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The Importance of a Comparative Characterization of *Saccharomyces Cerevisiae* and *Saccharomyces Pastorianus* Strains for Brewing

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Abstract: The volume and market share loss for classical beer types such as pils beer and wheat beer has been declining for several years, but the overall beer market remains almost unchanged as a result of the increasing interest in beer specialties Due to high biodiversity, the diversity of the strains, and the different flavor profiles, reliable and practical information regarding the characteristics of individual brewing strains is required to help brewers to find the right strain for their brewing purposes. This paper presents a comparison of 10 commercially available Technical University of Munich (TUM) brewing yeast strains. The strains were screened for genetic and phenotypic characteristics. After confirming the genetic distinctiveness by using species-specific real-time polymerase chain reaction (RT-PCR) systems and a strain typing method based on PCR-capillary electrophoresis of the partial intergenic spacer 2 (IGS2) fragment (IGS2-314 PCR-capillary electrophoresis), the strains were tested regarding phenotypic characteristics under controlled and identical fermentation conditions in small-scale brewing trials. Besides the fermentation performance, flocculation behavior, sugar metabolism and other phenotypic characteristics, the main focus was on the flavor and aroma profile of each investigated TUM yeast strain.

Keywords: *Saccharomyces pastorianus*; *Saccharomyces cerevisiae*; brewing yeasts; yeast characterization; top-fermenting yeast strains; bottom-fermenting yeast strains; culture yeast; brewing trials

1. Introduction

The Bavarian purity law of 1516 only permits barley, hops and water to be used to produce beer [1]. In 1993, the purity law was further clarified by the German "vorläufiges Biergesetz" (German preliminary beer law) and expanded in accordance with public perception that beer can only be produced using malt, hops and water. Even 477 years after the Bavarian purity law, there is still a lack of attention devoted to yeast as a raw brewing ingredient. Nowadays there is an increasing focus on yeast. MEIER-DÖRNBERG describes yeast as the flavor engine of the brewing industry [2,3]. Yeast metabolism during the fermentation and ripening process gives rise to approximately 80% of all aroma-active compounds in beer, thereby determining its aroma profile [4]. There are more than 300 different volatile and non-volatile fermentation by-products, which vary in their concentration from strain to strain. According to WHITE and ZAINASHEFF, brewer's yeast alone is able to produce about 500 different flavor and aroma compounds [5]. Yeast strains are as diverse as the resulting

flavors, and the choice of yeast strain is therefore directly linked to the individual and special flavors created when developing new beer types and styles.

To date, approximately 1,500 yeast species have been reported [6]. The most important yeast species for fermentation technology belong to the *Saccharomyces* genus and are taxonomically grouped in the *Saccharomyces sensu stricto* complex [7,8]. The *Saccharomyces sensu stricto* complex consists of *Saccharomyces cerevisiae*—the yeast used to produce top-fermented beers (often referred to as "ale"), wine, distillers' mash, sake and many other alcoholic beverages, *Saccharomyces bayanus*—used in wine, cider, cidre and apple wine production, and *Saccharomyces pastorianus*—the starter culture for bottom-fermented beer (lager) and apple wine production, as well as additional species (*S. cariocanus*, *S. jurei*, *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, *S. arboricolus* and *S. eubayanus*) which are not used industrially [7,9–13]. LIBKIND et al. published that the bottom-fermenting strains of the species *Saccharomyces pastorianus* used in the lager beer production are genetic hybrids of *Saccharomyces cerevisiae* and the Patagonian wild yeast *Saccharomyces eubayanus* [12]. DUNN and SHERLOCK claimed that there were at least two hybridization events and that all *Saccharomyces pastorianus* lager strains consist of at least two types [14]. The *S. pastorianus* strains they studied were divided into the groups Saaz and Frohberg. Some industrial strains exhibiting strong fermentation performance belong to the Frohberg group. PERES-TRAVEZ et al. investigated that *S. bayanus* strains are also hybrids, whereas the parental species are of an European linage of *S. eubayanus*-like strains and *S. bayanus* var. *uvarum* (*S. uvarum*) [15]. Rapid species identification within the *Saccharomyces sensu stricto* group plays a key role in verifying the purity of a species in a beer starter culture, and for detecting cross contamination. Some special beer styles also use non-*Saccharomyces* yeast species as starter cultures. *Schizosaccharomyces pombe* is found in some traditional African beers, and *Dekkera bruxellensis* in Belgian beers and in German Berliner Weiße. *Saccharomycodes ludwigii* is used to produce low-alcohol and non-alcoholic beer styles, while in the production of top-fermented wheat beer, *Torulaspora delbrueckii* can be used as a supplemental yeast strain to create a distinctive fruity aroma [16–18]. Spontaneous beer fermentation may utilize other non-*Saccharomyces* species such as *Debaryomyces* spp., *Meyerozyma guilliermondii*, *Pichia membranifaciens*, *Candida friedrichii*, *Naumovia castellii*, *Dekkera anomala*, and *Priceomyces* spp. in lambic beer [19], and *Cryptococcus keutzingii*, *Rhodotorula mucilaginosa*, *Candida krusei*, *Pichia fermentans*, and *Pichia opuntiae* in American coolship ale [20].

A brewing yeast strain should be taxonomically classified at the species and strain level using molecular biological methods. It should also be characterized in terms of its propagation and fermentation performance as well as its aroma profile. The Frisinga-TUM 34/70® strain is one of the most abundant lager yeast strains in the brewing industry, and is the reference lager strain when comparing the fermentation performance and final pure beer flavor of other lager strains. The genome of Frisinga-TUM 34/70® was also the first of the bottom-fermenting strains to be sequenced and published [21]. A study conducted by MÜLLER-AUFFERMANN used the characteristics of TUM 34/70 as a reference to develop a method to rapidly compare the performance of lager yeast strains [22]. MEIER-DÖRNBERG further optimized small-scale fermentation vessels used to conduct these trials, as well as the fermentation to make it possible to directly compare the investigated yeast strains [23]. These kinds of trials are very useful for breweries wishing to either replace their yeast strain or to introduce a second one to develop specialty beers with specific properties or to modify or improve existing beer styles.

The market share for such beer specialties is steadily increasing. The craft beer movement and the continuing interest in the variety of flavors mean that regional brewers in particular are benefiting from their willingness to experiment with a variety of different yeast strains. While the volume and market share loss of classical beer types such as pils beer and wheat beer has been declining for several years in Germany, the overall beer market remains practically the same [24]. The greater diversity of yeast that can be applied in brewing, along with an improved understanding of yeasts' evolutionary history and biology, is expected have a significant and direct impact on the brewing industry, with potential for improved brewing efficiency, product diversity and, above all, customer

satisfaction [25]. A lot of specialty beers with distinctive flavors are appearing on the market, especially for beers fermented with top-fermenting *Saccharomyces cerevisiae* strains. These strains produce intense flavors, and they are the focus of many craft- and microbreweries. They include Bavarian wheat beer, ales and Belgian specialty beers. The broad biodiversity and availability of different strains of *Saccharomyces cerevisiae* offer brewers a wealth of possibilities to create beers with unique attributes and flavor profiles. Descriptions of these top-fermenting specialty yeast strains are therefore of considerable importance when selecting suitable strains in the development of special products. The Bavarian wheat beer strain *Saccharomyces cerevisiae* LeoBavaricus-TUM 68® is a perfect example of a successful specialist yeast strain. It is phenolic-off-flavor (POF)-positive. Depending on the production process, Bavarian wheat beers can exhibit very strong fruity, clove like, estery flavors or a more neutral, yeasty, top-fermented character with a decent fruity note, or they can fall somewhere in between the two. The finished beer aroma is determined in part by the strain and how it is handled as well as by the process parameters. SCHNEIDERBANGER recently described the impact of the different wheat beer yeast strains on fermentation performance, and their respective aroma profiles [26]. SCHNEIDERBANGER et al. found that the Bavarian wheat beer strain LunaBavaria-TUM 127® used to ferment the first batch does not ferment maltotriose. This produces a different mouthfeel and aroma to wheat beer strains without this maltotriose gap [27]. Very different sensory impressions can be achieved by using other top-fermenting yeasts, as MEIER-DÖRNBERG recently showed. By investigating five different ale yeast isolates under the same fermentation and substrate conditions, entirely different and often surprising flavors could be identified in the finished beers. One yeast strain created citrus and fruity beer notes, whereas a second produced more floral flavors [23]. Describing both existing and new brewing yeast strains will help us understand their characteristics, and will pave the way for innovative brewers around the world to experiment and create novel products for the beer market. There is a more or less infinite potential for increasing biodiversity among brewing yeast strains.

2. Materials and Methods

The following methods were performed according to [23].

2.1. Yeast Isolates and Strains

A total of ten culture yeast strains were obtained in agar slants from the Yeast Center of the Research Center Weihenstephan for Brewing and Food Quality (BLQ) of the Technical University of Munich (TUM). These ten culture yeast strains included two bottom-fermenting *Saccharomyces pastorianus* and eight top-fermenting *Saccharomyces cerevisiae* brewing yeast strains commonly used to produce beer styles dependent on the industrial applications listed in the following Table 1.

Table 1. Technical University of Munich (TUM) culture yeast strains for industrial brewing.

TUM Yeast Strains			
TUM Yeast Strain	**Yeast Species**	**Industrial Application**	**Origin**
Frisinga-TUM 34/70®	*Saccharomyces pastorianus*	lager beer production	Freising-Weihenstephan, Germany
Securitas-TUM 193®	*Saccharomyces pastorianus*	lager beer production	Freising-Weihenstephan, Germany
LeoBavaricus-TUM 68®	*Saccharomyces cerevisiae*	wheat beer production	Freising-Weihenstephan, Germany
LunaBavara-TUM 127®	*Saccharomyces cerevisiae*	wheat beer production	Freising-Weihenstephan, Germany
Colonia-TUM 177®	*Saccharomyces cerevisiae*	kölsch beer production	Krefeld, Germany
Vetus-TUM 184®	*Saccharomyces cerevisiae*	alt beer production	Dusseldorf, Germany
Mel-TUM 211®	*Saccharomyces cerevisiae*	ale and stout beer production	region unknown, Great Britain
Monacus-TUM 381®	*Saccharomyces cerevisiae*	trappist beer production	region unknown, Germany
Tropicus-TUM 506®	*Saccharomyces cerevisiae*	ale beer production	region unknown, Great Britain
Harmonia-TUM 511®	*Saccharomyces cerevisiae*	ale beer production	region unknown, United States of America

2.2. Genetic Identification and Strain Determination

To confirm the genetic distinctiveness of each obtained TUM brewing yeast strain, a real-time polymerase chain reaction (RT-PCR) and a strain typing method based on a PCR-capillary

electrophoresis of partial intergenic spacer 2 (IGS2) fragment (IGS2-314 PCR-capillary electrophoresis) were used according to HUTZLER [28,29]. The RT-PCR was used in each case to identify the species the strain belonged to, and IGS2-314 PCR-capillary electrophoresis was used to confirm that the investigated yeast cultures of the same species represent different strains.

