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Обґрунтовано доцільність використання нетрадиційної рослинної сировини для збагачення пива, якою частково замінюють хміль. Досліджено параметри екстрагування (температура, тривалість, гідромодуль) біологічно-активних речовин із хвої сосни звичайної. Оцінено отримані водні екстракти за органолептичними та фізико-хімічними показниками. Розроблено рецептуру пива, до складу якого входить водний екстракт хвої сосни звичайної. Оцінено вплив пива з додаванням хвойного екстракту на антиоксидантну систему організму біологічних об'єктів

Ключові слова: рослинна сировина, антиоксиданти, хвойний екстракт, пивне сусло, математична модель, біологічні об'єкти

Обоснована целесообразность использования нетрадиционного растительного сырья для обогащения пива, которым частично заменяют хмель. Исследованы параметры экстрагирования (температура, продолжительность, гидромодуль) биологически активных веществ из хвои сосны обыкновенной. Оценены полученные водные экстракты по органолептическим и физико-химическим показателям. Разработана рецептура пива, в состав которого входит водный экстракт хвои сосны обыкновенной. Оценено влияние пива с добавлением хвойного экстракта на антиоксидантную систему организма биологических объектов

Ключевые слова: растительное сырье, антиоксиданты, хвойный экстракт, пивное сусло, математическая модель, биологические объекты

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RESEARCH INTO QUALITY OF BEER WITH THE ADDITION OF PINE NEEDLES EXTRACT

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1. Introduction

Beer is a rather popular beverage in many countries of the world that enjoys demand due to its flavor and aroma characteristics, especially among young people.

At present, technology of beer production is aimed at developing new varieties with the addition of alternative vegetable raw materials, which give the drink certain taste peculiarities and increases the demand for the produce [1, 2]. In addition, beer, made with the addition of vegetable raw materials, has its advantages: functional directed action, improved organoleptic, physical and chemical parameters, a longer shelf life [3, 4].

By adding antioxidants of vegetable raw materials to the formulation of beer, it is possible to reduce the oxidative and toxic effects of alcohol on the human organism [5]. Harmless

antimicrobial and antifungicide substances of natural origin, when added to beer, may improve its quality indicators; prevent contamination by microorganisms and application of preservatives in the production of the drink [4].

These benefits theoretically substantiate the feasibility of adding alternative vegetable raw materials to beer, namely the needles of Scots pine, which meets all the aforementioned requirements. Development of beer with the addition of pine extract gives an opportunity to obtain new original varieties of beer and expand the assortment of beer industry.

2. Literature review and problem statement

Cinnamon, coriander, ginger, nutmeg, pepper, vanilla, curry, various herbs, flowers of trees and bushes, leaves of coniferous trees, as well as mixed compositions of berries and fruit are most widely used in the production of beer.

The Czech breweries offer beer with horseradish, coriander, pear, elderberry and cinnamon. The Belarusian brewers created beer with extracts of cherry and cocoa beans. In China, bamboo beer, the so-called "green beer" with the addition of the extract and juice of young leaves of bamboo and lime, is rather popular.

Prominent experts in the field of brewing from various countries, such as Great Britain, Ireland, the USA and Finland, discuss innovations in the use of auxiliary materials and new technologies. The prospect of enhancing quality and safety of beer implies using only natural raw materials and controlling all stages of production [6, 7].

To obtain beer with the original organoleptic properties, the scientists proposed the cone berries of ordinary juniper, which partly replaced hops. The drink, containing a 3 % coniferous extract, is additionally enriched with biologically active substances and a sour-sweet refreshing tone [8].

A well-known way of producing beer is the one with the addition of a pine additive; in this case, the proportion of hops is 0.08–0.1 % and the proportion of pine additive is up to 0.8 % of beer wort. The additive, prepared from the powder of pine bark, cones and pine needles, is introduced at the stage of boiling with hops. This makes it possible to improve flavor properties of beer and enhance the biological value and medicinal properties of the finished drink [9].

Scientists consider walnut to be a promising plant raw material for the production of beer, because it has in its composition all the necessary substances that contribute to the stability of beer. The use of the walnut extract in brewing provides stability, medicinal properties of the drink, as well as gives it specific color and fragrance [10].

