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ANTIOXIDANT PROPERTIES OF SOME PLANT EXTRACTS AND EFFECT OF THEIR ADDITION ON THE OXIDATION STABILITY OF BIODIESEL

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Abstract. The extracts from the leaves of *Deschampsia antarctica* É. Desv., *Camelina sativa* (L.) Crantz, and *Camellia japonica* L. plants, as well as from defatted *Camelina sativa* and *Silybum marianum* seedcakes were investigated as potential additives for improvement of biodiesel stability against oxidation. Composition of the extracts was studied by means of HPLC, and antioxidant properties were evaluated using the Folin-Ciocalteu assay and the DPPH test. The oxidation of biodiesel was monitored during the accelerated procedure at 43°C, with the changes in the acid number of biodiesel samples being the criteria of this process. In spite of significant distinctions in the content of various phenolic compounds, all the extracts were found to possess high antioxidant activity and decelerate biodiesel oxidation by 9-26%. The data did not reveal a directly proportional relationship between the antioxidants content in the extract, on the one hand, and the enhancement in biodiesel stability, on the other hand; various extracts had different influence on the behaviour of biodiesel from rape and *Camelina* seed oils. The results obtained are consistent with the assumption that there is no universal stabilizer for different types of biodiesel and indicate the prospects on searching for novel antioxidants of natural origin to inhibit oxidative processes.

Keywords: plant extract, phenolic compound, antioxidant, biodiesel, oxidation stability.

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Introduction

Antioxidants (AOs) of natural origin are an important object of current research aimed at solving a number of problems in various fields of human activity [1]. Many of plant-derived phenols/polyphenols have high antioxidant properties and low toxicity, so the main field of their applications is herbal medicine and pharmacology [2]. Besides, plant extracts or individual AOs, available in the extracts, can be used, for example, for food preservation, in veterinary, in various processes of "green" synthesis, and, in general, in all the cases where there is a need to inhibit the oxidation of individual compounds or products [3-5].

The oxidation stability of biodiesel (BD) is an important property that affects the storage and performance of this fuel [6]. The use of AOs, *i.e.* the compounds capable to prevent or inhibit the oxidation of the principal components of BD (fatty acid esters, FAEs), is a common approach to improve biodiesel stability [7]. The data from the literature suggest that there are no universal AOs for different types of BD, so the search for new AOs, which can increase the storage stability of FAEs, remains an important task [8]. Up to now, synthetic AOs were mainly used for improving the BD stability, such as butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroquinone, propyl gallate, and ascorbyl palmitate [9,10]. However, the search for novel AOs of natural origin seems to be promising, as well [11-14]. Whole plants, their parts and remains may be used for AOs extraction, with the plant waste being especially attractive raw materials from an economic and

environmental point of view [15,16]. For example, the vegetable oil production wastes, in particular defatted seeds (seedcake), may be used for further extraction of AOs and other useful compounds.

In the previous studies, the antioxidant properties of extracts from a wide range of plants, both ordinary and more exotic, such as orchids, camellia, magnolia, and Antarctic hair grass were screened [17-19]. The extracts obtained from the plants grown *in situ* and from the same plants grown *in vitro* were also compared and it was found that in some cases such extracts possessed similar antioxidant properties in spite of significant distinction in the contents of various phenols. Among all the plants studied, a set of the promising ones was chosen for further investigations.

The aim of this work was to study the phenolic compounds available in *Deschampsia antarctica*, *Camelina sativa*, *Camellia japonica*, and *Silybum marianum* plants; to evaluate the antioxidant properties of plant extracts; to examine the possibility of using the extracts of these plants as inhibitors of biodiesel oxidation. The subject of the study may be of practical interest related to the production of efficient antioxidants of natural origin.

Experimental

Materials

All solvents, chemical compounds and reagents such as acetonitrile, ethanol, methanol, phosphoric acid, gallic, salicylic, vanillic, protocatechuic, *p*-hydroxybenzoic, syringic, *α*-resorcylic, *β*-resorcylic, *γ*-resorcylic, cinnamic, *o*-coumaric, *m*-coumaric, *p*-coumaric, caffeic, ferulic, sinapic, feruloylquinic, chlorogenic and fertaric acids, catechin, epicatechin, epigallocatechin gallate, ellagic acid, apigenin, quercetin, quercetin 3-*O*-glucoside, rutin, kaempferol, kaempferol 3-*O*-arabinoside, kaempferol 3-*O*-glucoside, vitexin, luteolin, orientin, 3-*O*-methyl quercetin, ethyl esters of oleic, stearic, linoleic, linolenic, and palmitic acids, 2,2-diphenyl-1-picrylhydrazyl (DPPH), the Folin-Ciocalteu reagent, were obtained from commercial sources (Merck, Germany) and used without further purification.

Samples of biodiesel BD1 (from rape seeds) and BD2 (from *Camelina* seeds) were obtained from the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of NAS of Ukraine.

