Antioxidant Activity of Osage Orange Extract in Soybean Oil and Fish Oil during Storage

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Abstract

The food industry is seeking natural antioxidants for edible oils that have comparable activity to synthetic counterparts. In this study, Osage orange extract (OOE) rich in osajin (42.9%) and pomiferin (30.0%) was obtained after hexane extraction of the fruit, and its antioxidant activity was examined in stripped soybean oil (SBO) and fish oil (FO), in which antioxidants and polar compounds were removed. The antioxidant activity of OOE was compared with commercial natural antioxidants (i.e., rosemary extract and mixed tocopherols) and a synthetic antioxidant, butylated hydroxytoluene (BHT), during storage at 25 and 40 . The 0.1% OOE had stronger antioxidant activity than 0.1% rosemary extract and 0.1% mixed tocopherols in both oils at 25 and 40 . Its activity was similar to 0.02% BHT in SBO and was similar or slightly stronger than 0.02% BHT in FO. When OOE was studied at 0.05, 0.1, and 0.2%, there was a weak dose-response in SBO but a stronger dose-response in FO. Headspace volatile analysis using solid phase micro-extraction (SPME) combined with GC-MS indicated that 0.1% OOE was very effective in preventing the formation of volatile oxidation products in both oils. Although it should be further tested for safety before the actual use, this study shows that OOE can be developed as an antioxidant for edible oils.

Introduction

Osage orange (Maclura pomífera (Raf.) Schneid., family Moraceae) is a tree native to Texas, Oklahoma and Arkansas and is also known as horse apple or hedge apple tree (Nečas et al., 2006). This tree is common throughout the midwestern and southwestern regions of the United States and also is grown in other parts of the world (Filip et al., 2015). Its use, however, is limited as a hedge, hardwood and an insect repellant around homes (Moser et al., 2011). Some studies were conducted to utilize it for industrial use such as composites (Tisserat & Harry-O'kuru, 2019). Extracts from fruit and other parts of the tree are of great interest due to biological activities such as anti-inflammatory and antinociceptive (Kupeli et al., 2006), antifungal (Peterson & Brockemeyer, 1953), cytotoxic (Jones & Soderberg, 1979), antimicrobial (Mahmoud, 1981), anti-tumor (Voynova et al., 1991), estrogenic (Maier et al., 1995), antiviral (Bunyapraphatsara et al., 2000), and antimalarial activities (Hay et al., 2004). Among a variety of phytochemicals found in the Osage orange fruit, the bioactivities of two major isoflavones, osajin and pomiferin, have been intensely studied. Osajin and pomiferin were shown to have anti-inflammatory (Abourashed et al., 2015), antidiabetic (Bartošíková et al., 2008) and cardioprotective (Florian et al., 2006; Nečas et al., 2006) activities. Pomiferin has also been shown to have anticancer (Yang et al., 2011) and antiulcer (Bozkurt et al., 2017) activities, to promote recovery of kidney functions (Bartošíková et al., 2010), and to inhibit intracellular oxidative stress (Abourashed et al., 2015). Other bioactive components in Osage orange fruit extracts that have been identified include scandenone, auriculasin (Kupeli et al., 2006) and other prenylated flavonoids (Orazbekov et al., 2018).

Edible oils such as vegetable oils and omega-3 rich oils and oil-containing food products generally require antioxidants to prevent their oxidation during manufacturing, transportation and storage. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert* -butylhydroquinone (TBHQ) have been employed for over 60 years to prevent oil oxidation (Shahidi & Ambigaipalan, 2015). However, several studies reported that these synthetic antioxidants may be toxic and could cause liver problems and cancer (Khan et al., 2014). Therefore, the use of these compounds in foods is strictly regulated by State and Federal agencies. For this reason, the food industry is seeking natural antioxidant replacements that have comparable activity to these synthetic counterparts.

Studies have shown that Osage orange fruit extracts and components in them have antioxidant activity. Pomiferin supported defensive reactions of the body against free radicals and decreased lipid peroxidation (Bartošíková et al., 2010; Bartošíková et al., 2007). Pomiferin and osajin had inhibition activity on lipid peroxidation in the rat liver microsomal fraction and scavenging ability for peroxynitrite and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radicals (Veselá et al., 2004). Orhan et al. (2016) also reported that pomiferin and osajin had DPPH radical scavenging ability, metal chelating capacity, ferric-reducing antioxidant power (FRAP), and phosphomolibdenum reducing antioxidant power (PRAP). Studies by Veselá et al. (2004) and Orhan et al. (2016) found that pomiferin had higher antioxidant activity than osajin, which is attributed to the additional hydroxyl group of pomiferin at the ortho position that stabilizes the phenoxy radical. Increased antioxidant activity in compounds with an additional hydroxyl group at the ortho position was also been observed in other antioxidant systems such as lignans (Hwang et al., 2012).

The previous studies, however, were *in vitro* antioxidant activity studies, which provide limited information about the antioxidant activity on edible oils. Although radical scavenging ability, reducing power, and metal chelation are the major antioxidant mechanisms, these*in vitro* methods often correlate poorly with the actual protection ability of an antioxidant because the test methods cannot reflect all of the environmental conditions of actual oil storage (Decker et al., 2005). Only a few studies have been conducted on the antioxidant activity of Osage orange fruit extracts in an oil or fat, but these studies were conducted at considerably higher temperatures (100-125) than the typical storage temperature (Clopton, 1953; Schall & Quackenbush, 1956). The oxidation mechanism and kinetics are different at different temperatures and, therefore, Decker et al. (2005) recommended using temperatures lower than 60 degC for storage studies. Budincevic & Vrbaski (1991) and Hamed & Hussei (2005) conducted studies on the antioxidant activity of Osage orange fruit extracts at 55-60 in linoleic acid emulsions and in purified sunflower triacylglycerols, respectively. However, they used only one indicator of oxidation that measures the concentration of hydroperoxides. Hydroperoxides are primary oxidation products, which are formed in the early stage of oil oxidation and decompose or react with other compounds to produce secondary products including aldehydes, ketones, alcohols, carboxylic acids, dimers, and polymers. Although the concentration of hydroperoxides is a good indicator at the early stage of oil oxidation, it cannot provide information after the value reaches a peak value. For this reason, both primary and secondary oxidation products should be measured for a better assessment of oil oxidation (Decker et al., 2005; Pignitter & Somoza, 2012).

In this study, hexane was used as a solvent to extract Osage orange fruit. The precipitate in the hexane extract, which was collected by filtering, had high contents of osajin and pomiferin. The precipitate, referred to as Osage orange fruit extract (OOE), was evaluated for its antioxidant activity at 25 and 40 in stripped soybean oil (SBO) and fish oil (FO), in which antioxidants and polar compounds were removed. Oxidation of oil was monitored with three different analytical methods, peroxide value (PV), conjugated diene value (CDV) and p-anisidine value (p -AV) to determine primary and secondary oxidation products. Headspace volatile analysis was also conducted to examine the effectiveness of OOE in reducing volatile oxidation products during storage. The activity of 0.1 wt.% OOE in oil was compared to a synthetic antioxidant, BHT, at its legal limit (0.02 wt.%), a leading commercial natural antioxidant, rosemary extract (RE), at the manufacturer's highest recommended concentration (0.1 wt.%), and a widely used natural antioxidant, mixed tocopherols (Toco), at 0.1 wt.%.

