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Valorisation of wine wastes by *de novo* biosynthesis of resveratrol using a recombinant xylose-consuming industrial *Saccharomyces cerevisiae* strain†

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Resveratrol is a stilbenoid with strong antioxidant activity and several beneficial properties for human health. Plant extraction of resveratrol from natural sources is expensive and non-sustainable, owing to the low quantity of biomass and generally restricted availability. Biotechnological production of resveratrol can overcome these drawbacks. Here, the heterologous resveratrol biosynthetic pathway (*via* phenylalanine) was expressed in a xylose-consuming *Saccharomyces cerevisiae* strain. We further elucidated the roles of the pentose phosphate pathway and nutritional supplementation in resveratrol titres. By simultaneous fermentation of glucose and xylose, a 1.31-fold increase in resveratrol titre was observed when compared with glucose-only cultivation at the same carbon molarity, achieving a titre of 388 mg L^{-1}. The recombinant strain was able to consume all sugars present in wine wastes, including non-naturally metabolised sugars like xylose. This allowed the valorisation of different vineyard residues, such as wine lees, grape must and hemicellulosic hydrolysate from vine pruning, achieving titres between 167.1 and 282.7 mg L^{-1} of resveratrol. The potential of environmental-friendly biotechnological processes over conventional processes like plant extraction using hazardous and polluting solvents is emphasised. This is the first report on the use of renewable carbon sources for resveratrol production from xylose and the use of winery by-products as a substrate to produce this stilbenoid. The expanded multi-sugar utilisation capacity of this yeast is valuable in a biorefinery context and obtaining high-value products such as resveratrol is critical to increasing process feasibility following a circular economy concept.

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1. Introduction

Resveratrol is a stilbenoid with strong antioxidant activity that plays a role in plant defence against environmental stresses.[1] Even though therapeutical effects of resveratrol in humans are still unclear regarding its mode of action and molecular target, several beneficial properties are attributed to resveratrol, such as treatment/prevention of cardiovascular diseases,[2] and anti-inflammatory[3] and anti-ageing properties.[4,5] Microbial production of resveratrol from carbon sources can be attained through tyrosine[6] or phenylalanine[7–9] *via* the shikimate pathway. In the latter, phenylalanine is converted into *p*-coumaric acid through the intermediary cinnamic acid. Coumaric acid is then converted into *p*-coumaroyl-CoA, which ultimately leads to resveratrol formation by condensation of this precursor with 3 molecules of malonyl-CoA[10] (Fig. 1). Resveratrol production has been previously reported in engineered *Saccharomyces cerevisiae* from different carbon sources such as glucose, lactose or ethanol.[6–9]

The current environmental and economic challenges have pushed the need for sustainable alternatives to produce energy and value-added products from renewable resources.[11,12] In this sense, using agro-industrial wastes has been gaining attention as possible feedstocks for biotechnological processes.[13] The Iberian Peninsula is an exceptional territory to produce a wide variety of wines. Both Portugal and Spain are among the main wine producers on the planet.[14] This extensive manufacture generates large quantities of wastes like vine pruning residues, grape pomace, or wine lees. Wine lees consist of residual fermentative yeast and other particles, and have high nitrogen and organic content, including acids,

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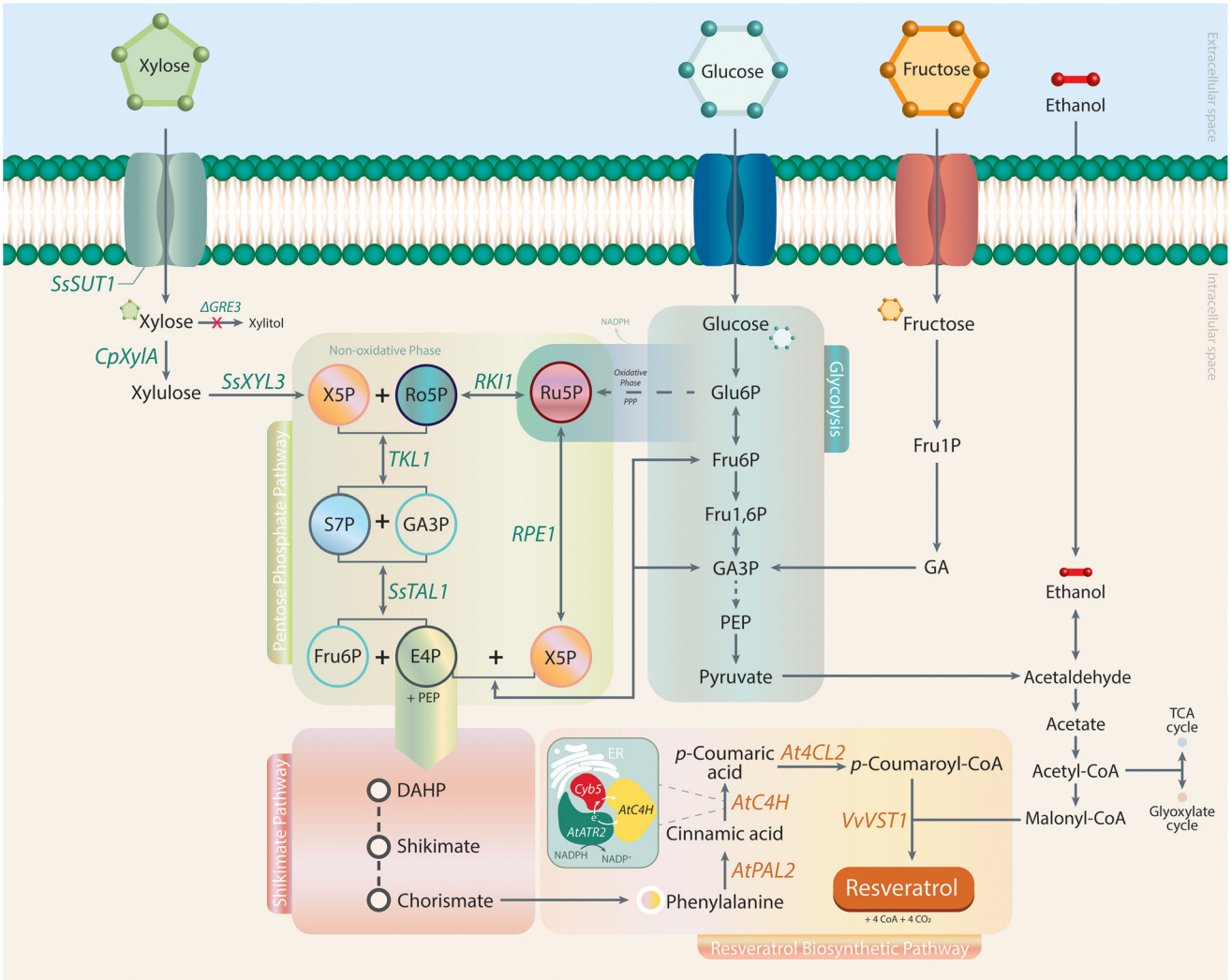

Fig. 1 Metabolic pathway for resveratrol production from several carbon sources. Single arrows represent single reaction steps and dashed arrows represent multiple reaction steps. Genes (over)expressed in the strain L543 are represented next to the reactions that are catalysed by them. *SsSUT1* encodes for a sugar transporter with a higher affinity for xylose from *Scheffersomyces stipitis*; *CpXylA*, xylose isomerase from *Clostridium phytofermentans*; *SsXYL3*, D-xylulokinase from *S. stipitis*. Glycolysis: Glu6P, glucose 6-phosphate; Fru6P, fructose 6-phosphate; Fru1,6P, fructose 1,6-biphosphate; GA3P, glyceraldehyde 3-phosphate; PEP, phosphoenolpyruvate. Fru1P, fructose 1-phosphate; GA, glyceraldehyde. Pentose Phosphate Pathway: X5P, xylulose 5-phosphate; Ro5P, ribose 5-pho 1-sphate; Ru5P, ribulose 5-phosphate; S7P, sedoheptulose 7-phosphate; E4P, erythrose 4-phosphate; *RPE1*, D-ribulose 5-phosphate 3-epimerase; *RKI1*, D-ribose-5-phosphate ketol-isomerase; *TKL1*, transketolase; *SsTAL1*, transaldolase from *S. stipitis*. DAHP, 3-deoxy-D-arabinoheptulosonate 7-phosphate resveratrol biosynthetic pathway: *AtPAL2*, phenylalanine ammonia-lyase from *Arabidopsis thaliana*; *AtC4H*, cinnamic acid hydroxylase from *A. thaliana*; *At4CL2*, *p*-coumaroyl-CoA ligase from *A. thaliana*; *VvVST1*, resveratrol synthase from *Vitis vinifera*.

