Antioxidant Activity of Sea Buckthorn Fruits Alcoholic Extract on Soy Bean Oil

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Free soy oil and soy oil treated with polyphenols extracted from sea buckthorn fruits, BHA and BHT synthetic antioxidants was submitted to thermal oxidation for 8 days at 62°C and to photo-oxidation for 30 min. at 254 nm wave-length light. Oxidation processes were evaluated by determining the peroxide value, para-anisidine value and the absorbance in UV domain. Using second derivative spectroscopy, it was determined the cis,trans/trans,trans conjugated dienes ratio. The concentration of 100 ppm sea buckthorn polyphenols determined the decrease of peroxide values and para-anisidine values after a pattern similar to BHT synthetic antioxidant and also the increase of cis,trans/trans,trans conjugated dienes ratio in case of soy oil submitted to thermal oxidation. The increase of cis,trans/trans,trans conjugated dienes ratio was also recorded in case of soy oil submitted to UV light oxidation, under the influence of sea buckthorn extract. The obtained results indicate that polyphenols extracted from sea buckthorn are able to protect soy oil against thermal and photochemical oxidation.

Keywords: antioxidant activity, Sea buckthorn, soy oil, peroxide value, para-anisidine value

Oils oxidation is a very complex process. Oxidation is started by the reaction of reactive oxygen species, especially hydroxil radical ($HO^{\cdot}$) and oxygen singlet ($^1O_2$), with methylene groups of polyunsaturated fatty acids (PUFAs) to form free radicals of unsaturated fatty acids ($L^{\cdot}$) and peroxyl radicals ($LOO^{\cdot}$). Unless they are stopped, the radicals are able to transfer themselves from molecule to molecule in a chain reaction. The results of oxidation and radicals reactions are oxygenated species such as hydroperoxides and subsequent degradation products [1]. Thus, the oxidation reaction of unsaturated fatty compounds affects the quality and utility of oils. Generally, the rate of oxidation is dependent of fatty acids composition; polyunsaturated fatty acids are more susceptible to oxidation.To stop chain reactions of radicals, lipophilic synthetic antioxidants are used (ex. 2,6-di-tert-butyl-p-hydroxytoluen - BHT or tert-butyl-4-hydroxyanisole - BHA). These antioxidants can block one or two of the major oxidation pathways, but they are suspected of being responsible for toxicity and carcinogenity [2-5].

Naturally occurring phenolic compounds (e.g. flavonoids and phenolic acids) have drawn attention because they have been ingested for centuries and are assumed to be relatively safe for human consumption [6-9]. Like synthetic antioxidants, plant polyphenols act as chelators of transition metals and/or as hydrogen donors and inhibitors of lipooxygenases. For these reasons, plant polyphenols can be used as natural preservatives, inhibiting the onset of oils peroxidation [10-16].

Pharmaceutical and antioxidant properties of sea buckthorn *(Hippophae rhamnoides)* have been reported by the last years' investigations [17-23].

Because soy oil is known for its instability due to the high content of unsaturated fatty acids, in this study it was investigated the ability of an alcoholic extract obtained from sea buckthorn *(Hippophae rhamnoides)* fruits to inhibit the oxidation of soy oil under thermal and UV light exposure.

Experimental part

Soy oil

The experiments were made on virgin soy oil obtained from non-genetically modified soy.

Obtaining vegetal extracts

In order to obtain vegetal extracts, dried sea buckthorn fruits were grounded and then subdued to a solid-liquid extraction with ethanol in a solvent extractor (VELP Scientifica). The extract was concentrated and used in different relations to determine the antioxidant activity against thermal and ultra-violet oxidation of soy bean oil.

