

## Study on antioxidants extraction from oak bark and their use for oxidation stability of sunflower oil

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### Abstract

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#### Keywords:

Oak  
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Oil

**Introduction.** Food industry requires the antioxidants obtained from natural raw materials. It is important to establish the methods for incorporation of hydrophilic antioxidants to oil and fat products.

**Materials and methods.** Oak bark was used as the raw material for the obtaining of antioxidants. The aqueous-ethanol solutions, alkali and acid addition, and microwave treatment were used for flavonoid extraction. The dry substances content in the extracts was determined gravimetrically. The kinetics of sunflower oil initiated oxidation was studied using a volumetric method in the presence of azobisisobutyric acid dinitrile.

**Results and discussion.** Solvents with different ratios of water: ethanol were used to obtain extracts from oak bark. Application of higher ethanol concentrations in solvent resulted in increase of extract concentration. The extract concentration increased by 45% with an increase of ethanol concentration from 50 to 80%. Addition of 1% ascorbic acid increased the yield of extractive substances from 2.3 to 3.1%; addition of 1% citric or lactic acid increased the yield up to 4.3 and 3%, respectively.

The microwave extraction does increase the extraction rate by 8 times and the yield of extractive substances by 1.5 times (from 2.3% dry substances in extract to 3.5%).

The water-alcohol extracts added to sunflower oil together with the emulsifier dispersed it to particle sizes not more than 2–3 μm. Water and alcohol were removed by distillation under reduced pressure. After evaporation of solvent, the diameter of the hydrophilic phase particles decreased to nano-size.

The dispersion of oak bark extracts in sunflower oil resulted in a 1.8-fold increase of the period of induction of sunflower oil oxidation, determined by the kinetics of accelerated oxidation and the accumulation of peroxides during the storage.

**Conclusions.** The use of food acids in combination with a water-alcohol solvent and a microwave treatment effectively increased the extraction of antioxidants from oak bark.

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## Introduction

Fats and oils are the products that have high rate oxidative deterioration due to the presence of unsaturated fatty acids (FA) (Javidipour et al., 2016). The interaction of fats with oxygen results in formation of a number of oxidation products (peroxide and hydroperoxide compounds, aldehydes, ketones, acids, etc.), which are considered to be toxic substances and they should not be present in food products. Rate of fats oxidation depends on many factors: degree of FA unsaturation, ions of polyvalent metals content, storage temperature, oxygen access, moisture content, etc (Xu et al., 2017). The main way to inhibit the rate of fat oxidation is using of antioxidants (Großhagauer et al., 2019).

Currently there is a number of antioxidants discovered in the world (Oswell et al., 2018). However, the list of healthy and officially legalized antioxidants is relatively short. When the antioxidants are used in medicine, pharmacy, or food technologies they have to satisfy to special requirements. Ionol, butylhydroxytoluene, propyl gallate, tocopherols, carotenoids are the most commonly used antioxidants in oil and fat industry (Gokoglu et al., 2006). Recent studies have shown that using of synthetic antioxidants can affect the human health (Morteza-Semnani et al., 2006), thereafter there is a need to search and implement the antioxidants obtained from natural raw materials (Olszowy et al., 2019). Perhaps the largest group of natural antioxidants is flavonoids, the most common group of polyphenolic compounds (Cisneros-Yupanqui et al., 2020).

Flavonoids are not synthesized in animal or human cells, and their presence in tissues depends entirely on plant foods consumption. It is proven that when the flavonoids are present in the human body, they are included in numerous processes of cell metabolism, gene expression and even protect the body against parasites and infections (Durazzo et al., 2019). They play a significant role in prevention of cardiovascular disease and cancer (Durazzo et al., 2019). Currently, the antioxidant effect of flavonoids obtained from different natural sources has been proved (Huyut et al., 2017). Therefore, the task of developing a variety of products, which contains flavonoids, is actual for the food industry. Flavonoids are phenolic compounds of different structure (known about eight thousand representatives) (Huyut et al., 2017), which are characterized by various physical and chemical properties like solubility in polar and nonpolar solvents. Therefore, despite the variety of known methods for flavonoids extraction, the easiest and the safest method is to extract them with two “edible” solvents of different polarity: water and ethanol. However, the amount of solvents and the extraction time are too high in this process. In addition, the alcoholic solutions with ethanol concentration less than 50% v/v cannot avoid oxidation processes in phenolic extracts during extraction (Shi et al., 2003). Therefore, the optimization of the flavonoids extraction process from plant and other raw materials have to be developed.

