Factorial design of phenolic extraction process from two phase olive mill waste

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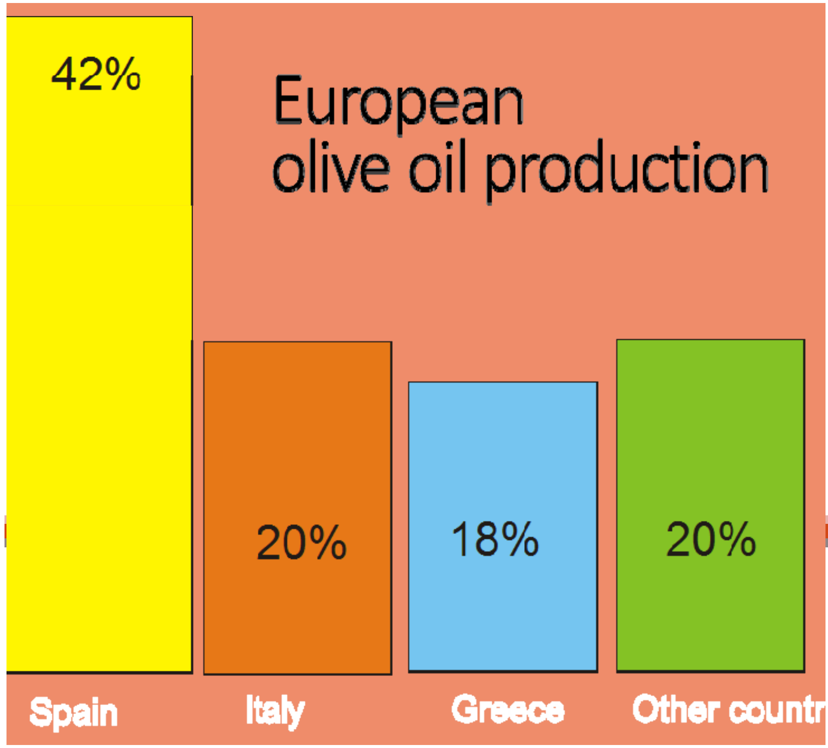
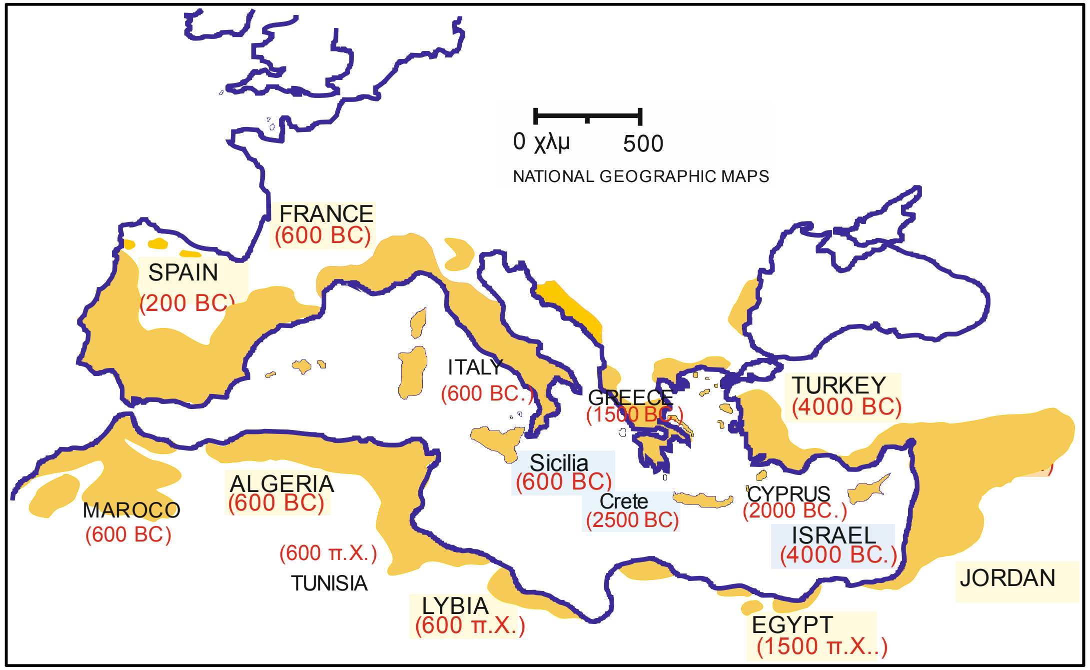
Scope of the study

