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Розроблено антиоксидантний комплекс для використання в оліях, жирах та продуктах харчування, що потребують збагачення біологічно активними речовинами рослинного походження. Досліджено раціональні умови одержання водно-етанольних екстрактів із рослинної сировини: кори дубу, листя евкаліпту та листя зеленого чаю. Отримано антиоксидант, що здатен запобігати окисненню жировмісних продуктів, зберігаючи їх високу поживну цінність. Антиоксидантні речовини природного походження дозволять створювати збалансовані за складом продукти з підвищеним строком придатності із збереженням початкового природного складу та структури компонентів. Також розроблений антиоксидант є додатковим джерелом речовин, що допомагають організму боротися з вільними радикалами, що утворюються внаслідок фізичних та психічних навантажень. До складу антиоксидантів рослинного походження входять антиоксидантні вітаміни (токофероли та аскорбінова кислота), рослинні феноли та тиолові антиоксиданти (глутатіон, ліпоєва кислота), мікроелементи. Ці складові беруть участь у процесах гальмування окиснення. Також до таких антиоксидантів входять селен, цинк, фолати та інші речовини.

Для планування експериментальних досліджень для кожного виду рослинної сировини стосовно антиоксидантної активності виділених речовин використано повний факторний експеримент першого порядку.

Виявлено синергізм дії антиоксидантних речовин при одночасному використанні екстрактів з кори дубу, листя евкаліпта, листя зеленого чаю.

Розроблений антиоксидант підвищує період індукції модельної речовини (олії соняшникової) у 2,7 рази, тоді як під час використання антиоксидантів окремо з кожного виду рослин найкращий показник збільшення періоду індукції склав 1,9. Отже, розроблений антиоксидант здатен сприяти збереженню якості і безпечності жировмісних продуктів харчування. Використання даного антиоксиданту може бути запропоновано для продуктів харчування людей, що потребують додаткового введення антиоксидантів та біологічно активних речовин до раціону харчування. Зокрема, це важливо для спортсменів

Ключові слова: антиоксидант, вільні радикали, рослинна сировина, окиснення, біологічно активні речовини, період індукції, екстрагування

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# **DEVELOPMENT OF A FOOD ANTIOXIDANT COMPLEX** OF PLANT ORIGIN

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#### 1. Introduction

Antioxidants are an important component of rational nutrition. These substances can slow down the oxidation

processes in lipid-containing products and fight free radicals formed in the body.

One of the important components in nutrition is fat-containing products. The problem related to using these components is their ability to oxidative and hydrolytic damage, resulting in harmful substances for human organism – free radicals. Taking this fact into consideration, there is an important issue regarding the production and use of fat-containing products – prevention of oxidation damage processes and extension of shelf life without the formation of free radicals. There are special categories of people who need in their diet foods enriched with plant-derived antioxidants, for example, special dietary nutrition for various diseases, sports nutrition. Under conditions of intense physical loads, the body undergoes significant biochemical changes. One of the factors limiting the activity of people associated with physical activity is the increased content of free radicals in the body as a result of intensive loads. In addition, free radicals can enter the body together with food containing oxidized lipids [1, 2].

At present, the task on protecting products from free radicals is solved by introducing predominantly synthetic antioxidants [3]. Synthetic antioxidants have a lower cost; however, from a hygienic point of view, it is advisable to use natural compounds derived from plants that are biologically valuable products and do not cause toxic effects on the human body. Among substances that are part of plant raw materials an antioxidant action is demonstrated by tocopherols, sesamol, carotenoids, phosphatides, chlorophyll, phenol, and other compounds. Plant-derived antioxidants are able not only to protect a product from oxidation, but to preserve the biological value of the product, and, given the presence of a series of biologically active substances, to ensure a positive effect on human health [4].

The current pressing issue is food quality and safety, preservation of their properties, production of foodstuffs using natural raw materials and components, as well as the development of products with an elevated content of exogenous antioxidants. Therefore, it is a relevant scientific task to develop highly effective plant-derived antioxidants; this study aims to address this issue.

