Rehydration of Active Dry Brewing Yeast and its Effect on Cell Viability

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ABSTRACT

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The use of active dry yeast (ADY) in the brewing industry is becoming increasingly attractive due to several key features, including its capacity to be stored conveniently and to be prepared rapidly for use. However, some studies have reported that undesirable effects may occur when employing lager strains in the ADY form, such as abnormal flocculation, haze formation and a less stable foam structure. These effects have been linked to reduced viability, caused by cell death during the drying and rehydration processes. It is known that the means by which yeast is rehydrated is important to maintain membrane integrity and to prevent potentially lethal damage from occurring. In order to determine the impact of rehydration conditions on yeast viability, three industrially manufactured ADY strains were examined. Each strain was rehydrated using a variety of parameters and monitored for cell viability using slide culture and a variety of brightfield and fluorescent stains.

INTRODUCTION

Active dried yeast (ADY) is widely used in industrial processes; in particular within the wine, distilling and baking industries, as well as the craft brewing sector. Some of the benefits associated with ADY include its capacity to be stored for extended periods of time and the speed at which it can be prepared for use. Furthermore, ADY has the potential to be used for primary and secondary (conditioning in bottle) fermentations, and to facilitate practices such as seasonal beer production and franchise brewing. Despite these characteristics, widespread adoption of ADY by larger brewing companies has not occurred due primarily to the absence of a driving force towards changing current practice within the brewing industry. In addition, this has been contributed to by previous studies which have suggested that some undesirable effects may be associated with the use of dried lager strains[4,11]. Although several reports have suggested that ADY performs in a similar fashion to brewery yeast

slurry[5,7] others have indicated that during laboratory scale fermentations, employing ADY may lead to abnormal flocculation, haze formation and a less stable foam structure[11]. These effects have been attributed to the reduced viability of ADY[11] and improvements to this parameter are important in order to increase consumer confidence in this product as a form of yeast supply.

The removal of water from yeast cells is a popular method of culture preservation but the process by which this is achieved can influence the viability of a yeast culture. Fluidised bed drying is the most common means of producing ADY for direct use in industrial fermentations, however other forms of drying such as freeze-drying and spray drying also exist. Freeze-drying consists of the removal of water by sublimination of a frozen culture under vacuum[18]. Viability of yeast preserved in this manner is sometimes as low as 0.1%[22] and consequently yeast preserved in this way is only appropriate for long term storage of strains. Spray-drying utilises a stream of hot air to rapidly dry droplets of a slurry solution, producing a powder[27]. Whilst industrial scale spray-drying is common for some bacterial species[3,26], it is not widely used for brewing yeast as it also produces low viability cultures[8]. Drying using a fluidised bed system[2,15] is less stressful to yeast cells than both freeze and spray drying and is furthermore capable of producing large quantities of biomass. A preliminary water removal stage based on vacuum filtration is used to convert yeast cream to a cake (approximately 30–32% dry weight) which is then extruded into noodle-like structures of approximately 0.2 mm in diameter. The remaining water is then removed in the fluidised bed drier, whereby a constant flow of dry air, maintaining the product at 35–37°C, is used to remove water until the dry weight of the yeast culture is approximately 93–95%.

Whilst the mode of dehydration can influence the capacity of a yeast cell to recover from desiccation, the process of rehydration is equally significant. Previous studies have investigated the effect of temperature[34], media composition and rehydration protocol[1,36,39] on the recovery of ADY. However, these studies have focused on the analysis of wine and baking yeast strains, which are typically more tolerant to stresses imposed by dehydration and rehydration than brewing yeast, and optimisation of the rehydration procedure for brewing yeast strains has only been the subject of limited investigation[12,14]. In this study we determine the effect of rehydration time and temperature on the viability of yeast cultures after rehydration and, in conjunction, assess methods to estimate live cells in rehydrated ADY cultures.

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MATERIAL AND METHODS

Yeast strains

Three strains of ADY brewing yeast; LAL1 (lager type yeast, commercial name Diamond), LAL2 and LAL4 (both ale type yeasts, commercial names Nottingham and Munich respectively) were provided by Lallemand Inc. (Montreal, Canada). Each strain was propagated using the industry standard protocol, involving batch and fed-batch growth on molasses based medium[10,17], before drying using a fluidised bed drier. ADY was provided in 20 g packets sealed under vacuum (LAL1) or 11 g nitrogen flushed packets (LAL2 and LAL4) and stored unopened at 4°C until required.

