Antioxidant activity of date (*Phoenix dactylifera* var. Khalas) seed and its preventive effect on lipid oxidation in model systems

Maqsood, S., Kittiphattanabawon, P., Benjakul, S., Sumpavapol, P. and Abushelaibi, A.

Department of Food Science, College of Food and Agriculture, United Arab Emirates University, Al-Ain, 17551, United Arab Emirates.

Department of Agro-Industry Technology and Management, Faculty of Agro-Industry, King Mongkut's University of Technology North Bangkok, Prachinburi, 25230, Thailand

Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand

Department of Food Science and Technology, Faculty of Technology and Community Development, Thaksin University, Phatthalung Campus, Phatthalung, 93110, Thailand

Abstract

Date seed extracts (DSE) obtained from ethanol and acetone extraction at different concentrations (0, 20, 40, 60, 80 and 100%, v/v) were characterized. Yield, phenolic and flavonoid contents increased as the concentration of ethanol and acetone increased up to 60 and 80% (v/v), respectively (P<0.05). The highest DPPH and ABTS radicals scavenging activities and ferric reducing antioxidant power (FRAP) were observed when ethanol and acetone were used at the concentrations of 60 and 80% (v/v) were used, respectively (P<0.05). However, the highest chelating activity was obtained when ethanol and acetone were used at a concentration of 20 and 40% (v/v), respectively (P<0.05). DSE prepared using 60% (v/v) ethanol (DSE-E60) and 80% (v/v) acetone (DSE-A80) were seen to have the tannic acid contents of 16.60 and 18.15 mg/g dry solid, respectively. Both extracts at levels of 100 and 200 ppm could inhibit lipid oxidation in both β-carotene linoleate and fish mince model systems. Therefore, it can be concluded that DSE-E60 and DSE-A80 can potentially be used as an alternative source of natural antioxidant.

Introduction

Lipid oxidation is a deteriorative reaction, where unsaturated fatty acids react with molecular oxygen via a free radical chain mechanism, forming fatty acyl hydroperoxides and non-volatile and volatile hydroperoxide breakdown products (Benjakul et al., 2013). Oxidation may occur in foods during harvesting, processing and storage, giving rise to the development of off-flavours, loss of essential fatty acids, fat-soluble vitamins and other bioactives, and formation of potentially toxic compounds, thus making the lipid or lipid-containing foods unsuitable for consumption (Shahidi and Zhong, 2010). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertbutylhydroquinone (TBHQ) are most widely used in foods because these synthetic antioxidants are often highly effective at controlling lipid oxidation. However, consumer demand for all-natural foods has prompted the food industry to look for more “label friendly” alternatives (McClements and Decker, 2000). As a consequence, much attention has been paid to alternative sources of natural antioxidant. So far, phenolic extract from several plant have been extracted and used as natural antioxidant such as lead seed (Benjakul et al., 2013), blueberries (Flores et al., 2014), grape seed (Shi et al., 2014) and Lamiaceae (Zhang et al., 2014).

Date (*Phoenix dactylifera* L.) palm is a principal fruit that is grown in many regions of the world, resulting in a surplus production. Currently, apart from being used as a whole fruit for human consumption, it is also used to produce different value added products such as date syrup, date confectionery, date sweets and candies. During this productions, a large quantity of date seeds or pits are generated as wastes after technological transformation of the fruits (Yousif and Alghamdi, 1999). At present, the date seeds are mainly used as an ingredient to prepare the feeds for the cattle, sheep, camel and poultry in United Arab Emirates. World production of dates is 7.5 million tons in 2012 (FAO, 2014). Date seeds, approximately 10-15% of the date fruit’s weight, depending on the variety and quality, are generated as waste from date production (Hussein et al., 1998). Al-Farsi and Lee (2011) reported that date seeds have been known that it contain valuable bioactive compounds, utilization
of this by-product is highly desirable for the date industry. Thus, utilization of such a waste is very important for date cultivation, in which an increased income can be achieved for the sector.

