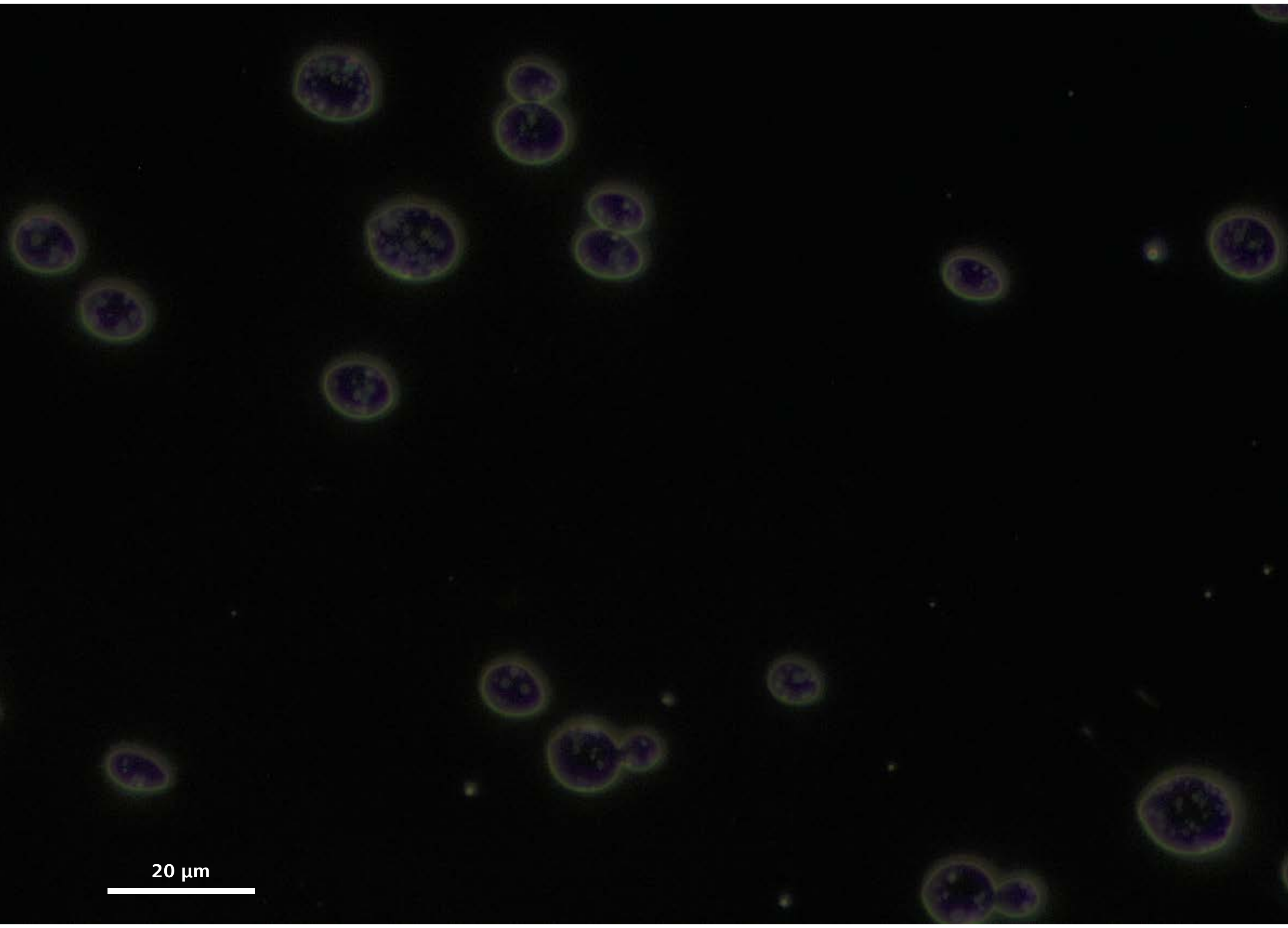

Baumgartner – the Beer from the Innviertel Region

Quality control with ZEISS Axiolab 5

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Seeing beyond

Baumgartner – the Beer from the Innviertel Region

Quality control with ZEISS Axiolab 5

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For the Austrian brewery Jos. Baumgartner GmbH, there are two key elements relating to the art of brewing: quality and taste. That is why Baumgartner only uses its own pure culture yeasts, which are checked on a regular basis using microscopes. There is no great science involved – it needs to be simple, and it has to taste good! The ideal field of activity for ZEISS's Axiolab 5 upright light microscope.

Jos. Baumgartner GmbH brewery

Huck di zuwa – is how curious customers are greeted on the brewery's website. And this also seems to be the philosophy of the company, which was founded as early as 1609 in Schärding (Figure 1). Independence is the basic principle of the brewery, which has been incorporated into the Baumgartner-Spanlang non-profit foundation after more than 400 years of family ownership. For this reason, Baumgartner brewery has also been committed to the foundation's charitable mission since 1989, which includes, for example, the promotion of facilities for disabled children and the elderly. This is a concept that has proven itself both in the past and the present. Today, Jos. Baumgartner GmbH is one of the largest medium-sized brewing companies in Austria, supplying mainly the Inntal.

Figure 1 *In 1954, the entire brewery was moved to the gates of Schärding. Nowadays, a brewery inn is located at the former site.*

The ingredients determine taste and quality

Tastes differ. That's why Baumgartner has the right drink for every taste (Figure 2). Whether a Zwicklbier, shandy or special beer for events – it has to taste good. New varieties and flavors are constantly being developed. For example, a separate wheat beer plant (Figure 4) was built in 2015.

"Brewing beer involves precision work for taste."

"What becomes of hops, malt and water is not determined by chance."
A key ingredient in beer production is yeast, which is responsible for the alcohol and carbon dioxide content of the beer – among other things. Good yeasts also provide a better flavor. Yeast is added during the brewing process (Figure 3) after mashing.

Figure 2 *The brewery offers the right product for every palate. Beer or lemonade – the most important thing is that it tastes good.*

