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STATE BIOTECHNOLOGICAL UNIVERSITY

**FORMATION OF BEER QUALITY WITH THE ADDITION  
OF NON-TRADITIONAL PLANT MATERIALS**

Monograph

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The monograph, based on experimental studies, scientifically substantiates and develops a new method of producing and enriching craft unfiltered beer with a natural herbal additive made from non-traditional plant material (pine needle extract) with antioxidant properties. A comprehensive commodity assessment of the developed beer enriched with pine needles, which differs from the traditional beer by its high content of natural plant biologically active substances, such as low- and high-molecular weight phenolic compounds and aromatic substances, was carried out. This allowed us not only to increase the biological value of the new beer, but also to double its shelf life. The reduction of the negative impact of the developed beer on the human body was proved in biological facilities. Data on the social and economic efficiency of the new products are presented.

Recommended for scientists, practitioners of the food industry, teachers who train specialists for the food processing industry and trade, postgraduate students and higher education students.

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## LIST OF ABBREVIATIONS USED

BAS – biologically active substances  
ATA – antioxidant activity  
MAC – maximum allowable concentrations  
TI – technological instruction  
DM – dry matter  
MF – mass factor  
SSRN – state sanitary rules and norms  
IPA (India Pale Ale) – Indian pale ale  
APA (American Pale Ale) – American pale ale  
EBC – European Brewing Convention  
ASBC – American Society of Brewing Chemists  
LP – lipid peroxidation  
DC – diene conjugates  
TBA – thiobarbituric acid  
RG – reduced glutathione  
CC – catalase activity  
DBZA – dithiobisbenzoic acid  
TRA – trichloroacetic acid  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> – sodium thiosulfate

## INTRODUCTION

**Relevance of the topic.** The problem of nutrition, which is relevant today in the country and in the world, is the imbalance of diets, the lack of vitamins, minerals and other biologically active substances (BAS), the content of which is almost twice lower than the recommended norms. It is known that this leads to a decrease in performance, a decrease in the activity of the immune system, and an increased risk of cardiovascular, oncological and other diseases. This is the reason for the widespread use of plant materials for the enrichment of food products with dietary supplements.

Recently, natural medicinal spice and flavouring raw materials have been replaced by synthetic additives that are harmful and can accumulate in the human body in the form of various allergens. The trend towards ensuring a healthy diet for the population is based on the naturalness of ingredients and the development of innovative technologies aimed at creating products with functional properties and increased nutritional value.

Beer is a promising product for enriching beverage bars, as it is a mass-consumption beverage. Today, mini-breweries focused on the production of craft beer using only natural ingredients are actively developing in Ukraine. The creation of new author's varieties with original organoleptic properties encourages brewers to search for new sources to expand their beer range. At the same time, there is a problem of supplying breweries with quality hop products. There is a shortage of domestic hops in Ukraine. In addition, the short shelf life of unfiltered beer causes many problems. Thus, to solve the above problem, the introduction of an additional component for beer production with partial replacement of hops with non-traditional plant materials that have antioxidant and preservative properties is an urgent task.

Improvement of consumer properties of low-alcohol beverages through the use of non-traditional raw materials and their extracts was investigated V. Domaretsky, V. Pribylsky, R. Pavlyuk, O.V. Bocharova, M. González-Gross, M. Lebrón, A. Marcos, J. Romeo, M. Kushad and others.

In view of the above, the use of additional natural ingredients for beer production will enrich the finished product with bioactive substances and extend its shelf life. Of particular interest is pine needles, which include a complex of the following BAS: low-molecular-weight phenolic compounds, flavonol glycosides, terpenoids, which can mobilise the body's defences and have antioxidant and preservative effects. The chemical composition and properties of pine needles are close to those of hop cones, the main raw material for brewing.

In view of the above, the study of the quality of non-traditional vegetable raw materials and the improvement of the consumer properties of craft beer using them is relevant.

**SECTION. 1**  
**SCIENTIFIC AND THEORETICAL ASPECTS**  
**FORMATION OF BEER QUALITY**

**1.1. Characteristics of the main raw materials for beer production and its impact on the quality of finished products**

Today, competition among brewers requires the use of only high-quality natural plant materials and strict compliance with regulatory documents [1]. The health benefits of beer depend on the chemical composition of the raw materials. The main raw materials for production are grain products (mainly barley), water, hops, and yeast.

According to the composition of extractives and their ability to ferment, barley is the most suitable cereal for malting operations. The main varieties are those that contain a lot of starch and a small amount of proteins, which leads to a better yield of extractives [2].

The most important requirements for the quality of grain used for malting are its active germination (90-95%), sufficient size and uniformity, moderate protein content (at least 8% and not more than 12%) and high starch content (up to 65%), and low film content (12-13%) [3].

Malt contains enzymes and starch. The starch is broken down by enzymes into simple sugars, which under the influence of yeast convert alcohol into carbon dioxide. Part of the malt protein is broken down by enzymes to feed and grow the yeast, while the rest remains in the beer, ensuring its extractability. The colour of beer is determined by the intensity of the Maillard reaction, which occurs during the drying of the malt. Depending on the malting process, there are different types of malt: pale, Pilsen, Vienna, Munich, caramel (dark), smoky, with active diastases, toasted, black, etc.

To increase the extractivity of beer and give it a certain taste, unsweetened raw materials (rice, corn, soya etc.). They are used to increase extractivity, create a certain taste and reduce the cost of beer.



In beer production, water is an important raw material and process product. Its chemical composition determines the organoleptic properties of beer. Water cations and anions affect the pH of the mash, wort and beer, which affects the fermentation processes during beer production. They also affect fermentation and subsequently the taste and stability of beer.

Water treatment plays an important role in beer production. The taste of the beverage is primarily influenced by the salt composition of the process water, which is subject to requirements for hardness, active acidity (pH), taste and smell, mechanical and microbiological purity. It is generally accepted that soft water (0.1–1.8 mg-eq/l) is advisable for light brews, and moderately hard water (1.8–3.5 mg-eq/l) for dark brews [4]. Water is used throughout the entire production process and is one of the most important elements in brewing.

Hops are an essential raw material for beer production. Hops contribute to the clarification of the beverage and the formation of foam, and increase its stability during storage. Not only the quality of beer, but also the efficiency of the brewing process as a whole depends on the rational use of hops. There are more than 100 varieties of hops, each with its own characteristics [5]. For a more complete use of hop extractives, it is used in ground briquetted form, in the form of hop granules, hop extracts. To enhance the beer aroma, the last portion of it is added at the end of wort boiling.

Hops contain essential oil (0.3–1.0%), bitter acids –  $\alpha$ ,  $\beta$ ,  $\gamma$  (10–20%), resins (12–20%) and tannins (2.0–6.0%). During the production of beer, hop bittering substances exhibit antibiotic activity against various microorganisms [6].

Hop polyphenols have high antioxidant properties, but some of them are lost due to their high reactivity. Tannins affect the taste of beer and form insoluble compounds with soluble proteins during brewing, contributing to clarification and stability of the drink [7].

Hops have beneficial and preventive properties [8, 9]. It is an indispensable component of any beer, but in case of excessive consumption, it has a negative impact on the human body. Hop cones contain 8-prenylnaringenin, a substance that

belongs to the class of phytoestrogens [10-12]. The content of phytoestrogens in raw materials ranges from 20 mg to 300 mg per 1 kg of plant mass. The effective concentration of the female hormone in 1 litre of beer can reach 0.15 mg in terms of estrogen. 90% of it is contained in beer in an inactive form, but under the influence of the intestinal microflora in the human body, it is converted to an active form [13].

From a botanical point of view, hemp is the closest relative of hops, and they can even be crossed to create hybrids. Hemp is a source of drugs such as marijuana and hashish. Hops also contain these drugs, but in a lower concentration. In addition, hops contain morphine, which is the active ingredient in opium and heroin [14].

German doctors have discovered carcinogenic substances in beer that come from hops. These are bitter substances that are classified as resins that contribute to the development of cancer. Hops are the most specific, indispensable and expensive raw material for beer production. Hops have a shelf life of 9 months in the refrigerator when not granulated. But the granulated product does not lose its quality for 1–2 years. Modern breweries do not use hop cones in their production, but rather use pellets made from hop extract.

Most breweries use imported hop products, as the market for granulated hops in Ukraine is underdeveloped. Of course, this leads to higher product prices, but such raw materials are of higher quality and sometimes indispensable when it comes to specific aromatic varieties that are only grown in certain areas. Today, Hopsteiner Ukraine is the only hop growing and processing company in Ukraine (80% of the country's total) that has its own pellet mill. Domestically produced granulated hops are used by mini-breweries, which account for 10% of the beer market. Large enterprises with foreign capital buy hop products abroad – in Germany, the UK, the US, Australia, etc.

A promising direction in beer production is the partial replacement of hops with natural plant materials (coniferous needles), which are close to hops in terms of their properties and chemical composition [15].

The yeast used in brewing belongs to the class Ascomycetes, order Endomycetales, family Saccharomycetaceae, genus *Saccharomyces*, species

*Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. Brewer's yeast is used for the fermentation of wort from fruit juices and honey, taking into account differences in carbohydrate composition and different levels of active acidity [5].

Based on the foregoing, it can be noted that the quality of raw materials primarily influences the formation of beer's consumer properties. Companies conduct studies of its compliance with regulatory requirements, as the safety of raw materials is the key to a high-quality beverage.

## **1.2. Formation of beer quality during production and its biological value**

### 1.2.1 Technological features of unfiltered beer production

The technological process of beer production is one of the factors that influences the quality of the finished product. The beverage production process includes preparation of the mash, boiling of wort with hops, fermentation of beer wort, beer fermentation and maturation, filtering and bottling of beer.

According to the technological requirements, the selection of malt and its milling ensure high quality of the wort and the finished beverage. The yield of malt extractives depends on the grinding: husk is 15–18%, coarse cereal 18–22%, small cereals – 30–35%, flour – 25–35% [16; 17].

There are two main methods of mashing: infusion and decoction. The first method is the simplest, but it requires highly fermentable malt. This produces a wort that has a light colour and a less pronounced taste. Boiled mashing methods are more common. Separation of the wort from the pellet is carried out on a filter press or filtration apparatus.

Filtered wort and wash water are collected in the wort brewhouse and boiled with hops. During boiling, the wort is boiled down to the solids concentration specified for each type of beer; aromatic and bitter substances are extracted from the hops; proteins are coagulated and the wort is sterilised. Coagulation of proteins is accelerated by tannins in hops. This process is important for the composition, fullness of flavour and colour of the beverage. The aroma of wort and beer is influenced by

hop oil, which is highly volatile. To enhance the aroma of beer, the last portion of hops is added before the end of boiling. During this time, the hop essential oil does not have time to fully evaporate and remains in the wort. It is at this stage that brewers achieve a predetermined density of the initial wort [18].

The hopped wort brought to the required concentration is filtered and cooled to 4...6 °C. Using separators, the wort is clarified, thus it is freed from proteins, suspended particles and saturated with air oxygen, necessary for normal function of yeast. The initial concentration of the cooled wort, its acidity and colour should correspond to the type of beer [18]. After this operation, the prepared beer wort is cooled to 6...7 °C and sent for fermentation.

The fermentation process consists of two stages: the main fermentation and the fermentation. During fermentation, most of the wort sugars are converted into alcohol and carbon dioxide, forming by-products that contribute to the taste and aroma of beer. Depending on the concentration of the initial wort, the duration of the main fermentation varies from 7 to 10 days. To saturate to a standard CO<sub>2</sub> concentration (0.3–0.35%), 1% of extractives are left in the young beer, and its solubility is increased by lowering the temperature to 0...2 °C and increasing the pressure to 0.03–0.07 MPa.

Clarification and loss of coarse bitterness during fermentation occurs after the end of fermentation, when the yeast settles and captures protein particles and hop resins as a precipitate [19]. During beer maturation, the content of aldehydes decreases, and the amount of esters, higher alcohols and acids increases. This enriches the taste and aroma of the drink. The duration of fermentation and aging depends on the type of beer (from 20 to 90 days) [20]. Unfiltered, unpasteurised beer in mini-breweries is stored in porcelain and delivered to the bar for bottling on an automatic line using special equipment. The finished product is poured into dark-coloured plastic bottles of various volumes and labelled.

Today, scientists are discussing the development of new technologies from fermentation to filtration, stabilisation, packaging, and analysing changes in beer safety and quality control, including chemical, microbiological and sensory analysis.

Brewers-technologists, without changing the technological parameters of the process and using flavour and aroma additives in the beer recipe, create new varieties with original organoleptic characteristics.

### 1.2.2 Classification of beer flavour components

New approaches to assessing the organoleptic and toxicological characteristics of the main beer fermentation products make it possible to identify substances that form the standard of permissible toxicity and the flavour and aroma bouquet of the drink.

The taste and aroma of beer are influenced by organic and inorganic compounds formed during fermentation and fermentation, but the substances whose concentration is greater than or equal to the threshold sensation value are important. The threshold of sensation is the value at which the aroma or taste of substances is detected by the taster in 50% of cases.

They produce substances that form the bouquet of young beer (diacetyl, aldehydes, sulphurous substances). They give beer a green, unripe taste and smell and, in high concentrations, adversely affect beer quality. These substances can be removed from beer by biochemical means during fermentation and maturation, which is the purpose of beer maturation. There are also substances that form the bouquet of finished beer (higher alcohols, esters). They largely determine the aroma of beer and, unlike the first group, cannot be removed from beer by technological means [20].

In recent years, the global brewing industry has been successfully using a system of terms and standard descriptions of the most important beer flavours and aromas developed by the European Brewing Convention (EBC) with the assistance of the American Society of Brewing Chemists (ASBC), which is accepted and understood internationally. This terminology system includes 144 beer flavours and aromas, grouped into 44 groups that form 14 main classes (Fig. 1.1). Depending on the amount and threshold of sensation, all beer components are divided into four groups [21].



The fourth group includes components present in concentrations more than 10 times lower than the threshold. It includes several hundred different substances that form the background taste of beer. All of these components interact with each other, and exceeding the concentration of one of them leads to defects in the finished product.

The most important substances of the carbonyl group are acetaldehyde, 2,3-butanedione (diacetyl) and trans-2-nonenal, which has a very low threshold of sensation and is the main substance characterising the flavour stability of beer. Beer contains flavour and aroma components that are in concentrations below the threshold of their sensation, as well as substances whose concentration may be above the threshold of sensation. The threshold values of organoleptic and toxicological characteristics of the main fermentation products that determine the taste and aroma of beer are given in Table 1.1.

Beer should have the right balance between bitterness, acidity, sweetness, alcohol content, concentration of esters, a pleasant hop aroma, and contain small amounts of many flavour components. Their concentrations should not exceed thresholds, but the sum will have a positive effect on the taste and aroma of beer [21].

Today, scientists are considering the need to introduce new methods of beer quality control. The international brewing community is offered generalised indicators: "biological value", "total dose of taste or toxicity", aimed at harmonising with international nutrition standards and bringing the beer quality and safety control system to the modern level.

The influence of the smell of a certain substance in the overall aroma is expressed by the aroma number. This concept was first introduced by scientists from the A.N. Bach Institute of Biochemistry. The fragrance number is the derivative of dividing the mass concentration of a substance by its threshold concentration.

Table 1.1

**Organoleptic and toxicological characteristics of the main fermentation products that determine the taste and aroma of beer [21]**

Substance	Limits of MPC, mg/l	Threshold value of taste and odour threshold, mg/l	LD50, mg/kg (lethal dose)	Toxicity class: experimental/calculated	Taste and aroma in beer that you can feel (EBC, ASBC*)
I. Restrictions based on toxicological criteria					
Ethanol	1000,0	2,5	800–900	3/4	Characteristic alcoholic (0110)
Butyl alcohol	0,1	1,0–2,0	603	3/2	Pleasantly oily (0640)
Acetic acid ethyl ester	0,2	1,0–10,0	7700	4/4	Fruit lollipop (0140)
Acetaldehyde	0,01	0,01–0,03	400	3/3	Sharp fruity (0150)
Methyl acetate	0,1	0,1	2900	4/2	Sharp fruity (0130)
Vinyl acetate	0,2	0,3	1613	4/3	Pleasant aroma (0131)
Diacetyl	0,1	0,1–0,3	1600–3400	4/3	Sweet, savoury (0620)
Acetoin	3,0	8,0–20,0	no data available	4/ no data available	Fruity (0140)
Higher alcohols C <sub>6</sub> –C <sub>10</sub>	10,0	10,0–15,0	1500	4/4	Pleasant floral (0161)
Octanol	10,0	10,0	4000	4/4	Floral (0160)
Methanol	3,0	0,1	50000–60000	3/3	Alcoholic taste and smell (0110)
Barbiturates	0,5	–	200	2/4	Odourless
II. Restrictions on flavour class and taste					
Amyl alcohol	1,5	0,0004	3000	4/4	Pungent odour of fusel oil **
Dimethyl sulphide	0,03	0,10–0,12	3700	4/4	Musty cucumber (0730)
Propyl alcohol	0,25	0,3	2260	4/3	Unpleasant pungent odour of fusel oil **
Allyl alcohol	0,1	0,003	66	3/2	Sharp irritant **

\* The taste and aroma of beer is indicated according to the classification of the European Brewery Convention (EBC) and the American Society of Brewing Chemists (ASBC). The classification is supported by two other national organisations in America and Japan: The Master Brewers Association of the Americas (MBAA) and the Brewery Convention of Japan (BCOJ).

\*\*Pungent off-flavours that go beyond the terminology system (the "wheel of beer flavours and aromas") agreed between the EBC, ASBC, MBAA, BCOJ.



The tasting assessment of beer is quite subjective, as it cannot fully characterise the influence of the qualitative composition and quantitative content of the aroma components of the raw materials used. Therefore, it is necessary to pay attention to determining the total amount of substances that form the taste and aroma of the finished product.

### 1.2.3 Quantitative and qualitative composition, nutritional and biological value of beer

The chemical composition of beer is of great importance for assessing its quality. This beverage is a rather complex system of organic and inorganic crystalloids and colloids in a weak water-alcohol solution, which includes more than 400 compounds. Beer, like other fermented beverages, is a natural product whose chemical composition varies widely. It depends on the type, production technology, and raw materials used. All beer components are divided into major and minor components [22].

The main components of beer are water (91–93%), carbohydrates (1.5–4.5%), ethyl alcohol (2.8–8.0%) and nitrogen-containing compounds, mainly amino acids and polypeptides (0.20–0.65%). It contains a small amount of higher alcohols (50–100 mg/l), methyl alcohol is almost absent. Minor components of beer include minerals, vitamins, organic acids, phenolic compounds, bitter and aromatic substances, biogenic amines, and estrogens.

The ethanol contained in beer, along with carbohydrates, is the main component of beer. The amount of ethanol can affect the taste of the drink. It should be noted that the carbon dioxide contained in beer encourages people to drink it to quench their thirst. The drink also stimulates gastric secretion and blood circulation in the muscles, brain, liver, lungs and kidneys [23–25].

Ethanol ingested with beer does not cause excessive negative effects due to the high water content of the drink. Despite the large amount of fluid consumed by beer drinkers, there are no significant disturbances in blood osmotic pressure due to the isotonic properties of the drink [26].

Beer's dietary supplements are amino acids, vitamins and minerals. Nitrogenous substances in beer are transferred to the drink from raw materials and yeast in the course of their vital activity. Typically, their content does not exceed 8-10% of the total extract. They consist mainly of protein breakdown products (albumin, peptides, polypeptides, amino acids and purine bases); they contain nitrogen and some enzymes and B vitamins. Most of the nitrogenous compounds in beer (40–50% of total nitrogenous substances) are medium-molecular compounds that stabilise the beer foam, 20–30% are high-molecular nitrogenous substances, and about 10–30% are low-molecular compounds, mainly amino acids.

Mineral compounds are added to the beverage from malt, other raw materials and water. Beer contains potassium, sodium, calcium, magnesium, phosphorus, sulphur and chlorine ions in biologically significant amounts. This beverage differs from other alcoholic beverages, in particular wine, in its high potassium content (160–450 mg/l). Beer consumed in the amount of 1 litre per day can meet the daily requirement for this element by about 30%. At the same time, beer is relatively low in sodium (about 120 mg/l). It is rich in calcium (about 80 mg/l), magnesium (about 80 mg/l), phosphorus (about 140 mg/l), and the content of iron, copper, zinc and other metals does not exceed 1 mg/l.

A 1-litre drink made from wort with a 10% concentration contains 20–50 mcg of vitamin B<sub>1</sub> (thiamine), 340–560 mcg of vitamin B<sub>2</sub> (riboflavin), 5800–9000 mcg of nicotinic acid, and about 110 mcg of folic acid. Consumption of 1 litre of beer per day can provide 40–60% of the daily requirement for these vitamins. A lot of thiamine is contained in malt and wort, but it is adsorbed by yeast. At the same time, a large amount of this vitamin in beer has a negative side, as it accelerates the degradation of phenolic compounds in the drink and causes precipitation. Beer contains 1–2 mcg of riboflavin per 1 g of barley, and its content doubles during malt germination, and this amount is retained in the finished product. The content of ascorbic acid in beer is 20–50 mg/l, and it is added during production to prevent spontaneous oxidation of other components. The beverage contains relatively small amounts of vitamin B<sub>6</sub> (0.4–1.7 mg/l), pantothenic acid (0.4–1.7 mg/l) and biotin

(about 5 mg/l). It should be noted that many vitamins have a phosphorylated form, so they are well absorbed [26].

Beer contains about 0.3% carbon dioxide, which affects the taste. The beverage contains volatile organic (acetic, formic, etc.) and non-volatile acids (citric, succinic, malic, fumaric, and pyruvic) ranging from 300 mg/l to 400 mg/l, as well as acidic phosphates and hop acids. This explains why the pH level of a fresh drink is in the range of 5.1–5.4. Beer contains salts of pyruvic (about 60 mg/l), acetic (about 90 mg/l), gluconic (about 30 mg/l) and oxalic (about 15 mg/l) acids. They are well absorbed in the intestines and are actively involved in metabolic processes. Organic acids are contained in beer in the form of salts. The largest amount is represented by citric acid salts (about 130 mg/l), which acts as a synergist of antioxidants and increases the stability of the drink [5].

Beer contains more than 20 amino acids: proline, glycine, alanine, phenylalanine, tyrosine, etc. Methionine was found in small amounts (14.8 mg/l), histidine (44.1 mg/l), valine (91.5 mg/l), leucine and isoleucine (91.9 mg/l), lysine (28 mg/l), threonine (91.6 mg/l), glutamic acid (38.9 mg/l). Scientists are conducting comprehensive research on the impact of the composition of the raw materials on the quality characteristics of the finished drink. The data obtained by the authors on the amount of amino acids in beer show that their total content, when the density of the initial wort is increased from 9.0% to 22.0% for light beer, ranges from 58.0 mg/l to 802.62 mg/l [27]. Phenolic compounds in beer are represented by tannins, flavonoids, anthocyanins, and anthocyanidins. Their total content is in the range of 150–300 mg/l (Table 1.2).

Approximately 80% of polyphenols are transferred to the beer wort from malt and 20% from hops. Beer contains the most anthocyanidins (14–77 mg/l), which include leucocyanidins, procyanidins and leucoanthocyanidins. Some beers contain quercetin, one of the most biologically active phenolic compounds. In addition to the above, beer contains other polyphenolic compounds (ellagic, procatechin, vanillic, salicylic, paraoxybenzoic acids, as well as phenol, orthocresol and coumarins) in concentrations of 1 mg% or less. Polyphenols have a positive effect on the intestinal microflora [28].

Table 1.2

**Phenolic substances in beer [39]**

№ з/п	Component	Average content, mg/l
1	Anthocyanidins	14–77
2	Catechins	5–55
3	Epicatechins	9–24
4	Rutin	1–6
5	Quercetin	5–125
6	Quercetin	1
7	Chlorogenic acid	2–20
8	Caffeic acid	2–20
9	Quinic acid	1–5
10	P-coumarinic acid	1–7
11	Ferulic acid	2–21
12	Synaptic acid	1–20
13	Camperol	5–20
14	Miricitrin	1
15	Gallic acid	5–29
Total number of		150–300

To date, about 70 components have been identified that are classified as aroma compounds in beer. They are divided into reduced and oxidised fractions. The reduced fraction includes monoterpenes (myrcene) and sesquiterpenes ( $\beta$ -caryophyllene, humulene, farnesin, etc.). The oxidised fraction consists of terpenes (linalool, geraniol) and other alcohols, aldehydes, ketones, esters and their derivatives.

Beer contains more than 30 macro- and microelements, most of which come from malt. About a third of the minerals are sodium, potassium and calcium salts, and another third are phosphoric acid salts. About one tenth is silicic acid salts.

Lipids are added to beer from malt or other grain raw materials, and are partly products of yeast fermentation. The bulk of them are fatty acids, which increase the nutritional value of the drink and its biological stability.

Beer contains a small amount of glycerol (about 0.1–0.3%). Another by-product is aldehydes, which are carbonyl substances that have a significant impact on taste and aroma. The following aldehydes have been found in beer: acetic, propionic, cinnamic, isobutyric, isovaleric, etc. The main aldehyde in beer is acetic

aldehyde (acetaldehyde), which gives a "grassy" or "pickle" flavour, typical of an immature drink. In young beer, its content ranges from 20 mg/l to 40 mg/l; in mature beer, it is less than 8–10 mg/l [21].

Bittering substances of hops are also important, with a content of 50 mg/l to 100 mg/l in the finished product. They have a positive effect on the digestive functions of the body [24]. Bittering substances come into beer from hops and give it a specific bitter taste. Depending on the technology of brewing and storage, they can undergo polymerisation, oxidation and, accordingly, change their original properties [29]. Low resinous substances, which are especially abundant in beer, consist mainly of humulone and lupulin. Humulone, which makes up 7% of the total amount of low resinous substances, provides only specific aromatic properties. An increase in the bittering content is accompanied by the appearance of bitterness in beer. Humulone is present in the form of  $\alpha$ -humulone, which is highly soluble and has a bitter taste. Another isomeric form is  $\alpha$ -co-humulones, which are primarily involved in the formation of beer aroma. However, when their content increases to 30% of the total humulones, they cause excessive bitterness. Lupulin has the same effect and is a natural preservative in beer [30].

Beer bittering agents, along with other hop extractives, are classified as psychoactive compounds. They have sedative, hypnotic, and, in large doses, hallucinogenic effects. In addition, bitter substances have bactericidal, antiseptic, bacteriostatic properties and have a stimulating effect on gastric juice secretion [30].

The content of phytoestrogens in beer is 1–36 mg/l. However, this amount is sufficient for a pronounced hormonal effect on the human body [14]. It is likely that changes in endocrine status (feminisation of men and masculinisation of women) in beer drinkers are mainly related to the influence of phytoestrogens.

The nutritional value of beer is determined mainly by carbohydrates. They contribute to the fullness of taste and consistency of the drink, form sorption complexes with aromatic substances that prevent negative changes during beer storage. Beer carbohydrates are mainly represented by short-chain dextrans

(75–85%) and simple sugars such as glucose, fructose, sucrose (10–15% of the total), which are easily absorbed by the body. Only 2–3% of carbohydrates are complex sugars (polysaccharides, etc.).

The energy value of beer ranges from 37 kcal to 78 kcal. The composition of extractive substances varies depending on the mass fraction of solids in the initial wort and the degree of its fermentation. A significant proportion is made up of maltodextrins (3.0–3.6%), as well as maltose (0.5–1.5%), glucose and fructose (1.2–1.6%). In addition, extractives contain gum-like substances close to carbohydrates, which come from yeast, resinous acids from hops and pectin from barley [27].

Beer is the only alcoholic beverage that contains hop bitterness, which activates the secretion of gastric juice and suppresses the undesirable effects of alcohol due to the content of natural antioxidants [31]. But its excessive consumption can lead to negative consequences for the human body [32]. Analysing the data of scientific studies [33; 34], it can be concluded that 330 ml of beer per day (about 13.2 ml of alcohol) can be considered a harmless dose of consumption. The optimal average consumption per year is between 40 litres and 60 litres per person per year. In Ukraine, 46.1% of the population drinks beer, of which 60% are men [35].

Beer contains a number of important components, among which vitamins, minerals and organic acids play a key role. This determines its high nutritional and energy value compared to other alcoholic beverages [36].

Modern consumers prefer drinks containing only natural ingredients. The trend towards ensuring a complete, rational diet for the population is the naturalness of ingredients and the development of innovative technologies aimed at creating products with certain functional properties and increased nutritional value. Therefore, the production of beer using additional vegetable raw materials or their extracts with a high content of dietary supplements is a topical issue for the modern food industry.