Materials & Methods

Materials

2,2,4-Trimethylpentane (isooctane), formic acid, *p* -anisidine, 4-methyl-1-pentanol, aluminum oxide (Brockman Grade 1), BHT, sand, volatiles standards, ammonium thiocyanate, ferrous chloride, and food grade mixed tocopherols (Toco) were purchased from Sigma–Aldrich (St. Louis, MO, USA). According to the manufacturer's report, Toco contained 74.0% total tocopherols including 13.2% α-, 0.16% β-, 42.9% γ-, and 16.3% δ-tocopherols in weight. A commercial rosemary extract (RE) was generously provided by DuPont (Wilmington, DE, USA), which contained the mono-diglycerides of fatty acids, acetic acid esters of mono- and diglycerides of fatty acids, propylene glycol, 3.8-4.7% carnosic acid and carnosol, and 4.0-4.7% phenolic diterpenes according to the product description. Soybean oil (SBO) was purchased from a local grocery store. Fish oil (FO) was purchased from Nordic Naturals (Watsonville, CA, USA). Hexane, methanol, acetic acid, isopropanol, acetonitrile, dichloromethane, and ethyl acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Extraction from Osage orange fruit

Osage orange fruits were collected from Tazewell and Peoria counties, Illinois, USA in 2018 and stored in a freezer at -20°C until processed. Fruits were sliced into 1-cm thick pieces and frozen for 4 h at -80 °C then immediately freeze-dried for 96 h using a Labconco freeZone 12L -50°C Console Freeze dryer (Labconco Corp., Kansas City, MO, USA). Freeze-dried fruit lost approximately 75-80% of their original weight. Freeze-dried fruits were sealed in vacuum bags until extracted with a retail food vacuum sealer (Model 4800, Foodsaver, Sunbeam Inc., Boca Raton, FL, USA). Freeze-dried fruits were ground using a food processor (Model BFP-703, Cuisinart, East Windsor, NJ, USA) to a powder. Approximately 230 g of powder was then transferred to a 90 mm diameter × 200 mm height × 1 mm thickness Cellulose Extraction thimble (LabExact, I.W. Tremont Co., Inc., Hawthorne, NJ, USA) and inserted in Soxhlet extractor system consisting of 8,000 ml of hexane in a 12,000 ml capacity round bottom boiling flask fitted into a Soxhlet extractor body (12.7 cm diameter × 48.2 cm height, Size 25, Kimble Chase, Vineland, NJ, USA) and a condenser. Heating and stirring were conducted with a Stirmantle equipped with a motor drive set at 70°C and 100 rpm (Model 100D EMS116, Glas-Col, Terre Haute, IN, USA). Condenser cooling was provided 8 °C antifreeze using a refrigerated recirculating chiller (Model 6000, PolyScience, Niles, IL, USA). After 72 hours, the heat was turned off while the stirring continued. Once room temperature was achieved, the condenser was disconnected, the thimble was removed, and another thimble containing freeze-dried fruit was inserted. Additional hexane was added in order to maintain the volume of hexane at 8,000 ml. Five thimbles of freeze-dried fruit (230 g) were extracted in the same hexane solvent. Yellowish precipitates accumulated in the solution and adhered to the glass walls. The extract solution was filtered through coffee filters fitted into Büchner funnel/flask filtration system to separate a yellow precipitate. 34 g (3.0% yield from dry fruit) of the precipitate, which is referred to as Osage orange fruit extract (OOE), was collected after drying under a fume hood. The filtrate was saved for further studies. Thin layer chromatography (TLC) was employed to initially identify the composition of OOE on silica glass plates using hexane: ethyl acetate: formic acid (60:20:3 ratio). At the conclusion of the TLC run, the plate was sprayed with a solution of 30% sulfuric acid in methanol. Spots were revealed by heating the plate in an oven at 100 °C for 3-5 min. Standards were run to identify the spots based on their R_f values. OOE contained two distinct spots at $R_f = 0.19$ and 0.31 for pomiferin and osajin, respectively.

Identification and determination of osajin and pomiferin

Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS) was used to identify osajin and pomiferin. A Thermo Scientific ACCELA series HPLC system (ACCELA 1250 UHPLC pump, ACCELA1 HTC cool stack autoinjector, and an ACCELA 80 Hz Photodiode Array (PDA) detector, Thermo Corp., Milford, MA, USA) running under Thermo Scientific Xcalibur 2.1.0.1140 LC-MS software equipped with a Thermo Electron LTQ Orbitrap Discovery Mass Spectrometer (MS) connected to a high precision electrostatic ion trap (Orbitrap) was used. The MS was run with the electrospray ionization (ESI) probe in the negative mode. The source inlet temperature was 300 @C, the sheath gas rate was set at 50 arbitrary units, the auxiliary gas rate was set at 5 arbitrary units, and the sweep gas rate was set at 2 arbitrary units.

The maximal mass resolution was set at 30,000, the spray voltage was 3.0 kV, and the tube lens was set at -100 V. Other parameters are determined and set by the calibration and tuning process. The column used was an Inertsil ODS-3 reverse phase C-18 column (3 μm, 150 x 3 mm from GL Sciences, Torrance, CA, USA). The initial solvent system was 30% acetonitrile verses water, with 0.1% formic acid in both solvents, at a flow rate of 0.25 ml per minute. After injection (15-25 μl) the column was developed in a linear gradient to 100% acetonitrile in 50 minutes. The column effluent was monitored at 280 nm in the PDA detector. The software package was set to collect mass data between 100-2000 AMUs. Generally, the most significant sample ions generated under these conditions were $[M-1]^-$ and $[M+HCOO]^-$. The two major mass peaks eluted at 42.21 and 46.56 minutes were with a m/z of 419.14984 $[M-H]^-$ and 403.15440 $[M-H]^-$, respectively. These correlate to a molecular formula of $C_{25}H_{23}O_6$ (calculated monoisotopic mass of 419.149465) with a delta mmμ of 0.28 and $C_{25}H_{23}O_5$ (calculated monoisotopic mass of (403.154550) with a delta mmμ of 1.015.

HPLC analysis to determine the percent concentrations of osajin and pomiferin was conducted on a Shimadzu LC-20 HPLC system (LC-20AT quaternary pump, DGU-20A5 degasser, SIL-20A HT autosampler, and a SPD M20A photodiode array detector, running under Shimadzu LCSolutions version 1.22 chromatography software, Columbia, MD, USA). The column used was an Inertsil ODS-3 reverse phase C-18 column (5 μm, 250 x 4.6 mm, GL Sciences, Torrance, CA). The initial conditions were 30% methanol with 0.25% trifluroacetic acid and 70% water with 0.25% trifluroacetic acid, at a flow rate of 1 ml per minute. The effluent was monitored at 250 nm on the VWD. After injection (typically 25 μL), the column was developed to 100% methanol with 0.25% trifluroacetic acid in a linear gradient over 55 minutes. Five-point standard curves for the evaluation of the concentration of osajin and pomiferin purified in this laboratory was used for the determination of extinction coefficients at 250 nm. Quantitation was based on conversion of the absorbance peak areas to mg/g based the extinction coefficient determined from the standard curves. OOE was determined to consist of 42.9% osajin and 30% pomiferin.

Preparation of stripped oils

To avoid the influence of the minor compounds in oil such as tocopherols, phospholipids, free fatty acids, and mono- and diacylglycerols, stripped oils were used in this study. Stripped oils were prepared using the previously reported method (Hwang et al., 2019). In brief, a column (5.5 cm i.d., 55 cm length) was prepared with hexane (500 ml) and dry aluminum oxide (333 g). Sand was added to make a thin layer on top of aluminum oxide. Oil (500 g) dissolved in hexane (about 500 ml) was added into the column and eluted to a 2 L round-bottom flask wrapped in aluminum foil under argon blown into the flask. After completion of elution, hexane (800 ml) was added into the column, and the oil/hexane mixture was collected in the flask. Hexane was removed using a rotary evaporator for 2 h at 30-35 ^{o}C followed by drying under high vacuum for 2 days. HPLC was used to confirm the removal of tocopherols and other polar compounds in oil. Stripped oils were stored at -20 ^{o}C under argon, and the storage studies started within a week.

Storage experiments

OOE (0.1 wt.%), mixed tocopherols (Toco, 0.1% wt.%), BHT (0.02 wt.%), or rosemary extract (RE, 0.1 wt.%) was weighed in an Erlenmeyer flask. Concentrations of OOE and Toco were based on the content of osajin and pomiferin (72.9%) and the content of total tocopherols (74.0%), respectively. Since RE was a commercial product containing other ingredients than antioxidants, the concentration of active components was not known. The concentration of RE recommended by the manufacturer was 0.02-0.1 wt.% in oil, and the maximum recommended concentration (0.1%) was used in this study. Stripped SBO or FO was added in the Erlenmeyer flask containing an antioxidant and stirred under argon until the antioxidant dissolved. Oil (3.0 g) was transferred to three vials (60 mm height, 26.2 mm i.d.) per treatment and per each time point. For OOE, which was not completely dissolved, 3.0 g of suspension of OOE in oil was transferred to vials while being stirred with a magnetic bar under argon. This is an accelerated storage test due to the relatively high surface area to volume ratio (1.65 cm^2/cm^3). Oil samples were stored at 25 and 40 , removed at each time point, transferred to smaller vials (45 mm height, 12 mm i.d.), and kept in a freezer (-20). PV was measured within 24 h, and then other values were determined after PV.

