Представлено дослідження ферментації відновлених сироватко-солодових сумішей з використанням лактозозброджувальних дріжджів та сахароміцетів. Застосування таких сумішей для виробництва напоїв бродіння з використанням відповідних видів дріжджів дає можливість підвищення біологічної цінності за рахунок поживних складових молочної сироватки та продуктів ферментації.

У результаті експериментів підібрано оптимальне співвідношення сухих солоду житнього та молочної сироватки для приготування сумішей. Під час досліджень враховували хімічний склад, розчинність сухих складових та можливості утилізації вуглеводів різними расами дріжджів. Виявлено, що раціональним співвідношенням сухих солоду до сироватки є 1:2. При ферментації сусел спостерігався найбільший приріст дріжджових клітин Kluyveromyces lactis 469 від 73 до 75,2 млн/см^3 за активної фази процесу з 4 до 16 год бродіння. Такі показники підтверджують високу бродильну активність при утилізації вуглеводів молочної сироватки.

За кількістю накопиченого етилового спирту та вмістом редукуючих речовин досліджено ефективність інших рас дріжджів. Для ферментації використовували сусло з відновленої сухої суміші з оптимальним співвідношенням складових. Виявлено, що найбільш активно спиртове бродіння проходило в суслі ферментованому дріжджами Saccharomyces lactis 95. Менш ефективними були дріжджі Saccharomyces cerevisiae P-87 у вище вказаному середовищі. При сумісному культивуванні лактозозброджувальних дріжджів та сахароміцетів процес бродіння значно не підсилюється. Синергізм мікроорганізмів не спостерігається.

За результатами газохроматографічних досліджень ідентифіковано побічні продукти бродіння ферментованих сироватко-солодових сусел. Встановлено, що концентрації метилацетату – (11,72±0,59) мг/дм^3 й етилацетату – (92,17±±4,61) мг/дм^3 у суслі, збродженому дріжджами Saccharomyces cerevisiae P-87 є достатніми для формування гармонійного смаку і аромату ферментованого напою

Ключові слова: суха молочна сироватка, сухий солод житній, лактозозброджувальні дріжджі, сахароміцети, сироватково-солодове сусло

UDC 663.86:637.344
DOI: 10.15587/1729-4061.2018.141974

RESEARCH ON FERMENTATION PROCESS OF RECONSTITUTED WHEY-MALT MIXTURES

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1. Introduction

The market segment of low-alcohol and soft beverages, including kvass, is constantly growing, which is emphasized in numerous scientific publications. There is a published research into quality indicators of beverages in which the main component of their formulation, rye malt, was replaced with wheat raw materials [1]. The information is given about the selection of strains of yeast, which ferment kvass wort at temperatures above 30...32 °C [2]. Other developments are aimed at special treatment of traditional components using the new biotechnological resources (xylanolytic enzymes and lactic acid bacteria) [3]. In general, scientific research in this field aims to find new alternative techniques to improve nutritional and biological value of kvass and to prolong the shelf life of such a type of beverages [4]. One of the possible ways to solve the above specified task is to enhance the extractivity of the starting wort up to 8 %, as compared with the traditional ones, with a mass fraction of dry substances of 2.5 % according to the normative documentation, through the use of milk whey as a raw material, as well as specially selected yeasts [5]. Milk whey is a complex biotechnological system that contains the protein substances, nitrogen compounds, carbohydrates, minerals, vitamins, organic acids, enzymes, macro- and micro-elements [6]. Such a structure predetermines its high biological value [7]. That could possibly ensure completeness of taste, improve the nutritional properties and the degree of saturation with carbon dioxide.

It is known, however, that the wide use of milk whey, a raw material or a water environment for beverages, is hindered by the short term of storage without an additional thermal treatment, as well as organoleptic properties (specific taste, smell, and color) [8]. Such sensory indicators are caused by a complex of substances, different in their chemical nature, which mainly form as a result of the action of enzymes on milk components during production of milk-protein products. Fermentation is accompanied by principal changes in the state of processes related to casein and fat [7, 9, 10]. These very components provide the bulk of volatile substances, such as peptides, free amino acids, aldehydes, ketones, volatile fatty acids such as butyric, propionic, acetic, formic acids, etc., which render the specific taste and smell [11, 12]. Prolonging the terms of storage can be achieved when using, as raw materials, the dried milk whey and malt, which could make it possible to produce dry concentrates for recombination and long-term storage.

A combination of dry whey with rye fermented malt as the base of the mixture for a beverage with the limited content of ethyl alcohol, that is kvass, would provide an opportunity to ensure high organoleptic indicators for reconstituted beverages. These mixtures have a long shelf life, they are easy to use both at home and at public catering establishments and in the industrial production of small scale.

Given the above, it is advisable to develop dry whey-malt mixtures and select the strains of yeast for fermentation after their recombination. It is required to identify the fermentation by-products of the fermented whey-malting wort. It is expedient to determine the influence of the type of yeast on the content of volatile substances and the formation of harmonious taste and aroma of beverages.

