# Prediction of the effects of environmental factors on the synthesis of fermentative aromas.

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# PREDICTION OF THE EFFECTS OF ENVIRONMENTAL FACTORS ON THE SYNTHESIS OF FERMENTATIVE AROMAS

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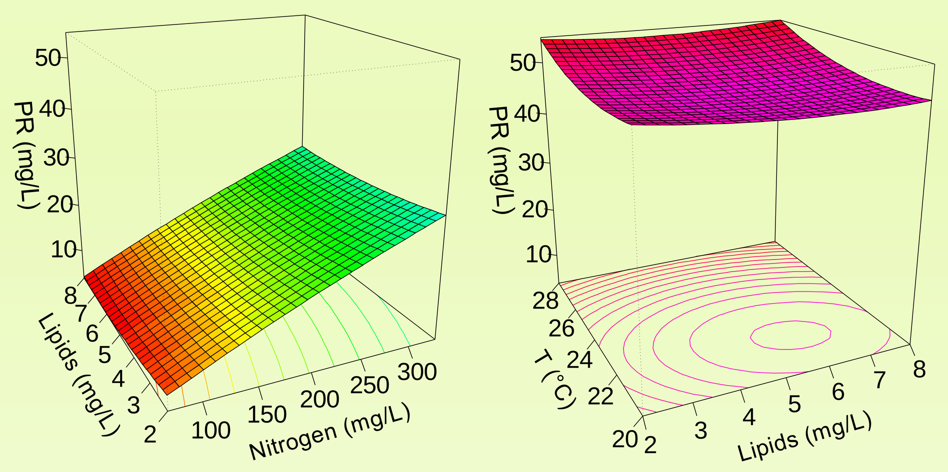
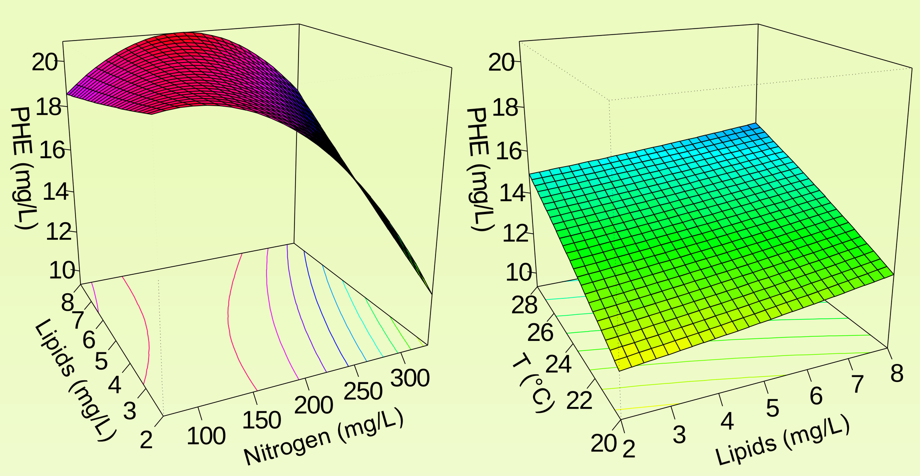
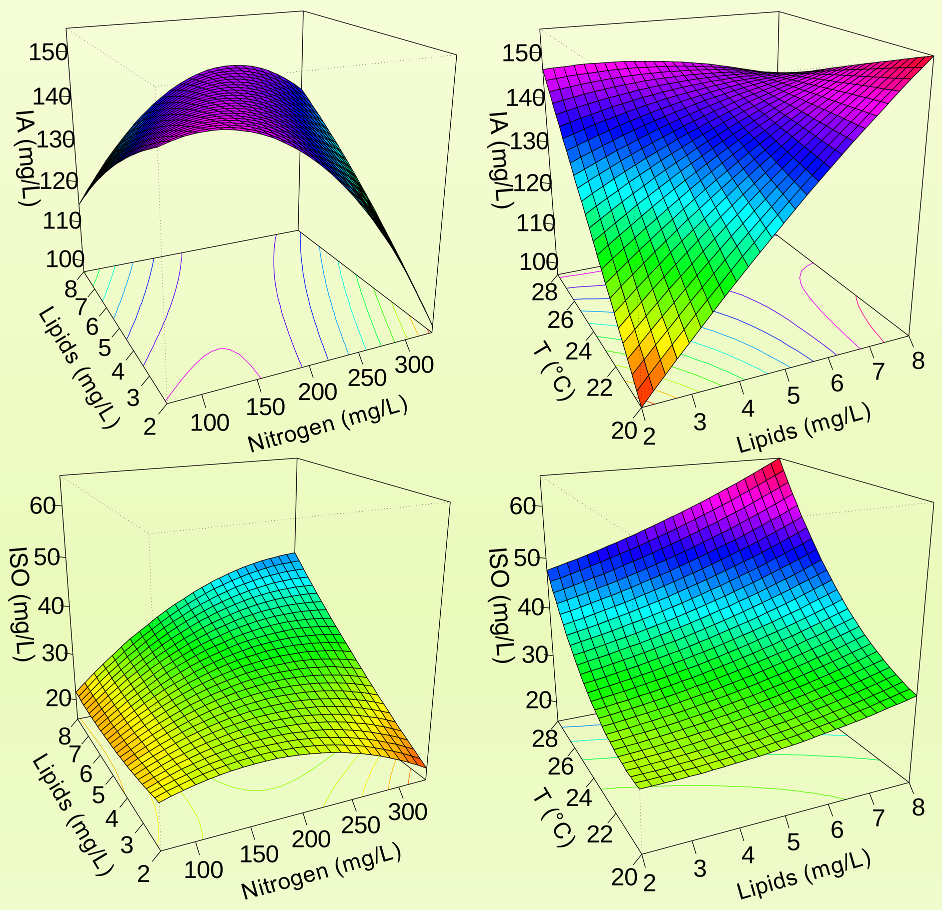
Most volatile compounds of organoleptic interest for young wines are synthesized by yeast metabolism, especially esters and higher alcohols. Their synthesis is highly influenced both environmental parameters and yeast strain. To determine the combined effects of temperature, initial assimilable nitrogen and phytosterol contents on the production of volatile compounds by Lalvin EC1118®, a response surface methodology was applied with a Box-Behnken experimental design. This plan allowed to classify these factors according to their impact on the synthesis of volatile compounds and to model these variations. Moreover, it was possible to estimate the percentage of loss in the gas of some volatile compounds and thus to recalculate their total production by yeast (liquid accumulation + losses). Finally, to better assess the impact of strain genetic background, this study was extended to other wine yeast strains under the two extreme conditions predicted by the model, leading to the highest and lowest production of esters.

