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DETERMINATION OF FAT-SOLUBLE DYES INFLUENCE ON THE OXIDATION INDUCTION PERIOD OF THEIR OIL SOLUTIONS

Pavlo Petik
Corresponding author
PhD, Director
Ukrainian Scientific Research Institute of Oils and Fats of the National Academy of Agricultural Sciences of Ukraine
Dziuba ave., 2-A, Kharkiv, Ukraine, 61019
E-mail: petik.pavlo.f@gmail.com
Serhii Stankevych
PhD*
Inna Zabrodina
PhD*
Oksana Zhulinska
PhD
Cycle Commission Hotel and Restaurant Business
Separate Structural Subdivision "Housing and Municipal Professional College of O. M. Beketov National University of Urban Economy in Kharkiv"
Shevchenka str., 233A, Kharkiv, Ukraine, 61033
Iryna Mezentseva
PhD, Associate Professor
Department of Occupational and Environmental Safety
National Technical University "Kharkiv Polytechnic Institute"
Kyrpychova str., 2, Kharkiv, Ukraine, 61002
Ivan Haliasnyi
PhD***
Tatyana Hontar
PhD***
Lidiia Shubina
PhD, Associate Professor
Cyclical Commission of Food Technology and Hotel-Restaurant Business
A separate structural unit "Kharkiv Trade and Economic Vocational College State University of Trade and Economics"
Otakara Yarosha side str., 8, Kharkiv, Ukraine, 61045
Oleh Kotliar
PhD
Department of Food Technology in the Restaurant Industry**
Svitlana Bondarenko
PhD
Department of Agrochemistry**
*Department of Zoology, Entomology, Phytopathology, Integrated Protection and Quarantine of Plants named after B. M. Litvynova**
**State Biotechnological University
Alchevskykh str., 44, Kharkiv, Ukraine, 61002
***Department of Restaurant, Hotel and Tourist Business
Ukrainian Engineering Pedagogics Academy
Universitetskaya str., 16, Kharkiv, Ukraine, 61003

The object of the study is the period of induction of accelerated oxidation of oil solutions of fat-soluble dyes chlorophyll and beta-carotene. The effect of the content of fat-soluble dyes on the period of induction of accelerated oxidation of their solutions in refined sunflower oil was studied. The peculiarity of the work consists in establishing approximate graphic and mathematical dependences of the term of the induction period of accelerated oxidation of refined sunflower oil on the content of chlorophyll and beta-carotene separately and together. This is important for predicting the shelf life of oil solutions of fat-soluble dyes.

It was determined that chlorophyll A practically does not show a pro-oxidant effect if its content is up to 0.05 g/l. The content of chlorophyll A at the level of 0.10 g/l leads to a reduction in the duration of the induction period by 14 %; 0.20 g/l – by 36 %; 0.30 g/l – by 48 %. The content of beta-carotene at the level of 0.10 g/l leads to an increase in the duration of the induction period by 35 %; 0.20 g/l – by 47 %; 0.30 g/l – by 54 %. The content in the oil system of 0.10 g/l of beta-carotene and 0.05 g/l of chlorophyll A leads to a reduction in the period of induction of accelerated oxidation by 8.4 % compared to the oil solution of 0.10 g/l of beta-carotene without chlorophyll A. The obtained data are explained by the fact that there is a compensatory effect of the antioxidant beta-carotene on the pro-oxidant effect of chlorophyll A in the oil solution. A feature of the obtained results is the possibility of predicting the shelf life of oil solutions of fat-soluble dyes. From a practical point of view, the results of the research allow to develop oil systems taking into account the separate and compatible features of reactivity to the oxidation of chlorophyll and beta-carotene. An applied aspect of using the scientific result is the possibility of expanding the assortment of oil products of increased nutritional value with different contents of chlorophyll and beta-carotene

Keywords: chlorophyll, beta-carotene, antioxidant, prooxidant, accelerated oxidation, differential scanning calorimetry

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1. Introduction

An important task of the oil and fat industry is to ensure the quality of vegetable oils, both during production and during storage. All oil-fat products are prone to oxidative deterioration. The main factor affecting the degree of oxidation of oils is the fatty acid composition, namely the presence of unsaturated fatty acids and their combined arrangement in the triacylglycerol molecule. The resistance to oxidation of fatty acids and oils in general is also affected by the presence of accompanying substances – phospholipids, tocopherols, carotenoids, chlorophyll. These compounds, depending on their composition and quantity, can inhibit the oxidation process or, on the contrary, accelerate it [1].

The biological effectiveness and nutritional value of oils depends on the composition, conditions and terms of storage. As a result of oxidation processes, primary (peroxides and hydroperoxides) and secondary (carbonyl compounds, polymers) oxidation products can accumulate in oils, which worsens the organoleptic and physicochemical parameters, as well as the stability of the oil during storage [2]. Oxidation products lead to a change in the main organoleptic characteristics of the product (taste, smell) and a decrease in its nutritional value. In addition, oxidation products can pose a danger to human health. Therefore, the speed of the oxidation process and the characteristics of the degree of oxidation of oils are determined not only by the indicator "peroxide number", which reflects the content of primary oxidation products in the oil, but also by a whole set of indicators [3, 4]. Therefore, research aimed at predicting the shelf life of oils and products based on them will allow to reveal the dependence of the duration of storage on the composition of raw materials, predict the shelf life of products, and optimize a number of production processes [2].