### **1.3. The importance of biologically active substances in nutrition and analysis of plant extracts in the food industry**

The problem that is relevant today in the country and in the world is the imbalance of diets, the lack of vitamins, minerals and other nutrients, which are almost twice less than the recommended norms. It is known that this leads to a decrease in performance, a decrease in the activity of the immune system, and an increased risk of cardiovascular, oncological and other diseases. This is the reason for the widespread use of plant materials for the enrichment of food products with dietary supplements.

Recently, beverages with the addition of medicinal and non-traditional plant materials containing a wide range of dietary supplements, such as vitamins, micro- and macroelements, phenolic compounds, aromatic substances, etc. have become popular. The use of local raw materials in production will reduce the cost of purchasing and delivering them and expand the industry's product range.

When processing raw materials and producing extracts from them, it is necessary to preserve the biologically active phytochemicals of the original raw materials as much as possible. The soluble BAS of plant materials include alkaloids, essential oils, tannins, phenolic compounds, glycosides (saponins, bittering agents, flavonol glycosides, etc.), vitamins (carotenoids, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, C, E, K, PP, choline, biotin, etc.), resins.

Consumption of synthetic food additives leads to a decrease in the body's defence functions, allergies, and various diseases. In this regard, it is important to search for non-traditional natural sources of plant materials containing a significant amount of BAS, develop innovative technologies for obtaining natural additives from them to enrich consumer products and give finished products a certain taste and aroma, the status of functional and health products [37–39].

The enrichment of food products with natural additives from plant materials in the form of pastes, powders, concentrates, extracts, fillers, etc. is widespread. Extracts derived from spice and aromatic, medicinal and technical raw materials,

wild berries are particularly popular, as they can enhance the antioxidant, immunomodulatory and preservative properties of the finished product. The extraction of substances is carried out taking into account the factors that determine the effective extraction, which will ensure a high level of transfer of extracted substances.

Scientists [40], using the developed unique methods of deep processing of food raw materials, for the first time found that fresh raw materials contain significant reserves of low-molecular weight food and biologically active substances. It has been proven that the bound form contains 2–5 times more biologically active substances than have been extracted from plant material using traditional processing methods so far. Using new methods of processing food raw materials, the authors managed to transform 50–70% of the substances that were in a latent form into an easily digestible nanoform. This makes it possible to obtain products and additives with fundamentally new consumer properties that cannot be achieved using traditional processing technologies, which are characterised by a significant loss of raw material nutrients.

The staff of the Scientific School for Fundamental and Applied Research of Prof. R. Pavliuk is developing the latest technologies for processing various plant raw materials to create new competitive functional health products in the form of multivitamin and antioxidant powders, pastes, etc. with fundamentally new consumer properties [41–46].

Scientists [47] have created new phytoconcentrates for beverages, phytosyrups, soft drinks and food supplements from medicinal and technical plant materials with immunomodulatory, radioprotective and antioxidant effects. New puree and powdered plant additives give the product fundamentally new properties compared to the raw materials: the content of low-molecular weight dietary supplements in the free state is 2–3 times higher than in the analogues and their solubility is 2–3 times higher than in the raw materials, while colloids are formed and their absorption by living organisms is 2–3 times higher. New technologies have been implemented at enterprises in Ukraine and Latvia.



A group of scientists from the scientific school [48] developed additives for health foods from natural plant spices. The content of BAS, such as essential oil, phenolic compounds with P-vitamin activity, tannins, vitamins,  $\beta$ -carotene, L-ascorbic acid. They are natural immunomodulators, antioxidants, have detoxifying and preservative effects, and can be used to enrich a wide range of health foods and have an extended shelf life.

The Institute of Technical Thermophysics of the National Academy of Sciences of Ukraine has developed a technology for producing food powders from vegetable raw materials. Drying as a method of preserving food products and subsequent production of powders allows for high quality products. According to the technology, new powders made from natural raw materials can be used in the food industry to enrich the dietary supplements [49].

Food additives made from plant raw materials (vegetables and fruits), made on the basis of powders, have been developed to create areas of dietary, children's, therapeutic and functional purposes in human nutrition, which affect the chemical composition of food, improve the organoleptic, physicochemical, structural and mechanical properties of the final product. New food additives are particularly valuable due to the content of ascorbic acid, folate, carotenoids, bioflavonoids, and are the main supplier of these to the body [49].

Thus, the use of plant extracts is aimed at obtaining functional products enriched with BAS, which have antioxidant and immunomodulatory properties necessary in the context of environmental pollution and a decrease in the defences of the human body.

There are large areas of coniferous forests in the Eastern region of Ukraine, so it is advisable to consider the possibility of using pine needles in the food industry, which contain many BAS, especially low- and high-molecular weight phenolic compounds, aromatic and bitter substances, essential oils, resins, etc.

#### **1.4. Natural vegetable raw materials in beer production**

In recent years, unfiltered craft beer has become particularly popular in Ukraine and many other countries. It differs from traditional beer in its special technology, innovative recipes that include various non-traditional natural plant ingredients, and is most often produced in mini-breweries.

There are several criteria for selecting non-traditional medicinal and technical spice and aromatic plant materials used in beer production: improving the flavour stability, antioxidant capacity and colloidal stability of the finished beverage, and preserving the bitter substances of hops contained in the final product. Additional raw materials can provide beer with therapeutic and prophylactic properties: stimulating metabolism, calming the nervous system and improving sleep, and reducing the effects of toxins.

Scientists also envisage a partial replacement of hops in beer production by introducing other plant-based raw materials. When replacing the main and most expensive ingredient, vegetable raw materials are close to hops in terms of chemical composition and properties.

Oxidation processes that occur in food products lead to a deterioration in quality. Even a slight occurrence of these processes in beer can lead to a significant deterioration in the quality of the beverage. Such processes are commonly referred to as oxidative or organoleptic aging. They include undesirable changes in all the properties of the beverage that are important to the consumer, such as changes in colour, transparency, foaming, taste and aroma. Modern breweries carefully control the oxidation of beer [20; 21].

Many beer aging reactions involve free radicals, the formation of which is enhanced by oxygen, and as a result of catalytic reactions involving iron and copper ions. Oxidation after fermentation impairs both the taste and colloidal stability of beer. Beer aging reactions include oxidation of higher alcohols, oxidative decomposition of isohumulones, Maillard reaction and Strecker decomposition, fatty acid oxidation, enzymatic decomposition of fatty acids,

secondary oxidation of aldehydes, and other processes. Changes in the taste and aroma of beer are caused by carbonyl compounds (aging carbonyls) formed as a result of the oxidation of unsaturated fatty acids, higher alcohols and other substances in beer. Currently, there are many ways to increase the antioxidant capacity of beer [50].

To prevent oxidation processes, reducing agents are added to beer - salts of sulfuric and thiosulfuric acids,  $\alpha$ -ascorbic and isoascorbic acids and their salts, sulphites, sodium dithionite. The use of sulphur-based reducing agents in brewing in large quantities worsens the taste during beer storage. It is proposed to use a balanced mixture of sulfur compounds with sodium erythroborate sodium [51].

The authors of scientific papers have concluded that oxidation processes are inevitable and the only thing that can be done is to increase the antioxidant resistance of the beverage, thus affecting the rate of their progression [52].

Naturalness and environmental friendliness of food products have recently become particularly relevant requirements. One of the most promising methods of beer production is the use of vegetable raw materials or their extracts. They should be harmoniously combined in taste and aroma with the beverage, have a positive impact on the quality of the finished product and provide it with certain beneficial properties. The use of plant materials with antioxidant properties is one of the ways to slow down the antioxidation processes in beer.

Antioxidants are substances that can inhibit the oxidation of organic and high molecular weight compounds, thereby reducing the yield of oxidation products. Free radicals actively react with almost all biomolecules and oxidise them using unpaired electrons. As a result, the membranes and walls of blood vessels are damaged, and the body experiences oxidative stress. Today, increasing the antioxidant capacity of beer is a rather urgent task.

Natural antioxidants contain phenolic and polyphenolic compounds, carotenoids that can react with peroxide radicals, destroy hyperoxides without the formation of free radicals, which leads to chain breakage and slows down the rate of oxidation. Scientists pay special attention to flavonoids [53]. The use of

antioxidants and plant extracts containing such substances is one of the ways to increase the antioxidant capacity of beer. Natural raw materials and their extracts give beer not only new original flavours, but also functional properties. In addition, the use of natural antioxidants increases the nutritional and biological value of the product. This helps to improve the organoleptic characteristics of the beverage, preserving its taste and aroma throughout the entire shelf life [54; 55].

To date, many studies have been conducted to investigate the antioxidant activity of plant materials and their extracts in beer production. Some authors believe that the use of amaranth leaves can produce functional beer enriched with antioxidant compounds [56]. It is known that the antioxidant activity of beer can be increased by adding green tea [57], elderberry [58], rose hips [59], cinnamon [60] etc.

Today, scientists are conducting research on the creation of new beer varieties with improved therapeutic and prophylactic properties and using a wide range of flavour and aroma additives from natural plant materials [60].

Scientists have developed a beer with improved antioxidant properties with the addition of oak bark to prevent colloidal clouding. The use of the antioxidant is based on its ability to instantly react with peroxide radicals formed during the chain free radical oxidation of beer components, as well as with sensitive beer proteins, forming more flakes that precipitate and are removed by filtration. The technology improves antioxidant properties, which have a positive effect on the colloidal stability and taste of beer [61].

H. Golikova, L. Drozdkova, Y. Dmitriev, and N. Skurikhina patented a beer production method that recommends replacing a part of the flavouring additive with bird cherry (up to 40%). The drink has an original taste and aroma, and partial replacement of hop products leads to significant savings in expensive hops and, consequently, to a reduction in the cost of the finished product [62].

Chinese scientists Shi Jingchun and Yu Zhenlong have patented Pomegranate Beer. It is rich in vitamins and minerals derived from plant materials (pomegranate, parsley root, corn leaves, chrysanthemum buds, etc.), easily

digestible, stable during storage, stimulates metabolism, improves blood circulation, heart and brain function, calms the nervous system and improves sleep, and reduces the effects of toxins [63].

Scientists Duk Ki Kim and Gi Jun Kim have developed a method for the production of health rice beer, which includes the following extracts: coniferous, ginseng and wild lanceolate root. The drink has an original taste and aroma, and partial replacement of hops reduces its negative impact on the human body [64].

Guo Peng and Lu Yingying proposed a technology for producing beer with the addition of green tea and alfalfa grass. The finished drink is rich in protein and has a therapeutic and preventive effect [65].

There is a well-known method of producing beer with the addition of milk thistle, which, due to changes in its composition, has a positive effect on the human body, namely the liver and kidneys, and has medicinal properties. The taste of the resulting beverage is characterised by medium bitterness and the aftertaste of raw materials [66].

A group of scientists has developed beer with a high content of dietary supplements and trace elements by replacing components. The use of chicory extract expands the biochemical composition of the drink, provides its health-protective properties, and improves its taste [67].

Kryshtaleve beer was introduced by scientists L. Moskaleva, V. Domaretsky and S. Udod. The technology involves the addition of ginseng root and echinacea herb extracts, which increases the content of dietary supplements and gives the finished drink tonic, therapeutic and prophylactic properties [68].

There is a known method of producing beer with the addition of a pine additive, the share of which is 0.8% of the beer wort. The additive, made from pine bark, cones and needles powder, is added at the boiling stage with hops. This allows to improve the taste properties of beer, increase the biological value and medicinal properties of the finished beverage [69].

Czech scientists Henry Štěpánek and Miroslav Tuček proposed a method of producing red beer with extracts from elderberry, juniper berries and Sudanese rose flowers, which would give the drink original organoleptic properties [70].

The results of the analysis of the presented developments indicate the use of a wide range of natural ingredients in beer production technology. It has been proved that the use of plant materials is a very promising and relevant area of research. Analysing the presented developments, it can be noted that various plant materials and their extracts are used as additional raw materials in beer production, which makes it possible to produce beverages with biologically functional properties. Natural raw materials, namely pine needles, are promising, as they have antioxidant properties, increase the antioxidant capacity of the finished product and form its original organoleptic properties.

### **1.5. Characteristics of biologically active phytocomponents and therapeutic and prophylactic properties of pine needles**

Recently, we have been searching for new raw materials to expand our range of herbal supplements. The integrated and rational use of raw materials, in particular scots pine, is important when harvesting wood. The high content of bioactive substances in pine needles determines its value as a raw material for various pharmaceuticals and other products.

Scots pine needles (*Pinus sylvestris*) contain vitamins A, E, C, trace elements (zinc, cobalt, copper, calcium), resins, fatty and organic acids, essential oils, glycosides and phenolic compounds [71]. Recently, a new way of using pine needles has emerged – the production of biologically active products for medicinal, food and feed purposes.

It has antioxidant properties, cleanses the body of accumulated toxins, helps to eliminate toxins and radionuclides, and strengthens the body's immune system. Pine needles also contain  $\alpha$ -pinene, which is the main active ingredient and component of the terpene fraction (7–16%). These same components are

responsible for the antioxidant, antitoxic and antimicrobial effects of scots pine needle extracts and oil [72; 73].

The phytoncides found in pine needles are important antimicrobial substances. They are part of the essential oil, a mixture of volatile aromatic compounds.

The pharmaceutical effect of flavonoids is to regulate the state of capillaries. In particular, they increase their permeability in case of atherosclerosis and thus contribute to reduction and normalisation of blood pressure. They have a diuretic and antispasmodic effect on the human body, dilate capillaries and coronary vessels, lower blood pressure, tone heart muscles, and reduce blood clotting.

Ascorbic acid, the amount of which reaches 600 mg/kg in raw materials, is involved in redox reactions and carbon metabolism [74]. The content of this vitamin is largely influenced by the season, light and age of the pine tree. The maximum ascorbic acid content is observed in winter and early spring. These factors should be taken into account when harvesting green needles. Ascorbic acid helps to accelerate the elimination of toxins from the body, reduce total cholesterol levels, affects protein and carbohydrate metabolism, and is widely used to prevent and treat hypo- and vitamin deficiencies.

The pine needles also contain a significant content of fat-soluble vitamins, among which  $\alpha$ -,  $\beta$ - and  $\gamma$ -carotenes are particularly important, with their amount being close to the carotene content of carrots. 1 kg of needles contains 140-320 mg of  $\beta$ -carotene. Vitamin E is also important (350–360 mg/kg), which consists mainly of  $\alpha$ -tocopherol, which has the highest physiological activity and is one of the most powerful antioxidants [71].

Pine needles are rich in ash elements, water-soluble and alcohol-soluble components, pectin substances and protein.

Hemicellulose, which is part of the cell walls of needles, contains two to three times less mannose than wood. Water- and ether-soluble components, which include a number of important BASs, are of particular interest in terms of the ways in which industrial greens can be used.

In addition to ascorbic acid, sucrose, glucose, fructose, pectin, tannins, glycosides, picein, coniferin, etc., the water-soluble part contains glycosides.

The fats and resins present in pine needles contain free oxypalmitic acid and esters of palmitic, oxypalmitic, stearic acids, as well as cetyl, ceryl and merisyl alcohols. This raw material also contains abetic and oleic acids, various terpenes and terpene alcohols, and phytosterols.

The composition of ash substances in pine needles is as follows: total ash 2.8%, calcium 0.55%, phosphorus 0.15%, magnesium 0.1%, iron 156 mg/kg, manganese 318 mg/kg, copper 7 mg/kg, zinc 30 mg/kg, cobalt 0.09 mg/kg in terms of dry matter [71].

Of the total protein in pine needles, 30% is insoluble nitrogenous substances, 70% of the protein is albumin, globulins, prolamines, glutamine and other soluble nitrogenous substances, with the largest amount extracted by water and alkali, and the smallest amount by alcohol.

Phenolic compounds are among the least studied classes of pine needles extractives, which have not been widely used in industry and have been studied in a fragmentary manner. They are valuable dietary supplements that have bactericidal and fungicidal effects, antiradical and antioxidant properties, and are the main components of pine needle-based medicines. The quantitative composition of phenolic compounds in pine needles requires further research.

Of particular interest for beverage enrichment is pine needles, which contain low-molecular-weight phenolic compounds, flavonol glycosides, terpenoids, which can mobilise the body's defences and have antioxidant and preservative effects. The needles have a pleasant taste and aroma, antioxidant and antimicrobial properties. Its extract is used to reduce the toxicity of chemical and pharmaceutical products and to reduce the toxicity of alcohol in beer.

In the Eastern region of Ukraine, pine plantations occupy sufficient areas. The raw material is harvested by special procurement centres and sold in herbal pharmacies and herbal medicine stores. Thus, this raw material is available in sufficient quantities on the consumer market for use in beer production.



Pine needles are used in the manufacture of medicines, bath infusions, in the cosmetics industry, etc. and are permitted for use in accordance with the State Pharmacopoeia.

The organoleptic properties of the beverage are the main quality criteria for the consumer, so one of the urgent tasks is to produce beer with the addition of plant materials with an original taste and aroma. To enrich craft beer, it is proposed to use an unconventional natural plant additive from pine needles in the form of an extract, which will allow to obtain a drink not only with a high content of bioactive substances, but also to extend its shelf life.

The aim of further research will be to develop beer with the addition of pine needles (in the form of an extract) with original taste, aroma, antioxidant properties, increased content of BAS and extended shelf life.

### **Conclusions to Chapter 1**

It has been substantiated that a promising additional raw material for the production of hops, with its partial replacement, is natural non-traditional plant material - pine needles, which is close to hop cones in chemical composition and properties. This will allow enriching the finished product with dietary supplements and extending its shelf life, as pine needles contain a complex of dietary supplements: low- and high-molecular-weight phenolic compounds that can mobilise the body's defences and have an antioxidant and preservative effect.

Thus, it is important to shape the taste, aroma and quality of a new craft beer with a high content of BAS, which has antioxidant properties, using a natural plant additive made from pine needles.

## **SECTION. 2**

### **STUDY OF THE INFLUENCE OF RAW MATERIAL QUALITY ON THE CONSUMER PROPERTIES OF BEER**

#### **2.1. Quality of key raw materials for beer production**

The wide range of domestic and foreign beers on the Ukrainian market makes producers very demanding of the quality of raw materials used for its production [75]. Otherwise, the drink will not be able to compete with a wide range of craft beers. The quality of the main raw materials – water, malt, yeast, and hops – affects the quality of beer.

During the All-Ukrainian Forum of Brewers and Restaurateurs "Mini Brewery as a Successful Business" [76], which was attended by about 120 owners and managers of breweries, mini-breweries and restaurateurs from different cities of Ukraine, the forum organisers together with representatives of the university conducted a questionnaire survey. Participants were asked to answer questions about the quality and benefits of the main raw materials used in the production of light beer. Ten varieties of malt and hop pellets were selected.

The quality of malt has a significant impact on the formation of beer quality, taste and colloidal characteristics of the finished beverage [77]. Since malt is made from barley of different varieties, the quality of which depends on the region and soil and climatic conditions of growth, we consider it appropriate to study its quality. The objects of the study were malt varieties preferred by craft brewing specialists. The experimental varieties of brewing light barley malt were evaluated by organoleptic and physicochemical parameters.

The organoleptic method was used to determine the indicators of appearance, smell and taste. The study found that the homogeneous grain mass of malt does not contain mouldy or damaged grains, has a colour from light yellow with a grey tint to yellow, a malt aroma and a sweet, malty taste. It can be used as a base malt - up to 100% in the backfill.

Physicochemical parameters of brewing barley malt depend on the technology of its production. These indicators were controlled in accordance with to DSTU 4282:2004 [78]. The results of the study of light brewing barley malt (hereinafter referred to as malt) are given in Tables 2.1, 2.2.

The results of the study revealed that all malt varieties do not contain any trash impurities. The indicator characterising the sieving of grains through a 2.2 x 20 mm sieve has slight differences among the presented varieties: from 1.2% (Bohemian Pilsner Weyermann) to 1.7% (Chateau Pilsen 2R, Ireks Pale Ale Malt and Castle Malting Chateau Pilsen), which is within the permissible limit (no more than 2%).

Flour content is a criterion for evaluating the dissolution of malt, in particular its endosperm. The uniformity of endosperm dissolution is the most important indicator of malt quality, which affects the process of producing wort and beer, namely the extract yield, wort clarification, fermentation and fermentation processes, filtration and colloidal stability [71]. Proper drying of malt is characterised by the amount of floury grains (at least 90%). According to the research results, the content of such grains in malt did not differ significantly: the highest value was found in Weyermann Munich type II (93.6%), the lowest – in Malteurop Vienna (90.2%).

For high quality malt, the amount of vitreous grains should not exceed 2%. An increase in their proportion leads to an increase in the difference in the mass fractions of fine and coarse malt extracts. This has a negative impact on the extract yield, wort filtration and clarification, fermentation, fermentation and clarification of finished beer [79]. It was determined that the amount of vitreous grains in all varieties does not exceed the permissible standards and ranges from 1.0–1.6%. No dark grains were found in them.

Table 2.1

**Physicochemical characteristics of light brewing barley malt varieties**  
(n = 5, P ≥ 0.95)

Name of the indicator	Requirements of the State Standard of Ukraine 4282:2004	Name of the variety of brewing barley malt				
		Chateau Pilsen 2RS	Malteurop Vienna	Weyermann Premium Pilsner	Malteurop Pilsen	Weyermann Munich type II
1	2	3	4	5	6	7
Sieving through a sieve (2.2x20 mm), %, not more than	2.0	1.70	1.40	1.30	1.40	1.10
Mass fraction of waste impurities, %	Not allowed	Not detected				
Number of grains, %: mealy, not less than vitreous, not more than dark	90.0 2.0 Not allowed	90.3 1.4 Not detected	90.2 1.6 Not detected	92.0 1.2 Not detected	90.5 1.3 Not detected	93.6 1.0 Not detected
Mass fraction of moisture, %, not more than	4.0	3.3	3.2	3.4	3.5	3.5
Mass fraction of the extract in the dry matter of fine malt, %, not less than	80.0	83.0	82.4	86.5	86.8	84.1
Difference in mass fractions of extracts in the dry matter of fine and coarse malt, %	No more than 1.5	1.1	1.0	1.0	1.1	1.0
Mass fraction of protein substances in malt dry matter, %, not more than	10.5	9.5	10.1	9.8	9.5	10.0

Continuation of the table 2.1

1	2	3	4	5	6	7
The ratio of the mass fraction of soluble protein to the mass fraction of protein substances in the dry matter of malt (Kolbach's number), %	39.0–41.0	39.5	40.0	39.3	39.7	39.2
Soluble nitrogen in malt (on a dry basis), %	0.75–0.70	0.74	0.72	0.73	0.71	0.70
Saccharification time, min, not more than	10	10	9	8	8	8
Laboratory wort: Colour, cm <sup>3</sup> of iodine solution with a concentration of 0.1 mol/dm <sup>3</sup> per 100 cm <sup>3</sup> of water	No more than 0.18	0.14	0.14	0.16	0.17	0.17
Acidity, cm <sup>3</sup> of sodium hydroxide solution with a concentration of 0.1 mol/dm <sup>3</sup> per 100 cm <sup>3</sup> of wort	0.9–1.1	0.94	1.00	1.00	1.00	0.96
Final degree of fermentation, %	79–81	79.4	79.6	80.0	79.3	80.3
Viscosity, MPa-s at 20 °C	1.45–1.54	1.47	1.43	1.43	1.50	1.42
Content of $\beta$ -glucans, mg/l, not more than	145.0	138.0	139.0	143.0	142.0	139.0

Table 2.2

**Physicochemical characteristics of light brewing barley malt varieties (n = 5, P ≥ 0.95)**

Name of the indicator	Requirements of the State Standard of Ukraine 4282:2004	Name of the variety of light brewing barley malt				
		Ireks Pale Ale Malt	Castle Malting Chateau Pilsen	Bohemian Pilsner Weyermann	BernarD	Castle Malting Pale Ale
1	2	3	4	5	6	7
Sieving through a sieve (2.2x20 mm), %, not more than	2.0	1.7	1.7	1.2	1.6	1.3
Mass fraction of waste impurities, %, not more than	Not allowed	Not detected				
Number of grains, %: mealy, not less than vitreous, not more than dark	90.0 2.0 Not allowed	91.5 1.2 not detected	92.1 1.1 not detected	93.4 1.3 not detected	92.6 1.0 not detected	91.7 1.1 not detected
Mass fraction of moisture, %, not more than	4.0	3.5	3.3	3.5	3.7	3.2
Mass fraction of extract in the dry matter of fine malt, %, not less than	80.0	82.5	82.6	87.5	84.5	85.3
The difference in mass fractions of extracts in the dry matter of fine and coarse malt,%	No more than 1.5	1.2	1.2	1.0	1.0	1.1
Mass fraction of protein substances in malt dry matter, %, not more than	10.5	9.5	9.3	9.1	10.0	9.6

Continuation of the table 2.2

1	2	3	4	5	6	7
The ratio of the mass fraction of soluble protein to the mass fraction of protein substances in the dry matter of malt (Kolbach's number), %.	39.0–41.0	39.6	39.7	40.2	39.3	39.1
Soluble nitrogen in malt (on a dry basis), %.	0.75–0.70	0.71	0.72	0.72	0.71	0.74
Saccharification time, min, not more than	10	9	10	8	9	10
Laboratory wort: Colour, cm <sup>3</sup> of iodine solution with a concentration of 0.1 mol/dm <sup>3</sup> per 100 cm <sup>3</sup> of water	No more than 0.18	0.13	0.14	0.16	0.17	0.13
Acidity, cm <sup>3</sup> of sodium hydroxide solution with a concentration of 0.1 mol/dm <sup>3</sup> per 100 cm <sup>3</sup> of wort	0.9–1.1	1.00	0.93	0.98	0.96	0.95
Final degree of fermentation, %.	79–81	79.2	79.9	80.2	79.6	80.0
Viscosity, MPa-s at 20 °C	1.45–1.54	1.49	1.45	1.43	1.49	1.48
Content of $\beta$ -glucans, mg/l, not more than	145.0	130.0	125.0	142.0	140.0	143.0

The duration of malt saccharification is significantly influenced by the moisture content of the germinated grains. With increasing moisture content, the duration of saccharification decreases. The analysis of the data in Tables 2.1 and 2.2 shows that malt of different varieties contains a moisture content ranging from 3.2% to 3.7%, which contributes to a short saccharification process. Malteurop Vienna and Castle Malting Pale Ale had the lowest value, while Bernard had the highest.

The mass fraction of extract in the dry matter (DM) of malt affects its extractability. This is related to such an important indicator of malt quality as the duration of saccharification. In the experimental varieties, the mass fraction of extract in the DM of fine malt ranged from 82.4% to 87.5%, which is within the permissible range (not less than 80%). The best result was achieved by the Bohemian Pilsner Weyermann malt variety.

The degree of malt dissolution is characterised by the difference in extract yield when mashing fine and coarse malt. Studies have shown that this indicator varies between different varieties within the permissible range (1-1.5%). The highest percentage was found in Ireks Pale Ale Malt and Castle Malting Chateau Pilsen – 1.2. The rest have an indicator in the range of 1.0–1.1.

To assess the quality of malt, the mass fraction of protein substances is important, which is determined by the malt germination regimes. A very high content leads to a deterioration in the quality of the finished beverage during production and storage. In all varieties, the mass fraction of protein substances in malt solids is in the range of 9.1–10.1%, i.e. does not exceed the permissible standards (no more than 10.5%). This indicator is important when considered in conjunction with the Kolbach number.

Kolbach's number shows the ratio of the mass fraction of soluble protein to the mass fraction of protein substances in the malt dry matter. According to the standard, the value of this indicator for high quality malt is 39–41%. Exceeding the permissible limits will result in a deterioration of the beer's aroma.



In the experimental malt samples, the Kolbach number ranges from 39.1% (Castle Malting Pale Ale) to 40.2% (Bohemian Pilsner Weyermann).

It is known that the amount of nitrogenous substances that dissolve during malt germination is much less than that formed during mashing [72]. Reducing their amount will prolong the fermentation process. The research revealed that the content of soluble nitrogen in malt of the experimental varieties did not have significant differences. Weyermann Munich type II malt contained the lowest amount of nitrogen (0.70%), while Chateau Pilsen 2RS and Castle Malting Pale Ale were characterised by the highest content (0.74%).

The most important indicators of malt quality are the duration of saccharification and the mass fraction of extractives. The Weyermann Premium Pilsner, Malteurop Pilsen, Weyermann Munich type II and Bohemian Pilsner Weyermann varieties have a saccharification time of 8 minutes; the Malteurop Vienna, Ireks Pale Ale Malt and Bernard varieties – 9 minutes; the Chateau Pilsen 2RS, Castle Malting Chateau Pilsen, Castle Malting Pale Ale varieties – 10 minutes; the requirements of regulatory documentation – no more than 10 minutes [77].

