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Evaluation of different hydration techniques on the viability of commercial dry brewing yeast

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ABSTRACT. The utilization of dry yeast is increasingly becoming established in the brewing context, owing to the ease of storage and handling, the long shelf life and the convenience of dispensing a delicate and costly propagation process for fresh liquid cultures, which would need specialized labor and equipment. Dried yeasts undergo a procedure that removes nearly all liquid contents from the cell, and rehydration is then recommended prior to inoculation into the wort. Hydration is a nuanced procedure that allows yeasts to regain their vitality and viability, however, if carried out improperly, it can adversely affect fermentation. This study aimed to evaluate the impact of four hydration techniques on the viability and vitality of dry commercial yeast cells, preliminarily evaluating the influence of hydration using distilled and filtered water. Two different methodologies were employed, including the assessment of viability, by counting live and dead cells in the Neubauer chamber and the evaluation of vitality, by measuring the CO_2 released after inoculation. The hydration techniques tested included hydration in water without stirring, hydration in water with stirring, and these two techniques replicated in dry malt extract (DME) wort with Specific Gravity (SG) 1040. Firstly, it was found that rehydrating the yeast in distilled water has a negative impact on cell viability. Also, the results indicated that initial agitation has a negative effect on yeast viability and vitality, while hydration in water without agitation produced the best results. Correct hydration of dry yeast is crucial for avoiding off-flavors due to accelerated or delayed fermentation.

Keywords: importance of rehydration; dry brewing yeast; vitality.

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Introduction

In the context of the brewing industry, the use of dry yeast has been growing due to several factors (Jenkins, Powell, Fischborn & Smart, 2011). Having fresh yeast available in the right quantity, condition and at the right time has proved problematic, especially for smaller scale producers, who always face a lack of resources to achieve satisfactory results in propagation, harvesting, storage and reinoculation (Van & Van, 2003).

The most common method for removing water from commercial brewer's yeasts is the fluidized drying bed. First, the culture of the strain in question is propagated in the laboratory until it reaches the desired quantity, using protectors to ensure viability whilst drying (Irina, Maria & Michael, 2009). After this, the preliminary removal of water by vacuum filtration takes place, causing the yeasts to change from a cream to cake consistency, with approximately 30% of the dry weight, then the culture is transferred to millimeter tubes (0.2 mm). The remaining water is removed in the fluidized drying bed, where a constant stream of dry air at 35-37°C is used to drain water until the dry weight of the culture reaches approximately 95% (Jenkins et al., 2011).

In this context, some drawbacks to using dried yeasts have been reported, such as abnormal flocculation, lower viability, turbidity, and foam instability (Jenkins et al, 2011). In addition, during drying, changes in humidity affect the functionality of the cell wall and plasma membrane, including a drop in the amount of phospholipids, a change in the degree of saturation of fatty acids, and peroxidation reactions, which destroy membrane lipids. These changes also affect the transport of substances into and out of the cell and the fatty acid reserve, which would be necessary for division and accelerated growth at the time of inoculation (Finn & Stewart, 2002).

Moreover, there are further advantages to using dry yeasts, such as their use in primary and secondary fermentations, the ease with which seasonal beers can be produced, the ease and speed with which they can be handled and the possibility of storing them for long periods of time (Jenkins et al., 2011). It can also replace

the propagation phase and, in some cases, can reduce the need for aeration of the wort. In addition, dry yeast has similar fermentation characteristics to liquid yeasts and the problem of lower viability, around 20 to 30 per cent when compared to liquid yeast, can be solved with the inoculation rate based on the amount of live and dead cells (Finn & Stewart, 2002).

Just as the way in which water is removed from cells influences their ability to recover from desiccation, the way in which rehydration is carried out is equally significant. In initial stages of hydration or still dehydrated, they may not respond to the variation in environment found at the time of wort insertion and in cell viability tests, due to the fragile conditions of the cell membrane, highlighting the importance of acclimatization and correct rehydration to maintain membrane integrity (Jenkins et al., 2011). Yeast cells need to repair damaged areas and eliminate synthesized products as a response to stress, recovering their ability to replicate when completely rehydrated (Irina et al., 2009). Careless hydration can result in an error in the rate of inoculation, delayed fermentation, turbidity, and inconsistent foam, due to the high quantity of dead cells (Jenkins et al., 2011).