Scientific studies on the identification of patterns of influence of substances accompanying vegetable oils on the oxidative deterioration of oil products are relevant. The results of such research are needed for production due to the need to predict the shelf life of such products, including unrefined oils, vitaminized oils and solutions of fat-soluble biologically active compounds.

2. Analysis of literary data and formulation of the problem

The process of oxidation of edible oils is influenced by the following factors [1]:

– incoming energy (light or heat);

– composition of fatty acids;

– degree of activation of oxygen atoms;

– the content of accompanying substances (tocopherols, phospholipids, free fatty acids, mono- and diglycerides, thermally oxidized compounds, chlorophylls, carotenoids).

It is interesting how the content of accompanying substances affects the stability of oils to oxidative deterioration.

The work [5] gives the results of studies on the influence of the fatty acid composition, as well as the composition of tocopherol isomers, on the stability to oxidative deterioration of the oils of sorghum and descourainia sofia in the dark and in the light. It was shown that an increase in the content of α–linolenic fatty acid in oil samples plays a negative role in terms of stability to oxidation in the presence and absence of ultraviolet radiation. The content of tocopherols has a positive effect on increasing the induction period under the condition of oxidation under light. However, questions related to the influence of other accompanying substances, in particular phospholipids and fat-soluble dyes (chlorophyll and carotenoids) on stability to oxidation, remained unresolved. The reason for this was the use of refined oils in the study. An option to overcome the relevant difficulties may be to change the research materials from refined to unrefined. This is the approach used in work [6], which presents an overview of the antioxidant properties of polar, non-polar and amphiphilic lipids, in particular phospholipids contained in edible oils. Probable mechanisms of interaction between different types of phospholipids were also clarified. In the work, the issue of complex mechanisms of antioxidant and pro-oxidant action of saponifiable accompanying substances (phospholipids) and unsaponifiable accompanying substances (phytosterols, vegetable dyes, etc.) remains undefined.

The question of the effect of the content of natural (tocopherol, anthocyanins) and synthetic (butyloxytoluene, butyloxyanisole, propyl gallate) antioxidants, heavy metals, as well as mono- and diacylglycerols on the period of induction of oxidation of triglycerides in soybean oil was considered in [7]. An overview of the mechanisms of reactions of natural and synthetic antioxidants with free radicals is presented. But in the study, the aspect of influence on stability to oxidation of fat-soluble dyes in unrefined or vitaminized oils remains uncertain. An attempt to investigate the effect of chlorophyll on the dynamics of pumpkin oil oxidation is based on the results of work [8], where the thermal oxidative stress of pumpkin oil at a temperature of 120 °C was simulated. Along with the high content of tocopherols, pumpkin oil is characterized by the presence of chlorophyll. This can reduce the antioxidant properties of the oil during storage or heat treatment, contributing to the formation of toxic oxidation products. IR spectroscopy was used to study changes in oil composition during oxidation. But in this work, the issue of clarifying the actual relationship between the content of accompanying substances and the stability of the oil to oxidative deterioration remained undefined.

In [9], the influence of biologically active substances of rice oil on its resistance to oxidation was investigated. The use of IR spectroscopy to determine the dynamics of rice oil oxidation made it possible to calculate oxidation coefficients that characterize the oxidation process by the stages of nucleation and chain breaking. It was established that the abnormally high content of vitamin E in rice oil, even with a significant amount of γ-oryzanol, exerts a pro-oxidant effect during accelerated oxidation. The lack of research on the content of tocopherol in rice oil samples of various types makes it impossible to comprehensively analyze its contribution, together with γ-oryzanol, to the oxidative stability of the oil.

According to the considered works, accompanying substances of oils, in particular fat-soluble dyes, have different effects on the stability to oxidation of lipids. Their effect can be both antioxidant and pro-oxidant.

Chlorophylls are pigments characteristic of oils, they are usually removed during the bleaching stage. Their content in crude olive oil is 12 ppm, in rapeseed 2–18 ppm [10]. In addition, the work determined that the oxidation of raw olive oil containing pheophytin is accelerated under the influence of light from fluorescent lamps. In crude and bleached soybean oil, the chlorophyll content is 0.25 and 0.10 ppm, respectively. In the work, the question of how the stability to oxidation of the oil depends on the chlorophyll content remains undefined.

In [11], it was shown that rapeseed oil purified on a silicic acid column did not contain any chlorophylls and did not form volatile compounds in the space above the oil under the influence of light at 10 °C. But refined rapeseed oil with chlorophyll addition and refined, bleached and deodorized rapeseed oil formed volatile compounds under the given experimental conditions. In the study [11], the question remains as to how chlorophyll interacts with other fat-soluble dyes during the oxidation of oils, in particular with such a common accompanying substance of oils as beta-carotene.

