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1. FOOD SCIENCE AND TECHNOLOGIES

USING OF IMMOBILIZED BEER YEAST FOR BREWING BEER WORT

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Abstract: *The article considers the possibility of using immobilized beer yeast of germinal fermentation, included in the matrix of polyvinyl alcohol gel, in the production of craft beer in restaurants. Methods of immobilization of microorganisms and carrier selection are analyzed. The efficiency of inclusion of cells in the gel is achieved with the optimal combination of pore size gel cells of the microorganism (yeast) and optimization of the microenvironment of the cell. In order to increase the mechanical strength of the carriers and to hold back the cells included in them, matrix processing with bifunctional cross-linking reagents that are able to interact with the functional groups that are part of the cell's shell is used. The advantage of the method are its simplicity, the possibility of obtaining preparations in any form (spherical particles, films, rolls, etc.), versatility, that is, the possibility of using any biologically active substances for the immobilization. The obtained samples of immobilized cells are stable, since they are protected by the gel from adverse external influences, including from bacterial contamination, because large bacterial cells can not penetrate into a small porous matrix. The advantages of using of polyvinyl alcohol as a carrier in comparison with other substances used for immobilization and the advantages of the method of immobilization of incorporation into a matrix structure differing in the strength of cell fixation have been established. Chemical methods of cell immobilization are not widely used. They are based on the formation of covalent bonds with the activated carrier, on the transverse crosslinking of cells due to active groups of amino acids and other compounds in the shell of cells with bifunctional reagents, which, in turn, have a toxic effect on cells, reducing their livelihoods. The prospects of application of immobilized cells in the processes of fermentation in obtaining alcohol-containing products are determined. The research of the process of main brewing of beer wort was carried out and a comparative characteristic of a qualitative indicator determined by the visible degree of fermentation, between a sample with the using of immobilized yeast and a sample with a direct yeast injection was carried out. Conclusions in favor of the using of the digestion method with the using of immobilized cells, which provides significant savings in material resources (yeast) compared with the classical technology of beer production are made. The proposed technology makes it possible to significantly reduce the cost of the process of lighting and filtering craft beer and kvass during production in restaurants, and to save material resources, since the use of yeast in immobilized form increases the multiplicity of their application.*

Keywords: kraft beer, immobilized beer yeast, polyvinyl alcohol, degree of fermentation.

Formulation of the problem and its relation to the most important scientific and practical tasks.

Beer has accompanied humanity throughout the history of civilization, that is, for about ten thousand years. In such a long time, much has changed in brewing technology. Beer production technologies are constantly being refined, especially for craft brewers, who seek not only to creatively revive forgotten technologies but also to innovate [1].

In recent years, new technologies have been introduced into beer production technology that allow to intensify production processes, and especially the most long-lasting ones - fermentation.

The tendency to accelerate the fermentation process has led to new methods of brewing wort - continuous, periodic in large capacity apparatus, fermentation by immobilized yeast.

The use of fermenters with immobilized yeast in the production of craft beer promises to increase and optimize the output of the finished product per unit volume of the column for fermentation of wort, which in this case becomes a continuous reactor, reducing current costs and automating the technological process of major production reconstruction costs. However, the benefits of yeast colonization by porous inorganic matrix carriers or stimulating their attachment to the modified carrier surface are still under discussion [2].

Immobilization of cells is understood as a restriction on the freedom of their physical movement in space [3], a material mediator that limits mobility is a carrier, and the resulting system of cells - a carrier is called an immobilized biocatalyst. Immobilization of cells provides the opportunity to create biotechnological processes of long-term use with high yield of the target product, with the technical solution of such processes is significantly simplified compared with the processes based on free cells [4].

Immobilization of microbial cells and enzymes is a widespread phenomenon in nature that plays an important role in the strategy of survival and preservation of the maximum number of catalytic functions. In nature, cells, as a carrier, are used insoluble materials to which cells are attached in real conditions (for example, wood, minerals, etc.). In this case, the functioning of the cell in the immobilized state occurs natively [3].

Immobilization helps to stabilize the enzymatic activity of cells, in principle increase the volume productivity, prolong the duration of enzymes inside and outside the cell, reduce the time of the process, achieve a more efficient technological transformation of the product (in our case, the transformation of beer wort into beer).

The use of insoluble carriers to immobilize cells allows to obtain a microbially quality product upon repeated application, and it is not technically difficult to separate the obtained products from the biocatalyst [4].

