Líneas Estratégicas de Investigación (LEIs)

Seminario: ***"Líneas estratégicas de investigación del IA2"***

Los pulsos eléctricos de alto voltaje: Una herramienta para mejorar la sostenibilidad de la industria alimentaria y biotecnológica

Javier Raso
Food Technology
IA2-University of Zaragoza (Spain)
jraso@unizar.es

Universidad Zaragoza

FOOD PROCESSING

- **Food processing**, involves a variety of operations conducted in the food industry by which raw foodstuffs are made suitable for consumption, cooking, or storage. ...

Unit Operations

- Material Handling
- Cleaning
- Separating
- Size reduction
- Fluid Flow
- Mixing
- Heat transfer
- Concentration
- Drying
- Forming
- Packaging
- Controlling

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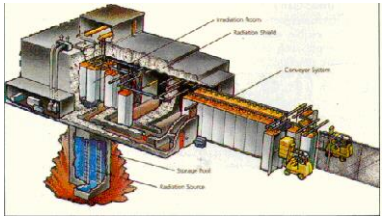
Problems

. Sensory and nutritional properties affected

- Energy consuption

- Waste generation

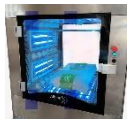
Nonthermal Processing Technologies



- Irradiation (IR)
- Ultra-violet ligth (UV)
- High Hydrostatic Pressure (HHP)
- Ultrasound (US)
- Pulsed Electric Fields

Improving Food Quality

New Products



Energetic Cost Reduction

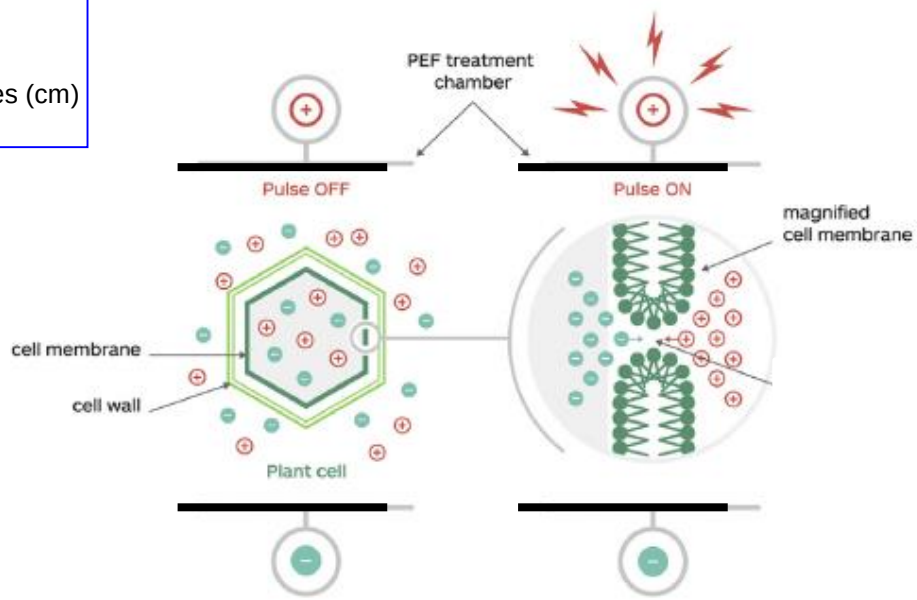
Definition of PEF

•**Application of pulses of high voltage (kV) and short duration (µs) to a biological material placed between two electrodes**

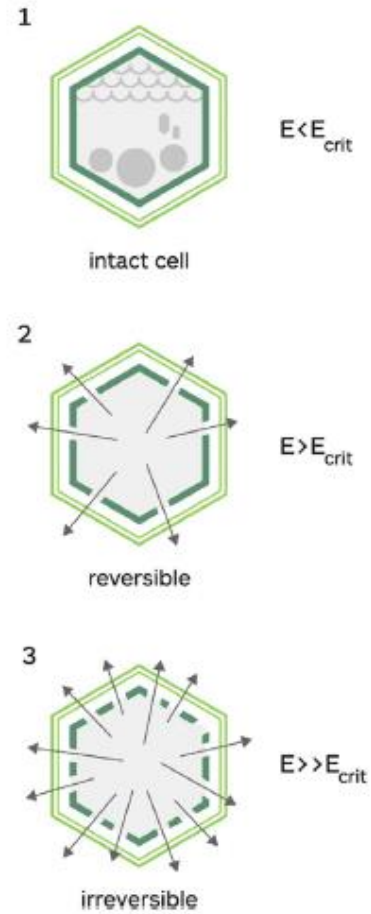
•**Electrical Field Strenght:**

$$E = V/d$$

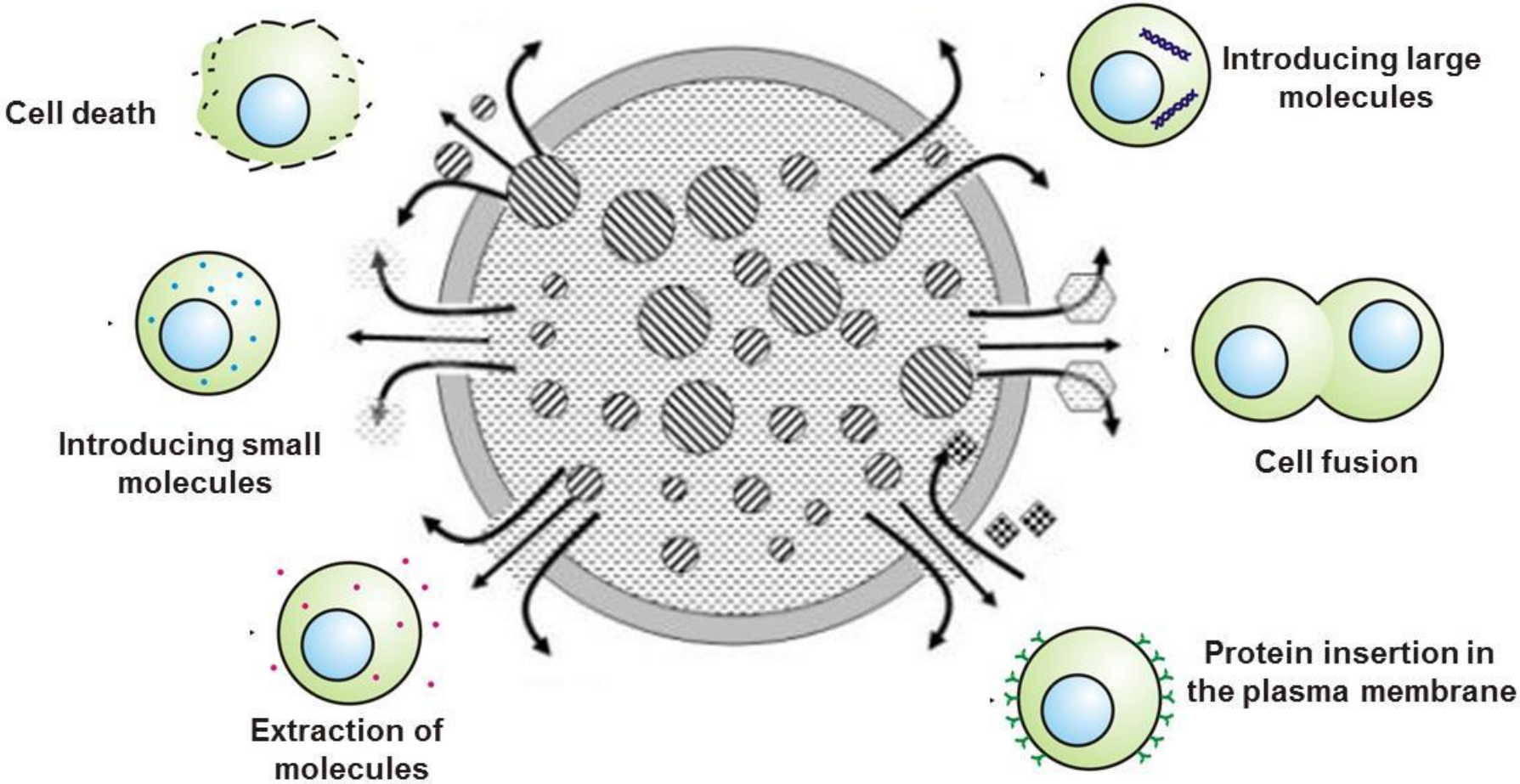
V= voltage (kV)
d= distance between electrodes (cm)



