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Split batch and coculture fermentation to regulate the organic acids and flavor profile of fruit wine-a case study of *Prunus mume* Sieb. et Zucc (greengage) wine

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Abstract

The higher acidity in fruits compared with grapes was one of the main obstacles to producing high-quality fruit wines. In the present research, the effect of the split batch fermentation pattern on the metabolites of greengage wines was investigated. This fermentation pattern included whole fruit immersion in the sucrose and coculturing *Saccharomyces bayanus* Y4 with *Torulaspora delbrueckii* Y7. These results showed that the contents of total acidity and dominant organic acids were significantly reduced among the batches. Aliphatic compounds were decreased with the batches, while aromatic compounds that contributed to flavor improvement were increased oppositely. The acetate esters, ketones, and terpenes contents were significantly increased in the first batch of coculture. In addition, acidic stress largely affected the kinetics characteristics of greengage wine on a pilot scale. Interestingly, the increment of total acidity was closely associated with the rate of total sugar consumption, which was affected by microbial activities. The acidity content was significantly declined and distinct flavour profiles were formed in split batch and coculture fermentation of greengage wines. It provides a new avenue to enhance the flavor and quality of fruit wines.

Keywords: wine acidity; greengage; split batch; coculture; flavor metabolite

Practical Application: The split batch based on whole fruit maceration and coculturing *Saccharomyces* yeasts with non-*Saccharomyces*, are beneficial to reducing the pre-treatments of deacidification and retaining the fruity aroma, which contributes to improving the quality and development of the fruit wines industry.

1 Introduction

Fruit wines are brewed from various fruits, except grapes, which not only have sensory characteristics but also is beneficial to health due to their rich secondary metabolites that enhance the unique nutrition function (Lu et al., 2022; Luo et al., 2022). Thus, it has been developing in many countries in the last decade (Velić et al., 2018). In contrast to the grapes, the acidity of other fruits is significantly higher (Miele, 2021; Velić et al., 2018), and the fruits may lose the unique varietal or "fruit" aroma when adjusting them for brewing fruit wines. Therefore, it is not suitable for the elaboration of high-quality fruit wines.

Prunus mume Sieb. et Zucc (greengage) is unique for its fruity aroma and is rich in organic acids (Han et al., 2020a), it is used to reduce fatigue and enhance appetite and shows the effect of antioxidant and antitumor (Jeong et al., 2006). It has been cultivated in East Asia for more than two thousand years, and the production of greengage wine could also be traced back to 1800 years ago (Zheng et al., 2014). The brewing technology included steeping and formulating wines. The varietal aroma is well retained in the former, but it is hard to coordinate it with the base wine aroma, and the fuel oil is higher (Zheng et al., 2014), while large amounts of exogenous additives are used in the latter. Fermented greengage wine gives a mellow and full mouthfeel. However, the acidic stress and antibacterial constituents in the fruit often lead to stuck fermentation and a low alcohol content compared with other fruit wines. So far, it is still challenging to solve the deacidification and reduce the influence of tannins on fermentation (Han et al., 2020b).

The whole grape fermentation wine based on carbonic maceration is effective to lower tannins and enhance the intensity of fruity aroma (Baiano et al., 2016). Split batch fermentation (SBF) is utilized in Chinese fermentation foods, such as vinegar and soy sauce to reduce the seed culture and enhance fermentation productivity (Jia, 2018). In contrast to the traditional batch fermentation of fruit wines, SBF is a valuable measure to eliminate substrate inhibition and helps to release constituents gradually. After SBF, most of the contents except core and peel are leached, which contributes to the full extraction of bioactive constituents in fruits.

Coculture of *Saccharomyces* yeasts with non-*Saccharomyces* has been applied to wine production, and the contents of terpenes, acetate and ethyl esters are increased for improving the quality

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(Izquierdo Cañas et al., 2014). Of the non-*Saccharomyces* used, *Torulaspora delbrueckii* is widely exploited since the contents of various flavor metabolites are enhanced, such as phenethyl alcohol, acetate and ethyl esters, *etc*. (Liu et al., 2018; Loira et al., 2014). However, there are no reports on the greengage fermentation technology based on combing SBF with the coculture of *Saccharomyces* yeasts with non-*Saccharomyces*.

This study investigated the influence of SBF and coculturing *Saccharomyces* yeast with *T. delbrueckii* on physicochemical properties, organic acids, and flavor metabolites for greengage wines. In addition, the fermentation technology was further validated on a pilot scale, and the kinetics and flavor profile were evaluated. It contributed to relieving acidic stress, regulating the flavor profile, and building a basis to develop high-quality fruit wines.

2 Materials and methods

2.1 Materials and strains

Fruits were purchased from the local greengage plantation in Dayi County, Chengdu City, Sichuan Province, China, with high maturity. The standards of organic acids, methyl octanoate, and 2-octanol were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Other chemicals were purchased from Jinshan Chemical Test Co. (Chengdu, China), and were anhydrous except $CuSO_4 \cdot 5H_2O$ and $C_4H_4O_6KNa \cdot 4H_2O$.

Saccharomyces bayanus Y4 and *T. delbrueckii* Y7 were isolated from the mash of the fruit wine factory (Chengdu, China) and identified according to the results of physiological and biochemical experiments, cell and colony morphology, ITS sequencing, and preservation in our laboratory. *S. bayanus* Y4 (CCTCCM2019522) and *T. delbrueckii* Y7 (CCTCCM2019523) were also preserved in the China Center for Type Culture Collection (Wuhan, China).

Inoculation of fermentation was performed by either single culture or coculture. For the single culture, the suspensions of *S. bayanus* Y4 were inoculated into the greengage maceration mash by the initial concentrations of 3×10^6 CFU/mL. While for the coculture, the suspensions of *T. delbrueckii* Y7 were simultaneously inoculated with *S. bayanus* Y4 at a ratio of 1:1, and the initial concentrations for each strain in the mash were 3×10^6 CFU/mL.

