Optimization of Pulsed Electric Fields-Assisted Extraction of phenolic compounds from sweet cherry press cake using Response Surface Methodology

• University of Salerno – Department of Industrial Engineering

• Ph. D. course in Innovative Engineering Technologies for Industrial Sustainability (IETIS)

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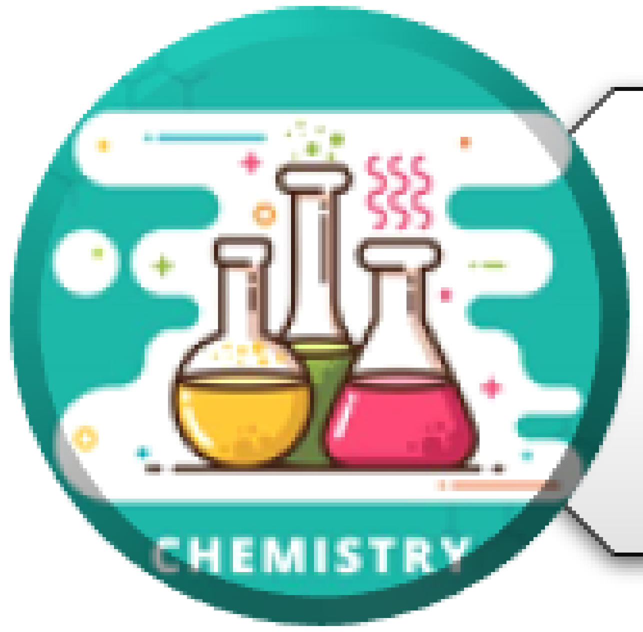
INTRODUCTION



STATE OF THE ART

OBJECTIVE OF THE WORK

RESULTS

VALICET
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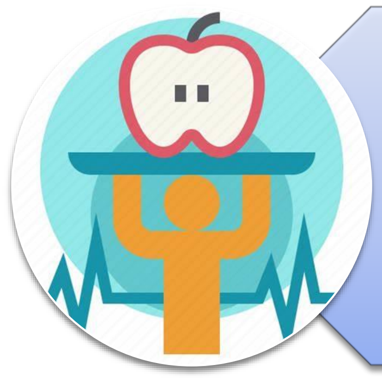
INTRODUCTION

Consumer food trends



Health/Nutrition

Economic

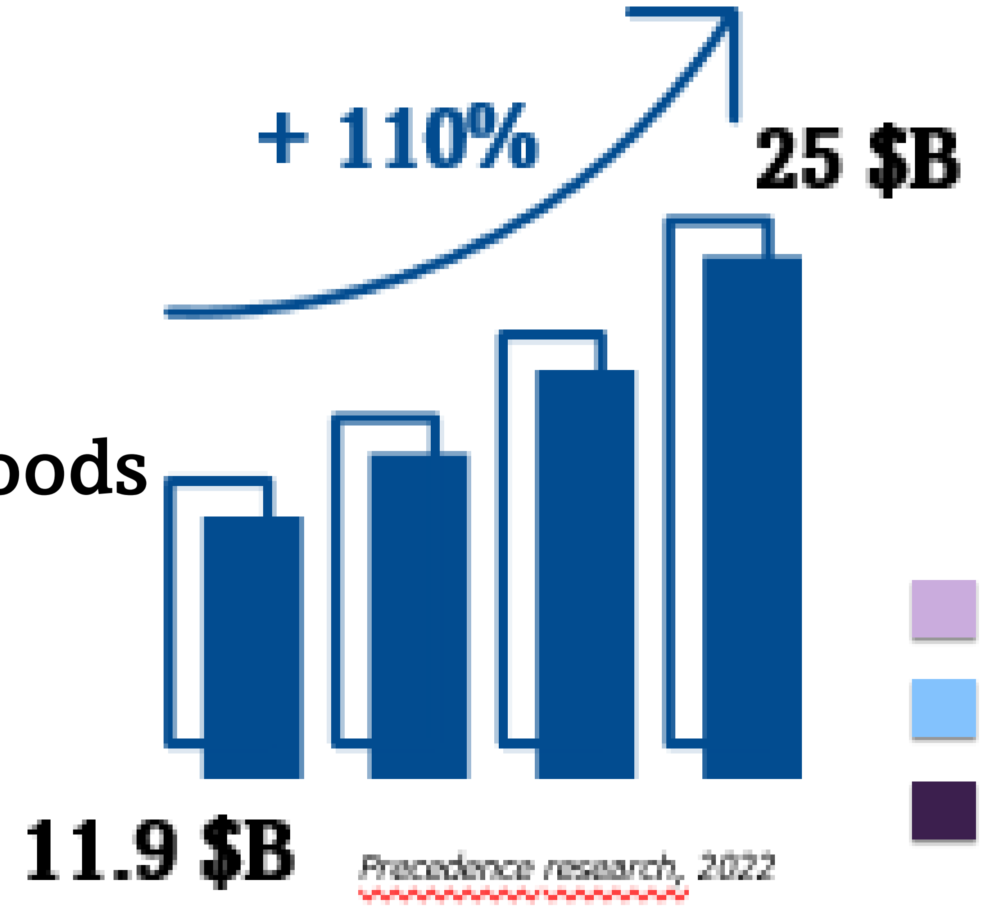
Environmental impact

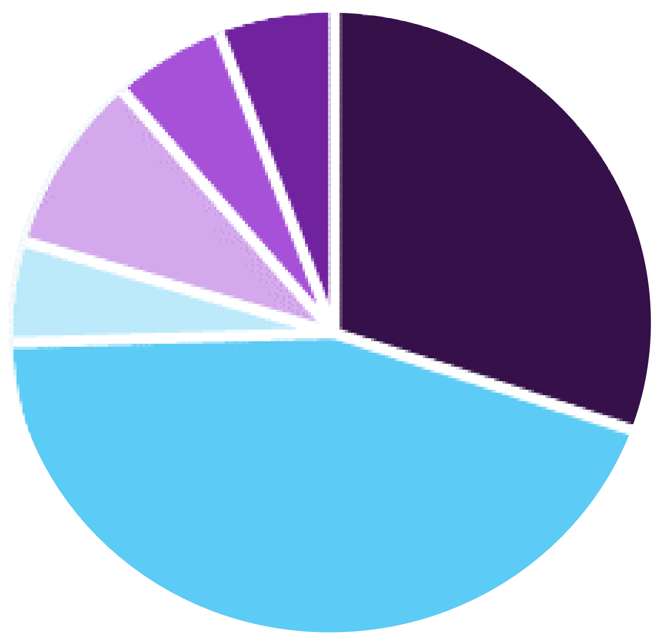
Socio/Cultural

Nutrient-rich diet

Consumption of polyphenol-rich foods

Global natural extracts market





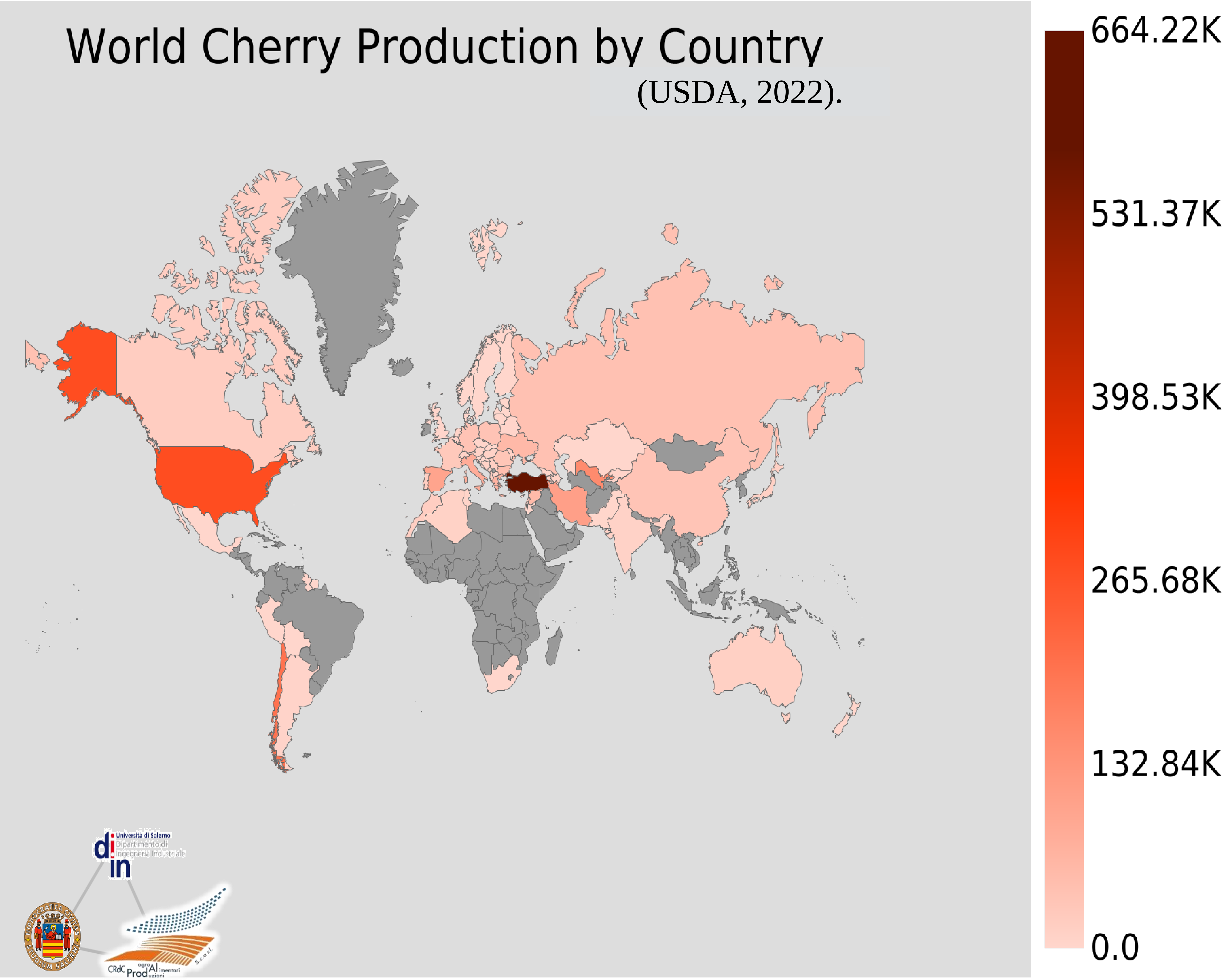
Food waste and by-products: a low-cost source of bioactive compounds

Around **88 million tonnes** of food waste are generated annually in the EU

- 42% Households
- 39% Manufacturing
- 14% Foodservice/Catering
- 5% Retail/ wholesale

50%
Call for a reduction of global food waste by 2030

Cherry production



Italy is the third largest cherry producer in the Mediterranean region with 98,600 tonnes production per year.

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Cherry production

Pomace

Stems

Seeds

Industry (Cherry juice production)

The nutritional composition

- **Dietary fiber:** lignin (69.4%), cellulose (18.4%), hemicellulose (10.7%), and pectin (1.5%).
- **Vitamin C**
- **Potassium**

Anthocyanins

Cyanidin 3-glucoside, cyanidin 3-rutinoside (**soluble in aqueous/organic** solvent mixture).

Flavon-3-ols

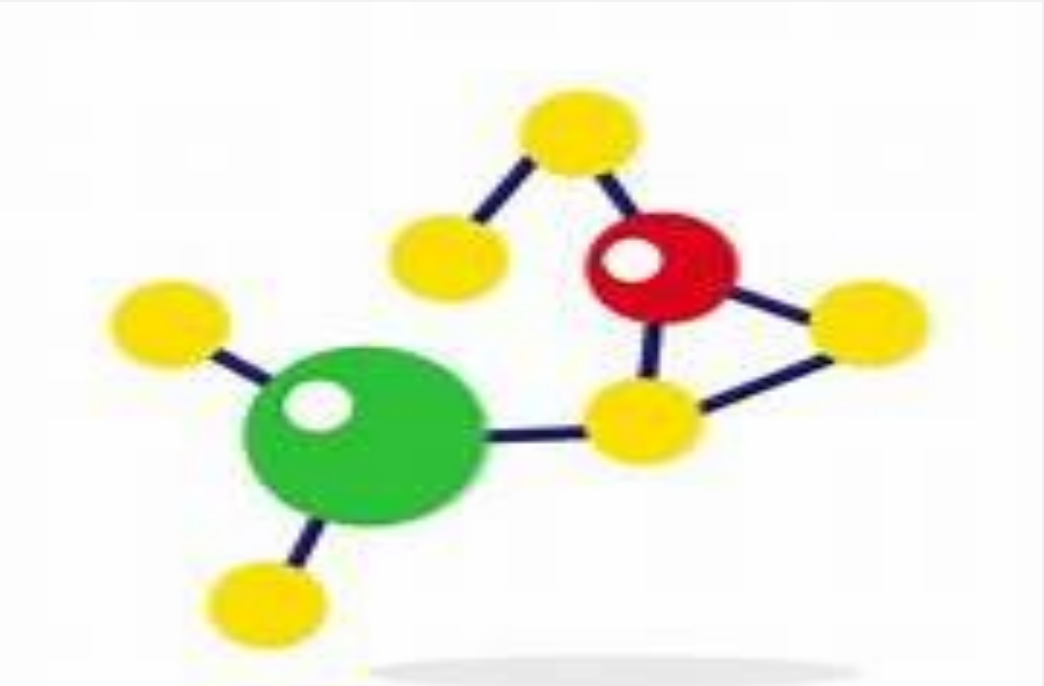
Catechin, Epicatechin (**soluble in ethanol).**
Phlorizin (soluble in ethanol and hot water)

Phenolic acids

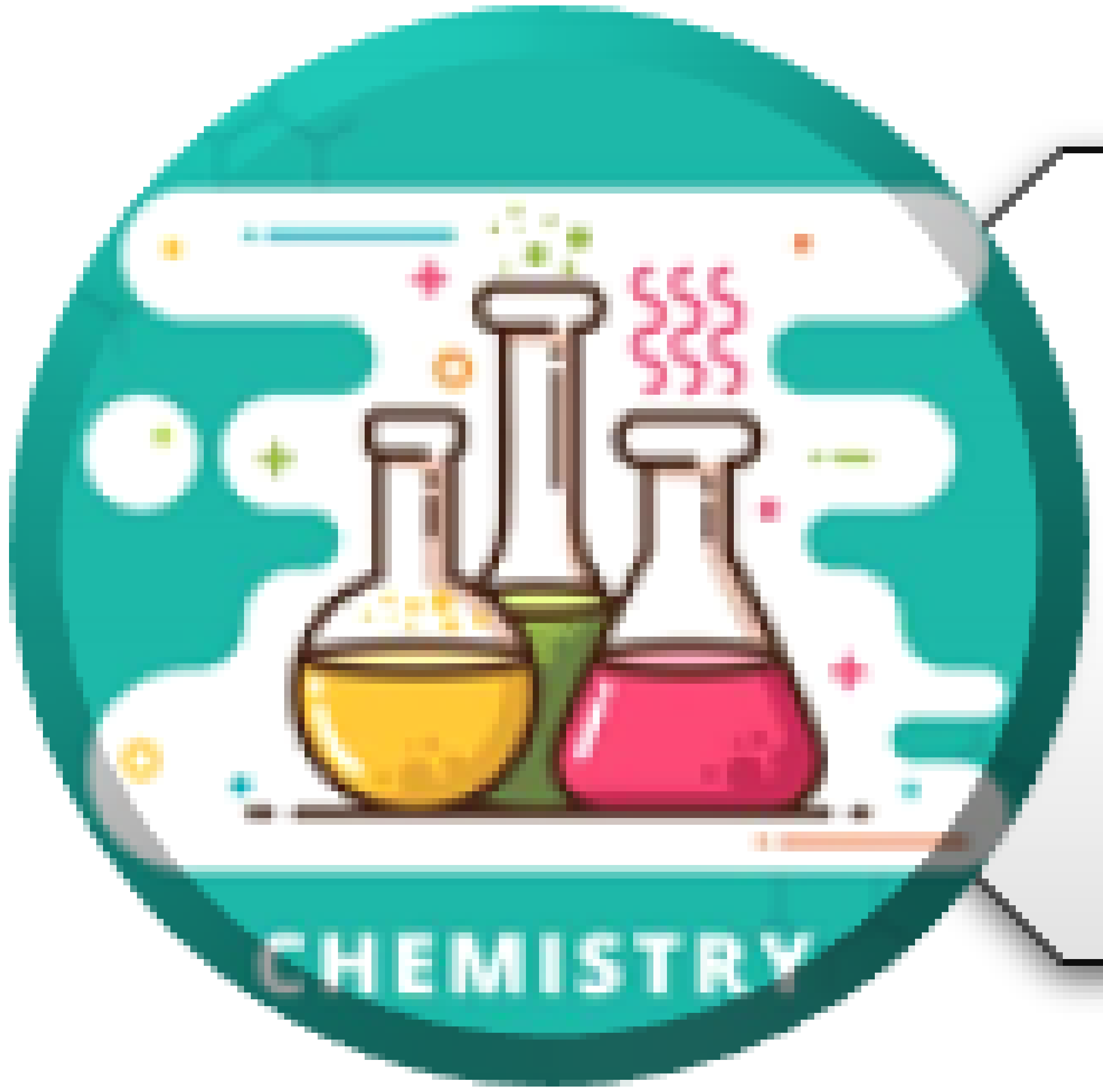
Neochlorogenic acid, chlorogenic acid, 4-p coumaroylquinic acids, 3,5-di-caffeoylquinic acid **(soluble in ethanol)**