There are two ways to immobilize enzymes and cells: physical and chemical methods. In physical methods, immobilization is achieved without the formation of covalent bonds between the matrix and the enzyme or cell of the microorganism. There are two subgroups of methods: 1) adsorption on insoluble carriers; 2) methods of inclusion in the matrix of the carrier - inclusion in the gel; microencapsulation;

inclusion in hollow filaments; in liposomes; inclusion in a medium with soluble enzymes or microorganisms. Immobilization by adsorption on insoluble carriers involves the interaction of the enzyme solution with a matrix of organic and inorganic nature. The interaction in this method is due to van der Waals forces, electrostatic interactions, hydrogen bonds and hydrophobic interactions of the carrier and proteins in the enzyme or microorganisms. Chemical methods of immobilization are based on the covalent bonding of molecules of a microorganism or enzyme. A positive feature of the immobilized enzymes obtained by the chemical method is their resistance to desorption, they are not "washed away" [5].

One way to increase productivity in biotechnological processes in food production is to use immobilized cells. Immobilized cells retain technological function, both immediately after immobilization and after a long period of use in biotechnological processes.

An important characteristic of immobilized cells, which distinguishes them from free cells, is prolonged functional activity [6].

Beer production technology requires significant financial costs for the purchase of yeast, which must be constantly updated after ten-fold generation. The use of immobilized yeast makes it possible to significantly reduce the cost of the technological process of beer and kvass production. The use of immobilized yeast at the stage of fermentation is promising, but at present such technology is under development.

Analysis of recent research and publications. The use of immobilized yeast is widely researched by both domestic scientists and foreign scientists. According to G.A. Ermolaeva [8], the advantages of using such technology are obvious:

- increasing the capacity of the main fermentation and fermentation areas, reducing the area for installation of equipment;
- the ability to create conditions for complete fermentation of sugars, the possibility of stabilizing the quality of finished products;
- complete separation of the fermented substrate from the cells of microorganisms, facilitates such a resource-intensive process as filtration;
- increasing the resistance of the yeast to the adverse effects of increased concentration of alcohol;
- the intensity of breeding of yeast cells decreases and as a consequence, reduction of waste;
- reducing resources (man-hours) while working with yeast;
- the possibility of using yeasts (to produce excellent organoleptic characteristics), regardless of their agglomeration capacity;
- the possibility of automation of the fermentation process.

The analysis of researches of scientists [7] shows that the search for effective methods of immobilization and carriers for fixing on them cells, is based on the methods of immobilization:

- adsorption of microorganisms on the surface of inert carriers;
- inclusion in the gel (carriers are calcium alginate, gelatin, carrageenan, agarose, chitosan, pectin, polyacrylamide, etc.);
- covalent bonding;

- membrane microencapsulation of microorganisms.

Each of these methods has its advantages and disadvantages.

Of most practical interest are three methods of immobilization - incorporation into the matrix, adsorption method and colonization by porous metal microorganisms.

In the scientific work [8], a method of incorporating low-fermentation brewer's yeast into the polyvinyl alcohol matrix was applied, so let us consider this method in more detail.

The essence of this method of immobilization is that the yeast cells are incorporated into a three-dimensional mesh of closely intertwined gel-forming polymer chains. The average distance between adjacent chains in the gel is smaller than the size of the included yeast cell, so the cell can not leave the polymer matrix and exit to the surrounding solution, ie in an immobilized state. Additional contribution to cell retention in the gel network can also be made by ionic and hydrogen bonds between the yeast cell and the surrounding polymer chains.

The peculiarity of the creation of polyvinyl alcohol cryogels is the presence of pores with an average size of 0.18-0.26 microns. The consequence of pore formation during the melting of crystals of the pore-forming agent (ice), which limits their size, is approximately the same pore size in the resulting gel. Thus obtained carrier does not create additional diffusion contamination for soluble substances [9].

Polymeric cryogels are called jelly-like materials formed in shallow-frozen solutions of polymeric or monomeric precursors. Shallow-frozen systems are systems at temperatures not lower than a few tens of degrees from the freezing point of a pure solvent. Such systems are usually two-phase, they contain solid phase polycrystals (porogens) and a small volume of non-crystallized liquid microphage where solutes concentrate and cryogel matrix is formed [9].

Polyvinyl alcohol (PVA) cryogels are formed as a result of freezing of concentrated PVA solutions, keeping them frozen for a certain time and thawing. The formation of the cryogel is based on the structuring of the polymer through the formation of hydrogen bonds. Immobilization of yeast into PVA cryogels includes: biomass accumulation to deep stationary growth phase, preparation of cell suspension in polymer solution, freezing in hydrophobic fluid, thawing.

This method is characterized by sufficient cell fixation strength. In this case, the cell is placed in a polymeric mesh, into which the molecules of the substrate molecule (wort of beer) enter.

Natural biopolymers are also used to create cryogels - polysaccharides with a carboxyl group (alginate, carrageenan, pectin) or with an amino group (chitosan).

