THE RELATIONSHIP BETWEEN YEAST VIABILITY AND CONCENTRATION IN THE FERMENTATION PROCESS OF WORT FOR BEER PRODUCTION

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ABSTRACT

Beer production depends on a controlled fermentation of wort by a known variety of yeast. Yeast quality, in terms of its viability and vitality, depends of the integrity of the yeast plasma membrane. The yeast cell membrane is affected by the stresses that occur during the brewing process and particularly during storage. The number of viable cells and the total concentration of yeast slurry are important indicators for the performance of the fermentation. Historically, these parameters have been measured using methylene blue staining of the yeast population. Methylene blue is an autoxidisable dye, once it enters into the cytoplasm of a living cell results in its oxidation to the colourless leuco-form. In brewing, yeast may be reused many times. A number of yeast repitchings differs significantly among the breweries. Adjusting the number of times a strain may be serially repitched is of great importance for quality and consistency of final products. In this study, the methylene blue staining method was applied for determining yeast viability and spin method for determining the cell concentration of yeast slurry. Samples were taken from *Saccaromyces uvarum* (*carlsbergensis*) lager yeast at different generations, from I to XI, after a serial repitching. Meanwhile, yeast slurry samples were analyzed for the concentration of cells. The focus of this study was to evaluate the relationship between these two parameters. Two trials were carried out in a 12 months period per each. Based on the obtained results, the first trial showed no correlation at all with an insignificant value (0.001). The second trial showed a negative correlation between yeast viability and concentration with a value -0.28.

Keywords: Viability, concentration, yeast, *S. cerevisiae*.

INTRODUCTION

Breweries have attempted to determine yeast quality by measurement of viability (the percentage of live cells within a population) and/or vitality (metabolically active yeast). Methods for testing the viability and vitality of yeast cells rely on three general principles: loss of replication capability, cell damage and loss of metabolic activity (White *et al.* 2008)
Inoculating the beer wort with the correct number of yeast cells is critical for the consistent fermentation performance. Under-pitching results in longer fermentations, which has negative economic impact. Over-pitching, which gives rise to a faster fermentation, can lead to lower viability yeast crops, loss of bitterness, filtration problems and increased risk of yeast autolysis. In order to inoculate at the correct rate, brewers must measure accurately two parameters – yeast cell number and yeast viability (Thornton, 2002).

On the completion of brewery fermentation yeast is harvested from the fermentation vessel and after a short period of time reinoculated into a fresh wort batch. A number of times, yeast

can be reused, depends on a variety of factors, but mainly on the individual strain, quality of the cropped yeast, original wort gravity and company policy. There is a big variation in a number of yeast repitchings among the breweries. In some breweries a lager brewing yeast culture is used 2–3 times while in others even 7–9 times for fermentation of wort at similar original gravity. It has also been reported that lager yeast can be reused even up to 20 times (Kordialik, 2013).

Viability is most frequently determined by direct observation using microscopy. Staining techniques are used to differentiate between viable and non-viable cells. The dye most widely used to measure yeast viability in breweries is methylene blue. The methylene blue assay involves mixing the dye with a yeast sample, with live cells either excluding or reducing the dye, such that only dead cells stain blue (Boyd, 2003). The sample is then analysed by light microscopy. Although relatively inexpensive and easy to perform, the assay may be subjective.

Correct pitching rates also require accurate determination of yeast cell number. Traditionally this is assayed with the 'spin' method, which involves centrifugation of the yeast slurry and measurement of the subsequent pellet volume. Although relatively simple to perform, it is subject to various errors. The assay assumes a constant cell size. Further, the spin method does not account for varying levels of wort particles present in the sample (Priest, 2003).

METHODOLOGY

The assay of yeast viability and concentration was performed at a private brewing company in Tirana, Albania. Brewer's yeast taken into consideration was lager strain *Saccharomyces uvarum* (*carlsbergensis*). A total of 95 samples were analyzed over a 12-months period during 2014, 231 samples during 2015, belonging to generations I - XI. Samples were all taken from storage tanks. Generations I to XI made up 8%, 10%, 13%, 16%, 20%, 19%, 5%, 2%, 0.8%, 0.8% and 1.7% of samples, respectively.

The method of methylene blue staining is described in Analytica Microbiologica EBC Method 2.2.2.3. The method aims to provide an estimate of the percentage of viable cells present in a sample of yeast. The methylene blue 0.01 g was dissolved in 10 ml distilled water and later 2 g of sodium citrate dehydrate was added to the solution. Yeast samples of 1 ml were diluted in 9 ml of distilled water. Solution of methylene blue and diluted yeast cells were placed on a glass slide under a coverslip. Examination under ten microscope fields was performed using 100x magnification. The viability was reported as the percentage of unstained cells.

The cell concentration was analyzed by spin method. The yeast slurry (47 ml) was centrifuged for 15 min in Rotofix 3500rpm, 22°C, in a 100 ml tube. The cell concentration percentage was reported as pellet volume compared to total volume.

The linear correlation between the viability percentage and the cell concentration percentage was calculated with Microsoft Excel program.