Determination of total phenolics compounds

The total phenolics content was estimated using Folin-Ciocalteu reagent based assay. To the mixture containing 500 μL plant extract and 4.5 mL of water, 0.2 mL Folin-Ciocalteu reagent was added. The mixture was kept for 5 min at room temperature and then 0.5 mL of 20% Na_2CO_3 were added. The mixture was allowed to stand at room temperature for 30 min and then the absorbance at 765 nm was recorded using an UV-VIS-NIR spectrophotometer (Jasco 670). Galic acid was used as standard for calibration curve [24].

Thermal oxidation assay

Soy oil was treated with sea buckthorn alcoholic extract in different relations (equivalent to 5 ppm, 25 ppm, 50 ppm, 100 ppm polyphenols). Also, Sigma BHT and BHA were added to soy oil in relation of 200 ppm. The oxidative stability of oil samples was determined during storage for 8 days at 62°C. Sea buckthorn extract and synthetic antioxidants were added directly to soy oil at room temperature and the dissolution was obtained by manual homogenization. All samples were evaluated in triplicate. A control (blank system, no extract and no antioxidants added), but with the same amount of ethanol, was always evaluated in triplicate.

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Ultra-violet oxidation assay

Soy oil samples treated with *Hippophae rhamnoides* fruits extract in different relations and synthetic antioxidants BHA and BHT (in relation of 200 ppm) were exposed at UV light (254 nm wave-length) for 30 min. All samples and control systems were evaluated in triplicate.

Determination of peroxide value

Peroxide value was determined according with Official American Oil Chemist's Society method (AOCS, 1985). The values were expressed as meq of peroxide O_2/kg oil.

Determination of para-anisidine value

Para-anisidine value was determined according with Official American Oil Chemist's Society methods (AOCS, 1997).

Determination of absorptivity in UV domain

2.5 μL oil sample exposed at 62°C were dissolved in 2 mL isooctane and UV-absorbance was scanned between 200 and 320 nm with a UV-VIS-NIR spectrophotometer (Jasco 670). Operating conditions were: the scanning speed – 100 nm/min; band width - 1.0 nm; data pitch - 0.2 nm. Oils exposed at UV light (254 nm wave-length) were investigated using the same protocol, with the difference that there were used 5μL oil.

Statistical analysis

The results were expressed as mean values (±SD) of 3 determinations. The mean value and standard deviations were calculated with Excel program from Microsoft Office package.

Results and discussions

Thermal oxidation

Total phenolics determination

In sea buckthorn crude extract, total pholyphenols were estimated at 84.81 mg galic acid equivalent/100 mL.

Peroxide value

Determination of lipid hydroperoxides, primary oxidation compounds, is used to monitor lipid oxidation in oils. The effect of sea buckthorn alcoholic extract upon soy oil, comparatively with synthetic antioxidants BHT and BHA, depending on the time of storage is shown in figure 1. During 8 days, BHA and BHT synthetic antioxidants protected soy oil against thermal oxidation, the most efficient being butylated hydroxytoluene (BHT). Sea buckthorn alcoholic extract showed antioxidant activity depending on the polyphenols concentration. Thus, for a polyphenols concentration of 5 ppm, it was recorded the incapacity of the extract to inhibit soy oil peroxidation process. In this case, during the 8 days of oil exposure at 62°C, it was noticed even o slight prooxidant action of the extract.

The treatment of soy oil with sea buckthorn alcoholic extract in concentration of 25 ppm polyphenols had as a result a protection against lipid peroxidation in a pattern similar to BHA synthetic antioxidant. Sea buckthorn alcoholic extract in concentration of 50 ppm and 100 ppm polyphenols inhibited lipid peroxides formation in the first 4 days in a manner similar to BHA, but inferior to BHT. After 6 days of thermal oxidation, in case of the soy oil samples treated with sea buckthorn extract, peroxide values were lower for 100 ppm polyphenols concentration (105 ± 12.1 mEq/kg) than the ones obtained for 50 ppm concentration (127 ± 9.6 mEq/kg), but higher than peroxide values obtained for BHT (79.0 ± 8.77 mEq/kg). After 8 days of exposure at 62°C, peroxide value was recorded at 111 ± 11.2 mEq/kg for 100 ppm relation, value similar to the one of BHT (115±13.1 mEq/kg).

