyth, Sharma, & Fitzgerald, 2016). Moreover, the pH is the main factor affecting lipid oxidation in mayonnaise enriched with fish oil. In mayonnaise, egg yolk, used as the emulsifier, contained a large amount of iron (734 µM) (Jacobsen, Timm, & Meyer, 2001). The iron forms cation bridges between the protein phosvitin and other components at pH 6. At the low pH (3.8-4) found in the mayonnaise, the iron bridges between phosvitin, lipovilelin and low-density lipoprotein (LDL) are destroyed and the iron is released, leading to the increased lipid oxidation (Jacobsen *et al*., 2001; Thomsen, Jacobsen, & Skibsted, 2000). When comparing PV of all samples containing antioxidants, the lowest increase in PV was found in the sample added with BHT at the concentration of 200 mg/kg for all storage time used ($P<0.05$), indicating the high efficiency in retarding lipid oxidation. Differences in PV between mayonnaises added with different antioxidants possibly resulted from differences in their polarity as well as localization of antioxidants in emulsion system. Recently, Chotphruethipong *et al*. (2017) reported that catechin and isoquercetin were found as the dominant phenolics in cashew leaf extract. Moreover, the other phenolic compounds including, gallic acid, hydroquinin, tannic acid and rutin were found in the extract from cashew leaves. These phenolics were more polar antioxidants, as compared to BHT. The hydrophobic antioxidants could have higher efficiency than hydrophilic antioxidant in the prevention of oxidation in oil-in-water emulsion systems by preferably orienting at oil-water interface and functioned as a hydrogen donor or radical scavenger (Yarnpakdee, Benjakul, & Kingwascharapong, 2015). Conversely, cashew leaf extracts might have higher polarity than BHT. As a result, cashew leaf extracts were more likely located at the aqueous phase. This might be associated with the lower preventive effect toward the oxidation of emulsion by the extracts. Moreover, the efficiency in inhibiting lipid oxidation was also dependent on the concentration of the extracts used. The results indicated that BHT and cashew leaf extract at 200 mg/kg lowered PV values more effectively than those at 100 mg/kg ($P<0.05$). Thus, the concentration of antioxidants used was an essential factor determining the efficiency in inhibiting lipid oxidation, especially at the early stage.

