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Cyclodextrins as co-solvents for the extraction of polyphenols from olive leaf

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Exploitation of plant by-products by the food industry

Food production

(raw materials, final products)

Food waste or by-products

(leaves, roots, seeds)

Phytochemicals

(antioxidants, antimicrobials)

Extraction of phytochemical with conventional and non-conventional techniques

Production of functional - novel foods

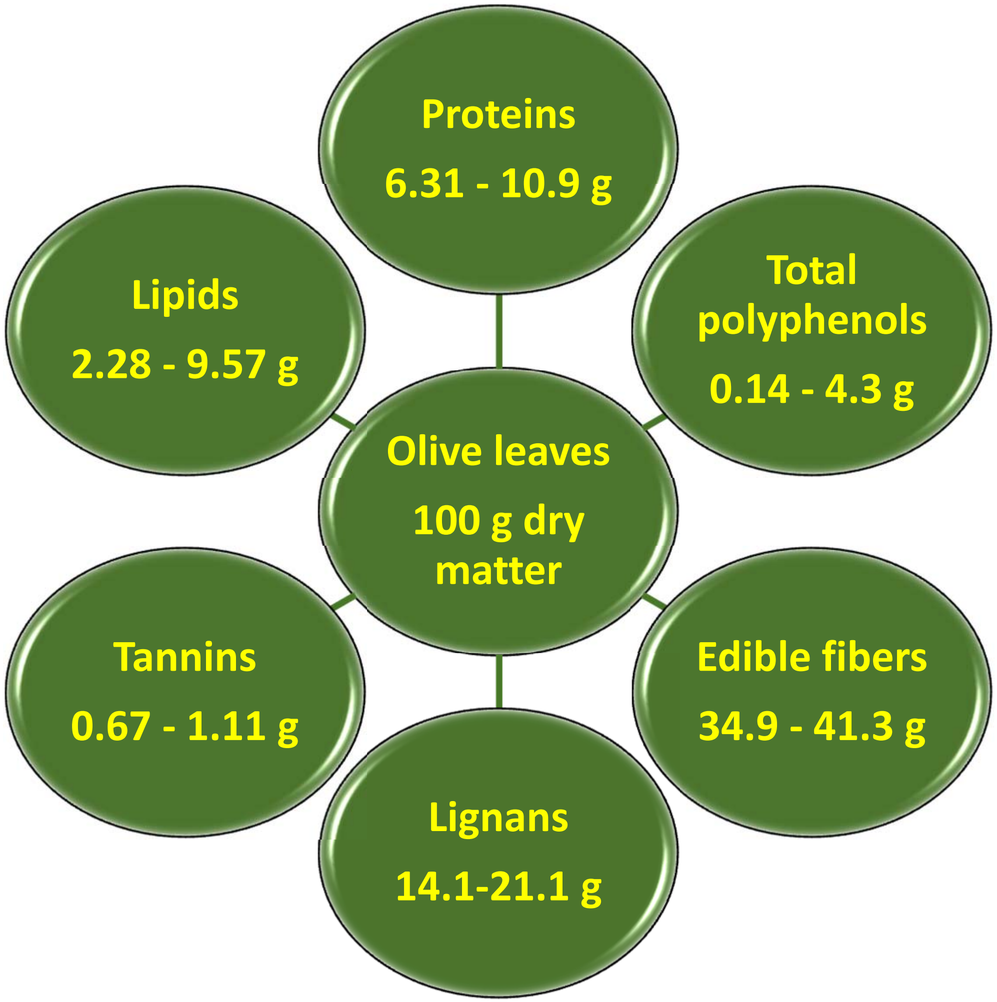
OPTIMIZATION OF A GREEN METHOD FOR THE RECOVERY OF HIGH-ADDED VALUE POLYPHENOLS FROM OLIVE LEAF USING CYCLODEXTRINS