2.2.1. Real-Time Polymerase Chain Reaction

RT-PCR (Light Cycler® 480 II. Roche Diagnostics Deutschland GmbH, Mannheim, Germany) was used to taxonomically classify the brewing yeast strains in species level. The primer and TaqMan® probe sequences used are listed in Table 2, and the RT-PCR procedure followed that of HUTZLER [28,29]. All RT-PCR systems listed in Table 2 are compatible and were performed using 10 µL 2x Mastermix (Light Cycler® 480 Probe Master, Roche, Germany), 1.4 µL dd (double distilled) H_2O PCR water, 0.8 µL (400 nM) of each primer (Biomers, Ulm, Germany), 0.4 µL (200 nM) probe (Biomers, Ulm, Germany; MGB probe from ThermoFisher scientific, Applied Biosystems®, USA), 0.5 µL IAC135-f (250 nM), 0.5 µL IAC135-r (250 nM), 0.4 µL IAC135-S (HEX) (200 nM), 0.1 µL IAC135 (dilution $1:10^{-13}$), 0.1 µL IAC135 rev (dilution $1:10^{-13}$), and 5 µL template DNA, with a total reaction volume of 20 µL, using the same temperature protocol: 95 °C/10 min; 40 cycles of 95 °C/10 s, 60 °C/55 s; 20 °C. IAC135 was developed by RIEDL at the Research Center Weihenstephan for Brewing and Food Quality of the Technical University Munich and is listed in Table 3. IAC (internal amplification control) is a control to confirm that the PCR reaction itself took place. If IAC is negative, the reaction has to be repeated. The yeast strains *S. cerevisiae* (LeoBavaricus-TUM 68®) and *S. pastorianus* (Frisinga-TUM 34/70®) were used as positive and negative controls respectively, according to the RT-PCR system tested.

Table 2. Qualitative real-time PCR systems for brewing yeast species differentiation [30,31].

Real-Time PCR Systems, Primer and Probe Sequences (5´–3´)	System Name	Reference	*S. cer.*	*S. cer.* var. *dia.*	*S. past.*
Sbp-f CTTGCTATTCCAAACAGTGAGACT Sbp-r1 TTGTTACCTCTGGGCGTCGA Sbp-r2 GTTTGTTACCTCTGGGCTCG Sbp ACTTTTGCAACTTTTTCTTTGGGTTTCGAGCA	Sbp	[32,33]	–	–	+
Sc-f CAAACGGTGAGAGATTTCTGTGC Sc-r GATAAAATTGTTTGTGTTTGTTACCTCTG Scer FAM-ACACTGTGGAATTTTCATATCTTTGCAACTT-BHQ1	Sce	[32,33]	+	+	+
Sc-GRC-f CACATCACTACGAGATGCATATGCA Sc-GRC-r GCCAGTATTTTGAATGTTCTCAGTTG Sc-GRC FAM-TCCAGCCCATAGTCTGAACCACACCTTATCT-BHQ1	Sc-GRC3	[30]	+	+	+
TF-f TTCGTTGTAACAGCTGCTGATGT TF-r ACCAGGAGTAGCATCAACTTTAATACC TF-MGB FAM-ATGATTTTGCTATCCCAAGTT-BHQ1 (MGB probe)	TF-COXII	[30]	+	+	–
BF300E CTCCTTGGCTTGTCGAA BF300M GGTTGTTGCTGAAGTTGAGA BF300 FAM-TGCTCCACATTTGATCAGCGCCA-BHQ1	BF-300	[32]	–	–	+
BF-LRE-f ACTCGACATTCAACTACAAGAGTAAAATTT BF-LRE-r TCTCCGGCATATCCTTCATCA BF-LRE FAM-ATCTCTACCGTTTTCGGTCACCGGC-BHQ1	BF-LRE1	[30]	–	–	+
Sd-f TTCCAACTGCACTAGTTCCTAGAGG Sd-r GAGCTGAATGGAGTTGAAGATGG Sdia FAM-CCTCCTCTAGCAACATCACTTCCTCCG-BHQ1	Sdia	[32]	–	+	–

Positive (+), negative (–).

Table 3. Primer, probe and target DNA sequences of the internal amplification control system (IAC135) used for real-time PCR systems.

Real-Time PCR Internal Amplification Control (IAC135)		
System Name	**Primer**	**Primer Sequence (5′–3′)**
IAC135	IAC135-f	TGGATAGATTCGATGACCCTAGAAC
	IAC135-r	TGAGTCCATTTTCGCAGATAACTT
	Probe	**Probe Sequence (5′–3′)**
	IAC135-S	HEX-TGGGAGGATGCATTAGGAGCATTGTAAGAGAG-BHQ1
	Target DNA	**DNA Sequence (5′–3′)**
	IAC135	TGCTAGAGAATGGATAGATTCGATGACCCTAGAACTAGTGGGAGGATGCATT AGGAGCATTGTAAGAGAGTCGGAAGTTATCTGCGAAAATGGACTCATTCGA GTGGCCTATTGACGGTCGCCCAAGGTGTCGCA
	IAC135-rev	TGCGACACCTTGGGCGACCGTCAATAGGCCACTCGAATGAGTCCATTTTCGC AGATAACTTCCGACTCTCTTACAATGCTCCTAATGCATCCTCCCACTAGTTCTA GGGTCATCGAATCTATCCATTCTCTAGCA

2.2.2. DNA Fingerprinting (PCR-Capillary Electrophoresis of the IGS2-314 Fragment)

In order to determine that the TUM brewing yeasts represented different strains, genetic fingerprints were generated using the IGS2-314 method [29]. The IGS2 is a spacer region within the ribosomal cluster. To amplify a partial sequence of the intergenic spacer 2 (IGS2-314) the specific primers IGS2-314f (5′-CGGGTAACCCAGTTCCTCACT-3′) and IGS2-314r (5′-GTAGCATATATTTCTTGTGTGAGAAAGGT-3′) (Biomers GmbH, Ulm, Germany) [34] were used at a concentration of 600 nM as described by HUTZLER [28]. PCR was performed with 22.5 µL RedTaq Mastermix (2x) (Genaxxon, Ulm, Germany) and 2.5 µL template DNA, with a total reaction volume of 25 µL. The Mastermix contained 12.5 µL buffer solution (RedTaq Mastermix), 7.0 µL DNA-free PCR water and 1.5 µL of each primer (Biomers, Munich, Germany). Cycling parameters were: A pre-denaturing step at 95 °C for 300 s, then 35 cycles for denaturing at 95 °C for 30 s, for annealing and elongation at 54 °C for 30 s and 72 °C for 40 s and for final elongation at 72 °C for 300 s. PCR was performed using a SensoQuest LabCycler48s (SensoQuest GmbH, Gottingen, Germany). Amplified fragments were analyzed using a capillary electrophoresis system (Agilent DNA 1000 kit) following the manufacturer's recommendations (lab on a chip, Bioanalyzer Agilent 2100, Agilent Technologies, Santa Clara, CA, USA).

2.3. Phenolic Off-Flavor Test

TUM yeast culture strains were taken from wort agar slopes and spread on a yeasts and mold agar plate (YM-agar) containing one of the following precursors: ferulic acid, cinnamic acid and coumaric acid. After three days of incubation at 24 °C, the three single agar plates per yeast isolate were evaluated by sniffing to detect any of the following aromas: ferulic acid becomes 4-vinylguajacol (4-VG, clove-like), cinnamic acid becomes 4-vinylstyrene (4-VS, styrofoam-like) and coumaric acid becomes 4-vinylphenol (4-VP, medicinal-like).

For the YM-agar plates a YM-media was prepared by adding distilled water to 3.0 g malt extract, 3.0 g yeast extract, 5.0 g peptone, 11.0 g glucose monohydrate, and 20.0 g agar to 1000 mL, and autoclaved. After autoclaving, an aliquot of the following stock solutions was added to the YM-media at 45–50 °C under sterile conditions. For the stock solution of coumaric acid, 100 mg of the instant were dissolved in 10 mL of 96% (*v/v*) ethanol. The stock solution of ferulic and cinnamic acid was made by dissolving 1 g in 20 mL of 96% (*v/v*) ethanol. 10 mL coumaric acid, 2 mL ferulic acid or 2 mL cinnamic acid stock solution was added for 1000 mL YM-media.

2.4. Brewing Trials

2.4.1. Wort

The wort characteristics used for propagation and the brewing trials are shown in Table 4. The wort was based on hopped barley malt concentrate (N53940; Döhler GmbH, Darmstadt, Germany). To achieve an original gravity of 12.4 °P, wort concentrate was diluted with distilled water and boiled for 5 min to guarantee sterile conditions. The same wort batch preparation was used for the propagation and brewing trials to ensure constant wort composition. Free alpha-amino nitrogen was quantified using the MEBAK II. 2.8.4.1 method. Sugar composition was determined using the HPLC MEBAK II. 3.2.2.1.2 method.

Table 4. Starting wort composition used for propagation and brewing trials (12.4 °P wort).

Wort composition	
Parameter	**Amount**
Original gravity (°P)	12.40
pH	5.19
Spec. weight SL 20/20 °C	1.05
Zinc (mg/L)	0.15
Free amino nitrogen (FAN) (mg/100 mL)	25.00
Total amino acid content (AS) (mg/100 mL)	203.22
Total sugar (g/L)	83.78
EBC-Bittering units	20.20
Glucose (g/L)	11.46
Fructose (g/L)	2.57
Saccharose (g/L)	1.12
Maltose (g/L)	53.65
Maltotriose (g/L)	14.98

2.4.2. Propagation

In order to propagate yeasts, yeast strains were inoculated from agar slants (yeast pure culture) into 60 mL of sterile wort medium in an 100 mL Erlenmeyer flask and incubated for 72 h at ambient temperature (20 °C) and pressure, then agitated at 80 rpm using a WiseShake 207 orbital shaker (Witeg Labortechnik GmbH, Wertheim, Germany). After incubation, yeasts were transferred to 4 kg of sterile wort medium and further propagated at the same conditions for an additional 72 hours. After allowing six hours for sedimentation, the supernatant was decanted and 2 kg of sterile wort medium at pitching temperature (20 °C) was added to the yeast sediment in each container. The yeast concentration was determined in terms of cells/g using a Thoma cell counting chamber with a chamber depth of 0.1 mm and an area of 0.00025 m^3 per square (Brand GmbH&Co.KG, Wertheim, Germany).

