Increasing the oxidative stability of soybean oil through fortification with antioxidants

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**Abstract**

Vegetable oils high in polyunsaturated fatty acids (PUFA), like soybean oil (SO), is known for lowering the risk of consumers for contracting cardiovascular disease as well as improving cognitive health. However, they are more susceptible to lipid oxidation than recently introduced high-oleic cultivars like high-oleic sunflower oil (HOSFO). Thus, the objective of this study is to increase the stability of PUFA oils to maintain the aforementioned health benefits by supplementing them with industrially relevant antioxidant compounds that prevent or delay oxidation during food production and storage. Herein, a variety of synthetic and natural antioxidants tested alone or in mixtures was screened to bring the stability of SO closer to that of HOSFO. Oils were stored under accelerated conditions (35 °C) in the dark for 28 weeks and the evolution of primary (hydroperoxides) and secondary (hexanal) lipid oxidation products was monitored. Oxidative stability index data showed that addition of 300 ppm of ascorbyl palmitate (AP) stabilized SO to the greatest magnitude. Further, a combination of AP (300 ppm) and M-TOC (1000 ppm) was able to limit hydroperoxide and hexanal formation in SO at 35 °C for 12 weeks. It was demonstrated that assessing multiple quality parameters for lipid stability are a necessary undertaking.

**Keywords**

High-oleic sunflower oil, soybean oil, oxidative stability, antioxidants, oil stability index (rancimat), hexanal

**Introduction**

Edible vegetable oils like soybean oil (SO) are rich in polyunsaturated fatty acids (PUFA) such as linoleic (18:2) and -linolenic (18:3) acids. These two fatty acids are essential to human health and development. Further, -linolenic acid (18:3) belongs to the omega-3 family and can be converted into eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) within the human body. -Linolenic acid, like other PUFA in the omega-3 family, is known for lowering the risk of consumers for contracting cardiovascular disease as well as improving cognitive health (Connor, 1999). It follows from this that consuming foods rich in linolenic acid, like SO, may bring health benefits to consumers.

Unfortunately, incorporating large amounts of SO into foods can lead to stability concerns due to susceptibility of PUFA to oxidative deterioration (Frankel, 2005). PUFA oxidation can be catalyzed by a variety of means such as exposure to light, heat, and transition metals, to name a few, and results in the generation of free radicals that can result in a deleterious chain reaction. Lipid hydroperoxides are generally identified as the primary products of autoxidation and further decomposition of this class of compounds can yield so-called ‘secondary’ oxidation products like volatile aldehydes (e.g. hexanal), ketones, alcohols, and organic acids (Shahidi & Wanasundara, 2008). These resultant end-products of lipid oxidation are of great concern in food industry because they can result in the development of undesirable off-flavors, odors (*e.g.* rancid), colors and potentially toxic compounds in food products.

Recently, high-oleic (18:1, or mono-unsaturated fatty acid [MUFA]) cultivars of typical edible oil crops such as sunflower oil (HOSFO) have seen widespread use in food industry due to their greater oxidative stability than traditional oils like SO. However, these high-oleic oils like HOSFO (in addition to being more expensive) often contain only small amounts of PUFA, and thus, they lack the aforementioned health benefits for consumers. In order to make a compromise and bring both the health benefits of PUFA as well as the stability of high-oleic oils, it is common practice to supplement soybean oil (SO) with antioxidant compounds that prevent or delay the oxidation process. Currently, various synthetic and natural antioxidant compounds are available in the marketplace and can be used to help in the stabilization of fats and oils.

Synthetic antioxidant compounds like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are often used due to their processing stability, high antioxidant activity, low cost and widespread availability. However, the safety of these synthetic antioxidants has often been questioned due to potential toxicological concerns (Yang *et al.*, 2002). Ascorbyl palmitate (AP) is a synthetic oil-soluble ester of ascorbic acid that has been proven to delay the onset of rancidity in edible oils (McMullen *et al.*, 1991). AP has also been proven to act as a synergist with tocopherol preserving its antioxidant efficacy (Beddows *et al.*, 2001). Nowadays, tocopherols are the most-used natural antioxidants in the food industry also for labeling reasons. They are often added as mixed isomers (i.e. or mixed tocopherols [M-TOC]) whose main function is termination of free-radical catalyzed oxidation reactions (Frankel, 2005). Citric acid (CA) is another well-known antioxidant used in food products that aids retarding the oxidative deterioration of lipids by chelating metal ions (Kirimura *et al.*,, 2011). Lastly, natural antioxidant extracts and preparations from spices and herbs such as rosemary (*e.g.* rosemary oil [RO]) are being used in food products. Rosemary extract, has in fact recently gotten approval for use as a food additive in the European Union (E#392; EU, 2010).

