Received: 12 September 2021 | Revised: 22 December 2021 | Accepted: 12 January 2022

DOI: 10.1111/1750-3841.16068

NEW HORIZONS IN FOOD RESEARCH

Journal of Food Science WILEY

Localization, purification, and characterization of a novel β-glucosidase from *Hanseniaspora uvarum* Yun268

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Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 31801528, 31972199

Abstract: β-Glucosidase is a key enzyme that hydrolyzes nonvolatile glycosylated precursors of aroma compounds and enhances the organoleptic quality of wines. In this study, a novel β-glucosidase from *Hanseniaspora uvarum* Yun268 was localized, purified, and characterized. Results indicated that β-glucosidase activity was mainly distributed within the cells. After purification via ammonium sulfate precipitation combined with chromatography, β-glucosidase specific activity increased 8.36 times, and the activity recovery was 56.90%. The enzyme had a molecular mass of 74.22 kDa. It has a Michaelis constant (K_m) of 0.65 mmol/L, and a maximum velocity (V_{max}) of 5.1 nmol/min under optimum conditions; and K_m of 0.94 mmol/L, and V_{max} of 2.8 nmol/min under typical winemaking conditions. It exhibited the highest activity at 50°C and pH 5.0 and was stable at a temperature range of 20–80°C and pH range of 3.0–8.0. The enzyme has good tolerance to Fe^{3+}, especially maintaining 93.68% of its activity with 10 mmol/L of Fe^{3+}. Ethanol (<20%) and glucose (<150 g/L) inhibited its activity only slightly. Therefore, β-glucosidase from *H. uvarum* Yun268 has excellent biochemical properties and a good application potential in winemaking.

KEYWORDS
biochemical properties, non-*Saccharomyces* yeast, varietal aroma, wine, β-glucosidase

Practical Application: Winemaking is a biotechnological process in which exogenous β-glucosidase is used to overcome the deficiency of endogenous β-glucosidase activity in grapes. By localizing, purifying, and characterizing of β-glucosidase from *Hanseniaspora uvarum* Yun268, it is expected to reveal its physical and chemical characteristics to evaluate its oenological properties in winemaking. The results may provide the basis for promoting the release of varietal aroma and improving wine sensory quality in the wine industry.

1 | INTRODUCTION

β-Glucosidase (EC 3.2.1.21) is a biologically important enzyme that hydrolyzes β-glucosidic linkage in aryl-, amino-, or alkyl-β-D-glucosides, cyanogenic glucosides, and disaccharides and short-chain oligosaccharides. It plays an essential role in the food, pharmaceutical, and biofuel industries (da Silva et al., 2019; Y. L. Li et al., 2018; J. Zhang, Zhao, et al., 2021). β-Glucosidase promotes the hydrolysis of aroma glycosides in winemaking and

significantly improves the aroma quality of wines (N. Li, Wang, Xu, et al., 2020).

The organoleptic properties of wine that reflect the typicality, style, and terroir are determined by the varietal aroma compounds in grapes, such as straight-chain alcohols, volatile terpenoids, shikimic acid metabolites, and norisoprenoids (Sun et al., 2018). However, many varietal aroma compounds exist as nonvolatile and odorless glycosides (Winterhalter & Skouroumounis, 1997). By enzymatic hydrolysis, these glycosides get volatilized during winemaking. Glycosides in the form of mono-glucoside are directly hydrolyzed by β-glucosidase, while those in the form of diglycoside-bound aglycones are sequentially hydrolyzed by α-L-arabinopyranosidase, α-L-rhamnopyranosidase, β-D-apiofuranosidase, or β-D-xylopyranosidase, followed by β-glucosidase (Claus & Mojsov, 2018; Y. H. Li, Wang, Xu, et al., 2020). Thus, β-glucosidase plays a significant role in the enzymatic hydrolysis of nonvolatile glycosides to release volatile aroma compounds in grapes and wine and improve the wine's quality (Dong et al., 2014; Wang et al., 2015).