2. Literature review and problem statement

There are widely applied dry mixes for fermented beverages the type of kvass, made from the classic raw materials of non-milk origin. There is a known technique to produce dry bread-based kvass from specially baked hardtacks [13]. Dry kvass has a wheat consistency, its taste is characteristic of custard rye bread, without bitter aftertaste, with the brightly expressed aroma typical for rye bread; brown in color with a red tint. It is the excessive and specific parameters that limit the common consumption of this type of beverages.

Based on an analysis of modern formulations of dry mixes for fermented beverages the type of kvass, it was found that most of them are supposed to be enriched with fruit and berry and plant raw materials. Thus, papers [14, 15] describe a technology for dry granulated kvass with the addition of an extract from roots of burdock, Hawthorn fruit and rose hips, nettle leaves. It was established that additional herbal ingredients do not exert any negative impact on the rate of wort fermentation and the physiological state of yeast. It should be noted that wild herbs have a chemical composition that varies depending on the period of a plant vegetation. Under industrial production there is a possibility that quality of the finished products may be unstable [16].

Paper [17] described a technology for the dry mixture of kvass and the powders of rose and carrot. When they are recovered, it is impossible to ensure the stability of beverages' indicators given different solubility of components and their impact on the fermentation agents.

There are data on the development of a semi-finished product of dry bread kvass [18]. The composition of the mixtures contains crumbs of dry hardtacks and rye flour in the ratio (1.5...4):1. Other formulation components include sugar, yeast, citric acid and an acidifier in the form of a 30...40 % solution of lactic acid. The latter is applied on the flour in the proportion 1:(4...5) and dried at a room temperature for 12...24 hours with humidity not exceeding 14.5 %. The resulting semi-finished product has a long shelf life – 6 months, which is a positive aspect. However, in general, the proposed technology is based on a multi-component formulation, which complicates the manufacturing process of preparing the components of different origin, their combination and fermentation. In addition, the recovery is guaranteed to be followed by losses with sedimentation of dry substances.

Using milk whey as a component of dry mixes for kvass is a promising direction in the development of technologies for recoverable fermented beverages.

The rational way to improve the organoleptic indicators of milk whey is the selection of aromatic substances, specialized food supplements, and plant raw materials. In addition, a promising technique to improve the organoleptic indicators and enhance the biological value of whey-based beverages is fermentation [19]. It is the selection of appropriate yeasts for the efficient fermentation of a mixture of whey and plant ingredients that forms a scientific task.

The result of the fermentation of sugars, in addition to basic products (ethyl alcohol and carbon dioxide), is the formation of side substances that are present in the substrate [20]. These include higher alcohols (propyl, isoamyl, tyrosyl, tryptophil). Determining the quantitative indicators of the above specified compounds would require additional research.

Milk whey, due to its high biological value, could have a positive impact on the growth and reproduction of yeast [21]. It is the amount of influence in the required direction that could be determined and further corrected according to technological tasks.

In addition to the amine nitrogen, yeast during life activity needs mineral substances, vitamins of group B, biotin. Some substances are present in milk whey in accessible form and could act as a nutrient medium for the yeast cell [22]. The scientific challenge is to determine the amount of malt, not only for the enrichment of the environment, but to increase the number of products of metabolism of microorganisms that form in the process of fermentation.

The selection of microorganism for the fermentation of whey-malt wort from a reconstituted mixture is determined primarily by technological factors and organoleptic indicators of finished beverages. The type and strain of yeast, as well as the conditions for cultivation, including the environment, affect the taste of the beverage and its stability during storage. The fermentation of whey-malt wort is carried out at the expense of biochemical activity of yeast, which is why the main criterion when choosing yeast is the respective fermenting activity in milk whey with rye malt, as well as the organoleptic indicators of finished beverages.

The organoleptic indicators of beverages, obtained from the reconstituted dry whey and malt, confirm the possibility for the fermentation of wort with lactose-fermenting yeast and saccharomyces [23]. To be studied is their joint cultivation in a whey-malt medium. It is required to determine the quantitative indicators of fermentation that confirm the effectiveness of a particular type.

One of the factors that influence the composition of higher alcohols and esters is the strain of yeast. The accumulation of the former at fermentation is defined by the intensity of metabolism in the yeast reproduction period and is associated with amination, the formation of keto acids form carbohydrate conversion products, and during the primary fermentation and post-fermentation – over-amination. Higher alcohols could also form without participation of amino acids, for example, via acetic acid in line with the pathway: acetic acid → acetoacetate → acetone → isopropanol. Higher alcohols are volatile substances with a specific smell and taste that manifest themselves after the formation of esters from them. Complex esters form as a result of esterification as the products of life activity of yeast from volatile or non-volatile organic acids and higher alcohols. Complex esters are the aromatic substances that characterize the taste and smell of the beverage [24]. Experimental confirmation of the above specified statements for the fermentation of whey-malt wort with special yeast is a research problem that needs a solution.