Date seed composed 3.1–7.1% moisture, 2.3–6.4% protein, 5.0–13.2% fat, 0.9–1.8% ash and 22.5–80.2% dietary fibre (Al-Farsi et al., 2007). Date seeds were also shown to have high levels of phenolics (31.02–44.30 g gallic acid equivalents/kg) and antioxidant activity (580–929 µmol Trolox equivalents/g) (Al-Farsi et al., 2007). Soong and Barlow (2004) reported that total phenolic content of seeds of several fruits, such as mango, avocado, and jackfruit, were higher than their edible flesh. Therefore, the seeds from different fruits could be considered a valuable source of phenolics. Al-Farsi and Lee (2008) reported that nine phenolic acids (free and liberated) were detected in date seeds and p-hydroxybenzoic (98.9 ppm), protocatechuic (88.4 ppm), and m-coumaric (84.2 ppm) acids were observed to be the dominant phenolics. From the result, date seed extract could serve as a potential source of natural antioxidant. Therefore, the aim of the present study is to evaluate the in vitro antioxidant activity of date seed extract and its efficacy in retarding lipid oxidation in different food model systems, as the study on this plant material is scarce.

Materials and Methods

Chemicals

All chemicals were of analytical grade. Ferulic acid, catechin, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyltriazine (TPTZ), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4’ ′, 4′ ′-disulphonic acid sodium salt (ferrozine), ethylenediaminetetraacetic acid disodium salt (Na₂ EDTA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tannic acid, β-carotene, Tween 40 and 1,1,3,3-tetramethoxypropane (TMP, a malondialdehyde, MDA, precursor) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Linoleic acid was purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). Folin–Ciocalteu, trichloroacetic acid (TCA) and ferrous chloride were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Lipoic acid was purchased from Sigma (St. Louis, MO, USA). Methanol, ethanol, acetone, chloroform and ammonium thiocyanate were obtained from Lab-Scan (Bangkok, Thailand).

Preparation of date seed extracts (DSE)

Date seeds obtained from date fruit (Phoenix dactylifera var. Khalas) were procured from Al- Foah date company, Al Ain, United Arab Emirates. Dry seeds were ground into a fine powder using a blender (Phillips, Guangzhou, China) and sieved through a stainless steel sieve of 40 mesh (Frilsch, Oberstein, Germany). The powder was placed in polyethylene bags and stored in a refrigerator until use.

Date seed powder (2 g) was mixed with 40 mL of distilled water, ethanol or acetone at different concentrations (20, 40, 60, 80 or 100%, v/v). Thereafter, the mixtures were stirred continuously for 12 h at room temperature (26-28°C). All mixtures were then filtered using a Buchner funnel with Whatman No.4 filter paper (Whatman International Ltd., Maidstone, England). Ethanol and acetone filtrates were evaporated at 40°C under vacuum using a rotary evaporator (EYELA N-100, Tokyo, Japan). All extracts were further subjected to freeze-drying using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lyngby, Denmark) and referred to as ‘date seed extract, DSE’. The resulting DSEs were analyzed for total phenolic and flavonoid contents and antioxidant activities. The yields of DSEs was expressed as g ferulic acid equivalents (FE)/kg dry date seed and calculated using the following equation:

\[
\text{Yield (g FE/kg date seed powder)} = \frac{\text{total phenolic content (g FE/kg dry date seed extract) } \times \text{weight of dry date seed extract (g)}}{\text{date seed powder (g)}}
\]

Determination of total phenolic content

Total phenolic contents of DSEs was determined using Folin–Ciocalteu reagent according to the method of Slinkard and Singleton (1977). The concentrations of total phenolic compounds for samples were calculated from the standard curve of ferulic acid in the range of 0 to 60 µg/mL and expressed as g ferulic acid equivalents (FE)/kg solid.

Determination of total flavonoid content

Total flavonoid content was determined according to the method of Zhishen et al. (1999). A standard curve was prepared using catechin in the range of 0–60 µg/mL. The activity was expressed as g catechin equivalents (CE)/kg solid.