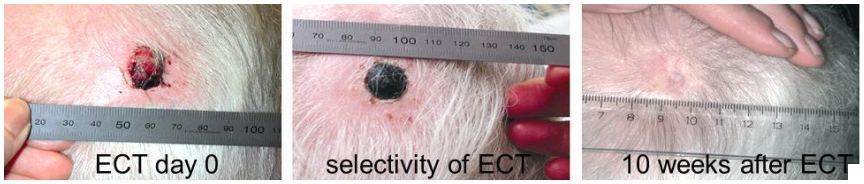
Electroporation

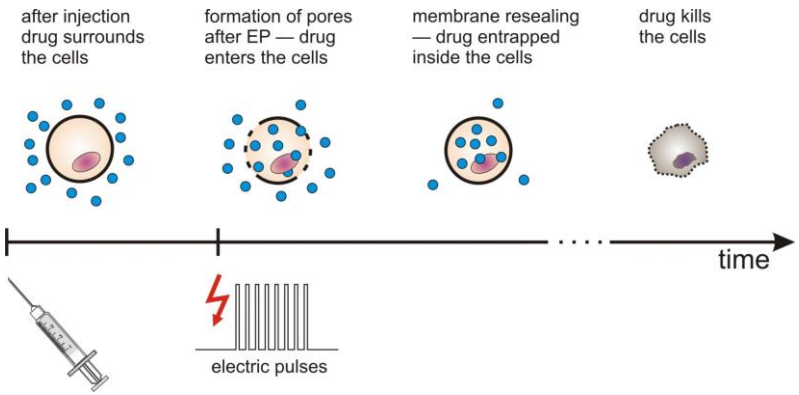


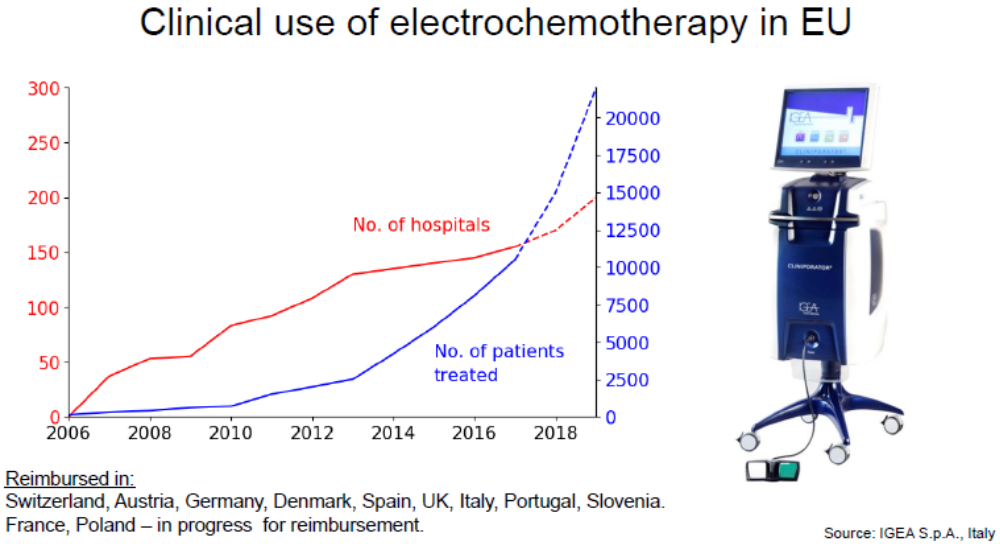
Electroporation



Electroporation







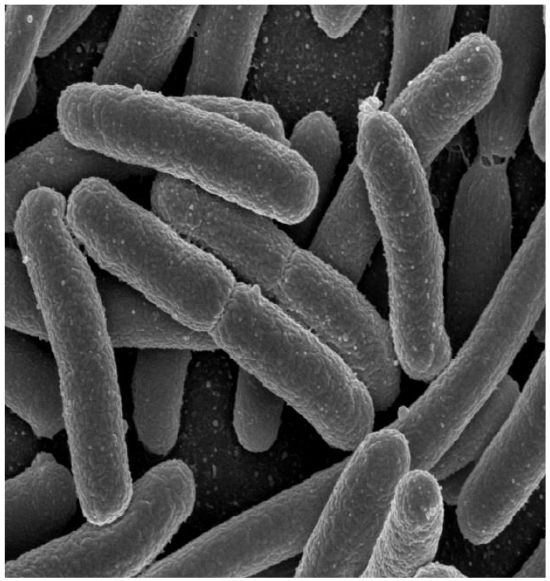
Julie Gehl.

Ugeskrift for Laeger 167(34): 3156-9, 2005.

Jarm, Cemazar, Miklavcic, Sersa.
Expert Rev. Anticancer Ther. 10: 729–746, 2010

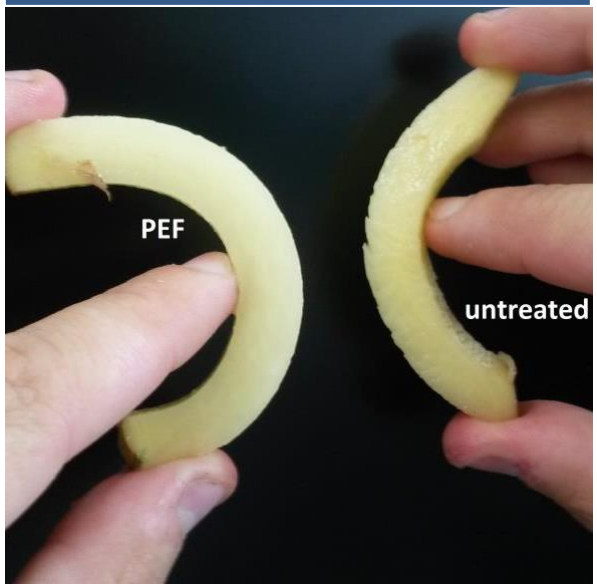
Applications of PEF in the Food Industry

Microbial inactivation

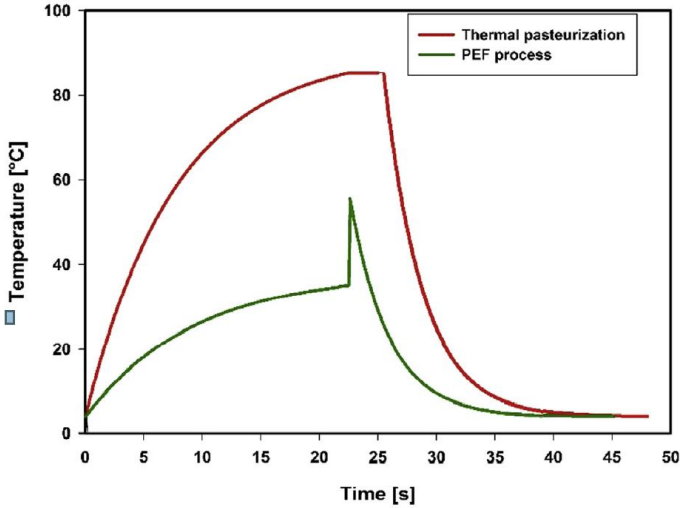


Improving Mass Transfer

Food Structure Modification



Microbial inactivation by PEF



These smoothies are slighty pasteurized. Pasteurization is a simple treatment that is known of milk, a perishable product as well. When Fruity King pasteurizes her products it is a mild pasteurisation, which won't affect the taste. The shelf life will be extended and therefore it becomes easier for kids to take it to school and drink it during the day.

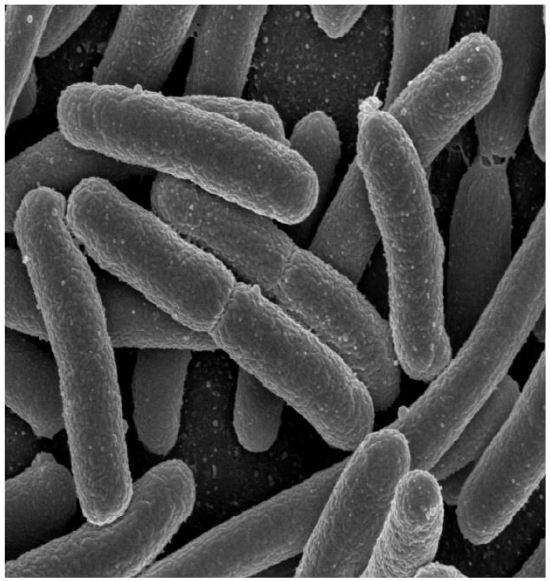
Untreated Thermal PEF

Figure 2.4 Color difference of untreated, thermally treated, and PEF-treated green smoothie (Elea, 2018). *PEF*, Pulsed electric field.

Producers	*Supermarkets*
FruityKing (Netherlands)	MARKS & SPENCER (UK)
Hoogstraten (Netherlands)	Albert Heijn (Netherlands)
true fruits 100% frucht – no tricks (Germany)	EDEKA (Germany)
ORCHARD HOUSE (UK)	REWE (Germany)
GROM IL GELATO COME UNA VOLTA (Italy)	

Applications of PEF in the Food Industry

Microbial inactivation



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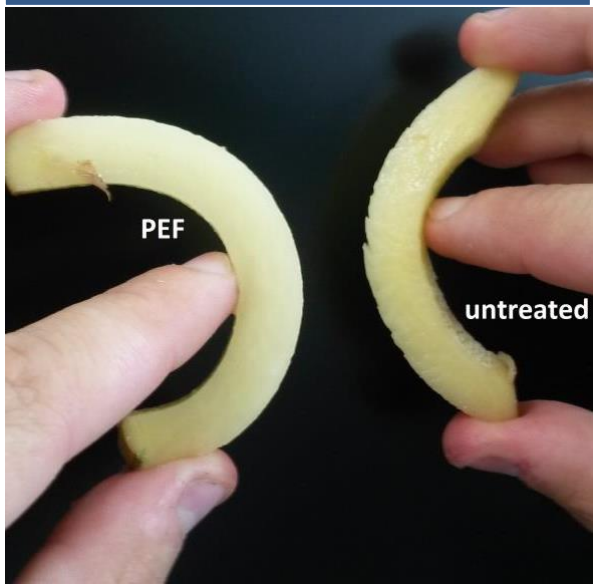
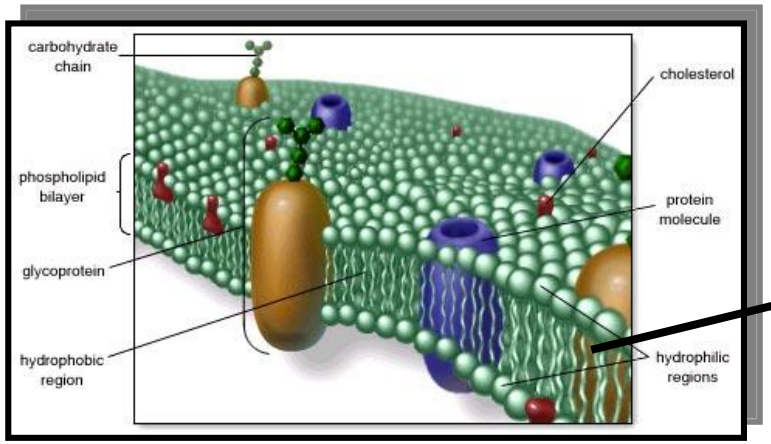
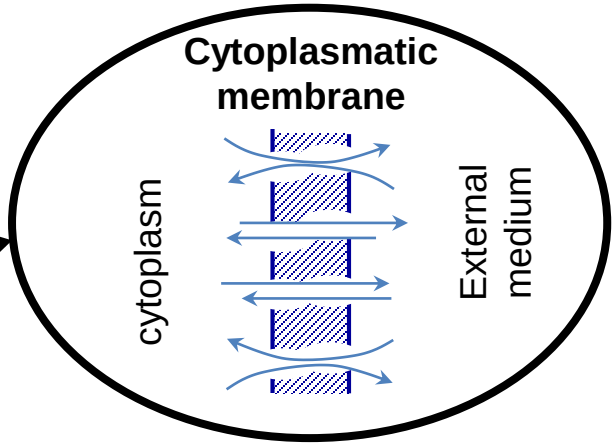