Peroxide value (PV)

A ferric-thiocyanate based spectrophotometric assay was used as previously reported (Shantha & Decker, 1994) for the determination of PV, with the following modifications: the assay volume was proportionally reduced to 3.0 ml, oil sample size was reduced to 10 mg, and a mixture of methanol/isopropanol (2:1 v/v) was used as the solvent to avoid using chloroform. Absorbances were measured using a PerkinElmer Lambda 35 UV/Vis spectrophotometer (Perkin Elmer, Waltham, MA, USA) at 510 nm. Ferric chloride solutions (1-15 μg Fe^{3+}/ml) in methanol/isopropanol (2:1 v/v) were used for the standard curve. Three experimental replicates were analyzed in duplicate.

Conjugated diene value (CDV)

The AOCS official method (Ti 1a-64) was used to determine CDV (AOCS, 1990). In brief, approximately 10 mg oil samples were weighed in test tubes (Pyrex, 16 × 125 mm) and dissolved in 10 ml isooctane. The solution (1.0 ml) was transferred to another test tube and diluted with 9 ml isooctane. The solution was added in a quartz cuvette and the absorbance was measured with the same spectrophotometer used for PV at 233 nm. Three experimental replicates were analyzed in duplicate.

p-Anisidine value (p-AV)

The AOCS standard method (Cd 18-90) was used to measure*p* -AV (AOCS, 2011). First, a*p* -anisidine solution was prepared by dissolving*p* -anisidine (25 g) in acetic acid (100 ml). Oil samples (about 0.5 g) were weighed into a 25 ml volumetric flask and isooctane was added to fill the flask. The sample solution (5 ml) was transferred into a test tube, the*p* -anisidine solution (1 ml) was added, and the solution was briefly vortexed. After 10 min, the absorbance was measured in a quartz cuvette with the same spectrometer used for PV at 350 nm. Isooctane (5 ml) and *p* -anisidine solution (1 ml) added in a test tube was used as a blank. When *p* -AV was over the measurement limit, the concentration of the sample was reduced to half and the value was measured again. *p* -AV of three experimental replicates were determined.

Headspace volatile analysis

Solid phase micro-extraction (SPME) combined with GC-MS was used to analyze headspace volatiles using a Thermo-Fisher ISQ GC-MS (Thermo-Fisher, Waltham, MA) with a Combi-Pal (CTC USA, MN) auto-sampler equipped with a shaking incubator, fiber cleaning station, and SPME holder, SPME injector insert, and Thermo-Fisher TG-WAXMS column (30 m x 0.25 mm x 0.25 μM). The SPME fiber was 1 cm, 50/30 μm divinlybenzene/carboxen® on polydimethylsiloxane on a StableFlex fiber (Millipore-Sigma, St. Louis, MO). An internal standard (IS) solution was prepared by dissolving 4-methyl-1-pentanol in stripped soybean oil to a concentration of 0.5 mg/g. Oil samples (0.1000 ± 0.0020 g) and IS (0.0100 ± 0.0012 g) were weighed in 2 ml amber headspace vials. Using the automated SPME headspace analyzer, samples were transferred to the incubator and held for 5 min at 60 °C with shaking (250 rpm). The SPME fiber was injected into the sample headspace and held for 30 min, then volatiles were desorbed by injecting the fiber into the GC-MS injector (250 °C, splitless mode) for 5 min at constant helium flow of 1.5 ml/min. The oven was held at 40 °C for 1 min, then heated to 240 °C at 10 °C/min where it was held for 2 min. The MS was operated in electron ionization mode at 70 eV and 250 °C and scanning mass/charge m/z 35 – 350. Volatiles were identified by comparison of retention time and mass spectra to commercial standards. Standard curves using the internal standard method were developed using 5 μg IS and mixtures (0.005 to 10 μg/g oil) of commercial standards, and analyzing the standard mixtures using the same procedure described above. Three experimental replicates were analyzed in duplicate.

Statistics

All the storage tests were carried out in triplicate and in randomized order. Data were analyzed by one-way analysis of variance (ANOVA) and all means of the oxidation data were compared by Tukey-Kramer HSD test with statistical significance at $P < 0.05$ using the program JMP 10.0.0 (SAS Institute, Cary, NC, USA).

Results and discussion

Comparison of extraction methods

A Soxhlet extractor with hexane as solvent was used to extract freeze-dried and ground fruit. This method conveniently provided an extract that contained 42.9% osajin and 30.0% pomiferin, which was collected by filtration of the precipitates from the hexane solution without further purification. Previously, Schall and Quackenbush (1956) reported that extractions of Osage orange fruit with petroleum ether, acetone, carbon tetrachloride, ethyl acetate, ethyl ether, and methanol produced extracts in 12.8, 22.8, 20.4, 23.9, 25.5, 18.2, 21.8, and 51.8% yields, respectively. However, yields were unreasonably high (e.g. 51.8% with methanol), and detailed information (e.g. contents of osajin and pomiferin) was not provided. Therefore, a literature search was conducted for more recent studies to compare the extraction method in the current study with other previous methods. Table 1 summarizes comparisons of the extraction yields and the contents of osajin and pomiferin, when reported. Among the solvents, 95% ethanol had the highest extraction yields ranging between 13.3 and 14.8% (Mahmoud, 1981; Su et al., 2017). However, Su et al. (2017) reported low contents of osajin and pomiferin (0.09 and 0.03%, respectively) in the extract while Mahmoud (1981) reported high contents of osajin (10.0%) and pomiferin (5.0%) in the extract. In another study using ethanol extraction of wet fruit, the extract (yield: 0.43%) contained 6.3% osajin and 6.3% pomiferin (Orazbekov et al., 2018). Methanol extraction produced an extract with relatively high contents of osajin (26.4%) and pomiferin (24.0%) in 5.6% yield from dry fruit (Abourashed et al., 2015). Methanol was also used in the study by Darji et al. (2013), which determined contents of osajin and pomiferin in dry fruit to be 0.6-2.1% and 1.6-5.0%, respectively, yet the extraction yield was not determined. The ethyl acetate gave an extract with higher contents of osajin (25.7%) and pomiferin (36.2%) compared to ethanol and methanol providing a 1.53% yield from wet fruit (Tsao et al., 2003). Similarly, Orhan et al. (2016) reported that their ethyl acetate extract of Osage orange fruit had higher total phenolic and flavonoid contents than ethanol, water and butyl alcohol extracts. To the best of our knowledge, no study has been conducted with hexane for Osage orange fruit. The hexane extraction of dried fruit in this study gave the extract containing 42.9% osajin and 30.0% pomiferin in a 3.0% yield. The total osajin and pomiferin content (72.9%) contained in this extract was the highest among the extraction methods reported so far.

Other solvents and such as supercritical CO_2 (Filip et al., 2015), chloroforms (Kupeli et al., 2006), and water (Kupeli et al., 2006) were used to give the yield of 5.3-7.9% from partially dried fruit, 4.03% from wet fruit, and 7.17% from wet fruit, respectively. However, the contents of osajin and pomiferin were not reported in these studies. Extraction methods using petroleum ether (Wolfrom & Mahan, 1942) and diethyl ether (Ribaudo et al., 2018) followed by the formation of lead complexes, separation and removal of lead were also developed to produce pure osajin and pomiferin.

Antioxidant activity of OOE in stripped SBO during storage

Antioxidant activity of OOE was evaluated in stripped SBO at two different temperatures: 25 ^{o}C as a typical storage temperature of oil and 40 ^{o}C as a possible high temperature during transportation and manufacturing processes. Figure 1 shows the oxidation of stripped SBO containing 0.1% OOE compared to control (stripped SBO without antioxidant), 0.02% BHT, 0.1% RE, and 0.1% Toco. As mentioned in the introduction section, to avoid relying on a single analytical method, PV, CDV and p -AV were used to determine oil oxidation. PV is an indicator for the early stages of oxidation, and p -AV is an effective indicator for secondary stages of lipid oxidation, while CDV is useful for early and intermediate stages.

