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Stability of rapeseed oil with horseradish *Amorica rusticana* L. and lovage *Levisticum officinale* L. extracts under medium temperature accelerated storage conditions

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Abstract. This study examined the antioxidant activity of horseradish leaves and lovage leaves and stems extracts added to crude rapeseed oil, under medium temperature accelerated storage conditions. To evaluate efficiency of plant extracts they were added to oil in different concentrations (0.25, 0.5, 1.0 and 1.5%). As a control rapeseed oil without extracts where analysed. For comparison 0.01% butylatedhydroxytoluene (BHT) were added to oil. Efficiency of extracts in oil where tested at +60 ± 1°C in the dark for 22 days. For all samples peroxide value, acid value and 2,2-diphenyl-1-picrylhydrozyl (DPPH•) activity were determined. In all steps of the experiments for samples with extract peroxide value was significantly ($P < 0.05$) lower comparing to the control. The control sample without extract reached 15meq O_2 kg^{-1} oil (maximal allowed value in Latvian legislation) in 3 days. The best results showed the horseradish leaves extract (1%) and the lovage leave extract (1.5%) reaching this value in 8.3 days and 7 days, respectively. DPPH• activity of the oil was compared after 3 days (when blank sample reached maximal allowed a peroxide value) and it shown that for all samples it was higher compared to the control sample. The highest activity showed the samples with horseradish leave extracts. A acid value in oil samples changed slightly. Lovage leave as stem and horseradish leave extracts could be successfully used for retarding of oxidation of rapeseed oil and in further experiments their activity in meat products will be tested.

Key words: horseradish, lovage, extract, rapeseed oil, oxidation.

INTRODUCTION

Antioxidants are used as additives in food for centuries. They are mainly used to prevent accumulation of the free radicals, strengthening the food oxidative stability (Halliwell, 1995; Giese, 1996). From the viewpoint of the nutrition science and the production benefits, it is highly desirable to control the oxidation by the addition of the antioxidants, providing appropriate food quality. Antioxidants are added to foods to control lipid oxidation and reduce the free radical concentration in human diet, as well as the consumption of the free radicals into the body (Pokorny, 2007).

In the recent years are a growing interest in plant origin supplements (Brielman et al., 2006; Naczk & Shahidi, 2006), and efforts have been dedicated to the research for finding the new sources of antioxidants, that could work as effectively as synthetic ones (Michiels et al., 2012). Substances occurring in nature could be used for enrichment of

foods and creation of functional foods that could prevent many diseases (Crozier et al., 2006). The phenolic compounds are widely distributed class of the biologically active substances in the plants that are known to be very effective natural antioxidants (Shahidi & Wanasundara, 1992; Tapeiro et al., 2002; Shahidi & Naczk, 2004).

The horseradish (*Armoracia rusticana* L.) and the lovage (*Levisticum officinale* L.) are perennial plants which belong to *Brassicaceae* and *Umbelliferae* families, respectively and are cultivated in temperate climate zone. For the horseradish the main culinary value has for roots due to its particularly pungent flavour (Raghavan, 2000). But for the lovage all plant parts (seeds, leaves, stems and roots) are strongly aromatic with a characteristic earthy, celery-like flavour and smell (Szebeni-Galambosi et al., 1992). Both plants contain the compounds that can act as natural antioxidants (Raghavan, 2000).

Since the phenolic compounds have antioxidant and anti-microbial properties, they can be used for the technological purposes as an alternative to the synthetic food additives. The foods phenol compounds show a protective and stabilizing effect on the lipids, the food colors and the flavors (Kammerer et al., 2011). The anticarcinogenic, antithrombotic, anti-inflammatory, antimicrobial, antidiabetic and antioxidant properties of the phenolic compounds are widely described, making these compounds very valuable. Structural diversity of the phenolic compounds is in accordance with the differences in their biofunctional properties, thus, the plants extracts differing in their phenolic profile may have different physiological effects (Valls et al., 2009; Kammerer et al., 2011).

