Time course of L-tryptophan metabolites when fermenting natural grape musts: effect of inoculation treatments and cultivar on the occurrence of melatonin and related indolic compounds

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Abstract

Background and Aims: Tryptophan is involved in the formation of bioactive compounds, such as melatonin (MEL) and 3-indoleacetic acid (3-IAA), by yeast. Melatonin is a neurohormone whose occurrence in wine has been widely reported in recent years. The occurrence, however, of MEL and other indolic compounds related to tryptophan metabolism by wine yeast strains has been scarcely reported in grape musts. This work examined the occurrence of these compounds during the alcoholic fermentation (AF) of musts from seven grape cultivars, Corredera, Chardonnay, Moscatel, Palomino Fino, Sauvignon Blanc, Tempranillo and Vijiriega.

Methods and Results: Must was fermented with three *Saccharomyces cerevisiae* strains and then in two cases an additional sequential inoculation with the non-*Saccharomyces* yeast *Torulaspora delbrueckii* was carried out. Fermented must samples were analysed by UHPLC/HRMS to determine the concentration of: L-tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, *N*-acetyl-5-hydroxytryptamine, MEL, 3-IAA, tryptamine, tryptophol and L-tryptophan ethyl ester. The profile of indolic compounds during AF with the Aroma White strain depended on the cultivar. The yeast strain did not influence the profile of indolic compounds; instead, fermentation time was found to be a more influential factor.

Conclusions: The production of indolic compounds during the AF depends largely on the cultivar used and the day of fermentation on natural grape musts.

Significance of the Study: This is the first study that quantifies 5-hydroxytryptophan and *N*-acetyl-5-hydroxytryptamine during the AF of grape must. The occurrence of compounds with bioactive potential, for example 3-IAA and ML, during fermentation with commercial yeast strains is also described.

Keywords: *3-indoleacetic acid, melatonin, qexactive,* Saccharomyces cerevisiae, *wine*

Introduction

The amino acid L-tryptophan (L-TRP) contains an indolic ring which confers a strong hydrophobic character (Palego et al. 2016). In addition to taking part in the biosynthesis of proteins, it is involved in the metabolism of several compounds of interest, such as 5-hydroxytryptamine (5-HT) and melatonin (MEL), their principal precursor being L-TRP (Murch et al. 2000). The occurrence of MEL has been reported in many plant-based foods and beverages, including grapes (Iriti 2009, Mercolini et al. 2012), strawberries (Stürtz et al. 2011), pomegranate wines (Mena et al. 2012), sweet cherries (Zhao et al. 2013) and peppers and tomatoes (Riga et al. 2014), amongst others (Feng et al. 2014).

Yeast such as *Saccharomyces cerevisiae* are capable of metabolising L-TRP to produce MEL. This was first reported for yeast grown in two media (Sprenger et al. 1999) and then in fermented products including beer (Maldonado et al. 2009), wine (Stege et al. 2010, Rodriguez-Naranjo et al. 2011a,b, Mercolini et al. 2012) and bread (Yılmaz et al. 2014). Different metabolic pathways have been proposed for the production of MEL from L-TRP. The synthesis of MEL by yeast was initially proposed to follow the same steps as those in animals, that is, via 5-hydroxytryptophan (5-HTRP), 5-HT and *N*-acetyl-5-hydroxytryptamine (NA5-HT) (Figure 1). A subsequent study, however, reported that the capacity of *S. cerevisiae* to synthesise 5-HT via 5-HTRP was limited (Park et al. 2008). This suggested that MEL formation via 5-HTRP was not the only viable pathway. An alternative pathway for synthesising MEL from L-TRP via 5-methoxytryptamine (Figure 1) has recently been proposed (Tan et al. 2016). Different *Saccharomyces* and non-*Saccharomyces* yeast have been reported to produce MEL during alcoholic fermentation (AF), for example in synthetic must (SM) by *S. cerevisiae* [Aroma White (AW), Red Fruit (RF), QA23, ES488, IGV-GRE and Uvaferm strains], *Torulaspora delbrueckii* or *Metschnikowia pulcherrima* (Rodríguez-Naranjo et al. 2012, Fernández-Cruz et al. 2017) and in orange juice by *Pichia kluyveri* (Fernández-Pachón et al. 2014).

Several previous studies have investigated the formation of L-TRP derivatives by the QA23 yeast strain, because of its ability to rapidly complete fermentation and its wide application in white, rosé and red winemaking (Rodríguez-Naranjo et al. 2012, Blanco et al. 2013, Fernández-Cruz et al. 2017, González et al. 2018). By comparison, *S. cerevisiae* strains, such as AW and RF, have been used only sporadically in laboratories, but have recently been studied

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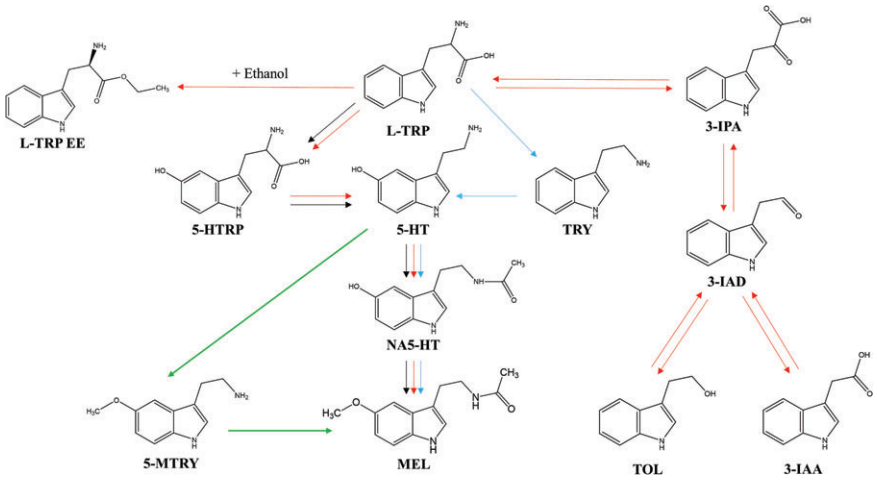

Figure 1. L-Tryptophan pathways showing formation of different indolic compounds by animals (——), plants (——) and yeast (——) and by alternative common pathways (——). [Adapted from Dickinson (2003), Simat et al. (2004) and Tan et al. (2016)]. 3-IAA, 3-indoleacetic acid; 3-IAD, 3-indole acetaldehyde; 3-IPA, 3-indolepyruvic acid; 5-HT, 5-hydroxytryptamine; 5-HTRP, 5-hydroxytryptophan; 5-MTRY, 5-methoxytryptamine; L-TRP, L-tryptophan; MEL, melatonin; NA5-HT, *N*-acetyl-5-hydroxytryptamine; TOL, tryptophol; TRY, tryptamine.

in SM (Fernández-Cruz et al. 2016, 2017, Muñíz-Calvo et al. 2017). Whereas AW is widely used in white winemaking, RF imparts appealing sensory properties to rosé and red wines. Moreover, the use of non-*Saccharomyces* yeasts in winemaking, such as *T. delbrueckii*, is considered an emerging biotechnological tool of great commercial interest (Puertas et al. 2017). *Torulaspora delbrueckii* is characterised by the production of low acetaldehyde, acetic acid, hydrogen sulfide and volatile phenols (Renault et al. 2009). As such, its sequential inoculation with *S. cerevisiae* is considered to improve wine quality (Bely et al. 2008, van Breda et al. 2013, Azzolini et al. 2014, González-Royo et al. 2015, Renault et al. 2015). It is therefore attracting the interest of winemakers to improve the fermentation process, which in turn will produce wines with improved characteristics, such as colour, aroma and better technological properties (Suárez-Lepe and Morata 2012).

