ISSN 2409-9066. Sci. innov. 2015, 11(4): 30—34 doi: http://dx.doi.org/10.15407/scine11.04.030

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KINETIC CHARACTERISTICS OF OIL NATURAL ANTIOXIDANTS

Oxidation parameters of sunflower, maize, walnut, palm, and palm kernel oils have been defined by Tsepalov`s graphical and DPPH methods. The influence of the oil natural antioxidants and the degree of unsaturation of fatty acids on the oxidation rate has been showed. The methods can be used to predict the oil oxidation stability in the course of storage.

Keywords: oils, oxidation, Tsepalov`s graphical method, kinetic parameters, DPPH method, and antioxidant activity.

THE STATEMENT OF THE PROBLEM

Many food products deteriorate during storage for their lipids interacting with oxygen. The oil and fat products are very important foodstuff, as they are a major source of energy for the human body.

The oxidation rate of oil and fat products depends on the structure of radicals of fatty acids that make up the triglycerides, the temperature conditions, the presence of catalysts and inhibitors of oxidation, and the action of light and radiation. The oxidation results in forming activated derivatives of molecular oxygen or reactive oxygen intermediates which are involved in free radical oxidation reactions, including lipid peroxidation that adversely affects the stability of products during their storage.

For improving the stability of oil and fat products it is necessary to study the activity and mechanism of action of natural antioxidants (tocopherols) containing in these products.

The antioxidants belong to the biologically active substances that bind excessive free radicals and prevent the accelerated oxidation of lipids and the formation of unwanted oxidation products [1].

There are direct and indirect methods for the identification of oil and fat oxidation products, e.g., chemiluminescence, gasometry, micro-calorimetry, determination of peroxide and acid numbers, model reactions of antioxidant interaction with radicals generated in various ways, as well as «electronic nose» and «electronic tongue» intelligent systems [2—5].

The aim of this research is to determine antioxidant activity and resistance to oxidation for some vegetable oils (sunflower, maize, walnut, palm, and palm kernel) by the *DPPH* (*2,2-diphenyl-1-picrylhydrazyl)* method and the Tsepalov graphical technique for forecasting the oxidative stability of oil and fat products during their storage.

THE RESEARCH RESULTS AND DISCUSSION

Lipid oxidation is a chain free-radical process consisting of three stages [2, 6, and 7]:

1) *Initiation*:

$$RH + O_2 \rightarrow R\cdot + OH;$$

2) *Propagation*:

$$R\cdot + O_2 \rightarrow ROO\cdot$$

$$ROO\cdot + RH \rightarrow ROOH + R\cdot;$$

3) *Termination*:

$$R\cdot + R\cdot \rightarrow RR$$

$$R\cdot + ROO\cdot \rightarrow ROOR$$

$$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2.$$

At the first phase of initiation, a free radical appears from lipid substrate under the action of initiator, with branching of chains being a result of the radical decay of hydroperoxides, the only primary products of oxidation.

In the presence of inhibitor in oil, the hydroperoxides can interact with it thereby slowing down or pausing for a while the further oxidation:

$$ROO\cdot + InH \rightarrow ROOH + In;$$

$$ROO\cdot + In\cdot \rightarrow \text{molecular products.}$$

For measuring the content of tocopherols that are natural inhibitors of chain processes, the high-resolution liquid chromatography method was used [8] (Table 1).

The data shows that the highest content of tocopherols have been found in the samples of refined maize oil. The lowest content of tocopherols are reported for the palm oil. The total concentration of tocopherols in the pressed sunflower oil does not exceed 95 mg %, with 90 % being α-tocopherol. The content of γ- and δ-isomers in the studied samples is insignificant, as it ranges from 5.6 % in the pressed peanut oil to 8.5 %, in the maize oil and depends on the natural features of oils.

The kinetics of oil oxidation was studied with free access of light and air (spontaneous oxidation) at 22 ± 2 °C by the accumulation of hydroperoxides therein and the change in peroxide number (PN) using the standard method [9].

According to the results of experiments (Fig. 1), the most stable among the oils of tropical origin are the palm and palm kernel oils: their PN is 2.36 and 3.23 mmol $^1/_2$O/kg, respectively. This can be explained by a low initial PN in the oils, within an induction period of oxidation. Similar results were obtained for the maize oil: with a low initial PN it was revealed a high stability on the 60th day of oxidation. The sunflower and walnut oils are oxidized in a similar way: an induction period within 30 days and a rapid accumulation of lipid hydroperoxides, at the end of oxidation.

The oxidation indicators of oils having been defined, the Tsepalov graphical method can be used to calculate the kinetic parameters of oxidation.

According to the theory of chain radical processes involving oxidation inhibitors the oxidation rate equation is as follows [10]:

$$W = \frac{k_{p2} \cdot [RH]}{k_7 \cdot f \cdot [lnH]} \cdot W_i, \qquad (1)$$

where k_7 is constant of the rate of chain breaking (constant of rate of interaction of peroxide radicals with natural antioxidants); f is inhibition factor (number of free radicals «dying» on one molecule of inhibitor); $[lnH]$ is concentration of antioxidant (inhibitor); W_i is initiation rate; k_{p2} is constant of chain expansion; $[RH]$ is product concentration.

