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PRESERVATION METHOD OF YEAST AND PERFORMANCE IN BEER FERMENTATION

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Abstract

Knowledge of properties of individual yeast strains is of major importance and is a main precondition for selection of the yeast as well as for technological conditions during fermentation. Loss of the desired brewing characteristics typical of a strain can have considerable commercial implications for a particular plant. The objective of the investigations is principally to get a better understanding of the influence of the preservation method relating to partial desiccation of yeasts on filter paper in terms of yeast performance and beer quality. The results are presented from fermentation tests with yeast strains partially desiccated on filter paper, lyophilized and deep-frozen. Major interests focused on whether and to what extend the level of survival rates of preserved yeasts influenced yeast vitality and beer quality.

Key words: *beer**,** yeast, fermentation, preservation, partially desiccated, viability, attenuation.*

Introduction

The quality of a beer is considerably influenced by the quality of its raw materials. Donhauser (1997) concluded that the character of a beer is not just determined by the condition of malt, hops and brewing liquor but also to a high degree by the yeast strain. Accordingly Wackenbauer (2003) intensity of propagation of yeast is important in commercial operations both for a rapid start to fermentation and also from a biological viewpoint. Rapid start to fermentation impedes the occurrence of beer-spoilage organisms. The aerobic and anaerobic phase must be well matched if formation of fermentation by-products is to be optimal (Van der Aar, 1996).

One of the most important technologically valuable characteristics of yeast is its ability to ferment the extract rapidly. The various yeast

strains behave in different ways as far as fermentative capability is concerned. The extract decrease is used for immediate control of the fermentation curve.

Storage and care of selected yeast strains play a significant role in the brewing sector. Loss of the desired brewing characteristics typical of a strain can have considerable commercial implications for a particular plant. A change especially in flocculation behavior of flocculent yeasts can make yeast harvesting more difficult, give rise to changes in beer aroma and influence the degrees of attenuation obtained and thus affect overall beer quality (Bassel, 1977).

Storing yeasts on slant agar, with regular subcultivation, represents the usual method of strain storage. The increased risk of contamination in transferring yeasts from one slant agar to another is the main drawback here (Kirsop, 1984). In addition, particularly with extensive strain collections, mix-ups may occur during subcultivation. The amount of work associated with this method is relatively large. Furthermore, with regular and frequent subcultivations, changes in yeast characteristics may be expected. Thus, various preservations methods present themselves as alternatives for strain storage. These are based on the principles of drying, freeze drying and freezing. With these methods, the objective is to suppress yeast growth and metabolism completely. It is also necessary to ensure that the strains are storable over many years, in particular in a contamination-free manner, under conditions of adequately high viabilities, even over a prolonged period of several years.

The objective of the investigations is principally to get a better understanding of the influence of the preservation method relating to partial desiccation of yeasts on filter paper in terms of yeast performance and beer quality. The results are presented from fermentation tests with yeast strains partially desiccated on filter paper, lyophilized and deep-frozen. Major interests focused on whether and to what extend the level of survival rates of preserved yeasts influenced yeast vitality and beer quality.

Experimental

Two yeast strains were used in the investigation: bottom flocculent yeast (FY) and bottom non-flocculent yeast (NFY).

Fermentation tests were carried out in the first test series in Erlenmeyer vessels (3 l) at 15°C with: reference flocculent yeast FY– stored on slant agar, deep frozen yeast FY (six years), lyophilized yeast FY (three and eleven years), as well as a yeast FY which prior to pitching, had been incubated in a 10% trehalose solution for 24 ore to increase the intracellular trehalose content.

In the second test, the bottom flocculent yeasts (FY) partially desiccated on filter paper was purposely used, while keeping tests conditions comparable to those described. Variant 1: 14 months old filter yeast; Variants 2 and 3: 4-month-old filter yeasts.

The third test series were carried out in industrial conditions, variants A, B, C, D represent yeast of a flocculent yeast (YF) and non-flocculent strain (NFY) which, prior to preservation, were treated in different ways in terms of pre-propagation and preservation conditions. The very heterogeneous selection in terms of viabilities was chosen on purpose to allow conclusions about the effect of different survival rates on the quality of the resulting beers. Reactivation of yeasts was followed by an aerobic propagation at 20°C and subsequent fermentation at 10°C in stainless steel cylindroconical tanks. Through this intermediate step, the influence of propagation on the flavor of beers of the first generation should be diminished, so that the influence of preservation could predominate. The wort used for each yeast strain originated from a brew was divided equally such that starting conditions were identical for each batch.

