Influence of the yeast aeration method on the quality characteristics of beer

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Abstract. Aeration of wort in brewing, as a traditional way to reduce the need for yeast oxygen, has several disadvantages associated with a change in the quality of the finished beverage. Another approach to solving this problem is the pre-fermentation preparation of brewer’s yeast, which consists in short-term aeration (30 min) of the inoculum followed by exposure without air for 2 hours. The effect of various methods of supplying the yeast culture with atmospheric oxygen on the formation of flavoring substances and change in physico-chemical characteristics of beer. Objects of research are bottom fermentation yeast Saccharomyces cerevisiae, young and finished beer. It was revealed that in young beer fermented with yeast after aeration, the content of sensory significant components (higher alcohols, acetaldehyde, diacetyl) is on average 27% lower than in the variant with wort aeration. A possible reason for this is the high activity in the prepared inoculum in comparison with the initial culture of enzymes (1.7-1.8 times increased) involved in the conversion of by-products. Stimulation of the metabolic processes of yeast with oxygen, especially in the sample with aeration of the wort, creates the conditions for reducing (from 7 to 23%) the main mutating components. However, pre-aeration of the inoculum allows you to get a ready drink with the best taste and aromatic characteristics, which is the advantage of this method in comparison with aeration of the wort.

1. Introduction

In brewing practice, wort aeration is used to provide yeast with oxygen at the fermentation stage, the need for which is expressed by conflicting opinions [1–4].

When the fermentation process is carried out in the classical periodic way, the required amount of dissolved oxygen in beer wort with an extractivity of 11–12% is determined by the range 6.0–8.0 mg/dm³ [1, 3]. Wort aeration (at the level of 10.0–18.0 mg O₂/dm³) is mandatory in the manufacture of high-density beers and / or in the preparation of a beverage using the “high-density brewing” technology: a significant content of active yeast cells intensifies the fermentation process and reduces the proportion of by-products that degrade the taste and aroma of beer [5].

Some researchers [6] note the multivariance of aeration of the fermentation medium (oxygen saturation of the wort of all brews or only part of them) with the process in cylindrical fermentation apparatus filled with the wort of several brews. At the same time, the absence of dependence of the kinetics of yeast development in the dynamics of medium fermentation on the methods of its aeration is ascertained, but fermentation without wort aeration gives the worst results in terms of reproduction rate and maximum cell growth.
On the one hand, oxygen, from the perspective of its consumption during fermentation, is important for yeast, mainly as a trace element that stimulates the formation of sterols and unsaturated fatty acids that are part of the structure of biological membranes [3, 5, 7]. At the same time, an excessive amount of dissolved oxygen in the medium can lead to negative processes: an increase in the redox potential; the predominance of the respiratory activity of yeast over fermentation ability; significant increase in biomass; synthesis in elevated concentrations of by-products and secondary fermentation products that adversely affect the organoleptic characteristics of beer; reduce the durability of the finished drink [1, 2, 4, 8, 9]. An alternative in this case may be the treatment of seed yeast, aimed at synthesizing an additional amount of anaerobic growth factors in the cells, by pre-saturation with atmospheric oxygen.

The proposed preparation of a yeast culture consists in short-term aeration of the inoculum in the presence of carbohydrate degradation products for the synthesis of sterols, followed by exposure without air to prevent the metabolism from fermentation to respiration [10].

The purpose of this work is a comparative study of various ways of providing brewer's yeast with oxygen for the chemical and physico-chemical characteristics of beer.

2. Materials and Methods

The object of the study was brewer's yeast Saccharomyces cerevisiae of lower fermentation of race 8(a) M of the third and fifth generations, young and finished beer, obtained in laboratory conditions from industrial wort with an extractivity of 11% according to the Zhigulevskoe technology.

The pre-fermentation inoculum preparation under optimized conditions consisted of aeration of a suspension of yeast in young beer (1:2) for 30 minutes at a temperature of 2–4 °С, followed by exposure without access of air (in a closed container) for 2 hours [10]. Saturation of the yeast suspension and the fermentation medium with atmospheric oxygen was carried out using a compressor.

Fermentation of 11% wort at 8–9 °C was carried out with yeast based on the inoculation rate of 20 million cells/cm³ of medium.