The inhibitor concentration changes with time according to the formula

$$f \cdot [lnH]_t = f \cdot [lnH]_0 - W_i \cdot t, \qquad (2)$$

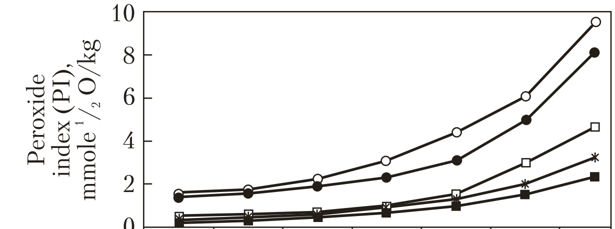
Where $[lnH]_t$ t is inhibitor concentration at time t; $[lnH]_0$ is initial inhibitor concentration (at the beginning of the process).

Induction period τ is an important parameter of the chain process. The higher is the constant of chain breaking and the lower is the rate of build-

Table 1

Tocopherol Content in the Oil Samples

Oil sample	Total content, mg %	Isomers, % of total content			Total content, mg%, (according to Codex Alimentarius)
		α	β	γ + δ	
Sunflower pressed	95	91.5	8.5	–	40.3–102.1
Walnut pressed	109	46.4	48.0	5.6	56.0–113.0
Maize refined deodorized	655	49.1	42.4	8.5	31.4–347.2
Palm	10	73.7	26.3	–	13.0–45.3
Palm kernel	56	27	73	–	28.9–130.1



	0	10	20	30	40	50	60
○ Sunflower oil	1.6	1.75	2.25	3.1	4.4	6.1	9.5
● Walnut oil	1.4	1.6	1.9	2.3	3.09	5	8.13
□ Maize oil	0.5	0.6	0.65	1	1.54	3	4.63
■ Palm oil	0.24	0.33	0.49	0.65	1	1.54	2.36
⁎ Palm kernel oil	0.31	0.45	0.6	1	1.3	2.04	3.23

Period of spontaneous oxydation, days

Fig. 1. Dynamics of spontaneous oxidation of oils

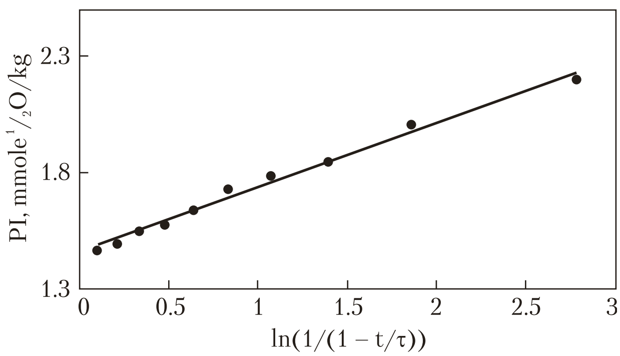


Fig. 2. Oxidation rate in logarithmic coordinates (pressed walnut oil)

up of chains, the longer is the induction period. Practically, the induction period is the guaranteed shelf life of fat-and-oil products.

The induction period τ (time) during which the chains are broken on inhibitor molecules is equal to:

$$\tau = f \cdot [lnH]_0 / W_i, \qquad (3)$$

provided $[lnH]_t = 0$ and $t = \tau$.

Based on the kinetic curves (Fig.1) the induction period was determined by approximation of tangent lines in the initial area for each sample.

For each moment within the induction period the value of ln $(1/(1 - t/\tau))$ was computed and PN — ln $(1/(1 - t/\tau))$ dependence was built (Fig. 2).

The parameter k_{p2}/k_7, (the ratio of constants of expanding and breaking of chains within the induction period) is calculated as tgα where α is angle of slope of PN – ln $(1/(1 - t/\tau))$. curve. The results are showed in Table 2.

The palm and palm kernel oils have the best parameters: k_{p2}/k_7 is equal to 1.84 та 2.66, respectively. This means that the rate of breaking of oxidation chain on inhibitor molecules exceeds the rate of expanding. The initiation rate during spontaneous oxidation of oils is determined by their composition in terms of fat acids (all other factors held equal), primarily, mono- and polyunsaturated acids [8]. As the content of saturated fatty acids in the glycerol structure increases, the initiation rate Wi slows down.

Since the initiation rate for linoleic acid is higher about ten times than that for oleic one [4, 7], the initiation rate for the oils having a higher content of polyunsaturated fatty acids should be higher, and, consequently, the induction period should be shorter. It has been reported for the oxidation of saturated tropical oils.