Growth media

YPD broth was composed of 1% [w/v] yeast extract, 2% [w/v] neutralised bacteriological peptone and 2% [w/v] glucose media. If required, YPD medium was solidified using 1.2% agar [w/v].

Rehydration

Rehydration was performed based on the manufacturer's guidelines (Lallemand Inc, Montreal, Canada) and samples for viability testing were recovered in triplicate at specific intervals. In each instance 1 g of ADY was sprinkled onto 10× its weight (10 mL) of temperature equilibrated (either 25 or 30°C as described below) tap water (pre-sterilised by autoclaving at 121°C and 15 psi for 15 min) in a 30 mL universal tube. The ADY was left to absorb water slowly for 15 min, mixed gently to form a slurry, and left undisturbed for a further 45 min. The temperature was maintained at either 25°C or 30°C throughout rehydration to determine the effect of this parameter on the viability of the final product once prepared for use. Samples for viability testing were recovered in triplicate immediately on suspension in water (Sample Point A), after initial mixing (Sample Point B) and subsequently at 15 min intervals for 1 h (Sample Points C1–C4).

Viability testing

The number of live cells in each population was estimated by microscopy using brightfield and fluorescent stains. In addition, a direct evaluation of the replicative capacity of cells was determined by slide culture. Irrespective of the method employed, triplicate samples were assessed and a minimum of 100 cells per sample were enumerated. The number of live (viable) cells is expressed as a percentage of the total population.

Methylene blue was dissolved in sodium citrate solution (2% w/v)[29] to a final concentration of 0.01%. Yeast cells were enumerated using a haemocytometer and diluted to a concentration of approximately 1×10^7 cells/mL. Yeast suspension (0.5 mL) was mixed with methylene blue (0.5 mL) and examined microscopically after a static incubation of 5 min at room temperature. Non-viable cells were stained blue and viable cells remained unstained.

MgANS (8-anilino-1-naphthalene-sulfonic acid hemi-magnesium salt hydrate) staining was performed according to the method of McCaig et al.[25] A 0.3 g aliquot of MgANS was dissolved in 2 mL of absolute ethanol and diluted in 98 mL of sterile water producing a final concentration of 0.3% (w/v). This was used as a stock concentration and stored at 4°C in a light protected container. Yeast cells were enumerated using a haemocytometer and diluted to a concentration of approximately 1×10^7 cells/mL. Yeast suspension (0.5 mL) was mixed with MgANS solution (0.5 mL) and incubated statically in a dark environment for 5 min at room temperature. Cells were examined using a fluorescence microscope (Optiphot-2, 100 W mercury lamp light source and a triple pass filter set for DAPI-FITC-TRITC, Nikon, Japan) at ×400 magnification. Non-viable cells appeared green and viable cells remained unstained[25].

Oxonol [bis-(1,3-dibutylbarbituric acid) trimethine oxonol (Dibac$_4$(3))] viability assessment was determined following the method of Lloyd and Dinsdale[21]. A 1 mg aliquot of oxonol dye (Invitrogen) was dissolved in 1 mL absolute ethanol and stored at –20°C in a dark environment. A 10 μL of the stock solution was diluted in 1 mL water to produce a working solution of 10 μg/mL. A 1×10^7 cells/mL yeast suspension (0.9 mL) was mixed with 100 μL of working solution of oxonol and incubated in a dark environment at room temperature for 5 min. Cells were examined using a fluorescence microscope (×400 magnification). Non-viable cells appeared green and viable cells remained unstained[21].

The slide culture technique used to assess viability was adapted from the American Society of Brewing Chemists Recommended Methods of Analysis[32]. In this instance YPD agar was used in preference to MYGP (malt extract, yeast extract, glucose and peptone) media. Using a pipette, 1 mL molten YPD agar was dispersed evenly over a 3 × 1 inch slide, previously sterilised by flaming. Once the agar had solidified, 10 μL yeast cell suspension (1×10^6 cells/mL) was pipetted onto the surface and a cover slip placed on top. Slides were incubated at 25°C for approximately 18 h. Cells were examined using a light microscope and individual cells giving rise to microcolonies were deemed viable, while single cells were scored as non-viable.

