

## Microbiologically controlled production of low-alcohol beer types

General Information about alcohol-free or low-alcohol beers:

Beer can be classified according to its alcohol content like the following:

1. Alcohol-free beer, possessing an alcohol content of up to 0.5%
2. Low-alcohol-beer, possessing an alcohol content of up to 1.5%
3. And beers possessing an alcohol content of above 1.5%, and therefore cannot be declared anymore as low- or alcohol-free

Besides of these cost-intensive possibilities in order to produce low-alcohol or alcohol-free beers (e.g., by vacuum distillation), there are also other ways that can be conducted to serve the same purpose: Into consideration comes an interruption of the fermentation process during a certain stadium (i.e. after a desired alcohol content was reached), or the use of special yeasts possessing a different degree of sugar fermentation, whereby there would be no need anymore to lower or eliminate the alcohol content. The way of disrupting the fermentation is normally chosen if one wants to produce nutrient-beers or malt beers.

Presuming however the different sugar fermentation capacities of certain yeasts, then it needs to be considered that the currently used top- and bottom-fermenting brewing yeasts are not only fermenting glucose and fructose contained in wort, but also the disaccharide sucrose, as well as maltose and maltotriose. Thereby, sucrose, in the contrary to maltose and maltotriose, is cleaved into glucose and fructose by the exoenzyme saccharase, already at the cell wall periphery, so that it is (alongside with the other hexoses present in the system) channeled into the inner yeast cell (through "eased diffusion") and fermented therein.

Information about the production of alcohol-free and low alcohol beer by *Saccharomyces ludwigii*:

Different species of yeasts are known that are only able to ferment certain single sugars contained in beer-wort. For our special case, this yeast is the so-called *Saccharomyces ludwigii*, which

possesses only a sugar fermentation capacity for glucose, fructose and sucrose, but not for maltose and maltotriose. By applying this yeast, only these primary sugars (glucose, fructose and sucrose, which are present only in small amounts), can be fermented, so that, depending on the original wort content, it is possible to produce alcohol-free or low-alcohol beers, without having to disrupt the fermentation process.

The content of hexoses can further be lowered by controlling the mashing process, so that it is possible to produce alcohol-free beers that have only an alcohol content of 0.5% alcohol, even if the original wort content was 7%, and without having to disrupt the fermentation process. At this 7%-level of original wort, already a reduction of the hexose content of ca. 0.2% becomes imperative. This way of controlling the sugar content of the hexoses (Glucose, Fructose and the hexoses that derive from sucrose) process does not play a role for low-alcohol beers, as the limit of 1.5% alcohol by using *Saccharomyces ludwigii* is not going to be exceeded. An additional controlling of the fermentation by eventually changing the pH of wort is not possible.

The possibility of using *Saccharomyces ludwigii* in order to produce low-alcohol beers is known since decades and has been partially practiced, for example, to produce the Bavarian nutrient-beer. Today the use of *Saccharomyces ludwigii* has received again attention, because of the subsequent alcohol elimination that is unfavorable due to the high costs that need to be defrayed. Some time ago, The Research Center for Brewing and Food Quality started again to concern the problem of producing low-alcohol beers by applying *Saccharomyces ludwigii* and conducted numerous of small-scale fermentation experiments in different varieties, involving later on some large-scale experiments to obtain praxis-relevant information.

At first, starting from pure laboratory culture, the yeast is cultivated afterwards. This cultivation/propagation scheme differs practically in no way from other commonly acknowledged cultivations schemes; only the cultivation temperature is increased to about ca. 20 °C. Within the time interval of 3 days, ca. five times the amount/volume of wort is added to the yeast culture in repeating cycles until the amount of yeast, or amount of fermenting wort, needed for the primary fermentation charge, is reached. The primary fermentation temperature is chosen at 15–20 °C. Also chosen are a pitching yeast volumes of ca. 0.3–0.5 l per hectoliter and a primary fermentation duration of ca. 4–5 days, because the final attenuation is under 20%. Afterwards, the fermentation-set is left for about 2 days at 15-20 °C due to enhanced degradation of diacetyl and 2-acetolactate, and is then cooled down to ca. 1 °C. At this temperature, the fermentation-set is again left for clarification (pre-stabilisation can also be applied to this production step) for about 2 days (or longer). Afterwards it is filtrated (with addition of stabilization agents), and filled and pasteurized afterwards, if not EK-filtered. Depending on the original wort content and bunging, a subsequent carbonization to a carbonic acid content of 0.5% is eventually necessary. Depending on the circumstances, bunging at ca. 3 bar should be conducted already when the primary fermentation step is introduced; this is in order to restrict furthest going the carbonization step, or to save it entirely.

During the production of this type of beer, best about one quarter of the fermented substrate is remained in the tank after the primary/main fermentation ended, and the fermentation tank is refilled with beer-wort, so that a fresh yeast culture is only needed after certain time points. The main concern is to avoid not only common infections, but also to keep away the commonly applied brewery-yeast, because if brewery yeast is anyhow transfused to the yeast *Saccharomyces ludwigii*, even in smallest amounts, then this inevitably is leading to an enforced increased formation of alcohol and therewith to an exceedance of the allowable limit of alcohol-free and low-alcohol beers.

### ***Saccharomyces ludwigii***

*Saccharomyces ludwigii* originally belongs to the Non-Saccharomyces wild yeasts in breweries. This kind of yeast can be described as weak-fermenting, with no capacity to ferment sugars such as maltose and maltotriose. From the sugars present in wort, only glucose, fructose and sucrose are fermented. During the fermentation of wort at different concentrations, the following values are obtained:

Average values of beer analysis:

<b>Original wort content %</b>	11,3	7,2	5,4
Alcohol content %	0,76	0,45	0,27
Bitter values	27,4	23,1	19,0
Final attenuation of the finished beer %	16,7	12,2	11,4
Final attenuation %	17,1	13,1	12,9
Final attenuation % (brewing yeast, <i>Saccharomyces pastorianus</i> ssp. <i>carlsbergensis</i> Frisinga - TUM 34/70)	82,4	78,4	78,5
pH	4,85	4,81	5,1

Fermentation by-products (mg/l), average values:

<b>Original wort content %</b>	11,3	7,2	5,4
Acetaldehyde	3,6	2,2	4,0
Ethylacetate	0,5	0,2	0,1
n-Propanol	1,6	1,6	0,9
i-Butanol	11,0	6,5	4,5
i-Amylacetate	0,1	Sp.	Sp.
i-Amylalcohols	20,2	13,3	8,9
Diacetyl	0,03	0,03	0,04
2,3-Pentandione	0,01	0,01	0,01

In tasting panels the beer with 7.2% of extract was evaluated as the preferred beer. The fermentation temperature of the analyzed beers was at 15 °C isotherm.