Evaluation Of The Effects Microcurrent In Saccharomyces Cerevisiae As An Experimental Biological Model

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ABSTRACT: Objective: The *Saccharomyces cerevisiae*, a species of yeast, is used as a model of study for several types of purposes, exceeding then, as a model of biological application to the glucose human metabolism. The microcurrent can produce the lesser amount of measurable electric chains with similar and compatible electric signals to the electromagnetic field of the human body when these are recovering from tissue injury or has disruption of its normal electric activity. Also, it would stimulate a metabolic alteration to the point where it produces significant amounts and until the modulating of energy in the form of Adenosine Triphosfate - (ATP). The goal of this study was to evaluate the effect of the microcurrent through the parameters of the absorbance and pH in the metabolism of the glucose to explore the human similarities and the capacity of inquiry that the Saccharomyces cerevisiae supplies. **Methods:** One of the methods of glucose determination in used liquid samples more in the world and widely used in assays biochemists of dosage of reducing sugars and for studies of kinetic enzymatic is of the acid dinitrosalicylic (DNS), discovered in the end of the decade of 50 (Miller, L., 1959). The pH measurements have been performed in the chemistry from the very beginning; in this case it was used for H^+ detection and the pH measured correlated to the total glucose concentration present in the sample. First, we apply the microcurrent of three distinct intensities in liquid samples of saccharomyces; later the glucose for the metabolization was added. Second, we submit the samples for the analysis of the absorbance and pH for possible verification of the metabolization of the glucose and evaluation of the results. **Results:** In this study, it could be observed that the treatment, depending on the intensity of the applied microcurrent, caused an increase of the absorbance and pH when observed for the intensities of 100µA, 500µA and 900µA, showing that the cells had absorbed little glucose.
Conclusion: From the analysis of the acquired results, it can be suggested that the evaluated microcurrent is capable of modifying the glucose and calcium captation in the leavenings with the increase of the absorbance and pH.
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Introduction

Paragraph Number 1 The microcurrent, low-frequency chain or Micro Electro Neuro Estimulation (MENS), as it is also called, became a widely used therapeutical method and aesthetic in the diverse biomedical areas. It's effects and applications are often cited and present in protocols of rehabilitation of injuries and recovery, as much in athletes as in patients of clinics and hospitals that long for faster recovery. They are also are used with the intention of face rejuvenation in aesthetic centers through the chemical substance introduction, using

polarized microcurrents aimed at potencializing it's effects, being able to characterize these methods as some between as many methods used for the microcurrent as observed until today (Wing, T., 1998). However, according to (Lambert, I., Marcus, P., Burgess, T., Noakes, D., 2002), scientific studies indicate that certain mechanisms of action of the microcurrent still are unknown and require further study for its understanding and application. The Saccharomyces cerevisiae is an organism eukaryote used as the model in the study of the biochemist, genetics and cellular biology. This is due to it's ease of maintenance in the laboratory and the biological knowledge on it is well- developed. It's genome was already sequenced and its metabolic and enzymatic characteristics can contribute in the study for the intrinsic understanding of its mechanisms, allies to the effect and mechanisms of action of the microcurrent therapy when the same ones are submitted, the metabolic dynamics reflected by the glucose consumption and the relation of the capacity to generate energy, (Goffeau, A., Barrel, G., Bussey, H., et al., 1996).

Paragraph Number 2 With the increasing necessity to understand the behavior and application of the organisms in function of the optimization of the used therapeutical resources for a homeostasis condition, as well as for the agreement of the complexity and biological influence of both, the developed study aims to try some of possible chains of application of the microcurrent and its effect in the metabolism of the glucose in leavening of the Saccharomyces type cerevisiae, exploring the primordial similarities of the metabolism eukaryote inside the studies and the research of a therapeutical resource and its possibilities, in favor of not only of the microcurrent and of a microorganism called "Saccharomyces cerevisiae" and in favor of the development of the models for the scientific knowledge.

