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# Influence of the ultrasounds stimulation on the quality-quantity structure of Candida yeast

***Abstract***. *Interactions between ultrasound and microorganisms are complex and not fully understood [1]. The purpose of the present study was to determine the effect of ultrasonic waves of different amplitudes and pulse durations on the cell geometry of selected microorganisms. The scope of work included exposing Candida krusei to ultrasonic waves of different ultrasonic wave amplitudes (2 µm, 6 µm and 10 µm) and pulse durations (5 min, 10 min and 15 min). For each of the above-mentioned combinations of interactions, the diameters and areas of yeast cells were measured. The experiments carried out made it possible to determine correlative and functional relationships between stimulation with different ultrasound wave amplitudes and organic matter, and enabled parameterization of ultrasound wave amplitude and pulse duration causing improvement or regression of growth of the microorganisms studied. The increase or decrease in growth capacity and cell size depended on the ultrasonic wave parameters used. The geometry of Candida krusei cells represented by radius length and cell surface area decreased*

***Streszczenie.*** *Interakcje między ultradźwiękami a mikroorganizmami są złożone i nie do końca poznane [1]. Celem niniejszego badania było określenie wpływu fal ultradźwiękowych o różnej amplitudzie i czasie trwania impulsu na geometrię komórek wybranych mikroorganizmów. Zakres prac obejmował poddanie Candida krusei działaniu fal ultradźwiękowych o różnych wartościach amplitudy fali ultradźwiękowej (2 µm, 6 µm i 10 µm) oraz czasu trwania impulsu (5 min, 10 min i 15 min). Dla każdej z wyżej wymienionych kombinacji oddziaływań zmierzono średnice i powierzchnie komórek drożdży. Przeprowadzone eksperymenty pozwoliły na określenie korelacyjnych i funkcjonalnych zależności pomiędzy stymulacją o różnej amplitudzie fali ultradźwiękowej a materią organiczną oraz umożliwiły parametryzację amplitudy fali ultradźwiękowej i czasu trwania impulsów powodujących poprawę lub regresję wzrostu badanych mikroorganizmów. Wzrost lub spadek zdolności wzrostu i wielkości komórek zależał od zastosowanych parametrów fali ultradźwiękowej. Geometria komórek Candidy krusei reprezentowana długością promienia i polem powierzchni komórek uległa zmniejszeniu. (**Wpływ stymulacji ultradźwiękowejna strukturę jakościowo-ilościową drożdży Candida**)*

**Keywords**: ultrasounds, yeast, *Candida* yeast
**Słowa kluczowe**: ultradźwięki, drożdże, drożdże *Candida*

## Introduction

Sound is the vibration of particles in an elastic medium. These vibrations move the particles around their rest position and along the direction of wave propagation caused by vibrational motions [1]. Ultrasound is defined as waves of a mechanical nature that require an elastic medium for propagation [2,3]. Ultrasound waves propagate at frequencies greater than 20 kHz (the upper limit of hearing for the human ear) to a frequency of 10 MHz, which then passes into the so-called hypersonic region. Ultrasound, which consists of mechanical waves, propagates through the medium by transfer of energy rather than particles. Vibrations propagate in the medium in different directions, so they are distinguished as longitudinal waves and transverse waves. In longitudinal waves, the oscillatory motion of particles in the transmission medium is parallel to the direction of propagation, while in transverse waves the motion is perpendicular. Longitudinal (or compressional) waves propagate in any medium, while transverse waves propagate only in solid media [4]. The chemical effects of ultrasonic waves vary, and three distinct phases have been identified in the reaction environment: the gas environment inside the bubble cavity, the liquid-bubble interface and the liquid. In the gas phase, pyrolysis reactions take place [5]. In the food sector, the use of ultrasonic waves has many advantages. In fact, ultrasonic waves have been used to extract phytocomplex extracts without changing their organoleptic properties, which has allowed researchers to understand their principles of action. Any solvent can be used for extraction, depending on the type of extract desired, and the process takes place only at room temperature, resulting in a significant reduction in the required extraction time, instruments, energy and human resources. In addition, the use of ultrasonic waves guarantees a reduction in the bacterial content of the final product due to the antibacterial effect of the ultrasonic waves. The extraction process has received biological certification for use in both the food and cosmetic industries [6]. The fields in which ultrasound is applied use both its mechanical and chemical effects. The importance of both effects varies depending on the frequency used [7,8]. At low frequencies (20-100 kHz), the mechanical effect due to unstable cavitation prevails, and as the frequency approaches 20 kHz, the bubbles collapse with increasing force. At medium frequencies (200-500 kHz), the chemical effect prevails, as more bubbles form and collapse less violently. At high frequencies (>1 MHz), both the chemical and physical effects associated with cavitation are minimal, while the acoustic flow effect dominates. High frequencies are typically used to clean delicate objects that can be damaged in the presence of cavitation [9].

