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Influence of methanolic extracts of soybean seeds and soybean oil on lipid oxidation in linseed oil

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Abstract

Methanolic extracts of the two varieties Giza 21 and Giza 35 of germinated soybean seed *Glycine max* and an extract of soybean oil of variety Giza 35 were examined for their antioxidant properties. The effect of the three extracts on lipid oxidation in linseed oil during storage at 60°C was monitored by the formation of primary and secondary lipid oxidation products in linseed oil. Conjugated diene hydroperoxides were determined by measuring the absorbance at 234 nm. The secondary products propanal, 1-penten-3-one, hexanal and octanal were selected based on their odour activity shown in gas chromatography–olfactometry analysis. The compounds were identified by gas chromatography–mass spectrometry and quantified by static headspace gas chromatography. Preliminary studies at 1% extract levels showed potential for the extracts of both seed varieties and to a lesser extent for the oil extract. In the main study, various concentrations (0, 0.5, 1, 2 and 4% v/v) of the seed extracts were assessed. Inhibition of lipid oxidation was related to the concentration of the extract added. The two seed extracts showed similar effects. Variety Giza 21 extracts (4% v/v) resulted in a 23% reduction in conjugated diene hydroperoxides, and 99, 86, 62 and 61% reductions in propanal, 1-penten-3-one, hexanal and octanal at the end of the storage study, respectively. The same concentration of the extract of variety Giza 35 reduced formation of the respective compounds by 30, 98, 70, 64 and 64%.

Keywords: Antioxidants; Linseed oil; Lipid oxidation; Soybean oil; Soybean seeds

1. Introduction

Oxidation of lipids is the main cause of quality deterioration in many food systems. Lipid oxidation may lead to off-flavours, formation of toxic compounds and lower the quality and nutritional value of foods. It is also associated with aging, membrane damage, heart disease, and cancer (Ramarathnam, Osawa, Ochi, & Kawakishi, 1995). Large efforts have been made to develop effective antioxidants for edible oils in order to retard lipid oxidation. The addition of synthetic antioxidants is effective in retarding oxidation. Antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*- butyl hydroquinone (TBHQ), are widely used. Their safety, however, has been questioned (Buxiang & Fukuhara, 1997).

In today's consumers' perception of agriculture and food production, aspects like health, safety and quality have become key words. In these circumstances, research on development of safer natural antioxidants is essential. Therefore, the interest in replacing synthetic antioxidants with natural components from oil seeds, spices and other plant materials has increased considerably. Environmental consciousness and cost factors have led to extraction of natural antioxidants from easily renewable sources, including plant waste materials. Extracts from plants, including waste materials such as old tea leaves (Zandi & Gordon, 1999) have shown antioxidant activity. Certain seeds have also shown to possess antioxidant activity: e.g. cowpea seeds (Ghazy, El-Sayed, Shaker, El-Sayed, Hassn & Darwesh, 2000) and rice seeds (Ramarathnam et al., 1995). Other examples are peanut hulls, which showed considerable antioxidant activity in soybean and peanut oils (Duh & Yen, 1997).

Phenolic compounds in plants are recognised as important compounds in conferring stability against

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auto-oxidation of vegetable oils. The diverse group of phenolic compounds in plants include simple phenolics, phenolic acids, anthocyanins, hydrocinnamic acid derivatives and flavonoids. All the phenolic classes have the structural requirements of free radical scavengers. However, the antioxidant activity of these compounds varies greatly, and some even exhibit pro-oxidant activity (Decker, 1998). Tocopherols are natural monophenolic compounds and although occurring as minor constituents in vegetable oils, they are among the best known and most widely used natural antioxidants. In addition, some of the other phenolic compounds may extend oxidative stability of oils. The presence of natural phenolic compounds is considered an important factor for the antioxidant activity of the plant materials described earlier.

