



The potential of spent coffee grounds hydrolysates fermented with *Torulaspora delbrueckii* and *Pichia kluyveri* for developing an alcoholic beverage: The yeasts growth and chemical compounds modulation by yeast extracts

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ABSTRACT

This study evaluated the effects of yeast extracts (YE) addition (0 % and 0.25 %, w/v) on the no-volatile and volatile compounds of spent coffee grounds (SCG) hydrolysates fermented with single-cultures of two non-*Saccharomyces* wine yeasts, *Torulaspora delbrueckii* and *Pichia kluyveri*. The added YE improved the growth of both *T. delbrueckii* and *P. kluyveri*, especially *P. kluyveri*, resulting in higher ethanol production (1.98 % vs 1.47 %, v/v) by the latter yeast. In addition, the added YE did not impact on most of the alkaloids production regardless of yeast type, while significantly decreasing the contents of chlorogenic, and caffeic acids in SCG hydrolysates fermented with *P. kluyveri*. Furthermore, more odor-active compounds such as acetate esters and 2-phenylethyl alcohol were produced when YE was added, and *P. kluyveri* generated significantly higher amounts of esters compared to that of *T. delbrueckii*. Moreover, YE addition showed a more noticeable effect on the fermentation performance of *P. kluyveri* relative to that of *T. delbrueckii*. These findings indicated the potential of SCG hydrolysates fermented with evaluated non-*Saccharomyces* yeasts and may expand the applications on utilizing SCG to develop new value-added alcoholic products.

1. Introduction

Spent coffee grounds (SCG) as a type of food grade waste is formed from the coffee bean powder (grounds) after hot water's extraction in the coffee industry such as manufacturing of instant coffees and coffee extracts/flavorings. Along with the rising consumption of coffee worldwide, SCG accumulation increases accordingly (Kovalcik et al., 2018). Meanwhile, an increasing concern on the serious environmental pollution and resource wastes due to SCG largely disposing of at the landfill appeared (Fernandes et al., 2017). However, SCG is rich in phenolics, carbohydrates, proteins, and lipids (Campos-Vega et al., 2015; Stylianou et al., 2018). Pre-treated SCG (e.g. sequential sulfuric acid hydrolysis (13.5 g/L) and enzymatic hydrolysis of a mixture of 4 % (v/v) Celluclast 1.5L, 4 % (v/v) β -glucosidase and 4 % (v/v) Viscozyme L, 100 rpm, 48 h) has been used as fermentation substrates to produce lactic acid with *Bacillus coagulans* (Hudeckova et al., 2018), or novel SCG

spirits fermented with *Saccharomyces cerevisiae* RL-11 strain (Sampaio et al., 2013; Machado et al., 2018). Therefore, it seems that the abundant carbohydrates and proteins in SCG after pre-treatment could serve as available carbon and nitrogen sources for the growth of microorganisms (e.g. bacteria and yeasts).

Yeasts belong to an important microbial group for fermentation, especially in wine industry, and are normally classified into *Saccharomyces* and non-*Saccharomyces* yeasts (Morata et al., 2020). *S. cerevisiae* as the most popular species in oenology has been well studied and is known for its easy adaptation to wine environment, excellent fermentation capacity, rapid growth and high tolerance to SO₂ (Parapouli et al., 2020). However, the products fermented with *S. cerevisiae* generally lack flavor complexity. In recent decades, researchers have more interested in seeking new features from non-*Saccharomyces* yeasts (Padilla et al., 2016). The advantages of using non-*Saccharomyces* yeasts in fermentation have been documented to reduce ethanol content, elevate glycerol

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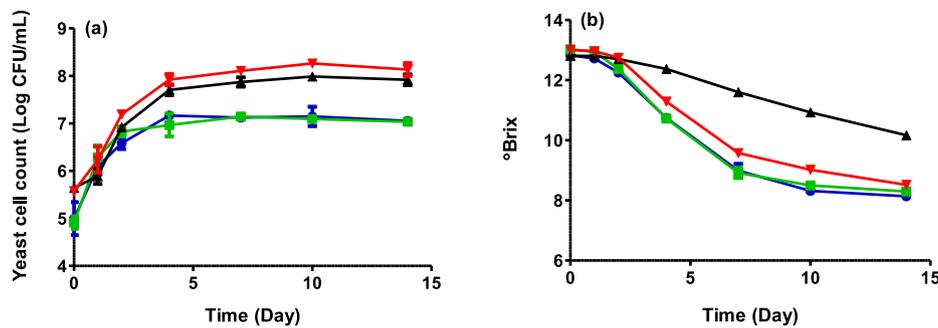


Fig. 1. Development of yeasts (a) and °Brix changes (b) of *T. delbrueckii* Biodiva with 0 % (●) and 0.25 % (w/v) (■) yeast extracts and *P. kluyveri* FrootZen with 0 % (▲) and 0.25 % (w/v) (▼) yeast extracts.

content, enrich aromatic complexity, increase antioxidant capacity, and produce polysaccharides and mannoproteins (Taillandier et al., 2014; Domizio et al., 2016; Benito, 2018; Mecca et al., 2020).

Torulasporea delbrueckii is one of the most studied non-*Saccharomyces* yeasts in winemaking (Benito et al., 2018; Ramírez and Velázquez, 2018). Although *T. delbrueckii* was previously deemed as a spoilage yeast, recent studies revealed that it produced lower contents of ethanol and generated more glycerol (Benito, 2018), helping to avoid problems caused by high sugar contents in grape musts grown at a rising climatic temperature. In addition, *T. delbrueckii* can generate more fruity esters, which increase the complexity of final products (Lu et al., 2016). *Pichia kluyveri*, another popular non-*Saccharomyces* yeast, is well known for its characteristics of low ethanol production but high acetate esters (e.g. 2-phenylethyl acetate) production (Vicente et al., 2021). Previous studies have demonstrated that *P. kluyveri* improved the aroma quality of various types of alcoholic beverages including fruits media (e.g. durian) and food byproducts media (e.g. soy whey) (Chua et al., 2018; Lu et al., 2016, 2017), enhanced chocolate aroma of fermented cocoa (Crafack et al., 2014) and coffee related aroma of fermented green coffee beans (Wang et al., 2020).

The amount of nitrogen compounds in the medium is one of the key factors for yeast fermentation. An amount of 140 mg N/L of yeast assimilable nitrogen (YAN) is recommended to complete fermentation in grape musts (Vilanova et al., 2007). The deficiency of YAN could result in sluggish or stuck grape wine fermentation (Bisson, 1999). Therefore, supplementation of nitrogen compounds into the medium is commonly adopted to boost or complete fermentation. The sources of nitrogen compounds include ammonia salts (e.g. diammonia phosphate) (Chen et al., 2019), a single amino acid (e.g. leucine) (Chua and Liu, 2020), or an amino acid mixture (e.g. yeast extracts, YE) (Su et al., 2020). Previous studies showed that the addition of nitrogen compounds (e.g. diammonia phosphate or amino acid mixture in synthetic must) ensured yeast growth (Chen et al., 2019; Su et al., 2020), enhanced the production of volatile compounds (Hazelwood et al., 2008), improved the generation of glycerol by *T. delbrueckii* (Chua et al., 2021), and even reduced the formation of undesirable volatile compounds by non-*Saccharomyces* during fermentation when the amount of supplementation was moderate (Kevvai et al., 2016; Taillandier et al., 2014). YE as a mixed nitrogen source is known for its rich amino acids, minerals, and vitamins (Li, Liao, Zhang, Du, & Chen, 2011).

