

# Aplicación de técnicas emergentes en vinificación: control microbiológico y extracción de color



**PTV**  
PLATAFORMA  
TECNOLÓGICA  
DEL VINO

**Iris Loira, Antonio Morata, Felipe Palomero & Jose Antonio Suárez-Lepe**  
**enotecUPM**  
Dpto. Química y Tecnología de Alimentos  
**ETSIAAB**  
Universidad Politécnica de Madrid

**REUNIÓN GRUPO DE I+D+i en PROCESO**

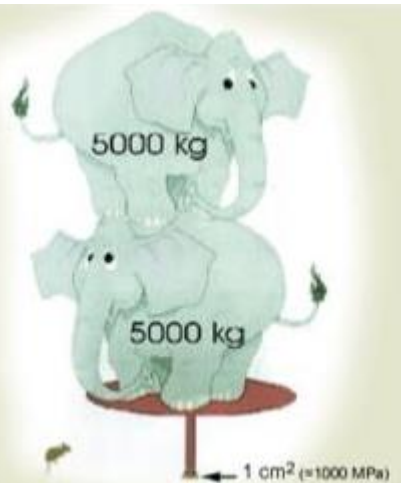
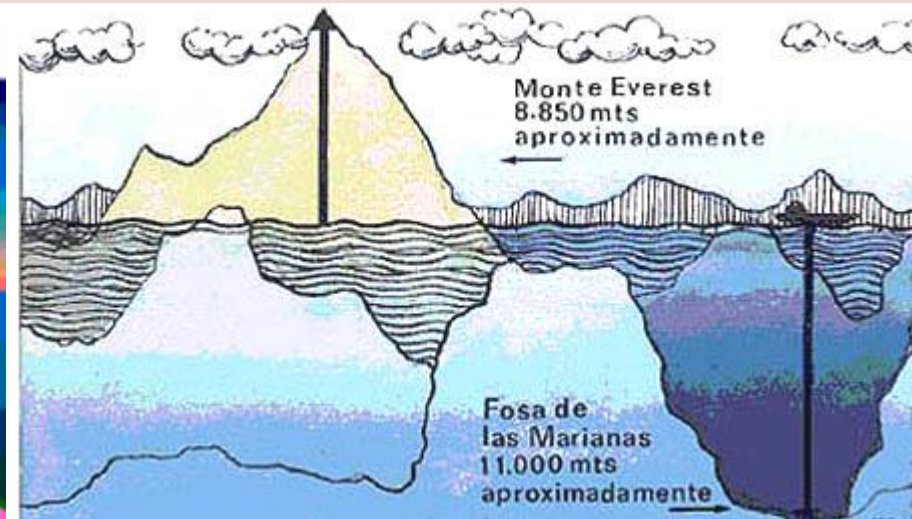
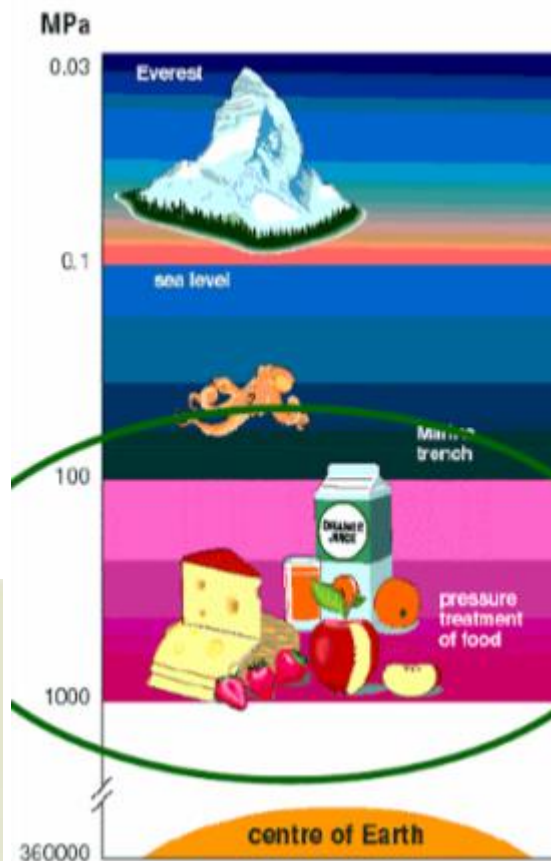
[iris.loira@upm.es](mailto:iris.loira@upm.es)

# Grape hygienization

- *Grapes are not sanitized*
  - *Grape composition: ↑↑ sugar, ↑ phenols, ↓↓ protein and fat, ↓pH*
  - *Wine composition: ↓↓ sugar, ↑ phenols, ↓↓ protein and fat, ↑ etOH, ↓pH*
- *No cleaning processes*
- *Only SO<sub>2</sub> (50 mg/L)*



# HHP. High hydrostatic pressure



**HHP. 400-600 MPa  $\approx$  4000-6000 bar**

## HHP. High hydrostatic pressure

HHP is a **non-thermal process** that subjects food under high hydrostatic pressure (which is transmitted by water) reaching up to 600 MPa

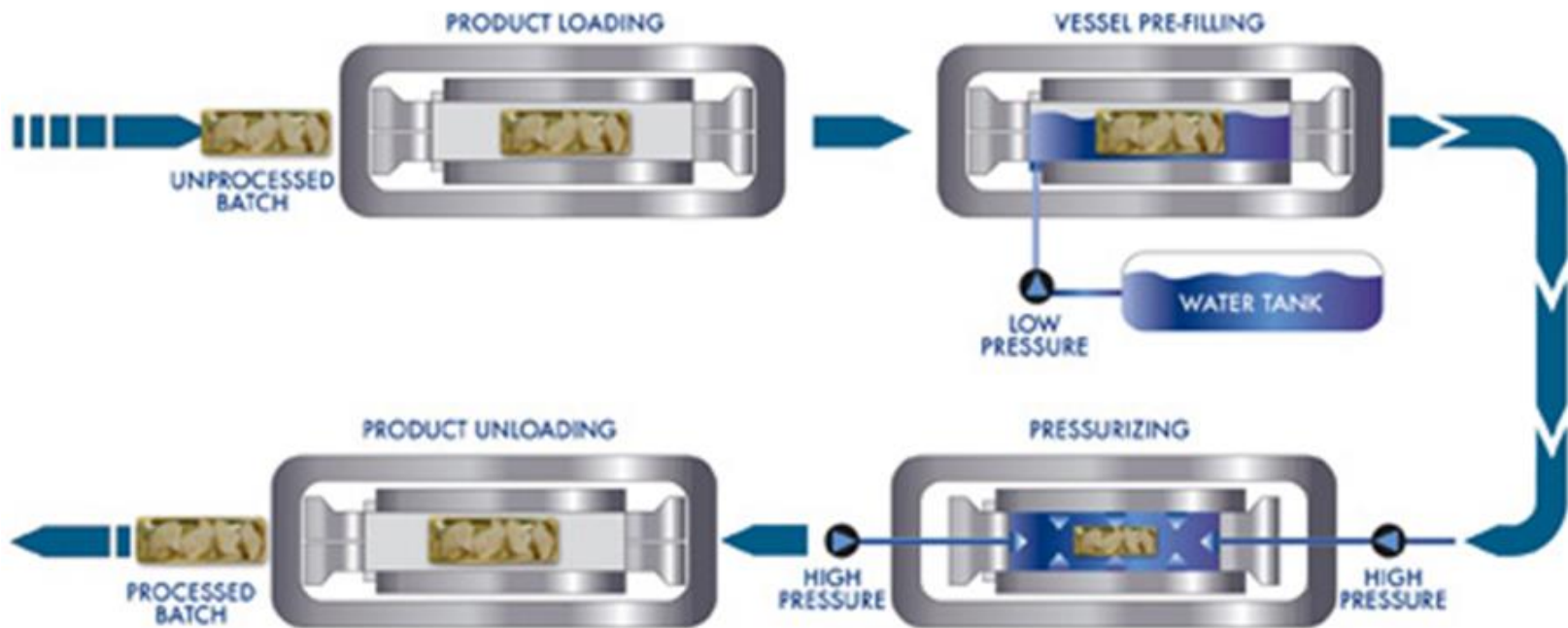


Diagram of operation of a HPP unit

## HHP. High hydrostatic pressure

# Improving microbiological quality

HHP.

