Journal of Functional Foods 19 (2015) 288–295

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Autochthonous yeasts with β-glucosidase activity increase resveratrol concentration during the alcoholic fermentation of *Vitis labrusca* grape must

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ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 11 September 2015
Accepted 14 September 2015
Available online

Keywords:
β-Glucosidase
Piceid
Wine yeast
Non-*Saccharomyces* selection
Hanseniaspora uvarum

ABSTRACT

Since red wine is the main dietary source of resveratrol, a well-known polyphenol that reduces coronary events in humans, different strategies have been employed in winery to achieve resveratrol-enriched wines. Yeasts-endowed β-glucosidase activity enhances free-resveratrol concentration in wine without modifying its composition or sensorial properties. Current assay screened 308 autochthonous yeast strains for β-glucosidase activity employing arbutin, esculin, cellobiose and piceid as substrates. The β-glucosidase-producer yeasts were evaluated in the must of *Vitis labrusca* Bordô grape to quantify resveratrol concentration before and after alcoholic fermentation. Fourteen yeasts increased the resveratrol concentration up to 102% without any significant difference and nine of these yeast strains also produced high ethanol contents. Four autochthonous *Hanseniaspora uvarum* β-glucosidase-producer strains showed adequate oenological characteristics and hydrolysed resveratrol-glucosides during the alcoholic fermentation of *V. labrusca* grape must.

1. Introduction

Although interest in the health benefits of red wine has increased over the last several years due to its phenolic compounds (Artero, Artero, Tarín, & Cano, 2015), most studies on the phenolic composition in wines are related to European grapevines (*Vitis vinifera*). However, several countries such as Brazil (de Sá Borges, da Silva, Roberto, de Assis, & Yamamoto, 2013), the United States (Muñoz-Espada, Wood, Bordelon, & Watkins, 2004), Korea (Hong & Park, 2013) and China (Zhang et al., 2011) also produce grapes, wines, and juices derived from American grapevines (*Vitis labrusca*). Further, 80% of wines in Brazil are produced from *V. labrusca* grapes, with great acceptance by the consumers and, consequently, underscoring the local economy (Biasoto, Netto, Marques, & da Silva, 2014).

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Chemical compounds: Resveratrol (PubChem CID: 445154); Piceid (PubChem CID: 5281718); Arbutin (PubChem CID: 440936); Esculin (PubChem CID: 5281417); Cellobiose (PubChem CID: 10712).

http://dx.doi.org/10.1016/j.jff.2015.09.041
1756-4646/

Resveratrol (3,5,4′-trihydroxystilbene or 3,5,4′-stilbenetriol; MW: 228.25) is a stilbene compound, catalogued as a polyphenol, that has attracted considerable attention due to several *in vitro* and *in vivo* studies which emphasize its health benefits (Poulsen et al., 2014; Wu, Yang, Wang, & Wang, 2013). Red wines are the most notable dietary source of resveratrol and usually contain an average resveratrol concentration of 1.9 ± 1.7 mg/L (Fernández-Mar, Mateos, García-Parrilla, Puertas, & Cantos-Villar, 2012), although its concentrations may range from undetectable to 14.3 mg/L (Mark, Nikfardjam, Avar, & Ohmacht, 2005). Differences in the resveratrol concentration in wines depend on grape variety, geographical region, plant stress and oenological practices (Stervbo, Vang, & Bonnesen, 2007).

Increase in the biologically active resveratrol concentration in wines, beginning with the precursor glucoside (polydatin/piceid), would enhance health assets without modifying the wine's original physical, chemical and sensory properties (Todaro, Palmeri, Barbagallo, Pifferi, & Spagna, 2008). Further, the study of the pharmacokinetics of resveratrol after its administration in grape juice, which predominantly contains glucosides such as piceid, and the resveratrol administration as the pure-free aglycone dissolved in alcoholic matrix, suggests that glucosidic forms of resveratrol are absorbed at a lesser extent than free aglycone (Meng, Maliakal, Lu, Lee, & Yang, 2004; Patel et al., 2011).

