

## Article

# Study on the Kinetic Model of Mixed Fermentation by Adding Glutathione-Enriched Inactive Dry Yeast

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**Abstract:** In order to investigate the impact of glutathione-enriched inactive dry yeast (g-IDY) on the co-fermentation process of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae*, different contents of g-IDY (0, 20, 40, and 100 mg/L) were added to the simulated liquid for fermentation. The yeast quantity, reducing sugar content, and ethanol volume fraction in the fermentation system were determined every 24 h. Nonlinear fitting of the measured values was carried out using classical Logistic, SGompertz, Boltzmann, and DoseResp models. Additionally, the aroma components of the wine were analyzed by GC-MS. The results indicate that the Logistic model performs best in terms of yeast growth kinetics, whereas the DoseResp and Boltzmann models exhibit the same fitting performance for reducing sugar consumption, both superior to the Logistic model, and the Boltzmann model shows the best-fitting performance for ethanol production. All optimal models have fitting coefficients ( $R^2$  values) above 0.99, demonstrating that different contents of g-IDY can effectively complete fermentation. Furthermore, all three fitting models can effectively describe the fermentation process using g-IDY. The use of g-IDY can increase the content of ethyl phenylacetate and phenylethanol, which can be employed to enhance the aroma of wine.

**Keywords:** glutathione-enriched inactive dry yeast; volatile components; fitting model



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## 1. Introduction

Mixed fermentation, which involves the use of multiple yeast strains, can enhance the aroma of fermented food, overcome the limitations of single-strain fermentation, and improve food flavor and quality. Therefore, mixed fermentation has important applications in various fields, such as food, health care products, and medicine. Recently, mixed fermentation has shown some progress in improving wine aroma [1], including promoting pyranoanthocyanin formation in blueberry wine [2], improving flavor and taste [3,4], decreasing volatile acidity [5], and increasing the content of phenylethyl acetate [5,6].

*Torulaspora delbrueckii* is a commonly used non-*Saccharomyces* yeast [7]. It can improve the aroma complexity of wine by increasing the concentrations of the volatile compounds that give wine its fruity aroma through the enzymatic hydrolysis of aroma precursors [8,9], reducing volatile acidity, enhancing the color, and raising the total ester contents [5,10]. These effects improve the sensory profile of the wine. However, the impact of *T. delbrueckii* on wine aroma is dependent on its interaction with *Saccharomyces cerevisiae*, and sometimes the results are inconsistent or even contradictory [7,11–13]. In addition, negative interactions between wine yeast and non-*Saccharomyces* yeast may occur, potentially impacting wine quality or leading to fermentation delays in multistarter fermentation processes [12].

Therefore, it is of great value to select suitable combinations of non-*Saccharomyces* yeasts and *S. cerevisiae* and to seek deeper and more innovative strategies to enhance wine aroma.

Glutathione-enriched inactive dry yeast (g-IDY) is a yeast preparation that is produced by growing yeast in a high-sugar culture medium and applying a specific winemaking process [14]. Compared to traditional yeast preparations, g-IDY offers several advantages, such as enhancing antioxidant properties [15], improving color and aroma profiles [16,17], and promoting the growth of lactic acid bacteria [18]. In a recent study, Naselli et al. [15] demonstrated that the addition of g-IDY, in combination with *Metschnikowia pulcherrima* and *S. cerevisiae*, increased the content of six ester compounds above sensory thresholds, thereby enhancing the complexity of wine aroma. Alfonzo et al. [19] found that the presence of g-IDY noticeably impacted the aroma of wine, favoring the formation of 2-hydroxypropyl acetate and markedly increasing the content of 3-methyl-1-butanol compared to the control group. In another study, the addition of g-IDY dramatically increased the total phenol content and positively affected most amino acids in kiwi fruit wine [20]. These findings suggest that studying the effects of g-IDY on wine aroma components will help address the challenge of wine aroma improvement. However, current research on g-IDY primarily focuses on aging processes [21], aroma components [22], phenols [23], and SO<sub>2</sub> replacement [24]. Giménez used g-IDY with *M. pulcherrima* to prevent browning and reduce the use of SO<sub>2</sub> [24]. Nioi et al. demonstrated an improvement in the oxidative stability of a model wine solution added with two yeast derivatives as antioxidants [25].

There are few studies on the effects of g-IDY on microbial growth and metabolism during fermentation. In addition, in the fermentation of fruit wines, the formation and alteration of flavors are closely related to yeast quantity [26]. Therefore, the addition of g-IDY, combined with the study of wine fermentation dynamics, may offer new research ideas for the development of wine aroma components. However, the mechanism of the g-IDY effect on wine aroma components, especially the effect of g-IDY addition on fermentation kinetics, and the interaction between g-IDY and wine aroma components remain unclear.

By establishing mathematical models to quantitatively describe the dynamics of the fermentation process, it is possible to predict or control microbial metabolic activities reasonably. This aims to achieve a more detailed understanding of the dynamic changes in crucial parameters during the fermentation process, ensuring fermentation success. Previous research has established fermentation kinetics models for various fruit wines, such as jackfruit wine [27] and sea buckthorn wine [28]. Such results are of scientific and practical importance for the rational control of the fermentation process and the regulation of product quality. In our previous study [29], we found that the addition of g-IDY could promote the fermentation process of pear wine, enhance the antioxidant property, and markedly increase the content of malic acid and total esters. Further research in this area will aid in the understanding of the relationship between g-IDY, mixed fermentation kinetics, and aroma components of wine.

Therefore, this study aims to investigate the impact of g-IDY on the dynamics of mixed fermentation. By establishing mathematical models to describe the dynamics of yeast growth, the consumption kinetics of reducing sugars, and the production kinetics of ethanol during fermentation, it becomes possible to predict changes in yeast quantity during the fermentation process. The importance of this work lies in the possibility of deciphering the effects of g-IDY on microbial growth and wine aroma during fruit wine fermentation and providing new ideas for improving the quality of fruit wine.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

*T. delbrueckii* CICC 33458 was obtained from China Center of Industrial Culture Collection, while *Saccharomyces cerevisiae* was purchased from Angel Yeast Co., Ltd. (Yichang, China). Glucose, fructose, peptone, agar, citric acid, tartaric acid, and L-malic acid were sourced from Macklin Biochemical Co., Ltd. (Shanghai, China); sodium chloride, sodium hydroxide, ammonium sulfate, magnesium sulfate, manganese sulfate, potas-

sium dihydrogen phosphate, and 3-methyl-5-dinitrosalicylic acid were all analytically pure, chronchem reagent (Chengdu, China); yeast extract powder and Lu's alkaline methylene blue dyeing solution were acquired from Solarbio Science & Technology Co., Ltd. (Beijing, China). Glutathione-enriched inactive dry yeast was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

### 2.2. Strain Inoculation Quantity and Inoculation Plan

The yeast was rehydrated before inoculation to reach the target yeast quantity. The inoculation rate was 3% of the synthetic grape must volume, with a yeast inoculation amount of  $10^6$  CFU/mL. Simultaneous inoculation and fermentation were performed at 22 °C. The groups were labeled as TS0, TS20, TS40, and TS100, corresponding to the addition of g-IDY at 0, 20, 40, and 100 mg/L, respectively. The fermentation conditions for wine samples used to determine aroma were the same as those mentioned earlier. All fermentations were carried out in triplicate, and each experiment was carried out in different containers under the same conditions.

