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Optimization of Pulsed Electric Fields-Assisted Extraction of phenolic compounds from white and red grape pomace using Response Surface Methodology

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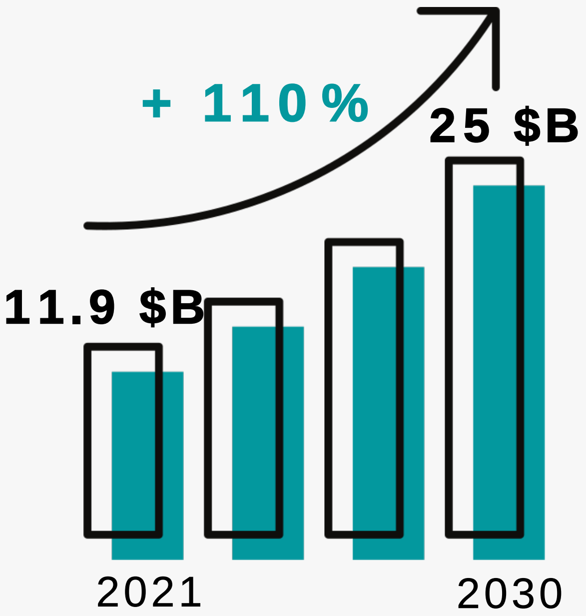
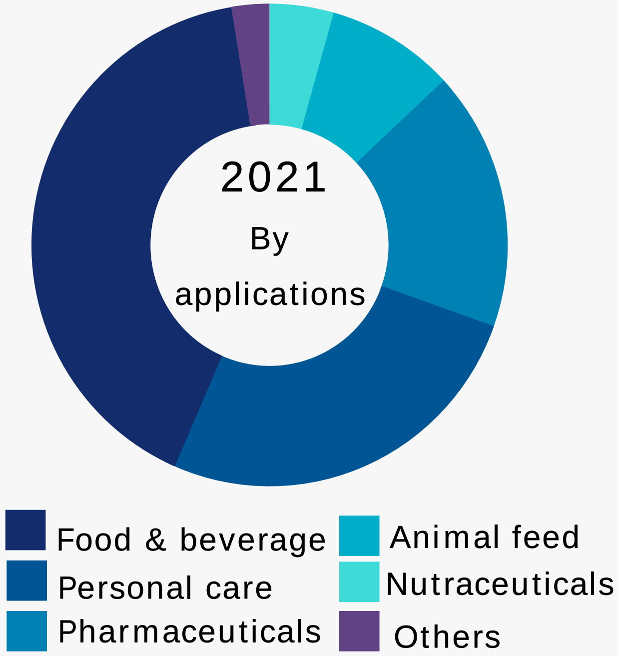
16/06/2022, Corfù, Greece

Global natural extracts market





Precedence research, 2022

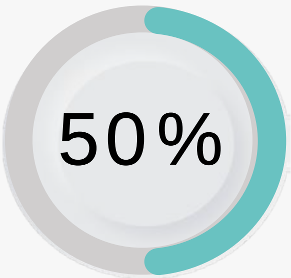
Food waste and by-products: a low-cost source of bioactive compounds

WRI analysis based on FAO

Eurostat, 2018

Intensive demand for natural ingredients

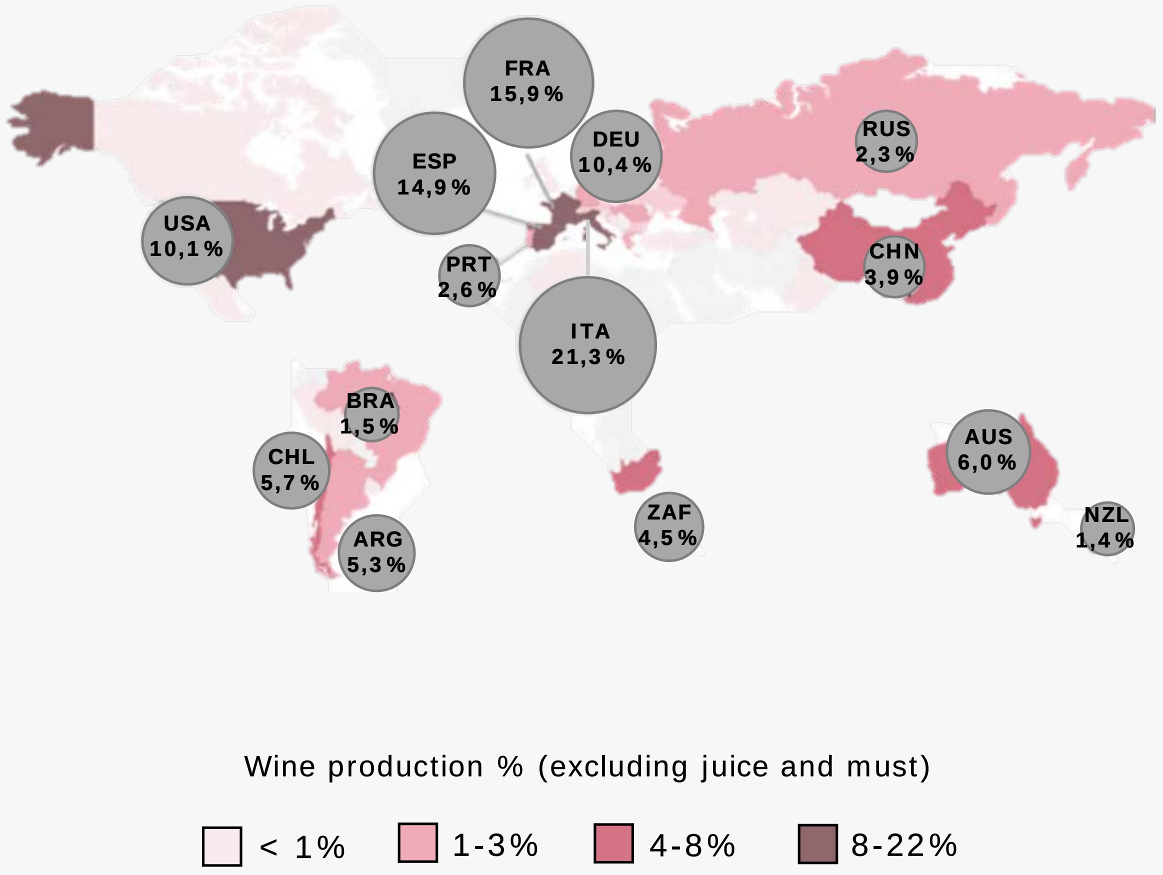
Innova Market Insights, Top Ten Trends for 2021