SCG is deficient in amino acids, vitamins, and other micronutrients. To ensure successful fermentation of pre-treated SCG, nutrient supplementation is deemed necessary. Therefore, in this study, we explored the effects of the addition of YE on the microbial and chemical components changes on SCG hydrolysates fermented with *T. delbrueckii* Biodiva and *P. kluyveri* FrootZen. Yeast growth and changes of chemical composition (e.g. sugars, organic acids, phenolics and volatile compounds) were evaluated with a view to develop a SCG-based alcoholic beverage.

2. Materials and methods

2.1. Preparation of SCG hydrolysates

The SCG hydrolysate was prepared by following a sequential acid-enzymatic hydrolysis method (Liu et al., 2020). Briefly, the oven dried SCG powder was defatted with hexane at a weight-volume ratio of 1:10 (w/v). The defatted SCG powder was dried for 12 h in a fume hood at room temperature before being ground to fine powder, which was then suspended in deionized (DI) water at a weight-volume ratio of 3:20 (w/v). The SCG suspension was hydrolyzed with 200 mM citric acid at 121 °C for 1 h before being further hydrolyzed with Viscozyme®L (6 %, v/w) at pH 5.0 for 24 h in a 50 °C water bath. During enzymatic hydrolysis, the SCG suspension was stirred at 350 rpm to improve efficiency.

The hydrolyzed SCG suspension was centrifuged (10,000 × g, 10 min, 20 °C) and the supernatant was collected. Sucrose (50 g/L) was added into the SCG hydrolysate before being divided into two groups: one group with the addition of 0.25 (w/v) YE (Oxoid, Basingstoke, Hampshire, England) and the second group did not add YE. The two groups of SCG hydrolysates were pasteurized (60 °C, 30 min) in a water bath and the effectiveness of pasteurization was confirmed by spread plating on potato dextrose agar (PDA, Oxoid, Basingstoke, Hampshire, England) and De Man, Rogosa and Sharpe (MRS) agar for yeast and bacteria, respectively. No visible cell was observed on both types' agar plates. The prepared SCG hydrolysates were stored at 4 °C and used within 3 days.

2.2. Yeasts and fermentation design

Freeze-dried cultures of *T. delbrueckii* Biodiva (Lallemand Inc, Brooklyn Park, Australia) and *P. kluyveri* FrootZen (Chr. Hansen, Hørsholm, Denmark) were propagated in a sterile nutrient broth (glucose 2:100, w/v, YE 0.25:100, w/v, bacteriological peptone 0.25:100, w/v, and malt extract 0.2:100, w/v, pH 5.0). They were cultured statically at 20 °C for 72 h. The cultured yeasts were used as pure cultures and stored in sterile nutrient broth (containing 30 % glycerol, v/v) at −80 °C before use.

The pre-cultures of the two yeast strains were prepared via inoculating the respective pure culture (5 %, v/v) into different SCG hydrolysates (with or without YE addition) and culturing at 20 °C for 3 days with the yeast cell counts reaching at least 7 Log CFU/mL.

The pasteurized SCG hydrolysates (300 mL) fermentation was performed in a 500-mL Erlenmeyer flask. Each treatment was conducted in triplicate and each flask was inoculated with 1 % (v/v, approximately 10⁵ CFU/mL) of each pre-culture. The SCG hydrolysates without and with supplementation of 0.25 % (w/v) YE fermented with *T. delbrueckii* Biodiva were defined as BC (control) and BY, and the SCG hydrolysates with or without YE fermented with *P. kluyveri* FrootZen were defined as FC and FY. The fermentation was performed statically at 20 °C for 14

Table 1
Enological parameters of SCG hydrolysates at day 0 and day 14.

	Day 0	Fermented SCG hydrolysates (Day 14)			
	Unfermented SCG hydrolysates	BC	BY	FC	FY
pH	5.04 ± 0.01c	4.99 ± 0.00b	5.01 ± 0.00b	4.95 ± 0.02a	4.95 ± 0.01a
°Brix	13.02 ± 0.01e	8.14 ± 0.05a	8.39 ± 0.05b	10.17 ± 0.01d	8.52 ± 0.07c
Ethanol (% v/v)	0.00 ± 0.00a	3.65 ± 0.10d	3.75 ± 0.02d	1.47 ± 0.06b	1.98 ± 0.10c
Yeast cell counts (Log CFU/mL)					
<i>T. delbrueckii</i>	4.99 ± 0.14a	7.05 ± 0.02b	7.03 ± 0.03b	\	\
Biodiva					
<i>P. kluyveri</i>	5.64 ± 0.08a	\	\	7.91 ± 0.11b	8.13 ± 0.13b
FrootZen					
Sugars (g/L)					
Fructose	14.41 ± 0.27d	0.00 ± 0.00a	0.00 ± 0.00a	11.98 ± 0.22c	4.33 ± 0.16b
Glucose	26.37 ± 0.47a	0.00 ± 0.00a	0.00 ± 0.00a	5.49 ± 0.17b	0.00 ± 0.00a
Sucrose	8.06 ± 0.06c	0.33 ± 0.01a	0.30 ± 0.00a	6.54 ± 0.33b	6.00 ± 0.23b
Mannose	7.69 ± 0.19c	2.32 ± 0.06b	2.46 ± 0.07b	2.16 ± 0.09b	1.35 ± 0.05a
Galactose	14.94 ± 0.20b	7.63 ± 0.51a	7.26 ± 0.30a	6.63 ± 0.19a	6.60 ± 0.19a
Arabinose	3.45 ± 0.04b	3.14 ± 0.04b	3.06 ± 0.07b	2.27 ± 0.12a	2.22 ± 0.12a
Total	74.98 ± 1.23d	13.40 ± 0.62a	13.08 ± 0.45a	35.06 ± 0.47c	20.49 ± 0.52b
Glycerol (g/L)	0.00 ± 0.00a	3.12 ± 0.15c	2.49 ± 0.14b	6.72 ± 0.28d	7.26 ± 0.26e
Organic acid (g/L)					
Citric acid	38.35 ± 1.13b	36.29 ± 0.22a	36.96 ± 0.22a	36.48 ± 0.60a	36.85 ± 0.35a
α-Ketoglutaric acid (mg/L)	30.89 ± 2.4c	23.05 ± 0.53b	16.59 ± 0.61a	34.96 ± 0.96d	36.45 ± 5.93d
Malic acid	0.97 ± 0.05c	0.29 ± 0.01 ab	0.24 ± 0.01a	0.33 ± 0.01b	0.25 ± 0.00a
Pyruvic acid	0.12 ± 0.01a	0.30 ± 0.01d	0.25 ± 0.00c	0.19 ± 0.00b	0.41 ± 0.02e
Succinic acid	1.83 ± 0.12a	2.40 ± 0.06c	3.05 ± 0.00d	2.18 ± 0.07b	3.17 ± 0.02d
Lactic acid	0.09 ± 0.00a	0.55 ± 0.04b	0.71 ± 0.05b	0.71 ± 0.06b	1.13 ± 0.12c
Acetic acid	0.04 ± 0.00a	0.58 ± 0.02d	0.57 ± 0.02cd	0.52 ± 0.02b	0.48 ± 0.03b
Total	41.30 ± 0.42a	40.13 ± 0.30a	41.55 ± 0.35a	40.24 ± 0.50a	41.91 ± 0.22a

Notes: a, b, c, d, e statistical analysis using ANOVA (n = 3) at 95 % confidence interval. The same letter indicates no significant difference between the groups. BC, BY: *T. delbrueckii* Biodiva with 0 % and 0.25 % (w/v) yeast extracts; FC, FY: *P. kluyveri* FrootZen with 0 % and 0.25 % (w/v) yeast extracts. 0.00 ± 0.00: not detected, it is used for statistical analysis; '\': blank.

days and monitored by yeast cell count, °Brix and pH. The enumeration of yeast cell count was counted by spread plating on PDA. The determination of °Brix and pH was conducted by TX-5000α refractometer (ATAGO, Tokyo, Japan) and pH meter (827 pH Lab, Metrohm, Herisau, Switzerland). Periodical samplings were taken at day 0, 1, 2, 4, 7, 10, 14 and stored at -20 °C before analysis, respectively.

