JAP&T TPA-Timişoara

Available on-line at www.japt.tpa.usab-tm.ro

Journal of Agroalimentary Processes and Technologies 2009, 15(3), 370-377

Journal of Agroalimentary Processes and Technologies

Influence of yeast drying process on different lager brewing strains viability

Irina C. Bolat[a*], Maria Turtoi[b], Michael C. Walsh[a***]**

[a] – *Heineken Supply Chain, Dept. Research & Innovation, Brewing Science Yeast, Burgemeester Smeetsweg 1, 2382 PH Zoeterwoude, The Netherlands, Phone: +31 611.076.750, Fax: +31 715.457.800*
[b] – *Sibiu Alma Mater University, 57 Somesului St., 550003 Sibiu, Romania Phone +40 269.216.490, Fax: +40 269.250.008*

Abstract

The potential for the application active dry yeast within brewing industry is supported by the advantages this type of yeast offers. Dried yeast is more robust and stable for transportation, distribution and storage. Further, there is no requirement for skilled laboratory staff as there is for yeast supplied on slope.

During the drying process though, performed at high temperatures, a considerable inactivation of the yeast culture occurs. Lager brewing yeast strains are more sensitive at high temperatures than ale yeast strains, the cell viability being affected at a greater extend as well as their flocculation properties.

The present study focuses on assessing the viability of three lager variant strains that were subjected to drying using the spray-drying approach, with further emphasis on only one variant against the initial mixture of WS34/70. This was part of a larger project concerning WS34/70 lager brewing yeast characterization. The variants analysed within this paper had been previously selected and isolated from the WS34/70 lager brewing yeast and stored at -80°C. The karyotype of the three variants, displayed using the short-run technique for low molecular chromosomes, differ in the ratio of two bands.

Thus the ratio is higher than 1 for the first variant (variant *a*), is equal to 1 for the second variant (variant *b*) and is lower than 1 for the third variant (variant *c*). All the analyses were performed against the WS34/70 lager yeast which underwent the same drying/rehydration treatment as the variants. The number of the total cells and dead cells, as well as viability, glycogen and trehalose content were evaluated for the dried strains, after their rehydration both in 15P wort and in phosphate buffered saline (PBS) with different amounts of emulsifier. The importance of treating the yeast suspension with an emulsifier before drying was showed by the positive evolution of yeast suspension viability after rehydration.

The number of dead cells after rehydration gradually decreased. This phenomenon was obvious when a high amount of emulsifier (10% v/v) was added in the yeast suspension, before drying. A low content of emulsifier (0.05% v/v) was not enough to protect the cell wall from degradation, high amounts of dead cells being recorded after rehydration. As a result the yeast suspension viability is much higher in the first case. Yeast suspension viability was lower when re-suspending the dried yeast in wort than in PBS, both at 1:20 and 1:10 dilution. Variant *a* displayed the highest increase in viability after rehydration, while the mixture of strains WS34/70 showed to be very resistant to the drying process with high viability values even after 1h of rehydration.

Keywords: brewing yeast, yeast drying, wort fermentation, viability.

* Corresponding author e-mail address: bolatirina@yahoo.com
** e-mail address: maria.turtoi@uamsibiu.ro
*** e-mail address: mike.walsh@heineken.com

1. Introduction

The drying process within the brewing industry had been initially applied to ale yeasts. In the last years this has been extended also to lager yeasts which are being called active dry lager yeasts [1, 2].

The main dried yeast suppliers are Fermentis (Division of S.I.Lesaffre) and Lallemand. The term active suggests a high vitality as well as a fermentation capacity of the dried yeast similar to that of slurry yeast.

The main disadvantage of the dried yeast cultures is represented by the pronounced inactivation of the culture during drying. To overcome this drawback the factors involved in the inactivation process should be studied and understood [3].

Luna-Solano and his team (2005) [4] proved that the outlet temperature in the spray drying process, the rotor speed and the concentration of the protecting agents used affect the specific growth rate but not the maximum growth value.

There are many papers suggesting the use of protecting agents during yeast drying in order to improve its viability, such as maltodextrins [5] or trehalose [6], sucrose [7]. Depending on the mechanism of protection applied on the yeast cells, two important groups of protecting agents could be mentioned: stabilization agents (trehalose, manitol, xylose) [8] and water activity reducing agents (glycerol, maltitol, xilitol) [6].