- An alternative approach for extraction of olive leaf polyphenols

- The solvent consists of glycerin an aqueous solution of cyclodextrins

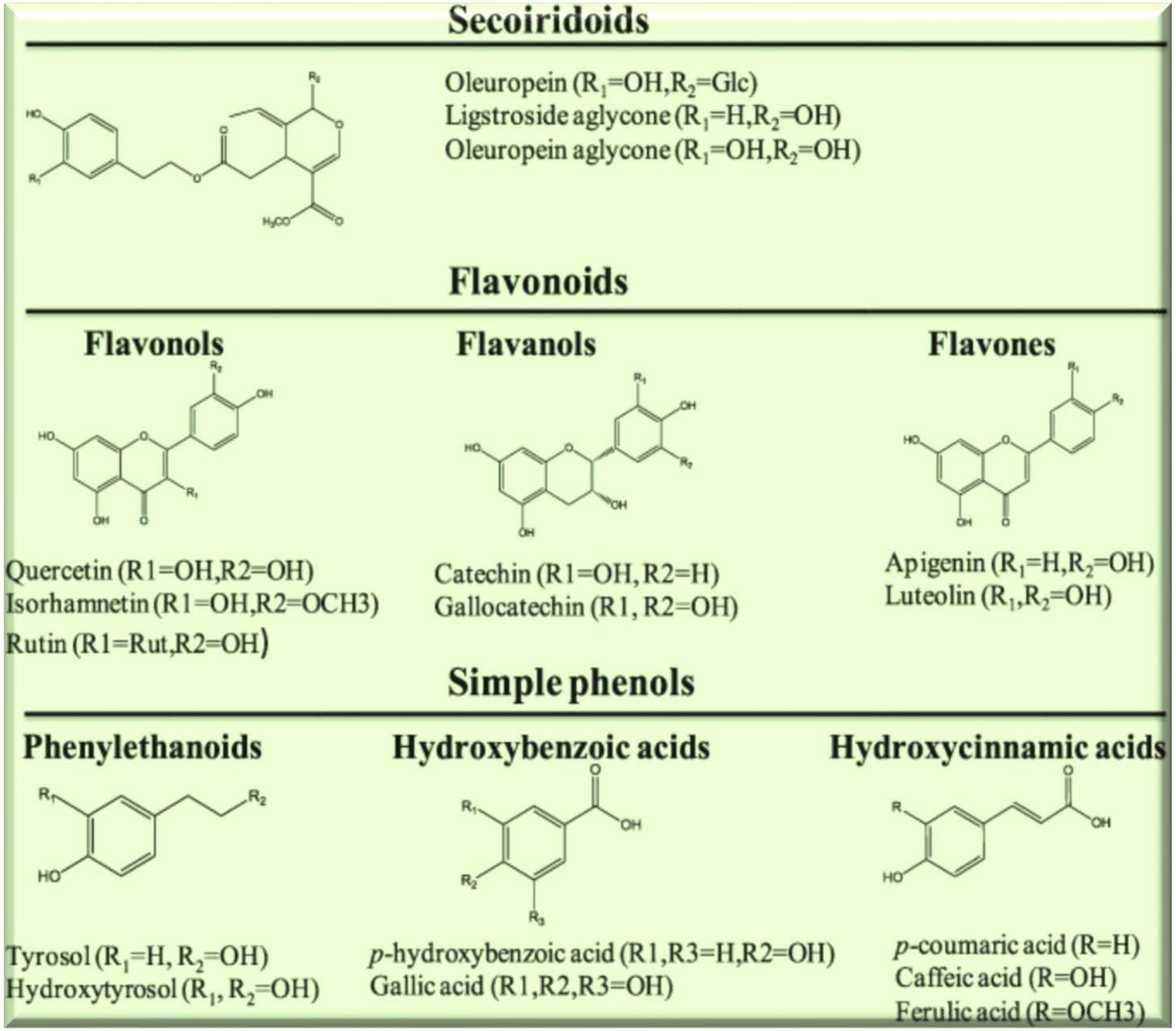
- Extracts could be used to fortify foods or as nutritional supplements

Olive leaves



Olive polyphenols

- ❖ ***Secoiridoids***
  - ❖ Oleuropein
  - ❖ Ligstroside
- ❖ **Flavonoids**
  - ❖ Apigenin
  - ❖ Luteolin
  - ❖ Kaempferol
- ❖ **Simple phenolics**
  - ❖ Tyrosol
  - ❖ Caffeic acid