Camellia japonica plants were grown in greenhouse of M.M. Gryshko National Botanic Garden of NAS of Ukraine. *Deschampsia antarctica* É. Desv., *Camelina sativa* (L.) Crantz plants were cultured *in vitro*. In the case of *Camelina sativa*, sterilized seeds were placed in glass flasks containing Murashige and Skoog basal medium [20] and exposed to artificial light of 2000 lx for 16 hours per day at temperature of 22-26°C and humidity of 70%. A sterile culture of *D. antarctica* was grown on solid agar medium based on Gamborg B5 medium [21]; the growing plants were exposed to artificial lighting of 3000-3500 lx for 16 hours per day at temperature of 13-18°C and humidity of 65-70%. Biowastes of vegetable oil production from *Camelina sativa* and *Silybum marianum* seeds (seedcakes) were obtained from a local company, which produces *Camelina* and *Silibum* seed oils in Kyiv (Ukraine).

Methods

Extraction procedures

The extracts were prepared from various types of plant raw materials using the following extraction procedures. To prepare the extracts of *D. antarctica*, the aerial parts of the plants were used. These plants were frozen to -20°C, then thoroughly ground and poured with methanol at a ratio of plant material to methanol of 1 g per 10 mL. The extraction was performed by maceration for 24 hours.

In the case of *C. japonica* and *C. sativa* plants, 100 mL of 96% ethanol were added to 1 g of finely chopped leaves, after that the mixtures were placed into the steam bath for 30 min. After cooling, the extracts were adjusted to the initial volume and filtered.

In the case of wastes from vegetable oil production, 10 g of grinded seedcake were additionally defatted using Soxhlet apparatus and hexane as extraction solvent. Then active compounds were extracted from defatted seedcake using 500 mL 96% ethanol. A rotary evaporator was used to decrease the volume of obtained extract down to 200 mL. To evaluate the effect of AOs concentration on BD oxidation stability, 4-fold concentration of extract from *C. sativa* seedcake was also performed *via* the solvent evaporation.

High performance liquid chromatography (HPLC) analysis

The composition of plant extracts and biodiesel samples was monitored by HPLC using an Agilent 1100 system with a diode array detector. The separations were carried out on the chromatographic column Poroshell 120 EC-C18 (150 mm×2.1 mm, 2.7 μm).

In the case of extracts from defatted seedcakes as well as from leaves of *C. japonica* and *C. sativa* the following gradient composition was used for each analysis: 0÷3 min – 89% A + 11% B at the flow rate 0.12 mL/min, 33 min – 34% A + 66% B at the flow rate 0.12 mL/min, 63 min – 0% A + 100% B at the flow rate 0.12 mL/min, 70 min – 0% A + 100% B at the flow rate increased to 0.35 mL/min, where A was aqueous solution of 0.05 M H_3PO_4 and B was methanol. The injection volume was 2 μL, initial column temperature was 20°C and it increased to 40°C from 40 min. Detection was performed at the wavelengths of 206, 254, 300, 350, and 450 nm.

In the case of extracts from *D. antarctica* the following gradient composition was used for each analysis: 0÷2 min – 99% A + 1% C at the flow rate 0.2 mL/min, 5 min – 90% A + 10% C at the flow rate 0.2 mL/min, 40 min – 60% A + 40% C at the flow rate 0.2 mL/min, 50 min – 30% A + 70% C at the flow rate 0.2 mL/min, 55 min – 1% A + 99% C at the flow rate 0.2 mL/min, 65 min – 1% A + 99% C at the flow rate increased to 0.6 mL/min, where A was aqueous solution of 0.05 M H_3PO_4 and C was acetonitrile. The injection volume was 5 μL, initial column temperature was 20°C and its increased to 40°C from 45 min. Detection was performed at the wavelengths of 206, 254, 300, 350, and 450 nm.

In the case of biodiesel fuels, the following gradient composition was used for each analysis: 0÷5 min – 30% A + 70% B at the flow rate 0.12 mL/min, 10 min – 0% A + 100% B at the flow rate 0.12 mL/min, 30 min – 0% A + 100% B at the flow rate increased to 0.5 mL/min, where A was aqueous solution of 0.05 M H_3PO_4 and B was methanol. The injection volume was 2 μL, the column temperature was 40°C. The detection was performed at the wavelength of 206 nm.

Individual compounds or classes of phenolic compounds available in the extracts were identified *via* comparing the retention time and UV–Vis spectra for respective signals with those for standard substances, such as gallic, salicylic, vanillic, protocatechuic, *p*-hydroxybenzoic, syringic, *α*-resorcylic, *β*-resorcylic, *γ*-resorcylic, cinnamic, *p*-coumaric, *m*-coumaric, *o*-coumaric, caffeic, ferulic, sinapic, feruloylquinic, chlorogenic and fertaric acids, catechin, epicatechin, epigallocatechin gallate, ellagic acid, apigenin, quercetin, quercetin 3-*O*-glucoside, rutin, kaempferol, kaempferol 3-*O*-arabinoside, kaempferol 3-*O*-glucoside, vitexin, luteolin, orientin, 3-*O*-methyl quercetin. Signals in chromatograms of BD samples were assigned with those in chromatograms for standards of ethyl esters of oleic, stearic, linoleic, linolenic, and palmitic acids. To estimate the quantity of phenols of various classes in the extracts, the integral intensities of respective signals were compared with those for reference substances (chlorogenic acid – for hydroxycinnamic acids, gallic acid – for hydroxybenzoic acids, rutin – for glucosides of flavones/flavonols, neohesperidin – for flavanones, catechin – for catechins, epigallocatechin gallate – for epigallocatechin gallate derivatives, ellagic acid – for ellagic acid derivatives). The amount of esters in initial and oxidized BD was estimated as integral intensity of all the peaks in chromatograms attributable to FAEs (using ethyl esters of oleic, stearic, linoleic, linolenic, and palmitic acids standards).