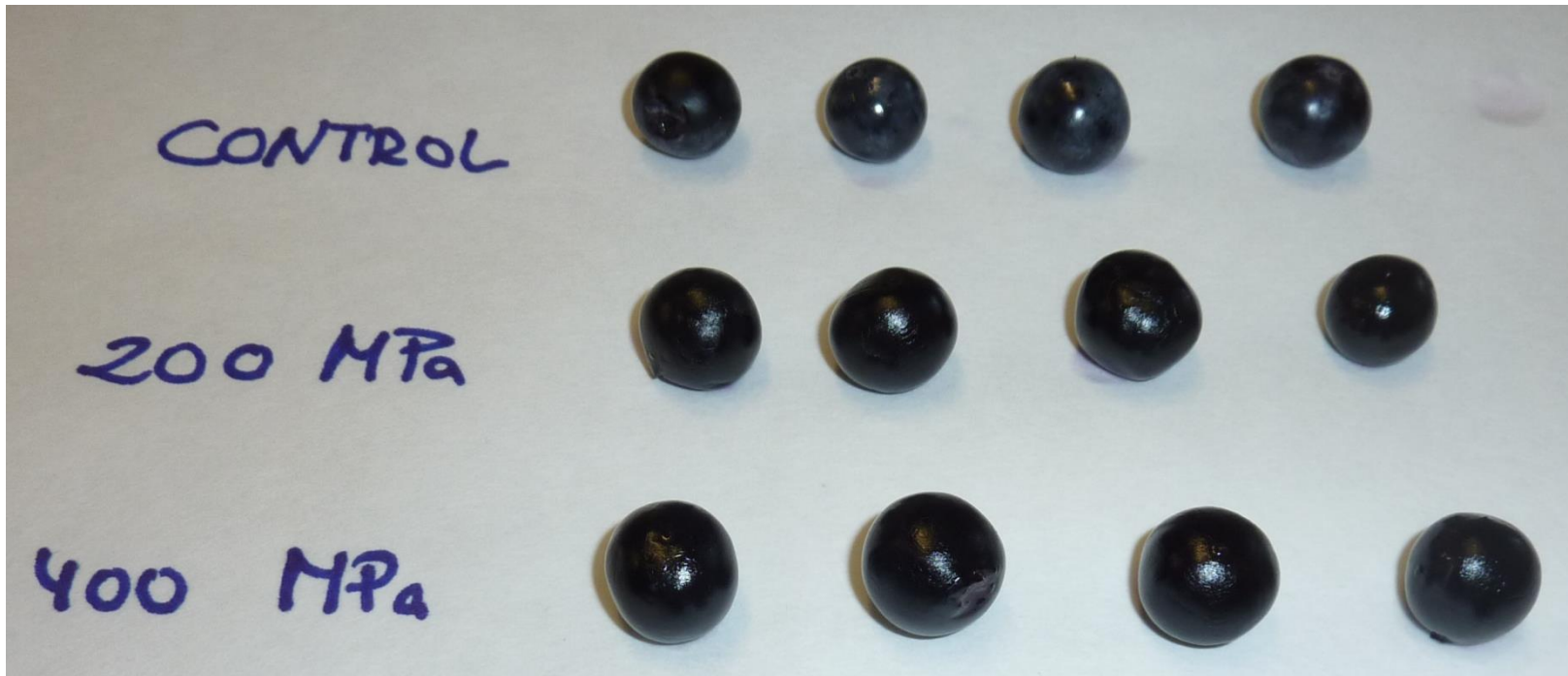
- $\Delta T^a$  adiabatic compression 2-3 °C/100 MPa
- Pressurization do not affect covalent bonds →  
Protects nutritional and sensory quality





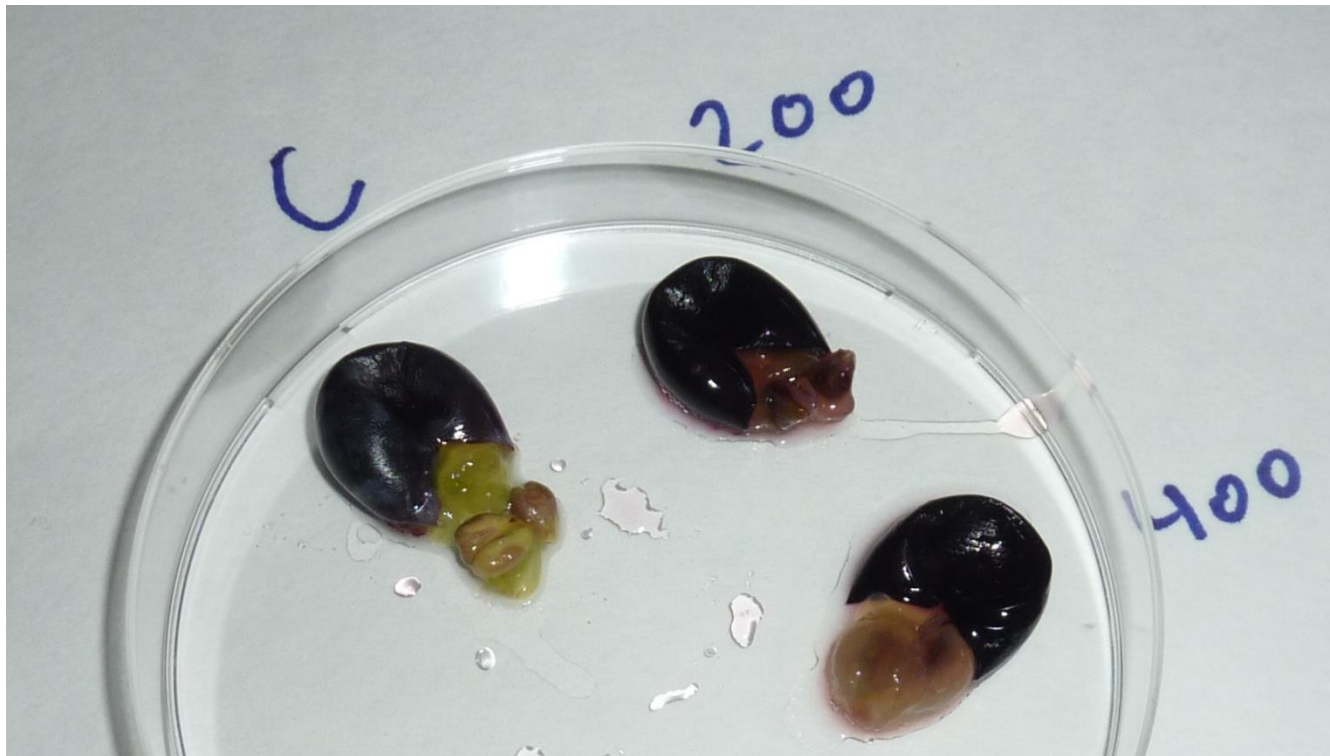
HHP. High hydrostatic pressure

## External shape and color unaffected



Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., & Suárez-Lepe, J. A. (2015). Grape processing by high hydrostatic pressure: Effect on microbial populations, phenol extraction and wine quality. *Food and Bioprocess Technology*, 8(2), 277-286.

## HHP. High hydrostatic pressure



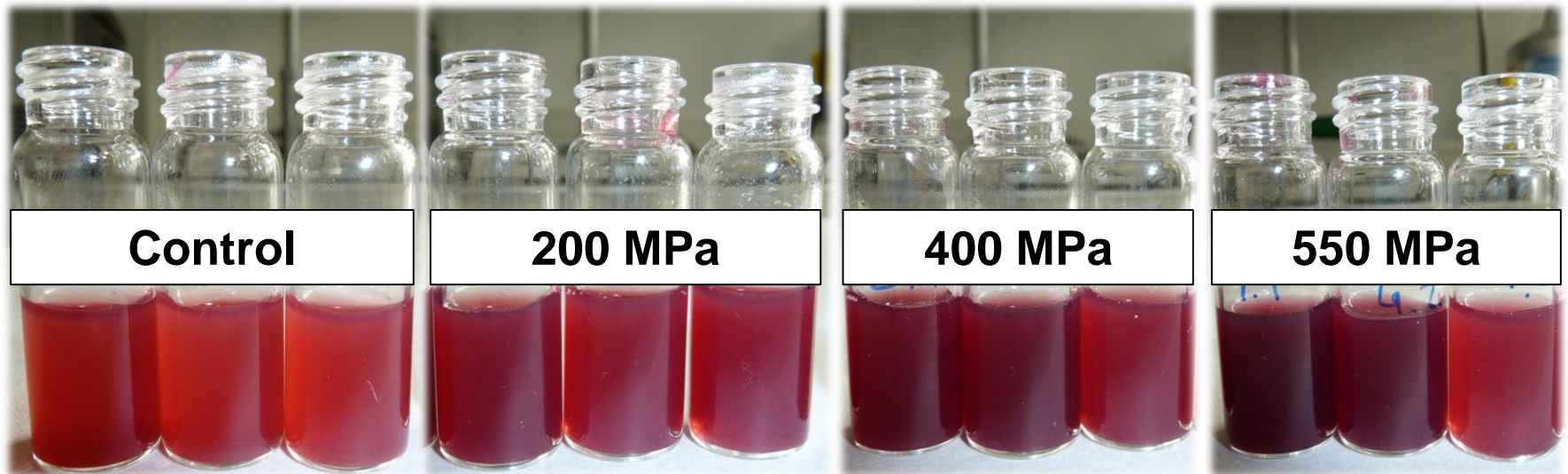
## Phenol extraction

Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., & Suárez-Lepe, J. A. (2015). Grape processing by high hydrostatic pressure: Effect on microbial populations, phenol extraction and wine quality. *Food and Bioprocess Technology*, 8(2), 277-286.

