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STUDY OF MICROWAVE RADIATION IN THE REDUCTION OF THE CONCENTRATION OF *Saccharomyces cerevisiae* IN THE SEARCH FOR A NEW RADIOTHERAPY FOR BACTERIAL ENDOCARDITIS

STUD OF THE *Saccharomyces cerevisiae* CONCENTRATION REDUCTION BY MICROWAVE RADIATION FOR THE SEARCH OF THE A NEW RADIOTHERAPY OF ENDOCARDITES

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Summary

In recent years, many efforts have been made to increase the tools for controlling the reduction of microorganisms through the use of microwave radiation, both in the health area and in the food industry. This study aimed to evaluate the lethal effect of microwave radiation on yeast cells *Saccharomyces cerevisiae*. A total of eight 20 mL aliquots of a suspension of *S. cerevisiae* in saline solution was used in the study, in which two of them were completely isolated from any type of electromagnetic radiation (control samples) and the others were bombarded in different scenarios, varying the power of the radiation in a resonance chamber at 2.45GHz for a period of 10 seconds. A reduction of around 102 was observed in the concentration of yeast suspensions exposed to electromagnetic radiation with 375 W and 750 W of power.

Key words: Microbial reduction; Microwave; Non-ionizing radiation; Radio-disinfection; Control of microorganisms.

Abstract

In recent years, many efforts have been made to increase the tools to control the reduction of microorganisms by the use of microwave radiation, both in the health area and in the food industry. This study aimed to evaluate the lethal effect of microwave radiation on Saccharomyces cerevisiae yeast cells. A total of eight 20-mL aliquots of a suspension of *S. cerevisiae* in saline solution were used in the study, in which two of them were completely isolated from any type of electromagnetic radiation (control samples) and the others were bombarded in different scenarios, varying the power of the radiation in 2.45GHz resonance chamber for a period of 10 seconds. A reduction of the order of 102 in the concentration of the yeast suspensions exposed to the electromagnetic radiation with 375 W and 750 W of power was observed.

Keywords: Microbial reduction; Microwave; Non-ionizing radiation; Radio-disinfection; Control of microorganisms.

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Introduction

The microwave reduction technique is performed by exposing microorganisms to non-ionizing electromagnetic radiation, whose oscillation frequency is between 300MHz and 300GHz, with a wavelength between 1m and 1mm and photonic energy from 1.24 μeV to 1.24 meV, respectively [1], [2], [3], [4].

In this exposure, two types of effects, thermal and non-thermal, causing microbiological reduction can occur. The thermal effects are caused by vibration through the interaction between dipoles of molecules and the electric field component (E-Field) of the electromagnetic wave and also by the appearance of electric currents whose carriers are free ions, causing the transfer of energy in the form of heat. . The non-thermal effects, in turn, are related to the transfer of energy, not in the form of heat, but as polar electrostatic effects, promoting the formation of hydrogen peroxide ($H_{two}O_{two}$), highly oxidizing and lethal for many microorganisms, in addition to breaking chemical bonds [5], [6], [7], [8].

The peculiar and deeply detailed cellular structure of*Saccharomyces cerevisiae*, commonly known as “brewer's yeast”, makes it one of the microorganisms of greatest interest to the scientific community as a model (Real Phantom) for study in areas related to Biology, such as Bioengineering, Biotechnology and Food Technology. It is found in the unicellular form, or in the form of pseudomycelia (filaments similar to the hyphae of molds). Its cellular reproduction occurs by budding. Its ability to ferment specific sugars is one of the main distinguishing factors among other yeasts [9].