2.2 The elaboration of greengage wines by SBF and coculturing S. bayanus Y4 with T. delbrueckii Y7

Fresh fruits (5.0 Kg) were washed without sulphiting and pasteurization, then mixed with the sugar layer by layer in a 15 L fermenter, with a ratio of 3.75:1 (w/w). Solid maceration was carried out at 25 °C for 2 days. After the sugar was melted, $(NH_4)_2HPO_4$ (150 mg/L) was added to the maceration mash, and pretreated cultures of yeasts by single culture and coculture were inoculated, respectively, and the fermentation was performed at 25 °C. After the alcohol reached about 10%vol, fresh wine was separated from the fruits. After that, the separated fruits of the first batch were blended with the 28% syrup (w/w) at a rate of 1:1 (w/w) and 150 mg/L $(NH_4)_2HPO_4$ was added into the mash, and no inoculation. The above operation was duplicated for the third batch, while the syrup was 23% (w/w). $NaHSO_3$ (120 mg/L) was supplemented immediately to fresh wine of each batch to end fermentation, and fresh wines were stored in the full and sealed tank for further analysis. The physicochemical properties were monitored during the fermentation.

To further validate the technology of SBF and coculture, the fresh fruits and sugar were added into an 8 m^3 fermentation pool (2744 Kg and 750 Kg), the fermentation was carried out by SBF and coculture, and under ambient (ca. 25 °C) at Sichuan Meihe Wine Industry Ltd. located in Chengdu City (Sichuan Province, China).

2.3 Physicochemical indices and organic acids analyzing

The contents of total sugar, total acidity, and alcohol were evaluated according to the National Standard (Standardization Administration of China, 2006). Total sugar and total acidity were titrated by Fehling reagent and NaOH solution, respectively, while the alcohol was determined by distillation and alcohol meter. The contents of organic acids were examined according to the method of Cocchi et al. (2002). Briefly, samples were centrifuged at 9,338×*g* for 10 min at 4 °C, the supernatant was purified by a solid-phase extraction column and filtered by a 0.22 μm filter. After that, the filtered samples were detected by the HPLC (1260, Agilent Technologies Inc., Santa Clara, CA, USA) combined with an organic acid column (Alltech OA-1000, 300 mm×6.5 mm, Alltech Associates Inc., Columbia, MD, USA). The chromatographic conditions were as follows: a UV detector with a wavelength of 215 nm, the column temperature was 75 °C, the mobile phase was 9 mM H_2SO_4, and the flow rate was 0.6 mL/min. The qualitative and quantitative analyses of organic acids were based on the retention time and calibration curve of the standards.

2.4 Volatile constituent analyzing

HS-SPME-GC–MS was performed to analyze the volatile constituent according to the method of Niu et al. (2017). GC–MS: Trace GC Ultra-DSQ II, (Thermo Electron Corp., Waltham, MA, USA) equipped with HP-Innowax (30 m ×0.25 mm ×0.25 μm, Agilent Technologies Inc.). HS-SPME was performed with DVB/CAR/PDMS fiber (Supelco Inc., Bellefonte, PA, USA). Volatile constituents were identified by comparing mass spectrometry with the standard library of the National Institute of Standards and Technology (2005) (Liu et al., 2020). Kováts retention indices (RI) that were calculated by C_8–C_{20} n-alkanes, were further verified with those reported in the literature. The area of methyl octanoate and 2-octanol in contrast to the total ion chromatogram was calculated to obtain the semiquantitation of volatile constituents.

2.5 Statistical analysis

The analysis was repeated three times, and the results were expressed as mean ± standard deviation. The difference analysis between the results was based on the one-way analysis of variance (ANOVA) and Duncan's multiple comparison test by IBM SPSS Statistics 25 (IBM Corp., Armonk, NY, USA), and $p < 0.05$ represented statistical significance. Odor activity value

(OAV) was calculated by dividing volatile constituent concentration by volatile odor threshold. Linear discriminant analysis Effect Size (LEfSe) was used to evaluate the volatile constituent differences between samples, and it was carried out on the Galaxy website (Afgan et al., 2018). Principal component analysis (PCA) was carried out in R 3.6.1. The kinetics characteristics were performed by Origin 2021b (OriginLab Corp., Northampton, MA, USA). Partial least square-discrimination analysis (PLS-DA) was employed to assess the correlation between volatile constituents and fermentation patterns by Simca 14.1 (Mks Umetrics Ab Corp., Malmö, Sweden).

3 Results and discussion

3.1 Differences in the metabolite profiles of SBF greengage wines

The total acidity was significantly reduced among the batches (Figure 1). Most of the organic acids were citric acid and L-malic acid, which originated from the fruits (Liu et al., 2021), and they changed similarly to that total acidity. Succinic acid and lactic acid synthesized by the yeasts were enhanced in the third batch, and their proportion was increased while the citric acid was decreased by 19.10–19.99%. Organic acids were naturally modified by microbial metabolism (Benito et al., 2013; Tristezza et al., 2016), but the changes in concentrations and varieties were limited. Thus, it was essential to develop a new process to ensure smooth fermentation under extreme conditions. For instance, the citric acid, L-tartaric acid, and L-ascorbic acid in prickly pear wine were 7.09, 4.77, and 0.05 mg/mL, while their contents in the prickly pear and Lantana camara blending wine were only 4.74, 1.06, and 0.12 mg/mL, which indicated that the organic acids were significantly influenced by the processes (Tsegay, 2020).

Fifty volatile constituents were identified in these samples, and total volatiles ranged from 3.62 mg/L to 4.65 mg/L (Table S1). They could be divided into six classes according to their chemical structures, including esters (26), alcohols (10), acids (6), aldehydes (2), ketones (4), and terpenes (2). The proportion of esters and aldehydes increased with the batches, but the acids and terpenes decreased. SBF significantly affected the species and contents of the volatiles. For example, twenty-nine, twenty-four, and thirty volatiles were identified among each batch of the single culture. In contrast to the first batch, ethyl lactate, ethyl dodecanoate, methyl salicylate, triethyl citrate, pentanol, hexanol, 3,4-dehydro-β-ionone, and linalool were undetected, while ethyl 3-hexenoate, tributyl phosphate, and isobutanol were newly examined in the second batch. Ethyl hexanoate, ethyl dodecanoate, methyl salicylate, triethyl citrate, and pentanol disappeared, but benzyl acetate, ethyl 9-decenoate, tributyl phosphate, isoamylol, 1,2-propanediol, and benzoic acid were newly detected in the third batch.