## HHP. High hydrostatic pressure

# Phenol extraction

Greater antioxidant capacity  
of the wine → ↓SO<sub>2</sub>



↑ IC  
↑ IPT

Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., & Suárez-Lepe, J. A. (2015). Grape processing by high hydrostatic pressure: Effect on microbial populations, phenol extraction and wine quality. *Food and Bioprocess Technology*, 8(2), 277-286.



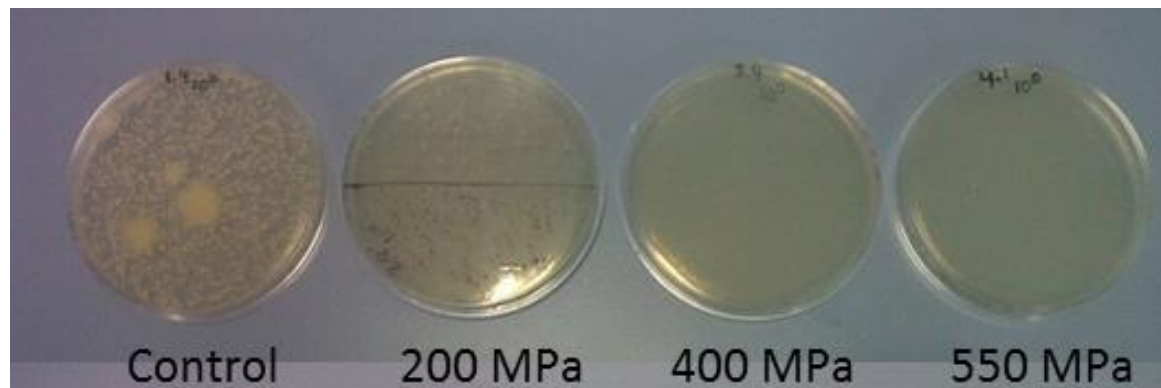
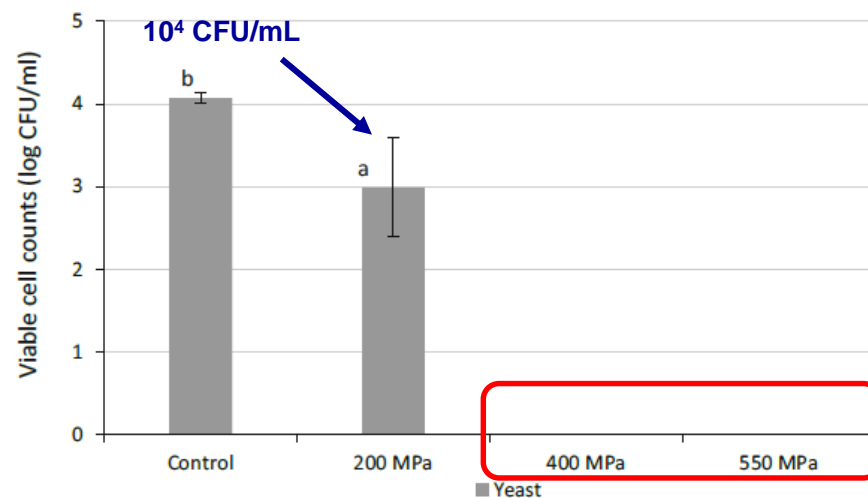


Fig. 3 Microbial counts in crushed control and HHP-treated grapes at the beginning of fermentation. a Yeast. b Bacteria. Values are means  $\pm$  standard deviations of four replicates. *Different letters* in the same series indicate significant differences between means ( $p < 0.05$ )

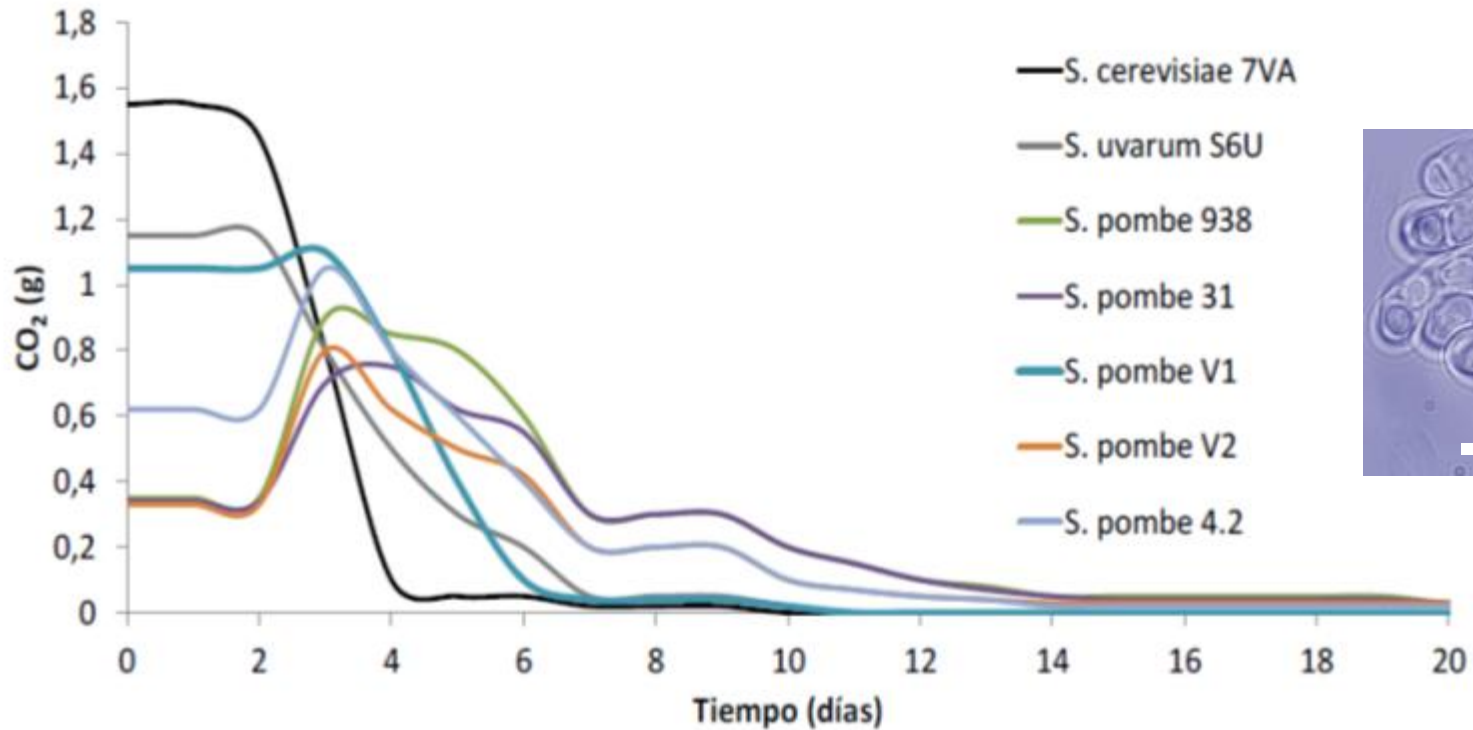


# Microbiological quality

Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., & Suárez-Lepe, J. A. (2015). Grape processing by high hydrostatic pressure: Effect on microbial populations, phenol extraction and wine quality. *Food and Bioprocess Technology*, 8(2), 277-286.



