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Rafael Jimenez-Lorenzo, Pascale Brial, Marc Perez, Christian Picou, Vincent Farines, Audrey Bloem, Carole Camarasa

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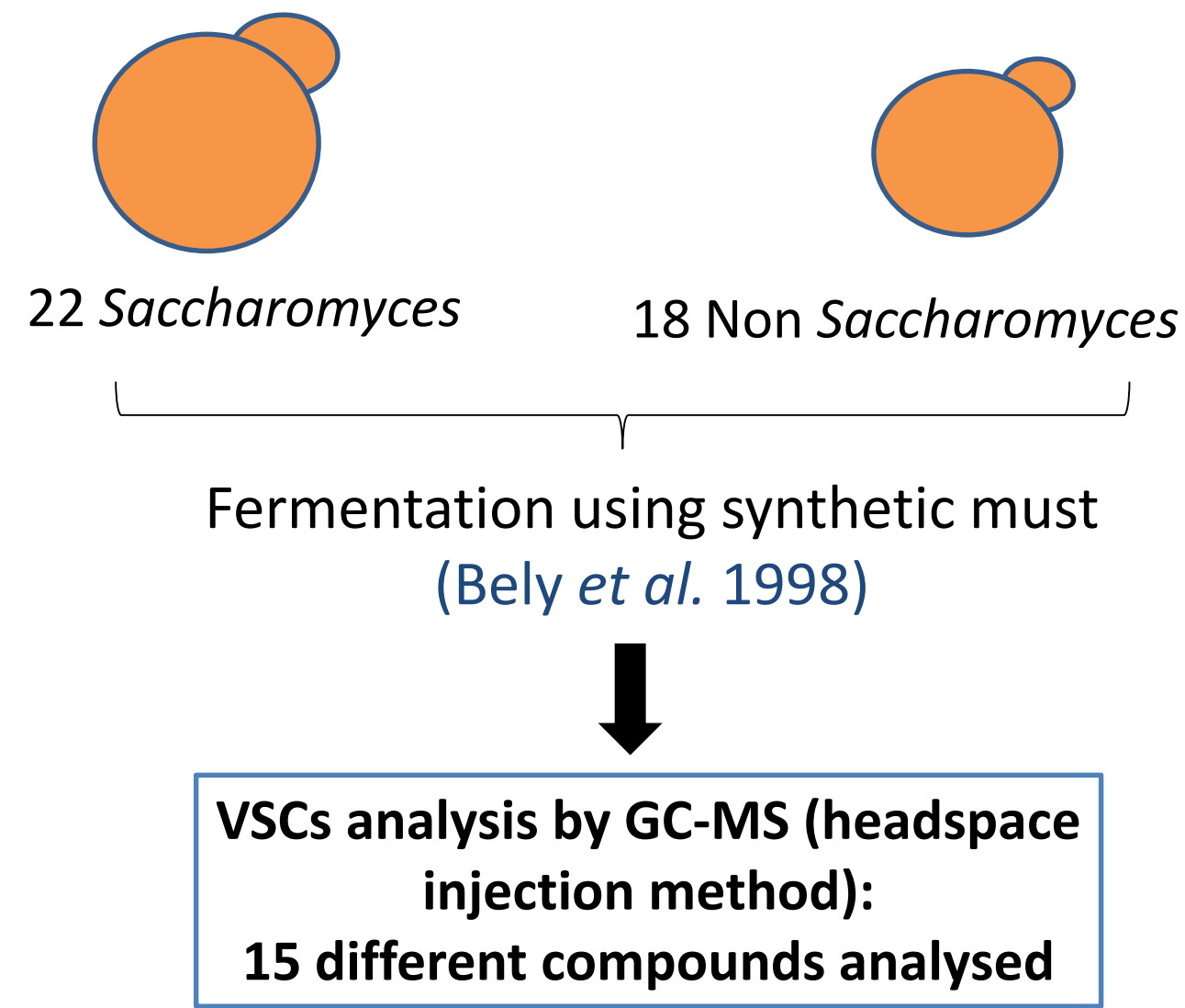
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# Environmental conditions can modulate the production of volatile sulfur compounds (VSC) during wine fermentation

JIMÉNEZ LORENZO R<sup>1</sup>, BRIAL P<sup>1</sup>, PEREZ M.<sup>1</sup>, PICOU C<sup>1</sup>, FARINES V<sup>1</sup>, BLOEM A<sup>1</sup>, CAMARASA C<sup>1</sup>.

