Implementation of Box-Behnken Experimental Design and Kinetics to Optimize Organic Solvent-Free Ultrasound Assisted Extraction of Red Grape Pomace Polyphenols and Pigments

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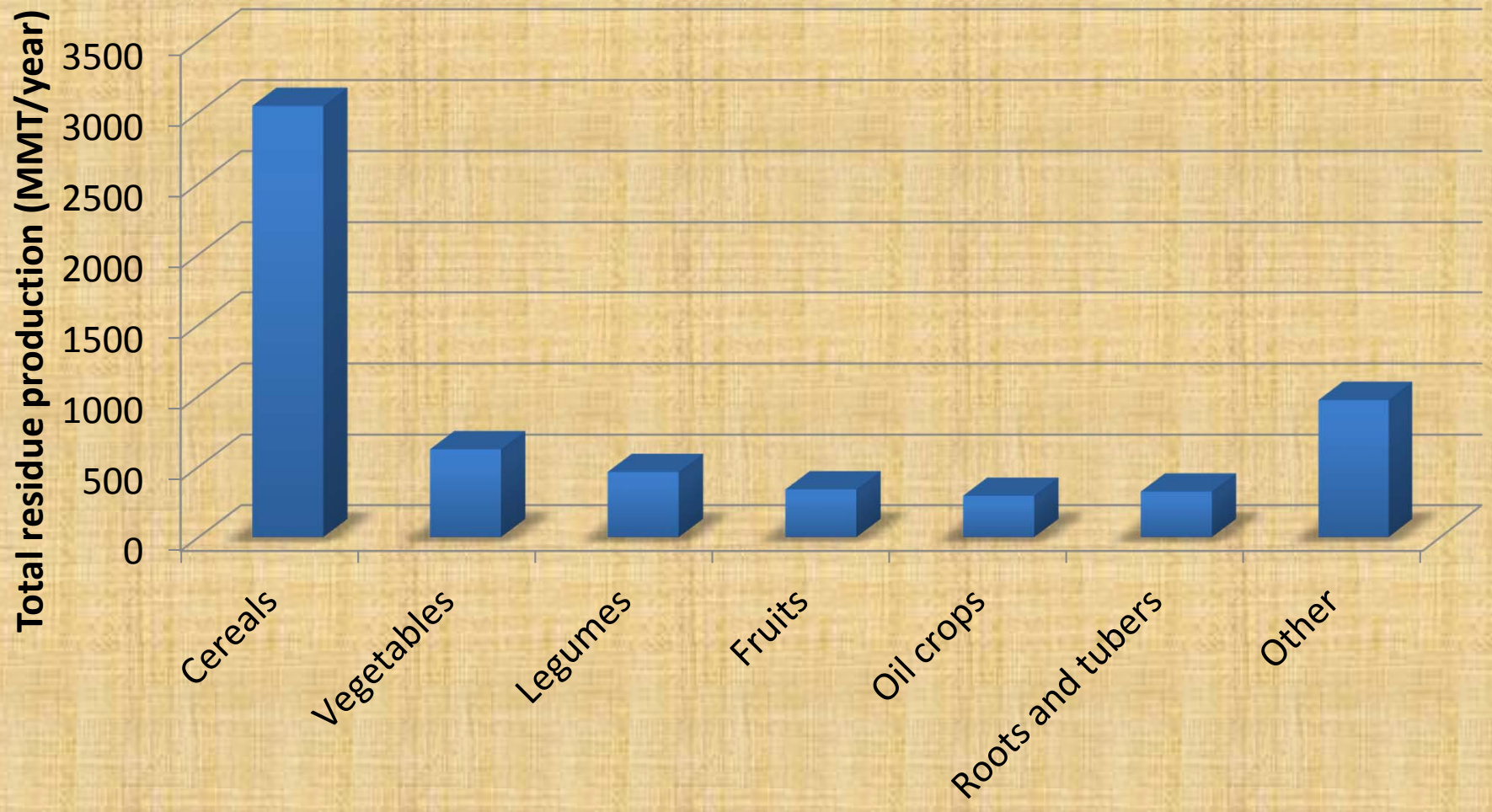
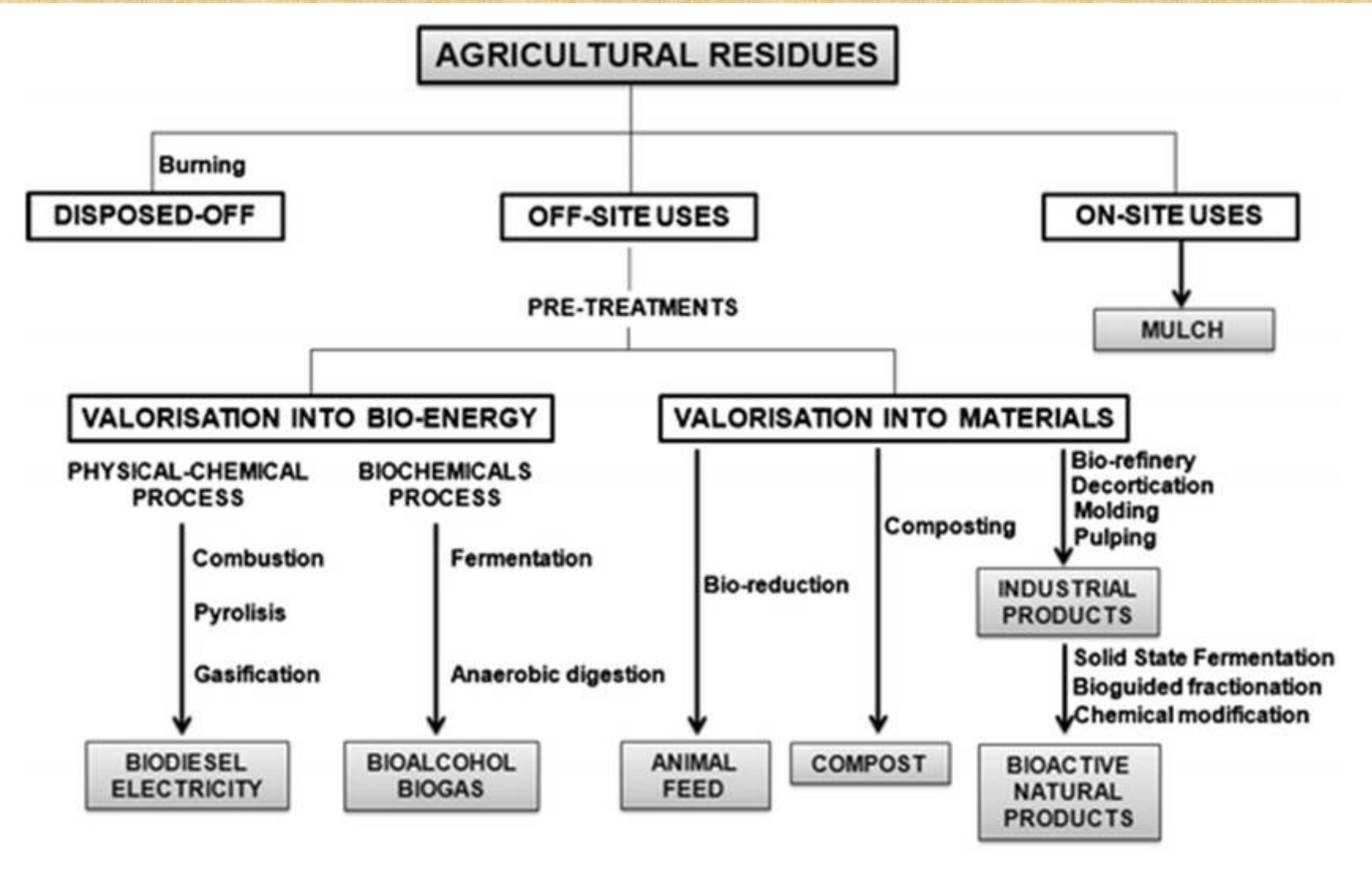
School of Environment