In addition to 5-HT and MEL, L-TRP metabolism encompasses other metabolites as shown in Figure 1. Different pathways have been proposed for the synthesis of indolic compounds such as 3-indoleacetic acid (3-IAA) (Maslov et al. 2011). This compound is a phytohormone that has been exhaustively described in both plants and microorganisms such as bacteria (Epstein and Ludwig-Muller 1993, Spaepen et al. 2007, Yu et al. 2014, Cook et al. 2016). Together with L-TRP metabolites, such as tryptophol (TOL), tryptamine (TRY) and L-TRP ethyl ester (L-TRP EE) (Gil-Agustí et al. 2007, Favre et al. 2014, Gardana et al. 2014, Tudela et al. 2016), these compounds are of interest as bioactives because of their potential as antioxidants (Bonnefont-Rousselot and Collin 2010), endothelial function protectors (Jadhav et al. 2012), antiangiogenic molecules (Cerezo et al. 2017) and neuroprotective agents (Hornedo-Ortega et al. 2018).

The present study tested whether different grape must substrates, yeast strains and inoculation procedures influence the formation of L-TRP metabolites in semi-industrial fermentations. In addition, the study aimed to ascertain the conditions which most efficiently produce L-TRP metabolites, some of which have bioactive potential.

Materials and methods

Reagents

The compounds, 5-HTRP, TOL, 3-IAA, 5-HT, NA5-HT, MEL, L-TRP EE and TRY of high-grade purity (>97%) were purchased from Sigma Aldrich (St Louis, MO, USA); L-TRP from Panreac (Darmstadt, Germany); analytical grade methanol for UHPLC analysis from Merck (Darmstadt, Germany) and formic acid from Prolabo (Obregón, Mexico).

Grapes

Grapes from six white cultivars, Corredera, Chardonnay, Moscatel, Palomino Fino, Sauvignon Blanc and Vijiriega, commonly grown in the south of Spain, and one red cultivar, Tempranillo, were harvested from vineyards located at the Instituto de Investigación y Formación Agraria y Pesquera (IFAPA) in Jerez de la Frontera, Spain (longitude 06:00:58 W, latitude 36:45:29 N).

Yeast strains

Yeasts were selected according to their efficiency in producing MEL and other L-TRP derived compounds reported in previous work (Fernández-Cruz et al. 2016, 2017) and included: *S. cerevisiae* AW (Enartis, Trecate, Italy), *S. cerevisiae* Lalvin YSEO QA23 (Lallemand, Bayern, Germany), *S. cerevisiae* RF (Enartis) and *T. delbrueckii* TD291 Biodiva (Lallemand). The yeasts were supplied as commercial active dehydrated yeasts (ADY).

Winemaking procedure

White winemaking. Grapes from the six white cultivars were harvested, destemmed, crushed and pressed. A pectolytic enzyme preparation (2.5 mL/hL, Enartis ZYM; Enartis, San Martino, Trecate, NO, Italy) and 40 mg/L of SO_2 (Sulfosol, Sepsa-Enartis; Enartis) were added to the must which was held for 24 h at 4°C. Subsequently, the clarified must was placed in a 100 L stainless steel vessel. The AF was induced by inoculation with the AW yeast strain and monitored at 18°C. Fermentations were in triplicate (n = 3) for each cultivar. Fermentation was considered finished when the concentration of residual sugar was less than 3 g/L.

Rose winemaking. Tempranillo must was produced with a pneumatic press. A pectolytic enzyme preparation (3 mL/hL, Enartis ZYM) and 40 mg/L of SO_2 were added. The must was then placed into 15 stainless steel 10 L vats.

The Tempranillo must was subjected to five inoculation treatments: (i) inoculation with *S. cerevisiae* QA23 yeast (CT-QA23); (ii) inoculation with *S. cerevisiae* RF yeast (CT-RF); (iii) sequential inoculation (SI-QA23) with *T. delbrueckii* TD291 and then with *S. cerevisiae* QA23, that is, when density had decreased by 15 units after the start of AF; (iv) sequential inoculation (SI-RF) with *T. delbrueckii* TD291 and then with *S. cerevisiae* RF, again when density had

decreased by 15 units; and (v) spontaneous fermentation (SP), that is without inoculation with commercial yeast. All fermentations were maintained at 16–18°C and were in triplicate (n = 3).

Sampling

The white and rose musts were settled for 24 h after which samples (15 mL) were taken. For white fermentations, additional samples (15 mL) were taken daily from inoculation until the end of AF, that is when the residual sugar concentration was less than 3 g/L. The Tempranillo fermentations, CT-QA23 and CT-RF, were sampled daily until day 6, while SP, SI-QA23 and SI-RF were sampled daily until day 9. Samples were also taken on day 13–14. The duration of AF and final residual sugar concentration for wines of each cultivar are provided in Table S1 and Figure S1. Samples were stored at −80°C prior to analysis.

Sample preparation

Samples were thawed and centrifuged for 10 min at 1500 *g* (Sorvall TC Dupont Centrifuge, Thermo Fisher Scientific, Barcelona, Spain) to remove lees. The resulting supernatant was collected in 15 mL falcon tubes and an aliquot (500 μL) extracted as described by Fernández-Cruz et al. (2017). Extracts were placed in a vacuum concentrator (HyperVACLITE, Gyrozen, Seoul, Korea) until total dryness was achieved (34°C, 2000 rpm in 740 g). Samples were subsequently reconstituted in aqueous methanol (1:1, 167 μL) to a 3:1 final concentration. Prior to UHPLC/HRMS analysis, samples were filtered (13 mm VWR syringe filters, 0.45 μm polytetrafluorethylene (PTFE)) and placed in dark coloured glass vials.

UHPLC/HRMS analysis

Extracts were analysed with a UHPLC system (Dionex Ultimate 3000, Thermo Fisher, San Jose, CA, USA) comprising an autosampler (WPS-3000RS), pump (HPG-3400RS) and column compartment (TCC-3000RS). Separation was achieved using a 2.1 × 100 mm SB-C18 column (Zorbax RRHD, 1.8 μm particle size) fitted with a guard column (2.1 × 5 mm, 1.8 μm particle size). The UHPLC system was coupled to a hybrid Quadrupole-Orbitrap Qexactive equipment (Thermo Scientific, Bremen, Germany). The UHPLC and HRMS conditions were set according to a previously validated method (Fernández-Cruz et al. 2016). Each sample replicate was injected in duplicate (n = 6). More information about the UHPLC/HRMS data is provided in Table S2. The system was controlled with Chromeleon Express software (Thermo Fisher Scientific, Bremen, Germany). Xcalibur software (v.3.0.63) and TraceFinder software (v.3.1), were used for data analysis. All software was provided by Thermo Scientific.

Statistical analysis

The data were subjected to Multiple linear regression, ANOVA and Tukey's honest significant difference (HSD) to determine any significant differences ($P < 0.05$). In addition, linear discriminant analysis (LDA) was also applied. Statistica software (StatSoft, Tulsa, OK, USA) v.7.0 was used for the statistical analyses.

Results and discussion

Composition of must from white grape cultivars during fermentation with S. cerevisiae *AW*

Must from six white grape cultivars, Corredera, Chardonnay, Moscatel, Palomino Fino, Sauvignon Blanc and Vijiriega, were analysed during AF with the *S. cerevisiae* AW strain to determine the occurrence of indolic compounds derived from L-TRP metabolism. It must be noted that, in all cases, the *S. cerevisiae* AW was the main strain inoculated during AF (data not shown).