Beta-carotene slows oil oxidation by partially absorbing light, inactivating sensitizers and neutralizing free radicals. Crude palm oil and red palm olein contain 450–750 ppm carotenoids. In crude olive oil, the content of beta-carotene is 1.2–2.9 ppm and lutein is 0.8–2.2 ppm [12]. The work does not determine which content of beta-carotene is effective in inhibiting oxidative processes.

In work [13] it is stated that the rate of oxidation of olive oil, which contains only beta-carotene, in the light at 25 °C decreased under the condition of using light filters that absorb light energy in the range of 380–520 nm. The antioxidant activity of carotenoids was not manifested when the oil was stored in the dark. It is worth noting that the complex effect of beta-carotene and other fat-soluble dyes, in particular chlorophyll, on oxidative processes occurring in oils was not investigated in [13].

In [14], it was determined that in the presence of chlorophylls, beta-carotene has an inhibitory effect on the oxidation processes of soybean oil stored in the light. It is assumed that such antioxidant processes occur mainly due to the transfer of singlet oxygen energy to carotenoids without the formation of oxidation products. But the question of the influence of different ratios of vegetable dyes on the oxidative stability of oils remained unresolved.

Thus, there is not enough scientific data on the influence of minor components on the oxidation stability of vegetable oils. There are no data on the effect of separate and combined content of fat-soluble dyes chlorophyll and beta-carotene on the induction period of accelerated oxidation of refined sunflower oil. Thus, it is expedient to determine the dependence of the influence of fat-soluble dyes on the oxidation stability of sunflower oil. Such development will allow to rationalize a number of technological processes of oil and fat production, to determine the dependence of the duration of storage of oil products on the composition and content of fat-soluble dyes. In addition, based on the obtained data, it will be promising to predict the shelf life of oil products enriched with fat-soluble dyes.

3. The aim and objectives of the study

The aim of the study is to determine the influence of the content of fat-soluble dyes on the period of induction of oxidation of their oil solutions. This will make it possible to predict the shelf life of oil solutions of fat-soluble dyes. Also, the obtained data will be useful for justifying the refining of oils by non-traditional methods to preserve biologically active substances in oils.

To achieve the set aim, it is necessary to solve the following problems:

– to determine the quality indicators of research materials – sunflower oil, chlorophyll oil concentrate, beta-carotene oil concentrate;

– to determine the influence of the content of individual chlorophyll and beta-carotene dyes on the induction period of accelerated oxidation of refined sunflower oil;

– to determine the effect of the combined content of chlorophyll and beta-carotene dyes on the induction period of accelerated oxidation of refined sunflower oil.

4. Research materials and methods

4. 1. Object and research hypothesis

The object of the study is the period of induction of accelerated oxidation of oil solutions of fat-soluble dyes chlorophyll and beta-carotene. The main hypothesis of the study is an increase in the period of induction of oxidation of vegetable oils during the addition of fat-soluble dyes chlorophyll and beta-carotene, which have a certain antioxidant effect on the process of free radical oxidation of triglycerides of oils and fats.

The research assumes that the period of induction of oxidation of oils and oil solutions of fat-soluble dyes under recommended storage conditions is directly proportional to their period of induction of oxidation under accelerated conditions. The recommended storage conditions are storage in closed darkened rooms in the temperature range from 0 °C to +20 °C [15]. Accelerated oxidation conditions are oxidation at a temperature of +110 °C with free access of oxygen.

The following simplifications are adopted in the study:

– the content of chlorophyll *A* (absorption spectrum at a wavelength of 665 nm) was determined in the composition of the chlorophyll oil concentrate without taking into account other forms of chlorophyll (absorption spectrum at a wavelength of 650 nm) [16]. In this way, the influence of the content of trace amounts of other forms of chlorophyll, predominantly hydrophilic, on the period of induction of oxidation of sunflower oil is ignored;

– in the beta-carotene oil concentrate, the content of beta-carotene was determined (absorption spectrum at a wavelength of 450 nm) without taking into account the accompanying carotenoids. In this way, the influence of the content of accompanying carotenoids in the oil extract of beta-carotene on the period of induction of oxidation of sunflower oil is ignored;

– the study did not take into account the effect of the presence of tocopherols on the oxidative stability of chlorophyll A and beta-carotene oil solutions. According to the results of screening studies, it is assumed that the content of tocopherols in oil solutions of fat-soluble dyes is in a constant amount at the level of 150...200 mg/kg;

– refined sunflower oil from different manufacturers and different batches of production has practically identical indicators of composition and quality, which affect stability to oxidative deterioration. Indicators of composition and quality are the fatty acid composition, the content of moisture and volatile substances, the content of free fatty acids, as well as primary and secondary oxidation products. This simplification should prove obtaining a certain repeatability of the dependences of the influence of the content of the specified fat-soluble dyes on the oxidation stability of sunflower oil determined during the research.

4. 2. Researched materials and equipment used in the experiment

The following materials were used during the research:

– refined, bleached and deodorized sunflower oil (produced in Ukraine), according to DSTU 4492/CAS 8001-21-6;

– chlorophyll oil concentrate (produced in Ukraine), according to registration certificate UA/1556/02/01/CAS 479-61-8, CAS 519-62-0;

– beta-carotene oil concentrate (produced in Ukraine), according to UA/2685/01/01/CAS:225234-03-7.

