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BIOTECHNOLOGICAL ASPECTS OF FERMENTED DRINKS PRODUCTION ON VEGETABLE RAW MATERIALS

Abstract. Fermented drinks are considered as the optimal form of a food product, used to enrich the diet with biologically active substances that have a beneficial effect on the metabolism and immunoresistance of the human body, which are provided not only by extraction, but also are formed as a result of the vital activity of various types of microorganisms. The latter, in turn, produce vitamins, amino acids, organic acids, etc., which gives fermented drinks a higher biological value. Drinks technology with the use of tea fungus (Medusomyces gisevii) is oriented for home cooking and it is not possible to apply it in an industrial environment. The relevance of this research work is to develop the technology of the drink, enriched with biologically active substances in the most accessible form to expand the range of functional beverages aimed at maintaining the health of various population groups. In order to solve this problem a combined yeast and lactic acid bacteria ferment was chosen, the optimal composition of the nutrient medium based on green tea and barley malt wort was determined, and the amino acid and vitamin activator was selected to reduce the fermentation process. The developed technology made it possible to obtain a drink with mixed leaven, harmonious in organoleptic characteristics, with a high content of amino acids, especially essential ones, which made it possible to recommend this functional drink for various groups of the population.

Keywords: tea, wort, microorganism cultures, yeast food, secondary fermentation products.

Introduction. Drinks are rightfully considered to be the most rational form of a food product for enriching the diet with biologically active substances and creating special-purpose products [1, 2]. The high popularity of beverages among the population suggests the constant development of technical, methodological, technological bases, including in the framework of the fight against fraud [3-7]. In recent years, the problem of their excessive consumption is actualized [8].

One of the priorities of the state policy of the Russian Federation in the field of healthy nutrition is the development of fermentation drink technologies, based on the use of plant raw materials and microorganism cultures, that produce a complex of substances with significant potential of useful properties [1]. According to many scientists, the use of fermentation drinks contributes to the correct metabolism and strengthens the immunoresistance of the human body, which are formed as a result of microorganism vital activity [1, 2, 9-11, 26].

One of the urgent tasks in the production of fermented drinks is the search for fermentation microorganisms, producing biologically active substances, which are necessary for the normal functioning of mankind during their life. The latter are most fully accumulated in the case of the symbiotic cultures use, which can be a single organism (for example, *Medusomyces gisevii*) or a cultures combination (for example, yeast and lactic acid bacteria) [1].

Literature review. The drink, obtained with use of *Medusomyces gisevii*, which consists of yeast cells and acetic acid bacteria, contains glucuronic, folic and citric acids, vitamins B_1 , B_2 , B_3 , B_6 , B_{12} , as well as difficult to determine substances with antibiotic action, including medusin. The viability of using

bacterial cellulose, obtained from *Medusomyces gisevii* as a hemostatic agent in veterinary medicine and humane medicine was confirmed [12]. A necessary condition for the life of this microorganism is the presence of tea in the raw materials composition.

Analysis of scientific and technical information suggests, that the creation of industrial technology, based on the use of cultures of microorganisms with known properties, that allow to control the process of drink production, is promising and relevant. At the same time, the conditions for obtaining a homemade drink described in the literature and patents for a method for producing a drink, based on tea fungus and attempts to create analogues with *Medusomyces gisevii* have not been continued [13-16].

Methods. To assess the effectiveness of technological methods, research methods used in the industry were used: determination of the volume fraction of alcohol - GOST 12787-81, the arbitration method, which is the separation of the alcohol fraction of a drink from extractive substances by distillation [17]; determination of titratable acidity - GOST 12788-87, which is a method for the titration of the acid content by neutralizing them with a 0.1 sodium hydroxide solution [18]; determination of the mass concentration of volatile components - a gas chromatography method with the Kristall 5000.1 flame ionization detector ("Khromatek", Russia) equipped with an automatic information collection and processing system [19]; determination of the mass concentration of organic acids – high-performance liquid chromatography with an "Agilent Technologies 1200" ("Agilent", USA) diode array detector [20]; determination of the mass concentration of sugars and glycerol - a method of high-performance liquid chromatography with a refractometric detector "Agilent Technologies 1200" ("Agilent", USA), equipped with an automatic system for collecting and processing information [21]; determination of the mass concentration of amino acids is a high performance liquid chromatography method with an "Agilent Technologies 1200" ("Agilent", USA) diode array detector, equipped with an automatic information collection and processing system [22].