In another way, for the adequate attenuation of the fermentable sugars of the wort in the production of beer, adapting to the desired style, the quantity of yeast cells required must be calculated. It is important to emphasize that the inoculated yeast must have good viability (number of live cells in relation to the number of total, dead and live cells, %) and cell vitality (metabolic capacity of the cells) to ensure good cell proliferation and proper fermentation (Bamforth, 2006; Hornink, 2024). Beyond that, it should be noted that under or over inoculation can result in unpleasant flavors (off-flavors) or other unwanted off-flavors (Künze, 2004; Hornink, Galembeck, & Muxel, 2024).

Elevated inoculum concentrations lead to high diacetyl (undesired flavor) amounts, increase susceptibility to autolysis, as well as raising the cost of yeast and the volume of cells to be removed. Additionally, a below-ideal inoculum volume produces an excess of oxygen in the environment related to the quantity of cells, when contrasting to the inoculum at the proper concentration. This excess generates lipid synthesis, a likely limiting factor in cell growth. Consequently, there is a decrease in wort attenuation, fermentation delay, and a favoring of the production of high quantities of SO_2, a compound frequently reported as an off-flavor (Künze, 2004; Hornink, Galembeck, & Muxel, 2024; Muxel, 2022).

Nowadays, Fermentis and Lallemand are one of the main manufacturers who produce dry yeast using fluidized drying bed, and Fermentis presented some evidence that the use of distilled water and agitation did not have a negative influence on rehydration, with regard to cell viability and vitality (Fermentis, 2021; Lallemand, 2022). Against this background, the aim of this study was to compare the impact of different hydration techniques on the viability and vitality of a Fermentis brewer's dry yeast combining different media (water and dry malt extract – DME), with and without initial agitation.

Materials and methods

The SafeAle™ S-04 commercial yeast was used as study material, which aimed to measure the influence of four forms of hydration of dry yeasts, combining two means, distilled water and DME Specific Gravity (SG) 1040 wort, with two hydration techniques, without agitation and with agitation. Thus, it is possible to measure which technique-medium combination provided the lowest mortality for the yeasts during cellular hydration, that is, the highest viability in the case of the Neubauer chamber cell count methodology. It is equally important evaluate the impact of the technique-medium combination on the vitality of the yeasts, through the controlled fermentation test, which related the cellular metabolic capacity with the amount of carbon dioxide produced and apparent attenuation (Boulton & Quain, 2001).

Preparation of the wort for the tests

The wort was prepared with DME (about 0.1089 g per mL of water to reach SG 1040) and all tests below were performed in triplicate, with the following procedures.

Pre-test with distilled and filtered water

To evaluate the impact on viability by rehydration in distilled and filtered water, 0.5 g of dry yeast was inoculated on the surface of 5 mL of water (distilled and filtered) disposed in a 10 mL beaker. After inoculation, the solution was left for 10 minutes at rest and then gently shaken, followed by a new 5-minute rest. The water was filtered using Colormaq filter, premium model, which contains activated carbon and propylene.

Afterwards, an aliquot of 1 mL was removed to evaluate cell viability by counting cells stained with methylene blue in a Neubauer chamber.

Characteristics of filtered water: pH = 7.12, hardness = 68 ppm, T = 25 °C.

Hydration techniques

The hydration techniques were performed in 25°C in a 10 mL beaker and, when subject to agitation, it was carried out at 72 rpm during 30 seconds. All subsequent tests were carried out with distilled water, for standardization criteria.

(i) Inoculation in water, without initial agitation: 0.5 g of yeast was added in 5 mL of distilled water. After inoculation, the solution was left to rest for 10 minutes and then gently shaken, followed by a further rest of 5 minutes;

(ii) Inoculation in water, with initial agitation: 0.5 g of yeast was inserted in 5 mL of distilled water. After inoculation, the solution was gently shaken, leaving it to rest for 10 minutes, followed by a further gentle stirring and another rest of 5 minutes;

(iii) Direct inoculation in wort, without initial agitation: 0.5 g of yeast was inserted in 5 mL of wort. After inoculation, the solution was left to rest for 10 minutes and then gently shaken, followed by a further rest of 5 minutes;