Soybeans are high in lipid content and contain considerable levels of phenolic compounds. When isolated, these natural antioxidants may have potential to prevent lipid oxidation in vegetable oils. In a preliminary study the extracts of the seeds and soybean oil were screened for their antioxidant properties. Subsequently, the effect of higher concentrations of two of the extracts on lipid oxidation in linseed oil were studied by following the formation of primary and secondary lipid oxidation products.

2. Materials and methods

2.1. Materials

2.1.1. Oils and seeds

Commercial linseed oil (LSO) was produced and supplied by Archer Daniels Midland Co. (Red Wing, MN, USA), and stored in a nitrogen gas atmosphere in the dark at 4°C until analysis (1 month max.). The fatty acid methyl ester composition of the oil determined by gas chromatography (GC) was 5.3% 16:0, 3.3% 18:0, 17.6% C18:1, 15.6% 18:2 and 57.6% 18:3. LSO contained 5.9 mg α-tocopherol/kg oil, 64.4 mg γ-tocopherol/kg oil and 2.9 g total phenols/kg oil. For oxidation experiments, the oil samples (3 ml) were stored in glass jars (22 ml) in the dark at 60°C. Three replicate oxidations were carried out for each treatment and each time an analysis was conducted.

The soybean seeds *Glycine max*, variety Giza 21 (SBseed21) and 35 (SBseed35) were grown by the Food Technology Institute Giza (Giza, Egypt). After harvesting in June 2000, the seeds were stored in the dark at 25°C until analysis (1 month max.).

Soybeans (*Glycine max*, variety Giza 35) were grown in Middle Egypt (same batch as SBseed35). The oil was produced by the Food Technology Institute Giza (Giza, Egypt) in a commercial scale plant. After production, the soybean oil (SBO35) was stored in stainless steel tanks in a nitrogen atmosphere in the dark at 25°C. The oil was supplied within 1 month and further stored at −10°C until analysis.

2.2. Methods

2.2.1. Methanolic extraction of soybean seeds and soybean oil

SBseeds (500 g) were germinated for 24 h at room temperature after addition of distilled water, which covered the seeds. The kernel (embryo) was separated from the rest of the seed, 2 g of which was ground with 20 ml of methanol. The mixture was stirred for 2 h at room temperature, centrifuged and the methanol phase concentrated to a volume of 5 ml by evaporation of the solvent under a nitrogen gas stream. In the main experiments, the SBseed extracts were added to the LSO in the following concentrations: 0, 0.5, 1, 2 and 4% v/v.

The SBO35 was stirred with methanol (30 ml; HPLC grade; Rathburn, Walkerburn, Scotland, UK) for 2 h at room temperature. Oil and methanol fractions were separated and the volume of the methanol fraction reduced to 5 ml following the procedure described for the SBseed extraction.

2.2.2. Determination of total phenols

Total phenols were measured in triplicate in the LSO, the extracts of the SBseeds 21 and 35, and the SBO35 extract, following a modified version of the method described by Montedoro, Servili, Baldioli, and Miniati (1992). For the LSO, a methanol–water mixture (4:1 v/v) consisting of 0.1% Tween 20 (Sigma-Aldrich, Steinheim, Germany) was mixed 1:1 with 10 g of the oil using an Ultra turrax T25 (15,000 rpm; Joe Walsh Scientific, Dublin, Ireland) for 1 min. The mixture was subsequently centrifuged (5000 ***g***, 10 min) and the supernatant collected and stored at −20°C. The oil sample was extracted twice. Water and methanol was added to the extracts of the seeds and the SBO35 extract to obtain a 4:1 methanol/water ratio (v/v). The extract of LSO, the seeds and SBO35 were mixed 1:1 (v/v) with the Folin & Ciocalteau's phenol reagent (BDH Laboratory Supplies, Poole, UK). The absorbance was measured at 765 nm. Five concentrations of gallic acid (Sigma-Aldrich, Steinheim, Germany) were measured in quadruplicate at the same wavelength for calibration.

