

## Article

# Brewing Efficacy of Non-Conventional *Saccharomyces Non-cerevisiae* Yeasts

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**Abstract:** Consumer demands for new sensory experiences have driven the research of unconventional yeasts in beer. While much research exists on the use of various common *Saccharomyces cerevisiae* strains as well as non-*Saccharomyces* yeasts, there exists a gap in knowledge regarding other non-*cerevisiae* *Saccharomyces* species in the fermentation of beer, in addition to *S. pastorianus*. Here, five distinct species of *Saccharomyces* from the UC Davis Phaff Yeast Culture Collection, as well as one interspecies hybrid from Fermentis, were chosen to ferment 40 L pilot-scale beers. *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, *S. bayanus*, and *S. uvarum* yeasts were used to ferment wort in duplicate pairs, with one fermenter in each pair receiving 10 g/L dry-hop during fermentation. Analytical measurements were made each day of fermentation and compared to controls of SafAle™ US-05 and SafLager™ W 34/70 for commercial brewing parameters of interest. Finished beers were also analyzed for aroma, taste, and mouthfeel to determine the flavor of each yeast as it pertains to brewing potential. All beers exhibited spicy characteristics, likely from the presence of phenols; dry-hopping increased fruit notes while also increasing perceived bitterness and astringency. All of the species in this study displayed great brewing potential, and might be an ideal addition to beer depending on a brewery's desire to experiment with flavor and willingness to bring a new yeast into their production environment.

**Keywords:** non-conventional yeasts; *Saccharomyces*; fermentation; beer; dry-hopping; brewing potential



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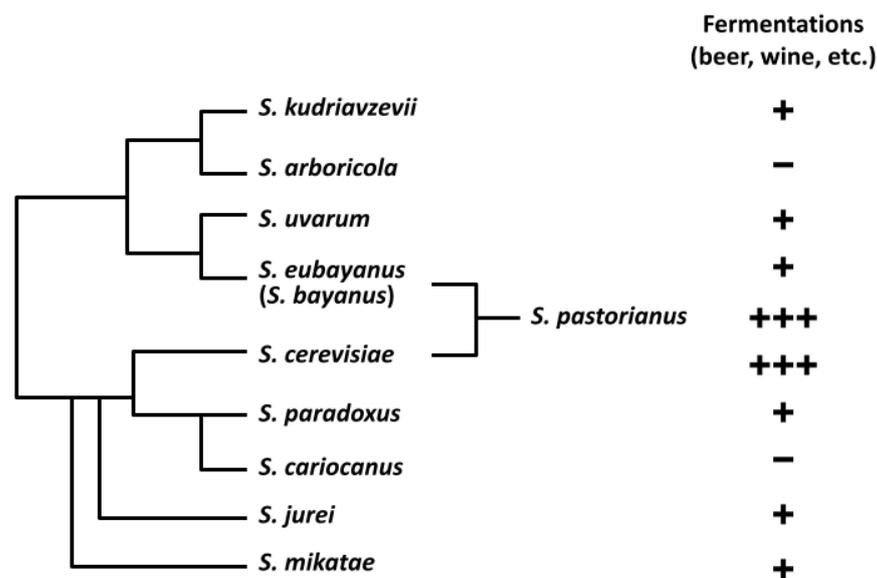
## 1. Introduction

Increasingly, changing demands by beer drinkers in search of new sensory experiences are driving research into novel fermentations [1–4]. Much of this research has utilized non-*Saccharomyces* yeast strains [5–12], which can be attributed to the rise in popularity of mixed-fermentation beers [13–15]. This pursuit of distinctive aromas and flavors has similarly driven the increased use of non-*cerevisiae* *Saccharomyces* species in the alcoholic fermentation of all beverages [16–22]. While much of this work has been focused on wine fermentations, the most widely used non-*cerevisiae* species in beer production is *S. pastorianus*, which has been used the world over in the production of lagers for centuries [20,23–26].

In addition to novel yeast-derived flavors, brewers are increasingly turning to dry-hopping to enhance their consumers' sensory experience. Historically, this procedure of adding hops (*Humulus lupulus*) cones to beer when fermentation is active or finished was performed to provide microbial stability in packaging and during transport [27,28]. Relatively more recently, with the rise of craft and micro brewers, dry-hopping with pellets or advanced hop products [29] has become a common tactic used by brewers desiring to add interesting flavors and aromas to their beer [30].

All *Saccharomyces* yeast species that have been found to produce ethanol from carbohydrate sugar sources have been classified as part of the *Saccharomyces sensu stricto* (*Sss*) complex [31–33]. While the *Sss* currently contains ten distinct species, only eight have been linked to alcoholic beverage fermentation (Figure 1). *S. cerevisiae* and *S. pastorianus*

have long been known for their use in alcoholic beverage production, but the *Sss* contains several non-conventional species. *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, *S. bayanus*, and *S. uvarum* are species that have already shown potential for alcoholic beverages, and have been identified in fermentations of wine, tepache, cider, chicha, palm wine, umqomboti, and other beverages around the world [19,34–39]. Many of these fermented beverages, however, contain mixed cultures of yeasts and sometimes bacteria, in addition to naturally formed interspecies hybrids between two or more different *Saccharomyces* species [24,40]. To date, none of these species have been evaluated in monoculture ale fermentations in a beer brewing context, but their efficacy has been previously reviewed [41]. Additionally, none of these yeasts have been studied for their use in the production of dry-hopped beer.



**Figure 1.** *Sss* phylogeny and extent of use in alcoholic beverage fermentations. *Saccharomyces bayanus* is listed in parenthesis to indicate it was derived from multiple hybridization events [42]. *S. pastorianus* is shown as a genetic hybrid of *S. eubayanus* and *S. cerevisiae* [21]. Use in fermented beverages is indicated with plus signs (+) for current commercial use, with *S. cerevisiae* and *S. pastorianus* exhibiting the most ubiquitous use in beer, and negative signs (–) for no known use. *S. cariocanus* is known to be harboring just four translocated chromosomes different than *S. paradoxus* [43]. *S. jurei* has very recently been proven to have brewing potential [44]. Figure previously used in 2020 review by Bruner and Fox [41] of yeasts’ use in alcoholic beverage fermentations.