Over the past few years, the properties of ultrasound have attracted growing interest in the food industry, as the induction of physical and chemical reactions can lead to a strategic advantage at various processing stages. Currently, ultrasound is considered an emerging and promising technology in the food processing industry because it induces permanent mechanical, chemical and biochemical changes in liquids and gases [10]. Ultrasound has been applied to food technology because of its mechanical and/or chemical effects on the processes of homogenization, mixing, extraction, filtration, crystallization, dehydration, fermentation, and degassing through antifoaming, particle size reduction, temporary or permanent viscosity modification, modulation of live cell growth, cell destruction and aggregate dispersion, inactivation of microorganisms and enzymes, and sterilization of equipment [11-14].

With ultrasound, the intense shearing action induced by high-frequency sound waves is used to disintegrate cell walls. The mechanism of cell disruption by ultrasound is usually related to the phenomenon of cavitation [15]. Cavitation is a combination of the formation, growth and collapse of gas and vapor bubbles that are induced by the action of intense sound waves [16]. The use of ultrasound leads to better cell disruption and faster mass transfer

between solvent and host material. ultrasounds is widely used to obtain intracellular proteins from microbial cells. The microbial inactivation activity induced by ultrasound is due to the interaction between cavitation bubbles and cells [17]. Many factors influence the effectiveness of ultrasound. Typically, the effectiveness increases exponentially as the acoustic intensity increases, until it reaches its maximum [18].

The purpose of the study was to determine the effect of ultrasonic waves of varying amplitude and pulse duration on the cell geometry of selected microorganisms. The experiments carried out made it possible to determine correlative and functional relationships between stimulation by varying the amplitude of the ultrasonic wave and organic matter, and allowed to parameterize the amplitude of the ultrasonic wave and the duration of pulses causing improvement or regression of growth of the studied yeast.

**Material and methods**

Candida krusei strain ATCC 14243, from the strain collection of the Laboratory for Experimental Research Techniques of Raw Materials and Biological Products, was used in the study. In order to restore vital functions, a reduction culture was performed on TSA solid medium in petri dishes. The plates were incubated for 48 hours at 37°C. Then suspensions of microorganisms were prepared with an optical density of 0.5 on the McFarland scale as in [19].

A Hielscher UP200St sonifier was used in the study (fig.1). The complete ultrasonic system - consisting of an ultrasonic transducer and a generator - converts electrical energy into mechanical vibrations and transmits them to the sonotrode. The mechanical amplitude of the processor is adjustable from 20% to 100%. The probotrodes are impedance-matched, so they can be used without amplitude limitation. Temperature-sensitive samples can be processed in high-intensity pulse mode.

Fig. 1. Hielscher UP200St sonifier

The test used an S26d26 sonotrode with a diameter of 26mm and a maximum amplitude of 20µm. The device is equipped with a color touch screen. The amplitude/power setting and pulse mode can be adjusted using the color touchscreen slider - figure 2.