As a response to dehydration yeast cells block their cellular division and activates certain genes that are essential for the stationary phase. Furthermore they start manifesting characteristics of the cells from the G0 phase. The transcription of a number of genes with activity on the cell wall is also modified [8].

During the rehydration process the cells need to repair their affected areas and to eliminate the products synthesized as stress response (such as trehalose). The surface of dried cells is wrinkled and crenelated, affecting the transport of different compounds in and out of the cell. The phospholipide content is lowered, the level of saturation of fatty acids is modified.

Also the flocculation characteristics of the dried yeast are affected [9].

Suppliers of active dried yeast claim that the active dried yeast avoids the necessity of propagation. Nevertheless they need to be rehydrated in wort or water before pitching, at 20…30°C [10].

2. Materials and methods

*Yeast variants.*Three variants selected from WS34/70 population were subjected to drying, along with the WS34/70 mixture itself. For the identification of the variants within the initial population pulsed field gel electrophoresis technique was used. As known this is a technique that allows separation of large DNA molecules, typically ranging in size from 50 to 10 000 kb (10 Mb) [11].

In Figure 1 the karyotype of three variants out of the WS34/70 population is displayed, with emphasis on the intensity of the chromosomes presented as band 8 and band 9. Each variant poses a different ratio between the two bands, as described in Table 1.

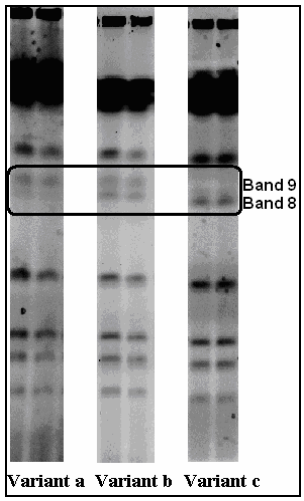

Figure 1. The short run karyotype of the three variants analysed

Table 1. Bands 9 and 8 ratio of each of the three variants

Variant	Band ratio
variant *a*	band 9 : band 8 > 1
variant *b*	band 9 : band 8 = 1
variant *c*	band 9 : band 8 < 1

Yeast drying technique. The drying process of the yeast was performed using the Mini Spray Dryer B-191.

The yeast suspension, atomized into millions of droplets by a nozzle, was sprayed into hot dry air circulating in counter-current. The water is vaporised immediately by the hot air and the product is transformed into a powder within seconds. This technique assures a gentle drying of the product without thermal shock.

The parameters used for drying the yeast suspensions are listed in Table 2.

Table 2. Spray-drying parameters

Parameter	Measure unit	Value
Inlet temperature	°C	90
Outlet temperature	°C	45
Feed pump speed	$mL \cdot min^{-1}$	15
Aspiration	%	100

Preparing the yeast suspension for drying. The yeast variants and the WS34/70 mixture were grown on 15Plato all malt wort, for 3 days, at 20°C, with continuous mixing at 120rpm. The emulsifier Struktol J673 (the citric ester of glycerine monostearat) was added to each yeast suspension at least 1 hour before drying. Variant *a* and WS34/70 suspensions were first dried with a very small amount of emulsifier (approx. 0.01% $w.v^{-1}$ or 0.05% $v.v^{-1}$). A second test was performed with an increased amount of 1.43% $w.v^{-1}$ (10% $v.v^{-1}$) emulsifier for all three variants and the mixed population WS34/70.

Dried yeast rehydration. The dried yeast (powder) was rehydrated in phosphate buffered saline (PBS) as well as in 15 Plato all malt wort, at two different concentrations of 5% $v.v^{-1}$ and 10% $v.v^{-1}$ with 30°C rehydration temperature, according to Table 3 and Table 4.

Yeast cell concentration. The total/dead cell count and the yeast viability during the rehydration process were determined using the NucleoCounter YC-100 System. The principle of this system consists in counting individual cells in suspension by detecting fluorescence signals of stained DNA in the cell nuclei. The staining dye used is propidium iodide (PI). The dye is immobilised in the Nucleo Cassette prior to analysis and it bounds to the cell DNA once in contact with a damaged cell. The staining of the cell nuclei as bright object can be seen in Figure 2.