The aim of this study was to evaluate the natural antioxidant effectiveness of the lovage leaves and stems and horseradish leaves extracts in unrefined rapeseed oil for the shelf life extension.

MATERIALS AND METHODS

Plant material

All samples were grown in Latvia and were harvested in September 2012. The horseradish (*Armoracia rusticana* L.) leaves were collected at the Pure Horticultural Research Centre collection field (latitude – 57° 03' N; longitude – 22° 91' E) and the lovage (*Levisticum officinale* L.) stems and leaves were grown in Jelgava, Latvia (latitude – 56° 39' N; longitude – 23° 44' E).

Herb extracts

From the fresh plants extracts were prepared using Soxhlet extraction method, and as a solvent ethanol was used. After extraction ethanol was evaporated and samples were kept at temperature +5 ± 1°C.

Rapeseed oil with extracts

In the study SIA 'Iecavnieks' (Latvia) crude canola oil was used. Initially oil peroxide value was not higher than 7 microequivalents active oxygen in kilogram of oil (meq O_2 kg^{-1}) and an acid value ranging from 1.27 to 1.32 milligrams of potassium hydroxide in gram of oil (mg KOH g^{-1}).

Requirements for food quality schemes, their implementation, operation, monitoring and control arrangements, Latvia Republic Cabinet of Ministers Regulation

Nr.461 states that in unrefined cold-pressed rapeseed oil peroxide number may not exceed 15 meq O_2 kg^{-1}, and acid number – 4 mg KOH g^{-1} oil (MKN Nr.461, 2014).

Three different extracts: the lovage leave and stem extracts and the horseradish leaves extract were added to the oil in four concentrations: 0.25, 0.5, 1.0 and 1.5% (w w^{-1}). As a control the unrefined rapeseed oil with no additives (control sample) were analyzed. Efficiency of natural antioxidants was compared with the oil sample containing 0.01% butylatedhydroxytoluene (BHT) in maximal allowed concentration for the oils (Regulation (EC) No. 1333/2008).

The unrefined rapeseed oil with added extract was placed in a glass conical flask sealed with a cork and was stirred with a magnetic stirrer (4.0 × magnets 0.5 cm) for 30 min in the dark at room temperature (20 ± 1°C). Prepared oil samples were stored in the glass bottles (50 mL) with HDPE cork (diameter 2.4 cm, material thickness 2 mm) and kept for 22 days at temperature 60 ± 2°C. In each step of experiment new bottle of oil was tested. Sample codes used in a study of added herbal extract efficiency in crude rapeseed oil are summarized in Table 1. Control sample without extract was marker with C, sample with BHT 0.01%–O_BHT.

Table 1. Sample codes used in a study of added herbal extract efficiency in crude rapeseed oil

Concentration of added extracts in oil, %	The added plant extract		
	lovage leaves	lovage stems	horseradish leaves
0.25%	O_LL_0.25%	O_LS_0.25%	O_HL_0.25%
0.5%	O_LL_0.5%	O_LS_0.5%	O_HL_0.5%
1.0%	O_LL_1%	O_LS_1%	O_HL_1%
1.5%	O_LL_1.5%	O_LS_1.5%	O_HL_1.5%

Chemicals

2,2-diphenyl-1-picrylhydraziyl ($DPPH^{\bullet}$) were purchased from Sigma-Aldrich (Switzerland). All other chemicals used in the research were obtained from Acros Organic (USA).

Determination of peroxide value

Peroxide value represents primary reaction products of lipid oxidation, which can be measured by their ability to liberate iodine from potasium iodide. Peroxide value of rapeseed oil samples was determined by the method as described in LVS EN ISO 3960:2010(2010).

Determination of acid value

Acid value indicates the proportion of free fatty acid present in oil or fat and may be defined as the number of milligrams of caustic potassium required to neutralize the acid in 1 g of the sample. Acid value of unrefined rapeseed oil samples was determined by the method as described in LVS EN ISO 660:2009 (2009).