Figure 1a shows PV of stripped SBO with four different treatments stored at 25 . Since the PV of 0.1% Toco and the control reached a peak value at day 14, reasonable comparisons between treatments could not be made beyond day 14. Therefore, we focused on the comparison among treatments during the lag phase, which were days 5 and 14. On day 5, SBO samples with 0.1% OOE and 0.02% BHT had lower PV (0.68 +- 0.09 and 0.73 +- 0.10 meq/Kg, respectively) than those with 0.1% RE (1.92 +- 0.20 meq/Kg) and 0.1% Toco (2.09 +- 0.03 meq/Kg) while the control had the highest PV (4.20 +- 0.19 meq/Kg). On day 14, the PV of the sample with 0.1% Toco sharply increased (12.09 +- 0.22 meq/Kg) and became close to that of the control (12.72 +- 0.34 meq/Kg). Oil samples with 0.1% OOE and 0.02% BHT had the lowest PV (2.62 +- 0.25 and 2.78 +- 0.07 meq/Kg, respectively) followed by 0.1% RE (6.02 +- 0.30 meq/Kg) on day

14. Figure 1b shows the CDV of SBO samples stored at 25 up to 50 days. CDV of the control sharply increased during storage up to day 21 and gradually increased between day 21 and day 50. All antioxidant treatments significantly lowered the CDV. Since differences between treatments became obvious on day 21, the Tukey-Kramer HSD test (at $P < 0.05$ for CDV) was used to compare mean CDV only for data from days 21 and 50. The 0.1% Toco was found to have significant antioxidant activity in comparison with the CDV of the control, but it had significantly higher CDV (5.38 +- 0.27 mmol/L) than 0.1% OOE, 0.02% BHT and 0.1% RE (3.23 +- 0.17, 2.72 +- 0.06 and 3.38 +- 0.18 mmol/L, respectively) on day 21. There was no significant difference between 0.1% OOE and 0.02% BHT throughout the study. The 0.1% RE treatment had a significantly higher CDV (4.64 +- 0.17 mmol/L) on day 50 compared to 0.02% BHT (2.93 +- 0.26 mmol/L), but didn't significantly differ from the 0.1% OOE treatment (3.42 +- 0.33 mmol/L). Figure 1c shows p -AV of SBO samples with statistical analysis of results for day 21 and day 50. While p -AV of the control increased to 134.05 +- 4.35 on day 50, those containing 0.1% OOE, 0.02% BHT, 0.1% RE, and 0.1% Toco did not significantly increase throughout the study. This indicates that all the antioxidants were very effective in preventing the formation of aldehydes, the secondary oxidation products, even though significant amounts of primary oxidation products, hydroperoxides and conjugated dienes, formed during storage up to 50 days.

Figure 2 shows the oxidation of SBO samples at 40 . The PV of the control and SBO with 0.1% Toco reached peak values on day 7, and so the treatment comparisons were focused on days 1, 3, and 7 (Fig. 2a). There was no significant difference between the 0.1% OOE and the 0.02% BHT treatments through Day 7. PV of SBO with RE was significantly higher than those with OOE and BHT on days 3 and 7. The PV of Toco increased more rapidly than the other three antioxidant treatments. CDV analysis also showed the antioxidant activity order of BHT [?] OOE > RE > Toco at 40 (Fig. 2b), which was observed with PV. On day 7, SBO samples containing 0.1% OOE, 0.02% BHT and 0.1% RE exhibited similar CDV (3.40 +- 0.05, 3.38 +- 0.12 and 3.90 +- 0.28 mmol/L, respectively) while CDV of SBO with 0.1% Toco (6.00 +- 0.12 mmol/L) was higher than those with other treatments indicating the weakest activity of Toco. On day 20, SBO with OOE and BHT had the lowest CDV (3.60 +- 0.10 and 3.27 +- 0.07 mmol/L, respectively) followed by RE (6.49 +- 0.25 mmol/L), and then Toco (13.81 +- 0.85 mmol/L). As observed at 25 , samples with all the treatments showed much lower p -AV than the control during the course of 20-day storage at 40 . Only at day 20, SBO containing 0.1% Toco showed a slightly, but not significantly, higher$p$ -AV (6.55 +- 0.55) than SBO with 0.1% OOE, 0.02% BHT, and 0.1% RE (1.38 +- 0.55, 1.19 +- 0.25 and 3.48 +- 0.10, respectively).

The trend observed with SBO samples at 25 was very similar to that at 40 . The overall antioxidant activity order was BHT [?] OOE > RE > Toco during storage of SBO at 25 and 40 . It is noteworthy that 0.1% OOE showed stronger antioxidant activity than the commercial natural antioxidants, RE at the manufacturer's recommended highest concentration (0.1%) and Toco at 0.1%.

Concentration effect of OOE in stripped SBO

Higher antioxidant concentrations do not necessarily provide greater antioxidant activity. In some cases, a higher concentration can result in a worse protection of oil. For example, it is well known that α-tocopherol exhibits pro-oxidant activity at highe concentrations (Huang et al., 1994). Another example is that 0.2 g/L and 0.25 g/L ethanol extract of Osage orange fruit did not show stronger antioxidant activity than 0.1 g/L in linoleic acid emulsion when monitored by a β-carotene discoloration method (Budincevic & Vrbaski, 1991). Therefore, a lower (0.05%) and higher (0.2%) concentration of OOE in SBO were compared with 0.1% OOE to determine the most effective concentration range of OOE.

As shown in Table 2, PV slightly lowered by increasing concentration of OOE from 0.05% to 0.2% at 25 . However, CDV slightly increased and$p$ -AV did not show a significant change with increasing concentration of OOE from 0.05 to 0.2%. At 40 , PV slightly increased while CDV slightly decreased with increasing concentration of OOE from 0.05-0.2%. Although p -AV seemed to increase with increasing concentration of OOE, the difference was not statistically significant. Therefore, at both temperatures (25 and 40), the activity of OOE at 0.05, 0.1 and 0.2% did not seem to be very different. This indicates that the protection

of SBO from oxidation at 25 and 40 can be achieved at a concentration as low as 0.05% OOE.

Antioxidant activity of OOE in stripped FO during storage

Antioxidant activity often depends on the type of oil used. For example, while 0.2% grapefruit seed extract had similar antioxidant activity to 0.01% TBHQ in palm oil, the extract had significantly lower activity than 0.01% TBHQ in FO (Safakar et al., 2016). As another example, 0.25% extract of spent coffee ground had stronger antioxidant activity than 0.02% BHT in stripped SBO, but it showed much weaker activity than 0.02% BHT in stripped FO (Hwang et al., 2019). The different activity of an antioxidant in different oils is attributed to different fatty acids composition and inherent antioxidants in oil (Safakar et al., 2016). Since differences in antioxidant activity were also observed between stripped SBO and stripped FO where inherent antioxidants were completely removed (Hwang et al., 2019), the fatty acid composition alone can affect the activity of an antioxidant.

To understand the antioxidant activity of OOE in different oils, the storage study was also conducted in stripped FO. As shown in Figure 3a, PV of the control FO and those of FO containing 0.1% Toco and 0.1% RE quickly increased and reached a peak value in 2 days at 25 . In contrast, PV of FO samples containing 0.1% OOE and 0.02% BHT increased more slowly. On day 2, while FO samples with 0.1% OOE and 0.02% BHT had PV of 4.90 ± 0.17 and 5.43 ± 0.12 meq/Kg, respectively, those with 0.1% Toco and 0.1% RE had significantly higher PV (12.33 ± 0.45 and 10.88 ± 0.0.35 meq/Kg, respectively). On day 5, while PV of FO with 0.02% BHT increased to 11.80 ± 0.78 meq/Kg, that with 0.1% OOE was significantly lower (8.89 ± 0.24 meq/Kg) indicating stronger antioxidant activity of 0.1% OOE than 0.02% BHT in FO. As shown in Figure 3b, CDV of FO with 0.1% OOE and 0.02% BHT were the lowest throughout the course of storage at 25 . FO with OOE had slightly, but not significantly, lower CDV (11.33 +- 0.24 mmol/L) than that with BHT (13.80 +- 0.07 mmol/L) at day 28. CDV at day 10 indicated that Toco had stronger antioxidant activity (12.87 +- 0.29 mmol/L) than RE (21.66 +- 1.31 mmol/L), which was opposite to the trend shown in SBO. CDV at day 28 also showed that Toco had stronger antioxidant activity (22.56 +- 0.42 mmol/L) than RE (45.68 +- 13.16 mmol/L) in FO at 25 . p -AV of FO with 0.1% RE also increased faster than that with 0.1% Toco confirming the stronger antioxidant activity of Toco than RE in FO (Fig. 3c). On day 28, FO with 0.1% OOE had significantly lower p -AV (32.49 +- 0.39) than that with 0.02% BHT (53.43 +- 0.61) indicating the stronger antioxidant activity of 0.1% OOE than 0.02% BHT. FO with RE had lower$p$ -AV (100.68 +- 10.88) than the control (218.45 +- 5.29) on day 10, but had even higher p -AV (564.02 +- 5.76) than the control (210.92 +- 0.65) on day 28. p -AV can reach a peak value and then decrease (Adjonu et al., 2019), and it is possible that the p -AV of the control might have reached its peak value and then decreased between days 10 and 28.