#### 2. Literature review and problem statement

The processes that form free radicals in the body are intensified during physical activity due to a rapid change in modes and intensity of energy supply. To fight free radicals, the body has the enzyme protection systems, but in some cases, in particular, during sports activities, it is necessary to introduce exogenous antioxidants [1, 2, 5].

The accumulation of endogenous toxic metabolic products due to intensive muscle work not only exerts a systemic effect on the progress of biochemical processes, but it also is one of the main causes for a decreased immunological reactivity in humans exposed to physical loads. In addition, endogenous intoxication adversely affects the processes of energy supply both at the tissue and cellular levels. Natural antioxidant processes are disrupted, thereby activating the processes of peroxide oxidation. This leads to the disorder of mitochondrial function, reducing resistance to damage of cell membranes and other structures, which contributes to the decreased functional activity of the state of vital organs and systems of the body [5].

Paper [6] shows the exceptionally important role of antioxidants that enter the human body with food. Antioxidants do not only perform certain functions as part of the products, but also take part in the metabolic processes in the body. However, there are no specific data on the composition

of antioxidants, specifically natural, in order to enable effective prevention of oxidation processes in food products.

Authors of [6] note that the antioxidant plant-derived preparations are well tolerated, they exert milder corrective influence on the human body, and demonstrate a wide range of regulative effects. However, in this case, there are no scientific data on the use of plant antioxidants for oils and fats, which are an important component of most food products for different purposes and designations.

Fats are one of the most important ingredients of normal functioning of the organism. During storage and use, fats undergo oxidative damage under the influence of oxygen, which leads to the formation of substances that are harmful to human health. In this regard, it is a relevant task to develop techniques to protect against the oxidative processes in fats, used directly in food, and are utilized in the production of fat-containing products. Lipid oxidation is one of the main factors limiting the shelf life of many food products. Special substances-antioxidants are used to inhibit the processes of oxidation and to extend the shelf life of products [8].

The natural fat-soluble antioxidants are tocopherols and tocotrienols. For most products, the dosage of  $0.02-0.06\,\%$  is sufficient to ensure effective anti-oxidation action.

Currently, food products include such widely used antioxidants of synthetic origin as propyl gallate, butylhydroxyanisole (BOA), butylhydroxytoluene (BOT), tert-butylhydroquinone (TBHQ).

Propyl gallate (3, 4, 5-trioxybenzoic acid n-propyl ester) is an effective antioxidant that is used to prolong the shelf life of oils under conditions of a (100–200) mg/kg dosing.

BOA and BOT are characterized by the property to withstand the high temperatures reached when making food, in particular, during baking and deep frying. However, these substances exhibit a rather low antioxidant efficiency in oils.

TBHQ is the most effective antioxidant for unsaturated plant oils and it has a series of advantages. However, the issue related to using TBHQ is a pink color that can be acquired under conditions of alkaline pH, in the presence of some proteins or sodium salts [8].

According to [8], synthetic antioxidants are a widespread means to inhibit oxidation processes in oils and fats, but they have a series of drawbacks and peculiarities in application. Therefore, it is an extremely important task to devise of new effective and safe antioxidants, based on plants, for oils and fats.

Plant raw materials contain a series of valuable antioxidant components, an important place among which is occupied by flavonoids. The efficacy of natural antioxidants in food and human body depends on many factors, including water or lipid solubility, stability, matrix interactions, and bioavailability [9]. However, there are no data on the effect of such antioxidants on the indicators of oxidation of oils and fats.

The processes of inhibition of lipid oxidation through various plant extracts are studied in paper [10]. The authors acquired data on the dynamics of accumulation of oxidation products over time and defined rational concentrations: dihydroquercetin (92 %) -0.05 %, extract of green tea -0.15%; extract of grape seed -0.05 and 0.1%; rosemary extract -0.15%. However, it was not shown how these antioxidants affect the indicators of lipid oxidation at elevated temperatures, which is a significant issue, since oils, fats, as well as products based on them, can be processed with heat treatment, for example when frying a product.