AGRICULTURAL WASTES



Santana-Méridas et al., 2012

AGRICULTURAL WASTES



AGRICULTURAL WASTES

- High process costs
- Destruction of abundant in wastes phytochemical compounds, with strong technological and functional properties
- Consumers disdain synthetic food additives

40% literature: **antioxidant recovery**

WINE INDUSTRY WASTES

- 70,000,000 tons of global production (*FAO, 2010*)
  - 14,500,000 tons of global wastes
    (↑organic compounds, seasonality)

LEAST IS UPGRADED OR RECYCLED!

WINE INDUSTRY WASTES

stalks

grape pomace

wine sludge

vine leaves

WINE INDUSTRY WASTES

stalks

2025%

grape pomace

wine sludge

vine leaves

WINE INDUSTRY WASTES

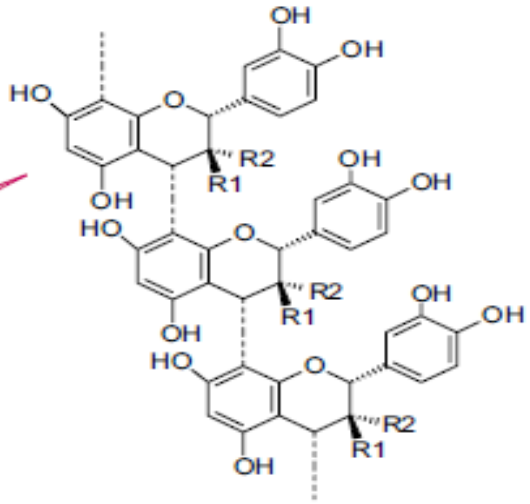
Grape pomace (↑BOD, COD and phenolic compounds):

