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Research Article

Effects of Process Variables on Fermentation Time and Vicinal Diketones Concentration for Beer Production

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Abstract

High quality market supplying is decisive for the beer industry due to the increasing competition and diversity in the sector. The beer-fermentation step represents 70% of all time spent on industrial beer production and it requires highly rigorous control instrumentation so it is not to become a process' bottleneck. Numerous by-products are formed during the fermentation step and when they are not under strict control, their excess may turn into unpleasant scent and flavour on the final product. Among the sub products formed during the fermentation stage, total vicinal diketones (diacetyl and 2,3pentanedione) are the main components, since they are limiting for subsequent processing steps, besides having a low perception threshold by the consumer and giving undesirable taste and odor. On this particular work, the effects of three process variables were analysed aiming to reduce vicinal diketones concentration on the final product, and to reduce fermentation time. After yeast acidification, with Phosphoric acid, a 50% increase on cell concentration on the fermentation stage, and 0.5 °C changes on fermentation temperature, the results showed a 38% reduction on fermentation hours and 66.3% reduction on vicinal diketones production over fermentation.

ABBREVIATIONS

VDK: Vicinal Diketones; FAN: Free Amino Nitrogen

INTRODUCTION

Beer is one of the longest appreciated beverages through history due to its usually simplistic formula and solid production techniques. Traditionally prepared from yeast-fermented malted barley, water and hops, increasing consumption has pressured beer industry on seeking new advanced technologies aiming lower costs and higher quality products [1]. Global beer production on 2014 had declined for the first time in 30 years, yet reaching 191 billion litters, and Brazil represented 7.4% of the global market share, holding a production volume around 14 billion litters [2].

Beer production can be understood as a series of chemical and biochemical reactions combined with solid-liquid unit operations for the separation and clarification of the brewing wort and the final beer [1]. Generally, traditional Pilsen beer production can be divided into five basic stages: preparation of brewing wort, fermentation, maturation, filtration and packing/filling. Under traditional methods, each one of these stages take 9, 192, 48 and 15 hours, respectively [3].

The fermentation step is critical and the most important part of the process and demands extensive control mechanisms since its success is directly affected by process conditions such as temperature, agitation, pH, oxygen input, nutrient and yeast concentrations [4]. Fermentation can be divided into two clearly separated stages: i) the first part occurs in the presence of oxygen and is characterised by an rapid increase on cell concentration and the formation of the vicinal diketones; i) second part occurs in the absence of oxygen and the yeast cells consume the vicinal diketones present in the wort [5,6]. Beer fermentation is responsible not only for the conversion of the wort's sugary content into beer's alcohol content, but also for the production of biochemical by-products responsible for flavour, for instance, vicinal diketones, superior alcohols, esters and carboxylic acids [3,7].

According to past studies, vicinal diketones (VDK) production is a result of extracellular natural and non-enzymatic oxidation of α-acetohydroxy acids, which are a by-product secreted by

yeast cells in the course of metabolic routes for biosynthesis of amino acids such as valine, leucine and isoleucine [8]. Diacetyl (2,3-butanedione) and 2,3-pentanedione, are the most significant for the process due to their low flavour thresholds, 0.1 and 0.9 mg/L, respectively, and the butterscotchand toffee-like flavour they give to beer [9,5]. Produced by yeast during the first part of beer fermentation, and enzymatically consumed during the second part of the process, VDK must be reduced through efficient and low cost process in order to ensure industry competitiveness as well as no sensorial impacts [7]. In larger beer production, maturation for diacetyl removal is one energy-demanding and time-consuming step, which needs to be over passed in order to produce economic profit with no repercussion on product quality [10].

Diverse methods for increasing diacetyl removal from final beer have been reported on the literature. Increasing viable yeast cell counting, applied for high gravity brewing, has produced considerable results on reducing both the build-up of VDK levels during fermentation, as well as the residual concentration once wort's fermentation is complete [8,11,12]. Reducing brewing wort's pH and increasing fermentation temperature have also been reported as having meaningful effects on VDK levels. More innovative techniques involve introducing alterations on wort's amino acid profile by supplementation and changing wort's free amino nitrogen (FAN) concentration. Genetic engineering has been cited as an option for addressing VDK levels, by constructing specific yeast strains with low VDK production rates [13,14]. For further reduction on VDK levels after fermentation step is over, the use of a packed bed reactor containing immobilized yeast cells has been reported as an alternative for additional conversion of diacetyl into acetoin, which has a more elevated flavour threshold [15].