TBARS

Changes in TBARS in mayonnaise enriched with

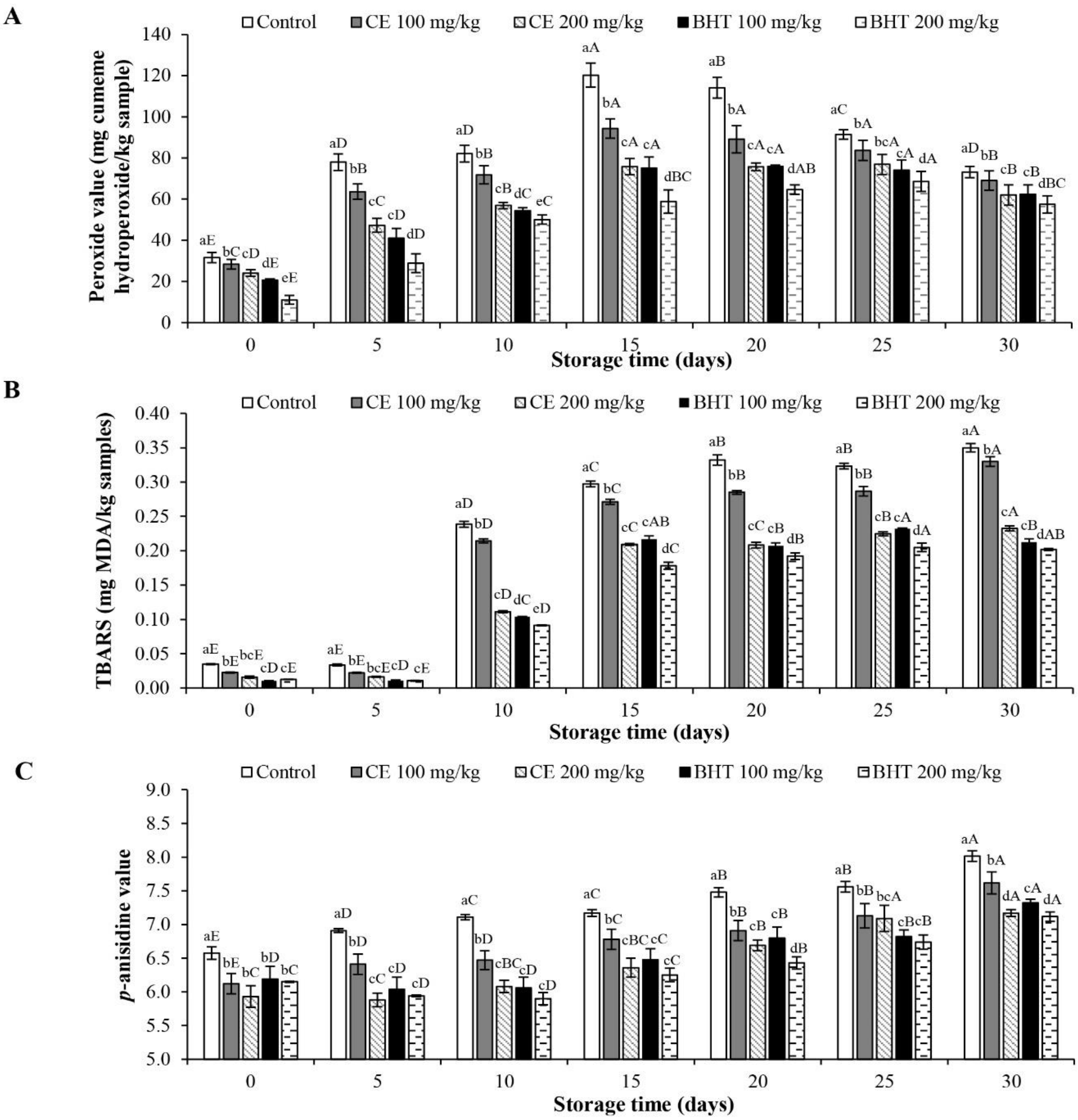

Figure 1. Changes in peroxide value (PV) (A), thiobarbituric acid reactive substances (TBARS) (B) and *p*-Anisidine value (AnV) (C) of mayonnaise enriched with fish oil without and with cashew leaf extract or BHT at various levels during the storage at 30±2ºC for 30 days. Bars represent standard deviation (n = 3). CE: cashew leaf extract and BHT: Butylated hydroxytolulene. Different lowercase superscripts within the same storage time denotes the significant difference (P<0.05). Different uppercase superscripts within the same sample denotes the significant difference (P<0.05). $CE_{100\ mg/kg}$ and $CE_{200\ mg/kg}$: mayonnaise added with cashew leaf extracts at the concentration of 100 mg/kg and 200 mg/kg, respectively. $BHT_{100\ mg/kg}$ and $BHT_{200\ mg/kg}$: mayonnaise added with butylated hydroxytoluene at the concentration of 100 mg/kg and 200 mg/kg, respectively.