- Distilleries
- Composting
- Animal feeding
- Methanisation or/and energy production
- Discharge into open areas

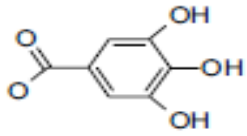
TOXICITY FOR FLORA AND FAUNA

CONTAMINATION OF GROUNDWATER

WINE INDUSTRY WASTES

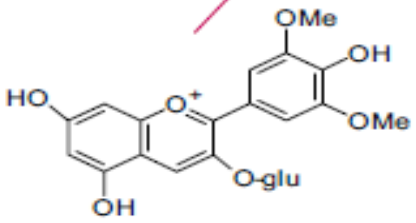


R1=H, R2=OH; R1=OH, R2=H; R1=H, R2=

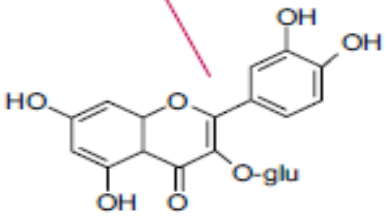


(Gal)

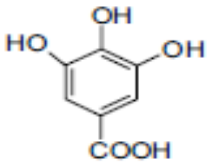
Proanthocyanidins
Polymers mainly consisting of of catechin, epicatechin and epicatechin gallate



Anthocyanins
e.g. Malvidin-3-glucoside



Flavonols
e.g. Quercetin glucoside



Hydroxy benzoates and Benzoic acids
e.g. Gallic acid

WINE INDUSTRY WASTES

RED GRAPE POMACE (SKINS)

POLYPHENOLS

POTENT ANTIOXIDANTS

NATURAL PIGMENTS

RECOVERY OF POLYPHENOLS FROM WINE INDUSTRY BY-PRODUCTS

- Conventional extraction solvents (organic):

  methanol, ethanol, ethyl acetate and methanol and aqueous ethanol solutions

  - expensive, toxic, flammable and environmentally hazardous
  - require further removal steps
  - the availability of ethanol is strictly controlled by state laws

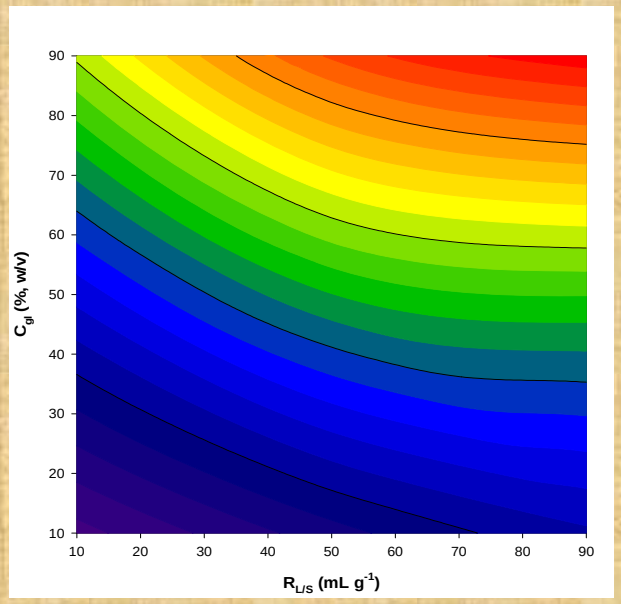
RECOVERY OF POLYPHENOLS FROM BY-PRODUCTS OF WINEMAKING INDUSTRY

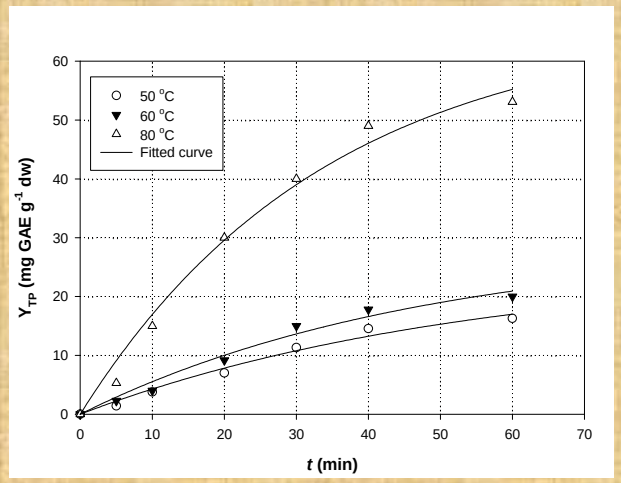
- Alternative, “green” extraction solvent:

glycerol

- cheap, abundant (biodiesel by-product), non-toxic, non-flammable
- no need for removal
- appropriate polarity for polyphenol extraction

