The use of ultrasound for development of baker's yeast activation technology

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Abstract. Various approaches are used to improve baker's yeast technological properties and final products quality. Because of low-frequency ultrasound does not have a destructive effect on yeast cells, the aim of our research is to test a new method of improving the technological properties of baker's yeast using low-frequency ultrasonic cavitation. The object of the study was active dried baker's yeast Saf-levure (France). All experimental and control samples were processed in a “Grad” ultrasonic bath (Russia) with different intensities and processing times at a frequency of 35 kHz. It was shown that preincubation of baker's yeast with ultrasound at a frequency of 35 kHz, an intensity of 0.5–1 W/cm² and an exposure of 1 and 3 minutes redound to improving the baker's yeast technological properties: the yeast specific growth rate increases by 1.8–2.7 times, and yeast rising power - by 1.2–1.6 times. At the same time, baked with ultrasonic-treated yeast bread has significantly improved physicochemical and organoleptic characteristics compared to traditional bread. Acidity and humidity of the bread crumb decreased by 1.2 and 1.3 times and porosity increased by 1.6–2.5 times with an increasing the time and intensity of the sonication. With increasing of sonication time and intensity, the bread crust became golden brown, the color of the crumb became lighter, and the loaf shape fluffier. The best results are obtained after preincubation of baker's yeast suspension with ultrasound at a frequency of 35 kHz, an intensity of 1 W/cm² and an exposure of 3 minutes.

1. Introduction
Baker's yeast is a technically pure culture of microorganisms belonging to the genus Saccharomyces. In wheat bread production, Saccharomyces cerevisiae is used. Different races of yeast - varieties that differ in one or more technological features, are used in production. The technological role of yeast is to carry out alcohol fermentation, producing a significant amount of carbon dioxide, forming alcohol and various aromatic substances, thereby affecting the technological process and final product sensory properties. The main technological requirement for baker's yeast is they must have high fermentation energy: maltase and zymase activity, characterizing the yeast ability to hydrolyze maltose and glucose of flour, respectively, which is expressed in an increasing of yeast rising power, that is, the lifting speed of the compiled according to specific recipe dough (GOST R 54845-2011).

Various approaches are used to improve baker's yeast technological properties and final products quality: flour mixture composition changing [1], genetic transformations and artificial selection of yeast [2], use of non-traditional vegetable raw materials [3], use of unconventional yeast strains in bakery [4],
a special way of forming and preparing the substrate, increasing the bioavailability of its components for yeast cells [5]. Pre-treatment is often carried out using ultrasound [6]. High-frequency ultrasound is used to destroy yeast cells in order to enrich wine and beer with biologically active substances of these microorganisms [7, 8]. Low-frequency ultrasound does not have a destructive effect on yeast cells, it is even used to measure the concentration of yeast in a liquid suspension [9].

Despite there are different ways to improve the technological properties of yeast, many of them are expensive, time-consuming or have an undesirable effect on the final product. The aim of the study is to test a new method for improving the technological properties of baker's yeast using low-frequency ultrasonic cavitation. The research objectives included studying the effect of ultrasonic cavitation on the specific growth rate and yeast rising power, as well as on the physicochemical and organoleptic properties of baked bread.

2. Materials and methods

The object of the study was active dried baker's yeast Saf-levure (France). To study the effect of cavitation on the yeast specific growth rate, 4 g of dry baker's yeast were suspended in flask with 210 ml of tap water at a temperature of 35°C and left for 10 minutes until the yeast was completely dissolved in water (5 flasks). Then the samples were processed in a “Grad” ultrasonic bath (Russia) with different intensities (I) and processing times (t) at a frequency (f) of 35 kHz (table 1).

| Sample No | 1 | 2 | 3 | 4 | 5 |
|-----------|---|---|---|---|---|
| f = 35 kHz|   |   |   |   |   |
| Processing options | t = 1 min | t = 3 min |   |   |   |
| I = 0.5 W/cm² | - | + | - | - | - |
| I = 1 W/cm² | - | - | + | - | - |

The specific yeast growth rate was calculated by the standard method. For this purpose, a series of serial dilutions in sterile saline were prepared from each sample before and after sonication. Three petri dishes with Saburo agar were inoculated with 0.2 ml 1x10⁻⁶ and 1x10⁻⁸ of dilutions. The plates were incubated in a thermostat at 35 ± 1°C. The final count of the yeast growth was carried out after 48 hours.