Gels are obtained from these carriers. Gels obtained during polymerization are not sufficiently resistant to mechanical influences, but inert to the substrate components and products of cell metabolism.

To obtain immobilized yeast cells in gel, they are first suspended in a solution of sodium alginate. This suspension is then sprayed with a solution of calcium chloride. The resulting granules have an insoluble layer of calcium alginate with the properties of a semipermeable membrane. But over time, the mechanical friction of the pellets between the frame layer is erased and this impedes the industrial application of such media.

A good alternative to polyacrylamide gels is polyvinyl alcohol hydrogels. They are obtained by freezing concentrated solutions of alcohol, which are highly resistant to corrosive solutions, non-toxic and mechanically resistant [10].

In [11] it is shown that immobilized cells included in the PVA cryogel have high fermentation activity, can be reused in the processes of alcoholic fermentation.

Saccharomyces cerevisiae cells immobilized in PVA become nearly twice as resistant to ethanol. Fermentation in immobilized cells occurs 1.5 times faster than in free cells [12].

The purpose of the study is to investigate the process of main fermentation of beer wort with immobilized yeast included in polyvinyl alcohol gel and to compare, by determining the apparent degree of fermentation, the workability of the proposed fermentation technology by classical technology, which involves direct contact with the yeast.

Materials and methods of research. The studies were conducted at the Department of Technology of Restaurant and Wellness Nutrition and the Department of Bioengineering and Water of ONAFT (Odessa, Ukraine).

The work used polyvinyl alcohol in the dry granular form of the brewer's yeast of fermentation of Saccharomyces cerevisiae lyophilically dried, and a beer wort made by infusion method using barley malt from Weyermann Munich. This method is very widely used in the manufacture of craft beer in brewery restaurants.

Immobilization of yeast was performed according to the developed method: to 100 ml of 10% aqueous solution of polyvinyl alcohol was added 5 g of yeast in dry form, with constant stirring until complete dissolution of the yeast and their uniform distribution in PVA solution (30 minutes).

To obtain the PVA solution, 10% of dry polyvinyl alcohol and 90% of distilled water were taken. The water was heated to a temperature of 74 ° C and polyvinyl alcohol was added, then thoroughly stirred until complete dissolution (gelation). The solution was cooled to a temperature of 40-45 ° C and added to the yeast in the amount indicated above. To form a work surface and increase contact between the carrier immobilized yeast and the beer wort, the obtained liquid gel with the included beer yeast was applied with a brush on sterile cotton carrier in the form of a tape (bandage), 400 x 150 mm, which was placed on the surface of the glass. The resulting structure was transferred to a freezer at minus 24 ° C and frozen for 24 h and then thawed, followed by re-freezing and thawing. They removed the structured ribbon of cryogel from glass and gave it the shape of a roll in order to be placed in a glass tube in the form of a diopter. After washing with water, 10 liters of beer wort was passed through the diopter and the main fermentation was carried out at a temperature of 5-7 ° C for 8 days.

During the fermentation process, the wort was determined by the apparent degree of fermentation and compared with the classical fermentation process.

Preparation of the wort by infusion involves the following technological operations [13]: - grinding malt with water at a temperature of 37-40 ° C, stirring for 20-30 minutes;

- raising the temperature to 50-52 ° C, pausing (protein) for 20 min without stirring;

- raising the temperature to 62-64 ° C with a speed of 1 ° C per min, withstand 10-30 min (maltose pause);

- raising the temperature to 70-72 ° C with stirring for the final saccharification of the mash;

- determination of the end of the process is determined by one sample;

- Sugar mash is heated to 75 ° C and filtration is carried out;

- boil congestion with hops to a solids content of 12% for about 1.5 hours;

- cooling the mash to a fermentation temperature of 5-7 ° C;

- for the 1st (1st) sample: the introduction of beer yeast grass bottom fermentation in the amount of 2.5 g / dal;

- for the 2nd sample: passing the wort through immobilized yeast;

- main fermentation: temperature 5-7 ° C, to achieve a visible degree of fermentation (attenuation) 65-67,5%.

The main fermentation takes place in several stages, which differ in appearance of the surface of the wort, which is fermented, as well as in the change of the extractivity and the degree of lightening of the young beer.

In the first stage of fermentation, which is called whitewash, a strip of white foam appears on the surface of the wort. This stage lasted for 1.5 days. The extractivity of the wort is reduced.

The second stage of fermentation - the period of low curls is characterized by a more intense release of carbon dioxide, the formation of thick compact foam. The foam, initially white, and then gradually darkened due to the oxidation of hop pitches. Extractivity is reduced by 1% per day. The duration of stage 2 days.