EXPERIMENTAL PROCEDURE



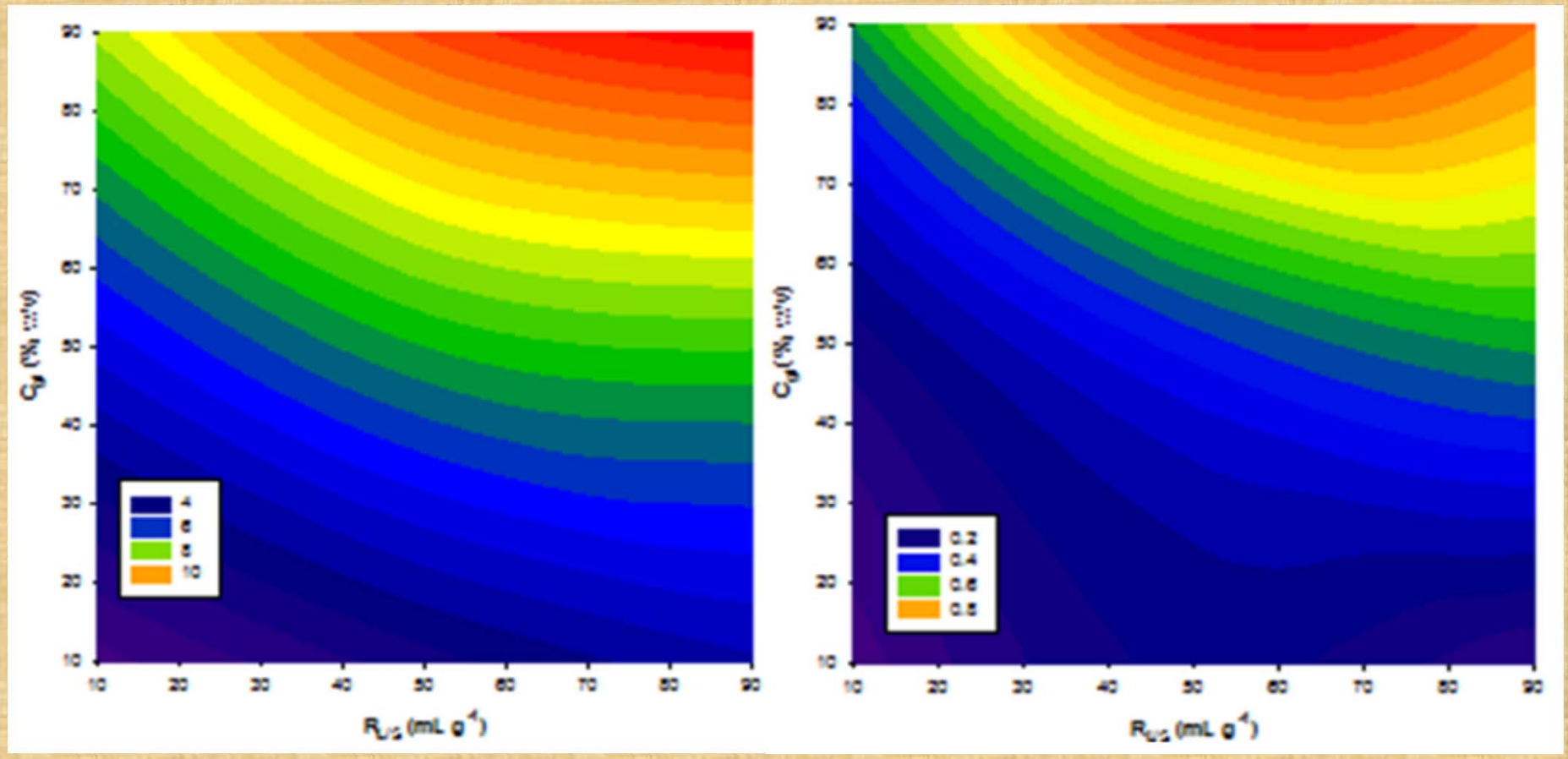


EXPERIMENTAL PROCEDURE

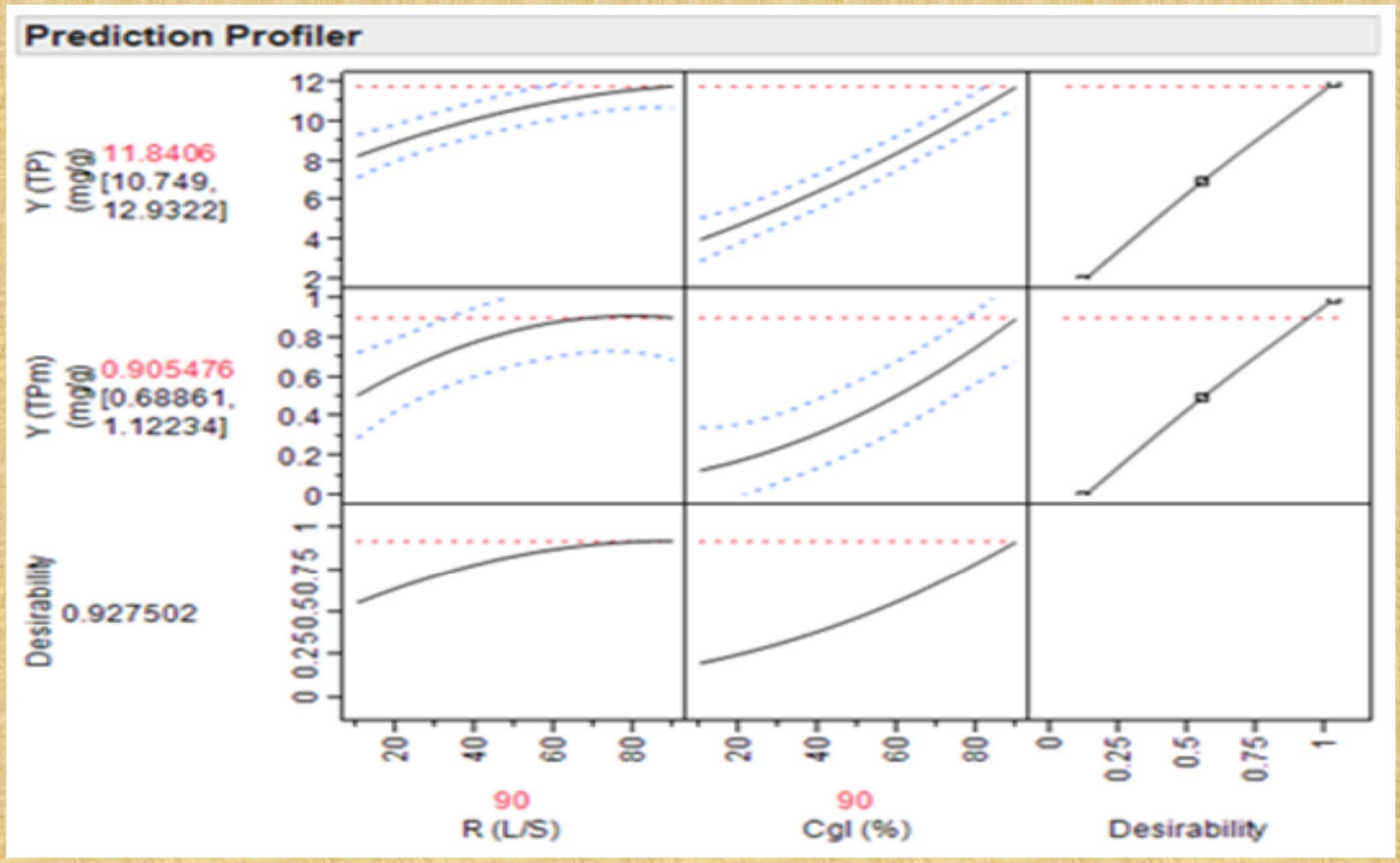
- Drying (65 °C, 48h) and pulverization (~0.3 mm) of RGP (skin) of *Vitis vinifera var. Agiorgitiko*
- Extraction assisted by ultrasounds (140 W, 37 kHz, 35 W L^{-1}, 60 min, 45 °C)
- Implementation of Box-Behnken design to optimise the concentration of glycerol (C_{gl}) and the liquid-to-solid ratio ($R_{L/S}$)
- Kinetics ⟶ determination of the effective diffusion coefficient (D_e)
- Determination of total polyphenols (TP) and total pigments (TP_m) yield, and reducing power (P_R) of the extracts