Determination of antioxidant activities

DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Binsan et al. (2008). A standard curve was prepared using Trolox in the range of 0 to 50 µM. The activity was expressed as µmol Trolox equivalents (TE)/g solid.
**ABTS radical scavenging activity**

ABTS radical scavenging activity was determined as described by Binsan et al. (2008). A standard curve of Trolox ranging from 0 to 500 µM was prepared. The activity was expressed as µmol Trolox equivalents (TE)/g solid.

**Ferric reducing antioxidant power (FRAP)**

FRAP was assayed according to the method of Benzie and Strain (1996). A standard curve was prepared using Trolox ranging from 0 to 500 µM. The activity was expressed as µmol Trolox equivalents (TE)/g solid.

**Chelating activity of ferrous ion**

Chelating activity toward Fe\(^{2+}\) was measured by the method of Thiansilakul et al. (2007). The Fe\(^{2+}\) chelating activity was expressed as EDTA equivalents (µmol EDTA equivalents (EE)/g solid) using a standard curve (0–50 µM EDTA). DSEs prepared using ethanol or acetone exhibiting the highest yield and antioxidant activity were used for determination of tannic acid content and antioxidant activity in model systems.

**Study on tannic acid content and antioxidant activity in model systems**

**Determination of tannic acid content**

Tannic acid content of DSE was determined using a high performance liquid chromatography (HPLC) equipped with variable wavelength detector following the method of Tian et al. (2009) with slight modifications. The HPLC system consisted of an Agilent 1100 series HPLC (Agilent Technologies, Waldbronn, Germany), quaternary pump with seal wash option, degasser, solvent, cabinet and preparative autosampler with thermostat equipped with a diode array detector. The separation was performed on a column (Hypersil ODS C18 4.0×250 mm, 5 µm, Cole-Parmer, Hanwell, London, UK). HPLC conditions were as follows: mobile phase: 0.4% (v/v) formic acid: acetonitrile (85:15); flow rate: 0.8 mL/min; temperature: 25°C. The detection was carried out at 280 nm. The concentration of extracts was 25 mg/mL, and the injection volume was 20 µL. Standard tannic acid was used for peak identification and quantification.

**β-Carotene-linoleate model system**

The antioxidant activity in β-carotene-linoleate model system was determined according to the method of Chandrasekara and Shahidi (2010) with slight modifications. To prepare β-carotene oil-in-water emulsion, 1 mg of β-carotene was dissolved in 10 mL of chloroform. The β-carotene solution (0.2 mL) was mixed with linoleic acid (20 mg) and Tween® 40 (200 mg). Chloroform was removed under a nitrogen stream. Oxygenated distilled water (50 mL) was added to the mixture which was then homogenized at a speed of 11,000 rpm for 1 min using a Ultra-Turrax T25 homogenizer (Janke & Kunkel, Staufen, Germany). The prepared emulsion was kept in the dark at room temperature (26-28°C). Date seed extracts dissolved in 50% (v/v) ethanol (500 µL) with different concentrations (1,000 and 2,000 ppm) were mixed with prepared emulsion (4.5 mL) to obtain final concentration of 100 and 200 ppm. The mixtures were incubated at 50°C in dark. After designated time (0, 10, 20, 30, 40, 60, 90, 120, 180, 240, 300 and 360 min), the mixture was randomly taken for monitoring the absorbance at 470 nm. The negative and positive controls were prepared in the same manner, except that distilled water and BHA (500 and 1,000 ppm), respectively, were used instead of the samples. The lower decrease in the absorbance at 470 nm indicated the ability to prevent oxidation of the system.

**Fish mince model system**

Fresh mackerel (Rastrelliger kanagurta) with an average weight of 100-150 g, off-loaded 24 h after capture, were purchased from the local market in Hat Yai, Thailand. Fish were kept in ice during transportation. Upon arrival, fish were washed, filleted, de-skinned and minced using a meat grinder with a hole diameter of 5 mm. Mince samples were placed in polyethylene bags and stored in ice during preparation.