In addition, according to results from work [11], a promising resource for obtaining natural antioxidants, in particular, flavonoids, as well as a valuable source of minerals, is the bark of oak. The high efficiency of oak bark extract in the process of inhibition of free-radical processes was shown, as well as the content of phenolic compounds in oak bark was analyzed, which amounted to (7.2–8.4) mg/g. However, the authors did not describe the effect of oak bark extracts on the degree of oxidation in oils and fats, specifically the peroxide number, which directly reflects the accumulation of oxidation products in a sample.

Another source of exogenous antioxidants of interest is the leaves of eucalyptus, whose extracts exert a series of health effects on the human body. The leaves of eucalyptus contain up to 3 % of the essential oil, whose main component is cineole. The leaves also yielded eucalyptol. The hypoglycemic, anti-diabetic effect of extracts from eucalyptus leaves is also known. The pain and anti-inflammatory properties of eucalyptus leaf extracts were determined. Eucalrobuzon C, isolated from the leaves of eucalyptus, has pronounced antitumor properties [12]. Authors of [13] established that extracts and essential oils from eucalyptus leaves are effective against an oxidative stress in the body, since they contain polysaccharides that provide inhibition of free radical processes. Paper [14] experimentally confirmed the high antioxidant effect of acetone, ethanol and methanol extracts from eucalyptus leaves against peroxidation in cells. Thus, the leaves of eucalyptus are promising raw materials in terms of their use as a source of antioxidants; however, the studies reported in papers [12–14] should have been supplemented with data on the influence of extracts from leaves of eucalyptus on peroxidase oxidation and to establish how this antioxidant affects the value of the peroxide number in oil over time.

One of the most common types of raw materials to obtain natural antioxidants is green tea. Thus, authors of [15] showed the prospects of using the anthocyanin fraction in green tea as a substitute for synthetic antioxidants in plant oils. The authors examined the effect of this plant-based antioxidant on the peroxide number of rapeseed oil after three months of storage, the result being that the peroxide number of oil with the addition of the developed antioxidant turned out to be lower by 12.6 times compared to the starting oil. This issue lacks enough data on the effectiveness of green tea antioxidants under the action of elevated temperatures. In addition, it is of interest to use such an antioxidant for sunflower oil, which is the most widespread and used type of oil.

The use of green, white, and black tea as components of a probiotic yogurt was investigated in paper [16]. A green teabased yoghurt demonstrated the highest content of phenols. Green tea is also used to prolong the shelf life of meat products; in this case, high efficiency is demonstrated by a green tea powder in the amount of 0.5 % and a green tea extract in the amount of 0.03 % [17]. That makes it possible to conclude that green tea is a promising raw material for obtaining antioxidants, but their effectiveness should have been checked on individual oils, the use of which would make it possible to assess the action of antioxidants against free radical oxidation processes.

In addition, a known kind of raw materials for obtaining substances-antioxidants is the bark of oak. Paper [18] examined a high antioxidant and antibacterial effect of oak bark extract at its application in yoghurt, cheese, and ice cream production. Author of [19] shows the prospects and expediency of using oak bark as a component of sweeteners to enrich the diet with polyphenols – the substances that

have an antiradical effect and help strengthen immunity. However, no study into the action of antioxidants in oils and fats was undertaken.

Thus, based on the scientific literary review, it was found that exploring various types of plant raw materials aimed at extracting antioxidant substances is a relevant field in the modern development of food industry. Another extremely important issue is the enrichment with antioxidant substances of certain types of foods, for example, for people with enhanced physical activity. These components would improve performance and endurance, prevention of diseases, strengthen the body.

At present, there are no enough data on the antioxidant effect of these substances, namely in the composition of pure oils and fats, which cause the processes of oxidative and hydrolytic process in products. The research into the activity of natural antioxidants in relation to oils and fats is an unresolved issue in this field since they are important elements of both food products and human body cells.

Thus, in order to devise effective natural antioxidants for oils and fats, three types of plant raw materials have been selected: green tea leaves, bark of oak, and eucalyptus leaves. Sunflower oil was chosen as a model substance to examine the antioxidant action of extracts as this is the most common and used kind of oil. Sunflower oil was selected as a model substance due to a high content of polyunsaturated fatty acids. Polyunsaturated fatty acids are also included in the human cell membranes. In addition, it is of interest to study the synergy of the antioxidants isolated from various types of raw materials.

