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Influence of Agitation on the Propagation of *Saccharomyces cerevisiae* in the Process of Manufacturing Artisan Beers

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Authors' contributions

This work was carried out in collaboration with all authors. Author ACC designed the study together with the authors FAPP and JRDF. The author FAPP carried out the laboratory, wrote the protocol and wrote the first draft of the manuscript. Author ACC managed the analysis of the study. Authors ACC, FAPP and JRDF assisted in the preparation of the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In view of the growth of the production of artisan beers and the influence of aeration on microbial growth, this work aimed to show the importance of approaching the transfer of oxygen in aerobic fermentation processes.
Study Design: In this study the agitation in the propagation of yeast *Saccharomyces cerevisiae* in the artisan brewing process was carried out, as it is of fundamental importance to consider the function of oxygen in the control of the metabolism and growth of the yeast.
Place and Duration of Study: Laboratory of Food Microbiology, University of Uberaba, between June 2016 and July 2016.
Methodology: The research was carried out at the Laboratory of Microbiology of Food and for the propagation, a dry malt extract (DME) was used, which is basically obtained by the hydrolysis of the raw and malted barley, and then subjected to concentration, vacuum dehydration and milling. For

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the fermentation process, the lyophilized yeast of *Saccharomyces cerevisiae*, Fermentis® brand, safarine strain US-05®, with the initial concentration of 19 x 109 cells per gram, was used in 11.5 g sachets, as indicated by provider. The strain was obtained from a specialist trade and is a neutral American yeast with no ester and high tolerance to alcohol. The wort, with an initial density of 1.035 g.cm-3, was prepared by dissolving 45 g of DME in 450 mL of boiling water and after 15 minutes of boiling it was cooled to 25℃. This procedure was repea ted twice by obtaining propagation means 1 and 2. The yeast 11.5 g volume was divided into two portions and each portion was hydrated in 50 mL of distilled water at room temperature and inoculated into the propagation media 1 and 2. Sample 1 was shaken on a Nova Ethics® Kline shaker with rotation set at 120 RPMs for 10 hours. Sample 2 was rested and homogenized for collection every 1 hour for 10 hours. The determination of the cellular concentration (cel/mL) was performed in Neubauer chamber (1/400 mm2 x 1/10 mm) and for the determination of viable and non-viable cells, the International Coloring Method was used, using methylene blue as described in the methodology proposed by ASBC (1996). Viability is given by the ratio of viable cells to total cells.

Results: The system that remained with constant agitation has a growth approximately 15% greater than the system without agitation.

Conclusion: From the results obtained, it can be concluded that the propagation of yeast cells plays a fundamental role in the production of beers, whether homemade or industrial, because it increases the amount of viable cells, making the fermentation more intense. Agitation accelerates the consumption of substrate and, consequently, cell multiplication, besides increasing cell viability, which is fundamental for fermentation.

Keywords: Kinetic study; fermentation; yeast; microbial multiplication; oxygenation.

1. INTRODUCTION

Historical data on alcoholic beverages are inaccurate, and it is difficult to know when the first fermented alcoholic beverages were obtained, although there are citations about their use before the Christian era. The most accurate descriptions come from Arab authors of the tenth century, assuming that they have created the terms alcohol and alembic [1]. The first known beer was made by the Sumerians, a people who lived in Mesopotamia, the Middle East [2]. Beyond this civilization, the Assyrians and Babylonians were also excellent brewers [2].

Industrial beers have established themselves worldwide and today, in parallel with the industry, the movement of artisanal breweries gains space in the Brazilian brewing sector [3]. The fans of this style search around history, culture and quality of the drink and is also seen as a great business opportunity that attracts many interested in investing in production [3]. What distinguishes artisanal beer from industrial, besides the size of production, is the best raw material quality and the addition of regional products, which generates more robust and unique flavors [3].

The growing demand for specialty beers has been driving the industry to seek innovations for its process. In the context of fermentation, yeasts are a key point, both regarding the tolerance to the stresses of the process, ensuring its efficiency and the production of the aromatic compounds of the beverage [4].