To prepare fish mince model system, fish mince was divided into 9 portions (180 g each). One portion was added with 20 mL of distilled water (control). Another six portions were added with 20 mL of selected DSEs (ethanol and acetone extract) to obtain final concentrations of 100, 200 and 500 ppm. The last two portions were added with BHA in the same manner to obtain final concentration of 100 and 200 ppm. All mixtures were thoroughly mixed in order to ensure uniformity. Different mince samples were packed in polyethylene bags, sealed and kept in ice using a mince/ice ratio of 1:2 (w/w). Molten ice was removed every day and the same quantity of ice was replaced. At designated times (0, 2, 4, 6, 8, 10 and 12 days), samples were taken for determination of peroxide value (PV) (Richards and Hultin, 2002) and thiobarbituric acid-reactive substances (TBARS) (Buege and Aust, 1978).
Statistical analysis
All experiments were carried out in triplicate. Data were subjected to the analysis of variance (ANOVA) and mean comparisons were performed using Duncan’s multiple range test (Steel and Torrie, 1980). Statistical analysis was carried out using the statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

Results and Discussions

Yield, total phenolic and flavonoid contents of date seed extract
Yield, total phenolic and flavonoid contents of DSE using water or ethanol or acetone as the extraction media at different concentrations are shown in Figure 1. The increase in the extraction yield of DSE was found with increasing concentration of the solvent up to 60 and 80% (v/v) when ethanol and acetone were used as extraction medium, respectively. Thereafter, the yield decreased (P<0.05). When water was used as an extraction medium, the lowest yield was obtained (P<0.05) (Figure 1A). The similar results were also obtained in total phenolic content (Figure 1B) and total flavonoid content (Figure 1C). The results indicated that non-polar compounds were dominated in date seed, which were extracted highly in ethanol and acetone, compared to water. Al-Farsi and Lee (2008) reported that when acetone (50%, v/v) and ethanol (50%, v/v) was used as extraction medium for date seeds, the extraction yield, total phenolic and total flavonoid contents were higher, compared to that of water extract (P<0.05). Sidduraju and Manian (2007) also reported that 70% (v/v) acetone was more efficient solvent for extracting the phenolic constituents in both raw and processed horse gram (Macrotyloma uniflorum) seeds of brown and black varieties than absolute methanol.

Among all DSEs, those obtained from 60% (v/v) ethanol and 80% (v/v) acetone extraction had the highest total phenolic and flavonoid contents (P<0.05) and these values were much higher than those obtained from both absolute solvents (P<0.05) (Figure 1B and 1C). The result was in accordance with plant extract from Orthosiphon stamineus (Chew et al., 2011). Al-Farsi and Lee (2008) reported that DSEs obtained from 50% (v/v) acetone and 50% (v/v) ethanol extraction showed higher total phenolic and flavonoid contents than those obtained from absolute acetone and ethanol extraction. In the present study, water DSE were seen to contain the lowest total phenolic and flavonoid contents (P<0.05). Water exhibited poor ability to extract phenolics (162 g/kg) and flavonoids (17 g/kg) in the date seeds due to the low solubility of these components in water (Al-Farsi and Lee, 2008). Moreover, proteins and polysaccharides could be co-extracted when water was used alone for extraction. These substances might cause the fouling for filtration. Also, the cumulative cost of the concentration operation would increase since water is more difficult to remove than acetone (Al-Farsi et al., 2007). In addition, acetone/ water or ethanol/water mixtures were more useful for extracting phenolics from protein matrices, since they
appear to dissolve the phenolic-protein complexes (Kallithraka et al., 1995). Therefore, acetone/water or ethanol/water mixture could be the better extracting media in terms of yield, phenolic content and cost.

