RESEARCH ARTICLE

Blending of Soybean Oil with Selected Vegetable Oils: Impact on Oxidative Stability and Radical Scavenging Activity

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Abstract

**Background:** Soybean oil may protect against cancer of the breast and prostate. It may also exert beneficial influence in combination with other oils. Here, blends (20%, v/v) of sea buckthorn oil (SEBO), camellia oil (CAO), rice bran oil (RBO), sesame oil (SEO) and peanut oil (PEO) with soybean oil (SBO) were formulated. **Materials and Methods:** Oxidative stability (OS) and radical scavenging activity (RSA) of SBO and blends stored under oxidative conditions (60°C) for 24 days were studied. By blending with different kinds oils, levels of polyunsaturated fatty acids (PUFA) decreased, while monounsaturated fatty acid (MUFA) content increased. Progression of oxidation was followed by measuring peroxide value (PV), p-anisidine (PAV), conjugated dienes (CD) and conjugated trienes (CT). **Results:** Inverse relationships were noted between PV and OS at termination of storage. Levels of CD and CT in SBO, and blends, increased with increase in time. The impact of SEO as additives on SBO oxidation was the strongest followed by RBO, CAO, SEBO and PNO. **Conclusions:** Oxidative stability of oil blends was better than SBO, most likely as a consequence of changes in fatty acids and tocopherols’ profile, and minor bioactive lipids found in selected oils. The results suggest that these oil blends could contribute as sources of important antioxidant related to the prevention of chronic diseases associated to oxidative stress, such as in cancer and coronary artery disease.

**Keywords:** Vegetable oil blends - soybean oil - oxidative stability - antiradical properties

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**Introduction**

Soybean consumption is associated with reduced risk of cancer of the breast and prostate and may enhance survival (Zhu et al., 2011; Kang et al., 2012; Zhang et al., 2012; Sugiyama et al., 2013). It is conceivable that its effects could be enhanced by mixing with other vegetable products, for example in oils. Commercial oils today are mainly extracted using a hexane solvent, which has environmental and safety problems, so that it is necessary to develop alternative methods to hexane extraction. Enzyme-assisted aqueous extraction processing (EAEP) has been studied, which uses water as an extraction and separation medium. EAEP has been applied to extract oil from several kinds of oil-bearing seeds, such as soybean, corn germ, rapeseed and peanut. Soybean oil with high levels of polyunsaturated fatty acids (PUFA) is the main oil used for cooking and frying. Soybean oil is considered superior to many vegetable oils, but it is inferior in thermal stability at high temperatures.

Oil oxidation is an autocatalytic reaction generating hydroperoxides from unsaturated acylglycerols. Oxidation of polyunsaturated food lipids often affect on development of unpleasant tastes and odors, characteristic of rancid fats and oils, as well as degradation of functional and nutritional properties. Lipid oxidation can directly reflect shelf life of a product. Environmental factors, such as air, light and temperature, accelerate oxidative reactions which might end in the production of off-flavors and odors associated with low molecular weight volatiles, discoloration (Navarro et al., 2012). Also, oxidation induce important chemical changes of the oils that may affect directly the quality of the edible oil, generates radical oxygen species that may cause irreversible damages when reacting with biological molecules such as DNA, proteins or lipids (Bansal et al., 2010; Cabiscol et al., 2010). Lipid oxidation has harmful effects on both food quality and human health. Then efforts must be made to minimize oxidation and improve oxidative stability of lipid products.

The kind of oils and their oxidative stability are very important indicators which can help to know how long oils can be used. To overcome the problem of poor oxidative stability (OS) of soybean oil, ways of modifying fatty acid composition and increasing natural antioxidants were sought, such as combining high-oleic sunflower oil with corn oil or hydrogenated soybean oil with soybean oil (Abulkarim et al., 2010; Naghshineh et al., 2010) or combining palm-olein with soybean oil (Warner and
The present study, therefore, aims to investigate the oils and fats besides enhancement in oxidative stability. To overcome the problem of poor stability of traditional soybean, ways of enhancing the oxidative stability and radical scavenging activity of high-linoleic soybean oil at an accelerated oxidative condition. The degradation of oxidized oils and blends was also investigated. Results from this study will aid the program of selecting unique oil blends for improved nutritional value and will provide insight into the mechanisms of the antioxidant.