The amount of the formed above-specified products depends mainly on the conditions for fermentation, as well as the strain of yeast used. It is known that higher alcohols, aldehydes and complex esters are the main background components of the flavor of all the products of fermentation, specifically in fermented beverages [25]. Human sensory organs are considered insufficiently sensitive and react differently to different quantities of aroma-forming substances. Therefore, the formation of a general aroma of the fermentation products of whey-malt wort is affected only by those substances that are present in the concentrations above the threshold value of sensitivity [25]. The identification of volatile compounds and determining their amount using instrumental methods, such as gas chromatography analysis, is necessary.

Given the above, it is possible to predict, in terms of beverages, the efficiency of using dry systems with a different degree of solubility and when selecting the special strains of yeast in order to solve technological problems. That could make it possible to obtain, following the recombination of such mixtures and fermentation under traditional conditions, beverages the type of kvass with preset functional properties.

Development of dry mixtures based on crushed fermented rye malt and clotted milk whey to be followed by the further recovery and fermentation would contribute to solving the above-specified scientific tasks. It is possible that concentrates would have a longer shelf life through the use of dry raw materials with constant parameters, while the obtained reconstituted beverages – the normalized content of ethyl alcohol, the taste and aroma close to the classical kvass. The affordability of technology would contribute to the effective performance of production system.

3. The aim and objectives of the study

The aim of this study is to develop dry mixes and to examine the process of fermentation of the whey-malt wort reconstituted with different types of yeast.

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

– to determine the solubility of the components of dry whey-malt mixtures;

– to establish a rational composition of whey-malt mixes based on estimating the productivity of yeast during fermentation of wort;

– to choose the strains of yeast based on the fermenting activity in whey-malt wort;

– to identify the fermentation by-products of the fermented whey-malt wort.

4. Materials and methods of research

In order to prepare whey-malt mixtures, we used dry whey and malt. Dry whey (DSTU 4552:2006, the European analog – "Sweet whey powder" ISO 9001; ISO 14001; FSSC 22000) had the following characteristics: mass fraction of moisture is (5.0±0.33) %; mass fraction of lactose is (50±2.5) %; mass fraction of fat is (2±0.05) %. Titrated acidity of the reconstituted whey to the mass fraction of dry substances of 6.5 % was (50±0.4) °T, the solubility index is (0.8±0.04) cm^3 of raw sediment. The latter indicator was determined by measuring the volume of the insoluble sediment in the reconstituted sample of the dry product [26, 27].

We used dry malt ("Malt for bread and kvass" (GOST 29272-92, the European analog – "Barley Malt Powder" ISO 9001, ISO 9000, ISO 14001, ISO 14000, ISO 20000) with the following parameters: mass fraction of moisture is (8.0±0.25) %; the extract in the dry substance of malt is (42±2.1) %; sodium hydroxide with a concentration of 0.1 mol/dm^3 per 100 g of dry matter of malt is (35±1.75) cm^3; iodine solution with a concentration of 1 mol/dm^3 per 100 g of dry matter of malt is (17±0.85) cm^3.

The course of the experiment, as well as the identification of the fermentation by-products of the fermented whey-malt wort, are described in more detail in paper [28].

5. Study results

5. 1. Determining the solubility of the components of whey-malt mixes

We reconstituted the mixtures at a temperature of 35...45 °C, by intensively stirring, by gradually increasing the temperature to 75...80 °C in order to transfer the extractive substances into solution (reducing sugars, soluble pentosanes, nitrogen-containing compounds, etc.). Next, the mixture, cooled to 25...30 °C, was sent for decanting in order to remove the remnants of denaturized proteins of milk whey and the malt sediment.

An important property of dry mixtures that are to be reconstituted for the preparation of beverages is the capability to dissolve. We therefore investigated the solubility index of dry whey, malt, and malt-whey mixtures with a varying ratio of malt to whey. Research results are given in Table 1.

Table 1

Comparative estimation of the solubility index of raw materials and other dry whey-malt mixtures, cm^3

Dry whey	Dry rye fermented malt	Ratio of dry malt to whey					
		1:1	1:1.5	1:2	1:2.5	1:3	1:3.5
0.75±0.04	2.23±0.11	1.92±0.1	1.80±0.09	1.55±0.08	1.4±0.07	1.35±0.07	1.21±0.06

According to data from Table 1, the addition of rye fermented malt reduces the mixtures' solubility index. This is probably the result of the presence of insoluble ballast substances (lignin, cellulose) in the plant component of the mixture – malt. In general, a difference in the values of solubility is within (1.21...1.92) cm^3, which is not critical and does not affect the overall solubility of the whey-malt mixture.