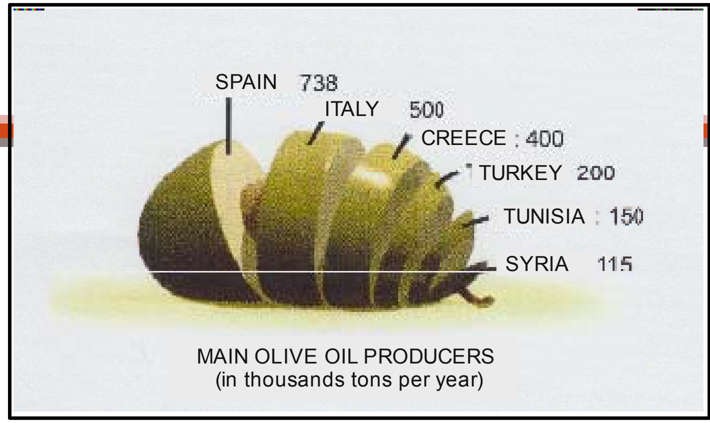
- Valorisation of the OMW from II-phase olive mills
  - Recovery of high added value compounds
    - Residual Oil
    - Phenolic compounds
- Decrease the environmental impact of this primary industrial field in Greece and in Mediterranean countries
- Necessary pretreatment steps to diminish inhibition phenomena to the biological processes that follow
  - Anaerobic digestion and/or composting
- Increase their sustainability
  - Developing novel processes leading to a range of added value products
  - Under the concept of zero waste biorefineries

INTRODUCTION

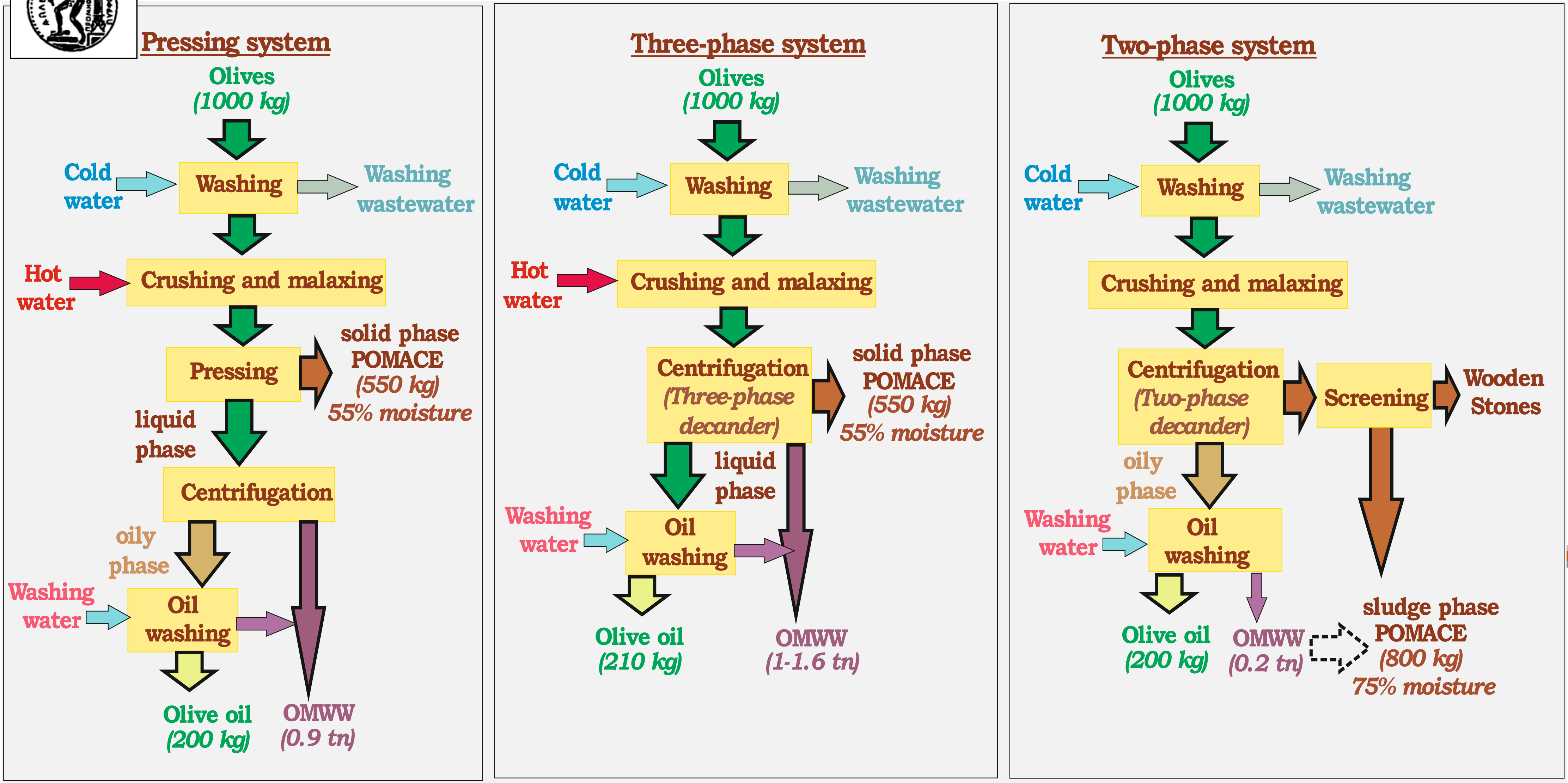
More than 95% of the global production derives from the Mediterranean area