fish oil in the absence and presence of different antioxidants at various concentrations are shown in Figure 1B. TBARS values of all samples increased when storage time increased (P<0.05). The control sample showed the higher TBARS values, compared to the samples containing antioxidants throughout 30 days of storage (P<0.05). After the first 5 days of storage, all samples had a marked increase in TBARS up to 15 days (P<0.05). However, no difference in TBARS was observed between the sample added with cashew leaf extract at 200 mg/kg and the sample added with BHT at 100 mg/kg at day 10 of storage (P>0.05). Subsequently, TBARS values of all samples were slightly increased up to day 25 of storage (P>0.05), except the samples added with cashew leaf extract at 100 mg/kg and the control sample. During days 25-30, there was no difference in TBARS value for the sample added with BHT at 200 mg/kg (P>0.05), while other samples showed slight increases in TBARS values (P<0.05). Increases in TBARS value indicated the decomposition of hydroperoxides into the secondary products (Coupland *et al*., 1996). Hydroperoxides are decomposed to malonaldehyde, which contributes to off-flavor of oxidized lipids (Zhang, Xiao, & Ahn, 2013). Among all samples containing antioxidants, the samples added with BHT at 200 mg/kg exhibited stronger oxidative stability than those added with BHT at 100 mg/kg or cashew leaf extracts at both concentrations (P<0.05). The differences in ability in inhibiting oxidation of the different antioxidants were probably owing to the differences in hydrophobicity/hydrophilicity balance as well as their localization in emulsion system (Yarnpakdee *et al*., 2015). When comparing TBARS values between the sample added with cashew leaf extract at 100 mg/kg and 200 mg/kg, the latter had lower TBARS than the former throughout 30 days of storage (P<0.05). Nevertheless, no difference in TBARS value was observed for both samples during the first 5 days of storage (P>0.05). The result suggested that the efficiency in inhibiting lipid oxidation depended on the concentration of extracts used. Chotphruethipong *et al*. (2017) reported that cashew leaf extract was able to donate hydrogen atom to free radicals, and could inhibit the propagation chain reaction during lipid oxidation process. Also, the extract was able to chelate the metal, known as prooxidant(Chotphruethipong*et al*., 2017). Thus, cashew leaf extract and BHT at the levels of 200 mg/kg were effective in retarding lipid oxidation in mayonnaise enriched with fish oil at 30°C for 30 days.

P-Anisidine Value (AnV)

AnV of mayonnaise enriched with fish oil without and with antioxidants at different concentrations during storage of 30 days at 30°C is shown in Figure 1C. The control sample had the highest AnV, as compared to the samples containing antioxidants throughout 30 days of storage (P<0.05). At the first 10 days of storage, there was no differences in AnV between the samples containing antioxidants (P>0.05), except the sample added with cashew leaf extract at 100 mg/kg, which showed higher AnV, compared to other samples (P<0.05). During day 10-15 of storage, all samples had the slight increase in AnV (P<0.05), except the sample added with cashew leaf extract at 200 mg/kg and the control sample. Thereafter, AnV of all samples increased up to day 20 of storage (P<0.05). However, no difference in AnV was found for the sample added with cashew leaf extract at 200 mg/kg during the storage of 15-20 days (P>0.05). Subsequently, the sample had the increase in AnV till day 25 of the storage, whereas there was no difference in AnV for other samples (P>0.05). At the end of storage, all samples possessed the highest AnV when compared with other storage time (P<0.05). Nevertheless, no differences in AnV were observed for the sample added with cashew leaf extract at 200 mg/kg during 25-30 days of storage (P>0.05). Among the samples tested, the control sample showed the highest AnV (P<0.05), whereas the lowest AnV was found for the samples added with BHT and cashew leaf extract at 200 mg/kg (P<0.05). Moreover, there were no differences in AnV between the samples added with BHT and cashew leaf extract at the concentration of 200 mg/kg at day 30 (P>0.05). Cashew leaf extract showed the antioxidative activity in mayonnaise in a dose dependent manner (P<0.05). The increase in AnV indicated the formation of the secondary lipid oxidation products, mainly non-volatile compounds (principally 2-alkenals and 2,4-alkadienals) in lipids (Choe & Min, 2006). Thus, cashew leaf extract, especially at 200 mg/kg, could be used as a natural antioxidant to retard the formation of non-volatile lipid oxidation products in mayonnaise enriched with fish oil during the extended storage.