4. 3. Methodology for determining physico-chemical parameters and indicators of the composition of oil samples

Determination of the acid value of sunflower oil samples, oil concentrates of chlorophyll and beta-carotene was carried out in accordance with DSTU ISO 660. The peroxide number of oil samples was determined according to DSTU ISO 3960. The mass fraction of moisture and volatile substances in oil samples was determined according to DSTU 4603. Chlorophyll content (in conversion to chlorophyll *A*) in samples of oils and oil solutions was determined according to ISO 10519. The content of beta-carotene in samples of oils and oil solutions was determined according to ISO 6558. The research used solutions of fat-soluble dyes in refined sunflower oil in the ranges of chlorophyll *A* content 0... 0.10 g/l and beta-carotene 0...0.1 g/l.

Methyl esters of fatty acids of the samples of the studied oils were prepared according to DSTU ISO 5509. The fatty acid composition of the samples of the studied oils was determined according to DSTU ISO 5508 on a Shimadzu chromatograph (Japan).

4. 4. Method of obtaining solutions of fat-soluble dyes in refined deodorized sunflower oil

Samples of solutions of fat-soluble dyes of different concentrations in refined deodorized sunflower oil were obtained by mixing the calculated amount of oil extracts of dyes and sunflower oil. The obtained solutions were subjected to exposure at a temperature of 40 °C for 10 minutes, followed by homogenization at a speed of at least 1000 rpm within 5 minutes.

4. 5. The method of determining the induction period of accelerated oxidation using differential scanning calorimetry

Determination of the induction period of the studied oil samples was carried out by the method of differential scanning calorimetry at a temperature of +110 °C in accordance with the recommendations [17]. This is an instrumental method that allows to track exothermic and endothermic changes in samples that occur as a result of chemical reactions of free radical oxidation of lipids.

The method consists in determining the time during which the antioxidant stabilizing system, present in the oil sample for testing, prevents oxidation under the condition of exposure of the sample in an isothermal mode at a given temperature in an oxidizing gas atmosphere. The time of the oxidation induction period is an estimate of the level (or degree) of stabilization of the tested oil material. High test temperatures result in a shorter oxidation induction period.

The weight of the sample for research is from 5 mg to 15 mg. The sample is weighed into a clean, dry crucible. Load the sample crucible and reference crucible at ambient temperature. Before the heating cycle, the device is purged with nitrogen for 5 minutes. The sample and the reference material are heated at a constant rate in an inert gas (nitrogen) medium at a rate of 20 °C/min to 110 °C. When the specified temperature is reached, the inert gas environment is replaced by an oxygen environment at a constant flow rate (50±5) cm^3/min. The sample is then kept at a constant temperature until the oxidation reaction is displayed on the thermal curve. The isothermal induction period is the time between the beginning of oxygen supply to the measuring cell and the beginning of the oxidation reaction. The beginning of oxidation is indicated by a sharp increase in the released heat, which can be observed on the curve of the dependence of heat flow on temperature. The end of the period of induction of oxidation of the sample substance is marked by an increase in reaction enthalpy due to an increase in the rate of oxidation of unsaturated lipids with oxygen molecules. The shelf life of an oil sample is directly proportional to the value of the oxidation induction period.

4. 6. Research planning and results processing

One-factor and two-factor experiments were used to determine the influence of the content of chlorophyll *A* and beta-carotene on the induction period of accelerated oxidation of sunflower oil. Each experiment was repeated three times. Statistical models of the specified dependencies were calculated using the approximation of experimental data by constructing a trend line. Processing of the obtained data and construction of graphical dependencies was performed using Microsoft Excel (USA) and Stat Soft Statistica v 6.0 (USA) packages.

The adequacy of the obtained regression equations (1) and (2) was checked by the coefficients of determination (R^2), which are equal to 0.982 and 0.964, respectively.

The significance of individual coefficients of the regression equation (3) was carried out using the Student's test (t) by testing the hypothesis that the corresponding parameter of the equation is equal to zero. The calculated absolute value of the Student's criterion t (8) when evaluating individual regression coefficients was compared with its critical tabular value $t_{table}(8)=2.31$ at the significance level $p=0.05$ and the number of degrees of freedom for multiple regression $df=8$. Data and conclusions regarding the determination of the significance of the coefficients of the regression equation (3) are given in Table 1.

Table 1

Conclusions on determining the significance of the coefficients of the regression equation (3)

The coefficient of the regression equation at	The value of the coefficient in natural quantities	t (8)	T_{table} (8)	Estimated probability of the null hypothesis for the coefficient of the regression equation (p-level)	Conclusion on the significance of the coefficient
Intercept	326.8056	8.59241	2.31	0.000263	Significant
c_{cp}	128.3333	5.38437		0.001854	Significant
c_{bc}	1091.6667	4.67115		0.008391	Significant

To assess the quality of the regression equation (3) and the completeness of the influence of the selected factors, the coefficient of determination R^2 was determined. The obtained value of $R^2=0.92$ allows to conclude about a very significant influence (greater than 92 %) of variations in the content of chlorophyll *A* and beta-carotene on variations in the value of the induction period of accelerated oxidation of oil solutions. To establish the significance of the regression model, Fisher's test (F) was calculated, based on the assumption that the equation is statistically insignificant ($R^2=0$;

null hypothesis). The calculated value of the Fisher test was $F(2,8)=14.372$ and was greater than its critical tabular value $F_{tab}(2,8)=4.46$ at the significance level $p=0.05$ and the number of degrees of freedom $df_1=2$ and $df_2=8$. This result allows to reject the null hypothesis and with a probability of 0.92 (or 92 %) to recognize the value of the coefficient of determination $R^2=0.92$ as significant, and the model as significant.