The activity of natural antioxidants was measured by the method with stable chromogen radical DPPH (2,2-diphenyl-1-picrylhydrazyl) [11—14]. To prepare the working solutions, 96 % methyl alcohol was used; initial concentration of DPPN in the reaction mixture was $7 \cdot 10^{-5}$ mole/l. The discoloration of DPPN solutions after introducing oils was determined by spectrophotometric method, at a wavelength of 515 nm. The reaction took place without access of light, in 10 mm thick quartz cuvettes, with α-tocopherol as reference substance. The DPPH radical dissolved in methanol reacts with antioxidant sample according to the scheme: DPPH•+AH= DPPH-H +A.

As a result of DPPH deoxidation by antioxidant from the sample, the purple blue DPPH discolors, with the reaction controlled by changing optical density. The measurements were made each 15 minutes during 1 hour.

The antioxidant activity of the samples was calculated by the formula:

$$AA = [1 - (A_1 - A_2) / A_3] \times 100\ \%, \qquad (4)$$

where A_1 is absorption of the sample with DPPH

ISSN 2409-9066. Sci. innov. 2015, 11(4)

solution, A_z is absorption of the sample with methanol solution, and A_z is absorption of DPPH solution in methanol.

The antioxidant activity of oil samples was determined using the calibration curve showed in Fig. 3.

The results are expressed through value of EC50 parameter that is antioxidant concentration at which 50 % inhibition of DPPH radical takes place. The results are given in Table 3.

According to the data of reaction with DPPH, the most stable to oxidation processes is refined maize oil. This can be explained by a high content of natural antioxidants (tocopherol isomers) that act as inhibitors of oxidation. Tropical oils characterized by a low content of natural antioxidants are oxidized rapidly at the early stages of reacting with DPPH, which is clearly seen from the comparison: the palm oil containing 10 mg % tocopherol deteriorates faster than the palm kernel oil containing 56 mg % tocopherols. The sunflower oil and the maize one differ insignificantly in terms of oxidation rate at the initial stages. This can be explained by both similar extraction method (pressing) and similar inhibitor contents.

CONCLUSIONS

The kinetic parameters of oxidation for several oils have been computed by the Tsepalov graphical method. The tocopherol isomers in vegetable oils have been established to compete with the fatty acids during the oxidation in the course of storage of the oils.

The antioxidant activity of oil natural antioxidants has been estimated using the DPPH radical method. The results correlate well with the content of tocopherols and indicate their dominant role in the total activity with respect to this radical.

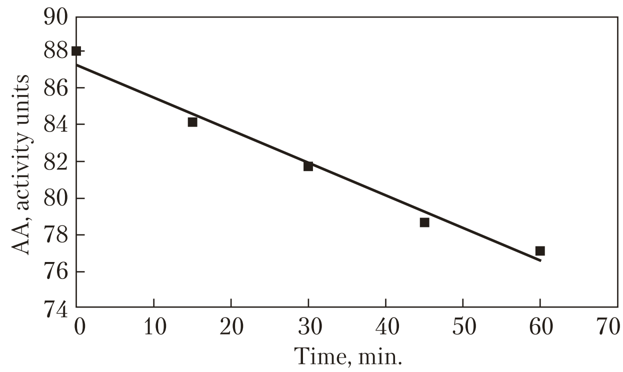


Fig. 3. Calibration curve of antioxidant activity (palm oil)

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Table 2

Kinetic Parameters of Oil Oxidation

Oil sample	$(k_{p2}/k_7) \cdot 10^2$, (l/mole s)
Sunflower pressed	2.82
Walnut pressed	3.07
Maize refined deodorized	3.88
Palm	1.84
Palm kernel	2.66

Table 3

Antioxidant Activity of the Samples

Oil sample	AA, activity units
Sunflower pressed	50
Walnut pressed	53.2
Maize refined deodorized	45
Palm	81.8
Palm kernel	77.3

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КІНЕТИЧНІ ХАРАКТЕРИСТИКИ ПРИРОДНИХ АНТИОКСИДАНТІВ РОСЛИННИХ ОЛІЙ

Проведено визначення показників окиснюваності соняшникової, кукурудзяної, горіхової, пальмової та пальмоядрової олій графічним методом Цепалова та методом DPPH. Показано вплив природних антиоксидантів олій та ступеня ненасиченості жирних кислот на швидкість процесу окиснення. Методи можуть бути використані з метою прогнозування окиснювальної стабільності олій при зберіганні.

Ключові слова: олії, окиснення, графічний метод Цепалова, кінетичні параметри, метод DPPH, антиоксидантна активність.

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КИНЕТИЧЕСКИЕ ХАРАКТЕРИСТИКИ ПРИРОДНЫХ АНТИОКСИДАНТОВ РАСТИТЕЛЬНЫХ МАСЕЛ

Определены показатели окисляемости подсолнечного, кукурузного, пальмового, пальмоядрового масел и масла грецкого ореха графическим методом Цепалова и методом DPPH. Показано влияние природных антиоксидантов растительных масел и степени ненасыщенности жирных кислот на скорость процесса окисления. Методы могут быть использованы для прогнозирования окислительной стабильности растительных масел при хранении.

Ключевые слова: растительные масла, окисление, графический метод Цепалова, кинетические параметры, метод DPPH, антиоксидантная активность.

Received 12.01.15