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Research article

Mango Seed Kernel Extract as A Natural Antioxidant in Minced Fish During Frozen Storage

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Abstract

Mango seed kernel extract (MSKE) has been reported to contain high content of phenolic compounds, and exhibit strong *in vitro* antioxidant activity. This study aimed to evaluate the application of MSKE as natural antioxidant in frozen minced fish products. The effect of MSKE at different concentrations (0%, 1%, 2% and 3% w/w) on retarding lipid and protein oxidation in minced fish samples during frozen storage at -18 ºC was investigated and compared to the chemical antioxidant, BHT (butylated hydroxytoluene) at 0.01% w/w. Physicochemical parameters related to the oxidative stability of minced fish samples including pH, color, peroxide value (PV), thiobarbituric acid reactive substances (TBARS), conjugated diene (CD), protein carbonyl, sulfphydryl groups (SH), and total volatile base nitrogen (TVB-N) were determined. The results showed that the pH values had significantly increased in all samples being treated with MSKE and BHT after 12 weeks of frozen storage. Samples treated with 3% MSKE or BHT were significantly lower in lightness (L^*) and redness (a^*) but presented no significant effects on yellowness (b^*). Moreover, MSKE at all concentrations studied, and BHT, significantly delayed the increase in values of PV, TBARS, CD, protein carbonyl, and TVB-N and the decrease in values of SH compared to the control sample. At the end of storage, no significant odd odor and flavor assessed by sensory test were detected in frozen minced fish. In conclusion, MSKE at 3% w/w proved to be as efficient as BHT in retarding lipid and protein oxidation in minced fish during frozen storage.

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1. Introduction

The pulp, peel and seeds of mango fruit (*Mangifera indica* L.) are a perfect source of polyphenols including kaempferol, anthocyanin, gallic and ellagic acid, propyl and methyl gallate, benzoic acid, and protocatechuic acid. Seeds and peel are the main by-products of the mango processing industry and account for 35%-60% of the fruit depending on cultivar [1, 2]. The phenolic constituents of mango seed kernel and their bioactivities including antioxidant activity have been extensively reported [2, 3]. The strong *in vitro* antioxidant properties and active phenolic components of Thai mango seed kernel extract (MSKE) from Kaew and Choke-Anan cultivars has previously been shown [4, 5]. In addition, the application of MSKE as a natural antioxidant used to protect against oxidation in sunflower seed oil under accelerated storage [6] and in bologna-type meat products during refrigerated storage [7] has been reported.

Fish and fish products contain large amounts of polyunsaturated fatty acids that can cause oxidative rancidity, and this process is affected by many factors such as light, heat, enzymes, metals, metalloproteins and microorganisms. This can lead to the deterioration of physicochemical properties and qualities of products. Rancidity is also involved in free radical production, which can cause adverse health effects for consumers [8]. In the food industry, products can be treated with chemical antioxidants to improve their oxidative stability, but there are legislative limits on certain types and amount of such substances. Moreover, the use of synthetic antioxidants such as BHT in food is not friendly with consumers due to concerns about health issues [9]. Numerous studies have reported on bioactive compounds from plants; compounds which can be used to retard quality deterioration of food. Extracts from different plants that contain polyphenols have been successfully applied to prevent lipid and protein oxidation of fish derived products during storage [10]. These include extracts from green tea leaves (*Camellia sinensis*) [11], grape seeds (*Vitis vinifera*), pomegranate rind (*Punica granatum* L., cv. Hicaznar) [12] and from different spices such as rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), turmeric (*Curcuma longa*), and thyme (*Thymus vulgaris*) [10]. However, no report could be found on the use of by-products from the mango processing industry to enhance oxidative stability and delay the deterioration of fish products. Hence, the present work is to evaluate the potential use of MSKE as a natural antioxidant capable of retarding lipid and protein oxidation of minced fish (Clown featherback and Striped catfish) samples during frozen storage at -18ºC and compared with the synthetic additive, BHT.

2. Materials and Methods

2.1 Preparation of mango seed kernel extracts (MSKE)

Mango fruits *cv.* Choke-Anan at the stage of maturity of around 110 days after the fruits were set, were obtained from an orchard in Nakornratchasima province, Thailand. Peel and pulp were removed from the fruits and the seeds were washed and stored at -18ºC. The preparation of mango seed kernel extract (MSKE) was done according to the method of Namngam *et al*. [5]. A 50 g sample was blended with 500 ml of 95% ethanol for 5 min in a blender (Moulinex, Mexico), then incubated in a sonication bath (JAC Ultrasonic 2010P; Jinwoo Engineering Co., Ltd., Korea) at 20 KHz and 25°C for 15 min, followed by another incubation in a water bath at 80°C for 1 h. The mixture was cooled and filtered through a filter paper and then evaporated in a rotary evaporator (Büchi Rotavapor R II; USA) at 50°C under vacuum to get an extract with 70% solids.