# HHP & non-*Saccharomyces*

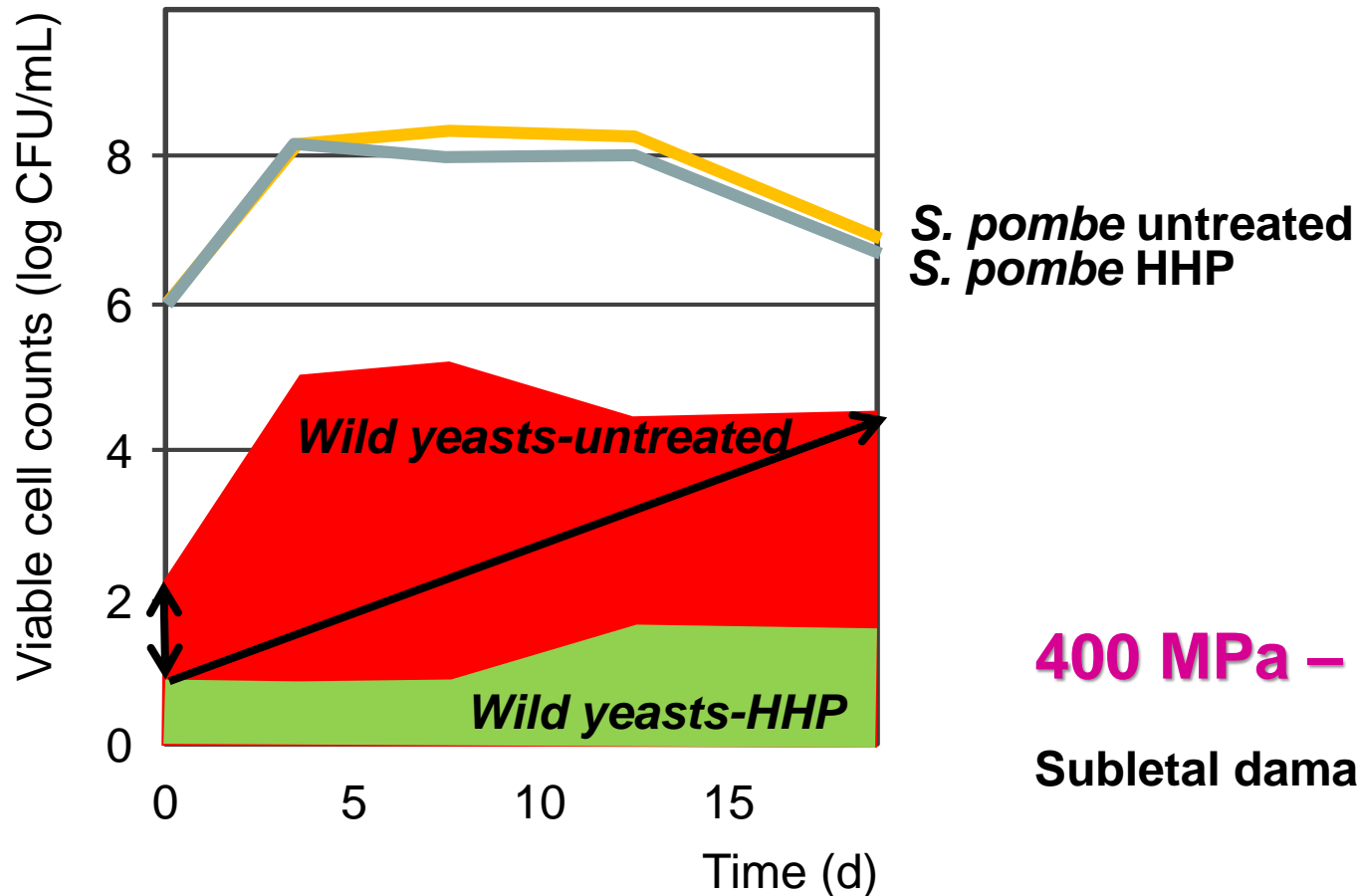


Average fermentation time *S. cerevisiae*

Average fermentation time *S. pombe*

*S. pombe*: High fermentation power (14 % v/v) but **slow fermentation rate**

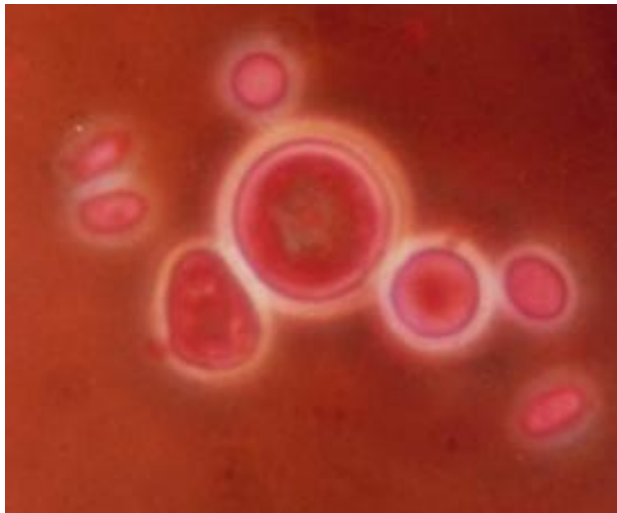
# *Schizosaccharomyces pombe*



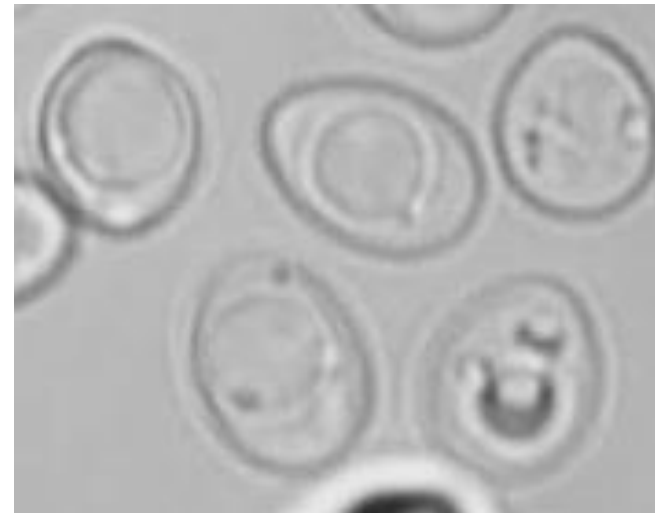
# *Metschnikowia pulcherrima*

*M. pulcherrima*: Low fermentation power (1-3 % v/v)

Really difficult development and competition in simultaneous growth with fermentative wild yeasts

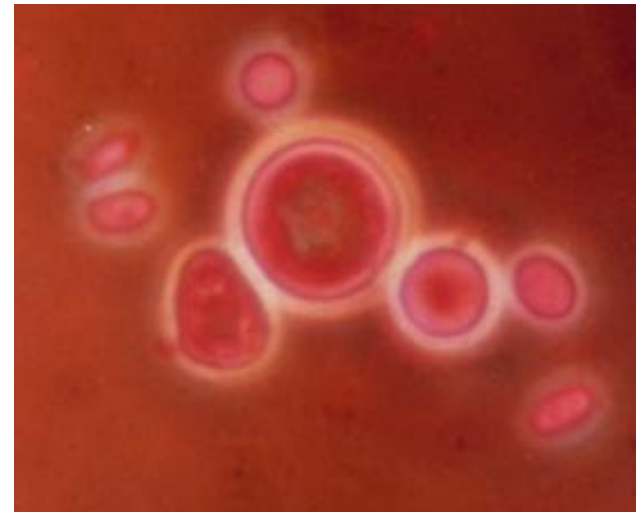
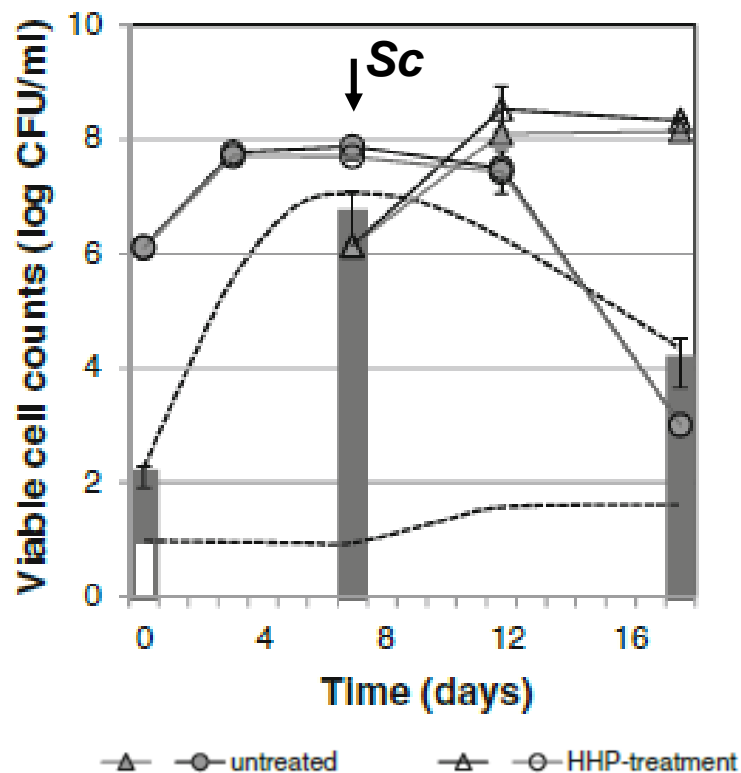