2.2.3. Determination of tocopherol

Tocopherol was measured in duplicate by high performance liquid chromatography performed with a Waters liquid chromatograph (Waters Corporation, Milford, MA, USA), consisting of a Waters 717 plus autosampler, a Waters model 510 pump and a model 486 detector set at 292 nm following the method described by Buttriss and Diplock (1984). A Machery-Nagel

Nucleosil 100-5 C18 column (250 μm, 8×4 mm i.d.; Waters Corporation, Milford, MA, USA) was used for analysis. The mobile phase was methanol/water (97:3 v/v) at a flow rate of 2.0 ml/min. Millennium 32 software (Waters Corporation, Milford, MA, USA) was used for integration of the chromatographic peaks, both for determination of the standard calibration curves (tocopherols: Sigma-Aldrich, Steinheim, Germany) and for calculation of the concentration of tocopherols in the samples.

2.2.4. Analysis of primary lipid oxidation products: conjugated diene hydroperoxides

Weighed oil samples were dissolved in 5 ml cyclohexane (Sigma-Aldrich, Steinheim, Germany), diluted, and the absorbance measured at 234 nm (Varian Cary 1E spectrophotometer; JVA Analytical Ltd, Dublin, Ireland). Absorbances were calculated as hydroperoxides in mmols per litre oil, using a molar absorptivity of 26,000 for linoleate hydroperoxides (AOCS Official Method Ce, 8-89, 1992).

2.2.5. Analysis of secondary lipid oxidation products: volatile compounds

For static headspace gas chromatography (SHGC), 2 ml of oil were transferred into a 10 ml vial and incubated at 60°C for 6 min in the automated headspace unit (Combipal-CTC Analytics system; JVA Analytical Ltd, Dublin, Ireland) of the gas chromatograph (Varian CP-3800; JVA Analytical Ltd, Dublin Ireland). The GC was equipped with a BPX5 capillary column (60-m length, 0.32 mm i.d. and 1.0-μm film thickness; SGE, Kiln Farm Milton Keynes, UK) and a flame ionisation detector (FID) at 275°C. An initial oven temperature of −30°C was used for 1 min, followed by a rate of 100°C/min to 60°C. The oven temperature was kept at 60°C for 5 min and was subsequently programmed to 110°C at 3°C/min, and further to 170°C at 4°C/min. After identification of the compounds by gas chromatography–mass spectrometry, peak areas were standardised with known concentrations of volatile compounds in air and in the various oil samples. Results were calculated as mmol/l oil. The air/oil partition coefficients determined and used for calculations were 5.18×10^{-3} for propanal, 1.29×10^{-2} for 1-penten-3-one, 4.17×10^{-5} for hexanal and $1.69\times a10^{-5}$ for octanal.

2.2.6. Analysis of aroma compounds

Aroma compounds were isolated in a model mouth system following the method published previously (van Ruth & Roozen, 2000). Volatile compounds trapped on Tenax TA 60/80 (Supelco, Bellfonte, PA, USA) were both analysed by gas chromatography–mass spectrometry (GC–MS) and gas chromatography–olfactometry (GC–O).

For GC–MS analysis, desorption of volatile compounds from Tenax (220°C, 4 min) was performed by a thermal desorption device (Tekmar Purge and Trap 3000 concentrator, JVA Analytical Ltd, Dublin, Ireland). Through a heated transfer line, the compounds were directed to the GC column of the GC–MS (GC: Varian Star 3400 CX, JVA Analytical Ltd, Dublin, Ireland; MS: Varian Saturn 3, JVA Analytical Ltd, Dublin, Ireland), where they were focussed by cryogenic means (−120°C). The GC column was identical to the column used for SHGC analysis. An initial oven temperature of 60°C was used for 5 min and the oven was subsequently programmed to 110°C at 3°C/min, and further to 170°C at 4°C/min. Mass spectra were obtained with 70 eV electron impact ionisation, while the mass spectrometer was continuously scanning from m/z 40 to 400 at a scan speed of 3 scans/s. Volatile compounds were identified by comparison of their spectra and their retention indices (RI; Van Den Dool & Kratz, 1963) with bibliographic data and with those of single authentic compounds.