In accordance with the requirements of the current standard, the laboratory wort was tested. It was found that the wort brewed from different types of malt was transparent in appearance. The wort colour index was almost the same for all varieties and was in the range of 0.13–0.17 units, i.e. did not exceed the requirements of the standard (no more than 0.18 units). The acidity of the laboratory wort in the experimental varieties did not differ significantly and ranged from 0.93 units to 1.0 units, i.e. was within the permissible limits (0.9–1.1 units). In order to regulate fermentation, the final degree of fermentation is determined, which is one of the priority indicators in quality assessment and is 79-81% for malt [110]. It was found that Bohemian Pilsner Weyermann has the highest rate (80.3%), and Ireks Pale Ale Malt has the lowest rate (79.2%).

The viscosity of wort depends on the content of reducing sugars, amine nitrogen and soluble protein [80]. This indicator in the malt of the experimental

varieties did not exceed the requirements of the standard (1.45–1.54 MPa-s) and ranged from 1.42 MPa-s (Weyermann Munich type II) to 1.50 MPa-s (Malteurop Pilsen).

To evaluate the quality of brewing barley malt according to DSTU 4282, it is recommended to comply with the requirements for the  $\beta$ -glucan content (no more than 145 mg/l), the physiological activity of which is their positive effect on carbohydrate metabolism. The research has shown that their quantitative content varies significantly between different varieties: the minimum amount is found in Castle Malting Chateau Pilsen malt (125 mg/l), and the maximum in Weyermann Premium Pilsner (143 mg/l).

Thus, according to the results of organoleptic and physicochemical studies, it was proved that the malt of the experimental varieties has a pronounced malt aroma and sufficient enzymatic activity to become the base mash, and fully meets the requirements of the current standard [77]. For beer production, the duration of saccharification and the mass fraction of extractives are important indicators, so the best malt varieties among the experimental samples are Bohemian Pilsner Weyermann and Malteurop Pilsen.

The safety of grain crops is ensured by determining the maximum permissible levels of safety indicators, including toxic elements and radionuclides. Among the heavy metals, lead, mercury, cadmium, zinc, and copper are particularly dangerous, as they are highly toxic and can accumulate in the body if consumed continuously. Therefore, it is advisable to study their content in brewing barley malt. The results of the study of the content of toxic elements are presented in Table 2.3.

The data obtained show that the indicators differ significantly, which can be explained by different places and conditions of plant cultivation. It was found that the mercury content in the malt of the experimental varieties does not exceed the maximum permissible concentration (0.03 mg/kg) and is in the range of 0.001–0.007 mg/kg. However, malt of the Bernard and Ireks Pale Ale Malt varieties is more prone to the accumulation of this trace element.

Table. 2.3

**Content of toxic elements in light brewing barley malt**

**(n = 5, P ≥ 0.95)**

Name of the variety of light brewing barley malt	Content, mg/kg					
	Mercury	Arsenic	Copper	Lead	Cadmium	Zinc
Requirements State Standard of Ukraine 4282:2004, permissible levels, mg/kg, not more than	0,03	0,2	10,0	0,5	0,1	50,0
Chateau Pilsen 2RS	0,004	0,013	3,81	0,054	0,018	20,0
Malteurop Vienna	0,001	0,021	3,73	0,042	0,011	20,0
Weyermann Premium Pilsner	0,001	0,011	3,45	0,036	0,013	19,0
Malteurop Pilsen	0,002	0,016	3,32	0,021	0,014	18,0
Weyermann Munich type II	0,001	0,012	3,38	0,027	0,011	18,0
Ireks Pale Ale Malt	0,005	0,015	3,19	0,021	0,012	18,0
Castle Malting Chateau Pilsen	0,001	0,013	3,62	0,020	0,010	19,0
Bohemian Pilsner Weyermann	0,003	0,014	3,27	0,027	0,017	17,0
BernarD	0,007	0,013	3,32	0,028	0,013	18,0
Castle Malting Pale Ale	0,001	0,011	3,10	0,023	0,012	20,0

Malt of different varieties has almost the same ability to accumulate arsenic and contains 0.01-0.03 mg/kg, which does not exceed the maximum permissible level (0.2 mg/kg). The most contaminated malt is Malteurop Vienna, Weyermann Premium Pilsner and Ireks Pale Ale Malt.

There were some differences in copper content: the highest amount was found in Chateau Pilsen 2RS (3.81 mg/kg) and Malteurop Vienna (3.73 mg/kg), and the lowest in Castle Malting Pale Ale (3.1 mg/kg), while the permissible value was 10.0 mg/kg. The lead content ranged from 0.020-0.054 mg/kg and does not exceed the requirements of the standard (0.5 mg/kg). The highest content was recorded in Chateau Pilsen 2RS and Malteurop Vienna (0.054 mg/kg and 0.042 mg/kg, respectively), while Castle Malting Chateau Pilsen had the lowest level.

Cadmium in malt is contained in amounts much lower than the established standards (0.1 mg/kg). Chateau Pilsen 2RS malt contains the most cadmium (0.018 mg/kg). Other varieties are characterised by approximately the same amount (0.010-0.014 mg/kg). The zinc content in malt varieties almost did not differ and ranged from 17.0 mg/kg (Bohemian Pilsner Weyermann) to 20.0 mg/kg (Chateau Pilsen 2RS, Malteurop Vienna and Castle Malting Pale Ale), while the permissible limit is 50.0 mg/kg.

Summarising the above, it should be noted that the malt of the experimental varieties contains heavy metals in amounts significantly lower than the maximum permissible concentrations.

Grain products are capable of accumulating strontium more than cesium, which is explained by the greater mobility of strontium in soils compared to cesium and ionic strontium in soil [81]. The content of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  radionuclides in malt is shown in Table 2.4.

The results obtained indicate that the radiological parameters of malt of different varieties differ slightly. The highest specific activity of radionuclides was found in the malt of Chateau Pilsen 2RS ( $^{137}\text{Cs}$  – 3.6 Bq/kg,  $^{90}\text{Sr}$  – 5.8 Bq/kg) and CastleMalting Chateau Pilsen ( $^{137}\text{Cs}$  – 2.57 Bq/kg,  $^{90}\text{Sr}$  – 5.8 Bq/kg).

**Radionuclide content in light brewing barley malt****(n = 5, P ≥ 0.95)**

Name of the variety of light brewing barley malt	Content, Bq/kg	
	<sup>137</sup> Cs	<sup>90</sup> Sr
Requirements of GN 6.6.1.1-130-2006, permissible levels of Bq/kg, not more than	50.0	20.0
Chateau Pilsen 2RS	3.60	5.80
Malteurop Vienna	1.22	4.50
Weyermann Premium Pilsner	1.03	4.10
Malteurop Pilsen	1.44	5.30
Weyermann Munich type II	1.15	4.32
Ireks Pale Ale Malt	1.31	4.70
Castle Malting Chateau Pilsen	2.57	5.80
Bohemian Pilsner Weyermann	1.33	4.60
BernarD	2.22	5.51
Castle Malting Pale Ale	1.03	4.96

The lowest values were recorded for malt of Weyermann Premium Pilsner (<sup>137</sup>Cs – 1.03 Bq/kg, <sup>90</sup>Sr – 4.1 Bq/kg), Weyermann Munich type II (<sup>137</sup>Cs – 1.15 Bq/kg, <sup>90</sup>Sr – 4.32 Bq/kg) and Bohemian Pilsner Weyermann (<sup>137</sup>Cs – 1.33 Bq/kg, <sup>90</sup>Sr – 4.6 Bq/kg).

Thus, the malt of the experimental varieties complies with the State Hygiene Standards "Permissible Levels of <sup>137</sup>Cs and <sup>90</sup>Sr Radionuclides in Food and Drinking Water" [82]. According to the results of the research, it was determined that the content of toxic substances and radionuclides in malt of all the experimental varieties does not exceed the permissible limits.

Hops are an indispensable component in beer production. Today, hop growing in Ukraine is underdeveloped, and malt production consists of many technological operations, so it is quite technological and needs to be brought to the highest possible level [83]. Breweries want to use raw materials mainly from foreign

producers [84], which are of high quality and have advantages during production. The next step was to study the quality of ten different varieties of hop pellets.

The type of hops (bitter, aromatic) is determined by the originator of the variety, which is indicated in the documents. Type-90 pellets are almost indistinguishable from hop cones in their composition; 90 kg of pellets of this type are obtained from 100 kg of cones by pressing [85]. The results of determining the quality indicators of hop pellets are shown in Table 2.5.

Table 2.5

**Quality indicators of aroma and bitter hop granules of different varieties.-90  
(n = 5, P ≥ 0.95)**

Name of the variety hop granules	Name of the indicator		
	Colour	Mass fraction of moisture,%	Mass fraction of $\alpha$ -acids, % in air-dry matter, not less than
Requirements of the State Standard of Ukraine 7028:2009	From light green to green on the surface and at the at the granule break	7.0–10.0	Hops aromatic – 2.5 bitter hops – 4.0
Clone-18, aromatic	Light green on the surface and at the break of the granules	8.1	4.9
Mandarina Bavaria, aromatic		7.5	7.4
Premiant, bitter	Green on the surface and at the granule break	8.0	9.0
Saaz, aromatic		8.1	3.1
Bramling Cross, aromatic		7.8	5.0
Sladek, bitter		8.3	5.2
Magnym, bitter		7.4	12.7
Hallertau Perle, bitter	Light green on the surface and at the break of the granules	7.9	8.4
Hallertau Hersbrucker, aromatic	Green on the surface and at the granule break	7.9	7.3
Williamette, aromatic		8.8	5.0

Each variety has a distinctive appearance and aroma. In appearance, they are cylindrical granules of different shades and approximately the same size. Clone-18,

Mandarina Bavaria, Magnym and Hallertau Perle hop pellets had a light green colour on the surface and at the break of the pellets. Premiant, Saaz, Bramling Cross, Sladek, Hallertau Hersbrucker, and Williamette were characterised by a darker shade of green on the surface and at the break. This indicates that the hop cones were harvested on time and all the requirements for drying raw materials were met.

An important indicator of pellet quality is the aroma. All varieties had a pure hop aroma characteristic of hop cones, without any other aroma. The most pronounced aroma is that of Mandarinina Bavaria and Hallertau Hersbrucker.

To evaluate the quality of hop granules, the mass fraction of moisture is important, as its content affects the processes that occur during storage and the quality of the finished beverage [84]. In all varieties, the moisture content is in the range of 7.4–8.8%, i.e. does not exceed the permissible standards (7–10%). The highest indicator is in Williamette hop pellets (8.8%), and the lowest is in Magnym (7.4%).

The source of hop bitterness in beer is soft resins, which contribute to foaming resistance, shape the aroma of beer and inhibit the growth of microorganisms, which ensures the stability of the drink during storage [84]. The main indicator of hop quality is the content of  $\alpha$ -acids. Hop bitterness in beer depends on the content of  $\alpha$ -acids. A high level of this indicator will provide a bitter taste and aroma to the finished product. The best hop pellets are Bramling Cross type-90, an aromatic hop variety, and Magnym, a bitter hop variety. It was found that among the aromatic varieties, the Mandarinina Bavaria variety has the highest mass fraction of  $\alpha$ -acids (7.4%), and the Clone-18 variety has the lowest (4.9%). The bitter varieties had indicators ranging from 5.2% (Sladek) to 12.7% (Magnym), which does not exceed the permissible standards.

Thus, the quality assessment of hop pellets revealed no violations of quality indicators. All varieties met the requirements of regulatory documents and had values within the permissible limits.

Beer is the product of the biochemical activity of yeast, which gives it its flavour. Along with the composition of the wort and technological conditions, they play an important role in the production of the beverage and affect the quality of the finished product. In addition, brewer's yeast enriches the beverage with B vitamins and minerals.

The organoleptic characteristics of beer, its taste and stability depend on the quality of water, which is the main ingredient in brewing. Any drinking water used in beer production must be treated and disinfected. After that, the necessary analyses and organoleptic evaluation are carried out. Therefore, the next stage of the study was to assess the quality of drinking water prepared for beer production. At this stage, the university department fruitfully cooperated with OLNA LLC (Kharkiv). At the enterprise, water undergoes detailed processing: it is filtered and softened. The water hardness and salt composition are regulated using a membrane water treatment method based on the principle of reverse osmosis. To remove unpleasant odours, the water is deodorised by passing it through a column filled with activated carbon. The treated water is then fed into storage tanks. The production laboratory checks the water quality for compliance with the standard requirements.

Water quality was determined according to TI 14297558-291-2003 [86]. The colour, turbidity, odour, taste, hydrogen index, total mineralisation, hardness, alkalinity, and content of mineral impurities were evaluated. The results of determining the indicators of the qualitative composition and quantitative content of mineral impurities in a batch of water prepared for beer production are given in Table 2.6.

Having analysed the results, we can say that the water meets the requirements of the current standard. It is clear (turbidity is  $0.05 \text{ mg/dm}^3$ ), colourless, tastes good, and is odourless. The chromaticity is 2 degrees and does not exceed the permissible limits (no more than 10 degrees).



Table 2.6

**Results of studying the qualitative composition and quantitative content of  
mineral impurities in water prepared for beer production**

**(n = 5, P ≥ 0.95)**

Name of the indicator	Requirements. TI 14297558-291-2003	Prepared water
<b>Organoleptic characteristics</b>		
Odor at temp: 20 °C, points	0	0
60 °C, not more than, points	1	0.50
Colour, no more than, degrees	10	2
Turbidity, not more than, mg/dm <sup>3</sup>	0.5	0.05
Taste and aftertaste, points	0	0
<b>Physical and chemical characteristics</b>		
Hydrogen index (pH), within, pH units	6.0–7.0	6.05
Dry residue (total mineralisation), not more than, mg/dm <sup>3</sup>	500.0	174.0
Total hardness, within, mmol/dm <sup>3</sup>	2.0–4.0	2.00
Total alkalinity, within, mmol/dm <sup>3</sup>	0.5–1.5	1.3
Sulphates, not more than, mg/dm <sup>3</sup>	150	25,0
Chlorides, not more than, mg/dm <sup>3</sup>	150	33,0
Total iron, not more than, mg/dm <sup>3</sup>	0.1	0.07
Manganese, not more than, mg/dm <sup>3</sup>	0.1	0.050
Copper, not more than, mg/dm <sup>3</sup>	1	0.005
Zinc, not more than, mg/dm <sup>3</sup>	1	0.0020
Calcium, within limits, mg/dm <sup>3</sup>	2.0–4.0	3.8
Magnesium, mg/dm <sup>3</sup>	Traces	Traces
Sodium, not more than, mg/dm <sup>3</sup>	150	36.0
Potassium, not more than, mg/dm <sup>3</sup>	150	50.0
<b>Sanitary and toxicological parameters, inorganic components</b>		
Aluminium, not more than, mg/dm <sup>3</sup>	0.2	Does not contain
Arsenic, not more than, mg/dm <sup>3</sup>	0.01	Does not contain

It is known that water hardness affects the quality of the finished beverage [82]. Our studies have shown that this indicator is within the permissible limits (2.0–4.0 mmol/dm<sup>3</sup>) and amounts to 2.1 mmol/dm<sup>3</sup>. The hydrogen index reached a value of 6.05 pH units (the norm is 6.0–7.0 pH units), and the amount of dry residue is 174 mg/dm<sup>3</sup> (the norm is 500 mg/dm<sup>3</sup>). Alkalinity has a significant impact on the taste of the beverage: its increased value will lead to a high pH value of wort and beer [87]. The total alkalinity of water is equal to 1.3 mmol/dm<sup>3</sup> (the permissible rate is 0.5–1.5 mmol/dm<sup>3</sup>).

Prepared water contains dissolved minerals (ions). Their type and concentration can affect the suitability of water for use in beer production, mashing performance, and the taste of the finished beverage [4]. According to the results of the study, the main minerals of interest for brewing are calcium (3.8 mg/dm<sup>3</sup>), magnesium (traces), sodium (36.0 mg/dm<sup>3</sup>), chlorides (33.0 mg/dm<sup>3</sup>) and sulphates (25.0 mg/dm<sup>3</sup>) are present in sufficient quantities and have a positive impact on the quality of the finished product.

The treated water also contains other compounds: total iron (0.07 mg/dm<sup>3</sup>), manganese (0.05 mg/dm<sup>3</sup>), copper (0.005 mg/dm<sup>3</sup>), zinc (0.002 mg/dm<sup>3</sup>). The potassium content in water affects the taste, adding saltiness to beer at excessive concentrations [87]. The amount of this component was determined to be 50 mg/dm<sup>3</sup>, which does not exceed the permissible limit (150 mg/dm<sup>3</sup>). Toxic elements of aluminium and arsenic were not detected in the test sample.

Thus, the water quality assessment did not reveal any violations of the quality indicators and the quantitative content of mineral impurities, and the water met the requirements of the NTC. Therefore, it will not affect the quality of the finished beverage during production and storage.

Today, beer production uses additional vegetable raw materials that shape the original consumer properties of the drink. Companies' innovation activities involve the development and introduction of new products, improving their quality and competitiveness. When developing new beer varieties, it is necessary to take into account consumers' requirements for their component composition.

## **2.2. Marketing rationale for the launch of Emerald beer with pine needle extract**

In the alcoholic beverages segment, beer is the market leader in terms of sales, accounting for more than 46%, and is consumed by the majority of the adult population. Today, Ukraine has prospects for the development of pubs and breweries focused on original drinks made from the best natural ingredients using an original approach.

The introduction of a newly developed beer to the consumer market necessarily involves preliminary market research on consumer preferences when buying a drink [87]. The most difficult thing in promoting craft beer is the product itself and the factor of fighting the mass market price environment. Therefore, it is necessary to justify the reason for the cost and promote the product using incentive marketing.

In order to identify new approaches to the formation of consumer properties of beer with the addition of vegetable raw materials, a marketing study of visitors to breweries in Kharkiv was conducted using a questionnaire survey. Out of a thousand questionnaires that were replicated, all those that were correctly filled out were selected for analysis, in the amount of 963 questionnaires. The empirical method determined that 630 questionnaires are sufficient to study the five age groups of the population we have identified by seven questions to ensure the representativeness of the sample. Since the study processed more than 900 questionnaires were processed, the results can be considered reliable.

The survey was conducted by questionnaire. The sample consisted of people of different genders, taking into account the specifics of the product, over 18 years old, with different income levels. The socio-demographic profile of the sample is shown in Table 2.7.

An important characteristic of consumer demand is the frequency of beer consumption. It was found that the majority of respondents, namely 31%, consume it 2–3 times a month, 45% – once a week, and 9% – 2–3 times a week (Fig. 2.1).

Table 2.7

### Characteristics of the socio-demographic profile of respondents

Characteristics of the socio-demographic portrait	Number of respondents, people	Share of respondents, persons
Gender: male	506	52.5
female	457	47.5
Age: 18 to 25 years old	241	25.0
from 26 to 35 years old	312	32.4
from 36 to 45 years old	212	22.0
from 46 to 55 years old	149	15.5
from 56 years and older	49	5.1
The level of average per capita family income per month:		
up to UAH 3000	65	6.7
from 3001 UAH to 5000 UAH	178	18.5
from 5001 UAH to 6000 UAH	216	22.4
more than 6000 UAH	504	52.3
<b>Total</b>	<b>963</b>	<b>100</b>

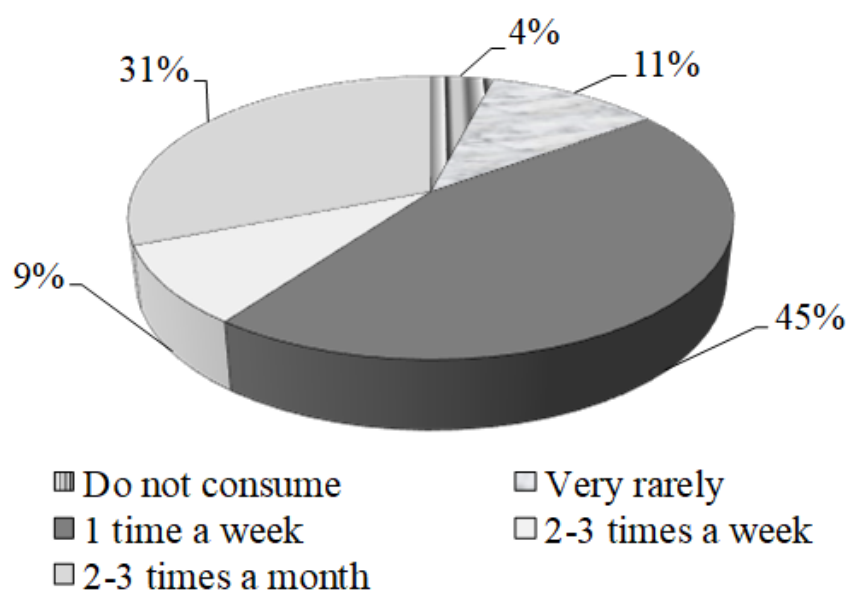


Fig. 2.1. Distribution of respondents' preferences by frequency of beer consumption in breweries

Very rarely, 11% of respondents drink beer. It should also be noted that almost 4% of respondents said they do not drink beer at all. There were no answers about drinking this drink on a daily basis.

Given the above data on preferences regarding the frequency of beer consumption, it is advisable to find ways to improve its consumer properties. This conclusion is also confirmed by the data on consumer satisfaction with the range of foamy beverages (Fig. 2.2).

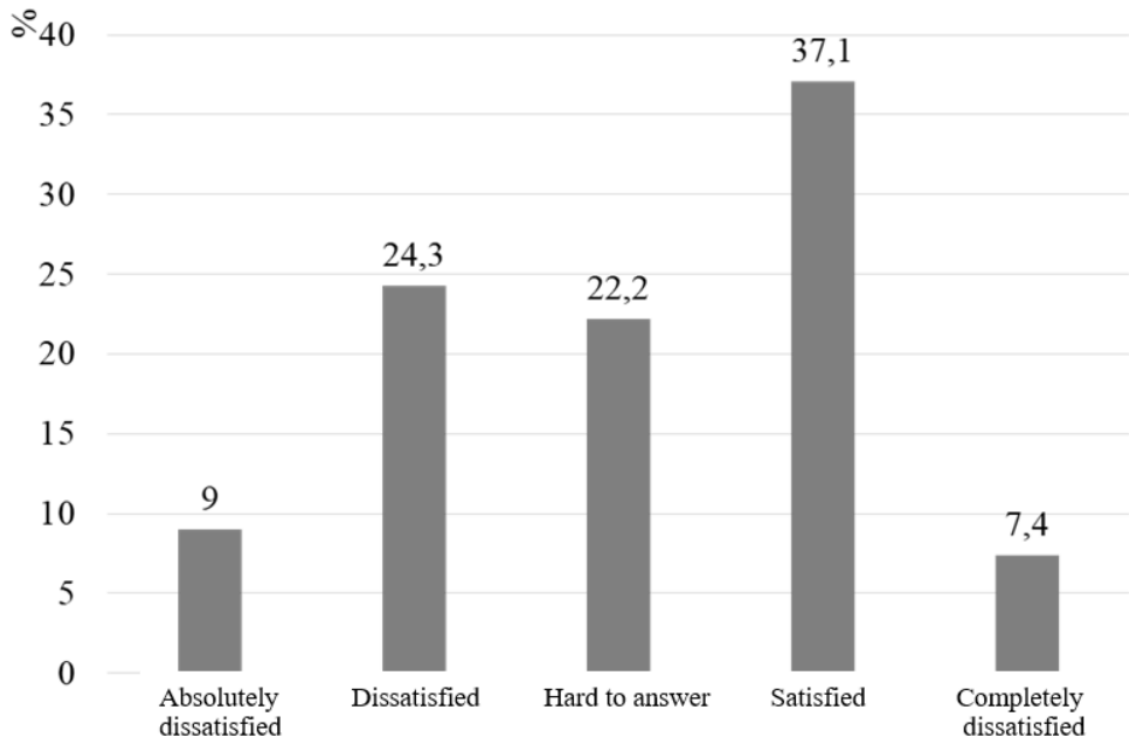


Fig. 2.2. Level of respondents' satisfaction with the range of beer in breweries

In total, less than half of the respondents (44.5%) are satisfied or absolutely satisfied with the range of beer, while 22.2% were undecided, which indicates a certain degree of dissatisfaction with the range of products available in breweries and beer restaurants. At the same time, 24.3% of respondents are dissatisfied with the assortment and 9% are completely dissatisfied.

In the survey, respondents were asked to choose the two most important factors among the following: original taste, aroma of the drink, composition of ingredients, price of the drink, alcohol content, and colour of the drink. The survey results (Fig. 2.3) show that the majority of respondents, namely 67.18%, focus on the original taste when choosing beer, 40.2% consider the composition of ingredients important, and 22% pay attention to the aroma of the drink. It is these

characteristics that shape the taste and nutritional value of the product, so in the context of finding opportunities to better meet the needs of beer consumers, it is necessary to search for and add ingredients to beer that will contribute to the formation of its original taste and aroma.

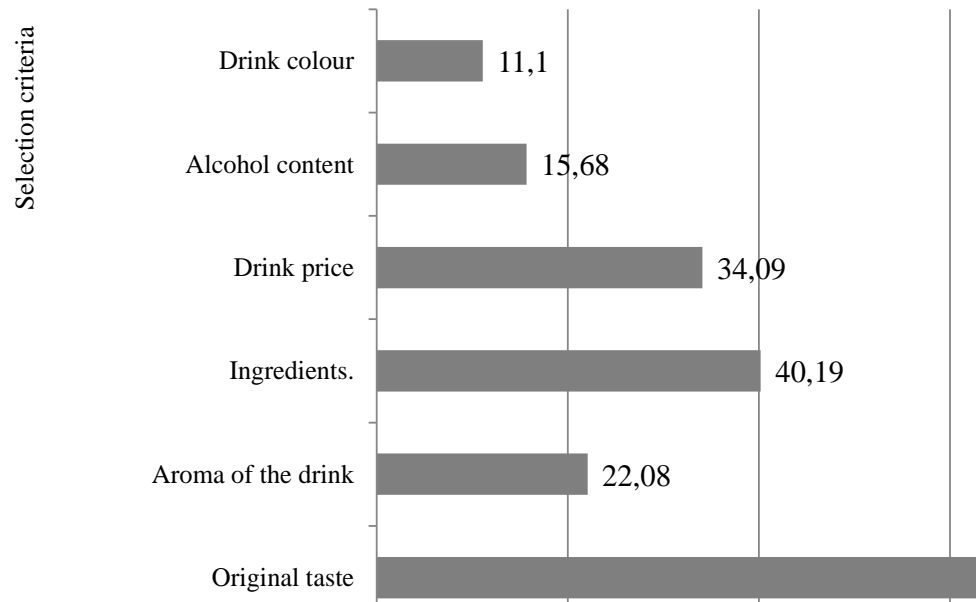


Fig. 2.3. Criteria for respondents' choice of beer consumer preferences

To ensure that a new product meets the needs and preferences of consumers, alcohol content is also an important factor, as it is preferred by more than 15% of respondents. It is also worth noting that 35% of respondents consider the price of the drink to be important when choosing a beer, so the choice of additional ingredients for a new product should be based on the cost of raw materials.

In order to determine the degree of risk of introducing a drink to the market that would not be in demand, respondents were asked to decide whether they would be willing to buy non-traditional beer. The introduction of innovative technologies and the addition of additional ingredients to beer recipes are stimulating factors for the growth of mini-breweries' profits. The survey results revealed a fairly high potential demand for beer with the addition of vegetable raw materials (Figure 2.4). About 65% of respondents expressed their willingness to buy such a drink.

It should be borne in mind that a significant proportion of respondents hesitated and could not answer convincingly to the question about their readiness to buy a new product (about 40%), while almost 23% of respondents indicated a probability of purchase (5 to 7 points). There is also a small proportion of people (2.4%) who had a negative attitude to this question, which requires the use of incentive marketing.

In order to identify the taste preferences of potential consumers regarding the type of herbal supplement, respondents were offered medicinal herbal spice and aromatic raw materials that have antioxidant properties. It should be harmoniously combined in taste and aroma with an intoxicating drink, positively affect the quality of the finished product and provide it with certain beneficial properties.