5. Results of research on the influence of fat-soluble dyes on the oxidation stability of sunflower oil

5. 1. Determination of quality indicators of sunflower oil and oil concentrates of fat-soluble dyes

The physico-chemical parameters of the samples of refined deodorized sunflower oil, oil concentrates of chlorophyll and beta-carotene were determined. The results of the study are shown in Table 2.

The content of fat-soluble dyes in the samples of refined deodorized sunflower oil, oil concentrates of chlorophyll and beta-carotene that were studied was determined. The results are given in the Table 3.

Table 2

Physico-chemical indicators of the investigated samples of oil raw materials

Physico-chemical indicators	Samples under study		
	Sunflower oil	Chlorophyll oil concentrate	Beta-carotene oil concentrate
Acid number, mg of KOH/g	0.100±0.005	0.85±0.04	1.05±0.05
Peroxide number, mmol ½O/kg	0.220±0.011	0.42±0.02	0.65±0.03
Mass fraction of moisture and volatile substances, %	0.0100±0.0005	0.120±0.005	0.080±0.004

Table 3

The content of fat-soluble dyes in samples of refined deodorized sunflower oil, oil concentrates of chlorophyll and beta-carotene

The name of fat-soluble dyes	Samples under study		
	Sunflower oil	Chlorophyll oil concentrate	Beta-carotene oil concentrate
Chlorophyll, g/l	–	10.50±0.51	0.010±0.005
Beta-carotene, g/l	–	0.020±0.008	3.56±0.17

According to the results of the experiments (Tables 2, 3), the studied samples of oil raw materials meet the requirements established in the relevant regulatory documentation – DSTU 4492/CAS 8001-21-6; UA/1556/02/01/CAS 479-61-8; UA/2685/01/01/CAS 7235-40-7.

The fatty acid composition of refined deodorized sunflower oil samples, chlorophyll and beta-carotene oil concentrates, which were selected for the study, was determined. The obtained data are shown in Table 4.

According to the results of experiments, the fatty acid composition of sunflower oil meets the requirements of DSTU 4492/CAS 8001-21-6. The results of studies of the fatty acid composition of oil concentrates of chlorophyll and beta-carotene confirm that the oil base is corn and sunflower oils, respectively.

Table 4

Fatty acid composition of samples of refined deodorized sunflower oil, oil concentrates of chlorophyll and beta-carotene

Name of fatty acids	Samples under study, % of the total amount		
	Sunflower oil	Chlorophyll oil concentrate	Beta-carotene oil concentrate
Myristic acid $C_{14:0}$	0.040±0.002	0.070±0.003	0.020±0.001
Palmitic acid $C_{16:0}$	6.480±0.324	8.320±0.416	5.700±0.285
Palmoleic acid $C_{16:1}$	0.120±0.006	0.260±0.013	0.160±0.008
Palmitic acid $C_{18:0}$	3.650±0.182	2.170±0.108	4.440±0.221
Oleic acid $C_{18:1}$	26.180±1.309	31.80±1.58	25.040±1.252
Linoleic acid $C_{18:2}$	62.880±3.144	56.51±2.52	64.030±3.201
Linolenic acid $C_{18:3}$	0.030±0.001	0.320±0.016	0.070±0.003
Arachic acid $C_{20:0}$	0.150±0.007	0.120±0.006	0.080±0.004
Gondoic acid $C_{20:1}$	0.070±0.003	0.120±0.006	0.020±0.001
Behenic acid $C_{22:0}$	0.40±0.02	0.310±0.015	0.440±0.022
Sum	100.00	100.00	100.00

5. 2. Determination of the influence of the content of individual dyes on the induction period of accelerated oxidation of their oil solutions

One-factor dependences of the induction period of accelerated oxidation of solutions of fat-soluble dyes in refined sunflower oil on the individual content of chlorophyll *A* and beta-carotene were studied.

Using equations (1) and (2), the approximate dependences of the induction period of accelerated oxidation of solutions of fat-soluble dyes in refined sunflower oil (*IP*, min.) are presented, respectively, on the content of chlorophyll *A* (c_{ch}, g/l) and beta-carotene (c_{bc}, g/l).

$$IP(c_{ch}) = -0.2619 \cdot c_{ch}^2 - 26.619 \cdot c_{ch} + 363.71, \quad (1)$$

$$IP(c_{bc}) = -5.2381 \cdot c_{bc}^2 + 71.476 \cdot c_{bc} + 260.71. \quad (2)$$

One-factor dependencies of the induction period of accelerated oxidation of solutions of fat-soluble dyes in refined sunflower oil on the individual content of chlorophyll *A* and beta-carotene were studied.

