Analyzing the minor volatilome of *Torulaspora delbrueckii* in an alcoholic fermentation

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Abstract

Torulaspora delbrueckii is an emerging yeast species in the beverage and food industry that is suitable for alcoholic fermentation and to improve the organoleptic quality of wine, beer, mead, and other beverages. Modern consumer preference toward new flavors and products drives the application of *T. delbrueckii* to ferment less traditional fruits and vegetables. Thus, it has become increasingly relevant to define those metabolites produced in minute quantities by *T. delbrueckii*, because they may have an impact when producing these new alcoholic beverages. In this study, we have identified metabolites of *T. delbrueckii* and have compared them with those of *Saccharomyces cerevisiae* in a controlled setting with a synthetic, high glucose medium using gas chromatography coupled to flame ionization detector (GC–FID) and stir bar sorptive extraction (SBSE) with GC coupled to mass spectrometry (MS). Results showed that *T. delbrueckii* produced metabolites with higher changes in odor activity complexes than *S. cerevisiae*: ethyl propanoate, 1,1-diethoxyethane, ethyl isobutyrate, ethyl butyrate, isoamyl acetate, ethyl heptanoate, nonanal, and decanal. We also report seven metabolites detected for the first time in *T. delbrueckii*. This datum serves to expand the knowledge of *T. delbrueckii* performance and shows that application of this yeast species is more suitable to a wide array of beverage producers.

Keywords *Torulaspora delbrueckii* · *Saccharomyces cerevisiae* · Metabolites · Volatile compounds · Fermentation

Abbreviations

ANOVA	Analysis of variance
EI	Electron impact
GC–FID	Gas Chromatography–Flame Ionization Detector
GC/MS	Gas Chromatography–Mass Spectrometry
HG	Homogenous groups
LRI	Linear retention indices
OAV	Odor Activity Value
OIV	International Organisation of Vine and Wine
PCA	Principle Component Analysis
PDMS	Polydimethylsiloxane
SBSE–TD–GC–MS	Stir Bar Sorptive Extraction–Thermal Desorption–Gas Chromatography–Mass Spectrometry
YPD	Yeast extract Peptone Dextrose
YAN	Yeast Assimilable Nitrogen
YNB	Yeast Nitrogen Base

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Introduction

Non-*Saccharomyces* yeast species have gained steady interest in the production of alcoholic beverages due to their contributions of increasing aromatic complexity and improving organoleptic properties. Naturally found on the skins of fruits, these indigenous yeasts refer to those species that have positive roles in beverage production, excluding *S. cerevisiae* [1]. Although non-*Saccharomyces* were once considered spoilage organisms, this belief has now changed and considers that these yeasts are fundamental for spontaneous fermentations, especially in winemaking where they are thought to be integral to determining the authenticity of natural wines by adding mouthfeel and improved flavor integration [2]. In addition, non-*Saccharomyces* yeasts have been utilized to obtain alcoholic beverage with lower ethanol yields to match the consumer trends of low alcohol drinks [3–10].

Out of the non-*Saccharomyces* yeasts, *Torulaspora delbrueckii* is considered the most common, commercially available yeast species. It is the most similar to *S. cerevisiae* in features required for alcoholic fermentation, making it an ideal candidate to be employed for production of a variety of beverages such as: wine, beer, mead, mescal, tequila, cider, etc. [11–16]. Thus far, *T. delbrueckii* has been utilized to ferment products made from uncommon fruits such as lychee, strawberry tree fruits, raspberries, etc. [17–20]. Depending on the strain, *T. delbrueckii* can ferment up to an ethanol concentration of 10% (v/v) while providing pleasant aromatic properties (fruity esters, lactones, thiols, and terpenes) as well as decreasing undesired compounds (higher alcohols) [20]. For beer, *T. delbrueckii* increases isoamyl alcohol and ethyl butanoate, as well as produced terpenes are absent in wort such as beta-caryophyllene and geranyl acetone [14, 21]. For mead production, *T. delbrueckii* has been proposed to be suitable for producing a sweet type of multifloral honey mead with low ethanol and high residual sugar content [13].