The content of the compounds in the samples is given in mean values ± SD for $p<0.05$.

Evaluation of antioxidant properties of the extracts

The antioxidant activity of extracts was evaluated using the reaction with 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical, according to the method described in detail elsewhere [22]. To prepare the reaction mixture, 1 mL of extract was poured into glass, followed by the addition of 2 mL of 70% ethanol and 2 mL of 0.15 mM $DPPH^{\bullet}$. The change in concentration of stable radicals in the mixtures during the reaction was determined from the change in absorption maximum at 520 nm for tested solutions as compared to the absorption value for the control solution. To prepare the control solution, 3 mL of 70% ethanol were mixed with 2 mL of 0.15 mM $DPPH^{\bullet}$ solution.

The antioxidant properties of the extracts were also tested using the Folin-Ciocalteu method. The total phenolic index was determined as follows: 9 mL of distilled water, 1 mL of Folin-Ciocalteu reagent, 4 mL of 20% sodium carbonate solution and 5 mL of distilled water were consecutively added to 1 mL of tested extract. The solution was stirred for 30 min in the dark, then the optical density at 750 nm (D_{750}) was measured and the total phenolic index was calculated [23].

UV-Vis spectra of solutions and reaction mixtures were recorded using a Lambda 35 spectrophotometer (Perkin Elmer, USA) in the wavelength range of 200÷800 nm.

Analysis of the biodiesel oxidation stability

The oxidation of BD was examined using the accelerated procedure at 43°C [24]. Glasses with a weight of 100 g of pure BD or with

100 g of biodiesel and 1 mL of extract (BD+AOs sample) were placed in an oven with air access and kept at a temperature of 43°C during 14 weeks (each week at 43°C tentatively corresponds to 1 month of fuel storage under normal conditions). Once a week, samples were taken out of the furnace and the acid number (AN) was determined by the titrometric method with visual indication [25]. Briefly, to measure AN, 5 g of the BD or BD+AOs samples were placed into glass, then 50 mL of a neutralized alcohol-chloroform mixture were added to the BD sample and then the mixture was quickly titrated with a 0.1 M potassium hydroxide solution under constant stirring until a stable within 30 s pink colour appeared. To prepare 50 mL of neutralized alcohol-chloroform mixture, equal parts of chloroform and ethyl alcohol were used with the addition of 5 drops of a 1% alcoholic solution of phenolphthalein. The mixture was neutralized with a 0.1 M potassium hydroxide solution to a faint pink colour.

The AN value was calculated using the Eq.(1) [25].

$$AN = \frac{5.61V}{M} \quad (1)$$

where, 5.61 is the amount of KOH contained in 1 mL of 0.1M solution, mg/mL;
V is the volume of 0.1M KOH used for the neutralization of free fatty acids, mL;
M is the weight of the sample taken for analysis, g.

The average AN values and the standard deviations were derived from triple measurements (p<0.05).

The change in AN of BD samples was used as a criterion of BD oxidation. The effect of antioxidants on BD stability was evaluated by comparing the AN values for oxidized BD samples stored with and without active additives ($(BD+AOs)_{ox}$ and BD_{ox} samples, respectively) [25]. The decrease in AN for the $(BD+AOs)_{ox}$ samples was calculated as follows (Eq.(2)).

$$\text{Decrease in AN} = \frac{AN(BD_{ox}) - AN(BD+AOs)_{ox}}{AN(BD_{ox})} 100\% \quad (2)$$

where, $AN((BD+AOs)_{ox})$ and $AN(BD_{ox})$ are AN for $(BD+AOs)_{ox}$ and BD_{ox} samples, respectively.

To characterize the effect of AOs addition on the oxidation behaviour of biodiesel, the ratio of the total amount of esters (Q_{FAEs}) preserved in $(BD+AOs)_{ox}$ sample as compared to BD_{ox} sample after oxidation in the presence and in the absence of AOs were also calculated for several samples. The decrease in consumption of FAEs in $(BD+AOs)_{ox}$ samples was calculated as follows (Eq.(3)).

$$\text{Decrease in consumption of FAEs} = \frac{Q_{FAEs}((BD+AOs)_{ox} - Q_{FAEs}(BD_{ox})}{Q_{FAEs}(BD_{ox})} 100\% \quad (3)$$

where, Q_{FAEs} ($(BD+AOs)_{ox}$) and Q_{FAEs} (BD_{ox}) are integral intensities of all the peaks attributed to FAEs in the chromatograms of $(BD+AOs)_{ox}$ and BD_{ox} samples, respectively.