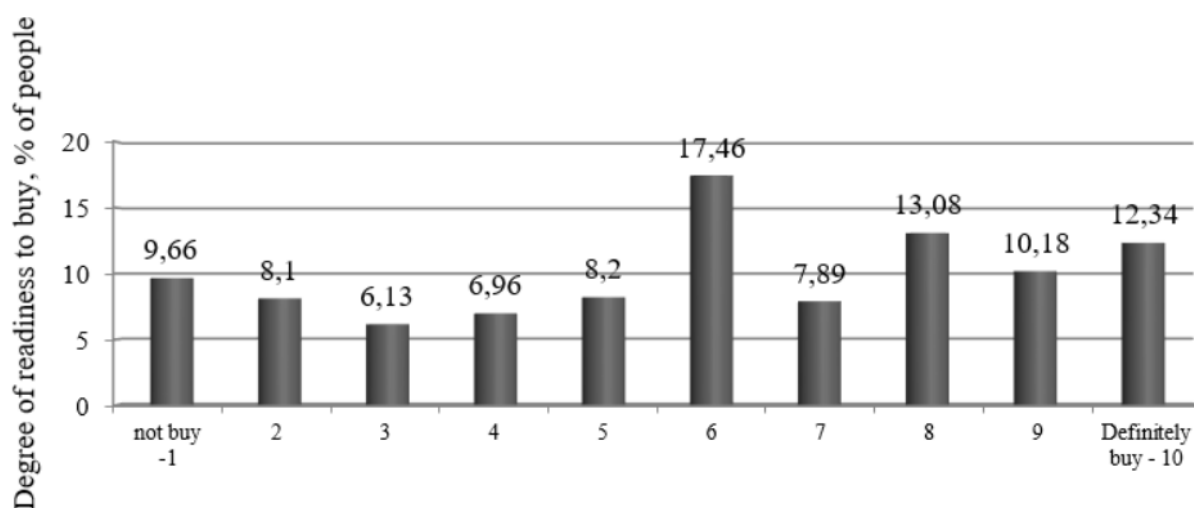


Fig. 2.4. The degree of respondents' readiness to buy beer with the addition of vegetable raw materials

Medicinal plant materials were selected for their pharmacological properties. According to the literature [5; 44–46], the presence of a complex of biologically active compounds in plants in a natural ratio helps to normalise metabolism, accelerates the excretion of toxic metabolites from the body, causes antioxidant activity, etc. The selected plant material belongs to the fifth class of toxicity – conditionally non-toxic raw materials.

Aromatic plant materials were selected for their high antioxidant properties: herbaceous plants (grasses) – lemon balm and zubrivka, which are intensely fragrant, and lemon wormwood; fruits – juniper berries, goji, blackthorn, rose hips, barberry; aromatic fragrant seeds – cardamom, coriander and pine needles [47; 88]. This will allow to achieve high organoleptic quality indicators of beer, to obtain a product with high nutritional value, in particular due to the high content of essential oils and phenolic compounds in spicy and aromatic raw materials that protect the human body from harmful environmental influences.

The respondents were asked to choose three herbal ingredients from among the proposed ones. According to the results obtained (Fig. 3.5), the majority of respondents preferred such herbal ingredients as barberry (66.1%) and pine needles (41.7%). The tastes and properties of these additives are clear to consumers, which explains their choice. Consequently, beer with the aforementioned additives will be in demand among the population. At the same time, consumers were distrustful and sceptical about such ingredients as cardamom and lemon wormwood.

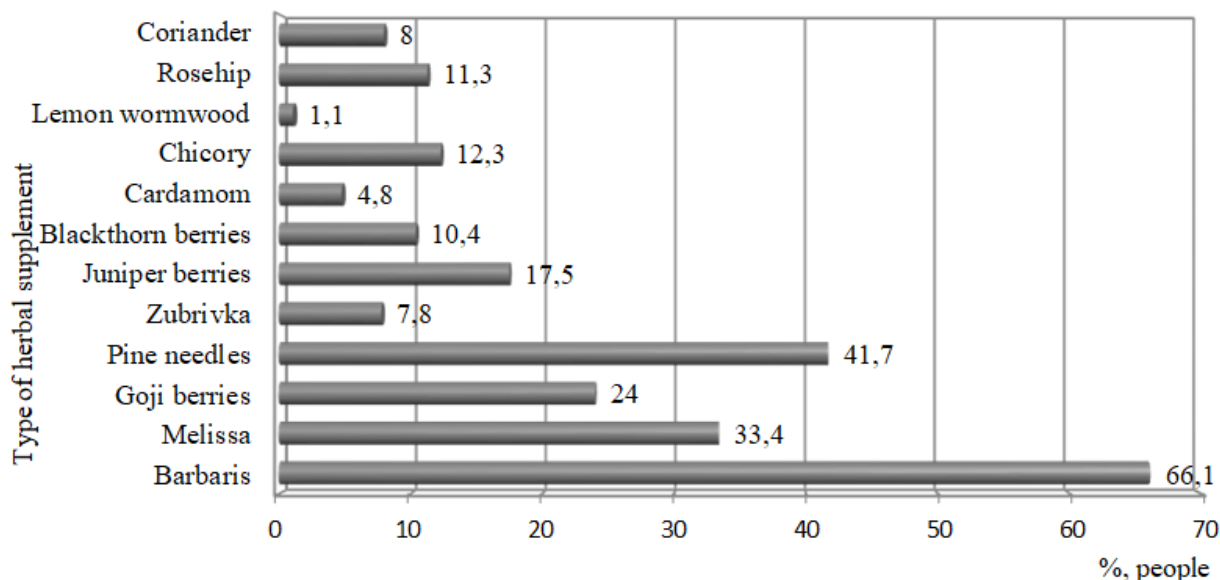


Fig. 2.5. Consumer preferences regarding the type of natural herbal supplement



Based on the results of market research, it was determined that it is advisable to search for original plant components that can improve the nutritional properties of beer.

In general, the identified potential demand is sufficient to ensure the success of the new beverage on the market, but this requires proper marketing support. The survey found that when buying beer with natural plant-based ingredients, 67% of respondents were motivated by the original taste, 40% by the composition of the ingredients, and 34% by the price of the drink.

In a highly competitive environment, only high quality products and an effective marketing strategy can ensure market success for mini-breweries. Based on the above data, when advertising a newly developed beer, consumers' attention should be drawn to the original taste and certain beneficial properties of the drink due to the addition of natural plant materials.

When choosing plant materials to create beer with original flavours and enhanced antioxidant properties, we relied on the results of a literature review and market research.

According to the research, the respondents preferred barberry when choosing raw materials for the beverage. Barberry fruit is widely used in the confectionery industry and alcoholic beverage production, and the pulp of mature berries has similar properties to lemon. But given that the main production of barberry is concentrated in the temporarily occupied territory of the Republic of Crimea and the Caucasus, adding it to beer will increase the price of the finished product.

The next ingredient preferred by more than a third of respondents was pine needles (41.7%). Adding pine needles to beer will contribute to the unique, original taste of the drink, which will provide new products with an exclusive competitive advantage.

So, based on the above, pine needles were chosen as a plant-based additive based on the results of market research. When choosing the raw material, we took into account that it should be economically feasible and occupy sufficient space for production. The most common raw material in the Eastern region is Scots pine

(*Pinus sylvestris*), whose physiological and pharmacological characteristics are described below.

Thus, the selected plant material contains a complex of dietary supplements: low molecular weight phenolic compounds, flavonol glycosides, terpenoids, which can mobilise the body's defences and have antioxidant and preservative effects. Adding pine needles as an additional ingredient to the beer recipe will have a positive impact on the formation of its original taste and aroma, while enriching the drink with dietary supplements and providing it with antioxidant properties.

### **2.3. Investigation of the content of BAS in hop cones and pine needles, determination of needle safety indicators**

In order to identify the main aspects of replacing hops, it is advisable to study the complex of biologically active phytochemicals of dried pine needles, compared to hop cones, as an unconventional spicy-aromatic plant material when used to form the quality of beer.

Pine needles are a medicinal raw material, the harvesting of which is recommended in winter [89], unlike hops, which is a seasonal product. It is known that during the harvesting of medicinal plant materials, there is a loss of bioactive substances [88]. For the study, we used hop cones crushed in accordance with State Standard of Ukraine 4098.1-2002 and medicinal plant material - dried pine needles sold in phytopharmacies in Kharkiv; hop granules (crushed hop cones pressed into granules) in accordance with State Standard of Ukraine 7028:2009. The results of the study of needle samples from different batches are shown in Table 2.8.

Based on the studies, it was found that the aroma and flavour complex of dried pine and hop needles includes unsaturated reactive substances such as essential oils, low molecular weight phenolic compounds (according to chlorogenic acid), flavonol glycosides (according to rutin), polyphenolic (tannins) substances, resins and bitter substances.

Polyphenolic (tannins) substances cause denaturation of proteins and clarification of beer [9; 90]. Their content in pine needles is 16.4%, which is

significantly higher than in hop cones and granules (5.2% and 5.8%, respectively). The content of low-molecular-weight phenolic compounds (by chlorogenic acid) in cones is 3.1%, in hop granules – 3.9%, in needles – 4.0%. The mass fraction of flavonol glycosides (by rutin) in hops is 1.3%, in hop granules – 1.6%, in needles – 2.7%. The studied medicinal plant material has a high content of phenolic compounds, which determines its antioxidant properties and will help reduce the lipid peroxidation reaction.

Table 2.8

**Content of biologically active substances of phenolic and terpenoid nature, %  
(n = 5, P ≥ 0.95)**

Biologically active substances	Spicy and aromatic medicinal plant raw materials		
	Pine needles	Hop cones	Hop pellets
Essential oil	3.8	0.55	0.62
Resin	27.3	7.0	7.3
Bitter substances	4.0	8.5	9.2
Polyphenolic (tannins) substances	16.4	5.2	5.8
Low-molecular-weight phenolic compounds (based on chlorogenic acid)	4.0	3.1	3.9
Flavonol glycosides (routine)	2.7	1.3	1.6
β-carotene, mg/100 g	15.6	4.8	5.7

Bitter substances inhibit the growth of microorganisms and ensure the stability of beer during storage [91]. This figure is 8.5% in cones, 9.2% in hop pellets, and 4.0% in needles. The content of β-carotene in pine needles is 15.6 mg/100 g, in hop cones – 4.8 mg/100 g, in hop granules 5.7 mg/100 g.

The formation of beer taste and aroma is influenced by essential oil [20]. The mass fraction of essential oil in pine needles is 3.8%, resin – 27.3%, which is higher than in cones – 0.5% and 7.0% and hop granules – 0.62% and 7.30%, respectively.

It was found that pine needles have a higher content of phenolic and terpenoidal substances (especially low- and high-molecular weight phenolic compounds and essential oils) – 1.5–5 times higher than in hop cones. The moisture content of hop cones and pine needles is 9–10%, and that of hop pellets is 7-8%, which explains the higher content of BAS in pellets than in hop cones.

Vitamins, micro- and macroelements of plant materials are very important components in beer production, as they shape its taste and enrich the finished beverage with these substances. In view of this, the main vitamin and mineral complex in hop cones and pine needles was determined (Table 2.9).

Table 2.9

**Vitamin and mineral complex, mg/100 g**  
(n = 5, P ≥ 0.95)

Nutrient	Spicy and aromatic medicinal plant raw materials		
	Pine needles	Hop cones	Hop pellets
Thiamine, B <sub>1</sub>	12.0	0.8	0.9
Riboflavin, B <sub>2</sub>	5.0	0.6	0.7
Pyridoxine, B <sub>6</sub>	1.8	0.4	0.5
Nicotinic acid, PP (B <sub>3</sub> )	26.0	7.0	8.4
Biotin, H (B <sub>7</sub> )	0.15	0.05	0.07
Ascorbic acid, vitamin C	335.5	15.7	16.4
Potassium	98.0	560.0	680.0
Phosphorus	0.92	388.0	423.3
Magnesium	10.12	303.60	383.1
Calcium	10.7	216.0	264.7
Sodium	4.5	9.5	10.1
Iron	2.2	3.2	3.5

Pine needles contain B vitamins, the amount of which is 3–10 times higher than in hops. Vitamin C in pine needles is 335.5 mg/100 mg, in cones 15.7 mg/100 g, and hop granules – 16.4 mg/100 g. Trace elements in plant materials are represented by potassium, calcium, magnesium, phosphorus, and iron. The amount of them in the granules is quite high (680 mg/100 g, 264.7 mg/100 g, 383.1 mg/100 g, 423.3 mg/100 g, 3.5 mg/100 g, respectively) compared to hop cones (560 mg/100 g, 216 mg/100 g, 303.6 mg/100 g, 388 mg/100 g, 3.2 mg/100 g,

respectively) and pine needles (98 mg/100 g, 10.7 mg/100 g, 10.12 mg/100 g, 0.92 mg/100 g, 2.2 mg/100 g, respectively).

According to the results of the research, it was determined that pine needles are close to hops in their properties and chemical composition and have advantages over cones and pellets in terms of the quantitative content of BAS: resins, essential oil, polyphenolic (tannins) and low-molecular-weight phenolic compounds that affect the formation of beer taste and aroma. Brewers prefer hop pellets because they have greater homogeneity, are easy to mix, and are more convenient to use. During hop pelleting, most of the lupulin grains are destroyed, so the bitter substances of hop pellets dissolve better during the wort hopping process compared to cone hops [81].

Based on the research conducted to identify the main aspects of replacing hops with pine needles, taking into account pharmacological data and literature, it is advisable to use pine needles (hereinafter referred to as needles) for further research, which will enrich beer with the maximum possible amount of BAS and extend the shelf life of the finished product.

The quality of raw materials determines the hygienic safety of food products. Given the unfavourable environmental situation and considering the prospect of food production in conditions of radioactive contamination, it is worth noting the urgency of the problem of healthy eating. Heavy metal compounds are the most dangerous because they accumulate in the body and cause various diseases and mutations [82]. Therefore, it is advisable to investigate the degree of accumulation of heavy metals and radionuclides in pine needles. The results of the study are presented in Table 2.10.

The analysis of the results shows that the lead content in the needles does not exceed the MPC (0.3 mg/dm<sup>3</sup>) and is 0.02 mg/dm<sup>3</sup>. The heavy metal mercury was not detected in the raw material, but there are traces of cadmium and arsenic.

**Results of studying the content of safety indicators in needles****(n = 5, P ≥ 0.95)**

Element name	Requirements Hygienic norms 6.6.1-130-2006, State Sanitary Rules and Regulations 4.2-180-2012, permissible level, not more than	Pine needles (dried)
Mercury, mg/dm <sup>3</sup>	0.01	Not detected
Lead, mg/dm <sup>3</sup>	0.3	0.02
Arsenic, mg/dm <sup>3</sup>	0.2	Traces
Cadmium, mg/dm <sup>3</sup>	0.03	Traces
<sup>137</sup> Cs, Bq/dm <sup>3</sup>	200.0	0.46
<sup>90</sup> Sr, Bq/dm <sup>3</sup>	50.0	0.81

Specific activity of radionuclides in the raw materials under study: <sup>137</sup>Cs – 0.46 Bq/dm<sup>3</sup>, <sup>90</sup>Sr – 0.81 Bq/dm<sup>3</sup>, which is significantly less than the permissible standards 200 Bq/dm<sup>3</sup> and 50 Bq/dm<sup>3</sup>, respectively.

The results of the study show that the content of toxic elements and radionuclides in pine needles is significantly lower than the Maximum Permissible Concentration.

Summing up the results of the research, it can be concluded that pine needles are a source of natural antioxidants and have a high content of biologically active substances. This makes it possible to use it as an auxiliary raw material with the simultaneous replacement of hops with pine needles in beer production, and thus contribute to the formation of the original taste and aroma of beer, enrich its composition, and increase its antioxidant properties.

**Conclusions to Section 2**

1. The quality of malt of different varieties was studied in terms of appearance, smell, taste, presence of impurities, content of floury and vitreous grains. The mass fraction of moisture (3.2–3.7%), the mass fraction of extract in the dry matter of fine malt (82.4–87.5%), the difference in the mass fractions of extracts in the dry matter of fine and coarse malt (1.0–1.2%), mass fraction of protein substances in the malt dry matter (9.1–10.1%), Kolbach number

(39.1–40.2%), mass fraction of soluble nitrogen (0.70–0.74%), saccharification time (8–10 min).

The laboratory wort of malt of individual varieties was studied in terms of transparency, colour (0.13–0.17 units), acidity (0.93–1.0 k.u.), final degree of fermentation (79.2–80.3%), viscosity (1.42–1.50 MPa·s), and the content of  $\beta$ -glucans (125–143 mg/l). The results confirm that the malt of all the experimental varieties meets the current requirements of the standard. Bohemian Pilsner Weyermann and Malteurop Pilsen were recognised as the best malt varieties.

2. It was found that malt contains salts of heavy metals and radionuclides in amounts significantly lower than the permissible concentrations. It was proved that the content of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  radionuclides in the varieties is less than the maximum permissible level. The lowest specific activity of radionuclides was found in malt of Weyermann Premium Pilsner ( $^{137}\text{Cs}$  – 1.03 Bq/kg,  $^{90}\text{Sr}$  – 4.1 Bq/kg), Weyermann Munich type II ( $^{137}\text{Cs}$  – 1.15 Bq/kg,  $^{90}\text{Sr}$  – 4.32 Bq/kg) and Bohemian Pilsner Weyermann ( $^{137}\text{Cs}$  – 1.33 Bq/kg,  $^{90}\text{Sr}$  – 4.6 Bq/kg).

3. The study of hop granules of different varieties in terms of appearance and aroma was carried out. The mass fraction of moisture (7.4–8.8%) and the mass fraction of  $\alpha$ -acids (5.2–12.7% in bitter varieties and 4.9–7.4% in aromatic varieties) were determined. The results confirm that all varieties meet the requirements of regulatory documents. The best varieties of aromatic hop granules were identified – Hallertau Hersbrucker and bitter hops – Magnym.

4. The prepared water was tested for turbidity, smell, taste, colour (2 degrees), hydrogen index (6.05 pH units), hardness (2.1 mmol/dm<sup>3</sup>), amount of dry residue (174 mg/dm<sup>3</sup>), total alkalinity (1.3 mmol/dm<sup>3</sup>). The content of mineral impurities was determined: Calcium (3.8 mg/dm<sup>3</sup>), sodium (36.0 mg/dm<sup>3</sup>), potassium (50.0 mg/dm<sup>3</sup>), chlorides (33.0 mg/dm<sup>3</sup>), sulphates (25.0 mg/dm<sup>3</sup>), total iron (0.07 mg/dm<sup>3</sup>), manganese (0.05 mg/dm<sup>3</sup>), copper (0.005 mg/dm<sup>3</sup>), zinc (0.002 mg/dm<sup>3</sup>). Toxic elements of aluminium and arsenic were not detected in the test sample. The results confirm that the treated water meets the current requirements.

5. Based on the literature review and market research, it has been proved that it is advisable to expand the range of beer by adding natural plant materials. The main criteria for choosing raw materials are their influence on the formation of consumer properties of the finished beverage, pharmacological properties, economic feasibility, content of bioactive substances, and the possibility of using them in production. As an additional raw material for beer production, pine needles were selected, which are similar in chemical composition and properties to hop cones.

6. The complex of biologically active phytocomponents of dried needles, in comparison with hops, as an unconventional spicy-aromatic plant material, was studied when used to form the quality of beer. It was found that the aromatic and flavour complex of biologically active phytocomponents includes unsaturated reactive substances such as essential oil (from 0.5% to 3.8%), low-molecular weight phenolic compounds (by chlorogenic acid) (from 3.1% to 4%), polyphenolic (tannins) substances (from 5% to 16%), resins (from 7% to 27%) and bitter substances (from 4% to 9.2%). At the same time, pine needles have a higher content of these BAS (especially low- and high-molecular weight phenolic compounds and essential oils) – by 1.5–5 times more than in hop cones and pellets.

7. It has been proved that the amount of toxic elements and radionuclides in the needles is much lower than the Maximum Permissible Concentration. The lead content is  $0.02 \text{ mg/dm}^3$ , and traces of arsenic and cadmium are present. The heavy metal mercury was not detected in the raw materials. Specific activity of radionuclides in raw materials:  $^{137}\text{Cs} - 0.46 \text{ Bq/dm}^3$ ,  $^{90}\text{Sr} - 0.81 \text{ Bq/dm}^3$ .

8. The prospects of forming consumer properties and expanding the range of beer through the use of pine needles in the production of beer have been substantiated, which will allow to obtain a drink with original taste and high antioxidant properties, enrich it with dietary supplements and extend the shelf life of the finished product.



### SECTION 3

## SUBSTANTIATION OF THE BEER PRODUCTION PROCESS WITH ADDITION OF AQUEOUS CONIFEROUS EXTRACT

### **3.1. Determination of optimal parameters of pine needle extraction and evaluation of pine needle extract quality**

At the present stage of development of the brewing industry, plant raw materials are used in the form of extracts. The study of the extraction process of bioactive substances from pine needles is the first stage in the development of the formulation composition of beer with the addition of pine needle extract.

For the preparation of pine needle extract, it is advisable to choose water as an extractant. The use of water-alcohol extraction of raw materials is impractical for beer production from an economic point of view, since alcohol infusions are material-intensive. It is known that extraction without grinding the needles is not possible due to their dense waxy shell. The needles were ground in a fine grinding mill to a particle size of 50–250  $\mu\text{m}$ , which allowed for deep processing of the raw material, accelerated the extraction process, and increased the yield of extractive substances [46–49].

The value of the antioxidant activity of the aqueous extract was taken as an indicator of the technological process. To conduct further research, taking into account the use of the MathCAD package, we introduce the following variables:  $x$  – extract temperature;  $y$  – hydromodule value;  $z$  – processing time.

According to the results of literature data and previous studies [93] of the technological process, it was considered expedient to set the following limits for changing input values:  $30\text{ }^{\circ}\text{C} \leq x \leq 80\text{ }^{\circ}\text{C}$ ;  $1:5 \leq y \leq 1:10$ ;  $10 \leq z \leq 60$ .

The purpose of further analysis and modelling is to determine such a set of input process parameters at which the maximum value of the antioxidant activity of coniferous extract can be achieved.

The determination of the sets of input variables that provide the maximum value of the output indicators of the extract was carried out by the Maximize

optimisation programme of the MathCAD package [80]. According to the results of the calculations, the optimal combinations of the input variables of the extract were found:  $x = 0.298$ ;  $y = - 0.012$ ;  $z = - 0.098$ . The found values are given in code form. The formula was used to convert to natural values:

$$X = \frac{x - \frac{x_{\max} + x_{\min}}{2}}{\frac{x_{\max} - x_{\min}}{2}}. \quad (3.1)$$

The model was used to find the theoretical value of the antioxidant activity of coniferous extract:  $Y = 201.3$  Cl/100 ml.

At the last stage, the input values were adjusted to take into account the further use of the obtained values in production. The extraction was carried out according to the following optimal conditions: the value of the hydromodulus of plant material and water in a ratio of 1:8; holding at a temperature of 60 °C for 30 min. The finished extract was infused for 8 h in an automatically controlled thermocontainer, cooled to a temperature of 8...10 °C, and filtered to remove impurities and suspended particles using diatomaceous earth.

The next stage of the work was to determine the organoleptic quality indicators of the aqueous pine needle extract: appearance, taste and aroma. The research results showed that the obtained extract has a refreshing rich taste with a pronounced aroma of pine needles, and its appearance is a transparent golden liquid (Table 3.1).

Table 3.1

**Organoleptic quality parameters of aqueous pine needle extract**

**(n = 5, P ≥ 0.95)**

Name of the indicator	Water extract of pine needles
Appearance	Transparent golden liquid
Flavour.	Clean, with a pronounced pine needle aroma
Taste	Full-bodied, refreshing, with a piney tone

The next stage of the work was to determine the content of bioactive substances and quality indicators of the aqueous pine needle extract. Since the

antioxidant activity of aqueous extracts of plant material is a quality indicator that characterises its reducing properties, i.e. the ability to react with free radicals - reactive oxygen species, it was advisable to determine this indicator in the developed extract. The value of bromine antioxidant activity reflects the total content of antioxidants in the extract [94]. The study was carried out by the coulometric method. The results are presented in Table 3.2.

Table 3.2

**Content of biologically active substances and quality indicators  
of aqueous pine needles extract (n = 5, P ≥ 0.95)**

Name of the indicator	Aqueous pine needle extract
Mass fraction of dry matter, %	3.8
Content of bitter substances, mg/100 ml	12.1
Polyphenolic (tannin) content, mg/100 ml	237.8
Content of low molecular weight phenolic compounds (by chlorogenic acid), mg/100 ml	289.4
Content of flavonol glycosides (by routine), mg/100 ml	194.2
Vitamin C content, mg/100 ml	27.8
Fragrance content (by number of fragrances), ml of sodium thiosulfate /100 ml	8000
Antioxidant activity, Cl/100 ml	202.3

Studies have shown that the mass fraction of dry matter in the extract is 3.8%. The content of polyphenolic (tannins) is 237.8 mg/100 ml, they give the extract a characteristic astringent taste. Due to the presence of a large number of alcohol groups, polyphenols form insoluble complexes with proteins, which is important for brewing during clarification of the finished beverage. In addition, tannins form insoluble complexes with heavy metal ions and contribute to their excretion from the body.

The mass fraction of bitter substances is 12.1 mg/100 ml. They protect the product from oxidation, which is likely to extend its shelf life. The amount of ascorbic acid is 27.8 mg/100 ml, the main property of which is antioxidant and antiradical action, which causes inhibition of the processes of lipid peroxidation and protection of body cells from damage.

The content of low molecular weight phenolic compounds in the coniferous extract (by chlorogenic acid) is 289.4 mg/100 ml, flavonol glycosides (by rutin) - 194.2 mg/100 ml. These compounds have an antioxidant and antimicrobial effect, which will positively affect the quality of the finished beverage and increase its shelf life. Since it is impossible to prevent the penetration of foreign microorganisms [130] into the aqueous extract during extraction, it is advisable to study its microbiological parameters (Table 3.3).

Table 3.3

**Microbiological parameters of aqueous pine needles extract (n = 5, P ≥ 0.95)**

Name of the indicator	Permissible level Sanitary Rules and Regulations 2.3.2 1078-01	Actual content in aqueous pine needle extract
Mesophilic Aerobic and facultatively Anaerobic Micro- organisms CFU/g	1,0x10 <sup>4</sup>	40
Bacterial coliforms (coliforms), CFU/cm <sup>3</sup>	not allowed	not detected
Pathogenic microorganisms, including Salmonella, in 25 cm <sup>3</sup>	not allowed	not detected

It was determined that the total number of mesophilic anaerobic and facultative anaerobic microorganisms in the aqueous extract was 40 CFU/g, which is within the permissible limits. Pathogenic microorganisms and bacteria of the *Escherichia coli* group were not detected.

To determine the total content of substances that determine the aroma of the extract, the aroma number was determined. The amount of aroma substances in the studied extract is quite significant – 8000 ml Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/100 ml. The flavour-forming substances of the coniferous extract have a positive effect on the overall taste and aroma of the beverage, since the organoleptic characteristics of beer are the main ones in the tasting assessment of its quality.

The antioxidant activity of the coniferous extract is 202.3 Cl/100 ml due to the content of polyphenolic (tannins) and bitter substances, low molecular weight phenolic compounds, flavonol glycosides, ascorbic acid.

It is known that pine needles are characterised by a high content of essential oils and resins, which are involved in the formation of aroma and can affect the consumer properties of the extract and beverage containing it. The study of these flavour-forming compounds is of great practical importance for the formation of the consumer characteristics of the finished product, as they may contain many substances, the minimum mass fraction of which can cause both qualitative and quantitative changes in flavour.

Taking into account the specific organoleptic properties of pine needles, the next stage of the work was to determine the component composition of aromatic substances of the obtained extract. The qualitative and quantitative composition of pine needles volatiles was studied on a gas chromatograph in the laboratory of the Department of Organic Materials Technology of the Scientific and Technical Complex "Monocrystals" of the National Academy of Sciences of Ukraine. The component composition of volatile substances is given in Table 3.4.

Table 3.4

**Component composition of volatile substances of aqueous coniferous extract  
( $n=5$ ,  $P \geq 0.95$ )**

№ 3/II	Retention time, min	Component name	Total number of %	No. s/n	Retention time, min	Component name	Total number, %.
1	3.934	Methanoic (formic) acid	0.046	11	12.380	2,2-Hydroxyethyl- 0,2-glycolamide	1.136
2	4.585	1-Isopropyl-4- methylene bicyclohexane (sabinene)	3.411	12	12.972	4-Heptanal	0.863
3	5.173	Pentadecanal	1.999	13	13.193	$\alpha$ -Terpinen	0.496
4	5.875	1,5- Cyclodextrin	7.358	14	14.439	$\alpha$ -Pinene	17.919
5	7.559	$\alpha$ -Cadinol t-Muuralol	0.906	15	14.866	1-Methyl-4-isopropenyl- cyclohexene-1 (limonene)	40.005
6	7.749	Tetracosan	0.719	16	16.411	2-Methyl-6-methyl- enooctadione-2,7 (myrcene)	0.578
7	8,477	Picein	1.767	17	17.386	2,8(9)-n-mentadiene (isolimonene)	0.983
8	9.657	Acetamide	11.180	18	18.223	1,4,7,10,13,16- Hexaoxacyclooctadecane	1.341
9	10.882	Conifer	4.193	19	18.539	1- $\alpha$ -Felandren	1.297
10	11.444	Borneol	3.617	20	19.226	2-Ethylidicyclopentane	0.185

The study revealed that the volatile substances of aqueous coniferous extract include 20 components. The complex volatile compounds isolated from the needles are represented by the following groups: esters, phenolic compounds (terpenes, monoterpenes, glucosides), aldehydes, organic acids, hydrocarbons and aromatic compounds.