Goji berries are added to beer to develop a drink with high antioxidant properties. These varieties of beer are characterized by intense coloration, coffee-like taste and high content of biologically active substances, such as rutin and ascorbic acid [11].

Hops are used in all varieties of beer and natural ingredients are added at different stages of production.

However, hop, which is an indispensable component of any beer, is the closest relative of hemp that is the source of such drugs as marijuana and hashish. Hops contain cannabidiol (the active substance of hashish and marijuana) and gumulin, the alkaloid-like substances with a narcotic effect. As a result of the fementation of hops, cadaverin, which is a ptomaine, is released.

Cones of hop contain 8-prenilnargenin – a substance that belongs to the class of phytoestrogens and surpasses all other estrogens by 10–100 times in terms of their hormone power. The content of phytoestrogens in hops reaches considerable values from 20 to 300 mg/kg. In the finished product – beer, their amount reaches up to 36 mg/l. However, this amount is sufficient to provide a pronounced hormone influence on the human organism. It is likely that the change of the endocrine status of a person (feminization of men and muscle increase in women), who abuses beer, is related mainly to the effects of phytoestrogens. It is impossible to purify beer from phytoestrogens that are analogues of female sex hormones because they are received by drink from vegetable raw materials [12].

German doctors found in beer carcinogenic substances, which are transferred to it from hops. These are bitter substances of hops, classified as resins. When it comes to tobacco, resins in it contribute to the development of cancer in smokers. No matter what healing properties are attributed to hops, at excessive use of alcohol, its resins will inevitably cause damage to human health [13].

Consequently, vegetable raw materials, which are a source of natural antioxidants, have a high nutritional and biological value and may be used as an alternative to hops. When choosing an additional component for the production of beer, we were guided by the high antioxidant properties of needles of Scots pine. Its chemical composition is the closest to the composition of hop cones (polyphenol, bitter and pectin substances, essential oils and the like) [14, 15].

Needle leaves contain not less than 200 mg % of the powerful antioxidant, vitamin C, which in winter reaches 600 mg %. This, for example, corresponds to its amount in black currant and sea buckthorn and is 7 times as high as in lemons. Vitamin C promotes accelerated removal of toxins from the body and reduction of total cholesterol level, regulates redox processes, and affects protein and carbohydrate metabolism. It is a booster of the immune system; it is not synthesized and stored by the organism, which makes it compulsory in the diet. It is widely used for the prevention and treatment of hypo– and deficiency of vitamin C.

The essential oils, contained in pine needles (up to 0.15–0.25 %), destroy the microorganisms that cause in humans inflammatory and infectious diseases of the respiratory tract, including lungs.

Pine needles contain 350–360 mg/kg of fat-soluble vitamin E (tocopherol). Its deficiency in the human organism causes disease changes in skeletal muscles, heart muscles, nerve cells and sex glands, leading to miscarriages and rapid aging.

Not less important antioxidant is provitamin A (carotene), which is also found in pine needles in sufficient quantities –140–320 mg/kg. Vitamin A provides good eyesight, promotes the body's resistance to infections.

Needles are the source of beta-carotene and have 140–320 mg of carotene per 1 kg. It is believed to be indispensable in the prevention of cancer.

Important components of the pine needles are flavonoids (rutin, quartzetin and its glycosides), which possess immunostimulating, immunomodelling and antioxidant properties. Flavonoids, along with vitamins, are involved in support of the functions of antioxidant protection for organism.

Phytoncides, which are contained in pine needles, are important anti-microbial substances; their composition includes essential oils – the mixtures of volatile aromatic compounds.

Based on the aforementioned, in order to reduce the negative effect of hops on the human organism and to enhance the drink by biologically active substances and extend the assortment, we proposed to use in the production of beer the needles of Scots pine, which partly replaced hops.

3. The aim and tasks of the study

The aim of the conducted studies was to determine the effect of the extract of Scots pine needles on the formation of quality in finished beer. This would make it possible to obtain a drink with high antioxidant properties and to decrease the negative effect of alcohol on the human body.