5. 2. Selection of the composition of whey-malt wort for the fermentation with the yeast *Kluyveromyces lactis 469*

The dynamics of accumulation of the yeast cells *Kluyveromyces lactis 469* at the fermentation of wort at a temperature of 30 °C for 36 hours from the reconstituted mixture with a different ratio of dry malt and whey is shown in Fig. 1.

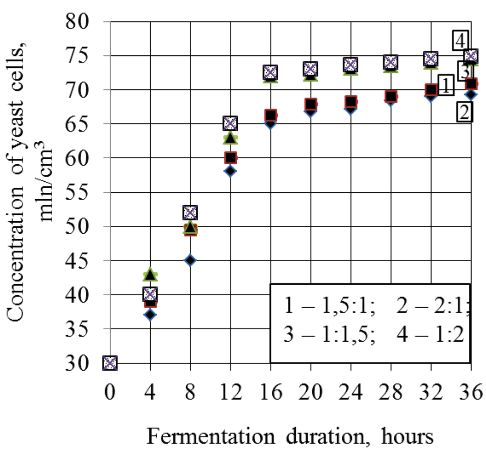

Fig. 1. The dynamics of accumulation of yeast cells at the fermentation of wort from the reconstituted mixture with a different ratio of malt and whey

A change in the number of yeast cells with glycogen (% of total concentration) in the same mixtures is shown in Fig. 2.

According to the plot from Fig. 1, the largest increase in the yeast cells *Kluyveromyces lactis 469* (72 and 73 million/cm^3, respectively) was observed for the ratio of malt:whey of 1:1.5 and 1:2 from hour 4 to 16 during fermentation. These values confirm the high fermenting activity of yeast to the carbohydrates of milk whey – lactose [29, 30]. In the fermented wort, prepared based on the reconstituted mixture with a ratio of dry malt to whey of 1.5:1 and 2:1, the cell growth was significantly lower and totaled, respectively, 45 and 57 million/cm^3, which is probably explained by the insufficient amount of carbohydrates of lactose for cell development and the specific action of this type of yeast.

An increase in the number of cells with glycogen (Fig. 2) was more intense in the samples with a whey to malt ratio of 1.5:1 and 2:1, respectively, fermented by the yeast *Kluyveromyces lactis 469*. Thus, at hour 36 of fermentation the number amounted to 67.2 and 68.9 %, respectively, of the total number of yeast cells.

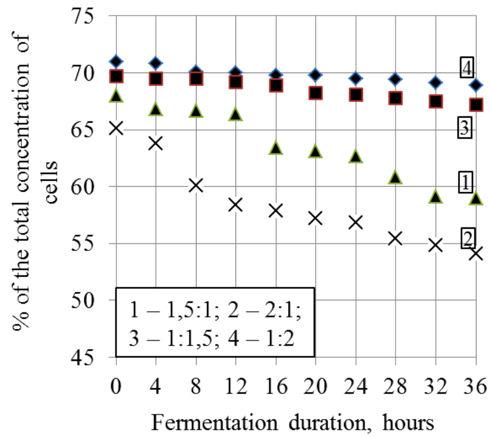

Fig. 2. The dynamics of change in the number of yeast cells with glycogen at the fermentation of wort from the reconstituted mixture with a different ratio of malt and whey

When investigating the rate of growth of the budding cells, it was revealed that the highest indicators were demonstrated by samples prepared based on the dry mixture at a ratio of malt to whey of 1:2 and 1:1.5 – 39.4, and, respectively, 37.9 % for those fermented by the yeast *Kluyveromyces lactis 469*. As is known, the development of yeast cells directly depends on the amount of sugars in wort; cells do not develop at a low amount of carbohydrates. Such an effect is observed in wort with a ratio of malt to whey of 1.5:1 and 2:1, in which at hour 36 of fermentation the number of the budding cells reduced, accordingly, to 31.9 and 31.0 %, which are the lowest indicators compared to other fermented worts. It should be noted that in all experimental samples the accumulation of cells takes place throughout the entire period of cultivation.

In order to examine the fermenting activity of different strains of yeast, we chose wort from the dry mix with the ratio malt:whey 1:2.

5. 3. Studying the fermenting activity of different strains of yeast in whey-malt wort

The amount of ethyl alcohol and the content of reducing substances in the fermented whey-malt wort when using different strains of yeast are shown in Fig. 3.

Fig. 3 shows that the greatest amount of ethyl alcohol was accumulated in the wort fermented by yeast of the strain *Saccharomyces casei* (1.8 % by volume) and *Saccharomyces cerevisiae M-5* (1.5 % by volume). Given the limit for the content of ethyl alcohol for fermented beverages the type of kvass, using the above-specified yeast is not appropriate. The relatively high content of ethyl alcohol was also observed in samples with the yeast *Saccharomyces cerevisiae P-87* (1.2 % by volume). When using other yeasts, the content of ethyl alcohol under the same conditions of fermentation is lower.

The metabolism of yeasts is associated with the course of chemical reactions, catalyzed by enzymes (present in yeast), and is related to the absorption of lactose and monosaccharides of malt as a source of carbohydrates. Given this, we determined the amount of reducing sugars remaining after the completion of the process of fermentation of malt-whey wort with various strains of yeast.