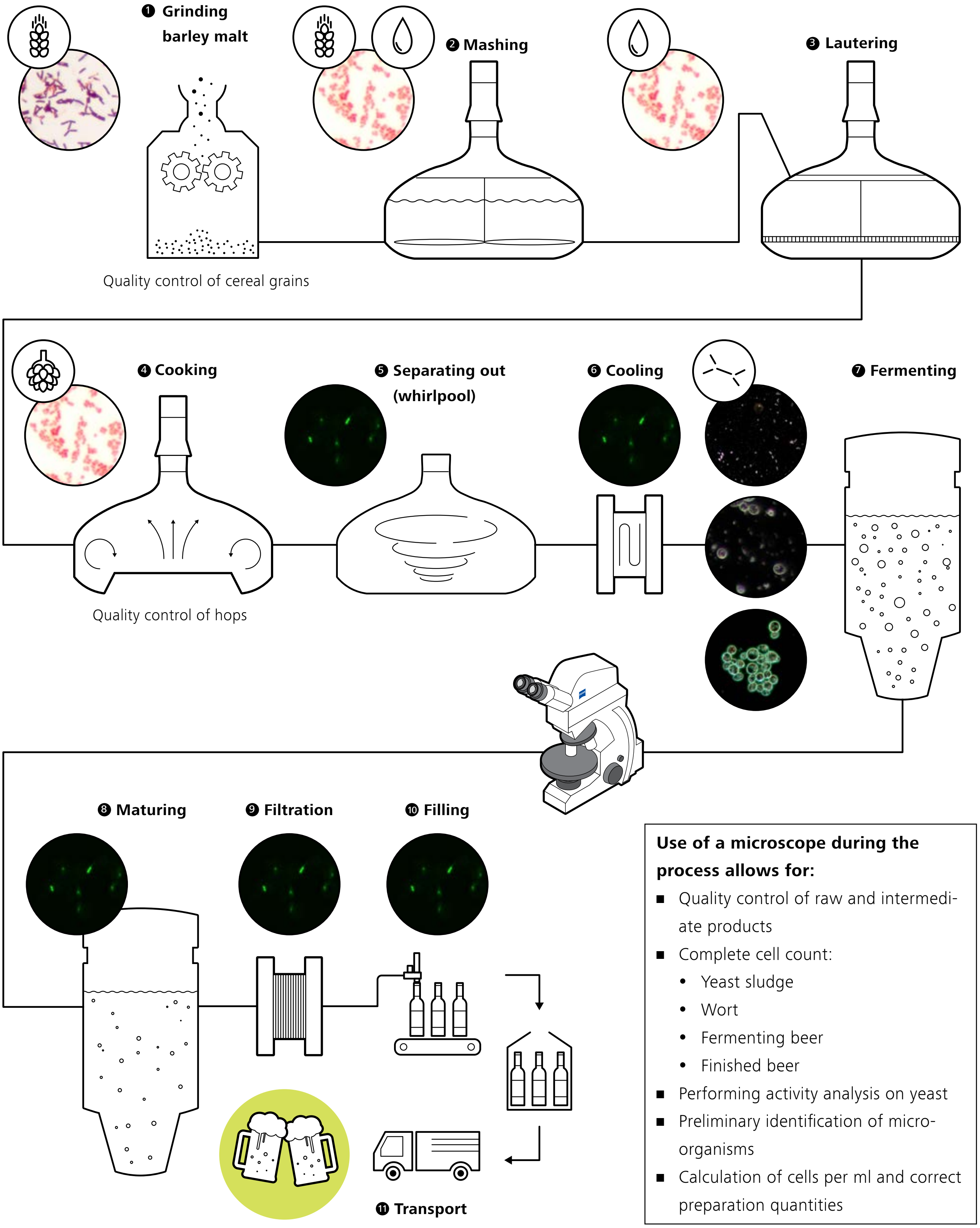

Figure 3 Brewing process

Use of a microscope during the process allows for:

- Quality control of raw and intermediate products
- Complete cell count:
  - Yeast sludge
  - Wort
  - Fermenting beer
  - Finished beer
- Performing activity analysis on yeast
- Preliminary identification of microorganisms
- Calculation of cells per ml and correct preparation quantities

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Figure 4 *Four tanks, each with a capacity of 120 hectoliters, make it possible to produce new wheat beer varieties separately.*

Yeasts are unicellular microorganisms which belong to the fungi genus. They are also often referred to as yeast fungi. Yeasts are eukaryotic cells (Figure 5) and, unlike bacteria, have a nucleus. They feed on sugar in particular and convert it into alcohol and carbon dioxide. Fermentation is therefore nothing more than the metabolic activity of yeast fungi. This is what gives beer its effervescence (Figure 6).

There are a variety of yeast strains that differ in their specific properties. However, the brewing process requires defined, predictable properties. This is the reason for the development of pure culture yeasts, such as those produced and used by the Baumgartner brewery. This allows the targeted use of yeasts that bring defined, stable properties and make it possible to produce large quantities of beer with consistent quality. The pure culture yeasts are initially grown as pitching yeast from the laboratory in yeast propagation plants to increasingly larger quantities until sufficient starter cultures are available. The number of yeast cells in one milliliter of wort is several million individuals, which multiply by a factor of 2 to 5 during fermentation and can thus be harvested at the end of the fermentation process and reused for the next brews. There is a natural limit to the propagation of yeast. It stops the fermentation process independently, which in turn also sets a natural limit to the alcohol content. For typical brewer's yeasts, this is around 8 % by volume.