2.3. Non-volatiles and volatile compounds analysis

Non-volatiles (e.g. sugars, organic acids, alkaloids) were analyzed through a high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan). A Zorbax carbohydrate column (150 mm × 4.6 mm,

Agilent, Santa Clara, CA, USA) connected to HPLC was used to detect sugars with an evaporative light scattering detector (ELSD). A Supelco C-610 H column (300 mm × 7.8 mm, Supelco, Sigma-Aldrich, Barcelona, Spain) was applied to quantify organic acids with a photodiode array (PDA) detector with UV-Vis wavelength at 210 nm. A Zorbax Eclipse C18 column related to HPLC was used to measure alkaloids and phenolic acids with a PDA at 320 nm as reported previously (Liu et al., 2020, 2021).

Ethanol was analyzed in a Sigma-Aldrich Supelcogel 300 × 7.8 mm C-610 column (Supelco, Barcelona, Spain) through a Waters HPLC system (MA, USA) connected to a refractive index detector (RID) (Liu et al., 2020). Amino acids were analyzed on MembraPure ARACUS Amino Acid Analyzer (Berlin, Germany). A lithium-cation exchange column with reagent A-F flowing through to detect amino acids (Liu et al., 2020, 2021). The concentrations of the detected amino acids were calculated by a calibration factor.

For volatiles, the fermented SCG hydrolysate (5 mL) was extracted by a Supelco carboxen/poly (dimethylsiloxane) fibre (85-µm coating, Sigma-Aldrich, Barcelona, Spain) using a headspace solid-phase micro-extraction (HS-SPME) method. The separated aroma compounds were delivered by helium and detected by gas chromatography-mass spectrometer and flame ionisation detector (GC-MS/FID) (Lu et al., 2016). The characterization and identification were performed by comparing mass file with Wiley MS library and NIST 14, and further confirmed by calculating the value of linear retention index (LRI) based on the retention time of alkanes standards (C7–C40). An internal standard (Butyl butyryl lactate, 1 ppm in methanol, Sigma-Aldrich, USA) was added into each sample to quantify the concentrations of volatiles.

2.4. Statistical analysis

SPSS 17.0 software (SPSS Corporation, Chicago, IL, USA) was applied to do statistical analysis on triple results of each treatment via a one-way analysis of variance (ANOVA) and Tukey's test. The results were deemed to be statistically significant with a confidence interval large than 95%. MATLAB R2013a (MathWorks, Natick, MA, USA) was used to perform principal component analysis (PCA) for volatile compounds.

3. Results and discussion

3.1. Yeast growth

There was a similar trend in growth of the two yeast strains (Fig. 1a). *T. delbrueckii* Biodiva grew faster in the first two days (early stage of exponential phase), slightly grew in the following five days (later stage of exponential phase), and then kept stable till day 14 (stationary phase). The growth of *T. delbrueckii* Biodiva with YE addition showed a similar trend to that without YE addition. *P. kluyveri* FrootZen showed faster growth in the first four days (early stage of exponential phase) and slight growth in the following three days (later stage of exponential phase), and with a stable population till day 14 (stationary phase). The cell counts increased by 2.06 Log CFU/mL (BC), 2.12 Log CFU/mL (BY), 2.28 Log CFU/mL (FC) and 2.46 Log CFU/mL (FY) at day 14, respectively. The cell count of *P. kluyveri* FrootZen in the fermented SCG with YE addition was obviously higher than that without YE addition from day 1 to day 14. In general, *P. kluyveri* FrootZen and *T. delbrueckii* Biodiva grew well in SCG hydrolysates regardless of YE addition. *P. kluyveri* FrootZen grew markedly better than *T. delbrueckii* Biodiva. In addition, it seemed that YE addition promoted the growth of *P. kluyveri* FrootZen, but not *T. delbrueckii* Biodiva (Fig. 1a).

3.2. Changes in °Brix, sugars, ethanol and glycerol

°Brix values decreased significantly from 13.02 (day 0) to 8.14 (BC), 8.39 (BY), 10.17 (FC) and 8.52 (FY) in SCG hydrolysates fermentation at

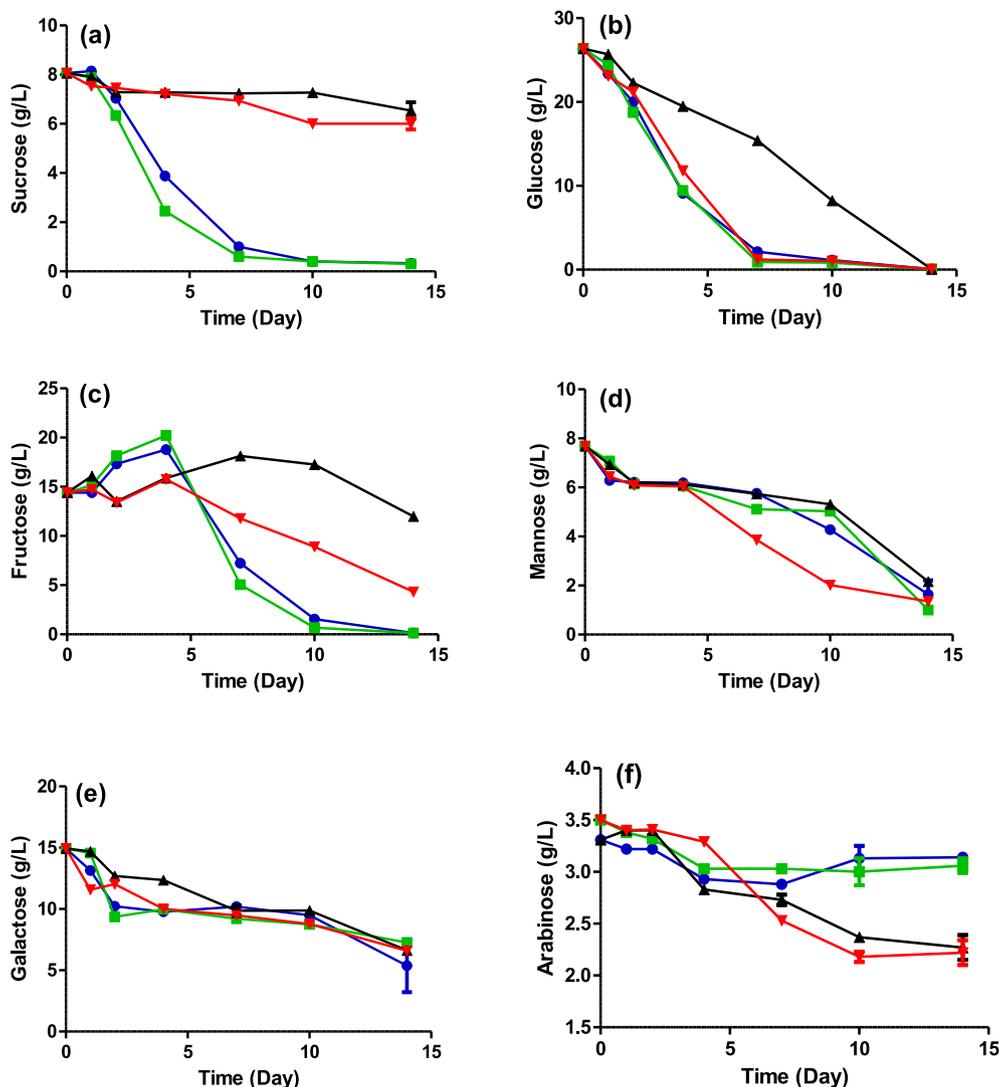


Fig. 2. Sugar utilization during SCG hydrolysates fermentation by *T. delbrueckii* Biodiva with 0 % (●) and 0.25 % (w/v) (■) yeast extracts and *P. kluyveri* FrootZen with 0 % (▲) and 0.25 % (w/v) (▼) yeast extracts.

day 14, respectively (Fig. 1b). The comparable °Brix values between BC and BY samples at day 14 could be ascribed to similar growth of *T. delbrueckii* Biodiva. However, the significantly higher decline of °Brix in FY samples than that of FC samples of *P. kluyveri* FrootZen was likely due to the enhanced growth by YE. Our results agreed with several previous studies that the added nitrogen sources (e.g. ammonium salts, YE) improved the growth of yeasts (e.g. *S. cerevisiae* MERIT.ferm, *L. thermotolerans* Concerto) and therefore, resulting in the significant decline of °Brix values in lychee wine (Chen et al., 2019) and fermented SCG hydrolysates (Liu et al., 2020).