It was found that the flavouring substances of the extract are components that form a specific tone of taste and aroma of pine needles. Among them, the glucoside picein (retention time 8.477 min; content 1.767%), which is a phenolic compound and forms a bitter taste, and the terpene pinene (retention time 14.439 min; content 17.919%), borneol (retention time 11.444 min; content 3.617%) and coniferol (retention time 10.882 min; content 4.193%), which have a pine and woody aroma, limonene ether (retention time 14.866 min; content 40.005%). In addition, there are myrcene (retention time 16.411 min; content 0.578%) and pinene, which are monoterpenoids with antiseptic properties.

The research suggests that the uniqueness of the composition of the coniferous extract lies in the harmonious combination of flavour and aroma components and functional ingredients, which, when added, will allow to produce beer with a high content of BAS and original organoleptic properties. In addition, the coniferous extract contains plant antioxidants, which will help to increase the antioxidant properties of the finished beverage and reduce the negative impact of beer on the human body.

In view of the above, it can be argued that it is expedient to introduce a natural plant additive from pine needles in the form of an extract to enrich craft beer with BAS and increase its shelf life.

### **3.2. Recipe for Emerald beer using aqueous coniferous extract**

In order to formulate the quality indicators of craft beer, it is necessary to develop and scientifically substantiate its recipe, to form the consumer properties of the finished product using a complex of dietary supplements from pine needles and hop cones extract and to determine their rational doses in the beverage recipe

using mathematical modelling and expert evaluation. At this stage of the study, the formulation composition of light beer using aqueous pine needle extract was developed.

Taking into account the experience of OLNA LLC, the most popular, especially among young people, is light beer with a mass fraction of alcohol of 3.0 vol%. Therefore, for further research, it was decided to brew light beer using the classical technology with a mass fraction of solids in the initial wort of 10%.

Hops are an indispensable raw material in brewing, as they give beer a specific aroma, promote foaming and stability during storage [96]. But this component is also the most expensive in beer production. Scientists are considering replacing hops with vegetable raw materials that have a high content of bioactive substances and will give the finished drink certain functional properties [97]. In addition, studies by scientists [11–14] show that in case of its excessive consumption, there are manifestations of the negative effects of hops on the human body.

The chemical composition of pine needles is closest to that of hop cones (polyphenolic (tannins), low molecular weight phenolic compounds, bitter substances, resins, essential oil, etc.) [98]. It is a source of natural antioxidants and has high nutritional and biological value [71; 74], so it can be used as an alternative to hops [16].

When developing the formulation composition of alcoholic beverages, the Maximum Allowable Concentration of medicinal plant material is of particular importance [44; 46; 49; 52]. The amount of beer consumed by a person at one time varies depending on individual sensitivity, physical activity, diet, gender, etc. Employees of the National University of Pharmacy (Kharkiv), taking into account the experience of studying phytoextracts [44–46], calculated the rational range of the amount of pine needles in the beer recipe, provided that its preventive effect is maintained and taking into account the maximum possible single consumption of the drink. The rational interval of the amount of needles was determined to be in the range of 0.02–0.05 kg per 1 dal of beer.

Classical light beer brewing technology was used as the basis for the production of a new beverage with the addition of pine needle extract. Aromatic hop granules (Hallertau Hersbrucker) were used, which give the beer a rich aroma, with a bittering rate of 0.4–0.7 g/dL of wort. At the same time, the beer recipe provided for the saving of hop granules and their partial replacement with coniferous extract in the range of 10–25% by weight in terms of freeze-dried raw materials (Table 3.5).

Table 3.5

**Raw material consumption per 1 litre of beer**

Name of raw material	Beer made according to using classical technology	Beer with replacement of hop granules for pine needles, %			
		10	15	20	25
Light brewing barley malt, kg	2.5	2.5			
Bottom fermentation yeast, kg	0.075	0.075			
Hop pellets, kg	0.180	0.160	0.150	0.140	0.130
Water extract of pine needles (with the corresponding amount of dry raw materials), l	-	0.190	0.270	0.350	0.430
Process water	Others				

The upper limit is determined by a certain rational amount of pine needles that can be used in the recipe to the maximum extent possible, and the lower limit is determined by its organoleptic characteristics. Beer with less than 10% of additional raw materials has no signs of pine flavour and aroma. The extract was added to the beer wort at the stage of main fermentation (at the end of fermentation), as this results in minimal loss of aromatic and tannins and does not affect the activity and reproduction of brewer's yeast. Malteurop Pilsen light barley malt was used, as well as Safbrew T-58 bottom fermentation brewer's yeast, 2nd and 3rd generation, produced by the OLNA brewery [99].

At the next stage, the beer was evaluated by tasting analysis on a 25-point scale, using the improved methodology for assessing the quality of beer "Emerald" [100]. The tasting was carried out by a group of experts, employees of the brewery of OLNA LLC. The summarised and processed results of the beer analysis are presented in Table 3.6.



Table 3.6

### Results of the tasting evaluation of beer samples

Sample name	Name of the indicator						
	Colour	Taste	Hop bitterness	Flavour	Foam formation	After taste	Total amount of points
Sample 1 according to the classical technology	3.0	4.6	4.9	3.5	5.0	3.0	24.0
Replacing hop pellets with pine needles							
Sample 2 – 10%	3.0	4.5	4.9	3.5	5.0	2.0	23.0
Sample 3 – 15%	3.0	4.8	4.9	4.0	5.0	3.0	24.7
Sample 4 – 20%	3.0	4.7	4.8	4.0	5.0	3.0	24.5
Sample 5 – 25%	3.0	3.0	4.2	3.0	5.0	1.7	19.9
Total score according to the improved methodology, not less than	3.0	5.0	5.0	4.0	5.0	3.0	25.0

It was found that all samples received the same scores for colour and foaming. This is because the addition of pine needle extract at the main fermentation stage (at the end of fermentation) did not reduce the activity of brewer's yeast and did not slow down the fermentation.

Beer with 15% and 20% hop substitution has a subtle, pleasant pine needle aftertaste that disappears quickly, and it received 3 points for aftertaste. For the sample brewed using the classic technology, the aftertaste was rated with the maximum number of points. This beer has a clean, fermented, malty taste with hop bitterness (24 points). The developed beverage with 10% hop substitution has an additional, subtle taste and aroma of pine needles and received 23 points. Samples with 15% and 20% hop substitution have a moderately refreshing pine needle aroma against a background of pronounced hop bitterness and malt flavour. None of the ingredients in these beverages stand out, they are balanced, harmonious and received a high overall score of 24.5 (sample 4) and 24.7 points (sample 3). The addition of coniferous extract to replace hops in the amount of 25% has a much

stronger effect on the taste and aroma of the beer. In the finished product, a distinct pine flavour prevailing over hop bitterness leaves a long aftertaste on the taste buds and is not harmoniously balanced (19.9 points).

According to the results of the tasting analysis, the samples received a total score in the range of 19.9–24.7 points, which corresponds to excellent and good quality. The difference in beer taste and aroma is explained by the inclusion of hops and pine needles in the recipe in different proportions. The main way to determine the quality of beer is through tasting analysis, which has shown that samples with 15% and 20% replacement of hop pellets with pine needles have the most complete and harmonious taste.

As the original taste is the top priority when choosing a beer according to the results of market research, its compliance with consumer requirements is a determining factor in the competitiveness of products. An objective assessment of the sensory characteristics of the developed beer was carried out using the descriptive expert method of profile analysis in accordance with the State Standard of Ukraine ISO 6564:2005 "Sensory testing. Methodology. Methods for creating flavour" [101].

The Flavor Profil method is a combined effect of taste, aroma perception and touch sensations in the mouth. It is based on two stages: building an organoleptic profile of a flavoured beverage and evaluating each ingredient in the recipe on a scale of desirability and intensity of sensation to describe the flavour of the product [102; 103].

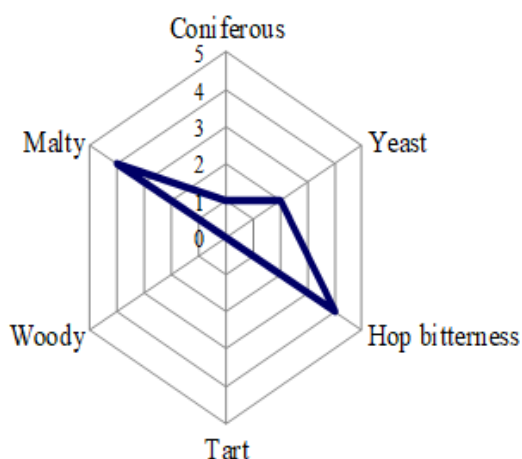
The tasting was carried out by a panel of six experts from OLNA's beer production facilities. The testers had previously compiled a list of terms and created a dictionary to describe the characteristics of the sample to be evaluated. The experts worked as a group using the consensus method, which aims to obtain a unanimous description of the product's flavour. The chairman of the committee was also one of the testers, leading the discussions until agreement was reached on each indicator, thus ensuring a complete description of the product's characteristics.

The following scale of desirability and intensity of feeling was used to assess the degree of intensity of the characteristic: 0 = absence; 1 = only recognition or threshold; 2 = weak intensity; 3 = moderate; 4 = strong; 5 = very strong. The taste descriptors that are important to consumers and are included in the comprehensive flavour profile of the hypothetical reference were evaluated: malt, pine, yeast, hop bitterness, astringent, woody.

The overall impression is an overall assessment of the product that takes into account the adequacy of the perceived characteristics, their intensity, identifiable background flavour and flavour mixing. The overall score was given on a three-point scale: 3 – good impression; 2 – average impression; 1 – poor impression.

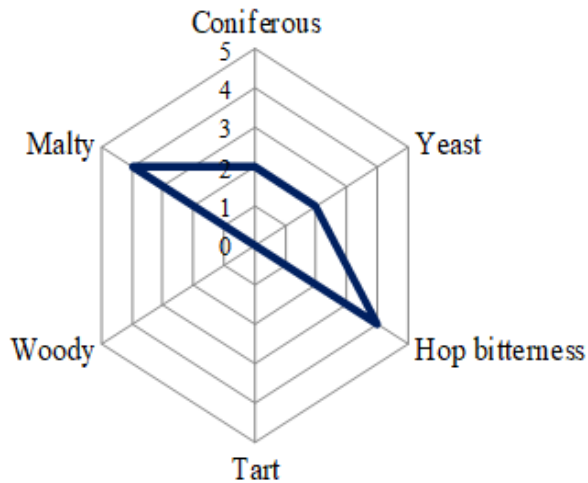
Based on the results of the tasting, we constructed circular flavour spectra of the developed beer samples with the addition of coniferous extract (Figs. 3.1–3.4).

All samples received the same score for malt flavour, which is explained by the use of the same amount of barley. It was found that the beverages have the same hop bitterness indicators, which confirms the feasibility and correct choice of raw materials to replace hops and preserve the taste characteristics of the beverage.



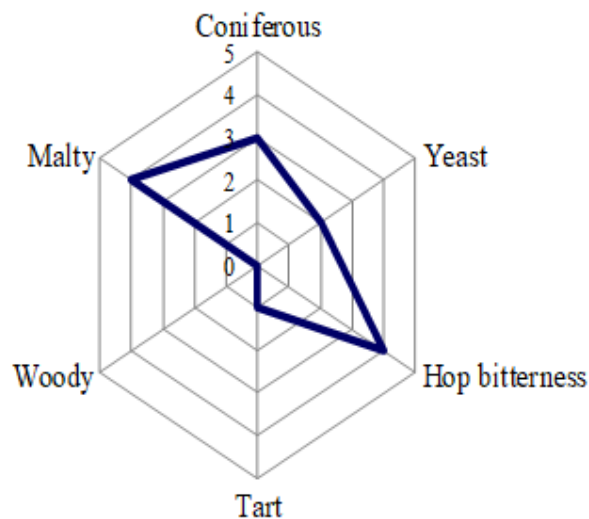
Residual aftertaste: barely perceptible coniferous  
 Persistence: disappears quickly  
 Overall impression: 2

Fig. 3.1. Pie chart of the flavour spectrum of the experimental beer sample with the addition of coniferous extract with 10% replacement of hops



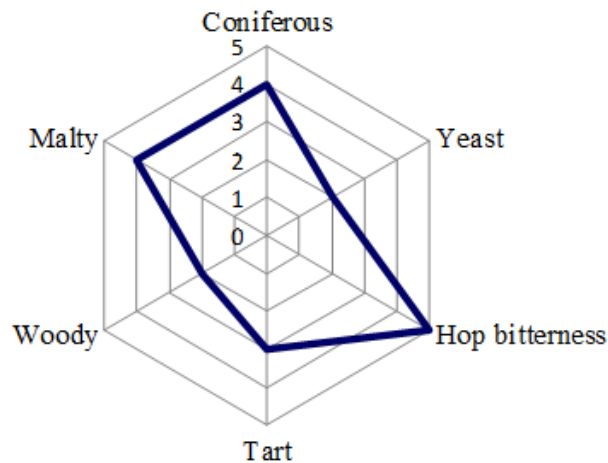
Residual aftertaste: noticeable coniferous  
 Persistence: disappears quickly  
 Overall impression: 3

Fig. 3.2. Pie chart of the flavour spectrum of the experimental beer sample with the addition of coniferous extract with 15% replacement of hops



Residual aftertaste: delicate coniferous  
 Persistence: disappears quickly  
 Overall impression: 3

Fig. 3.3. Pie chart of the flavour spectrum of the experimental beer sample with the addition of coniferous extract with 20% replacement of hops



Residual aftertaste: pronounced coniferous  
 Persistence: quite long lasting  
 Overall impression: 2

Fig. 3.4. Pie chart of the flavour spectrum of the experimental beer sample with the addition of coniferous extract with 25% replacement of hops

On the flavour spectrum profiles, the yeast flavour has a moderate intensity in all beer samples, indicating that the addition of coniferous extract does not change the fullness of the taste of the beverages.

On the contrary, the coniferous tone had some differences during the evaluation of the test samples. It was the last to be felt and only barely recognisable in the drink with 10% hop replacement, was slightly intense with 15% hop replacement, moderately intense with 20% hop replacement, and strongly intense with 25% hop replacement.

The sample with 20% hop substitution had an astringent taste with only recognition intensity and the sample with 25% substitution had moderate intensity. The taste of astringency is increased due to the high content of polyphenolic (tannins) in the coniferous extract. With an increase in the amount of additional

plant material to 25%, a faint woody flavour appears due to the content of terpenes in the coniferous extract, which form a specific taste.

The study found that the samples with 15% and 20% hop replacement were harmonious and balanced. The overall impression of these beverages was rated as three, with a subtle or perceptible piney aftertaste that quickly disappears.

The development and improvement of new beverage formulations is associated with the study of the ratio of the content of individual components in the finished product [100]. For further research and selection of the correct ratio of ingredients, a mathematical model was built that reproduces the dependencies between the variables under study and the quality indicators of the finished product. The quantitative ratio of hops and pine needles in the beer recipe was calculated by mathematical modelling, which allows to get as close as possible to the quality indicators of the finished product [104]. Malt and hop polyphenols largely determine the taste, colour, foaming properties and tendency to colloidal turbidity of the beverage. They are formed mainly by anthocyanogens contained in hop and malt tannins [9]. Thus, the risk of cloudiness in beer increases with the increase in the dose of hops. Since the coniferous extract also contains components that can affect the colloidal system of beer, the content of polyphenolic substances was chosen as an indicator of the technological process.

Taking into account the results of previous studies of the formulation composition [105], it can be concluded that the relative amount of pine needles cannot exceed 30% of the share of hops. This limitation is due to the results of previous studies of the finished product in terms of organolepticity. An excess of more than 30% of pine needles leads to a significant deterioration in beer quality.

Based on the results of the experiments, two vectors were created to assess the quality of the finished product (Table 3.7):  $Y_1$  – assessment by organoleptic indicators (on a 25-point scale),  $Y_2$  – assessment by the content of polyphenolic (tannins) substances (mg/100 g) – and a matrix of a two-factor experiment was constructed.

**Vectors for assessing beer quality by organoleptic indicators  
and polyphenolic (tannin) content**

Product quality assessment vector	Experiment number				
	1	2	3	4	5
$Y_1$	24.0	23.0	24.7	24.5	19.9
$Y_2$	114.3	125.4	139.7	153.9	164.5

In order to determine the ratio of pine needles and hops, the corresponding programs of the MathCAD package were used to find the maximum value of the organoleptic index and the minimum value of the polyphenolic content.

The calculations revealed that the optimal values in accordance with the quality criteria do not correspond to a single combination of the input parameters of the technological process. Thus, the maximum value for organoleptic evaluation was achieved at  $x_1=0.15$ ,  $x_2=0.5$ , and the minimum value for the content of polyphenolic substances – at  $x_1=0.15$ ,  $x_2=0.5$ . To calculate the final values of the content of pine needles and hops, it is necessary to combine these criteria into one comprehensive criterion. Thus, it is necessary to minimise two criteria for the quality of finished products at the same time.

The numerical values are the desired values of the quality indicators of the finished product. Finally, the formula used to determine the product parameters and the calculation results are presented in the form of a fragment of the MathCAD program:

$$R2 := \text{Minimize}(Q, x_1, x_2) \quad R2 = \begin{pmatrix} 0,2 \\ 0,8 \end{pmatrix}$$

$$M1(R2_1, R2_2) = 21,5 \quad M2(R2_1, R2_2) = 153,9.$$

To find the numerical values of the ratio of the amount of needles and hops, the standard Minimize programme of the MathCAD package was used.

The final values of the quantitative ratio of the beer recipe with the addition of pine extract were obtained:  $x_1=0.2$ ;  $x_2=0.8$ , which means that the pine needles content should be 20% by weight of the estimated hop rate.

The new products were brewed in accordance with the current "Technological Instructions for the Production of Malt and Beer" [106], which is based on the classic technological scheme of beer production. The principal technological scheme for the production of "Smaragd" beer is given in Appendix A. The implementation of this technology does not require additional hardware design of the process. Only the grinding operation and the pine needles extraction process need to be provided.

The production process consists of the following operations: preparation of mash, its saccharification, filtering, boiling of beer wort, cooling and introduction of yeast, fermentation of beer wort, fermentation of young beer and bottling of beer.

The wort was fermented with bottom fermentation brewer's yeast at a temperature of 8...10 °C. Aqueous coniferous extract in the amount of 300–350 ml per decalitre (dal) of wort was added at the stage of main fermentation (at the end of fermentation), which was carried out until the content of visible extract was 2.5–2.8%. Fermentation was carried out at a temperature of 0...2 °C in metal cylindrical tanks for 18 days without contact with air, under a CO<sub>2</sub> pressure of 0.04 MPa to 0.06 MPa. Fermentation and fermentation of beer lasted at least 25 days. Subsequently, the beer was pumped into the forfas for further storage and bottling.

The optimal ratio of ingredients was determined based on the results of mathematical modelling and organoleptic evaluation. The content of pine needles in terms of freeze-dried substance is 20% (35–40 g per dal of wort) by weight of the estimated hop rate. This amount is sufficient to preserve the bitterness and aroma of hops, while the beer has original organoleptic characteristics. The "Technological instruction for the production of 10% light beer "Emerald" TI 14297558-340:2016" was developed.



According to the developed recipe [107], a trial batch of "Emerald" beer was produced in the production conditions of the brewery of OLNА LLC, m. Kharkiv. A quality certificate was obtained. The developed beverage received a patent of Ukraine for utility model No. 109200 "Method of production of beer "Emerald"" [108].

Thus, the creation of a new beer variety enriched with BAS due to the introduction of aqueous coniferous extract will expand the range of high quality beverages with original organoleptic characteristics and enhanced antioxidant properties. In addition, the addition of an aqueous extract containing plant antioxidants to the beverage is one of the ways to increase the antioxidant capacity of the finished product.

### **Conclusions to Section 3**

1. The complex of bioactive substances in aqueous extracts from pine needles was determined, prepared using water as an extractant (with a 1:8 ratio of plant material and water and grinding to a particle size of 50–250  $\mu\text{m}$ ) and kept at 60 °C for 30 min, followed by infusion of the extract (8 h). The dry matter content of the extract is 3.8–3.9%.

2. It was found that the needles extract contains a biological complex consisting of aromatic substances (by the number of aroma) in the amount of 8000 ml of sodium thiosulfate in 100 ml, low molecular weight phenolic compounds (by chlorogenic acid) 289.4 mg per 100 ml, flavonol glycosides (by rutin) 194.2 mg per 100 ml, polyphenolic (tannins) substances 237.8 mg per 100 g and bitter substances 12.1 mg per 100 ml. The antioxidant activity of the coniferous extract was determined to be 202.3 Cl/100 ml and the content of ascorbic acid was 27.8 mg/100 ml.

3. The component composition of flavouring substances of the plant extract from pine needles, which forms the specific taste and aroma of pine needles and beer made with its use, was investigated by gas chromatography. It was found that the extract contains the highest amount of limonene ester (retention time – 14.866 min), which forms a lemon aroma, and also contains significant amounts of terpenes such as pinene (retention time – 16.411 min), borneol (retention time – 11.444 min) and coniferin (retention time – 10.882 min). Borneol and coniferin have a pine and woody aroma. In addition, the glycoside picein (retention time – 8.477 min) was detected, which forms a bitter taste.

4. A recipe was developed and the taste, aroma and quality of a new craft beer "Emerald" with a high content of BAS, which has antioxidant properties, were formed using a natural plant additive from pine needles. The dose and rational ratio of hops and dried pine needle extract were determined using mathematical modelling and expert opinion. The dose of hop pellets is 135–140 g per dal and is introduced during the boiling and wort production. Pine needle extract with a mass fraction of solids of 3.8–3.9% in the amount of 300–350 ml per dal of beer (or 35–40 g in solids) is added at the stage of main fermentation of beer (at the end of its fermentation).

## **SECTION 4**

### **CHANGES IN THE QUALITY OF "EMERALD" BEER IN THE PROCESS OF PRODUCTION AND STORAGE**

One of the factors that shapes the competitiveness of beer is the shelf life, during which the drink retains its transparency. Although the quality of beer depends on its quantitative composition, during storage, the consumer properties of beer change due to disruptions in the colloidal system, oxidative processes, protein denaturation, and microbial growth. The intensity of these processes depends on storage conditions and the brewing process. However, the chemical composition and functionality of the recipe components play an important role in increasing shelf life. It is known that the addition of herbal additives to beer increases its nutritional value and increases its shelf life [55; 97]. Since the developed aqueous pine needle extract has antioxidant properties, its introduction into the beer recipe can extend the shelf life of the finished product, so it is advisable to study its quality indicators during production and storage.

The commodity characteristics of unfiltered light beer with the addition of pine needle extract (hereinafter referred to as "Emerald" beer) and unfiltered light beer produced according to the classical technology (hereinafter referred to as "control" beer) were carried out after its fermentation and fermentation.

#### **4.1. Organoleptic quality indicators of "Emerald" beer**

At the next stage of the study, the organoleptic quality indicators of Emerald beer were evaluated and controlled for compliance with the requirements of State Standard of Ukraine 3888 [148]. In accordance with the regulatory document, the appearance, aroma, taste and foaming of the beverages were determined. Table 4.1 shows the characteristics of the organoleptic characteristics of the experimental samples.

Table 4.1

### Результати органолептичної оцінки якості пива

Name of the indicator	According to the State Standard of Ukraine 3888:2015	Beer control	Beer «Emerald»
Appearance	Opaque foamy liquid or clear with opalescence, without foreign inclusions that are not characteristic of beer. The presence of yeast sediment and particles of protein and tannin compounds is permissible	Transparent foamy liquid with opalescence, without foreign inclusions not typical for beer	
Flavour	Clean, fermented, malt, hop, without foreign odours. Faint yeast aroma is acceptable	Pure, fermented, malt, hop	Clean, fermented, malty, hop-like, with a light pine needle aroma
Taste	Clean, fermented, malty, with hop bitterness that corresponds to the type of beer, with a hint of yeast, without off-flavours	Clean, fermented, malty, with pronounced hop bitterness	Clean, fermented, malty, with pronounced hop bitterness and a refreshing piney tone
Foaming: foam height, mm	not less than 20.0	30.0	30.0
foam resistance, min	not less than 2.0	3.0	3.0

The analysis of the above data shows that the use of coniferous extract in beer production had a positive effect on its organoleptic characteristics. It is worth noting the bright, pronounced coniferous tone of Smaragd beer. The drink forms a thick and stable foam during pouring: the foam height is 30 mm, the foam resistance is 3 minutes, which is a sign of good quality beer with a fresh and full taste and sufficient carbon dioxide saturation.

According to the results of the organoleptic evaluation, it was found that the beer "Emerald" has a clean, fermented, malty taste, with a pronounced hop bitterness, a refreshing pine tone and a light aroma of pine needles, malt, and hops.

The results of the study proved that the developed beer meets the requirements of the current standard in terms of organoleptic characteristics.

The beer "Emerald" was tasted at the enterprise "OLNA" LLC (Kharkiv) and at a meeting of the Specialised Industry Tasting Commission for the assessment of the quality of beer, soft drinks, low-alcohol beverages, mineral and drinking waters, syrups and concentrates, Ukrpyvo, Kyiv.

At the next stage, the evaluation of Smaragd beer was carried out using the method of tasting analysis. The tasting was carried out by a group of experts (six people) - employees of the brewery of OLNA LLC. The maximum number of organoleptic evaluation points was 25 (based on the reference beer sample). The generalised and processed results of the analysis of the tasting evaluation of the developed beer are shown in Fig. 4.1.

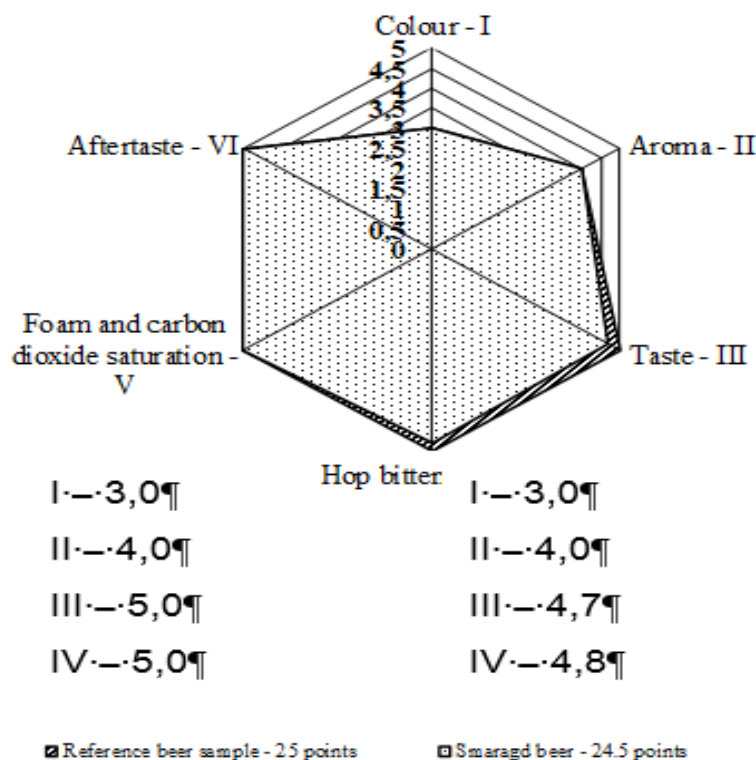


Fig. 4.1 Organoleptic evaluation of Emerald beer on a 25-point scale

As can be seen from the diagram above, Smaragd beer has sufficient carbon dioxide saturation and a harmonious taste. The drink received 4.7 points for taste

and 4.8 points for hop bitterness. Special attention was paid to the taste sensations from the first sip to the swallowing of the beer. The aroma and aftertaste of the beer received the maximum number of points - 4 and 3 points respectively.

The results of the study showed that Emerald beer is dominated by subtle hop bitterness, while extractives are almost imperceptible. The pleasant hop bitterness and light pine tone of the developed beer are harmoniously combined, leaving a subtle pine needle aftertaste on the tongue that quickly disappears. The drink has a balanced combination of taste and aroma, none of which is overpowering, which is evidence of its high quality. The extract content gives the finished product a refreshing and balanced taste.

Foam and carbon dioxide saturation are also important indicators of beer quality. The study observed a white, thick (compact), stable foam (foam resistance is 30 mm within 3 minutes) with a liquid and rapidly disappearing release of gas bubbles, which indicates that carbon dioxide in beer was formed naturally during fermentation and fermentation.

Thus, the addition of coniferous extract containing polyphenolic substances had a positive effect on the taste and foam stability of the finished drink. The developed sample has a total score of 24.5 points due to its original taste and aroma and excellent quality.