β-Glucosidase is widely found in plants, animals, bacteria, and fungi. However, the activity of β-glucosidase in grapes and *Saccharomyces* yeast (a dominant strain in winemaking) is strongly inhibited under winemaking conditions, such as low pH, low temperature, and high ethanol or glucose concentrations (Barbagallo, Spagna, Palmeri, Restuccia, et al., 2004). Several studies have analyzed the β-glucosidases of lactic acid bacteria in winemaking (*Oenococcus oeni*) and fungi (*Aspergillus niger*) (Dong et al., 2014; Gottschalk et al., 1934; J. Zhang, Zhao, et al., 2021). However, lactic acid bacteria, due to poor growth characteristics and sulfur dioxide tolerance, do not have wide industrial applications. β-Glucosidases from fungi have already been applied in wine production; however, they exhibit poor specificity, causing the hydrolysis of pigment glycosides to produce unpleasant flavor (volatile phenols) (Hu et al., 2016; Maicas & Mateo, 2005). Meanwhile, β-glucosidase from non-*Saccharomyces* yeast, existing on the surface of grape berries and in fresh must, exhibits high activity and reasonable specificity for glycosides under winemaking conditions (Kong et al., 2019; Wang et al., 2015). Recently, special attention has been given to explore the prospective role of non-*Saccharomyces* yeast for the production of β-glucosidases with convenient features.

Hanseniaspora uvarum, one of the most abundant non-*Saccharomyces* yeasts in fresh must, is an excellent producer of β-glucosidase (Belda et al., 2016; López et al., 2015). Mixed fermentation of *S. cerevisiae* and *H. uvarum* Yun268 increased volatile terpenes and C_{13}-norisoprenoids due to the high activity of β-glucosidase of *H. uvarum* Yun268, enhancing the floral and fruit aroma characteristics of wine (Hu et al., 2018; Sun et al., 2018; Wang et al., 2015). However, the enzymology of β-glucosidase is unclear, which limits its application in winemaking. In this study, the production and localization of β-glucosidase from the *H. uvarum* Yun268 were analyzed, and their physical and chemical characteristics were assessed to evaluate its oenological properties in winemaking.

2 | MATERIALS AND METHODS

2.1 | Strain and media

The *H. uvarum* Yun268, obtained from the China Center for Type Culture Collection (No: M 2013658; Wuhan city, Hubei province, China), was precultured in yeast peptone dextrose (YPD) broth for 3 days, and then the YPD culture was inoculated into the fermentation medium at a ratio of 10% (v/v) and then incubated in a shaking incubator (180 rpm) at 28°C. Three fermentation media were used in the experiment as follows: medium A containing 5 g/L NH_4SO_4, 3 g/L NH_4NO_3, 20 ml/L yeast nitrogen base without amino acids (YNB), 4 g/L KH_2PO_4, 0.5 g/L $MgSO_4$, and 20 g/L glucose; medium B containing 5 g/L NH_4SO_4, 3 g/L NH_4NO_3, 20 ml/L YNB, 4 g/L KH_2PO_4, 0.5 g/L $MgSO_4$, 20 g/L glucose, and 2 g/L cellobiose; medium C containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L NH_4NO_3, 4 g/L KH_2PO_4, 0.5 g/L $MgSO_4$, and 20 g/L glucose.

2.2 | Enzyme activity and protein content

The β-glucosidase activity was measured by the colorimetric method (Barbagallo, Spagna, Palmeri, & Torriani, 2004). The reaction mixture contained 0.75 ml of citrate phosphate buffer (pH 5.0, 125 mM), 0.25 ml of *p*-nitrophenyl-β-D-glucopyranoside (*p*-NPGlu, 1 mM, J&K Scientific Ltd., Beijing, China), and 0.2 ml of enzyme solution. The reaction was performed at 40°C for 30 min and stopped by adding 1 ml of Na_2CO_3 solution (1 M). The *p*-nitrophenol (*p*-NP) generated was measured using an ultraviolet-visible spectrophotometer (Cary 60; Agilent Technologies, Bayan Lepas, Penang, Malaysia) at 400 nm against a blank without substrates. One unit (U) of β-glucosidase activity is defined as the amount of β-glucosidase necessary to liberate 1 μmol of *p*-NP per min at 40°C. The Bradford method was used to determine the protein content at 595 nm (0.9 ml of distilled water, 0.1 ml of protein solution, and 5 ml of coomassie brilliant blue G-250) using the bovine serum albumin standard. The standard curves of *p*-NP and protein are shown in Figure S1.

2.3 | Enzyme localization

Enzymatic activity of the culture supernatant, cell wall, and cell fragmentation supernatant was evaluated. Approximately 1.5 ml of the culture (the fermentation medium containing *H. uvarum* Yun268, which was collected at 72 h) was centrifuged at 8000 rpm for 15 min to obtain the culture supernatant and cell pellet. The culture supernatant was filtered through a 0.45 μm filter before enzymatic activity determination. The cell pellet was washed twice in sterile saline, resuspended in 1.5 ml of PBS buffer (pH 7.5, 10 mM), and broken up with a grinder (JXFSTPRP-15; Jingxin Technologies, Shanghai, China). This mixture was centrifuged to yield the fragmentation supernatant and cell debris. The cell debris was washed twice in sterile saline and resuspended in 1.5 ml of PBS buffer to obtain the cell wall fraction. All fractions were stored at 4°C for further analysis (Arévalo Villena et al., 2005; Mcmahon et al., 1999).