On the other hand, the positive physiological effect of flavonoids, their effectiveness as antioxidants makes these compounds essential for the shelf life increase of oil and fat. But the problem is that the extracts of flavonoids or polyphenolic compounds are hydrophilic and insoluble in oils or fats, so their application is currently limited to water-soluble systems or emulsions (Belščak-Cvitanović et al., 2018). Thus, it is necessary to develop the technology of injection of such hydrophilic antioxidants to the fat.

In addition, the high prices of flavonoid extracts limit their using (Small et al., 2016). Therefore, oak bark was chosen as the cheap and common source (Skrypnik et al., 2019) for this study.

The aim of this work was to optimize the of water-alcohol extraction of flavonoids from oak bark and study the inhibition of sunflower oil oxidation by concentrates of oak bark antioxidants.

## **Materials and methods**

### **Antioxidants extraction from plant raw materials**

Oak bark was chosen as a raw material for antioxidants obtaining. It is a well-known source of flavonoids. Oak bark usually contains catechinic tannins (0.4%), free gallic and ellagic acids, galothannins (10–20%), quercetin, flobafen, resins, pectin substances (6%), sugars (levulosin and others), proteins, starch and minerals.

Oak bark was crushed using a laboratory mill RRH – 100 (Ukraine) at 28 000 rpm, after that the samples were sieved through a laboratory sieve with mesh size of 1 mm. The samples were stored at (-10)° C during four weeks (Ushkalova et al., 1016). Water and ethanol (96% vol.) mixtures were used to extract flavonoids. The dry oak bark in a mixture of solvents (at ratio of 1:3), respectively, was stirred at 100 rpm and (45±5) °C during 2 hr. The extracts were filtered and the dry substances content was determined gravimetrically.

### **Microwave extraction**

For microwave extraction the microwave chamber with the 2.450 GHz frequency and 300 W power was used (Li et al., 2011). The extraction was carried out during 10–100 min in the flask attached to the backflow condenser. The extracts were filtered and the dry substances content was determined gravimetrically.

### **Influence of pH on the extraction process**

Sodium hydroxide or edible acids (ascorbic, citric as a powder and lactic as 40% solution) were added to the aqueous-ethanol mixture to adjust alkali or acid medium.

The flavonoids extraction was carried out according to the above mentioned procedure.

### **Determination of sunflower oxidation rate**

Fully refined and dewaxed sunflower oil was used as a model system to study the inhibitors influence on oils and fats oxidation, since it tends to oxidative deterioration due to high content of unsaturated fatty acids.

The sunflower oil oxidation rate was studied by a volumetric method (Ghosh et al., 2019) in manometric device (Varburg type). The 5 ml reaction chamber was thermostated, equipped by manometer, source of oxygen (99%) and vacuum pump. At the beginning of the measuring the air was displaced by oxygen (the operation was repeated 5–8 times). The volume of oxygen absorbed by the sample was measured according to changes of level of stained fluid in a graduated tube connected with a reaction chamber.

The research was carried out at 70 °C under conditions of initiated oxidation. For oxidative reaction 2 g of oil mixed with 3 ml xylene and 0.3 ml of 0.1 mol/L solution of oxidation initiator azobisisobutyric acid dinitrile (AIBN) in xylene. Reaction mixture was blew by oxygen during 1 min and thermostated at 70 °C during 10 min before measurements. Oxidation curves at 70 °C were measured as volume of absorbed oxygen and plotted in coordinates: heating time ( $t$ , min) – height of absorbed oxygen column ( $H$ , mm).

Induction periods of oil oxidation were calculated from the curve of oxidation by the graphical method (Ghosh et al., 2019) as a segment of the abscissa axis cut by a perpendicular from the point of intersection of the tangents to the kinetic curve.

Periods of induction of sunflower oil oxidation without the addition of antioxidants and with antioxidant extract from oak bark were calculated.

The antioxidant activity was estimated as efficiency of the oxidation inhibition of sunflower oil and calculated according to:

$$AOA = \frac{\tau_i}{\tau_s},$$

where  $\tau_i$ ,  $\tau_s$  – the duration of induction period of sunflower oil with and without antioxidants.