Methods

Paragraph Number 3 The Saccharomyces Cerevisiae, which can be found in bakeries with its weight or amount measured in grams and standardized in accordance with the factory specifications, was used on experimentals procedures with 30 grams of leavening of the Fleischmann® mark (Brazil) macerated and diluted in 500ml of distilled water. Later, the solution was used for the viabilization of the experiment at 37° Celsius for 15 minutes for the activation of enzymes contained in the leavening. After the dilution and the heating, the treated group - (G100, G500 and G900) the microcurrent application of the mark Mesolifting® MS-80093310014 under the following characteristics was submitted: (composed exit of a wave of 500Hz, modulated for a square shaped carrier alternated of 100KHz) in the representative intensities respectively the denomination of the main groups in its numbers, which had been 100µA, 500 µA, and 900µA in 500ml total of solution ($H2O_{(Distilled)}$ +*Saccharomyces Cereviciae*) for each group, and divided into 5 sub-groups of each corresponding intensity, constituted of 100 ml. The experimental procedures for the controlled group have been the same ones, except for the absence of the applications of the microcurrents in the solution, in which case it was submitted with the off device, only simulating the procedure of the treated group.

Continuing the experiment, the 100ml was warmed and divided for each one of the 5 sub-groups of the 4 main groups GC, G100, G500 and G900, to leavening, 500ml total of the solution ($H2O_{(Distilled)}$ + *Saccharomyces Cereviciae*), was added of 25ml D (+) Glucose Anidra P.A Dextrose ($C6H12O6$) 2% VETEC® and submitted for a period of initial partial metabolization of 10 minutes. After 10 minutes referring to the initial partial metabolization of glucose, the solution was warm again for the period of 30 minutes at 37°C, and afterwards, there was a rest period of 1 hour for supposed stabilization of the reactions. During the 1 hour rest period, samples of 4ml of the solution of each one of the 5 conditioned sub-groups and in pipes of assay separately and centrifugation to the 1500 r.p.m had been collected, and later, made the withdrawal of the supernatant of the sample, the heating was added 1ml of DNS submitting it 100°C during the period of 5 minutes. To quantify the concentration of the sugars in the way, not metabolizing for the Saccaromyces cerevisiae, the method of the acid dinitro-salicílico was used (DNS), being one of the methods of glucose determination in used liquid samples widely used in assays biochemists of dosage of reducing sugars and for studies of kinetic enzymatic discovered in the end of the decade of 50 (Miller, L., 1959). It has as it's basis the reading of the absorbance 540nm of the formed complex (after heating), for the glucose with the DNS. The assay is based on a reaction of oxi-reduction between the reducing sugar and the acid dinitrosalicílico (DNS), being followed for spectrophonometer in adequate wave length. This complex confers to the solution how much bigger a reddish tone in the amount of applied glucose (Miller, L.,1959; Nirmala, M., Muralikrishna, G., 2003; Reguly, C., 1996; Wanderley, K., 2004). At the end of the 5 minutes of heating of the sample-supernatant at 100°C, each sample was diluted in 13ml of distilled H2O, and submitted to the reading in spectrophonometer Oleman® 33D in the band of

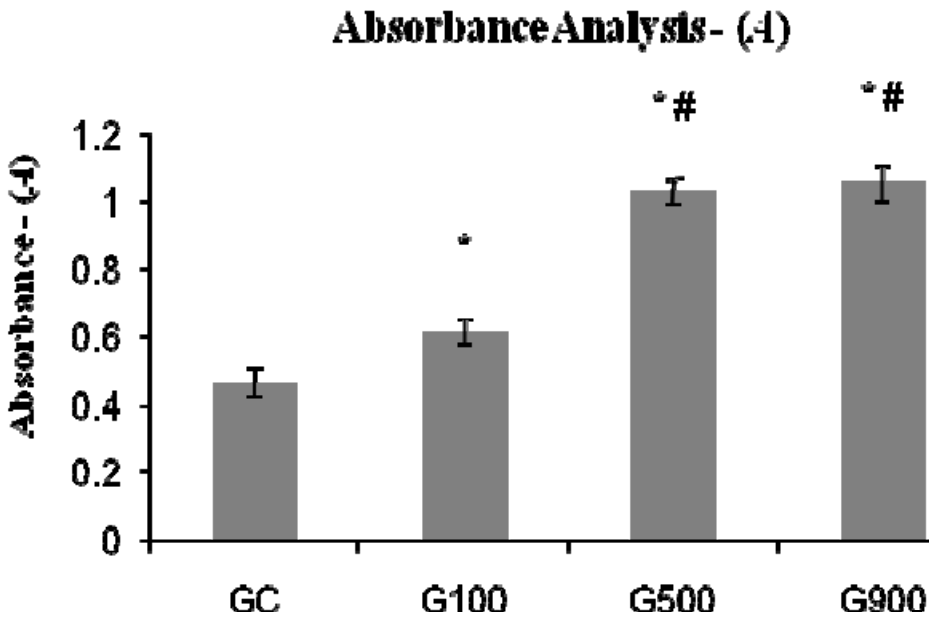
540nm and digital pHmetria Químis® for posterior evaluation of the results.