First isolated from oak trees of western Europe, *S. kudriavzevii* is a wild-type yeast that has been sequenced to contribute 23–96% of its genome to hybrids with *S. cerevisiae* [16,19,45]. While no commercial examples of its use in beer fermentation exist, *S. kudriavzevii* has been isolated from mixed cultures of farmhouse ciders in France and draft beer systems in Germany to New Zealand [46,47]. Due to its propensity to hybridize, this yeast has even been found as part of the genetic makeup in Belgian Trappist ale strains from Chimay, Westmalle, and Orval [48]. *S. kudriavzevii* is a cryophilic species and is currently used to ferment wines at lower temperatures (10 to 15 °C) in Europe and Australia [19,49]. Because it thrives at low temperatures and may have aromas similar to Belgian beers, *S. kudriavzevii* has potential for use in the production of hoppy lager beers in the brewing industry.

*S. paradoxus* has been found in African umqomboti [38] and white wine fermentations previously [50], but has only been studied for its beer brewing potential (at 15 °C) very recently, since the inception of this research [22]. *S. paradoxus* was one of the first species isolated as a member of the *Sss* in addition to *S. pastorianus* and *S. cerevisiae* and is typically found in tree sap of Northeastern Europe [51]. Being a wild-type yeast species suggests that *S. paradoxus* may produce interesting volatile aroma compounds at warmer (18 to 24 °C) ale temperatures [52].

*Saccharomyces mikatae* is a wild yeast that contributes to genetic hybrids from interspecies hybridization events with *S. cerevisiae* and *S. paradoxus* [53], and was first isolated from soil and decaying leaves in Japan [43]. *S. mikatae* was shown to form a biofilm on the surface of liquid media (pellicle) after twenty-five days at 20 °C, similar to wild-type strains [43]. It produced fruity, banana, floral, and sweet perfume aromas in white wine, and ferment slowly, perhaps all due to its diversion from the *S. cerevisiae* parent genome [54,55]. Both *S. paradoxus* and *S. mikatae* offer unique characteristics that might be of interest to craft brewers creating beer at ale fermentation temperatures.

*Saccharomyces bayanus* was previously thought to be the parent of the lager strain, *S. pastorianus* [21,47,56], but the hybridization event that produced lager brewing yeast is now proven to have occurred between *S. cerevisiae* and *S. eubayanus* [25,34,57,58]. *S. bayanus* has been characterized as its own species within the Sss, but in order delineate it from *S. eubayanus* and *S. uvarum*, it is commonly referred to as *S. bayanus* var. *bayanus* [21,42]. Genetic analysis of organisms in beer fermentations have identified *S. bayanus* as part of blended cultures due to its chromosomal similarity to *S. pastorianus* [26], but it is most common as a solitary species in wine fermentations [58].

A close relative, *Saccharomyces uvarum*, was once was thought to be a variant of *S. bayanus*, but has since been confirmed as a distinct species [59]. *S. uvarum* has been found to be part of the mixed culture of spontaneously fermented wines [36], as well as an interspecies hybrid known in some Norwegian kveik strains [17]. Both *S. bayanus* and *S. uvarum* exhibit increased levels of isoamyl acetate in wine and brandy [60,61], and might contribute similar flavor to beer.

Some commercial yeast suppliers are leveraging the power of interspecies hybrids through research and development (R&D) to create distinctive sensory experiences, including a *S. cerevisiae* × *S. bayanus* hybrid produced by Fermentis-LeSaffre (Marcq-en-Baroeul, France, EU; fermentis.com/en/) known as SafE<sup>no</sup>™ HDT18 [62]. This interspecies hybrid has been created through a LeSaffre R&D program to select a yeast strain that exhibits increased expression of aromatic terpenes. New research has identified these terpene compounds as some of the most impactful on dry-hopped beer aroma [30,63] through bio-transformation with glycosides and alcohols to produce unique aroma characteristics [64]. While this yeast was developed for wine fermentations, it may be of great interest to brewers making dry-hopped beers, and was therefore selected for this study.

While there is much research regarding the use of some of these species in a laboratory scale or wine fermentation, work remains for their efficacy and commercial use in the production of beer. Additionally, little to no sensorial analysis exists on the use of any of these *Saccharomyces* spp. in the fermentation of beer, most notably at ale fermentation temperatures (18–20 °C) or in dry-hopped beers. The aim of this study is to assess the brewing potential of the non-conventional non-*cerevisiae* *Saccharomyces* species outlined above by assessing fermentation dynamics and performance, yeast abundance and viability post-fermentation for serial re-pitching, as well as the flavor characteristics of the resultant beer. Beers in this study will be run as both dry-hopped and standard fermentations due to the pervasiveness of dry-hopping in the American craft brewing industry. While the most widely used non-*cerevisiae* *Saccharomyces* species is *S. pastorianus*, this species will only be used as an analytical control in this study as much research already exists on its brewing potential.

## 2. Materials and Methods

### 2.1. Experimental Beers

A total of eight all-malt pilot-scale brews were performed on the 1.8 hL Anheuser-Busch Research Pilot Brewery at the University of California, Davis. Brewing parameters, as well as the malt, hops, water chemistry, mashing regime, pH, boiling parameters, and knockout temperatures followed the same method as outlined in previous research [65]. The experimental beer recipe was similar to an American Pale Ale or Session IPA, with a target original gravity of 10 °P and 20 IBU from Centennial (8.3% AA, Hopsteiner,

New York, NY, USA) in the kettle, to yield a 4.2% (*v/v*) alcohol beer under standard ale fermentation conditions. All parameters were chosen to mimic typical production conditions in an American craft brewery. Wort from each 180 L brew was split evenly by volume between four 56 L fermenters, to fill each with approximately 40 L of cooled wort, allowing for each brew to be used for multiple distinct fermentations.