### 2.3. Preparation of Simulated Juice

The method for synthetic grape must to replicate the wine fermentation process is based on Li et al. [30] with slight modifications. The synthetic grape must consists of the following components per liter: glucose 100 g, fructose 100 g, yeast extract powder 1 g, ammonium sulfate 2 g, citric acid 0.3 g, malic acid 5 g, tartaric acid 5 g, magnesium sulfate heptahydrate 0.4 g, potassium dihydrogen phosphate 5 g, sodium chloride 0.2 g, manganese sulfate 0.05 g, with a pH adjusted to 3.9.

### 2.4. Analytical Method

The yeast quantity was measured using a Countstar Rigel S2, purchased from Shanghai Rui Yu Biotech Co., Ltd. (Shanghai, China). Every 24 h, a 20 µL sample of the fermentation broth was mixed with 20 µL of 0.4% Lu's alkaline methylene blue staining solution in a centrifuge tube. After thorough shaking, 20 µL of this mixture was transferred to a counting template after 3 min. The cell number was then determined using the cell counting mode, with a dilution ratio of 1:1.

The content of reducing sugar in the fermentation process was determined by 3,5-dinitrosalicylic acid (DNS). The regression equation of glucose standard curve was  $y = 13.721x - 0.0645$ , and the correlation coefficient was  $R^2 = 0.9944$ .

The alcohol content was determined by alcohol meter [31].

The aroma components were determined by headspace solid-phase microextraction and gas chromatography–mass spectrometry (HS-SPME-GC-MS). Wine sample (8 mL), internal standard (0.45 mg/L 2-octanol), and 2 g NaCl were added to a 15 mL headspace bottle. The HS-SPME conditions were as follows: extraction head, DVB/CAR/PDMS (50/30 µm, 1 cm). GC-MS analysis was performed with an Agilent 7890A Series gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) connected to an Agilent 5975B mass selective detector. The procedure involved preheating the wine sample at 50 °C for 10 min. Then, an extraction head was inserted into the headspace bottle to allow for adsorption for 35 min, with the fiber head positioned 1.5 cm from the liquid level. After adsorption, the fiber head was retrieved and quickly introduced into the GC injection port, followed by thermal analysis at 250 °C for 3 min.

GC conditions included the use of a DB-WAX capillary column (60 m × 0.25 mm × 0.25 µm, Agilent Technology, Santa Clara, CA, USA), an injection port temperature of 250 °C in non-shunt injection mode. The programmed temperature profile was as follows: initial temperature 40 °C, held for 5 min, increased at a rate of 2 °C/min to 60 °C, further increased at 5 °C/min to 180 °C, held for 5 min, and finally increased at 10 °C/min to 230 °C, where it was held for 10 min. High-purity helium served as the carrier gas with a constant flow rate of 1.2 mL/min.

MS conditions included an electron bombardment ionization source (EI) with an ionization source temperature of 230 °C, an electron energy of 70 eV, full scanning acquisition mode, an MS quadrupole temperature of 150 °C, and 3 min solvent delay.

For qualitative and semi-quantitative analysis of volatile substances, mass spectra corresponding to the chromatographic peaks were compared with the NIST/Wiley Database. Identification results with a matching degree of more than 80% were retained. The relative amount of each component was determined by comparing the peak area of the internal standard (2-octanol) with the peak area of each component in the wine. The calculation formula of Kovats index is as follows:

$$RI = 100 \times (N + (t - t_n)/(t_{n+1} - t_n))$$

where RI is the chromatographic retention index, n is the carbon number of the compound, t is the retention time of the compound under experimental conditions, and  $t_n$  and  $t_{n+1}$  are the retention times of two adjacent known standard samples.

### 2.5. Statistical Analysis

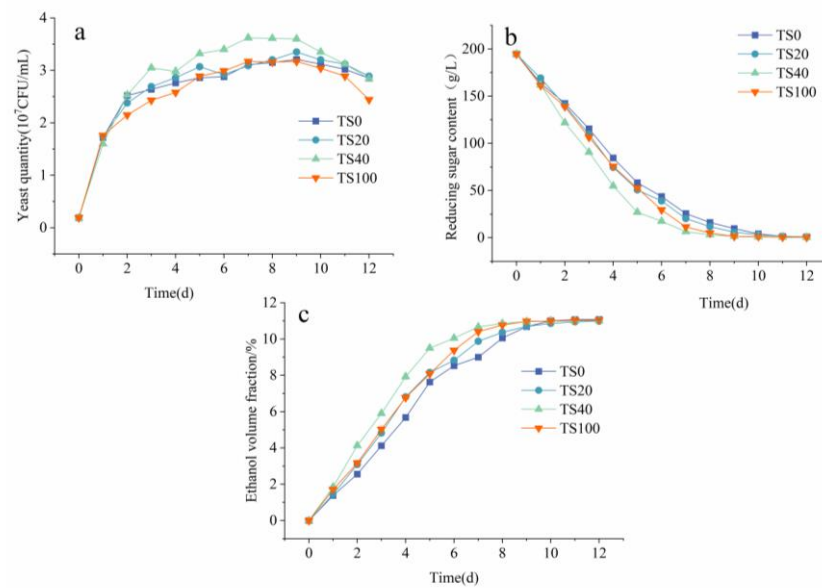
The data statistics and mapping analysis were carried out by using origin 2023b software, and the appropriate kinetic model was selected to non-linearly fit the number of yeast and the consumption of reducing sugar in the fermentation process, and the fitting coefficient  $R^2$  was used as the reliability evaluation to select the model with the best fitting effect in the fermentation process and quantitatively described and analyzed. SPSS Statistics (V17.0, Chicago, IL, USA) was used to deal with the aroma data, and the results t indicate mean value  $\pm$  standard deviation of three determinations. The significance was analyzed by Duncan test, and the results were expressed by the marked letter method.

## 3. Results

### 3.1. Changed Trend Chart of Yeast Quantity, Reducing Sugar Content, and Ethanol Volume Fraction during Fermentation

Yeast quantity, reducing sugar content, and alcohol content are important biochemical parameters of fruit wine, as shown by the changes in these three variables during fermentation in Figure 1. An increase in yeast quantity during fermentation implies a decrease in reducing sugar content and an increase in ethanol content. Yeasts grow and reproduce by metabolizing reducing sugars to produce ethanol and energy. The results showed that the use of g-IDY can increase the maximum amount of yeast, which may be due to the protective effect of GSH released by g-IDY on yeast. However, the yeast quantity did not increase proportionally with the addition of g-IDY, and the TS40 group had the largest yeast quantity in the experiment.

The content of reducing sugar decreased continuously with time, and the fastest decrease occurred from day 0 to 5. On day 8, the reducing sugar content of TS40 was 3.25 g/L in contrast to that of TS0 of 16.24 g/L. Notably, all experimental groups completed alcohol fermentation successfully, with reducing sugar contents dropping below 4 g/L. The results indicated that the addition of g-IDY could improve the fermentation rate, and the optimal effect was achieved when the addition amount was 40 mg/L. Ethanol, a byproduct of yeast's sugar metabolism during fermentation, exhibited an increase in volume fraction as fermentation progressed. Among the experimental groups, TS0 and TS20 displayed the most rapid growth in the first 9 days, whereas TS40 and TS100 exhibited the fastest growth during the initial 7 days. Ultimately, there was no significant difference in ethanol volume fractions among the four groups, with values of 11.08%, 10.99%, 11.02%, and 11.04% for TS0, TS20, TS40, and TS100, respectively. The production of ethanol almost corresponds to the consumption of reducing sugar and the growth of yeast.