Glycerol as co-solvent

Low cost, by-product of bio-diesel industry

Low dielectric constant

Ideal solvent for polyphenol extraction

3.6% glycerol (w/v)→ more efficient solvent than water

20% glycerol (w/v) → maximum efficiency of flavonoids extraction

Similar efficiency with water/ethanol mixtures

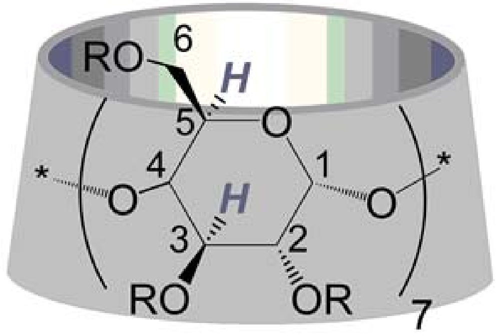
50% (v/v) ethanol, 50% (v/v) butanodiol και 70% (w/v) glycerol for the extraction of polyphenols

Cyclodextrins

Formation of inclusion complexes with polyphenols

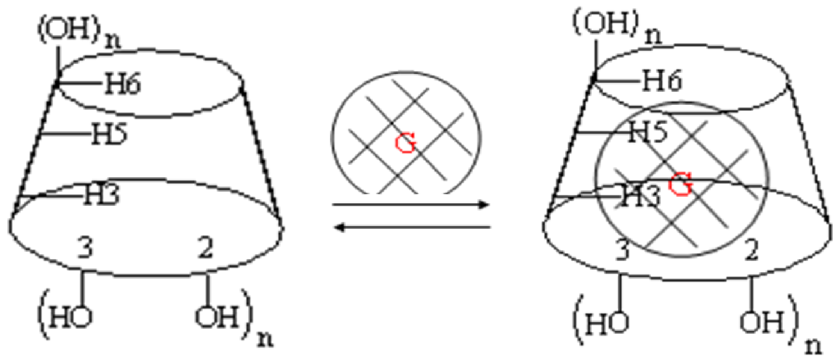
Aqueous solutions of cyclodextrins can be used as extraction solvents

Protection against oxidation and increased stability



R=H: β-CD

R=CH_3-CH(OH)-CH_2: HP-β-CD,
DS=0.5-1.3



Aim of the study

Optimization of an extraction process for efficient recovery of polyphenols from olive leaves, using 'green'' **water/glycerol/2-hydroxypropyl-β-cyclodextrin**

The optimization was based on a Box-Behnken experimental design

Independent variables

C_{CD}

T °C

C_{gl}

Responses measured

Y_{TP}

A_{AR}

Experimental values and coded levels of the independent variables used

Independent variables	Code units	Coded variable level		
		-1	**0**	**1**
C_{CD} (%,w/v)	X_1	1	7	13
C_{gl} (%, w/v)	X_2	0	30	60
T (°C)	X_3	40	60	80

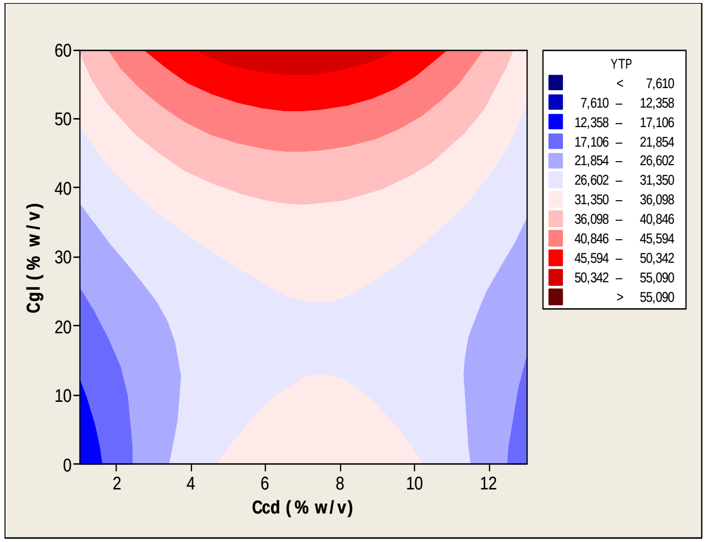
Polynomial equations and statistical parameters describing the effect of the independent variables considered on the responses (Y_{TP}) and (A_{AR})