## 2.2. Yeasts

Five experimental *Saccharomyces* yeasts sourced from the University of California, Davis, Phaff Yeast Culture Collection (UC Davis, Davis, CA, USA; phaffcollection.ucdavis.edu) included the type strains of *S. kudriavzevii*, *S. mikatae*, *S. paradoxus*, *S. bayanus*, and *S. uvarum*. Additionally, the analytical control *S. cerevisiae* and *S. pastorianus* species and one experimental *S. cerevisiae* × *S. bayanus* hybrid were provided by Fermentis (LeSaffre, Marcq-en-Baroeul, France) (Table 1). Yeasts from the Phaff Collection were revived from cryogenic storage and streaked onto potato dextrose agar (PDA) plates and incubated for 2 days at 30 °C before being moved to room temperature storage until propagation. Yeasts from Fermentis were provided as an active dry yeast with the emulsifier E491 (sorbitan monostearate) and stored at 4 °C until propagation.

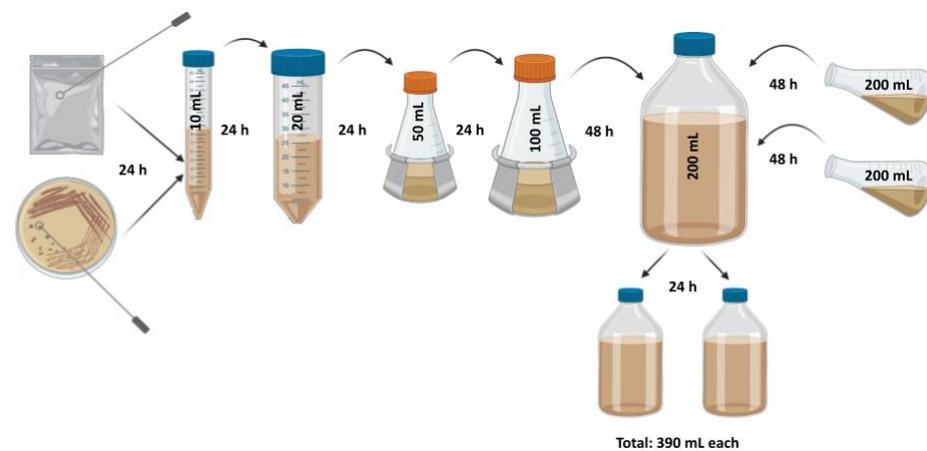
**Table 1.** Non-conventional non-*cerevisiae* *Saccharomyces* and control yeasts used in the fermentations of the experimental beer. Yeasts were sourced from either the Phaff Yeast Culture Collection at the University of California, Davis (UCD), or from Fermentis LeSaffre of Marcq-en-Baroeul, France (Saf). Type strain as defined in MycoBank (mycobank.org), origin, isolation, flocculation, and attenuation, as defined in the scientific or product literature. SafAle™ US-05 and SafLager™ W 34/70 are included as analytical controls.

Scientific Name	Yeast Name	Type Strain	Isolated from	Geographic Origin	Flocculation	Attenuation
<i>Saccharomyces kudriavzevii</i>	UCDFST 11-515	NCYC 2889T	oak tree bark	Western Europe	Medium High	Moderate
<i>Saccharomyces paradoxus</i>	UCDFST 01-161	DBVPG 6411	tree exudate	Northeast Europe	Medium	Moderate
<i>Saccharomyces mikatae</i>	UCDFST 11-510	NCYC 2888T	Soil	Japan	Medium	Moderate Low
<i>Saccharomyces bayanus</i>	UCDFST 01-135	CBS 380	turbid beer	Italy	Medium	Moderate
<i>Saccharomyces uvarum</i>	UCDFST 11-512	CBS 395	fruit and seeds	Scandinavia	High	Moderate
<i>Saccharomyces cerevisiae</i> × <i>Saccharomyces bayanus</i>	SafCeno™ HD T18	*	LeSaffre R&D	France	Medium	High
<i>Saccharomyces cerevisiae</i>	SafAle™ US-05 **	*	*	USA	Medium	78–82%
<i>Saccharomyces pastorianus</i>	SafLager™ W 34/70	W 34/70	Weihenstephan	Germany	High	80–84%

\* unknown; \*\* SafAle™ US-05 fermentations were performed in biological triplicate.

All yeasts were propagated according to the same procedure to ensure consistency throughout this study. Due to time constraints with research brewing at the UC Davis facility, only one yeast was chosen on which to perform three biological replicates to ferment from three separate brews: *S. cerevisiae* SafAle™ US-05. Yeasts were propagated in wort consisting of 10.0% *w/v* (10.0°P, 1.040 Specific Gravity) dried pilsner malt extract (Briess CBW® Pilsen Light; Chilton, WI, USA) in deionized water with 20 ppm CaCl<sub>2</sub> salts, targeting 5.2 pH, and 0.10% *w/v* yeast nutrient (Kerry Yeastex® 82; Beloit, WI, USA). Wort was boiled for ten minutes and sterilized via autoclave before being sterile filtered to remove protein and trub particulate. All transfers of yeast and wort were performed in a laminar flow hood or positive pressure room. Yeast colonies were transferred from PDA plate or package of active dry yeast via sterile inoculation loop to propagation wort and propagated stepwise over the course of 11 days following the methods outlined in previous research [65] as well as Figure 2. All growth took place at room temperature on a platform orbital shaker (Innova™ 2000, New Brunswick Scientific; Edison, NJ, USA) set to

150 rpm. Yeast cell counts and viability testing with methylene blue were performed on all propagations and fermentations according to standard methods [66].



**Figure 2.** Yeast propagation schematic following previous methods [65]. Yeasts were propagated to a final approximate total of  $40.0 \times 10^{10}$  cells in each bottle with a total of 390 mL of propagation wort, equivalent to the standard ale pitch rate of  $1.0 \times 10^6$  cells per mL per °P [67] for each 40 L, 10°P pilot fermentation. Figure created on [BioRender.com](https://www.biorender.com), not to scale.