(iv) Direct inoculation in wort, with initial agitation: 0.5 g of yeast was inserted in 5 mL of wort. After inoculation, the solution was gently shaken, leaving it to rest for 10 minutes, followed by a further gentle stirring and another rest of 5 minutes;

Viability assessment

After hydration of the brewing yeasts with a technique, the cells were stained with the insertion of 0.25% methylene blue in the previously diluted sample. This compound is able to stain dead cells, entering the compromised plasma membrane, allowing us to differentiate the living cells from the dead ones, once the living ones remain transparent, through the intact membrane. After staining, the solution was inserted into Neubauer's chamber and the living and dead cells were counted using light microscopy (White & Zainasheff, 2010).

Sample preparation for counting:

1. Perform the desired hydration technique;
2. Homogenize the sample containing the dried yeast with vortex for 3 seconds;
3. Remove 1 mL of the yeast and insert in the volumetric flask (100 mL) and perform the dilution, to facilitate the count 1:100;
4. Remove 1 mL of the diluted yeast and insert in the volumetric flask (10 mL);
5. Insert 6 mL of distilled water into the same flask and shake well;
6. Insert 1 mL of 0.25% methylene blue into the same flask and shake well;
7. Complete the volumetric flask with distilled water to the 10 mL mark;
8. Wait 2 minutes;
9. Gently clean the Neubauer coverslip and chamber with 70% alcohol and toilet paper;
10. Place the coverslip over the chamber and hold the side gently with your fingers;
11. Shake the flask and remove a small aliquot with a Pasteur pipette and insert a drop through the corner of the coverslip, in each area of the grid, taking care not to leave bubbles;
12. Perform the count in the Neubauer chamber.
13. Viability will be (White and Zainasheff, 2010): Viability (%) = (living cells/living cells + dead cells) * 100.

Controlled fermentation test

Controlled fermentation is used to compare the efficiency between hydration techniques in yeast vitality, assessing which combination among the techniques and the mean provided greater vitality for commercial yeast. With this experimental construction, the agitated fermentation system inoculated with the yeasts hydrated with the four techniques simultaneously, allows the exit of carbon dioxide as the fermentation occurs. In this context, the vitality of the yeast in question can be measured by observing that the yeasts consume the sugars present in the wort and produce, in an equimolar way, ethanol and carbon dioxide. Both carbon dioxide and the decay of gravity, measured in the experiment, are interconnected and are indicators of vitality (Boulton & Quain, 2001).

Preparation of samples for the test:

1. Add 30 mL of DME solution (SG 1040) in four Erlenmeyer flasks of 125 mL and incubate at 30°C for 10 minutes;
2. Weigh four aliquots of 1 g of dried brewing yeast in watch glasses;
3. Perform the four hydration techniques (i, ii, iii, iv) in 10 mL of distilled water;
4. Transfer the previously hydrated yeast to the Erlenmeyer;
5. Measure pH (digital pH meter) and specific gravity (digital light refractometer) - Brix;
6. Seal the neck of the flasks with the use of porous Kraft paper and string;
7. Place the Erlenmeyer vials on an analytical scale and note the total mass of the set of each vial;
8. Incubate in the incubator with orbital agitation at 30 °C and 150 rpm;
9. Allow the fermentation to take place, weighing and noting the dough of the set every 15 minutes up to 75 minutes from the beginning of the fermentation;
10. Perform calculations of carbon dioxide detached mass and apparent attenuation.

Statistical analysis

The assumptions of the analysis of variance were verified (Table 1), necessary for the data to fit into a parametric test model, using the Durbin-Watson test (Zeileis & Hothorn, 2002). To verify the independence of the errors of each observation, the Shapiro-Wilk test for the normal distribution of the residuals and the Bartlett test for the homogeneity of the variance of the errors (Ferreira, Cavalcanti & Nogueira, 2021). All these tests adopt a level of 5% significance, if the P value is lower in any of the tests, the data violate the assumption (Sheskin, 2011).

In case of violation of any of the three assumptions, the data were changed using the Box-Cox test (Venables & Ripley, 2002) for the transformation of linear models, which determines the exponent that accompanies the response variable, so that the data does not violate the assumptions of the analysis of variance when performing the tests again (Sheskin, 2011).

Table 1. Assumptions of analysis of variance of the data (ANOVA).