To determine the feasibility of aeration of the medium in comparison with the oxygen saturation of the inoculum with oxygen, the following options were compared: 1 – fermentation of the wort with the oxygen concentration, which is achieved due to its natural dissolution (4.0 ± 0.5 mg/dm³), with the initial yeast (control); 2 – fermentation of the wort with the same oxygen content inoculum after aerial treatment; 3 – fermentation of pre-aerated wort to the oxygen concentration required for normal yeast reproduction 8.0±0.5 mg/dm³ [1, 3]. In the latter case, yeast was used for inoculation without preliminary preparation.

The measurement in the wort of the amount of dissolved oxygen was carried out with a MARK-302E oxygen meter (Russia). The determination of titratable and active acidity, color, ethanol concentration, and the actual degree of fermentation in beer was carried out using standard methods adopted in brewing. The content of higher alcohols and acetaldehyde was evaluated by photocolorimetric methods based on a change in the color intensity of the solution in the reaction of higher alcohols with n-dimethylaminobenzaldehyde and acetic aldehyde with resorcinol, respectively; diacetyl content was determined by a modified Brenner method based on the reaction of diketone with hydroxylamine chloride to form dimethylglyoxime, followed by measuring its optical density on a spectrophotometer at 230 nm; acetoin content was determined after its oxidation by ferric chloride to diacetyl is similar to the content of the latter [11].

The analysis of the total concentration of polyphenols was carried out by the method of Jerumanis J., based on the reaction of these substances with ferric ammonium citrate (III) in an alkaline medium; anthocyanogens concentration was determined by the method of Steiner K., Stocker H.R. in the modification of Pfefger D. by the reaction of converting anthocyanogens to anthocyanidins by treating beer with a mixture of butanol and concentrated hydrochloric acid containing iron. The calculation of the content of high-molecular-weight proteins (fraction A according to Lundin) was carried out according to the tannin index, the determination of which is based on the ability of polypeptides to
react with tannin in an acidic environment. The finished beer was evaluated for its cold clouding tendency using the accelerated test 2/1 (measuring the turbidity after holding the beer for 24 h at 40 °C, then at 0 °C), which indirectly characterizes the colloidal resistance of the beverage [11].

To establish the relationship of the synthesis of by-products with other factors, the following indicators were additionally determined.

In the initial yeast, after processing, during the fermentation process, the activity of the enzymes was evaluated: β-fructofuranosidase, or invertase, which performs hydrolysis of sucrose, by polarimetric method; alcohol dehydrogenase (ADH), which catalyzes the final stage of alcoholic fermentation, using the spectrophotometric method for the recovery rate of NAD at a wavelength of 340 nm [12]. Enzyme activity was expressed in micromoles of a substrate (sucrose and NADH, respectively), hydrolyzed under the action of a catalyst in 1 min per 1 g of dry matter of yeast.

The total concentration of yeast and budding cells in the dynamics of the wort fermentation was determined by direct calculation in the Goryaev chamber.

All experiments studies were repeated three- or four-fold.

3. Results and Discussion
The taste and aroma characteristic of beer is largely determined by the products of yeast metabolism formed during the fermentation of wort and the fermentation of young beer. The difference in the supply of yeast with atmospheric oxygen can affect the quantitative and qualitative composition of the main and by-products of alcoholic fermentation.

The research results showed that during aerial treatment of wort and inoculum, the rate of formation of the main yeast metabolism product—ethyl alcohol—at the initial stage of fermentation is higher than in the control (Figure 1). The maximum rate in all cases is almost the same (7.4–7.8 mmol of alcohol/h), but in the sample with aeration of the inoculum it was reached a day earlier. In addition, by the end of the process of ethanol fermentation, the experimental samples contained 11–19% more compared to the control (Table 1).

![Figure 1. Effect of yeast aeration method on the rate of ethyl alcohol formation rate](image)

**Table 1. Content of yeast metabolism products in young beer depending on the conditions for oxygen supply**

| Variant            | Ethanol [wt%] | Higher alcohols [mg/dm³] | Acetaldehyde [mg/dm³] | Diacetyl [mg/dm³] | Acetoin [mg/dm³] |
|--------------------|---------------|--------------------------|-----------------------|------------------|-----------------|
| Control            | 2.7           | 69                       | 21.2/10.5             | 1.75/0.72        | 2.54/1.83       |
| Wort aeration      | 3.2           | 80                       | 25.4/13.6             | 2.10/0.66        | 2.85/1.66       |
| Yeast aeration     | 3.0           | 72                       | 23.0/8.2              | 2.32/0.50        | 2.65/1.50       |

*First digit in the fraction is the maximum amount of product formed during the fermentation, the second digit is the concentration of the product at the end of fermentation in young beer.*
The data obtained are consistent with a change in the activity of cellular alcohol dehydrogenase. After yeast aeration, the activity of ADH in yeast is 2.5 times higher than in the initial culture (Table 2). During fermentation, the maximum activity values of the studied enzyme in the yeast cells of the experimental variants are 13–24% higher than that of the control variant. A probable reason for this is the stimulation of ADH synthesis in the presence of alcohol [13].

