Changes in the biochemical properties of yeast during oxygen saturation of semi-finished bakery products

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Abstract. Microbiological and biochemical processes caused by yeast are the main factors affecting the quality of bread. The ability to manage the vital activity of yeast allows you to regulate these processes and contribute to the intensification of technological processes in baking. An important factor that affects the activity of yeast is oxygen. In bread-making technology, oxygen is mainly considered as a factor that affects oxidative processes. The effect of aeration of bakery semi-finished products on the properties of yeast has not been studied enough. In this work, we studied the enzymatic activity (zimase, maltase), morphological characteristics, viability and quantity of water-soluble sulfhydryl compounds released by yeast when the yeast suspension and liquid sourdough were saturated with oxygen in the range of 4-16 mg/l. The lifting force of yeast when the suspension was saturated with oxygen improved by 23 %. Similarly, the lifting force of liquid sourdough changed when it was saturated with oxygen. Increasing the oxygen content 3 times increased the lift of the liquid sponge by 20 %. It was found that the accumulation of total sulfhydryl compounds in the control was more intense than that in semi-finished products saturated with oxygen. In general, the oxygen content in the yeast suspension and in the liquid sponge significantly activates the vital activity of yeast and, accordingly, intensifies the process of their preparation.

1. Introduction

The quality of yeast largely determines the speed of microbiological and biochemical processes in the preparation of bread dough. Therefore, the study of mechanisms that regulate the growth and reproduction of yeast cells under the influence of environmental factors makes it possible to control the vital activity of yeast, increase their activity, and, consequently, contribute to the intensification of technological processes in baking. Increasing the activity of yeast leads to an intensification of the fermentation process of dough and semi-finished products, which helps to reduce the cost of dry substances for fermentation. Oxygen plays an important role in the life of yeast, since aerobic oxidation in the yeast cell contributes to their intensive reproduction. Similarly, the ability of yeast to ferment sugars in the absence of air is due to their accumulation of oxygen during reproduction [1]. The influence of aerobic conditions and other factors on the vital activity of yeast is actively studied on model systems [2, 3, 4, 5]. In baking technology, oxygen is mainly considered as a factor that affects the oxidation of gluten proteins, lipids, etc. [6]; however, there is no sufficient data on the effect of oxygen on yeast in the composition of bakery semi-finished products.

The effect of oxygen saturation of bakery semi-finished products on the biochemical properties of yeast was studied.
2. Materials and methods
The biochemical properties of yeast were evaluated by their enzymatic activity (zimase, maltase activity, lifting force), morphological characteristics, viability, and the number of water-soluble relatively low-molecular-weight highly reactive easily oxidizing sulfhydryl compounds (WRLMHREASC) released by them.

The lifting force that characterizes the total activity of yeast enzymes was determined using the “ball” method. Maltase and zimase activity of yeast was determined by the gasometric method based on the time of isolation of 10 ml of carbon dioxide by a yeast sample of 0.5 g during fermentation of sugar (maltose or sucrose).

In the study of morphological features, the yeast size was chosen as the basis, estimated by their diameter. During fermentation, the relative number of small, medium and large cells with diameters up to 3.3 microns, 3.3-5.8 microns, and 5.8-7.1 microns, respectively, was determined in the test sample. After isolation of yeast cells from the test, their number was counted in the Goryaev counting chamber with an increase of 400 times.

The total relative surface (per 100 yeast cells) of yeast cells was calculated, assuming the shape of the cell as an ideal ball. The total surface was calculated as the total surface of small, medium, and large cells.

The viability of yeast was determined in equal samples of liquid semi-finished product when diluted 10-7 times and seeded on dense must-agar nutrient media in Petri dishes. The number of cells in the liquid semi-finished product that give colonies was calculated after 48 hours of incubation of crops at a temperature of 30°C.