The obtained results demonstrate that sea buckthorn's polyphenols act protective upon polyunsaturated fatty acids from soy oil exposed to thermal oxidation in a manner dependent on the concentration. In case of 100 ppm concentration (polyphenols in oil), after 8 days of incubation at 62°C, antioxidant effect of sea buckthorn alcoholic extract was similar to the one of BHT and superior to the one of BHA.

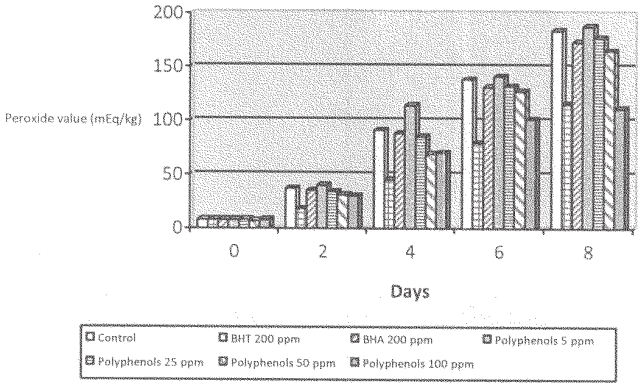

Fig. 1. Peroxide value of soy oil added with sea buckthorn alcoholic extracts, BHA and BHT, submitted to thermal oxidation at 62°C

Para-anisidine value

Oil samples' content in aldehydes, secondary products of oxidation process, was expressed by para-anisidine value; the obtained results are shown in figure 2. In case of sea buckthorn alcoholic extract used in concentration of 5 ppm polyphenols, para-anisidine values were slightly higher (1.56 ± 0.31, respectively 3.64 ± 0.82) than the ones obtained for control after 2 and 4 days of oil exposure at 62°C (1.37 ± 0.13, respectively 3.29 ± 0.75); after 6 and 8 days of exposure, para-anisidine values (5.12 ± 0.91, respectively 7.52 ± 1.07) were lower than the ones recorded for control (5.58 ± 1.87, respectively 7.68 ±2.03). In case of sea buckthorn alcoholic extract used in concentration of 25 ppm polyphenols, after 2 and 4 days of exposure at 62°C, the para-anisidine values (1.39 ± 0.91, respectively 3.53 ± 1.22) were comparable with the ones obtained for 5 ppm concentration (1.56 ± 0.31, respectively 3.64 ± 0.82); para-anisidine values recorded after 6 and 8 days (5.06 ± 1.31, respectively 7.06 ± 1.32) were similar to the ones obtained for BHA (5.32 ±1.08, respectively 7.06 ±2.30). For the 50 ppm polyphenols concentration, para-anisidine values were lower comparatively to BHA, but higher than the ones obtained for BHT. The lowest values of para-anisidine were obtained using sea buckthorn extract in a concentration of 100 ppm polyphenols, the values recorded during the 8 days of thermal exposure (day 2: 0.47 ± 0.11; day 4: 0.54 ± 0.13; day 6: 2.07 ± 0.41; day 8: 3.77 ± 0.76) being lower than the ones obtained for BHA and BHT.

These results are congruent to the results obtained for peroxide value and they reflect the capacity of sea buckthorn alcoholic extract used in relation of 100 ppm polyphenols to protect soy oil against thermal oxidation process.