In order to determine viability, the Koch pour plate method with wort agar was used. The wort agar was first liquefied and then tempered in a water bath at about 55°C. Parallel analyses were performed for each dilution step. The nutrient media were always incubated at 27°C for 72 h. The total cell count was determined using a Thoma cell counter. Dilution steps of between 20 and 300 CFU (colony forming units) were measured. On the basis of the relationship CFU: total cell count, the viability was calculated.

Beer analyses were carried out in accordance with MEBAK and using chromatographic tests.

Results and Discussions

The first fermentation test series concluded that the lyophilized yeast FY (three and eleven years) had very low viabilities immediately after reactivation (Table 1).

Table1. FY strain viability, %

Reference FY	Reference FY + trehalose	Deep frozen FY	Eleven years lyophilized FY	Three years lyophilized FY
98	98	89(*34)	74(*5)	71(*3)

*directly after reactivation

The worst fermentative performance is found to be associated with the yeast lyophilized three years previously; this has to be seen in the light of the degrees of attenuation obtained after the six-day main fermentation (Figure 1).

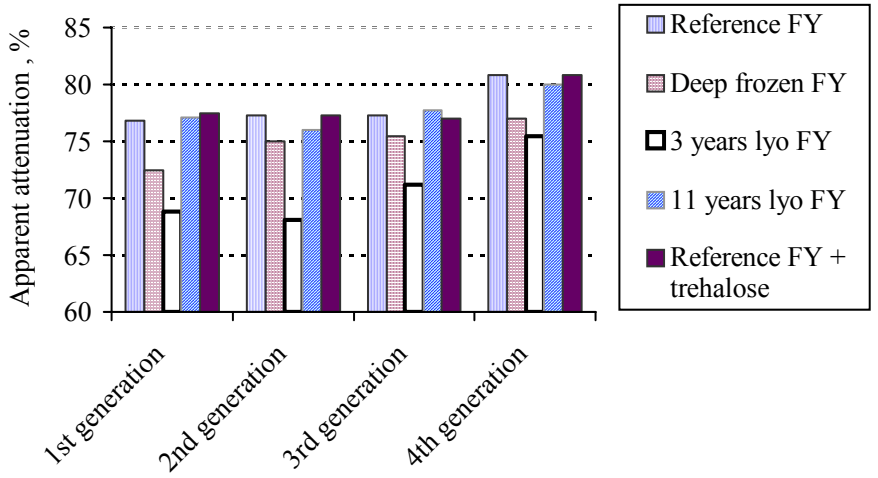

Fig. 1. Apparent attenuation after six days of main fermentation

This arises as a result of early sedimentation of cells in the fermentation vessels. Figure 2 and 3 shows this relationship on the basis of number of cells in suspension on the third fermentation day and the degree of fermentation achieved up to that point, compared to the higher attenuating reference yeast. The two non-preserved yeast as well as the 11 years lyophilized yeast previously fermented very uniformly over the four generations, the deep frozen yeast was not

quite as good by comparison. It may be noted in addition that an increase in intracellular trehalose content at the beginning of fermentation from 1.8% to 4.3% dry matter certainly does not produce better results in the first generation.

