YEAST SELECTION AND STORAGE THE KEY TO OPTIMAL PERFORMANCE IN FERMENTATION

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Rezumat

Cunoaşterea proprietăţilor şi a parametrilor tehnologici de fermentare are importanţă majoră in vederea selecţiei tulpinii de drojdie folosită pentru fabricarea berii. Metodele de conservare au de asemenea un rol important în industria berii. Pierderea proprietăţilor pentru care a fost selectată drojdia poate avea implicaţii considerabile. Obiectivul experimentelor constă în deplina înţelegere a influenţei metodei de conservare care are la bază desicarea parţială a culturii de drojdie pe hârtie de filtru asupra capacităţii de fermentare a drojdiei şi asupra calităţii berii. Testele de fermentaţie au fost efectuate cu culturi desicate parţial pe hârtie de filtru, liofilizate şi congelate la temperaturi scăzute. Atenţie deosebită s-a acordat măsurii în care viabilitatea populaţiei din cultura conservată influenţează capacitatea de fermentare, respectiv vitalitatea drojdiei si calitatea berii.

Résumé

La connaissance des propriétés et des paramètres technologiques de fermentation a une importance majeure en vue de la sélection de la cellule de levure utilisée pour la fabrication de la bière. Les méthodes de conservation ont aussi un rôle important dans l'industrie de la bière. Perdre les propriétés pour lesquels la levure a été sélectée peut avoir d'implications considérables. L'objectif des expérimentations réside en l'entière compréhension de l'influence de la méthode de conservation qui a à la base la dessiccation partielle de la culture de levure de bière sur du papier filtre sur la capacité de fermentation de la levure et sur a qualité de la bière. Les testes de fermentation ont été effectués avec des cultures partiellement dessiccatives sur du papier filtre, lyophilisés et congelés à des températures basses. Une attention particulière a été accordée à la mesure dont la viabilité de la population de la culture conservée influence la capacité de fermentation, respectivement la vitalité de la levure et la qualité de la bière.

Abriss

Die Erkenntnis den Eigenschaften sowie auch den technologischen Parameter der Gärung ist von hauptsächlicher Wichtigkeit bei der Selektion den Hefenstämme für die Bierherstellung. Die Konservierungsmethoden spielen ebenfalls eine wichtige Rolle in der Bierindustrie. Den Verlust den Eigenschaften bei den ausgewählten Hefestämme kann bedeutenden Nachfolgen haben. Das Ziel den Forschungen besteht in das vollständige Verständnis dem Einfluss der Konservierungsmethoden, was besteht in der partiellen Trocknung der Hefekultur auf Filterpapier, über der Gärungskapazität der Hefe und über der Qualität des Bieres. Die Gärungsproben wurden mit partiell getrocknete Hefekulturen, lyophilisiert und eingefroren. Besondere Aufmerksamkeit wurde gezeigt dem Studium des Einflusses der Lebensfähigkeit der konservierten Population auf der Gärungskapazität, sowie auch die Vitalität der Hefe und die Qualität des Bieres.

Introduction

The quality of a beer is considerably influenced by the quality of its raw materials. But 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.

The yeast strain must therefore be selected as carefully as the other raw materials. 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.

As the yeast not only influences the fermentation time but also formation of fermentations by-products such as higher alcohols, esters, acids, volatile sulphur compounds etc, it has a decisive influence on smell and taste of a beer.

Therefore, good yeast must meet very differentiated requirements. These mainly relate to: intensity of propagation and speed of start of fermentation, fermentative capability and extract decrease, break formation and clarification, concentration of hydrogen ions,

redox potential, utilization of carbohydrates and nitrogen, spectrum of fermentation by-products and taste, biological control.

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 anaerobic phase after pitching is very important for yeast propagation, vitality and for keeping the yeast healthy. The aerobic and anaerobic phase must be well matched if formation of fermentation by-products is to be optimal.