**Figure 1.** Changes in yeast quantity, reducing sugar content, and ethanol volume fraction in the fermentation process: (a) effect of g-IDY on yeast quantity, (b) effect of g-IDY on the reducing sugar content, (c) effect of g-IDY on the ethanol volume fraction.

### 3.2. Kinetic Model of Yeast Growth

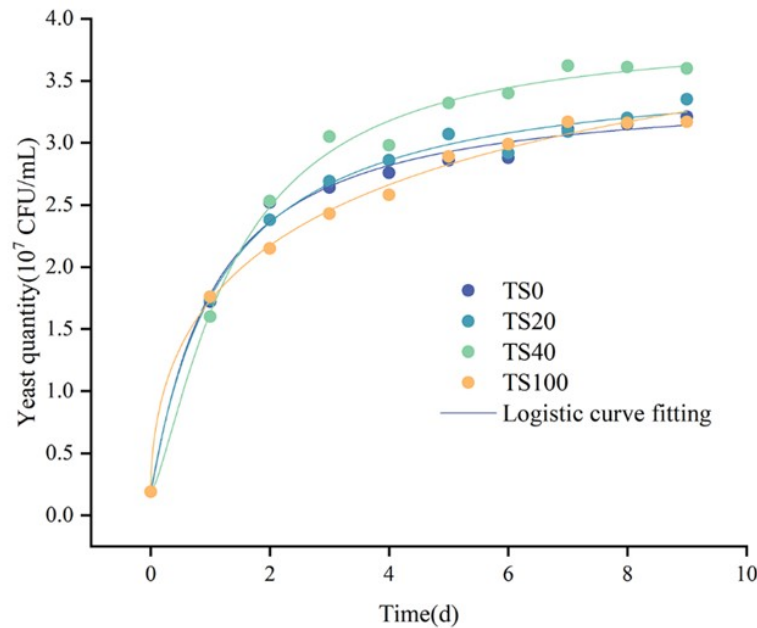
During the initial stages of alcohol fermentation, the yeast can grow and multiply normally in the nutrient-rich fermentation tank. The initial 9 days of fermentation correspond to the growth and stabilization phases. Consequently, this study applied nonlinear fitting techniques to model the yeast growth in the treatment groups from day 0 to day 9. We used the Logistic and SGompertz models to describe the dynamics of yeast quantity in all experimental groups. The fitting equations and fitting coefficients ( $R^2$  values) are shown in Table 1.

**Table 1.** Fitting equations and coefficients for yeast quantity.

Treatment Groups		Models	
		Logistic	SGompertz
Fitting equations	TS0	$y = 3.42764 - \frac{3.24034}{1 + (\frac{x}{1.02985})^{1.07655}}$	$y = 2.97886 \times e^{-e[-1.30788 \times (x - 0.63147)]}$
	TS20	$y = 3.62565 - \frac{3.43612}{1 + (\frac{x}{1.18591})^{1.02634}}$	$y = 3.08253 \times e^{-e[-1.10141 \times (x - 0.67808)]}$
	TS40	$y = 3.71758 - \frac{3.71758}{1 + (\frac{x}{1.20495})^{1.48494}}$	$y = 3.48346 \times e^{-e[-0.94436 \times (x - 0.85132)]}$
	TS100	$y = 4.16192 - \frac{4.15926}{1 + (\frac{x}{1.69503})^{0.68934}}$	$y = 3.05919 \times e^{-e[-0.79723 \times (x - 0.71808)]}$
$R^2$	TS0	0.99198	0.97062
	TS20	0.99412	0.97062
	TS40	0.99353	0.97928
	TS100	0.99600	0.94738

The Logistic model showed a better fitting performance than the SGompertz model, with  $R^2$  values above 0.99 for all groups. This indicates that the Logistic model can effectively simulate and predict the yeast growth patterns during mixed fermentation. The fitting curve of the Logistic model is shown in Figure 2. Moreover, we observed that the yeast population did not increase linearly with the g-IDY addition. The maximum population was achieved when g-IDY was added at 40 mg/L. This is consistent with the findings of Xu et al. [32]. These results suggest that the same GSH content may have different effects on yeast growth under different stress conditions. The possible differences

in yeast metabolic pathways under distinct conditions need further investigation to clarify the specific impact mechanisms.



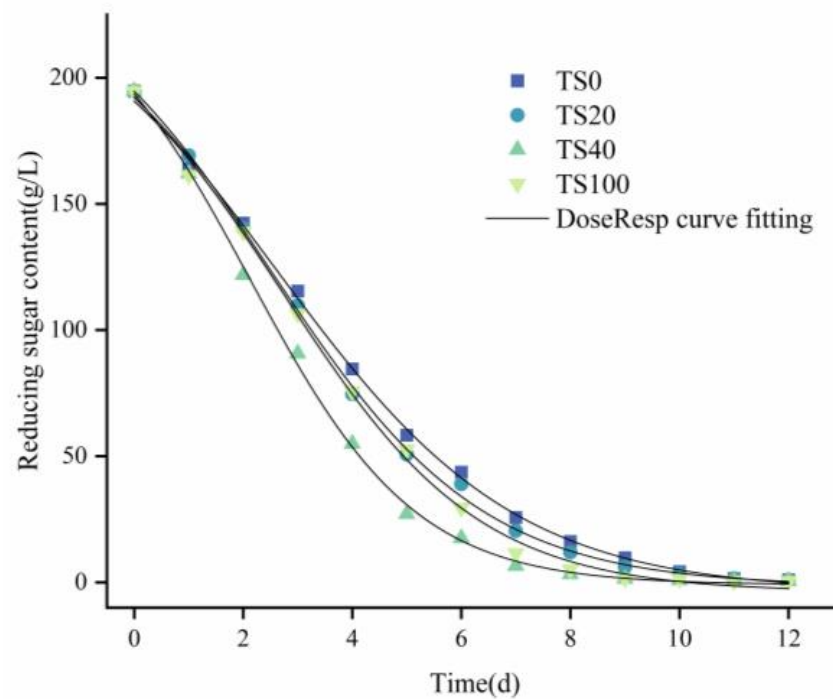
**Figure 2.** The fitting curves of yeast quantity under Logistic model.

**3.3. Kinetic Model of Reducing Sugar Consumption**

We used the Logistic, DoseResp, and Boltzmann models to analyze the reducing sugar consumption in the fermentation process. We selected the model with the highest R<sup>2</sup> value to describe the dynamics of reducing sugar consumption. Table 2 shows that both the DoseResp and Boltzmann models had excellent fitting performance for all experimental groups, with R<sup>2</sup> values above 0.99. Interestingly, the R<sup>2</sup> values for the DoseResp and Boltzmann models were identical, reaching 0.99901, 0.99920, 0.99900, and 0.99781 for all groups. This indicates that both models can accurately represent the consumption of reducing sugar during mixed fermentation with g-IDY addition. These results are consistent with the fermentation kinetics of pineapple wine reported by Wang et al. [27]. Figure 3 shows the fitting curve of the Boltzmann model. We observed a correlation between the content of reducing sugar and yeast quantity and another between the yeast quantity and ethanol volume fraction. Yeasts use reducing sugars to metabolize and produce ethanol, and they use the energy from metabolism for growth and reproduction. Thus, as the yeast quantity increases, the content of reducing sugars decreases, and the ethanol volume fraction increases. The content of reducing sugars in TS0 was below 4 g/L on day 12, and in the other groups, it was below 4 g/L on day 11.