Thus, Emerald beer has a harmonious, balanced taste with hop bitterness, a refreshing pine tone and a delicate aroma and aftertaste of pine needles, which is quite attractive to consumers.

#### **4.2. Component composition of volatile flavouring substances in "Emerald" beer**

The result of complex biochemical processes that occur during beer fermentation and fermentation is a beverage with a specific composition, taste and aroma, which are determined by volatile fermentation by-products. They are the basis for the formation of the sensory profile of the finished product, namely:

higher alcohols, esters, carbonyl and sulfur compounds, aldehydes, acids and other substances [21].

For the comprehensive study of beer, chromatography methods are used to determine the classes of components of interest for its production: carbohydrates, alcohols, organic acids, inorganic anions and inorganic cations [20].

The next stage of the work was to study the component composition of beer flavouring substances. The content of the compounds was determined by high-performance gas chromatography, which allows to determine the concentration of flavouring substances and to calculate them. The research was carried out in the laboratory of the Organic Materials Technology Department of the Institute of Single Crystals Scientific and Technological Complex.

Beer contains higher alcohols, esters, aldehydes, organic acids, etc. They are formed during the enzymatic decomposition of wort carbohydrates and form the aroma and taste of beer. Organic acids play the role of salts in beer. Their main function is to inhibit the growth and influence of many harmful microorganisms.

Beer Control contains ethyl ester (retention time – 19.927 min, 5.31% or 0.000326724 mg/100 ml), acetic acid (retention time 7.637 min; 13.05%; 0.0008026826 mg/100 ml), methyl ester (retention time 8.093 min; 8.58%; 0.0005275494 mg/100 ml), heptyl alcohol (retention time 16.527 min; 5.4%; 0.0003318789 mg/100 ml), 2-butyraldehyde (retention time 8.806 min; 6.87%; 0.0004224098 mg/100 ml), butenic acid (retention time 18.882 min; 1.82%; 0.0001118983 mg/100 ml), pentanal aldehyde (retention time 12.995 min; 3.27%; 0.0002013435 mg/100 ml), ethane methyl ester (retention time 13.732 min; 2.13%; 0.0001312468 mg/100 ml). A total of 32 flavour components formed as a result of fermentation were detected in the control beer sample.

Emerald beer contains butenic acid (retention time – 18.882 min, 2% of the total or 0.000087656 mg/100 ml), acetic acid (retention time 7.639 min; 16.04%; 0.0007033775 mg/100 ml), isopentanol (retention time 11.365 min; 5.18%; 0.002270767 mg/100 ml), pentanal (retention time 12.994 min; 7.18%; 0.0003148061 mg/100 ml), diethylamine (retention time 14.890 min; 4.2%;

0.0001840857 mg/100 ml), methyl ether (retention time 8.092 min; 11.06%; 0.000485071 mg/100 ml), ethyl ether (retention time 20.719 min; 0.28%; 0.0000120535 mg/100 ml), butanethiol (retention time 21.552 min; 1.59%; 0.0000698093 mg/100 ml). Additionally, the following substances were detected: cyclobutyl alcohol (retention time 5.821 min; 0.74%; 0.0000323096 mg/100 ml), methylbutane (retention time 10.170 min; 0.45%; 0.0000198783 mg/100 ml), methyl hydrosulfate (retention time 10.373 min; 0.2%; 0.000086374 mg/100 ml), butanoic acid (retention time 10.731 min; 0.27%; 0.0000116806 mg/100 ml). In total, 38 flavour components formed as a result of fermentation were identified in "Emerald" beer.

The factors that determine the taste and aroma of beer include alcohol fermentation by-products. The analysis of the study results shows that the content of flavour components in Emerald beer is higher than in the control.

To fully evaluate the aroma expression of Emerald beer, it was advisable to determine the total content of substances that determine the aroma of the drink (aroma number). This made it possible to fully characterise the effect on the finished beverage of the quantitative content of aroma-forming substances of the raw materials used [110].

The aroma number was determined by a method based on the ability of the chromium mixture to oxidise essential oils. The amount of potassium bichromate consumed was used to calculate the content of aromatic substances in beer. The research was carried out in the laboratory of the Organic Materials Technology Department of the Single-Crystal Institute Scientific and Technological Complex. The results of the aroma number study are shown in Table 4.2.

According to the results of the study, it was determined that the "Emerald" beer has an aroma number of 3361 ml of  $\text{Na}_2\text{S}_2\text{O}_3$ /100 ml, while the control beer has 2170 ml of  $\text{Na}_2\text{S}_2\text{O}_3$ /100 ml. Thus, it was proved that the developed beer contains a greater amount of substances that determine its aroma due to the addition of coniferous extract.



Table 4.2

**Results of determining the aroma number in beer 6(n = 5, P ≥ 0.95)**

Sample of beer	Amount of 0.1 M Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> solution used for titration 5 ml of chromium mixture, ml	Number of aroma (ml Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> /100 ml)
Control	9.4	2170
Emerald	15.1	3361

Since some classes of compounds were identified by gas chromatography, the quantitative chemical composition of the beer was studied (Table 4.3).

Table 4.3

**General chemical composition of beer (100 ml) (n = 5, P ≥ 0.95)**

Name of the indicator	Content in beer	
	Control	Emerald
Carbohydrates, g	4.63	4.61
Ethyl alcohol, g	3.00	2.90
Nitrogenous substances, g	0.30	0.30
Water, g	83.00	85.00
Organic acids, mg	36.00	32.70
Bitter substances, mg	3.10	2.90
Thiamine, B <sub>1</sub> , mg	0.003	0.004
Riboflavin, B <sub>2</sub> , mg	0.030	0.031
Pyridoxine, B <sub>6</sub> , mg	0.053	0.056
Nicotinic acid, PP, mg	0.78	0.87
Ascorbic acid, vitamin C, mg	0	3.50
Potassium, mg	48.60	48.00
Magnesium, mg	8.90	8.93
Calcium, mg	5.17	5.15
Sodium, mg	4.35	4.35
Iron, mg	0.02	0.02

The data presented in Table 4.3 show that "Emerald" beer has no significant differences from the control in terms of the main indicators of its composition. The ethyl alcohol content in the developed beverage is 3 g/100 ml vs. 2.9 g/100 ml in the control and almost the same bitterness in the case of replacing hops with pine needles in the amount of 20%.

Emerald beer contains mineral compounds, organic acids, B vitamins, and ascorbic acid. The content of bitter substances in the developed beer and the control beer is 2.9 mg/100 ml and 3.1 mg/100 ml, respectively.

The content of ascorbic acid in "Emerald" beer is 3.5 mg/100 ml. In the control, it is absent because the wort is boiled at a high temperature. In addition, OLNA does not use ascorbic acid in the production of traditional beer.

A comprehensive commodity assessment of the developed craft beer enriched with pine needles, which differs from traditional beer by its high content of natural plant-based biologically active substances, such as low- and high-molecular weight phenolic compounds, aromatic substances, etc.

It was found (Table 4.4), that craft beer, compared to traditional beer, contains 1.3–1.6 times more plant-based BAS, in particular low-molecular weight phenolic compounds (control – 90.6 mg/100 ml, "Emerald" – 133,8 mg/100 ml), polyphenolic (tannins) substances (control – 114.3 mg/100 ml, "Emerald" – 153.9 mg/100 ml), flavonoid glycosides (by routine) (control – 25.9 mg/100 ml, "Emerald" 40.8 mg/100 ml). This allows not only to produce a new beer with a high content of bioactive substances, but also to increase its shelf life.

Table 4.4

**Content of biologically active substances and antioxidant activity of beer**

**(n = 5, P ≥ 0.95)**

Name of the indicator Biologically active substance	Content in beer	
	Control	Emerald
Polyphenolic (tannin) substances, mg/100 ml	114.3	153.9
Low molecular weight phenolic compounds (by chlorogenic acid), mg/100 ml	90.6	133.8
Flavonol glycosides (routine), mg/100ml	25.9	40.8
Antioxidant activity, Cl/100 ml	156.1	178.1

To evaluate the quality of "Emerald" beer, it is advisable to use the value of antioxidant activity, which characterises the content and effect of all organic substances with reducing ability [111]. The antioxidant activity of "Emerald" beer

(178.1 Cl/100 ml) is 1.14 times higher than that of the control beer (156.1 Cl/100 ml).

The content of proteins, fats, carbohydrates, vitamins, minerals and other dietary supplements in foods determines their nutritional value. The term "nutritional value" has a broader meaning than the concepts of "biological value" (protein quality) and "energy value" (the amount of energy released in the body from food), as it reflects the full range of the product's beneficial properties. The study of the chemical composition of "Emerald" beer allowed us to determine the regulated indicators of its nutritional and energy value (Table 4.5).

Table 4.5

**Nutritional and energy value of beer, g/100 ml**

**(n = 5, P ≥ 0.95)**

Component name	Content in beer	
	Control	Emerald
Proteins	0.30	0.30
Carbohydrates	4.60	4.61
Energy value, kcal/100 ml	18.60	18.48

The nutritional value is calculated by the percentage of human satisfaction with the most valuable substances. The nutritional (food) value of beer is determined by the amount of carbohydrates in the drink due to the small amount of proteins and fats in the product.

It was found that the nutritional value of "Emerald" beer is 4.61 g/100 ml, the control – 4.63 g/100 ml. The energy value of the beverages was calculated, which is 18.48 kcal/100 ml in the new beverage and 18.60 kcal/100 ml in the control.

**4.3. Study of physicochemical and safety parameters of "Emerald" beer**

The next stage of the work was to study the physical and chemical characteristics of beer. According to the regulatory documentation, the mass fraction of solids in the initial wort, mass fraction of alcohol, acidity, colour, and

mass fraction of carbon dioxide were determined. The research results are shown in Table 4.6.

Table 4.6

**Results of the study of physical and chemical parameters of beer quality**

**(n = 5, P ≥ 0.95)**

Name of the indicator	According to the State Standard of Ukraine 3888	Control	Emerald
Mass fraction of alcohol, %, not less than	2.7	3.0	2.9
Mass fraction of dry matter in the initial wort, %.	10.0±0.3	10.0	10.3
Acidity, cm <sup>3</sup> , 0.1 mol/dm <sup>3</sup> of sodium hydroxide solution per 100 cm <sup>3</sup> of beer	1.2–2.8	1.8	1.7
Colour, cm <sup>3</sup> , 0.1 mol/dm <sup>3</sup> of iodine solution per 100 cm <sup>3</sup> of water	0.2–1.8	1.2	1.2
Mass fraction of carbon dioxide, %, not less than	0.3	0.3	0.3

According to Table 4.6, the mass fraction of alcohol in "Emerald" beer is 2.9%, which is 0.1 units less than in the control (3.0%). The content of SR in the initial wort in the developed beer is 10.3%, in the traditional beer – 10%. The acidity of "Emerald" beer is 1.7 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of sodium hydroxide solution per 100 cm<sup>3</sup> of beer, the control is 1.8 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of sodium hydroxide solution per 100 cm<sup>3</sup> of beer.

The difference can be explained by the addition of aqueous coniferous extract, which reduces the ethyl alcohol content and acidity in Emerald beer, and increases the content of dry matter in the initial wort.

The colour of the beer is 1.2 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of iodine solution per 100 cm<sup>3</sup> of water (or 17 European Brewery Convention colour units). The mass fraction of carbon dioxide is 0.3%, which indicates that the addition of coniferous extract at the stage of main fermentation (at the end of fermentation) did not reduce its intensity.

It was proved that the physicochemical parameters of Emerald beer are within the permissible limits and meet the requirements of the current standard.

Thus, the use of coniferous extract with the simultaneous replacement of hops with pine needles in the amount of 20% contributes to the improvement of antioxidant properties and organoleptic characteristics of beer. The results obtained are the basis for the introduction of "Emerald" beer with a high content of bioactive substances into production.

The main source of contamination of raw materials and food is the environment. In terms of radiation safety,  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  are the most dangerous pollutants today. The vegetable raw materials used for beer production are capable of accumulating radionuclides [112]. Beer must meet the established requirements for the quality and safety of food raw materials and food products in accordance with GN 6.6.1-130-2006 [82]. The results are presented in Table 4.7.

Table 4.7

**Beer safety indicators (n = 5, P ≥ 0.95)**

Element name	Requirements of GN 6.6.1-130-2006, Sanitary and Epidemiological Standards 4.2-180-2012, permissible level, not more than	Actual content in beer	
		Control	Emerald
Toxic elements, mg/dm <sup>3</sup>			
Mercury	0.05	0.0017	0.0011
Arsenic	0.20	0.0140	0.0150
Lead	0.30	0.0010	0.0018
Cadmium	0.03	0.0010	0.0016
Radionuclides, Bq/dm <sup>3</sup>			
Specific activity $^{137}\text{Cs}$	70	2.01	1.64
Specific activity $^{90}\text{Sr}$	100	2.11	1.76

It was proved that the content of toxic elements in Emerald beer is less than the Maximum Permissible Concentration. The amount of mercury is 0.0017 mg/dm<sup>3</sup> in the control and 0.0011 mg/dm<sup>3</sup> in "Emerald"; arsenic in the control is 0.014 mg/dm<sup>3</sup>, in "Emerald" 0.015 mg/dm<sup>3</sup>, which does not exceed the norm (0.2 mg/dm<sup>3</sup>). There are insignificant changes in the content of lead in beer –

0.0010 mg/dm<sup>3</sup> (control) and 0.0018 mg/dm<sup>3</sup> (Emerald) – and cadmium – 0.0010 mg/dm<sup>3</sup> (control) and 0.0012 mg/dm<sup>3</sup> (Emerald).

It was determined that the amount of radionuclides in beer is within the permissible concentrations. Their content in the control is as follows: <sup>137</sup>Cs – 1.64 Bq/dm<sup>3</sup>, <sup>90</sup>Sr – 2.01 Bq/dm<sup>3</sup>; in "Emerald" beer: <sup>137</sup>Cs – 1.76 Bq/dm<sup>3</sup>, <sup>90</sup>Sr – 2.11 Bq/dm<sup>3</sup>. The difference between the indicators of "Emerald" beer and the control can be explained by the introduction of additional plant material that is more capable of accumulating radionuclides. It has been proven that high-quality raw materials are used for beer production, which does not significantly increase the content of toxic elements. Based on the results of the study, we can speak of a high level of safety of the new product.

Microbiological cleanliness of the brewing process is a prerequisite for producing high-quality beer with excellent organoleptic properties and high biological stability. Unfortunately, it is difficult to completely exclude the penetration of foreign microorganisms into wort and beer, which can lead to cloudiness, changes in taste and aroma, etc. Therefore, it is advisable to study the microbiological parameters of beer [113].

Determination of the number of mesophilic aerobic and facultative anaerobic microorganisms in beer is not determined, since special microbial cultures are used during its production. The results of the study are presented in Table 4.8.

Table 4.8

**Microbiological indicators of beer (n = 5, P ≥ 0.95)**

Name of the indicator	Permissible level according to IK 00032744-4246-2006	Actual content in beer	
		Control	Emerald
Bacterial coliforms (coliforms), CFU/cm <sup>3</sup>	Not allowed	Not detected	Not detected
Pathogenic microorganisms, including Salmonella, in 25 cm <sup>3</sup>	Not allowed	Not detected	Not detected

It was proved that in terms of microbiological indicators, Emerald beer complies with the "Instructions for sanitary and microbiological control of brewing and non-alcoholic production".

#### **4.4. Toxicological and pharmacological study of the effect of Emerald beer on the organism of biological objects**

Alcohol in any form has a dose-dependent toxic effect on the human body. In case of excessive alcohol consumption, there is a progression of cardiovascular and chronic diseases of the digestive system, alcoholic psychosis, myorenal syndrome, brain swelling, pulmonary edema, etc. Alcohol is especially dangerous for adolescents, pregnant women, and the elderly. The toxic effects of alcohol can be reduced by adding antioxidants to drinks [114].

Toxicity studies are a mandatory stage of research on new medicines and food products, which allows assessing the health hazards of substances. Previous studies have shown the effect of beer on the body of biological objects [115; 116].

At this stage of the work, subacute toxicity was studied, which involves obtaining data on the toxicity of Emerald beer after its administration for a limited time. Studies on biological objects were conducted at the Central Research Laboratory of the National University of Pharmacy. The subacute toxicity of Emerald beer was studied in comparison with the control. The experiments were conducted on rats by intragastric administration, which implies the use of the drink in practice and is appropriate given the possibility of accidental situations that cause alcohol abuse.

The beer was administered to the animals by intragastric injection for 14 days, which corresponds to 2 months of human consumption. The study was conducted on 36 male and female white nonlinear rats. Before the study, the animals were divided into 6 groups of 6 animals each.

The maximum allowable volume of the drink was administered to rats – 32 ml/kg, i.e. 7.04 ml per animal weighing 220 g [158]. According to the literature,

the lethal dose of rectified ethyl alcohol of the highest purification LD50 is 9.5 ml/kg (7.71 g/kg) [118]. The amount of the substance administered was recalculated for the content of ethyl alcohol, the amount of which for a single administration was 7.5 times less than the average lethal dose. The intact animals were given an appropriate volume of purified water. After calculation, it was found that this indicator in animals of both sexes administered with "Smaragd" beer did not differ significantly from that of the intact group (Tables 4.9–4.10).

Table 4.9

**Weight of internal organs of white nonlinear rats (males) after 14-day intragastric administration of the test beverages (n = 6)**

The mass coefficient organs, 0.1 g/10 g	Experimental group		
	Intact	The one who was led away	
		Beer «Emerald»	Beer control
Liver	38.567±0.173	40.117±0.931	41.867±1.252*
Heart	3.333±0.021	3.333±0.021	3.400±0.110
Brain	16.567±0.148	16.567±0.152	16.500±0.073
Kidneys	9.667±0.042	9.633±0.165	10.233±0.056*
Adrenal glands	0.183±0.002	0.183±0.013	0.190±0.001
Spleen	3.813±0.008	3.700±0.110	3.813±0.008
Lungs	6.633±0.056	6.667±0.259	6.713±0.021
Thymus	0.933±0.003	0.943±0.003	0.957±0.004
Testes	4.100±0.037	3.913±0.076	3.767±0.021*

\* The change is significant relative to the values of the intact group (p < 0.05).

The biometric index of the relative weight of the liver of animals treated with beer control (41.867) was slightly higher than that of those treated with Emerald (40.117), indicating the beginning of the development of compensatory manifestations, in particular, organ hypertrophy.

The results of light microscopy showed that in intact rats (males and females) the condition of the liver parenchyma was typical for this species. The tissue pattern was indistinct. The boundaries of the lobules were found by triads. The triad zones are narrow. The radial orientation of the liver laminae was not disturbed. Hepatocytes had a characteristic shape and size, clear cell boundaries. The cell nuclei were oval, centrally located, moderately variable in size, contained



mostly one, rarely two nuclei. The cytoplasm of the cells was evenly coloured and did not contain any inclusions visible at the light level (Fig. 4.2).

Table 4.10

**Weight of internal organs in white nonlinear rats (females) after 14 days of intragastric administration of the test drinks (n = 6)**

The mass coefficient organs, 0.1 g/10 g	Experimental group		
	Intact группа	The one who was led away	
		Beer Emerald	Beer control
Liver	37.467±0.201	37.868±0.152	39.967±0.595*
Heart	3.500±0.146	3.500±0.112	3.600±0.073
Brain	16.100±0.123	16.133±0.220	16.167±0.259
Kidneys	9.533±0.076	9.367±0.092	10.267±0.117*
Adrenal glands	0.193±0.004	0.193±0.002	0.188±0.006
Spleen	3.767±0.092	3.800±0.110	3.800±0.037
Lungs	6.200±0.110	6.067±0.076	6.167±0.128
Thymus	0.960±0.011	0.950±0.022	0.980±0.038
Ovaries	0.263±0.006	0.273±0.011	0.275±0.012

\*The change is significant relative to the values of intact animals группы ( $p < 0.05$ ).

The analysis of microscopic data showed that the animals treated with beer control and "Emerald" (males and females) showed fatty infiltration, i.e. steatosis (fatty hepatosis) of the liver, which is the accumulation of fat in the liver parenchymal cells (Figs. 4.3, 4.4).

Most often, pathological fatty infiltration of the liver is a reaction of the organ to the effects of various toxic substances, including alcohol. In this case, hepatocytes lose their functions, gradually accumulating simple fats and degenerating into adipose tissue. Thus, this phenomenon is a reaction of the liver of biological objects to the toxic effects (exogenous intoxication) of the alcoholic beverages under study.

To a lesser extent, the intensity of fatty degeneration of hepatocytes was affected by "Emerald" beer, reducing their level. There were isolated accumulations of fat in the parenchymal cells of males and females, the characteristic areas of fatty degeneration were reduced compared to the control or the fatty degeneration was diffuse. According to histological assessment, the liver



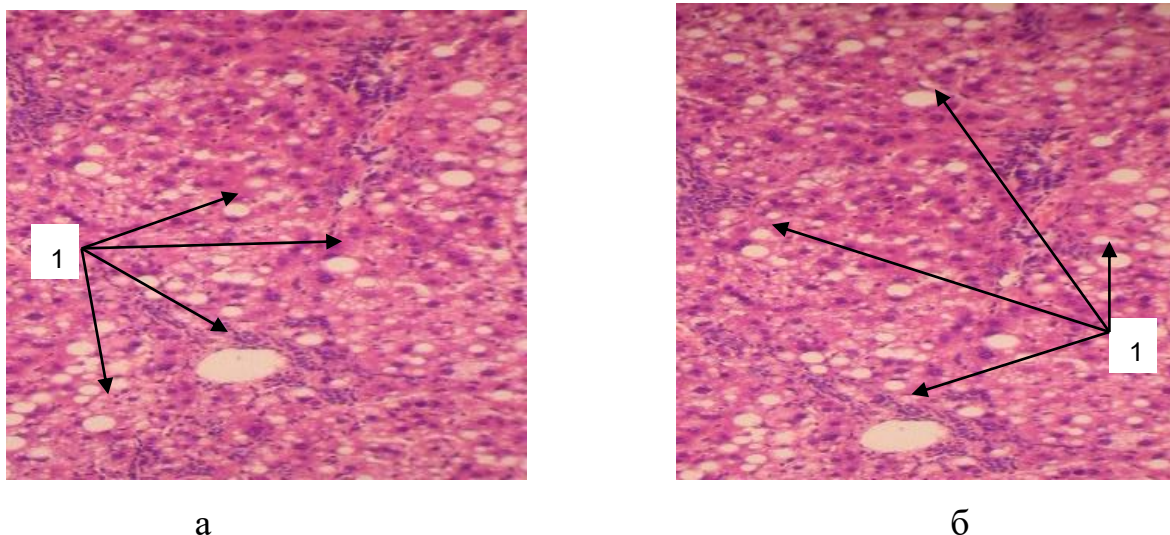


Fig. 4.4. Liver parenchyma of beer-control animals. Areas of fatty infiltration, small clusters, scattered, pronounced diffuse infiltration of fat in parenchymal cells (1). Hematoxylin-eosin stain, x250: a – males; b – females

Based on the research conducted to study the subacute toxicity of «Emerald» beer, it can be argued that the addition of coniferous extract to the drink's recipe reduces the negative effects of alcohol on the body of biological objects, which is primarily evidenced by the normalisation of the liver weight ratio of animals.

Frequent consumption of alcohol leads to chronic formation of free radicals and prooxidants. Ethyl alcohol primarily affects the liver, disrupts the antioxidant balance of hepatocytes and accelerates the formation of free radicals and prooxidants in them. Based on the research conducted to study the subacute toxicity of Emerald beer, it can be argued that the addition of coniferous extract to the drink's recipe reduces the negative effects of alcohol on the body of biological objects, which is primarily evidenced by the normalisation of the liver weight ratio of animals. Frequent consumption of alcohol leads to chronic formation of free radicals and prooxidants. Ethyl alcohol primarily affects the liver, disrupts the antioxidant balance of hepatocytes and accelerates the course of lipid peroxidation.

To investigate the effect of coniferous extract on the specific properties of beer, the antioxidant system of biological objects was evaluated. High values of

prooxidant balance markers (reduced glutathione and catalase) and low values of antioxidant markers (diene conjugates and thiobarbiturates of reactants) correspond to the normal status of the cell, otherwise, they indicate activation of lipid peroxidation and membrane destruction [119].

The experiment used a dose of 15 g/kg for intragastric administration to biological objects, which was equivalent to the average amount of alcoholic beverage (beer) consumed by a person at one time. Toxicological studies of beer "Emerald" were conducted in comparison with controls. The intact animals received an appropriate volume of purified water. The test beverages were administered for 14 days.

Since alcohol consumption often does not correlate with a healthy lifestyle and causes changes in the body with impaired intracellular homeostasis, metabolism, and pro-antioxidant balance, it was advisable to study the effect of the studied beverages on the pro-antioxidant balance of the liver in a model of oxidative stress [114, 120]. Thus, the effect of "Emerald" beer under conditions of increased oxidation (hypoxia) was studied.

We used the method of modelling glucocorticoid-induced oxidative stress described in [100]. The experiment was performed on 36 white nonlinear rats of both sexes, divided into 6 groups of 6 animals each.

In accordance with the general rules for conducting experiments with animals, the condition and behaviour of the rats were visually monitored during the study. During the experiment, the observed peculiarities of the behaviour and condition of the rats did not differ from the generally accepted data on the effects of alcohol on the body of biological objects.

The studied indicators of males and females changed proportionally and did not differ in dynamics, so it was advisable to compare the general indicators of animals by groups.

The results of biochemical parameters obtained from the liver homogenate of animals without pathology showed that consumption of control beer by animals significantly increased the content of prooxidant markers in the liver

homogenate: the content of diene conjugates increased by 10.2% and the content of thiobarbituric acid reagents by 35.5% compared to placebo animals (Table 4.11).

Table 4.11

**Content of pro-antioxidant markers in animal liver homogenate after 14 days of intragastric administration of the studied beverages under conditions of oxidative stress modelling, n = 6**

№ groups	Substance that was injected	Diene conjugates (µmol/g)	Thiobarbituric acid-reagents (µmol/g)	Reduced glutathione (µmol/g)	Catalase activity (mM/min.g)
Intact control animal groups					
1	Water purified	9.8±0.8	6.2±0.6	13.2±0.4	6.1±0.8
2	Beer control	10.8±0.5*	8.4±0.7*	12.6±1.2	3.6±1.1*
3	Beer «Emerald»	9.2±0.9	6.5±0.3	13.0±1.5	5.6±0.8
Groups of animals with modelled pathology					
4	Water purified	21.4±1.9	16.8±0.9	5.6±0.5	2.1±0.4
5	Beer control	24.5±1.5*	19.1±1.2*	4.2±0.8*	1.8±0.6*
6	Beer «Emerald»	22.3±0.7	16.5±1.4	5.3±0.4	2.0±0.5

\* The change is significant relative to the values of the groups where animals received placebo (purified water): for intact control animals - the value of № 1, for control pathology animals - the value of group № 4 ( $p < 0.05$ ).

The content of reduced glutathione (12.6 µmol/g) remained at the intact level (13.2 µmol/g), but catalase activity decreased significantly: by 41% compared to the intact control group. The data obtained indicate that even for a conditionally healthy person, long-term alcohol abuse can lead to disruption of the antioxidant system of the liver and the body as a whole.

It was found that Emerald beer has antioxidant potential and is able to protect the liver from the negative effects of alcohol in its composition under normal conditions (higher values of antioxidant markers: reduced glutathione by 3.0%, catalase activity by 32.8% and lower values of prooxidant markers: Diene conjugates by 4.1%, thiobarbituric acid reactive substances by 30.7% compared to the group of animals receiving beer control).

According to the results of the study, the liver homogenate markers of animals treated with Emerald beer were within the physiological norm. This suggests that the plant antioxidants contained in the coniferous extract compensate for the negative effects of alcohol and other harmful products in beer.

The damage to the liver antioxidant system in the biological objects treated with beer control was the greatest among all groups. The content of diene conjugates in the liver of these animals was 14.5% higher than in those who consumed purified water under conditions of oxidative stress. The content of thiobarbituric acid reactants was 13.7% higher than in rats from group 4. The rate of glutathione recovery in the liver of these animals decreased by 25%, catalase activity by 14.3% compared to the corresponding control group under conditions of increased oxidation.

In rats treated with Emerald beer, there were no significant changes in the oxidative stress compared to the corresponding intact group. All the studied markers of the liver pro-antioxidant system did not differ from those of animals that consumed purified water and were within the physiological norm.

The study of biochemical parameters obtained from the liver homogenate of animals that consumed Emerald beer in the oxidative stress model shows higher values of antioxidant markers: reduced glutathione – by 19.6%, catalase activity – by 9.5%; lower prooxidant markers: diene conjugates – by 10.3%, thiobarbituric acid-reactants – by 9.5% compared to the group of animals that received control beer.