Resveratrol concentration in musts and wines may be influenced by oenological practices, such as the use of β-glucosidase enzymes (Gerogiannaki-Christopoulou, Athanasopoulos, Kyriakidis, Gerogiannaki, & Spanos, 2006). β-Glucosidase (1,4-β-D-glucosidase, EC 3.2.1.21) catalyses the hydrolysis of alkyl and aryl β-glucosides and the disaccharide glucosides and short oligosaccharides of glucose (Rodríguez, Lopes, Valles, Giraudo, & Caballero, 2007). Most studies on β-glucosidase activity in winemaking are related to terpenyl glucoside hydrolysis to enhance wine aroma (Cordero Otero et al., 2003; González-Pombo, Fariña, Carrau, Batista-Viera, & Brena, 2011). The existence of β-glucosidases capable of hydrolysing natural glucosides with different aglycones may extend the range of musts and wines glucosidic compounds capable of being hydrolysed by wine-related β-glucosidase-producer yeasts (Rodríguez et al., 2007).

So that health and sensory attributes of wine could be enhanced, it is important to explore the potential of autochthonous yeasts biodiversity and perform screenings for β-glucosidase producer strains from specific oenological ecosystems. Since only few strains of *Saccharomyces cerevisiae* possess β-glucosidase activity, recent studies have focused on indigenous species of non-*Saccharomyces* strains to impart special characteristics to the wines (Comitini et al., 2011; Domizio et al., 2011; Hong & Park, 2013). The lack of screening methods for glucosidase activity which are able to correlate with real winemaking conditions is one of the limitations to identify proper wine-related β-glucosidase-producer yeasts (Pérez et al., 2011).

Current assay isolates autochthonous yeast strains from grapes and selects those with β-glucosidase activity suitable for winemaking and capable of increasing free-resveratrol concentration during the alcoholic fermentation of *V. labrusca* grape must.

2. Materials and methods

2.1. Chemicals and reagents

Glucose, peptone, yeast extract, agar and sodium metabisulfite were obtained from Merck (São Paulo, SP, Brazil). Cellobiose, fructose and ethanol were purchased from Fluka (São Paulo, SP, Brazil). YNB medium was purchased from Biosystems (São José dos Pinhais, PR, Brazil). Ferric citrate, glycerol, piceid, *trans*-resveratrol, arbutin and esculin were purchased from Sigma-Aldrich (São Paulo, SP, Brazil). Acetic acid, methanol and sulfuric acid were acquired from LAS do Brasil (Aparecida de Goiânia, GO, Brazil).

2.2. Isolation of wine-related yeasts

Yeasts were isolated on solid YPG medium, pH 4.5, (glucose 20 g/L, peptone 20 g/L, yeast extract 10 g/L, and agar 20 g/L) during spontaneous fermentation of *V. labrusca* Bordô grapes grown in the southern Brazilian state of Paraná. The isolated yeasts were maintained at –20 °C in cryoprotective liquid medium (YPG, pH 5.5, and glycerol 15 g/L). So that enzyme production of yeast in synthetic medium and in natural grape must could be investigated, the conserved yeast suspensions were thawed and each yeast strain was grown in liquid YPG medium, pH 5.5 (26 °C, 150 rpm, for 18 h), centrifuged and suspended in sterile distilled water. Recently prepared yeast suspensions were used as the inoculum in the assays described.

2.3. Identification

Yeasts were identified by molecular profiling using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS), described elsewhere (Agustini, Silva, Bloch, Bonfim, & da Silva, 2014).

2.4. Yeasts' β-glucosidase production assay

Isolated yeast strains were screened for β-glucosidase production with cellobiose as the substrate. A 10 μL-inoculation loop from each test-yeast suspension was streaked on agar plates containing cellobiose as single carbon source (YNB 6.7 g/L, cellobiose 10 g/L, agar 20 g/L; pH 5.5). Inoculated plates were maintained at 26 °C and examined daily for cell growth.

The production of the enzyme β-glucosidase was also verified in Erlenmeyer flasks with YNB 6.7 g/L liquid medium (pH 5.5) containing arbutin 0.75 g/L or esculin 0.94 g/L as enzyme substrates, with the addition of ferric citrate 0.1 g/L (Rosi, Vinella, & Domizio, 1994; Saqib & Whitney, 2006). The arbutin and esculin media were inoculated with 10^6 cells/mL from each test-yeast suspension and the flasks were maintained at 26 °C, 150 rpm, for 48 h. The assays were visually compared to arbutin and esculin media inoculated with *Candida wickerhamii* NRRL Y-2563 (Freer, 1993) and *S. cerevisiae* NRRL Y-12632 for positive and negative β-glucosidase production, respectively.