Much more is known about the use of *T. delbrueckii* in winemaking and the effect it has on final wine products. Their benefits have been suggested to solve current oenological challenges, where pure cultures of *T. delbrueckii* have been shown to produce lower levels of volatile acidity in high-sugar fermentations and less undesirable compounds like hydrogen sulfide and volatile phenols with respect to *S. cerevisiae* [22, 23]. They also contribute to mouthfeel, by producing more glycerol, polysaccharide, and mannoproteins and less toxic medium-chain fatty acids, resulting in a fuller and higher pallet weight [2, 24]. The use of *T. delbrueckii* has also been proposed to mitigate near future winemaking risks due to climate change. They contribute to the development of natural,

climate-smart wine systems that aim to prepare for problems such as increased proliferation of unwanted microorganisms and unintentional imbalance of sensory properties, and excess sugar in grapes and therefore elevated ethanol content [25, 26]. In this study, we focus on a strain of *T. delbrueckii* isolated from Montilla-Moriles in southern Spain because of its unique ability to ferment high concentrations of sugar and its significance and importance to this region.

While many reports have been made on the benefits of the use of *T. delbrueckii* for wine and beer, *production of these results* differs based on a variety of uncontrolled environmental factors such as the composition of the starting must or wort [20]. This can be distracting for producers looking to use *T. delbrueckii* to ferment their own fruits or vegetables different from traditional grapes or grains. In addition, there exists the possibility that *T. delbrueckii* may produce other metabolites in lower concentrations that have not yet been detected, but are able to influence positively or negatively the overall flavor and aroma of alcoholic beverages. Oftentimes, individual compounds that are linked to producing the aroma and flavor or off-flavor exist in concentrations ranging from ng/L (ppt) to mg/L (ppm) [27]. Analysis methods such as stir bar sorptive extraction (SBSE) coupled with gas chromatography/mass spectrometry (GC/MS) allows for a higher sensitive and improved compound identity system than conventional methodologies [28–30]. To focus on those compounds metabolized by *T. delbrueckii* that could influence sensory properties, we have focused on the volatile compounds secreted by *T. delbrueckii* and compare them with *Saccharomyces cerevisiae* under a controlled setting in synthetic, high glucose media using GC–FID and SBSE with GC–MS. The aim is to identify these compounds and uncover the potential of *T. delbrueckii* without the influence of uncontrolled environmental factors, so that the use of this species in modern production of alcoholic beverages can be improved. This unbiased data will be informative to producers of alcoholic beverages, including winemakers and brewers, so that they are able to make informative decisions on the use of *T. delbrueckii* to fulfill their individual fermentation conditions and improve their product quality.

Materials and methods

Microorganisms and experimental conditions

Two yeasts were used for this study: *S. cerevisiae* (ATCC-MYA-2451) as the control and *T. delbrueckii* (ATCC-MYA-426) as the experimental strain. Both originate from the Montilla-Moriles region in Spain and are from the yeast collection in the Department of Agricultural Chemistry, Edaphology and Microbiology at the University of Cordoba,

Spain. The strain of *T. delbrueckii* was selected for its unique ability to ferment high concentrations of sugar and its significance and importance to this region. The yeasts were separately pre-inoculated in YPD liquid (1% yeast extract, 2% peptone, and 2% glucose) for 24 h. Next, 1×10^6 cells/mL were inoculated in 250 mL flasks with 150 mL of sterile synthetic fermentation media supplemented with tartaric acid until pH was 3.1 (250 g/L glucose, 0.67% YNB without amino acids, titratable acidity 0.99 g/L, expressed as tartaric acid content and YAN 0.106 g/L). The high glucose media were chosen to mimic the high-sugar content of raw materials, such as grape must grow in warm grape growing regions of Southern Europe and the media volume to head-space ratio has been seen to induce desirable fermentation without oxidation, because oxygen is depleted by yeasts in the first 12 h of fermentation [31]. The flasks were closed with hydrophobic cotton and incubated at 28 °C with mild agitation (~100 rpm) to ensure homogenization of solutes for 10 days when the non-*Saccharomyces* are more metabolically active [32]. Each condition was performed in triplicate.