The ester contents in the last two batches of the single culture were enhanced by 25.51% and 26.91% compared to the first batch, due to the increment of ethyl benzoate by 2.46- and 3.86-fold, respectively. The fermentation aroma was weaker with the batches, but the varietal aroma was enhanced. For instance, ethyl hexanoate, ethyl lactate, ethyl succinate, ethyl glutarate, and ethyl dodecanoate decreased with the batches and were 21.32% and 78.13% higher in the first batch than in the second and third batches, respectively. The reduction of ethyl ester contents may attribute to the changes in the substrate nutrients, which were adverse to the overall metabolism of yeasts. However, the contents of benzyl acetate and ethyl benzoate in the third batch, were 3.88- and 0.41-fold higher than the first and second batches, respectively, which contributed to the varietal aroma (Kameoka & Kitagawa, 1976).

The alcohol contents of single culture reached 1.24–1.99 mg/L, and significant differences in alcohol constituents were observed among each batch. Pentanol, hexanol, and α-ionol were decreased

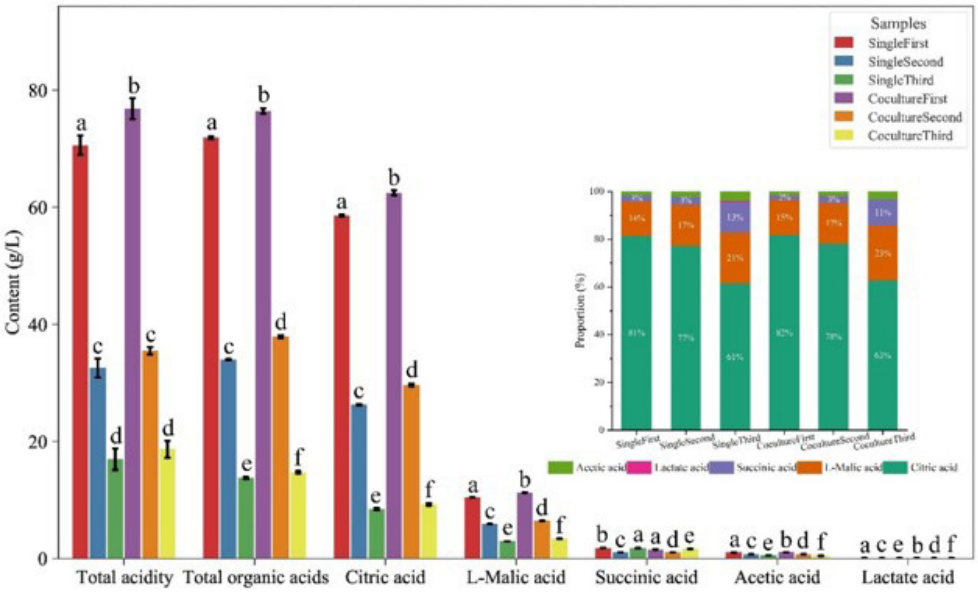

Figure 1. Total acidity and organic acids profiles of greengage wines fermented by split batch and coculturing *Saccharomyces bayanus* Y4 with *Torulaspora delbrueckii* Y7. Different letters on the graph indicate significant differences ($p < 0.05$, n = 3).

with the batches, while benzyl alcohol was increased. Among them, pentanol was not detected in the second and third batches. Benzyl alcohol gave the varietal aroma (Kameoka & Kitagawa, 1976), it was increased by 3.02- and 4.36-fold in the second and third batches, respectively, which led to high alcohol content. The increment in aroma volatile content might be related to the enzymes released by microorganisms (Padilla et al., 2016).

The acid constituents of the single culture decreased with the batches and ranged from 310.08 μg/L to 526.08 μg/L. The contents of fatty acids (hexanoate acid, octanoate acid, and decanoate acid) were decreased with batches. These volatile contents decreased by 38.63% and 58.31% in the second and third batches, respectively, but benzoic acid was only detected in the third batch. In addition, the contents of terpenes for single culture, such as linalool and α-terpineol, were decreased with batches, while benzaldehyde imparted varietal aroma was enhanced by 126.99% and 153.08% in the second and third batches, respectively. Typically, the varietal aroma was lower than the fermentative aroma in wines, but varietal constituent contents were increased among the batches for greengage wines. Thus, various flavor profiles were established in the SBF.

3.2 Differences in the metabolite profiles of coculture fermented greengage wines

Coculture significantly affected the species and contents of volatiles, but mainly in the first batch. Among the SBF, the contents of acetate esters, ketones, and terpenes were significantly increased in the first batch, which was in agreement with the results of high acidities fruit wines (Chen & Liu, 2016; Liu et al., 2020), and the contents of acids and aldehydes were decreased in the second batch, while only the acids were reduced in the third batch. These results revealed a continuous effect of coculture on volatile profiles in SBF. As such, the flavor profiles of greengage wines were developing. In addition, forty-five, twenty-six, and twenty-two volatiles were detected for coculture among three batches, respectively. Two volatiles of coculture disappeared, and eighteen volatiles including fifteen esters were newly detected in the first batch of coculture. The undetected and newly found volatiles were three and five in the second batch, while eight and zero in the third batch, respectively.

Acetate ester contents increased from 5.42 μg/L to 50.83 μg/L and ethyl esters enhanced from 1641.43 μg/L to 2232.92 μg/L in the first batch of coculture, in which benzyl acetate, phenethyl acetate, ethyl octanoate, ethyl decanoate, ethyl 9-decenoate, ethyl myristate, and ethyl hexadecanoate were newly found. The content of ethyl hexanoate was enhanced by 101.28%. The positive interaction between *S. bayanus* and *T. delbrueckii* contributed to the enhanced synthesis of flavor metabolites (Renault et al., 2015). However, differences in volatile contents in the second and third batches were minor, in which the content of ethyl benzoate was decreased by 22.13%, ethyl hexanoate disappeared, and ethyl lactate and ethyl 9-decenoate were newly detected in the second batch.

Significant differences in alcohol constituents were only observed in the first batch, the alcohol contents for coculturing enhanced from 1.24 mg/L to 1.45 mg/L, in which pentanol was undetected, isobutanol, isoamylol, and 1-nonanol were newly detected, and α-ionol increased by 78.57%. In addition, isobutanol disappeared, isoamylol and hexanol were newly detected in the second batch. The acid contents of coculture in the second and third batches decreased by 31.98% and 61.46%, respectively, which was attributed to the reduction of specific acid constituents. Among them, the contents of hexanoic acid and decanoic acid decreased by 22.43% to 33.49% in the second batch. While hexanoic acid, decanoic acid, and benzoic acid disappeared in the third batch.