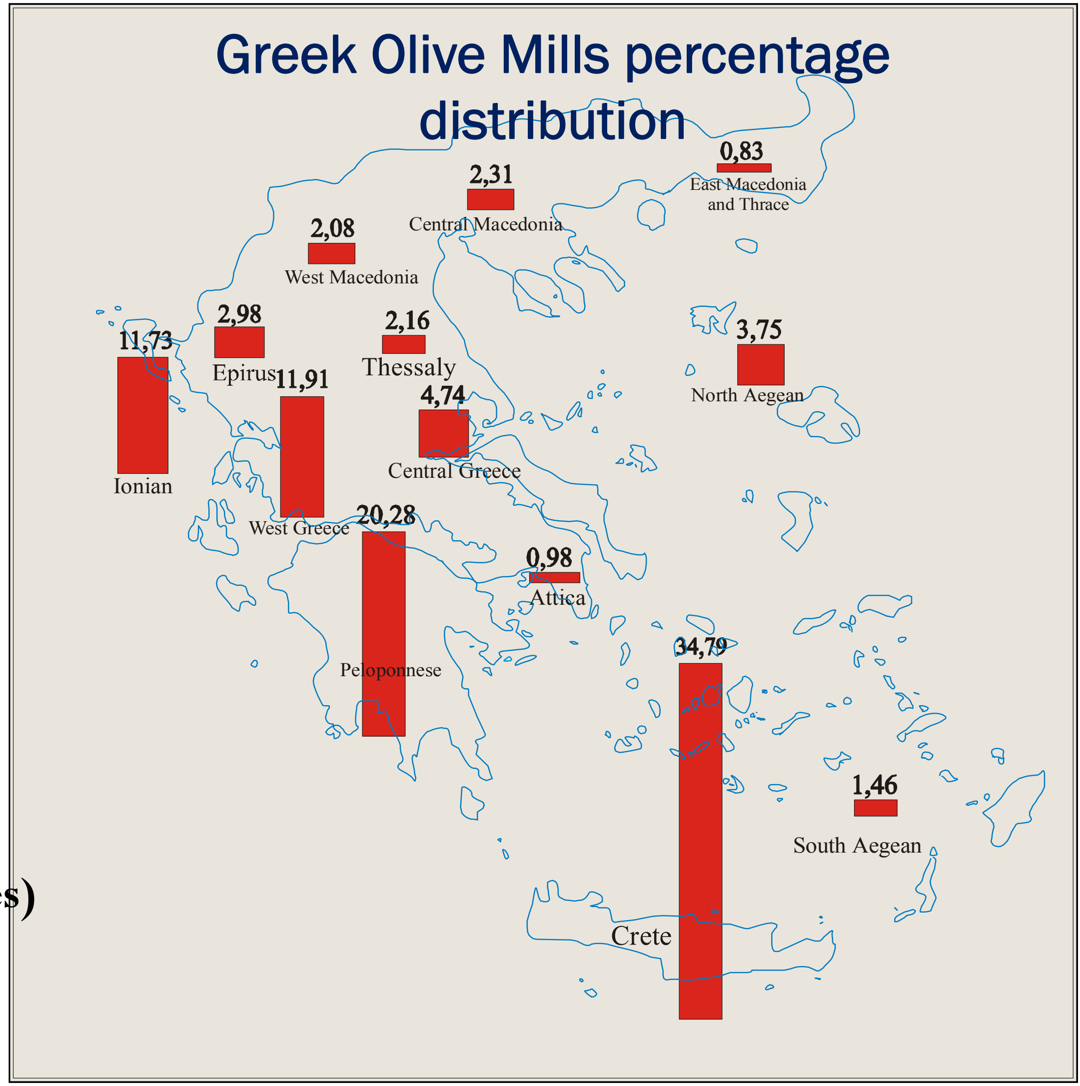
SYSTEMS FOR OLIVE OIL EXTRACTION



The Greek situation regarding the olive oil and OMW

- In Greece there are 150 M olive trees cultivated in ~765.000 hectares
- The annual production of each tree rises up to 300 kg of olives
- The 1/3 of Greek farmers are working on cultivation of olives
- Olives and olive oil production in Greece rise up 1.750.000 t and 400.000 t, respectively