Using equations (1) and (2), the approximate dependencies of the induction period of accelerated oxidation of solutions of fat-soluble dyes in refined sunflower oil (*IP*, min.) are presented, respectively, on the content of chlorophyll *A* (c_{ch}, g/l) and beta-carotene (c_{bc}, g/l).

Increasing the chlorophyll *A* content in refined sunflower oil leads to a decrease in the period of induction of accelerated oxidation relative to the period of induction of accelerated oxidation of the control sample (refined sunflower oil without the addition of dye). As evidenced by the results of research (Fig. 1), chlorophyll *A* practically does not show a pro-oxidant effect if its content is up to 0.05 g/l. Accordingly, the content of chlorophyll *A* in the oil at the level of 0.10 g/l leads to a reduction of the induction period by 14 %; 0.20 g/l – by 36 %; 0.30 g/l – by 48 %.

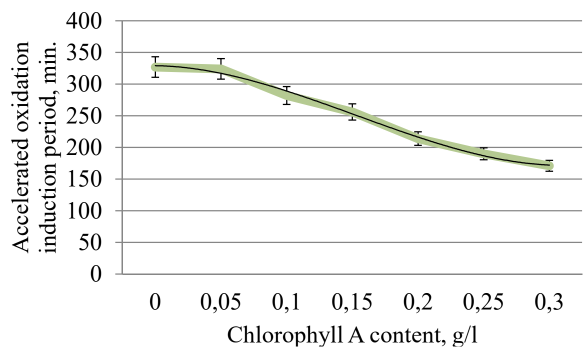

Fig. 1. Dependence of the induction period of accelerated oxidation of chlorophyll *A* solutions in refined sunflower oil (*IP*, min.) on the chlorophyll *A* content (c_{ch}, g/l)

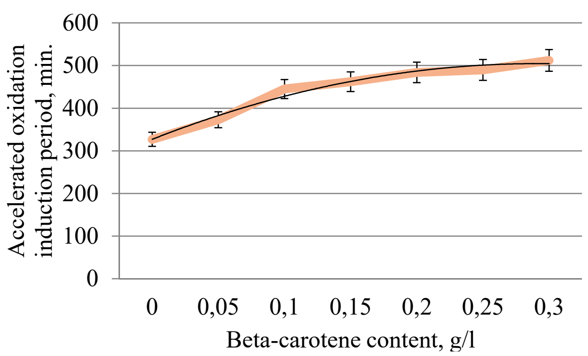

Fig. 2. Dependence of the induction period of accelerated oxidation of beta-carotene solutions in refined sunflower oil (*IP*, min.) on the content of beta-carotene (c_{bc}, g/l)

Increasing the beta-carotene content in the solution in refined sunflower oil, on the contrary, leads to an increase in the period of induction of accelerated oxidation relative to the period of induction of accelerated oxidation of the control sample (refined sunflower oil without beta-carotene). Accordingly, the content of beta-carotene in the oil at the level of 0.10 g/l leads to an increase in the induction period by 35 %; 0.20 g/l – by 47 %; 0.30 g/l – by 54 %.

Considering the obtained results of the experiments (Fig. 1, 2), it is of interest to study the influence of the combined content of chlorophyll *A* and beta-carotene dyes in oil solutions on the period of induction of accelerated oxidation.

5. 3. Determination of the influence of the compatible content of dyes on the induction period of accelerated oxidation of oil solutions

The two-factor dependence of the induction period of accelerated oxidation of oil solutions of fat-soluble dyes in refined sunflower oil on the content of chlorophyll *A* and beta-carotene was investigated.

Equation (3) shows the approximate dependence of the induction period of accelerated oxidation of oil solutions of fat-soluble dyes in refined sunflower oil (*IP*, min.) on the combined content of chlorophyll *A* (c_{ch}, g/l) and beta-carotene (c_{bc}, g/l):

$$IP(c_{ch},c_{bc}) = 326.8056 + 128.3333 \cdot c_{ch} + \\ +1091.6667 \cdot c_{bc} - 5333.3333 \cdot c_{ch}^2 - \\ -4700 \cdot c_{ch} \cdot c_{bc} + 66.6667 \cdot c_{bc}^2. \quad (3)$$

In the Table 5 shows the matrix of the planning of the experiment, as well as the experimental and calculated values of the induction period of accelerated oxidation of oil solutions of fat-soluble dyes in refined sunflower oil from the compatible content of dyes. Estimated values of the response function were obtained by regression equations (3).

Table 5

Dependence of the induction period of accelerated oxidation of oily solutions of chlorophyll *A* and beta-carotene in refined sunflower oil on the combined content of fat-soluble dyes

Sample No	The content of fat-soluble dyes in oil solutions		The induction period of accelerated oxidation, *IP* (c_{ch}, c_{bc}), min.	
	chlorophyll *A*, c_{ch}, g/l	beta-carotene, c_{bc}, g/l	Experiment	Calculation
1	0.00	0.00	327±13	327
2	0.00	0.05	373±15	380
3	0.00	0.10	445±18	438
4	0.05	0.00	324±13	320
5	0.05	0.05	371±15	363
6	0.05	0.10	408±16	411
7	0.10	0.00	282±11	286
8	0.10	0.05	318±13	318
9	0.10	0.10	353±14	350

The surface of the obtained dependence of the induction period of accelerated oxidation of oil solutions of fat-soluble dyes in refined sunflower oil on the compatible content of fat-soluble dyes is presented in Fig. 3.