The method used for GC–O of LSO has been published recently (van Ruth, Roozen, Posthumus, & Jansen, 1999). An identical analytical column was used as for the SHGC and GC–MS analyses. The oven temperature program was identical to the program used for GC–MS. Four trained assessors recorded the detection of an odour and provided odour descriptors. GC–O analysis was used to confirm the selection of secondary lipid oxidation products relevant to the aroma changes of the oil during storage.

2.2.7. Statistical analysis

Multivariate analysis of variance (MANOVA) was used to determine significant differences between the means of triplicate analysis of primary and secondary lipid oxidation products in the LSO for the treatments and storage times. If significant differences were found, Fisher's least significant difference tests (LSD test) were performed (O'Mahony, 1986). In order to determine significant differences in formation of lipid oxidation products between the three extracts after 3 days of storage, data were subjected to Student's t-tests (O'Mahony, 1986). Significance level is $P<0.05$ throughout the study.

Relative antioxidative effects were calculated as:

$$\frac{(\text{concentration compound A untreated oil} - \text{concentration compound A treated oil})}{\text{concentration compound A untreated oil}} \times 100$$

where compound A = conjugated diene hydroperoxides, propanal, 1-penten-3-one, hexanal or octanal.

3. Results and discussion

3.1. Preliminary study: effect of extracts of soybean seed 21, soybean seed 35 and soybean oil on lipid oxidation in linseed oil

Oxidative stability of the LSO was studied by following the formation of primary and secondary lipid oxidation products in the oil during 12 days of storage at 60°C. Primary products involved conjugated diene hydroperoxides, and secondary products involved volatile compounds formed in lipid oxidation. The volatile secondary lipid oxidation compounds of the untreated LSO after 9 days of storage at 60°C were isolated in the model mouth and analysed by GC–O and GC–MS. Four volatile compounds were perceived by each of the four GC–O assessors: propanal (RI = 560: chocolate, toffee, potato, solvent), 1-penten-3-one (RI = 659: apple, tyre, spicy, chemical, fruity), hexanal (RI = 781: green, grassy, rancid, aldehyde) and octanal (RI = 1037: fatty, leaf, oil, insecticide). The four compounds corresponded to odour active compounds of LSO or mixtures thereof determined in previous studies (van Ruth, Roozen, & Jansen, 2000). Hexanal and octanal have also been reported among the odour active compounds of soyabean oil (SBO) and rapeseed oil (Guth & Grosch, 1990). Propanal is one of the main volatile decomposition products of linolenate hydroperoxides, whereas hexanal is predominantly formed by cleavage of linoleate hydroperoxides (Frankel, 1991). Octanal is a specific breakdown product of oleate hydroperoxides (Labuza, 1971). The four odour active compounds propanal, 1-penten-3-one, hexanal and octanal were selected for monitoring formation of secondary lipid oxidation products.