The contents of ketones and terpenes increased by 402.44% and 39.16% in the first batch of coculture, respectively, due to the increment of β-ionone and α-terpineol. The significant increment of flavor constituents in the first batch of coculture revealed the excellent tolerance of *T. delbrueckii* to acidic stress, but the decreased initial concentrations had an adverse influence on the continued survival of non-*Saccharomyces* (Bagheri et al., 2015), which limited the synthesis of flavor metabolites in the second and third batches.

Forty-four volatiles contents have significant differences among samples based on the LEfSe analysis (Figure 2), in which two acetate esters, nine ethyl esters, and one methyl ester, β-ionone, neroliacetone, linalool, and α-terpineol were significantly increased in the first batch of coculture. In contrast, octanoic acid and decanoic acid were increased in the first batch of the single culture. The aromatic compounds and alcohols were enhanced in the third batch of coculture, including benzyl acetate, ethyl benzoate, benzaldehyde, benzyl alcohol, and isoamylol.

The PCA analysis showed that different samples were separated based on the specific volatile profiles (Figure 3). PC1 and PC2 explained the variation of 71.5% and 20.8%, respectively. The first batch of coculture was related to ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl 9-decenoate, phenethyl acetate, β-ionone, and linalool, and the third batch was related to isoamyl acetate and ethyl benzoic.

There were six volatiles with OAV>1 that contributed greatly to the overall aroma of greengage wines (Table S1). Among these, the first batch of coculture has the highest OAV except for ethyl benzoic, ethyl octanoate (49.09), ethyl hexanoate (17.86), and linalool (6.53) giving a strong fruity and floral aroma. In addition, the OAV of ethyl benzoic was increased, and others were decreased with the batches, which imparted a fruity, sweet, and citrus aroma to the first batch. In contrast, the third batch had a fruity and medicinal aroma.

3.3 Differences in kinetics and metabolites profiles of greengage wines on a pilot scale

The total sugar consumption of the first batch was rapid in the first 4 days and became slower between the 4th day and 8th day, and it got stable after the 8th day (Figure 4a). The alcohol accumulation was fast in the first 4 days, and became sluggish between the 4th day and 7th day, but it got delayed between the 7th day and 12th day and became stable subsequently. Total acidity increment was fast in the first 4 days and became slower between the 4th day and 9th day, and it got stable subsequently. Interestingly, even though the total acidity of greengage wine was

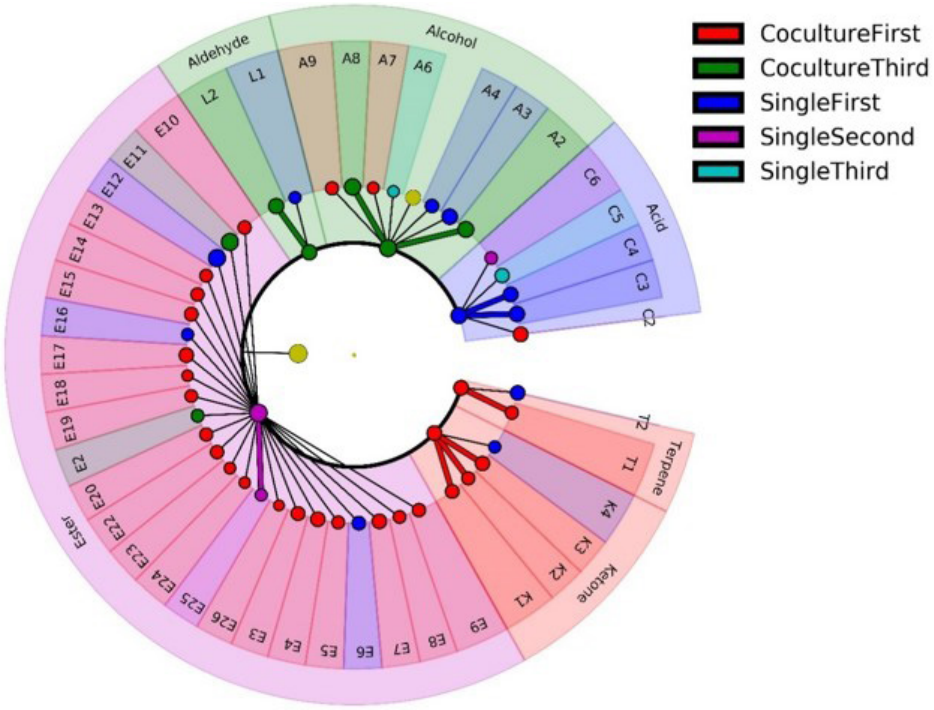

Figure 2. Linear discriminant analysis Effect Size (LEfSe) analysis of volatile constituents in greengage wines fermented by split batch and coculturing *Saccharomyces bayanus* Y4 with *Torulaspora delbrueckii* Y7 (Linear discriminant analysis value (LDA)>2, $p < 0.05$). The volatiles used for analysis were listed in Table S1.

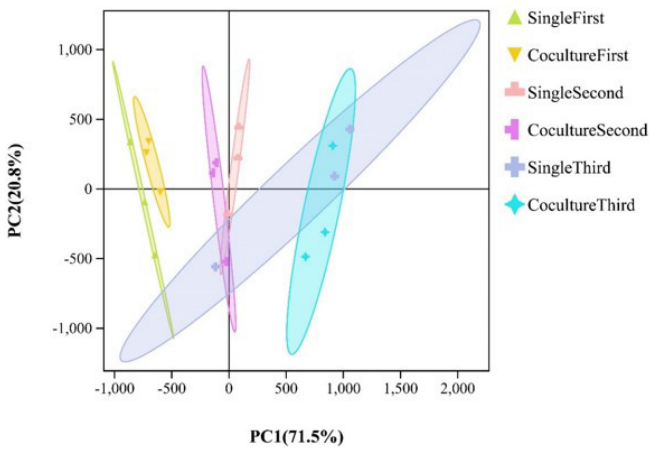

Figure 3. Principal component analysis (PCA) analysis of volatile constituents in greengage wines fermented by split batch and coculturing *Saccharomyces bayanus* Y4 with *Torulaspora delbrueckii* Y7 (Odor activity value (OAV)>0.1).

related to the fruit organic acids, its increment was also associated with the rate of total sugar consumption, which was affected by microbial activities. Based on the wine fermentation rate, the yeasts' growth cycle could be divided into the proliferation, stable, and decline stages (Lafon-Lafourcade et al., 1979), and the large increment of total acidity was before the decline stage of the yeasts. In addition, the total sugar of the second batch dropped rapidly in the first 7 days (Figure 4b). It became stable subsequently, and the changes period of alcohol and total acidity corresponded to the total sugar. The rate of total sugar consumption, alcohol accumulation, and total acidity increment in the third batch was fast in the first 5 days (Figure 4c), and it declined subsequently until the end of fermentation.