## HIGHER ALCOHOLS

| | Simple effects | | | Quadratic effects | | | Interaction effects | | |
|---|---|---|---|---|---|---|---|---|---|
| Higher alcohols | N | P | T | N | P | T | N : P | N : T | P : T |
| Propanol | + | | | | | | | | |
| Isobutanol | | + | + | | | | + | | |
| Isoamyl alcohol | | + | | - | | | + | | - |
| 2-Phenylethanol | | | | - | | | | | |







- **Propanol**:
  - Increase with nitrogen content
  - No impact of phytosterols and temperature

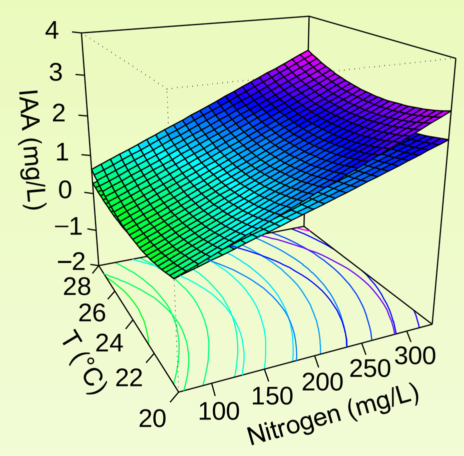
  → Consistent with its synthesis pathway

- **Phenylethanol**:
  - Favorable nitrogen content: 100-150 mgN/L
  - No impact of phytosterols and temperature

- **Isoamyl alcohol / Isobutanol:**
  - Increase with phytosterols
  - Interaction between nitrogen and phytosterols
  - Nitrogen and temperature: different impacts, despite their shared metabolic pathway

## ACETATE ESTERS

| | Simple effects | | | Quadratic effects | | | Interaction effects | | |
|---|---|---|---|---|---|---|---|---|---|
| Acetate esters | N | P | T | N | P | T | N : P | N : T | P : T |
| Ethyl acetate | + | | | | | | | | |
| 2-Phenylethylacetate | + | | | - | | | | | |
| Isobutyl acetate | + | | | | | + | | | |
| Isoamyl acetate | + | | | | | + | | | |
| Isoamyl acetate corrected | + | | | | | + | | | |