The influence of the extracts of the soybean seeds SBseed21 and SBseed35, as well as the extract of the soybean oil SBO35, on the oxidative stability of LSO during 12 days of storage is presented in Table 1. The formation of both conjugated diene hydroperoxides and volatile compounds increased rapidly during storage, showing a significant difference for the days of measurement for conjugated dienes [MANOVA, $F(4,13) = 26.1$, $P < 0.05$], propanal [MANOVA, $F(4,13) = 17.4$, $P < 0.05$] and octanal [MANOVA, $F(4,13) = 6.3$, $P < 0.05$]. Propanal was formed in larger quantities than the other compounds, which reflects the high concentration of linolenic acid (58%) in LSO (Mistry & Min, 1992). Generally from 6 days of storage concentrations of conjugated dienes and volatiles increased considerably in both treated and untreated samples. LSO is high in unsaturated fatty acids, and obviously concentrations of the extracts were not sufficiently high to exhibit an effect after 3 days of storage. Nevertheless, the extracts were effective during the first days of storage. A significant relative antioxidative effect (Fig. 1) with regard to formation of conjugated dienes and volatile lipid oxidation products (propanal, 1-penten-3-one, hexanal and octanal) was observed after 3 days of storage by addition of the SBseed21 and SBseed35 extracts (Student's t-test, $P < 0.05$). SBO35 reduced the formation of conjugated dienes, 1-penten-3-one and octanal significantly (Student's t-test, $P < 0.05$). Overall, SBseed21 reduced both primary and secondary lipid oxidation most efficiently, followed by SBseed35 and SBO35.

The tocopherol and total phenol content of the extracts of the SBseeds and SBO35 were determined and are listed in Table 2. Tocopherol levels were below detection in most of the extracts, due to the methanolic extraction. Total phenol concentrations were highest in the extract of SBO35, followed by the extracts of SBseed35, and SBseed21, respectively. Curiously enough, these concentrations are inversely proportional to the antioxidant activity of the extracts observed. Although some researchers established relationships between total phenols and oxidative stability of oils (Baldioli, Servili, Perretti, & Montedoro, 1996), it has been reported that phenolic compounds have different effects on oxidative stability in oils (Baldioli et al., 1996; Shahidi & Wanasundara, 1994). Therefore, for the present extracts the composition of the phenolic compounds is likely to be of greater importance than the total phenol concentration.

In this preliminary study, which was used for screening, especially the extracts of SBseed 21 and SBseed 35 showed potential for extension of the oxidative stability of LSO. It should be noted that in this preliminary experiment the antioxidative effect lasted only during a few days of storage of the oil. After 3 days, concentrations of lipid oxidation products increased considerably in the treated samples. For the following study higher concentrations of the extracts were examined.

3.2. Effect of various concentrations of soybean seed 21 and seed 35 extract on lipid oxidation in linseed oil

The extract of SBOseed21 was added in the concentrations 0, 0.5, 1, 2 and 4% v/v to LSO. The influence of the various concentrations added on conjugated diene hydroperoxide, propanal, 1-penten-3-one, hexanal and octanal levels in LSO during storage are presented in Table 3. A significant increase over time is observed for the conjugated diene hydroperoxides [MANOVA, $F(3,13) = 77.7$] and all the volatiles but propanal [MANOVA, 1-penten-3-one $F(3,13) = 5.1$, $P < 0.05$; hexanal $F(3,13) = 23.6$, $P < 0.05$; octanal $F(3,13) = 35.0$, $P < 0.05$]. A clear effect between treated and untreated LSO is shown for most lipid oxidation products. An overall significant difference between the treatments throughout the storage period was found for the conjugated diene hydroperoxides [MANOVA, $F(4,13) = 5.8$, $P < 0,05$] and propanal [MANOVA, $F(4,13) = 5.5$,

Table 1
Effect of addition of methanolic extracts of soybean seed 21 (SBseed21) and 35 (SBseed35), and soybean oil (SBO35) on formation of primary (conjugated dienes) and secondary lipid oxidation products (propanal, 1-penten-3-one, hexanal, octanal) in linseed oil during 12 days of storage in the dark at 60°C (mean, n = 3)