**Antioxidant activity of DSE**

**DPPH and ABTS radicals scavenging activities**

DPPH and ABTS radical scavenging activity of DSE obtained from water or ethanol or acetone extraction at different concentrations are shown in Figure 2A and 2B, respectively. DPPH and ABTS radicals scavenging activity of DSEs increased when the concentration of ethanol and acetone increased up to 60 and 80% (v/v), respectively ($P<0.05$). Thereafter, the activities decreased until the extraction solvent concentration increased up to 100% (v/v) ($P<0.05$). DPPH radical scavenging activity of plant extracts reflects their hydrogen donating ability (Siddhuraju and Becker, 2006). This indicated that the ethanol and acetone DSE had the pronounced effect on scavenging of free radicals. In general, phenolic compounds capable of donating hydrogen atom are more effective in scavenging DPPH and ABTS radicals (Leong and Shui, 2002). Higher antioxidant activity of 60% (v/v) ethanol and 80% (v/v) acetone DSEs might be due to their higher content of phenolics and flavonoids (Figure 1). Ethanol extracts of peanut seed testa showed 92.6% scavenging effect against the DPPH radical (Yen et al., 2005). Siddhuraju (2007) reported that the methanol and acetone extracts of dry heated tamarind (Tamarindus indica) seeds exhibited the highest hydroxyl radical scavenging activity of 56.6 and 45.7%, respectively. The extract of raw and dry-heated seeds Macrotyloma uniflorum using 70% (v/v) acetone were observed to show the higher DPPH radical-scavenging activity than those extracted with methanol (Siddhuraju and Manian, 2007).

The ability of the DSE to scavenge DPPH and ABTS radicals was found to be related to the total phenolic and flavonoid contents. The results were in accordance with the extracts from Helicteres isora fruit and Ceiba pentandra seeds (Loganayaki et al., 2013). Higher DPPH and ABTS radical scavenging activities of 60% (v/v) ethanol and 80% (v/v) acetone extracts were in agreement with the higher total phenolic (Figure 1B) and flavonoid contents (Figure 1C) in these extracts. The radical scavenging activity of ethanol and acetone DSEs could be related to the presence of phenolic compounds which can play a role as an antioxidant owing to their electron transfer/hydrogen donating ability (Brand-Williams et al., 1995).
Ferric reducing antioxidant power (FRAP)

FRAP of DSE obtained from water or ethanol or acetone extraction at different concentrations is shown in Figure 2C. FRAP increased with increasing concentration of ethanol and acetone up to 60 and 80% (v/v), respectively. Thereafter, there was a decrease in the reducing power ($P<0.05$). Increase in FRAP of DSE with increasing concentration of the extraction media could be related to the increase in total phenolic (Figure 1B) and flavonoid (Figure 1C) contents in DSE. Sidduraju (2006) reported that higher extractable total phenolics in the moth bean (*Vigna aconitifoia*) extract obtained from 70% (v/v) acetone extraction exhibited higher FRAP. Similar results were reported in guava fruit extracts (Jiménez-Esrig *et al*., 2001). DSE obtained from water extraction displayed lower activity compared to that obtained from ethanol and acetone extraction, except for that obtained from 100% (v/v) acetone extraction ($P<0.05$) (Figure 2C). DSE obtained from 60% (v/v) ethanol or 80% (v/v) acetone acetone extraction showed the highest FRAP, which were in accordance with their DPPH and ABTS radicals scavenging activities (Figure 2A and 2B, respectively). The extracts of stem bark of Indian bark laburnum and horse gram seed also showed FRAP (Sidduraju and Manian, 2007; Sidduraju *et al*., 2002). Therefore, 60% (v/v) ethanol and 80% (v/v) acetone DSE showed efficient ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) complex.