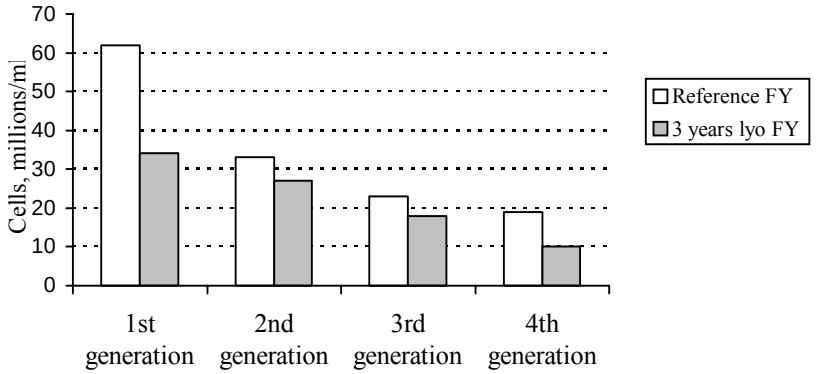

Fig. 2. Cell count in suspension after 3 days of main fermentation

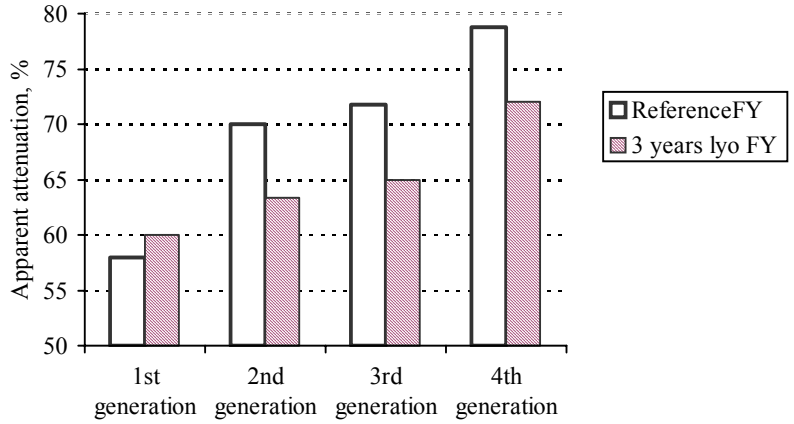

Fig. 3. The degree of attenuation after 3 days of fermentation

In the second test, variant 1yeast had a very low viability from the beginning and by the way of comparison, the other two variants demonstrated each, high viability, according table 2.

Over four generations, the degrees of attenuation achieved ranged in the region of 76 – 80%. Thou the reference yeast achieve a 1 - 2% higher degree of attenuation in the third and fourth generation, assisted by a slightly higher starting speed, the differences between the yeasts

are extremely small in this respect (figure 4). Flocculation behavior can be described as uniform in all batches (figure 5).

Table 2. FY strain, variants 1, 2, 3 viability, %

Reference FY	Variant 1	Variant 2	Variant 3
98	96 (*4)	98 (*68)	98 (*75)

*directly after reactivation

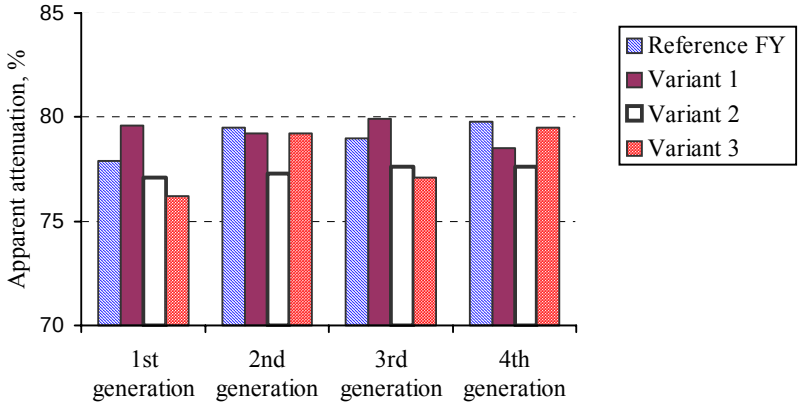

Fig. 4. Apparent attenuation of green beer in fermentations with preserved filter FY yeasts