Call for a reduction of global food waste by 2030

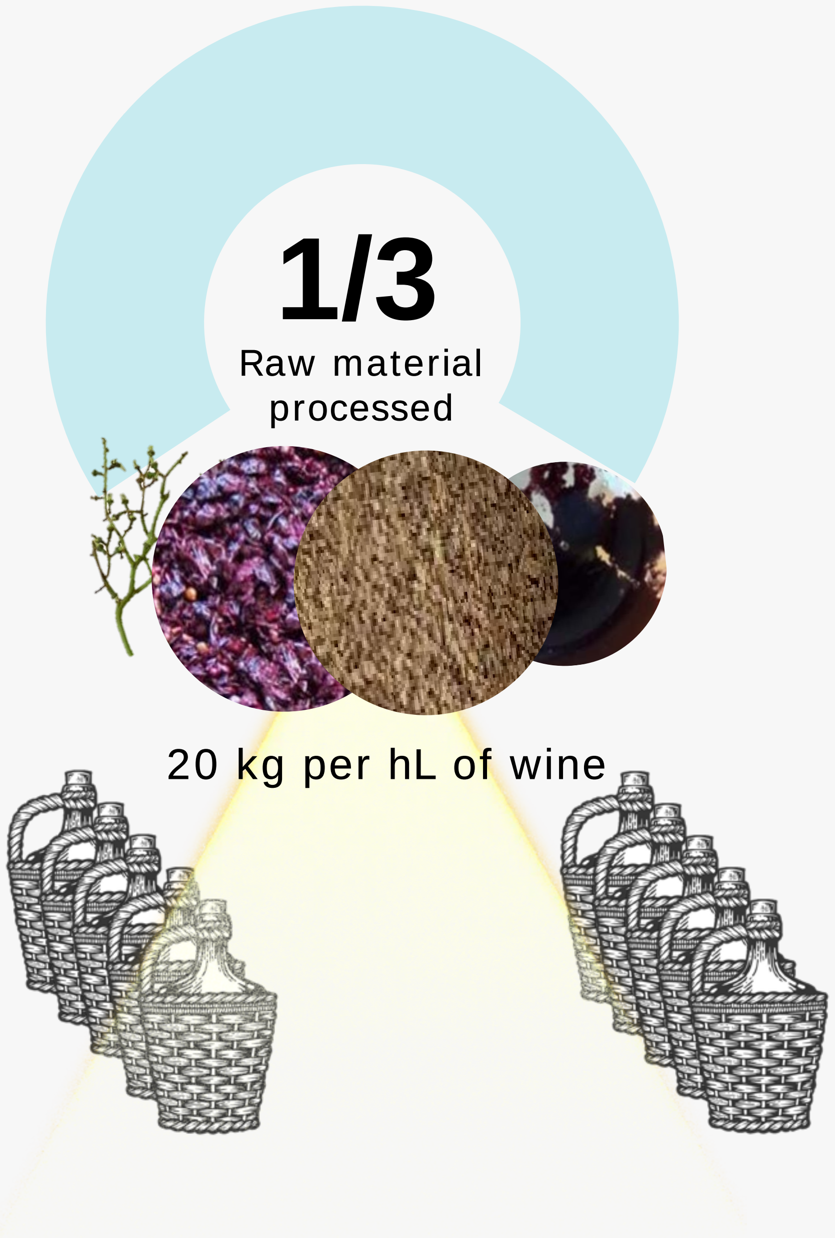
Wine production in 2021

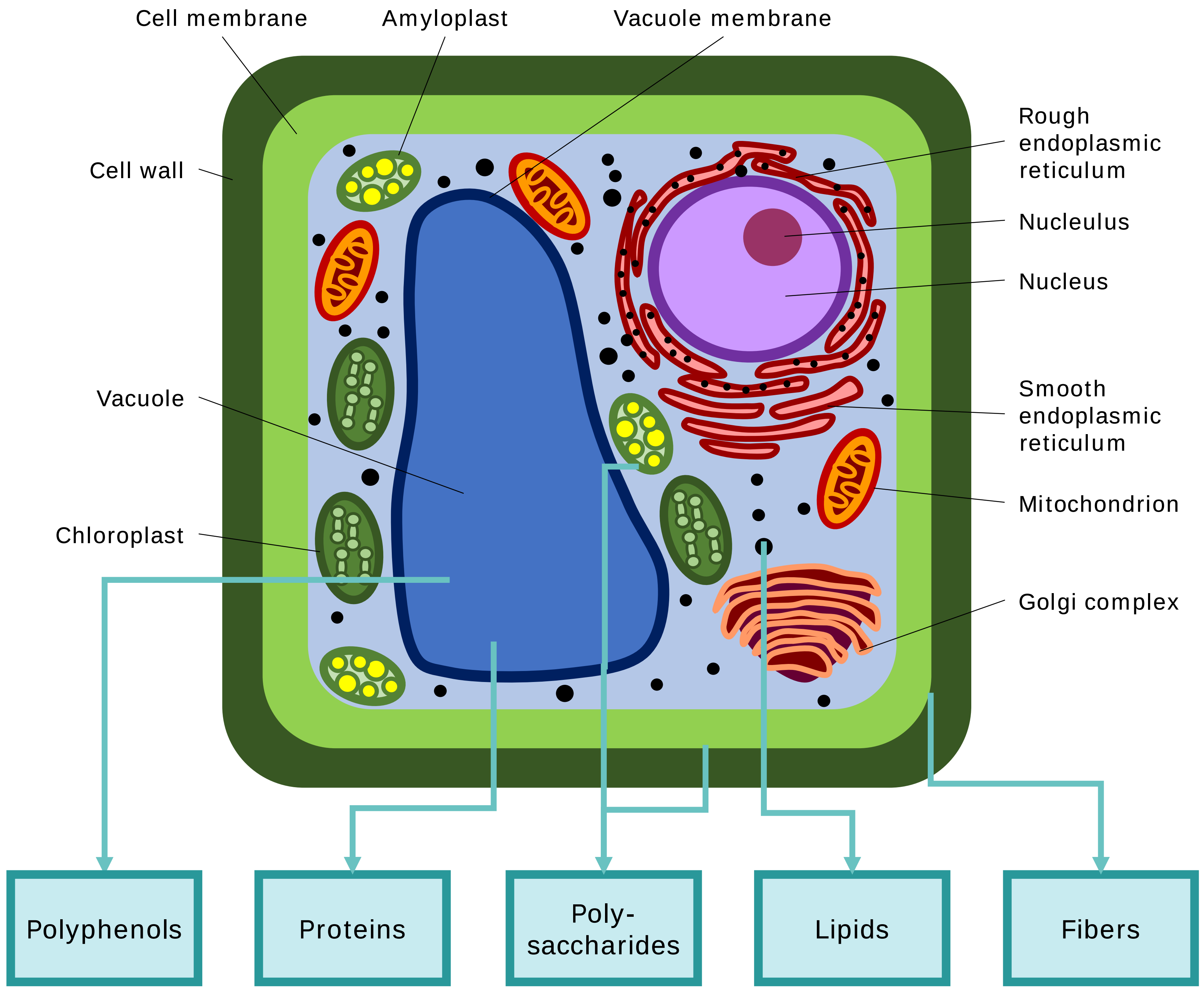
236 MhL of wine produced in 2021



OIV, International Organisation of Vine and Wine, 2020

Agri-food by-products: a rich and low-cost source of bioactive compounds




Cell membrane
Amyloplast
Vacuole membrane
Cell wall
Rough endoplasmic reticulum
Nucleulus
Nucleus
Vacuole
Smooth endoplasmic reticulum
Mitochondrion
Chloroplast
Golgi complex
Polyphenols
Proteins
Poly-saccharides
Lipids
Fibers

How to unlock and recover intracellular bioactives from these sources?

Need for cell disintegration pre-treatments

- Infusion
- Maceration
- Percolation
- Soxhlet extraction
- Decoction.

Strenght:
- Well-established techniques
- Widely spread techniques.

Weaknesses:
- Energy intensive
- High solvent consumption
- Long extraction times
- High temperatures
- Low extraction yields.

Conventional extraction techniques

- **Pulsed electric fields (PEF)-assisted extraction**
- High Pressure Homogenization (HPH)
- Ultrasound-assisted extraction (UAE)
- Supercritical fluid extraction (SFE)
- Microwave-assisted extraction (MAE)
- High Voltage Electrical Discharges (HVED)-assisted extraction.

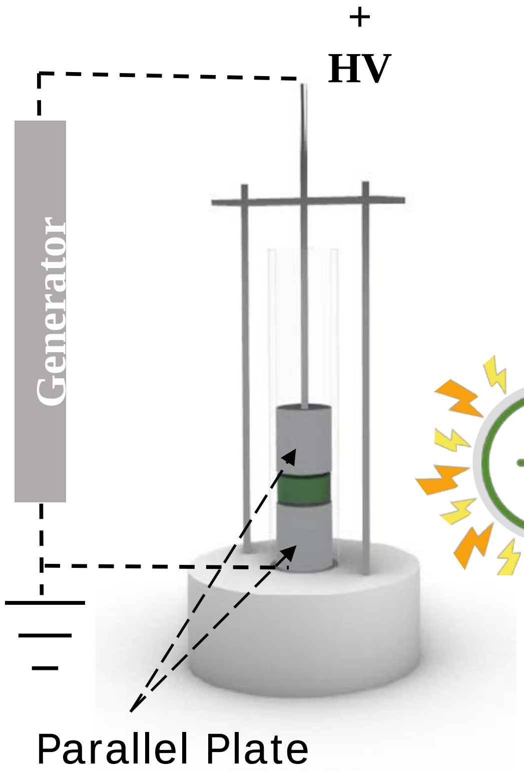
Strenght:
- Reduced residence times and solvent consumption
- Improved mass transfer
- Preserved functional properties
- Enhanced yields

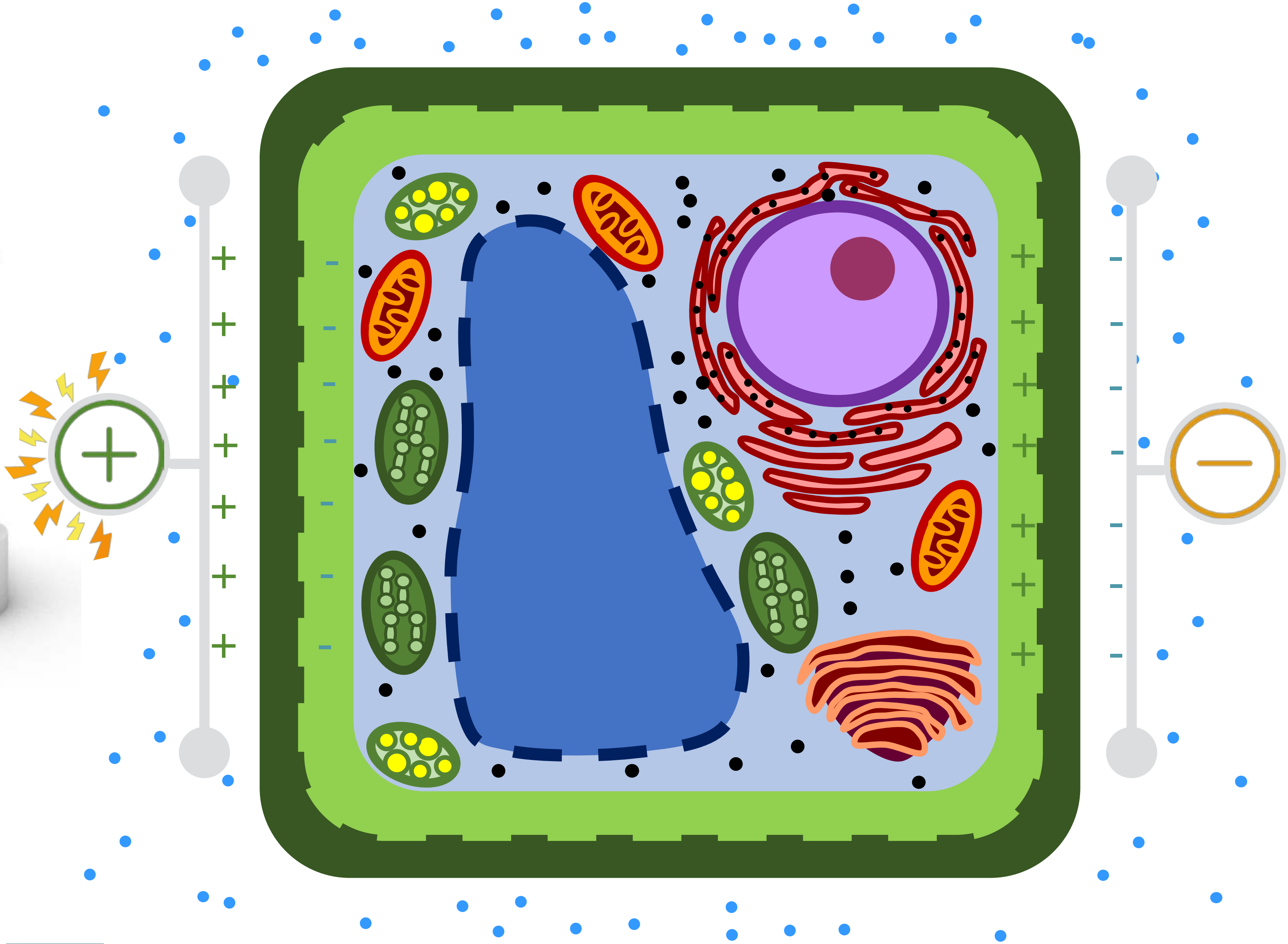
Weaknesses:
- Significant investment costs