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. This is given in ml CO_2/g of pitching rate and hour. The various yeast strains have different fermentative capabilities. The fermentative capability is a function of the special fermentative capability of the individual cell, of population density and of the activity of the maltase and maltotriose permease. The faster the enzyme induction and protein synthesis, the shorter will be the fermentation time. The extract decrease is used for immediate control of the fermentation curve.

When producing various beer types, use is made of different fermentation speeds. High attenuating flocculating yeasts and some very high attenuating powdery yeasts are generally used for pale lager, special, diet and strong beers because a rapidly progressing main and secondary fermentation will have a positive effect on smell, taste, aroma, liveliness and foam stability of a beer.

A vigorous secondary fermentation will lead to an intensive carbon dioxide wash whereby volatile fermentation by-products that are detrimental to taste and beer character will be washed out.

The type of yeast character – whether flocculating or powdery yeast – will have a strong influence on clarification processes. The yeast used should settle well in the fermenting room and once secondary fermentation is completed. Too early break formation will lead to premature completion of main fermentation; as a result, excessive extract would be introduced into the storage cellar during hosing. The consequences will be insufficient attenuation until retail degree, deficient leaching of unrefined fermentation by-product and biological susceptibility of the retail beer. A deficient break formation capability in turn would result in excessive yeast being introduced into the storage cellar. These beers can adopt a yeasty taste and smell.

The acid content of a beer is not only important for taste and lifeliness but also for excretion of proteic substances and hop bitter substances. Individual yeast strains differ in terms of their acid-forming properties.

Alcoholic fermentation leads to the formation of a number of by-products including organic acids such as citric acid, oxalic acid and others. Formation of these acids will raise the concentration of hydrogen ions considerably.

Although the absolute pH value of the finished beer is influenced by the composition of the wort, the fermentation process is highly significant for pH value.

The higher the fermentative intensity of a yeast strain and the more favourable the composition of the beer wort, the higher will be turnover and consumption of buffer substances. The concentration of hydrogen ions will rise.

The redox potential during fermentation and in the finished beer is of significance in terms of its physical-chemical stability. The various yeast strains show considerable differences. The various redox carriers of the wort such as sugar reductones, melanoidines, tannins, sulphhydryl groups are transformed during main and secondary fermentation so that

the initial redox potential will change. High attenuating yeast strains brew beers, which are rich in acid and with a high reductive power in the presence of a favourable wort composition.

Even small quantities of fermentation by-products have oftentimes a very significant influence on the character of a beer. This can be both positive and negative.

In addition, certain yeast properties can be steered by factors that can be technologically influenced. Diacetyl and acetoin have a very low taste threshold value so that even small quantities will affect taste properties.

Certain yeast strains tend to a more pronounced diacetyl formation, and not all strains can break down diacetyl equally well. The esters, an average of 30 mg/l, are among the most important aroma components that are desired to a certain exponents. In addition to ethyl acetate and other ethyl esters of a number of higher homologues of acetic acids, the acetates of practically all higher alcohols are represented. The main portion is accounted for by ethyl acetate with 50%. The yeast strains also differ in terms of ester formation so that ester formation can be also influenced by a suitable selection of the yeast strain, with an otherwise identical operation mode.

Raised isoamyl alcohol content will negatively affect wholesomeness and may cause as headache if consumed excessively. An increased content of aromatic alcohols such as tyrosol, tryptophol and phenyl ethanol will also have a negative effect on taste.

The individual yeast strains form different quantities of esters and higher alcohols so that the selection of a suitable yeast strain is of importance also for this.

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 behaviour 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.

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. 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.

Materials and methods

Two yeast strains were used in the investigation: a bottom flocculent yeast (FY) and a 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-month-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 flavour 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

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).

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.