Flavonols

Rutin, Isorhamnetin-3-O-rutinoside

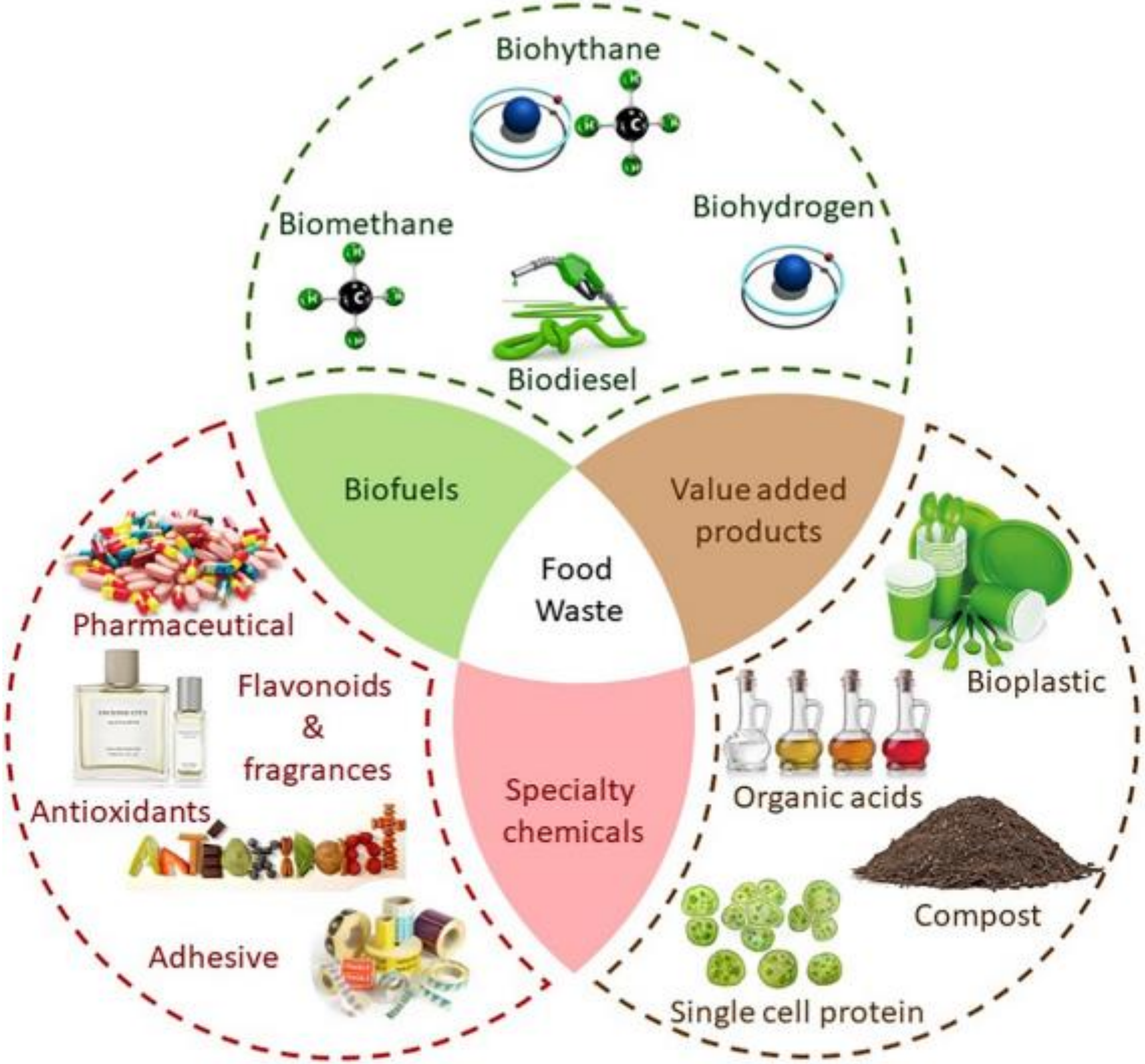


Bioactive compounds



STATE OF THE ART

Bio-conversion of food waste and their products



(Yukesh Kannah et al., 2020)

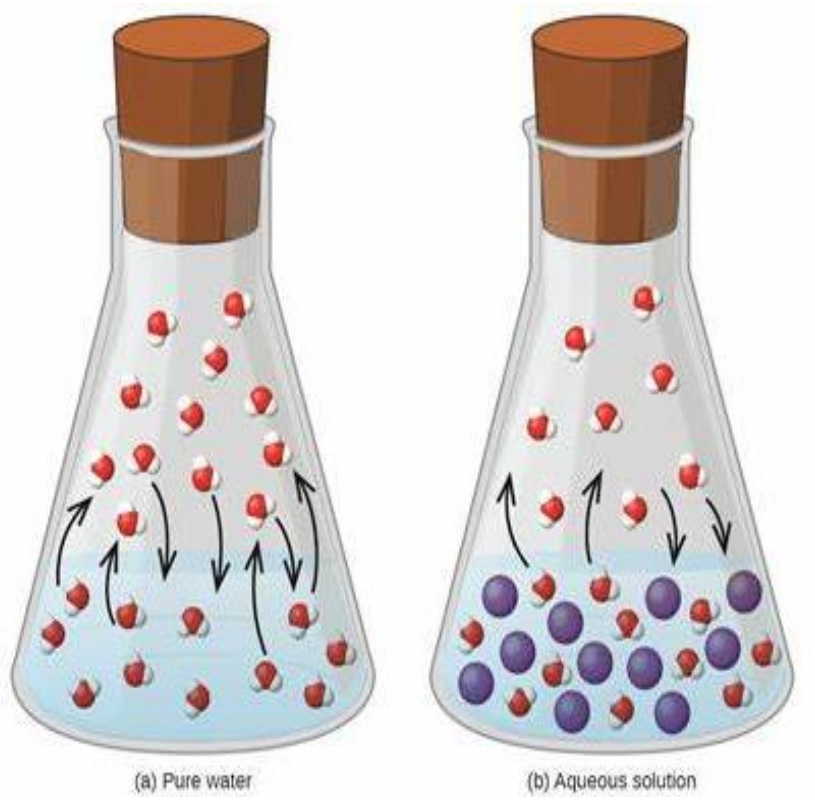
Extraction Process

Extraction Conditions → **Procedure**

Solvent

- ❖ “Low” of similarity and intervisibility
- ❖ Solvent Polarity Chart
- ➢ Polar
- ➢ Non-polar
- ❖ Green Solvent

- ❖ Low cost
- ❖ Recycle
- ❖ Non-toxic
- ❖ Non-flammable



- Temperature
- Time
- Solid/Liquid Ratio

Extraction Process

Extraction Conditions

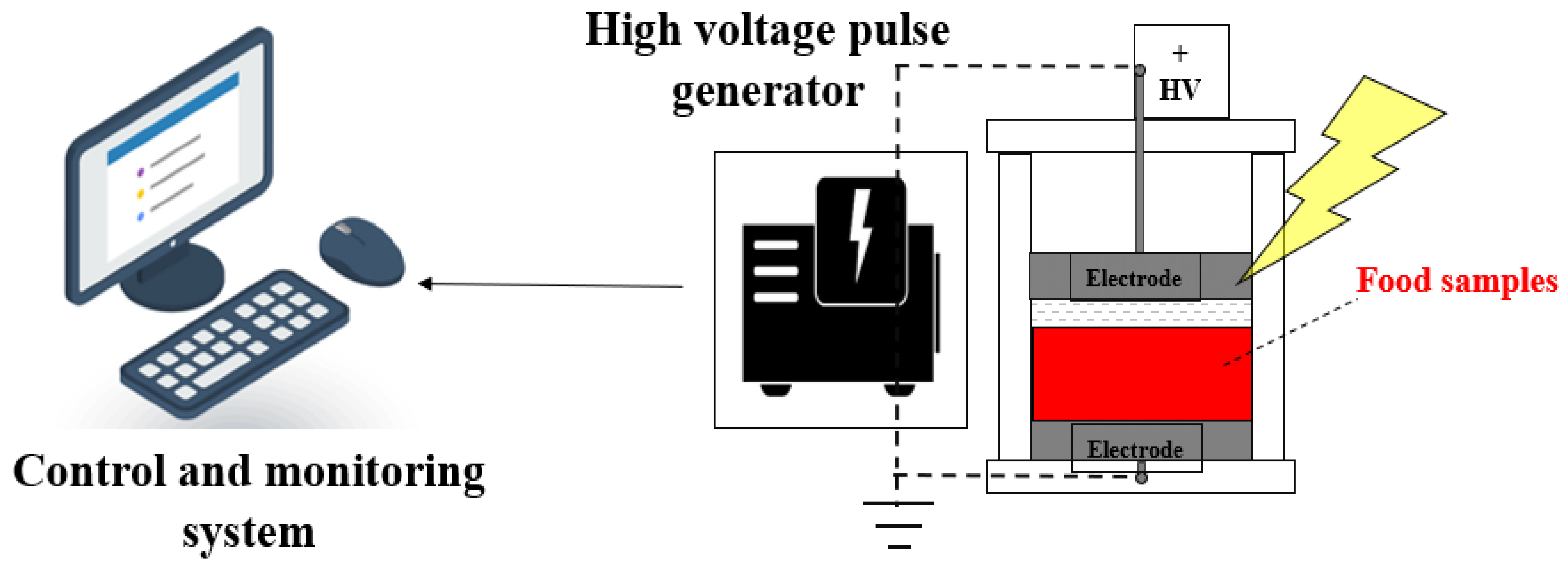
Procedure

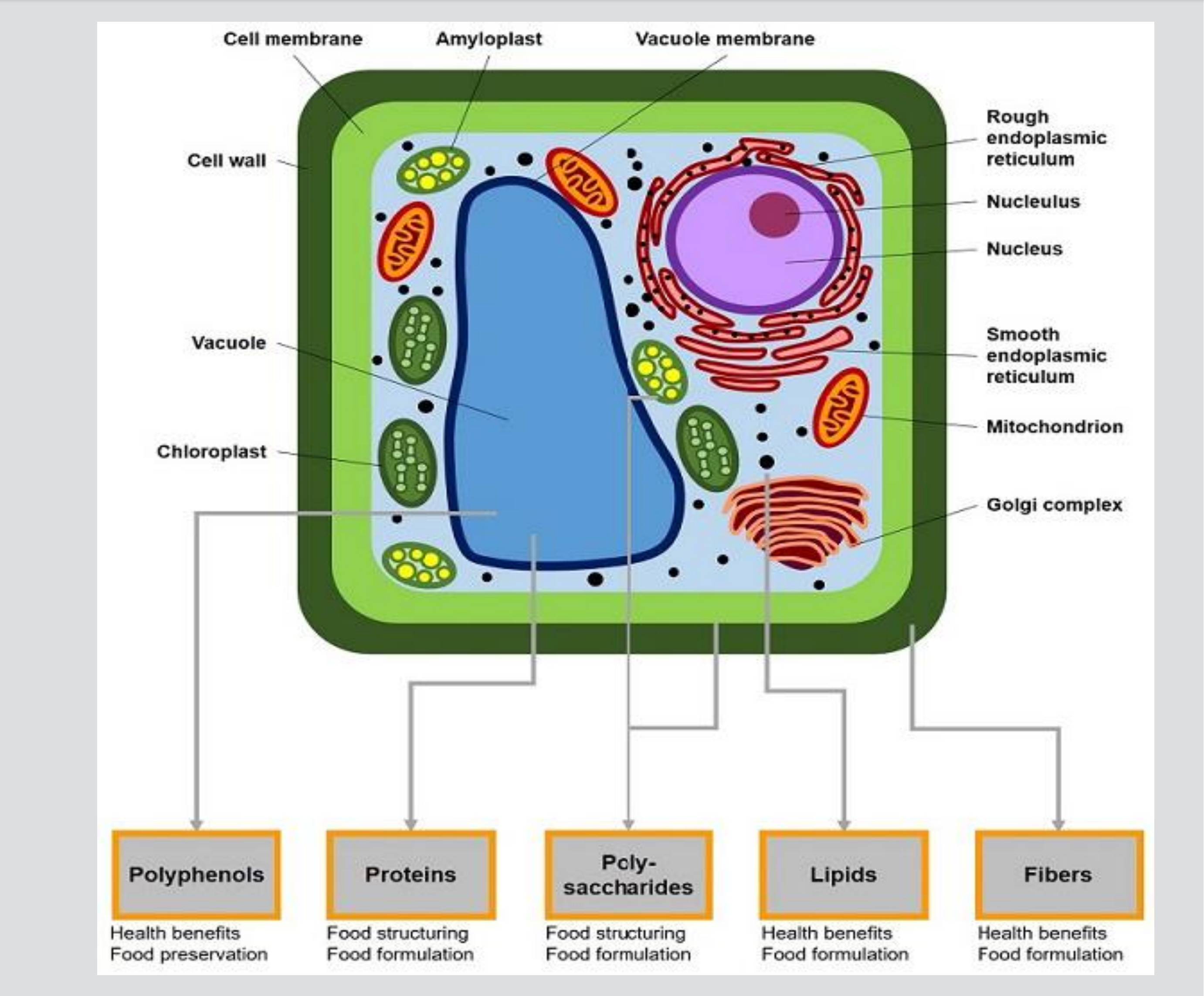
Conventional extraction methods

Extraction Process

Extraction

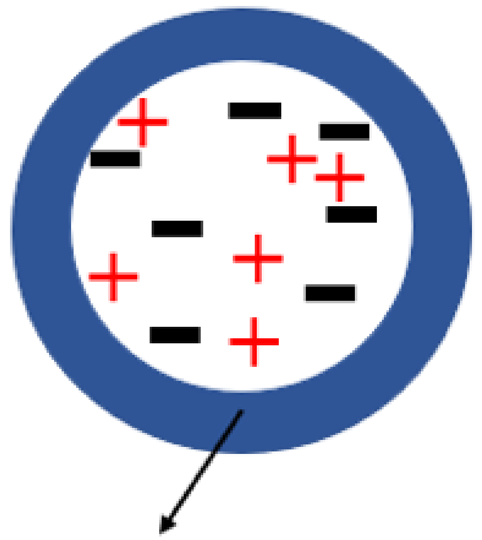
Procedure





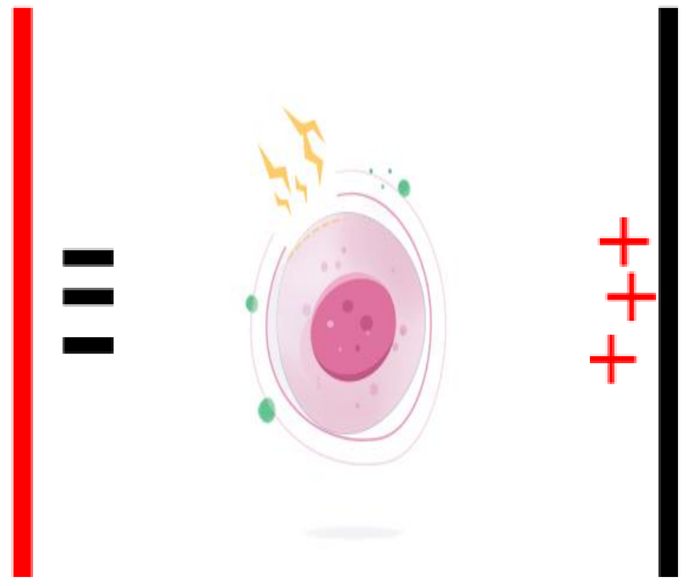
Input variables

- E (kV/cm)
- W_T (kJ/kg)