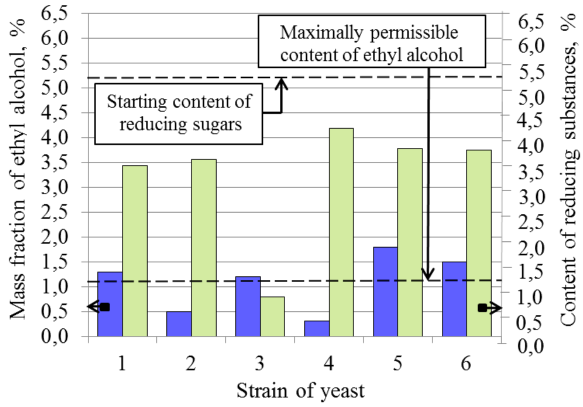

Fig. 3. The amount of ethyl alcohol and the content of reducing substances in the fermented whey-malt wort when using different strains of yeast: 1 — *Saccharomyces cerevisiae P-87*; 2 — *Kluyveromyces lactis 469*; 3 — *Saccharomyces lactis 95*; 4 — *Kluyveromyces lactis 2452*; 5 — *Saccharomyces casei*; 6 — *Saccharomyces cerevisiae M-5*

In all samples the content of reducing substances in the fermented whey-malt wort when using different strains of yeast did not differ substantially and ranged from 3.00 to 4.19 %, which was 20...43 % of the starting content. The obtained values confirm the low activity of enzymes that catalyze the hydrolysis of lactose. In the wort with the yeast *Saccharomyces lactis 95* we observed an almost fully completed fermentation process and the utilization of carbohydrates; the content of reducing substances is 0.8 %, which is significantly lower than that in other samples. The data acquired show that the yeast *Saccharomyces lactis 95* have the highest fermenting activity in terms of the whey carbohydrates. The fermentation of whey-malt wort with various types of yeasts demonstrated that the microorganisms actively developed over the first 12 hours of fermentation; they subsequently passed into the stationary phase of growth and completed the fermentation at hour 36.

By analyzing the fermenting activity of different strains of yeast for indicators of the content of ethyl alcohol and reducing sugars in the fermented whey-malt wort, we could conclude that the most active alcohol fermentation occurred, based on all indicators, in the wort fermented with the yeast *Saccharomyces lactis 95*, which demonstrated high capability to the utilization of lactose. The yeast *Kluyveromyces lactis 469* and *Saccharomyces cerevisiae P-87* revealed a slightly lower fermenting activity. However, the amount of the released ethanol at a level not exceeding 1.2 % by volume is sufficient according to the requirements from normative documentation regarding alcohol-free beverages (DSTU 4069:2002) to fermented beverages.

The starting concentration of yeast cells in all samples was 40 million/cm^3 of wort. The dependence of accumulation of yeast cells on cultivation temperature is given in Table 2.

Data from Table 2 confirm the significant effect of cultivation temperature on the development of the yeast cells. For the yeast *Saccharomyces casei, Kluyveromyces lactis 2452*, *Saccharomyces lactis 95*, *Kluyveromyces lactis 469*, the optimal temperature of fermentation is 30...32 °C, at which we observed the maximum accumulation of yeast – from 48 to 72 million cl/cm^3 of wort. For *Saccharomyces cerevisiae P-87* and *Saccharomyces cerevisiae M-5*, the temperature is at the level of 30...34 °C.

Table 2

Dependence of accumulation of yeast cells on the temperature of cultivation

Cultivation temperature, °C	Concentration of yeast cells in wort, million/cm^3					
	Saccharomyces		Lactose-fermenting			
	S. cerevisiae P-87	*S. casei*	*S. cerevisiae M-5*	*K. lactis 2452*	*K. lactis 469*	*S. lactis 95*
24	31	30	23	22	26	29
26	48	42	46	38	48	51
28	60	57	54	43	61	69
30	68	65	64	52	69	74
32	65	67	66	48	67	72
34	60	54	58	41	59	65
36	41	36	32	30	42	43

According to the results obtained, the future experiments to develop the whey-malt mixtures for recovery and fermentation could exploit both the lactose-fermenting microorganisms and saccharomyces. Although the lactose-fermenting yeast showed a lower result, the dynamics of the biomass accumulation is sufficient.

Thus, for the fermentation of whey-malt wort we have chosen the following strains of yeast: *Saccharomyces cerevisiae P-87* and *Kluyveromyces lactis 469,* based on the following parameters: generative ability (accumulation of yeast cells), fermenting activity (amount of ethyl alcohol and content of reducing sugars).

As is known, yeast influence not only the process of fermentation but the organoleptic estimation of fermented wort, the subsequent treatment of which leads to obtaining the beverages. At the next stage of research, we defined the organoleptic assessment of the reconstituted whey-malt mixes fermented with different strains of yeast.