Fig. 2. Hielscher UP200St sonification screen

Four samples each of suspensions of the test microorganisms were prepared: a control sample and three test samples. The samples were then exposed to ultrasonic waves at three variants of 2 µm, 6 µm and 10 µm amplitude and 25W power. Three variants of exposure time of 5, 10 and 15 minutes were used. The samples were then incubated in a hothouse at 35°C for 7 days, with optical density measurements taken every 24 hours. In addition, staining of the microbial preparation was performed immediately after the tests to determine the size of microbial cells.

Base slides were prepared and degreased beforehand. 0.01 ml of the tested suspensions of microorganism cells were transferred to the basic slides. A vivo preparation stained with lactophenol was prepared. Then, using Motic Plus 3.0 software compatible with the Motic Panthera Series microscope (figure 3), the diameter and area of Candida krusei cells were measured.

a) b)

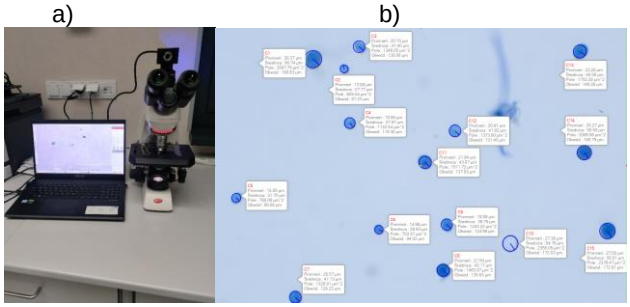

Fig. 3. a) Motic Panthera microscope; b) view of Motic Plus 3.0 program window.

**Results**

In the present study, the effect of ultrasonic stimulation on the geometry of Candida krusei yeast cells was investigated. Figures 4 - 9 show the geometric parameters of the cells after stimulation.

Figure 4 shows the radius of *Candida krusei* cells, obtained immediately after exposure to an ultrasonic wave with an amplitude of 2 µm. An alternating decrease and increase in the value of the cell radius was recorded. The highest value of cell radius was obtained for the control sample, which was 7.01 µm, while the lowest value of cell radius was obtained for the 15-minute exposure - 3.61 µm. The largest difference between the test samples and the control was obtained for the radius length obtained after a 15-minute exposure, which was 3.4 µm. The largest scatter between values was obtained for the sample of 15-minute exposure with ultrasonic waves (2.35 µm).

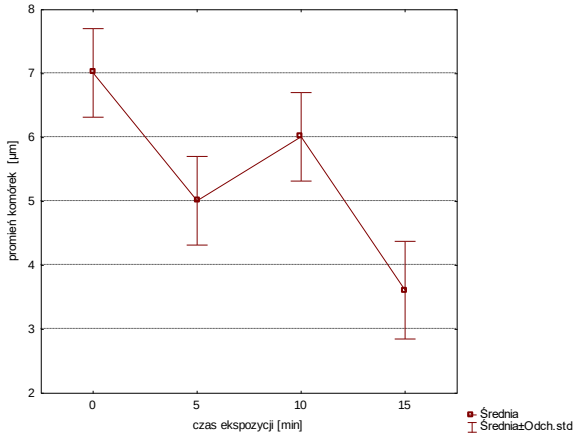


Fig. 4. Radius of *Candida krusei* cells obtained immediately after exposure to an ultrasonic wave with an amplitude of 2 µm

Figure 5 shows the radius of *Candida krusei* cells obtained immediately after exposure to an ultrasonic wave with an amplitude of 6 μm. An increase and then a decrease in the value of the cell radius was recorded. The highest value of cell radius was obtained for the sample of 5 minutes of ultrasonic wave exposure, which was 8.89 μm, while the lowest value of cell radius was obtained for 15 minutes of exposure - 3.65 μm. The largest difference between the test samples and the control was obtained for the radius length obtained after a 15-minute exposure, which was 3.36 μm. The largest scatter between the values was obtained for the sample of 5-minute exposure with ultrasonic waves (4.76 μm).