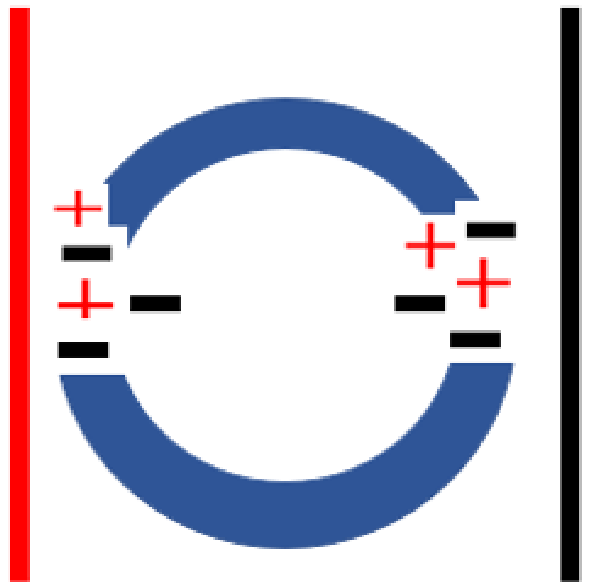
Cell before PEF application

Pulse electric field (E)



Polarisation (E<Ec)

Pulse electric field (E)



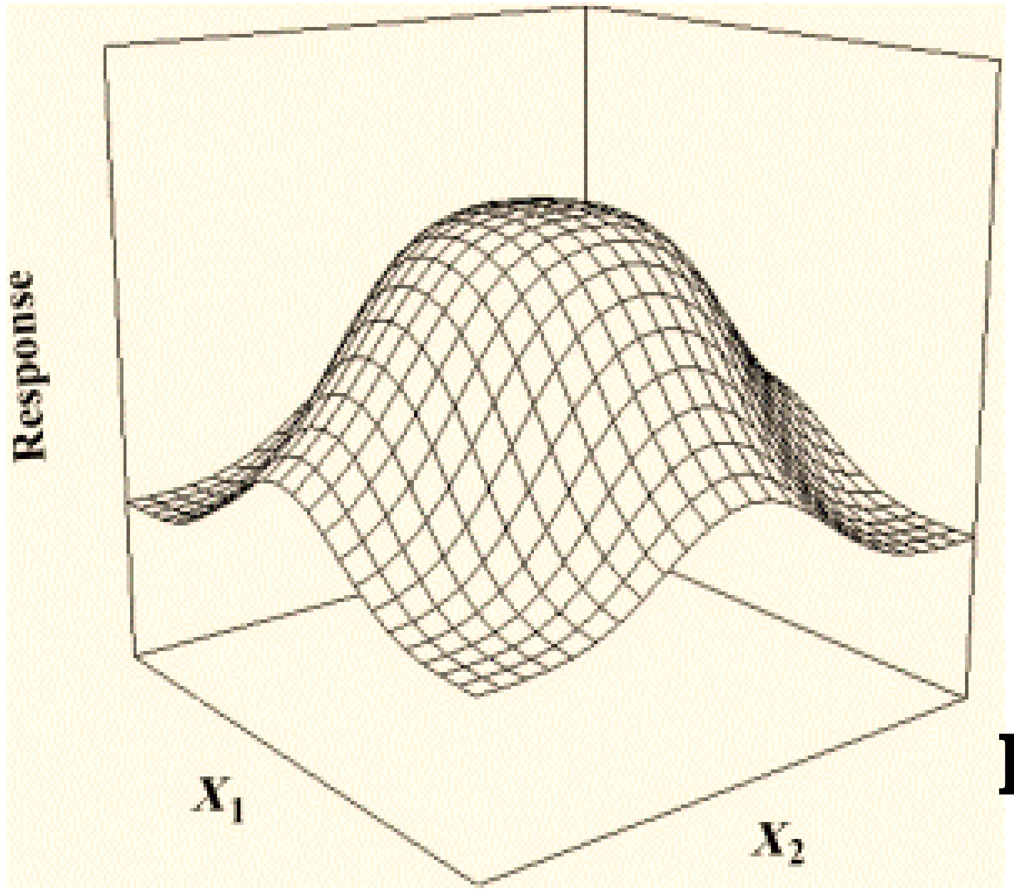
Pore Formation (E>Ec)

Electroporation phenomenon

OBJECTIVE OF THE WORK

The Objectives of the Work

Response surface for two factors (X_1 and X_2)



$$Yk = \beta 0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

Response variables

- Total phenolic compounds
- Total flavonoids
- Total anthocyanin content
- Antioxidant activity

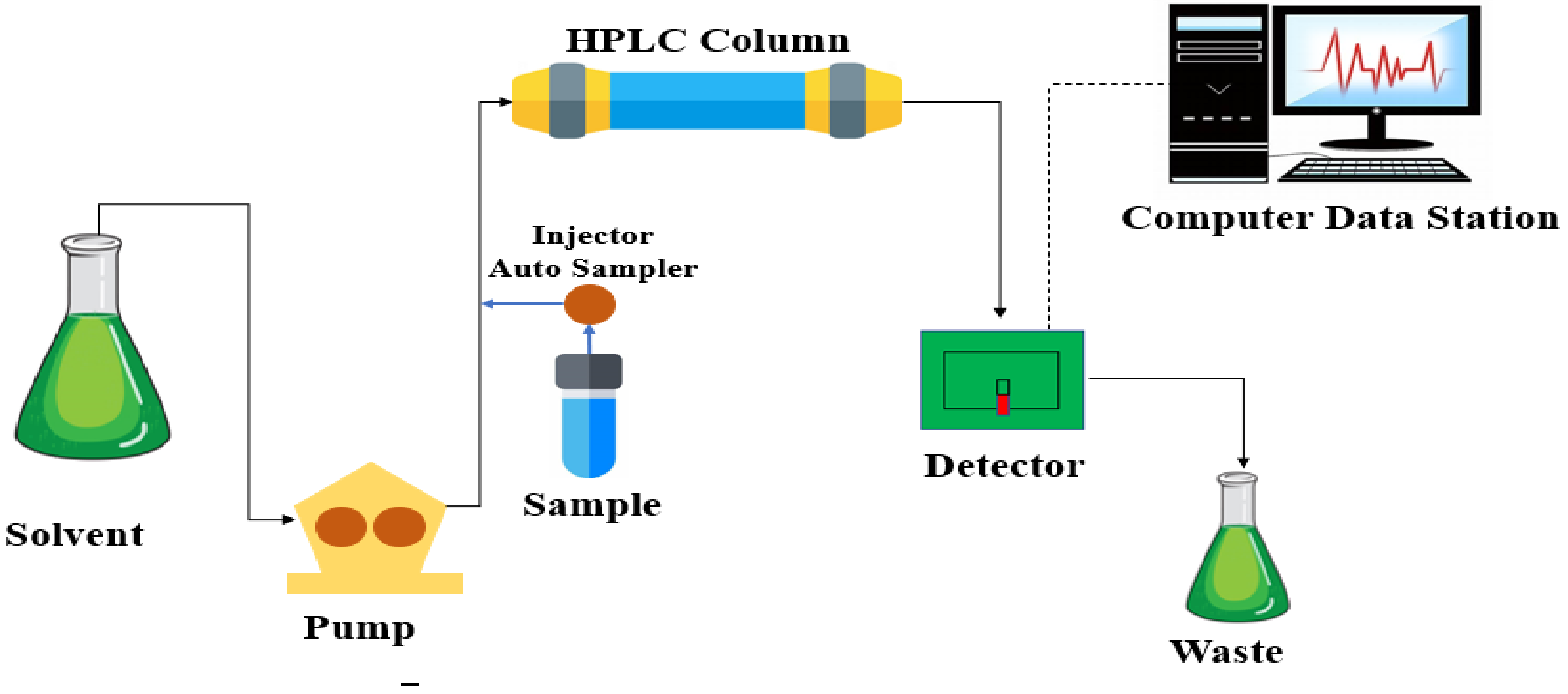
Input variables

- Time = (30–360 min)
- Temperature = (20–50 °C)
- Solvent = ethanol-water mixture (0-100%)

Optimization of the SLE conditions
Response surface methodology (RSM)

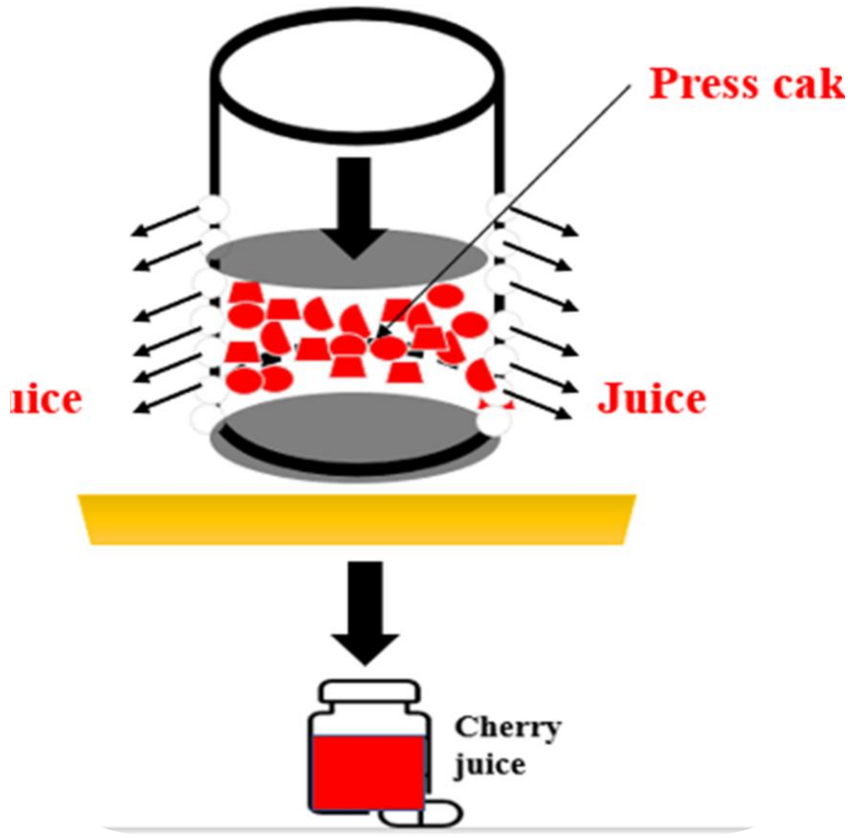
Quantification of the Phenolic Compounds via HPLC-PDA Analysis

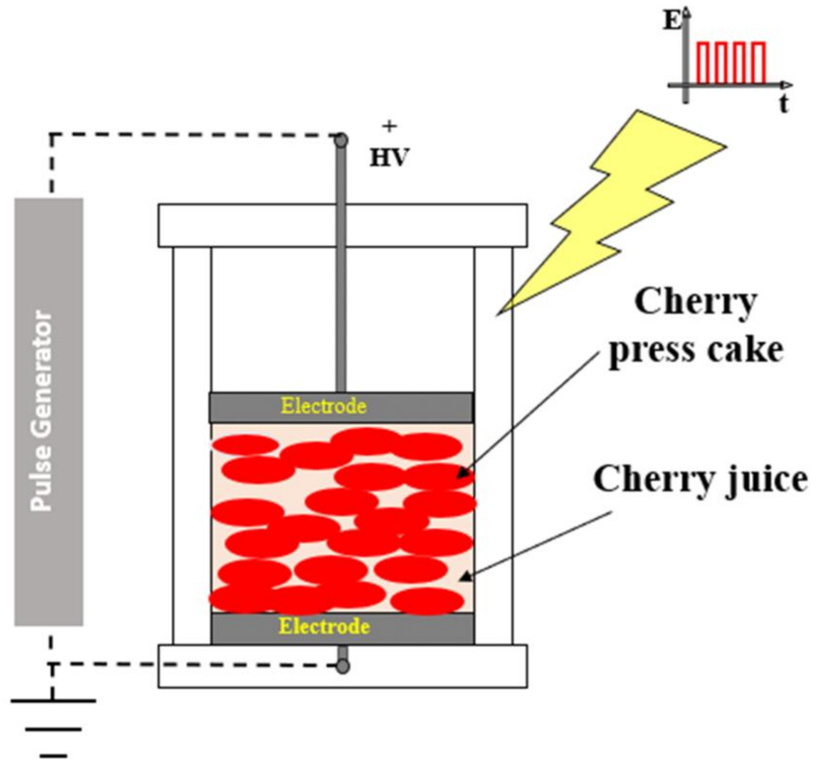
Potentially of PEF pre-treatment to enhance cherry press cake's ability to extract bioactive components

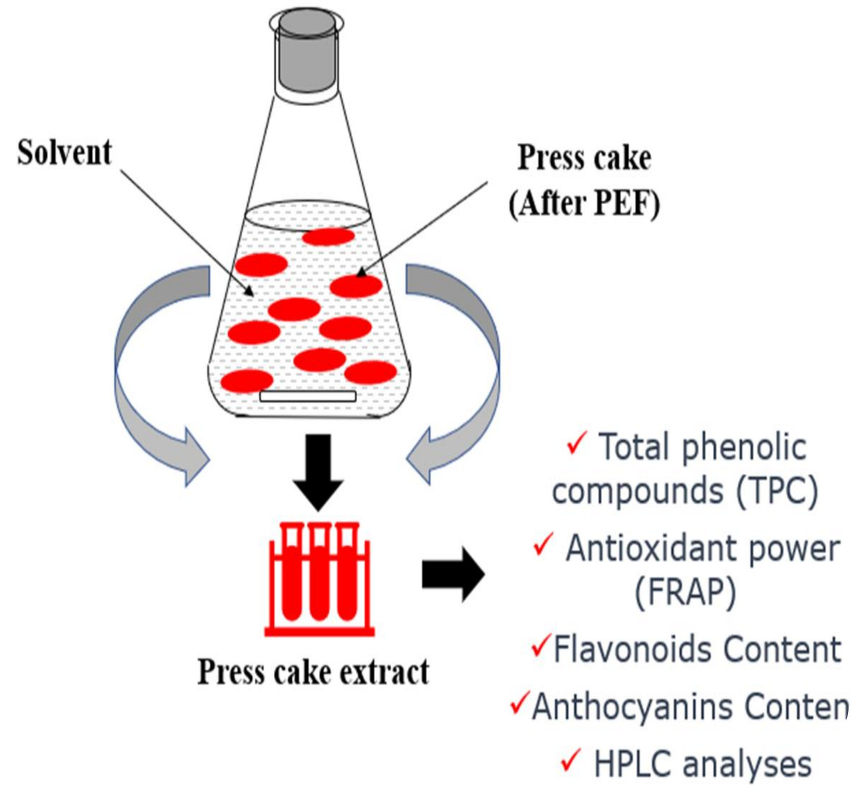


RESULTS

Work Plan







Sweet cherry "Ferrovia" Variety

Juice extraction by pressing P= 516.9 kPa for 8 min

PEF pre-treatment E=3kV/cm, Wp=10 kJ/kg

Optimization of SLE conditions

(Pataro et al., 2017): PEF pre-treatments were performed at constant specific energy input (WT = 10 kJ/kg) and different electric field strengths (E= 0.5-3 kV/cm) before applying a pressure of 1.64 bar for 5 min.