phenols, and ethanol.[15] They have been previously proposed as low-cost nutrients for microbial production of biochemical products,[16] but could also be used as substrate due to their ethanol content. Additionally, there are substantial volumes of grape must, rich in glucose and fructose, that are not used for wine production, due to being a surplus or having low quality. Grape must can be an interesting carbon source for biotechnological processes for value-added products generation, being already reported for the production of erythritol or mannitol.[16,17] Furthermore, vine pruning, which consists of the necessary removal of parts of a grapevine to renew its canes, generates significant amounts of lignocellulosic biomass, being the major by-product of viticulture, and their polysaccharides (cellulose and hemicellulose) constituents can be valuable substrates for biotechnological processes.[18] To attain fermentable sugars from vine pruning, pretreatment and hydrolysis are mandatory steps to break down the recalcitrant and typical structure of lignocellulosic biomasses.[19] Hydrothermal treatment (also known as autohydrolysis) of vine pruning has been employed as the first step of a biorefinery for the solubilisation of hemicellulose into xylooligosaccharides[20] and for ethanol production from cellulose.[21] The pentose sugar xylose is the most abundant sugar in the hemicellulosic fraction of vine pruning, followed by glucose. Xylose can comprise up to 20% of the total carbon content of

lignocellulose.[22] However, it is not naturally metabolised by *S. cerevisiae*, an industry workhorse for ethanol fermentation.[23]

Xylose metabolism in *S. cerevisiae* can be achieved by two different pathways, enabling the conversion of xylose into xylulose. The oxidoreductase pathway involves a two-step reaction: xylose reductase (XR) reduces xylose to xylitol, which is then oxidised to xylulose through the action of xylitol dehydrogenase (XDH). These two pathways can act independently or in concert.[24] A cofactor imbalance between the predominantly NADPH-dependent XR and the NAD+-dependent XDH leads to xylitol accumulation, thus capping the carbon flow to other metabolic routes such as ethanol or other value-added compounds production, consequently reducing fermentation yields.[25] Furthermore, an unspecific aldose reductase encoded by the endogenous *GRE3* gene can also convert xylose to xylitol, thus intensifying xylitol build-up.[26] Several studies have relied on the deletion of the *GRE3* gene to minimise xylitol accumulation,[27,28] while its overexpression has led to a xylitol high-production phenotype.[29–31] The isomerase pathway relies on a single reaction catalysed by xylose isomerase (XI), which can convert xylose directly into xylulose without requiring any cofactor.[32,33] Xylulose is then phosphorylated by xylulokinase (XK) to xylulose-5-phosphate (X5P) and shuffled to glycolysis *via* the pentose phosphate pathway (PPP). Several studies focused on the overexpression of XK, either the native *S. cerevisiae XKS1*[27,28,34] or the *Scheffersomyces stipitis XYL3*,[35] as well as the non-oxidative PPP genes (*RPE1*, *RKI1*, *TKL1*, *TAL1*) to enhance xylose assimilation.[27,28,33,36–38]

Besides its central role in xylose metabolism, the PPP is also vital to producing several valuable compounds like polyols, biofuels or phenylpropanoids. The PPP is tightly related to several metabolic steps of glycolysis, either by the assimilation of glucose-6-phosphate in the oxidative part of the PPP or the reversible reactions of the non-oxidative PPP to form fructose-6-phosphate or glyceraldehyde-3-phosphate.[39] Furthermore, the non-oxidative PPP is responsible for generating erythrose 4-phosphate (E4P) in the cell. E4P with phosphoenolpyruvate (PEP) from glycolysis condensate to form 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP), which flows into the shikimate pathway for the biosynthesis of aromatic amino acids like tyrosine and phenylalanine (Fig. 1). Malonyl-CoA is also an essential precursor to producing resveratrol. Furthermore, because malonyl-CoA is primarily employed as an important intermediary in fatty acid biosynthesis to maintain cell development, only a small amount of malonyl-CoA is available for resveratrol biosynthesis, which is a significant hurdle in resveratrol production.[40] Malonyl-CoA is converted from acetyl-CoA in a reaction catalysed by acetyl-CoA carboxylase (encoded by *ACC1*), which originated in the yeast cell from acetate. In its turn, acetate is derived from acetaldehyde that results from pyruvate or ethanol consumption, which can help to balance the supply of malonyl-CoA in the yeast cell (Fig. 1).

In this study, we focused on developing a resveratrol-producing recombinant industrial *S. cerevisiae*, able to metabolise all the sugars present in wine wastes, including the non-naturally metabolised xylose. Then, we aimed at the application of this recombinant strain for the valorisation of vineyard residues, such as hemicellulosic hydrolysate from vine pruning, grape must and wine lees. To accomplish that, we introduced a resveratrol biosynthetic pathway into a xylose-consuming strain, further elucidating the role of PPP in resveratrol production and fine-tuning the nutritional supplementation of the fermentation media for improved resveratrol titres. We also evaluated the impact of simultaneous fermentation of xylose and glucose. This is the first report on the use of renewable carbon sources for resveratrol production from xylose and the use of winery by-products as a substrate to produce this stilbenoid. The expanded multi-sugar utilisation capacity of this yeast is valuable in a biorefinery context, and the obtention of high-value products such as resveratrol is key to increasing the process feasibility following a circular economy concept.

2. Materials and methods

2.1. Plasmid construction

Plasmid construction in this study was accomplished by USER cloning, according to Jensen *et al.*[41] A list of plasmids, biobricks and primers used in this study is provided in Tables S1, S2 and S3,† respectively. Integrative vectors were generated using the EasyClone-MarkerFree vector set[42] to assemble the different biobricks, and the resultant plasmids were sequenced to confirm proper assembly. *E. coli* strains DH5α competent cells (NZYtech) were used for gene cloning tasks, and the *E. coli* transformants were selected and kept on Lysogeny Broth plates with 100 mg L^{-1} of ampicillin.

2.2. Yeast strains

All strains used in this study are derivatives from Ethanol Red®, an *S. cerevisiae* commercial strain developed by Fermentis, S.I. Lesaffre, mainly used for bioethanol fermentation. Yeast transformations were performed according to the lithium acetate protocol.[43] The parent strains were initially transformed with a Cas9-expressing plasmid,[44] followed by a subsequent transformation where the desired DNA fragment and the guide RNA (gRNA) plasmid targeting the required insertion site.[42] The strain L323 is described in Costa *et al.*,[27] expressing the resveratrol biosynthetic pathway (RBP), consisting of the genes *AtPAL2, At4CL2, AtC4H* and *VvVST1*. The strain L326 was developed by insertion of the RBP into a previously engineered xylose-consuming strain developed by Stovicek *et al.*[38] The strain L543 was developed by (over)expression of the genes *AtATR2* and *ScCYB5* in the strain L326. A detailed description of the genotype of all the strains is provided in Table S4.†

2.3. Media and cultivations

The recombinant yeast strains were kept on YPD plates (2% glucose, 2% peptone, 1% yeast extract, 1.5% technical agar). For all the experiments, cell pre-cultures were incubated overnight in 250 mL baffled shake flasks with a working volume of 50 mL of YPD20 (2% dextrose, 2% peptone, 1% yeast extract),

with orbital shaking (300 rpm), at 30 °C. Yeast grown overnight was collected at 4000 rpm for 5 min, subsequently washed with sterile deionised water and resuspended in 0.9% sodium chloride, for a final cell concentration of 300 g L^{-1} in fresh weight (FW). The initial inoculum was 8 g L^{-1} of cells (FW) for all fermentation assays, with biological duplicates. Percentages are weight per volume (w/v).

2.3.1. Synthetic media fermentation. Fermentations in synthetic media were carried out in 250 mL baffled shake flasks with a working volume of 50 mL, with orbital shaking (300 rpm), at 30 °C. Concentrated sugar solutions of glucose and xylose (200 g L^{-1}) were filter-sterilised and added to the media accordingly to the desired concentrations for each experiment, while the rest of the media components (water, yeast extract and peptone) were autoclaved separately at 121 °C for 15 min in the indicated concentrations. Standard YP supplementation was 10 g L^{-1} of yeast extract and 20 g L^{-1} of peptone and optimisation of nutritional supplementation ranged from 0 to 50 g L^{-1} of yeast extract with no peptone added.

2.3.2. Wine wastes fermentations

2.3.2.1. Hemicellulosic hydrolysate from vine pruning residue. Vine pruning residue (VPR), collected in May 2019, provided by the Department of Agriculture Research (ITACyL, Finca Zamadueñas, Valladolid, Spain), was used as raw material to obtain a hydrolysate enriched in xylose and glucose. The hemicellulosic hydrolysate of vine pruning residue (VPR) was obtained by hydrothermal treatment, followed by enzymatic hydrolysis of oligosaccharides to obtain fermentable sugars. Hydrothermal treatment was carried out using a liquid to solid ratio (LSR) of 8 kg of water/kg of dried vine pruning residue in a pressurised Parr reactor of 2 L volume in a non-isothermal regime at T_{max} of 215 °C (corresponding to a severity of 3.89), according to previous optimisation treatment by Jesus *et al.*[21] The hardness of hydrothermal treatments can be expressed in terms of "severity" (S_0), defined as the logarithm of the severity factor R_0.[45] After hydrothermal treatment, liquid and solid phases were separated by filtration to determine solid yield and analysed as described by Jesus *et al.*[21] The liquid phase (hemicellulosic hydrolysate) was subjected to enzymatic hydrolysis with commercial Cellic CTec2 (kindly supplied by Novozymes Bagsvaerd, Demark), with a xylanase activity of 626 U mL^{-1}, for 24 h in an orbital incubator at 45 °C. Xylanase activity was measured as described by Cunha *et al.*[46] The hydrolysate was detoxified with activated charcoal at a ratio of 10 g of hydrolysate per 1 g of activated charcoal for 1 h with agitation at room temperature. This step was attained to enable resveratrol on HPLC, due to the considerable phenolic content of hemicellulosic hydrolysates. The activated charcoal was removed by filtration and the hydrolysate was subsequently filter-sterilised. The detoxified VPR hemicellulosic hydrolysate was used as fermentation medium containing 14.0 ± 0.3 g L^{-1} of glucose, 15.5 ± 0.2 g L^{-1} of xylose and 6.1 ± 0.1 g L^{-1} of acetic acid, which was supplemented with 7.5 g L^{-1} of yeast extract.