*M. pulcherrima*



*S. cerevisiae*

# *Metschnikowia pulcherrima*



Bañuelos, M. A., Loira, I., Escott, C., Del Fresno, J. M., Morata, A., Sanz, P. D., ... & Suárez-Lepe, J. A. (2016). Grape processing by high hydrostatic pressure: effect on use of non-Saccharomyces in must fermentation. *Food and Bioprocess Technology*, 9(10), 1769-1778.



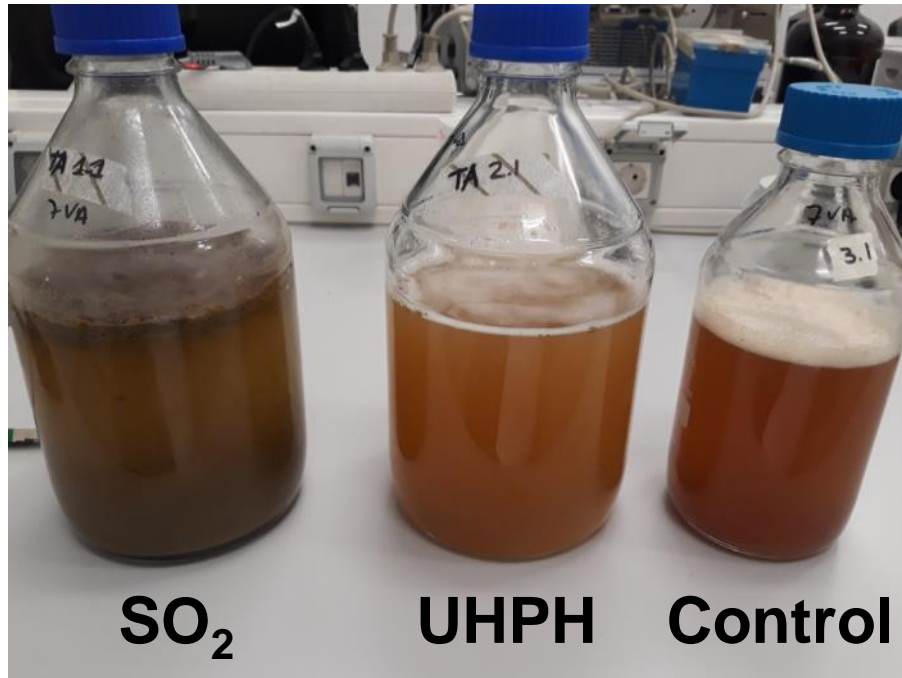
## Ultra High Pressure Homogenization for grape must



**Fricción, corte,  
cavitación, turbulencia,  
impacto**

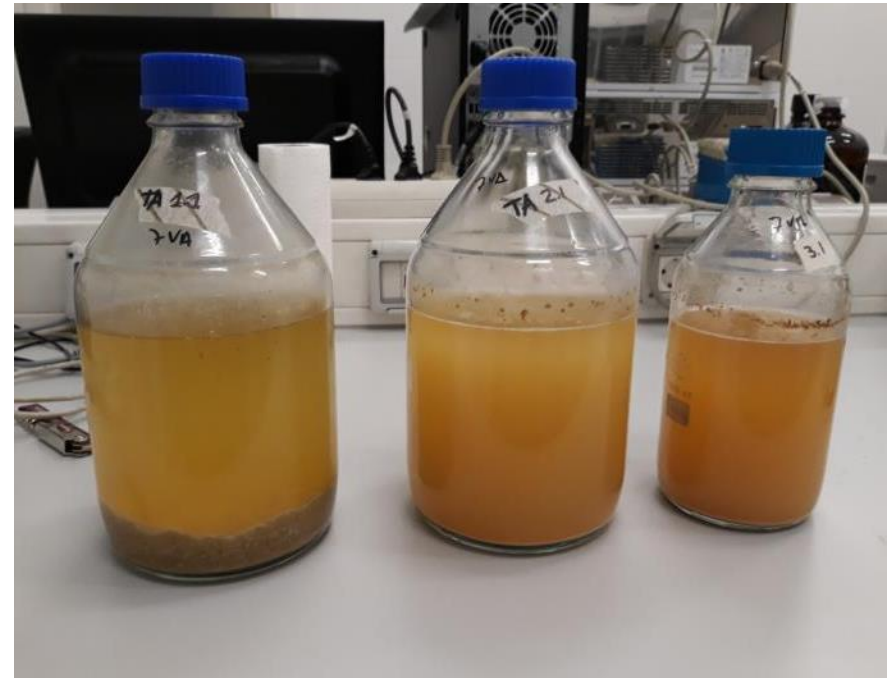
***200-400 MPa continuous tech.  
Ypsicon***

## Ultra High Pressure Homogenization for grape must



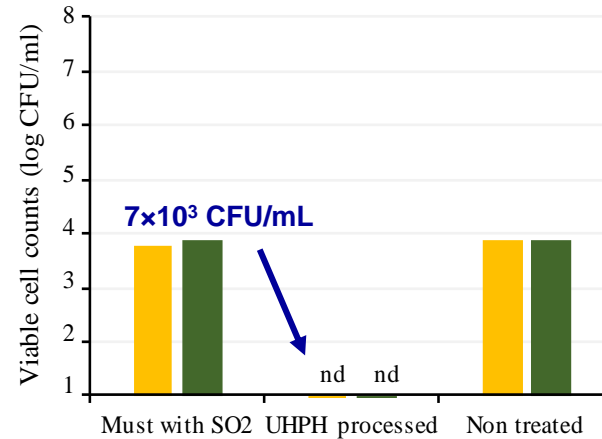
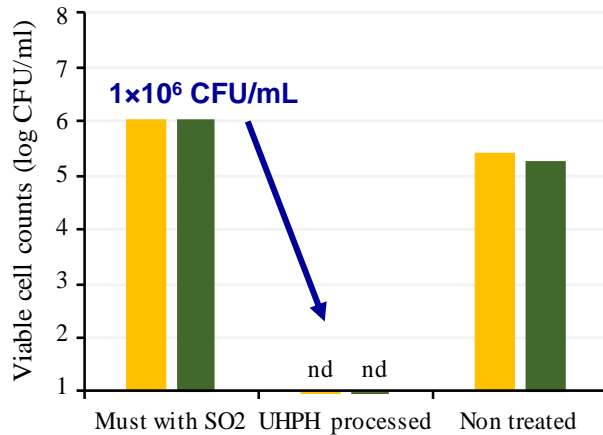
35 mg/L      300 MPa