2.4.3. Fermentation

Laboratory-scale brewing trials were performed using stainless steel vessels with dimensions of 10 cm diameter x 33 cm height (2.5 liters) with 20% headspace and clamped down lids, according to MEIER-DÖRNBERG [23]. The vessels were placed in a tempered cooling chamber (2023 Minicoldlab, LKB-Produkter AB, Bromma, Sweden) to guarantee a constant fermentation temperature. To imitate industrial brewery conditions during fermentation, a head pressure of 0.5 bar was applied to simulate a liquid height of 10 m (median hydrostatic pressure). Brewing trials were evaluated by pitching 8.5 L wort per yeast strain. Each batch was then divided into four fermentation vessels. By having four vessels, samples could be taken daily from one of the four vessels to estimate the specific gravity, cells in suspension and pH, while the other three vessels remained undisturbed. Yeast strains were added at an inoculation rate of 15 million cells/g of homogeneous mixed wort medium for the top-fermenting *Saccharomyces cerevisiae* yeast strains (LeoBavaricus-TUM 68®, LunaBavaria-TUM

127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506®, and Harmonia-TUM 511®) and an inoculation rate of 30 million cells/g of homogeneous mixed wort medium for bottom-fermenting *Saccharomyces pastorianus* yeast strains (Frisinga-TUM 34/70® and Securitas-TUM 193®). The wort was not oxygenated. Primary fermentation was maintained at 20 °C for the top-fermenting and 15 °C for the bottom fermenting TUM yeast strains. Fermentation was considered complete once the specific gravity remained constant for two consecutive days. An additional five days for maturation was given following primary fermentation at the same fermentation temperature, and seven days for lagering at 0 °C. The beers were then removed from the fermentation vessels, homogenized, and collected in sterile bottles. The specific gravity and pH of the samples were determined from the filtered fermentation samples using a DMA 35N (Anton-Paar GmbH, Graz, Austria) for specific gravity and a pH3210 (WZW, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) for pH measurement. The samples were filtered using Whatman® folded filter paper with a diameter of 320 mm (GE Healthcare Europe GmbH, Freiburg, Germany).

2.5. Analytical Methods

After lagering, the finished beers were analyzed for physical and chemical attributes, which included the following parameters: ethanol, pH, specific gravity, degree of attenuation, free amino nitrogen, amino acid composition, sugar composition, total SO_2, free and total dimethylsulfide, free vicinal diketones and the concentration of fermentation by-products. Ethanol, pH, specific gravity, and degree of attenuation were measured using an Anton Paar DMA 5000 Density Meter with Alcolyzer Plus measuring module, pH measuring module, and Xsample 122 sample changer (Anton-Paar GmbH, Ostfildern, Germany). Free amino nitrogen and amino acid composition were quantified using the HPLC MEBAK II (2.8.4.1) method. Residual sugar composition was determined using the HPLC MEBAK II (3.2.2.1.2) method. Total SO_2, free and total dimethylsulfide, and free vicinal diketones were quantified using a Clarus 500 gas chromatograph (Perkin-Elmer, Waltham, MA, USA) with a headspace unit and Elite-5 60 m × 0.25 mm, 0.5 µm column using a 2.3-hexandione internal standard. The final concentrations of fermentation by-products (e.g., acetaldehyde, ethyl acetate, n-propanol, i-butanol, isoamyl acetate, amyl alcohols, 4-vinylguajacol, diacetyl, 2.3-pentandione) were measured according to MEBAK II (3.2.21) methods using a gas chromatograph with a headspace unit, and an INNOWAX cross-linked polyethylene-glycol 60 m × 0.32 mm, 0.5 µm column (Perkin-Elmer, Waltham, MA, USA).

2.5.1. Determining the Cell Count (Cells in Suspension and Total Cell Count)

Cell counts for pitched yeast, cells in suspension until lagering, and total cell count after lagering were determined using a Thoma cell counting chamber with a chamber depth of 0.1 mm and an area of 0.00025 m^3 per square (Brand GmbH&Co.KG, Wertheim, Germany). Cells in suspension were analyzed every 24 h up to the start of lagering. To ensure cell count accuracy during fermentation and maturation, 20 mL of green beer was removed from the middle of the fermentation vessel by using a 10 mL volumetric pipette mounted on a stand. Prior to sampling, the head pressure in the vessel was released very slowly so that the cells in suspension were not affected by a pressure surge. The total cell count was determined after the lagering phase. Beers were removed from the fermentation vessels, and the decanted yeast masses were collected by suspending the yeast cells in a total of 50 g distilled water. The yeast cells were washed by centrifugation twice with 50 g distilled H_2O (5 min at 3000 rpm) and resuspended with distilled water up to a total of 100 g. Afterwards, distilled water was added to 1 g of the homogenous yeast suspension to make up to 100 mL. Total cell counts were determined in cells/g using the Thoma cell counting chamber.

2.6. Sensory Evaluation

Three single sensory tests were conducted which included: expected beer type test, DLG-scheme for beer (Deutsche Landwirtschafts-Gesellschaft) and a descriptive sensory evaluation. All beer samples were tasted and evaluated by a sensory panel of seven DLG-certified tasters with long-standing experience in the sensory analysis of beer at the Weihenstephan Research Center for Brewing and Food Quality. Accredited sensory evaluations were performed according to DIN EN 17025. Sensory evaluations were performed in individual walled tasting stations under controlled environmental conditions. Samples were provided in triplicate sets for all beers in dark glasses, each with a three digit code. All samples were served at 12 °C to guarantee optimal conditions to investigate the predominant flavor diversity. At first the panelists associated the beer samples with their expected beer type (e.g., ale, wheat-, Kölsch-, Alt-, stout, Berliner Weisse, porter-, lager-; Bock-; Märzen-, Rauch-, Schwarz-, Dunkles-, malt beer) followed by an examination of the beer samples according to the DLG-scheme. Secondly, a descriptive sensory evaluation was conducted during which trained panelists described specific flavors. Seven main categories were described (e.g., sweet, tropical fruity, fruity (other fruits), citrus, spicy, floral and other flavors). Every category was evaluated from 0, meaning not noticeable, to 5, extremely noticeable.

3. Results

3.1. Genetic Analysis

3.1.1. Real-Time PCR Assays and IGS2-314 PCR-Capillary Electrophoresis

RT-PCR results confirmed the species identity of the investigated TUM yeast strains of the TUM Yeast Center. In addition, the results of the DNA fingerprinting showed unique banding patterns, also confirming that each yeast represents a genetically different strain (Figure 1). Table 5 shows that the tested RT-PCR systems and the obtained results for all strains. All yeast strains were positive for the Sc-GRC3 and Sce loci. The RT-PCR systems Sc-GRC3 and Sce have positive signals when *S. cerevisiae* DNA is measured or the DNA of hybrid strains that contain these DNA loci. The top-fermenting yeast strains LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511® were also positive for the TF-COXII locus, suggesting that they belong to the *S. cerevisiae* strains. The bottom-fermenting yeast strains Frisinga-TUM 34/70® and Securitas-TUM 193® were also positive for loci that correlate with the PCR systems Sbp, BF-LRE1 and BF-300, which detect *S. bayanus*/*S. pastorianus* strains. In addition, all investigated TUM yeast strains were negative for the RT-PCR system Sdia which detects *S. cerevisiae* var. *diastaticus* strains.

Table 5. Qualitative results of the real-time PCR systems used for the investigated yeast strains and the reference strains to differentiate *Saccharomyces sensu stricto* species.

		RT-PCR-System						
Species Confirmation	**TUM Yeast Strain**	**Sc-GRC3**	**Sce**	**TF-COXII**	**Sbp**	**BF-LRE1**	**BF-300**	**Sdia**
S. pastorianus (*S. cerevisiae* hybrid strain)	Frisinga-TUM 34/70®	+	+	−	+	+	+	−
	Securitas-TUM 193®	+	+	−	+	+	+	−
S. cerevisiae	LeoBavaricus-TUM 68®	+	+	+	−	−	−	−
	LunaBavaricus-TUM 127®	+	+	+	−	−	−	−
	Colonia-TUM 177®	+	+	+	−	−	−	−
	Vetus-TUM 184®	+	+	+	−	−	−	−
	Mel-TUM 211®	+	+	+	−	−	−	−
	Monacus-TUM 381®	+	+	+	−	−	−	−
	Tropicus-TUM 506®	+	+	+	−	−	−	−
	Harmonia-TUM 511®	+	+	+	−	−	−	−

Positive (+), negative (−).

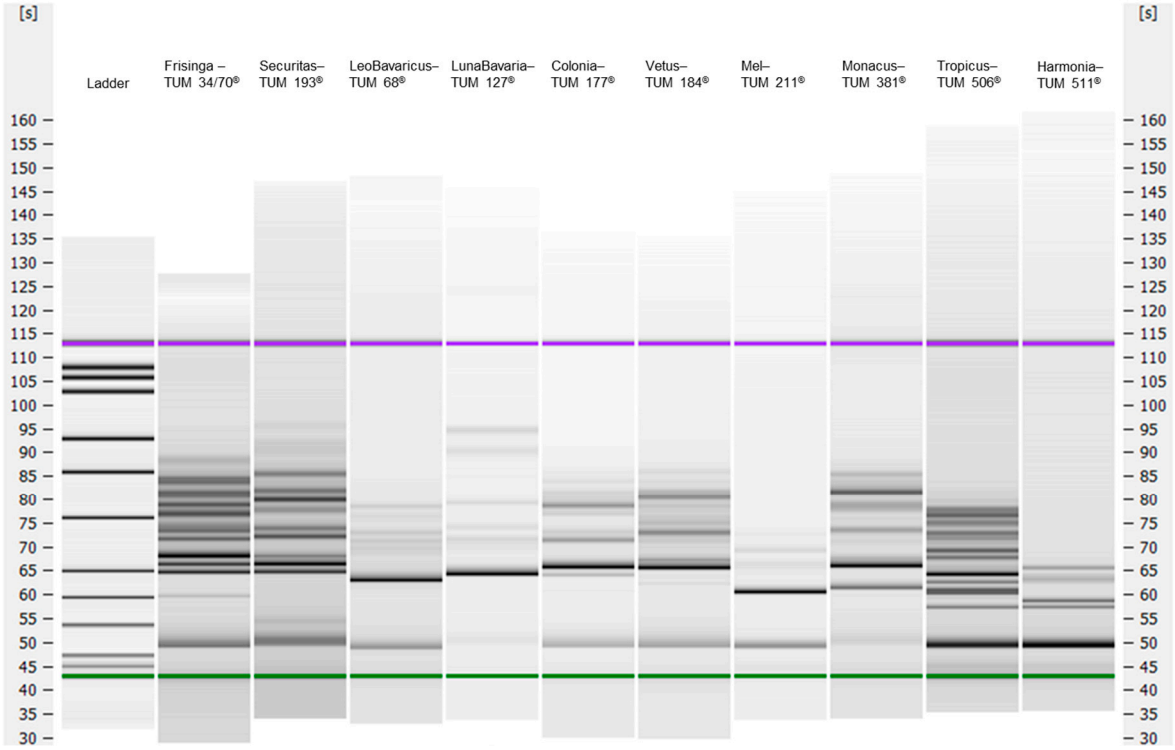


Figure 1. Capillary electrophoresis IGS2-314 ribosomal DNA (rDNA) patterns for the investigated yeast strains Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®; lower and upper internal marker are shown in green and purple.