RESULTS AND DISCUSSION

The viability of industrial ADY cultures is believed to be dependent on multiple factors including the strain employed[23], the method of drying[15,22], the temperature of drying[24], the temperature of rehydration[20,30], and the rehydration media[31]. In order to determine the effect of rehydration conditions on brewing yeast cells, the impact of rehydration time and temperature on the viability of three industrially manufactured dried brewing yeast strains was determined.

The effect of rehydration time on estimated cell viability

Typical protocol requires yeast to be rehydrated for up to an hour before utilisation. Because of operational constraints this practice is often not adhered to when ADY is employed on an industrial scale. To determine the effect of rehydration time on the viability of the subsequent culture, rehydration was conducted according to the protocol outlined above. The viability of each culture was assessed
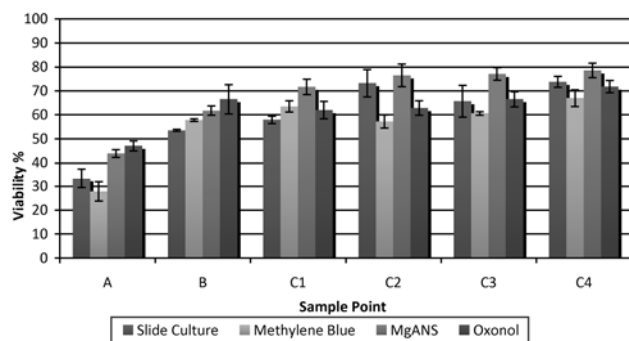
Fig. 1. Viability during rehydration of LAL1 at 25°C. Mean viabilities of ADY populations (triplicate samples with standard error shown).
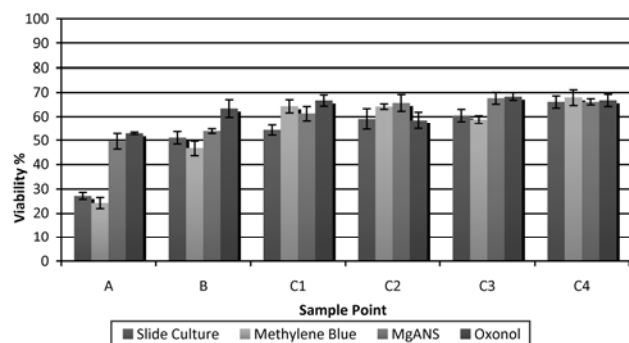
Fig. 2. Viability during rehydration of LAL1 at 30°C. Mean viabilities of ADY populations (triplicate samples with standard error shown).
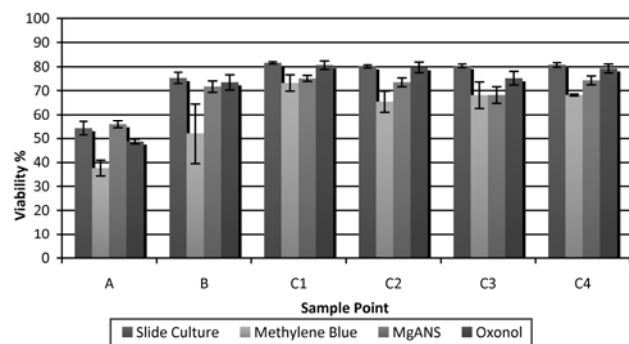
Fig. 3. Viability during rehydration of LAL2 at 25°C. Mean viabilities of ADY populations (triplicate samples with standard error shown).

Fig. 4. Viability during rehydration of LAL2 at 30°C. Mean viabilities of ADY populations (triplicate samples with standard error shown).

Fig. 5. Viability during rehydration of LAL4 at 25°C. Mean viabilities of ADY populations (triplicate samples with standard error shown).

Fig. 6. Viability during rehydration of LAL4 at 30°C. Mean viabilities of ADY populations (triplicate samples with standard error shown).

at specific time points using slide culture, bright field and fluorescent stains.