Paragraph Number 4 For the analysis of the collected data the descriptive statistics were used. In the analysis between the groups the variance analysis was used (ANOVA) and has tested F stops to compare the classrooms of the classified variable. For the localization of the differences, the test was used post-hoc of Bonferroni (P<0,05). The statistical package computational Graphpad Prism5® was used.

Results

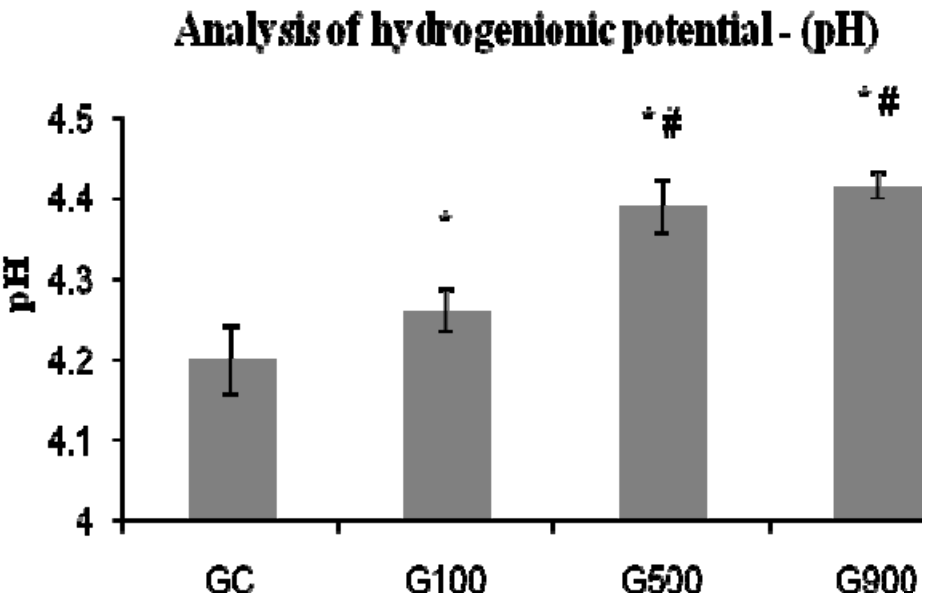
Paragraph Number 5 The graphs 1 and 2 represent the relative values to the average (x) and the shunting line standard (DP) of the studied groups which had been GC (Controlled Group), G100 (Group submitted to the microcurrent treatment 100µA), G500 (Group submitted to the microcurrent treatment 500µA) and G900 (Group submitted to the microcurrent treatment 900µA).

Paragraph Number 6 In accordance with the analysis statistics of the results, it can be observed that in relation to the analysis of the absorbance, there was a significant difference between G100, G500 and G900 (p<0,05) when compared with GC (Controlled Group). Still analyzing the above-mentioned groups with respect to absorbance, an alteration was observed statistically between the groups G500 and G900 when compared with G100 (p<0,05). In regards to the analysis of the hydrogenic potential - pH, produced different statistics (p<0,05) for the G100 groups, G500 and G900 when compared with GC (Controlled Group). However, in the referring analysis to the groups G500 and G900, a significant difference statistically when compared with G100(p<0,05) was observed. (Figure 1, Figure 2).



* Statistical difference in relation to GC (p < 0,05).
\# Significant difference in relation to G100 (p < 0,05).

Figure 1. Absorbance analysis



* Statistical difference in relation to GC ($p < 0,05$).
\# Significant difference in relation to G100 ($p < 0,05$).

Figure 2. Analysis of hydrogenionic potential

Discussion

Paragraph Number 6 In this study, it could be observed that the treatment, to depend on the intensity of the applied microcurrent, caused an increase the absorbance when observed for the intensities of 100µA., 500µA. and 900µA. This result demonstrated that the cells had absorbed little glucose, which could be related to the alteration in the potential of the membrane, causing a decrease of the activity of fosfolipase C (Plc1) reflecting the reduction of intake of citosólico Calcium, this leading to a decrease of the H (+) - ATPase and a possible minor glucose consumption. In fact, the possible increase in the Hydrogen concentration in the intermembrane space, would make possible a displacement of this ion for the half extracellular one, which would explain the increase of pH for the cited microcurrent intensities analysed.