Figure 4a shows PV of FO samples at 40 . The PV of the control and FO samples with 0.1% Toco and 0.1% RE reached peak values in 1 day (11.26 +- 1.03, 10.91 +- 0.82 and 11.31 +- 0.40 meq/Kg, respectively). FO with 0.1% OOE had the lowest PV on day 1 (7.18 +- 0.21 meq/Kg) followed by 0.02% BHT (9.58 +- 0.05 meq/Kg) indicating stronger antioxidant activity of 0.1% OOE compared to 0.02% BHT in FO at 40 . FO with 0.1% RE had a higher CDV (24.23 +- 1.06 mmol/L) than FO with 0.1% Toco (12.45 +- 0.45 mmol/L) on day 5 as well as day 10 (28.84 +- 3.13 and 18.03 +- 0.21 mmol/L, respectively) (Fig. 4b) indicating stronger antioxidant activity of Toco than RE in FO. FO samples with 0.1% OOE and 0.02% BHT had the lowest CDV throughout the course of storage at 40 . 0.1% RE did not show very strong activity in preventing p -AV of FO at 40 while all other treatments had significantly lower p -AV compared to the control FO (Fig. 4c). FO with 0.1% OOE had slightly, but not significantly, lower p -AV (23.15 +- 0.77) than those with 0.02% BHT and 0.1% Toco (33.76 +- 0.38 and 33.83 +- 0.97, respectively) on day 28. In general, the order of antioxidant activity in FO was OOE [?] BHT > Toco > RE, which was somewhat different from the trend with SBO. Since stripped oils were used in this study, the different activity trends in the two different oils is attributed to their fatty acid compositions.

Concentration effect of OOE in stripped FO

Table 3 shows PV, CDV and p -AV of FO samples with 0.05, 0.1 and 0.2% OOE after 10 days at 25 and

after 5 days at 40 . At 25 , PV, CDV and p -AV of FO slightly lowered by increasing concentration of OOE from 0.05% to 0.2%. At 40 , PV did not show the differences between OOE concentrations. This is because that PV of FO samples increased so quickly at 40 and FO samples with 0.05-0.2% OOE might have already reached the peak PV in 2 days as shown in Figure 4. CDV decreased from 10.19 +- 0.24 mmol/L to 8.43 +- 0.14 mmol/L as OOE concentration increased from 0.05% to 0.2%. p -AV of FO with 0.2% OOE was significantly lower (16.23 +- 0.08) than that with 0.05% OOE (36.77 +- 0.70). Overall results in Table 3 indicate that, unlike in SBO, the protection effect of FO can be improved by increasing concentration of OOE from 0.05% to 0.2%.

Headspace volatile analysis

While most analytical methods give information on nonvolatile oxidation products such as hydroperoxides, conjugated dienes and nonvolatile aldehydes in oil, the headspace volatile analysis determines volatile oxidation products formed in the headspace of oil sample. In fact, volatile oxidation products are very important for perception of oil quality because they are responsible for off-odors and flavors (Kerrihard et al., 2015). Therefore, in this study, headspace volatiles were analyzed to investigate the effect of 0.1% OOE on preventing the formation of volatile oxidation products in SBO and FO.

Table 4 shows headspace volatile analysis results for stripped SBO and stripped FO with the four antioxidants. SBO samples were analyzed after storing for 21 days at 25 oC and for 7 days at 40 oC, and FO samples were analyzed after 10 days at 25 oC and 5 days at 40 oC. The instrument was set to monitor 22 headspace volatiles including pentanal, hexanal, heptanal, octanal, nonanal, 2-propenal, t -2-pentenal,t -2-hexenal, t -2-heptenal, t -2-octenal,t -2-decenal, t -2-undecenal, t,t -2,4-heptadienal,t,t -2,4-nonadienal, t,t -2,4-decadienal, 1-pentanol, 1-hexanol, 1-octen-3-ol, n-butyl methacrylate, 2-ethylfuran, 2-pentylfuran, and decane. There were no detectable lipid oxidation-related volatiles in the initial stripped SBO. After storage at 25 for 21 days, pentanal, hexanal, t -2-pentenal,t -2-heptenal, t -2-octenal, 1-octen-3-ol, andt,t -2,4-heptadienal were observed in control stripped SBO, while pentanal, hexanal, t -2-pentenal, and t -2-octenal were detected in the stripped soybean oil with antioxidant treatments. It is worthwhile to note that all four treatments were very effective in preventing the formation of t -2-heptenal, which was the most abundant volatile (12.30 +- 0.64 μg/g) in the control stripped SBO and is known to be the key odorant in edible oils along with pentanal and hexanal (Xu et al., 2017). Formation of 1-octen-3-ol and$t$,t -2,4-heptadienal was also effectively prevented by all four antioxidants. Other volatiles including pentanal, hexanal,t -2-pentenal, and t -2-octenal were significantly reduced by the four antioxidants. The overall effectiveness of 0.1% OOE in reducing volatile oxidation products of SBO at 25 was very similar to 0.1% Toco, 0.02% BHT and 0.1% RE. After 7 days at 40 , in addition to the 7 volatiles observed at 25 , t -2-hexenal was also detected in the headspace of the control, and hexanal was the most abundant volatile (10.77 ± 0.34 μg/g) followed by$t$ -2-heptenal (6.97 ± 0.97 μg/g). All four antioxidants prevented the formation of these volatiles, as only hexanal was detected in the samples at significantly lower levels compared to the control, and their effectiveness was very similar.

The control stripped FO at day 0 did not have any detectable volatiles, however after storage at 25 for 10 days, five volatiles, pentanal, hexanal, t -2-pentenal, t -2-heptenal, and$t$,t -2,4-heptadienal were detected.t ,t -2,4-Heptadienal was detected at the highest concentration, which was consistent with the earlier studies with FO heated at 80 (Yang et al., 2017) and with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) heated at 110 °C (Peinado et al., 2016).t ,t -2,4-Heptadienal is known to be one of the major volatiles associated with oxidized FO odor (Sullivan & Budge, 2012; Venkateshwarlu et al., 2004). 0.1% OOE, 0.02% BHT and 0.1% Toco completely prevented the formation of$t$,t -2,4-heptadienal during storage of FO for 10 days at 25 , while 0.1% RE was not very effective in preventing this volatile. Hexanal was not detected in FO samples with 0.1% OOE and 0.1% Toco while it was present in FO samples with 0.02% BHT and 0.1% RE at 0.07 +- 0.00 and 0.18 +- 0.02 μg/g, respectively, after 10 days at 25 . FO with 0.1% RE had higher amounts of t -2-pentenal (1.29 ± 0.32 μg/g), hexanal (0.18 ± 0.02 μg/g) and t ,t -2,4-heptadienal (2.09 ± 0.79 μg/g) compared to FO with other antioxidants. The lower antioxidant activity of 0.1% RE than other antioxidants in FO was also observed with PV, CDV and p -AV. One more volatile,t -2-octenal, was

observed in the control FO after 5 days at 40 . OOE effectively prevented the formation of all the volatiles except for pentanal, which was detected at 0.02 ± 0.01 μg/g. 0.1% RE was found to be the least effective in preventing the formation of pentanal, hexanal,*t* -2-pentenal, and *t,t* -2,4-heptadienal during storage of FO at 40 among the four antioxidants. Overall results showed that 0.1% OOE was very effective in preventing the formation of volatile oxidation products during storage of FO at 25 and 40 , which was similar to 0.02% BHT and 0.1% Toco, and better than 0.1% RE.

Conclusions

In this study, hexane extraction of Osage orange fruit produced an extract rich in osajin (42.9%) and pomiferin (30.0%). The antioxidant activity of the extract (OOE) was examined in stripped SBO and FO at 0.1 wt.% during storage at 25 and 40 using PV, CDV and *p* -AV. The overall antioxidant activity order was BHT [?] OOE > RE > Toco in SBO at 25 and 40 . In contrast, the activity order was OOE [?] BHT > Toco > RE in FO at both temperatures, 25 and 40 . The activities of 0.05, 0.1 and 0.2% OOE in SBO were not very different indicating that the protection of SBO from oxidation can be achieved at a concentration of OOE as low as 0.05%. Unlike in SBO, the antioxidant activity of OOE increased with increasing concentration from 0.05% to 0.2% in FO. Headspace volatile oxidation products were analyzed using SPME combined with GC-MS. 0.1% OOE was effective in preventing the formation of volatile oxidation products in SBO and FO. While all four antioxidants had similar effects in SBO, 0.1% OOE prevented the formation of volatile oxidation products better than 0.1% RE and similar to 0.02% BHT and 0.1% Toco in FO. Although it should be further tested for safety before the actual use, the strong antioxidant activity of OOE in SBO and FO found in this study indicates the high potential of OOE as a natural antioxidant for edible oils and oil-containing food products.