The strains which developed on cellobiose agar and hydrolysed at least one other substrate (either arbutin or esculin) were positive to β-glucosidase production.

The piceid hydrolysis to resveratrol and glucose was verified in Erlenmeyer flasks with YNB 6.7 g/L liquid medium (pH 5.5) containing piceid 0.54 g/L, inoculated with 10^6 cells/mL of each selected test-yeast suspension. The flasks were maintained at 26 °C, 150 rpm, for 36 h. The media were centrifuged at 14 500 × *g* for 10 min to get cell-free broth and the supernatant analysed by HPLC to evaluate the presence of piceid and resveratrol.

2.5. *β-Glucosidase activity of yeasts in winemaking conditions*

β-Glucosidase activity in winemaking conditions and the hydrolysis of the piceid present in grapes were verified in 300 mL Erlenmeyer flasks containing 100 mL of *V. labrusca* Bordô grape must, without the skins, with the addition of sodium metabisulfite 50 mg/L. Five hours after the addition of SO_2, the grape must was inoculated with 10^7 cells/mL of each test-yeast suspension. Fermentation assays were carried out under static conditions at 18 °C, for 5 days. The supernatant was centrifuged at 14 500 × *g* for 10 min and analysed to verify resveratrol, glucose, fructose, acetic acid, glycerol and ethanol concentrations before and after fermentation.

2.6. *Chemical analysis*

Quantification analysis was performed in high-performance liquid chromatography (HPLC) on a Varian ProStar HPLC (Varian Inc, Walnut Creek, CA) comprising a ProStar 230 ternary pump.

The chromatographic separation of stilbenes was performed in a Varian Microsorb MV 100-5 C-18 (250 × 4.6 mm, 5 μm) column using a 20 μL injection sample volume. The column was thermostatically controlled to maintain a temperature of 40 °C. The eluents were (A) water : acetic acid (98:2) and (B) methanol, whilst the mobile phase flux was 1 mL/min. The linear gradient for the mobile phase (A) followed the protocol: 0 min, 100%; 10 min, 85%; 15 min, 75%; 20 min, 70%; 25 min, 65%; 35 min, 50%, 40 min, 40%, 45 min, 20%, 55 min, 100%. The eluent was monitored at 288 nm by an UV-Vis detector (Varian ProStar, Lake Forest, CA). *trans*-Resveratrol concentrations were determined by standard calibration curve (1–15 μg/mL).

The chromatographic separation of glucose, fructose, glycerol, acetic acid and ethanol was performed on an Agilent Hi-Plex H ion-exchange analytical column (300 mm × 7.7 mm, 8 μm) with an injection sample volume of 20 μL. As the isocratic mobile phase, H_2SO_4 8 mM had a flow rate of 0.4 mL/min at room temperature (25 °C). The compounds were detected by a refractive index detector (Detector 350 RI, Varian ProStar, Lake Forest, CA). Glucose, fructose, acetic acid, glycerol, and ethanol concentrations were determined by standard calibration curves (0.72–2.4 mg/mL).

2.7. *Statistical analysis*

Rates were given as means ± 1-standard deviation (SD) for three independent experiments analysed in triplicate. Comparisons among autochthonous yeasts were tested with one-way ANOVA, whilst P-value < 0.05 was considered significant. Tukey's HSD post-hoc correction test was applied. Correlations among the data obtained were calculated by Pearson's correlation coefficient. Statistica for Windows version 8.0 (StatSoft Inc., Tulsa, OK, USA) was used for all analyses. All data showed a normal distribution without outliers.

3. Results and discussion

3.1. *Isolation and identification of yeasts, and β-glucosidase production screening*

Three hundred and eight autochthonous yeast strains isolated from *V. labrusca* Bordô grapes were evaluated for β-glucosidase production, taking into consideration cellobiose and arbutin and/or esculin hydrolysis. Further, 18.8% provided a positive β-glucosidase result. The frequency of autochthonous β-glucosidase-producer yeasts in current study was similar to that reported by Spagna, Barbagallo, Palmeri, Restuccia, and Giudici (2002) who studied β-glucosidase activity in 361 yeasts isolated from samples of Sicilian wine must and reported that 18% of yeasts were positive for β-glucosidase activity.