The amount of WRLMHREASC released by yeast, with simultaneous determination of reduced glutathione in one sample, was controlled by the method. In sum WRLMHREASC includes restored glutathione and easily oxidizing sulfhydryl compounds: glutamylcysteine, cysteamine, contingency, cysteine, homocysteine (a derivative of methionine). The method for determining WRLMHREASC in the sample allowed the deposition of the restored glutathione sulfate cadmium and iodometric titration. The number of recovered glutathione was calculated by difference WRLMHREASC before and after sedimentation of sulphate of cadmium.

3. Discussion of the results
Such qualitative indicators as fermentation activity and lifting force give the most complete idea of the biochemical properties of yeast, expressed in total. With an increase in the amount of oxygen in the liquid brew, the fermentation activity of yeast in it improved and had a maximum value at an oxygen content of 12 mg/l, i.e., there was an improvement in the fermentation activity almost 2 times (table 1, 2).

The lifting force of yeast when the suspension was saturated with oxygen improved and was 14 minutes against 18 minutes in the control. When the oxygen content was increased by 2 times, the lifting force of yeast improved by 23%. Similarly, there is a change in the lifting force of liquid sourdough when it is saturated with oxygen. A growth in the oxygen content of 3 times increased the lift of the liquid sponge by 20% (tables 1, 2).

| Oxygen concentration, mg/l | “Lifting force”, min | Maltase activity, min | Zimase activity, min |
|---------------------------|---------------------|-----------------------|---------------------|
| 7 (control)               | 18                  | 245                   | 51                  |
| 10                        | 17                  | 213                   | 47                  |
| 13                        | 16                  | 208                   | 46                  |
| 16                        | 14                  | 198                   | 44                  |
To explain the effect of improving the quality indicators of yeast, the effect of oxygen saturation of the yeast suspension on the maltase and zimase activity of yeast was studied. From the data in table 1, it can be seen that with an increase in the amount of oxygen in the yeast suspension, the maltase and zimase activity of yeast increased by 20.0 and 13.6%, respectively.

Table 2. Changes in yeast activity in liquid sourdough when saturated with oxygen

| Oxygen concentration, mg/l | Fermentation activity, ml | “Lifting force”, min |
|----------------------------|---------------------------|---------------------|
| 4 (control)                | 6                         | 29                  |
| 6                          | 7                         | 25                  |
| 9                          | 9                         | 24                  |
| 12                         | 12                        | 23                  |

Thus, oxygen saturation improved quality indicators of yeast in the semi-lift and fermentation activity by increasing the activity of sinusoidal and maltego enzyme complexes of yeast.

Data from the study of the viability of yeast cells when seeded on dense wort-agar media showed that with an increase in the oxygen content in the liquid brew, the number of grown yeast colonies increased. It amounted to $217 \times 10^7$ in terms of 1 g of brew in the control, in experimental samples with an oxygen content of 6; 9 and 12 mg / l during cultivation, $300 \times 10^7$; $400 \times 10^7$ and $425 \times 10^7$ of yeast colonies, respectively. As you can see, yeast cells in liquid sourdough are more viable when saturated with oxygen, the number of yeast colonies that have grown increased in comparison with the control.

Many researchers [1,7] associate the quality of yeast with the presence in them and the ability to secrete water-soluble relatively low-molecular-weight highly reactive easily oxidizing sulfhydryl compounds (WRLMHREASC), among which a special role is assigned to glutathione. We have studied the effect of oxygen saturation of yeast suspension on the process of excretion of reduced glutathione and other WRLMHREASC by yeast. The change in the content of WRLMHREASC was studied for 6 hours in a control sample of yeast suspension without oxygen saturation and in a saturated one up to 16.8 mg / l. The results are shown in figure 1.

Figure 1. Changes in the content of sulfhydryl compounds (5, 6 - general, 3, 4 – reduced glutathione, 1, 2 - others) in the yeast suspension over time: 2, 4, 6 - control; 1, 3, 5 – oxygen - saturated to 16.8 mg/l.
As can be seen from figure 1, the total amount of WRLMHREASC increased both in the control and in the experiment and after 6 hours corresponded to 19.9 and 15.7 mg%, respectively. At the same time, the initial content of total WRLMHREASC in the experimental sample was 1.9 mg% less than in the control. The change in the overall WRLMHREASC in the control over time was more intense than in the experiment. So, for 6 hours, the number of total WRLMHREASC increased in the control sample by 4.0 mg%, and in the experiment - by 1.8 mg%.