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Figure Captions

Figure 1. Oxidation of stripped soybean oil (SBO) containing 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE) at 25 ^{o}C: a) peroxide value (PV), b)

conjugated diene value (CDV), and c) *p* -anisidine value (p-AV). Error bars represent standard deviations. Means not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).

Figure 2. Oxidation of stripped soybean oil (SBO) containing 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE) at 40 ºC: a) peroxide value (PV), b) conjugated diene value (CDV), and c) *p* -anisidine value (p-AV). Error bars represent standard deviations. Means not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).

Figure 3. Oxidation of stripped fish oil (FO) containing 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE) at 25 ºC: a) peroxide value (PV), b) conjugated diene value (CDV), and c) *p* -anisidine value (p-AV). Error bars represent standard deviations. Means not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).

Figure 4. Oxidation of stripped fish oil (FO) containing 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE) at 40 ºC: a) peroxide value (PV), b) conjugated diene value (CDV), and c) *p* -anisidine value (p-AV). Error bars represent standard deviations. Means not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).

Table 1. Comparison of the extraction method in the current study with previously reported methods

Solvent	Fruit	Procedure
Hexane	Dried	Soxhlet extraction followed by filtration
95% Ethanol	Dried	Soaking at room temperature (3 times), partitioning between water and ethyl acetate
95% Ethanol	Dried	Percolation, partitioning in chloroform and water and drying the chloroform layer.
Ethanol	Wet	Maceration at 25 (twice), evaporation to concentrate and filtration.
Methanol	Dried	Ultrasonication for 30 min, filtration and evaporation.
Methanol	Dried	Ultrasonication for 15 min at room temperature and centrifugation.
Ethyl acetate	Wet	Soaking overnight at room temperature, drying with anhydrous sodium sulfate, and f
Supercritical CO2	12.7% moisture	210 bar at 40 or 350 bar at 60
Chloroform	Wet	Soaking at room temperature for 2 days and evaporation
Water	Wet	Soaking at room temperature for 2 days and evaporation

*The yield was not determined by the authors, but calculated in this study from the procedure written in the article.

Table 2. Peroxide value (PV), conjugated diene value (CDV) and p-anisidine value (p-AV) of stripped soybean oil (SBO) with Osage orange fruit extract (OOE) at three different concentrations (0.1%, 0.25%, 0.5%) during storage at 25 ºC for 21 days and at 40 ºC for 7 days. Values in a row not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).

	After 21 days at 25 ºC	*After 21 days at 25 ºC*	*After 21 days at 25 ºC*
Concentration of OOE	*0.05%*	*0.1%*	*0.2%*
PV	3.12 ± 0.31 a	2.81 ± 0.13 ab	2.50 ± 0.22 b
CDV	3.01 ± 0.08 c	3.23 ± 0.17 b	3.55 ± 0.55 a
p-AV	0.37 ± 0.06 a	0.31 ± 0.14 a	0.22 ± 0.18 a
	After 7 days at 40 ºC	*After 7 days at 40 ºC*	*After 7 days at 40 ºC*
Concentration of OOE	*0.05%*	*0.1%*	*0.2%*
PV	3.11 ± 0.12 b	4.26 ± 0.66 a	4.56 ± 0.14 a

	After 21 days at 25 ºC	*After 21 days at 25 ºC*	*After 21 days at 25 ºC*
CDV	4.40 ± 0.19 a	3.40 ± 0.05 b	3.57 ± 0.12 b
p-AV	0.80 ± 0.08 a	1.20 ± 0.05 a	1.79 ± 3.62 a

Table 3. Peroxide value (PV), conjugated diene value (CDV) and p -anisidine value (p-AV) of stripped fish oil (FO) with Osage orange fruit extract (OOE) at three different concentrations (0.1%, 0.25%, 0.5%) during storage at 25 ºC for 10 days and at 40 ºC for 5 days. Values in a row not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).

	After 10 days at 25 ºC	*After 10 days at 25 ºC*	*After 10 days at 25 ºC*
Concentration of OOE	*0.05%*	*0.1%*	*0.2%*
PV	11.49 ± 0.34 a	11.37 ± 0.69 a	9.06 ± 0.42 b
CDV	7.99 ± 0.34 a	7.35 ± 0.29 b	7.58 ± 0.06 b
p-AV	15.50 ± 0.63 a	11.01 ± 0.61 b	9.96 ± 0.23 b
	After 5 days at 40 ºC	*After 5 days at 40 ºC*	*After 5 days at 40 ºC*
Concentration of OOE	*0.05%*	*0.1%*	*0.2%*
PV	10.77 ± 0.55 a	10.40 ± 0.84 a	10.38 ± 0.44 a
CDV	10.19 ± 0.24 a	8.80 ± 0.29 b	8.43 ± 0.14 c
p-AV	36.77 ± 0.70 a	23.15 ± 0.22 b	16.23 ± 0.08 c

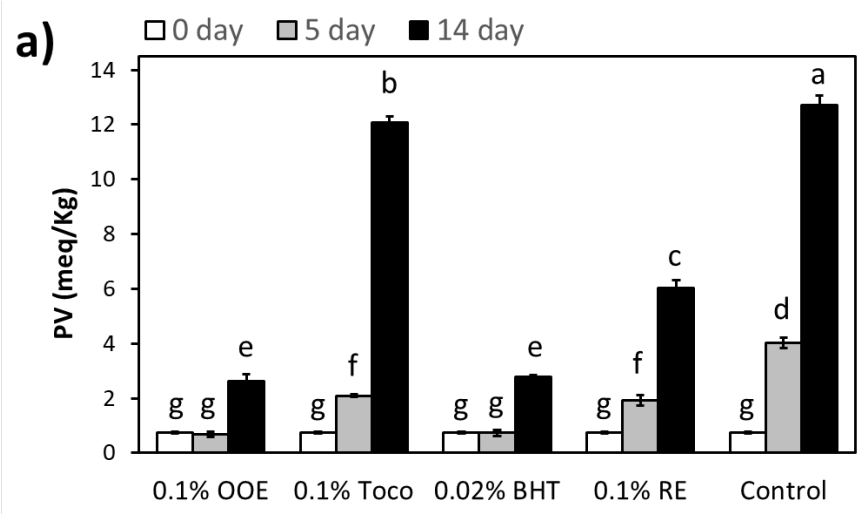
Table 4. Headspace volatiles (µg/g oil) of stripped soybean oil (SBO) stored at 25 ºC for 21 days and at 40 ºC for 7 days, and those of stripped fish oil (FO) stored at 25 ºC for 10 days and at 40 ºC for 5 days. Oil samples contained 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE). Values in a row not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).

SBO, 25 ºC for 21 days	*SBO, 25 ºC for 21 days*	*SBO, 25 ºC for 21 days*	*SBO, 25 ºC for 21 days*	*SBO, 25 ºC for 21 days*	*SBO, 25 ºC for 21 days*
	0.1% OOE	*0.1% Toco*	*0.02% BHT*	*0.1% RE*	*Control*
Pentanal	0.04 ± 0.00 bc	0.04 ± 0.01 bc	0.06 ± 0.01 b	0.03 ± 0.00 c	0.27 ± 0.01 a
Hexanal	0.19 ± 0.01 b	0.16 ± 0.00 b	0.17 ± 0.00 b	0.22 ± 0.03 b	6.87 ± 0.63 a
t-2-Pentenal	0.17 ± 0.00 b	0.18 ± 0.02 b	0.18 ± 0.01 b	0.19 ± 0.01 b	0.47 ± 0.01 a
t-2-Heptenal	nd*	nd	nd	nd	12.30 ± 0.64
t-2-Octenal	0.22 ± 0.01 c	0.22 ± 0.02 c	0.22 ± 0.00 c	0.26 ± 0.01 b	1.03 ± 0.04 a
1-Octen-3-ol	nd	nd	nd	nd	5.61 ± 0.15
t,t-2,4-Heptadienal	nd	nd	nd	nd	1.26 ± 0.03
SBO, 40 ºC for 7 days	*SBO, 40 ºC for 7 days*	*SBO, 40 ºC for 7 days*	*SBO, 40 ºC for 7 days*	*SBO, 40 ºC for 7 days*	*SBO, 40 ºC for 7 days*
	0.1% OOE	*0.1% Toco*	*0.02% BHT*	*0.1% RE*	*Control*
Pentanal	nd	nd	nd	nd	0.41 ± 0.06
Hexanal	0.09 ±0.01 b	0.07 ±0.00 b	0.07 ± 0.02 b	0.09 ±0.01 b	10.77 ± 0.34 a
t-2-Pentenal	nd	nd	nd	nd	0.22 ± 0.03
t-2-Hexenal	nd	nd	nd	nd	0.13 ± 0.01
t-2-Heptenal	nd	nd	nd	nd	6.97 ± 0.97
t-2-Octenal	nd	nd	nd	nd	0.76 ± 0.07
1-Octen-3-ol	nd	nd	nd	nd	4.74 ± 0.40