2.2 Preparation of mixed minced fish

Fresh Clown featherback (*Chitala ornate*) and Striped catfish (*Pangasianodon hypopthalmus*) weighing between 300-500 g per fish were purchased from the fish market in Bangkok within 12 h of being killed and stored on ground ice before being transported to the laboratory. The samples were rapidly washed with cold water, gutted, skinned, boned and minced at room temperature in a mincer, mixing 70:30 (w/w) of Clown featherback:Striped catfish. The minced fish were divided into 500 g samples and each sample was mixed well with 1, 2 and 3% (w/w) MSKE and without MSKE as a control (0% MSKE) or 0.01% w/w BHT (butylated hydroxytoluene) as standard reference. There were 18 samples prepared for each treatment, giving 6 sampling dates (0, 3, 6, 9, 12 or 15 weeks) and 3 replicates for each treatment. The samples were stored at -18ºC, and then were picked up and thawed at 4ºC for 6 h before analysis, Details of the analysis are as follows:

2.2.1 pH determination

Each 1 g of sample was homogenized with 10 ml distilled water for 1 min, and then the pH was measured using a digital pH meter (Digi-Sense 5938-10, Chicago, USA).

2.2.2 Color determination

Color parameters of the thawed samples were measured in a colorimeter (Minolta CR 400, Japan) at room temperature and operated in the CIE system; L^* is lightness (0 = black; 100 = white), $+a^*$ is redness and $+b^*$ is yellowness.

2.2.3 Lipid extraction

Total lipids of the minced fish samples were extracted according to the method of Bligh and Dyer [13] and the percentage of total lipids was calculated.

2.2.4 Peroxide value (PV) determination

Peroxide values of the minces were determined with a slight modification of the method of Grunwald and Richards [14] by mixing 50 µl of sample with 2.35 ml of 75% ethanol, 50 µl of 30 % ammonium thiocyanate, and 50 µl of 20 mM ferrous chloride solution in 3.5% HCl for 3 min. The absorbance at 500 nm was then measured on a spectrophotometer and reported as relative PV.

2.2.5 Thiobarbituric acid reactive substances (TBARS) determination

TBARS were measured using the method described by Maqsood *et al*. [15]. A standard curve was constructed using 1,1,3,3-tetramethoxypropane (malondialdehyde, MAD). TBARS were expressed as mg of MAD equivalents kg^{-1} sample.

2.2.6 Conjugated diene (CD) determination

Conjugated determination was measured according to the method of Frankel *et al*. [16]. The CD amount of each sample was measured as the increase of absorbance at 234 nm and reported as relative CD.

2.2.7 Protein oxidation by total carbonyls determination

Protein carbonyls were measured by 2,4 dinitrophenyl-hydrazones determination using the method described by Levine *et al*. [17]. The carbonyl content was determined by measuring the absorbance at 370 and protein concentration was measured at 280 nm with BSA as a standard. Results were expressed in micromoles of carbonyls to mg^{-1} of soluble protein.

2.2.8 Protein oxidation by Sulphydryl groups (SH) determination

Total sulphydryl content was determined using 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) according to the method of Ellman [18]. The absorbance was measured at 412 nm, and the SH content was calculated using a molar extinction coefficient of 13,600 M^{-1} cm^{-1}. Results were expressed in micromoles of SH to g^{-1} of sample.

2.2.9 Total volatile base nitrogen (TVB-N) determination

TVB-N values were determined according to the method described by Ingridy *et al*. [19]. Each minced fish sample of 50 g was homogenized in trichloroacetic acid (150 ml) to precipitate the protein nitrogen. The volatile nitrogen in the supernatant that had been converted to alkalized steam by distillation was collected in boric acid solution. The solution was then titrated with sulfuric acid and TVB-N values were expressed as mg of nitrogen 100 g^{-1} sample.

2.3 Sensory evaluation

Samples of minced fish treated with 3% MSKE or without MSKE (control) both at the initial and the end of 15 weeks frozen storage, after thawing, were shaped into 2 cm flat round shapes and cooked by steaming at 100+5ºC for 10 min. After cooling at room temperature for 5 min, the samples were ready for sensory evaluation by 20 untrained healthy panelists aged between 18 and 30 years. The attributes evaluated were color, odor, flavor, texture, and overall acceptance using a 9-point hedonic scale test; where: 1 = extremely dislike to 9 = extremely like. The experiment design was a balanced complete block [20].

2.4 Experimental design

The experiment was a randomized block design with 6 storage times (0, 3, 6, 9, 12 and 15 weeks) x treatments (1, 2 and 3% MSKE + BHT at 0.01% as a treated control + an untreated control) and replicated 3 times. Analysis of variance was performed on the data and differences between means were evaluated using the Duncan's Multiple Range Test at $p \leq 0.05$.