Therefore, the present study investigated a continuous process of optimization of a Pilsen beer production process already implemented on a local brewer by analysing the impact of three process variables, namely previous yeast acidification, increase of yeast cells concentration and adjusting fermentation temperature, on reducing both fermentation hours and vicinal diketones levels for Pilsen beer production.

MATERIALS AND METHODS

Yeast propagation and growth medium

*Saccharomyces cerevisia*e pure strains were propagated via aseptic conditions into brewing wort at 10 °C, under intense aeration for 2 to 4 hours, with 3 kgf/cm^2 pressure of dry sterile compressed air, generating a pasty-like mix of growth medium and cell content.

Cell's concentration and viability

After inoculation, the yeast cell's concentration per mL in the starchy-like ferment was determined through specific instrumentation (Cell Counter model 871, Hook and Tuccker). The viability was defined as the fraction per cent of cells in reproducing conditions and perfect physiological status, which will be able to carry the beer fermentation. Viability was assessed through qualitative microscopic Gram analysis.

Fermentation

Pasty ferment was appropriately quantified and carried into conic stainless steel tank (capacity 4000 hL), which as thermic isolated and connected to a cooling system with four zones of coolant fluid (alcoholic solution) circulation – one situated on the conic area, and the other three equally distributed along the tank. For Pilsen beer, yeast concentration is 10 to 40 million cells per mL of brewing wort [9]. During ferment dosing step and throughout fermentation process, pH was assessed. Samples were collected from the tank and analysed with digital pH meter (*Mettler-Toledo*).

Yeast acidification

Pasty-like yeast solution received Phosphoric Acid solution (food grade) and Zinc SulphateHeptahydrate solution (food grade) before it was added into the brewing tank. Acidified ferment was mixed into wort through intense aeration (3 kgf/cm^2 pressure of dry sterile compressed air) for 2 to 4 hours. Phosphoric acid solution was prepared in cold water in the concentration of 17±1%, aiming to reduce wort's pH to 2,3 ± 0,1. Zinc Sulphate solution was prepared at the concentration to assure the concentration of 0,5 ppm on the brewing mix.

Cell concentration and temperature effects

Yeast concentration was increased from 1,0 million cells/mL/°P to 1,5 million cells/mL/°P in the brewing wort, at the same time as total aeration was decreased from 75% to 55% of the fermentation tank total capacity, aiming to reduce yeast reproduction frequency down to twice during the fermentation step. Two changes in temperature (9 °C to 8,5 °C; 15 °C to 15,5 °C) were introduced aiming to control wort's cooling stages during fermentation to observe the effects of different cooling conditions on fermenting hours.

Vicinal diketones determination

Vicinal diketones concentration was determined through spectrophotometric methods. Samples were collected during fermentation and maturation stages, as well as from final products. 100 mL samples were transferred to a distillation pot still with 25 mL of distilled water, and the distillation occurred until 24 ± 0,5mL of product were collected. The volume of the product was corrected to 25 mL with distilled water, and 10 mL were transferred to two test tubes (sample test and blank). The sample test tube received 0,5 mL of ortho-phenylenediamine, and was reserved to a dark area. After 35 ± 5 minutes the sample test tube and the blank tube received, 2 and 2,5 mL of Hydrochloric acid solution (4 N). Absorbance was read at 335 nm, using the blank tube as reading blank. Vicinal diketones concentration was determined through Equations 1 and 2, and the results were expressed in mg.L^{-1}, with two decimals and ± 0,01 mg.L^{-1} standard deviation.

$$Totalvicinaldiketons\left(mg.L^{-1}\right) = A_{335} * 2,4 * f \quad (1)$$

Where A_{335} is the absorbance value read at 335 nm, and *f* is a calibration factor for the spectrophotometer.

$$PVD\left(mg.L^{-1}\right) = TVD - FVD \quad (2)$$

Where PVD are the potential vicinal diketones, TVD are the total vicinal diketones and FVD are the free vicinal diketones, all expressed in $mg.L^{-1}$.

The optimization process was tested through continuous improvement on the process variables. For assessing the continuous improvement on reducing VDK levels, five conditions were tested: current conditions already used by the local brewer (BrT-1); yeast acidification applied simultaneously with zinc addition (BrT-2); zinc treatment followed by yeast acidification (BrT-3); increase on cell concentration of previously zinc treated and acidified yeast (BrT-4); and more cell concentrated, previously zinc treated and acidified yeast with temperature adjusts (BrT-5). Three independent fermentations were carried (n = 3) for each one of the conditions tested, and the results presented represent average values.