**Table 2.** Fitting equations and coefficients for reducing sugar content.

Treatment Groups		Models		
		Logistic	DoseResp	Boltzmann
Fitting equations	TS0	$y = -33.01765 + \frac{222.17907}{1 + (\frac{x}{3.16417})^{1.79267}}$	$y = -3.81530 + \frac{257.47476}{1 + 10^{-0.19598 \times (2.57223 - x)}}$	$y = -3.81531 + \frac{257.4748}{1 + e^{\frac{x - 3.57223}{2.21602}}}$
	TS20	$y = -21.91715 + \frac{212.60318}{1 + (\frac{x}{3.64462})^{1.97183}}$	$y = -1.57477 + \frac{250.6648}{1 + 10^{-0.22305 \times (2.49817 - x)}}$	$y = -1.57477 + \frac{250.66579}{1 + e^{\frac{x - 2.49817}{1.94709}}}$
	TS40	$y = -14.00801 + \frac{203.9783}{1 + (\frac{x}{2.82101})^{2.10633}}$	$y = -1.02878 + \frac{243.02067}{1 + 10^{-0.28527 \times (2.11569 - x)}}$	$y = -1.02878 + \frac{243.0207}{1 + e^{\frac{x - 2.11569}{1.52238}}}$
	TS100	$y = -23.12242 + \frac{209.81582}{1 + (\frac{x}{3.63349})^{2.05496}}$	$y = -3.88435 + \frac{238.06253}{1 + 10^{-0.23987 \times (2.71467 - x)}}$	$y = -3.88436 + \frac{238.06254}{1 + e^{\frac{x - 2.71467}{1.31036}}}$
R2	TS0	0.99601	0.99901	0.99901
	TS20	0.99738	0.99920	0.99920
	TS40	0.99456	0.99900	0.99900
	TS100	0.99214	0.99781	0.99781



**Figure 3.** The fitting curves of reducing sugar content under Boltzmann model.

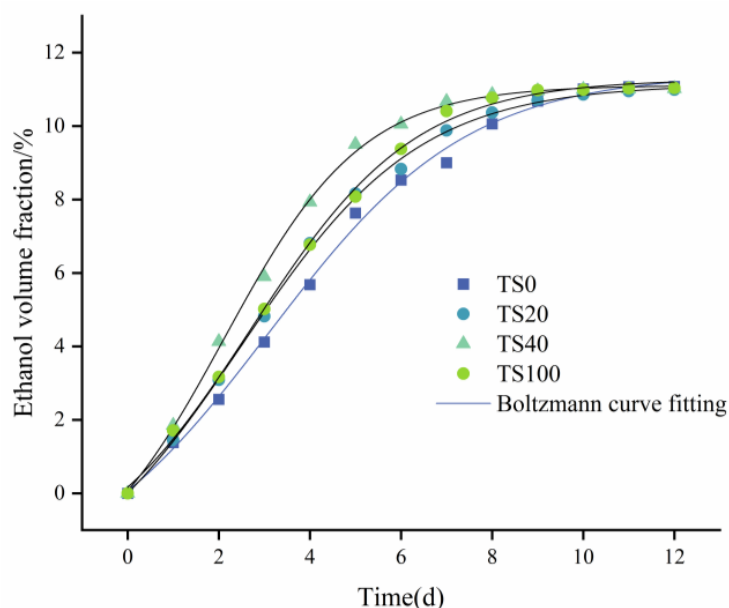
### 3.4. Kinetic Model of Ethanol Formation

We used the Logistic and Boltzmann models to fit the ethanol production in the fermentation process. Table 3 shows the fitting equations and fitting coefficients ( $R^2$  values) for both models. The Boltzmann model had a better fitting performance than the Logistic model for all experimental groups, with  $R^2$  values ranging from 0.99780 to 0.99921.

**Table 3.** Fitting equations and coefficients for ethanol volume fraction.

Treatment Groups	Models		
	Logistic	Boltzmann	
Fitting equations	TS0	$y = 13.01621 - \frac{12.7151}{1 + \left(\frac{x}{4.46797}\right)^{1.94514}}$	$y = 11.42833 - \frac{14.10699}{1 + e^{\frac{x-3.10192}{2.18142}}}$
	TS20	$y = 12.29294 - \frac{12.06585}{1 + \left(\frac{x}{3.64777}\right)^{1.96796}}$	$y = 11.13407 - \frac{14.23863}{1 + e^{\frac{x-2.49238}{1.95195}}}$
	TS40	$y = 11.84205 - \frac{12.10946}{1 + \left(\frac{x}{2.82231}\right)^{2.10474}}$	$y = 11.10408 - \frac{13.79547}{1 + e^{\frac{x-2.11424}{1.52429}}}$
	TS100	$y = 12.33019 - \frac{11.93593}{1 + \left(\frac{x}{3.60468}\right)^{2.07818}}$	$y = 11.27388 - \frac{13.5504}{1 + e^{\frac{x-2.71933}{1.79275}}}$
R2	TS0	0.99586	0.99780
	TS20	0.99740	0.99921
	TS40	0.99462	0.99902
	TS100	0.99347	0.99844

Figure 4 shows the change in the ethanol volume fraction during the fermentation process. The measured value and predicted values had a good fit. Ethanol is produced by yeast from reducing sugars. In the middle stage of fermentation, the ethanol volume fraction of TS40 increased the fastest among the four groups, which may be related to its high yeast quantity. In the later stage of fermentation, the yeast quantity decreased, the sugar conversion rate was low, and the ethanol volume fraction increased slowly.



**Figure 4.** The fitting curves of ethanol volume fraction under Boltzmann model.

### 3.5. Volatile Aroma Compounds

Aroma is a crucial quality indicator of fruit wine, and esters and higher alcohols are the main contributors to the aromatic profile during fruit wine fermentation. In our study, we used HS-SPME-GC-MS to preliminarily identify 20 volatile aroma compounds in the wine, including 9 esters, 7 higher alcohols, and 4 aldehydes/ketones, as shown in Table 4.

**Table 4.** The contents of volatile aroma compounds. Different letters represent significant differences after Duncan test, n = 3 and p-value < 0.05.