Metal chelating activity

Metal chelating activity of DSEs obtained from water or ethanol or acetone extraction at different concentrations is shown in Figure 2D. DSEs displayed different trends in metal chelating activity when compared to their scavenging of free radicals (Figure 2A and 2B) and reducing power (Figure 2C). DSE obtained from 20% (v/v) ethanol and 40% (v/v) acetone acetone extraction showed the highest metal chelating activity ($P<0.05$), while the lowest metal chelating activity was observed in those obtained by 100% (v/v) ethanol and acetone extraction ($P<0.05$). DSE obtained using water showed higher metal chelating activity than that extracted with 20% (v/v) acetone and those extracted with 60, 80 and 100% (v/v) ethanol and acetone ($P<0.05$). This could be due to the fact that polar compounds being extracted from the date seed could play the major role in metal chelating. In general, DSE obtained from 60% (v/v) ethanol and 80% (v/v) acetone acetone extraction showed higher yield and phenolic and flavonoid contents as well as higher activity in most of the in vitro antioxidant activities. Therefore, DSE obtained from 60% (v/v) ethanol (DSE-E60) and 80% (v/v) acetone (DSE-A80) extraction were selected for evaluation of their effect in retardation of lipid oxidation in β-carotene-linoleate and fish mince model systems.

Tannic acid content of DSEs

DSE-E60 and DSE-A80 were analyzed for their tannic acid contents by HPLC-diode array detection (DAD) analysis. Tannic acid content of DSE-E60 and DSE-A80 were found to be 16.60 and 18.15 mg/g solid, respectively. The content of tannic acid in date seed extracts was correlated well with retardation of lipid oxidation in β-carotene-linoleate and fish mince model systems (Figure 3 and 4, respectively). Maqsood and Benjakul (2010) reported that tannic acid exhibited superior radical scavenging activities as well as reducing power and effectively inhibited lipid oxidation in fish mince and emulsion model systems.

Effect of DSE on lipid oxidation of β-carotene-linoleate model system

Changes in lipid oxidation in β-carotene-linoleate model system containing DSE-E60 or DSE-A80 at concentrations of 100 and 200 ppm in comparison with the system added with BHA (50 and 100 ppm) are shown in Figure 3. Absorbance at 470 nm (A470) of all samples generally decreased as incubation time increased ($P<0.05$). The decrease in A470 indicates the oxidation of β-carotene in the system caused by free radicals from oxidation of linoleic acid (Chandrasekara and Shahidi, 2010). In the presence of antioxidants, β-carotene bleaching was retarded mainly due to free radicals scavenging activity of antioxidants (Kittiphattanabawon *et al*., 2012). A470 of the system containing DSE and BHA was higher.

![Figure 3](image-url) Changes in lipid oxidation of β-carotene-linoleate model system added with DSEs at different concentrations. DSE-A80, DSE-E60 and BHA denote date seed extracts extracted with 80% (v/v) acetone, 60% (v/v) ethanol and butylated hydroxyanisole, respectively. Bars represent standard deviation ($n=3$).
than that of the system without antioxidant ($P<0.05$). No differences in A470 among the systems containing 100 ppm DSE-A80, 200 ppm DSE-E60 and BHA were observed. The results indicated that DSE, especially DSE-A80 at the concentration of 200 ppm, effectively retarded lipid oxidation in β-carotene-linoleate model system. The ability of DSE to retard lipid oxidation in the system might be caused by their phenolic and flavanoid contents (Figure 1) and related with their ability to donate electron and/or hydrogen atom (Figure 2). When comparing antioxidant activity of DSE at the same concentration added in the system, DSE-E60 showed the lower antioxidant activity than DSE-A80 ($P<0.05$). The difference in lipid oxidation between the system containing ethanol extract and acetone extract was most likely due to the difference in polarity, which might have influenced the extraction of compounds responsible for antioxidant activity of the extract. Since the polarity of acetone was lower than that of ethanol, DSE obtained from acetone extraction might show lower polarity than that obtained from ethanol extraction. Zhong and Shahidi (2012) reported that non-polar antioxidants are more effective than polar ones in emulsions. Therefore, acetone extract was effective in prevention of lipid oxidation in the β-carotene-linoleate model system.