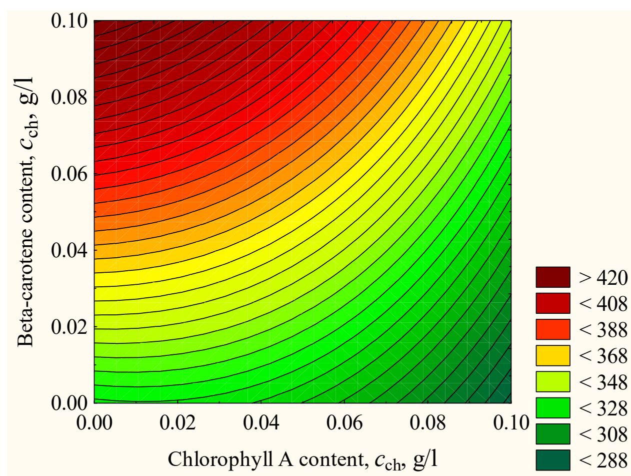

Fig. 3. Dependence of the period of induction of accelerated oxidation of oil solutions of fat-soluble dyes on the compatible content of fat-soluble dyes

Approximation dependence (3) adequately describes the periods of induction of accelerated oxidation of solutions of fat-soluble dyes in refined sunflower oil in the ranges of chlorophyll *A* content 0...0.10 g/l and beta-carotene 0...0.1 g/l.

The results of the conducted studies indicate a certain compensatory effect of beta-carotene on the pro-oxidant effect of chlorophyll *A* in a solution of refined sunflower oil. For example, the presence of 0.05 g/l of chlorophyll *A* in an oil solution slightly affects the antioxidant effect of beta-carotene. If 0.05 g/l beta-carotene is added to this system, the induction period of accelerated oxidation will be the same as in the oil solution without chlorophyll *A* (371 and 373 min, respectively). If 0.10 g/l beta-carotene is added to this system, the induction period of accelerated oxidation will be 8.4 % shorter than in the oil solution without chlorophyll *A* (408 and 445 min, respectively).

Under the condition of a separate content of chlorophyll *A* at the level of 0.10 g/l, the period of induction of accelerated oxidation of the solution is reduced by 14 % relative to the control sample. If 0.5 g/l of beta-carotene is added to this solution, the induction period practically does not decrease compared to the control sample (3 % decrease). In the case of increasing the concentration of beta-carotene in the solution to 0.10 g/l, the induction period increases by 8 % compared to the control sample.

6. Discussion of the results of the analysis of the influence of the content of fat-soluble dyes on the oxidation stability of sunflower oil

The influence dependence of the plant origin fat-soluble dyes content (chlorophyll *A* and beta-carotene) on the oxidation stability of solutions in refined sunflower oil was investigated. The obtained research results, namely the approximate dependences (1)–(3), make it possible to predict the shelf life of oil solutions of fat-soluble dyes. In addition, the obtained dependencies (1)–(3) are useful for justifying the refining of oils by non-traditional methods in order to preserve biologically active substances in them.

According to the data in the Table 2, the indicators of the quality of the studied raw materials indirectly testify to the absence of a significant influence on the process of oxidative deterioration of such factors as the content of free fatty acids, primary oxidation products, and moisture. This makes it possible to ensure the adequacy of the obtained dependences of the effect of selected fat-soluble dyes on the oxidative stability of their oil solutions.

According to the data given in the Table 3, the display of fat-soluble dyes in refined sunflower oil is proven, and their content in the corresponding oil extracts is also determined. This made it possible to ensure the concentration of fat-soluble dyes in model oil solutions (Fig. 1–3, Table 5) by mixing. Also, the obtained data prove the exclusion of mutual influence of selected biologically active substances in the case of separate use of extracts as sources of fat-soluble dyes.

The obtained results of the study are shown in the Table 4, testify that the oil base of the studied samples (sunflower and corn oil) belongs to the linoleic oil group. This ensures the exclusion of the fatty acid composition as a factor that unevenly affects the oxidized stability of the studied oils and oil extracts.

As a result of studies of one-factor dependences of the induction periods of accelerated oxidation of refined sunflower oil on the individual content of chlorophyll *A* and beta-carotene, such a dependence was simulated for a fairly wide range of fat-soluble dyes content – 0.00...0.30 g/l. The obtained data (equations (1), (2), Fig. 1, 2) allow to determine the ranges of the content of chlorophyll *A* and beta-carotene with different levels of pro-oxidant and antioxidant effects on the control system – refined sunflower oil. In particular, it is interesting that there is no pro-oxidant effect of chlorophyll *A* in the range of concentrations up to 0.05 g/l and a significant pro-oxidant effect – with an increase in its concentration. Beta-carotene exhibits the greatest antioxidant effect in the concentration range up to 0.1 % (an increase in the induction period of the oil solution by 35 % compared to the control).