No	RI	Aroma Compounds	Mass Contents/(µg/L)			
			TS0	TS20	TS40	TS100
1	714	Acetaldehyde	nd	15.18 ± 0.38 b	18.04 ± 0.27 c	18.43 ± 0.58 c
2	880	Ethyl acetate	1661.96 ± 0.12 a	1980.23 ± 0.21 d	1756.53 ± 0.08 c	1719.37 ± 0.09 b
3	898	Acetal	50.25 ± 1.27 a	91.15 ± 0.89 b	nd	nd
4	1094	Isobutanol	165.67 ± 0.19 a	185.94 ± 0.35 b	195.88 ± 0.11 c	198.75 ± 0.24 d
5	1126	Isoamyl acetate	75.62 ± 0.13 b	46.81 ± 0.1 a	93.14 ± 0.16 c	113.84 ± 0.12 d
6	1211	Isoamyl alcohol	2222.00 ± 1.56 a	2449.52 ± 2.54 b	2491.72 ± 1.35 c	2578.77 ± 1.69 d
7	1227	Ethyl hexanoate	68.96 ± 0.05 a	96.22 ± 0.08 d	82.62 ± 0.11 b	84.07 ± 0.09 c
8	1283	2-Octanone	22.05 ± 0.21 b	21.20 ± 0.31 a	21.44 ± 0.29 a	22.19 ± 0.19 b
9	1359	N-hexanol	nd	39.07 ± 1.05 a	40.18 ± 0.33 b	41.73 ± 0.38 c
10	1382	Hexyl formate	36.22 ± 0.63	nd	nd	nd
11	1441	Ethyl octanoate	65.37 ± 0.19 a	65.46 ± 0.15 a	69.97 ± 0.42 b	69.77 ± 0.23 b
12	1528	Benzaldehyde	10.14 ± 0.28 a	10.18 ± 0.19 a	20.67 ± 0.24 c	15.14 ± 0.14 b
13	1541	Ethyl nonanoate	nd	22.68 ± 0.004	nd	nd
14	1564	1-Octanol	nd	16.11 ± 0.38 b	nd	14.43 ± 0.65 a
15	1580	2,3-Butanediol	32.72 ± 0.64 b	nd	13.96 ± 0.35 a	13.37 ± 0.28 a
16	1643	Ethyl decanoate	27.68 ± 0.19 b	14.64 ± 0.21 a	34.42 ± 0.15 c	35.97 ± 0.31 d
17	1702	3-methylthiopropyl alcohol	12.65 ± 0.39 a	13.12 ± 0.24 b	17.20 ± 0.15 d	14.47 ± 0.39 c
18	1826	Phenylethyl acetate	66.06 ± 0.35 a	74.51 ± 0.54 c	71.39 ± 1.02 b	88.49 ± 0.18 d
19	1847	Ethyl Laurate	10.20 ± 0.38 b	nd	13.65 ± 0.26 c	29.86 ± 0.24 d
20	1923	Phenylethanol	1705.21 ± 1.25 a	2398.63 ± 1.89 b	2466.49 ± 2.18 c	2682.46 ± 1.64 d



Esters are the main contributors to wine aroma, imparting floral and fruity notes and influencing the aroma profile and style of the wine. The relative number of esters ranged from 2012.07 to 3000.55  $\mu\text{g/L}$ , and TS20 and TS40 had 14.34% and 5.45% higher contents than TS0, respectively. The content of ethyl acetate and isoamyl acetate increased first and then decreased with increasing g-IDY content, reaching the maximum at 20 mg/L. This is consistent with the findings of Qi et al. [33], indicating that g-IDY treatment can increase the content of phenylethyl acetate and improve wine aroma quality.

Higher alcohols are produced by yeast through the Ehrlich pathway during amino acid degradation [34] and can enhance the complexity of fruity aromas and the overall taste harmony. In the present study, the higher alcohols showed significant variations in total contents among wines fermented with different g-IDY contents. The relative amount of total higher alcohols in TS100 was the highest among the four groups and reached 5543.98  $\mu\text{g/L}$ , 33.97% higher than that in TS0. The relative amounts of phenylethanol and isobutanol increased by 57.31% and 16.06%, respectively. The content of higher alcohols also increased with g-IDY content, which may be due to g-IDY containing amino acids, thereby increasing the amino acid contents in the wine and promoting higher alcohol production by yeast metabolism [35].

Aldehyde compounds add grassy aromas to the wine. Benzaldehyde imparts cherry and nutty aromas, even in small amounts, adds a unique character to the wine, and has antifungal properties. Acetaldehyde, a key aldehyde compound in fruit wine, affects wine aroma and color. During aging, acetaldehyde helps tannin condensation, reduces wine sediment, and stabilizes wine color. We detected three aldehyde compounds, with contents from 38.17 to 60.39  $\mu\text{g/L}$ . Among the four groups, TS40 had the highest relative amounts of benzaldehyde and acetaldehyde, with 20.67 and 18.04  $\mu\text{g/L}$ , respectively.

#### 4. Discussion

The increasing recognition of *T. delbrueckii*'s role in wine aroma has stimulated research on its fermentation products, metabolic and evolutionary pathways, and potential synergies with other yeasts to enhance fermentation performance [36,37]. Multistarter fermentations with non-*Saccharomyces* and *Saccharomyces* strains can simulate natural fermentation [38,39], reduce the risk of fermentation failure, and facilitate the study of strain interactions, overcoming the variability of natural fermentation. The interactions among different yeasts influence the aroma compounds and the metabolic pathways of yeast. These interactions are also modulated by the fermentation environment [40]. Yeast derivatives can alter some components of the fermentation medium, so their effects on microbial growth need to be investigated. Unlike previous studies, we used simulated grape juice to minimize the influence of other factors, and we explored the effects of different doses of g-IDY on wine aroma, yeast growth, and metabolic kinetics. Prior et al. [41] investigated the nitrogen source preference of commercial non-*Saccharomyces* yeasts by conducting pure culture and sequential culture fermentations in synthetic grape musts with adjusted nitrogen contents. Kosel et al. [42] studied the effects of *S. cerevisiae* and *Dekkera bruxellensis* on wine aroma by constructing a double-compartment membrane system with synthetic wine must and found that it could reduce the content of ethylphenols and enhance the formation of aromatic esters.

Wine fermentation is a stressful environment for yeast. Several environmental stresses, including pH shock, ethanol toxicity, nutrient starvation, and oxidative stress, can individually or collectively affect yeast physiology deleteriously. Under these conditions, genomic adaptation of yeast to cope with the adverse effects of the stress factors occurs [43–45]. Ethanol stress can increase the production of reactive oxygen species (ROS) in yeast, which can damage the yeast structure and cause cell death [46]. GSH is a key molecule that protects yeast from oxidative damage. It scavenges ROS, such as  $\text{H}_2\text{O}_2$  non-enzymatically, and acts as a cofactor for various antioxidant enzymes to cope with stressful environments. GSH also helps yeast maintain the integrity and adaptation of the mitochondrial genome [43] and serves as a source of sulfur or nitrogen under nutrient limitation [47,48]. GSH exhaustion negatively impacts both the electron transport chain function and the

chronological life span of yeast [49]. However, the GSH level in yeast varies depending on the experimental conditions [50–52]. The yeast population may increase due to the uptake of GSH from g-IDY by yeast cells, which protects the yeast structure and reduces the oxidative stress in yeast [49]. Moreover, g-IDY can also release polysaccharides and peptides that affect yeast metabolism and the aroma profile of wine [33].

The aroma compounds generated during fermentation influence the perception of the final wine aroma. Adding g-IDY can increase the content of phenylethanol, a floral compound, by enhancing the GSH-mediated reduction in phenylacetaldehyde by alcohol dehydrogenase [53–55]. Phenylethyl acetate, another floral compound, is formed by the esterase-catalyzed reaction of acetic acid and phenylethanol [56]. It may be that the yeast synthesizes more phenylethanol, which is subsequently converted by esterase into more phenylethanol acetate.

We used nonlinear regression models to fit the yeast growth, sugar consumption, and ethanol production data during wine fermentation. The Logistic model fitted the yeast growth better, and the Boltzmann model fitted the sugar consumption and ethanol production better. Both models captured the kinetics of co-fermentation well. Our results agreed with those of Qi et al. [57] for the Logistic model and Wang et al. [27] for the Boltzmann model, with  $R^2$  values above 0.99. However, our models were empirical and did not account for the possible metabolic changes in yeast. Therefore, we suggest integrating the kinetic model with the formation mechanism of matter for an improved understanding of the wine fermentation process [58].