To achieve the set goal, the following tasks were to be solved:

– to determine optimal parameters of extraction of pine needles and to explore organoleptic and biological parameters and biological value of the pine extract;

– to construct a mathematical model for determination in beer the quantitative composition of pine needles, which partly replaced hops;

– to assess the quality of developed beer with the addition of pine extract;

– to explore the impact of beer with the addition of pine extract on the organism of biological objects.

4. Materials and methods for studying the impact of Scots pine needles on the quality indicators of beer

4. 1. The examined plant raw material in the experiment

For a partial replacement of hops and enrichment of beer with biologically active substances, we selected water extract of alternative vegetable raw materials – Scots pine needles (Pinus sylvestris).

Scots pine needles for the experiment were gathered in winter, as, according to preliminary research, at this particular time, pine needles accumulate the highest amount of biologically active substances. The raw material met the requirements of TU U 15.8-31062507-022:2009 "Vegetable raw materials, natural, for the production of dietary additives".

It is possible to get acquainted in more detail with the technology of obtaining pine extract, with the research into qualitative indicators of pine extract and the developed beer with its addition, as well as with the research into the influence of quality parameters of pine extract and the developed beer with its addition, on the organism of biological objects, in paper [16].

5. Results of research into the influence of Scots pine needles on the quality of the finished beer

5. 1. Optimal parameters of extraction of Scots pine needles, research into parameters and biological value of pine extract

To obtain water extract, Scots pine needles were crushed to the dimensions of particles of 3.0–5.0 mm. As an indicator of technological process, the value of antioxidant ability of aqueous extract was accepted.

The technological process of extracting antioxidants is characterized by three input variables that determine the technological process of treatment. The purpose for constructing a mathematical model is to find appropriate dependences between the input and output parameters of the technological process. To conduct further studies taking into account of applying the MathCAD package, we will introduce the following variables: x – temperature of the extract; y – value of the hydrological module; z – duration of treatment.

To describe the dependences between the output variable and input parameters, a quadratic model of the following form was selected:

$$M_i(x,y,z) = a_{0,j} + a_{1,i}x + a_{2,i}y + a_{3,i}z + a_{4,i}x^2 + \\ + a_{5,i}y^2 + a_{6,i}z^2 + a_{7,j}xy + a_{8,j}xz + a_{9,j}yz, \quad (5)$$

where $a_{i,j}$ are the coefficients of mathematical model; j is the relation to the specific quality parameters of the pine needles extract.

The use of the model of type (5) gives the possibility to find, in a certain sense, the best ratio in terms of the indicators of technological process in relation to the quality parameters.

According to results of the scientific literature data and of previous research [17] into the technological process, it was found appropriate to set the following boundaries of changes in the input magnitudes: 40 °C≤x≤100 °C; 1:10≤y≤1:40; 10≤z≤100.

Encoded values of variables, for example x, are found from expression:

$$X = \frac{x - \frac{x_{max} + x_{min}}{2}}{\frac{x_{max} - x_{min}}{2}}. \quad (6)$$

Upon defining the experiment table, the matrix of experiment F was created based on it, which takes into account selected type of the mathematical model. After carrying out the research, the matrix of data on experiment Y was constructed. Determining the coefficients of the model was conducted by a general formula:

$$k = (F^T F)^{-1} F^T Y, \quad (7)$$

where k is the matrix of coefficients of the model.

Thus, for this technological process, we obtained the model of form (5) for the quality indicator of the extract of Scots pine needles:

$$Y1 = (x,y,z) = a_1 + a_2 \cdot x + a_3 \cdot y + a_4 \cdot z + a_5 \cdot x^2 + \\ + a_6 \cdot y^2 + a_7 \cdot z^2 + a_8 \cdot x \cdot y + a_9 \cdot x \cdot y + a_{10} \cdot y \cdot z. \quad (8)$$

The purpose of further analysis and modeling is to define such a totality of the input parameters of the process, at which it is possible to achieve maximum value of antioxidant ability of the pine extract.