The most complete and pronounced bouquet was demonstrated by sample, which was fermented with the yeast *Saccharomyces cerevisiae P-87* in combination with *Kluyveromyces lactis 469*. This sample has the most harmoniously balanced taste and the distinct refreshing aroma of rye bread with fruit tones. We observed an increase in the intensity of aroma complex, the indicator of organoleptic properties of beverages. The taste-aroma portrait of these beverages is characterized by the harmonious combination of the taste of rye malt and whey and has a sour-fruity aroma. The whey beverages fermented with the yeast *Saccharomyces casei* and *Kluyveromyces lactis 2452* differ from other samples in a barely noticeable smell of fusel oils with a note of fermentation and a poorly pronounced flavor. The character of the given profile of taste and aroma shows that a given beverage is distinguished among other samples by the specific taste of milk whey and saturation with carbon dioxide.

The results of determining the physical-chemical indicators of whey-malt wort, fermented with monocultures of the yeast *Saccharomyces cerevisiae P-87* and *Kluyveromyces lactis 469*, and when combined, are given in Table 3.

The comparative characteristics of the physical-chemical indicators of quality for the fermented whey-malt wort with the standards that are given in Table 5 testify to the highest fermenting activity of the yeast Saccharomyces cerevisiae P-87 and compliance with the indicators of quality such as the content of ethyl alcohol, 1.0 % by volume; acidity of a sodium hydroxide solution with a concentration of 1 mol/dm^3 per 100 cm^3 of beverage, 3.5 cm^3; stability of beverage at 20 °C, 5 days. The physical-chemical indicators of wort, fermented with the yeast Kluyveromyces lactis 469, are considerably lower than the standard, indicating the low activity of enzymes that catalyze the hydrolysis of lactose. The indicators for the whey-malt wort, fermented with the yeast Kluyveromyces lactis 469 + Saccharomyces cerevisiae P-87, were at the sufficient level.

Table 3

Physical-chemical indicators of whey-malt wort

Indicator	According to DSTU 4069:2002	Whey-malt wort, fermented with yeast		
		Saccharomyces cerevisiae P-87	Kluyveromyces lactis 469	Kluyveromyces lactis 469 + Saccharomyces cerevisiae P-87
Content of ethyl alcohol, % by volume, not exceeding	1.2	1.0	1.3	1.2
Acidity, cm^3 of a sodium hydroxide solution with a concentration of 1 mol/dm^3 per 100 cm^3 of beverage	3.5	3.5	3.0	3.3
Mass fraction of dry substances, %	0.3–7.5	7.3	8.2	7.5
Mass fraction of carbon dioxide, %, not less	0.3–0.4	0.3	0.3	0.3
Stability of beverage at 20 °C, days	7	7	6	7

Physiological state of yeast was assessed based on total number of cells. The growth of cells for different variations of yeast in the process of fermentation of whey-malt wort is shown in Fig. 4.

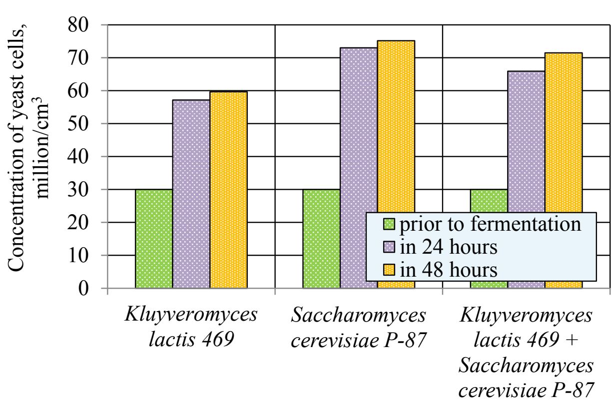

Fig. 4. Growth of the number of yeast cells during fermentation of wort with different variations of yeast strains

The results obtained indicate the advantage of saccharomyces compared with the lactose-fermenting yeasts in the growth of yeast cells during fermentation of the malt-whey wort. The non-lactose-fermenting culture *Saccharomyces cerevisiae P-87* developed well in the reconstituted mixture obviously due to the presence of the fermented rye malt with easily-metabolizing carbohydrates, and the number of microorganisms was 75.2 million/cm^3 of wort at hour 36 of cultivation. The lactose-fermenting micro-organisms accumulated the yeast cells less intensively; the number is 59.7 million/cm^3 of wort at hour 36 of fermentation. At the same time, the combination of two different strains of yeast did not positively affect the dynamics of accumulation of the biomass of microorganisms, although the number of cells increased by 11.8 million/cm^3 of wort compared with the sample fermented by the lactose-fermenting yeast. Thus, the fermentation process does not significantly intensify at the joint cultivation of yeast. No synergism between microorganisms was observed.