- **Liquid concentrations**:
  - Increased with nitrogen content

  → Consistent with their production via Ehrlich pathway
  - No impact of phytosterols
  - Temperature: minimal concentration at 24°C

- **Total production of isoamyl acetate**:
  Quadratic effect of temperature still significant after calculation of total production

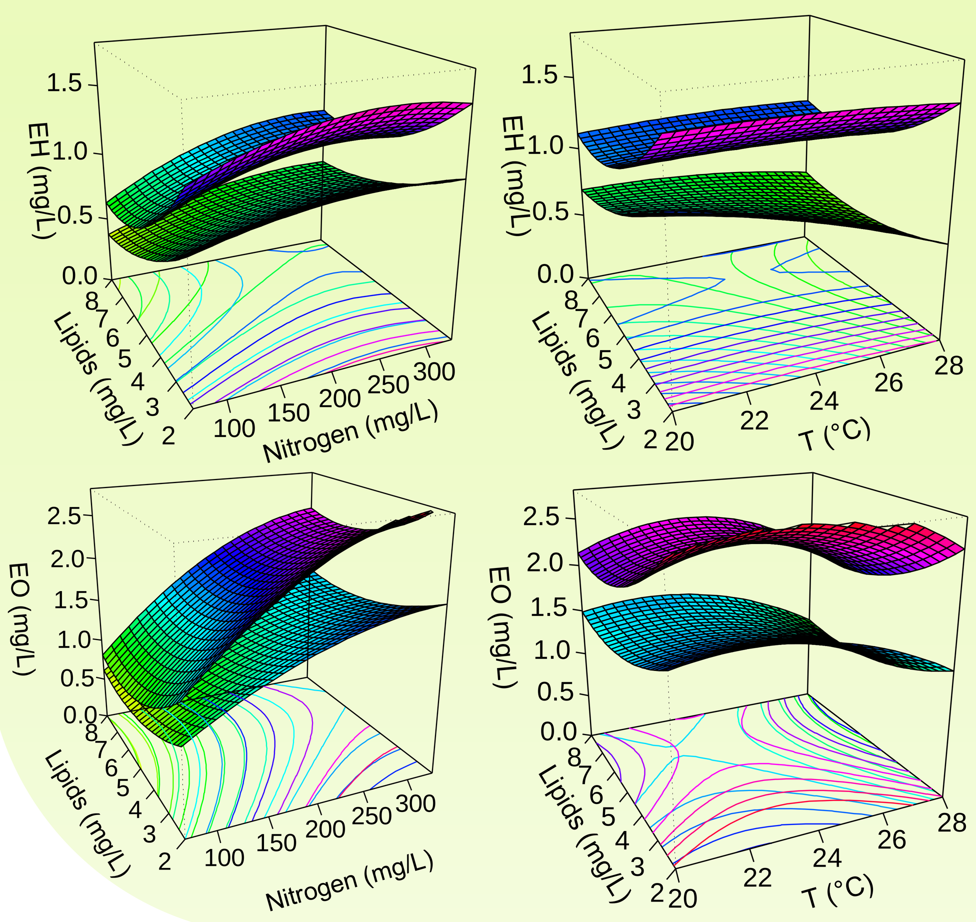
  → Major impact of temperature on isoamyl acetate synthesis

## ETHYL ESTERS

| | Simple effects | | | Quadratic effects | | | Interaction effects | | |
|---|---|---|---|---|---|---|---|---|---|
| Ethyl esters | N | P | T | N | P | T | N : P | N : T | P : T |
| Ethyl hexanoate | + | - | - | | | | | | |
| Ethyl hexanoate corrected | + | - | | | + | | | | |
| Ethyl octanoate | + | - | - | | | - | | | |
| Ethyl octanoate corrected | + | - | | | | - | | | |
| Ethyl butanoate | + | - | - | - | | | | | |

- **Liquid concentrations**:
  - Decrease with phytosterols
  - Increase with nitrogen content
  - Decrease with temperature

  → Consistent with their synthesis pathway and literature



- **Total production of ethyl hexanoate**:
  No temperature effect after calculation of total production