Determination of antiradical activity of oil

Antiradical activity of oil was measured by $DPPH^{\cdot}$ free radical scavenging method (Ha, et al., 2012). Oil samples (0.15 mL) were dissolved in 3.75 mL of isooctane. Then 1 mL of solution was mixed with 3 mL of a freshly prepared solution of 0.1 mM $DPPH^{\cdot}$ solution in isooctane. After storage for 30 minutes in the dark the results were read using spectrophotometer 'JENWAY 6300' ('Baroworld Scientific' Ltd., UK) at a wavelength of 509 nm. The following formula (1) was used for calculation of the oil antiradical activity sample.

$$ARA,\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100, \quad (1)$$

where: $A_{control}$ – absorbtion of control sample (isooctane with $DPPH^{\cdot}$); $A_{paraugs}$ – absorbtion of analysed sample.

Statistical analysis

Experimental results were means of three parallel measurements and were analyzed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey test were used to determine differences among samples.

RESULTS AND DISCUSSION

Peroxide value

The peroxide value describes peroxide and hydroperoxide concentration during the first stages of lipid oxidation. An analysis of the peroxide value is one of the most widely used methods for determination of the degree of the oil and fats oxidation (Gertzetal, 2000). In the current study the degree of oxidation of the rapeseed oil with and without antioxidant additives stored at elevated temperature in the dark was determined. The initial rapeseed oil peroxide value was 6.14 meq active O_2 kg^{-1} oil (or 3.07 mmol O_2 kg^{-1} oil), which is in accordance consistent with other studies of the unrefined rapeseed oil with a peroxide value 3.9 mmol O_2 kg^{-1} oil (Kleinová et al., 2013). Requirements for food quality schemes, their implementation, operation, monitoring and control arrangements, Latvia Republic Cabinet of Ministers Regulations Nr.461 states that unrefined cold-pressed rapeseed oil, peroxide value may not exceed 15 meq active O_2 kg^{-1} (MKN Nr.461 from 2014). The effect of the added antioxidant on rapeseed oil peroxide value during the experiment in accelerated storage conditions are shown in Figure 1. The control sample maximal allowed peroxide value reached during the period from the first to third days of storage, while the samples with added plant extracts at a concentration of 1% during the period from 5th to 8th storage day, suggesting that the added antioxidants retard oil oxidation

Under the medium temperature and as well as due to the relatively high number of unsaturated fatty acids with double bonds, the peroxide value in all samples increased rapidly. The analyzed rapeseed oil samples under thermal stress conditions reached maximal peroxide number after 22 days of storage and the highest peroxide value for control sample was determined (103 ± 2 meq O_2 kg^{-1} of oil).

After 5 days of storage the peroxide value of the control was 64% higher compared to the sample with lovage leaves extract (1.5%), and it was the biggest difference between the analyzed samples in this experiment. Also tendency that higher concentration of the extracts better retard oxidation process showing positive impact of the extracts on the stability of the oil by preventing oxidation was observed.