In the yeast suspension, the total WRLMHREASC was 60% represented by reduced glutathione. In fig. 1 it can be seen that the accumulation of reduced glutathione in yeast suspension samples over time is similar to the change in the total amount of WRLMHREASC. The initial content of reduced glutathione in an oxygen-rich yeast suspension is 0.8 mg% lower and during the study time it accumulated 2 times less than in the control sample of the yeast suspension. Thus this changed the contents of the other WRLMHREAS in the samples.

Consequently, the amount of WRLMHREASC and the ability of yeast to release them due to oxidation decreased in the yeast suspension saturated with oxygen.

Morphological changes of yeast were studied in the fermentation test. To do this, small, medium and large cells were counted and their relative number and total specific surface area (per 100 yeast cells) were calculated using the method described in the research methodology chapter. The dough was prepared on a liquid sponge using an oxygen-rich yeast suspension with an oxygen content of 16.8 mg/l and a liquid sponge saturated to 12 mg/l of oxygen, the control was the dough prepared on a liquid sponge without saturation. Microscopy revealed that the number of dead cells in the liquid brew remained unchanged when saturated with oxygen in comparison with unsaturated semi-finished products and amounted to no more than 9% of the total number of yeast cells. During the fermentation of the test in the first two hours, both in the control and in the test samples, yeast reproduction practically did not occur. The number of yeast cells in 1 g of the test was $510 \times 10^6 - 520 \times 10^6$ pcs. By the third hour of fermentation, the number of cells in the test increased: in the control up to $555 \times 10^6$ in 1 g of dough, in the test on a liquid sponge using an oxygen-saturated yeast suspension – up to $625 \times 10^6$ and in the test on a saturated liquid sponge – up to $695 \times 10^6$ cells.

Figure 2 shows the change in the relative number of test samples during fermentation of small (diameter of 0-3.3 microns), medium (diameter of 3.3-5.8 microns) and large (diameter of 5.8-7.1 microns) cells. In the dough during fermentation, the yeast cells have increased in size. The relative number of medium and large cells increased in the control by 9% and 5%, respectively, in experimental test samples – using an oxygen-rich yeast suspension by 10 % and 11 %, and using the oxygen-rich liquid sponge – by 11% and 14 %.

![Figure 2. Changes in the percentage of yeast cells in the test during fermentation: (a) – large cells (diameter 5.8 – 7.1 microns); (b) – medium cells (diameter 3.3 – 5.8 microns); (c) – small cells (diameter 0 – 3.3 microns). 1 - control; 2 - on a yeast suspension saturated with oxygen up to 16 mg/l; 3 - on a liquid sponge saturated with oxygen up to 12 mg/l.](image-url)
At the same time, the relative number of small yeast cells in the test during fermentation decreased accordingly.

The calculation of the specific surface of yeast cells showed (Figure 3) that during fermentation, the total specific surface area of yeast cells in the control sample increased from 5680 to 7116 µm², in experimental test samples in the case of using an oxygen-rich yeast suspension – from 5883 to 7924 µm², oxygen-rich liquid sponge – from 5883 to 8570 µm². During fermentation, the increase in the total specific surface area of yeast cells in the test samples in comparison with the control exceeded by 605 and 1251 µm², respectively.

Therefore, when preparing the dough on oxygen-saturated semi-finished products, the yeast multiplies more actively, becomes larger, and their contact with nutrients increases.

6. Conclusion

The influence of the oxygen content in the yeast suspension and in the liquid sponge on the enzymatic activity of yeast, their viability, production of the most significant metabolites and their morphological changes was studied. It was found that an increase in the oxygen content in bakery semi-finished products significantly activates the vital activity of yeast and, accordingly, intensifies the process of their preparation.

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