Materials and Methods

Samples and reagents

Full-fat soybean flakes, sea buckthorn oil, camellia oil, rice bran oil, sesame oil and peanut oil were obtained from a local market (Harbin, China). Protease 6L (2.4 AU/g, from Bacillus licheniformis) was purchased from Novo-Nordisk A/S (Bagsvaerd, Denmark). The fatty acid methyl ester (FAME) standards and 1, 1-Diphenyl-2-picrylhydrazyl (DPPH, approximately 90%) were purchased from Sigma (St. Louis, MO, USA). All solvents and reagents from various suppliers were of the highest purity needed for each application and used without further purification.

Extraction of vegetable oils

The Enzyme-assisted aqueous extraction processing was performed with minor modifications (Li et al., 2013). The extruded seed mixtures were collected into a beaker, and additional water was added to achieve a 1:6 solid:water (wt/vol) ratio. The beaker was then incubated at 55°C in a water bath, and the pH of the slurry was adjusted to 9 by the addition of 2 N NaOH. The enzyme dosage of Alcalase 2.4 L was 1.85 % (v/w, based on the dry weight of the samples). A continuous-stirring device was used to disperse the mixture during the enzymatic hydrolyze process. The reactions were maintained at the stable temperature and pH mentioned above for 3 h.

As food habits of most countries are based on fried foods, oxidative-resistant oils are needed. Therefore, the use of more stable frying oils of comparatively low price would be desirable. To overcome the problem of poor stability of traditional soybean, ways of enhancing the levels of bioactive lipids and natural antioxidants were sought. Blending different kinds of vegetable oils can modify the physicochemical characteristics of vegetable oils and fats besides enhancement in oxidative stability. The present study, therefore, aims to investigate the effects of blending selected oils with soybean oil on the oxidative stability and radical scavenging activity of high-linoleic soybean oil at an accelerated oxidative condition.

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At the end of the EAEP, the slurry was centrifuged in a 50 mL centrifuge tube at multiples of the force of gravity and express as 2, 372 g (TGL-16G, China) for 20 min at 20°C. Four distinct layers (oil, cream, skim and insoluble) were obtained after the centrifugation. The cream layer is an oil rich fraction (oil-in-water emulsion), whereas the skim fraction is an oil-lean and protein and sugar-rich soluble aqueous fraction. The insoluble layer is a fiber-rich insoluble fraction. The cream was collected in centrifuge tube and stored in refrigerator for 12 h, then centrifuged. The upper oil layer was carefully collected using a Pasteur pipette.

Blending of vegetable oils

The vegetable oil blends were formulated by blending with preheated (60°C) soybean oil (SBO) in proportions of 80:20 (w/w). The oils were thoroughly mixed to form uniform blends. Quality evaluation of the oil blends was by employing storage ability and thermal stability tests.

Fatty acid composition analysis

The fatty acid methyl esters (FAMEs) were prepared by the following two steps: (1) oils were saponified with 0.5 M KOH; (2) later methylated with 40% BF3 in methanol.
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Gas chromatography mass spectrometry (GC/MS) analysis was carried out with an Agilent 6890-5973 (Agilent Technologies, CA, USA) instrument. Separating procedure was achieved on an Agilent HP-88 capillary column (100x0.25 mm i.d., film thickness 0.2 μm). The operating conditions were as follows: carrier gas pressure, 100 kPa; carrier gas, helium; split ratio was 1:30; injection temperature, 250°C; scanning scope: 50-550 amu; ionization voltage: 70 eV. Oven temperature was programmed as follows: held at 80°C for 5 min, and then rising to 150°C at 10°C/min, and held for 2 min at 150°C; then continuously rising to 230°C at 5°C/min and held for 10 min. The individual fatty acids were identified and quantified by comparing their retention times with external standards.