In addition, the degree of sunflower oil oxidation rate was determined by changes of peroxide values (Xu et al., 2017). Two samples of refined, deodorized and winterized sunflower oil with and without addition of 2% of aqueous alcohol solution of oak bark extracts were stored in opened flasks for 50 days at 25 °C with free access of air and light. The peroxide value of oil was determined every 2 days. The kinetic curves of samples oxidation were plotted and the duration of the induction period of hydroperoxide accumulation was determined (Ghosh et al., 2019).

### **Determination of peroxide value**

Determination of peroxide value of the extracted oil was carried out according to the standard IUPAC methods (IUPAC, 1987).

### **Particles size determination**

The particles size of the obtained mixture “water-soluble antioxidant – oil” were determined under light microscope. The hydrophilic part was dyed with a water-soluble dye (methyl-orange). The maximum resolution of the microscope was adjusted as 0.2 microns.

### **Statistical analysis**

Samples were analyzed in triplicate. Statistical analysis was performed using Microsoft Excel 2007 (Microsoft, City of Redmond, USA). The results were reported as mean±SD. Differences were considered to be significant at validity of  $\alpha=0.95$ .

## **Results and discussion**

### **Effect of ethanol concentration on the extraction of oak bark antioxidants**

The extraction of oak bark under different water:ethanol ratio demonstrated that higher ethanol content in solvent had resulted in increase of extractive substances concentration (Table 1). The extract concentration had increased by 45% under increasing of ethanol concentration from 50 to 70%. Simultaneously, the induction period of sunflower oil oxidation with addition of 2% of extract had increased (Table 1).

Table 1

Influence of water: ethanol ratio on the extracts concentration and their antioxidant activity

N <sup>o</sup> of sample	Water:ethanol (96% v/v) ratio	Extract concentration, % of dry substances	Sunflower oil induction period of oxidation with addition of 2% of extract, min
1	50:50	1,57 ±0.014	46 ±2.8
2	40:60	1,95 ±0.042	50 ±3.5
3	30:70	2,30 ±0.035	57 ±1.4
4	20:80	2,27 ±0.021	52 ±1.4

Thus, the optimal parameters of antioxidants extraction from oak bark (Table 1): water: ethanol ratio 30:70, solids-to-solvent ratio 1:3, temperature – (45±5) °C, duration – 2 hours. These conditions were chosen for the next experiments.

It was shown (Piccand et al., 2019) that there is a significant correlation between the composition of oak bark extract and its antioxidant activity. We have investigated the antioxidant activity of the sunflower oil under addition of the obtained oak bark extracts. The duration of induction period of oil initiated oxidation is commonly used as the measure of oil oxidation stability (18). The oxidation curve of sunflower oil with (sample 2) and without (sample 1) addition of 2% of oak bark extract is shown on the Figure 1. The induction period of sunflower oil initiated oxidation had been increased from 31 to 57 minutes under addition of extract, that proved the high antioxidant properties of obtained extracts (Table 1). The value of antioxidant activity with the addition of extracted from oak bark substances was AOA = 1.8. This result indicates the effectiveness of the extracted from oak bark antioxidants (Li et al., 2012).

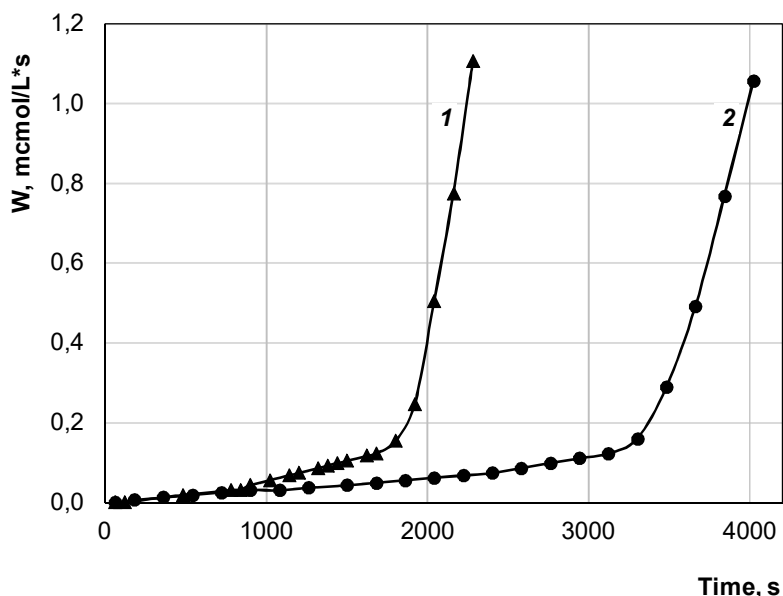


Figure 1. Absorption of oxygen by sunflower oil under initiated oxidation without (1) and with (2) addition of 2% oak bark extract

### Effect of pH on the extraction of oak bark antioxidants

It was reported (Corbin et al., 2015) that using of alcoholic solution of sodium hydroxide had increased in the concentration of flavonoids in extracts compared to the "non-alkali" alcoholic solution.