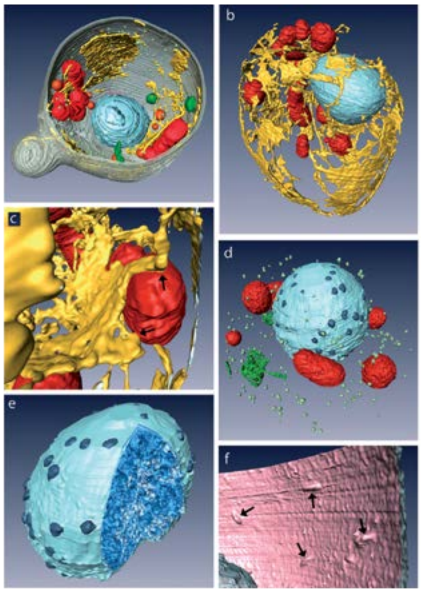


Figure 5 *3D reconstruction of a yeast cell, 3.72 × 3.72 × 15 nm volume showing ER, nucleus, cisternae, vesicles, lipid droplets, microtubules, mitochondria, cell walls. Block sample, FIB-SEM. Courtesy of J. Caplan, Delaware Biotechnology Institute, University of Delaware, USA. Biotechniques 53: 41 – 48 (July 2012) doi 10.2144/000113850*

Figure 6 *The carbonic acid released by the fermentation process is responsible for the formation of the beer froth and the effervescence of the beer.*

The discovery of yeast

Around the 16th century, people found out that the perfect beer needs alcoholic fermentation. In 1789, it was discovered that the yeast creates the alcohol and thus also a longer shelf life as well as the sparkling effervescence. Further research in the 19th century led to even more profound findings, especially with regard to the use of different yeast strains in the brewing process.

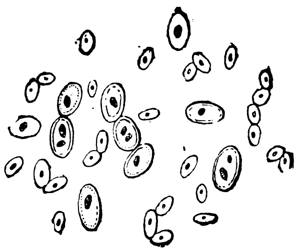
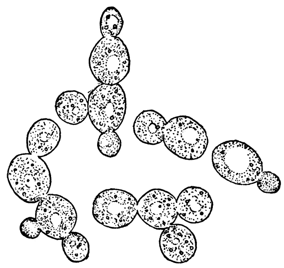
Emil Christian Hansen and Louis Pasteur in particular made a significant contribution to the development of beer through the development of yeast breeding and the isolation of yeast strains, respectively. They used microscopes to detect different yeast strains. People started to grow and cultivate pure yeast. During this time, it was also included and targeted as an ingredient in the German purity law for beer.

Microscopic quality control

Regular checks during the brewing process are not required by law. Nevertheless, it is a good idea to examine various parameters, and not only for economic reasons. And that is why, at the Jos. Baumgartner GmbH brewery, three laboratory employees in the brewery's own laboratory are also charged with microscopic quality control.

The following are primarily assessed:

1. The type and quality of the yeast.
2. Bacterial contamination by beer-spoilage bacteria.

Type	Top-fermented yeast	Bottom-fermented yeast
Representative	Saccharomyces cerevisiae	Saccharomyces carlsbergensis, Saccharomyces pastorianus
Morphology	Large cell surface, round to ovoid, diameter 5 – 10 µm	Small cell surface area
Optimum fermentation temperature	15 – 20 °C	4 – 9 °C
Behavior	Rises to the top, faster, works for 3 – 6 days	Sinks to the bottom, slower, works for 8 – 10 days
Table of contents	Wheat, rye, spelt malt	Barley malt
Taste	Fruity	Lean, pure
Typical types of beer	Ale, wheat beer, Altbier, Kölsch	Pilsner, lager, export, Märzen, bock
Example		

In addition to alcohol, the yeast cells produce up to 300 other by-products during fermentation, such as alcohols, esters and aldehydes, which play a key role in determining the taste of the beer. It is therefore possible to influence the taste – and also the smell – by means of the specific combination of different yeasts.