### 2.3. Pilot-Scale Fermentations

Pilot fermentations were performed in 56.0 L glycol-cooled cylindroconical fermenters (JV Northwest; Canby, OR, USA) filled to 40.0 L and set to a standard ale temperature of 20.0 °C. Each unique *Saccharomyces* species (Table 1) was pitched to its own fermenter of wort in duplicate pairs, with the analytical control *S. cerevisiae* US-05 pair fermented in biological triplicate for quality assurance, totaling twenty distinct fermentations. One fermenter in each yeast pair received 10.0 g/L Centennial (8.3% AA, Hopsteiner, New York, NY, USA) T-90 hop pellets as a dry-hop when the measured gravity decreased to below 4.0°P or at seven days into fermentation, whichever occurred first, while the other was fermented traditionally. This amount of dry-hopping has become standard practice among craft breweries today, with many brewers far exceeding this amount at times [29,30,68,69]. End of fermentation or “terminal gravity” [70] was defined here as a change of less than 0.10°P gravity for two simultaneous days following dry-hop.

After fermentation was completed, all beer in all fermenters except the *S. cerevisiae* and *S. pastorianus* analytical controls were cold conditioned at 0.0 °C for two days to allow for natural clarification. The analytical controls were not conditioned for clarity because sensory analysis was not performed on these beers. Yeast and hops were removed from the bottom of the cylindroconical fermenter before the beer was transferred to a 19.6 L Sankey keg for carbonation. All were packaged from the kegs into CO<sub>2</sub>-purged 0.95 L (32 oz.) “Crowler™” cans (Ball Corporation; Westminster, CO, USA) and stored below 4.0 °C until sensory analysis and shipping.

### 2.4. Sample Collection and Preparation

Fermenting beers were aseptically sampled daily within a two-hour window of the time of knockout transfer of wort to fermenter. Using conical tubes, 50 mL of each sample was centrifuged (ThermoFisher Scientific; Waltham, MA, USA) at 20 °C and  $3000 \times g$  RCF for five minutes. The clarified supernatant was then degassed for five minutes using the degas setting on a VWR B1500A-DTH 1.90 L ultrasonic cleaner (Radnor, PA, USA). Degassed samples were then decanted into the sample tubes of the Anton Paar (Graz, Austria, EU) auto-sampling carousel for immediate analysis. Samples were then measured for extract, gravity, alcohol [71], real degrees of fermentation (RDF), and calories using an Anton Paar Density Meter (DMA 5000 M) and alcolyzer (Alcolyzer Beer M) (Anton Paar USA Inc., Torrance, CA, USA). The DMA 5000 M has a repeatability within 0.000001 g/mL and the Al-

colyzer Beer M has a repeatability within 0.03 °P and 0.01 % *v/v* alcohol. pH was measured on a ThermoFisher benchtop pH meter (ThermoFisher Scientific, Waltham, MA, USA) that received a weekly three-point calibration.

### 2.5. Sensory Analysis

Each set of packaged beer from an individual fermentation was assigned a randomly selected three-digit code in order to ensure blind analysis of experimental samples. The willing members of the UC Davis Brewing and Malting Science laboratory team ( $n = 7$ ) used a modified consensus method [72] with check-all-that-apply (CATA) [73] in two tastings to choose appropriate aroma descriptor terms from the DraughtLab Beer Flavor Map<sup>®</sup> (Figure S1) for the twelve beers being analyzed. *S. cerevisiae* and *S. pastorianus* controls fermentations were not included for sensory analysis due to their ubiquity in the brewing industry and known flavor potential for craft beer production. The lab members assessed beers served in 60 mL volumes in clear straight sided glasses, after being removed from cold storage (4.0 °C), under white light. Consensus panelists were instructed to cleanse their palates with water and unsalted crackers between each sample. The common aroma descriptors were parsed down to the twelve most recurrent amongst the experimental beers. Each of these twelve descriptors, the five accepted taste modalities, and three recurrent mouthfeel descriptors from the consensus panel were placed on a 9-point intensity scale for scoring by the local brewery panelists (Table 2).

**Table 2.** Sample ballot given to brewery taste panels accompanying the beer for sensory analysis. Aroma attributes determined from consensus method with CATA performed by UC Davis Brewing Lab members.

Beer:	XXX	Sex: M/F	Age:
Score each attribute by choosing a number, with 0 = none to 9 = extremely strong			
<i>Aroma:</i>			
<b>Cereal:</b> Grainy, Biscuit, Cracker, Wort	0 1 2 3 4 5 6 7 8 9		
<b>Earthy:</b> Musty, Barnyard, Mushroom	0 1 2 3 4 5 6 7 8 9		
<b>Spicy:</b> Clove, Black Pepper, Ginger	0 1 2 3 4 5 6 7 8 9		
<b>Grassy:</b> Fresh Cut, Dry Leaves, Green, Hay	0 1 2 3 4 5 6 7 8 9		
<b>Citrus:</b> Grapefruit, Orange, Lemon, Lime	0 1 2 3 4 5 6 7 8 9		
<b>Tropical:</b> Mango, Papaya, Guava, Banana	0 1 2 3 4 5 6 7 8 9		
<b>Stone Fruit:</b> Apricot, Nectarine, Peach	0 1 2 3 4 5 6 7 8 9		
<b>Stale:</b> Cardboard, Goat Hair, Oxidation, Meaty	0 1 2 3 4 5 6 7 8 9		
<b>Vegetal:</b> Cooked Vegetable, Onion, Celery	0 1 2 3 4 5 6 7 8 9		
<b>Solvent:</b> Chemical, Paint Thinner, Nail Polish Remover	0 1 2 3 4 5 6 7 8 9		
<b>Rotten:</b> Baby Vomit, Sweat, Boiled Egg	0 1 2 3 4 5 6 7 8 9		
<b>Metallic</b>	0 1 2 3 4 5 6 7 8 9		
<b>Other:</b> (Write In)	0 1 2 3 4 5 6 7 8 9		
<i>Taste:</i>			
<b>Sweet</b>	0 1 2 3 4 5 6 7 8 9		
<b>Bitter</b>	0 1 2 3 4 5 6 7 8 9		
<b>Sour</b>	0 1 2 3 4 5 6 7 8 9		
<b>Salty</b>	0 1 2 3 4 5 6 7 8 9		
<b>Umami</b>	0 1 2 3 4 5 6 7 8 9		
<i>Mouthfeel:</i>			
<b>Body</b>	0 1 2 3 4 5 6 7 8 9		
<b>Alcohol</b>	0 1 2 3 4 5 6 7 8 9		
<b>Astringency</b>	0 1 2 3 4 5 6 7 8 9		