Our results have shown that alkali solvent under extraction did not increase the extractive substances yield, their amount remained approximately at 2,3% (Table 2). Extracts, obtained in the alkaline solvents, did exhibit the antioxidant activity, mainly addition of 2% of the extract resulted in 52 minutes induction period of sunflower oil oxidation (for a sample with 5% of sodium hydroxide in extraction solvent). However, the increase in the pH of the medium did not enhance the efficiency of extraction of antioxidants from oak bark and inhibition of sunflower oil oxidation by the obtained extract.

Table 2

Influence of alkali addition on the extractive substances yield

Amount of NaOH (%) relatively to the mass of the oak bark	Yield of extractives (%) relatively to the oak bark sample weight
1	2,30±0.023
2	2,30±0.018
5	2,32±0.017
10	2,44±0.026

The effect of acid medium on the oak bark flavonoids extraction was studied also with using ascorbic, citric, lactic acids. Addition of food acids to a water-ethanol mixture had shown a positive effect on the process of oak bark extraction. The effect of the acid addition on the yield of the extractive substances and the induction period of the oil oxidation in the presence of the obtained antioxidants are shown in Table 3. It was demonstrated that increase of acid concentration had resulted in the higher extract concentration. Addition of 1% ascorbic acid (relatively to the oak bark) increased the yield of extractive substances from 2.3 to 3.1%, addition of 1% citric and lactic acid increased the yield up to 4,3% and 3%, respectively (Table 3). Further increase of food acids content also slightly increased the yield of extractive substances, but the maximum growth of extraction rate was observed when the content of food acids is close to 1%, so this content can be recommended for practice use. Citric acid (at all introduced acid concentrations) had the most prominent effect on the process of extract obtaining from oak bark. Addition of 1% citric acid had showed a highest yield of extractive substances (increased from 2.3 to 4.3%).

Table 3

Influence of acid addition on the oak bar extractive substances yield

Food acid	Yield of extractive substances (%) of oak bark			
	Amount of acid (%) relatively to the mass of oak bark			
	0,5	1	2	3
Ascorbic acid	2.6±0.034	3.1±0.033	3.2±0.024	3.35±0.022
Citric acid	3.1±0.029	4.3±0.040	4.5±0.044	4.8±0.036
Lactic acid	2.7±0.019	3.0±0.025	3.1±0.010	3.4±0.030

Our results are in accordance with the data obtained in (Halee et al., 2018) on the maximum yield of the flavonoids from colored rice under adding of citric acid to the solvent at concentration 0.1 mol/dm<sup>3</sup>.

In addition, in the presence of antioxidant extracts, obtained in 1% acid water: ethanol solution, the induction period of sunflower oil oxidation was prolonged (Table 4). However, the highest effect on the prolongation of the induction period of sunflower oil oxidation had the extract, obtained with ascorbic acid. The induction period of sunflower oil oxidation with addition of the extracts, obtained in acid medium, changed as follows: lactic acid – from 52 (extract without acid, extractives content 2,3%) to 60 min (extract containing lactic acid, extractive content 3%), citric acid – from 52 to 70 min, ascorbic acid – from 52 to 80 min. This effect is probably due to synergic effect of ascorbic acid that itself is an antioxidant and is able to inhibit the oxidation of fats (Wang et al., 2019).