2.3.2.2. Grape must. Grape must (GM) used in this study are derived from white grapes (variety Verdejo), collected in September 2020, and were provided by the Oenological Station of Castile and Leon – ITACyL (Rueda, Spain) and stored at −20 °C until used. GM density is 1.09 kg L^{-1} and contained composed of 111 g L^{-1} glucose, 116 g L^{-1} fructose 0.69 g L^{-1} total Kjeldahl nitrogen and 0.15 g L^{-1} total phenolic compounds, previously characterised by Hijosa-Valsero *et al.*[16] Grape must pH was adjusted to 6 prior sterilisation, then autoclaved at 121 °C for 15 min. Grape must was dissolved in the fermentation media in percentages from 12.5% to 90% (v/v) and supplemented with 7.5 g L^{-1} of yeast extract.

2.3.3. Wine lees. Wine lees (WL) are derived from red wine, were collected between September and November 2020, and were provided by the Oenological Station of Castile and Leon – ITACyL (Rueda, Spain). WL and have a density of 1.05 kg L^{-1}, containing 99.3 g L^{-1} ethanol, 12.2 g L^{-1} total Kjeldahl nitrogen and 1.51 g L^{-1} phenolic compounds (characterised by Hijosa-Valsero *et al.*[16]). Wine lees were sterilised by pasteurisation for 1 hour at 60 °C to avoid loss in ethanol content and subsequently sonicated for 30 min to disrupt yeast cells, releasing cytoplasmic contents that act as nitrogen sources for nutritional supplementation of the media, not requiring the addition of commercial yeast extract. Wine lees were dissolved in the fermentation media in percentages from 40% to 80% (v/v).

2.4. Analytical methods

Fermentations were monitored by collecting 500 μL of the sample for analysis of sugars, acetic acid, and ethanol, and another 500 μL of the sample was mixed with an equal volume of ethanol (>99% purity), vortexed for 10 s, for the quantification of resveratrol and *p*-coumaric acid. Samples were centrifuged at 13 000 rpm for 5 min, and the supernatants were stored until analysed by HPLC. For sugars, acetic acid, and ethanol, a BioRad Aminex HPX-87H column, at 60 °C, using 5 mM H_2SO_4 as mobile phase and a flow rate of 0.6 mL min^{-1}, was used. Peak detection was accomplished using a Knauer-IR intelligent refractive index detector. Resveratrol and *p*-coumaric acid were quantified using a Discovery® HS F5 150 mm × 2.1 mm column (particle size 3 mm), with a flow rate was 0.7 mL min^{-1}. A linear gradient was set from 5% to 60% of acetonitrile over 10 mM ammonium formate (pH adjusted by formic acid to 3.0) from 0.5–9.5 min. Resveratrol and *p*-coumaric acid detection were made by absorbance at 333 nm, with retention times of 7.1 min and 6.0 min, respectively, using a diode-array detector (Fig. S1†). Biomass dry weight (BDW) quantification was accomplished by collecting 1 mL of fermentation broth in previously dried and weighed tubes. The pellet was washed out twice in ethanol and deionised water, sequentially, to remove precipitated resveratrol and residual fermentation media, and incubated at 105 °C for 24 h before being weighed again.

2.5. Determination of fermentation parameters

Resveratrol yield on carbon source ($Y_{R/S}$) was calculated as the ratio between the resveratrol concentration at the end of fer-

mentation and the total amount of carbon source consumed over the fermentation. Resveratrol yield on biomass in dry weight ($Y_{R/BDW}$) was determined as the ratio between resveratrol and biomass dry weight concentrations at the end of fermentation. Xylose consumption rates were calculated as the ratio between xylose consumed in a defined period and the duration of that specific period.

2.6. Statistical analysis

Statistical analyses were performed using GraphPad Prism. Statistically significant differences between fermentation parameters were tested by repeated measures one-way ANOVA, followed by Tukey *post hoc* test. Statistically significant differences were established at p-value < 0.05. Significance levels: "ns" (non-significant) – $p > 0.05$; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$; **** – $p < 0.0001$.

3. Results and discussion

3.1. Improvement of resveratrol titre by metabolic engineering

The pentose phosphate pathway plays an important role in both xylose metabolism and resveratrol biosynthesis. We started by introducing the heterologous resveratrol biosynthetic pathway (RBP) in a xylose-consuming recombinant Ethanol Red strain, generating the strain L326. In this strain, the 4 genes involved in the non-oxidative PPP reactions (*TAL1, RPE1, RKE1* and *TKL1*) were previously overexpressed.[47] When compared to L323, which is derived from the same Ethanol Red parent strain with the sole expression of the RBP, L326 produced 107.3 mg L^{-1} of resveratrol from 20 g L^{-1} of glucose, 22% higher than the 88.0 mg L^{-1} titre obtained with L323 (Fig. 2). Previous studies have shown that directing the carbon flux to the formation of erythrose 4-phosphate (E4P) through the PPP increases the precursor supply in the shikimate pathway and the aromatic amino acid supply, which are key to resveratrol formation.[48,49] In particular, overexpression of *RKI1* has previously been shown to guide carbon flow toward E4P production while avoiding its recirculation back into glycolysis, and its combination with *TKL1* overexpression further increased shikimate titres.[49]

Several cytochrome P450 monooxygenases, such as cinnamate-4-hydroxylase (C4H), a central enzyme in the resveratrol pathway for the conversion of cinnamic acid into *p*-coumaric acid, have known low activity, are dependent on NAD(P)H and require auxiliary proteins for electron transfer.[50] In this sense, we subsequently enhanced the activity of cytochrome P450 by expression of cytochrome B5 (*ScCYB5*) and cytochrome P450 reductase (*AtATR2*) originating the strain L543. This improvement resulted in a resveratrol titre of 136.8 mg L^{-1}, an additional increase of 28% compared to the strain L326 (Fig. 2). Overexpression of *CYB5* from *S. cerevisiae* and *ATR2* from *A. thaliana* has previously been reported to improve resveratrol production in a laboratory *S. cerevisiae* strain,[8] and our data is in accordance with this previous report. Biomass formation, critical for resveratrol production,[10] does not show statistically significant differences between the 3 strains, hence, resveratrol yield on biomass increased from 13.2 mg g^{-1} (L323) to 21.9 mg g^{-1} (L543). Therefore, we used the strain L543 in the subsequent fermentations in this study.

3.2. Resveratrol production from xylose as sole carbon source

Due to its ability to metabolise xylose, the production of resveratrol exclusively from this pentose sugar was assessed with the previously selected recombinant strain L543. This strain produced 61.3 mg L^{-1} of resveratrol from a medium with an initial xylose concentration of approx. 20 g L^{-1} (YPX20).

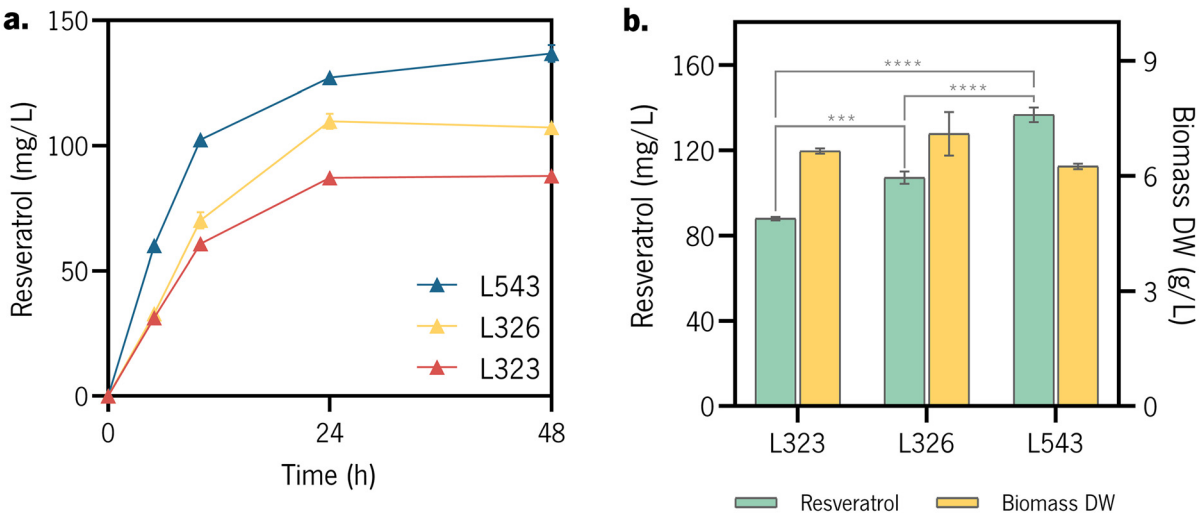

Fig. 2 Comparison between the resveratrol-producing strains L323 (Ethanol Red with the resveratrol biosynthetic pathway – RBP), L326 (xylose-consuming Ethanol Red with the RPB) and L543 (L326 with overexpression of *CYB5*/*ATR2*) in YPD20 medium. (a) Resveratrol production; (b) Biomass dry weight and maximum resveratrol production at the end of the fermentation.