Beers were cold transferred to local breweries within three weeks of packaging for sensory analysis with the descriptors previously determined via consensus. Trained beer sensory taste panels at Lagunitas Brewing Company (Petaluma, CA, USA), Deschutes Brewery (Bend, OR, USA), Russian River Brewing Company (Windsor, CA, USA), Sierra Nevada Brewing Company (Chico, CA, USA), Budweiser Brewery (Fairfield, CA, USA), and Sudwerk Brewing Company (Davis, CA, USA) used the descriptors determined previously by consensus method and rated each on a 9-point intensity scale from “none” to “extremely strong” [74,75]. Training, methods and frequency of sensory panels varied from brewery to brewery. However, it was minimally required that the panelists were able to accurately distinguish dry-hopped from non-hopped beer and identify German, Belgian, and American ale strain characteristics. Panelists from breweries were used due to the inability to train a sensory panel in person during the global COVID-19 pandemic. The total sample group to perform sensory analysis on the experimental beers consisted of 51 panelists (36 male and 15 female), ranging in age from 24 to 61. No panelists had medical reasons for not consuming alcohol.

### 2.6. Statistical Analysis

Standard deviation values were determined for US-05 analytical data and viability measurements. Two-tailed statistical analysis (*t*-test) of fermentation data with corresponding *p*-values, as well as two-way analysis of variance (ANOVA) and coefficients of variance for sensory data were performed in Microsoft<sup>®</sup> Excel 2019, Version 2102 (Build 13801.20360) to determine the statistical difference between dry-hopped and traditional fermentations for all beers compared to each other, as well as compared to the analytical control.

## 3. Results and Discussion

### 3.1. Pilot Fermentations

Twenty fermentations were performed, with a mean original gravity (O.G.) of 10.2° Plato ( $\pm 0.36$ ) due to a higher brewhouse efficiency than expected for the recipe designed at 10.0°P (Figure S2). Fermentations were carried out at 20.0 °C, standard ale temperatures, and analytical parameters were measured on each day of fermentation. Results for the six experimental strains were compared with the two analytical control strains, *S. cerevisiae* US-05 (performed in triplicate) and *S. pastorianus* W 34/70. Vigorous and complete fermentations of the two analytical control species in this study suggest that an adequate yeast pitching rate, sufficient nutrients levels, and proper wort aeration procedures were utilized for all fermentations.

All fermentations reached terminal gravity within two weeks, with the exception of *S. uvarum* UCDFST 11-512, which took fifteen days for the non-hopped fermentation but only eleven days for the dry-hopped fermentation (Table 3). However, all average fermentation lengths were not shown to be statistically different between dry-hopped and non-hopped fermentations ( $p > 0.05$ ). These fermentation lengths indicate all the yeasts studied here are viable candidates for production breweries that normally ferment lagers, but perhaps too long for breweries that normally produce ales. Conditioning time was not accounted for in this study as all fermentations were deemed terminal based on gravity determinations. This is opposed to commercial beer production settings that would instead deem beers terminal from the presence or absence of secondary metabolites such as diacetyl or acetaldehyde.

All yeasts measured for viability showed greater than 80.0% living cells at the end of fermentation, signifying a potential for serial re-pitching in a commercial setting. Viability was not measured on the two analytical control strains, US-05 and W 34/70, as their ability for propagation and serial re-pitching has been extensively studied [76–78]. Viability data for the interspecies hybrid *S. bayanus*  $\times$  *S. cerevisiae* HD T18 were not available and should be further evaluated as it is not standard practice to re-pitch wine yeasts due to ethanol toxicity [79].