Moreover, 57% of the 58 yeast strains that could hydrolyse cellobiose, and arbutin and/or esculin, were able to hydrolyse piceid to free resveratrol in the synthetic media. Yeasts were identified by MALDI–TOF MS as belonging to the species *Hanseniaspora uvarum* (n = 23), *Hanseniaspora opuntiae* (n = 4), *Candida zemplinina* (n = 3), *S. cerevisiae* (n = 1), *Zygoascus meyerae* (n = 1) and *Zygosaccharomyces bailii* (n = 1).

It was expected that a smaller number of isolated yeasts hydrolysed piceid, since the β-glucosidase enzyme had different substrate specificity (Fia, Giovani, & Rosi, 2005), and some species behaved differently due to their strain (Rosi et al., 1994; Villena, Iranzo, & Pérez, 2007). Substrate specificity and differences among strains were more evident to the isolated yeasts of *S. cerevisiae*. Only one strain out of eleven *S. cerevisiae* β-glucosidase-producers hydrolysed piceid in synthetic media.

Although arbutin and esculin derivatives were the most reliable β-glucosidic grape analogues, no studies have correlated these assays with the natural grape glucosides hydrolysis capability by wine-related yeasts (Pérez et al., 2011), as the piceid substrate.

In general, β-glucosidase enzymes cleave the β(1→4) glucosidic bonds in several substrates, but little is known about the β-glucosidases interaction with their substrates, especially with regard to the aglycone chemical structure moiety that is the basis of the diversity of compounds able to be hydrolysed and which cause subtle differences in substrate specificity (Singhania, Patel, Sukumaran, Larroche, & Pandey, 2013).

Pérez et al. (2011) showed that it is important to create collections of native wine yeasts, since very few strains with β-glucosidase activity have been identified. In fact, the above restricts knowledge of their winemaking potential. Therefore, it is necessary to investigate the capacity of wine-related β-glucosidase-producer yeasts on natural substrates.

3.2. *Alcoholic fermentation of* V. labrusca *grape must*

The 33 wine-related β-glucosidase-producer yeasts that hydrolysed the substrate piceid in the synthetic media were used