Kinetic models are increasingly used to optimize alcoholic beverage production. Fermentation efficiency depends not only on the inherent fermentation capacity of microorganisms but also on factors such as microbial interactions, fermentation processes, and conditions. By using kinetic models to monitor key indicators in the fermentation process, the final levels of relevant indicators in the wine production process can be accurately predicted, which has important implications for industrial production. Future research should focus on elucidating the molecular complexity of g-IDY, considering factors such as gene expression and enzyme activity, and using genomic and molecular techniques to study its effects on yeast metabolism and interactions, offering more comprehensive insights into the application of g-IDY in wine production. This study improved the understanding of the effects of g-IDY on the fermentation process and the aroma of wine and could ultimately help improve wine production.

## 5. Conclusions

In this study, we utilized four well-established fermentation kinetic models, namely, Logistic, SGompertz, Boltzmann, and DoseResp models, to perform nonlinear fitting of yeast cell growth, reducing sugar content, and ethanol production during the mixed fermentation of grape wine with the addition of g-IDY. To assess the reliability of these fitting models, we evaluated them using the fitting coefficients ( $R^2$  values). These models effectively capture the kinetic characteristics of the fermentation process, with the Logistic, DoseResp, and Boltzmann models emerging as particularly suitable for simulating yeast cell growth, reducing sugar content, and ethanol production, respectively. Additionally, by conducting GC-MS analysis of wine aromas, we found that the addition of g-IDY increased the aroma content of phenethyl acetate, phenylethanol, and isoamyl alcohol. These findings not only contribute to the understanding of the impact of g-IDY on fruit wine production but also provide a valuable theoretical foundation and practical guidance for its application in the industry.

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## References

1. Fernandes, T.; Silva-Sousa, F.; Pereira, F.; Rito, T.; Soares, P.; Franco-Duarte, R.; Sousa, M.J. Biotechnological Importance of *Torulaspora delbrueckii*: From the Obscurity to the Spotlight. *J. Fungi* **2021**, *7*, 712. [[CrossRef](#)] [[PubMed](#)]
2. Jianli, Z.; Chuqi, T.; Shuliang, Z.; Liangbo, L.; Yuangen, W.; Wenhua, Y.; Jean Damascene, H.; Jiang, Z.; Wenwen, Z.; Dan, D.; et al. Enhancement of pyranoanthocyanin formation in blueberry wine with non-*Saccharomyces* yeasts. *Food Chem.* **2024**, *438*, 137956. [[CrossRef](#)]
3. Azzolini, M.; Tosi, E.; Lorenzini, M.; Finato, F.; Zapparoli, G. Contribution to the aroma of white wines by controlled *Torulaspora delbrueckii* cultures in association with *Saccharomyces cerevisiae*. *World J. Microbiol. Biotechnol.* **2015**, *31*, 277–293. [[CrossRef](#)] [[PubMed](#)]
4. Wu, Y.; Li, Z.; Zou, S.; Dong, L.; Lin, X.; Chen, Y.; Zhang, S.; Ji, C.; Liang, H. Chemical Composition and Flavor Characteristics of Cider Fermented with *Saccharomyces cerevisiae* and Non-*Saccharomyces cerevisiae*. *Foods* **2023**, *12*, 3565. [[CrossRef](#)] [[PubMed](#)]
5. Bely, M.; Stoeckle, P.; Masneuf-Pomarede, I.; Dubourdiou, D. Impact of mixed *Torulaspora delbrueckii*-*Saccharomyces cerevisiae* culture on high-sugar fermentation. *Int. J. Food Microbiol.* **2008**, *122*, 312–320. [[CrossRef](#)] [[PubMed](#)]
6. Vaquero, C.; Escott, C.; Heras, J.M.; Carrau, F.; Morata, A. Co-inoculations of *Lachancea thermotolerans* with different *Hanseniaspora* spp.: Acidification, aroma, biocompatibility, and effects of nutrients in wine. *Food Res. Int.* **2022**, *161*, 111891. [[CrossRef](#)]
7. Silva-Sousa, F.; Fernandes, T.; Pereira, F.; Rodrigues, D.; Rito, T.; Camarasa, C.; Franco-Duarte, R.; Sousa, M.J. *Torulaspora delbrueckii* Phenotypic and Metabolic Profiling towards Its Biotechnological Exploitation. *J. Fungi* **2022**, *8*, 569. [[CrossRef](#)]
8. Belda, I.; Navascues, E.; Marquina, D.; Santos, A.; Calderon, F.; Benito, S. Dynamic analysis of physiological properties of *Torulaspora delbrueckii* in wine fermentations and its incidence on wine quality. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 1911–1922. [[CrossRef](#)]
9. Renault, P.; Coulon, J.; Moine, V.; Thibon, C.; Bely, M. Enhanced 3-Sulfanylhexan-1-ol Production in Sequential Mixed Fermentation with *Torulaspora delbrueckii* / *Saccharomyces cerevisiae* Reveals a Situation of Synergistic Interaction between Two Industrial Strains. *Front. Microbiol.* **2016**, *7*, 293. [[CrossRef](#)]
10. Zhang, B.Q.; Luan, Y.; Duan, C.Q.; Yan, G.L. Use of *Torulaspora delbrueckii* Co-fermentation With Two *Saccharomyces cerevisiae* Strains With Different Aromatic Characteristic to Improve the Diversity of Red Wine Aroma Profile. *Front. Microbiol.* **2018**, *9*, 606. [[CrossRef](#)]
11. Renault, P.; Miot-Sertier, C.; Marullo, P.; Hernández-Orte, P.; Lagarrigue, L.; Lonvaud-Funel, A.; Bely, M. Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: Potential applications in the wine industry. *Int. J. Food Microbiol.* **2009**, *134*, 201–210. [[CrossRef](#)] [[PubMed](#)]
12. Sadoudi, M.; Tourdot-Marechal, R.; Rousseaux, S.; Steyer, D.; Gallardo-Chacon, J.J.; Ballester, J.; Vichi, S.; Guerin-Schneider, R.; Caixach, J.; Alexandre, H. Yeast-yeast interactions revealed by aromatic profile analysis of Sauvignon Blanc wine fermented by single or co-culture of non-*Saccharomyces* and *Saccharomyces* yeasts. *Food Microbiol.* **2012**, *32*, 243–253. [[CrossRef](#)]
13. Comitini, F.; Gobbi, M.; Domizio, P.; Romani, C.; Lencioni, L.; Mannazzu, I.; Ciani, M. Selected non-*Saccharomyces* wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiol.* **2011**, *28*, 873–882. [[CrossRef](#)] [[PubMed](#)]
14. Rodríguez-Bencomo, J.J.; Andújar-Ortiz, I.; Moreno-Arribas, M.V.; Simó, C.; González, J.; Chana, A.; Dávalos, J.; Pozo-Bayón, M.Á. Impact of Glutathione-Enriched Inactive Dry Yeast Preparations on the Stability of Terpenes during Model Wine Aging. *J. Agric. Food Chem.* **2014**, *62*, 1373–1383. [[CrossRef](#)]
15. Naselli, V.; Prestianni, R.; Badalamenti, N.; Matraxia, M.; Maggio, A.; Alfonzo, A.; Gaglio, R.; Vagnoli, P.; Settanni, L.; Bruno, M.; et al. Improving the Aromatic Profiles of Catarratto Wines: Impact of and Glutathione-Rich Inactivated Yeasts. *Antioxidants* **2023**, *12*, 439. [[CrossRef](#)]
16. Liu, D.; Xu, J.; Cao, Y.; Qi, Y.; Yang, K.; Wei, X.; Xu, Y.; Fan, M. Effect of glutathione-enriched inactive dry yeast on color, phenolic compounds, and antioxidant activity of kiwi wine. *J. Food Process. Preserv.* **2020**, *44*, e14347. [[CrossRef](#)]
17. Pozo-Bayón, M.Á.; Andujar-Ortiz, I.; Alcaide-Hidalgo, J.M.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V. Characterization of Commercial Inactive Dry Yeast Preparations for Enological Use Based on Their Ability To Release Soluble Compounds and Their Behavior toward Aroma Compounds in Model Wines. *J. Agric. Food Chem.* **2009**, *57*, 10784–10792. [[CrossRef](#)] [[PubMed](#)]
18. Andujar-Ortiz, I.; Pozo-Bayon, M.A.; Garcia-Ruiz, A.; Moreno-Arribas, M.V. Role of specific components from commercial inactive dry yeast winemaking preparations on the growth of wine lactic acid bacteria. *J. Agric. Food Chem.* **2010**, *58*, 8392–8399. [[CrossRef](#)]

