

INFLUENCE OF 2.45 GHZ EMF ON YEAST – ANTIOXIDANT DEFENCE AND CELL WALL

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Abstract

The study of the non-thermal effects of 2.45 GHz electromagnetic fields on living organisms is essential, as in recent years the use of devices that emit them, albeit at low intensity, has significantly increased. The aim of our study is to test whether 2.45 GHz electromagnetic radiation has an effect on the antioxidant system of the cell and whether it affects the structure of the cell wall of the yeast *Saccharomyces cerevisiae*. We have investigated the intracellular content of reduced glutathione, total antioxidant and superoxide dismutase activities 1 and 4 h after exposure, as well as 24 h after inoculation of the treated cells in order to monitor delayed effects. A lyticase test was performed to assess changes in the structure of the cell wall. Following a 2 h EMF exposure with 2.45 GHz electromagnetic wave with electric field of 190 V/m glutathione levels decreased and total antioxidant capacity increased 4 h after treatment. A slight trend towards a decrease in superoxide dismutase activity was observed. No significant changes in yeast cell wall integrity were observed under the applied treatment conditions.

Key words: radiofrequency EMF, microwaves, *Saccharomyces cerevisiae*, glutathione, antioxidant activity, superoxide dismutase activity, lyticase test, non-thermal effects

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Abbreviations: ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), EDTA – Ethylenediaminetetraacetic acid, EMF – Electromagnetic field/s, GSH – reduced glutathione, OD_x – optical density at wavelength x nm, PPB – Potassium phosphate buffer, RF – Radiofrequency, ROS – Reactive oxygen species, RPM – Revolutions per minute, SEM – Standard error of mean, SOD – Superoxide dismutase, TEAC – Trolox equivalent antioxidant capacity, WW – Wet weight, YPD – Yeast extract, Peptone and Dextrose

Introduction. Radiofrequency (RF) electromagnetic fields (EMF) are unavoidable environmental factor, only expanding in quantity (intensity) and quality (diverse frequency bands in the microwave region) with the development of communication technologies and their increasing application in everyday life. Therefore, the question of the impact of man-made high-frequency RF EMF on humans and living organisms in general gains significance. The main mechanism of biological action of microwaves is tissue heating, hence it currently defines the non-ionizing EMF levels permitted by regulations. Even though daily exposure rarely causes heating, other microwave effects cannot be excluded. Therefore, non-thermal biological effects caused by high-frequency RF EMF are of particular scientific interest.

Oxidative stress is defined as an imbalance between cell oxidants and antioxidants in favour of oxidants, which causes disruptions in signalling processes and damage at the molecular level. Cellular defence against oxidants primarily involves antioxidant enzymes as well as low molecular weight compounds [1]. Environmental stress factors often increase the production of reactive oxygen species (ROS) in the cell, which leads to oxidative damage of cellular structures [2,3]. A significant body of research has shown that non-thermal effects of low-intensity RF EMF, including 2.45 GHz microwave radiation emitted by devices such as Wi-Fi routers and microwave ovens, are largely due to induced oxidative stress in biological systems [4]. Exposure to 2.45 GHz radiation increases lipid peroxidation and cytosolic free calcium ion concentrations in human leukaemia cells [5]. In rats, 2.45 GHz EMF leads to increased levels of malondialdehyde, a marker of lipid peroxidation, and reduced activities of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase [6]. Although studies on yeast at the particular frequency of 2.45 GHz are limited, studies with other microwaves have shown similar observations. Irradiation of yeast with pulsed EMF at a frequency of 9.71 GHz leads to increased production of free radicals and reduced membrane fluidity as a result of oxidative stress [7]. It is also interesting that exposure to a magnetic field during cultivation increases bioproducts such as the antioxidants glutathione and peroxidase in *Saccharomyces cerevisiae* [8].