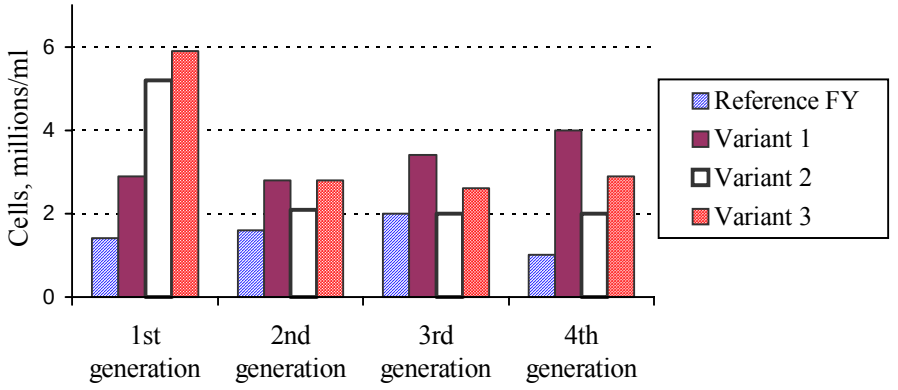

Fig. 5. Cells in suspension in fermentation with preserved filter FY yeasts

In the third test series, variants A – D illustrate a very heterogeneous selection in terms of viabilities (table 3), for the both yeast strains tested. The tests related with main fermentation, looking

at the viabilities of the FY and NFY yeasts after the reactivation and the corresponding vitality during fermentation, shown here as the degree of attenuation achieved up to the four fermentation day, indicate that no relationship exists.

Table 3. FY and NFY yeast strains viabilities (%), after reactivation

Variant A		Variant B		Variant C		Variant D	
FY	NFY	FY	NFY	FY	NFY	FY	NFY
29	56	16	16	62	60	59	61

After chemical – technical beers analysis, FY beers (table 4) show very uniform levels of vicinal diketones, the higher aliphatic alcohols 70.6 – 80 ppm and their esters ranges 16 – 20 ppm. With certain reservations, the NFY beers can also be regarded analytically as homogenous (table 5). Under identical conditioning conditions, none of the five beers was over the target limit value of 0.1 ppm in terms of overall diacetyl content. As in the FY beers, the NFY beers had maximum 10 ppm differences in terms of higher aliphatic alcohols.

Table 4. YF strain – analyses of filtered beer

Parameters	Reference FY	Variant A	Variant B	Variant C	Variant D
Attenuation, %	79.9	78.6	78.4	79.2	77.1
Ethanol, %	3.9	3.9	3.9	4	3.9
pH	4.6	4.5	4.4	4.4	4.5
Diacetyl, ppm	0.04	0.05	0.05	0.04	0.05
Pentadione, ppm	0.03	0.04	0.04	0.03	0.04
Higher aliphatic alcohols, ppm	79	70.8	70.6	78.5	80
Esters, ppm	16.8	16.6	16	20	19.5

Table 5. NYF strain – analyses of filtered beer

Parameters	Reference NFY	Variant A	Variant B	Variant C	Variant D
Attenuation, %	80	81.1	79.8	80	81
Ethanol, %	4.1	4.2	4.2	4.4	4.4
pH	4.6	4.5	4.5	4.5	4.4
Diacetyl, ppm	0.1	0.1	0.1	0.1	0.08
Pentadione, ppm	0.04	0.04	0.04	0.04	0.03
Higher aliphatic alcohols, ppm	73	70.9	68.7	70	71.6
Esters, ppm	33.2	30.4	30.3	31.8	34.3

Conclusions

The results of detailed observations of yeasts partially desiccated on filter paper show very clearly in the first instance that no relationship exists between viability or survival rate of preserved yeasts and the vitality of previously propagated yeasts during fermentation. This lends added emphasis to the fact that, when looking simply at yeast vitality, the highest possible survival rate after preservation is not an absolute priority. As the stains should be suitable for storage over many years, it is, however, to be recommended that the starting viability be as high as possible immediately after preservation.

It was found repeatedly that flocculent yeast lyophilized three years previously showed a markedly reduced fermentative performance compared to other batch. Similar related data (Van der Aar, 1996), the cause of this can relate to a premature initiation of flocculation, possibly as preservation – related restricted or delayed uptake or utilization of maltotriose or increased formation of manopreoteins or lectins. It is accepted that the synthesis of esters starts when the increase in the cell count slows down. Immediately as of the start of break formation, contact of flocculating cells with the substrate is diminished and thus formation of esters within the yeast is significantly reduced. This provides an explanation for the generally highest concentration of vicinal diketones in the beers. Lyophilisation is therefore unsuitable as a preservation method. Significantly more uniform results can be achieved using the methods of deep-freezing in liquid nitrogen and, in particular, partial desiccation on filter paper.

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