RESULTS AND DISCUSSION

1. Response surface optimisation



RESULTS AND DISCUSSION



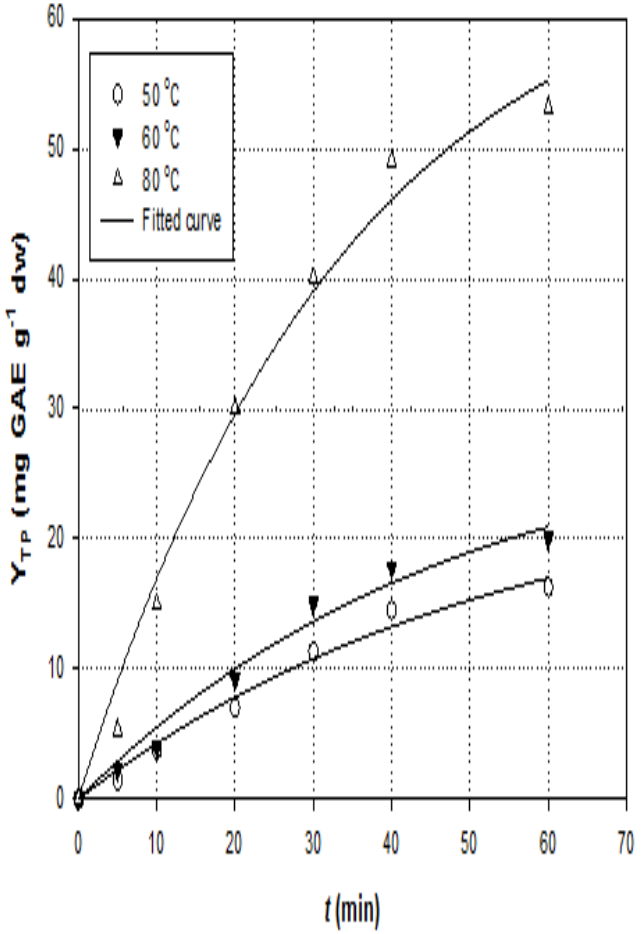
RESULTS AND DISCUSSION

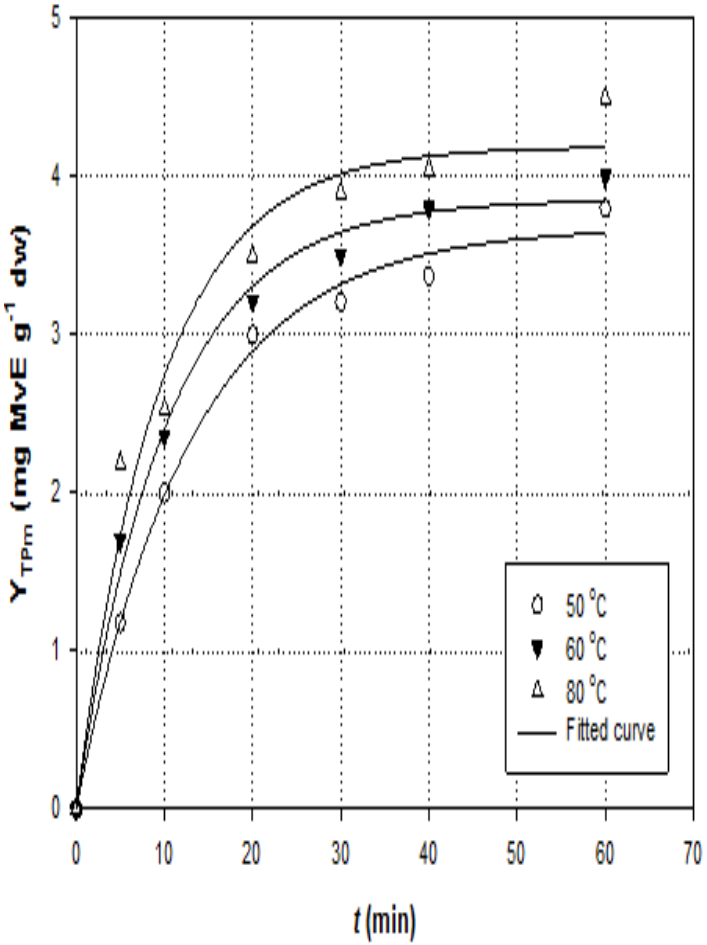
Response variables	Polynomial equations	R^2	p
Y_{TP} (mg GAE g^{-1}dw)	$0.710 + 0.034R_{L/S} + 0.087C_{gl}$	0.99	0.0003
Y_{TPm} (mg MvE g^{-1}dw)	$-0.118 + 0.003R_{L/S} + 0.078C_{gl}$	0.96	0.0066

Response variables	Maximal predicted value	Optimal conditions	
		C_{gl} (w/v, %)	$R_{L/S}$ (mL g^{-1})
Y_{TP} (mg GAE g^{-1}dw)	11.84±1.09	90	90
Y_{TPm} (mg MvE g^{-1}dw)	0.91±0.09	90	79

RESULTS AND DISCUSSION

2. Extraction kinetics and the effect of temperature





$$Y_t = Y_s\left(1 - e^{-kt}\right)$$

$$\frac{Y_t}{Y_s} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n}e^{-\frac{D_e n^2 \pi^2 t}{r^2}}$$