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

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

TPC: +26%
FC:+27 %
AC:+42 %
FRAP: +44%

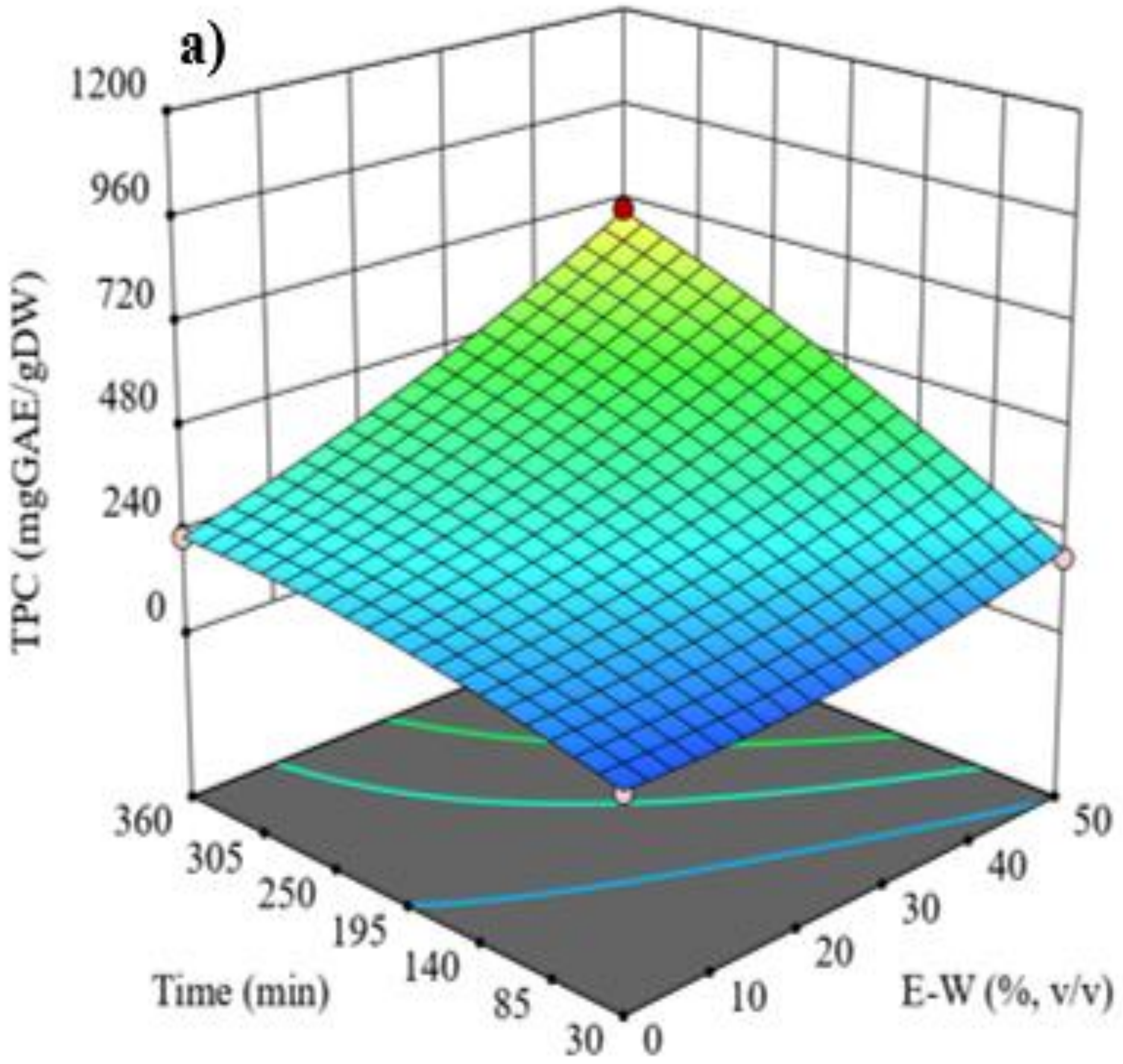
Coefficients	SLE				PEF-assisted extraction			
	TAC ($mgC3G/g_{DW}$)		FRAP ($mgAAE/g_{DW}$)		TAC ($mgC3G/g_{DW}$)		FRAP ($mgAAE/g_{DW}$)	
β0	+5.05609		+259.105		+2.38321		+198.245	
β_1 (T)	-0.055280	0.0456	-17.9103	*	+0.287320	**	-28.0091	*
β_2 (E-W %)	-0.084171	***	-0.364455	***	+0.074419	***	-4.12032	***
β_3 (S/L)	-82.66792	***	+421.709	**	-145.669	***	+5502.52	***
β_4 (t)	+0.000522	***	+0.937563	***	+0.004825	***	+1.17821	***
β_{12} (T x E-W)	-0.000129	ns	-0.018112	ns	+0.000129	ns	+0.096241	*
β_{13} (T x S/L)	-0.028770	ns	-0.178920	ns	-0.299315	ns	-18.08076	ns
β_{14} (T x t)	+0.000053	ns	+0.001744	ns	+0.000179	ns	-0.003363	ns
B_{23} (E-W x S/L)	+0.580072	**	+3.61471	ns	+0.521672	*	-1.48133	ns
B_{24} (E-W x t)	+0.000411	***	+0.012425	***	+0.000246	*	+0.020849	***
B_{34} (S/L x t)	+0.090628	**	+1.05870	ns	+0.134985	**	+1.77216	ns
B_{11} (T x T)	+0.001433	ns	+0.281959	*	-0.002847	ns	+0.439359	**
β_{22} (E-W x E-W)	+0.000346	ns	+0.049488	ns	-0.002320	ns	+0.084158	ns
β_{33} (S/L x S/L)	+280.333	ns	-1057.03	ns	+571.211	**	-17286.8	***
β_{44} (t x t)	0.000025	ns	0.002048	ns	0.000027	ns	0.002378	ns
The p-value of the model	≤ 0.0001	significant	< 0.0001	significant	≤ 0.0001	significant	≤ 0.0001	significant
R^2	0.919		0.945		0.933		0.971	
RMSE	0.815		0.815		0.200		0.192	

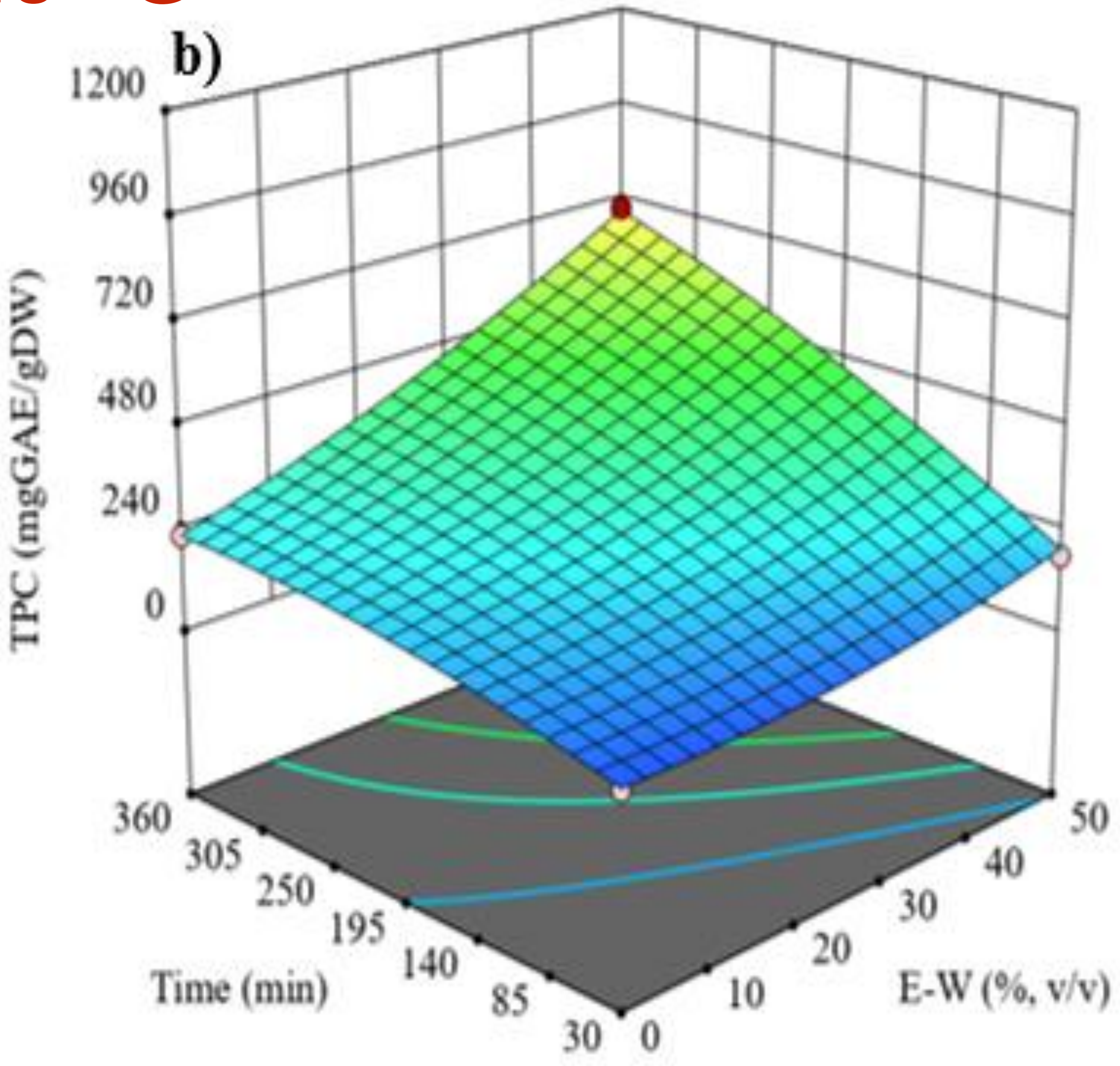
Coefficients	SLE				PEF-assisted extraction			
	TPC ($mgGAE/g_{DW}$)		FC ($mgQE/g_{DW}$)		TPC ($mgGAE/g_{DW}$)		FC ($mgQE/g_{DW}$)	
β0	+229.916		+308.54073		+303.895		+105.268	
β_1 (T)	-18.2725	**	-18.91065	**	-23.2728	**	-7.81384	**
β_2 (E-W %)	-4.27231	***	+1.04473	***	-5.29687	***	-0.457265	***
β_3 (S/L)	+1698.72	***	+65.48101	***	+2523.35	***	+1814.61	***
β_4 (t)	+0.260611	***	+0.158169	***	-0.015500	***	+0.786917	***
β_{12} (T x E-W)	+0.050870	ns	+0.020603	ns	+0.144746	*	+0.078691	**
β_{13} (T x S/L)	+0.179421	ns	-9.00317	ns	-23.3756	ns	-15.1850	*
β_{14} (T x t)	+0.010823	ns	+0.007007	ns	+0.009599	ns	+0.000247	ns
B_{23} (E-W x S/L)	+9.37632	ns	+9.18320	ns	+10.8858	ns	-7.23572	ns
B_{24} (E-W x t)	+0.021752	***	+0.013378	***	+0.027195	***	+0.013413	***
B_{34} (S/L x t)	+2.12559	ns	+0.537662	ns	+2.95764	ns	+1.32383	ns
B_{11} (T x T)	+0.257754	ns	+0.301853	0.0223	+0.352478	ns	+0.129137	ns
β_{22} (E-W x E-W)	+0.057283	ns	-0.007623	ns	+0.018122	ns	+0.040554	ns
β_{33} (S/L x S/L)	-6160.98	ns	+2163.57416	ns	-5754.01	ns	-2573.49	ns
β_{44} (t x t)	-0.001235	ns	-0.000424	ns	-0.000452	ns	-0.001349	ns
The p-value of the model	< 0.0001	significant	< 0.0001	significant	< 0.0001	significant	< 0.0001	significant
R^2	0.968		0.97		0.957		0.984	
RMSE	0.0853		0.007		0.076		0.291	

Total phenolic compounds (TPC)