3.2. Brewing Trials

3.2.1. Sugar Utilization

As Table 6 shows, not all of the strains were able to metabolize all major wort sugars (e.g., glucose, fructose, sucrose, maltose, maltotriose). Maltotriose could not be utilized by all strains. The top-fermenting yeast strain LunaBavaria-TUM 127® could not ferment maltotriose (1.05%), while the culture yeast strains Mel-TUM 211®, Tropicus-TUM 506® and Vetus-TUM 184® only fermented a small amount of maltotriose (26.66%, 59.28% and 60.92%). Variations in the maltotriose utilization for all other strains were above 83%. The results suggested that the utilization degree as well as the ability to utilize maltotriose is strain dependent.

Table 6. Mean percentage of total wort sugar utilization in beer, measured in triplicate after lagering; confidence level 95%.

TUM Yeast Strain	Glucose	Fructose	Sucrose	Maltose	Maltotriose
Frisinga-TUM 34/70®	98.61 ± 0.00	96.15 ± 0.00	100.00 ± 0.00	99.02 ± 0.12	94.06 ± 0.91
Securitas-TUM 193®	98.70 ± 0.14	96.15 ± 0.00	100.00 ± 0.00	96.89 ± 0.12	86.60 ± 0.52
LeoBavaricus-TUM 68®	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.92 ± 0.02	99.65 ± 0.28
LunaBavaria-TUM 127®	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.07 ± 0.10	01.05 ± 2.89
Colonia-TUM 177®	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.85 ± 0.02	94.80 ± 0.78
Vetus-TUM 184®	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	98.00 ± 0.53	60.92 ± 5.87
Mel-TUM 211®	98.55 ± 0.47	98.57 ± 0.21	98.51 ± 0.48	87.23 ± 0.82	26.66 ± 0.26
Monacus-TUM 381®	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.65 ± 0.14	97.75 ± 0.09
Tropicus-TUM 506®	98.23 ± 0.12	98.05 ± 0.36	99.11 ± 0.00	98.48 ± 0.93	59.28 ± 0.81
Harmonia-TUM 511®	99.42 ± 0.12	98.05 ± 0.00	95.54 ± 0.00	99.28 ± 0.09	83.91 ± 0.71

3.2.2. Amino Acid Utilization

The mean amino acid uptake in the finished beers after lagering using the investigated *S. pastorianus* and *S. cerevisiae* yeast strains is shown in Supplementary Material Tables S1 and S2. The commonly accepted amino acid uptake classification is indicated with shading according to JONES and PIERCE [22,23,35,36].

As shown in Tables S1 and S2, the total amino acid utilization followed no defined process and was different for each of the investigated TUM yeast strains. As MEIER-DÖRNBERG previously described using five tested *S. cerevisiae* ale yeast strains, the exact course of absorption and the sequence varies, even if specific amino acids were preferred by the yeast [23]. However, in contrast to the tested top-fermenting *S. cerevisiae* ale yeast strains, the bottom-fermenting *S. pastorianus* strains metabolized the single amino acids in a similar order. This might depend on the same industrial application (lager and export beer production) for which both strains were commonly used. Fermentation conditions are similar between lager and export beer production, and the Frisinga-TUM 34/70® and Securitas-TUM 193® strains may adapt to the same circumstances and react with similar cell metabolisms, even if they are genetically different and show unique sensory profiles. Figures 2 and 3 show the free amino nitrogen (FAN) and the total amino acid (AS) utilization of each yeast strain in comparison with the corresponding residual contents. The utilization rate of FAN and AS was correlated for the same yeast strain, but different across strains.

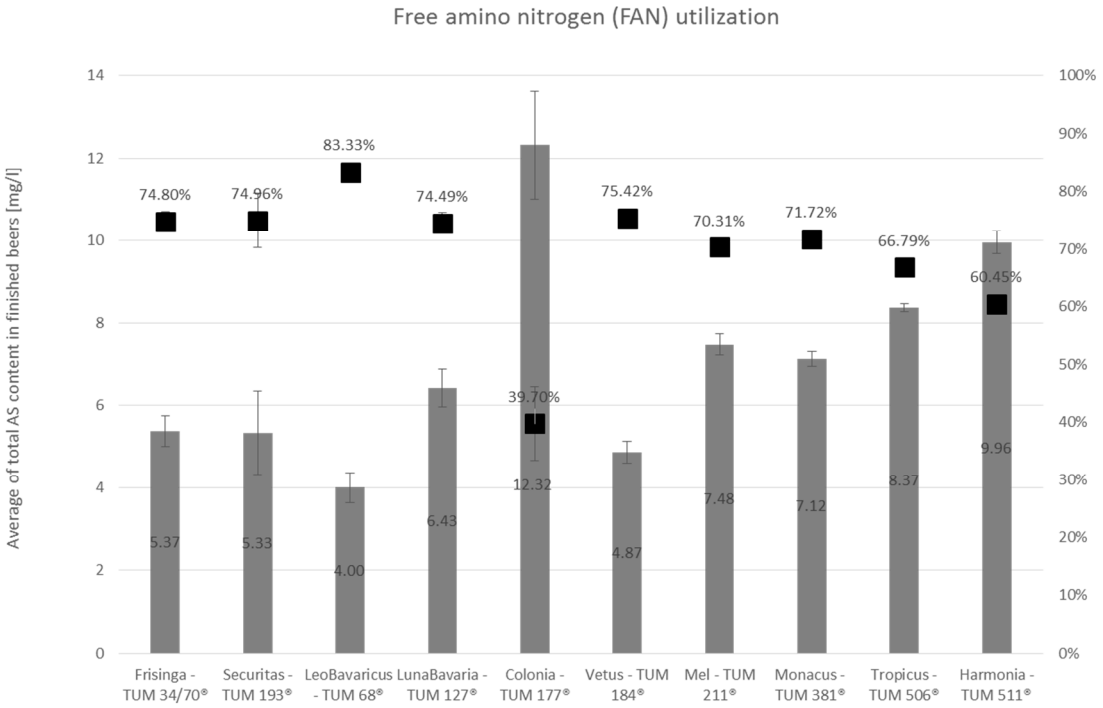


Figure 2. Average of metabolized and free amino nitrogen (FAN) content in finished beers produced with the yeast strains Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®; confidence level 95%.

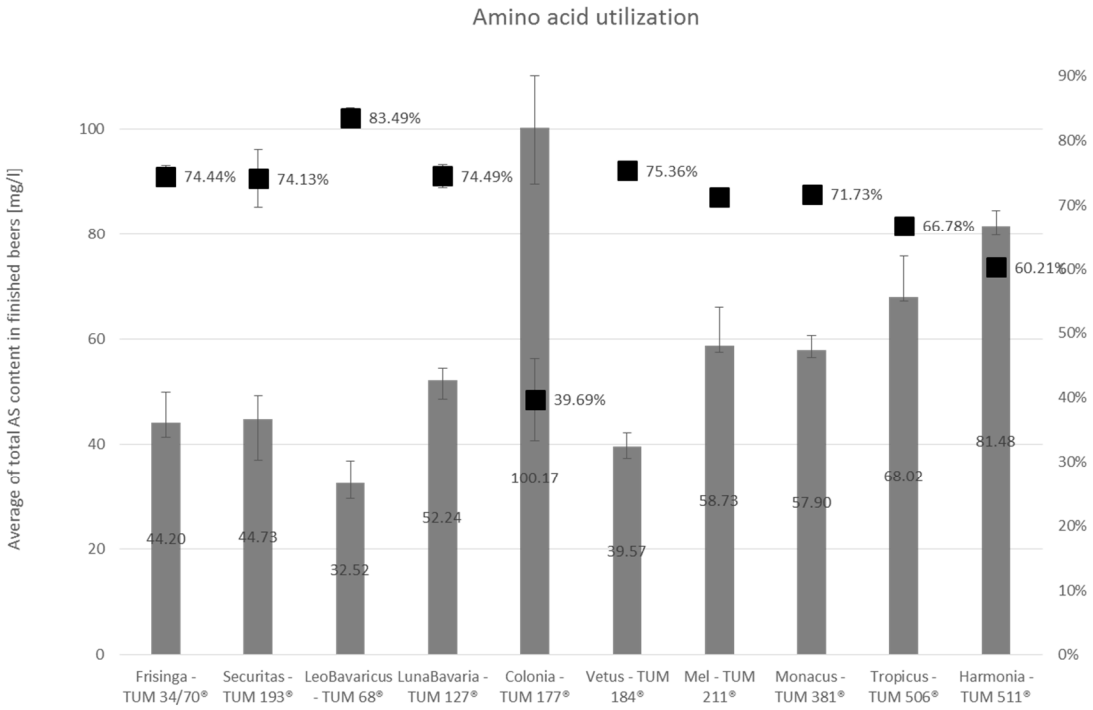
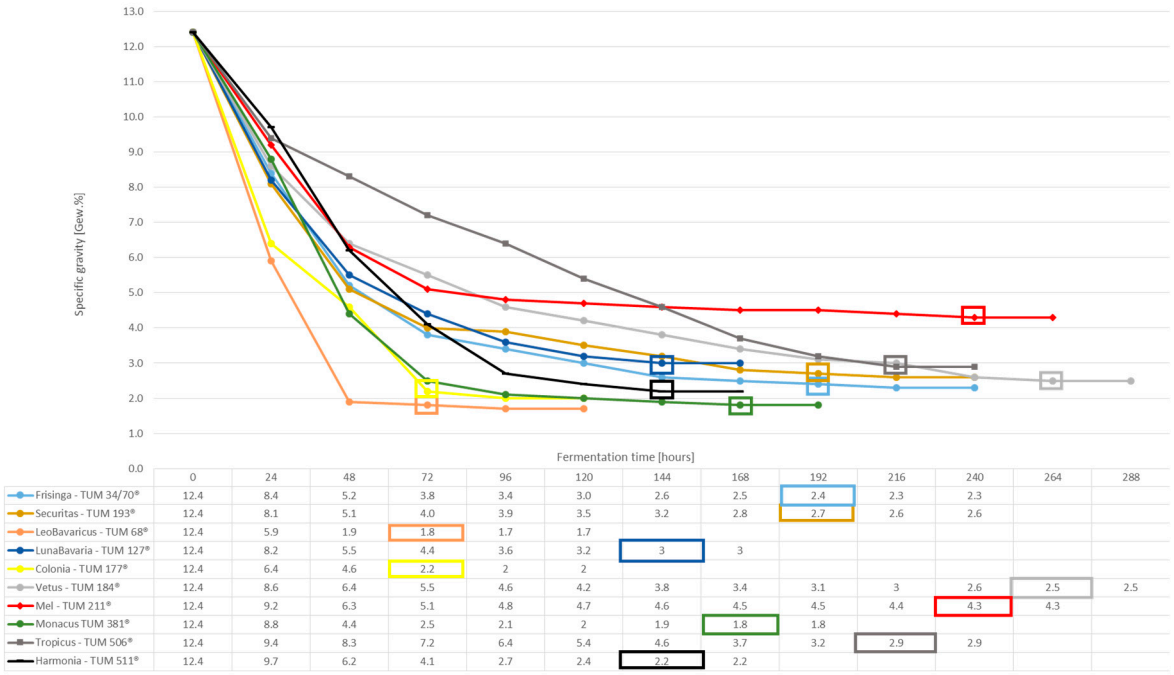


Figure 3. Average of metabolized and total amino acid (AS) content in finished beers produced with yeast strains Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®; confidence level 95%.