Figure 2 shows changes in the concentration of the precursor amino acid L-TRP, together with metabolites of the MEL pathway, 5-HTRP, 5-HT, NA5-HT and MEL, the higher alcohol TOL and other compounds of interest (3-IAA and L-TRP EE), during AF. The initial concentration of L-TRP in must ranged from 0.4 to 2.3 mg/L (416–2313 ng/mL), with Corredera and Chardonnay containing the lowest and highest concentration, respectively (Figure 2a). The amino acid profile is influenced by several parameters, such as the soil composition of the area where the grapes were cultivated, the cultivar employed (Garde-Cerdán et al. 2009), irrigation (Bouzas-Cid et al. 2018), leaf treatments (Ruiz-Rodríguez et al. 2017) and terroir (Moreno et al. 2015, Uriarte et al. 2016). Despite it being one of the main amino acids, however, little attention has been paid to L-TRP; a few studies report the L-TRP concentration of grapes and must in different cultivars. The concentration measured in the current study is much lower than that previously reported (Table 1). At the end of AF, L-TRP values had decreased significantly, ranging from 4.2 ng/mL for Vijiriega to 1902 ng/mL for Moscatel.

The first intermediate metabolite in the MEL pathway, described previously for animals, plants and yeasts, is 5-HTRP (Figure 1). This study is the first to report the occurrence of 5-HTRP in grape must during AF. As shown in Figure 2b, 5-HRTP was initially present at a low concentration (<2.5 ng/mL) in must prior to AF for all cultivars; a significant quantity was then consumed by the end of AF. The progressive decrease in 5-HTRP concentration ranged from 55% for Corredera to 99% for Sauvignon Blanc. It appears that yeast are capable of consuming 5-HRTP quickly during the early stages of AF (i.e. during days 1–3). A similar trend was previously observed during the AF of SM (Fernández-Cruz et al. 2016).

The subsequent MEL pathway intermediates, 5-HT and NA5-HT, were not detected in the white musts, however, the final metabolite, MEL, was quantified during AF of all samples, except Palomino Fino. Melatonin was measured at a concentration ranging from 0.10 to 0.45 ng/mL in the initial must samples (i.e. prior to AF) (Figure 2c). Accumulation of MEL followed two trends during AF, despite the use of the same yeast strain. A zigzag plot trend was observed for the MEL concentration in Corredera and Moscatel, with significant change observed during AF. In contrast, MEL concentration remained constant during AF of Chardonnay, Sauvignon Blanc and Vijiriega (Figure 2c). A previous study reported the maximum production of MEL on day 2 for a SM fermentation with the *S. cerevisiae* AW strain (Fernández-Cruz et al. 2016). In this study, however, more than one peak in MEL production was found during AF (Figure 2c). There was a maximum peak for MEL at the end of AF (i.e. on day 9) for Chardonnay, while other cultivars, for example Corredera and Moscatel, gave multiple peaks, especially during the first stages of AF (i.e. between days

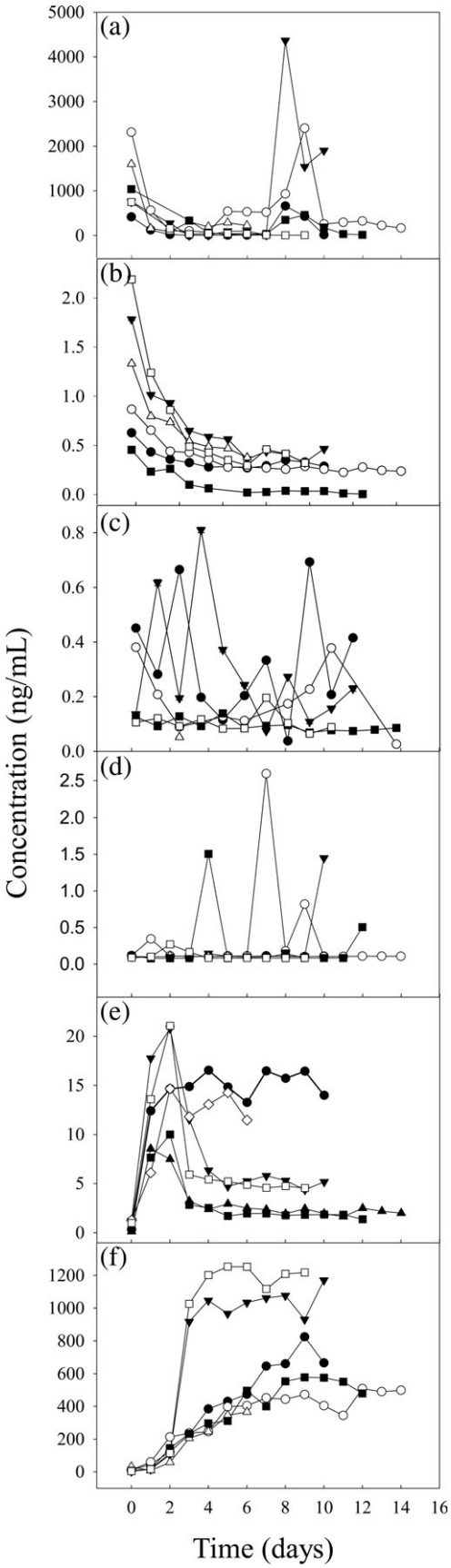

Figure 2. Effect of *Saccharomyces cerevisiae* Aroma White strain on the concentration of (a) L-tryptophan, (b) 5-hydroxytryptophan, (c) melatonin, (d) L-tryptophan ethyl ester, (e) 3-indoleacetic acid and (f) tryptophol during the fermentation of musts of the white grape cultivars, Corredera (●); Chardonnay (▲); Moscatel (▼); Palomino Fino (◇); Sauvignon Blanc (■) and Vijiriega (□). Values are means of six replicates (n = 6) ± standard deviation.

Table 1. L-Tryptophan concentration reported in white and red grape cultivars.

Type of grape and cultivar	Concentration (ng/mL)	References
White		
Kerner	2000–80 000	Hoenicke et al. (2001)
Riesling	4000–35 000	Linsenmeier et al. (2004)
White grapes from different countries	200–11 000	Bell and Henschke (2005)
White grapes from Alentejo region (Portugal)	5500–17 800	Herbert et al. (2006)
Albana	70–117	Mercolini et al. (2012)
Emir	108–760	Ünal et al. (2015)
Narince	22–410	Ünal et al. (2015)
Sultaniye	34–520	Ünal et al. (2015)
Verdejo	2500–4000	Ruiz-Rodríguez et al. (2017)
Treixadura	8000–18 000	Bouzas-Cid et al. (2018)
Chardonnay	5000–45 000	Meng et al. (2018)
	3–400	Vigentini et al. (2015)
Red		
Sangiovese	70–133	Mercolini et al. (2012)
Croatina	5–200	Vigentini et al. (2015)
Tempranillo	9410–17 630	Pérez-Álvarez et al. (2017)
	23–380	Garde-Cerdán et al. (2017)
	25–810	Gutiérrez-Gamboa et al. (2017)
	30 000	González-Santamaría et al. (2018)
Monastrell	33 420	Garde-Cerdán et al. (2017)
Carignan Noir	6240–15 540	Gutiérrez-Gamboa et al. (2018a,b)
Garnacha	13–100	Gutiérrez-Gamboa et al. (2018a, b)

1–6). These results show a remarkable difference among the substrates studied, resulting in each cultivar displaying a different trend in MEL production.