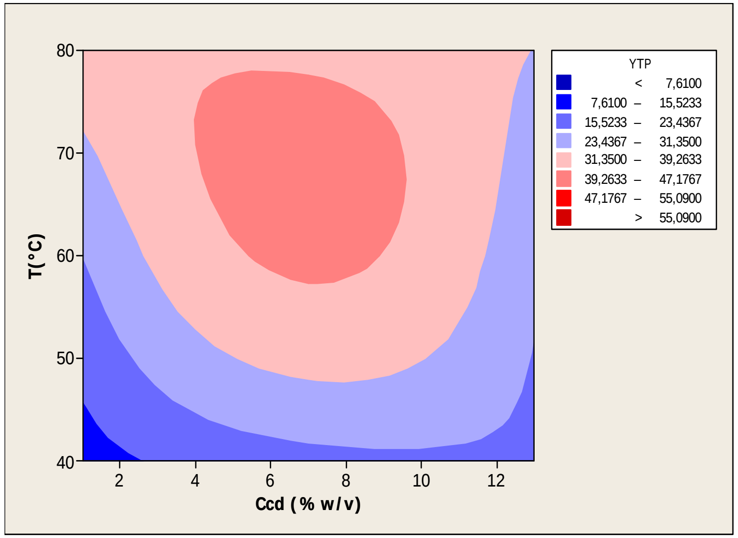
Response	Polynomial equation	R^2	*p*
Y_{TP}	$36.43 + 9.55X_2 + 8.47X_3 + 6.71X_2X_3 - 12.10X_1^2 + 8.35X_2^2 - 7.19X_3^2$	0.96	0.0012
A_{AR}	$276.38 + 14.29X_1 + 16.43X_3 + 23.02X_2X_3 - 65.20X_1^2 + 36.39X_2^2$	0.95	0.0033

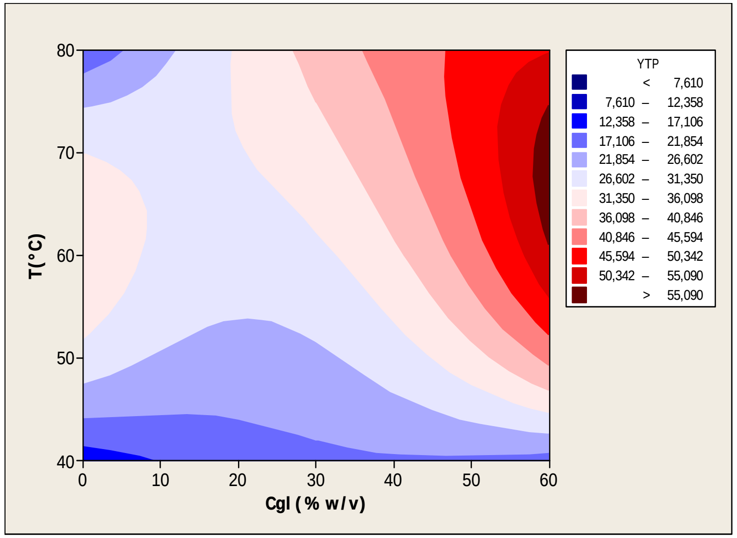
Measured and predicted values of Y_{TP} and A_{AR}, determined for individual design points, for the extractions performed with water/glycerol mixtures

Design point	Independent variables			Response (Y_{TP}, mg GAE g^{-1} dw)		Response (A_{AR}, µmolTR dw)	
	X_1	X_2	X_3	Measured	Predicted	Measured	Predict
1	-1	-1	-1	9.69	7.61	222.55	216.75
2	-1	-1	1	18.96	18.37	202.14	201.98
3	-1	1	-1	14.42	17.52	251.23	238.82
4	-1	1	1	56.78	55.10	311.49	316.14
5	1	-1	-1	19.51	20.74	235.67	231.96
6	1	-1	1	20.6	17.05	207	220.35
7	1	1	-1	22.05	22.19	276.5	277.60
8	1	1	1	43.69	45.317	351.35	358.09
9	-1	0	0	22.24	23.49	183.18	196.90
10	1	0	0	24.6	25.16	242.96	225.47
11	0	-1	0	30.24	35.23	276.5	272.82
12	0	1	0	57.51	54.32	352.81	352.72
13	0	0	-1	23.15	20.76	249.28	270.09
14	0	0	1	33.51	37.70	327.53	302.95
15	0	0	0	40.42	36.42	269.7	276.38
16	0	0	0	36.05	36.42	275.53	276.38