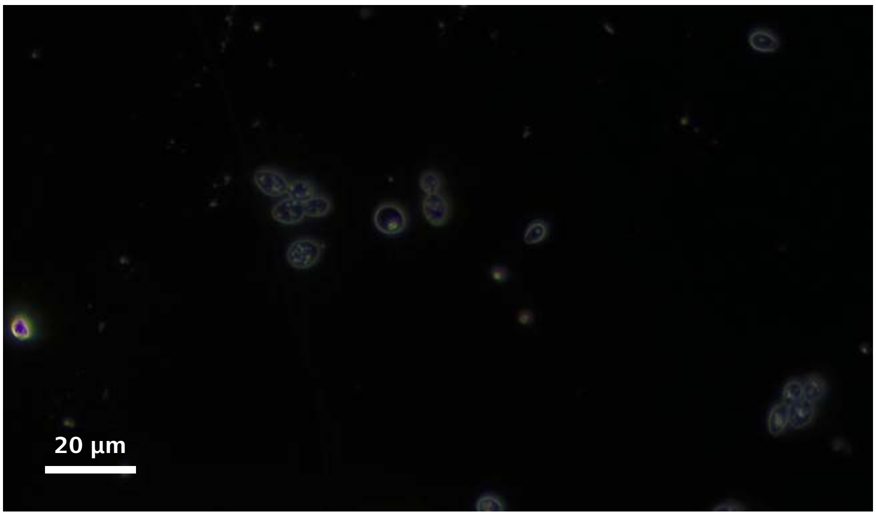


Figure 7 *Brewing yeast in darkfield imaging*

1. Top-fermented or bottom-fermented yeasts
Use of top-fermenting or bottom-fermenting yeast strains (Figure 7) results in different types of beer. With top-fermenting yeasts, yeast harvesting takes place from the top, while bottom-fermenting yeasts settle more quickly and can be harvested from the bottom of the fermentation vessels.

2. Bacterial contamination by beer-spoilage bacteria
So-called beer-spoilage bacteria can pose a problem that could threaten the existence of any brewery. This refers to microorganisms that enter the beer during the brewing process. Although most bacteria cannot survive due to the few nutrients, alcohol, bitterness, high carbon dioxide content and low pH value, some species of bacteria have managed to adapt and grow in beer.