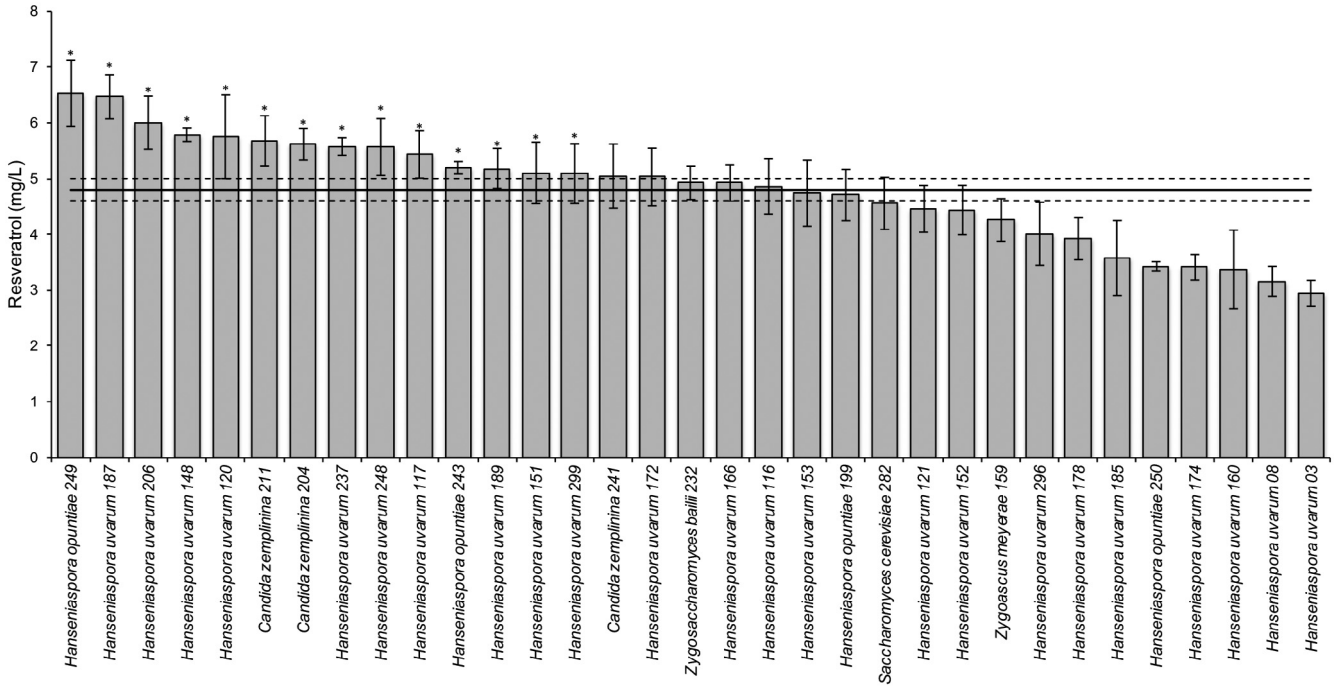

Fig. 1 – Resveratrol concentrations (mg/L) after a five-day fermentation of *Vitis labrusca* grape must, inoculated with autochthonous wine-related yeast strains. Bars show mean ± standard deviation. Resveratrol concentrations are given in descending order for the evaluated strains. Asterisk (*) above the bars represent no significant difference (ANOVA with Tukey's HSD correction, P > 0.05). Solid line (4.8 mg/L) represents resveratrol average rates; dashed lines represent upper (5.0 mg/L) and lower (4.6 mg/L) 95% confidence interval.

as starters in the alcoholic fermentation of grape must to verify their enzyme's capability to hydrolyse the natural piceid in *V. labrusca* Bordô grapes under winemaking conditions.

The inoculated grape must had a resveratrol concentration of 3.18 ± 0.01 mg/L, fructose and glucose concentrations of 100.01 ± 20.70 g/L and 38.23 ± 8.38 g/L, respectively, and pH of 3.2 ± 0.2 at the beginning of fermentation.

The highest resveratrol concentration was reported in fermentations carried out by 14 yeast strains without any significant difference (ANOVA, P > 0.05). Resveratrol concentrations above the 95% confidence interval showed higher resveratrol concentrations when compared to all studied yeast strains (Fig. 1).

Although several studies reported that β-glucosidase activity was inhibited under winemaking conditions due to low pH value, high initial glucose concentration and low aeration caused by the static condition of fermentation (Rodríguez et al., 2007; Rosi et al., 1994; Villena Arévalo, Iranzo Úbeda, Otero Cordero, & Pérez Briones, 2005), the β-glucosidase activity could be identified in most of the wine-related studied yeasts since there was an increase in the initial resveratrol concentration, reaching 102% after five days of the grape must fermentation. The four *H. opuntiae* strains increased the resveratrol concentration but at significantly different levels (ANOVA, P < 0.05): *H. opuntiae* strains 249 and 243 increased the resveratrol to an average of 5.76 mg/L, *H. opuntiae* 199 increased the resveratrol to an average of 4.71 mg/L and *H. opuntiae* 250 increased the resveratrol concentration to an average of 3.42 mg/L. This fact suggested differences in the enzyme activity amongst β-glucosidase-producer strains belonging to the same species.

The yeast species *H. uvarum* also revealed significant differences in the final free-resveratrol concentrations (ANOVA, P < 0.05): *H. uvarum 187* increased the initial resveratrol concentration to 6.5 ± 0.4 mg/L and *H. uvarum 03* maintained the initial resveratrol concentration. Consequently, only the β-glucosidase enzyme produced by this yeast was probably inactivated by winemaking conditions.

As demonstrated in Table 1, after five days of alcoholic fermentation, the highest ethanol concentrations ranged between 33.7 ± 7.1 g/L and 48.8 ± 2.7 g/L, without significant differences (ANOVA, P > 0.05); the lowest residual glucose concentrations ranged between 1.2 ± 0.1 g/L and 5.9 ± 1.4 g/L, without significant differences (ANOVA, P > 0.05); and the lowest residual fructose concentrations ranged between 3.0 ± 0.5 g/L and 11.6 ± 1.8 g/L without significant differences (ANOVA, P > 0.05). They were detected in the fermented must inoculated with the yeast *S. cerevisiae* 282 and with eight non-*Saccharomyces* yeasts out of the 33 studied yeasts. High ethanol formation by the isolated yeast strains *H. uvarum* 117, 121, 148, 166, 172, 187, 189, and 206 corroborated studies that verified whether apiculate yeasts might last throughout the alcoholic fermentation for longer periods than has been previously surmised, survive high ethanol concentrations and even produce alcohol concentrations similar to *S. cerevisiae* industrial wine yeasts (Hong & Park, 2013; Moreira et al., 2011). The β-glucosidase activity in *S. cerevisiae* strains has been formerly found in small numbers. When present, it was very

Table 1 – Resveratrol, glucose, fructose, ethanol, acetic acid and glycerol concentrations after five-day fermentation of *Vitis labrusca* grape must inoculated with autochthonous wine-related yeast strains that formed the highest ethanol contents.

