Innovations in wine technol. & biotechnol.

Tecnologías emergentes no térmicas y aplicaciones biotecnológicas en enología

Antonio Morata, Catedrático de la **UPM** (química y tecnología de los alimentos)

"Tecnologías emergentes no térmicas y aplicaciones biotecnológicas en enología"

Universidad Politécnica de Madrid
Spain

antonio.morata@upm.es
https://blogs.upm.es/wineprof/antonio-morata/
https://www.researchgate.net/profile/Antonio-Morata
https://www.linkedin.com/in/antonio-morata-barrado-00b07a82/

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Interprofesional del VINO DE ESPAÑA

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Biotech/InstAnalysis/Emerging Tech

enotecUPM

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POLITÉCNICA "Engineering the future"

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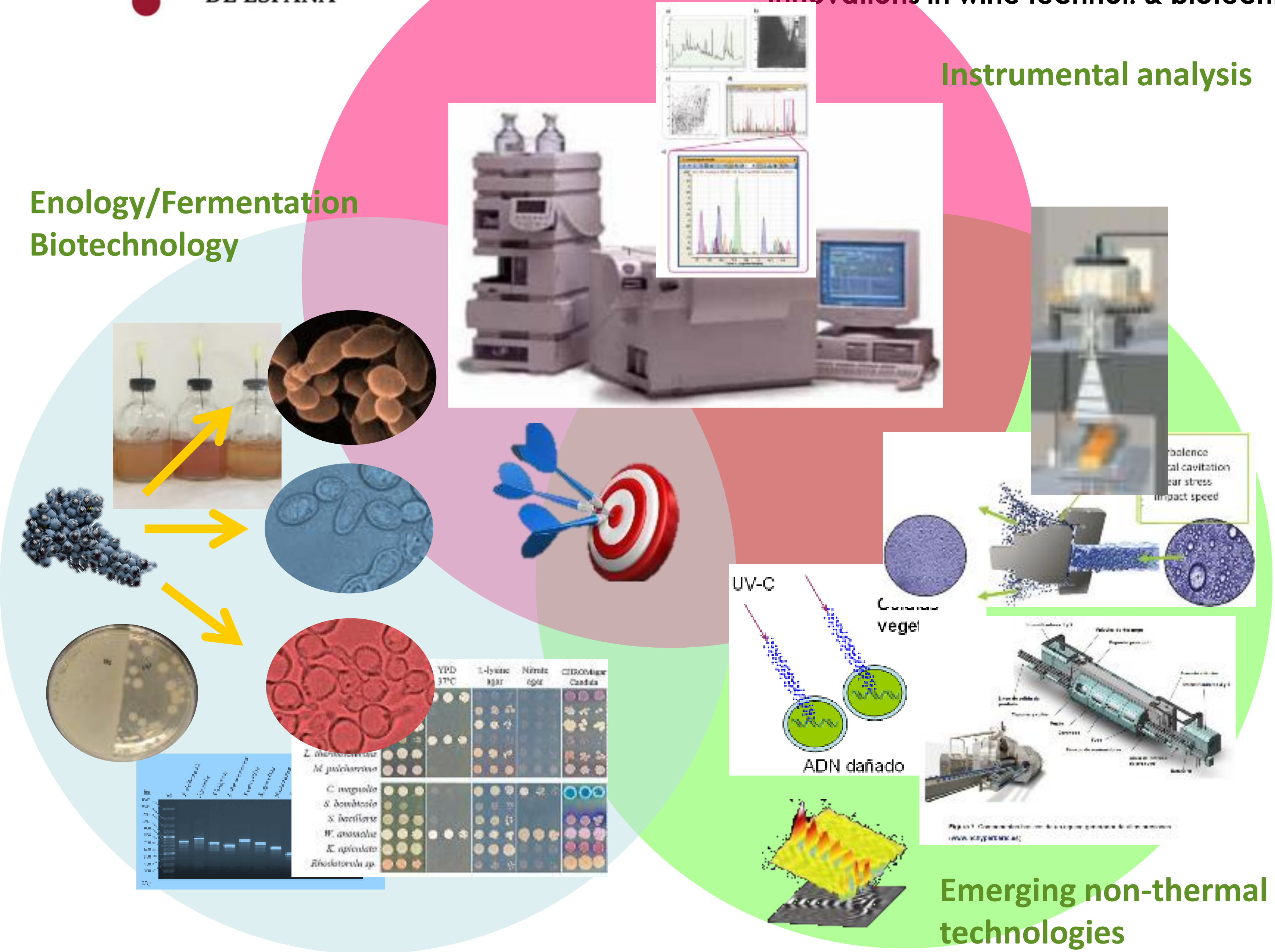
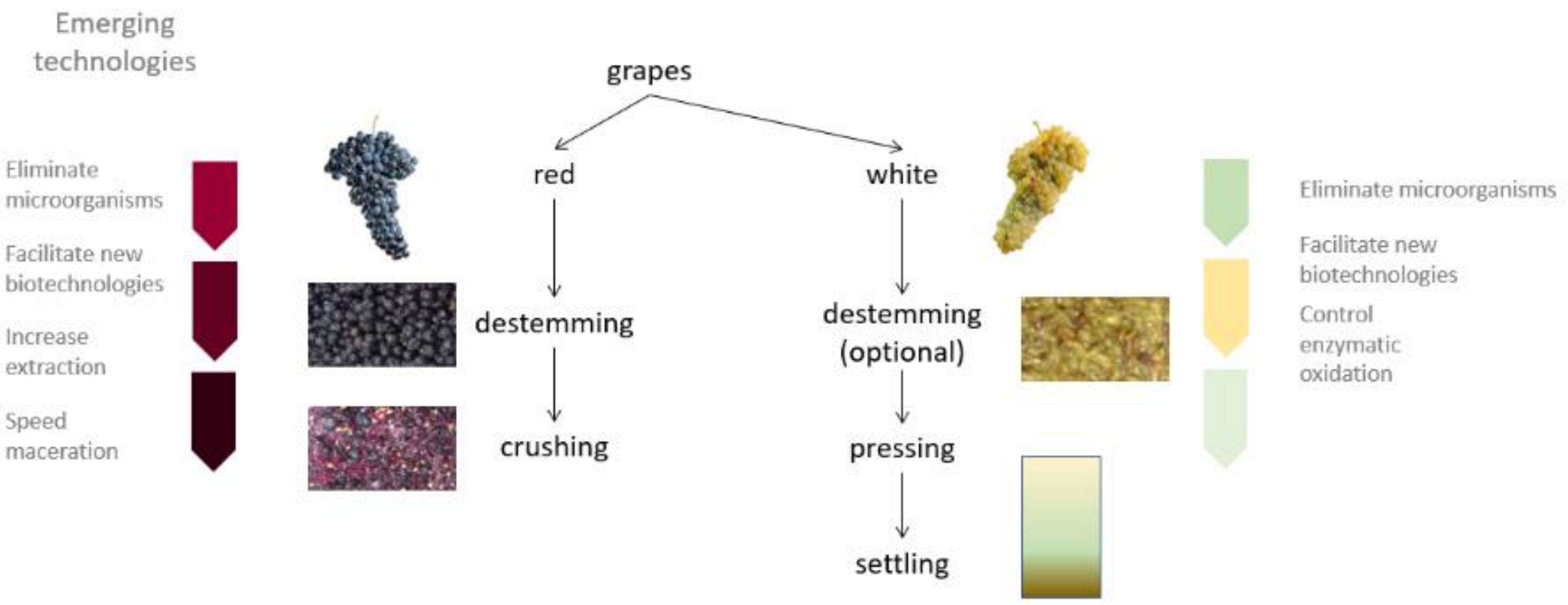
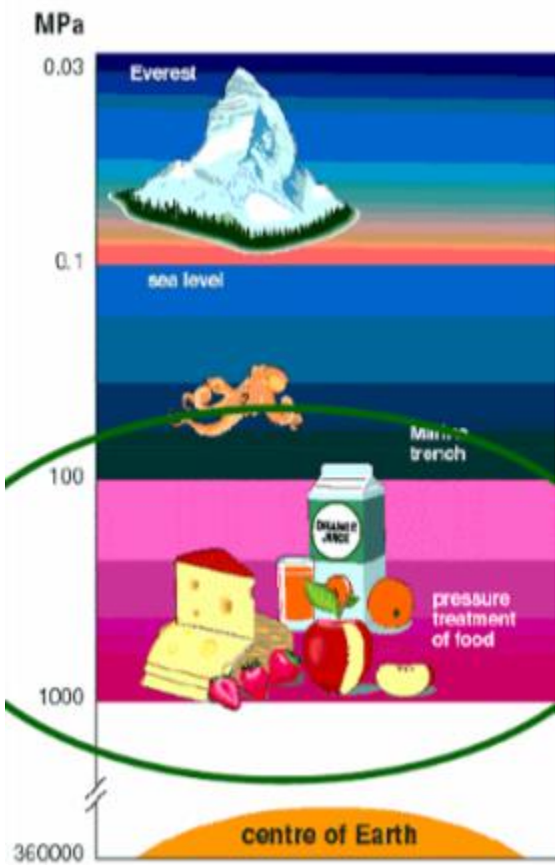


Emerging technologies



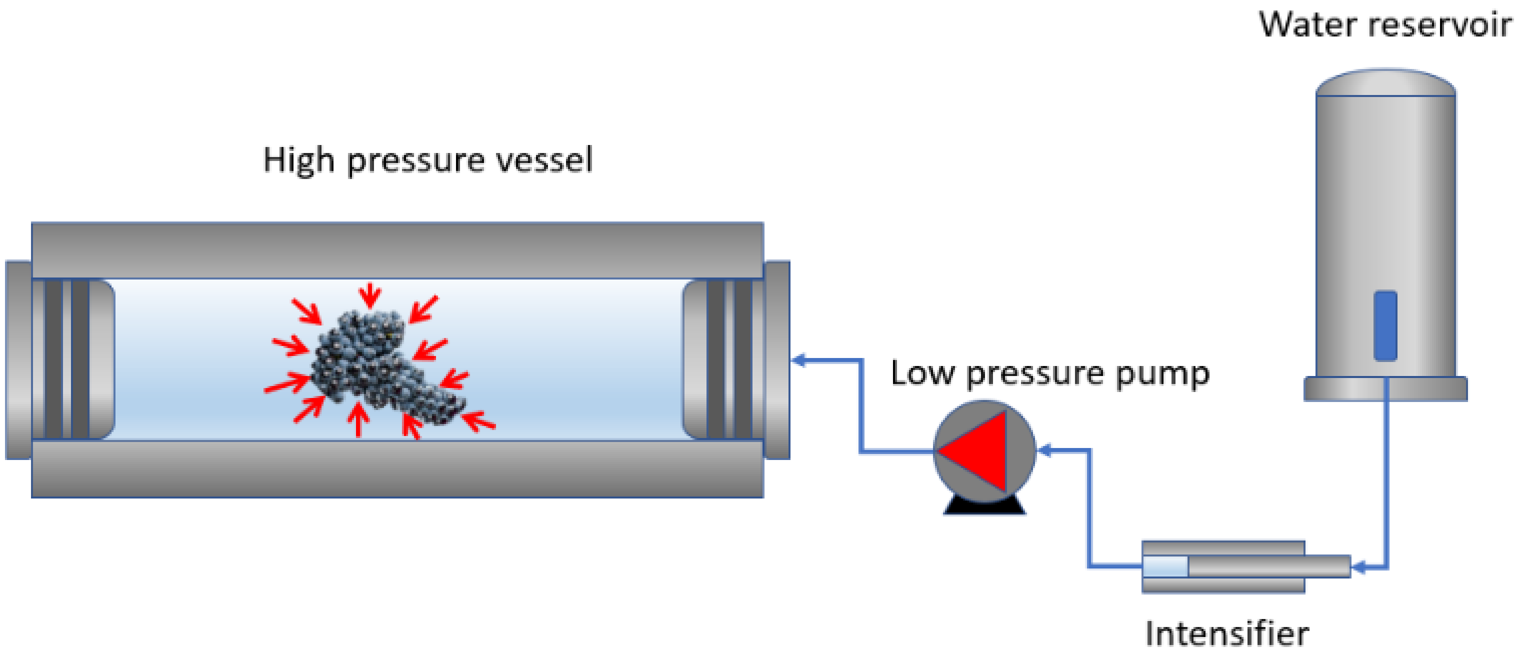
HHP, UHPH, irradiation, PL, PEF, US

HHP. High hydrostatic pressure



HHP. 400-600 MPa ≈ 4000-6000 bar

HPP is a non-thermal process that keep food under high hydrostatic pressure (transmitted by water) reaching up to 600 MPa



RESOLUTION OIV-OENO 594A-2019

REDUCTION OF INDIGENOUS MICROORGANISMS IN GRAPES AND MUSTS BY DISCONTINUOUS HIGH PRESSURE PROCESSES (HIGH HYDROSTATIC PRESSURE – HHP)

THE GENERAL ASSEMBLY,

IN VIEW of article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

ON THE PROPOSAL of the "Microbiology" expert group,

CONSIDERING the importance of new physical preservation technologies able to protect the sensory properties of grapes and also allowing for a reduction in SO_2 levels,

CONSIDERING that new fermentation biotechnologies like the use of non-*Saccharomyces* yeasts or the application of co-inoculations with lactic-acid bacteria and yeasts to perform simultaneous malolactic and alcoholic fermentations can be promoted by the reduction of initial counts of indigenous microorganisms in grapes,

CONSIDERING the work of the "Technology" and "Microbiology" expert groups,

CONSIDERING that high hydrostatic pressure (HHP) can be applied to reduce wild yeast and

Presenta una capacidad de producción de hasta 50 equipos HPP por año. Existen más de 60 equipos industriales de altas presiones en producción distribuidos mundialmente. NC Hyperbaric ha instalado el 35% de los equipos de altas presiones en producción a nivel mundial, y el 80% desde 2005 (**Figura 40**).

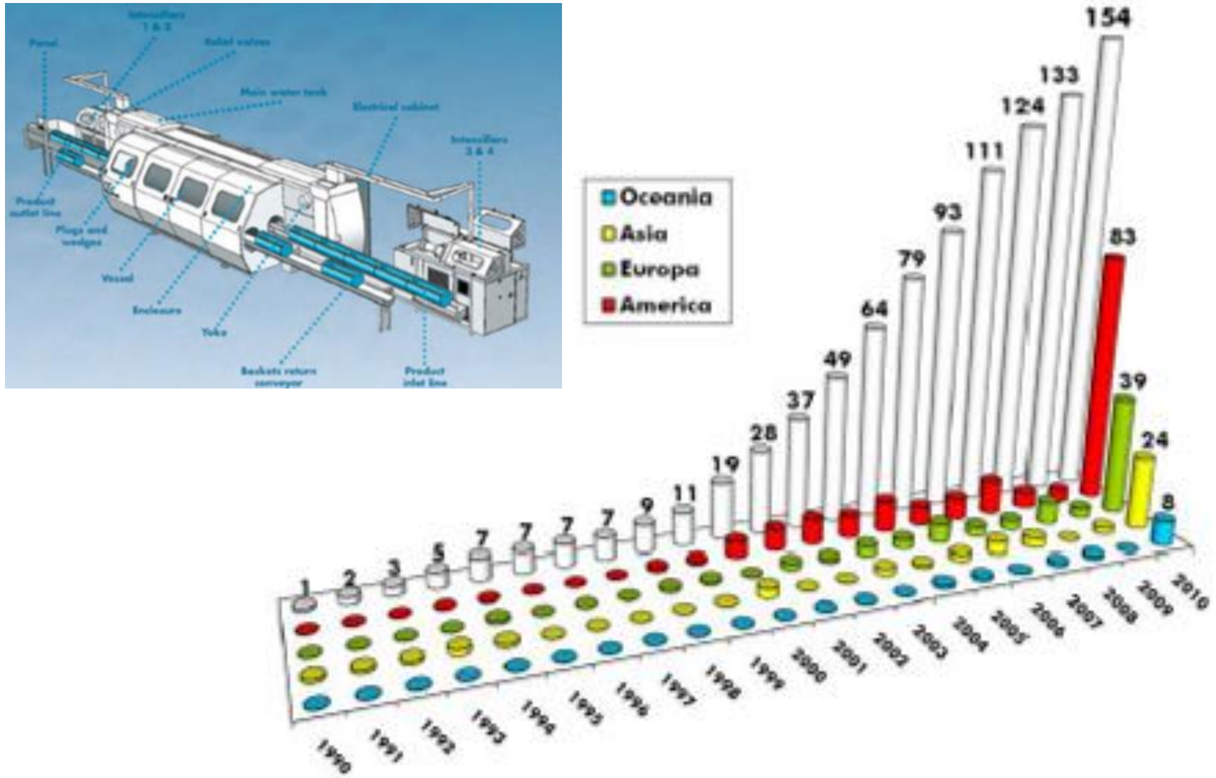
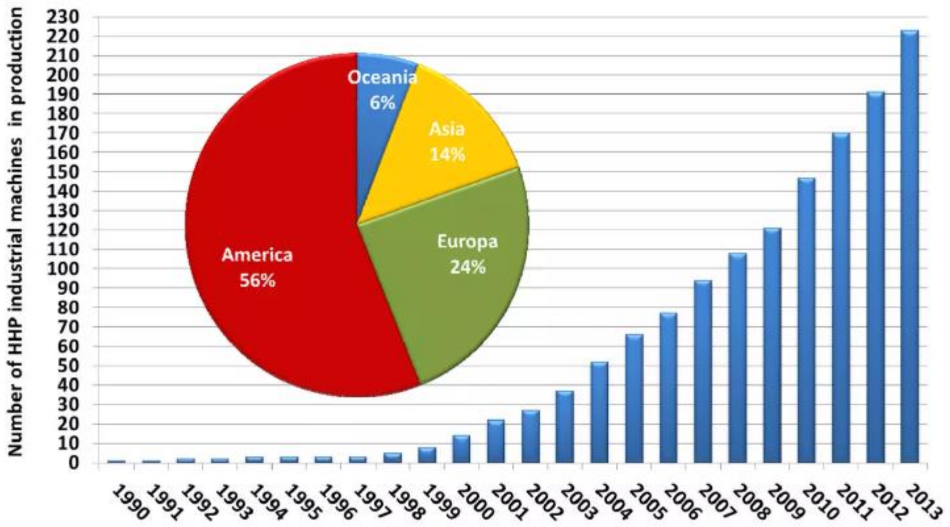


Figura 40. Número de equipos industriales versus año de instalación y continentes (NC Hyperbaric, 2010).