It has been proven that Emerald beer has an antioxidant potential that can protect the liver from the negative effects of alcohol in its composition both under normal conditions and in conditions of increased oxidation of the body. At the same time, beer control enhances pro-oxidative processes caused by prednisolone, damaging hepatocytes.

According to the results of the study, it can be concluded that "Emerald" beer is by no means a corrector of the impaired antioxidant status of the body. It was found that the addition of coniferous extract to the beer recipe can reduce the

negative effects of alcohol, which is an undoubted advantage when introducing a new product into production.

#### **4.5. Changes in the quality of beer «Emerald» during storage**

The modern market requires brewers not only to produce high quality products, but also to maintain the stability of the beverage for a long time [164]. During storage, the consumer properties of beer change due to the chemical composition, functionality of the recipe components, technological mode of preparation and storage conditions. It is known that the inclusion of natural plant materials with a high content of bioactive substances with antioxidant effects in the recipe reduces oxidative processes and, accordingly, increases the shelf life of the finished product [54].

In beer, most extractive substances are in the form of colloidal solutions, which affect the quality and properties of the product. During fermentation and aging, the colloidal system is in equilibrium. However, it is easily disturbed by colloidal aging, protein denaturation and the formation of adsorption compounds. In this case, colloidal particles gradually increase, forming turbidity. The shelf life for each variety is determined individually according to the technological instruction. Therefore, it was advisable to study the change in beer quality indicators during storage.

After maturation, the beer is pumped into bottles for further storage and bottling. Storage takes place at a temperature of 0°C to 2°C under a pressure of 1.5–2.0 atm. OLNA LLC produces unfiltered, unpasteurised beer, which is supplied to the bar via a communication system. The predicted shelf life of unfiltered beer is 3–5 days.

The beer was packaged in dark-coloured thermoplastic bottles with a capacity of 1.0 litres and stored in a darkened, uncooled room at a temperature of (20±2) °C. At the first stage, we studied changes in the organoleptic quality indicators of Emerald beer and the control, such as appearance, aroma, taste, and

foaming. According to the technological instruction for unfiltered light beer produced using classical technology, its shelf life is 3–5 days. The quality of the beverages was monitored immediately after bottling (as an initial indicator) and then daily for 12 days.

Changes during storage in the control began on the 5th day, and in Emerald beer – only on the 10th day. This indicates that the antioxidants of the coniferous extract reduce oxidative processes and prevent the formation of opalescence and cloudiness, which negatively affect the consumer properties of the beverage.

The changes in both samples are associated with a slight loss of transparency, deterioration of taste characteristics, and weakening of foaming, but the beer control has undergone more significant changes. This is due to the addition of a coniferous extract with antioxidant and bactericidal properties to the recipe of the developed beer. The changes that occur during the entire shelf life are also due to the permeability of the container material, but the polymer bottle is able to maintain the quality of the beer during this period.

Changes in microbiological indicators of beer quality were studied during storage. The results of the study showed that no pathogenic microorganisms and bacteria of the *E. coli* group were detected during the entire period. It was proved that in terms of microbiological indicators, Emerald beer complies with the "Instructions for sanitary and microbiological control of brewing and non-alcoholic production".

Throughout the entire shelf life, changes in the main physicochemical parameters were monitored: the mass fraction of alcohol and carbon dioxide, acidity, and colour. Additionally, the tannin index was determined, which characterises the protein-colloid stability of beer and the content of bitter substances. According to the regulatory document [111], the stability of unfiltered beer is determined by the increase in acidity to the maximum permissible values (2.8 units). The results of the study of acidity changes are shown in Fig. 4.5.



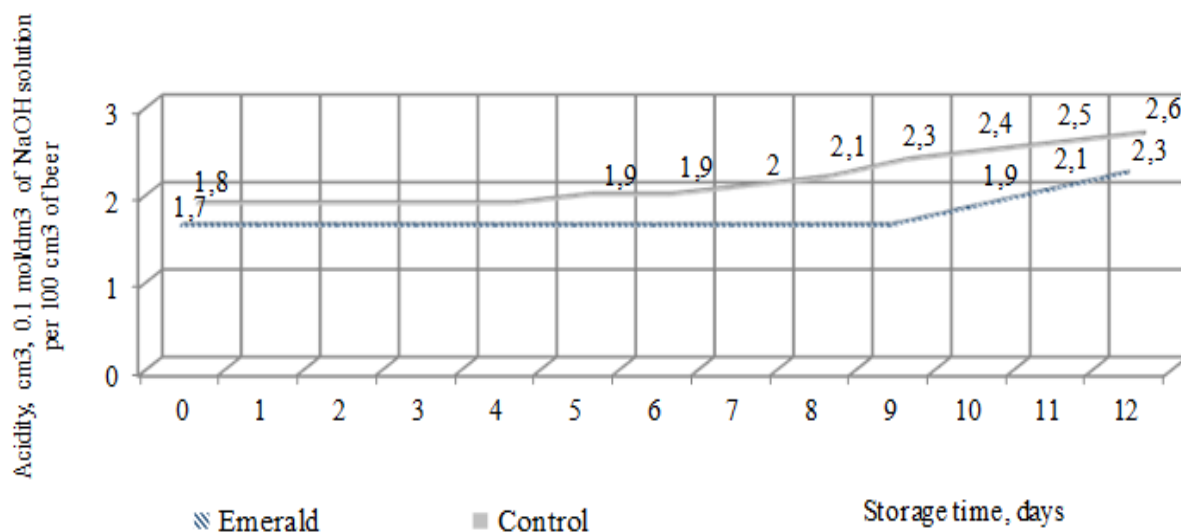


Fig. 4.5. Dynamics of changes in acidity of "Emerald" beer compared to with the control during storage

The results of the study indicate that the acidity of beer during storage varied within 1.7–2.6 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of sodium hydroxide solution per 100 cm<sup>3</sup> of beer. This figure in the control was 2.6 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of sodium hydroxide solution per 100 cm<sup>3</sup> of beer, and in "Emerald" beer – 2.3 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of sodium hydroxide solution per 100 cm<sup>3</sup> of beer. Changes in acidity occur as a result of the oxidation of various functional compounds in beer, primarily due to aldehyde oxidation and natural oxidation. It was noted that less significant changes in acidity occurred in Emerald beer, which can be explained by the content of coniferous extract in the recipe, which has antioxidant properties and helps to reduce oxidative processes during storage.

As can be seen from the data in Table 4.12, the addition of the extract does not significantly affect the colour during storage. The colour of the control changed by 0.1 units on the fifth day, which is associated with the oxidation of polyphenols. This indicator of Emerald beer remained stable until the end of the shelf life, which indicates the stability of the finished drink. The study proved that the addition of

coniferous extract, which has antioxidant properties, does not significantly change the colour of the developed beer during storage.

Table 4.12

**Colour change dynamics of «Emerald» beer compared to the control during storage (n = 5, P ≥ 0.95)**

Name of beer	Duration of storage, days												
	0	1	2	3	4	5	6	7	8	9	10	11	12
	Colour, cm <sup>3</sup> , of iodine solution with a concentration of 0.1 mol/dm <sup>3</sup> per 100 cm <sup>3</sup> of water												
Control	1.2	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Emerald	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.1	1.1

The most valuable substances in beer are bittering agents, which affect the fullness and purity of the taste of the drink, its stability and colour. They give beer its bitterness, have antiseptic properties, and are involved in the formation of foam. Plant antioxidants added to beer protect the bitter substances in beer from oxidative degradation [30; 91]. At the initial stage, the content of bitter substances in the control was 2.9 mg/100 ml, and in the "Emerald" beer – 3.1 units. During storage, this indicator changed in the "Emerald" beer by 0.9 units and in the control by 1.9 units (Fig. 4.6).

At the end of storage, the content of bitter substances in "Smaragd" beer decreased by 2.1 times compared to the control (1.2 mg/100 ml) and amounted to 2 mg/100 ml. The results obtained indicate that the addition of the extract helps protect bitter substances from oxidative degradation, which is likely to increase the shelf life of the finished product.

The tannin index characterises the protein-colloidal stability of beer. Fig. 4.7 shows that the value of this indicator in beer varied between 0.300 D and 0.355 D. This can be explained by the fact that during storage, proteins aggregate, which increases their molecular weight and decreases their solubility, leading to cloudiness [90].

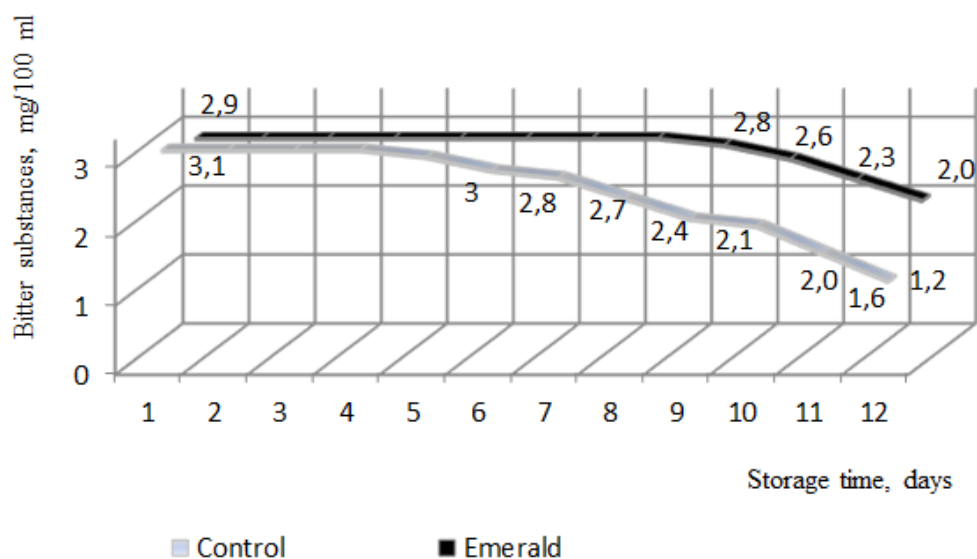


Fig. 4.6. Bitter substance content of "Emerald" beer compared to the control during storage

The highest stability of the tannin index was observed in the beer "Emerald" – 0.312 D. During the entire shelf life, this indicator in the developed beer decreased by half compared to the control (0.355 D), which indicates resistance to changes in its redox properties caused by plant antioxidants of coniferous extract and contributes to the stability of the finished product.

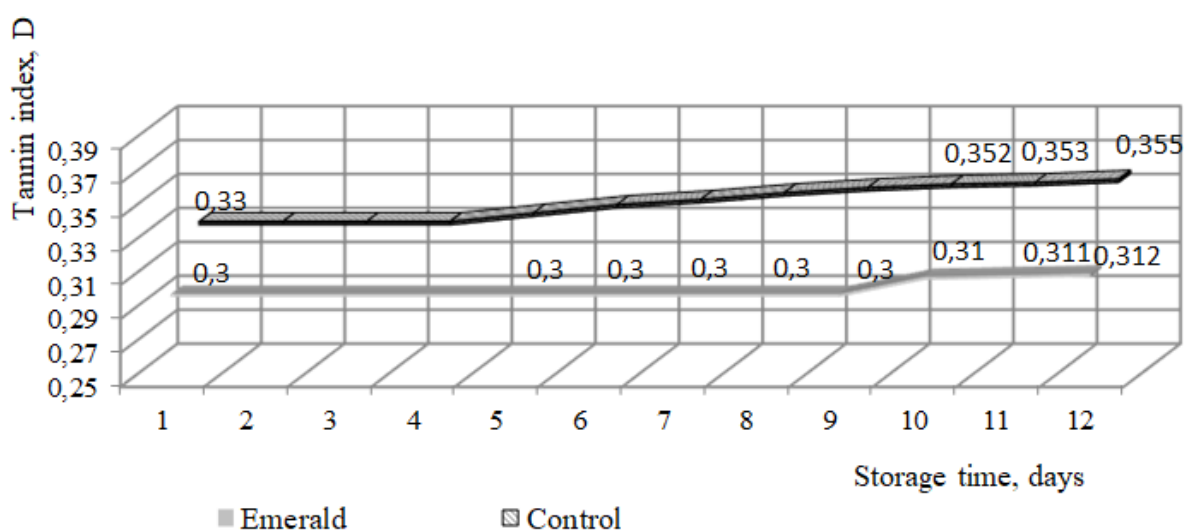


Fig. 4.7. Dynamics of changes in the tannin index of beer samples during storage

It was found that the tannin index of the Emerald beer sample changed more significantly than that of the control. This can be explained by the addition of a coniferous extract to the formulation of the developed beverage, which, due to its antioxidant properties, helps to protect bitter substances from oxidative degradation and increases the antioxidant capacity of the finished product.

It was found that during storage, the developed craft beer, compared to traditional beer, has a lower loss of acidity – by 2 times, bitterness – by 2.5 times, and tannin – by 2.1 times. This made it possible to extend the shelf life of Emerald beer by 2 times (respectively, the shelf life of the control beer on hop cones is 5 days, and with its partial replacement with pine needle extract – 10–11 days).

Thus, the expediency of introducing a bioactive substance of a natural plant additive from pine needles in the form of an extract to enrich craft beer to increase its shelf life has been proved.

Since changes in the chemical composition of beer occur during storage, it is advisable to determine the quantitative composition of its main components. The results of the study are shown in Table 4.13.

It has been established that during storage, the content of ethyl alcohol and carbon dioxide increases and carbohydrates decrease due to the growth of microorganisms and the residue of cultured yeast. Opalescence is formed, which leads to a decrease in the biological stability of the product.

Achieving the maximum degree of fermentation, the content of alcohol and hop substances, high acidity and bottling conditions do not fully protect the vital activity of microorganisms. All of these processes are slower in Emerald beer: the carbohydrate content is reduced by 1.0–1.5 times, carbon dioxide by 2.0–2.5 times and ethyl alcohol by 1.3–1.5 times.

The content of ascorbic acid in Emerald beer decreases by 50% during storage. On the 12th day, its mass fraction is 1.9 mg/100 ml.

As can be seen from Figs. 4.8–4.10, the content of polyphenolic substances in the developed beer decreased by 1.6 times, low-molecular phenolic compounds

by 2.0 times, and flavonol glycosides (by routine) by 2.0–2.2 times compared to the control.

Table 4.13

**Chemical composition of «Emerald» beer compared to the control during storage, 100 ml**

Shelf life, days	Component name				
	Carbohydrates, g	Ethyl alcohol, g	Nitrogenous substances, g	Carbon dioxide, g	Ascorbic acid, C, mg
<b>Beer «Emerald»</b>					
0	4.61	2.90	0.30	0.30	3.5
1	4.61	2.90	0.30	0.30	3.5
2	4.61	2.90	0.30	0.30	3.5
3	4.61	2.90	0.30	0.30	3.5
4	4.61	2.90	0.30	0.30	3.5
5	4.61	2.90	0.30	0.30	3.5
6	4.61	2.90	0.30	0.30	3.5
7	4.61	2.90	0.30	0.30	3.5
8	4.57	2.99	0.30	0.30	3.5
9	4.54	2.99	0.30	0.30	3.5
10	4.50	2.99	0.28	0.31	3.0
11	4.48	3.00	0.26	0.32	2.7
12	4,42	3,05	0,25	0,32	1,9
<b>Beer-control</b>					
0	4.63	3.00	0.30	0.30	0
1	4.63	3.00	0.30	0.30	0
2	4.63	3.00	0.30	0.30	0
3	4.63	3.00	0.30	0.30	0
4	4.63	3.00	0.30	0.30	0
5	4.58	3.03	0.30	0.31	0
6	4.54	3.03	0.29	0.31	0
7	4.49	3.05	0.29	0.32	0
8	4.46	3.07	0.28	0.32	0
9	4.42	3.08	0.26	0.33	0
10	4.38	3.10	0.25	0.34	0
11	4.32	3.15	0.25	0.34	0
12	4.30	3.20	0.24	0.35	0

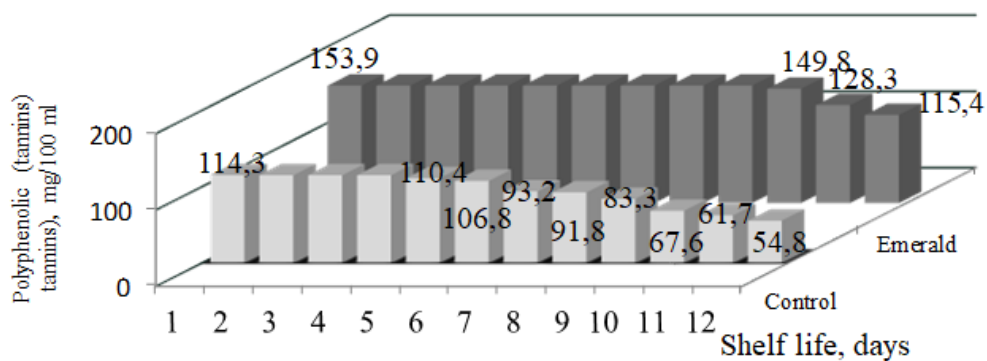


Fig. 4.8. Changes in the content of polyphenolic (tannin) substances in Emerald beer compared to the control during storage.

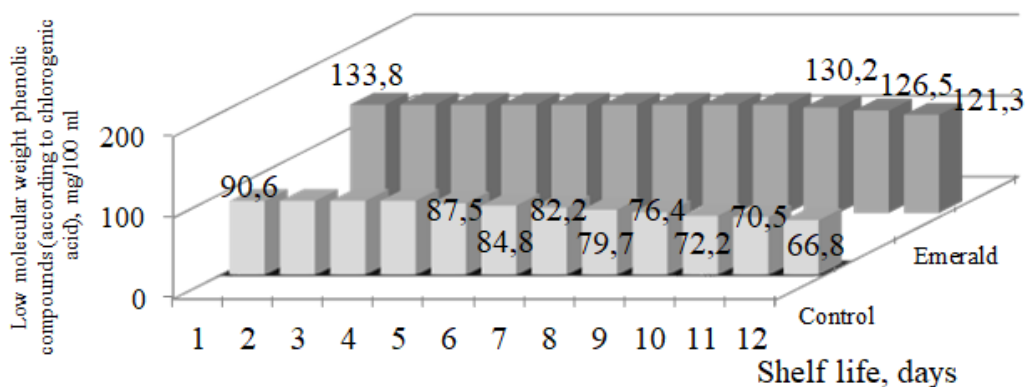


Fig. 4.9. Changes in the content of low molecular weight phenolic compounds (by chlorogenic acid) in Emerald beer compared to the control during storage

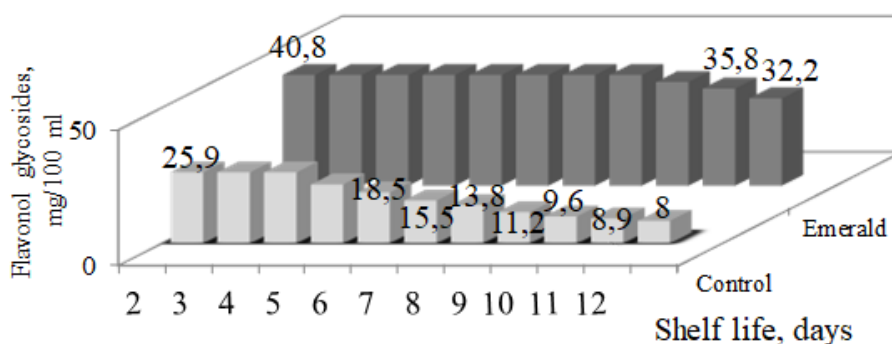


Fig. 4.10. Changes in the content of flavonol glycosides (by routine) in Emerald beer compared to the control during storage

As can be seen from Figs. 4.8–4.10, the content of polyphenolic substances in the developed beer decreased by 1.6 times, low-molecular phenolic compounds by 2.0 times, and flavonol glycosides (by routine) by 2.0–2.2 times compared to the control.

The content of polyphenolic compounds in beer of both samples decreases during storage. They disrupt the colloidal structure, causing the formation of sediment and cloudiness. At the early stages of storage after beer bottling, sediment does not appear because the concentration of insoluble complexes is still low and relatively low-molecular-weight polyphenols are still involved in their formation. With increasing storage time, when polyphenolic compounds gradually polymerise under the catalytic influence of an acidic environment and oxygen dissolved in beer contained in the upper part of the container, the formation of polypeptide-polyphenolic complexes reaches a level where their concentration and size are sufficient for the formation of a precipitate [10].

The amount of organic acids increased in the developed sample twofold compared to the control (Fig. 4.11). Slowing down the oxidation processes and increasing colloidal stability is due to the natural plant additive from pine needles in the form of an extract, which proves the extension of the shelf life of the developed beverage.

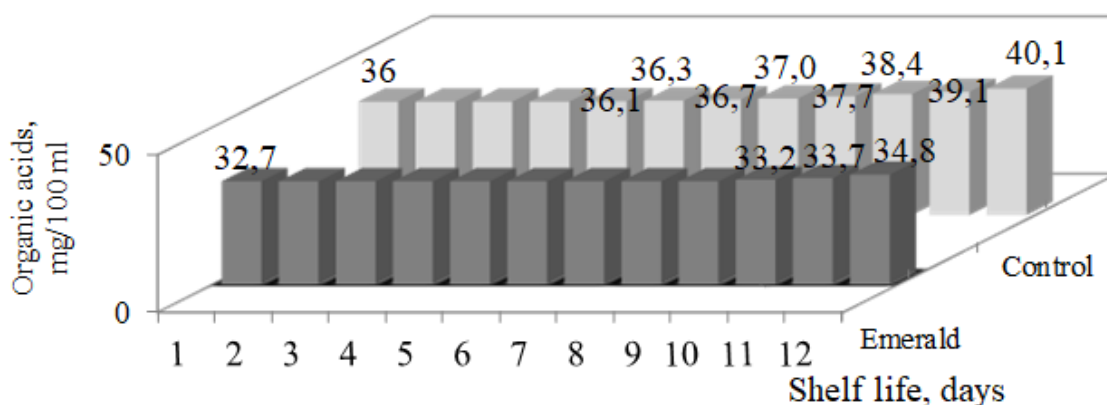


Fig. 4.11. Changes in the content of organic acids in beer "Emerald" compared to with the control during storage

It was found that during storage, the developed craft beer had less loss of the main chemical composition, in particular polyphenolic (tannins) substances by 2 times, low-molecular phenolic compounds (by chlorogenic acid) by 2.0–2.2 times, flavonol glycosides (by routine) by 2.0–2.5 times, compared to the control. This allowed not only to increase the biological value of Emerald beer, but also to extend its shelf life by 2 times (respectively, the shelf life of the control beer on hop cones is 5 days, and with its partial replacement with pine needle extract – 10–11 days).

#### **4.6. Comprehensive commodity assessment of the quality of "Emerald" beer**

When assessing product quality, the principles of qualimetry are used to obtain comprehensive information about product quality, taking into account all its properties. For the completeness of the quality assessment and control of Emerald beer, a comprehensive quality indicator was determined.

At the first stage, in order to rank the indicators, a "tree of properties" was built, the structure of which consists of several levels (Fig. 4.12).

At the zero level is a comprehensive indicator of beer quality (Co). At the first level, the set of properties is divided into the following groups:

- organoleptic quality indicators of beer;
- physicochemical quality indicators, including the mass fraction of solids in the initial wort, mass fraction of alcohol, acidity, colour, mass fraction of carbon dioxide;
- content of dietary supplements: polyphenolic (tannins) substances, low molecular weight phenolic compounds, bitter substances, flavonol glycosides, aromatic substances (by the number of aromas);
- shelf life indicator.



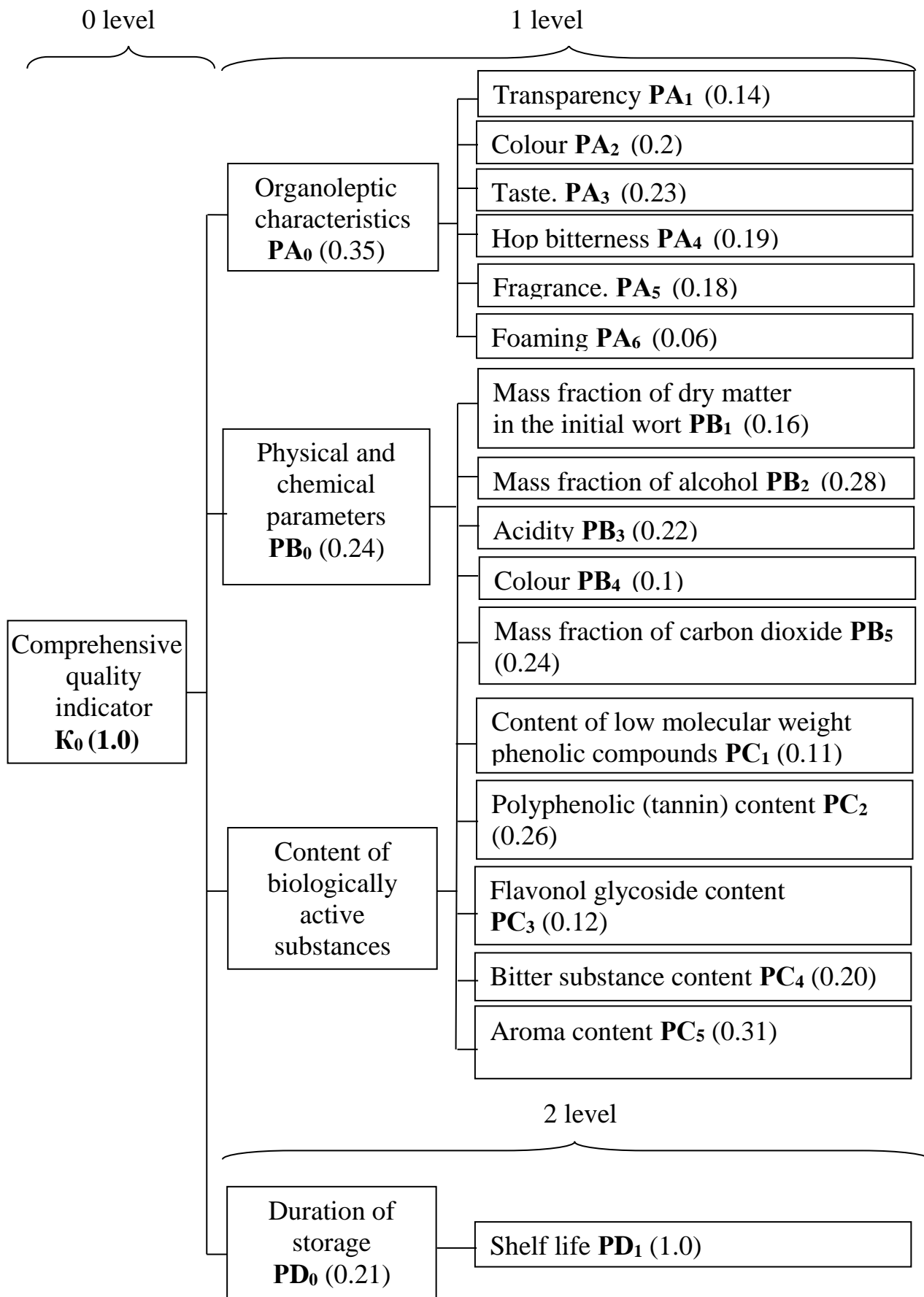


Fig. 4.12. "Property tree" for assessing the quality of «Emerald» beer

The expert group of employees of the brewery LLC determined intergroup and intragroup weighting indicators. To determine the relative quality indicators, the data of absolute and baseline values were used.

Absolute quality indicators within the group of properties A, B, C, D are defined in Section 4. Next, we determined the baseline indicators for assessing property groups. Baseline indicators ( $P_{bas}$ ) are those regulated by regulatory documents or those that have been studied in products in practice. The best indicators  $P_{bas}$  among the prototypes were selected as the  $R_{bas}$  for the property groups.

Baseline indicators:

$PA_{1bas}$  – 3.0 points;  $PA_{2bas}$  – 3.0 points;  $PA_{3bas}$  – 5.0 points;  $PA_{4bas}$  – 5.0 points;  $PA_{5bas}$  – 4.0 points;  $PA_{6bas}$  – 5.0 points.

$PB_{1bas}$  – 10.0%;  $PB_{2bas}$  – 2.7%;  $PB_{3bas}$  – 1.7 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of sodium hydroxide solution per 100 cm<sup>3</sup> of beer;  $PB_{4bas}$  – 1.2 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of iodine solution per 100 cm<sup>3</sup> of water;  $PB_{5bas}$  – 0.3%.

$PC_{1bas}$  – 33.8 mg/100 ml;  $PC_{2bas}$  – 29.8 mg/100 ml;  $PC_{3bas}$  – 15.9 mg/100 ml;  $PC_{4bas}$  – 3.1 mg/100 ml;  $PC_{5bas}$  – 3361 ml of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>/100 ml.

$PD_{1bas}$  – 10 days.

The calculations were carried out by the method of comprehensive assessment of beer quality [137]. The results of determining the complex quality indicators by groups of properties for beer are given in Table 4.14.