In the brewing process, fermentation is important and *Saccharomyces cerevisiae* is a yeast that has shown a prominence in the various fermentative processes due to its ability to convert sugars into ethanol, organic acids and carbonic gas [4]. The performance of brewer's yeast in the fermentation is influenced and controlled by several factors: yeast strain employed, yeast cell stress tolerance, cell viability and vitality, cell concentration of the inoculum, concentration and nature of nitrogen the variety and concentration of sugars in the must, the availability of metallic ions, temperature, pH, dissolved oxygen and must density [4].

The aeration in fermentative processes is fundamental and essential to favor the growth conditions of the microorganism, and the aim of the agitation and aeration conditions are the dispersion of the air bubbles, consequently the oxygen supply to the microorganisms. Upon shaking the microbial cells remain in suspension with increasing heat transfer and mass in the medium [4].

In view of the growth of the production of artisan beers and the influence of aeration on microbial growth, this work aimed to show the importance

of approaching the transfer of oxygen in aerobic fermentation processes.

2. MATERIALS AND METHODS

The research was carried out at the Laboratory of Microbiology of Food of and for the propagation, a dry malt extract (DME) was used, which is basically obtained by the hydrolysis of the raw and malted barley, and then subjected to concentration, vacuum dehydration and milling. For the fermentation process, the lyophilized yeast *Saccharomyces cerevisiae*, brand Fermentis®, safarine strain US-05®, with the initial concentration of 19 x 10^9 cells per gram, was used in sachets of 11.5 g, as indicated by provider. The strain was obtained from a specialist trade and is a neutral American yeast with no ester and high tolerance to alcohol.

The wort, with an initial density of 1.035 g.cm-3, was prepared by dissolving 45 g of DME in 450 mL of boiling water and after 15 minutes of boiling it was cooled to 25℃.This procedure was repeated twice by obtaining propagation means 1 and 2.

The yeast 11.5 g volume was divided into two portions and each portion was hydrated in 50 mL of distilled water at room temperature and inoculated into the propagation media 1 and 2.

Sample 1 was shaken on a Nova Ethics® Kline shaker with rotation set at 120 RPMs for 10 hours. Sample 2 was rested and homogenized for collection every 1 hour for 10 hours.

The determination of the cellular concentration (cel/mL) was performed in Neubauer chamber (1/400 mm2 x 1/10 mm) and for the determination of viable and non-viable cells, the International Coloring Method was used, using methylene blue according to ASBC [5]. Cell viability was calculated from the equation below. Viability is given by the ratio of viable cells to total cells [6].

$$\text{Viabilidade celular (\%)} = \left(\frac{\textit{número de células viáveis}}{\textit{número de células totais}}\right) x\ 100$$

3. RESULTS AND DISCUSSION

Fig. 1. shows cell growth over 10 hours of propagation of the cellular systems with and without agitation.

The two populations depart from approximately 200 million cells per milliliters, and it can be seen that the system which remained with constant agitation has a growth approximately 15% greater than the system without agitation. This difference would have been greater if the non-agitated system was not homogenized every 1h for collection, thus the decanted cells were suspended, which increased the contact with the substrates and accelerated the growth. The agitation and consequent aeration of the medium favors the dispersion of the air bubbles and the supply of oxygen to the microbial cells and also their suspension by increasing the transfer of heat and mass in the medium.