Must  
All in triplicate

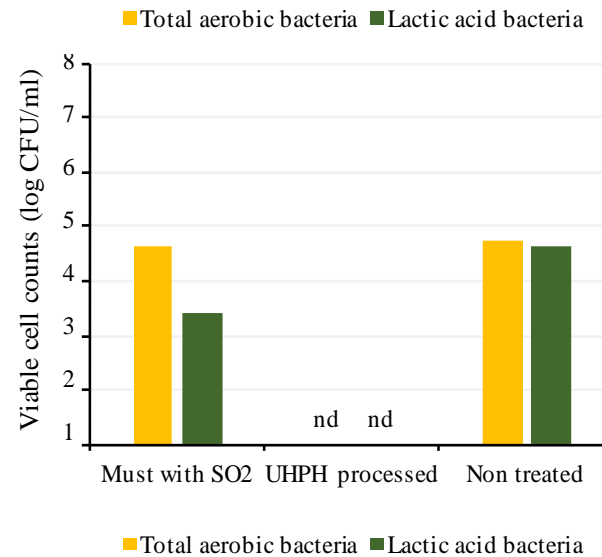
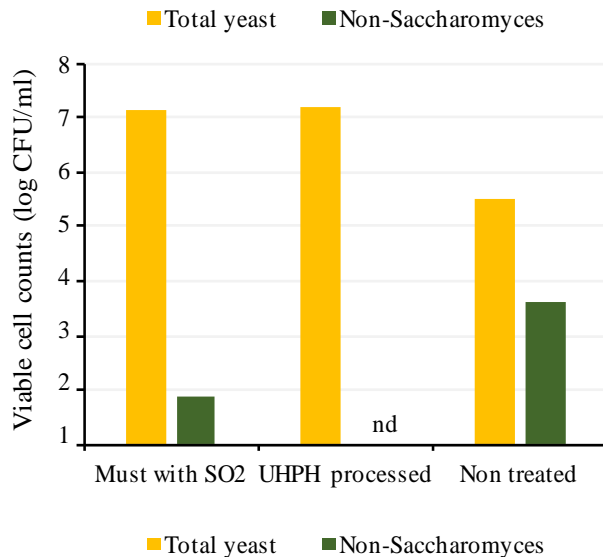


End of fermentation  
*S. cerevisiae* strain 7VA

# UHPH Yeast & bacteria populations

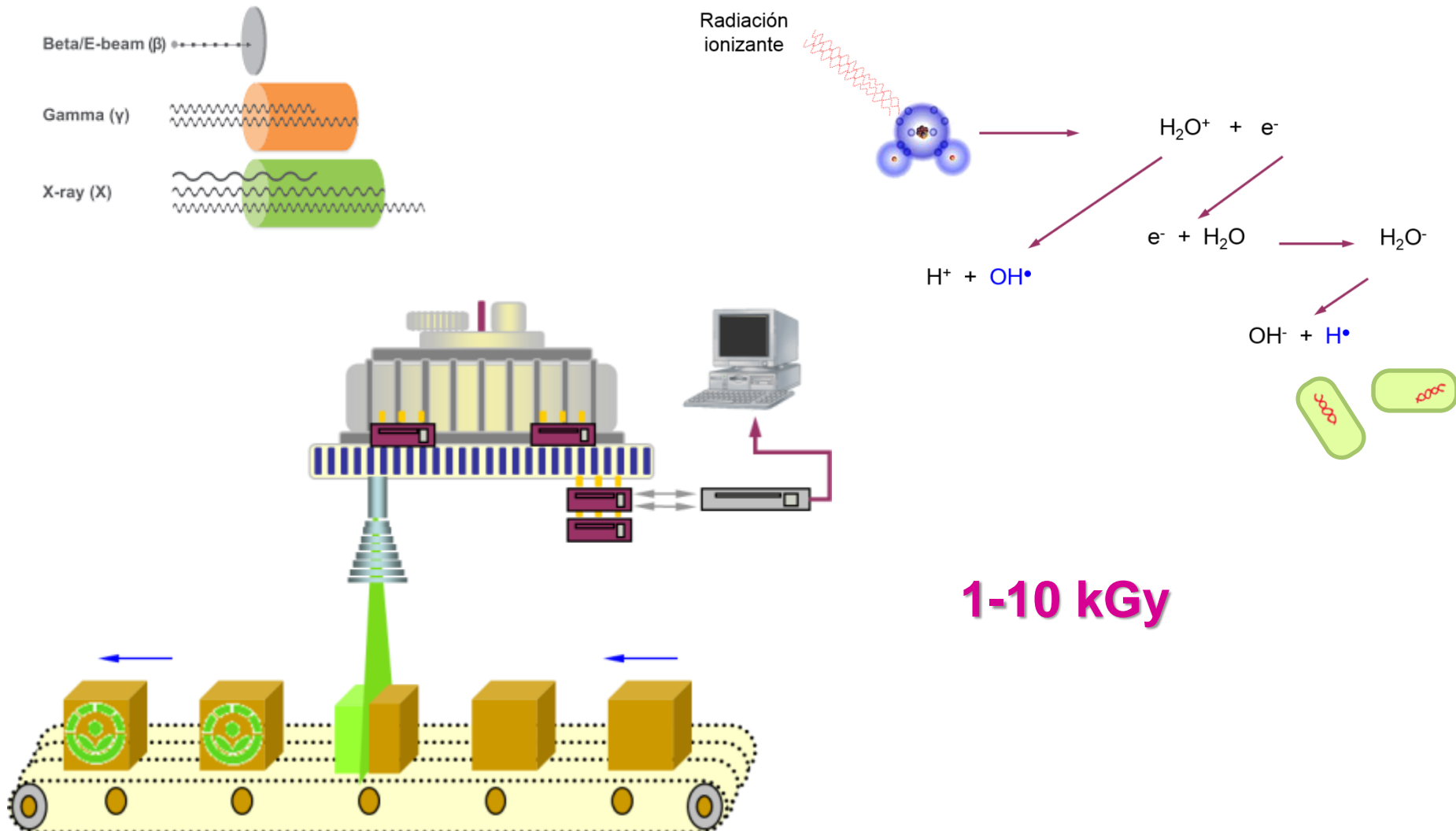


Must



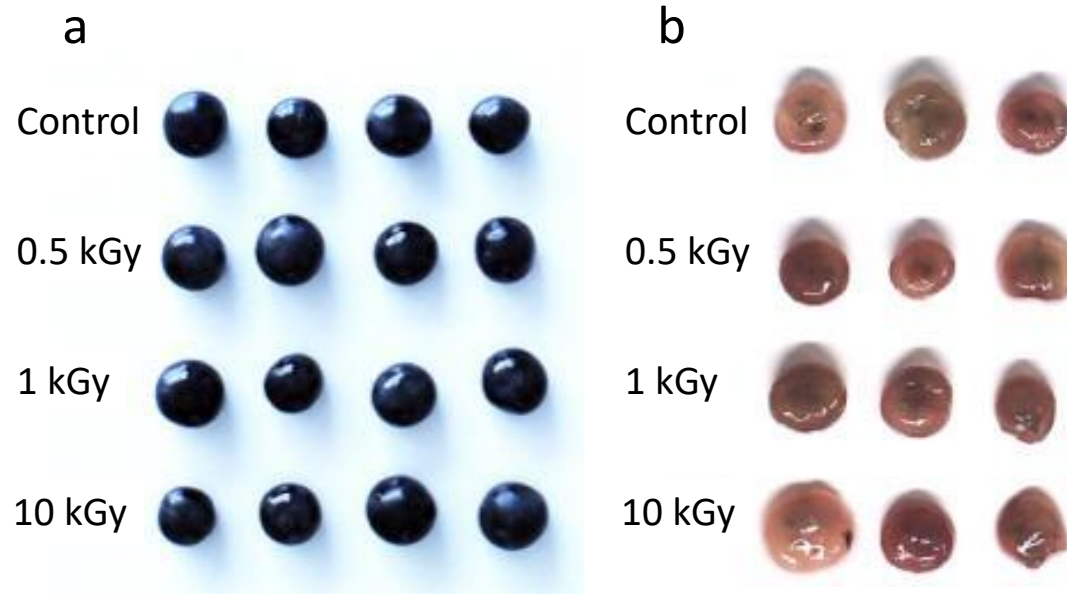
End of  
fermentation  
Yeast 7VA

# e-beam irradiation



## e-beam irradiation

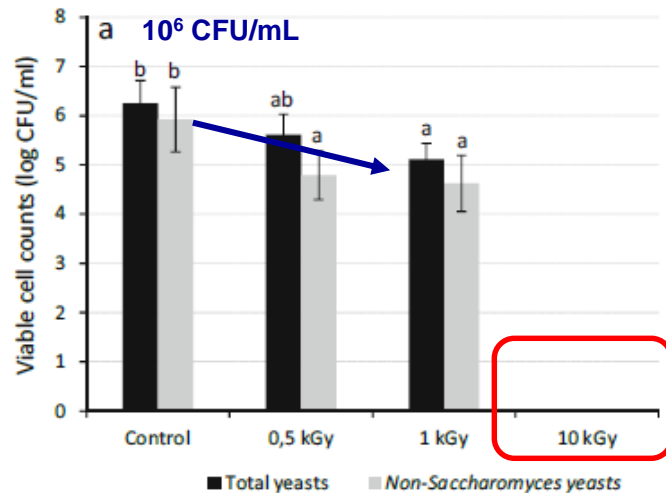
**Fig. 1** Appearance of irradiated grapes. **a** External surface. **b** Peeled



