IDENTIFICATION OF γ-NONALACTONE PRECURSOR IN MERLOT AND CABERNET SAUVIGNON GRAPES

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INTRODUCTION

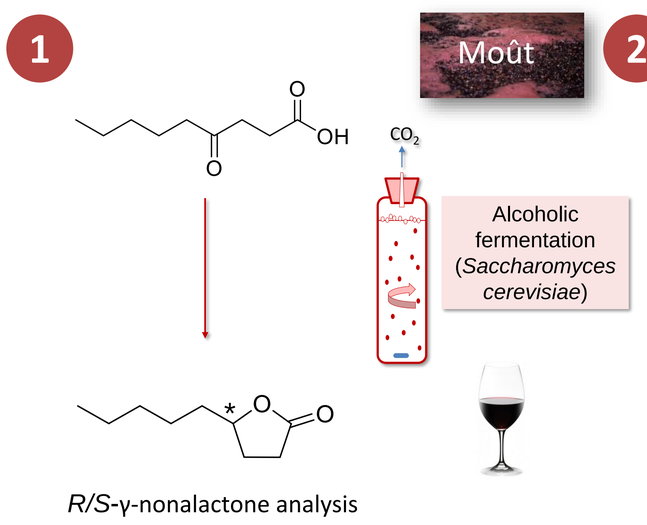
The fruity flavor of cooked apricot and peach, dried fig and prune, characteristic of grapes and red wines resulting from hot vintages, was recently associated with the high content of varietal lactones, especially (*R/S*)-γ-nonalactone.[1] With a detection threshold of 27 µg/L and wine concentrations ranged between 1.5 µg/L to more than 64 µg/L, this lactone was one of the most important contributors of these aromatic nuances of many grape cultivars, like Shiraz, Pinot, Grenache, and recently in Merlot and Cabernet Sauvignon.[2,3] Despite of several agronomical factors known to influence γ-nonalactone content in wine, such as the over ripening leading to berries shriveling or necrosis[4], little is known about its formation pathways in grapes and wines.

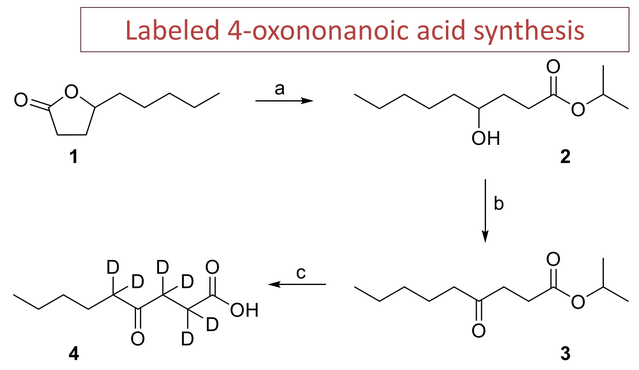
This project aims to identify γ-nonalactone formation pathways in red wines. Our study is based on previous work in beer hypothesizing 4-oxononanoic acid biotransformation to γ-nonalactone during alcoholic fermentation (*S. cerevisiae).*[5] This hypothesis was extended to study the occurrence of 4-oxononanoic acid in Bordeaux *V. Vinifera* red cultivar musts. Once this keto acid was identified and quantified, its biotransformation by *S. cerevisiae* into (*R*)-γ-nonalactone and (*S*)-γ-nonalactone was subsequently studied.

MATERIAL AND METHODS

Analysis and Quantification

	4-oxononanoic acid	γ-nonalactone	*R/S*-γ-nonalactone
Extraction Protocol	Solid Phase Extraction		
Instrument	LC-MS/MS	GC-MS/MS	GC-MS
Ionisation	ESI	EI	NCI
Column	C18 100mm×2.1mm ×5µm	WAX 60m×0.25mm×0.25 µm	Chiral (acetyl *tert*-butylsilyl-β-cyclodextrin) 25m×0.25mm×0.25 µm
Detection	SRM	SRM	SIM
Quantification ion (*m/z*)	173 ->137	85 -> 57	155