Nevertheless, the strain could only consume 53% of the xylose present in the fermentation medium, with a residual xylose concentration of 9.1 g L^{-1} (Fig. 3).

The parent strain of L543, a xylose-consuming Ethanol Red strain without the RBP (strain XylC2 V1), was previously shown to have high xylose metabolisation capacity in the same medium (YPX20), being able to consume nearly all xylose in the medium in approx. 48 h.[47] The reduced xylose consumption observed in strain L543 might be associated with the metabolic burden caused by the multiple gene integrations in this strain, which has a negative impact on its physiology.[51] Furthermore, high-level expression of the resveratrol biosynthetic pathway genes was previously found to prejudice yeast cell growth.[8,10] Additionally, intracellular accumulation of xylose in yeast cells which are actively metabolising this pentose sugar activates cell mechanisms comparable to carbon limitation, which is often associated with low assimilation rates.[52] Numerous yeast cell defence mechanisms are also triggered in *S. cerevisiae* when in xylose cultivations, such as the upregulation of genes involved in cell starvation, lipid metabolism, stress response and DNA damage.[53] While xylose assimilation capacity does not hinder xylose uptake on the parent strain XylC2 V1, the combination of both RBP and xylose metabolism appears to prejudice xylose consumption by the resveratrol-producing strain L543. No ethanol was observed at any time point probably because xylose consumption was very slow, with an average uptake rate of 0.11 g L^{-1} h^{-1} for the 96 h of fermentation. As the strain L543 also utilises ethanol to produce resveratrol, it is most likely that the rate at which ethanol is being produced from xylose is lower than the rate of ethanol conversion into resveratrol. Nevertheless, even though the strain was not able to consume all xylose and achieved only 45% of the resveratrol titre on glucose, its yield of carbon consumed was approx. 80% of the yield on glucose (5.6 mg g^{-1} of xylose against 6.8 mg g^{-1} of glucose).

3.3. Evaluation of the effect of nutritional supplementation of the fermentation media on resveratrol production

For increased resveratrol titres, optimisation of the fermentation media supplementation was evaluated. The standard YP supplementation (10 g L^{-1} of yeast extract and 20 g L^{-1} of peptone) was compared against increasing concentrations of yeast extract (YE) from 0 to 50 g L^{-1} (without peptone) in glucose media (Fig. 4). Interestingly, the maximum resveratrol concentration of 259.2 mg L^{-1} was obtained with supplementation of 7.5 g L^{-1} of yeast extract (a 1.87-fold increase compared to YP supplementation), giving a yield of 11.6 mg of resveratrol per g of glucose (Fig. 4a). Fine-tuning of the yeast extract supplementation has a high impact on resveratrol production, as the decrease in YE concentration from 7.5 to 5 g L^{-1} reduced resveratrol titre by near 4-fold to only 66.8 mg L^{-1}. On the other hand, an increase in YE above 7.5 g L^{-1} causes a decrease in resveratrol production, with resveratrol titres declining gradually to 40.6 mg L^{-1} when the medium is supplemented with 50 g L^{-1} of YE. Biomass formation is, as expected, correlated to an increase in YE supplementation, as YE is commonly used as supplementation for yeast growth. Even though resveratrol production is highly dependent on biomass, a balance between both appears mandatory for increased yield, as an excessive direction of the carbon to biomass formation penalises metabolite production.

We further supplemented a xylose media with these optimal conditions. Supplementation with 7.5 g L^{-1} of YE led to a resveratrol titre of 223.6 mg L^{-1}, 3.65-fold higher when compared to the control supplementation with 20 g L^{-1} of peptone and 10 g L^{-1} of yeast extract. This titre is comparable to the one attained in the only previous report on resveratrol production from xylose, which was made using *S. stipitis*, a yeast naturally capable of fermenting this pentose sugar. However, the yield reported in this study is higher. The *S. stipitis* strain produced 248.6 mg L^{-1} of resveratrol from 50 g

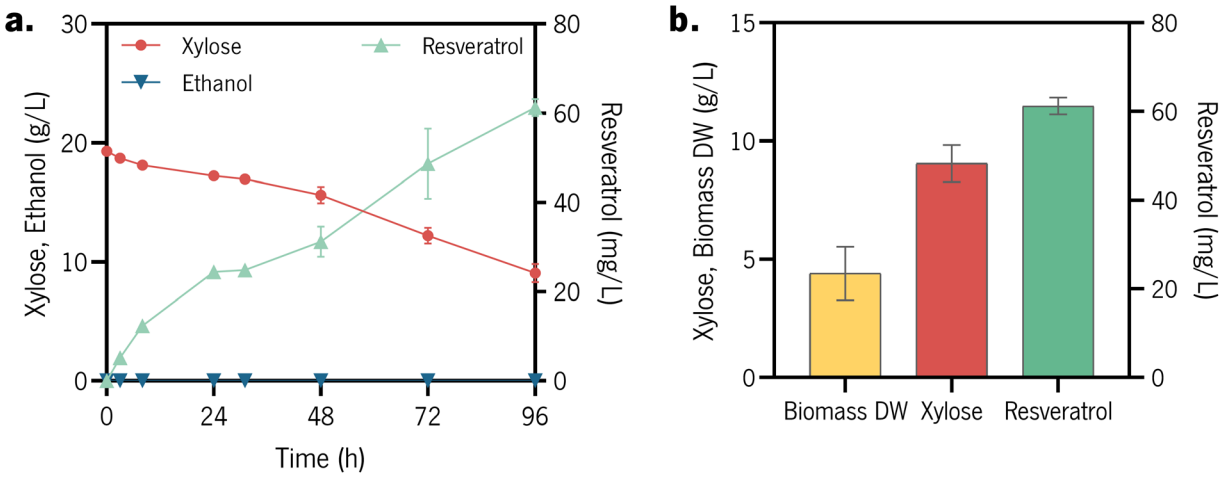

Fig. 3 (a) Fermentation profile of xylose synthetic media by the strain L543; (b) biomass dry weight, residual xylose and maximum resveratrol concentrations at the end of the fermentation.

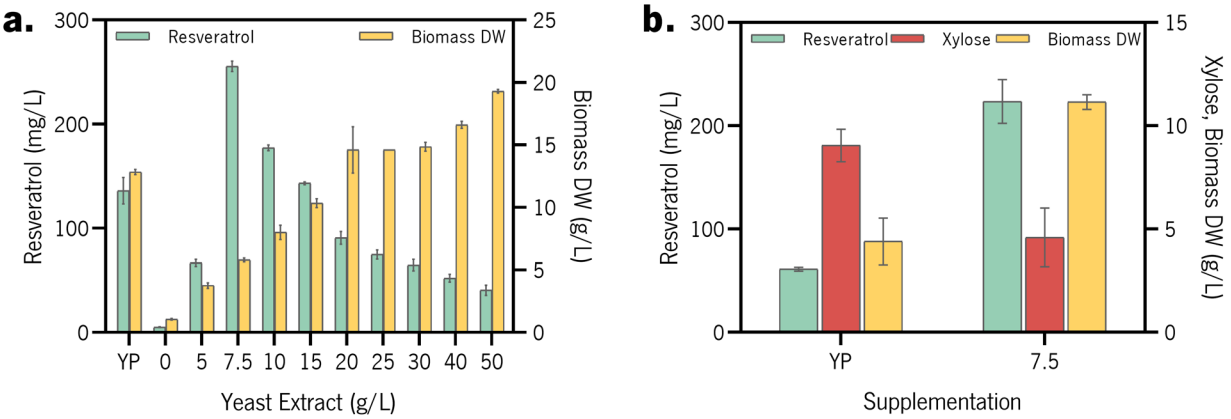

Fig. 4 Optimisation of media supplementation. (a) Resveratrol and biomass in dry weight concentrations at the end of fermentation (96 h) in glucose media (20 g L^{-1}) supplemented with different concentrations of yeast extract (from 0 to 50 g L^{-1}) against the control standard YP supplementation (10 g L^{-1} of yeast extract and 20 g L^{-1} of peptone); (b) resveratrol, xylose and biomass dry weight concentrations at the end of fermentation (96 h) of xylose media (20 g L^{-1}) supplemented with 7.5 g L^{-1} of yeast extract against the control standard YP supplementation.