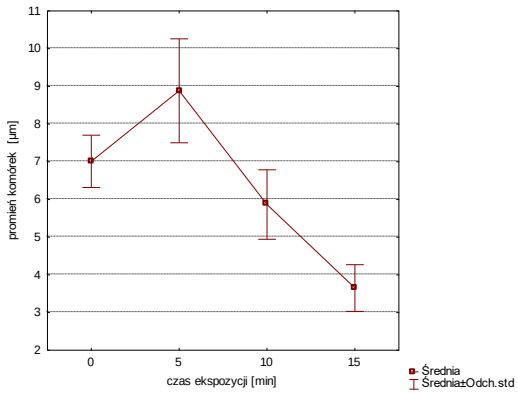


Fig. 5. Radius of *Candida krusei* cells obtained immediately after exposure to an ultrasonic wave with an amplitude of 6 μm

Figure 6 shows the radius of *Candida krusei* cells obtained immediately after exposure to an ultrasonic wave with an amplitude of 10 μm. An increase and then a decrease in the value of the cell radius was recorded. The highest value of cell radius was obtained for the sample of 5 minutes of ultrasonic wave exposure, which was 9.31 μm, while the lowest value of cell radius was obtained for 15 minutes of exposure - 4.56 μm. The largest difference between the test samples and the control was obtained for the radius length obtained after a 15-minute exposure, which was 2.45 μm. The largest scatter between values was obtained for the sample of 5-minute exposure to ultrasonic waves (11,06 μm).

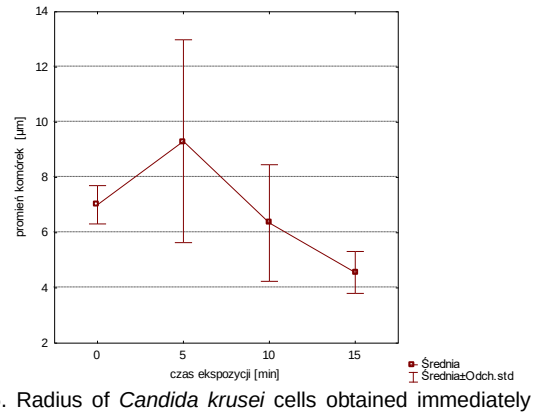


Fig. 6. Radius of *Candida krusei* cells obtained immediately after exposure to an ultrasonic wave with an amplitude of 10 μm

Figure 7 shows the cell surface area of *Candida krusei* cells obtained immediately after exposure to an ultrasonic wave with an amplitude of 2 μm. An alternating decrease and increase in the value of the cell area field was recorded. The highest cell area value was obtained for the control sample, which was 155.86 μm$^2$, while the lowest cell area value was obtained after a 15-minute ultrasonic wave exposure - 46.42 μm$^2$. The largest difference between the test samples and the control was obtained for the area obtained after a 15-minute exposure, which was 109.44 μm$^2$. The largest scatter between the values was obtained for the control sample (86,59 μm$^2$).

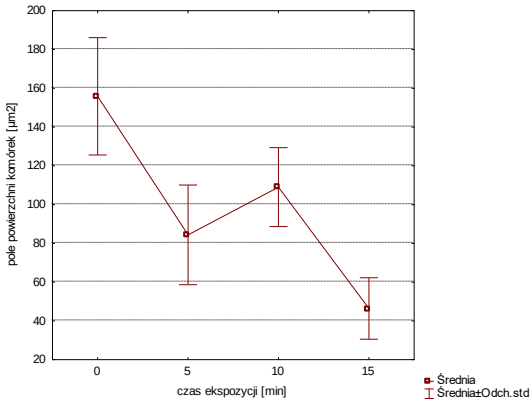


Fig. 7. The surface area of *Candida krusei* cells obtained directly after exposure of an ultrasonic wave with an amplitude of 2 μm