For each output magnitude of antioxidant ability Yi, we will find such a totality of input parameters that provide for its maximum value for the pine extract. Finding such a totality will be conducted based on the obtained model (5). The criterion for finding a totality of input variables x_i will be the following equation:

$$x_i = \max_{x_i \in x} y_i, \quad (9)$$

where y_i is the value of the antioxidant ability of the extract; x_D is the permissible range of change in input variables.

Determining the totalities of input magnitudes that provide for the maximum value of output parameters of the extract is performed using the optimization program Maximize from the MathCAD package [18].

Based on results of the calculations, we found the optimum combinations of input variables and values of indicators of antioxidant capacity of the extract. For the extract of Scots pine needles, it is necessary to establish the values of the input magnitudes, which are presented in Fig. 1 in the form of a fragment of the MathCAD package.

$$\begin{pmatrix} x \\ y \\ z \end{pmatrix} := \begin{pmatrix} -0.419 \\ 1 \\ -0.573 \end{pmatrix}$$

Fig. 1. Values of input magnitudes for pine needles extract

The found values are presented in the encoded form. To convert them to natural values, we used formula (6).

By applying the model, we found the following theoretical values of antioxidant activity of the extract of pine needles: Y=223 mg/dm^3. It shows the feasibility of using mathematical modeling, since the found values do not exceed the values that were obtained based on the results of the experiment.

At the last stage, we performed correction of the input magnitudes with regard to subsequent use of the obtained values during production. The extraction was carried out in accordance with the obtained optimal conditions: hydromodule – 1:20; temperature – 60 °C, extraction time – 30 min.

The finished extract was cooled to temperature 8...10 °C and filtered. The mass fraction of solids in the finished pine needles extract is 3.25 %.

The biological value and organoleptic parameters of the pine extract were determined (Table 1).

Table 1

Organoleptic parameters and biological value of aqueous extract of pine needles

Parameter	Characteristic
Exterior	Transparent liquid of golden color
Fragrance	Clear, with pronounced pine fragrance
Flavor	Harmonious, refreshing with a pine tone
Content of ascorbic acid, mg/100 g	0.275
Antioxidant activity, Kl/100 g	202.3

The extract was added to beer wort at the stage of main fermentation, because it ensures minimum loss of aromatic substances. An addition of the pine extract will make it possible to enrich the drink with biologically active substances and to obtain new original beer with a refreshing pine tone.

5. 2. Construction of a mathematical model for determining the quantitative composition of pine needles in beer, which partly replaced hops

A quantitative ratio of hops and pine needles in the formulation of beer was found by mathematical modeling, which makes it possible to maximally approach quality parameters of the finished product [19]. According to modern perceptions, polyphenols of malt and hops largely determine the taste, color, foamy properties of beer, as well as a tendency of the finished drink to colloidal turbidity. Colloidal turbidity is mainly formed by antocianogenes, contained in hops and malt tanning materials [20, 21]. Therefore, the risk of turbidity in beer increases with an increase in the share of hops.

A task to be solved when constructing a mathematical model is to assign its order. Taking into account that there is no preliminary information about the nature of relationship between the assigned magnitudes x_1 (amount of pine needles), and x_2 (amount of hops), we proposed as a quality parameter of the finished product a mathematical model of fourth order, which takes the following form:

$$\hat{y}(x_1, x_2) = a_1x_1 + a_2x_2 + a_3x_1x_2 + \\ + a_4x_1x_2(x_1 - x_2) + + a_5x_1x_2(x_1 - x_2)^2. \quad (10)$$

Another issue that needs to be solved is to determine the range of change in the input components with regard to equation:

$$\sum_{i=1}^{2} x_{1u} = 1, \; x_{iu} \geq 0 \quad (i = 1, 2, u = 1,2,\ldots,N), \quad (11)$$

where N in the number of points of planning.

Given the results of earlier studies [22] on the formulation composition, we may conclude that the the relative proportion of pine needles may not exceed 0.3 of the proportion of hops. This limitation is based on the results of previous studies of the finished product in terms of organoleptics. Excession of proportion of pine needles larger than by 0.3 leads to a significant deterioration in beer quality. We proposed numerical data for conducting the experiment, presented in Table 2.