These results concur with the findings of (Tökés, M., Bedwell, M., Repa, I., et al., 2002), which suggest a correlation of fosfolipase C (Pcl 1) with canals and subunits of high affinity for Calcium, present in the membrane called Cch1/Mid1 which could be dependent voltage. In relation to the analysis of the acquired results, it can be suggested that would have a bigger energy income in the treatment with 100 µA, a time that the comparative cells had absorbed more glucose when with chains of 900 and 500µA, a fact which could be explained by the diauxism.

It is known that the repression for glucose is responsible for the sprouting of this diauxism. In the presence of high glucose levels (above of the Ccrit), the genes that codify enzymes necessary to constitute the aerobic way, are restrained. Thus, the glucose is leavened producing etanol. The repression mechanism is based on the interaction between a signal, decurrent of the glucose, and the protein(s) regulatory(s) of the genic expression, activating repressed proteins or inhibiting activator proteins.

When being depleted of glucose, these genes are released and create the conditions so that now the

present carbon source (etanol - produced by the proper S. cerevisiae) either "canalized" or just reconstituted by aerobic (Ishtar, S., Yde, S., 2007).

It can be speculated that the microcurrent application in the studied intensities would consequently inhibit the activity of the Plc1 and of the opening of the Calcium canals and the glucose captation associated with a possible mechanism of draining of H+ of citosol decurrent of the accumulation of these in the mitocondrial intermembrana space from the activation of the H (+) - ATPase of the plasmic membrane.

The H (+) - ATPase constitutes one of most abundant proteins of the cytoplasmic membrane of fungii and has an essential paper in the physiology of the cell. The basic function of this bomb of prótons consists of creating essential an electrochemical gradient for the captation of nutrients and the maintenance of pH intracellular. It was demonstrated recently, the envolvement of the glucose sensor Snf3 p, protein Gpa2 p and of the protein kinase C in the way of transducing of signal, induced for glucose, involved in the regulation of cytosolic calcium and activation of the H (+) - ATPase (Lambert, I., Marcus, P., Burgess, T., Noakes, D., 2002).

However it was not possible, still, to elucidate at great length this finding, since it is necessary to explain as these different elements are integrated to detect and to transmit the signal generated for the glucose. In this work, the activation of the H was suggested that H(+) - ATPase could be inhibited by a difference of external electric potential, beyond being dependent of the extracellular calcium availability, even so the canal of membrane Mid1 p is not involved, and that the accumulation of IP3 intensifies the activity of the enzyme as suggested by (Belde, P., Vossen, J., Borst, G., Theuvenet, A., 1993).

Moreover, the Ca2++H (+) - ATPase was demonstrated that to vacuolar way Pmc1p is important for the control of the cytosolic intracellular calcium signalling and consequently for the regulation after-transcricional induced for sugar of the H (+) - ATPase (Trópia, J., Cardoso, S., Tisi, R., et al., 2006).

Works carried through for (Tökés, M., Bedwell, M., Repa, I., et al., 2002) had also shown evidence that hexokinase is not involved directly in the signalling process, that probably is mediated by the levels of phosphorylation sugars (Glucose 1 phosphate and Glucose 6 phosphate). With regard to the envolvement of Snf3p, it was demonstrated that this sensor functions in synergy with Gpa2 p, since in a mutant with deletion the genes that codify for these two proteins the activation of the H (+) - ATPase is practically absent. The results that had been presented by (Klochow, C., Stahl, F., Scheper, P., Hitzmann, B., 2008) suggest despite Snf3 p by way domain C-terminal, would be responsible for the detention of the internal signal of this (probably phosphorylation sugars) connecting the rise of the calcium levels with the activation of the H (+) - ATPase.

In our study we can speculate on a correlation of the chain intensities studied with the inhibition of the Calcium canals and of glucose, is possibly related with the inhibition of the H-ATPase, resulting in a bigger increase of pH extracellular.

Conclusions

From the analysis of the gotten results it can be suggested that the evaluated microcurrents had been capable of modifying the glucose and calcium captation in the leavenings with relation to the increase of the absorbance and pH.

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