The occurrence of L-TRP EE during AF of white grape must was highly variable (Figure 2d). In five of the six initial samples, L-TRP EE was present. The exception, Corredera, L-TRP EE did not appear until day 3. Moreover, Corredera, Palomino Fino and Vijiriega gave relatively constant L-TRP EE values, with little change during AF (ca. 0.1 ng/mL). In Chardonnay and White Muscat, L-TRP-EE concentration to slightly increased up until the end of AF (0.5 ng per 10^6 CFU), when it then diminished (Vigentini et al. 2015). In an earlier study, we reported an increase in L-TRP EE concentration in SM during AF using the *S. cerevisiae* AW strain under laboratory conditions, with a final concentration of 17.5 ng/mL at the end of AF (Fernández-Cruz et al. 2016). Other researchers have also reported the formation of L-TRP EE in growth medium and grape musts, under laboratory conditions. In growth medium, L-TRP EE increased in concentration 24 h after inoculation (12.0–-208.1 ng/mL) with different strains of *S. cerevisiae* (UMY255, EC1118 and IOC18-2007), but it decreased to a negligible concentration (0.3–1.6 ng/mL) 72 h after inoculation (Vigentini et al. 2015). In the current study, the maximum concentration of L-TRP EE found in white grape must (0.1 ng/mL) was lower than that reported in SM (17.5 ng/mL). Apparently, yeast produce ethyl esters from amino acids part way through AF, when the concentration of alcohol in the fermenting medium is high (Lambrechts and Pretorius 2000). The L-TRP EE appears to be formed from L-TRP (Figure 1) in a one-step reaction (Arapitsas et al. 2018).

The initial concentration of 3-IAA observed in the six white grape musts was negligible (<1 ng/mL), in agreement with that previously observed in the initial must obtained from Kerner and Malvasia grapes, that is less than 5 ng/mL (Simat et al. 2004, Maslov et al. 2011). Our results show a remarkable increase in 3-IAA during the early stages of AF for all must samples (Figure 2e), indicating production because of yeast metabolism. In yeast, the occurrence of 3-IAA is reported to be dependent on L-TRP (Contreras et al. 2014, Nutaratat et al. 2016). This relationship was not found in the current study, even though two accumulation trends were observed during AF. Chardonnay, Moscatel, Sauvignon Blanc and Vijiriega showed a significant decrease in 3-IAA at day 3, relative to their initial 3-IAA concentration. A similar trend was previously reported for Müller-Thurgau grape must fermented with *S. cerevisiae* Uvaferm CM, where the highest concentration of 3-IAA (being 70 ng/mL) occurred during turbulent fermentation, with the concentration diminishing at the end of AF (Simat et al. 2004). In the case of SM fermented by the *S. cerevisiae* AW strain, the main trend observed was an initial increase, and then a decrease in 3-IAA concentration, from 83.8 ng/mL at day 1 to 0.9 ng/mL by the end of AF (Fernández-Cruz et al. 2016). In contrast, Corredera and Palomino Fino gave a relatively consistent 3-IAA concentration, being 16.5 and 14.8 ng/mL, respectively, at the end of AF, that is the concentration was significantly higher than that prior to AF (Figure 2e). Similarly, a study involving fermentation of Riesling grape must by four commercial *S. cerevisiae* strains (Uvaferm CEG, Lalvin Cross, Anchor VIN and Anchor Exotics SPH) reported an increase in 3-IAA during AF of 10–35 ng/mL, but the concentration did not diminish at the end of fermentation (Mihaljević Žulj et al. 2015). These data suggest that the combination of yeast strain and grape cultivar play a decisive role in the accumulation of 3-IAA.

Tryptophol was the compound with the highest concentration during the AF of must from the six white grape cultivars (Figure 2f). Tryptophol was present in the initial must at a concentration of 3.3 and 31.3 ng/mL for Moscatel and Palomino Fino, respectively. Two accumulation patterns were then observed. Corredera, Chardonnay, Palomino Fino and Sauvignon Blanc accumulated TOL weakly during AF with a final concentration ranging from 365 to 666 ng/mL. In contrast, TOL increased sharply after 1–2 days of AF of Moscatel and Vijiriega (1170–1217 ng/mL) and was constant at the end of AF, giving a significantly higher concentration of TOL compared with that observed for other cultivars. The same trend was reported for AF of must from Müller-Thurgau grapes, with TOL being synthesised during the first stages of the fermentation process and increased until the end of AF (Simat et al. 2004). In SM fermented with *S. cerevisiae* AW strain, TOL also increased in concentration during the first 3 days of AF (Fernández-Cruz et al. 2016). It is well established that TOL is the fusel alcohol of the amino acid L-TRP and it is produced by yeast during AF via the Ehrlich pathway (Hazelwood et al. 2008, Mas et al. 2014). Synthesis of TOL occurs after the formation of two intermediate metabolites, 3-indolpyruvic acid (3-IPA) and 2-indolacetaldehyde (3-IAD), which are derived from L-TRP (Dickinson 2003) (Figure 1). In our samples, this pathway appears to be the one that yeast preferred during AF, because of most of the L-TRP present in the initial must was transformed into TOL.

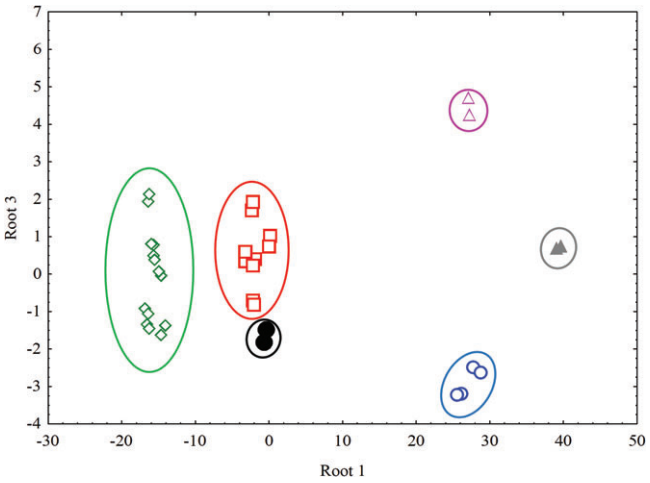

Figure 3. Linear discriminant analysis performed on must of the white grape cultivars Corredera (○), Chardonnay (□), Moscatel (◇), Palomino Fino (△), Sauvignon Blanc (●) and Vijiriega (▲) at different points during alcoholic fermentation by *Saccharomyces cerevisiae* Aroma White.

Linear discriminant analysis was performed with cultivar selected as the independent variable. Forward stepwise analysis included the following variables: 5-HTRP, MEL, 3IAA, TRYPT, TOL, L-TRP EE in the model. 5-Hydroxytryptamine and NA5-HT could not be included, as they were not detected in a sufficient number of samples. Classification rates of 100% were achieved. Figure 3 shows that samples were grouped according to cultivar using factors 1 and 3. This indicates the production of indolic compounds depends largely on the grape cultivar being fermented, in addition to the yeast strain.

Effect of yeast strain on the composition of Tempranillo must during fermentation

Tempranillo must was settled for 24 h and then inoculated with several yeast strains. Figure 4 shows the compositional changes observed during the five different fermentations carried out with Tempranillo must. Figure 4a shows an initial L-TRP concentration of 3492 ng/mL, which was considerably lower than the concentration previously reported for must of different red grape cultivars, being 9410–30 000 ng/mL (Table 1). This may reflect differences in soil type and climate, as previously suggested (Fernández-Marín et al. 2013). Compared with the results obtained, however, for the white grape cultivars (Figure 2a), the initial concentration of L-TRP was highest in Tempranillo must. The accumulation of L-TRP remained similar to that observed for the white must, that is significant consumption of L-TRP was observed between days 1 and 4, irrespective of the inoculation treatment; with negligible concentration detected from day 5. The same trend was previously observed in SM (Fernández-Cruz et al. 2017).