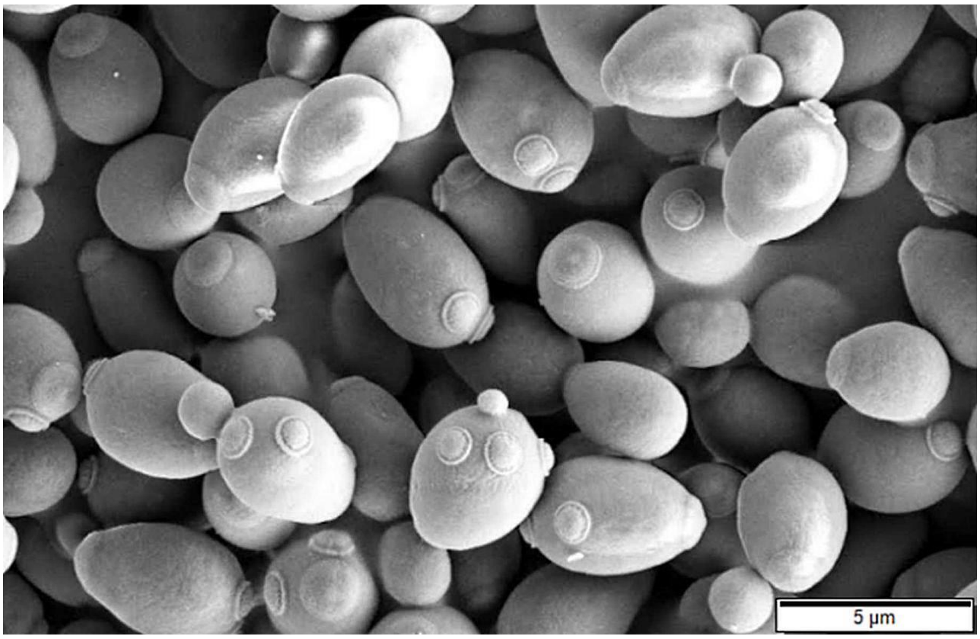

Figure 1: Specimens of Saccharomyces cerevisiae [10].

Development

An inoculum was transferred from*S. cerevisiae*to 100 ml tryptone soy broth enriched with 1.5% glucose, previously sterilized, followed by incubation at 35ºC for 24 hours. After incubation, a 20 -L aliquot of the culture was added to 200 mL of sterile 0.85% saline solution, obtaining the working suspension.

20 ml of the working suspension was added to eight sterile Petri dishes. Three pairs of plates containing the working suspension were subjected to microwave radiation for a period of 10 seconds in an equipment for domestic use, LG, model MS3048GA, with

input power of 1250 W and three different output powers: minimum (10% or 75W), medium (50% or 375W) and maximum (100% or 750 W). A pair of Petri dishes was not subjected to radiation, being considered the control group.

In this experiment, we sought to mitigate the possibility of reduction due to thermal effects, so preliminary tests were carried out with 20 mL of water in order to determine the final temperature to which the yeasts would be submitted during the entire process.

Table 1 presents the temperatures reached by samples of 20 mL of water after exposure for 10 seconds with radiation occurring under conditions of minimum, average and maximum power. A process of repetition occurred three times to obtain the average temperature of the 20 mL of water at the end of the exposure.

TABLE 1 - Mean values of water temperature after exposure to three different potencies for 10 seconds. n = 3

power (W)	temperature (oW)
75	38.3
375	50
750	51

From this first experiment, and based on the ambient temperature of the room, which was at 30°C, it was verified that the final average temperature obtained was only 23°C above the ambient temperature, minimizing the probability that the reduction would occur due to thermal effects.

Immediately after the irradiation tests, one milliliter (1 mL) of all samples of yeast suspensions, irradiated and control, was transferred to 9 mL of saline, resulting in a 1:10 dilution (10^{-1}). Next, 1 mL of this dilution was transferred to another tube containing 9 mL of saline solution, making the dilution 1:100 (10^{-two}). Finally, a 1:1000 dilution (10^{-3}) from the transfer of 1 mL of dilution $10^{-two}$to 9 mL of saline solution.

1 mL aliquots of dilutions $10^{-two}$and $10^{-3}$of all samples were inoculated in depth (pour plate) in tryptone soy agar supplemented with 1.5% glucose, followed by incubation at 35° C for 72 hours.

After the incubation period, colony forming units (CFU) counts of the yeasts on the plates were performed, using the procedure assisted by computer vision proposed by GEISSMANN [11].

Next, the possible effects of exposure to microwave radiation, suffered by the microorganisms described in this study, are presented.

Effects of microwave radiation

The main effect of microwave radiation in a medium is thermal, and – unlike radiation with frequencies from the visible range upwards – its interaction with matter does not occur through the absorption of the wave by an electron or by the nucleus of a medium. an atom, but by the action of the electric field of electromagnetic waves on the dipoles existing between polar molecules. The electric field of the wave interacts with the dipole causing the molecules to rotate when seeking alignment with the electric field and this movement is heat [12] [13]. Therefore, the interaction effectiveness of each medium with a given microwave frequency is directly related to its dielectric constant.

Microwave heating has some notable differences compared to conventional heating:

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1- For a medium containing a component capable of coupling with an incident wave frequency, much higher heating rates can be achieved.

2 – The energy source can be remote, that is, it can be far from the point of application/use.

3 – Each material interacts with different intensity and, thus, selective heating can be achieved within the same system [14].

In addition to these, non-thermal effects may also occur, such as an increase in the yield of reactions, conducted in a non-polar medium or without solvent, which have polar transitions [15], displacement of electrons within a molecule [16], reduction of the activation energy of some reactions [17], increased probability of contact between molecules of reagents – thus increasing the reaction speed – due to the friction and mobility caused by the microwave [18].

Other possibilities offered by microwaves are:

1 - Catalyst selectivity: Polar catalysts absorb energy differently and reach temperatures higher than the global temperature of the reaction medium [19], [20].

2 – Use of susceptors: Susceptors are materials that absorb microwaves efficiently and transfer the thermal energy generated to molecules in the neighborhood that do not have this efficiency. Although energy transmission occurs in a conventional way, it is faster than conventional heating [21], [22].