The kinetics characteristics were similar to that of the single culture of traditional batch fermentation (Sener et al., 2007), due to an intense competition of *Saccharomyces* yeasts over non-*Saccharomyces* at the late stage of alcohol fermentation. However, the residual sugar at the end of each batch was higher, which indicated that the end of fermentation was not induced by the consumption of total sugar. Even though yeast metabolism was influenced by the high concentrations of sugar and alcohol, the kinetics characteristics were mainly affected by the acidic stress. The fruit specifics and maceration patterns among each batch also led to differences in kinetics characteristics. Among the SBF, the first batch was markedly affected by the acidic stress. Its alcohol content before the yeast decline stage (4th day) reached 5.3% in contrast to 11.5% of the fresh wine (Figure 4a). After that, the growth of yeasts was stopped, and the rate of total sugar consumption, alcohol accumulation, and total acidity increment was dropped. Similarly, the alcohol content reached 10.9% (6th day) compared to 11.8% of the second batch (Figure 4b), while it reached 8.4% (5th day) in contrast to 12.5% of the third batch (Figure 4c). The acidic stress was the lowest in the third batch, but the contents of nitrogen, lipids, and minerals in the fermentation substrates also declined.

The acidic stress of greengage mash on yeasts usually led to a stuck of liquid batch fermentation, while the SBF based on whole fruit maceration was beneficial to regulate the acidity

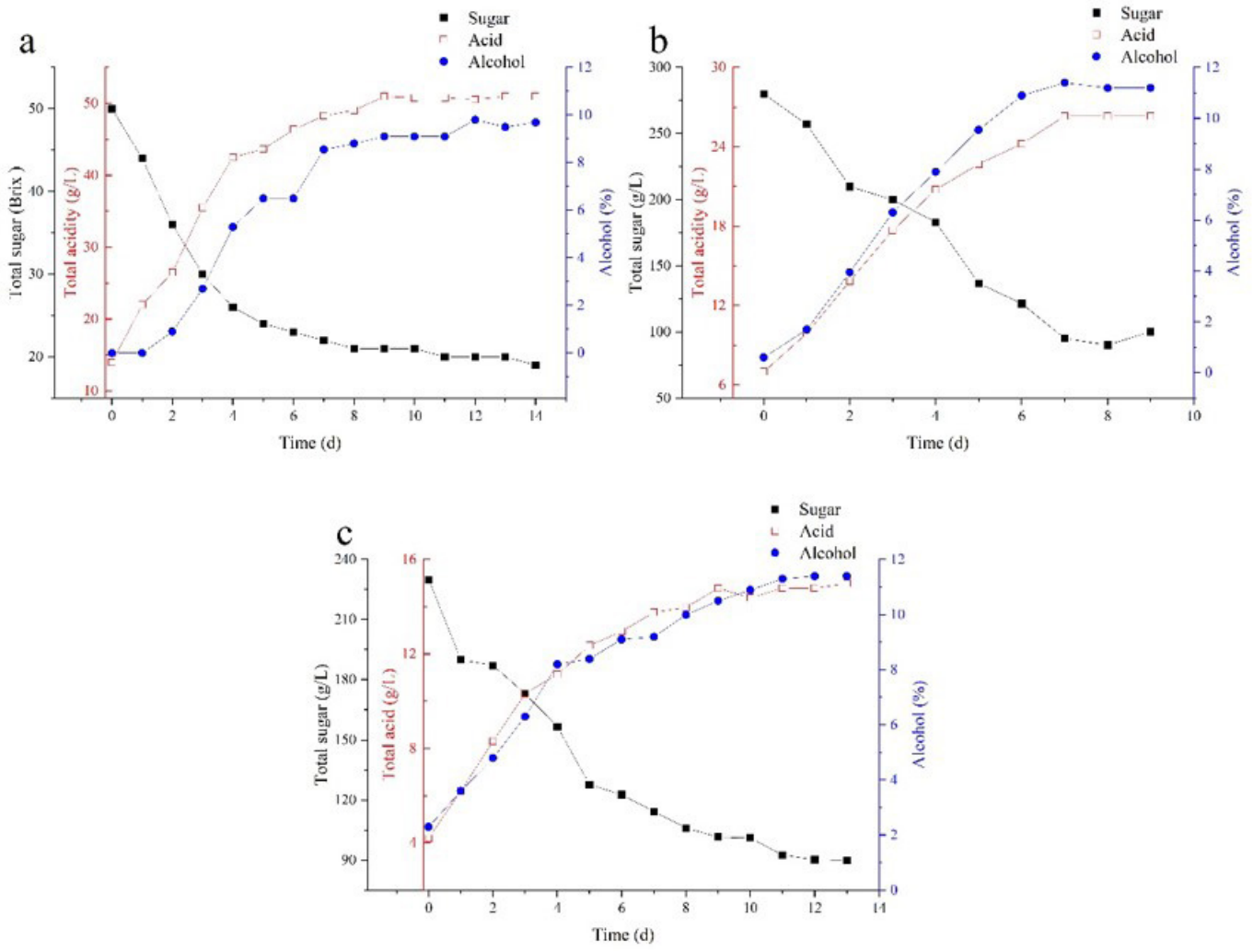

Figure 4. Kinetics characteristics of greengage wines on a pilot scale. (a) first batch, (b) second batch, (c) third batch.