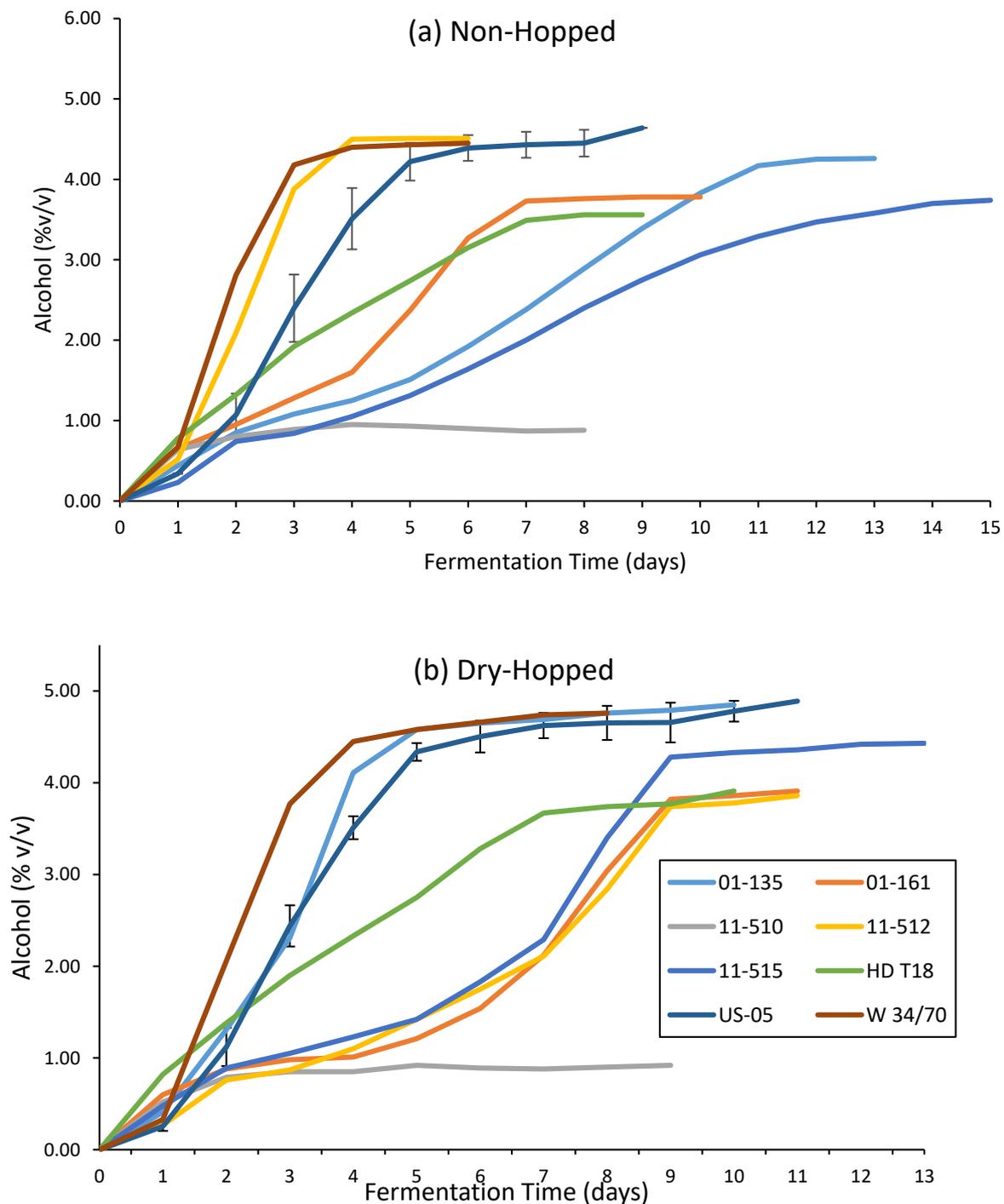
**Table 3.** Terminal fermentation characteristics of *Saccharomyces* species and reference strains used to ferment all-malt wort at 40.0 L pilot scale under two different conditions: non-hopped or dry-hopped during fermentation. Measurements of original gravity (O.G.), final gravity (F.G.) as %w/w of apparent extract, alcohol by volume (ABV), real degree of fermentation (RDF), and calories (Cal) performed on Anton Paar Alcozyler Beer M. Viability was performed from cells in suspension on non-hopped beers on day of terminal gravity, stained with methylene blue, as per standard procedure (69). Viability was not performed on dry-hopped beers due to interference from hops in suspension. Fermentation length as given in days to achieve final gravity. Strain listed as “Hybrid” is the HDT18 interspecies hybrid of *S. bayanus* × *S. cerevisiae* from LeSaffre R&D. US-05 analytical control is listed with standard deviation.

Species	<i>S. cerevisiae</i>	<i>S. pastorianus</i>	<i>S. kudriavzevii</i>	<i>S. paradoxus</i>	<i>S. mikatae</i>	<i>S. bayanus</i>	<i>S. uvarum</i>	Hybrid
Code Name	US-05 *	W 34/70	11-515	01-161	11-510	01-135	11-512	HD T18
<i>Non-Hopped</i>								
O.G. (°P)	10.3 ± 0.6	10.4	10.0	10.1	10.1	10.3	10.4	9.80
F.G. (°P)	1.99 ± 0.36	1.84	1.93	3.43	8.75	2.10	3.33	3.32
ABV (%v/v)	4.44 ± 0.19	4.45	4.26	3.78	0.95	4.51	3.74	3.56
RDF (%)	66.6 ± 1.63	67.5	66.4	55.9	14.2	66.1	56.2	55.4
Cal (kJ/100 mL)	156 ± 10.6	154	150	160	163	160	157	152
Final pH	4.36 ± 0.06	4.42	4.24	4.45	4.60	4.31	4.48	4.16
Viability (%)	**	**	80.7 ± 2.4	97.1 ± 0.8	99.0 ± 0.5	83.7 ± 1.9	81.6 ± 4.5	**
Ferm. Length (days)	8.33 ± 0.58	6	13	10	8	6	15	9
<i>Dry-Hopped</i>								
O.G. (°P)	10.3 ± 0.6	10.4	10.0	10.1	10.1	10.3	10.4	9.80
F.G. (°P)	1.69 ± 0.42	1.57	1.79	3.44	8.96	1.64	3.39	3.09
ABV (%v/v)	4.69 ± 0.20	4.76	4.43	3.91	0.92	4.85	3.86	3.91
RDF (%)	69.2 ± 2.19	70.0	67.8	56.5	13.5	69.8	56.5	58.2
Cal (kJ/100 mL)	159 ± 11.5	159	153	163	165	162	161	158
Final pH	4.64 ± 0.02	4.70	4.47	4.68	4.75	4.47	4.55	4.45
Ferm. Length (days)	10.0 ± 1.0	8	13	11	9	8	11	10

\* indicates the mean of the three biological replicates; \*\* data not recorded.

Differences between the average values for alcohol, calories, pH, and RDF when comparing dry-hopped and non-hopped fermentations for all yeast species were highly significant ( $p < 0.05$ ). Dry-hopping has been shown to biochemically change the composition of wort during fermentation, allowing yeast access to a greater amount of fermentable sugars and subsequent additional fermentative capacity, a phenomenon known as hop creep [80–84]. A previous study was performed to determine if a yeast species or strain could be used to diminish the biochemical change of hop creep observed during fermentation with dry-hops [65]. Most of the novel yeasts shown here show no ability to mitigate the hop creep phenomenon in an effective manner, as all yeasts, with the exception of *S. mikatae* UCDFST 11-510, showed increases in RDF (Table 3) and alcohol (Figure 3) from the addition of dry-hops during fermentation.