2.4 | Enzyme production

The optimal medium was identified based on the β-glucosidase activity of cell fragmentation supernatant (the highest enzyme activity fraction in Section 2.3)of the different medium. The *H. uvarum* Yun268 was cultured in a fermentation medium to produce β-glucosidase. The visible cells in the fermentation medium were collected 8 times (0, 17, 28, 40, 52, 64, 76, and 88 h) and then were counted using Wallerstein nutrient (WLN) agar (Hu et al., 2018) during the fermentation. The activity of β-glucosidase in fragmentation supernatant was determined at the end of fermentation (88 h) to evaluate the amount of enzyme produced in the media. Further, the cells in the optimal medium were harvested 7 times (24, 48, 60, 72, 84, 96, and 120 h), and the β-glucosidase activity was determined to ascertain the timing of peak enzyme synthesis.

2.5 | Enzyme purification

Here, $(NH_4)_2SO_4$ was added to the crude enzyme extract on ice (the fraction with the highest β-glucosidase activity in Section 2.3) to 80% saturation to purify β-glucosidase from *H. uvarum* Yun268. The mixture was incubated for 8 h at 4°C and then centrifuged at 8000 rpm for 10 min to obtain the precipitate, which was dissolved in acetic acid-sodium acetate buffer (pH 5.0, 50 mM). The $(NH_4)_2SO_4$ precipitate was desalted with a dialysis bag (cutoff 14 kDa) using acetic acid-sodium acetate buffer (pH 5.0, 50 mM) at 4°C, with four buffer changes at 4 h intervals. The solution was concentrated with PEG 20,000 (4°C, 30 min) and loaded onto a DEAE-Sepharose fast flow column (1.0 cm × 20 cm) at a flow rate of 1.33 ml/min (Hu et al., 2018; Zhuang et al., 2016). The elution (protein peak elution was obtained at 280 nm) was carried out using an increasing gradient of NaCl (0–1 M) in five CV of phosphate buffer (pH 6.0, 50 mM). Fractions were collected and analyzed for β-glucosidase activity. The active fractions were pooled, concentrated with PEG 20,000 (4°C, 30 min), loaded onto a Sephadex G-200 column (1.6 cm × 40 cm), and eluted with citrate phosphate buffer (pH 6.5, 50 mM) at 0.4 ml/min flow rate to obtain protein peak elution at 280 nm. Each fraction was used for enzyme assay. The fractions with peak enzyme activity were pooled for further analysis.

2.6 | Electrophoresis and molecular weight determination

The protein samples for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were prepared, electrophoresed, and visualized following the previously reported method (Dong et al., 2014). The protein samples were prepared by mixing the same volume of purified β-glucosidase (Sephadex G-200 column purified fraction) and gel loading buffer, boiled for 3 min, and loaded onto the gel, along with a molecular weight marker. After electrophoresis, the gel was stained for 12 h with 0.25% Coomassie brilliant blue R-250 on a rocker. Later, the gel was destained with acidified methanol-water (45% methanol and 10% acetic acid) until the protein bands became distinguishable and prominent.

The molecular weight of the purified β-glucosidase was determined using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Lippens et al., 2018). The chromatographic separation was carried out on a Shimadzu UPLC system (Nexera UHPLC LC-30A, Kyoto, Japan). Mass spectrometry determination was carried out on an AB SCIEX Triple TOF 4600 (Foster City, CA, USA).

2.7 | Effect of temperature and pH

The effects of pH on the enzyme activity were evaluated by incubating the purified enzyme in citrate-phosphate buffer at pH ranging from 3.0 to 8.0. After setting the enzyme in different buffers at 4°C for 12 h, the stability was evaluated. Meanwhile, the effect of temperature (20−80°C) on the enzyme activity was investigated at pH 5.0. The thermal stability of β-glucosidase was determined after incubating the purified enzyme solutions at temperatures ranging from 20 to 80°C for 1 h and in an ice bath for 30 min.