Methodology	Test	P value	Situation	Transformation P value
Viability	Durbin-Watson	0,5039	independent errors	-
	Shapiro-wilk	0,6658	normal residues	-
	Bartlett	0,2754	homogeneous variances	-
Viability: Distilled Water x Filtered Water	Durbin-Watson	0,1049	independent errors	-
	Shapiro-wilk	0,7091	normal residues	-
	Bartlett	0,89	homogeneous variances	-
Release of carbon dioxide (mass)	Durbin-Watson	0,073	independent errors	0,081
	Shapiro-wilk	0,01	abnormal residues	0,102
	Bartlett	0,1864	homogeneous variances	0,388
Release of carbon dioxide (Apparent attenuation)	Durbin-Watson	0,001	dependent errors	0,081
	Shapiro-wilk	0,019	abnormal residues	0,059
	Bartlett	0,439	homogeneous variances	0,257

The analysis of variance of the data (ANOVA) was performed according to a completely randomized design for the two methodologies, considering the disposition treatment – response variable, at the level of 5% significance. In the case of significant difference $P<0.05$, the Tukey test (Ferreira et al, 2021) was used to analyze and compare the averages of the treatments (Sheskin, 2011). The entire data analysis procedure was done through the R software (R Core Team, 2023).

Results and discussion

Distilled water x Filtered water

It was found out that distilled water promotes negative impacts on the viability of brewing yeasts, when observing the low viability presented by cells hydrated with distilled water compared to cells hydrated with filtered water (Figure 1). This comparison is validated by the difference found in the statistical analysis of the data (Table 2).

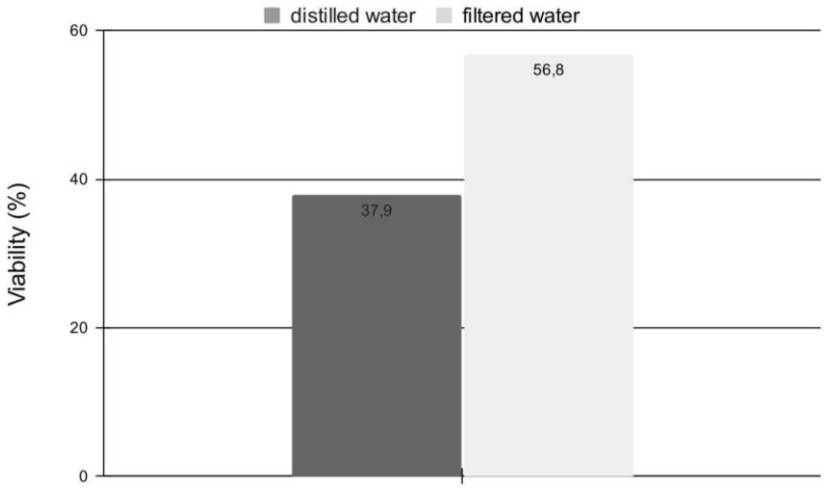

Figure 1. Viability (%) of yeast cells using distilled water and Filtered water in hydration.

Table 2. Statistical analysis of the data (ANOVA) and post-hoc test.

Methodology	P value	Tukey's test		
		Treatments	Groups	Averages
Viability: Distilled Water x Filtered Water	0,013	FW	a	56,8
		DW	b	37,9
Viability	0,16	-	-	-
Release of carbon dioxide (mass)	0,0017	MS	a	0,1
		AS	a	0,098
		MA	ab	0,089
		AA	b	0,084
Release of carbon dioxide (Apparent attenuation)	0,067	-	-	-

FW: Filtered water; DW: Distilled water. MS: Wort without agitation; AS: Water without agitation; MA: Wort with agitation; AA: Water with agitation.

The phenomena occurring in the plasma membrane during cellular dehydration and rehydration play a significant role in the discussion of the data. Under normal conditions, the phospholipid bilayer of biological membranes is in a fluid lamellar liquid-crystalline phase. Upon dehydration, as water molecules are removed, phospholipids come closer together, increasing the strength of Van Der Waals interactions between fatty acid chains, forcing the membrane into a gel phase. Rehydration allows the membrane to return to a liquid-crystalline state after the phase transition, potentially exhibiting both gel and liquid-crystalline phases simultaneously in the transient stage. The coexistence of these states can cause packaging problems in interactions between phase boundaries, leading to significant alterations in membrane permeability, possibly resulting in leakage of cytoplasm constituents and consequently cell death (Laroche & Gervais, 2003).