Results and discussion

In order to determine the phenolic constituents of plant extracts, HPLC studies were carried out. The examples of chromatograms (for *C. japonica* and *D. antarctica* extracts) are shown in Figures 1 and 2. The designations for the compounds or the groups of compounds identified in the extracts are given in the legends for Figures 1 and 2. The results of quantitative analysis of the composition for all the extracts under study are presented in Table 1. As the extracts were found to contain a large number of various phenols, the data presents only the amounts of phenolic compounds grouped by different subclasses, such as hydroxycinnamic acids, flavones, flavanones, catechins, etc.

The results (Table 1) show that all the extracts contain a significant amount of phenolic AOs, while the quantity of various phenols in the extracts differs from each other. Phenolic (hydroxybenzoic and hydroxycinnamic) acids and flavones/flavonols, as well as the derivatives of these compounds, predominate in the extract from *D. antarctica* and in all the extracts from *C. sativa*, with luteolin and quercetin being the most abundant aglycones for *D. antarctica* (see Figure 1) and *C. sativa* extracts (the chromatogram is not shown), respectively. For the extracts from the plants grown *in vitro*, the ratio of phenolic acids to flavonoids is higher than that for the extract from the plants growing in nature, and that for the ones from the defatted seedcakes (Table 1). For *C. japonica* extract, hydroxycinnamic acids are not detected, and flavones/flavonols are found in little quantity, while hydroxybenzoic acids are the main phenolic acids and catechins are the main flavonoids. The highest number of various phenolic compounds was registered in the extract from the defatted *S. marianum* seedcake, with the flavanones or their derivatives being the most abundant flavonoids.

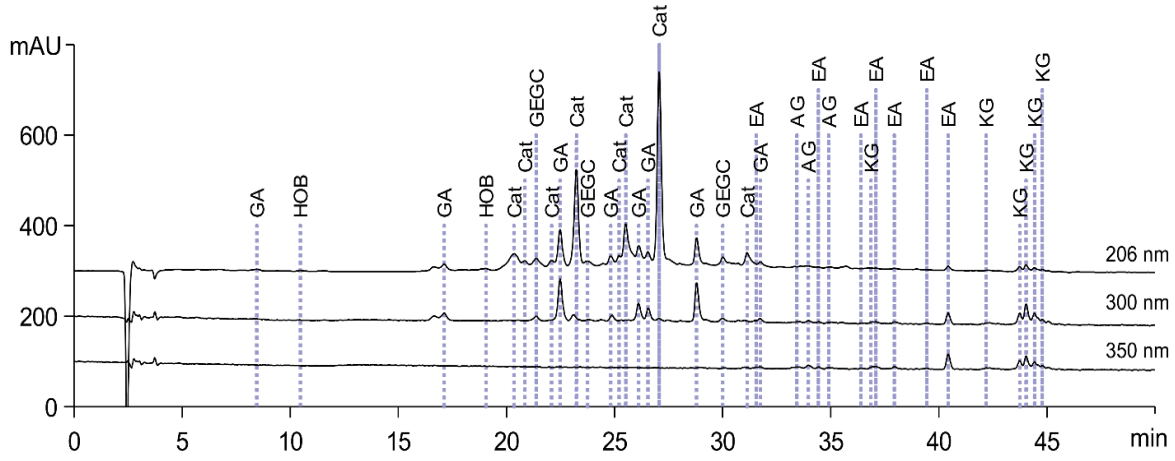


Figure 1. Fragments of chromatograms for the extracts from *C. japonica* plants. Identified compounds/groups of compounds: HOB – hydroxybenzoic acids/simple phenols and their derivatives, HOC – hydroxycinnamic acids and their derivatives, GA – gallic acid derivatives, EA – ellagic acid derivatives, Cat – catechins, GEGC –epigallocatechin gallates, KG – kaempferol glycosides, AG – apigenin glycosides.