3. Results and Discussion

3.1 Effect of MSKE on pH of mince fish during frozen storage

Results in Table 1 clearly showed that addition of MSKE at all concentrations studied had no effect on the pH of initial minced fish samples compared with the control and sample with BHT added. The pH of the control progressively increased during storage and was significantly higher ($p \leq 0.05$)

Table 1. Changes of pH values during frozen storage at -18°C of minced fish (MF) samples treated with different concentrations of MSKE

Samples	Storage Time (weeks)					
	0	3	6	9	12	15
Control MF	6.33 ± 0.03^{Ac}	6.34 ± 0.03^{Ac}	6.98 ± 0.02^{Ab}	6.84 ± 0.07^{Ab}	7.45 ± 0.01^{Aa}	7.89 ± 0.01^{Aa}
MF + BHT	6.31 ± 0.04^{Ab}	6.32 ± 0.17^{Ab}	6.34 ± 0.05^{Bb}	6.37 ± 0.03^{Bb}	6.57 ± 0.02^{Ba}	6.57 ± 0.05^{Ba}
MF + 1% MSKE	6.36 ± 0.05^{Ab}	6.38 ± 0.02^{Ab}	6.33 ± 0.05^{Bb}	6.37 ± 0.03^{Bb}	6.54 ± 0.01^{Ba}	6.55 ± 0.02^{Ba}
MF + 2% MSKE	6.35 ± 0.02^{Ab}	6.36 ± 0.15^{Ab}	6.35 ± 0.05^{Bb}	6.33 ± 0.05^{Bb}	6.54 ± 0.02^{Ba}	6.56 ± 0.05^{Ba}
MF + 3% MSKE	6.37 ± 0.02^{Ab}	6.31 ± 0.04^{Ab}	6.35 ± 0.01^{Bb}	6.32 ± 0.02^{Bb}	6.53 ± 0.02^{Ba}	6.57 ± 0.02^{Ba}

Values are means ± SD. Different lower-case letters indicate significant differences ($p \leq 0.05$) within each row and different capital letters indicate significant differences ($p \leq 0.05$) within each column.

than other samples after 6 weeks (Table 1). Samples treated with MSKE and BHT had relatively stable pH values with slight but significant increases ($p \leq 0.05$) after 12 weeks storage in all cases. The pH rise has also been reported in Bologna-type mortadella samples treated with 0.01% BHT or 0.1-0.2% mango seed extract during chilled storage [7].

The elevated pH observed in minced fish during chilled and frozen storage could be related to the generation of alkaline compounds as a decomposed products such as volatile bases [21]. Viji *et al*. [22] also reported the increase in pH during chilled and iced storage of Sutchi catfish steaks from an initial value of 6.35 and 6.21 to a final value of 6.64 and 6.62, respectively.

3.2 Effect of MSKE on color values of minced fish during frozen storage

It is clear from the results in Table 2 that MSKE had no impact on the color parameters (L^*, a^*, b^*) of the initial minced fish samples. During the frozen storage, there were no significant changes in lightness (L^*) observed for all minced fish samples from 0 to 6 weeks; however, the control sample became significantly ($p \leq 0.05$) darker from 9 weeks through the end of storage. In addition, samples treated with BHT or 2-3% MSKE showed significantly ($p \leq 0.05$) delayed lightness reduction during frozen storage.

The change in redness values (a^*) was lowest for BHT treated minced fish and 1-3% MSKE addition could significantly ($p \leq 0.05$) slow down the reduction of the redness of samples compared with the control. This observation could be due to the preventive effect of BHT and MSKE against the oxidation of heme proteins, haemoglobin and myoglobin, which change from red in their reduced ferric stage to brown in their oxidized counterpart [23].

Considering the changes of yellowness values (b^*), the control minced fish samples obviously showed an increase of yellowness after 6 weeks during frozen storage, while BHT and all MSKE treated samples exhibited singnificantly ($p \leq 0.05$) lower increments of b^* values. Moreover, 3% MSKE was as efficient as BHT in retarding the rise of yellowness found in minced fish samples during frozen storage for 15 weeks. The increase in yellowness observed in the minced fish samples could have been due to lipid oxidation, which also correlated well with the changes of PV values (Figure 1). The aldehydes produced from lipid oxidation can rapidly affect amino acid residues, such as lysine, producing pyrroles, which are responsible for the color changes [24]. Decreases in lightness and redness and increases in yellowness during frozen storage were also observed in Baltic cod fillets [25], and similar protective effects of MSKE and BHT on color of bologna-type mortadella during chilled storage were also reported by Pereirai *et al*. [7].