and smooth fermentation. As the kinetic curves demonstrated, the total acidity of greengage wine was affected not only by the fruits but also by yeast metabolism. Total acidity content was associated with the fruit organic acids diffusion and solvent (water) penetration during SBF, and the rate of total acidity increment was related to the yeast growth cycle. For example, the total acidity of the first batch before the yeast decline stage compared with the initial was increased by 203.64%, while the end of fermentation compared with the former was only raised by 19.90% (Figure 4a). Similarly, the second batch was 246.14% and 8.67% (Figure 4b), while the third batch was 194.29% and 21.28% (Figure 4c), respectively. The diffusion rate of organic acids was positively related to the diffusion coefficient, diffusion area, and concentration gradient, while the diffusion coefficient was a positive correlation with temperature and negatively correlated with particle diameter and medium viscosity (Chen et al., 2007). With a high rate of total sugar consumption before the yeast decline stage, the fermentation temperature raised and mash viscosity dropped considerably, and the diffusion coefficient of solute in water solution could be enhanced by 2.0–3.5% when the temperature was increased by 1 °C. Therefore, the rate of total acidity increment was higher. In addition, the penetration rate was a positive correlation with temperature and solution concentration. Before the yeast decline stage, the fermentation temperature was raised due to the vigorous metabolism, the penetration rate of fruit water molecules and the folds on the fruit surface were increased, and the diffusion area of organic acids and diffusion rate were also enhanced. These results revealed that a shorter period before the yeast decline stage and a lower temperature were all beneficial to regulate the acidity in SBF. Schmidt found that the pH and potassium concentration of Chardonnay must were the key factors affecting the fermentation kinetics of *S. cerevisiae*, and the fermentation may not be ideal even though other parameters such as yeast assimilable nitrogen were optimized (Schmidt et al., 2011).

The contents of total acidity and dominant organic acids were also significantly decreased on a pilot scale. The contents of alcohol, succinic acid, and propionic acid were increased in contrast to the lab scale (Table S2), while the total acidity and L-malic acid were decreased in the second and third batches,

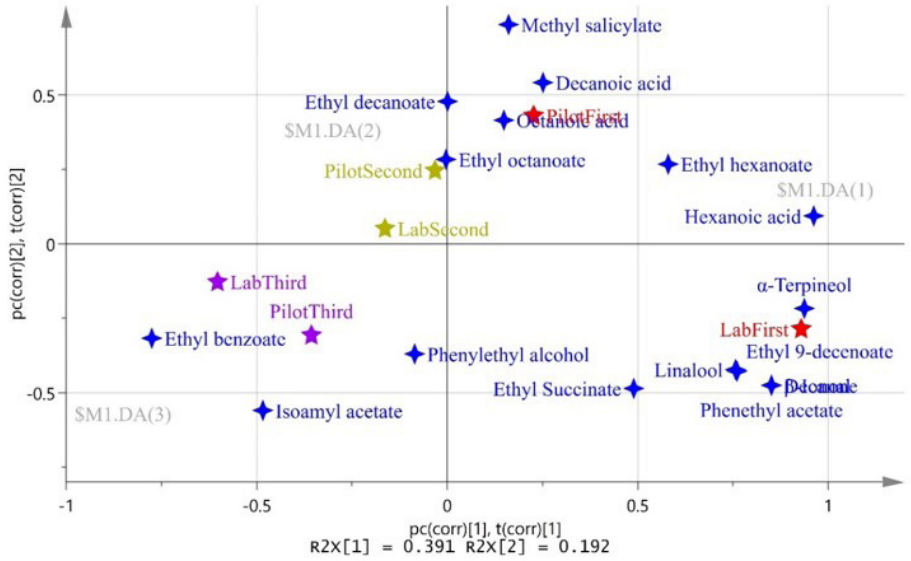

Figure 5. Partial least square-discrimination (PLS-DA) analysis of volatile constituents in greengage wines on a pilot scale (Odor activity value (OAV)>0.1).

and total acidity content was reduced, it may be related to the large specific surface area of fermentation pool, which facilitated the fruits to be floated. In addition, a total of thirty-nine volatiles were identified in the greengage wines fermented on a pilot scale (Table S2), and the total volatiles ranged from 5.66 mg/L to 6.88 mg/L, including esters (20), alcohols (7), acids (6), aldehydes (2), ketones (1), phenols (2), and terpenes (1). The change law of the volatiles profile was consistent with the lab scale. However, there were differences in the contents of esters, alcohols, and acids.

The contents of fatty acid ethyl esters were decreased with the batches, and ethyl benzoic was increased, but the reduction of ethyl hexanoate, ethyl octanoate, and ethyl decanoate was smaller than that of the lab scale. Among them, ethyl octanoate, ethyl decanoate, and methyl salicylate were increased by 2.01-, 6.22- and 8.23-fold in the first batch, while ethyl hexanoate, ethyl octanoate, ethyl decanoate, and methyl salicylate were newly found in the second and third batches. Ethyl ester contents were influenced by yeast, temperature, aeration, and sugar concentration (Duarte et al., 2010). The increment of ethyl esters may associate with a high temperature due to the fruit accumulation above the mash and difficulty to emit the heat immediately, as well as the enhancement of dissolved oxygen in the mash by pumping.

The fatty alcohol contents decreased with the batches, while benzyl alcohol and phenylethyl alcohol increased resulting in high alcohol content, and benzyl alcohol in the third batch was increased by 42.96% compared with the lab scale. Isoamyl alcohol, benzyl alcohol, and phenethyl alcohol were dominant, both in the two fermentation scales. The high fermentation temperature led to an increment of isoamyl alcohol (Pérez et al., 2018).

Similar to the lab scale, fatty acids were decreased with batches, but the contents of mid-chain fatty acids (hexanoic acid, octanoic acid, and decanoic acid) were increased by 3.65- and 15.72-fold in the second and third batches, respectively. The fatty acid contents were significantly affected by the fermentation temperature when the strains and raw materials were consistent (Molina et al., 2009). As the availability of fatty acids was the main limiting factor for the biosynthesis of fatty acid ethyl esters (Rollero et al., 2015), therefore, the fatty acids and corresponding ethyl esters were all enhanced.

PLS-DA analysis of volatiles showed that two fermentation scales shared a similar volatile profile in SBF and coculture (Figure 5), and PC1 and PC2 explained 58.3% of the total variations. Among them, the first batch was associated with phenethyl acetate, methyl salicylate, fatty acids (hexanoic acid, octanoic acid, and decanoic acid) and their corresponding ethyl esters, α-terpineol, linalool, β-ionone, and decanal, and the third batch was related to ethyl benzoate, isoamyl acetate, and phenethyl alcohol.