VDK concentration data were evaluated using one-way analysis of variance (ANOVA) and Tukey HSD post hoc test (95% of probability) was used to determine statistical significance (p< 0,05). Statistical analysis was performed using STATISTICA® 7.0 software.

RESULTS AND DISCUSSION

Vicinal diketones concentration (ppm) was assessed throughout the fermentations and the results can be seen on (Figure 1).

Yeast acidification

Yeast acidification aims to reduce viscosity of the pasty-like ferment solution, via pH decrease, when it goes into fermentation tanks, preventing yeast cells to early flocculating. Flocculation is a reversible mechanism during beer fermentation as well as a significant characteristic of yeast cells, which benefits cells separation process after complete wort-fermentation. However, early flocculation is undesirable since it affects alcohol production efficiency and may represent unwanted impacts on flavour [16,17].

Once acidified, yeast cells assumed a more granular, dusty aspect (pulverulent aspect), increasing cell dispersion rate along the fermentation tank, boosting vicinal diketones reduction rates during fermentation. The results show that the dynamic of vicinal diketones formation and consequently consumption is directly affected by the reduced pH. There was a lower peak, with statistical significance (p <0,05), on vicinal diketones production (1,99 ppm for BrT-1; 1,67 ppm for BrT-2) and the velocity of the curve's decrease is considerably higher for the acidified yeasts.

Meanwhile yeast acidification process, a Zinc Sulphate solution was also used for cell treatment before fermentation. Zinc Sulphate works as enzymatic co-factor during yeast fermentation [5]. However, different fermentation behaviour was observed when the Zinc solution was added before and during acidification step.

When Zinc Sulphate and Phosphoric acid solutions were used simultaneously (BrT-2) on the yeast cells, a chemical reaction promoted Zinc precipitation, sensibly increasing the pasty aspect of the ferment solution and, consequently, fermentation hours. Alternatively, when Zinc sulphate solution was used prior cell acidification (BrT-3), precipitation was not observed and ferment solution preserved the intended lower viscosity, and the result was detected on fermentation time.

Fermentation hours were reduced for both cases of Zinc solution application. For the first case (Zinc and acid solutions used at the same time), there was a decrease of 49 hours on fermentation (from 266 to 217 hours); on the second case, additional 22 hours were reduced (from 217 to 195 hours), 71 hours in total. The results show that the dynamic of vicinal diketones production and consumption was not greatly disrupted by the order of Zinc utilization, but peak concentration was slightly lower for the second case (1,67 ppm for BrT-2; 1,55 ppm for BrT-3).

Once pH reduction prevents yeast undesired flocculation, it is expected an increase of cell concentration in the fermentation tank, since the pulverulent aspect of the acidified cells increment dispersion rates (Figure 2). demonstrate the boost on cell's distribution by the reduction on ferment solution's pH (average values for a number of fermentations). When pH declines from 5,22 to 2,35, yeast cell's scattering concentration grow from 2,45 x 10^6 to 16,38 x 10^6 cells/mL, which represents an 85% increase.

It is important to highlight that cell's viability was not affected by acidification treatment. There was a slight decrease on viability (from 97% to 95%) which was not significant when it comes to yields on beer final production.

Yeast acidification is an interesting process change when it comes to controlling the production of biochemical compounds of interest on beer fermentation. Reducing pH of beer has been reported as a mechanism for decreasing VDK residual levels after fermentation since more acid pH favours the conversion rate of diacetyl into acetoin, which does not affect flavour. However the decrease in pH, by itself, may carry flavour impact, once this acidification would have to go through pH levels below 3.5 [18].

Yeast cell's concentration during fermentation

VDK production during beer fermentation is related to yeast cells metabolism, since it is a by-product associated with the production of the aminoacids valine and isoleucine. Previous studies have shown that increasing cell count during brewing fermentations has direct impact both on increasing VDK peak concentration, as well as on reducing residual concentrations, at the end of fermentation, since the more viable cells present in the wort, higher is the biosynthesis of essential amino acids, and more efficient is the consumption of VDK [15].