Absorptivity in UV domain

Oxidation of polyunsaturated fatty acids can also be analyzed through the absorptivity increase in UV spectrum. During the oxidation process, lipids that contain polyunsaturated fatty acids show a change in the double

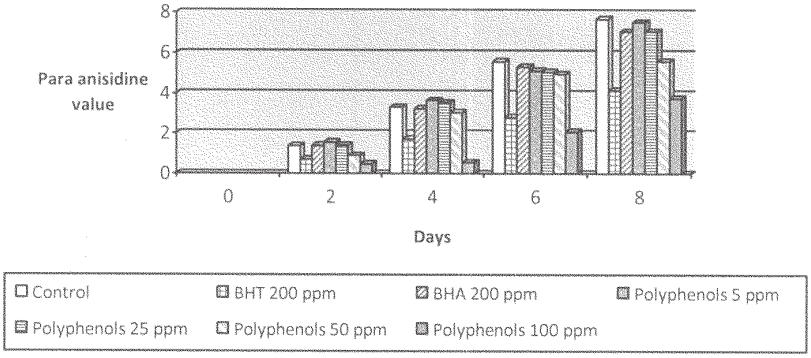

Fig. 2. Para-anisidine values of soy oil added with sea buckthorn alcoholic extracts, BHA and BHT, submitted to thermal oxidation at 62°C

bonds positions, due to the isomeration and the conjugation in the molecule. The specific absorptivity, in terms of conjugated dienes (CD) at 232 nm and conjugated trienes (CT) at 268 nm, is considered an important parameter for the investigation of oxidative process in the oils [25].

The absorption spectrum obtained after 8 days of soy oil exposure at 62°C is congruent with the results obtained after peroxide and para-anisidine assays (fig. 3).

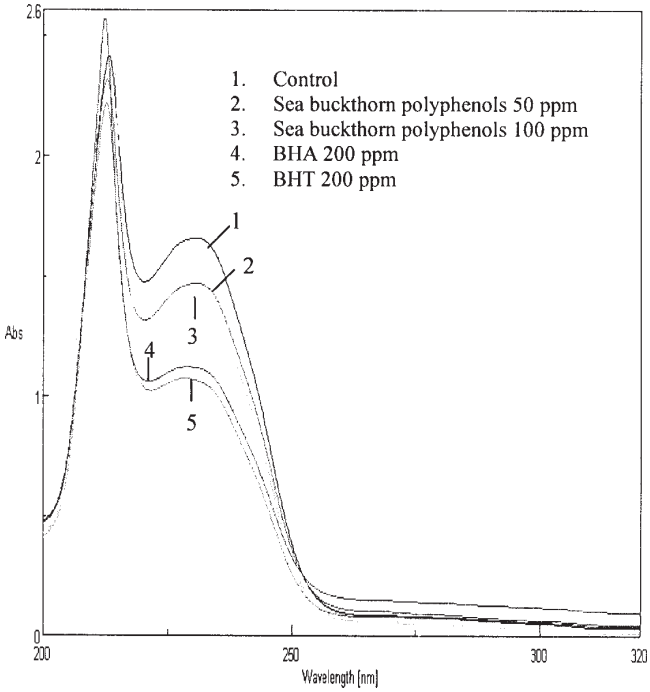

Fig. 3. UV absorbance scanning between 200 and 320 nm of soy oil exposed for 8 days at 62°C

The lowest values of absorptivity at 232 nm were recorded for oil samples added with BHT, while the highest values were recorded for control. Conjugated trienes, expressed as absorptivity at 268 nm, showed the lowest values in case of soy oil added with sea buckthorn polyphenols in concentration of 100 ppm (table 1).

Table 1
ABSORPTIVITIES AT 232 nm AND 268 nm OF SOY OIL ADDED WITH BHT, BHA AND SEA BUCKTHORN POLYPHENOLS IN DIFFERENT CONCENTRATIONS, AFTER EXPOSURE AT 62°C FOR 8 DAYS

Sample	A_{232nm}	A_{268nm}
Control	1.645	0.146
BHT 200 ppm	1.050	0.070
BHA 200 ppm	1.104	0.084
Polyphenols 50 ppm	1.458	0.100
Polyphenols 100 ppm	1.408	0.051

Measurement of UV absorbance of conjugated dienes at 232 nm may be partially masked by the unoxidized fatty acids themselves or by some extract compounds that absorb at wavelength nearby that of conjugated dienes [26-29]. For this reason, table 2 shows minima at 233 nm (*trans,trans* CD) and 242 nm (*cis,trans* CD) obtained by second derivative spectroscopy, and also the ratio *cis,trans/trans,trans*.