Hiperbaric

Evolución del total de equipos HPP instalados en el mundo



Número total de máquinas en producción a mediados de 2013:222

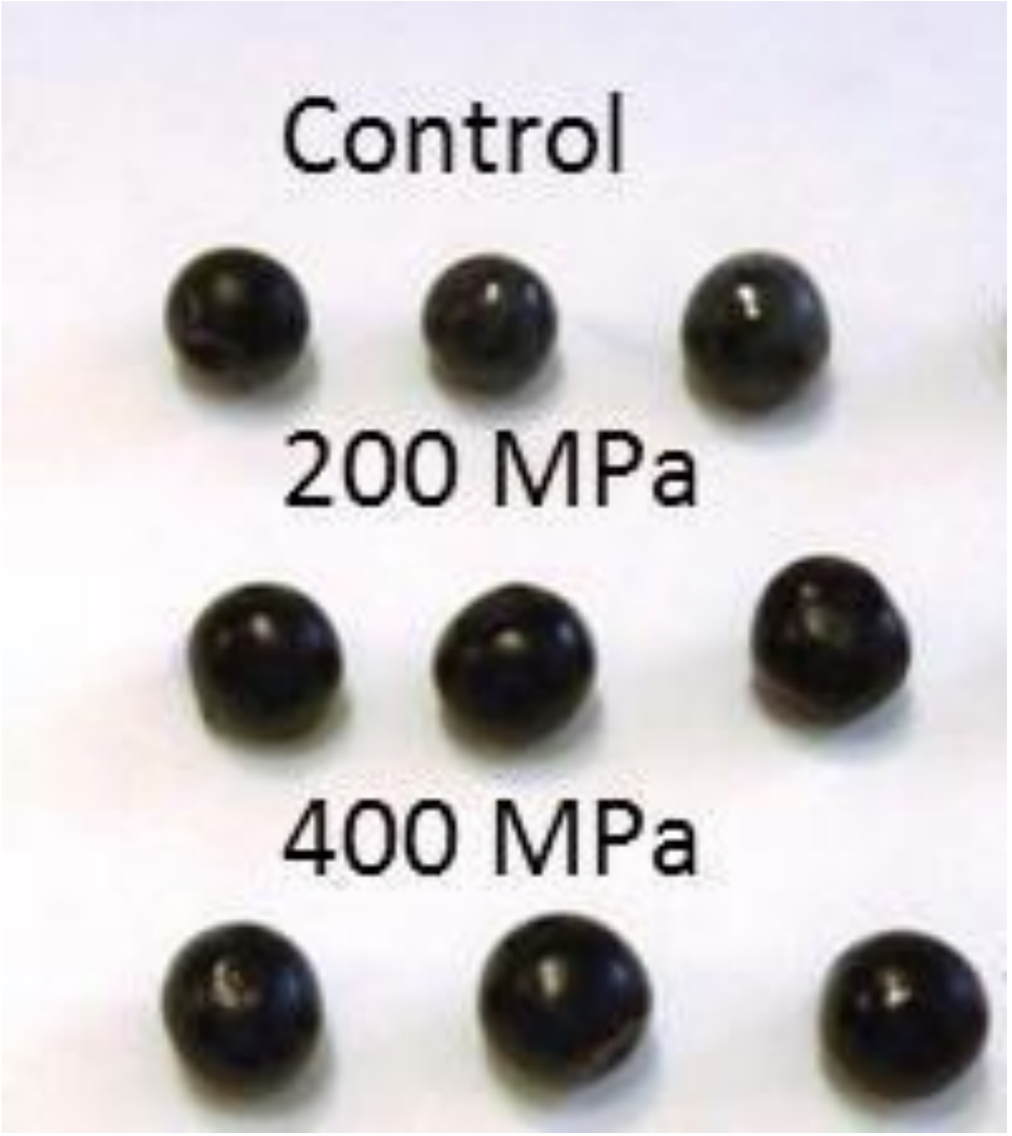
Improving microbiological quality

HHP.

- ΔTª adiabatic compression 2-3 ºC/100 Mpa

- Pressurization do not affect covalent bonds. Protect sensory quality.

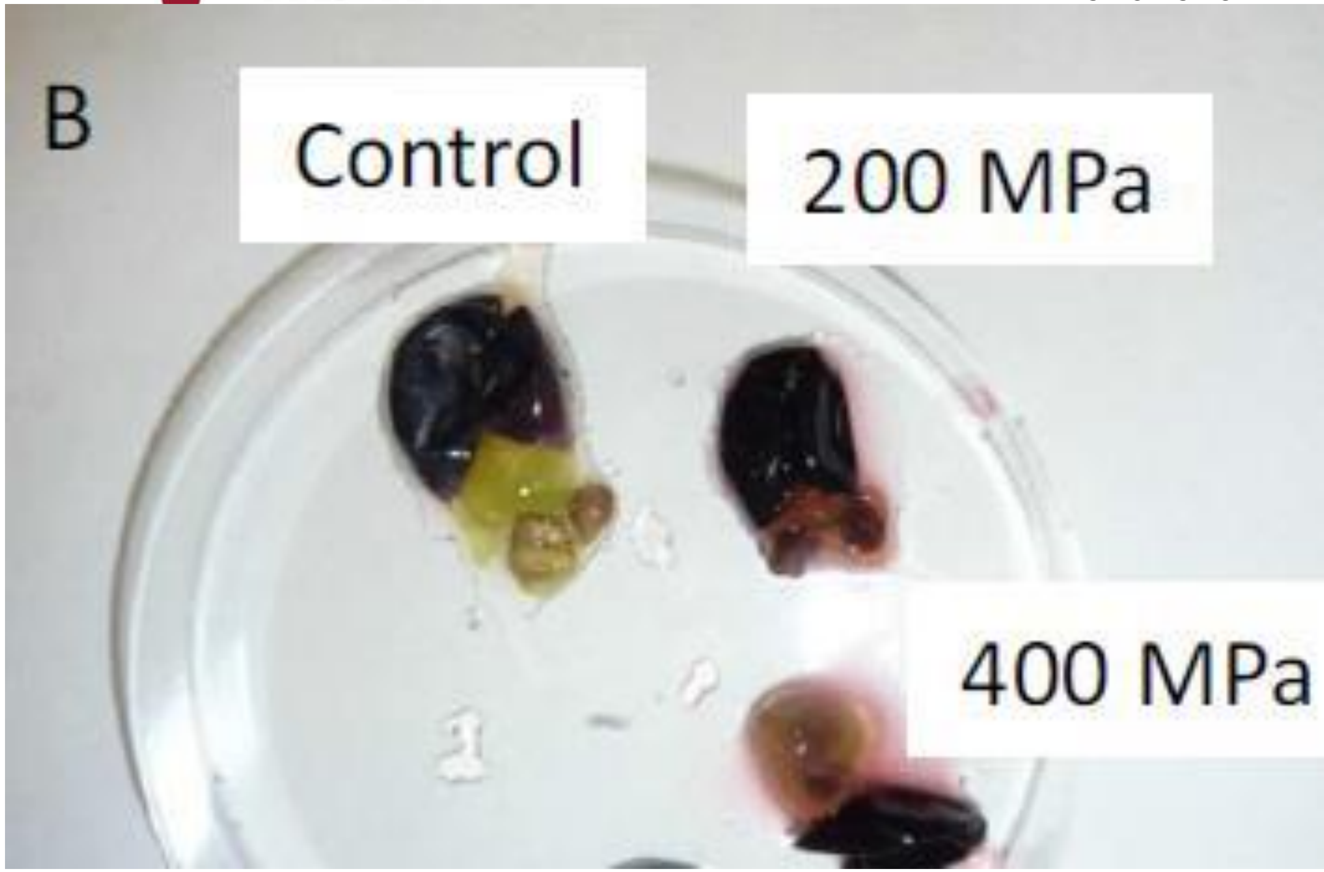
-Water is liquid at -20 ºC / 200 MPa



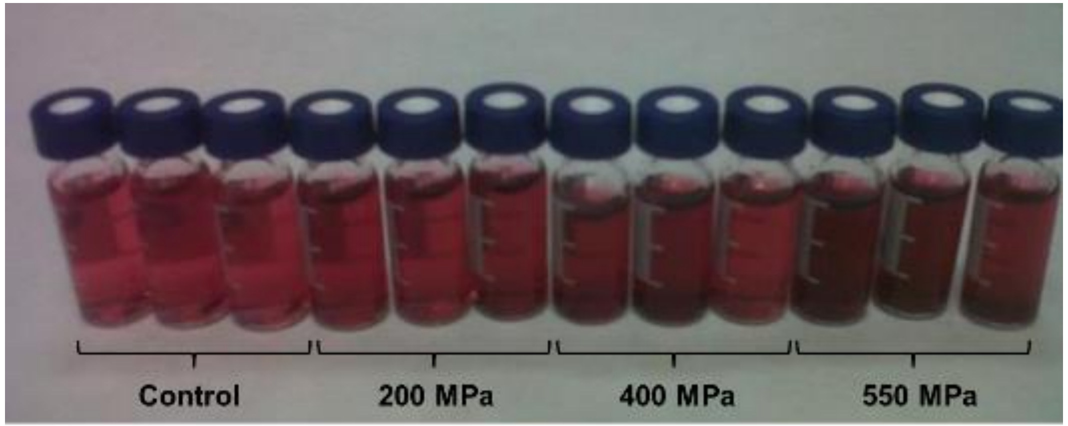
External shape and color unaffected

Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., Suárez-Lepe, J. A. Grape Processing by High Hydrostatic Pressure: Effect on Microbial Populations, Phenol Extraction and Wine Quality. Food Bioprocess Technol. Food and Bioprocess Technology 2015, 8, 277-286.

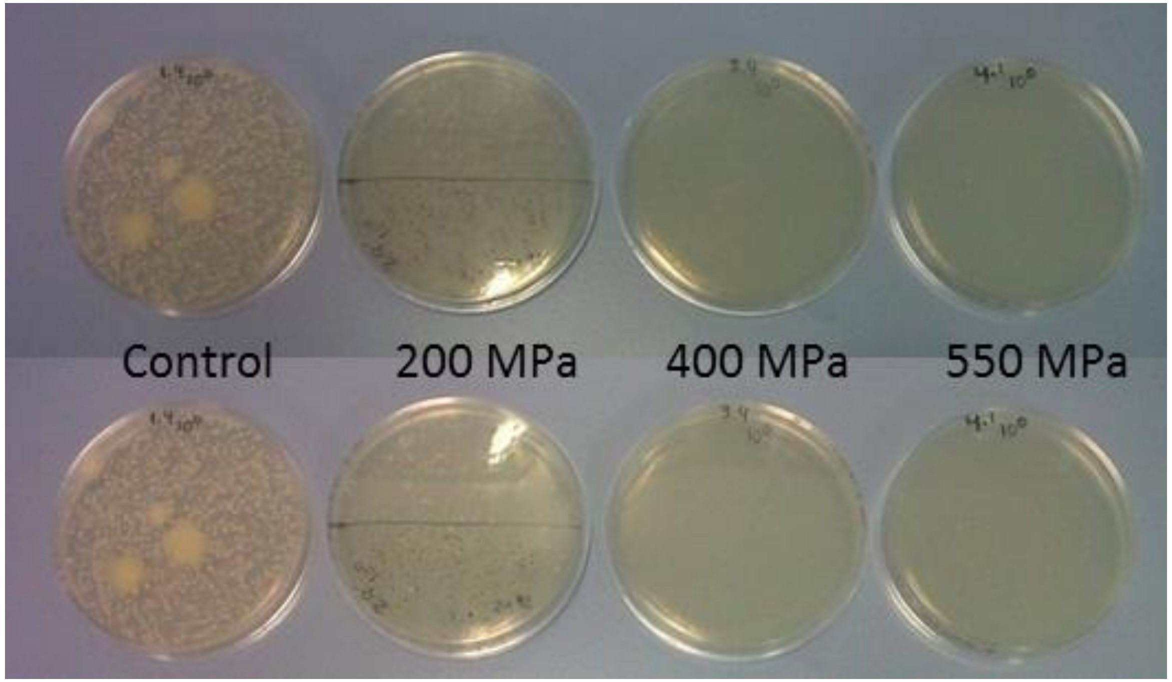
Innovations in wine technol. & biotechnol.



Phenol extraction

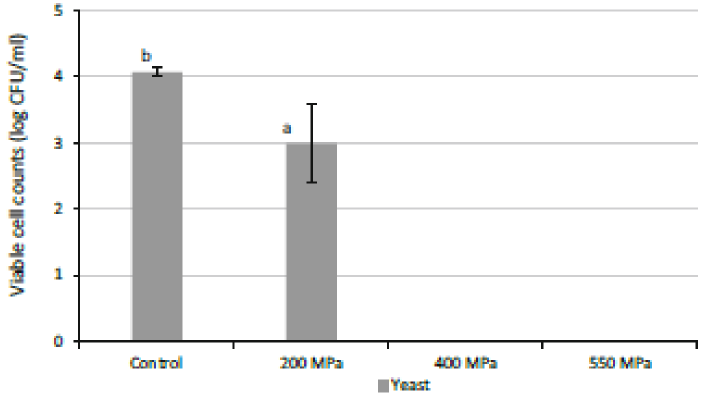


Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., Suárez-Lepe, J. A. Grape Processing by High Hydrostatic Pressure: Effect on Microbial Populations, Phenol Extraction and Wine Quality. Food Bioprocess Technol. Food and Bioprocess Technology 2015, 8, 277-286.



Food Bioprocess Technol

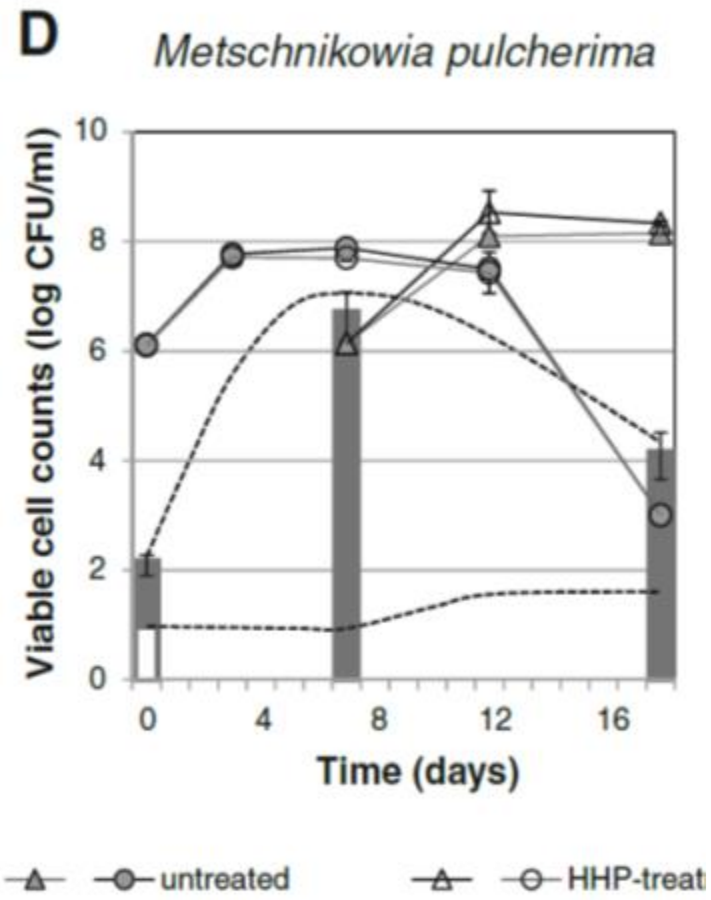
Fig. 3 Microbial counts in crushed control and HHP-treated grapes at the beginning of fermentation. a Yeast. b Bacteria. Values are means±standard deviations of four replicates. *Different letters* in the same series indicate significant differences between means ($p<0.05$)

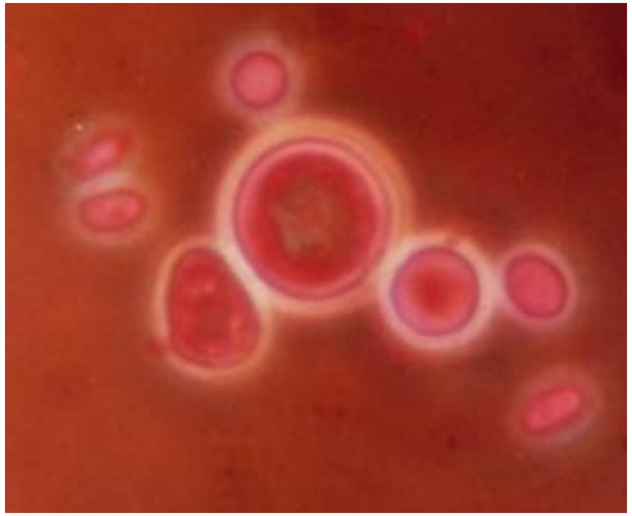


microbiological quality

Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., Suárez-Lepe, J. A. Grape Processing by High Hydrostatic Pressure: Effect on Microbial Populations, Phenol Extraction and Wine Quality. Food Bioprocess Technol. Food and Bioprocess Technology 2015, 8, 277-286.