L^{-1} of xylose, corresponding to a yield of 4.97 mg of resveratrol per gram of xylose consumed,[54] against 14.5 mg g^{-1} achieved using the *S. cerevisiae* L543 (2.92-fold higher).

Interestingly, with the fine-tuning of supplementation, the resveratrol yield on xylose was higher than the resveratrol yield on glucose (14.5 mg g^{-1} of xylose consumed against 11.6 mg g^{-1} of glucose consumed). Even though resveratrol titres on glucose medium were 1.16-fold higher with the supplementation of 7.5 g L^{-1} of YE compared to xylose fermentation, the differences were minimised compared to the fermentation with standard YP supplementation (resveratrol titre on YPD20 was 2.23-fold higher than on YPX20). The enhanced xylose consumption observed after the fine-tuning of the supplementation (1.41-fold higher) can help to explain this. Also, contrarily to glucose fermentation, biomass formation with 7.5 g L^{-1} of YE supplementation in xylose medium was 2.53-fold higher than with YP supplementation. Another possible explanation for the different behaviour between glucose and xylose fermentation may rely on the fact that *S. cerevisiae* is Crabtree-positive, fermenting glucose into ethanol under aerobic conditions. This effect does not occur in xylose fermentation in yeast due to the requirement of high metabolic flux.[55] This means that in high aeration conditions (such as resveratrol production processes), glucose fermentation is more prone to direct the carbon flow to other metabolic routes than xylose fermentation. Sun *et al.*[56] reported that, for the production of isoprenoids (also derived from acetyl-CoA), the lack of Crabtree-effect in xylose cultivation led to an increase of 53% in biomass formation, with higher yields on carbon when compared to glucose cultivation. Improved biosynthesis of acetyl-CoA-derived products was also attained by exploiting xylose as a carbon source, due to the facilitated supply of acetyl-CoA in the cytosol.[57] Data in Fig. 4 shows that supplementation of the medium with 7.5 g L^{-1} of yeast extract is more favourable to resveratrol production than the standard YP supplementation in both glucose and xylose cultivations. The fine-tuning of the media supplementation is attractive for the increase of resveratrol titres causing a reduction in operation costs, which is mandatory in an industrial context. Therefore, we used 7.5 g L^{-1} of yeast extract in further experiments of this work where supplementation was necessary.

3.4. Co-fermentation of xylose and glucose for increased resveratrol titres

The Pentose Phosphate Pathway (PPP) is crucial for both resveratrol production and xylose assimilation (Fig. 1). We hypothesised that co-fermentation of xylose and glucose in the medium might benefit the resveratrol production by pushing the metabolic flux through PPP, therefore improving the resveratrol producing pathway. We compared a fermentation medium with 62 g L^{-1} (YED) against a medium with 50 g L^{-1} of glucose and 10 g L^{-1} of xylose (YEDX), a common concentration found in lignocellulosic whole slurry fermentations,[58] for a matched total carbon molarity of 344 mM in both media. Simultaneous fermentation of glucose and xylose led to an increase of 1.31-fold in resveratrol production when compared to sole glucose fermentation (Fig. 5).

In both fermentations, all glucose was consumed in the first 12 h, with an accumulation of ethanol in the fermentation medium of around 17 g L^{-1} in both conditions. Interestingly, resveratrol concentration at 12 h in YED fermentation was only 69.2 mg L^{-1}, while YEDX fermentation produced 186.2 mg L^{-1} in the same timeframe, with a total carbon consumption of 53.8 g L^{-1} (50.1 g L^{-1} of glucose plus 3.7 g L^{-1} of xylose). Resveratrol production in glucose fermentation relies mainly on the ethanol phase. The ethanol produced from glucose fermentation is consumed and converted into resveratrol, representing approx. 76% of the overall resveratrol production in this condition. Our observation is in accordance with previous studies on *de novo* resveratrol production from glucose.[7,8] On the other hand, while simultaneously fermenting glucose and xylose, the yeast achieved approx. 48% of the overall pro-

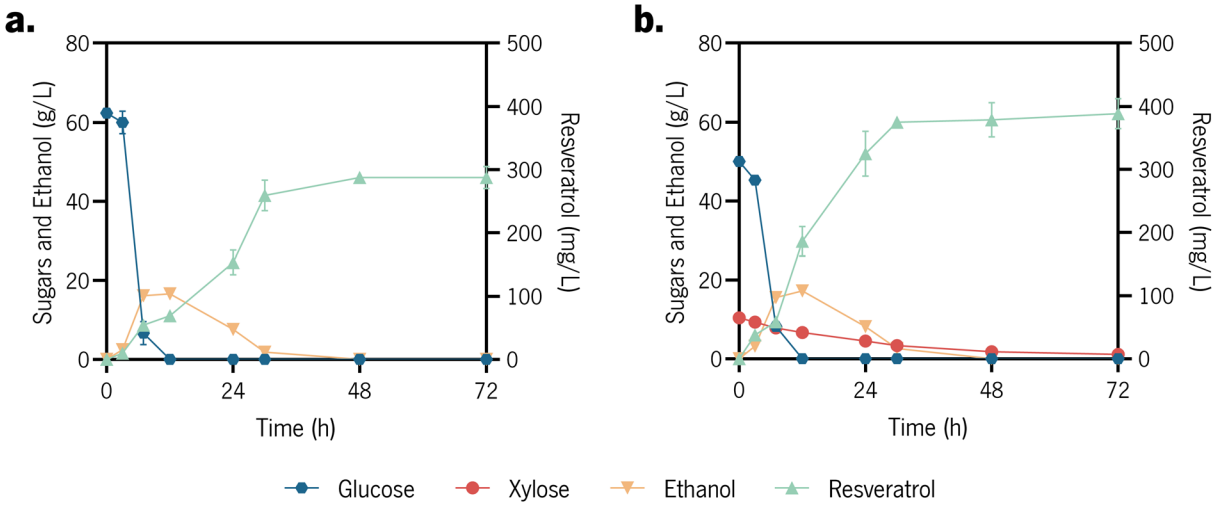

Fig. 5 Fermentation profiles of YE media with (a) 62 g L^{-1} of glucose and (b) 50 g L^{-1} of glucose together with 10 g L^{-1} of xylose both fermentations with total carbon molarity of 344 mM.

duction in the first 12 h, increasing the resveratrol yield of the sugar phase. After glucose depletion, both conditions produced similar resveratrol concentrations of approx. 200 mg L^{-1}. The increased resveratrol titre associated with YEDX fermentation is probably related to the abovementioned influence of the PPP in the resveratrol pathway. It is estimated that only up to 2.5% of the glucose is metabolised *via* the oxidative PPP by *S. cerevisiae*, and another 10 to 20% can enter the non-oxidative PPP *via* the glycolytic metabolites (Fig. 1).[59,60] On the other hand, xylose enters the cell metabolism *via* xylulose-5-phosphate, part of the non-oxidative PPP, essential in generating E4P for the shikimate pathway and, ultimately, in phenylalanine formation. In this sense, it is most likely that the presence of xylose in the fermentation media pushes the PPP activity in the yeast, increasing resveratrol titres (Table 1).

Even though a higher resveratrol titre was achieved in YEDX fermentation, a residual concentration of 1.1 g L^{-1} of xylose was observed at the end of fermentation (89% of the xylose consumed). Xylose uptake in yeast cells is facilitated by hexose transporters, unspecific for pentose sugars,[61] but high concentrations of glucose outcompete xylose, which represses the simultaneous co-consumption of glucose and xylose.[62] Glucose is the preferred source of energy for *S. cerevisiae*, being usually consumed prior to any other carbon source.[63] This results in diauxic growth, with an initial fast assimilation of glucose followed by a slower uptake of other carbon sources.[64] Furthermore, a previous study reported that, in a medium with both glucose and xylose present (common in lignocellulosic fermentation), after glucose depletion, the xylose consumption rate is considerably reduced to rates lower than the ones observed in xylose-only media.[65] Indeed, we observed that, after glucose depletion, the xylose consumption rate drops from 0.31 g L^{-1} h^{-1} to 0.09 g L^{-1} h^{-1}. Only 2.3 g L^{-1} of xylose were consumed after 30 h of fermentation, which did not lead to an increase in resveratrol concentration and was probably channelled to cell maintenance. A glucose-limited fed-batch fermentation strategy, used, *e.g.*, to increase xylitol productivity,[29,66] could be an interesting approach to aid in the full consumption of xylose, by avoiding catabolite repression, possibly leading to even higher resveratrol titres.