Pulsed electric fields: mass transfer

•Migration of a substance between two phases under the influence of a concentration gradient in order to reach chemical equilibrium



PEF



Electroporation

Extraction of intracellular compounds

- Juices from fruits (Apple juice, Carrot juice)
- Sugar from beets
- Pigments from plants (Anthocyanins, betains)
- Phenolic components (Winemaking)

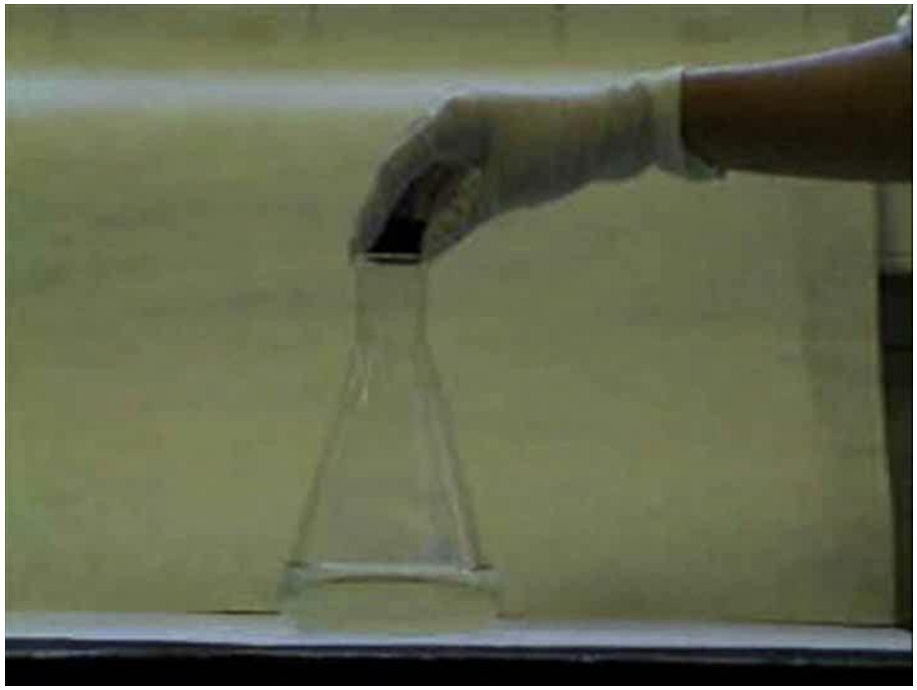
Removing water

-Drying

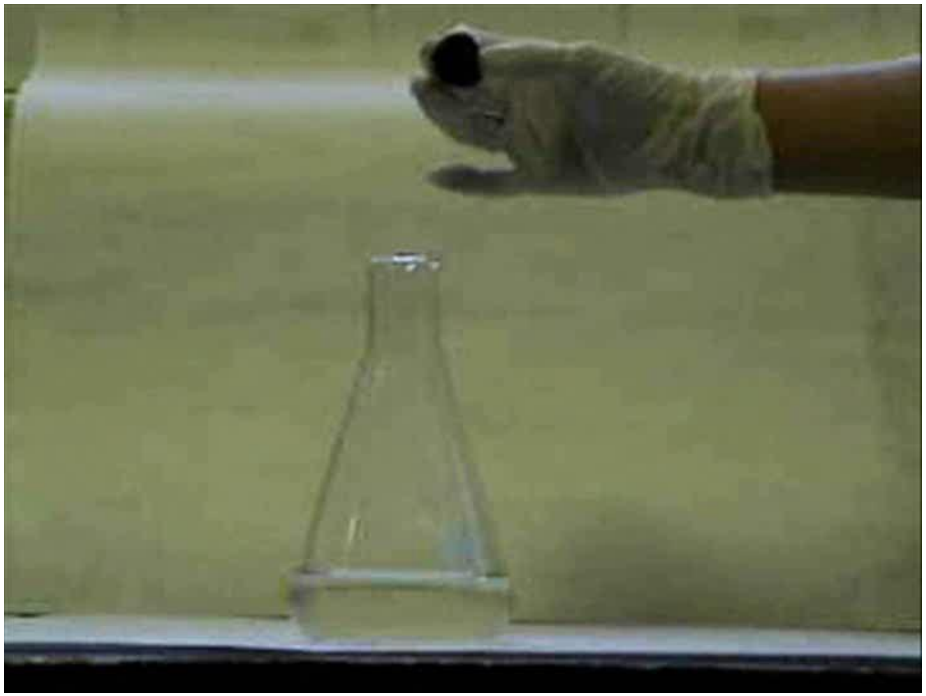
Introduction of a substance into the food matrix

- Osmotic dehydration (apple, pepper)
- Salting (Serrano type ham)
- Brining (cooked ham)

Improving Mass Transfer by PEF



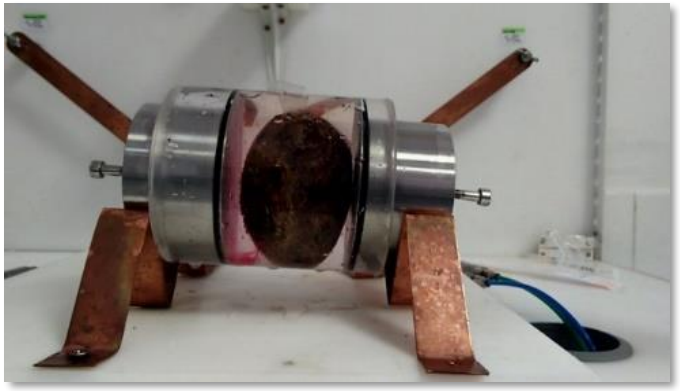
Control

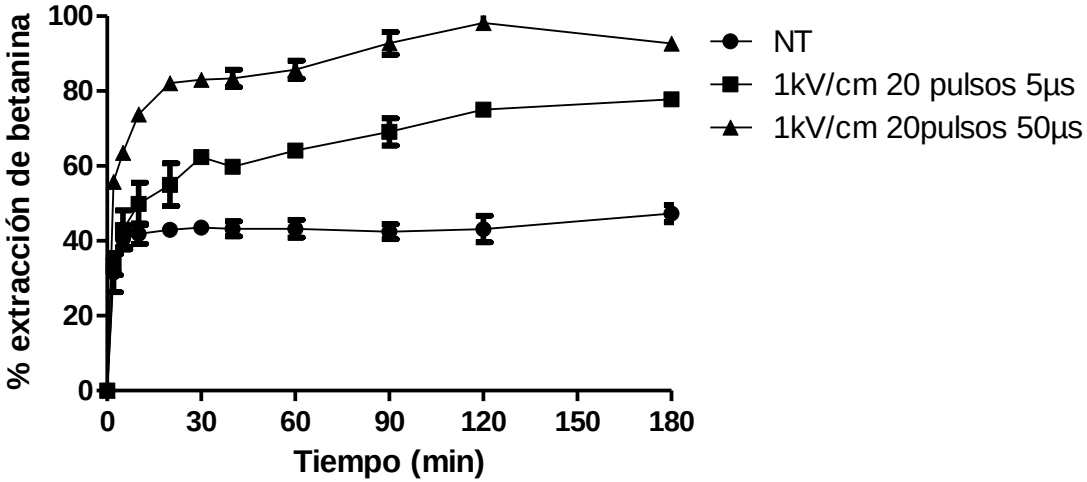


PEF treated

PEF : transferencia de masa

Extracción de BETANINA (E-162) de Remolacha roja:

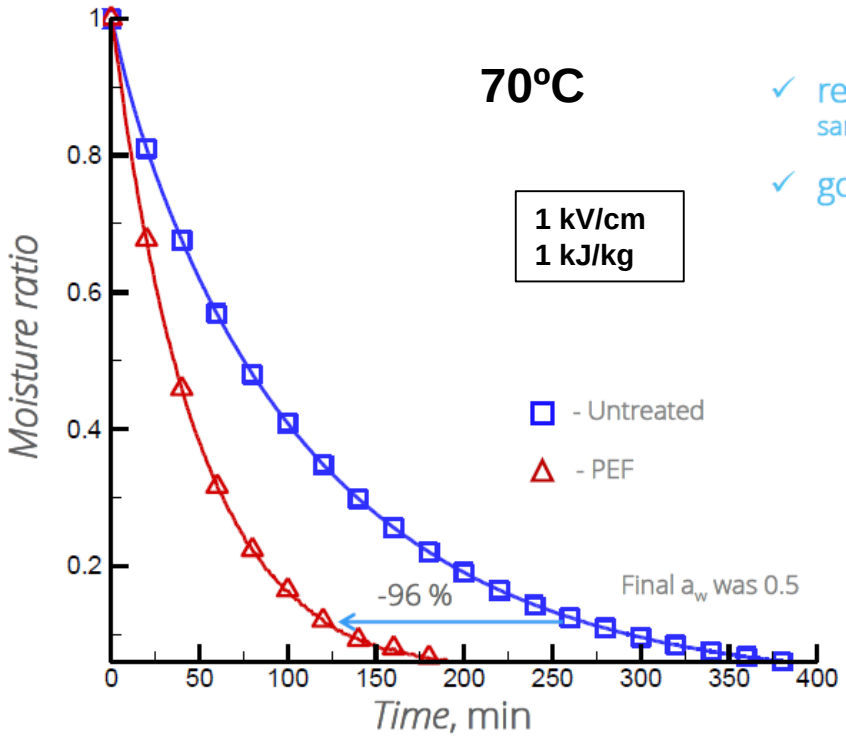




Reduction energetic consumption

Drying

Hot air drying - mango

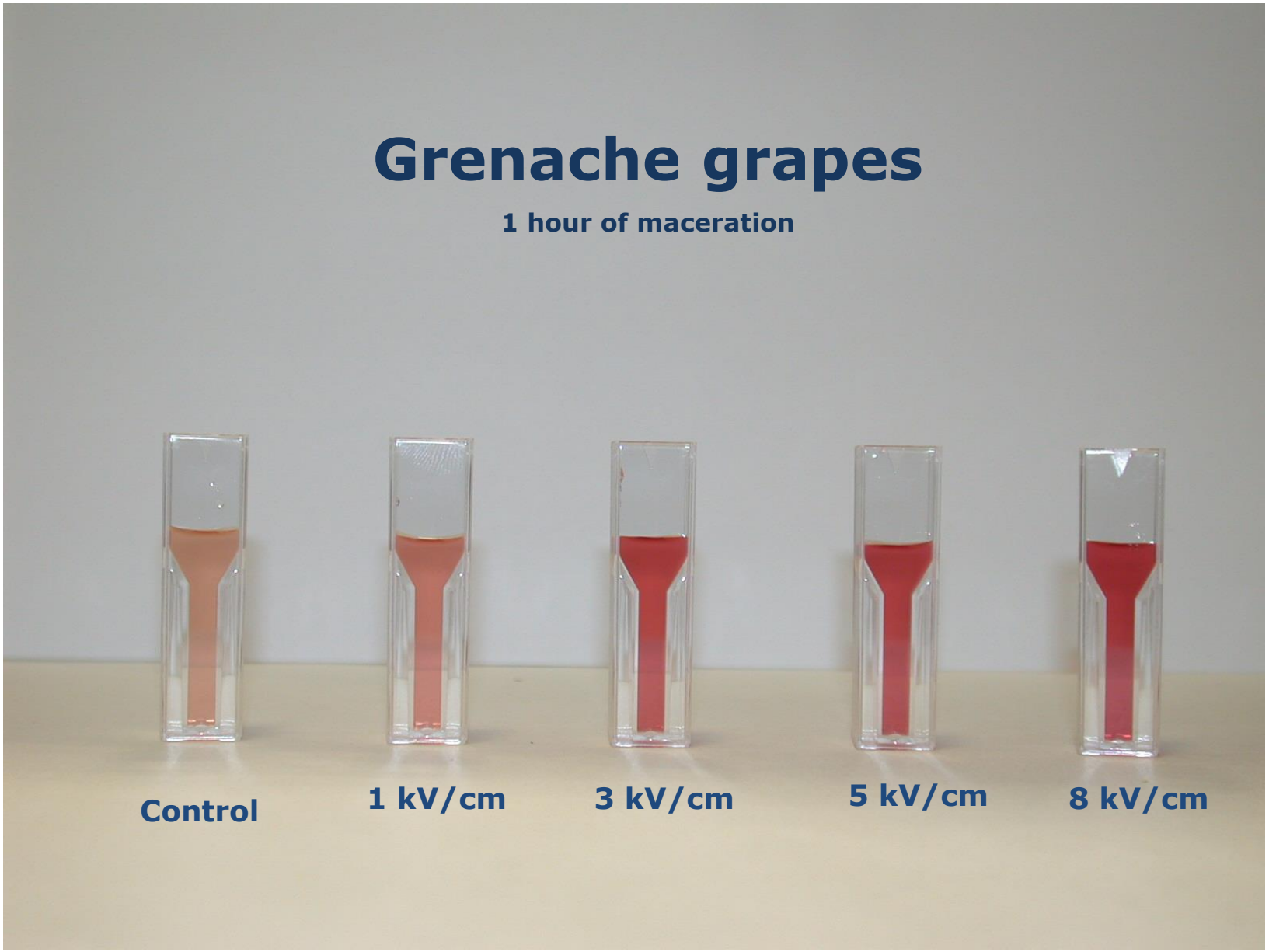


PEF resulted in

- ✓ reduction of drying time by 50% (to the same water content MR=0.06)
- ✓ good colour retention

Grenache grapes

1 hour of maceration



Reduction energetic consumption

Improving red winemaking

	Total production (tons)	Duration maceration (days)	Energy consumption (kWh/t)	Total Energy consumption kWh	Energy cost €/kWh	Total cost €	Saving costs €
Control	13000	10	23.23	302043	0.09	27184	0
PEF	13000	8	21.86	284173	0.09	25576	1608
PEF	13000	7	19.54	253969	0.09	22857	4237
PEF	13000	6	17.21	223764	0.09	20139	7045
PEF	13000	5	14.89	193560	0.09	17420	9763

Table 3.- Comparison of the annual total **energy consumption** and its cost in the maceration/fermentation step to process 13.000 tons of untreated grapes (10 days of maceration) or of grapes treated by PEF (maceration ranging from 8-5 days).

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elea

COGNIT

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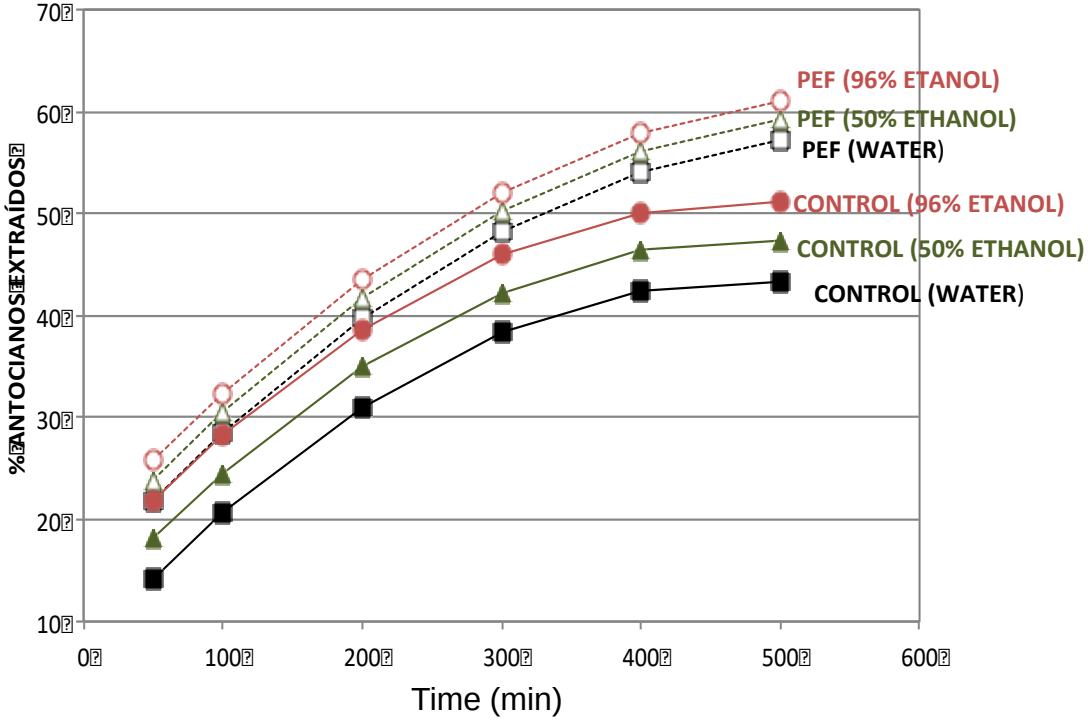
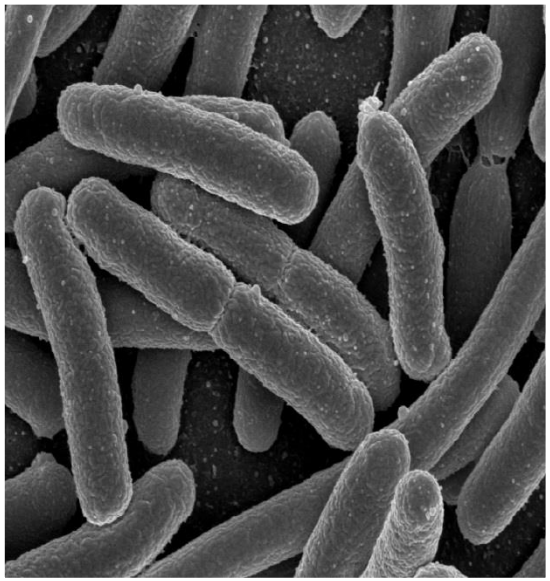
Reduction the use of organic solvents