2.8 | Inhibitory test

The activity of β-glucosidase from *H. uvarum* Yun268 was assayed in varying concentrations of ethanol, sugars (glucose, fructose, and sucrose), and chloride salts ($FeCl_3$, $MnCl_2$, $MgCl_2$, KCl, and $CaCl_2$) to determine the influence of inhibitors. The inhibitor was added to the solution (0.75 ml of citrate phosphate buffer [pH 5.0, 125 mM] and 0.2 ml of the enzyme solution) and incubated at 30°C for 1 h, and the β-glucosidase activity was measured immediately. The β-glucosidase activity measured without the inhibitor was used as a control.

2.9 | Kinetic parameters

Different concentrations of *p*-NPGlu (0.25 to 3 mmol/L) were used to determine the kinetic parameters (K_m and V_{max}) of β-glucosidase under optimum conditions (optimum pH and optimum temperature) and under typical winemaking conditions (at 25°C, pH 3.5 and 15% ethanol) following the previously reported method by drawing the Lineweaver–Burk plot (Dong et al., 2014). The reciprocal plot (1/[V] vs. 1/[S]) was plotted, and the K_m and V_{max} were determined from the slope and intercept of the straight line (Mishra et al., 2012).

3 | RESULTS AND DISCUSSION

3.1 | Localization and production of β-glucosidase

Three fractions were analyzed (culture supernatant, cell wall, and cell fragmentation supernatant) to determine β-glucosidase localization. As shown in Figure 1, β-glucosidase activity was detected in all three fractions; however, enzyme activity and specific activity were significantly higher in the cell fragmentation supernatant than in the other two fractions, suggesting β-glucosidase distribution inside *H. uvarum* Yun268 cells. Enzyme activity in the culture supernatant and the cell wall might have derived via the cell autolysis during growth. The localization of β-glucosidase in *H. uvarum* Yun268 is consistent with that in other *H. uvarum* strains (Arévalo Villena et al., 2005; Swangkeaw et al., 2010).

The composition of the medium (carbon source, nitrogen source, or metal ions) often affects enzyme production. As shown in Figure 2a,b, no significant difference in viable cell number and β-glucosidase activity in cell fragmentation supernatant was observed between medium B (2 g/L cellobiose) and medium A after 88 h, indicating no inductive effect of cellobiose on β-glucosidase production. Meanwhile, viable cell number in cell fragmentation supernatant of *H. uvarum* Yun268 cultured in medium C (containing organic nitrogen: 10 g/L yeast extract, 20 g/L peptone) for 88 h was 4.5 times more than that grown in medium A (inorganic nitrogen as the sole nitrogen), and β-glucosidase activity in cell fragmentation supernatant of *H. uvarum* Yun268 cultured in medium C for 88 h was 3.8 times more than that cultured in medium A. The addition of organic nitrogen significantly promoted the growth of *H. uvarum* Yun268 and the production of β-glucosidase. Similarly, the highest β-glucosidase activity in *Aspergillus awamori* 2B.361 U2/1 was observed when yeast extract was present in the medium (Gottschalk et al., 1934). However, the mechanism by which the organic nitrogen affected β-glucosidase production remains unclear (Mateo Tolosa, 2019). In addition, β-glucosidase activity attained a peak after 60 h growth of *H. uvarum* Yun268 in medium C (Figure 2c). The cell fragmentation supernatant at this time was used for the subsequent experiments.

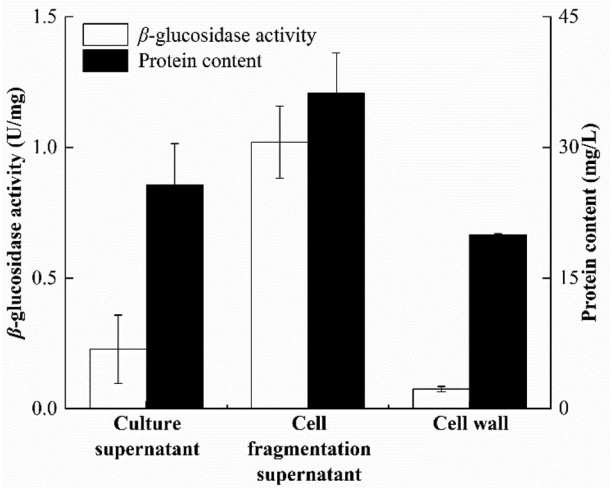

FIGURE 1 β-Glucosidase localization in *H. uvarum* Yun268

3.2 | β-Glucosidase purification

The β-glucosidase from *H. uvarum* Yun268 was purified by $(NH_4)_2SO_4$ precipitation, ion exchange, and gel-filtration chromatography. The results of β-glucosidase purification are presented in Table 1, and the electrophoresis of enzyme solution was shown in Figure 3. After $(NH_4)_2SO_4$ precipitation, the β-glucosidase specific activity was 0.24 U/mg, and the purification factor was 1.09, similar to the crude extract. This observation suggested that β-glucosidase was not effectively separated from the other proteins through $(NH_4)_2SO_4$ precipitation. Subsequently, two distinct fractions were eluted from the DEAE-Sepharose FF column until 2 h 44 min (Figure 3a), of which the second peak