3.5. Valorisation of wine wastes as carbon sources for resveratrol production

As abovementioned, the wine industry generates multiple wastes on a large scale, which can be used for several biotechnological processes. Among them, it is possible to obtain several different carbon sources. Grape must (GM) has a high content of glucose and fructose (approx. 11% (w/v) of each) and wine lees (WL) are rich in ethanol (approx. 10% (w/v)). Additionally, the hemicellulosic hydrolysate from hydrothermal treatment of vine pruning residues (VPR) has similar content of glucose (14 g L^{-1}) and xylose (15.5 g L^{-1}). The strain

Table 1 Fermentation parameters of glucose and co-fermentation of glucose and xylose in YE media

Condition	G_0 (mM)	X_0 (mM)	R_{max} (mg L^{-1})	Biomass DW (g L^{-1})	$Y_{R/S}$ (mg g^{-1})	$Y_{R/BDW}$ (mg g^{-1})
D62	344	0	287.5 ± 17.7	10.6 ± 0.0	4.6 ± 0.3	27.1 ± 1.7
D50X10	277	67	388.4 ± 23.9	11.8 ± 0.4	6.5 ± 0.0	31.8 ± 1.3

G_0, initial glucose concentration; X_0, initial xylose concentration; R_{max}, resveratrol concentration at the end of the fermentation; biomass DW, biomass concentration in dry weight; $Y_{R/S}$, yield of resveratrol on carbon source; $Y_{R/BDW}$, yield of resveratrol on biomass dry weight.

L543 can use all these carbon sources to produce resveratrol (Fig. 1) and, therefore, we applied it to different wine valorisation processes.

3.5.1. Resveratrol production from hemicellulosic hydrolysate derived from hydrothermally pretreated vine pruning residue. As shown in data from Fig. 5, simultaneous consumption of glucose and xylose favours resveratrol production. In this sense, vine pruning residue (VPR) hydrolysate is a very promising substrate for resveratrol production, as it has a relatively high amount of glucose (14 g L^{-1}) when compared to other hydrolysates like eucalyptus wood or corn cob,[27] allied to a xylose concentration of 15 g L^{-1}. The acetic acid present in the VPR hydrolysate (approx. 6.1 g L^{-1}) can also be used for resveratrol production through acetate metabolism (Fig. 1), but a concentration in this range is known to have a negative impact on yeast physiology.[67] This attributes even more importance to the choice of a robust chassis, such as Ethanol Red, that has demonstrated effective usage of lignocellulosic substrates.[46,68–70] Due to the presence of several phenolic compounds in the hemicellulosic hydrolysate of the VPR, these compounds were removed by activated charcoal adsorption to enable resveratrol quantification by UHPLC. When the non-detoxified hydrolysate was used, quantification of resveratrol concentration was not possible due to the presence of other compounds, even though the strain was able to ferment the hydrolysate, with a similar content of sugars, acetic acid and ethanol at the end of the fermentation (data not shown). In Fig. 6, we see that the strain L543 is capable of producing 167.1 mg L^{-1} of resveratrol after 96 h of fermentation in detoxified VPR hemicellulosic hydrolysate (93% of fermentation medium volume, plus inoculum and supplementation), accumulating 42.3 mg L^{-1} of *p*-coumaric acid at the end of fermentation. All glucose in the medium was consumed, as expected, but only 8.4 g L^{-1} of xylose was consumed (residual 6.6 g L^{-1}). This is aligned with the previous experiments in this study, where this strain only seemed to be capable of consuming up to 10 g L^{-1} of xylose in the medium in 96 h. A concentration of 1.97 g L^{-1} of acetic acid was observed at the end of fermentation, meaning that the strain partially utilised the acetic acid present in the hydrolysate. No ethanol was observed at the end of fermentation, which again may be related to the accumulation of *p*-coumaric acid after ethanol depletion. Here we demonstrate the feasibility of a hemicellulose-to-resveratrol process, and possible combinations between the different wine residues might further contribute to increased resveratrol yields.

3.5.2. Resveratrol production from glucose and fructose mixture derived from grape must. Initially, the maximum volume possible of grape must dissolved in the fermentation medium was attempted, using 95% of grape must (GM), containing 103.7 g L^{-1} of glucose and 111.5 g L^{-1} of fructose, with the remaining volume accounted for inoculum and medium supplementation. A resveratrol production of only approx. 50 mg L^{-1} was attained, which is relatively low when considering a total initial carbon availability of more than 200 g L^{-1} of both glucose and fructose. This was mainly due to the accumulation of high levels of glycerol and acetic acid. After 24 h almost 10 g L^{-1} of acetic acid were found in the medium, which practically ceased ethanol consumption and *p*-coumaric acid conversion (Fig. S2†). Grape must is very acidic, and even though the initial pH was adjusted to 6 and calcium carbonate was added to neutralise the medium, the final pH was still very low (approx. 3). Given this, we reduced the GM content in the medium to half to have milder conditions, and this led to an over 4-fold increase in resveratrol titre, to 212.3 mg L^{-1}, using 50% of GM in the fermentation medium (54.7 g L^{-1} of

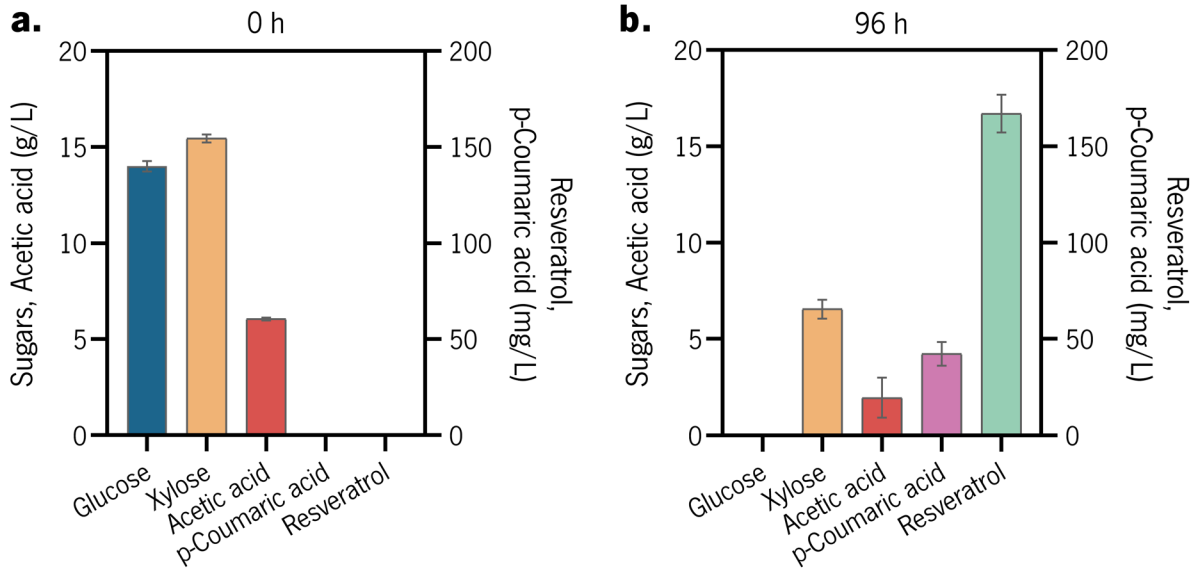

Fig. 6 Vine pruning residue fermentation data points of (a) sugars and acetic acid at the beginning of the fermentation and (b) sugars, acetic acid, *p*-coumaric acid and resveratrol at the end of fermentation.

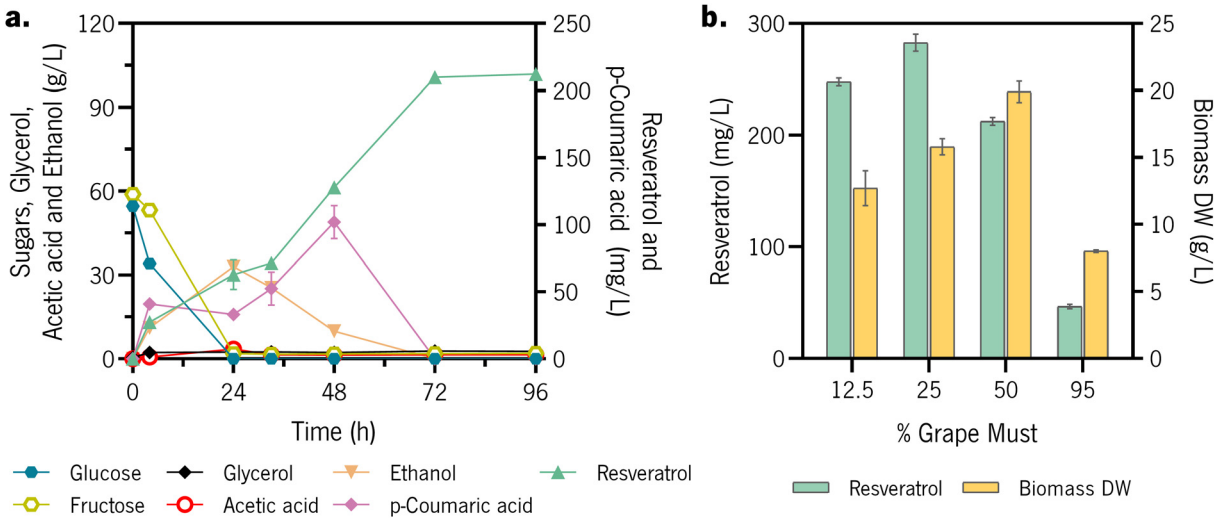

Fig. 7 Fermentation of grape must. (a) Fermentation profile of a medium with 50% grape must (v/v); (b) resveratrol concentration at the end of fermentation (96 h) using a range of grape must dissolved in the media from 12.5 to 95%.

glucose and 58.9 g L^{-1} of fructose). In this case, no accumulation of by-products, ethanol or *p*-coumaric acid was observed at the end of fermentation, with a final pH of 4.22 (Fig. 7a).