In yeast, one of the most environmentally sensitive structures is the cell wall, which dynamically remodels under stress to maintain cell integrity. Thermotolerant strains of *S. cerevisiae*, compared to controls, exhibit resistance to multiple stresses, low levels of intracellular ROS, and efficient cell wall remodelling upon

exposure to stressors [9]. Irradiation of yeast with 40.68 MHz EMF resulted in changes in the cell surface (additional polysaccharide layer) and consequently altered the susceptibility of the organisms to antifungal antibiotics [10]. Exposure to electric fields, especially pulsed ones, can affect the cell wall of yeast. A study showed that application of rectangular electric pulses (0.1–2 ms duration, 2.5–4.5 kV/cm) to yeast suspensions increased their susceptibility to lyticase degradation, indicating improved cell wall porosity [11]. Structural changes in the cell surface (cells appearing rough) were also observed by scanning electron microscopy. Therefore, the pulsed electric field not only permeabilized the plasma membrane, but also affected the cell wall.

The aim of our work was to investigate the effect of high-frequency RF EMF on yeast *S. cerevisiae* as a model system of eukaryotic cell at 2.45 GHz as major frequency band applied in telecommunications. Since increased oxidative stress is one of the main non-thermal biological effects of RF EMF reported, we focused on the cellular antioxidant system – glutathione, total antioxidants and SOD, whose response was monitored in time – 1 and 4 h after exposure, as well as 24 h after inoculation of the treated cells in order to test for delayed effects. Further, we investigated the influence of the EMF on the porosity of the cell wall since there is no data in the literature on it at 2.45 GHz.

The study emphasizes the non-thermal effects of RF EMF exposure, as these often fall outside the scope of standard safety assessments. The focus on glutathione, total antioxidant capacity, and SOD provides valuable insights into potential cellular defense and compensatory mechanisms following exposure. Monitoring these parameters over time after inoculation represents an important step toward evaluating possible long-term and cumulative effects of RF EMF. The additional investigation into yeast cell wall porosity contributes new knowledge to an area for which data at 2.45 GHz frequency are currently lacking, highlighting a potentially novel direction for exploring structural cellular changes. Thus, the present work not only complements existing scientific literature with new experimental data but also underscores the need for systematic, interdisciplinary research into the biological relevance and potential risks of chronic low-intensity RF EMF exposure in the context of an increasingly EMF-saturated environment.

Materials and methods. Yeast material. *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, strain U1, reference number 584 of NBIMCC (National Bank for Industrial Microorganisms and Cell Cultures, University of Chemical Technology and Metallurgy, Sofia, Bulgaria) was used in the study. The cells were cultured in an orbital shaker-incubator ES-20 Grant-bio (Grant Instruments Europe B.V., Amsterdam, The Netherlands) in YPD (10 g/L Yeast extract, 20 g/L Peptone, 20 g/L Dextrose) medium at 30 °C and 180 revolutions per minute (RPM) for 24 h to late exponential phase ($OD_{660} = 35$), with 1.5 mL of cell suspension inoculated into 60 mL of fresh medium. Before each experiment 2 fresh consecutive cultivations were prepared and the cells from the second culture were

washed with distilled water by centrifugation at 4200 RPM for 10 min (MPW-360, Mechanika Precyzyjna, Warsaw, Poland) and resuspending, three times at total. Finally, the cells were concentrated to 100 mg wet weight per mL (WW/mL) and that suspension was distributed in 2 mL tubes used as experimental samples.

Treatment. Half of the samples were irradiated with 2.45 GHz electromagnetic wave with electric field 190 V/m in the far-field of the antenna for 2 h. The other half was held in the same room (semi-anechoic chamber) but these samples were wrapped in tin foil to shield them from the applied EMF. It was emitted by horn antenna connected through power amplifier FLG-50F (Frankonia, Heideck, Germany) with signal generator SMB-100A (Rohde & Schwarz, Munich, Germany) by coaxial cable. The electric field at the place of the samples was set by tuning the generator output power while monitoring the electric field with LSProbe 1.2 Field Probe System (LUMILOOP GmbH, Dresden, Germany). The 190 V/m electric field of the electromagnetic wave was chosen as a conservative safety value since its corresponding incident power (96 W/m^2) is almost 2-times higher than the reference level for occupational exposure, averaged over 30 min and the whole body, to EMF from 2 to 300 GHz – 50 W/m^2 [12]. Specific absorption rate in the samples was simulated to be 10.37 W/kg on average. The temperature of the irradiated samples was monitored during the treatment, as well as that of the controls over the irradiation period. In the first case, it increased by 3.29°C , and in the second – by 1.28°C , not exceeding 26°C in both cases.