In a previous study, the oxidative stability of conventional and high-oleic varieties of commercial vegetable oils, with and without added antioxidants, was studied using the oil stability index (OSI) (Merrill et al., 2008). Authors reported that addition of TBHQ (tertiary-butylhydroquinone), alone and in combination with other antioxidants such as ascorbyl palmitate, resulted in the significant in oxidative stability of high-oleic oils. Considering the questionable regulatory status of TBHQ in many markets, it is critical for industries to evaluate other relevant antioxidants which can improve the oxidative stability of PUFA rich oils. As lipid oxidation often involves a complex series of parallel reactions, a single strategy or approach for stabilization might not be effective. .

In this study, we aimed to evaluate industrially relevant synthetic and natural antioxidants (see **Table 1** for the list of compounds tested), and mixtures there ofto improve the stability of SO more towards that of HOSFO in similar storage conditions. Furthermore, the secondary objective was also to evaluate whether screening oils with only one quality parameter (e.g. OSI) is sufficient or not, as currently practiced in most industries. Hence, together with oxidative stability index (OSI test),storage tests were also conducted under mildly accelerated conditions (35 °C) in the dark for up to 30 weeks and the evolution of primary (hydroperoxides) and secondary (hexanal) lipid oxidation products were monitored throughout.

**Materials and Methods**

Ascorbyl palmitate (AP), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), mixed tocopherols (M-TOC), citric acid (CA) and rosemary extract (RO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hexanal (purity 98%) and hexanal-d12 isotopic enrichment (minimum 98% atoms) were purchased from CDN Isotopes (Quebec, Canada). Methyl tridecanoate (purity 99.5%) and quantitative calibration standard mixture of fatty acid methyl esters (GLC-Nestle 36) were purchased from Nu-Chek-Prep (Elysian, MN, USA). All other chemicals used in this work were of analytical grade and purchased from Sigma-Aldrich, unless otherwise specified. Commercial soy oil (SO) and high oleic sunflower oil (HOSFO) were procured from a Nestlé factory and were sourced from a global fat and oil supplier at the beginning of the experimental design. The native SO and HOSHO had tocopherol content of 9.4 and 36.6 α-tocopherol euivalents/ 100g, respectively. Upon receipt, oils were stored in their original containers in the dark at 4 °C until evaluation of fatty acid composition, as well as initial quality (hydroperoxides, hexanal) and stability (oxidative stability index). Where applicable, antioxidant(s) were dissolved stepwise in the vegetable oils (with up to 30 min stirring) just before analyses, or prior to incubation for subsequent analysis in the case of the storage test samples.

*Fatty acid composition*

Fatty acids (FA) profile was determined using a modified version of the ISO-15884 (2002) for preparing fatty acid methyl esters for analysis, followed by quantification using gas chromatographic (GC) detection described in AOAC 2012.13 – First Action (2012). The internal standard solution was prepared in a 100 mL volumetric flask by dissolving methyl tridecanoate (200±0.1 mg) in n-hexane. A 100 mg test portion was weighed (to the nearest 0.1 mg) into a centrifuge tube (10 mL) with a screw cap. The transesterification reaction was conducted at room temperature for 3.5–4.0 min after adding the 2 mL of internal standard solution and 0.2 mL of methanolic potassium hydroxide (2 N) to the sample vessel followed by vortexing for 2 min. After centrifugation for 5 min at 2000 rpm, the upper organic layer was collected and diluted with a subsequent 1 mL of hexane before GC analysis. Analysis of FA profile were performed on a gas chromatograph (Agilent 6890N), equipped with a fused-silica CP-Sil 88 capillary column (100% cyanopropylpolysiloxane, 100 m×0.25 mm internal diameter, and a 0.25 m film thickness; Agilent Technologies Netherlands B.V., Groenelaan 5 Amstelveen 1186 AA, Netherlands). The split injector temperature was set at 220 °C and the flame-ionization detector was set at 280 °C.