Further on, we attempted to use other percentages of GM in the media. A maximum resveratrol titre of 282.7 mg L^{-1} was obtained using 25% of GM after 96 h of fermentation (27.5 g L^{-1} of glucose and 29.3 g L^{-1} of fructose), corresponding to an initial carbon source availability of nearly 60 g L^{-1} total (Fig. 7b). Nevertheless, the highest resveratrol yield of 8.8 mg g^{-1} of sugar was obtained using 12.5% of GM (13.7 g L^{-1} of glucose and 14.4 g L^{-1} of fructose), against the yields of 5.0 mg g^{-1} using 25% and 1.9 mg g^{-1} using 50%. This follows the data obtained in synthetic media where, despite an increase in resveratrol titres by increasing glucose concentration from 20 (section 3.3) to 62 g L^{-1} (section 3.4), resveratrol yield on glucose decreased from 11.6 mg g^{-1} to 4.6 mg g^{-1}. The increased yields associated with a lower percentage of GM dissolved in the media are also valuable from the point-of-view of cost reduction and optimisation of process conditions. Using lower concentrations of GM also eliminated the need to add calcium carbonate ($CaCO_3$) to neutralise the fermentation media. The final pH in the fermentation with 12.5 to 50% of GM was between 4.36 and 4.22, respectively. This also helps in preserving resveratrol stability, as resveratrol is more stable under acidic conditions, up to pH 6.[71] On the other hand, fermentation of 95% of GM without $CaCO_3$ is not possible, as the pH drops very sharply, and the strain is not able to produce resveratrol (data not shown). Even with the addition of 5 g L^{-1} of $CaCO_3$, the final pH for the fermentation of 95% was 4.97. Interestingly, with a lower percentage of GM in the media (up to 25%), when $CaCO_3$ was added, resveratrol production slightly decreased (Fig. S3†).

3.5.3. Resveratrol production exclusively from ethanol derived from wine lees. Wine lees (WL) are an interesting substrate for resveratrol microbial production due to their high content of ethanol (around 100 g L^{-1}). Ethanol can act as a carbon source for resveratrol production, increasing the pool of malonyl-CoA, a direct precursor of resveratrol (Fig. 1), and its use as a carbon source, always coupled with glucose, has been already demonstrated in *S. cerevisiae*.[6–8] This yeast is Crabtree-positive, therefore favouring the conversion of pyruvate into ethanol in glucose fermentation. When glucose or other preferred carbon sources are depleted a diauxic shift occurs, switching to aerobic respiration, therefore using ethanol as carbon source instead.[72] Ethanol degradation consists of a three-step pathway, from ethanol to acetyl-CoA, with acetaldehyde and acetate as intermediaries (Fig. 1). Acetyl-CoA can be converted into malonyl-CoA (direct precursor of resveratrol) but is also directed to the tricarboxylic acid (TCA) cycle for aerobic respiration and the glyoxylate cycle, which is activated in the absence of glucose to satisfy the carbon requirements of the yeast cell (Fig. 1). The glyoxylate cycle plays an anaplerotic role in the supply of precursors for biosynthesis, being responsible for the creation of sugars and other essential organic compounds. From here, the cell is able to generate phenylalanine, which generates the also needed *p*-coumaric acid for the resveratrol biosynthetic pathway.[73] Here, we show the feasibility of using WL as a cheap substrate to produce resveratrol exclusively from ethanol, which has not been demonstrated before. A maximum resveratrol titre of 263.9 mg L^{-1} was attained with 50% of WL in the fermentation medium (49.4 g L^{-1} of ethanol). Above 70% of WL (68.4 g L^{-1} of ethanol) dissolved in the fermentation media no resveratrol production was observed (Fig. 8a). Dissolution of 80% of WL resulted in excessive viscosity of the fermentation medium, which created constrains in mass transfer. Furthermore, the initial ethanol concentration available in this condition was 78.5 g L^{-1}, which was previously found to be inhibitory for cell

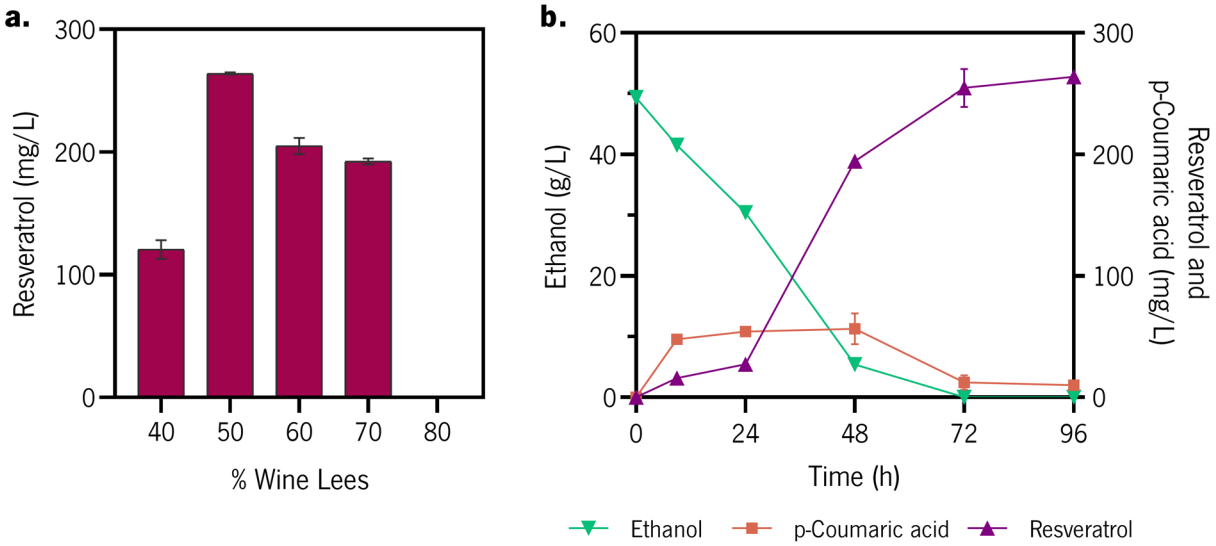

Fig. 8 Fermentation of wine lees using ethanol as the sole carbon source. (a) Resveratrol concentration after 96 h in media with a percentage of dissolved wine lees from 40 to 80% (v/v); (b) fermentation profile of a medium with 50% (v/v) of wine lees dissolved.

growth when using the strain L323, the background strain of L543.[7] Given this, no resveratrol production was attained at 80% (or above) of WL dissolved in the media.

Data in Fig. 8b shows that all ethanol in the medium with 50% of WL is consumed in 72 h. A residual concentration of 10 mg L^{-1} of *p*-coumaric acid was observed at the end of fermentation (96 h). It is worth noticing that, after ethanol depletion, *p*-coumaric acid conversion ceased and no additional resveratrol was produced. In a parallel experiment, the addition of 10% of GM to provide a source of glucose and fructose showed no considerable difference in resveratrol production (approx. 270 mg L^{-1}) but rather increased the residual *p*-coumaric acid at the end of fermentation. Again, we observed that after ethanol depletion, the conversion of *p*-coumaric acid into resveratrol stops, which ultimately led to its accumulation (Fig. S4†). One possible explanation for this might hinge on an imbalance between the precursors needed for resveratrol formation. Resveratrol formation depends directly on the precursors *p*-coumaroyl-CoA and malonyl-CoA. The first one is obtained through phenylalanine-derived *p*-coumaric acid, while malonyl-CoA is derived from acetate, which by its turn can be obtained through ethanol metabolism (Fig. 1). To generate 1 molecule of resveratrol, 1 molecule of *p*-coumaroyl-CoA and 3 molecules of malonyl-CoA are necessary,[10] and the lack of malonyl-CoA supply after ethanol depletion most likely cease *p*-coumaric conversion to resveratrol to the imbalance of both branches. This way, fine-tuning the balance between both branches of the precursor supply of the resveratrol biosynthetic pathway is likely necessary for optimal resveratrol yields. Table 2 compiles the main fermentation parameters of the experiments using wine wastes as substrate.