Microbiological Load

TVC of all samples was below the recommended values of the Thai Industrial Standard (1997) (TVC <10^3 CFU/g) during 30 days of the storage (data not shown). Organic acids used as ingredients in mayonnaise including acetic acid and citric acid could lower the pH and acted as antimicrobial agents (Ma & Boye, 2013). The concentration of acids used was more likely sufficient to inhibit the microbial growth. In general, organic acids are effective food preservatives by lowering the pH of food products to levels that can inhibit bacterial growth (Hinton Jr, 2006). Organic acids are able to penetrate cell membranes of microorganisms and intracellular dissociation, resulting in acidification of the cytoplasm and intracellular acid anion accumulation to toxic levels (Beales, 2004). Moreover, the addition of cashew leaf extract in mayonnaise might result in the reduction of microbial count. It has been reported that cashew leaf extract had antimicrobial activity against *Porphyromonasgingivalis*, *Prevotella intermedia*, *Escherichia coli*, *Shigelladysenteriae, Salmonella* typhimurium, *Stapphylococcus aureus* and *Pseudomonas auroginosa*(Omojasola& Awe, 2004; Varghese, Tumkur, Ballal, & Bhat, 2013). Thus, the use of organic acids such as citric acid and acetic in combination with cashew leaf extract plausibly inhibited the microbial growth, leading to the extension of shelf-life and quality maintenance of mayonnaise enriched with fish oil.

Color and Sensory Property

Color changes in lightness (L*), redness (a*), yellowness (b*) and total color difference (ΔE*) of fish oil enriched mayonnaise added without and with antioxidants at various concentrations during storage of 30 days at 30°C are shown in Figure 2. An increase in the storage time resulted in the decrease in L* value of all samples tested (P<0.05). On the other hand, a*, b*