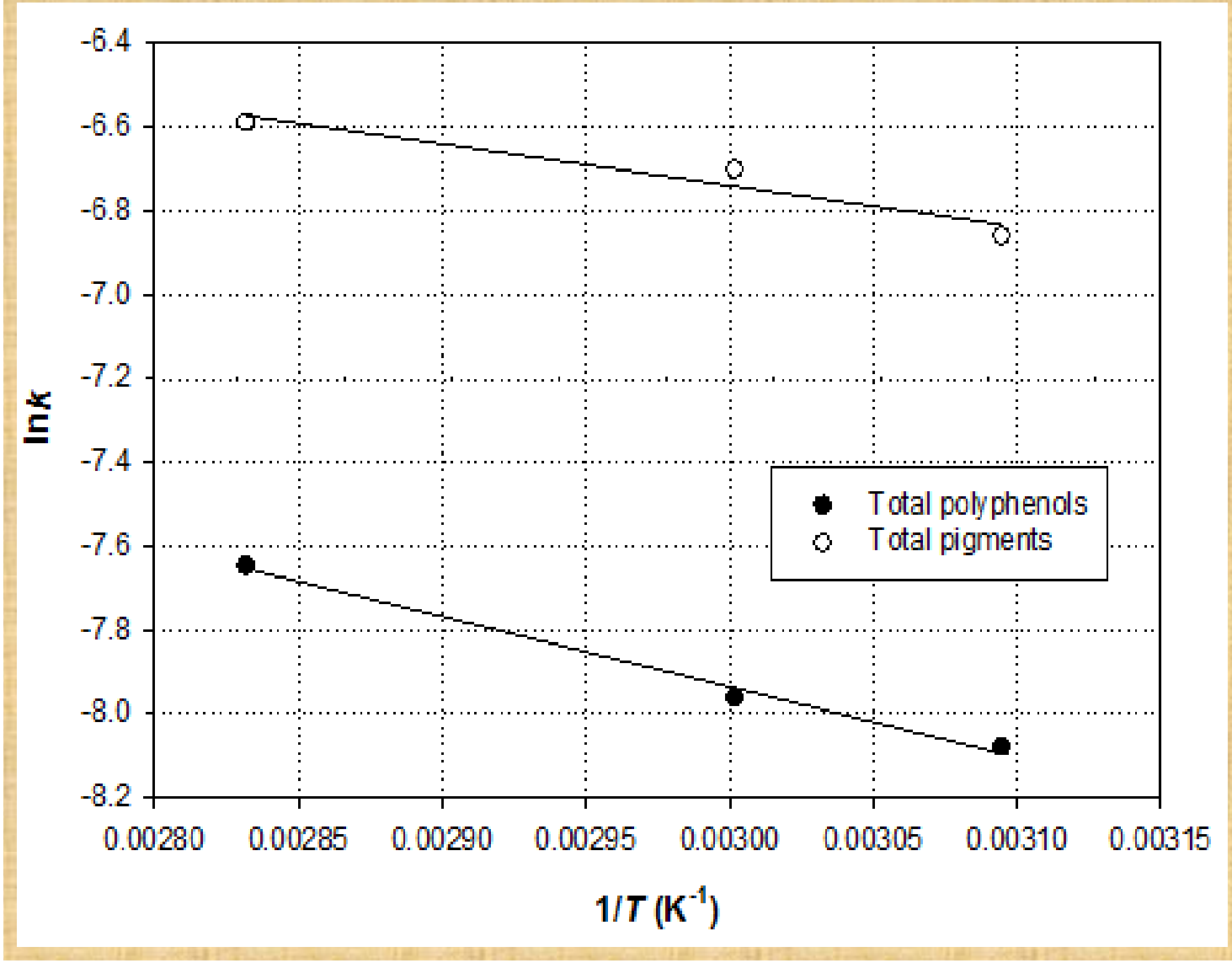
$$ln\left(\frac{Y_s}{Y_s - Y_t}\right) = ln\frac{\pi^2}{6} + \frac{D_e \pi^2 t}{r^2}$$

$$slope = \frac{D_e \pi^2}{r^2}$$

RESULTS AND DISCUSSION

Kinetic parameters	Temperature (°C)		
	50	60	80
Total polyphenols			
k (min^{-1})	0.019	0.021	0.029
D_e ($m^2\ s^{-1}$) × 10^{-12}	2.73	3.07	4.22
$Y_{TP(s)}$ (mg GAE g^{-1})	25.36	29.25	66.70
Total pigments			
k (min^{-1})	0.063	0.078	0.083
D_e ($m^2\ s^{-1}$) × 10^{-12}	9.59	11.89	12.59
$Y_{TPm(s)}$ (mg MvE g^{-1})v	3.68	3.86	4.19