Thus, investigating the antioxidation properties of plant extracts using sunflower oil is appropriate and could provide an opportunity to substantially complement and extend current ideas about the antioxidant properties of extractive substances of plant origin.

#### 3. The aim and objectives of the study

The aim of this study was to devise a plant-based antioxidant with the combined composition of antioxidation substances for use in fat-containing food products, in particular sports nutrition.

To accomplish the aim, the following tasks have been set:

- to select plant raw materials with a high content and a wide range of antioxidant substances;
- to experimentally investigate the effectiveness of antioxidant extracts from plant raw materials at elevated temperatures using a model substance (sunflower oil);
- to define rational conditions for the extraction of substances with an antioxidant effect using a model substance (sunflower oil);
- to explore the synergy of action of a mixture of antioxidants from the selected types of plant raw materials.

# 4. Materials and methods to study the development of an antioxidant for use in sports practice

### 4.1. Examined materials and equipment used in the experiment

The following reagents and materials were used in this study:

- bark of oak, green tea leaves, leaves of *Eucalyptus viminalis*, according to acting normative documentation;

- refined deodorized winterized sunflower oil, grade P, according to DSTU 4492:2017;
  - ethyl rectified alcohol, according to DSTU 4221:2003;
- distilled water, according to acting normative documentation;
- potassium iodide, grade "pure chemical", according to acting normative documentation;
- soluble starch, according to acting normative documentation;
- sodium thiosulfate, according to acting normative documentation;
- acetic acid, according to acting normative documentation:
- chloroform, according to acting normative documentation.

### 4. 2. Procedure for obtaining water-ethanol extracts from plant raw materials

Plant raw materials, crushed to the particle size of 2 mm, are placed into a flask, poured with a solvent, closed with a cork, aged at stirring and at a predetermined temperature over a required time.

After the end of the extraction, the extract is filtered through a folded filter and poured to a dark glass. The extract is kept in a cool, dark place.

In the finished extract, one defines a mass fraction of solids. To this end, the porcelain bowl with 2 g of the extract is weighed. The bowl is placed inside a drying chamber, with a temperature of  $105\,^{\circ}\mathrm{C}$ , for  $45\,\mathrm{minutes}$ . After that, the bowl is placed in a desiccator for  $15\,\mathrm{minutes}$ . After 15 minutes, it is weighed with an accuracy of up to 0.001. The drying cycles are repeated until the mass becomes constant.

### 4. 3. Procedure for determining the stability of samples of sunflower oil to oxidation

The oxidation stability of sunflower oil samples was determined according to the accelerated method of the "active oxygen", which implies determining an induction period based on a change in peroxide number when oil is aged under conditions of the predefined temperature. We put 10 g of an oil sample into glass containers, placed it inside a drying chamber heated to the set temperature. To determine the peroxide number, we took the appropriate container out of the dryer. A value for the period of sunflower induction was determined graphically, based on the growth curves of peroxide numbers.

The peroxide number was determined according to a standard procedure in line with DSTU 4350:2004.

# 4. 4. Planning an experimental research and processing the results

In order to plan our study and process the results obtained, a full first-order factorial experiment was applied; the calculation was performed in the Microsoft Office Excel 2003 (USA) and Stat Soft Statistica v6.0 (USA) software packages. The experiments were repeated twice.

# 5. Results of studying the development of an antioxidant for athletes

# 5. 1. Examining the effectiveness of antioxidant extracts from plant raw materials at elevated temperatures

In order to establish the study parameters in advance, we determined an increase in the oil peroxide number over

- 1 hour for each type of examined plant raw materials at different temperatures. Two samples of extracts were used for research:
- 1 corresponds to the values for extraction parameters: extraction temperature: is 50 °C; the ratio of the amount of an extractant and a plant raw material is 1:10; the concentration of ethanol in the extractant is 30 %;
- 2 corresponds to the values for extraction parameters: extraction temperature is 50 °C; the ratio of the amount of an extractant and a plant raw material is 1:10; the concentration of ethanol in the extractant is 70 %.