The oven temperature programming was as follows: isothermal at 60 °C for 1 min, increased to 165 °C at 15 °C/min, isothermal for 1 min at this temperature then increased to 225 °C at 3 °C/min and held isothermal at 225 °C for 18min. The inlet pressure of the carrier gas (hydrogen purity >99.9%) was fixed at 350 kPa, the split flow was set at 80 mL/min, and the corresponding split ratio was 35:1. A 1.0 µL aliquot of each sample and standard solution were injected for analysis. FA methyl ester response factors relative to methyl tridecanoate used as internal standard were determined using a calibration standard solution (GLC-Nestle36 from Nu-Chek-Prep) and corresponding peaks were identified in samples by the comparison of retention times (RT) determined with the calibration standard. The FA profile was then calculated using methyl tridecanoate as internal standard and results were expressed in grams of FA/100 g total FA.

*Oil stability index (OSI) test*

Oil stability index, or OSI (also called Rancimat), of the bulk oils with or without added antioxidants was measured at 110 °C in triplicates with a Rancimat instrument (Model 743; Metrohm Ltd., Herisau, Switzerland) according to ISO-6886 (2006). Only the supplemented SOs that exhibited a marked ‘stabilization effect’ in the OSI test were then submitted to storage stability testing in order better understand the mechanism of action.

*Storage Stability Test*

The bulk HOSFO and SO, as well as the supplemented SOs, were individually aliquoted into 50 g amber bottles for hydroperoxide tests. Further, the oils were transferred into 20 mL headspace solid-phase microextraction (SPME) amber glass vials for volatile (hexanal) analyses via GC tandem mass spectrometry (or GC-MS). Special attention was given during the manual sampling in order to allow for a similar headspace in the containers, and thus, the same oxygen availability. Further, a special aluminum membrane was inserted between the top of the headspace SPME vial and the septa to prevent loss of volatiles during the storage study. The amber bottles and SPME vials were stored in triplicates at 35 °C (accelerated conditions) for up to a 28 week storage period, and sampled each 4 weeks (normally). The storage period was chosen based on the normal maximum time limit (6-7 months) that lipid raw materials are stored at food manufacturing facilities before inclusion into finished products. Samples for hydroperoxide and hexanal analyses were staggered by 1 week when put into the storage ovens to allow for constant sampling and analysis over the storage period.

Peroxide value (PV) test

Peroxide value (PV) was measured in triplicates by titration with 0.1 N sodium thiosulphate, using starch indicator according to AOCS Cd 8b-90 (2011). Results of this assay were reported in milli-equivalents of peroxides per kg of oil sample (or meq O2/kg oil).

Hexanal quantification via SPME GC-MS

Hexanal concentrations in the challenged lipids were determined on triplicate samples following a method previously described in the literature (Giuffrida *et al.*, 2005). Quantification using isotopic dilution method (*i.e.* with labeled hexanal-d12 as internal standard) was deemed necessary in order to avoid competition on the SPME fiber with other volatiles present in the headspace. Samples were weighed (2±0.01 g) in a headspace SPME amber glass vial (20 ml) and spiked with hexanal-d12 (120 μg) as internal standard using a calibrated syringe before the analysis. The SPME conditions were the following: the fiber selected was a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; Supelco, Palo Alto, USA) with an equilibration temperature of 37 °C, incubation time 30 min, and a desorption time of 30 s.

The GC-MS analyses were performed on a Thermo Finningan GC TOP 8000 coupled to a Thermo Finningan Voyager quadrupole mass spectrometer and equipped with a CTC PAL injector configured for SPME experiments (Brechbühler Schlieren, Switzerland). The GC injection was performed on a split-less injector with linear velocity of 30 mL/min. Helium was selected as carrier gas at constant pressure of 150 kPa. A DB-5MS (5% phenyl 95% dimethyl arylene siloxane stationary phase; Brechbühler, Schlieren, Switzerland) capillary column with 60 m length, 0.25 mm internal diameter and 0.25 μm film thickness was used. The column temperature was held at 50 °C for 10 min and increased to 250 °C at 10 °C/min and maintained at 250 °C for 10 min. The temperatures of the ion source and transfer line were 200 and 220 °C, respectively. Electron impact mass spectra were recorded at 500 V in the 35-250 micron mass range, at two scans/s. The concentration of hexanal was determined in triplicate samples from the peak area of the labeled internal standard (hexanal-d12). In explanation, the quantification of hexanal was performed by making a calibration curve of hexanal in oil against the internal standard. The amount of volatile hexanal (expressed in μg/g, or ppm) was calculated according to the following equation:



*[Note: AA = area of hexanal in the sample, AIS = area of internal standard in sample, I = intercept of the calibration curve’s equation, S = slope of the calibration’s curve equation, m = amount of internal standard (μg), and SW = sample weight (g)]*

*Statistical analyses*

The results were analysed statistically by analysis of variance (ANOVA) for significance (p ≤ 0.05) using Minitab 15.1.0 software (Minitab Inc., State College, PA, USA). All results were reported as the mean and standard deviation determined from at least six measurements on individual samples.

**Results and Discussion**

*Fatty acid composition*

Fatty acid composition data from the commercial HOSFO and SO were summarized and appear in **Table 2**. As seen in **Table 2** linoleic acid (18:2, 6) was the most abundant unsaturated fatty acid present in SO (52.4 g FA/100 g total FA) followed by oleic acid (18:1, 9; 23.7 g/100 g total FA) and -linolenic acid (18:3, 3; 5.4 g/100 g total FA) acids in SO. Being a high-oleic variant of a vegetable oil, HOSFO had a similar distribution of fatty acid classes, but much higher amounts of oleic acid (80.3 g/100 g total FA). Linoleic acid and -linolenic acid contents in HOSFO were also quite lower than compared to SO (*i.e.*, at 10.7 and 0.10 g/100 g total FA, respectively). In general, it is quite well accepted that as the level (concentration) and extent (number of double bonds) of the unsaturation of lipids in oils increases, the more rapidly they will undergo autoxidation. Martin-Polvillo *et al.* (2004) demonstrate this while comparing the oxidative stability of native and high-oleic sunflower oil during long-term storage at ambient temperatures. Given that the SO has a total polyunsaturated lipid content of 58.2% and HOSFO only 10.8%, it is logical that SO would be much less stable to lipid oxidation.

*Oil stability index (OSI) test*

Determination of the induction period, or overall oxidative stability, of oil samples by the Rancimat method is the most widely applied industrial standard currently in use. As such, OSI scores are often mandatory criteria within purchasing specifications of food companies whilst procuring large shipments of oils for food production. The OSI scores from the HOSFO, SO and SO containing various synthetic and natural antioxidant mixtures appear in **Table 1**. The majority of the antioxidant concentrations, mixtures and proportions included in this study were chosen based on previous screening experiments using the OSI for reference to the efficacy of such mixtures in limiting lipid oxidation in bulk oils (data not shown). In the case of AP and BHA/BHT, more practical factors limited their usage herein such as solubility in oil (300ppm for AP) and legal limits for incorporation (< 200ppm combined for most countries; Shahidi & Zhong, 2005), respectively.

As suspected, HOSFO exhibited the highest oxidative stability of the oils tested with an induction period of 18.8 h. Native SO had the lowest OSI score with 5.9 h. The addition of commercial synthetic antioxidants BHA and BHT alone in the concentrations tested did not improve the stability of SO compared to the reference. The addition of BHA and BHT together, however, improved the stability of SO from 5.9 h to 8.7 h. A synergistic effect of BHA and BHT in the stabilization of radical-catalyzed autoxidation has long been known. Omura (1995) demonstrates the synergism between BHA and BHT through synthesis of their radicals *in vitro*. Ascorbyl palmitate (AP) when added alone was shown to have a promising effect on improving the stability SO. In fact, use of up AP up to 300 ppm in SO resulted in an induction period of 12.2 h, which is more than twice that of SO alone without added antioxidants. The addition of low concentrations (100-1000 ppm) of M-TOC, however, was not shown to be significantly effective at increasing the induction period of SO within the OSI test. Further, the combination of the 300 ppm of AP and 1000 ppm of M-TOC, as well as 20 ppm CA in addition to the former, did not show any synergism in stabilizing SO as was the case with the synthetic antioxidants BHA and BHT. Lastly, addition of rosemary extract up to 500 ppm had no effect on OSI value of SO. .