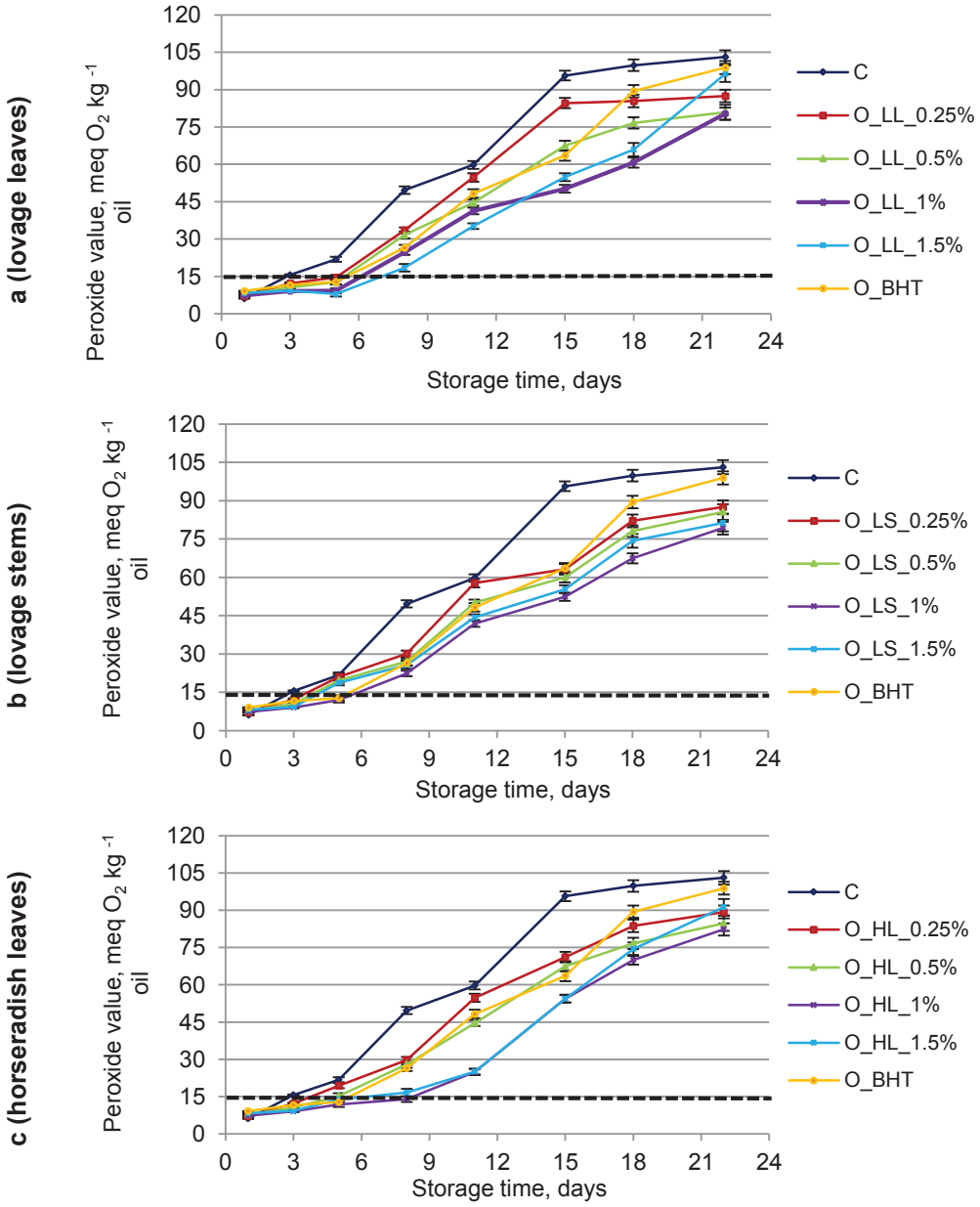

Figure 1. Changes of rapeseed oil peroxide value stored in dark at 60 ± 1°C temperature.

The oil samples with added the lovage stem and the horseradish leaf extracts, as well as synthetic antioxidant BHT, showed the biggest difference with the control after 8 days of storage, reaching up to 72% in sample with 1% horseradish leaf extract.In the following days of storage, the difference significantly ($P < 0.05$) decreases, and after 22 days of storage the rises again reaching a difference of 23%. The peroxide value of rapeseed oil with BHT after 22 days of storage was 98.87 meq active O_2 kg^{-1} of oil which is only 4.09% less than the control sample. About similar trends also reported Pukalskas et al. (2010) who found that BHT added to rapeseed oil and kept at 80°C did not prevent oxidation significantly ($P < 0.05$). After 22 days storage of the rapeseed oil samples with the plant extracts, common trend was observed: oil samples with 1% natural antioxidant extracts had lower peroxide values. Additionally, efficiency of plants was not significantly different ($P < 0.05$).