	Storage (days)					CV (%)[a]
	0	3	6	9	12	
CD[b]						
LSO	22.1	52.9	51.7	53.8	53.4	2
+ SBseed21	26.1	42.6	52.2	54.8	55.7	3
+ SBseed35	24.6	45.9	53.9	54.7	56.2	3
+ SBO35	24.1	47.6	53.7	56.0	56.3	2
Propanal[b]						
LSO	0.6	10.3	20.3	26.3	34.0	13
+ SBseed21	1.0	5.7	25.9	45.5	75.0	17
+ SBseed35	1.0	7.9	34.4	63.1	96.1	16
+ SBO35	0.9	8.9	38.0	89.5	106.5	22
1-Penten-3-one[b]						
LSO	0.2	0.6	3.4	10.7	7.5	24
+ SBseed21	BD	0.3	1.0	2.0	6.6	24
+ SBseed35	BD	0.3	1.5	4.6	8.0	12
+ SBO35	BD[c]	0.2	1.8	7.4	10.1	15
Hexanal[b]						
LSO	0.5	0.8	2.0	3.2	4.4	18
+ SBseed21	0.5	0.4	2.3	3.2	6.5	19
+ SBseed35	0.4	0.5	2.3	5.2	9.5	21
+ SBO35	0.5	1.1	2.8	10.6	16.5	25
Octanal[b]						
LSO	0.2	3.2	6.3	9.5	12.7	15
+ SBseed21	BD	1.2	4.7	9.3	13.0	16
+ SBseed35	BD	1.8	6.4	11.1	15.3	12
+ SBO35	BD	2.0	7.7	16.9	15.5	8

[a] CV, average coefficient of variance (%).
[b] Concentration conjugated diene, propanal, 1-penten-3-one, hexanal and octanal in mmol/l oil.
[c] BD is below detection level (<0.1 mmol/l).

$P < 0.05$]. In particular, the treatments after 3 days differed significantly from each other (LSD test, $P < 0.05$). The 4% level was significantly different from the others throughout the study (LSD test, $P < 0.05$). The propanal formation in the untreated LSO was significantly higher than in the treated samples (LSD test, $P < 0.05$). Generally, the extract of SBseed21 extended oxidative stability of the LSO sufficiently, the effect of which was related to the concentration applied. The relative antioxidative effect of the highest concentration at the end of the storage period for the 4% concentration level for the conjugated diene hydroperoxides, propanal, 1-penten-3-one, hexanal and octanal was 23, 99, 86, 62 and 61%, respectively.

A similar experimental design as for the SBseed 21 was applied for the SBseed35 extract, the results of which are presented in Table 4. The differences between the days and treatments were more pronounced with the addition of SBseed35 in comparison with SBseed21. Significant increase during storage was shown for the conjugated diene hydroperoxides [MANOVA, $F(3,13) = 68.5$,

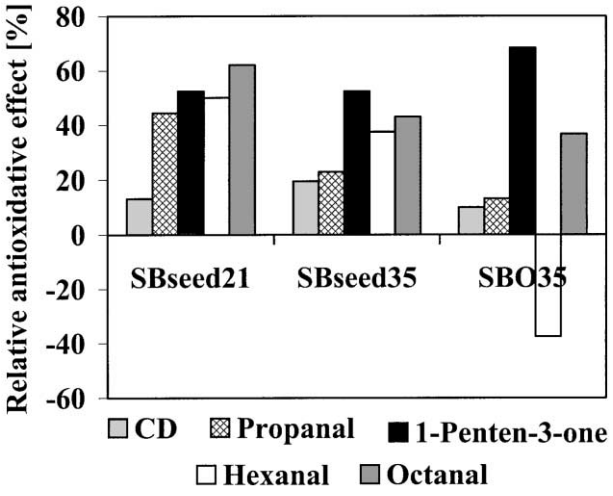

Fig. 1. Relative effect of methanolic extracts of two soybean seeds (SBseed21 and SBseed35), and soybean oil (SBO35) on formation of primary (conjugated diene hydroperoxides: CD) and secondary lipid oxidation products (propanal, 1-penten-3-one, hexanal, octanal) in linseed oil after 3 days of storage in the dark at 60°C.