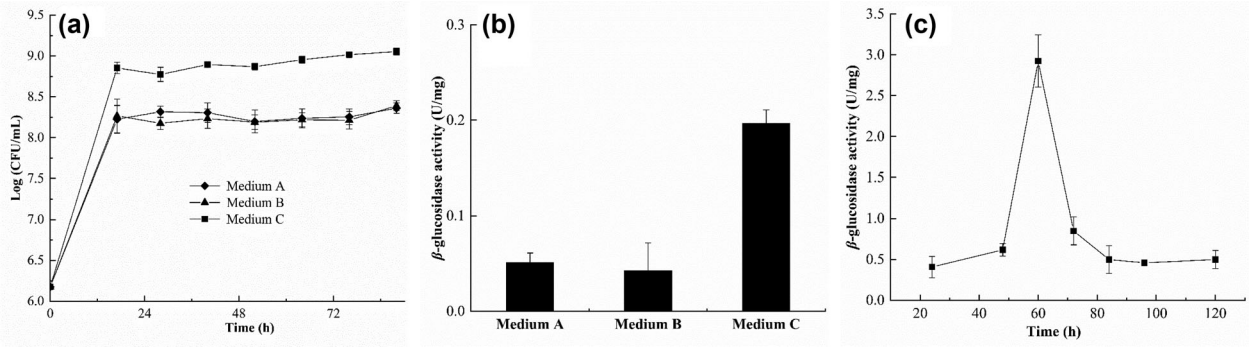

FIGURE 2 Growth of *H. uvarum* Yun268 and production of β-glucosidase derived from cell fragmentation supernatant. (a) Biomass during fermentation and (b) β-glucosidase activity at the end of fermentation (88 h) under different media. (c) β-Glucosidase activity during fermentation in medium C

TABLE 1 Purification protocol for β-glucosidase from *H. uvarum* Yun268

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification factor	Yield (%)
Crude extract	2.97	13.69	0.22	1.00	100.00
$(NH_4)_2SO_4$ precipitation	2.53	10.58	0.24	1.09	85.19
DEAE-Sepharose FF	2.50	2.43	1.03	4.68	84.18
Sephadex G-200	1.69	0.92	1.84	8.36	56.90

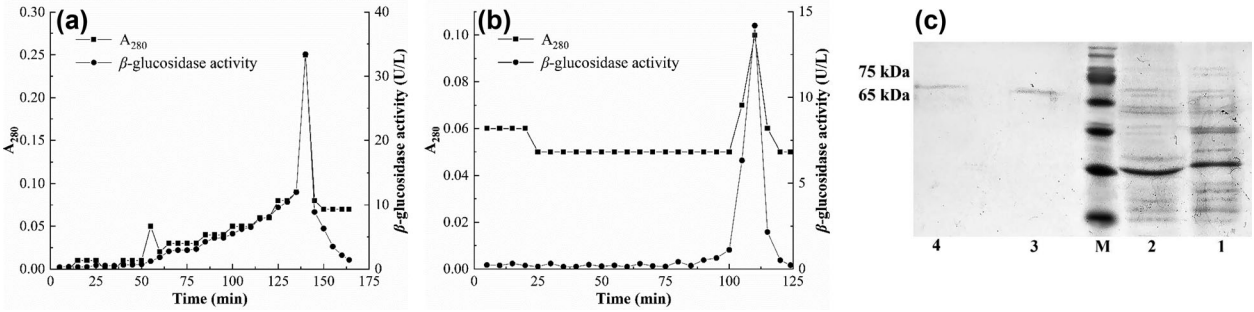

FIGURE 3 The result of β-glucosidase purification. (a) Chromatography spectrogram of DEAE-Sepharose FF. (b) Chromatography spectrogram of Sephadex G-200. (c) SDS-PAGE analysis of purified β-glucosidase. Lane assignment: lane M, protein marker; lane 1, Crude extract; lane 2, $(NH_4)_2SO_4$ precipitation; lane 3, DEAE-Sepharose FF; lane 4, Sephadex G-200