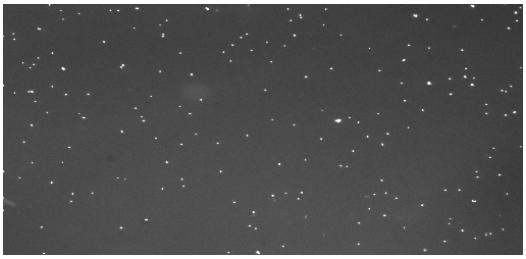

Figure 2. A fluorescent microscopy image of a sample for total cell count with NecleoCounter YC-100

3. Results and discussion

Rehydration behaviour of variant a and WS34/70. The results of the rehydration process, with respect to the dead cell profile, performed in wort and PBS of both variant *a* and the mixed population represented by WS34/70, are shown in Figure 3.

Table 3. Rehydration parameters applied to variant *a* and the WS34/70 population.

Rehydration temperature	variant *a*		WS34/70		Emulsifier*
	wort % (w.v-1)	PBS % (w.v-1)	wort % (w.v-1)	PBS % (w.v-1)	% (w.v-1)
30°C	5	5	5	5	0.007
30°C	10	10	10	10	0.007

* the emulsifier was added in the yeast suspension before drying.

Table 4. Rehydration parameters applied to all three variants and WS34/70 population.

Rehydration temperature	WS34/70	variant *a*	variant *b*	variant *c*	Emulsifier*
	wort % (w.v-1)				% (w.v-1)
30°C	5	5	5	5	1.43

* the emulsifier was added in the yeast suspension before drying.

Before the spray-drying of the two yeasts a small amount of emulsifier was added (0.007% w.v^{-1}) into the yeast suspensions.

Nevertheless the amount was far too low for a significant improvement of the number of dead cells in dried yeast. When PBS was used as rehydration medium no decrease in the amount of dead cells was noticed after 7 hours of rehydration. When wort was used as rehydration medium the amount of dead cells decreased with approximately 30%. Variant *a* proved to be more sensitive to the drying process as no improvement could be detected during rehydration.

The test was also performed for a higher concentration of cells (10% w.v^{-1}) both in wort and PBS (data not shown) but the number of cells was even higher respecting the same pattern during 7h rehydration.

When repeating the test with dried yeast obtained from yeast suspension previously treated with 1.43% emulsifier, the dead cells count displayed a different and more pronounced evolution (Figure 4). The cells recover within the first 3 hours of rehydration, emphasizing the positive impact of emulsifier on dried yeast viability.

The overall gain brought by adding emulsifier in a yeast suspension before drying can be observed in the increase of the viability of the dried cells during rehydration. Figure 5 points out the viability evolution of variant *a*, and WS34/70 population during rehydration. When prolonging the rehydration time to 24 hours, both variant *a* and WS34/70 population displayed a significant improvement of their viability (Figure 6), even if this could not be observed during 6 hours rehydration (as shown in Figure 5).

When using more emulsifier, the viability of the cells after only 1h rehydration was high leaving space for little improvement. Therefore, after 24 hours of rehydration, the viability of both strains exceeded 99% (Figure 7).

Regardless the amount of emulsifier used, after 24 hours of rehydration variant *a*, which is more sensitive to the high temperature that accompanies the drying process, had the ability to recover extremely well.

This feature makes it a good candidate for using the strain as active dry yeast.

Rehydration behaviour of three variants and WS34/70. Being aware of the importance of treating the yeast suspension prepared for spray-drying with a protective agent, a second experiment was performed using the 1.43% (w.v^{-1}) emulsifier for drying two more strains (variant *b*, variant *c*) selected from the WS34/70 population along with the variant *a*. The behaviour of these three variants was studied together with WS34/70, during rehydration in wort at 30°C. The evolution of the number of dead cells was analysed for each strain (Figure 8). The trend for all strains was a gradual decrease in the number of dead cells during the rehydration process.