Figure 1: Apparent attenuation after six days of main fermentation

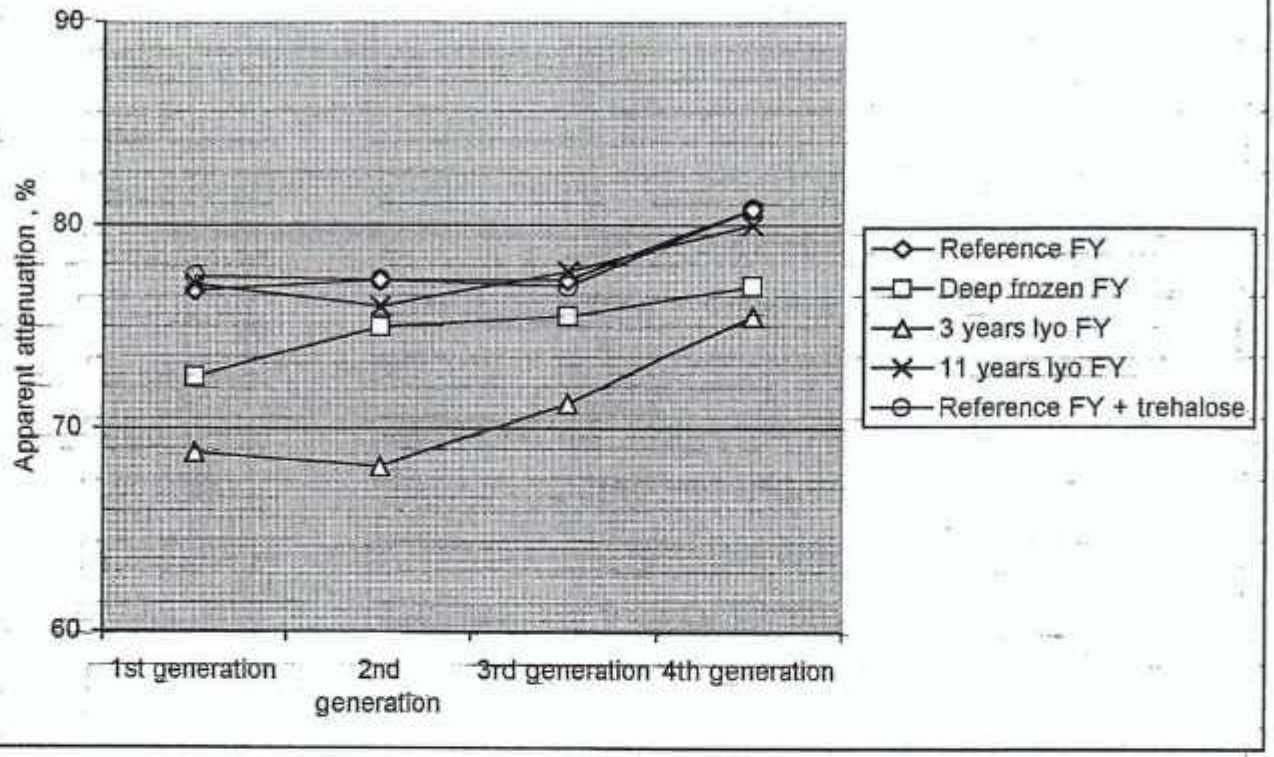


Figure 2: Cell count