

# Revisiting the role of glycosidic aroma precursors on wine aroma: Effects of microorganisms and of slow hydrolytical processes

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## Abstract

Grapes are determinant for the quality of the final wine since they not only provide a specific profile of nutrients which strongly determines the production of secondary metabolites by yeast, but they provide precursors of key aroma components. Glycosidic precursors were the first category of aroma precursors discovered and for long it has been known that they constitute the main source of relevant wine aroma molecules such as linalool and  $\beta$ -damascenone, but also main sources of some potential off-flavours such as 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN).

In spite of their known importance, their role in wine aroma formation is not completely understood due to the complexity of their genesis. Difficulties arise because many aglycones undergo different chemical rearrangements to produce the aroma molecule.

In order to better understand the role of yeast and of aging two large fractions of precursors from highest quality Garnacha grapes were obtained by SPE, and used to prepare model musts. The musts have been fermented by different yeasts and resulting wines have been subjected to accelerated aging under strict anoxic conditions. Analytical controls have been included all throughout the process in order to ensure an efficient control of the mass balance.

Results show that aromas formed from different grape varieties and in combination with different microorganisms lead to high aroma diversity. Besides, this study has allowed to differentiate the aroma formation influenced by enzymatic or hydrolytic activity, as well as their evolution during bottle aging, enlightening the principal formation mechanism and the fate of these aromas during the shelf-life of wines.

## Introduction

Most wine grapes are aromatically neutral, nonetheless, they are important providers of aroma precursors that can be released during wine making and wine aging. Different families of odorants can be formed from precursors such as polyols by chemical rearrangements, glycosides by enzymatic or acidic hydrolysis, or cysteinyl derivatives by the action of yeast  $\beta$ -lyases. Glycosides are related with the genesis of important aroma volatiles, some of them are considered as varietal wine aroma compounds [1][2]. Since different strains of *Saccharomyces* contain different types and activities of glycosidases, some specific strains have been proposed as having abilities to enhance the varietal aroma of wines made of grapes from a single variety. Moreover, different *genera* of yeast can lead to very different fermentative outcomes due to their diverse genetic pool and that is why the usage of non-*Saccharomyces* yeast strains as enhancers of the organoleptic properties of wine is gaining more and more attention [3][4].

The processes that lead to the release of aroma from non-floral grape precursors by yeast is not yet fully understood, since large amount of reactions and interconversions

take place during this stage of winemaking. Particularly, the bound fraction of grape glycosides can be source of several families of odorants, such as monoterpenes, volatile phenols, C<sub>13</sub>-norisoprenoids or vanillin derivatives. These compounds are very important to establish the varietal character of wines and some can be very powerful odorants, even at small concentrations. While the release of some compounds can occur by direct enzymatic or acidic hydrolysis of the glycosylated bound between the glycone (sugar moieties) and the aglycone of volatile aroma compounds, others require more complex reactions or even chemical rearrangements. In addition, wine aging can also be determinant to the appearance of certain odours, positive or negative, which can further contribute to the development of the varietal character of wine [1][2].

The present research intends to further investigate the effects of yeast (*S. cerevisiae* and non-*Saccharomyce*) on the formation of aroma compounds derived from glycosidic precursors taking into account aging time.

## Experimental

Glycosidic precursors fraction was extracted from 23 kg of Garnacha grapes from Spain. After grape crushing and addition of SO<sub>2</sub> (5 mg/kg) and pectolitic enzymes (Lafazym, 127 mg/kg), cold maceration took place during 48 hours inside a closed recipient. Grapes were then pressed and the liquid obtained was sulfited (90 mg/L) and let to sediment at 4°C for 24hours, after which the clean must was further filtered, divided into two 5L-batches and sulfited again (90 mg/L). Five grams of conditioned LiChrolut-EN resins were added to each batch and kept under magnetic stirring for 48 hours at 10°C. Resins were further recovered using paper filter, washed with water and re-packed into beds. Free aroma compounds were washed out with 45ml of DCM and the glycosidic fraction was eluted with 90ml of Ethyl acetate-methanol (95:5, v/v). The extracted must was further sulfited (50 mg/L) and re-extracted with a second 5g-batch of clean resins, which were similarly processed to obtain the glycosidic fraction which was collected with the previous one and evaporated to dryness under Nitrogen.

A complex synthetic grape must with pH 3.5 containing oligoelements, vitamins, glucose, fructose, Tween, and amino acids imitating Garnacha grapes was prepared under aseptic conditions. Three-hundred and fifty mL volumes of synthetic must, containing or not glycosidic precursors were inoculated with non-*Saccharomyces* yeast strains at day 0 and with *S. cerevisiae* after 4 days. Control samples fermented only with *S. cerevisiae* were also prepared. The strains used were *Pichia kluyveri* (Frootzen), *Lachancea thermotolerans* (Concerto), *Torulaspota delbruekii* (Prelude), all from Chr. Hansen. Air locks were used to seal the fermenters and fermentation was carried out at 21°C.

Once the fermentation was over, the wines were centrifuged, introduced into an anoxic chamber, aliquoted into three air tight tubes and further bagged in high density plastic bags containing oxygen scavengers. The tubes were subjected to anoxic accelerated aging at 50°C for 1, 2 and 5 weeks.

Resulting wine samples were characterized according to their general enological parameters; major volatiles were analysed by liquid-liquid microextraction followed by a GC-FID analysis [5], and minor volatiles were isolated by SPE and determined by GC-MS [6].