**Accelerated oxidation**

SBO and oil blends were placed in a series of dark glass bottles with a volume 50 ml each. The bottles were completely filled with SBO, oil blends and sealed. No headspace was left in the bottles. The oxidation reaction was accelerated in a forced draft air oven, set at 60±2°C for up to 0, 4, 8, 12, 16, 20 and 24 days. Immediately after storage period, oil samples were withdrawn for triplicate analyses.

**Determination of peroxide value (PV)**

Accurate weigh of oil (300 mg) was dissolved in 9.9 ml of chloroform: methanol (7:3, v/v), then added 50 μL of 10 mM xylenol orange and 50 μL of iron (II) chloride solution (Pegg, 2001). The mixture solution was incubated at room temperature for 5 min and then centrifuged at 1000 g for 5 min at 5°C. The supernatant was used for measurement of absorbance at 560 nm with a UV-260 visible recording spectrophotometer. The iron (III) chloride solution was used as standard curve.

**Determination of p-anisidine value (PAV)**

P-anisidine value of oxidized oil were determined according to AOCS Recommended Practice Ti la-64 (1998). Accurate weigh of oil (100 mg) was dissolved in 25 ml and measured at 350 nm. This solution (2.5 ml) was mixed with 0.5 ml of 0.5% (w/v) p-anisidine in acetic acid for 10 min.

**Determination of the absorptive values**

The absorptivity values at 232 and 270 nm were recorded by spectrophotometry (UV-260, Japan), following the analytical methods described by IUPAC (1979), method II.D.23. The contents of conjugated diene (CD) and conjugated trienes (CT) were expressed as absorptivities of the 1% oil in 2, 2, 4-trimethylpentane.

**Determination of free radical scavenging activity (DPPH) assay**

Radicals were dissolved in toluene according to the procedure described in (Ramadan et al., 2010). Radical Scavenging Activity (RSA) and the presence of hydrogen donors in SBO and the oil blends were examined by reduction of DPPH in toluene during the accelerated oxidation test. Toluene solutions of DPPH radicals were freshly prepared at concentrations of 10-4 M. The radical, in the absence of antioxidant compounds, was stable for more than 2 h of normal kinetic assay. For evaluation, 10 mg of SBO or oil blends sampled during the Shaal oven test (in 100 μL toluene at room temperature) was mixed with 390 μL toluene solution of DPPH radicals and the mixture was vortexed for 20 sec. at ambient temperature.

The decrease in absorption at 515 nm was measured after 60 min against a toluene blank (without DPPH) in 1 cm quartz cells using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan).

**Statistical analysis**

Samples of pure oils and various blends were taken in triplicate. Data from the replications of all kinds were subjected to a variance analysis (ANOVA) using SPSS 16.0 for Windows. Significant differences between the means were determined by Duncan’s new multiple range test (P<0.05). The correlation between all the studied parameters was determined by the principal component analysis (PCA) using XLSTAT software.

**Results and Discussion**

**Fatty acid composition**

An examination of FAME derivatives showed thirteen fatty acids (Table 1). The total saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (PUFA) showed significant variation in their contents. Linoleic acid was the major fatty acid in soybean oil, followed by oleic acid (22.68%) and palmitic acid (16.66%). Similar fatty acid profiles for these oils have been also shown in several works (Reena and Lokesh, 2007; Haiyan et al., 2007; Sharif et al., 2009). Except SEO all selected oils under study contained more PUFA than MUFA.