Table 2. Instrumental color values of minced fish samples treated with different concentrations of MSKE compared to BHT during storage at -18°C

Color Analysis	Treatments	Storage Time (weeks)					
		0	**3**	**6**	**9**	**12**	**15**
*L**	Control	55.19 ± 0.65^{Aa}	55.16 ± 0.66^{Aa}	55.18 ± 0.35^{Ab}	52.26 ± 0.35^{Cc}	48.99 ± 0.81^{Dd}	45.15 ± 0.30^{Dd}
	BHT	55.24 ± 0.72^{Aa}	55.23 ± 0.73^{Aa}	55.21 ± 0.45^{Aa}	55.22 ± 0.26^{Aa}	53.90 ± 0.65^{Bb}	53.10 ± 0.33^{Ab}
	1% MSKE	55.21 ± 0.39^{Aa}	55.20 ± 0.55^{Aa}	55.23 ± 0.42^{Aa}	54.25 ± 0.31^{Bb}	51.97 ± 0.63^{Cc}	48.18 ± 1.20^{Cc}
	2% MSKE	55.24 ± 0.78^{Aa}	55.15 ± 0.24^{Aa}	55.14 ± 0.29^{Aa}	55.18 ± 0.40^{Aa}	53.90 ± 0.69^{Ab}	48.14 ± 0.19^{Cc}
	3% MSKE	55.17 ± 0.40^{Aa}	55.23 ± 0.43^{Aa}	55.23 ± 0.22^{Aa}	55.21 ± 0.49^{Aa}	53.97 ± 0.42^{Ab}	53.17 ± 0.34^{Ac}
*a**	Control	10.71 ± 0.68^{Aa}	10.79 ± 0.31^{Aa}	10.70 ± 0.32^{Aa}	7.67 ± 0.28^{Bb}	5.07 ± 0.623^{Cc}	5.08 ± 0.46^{Cc}
	BHT	10.72 ± 0.33^{Aa}	10.78 ± 0.44^{Aa}	10.63 ± 0.24^{Aa}	10.68 ± 0.22^{Aa}	8.85 ± 0.24^{Ab}	8.81 ± 0.29^{Ab}
	1% MSKE	10.72 ± 0.25^{Aa}	10.76 ± 0.71^{Aa}	10.17 ± 0.37^{Aa}	10.65 ± 0.44^{Ab}	7.57 ± 0.47^{Bb}	7.55 ± 0.45^{Bc}
	2% MSKE	10.70 ± 0.27^{Aa}	10.72 ± 0.28^{Aa}	10.68 ± 0.28^{Aa}	10.66 ± 0.44^{Ab}	7.67 ± 0.21^{Bb}	7.52 ± 0.48^{Bc}
	3% MSKE	10.74 ± 0.31^{Aa}	10.71 ± 0.29^{Aa}	10.74 ± 0.27^{Aa}	10.66 ± 0.26^{Ab}	7.61 ± 0.30^{Bb}	7.64 ± 0.48^{Bc}
*b**	Control	5.66 ± 0.45^{Ae}	5.61 ± 0.43^{Ae}	6.15 ± 0.27^{Ad}	7.72 ± 0.31^{Ac}	8.95 ± 0.67^{Ab}	9.83 ± 0.31^{Aa}
	BHT	5.69 ± 0.33^{Aa}	5.68 ± 0.29^{Aa}	5.71 ± 0.37^{Ba}	5.68 ± 0.12^{Ba}	5.72 ± 0.15^{Ca}	5.74 ± 0.24^{Ca}
	1% MSKE	5.62 ± 0.47^{Ab}	5.68 ± 0.48^{Ab}	5.70 ± 0.11^{Bb}	5.65 ± 0.12^{Bb}	6.47 ± 0.41^{Ba}	6.48 ± 0.16^{Ba}
	2% MSKE	5.64 ± 0.22^{Ab}	5.68 ± 0.23^{Ab}	5.65 ± 0.40^{Bb}	5.70 ± 0.11^{Bb}	6.48 ± 0.18^{Ba}	6.45 ± 0.13^{Ba}
	3% MSKE	5.69 ± 0.21^{Aa}	5.69 ± 0.51^{Aa}	5.63 ± 0.27^{Ba}	5.71 ± 0.36^{Ba}	5.67 ± 0.30^{Ca}	5.70 ± 0.58^{Ca}

Values are means ± SD. Different lower-case letters indicate significant differences ($p \leq 0.05$) within each row and different capital letters indicate significant differences ($p \leq 0.05$) within each column.

3.3 Effect of MSKE on peroxide value (PV) of minced fish during frozen storage

Peroxide value is an index commonly used to determine the degree of lipid oxidation and reflects the quality of fat and oil or food containing high content of fat and oil. Sharp increases in PV have been taken as a measure of shelf-life ending for fish and fish products since they correlate with the development of off-flavors, and changes in color and nutritional value [26].