Non-conventional extraction techniques

Kumar et al., 2015

Pulsed Electric Fields: an effective cell permeabilization approach





E: 0.5–10 kV/cm
W_T: 1–20 kJ/kg

Selective membrane permeabilization

Main challenges to face

- Few works demonstrated the feasibility of PEF technology to intensify the recovery yield of phenolic compounds from winery by-products

- None of them focused on the extractability of phenolic compounds from white grape pomace

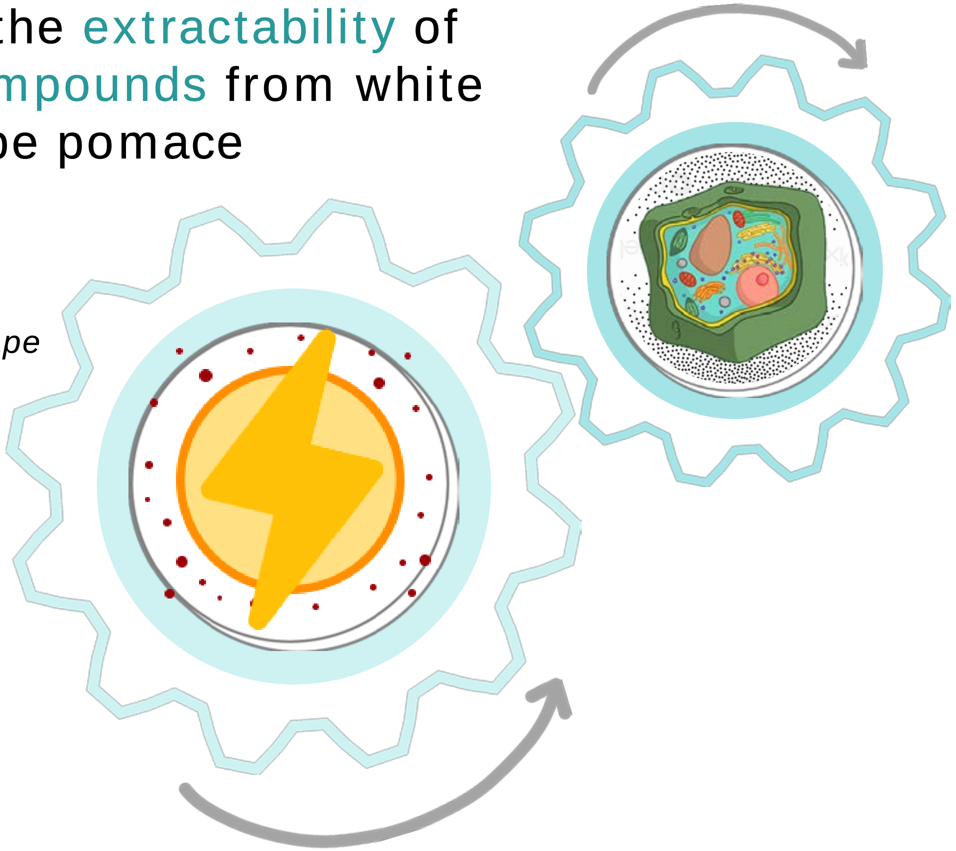
- To fully exploit the advantages deriving from the application of PEF-assisted extraction over the conventional SLE process, an optimization step is required

- No study on the optimization of the whole innovative extraction process made of a PEF pre-treatment stage followed by a subsequent SLE step

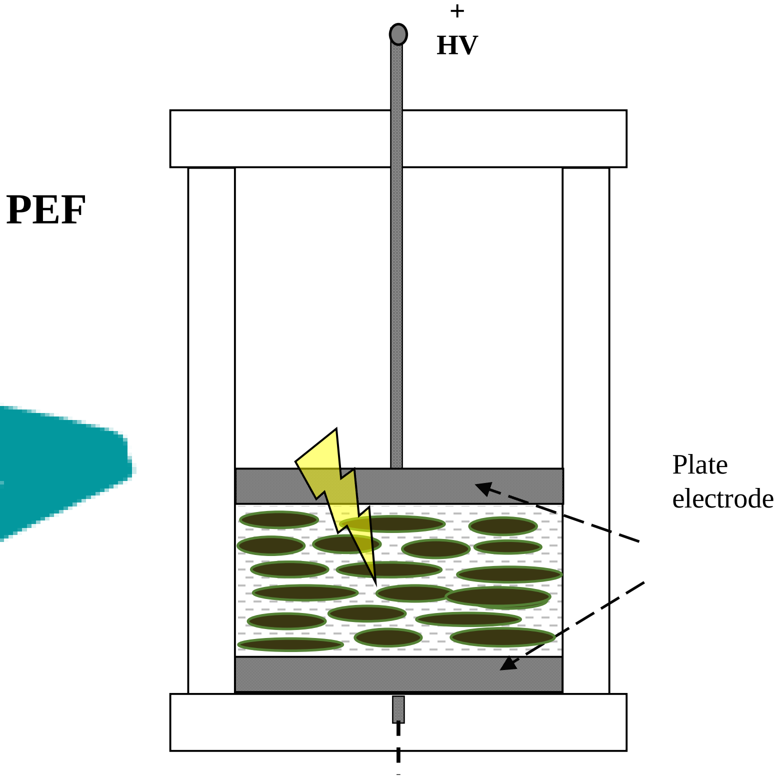
Objective of the work

Potentiality of PEF pre-treatment to intensify the extractability of bioactive compounds from white and red grape pomace

White and Red grape pomace



Optimization of the PEF processing conditions based on cell disintegration index evaluations



Input variables

- E=0.5-5 kV/cm
- W_T=1-20 kJ/kg

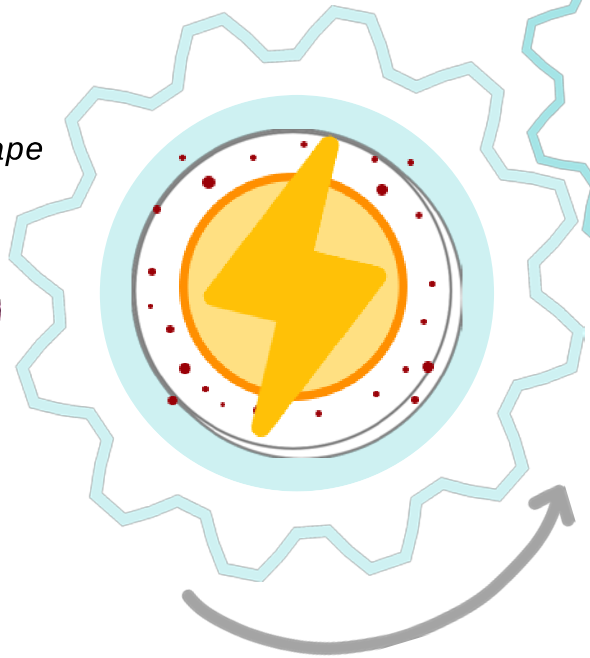
Response variable

- Z_p

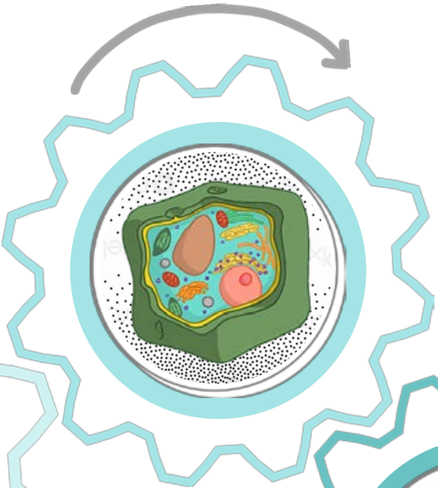
Objective of the work

Potentiality of PEF pre-treatment to intensify the extractability of bioactive compounds from white and red grape pomace