4 Conclusions

The content of total acidity and the dominant organic acids were significantly reduced among the batches. SBF affected the species and contents of specific volatiles. Among them, the contents of aliphatic compounds, which gave fermentation aroma, were decreased with the batches, while aromatic compounds with varietal aroma were enhanced. Coculture mainly enhanced the acetate esters, ketones, and terpenes contents of the first batch. In addition, kinetics characteristics were greatly influenced by the acidic stress, and it was interesting that the increment of total acidity was closely related to the rate of total sugar consumption. The change law of flavour metabolites was consistent between the pilot and lab scales, but there were differences in their contents attributed to the variations of the fermenter, temperature, and dissolved oxygen.

Conflict of interest

The authors declare no conflicts of interest.

References

Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Cech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B. A., Guerler, A., Hillman-Jackson, J., Hiltemann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., & Blankenberg, D. (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Research*, 46(W1), W537-W544. http://dx.doi.org/10.1093/nar/gky379.

Bagheri, B., Bauer, F. F., & Setati, M. E. (2015). The diversity and dynamics of indigenous yeast communities in grape must from vineyards employing different agronomic practices and their influence on wine fermentation. *South African Journal of Enology and Viticulture*, 36(2), 243-251.

Baiano, A., Scrocco, C., Sepielli, G., & Del Nobile, M. A. (2016). Wine processing: a critical review of physical, chemical, and sensory implications of innovative vinification procedures. *Critical Reviews in Food Science and Nutrition*, 56(14), 2391-2407. http://dx.doi.org/10.1080/10408398.2013.842886. PMid:25629416.

Benito, S., Palomero, F., Morata, A., Calderón, F., Palmero, D., & Suárez-Lepe, J. A. (2013). Physiological features of *Schizosaccharomyces pombe* of interest in making of white wines. *European Food Research and Technology*, 236(1), 29-36. http://dx.doi.org/10.1007/s00217-012-1836-2.

Chen, D., & Liu, S. Q. (2016). Impact of simultaneous and sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on non-volatiles and volatiles of lychee wines. *Lebensmittel-Wissenschaft + Technologie*, 65, 53-61. http://dx.doi.org/10.1016/j.lwt.2015.07.050.

Chen, J., Xia, W., & Chen, Z. (2007). Pickling fermentation and smoking of food. In W. Xia (Ed.), *Food technology* (pp. 187-190). Beijing: China Light Industry Press.

Cocchi, M., Lambertini, P., Manzini, D., Marchetti, A., & Ulrici, A. (2002). Determination of carboxylic acids in vinegars and in Aceto Balsamico Tradizionale di Modena by HPLC and GC methods. *Journal of Agricultural and Food Chemistry*, 50(19), 5255-5261. http://dx.doi.org/10.1021/jf020155l. PMid:12207457.

Duarte, W. F., Dias, D. R., Oliveira, J. M., Vilanova, M., Teixeira, J. A., e Silva, J. B. A., & Schwan, R. F. (2010). Raspberry (*Rubus idaeus* L.) wine: yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds. *Food Research International*, 43(9), 2303-2314. http://dx.doi.org/10.1016/j.foodres.2010.08.003.

Han, X., Peng, Q., Yang, H., Hu, B., Shen, C., & Tian, R. (2020a). Influence of different carbohydrate sources on physicochemical properties and metabolites of fermented greengage (*Prunus mume*) wines. *Lebensmittel-Wissenschaft + Technologie*, 121, 108929. http://dx.doi.org/10.1016/j.lwt.2019.108929.

Han, X., Zhao, Y., Hu, B., Yang, H., Peng, Q., & Tian, R. (2020b). Influence of different yeast strains on the quality of fermented greengage (*Prunus mume*) alcoholic beverage and the optimization of fermentation conditions. *Lebensmittel-Wissenschaft + Technologie*, 126, 109292. http://dx.doi.org/10.1016/j.lwt.2020.109292.

Izquierdo Cañas, P. M., García-Romero, E., Heras Manso, J. M., & Fernández-González, M. (2014). Influence of sequential inoculation of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae* in the quality of red wines. *European Food Research and Technology*, 239(2), 279-286. http://dx.doi.org/10.1007/s00217-014-2220-1.

Jeong, J. T., Moon, J. H., Park, K. H., & Shin, C. S. (2006). Isolation and characterization of a new compound from *Prunus mume* fruit that inhibits cancer cells. *Journal of Agricultural and Food Chemistry*, 54(6), 2123-2128. http://dx.doi.org/10.1021/jf0523770. PMid:16536585.

Jia, S. (2018). *The map of traditional fermented food in China*. Beijing: China Light Industry Press.

Kameoka, H., & Kitagawa, C. (1976). The constituents of the fruits of *Prunus mume* Sieb. et Zucc. *Nippon Nogeikagaku Kaishi*, 50(9), 389-393. http://dx.doi.org/10.1271/nogeikagaku1924.50.9_389.

Lafon-Lafourcade, S., Larue, F., & Ribereau-Gayon, P. (1979). Evidence for the existence of "survival factors" as an explanation for some peculiarities of yeast growth, especially in grape must of high sugar concentration. *Applied and Environmental Microbiology*, 38(6), 1069-1073. http://dx.doi.org/10.1128/aem.38.6.1069-1073.1979. PMid:16345473.

Liu, J., Liu, M., Ye, P., He, C., Liu, Y., Zhang, S., Huang, J., Zhou, J., Zhou, R., & Cai, L. (2021). Characterisation of the metabolite profile and microbial community of repeated batch and coculture-fermented greengage wine. *Process Biochemistry*, 109, 117-129. http://dx.doi.org/10.1016/j.procbio.2021.06.016.

Liu, J., Liu, M., Ye, P., Lin, F., Huang, J., Wang, H., Zhou, R., Zhang, S., Zhou, J., & Cai, L. (2020). Characterization of major properties and aroma profile of kiwi wine co-cultured by *Saccharomyces* yeast (*S. cerevisiae, S. bayanus, S. uvarum*) and *T. delbrueckii*. *European Food Research and Technology*, 246(4), 807-820. http://dx.doi.org/10.1007/s00217-020-03439-6.