Fermentation dynamics were grouped more closely in the dry-hopped fermentations compared to the non-hopped treatment (Figure 3), with *S. bayanus* UCDFST 01-135 showing the most similar fermentation profile to both of the control strains, and *S. kudriavzevii* UCDFST 11-515, *S. paradoxus* UCDFST 01-161, and *S. uvarum* UCDFST 11-512 showing slower, yet steady fermentation. *S. paradoxus* UCDFST 01-161 showed decreased dynamics with the addition of dry-hops (Figure 3b), but was still a slower fermenter than the control strains in both treatments. The *S. cerevisiae* × *S. bayanus* hybrid HD T18 showed no change in dynamics with the addition of dry-hops, showing moderate and steady fermentative capacity, with a terminal RDF similar to *S. paradoxus* UCDFST 01-161 and *S. uvarum* UCDFST 11-512. The *S. cerevisiae* × *S. bayanus* hybrid HD T18 fermented to a lower relative alcohol content than these other strains due to it starting from a brew with the lowest O.G.



**Figure 3.** Alcohol content by volume measured daily on the Anton Paar Alcolyzer Beer M, as reported for both (a) non-hopped and (b) dry-hopped fermentations of all yeasts in this study. Results for US-05 are reported as the mean of three biological replicates with error bars for standard deviation at each day of fermentation.

Of note is the strain UCDFST 11-510, *S. mikatae*, as it was an outlier from the group with the lowest RDF (Table 3) and final amount of alcohol produced, whether dry-hopped or not (Figure 3). UCDFST 11-510 recorded  $99.0 \pm 0.5\%$  yeast viability in suspension at the end of fermentation, yet only 14.2% RDF in the non-hopped treatment. This indicates the strain is a potential candidate for low- or no-alcohol beer fermentations if brewing parameters are adjusted to get the final alcohol below 0.5% (*v/v*) and considerations are taken for microbial stability. Analysis of the sugars remaining in this beer may aid in determining

which carbohydrates this *S. mikatae* strain was able to assimilate during fermentation and should be performed with future research. Additionally, this species has been shown to form a pellicle on top of fermenting beer after twenty-five days at 20 °C [43], suggesting that it may ferment comparably slowly as wild-type yeasts, such as *Brettanomyces* or *Hanseniaspora* spp. Further research regarding *S. mikatae* in fermentation for the production of low- and no-alcohol beers should be performed.