White and Red grape pomace



Optimization of the PEF processing conditions based on cell disintegration index evaluations

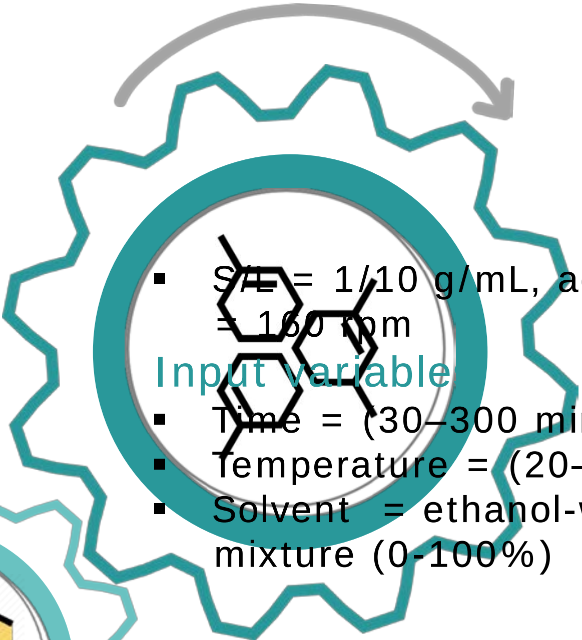


Optimization of the SLE conditions

▪ S/L = 1/10 g/mL, agitation = 160 rpm

Input variables
- Time = (30–300 min),
- Temperature = (20–50 °C),
- Solvent = ethanol-water mixture (0-100%)

Response variables
- Total phenolic compounds
- Total flavonoids
- Antioxidant activity
- Anthocyanin content



Quantification of the Phenolic Compounds via HPLC-PDA Analysis

RESULTS

Optimization of PEF processing conditions
Cell Membrane Permeabilization of Grape Pomace Tissues

$$Y_k = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

Xi and Xj: independent variables; b0, bi, bii, and bij: intercept, regression coefficients of the linear, quadratic, and interaction terms of the model, respectively.

Coefficients	Response Zp [-]			
	White grape pomace		Red grape pomace	
β_0	-0.123806		-0.148433	
β_1 (E)	0.349245	***	0.240292	***
β_2 (W_T)	0.017540	***	0.018985	***
β_{12} (E x W_T)	-0.001756	**	0.002172	ns
β_{11} (E x E)	-0.035406	***	-0.028986	**
β_{22} (W_T x W_T)	-0.000229	ns	-0.000562	ns
p value of the model	< 0.0001	***	< 0.0001	***
R^2	0.9956		0.9545	
RMSE	0.0067		0.0071	

ns, not significant for $p > 0.05$.
**significant for $p \leq 0.01$; ***significant for $p \leq 0.001$.
RMSE, Root Mean Square Error.

White grape pomace: E = 3,8 kV/cm; Wt = 10 kJ/kg

Red grape pomace: E = 4,6 kV/cm; Wt = 20 kJ/kg

Optimization of SLE conditions

$$Y_k = \alpha_0 + \sum_{i=1}^{3} \alpha_i X_i + \sum_{i=1}^{3} \alpha_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{4} \alpha_{ij} X_i X_j + \sum_{i=1}^{3}\sum_{j=i+2}^{5} \alpha_{ij} X_i X_j + \sum_{i=1}^{3} \alpha_{iii} X_i^3 + \sum_{i=1}^{3}\sum_{j=i+1}^{4} \alpha_{iij} X_i^2 X_j + \sum_{i=1}^{3}\sum_{j=i+1}^{4} \alpha_{ijj} X_i X_j^2$$

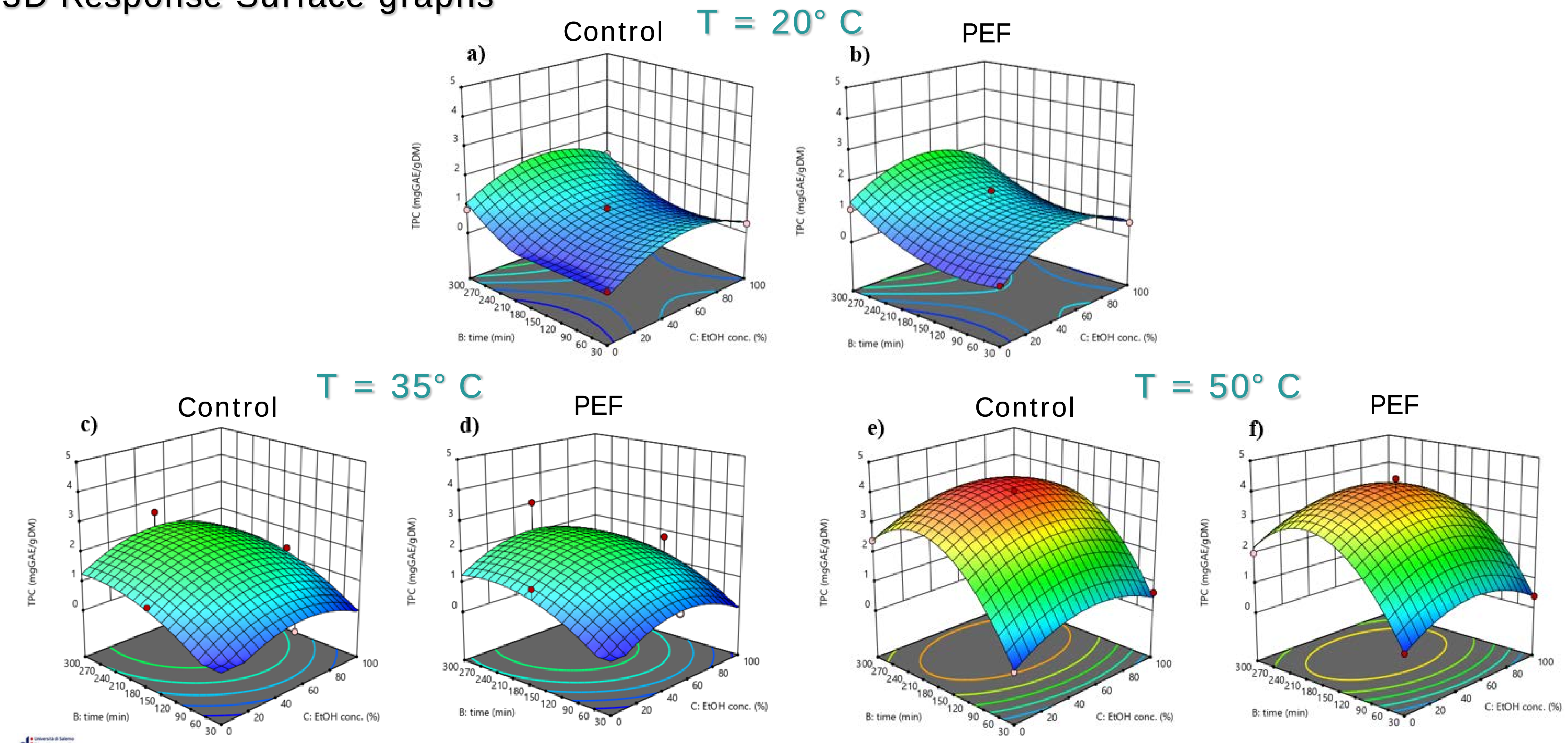
Yk: predicted response variables; Xi and Xj: independent variables; a0, ai, aii, aij, aiij, and aijj: intercept, regression coefficients of the linear, quadratic, and interaction terms of the model, respectively

Coefficients	SLE						PEF-assisted extraction					
	TPC ($mgGAE/g_{DM}$)		FC ($mgQE/g_{DM}$)		FRAP ($mgAAE/g_{DM}$)		TPC ($mgGAE/g_{DM}$)		FC ($mgQE/g_{DM}$)		FRAP ($mgAAE/g_{DM}$)	
β_0	3.00885		5.60444		5.27926		3.3065		11.29163		6.57299	
β_1 (T)	-0.187327	***	-0.380323	***	-0.346407	***	-0.198687	***	-0.708839	***	-0.414410	**
β_2 (time)	-0.041053	***	-0.054257	***	-0.050758	***	-0.036188	***	-0.082916	***	-0.056876	**
β_3 (EtOH)	0.038154	***	0.022133	***	0.027234	***	0.04076	**	0.040509	**	0.036218	ns
β_{12} (T x t)	0.001647	**	0.022157	**	0.003027	*	0.001539	ns	0.003215	ns	0.002992	ns
β_{13} (T x EtOH)	-0.000041	ns	0.000332	ns	0.000214	**	-0.000094	ns	0.000371	ns	-0.000039	ns
β_{23} (t x EtOH)	-0.000012	ns	0.000015	ns	-5.72614E-6	ns	-8.88358E-6	ns	0.000034	ns	-8.6925E-6	ns
β_{11} (T x T)	0.002114	***	0.004863	***	0.004787	*	0.002297	**	0.008894	**	0.005638	ns
β_{22} (t x t)	0.000129	**	0.000164	**	0.000059	**	0.000118	*	0.000253	*	0.000090	ns
β_{33} (EtOH x EtOH)	-0.000321	*	-0.000202	*	-0.000301	***	-0.000345	*	-0.000433	*	-0.000327	**
β_{112} (T x T x t)	-	-	-	-	-0.000034	***	-	-	-	-	-0.000027	*
β_{122} (T x t x t)	-4.55189E-6	***	-5.88399E-6	***	-2.15914E-6	***	-4.39068E-6	**	-9.22899E-6	**	-3.04141E-6	*
p value of the model	<0.0001	***	<0.0001	***	<0.0001	***	0.0076	**	0.001	***	0.0042	**
R^2	0.967		0.977		0.990		0.863		0.916		0.912	
RMSE	0.057		1.193		0.095		0.345		0.651		0.153	