3.2.3. Fermentation Kinetics

Figure 4 shows the drop in specific gravity during fermentation by the investigated yeast strains. As shown in Figure 4, LeoBavaricus-TUM 68® has the quickest drop in specific gravity, followed by Colonia-TUM 177®. Both strains reached their final gravity after 96 hours of fermentation. Monacus-TUM 381® needed 72 hours more to reach the final gravity of 1.8 °P after 168 hours. Mel-TUM 211® fermented the wort slower than the other strains but did so continuously until it reached the lowest apparent attenuation of all investigated yeast strains at 66.13% after 216 hours of fermentation.

Table 7 shows the apparent attenuation compared with the fermentation time required by the isolated strains. The different fermentation rates and degrees of apparent attenuation are due to their ability to ferment maltose and maltotriose (see Table 6). LeoBavaricus-TUM 68® and Monacus-TUM 381® reached the attenuation limit of the wort used at an apparent attenuation of 86.17%. The attenuation limit was previously tested according to MEBAK Bd. II and was achieved by using the top-fermenting *Saccharomyces cerevisiae* brewing yeast strain LeoBavaricus-TUM 68® and the same wort used in this trial. The low apparent attenuation of 76.2% by LunaBavaria-TUM 127® was due to the unique strain property of not fermenting one of the major wort sugars, namely maltotriose. The low drop in specific gravity over 144 h was not due to the maltotriose gap in the strain specific sugar metabolism, because maltotriose was taken up by the yeast cell as the last wort sugar, and was therefore not necessary for sufficient cell growth.



	0	24	48	72	96	120	144	168	192	216	240	264	288
Frisinga - TUM 34/70®	12.4	8.4	5.2	3.8	3.4	3.0	2.6	2.5	2.4	2.3	2.3		
Securitas - TUM 193®	12.4	8.1	5.1	4.0	3.9	3.5	3.2	2.8	2.7	2.6	2.6		
LeoBavaricus - TUM 68®	12.4	5.9	1.9	1.8	1.7	1.7							
LunaBavaria - TUM 127®	12.4	8.2	5.5	4.4	3.6	3.2	3	3					
Colonia - TUM 177®	12.4	6.4	4.6	2.2	2	2							
Vetus - TUM 184®	12.4	8.6	6.4	5.5	4.6	4.2	3.8	3.4	3.1	3	2.6	2.5	2.5
Mel - TUM 211®	12.4	9.2	6.3	5.1	4.8	4.7	4.6	4.5	4.5	4.4	4.3	4.3	
Monacus TUM 381®	12.4	8.8	4.4	2.5	2.1	2	1.9	1.8	1.8				
Tropicus - TUM 506®	12.4	9.4	8.3	7.2	6.4	5.4	4.6	3.7	3.2	2.9	2.9		
Harmonia - TUM 511®	12.4	9.7	6.2	4.1	2.7	2.4	2.2	2.2					

Figure 4. Drop in specific gravity measured in a single reference vessel compared with the average in final gravity (marked with box) measured in triplicate in final beers for the tested yeast strains Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®; confidence level 95%.

Table 7. Apparent attenuation (AA%) of the final beer compared with specific time for primary fermentation for the investigated yeast strains Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®; confidence level 95%.

Apparent attenuation (AA %) of the final beer		
TUM Yeast Strain	**AA (%)**	**Fermentation time (hours)**
Frisinga-TUM 34/70®	81.63 ± 0.51	216
Securitas-TUM 193®	79.30 ± 0.51	216
LeoBavaricus-TUM 68®	86.17 ± 0.05	96
LunaBavaria-TUM 127®	76.20 ± 1.76	144
Colonia-TUM 177®	84.93 ± 0.37	96
Vetus-TUM 184®	80.97 ± 3.02	264
Mel-TUM 211®	66.13 ± 0.51	240
Monacus-TUM 381®	86.17 ± 0.11	168
Tropicus-TUM 506®	77.37 ± 1.34	216
Harmonia-TUM 511®	82.70 ± 0.42	144

3.2.4. Flocculation (Cell Count)

The flocculation behavior of a yeast strain is an important selection criterion to ensure reliable product quality in industrial brewing processes. Besides the genetic background of the yeast strain (e.g., variation in FLO genes), the flocculation behavior is affected by the physiological environment (e.g., the pH and availability of metal ions and nutrients of the wort), by the physical environment (e.g., soluble oxygen, hydrodynamic conditions and low agitation), and the fermentation temperature, as well as a sufficient concentration of yeast cells in suspension [37–39]. In this research, all environmental and fermentation conditions were kept constant, in order to investigate and classify the investigated

strains according to their specific flocculation behavior in flocculent and non-flocculent ("powdery") yeast strains (see Table 8). According to ANNEMÜLLER, a flocculent yeast strain accumulates to flocs and settles at the bottom of the fermentation vessel when the nutrients present in brewers wort are largely consumed [40].

As Figure 5 shows, in contrast to the eight top-fermenting TUM yeast strains, the bottom-fermenting yeast strains Frisinga-TUM 34/70® and Securitas-TUM 193® flocculated very rapidly in the first three days of the main fermentation and reached a concentration of cells in suspension below the pitching rate. By reaching their apparent attenuation both strains shows cell concentrations below two million yeast cells/mL (e.g., 1.8 and 1.6 million cells/mL), and could therefore be classified according to their flocculation behavior as flocculent yeast strains (see Table 8). With the exception of the LunaBavaria-TUM 127® and Vetus-TUM 184® yeast strains, the top-fermenting TUM yeast strains LeoBavaricus-TUM 68®, Colonia-TUM 177®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511® were largely dispersed and remained in a suspension that is close to the pitching concentration, even once they have reached their apparent attenuation. According to BÜHLINGEN et al., the strains exhibited a non-flocculent ("powdery") behavior [41]. In contrast, LunaBavaria-TUM 127® and Vetus-TUM 184® showed similar flocculation behavior to the bottom-fermenting yeast strains and flocculated below four million cells/mL (e.g., 3.5 and 0.5 million cells/mL) by reaching their apparent attenuations. LeoBavaricus-TUM 68® also showed noticeable flocculation behavior, but also had the greatest increase in the number of cells, so that, similarly to Colonia-TUM 177®, the concentration of cells in suspension at the time of apparent attenuation was still above the pitching concentration. The flocculation behavior of both strains was therefore classified as powdery, but showed rapid flocculation, with a final concentration of cells in suspension below one million cells/mL at the end of maturation. With the exception of Mel-TUM 211® and Tropicus-TUM 506®, the "powdery" yeast strains fermented beer faster than the "flocculent" yeast strains, but both resulted in similar final attenuations.

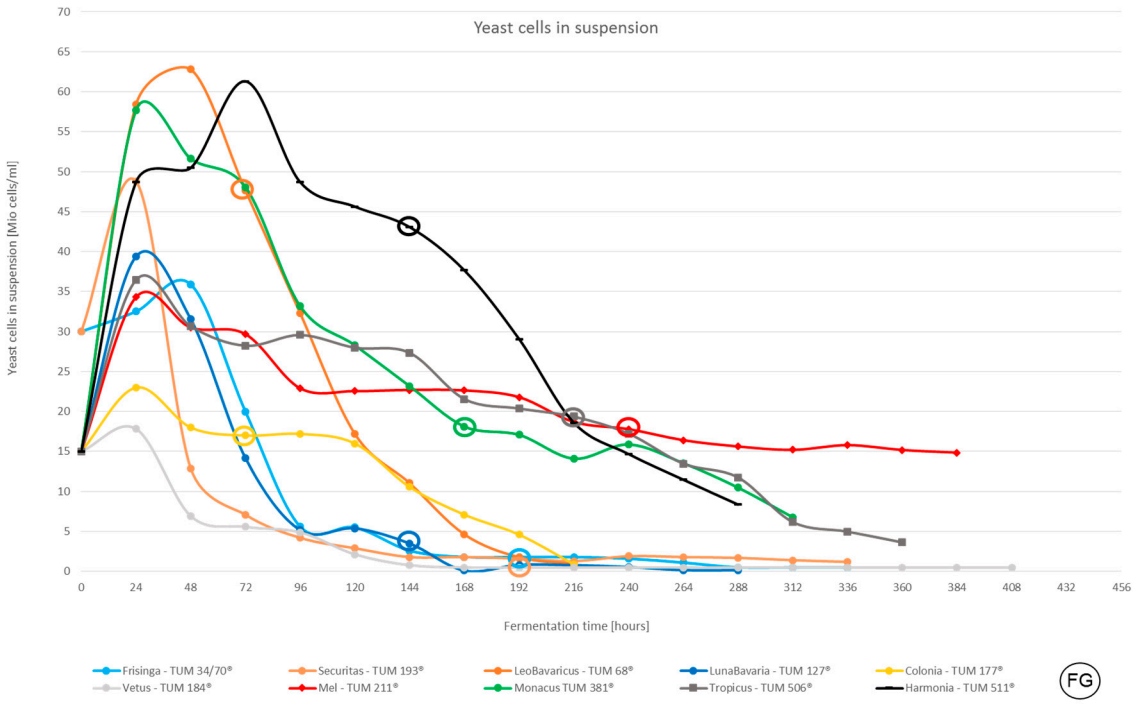


Figure 5. Yeast cells in suspension during the main fermentation and maturing phase. The circle marks the specific final gravity (FG) of the investigated yeast strains Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®.

Table 8. Difference in maximum yeast cell concentration during primary fermentation and yeast cell concentration by reaching the specific final gravity (FG) and the flocculation behavior of Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®.

Yeast Cell Sedimentation at the end of Primary Fermentation				
TUM Yeast Strain	**Max. Cell Conc.**	**Cell Conc. FG**	**Difference**	**Flocculation Behavior**
Frisinga-TUM 34/70®	35.90	1.80	−34.10	flocculent
Securitas-TUM 193®	48.80	1.30	−47.50	flocculent
LeoBavaricus-TUM 68®	62.81	32.29	−30.52	powdery
LunaBavara-TUM 127®	39.38	7.50	−31.88	flocculent
Colonia-TUM 177®	23.00	17.20	−5.8	powdery
Vetus-TUM 184®	17.83	0.50	−17.33	flocculent
Mel-TUM 211®	34.30	17.80	−16.50	powdery
Monacus-TUM 381®	57.65	18.12	−39.53	powdery
Tropicus-TUM 506®	36.45	19.36	−17.09	powdery
Harmonia-TUM 511®	61.30	43.07	−18.23	powdery

3.2.5. Change in pH Value

Table 9 shows the drop in pH during the first 96 hours of primary fermentation, the pH value after the maturation phase, and the average pH value of the final beer. As shown in Table 9, the drop in pH and the time taken to reach the minimum pH value for primary fermentation is different for all the investigated yeast strains.