The initial concentration of 2.3 ng/mL and the accumulation trend of 5-HTRP (Figure 4b) were similar to those observed for white grape must (Figure 2b). All fermentations followed the same pattern, regardless of the yeast strain inoculated, even the spontaneous fermentation. As for L-TRP, 5-HTRP was exhausted during the early stages of AF, but it was not produced in either the white or Tempranillo grape musts. We reported the same trend for the occurrence of 5-HTRP in SM fermented with *S. saccharomyces* RF, QA23 and *T. delbrueckii* under laboratory conditions (Fernández-Cruz et al. 2017). These results show that use of the same yeast strains under

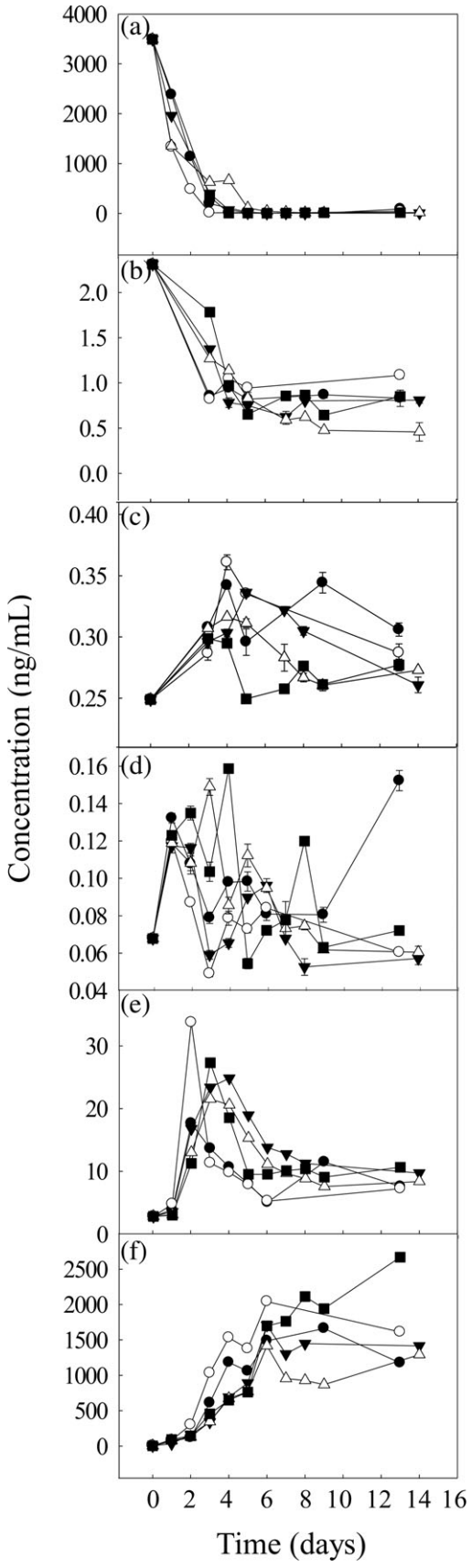

Figure 4. Effect of yeast on the concentration of (a) L-tryptophan, (b) 5-hydroxytryptophan, (c) melatonin, (d) L-tryptophan ethyl ester, (e) 3-indoleacetic acid and (f) tryptophol in Tempranillo grape must fermented with: *Saccharomyces cerevisiae* QA23 (●); *S. cerevisiae* Red Fruit (○); sequential inoculation with *Torulaspora delbrueckii* and *S. cerevisiae* QA23 (▼); sequential inoculation with *T. delbrueckii* and *S. cerevisiae* Red Fruit (△); spontaneous fermentation (■). Mean ± standard deviation (SD), values are means of six replicates (n = 6) ± SD.

either semi-industrial or laboratory conditions gave similar patterns of 5-HTRP consumption. Thus, it appears that the inoculation method does not significantly affect 5-HTRP consumption during AF, regardless of fermentation scale.

This is the first time, to our knowledge, that NA5-HT has been quantified during AF of Tempranillo must. The initial must contained 0.25 ng/mL NA5-HT at (Figure 4c). During AF, NA5-HT concentration peaked between days 3 and 4 and then decreased substantially until the end of AF, to give final concentration of 0.3 ng/mL. In an earlier study, we reported NA5-HT at a negligible concentration (0.1 ng/mL) at the start of fermentation in SM, with NA5-HT disappearing during AF (Fernández-Cruz et al. 2017). In commercial sparkling wines, such as Cava, Reserva, and Gran Reserva, NA5-HT has been quantified at a higher concentration of 0.3–2.0 ng/mL (Tudela et al. 2016).

Following the corresponding pathway (Figure 1) MEL is one of the final L-TRP metabolites. In this study, MEL was present in Tempranillo must at a low concentration, that is 0.07 ng/mL (Figure 4d). A considerably higher MEL concentration has been reported in red grape must after pressing, that is 74.1, 77.7, 241.2 and 322.7 ng/L for Cabernet Sauvignon, Tempranillo, Merlot and Tintilla de Rota, respectively (Rodriguez-Naranjo et al. 2011a). During AF,

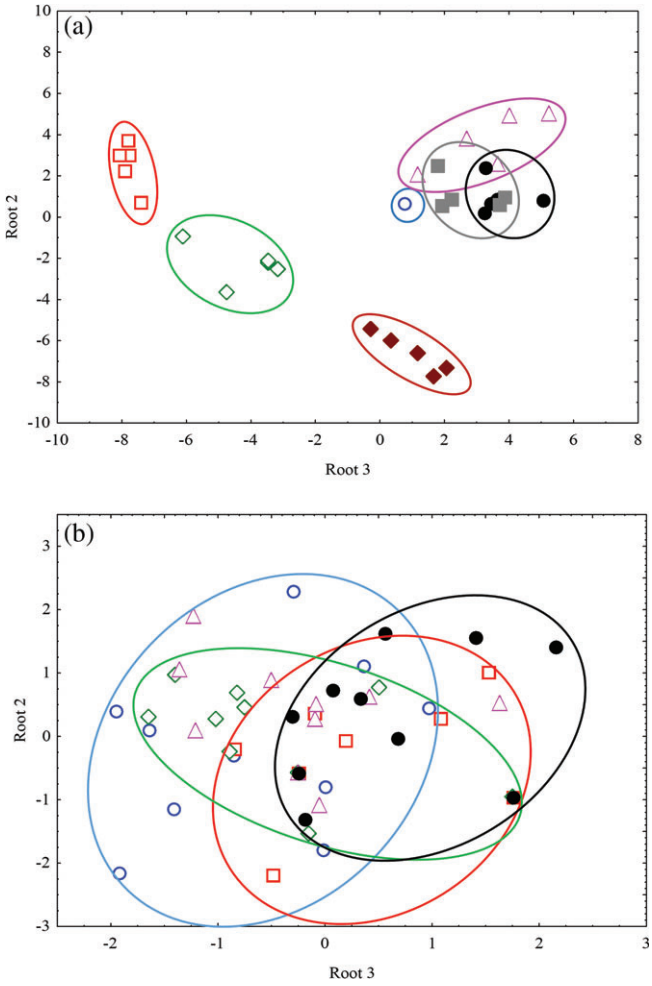

Figure 5. Linear discriminant analysis performed on must of the red grape cultivar Tempranillo fermented (a) with *Saccharomyces cerevisiae* QA23/Red Fruit (RF) at day 0 (○), day 1 (□), day 2 (◇), day 3 (△), day 4 (●), day 5 (■) and day 6 (◆) and (b) with inoculation of SP (○), CT-RF (□), CT-QA23 (◇), SI-RF (△) and SI-QA23 (●).

considerable variation in MEL was observed, similar to that described above during AF of white grape must (Figure 2c).

The concentration of 3-IAA in Tempranillo must (Figure 4e) also followed the trend described during AF of white grape must (Figure 2e). 3-Indole acetic acid was initially present at a concentration of 2.8 ng/mL, but the concentration subsequently increased during the first few days of fermentation, reaching a maximum (irrespective of inoculation treatment) between days 2 and 4. The concentration of 3-IAA then decreased significantly until the end of AF, with the final concentration ranging from 7.2 to 10.8 ng/mL for the CT-RF and SP samples, respectively. Similar trends were seen for SM fermented with RF and QA23 yeast strains (Fernández-Cruz et al. 2017).

