Frozen bottom-fermenting yeast – the format of the future

STERILE PITCHING YEAST | Yeast quality exerts a huge influence on beer quality. A flawless and uncomplicated fermentation can only be ensured if wort is pitched with vital yeast. However, this is easier said than done – in reality, yeast management and propagation require significant investments in both time and money. Furthermore, this essential aspect of brewing does not always receive the attention it deserves. A new product from the Yeast Center at the Research Center Weihenstephan for Brewing and Food Quality not only lessens the workload but also guarantees microbiological safety.

THE RESEARCH CENTER for Brewing and Food Quality (BLQ) at the Technical University of Munich-Weihenstephan is offering its world-famous bottom-fermenting yeast strain TUM 34/70, *Saccharomyces pastorianus* Frisinga, in a new frozen format. The yeast has been available in this format since December of 2021.

This frozen yeast product is named SmartBev™ Lager – TUM 34/70. It was developed in collaboration with the company Chr. Hansen, Hørsholm, Denmark, and has been sanctioned for use in beer production.

The following article describes the advantages of this new yeast product and its use in the brewery. This yeast enables breweries to bypass the entire yeast cultivation process in the laboratory and to pitch directly in a microbiologically pure form (into a propagator or fermentation tank).

A cosmopolitan yeast

Over 90 percent of the beer brewed around the world is bottom-fermented, and it is produced using *Saccharomyces pastorianus* lager yeast strains. The Frohberg type of bottom-fermenting yeast is characterized by its cryotolerance and that it is highly attenuative. Almost all bottom-fermenting yeasts employed for industrial applications belong to the Frohberg type, which, in contrast to the Saaz type, is highly attenuative. The properties of bottom-fermenting yeast during fermentation under cold conditions ensure that the yeast forms low concentrations of fermentation by-products at low fermentation temperatures. This, combined with the fact that the yeast is capable of reaching a high degree of attenuation, produces beers which are neutral in flavor with a high drinkability.

The best-known and most widely used bottom-fermenting yeast strain in the world is *Saccharomyces pastorianus* Frisinga – TUM 34/70. This strain was characterized in 1956 by Prof. Ludwig Narziß together

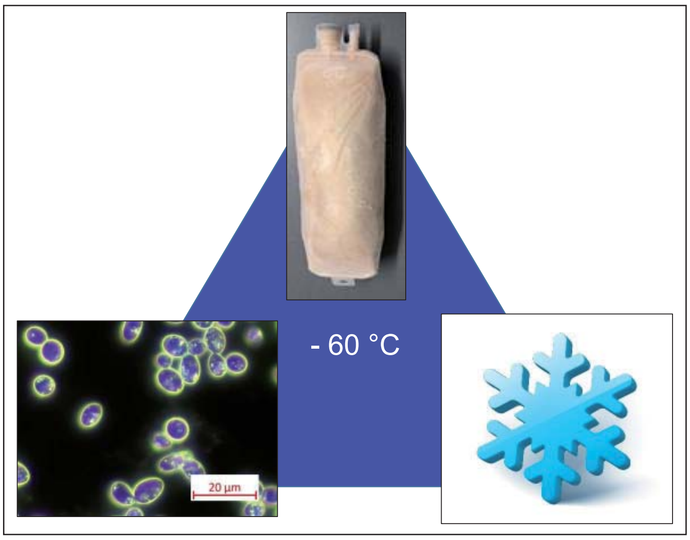

Fig. 1
The frozen bottom-fermenting yeast product SmartBev™ Lager – TUM 34/70; the 1 kg pouch is frozen at – 60 °C. It can be stored and has a cell density of > 1 billion cells per ml

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with other yeast strains in his dissertation. In this academic treatise, the yeast strain was noted to exhibit excellent properties during fermentation and a balanced aroma profile. Since then, this yeast strain has spread to breweries at the far corners of the Earth, no doubt fostered, in part, by the international consulting performed by the Technical University of Munich. *Saccharomyces pastorianus* Frisinga – TUM 34/70 also serves as a scientific reference strain (e.g., in fermentation trials). In addition, it was the first bottom-fermenting yeast strain to have its entire genome sequenced. Frisinga – TUM 34/70 is sent by the Research Center Weihenstephan for Brewing and Food Quality at the Technical University of Munich either as agar slant culture or as a 500 ml liquid culture to breweries worldwide and is used in almost every country in the world.