Acid value

The acid value of the analyzed oil samples ranged from 1.27 mg KOH g^{-1} oil at the first day of experiment to 4.17 mg KOH g^{-1} oil after 22 days of storage (Fig. 2.).

During the all storage period (22 days) acid value in analyzed oil samples increased for three times, but at the end of this period, only one half of the analyzed samples exceeded the allowed limit of the Latvian legislation - 4 mg KOH g^{-1} (Requirements for food quality schemes, their implementation, operation, monitoring and control arrangements, MKN Nr.461, 2014).

During accelerated storage conditions for all samples the acid value increased steadily. Also, in peanut and sunflower oil samples with the added natural antioxidants stored for 40 days acid value increased slightly (Miguel et al., 2005). After 22 days of storage in the dark at +60 ± 1 °C temperature, the lowest acid value was found in the oil sample with BHT (3.84 mg KOH g^{-1} oil), while the highest for the control sample (4.17 mg KOH g^{-1} oil). Also comparing the oil samples with added the plant extracts in the same concentration, no significant differences ($P < 0.05$) throughout the storage period were observed.

In samples with higher concentration of the plant extracts, lower acid value was detected. This means that the added plant extracts affects the inhibition of fat decomposition and the formation of free fatty acids.

Compared the effectiveness of added natural antioxidants, it could be concluded that after 22 days of storage, the lowest acid value (3.87 mg KOH g^{-1} oil) was in the oil with 1.5% lovage stem extract. Also the oil samples with added 1.5% horseradish leaves and 1.5% lovage leaf extracts acid value were lower than the in control sample. It could be concluded that in the dark under the accelerated storage conditions, natural antioxidants significantly ($P < 0.05$) reduced free fatty acid formation. However, the experiments showed that the most effectively the formation of free fatty acid prevented synthetic antioxidant (BHT).