A comparison of the number of dead cells after 7 hours and 24 hours respectively of rehydration underlines the remarkable improvement of the dried cells (Figure 9). Among the three variants the most notable recovery could be seen for variant *a*, which started with the highest number of dead cells only to end up with the lowest amount after 24 hours of rehydration (Figure 9).Table 5 contains the quantification of the improvement in the dead cells number of the rehydrated strains, as percentage.The values for variant *a* place it on top in terms of rehydration efficiency while variant *b* is the slowest. These results support the potential of using variant *a* as a single strain in the process of brewing when utilizing dried yeast for pitching, but at the same time confirm the inefficiency of variant *b* as dried yeast for industrial use.

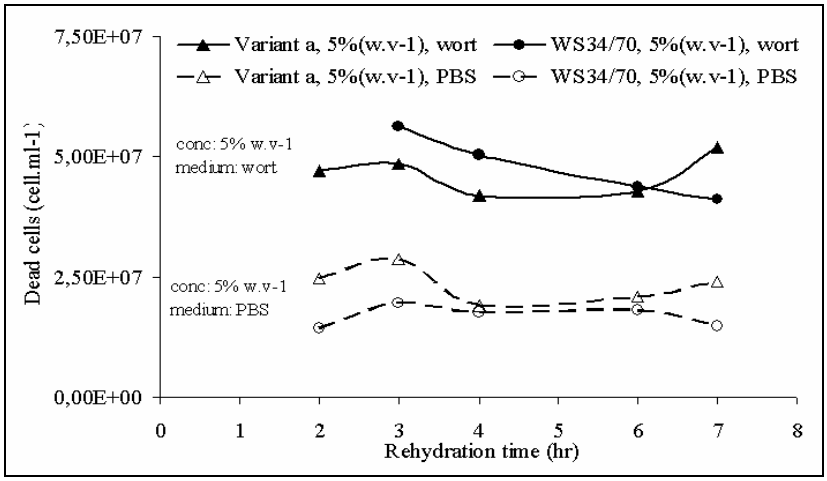

Figure 3. The evolution of the dead cells count during 7 hours of rehydration in wort and in PBS at a concentration of 5% and 10% respectively

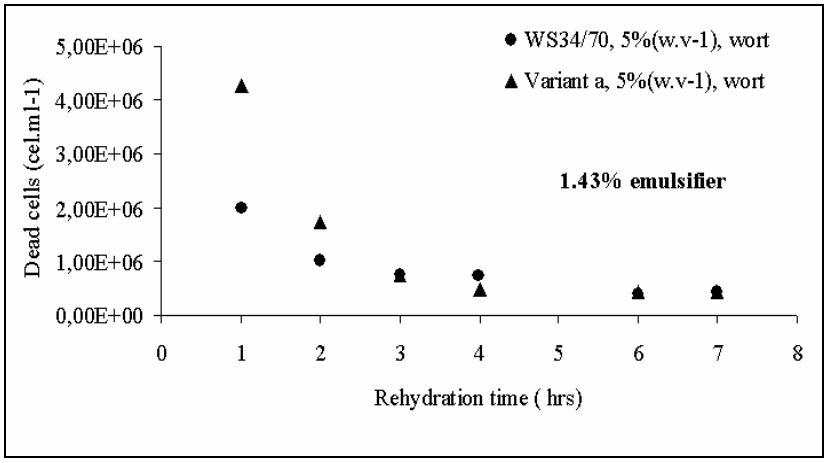

Figure 4. Dead cell count during rehydration of variant x, and WS34/70 population treated with 200 times higher amount of emulsifier previous to drying.