Analysis of samples at time point A revealed that although some variation in viability was observed according to the method employed (as discussed below). Viability was exclusively lower (25–50%, 40–55% and 20–55% for strains LAL1, LAL2 and LAL4 respectively) than at subsequent sample points for each of the strains analysed (Figs. 1–6). At sample point C4, when rehydration was deemed to be complete, the viability was observed to range from approximately 65–75% for strain LAL1 (Figs. 1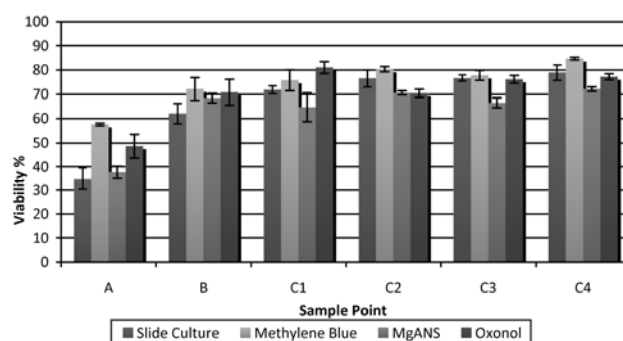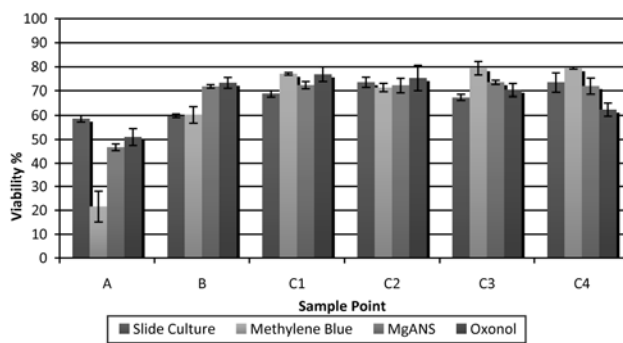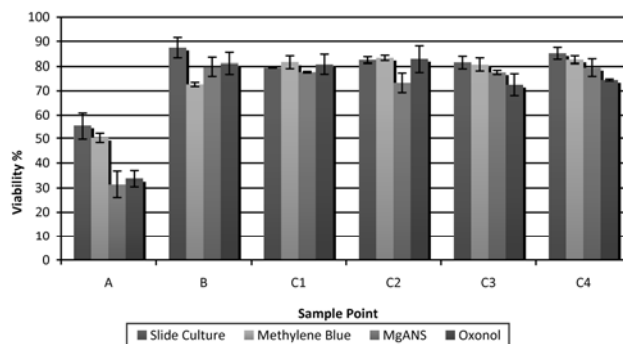 and 2) to 75–85% for strains LAL2 and LAL4. This suggests that the use of viability tests to assess ADY quality before rehydration has been completed may provide unreliable data and a more accurate estimation of cell viability can only be obtained once a culture has been fully rehydrated. However, it is also important to consider that the lower apparent viability at Sample Point A may indicate that there are differences in the physiological and metabolic state of yeast populations at this stage of the process. Viability analysis by means of slide culture, as well as the different staining methods, demonstrated an increase in the estimated number of live cells throughout rehydration (Figs. 1–6). Consequently, although it was

evident that cells were able to recover their capacity to replicate when fully rehydrated, these data suggest that yeast cells at an early stage of rehydration may not be able to respond to the change in environmental conditions required for viability assessment, perhaps reflecting the fragile condition of the cell membrane at this point. This hypothesis is supported by previous data indicating that direct pitching of yeast into wort can be detrimental and that pre-incubation is important to ensure fermentation performance (unpublished data). While the current reported data indicates that a shorter incubation period than previously suggested by the manufacturer (20–60 min) may be possible for the strains analysed here, it should be noted that the current measure of yeast condition (viability) does not guarantee fermentation performance[19]. Although viability did not appear to change significantly during the latter stages of rehydration, it is possible that this time period is required to remove the products of dehydration stress response, such as trehalose, which can cause inhibition of yeast metabolic pathways[13,33]. Consequently, although the precise benefits of allowing yeast to slowly acclimatise to the environment have not been directly assessed here, it is suggested that this period may play a significant role in determining how ADY performs in industrial scale fermentations.