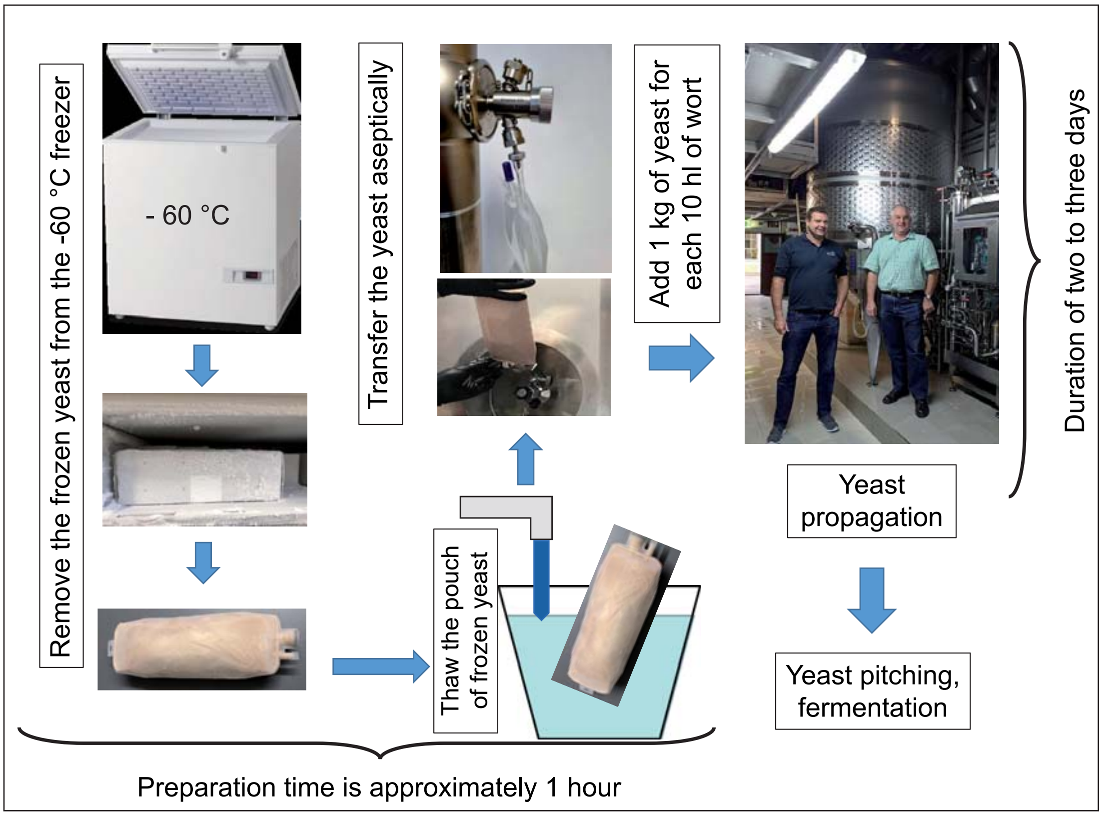

Fig. 2 Instructions for use (removal from the freezer, thawing, aseptic transfer, propagation) of the SmartBev™ Lager –TUM 34/70 bottom-fermenting frozen yeast product, spanning the period until yeast propagation begins through subsequent pitching to initiate fermentation (total time until the yeast is ready to pitch: two to three days, preparation time of 1 h)

Yeast care and cultivation – a full-time job

Once a Frisinga – TUM 34/70 agar slant culture or a 500 ml liquid culture arrives in a brewery laboratory, the yeast is grown up under aseptic conditions to the pitching volume required for the yeast propagator. The aim is to produce yeast with a high degree of vitality and in the purest microbiological state. Depending upon the process, cultivation of this pure culture takes about three to seven days and involves several operational steps. Even if the highest hygiene standards are observed when working with pure brewing cultures, there is always a risk of contamination in the laboratory. In addition to growing up the yeast in a Carlsberg flask or in a sterilized transfer keg (volume: 5 to 30 l) in the laboratory, there are still many other operational aspects to consider when striving for best practices in yeast management.