The replication of experiments at all stages of the experiment - not less than 3. The results of experimental studies were processed by methods of mathematical statistics using Student's criterion.

In order to conduct a study on the production of fermented drinks, the following materials were used: barley malting brewing malt; brewing tricyclic malt light; black tea and green leaf; mixed ferment on the basis of dry brewing yeast S. cerevisiae and lactic acid bacteria *Betabacterium breve*.

Results. As part of the study, the following tasks were set: to investigate the possibility of using various plant raw materials and mixed cultures of microorganisms traditional for the brewing and non-alcoholic industries in the technology of fermented drink; select the optimal fermentation conditions; get a drink with stable performance over a long shelf life without loss of compounds, responsible for the functional orientation of the finished drink.

To identify the optimal composition of the nutrient medium, allowing to obtain a harmonious drink from the point of organoleptic and physicochemical parameters view, provided that yeast and lactobacilli are used, the following plant materials were used in the study: leaf tea (black and green), barley based wort and tritium-potassium malt brewing, as well as granulated sugar, in various ratios, and the fermentation was carried out by making mixed yeast brewer's yeast and lactic acid bacteria d at the rate of 4% by volume of the nutrient medium.

During the experiments, the duration of fermentation, controlled by loss of dry substances, using black and green tea coincided and amounted to 141 hours with barley malt wort and 114 hours with triticali wort, which is explained by the rich amino-acid composition of triticali malt wort, which accelerates enzymatic processes associated with the activity of yeast. However, the organoleptic evaluation of all analyzed samples using a 25-point system showed, that the most balanced indicators were fermented drink using a carbohydrate-tea base (based on green tea) and barley malt wort, in particular, a variant of the nutrient medium, based on green tea and wort from barley malt in a ratio of 75% and 25%, the data are presented in table 1.

In all other samples, the alcohol content and acidity were at 5–10% higher than the optimal sample, but an autolysis tone was present, which is unacceptable.

However, the duration of cultivation was quite long, so the next stage of our work was the use of various activators to shorten the process. For this purpose, the most productive amino acid-vitamin activator (AVA), obtained according to the technology [23] and containing 40-42% of soluble protein, including 13-15% of amine nitrogen, and vitamins (thiamine (B₁), pantothenic acid (B₃), pyridoxine (B₆), biotin (B₇), inoside (B₈) - 17 mg/kg. The share of AVA was 2.5-10 ml/hl or 0.5-2% by volume of the fermented wort (table 2).

Indicators

Characteristics of Fermented Green Tea and Barley Malt Wort

Organoleptic Indicators

Taste

fermented drink with slightly sour

Colour

straw

Aroma

pleasant aroma of a well fermented fruit drink

Physicochemical Indicators

Alcohol content, vol.%

3,30±0,2

Acidity, a.u.

11,00±0,3

Table 1 – Organoleptic and physicochemical indicators of the fermented wort optimal variant

Table 2 – Applied feedings doses for fermentation

	Nutrient medium variant					
Indicator name	Control	with Feedings of AVA, ±0,05				
	I	II	III	IV	V	
Applied feedings doses, %	-	0,5	1	1,5	2,0	

The dose of dressing with the use of selected nutrient medium (75% of tea and 25% of barley malt wort) is presented in table 2, the dynamics of acidity accumulation during fermentation - in figure 1, the content of secondary fermentation products – in the table 3. It should be noted that we previously studied other supplements, but they did not show any tangible effect [24].