In this context, yeast cells are exposed to a combination of membrane instability during the transition phase and an osmotic pressure gradient. This gradient arises from the negligible intracelullar solvent concentration compared to the extracellular environment, which contains only solvent, in the case of distilled water. Consequently, as the extracellular medium has no solute concentration to balance osmotic pressure, a water flow into the cell occurs. This inflow, manifesting simultaneously with the conformational changes of phospholipids, induced by the membrane during the transitional phase, results in cytoplasmic leakage, followed by cell death (Laroche & Gervais, 2003).

Comparison between hydration techniques: viability

In relation to the comparison between hydration techniques, the combinations between medium – hydration technique were considered equal by the statistical analysis (Table 2). However, the combination 'water without initial agitation' was the technique which killed the least cells (Figure 2), 19% less when compared to 'water with agitation' even using distilled water. As previously discussed, distilled water can damage the dry cell membrane and, when combined with early agitation, could provoke even more impacts on the viability of yeasts. Previous studies showed the negative effect when agitation exceeds that necessary to homogenize the cells with the medium (Soubeyrand, Julien & Sablayrolles, 2006). In addition, other studies reported a decrease of 5 to 7%, in viability, related to mechanical stirring (Ferrarini, Bocca, & Cavazza, 2007).

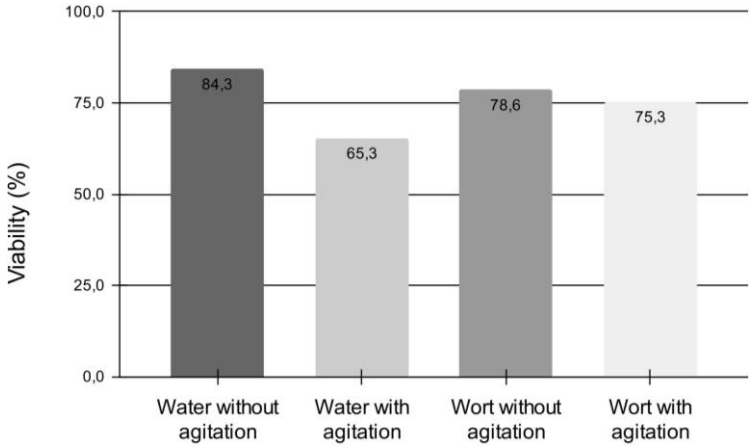

Figure 2. Viability (%) of yeast cells mediated by hydration techniques.

Hydration media containing DME (‘wort without initial agitation’ and ‘wort with initial agitation’) also perform good viability rates (Figure 2), even with early mechanical stress slightly reducing cell viability, in the case of the ‘wort with initial agitation’ treatment. This demonstrates the importance of the plasma balance between the intracellular and extracellular environment, when realizing that the DME provides to the extracellular environment a good concentration of solute with mineral salts and carbohydrates.

Comparison between hydration techniques: vitality

In regards to the mass of carbon dioxide released, the combinations of technique - medium ‘wort without initial agitation’ and ‘water without initial agitation’, were considered statistically equal, while the combination ‘wort with initial agitation’ fit into the two groups of averages (Table 2).

The combination ‘water with initial agitation’ showed lower mass detachment, therefore, lower yield, linked to factors such as cell death and damage from osmotic pressure and agitation (Figure 3). In addition, the treatments that presented higher yields in fermentation (‘water without initial agitation’, ‘wort without initial agitation’), are motivated by the attenuation of the damage and cell death, in both cases, due to the absence of early mechanical stress and osmotic stability, in relation to ‘wort without initial agitation’. Finally, referring to ‘wort with initial agitation’, there was a slight decrease in vitality due to stirring, however, it still maintained good performance with the balance of osmotic pressure, promoted by DME.