  → Impact of temperature on liquid accumulation only due to evaporation

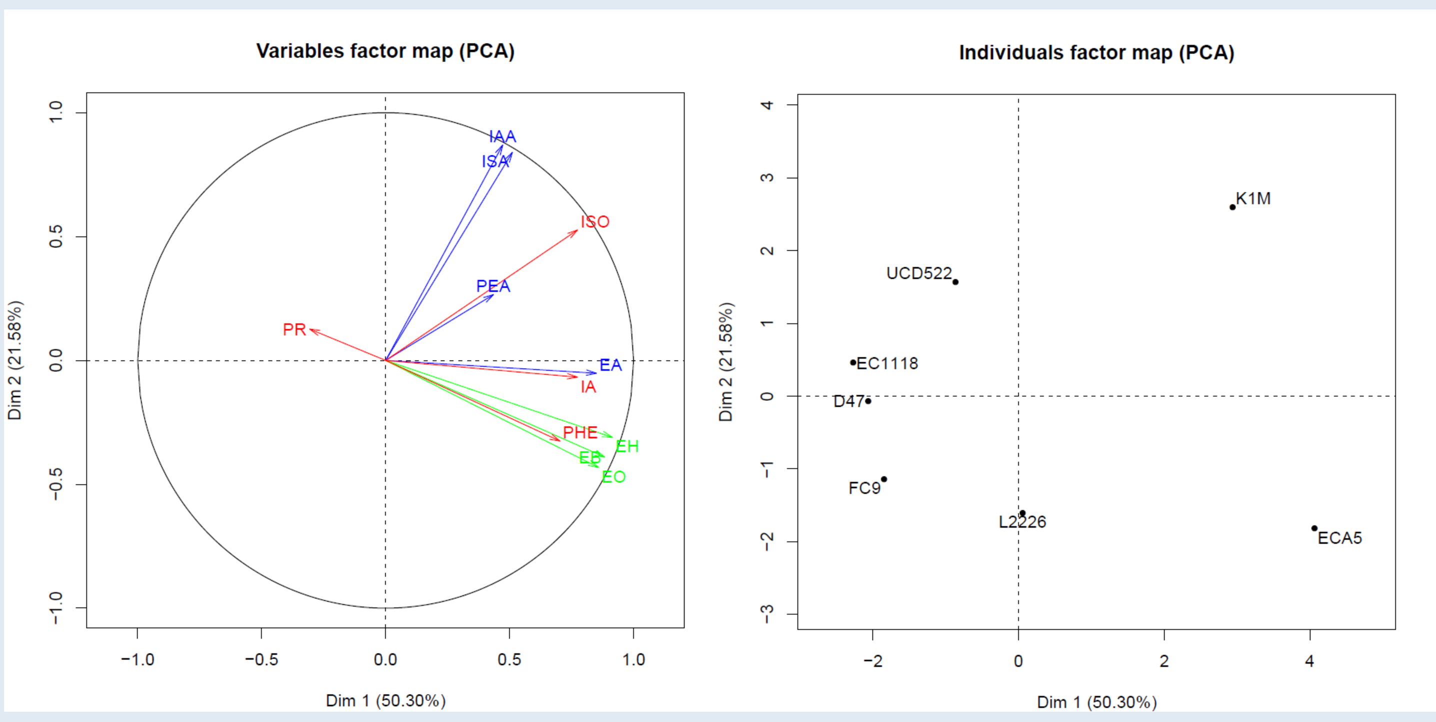
- **Total production of ethyl octanoate:**
  No simple effect of temperature after calculation of total production but quadratic effect still present

  → Impact of temperature on liquid accumulation mainly due to evaporation

  → Minor modification of yeast metabolism

## EFFECT OF GENETIC BACKGROUND

- PCA performed with the percentage of variation of volatile compound production between two extreme conditions




- Correlation between isobutyl and isoamyl acetate but not between isobutanol and isoamyl alcohol

  → Higher alcohols affected differently by changes in fermentation parameters, similar responses for their acetates
- K1M and ECA5 (an aromatic strain obtained by adaptive evolution): separated from the other strains → Atypical regulation

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- The initial nitrogen content has the greatest impact on the production of volatile compounds while phytosterols and temperature have only moderate effects on a few number of molecules.
- Acetate esters are similarly affected by fermentative parameters, while each higher alcohol is differently impacted. This is in line with a major role of alcohols acetyl-transferases in the control of the production of acetate esters.
- By calculating the total production of esters, we showed that temperature effect is mainly explained by evaporation, but a true effect on yeast metabolism was observed for some compounds (en citer?).
- The study of 7 different wine yeast strains in 2 extreme conditions revealed a similar response of 5 strains to environmental factors and highlighted an atypical regulation for 2 strains, K1M and ECA5, an evolved strain overproducing esters.

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