Changes in yeast cell's concentration for the fermentation stage were carried associated with aeration changes aiming to reduce cell's reproduction rate over fermentation. Increasing cells concentration from 1 million cells/mL/°P (BrT-3) to 1,5 million cells/mL/°P (BrT-4), and reducing wort aeration from 10 to 8 hours, resulted in a decrease of reproduction cycles for yeast cells, from 3,0 ± 0,3 to 2,0 ± 0,3 (Figure 3), which represented a lower peak on vicinal diketones production (1,55 ppm for BrT-3; 0,87 ppm for BrT-4), due to the minor need of valine processing

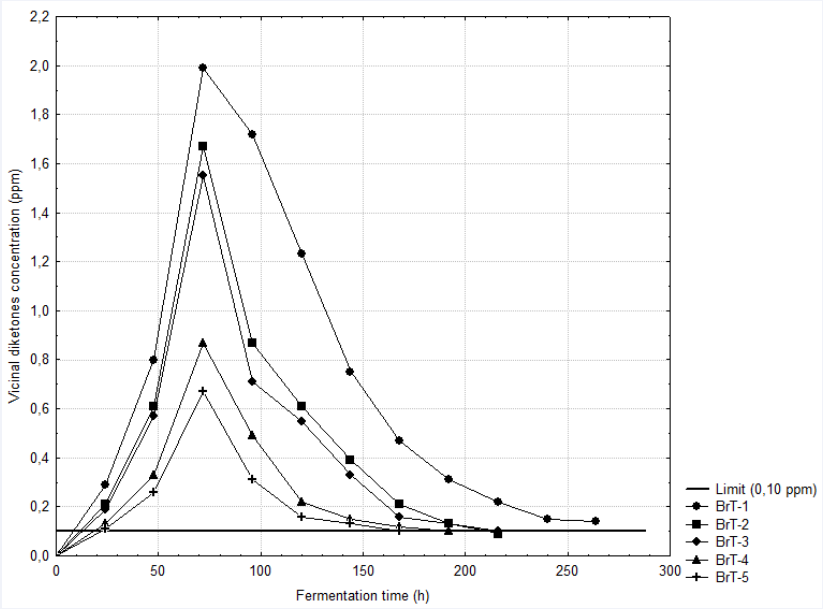

Figure 1 Vicinal diketones concentration (ppm) over fermentation hours for all fermentation conditions tested.

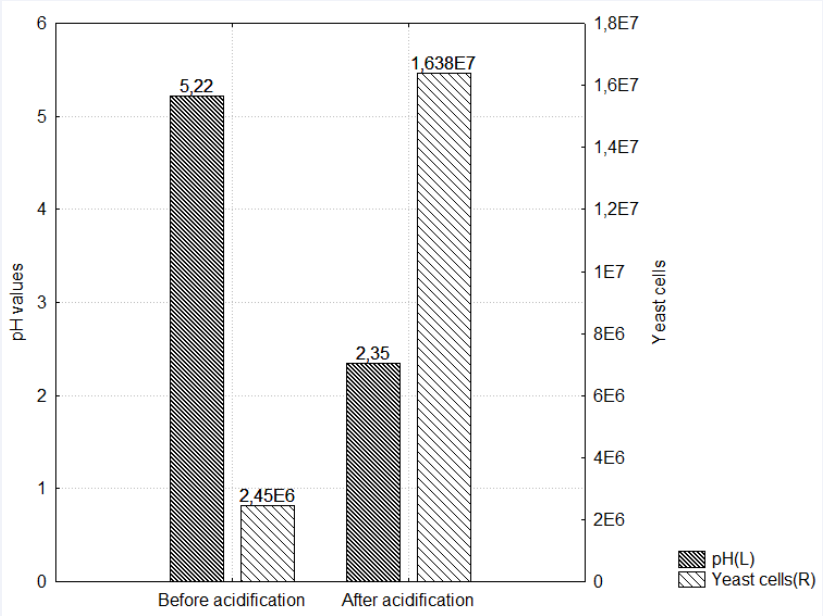

Figure 2 Increase of yeast cell dispersion by ferment pH reduction via acidification.

by the cells [19], as well as fermentation hours from 195 to 174 hours (92 hours in total).

Erten et al. (2007), reported achieving residual VDK significant (p < 0,05) reductions by increasing yeast cell concentrations in several-fold, but did not mention its impact on peak concentrations during the initial stages of fermentation [8]. Combining the increase on cell count with the reduction on aeration time avoided possible elevations on VDK maximum concentrations and secured the decrease expected on residual VDK.