Growth rate and fermentation rate

Yeast cell growth was monitored every 2 h until the stationary phase was achieved by measuring growth at OD580 using the Beckman Coulter™ DU 640 Spectrophotometer. Yeast cells range from 5 to 10 µm in size [20, 33]. Fermentation was tracked by monitoring the CO₂ release as a measure of weight loss until constant [34].

Metabolome analysis

At the beginning (day 0) and end (day 10) period from the growth medium of yeast metabolism during fermentation, 35 mL of sample was taken and yeast cells were removed by centrifugation at 5000 rpm, 22 °C for 10 min (Thermo Scientific TX-400 Rotor). The supernatant was then analyzed for metabolites. Fermentable sugars, titratable acidity, volatile acidity, pH, and ethanol were also measured by the methods of the International Organisation of Vine and Wine [35]. Fixed acidity was calculated by equivalents of tartaric acid expressed in Table 1. Metabolites related to the sensory properties of wine and fermented beverages, such as major volatiles and polyols, were identified and quantified by gas chromatography (GC) (model 6890 gas chromatograph from Agilent, Palo Alto, CA, USA) with a CP-WAX 57-CB capillary column (60 m long $\times$ 0.25 mm i.d., 0.4 µm film thickness) and a flame ionization detector (FID). Each injection contained 0.5 µL aliquots of liquid sample. Further details of the metabolite procedure are outlined in [36].

Volatile compounds in a concentration of less than 10 mg/L (ppm) were analyzed using SBSE-TD-GC-MS (Stir Bar Sorptive Extraction–Thermal Desorption–Gas

Chromatography–Mass Spectrometry). The GC-7890A chromatograph with MSD 5975 detector from Agilent Technologies (Wilmington, DE, USA) and a Multi-Purpose-Sampler from Gerstel (Mülheim an der Ruhr, Germany) all utilized the software of Chemstation (Agilent Technologies) and Maestro (Gerstel). The compounds were extracted by SBSE with a Twister coated with 10 mm long, 0.5 mm-thick film of polydimethylsiloxane (PDMS). Next, method outlined in (Tredoux et al., 2008), 10 mL vial was filled with 5 mL of sample and 0.1 mL of internal standard solution (0.4464 mg/L ethyl nonanoate in pure ethanol) plus 12% (v/v) ethanol adjusted to pH 3.5, 2.6 g/L tartaric acid, and 2.2 g/L potassium bitartrate to a final volume of 10 mL. The Twister was put into this vial and stirred at 1200 rpm at 20 °C for 100 min on a Variomag Multipoint 15 magnetic stirrer from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). After the cycle was complete, the Twister was removed, rinsed, and dried, and then placed in a Thermal Desorption Unit in a desorption tube from Gerstel. Compounds were thermally desorbed at an initial temperature of 35 °C for 0.1 s and increased to 280 °C at 120 °C/min for 10 min followed by a helium stream at 16 mL/min in splitless mode into a Cooled Injection System (CIS-4) from Agilent Technologies furnished with an inlet liner packed with Tenax® (3 $\times$ 2 mm). The CIS-4 had the following program: 25 °C (hold 0.05 s), 12 °C/s ramp to 280 °C (7 min); 16 mL/min helium inlet flow. The 7890 GC instrument was set with an HP-5MS-fused silica capillary column (30 m $\times$ 0.25 mm i.d., 0.25 µm film) from Agilent Technologies (Wilmington, DE, USA). Initial oven temperature was set to 50 °C (2 min) and then raised 4 °C/min to 190 °C (10 min). The MSD was set at 70 eV in the electron impact mode (EI), with the mass range at 35–550 Da at 150 °C. All samples were measured in triplicate. Identification of compounds was conducted by comparing peaks of aroma compounds with mass spectra in the NIST08 and Wiley7 libraries. Linear retention indices (LRI) for standards compiled in the NIST Web Book of Chemistry (2014) were used to confirm the compounds. Next, quantification was accomplished based on response factors, where the standard solutions of each compound with known concentration were subjected to the same procedures as the samples and using the target and qualifier ions from their respective mass spectra from the Chemstation.