Table 2

Numerical data of the experiment

No. of experiment	1	2	3	4	5	6	7
X_1	0	0.1	0.15	0.2	0.25	0.12	0.22
X_2	1	0.9	0.85	0.8	0.75	0.88	0.78

According to the results of the conducted experiments, two vectors for quality evaluation of the finished product were created (Table 3): Y_1 – the score by organoleptic parameters (a 25– point scale), Y_2 – the score by the content of tannins (mg/dm^3), and the matrix of the two-factor experiment was constructed.

Coefficients of the model were found by formula:

$$a_i = (F^TF)^{-1}F^TY_i, \quad (12)$$

where F is the matrix of the experiment, constructed based on data in Table 1.

Table 3

Results of the study

Parameter	Number of experiment				
	1	2	3	4	5
Y_1	24,0	24,0	24,8	24,5	22,6
Y_2	239,0	236,0	232,0	229,0	225,0

A criterion of least squares was accepted as the criterion for approaching the parameters of product quality as it allows us to approach the assigned value without taking into account possible large deviations from the set values on a small interval of fluctuations in the values of parameters of the technological mode:

$$q = (y_i - y_{is})^2, \quad (13)$$

where y_i is the quality indicator whose value is determined by the results of computing a mathematical model; y_{is} is the assigned quality indicator of the corresponding parameter.

To determine the ratio of pine needles and hops for finding the maximum value of organoleptic indicator and the minimum value of the content of tannins, the appropriate programs from the MathCAD package were employed.

In the course of calculation, it was found that the optimal values relative to the quality criteria do not correspond to the uniform combination of input parameters of the technological process. Thus, the maximum value in terms of organoleptics was provided at x_1=0.165, x_2=0.835. The minimum value in terms of tannic substances was provided at x_1=0.25, x_2=0.75. To find the final values of indicators of content of pine needles and hops, it is necessary to integrate these criteria into one comprehensive criterion. Therefore, it is necessary to simultaneously minimize two criteria of quality indicators of the finished product.

The criterion, which is presented in the form of the following formula, was selected as a generalized quality criterion:

$$Q(x_1, x_2) = \\ = \sum_{i=1}^{2}\left[(24{,}86 - Y1(x_1, x_2))^2 + (225 - Y_2(x_1, x_2))^2\right]. \quad (14)$$

Numerical values are the desired values of quality indicators of the finished product. The final formula by which parameters of a product are determined, and results of the calculation, are shown in Fig. 2 in the form of the MathCAD program fragment:

$$R2 := \text{Minimize}(Q, x_1, x_2) \quad R2 = \begin{pmatrix} 0{,}192 \\ 0{,}808 \end{pmatrix}$$

$$M1(R2_1, R2_2) = 24{,}651 \qquad M2(R2_1, R2_2) = 29{,}462$$

Fig. 2. Final formula for determining the parameters of the finished product

To find the numerical values for the ratio of amount of pine needles and hops, we used a standard program Minimize from the MathCAD package.

The following final values of quantitative ratio in the formulation of beer with the addition of pine extract were obtained: x_1=0.2; x_2=0.8; thus, the content of pine needles may not exceed 20 % by weight of the estimated norm of hops.

5. 3. Description of technological process for brewing beer with the addition of pine needles extract

The technological process consists of the following operations: preparation of mash, its saccharification, filtering, wort boiling, cooling and introduction of yeast, wort fermentation, additional fermentation of young beer and bottling of beer "Smaragd" (Ukrainian: emerald). Making beer is carried out according to the acting "Technological instruction on malt and beer production" TI 18-6-47-85 and "Technological instruction on the production of 10 % lager beer "Smaragd", TI 14297558-340:2016, developed by Kharkiv University of Food Technology and Trade (Ukraine). The aqueous extract of pine needles was added by the standard of 600–620 ml/dal of malt and it was fermented by beer yeast of bottom fermentation at temperature 8...10 °C. The main fermentation was carried until the content of the visible extract was 2.5–2.8 %. Fermentation and final fermentation of beer lasted not less than 25 days.