1.5 mL of control and treated cells were inoculated into 300 mL flasks with 60 mL of YPD broth. They were cultured for 24 h at 30°C , 180 RPM, after which the cells were washed twice with distilled water, and a suspension for analysis with a concentration of 100 mg WW/mL was prepared.

Mechanical disruption of yeast. Prior to all but cell wall analyses yeast lysates were prepared using a Digital Disruptor Genie (Scientific Industries Inc., Bohemia, NY, USA) by adding 0.666 g of quartz sand to 1 mL of yeast suspension. The treatment was performed at 2700 RPM for 10 min. The lysates were centrifuged at 12000 RPM for 2 min (Minispin, Eppendorf, Hamburg, Germany) and the supernatant was extracted for analyses.

Analyses. The low molecular weight antioxidants were evaluated by the reduced glutathione (GSH) content in and Trolox equivalent antioxidant capacity (TEAC) of cell lysates. GSH content was determined by OD_{412} of TNB (2-nitro-5-thiobenzoate) chromophore produced by the reaction of GSH with DTNB (5,5'-dithiobis-(2-nitrobenzoic acid); D8130, Sigma Chemical Co., St. Louis, MO, USA) [13]. GSH (120000050, Thermo Scientific Chemicals, MA, USA) was used as standard in the $0\text{--}20 \text{ }\mu\text{g/mL}$ concentration range. To 980 μL 0.1 M potassium phosphate buffer (PPB) containing 5 mM EDTA, pH 7.5, 20 μL DTNB and 20 μL sample were added. OD_{412} was measured followed by its conversion to GSH concentration by a constructed standard curve. TEAC was established following the improved ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid);

194430, Sigma-Aldrich Co., St. Louis, MO, USA) radical cation decolourization assay measuring OD₇₃₄ [14]. Trolox (218940010, Thermo Scientific Chemicals, MA, USA) was used as standard in the 0–17.5 µmol/L concentration range. To obtain the ABTS working solution the stock solution (7 mM aqueous solution of ABTS with 2.45 mM potassium persulphate) was diluted with 50 mM PPB, pH 7, to OD₇₃₄ of 0.7. A 4 µL of each sample were mixed with 1 mL of the working solution. The decrease of the OD₇₃₄ was measured after 30 min and used to determine the Trolox equivalent by a standard curve.

SOD activity in cell lysates was determined spectrophotometrically using the SOD Assay Kit, 19160, Sigma-Aldrich. WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, mono-sodium salt) forms a water-soluble formazan dye upon reduction with superoxide radical. The rate of reduction of WST-1 is linearly dependent on the activity of xanthine oxidase. This reduction is inhibited by SOD since it converts superoxide radical. The percentage inhibition is calculated by preparing a standard curve for determining SOD activity. The measurements were made as described in the assay kit instructions [15]. All antioxidant analyses were performed 1 and 4 h after irradiation, as well as after 24 h of cultivation.

To study the porosity of the cell wall, a lyticase test was performed [11]. Cell suspensions with a concentration of 100 mg WW/mL were diluted 5 times in 250 mM PPB with pH 7. To 200 µL of the thus prepared cell suspensions, 5 µL of 2000 U/mL lyticase was added. They were incubated at 30 °C. Immediately after the enzyme addition, OD₆₆₀ was read, and then – at different time intervals up to 90 min, with 5 µL of the suspension being resuspended in 1 mL of distilled water. The initial OD₆₆₀ value for all suspensions was about 0.3, where there is a linear relationship between OD₆₆₀ and cell number. The decrease in OD₆₆₀ directly reflects the degree of cell lysis. The change in OD₆₆₀ was calculated as a percentage, with OD₆₆₀ for each sample before addition of lyticase being taken as 100%.