Statistical analysis

Statistical analysis was performed using the software package Statgraphics® Centurion XVI from Stat Points Technologies, Inc. (Warrenton, Virginia, USA) for the following methods: ANOVA (Analysis Of Variance) and Fisher test for the establishment of Homogeneous Groups (HG) at a significance level $p \leq 0.05$ and Principal Component Analysis

Table 1 Concentration differences of metabolites detected in media fermented with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*

Parameters/sample	<i>Saccharomyces cerevisiae</i> (flor yeast)				<i>Torulaspora delbrueckii</i>			
	$\Delta[T1 - T0]$		$\Delta[T1 - T0]$		$\Delta[T1 - T0]$		$\Delta[T1 - T0]$	
	Avg	SD	HG	OA	Avg	SD	HG	OA
Library/ID	Method of detection	CAS	OT (mg/L)	Odor/flavor description	Avg	SD	HG	OA
Ethanol (g/L)	Parameters measured according to OIV	64-17-5	10	Alcoholic	52.15	0.20	b	5215.00
Volatile acidity (acetic acid) (g/L)		64-19-7	200	Vinegar	2.32	0.06	b	11.60
Titrate acid (g/L)					2.77	0.06	b	–
pH					-0.78	0.03	a	–
Fixed acidity (equiv/L)					0.00	0.00	a	–
Glucose (g/L)		50-99-7			-115.00	11.00	b	–
Acetaldehyde (mg/L)		75-07-0	10	Over-ripe apple	375.30	15.00	b	37.53
Ethyl acetate (mg/L)		141-78-6	7.5	Pineapple, varnish, balsamic	36.00	1.00	b	4.80
Methanol (mg/L)		67-56-1	668	Chemical, medicinal	3.20	0.80	a	0.00
1-Propanol (mg/L)		71-23-8	830	Ripe fruit, alcohol	31.84	0.90	b	0.04
Isobutanol (mg/L)	GC-FID	78-83-1	40	Alcohol, wine like, nail polish	22.60	0.20	b	0.57
2-Methylbutanol (mg/L)		137-32-6	N.f	N.f	18.46	0.15	b	–
Isoamyl alcohol (mg/L)		123-51-3	30	Alcohol, nail polish	22.00	0.20	b	0.73
Acetoin (mg/L)		53,584-56-8	30	Buttery, cream	1742.00	124.00	b	58.07
Ethyl lactate (mg/L)		97-64-3	100	Strawberry, raspberry, buttery	35.00	4.00	b	0.35
Diethyl succinate (mg/L)		123-25-1	100	Over-ripe, lavender	3.40	0.40	b	0.03
2-Phenylethanol (mg/L)		60-12-8	10	N.f	3.60	1.50	b	0.36
2,3-Butanediol I (mg/L)		24,347-58-8	668	Buttery, creamy	1177.00	108.00	b	1.76
2,3-Butanediol m (mg/L)		5341-95-7	668	Buttery, creamy	413.00	38.00	b	0.62
2,3-Butanediol m+1 (mg/L)					3714.00	321.00	b	–
Glycerol (g/L)		56-81-5		Confers body and smoothness and a sweet taste	17.50	1.85	b	–

Table 1 (continued)