Extracción of Antocianos (E-162) de patata morada



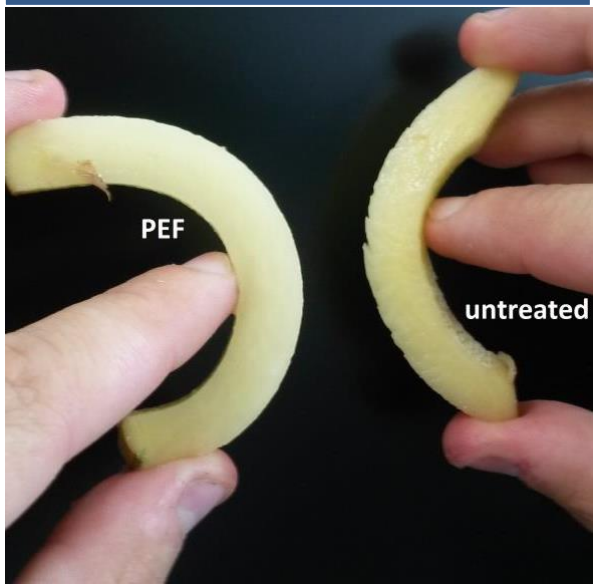
Applications of PEF in the Food Industry

Microbial inactivation



Improving Mass Transfer

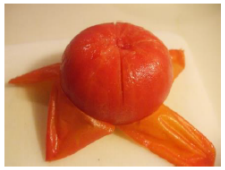
Food Structure Modification

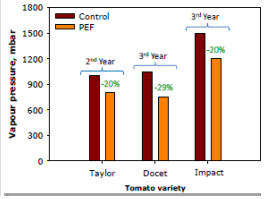


Reduction energetic consumption

Improving Tomato Peeling by PEF

PEF pre-treatment can improve the efficiency of industrial processing of tomato fruits





Improving peelability

- ✓ Reduction of peel resistance and adhesiveness
- ✓ Promoting cracking formation
- ✓ Reduction of peeling index
- ✓ Improving peeling performance

Lowering energy costs of the process

- ✓ Steam saving (20-30%) during the thermophysical peeling phase

Improving potato processing by PEF

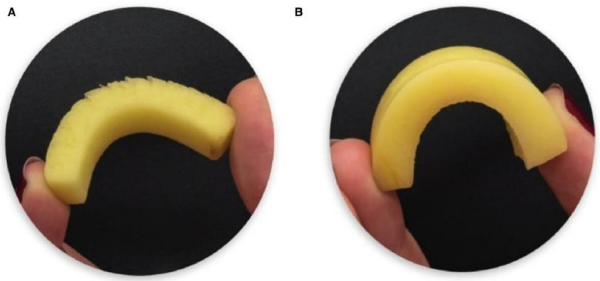
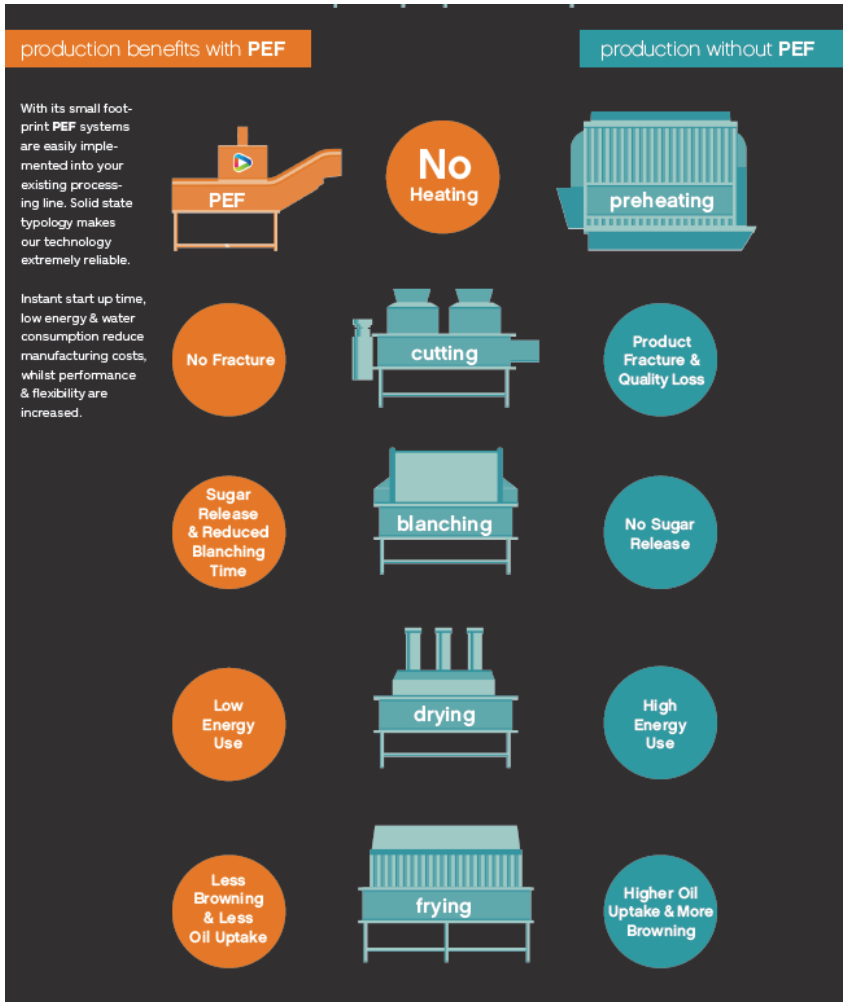


Figure 3 Typical rough and scaly surface appearance of untreated potato sticks (a) and smooth surface of PEF pretreated sticks. (
(Elea GmbH, 2019).



Motivación

- Técnica que puede contribuir a dar solución a algunos retos actuales de la industria alimentaria relacionados con la sostenibilidad y la economía circular
- Posibilidad de transferir las investigaciones a la industria
- Posibilidad de crear un grupo de trabajo multidisciplinar

Miembros

Tecnología PEF

- *Grupo de Electrónica de Potencia y Microelectrónica (*GEPM*)*

Procesos de la industria alimentaria y biotecnológica

- *Grupo de Nuevas Tecnologías de Procesado de los Alimentos*
- *Alimentos de Origen Vegetal* (GIAOVE),
- *Laboratorio de Análisis de Aroma y Enología* (LAAE)

Desarrollo de disolventes con mejores prestaciones y menor impacto medioambiental

- PLATON

Nutrición

- *Growth, Exercise, Nutrition and Development* Group (GENUD)

Producción de inmunotoxinas basadas en la granulolisina a partir de levaduras

- *Apoptosis, Inmunidad y Cáncer*

Evaluación del impacto económico y medioambiental de los procesos agroalimentarios

- *Economía Agroalimentaria y de los Recursos Naturales,*
- *Crecimiento, Demanda y Recursos Naturales* (CREDENAT)

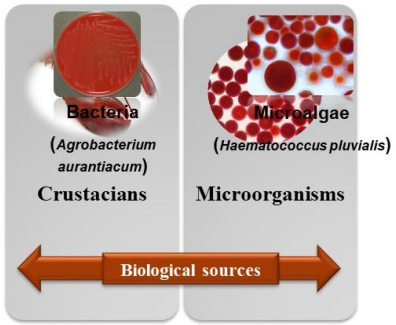
Objetivos

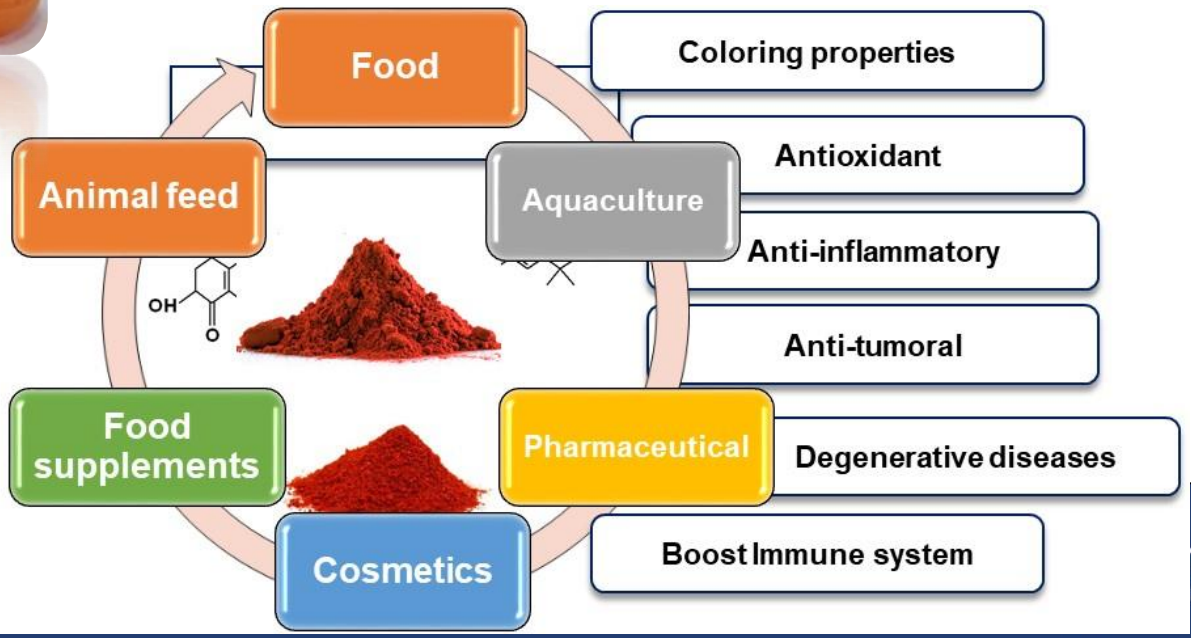
- Optimización de procesos de elaboración de alimentos con objeto de mejorar rendimientos y reducir costes energéticos manteniendo o mejorando la calidad del producto final.
- Desarrollo de procesos de extracción de componentes de interés producidos por microorganismos o plantas más eficientes y menos contaminantes mediante el uso de disolventes con mejores prestaciones y menor impacto medioambiental.
- Puesta a punto de procesos para el aprovechamiento de subproductos de la industria alimentaria para la obtención de productos para la alimentación humana y animal.
- Caracterización de los cambios físico-químicos que ocurren en el producto tratado con influencia en las propiedades sensoriales, nutritivas y funcionales
- Evaluación económica y medioambiental de los procesos desarrollados.