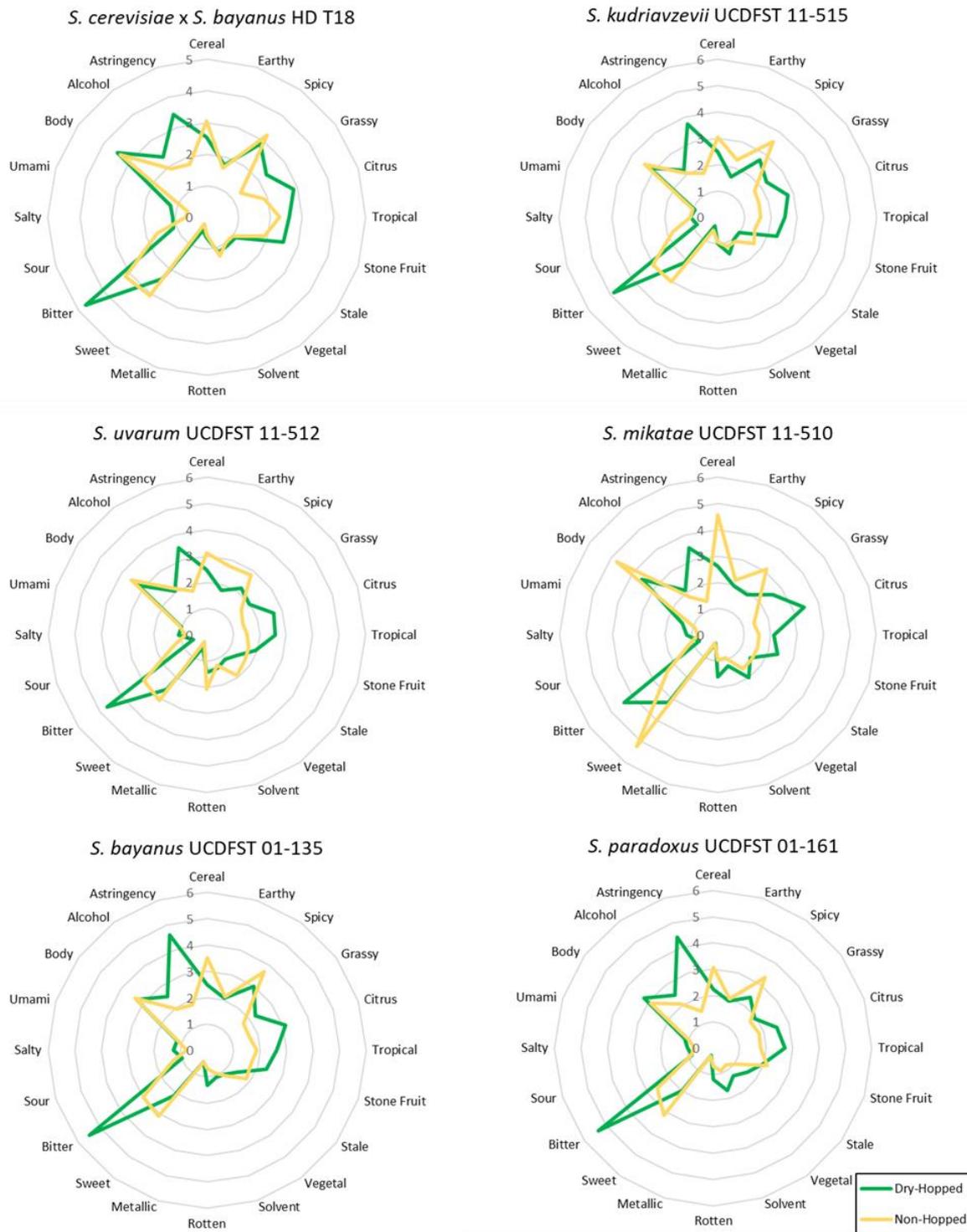
### 3.2. Sensory Analysis

The flavor of the beers from these fermentations was investigated for aroma, taste, and mouthfeel in order to further qualify the brewing potential of these non-conventional *Saccharomyces* yeasts. *S. cerevisiae* and *S. pastorianus* controls fermentations were not included for sensory analysis due to their ubiquity in the brewing industry and known flavor potential for craft beer production. Modified consensus method with CATA from the UC Davis Brewing and Malting Science lab members yielded twelve aroma and three mouthfeel descriptors that were deemed most discriminant and non-redundant from the Beer Flavor Map© as provided by DraughtLab©. The most commonly agreed upon descriptors included Cereal, Earthy, Spicy, Grassy, Citrus, Tropical, Stone Fruit, Stale, Vegetal, Solvent, Rotten, and Metallic for aroma, with additional descriptors within each aroma category outlined above (Table 2). DraughtLab© sensory software (version 3.18.0, DraughtLab, LLC: Webster, NY, USA, 2021) was used to confirm that statistically significant differences were observed for all of the consensus CATA terms after accounting for both panelist and replication effects. Body, Alcohol, and Astringency were selected as the most common mouthfeel descriptors.

From the panelists at participating breweries, all beers showed increases in bitterness and astringency from the high level of dry-hopping (Figure 4), suggesting that beer clarification prior to packaging may have been necessary to fully distinguish the effects of the hops without particulates in suspension effecting flavor. The base beer was also of relatively low alcohol (0.9 to 4.5% abv) and bitterness (20 calculated IBU) content, which could contribute to perceived bitterness from the increase in humulinones from dry-hopping a low IBU beer [85], or perceived astringency from the increase in polyphenol content [80]. Dry-hopping increased the fruit (Citrus, Tropical, and Stone Fruit) perception on all beers as expected from the Centennial cultivar used here [86], with the exception of Stone Fruit in UCDFST 01-161 *S. paradoxus*.

All experimental beers displayed Spicy aromas, likely from the expression of phenols, but genetic testing for the POF phenotype should be performed to confirm [87]. Interestingly, these Spicy aromas were perceived lower in the dry-hopped beers, in contrast to expectations, as resinous and spicy characteristics are also noted as aroma characteristics of Centennial hops. On average, many of the unique attributes perceived in the beers fermented with these yeasts can be generally considered as off-flavors in beer (Solvent, Metallic, Vegetal, Rotten, or Stale). Trained panelists perceived these descriptors in very low amounts, with no off-flavor characteristic achieving an average greater than 2 on the 9-point intensity scale. All attribute averages did not exceed a 6 on the 9 point scale and were thus displayed as such in Figure 4 for ease of analysis.

Other descriptors were written in on the ballot (Table 2) by the trained panelists at breweries. Beers made with *S. uvarum* UCDFST 11-512 commonly had notes of diacetyl in the dry-hopped treatment and sulfur in the non-hopped fermentation. Beers made with *S. kudriavzevii* UCDFST 11-515 were described as having distinct phenolic and sulfur characteristics in the non-hopped treatment. The non-hopped beer fermented with *S. mikatae* UCDFST 11-510 was perceived as being wort-like, likely due to its low attenuation. Write-in descriptors are presented here only if more than 10% of panelists ( $n = 5$ ) reported a given characteristic.



**Figure 4.** Radar charts of attributes for each experimental yeast fermentation in this study. Dry-hopped treatments are shown in green, while non-hopped are shown in yellow. ( $n = 51$ , with 36 male and 15 female).

#### 4. Conclusions

Fermentation dynamics and yeast viabilities here suggest that appropriate pitching rate, adequate nutrients, and proper wort aeration from the brewhouse were achieved on all brews and fermentations. All yeasts reached terminal gravity in under two weeks, with the exception of *S. uvarum* UCDFST 11-512, which took fifteen days for the non-hopped fermentation. These dynamics make all the yeasts studied viable candidates for production breweries; conditioning time should be accounted for but were not studied

here. All fermentations in this study were deemed terminal based on gravity as opposed to metabolite production like in most production breweries, so further analysis and brewer-specific standards are required before recommendations of full-scale production. All yeasts displayed high potential for re-pitching in a commercial setting with high viabilities at the end of fermentation in the non-hopped fermentations. These high numbers are promising, but viability should be assessed during fermentation and prior to re-pitch in order to ensure adequate cell count for vigorous growth in a commercial setting.

With the exception of *S. mikatae* UCDFST 11-510, all yeasts displayed increased RDF and alcohol with the addition of dry-hops during fermentation, as was expected due to hop creep. Further research should be pursued in the use of *S. mikatae* UCDFST 11-510 and other strains of this species for the production of low- and no-alcohol beers and its possible resistance to hop creep. Strong phenolic characteristics were perceived in the flavor of beers fermented with all non-conventional yeasts, but dry-hopping, in this case with Centennial, decreased this aroma while increasing all fruit aromas, as well as bitterness and astringency. No flavors that are generally associated with poor fermentation scored high among trained sensory panels. Comparisons to the standard control beer yeast fermentations should have been performed in sensory analysis as well, but experimental time constraints did not allow this. Previous research has shown these yeasts' ability to co-ferment with standard *S. cerevisiae*, and flavor analysis should also be performed on these potential combinations. All of these species displayed great brewing potential given a brewery's desire to experiment with flavor and willingness to bring in a new yeast.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/beverages7030068/s1>, Figure S1: The Beer Flavor Map<sup>®</sup>, as provided by DraughtLab<sup>®</sup>, which outlines the flavor descriptors common to beer and was used to determine terms for consensus method and subsequent descriptive analysis. Figure S2: The average of the standard brew day analytical parameters documented in this experiment, with error bars representing standard deviation.

**Author Contributions:** J.B. conceived this study, performed the bulk of the research, gathered and transcribed data, and wrote the original manuscript. A.M. assisted in the brewing of beer and brew day sample collection, data curation with figure manipulation and statistics, and assisted with the final editing of the manuscript. G.F. supervised the work, offered insight, and assisted with final editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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