$P<0.05$], propanal [MANOVA, $F(3,13)=4.7$, $P<0.05$], 1-penten-3-one [MANOVA, $F(3,13)=13.7$, $P<0.05$], hexanal [MANOVA, $F(3,13)=13.8$, $P<0.05$] and octanal [MANOVA, $F(3,13)=26.6$, $P<0.05$]. Significant differences between the samples in oxidative stability throughout the storage period was found for conjugated diene hydroperoxides [MANOVA, $F(4,13)=5.4$, $P<0.05$], propanal [MANOVA, $F(4,13)=7.6$, $P<0.05$], hexanal [MANOVA, $F(4,13)=7.9$, $P<0.05$] and octanal [MANOVA, $F(4,13)=6.5$ $P<0.05$]. Formation of

Table 2
Total phenol and tocopherol content of methanolic extracts of soybean seeds 21 (SBseed21) and 35 (SBseed35) and soybean oil (SBO35) (mean±S.D.; total phenol analysis: $n=3$; tocopherol analysis: $n=2$)

	Total phenols[a]	Tocopherols[b]		
		α-Tocopherol	β-Tocopherol	γ-Tocopherol
SBseed21	2.5±0.3	BD[c]	BD	BD
SBseed35	4.0±0.4	BD	BD	BD
SBO35	4.7±0.7	BD	BD	4.8

[a] Total phenols in mg/g extract.
[b] Tocopherols in mg/kg extract.
[c] BD is below detection level (<2.5 mg/kg extract).

Table 3
Effect of addition of various concentrations methanolic extracts of soybean seed 21 (SBseed21) on formation of primary (conjugated dienes) and secondary lipid oxidation products (propanal, 1-penten-3-one, hexanal, octanal) in linseed oil during 9 days of storage in the dark at 60°C (mean, $n=3$)

	Storage (days)				CV (%)[a]
	0	3	6	9	
CD[b]					
LSO	12.7	41.1	54.0	57.9	6
+0.5% SBseed21	13.3	42.6	54.8	55.3	4
+1.0% SBseed21	15.0	34.3	55.4	56.8	10
+2.0% SBseed21	12.7	31.4	56.9	50.6	5
+4.0% SBseed21	10.3	26.6	30.7	44.6	8
Propanala[b]					
LSO	0.80	8.2	19.5	28.3	18
+0.5% SBseed21	0.6	1.3	3.1	5.3	23
+1.0% SBseed21	0.9	1.7	3.5	5.4	15
+2.0% SBseed21	0.4	1.1	1.3	2.6	35
+4.0% SBseed21	1.1	0.2	0.1	0.4	44
1-Penten-3-one[b]					
LSO	1.9	0.2	3.9	20.1	24
+0.5% SBseed21	BD[c]	0.3	1.0	4.2	19
+1.0% SBseed21	0.1	0.5	1.0	3.2	21
+2.0% SBseed21	BD	1.0	2.3	6.5	28
+4.0% SBseed21	0.1	0.1	0.4	2.9	41
Hexanal[b]					
LSO	0.4	0.2	2.3	5.0	10
+0.5% SBseed21	0.4	0.4	1.6	2.8	18
+1.0% SBseed21	0.4	0.4	2.1	2.4	15
+2.0% SBseed21	0.4	0.1	1.7	2.4	8
+4.0% SBseed21	0.4	0.2	1.4	1.9	35
Octanal[b]					
LSO	BD	2.1	5.5	12.7	5
+0.5% SBseed21	BD	1.0	3.2	7.7	7
+1.0% SBseed21	BD	1.1	2.8	7.4	14
+2.0% SBseed21	BD	1.8	5.1	7.2	5
+4.0% SBseed21	BD	1.6	3.1	5.0	31

[a] CV, average coefficient of variance (%).
[b] Concentration conjugated diene, propanal, 1-penten-3-one, hexanal and octanal in mmol/l oil.
[c] BD is below detection level (<0.1 mmol/l).

propanal and octanal were clearly extract concentration related. High concentrations reduced the formation of both primary and secondary lipid oxidation products considerably. The antioxidant effects of the 4% concentration level of the SBseed35 extract were: conjugated diene hydroperoxides 30%, propanal 98%, 1-penten-3-one 70%, hexanal 64% and octanal 64%.