Grupo de Nuevas Tecnologías de Procesado de los Alimentos y Platon

Actividades:

Desarrollo de procesos de extracción más sostenibles basados en el uso de la tecnología PEF en combinación con solventes eutécticos





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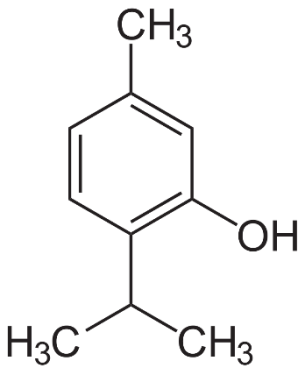
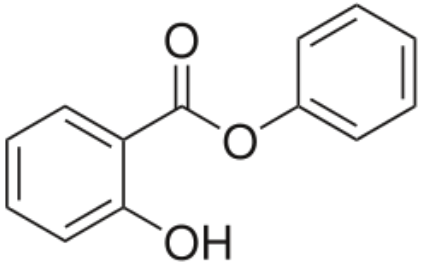
Los **disolventes eutécticos** profundos (DESs) son mezclas de al menos un aceptor de enlaces de hidrógeno (HBA) y un donante de enlaces de hidrógeno (HBD),

- **No son volátiles,**
- **Baja toxicidad**
- **Alta biodegradabilidad**
- **Baratos**
- **Renovables**



Timol



Salol

Grupo de Nuevas Tecnologías de Procesado de los Alimentos y Platon

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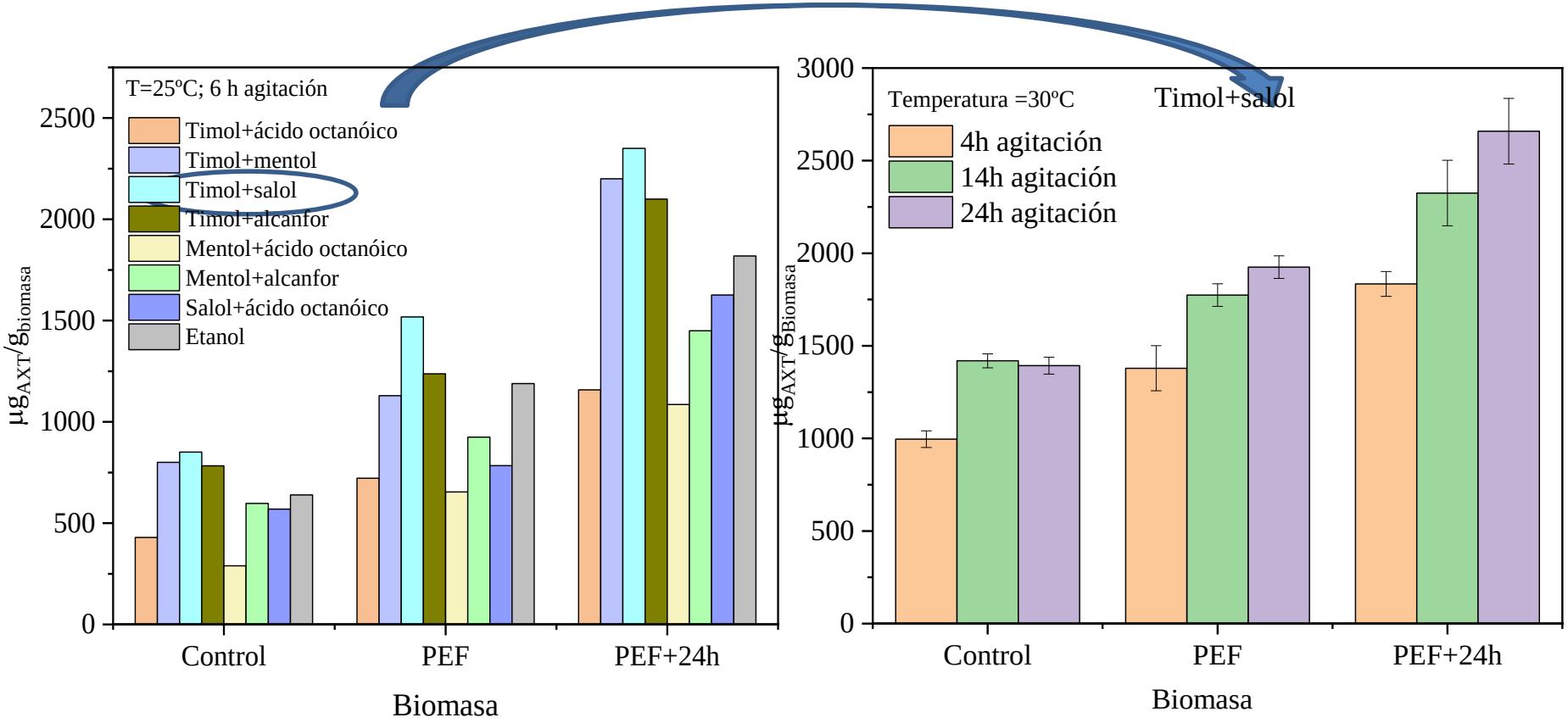

- *Nuevas Tecnologías de Procesado de los Alimentos*
- *Apoptosis, Inmunidad y Cáncer*

Actividades:

Mejora en el rendimiento de obtención de inmunotoxinas basadas en la granulolisina a partir de *Pichia pastoris*

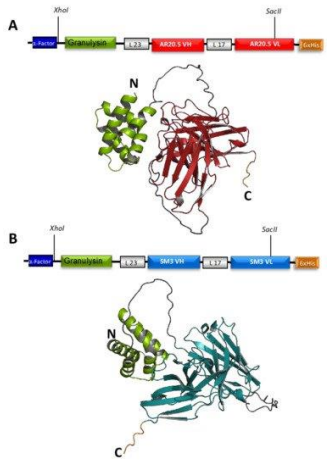

Figure 1. Structural features of immunotoxins. Plasmid constructution and Alpha-Fold structural model of the chimeric protein of the (**A**) immunotoxin AR20.5GRNLY (Granulysin-AR20.5_ScFv-HisTag) and (**B**) SM3GRNLY (Granulysin-SM3_ScFv-HisTag)

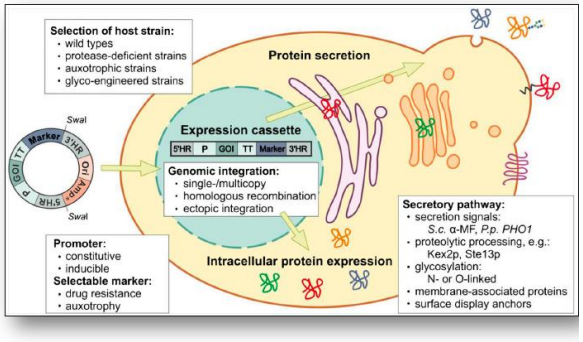

Fig. 14 Esquema sobre las ventajas de P.pastoris para la producción de proteínas recombinantes. (Ahmad et al., 2014).

Tabla 4 Rendimiento de la producción de las proteínas recombinantes usando diferentes técnicas de purificación. Los datos se muestran como la media en mg o nmol de proteína producida por litro de cultivo de P.pastoris.

Protein	Source	Yield (mg/l)	Molar yield (nmols/l)	N° productions
GRNLY	Extracellular	5.040 mg	458 nmol	5
SM3GRNLY	Extracellular	2.115 mg	45 nmol	6
iSM3GRNLY	Intracellular	8.260 mg	176 nmol	7
AR20.5GRNLY	Extracellular	555 mg	12 nmol	16
iAR20.5GRNLY	Intracellular	12.891 mg	292 nmol	7

- *Nuevas Tecnologías de Procesado de los Alimentos*
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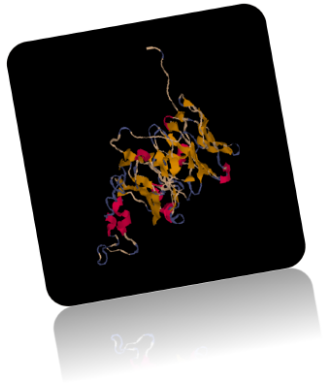