Metschnikowia pulcherrima





Food Bioprocess Technol (2016) 9:1769–1778
DOI 10.1007/s11947-016-1760-8

ORIGINAL PAPER

Grape Processing by High Hydrostatic Pressure: Effect on Use of Non-*Saccharomyces* in Must Fermentation

Maria Antonia Bañuelos[1] · Iris Loira[2] · Carlos Escott[2] · Juan Manuel Del Fresno[2] · Antonio Morata[2] · Pedro D. Sanz[3] · Laura Otero[3] · Jose Antonio Suárez-Lepe[2]

UHPH. Ultra High-Pressure Homogenization

YPSICON

SEAL OF EXCELLENCE
European Commission
PYME INNOVADORA

PTV PLATAFORMA TECNOLÓGICA DEL VINO

Interprofesional del VINO DE ESPAÑA

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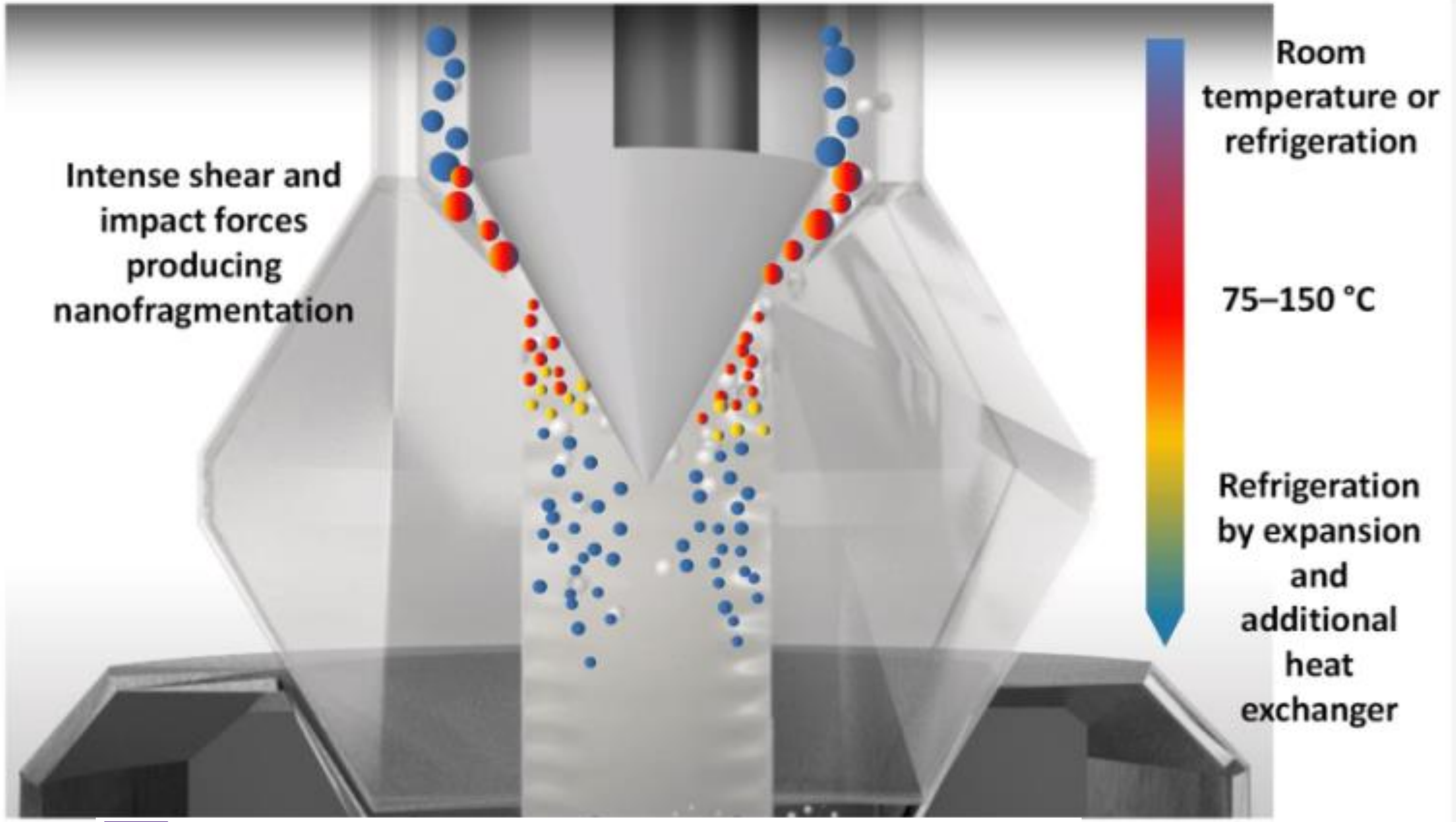
Figure 3. Effect of impact and shear forces on colloid nanofragmentation and temperature in a UHPH valve (adapted from **http://www.ypsicon.com/** (accessed on 15 July 2023)).



-Pasteurization/sterilization
-Nano-fragmentation
-Enzyme inactivation
-Nano-covering
-Nano-encapsulation

3xsound speech (Mach 3)

antioxidants MDPI

Review

Effect of HHP and UHPH High-Pressure Techniques on the Extraction and Stability of Grape and Other Fruit Anthocyanins

Antonio Morata [1,*], Juan Manuel del Fresno [1], Mohsen Gavahian [2], Buenaventura Guamis [3], Felipe Palomero [1] and Carmen López [1]

https://doi.org/10.3390/antiox12091746

RESOLUTION OIV-OENO 594B-2020

ELIMINATION OF WILD MICROORGANISMS IN MUSTS BY CONTINUOUS HIGH PRESSURE PROCESSES (ULTRA HIGH PRESSURE HOMOGENISATION – UHPH)

THE GENERAL ASSEMBLY,

IN VIEW OF THE ARTICLE 2, paragraph 2 b) iv of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

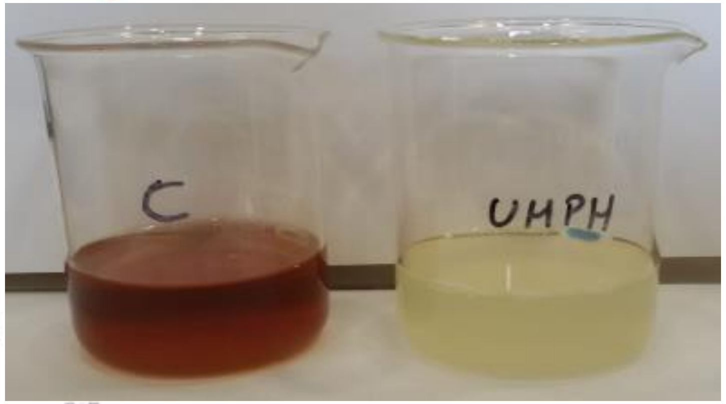
AT THE PROPOSAL of the "Microbiology" Expert Group,

CONSIDERING the importance of new physical preservation technologies able to protect the sensory properties of musts and also allowing for a reduction in SO_2 doses,

CONSIDERING that new fermentation biotechnologies like the use of non-*Saccharomyces* yeasts or the application of co-inoculations with LABs and yeasts to perform simultaneous malolactic and alcoholic fermentations can be favored by the reduction of initial counts of wild microorganisms in musts,

CONSIDERING the work of the of "Technology" and "Microbiology" Expert Groups,

CONSIDERING that ultra high pressure homogenization (UHPH) can be applied to strongly decrease or eliminate wild yeast and bacteria populations in musts,



Food Chemistry 332 (2020) 127417
Contents lists available at ScienceDirect
Food Chemistry
journal homepage: www.elsevier.com/locate/foodchem

White wine processing by UHPH without SO_2. Elimination of microbial populations and effect in oxidative enzymes, colloidal stability and sensory quality

Mª Antonia Bañuelos[a], Iris Loira[b], Buenaventura Guamis[c], Carlos Escott[b], Juan Manuel Del Fresno[b], Idoia Codina-Torrella[c], Joan Miquel Quevedo[d], Ramon Gervilla[d], Jesús María Rodríguez Chavarría[c], Sergi de Lamo[e], Raúl Ferrer-Gallego[f], Rocío Álvarez[a], Carmen González[b], José Antonio Suárez-Lepe[b], Antonio Morata[b,*]

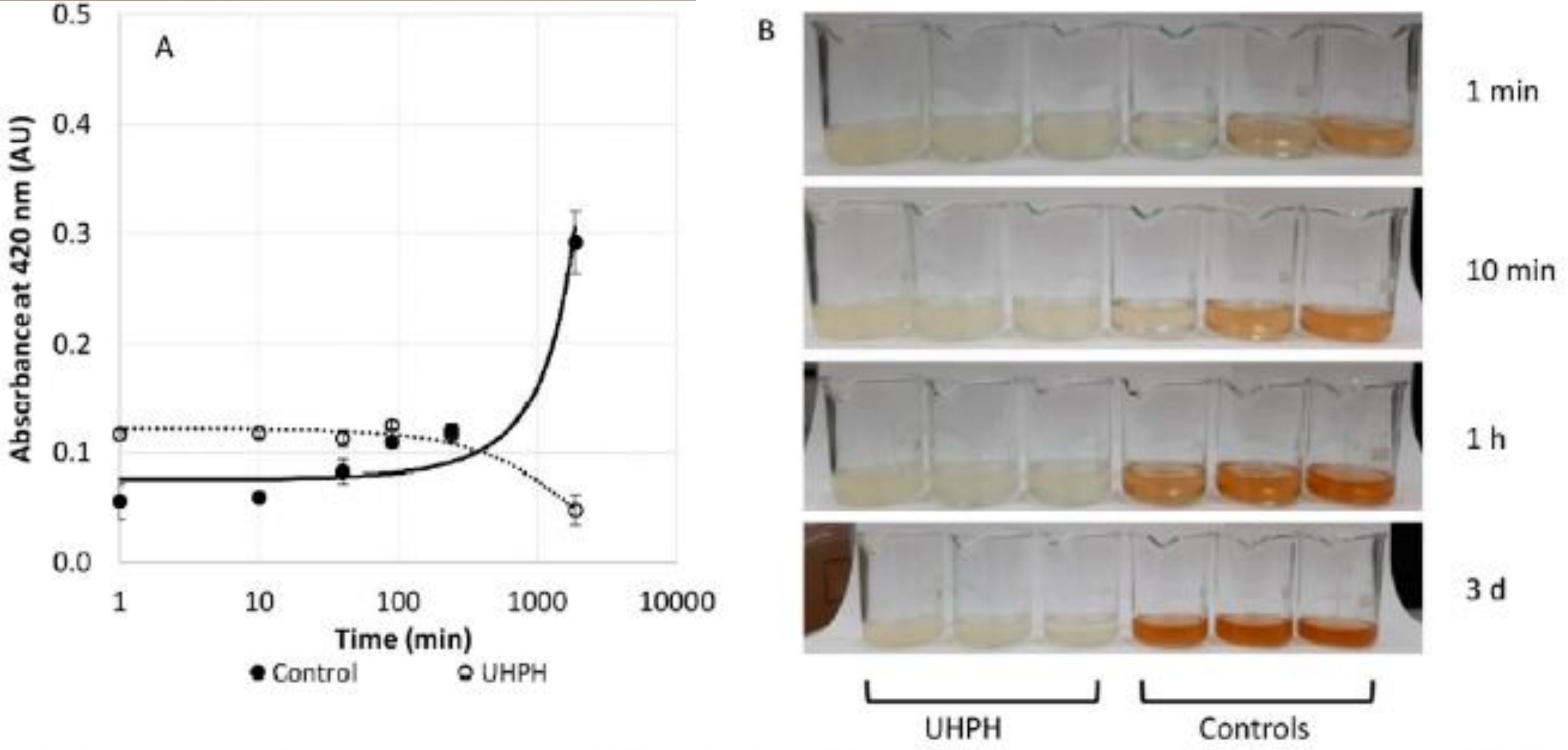


Fig. 2. Absorbance at 420 nm in control and UHPH-processed musts (a) and colour changes by enzymatic oxidative browning in triplicate (b), UHPH-processed (left) and controls (right).

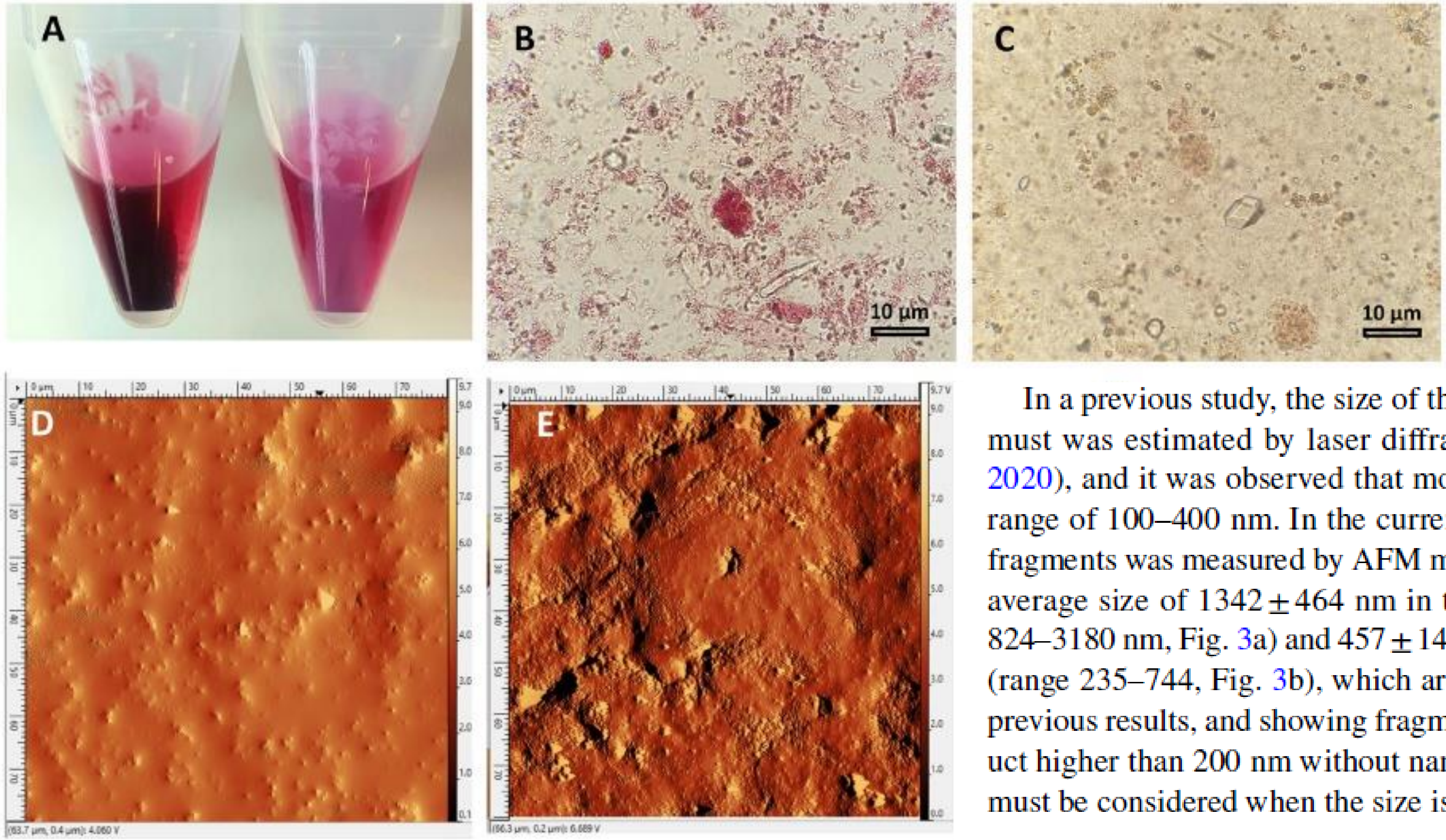


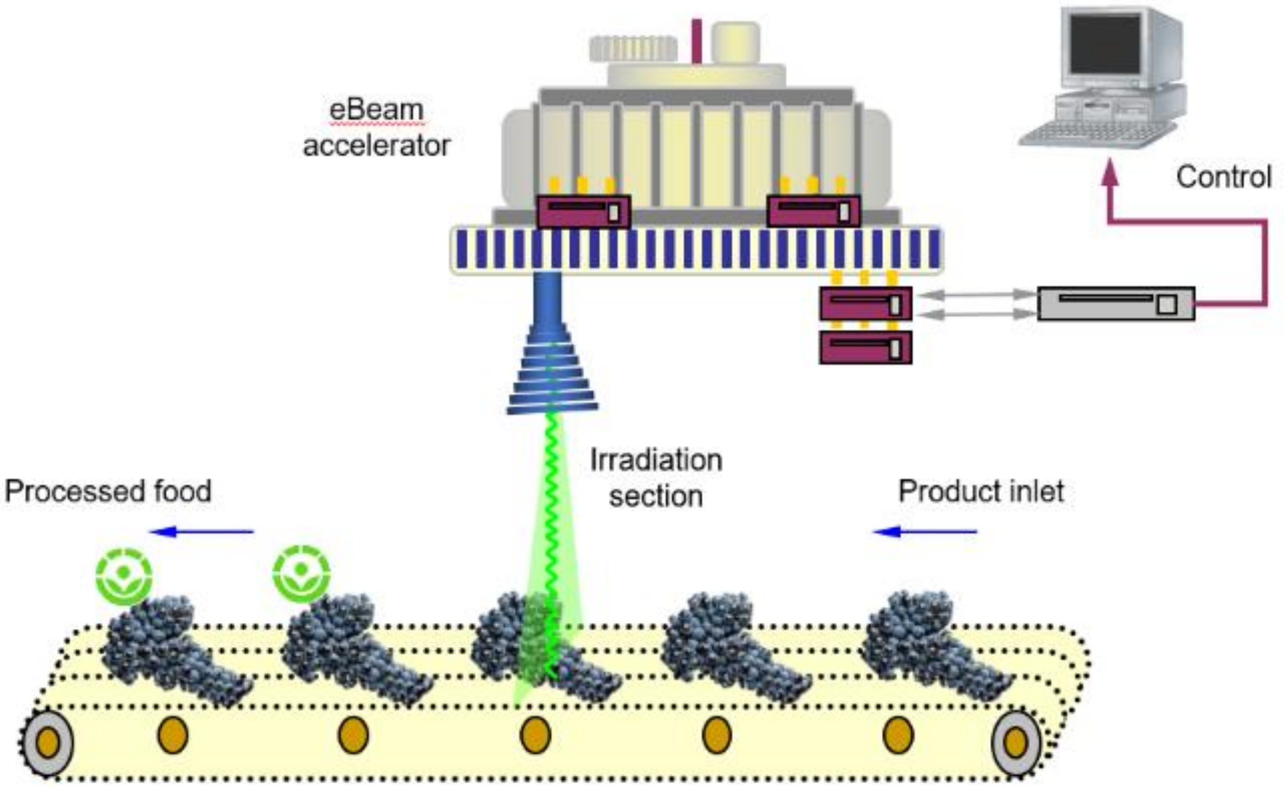
Fig. 2 External appearance of the control and UHPH-processed must after centrifugation **A**, optical microscopy (600×) of a centrifuged drop of cabernet sauvignon must **B**, optical microscopy (600×) of the UHPH centrifuged must **C**, AFM scanning in resonant mode of a drop of dried cor graphic renders of UHPH dried drops

In a previous study, the size of the colloids in the UHPH must was estimated by laser diffraction (Bañuelos et al., 2020), and it was observed that most particles were in the range of 100–400 nm. In the current study, the size of the fragments was measured by AFM microscopy, obtaining an average size of 1342 ± 464 nm in the control must (range 824–3180 nm, Fig. 3a) and 457 ± 140 nm in the UHPH must (range 235–744, Fig. 3b), which are relatively close to our previous results, and showing fragments in the UHPH product higher than 200 nm without nano-safety impact (which must be considered when the size is < 100 nm).