This includes keeping to a tight schedule, i.e., the pure brewing yeast culture must be ordered far enough in advance that there is still time to hygienically propagate it to the required volume. If the brewery laboratory stores its own pure yeast culture on an agar slat, the agar must be prepared with wort which contains a sufficient amount of nutrients. Moreover, it must be transferred to a fresh agar slant every four to six weeks and stored at 2 – 4 °C. If a pure brewing yeast culture is not available at the requisite time, the propagator cannot be started, and there

MICROBIOLOGICAL SPECIFICATIONS FOR *SACCHAROMYCES PASTORIANUS* SMARTBEV™ LAGER – TUM 34/70

Analysis method	SmartBev™ Lager – TUM 34/70
Beer-spoiling bacteria – microscopy and enrichment in NBB broth	absent (microscope examination), negative in 1.0 ml (NBB-B)
Top-fermenting yeast in bottom-fermenting yeast – the 37 °C method	negative in 0.1 ml
Wild yeast strains – enrichment in YM broth + $CuSO_4$	negative in 0.1 ml
Bacterial contamination of pure brewing yeast – enrichment in liquid yeast extract	negative in 0.1 ml
PCR identification of *S. cerevisiae* var. *diastaticus*	negative in 0.1 ml
Aerobic bacteria – enrichment in wort + actidione	negative in 1.0 ml
Aerobic bacteria – cultivation on WLD agar	negative in 0.1 ml

Table 1

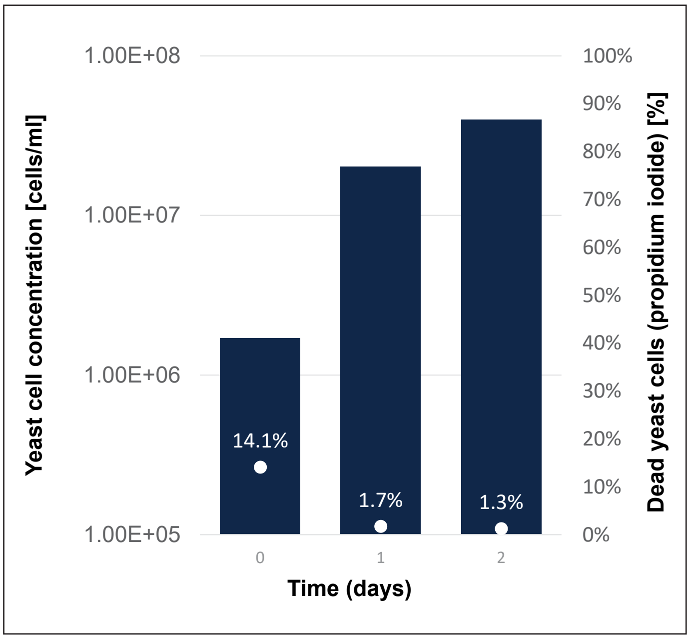

Fig. 3
Change in the yeast cell concentration per ml (blue columns) and the proportion of dead cells, expressed as a percentage in the yeast population (white dots) over the course of two days after inoculation of the wort

will be no yeast available for fermentation. Cropped yeast from a previous fermentation must then be used. In short, the challenge is to have the laboratory culture bottom-fermenting yeast ready in order to make it available at the right moment in time. The yeast must be in a microbiologically pure form, with the yeast cells exhibiting a high degree of vitality and in the right volume, precisely at the time it needs to be transferred to the yeast propagator. The new frozen yeast product SmartBev™ Lager – TUM 34/70 can lessen the workload at the brewery, and depending upon the conditions in the laboratory, the frozen yeast may be able to offer a brewery a wealth of advantages. These aspects of yeast in this new format will be discussed in detail below.