Fermentation temperature

Controlling fermentation temperature is essential for assure a disciplined vicinal diketones dynamic over fermentation. Temperature change was carried (BrT-5) aiming to decrease vicinal diketones production on the first part of the fermentation – by reducing temperature in 0.5 °C, from 9 °C to 8,5 °C; and to increase vicinal diketones consumption on the second part – by elevating temperature in 0.5 °C, from 15 °C to 15.5 °C. These changes were expected to initially slower yeast metabolism and posteriorly expand it [5,4].

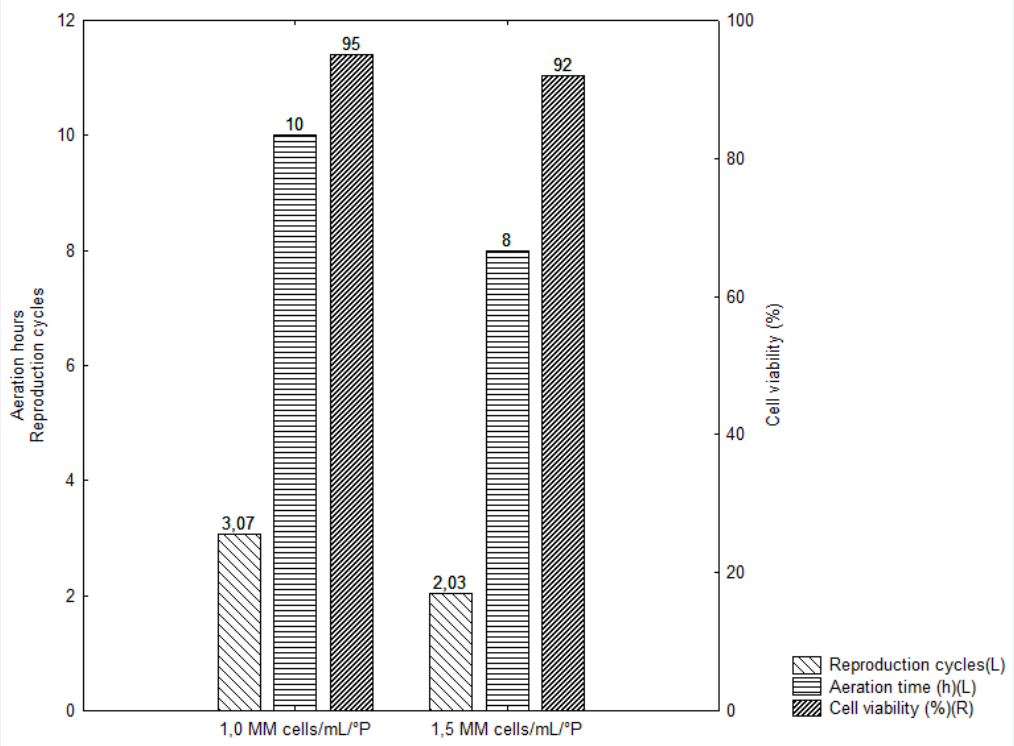

Figure 3 Results of cell's reproduction cycles and viability for different cell concentrations and reduced aeration hours.

Table 1: Vicinal diketones (VDK) peak concentration for each one of the fermentation conditions tested. Mean ± standard deviation (n = 3). *Different letters* (*a, b, c, d*) indicate significant differences by Tukey HSD post hoc test (p <0,05).

Fermentation condition tested	Vicinal diketones peak concentration (ppm)
BrT-1	1,99 ± 0,10[d]
BrT-2	1,67 ± 0,08[a]
BrT-3	1,55 ± 0,08[a]
BrT-4	0,87 ± 0,06[c]
BrT-5	0,67 ± 0,03[b]

The results showed an even lower peak of vicinal diketones production, from 0.87 ppm to 0.67 ppm, as expected, and an additional reduction of 9 hours on fermentation time, from 174 to 165 hours (101 hours in total).

The contraction on VDK peak concentrations may be credited to the slowed metabolic reactions in the first stage of fermentation, due to lower temperature. More moderated metabolic rates require a downgrade on biosynthesis of essential amino acids by yeast, reducing its by-products, which are the precursors of VDK. Krogerus and Gibson (2013) also minimized VDK spike concentrations on fermentation preventing the yeasts of undertaking the metabolic routes for biosynthesising the required amino acids, using the strategy of supplementing the brewer wort with amino acids [10].

Statistical analysis

Average values for vicinal diketones peak concentrations along with standard deviations (SD) are presented on (Table 1). ANOVA and Tukey HSD test showed that the modifications on process variables significantly (p <0,05) changed vicinal diketones levels on the fermentations carried. Among the four conditions tested, only the Zinc sulphate addition order did not represent a significant (p <0,05) change on VDK concentrations.