Library/ID	Parameters/sample	Method of detection	CAS	OT (mg/L)	Odor/flavor description	<i>Saccharomyces cerevisiae</i> (flor yeast) $\Delta[T1 - T0]$				<i>Torulaspora delbrueckii</i> $\Delta[T1 - T0]$			
						Avg	SD	HG	OAV	Avg	SD	HG	OAV
Ethyl propanoate (mg/L)		SBSE-GCMS	105-37-3	0.055	Sweet, fruity, pineapple	27.88	4.54	b	506.91	18.31	0.22	a	332.91
1,1-Diethoxyethane (mg/L)			105-57-7	1	Green fruit, liquorice	25.61	2.22	b	25.61	12.19	0.58	a	12.19
Ethyl isobutyrate (mg/L)			97-62-1	0.015	Sweet, rubber	0.46	0.05	a	30.67	3.33	1.45	b	96.67
Ethyl butyrate (mg/L)			105-54-4	0.02	Fruity, sweet, apple	1.72	0.20	a	86.00	10.00	3.05	b	152.50
Butyl acetate (mg/L)			123-86-4	4.6	Banana, ripe pear, glue	0.02	0.01	a	0.00	0.00	0.00	b	0.01
Furfural (mg/L)			98-01-1	0.77	Burn almond, incense, floral	0.00	0.01	a	0.00	0.01	0.15	b	0.19
Hexanol (mg/L)			111-27-3	2.5	Grass, resinous, cream	9.60	0.86	b	3.84	0.34	0.13	a	0.05
Isoamyl acetate (mg/L)			123-92-2	0.03	Banana	1.36	0.14	b	45.33	4.67	0.57	a	19.00
2(5H)-furanone (mg/L)			497-23-4	1	Toasty, caramel	0.04	0.01	a	0.04	0.01	0.17	b	0.17
Benzaldehyde (mg/L)			100-52-7	5	Bitter almond, nutty, smoky	0.08	0.00	a	0.02	0.00	0.08	a	0.02
Ethyl hexanoate (mg/L)			123-66-0	0.005	Banana, green apple	0.00	0.00	a	0.00	0.00	0.00	b	0.00
Limonene (mg/L)			138-86-3	0.01	N.f	0.01	0.00	a	1.00	0.00	0.01	a	1.00
Ethyl heptanoate (mg/L)			106-30-9	0.0022	Fruity, pineapple, sweet, banana	0.03	0.00	a	13.64	0.00	0.04	b	18.18
Nonanal (mg/L)			124-19-6	0.0011	Floral, orange-rose odor or waxy and green	0.00	0.00	a	0.00	0.00	0.01	b	9.09
2-Phenylethanol (mg/L)			60-12-8	N.f	N.f	3.41	0.18	a	–	23.33	1.04	b	–
Diethyl succinate (mg/L)			123-25-1	100	Over-ripe, lavender	2.25	0.18	b	0.02	0.00	0.02	a	0.00
Ethyl octanoate (mg/L)			106-32-1	0.005	Pineapple, pear, soapy	0.01	0.00	b	2.00	0.00	0.00	a	0.00
Decanal (mg/L)			112-31-2	0.0005	N.f	0.07	0.00	b	140.00	0.00	0.06	a	120.00
Geraniol (mg/L)			106-24-1	0.0575	N.f	0.01	0.00	a	0.17	0.00	0.02	b	0.35
2-Phenethyl acetate (mg/L)			103-45-7	0.25	Fruity, rose, sweet, honey	0.05	0.01	a	0.20	0.04	0.18	b	0.72
Decanoic acid (mg/L)			334-48-5	1	Rancid, waxy	0.01	0.00	a	0.01	0.00	0.04	b	0.04
Ethyl decanoate (mg/L)			110-38-3	1.1	Sweet, fruity, nuts and dried fruit	0.00	0.00	a	0.00	0.00	0.00	a	0.00
Dodecanoic acid (mg/L)			0143-07-07	10	Waxy, soapy	0.00	0.00	a	0.00	0.00	0.01	b	0.00
Palmitic acid (mg/L)			57-10-3	10	N.f	0.01	0.00	b	0.00	0.00	0.01	a	0.00

Odorant descriptor and odor threshold were taken from Zea et al. [57], Welke et al. [63], Peinado et al. [64], Ruiz et al. [65], Katarina et al. [66], and www.leffingwell.com.

GC–FID Gas Chromatography–Flame Ionization Detector, HG Homogenous groups, N.f. not found in the bibliography, OAV Odor Activity Value, OIV International Organisation of Vine and Wine, OT Odor Threshold, SBSE–TD–GC–MS Stir Bar Sorptive Extraction–Thermal Desorption–Gas Chromatography–Mass Spectrometry, SD Standard Deviation, T0 metabolite concentration at before fermentation, T1 metabolite concentration after 10 days of fermentation

Fig. 1 Growth rate of *Saccharomyces cerevisiae* (blue) and *Torulaspora delbrueckii* (orange)

Fig. 2 Fermentation kinetics of *Saccharomyces cerevisiae* (blue) and *Torulaspora delbrueckii* (orange)

(PCA). Sunray plots were made by Statgraphics® Centurion XVI, while PCAs were made using the software PAST [37].