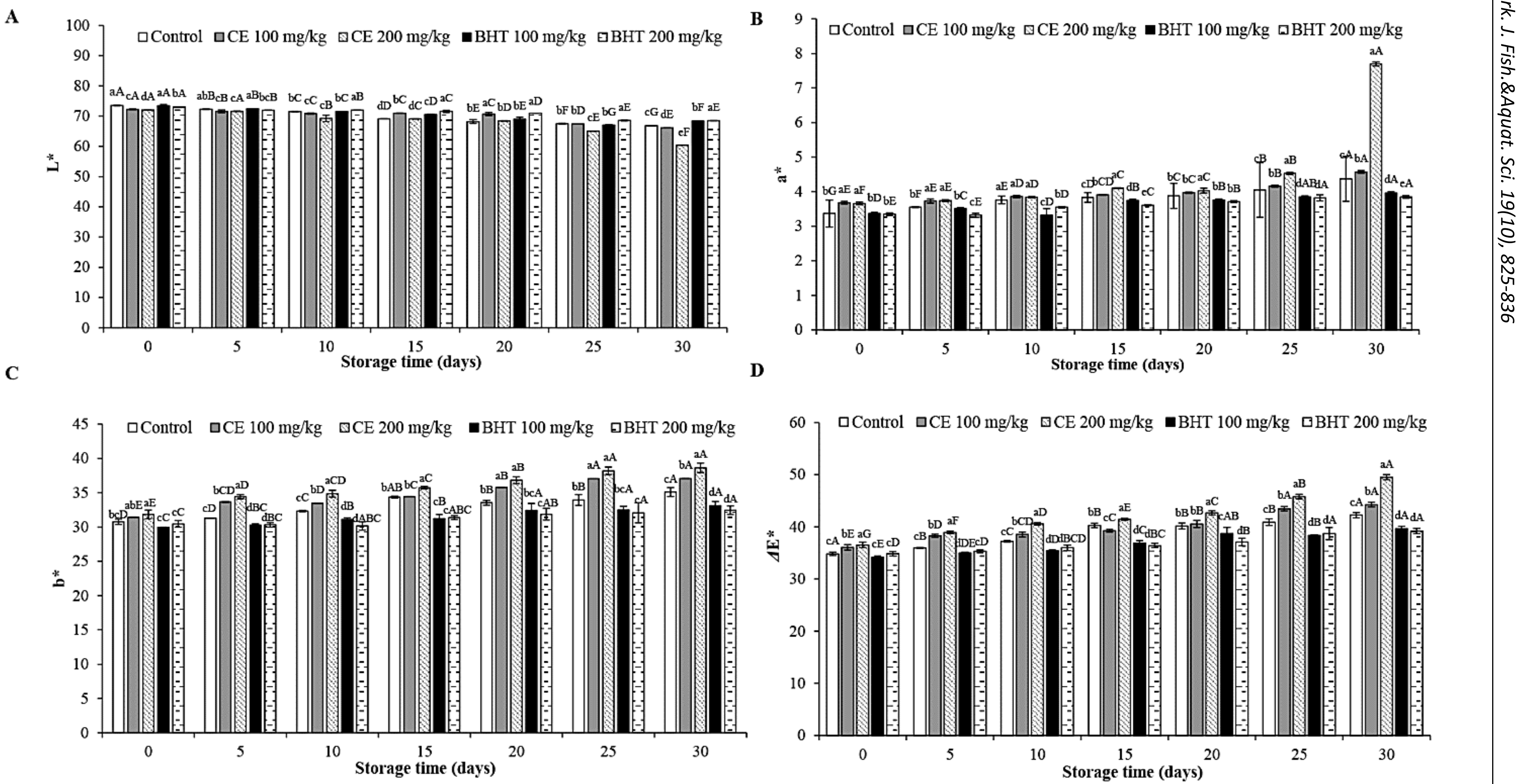

Figure 2. Changes in L* (A), a* (B), b* (C) and ΔE* (D) values of mayonnaise enriched with fish oil without and with cashew leaf extract or BHT at various levels during storage of 30 days at 30 ˚C. Bars represent standard deviation (n = 3). Different lowercase superscripts within the same storage time denotes the significant difference (P<0.05). Different uppercase superscripts within the same sample denotes the significant difference (P<0.05). $CE_{100\ mg/kg}$ and $CE_{200\ mg/kg}$: mayonnaise added with cashew leaf extracts at the concentration of 100 mg/kg and 200 mg/kg, respectively. $BHT_{100\ mg/kg}$ and $BHT_{200\ mg/kg}$: mayonnaise added with butylated hydroxytoluene at the concentration of 100 mg/kg and 200 mg/kg, respectively.