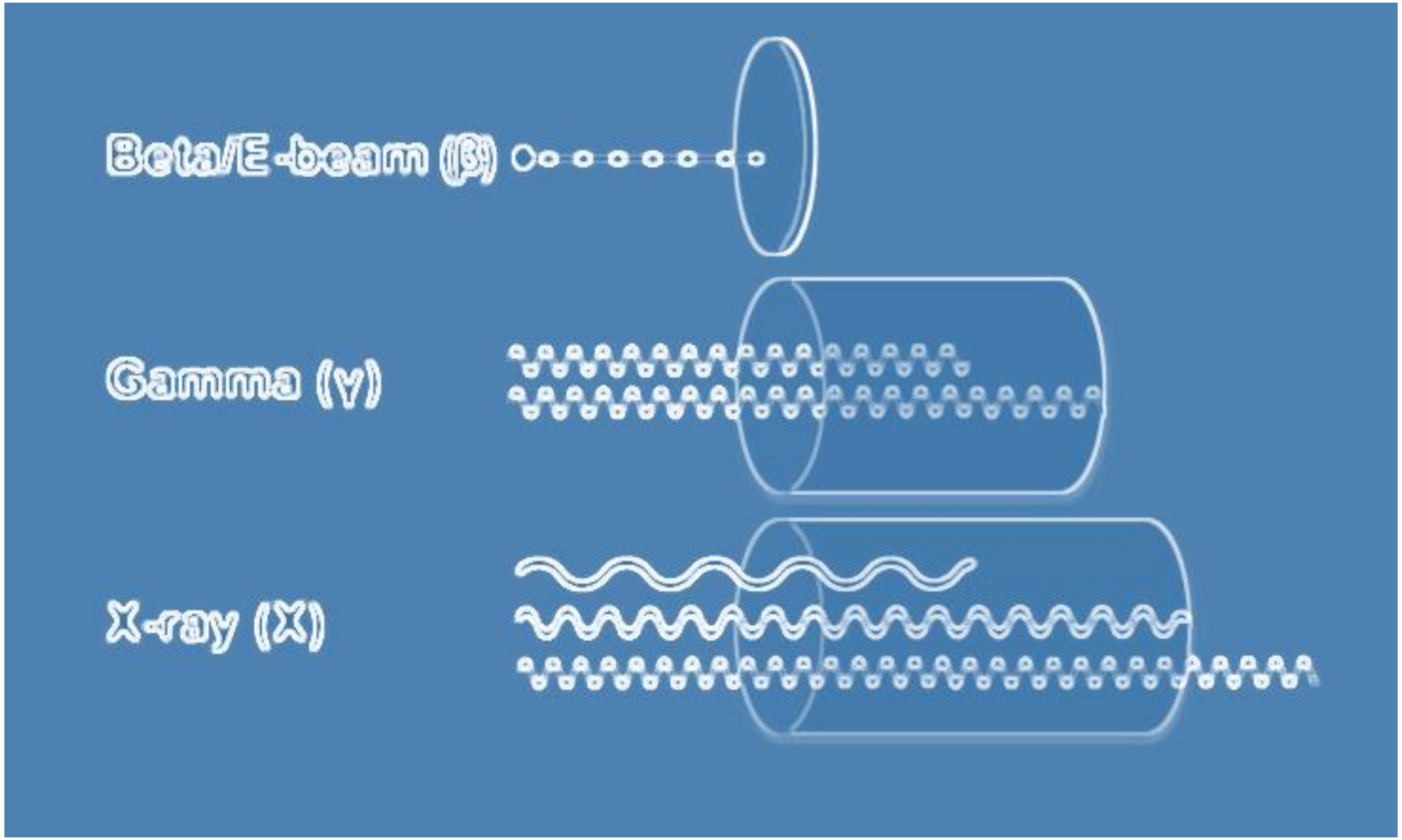
https://doi.org/10.1007/s11947-022-02766-8

Springer

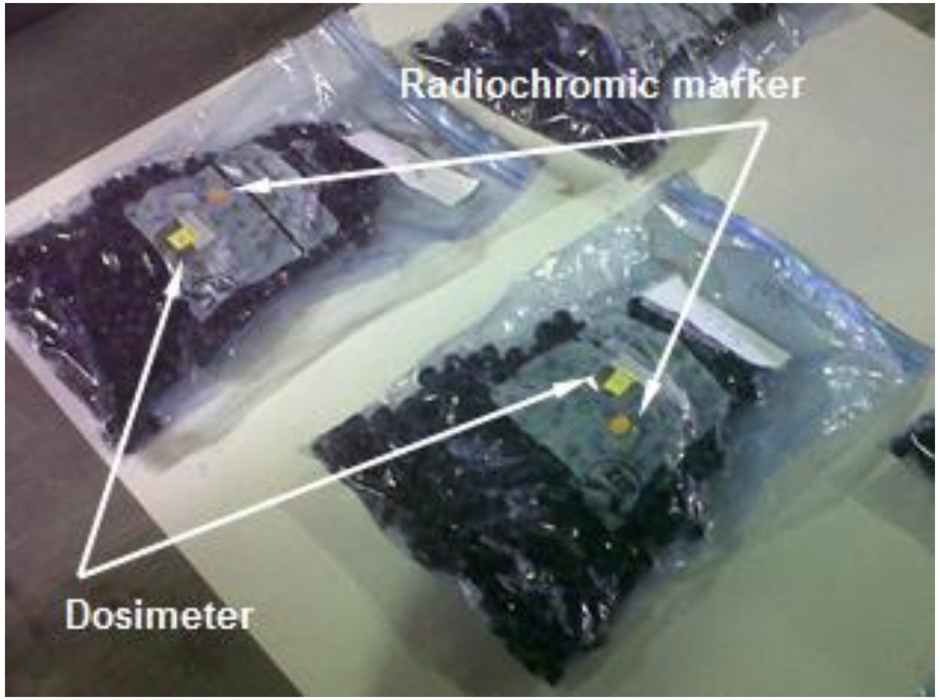
e-Beam irradiation

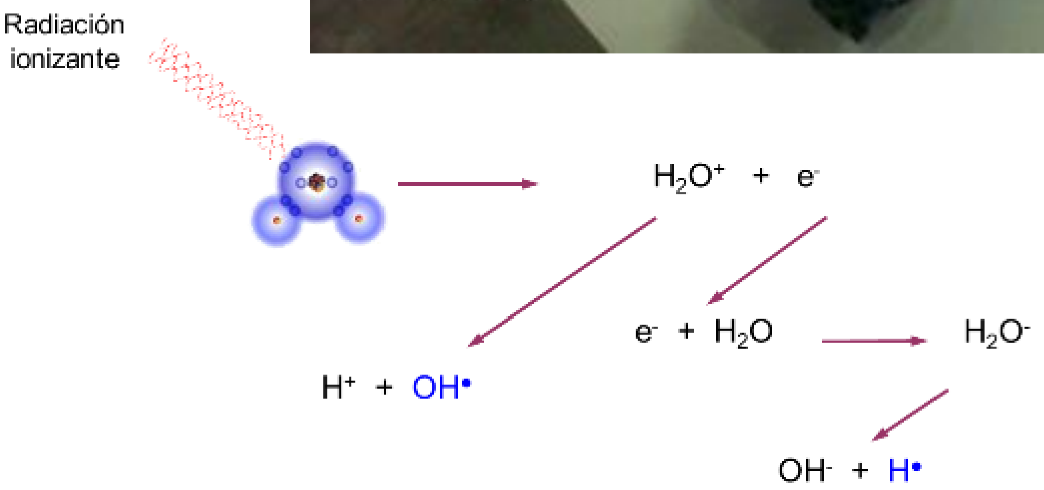


e-beam irradiation



e-beam irradiation





e-beam irradiation

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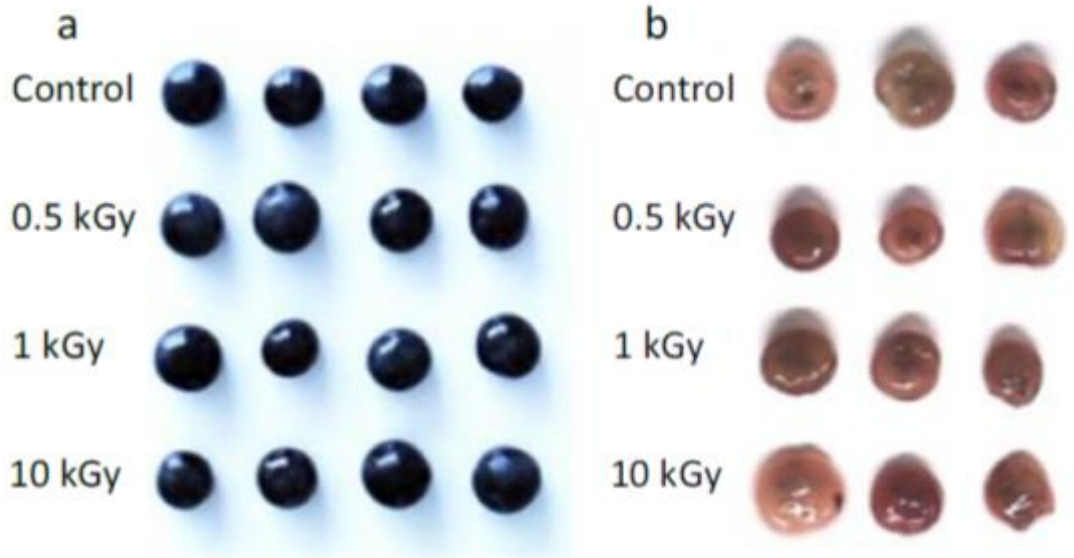
Fig. 1 Appearance of irradiated grapes. a External surface. b Peeled



Food Bioprocess Technol
DOI 10.1007/s11947-015-1540-x

ORIGINAL PAPER

Electron Beam Irradiation of Wine Grapes: Effect on Microbial Populations, Phenol Extraction and Wine Quality

Antonio Morata[1] · María Antonia Bañuelos[2] · Wendu Tesfaye[1] · Iris Loira[1] · Felipe Palomero[1] · Santiago Benito[1] · María Jesús Callejo[1] · Ana Villa[2] · M. Carmen González[1] · Jose Antonio Suárez-Lepe[1]

e-beam irradiation

Food Bioprocess Technol
DOI 10.1007/s11947-015-1540-x

ORIGINAL PAPER

Electron Beam Irradiation of Wine Grapes: Effect on Microbial Populations, Phenol Extraction and Wine Quality

Antonio Morata[1] · María Antonia Bañuelos[2] · Wendu Tesfaye[1] · Iris Loira[1] · Felipe Palomero[1] · Santiago Benito[1] · María Jesús Callejo[1] · Ana Villa[2] · M. Carmen González[1] · Jose Antonio Suárez-Lepe[1]

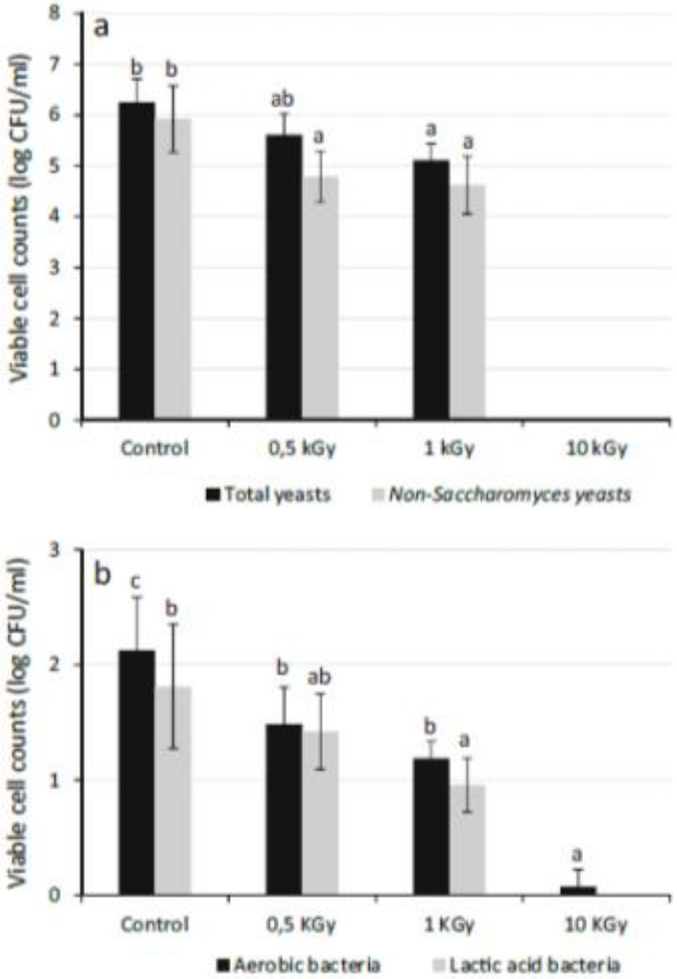
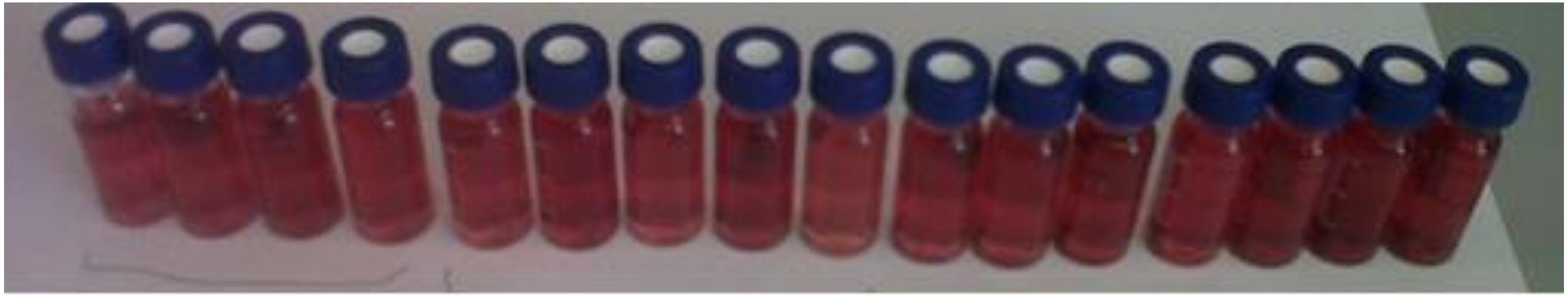


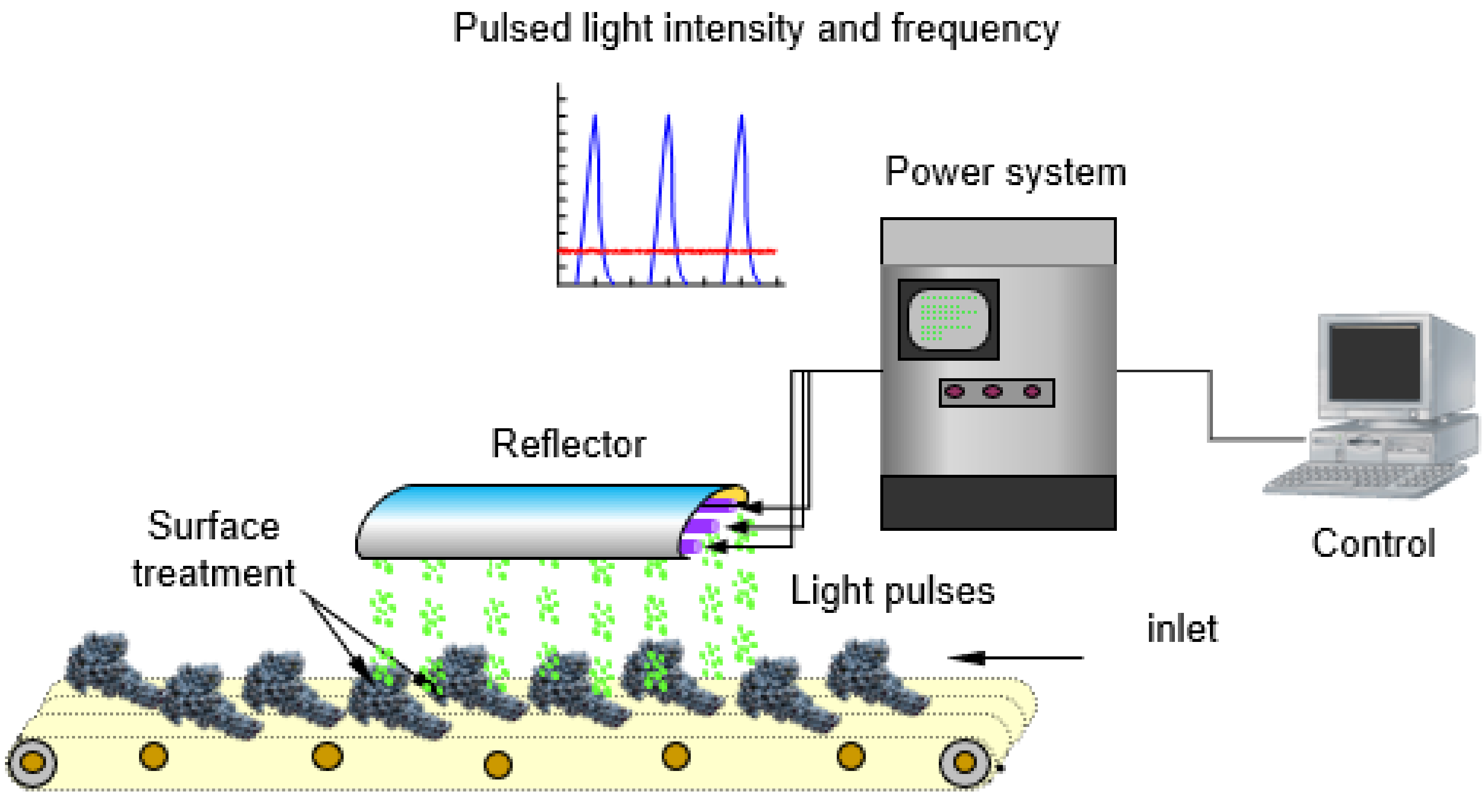
Fig. 2 Microbial counts (viable cells) in the must from crushed control and irradiated grapes (log cfu/mL). **a** Yeasts. **b** Bacteria. *Different letters in the same series* indicate significant differences between means ($p<0.05$)