Clarification (filtering) of beer was carried out by the kieselguhr filter. In case of insufficient saturation of beer with carbon dioxide, there was additional carbonization at temperature 0...2 °C. After maturing, beer was pumped to forfases for further storage and bottling. Pasteurization is carried out under automated mode according to the set program of the process.

5. 4. Development of beer formulation with the addition of the pine needles extract

The formulation of 10 % lager beer "Smaragd" (Ukr. emerald) with the addition of aqueous extract of pine needles was developed (Table 4).

Table 4

Formulation composition of 10 % lager beer "Smaragd", consumption of raw materials per 1 dal of beer

Ingredients	Content of ingredients
Brewing light barley malt	100 %
Yeast of bottom fermentation	50–100 g/dal
Hop granules, norm of bitter substances	Bs=0.4–0.7 g/dal
Extract of pine needles	600–620 ml/dal
Technical water	by consumption

The decision on the optimum ratio of ingredients was made by the results of mathematical modeling and organoleptic evaluation. The content of pine needles, converted to sublimation substance, is not higher than 20 % by weight of the estimated norm of hops. This amount is sufficient to keep bitterness and aroma of hops and saturation of the developed new drink with biologically active substances.

5. 5. Quality assessment of the developed beer with the addition of pine needles extract

The quality of the finished drink was assessed by organoleptic indicators, which are listed in Table 5.

The foaming of the finished beer was determined as follows: the foam height is 30 mm, foam stability is 3.0 min. Nutritional value of the beer "Smaragd" is 4.6 g/100 g, energy value is 42 kcal/100 g.

Table 5

Organoleptic indicators of 10 % lager beer "Smaragd"

Indicator	Characteristic of indicator	
	Filtered beer	Non-filtered beer: clarified, non-clarified
Exterior	Clear foamy liquid, without sediment and outer impurities not inherent in beer	Clear foamy liquid, without sediment and outer impurities not inherent in beer. Permissible presence of yeast sediment and particles of protein-tanning compounds
Fragrance	Pure malt, hops, with light scent of pine needles, without any outside smells	Clear, fermented, malt, hops, with light scent of pine needles, without any outside smells Permissible weak yeast aroma
Taste	Clear, malt, with pronounced hops bitterness and refreshing pine tone, without any outside after-tastes	Clear, fermented, with pronounced hops bitterness and refreshing pine tone, without any outside extra after-tastes

All-purpose chromatographic method of research allowed us to obtain the typical chromatograms, derived by the method of absolute calibration, which are required for the identification of microcomponents. Calculations of chromatograms of two beer samples were performed: one was made with the addition of the extract of pine needles; the other was made according to the classical technology, by groups of chemicals.

The content of methanol, which belongs to the group of one-atom alcohols, is:

– in the sample with the addition of pine needles – 0.004 %, which corresponds to the peak area of 2.850;

– in the sample, produced by the classical technology – 0.010 %, which corresponds to the peak area of 8.352.

Based on the obtained results, the content of methanol in the sample with the addition of pine extract is 2.5 times less than in the sample, produced by the classical technology.

Methanol is known to be the most toxic compound of all alcohols. It is oxidized in the human body much slower than ethyl alcohol and, during its oxidation, a variety of poisonous substances are formed. Methyl alcohol is quickly absorbed through the stomach and small intestine. Methanol is almost entirely (90 %) metabolized in the liver via enzyme alcoholdehydrogenase, resulting in the formation of formaldehyde and formic acid that are highly toxic. Kidneys remove metabolites of methanol, and the unchanged smaller part (15 %) is released through lungs. Thus, the addition of pine needles extract reduces the content of methanol in beer and decreases its adverse effects on the human body.

The next stage of the study was examining the physical and chemical indicators and biological value of the finished drink (Table 6).