- **There are around 2500 olive oil mills in Greece**
    - **2100 centrifugal systems (most of them III-phases)**
    - **Replaced from II-phases**
- **There are 20 pomace processing plants**



Composition of II-phase OMW

- Moisture: 67.5%
- COD: 30 kg/m^3
- Oil content: 10% (db)
- TPC: 30.5 mg/g db

Experimental Methodology for extracting the phenolic compounds

- Acid hydrolysis process
- Lab scale experiments with initial amount of OMW 100 g (67.5% of moisture)
- Design of a 2^4 factorial experiment in order to measure the effect of four important process parameters
  - Dilution of OMW (X1)
  - Quantity of a strong acid (X2)
  - Hydrolysis Time (X3)
  - Temperature (X4)

Level	Dilution (v/w)	H_2SO_4 % (v/w)	Time (min)	Temperature (°C)
1	3	3	60	70
0	2	2	45	60
-1	1	1	30	50

At the end of each run the aqueous phase was separated and analyzed

- TPC (Folin method)
- Phenolic compounds (HPLC)

Hydroxytyrosol - Tyrosol - Oleuropein

Experimental Results

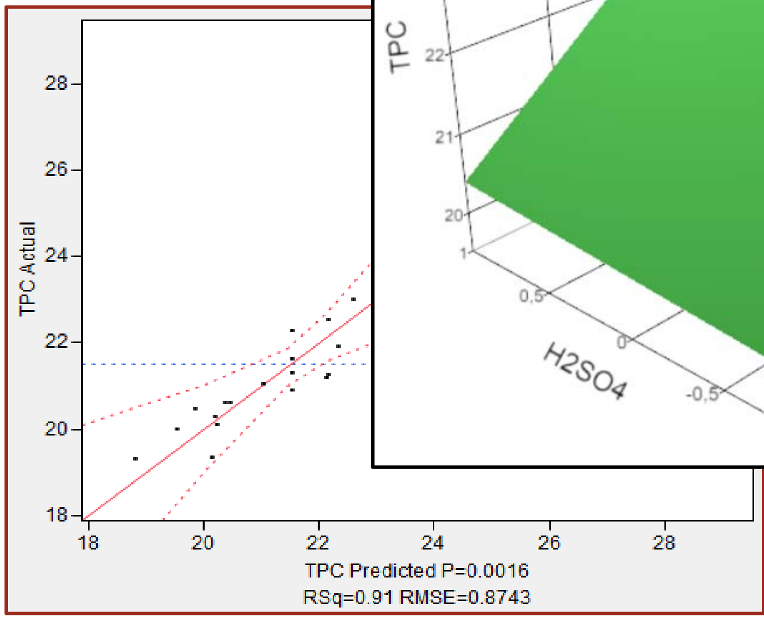
Run	X1 Dilution (mL)	X2 Strong Acid (mL)	X3 Time (min)	X4 Temperature (oC)	HYDROXY-TYROSOL mg/g Dry	TYROSOL mg/g Dry	OLEUROPEIN mg/g Dry	TPC (mg/g)
1	100 (-1)	1 (-1)	30 (-1)	50 (-1)	5.12	3.61	1.24	19.33
2	100 (-1)	1 (-1)	30 (-1)	70 (+1)	5.93	3.94	1.27	20.61
3	100 (-1)	1 (-1)	60 (+1)	50 (-1)	5.82	3.76	1.36	19.97
4	100 (-1)	1 (-1)	60 (+1)	70 (+1)	6.01	3.56	1.15	20.59
5	100 (-1)	3 (+1)	30 (-1)	50 (-1)	6.34	3.29	1.16	19.31
6	100 (-1)	3 (+1)	30 (-1)	70 (+1)	6.26	3.74	1.33	20.29
7	100 (-1)	3 (+1)	60 (+1)	50 (-1)	6.74	3.81	1.42	20.10
8	100 (-1)	3 (+1)	60 (+1)	70 (+1)	6.83	3.99	1.33	21.90
9	300 (+1)	1 (-1)	30 (-1)	50 (-1)	5.40	3.92	1.38	21.02
10	300 (+1)	1 (-1)	30 (-1)	70 (+1)	5.14	3.86	1.37	20.46
11	300 (+1)	1 (-1)	60 (+1)	50 (-1)	5.24	4.30	1.27	22.99
12	300 (+1)	1 (-1)	60 (+1)	70 (+1)	6.01	3.95	1.22	21.17
13	300 (+1)	3 (+1)	30 (-1)	50 (-1)	6.27	3.61	1.41	22.52
14	300 (+1)	3 (+1)	30 (-1)	70 (+1)	6.25	3.47	1.34	21.25
15	300 (+1)	3 (+1)	60 (+1)	50 (-1)	6.64	4.85	1.64	25.05
16	300 (+1)	3 (+1)	60 (+1)	70 (+1)	6.41	5.14	1.73	27.73
17	200 (0)	2 (0)	45 (0)	60 (0)	6.04	3.89	1.36	21.28
18	200 (0)	2 (0)	45 (0)	60 (0)	6.08	3.76	1.41	22.27
19	200 (0)	2 (0)	45 (0)	60 (0)	6.00	3.92	1.37	20.90
20	200 (0)	2 (0)	45 (0)	60 (0)	6.07	3.93	1.30	21.62