In the increasingly competitive worldwide market of wines, a current challenge is to offer products with style and character to meet the consumer's expectations. In this context, controlling the production of volatile compounds during the fermentation, which may have a positive or negative incidence on the wine aroma, is essential. This project focuses on the understanding of the formation of volatile sulfur compounds (VSCs) deriving from yeast metabolism and described as unpleasant aromas in wines. The yeast metabolic network underlying the synthesis of these molecules remains until now poorly elucidated as well as the incidence on the activity of these pathways of many environmental and technological parameters including pH, sugars, sugar concentration, nitrogen resource composition and availability or SO<sub>2</sub> addition.

## Screening of 40 different strains on the basis of VSCs production



## Materials and Methods

Selection of strains displaying a high capacity to produce VSCs

- 3 levels of :
- sugar (glucose + fructose)
  - pH
  - SO<sub>2</sub>

### Box-Behnken design:

- 15 fermentations with central point in triplicate
- Determination of the effects of parameters on VSC production

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{ij} X_i X_j$$

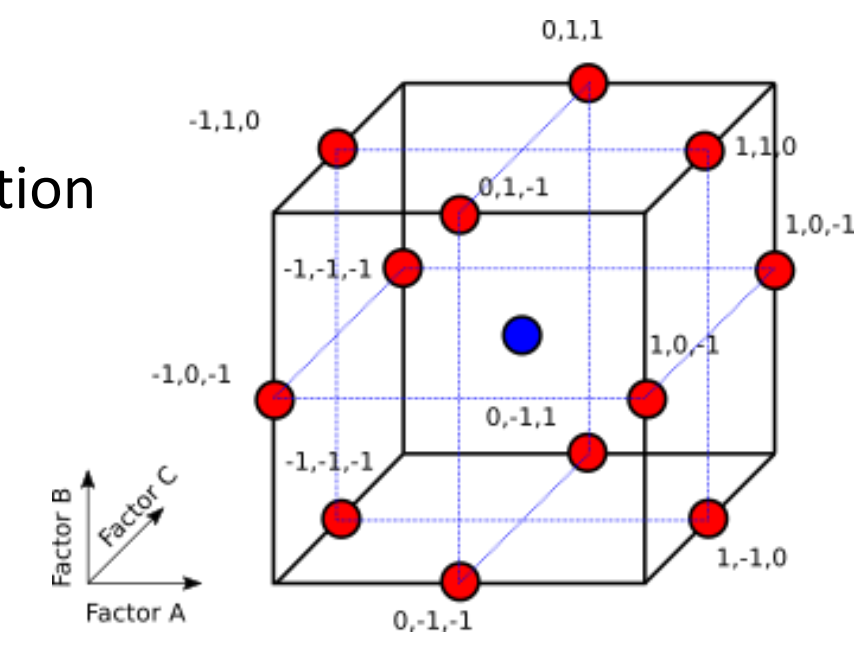
Simple effect

Quadratic effect

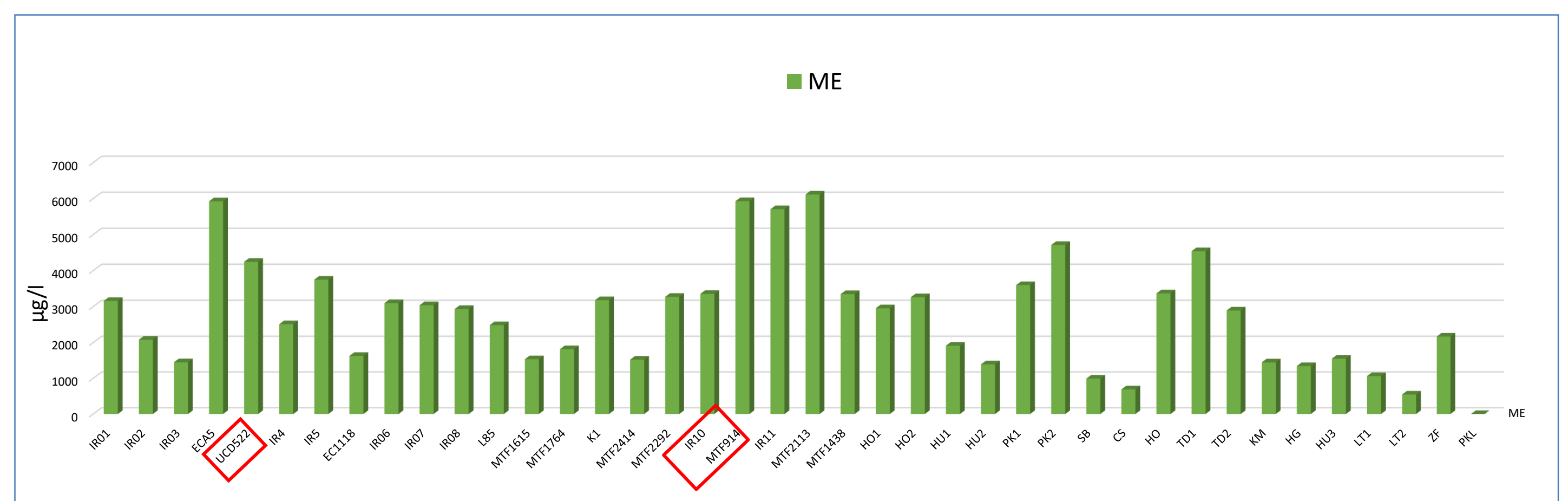
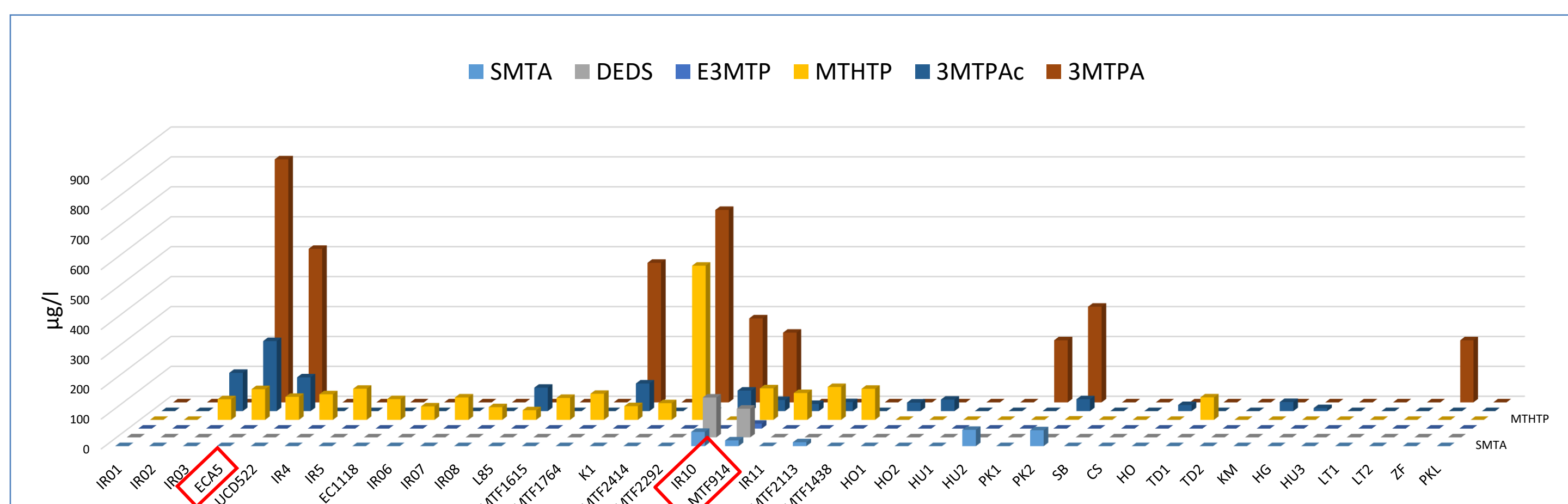
Interactions

## Incidence of environmental parameters

Variables Levels	Sugar (g/L)	pH	SO <sub>2</sub> (mg/L)
-1	180	3	30
0	210	3.2	45
+1	240	3.4	60