19. Alfonzo, A.; Prestianni, R.; Gaglio, R.; Matraxia, M.; Maggio, A.; Naselli, V.; Craparo, V.; Badalamenti, N.; Bruno, M.; Vagnoli, P.; et al. Effects of different yeast strains, nutrients and glutathione-rich inactivated yeast addition on the aroma characteristics of Catarratto wines. *Int. J. Food Microbiol.* **2021**, *360*, 109325. [[CrossRef](#)]
20. Liu, D.; Qi, Y.M.; Zhao, N.; Cao, Y.F.; Xu, J.N.; Fan, M.T. Multivariate analysis reveals effect of glutathione-enriched inactive dry yeast on amino acids and volatile components of kiwi wine. *Food Chem.* **2020**, *329*, 127086. [[CrossRef](#)]
21. Andújar-Ortiz, I.; Chaya, C.; Martín-Álvarez, P.J.; Moreno-Arribas, M.V.; Pozo-Bayón, M.A. Impact of Using New Commercial Glutathione Enriched Inactive Dry Yeast Oenological Preparations on the Aroma and Sensory Properties of Wines. *Int. J. Food Prop.* **2014**, *17*, 987–1001. [[CrossRef](#)]
22. Gabrielli, M.; Aleixandre-Tudo, J.L.; Kilmartin, P.A.; Sieczkowski, N.; du Toit, W.J. Additions of Glutathione or Specific Glutathione-rich Dry inactivated Yeast Preparation (DYP) to Sauvignon blanc Must: Effect on Wine Chemical and Sensory Composition. *S. Afr. J. Enol. Vitic.* **2017**, *38*, 18–28. [[CrossRef](#)]
23. Ortiz, I.A.; Bayón, M.Á.P.; Garrido, I.; Álvarez, P.J.M.; Bartolomé, B.; Arribas, M.V.M. Effect of using glutathione-enriched inactive dry yeast preparations on the phenolic composition of rosé Grenache wines during winemaking. *OENO One* **2012**, *46*, 241–351. [[CrossRef](#)]
24. Giménez, P.; Just-Borras, A.; Pons, P.; Gombau, J.; Heras, J.M.; Sieczkowski, N.; Canals, J.M.; Zamora, F. Biotechnological tools for reducing the use of sulfur dioxide in white grape must and preventing enzymatic browning: Glutathione; inactivated dry yeasts rich in glutathione; and bioprotection with *Metschnikowia Pulcherrima*. *Eur. Food Res. Technol.* **2023**, *249*, 1491–1501. [[CrossRef](#)]
25. Nioi, C.; Lisanti, M.T.; Meunier, F.; Redon, P.; Massot, A.; Moine, V. Antioxidant activity of yeast derivatives: Evaluation of their application to enhance the oxidative stability of white wine. *LWT-Food Sci. Technol.* **2022**, *171*, 114116. [[CrossRef](#)]
26. Xu, W.; Liu, B.; Wang, C.; Kong, X. Organic cultivation of grape affects yeast succession and wine sensory quality during spontaneous fermentation. *LWT* **2020**, *120*, 108894. [[CrossRef](#)]
27. Wang, S.; Zhang, Y.; Wu, G.; Xu, F.; Hu, R.; Tan, L. Kinetics model of jackfruit wine in batch fermentation. *Food Ferment. Ind.* **2021**, *47*, 74–79. [[CrossRef](#)]
28. Zhang, Q.; Zhu, D.; Niu, G.; Wei, W.; Yan, F. Fermentation kinetics and antioxidant activity of sea buckthorn wine. *Food Ferment. Ind.* **2019**, *45*, 53–58. [[CrossRef](#)]
29. Chen, X.; Diao, T.; Lai, X.; Wei, X.; Leng, Y.; Ma, Y. Effects of glutathione-enriched inactive dry yeast addition during different brewing stages on the qualities of pear wine. *Food Sci.* **2022**, *44*, 132–142. [[CrossRef](#)]
30. Li, Y.-Q.; Hu, K.; Xu, Y.-H.; Mei, W.-C.; Tao, Y.-S. Biomass suppression of *Hanseniaspora uvarum* by killer *Saccharomyces cerevisiae* highly increased fruity esters in mixed culture fermentation. *LWT* **2020**, *132*, 109839. [[CrossRef](#)]
31. GB 5009.225-2023; National standard for Food Safety-Determination of Ethanol Concentration in Wine and Edible Alcohol. State Health and Wellness Commission, State Administration of Market Supervision: Beijing, China, 2023.
32. Xu, J.N.; Qi, Y.M.; Zhang, J.; Liu, M.M.; Wei, X.Y.; Fan, M.T. Effect of reduced glutathione on the quality characteristics of apple wine during alcoholic fermentation. *Food Chem.* **2019**, *300*, 125130. [[CrossRef](#)] [[PubMed](#)]
33. Qi, Z.; Xu, Y.; An, C.; Peng, B.; Wang, J. Impacts of glutathione-enriched inactive dry yeast preparations on the quality of 'viognier' dry white wine. *Food Ferment. Ind.* **2019**, *45*, 157–164. [[CrossRef](#)]
34. Qin, Z.; Petersen, M.A.; Bredie, W.L.P. Flavor profiling of apple ciders from the UK and Scandinavian region. *Food Res. Int.* **2018**, *105*, 713–723. [[CrossRef](#)] [[PubMed](#)]
35. Zhu, Z.; Hu, K.; Chen, S.; Xiong, S.; Tao, Y. Increase in Fruity Ester Production during Spine Grape Wine Fermentation by Goal-Directed Amino Acid Supplementation. *Fermentation* **2021**, *7*, 231. [[CrossRef](#)]
36. Silva, M.; Pontes, A.; Franco-Duarte, R.; Soares, P.; Sampaio, J.P.; Sousa, M.J.; Brito, P.H. A glimpse at an early stage of microbe domestication revealed in the variable genome of *Torulaspora delbrueckii*, an emergent industrial yeast. *Mol. Ecol.* **2023**, *32*, 2396–2412. [[CrossRef](#)] [[PubMed](#)]
37. Santiago, C.; Rito, T.; Vieira, D.; Fernandes, T.; Pais, C.; Sousa, M.J.; Soares, P.; Franco-Duarte, R. Improvement of *Torulaspora delbrueckii* Genome Annotation: Towards the Exploitation of Genomic Features of a Biotechnologically Relevant Yeast. *J. Fungi* **2021**, *7*, 287. [[CrossRef](#)]
38. Liu, W.; Li, H.; Jiang, D.; Zhang, Y.; Zhang, S.; Sun, S. Effect of *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and malolactic fermentation on fermentation kinetics and sensory property of black raspberry wines. *Food Microbiol.* **2020**, *91*, 103551. [[CrossRef](#)]
39. Fairbairn, S.; Engelbrecht, L.; Setati, M.E.; du Toit, M.; Bauer, F.F.; Divol, B.; Rossouw, D. Combinatorial analysis of population dynamics, metabolite levels and malolactic fermentation in *Saccharomyces cerevisiae*/ *Lachancea thermotolerans* mixed fermentations. *Food Microbiol.* **2021**, *96*, 103712. [[CrossRef](#)]
40. Taillandier, P.; Lai, Q.P.; Julien-Ortiz, A.; Brandam, C. Interactions between *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in wine fermentation: Influence of inoculation and nitrogen content. *World J. Microbiol. Biotechnol.* **2014**, *30*, 1959–1967. [[CrossRef](#)]
41. Prior, K.J.; Bauer, F.F.; Divol, B. The utilisation of nitrogenous compounds by commercial non-*Saccharomyces* yeasts associated with wine. *Food Microbiol.* **2019**, *79*, 75–84. [[CrossRef](#)]
42. Kosel, J.; Čadež, N.; Schuller, D.; Carreto, L.; Franco-Duarte, R.; Raspor, P. The influence of *Dekkera bruxellensis* on the transcriptome of *Saccharomyces cerevisiae* and on the aromatic profile of synthetic wine must. *FEMS Yeast Res.* **2017**, *17*, fox018. [[CrossRef](#)] [[PubMed](#)]