All the analyses were performed with UV-21 and V-10 Plus ONDA spectrophotometers (Giorgio Bormac S.r.l., Carpi, Italia).

Statistics. The data presented are mean with standard error of mean (SEM) values calculated from 4 independent experiments ($n = 4$). Two-way ANOVA with factors experimental variant and experimental repetition, followed by Holm–Sidak test, was performed to differentiate the statistically significant differences ($p < 0.05$) between control and EMF variants from those due to variations among individual experiments. SigmaPlot 11 program (Systat Software, Inc., USA) was used for the statistics procedures.

Results and discussion. Changes in the GSH content in the yeast cells after exposure are presented in Fig. 1A. GSH levels in EMF-treated samples were slightly lower than the controls 1 h after treatment but that was not statistically significant. Real reduction in GSH due to irradiation was observed only 4 h after it.

Interestingly, lower-than-control GSH levels were spotted also after cultivating the treated cells but random sampling variability could not be excluded as underlying reason (Fig. 1B).

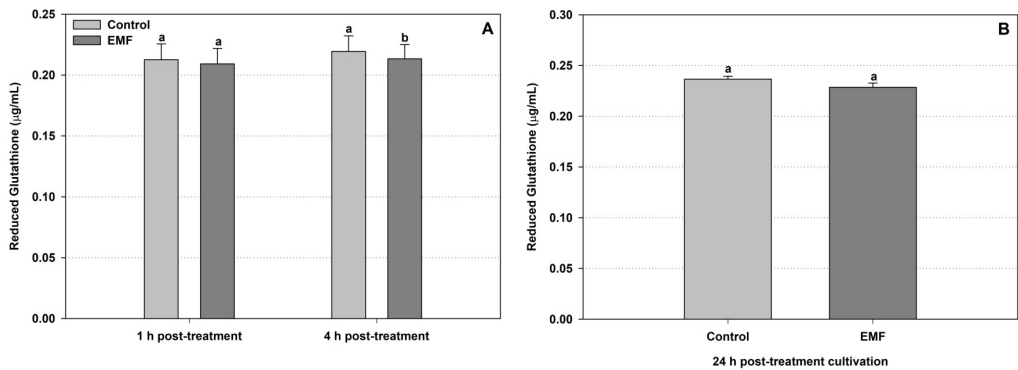


Fig. 1. GSH of irradiated with 2.45 GHz EMF (EMF) and non-treated (Control) *S. cerevisiae* suspensions 1 and 4 h after exposure (A) and in cells cultivated for 24 h from those suspensions (B). Average $\pm$ SEM values from 4 independent experiments are displayed ($n = 4$). Distinct letter labels denote significantly different experimental variants according to Holm–Sidak test ($\alpha = 0.05$)

Alterations in the total antioxidant activity of the yeast cells after EMF-treatment are presented as TEAC in Fig. 2A. TEAC was slightly but insignificantly higher in the exposed samples 1 h after treatment compared to the controls but it increased significantly 4 h after irradiation. A non-significant trend toward increased TEAC was observed after 24 h post-treatment cultivation (Fig. 2B).