TPC: +8%
FC: +31%
AA: +36%

Total phenolic compounds (TPC)

3D Response Surface graphs

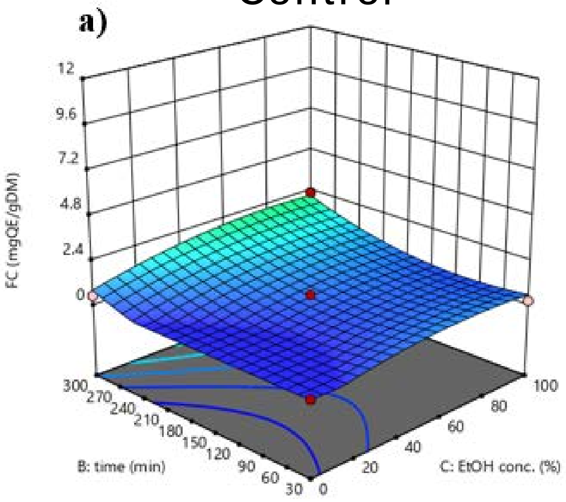


Flavonoid content (FC)

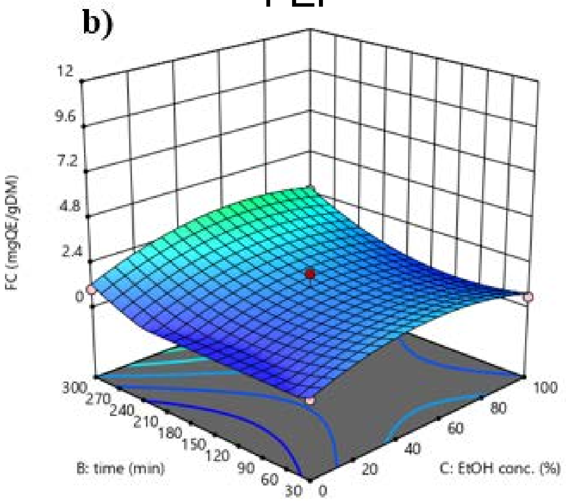
3D Response Surface graphs

T = 20° C

Control

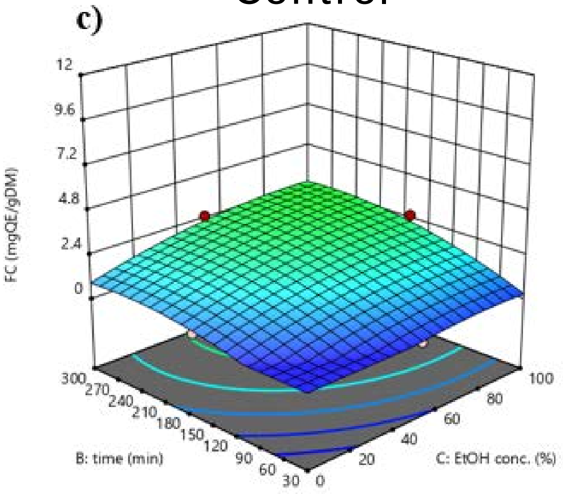


PEF

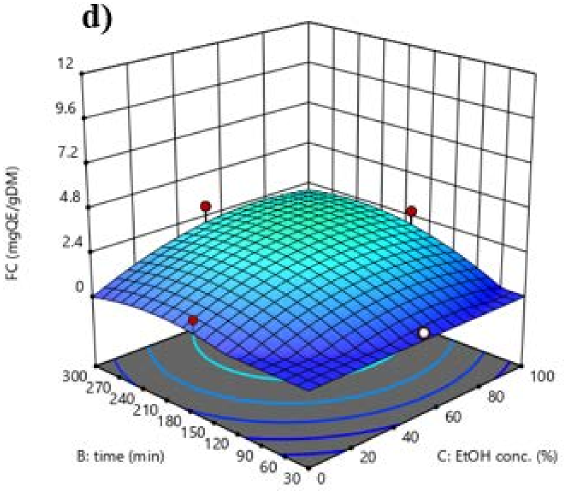


T = 35° C

Control

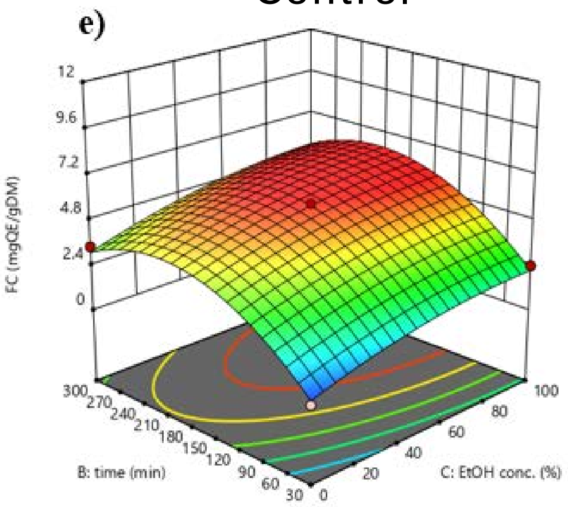


PEF

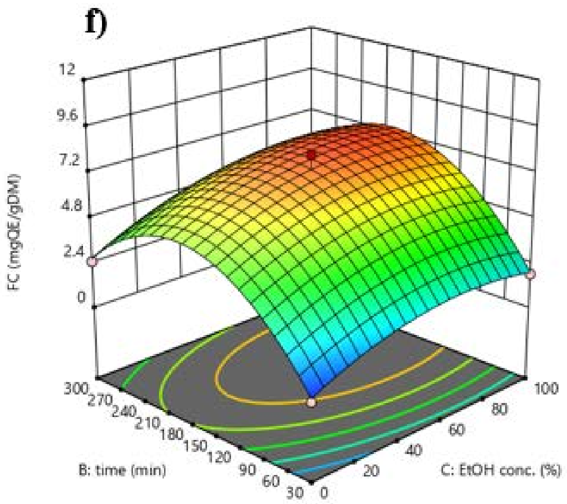


T = 50° C

Control



PEF

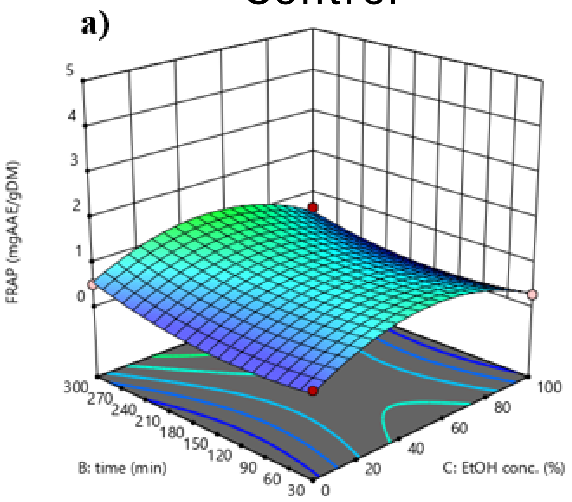


Antioxidant activity (FRAP)