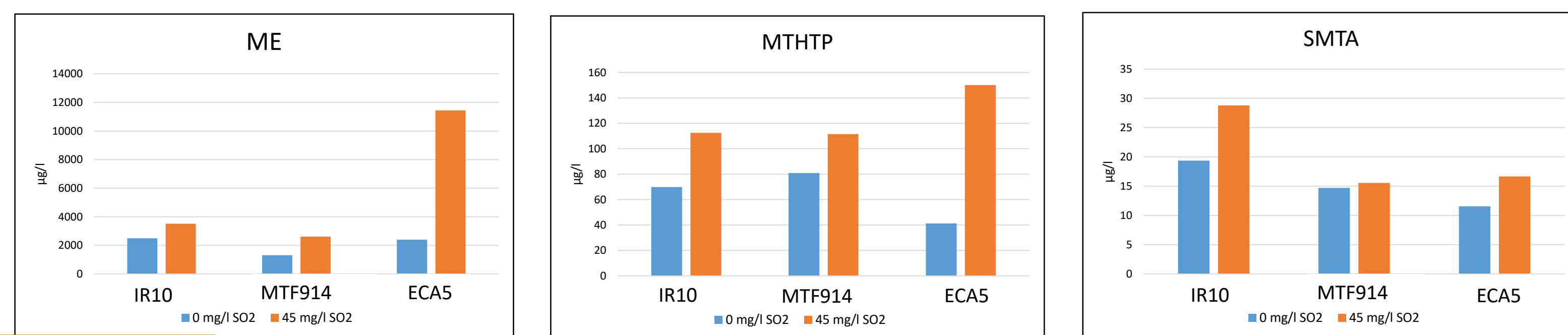
## Screening of wine yeast collection to select high VSCs producers



SMTA: S-methylthioacetate; DEDS: Diethyl disulfide; E3MTP: 3-Methylthio propanoic acid ethyl ester; MTHTP: 2-methyltetrahydrothiophenone; 3MTPAc: 3-Methylthio propyl acetate; 3MTPA: 3-Methylthio propanoic acid; ME: methionol

- Substantial differences between strains in their capacity to produce VSCs, with in general, lower productions by non-Saccharomyces strains
- Most of the molecules produced by a limited number of strains, apart from methionol, produced by all the studied yeasts