Furthermore, while the data presented here indicates that the ADY populations in their dry form and during the initial stages of rehydration may demonstrate different physiological properties to fully rehydrated or wet yeast slurries, it is suggested that they should not immediately be considered to be non-viable, as the apparent viability increases during rehydration. Whilst drying results in cells in a dormant state that have non-viable characteristics, viable characteristics can be recovered during rehydration. This highlights the difficulties in assessing viability as an absolute phenotype. Practically, this is of significance given that an accurate assessment of the viable state of yeast cultures is important to ensure that the correct pitching rate is achieved. It is proposed that measurements of ADY viability should be treated with caution if taken at an early point during the rehydration process. A more accurate estimation can only be achieved once the yeast has been fully rehydrated.

The effect of rehydration temperature on cell viability

Laroche and Gervais[20] suggested that survival during rehydration is determined by the osmotic pressure and temperature of the yeast cell and the surrounding medium, and that rehydration across an unstable membrane is a significant cause of cell death. In order to determine the effect of rehydration temperature on cell viability, each yeast strain was rehydrated at both 25°C and 30°C and analysed using the techniques described above.

For the lager strain LAL1, it can be seen that cell survival was dependent on the temperature of rehydration, with a greater viability at time point C4 (complete rehydration) when 25°C was applied compared to rehydration at 30°C (Figs. 1 and 2). Although some variation was observed according to the method by which viability was estimated, at 25°C a viability of approximately 73% could be achieved compared to that of 67% at 30°C. This pattern of results was not observed for the other strains analysed, with the ale strain LAL4 exhibiting a higher viability when rehydrated at 30°C (approximately 80%) than at 25°C (approximately 72%). Interestingly, the viability of ale strain LAL2 did not appear to be as temperature dependent, with similar viabilities observed at both 25°C and 30°C (approximately 75% and 78% respectively).

The temperature of rehydration is believed to be particularly important in avoiding structural damage as a result of phase transition events (characterised by a loss of fluidity) within the plasma membrane[34]. Such events may be avoided by rehydration at a temperature above the phase transition temperature of the cell membrane[30], which may lead to improved viability in certain yeast strains. However, thermal tolerance in yeast is also known to be strain dependent and determined by factors such as the presence of protectants[6] and synthesis of heat shock proteins[37]. Therefore the benefit of increased rehydration temperatures in terms of membrane stability may be reduced in some yeast strains due to poor heat resistance. Lager yeast are known to have an optimum growth temperature that is considerably lower than those of ale yeast, the former being unable to grow at temperatures of above 34°C[40]. This suggests that the optimum temperatures for not only yeast function, but also rehydration, may be lower for lager strains than for ale yeast. This hypothesis is supported by our observation that higher viabilities were obtained for LAL1 (lager strain) at 25°C, while strains LAL2 and LAL4 (ale strains) were able to be rehydrated with good results at the higher temperature of 30°C. As a consequence, it is proposed that the rehydration temperatures selected for ADY should be aligned with the optimum functional temperature of each yeast strain and that deviation from these temperatures may adversely affect viability, a key factor in achieving a successful fermentation. Furthermore, it is anticipated that correct preparation of ADY may prevent excessive cell death (some cell death must be anticipated when using ADY) and that as a result the abnormal characteristics reported to be associated with using yeast in this form may be alleviated.

Methods for assessing ADY viability

The basic criterion for a yeast cell to be considered viable is that it has the ability to reproduce. Although techniques based on cultivation are able to directly assess the capacity of cells to divide, such methods are typically time consuming. Alternative methods based on cell staining are frequently adopted within industry as a means of estimating brewing yeast viability. Staining protocols are typically used to provide an indication of viability based on either membrane exclusion, the staining of cellular compounds, or by the ability of cells to convert or degrade the stain during cellular metabolism. Consequently, although such stains do not directly determine the capacity of cells to divide, they assess specific aspects of the cell which are critical to function and as such may be used as a measure of viability[38].