Antioxidant activity of the developed beer is 178.1 Kl/100 g, which defines its high biological value. Addition of the pine needles extract at the stage of the head end fermentation not only enhances the gustatory properties of the finished drink, but also reduces the content of methanol and increases its biological value. The introduction to beer of the extract, which has plant-based antioxidants, is only one way of suppressing the growth of microorganisms and enhancing antioxidant ability of the finished drink. This opens up the prospect of research, aimed at developing the measures of antioxidant stabilization of different varieties of beer that might be implemented into production, which is sure to have a positive impact on the quality of this food product.

Table 6

Physical and chemical indicators and biological value of 10 % lager beer "Smaragd"

Indicator	Value
Mass fraction of dry substances in primary wort, %	10.3
Mass fraction of alcohol, %	2.9
Acidity, cm^3, 0.1 mol/dm^3 of solution of sodium hydroxide per 100 cm^3 of beer	1.7
Color, cm^3, 0.1 mol/dm^3 of iodine solution per 100 cm^3 of water	1.2
Mass fraction of carbon dioxide, %	0.3
Stability, days:	
filtered pasteurized	35
Non-filtered non-pasteurized clarified	5
Non-filtered non-pasteurized non-clarified	3
Content of ascorbic acid, mg/100 g	3,52
Antioxidant activity, Kl/100 g	178.1

5. 6. Study of influence of beer with the addition of pine needles on the organism of biological subjects

To study the influence of the pine needles extract on specific properties of beer, the antioxidant system of the organism of biological subjects was assessed. The studied indicators for males and females changed proportionally, and did not differ by dynamics, so it was appropriate to compare overall indicators in the groups of animals.

White nonlinear rats received beer at the dose of 15 g/kg for 14 days. The introduction of beer to animals that was prepared according to the classical technology probably increased the content of pro-oxidation markers in the liver homogenate when compared with animals that received placebo: DC by 20.4 %, TBA-reagents by 51.6 %. At the same time, the activity of catalase decreased on the contrary by 40.9 %. Homogenate markers of liver of animals that received beer with the pine needles extract were within the physiological norm (Table 7).

Under the influence of prednisolonum (50 mg/kg), the content of diene conjugates in the liver of animals that received the indicated beer increased by 23.8 %. A comparison was carried out to the animals, which, under conditions of oxidation stress, consumed purified water. The content of TBA-reactants was by 13.7 % higher, the content of reduced glutathione was by 25.0 % lower, compared with the placebo group. For the animals that received beer with the pine needles extract, all markers of anti-prooxidation liver system and catalase activity did not differ credibly from the indicators of animals that consumed purified water (Table 7).

Table 7

Content of pro-antioxidant markers in liver homogenate of mice after 14 days of inter-gastric introduction of the examined beverages under conditions of modeling of oxidation stress, n=6

No. of the group	Diene conjugates (μmol/g)	TBA reactants (μmol/g)	Reduced glutathione (μmol/g)	Catalase activity (μmol/g)
Groups of animals of intact control				
1	9,8±0.8	6.2±0.6	13.2±0.4	6.1±0.8
2	11.8±0.5*	9.4±0.7*	12.4±1.2	3.6±1.1*
3	9.2±0.9	6.9±0.3	13.6±1.5	5.6±0.8
Groups of animals with modeled pathology				
4	21.4±1.9	16.8±0.9	5.6±0.5	2.1±0.4
5	26.5±1.5*	19.1±1.2*	4.2±0.8*	1.8±0.6
6	22.3±0.7	16.5±1.4	5.3±0.4	2.0±0.5

*Note: * – the change is probable, with respect to the values of animals in the groups receiving placebo (water): for the animals of intact control – indicator of group No. 1, for the animals of reference pathology – indicator of group No. 4 ($p<0.05$)*

Since we conducted postmortal liver tests of animals for the preparation of homogenate, it was impossible to examine the behavior and condition of rats after the end of the experiment. According to the general rules of performing experiments involving animals, we were visually examining the state and behavior of animals in the process of the study. In the course of the experiment, the observed peculiarities of behavior and condition of the rats did not differ from generally accepted data on the effects of alcohol on the organism of biological objects.