5. 4. Identification of fermentation by-products from the fermented whey-malt wort

To conduct a gas chromatographic study, we took the samples of whey–malt wort, fermented by the yeast strains *Saccharomyces cerevisiae P-87* and *Kluyveromyces lactis 469*, at the end of the process. Next, we obtained the distillates that characterized the content of all by-products of fermentation. The result of examination of the qualitative and quantitative composition is the identified compounds such as acetaldehyde, higher alcohols, ethers. Characteristic chromatograms acquired when determining the by-products of fermentation in the distillates of whey–malt wort, fermented with the strains of microorganisms *Saccharomyces cerevisiae P-87* and *Kluyveromyces lactis 469*, are shown in Fig. 5, 6.

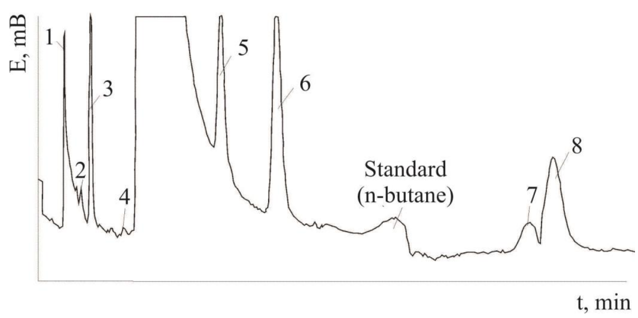

Fig. 5. Chromatogram of higher alcohols of the whey-malt wort, fermented with the yeast *Saccharomyces cerevisiae P-87*: 1 – acetaldehyde; 2 – methyl acetate; 3 – ethyl acetate; 4 – methanol; 5 – n-propanol; 6 – isobutane; 7 – 2-methyl-1-butanol; 8 – 3-methyl-1-butanol

The results of determining the content of volatile substances in the fermented whey-malt wort when using different strains of yeast (*Saccharomyces cerevisiae P-87* and *Kluyveromyces lactis 469*) are given in Table 4.

Given the data from Table 4 and the above-specified information about the threshold concentrations of basic volatile components, it could be argued that the concentrations of methyl acetate of (11.72±0.59 in) mg/dm^3 and ethyl acetate of (92.17±4.61) mg/dm^3 in the wort, fermented with the yeast *Saccharomyces cerevisiae P-87*, are optimal for the formation of a harmonious aroma of a fermented beverage. In the wort fermented with the yeast *Kluyveromyces* *lactis 469* we observed the low concentration of methyl acetate, (8.03±.0.40) mg/dm^3, compared with the threshold one; the fruity floral aroma was very weak, and, conversely, the concentrations of ethyl acetate were significantly higher than the threshold one, which gives the beverage a sharp scent of the artificial fruit essence.

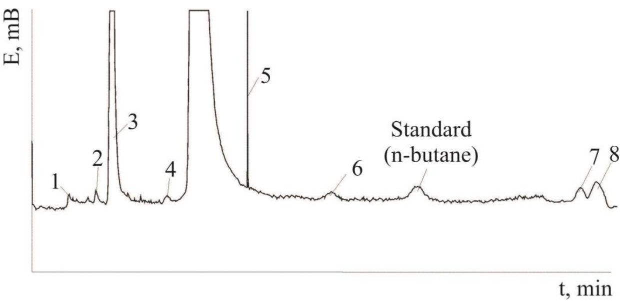

Fig. 6. Chromatogram of volatile components in the whey-malt wort, fermented with the yeast *Kluyveromyces lactis 469*: 1 — acetaldehyde; 2 — methyl acetate; 3 — ethyl acetate; 4 — methanol; 5 — n-propanol; 6 — isobutane; 7 — 2-methyl-1-butanol; 8 — 3-methyl-1-butanol

Table 4

Content of volatile substances in the fermented whey-malt wort when using different strains of yeast

Volatile component, mg/dm^3	Yeast strain	
	Saccharomyces cerevisiae P-87	Khuyveromyces lactis 469
acetaldehyde	32.05±1.60	172.48±8.62
methyl acetate	11.72±0.59	8.03±0.40
ethyl acetate	92.17±4.61	498.17±24.91
n-propanol	9.89±0.50	33.29±1.66
isobutane	27.39±1.40	32.27±1.61
2-methyl-1-butanol	52.29±2.61	6.24±0.31
3-methyl-1-butanol	207.19±10.36	17.56±0.88

Thus, in terms of the content of higher alcohols and aldehydes, the best is the wort that was fermented with the yeast *Saccharomyces cerevisiae P-87*, characterized by the low concentrations of n-propanol, (9.89±0.50) mg/dm^3, isobutane, (27.39±1.40) mg/dm^3, acetaldehyde, (32.05±±1.60 mg/dm^3), and the high concentrations of 2-methyl-1-butanol, (52.29±2.61) mg/dm^3, and 3-methyl-1-butanol, (207.19±10.36) mg/dm^3. The wort fermented with the yeast *Kluyveromyces lactis 469* is characterized by the accumulation of high concentrations of both n-propanol, (33.29±±1.66) mg/dm^3), isobutane, (32.27±1.61) mg/dm^3), acetaldehyde, (172.48±8.62) mg/dm^3), and low concentrations of 2-methyl-1-butanol, (6.24±0.31) mg/dm^3, and 3-methyl-1-butanol, (17.56±0.88) mg/dm^3), which influence the formation of the general aroma in a fermented beverage.