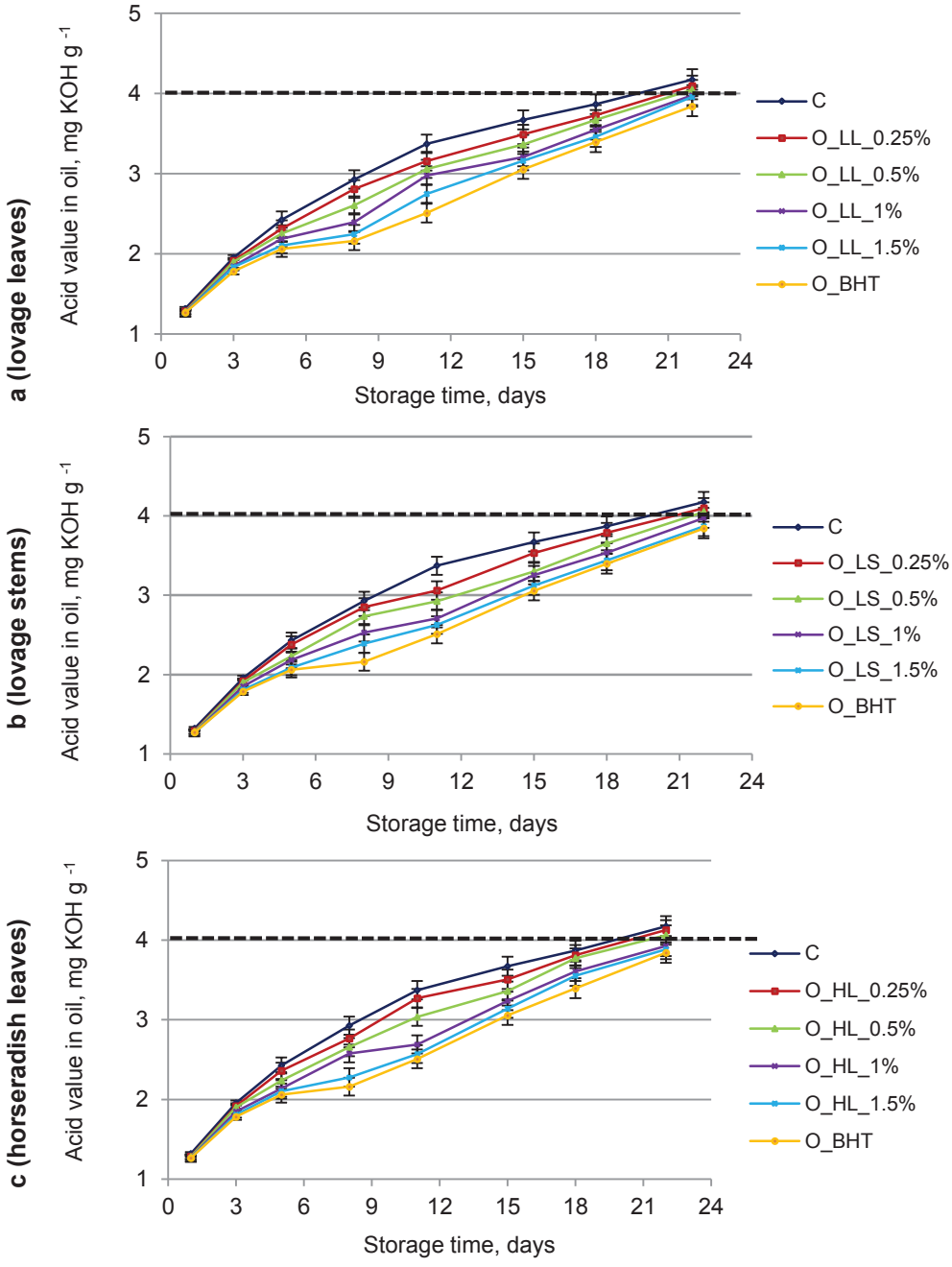

Figure 2. Changes of rapeseed oil acid value stored in dark at 60 ± 1 °C temperature.

Antiradical activity of oil

In present antiradical activity assessment method nonpolar solvent isooctane was used. The extracts added to the oil were rich in polar compounds therefore, in these experiments activity of the added compounds possible, could not be seen.

Crude rapeseed oil already contains compounds that are able to scavenge DPPH radicals. One of the most important compounds are tocopherols, that are found in most cold-pressed, fruit and nut oils and can act as a natural antioxidant and the synergist (Rodrigues et al., 2015).

Presence of these compounds may approve fact that in the control sample at the beginning of the experiment DPPH radicals scavenging activity was 80.30% (Fig.3). Activity during storage in the dark at the +60 ± 1°C temperature decreased sharply and after 22 days of storage was only 26.95% (for control).