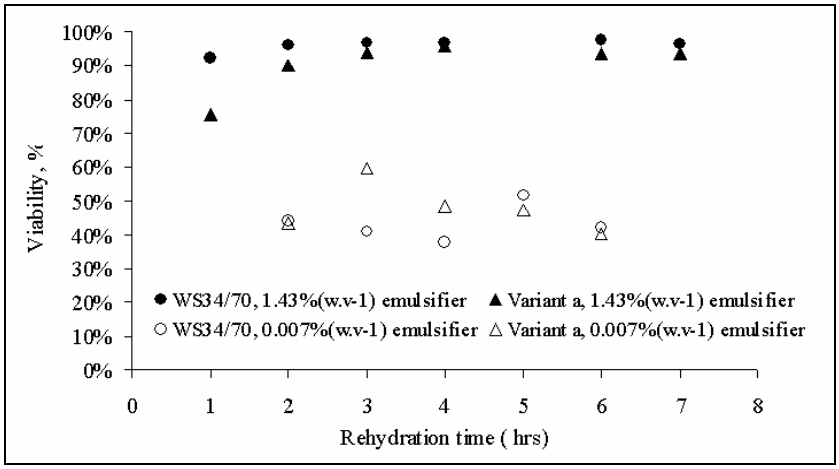

Figure 5. Viability profile of the dried yeast cells during 7 hours of rehydration, cells previously treated with two different amounts of emulsifier

Table 5. The rehydration efficiency after 7 hours and 24 hours as percentage of cells that recovered their cell wall permeability.

Yeast strain	Rehydration tine (hrs)	
	7	24
WS34/70	79%	91%
Variant *a*	90%	96%
Variant *b*	66%	81%
Variant *c*	76%	93%

*Glycogen and trehalose within the dried yeast strains.*Glycogen is the major storage carbohydrate in brewing yeasts, the amount accumulated being strain specific [12]. This compound is utilised for maintenance functions [13] its degradation being triggered by different stress factors that might affect the physiological state of the yeast cell [14]. In Figure 10 the glycogen content of the dried strains is displayed. The dried variant *a* and WS34/70 contain higher amounts of glycogen within the cells than variant *b* and variant *c*.

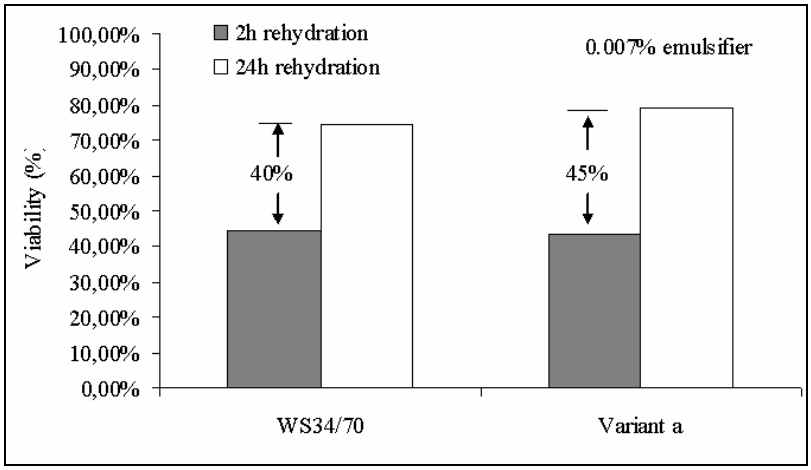

Figure 6. The improvement of viability after 24 hours of rehydration at 30°C, with low amount of emulsifier.

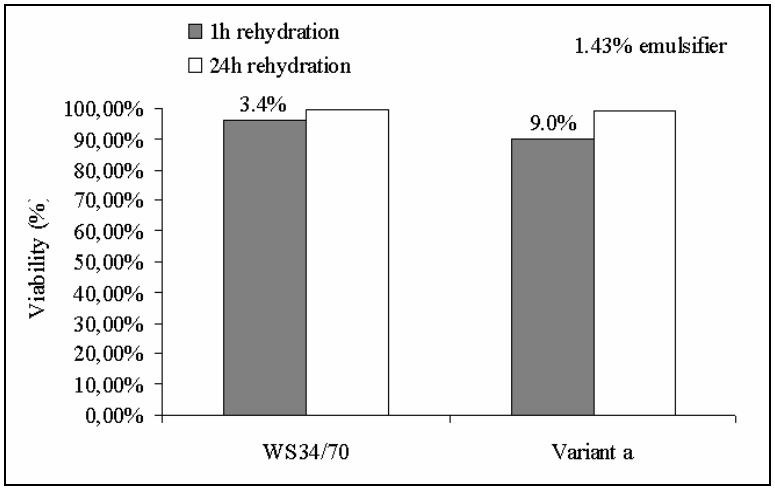

Figure 7. The improvement of viability after 24 hours of rehydration at 30°C, with higher amount of emulsifier.