6. Discussion of results of studying the process of fermentation of the reconstituted whey-malt mixtures

The reported result is continuation of our study into the impact of plant components on the process of fermentation of milk whey. The lactose-fermenting yeast *Kluyveromyces lactis 469* showed during fermentation of a whey-plant wort a sufficient level of fermenting activity for the indicator of accumulation of ethyl alcohol at the level of 0.64...0.69 % by volume, which, according to the normative documentation, will suffice for fermented beverages.

Based on the results of experiments, we have developed dry mixes from rye malt and milk whey for recovery and fermentation. The selection of components of the mixtures was carried out by taking into consideration the chemical composition, solubility, and the sources of carbohydrate supply for different kinds of yeast. It was discovered that the rational ratio of dry malt to whey is 1:2.

We have selected yeast for the fermentation of the reconstituted wort: lactose-fermenting *Saccharomyces cerevisiae M-5*, *Kluyveromyces lactis 2452*, *Kluyveromyces lactis* 469, *Saccharomyces 95* – for the utilization of a carbohydrate component of milk whey (lactose); and saccharomyces *Saccharomyces casei* та *Saccharomyces cerevisiae P-87* – for the carbohydrates of rye malt. Based on such fermentation indicators as the content of ethyl alcohol and reducing sugars during fermentation of whey-malt wort malt, we have chosen the following strains of yeast: *Saccharomyces cerevisiae P-87* and *Kluyveromyces lactis 469*. The fermentation process does not significantly intensify at a joint cultivation.

The results of gas chromatographic analysis of determining the by-products of fermentation made it possible to objectively evaluate the advantages of the yeast strain *Saccharomyces cerevisiae P-87*. As shown by the experimental study, the biosynthesis of fermentation by-products could be determined as a result of regulatory functions of the yeast cell [31]. The application of the yeast strain *Saccharomyces cerevisiae P-87* for the fermentation of whey-malt wort positively affects the metabolism of the producer by stimulating the biosynthesis or transformation of aromatic substances of the nutrient environment. The fermented wort was characterized by the aroma of rye bread with fruity tones, which is important for the characteristics of beverages.

The prospects for further research relate to the selection of other strains of yeast in order to increase the ethanol content in brews, to adjust the composition of wort by introducing malts from other types of grain with a varying degree of moisture content.

The practical outcome of present research is the development of the technology of dry whey-malt mixtures for recovery and fermentation with the refined specification of technological modes to produce a beverage based on them. The use of milk whey helps enrich the ready beverage with such valuable components as protein substances, nitrogen compounds, carbohydrates, minerals, vitamins, organic acids, enzymes, macro- and microelements.

The results of our research constitute one of the directions for the comprehensive work to exploit milk whey. Implementation of technological solutions aimed at complete processing of secondary raw materials at existing equipment at enterprises is relevant for the dairy industry.

7. Conclusions

1. We have determined a degree of solubility of the components of whey-malt mixtures for recovery and fermentation. It was established that the fluctuations in the values for solubility of malt and whey are within

(1.21...1.92) cm^3, which is not critical and does not degrade the overall solubility of a whey-malt mixture.

2. We have estimated the productivity of the yeast Kluyveromyces lactis 469 at the fermentation of wort from the reconstituted mixture of whey and the fermented rye malt. It was established that the most active growth of yeast cells was observed in the fermented whey-malt wort from the reconstituted mixture at a ratio of malt to whey of 1:2.

3. The fermenting activity of different strains of yeast was investigated during fermentation of whey-malt wort based on the main physical-chemical and biochemical indicators. It was established that the most active alcohol fermentation occurred in the wort fermented with the yeast Saccharomyces lactis 95. A slightly lower fermenting activity was demonstrated by the yeast Kluyveromyces lactis 469 and Saccharomyces cerevisiae P-87. It was established that the whey-malt wort, fermented with the yeast Saccharomyces cerevisiae P-87+Kluyveromyces lactis 469, had the most harmoniously balanced taste and the distinct refreshing aroma of rye bread with fruit tones.

4. The results from a gas chromatographic analysis for determining the volatile substances in the fermented whey beverages made it possible to objectively estimate the advantage of the yeast strain Saccharomyces cerevisiae P-87. It was established that the concentrations of methyl acetate, (11.72±0.59) mg/dm^3, and ethyl acetate, (92.17±4.61) mg/dm^3, in the wort fermented with the yeast Saccharomyces cerevisiae P-87, are optimal for the formation of a harmonious taste and aroma of the fermented beverage.

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