Sugars changes are presented in Table 1 along with Fig. 2a–f. The low initial concentration of sucrose (8.06 g/L) at day 0 could be due to the enzymatic action from the added enzyme Viscozyme (e.g. the side activity of invertase) because the SCG hydrolysate was not heat treated after enzyme treatment (Liu et al., 2020). The initial increases in fructose in the strain *T. delbrueckii* Biodiva fermented SCG hydrolysate were attributed to sucrose hydrolysis and slower utilization of fructose, while the glucose released was rapidly used by the glucophilic yeast. Sucrose, glucose, fructose, mannose and galactose were consumed by both yeasts (Fig. 2). *T. delbrueckii* Biodiva did not utilize arabinose but *P. kluyveri* FrootZen did (Fig. 2f). In addition, the two yeast strains exhibited different consumption rates to different sugars. *T. delbrueckii* Biodiva utilized all the fructose, glucose and sucrose, then consumed mannose,

galactose and arabinose when glucose and fructose were insufficient. In comparison, *P. kluyveri* FrootZen consumed more glucose, mannose and arabinose but less fructose and sucrose. The SCG hydrolysates fermented with *P. kluyveri* FrootZen had significantly higher total residual sugar contents than that of *T. delbrueckii* Biodiva at day 14 (Table 1).

It was reported that mannose could be consumed by *T. delbrueckii* Biodiva to produce mannoprotein (Benito, 2018). Galactose might be assimilated in the Leloir pathway as revealed in *S. cerevisiae* (Timson, 2007) and arabinose might be transported by transporter Gal2p and metabolized to produce ethanol or support growth of some yeasts including a few strains of *S. cerevisiae* (Oehling et al., 2018). Furthermore, the supplementation of YE accelerated, to a limited degree, the utilization of fructose and sucrose by *T. delbrueckii* Biodiva (Fig. 2a, c). In contrast, the added YE dramatically promoted the consumption of almost all sugars (except galactose) especially glucose, fructose, and mannose by *P. kluyveri* FrootZen (Fig. 2b, c, d). Correspondingly, the added YE significantly promoted ethanol production by *P. kluyveri* FrootZen in FY samples (1.98 %, v/v) than FC samples (1.47 %, v/v), while there was no discernible effect on ethanol production by *T. delbrueckii* Biodiva (Table 1).

The addition of YE decreased glycerol production by *T. delbrueckii* Biodiva in BY samples (2.49 g/L) compared to BC samples (3.12 g/L). The higher glycerol production in SCG hydrolysates without YE might be

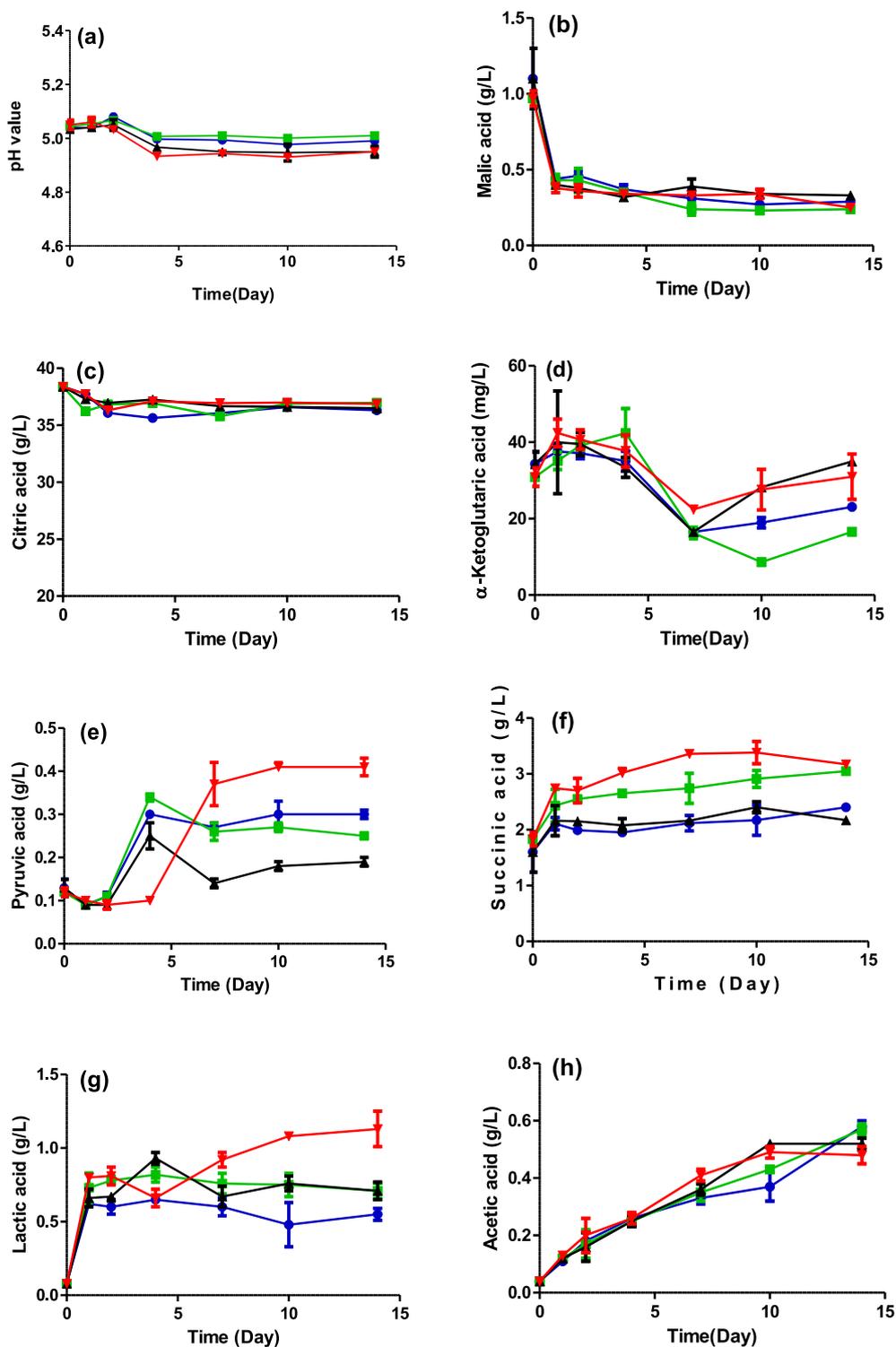


Fig. 3. Changes in pH (a) and organic acids (b)–(h) during SCG hydrolysates fermentation by *T. delbrueckii* Biodiva with 0% (●) and 0.25% (w/v) (■) yeast extracts and *P. kluyveri* FrootZen with 0% (▲) and 0.25% (w/v) (▼) yeast extracts.

triggered by its higher osmotic environment due to slower fructose consumption (Fig. 2c). However, it is interesting to note that the addition of YE significantly increased glycerol production by *P. kluyveri* FrootZen in FY samples (7.26 g/L) compared to FC samples (6.72 g/L). This could be caused by the higher biomass of *P. kluyveri* FrootZen to generate more glycerol in FY samples. Regardless of YE, *P. kluyveri* FrootZen produced 2 times more glycerol than *T. delbrueckii* Biodiva. Glycerol plays a significant role in cell osmotic regulation as well as

redox balance and may affect taste and mouthfeel (Zhao et al., 2015).