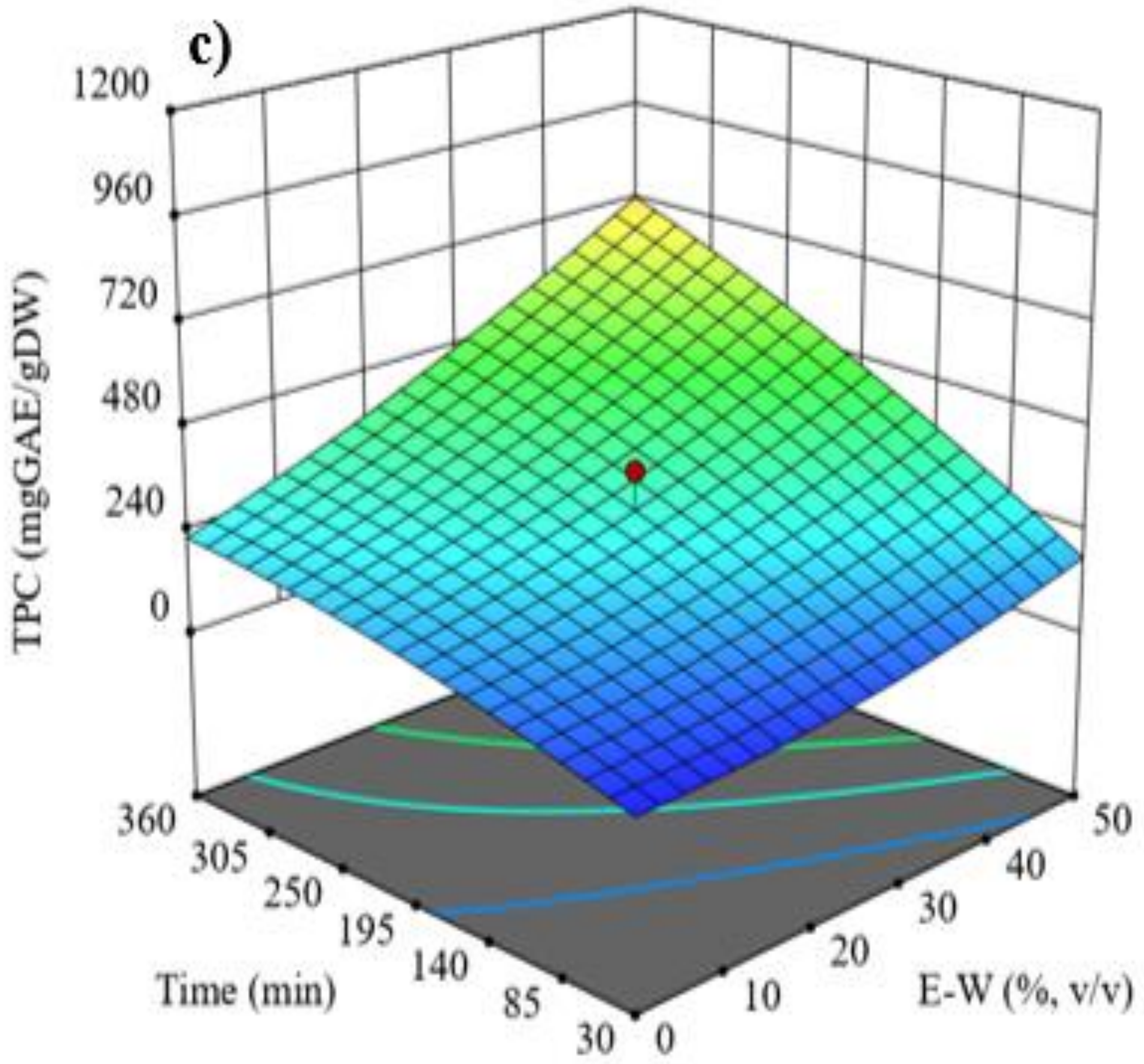
3D Response Surface graphs

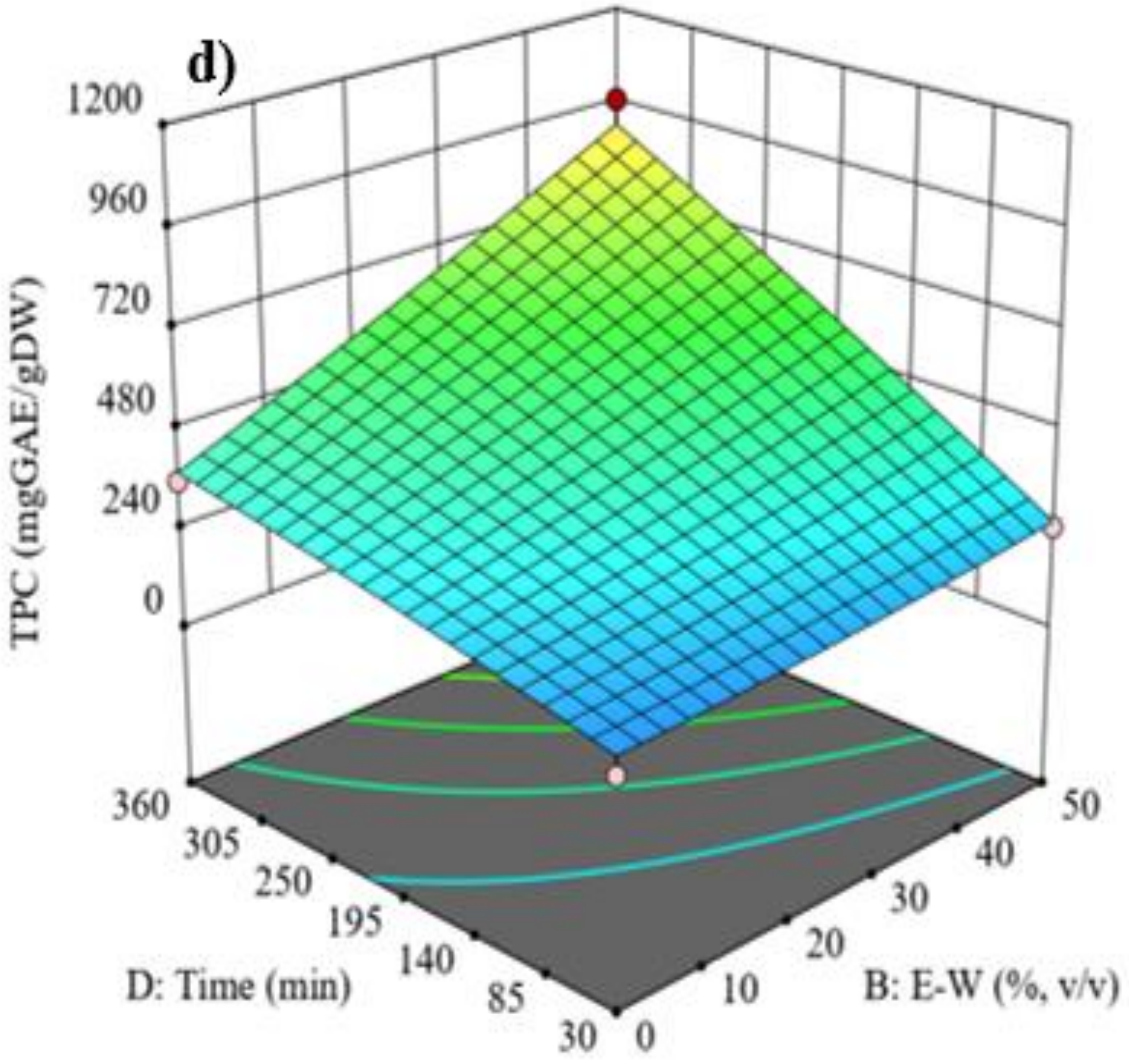
Control **T = 20° C** **PEF**



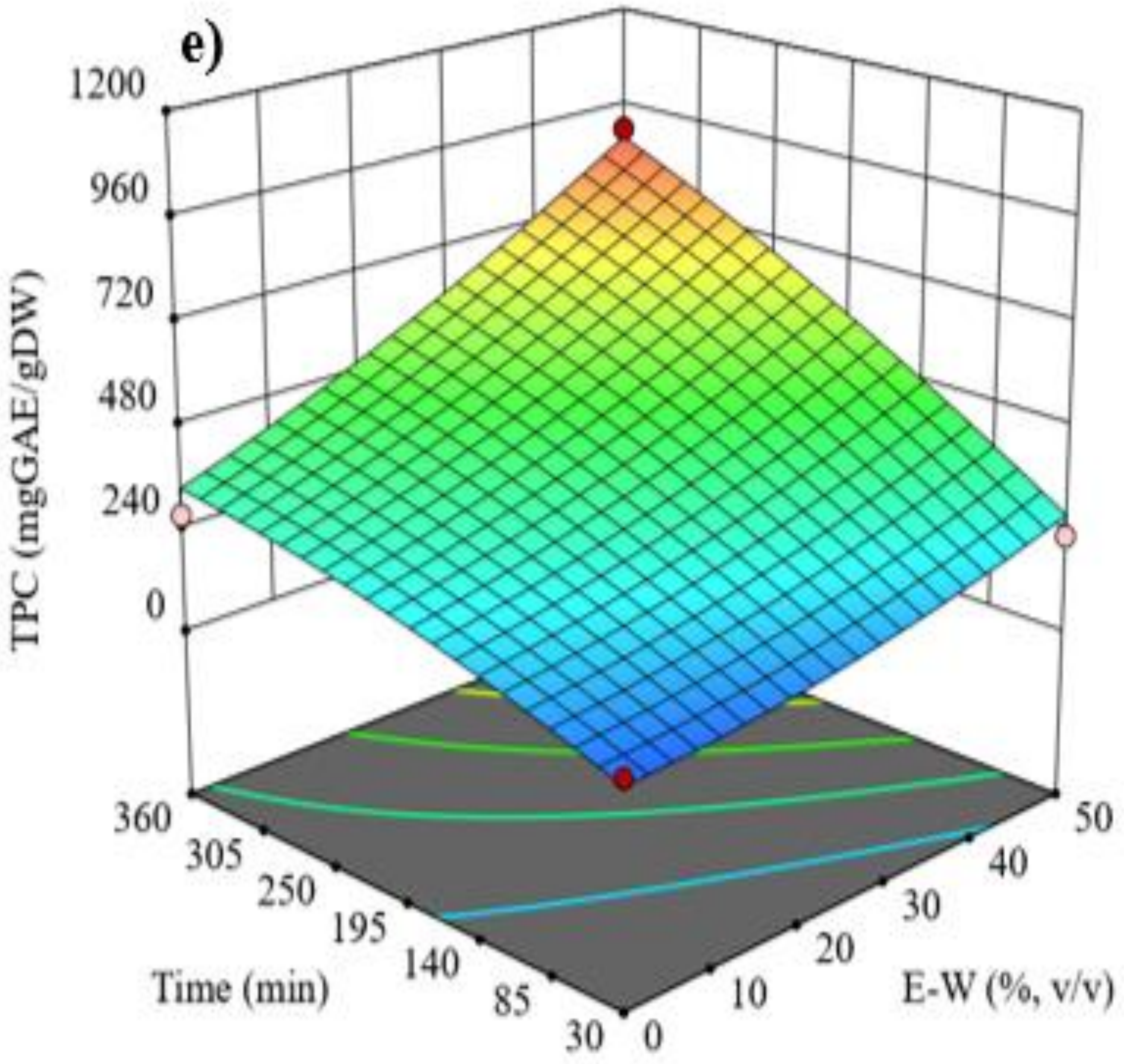


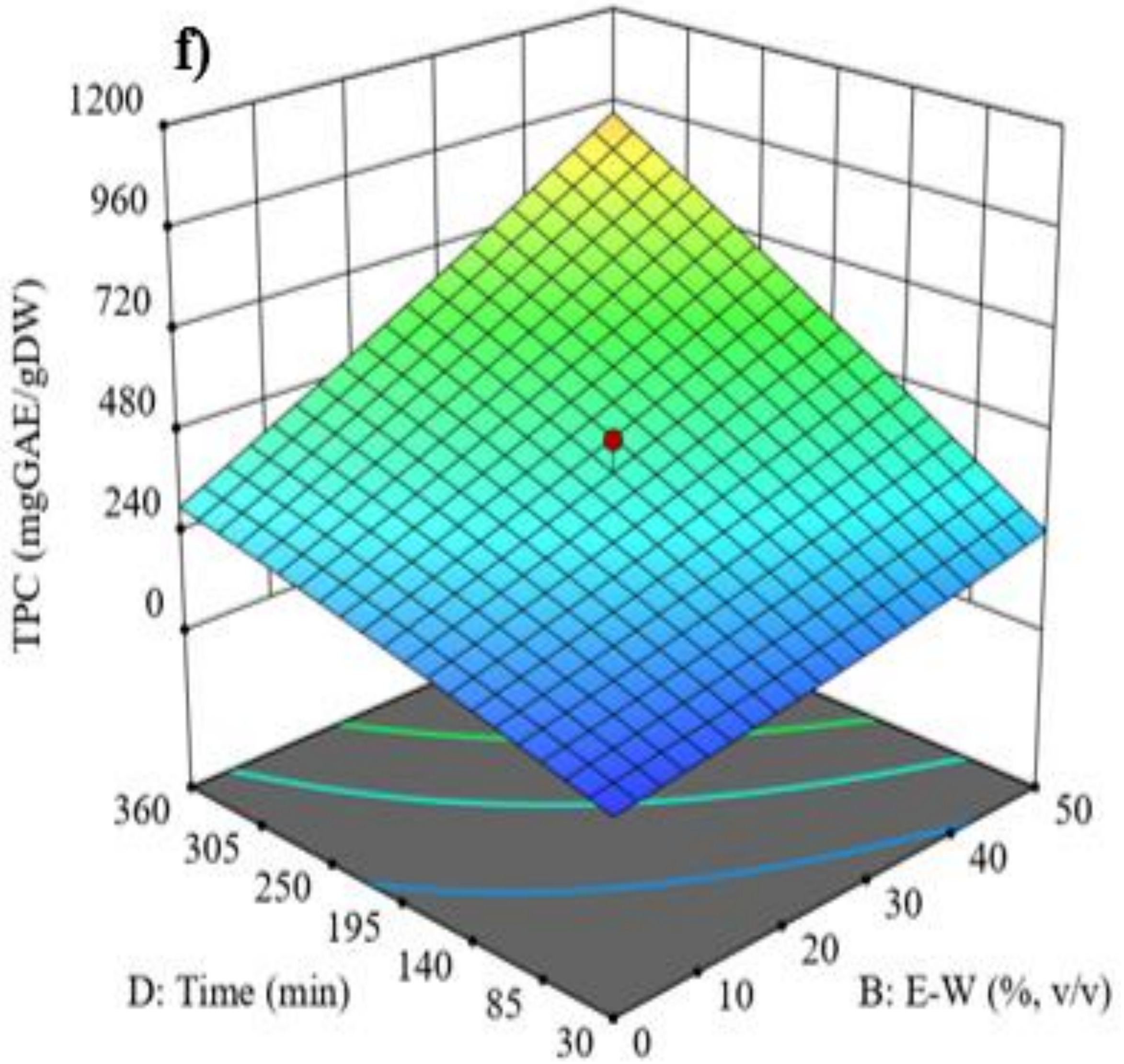
Control **T = 35° C** **PEF**





Control **T = 50° C** **PEF**

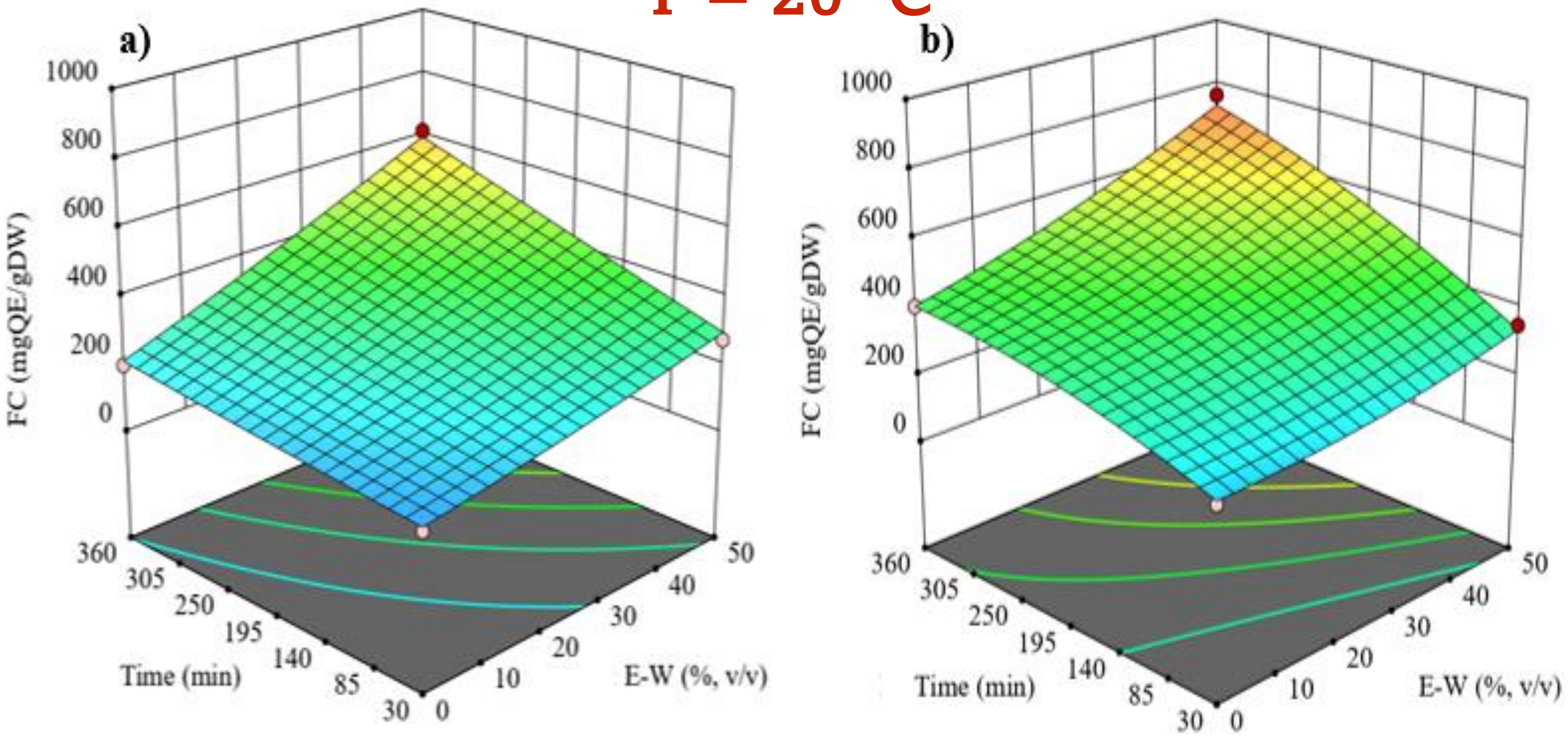