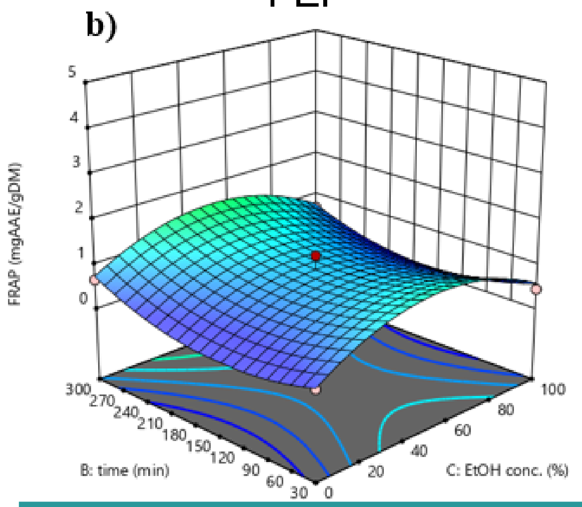
3D Response Surface graphs

T = 20° C

Control

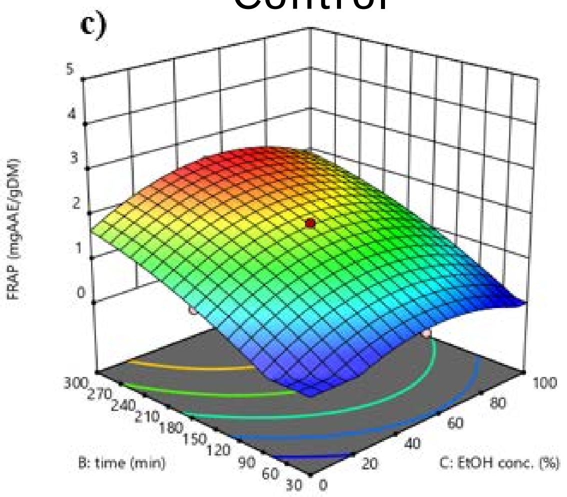


PEF

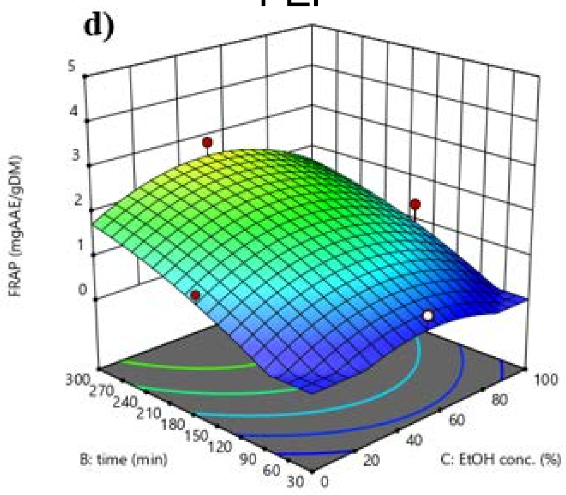


T = 35° C

Control

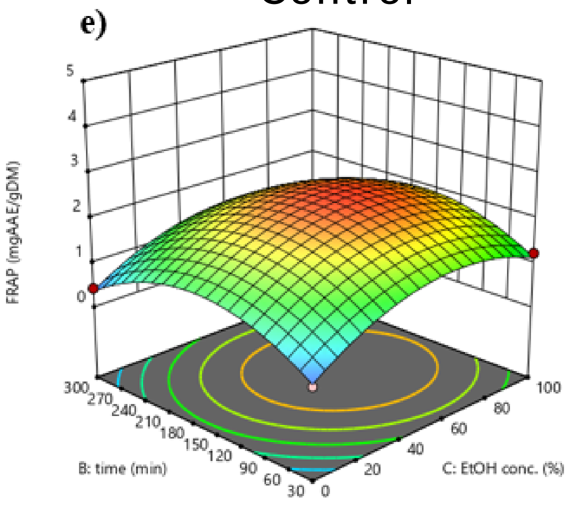


PEF

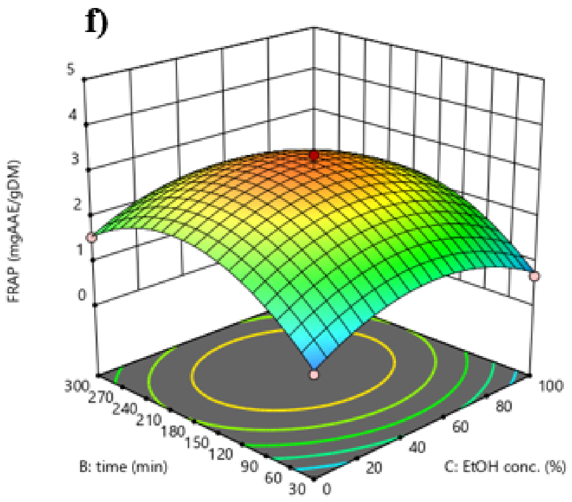


T = 50° C

Control

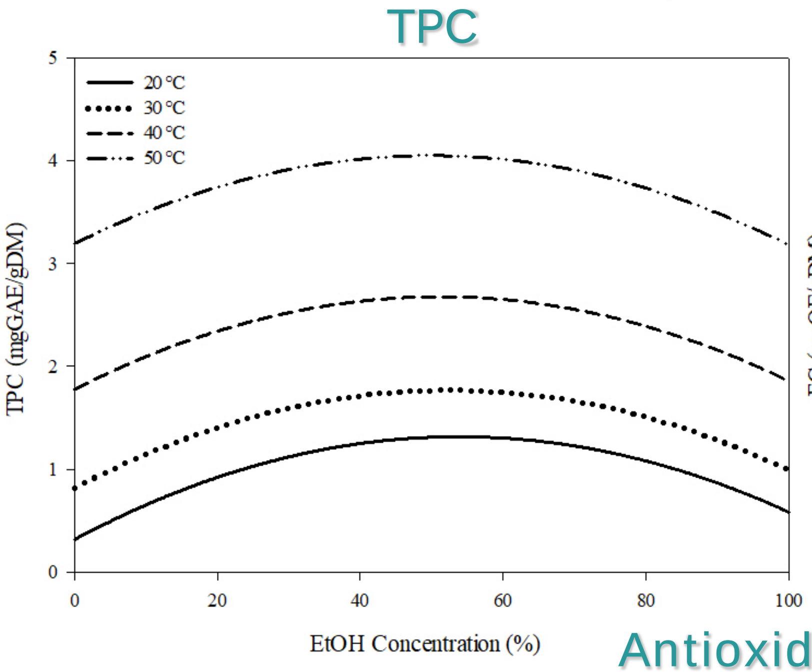


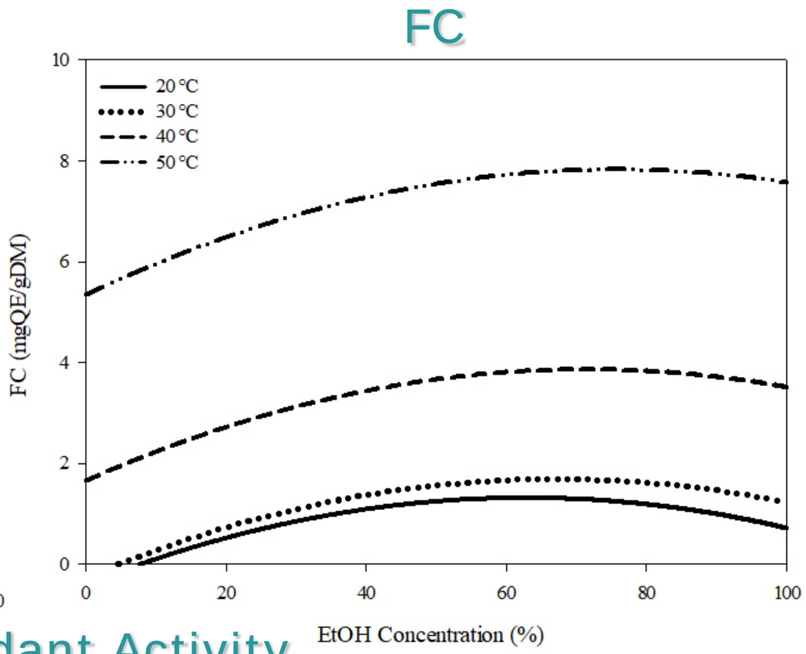
PEF

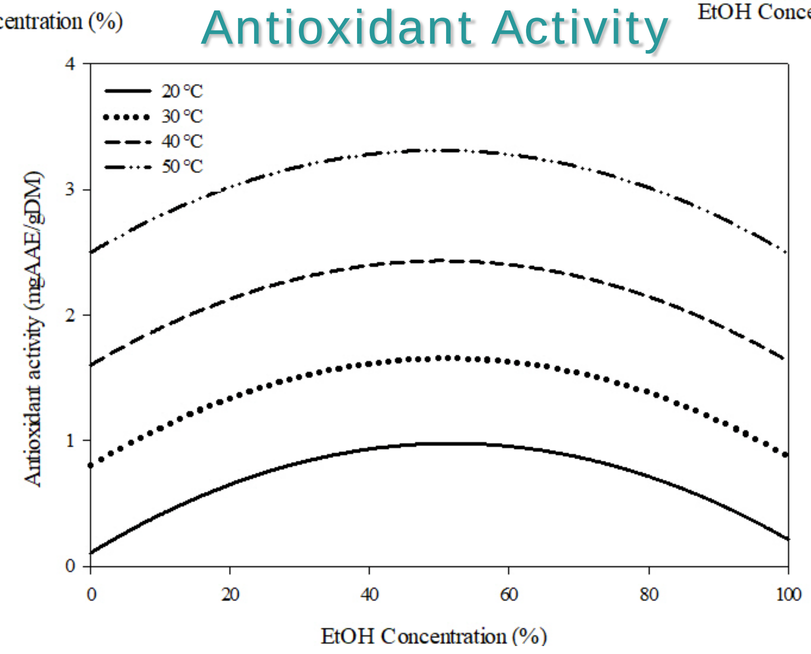


Pulsed Electric Fields (PEF): white and red grape pomace

Effect of the ethanol concentration on the response variables