Fatty acid composition of SBO and oil blends is presented in (Table 1). Blending of selected oils with SBO non-significantly changed the kind of main fatty acids in the blends. While major changes were noted in the contents of oleic acid (C18:1) and linoleic acid (C18:2) of blended oils. SBO had the highest percentage of PUFA and the lowest MUFA. Blending with selected oil didn’t lead to significant modification in SFA. Several studies mentioned the effect of blending oils; Mariod et al., (2004) found that blending of sunflower kernel oil with Sclerocarya birrea oil could modify the fatty acid profile of the blends. Farooq et al., (2007) reported that blending Moringa oleifera oil with sunflower oil and soybean oil in different proportions can result in an increase in oleic acid and reduction of linoleic acid. Ramadan and Wahdan, (2012) studied that blending black cumin seed oil and coriander seed oil with corn oil could change the fatty acid profile of the blends; while blending with coriander seed oil has the distinguished impact. In general, it could be noted that the impact of blending SBO with CAO and SFO on fatty acid profile was more marked than that of the blending with others.

**Principal component analysis**

Principal Component Analysis was then carried out.
Acylglycerols are isomerized to more stable conjugated double reactive substances to a certain degree. Blends seem to be successful in reducing the formation of p-anisidine was the strongest followed by PNO, SEBO, CAO, RBO and SEO. All PA V of oil samples increased with increase of storage period. SBO during storage in the dark (Choe and Min, 2007). Results indicate that and 20% (v/v) improved the lipid oxidative stability of fried products dark and found that the addition of sesame oil to soybean oil at 10% Jissang al. investigated the effects of adding sesame oil to soybean oil 26.23 mg/g for SBO blends enriched with RBO and SEO, respectively. The PA Vs of EAEP oils were much on the development of PA V during 160°C frying and storage in the was attributed to roasted sesame oil originally contains many volatiles such observed after 16th day, which was mainly associated with the rate of hydroperoxides decomposition. Unstable hydroperoxides formed in the initial stage of storage decomposed result in the formation of secondary products of oxidation. Except blending with SFO, the blends showed a marked decline in their PV during storage, thus result in enhancement of the OS of SBO. Padmavathy et al., (2001) reported that blending vegetable oils not only improve sensuous quality, also enhance the storage stability of the edible oils (Gulla et al., 2010). On the basis of PV, the OS of SBO and oil blends varied significantly, while the blends enriched with SEO being most stable. At the end of storage period, SBO control was oxidized rapidly and had the highest PV (28.33 meq/kg).

P-anisidine value: Unstable hydroperoxide occurs with β-scission readily decomposed to alkoxy radicals, resulting in formation of unsaturated aldehydes, nonvolatile aldehydes, ketones, acids, esters, alcohols and short-chain hydrocarbons at high temperature (Choe and Oh, 2013). These compounds produce rancid odors and flavors. Table 4 presents the changes in the level of PAV in SBO and oil blends after 24 days of storage under oxidation conditions. PAVs of SBO and blends increased with the increase of storage period. The PV of SBO was in a range of (4.75 to 44.07 mg/g). The PAVs of EAEP oils were much higher than those of blends over storage up to 24th day, indicating that lipid oxidation was more extensive in EAEP oil (with higher levels of polyunsaturated fatty acids). PAVs of SBO and SEO blend were significantly higher than other blends without treatment, which attributed to roasted sesame oil originally contains many volatiles such as aldehydes, ketones, alcohols, pyrazines, furans and pyrroles (Chung et al., 2006). At the end of storage period, PAV recorded 27.13 and 26.23 mg/g for SBO blends enriched with RBO and SEO, respectively. Jissang al. investigated the effects of adding sesame oil to soybean oil on the development of PA during 160°C frying and storage in the dark and found that the addition of sesame oil to soybean oil at 10% and 20% (v/v) improved the lipid oxidative stability of fried products during storage in the dark (Choe and Min, 2007). Results indicate that PAV of oil samples increased with increase of storage period. SBO was the strongest followed by PNO, SEBO, CAO, RBO and SEO. All blends seem to be successful in reducing the formation of p-anisidine reactive substances to a certain degree.