Securitas-TUM 193®, Monacus-TUM 381® and Mel-TUM 211® reached their minimum and final beer pH value after 48 hours. Frisinga-TUM 34/70® showed a similar change in pH value to the ale yeast strains Mel-TUM 211®, Tropicus-TUM 506® and Harmonia-TUM 511®, which was already shown by MEIER-DÖRNBERG [23]. These four strains reached their minimum pH value for primary fermentation after 48 hours, and recorded a pH value increase of 0.1 after the maturation and lagering phase. LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177® and Vetus-TUM 184® needed 96 hours to reach their minimum pH value for primary fermentation. Colonia-TUM 177® recorded a pH value increase of 0.1 as measured in triplicate in the final beer, while an increase of 0.2 could be registered in the produced beers with LeoBavaricus-TUM 68® and LunaBavaria-TUM 127®. According to ANNEMÜLLER and MANGER, an increase in the pH value of more than 0.1 could indicate cell autolysis, and might be due to the excretion of yeast metabolites and the uptake and metabolization of pyruvate [42]. Frisinga-TUM 34/70®, LeoBavaricus-TUM 68®, and Harmonia-TUM 511® exhibited the strongest capacity for acidification (ΔpH 0.8) compared with the other yeast strains. Mel-TUM 211® and Tropicus-TUM 506® exhibited the weakest capacity for acidification (ΔpH 0.5), which might be due to cell autolysis caused by low fermentation performance (see Section 3.2.3).

Table 9. Change in pH value during primary fermentation, after the maturation and lagering phase, rounded to two decimal figures, for Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®; confidence level 95%.

pH Value Decrease during Primary Fermentation									
TUM Yeast Strain	0 h	24 h	48 h	72 h	96 h	After primary fermentation	After maturation	Final beer (after lagering)	ΔpH
Frisinga-TUM 34/70®	5.2	4.6	4.5	4.5	4.5	4.5	4.5	4.4 ± 0.04	0.8
Securitas-TUM 193®	5.2	4.6	4.5	4.5	4.5	4.5	4.5	4.5 ± 0.01	0.7
LeoBavaricus-TUM 68®	5.2	4.8	4.3	4.3	4.2	4.2	4.2	4.4 ± 0.01	0.8
LunaBavaria-TUM 127®	5.2	4.6	4.5	4.5	4.4	4.4	4.4	4.6 ± 0.01	0.6
Colonia-TUM 177®	5.2	5	4.8	4.8	4.7	4.7	4.7	4.6 ± 0.02	0.6
Vetus-TUM 184®	5.2	4.7	4.6	4.6	4.5	4.5	4.6	4.5 ± 0.04	0.7
Mel-TUM 211®	5.2	4.5	4.4	4.4	4.4	4.4	4.5	4.7 ± 0.01	0.5
Monacus-TUM 381®	5.2	4.6	4.5	4.5	4.5	4.5	4.5	4.5 ± 0.01	0.7
Tropicus-TUM 506®	5.2	4.6	4.5	4.5	4.5	4.5	4.6	4.7 ± 0.01	0.5
Harmonia-TUM 511®	5.2	4.6	4.4	4.4	4.4	4.4	4.4	4.4 ± 0.01	0.8

3.3. Flavor Characterization

3.3.1. Phenolic Off-Flavor

Table 10 shows the results of the POF-tests evaluated by sniffing. As shown in Table 10, not all of the investigated yeast strains were capable of building phenolic flavors. The panelists could only detect aroma active components formed by the top-fermenting yeast strains LeoBavaricus-TUM 68® and LunaBavaria-TUM 127®, commonly used for wheat beer production, and Harmonia-TUM 511® (ale beer production) and for Monacus-TUM 381® (trappist beer production). For these yeasts, all three corresponding POF-flavors were detected by sniffing.

Both bottom-fermenting yeast strains Frisinga-TUM 3470® and Securitas-TUM 193®, as well as the four top-fermenting yeast strains Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, and Tropicus-TUM 506® were POF negative. These strains were normally used for the production of lager beer (Frisinga-TUM 3470®, Securitas-TUM 193®), kölsch (Colonia-TUM 177®), and alt (Vetus-TUM 184®), as well ale beer production (Mel-TUM 211® and Tropicus-TUM 506®). Phenolic off-flavors are typically not desired in these classic beer styles. These three yeast strains cannot decarboxylate any of the precursor acids. Therefore the phenylacrylic acid decarboxylase (*PAD1*) and/or ferulic acid decarboxylase (*FDC1*) gene activity might be inactive or blocked [43–45].

Figure 6 shows the concentrations of 4-vinylguajacol measured in the finished beers after lagering. According to the evaluation by sniffing, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Monacus-TUM 381®, and Harmonia-TUM 511® were POF-positive, with detected concentrations of 4-vinylguajacol above the individual threshold for 4-vinylguajacol of 0.3 mg/L [46].

Table 10. Phenolic off-flavor (POF) test results for the investigated yeast strains.

	POF-Test/Sniffing Perception of:		
TUM Yeast Strain	**4-Vinylguajacol/ Ferulic Acid**	**4-Vinylphenol/ Coumaric Acid**	**4-Vinylstyrene/ Cinnamic Acid**
Frisinga - TUM 3470®	–	–	–
Securitas-TUM 193®	–	–	–
LeoBavaricus-TUM 68®	+	+	+
LunaBavaria-TUM 127®	+	+	+
Colonia-TUM 177®	–	–	–
Vetus-TUM 184®	–	–	–
Mel-TUM 211®	–	–	–
Monacus-TUM 381®	+	+	+
Tropicus-TUM 506®	–	–	–
Harmonia-TUM 511®	+	+	+

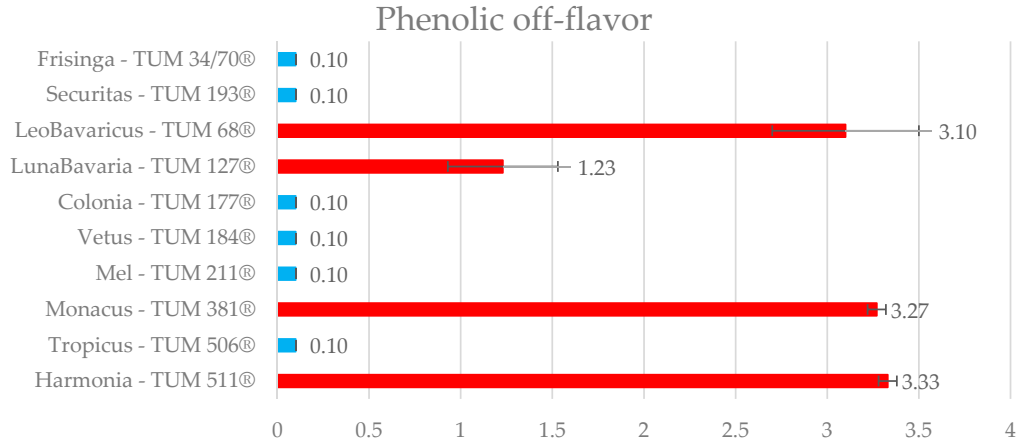


Figure 6. Phenolic off-flavor ability of the investigated yeast strains; confidence level 95%.

3.3.2. Fermentation by-Products

There was a variation in the production of fermentation by-products for all of the yeast strains (Tables 11 and 12). The beers produced with LeoBavaricus-TUM 68®, Luna Bavaria-TUM 127®, Monacus-TUM 381® and Harmonia -TUM 511® had the highest levels of isoamyl acetate and 4-vinylguajacol, with concentrations above 2.9 mg/L for isoamyl acetate (Harmonia-TUM 511®) and above 1.2 mg/L for 4-vinylguajacol. The concentration of these esters specific to the production of wheat beers were within the average reference values for regular wheat beers (2–8 mg/L isoamyl acetate and 1–4 mg/L of 4-vinylguajacol) according to Back [47]. LeoBavaricus-TUM 68® had the highest concentration of higher alcohols (212.07 ± 13.15 mg/L), and the highest level of esters was detected in the beer produced by Harmonia-TUM 511®, with a concentration of 57.33 ± 0.65 mg/L.

Acetaldehyde, 2.3-pentanedione and diacetyl are associated with unmatured beer, and can result in an unpleasant flavor if the concentrations are above their individual thresholds. The concentration of acetaldehyde is below their individual thresholds of 25 mg/L for all strains. Frisinga-TUM 34/70®, LunaBavaria-TUM 127® and Colonia-TUM 177® showed concentrations of diacetyl above the individual threshold of 0.15 mg/L [46]. The production as well as the degradation of diacetyl is strain dependent, and were influenced by fermentation conditions and the yeast management (pitching rate, vitality and viability).

Table 11. Average of important fermentation by-products (FBP) measured in triplicate of the final beers produced with Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127® and Colonia-TUM 177®; confidence level 95%.

Fermentation by-Products (mg/L)					
FBP	**Frisinga-TUM 3470®**	**Securitas-TUM 193®**	**LeoBavaricus-TUM 68®**	**LunaBavaria-TUM 127®**	**Colonia-TUM 177®**
Isoamyl acetate	0.63 ± 0.19	1.38 ± 0.05	4.07 ± 0.46	3.97 ± 2.30	2.40 ± 0.16
Ethyl acetate	19.77 ± 2.50	26.07 ± 1.89	32.50 ± 2.97	36.97 ± 2.93	32.87 ± 1.68
∑ Ester (E)	20.40 ± 2.69	27.90 ± 1.94	36.57 ± .3.43	40.93 ± 3.16	35.27 ± 1.83
n-Propanol	11.23 ± 0.77	13.43 ± 0.72	22.77 ± 2.37	15.93 ± 0.70	21.30 ± 1.25
i-Butanol	10.63 ± 0.71	14.27 ± 0.47	62.30 ± 3.51	43.70 ± 3.42	10.53 ± 0.11
Amyl alcohols	60.53 ± 3.31	82.60 ± 2.68	127.00 ± 7.33	91.60 ± 3.34	80.77 ± 1.06
∑ Higher alcohols (HE)	82.40 ± 4.76	110.30 ± 3.86	212.07 ± 13.15	151.23 ± 6.25	112.60 ± 2.32
4-Vinylguajacol	0.10 ± 0.00	0.10 ± 0.00	3.10 ± 0.40	1.23 ± 0.30	0.10 ± 0.00
Diacetyl	0.18 ± 0.07	0.10 ± 0 01	0.11 ± 0.01	0.17 ± 0.03	0.14 ± 0 03
2,3-Pentandione	0.00 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.01
∑ Vicinal diketones	0.18 ± 0.07	0.12 ± 0.02	0.12 ± 0.01	0.19 ± 0.03	0.15 ± 0.04
Acetaldehyde	1.33 ± 0.47	11.17 ± 1.46	7.27 ± 1.80	2.80 ± 0.94	8.23 ± 2.02
Ratio (∑E:∑HE)	4.04	3.95	5.80	3.69	3.19

Table 12. Average of important fermentation by-products (FBP) measured in triplicate of the final beers produced with Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®; confidence level 95%.