Contour plots illustrating the effect of the independent variables examined on the YTP





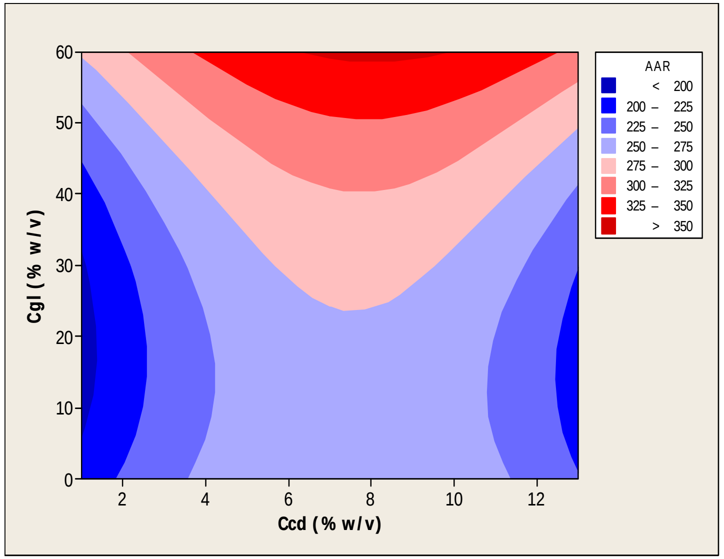


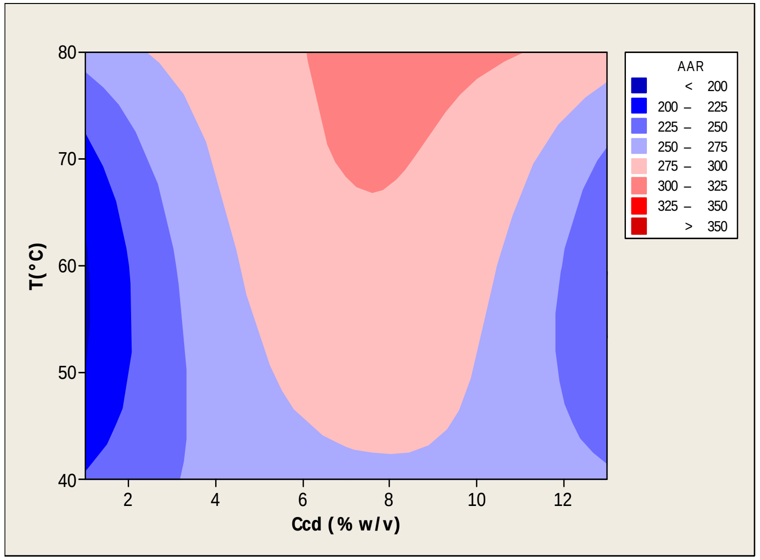
CCD = 7% w/w

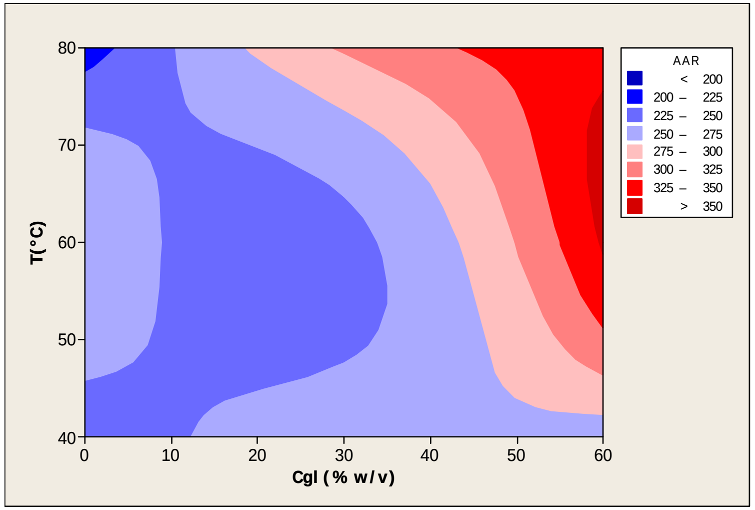
Cgl = 60% w/w

T = 70 °C