*Storage Stability Test*

According to the Arrhenius equation, storage stability of an edible oil at 35 °C for ‘1 week’ is approximately equivalent to ‘1 month’ at ambient temperatures (~18 °C; Taoukis & Labuza, 1996). This type of approach can be useful in order to put the combined hydroperoxide and hexanal data contained herein into the context of food product ‘shelf-life.’ Storage stability study in terms of hydroperoxide evolution and generation of hexanal is only shown for the SOs with or without containing antioxidants, which showed a marked ‘stabilization effect’ (i.e., those that exhibited OSI values above 8 h for all replicates).

Peroxide value (PV) test

Results of the peroxide value (PV) tests (in meq O2/kg oil) of the oils stored at 35 °C for 28 weeks were plotted and appear in **Figure 1**. As seen in **Figure 1**, HOSFO did not demonstrate an increase in hydroperoxides (5.9 meq O2/kg oil) until after 25 weeks storage at 35 °C. In comparison, the native SO showed an increase in hydroperoxide formation just after 5 weeks of the storage study and finished with a level of 38.8 meq O2/kg oil at the final measurement. As hydroperoxides are markers of early oxidation progress, these results further demonstrate the instability of native SO, in comparison oils with significantly lower polyunsaturated fatty acids contents like HOSFO *(p < 0.05)*. Martin-Polvillo *et al.* (2004) showed that in the initial stages of oxidation, oxidized triglyceride monomers are the most predominant compounds and their presence is well correlated with an increase in hydroperoxide levels as well as a decrease in antioxidant (tocopherol) concentrations. Further, hydroperoxides are considered to be a main contributer to the propagation of autoxidation in oils acting as a proxidant compound once formed (Kim *et al.*, 2007). This helps to explain the exponential increase in PV exhibited by the SOs in **Figure 1** after a lag period during which the oils remain relatively stable to oxidation.

All the antioxidant mixtures tested (*i.e.* that performed well in stabilizing the SO within the OSI test, see above), resulted in a reduction of the onset of hydroperoxide formation in the challenged SO. The antioxidants stabilized the SO according to the following relationship: 300 ppm AP + 1000 ppm M-TOC **>** 300 ppm AP **>** 20 ppm CA + 300 ppm AP + 1000 ppm M-TOC **>** 125 ppm BHA + 75 ppm BHT. These results are roughly in accordance with the relationships present in the OSI data shown in **Table 1**. It should be noted, however, that unlike the OSI data, the combination of 300 ppm AP + 1000 ppm M-TOC far outperformed the 300 ppm AP alone at stabilizing the SO against hydroperoxide onset. The final hydroperoxide levels for these two samples were 21.7 and 33.3 meq O2/kg oil, respectively, at the last measurement in the storage study. These results confirm a potential synergism between AP and M-TOC in stabilizing unsaturated lipids in SO, which was not obvious in the OSI data alone. Such synergisms are often widely mentioned in the literature (Wanasundara & Shahidi, 2005), but as seen herein, only specific oxidation assays are useful to demonstrate such relationships in oils.

Hexanal quantification via SPME GC-MS

As mentioned previously, hexanal is a byproduct of the secondary oxidation of lipids in foods. More specifically, hexanal has been established as a ‘marker’ compound for the decomposition of n-6 (or 6) fatty acids such as linoleic (18:2) and arachidonic (20:4) acids (Shahidi & Pegg, 1994; Leufven *et al.*, 2010; Kittipongpittaya *et al*., 2012). **Figure 2** is a summary plot of hexanal accumulation (in μg/g or ppm) in the HOSFO and SOs submitted to the storage study at 35 °C for up to the 28 week period (data shown only until week 20). As seen in **Figure 2**, a significant increase in hexanal concentrations was apparent in the native SO at the 4th week of the storage study. Conversely, the HOSFO exhibited a near lack of hexanal formation (<1 ppm) over an entire 20 weeks of storage at 35 °C *(p > 0.05)*. These results are quite in accordance with the PV data presented in **Figure 1**. As hydroperoxides are formed in the case of the native SO at around 5 weeks of accelerated storage, they rapidly decompose and result in the evolution of hexanal in a similar time frame (as seen in **Figure 2**) due to the high concentration of linoleic acid present therein (*i.e.* refer back to **Table 2**). The HOSFO, however, contains significantly less linoleic and -linolenic acids and is much less vulnerable to lipid autoxidation.