Fermentation by-Products (mg/L)					
FBP	**Vetus-TUM 184®**	**Mel-TUM 211®**	**Monacus-TUM 381®**	**Tropicus-TUM 506®**	**Harmonia-TUM 511®**
Isoamyl acetate	1.80 ± 0.09	2.03 ± 0.05	4.33 ± 0.05	1.53 ± 0.11	2.93 ± 0.05
Ethyl acetate	37.40 ± 0.58	37.93 ± 0.11	50.60 ± 0.85	22.57 ± 1.31	54.40 ± 0.61
∑ Ester (E)	39.20 ± 0.61	39.97 ± 0.14	54.93 ± 0.84	24.10 ± 1.36	57.33 ± 0.65
n-Propanol	15.40 ± 0.46	18.30 ± 0.16	18.30 ± 0.16	20.67 ± 1.01	20.77 ± 0.53
i-Butanol	11.37 ± 0.14	16.20 ± 0.37	24.17 ± 0.66	20.90 ± 1.40	13.13 ± 0.28
Amyl alcohols	67.37 ± 0.91	59.27 ± 1.32	101.00 ± 4.23	88.50 ± 5.90	74.97 ± 1.24
∑ Higher alcohols (HE)	94.13 ± 1.43	93.77 ± 1.83	142.23 ± 5.26	130.07 ± 8.12	108.87 ± 1.23
4-Vinylguajacol	0.10 ± 0.00	0.10 ± 0.00	3.27 ± 0.05	0.10 ± 0.00	3.33 ± 0.05
Diacetyl	0.06 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.12 ± 0.01	0.06 ± 0.00
2,3-Pentandione	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
∑ Vicinal diketones	0.06 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	0.14 ± 0.01	0.07 ± 0.00
Acetaldehyde	6.80 ± 0.80	4.60 ± 0.48	6.23 ± 0.77	5.93 ± 0.93	4.03 ± 0.46
Ratio (∑E:∑HE)	2.40	2.35	2.59	5.40	1.90

3.3.3. Sulfur Dioxide

Table 13 shows the SO_2 concentration of the finished beers produced by the investigated TUM yeast strains. As shown in the table, all of the investigated yeast strains form sulfur dioxide (SO_2) during fermentation. The concentration produced varies from strain to strain and differs by up to a about 9 mg/L SO_2 in the finished beers. This can be confirmed by SWIEGERS and ANNEMÜLLER, who reported that differences between 2 and 10 mg/L SO_2 can be detected in the finished beer under identical fermentation conditions [40,48]. In conclusion and also according to the results obtained by MEIER-DÖRNBERG in 2017, the SO_2 formation is mainly influenced by the used yeast strain and is strain dependent [23]. As Table 13 shows, Securitas-TUM 193® produced the highest quantity of SO_2 at a total amount of 9.47 ± 0.68 on average. According to BACK, each additional mg/L SO_2 below 5 mg/L prolongs the flavor stability of beer by about one month (Back Technologisches Seminar Weihenstephan 2015). Therefore TUM yeast strain Securitas-TUM 193® could be very suitable for producing lager beers with a long-term flavor stability. The lowest concentration was produced by yeast strain Monacus-TUM 381® and Harmonia-TUM 511® at a concentration of 0.5 mg/L. The second lowest concentration in the finished beer of 1.63 ± 0.51 mg/L SO_2 was produced by the top-fermenting wheat beer yeast strain LunaBavaria-TUM 127®.

Table 13. SO_2 concentration of the final beers produced using Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Colonia-TUM 177®, Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511®; confidence level 95%.

SO2 Concentration of the Finished Beers	
TUM Yeast Strain	**SO2 (mg/L)**
Frisinga-TUM 34/70®	6.03 ± 0.30
Securitas-TUM 193®	9.47 ± 0.68
LeoBavaricus-TUM 68®	2.87 ± 0.30
LunaBavaria-TUM 127®	1.63 ± 0.51
Colonia-TUM 177®	3.80 ± 0.79
Vetus-TUM 184®	3.10 ± 0.16
Mel-TUM 211®	2.60 ± 0.98
Monacus-TUM 381®	0.50 ± 0.00
Tropicus-TUM 506®	2.23 ± 1.02
Harmonia-TUM 511®	0.50 ± 0.00

3.3.4. Sensory Evaluation

Sensory analysis of the beers was conducted after maturation and lagering. All of the beers produced had no prevailing off-flavors and were rated with a four or a five in every category of the DLG scheme for beer (data not shown). In terms of the descriptive sensory evaluation, the following Figures 7–11 show the aroma profile of each investigated TUM yeast strain. The overall flavor impression is shown in orange, and the most distinct individual flavor attributes are shown in blue. The individual flavor attributes represent the most noted and highest rated flavors by all panelists within the seven main aroma categories. The average values of the single flavor attributes are summarized in main categories and represent the overall flavor impression. As shown in the figures, LeoBavaricus-TUM 68® and LunaBavaria-TUM 127® had a very distinct clove-like aroma. In addition to Monacus-TUM 381® and Harmonia-TUM 511®, all four *Saccharomyces cerevisiae* yeast strains were POF positive, with analytically detected concentrations above the individual threshold of 4-vinylguajacol. A clove-like aroma is the main aroma compound in German wheat beers, and probably the reason for why over 90% of the tasters associated the produced and tasted beers with wheat beer. In contrast to LeoBavaricus-TUM 68®, LunaBavaria-TUM 127® and Monacus-TUM 381®,

the clove-like flavor was not recognized by the panelists for Harmonia-TUM 511® (flavor intensity of 0.14, e.g., Figure 11), even if this strain produced the highest concentration of 4-VG at 3.33 mg/L.

According to MEIER-DÖRNBERG [23], this may be caused and suppressed by synergistic effects and due to the well-balanced flavor profile with citrus and fruity flavors, which were perceived as being slightly more distinct. In conclusion, 28.5% of the panelists could not clearly assign this beer to a wheat nor to an ale style. MEIER-DÖRNBERG suggested that this strain was particularly suitable for brewing a beer with the fruitiness of an ale style brewed beer, underlined by the slightly spicy and yeasty flavors of a wheat beer and proposed calling it "Bavarian Ale". The brewing yeast Mel-TUM 211® and Tropicus-TUM 506® were an ale beer style, and the beers produced were very fruity. Tropicus-TUM 506® has fruity flavors, especially within the tropical fruit category. Vetus-TUM 184® also produces fruity and sweet flavors as well as a flavor reminiscent of wine, which was described as dry, and could be responsible for the drier beer flavor expected in beers of the alt type. Colonia-TUM 177® seemed to be suitable for more than one beer type. The panelists assigned the beer produced using Colonia-TUM 177® to a kölsch and an alt style (27.27% kölsch and alt). The produced beer had a sweet and yeasty flavor with aromas slightly reminiscent of citrus fruits, such as grapefruit. The bottom-fermenting yeast strain Frisinga-TUM 34/70® had a well-balanced aroma profile with a tendency towards floral and fruity flavor impressions. The produced beers were clearly described as being lager beers. In comparison, the beers produced using Securitas-TUM 193® were also assigned to fruity beer types such as ale and kölsch (28.57% Lager, 14.28% Ale and 14.28% kölsch). This could be confirmed by the specific aroma profile. Securitas-TUM 193® was characterized by plenty of fruity flavors, particularly reminiscent of berries, with additional fresh yeasty and sulfuric flavors, typically for lager beers, and a sweet body.

Figure 7. Comparison of the flavors grouped according to the main categories and the respective main aroma attributes for the bottom-fermenting *Saccharomyces pastorianus* yeast strains Frisinga-TUM 34/70® and Securitas-TUM 193®.

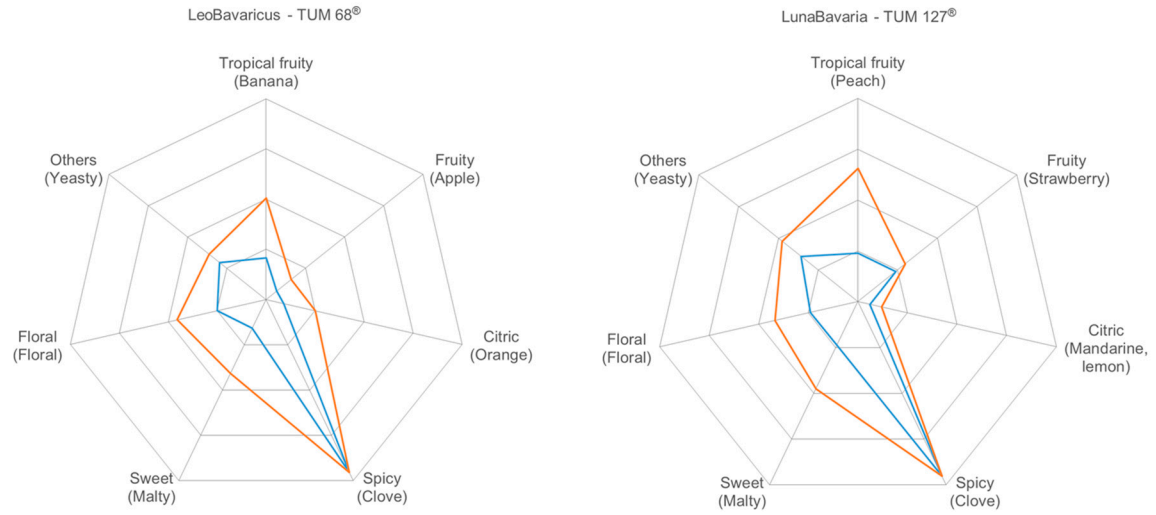


Figure 8. Comparison of the flavors grouped according to the main categories and the respective main aroma attributes for the top-fermenting *Saccharomyces cerevisiae* yeast strains LeoBavaricus-TUM 68® and LunaBavaria-TUM 127®.