3.3. Changes in pH and organic acids

pH changes followed a similar trend of significant reduction in all samples (with or without YE) regardless of yeast type (Table 1, Fig. 3a). The significantly lower pH values in *P. kluyveri* FrootZen-fermented samples (FC, 4.95; FY, 4.95) than that of *T. delbrueckii* Biodiva-

Table 2
Phenolic compounds and alkaloids in the SCG hydrolysates at day 0 and day 14.

Compounds (mg/L)	Day 0	Fermented SCG hydrolysates (Day 14)			FY
	Unfermented SCG hydrolysates	BC	BY	FC	
Alkaloids					
Trigonelline	523.19 ± 15.71a	699.28 ± 6.07b	725.95 ± 31.53b	716.28 ± 18.57b	594.11 ± 12.62a
Theobromine	126.01 ± 3.33b	103.18 ± 2.90a	108.20 ± 4.63ab	111.95 ± 3.61ab	99.89 ± 1.67a
Theophylline	73.23 ± 2.17c	54.87 ± 1.89a	57.93 ± 2.48a	60.48 ± 1.94b	53.29 ± 1.01a
Caffeine	1386.29 ± 39.10a	1328.01 ± 47.39a	1435.86 ± 25.26a	1412.35 ± 21.69a	1395.78 ± 53.78a
Phenolic acids					
Chlorogenic acid	120.86 ± 2.27c	92.51 ± 5.09b	100.69 ± 4.66b	99.57 ± 9.53b	66.11 ± 3.77a
Caffeic acid	469.88 ± 8.27c	874.83 ± 13.01d	896.66 ± 30.67d	340.83 ± 17.73b	219.57 ± 6.62a
Ferulic acid	11.66 ± 0.85a	18.71 ± 0.70b	19.41 ± 0.67b	16.83 ± 1.63b	11.96 ± 0.32a
p-Coumaric acid	7.40 ± 0.21a	7.13 ± 0.29a	7.74 ± 0.13a	7.44 ± 0.17a	7.43 ± 0.34a

Notes: a, b, c, d statistical analysis using ANOVA (n = 3) at 95 % confidence interval. The same letter indicates no significant difference between the groups. BC, BY: *T. delbrueckii* Biodiva with 0 % and 0.25 % (w/v) yeast extracts; FC, FY: *P. kluyveri* FrootZen with 0 % and 0.25 % (w/v) yeast extracts.

fermented samples (BC, 4.99; BY, 5.01) correlated with the enhanced higher generation of succinic and lactic acids by *P. kluyveri* FrootZen (Fig. 3f and g).

The kinetics of organic acids are illustrated in Fig. 3b-h. In general, the added YE did not affect the changes of malic, citric, and acetic acids (Fig. 3b, c, h) regardless yeast type. The decrease of malic acid should be due to the passive diffusion into yeasts although some researchers reported that *P. kluyveri* may partially consume L-malic acid such as in durian wine fermentation (Lu et al., 2016, 2017). Citric acid was introduced into SCG hydrolysates during acid hydrolysis and kept relatively stable (Fig. 3c). Acetic acid increased gradually with significantly higher contents in *T. delbrueckii* Biodiva-fermented samples (BC, 0.58 g/L; BY, 0.57 g/L) than that in *P. kluyveri* FrootZen-fermented samples (FC, 0.52 g/L; FY, 0.48 g/L) (Table 1). The amount of acetic acid in the range of 0.2–0.7 g/L is generally considered to be acceptable to red wine flavor (Vilanova et al., 2007).

On the other hand, the added YE markedly increased the generation of pyruvic, succinic and lactic acids by both yeast strains (Table 1; Fig. 3e, f, g). The higher production of these organic acids might be the result of improved metabolic activity of the yeasts associated with TCA cycle, glycolysis and amino acids in the presence of added YE.

3.4. Changes in alkaloids and phenolic acids

The contents of alkaloids (theobromine, trigonelline, theophylline and caffeine) and four phenolic acids before and after fermentation are shown in Table 2. It seems that the added YE did not impact on the changes of alkaloids regardless of yeast type. Theobromine and theophylline significantly decreased in all fermented samples, whereas trigonelline significantly increased after fermentation except for FY samples (also increased albeit statistically insignificant) (Table 2). In addition, there were no significant changes in caffeine in all samples. The decrease of theobromine and theophylline could be ascribed to their degradation to xanthine in most fungi (Dash and Gummadi, 2006). The increase of trigonelline could stem from the methylation of the nitrogen atom of niacin (vitamin B3) as reported previously (Garg, 2016), although we did not measure the contents of niacin. The changes in

alkaloids in the present study were different from a previous work, in which SCG hydrolysates were fermented with *L. thermotolerans* Concerto and *S. cerevisiae* MERIT. This could be ascribed to yeast species and strain variations (Chan et al., 2020; Liu et al., 2020).

The changes of most phenolic acids showed differences between the two yeast strains. In *T. delbrueckii* Biodiva-fermented samples, there were significant reductions in chlorogenic acid but increases in caffeic acid and ferulic acid, while there was no change to *p*-coumaric acid; the impact of YE was not evident. In *P. kluyveri* FrootZen-fermented samples, both chlorogenic and caffeic acids decreased significantly especially in the presence of YE; ferulic acid increased in samples without YE; *p*-coumaric acid remained unchanged and the effect of YE was not observable.

The decrease of chlorogenic acid could be due to its hydrolysis into quinic acid and the other phenolic acids like caffeic and ferulic acids. Ferulic acid and caffeic acid could be converted via decarboxylation into 4-ethylguaiacol and 4-ethylcatechol, respectively (Devi & Anu-Appaiah, 2018), although the two volatile phenols were not found in the fermented samples (Table 3). In addition, ferulic acid and caffeic acid could be released from its ester forms. The final amounts of caffeic acid and ferulic acid would be the net balance of production and utilization during fermentation.

3.5. Changes in free amino acids

The total yeast assimilable nitrogen (YAN) in unfermented SCG hydrolysates was 64.44 N mg/L (primary amino acid nitrogen 29.52 N mg/L and ammonia 34.92 N mg/L) and increased to 161.51 N mg/L (primary amino acid nitrogen 125.02 N mg/L and ammonia 36.49 N mg/L) after adding 0.25 % (w/v) YE (Fig. 4, Table S1). The addition of YE boosted SCG hydrolysates YAN to meet the level of up to 140 N mg/L, which is used in grape wine fermentation (Vilanova et al., 2007). YE enriched almost all amino acids that endogenous in raw SCG hydrolysates, especially increasing the contents of aspartic acid, glutamic acid, alanine, leucine and phenylalanine.