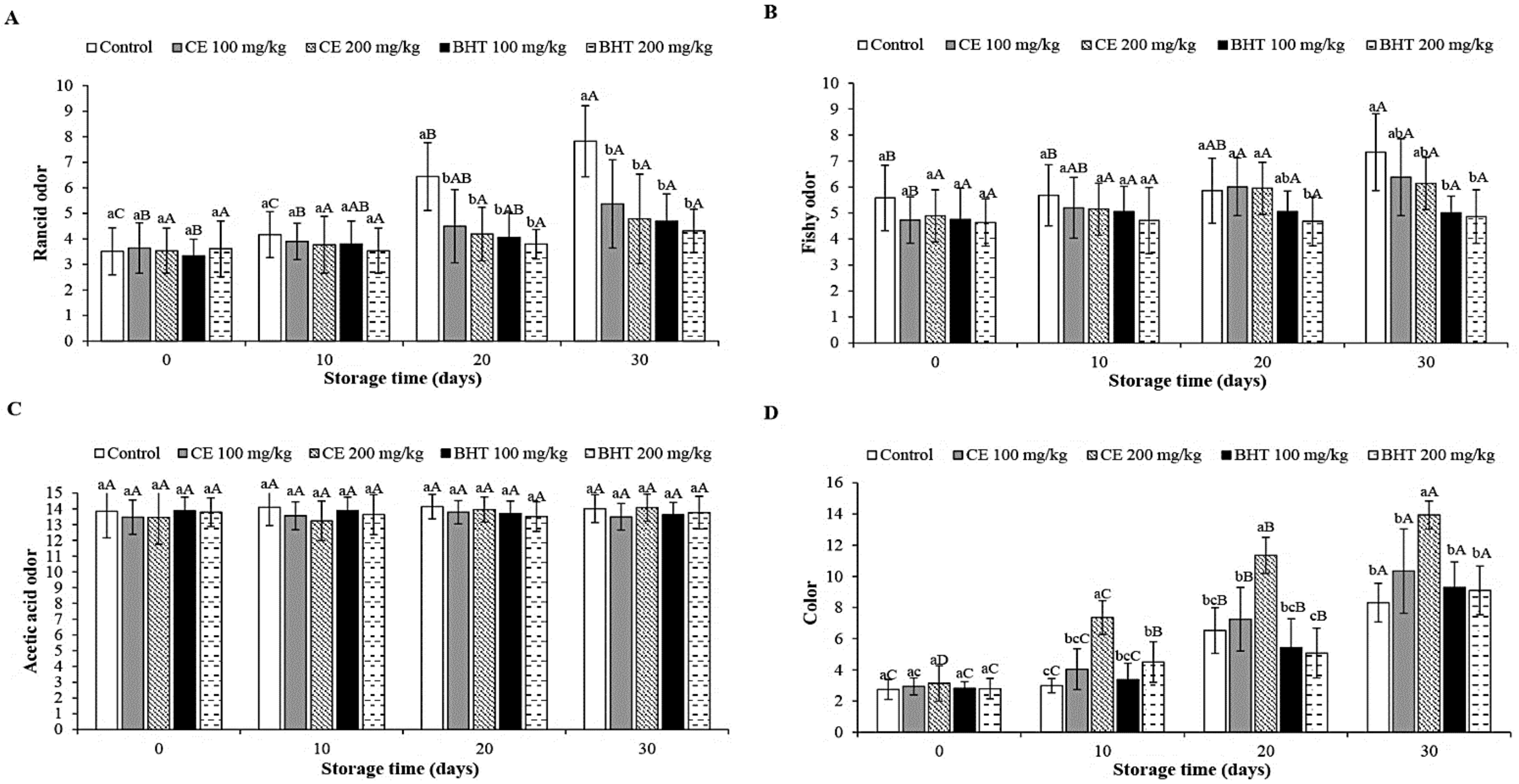

Figure 3. Changes in sensorial characteristics; (A) rancid odor, (B) fishy odor, (C) acetic odor and (D) color of mayonnaise enriched with fish oil without and with cashew leaf extract or BHT at various levels during storage of 30 days at 30 ˚C. Bars represent standard deviation (n = 12). Different lowercase superscripts within the same storage time denotes the significant difference (P<0.05). Different uppercase superscripts within the same sample denotes the significant difference (P<0.05). $CE_{100\ mg/kg}$ and $CE_{200\ mg/kg}$: mayonnaise added with cashew leaf extracts at the concentration of 100 mg/kg and 200 mg/kg, respectively. $BHT_{100\ mg/kg}$ and $BHT_{200\ mg/kg}$: mayonnaise added with butylated hydroxytoluene at the concentration of 100 mg/kg and 200 mg/kg, respectively. Score are based on scale of 0 - 15 cm, where 0 is the absence and 15 is the strongest intensity.

and ΔE*values of all samples increased when the storage time increased (P<0.05). These discolorations were probably associated withlipid oxidation products generated during storage, which might serve as the source of carbonyl compounds for non-enzymatic browning reaction as well as polymerization of oxidized phenolic compounds (Bharate & Bharate, 2014). Moreover, the addition of cashew leaf extract resulted in the decrease in lightness and an increase in redness as well as ΔE* values (P<0.05). ΔE* values of the sample containing cashew leaf extract were higher than those of other samples (P<0.05). This might be due to pigments present in cashew leaf extract, which most likely contributed to the changes in color of mayonnaise during storage. Altunkaya *et al*. (2013) reported that the addition of grape seed extract decreased in lightness and yellowness of the mayonnaise samples, while increased redness. Moreover, the intensity of color might depend on the amount of the extract added. The result revealed that the mayonnaise added with 200 mg/kg cashew leaf extract had higher decrease in L* value and higher increases in a* and b* values than those of the mayonnaise containing cashew leaf extract at the lower concentration. At the end of storage, the highest L* value and the lowest a* value were observed in mayonnaise added with BHT at the level of 200 mg/kg (P<0.05), while the lowest L* value and the highest a*, b* and ΔE* values were found in the sample added with cashew leaf extract at 200 mg/kg (P<0.05). No difference in b* and ΔE* values between the samples containing BHT at both levels was found (P>0.05). Although, cashew leaf extract at high concentration could retard the lipid oxidation product as indicated by the lower PV and TBARS formations, the pigments in the extract might impact on color changes of mayonnaise, especially with extended storage.