### Table 2. Enzyme activity and growth of yeast biomass under different aeration conditions

| Indicators                          | Control          | Wort aeration | Yeast aeration |
|------------------------------------|-----------------|---------------|----------------|
| Invertase activity $^a$            | 39.3±0.5/       | 39.3±0.5/     | 70.5±0.5/      |
| [μmol sucrose/g DS · min]          | 60.4±0.5/       | 71.7±0.5/     | 78.6±0.5/      |
| ADH activity                       | 3.1±0.3/        | 3.1±0.3/      | 5.2±0.3/       |
| [μmol NADH/g DS · min]             | 6.3±0.3/        | 7.1±0.3/      | 7.8±0.3/       |
| Growth of budding cells            | 32              | 54            | 40             |
| $(C_{MAX} - C_{INIT.})$ [%]        |                 |               |                |

$^a$ first digit in the fraction is the activity of the enzyme in the initial yeast (after treatment in the variant with inoculum aeration), second digit is the maximum enzyme activity during fermentation.

Although the ADH activity of yeast in the sample with aeration of the wort is slightly lower; however, alcohol accumulates in young beer more than in the case of medium fermentation with a previously prepared inoculum. This fact can be associated with a high increase in yeast biomass in the first case (Table 2) due to the increased level of dissolved oxygen in the wort, and indicates the intensification of metabolic processes in cells.

In the sample of young beer with pre-fermentation treatment of the inoculum, the amount of higher alcohols is at the level of the control variant, while aeration of the wort leads to a more significant (16%) synthesis of these metabolites, which is understandable.

The formation of higher alcohols is the result of a constructive exchange of yeast cells, and stimulation of the propagation of the culture increases the content of this group of by-products [1, 2, 3, 9, 13, 14]. The low concentration of higher alcohols compared to wort aeration in the sample with processed yeast is associated not only with a smaller increase in cell biomass, but, possibly, with higher than in other variants, the values of ADH and β-fructofuranosidase activity. The first enzyme promotes less accumulation of pyruvate, the central metabolite in the biosynthesis of higher alcohols [13]. Invertase can exhibit along with hydrolytic and transferase function [15]. This biocatalyst, transferring the remaining sugars to alcohols, binds the latter to alkyl fructosides, thereby reducing the concentration of higher alcohols in the medium.

Acetaldehyde accumulates at the beginning of fermentation as an intermediate product of cell metabolism along the path of glycolytic conversion of carbohydrates to ethanol. An increased concentration of this metabolite in beer is undesirable, because gives the drink an immature flavor.

In the sample with aeration of the wort, the maximum amount of acetic aldehyde is 20% higher than in the control, and with yeast processing this increase is only 8%. In the latter case, in comparison with other options, the active reduction of acetaldehyde to ethanol is also observed. In young beer obtained using aero-processed yeast, the content of acetic aldehyde is 22 and 40% lower than in the control and in the sample with aeration of wort, respectively.

The increased activity of ADH accelerates the reaction of reduction of acetaldehyde to ethanol, accompanied by the regeneration of NAD, necessary for the further oxidation of triose phosphates, and the rate of glycolysis is known to be limited by the lack of oxidized NAD [3, 13].

The conditions of yeast aeration affect the dynamics of diacetyl changes during fermentation [9]. In the presence of atmospheric oxygen (both in the case of inoculum aeration treatment and in wort
aeration), the yeast is more active and, in comparison with the control, the vicinal diketon is intensely synthesized, but for the same reason it is rapidly reduced to acetoin. This ensures that prototype samples in young beer, especially with inoculum aeration, have a lower concentration of diacetyl (9–30%) and acetoin (10–20%) than in the control variant.

Diacetyl reduction is an enzymatic process catalyzed by diacetyl reductase [3, 9]. Along with this enzyme, alcohol dehydrogenase also takes part in the diacetyl reduction reaction [13]. Perhaps the high activity of ADH in the treated yeast intensifies the process of diketone reduction.

Samples of young beer (experimental after 6 days, control after 7 days) were fermented for 21 days at a temperature of 2 °C.