The data are given in Table 1.

Table 1
Increase in the peroxide number of a model substance over 1
hour at different temperatures

C1-	Increase in PN over 1 hour, 1/2 O mmol/kg						
Sample	70 °C	90 °C	110 °C				
Starting oil	0.28	1.0	2.72				
Oak bark extract added							
Point 1	0.20	0.64	2.13				
Point 2 0.28		0.52	2.05				
Eucalyptus leaves extract added							
Point 1	0.25	0.57	2.14				
Point 2	0.25	0.64	2.18				
Green tea extract added							
Point 1	Point 1 0.28		1.63				
Point 2	Point 2 0.28		1.79				

Thus, we have experimentally confirmed the inhibiting capability of extracts from the selected raw materials at elevated temperatures, including 90 °C and 110 °C. to investigate effectiveness of the obtained plant antioxidants, the accelerated oxidation of sunflower oil at a temperature of 110 °C was used. At this temperature there is a significant increase in the values of peroxide numbers, which would make it possible to promptly monitor the intensity of oxidation processes in a model substance and to determine the corresponding induction periods. And, consequently, to assess antioxidant efficiency for athletes.

#### 5. 2. Determining the rational conditions of extraction of substances with an antioxidant effect

The full factorial experiment of first order implies the simultaneous variation of all factors while carrying it out in line with a certain plan, derivation of a mathematical model in the form of a linear polynomial and examination of the latter using the methods of mathematical statistics. The response function was a period of induction defined based on a change in peroxide number when the examined model substance, sunflower oil, was exposed to an elevated temperature.

Intervals of factors variation are:

- $x_1$  ratio of the amount of an extractant and a plant raw material: from 1:10 to 1:5;
- $x_2$  concentration of ethanol in the extractant: from 30 to 70 %;
  - $x_3$  extraction temperature: from 50 °C to 75 °C.

Rational conditions are the conditions under which the obtained extracts have the largest antioxidant power. The greatest antioxidant power is demonstrated by extracts whose content of the oxidation inhibitors is the largest.

When adding extracts that have the highest antioxidant power to a model substance, its rate of oxidation is the lowest. This means that the oil, which has the lowest oxidation rate, is more stable to oxidation.

Induction periods in all cases were defined at a temperature of 110  $^{\circ}\mathrm{C}.$ 

The period of oil induction in the starting form (without the addition of antioxidants) amounted to 160 minutes. In all experiments, the total concentration of antioxidant extracts in oil was  $0.02\,\%$  in terms of dry matter.

The rational conditions for the extraction of substances with antioxidant properties from plant raw materials were determined as follows.

Tables 2–4 give experimental and estimated values for a response function – the period of induction of a model substance with the addition of extracts of oak bark, eucalyptus leaves, and green tea leaves.

Table 2 Experimental  $(y_{exp})$  and estimated  $(y_{estimated})$  values for a response function for extracts from oak bark (min.)

No.	1	2	3	4	5	6	7	8
$y_{exp.}$	234.5	236	237.5	214	192	304	179	221
$y_{estimated}$ .	234.4	236.1	237.6	214.1	191.9	303.9	178.9	221.1

Table 3 Experimental  $(y_{exp})$  and estimated  $(y_{estimated})$  values for a response function for extracts from eucalyptus leaves (min.)

-								
No.	1	2	3	4	5	6	7	8
$y_{exp.}$	214	207.5	194	238.5	229.5	181.5	178.5	207
Uestimated	213.3	208.2	193.3	237.8	230.2	180.8	179.2	207.7

Table 4 Experimental  $(y_{exp})$  and estimated  $(y_{estimated})$  values for a response function for green tea extracts (min.)

No.	1	2	3	4	5	6	7	8
$y_{exp.}$	288	295.5	303.5	290.5	342.5	306.5	327.5	289
Uestimated	286	293.5	301.5	292.5	340.5	308.5	329.5	291

The result of obtained data processing is the calculated regression dependences between the period of induction of a model substance and the extraction parameters for each kind of a plant raw material.