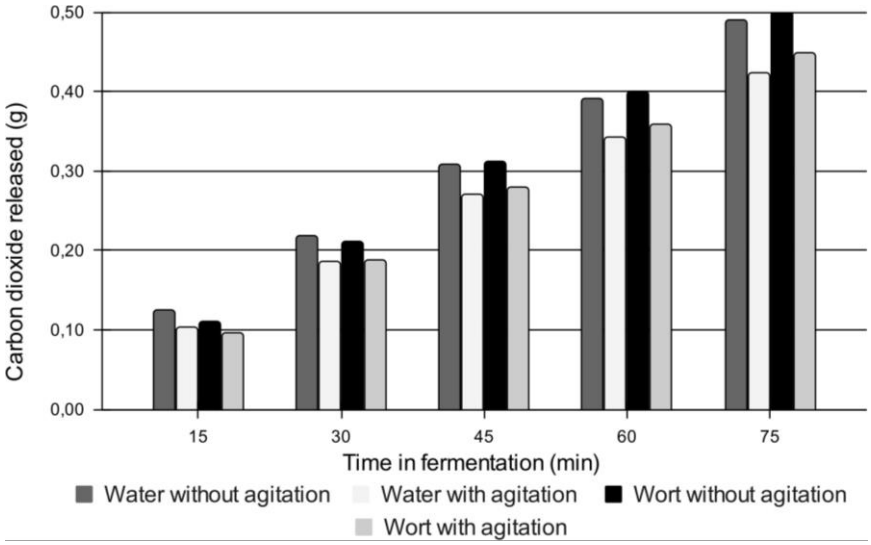

Figure 3. Mass of carbon dioxide released (g) by yeast cells influenced by hydration techniques.

Concerning the apparent attenuation, even though the statistical similarity of the data (Table 2), greater vitality is observed in the treatment ‘water without initial agitation’ (Figure 4). This indicates the efficiency of the use of water as a means for hydration and the outcomes of mechanical stress before rehydration, as observed in the ‘water with initial agitation’ treatment.

Not only, comparing the treatment 'wort without initial agitation' with the 'wort with initial agitation' and with the others, it is noticed that the use of wort for hydration provided good attenuation in both cases. Sugars, such as DME, have the effect of activating transcription and translation, as well as mobilizing trehalose, which is responsible for the negative regulation of the general stress responses. Transcripts related to ribosomal biogenesis together with trehalose are fundamental for the early response of yeast to hydration, resulting in great vitality (Schmidt & Henschke, 2015). Besides this fact, related to the treatment 'wort with initial agitation', the dispersion of nutrients in the hydration solution caused by the initial agitation also provides better adaptation of the yeasts to the wort, increasing fermentation yield when confronting wort without initial agitation. Worth highlighting that these facts are valid for both the methodologies that assess vitality.

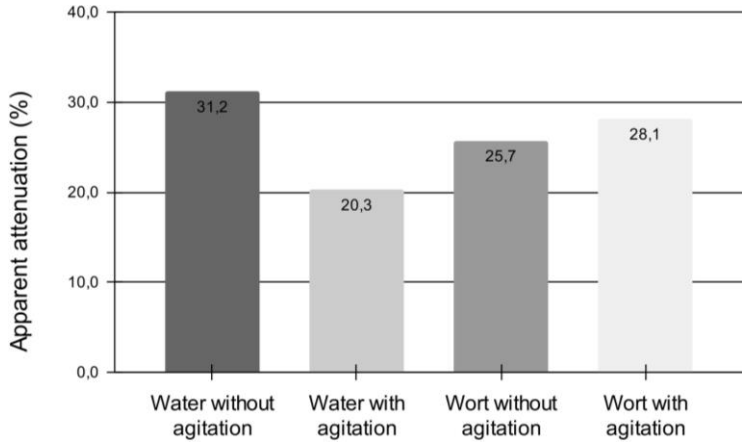

Figure 4. Apparent attenuation generated by yeast cells using each hydration technique.

Conclusion

From the results of cell viability and fermentation, it is possible to infer the hydration technique that provided better viability and vitality for the dry yeasts, which probably generated less possible stress and harm in the process of rehydration of the cells. In this case, the most efficient combination was that of water with absence of initial agitation, which presented best performance in viability, highest apparent attenuation and similar value of carbon dioxide detachment, all variables interconnected to vitality.

Finally, it is noteworthy that this study may have implications for beer production, since the refinement of the hydration technique of dry yeasts can increase cell viability and vitality, improving the fermentation process, reducing yeast costs and increasing beer quality.

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