Autochthonous yeast strain evaluated	Resveratrol (mg/L)	Glucose residue (g/L)	Fructose residue (g/L)	Ethanol (g/L)	Acetic acid (g/L)	Glycerol (g/L)
Saccharomyces cerevisiae 282	4.6 ± 0.5[b,c]	4.2 ± 1.9	4.5 ± 1.0	48.8 ± 2.7	9.5 ± 3.8[b,c]	5.1 ± 0.4
	4.4 (4.2–5.1)	4.3 (2.4–5.9)	4.5 (3.6–5.4)	48.7 (45.6–52.0)	9.3 (5.8–13.5)	5.0 (4.8–5.6)
Hanseniaspora uvarum 117	5.4 ± 0.4[a,b]	5.5 ± 2.6	5.6 ± 2.3	40.5 ± 3.2	13.2 ± 4.2[c]	4.7 ± 0.6
	5.5 (5.0–5.9)	4.7 (3.5–9.0)	4.9 (3.8–9.0)	41.3 (35.9–43.2)	12.0 (5.9–22.9)	4.7 (3.9–5.4)
Hanseniaspora uvarum 121	4.5 ± 0.4[b,c]	5.9 ± 1.4	11.6 ± 1.8	40.0 ± 1.8	5.5 ± 2.3[a,b]	7.7 ± 0.9
	4.5 (4.0–4.9)	6.2 (4.0–7.1)	11.5 (9.6–13.6)	40.1 (38.0–42.1)	5.6 (3.0–7.5)	7.7 (6.7–8.8)
Hanseniaspora uvarum 148	5.8 ± 0.1[a,b,c]	3.2 ± 0.5	4.1 ± 0.7	42.8 ± 2.5	5.4 ± 2.0[a,b]	7.3 ± 0.2
	5.7 (5.7–5.9)	3.1 (2.8–4.0)	3.8 (3.7–5.2)	42.6 (40.0–46.1)	5.4 (3.3–7.6)	7.3 (7.1–7.5)
Hanseniaspora uvarum 166	4.9 ± 0.3[b,c]	2.1 ± 1.3	5.1 ± 2.1	38.0 ± 4.2	2.4 ± 0.6[a]	5.7 ± 1.6
	5.0 (4.6–5.2)	1.9 (1.0–3.6)	4.7 (3.2–8.0)	41.4 (25.8–43.4)	2.2 (1.9–3.4)	6.4 (3.4–6.7)
Hanseniaspora uvarum 172	5.0 ± 0.5[b,c]	2.6 ± 1.3	4.9 ± 3.6	33.7 ± 7.1	0.8 ± 0.7[a]	7.0 ± 1.4
	5.2 (4.5–5.5)	2.1 (1.6–4.4)	3.6 (2.3–10.0)	36.3 (15.5–46.5)	0.4 (0.4–1.8)	7.9 (3.0–9.2)
Hanseniaspora uvarum 187	6.5 ± 0.4[a]	3.0 ± 1.3	4.2 ± 0.6	45.1 ± 1.8	1.5 ± 0.2[a]	6.8 ± 0.5
	6.5 (6.1–6.9)	3.1 (1.5–4.3)	4.3 (3.7–4.7)	44.7 (43.3–47.7)	1.4 (1.3–1.7)	7.0 (6.0–7.1)
Hanseniaspora uvarum 189	5.2 ± 0.4[a,b]	1.2 ± 0.1	3.0 ± 0.5	35.4 ± 6.0	2.0 ± 0.5[a]	5.4 ± 0.8
	5.0 (5.0–5.6)	1.5 (0.9–1.8)	3.4 (3.1–3.7)	37.3 (26.7–40.4)	1.8 (1.6–2.7)	5.6 (4.2–6.0)
Hanseniaspora uvarum 206	6.0 ± 0.5[a,b]	1.8 ± 0.7	4.3 ± 1.3	37.0 ± 6.4	1.6 ± 0.9[a]	5.8 ± 0.9
	5.9 (5.6–6.5)	1.6 (1.3–2.8)	3.9 (3.1–6.1)	37.4 (28.8–44.5)	1.6 (0.6–2.6)	5.6 (5.1–7.1)
All strains data combined	4.8 ± 1.0	9.6 ± 6.8	22.4 ± 17.4	28.2 ± 10.6	10.6 ± 9.6	4.7 ± 2.3
	4.9 (2.7–7.2)	8.8 (0.9–36.5)	20.7 (2.2–71.7)	26.1 (6.5–52.0)	7.9 (0.4–42.2)	4.6 (0.0–12.5)