In the white non-linear mice that received classic beer by the model of normobaric hypoxia, there was a reliable decrease in the average life span by 313.1 s under conditions of oxygen deficiency compared with the animals that received purified water. This may be explained by the fact that against the background of alcohol abuse, the negative effect of hypoxia on the brain increases and the processes of lipid peroxidation in brain tissues are potentialized. In animals, which were introduced with beer with the pine extract, the average life span remained at the level of intact indicators and had no reliable deviations (Table 8).

Table 8

Results of examining the influence of the investigated drinks on the model of normobaric hypoxia

Experimental group (n=6)	Average life span of animals (M±m), s
Intact control (purified water)	1523.5±67.1
Beer, produced by classical technology	1210.4±45.9*
Beer with extract of pine needles	1498.6±52.4

*Note: * – change is probable, with respect to the values of animals from the intact control group ($p<0.05$)*

Assessment of the impact of adding the extract of pine needles on the beer properties under conditions of acute pathological state suggests that the consumption of this product instead of beer, brewed according to the classical technology, may decrease the oxidation effect of alcoholic beverage on the brain.

6. Discussion of the impact of the pine needles extract on the finished beer quality

The proposed technique for preparing the pine extract may be applied at breweries and enterprises of light-alcohol production, because its implementation does not require any additional means.

The content of pine needles in the formulation of beer "Smaragd", converted to sublimation substance, does not exceed 20 % by weight of the calculated norm of hops, which is enough to maintain bitterness and aroma of hops.

An addition of pine extract enhances the taste of beer, reduces the content of methanol and increases the content of biologically active substances in the finished drink.

Under acute pathological condition, beer with the addition of pine needles extract reduces the oxidation influence of alcoholic beverage on the brain of biological objects. The "Smaragd" beer reduces toxic effect of alcohol on the organism of a living creature due to its antioxidant properties.

The formulation composition of beer "Smaragd" was approved at the meeting of Specialized sectoral tasting board on quality evaluation of beer, non-alcoholic, low-alcohol beverages, mineral and drinking waters, syrups and concentrates, Ukrpivo. The patent of Ukraine was received for the useful model No. 109200 "A technique for producing beer "Smaragd".

Introduction of the pine needles extract to beer, due to the plant-based antioxidants of the former, is one of the ways

to enhance antioxidation capacity of the finished drink. This opens up the prospect for research aimed at developing measures to stabilize different varieties of beer that can be implemented in production, which will have a positive impact on the quality of this food product.

7. Conclusions

1. Optimum parameters of extraction of pine needles relative to the indicator of antioxidant activity of the extract were determined: hydromodule is 1:20; temperature is 60 °C, extraction duration is 30 min. Pine extract has clear aroma and harmonious, refreshing taste with a pine tone. The content of ascorbic acid in the extract is 0.275 mg/100 g, antioxidant activity – 202.3 Kl/100 g.

2. A mathematical model was constructed and quantitative composition of pine needles in beer was determined that does not exceed 20 % by weight of the estimated norm of hops, which is sufficient to maintain bitterness and aroma of hops.

3. The formulation of 10 % lager beer "Smaragd" was developed. In terms of organoleptic, physical and chemical indicators, the developed beer meets the requirements of DSTU 3888:2015. The finished drink has clear, malt flavor, with pronounced hop bitterness and a refreshing pine tone. The content of ascorbic acid in beer is 3.52 mg/100 g, antioxidant activity is 178.1 Kl/100 g.

4. An influence of beer with the addition of pine needles extract on the organism of biological objects was explored. Markers of the liver anti-prooxidant system in rats that received beer "Smaragd" against the background of oxidation stress are equal to: DC=22.3 μmol/g, TBA-reagents=16.5 μmol/g, RG=5.3 μmol/g and catalase activity=2.0 μmol/min·g and do not differ credibly from the indicators of animals that consumed purified water. The mice that consumed beer, brewed by the classical technology, on the model of normobaric hypoxia, had a probable reduction in the average life span by 313.1 s compared with the animals that received purified water (1523.5 s). For the animals that were given beer with the pine needles extract, the average life span remained at the level of intact indicators – 1498.6 s. According to the biochemical studies, beer "Smaragd" minimizes the negative impact of alcohol on the body due to its antioxidant properties.

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