Ultraviolet absorptive: Nonconjugated double bonds in unsaturated acylglycerols are isomerized to more stable conjugated double
bonds when reacting with oxygen during storage at 60°C, indicating the quality status of SBO and blends (Mohdaly et al., 2010). The changes in UV absorbance at 232 and 268 nm, quantified by K232 and K268 have been used to evaluate the occurrence and extension of oxidative reactions. The uptake of oxygen and formation of peroxides in the early stages of lipid peroxidation reactions, as well as with the degradation of linoleic and linolenic acids lead the increase in K232 and K268. A significant negative correlation between oxidative stability and absorptivity at 232 and 268 nm in all oils (Zhu et al., 2013). K232 and K268 of SBO and blends during storage and absorptivity at 232 and 268 nm in all oils (Zhu et al., 2013). K232 and K268 of SBO and blends during storage and absorptivity at 232 and 268 nm in all oils (Zhu et al., 2013). K232 and K268 of SBO and blends during storage and absorptivity at 232 and 268 nm in all oils (Zhu et al., 2013).

The inhibition of CD contents of all oils increased slowly in the beginning of storage and the increase became faster thereafter. Moreover, the rate of formation of conjugated dienes and polymers reach an equilibrium, leading non-significant change in K232 (Nystrom et al., 2005). So, there was no significantly different between SBO and oil blends before 4th day; while, K232 values in SBO increased significantly. The K232 increased gradually with the increase in time (0-24 days) with a greater rate for the SBO (percentage increase of 366). The SBO stabilized with the oils showed lower levels of the K232 after 24 days. The formation of CT as well as unsaturated ketones and aldehydes result in increase of K268, showed a pattern similar to K232. SBO had the highest content of CT by addition of selected oils achieved by means of preventing the subsequent formation of reactive lipid radicals, and extending the induction period of the lipid oxidation of fried products during storage in the dark. The inhibitory effect of selected oils on CD and CT formation was time-dependent.

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Table 2. Discriminate Variables Factors of Principal Components Analysis

| Variables (axes F1 and F2: 72.21 %) | F1 | F2 |
|-----------------------------------|----|----|
| Eigen value                       | 5.27 | 3.4 |
| Variability (%)                   | 43.89 | 28.32 |
| Cumulative (%)                    | 43.89 | 72.21 |
| Myristic acid (C14:0)             | - | -18.62 |
| Palmitic acid (C16:0)             | -13.61 | - |
| Palmitoleic acid (C16:1)          | -10.97 | - |
| Stearic acid (C18:0)              | -13.26 | - |
| Linoleic acid (C18:2)             | -3.75 | - |
| Linolenic acid (C18:3)            | - | -3.75 |
| Arachidic acid (C20:0)            | 16.32 | - |
| Gadoleic acid (C20:1)             | 18.04 | - |
| Arachidic acid (C20:2)            | 10.01 | - |
| Behenic acid (C22:0)              | 15.19 | - |
| Lignoceric acid (C24:0)           | - | 3.68 |