The initial concentration of TOL in Tempranillo must (Figure 4f) was low, at 4.9 ng/mL, compared with the level (123 ng/mL) reported for Monastrell grape must (Bordiga et al. 2016). The concentration of TOL then increased significantly during AF, reaching a final concentration of between 1979 for CT-QA23 and 2668 ng/mL for SP.

L-Tryptophan ethyl ester was not detected in the initial Tempranillo must, but it accumulated in all fermentations, irrespective of inoculation treatment, from day 1 at a constant concentration (data not shown), with no change in concentration observed during AF (0.032–0.042 ng/mL).

Linear discriminant analysis was performed to compare the occurrence of indolic compounds as a function of both inoculation treatment and the duration of fermentation, that is, from the initial must (i.e. at day 0) to day 6. All the quantified compounds were included in the analysis. Figure 5 shows the plots obtained with a forward stepwise method in which 35 samples were analysed. Four groups, representing the initial must, and the must after 1, 5 and 6 days of fermentation, were clearly differentiated (Figure 5a). Samples collected on days 2–4, representing the midpoint of AF, formed one uniform group, with few noticeable differences. The greatest change was therefore observed during the early stages of AF, where L-TRP metabolism by yeast was more noticeable. In contrast, the production of indolic compounds during AF was not strongly influenced by the inoculation treatment (Figure 5b); that is separation of samples was achieved only when the duration of fermentation was the variable.

Conclusion

Must from several cultivars gave different profiles of the indolic compounds derived from the amino acid L-TRP. Although the presence of L-TRP in the initial must is essential, the occurrence of individual indolic compounds was not always related to the initial concentration of L-TRP. Changes in the concentration of 5-HTRP and NA5-HT, intermediates of the MEL pathway in yeast metabolism, were described for the first time during the AF of grape must fermented by different yeast strains. The accumulation of MEL and 3-IAA, compounds considered to have bioactivity potential, was also reported during AF, with an especially high concentration observed for Corredera. The higher alcohol, TOL, was the L-TRP metabolite present at the highest final concentration in all white fermentations, with L-TRP being the pathway preferred by yeast. In a common substrate such as Tempranillo must, inoculation treatments involving different yeast strains did not affect the occurrence of indolic compounds during AF. Their occurrence was, however, influenced by the duration of fermentation. Further studies are required to ascertain the combination of grape cultivar and inoculation strategy that optimises the concentration of indolic compounds so as to realise their potential bioactivity in wine.

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References

Arapitsas, P., Guella, G. and Mattivi, F. (2018) The impact of SO_2 on wine flavanols and indoles in relation to wine style and age. Scientific Reports **8**, 858.

Azzolini, M., Tosi, E., Lorenzini, M., Finato, F. and Zapparoli, G. (2014) Contribution to the aroma of white wines by controlled *Torulaspora delbrueckii* cultures in association with *Saccharomyces cerevisiae*. World Journal of Microbiology & Biotechnology **31**, 277–293.

Bell, S.J. and Henschke, P.A. (2005) Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research **11**, 242–295.

Bely, M., Stoeckle, P., Masneuf-Pomarède, I. and Dubourdieu, D. (2008) Impact of mixed *Torulaspora delbrueckii–Saccharomyces cerevisiae* culture on high-sugar fermentation. International Journal of Food Microbiology **122**, 312–320.

Blanco, P., Mirás-Avalos, J.M., Pereira, E. and Orriols, I. (2013) Fermentative aroma compounds and sensory profiles of Godello and Albariño wines as influenced by *Saccharomyces cerevisiae* yeast strains. Journal of the Science of Food and Agriculture **93**, 2849–2857.

Bonnefont-Rousselot, D. and Collin, F. (2010) Melatonin: action as antioxidant and potential applications in human disease and aging. Toxicology **278**, 55–67.

Bordiga, M., Lorenzo, C., Pardo, F., Salinas, M.R., Travaglia, F., Arlorio, M., Coïsson, J.D. and Garde-Cerdán, T. (2016) Factors influencing the formation of histaminol, hydroxytyrosol, tyrosol, and tryptophol in wine: temperature, alcoholic degree, and amino acids concentration. Food Chemistry **197**, 1038–1045.

Bouzas-Cid, Y., Falqué, E., Orriols, I. and Mirás-Avalos, J.M. (2018) Effects of irrigation over three years on the amino acid composition of Treixadura (*Vitis vinifera* L.) musts and wines, and on the aromatic composition and sensory profiles of its wines. Food Chemistry **240**, 707–716.

van, Breda, V., Jolly, N. and van, Wyk, J. (2013) Characterisation of commercial and natural *Torulaspora delbrueckii* wine yeast strains. International Journal of Food Microbiology **163**, 80–88.

Cerezo, A.B., Hornedo-Ortega, R., Álvarez-Fernández, M.A., Troncoso, A.M. and García-Parrilla, M.C. (2017) Inhibition of VEGF-induced VEGFR-2 activation and HUVEC migration by melatonin and other bioactive indolic compounds. Nutrients **9**(3), 249–265.

Contreras, A., Curtin, C. and Varela, C. (2014) Yeast population dynamics reveal a potential 'collaboration' between *Metschnikowia pulcherrima* and *Saccharomyces uvarum* for the production of reduced alcohol wines during Shiraz fermentation. Applied Microbiology and Biotechnology **99**, 1885–1895.

Cook, S.D., Nichols, D.S., Smith, J., Chourey, P.S., Mcadam, E.L., Quittenden, L. and Ross, J.J. (2016) Auxin biosynthesis: are the indole-3-acetic acid and phenylacetic acid biosynthesis pathways mirror images? Plant Physiology **171**, 1230–1241.

Dickinson, J.R. (2003) The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. Journal of Biological Chemistry **278**, 8028–8034.

Epstein, E. and Ludwig-Muller, J. (1993) Indole-3-butyric acid in plants: occurrence, synthesis, metabolism and transport. Physiologia Plantarum **88**, 382–389.

Favre, G., Peña-Neira, Á., Baldi, C., Hernández, N., Traverso, S., Gil, G. and González-Neves, G. (2014) Low molecular-weight phenols in Tannat wines made by alternative winemaking procedures. Food Chemistry **158**, 504–512.

Feng, X., Wang, M., Zhao, Y., Han, P. and Dai, Y. (2014) Melatonin from different fruit sources, functional roles, and analytical methods. Trends in Food Science & Technology **37**, 21–31.

Fernández-Cruz, E., Álvarez-Fernández, M.A., Valero, E., Troncoso, A.M. and García-Parrilla, M.C. (2016) Validation of an analytical method to determine melatonin and compounds related to L-tryptophan metabolism using UHPLC/HRMS. Food Analytical Methods **9**, 3327–3336.

Fernández-Cruz, E., Álvarez-Fernández, M.A., Valero, E., Troncoso, A.M. and García-Parrilla, M.C. (2017) Melatonin and derived tryptophan metabolites produced during alcoholic fermentation by different yeast strains. Food Chemistry **217**, 431–437.

Fernández-Marín, M.I., Guerrero, R.F., García-Parrilla, M.C., Puertas, B., Ramírez, P. and Cantos-Villar, E. (2013) Terroir and variety: two key factors for obtaining stilbene-enriched grapes. Journal of Food Composition and Analysis **31**, 191–198.

Fernández-Pachón, M.S., Medina, S., Herrero-Martín, G., Cerrillo, I., Berná, G., Escudero-López, B., Ferreres, F., Martín, F., García-Parrilla, M.C. and Gil-Izquierdo, A. (2014) Alcoholic fermentation induces melatonin synthesis in orange juice. Journal of Pineal Research **56**, 31–38.