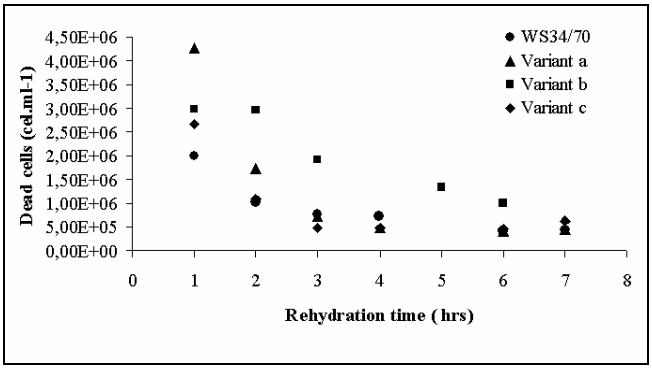

Figure 8. Evolution of the amount of dead cells during rehydration of three strain variants and the initial mixture WS34/70.

This confirms the robustness of variant *a* in comparison with the other two variants.

Trehalose accumulation is known to be a general response to stress such as dehydration, acting as a membrane stabilizer and protecting agent against the applied stress [15]. Studying the trehalose content of the three variant strains and the initial mixture WS34/70 (Figure 11) higher levels were detected in variant *b* and variant *c* suggesting that these two strains are more sensitive to dehydration.

As WS34/70 displayed a lower content of trehalose, it could be presumed that within this mixture of strains there are other variants more resistant to stresses.

The amount of glycogen within yeast cells is in inverse proportion with the amount of trehalose.

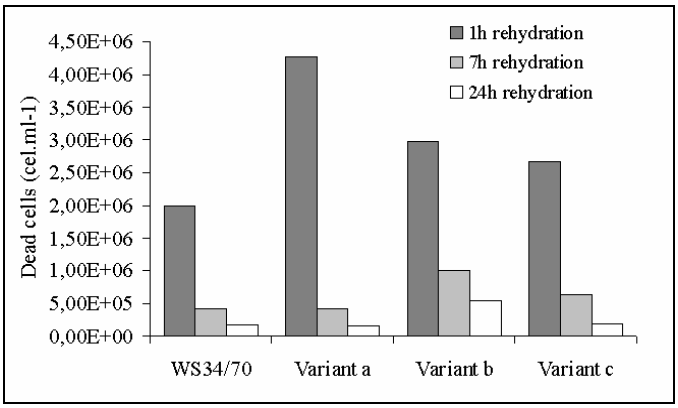

Figure 9. Dead cell count comparison after 1hour, 7 hours and 24 hours of rehydration in all malt 15 Plato brewing wort.

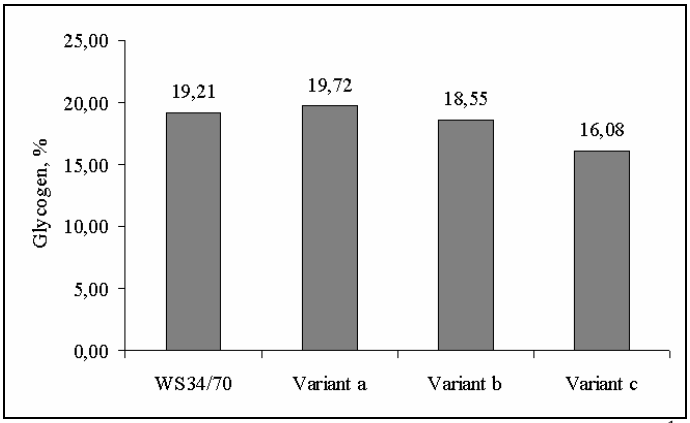

Figure 10. The glycogen content of the dried yeast pretreated with 1.43% $w.v^{-1}$ protecting agent.

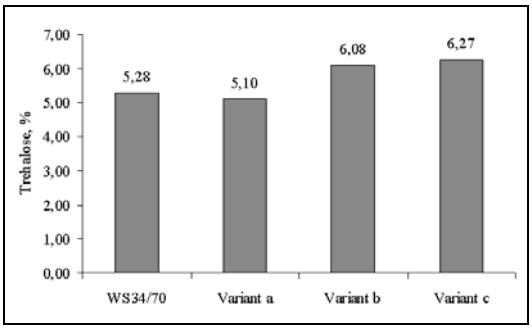

Figure 11. The trehalose content of the dried yeast pretreated with 1.43% w.v^{-1} protecting agent.