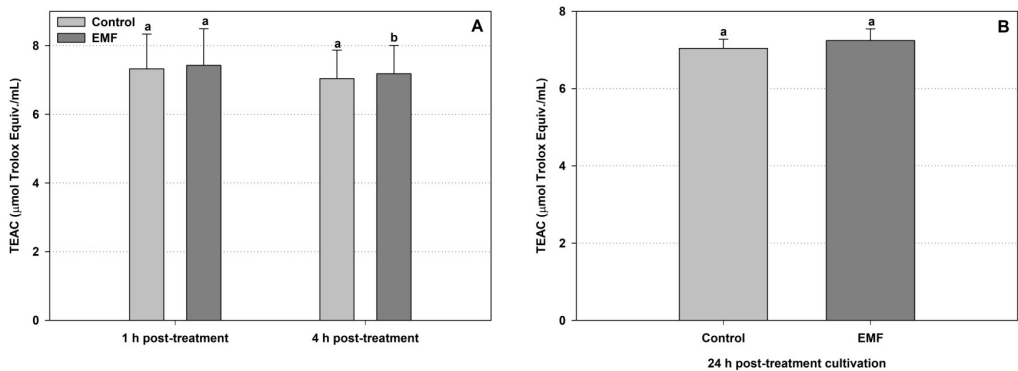


Fig. 2. TEAC of irradiated with 2.45 GHz EMF (EMF) and non-treated (Control) *S. cerevisiae* suspensions 1 and 4 h after exposure (A) and in cells cultivated for 24 h from those suspensions (B). Average $\pm$ SEM values from 4 independent experiments are displayed ($n = 4$). Distinct letter labels denote significantly different experimental variants according to Holm–Sidak test ($\alpha = 0.05$)

The results showed that there was no significant disturbance in antioxidant content 1 h after the treatment. However, minor disturbances in key cell homeostasis-supporting pathways could be triggered by EMF radiation [16]. Those disturbances could cause redox imbalance increasing in time. Indeed, EMF-related changes in antioxidant levels were registered on the 4th hour after irradiation, even though they were subtle. GSH was suppressed while TEAC was induced by EMF. GSH is a major water soluble antioxidant in the cell involved in a diverse set of ROS-neutralizing reaction. So GSH reduction is a logical effect if the applied irradiation conditions were able to cause elevated ROS formation as oxidative stress due to RF EMF was reported in many studies previously even at low-intensities, and we applied a relatively high electric field [4]. On the other hand, the rise in TEAC could be explained as a result of up-regulation of genes responsible for antioxidant homeostasis of the cell. Moreover, specific interaction between the EMF and DNA influencing gene expression have been reported [16]. It should be noted that TEAC measured by ABTS is determined by various antioxidant substances but only those that are neutral and negatively charged since ABTS is a cation [14].

SOD is an enzyme found in almost all cells and tissues that plays a key role in protecting cells from oxidative stress. It catalyses the conversion of the superoxide radical, a highly reactive and harmful form of oxygen, into more harmless molecules – hydrogen peroxide and molecular oxygen. MIGDAŁ et al. [17] showed that after 12 h of exposure of honey bees to 50 Hz EMF at different intensities, the activity of SOD increased up to fourfold, as well as other antioxidant enzymes in their haemolymph. LIAN et al. [18] observed significantly increased activity of SOD and catalase after short-term exposure to 50 Hz and 2 GHz EMF, but not

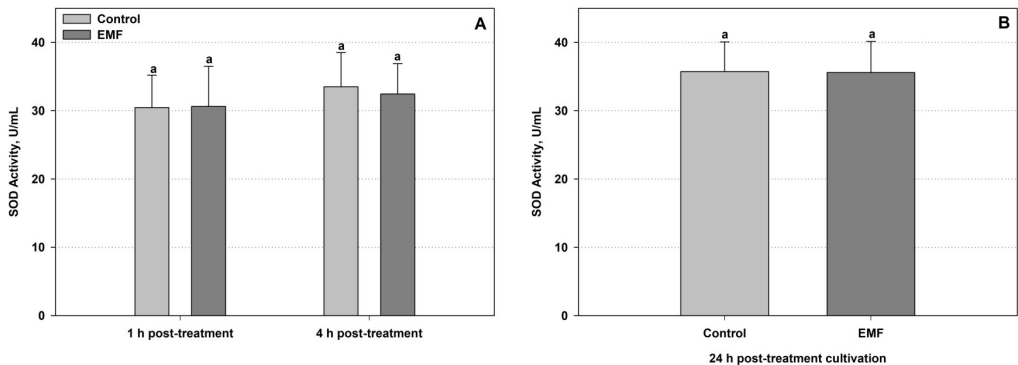


Fig. 3. SOD activity of irradiated with 2.45 GHz EMF (EMF) and non-treated (Control) *S. cerevisiae* suspensions 1 and 4 h after exposure (A) and in cells cultivated for 24 h from those suspensions (B). Average $\pm$ SEM values from 4 independent experiments are displayed ($n = 4$). Distinct letter labels denote significantly different experimental variants according to Holm–Sidak test ($\alpha = 0.05$)

after long-term exposure of yeast. However, irradiation of albino rats with 2.45 GHz EMF for 60 min daily for 4 weeks led to decreased SOD activity in skin cells [6].