Results and discussion

The growth pattern of *T. delbrueckii* exhibited an initial start later than *S. cerevisiae*, where the former culture had a lag phase of 21 h and the latter culture of only 8 h (Fig. 1). Once the exponential phase was reached, the growth rate appeared to be similar, and both reached the same final cell concentration of around 48 h. Interestingly, fermentation kinetics displayed different patterns between the two yeasts where *T. delbrueckii* fermented at a much more rapid rate than *S. cerevisiae*, reaching a peak of over 13 g/day in little over 24 h (Fig. 2). After 1.5 days, there appeared to be no more CO₂ significant release with *T. delbrueckii*. *S. cerevisiae* peaked later at around day 2, but the CO₂ release was less than two-thirds (4 g/day) of *T. delbrueckii*. Fermentation rate continued at a gradual pace until the end of day 6. *T. delbrueckii* has been reported to have higher specific rates

of CO₂ release and O₂ consumption than *S. cerevisiae* [20, 36], matching results of this study.

When observing total glucose consumption and product yield, *T. delbrueckii* consumed less glucose (84 g/L) than *S. cerevisiae* (137 g/L) and produced less ethanol 39 g/L vs 52 g/L, respectively (Fig. 3). Glucose consumption is within the range reported in the previous literature [13, 38]. However, if the product yield was expressed as a percentage (m/m), *T. delbrueckii* converted 46% of the consumed glucose to ethanol, whereas *S. cerevisiae* converted 45% of the glucose. While both yeasts have similar glucose to ethanol metabolizing efficiency, *S. cerevisiae* may be able to produce more ethanol, because it is able to switch to respiro-fermentative metabolism faster than *T. delbrueckii* during decreasing the oxygen feed rate conditions [39]. *T. delbrueckii* is also known to be sensitive to a low oxygen content, and perhaps, this stress could impact the rate of glucose uptake unlike *S. cerevisiae* [40, 41].

Metabolism differences between *T. delbrueckii* and *S. cerevisiae*

Within the parameters measured according to OIV (2012), five out of the six were significantly different (p value ≤ 0.05) between the two yeasts, except for the pH. Significant pH drops were also observed in the previous studies using these strains [13, 42, 43]. In *S. cerevisiae*, more glucose was consumed and more ethanol produced compared to *T. delbrueckii* (Fig. 3). These match with the previous reports, where *T. delbrueckii* consistently produced lower ethanol concentration compared to traditional fermentations [44]. Likewise, volatile acidity was significantly lower in *T. delbrueckii*, 1/5 of the amount produced by *S. cerevisiae*, which also lines up with the previous reports [8, 34, 45]. *T. delbrueckii* has the tendency to not produce excessive acetic acid in response to hyper-osmotic conditions, unlike *S. cerevisiae*. This feature is especially attractive in winemaking, because the volatile acidity concentration is often typically tied to the measure of wine quality, where concentrations above 0.8 g/L are considered higher than normal and can lead to a vinegar-like odor [2].

Fourteen major volatile compounds were identified and quantified by GC–FID: acetaldehyde, ethyl acetate, methanol, 1-propanol, isobutanol, 2-methylbutanol, isoamyl alcohol, acetoin, ethyl lactate, diethyl succinate, 2-phenylethanol, 2,3-butanediol 1 + m, and glycerol. All metabolites presented significant changes (p value ≤ 0.05) between strains and were produced in higher quantities by *S. cerevisiae* than *T. delbrueckii* except for methanol (Table 1) and match with those of previous studies [46]. The sunray plot (Fig. 4B) visually represents unique differences in the volatile footprint where the end of the ray is the mean value plus three standard deviations and the origin is the mean

Fig. 3 Glucose consumed by yeasts *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* (center circle) and percentage of glucose metabolized into the measured parameters (outer circle)