Table 3. Changes in Peroxide Values of SBO and Oil Blends during Oven Test

| Time (days) | CONTROL | SBO:CAO | SBO:SEO | SBO:RBO | SBO:PNO | SBO:SEBO |
|------------|---------|---------|---------|---------|---------|---------|
| 0          | 2.34±0.04Aa | 2.15±0.04Aa | 2.51±0.17Aa | 2.33±0.07Aa | 3.79±0.17Aa | 2.42±0.27Aa |
| 4          | 7.93±0.01Bc | 6.51±0.14Bb | 4.32±0.24Ba | 5.21±0.05Ba | 16.08±0.62Bd | 5.55±0.07Ba |
| 8          | 13.7±0.30Cc | 8.51±0.19Cb | 6.22±0.09Ca | 8.81±0.03Cb | 21.33±0.19Cd | 9.21±0.08Cb |
| 12         | 17.45±0.18Dc | 13.21±0.08Db | 8.32±0.05Da | 8.7±0.21Ca | 23.37±0.23Dd | 11.51±0.21Da |
| 16         | 20.1±0.07Ed | 17.27±0.10Ec | 12.49±0.07Ea | 15.34±0.09Db | 25.17±0.03Ee | 12.54±0.07Ed |
| 20         | 25.35±0.06Fd | 20.62±0.23Fb | 16.36±0.10Fa | 22.14±0.08Ec | 27.38±0.01Fd | 16.65±0.24Fa |
| 24         | 28.3±0.12Gd | 22.74±0.24Gd | 18.87±0.04Ga | 20.6±0.05Fc | 37.58±0.16Ge | 19.59±0.14Gb |

Table 4. Changes in p-anisidine Values of SBO and Oil Blends during Oven Test

| Time (days) | CONTROL | SBO:CAO | SBO:SEO | SBO:RBO | SBO:PNO | SBO:SEBO |
|------------|---------|---------|---------|---------|---------|---------|
| 0          | 4.75±0.43Ac | 4.27±0.08Aa | 4.74±0.05Ac | 4.32±0.05Aa | 4.45±0.03Ab | 4.41±0.04Ab |
| 4          | 7.71±0.13Bc | 5.89±0.11Bb | 6.16±0.40Bb | 7.90±0.04Bcd | 5.09±0.53Ba | 8.68±0.18Bd |
| 8          | 10.8±0.36Cc | 12.23±0.30Cd | 13.64±0.46Ccd | 9.78±0.04Cbc | 6.36±0.16Ca | 12.04±0.04Cd |
| 12         | 14.9±0.23Db | 21.93±0.28Dd | 13.23±0.16Cab | 14.58±0.06Db | 10.48±0.02Da | 18.31±0.06Dc |
| 16         | 27.0±0.69Fd | 20.71±0.82Db | 22.76±0.11Dc | 18.04±0.04Ea | 18.98±0.05Ea | 18.89±0.03Ea |
| 20         | 39.1±0.27Ff | 28.68±0.20Ed | 25.54±0.03Ec | 18.38±0.02Ea | 21.85±0.21Fb | 30.12±0.18Fb |
| 24         | 44.0±0.13Ge | 34.25±0.23Gb | 27.13±0.06Ff | 26.23±0.42Fa | 40.59±0.51Fd | 36.69±0.09Gc |
conjugated oxidative products, owing to its high linoleic acid content.

Radical scavenging activity (DPPH assay)

Blending selected oil with SBO increased antiradical action of SBO (Figure 4). The impact of SEO as additives on SBO oxidation was the strongest followed by RBO, CAO, SEBO and PNO. Rice bran oil has higher oxidative stability because it comprises not only tocopherol but also gamma-oryzanol. Therefore, the presence of gamma-oryzanol is possible to inhibiting the lipid peroxidation in several oil modes. Lignans and tocopherols are well known naturally occurring antioxidant components present in SEO, with sesamin and sesamolin being the predominant sesame lignans (Podloucka et al., 2013). It is also well known that c-oryzanol together with tocopherols are responsible for the high antioxidative strength of the RBO (Debnath et al., 2012). Seabuckthorn seed oil is a rich source of tocopherols and carotenoids, which are known to have significant antioxidant activities (Ting et al., 2011).

In summary, several of oil blends proved to be highly active and raise the nutritional values. While sesame oil was capable of high inhibition in the formation of oxidation compounds followed by rice bran oil, camellia oil, seabuckthorn oil and peanut oil. Nevertheless, the quality, composition, bioactive properties and oxidative stability of all the studied oils were greatly affected by the storage time. Thus, a long-term storage of these oils should be avoided.

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