The estimation of significance of coefficients for the regression equations was carried out according to the Student criterion (a tabular value under condition of the significance level of 0.05 is 2.31 [16]). The condition for a coefficient significance is the value for the calculated Student coefficient that is greater than the tabular one.

The calculated values for a Fisher criterion were:

- for oak bark 0.03;
- $\ for \ eucalyptus \ leaves -0.511;$
- − for green tea leaves − 4.613.

The tabular value for a Fisher criterion in each case is 5.32. Because the calculated values for a Fisher criterion are smaller than the tabular ones, the resulting equations adequately describe the examined process.

The derived regression equations after decoding take the following form:

- for oak bark:

$$y = -23.625 + 1,391.25 \cdot x_1 + 8.0875 \cdot x_2 +$$

$$+6.385 \cdot x_3 - 37.875 \cdot x_1 \cdot x_2 - 28.4 \cdot x_1 \cdot x_3 -$$

$$-0.1415 \cdot x_2 \cdot x_3 + 0.66 \cdot x_1 \cdot x_2 \cdot x_3,$$

$$(1)$$

for eucalyptus leaves:

$$y = -70.323 + 1,305.66 \cdot x_1 - 0.0684 \cdot x_2 +$$

$$+4.98512 \cdot x_3 + 7.938 \cdot x_1 \cdot x_2 -$$

$$-25.5008 \cdot x_1 \cdot x_3 - 0.0153 \cdot x_2 \cdot x_3;$$
(2)

- for green tea leaves:

$$y = -23.625 + 1,391.25 \cdot x_1 + 8.0875 \cdot x_2 +$$

$$+6.385 \cdot x_3 - 37.875 \cdot x_1 \cdot x_2 - 28.4 \cdot x_1 \cdot x_3 -$$

$$-0.1415 \cdot x_2 \cdot x_3 + 0.66 \cdot x_1 \cdot x_2 \cdot x_3,$$
(3)

where  $x_1$  is the ratio of a plant raw material to a solvent;  $x_2$  is the volumetric proportion of ethyl alcohol in an extractant, %;  $x_3$  is the extraction temperature, °C.

Based on the derived mathematical models, we built graphic dependences – the response surfaces and the projections of response surfaces. Fig. 1–3 show projections of the response surface that reflect the dependence of an induction period of sunflower oil on the parameters of extraction of substances with an antioxidant effect from the examined samples of plant raw materials: the ethanol concentration and the temperature of extraction. In this case, the registered value for the ratio of a plant raw material to a solvent  $(x_1)$  is 0.1 (1:10).

The introduction of antioxidants is aimed at prolonging the induction period. The greater the value of an induction period, the more stable to oxidation a sample is. However, when analyzing and selecting the rational values for input variables, one should also be guided by the extent to which the induction period grows during the further increase in parameters values and whether it is expedient to enhance temperature, alcohol concentration and the ratio of a plant raw material to an extractant. Adequacy of the constructed mathematical model was tested within the range of the established region of factor variation.

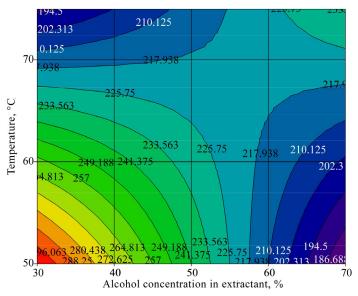


Fig. 1. Dependence of the induction period of sunflower oil on the extraction parameters of substances with an antioxidant effect from oak bark: ethanol concentration and temperature of extraction

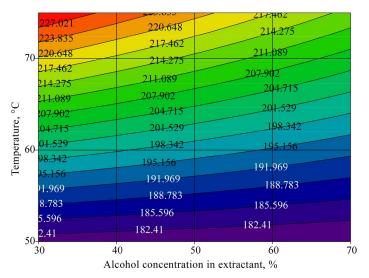


Fig. 2. Dependence of the induction period of sunflower oil on the extraction parameters of substances with an antioxidant effect from eucalyptus leaves: ethanol concentration and temperature of extraction