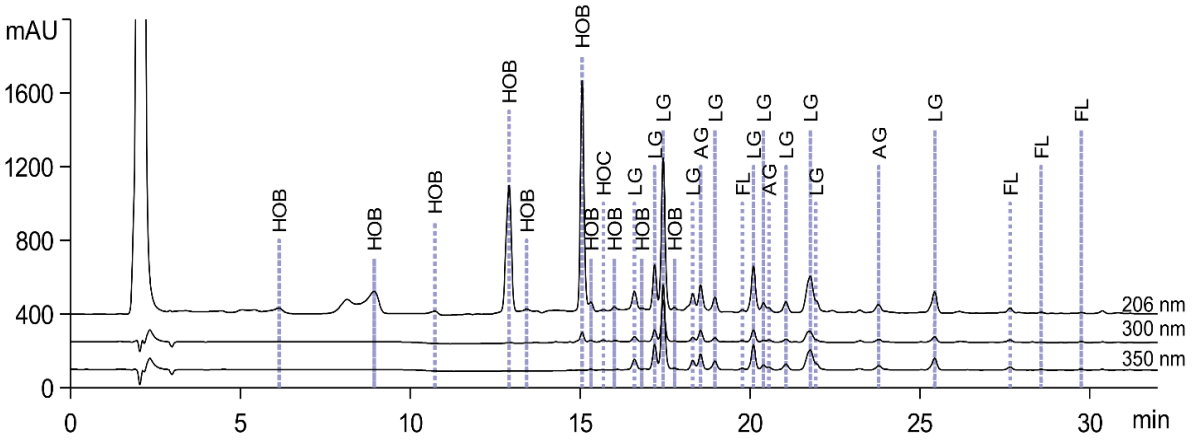


Figure 2. Fragments of chromatograms for the extracts from *D. antarctica*. Identified compounds/groups of compounds: HOB – hydroxybenzoic acids/simple phenols and their derivatives, HOC – hydroxycinnamic acids and their derivatives, LG – luteolin glycosides, AG – apigenin glycosides, FL – other flavonoids.

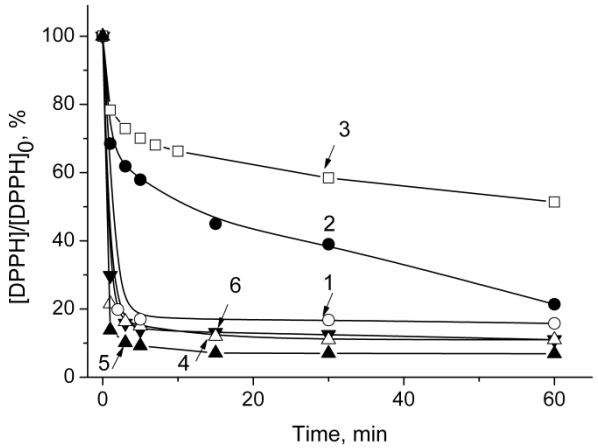
Table 1

The content of various phenols and total phenolic index for the studied extracts.

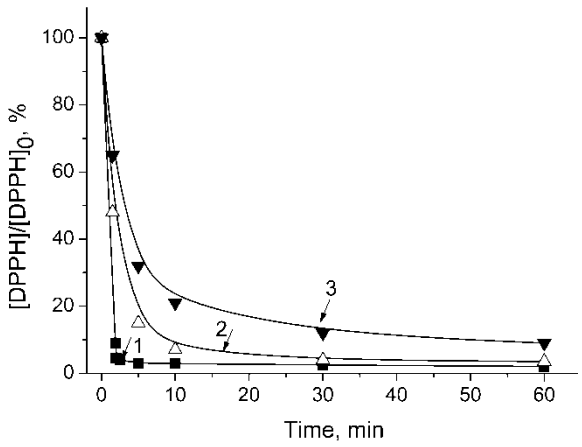
Extract	*Total phenolic index*	*Hydroxybenzoic acids, mg/L*	*Hydroxycinnamic acids, mg/L*	*Flavones/ Flavonols, mg/L*	*Flavanones, mg/L*	*Catechins, mg/L*	*Sum of phenols, mg/L*
C. japonica, in situ	6.6	186±9	n.d.	40±4	n.d.	196±18	422±31
D. antarctica, in vitro	6.6	171±8	8.0±0.4	136±12	n.d.	n.d.	315±20
C. sativa, in vitro	0.4	27.0±1.3	4.00±0.18	4±0.39	n.d.	n.d.	35.0±1.9
Defatted *C. sativa* seedcake	4.2	122±6	176±8	566±52	n.d.	41±4	905±70
Defatted *S. marianum* seedcake	10.8	10.0±0.5	6.00±0.32	12.0±1.1	585±57	n.d.	613±59

n.d. = not detected. SD was calculated for $p<0.05$

Figure 3*(a)* presents the data on the DPPH radicals inhibition by the extracts mentioned in Table 1 as well as by the 4-fold concentrated extract from defatted *C. sativa* seedcake. The antioxidant/reducing properties of the extracts were also studied using the Folin-Ciocalteu method; the total phenolic index values are presented in Table 1. The results show that 5 out of 6 extracts are characterized by a high value of total phenolic index and by a very high ability to inhibit DPPH radicals. Because almost instantaneous disappearance of the colour of the DPPH solution was observed while using these initial extracts in the DPPH test, the extracts had to be diluted by 10 times before the testing in order to register the reaction kinetics.



(a)



(b)

Figure 3. Time dependence of DPPH radicals inhibition: for the extracts from plants of *C. japonica* (1), *D. antarctica* (2), and *C. sativa* (3), from defatted seedcakes *of C. sativa* (initial (4) and 4-fold concentrated (5)) and *S. marianum* (6). All the extracts, except extract (3), were diluted by 10 times before measurements *(a)*; for 1 mM solutions of ascorbic acid (1), quercetin (2), and rutin (3) *(b)*.

Only the extract from *C. sativa* plants grown *in vitro* had comparatively low activity in the reaction, although even in this case the inhibition of 40% of DPPH radicals by undiluted extract was observed. For comparison, Figure 3*(b)* shows the data on the DPPH radicals inhibition for some standard AOs, such as ascorbic acid, quercetin and rutin.