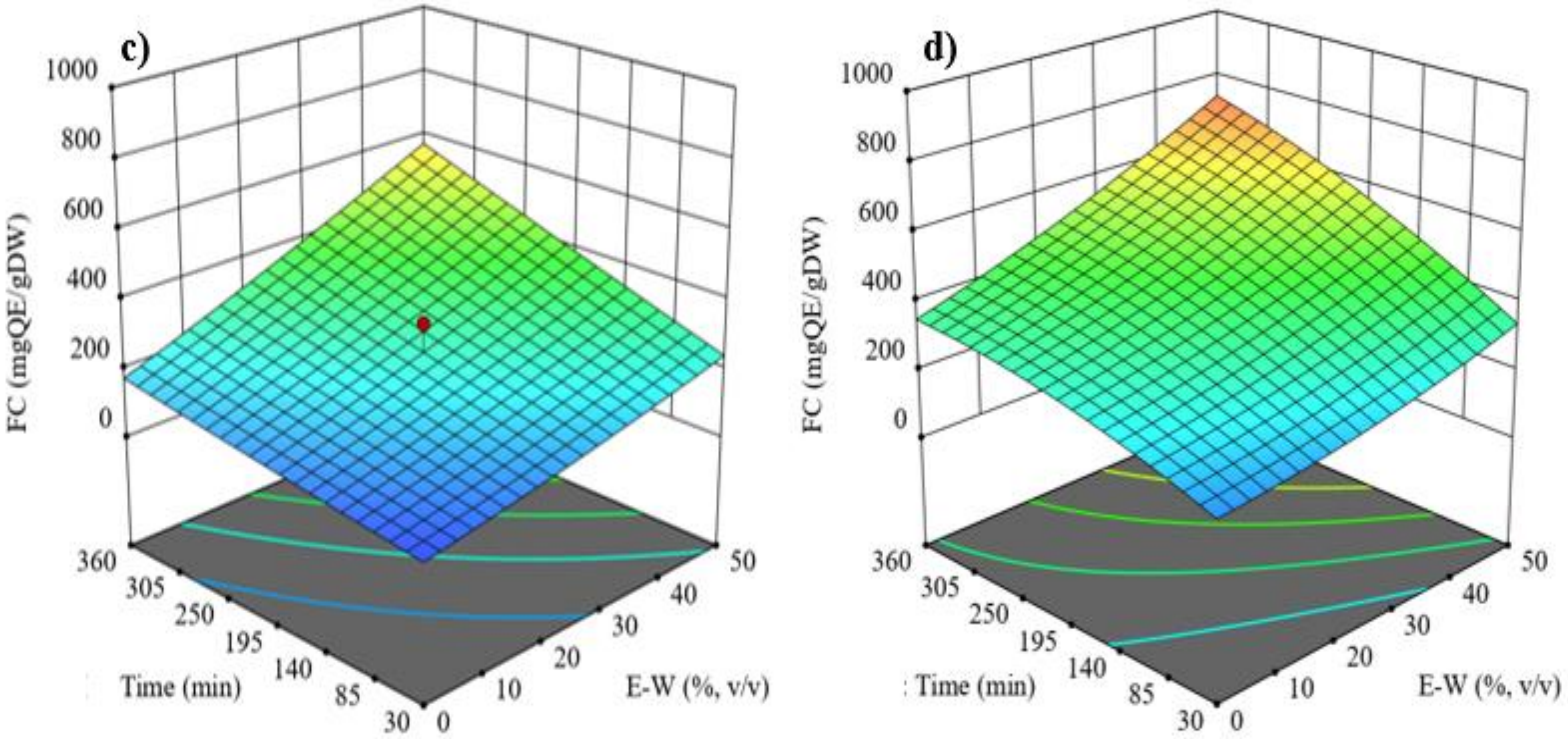
Flavonoids Content (FC)

3D Response Surface graphs

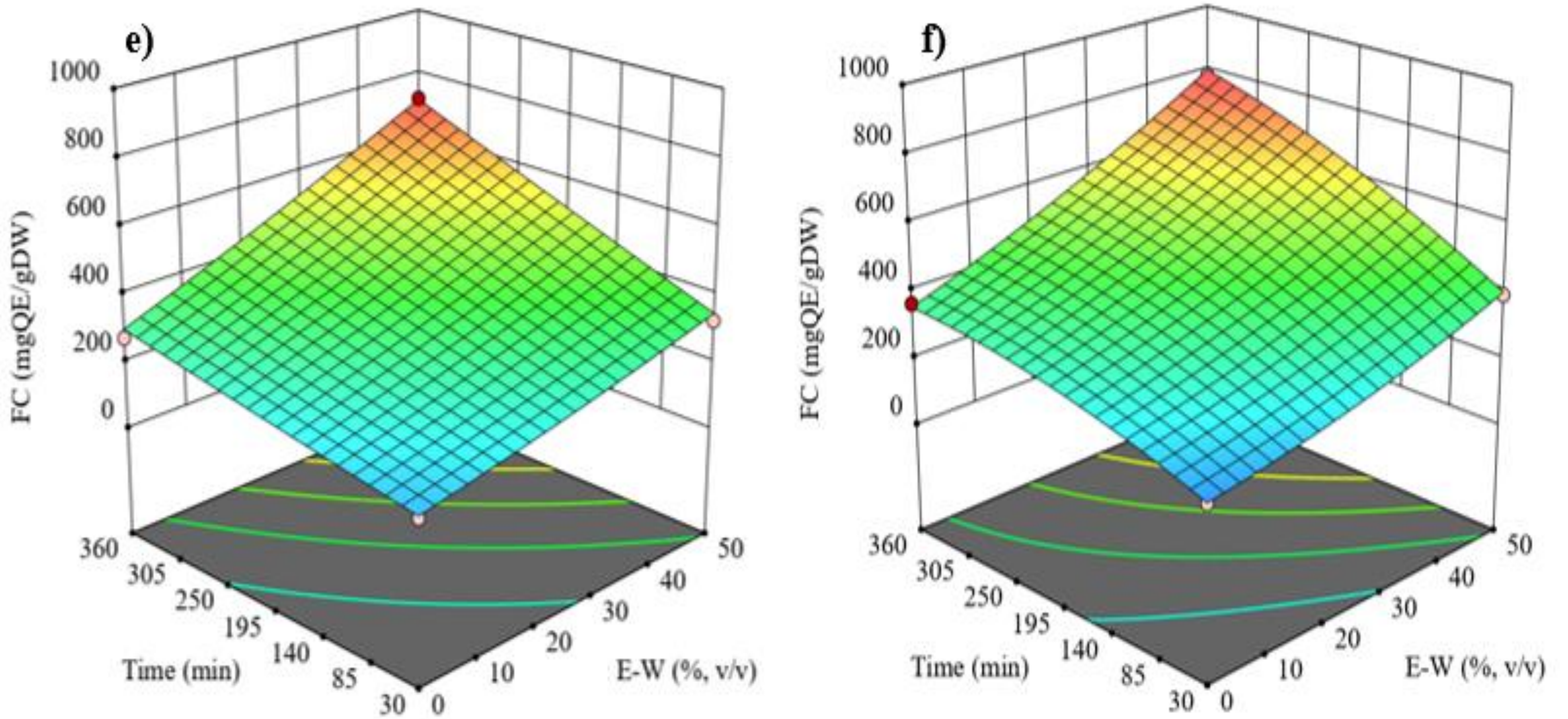
Control T = 20° C PEF



Control T = 35° C PEF



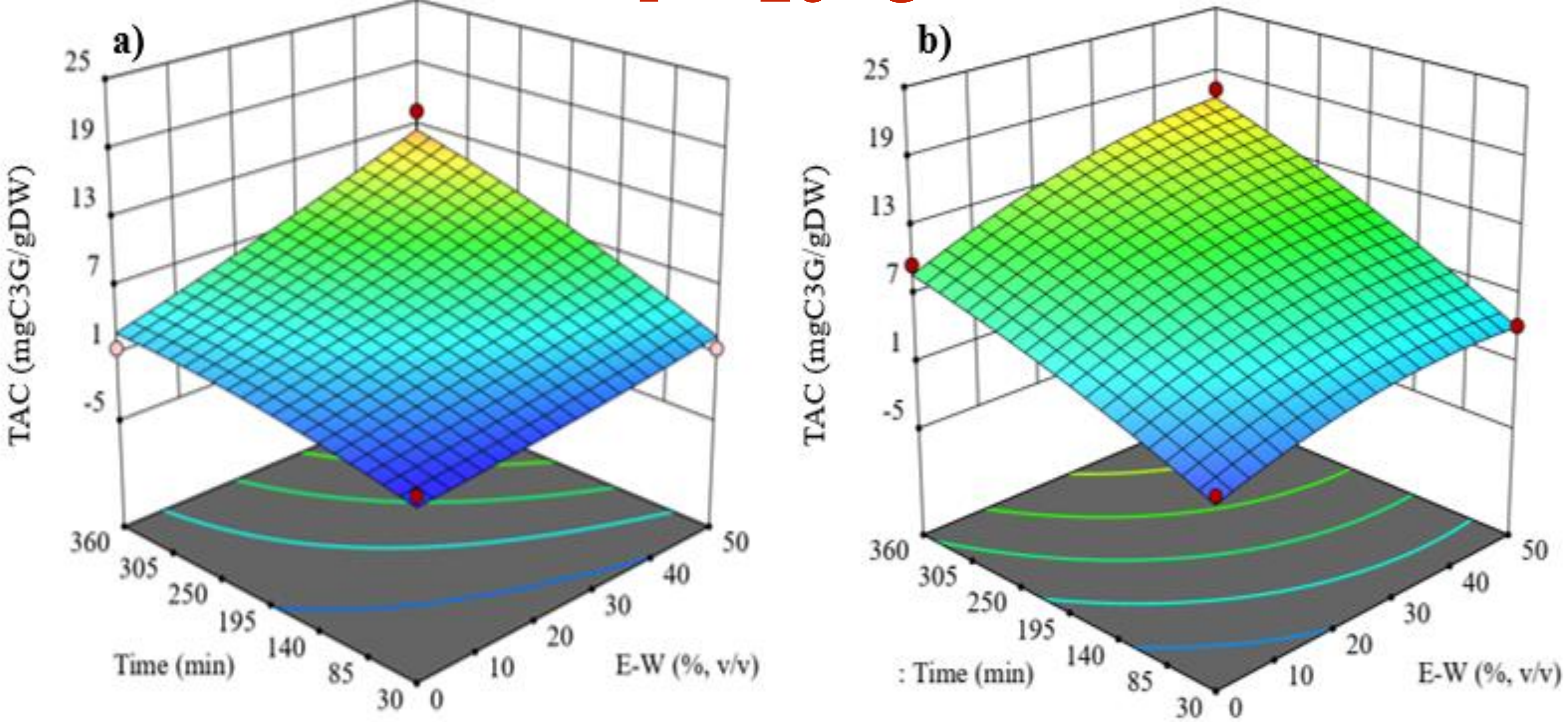
Control T = 50° C PEF



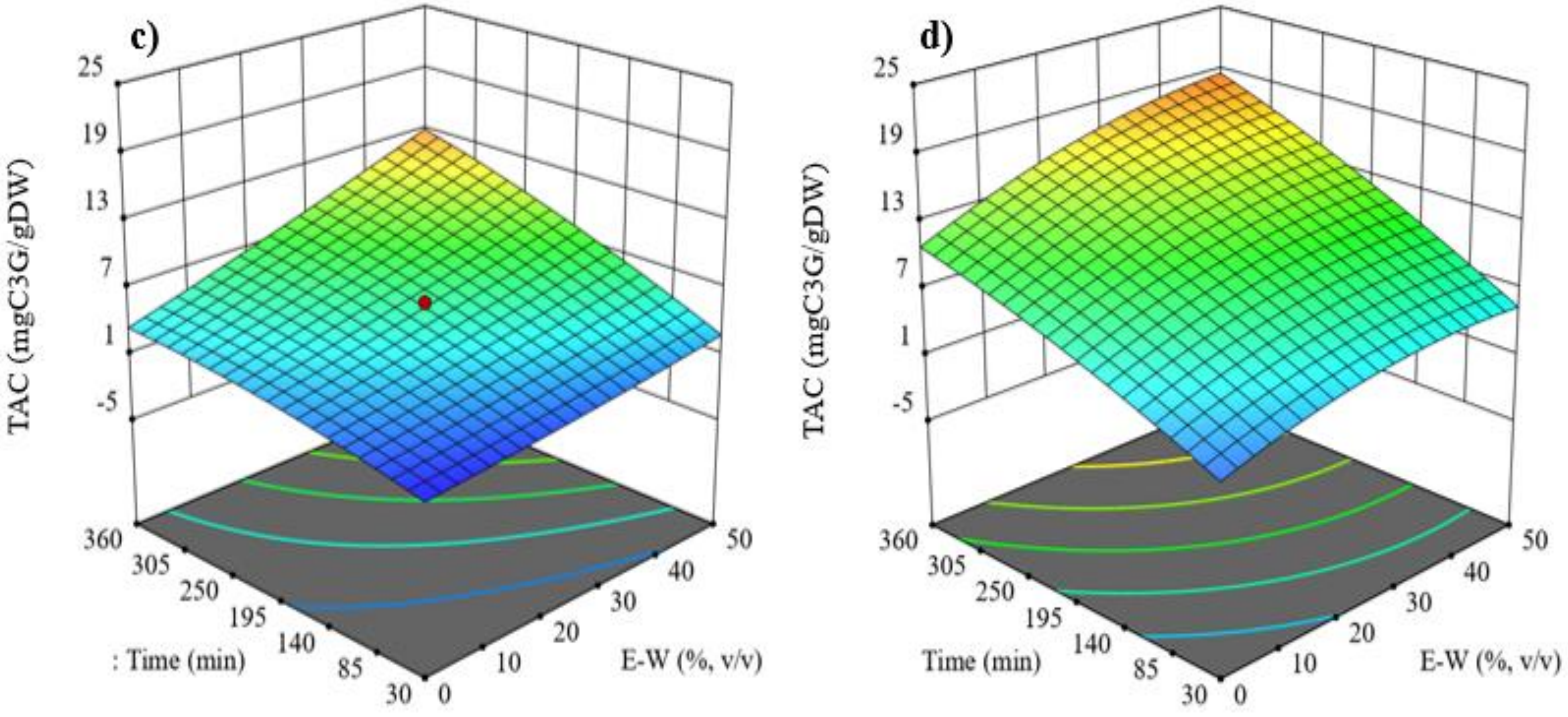
Anthocyanins Content (FC)