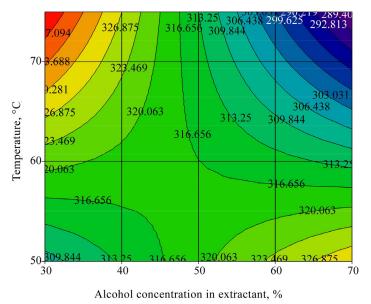


Fig. 3. Dependence of the induction period of sunflower oil on the extraction parameters of substances with an antioxidant effect from green tea: ethanol concentration and temperature of extraction

We have analyzed the data obtained for periods of a model substance's induction, as well as mathematical and graphic dependences, and established the following.

At all examined points, under condition of fixing the value for the ratio of a plant raw material to a solvent ( $x_1$ ) at 0.1 (1:10), the induction period in cases with the addition of extracts is greater than that for the starting model substance (160 min.).

Changing the extraction parameters affects differently the period of induction of the model substance and, consequently, the antioxidant activity of the extracted substances. Each type of a raw material demonstrates different patterns in the influence of extraction parameters on the efficiency of antioxidants.

In all cases, the highest values for induction periods were observed for green tea leaves.

Increasing the temperature of extraction and the concentration of ethanol leads to a decrease in the effectiveness of extractive substances from the bark of oak. The ratio of a plant raw material to an extractant almost does not affect this indicator. In general, oak bark demonstrated more efficacy regarding the antioxidant activity of extractive substances than the eucalyptus leaves.

The effectiveness of an oak bark extract is most significantly affected by the temperature of extraction; under experimental conditions the most effective use of this raw material is at a temperature of 50  $^{\circ}$ C (221.125 min). The best result was shown by ethanol concentration of 30 % (303.875 min).

As regards the extract from eucalyptus leaves, its efficiency is increased under conditions of a temperature rise and a decrease in ethanol concentration. The ratio of eucalyptus leaves to an extractant showed the best result at a value of 1:10.

In general, as regards green tea leaves, there is an increase in the induction period when the extraction parameters are increased, but, given the induction period growth intensity, the optimal values are not maximal. Based on a graphic data analysis, we defined regions of the rational values for extraction parameters, which are given in Table 5.

Table 5
Rational conditions for the extraction of substances
with antioxidant properties from plant materials

	Rational conditions						
Plant raw material	Ratio of plant raw material to solvent x <sub>1</sub>	Volumetric share of eth- yl alcohol in extractant x <sub>2</sub> , %	Extraction temperature $x_2$ , °C				
Oak bark	1:10	30	50				
Eucalyptus leaves	1:10	30	60-70				
Green tea leaves	1:10	30-50	50-60				

# 5. 3. Studying the synergistic action of antioxidants from oak bark, green tea leaves, and eucalyptus leaves

The synergistic effects of antioxidants from the three examined types of plant raw materials have been examined. We determined the period of induction of the model substance under conditions of introducing the extracts from oak bark, green tea leaves, and eucalyptus leaves in a ratio of (33:33:33) %. The total content of this mixture in the model substance amounted, similarly to earlier studies, to 0.02 % in terms of dry matter. The induction period of the model substance was 425 min., which is 2.7 times larger than the period of induction of the model substance without the addition of an antioxidant. Furthermore, the induction period magnitude, with the addition of all three extracts, is higher than in the case of each of them individually. This fact indicates that a mixture of antioxidants has a more effective antioxidant action.

### 6. Discussing the results of developing antioxidants for use in sports practice

The result of our study is the devised antioxidants from plant raw materials: oak bark, eucalyptus leaves, green tea leaves. The extracted antioxidants are effective at inhibiting the processes of sunflower oil oxidation both individually and under the conditions of a simultaneous action. Thus, we have obtained the antioxidant complex with a combined composition of antioxidant substances. The devised antioxidant increases the period of sunflower oil induction by 2.7 times. Lipid components are the main factor for the shelf life of food products. The use of such an antioxidant is appropriate not only in oils, fats, but also in products containing these components. The introduction of a plant antioxidant complex would enrich a product with biologically active components and antioxidants, which is important in the development of products to strengthen and enhance endurance of the body.