Both SBseed21 and SBseed35 extended oxidative stability of the LSO. Only a few studies have been published using waste materials or easily renewable resources for reduction of lipid oxidation. The varying oils and methods used in those studies complicate comparison. For example, Shahidi and Wanasundara (1994) reported an inhibition of formation of 2-thiobarbituric acid of 70% for the highest concentrations of canola seed extract added to canola oil. A similar experiment with a methanolic extract of peanut hulls added to peanut oils, showed a reduction in acid value of 80% during a 40 days storage period (Duh & Yen, 1997). Antioxidant activity of potato peel extracts was reported to be 60%, expressed as inhibition of β-carotene degradation relative to a control (Mansour & Khalil, 2000). Old tea leaves also showed great potential and reduced *p*-anisidine values up to 88% (Zandi & Gordon, 1999). Despite the different methods it can be concluded that, in comparison with other studies, the extracts of the soybean seeds in the present study extended oxidative stability considerably.

4. Conclusions

Extracts of soybean seeds *Glycine max*, variety Giza 21 and Giza 35 reduced formation of primary oxidation products up to 30% and secondary lipid oxidation products up to 99%, resulting in significantly increased oxidative stability of LSO.

Table 4
Effect of addition of various concentrations methanolic extracts of soybean seed 35 (SBseed35) on formation of primary (conjugated dienes) and secondary lipid oxidation products (propanal, 1-penten-3-one, hexanal, octanal) in linseed oil during 9 days of storage in the dark at 60°C (mean, $n=3$)

	Storage (days)				CV (%)[a]
	0	3	6	9	
CD[b]					
LSO	16.2	51.8	51.3	52.1	9
+0.5% SBseed35	13.4	48.2	40.6	49.6	8
+1.0% SBseed35	12.6	39.8	39.5	44.9	13
+2.0% SBseed35	13.1	36.7	33.8	47.8	11
+4.0% SBseed35	16.7	40.1	33.3	36.4	6
Propanal[b]					
LSO	0.1	8.8	12.9	16.0	34
+0.5% SBseed35	0.2	4.8	5.8	9.2	20
+1.0% SBseed35	0.1	2.0	3.9	5.9	17
+2.0% SBseed35	0.1	1.1	0.7	0.7	38
+4.0% SBseed35	0.3	0.1	0.1	0.4	17
1-Penten-3-one[b]					
LSO	0.2	1.4	5.3	10.6	23
+0.5% SBseed35	0.2	1.1	3.0	6.5	14
+1.0% SBseed35	0.2	1.7	3.3	5.2	22
+2.0% SBseed35	0.1	3.4	4.2	4.0	14
+4.0% SBseed35	0.1	0.9	1.2	3.2	21
Hexanal[b]					
LSO	0.8	1.5	1.9	2.3	15
+0.5% SBseed35	0.7	1.9	1.9	2.7	20
+1.0% SBseed35	0.6	2.6	1.9	2.1	21
+2.0% SBseed35	0.6	1.9	1.4	1.8	11
+4.0% SBseed35	0.5	0.6	0.7	0.8	12
Octanal[b]					
LSO	0.5	4.9	7.7	8.8	8
+0.5% SBseed35	0.4	2.6	4.7	8.0	10
+1.0% SBseed35	0.4	2.9	4.9	6.9	12
+2.0% SBseed35	0.5	2.4	3.7	4.9	14
+4.0% SBseed35	0.6	1.1	1.8	3.2	10

[a] CV, average coefficient of variance (%).
[b] Concentration conjugated diene, propanal, 1-penten-3-one, hexanal and octanal in mmol/l oil.

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