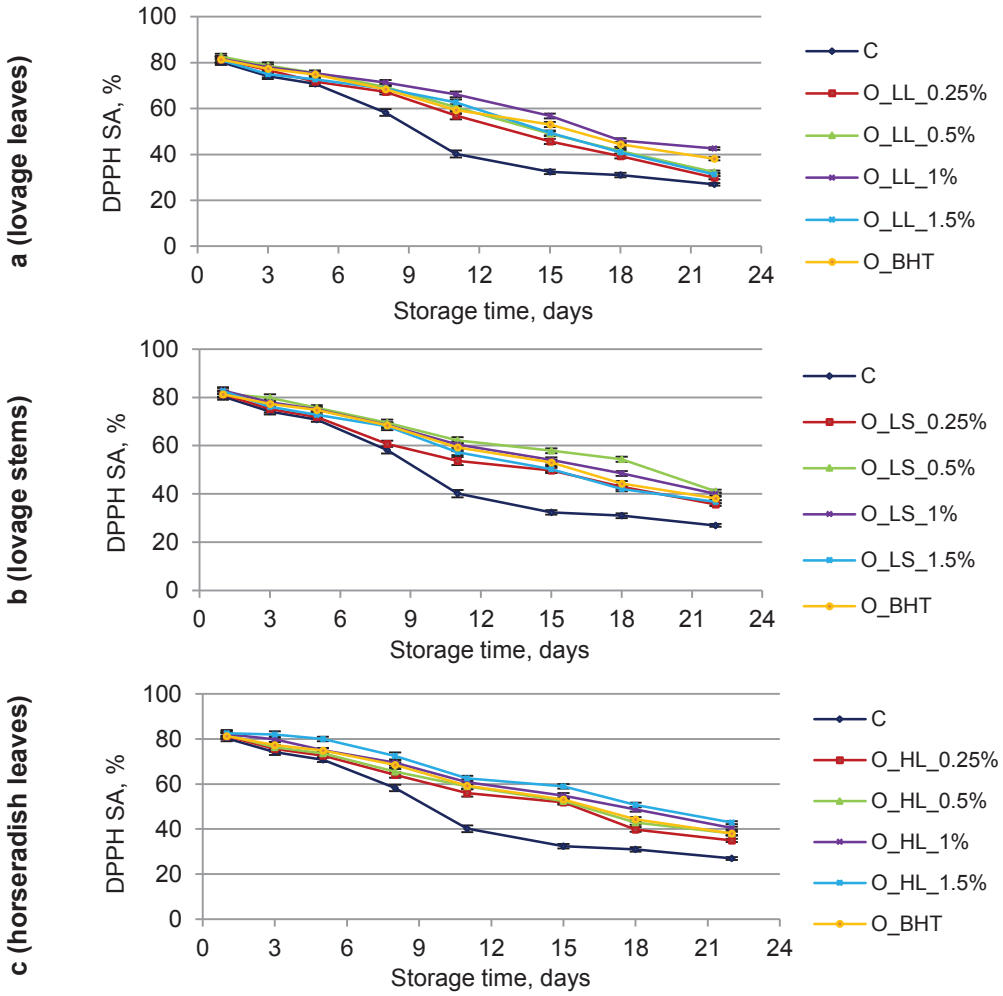

Figure 3. Changes of scavenging activity (SA) of rapeseed oil stored in dark at 60 ± 1 °C temperature.

In the oil samples with the added lovage stems, lovage leaves and horseradish leaves extracts and also BHT DPPH radicals scavenging activity during storage significantly ($P < 0.05$) decreased. After 22 days of storage, in the oil sample with 1% lovage leaves extract antiradical activity reduced for 1.92 times, and it was 1.58 times higher compared with the control. Also, activity of oil samples with added 1.5% horseradish leaves and 0.5% lovage stem extract after 22 days of storage decreased by 1.93 and 1.98 times, respectively.

The results shows that during almost all experiment the highest antiradical activity demonstrates oil with added 1% of lovage leaves (Fig.3.a) as well as 0.5% lovage stem (Fig.3.b) and 1.5% horseradish leaf extract in oil (Fig.3.c).

In the unrefined rapeseed oil with BHT antiradical activity decreased 2.13 times, and compared to other samples it is average result.

Comparing analyzed oil samples with sample with synthetic antioxidant, it can be seen that better results demonstrated oil samples with added 1.5% and 1% of horseradish leaves, 0.5% and 1% of lavage stems, as well as 1% lovage leaves extract.

CONCLUSIONS

Addition of the lovage leaves, lovage stems or horseradish leaves extracts to unrefined rapeseed oil can prevent the oxidation of unrefined rapeseed oil in accelerated storage conditions in the dark. Increased efficiency was for oil samples with added 1% of the natural antioxidant extract and there were not significant differences ($P < 0.05$) between lovage leaves, lovage stems or horseradish leaves extracts.

In oil samples with added natural antioxidant extracts acid value was less than the control sample. The lovage leaves, lovage stems and horseradish leaves extracts added to unrefined rapeseed oil during storage significantly ($P < 0.05$) increased the oils ability to scavenge DPPH free radicals. Besides in the oil samples with 1.5% and 1% horseradish leaves extract, 0.5% and 1% with lovage stem extract and 1% lovage leaves extract antiradical activity was higher compared to synthetic antioxidant BHT.

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