showed higher β-glucosidase activity (33.43 U/L). DEAE-Sepharose FF column chromatography improved the β-glucosidase specific activity and purification factor by 4.68 times, while the yield of enzyme activity was 84.18%. Only one fraction was eluted from the Sephadex G-200 column until 2 h 4 min (Figure 3b). This fraction had a β-glucosidase activity of 14.19 U/L. Sephadex G-200 column chromatography increased β-glucosidase specific activity and purification factor by 8.36 times; however, the yield of enzyme activity decreased by 56.90%. The SDS-PAGE gel electrophoresis showed many bands for the crude enzyme solution and $(NH_4)_2SO_4$ precipitation (Figure 3c), indicating the presence of several miscellaneous proteins. Meanwhile, a single band was obtained after ion-exchange and gel-filtration chromatography. UPLC-MS/MS was used to analyze the purified β-glucosidase, and the result is shown in Figure S2. The intensity of the purified β-glucosidase was significantly higher than that of the miscellaneous proteins, indicating that the β-glucosidase, with molecular mass 74.22 kDa, was highly purified. The β-glucosidase purification factor and enzyme activity yield obtained in this study were consistent with the earlier reports (Choi et al., 2011; Dong et al., 2014). In this study, after three purification steps, the purification factor and enzyme

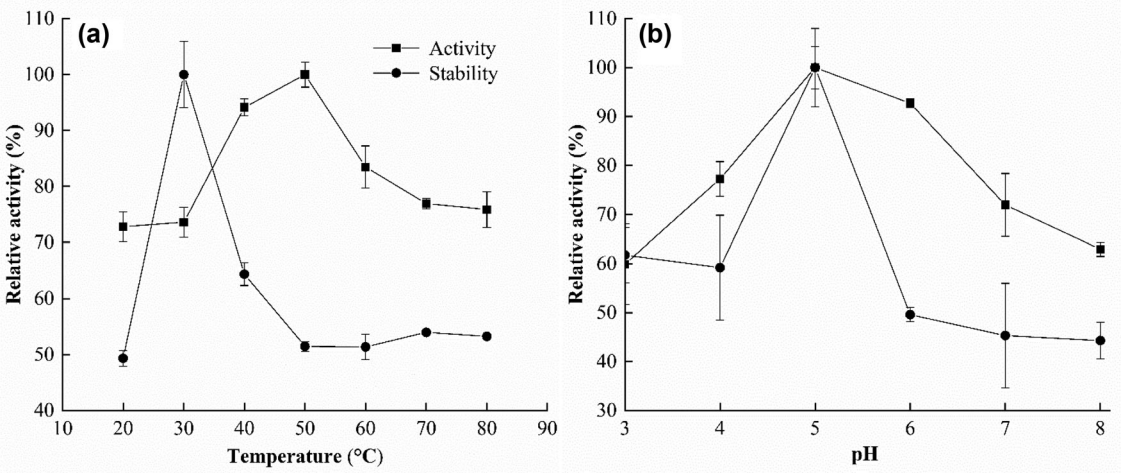

FIGURE 4 Effect of (a) temperature and (b) pH on β-glucosidase

activity yield of β-glucosidase from *H. uvarum* Yun268 were 8.36 and 56.90%, respectively, indicating well-purified β-glucosidase.

3.3 | Biochemical properties of β-glucosidase

3.3.1 | Effects of temperature and pH on β-glucosidase activity and stability

Temperature and pH are essential factors that affect the activity and stability of β-glucosidase, which determines its application in winemaking. The β-glucosidase from *H. uvarum* Yun268 showed the highest activity at pH 5.0 and 50°C and was the most stable at pH 5.0 and 30°C (Figure 4). Besides, the enzyme remained stable in the temperature range of 20–80°C, conserving more than 50% of the original activity. It also remained stable in the pH range of 3.0–8.0, preserving more than 45% of the initial activity. The optimal temperature and pH recorded in this study were consistent with the β-glucosidase isolated from *Pichia guilliermondii* G1.2, *P. guilliermondii* K123-1, *O. oeni* ATCC BAA-1163, and *Lactobacillus plantarum* USC1 (da Silva et al., 2019; Michlmayr et al., 2010; Sestelo et al., 2004). However, the activity of most β-glucosidases reported previously was inhibited under the winemaking conditions (pH = 3.0–3.5, temperature = 18–30°C), which urged researchers to explore the prospective role of microorganisms with stable β-glucosidase activity during winemaking. The β-glucosidase activity from *Wickerhamomyces Anomalus* AL112 and MDD24 maintained 30% and 10% at pH 3.0, respectively. The β-glucosidase activity from *Sporobolomyces Pararoseus* SP8A, *W. Anomalus* 6 and Wa1 showed high activity at pH 4.0, but decreased dramatically at pH 3.0 (P. Zhang, Zhang, et al., 2021). In the present study, the β-glucosidase from *H. uvarum* Yun268 was stable and maintained an activity above 50% at 20–30°C; its activity was above 60% at pH 3.0–3.5, indicating β-glucosidase from *H. uvarum* Yun268 as a good candidate for the wine industry.