It is important to note that all the antioxidant mixtures/concentrations tested were successful in reducing hexanal formation in the SO over the storage period as seen in **Figure 2**. Globally, this result is in line with the OSI data presented in **Table 1**. Antioxidants stabilized SO from lipid oxidation, and subsequent hexanal formation, according to the following ranking: 300 ppm AP + 1000 ppm M-TOC **>** 20 ppm CA + 300 ppm AP + 1000 ppm M-TOC **>** 125 ppm BHA + 75 ppm BHT **>** 300 ppm AP. The SO sample supplemented with the 300 ppm AP + 1000 ppm M-TOC showed little hexanal formation until at least the 16th week in storage at 35 °C. In comparison with the PV data from **Figure 2**, this combination of antioxidants was able to limit both hydroperoxide and hexanal (**Figure 3**) formation in SO at 35 °C for up to 12 weeks. According to the Arhenius equation aforementioned, 12 weeks is roughly equivalent to ~12 months storage at ambient temperatures (~18 °C).

The kinetic curves for hexanal formation over the storage period for the other three SO samples supplemented with antioxidants were quite similar exhibiting an increase in hexanal formation from after 12 weeks of storage *(p > 0.05)*. These three samples finished the storage study with between 3-4.5 ppm hexanal accumulated within the vial headspace. Interestingly, while the SO sample supplemented with AP exhibited a longer induction period (22 weeks) than the SO with added BHA and BHT (7 weeks) in the PV results in **Figure 1**, this was not observed in the hexanal formation data for the two samples in **Figure 2**. This can be explained by the presence of -linolenic acid in the SO; *i.e.*, which is essentially absent in the HOSFO (see **Table 2**). While the formation of hydroperoxides of oleic, linoleic, and -linolenic acids can be quantified in the PV assay, hexanal is an oxidation marker for 6 linoleic acid only. This may suggest that whilst both the AP and the BHA/BHT are capable of stabilizing linoleic assay (as observed by the induction period for hexanal accumulation in **Figure 2**), the AP is much more efficient at preventing the oxidation of -linolenic acid (as observed by the induction period for hydroperoxides in **Figure 1**). The kinetics of -linolenic acid oxidation are known to be much faster than linoleic acid (and even more so than oleic acid). Min & Bradley (1992) determined the relative autoxidation rates of oleic (18:1) to linoleic (18:2), and linoleic (18:2) to a-linolenic (18:3) acids to be 1:40 and 50:100, respectively.

**Conclusions**

It was shown herein that highly unsaturated oils like SO are quite vulnerable to lipid oxidation when compared to HOSFO, which contain much lower quantities of linoleic acid and negligible amounts of -linolenic acid. Various antioxidant concentrations/mixtures were demonstrated to prevent or delay the oxidation of SO during accelerated storage tests, but none were shown to be as stable as HOSFO during the storage period. A combination of AP (300 ppm) and M-TOC (1000 ppm) was able to limit both hydroperoxide and hexanal formation in SO stored at 35 °C for up to 12 weeks (equivalent to ~ 12 months at ambient temperatures) making this a promising industrially relevant antioxidant mixture for SO. Lastly, the results of the various lipid oxidation assays employed herein demonstrate that when assessing the efficacy of approaches for lipid stabilization, a variety of analytical techniques must be employed in order to determine their mechanism of action. In other words, as there is no ‘universal’ marker to explain such a complex process as lipid oxidation, screening oils according to only one quality parameter (*e.g.* Rancimat) is insufficient.

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**Legends to Figures**

**Figure 1.** Mean (*n*=3) formation of hydroperoxides during the storage of oils (*i.e.* HOSFO, SO, and SOs with added antioxidants) incubated at 35 °C for 28 weeks. Refer to **Table 1** for the abbreviations used in this study.

**Figure 2.** Mean (*n*=3) accumulation of hexanal in the headspace during the storage of oils (*i.e.* HOSFO, SO, and SOs with added antioxidants) incubated at 35 °C for 20 weeks. Refer to **Table 1** for the abbreviations used in this study.