Therefore, we decided to examine SOD activity in control and treated yeast suspensions 1 and 4 h after exposure (Fig. 3A). In both cases, we did not observe a statistically significant difference in the enzyme activity of the cells, but at 4 h there was a minimal trend towards a decrease in EMF. We also checked the SOD activity in a 24 h culture grown from the irradiated cells, but we did not find a difference compared to a control cell culture (Fig. 3B).

To test whether 2.45 GHz RF EMF affects the cell wall, we performed a lyticase assay on a cell suspension 4 h after exposure, as well as on a suspension obtained from cultivating the irradiated cells. In both cases, we found no difference in sensitivity to lytic enzymes (Fig. 4), indicating that the cell wall was not altered after treatment.

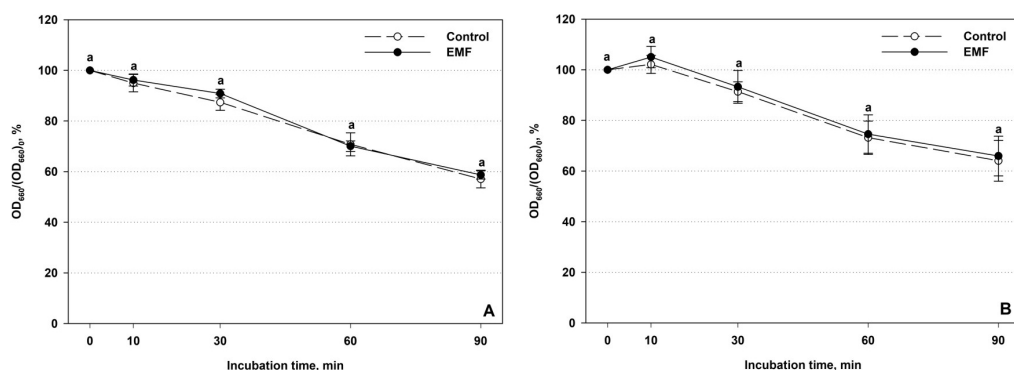


Fig. 4. Time course of relative (to the initial moment) OD₆₆₀ during 90-min lyticase incubation of irradiated with 2.45 GHz EMF (EMF) and non-treated (Control) *S. cerevisiae* cells 4 h after exposure (A) and of cells cultivated for 24 h from the treated and control cells suspensions (B). Average $\pm$ SEM values from 4 independent experiments are displayed ($n = 4$). Distinct letter labels denote significantly different experimental variants according to Holm–Sidak test ($\alpha = 0.05$)

The changes in the cell wall that VOYCHUK et al. [10] observed were at a much lower frequency EMF, which does not necessarily mean that changes are not possible at the one we used. As we noted, in the study by Lian et al. [18] both low-frequency and high-frequency EMF equally affected SOD activity so similar effects of both frequency bands on other parameters could be expected. The effects on the cell wall observed following yeast treatment with a very high impulse electric field were also accompanied by permeabilization of their cell membrane [11], which could suggest a different mechanism for the changes in the cell wall compared to the effects resulting from exposure to lower frequency in [10]. However, under the applied RF EMF conditions no changes in the structure of the cell wall were detected.

Conclusion. Treatment of yeast cells with 2.45 GHz EMF for 2 h resulted in a slight decrease in intracellular GSH and an increase in total antioxidant activity compared to control untreated cells on the 4th hour after exposure. A similar weak trend is observed after 24 h cultivation of irradiated cells. The applied treatment had no effect on SOD activity 1 h after treatment and after 24 h of cultivation, just a slight tendency towards a decrease was observed 4 h after treatment. Under the applied irradiation conditions, effects on the yeast cell wall were not detected. These results suggest that RF EMFs may subtly shift redox homeostasis without causing structural cellular damage under the conditions tested. Based on these findings, future research could focus on EMF-related genetic alterations, as well as on longer-term exposures, in order to clarify the potential long-term consequences of chronic RF EMF irradiation.