Figure 8 shows the surface area of *Candida krusei* cells obtained immediately after exposure of an ultrasonic wave with an amplitude of 6 μm. An increase and then a decrease in the value of the cell surface area. The highest value of cell surface area was obtained for the sample of 5 minutes of ultrasonic wave exposure, which was 253.34 μm$^2$, while the lowest value of cell surface area was obtained after 15 minutes of exposure - 42.99 μm$^2$. The largest difference between the test samples and the control was obtained for the area obtained after a 15-minute exposure, which was 112.87 μm$^2$. The largest scatter between values was obtained for the 5-minute sample (230,86 μm$^2$).

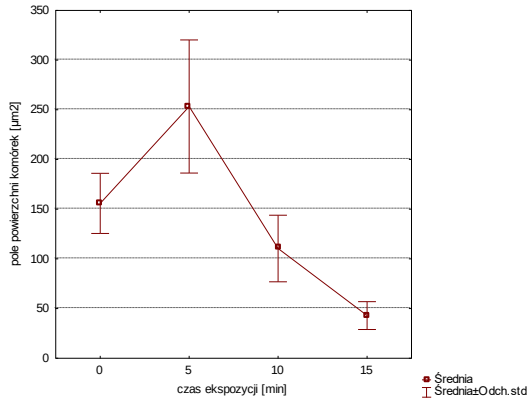


Fig. 8. The surface area of *Candida krusei* cells obtained directly after exposure of an ultrasonic wave with an amplitude of 6 μm.

Figure 9 shows the cell surface area of *Candida krusei* cells obtained immediately after the exposure of an ultrasonic wave with an amplitude of 10 μm. An increase and then a decrease in the value of cell surface area was recorded. The highest value of cell surface area was obtained for the sample subjected to a 5-minute ultrasonic wave exposure, which was 310.77 μm$^2$, while the lowest value of cell surface area was obtained after a 15-minute exposure - 66.93 μm$^2$. The largest difference between the test samples and the control was obtained for the area obtained after a 5-minute exposure, which was 154.91 μm$^2$. The largest scatter between values was obtained for the 5-minute sample (594,12 μm$^2$).

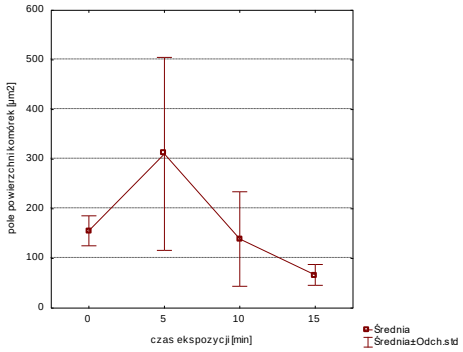


Fig. 9. The surface area of *Candida krusei* cells obtained immediately after exposure to an ultrasonic wave with an amplitude of 10 µm

Figure 10 shows the relationship between the amplitude and exposure time of the ultrasonic wave and the radius of *Candida krusei* cells. In the case of *Candida krusei*, in order to obtain the largest cell radius, it is necessary to select the parameters of exposure time and ultrasonic wave amplitude so that the combination of parameters situates the system in the red color region and does not exceed the yellow color limit line. Using combinations of exposure time parameters of more than 11 minutes regardless of the amplitude, a cell radius not exceeding 6 µm was registered, which reached the smallest values (less than 4 µm) with parameters of more than 14 minutes and amplitude up to 6 µm.