Fig. 4 Principal component analysis (A) and Sunray Plots (B) of compounds quantified by the GC–FID from the metabolism of *Saccharomyces cerevisiae* (blue) and *Torulaspora delbrueckii* (orange). 1: acetaldehyde; 2: ethyl acetate; 3: methanol; 4: 1-propanol; 5: isobutanol; 6: 2-methylbutanol; 7: isoamyl alcohol; 8: acetoin; 9: ethyl lactate; 10: diethyl succinate; 11: 2-phenylethanol; 12: 2,3-butanediol l; 13: 2,3-butanediol m; 14: 2,3-butanediol 1+ m; 15: glycerol

minus three standard deviations. This plot shows that *T. delbrueckii* is distinctly different from *S. cerevisiae*, because the methanol sharply juts out. The PCA (PC1 96.50% and PC2

2.26%), also reflects a similar pattern, where methanol is the only variable on the left side of the y-axis, closest to the *T. delbrueckii* cluster, while all other variables project over

the right side of the y-axis toward *S. cerevisiae* (Fig. 4A). Methanol is an alcohol that is scarcely produced by yeasts [13]. Synthesis occurs during the reversible demethylation of methyl esters by a carboxyl methyl esterase [47, 48]. The odor of methanol is pungent and is described to be alcoholic odor when pure [49]. However in this study, the concentration of methanol produced by *T. delbrueckii* was well below its detection threshold of 668 mg/L, at 10 mg/L.

Twenty-four total minor volatile metabolites were identified, and out of these, 20 were significantly different (p value ≤ 0.05) (Table 1). The metabolites are represented on a PCA (PC1 83.41% and PC2 10.03%), where decanal, palmitic acid, diethyl succinate, ethyl octanoate, 1,1-diethoxyethane, ethyl propanoate, hexanol, and isoamyl acetate project over the left side of the y-axis, closest to the cluster of *S. cerevisiae*. Ethyl isobutyrate, ethyl butyrate, butyl acetate, furfural, 2(5H)-furanone, benzaldehyde, ethyl hexanoate, limonene, ethyl heptanoate, nonanal, 2-phenylethanol, geraniol, 2-phenethyl acetate, decanoic acid, ethyl decanoate, and dodecanoic acid projected on the right side of the y-axis, closest to the cluster of *T. delbrueckii* (Fig. 5A). These same metabolites that projected toward the right side of the y-axis in the PCA were also distinctive in determining the profile of *T. delbrueckii* in the sunray plot (Fig. 5B). Those compounds that had the most impact on aroma profile are discussed in greater depth in the following section.

Compounds affecting aroma

Assessment of the potential impact of these volatile compounds on aroma can be determined by the Odor Activity

Value (OAV). The OAV is calculated as the concentration: odor threshold ratio, which allows an objective comparison of volatile compounds that could influence the aroma of beverages. Figure 6 shows the OAV changes of metabolites produced by *T. delbrueckii* and *S. cerevisiae*, where the larger the OAV, the greater the impact of aroma of that compound. The compounds produced by *T. delbrueckii* that were the greatest contributors to aroma are ethanol (OAV = 3863.00), ethyl propanoate (332.91), ethyl butyrate (152.50), decanal (120.00), ethyl isobutyrate (96.67), isoamyl acetate (19.00), ethyl heptanoate (18.18), 1,1-diethoxyethane (12.19) and nonanal (9.09), acetaldehyde (5.00), ethyl acetate (1.33), acetoin (2.33), and limonene (1.00).

Out of these compounds, *T. delbrueckii* presents a greater change in OAV compared to *S. cerevisiae* for ethyl isobutyrate, ethyl butyrate, ethyl heptanoate, and nonanal. Each metabolite is associated with an aroma descriptor which could contribute to providing a unique character to the medium. Ethyl isobutyrate is reminiscent of a sweet and rubber smell. Ethyl butyrate is characterized to have fruity, sweet, and apple like aromas and pineapple tastes [50]. Ethyl heptanoate is described to contribute aromas that are fruity, pineapple, sweet, and banana. Nonanal is associated with floral, orange-rose odor or waxy and green [51]. Overall, the compounds with higher OAV in *T. delbrueckii* are sweet or fruity, which suggests that the final product when fermented with *T. delbrueckii* will most likely reflect these aromas.