3D Response Surface graphs

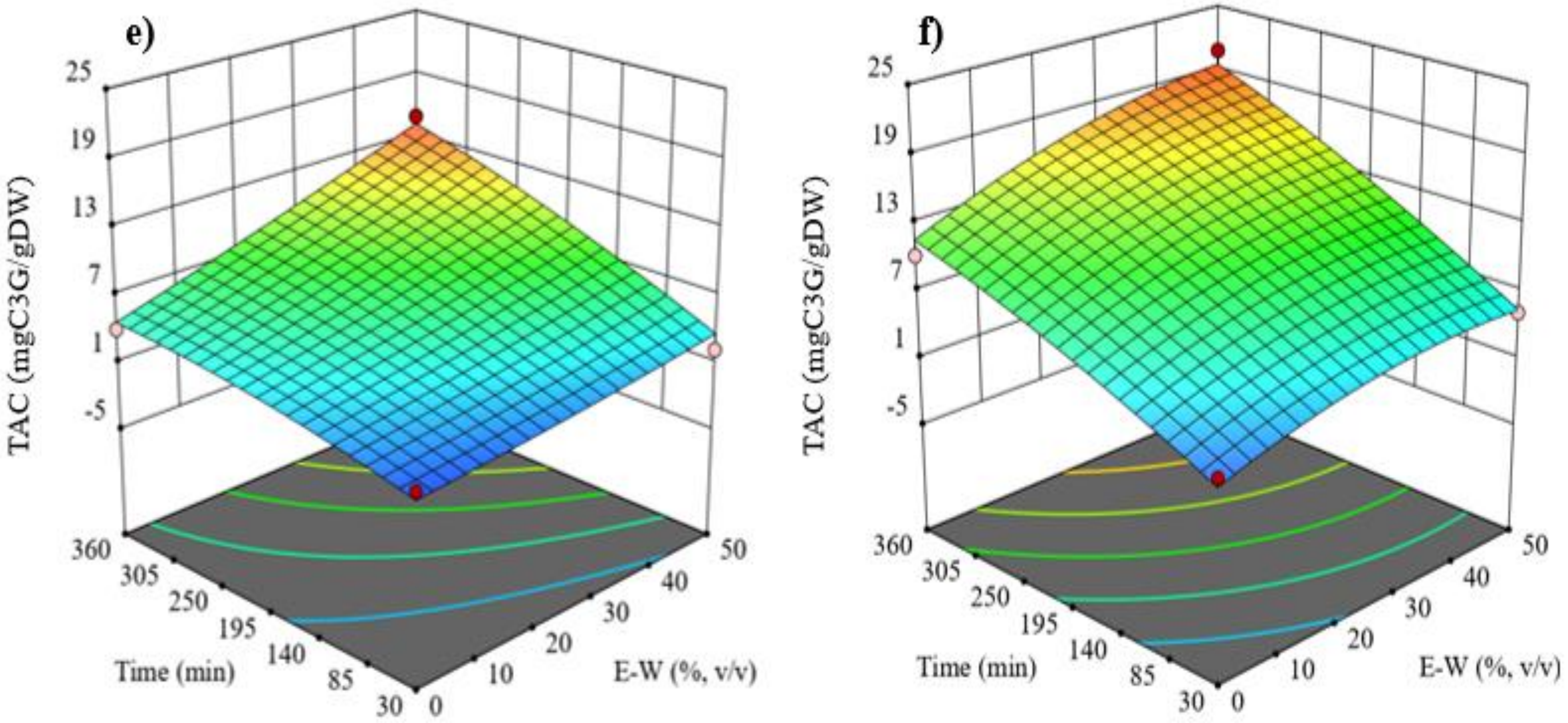
Control **T = 20° C** PEF



Control **T = 35° C** PEF



Control **T = 50° C** PEF



Antioxidant Activity (FRAP)

3D Response Surface graphs

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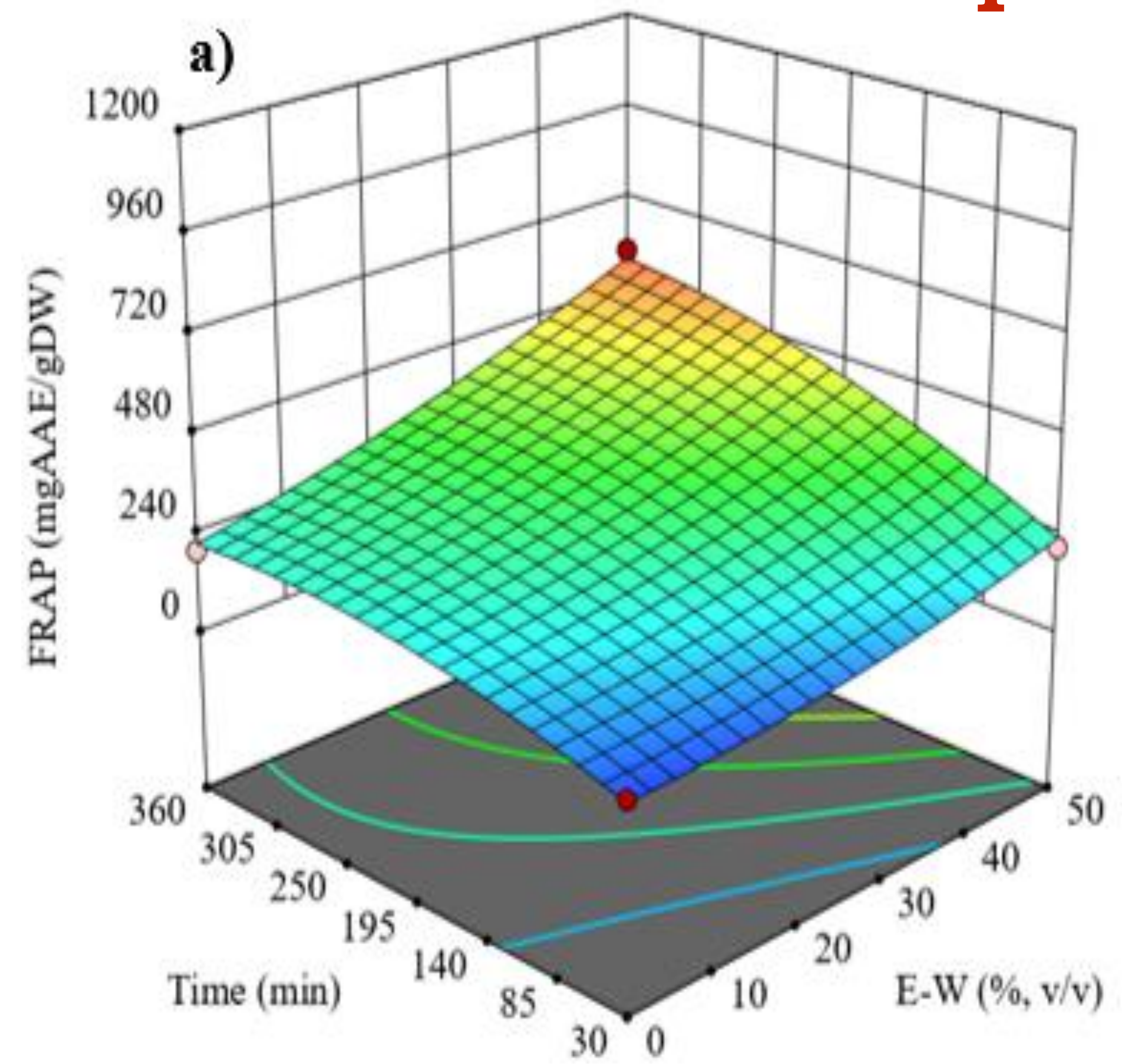
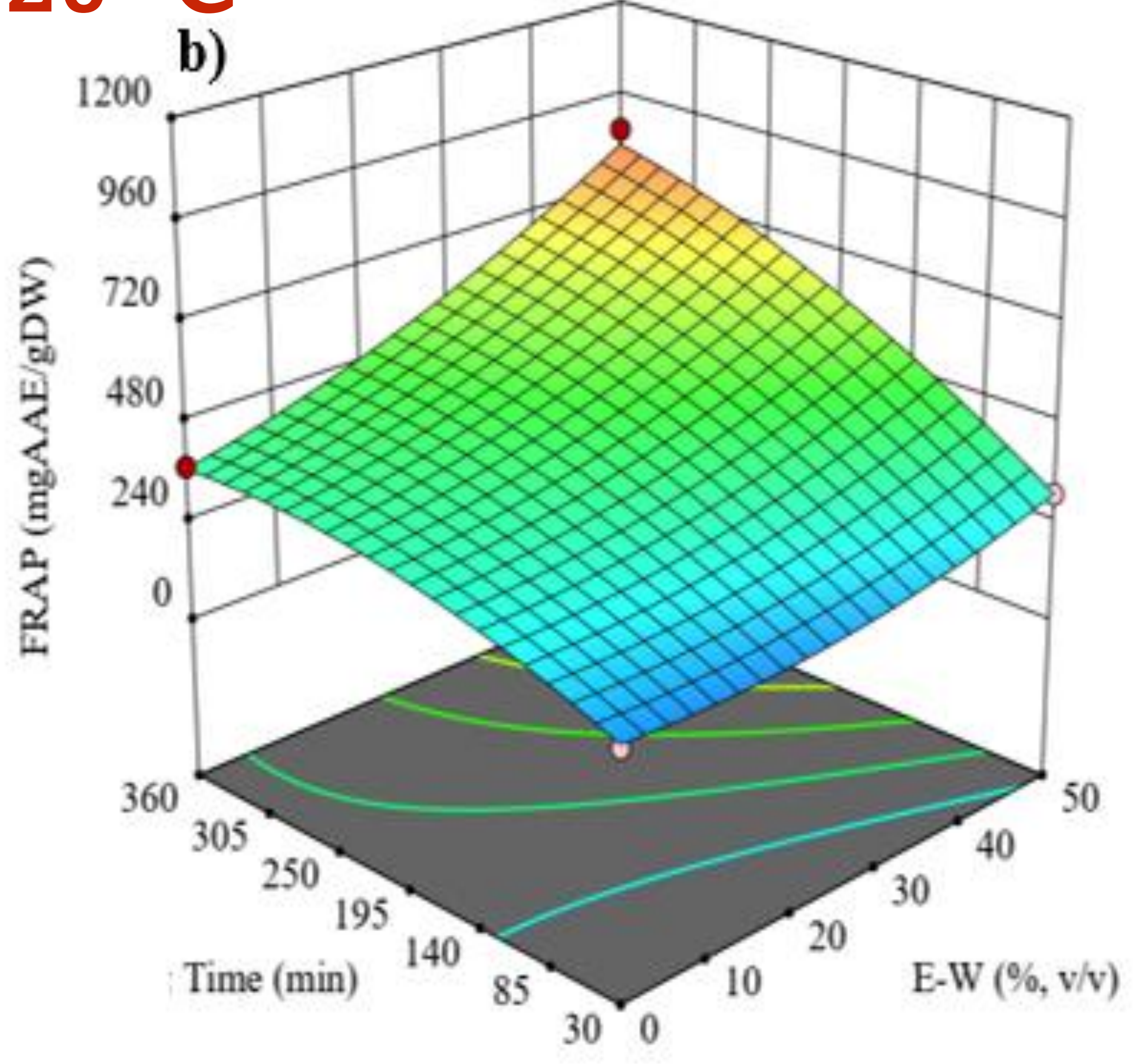
T = 20° C