Table 3 – The Content of Organic Acids (OA), Sugars and Glycerin in the Drink

Component	Content, mg/dm ³	Component	Content, mg/dm ³	Component	Content, mg/dm ³	
OA±5,0%	**	Amino Acids ± 5,0%**				
Oxalic acid	0,070	Aspartic	27,0	Tryptophan*	25,5	
Wine acid	0,104	Glutamic	29,2	Isoleucine*	36,0	
Malic acid	0,303	Asparagine	36,8	Phenylalanine*	17,5	
Lactic acid	4,614	histidine*	20,0	Leucine*	65,0	
Citric acid	0,147	Serine	48,0	Lysine*	5,0	
Succinic acid	0,257	Glutamine	22,0	Alanine	22,0	
The amount of volatile acids, in terms of acetic	0,18±2%	Arginine*	17,5	Tyrosine	16,8	
		Threonine*	126,5	Valine*	41,0	
		Glycine	40,0	Methionine*	49,0	
Sugars and Glycerin ± 4,0%**						
Fructose	10,480	Glucose	5,310	Sucrose	2,7000	
* - irreplaceable amino acids; ** - method reproducibility.						

The data in table 3 show, that the resulting drink had a wide range of functional compounds, responsible for functional orientation, and more significant accumulation of lactic acid occurs, as compared with the control, since the lactic acid bacteria Betabacter breve was used during fermentation. Sugar and glycerin in the finished drink is formed less than in the control, which is explained by a more intensive activity of microorganisms that consume sugar for reproduction. The composition of amino acids are also essential in 2 times more, than the control, which is explained by the most active metabolism of microorganisms.

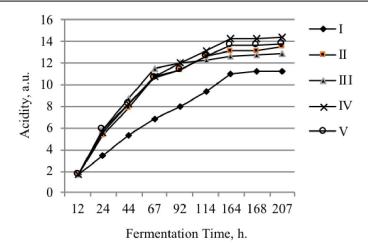


Figure 1 – Dynamics of acidity accumulation in the samples when adding feedings to the nutrient medium during fermentation

Comparative studies have been carried out on fermented drink with use of tea fungus and mixed teabased leaven.

The composition of volatile compounds and sugars are presented in tables 4, 5. It should be noted that the higher alcohols in the resulting drink, compared with the drink on the tea fungus, accumulate much more (except for l-propanol), and the content of esters is not so different.

Table 4 – Content of volatile compounds, organic acids and sugars in drink with use of the microorganism medusomyces gisevii

Component	Content mg/dm ³	Component	Content mg/dm ³	Component	Content mg/dm ³	
Ethers, $mg/dm^3 \pm 10\%$ **	Amino Acids ± 5,0%**					
Ethyl acetate	2,89	Aspartic	10,9	Valine*	1,44	
Ethyl lactate	5,79	Glutamic	1,8	Methionine*	-	
Organic Acids, g/dm ³ ± 5,0%**		Аспарагин	Asparagine	Трипто-фан*	Tryptophan*	
Oxalic acid	_	histidine*	0,8	Isoleucine*	-	
Wine acid	-	Serine	1,6	Phenylalanine*	2,0	
Malic acid	0,021	Glutamine	_	Leucine*	0,9	
Lactic acid	0,059	Arginine*	1,0	Lysine*	0,8	
Citric acid	0,0083	Glycine	1,2	Tyrosine	-	
Succinic acid	0,061	Threonine*	4,5	Totally Irreplaceable	11,4	
		Alanine	1,3			
Sugars, $mg/cm^3 \pm 4.0\%$ **		•	•	•		
Fructose	3,39	Glucose	2,74	Sucrose	17,33	
* - irreplaceable amino acids	; ** - method re	eproducibility.				

According to the content of amino acids, more intensive accumulation occurs in the drink with use of mixed yeast leaven and lactic acid bacteria, including irreplaceable bacteria, accumulates 36 times more compared to the drink on the tea fungus.

Component	Content, mg/dm ³	Component	Content, mg/dm ³	Component	Content, mg/dm ³		
Ethers, mg/dm ³ ±10%**	Ethers, $mg/dm^3 \pm 10\%$ **		Amino Acids ± 5,0%**				
Ethyl acetate	1,3	Aspartic	27,0	Valine*	41,0		
Ethyl lactate	3,3	Glutamic	29,2	Methionine*	49,0		
Organic Acids, $g/dm^3 \pm 5.0\%$	Organic Acids, $g/dm^3 \pm 5,0\%$ **		36,8	Tryptophan*	25,5		
Oxalic acid	histidine*	histidine*	20,0	Isoleucine*	36,0		
Wine acid	0,373	Serine	48,0	Pheny lalanine*	17,5		
Malic acid	4,83	Glutamine	22,0	Leucine*	65,0		
Lactic acid	0,059	Arginine*	17,5	Lysine*	5,0		
Citric acid	0,12	Glycine	40,0	Tyrosine	16,8		
Succinic acid	0,331	Threonine*	126,5	Totally Irreplaceable	403		
		Alanine	1,3				
Sugars, $mg/cm^3 \pm 4.0\%$ **							
Fructose	19,9	Glucose	11,97	Sucrose	2,36		
* - irreplaceable amino acids; ** - method reproducibility.							