3.6. Overall balance of wine wastes processing for resveratrol production

According to the Food and Agriculture Organization of the United Nations (FAO) and the OIV, on average, approximately 75 million tonnes of grapes are produced every year. Roughly,

Table 2 Fermentation parameters from experiments using wine waste as substrate

	Carbon source (g L^{-1})					
Residue	**Glucose**	**Xylose**	**Fructose**	**Ethanol**	R_{max} (mg L^{-1})	$Y_{R/S}$
Vine pruning residue	13.0 ± 0.3	14.4 ± 0.2	—	—	167.1 ± 9.8	7.3 ± 0.4
Grape must						
12.5%	13.7 ± 0.2	—	14.4 ± 0.6	—	247.6 ± 3.5	8.8 ± 0.0
25%	27.5 ± 0.6	—	29.3 ± 0.9	—	282.7 ± 7.6	5.0 ± 0.3
50%	54.7 ± 1.8	—	58.9 ± 1.8	—	212.3 ± 3.5	1.9 ± 0.0
95%	103.7 ± 2.3	—	111.5 ± 2.7	—	46.5 ± 2.0	0.2 ± 0.0
Wine lees						
40%	—	—	—	40.3 ± 0.4	120.6 ± 7.6	3.0 ± 0.2
50%	—	—	—	49.4 ± 1.1	263.9 ± 0.9	5.3 ± 0.1
60%	—	—	—	60.2 ± 2.6	205.0 ± 6.6	3.4 ± 0.0
70%	—	—	—	68.4 ± 2.7	192.4 ± 2.4	2.8 ± 0.1
80%	—	—	—	78.5 ± 0.8	0.0 ± 0.0	0.0 ± 0.0

R_{max}, resveratrol concentration at the end of the fermentation; $Y_{R/S}$, yield of resveratrol on carbon source.

for each hectare of vineyard surface area, it is possible to obtain 10 tonnes of grapes.[74] The mandatory pruning of vine trees generates around 5 tonnes per hectare of vineyard per year.[75] From the total grape production, approximately 4.79 tonnes of grapes per hectare of surface area are pressed for wine production, while 493 kg of pressed grapes per hectare are not used for wine production, but rather used for grape must and juice production.[74] On average, 1.3 kg of grapes are necessary to produce 1 L of wine, and 6% of the total grape content is wasted in the form of wine lees,[75] resulting in the accumulation of around 287 kg of wine lees per hectare of vineyard, which can contain approx. 27 kg of ethanol (depending on the type of wine).

Therefore, taking into account the results obtained for resveratrol production from the abovementioned wine wastes (Table 2) and considering the amount of vine pruning residue, grape must and wine lees that are generated, Fig. 9 shows the kg of resveratrol that can be produced per hectare of vineyard. As seen, approx. 7 kg of resveratrol could potentially be produced from the vine pruning residues generated by each hectare cultivated. On the other hand, grape must (not used for wine production) and wine lees could yield 1.11 kg and 0.148 kg per ha, respectively. Nevertheless, the highest yield (measured as g of resveratrol per kg of biomass) was obtained for grape must (2.26 g resveratrol per kg grape must), followed by 1.40 g and 0.52 g of resveratrol per kg of vine pruning residue and wine lees, respectively. Table 3 compares yields obtained from different extraction processes from the vine, *Vitis vinifera*, expressed in grams of resveratrol per kg of raw material in fresh weight, against the yields per kg of substrate reported in this study. While there is a large room to improve its titres, the resveratrol yields attained by microbial production are already very competitive in comparison with the ones obtained from extraction from the vine, being on average an order of magnitude higher. Additionally, it is worth mentioning that both these processes can be complementary, as the wine-derived wastes here used as a substrate to produce resveratrol do not contain grape skin, the main source of resveratrol extracted from *Vitis vinifera.*

Table 3 Comparison between yields of plant extraction processes against microbial production from wine wastes

Source/substrate	Extraction method	Yield ($g\ kg^{-1}$)	Ref.
Plant extraction			
Vitis vinifera	Pressurised liquid extraction	0.002	78
	Supercritical fluid extraction	0.04–0.17	79
	Organic solvent extraction	0.006	80
Microbial synthesis			
Vine pruning residue hemicellulosic fraction	—	1.40	This study
Grape must	—	2.46	This study
Wine lees	—	0.52	This study

The most conventional methods currently used to extract and purify resveratrol from plants comprise heating under reflux with ethanol, filtration, concentration due to its low content and, lastly, purification. This is time-consuming and labour-intensive, requiring large volumes of organic solvents.[76] Other methods like supercritical fluid extraction, using CO2 as extraction medium, are also reported in the literature to be effective, but they are both time-consuming and expensive.[76] An additional drawback associated with extraction processes from plants is the presence of contaminants like emodin or polycyclic aromatic hydrocarbons in many Plant species. By its

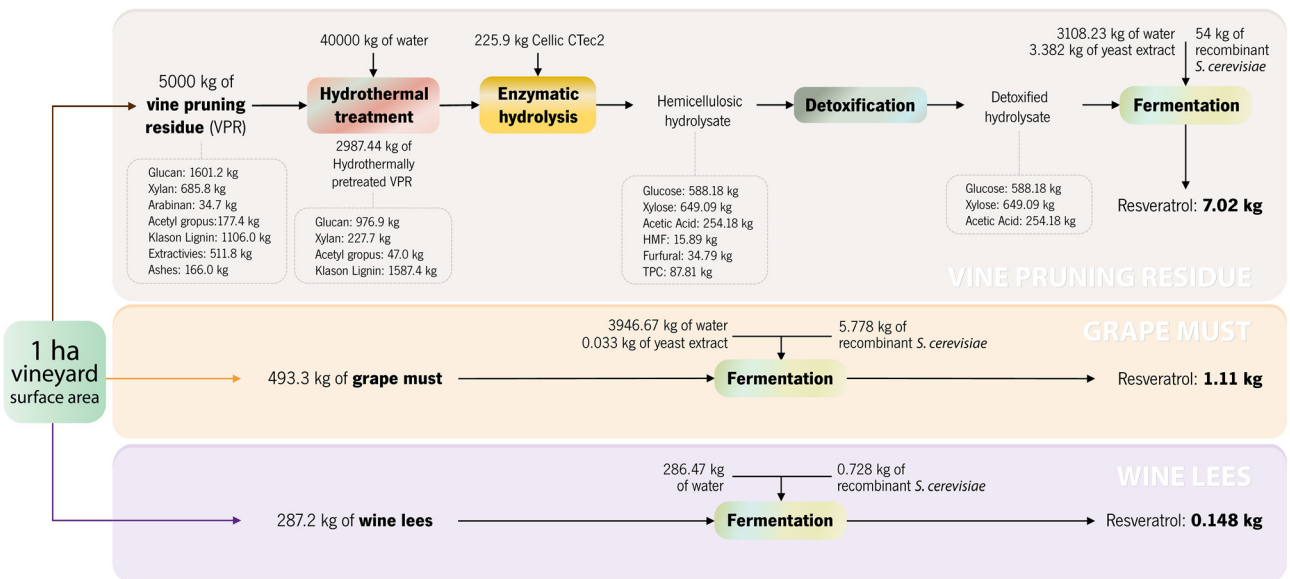

Fig. 9 Overall balance of resveratrol biosynthesis by recombinant *S. cerevisiae* from vine pruning residues, grape must and wine lees produced by each hectare of vineyard cultivated.

turn, microbial production of resveratrol not only has high stereoselectivity (>99% of *trans*-resveratrol) but also a much lower amount of contaminants.[77] In contrast, microbially produced resveratrol can be relatively easily recovered with high efficiency, for example, by dissolving resveratrol at high pH (above 11), removing the fermentative microorganism (*e.g.* micron-range pressure filter), followed by acid precipitation of resveratrol, filtration and crystallization.[77]

4. Conclusions

In this work, an *S. cerevisiae* strain was, for the first time, utilised as a host for resveratrol production from xylose, the second most abundant sugar in nature, which enabled the usage of a wide range of substrates. The significant role of the non-oxidative part of the pentose phosphate pathway in the resveratrol biosynthesis was shown. Valuable insights on the effect of coupling xylose with glucose fermentation are provided, where the benefits of simultaneous fermentation of xylose and glucose over glucose-only media are highlighted by a 1.31-fold increase in resveratrol production, reaching a titre of 388 mg L^{-1} from a mixture of both carbon sources. Here, following the circular bioeconomy concept, the palette of possible substrates for resveratrol production was expanded by repurposing several residues from the wine industry. Resveratrol production exclusively using ethanol obtained from wine lees, as well as the simultaneous use of glucose and fructose from grape must, were achieved, attaining competitive titres above 250 mg L^{-1}. Additionally, this is also the first report on the development of a hemicellulose-to-resveratrol process, by fermentation of a detoxified vine pruning residue hydrolysate. At its core, the concept of biorefinery comprehends the conversion of renewable materials into value-added compounds, allowing for full resource usage. This work contributes significantly to further advances toward the integration of industrial residues for high-value compound production promoting a greener future in bioprocess development.

Author contributions

Carlos E. Costa: conceptualization, investigation, visualisation, writing – original draft. Aloia Romaní: conceptualization, supervision, writing – review & editing. Iben Møller-Hansen: supervision, writing – review & editing. José A. Teixeira: supervision, funding acquisition, writing – review & editing. Irina Borodina: supervision, writing – review & editing. Lucília Domingues: conceptualization, supervision, funding acquisition, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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