The comparison of DPPH test results with the data on the extracts composition (Table 1) shows that there is no direct relation between the antiradical activity of the extracts and AOs concentration or total phenolic index. For example, the antiradical activity of the original and concentrated extracts from defatted *C. sativa* seedcakes (curves 4 and 5, Figure 3) is almost the same despite a 4-fold difference in AOs content. Apparently, this is because of excess of AOs already present in the initial extract, so the increase in AOs content does not lead to further increase in antioxidant activity. Extracts from the plants grown *in vitro* differed from the others by slower kinetics of DPPH inhibition (curves 2 and 3, Figure 3). According to the previous data, such behaviour is typical for most extracts from the plants grown *in vitro* and is probably caused by relatively high content of hydroxybenzoic and hydroxycinnamic acids [18].

Figure 4 shows the chromatograms of two types of biodiesel, BD1 and BD2, before *(a,b)* and after *(c-f)* accelerated oxidation at 43°C for 14 weeks. The main E1, E2, E3 peaks in the chromatograms of both initial biodiesels can be attributed to linolenic, linoleic and oleic ethyl esters, respectively; the chromatogram of BD2 sample also contain minor E4-E6 signals, probably originated from other ethyl esters of fatty acids, such as palmitic, arachidic, and erucic acids, typical for *C. sativa* oil [26]. For oxidized $BD1_{ox}$ and $BD2_{ox}$ samples, the decrease in the intensity of E1-E3 peaks or even disappearing of E1 peak is observed (see Figure 4*(c)* for $BD1_{ox}$ sample), while new signals from the products of FAEs oxidation were detected in the chromatograms. The oxidation products can be divided into two groups: relatively more hydrophilic compounds with a retention time much lower than that for the initial FAEs (O1 signals), and the compounds with a retention time close to that for the initial FAEs (O2 signals). The chromatograms of biodiesels oxidized in the presence of extracts $((BD1+AOs)_{ox}$ and $(BD2+AOs)_{ox}$ samples, Figures 4*(e-f)* are of the same profile, but differed in the intensity of the signals of the initial FAEs

and their oxidation products. Quantitative estimation of the total amount of FAEs preserved in biodiesels after oxidation showed that in the presence of AOs the consumption of esters during oxidation was decreased. The respective values for several samples are given in Table 2.

The FAEs oxidation is known to occur *via* both the hydrolysis of initial esters (to produce free fatty acids) and the oxidation of unsaturated sites of hydrocarbon chain, followed by the chain scission and the formation of various hydrophilic groups at the scission sites with possible eventual transformation into carboxylic groups [27,28]. The presence of O1 and O2 groups of the signals with different retention times in the chromatograms of oxidized biodiesels presumably corresponds to the formation of two groups of the BD oxidation products with the molecules of different chain length.

Table 2

Some characteristics of the extracts and their effect on the oxidative behaviour of biodiesel (BD).

Extract	*Total phenolic index*	*Antioxidants content in biodiesel, ppm*	*Decrease in acid number for oxidized biodiesel due to the extract addition, %*		*Decrease in consumption of fatty acid esters due to the extract addition, %*	
			BD1	BD2	BD1	BD2
C. japonica, in situ	6.6	422	17±2	9±1	8±1	4±1
D. antarctica, in vitro	6.6	315	14±1	20±2		
C. sativa, in vitro	0.4	35	10±1	11±1		
Defatted *C. sativa* seedcake	4.2	905	12±1	18±2	8±1	15±2
Defatted *C. sativa* seedcake, 4-fold concentrated	17.0	3620	11±1	19±2		
Defatted *S. marianum* seedcake	10.8	613	17±2	26±2	10±1	16±2