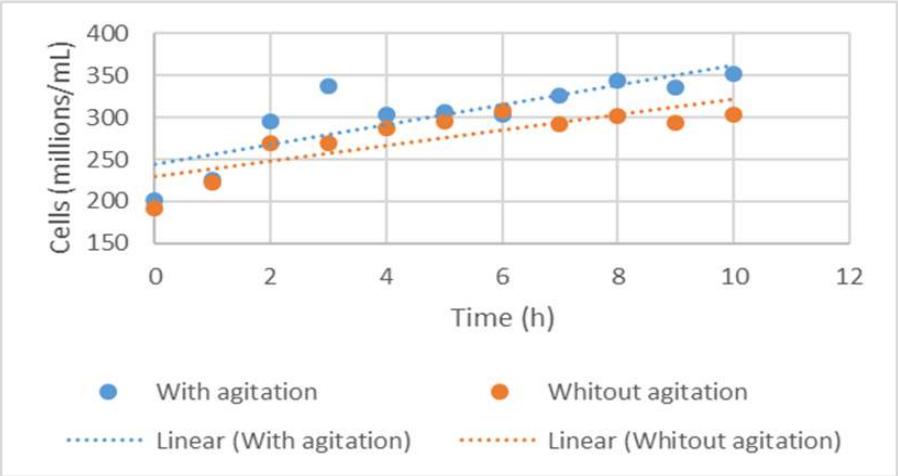

Fig. 1. Cell growth of systems with and without agitation

For facultative microorganisms, such as *Saccharomyces cerevisiae*, oxygen plays a crucial role in all metabolism because it participates in the generation of energy through the chain of respiration within the mitochondria, which is fundamental to obtain a specific specific growth rate [7].

The aeration of the must is one of the factors that affect the fermentation stage [8]. The amount of dissolved oxygen as well as the decrease in substrate concentration directs the assimilation sequence of the components of the fermentative medium as well as the formation of metabolic by-products in a coordinated manner. The flavor of beer results from the mixture of compounds derived from aerobic and anaerobic metabolism from the different stages of fermentation. The dissolved oxygen affects the production of fermentation by-products such as diketones, higher alcohols, esters and acetaldehyde [9]. In relation to the consumption of substrates, the results obtained are in Fig. 2, where the decrease of the density of the two systems can be observed.The musts that are obtained only from the malt contain as carbon source the following sugars: sucrose, glucose, fructose, maltose, maltotriose and dextrins [10]. The sucrose is hydrolyzed by an invertase enzyme which is secreted into the yeast periplasm, thereby increasing the concentration of glucose and fructose, sugars which are first assimilated [11] and when 50% of the glucose was consumed by the yeast, the maltose and maltotriose assimilation begin respectively [10].

The maltose that is the carbohydrate of greater quantity in the must, begins to be metabolized and when this reaches an undetectable level, maltotriose begins to be assimilated [11]. That maltose and maltotriose are ultimately used because glucose is the main source of carbon and energy for yeast [12]. The absorption of maltose involves two systems: energy-dependent maltose (ATP converted to ADP) transporters, which carry intact maltose through the cell membrane and maltase (α-glucosidase), which hydrolyzes maltose internally in order to obtain two glucose molecules. Maltotriose, on the other hand, has an independent carrier which depends on energy for intact transport, but shares α-glucosidase, which hydrolyses sugar into three glucose units [13].

From the results obtained it is possible that the lag time period ends first in the sample with agitation and it is noticed that from the second hour there is consumption of substrate. In sample 2, without stirring, this occurs only in the third hour. This higher consumption rate in sample 1 remains throughout the entire process, as the cells do not decant and remain in contact with the substrate for longer.

Through the graphical representation of cell viability as a function of the fermentation time presented in Fig. 3, it was observed that in the first hour, after inoculating the cells in the must, there is a drop in viability due to the osmotic pressure gradient. After 10 hours, viability is approximately 10% greater than the initial one in the two samples.