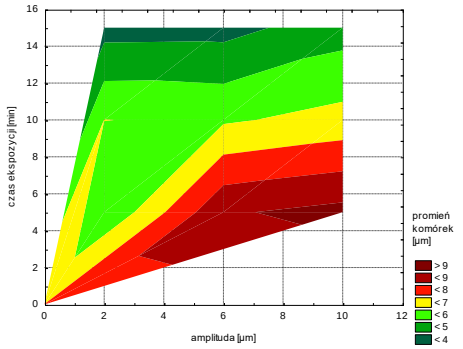


Fig. 10. Relationship between the amplitude and exposure time of the ultrasonic wave and the radius of *Candida krusei* cells

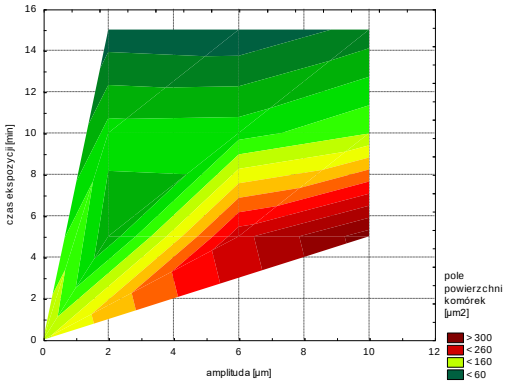


Fig. 11. Relationship between the amplitude and exposure time of the ultrasonic wave and the cell surface area of *Candida krusei* cells

Figure 11 shows the relationship between the amplitude and exposure time of the ultrasonic wave and the cell surface area of Candida krusei cells. In the case of *Candida krusei*, in order to obtain the largest cell surface area, the parameters of exposure time and ultrasonic wave amplitude should be selected so that the combination of parameters situates the system in the red color area and does not exceed the yellow color limit line. Using combinations of exposure time parameters of more than 10 minutes regardless of the amplitude, a cell surface area of less than 100 µm was registered, which reached the smallest values (less than 60 µm) with parameters of more than 14 minutes and an amplitude of up to 6 µm.

A Pearson's correlation analysis was performed to determine the interrelationships between the various parameters studied. The obtained results of the analysis were statistically significant. The correlation matrix of the individual factors is shown in Table 1.There was a significant effect of both the amplitude of the ultrasound wave and the and the time of its exposure on the radius and area of *Candida krusei* cells. The value of the correlation coefficient between the amplitude of the ultrasonic wave and the geometry of *Candida krusei* cells was 0.19 for radius and 0.25 for area. On the other hand, between the exposure time of the ultrasonic wave and the size of *Candida krusei*, the correlation coefficients reached -0.58 for cell radius and -0.5 for area. The values of correlation coefficients between ultrasound wave amplitude and cell geometry were positive, but at a low level. This means that as the amplitude increases, the radius of the cells and their surface area also increase slightly. On the other hand, between ultrasonic wave exposure time and *Candida krusei* size, the correlation coefficients were negative, indicating that cell radius and surface area increase with decreasing ultrasonic wave amplitude and exposure time.

Table 1 Correlation coefficients between amplitude and exposure time of the ultrasound wave and the radius and surface area of *Candida krusei*

| | Amplitude [µm] | Exposure time [min] | Cell radius [µm] | Cell surface area [µm$^2$] |
|---|---|---|---|---|
| Amplitude [µm] | 1 | | | |
| Exposure time [min] | 0,31* | 1 | | |
| Cell radius [µm] | 0,19 | -0,58* | 1 | |
| Cell surface area [µm$^2$] | 0,25* | -0,5* | 0,97* | 1 |

*- calculations performed at α=0.05 level of significance*
** correlation coefficients statistically significant*

## Conclusion

Subjecting Candida krusei yeast to ultrasonic exposure affected its qualitative and quantitative structure by increasing or decreasing the geometric parameters of the cells of these microorganisms. Increasing or decreasing the size of the cells was dependent on the ultrasonic wave parameters used. The geometry of *Candida krusei* cells represented by radius length and cell surface area decreased. The exceptions were samples exposed to an ultrasonic wave with an amplitude of 6 µm during 10 minutes and with an amplitude of 10 µm during 5 minutes of exposure, where an increase in the size of *Candida krusei* cells was observed.

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