## Results and discussion

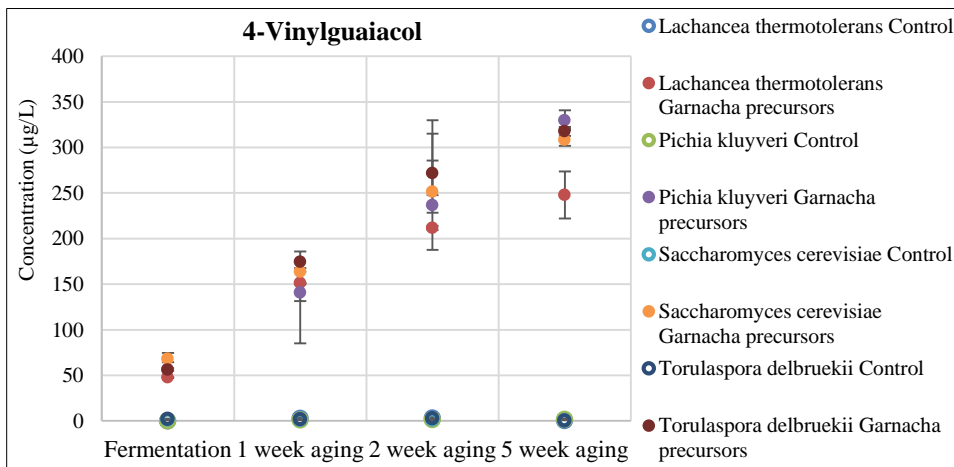
The effects of the presence of glycosidic precursors on the aroma profile were assessed by comparing the odorant profiles (paired t-test) of corresponding ferments with

or without precursor fractions. Overall, the presence of glycosidic precursors caused significant increases in the levels of 28 compounds, 9 of which were above or close to the odour threshold. Eight out of the 28 compounds were in fact fermentative compounds, which suggest that the precursor fraction –in synthetic must- exert a general effect on the secondary metabolism of yeast.

**Table 1:** Aroma compounds whose levels significantly increase in the presence of glycosidic precursors. Compounds marked with \* are at levels above or close to the odour threshold.

<i>Varietal odorants</i>	
<i>Monoterpenols</i>	Linalool, $\alpha$ -Terpineol, Geraniol*
<i>Norisoprenoids</i>	$\beta$ -Damascenone*
<i>Volatile Phenols</i>	4-Vinylphenol, E-Isoeugenol*, Eugenol, 4-Vinylguaiacol*, Guaiacol*, 2,6-Dimethoxyphenol, 4-Ethylguaiacol, 4-Allyl-2,6-dimethoxyphenol
<i>Lactones</i>	$\gamma$ -Nonalactone, $\gamma$ -Butyrolactone
<i>Vanillin derivatives</i>	Methyl vanillate, Ethyl vanillate, Acetovanillone, Syringaldehyde, Vanillin
<i>Cinnamates</i>	Ethyl dihydrocinnamate*
<i>Fermentative odorants</i>	
<i>Higher alcohols</i>	1-Hexanol, Benzyl alcohol, Methionol*
<i>Ethyl esters</i>	Ethyl hexanoate*, Ethyl octanoate*, Ethyl decanoate*
<i>Acetate esters</i>	Butyl acetate
<i>Carbonyl compounds</i>	Acetoine

In order to further investigate the role of the different strains of yeasts and the effects of accelerated wine aging on the formation of these aroma compounds a two-way ANOVA was carried out. As summarized in Figure 1 for the particular case of 4-vinylguaiacol, the effects of the precursor fraction increased with aging time and were just slightly dependent on the strain of yeast which conducted the fermentation, in apparent disagreement with previous results [3][4].



**Figure 1:** 4-Vinylguaiacol content in fermentations carried out by *L. thermotolerans*, *P. kluyveri* and *T. delbrueckii*, sequentially inoculated with *S. cerevisiae* and *S. cerevisiae*, individually inoculated (yeast control) in control must and in musts spiked with glycosidic precursors fraction. Samples were analysed at the end of fermentation and after 1, 2 and 5 weeks of accelerated wine aging under strict anoxia conditions.

Two relevant exceptions to this general pattern of low yeast dependence were the cases of ethyl dihydrocinnamate and of geraniol, for which levels were significantly influenced by the strain of yeast. In the cases of ethyl dihydrocinnamate, *T. delbruekii* seems to be able to produce this compound at relatively large levels regardless of the presence of glycosidic precursors.

By contrast, wines made with the other yeasts contained low levels of this important aroma compound, just slightly higher in samples fermented in the presence of glycosidic precursors.

The case of geraniol was still more challenging. In wines obtained from *L. thermotolerans* and *T. delbruekii*, highest levels of this compound were observed by the end of fermentation in those samples containing glycosidic precursors. Levels of this unstable compound later decreased throughout aging. Contrarily, wines fermented with *P. kluyveri* and *S. cerevisiae* did not contain any geraniol by the end of fermentation, but its levels later increased during aging, regardless of the presence of precursors. Again, this suggests that both yeasts are able to form *de novo* a precursor of this odorant.

In conclusion, while this research confirms that the glycosidic aroma precursor fraction has an important effect on the levels of many wine aroma components, it also revealed that the effects of the yeast carrying out the first step of fermentation on the levels of most aromatic aglycones were surprisingly low. In contrast, some yeasts showed a specific activity to form *de novo* aroma molecules or aroma precursors. All this suggests that the different role played by yeast are more related to their specific secondary metabolism and not to their differential glycosidase activities.

## References

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