Using the obtained data, the specific yeast growth rate or growth coefficient (GC) was calculated using the following formula:

$$GC = 2.303(\log a_2 - \log a_1)/(t_2 - t_1),$$

where: $a_1$ – the yeast cells number at the first inoculation (CFU/ml), $a_2$ - the yeast cells number at the second inoculation (CFU/ml), $(t_1 - t_2)$ – time interval between inoculation (h).

The yeast rising power was determined according to GOST R 54731-2011. For that purpose the dry yeast mass, corresponding to the weight of compressed yeast, was calculated by the formula:

$$m = m_{pr}(100 - W_{pr})/(100 - W),$$

where: $m_{pr}$ – compressed yeast mass, g, $W_{pr}$ – compressed yeast moisture, %, $W$ – dried yeast moisture, %.

Then, all bread samples were baked and their physicochemical and organoleptic properties were analyzed. The following ingredients were used to prepare the dough using the sourdough method: tap
water 210 ml, premium wheat flour 450 g, salt 4 g, sugar 37 g, refined vegetable oil 17 g, active dry yeast 4 g.

After baking in a UNOX oven (Italy) at temperatures of 160°C (top) and 220°C (bottom) for 30-40 minutes and the cooling of bread, its moisture was determined according to GOST 21094-75, the acidity of the bread crumb was determined according to GOST 5670-96 and porosity - according to GOST 5669-96. Organoleptic indicators of bread were evaluated according to GOST 5667-65.

Specific yeast growth rate data under different sonication conditions were calculated (table 2).

3. Results and discussions
Specific yeast growth rate data under different sonication conditions were calculated (table 2).

Table 2. Specific yeast growth rate.

| Sample No | Processing options | GC, CFU/h |
|-----------|--------------------|-----------|
| 1         | No processing      | 2.04±0.19 |
| 2         | I=0.5 W/cm²; t=1 min; f=35 kHz | 3.61±0.36* |
| 3         | I=0.5 W/cm²; t=3 min; f=35 kHz | 3.63±0.36* |
| 4         | I=1 W/cm²; t=1 min; f=35 kHz | 3.79±0.38* |
| 5         | I=1 W/cm²; t=3 min; f=35 kHz | 5.44±0.32*# |

* - the difference is statistically significant between the experimental and control samples; # - the difference is statistically significant between the experimental samples; (P ≤ 0.05 at t critical 2.10).

As it follows from the presented in table 2 data, the effect of cavitation stimulates the growth and reproduction of yeast. After ultrasound exposure the specific yeast growth rate (GC) increased by 1.8 - 2.7 times. This is due to stimulating the yeast vital activity because of mechanical separation of their clusters with formation of separate viable cells, giving rise to new colonies. The best specific growth rate data were observed at an intensity of 1 W/cm² and an exposure time of 3 minutes. Moreover, judging by the dynamics, the limiting factor in this case is the exposure time. It can be explained by the fact that ultrasonic energy is spread in fluid from a localized zone. Accordingly, the volumetric density of ultrasound energy is uneven in the irradiated space. Yeast cells are rapidly stimulated near the source of ultrasonic vibrations. For effect on cells in the entire irradiated volume, some time is needed.

The yeast rising power data under different sonication conditions are illustrated in figure 1.