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REFERENCES

- [1] SIES H. (2020) Oxidative stress: Concept and some practical aspects, *Antioxidants* (Basel), **9**(9), 852.
- [2] MILLER G., V. SHULAEV, R. MITTLER (2008) Reactive oxygen signaling and abiotic stress, *Physiol. Plant.*, **133**(3), 481–489.
- [3] NUSZKIEWICZ J., A. WOŹNIAK, K. SZEWCZYK-GOLEC (2020) Ionizing radiation as a source of oxidative stress – the protective role of melatonin and vitamin D, *Int. J. Mol. Sci.*, **21**(16), 5804.
- [4] YAKYMENKO I., O. TSYBULIN, E. SIDORIK et al. (2016) Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation, *Electromagn. Biol. Med.*, **35**(2), 186–202.
- [5] NAZIROĞLU M., B. ÇIĞ, S. DOĞAN et al. (2012) 2.45-Gz wireless devices induce oxidative stress and proliferation through cytosolic Ca^{2+} influx in human leukemia cancer cells, *Int. J. Radiat. Biol.*, **88**(6), 449–456.
- [6] CEYHAN A. M., V. B. AKKAYA, Ş. C. GÜLEÇOL et al. (2012) Protective effects of β -glucan against oxidative injury induced by 2.45-GHz electromagnetic radiation in the skin tissue of rats, *Arch. Dermatol. Res.*, **304**(7), 521–527.
- [7] CROUZIER D., A. PERRIN, G. TORRES et al. (2009) Pulsed electromagnetic field at 9.71 GHz increase free radical production in yeast (*Saccharomyces cerevisiae*), *Pathol. Biol. (Paris)*, **57**(3), 245–251.
- [8] MACHADO B. R., P. G. P. SILVA, J. GARDA-BUFFON et al. (2022) Magnetic fields as inducer of glutathione and peroxidase production by *Saccharomyces cerevisiae*, *Braz. J. Microbiol.*, **53**(4), 1881–1891.
- [9] KITICHANTAROPAS Y., C. BOONCHIRD, M. SUGIYAMA et al. (2016) Cellular mechanisms contributing to multiple stress tolerance in *Saccharomyces cerevisiae*

- strains with potential use in high-temperature ethanol fermentation, *AMB Express*, **6**(1), 107.
- [10] VOYCHUK S. I., E. N. GROMOZOVA, P. M. LYTVYN et al. (2005) Changes of surface properties of yeast cell wall under exposure of electromagnetic field (40.68 MHz) and action of nystatin, *Environmentalist*, **25**, 139–144.
 - [11] GANEVA V., B. GALUTZOV, J. TEISSIE (2014) Evidence that pulsed electric field treatment enhances the cell wall porosity of yeast cells, *Appl. Biochem. Biotechnol.*, **172**(3), 1540–1552.
 - [12] ICNIRP (2020) Guidelines for Limiting Exposure to Electromagnetic Fields (100 kHz to 300 GHz), *Health Phys.*, **118**(5), 483–524.
 - [13] RAHMAN I., A. KODE, S. K. BISWAS (2006) Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method, *Nat. Protoc.*, **1**(6), 3159–3165.
 - [14] RE R., N. PELLEGRINI, A. PROTEGGENTE et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.*, **26**(9–10), 1231–1237.
 - [15] Sigma-Aldrich (2018) 19160 SOD Determination Kit <https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/254/301/19160dat.pdf>.
 - [16] BLANK M., R. GOODMAN (2009) Electromagnetic fields stress living cells, *Pathophysiology*, **16**(2–3), 71–78.
 - [17] MIGDAL P., A. MURAWSKA, A. STRACHECKA et al. (2020) Changes in the honeybee antioxidant system after 12 h of exposure to electromagnetic field frequency of 50 Hz and variable intensity, *Insects*, **11**(10), 713.
 - [18] LIAN H. Y., K. W. LIN, C. YANG et al. (2018) Generation and propagation of yeast prion [URE3] are elevated under electromagnetic field, *Cell Stress Chaperones*, **23**(4), 581–594.

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