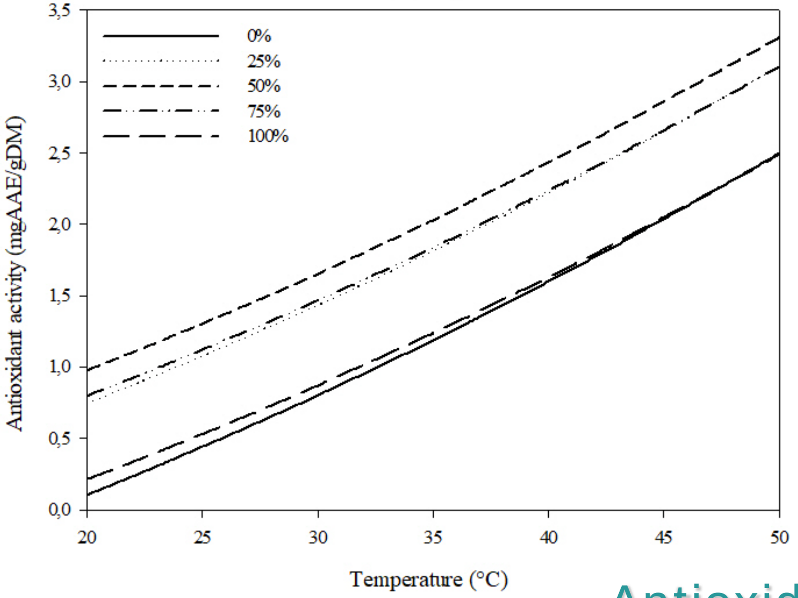




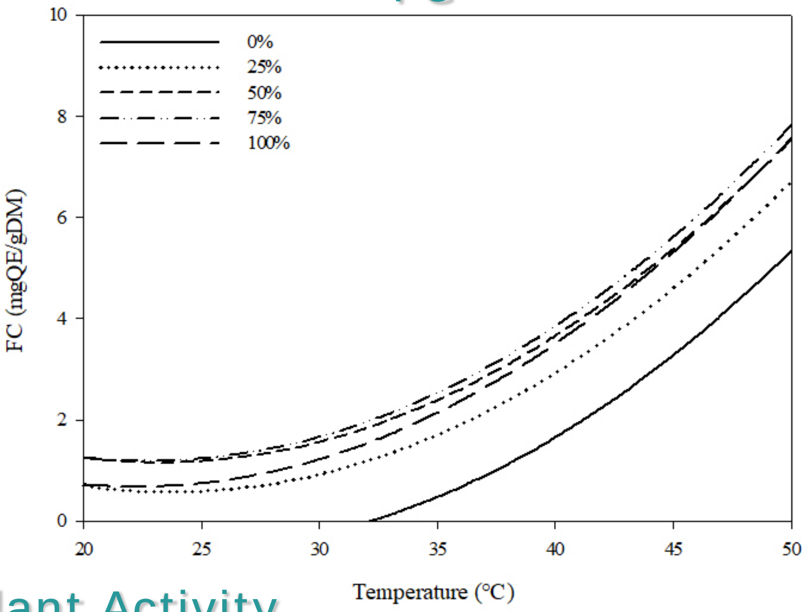
Pulsed Electric Fields (PEF): white and red grape pomace

Effect of the temperature on the response variables

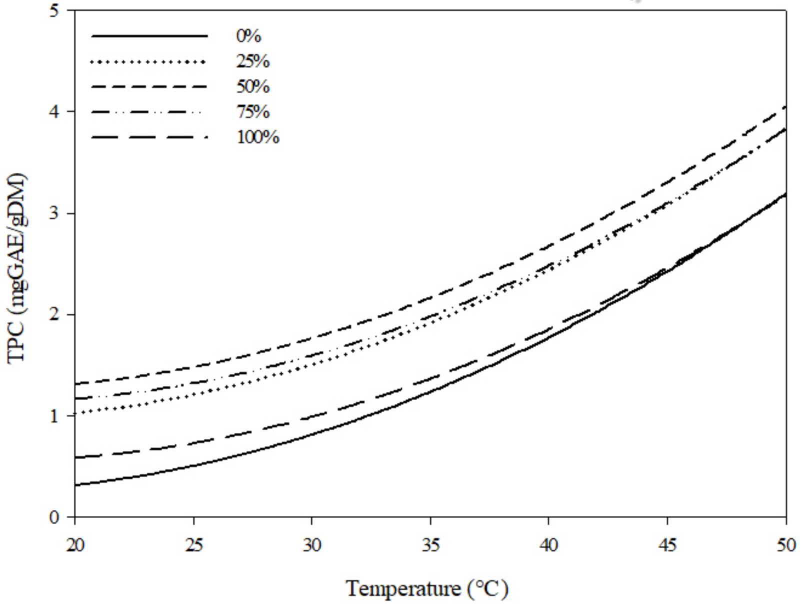
TPC



FC



Antioxidant Activity



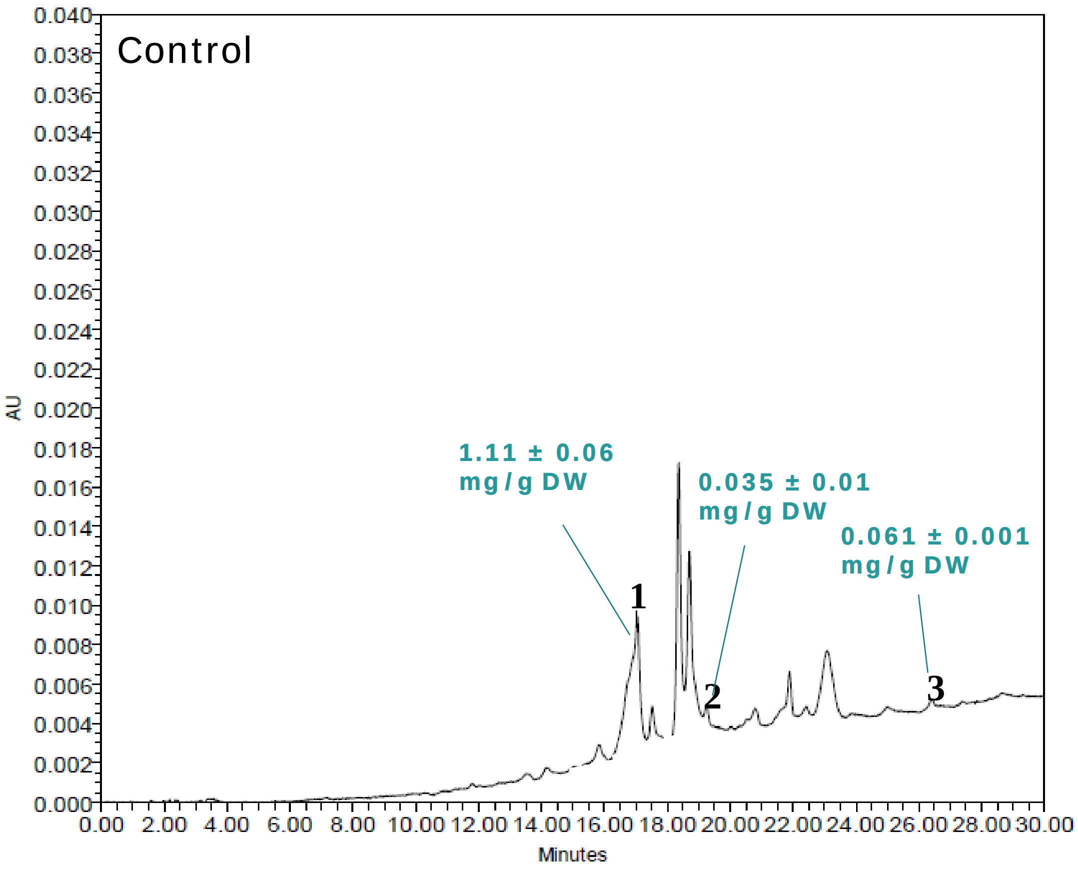
Pulsed Electric Fields (PEF): red grape pomace

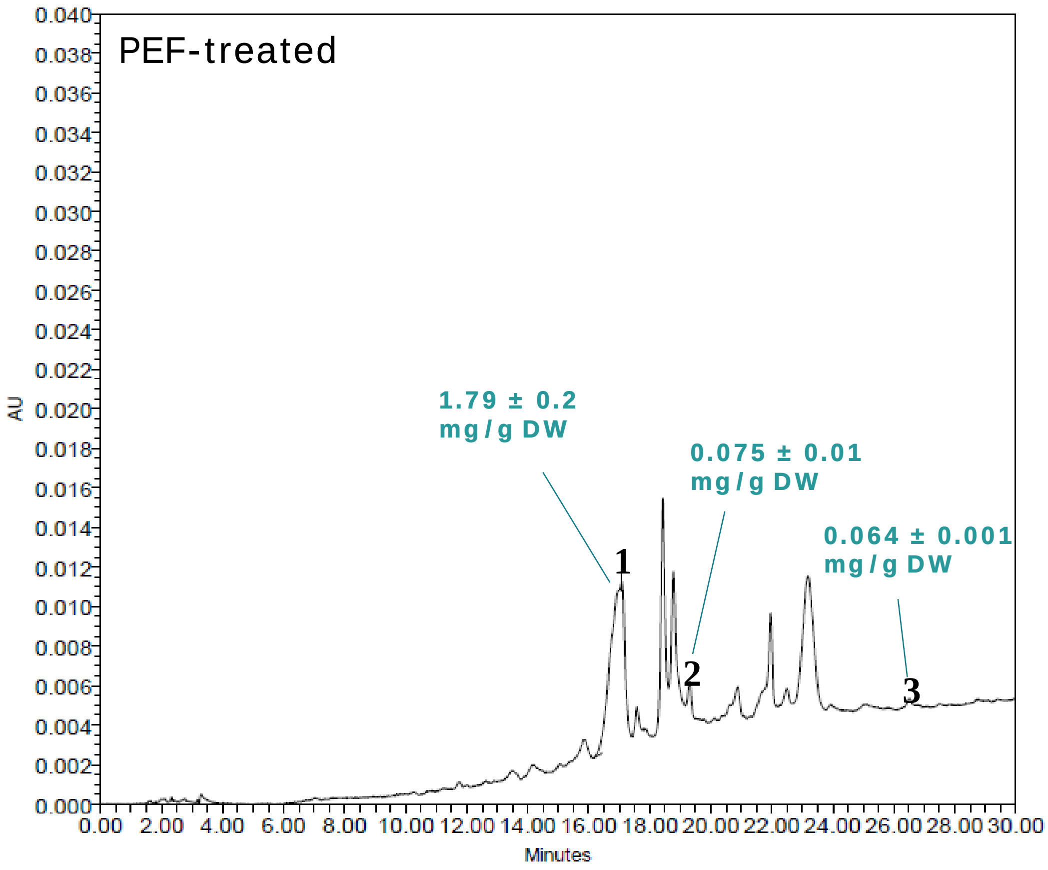
Response	Temperature [°C]	Ethanol concentration [%]	Time [min]	Increment over SLE [%]
TPC	50	50	300	+6%
FC	50	50	300	+25%
FRAP	50	50	300	+12%
TAC	50	50	300	+54%