As it shown in figure 1, the yeasts’ rising power significantly increased after ultrasound exposure. After ultrasound exposure at intensity of 0.5 W/cm² in samples 2 and 3, the yeasts’ rising power increased by 1.2 and 1.3 times, respectively. Moreover, indicators of 2 and 3 samples did not have significant differences between themselves, but had significant differences from the control and from...
the yeasts’ rising power indicators of 4 and 5 samples. The yeasts’ rising power of 4 and 5 samples was significantly different from the control, this indicator was 1.5 and 1.6 times higher for 4 and 5 samples, respectively. Therefore, the limiting factor for this indicator is ultrasound intensity. It can be explained by the fact that water, due to ultrasonic cavitation, dissociates into H$^+$ and OH$^-$ ions with the subsequent formation of active molecules, which promote starch sugars hydrolysis and create conditions for yeast life-sustaining activity, including the pH of the medium. In addition, ultrasound restructures water, breaks hydrogen linkages between molecules in it, which makes water molecules more active to accelerate the hydration of flour biopolymers.

Since we performed sonication in the aqueous phase, which was then used to dough preparing, yeasts’ rising power increased, and, accordingly, the dough maturing time decreased. This is due not only to an increase in the number of activated yeast, but also to an increase in water activity.

After baking, the physicochemical and organoleptic parameters of the final product were studied. The results of the study are shown in table 3 and figure 2.

### Table 3. Physical and chemical parameters of bread crumb.

| Sample No | Processing options | Acidity, ° | Humidity, % | Porosity, % |
|-----------|--------------------|------------|-------------|-------------|
| 1         | No processing      | 1.8±0.2    | 40.0±3.9    | 20.1±1.9    |
| 2         | I=0.5 W/cm$^2$; t= 1 min; f=35 kHz | 1.7±0.1    | 34.6±3.4*   | 32.7±3.2*   |
| 3         | I=0.5 W/cm$^2$; t= 3 min; f=35 kHz | 1.6±0.1*   | 33.8±3.3*   | 45.4±4.4#   |
| 4         | I=1 W/cm$^2$; t= 1 min; f=35 kHz | 1.5±0.1*   | 32.7±3.2*   | 47.6±4.6*   |
| 5         | I=1 W/cm$^2$; t= 3 min; f=35 kHz | 1.5±0.1*   | 31.5±3.1*   | 50.2±4.9#   |

* - the difference is statistically significant between the experimental and control samples; # - the difference is statistically significant between the experimental samples; ($P \leq 0.05$ at $t$ critical 2.10).

As it follows from the presented in table 3 data, the product's acidity and humidity decreased by 1.2 and 1.3 times as maximum, with an increasing the time and intensity of the sonication. While the porosity of the bread crumb increased by 1.6-2.5 times depending on the conditions of ultrasound action. The first factor explains blander bread taste and may be due to reducing of the dough maturing time. The second and third factors may be due to more intensive process of gas formation in the dough, they contribute to a more complete bread digestibility as a result of easier chewing and soaking it with saliva.

![Figure 2. Bread organoleptic properties (1 - control; 2 - 1 min 0.5 W/cm$^2$; 3 - 3 min 0.5 W/cm$^2$; 4 - 1 min 1 W/cm$^2$; 5 - 3 min 1 W/cm$^2$).](image)
With increasing of sonication time and intensity, the bread crust became golden brown, the color of the crumb became lighter, and the loaf shape fluffier. The bread with the highest acceptability was produced using yeast after sonication with an ultrasound intensity of 1 W/cm$^2$ and an exposure time of 3 minutes. Ultrasonic processing gives another positive effect, which is increasing the whiteness of the bread crumb, prepared using an ultrasound-treated yeast water suspension. With the gradual decomposition of hydrogen peroxide, forming during ultrasonic processing, oxygen is formed, which has whitening effect when interacting with flour pigments.

4. Conclusion

Thus, pre-treatment with low-frequency ultrasound improves the technological properties of baking yeast: the yeast specific growth rate increases by more than 2 times, on average, and yeasts’ rising power increases by more than 1.5 times. At the same time, baked with ultrasonic-treated yeast bread has significantly improved physicochemical and organoleptic characteristics compared to traditional bread.

The obtained data are confirmed by the opinion of researchers who showed that generated by low-frequency ultrasound cavitation reduces the particle size of yeast β-glucan, which is a major component of the yeast cell wall. That is ultrasonic induced cavitation promotes better enzymolysis by increasing the total surface area of yeast cells and increasing the formation of cell enzymes [10].

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