SBO, 25 ºC for 21 days	*SBO, 25 ºC for 21 days*	*SBO, 25 ºC for 21 days*	*SBO, 25 ºC for 21 days*	*SBO, 25 ºC for 21 days*	*SBO, 25 ºC for 21 days*
t,t-2,4-Heptadienal	nd	nd	nd	nd	1.06 ± 0.16
FO, 25 ºC for 10 days	*FO, 25 ºC for 10 days 0.1% OOE*	*FO, 25 ºC for 10 days 0.1% Toco*	*FO, 25 ºC for 10 days 0.02% BHT*	*FO, 25 ºC for 10 days 0.1% RE*	*FO, 25 ºC for 10 days Control*
Pentanal	nd	nd	nd	nd	1.35 ± 0.09
Hexanal	nd	nd	0.07 ± 0.00 c	0.18 ± 0.02 b	0.33 ± 0.02 a
t-2-Pentenal	0.04 ± 0.00 c	0.09 ± 0.04 c	0.04 ± 0.02 c	1.29 ± 0.32 b	2.54 ± 0.31 a
t-2-Heptenal	nd	nd	nd	nd	2.15 ± 0.31
t,t-2,4-Heptadienal	nd	nd	nd	2.09 ± 0.79 b	3.47 ± 0.30 a
FO, 40 ºC for 5 days	*FO, 40 ºC for 5 days 0.1% OOE*	*FO, 40 ºC for 5 days 0.1% Toco*	*FO, 40 ºC for 5 days 0.02% BHT*	*FO, 40 ºC for 5 days 0.1% RE*	*FO, 40 ºC for 5 days Control*
Pentanal	0.02 ± 0.01c	nd	nd	0.65 ± 0.11 b	1.58 ± 0.19 a
Hexanal	nd	nd	nd	0.04 ± 0.11 b	0.44 ± 0.03 a
t-2-Pentenal	nd	nd	nd	1.52 ± 0.16 b	2.21 ± 0.19 a
t-2-Heptenal	nd	nd	nd	nd	2.56 ± 0.26
t-2-Octenal	nd	nd	nd	nd	0.61 ± 0.28
t,t-2,4-Heptadienal	nd	nd	nd	5.51 ± 0.16 b	7.73 ± 0.21 a

*nd: not detected.

Figure 1. Oxidation of stripped soybean oil (SBO) containing 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE) at 25 ºC: a) peroxide value (PV), b) conjugated diene value (CDV), and c) *p* -anisidine value (p-AV). Error bars represent standard deviations. Means not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).



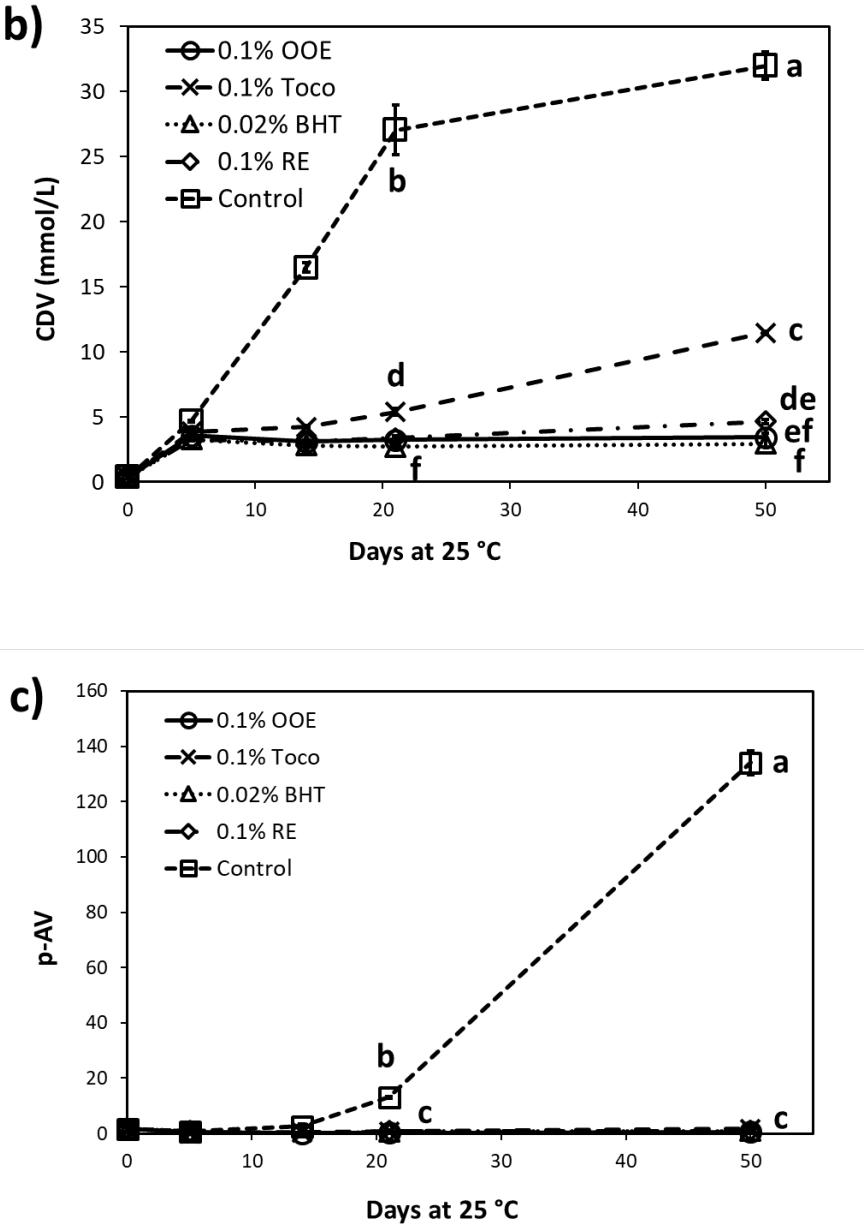
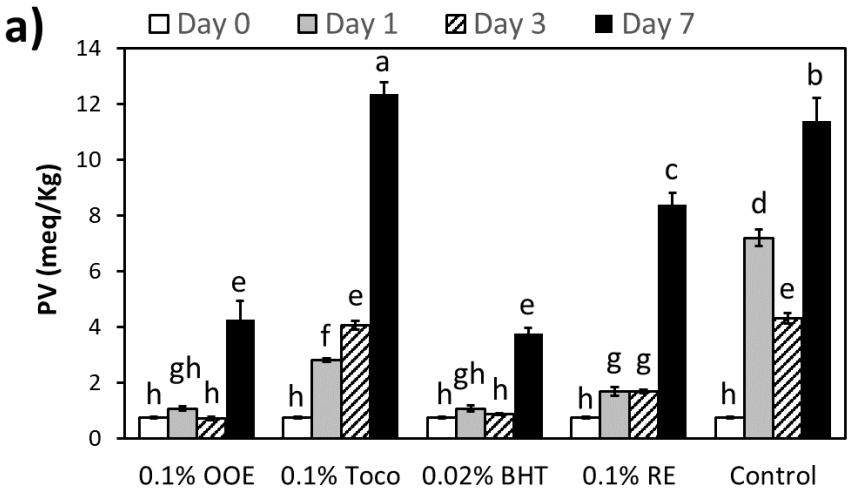
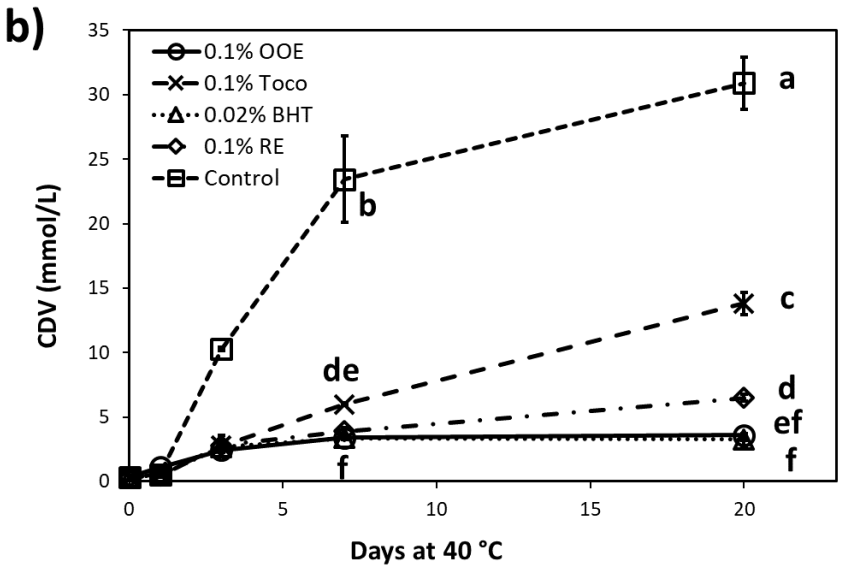