The finished beer of the experimental variants is fermented deeper than the control sample and contains 13–18% more alcohol (Table 3). At the same time, the drink where wort aeration was used, in contrast to the control beer and made using aero-processed yeast, is characterized by an increased concentration of metabolites that adversely affect the taste and aroma of the product, which is confirmed by the results of tasting analysis.

Changes in the redox conditions in the fermentation medium, and especially in the finished beer, significantly affect the processes of transformation of colloidal substances and the formation of turbidity. The oxidative polymerization of polyphenols, leading to the formation of complex insoluble complexes with polypeptides, plays a significant role in the stability of the colloidal system. In addition, the oxidation of polyphenols impairs the taste of beer. Oxygen probably oxidizes sulfhydryl groups in polypeptides, which promotes the binding of several chains of polypeptides by disulfide bridges, their enlargement, and decrease solubility [9].

| Indicator                   | Control | Wort aeration | Yeast aeration |
|-----------------------------|---------|---------------|----------------|
| Ethanol [wt%]               | 2.92    | 3.45          | 3.31           |
| Actual fermentation degree [%] | 51.4   | 61.1          | 58.5           |
| Acidity                     | 2.50    | 2.62          | 2.60           |
| Color                       | 0.70    | 0.64          | 0.67           |
| pH                          | 4.46    | 4.30          | 4.38           |
| Higher alcohols [mg/dm³]    | 80.0    | 99.0          | 86.0           |
| Diacetyl [mg/dm³]           | 0.46    | 0.55          | 0.37           |
| Acetoin [mg/dm³]            | 1.21    | 1.44          | 1.16           |
| Acetaldehyde [mg/dm³]       | 7.4     | 8.5           | 6.6            |
| Total polyphenols [mg/dm³]  | 202     | 181           | 189            |
| Anthocyanogens [mg/dm³]     | 121     | 105           | 110            |
| Protein fraction A [mg/100 cm³]  | 10.4  | 8.0           | 9.1            |
| Test 2/1 [unit opt. dens.]  | 0.38    | 0.31          | 0.33           |
| Head height [mm]            | 40      | 38            | 41             |
| Head retention [min]        | 3.7     | 3.5           | 3.7            |
| Tasting assessment [points] | 24.0    | 23.4          | 24.5           |

In the samples of finished beer obtained using processed yeast and aerated wort, the content of polyphenols is lower than in the control variant by 7–10%, that of anthocyanogens by 9–13%, and that of high molecular weight proteins by 13–23% (Table 3).

The most adequate information on the stability of the colloidal system of beer is provided by the method based on determining the intensity of the turbidity of the drink in the cold - test 2/1 [9, 11]. Samples of experimental finished beer are characterized by low values of this indicator in comparison with the control.
The above results indicate that, despite the different initial level of oxygen in the medium, the finished beer of the experimental variants in terms of indicators characterizing the tendency of the drink to colloidal turbidity are close to each other. It is likely that the initial concentration of oxygen in the wort does not have a significant effect on the colloidal resistance of beer due to its rapid consumption by yeast, but the indirect effect of oxygen is obviously manifested.

The intensification of the metabolic processes of yeast culture with any method of oxygen supply leads to a higher content of alcohol and other fermentation products (higher alcohols, aldehydes, ketones) with which polyphenols can interact. As a result, the properties of phenolic substances change, they are more likely to exhibit polycondensation and polymerization, enlargement of molecules and precipitation. Low values of active acidity in experimental beer variants affect the dispersion and solubility of proteins, contributing to their isolation from solution, including in the form of protein-phenolic complexes. All this, in turn, stabilizes the colloidal composition of beer. It can be assumed that the pre-fermentation treatment of yeast and aeration of the wort will have a positive effect on the colloidal resistance of the finished drink.

One of the important properties of beer is frothing. According to the value of this indicator, the drink obtained using processed yeast is close to the control, while in the sample with aeration of the wort the head height is somewhat lower and less stable. Perhaps this is due to the large removal of polypeptides from beer, which play a significant role in the formation of a strong head [1, 2].

4. Conclusions
The obtained experimental data indicate that stimulation of the metabolic processes of yeast with oxygen both at the stage of fermentation of the medium and at the stage of preparation of the inoculum intensifies the fermentation of beer wort, the formation of alcohol, and creates conditions for better isolation of the main mutating substances from beer. However, aerial processing of yeast culture contributes to moderate synthesis of metabolites adversely affecting the taste and aromatic profile of beer, which makes this method of yeast aeration more preferable as an alternative to wort aeration.

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