Figure 1A shows the progressively increasing PV in all minced fish samples during storage at -18ºC with the lowest rise being for BHT treated samples and the highest for the control samples. The ability of MSKE to slow down the increment of PV in minced fish during frozen storage was concentration dependent and 3% of MSKE addition was comparable to 0.01% BHT. The results were similar to those described by Tang *et al*. [27], who found that the incorporation of green tea catechins in mackerel mince was effective in lowering PV and TBARS values compared to the control during frozen storage. Treatment of fish products with plant extracts has previously been shown to delay changes in PV. For examples, Banani and Suchandra [28] found that minced Tilapia fish treated with fennel, pepper or cinnamon showed significantly ($p \leq 0.05$) lower PV changes compared to control samples during refrigerated storage for 28 days. Sarabi *et al*. [29] reported that fried escolar (*Lipidocybium flavobrunium*) fish fillet that had been treated with rosemary extract at 0.1, 0.2, 0.3% or 0.1% BHT had slower PV development during 5 months of frozen storage. The use of MSKE has also been confirmed for its ability to delay the change of PV in sunflower oil [6] and in bologna-type mortadella [7] during storage. Our results indicate that the MSKE from Choke-Anan cultivar, especially at 3 % w/w, has the potential to be used to retard PV formation in minced fish.

3.4 Effect of MSKE on thiobarbituric acid reactive substances (TBARS) of minced fish during frozen storage

The progression of secondary lipid oxidation in frozen minced fish samples during storage was evaluated based on the TBARS value, which is an index of malonaldehyde (MDA) concentration. The presence of TBARS in a sample of minced fish has been shown to indicate that lipid peroxidation had taken place [30]. All samples showed increases in TBARS during frozen storage with the control minced fish sample having the highest rise, especially after 12 weeks. However, no significant differences in TBARS were found between minced fish treated with 3% MSKE and 0.01% BHT after 15 weeks storage (Figure 1B). These results indicate that the lipid oxidation causing the racidity of minced fish was greater in the control sample throughout storage than that in samples treated with MSKE and BHT.

Ahmadi *et al*.[8] reported that there was no significant difference between the effects of 200 and 400 ppm of hydroalcoholic and water extracts of nettle leaves (*Urtica dioica*) on changes in TBARS of minced kilka fish (*Clupeonella cultriventris*) samples during storage at -2°C, and both showed significantly lower TBARS values when compared to the control. Many researchers have demonstrated that phenolic compounds or plant extracts containting polyphenols were effective in retarding the generation of MDA in minced fish during chilled or frozen storage [31, 32]. Our results also confirmed that the MSKE especially at 3% w/w can potentially prevent MDA formation in minced fish.