Gardana, C., Iriti, M., Stuknyté, M., De Noni, I. and Simonetti, P. (2014) "Melatonin isomer" in wine is not an isomer of the melatonin but tryptophan-ethylester. Journal of Pineal Research **57**, 435–441.

Garde-Cerdán, T., Gutiérrez-Gamboa, G., Portu, J., Fernández-Fernández, J.I. and Gil-Muñoz, R. (2017) Impact of phenylalanine and urea applications to Tempranillo and Monastrell vineyards on grape amino acid content during two consecutive vintages. Food Research International **102**, 451–457.

Garde-Cerdán, T., Lorenzo, C., Lara, J.F., Pardo, F., Ancín-Azpilicueta, C. and Salinas, M.R. (2009) Study of the evolution of nitrogen compounds during grape ripening. Application to differentiate grape varieties and cultivated systems. Journal of Agricultural and Food Chemistry **57**, 2410–2419.

Gil-Agustí, M., Carda-Broch, S., Monferrer-Pons, L. and Esteve-Romero, J. (2007) Simultaneous determination of tyramine and tryptamine and their precursor amino acids by micellar liquid chromatography and pulsed amperometric detection in wines. Journal of Chromatography A **1156**, 288–295.

González, B., Vázquez, J., Morcillo-Parra, M.Á., Mas, A., Torija, M. J. and Beltran, G. (2018) The production of aromatic alcohols in non-*Saccharomyces* wine yeast is modulated by nutrient availability. Food Microbiology **74**, 64–74.

González-Royo, E., Pascual, O., Kontoudakis, N., Esteruelas, M., Esteve-Zarzoso, B., Mas, A., Canals, J.M. and Zamora, F. (2015) Oenological consequences of sequential inoculation with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii* or *Metschnikowia pulcherrima*) and *Saccharomyces cerevisiae* in base wine for sparkling wine production. European Food Research and Technology **240**, 999–1012.

González-Santamaría, R., Ruiz-González, R., Nonell, S., Garde-Cerdán, T. and Pérez-Álvarez, E.P. (2018) Influence of foliar riboflavin applications to vineyard on grape amino acid content. Food Chemistry **240**, 601–606.

Gutiérrez-Gamboa, G., Portu, J., López, R., Santamaría, P. and Garde-Cerdán, T. (2018b) Effects of a combination of elicitation and precursor feeding on grape amino acid composition through foliar applications to Garnacha vineyard. Food Chemistry **244**, 159–163.

Gutiérrez-Gamboa, G., Portu, J., Santamaría, P., López, R. and Garde-Cerdán, T. (2017) Effects on grape amino acid concentration through foliar application of three different elicitors. Food Research International **99**, 688–692.

Gutiérrez-Gamboa, G., Carrasco-Quiroz, M., Martínez-Gil, A.M., Pérez-Álvarez, E.P., Garde-Cerdán, T. and Moreno-Simunovic, Y. (2018a) Grape and wine amino acid composition from Carignan noir grapevines growing under rainfed conditions in the Maule Valley, Chile: effects of location and rootstock. Food Research International **105**, 344–352.

Hazelwood, L.A., Daran, J.-M., van, Maris, A.J.A., Pronk, J.T. and Dickinson, J.R. (2008) The Ehrlich pathway for fusel alcohol production: a century of research on *Saccharomyces cerevisiae* metabolism. Applied and Environmental Microbiology **74**, 2259–2266.

Herbert, P., Cabrita, M.J., Ratola, N., Laureano, O. and Alves, A. (2006) Relationship between biogenic amines and free amino acid contents of wines and musts from Alentejo (Portugal). Journal of Environmental Science and Health – Part B Pesticides, Food Contaminants, and Agricultural Wastes **41**, 1171–1186.

Hoenicke, K., Simat, T.J., Steinhart, H., Köhler, H.J. and Schwab, A. (2001) Determination of free and conjugated indole-3-acetic acid, tryptophan, and tryptophan metabolites in grape must and wine. Journal of Agriculture and Food Chemistry **49**, 5494–5501.

Hornedo-Ortega, R., Da Costa, G., Cerezo, A.B., Troncoso, A.M., Richard, T. and García-Parrilla, M.C. (2018) In vitro effects of serotonin, melatonin, and other related indole compounds on amyloid-β kinetics and neuroprotection. Molecular Nutrition and Food Research **62**, 1–12.

Iriti, M. (2009) Melatonin in grape, not just a myth, maybe a panacea. Journal of Pineal Research **46**, 353.

Jadhav, A., Liang, W., Balsevich, J., Bastin, G., Kroetsch, J., Heximer, S., Backx, P.H. and Gopalakrishnan, V. (2012) L-Tryptophan ethyl ester dilates small mesenteric arteries by inhibition of voltage-operated calcium channels in smooth muscle. British Journal of Pharmacology **166**, 232–242.

Lambrechts, M.G. and Pretorius, I.S. (2000) Yeast and its importance to wine aroma – a review. South African Journal of Enology and Viticulture **21**, 97–129.

Maldonado, M.D., Moreno, H. and Calvo, J.R. (2009) Melatonin present in beer contributes to increase the levels of melatonin and antioxidant capacity of the human serum. Clinical Nutrition **28**, 188–191.

Linsenmeier, A., Löhnertz, O. and Schubert, S. (2004) Effect of different N fertilization of vine on the tryptophan, free and total indole-3-acetic acid concentrations. Vitis **43**, 157–162.

Mas, A., Guillamon, J.M., Torija, M.J., Beltran, G., Cerezo, A.B., Troncoso, A.M. and García-Parrilla, M.C. (2014) Bioactive compounds derived from the yeast metabolism of aromatic amino acids during alcoholic fermentation. BioMed Research International **2014**, 1–7.

Maslov, L., Jeromel, A., Herjavec, S., Korenika, A.-M.J., Mihaljević, M. and Plavša, T. (2011) Indole-3-acetic acid and tryptophan in Istrian Malvasia grapes and wine. Journal of Food, Agriculture and Environment **9**, 29–33.

Mena, P., Gil-Izquierdo, Á., Moreno, D.A., Martí, N. and García-Viguera, C. (2012) Assessment of the melatonin production in pomegranate wines. LWT–Food Science and Technology **47**, 13–18.

Meng, N., Ren, Z.-Y., Yang, X.-F. and Pan, Q.-H. (2018) Effects of simple rain-shelter cultivation on fatty acid and amino acid accumulation in 'Chardonnay' grape berries. Journal of the Science of Food and Agriculture **98**, 1222–1231.

Mercolini, L., Mandrioli, R. and Raggi, M.A. (2012) Content of melatonin and other antioxidants in grape-related foodstuffs: measurement using a MEPS-HPLC-F method. Journal of Pineal Research **53**, 21–28.

Mihaljević Žulj, M., Tomaz, I., Bandić, L.M., Puhelek, I., Korenika, A.M.J. and Jeromel, A. (2015) Influence of different yeast strains on metabolism of tryptophan and indole-3-acetic acid during fermentation. South African Journal of Enology and Viticulture **36**, 44–49.

Moreno, D., Vilanova, M., Gamero, E., Intrigliolo, D.S., Talaverano, M.I., Uriarte, D. and Valdés, M.E. (2015) Effects of preflowering leaf removal on phenolic composition of Tempranillo in the semiarid terroir of Western Spain. American Journal of Enology and Viticulture **66**, 204–211.

Muñíz-Calvo, S., Guillamón, J.M., Domínguez, I. and Doménech-Carbó, A. (2017) Detecting and monitoring the production of melatonin and other related indole compounds in different *Saccharomyces* strains by solid-state electrochemical techniques. Food Analytical Methods **10**, 1408–1418.

Murch, S.J., KrishnaRaj, S. and Saxena, P.K. (2000) Tryptophan is a precursor for melatonin and serotonin biosynthesis in in vitro regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. Plant Cell Reports **19**, 698–704.