in suspension after 3 days of main fermentation

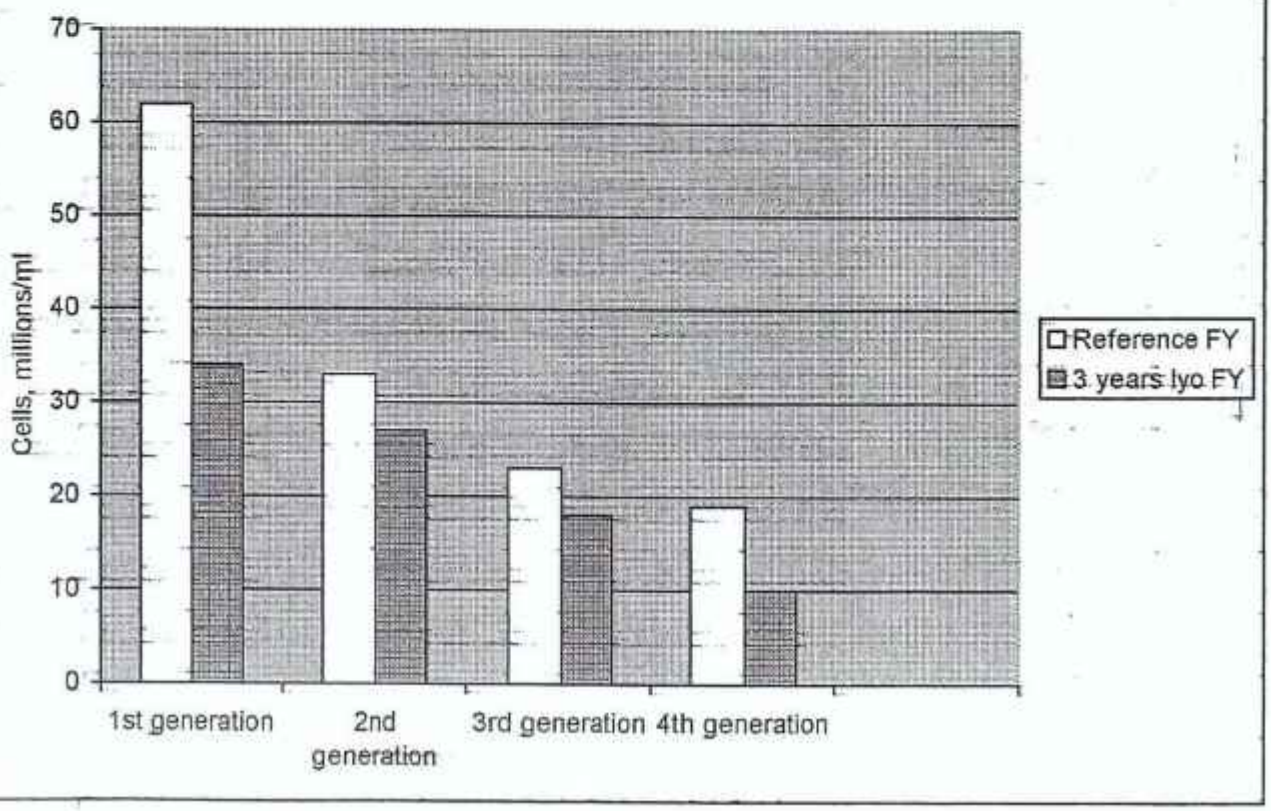
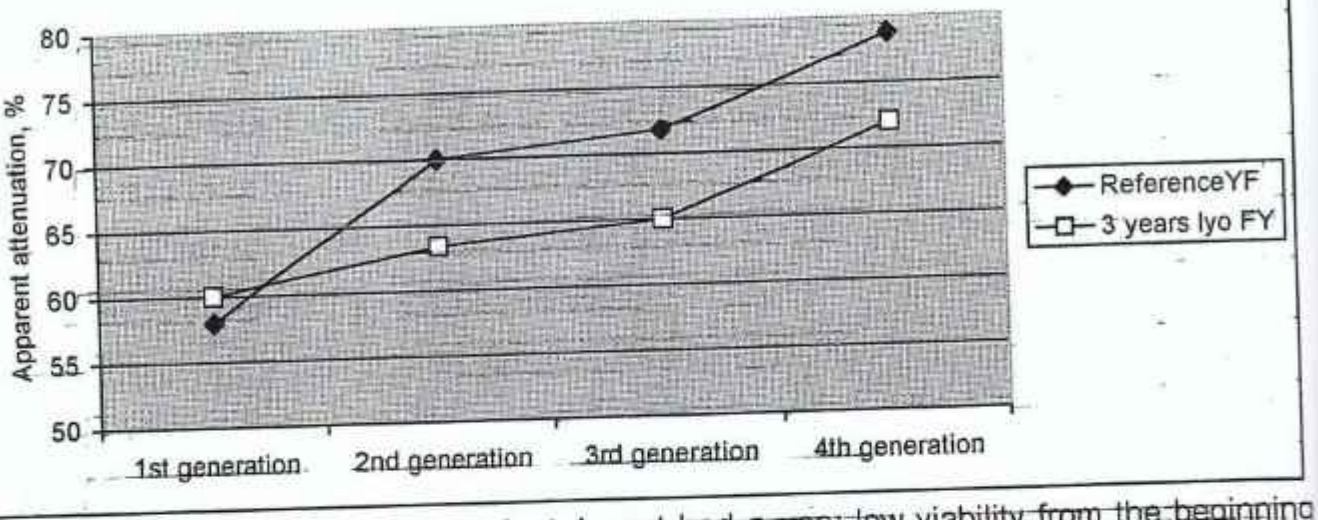