Overall, four different beer fermentation conditions were tested and compared to the current applied process, optimizing three different process variables, resulting on a reduction of 101 fermentation hours, satisfying the desired limit for vicinal diketones concentration on the final product as well as reducing vicinal diketones total production throughout fermentation.

CONCLUSION

The process variables chosen for this study were satisfactory on achieving its prime goal, which was to guarantee that vicinal diketones concentration restrictions on Pilsen beer production. The combination of yeast acidification, increase on yeast cell's concentration and fermentation temperature optimization significantly (p <0,05) yielded on a 38% reduction on fermentation hours (from 266 hours to 165 hours), a 66.3% reduction on the peak concentration production of vicinal diketones during the first step of fermentation (from 1.99 ppm to 0.67 ppm), and

secured vicinal diketones limits on the final product. These referred changes can be easily reproduced on breweries which are currently facing problems related to vicinal diketones levels.

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REFERENCES

1. Ambrosi A, Cardozo NSM, Tessaro IC. "Membrane Separation Processes for Beer Industry: a Review and State of the Art." Food Bioprocess Technol. 2014; 7: 921-936.
2. Kirin Holdings Company. "Kirin Beer University Report." 2015.
3. C. Banforth. Beer: Tap into the Art and Science of Brewing, 2nd edition. Oxford University Press. 2003.
4. Bokulich NA, Bamforth CW. "The Microbiology of Malting and Brewing." Microbiol Mol Biol Rev. 2013; 77: 157-172.
5. Kunze W. Technology Brewing and Malting, Berlin: VLB. 1999.
6. Brown AK, Hammond JRM. "Flavour Control in Small-scale beer fermentations." Trans IChemE. 2003; 81: 40-49.
7. Noman Cyr, Blanchette M, Prince SP, Sheppard JD. "Vicinal Diketones Production and Amino Acid Uptake by Two Active Dry Lager Yeasts During Beer Fermentation." J Am Soc Brew Chem. 2007; 138-144.
8. Erten H, Tanguler H, Cakiroz H. "The Effect of Pitching Rate on Fermentation and Flavour Compounds in High Gravity Brewing." J Inst of Brew. 2007; 113: 75-79.
9. Vogel HC, Todaro CL. Fermentation and Biochemical Engineering Handbook: Principles, Process Design, and Equipment, 2a edition. New Jersey: Noyes Publications. 1997.
10. Krogerus K, Gibson R. "Influence of valine and other amino acids on total diacetyl and 2,3-pentanedione levels during fermentation of brewer's wort." Appl Microbiol Biotechnol. 2013; 97: 6919-6930.
11. Verbelen PJ, Mulders SV, Saison D, Laere SV, Delvaux FR. "Characteristics of High Cell Density Fermentations with Different Larger Yeast Strains." J Inst Brew. 114: 127-133.
12. Nguyen TH, Viet Man V. "Using high pitching rate for improvement of yeast fermentation performance in high gravity brewing," Int Food Res J. 2009; 16: 547-554.
13. Kusunoki K, Ogata T. "Construction of self-cloning bottom fermenting yeast with low vicinal diketone production by the homo-integration of ILV5." Yeast. 2012; 29: 435-442.
14. Jun Lu, Jian Dong, Deguang Wu, Yefu Chen, Xuewu Guo, Yu Shi, et al. "Construction of recombinant industrial brewer's yeast with lower diacetyl production and proteinase A activity," Euro Food Res and Technol. 2012; 235: 951-961.
15. Krogerus K, Gibson BR. "125th Anniversary Review: Diacetyl and its control during brewery fermentation." J Inst Brew. 2013; 119: 86-97.
16. Verstrepen KJ, Derdelinckx G, Verachtert H, Delvaux FR. "Yeast flocculation: what brewers should know." Appl Microbiol Biotechnol. 2003; 61: 197-205.
17. Bergen BV. Diacetyl: identification and characterisation of molecular mechanisms for reduction in yeast and ther application in a novel enzyme based assay for quantification in fermentation systems, Montreal: McGill University. 2006.
18. Bamforth CW, Kanauchi M. "Enzymology of vicinal diketone reduction in brewer's yeast." J Inst of Brew. 2004; 110: 83-93. 2004.
19. Boulton CA, Box WG, Quain DE, Mouzahn SW. "Vicinal Diketone Reduction as a Mesure of Yeast Vitality." MBAA Technical Quarterly. 2001; 38: 89-93.

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