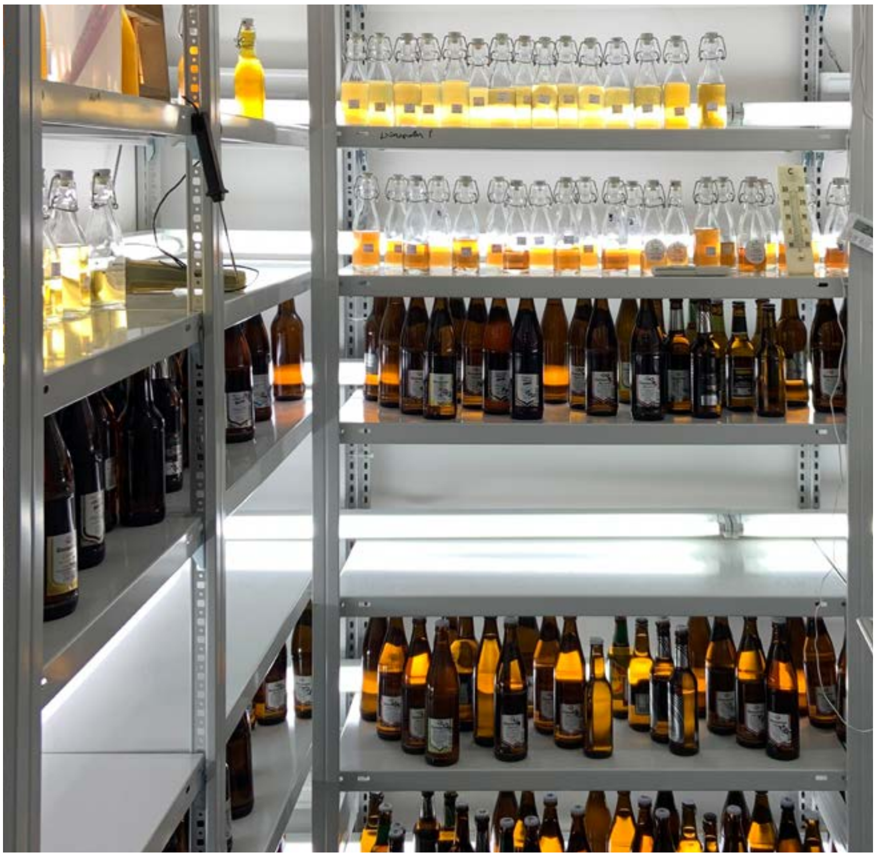


Figure 8 *Retention samples serve as proof of the purity of the beer, which may be required at a later date.*

A distinction is made between different categories of beer spoilage and groups of bacteria. The most common beer-spoilage bacteria are:

- *Lactobacillus brevis*, which makes the beer cloudy and sour
- *Pediococcus damnosus*, which gives the beer a buttery taste
- *Lactobacillus lindneri*
- *Megasphaera cerevisiae*
- *Pediococcus inopinatus*

These germs always lead to spoilage of the beer. Other microorganisms, such as various lactic acid bacteria, and also foreign yeasts such as *Saccharomyces diastaticus* only grow under certain conditions, e.g. low hop or alcohol content or an increased pH value. Although most of the germs in beer are apathogenic – i.e. do not pose a risk to humans – they can, however, significantly impair the taste and smell of the product. In the worst case scenario, contamination with bacteria can lead to the recall and discarding of entire batches – with financial consequences for the brewery and damage to the brand. Reliable, rapid detection of beer-spoilage bacteria is therefore of major importance for further beer production.

In the brewery’s own laboratory, beer-spoilage bacteria detected using microscopes, among other things. The product or rinse water samples are first treated by membrane filtration and incubation of the filter in a nutrient medium at a temperature of approximately 28 °C to enrich contaminating germs. The growth or multiplication of any microorganisms from a visible colony size can thus be observed. This method makes a quick positive/negative control possible. This is followed by a microscopic examination of the enriched sample, which allows a definite conclusion to be reached.

Retention samples (Figure 8) from the various brewing phases are kept until the beer’s expiration date and thus serve as evidence in the event of any complaints.

Figure 9 *The ZEISS Axiolab 5 laboratory microscope provides high-contrast images of beer yeasts.*

Requirements for the microscope and equipment used

The assessment takes place using an upright light microscope in darkfield imaging. Simple operation and few adjustment options are the main requirements for the microscope. Documentation is not absolutely necessary.

In the Jos. Baumgartner GmbH brewery's laboratory, the employees work with ZEISS Axiolab 5 in stand-alone mode (Figure 9). Axiolab 5 is made for routine work that goes on every day in the lab. The microscope offers easy handling and an ergonomic user concept that is adapted to the lab work. The smart microscope system automatically adjusts the parameters for image acquisition. Position the sample, focus and acquire the image directly on the device – it could hardly be easier. You access all the control elements with just one hand, including the snap button, stage drive, focus adjustment, and brightness control. The microscope is equipped with darkfield imaging and allows yeast cells, for example, to appear in high contrast against the dark background. The basic equipment also includes the LD A-Plan 63× objective. Compared to the previously used oil lens, this one provides high-quality images without the need to work with an immersion medium.

ZEISS Axiolab 5 operates independently of a computer system. Used in combination with the Axiocam 208 color microscope camera, it offers the full advantage of a smart stand-alone microscope system:

- Snap images and record videos directly from the stand
- Use the mouse (and optionally, a keyboard) to control the camera via the on-screen display
- Save settings
- Store images with all meta data of the microscope and camera as well as scaling information
- Predefine the name or rename your image

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