Contour plots illustrating the effect of the independent variables examined on the AAR





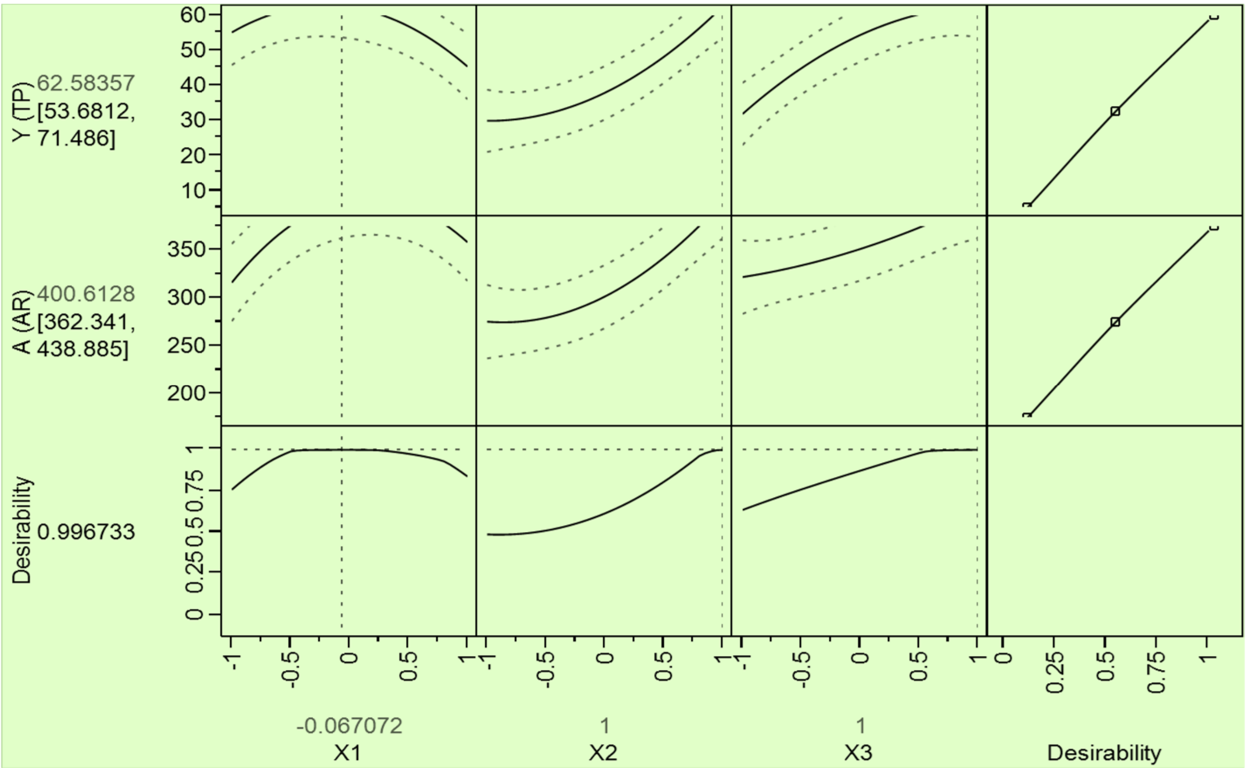


CCD = 7% w/w

Cgl = 60% w/w

T = 70 °C

Prediction profiler displaying the overall desirability of the model, following adjustment of the independent variables at their optimal values