e-beam irradiation



Control 0,5 kGy 1 kGy 10 kGy

Pulsed Light



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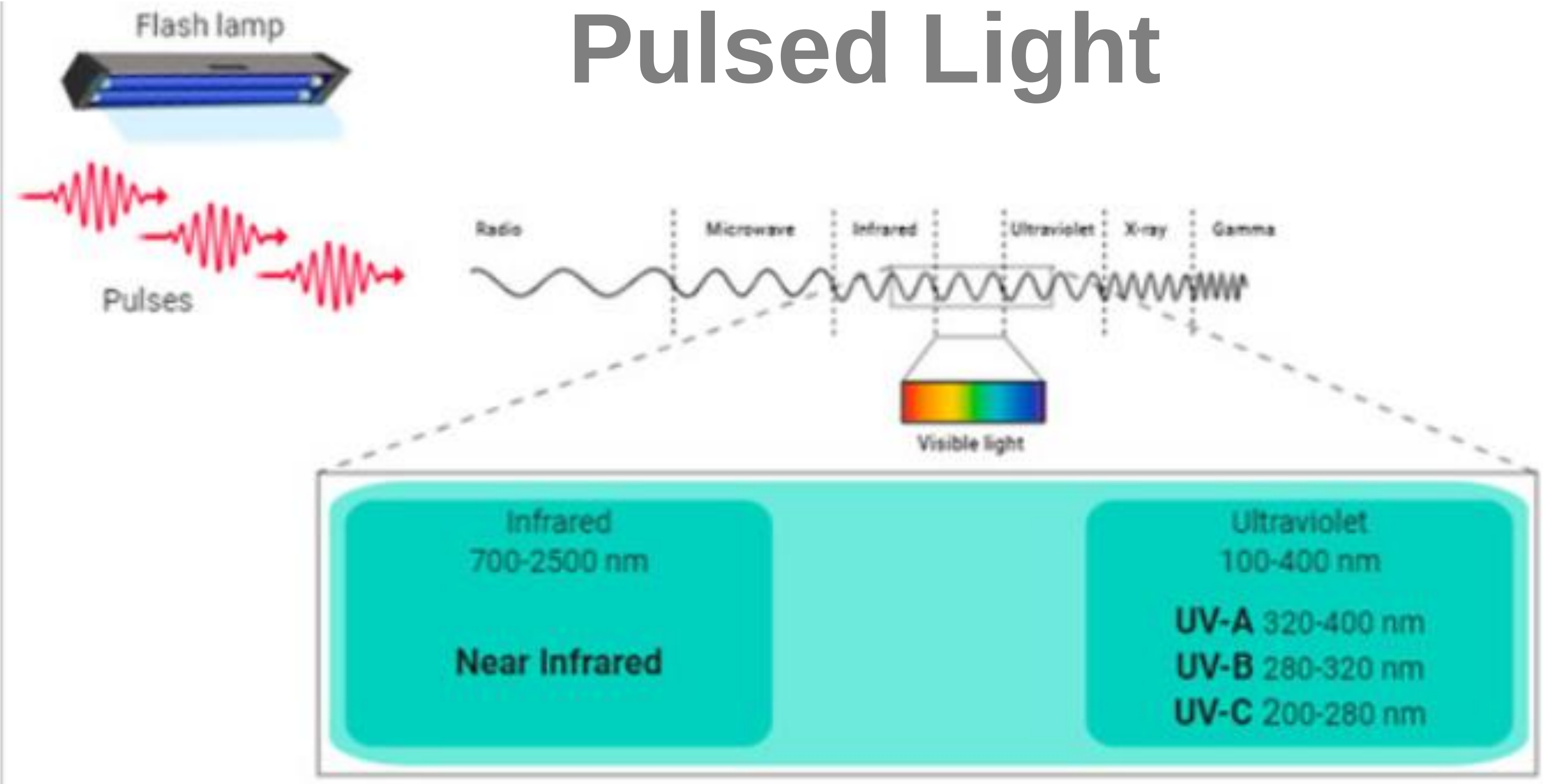
Pulsed Light



https://doi.org/10.3390/beverages6030045


beverages

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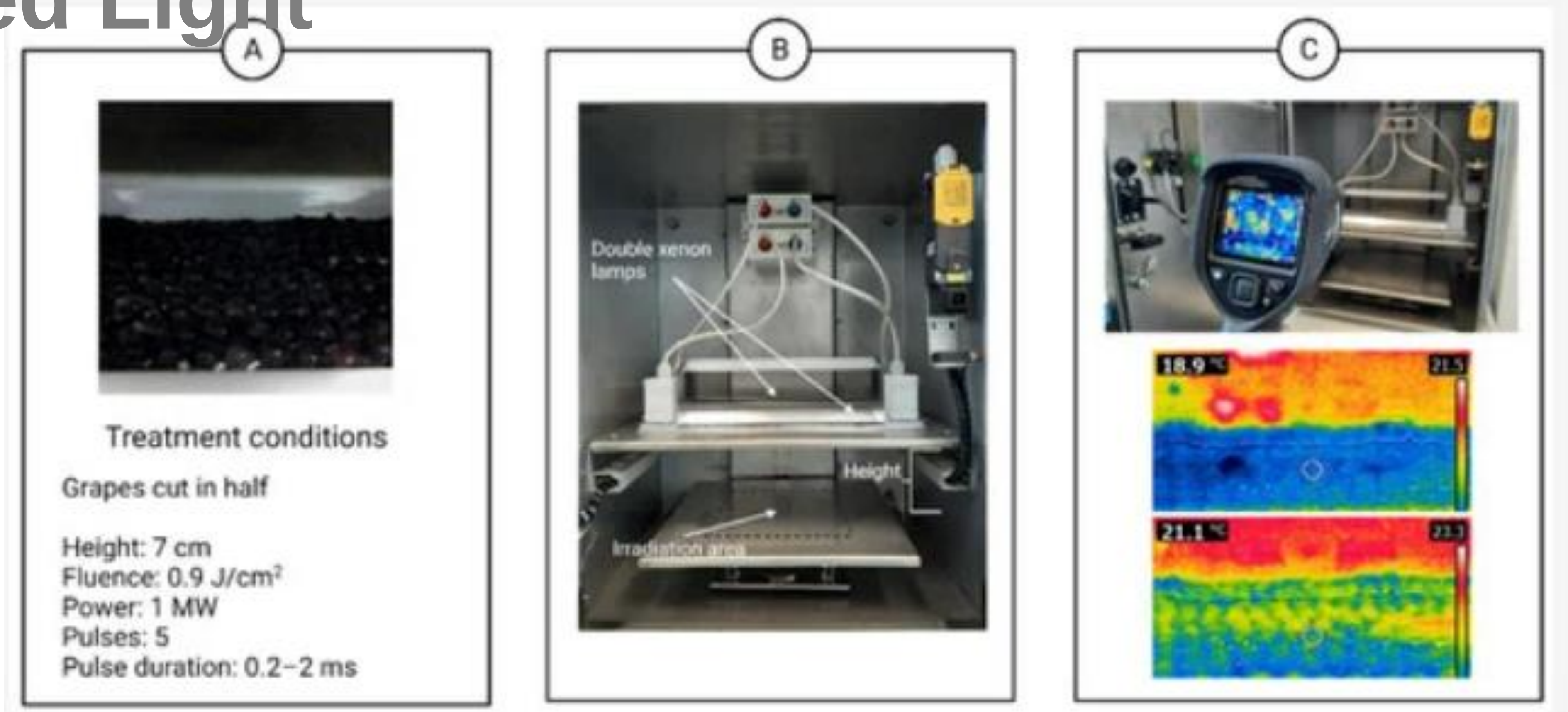
Pulsed Light: Challenges of a Non-Thermal Sanitation Technology in the Winemaking Industry

by Aitana Santamera, Carlos Escott, Iris Loira, Juan Manuel del Fresno, Carmen González and Antonio Morata *

EnotecUPM, Chemistry and Food Technology Department, Universidad Politécnica de Madrid, Avenida Puerta de Hierro 2, 28040 Madrid, Spain

Pulsed Light

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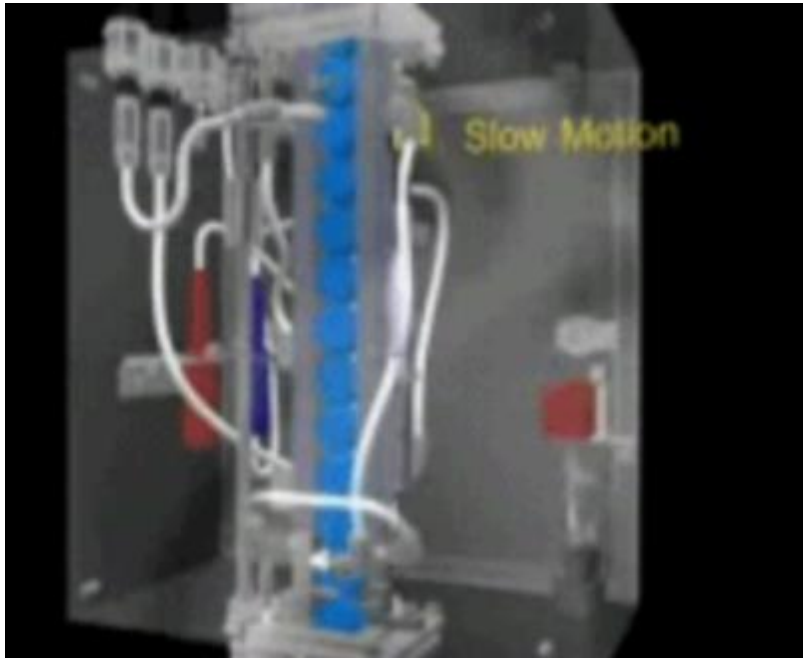
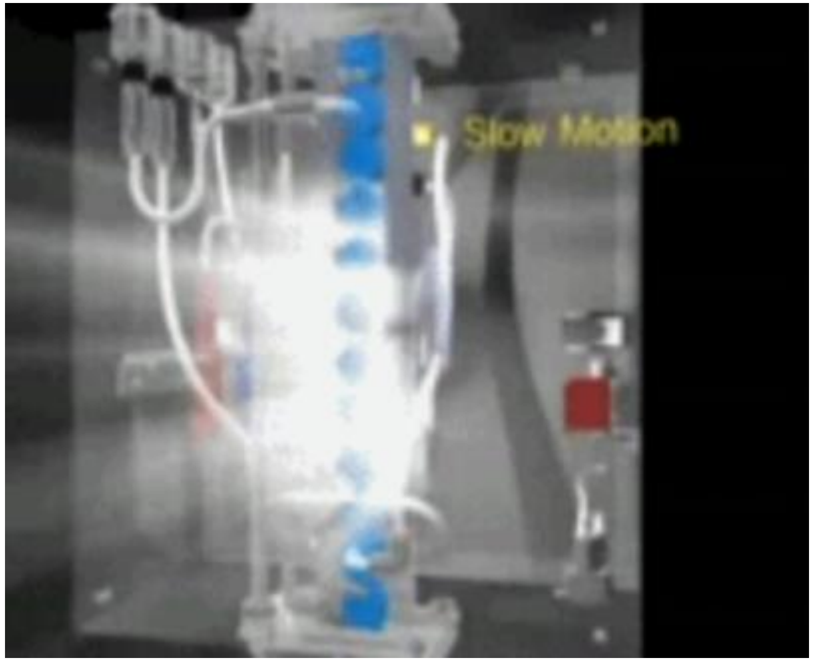
Improvement of Must Fermentation from Late Harvest cv. Tempranillo Grapes Treated with Pulsed Light

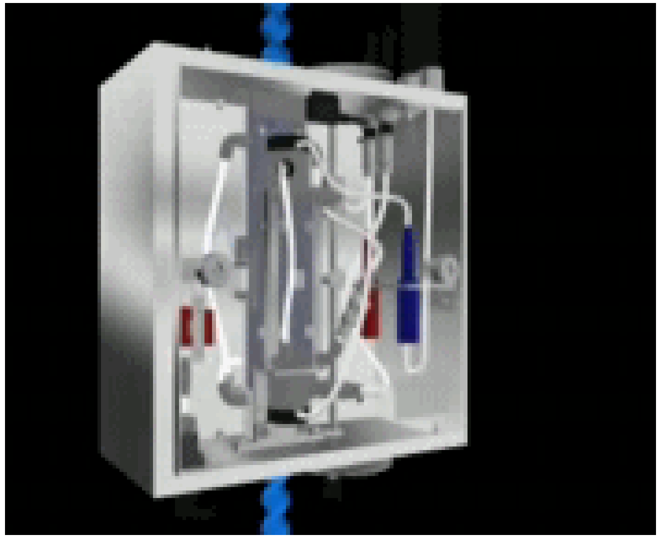
by Carlos Escott [1,*], Carmen López [1], Iris Loira [1], Carmen González [1], María Antonia Bañuelos [2], Wendu Tesfaye [1], José Antonio Suárez-Lepe [1] and Antonio Morata [1]

https://doi.org/10.3390/foods10061416





Pulsed Light



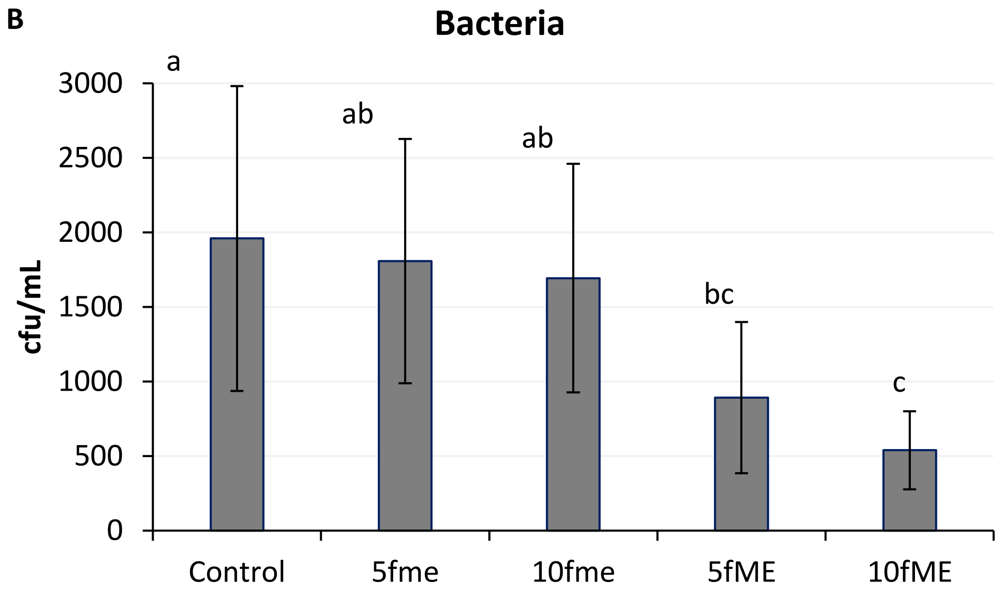
Pulsed Light

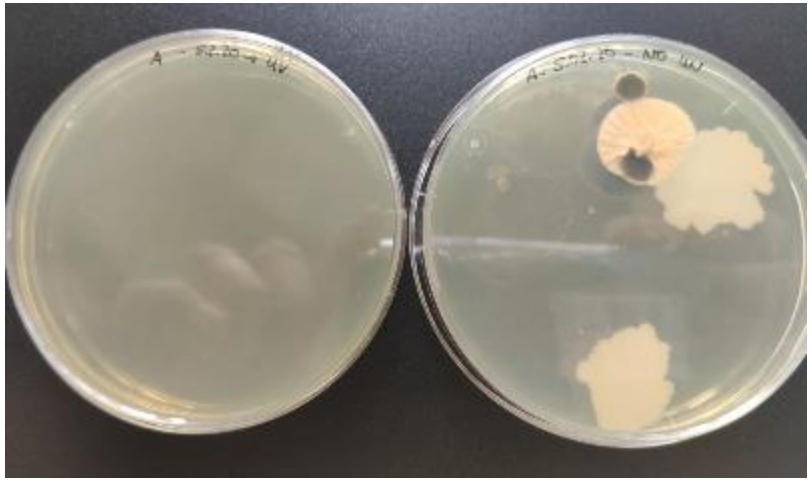
Control

5 pulses

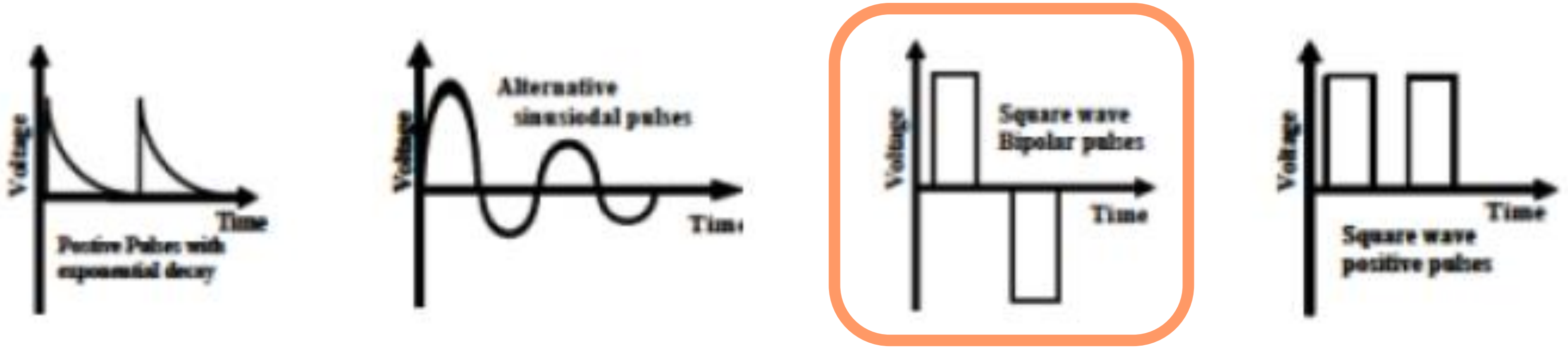
10 pulses

Pulsed Light





Pulsed electric fields



10-40 kV/cm

40-60A

us

RESOLUTION OIV-OENO 634-2020

TREATMENT OF GRAPES BY PULSED ELECTRIC FIELDS - (PEF)