Fig. 5 Principal component analysis (A) and Sunray Plots (B) of compounds quantified by the SBSE–GC–MSD from the metabolism of *Saccharomyces cerevisiae* (blue) and *Torulaspora delbrueckii* (orange). 1: ethyl propanoate; 2: 1,1-diethoxyethane; 3: ethyl isobutyrate; 4: ethyl butyrate; 5: butyl acetate; 6: furfural; 7: hexanol; 8:

isoamyl acetate; 9: 2(5H)-furanone; 10: benzaldehyde; 11: ethyl hexanoate; 12: limonene; 13: ethyl heptanoate; 14: nonanal; 15: 2-phenylethanol; 16: diethyl succinate; 17: ethyl octanoate; 18: decanal; 19: geraniol; 20: 2-phenethyl acetate; 21: decanoic acid; 22: ethyl decanoate; 23: dodecanoic acid; 24: palmitic acid

Fig. 6 Odor activity values (OAV) of compounds anabolized by *Saccharomyces cerevisiae* (blue) and *Torulaspora delbrueckii* (orange)

Compounds identified for the first time in *T. delbrueckii*

While all compounds mentioned in this study have been reported in *S. cerevisiae*, this study has reported metabolites, identified as far as our knowledge, for the first time by *T. delbrueckii*. These metabolites are: 1,1-diethoxyethane, 2(5H)-furanone, limonene, ethyl heptanoate, dodecanoic acid, and palmitic acid. The metabolite found in the highest concentration in this group from this study was 1,1-diethoxyethane at 12.19 mg/L with a significant OAV change that would impact aroma. This metabolite is known to be a key factor in giving sherry wine and its unique characteristic aroma [52–56]. Standing alone, 1,1-diethoxyethane has an active odorant profile of green fruit and liquorice, as well as balsamic notes [54, 56]. Zea, Serratos, Mérida, and Moyano [57] claimed that acetals combined with other alcohols produce strong woody notes; however, Etiévant [58] described 1,1-diethoxyethane as the only acetal that can contribute to wine aroma.

Most of the other compounds that were identified in *T. delbrueckii* were not present in high amounts to evoke strong impacts on the sensory property; however, many have added benefits apart from an aroma profile. For example, dodecanoic acid and palmitic acid are fatty acids that have antimicrobial properties. Especially, dodecanoic acid, or lauric acid, can be converted to monolaurin, which is able to destroy the lipid membrane of bacteria, making it a natural food preservative [59]. Another metabolite is

2(5H)-furanone, which belongs to the furanone subclass of organic compounds and has an easily oxidized nature that allows them to be important signaling molecules. The furanones have an important role as a pharmaceutical for their anti-carcinogenic activity as well as their antioxidant quality that is comparable to ascorbic acid [60]. Furanones are produced by yeasts are thought to be an indirect metabolite through a spontaneous reaction between acetaldehyde and α -ketobutyrate [61]. In wines, they contribute to a “nutty” aroma first discovered in sweet botrytized wines [62].

Conclusion

Overall, this study presents a general, untargeted volatilome profile of *T. delbrueckii* from the Montilla-Moriles region of Spain when subjected to a high glucose fermentation medium. We can detect low concentration, but aromatically impactful metabolites that otherwise may have gone unnoticed in complex, natural media. We were also able to find new metabolites of *T. delbrueckii* which were 1,1-diethoxyethane, 2(5H)-furanone, limonene, ethyl heptanoate, 2-phenethyl acetate, dodecanoic acid, and palmitic acid. While the concentrations of these newly detected metabolites in *T. delbrueckii* were small, it is important to note here that environmental and conditional differences and parameters like: nitrogen sources use, grape variety, and oxygen addition can influence yeast activity. However, these preliminary data can be valuable in designing the next steps in

enhancing the metabolite production of this lesser-known yeast. From here, wine producers, brewers, and fermented beverage producers can regulate fermentation conditions to adjust the production of desired metabolites to their needs.

In today's growing market for alcoholic beverages, consumer demand is shifting toward new, unique aroma/taste. Application of *T. delbrueckii* will likely expand and grow as this trend evolves in the near future. With this in mind, the data in this study are widely applicable to benefit those producers looking to make new products as well as improving existing ones by fermentation with the less traditional, but an up-and-coming yeast—*T. delbrueckii*.

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Availability of data and materials Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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