As a result of the study of the two-factor dependence of the induction period of accelerated oxidation of refined sunflower oil on the joint content of chlorophyll *A* and beta-carotene, the following dependence was modeled for the range of fat-soluble dyes content – 0.00...0.10 g/l. This range was chosen for the purpose of researching the concentrations of these biologically active substances, which are most widely used in the production of vitaminized oils in particular. The obtained data (equation (3), Table 5, Fig. 3) allow to visually present the mutual influence of these minor components accompanying vegetable oils on the period of induction of accelerated oxidation.

The development differs from [12, 14], where the influence of minor components (including chlorophyll *A* and beta-carotene) in individual oils is studied. This significantly narrows the range of content of the specified dyes in the studied oil systems. In addition, factors such as the content of accompanying substances characteristic of unrefined oils, including water, primary oxidation products, etc., have an increased influence on the process of oxidative deterioration of samples, described in studies [12, 14]. Thus, the study of the mutual influence of selected fat-soluble dyes, which are basic for vegetable oils, on the oxidative deterioration of the model system, where other factors are practically excluded, is interesting from both a scientific and a practical point of view.

The results of the conducted research (Fig. 1–3) make it possible to effectively take into account the content of chlorophyll *A* and beta-carotene when justifying the expediency of oil refining by non-traditional methods. In addition, the obtained dependencies (1)–(3) are useful for predicting the shelf life of a number of options for the vitaminization of oils, in particular sunflower oil. The use of a differential scanning calorimeter during the determination of periods of induction of accelerated oxidation of oil systems made it possible to unify and intensify the experimental process.

The limitation of the use of the obtained results (Fig. 1–3) is that the experimental studies used refined sunflower oil, which is a fairly common basis for the production of vitaminized oils. Therefore, in the case of the use of other types of oils in the vitaminization technology, especially oils that belong to other groups in terms of fatty acid content, it is necessary to take into account the specified factors for correcting the expiration dates of products.

The lack of data on induction periods of oxidation of model oil solutions of fat-soluble dyes at the storage temperature of such products, namely 0...+20 °C, can be called a drawback of the study. In addition, the study did not take into account the influence of the presence of tocopherols on the oxidative stability of oil solutions of chlorophyll *A* and beta-carotene.

It is worth noting promising directions of work on the study of the influence of fat-soluble dyes chlorophyll *A* and beta-carotene on the period of induction of oxidation of refined sunflower oil. This is, first of all, a study of the influence of light (ultraviolet) on the separate and combined properties of chlorophyll *A* as a prooxidant and beta-carotene as an antioxidant in model oil systems.

7. Conclusions

1. Quality indicators (acid number, peroxide number, mass fraction of moisture and volatile substances), as well as the content of fat-soluble dyes in the objects of the study – sunflower oil, oil concentrates of chlorophyll and beta-carotene – were determined. The samples of oil raw materials that were studied meet the requirements established in the relevant regulatory documentation – DSTU 4492/CAS 8001-21-6; UA/1556/02/01/CAS 479-61-8; UA/2685/01/01/CAS 7235-40-7. In terms of fatty acid composition, the sunflower oil sample meets the requirements of DSTU 4492/CAS 8001-21-6. According to the fatty acid composition of the oil concentrates of chlorophyll and beta-carotene, the oil bases of the concentrates were identified – these are corn and sunflower oils, respectively.

2. The influence of the content of individual chlorophyll and beta-carotene dyes on the induction period of accelerated oxidation of their oil solutions in refined sunflower oil was determined. Chlorophyll *A* practically does not show a pro-oxidant effect if its content is up to 0.05 g/l. The content of chlorophyll *A* at the level of 0.10 g/l in the oil solution leads to a reduction of the induction period by 14 %; 0.20 g/l – by 36 %; 0.30 g/l – by 48 %. This can be explained by the fact that chlorophyll contains a magnesium ion in its molecule, which can easily lose electrons and become an oxidant. In addition, chlorophyll contains pyridine rings, which can also participate in redox reactions. The content of beta-carotene at the level of 0.10 g/l leads to an increase in the induction period by 35 %; 0.20 g/l – by 47 %; 0.30 g/l – by 54 %. The obtained results are explained by the presence of numerous double bonds in the beta-carotene molecule, which have a high electron density and donate electrons to neutralize free radicals.

3. The effect of the combined content of chlorophyll and beta-carotene dyes on the induction period of accelerated oxidation of refined sunflower oil was determined. The compensatory effect of beta-carotene on the pro-oxidant effect of chlorophyll *A* in a solution of refined sunflower oil has been proven. The content of 0.05 g/l of chlorophyll *A* in the oil solution slightly affects the antioxidant effect of 0.05 g/l of beta-carotene (induction period 371 and 373 min, respectively). The content in the oil system of 0.10 g/l of beta-carotene and 0.05 g/l of chlorophyll *A* leads to a reduction in the induction period of accelerated oxidation by 8.4 % compared to the oil solution of 0.10 g/l of beta-carotene without chlorophyll *A* (induction period 408 and 445 min. respectively).

Conflict of interest

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

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Availability of data

The manuscript has no associated data.

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