The amounts of resveratrol and sugar were respectively 3.18 mg/L and 170 g/L at the start.

All rates are given as mean ± standard deviation, median (minimum–maximum) of three independent experiments. Rates displaying similar superscript letters ([a, b, c]) within each column are not different (ANOVA with Tukey's HSD correction, $P > 0.05$).

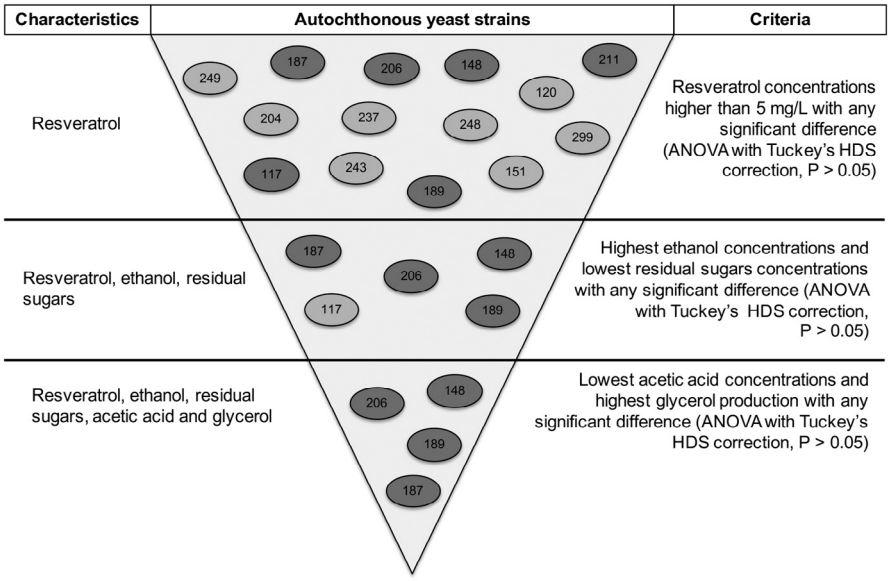

Fig. 2 – Criteria employed to select autochthonous yeast strains based on resveratrol concentration and oenological metabolites. Numbers inside each circle indicate the evaluated yeast strain: 117: *Hanseniaspora uvarum* 117, 120: *H. uvarum* 120, 148: *H. uvarum* 148, 151: *H. uvarum* 151, 187: *H. uvarum* 187, 189: *H. uvarum* 189, 204: *Candida zemplinina* 204, 206: *H. uvarum* 206, 211: *C. zemplinina* 211, 237: *Hanseniaspora opuntiae* 237, 243: *H. opuntiae* 243, 248: *H. uvarum* 248, 249: *H. opuntiae* 249, and 299: *H. uvarum* 299.

limited and easily inhibited by high glucose and ethanol concentrations, anaerobiosis and sulfur dioxide (Cordero Otero et al., 2003; Villena et al., 2007).

In the case of other oenological metabolites produced during alcoholic fermentation, the lowest concentrations of acetic acid ranged between 0.8 ± 0.7 g/L and 5.5 ± 2.3 g/L, without significant differences (ANOVA, $P > 0.05$) and the highest glycerol concentrations ranged between 4.6 ± 0.7 g/L and 7.7 ± 0.9 g/L, without significant differences (ANOVA, $P > 0.05$), measured in 14 and 18 fermentations, respectively. Acetic acid is a secondary metabolite derived from pyruvic acid, which is always formed during alcoholic fermentation. Beyond a certain limit, it has a detrimental organoleptic effect on the product's quality since it provides a bitter taste and a vinegar-like smell (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006). Glycerol is a by-product of the fermentation of sugar to ethanol, mainly produced during glyceropyruvic fermentation at the start of alcoholic fermentation. In fact, it is the third most common constituent of wine, after water and ethanol. Glycerol is an interesting metabolite for wines due to its positive contribution to taste sensations, such as sweetness, softness, silkiness and thickness. Glycerol concentration in wines usually has a 1:10 ratio of the ethanol produced, whilst wine-related yeast varies widely in glycerol production, ranging from 4 g/L to 10 g/L (Loira et al., 2014; Moreno-Arribas & Polo, 2009).