Universidad Zaragoza

FACULTAD DE CIENCIAS

DEPARTAMENTO DE BIOQUÍMICA Y BIOLOGÍA MOLECULAR Y CELULAR

EFECTO ANTITUMORAL DE INMUNOTOXINAS ANTI-TN CONJUGADAS CON GRANULISINA

Tesis doctoral

Patricia A. Guerrero Ochoa

2021

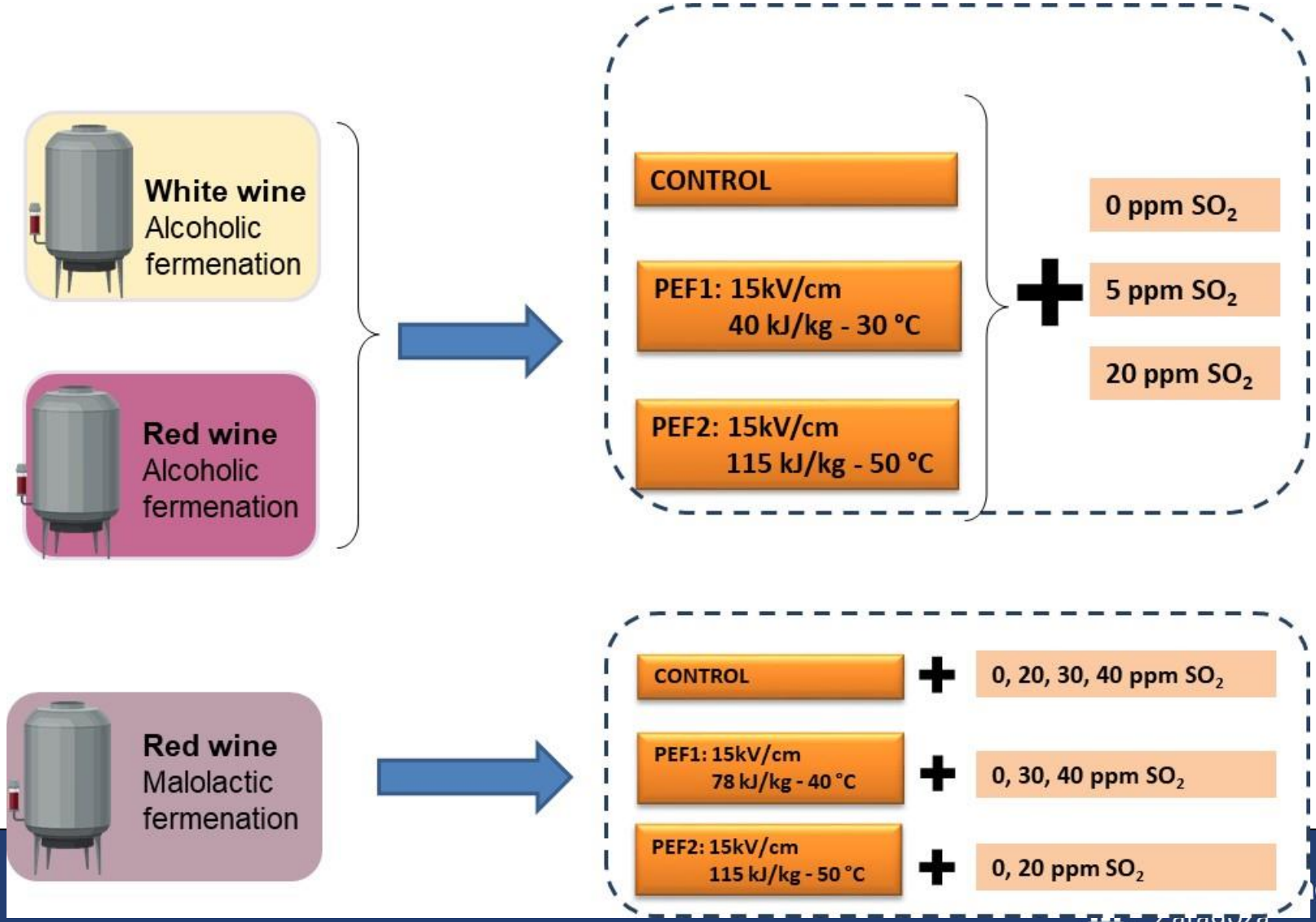
Guerrero-Ochoa, P.; Aguilar-Machado, D.; Ibáñez-Pérez, R.; Macías-León, J.; Hurtado-Guerrero, R.; Raso, J.; Anel, A. Production of a Granulysin-Based, Tn-Targeted Cytolytic Immunotoxin Using Pulsed Electric Field Technology. *Int. J. Mol. Sci.* **2020**, *21*, 6165

Guerrero-Ochoa, P.; Ibáñez-Pérez, R.; Berbegal-Pinilla, G.; Aguilar, D.; Marzo, I.; Corzana, F.; Minjárez-Sáenz, M.; Macías-León, J.; Conde, B.; Raso, J.; Hurtado-Guerrero, R.; Anel, A. Preclinical Studies of Granulysin-Based Anti-MUC1-Tn Immunotoxins as a New Antitumoral Treatment. *Biomedicines* **2022**, *10*, 1223. https://doi.org/10.3390/biomedicines10061223

Actividades:

- *Nuevas Tecnologías de Procesado de los Alimentos*
- *Laboratorio de Análisis de Aroma y Enología*

Mejora en el proceso de elaboración de vino mediante la tecnología PEF



Actividades:

- *Nuevas Tecnologías de Procesado de los Alimentos*
- *Laboratorio de Análisis de Aroma y Enología*

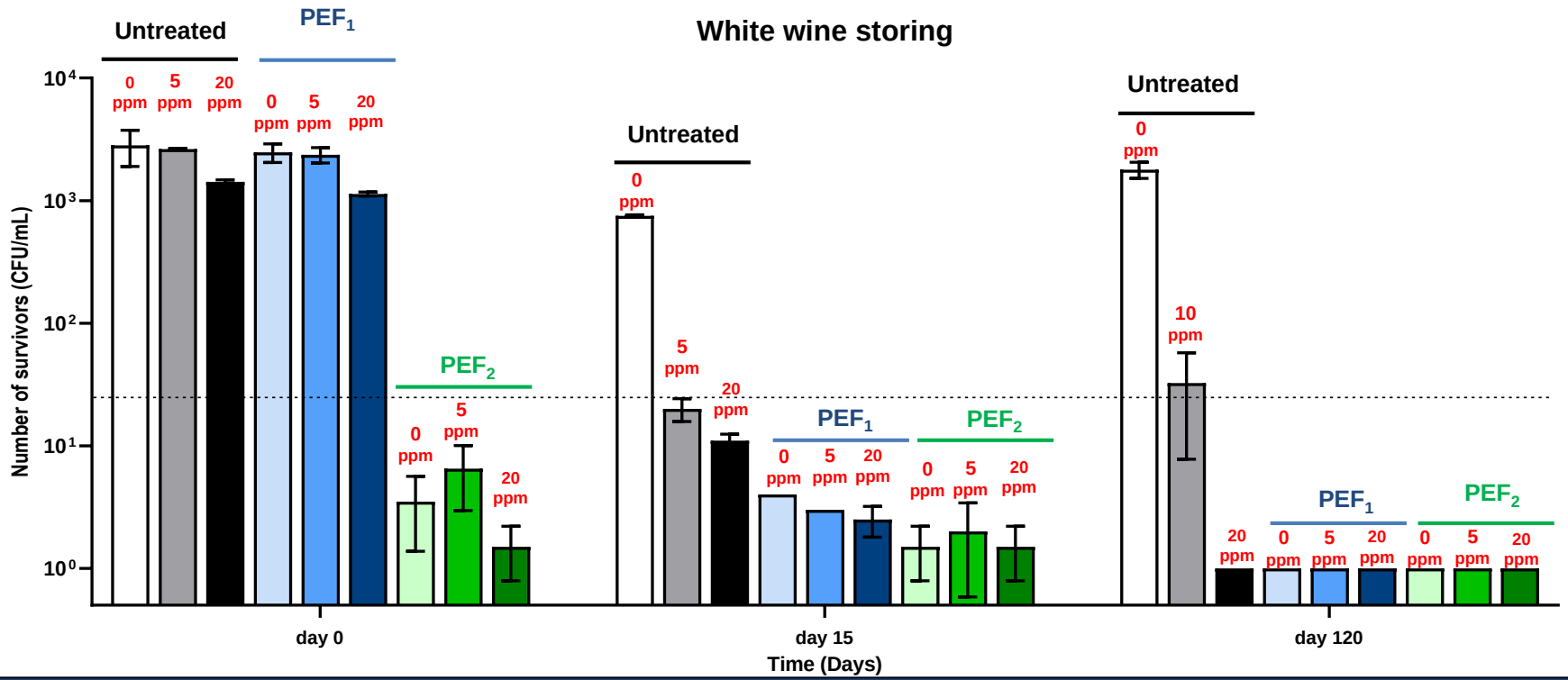
Mejora en el proceso de elaboración de vino mediante la tecnología PEF

PEF1: 15kV/cm - 108 µs - 43 kJ/kg - outlet Tª: 30 °C

PEF2: 15kV/cm - 177 µs – 97 kJ/kg - outlet Tª: 45 °C

Chardonnay wine after alcoholic fermentation

Saccharomyces bayanus



Actividades:

- *Nuevas Tecnologías de Procesado de los Alimentos*
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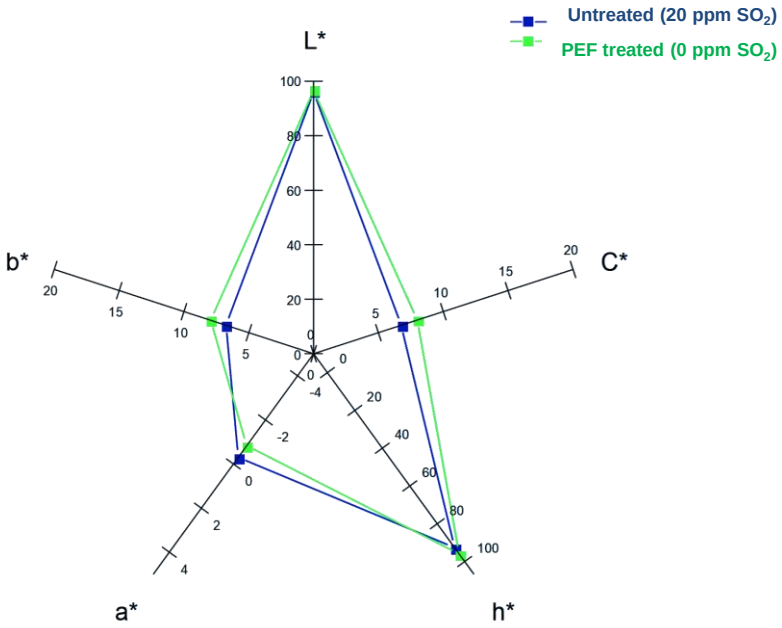
Mejora en el proceso de elaboración de vino mediante la tecnología PEF