THE GENERAL ASSEMBLY,

IN VIEW OF THE ARTICLE 2, paragraph 2 b) ii of the Agreement of 3 April 2001 establishing the International Organization of Vine and Wine,

CONSIDERING the work of the "Technology" Expert Group,

CONSIDERING the importance of new physical methods for improving the extraction of grape compounds located inside the cells that may improve the wine processing,

CONSIDERING that pulsed electric fields increases the permeability of the cell membranes,

DECIDES, at the proposal of Commission II "Oenology", to introduce the following oenological practices and treatments into part II, chapter 2 of the *International Code of Oenological Practices*,

Part II

Chapter 2: MUSTS

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)

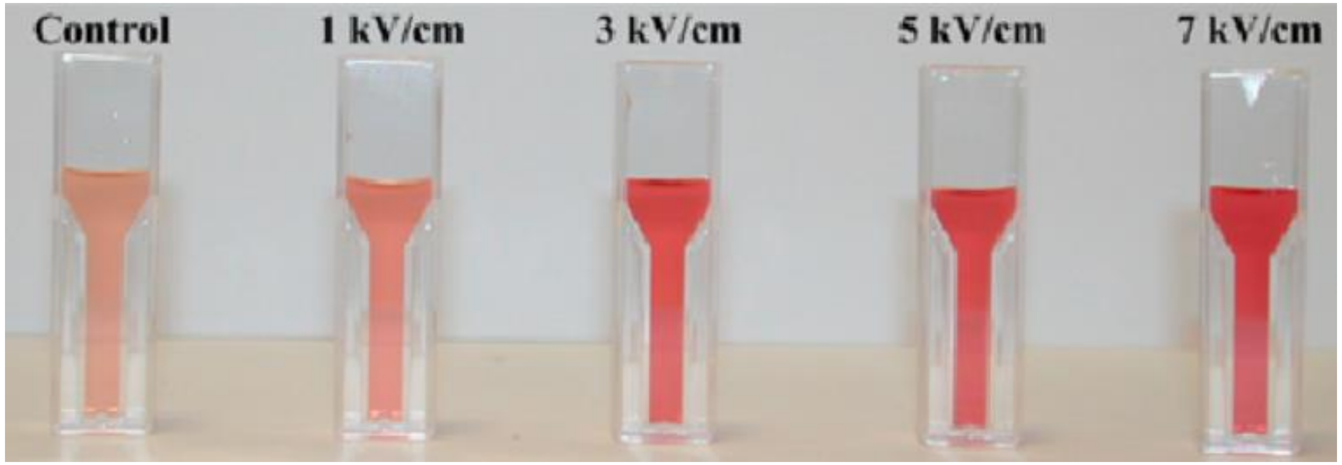
Treatments	*Microbial log reductions, mean and standard deviation*
Control, no pulse	1·5±1·1[a]
20 pulses	4·0±0·3[b]
30 pulses	4·1±0·2[b]
40 pulses	4·2±0·5[b]

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

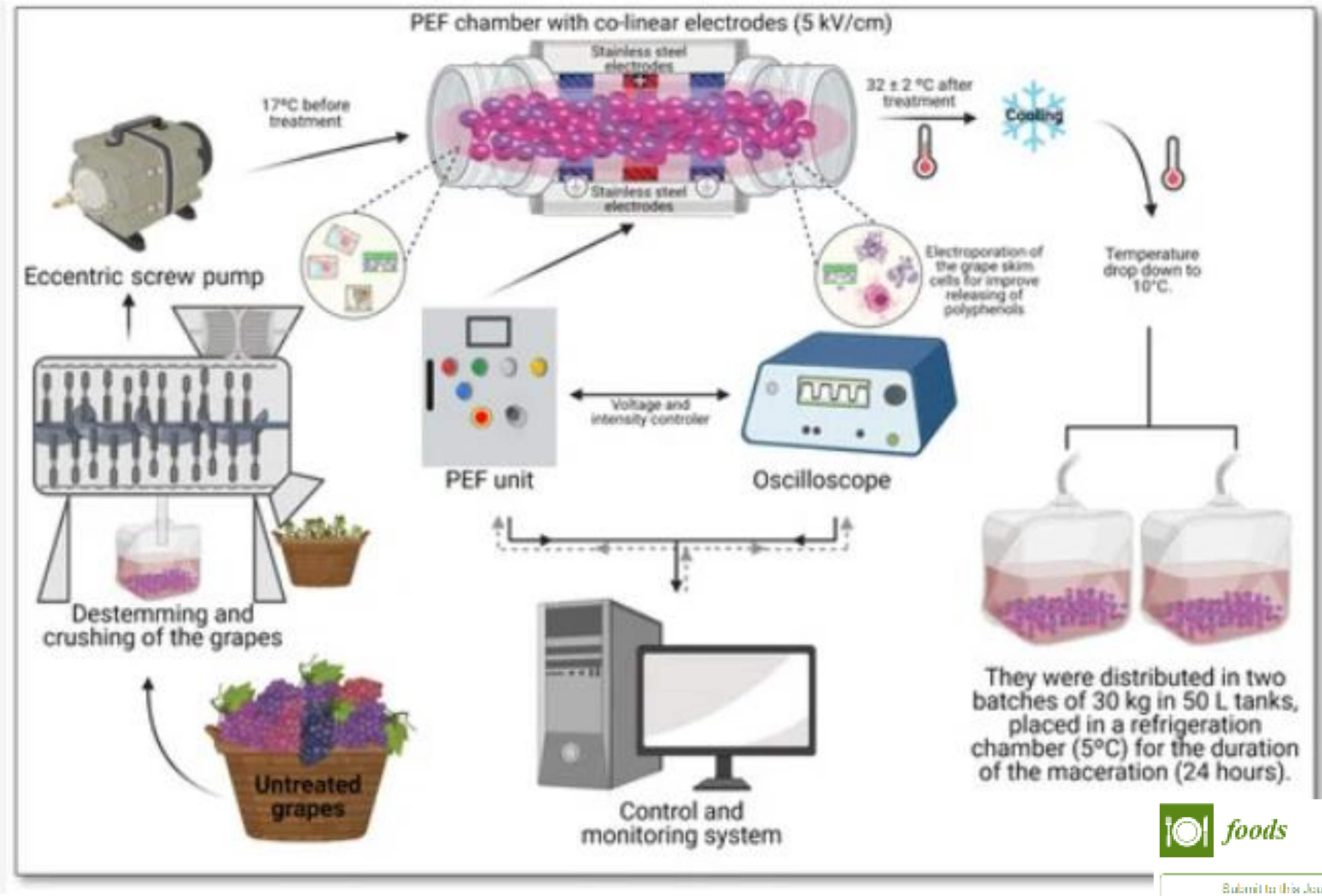
Maceration time: 1 hour
Low temperature

Control | **1kV/cm- 50 pulses** | **3kV/cm- 50 pulses** | **5kV/cm- 50 pulses** | **8kV/cm- 50 pulses**



Garnacha must after 1 h of maceration with grapes untreated and treated by PEF (50 exponential decay pulses; 1–7 kV/cm; 0.4–4.1 kJ/kg)

E. **Puértolas**, G. Saldaña, S. Condón, I. Álvarez, J. Raso. Evolution of polyphenolic compounds in red wine from Cabernet Sauvignon grapes processed by pulsed electric fields during aging in bottle. Food Chemistry 119, 1063–1070 (**2010**)



Pulsed electric fields

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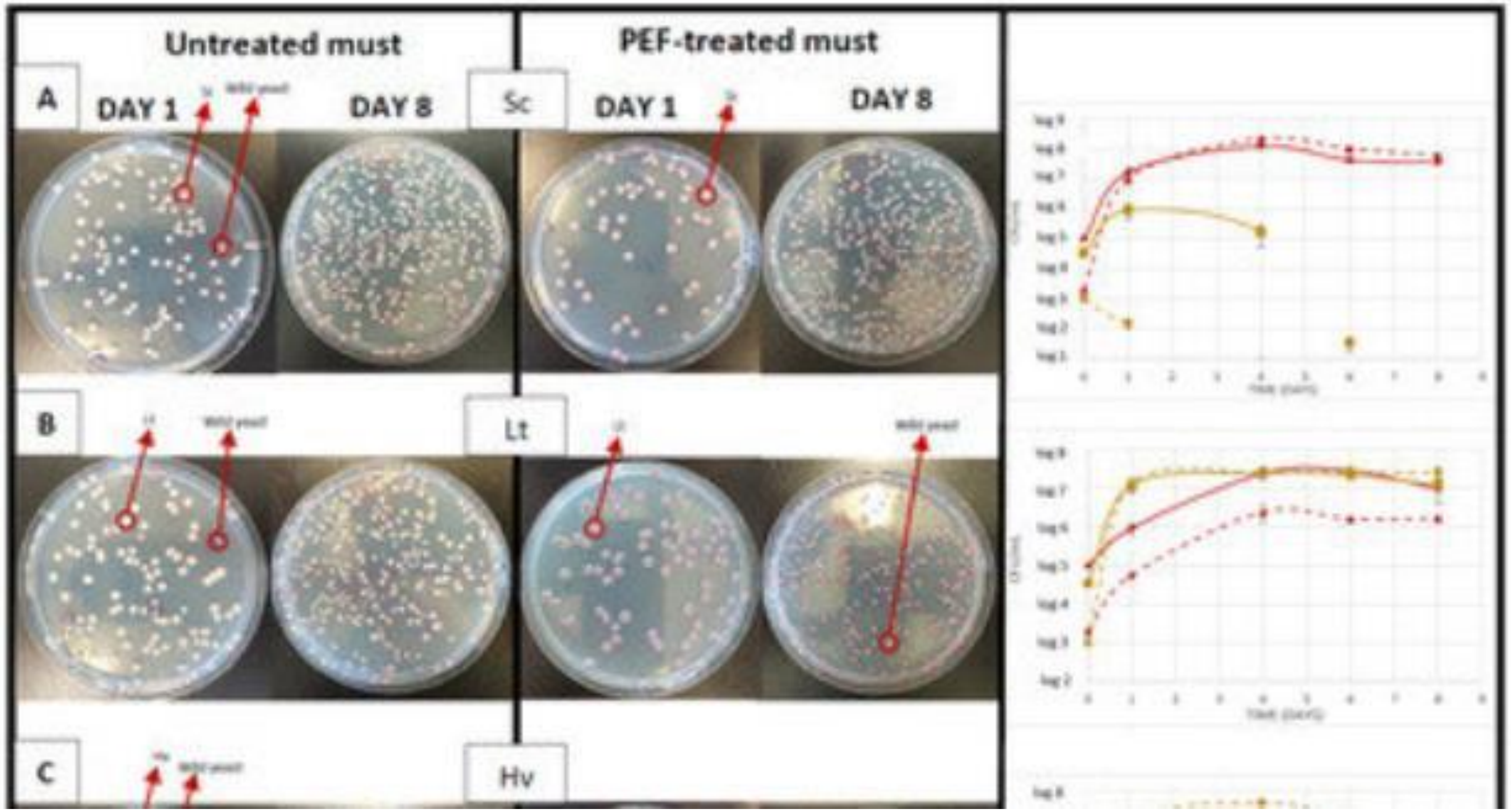
Pulsed Electric Fields to Improve the Use of Non-*Saccharomyces* Starters in Red Wines

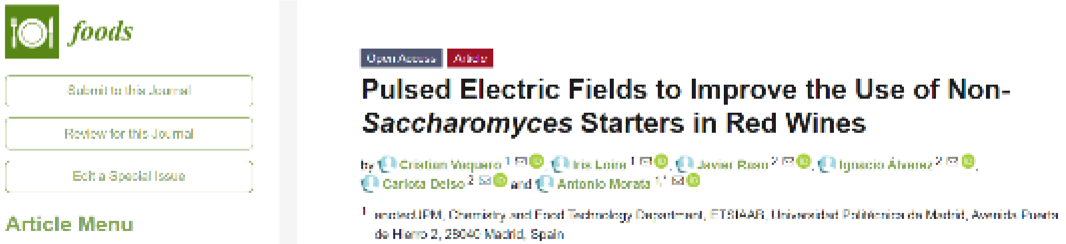
by Cristian Vaquero [1], Iris Loira [1], Javier Raso [2], Ignacio Álvarez [2], Carlota Delso [2] and Antonio Morata [1,*]

[1] enotecUPM, Chemistry and Food Technology Department, ETSIAAB, Universidad Politécnica de Madrid, Avenida Puerta de Hierro 2, 28040 Madrid, Spain

https://doi.org/10.3390/foods10071472



Pulsed electric fields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Pulsed Electric Fields to Improve the Use of Non-*Saccharomyces* Starters in Red Wines

by Cristian Vaquero [1], Iris Loira [1], Javier Raso [2], Ignacio Álvarez [2], Carlota Delso [2] and Antonio Morata [1,*]

[1] enotecUPM, Chemistry and Food Technology Department, ETSIAAB, Universidad Politécnica de Madrid, Avenida Puerta de Hierro 2, 28040 Madrid, Spain

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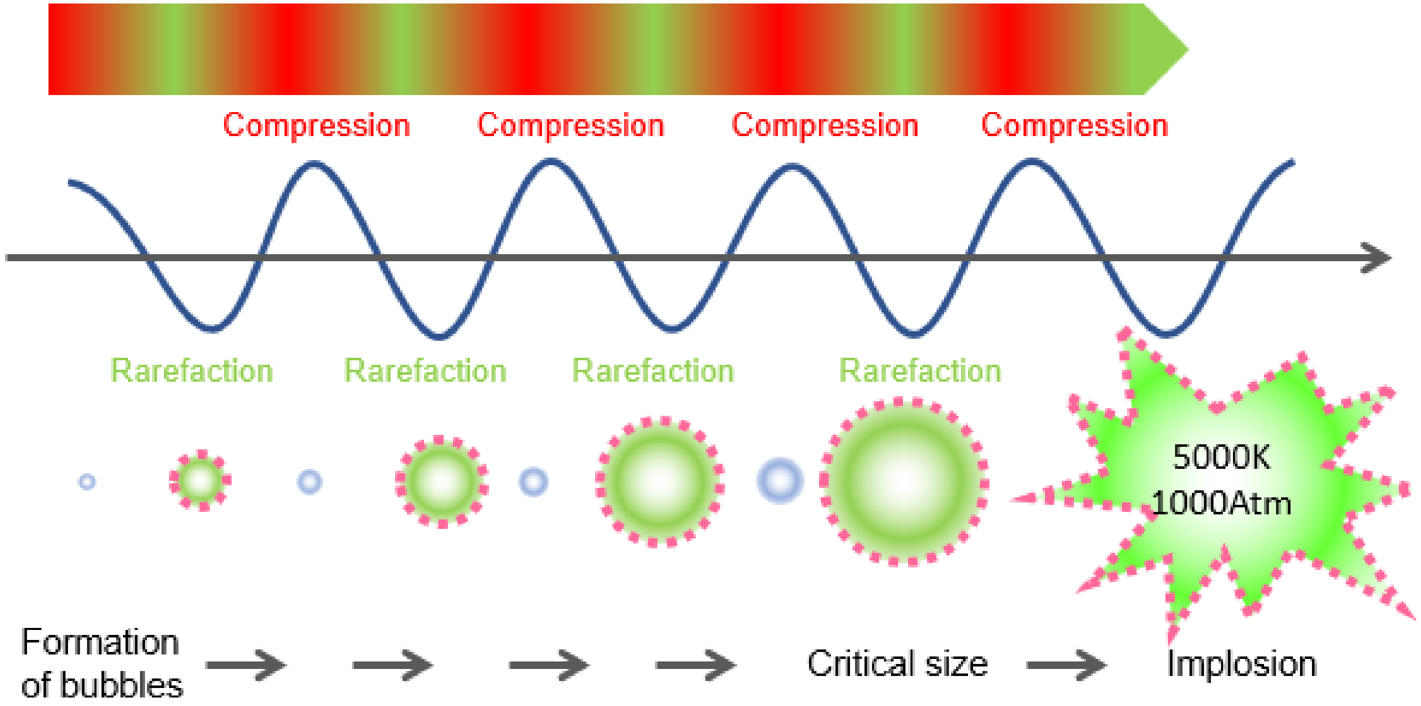
Ultrasounds



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Interprofesional del VINO DE ESPAÑA