Figure 2. Oxidation of stripped soybean oil (SBO) containing 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE) at 40 ºC: a) peroxide value (PV), b) conjugated diene value (CDV), and c) *p* -anisidine value (p-AV). Error bars represent standard deviations. Means not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).





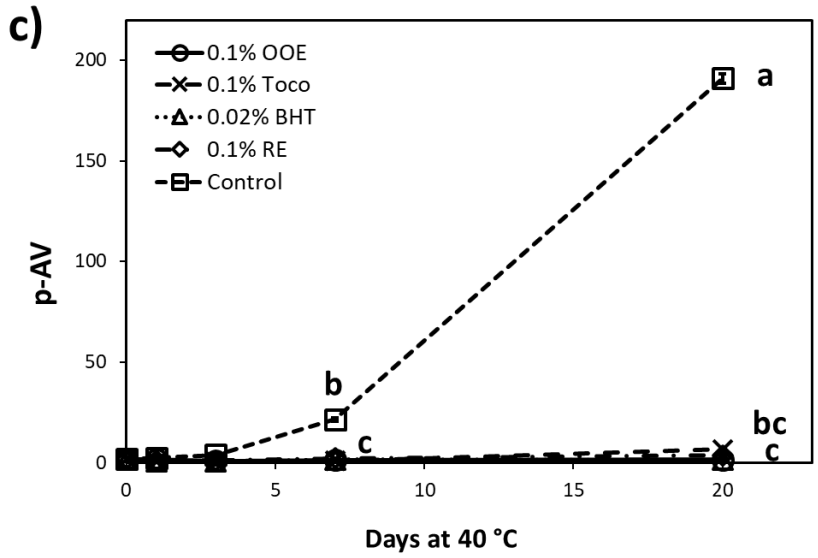
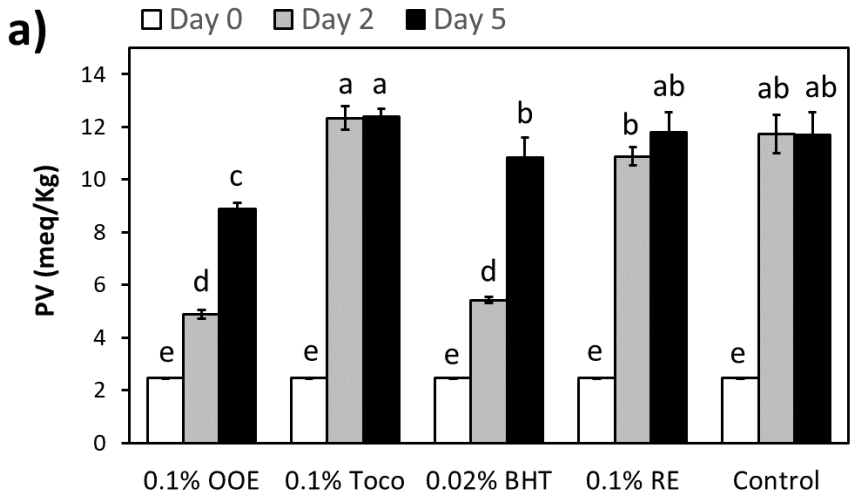


Figure 3. Oxidation of stripped fish oil (FO) containing 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE) at 25 ^{o}C: a) peroxide value (PV), b) conjugated diene value (CDV), and c) p -anisidine value (p-AV). Error bars represent standard deviations. Means not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).



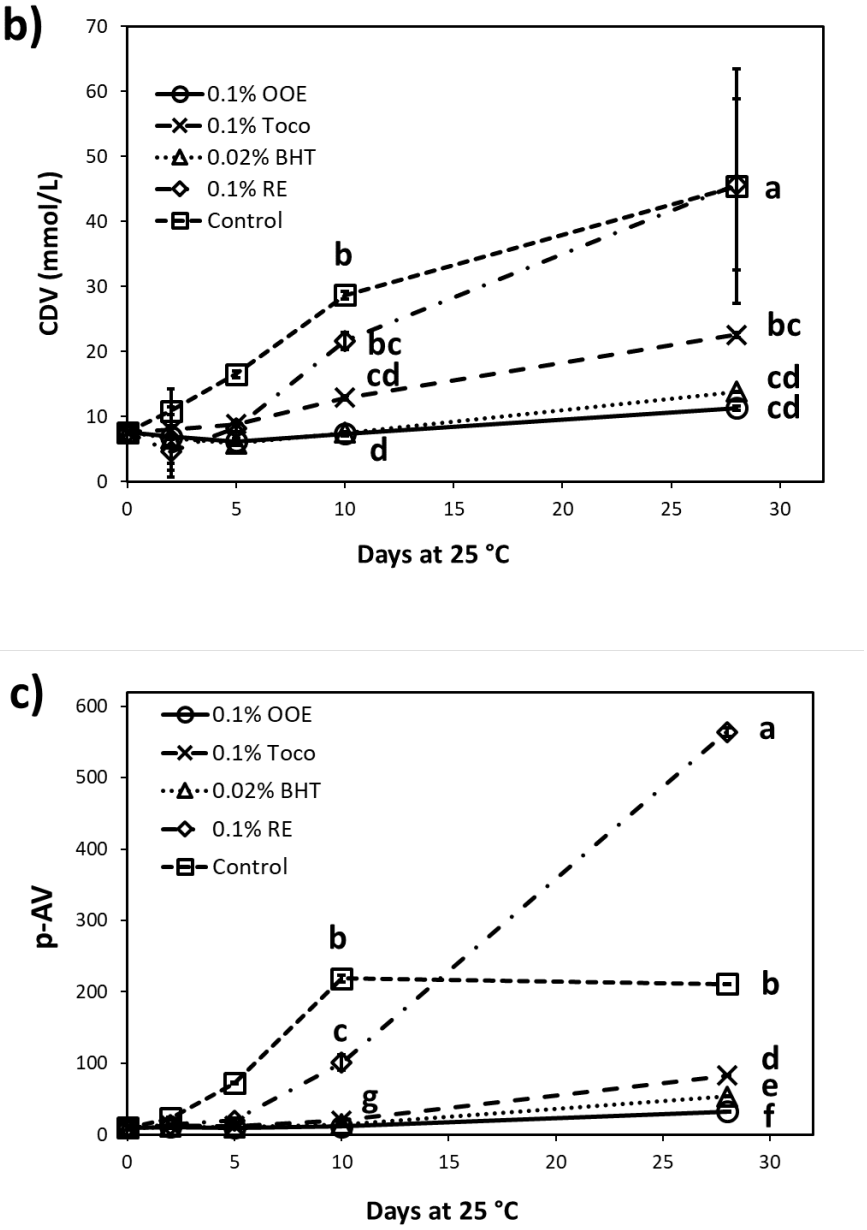


Figure 4. Oxidation of stripped fish oil (FO) containing 0.1% Osage orange fruit extracts (OOE), 0.1% tocopherols (Toco), 0.02% BHT, or 0.1% rosemary extract (RE) at 40 ºC: a) peroxide value (PV), b) conjugated diene value (CDV), and c) p -anisidine value (p-AV). Error bars represent standard deviations. Means not sharing the same letter (s) are significantly different by Tukey-Kramer Honestly Significant Difference test ($P < 0.05$).