Despite its widespread use within the brewing industry for viability assessment, methylene blue is known to be inaccurate for the assessment of cultures with a viability of less than 90%[16,28,35] and may not represent the most

appropriate test for use with ADY, while other stains such as Oxonol and MgANS may be more suitable. Oxonol is a potentiometric fluorescent stain that is excluded from viable yeast, but enters cells when the trans-membrane potential is lost[21]. On entry, the dye binds to intracellular lipids and proteins and fluoresces, indicating that the cell is non-viable[9]. The dye MgANS is excluded from viable cells by the cell membrane, but can enter dead cells, where it binds to cytoplasmic proteins forming a highly fluorescent complex[25]. With the differing modes of action for each of these methods, it was anticipated that the results obtained might provide a more accurate reflection of the viable state of ADY cells.

Analysis of samples at time point A revealed that a large variation in viability was obtained when different methods were applied (Figs. 1–6). This variation may reflect the period of most change within the yeast cells, and population as a whole, which displays greater variation with respect to viability assessment. As suggested previously, this indicates that the use of viability tests to assess ADY quality before rehydration has been completed may yield unreliable data, and a more accurate estimation of cell viability can be obtained once a culture has been fully rehydrated. At sample point C4, when rehydration was deemed to be complete, the discrepancies between the percentage viability when different assessment techniques were applied was considerably reduced. Irrespective, although the means by which the viability of the yeast slurry was determined indicated variation between methodologies, no specific trends were observed and no individual technique gave consistently high or low estimates. Consequently no single viability technique can be recommended for analysis of rehydrated ADY cultures. This was perhaps surprising given that the mode of action of each of the methods employed differed, however the current data suggests that standard viability techniques employed in breweries are adequate for analysis of ADY, as long as the yeast population is completely rehydrated, as discussed earlier.

CONCLUSIONS

ADY cultures have been reported to exhibit lower cell viabilities than wet yeast populations of the same strain, leading to altered fermentation performance. Consequently it is important to ensure that ADY viability is maximised prior to inoculating a fermentation vessel. It is clear from this study that the method of rehydration may play a particularly important role in maintaining population health and yeast slurry viability. Specifically, incomplete rehydration, or rehydration at a sub-optimal temperature, is likely to result in impaired viability. While the current study has focused on the rehydration of yeast under laboratory conditions, these results may indicate that directly pitching ADY into wort (particularly cooler, lager type worts) could potentially result in viability loss and negatively influence fermentation performance. Furthermore, it is suggested that the optimum temperature of rehydration should be determined for each individual ADY strain and that these guidelines should be applied in the brewery in order to help ensure yeast viability and final product quality.