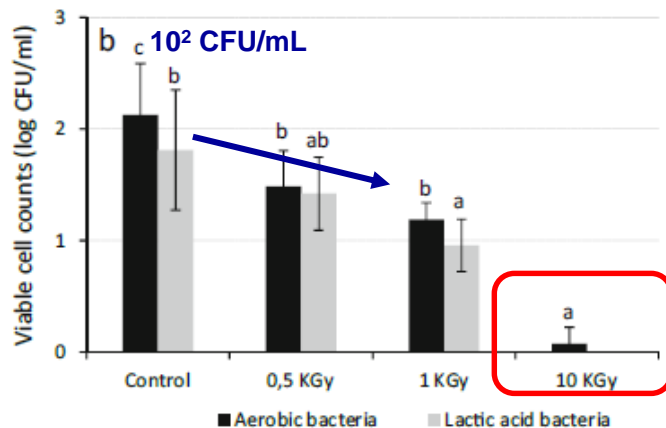
Morata, A., Bañuelos, M. A., Tesfaye, W., Loira, I., Palomero, F., Benito, S., ... & Suárez-Lepe, J. A. (2015). Electron beam irradiation of wine grapes: effect on microbial populations, phenol extraction and wine quality. *Food and bioprocess technology*, 8(9), 1845-1853.



## e-beam irradiation



**Yeast counts:** 1 kGy → ↓ 1 log CFU/mL  
10 kGy → total elimination



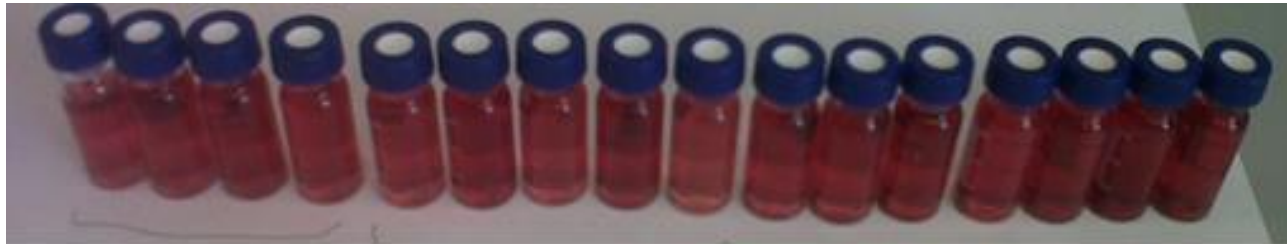
**Bacteria counts:** 1 kGy → ↓ 1 log CFU/mL  
10 kGy → <10 CFU/mL

Fig. 2 Microbial counts (viable cells) in the must from crushed control and irradiated grapes (log cfu/mL). a Yeasts. b Bacteria. Different letters in the same series indicate significant differences between means ( $p < 0.05$ )

Morata, A., Bañuelos, M. A., Tesfaye, W., Loira, I., Palomero, F., Benito, S., ... & Suárez-Lepe, J. A. (2015). Electron beam irradiation of wine grapes: effect on microbial populations, phenol extraction and wine quality. Food and bioprocess technology, 8(9), 1845-1853.

# e-beam irradiation

## Mosto Tempranillo

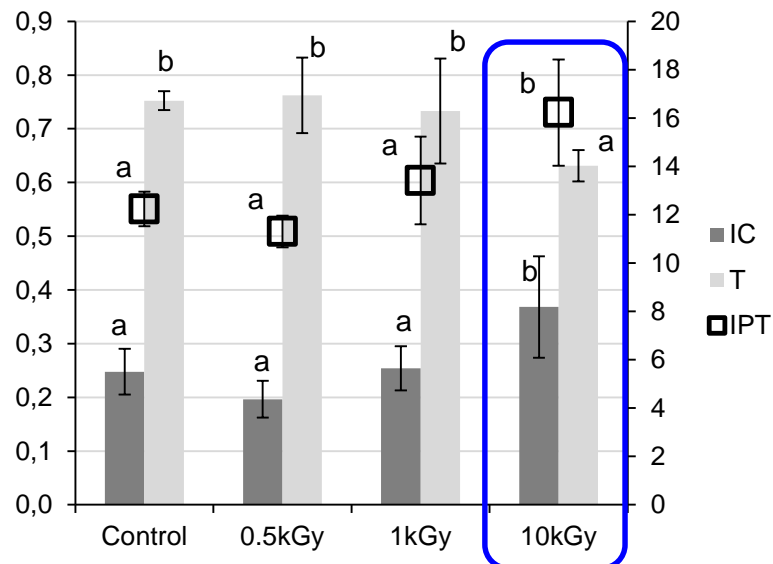


Control

0,5 kGy

1 kGy

10 kGy



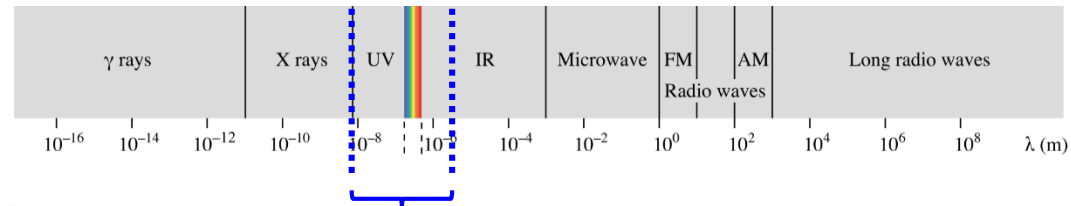
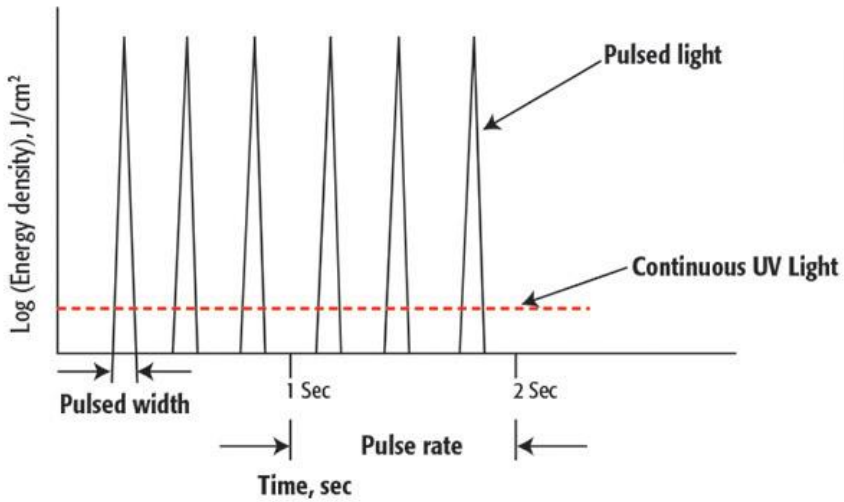
**10 kGy** → ↑ IC ↓ T ↑ IPT