RESULTS AND DISCUSSION



$$k = k_0 e^{-\frac{E_a}{RT}}$$

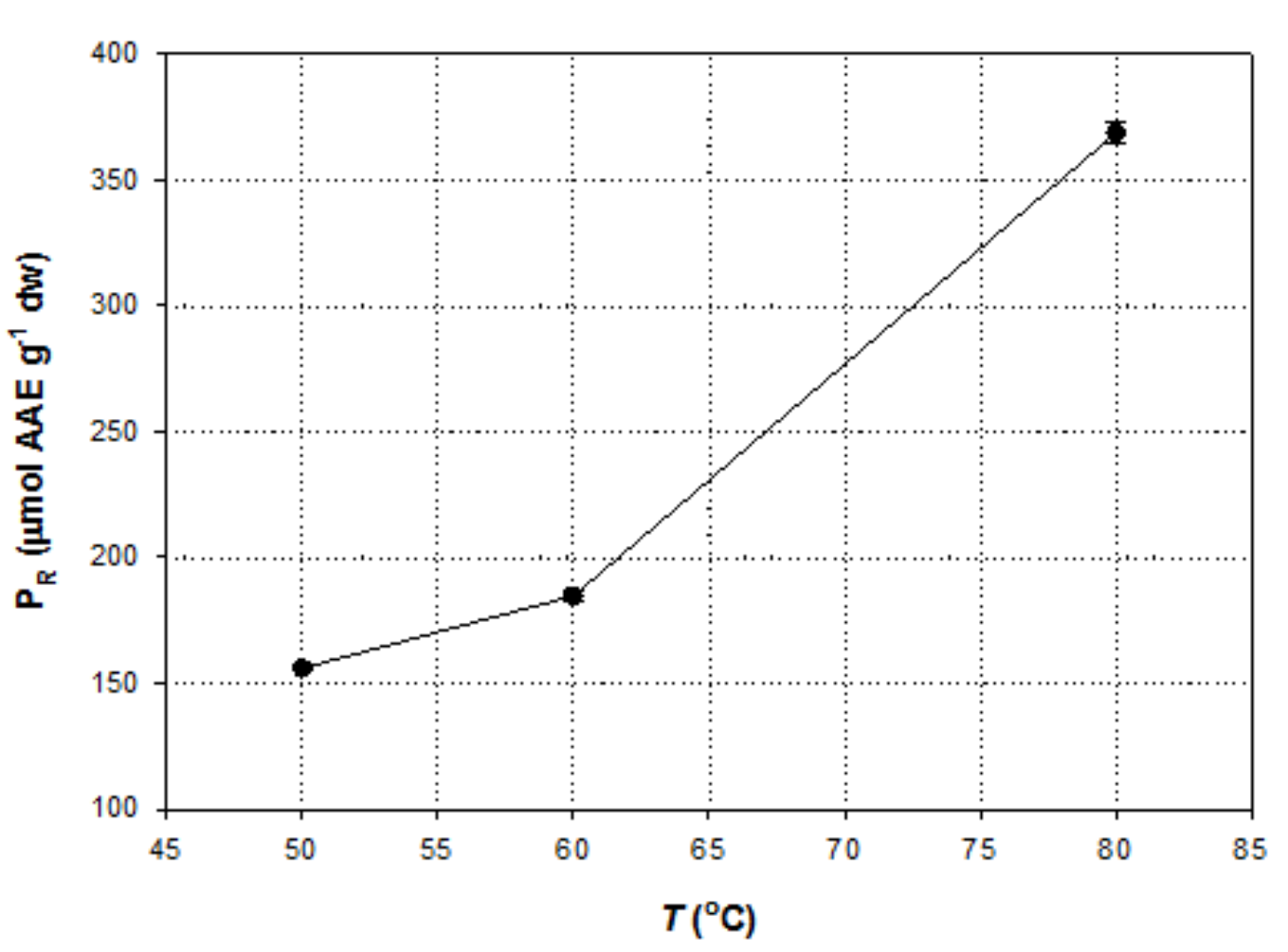
$$slope = -\frac{E_a}{R}$$

$E_a^{TP} = 13.94\ kJ\ mol^{-1}$

$E_a^{TPm} = 8.22\ kJ\ mol^{-1}$

RESULTS AND DISCUSSION

3. Reducing power (P_R)



CONCLUSIONS

- ✓ This study demonstrated for the first time that an extraction medium composed of 90% (w/v) aqueous glycerol can efficiently extract polyphenols and pigments from red grape pomace, with the assistance of ultrasonication
- ✓ The relatively low activation energies for the extraction of total polyphenols and total pigments were ascribed to the effect of ultrasounds
- ✓ The effect of the temperature on the antioxidant activity of the extracts obtained, was positive

CONCLUSIONS

- ✓ The adoption of similar processes by the industries would be expected to form the basis for the development of green procedures, aimed at the valorisation of food industry waste streams and the sustainable production of value-added commodities, such as food additives, food supplements, pharmaceutical formulations and cosmetics


Thank you!