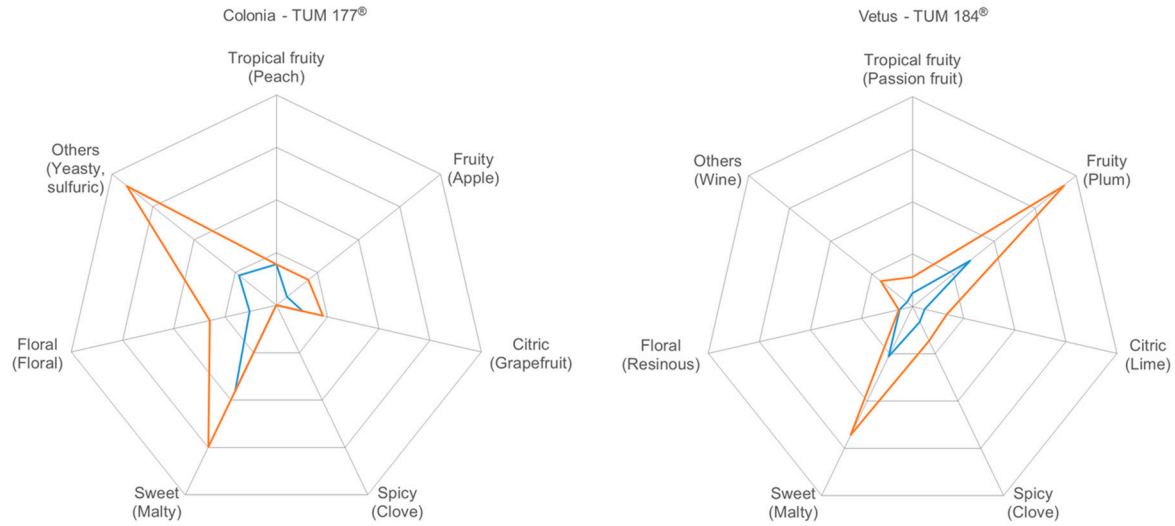


Figure 9. Comparison of the flavors grouped according to the main categories and the respective main aroma attributes for top-fermenting *Saccharomyces cerevisiae* yeast strains Colonia-TUM 177® and Vetus-TUM 184®.

Figure 10. Comparison of the flavors grouped according to the main categories and the respective main aroma attributes for top-fermenting *Saccharomyces cerevisiae* yeast strains Mel-TUM 211® and Monacus-TUM 381®.

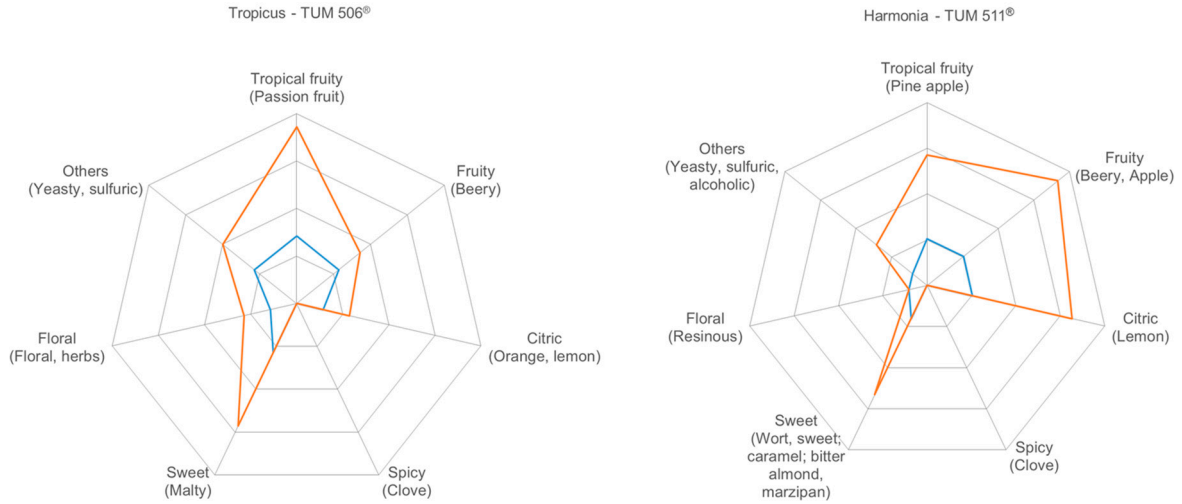


Figure 11. Comparison of the flavors grouped according to the main categories and the respective main aroma attributes for top-fermenting *Saccharomyces cerevisiae* yeast strains Tropicus-TUM 506® and Harmonia-TUM 511®.

4. Conclusions

All 10 investigated TUM brewing yeast strains showed different phenotypic characteristics and flavor profiles. The most interesting differences are presented in the following Table 14 and highlighted in red, yellow, or green according to their performance and brewing characteristics. Based on the results of the genetic analysis, the species identity as well as the genetic distinctness of the investigated TUM yeast strains of the TUM Yeast Center could be confirmed. Except Frisinga-TUM 34/70® and Securitas-TUM 193®, which belong to the *Saccharomyces pastorianus* species, all other TUM yeast strains belonged to *Saccharomyces cerevisiae*. All 10 TUM yeast strains showed different fermentation rates and degrees of apparent attenuation and can be explained by their different ability to ferment maltotriose. The top-fermenting yeast strain Mel-TUM 211® only fermented a low level of maltotriose (26.66% ± 0.26%), while the LunaBavaria-TUM 127® yeast strain could not ferment maltotriose at all (01.05% ± 2.89%). In the case of non-fermentation of maltotriose, LunaBavaria-TUM

127® beers reached their apparent attenuation faster than Mel-TUM 211®, and needed less time for fermentation. Except for the strains Mel-TUM 211® and Tropicus-TUM 506®, the pH of the final beer was within the range of 4.4 to 4.6 [49]. Mel-TUM 211® and Tropicus-TUM 506® exhibited the weakest capacity for acidification (ΔpH 0.5) of all the investigated strains, which might be due to cell autolysis caused by the low fermentation performance. The total amino acid utilization was also different for each investigated TUM yeast strain, and no conclusion can be drawn as to cell growth. The cell concentration was measured during the main fermentation phase and maturation phase to classify the investigated strains according to their specific flocculation behavior. Not every top-fermenting yeast strain shows powdery behavior. LunaBavaria-TUM 127® and Vetus-TUM 184® showed a flocculent behavior similar to the bottom-fermenting yeast strains Frisinga-TUM 34/70® and Securitas-TUM 193®. As expected, some of the yeast strains most commonly used in industry Frisinga-TUM 34/70® and LeoBavaricus-TUM 68® showed the best phenotypic characteristics, thereby standing out from the other investigated yeast strains. However, every brewer's ultimate goal is the final desirable taste of the produced beer. In this respect, the main focus in this study was on the individual and main flavor impression of the final beers. Only the top-fermenting yeast strains LeoBavaricus-TUM 68®, LunaBavaria-TUM 127®, Harmonia-TUM 511®, and Monacus-TUM 381® were capable of building phenolic off-flavors, which was confirmed by the concentrations of 4-vinylguajacol in the finished beers, which were above the individual threshold. Except for the Monacus-TUM 381® strain, all panelists recognized the clove like flavor and therefore referred to these beers as wheat style beers. The production of fermentation by-products, as well as the resulting flavor composition in the finished beers was strain-dependent and followed no defined order. The formation of sulfur dioxide (SO_2) during fermentation could be detected in all strains, but concentrations differed from 0.50 ± 0.00 mg/L for Monacus-TUM 381® and Harmonia-TUM 511®, to a considerably higher level on average of 9.47 ± 0.68 mg/L for Securitas-TUM 193®. The bottom-fermenting yeast strain Securitas-TUM 193® could therefore be very suitable for the production of lager beers with high flavor stability. This characterization model for yeast strains allows brewers around the world access to a simplified and targeted selection of brewing yeast strains suitable for their specific purposes. By analyzing and comparing different yeast strains, breweries can be given customized advice when selecting a yeast strain suitable for their brewing process or type of beer, irrespective of whether they want to replace their existing yeast strain to improve the aroma profile of existing beer styles, develop new beer styles, or optimize the fermentation process by selecting a strain with the corresponding fermentation characteristics. Knowledge about the different yeast strain characteristics can, in particular, promote the competitiveness of small and medium-sized breweries and, if necessary, secure their existence by being part of the steadily increasing market for beer specialties [24].

Table 14. Comparison of the investigated 10 TUM yeast strains with the focus on recommended beer style, POF, flocculation behavior, maltotriose utilization, pH drop, SO_2, apparent attenuation and time to reach the final gravity (red=weak, yellow=normal, green=strong).

TUM Yeast Strains									
TUM Yeast Strain	**Yeast Species**	**Recommended Main Beer Style**	**Phenolic Off-Flavor**	**Flocculation Behavior**	**Maltotriose-Utilization (%)**	**ΔpH**	**SO_2(mg/L)**	**AA(%)**	**Fermentation Time (days)**
Frisinga-TUM 34/70®	*S. pastorianus*	lager beer	–	flocculent	94.06 ± 0.91	0.8	6.03 ± 0.30	81.63 ± 0.51	9
Securitas-TUM 193®	*S. pastorianus*	lager bee	–	flocculent	86.60 ± 0.52	0.7	9.47 ± 0.68	79.30 ± 0.51	9
LeoBavaricus-TUM 68®	*S. cerevisiae*	wheat beer	+	powdery	99.65 ± 0.28	0.8	2.87 ± 0.30	86.17 ± 0.05	4
LunaBavaria-TUM 127®	*S. cerevisiae*	wheat beer	+	flocculent	01.05 ± 2.89	0.6	1.63 ± 0.51	76.20 ± 1.76	6
Colonia-TUM 177®	*S. cerevisiae*	kölsch and alt beer	–	powdery	94.80 ± 0.78	0.6	3.80 ± 0.79	84.93 ± 0.37	4
Vetus-TUM 184®	*S. cerevisiae*	alt beer	–	flocculent	60.92 ± 5.87	0.7	3.10 ± 0.16	80.97 ± 3.02	11
Mel-TUM 211®	*S. cerevisiae*	ale beer	–	powdery	26.66 ± 0.26	0.5	2.60 ± 0.98	66.13 ± 0.51	10
Monacus-TUM 381®	*S. cerevisiae*	wheat beer	+	powdery	97.75 ± 0.09	0.7	0.50 ± 0.00	86.17 ± 0.11	7
Tropicus-TUM 506®	*S. cerevisiae*	ale beer	–	powdery	59.28 ± 0.81	0.5	2.23 ± 1.02	77.37 ± 1.34	9
Harmonia-TUM 511®	*S. cerevisiae*	ale and wheat beer	+	powdery	83.91 ± 0.71	0.8	0.50 ± 0.00	82.70 ± 0.42	6

Supplementary Materials: The following Tables are available online at www.mdpi.com/2311-5637/3/3/41. Supplementary Tables: Table S1. Mean percentage of amino acid uptake of the yeast strains Frisinga-TUM 34/70®, Securitas-TUM 193®, LeoBavaricus-TUM 68®, LunaBavaria-TUM 127® and Colonia-TUM 177® after lagering measured in the finished beers (Group A = light gray, Group B = dark gray, Group C = no shading); confidence level 95%; Table S2. Mean percentage of amino acid uptake of the yeast strains Vetus-TUM 184®, Mel-TUM 211®, Monacus-TUM 381®, Tropicus-TUM 506® and Harmonia-TUM 511® after lagering measured in the finished beers (Group A = light gray, Group B = dark gray, Group C = no shading); confidence level 95%.

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