Chardonnay wine after alcoholic fermentation

PEF1: 15kV/cm - 108 µs - 43 kJ/kg - outlet Tª: 30 °C

	Untreated $S0_2$ (20 ppm)	PEF_2 $S0_2$ (0 ppm)
pH	3.33 ±0.05	3.34 ±0.02 a
Glucose-Fructose (g/L)	0.32 ±0.02	0.30 ±0.02
% Ethanol (v/v)	13.43 ±0.11	13.07 ±0.21
Total acidity (g/L)[a]	5.45 ±0.19	6.12 ±0.22
Volatile acidity (g/L)[b]	0.1 ±0.00	0.1 ±0.00
Malic acid (g/L)	1.93 ±0.01	1.94 ±0.02
Free SO_2 (mg/L)*	9.6 ±3.2	3.2 ±3.2
Total SO_2 (mg/L)*	35.2 ±3.2	22.4 ±3.2
Abs 420 nm	0.127 ± 0.001	0.135 ± 0.018



Actividades:

- *Nuevas Tecnologías de Procesado de los Alimentos*
- *Laboratorio de Análisis de Aroma y Enología*

Mejora en el proceso de elaboración de vino mediante la tecnología PEF

Chardonnay wine after alcoholic fermentation

Concentration of esters (µg/L) detected in untreated (20 ppm SO_2) and PEF (15kV/cm - 177 µs – 97 kJ/kg - outlet Tª: 45 °C) wine

Compound	Odour threshold	Control SO_2 (20 ppm)	PEF_2
Branched ethyl esters			
Ethyl isobutyrate	15	82.90 ± 3.68	83.84 ± 1.93
Ethyl 2-methylbutyrate	18	6.01 ± 0.39	6.32 ±0.07
Ethyl isovalerate	3	7.21 ± 0.70	8.29 ± 1.81
Acetates			
Isobutyl acetate	1600	130.60 ± 4.06	143.45 ± 2.28
Butyl acetate	1800	17.48 ± 0.27	17.37 ± 1.11
Phenylethyl acetate	250	672.09 ± 6.06	736.90 ± 9.19
Carbonyl compound			
Benzaldehyde	2000	2.40 ± 0.10	2.22 ± 0.12
Lineal ethyl esters			
Ethyl propanoate	5.5	0.10 ± 0.02	0.11 ± 0.00
Ethyl butyrate	0.125	0.18 ± 0.01	0.23 ± 0.03
Ethyl hexanoate	0.062	0.63 ± 0.03	0.73 ± 0.19
Ethyl octanoate	0.58	0.77 ± 0.05	0.83 ± 0.14
Ethyl decanoate	0.2	0.40 ± 0.05	0.45 ± 0.12

Concentration (µg/L) of terpernes and norisoterpenes detected in untreated (20 ppm SO_2) and PEF (15kV/cm - 177 µs – 97 kJ/kg - outlet Tª: 45 °C) wine

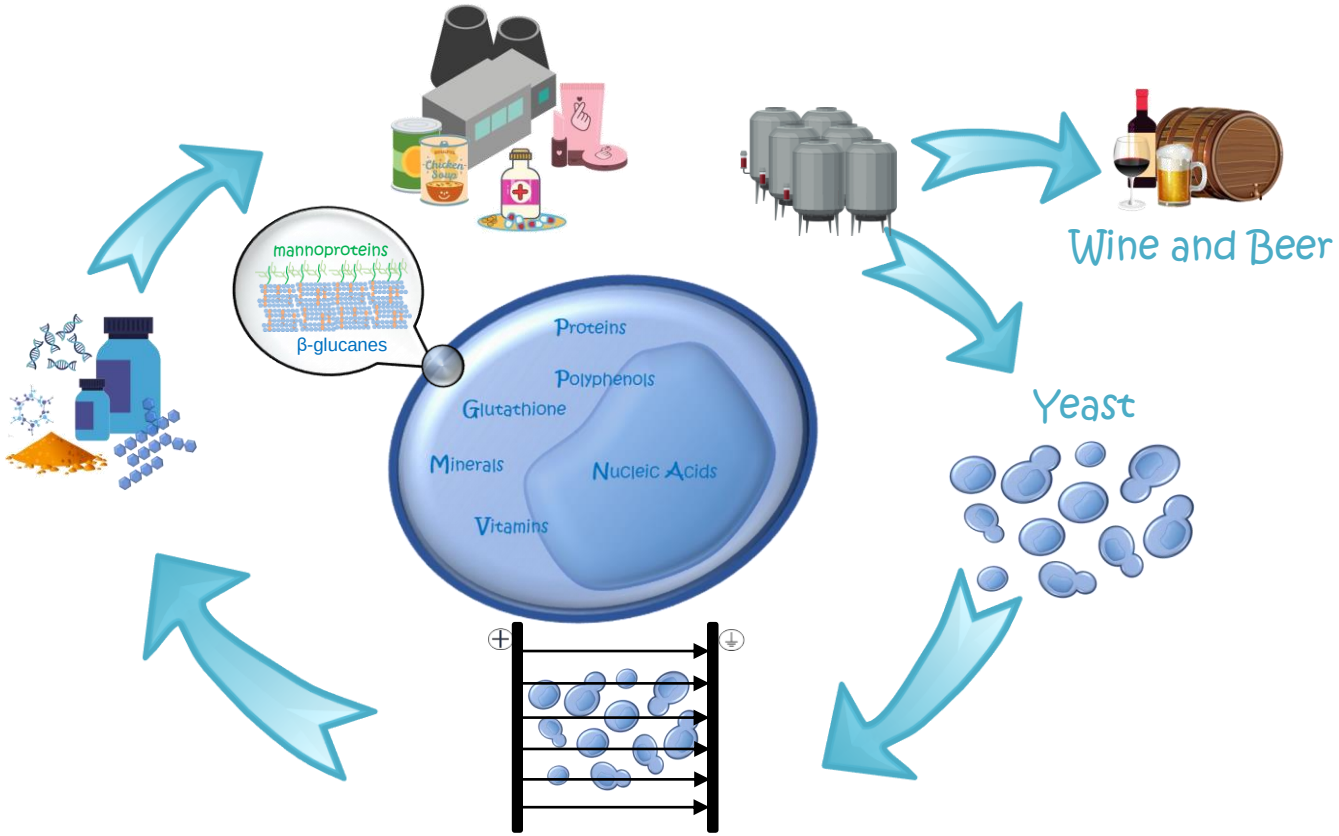
Compound	Odour threshold	Control SO_2(20 ppm)	PEF_2
Monoterpenols			
Linalool	25	6.50 ± 0.08	6.67 ± 0.18
Linalool acetate		0.14 ± 0.01	0.12 ± 0.01
α-Terpineol	250	2.07 ± 0.10	2.10 ± 0.12
β-Citronelol	100	1.95 ± 0.18	1.72 ± 0.06
Geraniol	20	9.33 ± 0.08	8.17 ± 2.03
Norisoprenoids			
β-Damascenone	0.05	14.44 ± 1.19	14.95 ± 1.20
α-Ionone	2.60	0.61 ± 0.04	0.65 ± 0.02
β-Ionone	0.09	0.23 ± 0.10	0.25 ± 0.15
Phenols			
Guaiacol	9.5	4.69 ± 0.50	5.53 ± 0.11
o-Cresol	31	0.30 ± 0.01	0.34 ± 0.04
4-Ethylguaiacol	33	0.23 ± 0.04	0.28 ± 0.08
m-Cresol	68	0.17 ± 0.08	0.17 ± 0.01
4-Propylguaiacol	10	< DL	< DL
Eugenol	6	1.27 ± 0.08	1.17 ± 0.05
4-Ethylphenol	35	0.64 ± 0.01	0.65 ± 0.03
4-Vinylguaiacol	40	350.20 ± 8.20	406.33 ± 16.28
E-Isoeugenol	6	0.75 ± 0.03	0.83 ± 0.07
2,6-Dimethoxyphenol	570	3.89 ± 1.25	4.06 ± 1.09
4-Vinylphenol	180	311.08 ± 10.30	345.49 ± 4.22
4-Alyl-2,6-dimethoxyphenol	1200	5.73 ± 0.57	5.90 ± 0.38

Actividades:

- *Nuevas Tecnologías de Procesado de los Alimentos*
- *Alimentos de Origen Vegetal* (GIAOVE),

Aprovechamiento de subproducts mediante la tecnología PEF

PID2020-113620RB-I00, IMPLEMENTACION DE LA TECNOLOGIA DE LOS PULSOS ELECTRICOS DE ALTO VOLTAJE PARA LA REVALORIZACION DE LEVADURAS DEL SECTOR CERVEZERO Y ENOLOGICO



Actividades:

7th School on Pulsed Electric Field Applications in Food and Biotechnology

Zaragoza, Spain **31 May - 2 June, 2021**

ISEBTT International Society for Electroporation-Based Technologies and Treatments

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