RESULTS

The accepted level of viability is 80% of viable cells, meanwhile the second and the third generations, which are highly adaptive rather than the first generation, may reach a vitality level up to 90%. The values have been calculated according to:

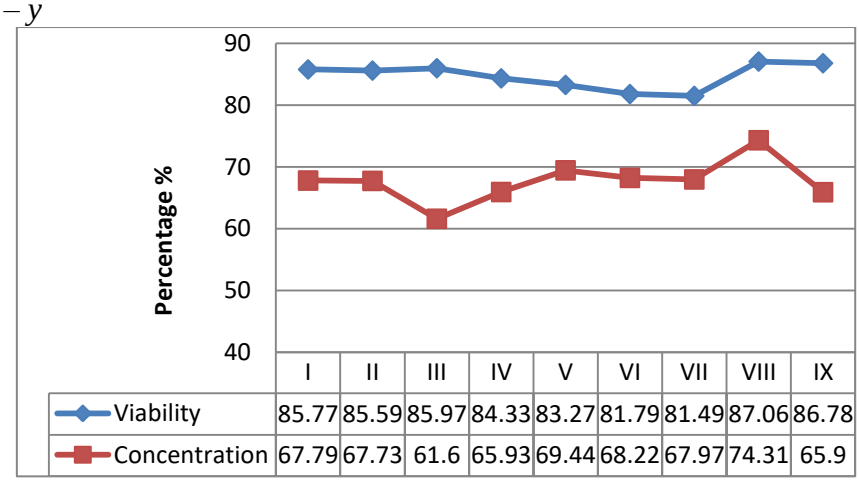
$$\textbf{\textit{Viability}}\ \% = \frac{\textbf{\textit{total counted cells} – \textit{total counted of dead cells}}}{\textbf{\textit{total counted cells}}} \cdot \mathbf{100}$$

Considering the cell concentration in the yeast slurry, the accepted level is 60%. The estimated percentage is calculated according to the following formulation:

Initial weight – *x*

Final weight – *y*

$$\textbf{\textit{Cell concentration}}\ \% = \frac{\boldsymbol{Y}}{\boldsymbol{X}} \cdot \mathbf{100}$$

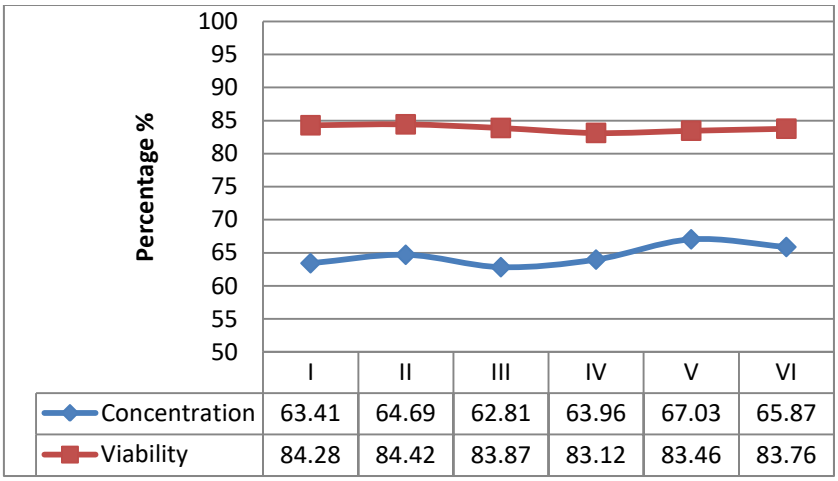


	I	II	III	IV	V	VI	VII	VIII	IX
Viability	85.77	85.59	85.97	84.33	83.27	81.79	81.49	87.06	86.78
Concentration	67.79	67.73	61.6	65.93	69.44	68.22	67.97	74.31	65.9

Figure 1. The average values of yeast viability at different generations during 2014

The results obtained for the evaluation of viability demonstrated a constant level above 80% (for the period of 2014). In the first three generations the average viability was almost the same, 85%. There was a slight decline from the generation III to generation VII, 85% to 81%, and a sharp increase to generation VIII and IX, 87% and 86%, respectively.

Concerning the yeast cell concentration, the average value for all the generations was above 60%. The results showed fluctuating data, starting from 67% in the first generations, followed by a sharp decrease in the third generation. However, the correlation between these parameters was very weak, showing a value of 0.001.



	I	II	III	IV	V	VI
Concentration	63.41	64.69	62.81	63.96	67.03	65.87
Viability	84.28	84.42	83.87	83.12	83.46	83.76

Figure 2. The average values of yeast viability at different generations during 2015

The viability results for the period of 2015 are almost constant for all the generations, showing an approximate level of 83% 84%. The obtained results for the evaluation of yeast cell concentration showed that the level never fell below 60%. In the first generations there

was a constant concentration of about 63%, meanwhile there was a slight increase in the fifth generation with 67%, following a decrease to 65%. The correlation rate in this trial between the two parameters was negative, showing a value of – 0.28.

DISCUSSION

Methylene blue staining has been the standard for assessing yeast viability since the 1920s. However, this method has recently been questioned given its poor reproducibility and inaccuracy with apparent viability below 90%. It is further suggested that living but damaged cell membranes may result in the occurrence of variable cell shading, which is likely to be responsible for inconsistent viability counts. The accuracy of the methylene blue procedure has been reported by some researchers to be reliable only at viabilities greater than 90%. It has also been reported that methylene blue will yield viabilities as high as 30–40% at 0% true viability (Smart, 2003).

CONCLUSIONS

The evaluation of yeast viability during the fermentation process of beer using methylene blue staining technique showed different levels of viability among the generations. The first generations showed an average viability about 85%, followed by a slight decrease in the later generations. But the difference was not significant, which means it is not noted any important changes in the biotechnological abilities of the fermentation yeast strain. Concerning the yeast slurry, all the generation showed a constant concentration with no significant differences among them. The average values were above the accepted limit. The first trial showed a very weak correlation (0.001) between viability and concentration which means that there is no effect on these two parameters. Meanwhile the second trial, carried out in a later period, showed a negative weak correlation (–0.28), which means that the concentration in generation IV, V and VI increases while the viability remains unchanged.

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