TUM 34/70 goes frozen

A pouch containing 1 kg of SmartBev™ Lager –TUM 34/70 yeast is shown in fig. 1. The company Chr. Hansen of Hørsholm, Denmark, produces this frozen bottom-fermenting brewing yeast at the Pohlheim facility in the state of Hesse in Germany. It is distributed to German-speaking countries by the Research Center Weihenstephan for Brewing and Food Quality (BLQ) of the Technical University of Munich.

Fig. 2 shows the instructions for handling SmartBev™ Lager – TUM 34/70 yeast. The yeast can be stored at – 60 °C for up to 18 months, which would thus correspond to its shelf life.

This type of storage requires a special – 60 °C freezer, which is available in various sizes to accommodate different volumetric capacities. The net cost associated with this one-time investment is between about 1200 EUR and 2000 EUR, depending upon the amount of storage space (10 – 150 pouches). Before the yeast can be used, it must be thawed for about one hour in a container filled with water (e.g., a 30-l bucket). The frozen yeast melts and turns to liquid during this period. The liquid yeast suspension can be punctured aseptically through a septum in the bag. There are currently two different aseptic transfer systems on the market. The liquid yeast in the pouch can be pressed into a pipe or into a vessel (e.g., Carlsberg flask or propagator). After the yeast is transferred, propagation can commence. At a dosing rate of 1 kg SmartBev™ Lager – TUM 34/70 per 10 hl (e.g., 3 × 1 kg pouch per 30 hl of wort), propagation takes about two to three days to reach a cell density of 80 – 100 million yeast cells per ml in the propagator (depending upon the temperature, aeration intervals and circulation of the yeast suspension). Subsequently, wort can be pitched with this propagated yeast to initiate fermentation. Recommended cell counts for pitching are 10 to 20 million cells per ml to ensure a rapid onset of fermentation.

Microbiological specifications

The basic prerequisite for smooth yeast propagation and fermentation is a pure culture of brewing yeast with no contaminants. This ensures that the propagated yeast is also of equal microbiological purity.

Table 1 lists relevant microbiological parameters in the brewery and the corresponding specifications for the SmartBev™ Lager – TUM 34/70 product. Only after a batch of SmartBev™ Lager – TUM 34/70 yeast meets these specifications will it be released for sale. Furthermore, the yeast is screened for relevant food pathogens, and the yeast vitality and viability are also tested.

Using frozen yeast for propagation

Fig. 3 shows utilization of SmartBev™ Lager – TUM 34/70 for a 30-hl propagation. The graph depicts the rapid decrease in the fraction of dead cells (white dots). One should note that in this measurement, the dead cells were stained with propidium iodide, an intercalating dye, which detects more dead cells than methylene blue. Therefore, the fraction of dead cells is higher with propidium iodide than if the cells had been stained with methylene blue. The proportions of dead cells on days 1 and 2 of propagation were still less than two percent.

In addition, fig. 3 shows the expected increase in the concentration of yeast cells, which in this case corresponds to an approximately 25-fold increase in the yeast population within 48 h.

The yeast can be used for pitching after 48 h in the propagator, or if necessary, it can be propagated a little longer if even higher cell densities are required for pitching (depending upon the volume used to pitch the wort). Propagation of the SmartBev™ Lager – TUM 34/70 yeast can be adapted to site-specific production operations in order to achieve the required cell count with a high level of vitality for pitching.

Beer quality and repitching cropped yeast

In addition to propagated yeast, the ability to utilize cropped yeast is an important economic factor and impacts process technology as well. Table 2 shows the parameters used to measure bottom-fermented beers (industrial, commercially produced beers) produced with SmartBev™ Lager – TUM 34/70 propagated yeast and the resultant yeast harvested from the first and third batches. Both the propagated yeast and cropped yeast produced beers with a high level of sensory acceptance (the DLG score of 5 is the maximum possible). The beers were evaluated sensorially based on the DLG scheme and scored 4.3 points or higher for all of the sensory attributes. Moreover, table 2 shows that the chemical measurements of the fermentation by-products (aroma compounds typically found in green beer and in matured beer) vary within a narrow range, allowing the beers to be fundamentally compared on a sensory level. The substances, which are responsible for the green beer profile (e.g., diacetyl and acetaldehyde), were reduced by the cropped yeast in the different batches to such an extent that they had no negative sensory impact (keyword: off-flavor) on the beer. The concentrations of aroma and flavor compounds contributing to the sensory profile of the matured beer exhibited only a small amount of variation. In summary, the use of SmartBev™ Lager – TUM 34/70 propagated yeast and the resultant cropped yeast allows beer of a very high sensory quality to be produced in a reproducible manner.