Figure 3: The degree of attenuation after 3 days of fermentation



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:

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			

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 behaviour can be described as uniform in all batches (figure 5).

Figure 4: Apparent attenuation of green beer in fermentations with preserved filter YF yeasts

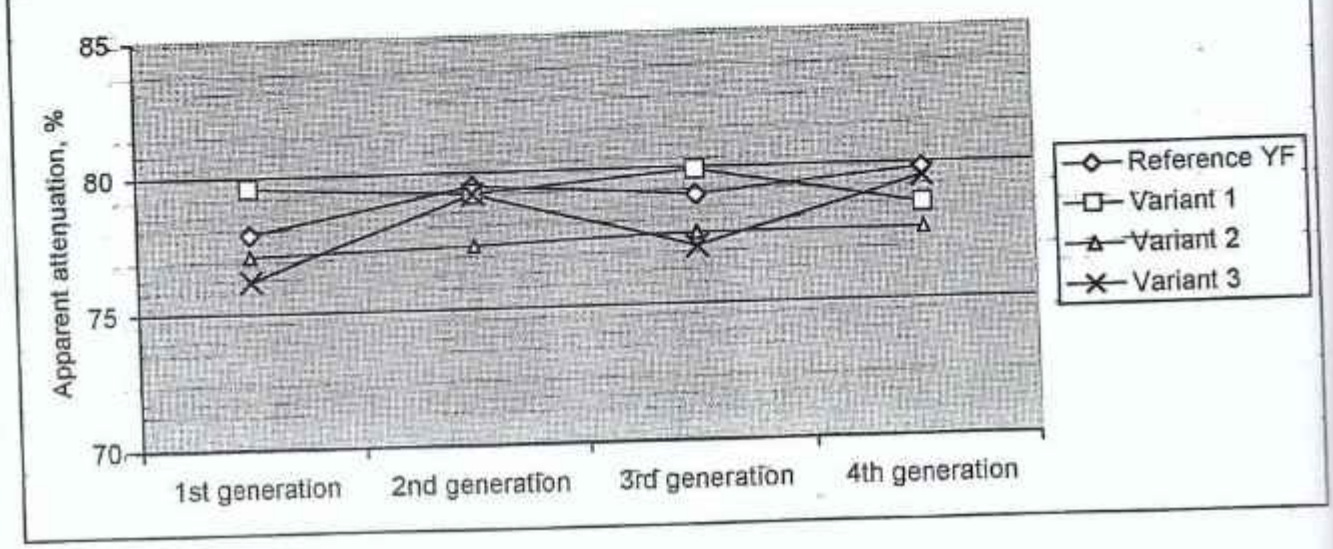
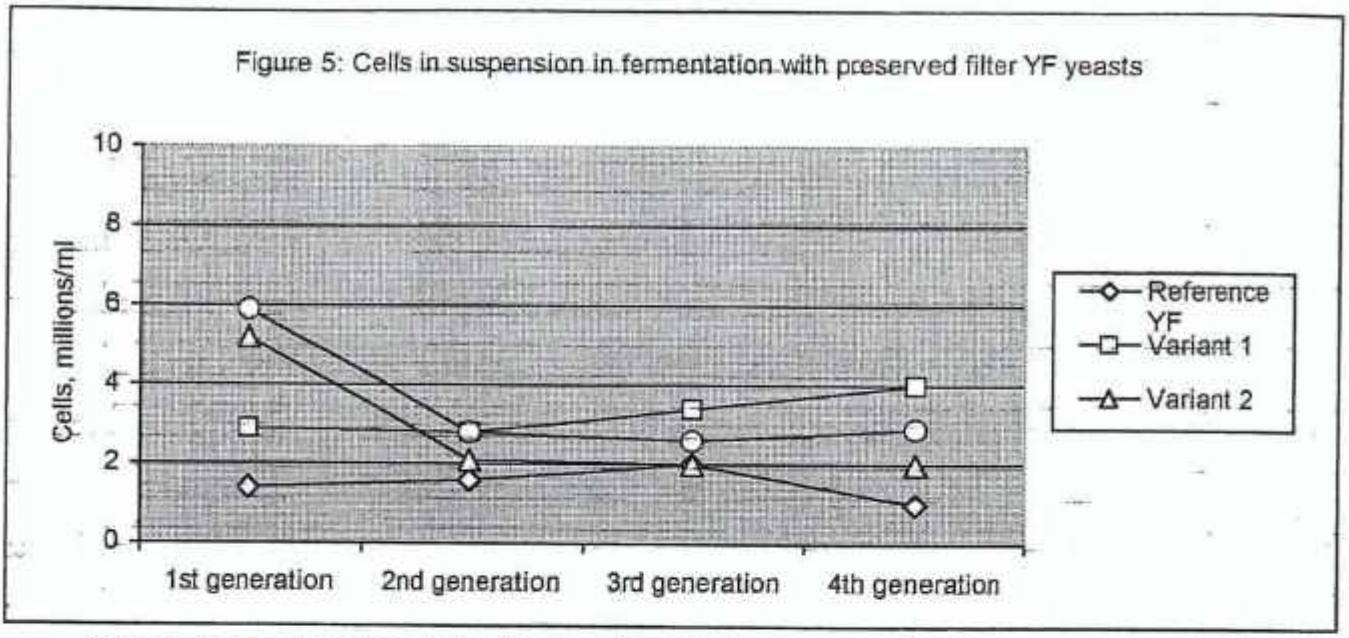


Figure 5: Cells in suspension in fermentation with preserved filter YF yeasts



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:

Table 3

YF and NYF yeast strains viabilities (%), after reactivation:

Variant A		Variant B		Variant C		Variant D	
YF	NYF	YF	NYF	YF	NYF	YF	NYF
29	56	16	16	62	60	59	61

The tests related with main fermentation, looking at the viabilities of the YF and NYF 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.

After chemical – technical beers analysis, YF 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 YF beers, the NYF beers had maximum 10 ppm differences in terms of higher aliphatic alcohols.

Table 4

YF strain – analyses of filtered beer:

Parameters	Reference YF	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 YF	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. 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 may be pointed out that the decrease in esters is represented in particular by a restricted formation of ethyl acetate, with the level of isoamyl acetate practically unchanged. What is happening here is that start of fermentation, including cell propagation, runs along similar lines, leading to comparable levels of higher alcohols and isoamyl acetate until such time as pronounced settling of the cells gives rise of the decrease in ester formation primarily via ethanol and acetyl-CoA. 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. The influence that the flocculation behavior of the yeast has on the later composition of the beer is clearly established on the basis of this causal chain.

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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