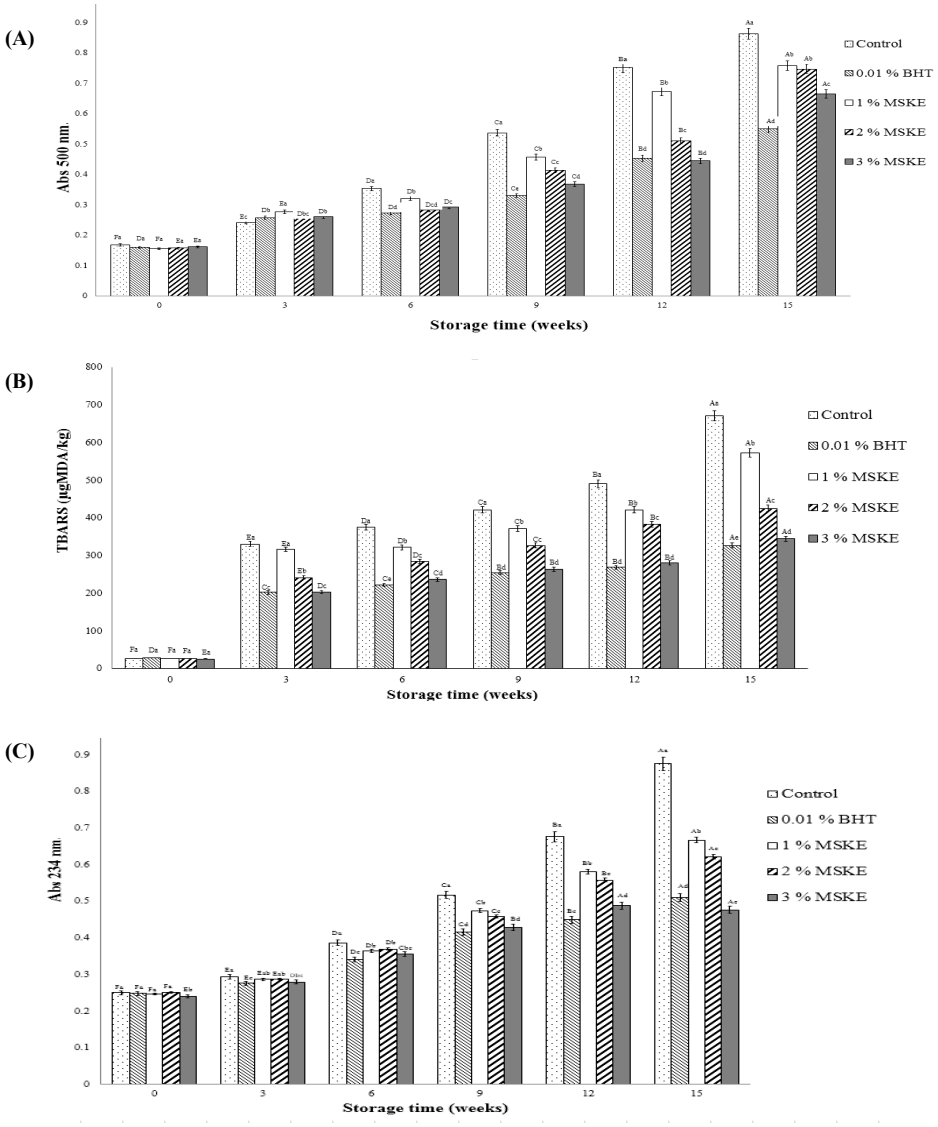

Figure 1. Changes in relative peroxide values (A), thiobarbituric acid reactive substances (B) and relative conjugated diene (C) of minced fish treated with different concentrations of MSKE compared to BHT during storage at -18°C

3.5 Effect of MSKE on relative conjugated diene (CD) of minced fish during frozen storage

The investigation of another common index of primary lipid oxidation in frozen minced fish samples during storage is the formation of CD from polyunsaurated fatty acids. Results in Figure 1C showe that relative CD values of all samples increased gradually during 15 weeks of storage at -18°C with the control samples showing the highest CD formation among all treatments tested. The samples treated with 3% MSKE and 0.01% BHT showed the highest activity in preventing formation of CD.

Our finding was in agreement with several reports. For example, Banani and Suchandra [28], who demonstrated that the CD level in minced Tilapia treated with extracts of spices (fennel seeds, black pepper or cinnamon) or BHT, was significantly ($p \leq 0.05$) lower than the control during 35 days of refrigerated storage. Maqsood and Benjakul [32] reported that among the different phenolic compounds tested, tannic acid showed the highest activity in preventing CD formation in minced Mackerel over 15 days storage in ice. Papuc *et al*. [31] also showed that the CD level of carp muscle (*Cyprinus carpio*) was lower for those samples treated with extracts from sea buckthorn fruits (*Hippophae rhamnoides*) during frozen storage for 6 weeks compared to the control.

3.6 Effect of MSKE on protein carbonyls of minced fish during frozen storage

Two oxidative processes including lipid oxidation and protein oxidation can readily occur in meat and fish muscles during storage, resulting in the formation of radicals, hydroperoxides and secondary compounds that lead to the loss of protein functionality [33]. Protein modification caused by the interaction of reactive oxygen species and proteins can affect muscle food qualities such as texture, aroma, water holding capacity, color and functionality [34]. These protein oxidative modifications in fish muscle can generate protein carbonyls.

Figure 2A shows no significant differences in protein carbonyl formation between BHT and all MSKE treated minced fish samples during frozen storage. However, the control sample possessed a sharp increase of protein carbonyls after 6 weeks of storage. Our results were in agreement with many previous findings such as those of Baron *et al*. [35], who found a significant increase in the carbonyl content of rainbow trout during storage at -20°C for up to 13 months. Sabeena *et al*. [36] reported that minced horse mackerel (*Trachurus trachurus*) treated with potato peel extracts showed significantly ($p \leq 0.05$) lower protein carbonyl formation compared to the control during storage at 5°C. Siebet *et al*. [37] explained that polyphenol compounds can inhibit the oxidation of proteins in muscle products. The bioactive polyphenols that are found mainly in MSKE such as flavonols, xanthones and gallotannins [3], possibly play a major role in retarding protein oxidation.

3.7 Effect of MSKE on sulfhydryl groups (SH) of minced fish during frozen storage

The decrease in total SH content has been known to reflect the formation of disulfide bonds and other oxidized species due to the oxidation of sulfhydryl groups or disulfide interchanges occurring in proteins [38]. As shown in Figure 2B, the SH group content of minced fish gradually decreased during the entire frozen storage period in all samples, with that of the control sample decreasing the most. MSKE, with a concentration dependence, exhibited a protective effect in SH group reduction of minced fish during frozen storage, and the samples treated with 3 % MSKE were protected as well as those treated with 0.01 % BHT. A similar finding was documented by Özen and Soyer [12], who used extracts from green tea, grape seed, and pomegranate rind in frozen mackerel mince to slow down the loss of SH groups in samples treated with all plant extracts. Treating processed fish with potato peel extracts and brown seaweed was previously shown to protect the reduction of SH groups in the samples during extended storage [36, 39]. These results suggested that the MSKE showed a protective effect against protein oxidation in minced fish during frozen storage.