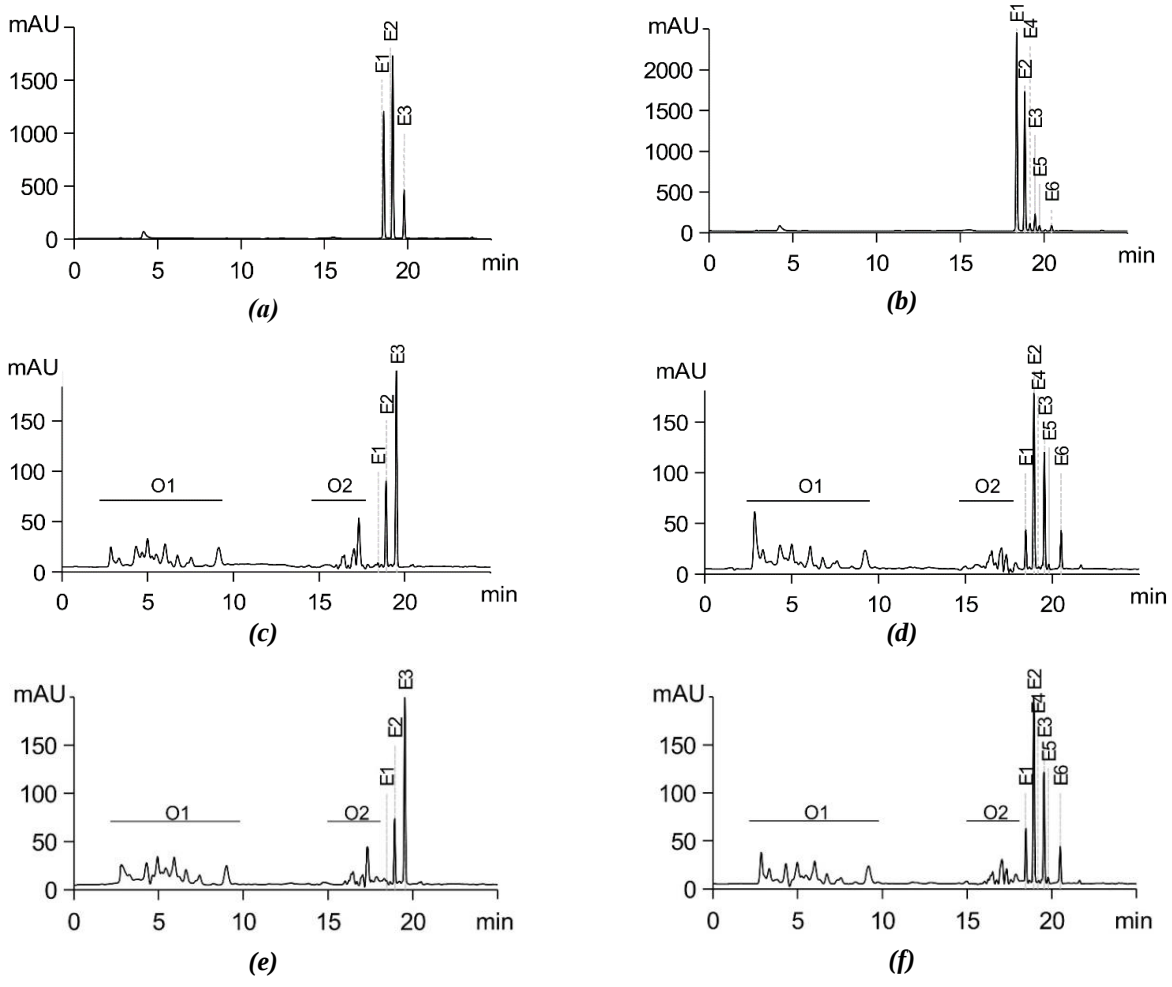


Figure 4. Chromatograms for initial BD1 *(a)* and BD2 *(b)* biodiesels, for oxidized $BD1_{ox}$ *(c)* and $BD2_{ox}$ *(d)* samples, and for oxidized $(BD1+AOs)_{ox}$ *(e)* and $(BD2+AOs)_{ox}$ *(f)* samples.

The formation of carboxylic groups through both the oxidation pathways mentioned above leads to an increase in AN of BD. The dependence of AN values on the time of oxidation for initial BD samples and for the BD+AOs biodiesels with added extracts from *C. japonica* and *D. antarctica* plants and from defatted *S. marianum* seedcake is shown in Figure 5. As one can see from Figure 5, a relatively slow BD oxidation occurred during the first 4 weeks of keeping at 43°C, while a significant acceleration of the oxidation process was observed during next 10 weeks. The results in Figure 5 demonstrate that the addition of extracts inhibits the biodiesel oxidation, which appears as a relative decrease in the AN values for $(BD+AOs)_{ox}$ samples in comparison with BD_{ox} ones. The decrease in AN values for all six extract additives is shown in Table 2.

As one can see from the Figure 5 and the Table 2, all extracts indeed decelerate the BD oxidation, since the AN value for oxidized samples is decreased by 9-26%. These results correlate with HPLC data on the higher (by up to 16%) FAEs content in BD samples oxidized in the presence of AOs. However, there was no direct correlation between the AN value decrease and phenol content increase (Table 2). For instance, the 4-fold concentrated extract from defatted *C. sativa* seedcake did not improve the resistance of BD against oxidation in comparison to the non-concentrated extract. Probably, there is a limit in the efficiency of inhibition of FAEs oxidation by AOs, so that a further increase in the AOs concentration does not increase the BD stability [8].

The extract from defatted *S. marianum* seedcake appeared to be the most effective additive for inhibition of BD oxidation, with the higher effect being observed for BD2 biodiesel. On average, the addition of this extract leads to a decrease in the AN value by 17% and 26% for BD1 and BD2 samples, respectively. Being added to diesel samples in concentration of about 600 ppm, this extract showed a stabilizing effect, commensurable with the effect of 1000 ppm of *α*-tocopherol in soybean-oil-based biodiesel (where the decrease in AN value by about 20% was observed [10]). The extract from *D. antarctica* and all the extracts from *C. sativa* also have more pronounced influence on the oxidation of BD2 biodiesel. The least distinction in the effect of the additive on the two types of BD was observed for extract from *C. sativa* plants grown *in vitro* (Table 2). The *C. japonica* extract, in contrast to all other additives, had a greater influence on BD1 biodiesel: AN for the oxidized sample was decreased by 17%.