The total free amino acids significantly decreased from 1199.20 mg/L to 120.13 mg/L (BC), 663.88 mg/L (BY) and 92.12 mg/L (FC), 11.36 mg/L (FY) after fermentation, respectively. In general, a declining trend was observed among all detected amino acids and ammonium. Interestingly, *T. delbrueckii* Biodiva and *P. kluyveri* FrootZen showed different utilization rates and preferences for amino acids. *P. kluyveri* FrootZen had a higher consumption of all amino acids regardless of YE compared to that of *T. delbrueckii* Biodiva (Fig. 4, Table S1). *T. delbrueckii* Biodiva preferred glutamic acid, leucine and lysine, while *P. kluyveri* FrootZen preferred aspartic acid, alanine, isoleucine, glutamic acid, leucine, phenylalanine and lysine. Previous studies demonstrated that the higher assimilation of leucine, isoleucine and phenylalanine contributed to the generation of higher alcohols (e.g. isoamyl alcohol and 2-phenylethanol) under the Ehrlich pathway, which also provided more substrates for the formation of esters (Walker and Stewart, 2016) (Table 3).

After fermentation, the total YAN of the remaining nitrogen compounds decreased to the range of 11.36–92.12 N mg/L for BC, BY samples and 17.74–27.37 N mg/L for FC, FY sample, respectively (Table S1), manifesting that most free amino acids from YE were metabolized. The residual amino acids should be a net balance of consumption and release from yeasts during the growth and autolysis (Chua and Liu, 2020).

3.6. Changes in volatile components

As shown in Table 3, volatile components in raw SCG hydrolysates (with or without YE) at day 0 and in *T. delbrueckii* Biodiva-fermented samples (BC and BY) or *P. kluyveri* FrootZen-fermented samples (FC and FY) at day 14 were detected and quantified. In general, the added YE affected the generation of higher alcohols (e.g. 1-pentanol and 2-phenylethyl alcohol), esters (e.g. ethyl acetate, 2-phenethyl acetate, and isoamyl acetate) and aldehydes (e.g. benzeneacetaldehyde).

Table 3Main selected aroma compounds and their concentrations ($\mu\text{g/L}$) in SCG hydrolysates at day 0 and day 14.

Compound	Identification	LRI	Unfermented SCG hydrolysates (Day 0)		Fermented SCG hydrolysates (Day 14)			
			methods	0 % YE	0.25 % YE	BC	BY	FC
Aldehydes								
Benzaldehyde	MS, LRI	1540	127.92 \pm 21.65c	125.21 \pm 4.36c	73.53 \pm 4.86b	80.83 \pm 13.49b	40.19 \pm 7.43a	120.71 \pm 11.69c
Benzeneacetaldehyde	MS, LRI	1629	0.00 \pm 0.00a	0.00 \pm 0.00a	30.41 \pm 3.59b	50.95 \pm 0.04b	141.31 \pm 21.10c	401.67 \pm 20.10d
Furfural	MS, LRI	1488	14.25 \pm 2.09a	13.63 \pm 2.65a	23.17 \pm 2.09b	21.48 \pm 4.27b	14.19 \pm 1.67a	15.86 \pm 0.54a
(<i>E,E</i>)-2,4-Decadienal	MS, LRI	1866	99.54 \pm 10.02b	92.14 \pm 10.29b	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a
Acids								
Pentanoic acid	MS, LRI	1623	0.00 \pm 0.00a	0.00 \pm 0.00a	48.69 \pm 3.91b	46.51 \pm 7.99b	48.86 \pm 4.41b	73.40 \pm 5.03c
4-Hexenoic acid	MS, LRI	1918	0.00 \pm 0.00a	0.00 \pm 0.00a	26.12 \pm 2.74b	27.75 \pm 0.78b	0.00 \pm 0.00a	0.00 \pm 0.00a
Hexanoic acid	MS, LRI	1834	0.45 \pm 0.05a	14.88 \pm 1.76b	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00b	0.00 \pm 0.00b
Benzoic acid	MS, LRI	2452	8.89 \pm 0.52b	36.98 \pm 3.34c	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a
Octanoic acid	MS, LRI	2038	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	30.84 \pm 1.70b	93.64 \pm 5.45c
Alcohols								
1-Pentanol	MS, LRI	1228	0.00 \pm 0.00a	0.00 \pm 0.00a	172.18 \pm 14.38c	183.43 \pm 10.44c	0.00 \pm 0.00a	51.06 \pm 2.95b
2-Phenylethyl alcohol	MS, LRI	1934	34.27 \pm 2.70a	40.94 \pm 5.17a	861.22 \pm 20.02d	909.25 \pm 61.06d	267.93 \pm 12.26c	195.91 \pm 11.17b
Esters								
Ethyl acetate	MS, LRI	–	0.00 \pm 0.00a	0.00 \pm 0.00a	215.30 \pm 9.80a	277.12 \pm 13.11a	5850.99 \pm 337.23b	10482.89 \pm 966.95c
Ethyl hexanoate	MS, LRI	1252	0.00 \pm 0.00a	0.00 \pm 0.00a	59.30 \pm 8.92b	88.97 \pm 3.88c	54.40 \pm 6.82b	84.83 \pm 6.53c
Ethyl (<i>E</i>)-4-hexenoate	MS, LRI	1298	0.00 \pm 0.00a	0.00 \pm 0.00a	56.80 \pm 8.15b	82.16 \pm 10.35c	0.00 \pm 0.00a	0.00 \pm 0.00a
Ethyl octanoate	MS, LRI	1447	0.00 \pm 0.00a	0.00 \pm 0.00a	66.95 \pm 6.80b	141.92 \pm 18.68c	40.99 \pm 5.54 ab	321.14 \pm 41.91d
Ethyl decanoate	MS, LRI	1653	0.00 \pm 0.00a	0.00 \pm 0.00a	84.82 \pm 9.57c	292.67 \pm 31.48d	125.66 \pm 11.01c	292.22 \pm 25.92d
Ethyl (<i>Z</i>)-4-decenoate	MS, LRI	1601	0.00 \pm 0.00a	0.00 \pm 0.00a	10.05 \pm 0.96b	13.80 \pm 0.30c	0.00 \pm 0.00a	0.00 \pm 0.00a
Ethyl dodecanoate	MS, LRI	1829	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	12.43 \pm 1.18b	0.00 \pm 0.00a	50.11 \pm 1.32c
Ethyl 2,4-hexadienoate	MS, LRI	1509	0.00 \pm 0.00a	0.00 \pm 0.00a	85.51 \pm 5.52c	106.50 \pm 10.08d	67.62 \pm 5.12b	80.19 \pm 1.90c
Isoamyl acetate	MS, LRI	1147	0.00 \pm 0.00a	0.00 \pm 0.00a	23.56 \pm 1.45a	21.61 \pm 2.13a	1713.15 \pm 121.23b	3387.50 \pm 534.03b
Propyl acetate	MS, LRI	–	0.00 \pm 0.00a	0.00 \pm 0.00a	216.41 \pm 4.40b	190.83 \pm 14.60c	0.00 \pm 0.00a	51.07 \pm 3.09d
Octyl acetate	MS, LRI	1477	0.00 \pm 0.00a	0.00 \pm 0.00a	66.95 \pm 6.80c	144.90 \pm 17.49d	18.88 \pm 2.49 ab	21.19 \pm 3.06b
Benzyl acetate	MS, LRI	1744	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	48.48 \pm 7.02b	50.48 \pm 5.42b
2-Phenethyl acetate	MS, LRI	1817	0.00 \pm 0.00a	0.00 \pm 0.00a	33.37 \pm 2.57a	42.61 \pm 6.57a	20470.10 \pm 925.96b	30988.75 \pm 1127.51c
2-Phenethyl propionate	MS, LRI	1882	0.00 \pm 0.00a	0.00 \pm 0.00a	24.53 \pm 0.96b	38.35 \pm 5.33c	79.53 \pm 6.20d	86.53 \pm 5.12d
Furfuryl acetate	MS, LRI	1520	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	315.74 \pm 46.15b	286.55 \pm 10.12b
Furans								
2,5-Dimethylfuran	MS, LRI	1033	0.00 \pm 0.00a	0.00 \pm 0.00a	58.73 \pm 2.12c	62.44 \pm 4.50c	43.17 \pm 3.81b	74.79 \pm 4.27d
2-Pentylfuran	MS, LRI	1247	1.95 \pm 0.15b	2.16 \pm 0.16b	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a	0.00 \pm 0.00a
2-Vinylfuran	MS, LRI	2001	15.15 \pm 1.80a	11.72 \pm 2.33a	11.15 \pm 0.63a	10.07 \pm 0.51a	13.21 \pm 0.95a	17.26 \pm 1.16a
2-Formyl-5-methylfuran	MS, LRI	1574	6.03 \pm 0.59b	5.75 \pm 0.76b	11.29 \pm 1.15c	8.12 \pm 0.51c	0.00 \pm 0.00a	0.00 \pm 0.00a
Others								
Guaiacol	MS, LRI	1892	12.70 \pm 2.23c	10.23 \pm 1.20bc	7.06 \pm 1.09a	7.39 \pm 1.00a	5.67 \pm 0.54a	8.49 \pm 0.56a
3-Acetylpyrrole	MS, LRI	1992	20.56 \pm 2.79bc	19.48 \pm 1.55b	12.94 \pm 1.87a	10.92 \pm 1.67a	17.84 \pm 1.16b	24.19 \pm 0.51c