3 – Durability/stability of the catalyst: As microwave-assisted reactions are faster, this reduces the time of exposure of catalysts to high temperatures. Catalytic processes with shorter reaction times preserve the catalyst from deactivation and decomposition, thus increasing its useful life.

Thus, in the next Section, the results of tests on exposure to non-ionizing microwave radiation of microorganisms and their direct effect on reducing colony formation will be presented.

Results and discussions

After exposure to microwave radiation, a reduction in the concentration of viable cells of *S. cerevisiae*of suspensions exposed to electromagnetic bombardment for a period of 10 seconds, at the three different output powers in relation to the control, as shown in Table 2.

TABLE 2 - Concentrations of*Saccharomyces cerevisae*in the control sample and in the plates submitted to different output powers for 10 seconds.

		Powers (W)		
	Control	**75**	**375**	**750**
Concentrations (CFU/mL)	3.20×10^5	2.6×10^4	1.0×10^3	2.6×10^3

The results obtained in this study showed that the non-thermal effects of microwave radiation were efficient in microbial control, with emphasis on medium (375 W) and high (750 W) power, which caused a reduction of the order of 10^{two}or 2 log cycles in yeast suspension concentration.

The microwave reduction technique can be presented as a viable method for future developments, especially in the food industry, not discarding its use as a means of disinfection or even sterilization in health care procedures, sometimes in medical treatment such as radiotherapy , sometimes in the deactivation of microorganisms for the production of vaccines.

Non-ionizing electromagnetic radiation has as a residue, arising from non-thermal effects, hydrogen peroxide, which is lethal to bacteria of the genus*Streptococcus*, as they are incapable of producing the enzyme catalase, responsible for the cleavage of this substance.

The species *mutans*, *viridans* The *bovis*, of this genus are important etiological agents of bacterial endocarditis [23], [24]. It is inferred, therefore, the possibility of applying the microwave reduction technique, the objective of this study, as an auxiliary treatment for this pathology.

bacterial endocarditis

Bacterial Endocarditis (EB) is an infectious disease where pathogens invade the endocardial surface, causing damage and inflammation. Normally, a biota (vegetation) composed mainly of fibrin and platelets, in addition to infectious agents, is established in the heart valves.

The diagnosis is performed by transthoracic or transesophageal echocardiography, which is the second with better sensitivity and specificity results [25]. Despite advances in chemotherapy, the lethality of EB is between 13 and 40%. The variation in lethality can be attributed to the variable and dynamic profile of the vegetation, the causes and clinical complications, in addition to the diagnosis by echocardiogram. The incidence of EB varies between 1.7 and 6.2/100,000 people/year, aged between 30 and 40 years, affecting more men than women.

Degenerative valve alterations and prostheses are the most common causes.

Age above 40 years, class IV heart failure or cardiovascular shock, uncontrolled sepsis, conduction disturbances and arrhythmia are predictive factors of higher mortality [26].

Streptococcus It is *Staphylococcus* are bacteria that figure as the main causes of EB. These bacteria are widely found in the oral and intestinal biota, normally not causing disease, but when combined with risk factors, they can lead to EB and later death [27].

A *Streptococcus* It is a group D bacteria that can cause endocarditis. This is responsible for 14% of EB cases [28]. A *Streptococcus bovis* is among the main causes of EB and is associated with colonic neoplasia. Its action typically affects the aortic valve, with congestive heart failure as a consequence and complication, accompanied by valve abscess and systemic thromboembolism [24].

Alternative or complementary treatment methods to chemotherapy, such as the method proposed in this work using microwave reduction, are important to prevent or mitigate EB heart failure.

Conclusion

Through this study, it was possible to evaluate the lethal effect of microwave radiation on yeast cells *Saccharomyces cerevisiae*, generally regarded in the literature as Real Phantom.

A total of eight 20 mL aliquots of a suspension of *S. cerevisiae* in saline solution were used in this study, two of which were completely isolated from any type of electromagnetic radiation (control samples) and the others were electromagnetically bombarded in different scenarios, varying the power of the radiation in the resonance chamber at 2.45GHz for one period of 10 seconds.

There was a reduction of the order of 10two in the concentration of yeast suspensions exposed to electromagnetic radiation with 375 W and 750 W of power, leading us to believe that: Due to non-thermal effects, there was an alteration in the metabolism of microorganisms, specifically due to the synthesis of hydrogen peroxide , which led them to a very limited condition for life support.

In this way, it is concluded that exposure to non-ionizing radiation of 2.45 GHz, at a power of up to 750W, for no longer than 10 seconds, is lethal for microorganisms such as yeast cells *Saccharomyces cerevisiae*, thus inferring this condition of radio disinfection to bacteria of the genus *Streptococcus*, which in the species *mutans* It is *viridans* are etiological agents of endocarditis

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bacterial infection, opening a new horizon for the search for radiotherapy for this type of cardiac pathology.

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