Table 4.14

**The results of determining group comprehensive quality indicators of Emerald beer in comparison with the control**

Name of beer	Comprehensive metrics for property groups			
	PA	PB	PC	PD
Control	0.93	0.95	0.72	0.5
Emerald	0.95	0.98	1	1

The data shows that in groups A, B and D, whose properties are related to organoleptic and physicochemical indicators, almost the same numerical values were determined. The highest score was given to the beer "Emerald". In groups C and D, it also received the highest score, which is due to the content of bioactive substances of phenolic and terpenoid nature with antioxidant and bactericidal effects (group C) and shelf life (group D).

The value of the complex beer assessment was obtained by combining the group property assessments. The complex indicator was calculated using the additive model of complex evaluation according to the formula:

$$M_j(x, y, z) = a_{0,j} + a_{1,i}x + a_{2,i}y + a_{3,i}z + a_{4,i}x^2 + a_{5,i}y^2 + a_{6,i}z^2 + a_{7,j}xy + a_{8,j}xz + a_{9,j}yz, \quad (4.1)$$

where  $a_i, j$  – coefficients of the mathematical model;

$j$  – the relation to specific extract quality indicators.

For beer control:  $K_0 = 0.35 \times 0.93 + 0.24 \times 0.95 + 0.20 \times 0.72 + 0.21 \times 0.5 = 0.8$ .

For beer «Emerald»:  $K_0 = 0.35 \times 0.95 + 0.24 \times 0.98 + 0.20 \times 1 + 0.21 \times 1 = 0.98$ .

The rating scale from 1 to 0 is divided into five intervals: 1.00–0.80 – very good; 0.80–0.63 – good; 0.63–0.37 – satisfactory; 0.37–0.20 – poor; 0.20–0.00 – very poor.

According to the calculation results, it was determined that the "Emerald" beer has a comprehensive quality indicator of 0.98, which corresponds to the "very good" rating and is 1.2% higher than the control. This indicates the predicted competitiveness of the new product with its original flavour characteristics and high content of bioactive substances.

## Conclusions to Chapter 4

1. According to the results of the organoleptic analysis, Emerald beer is dominated by subtle hop bitterness and a refreshing pine tone, while the taste of extractives is almost imperceptible. The height of the foam is 30 mm, and the foam

resistance of the drink is 3 minutes. The beer has a harmonious combination of taste and aroma, none of these characteristics prevails, which indicates its high quality.

2. The component composition of volatile flavour and aroma substances in beer was investigated. It was found that "Emerald" beer contains such components as butenic acid (0.000087656 mg/100 ml), acetic acid (0.0007033775 mg/100 ml), isopentanol (0, 002270767 mg/100 ml), pentanal (0.0003148061 mg/100 ml), diethylamine (0.0001840857 mg/100 ml), methyl ether (0.000485071 mg/100 ml), ethyl ether (0.0000120535 mg/100 ml), butanethiol alcohol (0.0000698093 mg/100 ml). Additionally, the following substances were detected: cyclobutyl alcohol (0.0000323096 mg/100 ml), methylbutane (0.0000198783 mg/100 ml), methyl hydrosulfate (0.000086374 mg/100 ml), and butanoic acid (0.0000116806 mg/100 ml). A total of 38 flavour components were identified in the Emerald beer, while in the control 32 components formed as a result of fermentation.

It was proved that Emerald beer contains a higher amount of volatile flavour-forming substances due to the use of a natural plant additive from pine needles in the form of an extract.

3. The physicochemical parameters of beer quality were investigated. It was determined that the mass fraction of alcohol in the developed beer is 2.9%, in the control – 3.0%. The content of SR in the initial wort of "Emerald" beer is 10.3%, in the control – 10%. The acidity of the developed beverage is 1.7 cm<sup>3</sup>, 0.1 mol/dm<sup>3</sup> of sodium hydroxide solution per 100 cm<sup>3</sup> of beer. It has been established that all the studied parameters are within the permissible limits and meet the requirements of the current standard.

It was proved that the content of toxic elements in the beverages is less than the MPC. The amount of mercury is 0.0017 mg/dm<sup>3</sup> in the control and 0.0011 mg/dm<sup>3</sup> in the "Emerald" beer; arsenic in the control – 0.014 mg/dm<sup>3</sup>, in the "Emerald" beer 0.015 mg/dm<sup>3</sup>, which does not exceed the standard (0.2 mg/dm<sup>3</sup>). The changes in the content of lead in the beverages are insignificant

– 0.001 mg/dm<sup>3</sup> (control) and 0.0018 mg/dm<sup>3</sup> (Emerald) and cadmium – 0.001 mg/dm<sup>3</sup> (control) and 0.0016 mg/dm<sup>3</sup> (Emerald). The difference can be explained by the addition of pine needles to the developed beer, which has the ability to accumulate toxic elements. Pathogenic microorganisms and bacteria of the E. coli group were not detected in the beer.

4. Subacute toxicity studies on biological objects have shown a reduction in the negative impact of Emerald beer. The new product reduces the negative impact due to the content of a natural plant additive with antioxidant properties, which primarily leads to the normalisation of the liver mass index of animals, which is as close as possible to that of intact animals (an increase in the mass index in males by 4%, in females – by 1%). The mass index of kidneys increased in the case of administration of beer control (in males by 5.8%, in females – 7.7% compared to intact animals). The histological assessment confirmed that the liver parenchyma of animals treated with Emerald beer was the closest to the liver parenchyma of intact animals.

It was proved that Emerald beer has antioxidant potential and is able to protect the liver from the negative effects of alcohol in its composition under normal conditions (higher values of antioxidant markers: reduced glutathione – by 3%, catalase activity – by 32.8%; lower prooxidant markers: diene conjugates – by 4.1%, thiobarbituric acid reactants – by 30.7% compared to the group of animals receiving control beer).

It has been established that the results of the study of biochemical parameters obtained from the liver homogenate of animals that consumed "Emerald" beer on the model of oxidative stress differed from the group of animals that consumed control beer: higher values of antioxidant markers: reduced glutathione – by 19.6%, catalase activity – by 9.5% and lower prooxidant markers: dinic conjugates – by 10.3%, thiobarbituric acid-reactants – by 9.5%.

4. The organoleptic characteristics of beer during storage were determined. Beer "Emerald" has less changes in taste and aroma characteristics. It was found that the developed craft beer, compared to traditional beer, has a lower loss of acidity – by 2 times, bitterness – by 2.5 times, and tannin – by 2.8 times during

storage. This made it possible to extend its shelf life by 2 times (respectively, the shelf life of the control beer on hop cones is 5 days, and with its partial replacement with pine needle extract – 10–11 days).

5. It was found that during storage, the content of carbohydrates in "Emerald" beer decreased by 1.0–1.5 times, carbon dioxide – by 2.0–2.5 times, and ethyl alcohol – by 1.3–1.5 times. The analytical index of the content of polyphenolic (tannins) substances in the developed beer decreased by 1.6 times, low molecular weight phenolic compounds (by chlorogenic acid) – 2.0 times, flavonol glycosides (by rutin) – 2.0–2.2 times compared to traditional beer. The amount of organic acids in the developed beverage increased by 2 times compared to the control. The content of ascorbic acid in "Emerald" beer decreased by 50% during storage. Slowing down the oxidation processes and increasing colloidal stability are due to the content of the natural pine needle extract, which not only increased the biological value of the new product, but also extended its shelf life by 2 times.

6. A comprehensive commodity assessment of "Emerald" beer enriched with pine needles, which differs from the traditional beer by its high content of natural plant-based biologically active substances, such as low- and high-molecular weight phenolic compounds, aromatic substances, etc. It has been established that the developed craft beer contains significantly more plant-based bioactive substances, in particular low-molecular weight phenolic compounds by 1.4–1.5 times (control – 90.6 mg/100 ml, "Emerald" – 133.8 mg/100 ml), polyphenolic (tannins) substances by 1.3–1.4 times (control – 114.3 mg/100 ml, "Emerald" – 153.9 mg/100 ml), aromatic substances by 1.5... 1.6 times (control – 2170 ml of  $\text{Na}_2\text{S}_2\text{O}_3$ /100 ml, "Emerald" – 3361 ml of  $\text{Na}_2\text{S}_2\text{O}_3$ /100 ml). This made it possible not only to produce a new beer with a high content of dietary supplements, but also to increase its shelf life. The content of ascorbic acid in Emerald beer is 3.5 mg/100 ml. According to the results of the integral index calculation, the developed product meets the "very good" rating (0.98). It was proved that "Emerald" beer has the predicted competitiveness.

**SECTION 5**  
**ECONOMIC EFFICIENCY OF RESEARCH**  
**AND THEIR IMPLEMENTATION**

**5.1. Calculation of the economic effect from the production of "Emerald" beer**

The feasibility of bringing innovative developments to the market and introducing them into production is justified in the process of assessing the economic efficiency of production.

The innovative component of the research is focused on improving the consumer characteristics of beer by adding a coniferous extract to its recipe, which allows for the production of high quality products and expanding their range.

The principle of evaluating the effectiveness of scientific developments is to compare the results and their corresponding costs. The variety of results and costs associated with them defines a certain range of approaches to efficiency assessment. In particular, the focus of the research on improving the quality of beverages leads to the distinction between the economic and consumption effects.

The excess of income from the production and sale of products over expenses in value terms compared to traditional products characterises the economic efficiency and competitiveness of innovative goods.

Expanding the range of beverages that are affordable and have improved quality characteristics, as well as reducing the negative impact on consumers' health, can be considered positive results in the field of alcohol consumption.

The economic justification for widespread implementation of developments to improve the formulation of beverages is based on a comparison of future benefits and costs associated with the manufacture of these products.

Price is the most important factor that determines sales revenues and profits. Therefore, at the first stage, the price of the proposed beverage is calculated to compare it with a similar beverage that meets the needs of the same consumer market segment.

The basis for wholesale prices of manufacturing companies is the cost of production, which is determined by Accounting Regulation (Standard) 16 "Expenses" approved by Order of the Ministry of Finance of Ukraine, as well as by the methodological recommendations on the formation of the cost of production (works, services) in industry approved by Order of the Ministry of Industrial Policy of Ukraine.

The starting point for determining the cost of production is the calculation of the cost of raw materials and inputs that form the basis of the product (Table 5.1).

Table 5.1

### Calculating the cost of raw materials for beer production

Name of raw material	Wholesale price 1 kg, UAH	Name of the beer sample			
		Control		«Emerald»	
		Raw material consumption per 1 dal, kg	Cost of raw materials, UAH	Raw material consumption per 1 dal, kg	Cost of raw materials, UAH
Malt Pilsner	43.00	2.5	107.5	2.5	107.5
Yeast Safbrew T-58	3672.00	0.075	68.85 (275.4/4)	0.075	68.85 (275.4/4)
Granulated hops Hallertau Hersbrucker	890.00	0.180	160.20	0.140	124.60
Pine needles (dried)	140.00	–	–	0.040	5.60
Water, л	0.009	7.245	0.065	7.245	0.065
Total		–	336.62	–	306.62
Cost of raw materials per 1 litre of beverage		–	33.66	–	30.66

Since the developed method of producing Smaragd beer involves the addition of aqueous pine needle extract to the recipe, the cost of raw materials and the cost of its production are included in the cost of the main product.

The cost of raw materials is the most significant cost component, accounting for 60.0–65.0% of the total cost of beer production and sales.



Due to the lack of accurate information on such cost elements as labour remuneration, social contributions, fuel and electricity for production needs, depreciation charges, maintenance and operation of fixed assets and rent, general and administrative expenses, calculations were made using aggregate indicators based on the cost of production of analogue products and taking into account changes in the technological process. At the same time, variable and fixed costs were separated in accordance with changes in production volumes and operating conditions.

The share of other beer production and sales costs in the total cost of production is on average 38.0% (35.0% of which are variable), which is taken into account in its calculations for the analogue.

The introduction of coniferous extract into the recipe composition of Emerald beer determines the relevance of labour and energy costs associated with the production of this extract. The increase in these costs was calculated based on the specifics of the technological process, the time required for grinding and extraction operations, heating temperature, energy capacity of the equipment, and the share of these costs in the cost price, which is generally equal to UAH 0.9 per 1 dal. The variable costs of producing Emerald beer were adjusted by this amount. In calculating the price of beer, the profit margin was assumed to be 18.0% of the cost price, as it is in similar production facilities.

The results of calculating the cost and selling prices of beer are shown in Table 5.2.

The calculations showed that the price of Emerald beer is 1% lower than the price of control beer. Therefore, the sale of the developed beverage with enhanced quality characteristics and enriched with dietary supplements at the price obtained will have a positive impact on consumer demand.

The calculated price of Emerald beer is the lowest price at which the manufacturer can sell its products. The focus of the pricing policy on the level of profitability prevailing in this segment of the consumer market provides the

manufacturer of new products with certain competitive advantages over similar products due to high quality parameters and prices within the market range.

Table 5.2

**Calculations of the cost price and price of beer "Emerald", UAH per 1 dal**

Name of the indicator	Sample of beer	
	Control	Emerald
Cost of raw materials	336.62	306.62
Other production and selling expenses	127.91	128.81
including variables	44.77	45.67
permanent	83.14	83.14
Total cost of sales	464,53	435,43
Profit	83,62	78,38
Cost at wholesale prices	548,15	513,81
Wholesale price 1 litre	54,82	51,38
Value added tax (VAT)	10,96	10,28
Selling price 1 litre	65,78	61,66

The economic effect of the introduction of the "Emerald" beer production method with such a pricing policy can be achieved through:

- increase in revenue from beer sales, provided that demand is elastic in terms of price and quality;
- increase in profit (assuming unchanged profitability) as a result of an increase in sales volume;
- increased profitability due to a decrease in specific fixed costs in connection with a possible increase in sales.

Since the study was conducted to determine the complex quality indicator of "Emerald" beer according to the main organoleptic, physicochemical and safety indicators, it is advisable to determine an integral (generalised) quality indicator that takes into account economic efficiency, antioxidant activity and biochemical studies (pro-antioxidant balance of liver tissue), which characterise the toxic

effects of the product, based on a multiplicative model. The integral Kint index comprehensively characterises the quality of the new beverage compared to the control beer.

The following indicators were used to assess the toxic effects of beer on the body: indices of the content of diene conjugates, thiobarbituric acid-reactants, reduced glutathione, and catalase activity.

When choosing indicators for assessing the quality of the beverage, we took into account their significance and adequacy of process reflection, the availability of information support for calculating indicators, the nature and directions of influence of the selected characteristics on the quality of the beverage, the possibility of accurately determining the calculation algorithm, which ensures unambiguous understanding and interpretation of the result by different specialists.

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**Calculation of the integrated quality indicator of "Emerald" beer  
in comparison with the analogue**

Name of the indicator	Meaning.
$K_1$ – Comprehensive quality index	1.22
$K_2$ – antioxidant activity index	1.140
$K_3$ – is a local integral indicator that characterises the toxic effects of a beverage on the body: diene conjugate index	0.779
thiobarbituric acid index of reactants	0.734
reduced glutathione index	1.096
catalase activity index	1.156
$K_3 = \sqrt[4]{1/0.779 \times 1/0.734 \times 1.096 \times 1.156}$	1.314
$K_4$ – economic indicator index	1.067
$K_{int}$ – integral indicator of beer quality «Emerald»	
$K_{int} = \sqrt[4]{1.22 \times 1.14 \times 1.314 \times 1.067}$	1.14

Improvements in quality characteristics also lead to an increase in sales of elastic demand goods, as consumers perceive an increase in quality as a corresponding decrease in price. The increase in sales volume due to improved quality was determined by taking into account the elasticity of demand with respect to quality. The coefficient of elasticity of demand for beer with respect to quality is 1.2.

Based on the results of the study, a possible increase in sales (13.43%) was calculated, which was obtained by reducing the price by 1.77% per 1 litre and improving the quality by 11.66% per 1 litre (Table 5.4). An increase in the volume of products sold leads to such an economic effect as an increase in profit, provided that profitability remains unchanged.

Table 5.4

**Calculations of sales volume growth and profitability from the production of Emerald beer (relative to the analogue)**

Indicator	Value
Wholesale price 1 litre, UAH	51.38
Coefficient of price elasticity of demand	3.2
Coefficient of elasticity of demand by quality	1.2
Quality factor	1.28
Possible increase in sales, % (total)	13.43
including by reducing the price	1.77
quality improvement	11.66
Share of fixed costs in the price, %	16.18
Increase in profitability due to the level of fixed costs, %	3.14

The increase in sales volume also contributes to a reduction in the level of fixed costs, which is a factor in increasing profitability. It has been determined that the increase in profitability due to a decrease in the level of conditionally fixed costs for the production of "Emerald" beer is 3.14%.

Improved quality characteristics and a slightly lower price of the developed beer are also a positive effect for consumers. The relative benefit of customers from purchasing better quality products, if sold at the calculated prices, was calculated to be 23.0% (Table 5.5).

Table 5.5

**Effect of the introduction of the production of "Emerald" beer compared to the analogue (per 1 dal)**

Type of effect	Value
Economic effect	
Increase in the volume of products sold, UAH	39.26
Profit growth, UAH	5.99
Increase in profitability, %.	3.14
Effect for consumers	
Absolute savings per 1 litre, UAH	4.12
Relative consumer benefit from quality improvement, %.	23.0
Quality improvement, %.	26.2
Reduction of toxic impact, %.	13.7

The reduction in the price of "Emerald" beer results in savings in consumer spending on its purchase compared to similar products. The article shows absolute savings of money for the purchase of products (release of consumers' funds) in the amount of UAH 4.12 per 1 litre of drink.

On the basis of the calculations carried out, it is proved that the economic effect of the introduction of "Emerald" beer into production reflects the value indicators of the volume of products sold, the amount of profit received, and the total savings from reducing the cost of production.

It is shown that the lower price level for a similar product and better quality allow obtaining an economic effect from the introduction of the developed beer into production by increasing the volume of sold products (UAH 39.26 per 1 dal), increasing profits (UAH 5.99 per 1 dal) and increasing profitability (3.14%).

It has been proved that the social effect is to save money (UAH 4.12 per 1 litre), improve quality (26.2%) and reduce the toxic effect (13.7%) by purchasing the developed beverage at the calculated prices.

Thus, the calculations confirm the overall economic effect of the introduction of a new craft beer with a high content of BAS, which has antioxidant properties using a natural plant additive made from pine needles. The design of the label and counter-label of the "Emerald" beer was developed.

## **5.2. Practical implementation of research results**

At the final stage of the study, a number of measures were taken to implement the results into production. The research results were put into practice with the aim of launching a beer with the addition of vegetable raw materials on the market. In this regard, the Company has previously obtained a declarative patent of Ukraine for the developed beverage and approved the technical specifications for production with the recipe.

The developed technology was tested and implemented at the following enterprises Demeter Trading House LLC, TD-Centre LLC, OLNA LLC and in the educational process of Kharkiv State University of Food Technology and Trade. A

pilot batch of beer was produced and tasted at the OLNA brewery. The technological instruction with the recipe of "Emerald" beer was approved at a meeting of the Specialised Industry Tasting Commission for the assessment of the quality of beer, soft drinks, low-alcohol beverages, mineral and drinking waters, syrups and concentrates, Ukrpyvo, Kyiv. The results of the study and Emerald beer were presented and received positive feedback at exhibitions of scientific developments.

### **Conclusions to Chapter 5**

1. As a result of determining the economic efficiency of introducing the production of "Emerald" beer, it was proved that the production of a new craft beer with a high content of bioactive substance, which has antioxidant properties, using a natural plant additive from pine needles, leads to an economic effect.

2. It has been determined that the economic effect of the introduction of "Emerald" beer into production is due to an increase in the volume of sold products (UAH 39.26 per 1 dal), increase in profit (UAH 5.99 per 1 dal) and increase in profitability (3.14%). It is proved that the social effect consists in saving money (UAH 4.12 per 1 litre), improving quality (26.2%), reducing toxic effects (13.7%) and expanding the range of beer. The integral quality index of the developed beverage was calculated and was 1.14. A new product label design was developed.

3. Developed and approved in accordance with the established procedure TI 14297558-340:2016 "Technological instruction for the production of 10% light beer "Smaragd" with a recipe" was developed and approved in accordance with the established procedure. A set of organisational activities was carried out to introduce the new craft beer "Emerald" into production and the educational process.

## CONCLUSIONS

1. The complex of biologically active phytochemicals of dried pine needles as an unconventional spicy-aromatic plant raw material in comparison with hops, when used to form the quality of beer, was investigated. It was found that the aromatic and flavour complex of pine needles and hops includes unsaturated reactive substances such as essential oil (from 0.5% to 3.8%), low molecular weight phenolic compounds (by chlorogenic acid) (from 3, 1% to 4.0%), flavonol glycosides (by rutin) (1.3% to 2.7%), polyphenolic (tannins) substances (5.2% to 16.4%), resins (7.0% to 27.3%) and bitter substances (4.0% to 9.2%). At the same time, pine needles have a higher content of these bioactive substances (low- and high-molecular weight phenolic compounds, essential oil) – 1.5...5.0 times higher than hops.

2. The complex of bioactive substance in the aqueous extract from pine needles, made using water as an extractant (1:8 ratio of plant material and water, grinding of needles to a particle size of 50...250  $\mu\text{m}$ ); kept at 60 °C for 30 min followed by infusion for 8 h was determined. It was found that the pine needle extract includes a biological complex consisting of aromatic substances (by the number of aroma) in the amount of 8000 ml of sodium thiosulfate in 100 ml, low-molecular-weight phenolic compounds (by chlorogenic acid) – 289.4 mg per 100 ml, flavonol glycosides (by rutin) – 194.2 mg per 100 ml, polyphenolic (tannins) substances – 237.8 mg per 100 ml and bitter substances – 12.1 mg per 100 ml.

3. The component composition of flavouring substances of the plant extract from pine needles, which forms the specific taste and aroma of pine needles and beer made with its use, was investigated by gas chromatography. It was determined that the extract has the highest content of limonene ether (40.005%), which forms the lemon aroma, and also contains terpenes such as pinene (17.919%), borneol (3.617%) and coniferin (4.193%) in significant quantities. Pinene and coniferin



have a pine and woody aroma. The glycoside picein (1.767%) was detected, which forms a bitter taste.

4. The expediency of introducing a biological substance of a natural plant additive from pine needles in the form of an extract for the enrichment of craft beer and extending its shelf life has been proved.

5. The taste, aroma and quality of a new craft beer with a high content of bioactive substances with antioxidant properties using a natural plant additive from pine needles were formed. The dose and rational ratio of hops and dried pine needles extract were determined using mathematical modelling and expert opinion. The dose of hop pellets is 135...140 g per dal of wort and is introduced during boiling and wort production. The pine needle extract with a mass fraction of solids 3.8...3.9% in the amount of 300...350 ml per dal of beer (or in solids 35...40 g) is added at the end of the main beer fermentation.

6. A new method of producing and enriching a mass consumption beverage - craft unfiltered beer – with a natural herbal additive made from non-traditional plant material (pine needle extract) with antioxidant properties has been scientifically substantiated and developed.

7. A comprehensive commodity assessment of the developed craft beer enriched with pine needles, which differs from the traditional one in its high content of natural plant bioactive substances, such as low- and high-molecular weight phenolic compounds and aromatic substances, was carried out. It has been proved that the developed craft beer, compared to traditional beer, contains significantly more plant-based bioactive substances, in particular low-molecular weight phenolic compounds in 1.5...1.6 times, polyphenolic (tannins) substances – 1.3...1.4 times, aromatic substances – 1.5...1.6 times. This allowed not only to increase the biological value of the new beer, but also to double its shelf life (the shelf life of the control beer with hops is 5 days, and with its partial replacement with pine needle extract – 10...11 days).

8. It was determined that the content of toxic elements and radionuclides in the new beer is less than the Maximum Permissible Concentration. The reduction

of the negative impact of the developed beer on the body was proved on biological objects and it was found that it has antioxidant potential and is able to protect the liver from the negative effects of alcohol in its composition (higher values of antioxidant markers: reduced glutathione – by 3.0%, catalase activity – by 32.8%; lower values of prooxidant markers: diene conjugates – by 4.1%, thiobarbituric acid-reactants – by 30.6% compared to the group of animals receiving control beer).

9. The Technological Instruction for the production of 10% light beer "Emerald" with the recipe TI 14297558-340:2016 with a natural plant additive made from non-traditional plant materials (pine needles extract) was developed and approved. The economic effect of the implementation was calculated, which consists in an increase in profit from sales (UAH 5.99 per 1 dal), an increase in the volume of sales (UAH 39.26 per 1 dal) and an increase in profitability (3.14%). The recommended selling price of the new product per 1 litre is UAH 61.66. The integrated quality indicator of "Emerald" beer was calculated and was 1.14. The technology of the new product was tested in production conditions and pilot batches were produced (Demeter Trading House LLC, TD-Centre LLC, OLNA LLC). The results of the research work have been implemented in the educational process of the Kharkiv University of Food Technology.

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## **APPENDICES**

**Schematic flow chart of production of "Emerald" beer**



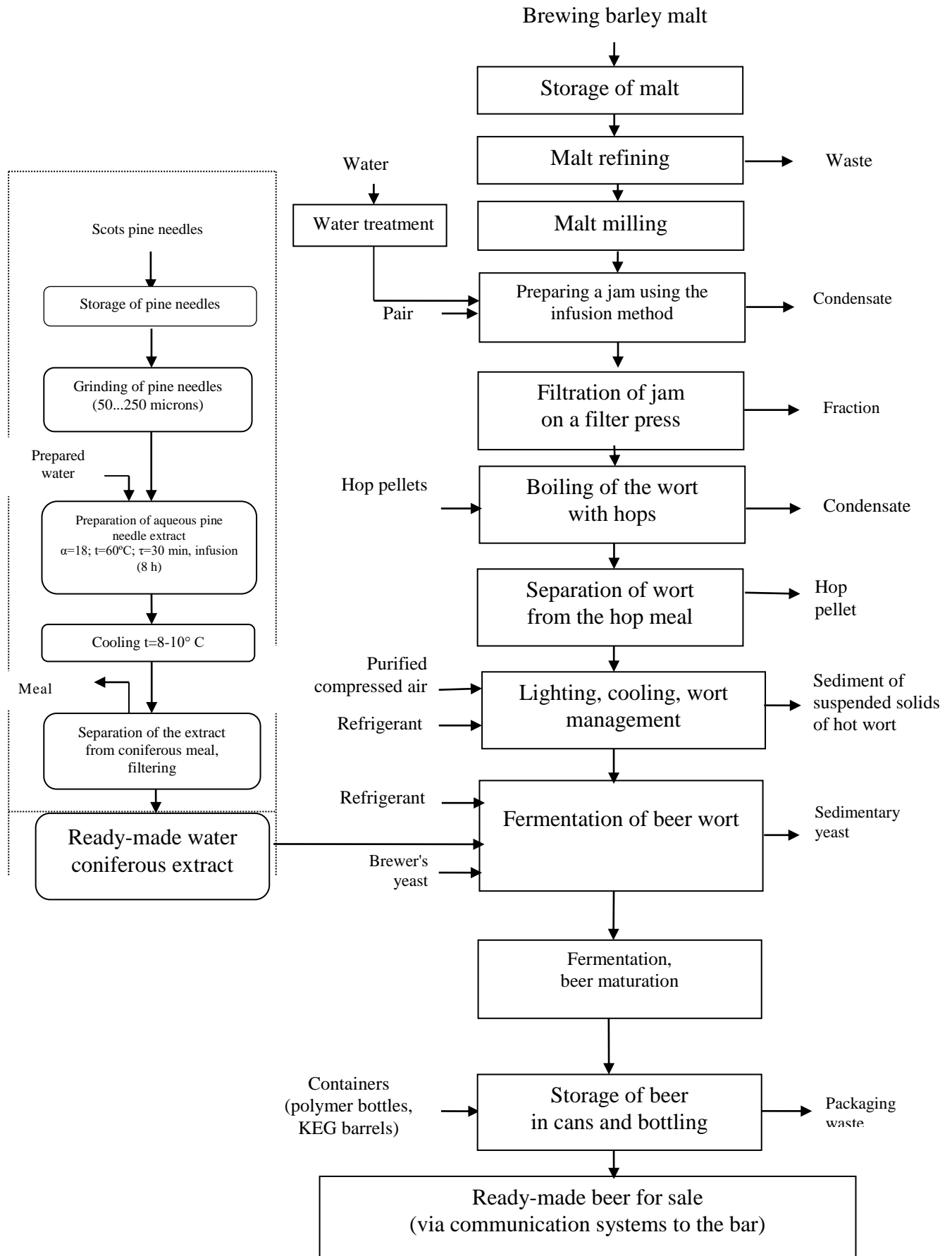


Fig. Schematic diagram of the production process of "Emerald" beer

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