Notes: LRI: linear retention index; determined on a DB-FFAF column relative to C7–C40 hydrocarbons. YE: yeast extract. a, b, c, d, e Statistical analysis using ANOVA ($n = 3$) at 95 % confidence interval. Same letter indicates no significant difference between samples. BC, BY: *T. delbrueckii* Biodiva with 0 % and 0.25 % (w/v) yeast extracts; FC, FY: *P. kluyveri* FrootZen with 0 % and 0.25 % (w/v) yeast extracts. 0.00 \pm 0.00: not detected, it is used for statistical analysis.

Among the aldehydes, the added YE significantly enhanced the production of benzeneacetaldehyde in BY and FY samples, in which *P. kluyveri* FrootZen (FY) produced significantly higher amounts than *T. delbrueckii* Biodiva. In addition, the production of furfural by *T. delbrueckii* Biodiva significantly increased regardless of YE, while it kept stable in SCG hydrolysates fermented with *P. kluyveri* FrootZen. The increase of furfural could be ascribed to the release from its bound form due to yeast metabolism. (*E,E*)-2,4-Decadienal was totally consumed in all fermented samples, likely reduced to its alcohol form ((*E,E*)-2,4-decadienol). Benzaldehyde was significantly decreased in almost all samples except FY, possibly reduced to benzyl alcohol or oxidized to benzoic acid. *P. kluyveri* FrootZen might have the ability to enhance the generation of benzaldehydes in SCG hydrolysate as this strain increase the production of acetaldehyde in the fermentation of Shiraz wine (Whitener et al., 2017).

Volatile acids were dominated by pentanoic, 4-hexenoic and octanoic acids in the fermented SCG hydrolysates. Pentanoic acid was produced by the two yeast strains, while 4-hexenoic acid was only produced by *T. delbrueckii* Biodiva and octanoic acid was merely detected in SCG hydrolysates inoculated with *P. kluyveri* FrootZen. It is clear that the addition of YE significantly increased the generation of pentanoic acid by *P. kluyveri* FrootZen, but not by *T. delbrueckii* Biodiva (Table 3).

Alcohols were dominated by 2-phenylethyl alcohol in all fermented SCG hydrolysates. The amount of 2-phenylethyl alcohol was markedly higher in SCG hydrolysates fermented with *T. delbrueckii* Biodiva than that of *P. kluyveri* FrootZen. It seems that the added YE slightly increased the production of 2-phenylethyl alcohol by *T. delbrueckii* Biodiva but significantly decreased its generation in SCG hydrolysates fermented with *P. kluyveri* FrootZen, possibly because of the diversion of this alcohol to its ester, 2-phenylethyl acetate, which was consistent with the

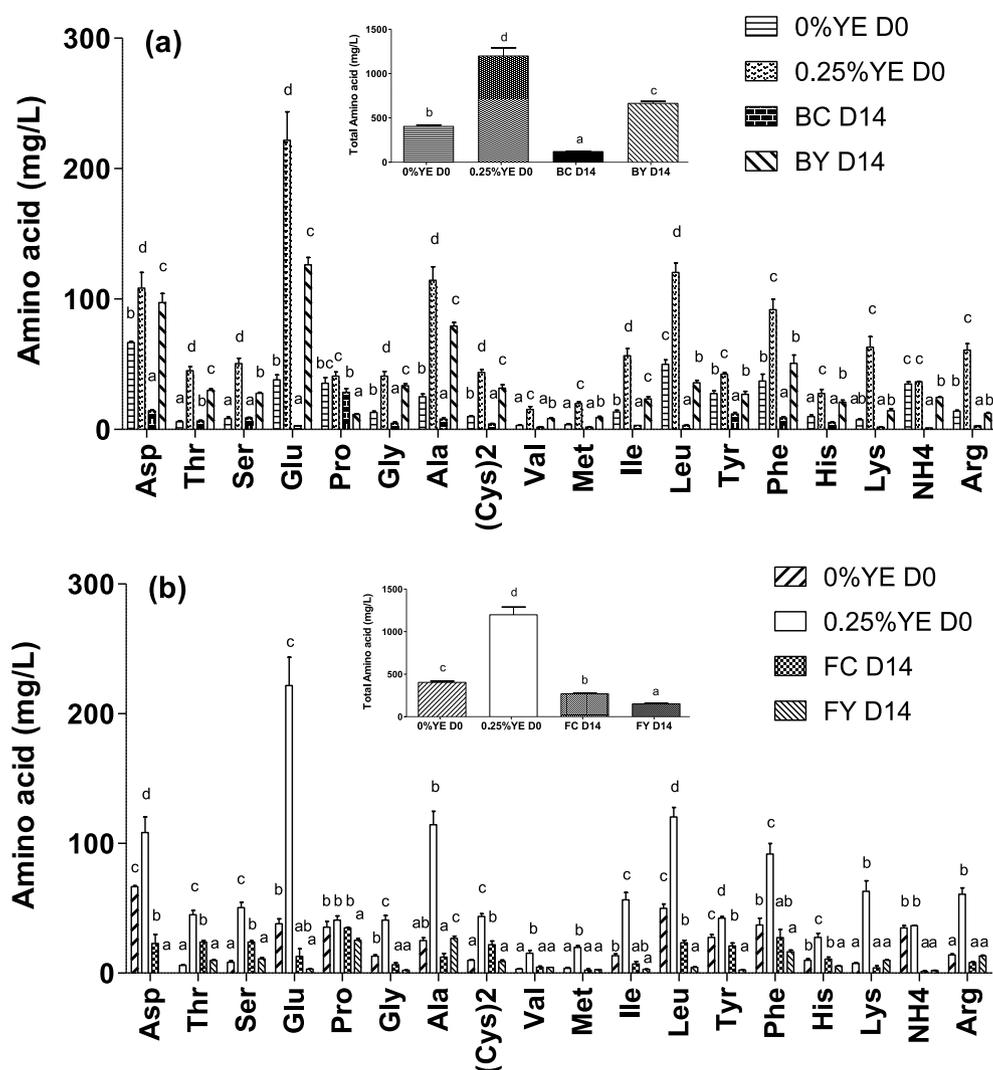


Fig. 4. (a) Free amino acids and ammonia in SCG hydrolysates with/without added yeast extracts with inoculation of *T. delbrueckii* Biodiva at day 0 and day 14; (b) Free amino acids and ammonia in SCG hydrolysates with/without addition of yeast extracts with inoculation of *P. kluyveri* FrootZen at day 0 and day 14. 0% YE stands for SCG hydrolysates without yeast extracts at day 0; 0.25% YE: SCG hydrolysates with 0.25% (w/v) yeast extracts at day 0; BC: *T. delbrueckii* Biodiva with 0% (w/v) yeast extracts; BY: *T. delbrueckii* Biodiva with 0.25% (w/v) yeast extracts; FC: *P. kluyveri* FrootZen with 0% (w/v) yeast extracts; FY: *P. kluyveri* FrootZen added with 0.25% (w/v) yeast extracts; D14: Day 14 of the fermentation.