The results presented in table 2 reflect the efficacy of polyphenols from sea buckthorn alcoholic extract to inhibit lipid peroxidation of PUFAs and to reduce *trans,trans* CD concentration. A high ratio of *cis,trans/trans,trans* CD due to the inhibition of *trans,trans* isomers production by hydrogen donors reflects an efficient activity of radical termination by antioxidants [30]. The treatment of soy oil with sea buckthorn polyphenols in 100 ppm concentration determined, after soy oil exposure at 62°C for 8 days, a 22% decrease of *cis,trans/trans,trans* CD ratio comparatively with the control. Thus, for control, *cis,trans/trans,trans* CD ratio was 0.45 ± 0.11, while for soy oil added with sea buckthorn polyphenols in 100 ppm concentration, *cis,trans/trans,trans* CD ratio was 0.55 ± 0.13. As a remark, the lowest value of *cis,trans/trans,trans* CD ratio was recorded in case of BHT synthetic antioxidant.

Sample	Minima at 233 nm	Minima at 242 nm	*cis,trans/trans,trans* ratio
Control	-0.0011	-0.0005	0.45 ± 0.11
BHT 200 ppm	-0.0015	-0.0005	0.33 ± 0.10
BHA 200ppm	-0.0020	-0.0010	0.50 ± 0.17
Polyphenols 50 ppm	-0.0019	-0.0010	0.52 ± 0.29
Polyphenols 100 ppm	-0.0018	-0.0010	0.55 ± 0.13

Table 2
SECOND DERIVATIVE SPECTROSCOPY. MINIMA AT 233 nm AND 242 nm OF SOY OIL ADDED WITH BHT, BHA AND SEA BUCKTHORN POLYPHENOLS IN DIFFERENT CONCENTRATIONS, AFTER EXPOSURE AT 62°C FOR 8 DAYS

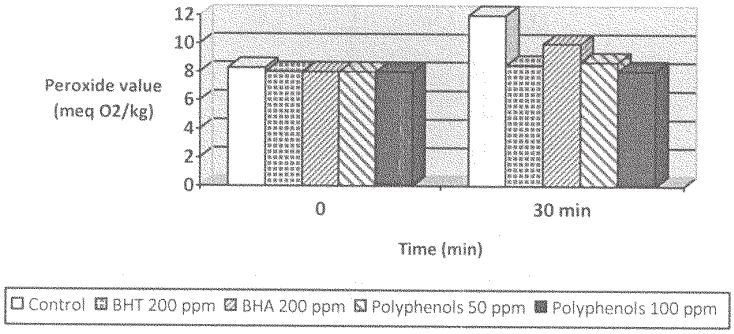

Fig. 4. Peroxide value of soy oil added with sea buckthorn alcoholic extracts, BHA and BHT submitted to UV radiation (254 nm)