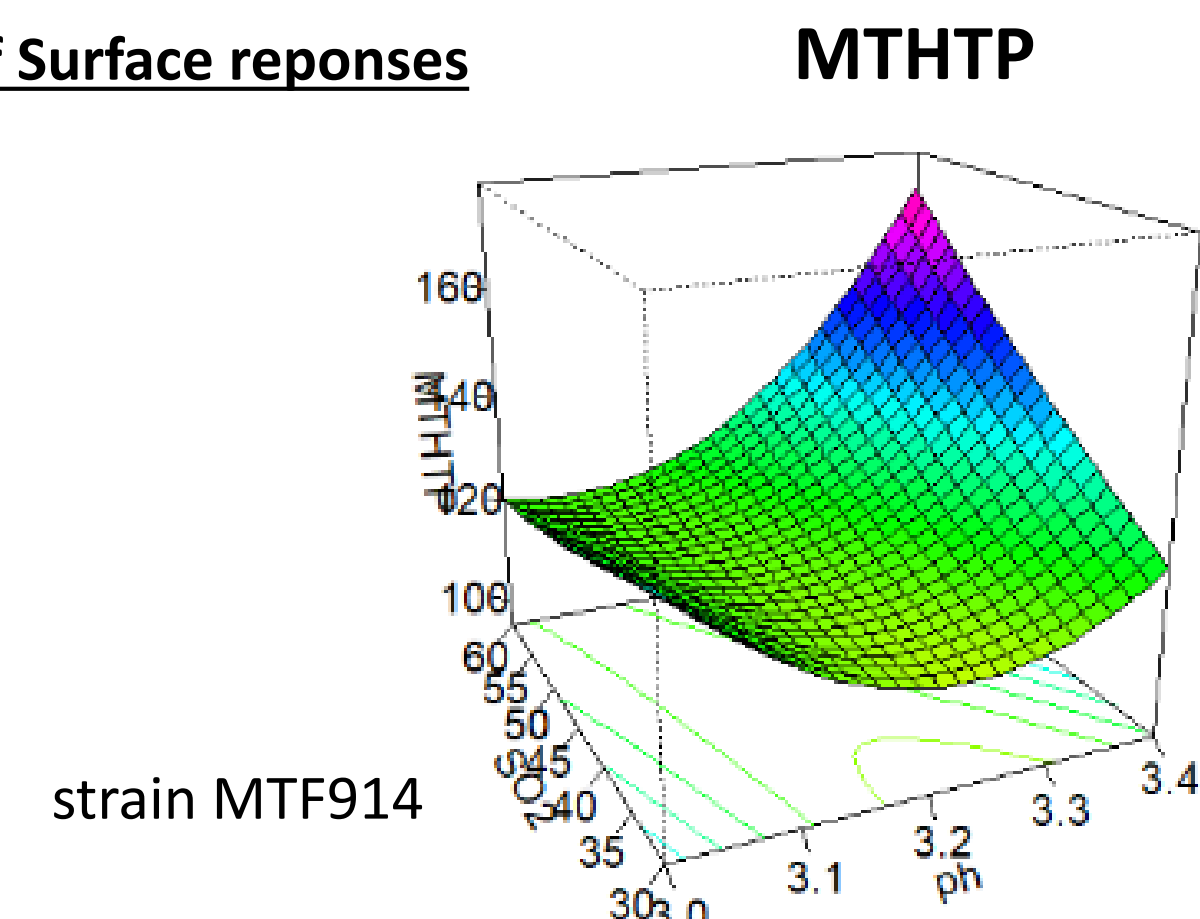
## Impact of SO<sub>2</sub> addition



- Overall, increase in VSCs production by adding of 45 mg/l SO<sub>2</sub>
- Extent of the response to SO<sub>2</sub> addition depending on both the strain and the nature of the compound

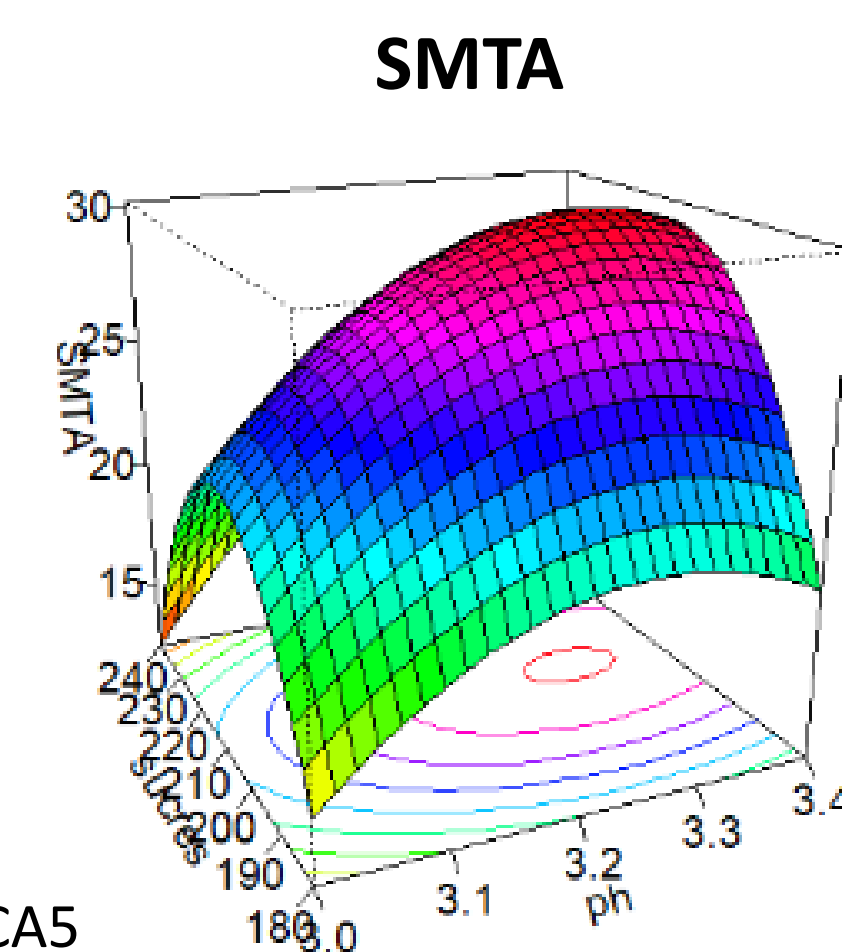
## Combined impact of SO<sub>2</sub>, sugar and pH