4.Conclusions

Dried yeast became increasingly used in the brewing industry due to many advantages it offers. The experiments conducted during this study confirmed the importance of a protecting agent added in the yeast suspension subjected to dehydration. The effect of the drying process on yeast viability is strain dependent. Care must be taken when using lager dried yeast in the brewing process as the inoculation of a high number of dead cells occurs. In order to have similar results as with yeast slurry, the inoculation should be performed according to the amount of viable cells.

Acknowledgments. The authors wish to thank Heineken Supply Chain for their support of this work.

Abbreviations. DNA – deoxyribonucleic acid; PBS – phosphate buffered saline

References

1. Cyr, N.; Blanchette, M., Price, S.P., 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(3), 138–144.
2. Finn, D.A.; Stewart, G.G., Fermentation Characteristics of dried brewers yeast : effect of drying on flocculation and fermentation. *Journal of ASBC*, 2002, 60(3), 135-139.
3. Lievense, L.C.; Riet, van't K., Convective Drying of Bacteria, I. The Drying Processes, *Advances in Biochemical Engineering/ Biotechnology*, 1993, 50, 45-64.
4. Luna-Solano, G.; Salgado-Cervantes, M.A., Rodriguez-Jimenes, G.C., Garcia-Alvarado, M.A., Optimization of brewer's yeast spray drying process, *Journal of Food Engineering*, 2005, 68, 9-18.
5. Luna-Solano, G.; Salgado-Cervantes, M.A., Garcia-Alvarado, M.A., Rodriguez-Jimenes, G., Improved Viability of Spray Dried Brewer's Yeast by Using Starch (Grits) and Maltodextrin as Processing Aids. *Journal of Food Process Engineering*, 2000, 23, 453-462.
6. Oshita, K.; Fukui, N., Yomo, H., Umezawa, Y, Method of Producing Active Dry Yeast, *Patent Application Publication*, 2004, 1-9.
7. Leslie, S.B.; Israeli, E.; Lighthart, B.; Crowe, J.H.; Crowe, L.M., Trehalose and Glucose Both Protect Membranes and Proteins in Intact Bacteria during Drying, *Applied and Environmental Microbiology*, 1995, 61(10), 3592-3597.
8. Gibson, B.; Lawrence, S., Leclaire, J., Powell, C., Smart, K.A., Yeast responses to stresses associated with industrial brewery handling, *FEMS Microbiol Rev*, 2007, 31 (5), 535-569.
9. Finn, D.A.; Stewart, G.G. Fermentation Characteristics of dried brewers yeast : effect of drying on flocculation and fermentation, *Journal of ASBC*, 2002, 60(3), 135-139.
10. Reckelbus, B.; Fels, S., Gosselin, Y., Debourg, A., Optimising the use of dried yeasts in breweries. *MBAA Technical Quarterly*, 2000, 37 (1), 21-25.
11. Johnston, J.R., *Molecular Genetics of Yeast-A Practical Approach*, Oxford University Press, 1994.
12. O'Connor-Cox, E.S.C.; Majara, M., Lodolo, E.J., Mochaba, F.M. and Axcell B.C. The Use of Yeast Glycogen and Trehalose Contents as Indicators for Process Optimisation. *Ferment*, 1996, 9, 321–328.
13. Steward, G.; Russell, I., Fermentation – The „Black Box" of the Brewing Process. *MBAA Technical Quarterly*, 1993, 30, 159–168.
14. Quain, D.E., Stress and the Yeast Cell, Bass Brewers Limited-Research Forum, *Ferment.*, 1991, 4(3), 155-156.
15. O'Connor-Cox, E.S.C.; Majara, M.; Lodolo, E.J.; Mochaba, F.M.; Axcell, B.C., The Use of Yeast Glycogen and Trehalose Contents as Indicators for Process Optimisation, *Ferment.*, 1996, 9, 321–328.