**Shorter maceration times**

**Wines:** no significant differences

**Oxidative damage**

# Pulsed light

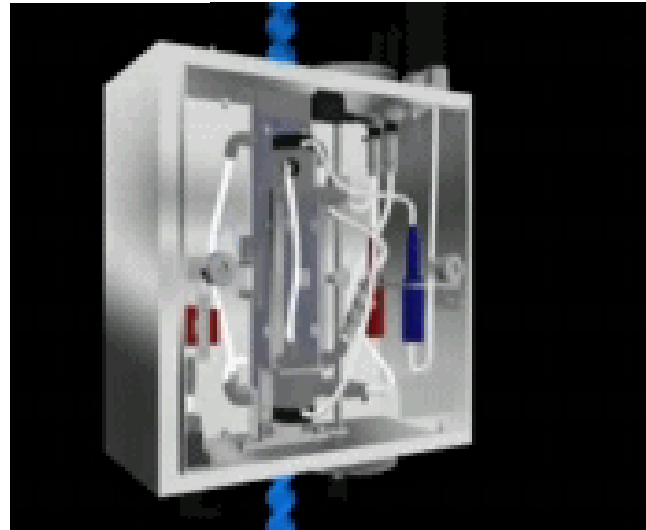


**170 nm UV – 2600 nm IR cercano**



**DNA damage (254 nm)  
Structural damage**

**35 MW  
Xenon lamps**

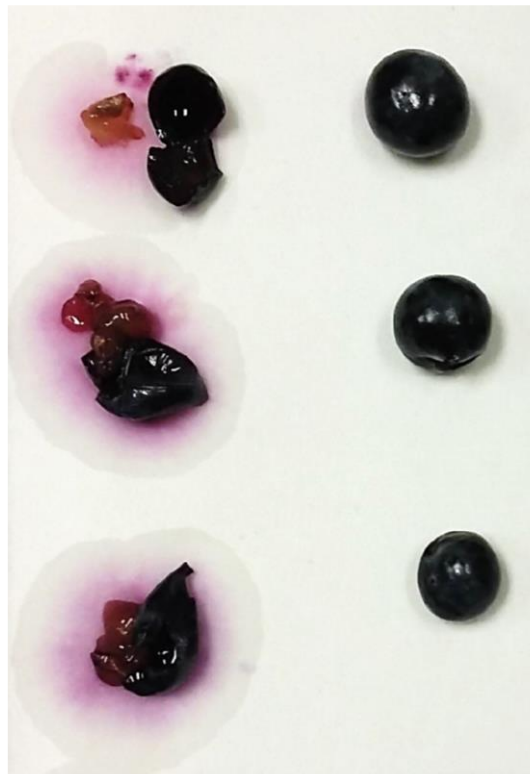


## Pulsed light

Control

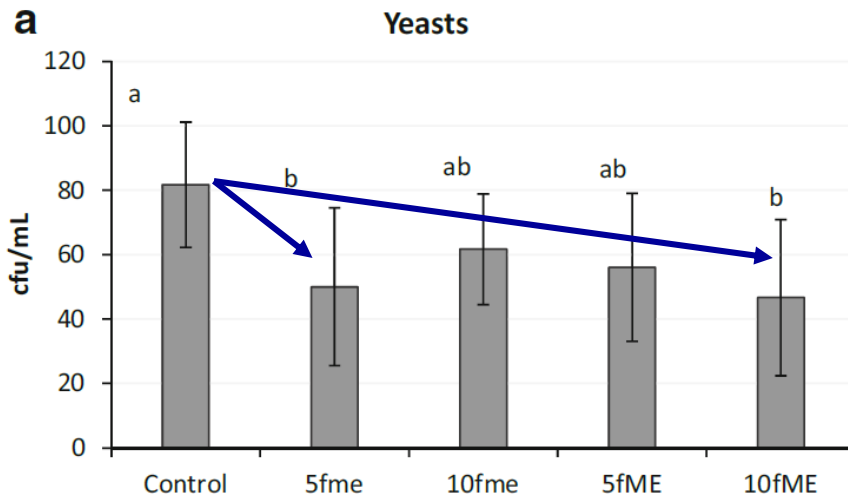
5 pulses

10 pulses



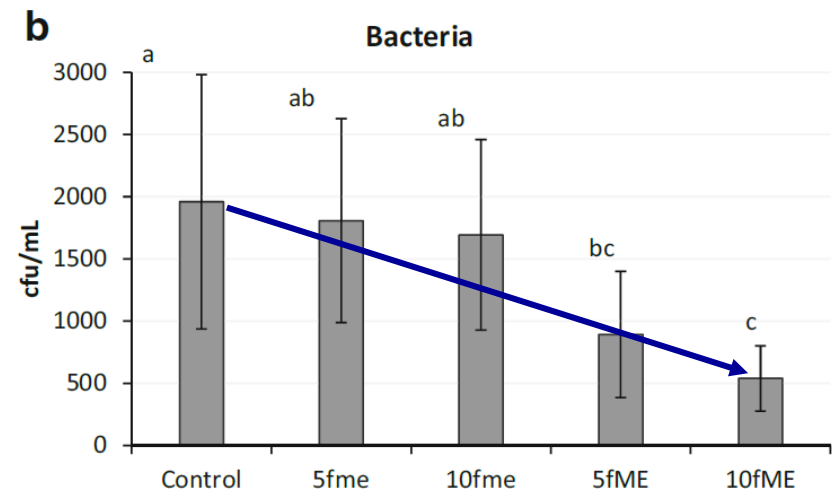
**600 J each pulse**

# Pulsed light



Yeast counts: ↓ 50%

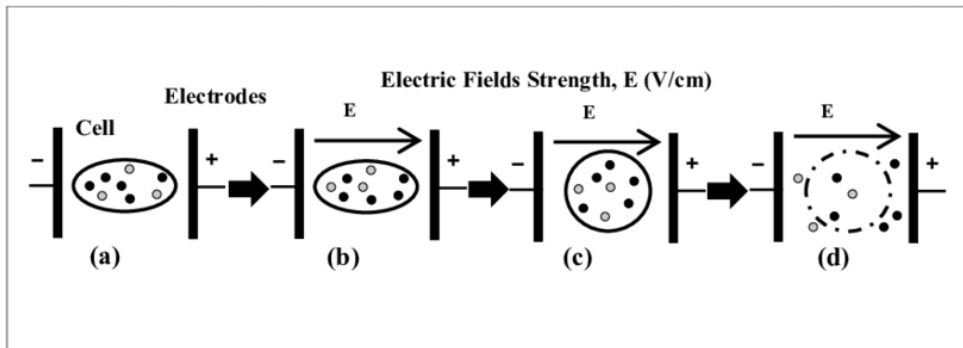
Shadow areas  
Low penetration power



Bacteria counts: ↓ 75%



# Pulsed electric fields

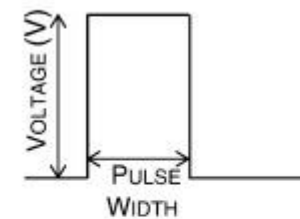


- (a) At zero potential
- (b) Osmotic imbalance
- (c) Swelling
- (d) Membrane ruptures

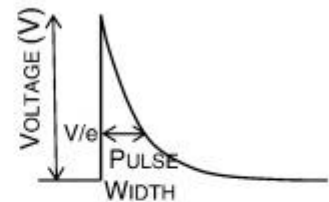
**10-40 kV/cm**  
**40-60A**

## PULSE SHAPE

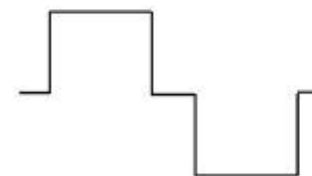
UNIPOLAR  
SQUARE WAVE PULSE



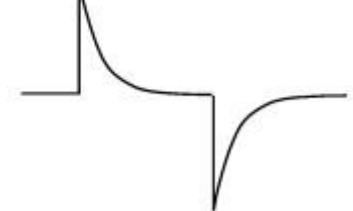
UNIPOLAR  
EXPONENTIAL PULSE



BIPOLAR  
SQUARE WAVE PULSE



BIPOLAR  
EXPONENTIAL PULSE



TIME ( $\mu$ s OR ms)

## Pulsed electric fields

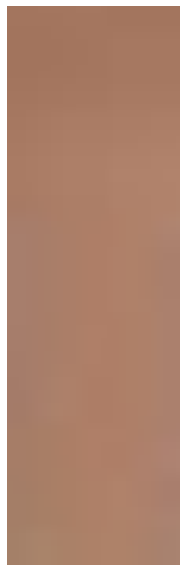
Effects of antimicrobials with and without pulsed electrical field (PEF) treatment on microbial reduction (log values) in white grape juice at 50 °C (4–8 replications, 65 kV/cm field, peak-to-peak, and 4 mm electrode gap)

<i>Treatments</i>	<i>Microbial log reductions, mean and standard deviation</i>
Control, no pulse	1.5±1.1 <sup>a</sup>
20 pulses	4.0±0.3 <sup>b</sup>
30 pulses	4.1±0.2 <sup>b</sup>
40 pulses	4.2±0.5 <sup>b</sup>

Wu, Y., Mittal, G. S., Griffiths, M. W. 2005. Effect of Pulsed Electric Field on the Inactivation of Microorganisms in Grape Juices with and without Antimicrobials. *Biosystems Engineering*, 90, 1-7

# Pulsed electric fields

**Maceration time: 1 hour**  
**Low temperature**



**Control**



**1kV/cm-  
50 pulses**



**3kV/cm-  
50 pulses**



**5kV/cm-  
50 pulses**



**8kV/cm-  
50 pulses**

## Conclusions

- Non-thermal** technologies: Gentle with pigments and aromatic molecules
- Antimicrobial** effectivity: SO<sub>2</sub> reduction
- Accelerated **phenol extraction**
- Facilitate **new biotechnologies**:
  - Use of non-*Saccharomyces*
  - Coinoculation (Yeast-Bacteria)

# Muchas gracias!

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