### Examples of Surface responses

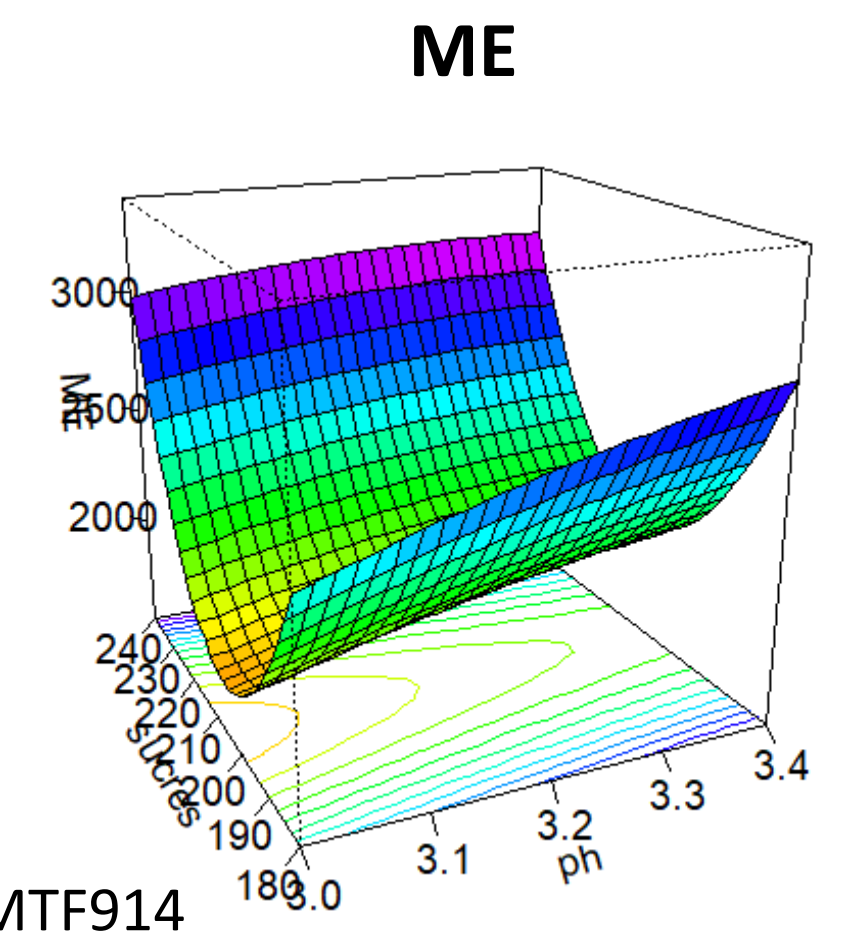


- Interaction between pH and SO<sub>2</sub> in the production of MTHTP by the strain MTF914:
  - Positive SO<sub>2</sub> effect of at high pH
  - Negative SO<sub>2</sub> effect at low pH

- One parameter is fixed, the others two are represented on x and y axis
- The studied aroma is represented on the z axis



- Quadratic effect of sugar
- Positive impact of pH



- Quadratic effect of sugar
- No impact of pH

## Summary table

Souche	Compound	Simple effects			Quadratic effects			Interactions		
		P	S	SO	P <sup>2</sup>	S <sup>2</sup>	SO <sup>2</sup>	S:P	P:SO	S:SO
IR10	SMTA									
	MTHTP									
	ME									
ECAS	SMTA	+								+
	MTHTP									
	ME									
MTF914	SMTA	+	+	-	+	-	+	-	+	+
	MTHTP		-		+			-		+
	ME					+				

+ : positive effect; - : negative effect  
N : nitrogen; S : sugar; L : lipids  
Green : p<0.001; blue : p<0.01; orange : p<0.05, white : NA

- Diversity of the response depending of the stain and the compound concentration

- Sugars, pH, and SO<sub>2</sub> act in a complex way on VSCs production (quadratic effect – interaction effect)

Exploring the profile of VSCs formation within a collection of 40 yeasts (*Saccharomyces* and non *Saccharomyces* species) commonly encountered in wine environment revealed an important variability between strains in their abilities to produce VSCs. Furthermore, using a Box-Behnken experimental design, we were able to show pH, sugar, and SO<sub>2</sub> concentrations modulate the production of VSCs, with a strain-dependent response.