3.3.2 | Inhibitory tests

Few compounds (ethanol, sugars, and metal ions) inhibit β-glucosidase activity, limiting the application of β-glucosidase (Cota et al., 2015; Dong et al., 2014; J. Zhang, Zhao, et al., 2021). As shown in Table 2, ethanol slightly inhibited the activity of β-glucosidase from *H. uvarum* Yun268. Meanwhile, glucose at 50 g/L concentration slightly promoted β-glucosidase activity while at 100 and 150 g/L concentrations slightly inhibited; glucose at 200 g/L concentration almost inactivated the enzyme. β-Glucosidase was more sensitive to fructose and sucrose than glucose when the sugar concentration was less than 150 g/L; both fructose and sucrose at 50 g/L remarkably inhibited the enzyme activity. Among the metal ions investigated, Mn^{2+}, Mg^{2+}, K^{+}, and Ca^{2+} remarkably reduced β-glucosidase activity, while Fe^{3+} had very little inhibitory effect. Especially the enzyme maintained 93.68% of the activity with 10 mmol/L of Fe^{3+}.

Sugars (fructose, glucose, and sucrose) in fruit juices generally inhibit β-glucosidase activity, limiting the application of β-glucosidase in winemaking (Cota et al., 2015; Swangkeaw et al., 2010). Grape must contains a large amount of glucose, so the tolerance level of β-glucosidase to glucose is considered as an important criterion for the selection of β-glucosidase in wine production. Generally, the β-glucosidase activity decreases and the substrate hydrolysis slows down with glucose at a high level. In addition, yeast growing at high level of glucose concentration

TABLE 2 The result of inhibitory and substrate specificity tests

Compound	Concentration	Relative activity (%)
Control	0	100 ± 0[a]
Ethanol (%, v/v)	5	86.75 ± 5.80[b]
	10	81.39 ± 4.02[b]
	15	79.97 ± 2.01[b]
	20	89.12 ± 2.90[b]
Control (g/L)	0	100 ± 0[a]
Glucose	50	116.56 ± 7.81[b]
	100	92.59 ± 3.35[ac]
	150	82.97 ± 5.80[c]
	200	3.94 ± 2.90[d]
Control (g/L)	0	100 ± 0[a]
Fructose	50	64.80 ± 3.48[b]
	100	49.81 ± 4.24[c]
	150	42.75 ± 0.82[d]
	200	34.43 ± 3.01[e]
Control (g/L)	0	100 ± 0[a]
Sucrose	50	50.39 ± 4.51[b]
	100	38.97 ± 1.78[c]
	150	51.06 ± 2.04[b]
	200	48.81 ± 2.03[b]
Control (mmol/L)	0	100 ± 0[a]
Fe^{3+}	10	93.68 ± 5.75[a]
Mn^{2+}	10	57.00 ± 8.27[b]
Mg^{2+}	10	55.77 ± 8.57[b]
K^{+}	10	61.25 ± 6.40[b]
Ca^{2+}	10	59.19 ± 7.92[b]

Note: Values represent means ± standard error ($n = 3$). Different letters in different treatments denote a significant difference ($p < 0.05$).