Control

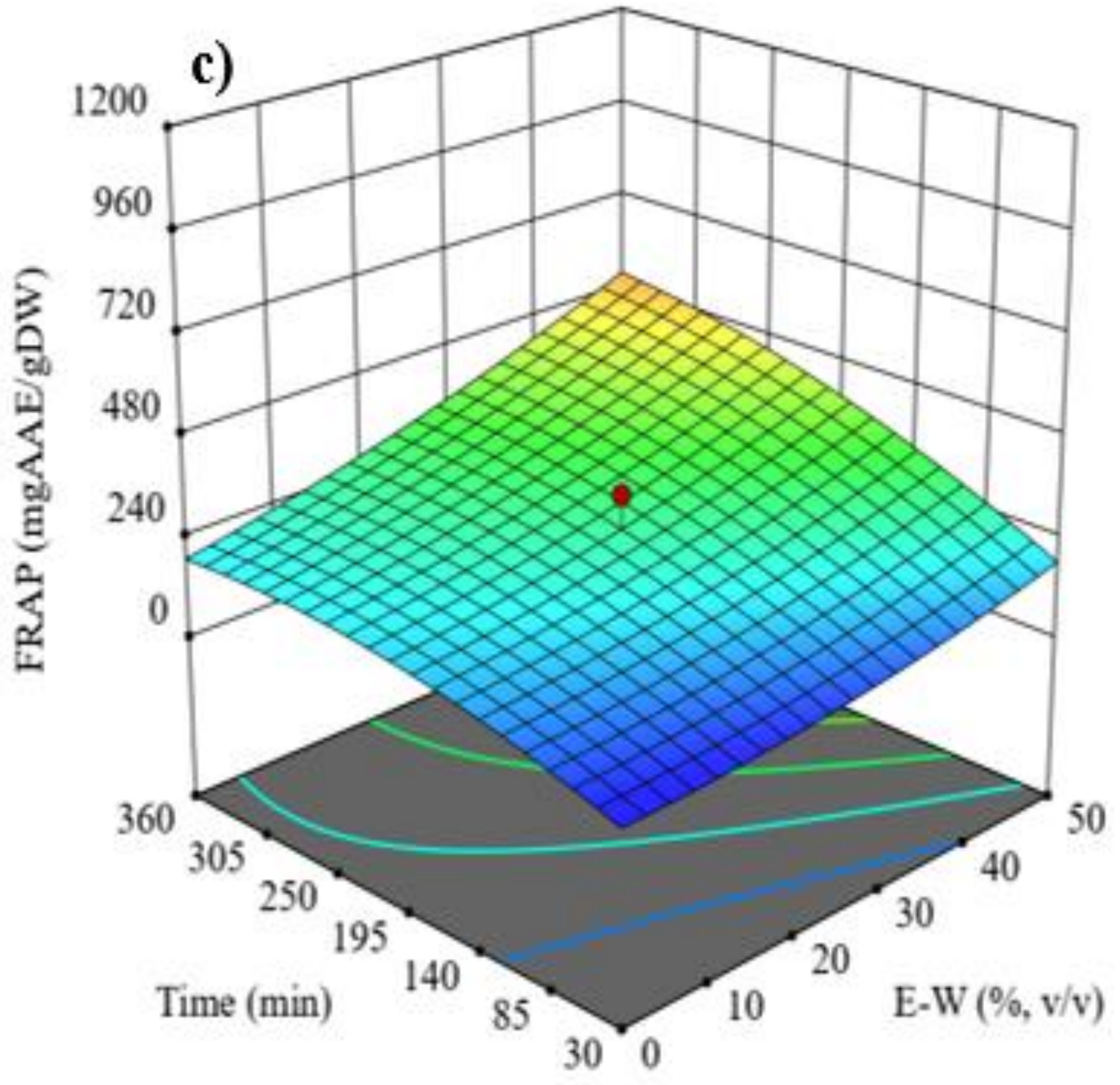


PEF

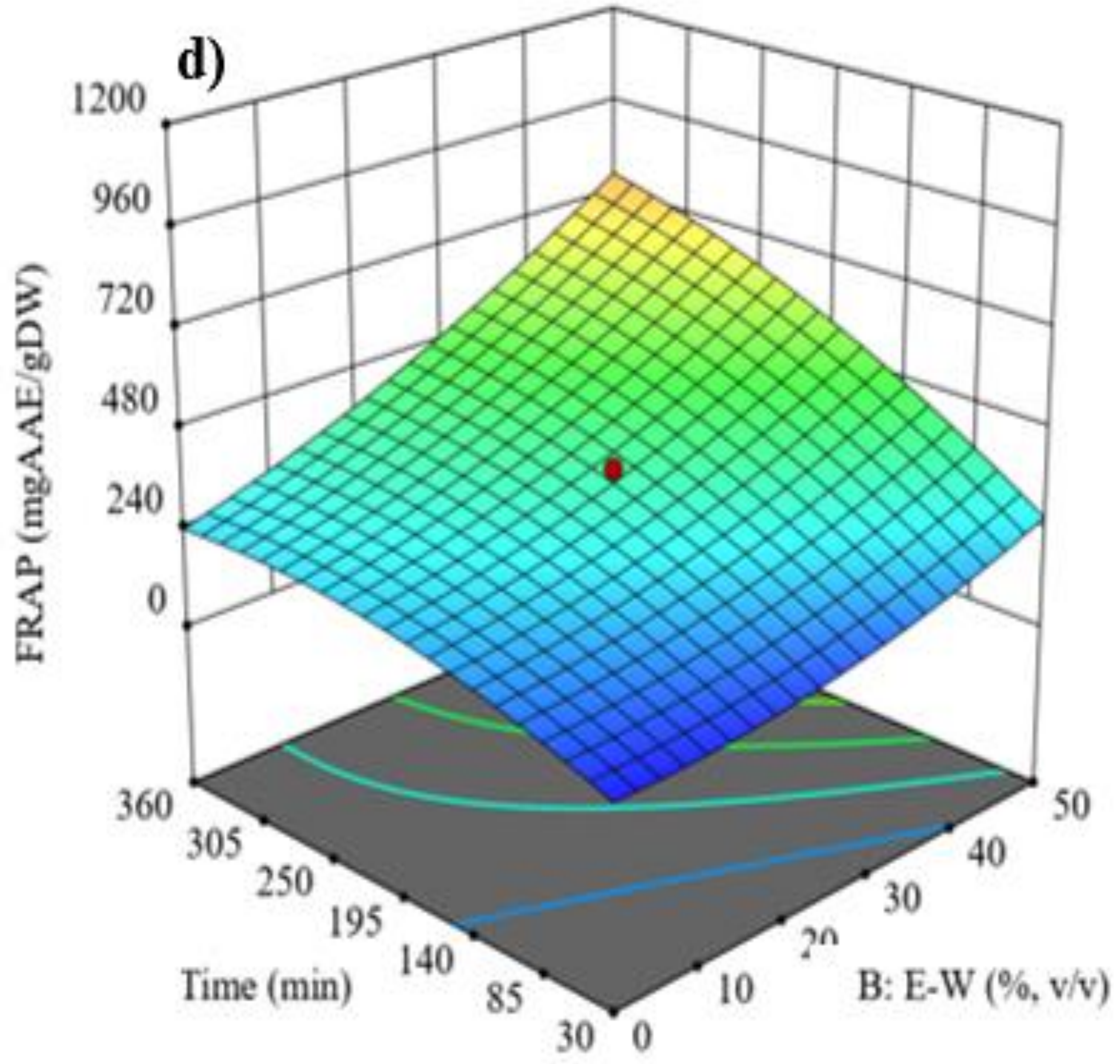


T = 35° C

Control

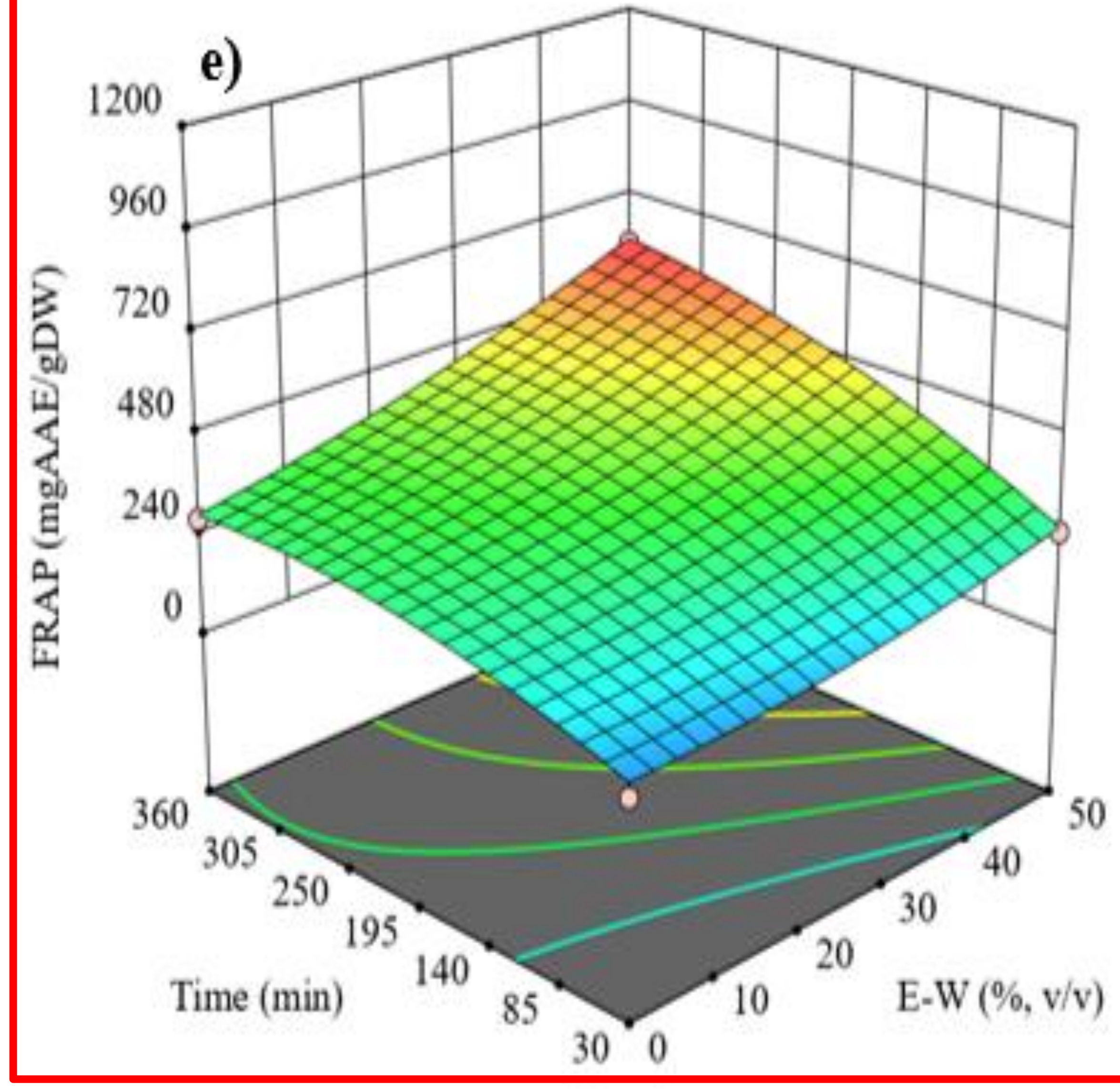


PEF

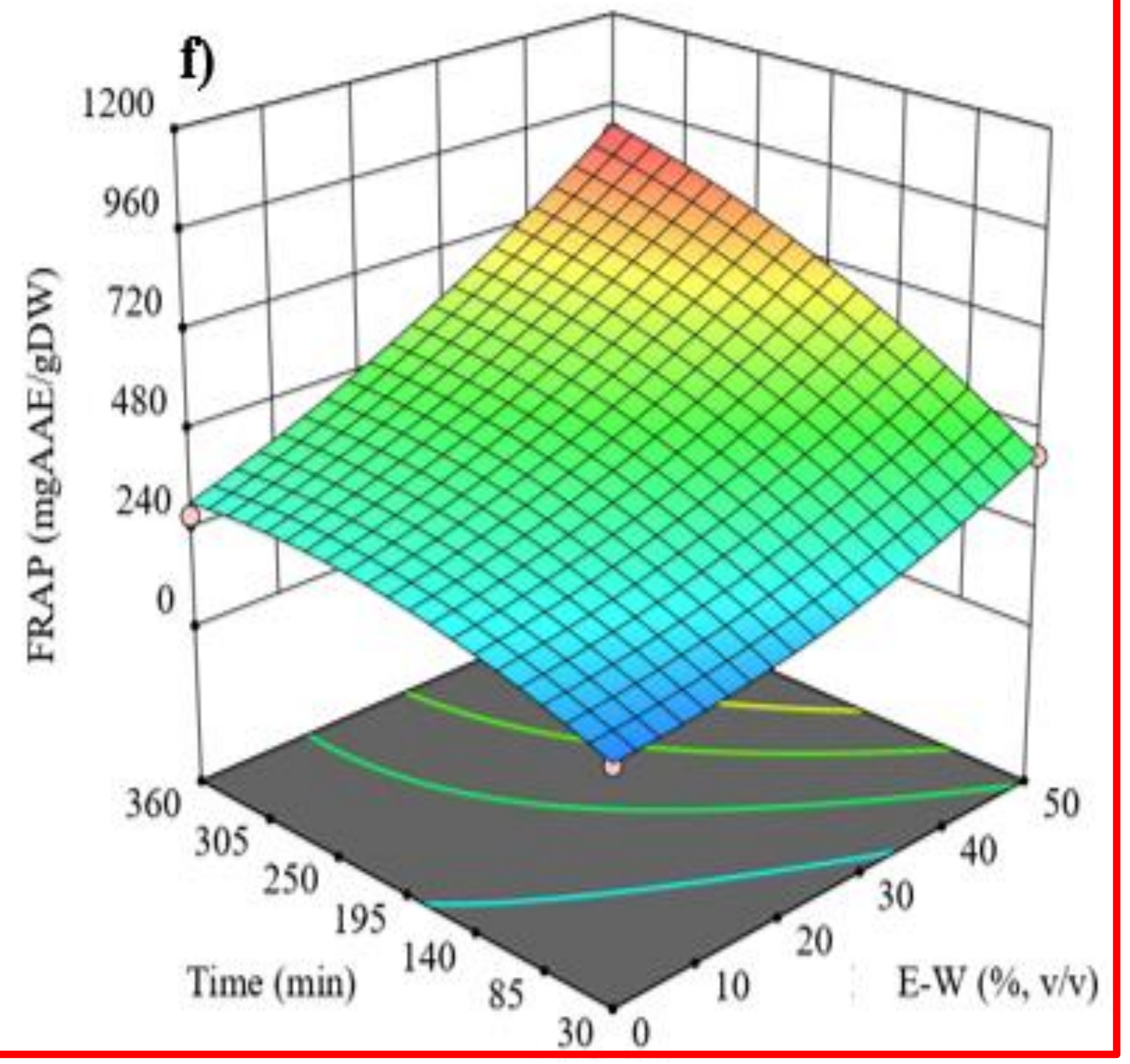


T = 50° C

Control



PEF



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Pulsed Electric Fields (PEF): Sweet cherry press cake

Response variables at the optimal PEF-assisted extraction conditions

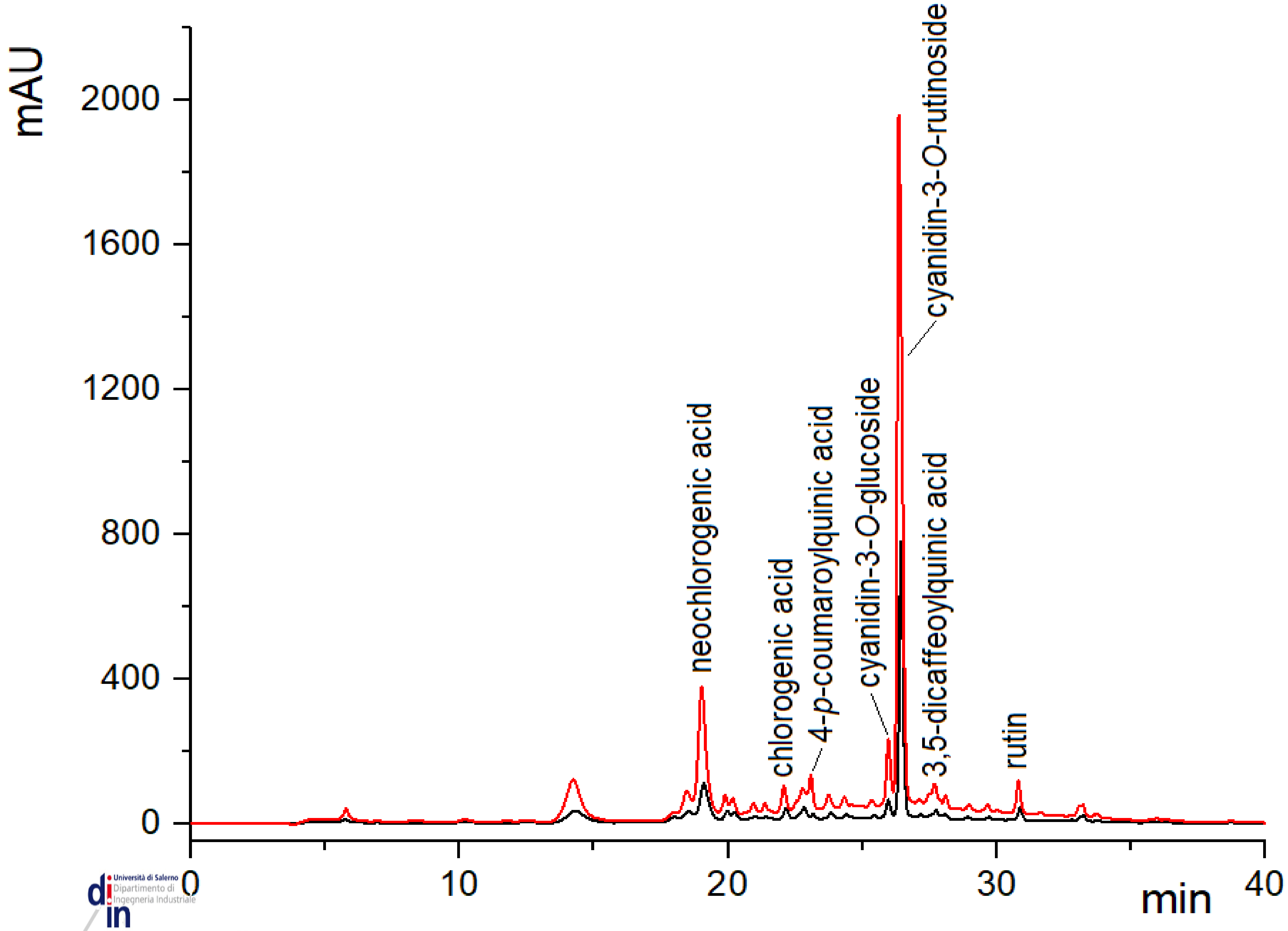
Response	Ethanol –Water		Temperature		Time		S/L		Increment over SLE [%]
	SLE	PEF	SLE	PEF	SLE	PEF	SLE	PEF	PEF
TPC (mgGAE/g_{DW})	50	49	50	50	360	360	0.2	0.2	+26
FC (mgQE/g_{DW})	50	50	50	48	360	356	0.2	0.2	+27
AC (mgC3G/g_{DW})	50	50	50	50	360	360	0.2	0.2	+42
FRAP (mgAAE/g_{DW})	50	50	50	50	360	342	0.2	0.17	+44

Extraction time **(4-18 min)**
Solid/ liquid ratio **2%**

Selected overall optimal extraction conditions:
EtOH-Water = 50 % (v/v), T = 50 °C, Time = 360 min, S/L= 0.2

Pulsed Electric Fields (PEF): Sweet cherry press cake

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

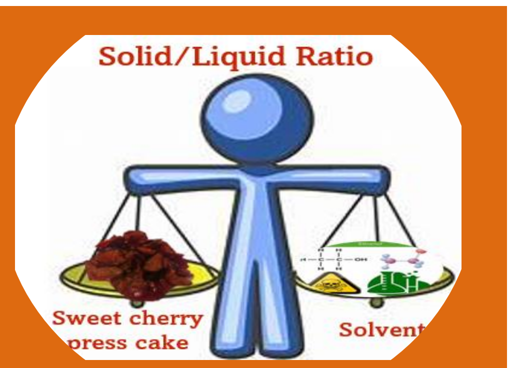


Compound (mg/L)	Concentration (mg/g FW)		Increment (%)
	Control	PEF	
Neochlorogenic acid	0.085	0.276	**+224.7**
Chlorogenic acid	0.019	0.059	**+210.5**
4-p-coumaroylquinic acid	0.01	0.061	**+510**
Cyanidin 3-glucoside	0.009	0.038	**+322.2**
Cyanidin 3-rutinoside	0.087	0.283	**+225.3**
3,5-dicaffeoylquinic acid	0.016	0.065	**+306.3**
Rutin	0.032	0.164	**+412.5**

Conclusion

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

PEF is effective in intensifying the recovery yield of total **polyphenols content (+26%), flavonoids (+27%), anthocyanin content (+42%). and antioxidant power (+44%),** from **the sweet cherry press cake**



Has the potential to **reduce the solid/liquid ratio (2%)** and **shorten the extraction time (18 min)** to achieve the **same recovery yield** of phenolic compounds

Higher release of different phenolic compounds, including **Cyanidin 3-glucoside (+322.2%)**, and **Cyanidin 3-rutinoside (+225.3%)** upon PEF compared to the control.

No degradation phenomenon was observed.

Questions, ideas, and suggestions suggestion are welcome

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