Table 5 – Content of volatile compounds, organic acids and sugars in the drink with use of mixed yeast leaven and lactic acid bacteria

Particular attention should be paid to the threonine amino acid, which accumulates 3 times more than in the drink with tea fungus (table 4, 5). From the point of view of the functional load, threonine is very useful for the body, since it is responsible for the protein metabolism in the body, is part of collagen, elastin and tooth enamel protein, positively affects the person's psycho-emotional state [25]. We assume that this drink, obtained by us with the help of a mixed leaven, can be recommended for a wide range of the population of Russia.

The third task to get a drink with stable performance over a long shelf life without losing the compounds, responsible for the functional orientation of the finished drink was solved using pasteurization techniques at a maximum processing temperature of 72 °C, due to which it was possible to extend the shelf life of the drink for 6 months.

Analysis of the secondary fermentation products in mixed leaven in a drink showed, that their concentration differs from that in a drink made on the basis of a tea fungus, which is explained by a different metabolic set of enzymes in two cases.

On the basis of the data, obtained in the study, the optimal organoleptic and physicochemical indicators of fermented drinks, prepared from pasteurized fermented bases were determined, which made it possible to develop a draft regulatory documentation for fermented drinks on plant raw materials.

Conclusions. Studies have allowed picking up planting materials and microbiological cultures, working out the technological parameters of the process, including fermentation modes - the optimal composition for the drink technology was the ratio of green tea extract and barley malt wort 3:1, as well as the use of an additive ava in the amount of 1%, which made it possible to reduce the fermentation time to 93 hours and to obtain a drink with high organoleptic and physicochemical parameters.

On the basis of a comparison of the drink we developed, with based on tea fungus drink, at the main functional components, it was concluded that the content of amino acids was 4 times higher than the total amount and 3 times threonine, which is probably the basis recommend a drink for food of various groups of the population of the Russian Federation.

Discussion. The conducted literary search on the studied problem revealed a diverse composition of vegetable raw materials in order to apply it in the drink technology. This type of raw material is traditionally used in fermented drinks, based on the microorganism *Medusomyces gisevii* [1-3, 18]. However, this microorganism did not find wide application in industry due to insufficient knowledge of catabolic reactions.

Therefore, we were faced with the task of developing a technology for a drink, that would be an analogue of the "Kombucha" drink. For this purpose, were used yeast and lactic acid bacteria, traditionally used in the production of kvass.

The use of plant materials, in particular tea, and barley malt wort and mixed microbiological cultures, made it possible to obtain a fermented drink enriched with functional compounds such as organic acids, amino acids, ethers and other volatile compounds in quantities exceeding drinks with use of tea fungus culture. This fact is explained by the difference in the metabolism and the set of enzymes of the two microorganism cultures, since the in technology were used lactic acid bacteria together with yeast, which is characterized by its own set of secondary products of metabolism compared to the culture of *Medusomyces gisevii* [19].

The obtained data on the composition and content of alcohols in the drink confirm the correctness of the choice of the malting wort as a source of nitrogenous nutrition for yeast, since it is known that it is the amino acids, that participate in the formation of higher alcohols as a result of catabolic reactions. The use of yeast and lactic acid bacteria, as well as a balanced plant substrate (tea and malt wort) made it possible to obtain a harmonious drink, rich in functional compounds. Accelerated fermentation metabolism was promoted by the use of an amino acid activator in the above-mentioned concentration, since the activator influenced the growth and reproduction of yeast. This is of great importance, since the duration of fermentation has decreased to 93 hours. The use of pasteurization techniques has made it possible to increase the shelf life without loss of useful compounds.