The third stage of fermentation - the stage of high curls is characterized by the highest intensity of digestion, the maximum process temperature. Extractivity drop from 1 to 1.5% per day. The foam becomes flaky, voluminous, the curls reach the highest value, the upper curls are brown, the bottom is white, the pH is lowered. The stage lasts 3-4 days. At the beginning of this stage, the wort was cooled.

In the fourth stage, called the curl fall or the formation of the deck, the foam drops, the curls disappear, leaving the wort surface covered with a thin layer of deck. Curls last for 2 days. The extractivity of the wort is reduced and fermentation is stopped.

During the main fermentation, most of the extractive substances are converted into fermentation products. The progress of this process is controlled by the degree of fermentation. Distinguish between visible and true degree of origin. If the content of the extract in beer is determined in the presence of alcohol and carbon dioxide, it is a visible extract. Using these values determine the apparent degree of digestion. The actual degree of fermentation is found after the removal of alcohol and carbon dioxide by pycnometric method (according to the relative density of the wort or beer).

After each day, the apparent degree of main fermentation was determined according to the sugar meter. The apparent degree of fermentation is an indicator that determines the ratio of visible beer extract to the extractability of the initial wort expressed as a percentage.

In the wort with a solids content of 12% sugar, in the first sample was made dry yeast grass bottom fermentation in the amount of 2.5 g / dal, the second sample was

passed through immobilized yeast. The contact time of the wort with the immobilized yeast was 7 s. Before making the yeast, the wort was aerated to saturate it with oxygen.

The fermentation process was carried out at a temperature of 5-7 ° C, in a tank with air access, for (for) 8 days. For such a wort, the degree of fermentation should be 65-67.5%. The apparent degree of fermentation (attenuation) was determined by the formula:

$$V = C_2 \cdot 100 / C_1$$

here, C_1 - is the concentration of solids in the initial wort,%;

C_2 - is the difference between the dry solids concentration of the initial wort and the dry solids concentration of the wort,%.

Results of the study and their discussion.

The results of the study are shown in the table.

Table 1 - Results of experimental studies of the kinetics of the apparent degree of digestion of beer wort 12%

Fermentation time, days	Visible extract by sugar meter,%		The apparent degree of fermentation,%		pH	
	1 sample	2 sample	1 sample	2 sample	1 sample	2 sample
Before fermentation	12	12	-	-	5,4	5,4
The first one	11,8	11,9	1,7	0,83	5,2	5,3
Another	10,9	11	9,2	8,3	5	5,1
Third	8,6	8,8	28,3	26,7	4,75	4,77
Fourth	7,1	7,2	40,8	40	4,62	4,63
Five	5,6	5,8	53,3	51,7	4,63	4,65
Sixth	4,9	5	59,2	58,3	4,48	4,48
Seventh	4,5	4,6	62,5	61,7	4,42	4,45
Eight	4,1	4,2	65,8	65	4,42	4,45

On the eighth day the apparent degree of fermentation of the 1st sample was 65.8% and the 2nd sample 65%. The results obtained fit into the interval of the degree of fermentation for this type of wort (65-67.5%).

The degree of fermentation of the beer determines a large number of its final properties after the fermentation process, which include the taste sensation, fullness of the body and the content of ethyl alcohol in the beer. Beer that has a very high apparent degree of fermentation (attenuation) will eventually be very dry after fermentation. Such beer, as a rule, with a lighter body and taste than a beer with a lower apparent degree of digestion, will also contain more alcohol, provided that the wort has the same initial density.

The greatest influence on the fermentation process is the temperature and the amount of yeast. It is best fermented wort with an initial density of 10-12%. The apparent degree of fermentation of young beer should be 59.1… 67.5%.

Conclusions. The results show that in the 2nd sample, with the immobilized yeast, fermentation is slower, and than in the 1st sample, but there are no significant

deviations from the indicators given in the table. It can be argued that the method of fermentation of beer wort using immobilized yeast does not affect the course of the technological process. The slight lag in the dynamics of fermentation of the second sample can be explained by the increase in the latent phase of immobilized yeast. To avoid this, it is necessary to activate the immobilized yeast before use, for example, to keep them in distilled water for a while.

The samples differed significantly in transparency during being in glass containers. The 2nd sample, which was treated with immobilized microorganisms, had a much more transparent and glossy appearance. This indicates that in this sample, a significantly smaller number of cells of microorganisms that are in a suspended state.

The proposed technology, which involves the use in the production of craft beer immobilized yeast, makes it possible to significantly reduce the cost of the process of clarification and filtering of craft beer and kvass in the production of restaurants, and save material resources, because the use of yeast in the form of immobilized yeast application.

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