Selected overall optimal extraction conditions:
EtOH-Water = 50 % (v/v), T = 50 °C, Time = 300 min

Pulsed Electric Fields (PEF): white grape pomace

HPLC-PDA analysis: effect of PEF pre-treatment on the phenolic composition



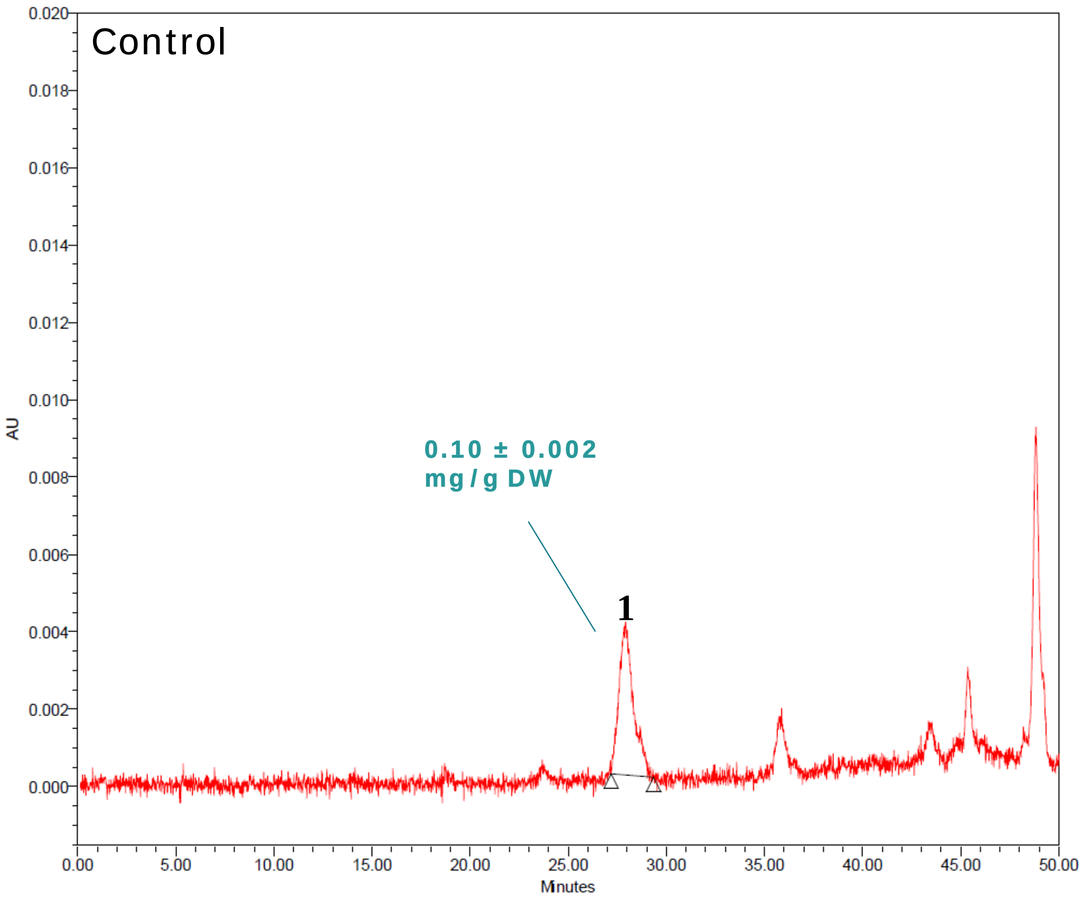


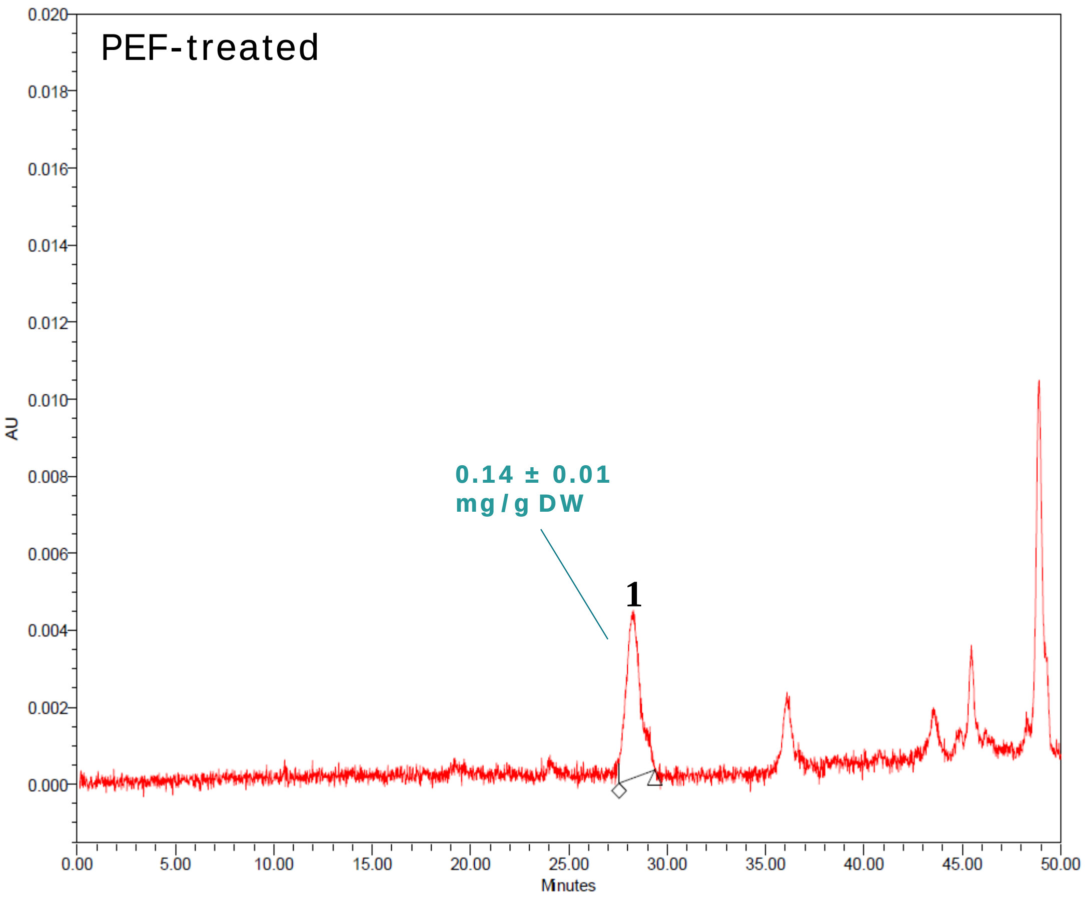
Identified compound	Increment (%)
Epicatechin	+61
p-coumaric acid	+114
Quercetin	+5

1 = epicatechin; **2** = p-coumaric acid; **3** = quercetin

Pulsed Electric Fields (PEF): red grape pomace

HPLC-PDA analysis: effect of PEF pre-treatment on anthocyanin composition





Identified compound	Increment (%)
Peonidin-3-O-glucoside	+40

1 = Peonidin-3-O-glucoside

Conclusions

Optimization step: the variables were significant, and the model accurately predicted the investigated responses for both PEF treatment and extraction step

Higher release of different phenolic compounds, including epicatechin (+61%), and p-coumaric acid (+114%) upon PEF compared to the control

PEF effective in intensifying the recovery yield of flavonoids (+24-31%), antioxidant power (+9-36%), and anthocyanin content (+40%) from grape pomace

Potentiality to reduce, the solvent consumption (3-12%) and shorten the extraction time (23-103 min) to achieve the same recovery yield of phenolic compounds

Thank you for you attention!