Summary

In closing, the advantages of the SmartBev™ Lager – TUM 34/70 yeast product are listed below along with a description of how it simplifies operations in the brewery:

- The entire yeast propagation process in the laboratory is eliminated along with the associated risk of contamination;
- the yeast supplied always has a high level of vitality – 10 hl wort can be directly inoculated with 1 kg of SmartBev™ Lager – TUM 34/70 yeast;
- the yeast is ready for use within one hour: the frozen product is thawed in a 20 – 30 °C water bath, no further activation is necessary;
- the frozen yeast is certified and is of a consistent purity, quality and stability (guaranteed pure culture, free of microbial contaminants and of a consistent quality);
- the product has an 18-month shelf life when stored at a temperature of – 50 °C or lower (specially designed freezers, net cost ranging from 1200 – 2000 EUR, allow on-site storage);
- the product is available from reliable supply chains around the world;
- expert customer support is available – from implementation to optimization and beyond;
- the yeast can be transferred with a special aseptic device directly from the pouch to inoculate the wort.

CHEMICAL AND SENSORY QUALITY ATTRIBUTES OF BOTTOM-FERMENTED BEER...

...of the style Bavarian Helles with an original gravity of 11.2 % w/w and 4.9 % alcohol by volume. This beer was fermented with SmartBev™ Lager – TUM 34/70 propagated yeast as well as with yeast cropped from the first and third fermentations

	Bottom-fermented Helles (propagated yeast)	Bottom-fermented Helles (cropped yeast after 1st fermentation)	Bottom-fermented Helles (cropped yeast after 3rd fermentation)
DLG score for aroma	4.4	4.5	4.5
DLG score for flavor	4.4	4.4	4.5
DLG score for body	4.6	4.6	4.6
DLG score for liveliness	4.6	4.6	4.6
DLG score for quality of the bitterness	4.3	4.4	4.4
pH	4.5	4.5	4.5
Acetoin	1.9	3.0	2.8
Diacetyl	0.02	0.02	0.02
2,3-pentanedione, total [mg/l]	0.01	0.01	0.01
DMS [µg/l]	84	64	74
Butyric acid ethyl ester [mg/l]	0.13	0.14	0.14
Decanoic acid ethyl ester [mg/l]	0.06	0.04	0.03
Acetic acid -2-phenyl ethyl ester [mg/l]	0.55	0.6	0.82
Acetic acid-isobutyl ester [mg/l]	0.06	0.07	0.08
Hexanoic ethyl ester [mg/l]	0.25	0.21	0.32
Octanoic acid ethyl ester [mg/l]	0.3	0.23	0.22
Isovaleric acid [mg/l]	1.1	1.3	1.2
Hexanoic acid [mg/l]	3.8	2.9	2.8
Octanoic acid [mg/l]	4.4	4.2	4.0
Decanoic acid [mg/l]	1.0	1.0	1.0
Acetaldehyde [mg/l]	3.5	7.8	5.6
Ethyl acetate [mg/l]	16.2	22.7	18.4
n-propanol [mg/l]	10.6	11.6	11.5
i-butanol [mg/l]	10.5	12.7	12.8
Isoamyl acetate [mg/l]	1.6	2.2	2.0
Amyl alcohols (2-, 3-methylbutanol) [mg/l]	60.0	74.0	68.1
Phenylethanol [mg/l]	22.8	22.9	27.1

Table 2

The Research Center Weihenstephan (BLQ) of the Technical University of Munich and in particular Dr. Mathias Hutzler with his team at the Yeast Center are available to assist breweries with the implementation of this new trend-setting technology. The use of the frozen SmartBev™ TUM yeast marks a significant advance in microbiologically safe fermentation technology with the highest levels of consistency. ■

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