This fact allows to introduce this technology in the enterprises of the beer and non-alcoholic industry, since the microorganisms used are well studied and their livelihoods can be regulated, that is, they are technological.

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ӨСІМДІК ШИКІЗАТЫН ҚОЛДАНЫП АШЫТЫЛАТЫН СУСЫНДАР ӨНДІРІСІНІҢ БИОТЕХНОЛОГИЯЛЫҚ АСПЕКТІЛЕРІ

Аннотация. Ферменттелген сусындар биологиялық белсенді заттармен тағам рационын байыту үшін пайдаланылатын тамақ өнімдерінің оңтайлы түрі болып табылады, олар адам ағзасының зат алмасуына және иммундык тұрақтылығына оң әсерін тигізеді, олар экстракция арқылы ғана емес, сондай-ақ түрлі микроорганизмдердің өмірлік белсенділігі нәтижесінде пайда болады. Олар өз кезегінде витаминдерді, аминқышқылдарын, органикалық қышқылдар және тағы басқа заттарды түзеді, бұл ферменттелген сусындардың биологиялық құндылығын арттырады. Шай саңырауқұлағы (Medusomyces gisevii) қолданылған сусындардың технологиясы үйде алуға арналған және оны өнеркәсіптік жағдайда қолдану мүмкін емес. Осы ғылымизерттеу жұмысының өзектілігі әртүрлі халық топтарының денсаулығын сақтауға бағытталған функционалдық сусындардың ауқымын кеңейту үшін барынша қолжетімді түрде биологиялық белсенді заттармен байытылған сусынның технологиясын жасау болып табылады. Бұл мәселені шешу үшін аралас ашытқылар мен сүт қышқылдары бактерияларының ұйытқысы таңдап алынды, көк шай және арпа уыты негізіндегі қоректік ортаның оңтайлы құрамы анықталды, аминқышқылы мен витаминді активатор ашыту үрдісін азайту үшін таңдалды. Алынған технология құрамында ауыстырылмайтын амин қышқылдарының көп мөлшері бар, органолептикалық көрсеткіштері үйлесімді аралас ашытқы негізіндегі сусын алуға мүмкіндік берді, бұл халықтың түрлі топтары үшін осы функционалдық сусындарды ұсынуға мүмкіндік берді.

Түйін сөздер: шай саңырауқұлағы, арпа уыты, ашытқылар мен сүтқышқылды бактериялардың біріктірілген ұйытқысы.

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БИОТЕХНОЛОГИЧЕСКИЕ АСПЕКТЫ ПРОИЗВОДСТВА НАПИТКОВ БРОЖЕНИЯ С ПРИМЕНЕНИЕМ РАСТИТЕЛЬНОГО СЫРЬЯ

Аннотация. Напитки брожения рассматриваются как оптимальная форма пищевого продукта, используемого для обогащения рациона питания биологически-активными веществами, благотворно влияющими на обмен веществ и иммунорезистентность организма человека, которые обеспечиваются не только за счет экстракции, но и формируются в результате жизнедеятельности различных видов микроорганизмов. Последние, в свою очередь, продуцируют витамины, аминокислоты, органические кислоты и др., что и придает ферментированным напиткам повышенную биологическую ценность. Технология напитков с применением чайного гриба (Medusomyces gisevii) предназначена для домашнего приготовления и не представляется возможным применить ее в промышленных условиях. Актуальность данной исследовательской работы заключается в разработке технологии напитка, обогащенного биологически активными веществами в наиболее доступной форме для расширения ассортимента функциональных напитков, направленных на поддержание здоровья различных групп населения. С целью решения данной задачи была выбрана комбинированная закваска дрожжей и молочнокислых бактерий, определен оптимальный состав питательной среды на основе зеленого чая и ячменного солодового сусла, для сокращения процесса брожения был подобран аминокислотно-витаминный активатор. Разработанная технология позволила получить напиток на смешанной закваске, гармоничный по органолептическим показателям, с повышенным содержанием аминокислот. особенно незаменимых, что позволило рекомендовать этот функциональный напиток для различных групп населения.

Ключевые слова: чайный гриб, сусло, комбинированная закваска дрожжей и молочнокислых бактерий.

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