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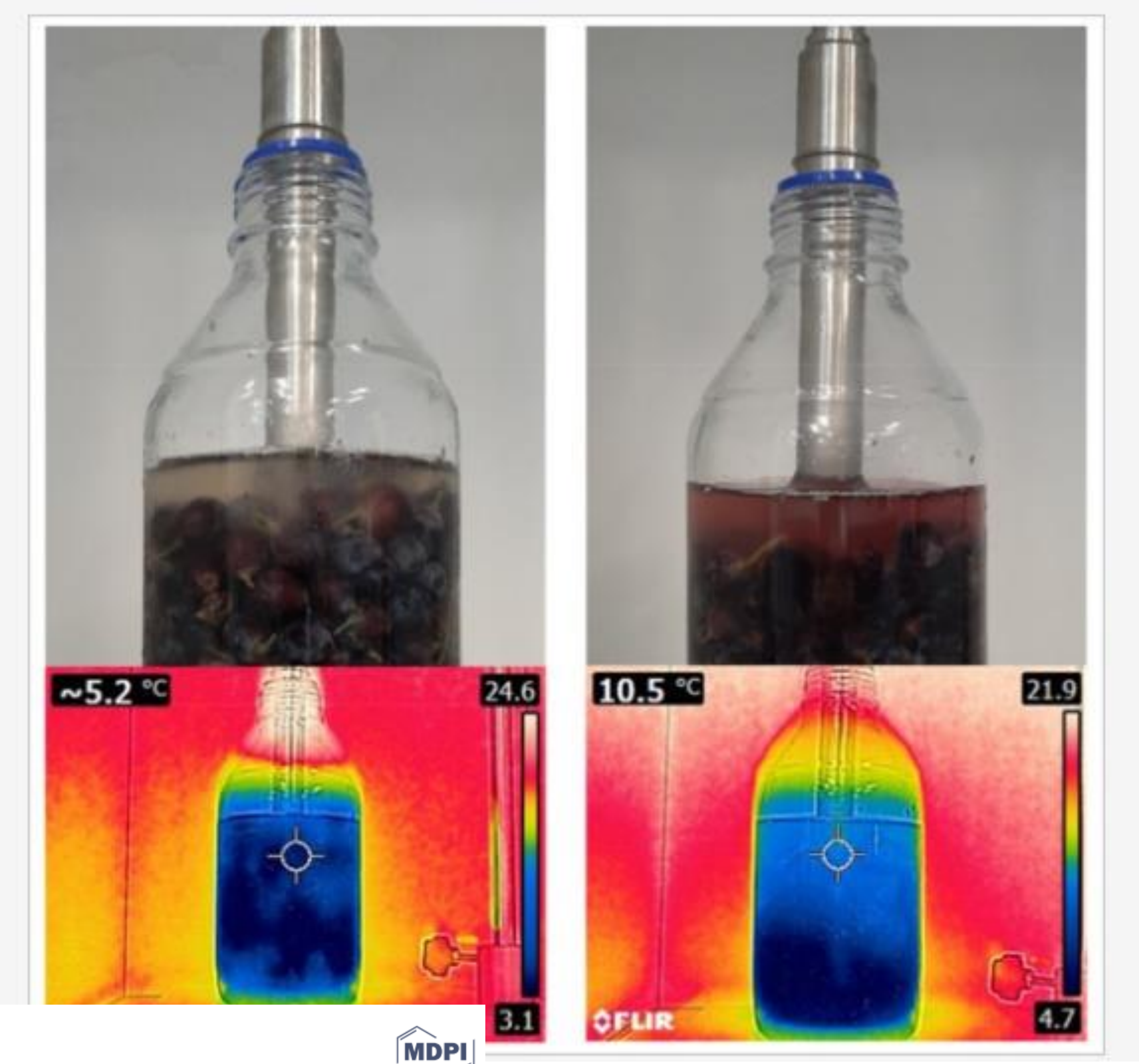


antioxidants MDPI

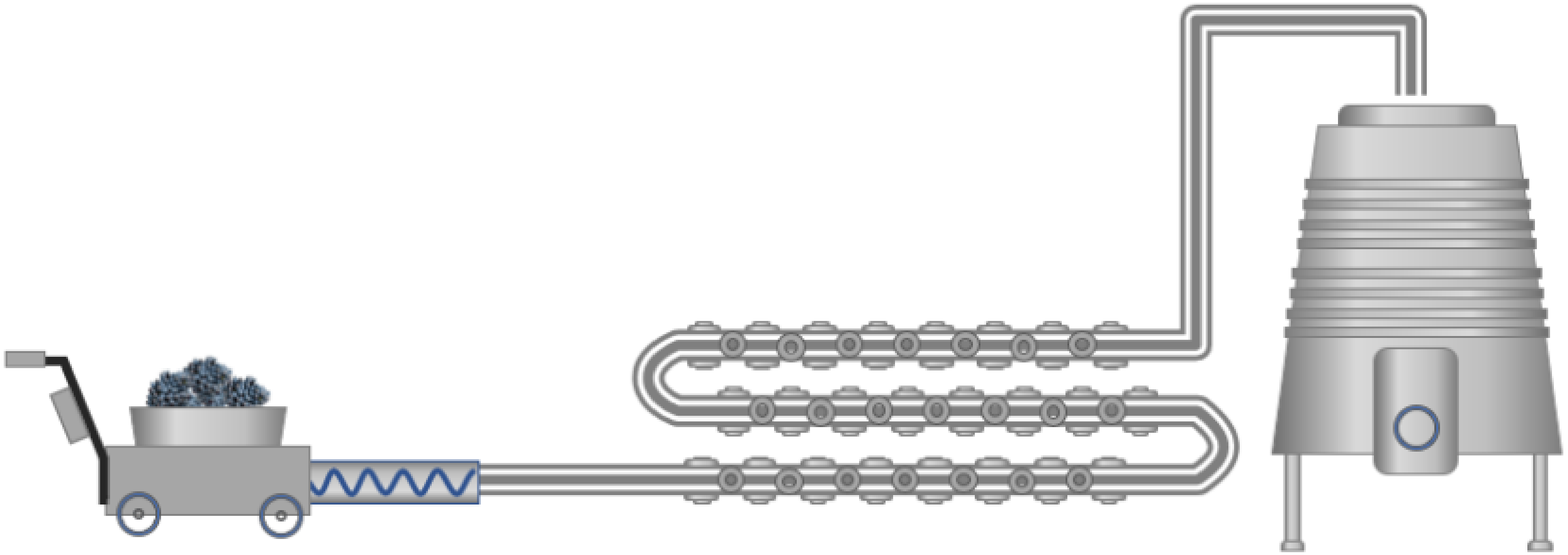
Review

Emerging Non-Thermal Technologies for the Extraction of Grape Anthocyanins

https://doi.org/10.3390/antiox10121863

Antonio Morata *, Carlos Escott, Iris Loira, Carmen López, Felipe Palomero and Carmen González

Ultrasounds



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L. thermotolerans

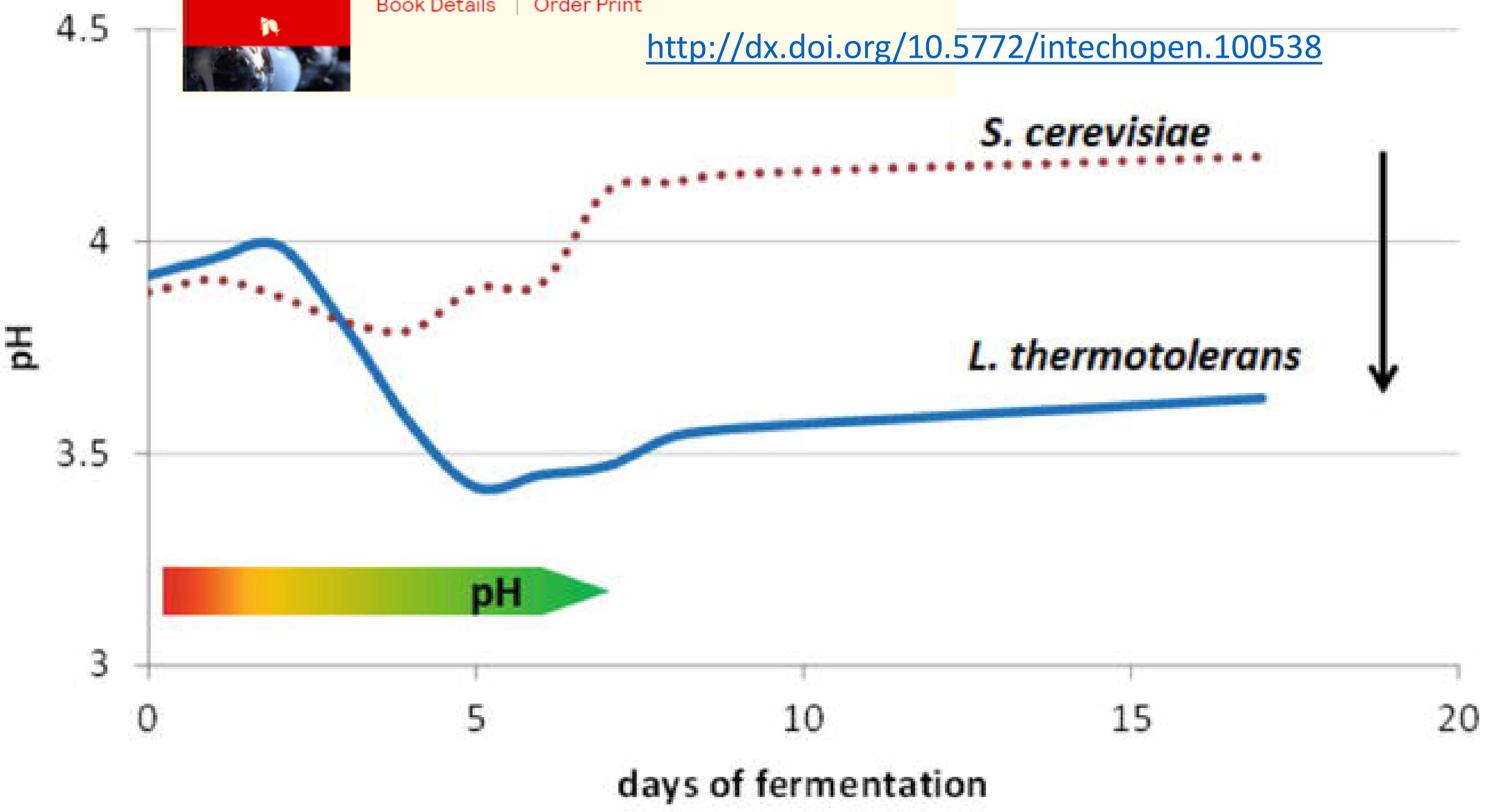

Figure 5.

Typical pH evolution in industrial fermentations driven by Lachancea thermotolerans. The gradient color scale shows the safety of wines in terms of microbial and chemical stability as a function of pH.

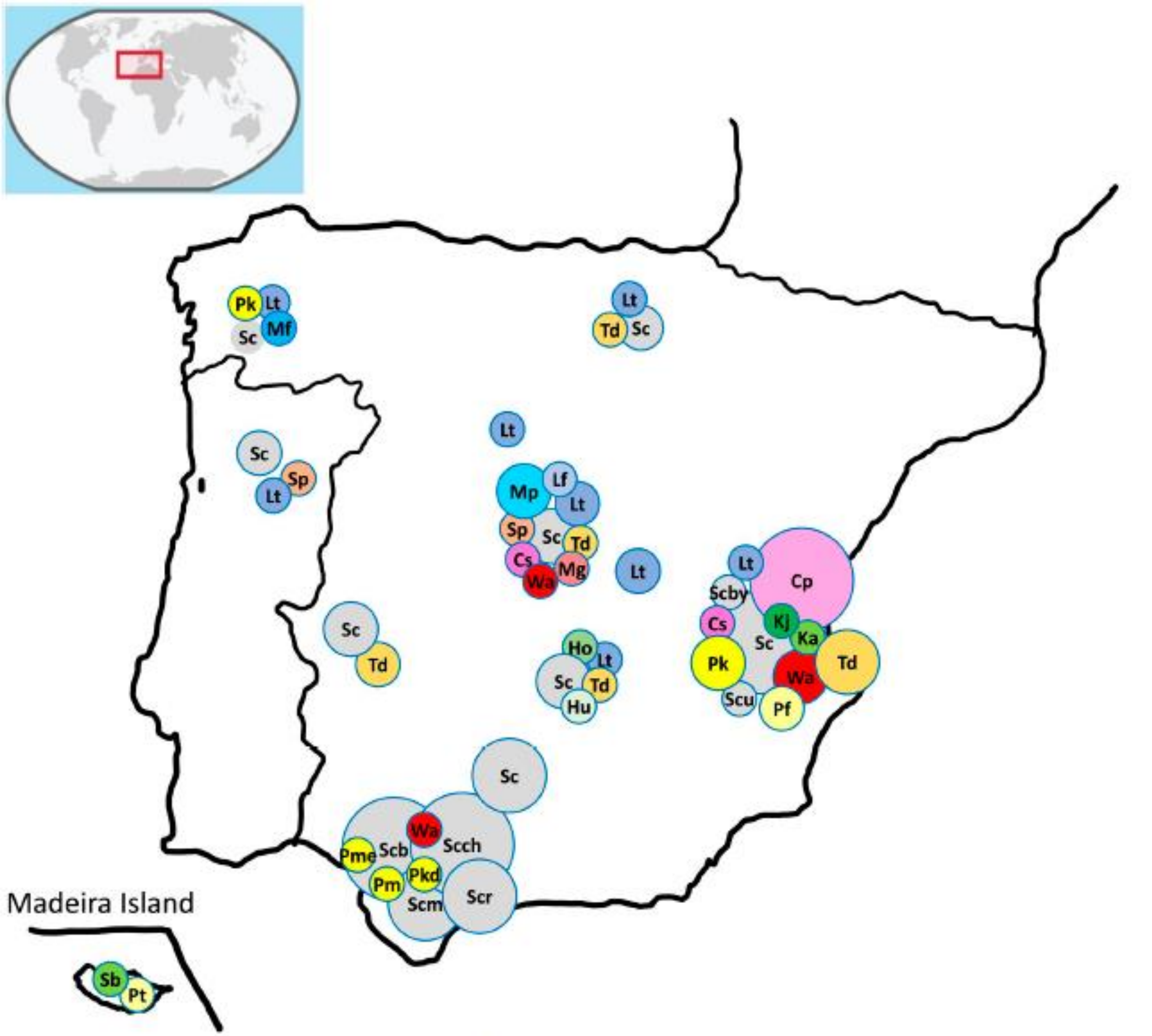

Figure 1. Yeast strains selections in several Iberian wine regions. The area of circles is proportional to the number of selected strains. Sc: *Saccharomyces cerevisiae*, Scby: *Saccharomyces cerevisiae* var. bayanus, Scb: *Saccharomyces cerevisiae* var *beticus*, Scm: *Saccharomyces cerevisiae* var *montuliensis*, Scch: *Saccharomyces cerevisiae* var *cheresiensis*, Scr: *Saccharomyces cerevisiae* var *rouxii*, Scu: *Saccharomyces uvarum*, Cp: *Candida pulcherrima*, Cs: *Candida stellata*, Ho: *Hanseniaspora opuntiae*, Hu: *Hanseniaspora uvarum*, Ka: *Kloeckera apis*, Kj: *Kloeckera japonica*, Lf: *Lachancea fermentati*, Lt: *Lachancea thermotolerans*, Mf: *Metschnikowia fructicola*, Mp: *Metschnikowia pulcherrima*, Mg: *Meyerozyma guilliermondii*, Pf: *Pichia fermentans*, Pk: *Pichia kluyveri*, Pkd: *Pichia kudriavzevii*, Pm: *Pichia manshurica*, Pme: *Pichia membranaefaciens*, Pt: *Pichia terricola* Sp: *Schizosaccharomyces pombe*, Sb: *Starmerella bacillaris*, Td: *Torulaspora delbrueckii*, Wa: *Wickerhamomyces anomalus*. Madeira Islands are not located in the real geographic position.

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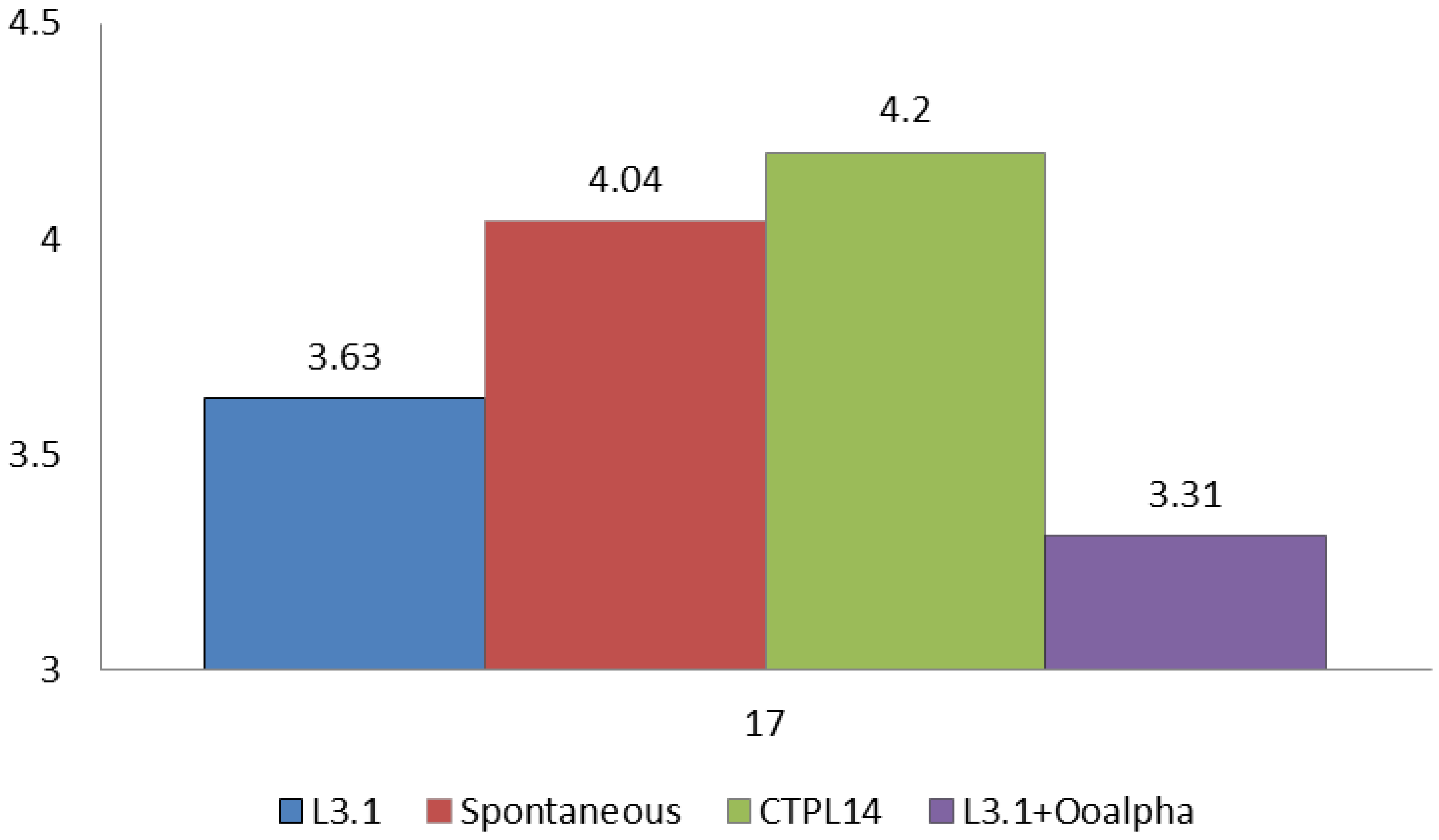
L. thermotolerans L3.1

Tempranillo; sugars 252 g/L; pH=3.9



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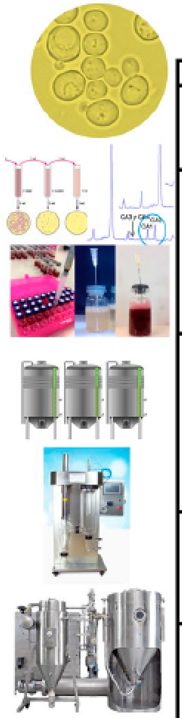
Lachancea thermotolerans wild strains
Selected in:



***Ribera de Duero* - strain L31**
2015
Lactic acid production 3-9 g/L (High)

Lab evaluation
2015-19
Fermentative performance 7-9 % EtOH
Temperatures 17-27 ºC
Nutritional requirements
Volatiles
Biochemical and molecular profiling

Pilot scale at winery-Liquid inoculum
2016
White grape (Albariño)-scale 500 L
pH reduction 3.1→ 2.85 (Lactic acid 2.7 g/L)
2017
Red grape (Tempranillo)- scale 1000 kg
pH reduction 4.2→ 3.66 (Lactic acid 6.6 g/L)

Drying test small scale-sample 50g
2019-Lallemand
Red grape (Tempranillo)
pH reduction 3.96→ 3.09 (Lactic acid 8.4 g/L)

Drying test medium scale-sample 50kg
2020-Lallemand
Red grape (Tempranillo)- La Mancha 8,000 kg
pH reduction 3.84→ 3.34 (Lactic acid 9.4 g/L)
Red grape (Tempranillo)- Ribera del Duero 15,000 kg
pH reduction 3.8→ 3.66 (Lactic acid 2.3 g/L)

***Mancha* – strain A54**
2018
Lactic acid production 2-8 g/L (High)

Lab evaluation
2018-20
Fermentative performance 7-9 % EtOH
Temperatures 17-27 ºC
Nutritional requirements
Volatiles
Biochemical and molecular profiling

Pilot scale at winery-Liquid inoculum
2020
White grape (Airén)-scale 12,000 L
pH reduction 3.75→ 3.47 (Lactic acid 2 g/L)
2021
Red grape (Airén)- scale 12,000 L
pH reduction 3.85→ 3.75 (Lactic acid 0.5x g/L)

***Uclés* – strains F108 & F111**
2018
Lactic acid production 0.5-2 g/L (Low)
Sauvignon Blanc & Tempranillo

Lab evaluation
2018-20
Fermentative performance 7-9 % EtOH
Temperatures 17-27 ºC
Nutritional requirements
Volatiles
Biochemical and molecular profiling

Pilot scale at winery-Liquid inoculum
2019
White grape (Airen)-scale 1000 L
pH reduction 3.1→ 2.85 (Lactic acid 2.7 g/L)
2020
White grape (Macabeo)- scale 1000 L
pH reduction 4.2→ 3.66 (Lactic acid 6.6 g/L)

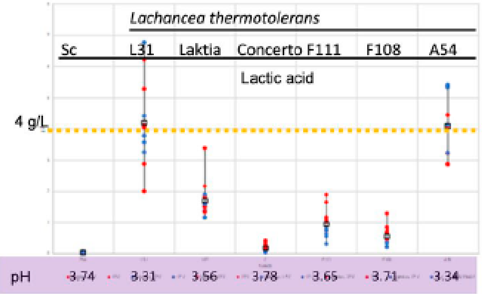


Figure 5. Yeast selection processes of several strains of the species *Lachancea thermotolerans* from grape to pilot and industrial fermentation as liquid cultures and dried yeast. Also, it is shown the industrial performance for wine acidification compared with the commercial strains Laktia™ and Concerto™ (Vaquero et al. 2020).

GROUPE ICV
L'art & l'expertise du vin

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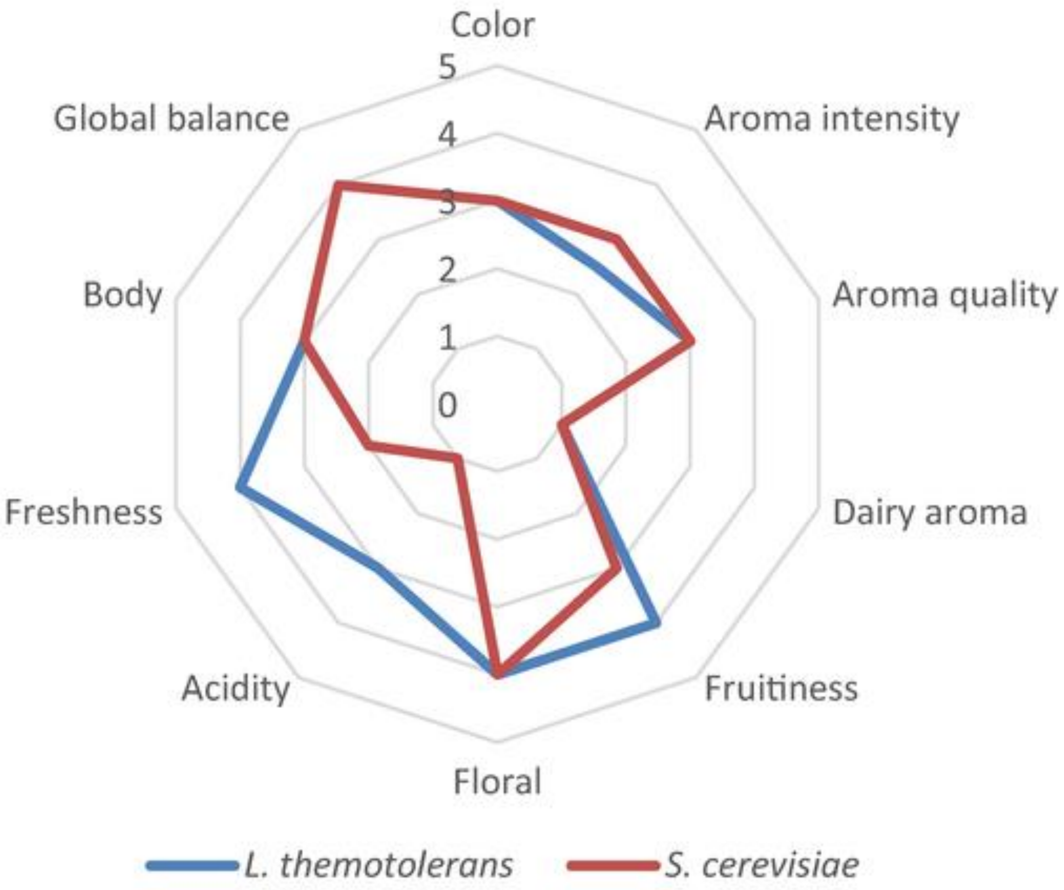
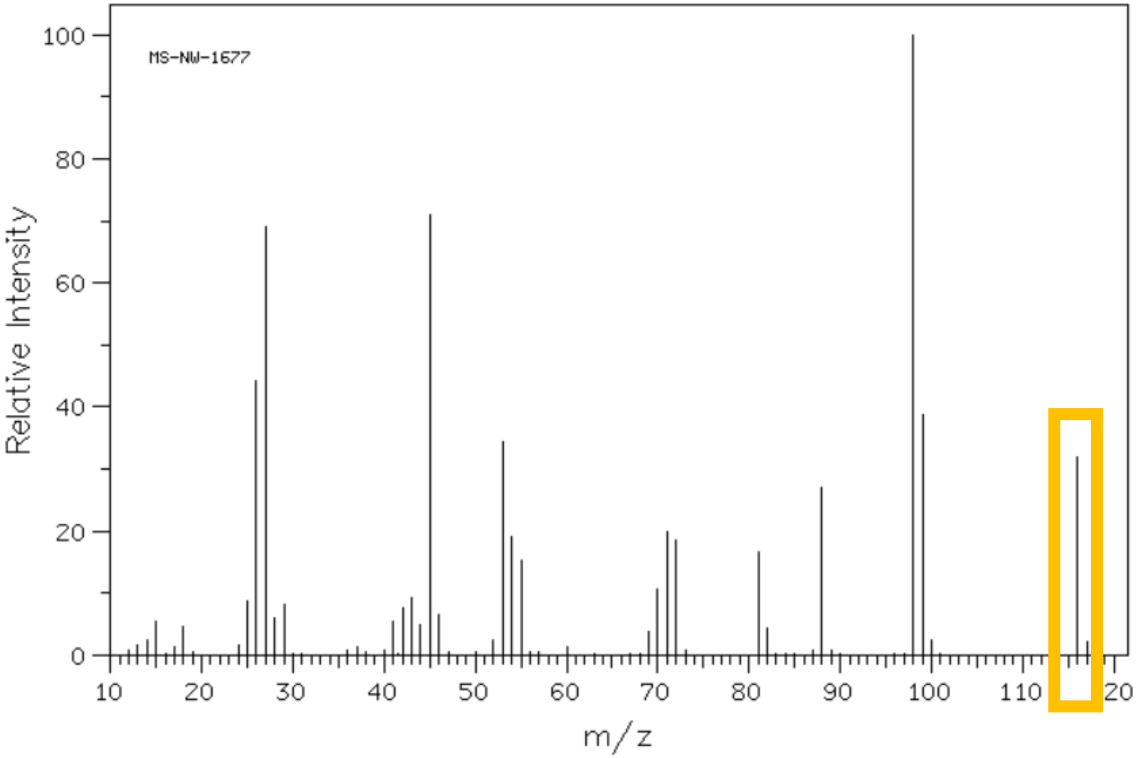


Figure 6.
Comparative sensory spider net of fermentations with Lachancea thermotolerans and Saccharomyces cerevisiae.

Molar mass, 116.072 g·mol−1
Appearance, White solid
Density, 1.635 g/cm3
Melting point, 287 °C (549 °F; 560 K) (decomposes)
Solubility in water. 4.9 g/L at 20ºC



Mass
MS-NW-1677
fumaric acid
C4H4O4 (Mass of molecular ion: 116)

Source Temperature: 190 °C
Sample Temperature: 170 °C
Direct, 75 eV

-Organic acids such as fumarate are commonly used as antimicrobials in foods.

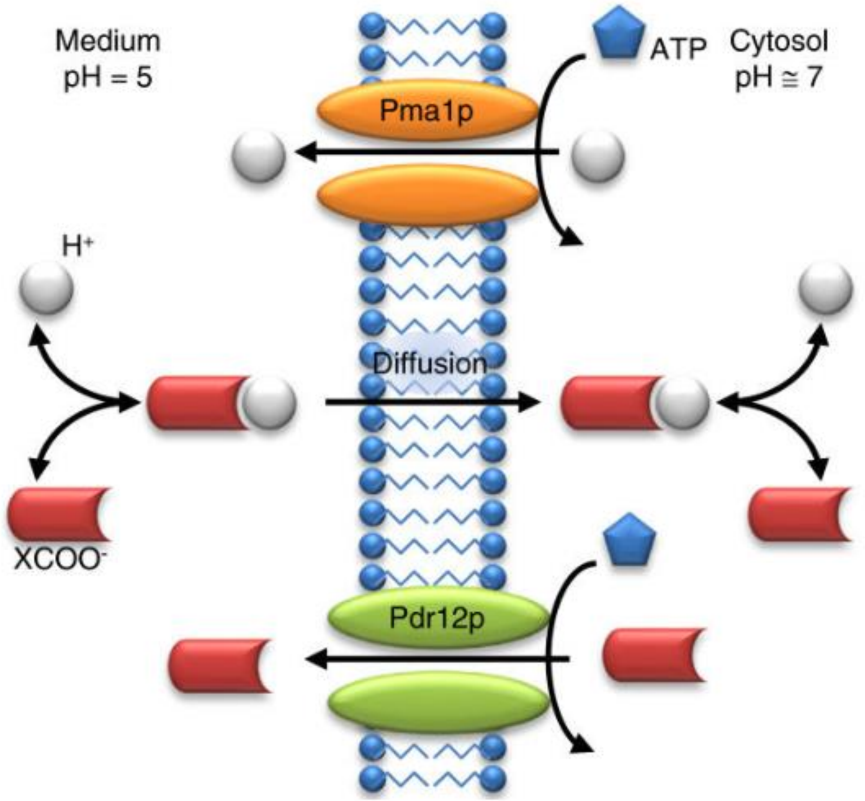
-Classical mechanism of **intracellular dissociation and release of protons**.

-FH2: high effectivity against: *E. coli*, *L. monocytogenes* and *Salmonella* sp. Also LAB.

-FH2: high effectivity with acetic acid bacteria

Intracellular GAD system (GADi) or other acid resistance systems

The GAD system converts glutamate to γ-amino butyric acid (GABA) with the removal of a proton resulting in an increase in the intracellular pH



Vital-Lopez, F.G., Wallqvist, A. & Reifman, J. Bridging the gap between gene expression and metabolic phenotype via kinetic models. BMC Syst Biol 7, 63 (2013). https://doi.org/10.1186/1752-0509-7-63

Antimicrobial effect of weak organic acids (WOAs) and resistance mechanisms of S. cerevisiae . At low extracellular pH, WOAs are mainly in their undissociated form, which can diffuse through the cellular membrane. The WOAs dissociate in the cytosol and the cell responds by upregulating transporter proteins, such as Pma1 and Pdr12, to secrete protons and carboxylate anions (XCOO-), respectively, to avoid toxicity.

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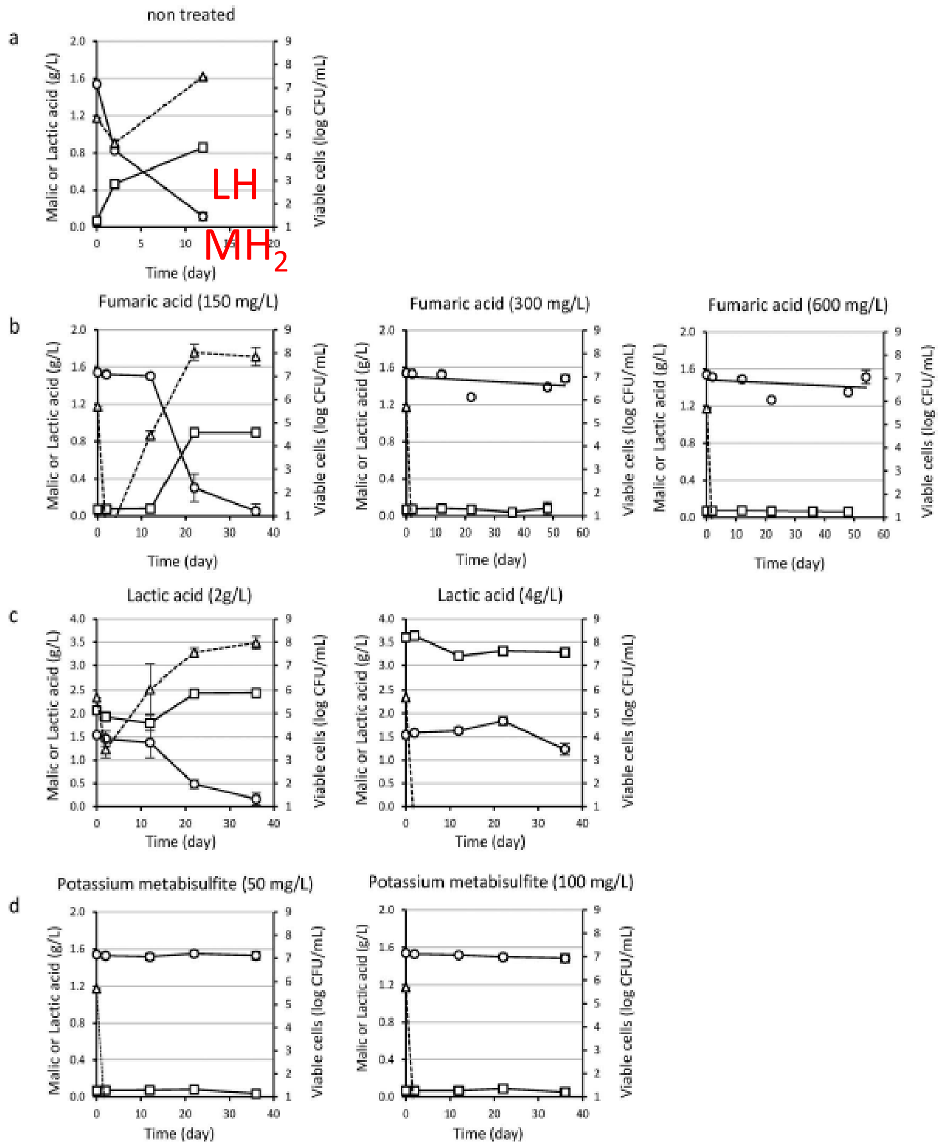
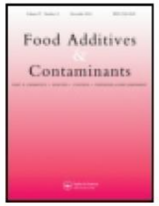


Figure 3. Malic and lactic acid concentrations (circles and squares, respectively) and viable cells of *O. oeni* (triangles and dotted line) during malolactic fermentation of wine in control (a), treated with different fumaric acid concentrations (b), lactic acid concentrations (c) or potassium metabisulfite concentrations (d). Means ± standard deviation of three replicates.

Inhibitory FML 300-600 mg/L
Some effect 150 mg/L
Lactic acid needs 4 g/L



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Use of fumaric acid to control pH and inhibit malolactic fermentation in wines

Antonio Morata, María Antonia Bañuelos, Carmen López, Chenli Song, Ricardo Vejarano, Iris Loira, Felipe Palomero & Jose Antonio Suarez Lepe

Conclusions

-Non-thermal technologies
-Accelerated phenol extraction
-Gentle with pigments and aromatic molecules
-Antimicrobial effectivity
-PPO control
-SO2 reduction

-Facilitate new biotechnologies
 -Use of non-*Saccharomyces*
 -Coinoculation (Yeast-Bacteria)

Emerging Technologies to Increase Extraction, Control Microorganisms, and Reduce SO2
https://www.intechopen.com/chapters/71684

Use of UHPH to Obtain Juices With Better Nutritional Quality and Healthier Wines With Low Levels of SO2
https://www.frontiersin.org/articles/10.3389/fnut.2020.598286/full

Effect of HHP and UHPH High-Pressure Techniques on the Extraction and Stability of Grape and Other Fruit Anthocyanins
https://doi.org/10.3390/antiox12091746

Emerging Non-Thermal Technologies for the Extraction of Grape Anthocyanins
https://doi.org/10.3390/antiox10121863

Emerging biotechnologies and non-thermal technologies for winemaking in a context of global warming
https://doi.org/10.3389/fmicb.2023.1273940

White Wine Technology

1st Edition

Write a review

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Muchas gracias!

Antonio Morata
Universidad Politécnica de Madrid
Spain
antonio.morata@upm.es
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