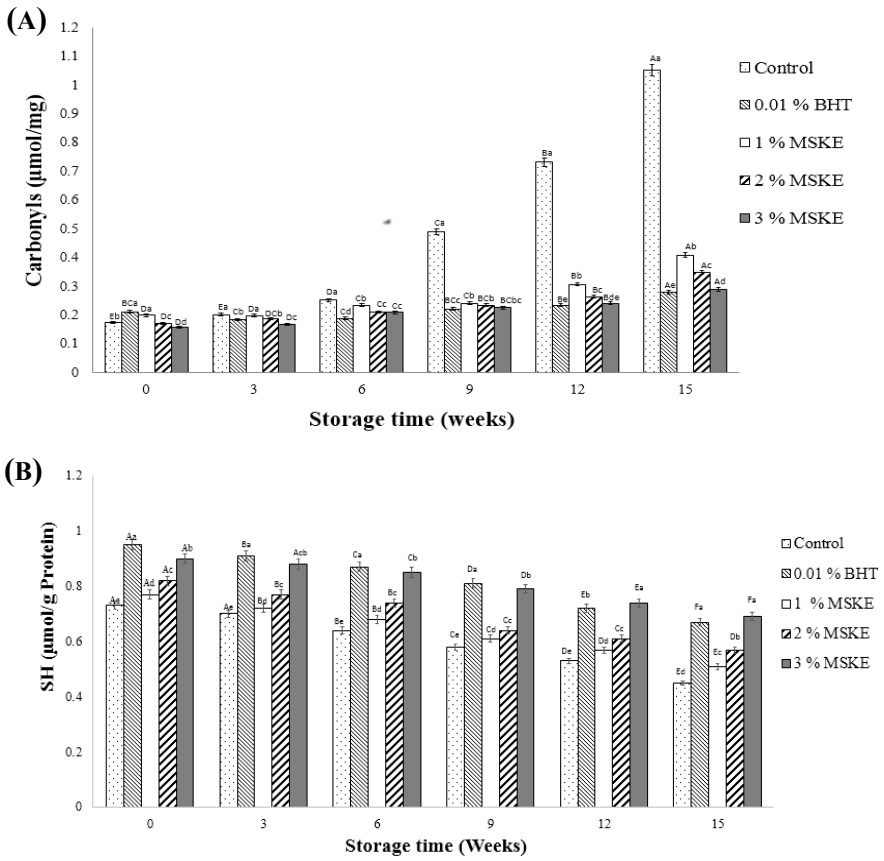

Figure 2. The formation of protein carbonyls (A) and the changing of sulfhydryl groups (B) of minced fish treated with different concentrations of MSKE compared to BHT during storage at -18°C.

3.8 Effect of MSKE on total volatile base nitrogen (TVB-N) of minced fish during frozen storage

TVB-N content has been known to be related to microbial growth and can be used as a quality indicator for spoilage of muscle foods such as fish, other sea foods, meats and their products [40]. In general, the acceptable limit of TVB-N for marine fish is in the range of 30 mg N 100 g^{-1} for marine fish and 15 mg N 100 g^{-1} for fresh water fish [41]. Table 3 shows the changes of total volatile base nitrogen of minced fish samples treated with different concentrations of MSKE compared to BHT during extended storage at -18°C. The TVB-N level was low, as would be expected, for the initial minced fish; however, the values continue to increase during frozen storage, especially with the rapid rise for the control sample. The minced fish used in this study was prepared from fresh water fishes; therefore, the TVB-N limit for acceptability should not be over 15 mg N 100 g^{-1}. All minced fish samples treated with MSKE gave the TVB-N content at the end of frozen storage that was lower than 15 mg N 100 g^{-1}.