REFERENCES

1. Attfield, P. V., Kletsas, S., Veal, D. A., van Rooijen, R. and Bell, P. J. L. Use of flow cytometry to monitor cell damage and predict fermentation activity of dried yeasts. *J. Appl. Microbiol.*, 2000, **89,** 207-214.
2. Bayrock, D. and Ingledew, W. M., Fluidized bed drying of baker's yeast: moisture levels, drying rates, and viability changes during drying. *Food Res. Int.*, 1997, **30,** 407-415.
3. Corcoran, B. M., Ross, R. P., Fitzgerald, G. F. and Stanton, C., Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. *J. Appl. Microbiol.*, 2004, **96,** 1024-1039.
4. Cyr, N., Blanchette, M., Price, S. P. and Sheppard, J. D., Vicinal diketone production and amino acid uptake by two active dry lager yeasts during beer fermentation. *J. Am. Soc. Brew. Chem.*, 2007, **65,** 138-144.
5. De Rouck, G., De Clippeleer, J., Poiz, S., De Cock, J., van Waesberghe, J., De Cooman, L. and Aerts, G., Prolonged flavour stability by production of beer with low residual FAN using active dried yeast. Proc. Eur. Brew. Conv. Congr., Venice, Fachverlag Hans Carl: Nürnberg, 2007, pp. 455-467.
6. De Virgilio, C., Burckert, N., Bell, W., Jeno, P., Boller, T. and Wiemken, A., Disruption of TPS2, the gene encoding the 100-kDa subunit of the trehalose-6-phosphate synthase/phosphatase complex in *Saccharomyces cerevisiae*, causes accumulation of trehalose-6-phosphate and loss of trehalose-6-phosphate phosphatase activity. *Eur. J. Biochem.*, 1993, **212,** 315-23.
7. Debourg, A. and Van Nedervelde, L., The use of dried yeast in the brewing industry. Proc. Eur. Brew. Conv. Congr., Cannes, IRL Press: Oxford, 1999, pp. 751-760.
8. Elizondo, H. and Labuza, T. P. , Death kinetics of yeast in spray drying. *Biotechnol. Bioeng.*, 1974, **16,** 1245-1259.
9. Epps, D. E., Wolfe, M. L. and Groppi, V., Characterization of the steady-state and dynamic fluorescence properties of the potential-sensitive dye bis-(1,3-dibutylbarbituric acid)trimethine oxonol (Dibac4(3)) in model systems and cells. *Chem. Phys. Lipids*, 1994, **69,** 137-150.
10. Ertugay, N., Hamamci, H. and Bayindirli, A., Fed-batch cultivation of bakers' yeast: Effect of nutrient depletion and heat stress on cell composition. *Folia Microbiol.*, 1997, **42,** 214-218.
11. Finn, D. A. and Stewart, G. G., Fermentation characteristics of dried brewers yeast: Effect of drying on flocculation and fermentation. *J. Am. Soc. Brew. Chem.*, 2002, **60,** 135-139.
12. Fischborn, T., Cyr, N. Wardrop, F. and Gauthier, M., Dry beer yeast- New aspects of rehydration, storage and shelf life. World Brewing Congress 2004, San Diego, U.S.A., Poster 83 Abstract.
13. Gancedo, C. and Flores, C. L., The importance of a functional trehalose biosynthetic pathway for the life of yeasts and fungi. *FEMS Yeast Res.*, 2004, **4,** 351-359.
14. Gosselin, Y. and Fels, S., Fermentation characteristics from dried ale and lager yeasts. *Tech. Q. Master Brew. Assoc. Am.*, 1998, **35,** 129-132.
15. Grabowski, S., Mujumdar, A. S., Ramaswamy, H. and Strumillo, C., Evaluation of fluidized versus spouted bed drying of baker's yeast. *Dry. Technol.*, 1997, **15,** 625-634.
16. Jones, R. P., Measures of yeast death and deactivation and their meaning. Parts I and II. *Process Biochem.*, 1987, **22,** 118-134.
17. Jorgensen, H., Olsson, L., Ronnow, B. and Palmqvist, E. A., Fed-batch cultivation of baker's yeast followed by nitrogen or carbon starvation: effects on fermentative capacity and content of trehalose and glycogen. *Appl. Microbiol. Biotechnol.*, 2002, **59,** 310-317.

18. Kawamura, S., Murakami, Y., Miyamoto, Y. and Kimura, K., Freeze-drying of yeasts. *Methods Mol. Biol.*, 1995, **38,** 31-37.

19. Kobayashi, M., Shimizu, H. and Shioya, S., Physiological analysis of yeast cells by flow cytometry during serial-repitching of low-malt beer fermentation. *J. Biosci. Bioeng.*, 2007, **103,** 451-456.

20. Laroche, C. and Gervais, P., Achievement of rapid osmotic dehydration at specific temperatures could maintain high *Saccharomyces cerevisiae* viability. *Appl. Microbiol. Biotechnol.*, 2003, **60,** 743-747.

21. Lloyd, D. and Dinsdale, G., From bright field to fluorescent and confocal microscopy. In: Brewing Yeast Fermentation Performance, K.A. Smart, Ed., Blackwell Science: Oxford, 2000, pp. 3-9.

22. Lodato, P., de Huergo, M. S. and Buera, M. P., Viability and thermal stability of a strain of *Saccharomyces cerevisiae* freeze-dried in different sugar and polymer matrices. *Appl. Microbiol. Biotechnol.*, 1999, **52,** 215-220.

23. Luna-Solano, G., Salgado-Cervantes, M. A., Garcia-Alvarado, M. A. and Rodriguez-Jimenes, G., Improved viability of spray dried brewer's yeast by using starch (grits) and maltodextrin as processing aids. *J. Food Process Eng.*, 2000, **23,** 453-462.

24. Luna-Solano, G., Salgado-Cervantes, M. A., Ramierez-Lepe, M., Garcia-Alvarado, M. A. and Rodriguez-Jimenes, G. C., Effect of drying type and drying conditions over fermentative ability of brewer's yeast. *J. Food Process Eng.*, 2003, **26,** 135-147.