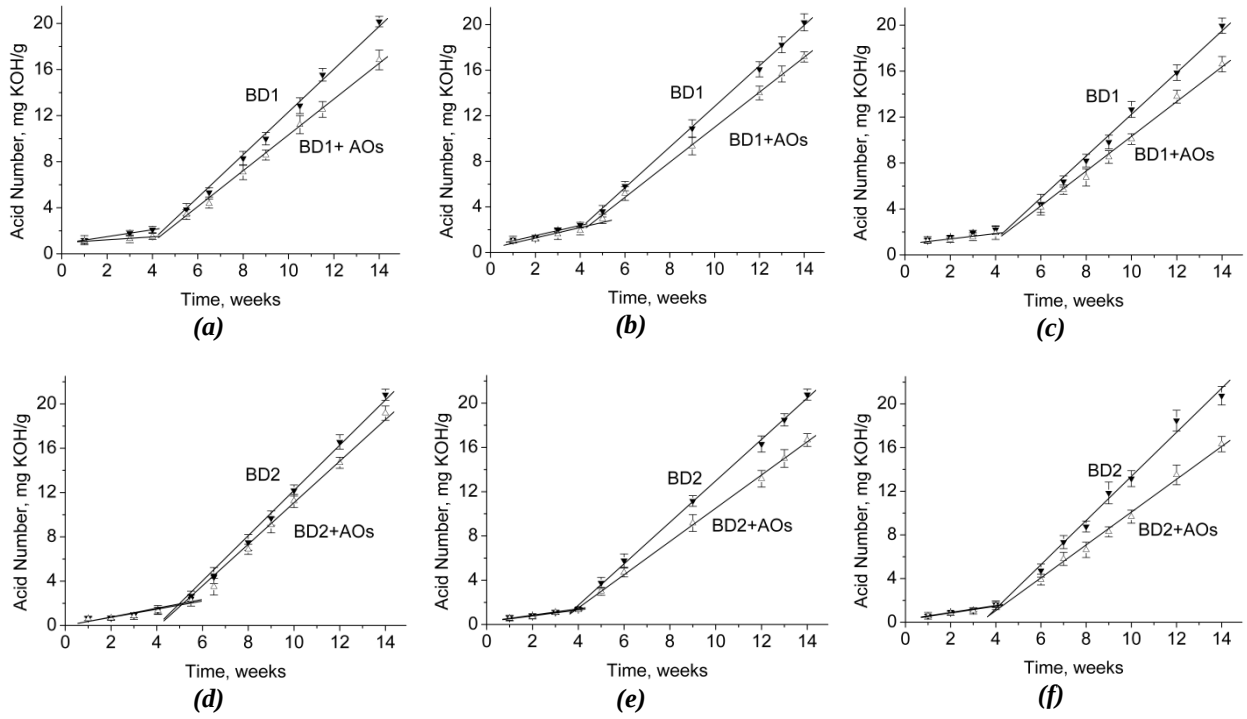


Figure 5. Acid number for BD and BD+AOs samples *vs* time of oxidation at 43°C. AOs are extracts from *C. japonica (a, d)* and *D. antarctica (b, e)* plants and from defatted *S. marianum* seedcake *(c, d)*.

The different effect of the active additives on the oxidation of BD1 and BD2 biodiesels is probably associated with various compositions of the extracts. According to HPLC data, the characteristic feature of *C. japonica* extract is the absence of hydroxycinnamic acids and prevalence of catechins among the flavonoids. The peculiarity of the extracts from the plants grown *in vitro* is a relatively high amount of low-molecular-weight phenols, such as phenolic acids. The different influence of various extracts on the BD1 and BD2 samples oxidation is in agreement with the assumption that there is no universal stabilizer for different types of biodiesel [8].

Conclusions

The composition and antioxidant properties of the extracts from the leaves of *Camellia japonica* L., *Deschampsia antarctica* É. Desv., and *Camelina sativa* plants grown *in situ* or *in vitro*, as well as of the extracts from the defatted seedcakes of *Silybum marianum* and *Camelina sativa*, remaining after the production of vegetable oil, were studied**.** For the first time, the effect of these extracts as additives to inhibit the oxidation of biodiesels from rape and *Camelina* seed oils was evaluated.

All the extracts were found to effectively scavenge DPPH radicals (45-95% for 60 min) and decelerate the transformation of fatty acid esters into organic acids by 9-26%. The antioxidants of extract from defatted *S. marianum* seedcake, being added to diesel samples in a concentration of about 600 ppm, showed the best stabilizing effect towards the two types of biodiesel (17 and 26% respectively for biodiesel from rape and *Camelina* seed oil) and appeared to be a promising additive to improve resistance of the fuel against oxidation.

Various extracts were found to have different influence on the oxidation stability of biodiesels from rape and *Camelina* seed oils. The extract from *C. japonica*, which contained mainly catechins and hydroxybenzoic acids (196 and 186 mg/L, respectively), affected in the most prominent way the stability of biodiesel from rapeseed oil. All other extracts, containing higher amounts of hydroxycinnamic acids (up to 176 mg/L) and flavones, flavonols or flavanones (up to 566 mg/L) were more effective in stabilization of biodiesel from *Camelina* seed oil. The obtained results are consistent with the assumption that there is no universal stabilizer for different types of biodiesel and indicate the prospects on searching for novel antioxidants of natural origin to inhibit oxidative processes.

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