According to the results shown in Table 3, the increased rate of TVB-N in the MSKE treated samples was significantly ($p \leq 0.05$) lower compared to the control. This effect of MSKE

Table 3. Changes of total volatile base nitrogen (mg N 100 g^{-1}) of minced fish (MF) treated with different concentrations of MSKE compared to BHT during storage at -18°C

Samples	Storage Time (weeks)					
	0	**3**	**6**	**9**	**12**	**15**
Control MF	3.17 ± 0.60^{Af}	5.24 ± 0.51^{Ae}	7.59 ± 0.02^{Ad}	11.46 ± 0.07^{Ac}	15.19 ± 0.01^{Ab}	16.85 ± 0.23^{Aa}
MF + BHT	3.31 ± 0.04^{Af}	5.32 ± 0.17^{Ae}	7.94 ± 0.05^{Ad}	11.33 ± 0.03^{Ac}	15.57 ± 0.02^{Ab}	16.57 ± 0.05^{Aa}
MF + 1% MSKE	3.36 ± 0.05^{Af}	5.38 ± 0.02^{Ae}	7.73 ± 0.05^{Ad}	10.37 ± 0.03^{Bc}	13.54 ± 0.01^{Bb}	14.52 ± 0.02^{Ba}
MF + 2% MSKE	3.35 ± 0.02^{Af}	5.36 ± 0.15^{Ae}	6.35 ± 0.05^{Bd}	8.33 ± 0.05^{Cc}	10.54 ± 0.02^{Cb}	12.56 ± 0.05^{Ca}
MF + 3% MSKE	3.47 ± 0.02^{Af}	5.31 ± 0.04^{Ae}	6.35 ± 0.01^{Bd}	7.32 ± 0.02^{Dc}	9.53 ± 0.02^{Db}	10.57 ± 0.02^{Da}

Values are means ± SD. Different lower-case letters indicate significant differences ($p \leq 0.05$) within each row and different capital letters indicate significant differences ($p \leq 0.05$) within each column.

was proportional to its concentration with it being more effective at the higher concentration used. On the other hand, BHT was totally ineffective in retarding the increase of TVB-N content in minced fish and was not significantly different to the control throughout the frozen storage period. A possible explanation of this finding could be due to the MSKE that was more effective in inhibiting the growth of microorganisms than BHT at the level tested. The antioxidant and antibacterial activity of Thai mango seed extract was previously reported by Khammuang and Sarnthima [42].

3.9 Sensory evaluation of MSKE treated minced fish

Preliminary sensory test was performed in order to investigate the impact of MSKE on the organoleptic quality of minced fish samples at the initial and the end of frozen storage. There was no serious deterioration observed after 15 weeks frozen storage of minced fish samples, an observation supported by the TVB-N values obtained in the previous experiment. Furthermore, the cytotoxicity of MSKE was examined and the negative results were obtained (data not shown). Table 4 shows the results of sensory test and there was no significant effect of MSKE addition on the odor and flavor of the minced fish samples. However, color, texture and overall acceptance scores were significantly ($p \leq 0.05$) higher for both control and 0.3% MSKE treated samples at the initial stage compared to those at 15 weeks storage with no difference between the samples. The decreased color, texture and overall acceptance scores observed in both control and MSKE treated minced fish samples after 15 weeks of frozen storage was likely due to the effect of storage time, not the added MSKE. It is suggested that MSKE treatment could be a suitable natural alternative antioxidant for controling lipid and protein oxidation in minced fish with no interference on sensory qualities.

Table 4. Sensory scores by 9-point hedonic scale test of steamed minced fish samples evaluated by panelists (n=20)

Samples Formula	Color	Odor ^{ns}	Flavor ^{ns}	Texture	Overall Acceptance
Control 0 day	8.35 ± 0.56^{a}	7.45 ± 0.60	7.15 ± 0.15	8.02 ± 0.73^{a}	8.05 ± 0.60^{a}
MSKE 0 day	8.50 ± 0.61^{a}	7.25 ± 0.72	7.10 ± 0.64	8.20 ± 0.62^{a}	8.10 ± 0.62^{a}
Control 15 Weeks	6.40 ± 0.60^{b}	7.35 ± 0.56	7.15 ± 0.67	6.00 ± 0.79^{b}	5.85 ± 0.67^{b}
MSKE 15 Weeks	6.55 ± 0.60^{b}	7.30 ± 0.66	7.30 ± 0.73	5.95 ± 0.80^{b}	6.10 ± 0.55^{b}

Values are expressed as means ± standard deviation (n=20). Different letters in the same column indicate significant differences ($p \leq 0.05$). ns = non-significant differences.

4. Conclusions

Mango seed kernel extract (MSKE) from Choke-Anan cultivar (1-3 % w/w) showed significant protective effect against lipid and protein oxidation in minced fish during frozen storage at -18°C for 15 weeks. The ability of MSKE to inhibit oxidative deterioration of minced fish samples was concentration dependent, with the level at 3% w/w being as effective as 0.01% BHT in retarding changes in pH, color, PV, TBARS, CD, protein carbonyls, SH groups and TVB-N content in the samples. This finding indicates that MSKE derived from by-products of the mango processing industry can be an alternative natural antioxidant with a promising application in controlling oxidative deterioration of fish products. Further study should focus on the development of MSKE in a more friendly usage form, its stability, and its application in other food products.

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