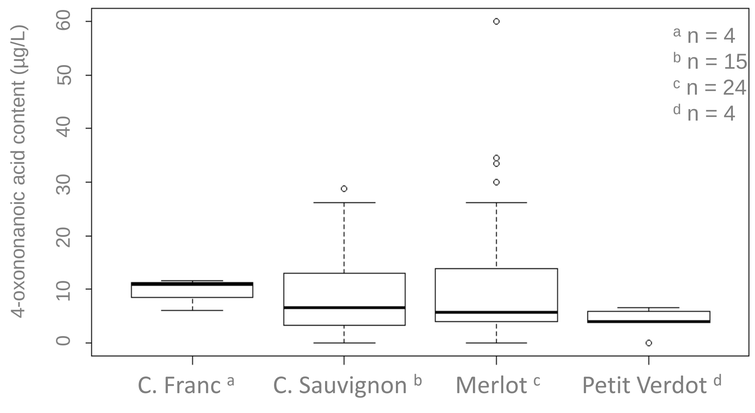




Ferron *et al.*, *J. Agric. Food Chem.*, **2020**, 68, 13344

RESULTS AND DISCUSSION

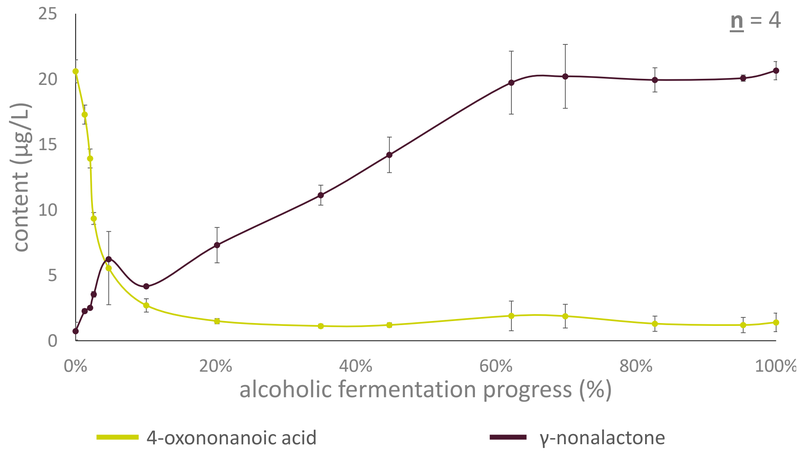
1 Quantification of 4-oxononanoic acid in musts from Bordeaux



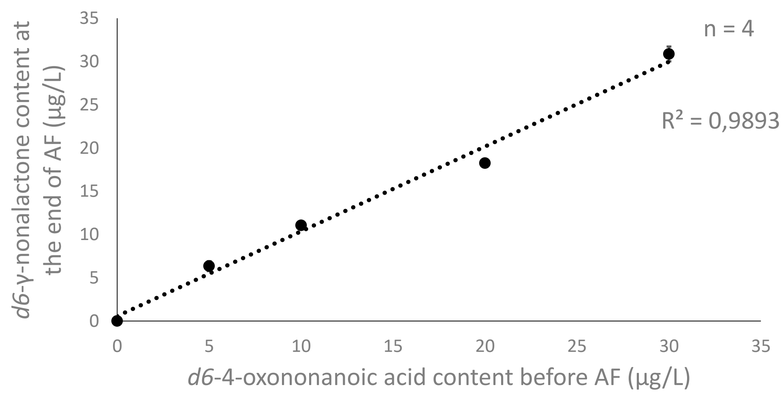
- First identification and quantification of 4-oxononanoic acid in must
- Highest 4-Oxononanoic acid concentrations were 60 µg/L in Merlot.

2 Impact of alcoholic fermentation on the formation of γ-nonalactone from 4-oxononanoic acid

Monitoring during alcoholic fermentation progress



d6-4-Oxononanoic acid biotransformation into d6-γ-nonalactone



→ **4-oxonanoic acid is biotransformed into γ-nonalactone by *S. cerevisiae* during alcoholic fermentation**

→ **Using deuteured analogs confirms that 4-oxononanoic acid is a precursor of γ-nonalactone**

3 Distribution of γ-nonalactone enantiomers in red wines

Samples	Varieties	Vintage	(*R*) (%)	(*S*) (%)
Wine	Merlot	2010	60	40
	C. Sauvignon	2014	62	38
	Merlot	2010	67	33
	C. Sauvignon	2012	67	33
	Merlot	2019	70	30

4 Impact of alcoholic fermentation on the enantioselective biotransformation of 4-oxononanoic acid into (*R*)-γ-nonalactone

Modalities	γ-nonalactone (*R/S*) (%)	RSD (%)
Grape juice Control	**77/23**	6.1
Grape juice + 60 µg/L [a]	**94/6**	2.6
Synthetic must + 60 µg/L [a]	**99/1**	0.06

[a] samples spiked with 60 µg/L of 4-oxononanoic acid

→ **In wine (*R/S*)-γ-nonalactone ratio is approximately 60/40**

→ **The biotransformation of 4-oxononanoic acid into γ-nonalactone is *R*-enantioselective**

→ **Considering γ-nonalactone *R/S* ratio found in wine, this result is sheding light on other formation pathways**

CONCLUSION

4-Oxononanoic acid has been identified and quantified for the first time in musts. Thanks to its monitoring during alcoholic fermentation (*S. cerevisiae*) we demonstrated that yeast enzyme activities were able to reduce and cyclize this keto acid into γ-nonalactone. We confirm that 4-oxononanoic acid is a precursor of γ-nonalactone. However, enantioselective analysis of (*R/S*)-γ-nonalactone demonstrated that 4-oxononanoic acid biotransformation is responsible for *R*-form formation. Other formation pathways of γ-nonalactone, particularly in favor to *S*-form, should be considered and explored.

To learn more: de Ferron. P. *et al*. Aromatic Potential of Bordeaux Grape Cultivars: identification and assays on 4-oxononanoic acid, a γ-nonalactone precursor. *J. Agric. Food Chem*., **2020**, 68, 13344-13352

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2 – Czerny *et al., Eur. Food Res. Technol.* 2008, 228(2), p. 265-273
3 – Allamy, *Food Chem*, 2018, 266, p. 245-263
4 – Suklje *et al., J. Agric. Food Chem*., 2016, 64(4), p. 870-880
5 – Tressl *et al., Biogeneration of aroma*, 1986, 10, p. 114-113