Further, the resveratrol and the alcoholic fermentation metabolite concentrations measured at the end of fermentations inoculated with each of the thirty-three studied yeasts underwent Pearson's linear correlation test. Correlation was reported when comparing total sugar consumption to ethanol, glycerol and acetic acid formation, as expected. Data also showed a small (27%) but statistically significant ($P = 0.006$) correlation when ethanol formation and the resveratrol increasing concentrations were compared. Therefore, the final concentration of resveratrol in wines rises when there is ethanol formation too, possibly contributing to improve the free-resveratrol solubility in the fermented must (Bavaresco, Mattivi, De Rosso, & Flamini, 2012). The above corroborates the fact that β-glucosidase activity and adequate alcoholic fermentation characteristics should be present to select the appropriate wine yeast for the increase of resveratrol concentration in wines.

Figure 2 demonstrates that 14 out of the 33 yeast strains that hydrolysed piceid increased the initial resveratrol concentration in grape must of *V. labrusca* to approximately 5.64 mg/L without significant differences (ANOVA, $P > 0.05$). Five autochthonous yeast strains were also able to increase resveratrol concentration, whilst consuming sugars and producing the highest concentrations of ethanol measured. Four out of the five strains produced minimum acetic acid and glycerol concentrations at the expected range.

The autochthonous wine-related yeast strains *H. uvarum* 148, 187, 189, and 206 provided the most adequate oenological characteristics without any significant difference (ANOVA, $P > 0.05$) when the oenological metabolites, such as ethanol, glycerol and acetic acid, were taken into account; similarly, resveratrol increased at the end of the alcoholic fermentation of the winemaking process.

Although it is expected and desired that a *S. cerevisiae* conducts the alcoholic fermentation, the non-*Saccharomyces* wine yeasts are usually adapted to the specific grape environment (Jolly, Augustyn, & Pretorius, 2006) and experimental evidence has emphasized the positive role of non-*Saccharomyces* yeasts in wine composition. Consequently, a re-evaluation of their role in the winemaking process has occurred (Ciani, Comitini, Mannazzu, & Domizio, 2010; Comitini et al., 2011).

A study focusing on the potential market for functional wines in which resveratrol content was enhanced revealed that consumers were willing to pay 55% more for a bottle of wine with increased resveratrol concentration (Barreiro-Hurlé, Colombo, & Cantos-Villar, 2008).

So that functional wines enriched with resveratrol could be obtained, it is important to bear in mind in wine-related yeast selection that the yeast's enzyme should be capable of hydrolysing the synthetic grape glucosidic analogues during the screening selection stage and the natural resveratrol-glucosides present in the grape berries during the fermentation procedure. Further, the yeast should be equally able to ferment the sugars in the grape must which produces ethanol, so the ethanol in the wine maintains the phenolic compounds soluble, both in the wine and in the human intestine (Goldberg, 1995; Gürbüz et al., 2007).

4. Conclusion

During screening to select β-glucosidase-producer autochthonous yeast strains using grape-related glucoside substrates, such as arbutin and esculin, 57% of the yeasts that hydrolyse these substrates were also able to hydrolyse the resveratrol-glucoside piceid. The results suggest that wine-related yeasts endowed with β-glucosidase activity do not merely release aroma precursors throughout the alcoholic fermentation of grape must but may also hydrolyse resveratrol-glucosides which are naturally present in the grapes. It is desired that the selected yeast strain would have adequate alcoholic fermentation characteristics and β-glucosidase activity in winemaking conditions to guarantee free-resveratrol solubility in the fermented grape must of *V. labrusca*.

Acknowledgements

Current study was funded by the Brazilian Coordination for the Upgrading of Higher Education Personnel (CAPES) (Capes - Embrapa 001/2011). Brazilian CGEN authorization 010198/2013-0.

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