43. Legras, J.L.; Galeote, V.; Bigey, F.; Camarasa, C.; Marsit, S.; Nidelet, T.; Sanchez, I.; Couloux, A.; Guy, J.; Franco-Duarte, R.; et al. Adaptation of *S. cerevisiae* to Fermented Food Environments Reveals Remarkable Genome Plasticity and the Footprints of Domestication. *Mol. Biol. Evol.* **2018**, *35*, 1712–1727. [[CrossRef](#)] [[PubMed](#)]
44. Galafassi, S.; Toscano, M.; Vigentini, I.; Piškur, J.; Compagno, C. Osmotic stress response in the wine yeast *Dekkera bruxellensis*. *Food Microbiol.* **2013**, *36*, 316–319. [[CrossRef](#)] [[PubMed](#)]
45. Wenwen, Z.; Mengyang, B.; Zufang, W.; Peifang, W.; Yingjie, M. Research Progression in Mixed Culture Fermentation of Fruit Wine with Yeasts. *Food Sci.* **2018**, *39*, 252–259. [[CrossRef](#)]
46. Sun, M.; Shi, F.; Wang, X. The Role of *Saccharomyces cerevisiae* NAD(H) Kinase Pos5p on the Defense of Oxidative Stress. *Microbiol. China* **2010**, *37*, 1740–1746. [[CrossRef](#)]
47. Kritzinger, E.C.; Bauer, F.F.; Du Toit, W.J. Influence of yeast strain, extended lees contact and nitrogen supplementation on glutathione concentration in wine. *Aust. J. Grape Wine Res.* **2013**, *19*, 161–170. [[CrossRef](#)]
48. Lee, J.-C.; Straffon, M.J.; Jang, T.-Y.; Higgins, V.J.; Grant, C.M.; Dawes, I.W. The essential and ancillary role of glutathione in *Saccharomyces cerevisiae* analysed using a grande gsh1 disruptant strain. *FEMS Yeast Res.* **2001**, *1*, 57–65. [[CrossRef](#)]
49. Tello-Padilla, M.F.; Perez-Gonzalez, A.Y.; Canizal-García, M.; González-Hernández, J.C.; Cortes-Rojo, C.; Olivares-Marin, I.K.; Madrigal-Perez, L.A. Glutathione levels influence chronological life span of *Saccharomyces cerevisiae* in a glucose-dependent manner. *Yeast* **2018**, *35*, 387–396. [[CrossRef](#)] [[PubMed](#)]
50. Rodríguez-Bencomo, J.J.; Andújar-Ortiz, I.; Sánchez-Patán, F.; Moreno-Arribas, M.V.; Pozo-Bayón, M.A. Fate of the glutathione released from inactive dry yeast preparations during the alcoholic fermentation of white musts. *Aust. J. Grape Wine Res.* **2016**, *22*, 46–51. [[CrossRef](#)]
51. Andújar-Ortiz, I.; Pozo-Bayón, M.A.; Moreno-Arribas, M.V.; Martín-Álvarez, P.J.; Rodríguez-Bencomo, J.J. Reversed-phase high-performance liquid chromatography-fluorescence detection for the analysis of glutathione and its precursor  $\gamma$ -glutamyl cysteine in wines and model wines supplemented with oenological inactive dry yeast preparations. *Food Anal. Methods* **2012**, *5*, 154–161. [[CrossRef](#)]
52. Kritzinger, E.C.; Stander, M.A.; Du Toit, W.J. Assessment of glutathione levels in model solution and grape ferments supplemented with glutathione-enriched inactive dry yeast preparations using a novel UPLC-MS/MS method. *Food Addit. Contam. Part. A* **2013**, *30*, 80–92. [[CrossRef](#)] [[PubMed](#)]
53. Etschmann, M.M.W.; Bluemke, W.; Sell, D.; Schrader, J. Biotechnological production of 2-phenylethanol. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 1–8. [[CrossRef](#)] [[PubMed](#)]
54. Qi, Y.; Cheng, Z.; Fan, M. Effect of glutathione addition on the aroma components of stored kiwi fruit wine. *Sci. Technol. Food Ind.* **2017**, *08*, 183–188. [[CrossRef](#)]
55. Webber, V.; Dutra, S.V.; Spinelli, F.R.; Marcon, Â.R.; Carnieli, G.J.; Vanderlinde, R. Effect of glutathione addition in sparkling wine. *Food Chem.* **2014**, *159*, 391–398. [[CrossRef](#)] [[PubMed](#)]
56. Callejón, R.M.; Tesfaye, W.; Torija, M.J.; Mas, A.; Troncoso, A.M.; Morales, M.L. Volatile compounds in red wine vinegars obtained by submerged and surface acetification in different woods. *Food Chem.* **2009**, *113*, 1252–1259. [[CrossRef](#)]
57. Zhang, Q.; Zhu, L.; Zhu, D. Fermentation kinetics of blackcurrant fruit wine. *China Brew.* **2020**, *39*, 125–128. [[CrossRef](#)]
58. Moimenta, A.R.; Henriques, D.; Minebois, R.; Querol, A.; Balsa-Canto, E. Modelling the physiological status of yeast during wine fermentation enables the prediction of secondary metabolism. *Microb. Biotechnol.* **2023**, *16*, 847–861. [[CrossRef](#)]

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