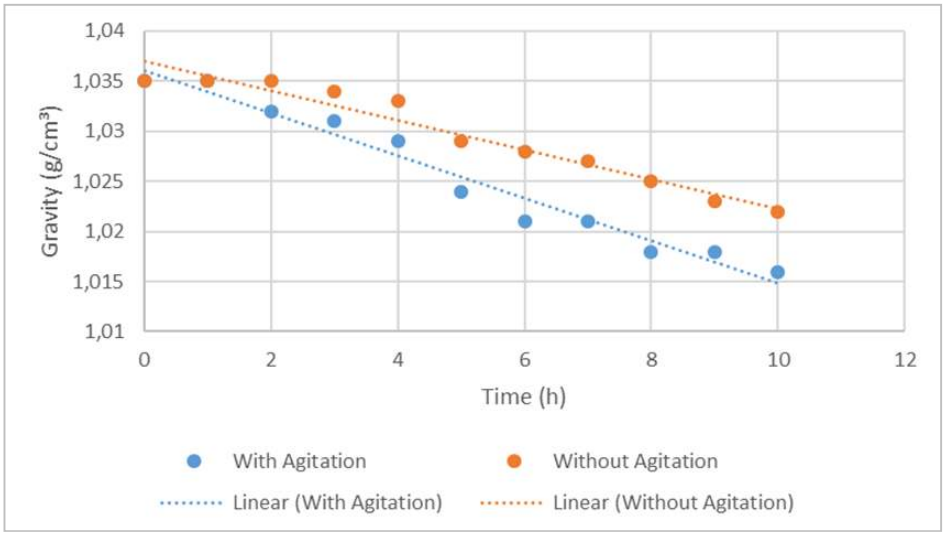

Fig. 2. Consumption of substrates over time

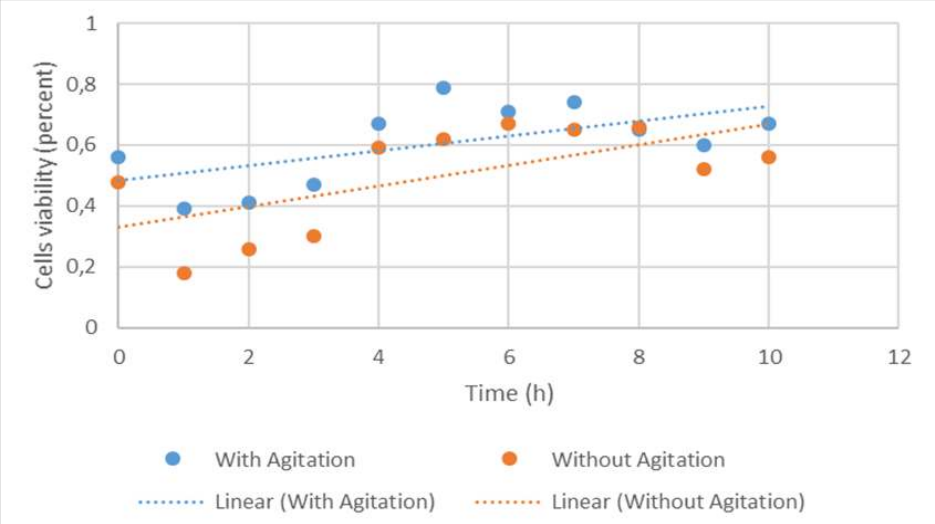

Fig. 3. Cell viability over time

The logarithmic or exponential phase, in which the cell growth is quite expressive, and consequently, there is higher consumption of the substrate and formation of the product (ethanol), was reached between 4h and 6h, for both experiments. The viability values were elevated throughout the fermentation, but there is a decrease between the eighth and tenth hours, which can be explained by the toxicity of ethanol, which is one of the main factors that cause instability in the fermentation process and also the reduction of available substrate [14].

It is important to emphasize that the fermentation stage depends not only on the aeration of the must, but also on several other characteristics of the medium, such as temperature, chemical composition and extract concentration in the must, quantity and mode of inoculation of the yeast in the must, geometry and dimensions two fermentation tanks. The aeration of the wort is not a determinant factor for the stability of the flavor, but an inefficient aeration can have a negative impact on the process [15].

4. CONCLUSION

From the results obtained, it can be concluded that the propagation of yeast cells plays a fundamental role in the production of beers, whether homemade or industrial, because it increases the amount of viable cells, making the fermentation more intense. Agitation accelerates the consumption of substrate and, consequently, cell multiplication, besides increasing cell viability, which is fundamental for fermentation. The fact that viability has increased less than that described in the literature shows that only the agitation is not enough for a more extensive multiplication, which can be improved with oxygenation of the used wort.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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