may produce too many alcohols that inhibit β-glucosidase activity. López et al. (2015) found the β-glucosidase activity from *Pichia membranifaciens* Pm7, *Hanseniaspora vineae* Hv3, and *Wickerhamomyces anomalus* Wa1 maintained lower than 40% of the maximum activity when glucose at 36 g/L. It has also been reported that low concentrations of glucose increase β-glucosidase activity. Corrêa et al. (2021) reported that glucose improves the β-glucosidase activity by changing the size of the activity site entrance. Ethanol could inhibit β-glucosidase activity, probably due to denaturation (Gueguen et al., 1997). Swangkeaw et al. (2010) found that β-glucosidase from *Hanseniaspora* sp. BC9 was substantially inhibited in the presence of 4%–12% (v/v) ethanol. Barbagallo Spagna, Palmeri, and Torriani (2004) found that β-glucosidase of commercial strain DSTVR 1 maintained 50% and 25% of the activity at 8% and 16% ethanol (v/v), respectively. β-Glucosidase with low tolerance to ethanol has low activity or even inactivation in the middle or late alcohol fermentation period, which is not conducive to the release of wine aroma. In the present study, β-glucosidase from *H. uvarum* Yun268 had the excellent tolerance to glucose and ethanol, suggesting its good performance in the winemaking.

3.3.3 | Kinetic parameters

K_m and V_{max} values are the characteristic constants of the enzyme under the certain condition. For β-glucosidase from *H. uvarum* Yun268, the K_m and V_{max} values for *p*-NPGlu were 0.65 mmol/L and 5.1 nmol/min under optimum conditions, respectively. The Lineweaver–Burk plot is shown in Figure S3. The K_m of this β-glucosidase was lower than those of β-glucosidases from *Aspergillus niger* CDBB-H-175 (2.7 mmol/L), *A. niger* CDBB-H-176 (2.2 mmol/L), and *O. oeni* 31MBR (1.05 mmol/L) (Alarid García & Escamilla Silva, 2017; Dong et al., 2014), which indicates this enzyme has a higher binding force to aroma glycosides than the enzymes mentioned above. Besides, The V_{max} was higher than that of β-glucosidase from *O. oeni* 31MBR (0.957 nmol/min), which indicates that this enzyme in our study has a better catalytic activity. In order to investigate the application potential of this enzyme, the kinetic parameters were determined under typical winemaking conditions (25°C, pH 3.5, and 15% ethanol). It was found that its K_m (0.94 mmol/L) increased by 44.6% and its V_{max} (2.8 nmol/min) decreased by 45.1% compared with those under optimum conditions. It indicates that winemaking conditions limited the catalytic ability of this enzyme greatly. However, its K_m was still lower than the K_m of enzymes from *Candida glabrata* strain D18 and *H. uvarum* strain H30, and its V_{max} was similar to those enzymes under typical winemaking conditions (Han et al., 2021). It was reported that the enzymes from the strains D18 and H30 both showed high β-glucosidase activity during 16 days of wine fermentation and finally significantly increased the aroma of wine, suggesting that β-glucosidase in the present study may exhibit high catalytic efficiency in releasing wine aroma in winemaking.

4 | CONCLUSION

β-Glucosidase, a protein with an estimated molecular mass of 74.22 kDa, was purified from *H. uvarum* Yun268 cells using a combination of $(NH_4)_2SO_4$ precipitation and chromatographic methods. It conserved more than 50% of the original activity at the temperature and pH range investigated. Furthermore, ethanol and glucose showed only a slight inhibitory effect on the enzyme activity. Its K_m and V_{max} values were 0.94 mmol/L and 2.8 nmol/min under typical winemaking conditions, respectively. These

findings indicate the great potential of β-glucosidase from *H. uvarum* Yun268 in the wine industry due to its excellent tolerance to winemaking conditions. However, the action mechanism of this β-glucosidase on the glycosides of aroma compounds in grapes and wines needs to be investigated in the future.

ACKNOWLEDGMENTS

This study was financially supported by the National Natural Science Foundation of China (31801528 and 31972199), Chinese Postdoctoral Science Foundation (2019M663833), the Key Research and Development Project of Xinjiang Uygur Autonomous Region in the 14th Five Year Plan (2020B01005-2), and the Basic Research Program of Natural Science in Shaanxi Province (2021JQ-147).

AUTHOR CONTRIBUTIONS

Tongtong Fan: writing—original draft–Equal. Siyu Jing: data curation–equal. Hongyan Zhang: formal analysis–equal. Xiaobing Yang: validation–equal. Guojie Jin: project administration–equal; writing—original draft–equal; writing—review & editing–Equal. Yongsheng Tao project administration–equal; resources–equal; supervision–equal; writing—review & editing–equal.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Fan, T., Jing, S., Zhang, H., Yang, X., Jin, G., & Tao, Y. (2022). Localization, purification, and characterization of a novel β-glucosidase from *Hanseniaspora uvarum* Yun268. *Journal of Food Science*, *87,* 886–894. https://doi.org/10.1111/1750-3841.16068