**Table 1.**  Mean (*n*=3) Oil stability index (OSI) values at 110 °C for HOSFO, as well as SO containing various mixtures of added antioxidants (Soy bean Oil, SO; High oleic sunflower oil, HOSFO; Ascorbyl palmitate, AP; Butlylated hydroxyanisole, BHA; Butylated hydroxytoluene, BHT; mixed tocopherols, M-TOC; Rosemary extract, RO; Citric acid, CA.

|  |  |
| --- | --- |
| Oils containing various mixtures of added antioxidants | OSI values values at 110 °C  |
| HOSFO | 18.83 + 0.14 |
| SO  | 5.85 + 0.28 |
| SO + 100 ppm AP | 8.48 + 0.88  |
| SO + 300 ppm AP | 12.15 + 1.04 |
| SO + 100 ppm M-TOC | 5.73 + 0.49 |
| SO + 1000 ppm M-TOC | 6.05 + 0.18 |
| SO + 300 ppm AP + 1000 ppm M-TOC | 11.45 + 0.07 |
| SO + 20 ppm CA | 7.13 + 0.14 |
| SO + 20 ppm CA + 300 ppm AP + 1000 ppm M-TOC | 8.9 + 0.07 |
| SO + 50 ppm BHA | 6.01 + 0.07 |
| SO + 125 ppm BHA | 6.05 + 0.14 |
| SO + 50 ppm BHT | 5.95 + 0.28 |
| SO + 75 ppm BHT | 6.03 + 0.11 |
| SO + 50 ppm BHA + 50 ppm BHT | 6.45 + 0.07 |
| SO + 125 ppm BHA + 75 ppm BHT | 8.65 + 0.14 |
| SO + 100 ppm RO | 6.23 + 0.04 |
| SO + 500 ppm RO | 6.13 + 0.04 |

**Table 2.** Fatty acid (FA) composition results (g FA/100 g total FA) for the commercial HOSFO and SO used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Fatty Acids** |  | **(HOSFO)** | **(SO)** |
|  |  | High-oleic Sunflower Oil | Soybean Oil |
| 4:0 | Myristic | nd† | 0.10 |
| 16:0 | Palmitic | 3.83 | 11.18 |
| 16:1 n-9 cis | Palmitoleic | 0.10 | 0.09 |
| 17:0 | Margaric | nd | 0.09 |
| 18:0 | Stearic | 2.89 | 3.56 |
| 18:1 *trans* | total 18:1 *trans* isomers | nd | nd |
| 18:1 n-9/n-7 | Oleic & other *cis* | 80.28 | 23.74 |
| 18:2 *trans* | total 18:2 *trans* isomers | 0.13 | 0.54 |
| 18:2 n-6 *cis* | Linoleic | 10.65 | 52.44 |
| 18:3 *trans* | total 18:3 *trans* isomers | nd | 0.89 |
| 18:3 n-3 *cis* | alpha-Linolenic | 0.10 | 5.73 |
| 20:0 | Arachidic | 0.26 | 0.34 |
| 20:1 n-9 *cis* | Eicosenoic | 0.27 | 0.24 |
| 22:0 | Behenic | 0.83 | 0.44 |
| 24:0 | Lignoceric | 0.30 | 0.15 |
| OFA | Other Fatty Acids (sum) | 0.38 | 0.48 |
| **Total Fatty Acids** |  | **100.00** | **100.00** |
| Saturated fatty acids | 8.10 | 15.86 |
| Monounsaturated fatty acid | 80.65 | 24.07 |
| Polyunsaturated fatty acid | 10.75 | 58.17 |
| Other fatty acids (OFA) | 0.38 | 0.48 |
| Total *trans* fatty acids | 0.13 | 1.42 |
| **Total Fatty Acids** |  | **100.00** | **100.00** |

†nd = not detectable (≤ 0.05)



Figure 1. Mean (*n*=6) formation of hydroperoxides during the storage of oils (*i.e.* HOSFO, SO, and SOs with added antioxidants) incubated at 35 °C for 28 weeks. Error bars represent standard deviations. Refer to Table 1 for the abbreviations used in this study.



Figure 2. Mean (*n*=6) accumulation of hexanal in the headspace during the storage of oils (*i.e.* HOSFO, SO, and SOs with added antioxidants) incubated at 35 °C for 20 weeks. Error bars represent standard deviations. Refer to Table 1 for the abbreviations used in this study.