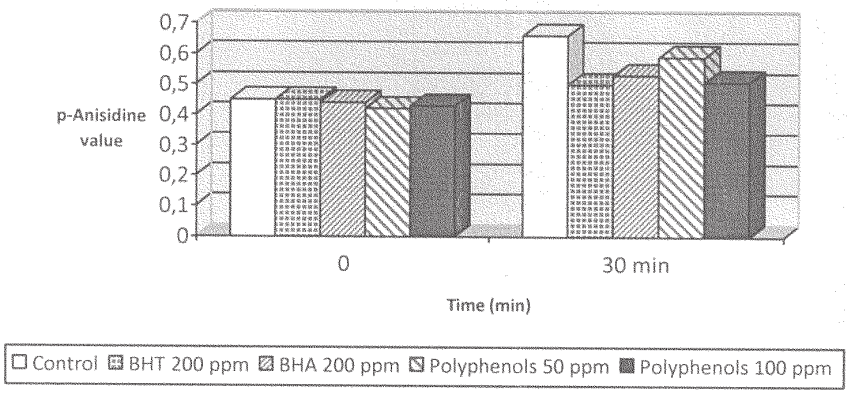

Fig. 5. Para-anisidine values of soy oil added with sea buckthorn alcoholic extracts, BHA and BHT, submitted to UV radiation (254 nm)

Ultra-violet oxidation

Peroxide value

The peroxide values obtained after the exposure to 254 nm wave-length monochromatic radiation for 30 min of soy oil treated with synthetic antioxidants and sea buckthorn alcoholic extracts revealed the protective effect against lipid peroxidation of sea buckthorn polyphenols (fig. 4). For a 100 ppm polyphenols concentration, the protective effect was similar to the one induced by BHT (8.1 ±1.9 mEq/kg, respectively 8.5 ± 2.3 mEq/kg). The protection offered by sea buckthorn polyphenols in concentration of 100 ppm against lipid peroxidation was also similar with the one exhibited by 50 ppm polyphenols concentration (8.1 ± 1.9 mEq/kg, respectively 8.7 ± 2.6 mEq/kg). The close peroxide values obtained in case of soy oil treated with 50 ppm and 100 ppm polyphenols are probably due to the existence of small quantities of photosensitizers in sea buckthorn alcoholic extract (e.g. riboflavin).

Para-anisidine value

Para-anisidine values showed, after the exposure to UV radiation for 30 min, a similar evolution with the one recorded for peroxide values assay. In this assay, the smallest para-anisidine value was recorded in the case of oil samples treated with 100 ppm polyphenols. (0.51 ± 0.17) (fig. 5).

UV absorptivity assay

Scanning between 200 and 320 nm of oils treated with synthetic and natural antioxidants after exposure to UV radiation is showed in figure 6. At 232 nm wave-length, the smallest absorbance was recorded for the soy oil samples treated with 100 ppm polyphenols, while the highest absorbance was recorded for control. At this wave-length, the absorbance of oil samples treated with synthetic antioxidants was lower than the one of control, but higher than the ones of oils treated with sea buckthorn polyphenols. At 268 nm wave-length, the highest absorbance was recorded for control, while the lowest absorbance was recorded for oil samples treated with 100 ppm polyphenols (table 3). Table 4 shows minima at 233 and minima at 242 nm obtained by second derivative spectroscopy and also the *cis,tran/trans,trans* CD ratio.