Changes in sensorial characteristics including rancid, fishy and acetic acid odors as well as color of mayonnaise enriched with fish oil without and with antioxidants at different concentrations are shown in Figure 3. Within the first 10 days of storage, there were no differences in scores between samples for all odors evaluated (P>0.05). However, the sample containing cashew leaf extract at the level of 200 mg/kg showed the highest score of color, compared to other samples (P<0.05). The result indicated the changes in color of sample, in which brown color was developed. This was in accordance with the decrease in L* value of sample (Figure 2A). After 20 days of storage, the highest score of rancidity was observed in the control, as compared to other samples (P<0.05), indicating that lipid oxidation proceeded to higher extent. The result was in agreement with the TBARS values (Figure 1B), in which the higher value was found in the control sample (P<0.05). No difference in score of acetic acid odor was found among all samples tested throughout 30 days of storage (P>0.05). At the end of storage, the sample containing BHT or cashew leaf extract at both levels had lower intensity of fishy and rancid odors than the control (P<0.05). Also, there was no difference in both attributes among samples containing BHT and cashew leaf extracts at all levels added (P>0.05). Off-odors were generally in accordance with the deteriorative reactions of lipids. It was reported that lipid oxidation of fish lipid contributed to development of fishy odor (Yarnpakdee *et al*., 2012). Thus, the addition of cashew leaf extracts in mayonnaise could reduce off-odor, especially rancidity. Nevertheless, the addition of cashew leaf extract negatively, affected the color of mayonnaise, particularly when the extract at high level was incorporated.

Volatile Compounds

Selected volatile compounds in fish oil enriched mayonnaise without and with cashew leaf extract at 200 mg/kg at day 0 and day 30 of storage at 30°C are presented in Table 1. Fish oil enriched mayonnaise had high content of linoleic acid (18:2), linolenic acid (18:3) eicosapentanoic acid (20:5) and docosahexaenoic acid (22:6), which are susceptible to oxidation (Depree & Savage, 2001). Thanasak Sae-leaw and Benjakul (2017) reported that oil extracted from seabass visceral depot fat contained oleic acid (25.49%), palmitic acid (21.8%), linoleic acid (13.84 %), DHA (6.91%) and EPA (2.09%). In general, volatile compounds detected in mayonnaise at day 0 of storage were lower in abundance than those found at the end of storage (30 days). After storage, aldehydes were the most prominent volatiles detected in all samples. At day 0 of storage, pentanal and hexanal were found as the major compounds in the control sample. The result suggested that oxidation had already taken place before or during mayonnaise preparation. Other aldehydes including benzaldehyde, (*E*)-2-decenal, heptenal and (*E*)-2-hexenal were also detected at the low levels. Hexanal and heptenal were major compounds, which contributed to rancid odor and fishy odor (Yarnpakdee *et al*., 2012). Moreover, volatile alcohols (1-methyl-4-(1-methylethenyl)-cyclohexanol), ketones (1-penten-3-one) and furans (2-ethyl-furan) were found at low contents at day 0. At the end of storage, lower formation of volatile compounds was observed in the sample containing cashew leaf extract, compared with the control sample. This result was coincidental with lower TBARS formation (Figure 1B), indicating that cashew leaf extract at a level of 200 mg/kg had high efficiency in preventing the formation of volatile lipid oxidation compounds. In the control, pentanal and hexanal were the predominant aldehydes. 2-hydroxy-3-pentanane and 1-octen-3-ol were the prevalent ketone and alcohol, respectively. Additionally, some additional volatile compounds were detected in the samples after storage. For the sample containing the cashew leaf extract, the lower abundance of