25. McCaig, R., Evaluation of the fluorescent dye 1-anilino-8-naphthalene sulfonic acid for yeast viability determination. *J. Am. Soc. Brew. Chem.*, 1990, **48,** 22-25.

26. Millqvist-Fureby, A., Malmsten, M. and Bergenstahl, B., An aqueous polymer two-phase system as carrier in the spray-drying of biological material. *J. Colloid Interface Sci.*, 2000, **225,** 54-61.

27. Morgan, C. A., Herman, N., White, P. A. and Vesey, G., Preservation of micro-organisms by drying; A review. *J. Microbiol. Methods*, 2006, **66,** 183-193.

28. O'Connor-Cox, E. S. C., Mochaba, F. M., Lodolo, E. J., Maraja, M. and Axcell, B. C., Methylene blue staining: use at your own risk. *Tech. Q. Master Brew. Assoc. Am.*, 1997, **34,** 306-312.

29. Pierce, J., Institute of Brewing Analysis Committee: Measurement of yeast viability. *J. Inst. Brew.*, 1970, **76,** 442-443.

30. Poirier, I., Marechal, P. A., Richard, S. and Gervais, P., *Saccharomyces cerevisiae* viability is strongly dependant on rehydration kinetics and the temperature of dried cells. *J. Appl. Microbiol.*, 1999, **86,** 87-92.

31. Rodriguez-Porrata, B., Novo, M., Guillamon, J., Rozes, N., Mas, A. and Otero, R. C., Vitality enhancement of the rehydrated active dry wine yeast. *Int. J. Food Microbiol.*, 2008, **126,** 116-122.

32. Russell, I., Berndt, R. L., Blenkinship, B. K., Casey, G. P., Coutts, A. D., Crabtree, D., Fernandez, S., Frati, M., Gould, A. M., Helbert, J. R., Krause, J., Lin, Y., Martin, P. A., McCaig, R., Middlekauff, J. E., Taparowsky, J. A., Engel, E. L. V., Wallin, C. E. and Hysert, D. W., Report of the Subcommittee on Microbiology. *J. Am. Soc. Brew. Chem.*, 1984, **42,** 132-134.

33. Sebollela, A., Louzada, P. R., Sola-Penna, M., Sarone-Williams, V., Coelho-Sampaio, T. and Ferreira, S. T., Inhibition of yeast glutathione reductase by trehalose: possible implications in yeast survival and recovery from stress. *Int. J. Biochem. Cell Biol.*, 2004, **36,** 900-908.

34. Simonin, H., Beney, L. and Gervais, P., Controlling the membrane fluidity of yeasts during coupled thermal and osmotic treatments. *Biotechnol. Bioeng.*, 2008, **100,** 325-33.

35. Smart, K. A., Chambers, K. M., Lambert, L. and Jenkins, C., Use of methylene violet staining procedures to determine yeast viability and vitality. *J. Am. Soc. Brew. Chem.*, 1999, **57,** 18-23.

36. Soubeyrand, V., Julien, A. and Sablayrolles, J. M., Rehydration protocols for active dry wine yeasts and the search for early indicators of yeast activity. *Am. J. Enol. Vitic.*, 2006, **57,** 474-480.

37. Trott, A. and Morano, K. A., The yeast response to heat shock. In: Yeast Stress Responses. S. Hohmann and W.H. Mager, Eds., Springer: Berlin, 2003, pp. 71-119.

38. Van Zandycke, S. M., Simal, O., Gualdoni, S. and Smart, K. A., Determination of yeast viability using fluorophores. *J. Am. Soc. Brew. Chem.*, 2003, **61,** 15-22.

39. Vaudano, E., Costantini, A., Cersosimo, M., Del Prete, V. and Garcia-Moruno, E., Application of real-time RT-PCR to study gene expression in active dry yeast (ADY) during the rehydration phase. *Int. J. Food Microbiol.*, 2009, **129,** 30-36.

40. Walsh, R. M. and Martin, P. A., Growth of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in a temperature gradient incubator. *J. Inst. Brew.*, 1977, **83,** 169-172.

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