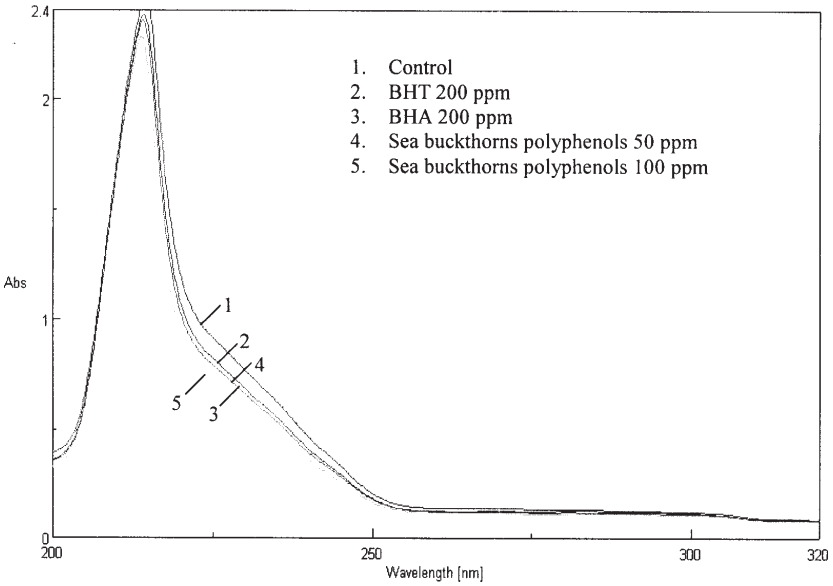

Fig. 6. UV absorbance scanning between 200 and 320 nm of soy oil exposed for 30 min at 254 nm UV radiation

Table 3
ABSORPTIVITIES AT 232 nm AND 268 nm OF SOY OIL ADDED WITH BHT, BHA AND SEA BUCKTHORN POLYPHENOLS IN DIFFERENT CONCENTRATIONS, AFTER EXPOSURE AT 254 nm UV RADIATION FOR 30 min

Sample	A_{232nm}	A_{268nm}
Control	0.714	0.137
BHT 200 ppm	0.704	0.136
BHA 200ppm	0.603	0.123
Polyphenols 50 ppm	0.626	0.121
Polyphenols 100 ppm	0.544	0.114

Table 4
SECOND DERIVATIVE SPECTROSCOPY. MINIMA AT 233 nm AND 242 nm OF SOY OIL ADDED WITH BHT, BHA AND SEA BUCKTHORN POLYPHENOLS IN DIFFERENT CONCENTRATIONS, AFTER EXPOSURE AT 254 nm UV RADIATION FOR 30 min

Sample	Minima at 233nm	Minima at 242nm	*cis,trans/trans,trans* ratio
Control	-9.47	-0.00030	$3.16x10^{-5}$
BHT 200 ppm	-5.84	-0.00027	$4.62x10^{-5}$
BHA 200 ppm	-3.03	-0.00024	$7.92x10^{-5}$
Polyphenols 50 ppm	-2.42	-0.00018	$7.43x10^{-5}$
Polyphenols 100 ppm	-3.07	-0.00015	$4.88x10^{-5}$

The results showed in table 4 indicate the fact that the lowest value of *cis,trans/trans,trans* CD ratio was recorded for control, while the highest *cis,trans/trans,trans* CD ratio was recorded for soy oil treated with BHA synthetic antioxidant. These values indicate the highest concentration of *trans/trans* conjugated dienes in control and the lowest concentration of *trans/trans* conjugated dienes in soy oil samples treated with BHA synthetic antioxidant. In case of soy oil samples treated with polyphenols extracted from sea buckthorn, the value of *cis,trans/trans,trans* CD ratio was higher for soy oil samples treated with 50 ppm polyphenols comparatively with soy oil samples treated with 100 ppm polyphenols. These observations are in concordance with the obtained peroxide values and para-anisidine values and they demonstrate the fact that the presence of some photosensitizers in sea buckthorn alcoholic extract can manifest a negative effect of soy oil oxidation at UV exposure.

Conclusions

The polyphenols extracted from sea buckthorn protected soy oil against thermal oxidation after exposure at 62°C for 8 days. The concentration of 100 ppm sea buckthorn polyphenols determined the decrease of peroxide values and para-anisidine values after a pattern similar to BHT synthetic antioxidant. At this concentration, sea buckthorn polyphenols induced the increase of *cis,trans/trans,trans* conjugated dienes ratio.

Also, the polyphenols extracted from sea buckthorn protected soy oil against oxidation induced by the exposure at 254 nm UV radiation. The protective effect of 50 ppm polyphenols was similar to the one manifested by 100 ppm polyphenols. After the exposure of soy oil to 254 nm UV radiation for 30 min, for the concentration of 100 ppm polyphenols it was recorded a lower level of *cis,trans/trans,trans* conjugated dienes ratio comparatively with the concentration of 50 ppm polyphenols.

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