Nutaratat, P., Srisuk, N., Arunrattiyakorn, P. and Limtong, S. (2016) Indole-3-acetic acid biosynthetic pathways in the basidiomycetous yeast *Rhodosporidium paludigenum*. Archives of Microbiology **198**, 429–437.

Palego, L., Betti, L., Rossi, A. and Giannaccini, G. (2016) Tryptophan biochemistry: structural, nutritional, metabolic, and medical aspects in humans. Journal of Amino Acids **2016**, 1–13.

Park, M., Kang, K., Park, S. and Back, K. (2008) Conversion of 5-hydroxytryptophan into serotonin by tryptophan decarboxylase in plants, *Escherichia coli*, and yeast. Bioscience, Biotechnology, and Biochemistry **72**, 2456–2458.

Pérez-Álvarez, E.P., Garde-Cerdán, T., García-Escudero, E. and Martínez-Vidaurre, J.M. (2017) Effect of two doses of urea foliar application on leaves and grape nitrogen composition during two

vintages. Journal of the Science of Food and Agriculture **97**, 2524–2532.
Puertas, B., Jiménez, M.J., Cantos-Villar, E., Cantoral, J.M. and Rodríguez, M.E. (2017) Use of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* in semi-industrial sequential inoculation to improve quality of Palomino and Chardonnay wines in warm climates. Journal of Applied Microbiology **122**, 733–746.
Renault, P., Coulon, J., de, Revel, G., Barbe, J.C. and Bely, M. (2015) Increase of fruity aroma during mixed *T. delbrueckii*/*S. cerevisiae* wine fermentation is linked to specific esters enhancement. International Journal of Food Microbiology **207**, 40–48.
Renault, P., Miot-Sertier, C., Marullo, P., Hernández-Orte, P., Lagarrigue, L., Lonvaud-Funel, A. and Bely, M. (2009) Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: potential applications in the wine industry. International Journal of Food Microbiology **134**, 201–210.
Riga, P., Medina, S., García-Flores, L.A. and Gil-Izquierdo, A. (2014) Melatonin content of pepper and tomato fruits: effects of cultivar and solar radiation. Food Chemistry **156**, 347–352.
Rodriguez-Naranjo, M.I., Gil-Izquierdo, A., Troncoso, A.M., Cantos-Villar, E. and García-Parrilla, M.C. (2011a) Melatonin is synthesised by yeast during alcoholic fermentation in wines. Food Chemistry **126**, 1608–1613.
Rodriguez-Naranjo, M.I., Gil-Izquierdo, A., Troncoso, A.M., Cantos, E. and García-Parrilla, M.C. (2011b) Melatonin: a new bioactive compound in wine. Journal of Food Composition and Analysis **24**, 603–608.
Rodríguez-Naranjo, M.I., Torija, M.J., Mas, A., Cantos-Villar, E. and García-Parrilla, M.C. (2012) Production of melatonin by *Saccharomyces* strains under growth and fermentation conditions. Journal of Pineal Research **53**, 219–224.
Ruiz-Rodríguez, A., Carrera, C.A., Setyaningsih, W., Barbero, G.F., Ferreiro-González, M., Palma, M. and Barroso, C.G. (2017) Tryptophan levels during grape ripening: effects of cultural practices. Molecules **22**, 1–9.
Simat, T., Hoenicke, K., Gessner, M. and Christoph, N. (2004) Metabolism of tryptophan and indole-3-acetic acid formation during vinification and its influence on the formation of 2-aminoacetophenone. Mitteilungen Klosterneuburg, Rebe und Wein. Obstbau und Fruchteverwertung **54**(1/2), 43–55.
Spaepen, S., Vanderleyden, J. and Remans, R. (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews **31**, 425–448.
Sprenger, J., Hardeland, R., Fuhrberg, B. and Han, S. (1999) Melatonin and other 5-methoxylated indoles in yeast: presence in high concentrations and dependence on tryptophan availability. Cytologia **64**(2), 209–213.
Stege, P.W., Sombra, L.L., Messina, G., Martinez, L.D. and Silva, M. F. (2010) Determination of melatonin in wine and plant extracts by capillary electrochromatography with immobilized carboxylic multi-walled carbon nanotubes as stationary phase. Electrophoresis **31**, 2242–2248.
Stürtz, M., Cerezo, A.B., Cantos-Villar, E. and García-Parrilla, M.C. (2011) Determination of the melatonin content of different varieties of tomatoes (*Lycopersicon esculentum*) and strawberries (*Fragaria ananassa*). Food Chemistry **127**, 1329–1334.
Suárez-Lepe, J.A. and Morata, A. (2012) New trends in yeast selection for winemaking. Trends in Food Science & Technology **23**, 39–50.
Tan, D.-X., Hardeland, R., Back, K., Manchester, L.C., Alatorre-Jimenez, M.A. and Reiter, R.J. (2016) On the significance of an alternate pathway of melatonin synthesis via 5-methoxytryptamine : comparisons across species. Journal of Pineal Research **61**, 27–40.
Tudela, R., Ribas-Agustí, A., Buxaderas, S., Riu-Aumatell, M., Castellari, M. and López-Tamames, E. (2016) Ultrahigh-performance liquid chromatography (UHPLC) tandem mass spectrometry (MS/MS) quantification of nine target indoles in sparkling wines. Journal of Agricultural and Food Chemistry **64**, 4772–4776.
Ünal, M.Ü., Şener, A., Şen, K. and Yilmaztekin, M. (2015) Seasonal variation in amino acid and phenolic compound profiles of three Turkish white wine grapes. Turkish Journal of Agriculture and Forestry **39**, 984–991.
Uriarte, D., Sebastiano, D., Alberto, L., Valdés, E., Gamero, E. and Henar, M. (2016) Combined effects of irrigation regimes and crop load on 'Tempranillo' grape composition. Agricultural Water Management **165**, 97–107.
Vigentini, I., Gardana, C., Fracassetti, D., Gabrielli, M., Foschino, R., Simonetti, P., Tirelli, A. and Iriti, M. (2015) Yeast contribution to melatonin, melatonin isomers and tryptophan ethyl ester during alcoholic fermentation of grape musts. Journal of Pineal Research **58**, 388–396.
Yılmaz, C., Kocadağlı, T. and Gökmen, V. (2014) Formation of melatonin and its isomer during bread dough fermentation and effect of baking. Journal of Agricultural and Food Chemistry **62**, 2900–2905.
Yu, P., Hegeman, A.D. and Cohen, J.D. (2014) A facile means for the identification of indolic compounds from plant tissues. The Plant Journal **79**, 1065–1075.
Zhao, Y., Tan, D.-X., Lei, Q., Chen, H., Wang, L., Li, Q., Gao, Y. and Kong, J. (2013) Melatonin and its potential biological functions in the fruits of sweet cherry. Journal of Pineal Research **55**, 79–88.

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Supporting information

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Figure S1. Sugar consumption during alcoholic fermentation of must from (a) the white grape cultivars Corredera (●), Chardonnay (■), Moscatel (▲), Palomino Fino (▼), Sauvignon Blanc (◆) and Vijiriega (□) and from (b) Tempranillo following inoculation with *Saccharomyces cerevisiae* QA23 (◆) and *S. cerevisiae* Red Fruit (RF) (□), sequential inoculation with *Torulaspora delbrueckii* and *S. cerevisiae* QA23 (■) and with *T. delbrueckii* and *S. cerevisiae* RF (●), and spontaneous fermentation (▲).

Table S1. Effect of grape cultivar on the duration of alcoholic fermentation and residual sugar concentration at the end of fermentation.

Table S2. Retention time, protonated ion, main fragment, theoretical fragment, relative intensity, formula and error of the different indolic compounds analysed during the alcoholic fermentation.