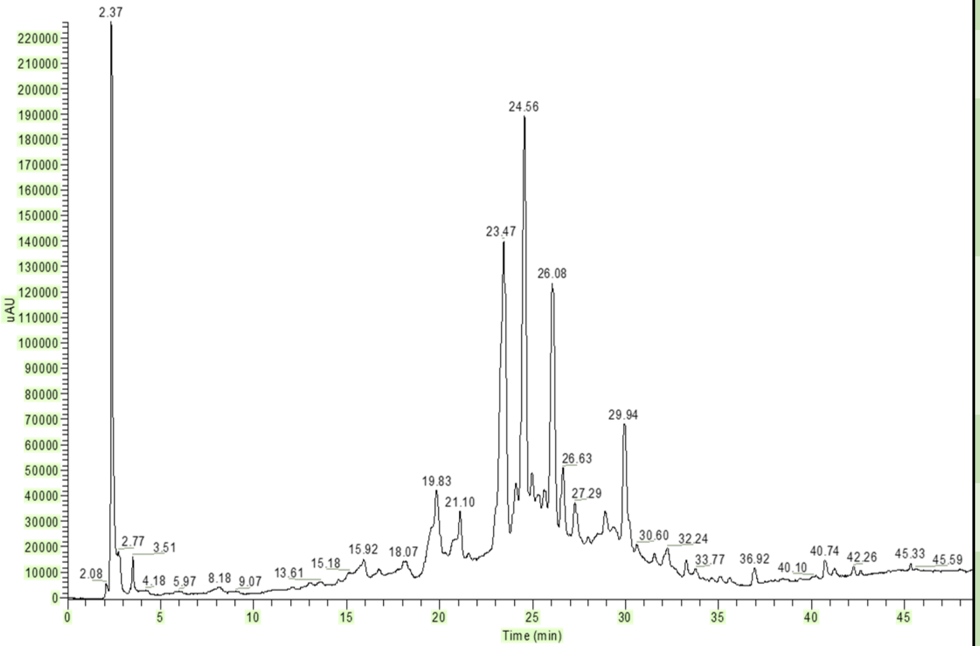


YTP = 54,33 mg GAE/g dw

&

AAR = 352,72 µmol TRE/g dw

LC – MS analysis



UV-Vis and mass spectral characteristics of the main phytochemicals detected in the optimally obtained olive leaf extract

Peak	Rt (min)	λ_{max} (nm)	$[M+H]^+$	Other ions (*m/z*)	Compound
1	20.15	244, 274, 336	611	287 [M – 2 glucosylunits + H] $^+$	Luteolin di glucoside
2	23.75	252, 264, 348	449	287 [M – glucosyl unit + H]$^+$	Luteolin glucoside
3	24.38	254, 356	611	303 [M – rutinosyl unit + H] $^+$	Rutin (quercetin 3-*O*-rutinoside)
4	24.74	248, 280	541	563 [M + Na]$^+$, 361 [M – glucosyl unit + H]$^+$, 137 [hydroxytyrosyl unit]$^+$	Oleuropein isomer
5	25.23	252, 350	579	433 [M – rhamnosyl unit + H]$^+$, 271[M – rutinosyl unit + H]$^+$	Apigenin rutinoside
6	25.64	252, 350	433	271	Apigenin rhamnoside
7	26.36	268, 344	449	287 [M – glucosyl unit + H]$^+$	Luteolin glucoside
8	26.87	248, 280	541	563 [M + Na]$^+$, 361 [M – glucosyl unit + H]$^+$, 137[hydroxytyrosyl unit]$^+$	Oleuropein
9	27.48	268, 344	449	287 [M – glucosyl unit + H]$^+$	Luteolin glucoside
10	30.46	252, 264, 352	625	287	Luteolin derivative
11	33.60	254, 264, 352	617	287	Luteolin derivative

Conclusions

- Development of a novel approach for more efficient extraction of polyphenols from olive leaves leading to eco-friendly extracts and processes.

- Green-extraction techniques minimize the use of petrochemicals.

- Liquid extracts of plant polyphenols could become attractive and safe vehicles of these compounds to fortify food products or used as nutritional supplements to enhance the antioxidant and antimicrobial potency of a daily diet.

- Extracts should be also tested for their stability upon storage to maximize their effectiveness in a real food matrix.

Thank you for your attention !