generation trends of high alcohols not only in durian wine fermented with *T. delbrueckii* Biodiva and *P. kluyveri* FrootZen (Lu et al., 2016), but also in Riesling wine fermented with sequential inoculation of *P. kluyveri* FrootZen and *S. cerevisiae* Level 2 (Dutraive et al., 2019). Meanwhile, 1-pentanol was detected in BC, BY and FY samples. The addition of YE resulted in the production of 1-pentanol in SCG hydrolysates fermented with *P. kluyveri* FrootZen, while its production in SCG hydrolysates fermented with *T. delbrueckii* Biodiva was not affected regardless of YE.

The added YE significantly improved the production of ethyl octanoate and ethyl decanoate in SCG hydrolysates regardless of yeast type. Although the addition of YE only significantly increased the production of ethyl hexanoate and ethyl (*E*)-4-hexenoate by *T. delbrueckii* Biodiva (BY samples), the production of acetate esters (e.g. ethyl acetate, 2-phenethyl acetate and isoamyl acetate) was only elevated in SCG hydrolysates fermented with *P. kluyveri* FrootZen (FY samples). Our results partially agreed with the findings of Mecca et al. (2020), where nitrogen addition also significantly enhanced the production (21–44%) of ethyl esters (especially ethyl propionate and ethyl decanoate) in grape wines fermented with *T. delbrueckii* (Biodiva and Prelude). Several studies have found that *P. kluyveri* FrootZen is a good producer of acetate esters (Lu et al., 2016, 2017), however, few studies reported the nitrogen supplementation increases its generation of volatile compounds and this needs further investigation.

Four detectable furans were quantified in SCG hydrolysates (Table 3). The production of 2,5-dimethylfuran was significantly

enhanced in SCG hydrolysates fermented with *P. kluyveri* FrootZen when YE was added. Although there was no statistically significant difference, the added YE slightly enhanced the production of 2,5-dimethylfuran by *T. delbrueckii* Biodiva. 2-Pentylfuran was totally metabolized after fermentation regardless of *T. delbrueckii* Biodiva or *P. kluyveri* FrootZen. 2-Vinylfuran kept stable in all fermented SCG hydrolysates. The amounts of 2-formyl-5-methylfuran in SCG hydrolysates fermented with *T. delbrueckii* Biodiva were significantly higher than their initial samples (day 0), while the added YE did not affect its production. Furans could be derived from SCG hydrolysates fermentation and could impact coffee related flavor.

Guaiacol belonging to volatile phenols was detected in all samples, while its contents in fermented SCG hydrolysates were significantly lower than that in unfermented SCG hydrolysates (day 0). 3-Acetylpyrrole was also detected in SCG hydrolysates; it was decreased by *T. delbrueckii* Biodiva regardless of YE, but increased by *P. kluyveri* FrootZen in the presence of YE.

3.7. Yeast extract and strain impact on volatile compounds profile

The relationship between YE and main volatile compounds was elucidated by PCA (Fig. 5). A total of 30 compounds including 28 aroma compounds listed in Table 3, and two compounds (ethanol, acetic acid) listed in Table 1 were applied for PCA with the first two principal components (PC1, PC2) accounting for 89.37% of the total variance

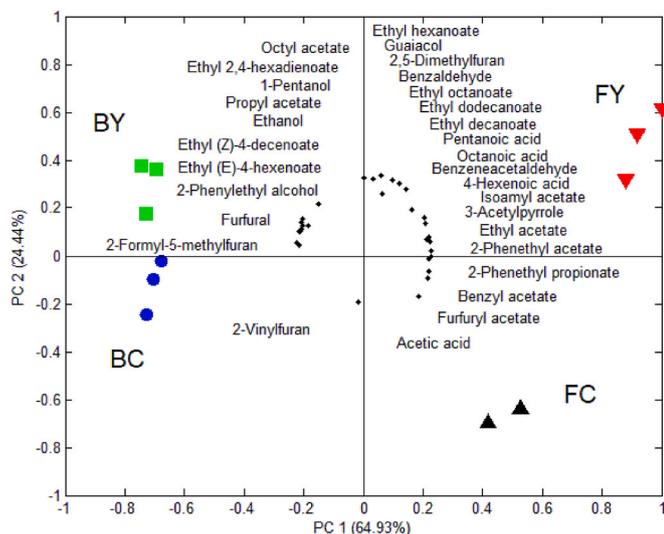


Fig. 5. Principal component analysis (PCA) of SCG hydrolysates with/without added yeast extracts at day 14. The data of ethanol and acetic acid in Table 1 were also used in PCA analysis. BC (●): SCG hydrolysates with inoculation of *T. delbrueckii* Biodiva with 0 % (w/v) yeast extracts; BY (■): SCG hydrolysates with inoculation of *T. delbrueckii* Biodiva with 0.25 % (w/v) yeast extracts; FC (▲): SCG hydrolysates with inoculation of *P. kluyveri* FrootZen with 0 % (w/v) yeast extracts; FY (▼): SCG hydrolysates with inoculation of *P. kluyveri* FrootZen added with 0.25 % (w/v) yeast extracts.

(Fig. 5). PC1 held 64.93 % and distinguished the SCG hydrolysates inoculated with *P. kluyveri* FrootZen (FC, FY samples) from those inoculated with *T. delbrueckii* Biodiva (BC, BY samples).

Fermented SCG hydrolysates with or without YE were distinguished by PC2 accounting for 24.44 % of the total variance. SCG hydrolysate with 0.25 % (w/v) YE and inoculated with *T. delbrueckii* Biodiva was in the positive part of PC2, because there was more ethanol, 2-phenylethyl alcohol, furfural, ethyl 2,4-hexadienoate, octyl acetate, propyl acetate, ethyl (Z)-4-decenoate, ethyl (E)-4-hexenoate and 2-formyl-5methylfuran in BY samples than that in BC samples (Fig. 5). The SCG hydrolysates with YE and fermented with *P. kluyveri* FrootZen (FY samples) contained more ethyl dodecanoate, ethyl acetate, isoamyl acetate, furfuryl acetate, 2-phenethyl acetate, guaiacol, and 2-vinylfuran than that in FC samples. These aroma compounds could impart pleasant fruity and floral flavor to FY samples. The PCA results indicated that the supplementation of YE did modulate aroma compounds and the supplementation effects varied with yeasts.

4. Conclusions

The added YE enhanced the growth of *P. kluyveri* but not *T. delbrueckii* in SCG hydrolysates fermentation. The addition of YE also significantly improved the generation of succinic and lactic acids. More odor-active compounds such as short-chain esters were produced when YE was added. YE exhibited a more obvious effect on the fermentation performance (e.g. amino acids consumption) of *P. kluyveri* than that of *T. delbrueckii*. The addition of YE could be an efficient way to promote the oenological potential SCG hydrolysates fermentation by selected non-*Saccharomyces* yeasts so as to develop novel fermented SCG-based products and enable SCG valorization.

CRediT authorship contribution statement

Yunjiao Liu: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. **Yuyun Lu:** Conceptualization, Validation, Writing – review & editing, Supervision. **Shao Quan Liu:** Conceptualization, Validation, Resources,

Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crfs.2021.07.004>.

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