Liu, S., Laaksonen, O., Kortesniemi, M., Kalpio, M., & Yang, B. (2018). Chemical composition of bilberry wine fermented with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii* and *Schizosaccharomyces pombe*) and *Saccharomyces cerevisiae* in pure, sequential and mixed fermentations. *Food Chemistry*, 266, 262-274. http://dx.doi.org/10.1016/j.foodchem.2018.06.003. PMid:30381185.

Loira, I., Vejarano, R., Bañuelos, M. A., Morata, A., Tesfaye, W., Uthurry, C., Villa, A., Cintora, I., & Suárez-Lepe, J. A. (2014). Influence of sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on wine quality. *Lebensmittel-Wissenschaft + Technologie*, 59(2), 915-922. http://dx.doi.org/10.1016/j.lwt.2014.06.019.

Lu, L., Mi, J., Chen, X., Luo, Q., Li, X., He, J., Zhao, R., Jin, B., Yan, Y., & Cao, Y. (2022). Analysis on volatile components of co-fermented fruit wines by *Lycium ruthenicum* murray and wine grapes. *Food Science and Technology (Campinas)*, 42, e12321. http://dx.doi.org/10.1590/fst.12321.

Luo, J., Fan, Z., Yang, X., Bao, Y., Liang, M., & Guo, Y. (2022). Anthocyanins and antioxidant activity of *Lonicera caerulea* berry wine during different processes. *Food Science and Technology (Campinas)*, 42, e25121. http://dx.doi.org/10.1590/fst.25121.

Miele, A. (2021). Wine composition of Merlot and Cabernet Sauvignon vine clones under the environmental conditions of Serra Gaúcha, Brazil. *Food Science and Technology (Campinas)*, 41(suppl 1), 116-122. http://dx.doi.org/10.1590/fst.10520.

Molina, A. M., Guadalupe, V., Varela, C., Swiegers, J. H., Pretorius, I. S., & Agosin, E. (2009). Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. *Food Chemistry*, 117(2), 189-195. http://dx.doi.org/10.1016/j.foodchem.2009.03.116.

Niu, M., Huang, J., Jin, Y., Wu, C., & Zhou, R. (2017). Volatiles and antioxidant activity of fermented Goji (Lycium Chinese) wine: effect of different oak matrix (barrel, shavings and chips). *International Journal of Food Properties*, 20(suppl 2), 2057-2069. https://doi.org/10.1080/10942912.2017.1362649.

Padilla, B., Gil, J. V., & Manzanares, P. (2016). Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Frontiers in Microbiology*, 7, 411. http://dx.doi.org/10.3389/fmicb.2016.00411. PMid:27065975.

Pérez, D., Assof, M., Bolcato, E., Sari, S., & Fanzone, M. (2018). Combined effect of temperature and ammonium addition on fermentation profile and volatile aroma composition of Torrontés Riojano wines. *Lebensmittel-Wissenschaft + Technologie*, 87, 488-497. http://dx.doi.org/10.1016/j.lwt.2017.09.020.

Renault, P., Coulon, J., de Revel, G., Barbe, J. C., & Bely, M. (2015). Increase of fruity aroma during mixed *T. delbrueckii*/*S. cerevisiae* wine fermentation is linked to specific esters enhancement. *International Journal of Food Microbiology*, 207, 40-48. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.04.037. PMid:26001522.

Rollero, S., Bloem, A., Camarasa, C., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J. M., Dequin, S., & Mouret, J. R. (2015). Combined effects of nutrients and temperature on the production of fermentative aromas by *Saccharomyces cerevisiae* during wine fermentation. *Applied Microbiology and Biotechnology*, 99(5), 2291-2304. http://dx.doi.org/10.1007/s00253-014-6210-9. PMid:25412578.

Schmidt, S. A., Dillon, S., Kolouchova, R., Henschke, P. A., & Chambers, P. J. (2011). Impacts of variations in elemental nutrient concentration of Chardonnay musts on *Saccharomyces cerevisiae* fermentation kinetics and wine composition. *Applied Microbiology and Biotechnology*, 91(2), 365-375. http://dx.doi.org/10.1007/s00253-011-3197-3. PMid:21476141.

Sener, A., Canbas, A., & Ünal, M. Ü. (2007). The effect of fermentation temperature on the growth kinetics of wine yeast species. *Turkish Journal of Agriculture and Forestry*, 31(5), 349-354.

Standardization Administration of China. (2006). *Analytical methods of wine and fruit wine* (GB/T 15038-2006). China: Standardization Administration of China.

Tristezza, M., Tufariello, M., Capozzi, V., Spano, G., Mita, G., & Grieco, F. (2016). The oenological potential of *Hanseniaspora uvarum* in simultaneous and sequential co-fermentation with *Saccharomyces cerevisiae* for industrial wine production. *Frontiers in Microbiology*, 7, 670. http://dx.doi.org/10.3389/fmicb.2016.00670. PMid:27242698.

Tsegay, Z. T. (2020). Total titratable acidity and organic acids of wines produced from cactus pear (*Opuntia-ficus-indica*) fruit and Lantana camara (*L. Camara*) fruit blended fermentation process employed response surface optimization. *Food Science & Nutrition*, 8(8), 4449-4462. PMid:32884725.

Velić, D., Velić, N., Amidžić Klarić, D., Klarić, I., Petravić Tominac, V., Košmerl, T., & Vidrih, R. (2018). The production of fruit wines – a review. *Croatian Journal of Food Science and Technology*, 10(2), 279-290. http://dx.doi.org/10.17508/CJFST.2018.10.2.19.

Zheng, X., Zhang, M., Fang, Z., & Liu, Y. (2014). Effects of low frequency ultrasonic treatment on the maturation of steeped greengage wine. *Food Chemistry*, 162, 264-269. http://dx.doi.org/10.1016/j.foodchem.2014.04.071. PMid:24874386.

Supplementary Material

Table S1. Physicochemical properties and volatile constituents (μg/L) of the split batch and coculturing Saccharomyces bayanus Y4 with Torulaspora delbrueckii Y7 fermented greengage wines.

Table S2. Physicochemical properties (g/L) and volatile constituents (μg/L) of the split batch and coculture fermented greengage wines on a pilot scale.