**Effect of DSE on lipid oxidation of fish mince**

Impact of DSE-E60 and DSE-A80 on hydroperoxide formation in fish mince during 12 days of ice storage is shown in Figure 4A. There was a slight increase in PV of all samples up to 4 days of storage ($P<0.05$). Thereafter, a slight decrease in PV for all samples was observed, except that without antioxidant (control), which showed a sharp increase in PV until day 8. Nevertheless, a decrease was noticeable up to the end of ice storage ($P<0.05$). Hydroperoxides were formed at higher rate in the control and were decomposed rapidly during the extended storage of 12 days. Hydroperoxides formed in the control underwent decomposition at a higher rate than the rate of formation, resulting in the lower PV until the end of storage. In general, decrease in PV with increasing storage time was most likely caused by decomposition of hydroperoxide formed into the secondary oxidation products (Boselli et al., 2005). When comparing PV of all samples, it was found that the control had higher PV than the samples containing DSEs and BHA ($P<0.05$). The lowest PV was found in the sample containing 100 ppm BHA ($P<0.05$). The lower formation of hydroperoxides was found in the samples containing DSE-E60 and DSE-A80 at different concentrations, compared to the control ($P<0.05$). Among the samples containing DSE, those containing 200 ppm DSE-A80 exhibited the lower PV, compared with that containing 200 ppm DSE-E60, 100 ppm DSE-A80 and 100 ppm DSE-E60, respectively ($P<0.05$). Also DSE at higher concentration (200 ppm) was more effective in lowering the formation of PV, compared with that with lower concentration (100 ppm). Therefore, acetone DSE was more effective in delaying the formation of hydroperoxides in fish mince, compared to ethanol DSE ($P<0.05$).

Impact of DSE-E60 and DSE-A80 on TBARS formation in fish mince during 12 days of ice storage is shown in Figure 4B. TBARS values of control increased continuously during 12 days of ice storage ($P<0.05$), which indicates that the control underwent lipid oxidation at a higher degree. When the fish mince was incorporated with DSE-E60 and DSE-A80, the TBARS formation was inhibited greatly, compared to control ($P<0.05$), indicating that DSE could effectively retard lipid oxidation in the fish mince. Extracts produced from different

![Figure 4. Changes in lipid oxidation products of fish mince model system added with DSEs at different concentrations as monitored by peroxide value (A) and thiobarbituric acid-reactive substances (TBARS) values (B). DSE-A80, DSE-E60 and BHA denote date seed extracts extracted with 80% (v/v) acetone, 60% (v/v) ethanol and butylated hydroxyanisole, respectively. Bars represent standard deviation (n=3).](image-url)
leaves and fruit seed were reported to have inhibitory effect on the formation of TBARS in fish and meat model system (Arun and Rajkumar, 2011; Brannan and Mah, 2007; Pazos et al., 2005). Among the system containing DSEs, the system containing 200 ppm DSE-A80 showed the lower formation of TBARS from 6 days of iced storage onwards, compared to others (P<0.05). The inhibitory effect of DSE on the formation of TBARS was found to be concentration dependent. Higher concentration (200 ppm) of DSE-E60 and DSE-A80 was more effective than lower concentration (100 ppm) in retarding the formation of lipid oxidation products. The preventive effect of DSE on lipid oxidation could be attributed to the presence of different phenolic compounds. Therefore, DSE containing different phenolic compounds could be a promising antioxidant and play an important role in preventing lipid oxidation in fish mince products.

Conclusions

Yield, phenolic and flavanoid contents and antioxidant activities of DSE depended on type and concentration of solvent extraction. Use of 60% (v/v) ethanol or 80% (v/v) acetone rendered the extracts with the highest yield, phenolic and flavanoid contents and antioxidant activity. Both extracts effectively retarded lipid oxidation in β-carotene-linoleate and fish mince model systems. Therefore, they might serve as a potential source of natural antioxidant to retard lipid oxidation in different foods, especially oil in water emulsion and fish or meat mince products.

Acknowledgements

This research was funded by King Mongkut’s University of Technology North Bangkok with contract no. KMUTNB-NEW-57-06. We would like to thank Prince of Songkla University for the equipments support.

References