For example, the use of such an antioxidant complex is relevant in the development of nutrition for professional athletes. This relates to the formation of free radicals in the body during intensive training, as well as the need for rapid recovery of the body under conditions of mental and physical loads. Simultaneous introduction of the extract from green tea, extracts from oak bark and eucalyptus in equal proportions produces a synergetic antioxidant effect; in this case, it is possible to create products with a mixture of different antioxidants, which are extremely important components in the rational sports nutrition.

Our study has shown a high antioxidant effect of extracts on the model substance, sunflower oil. This type of oil is the most widespread and used, and is a component of many foodstuffs, including for a certain purpose diet (dietary, sports, wellness, etc.).

The composition of the selected raw materials includes many anti-oxidants, which are soluble in polar environments, hence a mixture of water and ethanol is the optimum extractant to derive antioxidants. A temperature of  $50-60\,^{\circ}\mathrm{C}$  intensifies the extraction of antioxidants through the activation of contact between a solvent and extractive substances. Further rise in temperature is impractical, because it begins to partially influence the structure of antioxidants, thereby reducing their antioxidative effect in the subsequent use.

The advantages of the devised plant antioxidant are achieved due to the quality of extraction of anti-oxidation substances in the composition of raw materials and synergy between them in the finished antioxidant.

The merits of this study, in comparison with similar known ones, are in that the plants used for the extraction contain not only antioxidants, but also a series of biologically active substances.

The devised antioxidant is recommended to use not only for the purpose of preserving the quality of fat-containing food, but also as an additional source of exogenous antioxidants. In the further studies involving the devised antioxidant it is promising to add to its composition extracts from other plants and to investigate synergy more broadly. In addition, it is of interest to use plant-derived antioxidants for inhibiting the oxidative, hydrolytic and microbiological spoilage of food products with a multicomponent composition.

The advancement of our study might be to use a given antioxidant not only in foods, but also in the development of special dietary supplements to food products with a high content of antioxidants.

#### 7. Conclusions

- 1. To devise a dietary antioxidant complex, we have examined the following plant raw materials with a wide content of antioxidizing substances: green tea leaves, eucalyptus leaves, bark oak, which are valuable sources of biologically active and antioxidant substances. The selected raw materials were studied in terms of inhibiting the processes of oxidation of a model substance, sunflower oil, using an extract from each type of plants, as well as in the form of a mixture, that is the phenomenon of synergy of antioxidant action has been established.
- 2. The efficiency of antioxidant extracts from the selected raw materials has been investigated at temperatures 70 °C, 90 °C, 110 °C. Based on an increase in the peroxide number of the model substance (sunflower oil) we have established that under all specified temperatures plant-derived antioxidants demonstrate high efficiency, thereby lowering the values of peroxide numbers in all experiments.
- 3. The character of effect exerted by each extraction parameter on the period of a model substance's induction has been defined, and the rational conditions for extraction have been established. The rational ratio of a plant raw material to a solvent for all types of raw materials was 1:10; the volumetric share of ethyl alcohol in the extractant for oak bark is 30 %, eucalyptus leaves and green tea leaves— (30-50)%; the extraction temperature for oak bark is 50 °C, eucalyptus leaves and green tea leaves— (50-60) °C.
- 4. Among the samples with the addition of a plant antioxidant the greatest antioxidant efficiency was demonstrated by a mixture of antioxidants from the three examined types of raw materials. When introducing extracts from green tea, bark of oak and eucalyptus in equal proportion in the amount of 0.02 % per dry matter the induction period of a model substance (sunflower oil) increases by 2.7 times compared to a model substance without adding antioxidants. The obtained antioxidants-extracts are a valuable source of biologically active substances, which exhibit a stable antioxidant effect on food products and demonstrate synergy between each other. This could not only protect the fat-containing products from oxidation, but also to additionally introduce natural antioxidants into the body.

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