**Table 4**

**Influence of acid oak bark extracts sunflower oil oxidation**

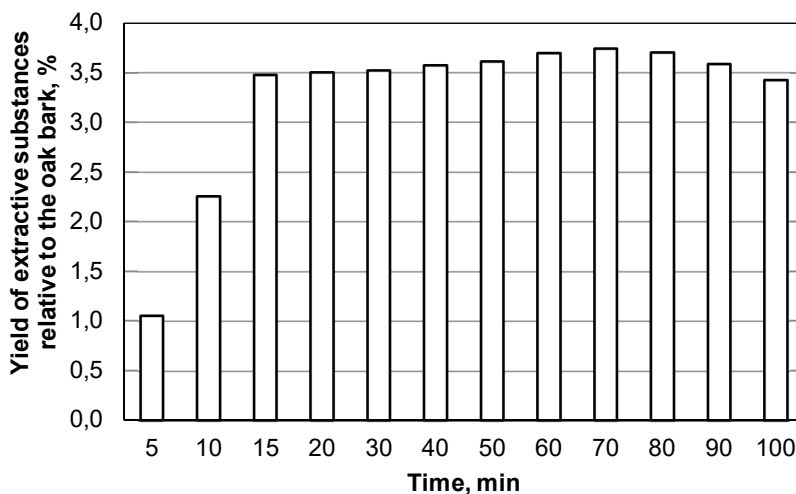
<b>Food acid</b>	<b>Sunflower oil induction period of oxidation in the presence of 2% of extract (1% of food acid), min.</b>	<b>Antioxidation activity of the extracts</b>
Ascorbic acid	78±3,1	2,5
Citric acid	70±1,3	2,2
Lactic acid	60±2,5	1,9

In general, all studied food acids significantly increase the yield of extractive substances and extend the shelf life of fat. Increasing induction period of sunflower oil oxidation by extracts of antioxidants containing ascorbic acid by 2.5 times (Table 4) or citric acid – by 2.2 times indicates the suitability of extraction of antioxidants in the presence of these substances (23). This approach to antioxidant extraction is the most promising due to its simplicity, low cost and safety.

#### **Influence of the microwave treatment on the extractive substances yield from oak bark**

The well-known method of extraction acceleration is a microwave extraction. It was shown (Li et al., 2012) that microwave treatment is an effective method to destroy the cell walls without damage of the plant substances. The main advantage of microwave extraction is significant reduce of the extraction time: from seconds to 15–20 minutes compared to 1.5–6 hours for traditional extraction. It was shown that the properties of biologically active substances are preserved and their content in the extract is higher comparing to traditional extraction (Li et al., 2012).

Study of the influence of microwave extraction time on the extractive substances yield had shown that yield maximum had reached after about 15 min extraction. There was not substantial increase of extractive substances yield from 15 to 100 min (Figure 2). Thus, there is no need to carry out extraction under microwave irradiation longer than 15 minutes. The microwave extraction does increase the extraction rate by eight times (2 hours for a traditional method vs. up to 15 min. Solids-to-solvent ratio and solvent composition remained the same). Microwave extraction also increased the yield of extractive substances by 1.5 times (from 2.3 to 3.5% of dry substances in the extract). Therefore, the microwave treatment has positively influence on the extraction rate and the extraction yield.



**Figure 2. Influence of microwave extraction time on the extractives substances yield**

#### **Development of the method for stabilization of hydrophilic extracts in oil and fat**

The extracts obtained are hydrophilic systems and are insoluble in fats. It is impossible to form a stable emulsion of water-alcohol extracts in oil and fat (Castro et al., 2020) and, consequently, the extracted antioxidants are not able to protect fats and oils from oxidative deterioration during storage.

Therefore, we had forwarded the working hypothesis of the possibility to obtain a stable system by dispersion of aqueous-alcoholic antioxidant solution in the oil phase with a particle size up to 100 nm. Such systems with an extremely high degree of dispersion are known as ultradisperse systems (the particle size in such systems is in the range from 1 to 100 nm) (Castro et al., 2020). Recently numerous studies have shown that under transition from micro- to nanoparticles the qualitative changes of number of physical and chemical properties of systems, including their solubility, occur (Castro et al., 2020; Summ et al., 2001). This happens due to the fact that for particles which dimension at least one of the directions are comparable or smaller than the correlation radius of any physical or chemical property (for example, the size of the new phase nucleation), the dimensional effects occur. These specific properties are the basis for considering the ultradisperse state as the fifth state of the matter.

To obtain the ultradispersed system the water-alcohol solution of flavonoids was added to the oil or fat together with the emulsifier, the system was dispersed to particle sizes not more than 2-3  $\mu\text{m}$ . The water and alcohol had been distilled under reduced pressure. The antioxidant molecules remain in the volume of the fat. They are dispersed so tightly that they cannot be removed from the hydrophobic system. As a result, after evaporation of solvent molecules, the diameter of the hydrophilic phase particles were decreased to nano-sizes.