Table 1. Volatile compounds in mayonnaise enriched with fish oil without and with 200 mg/kg cashew leaf extract at day 0 and day 30 of storage at 30 ˚C

Volatile compounds	Peak area (Abundance) x10^5		
	Control day 0	day 30	
		Control	CE_{200}
Furans			
2-Ethyl-furan	29	330	143
2-Pentyl-furan	ND	33	ND
Aldehydes			
Propanal	ND	49	50
2-Methyl-butanal	ND	ND	56
3-Methyl-butanal	ND	295	25
Pentanal	119	697	219
Hexanal	162	695	574
(E)-2-Hexenal	5	ND	ND
Heptenal	10	273	70
(E, E)-2,4-heptadienal	ND	125	95
Octanal	ND	52	ND
(E)-2-decenal	15	ND	ND
Benzaldehyde	22	215	50
Ketones			
1-phenyl-1,2-propandione	ND	ND	71
1-Penten-3-one	5	169	ND
2,3-Pentanedione	ND	49	ND
2-Hydroxy-3-pentanone	ND	1940	ND
Alcohols			
1-Cyclobutylcyclopropanol	ND	44	ND
1-Penatanol	ND	107	ND
(Z)-2-pentenol	ND	40	56
(E)-2-hexenol	ND	64	ND
1-Methyl-4-(1-methylethenyl)-cyclohexanol	9	ND	ND
1-Octen-3-ol	ND	2712	ND
(Z)-1,5-octadien-3-ol	ND	42	ND

ND: not detectable.
CE_{200}: mayonnaise added with cashew leaf extracts at the concentration of 200 mg/kg.

aforementionedcompounds was noticeable. Aldehydes, ketones and alcohols have been known to be associated with lipid oxidation (Sae-leaw & Benjakul, 2014), which might occur during storage. Several volatiles compounds in both samples have been shown to correlate with increased fishy and rancid off-flavor in mayonnaise enriched with fish oil. Those included 3-methyl-butanal, 2-ethyl-furan, 1-penten-3-one, 1-octen-3-ol and *(E, E)*-2,4-heptadienal (Jacobsen *et al*., 2000). Hexanal was a degradation product of linoleic acid hydroperoxide, which was responsible for grassy and fatty odor (Yu, Chen, Sheng, & Tong, 2016). Unsaturated alcohols including 1-octen-3-ol and 1,5-octadien-3-ol also had impact on off-flavors, mushroom-like odor (Bader, Czerny, Eisner, & Buettner, 2009). Furans were increased after 30 days of storage, especially for the control. Furans and their derivatives such as 2-ethylfuran and 2-pentylfuran are formed by the decomposition of 12-hydroperoxide of linolenate (18:3n-3), 14-hydroperoxide of eicosapentaenoate (20:5n-3) and 16-hydroperoxide of docosahexaenoate (22:6n-3)(Maqsood & Benjakul, 2011). Thus, volatile compounds presented in mayonnaise enriched with fish oil more likely affected their sensory properties, particularly odors and flavors. Nevertheless, their formation could be retarded by the addition of cashew leaf extract.

Conclusion

Cashew leaf extract and BHT at the concentration of 200 mg/kg effectively retarded lipid oxidation of mayonnaise enriched with fish oil during storage as indicated by lower PV, TBARS and AnV values. However, addition of cashew leaf extract at both levels (100 mg/kg and 200 mg/kg) decreased L* but increased a*, b* and ΔE* values. Rancidity and fishy odor of the samples containing cashew leaf extract at 200 mg/kg were lower than control sample. This was related with the decrease in volatile secondary lipid oxidation products in mayonnaise. TVC of all samples was below 10^3 CFU/g throughout 30 days of the storage. Thus, cashew leaf extract, especially at the level of 200 mg/kg, could be used as a natural antioxidant for retardation of lipid oxidation in mayonnaise enriched with fish oil during the extended storage at room temperature.

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