It was shown that the size of the particles of the emulsion (water-alcohol solution of antioxidant – oil) was 2–3  $\mu\text{m}$ . The size of the antioxidant particles after water and alcohol evaporation was determined mathematically: the content of antioxidant (flavonoids) in the aqueous alcohol solution was approximately 3%, so, after evaporation of the solvents, the

particles diameter of the dispersed phase decreased by more than 2 orders and was equal to the value less than 100 nm.

It is known that when the size of the disperse system's particles is reduced, two opposite processes compete. They are dispersion and aggregation of the particles. It was noted already that ultradispersed systems are characterized by a highly developed interphase surface and, respectively, a significant excess of the Helmholtz energy  $\Delta F_s$ , i.e. they tend to aggregation (Castro et al., 2020; Summ et al., 2001). The aggregation of particles will also occur during storage of the disperse system. Emulsifiers are commonly used to maintain the stability of the obtained disperse system during storage (Cassiday et al., 2016).

In this study it was proposed to use the emulsifier E 471 (mono- and diglycerides of fatty acids) in order to reduce energy consumption on dispersion and to increase the dispersion ability of water-soluble antioxidants.

We had not observed the gravitation segregation of the resulting systems during the whole period of their storage (2 months). The rate of hydroperoxide accumulation in sunflower oil under addition of oak bark antioxidant nanoparticles had decreased dramatically. The oxidation kinetics for refined deodorized sunflower oil during storage without and with addition of flavonoids (2% of aqueous-alcoholic solution relatively to oil) are shown in Figure 3. The induction period of sunflower oil oxidation was 21 days (without antioxidant) and 37 days (with antioxidant) that is, the oil's shelf life increased by 1.8 times, that correlates with another method of oxidation kinetics (data given in Figure 1). Similarly, it was reported a 2.5-fold increase in the period of biodiesel (that is methyl esters of fatty acids) oxidation induction with the addition of green tea leaf extract (one of the most flavonoid-rich plant materials) (Bharti et al., 2020).

In addition, fats usually contain natural oxidation inhibitors – tocopherols (Jung et al., 1990). It was previously shown a synergic effect of tocopherols and flavonoid extracts (Demidova et al., 2016), that also indicates that injection of flavonoid extracts is reasonable to inhibit the oxidation of oil and fat products.

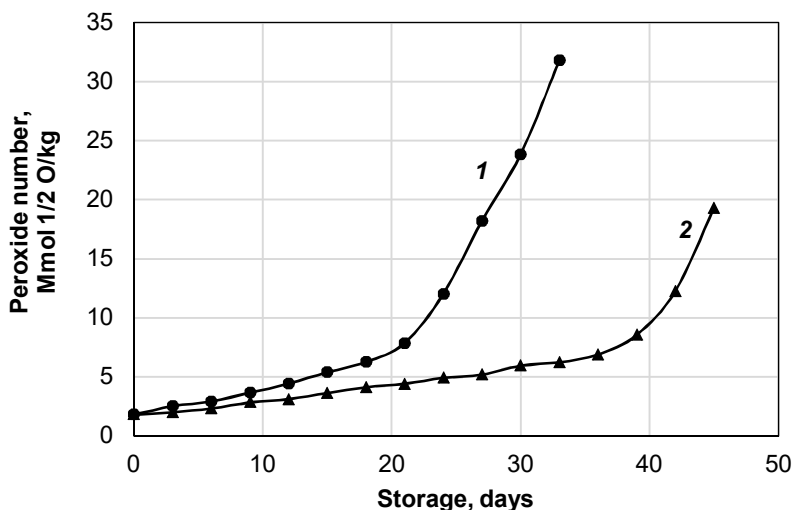


Figure 3. Oxidation rates of refined deodorized sunflower oil without (1) and with (2) addition of the developed antioxidant extracts according to the peroxide values

## Conclusions

Suitable conditions for polyphenols extraction from oak bark were established in this study in order to use them as inhibitors of vegetable oil oxidation. It was shown that polyphenols of oak bark were effective as natural inhibitors of oil oxidation that makes these compounds promising to use in various fat-containing foods. The mixture of extractives of oak bark and ascorbic acid has the most significant effect on prolongation of sunflower oil shelf life.

The use of food acids in combination with a water-alcohol solvent and microwave field were effective for the enhancing of the extraction process of oak bark. The method of creation of the stable fat and hydrophilic antioxidants containing system was developed. Its efficiency was proved by the significant prolongation of induction period of sunflower oil oxidation during storage.

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