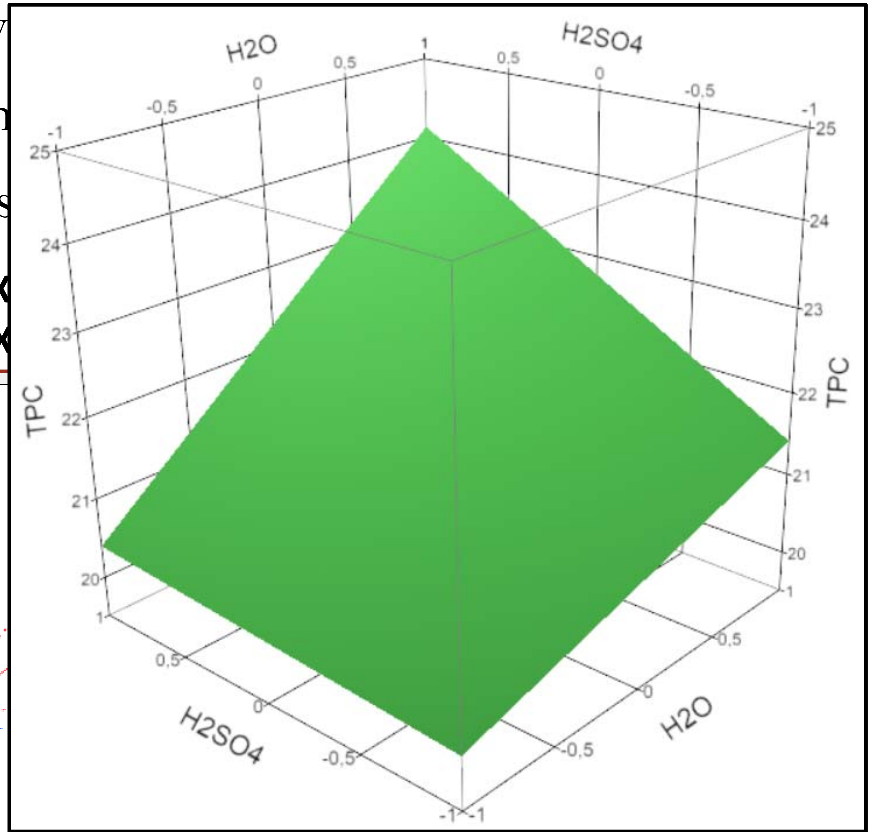
Modelling Results from the Factorial Design

- Regarding the TPC, the significance of the model w
- R^2 between the experimental and model prediction
- Important parameters are the dilution, the hydrolys

Parameter	Estimate	t Ratio
β0	21.52	
β1	1.255	5.74
β2	0.75	3.43
β3	0.91875	4.2
β4	0.23125	1.06
β5	0.6125	2.8
β6	0.54125	2.48
β7	0.50625	2.32
β8	-0.35375	-1.62
β9	0,.9125	1.33
β10	0.1775	0.81

Y_{TPC} = β0 + β1 X1 + β2 X
X3 + β8 X1 X4 + β9 X2 X





Central composite design for the most important parameters

•Finally, a central composite design was implemented 3^2 examining the most important parameters of the factorial design

- Dilution of OMW (X1)
- Addition of strong acid (X2)

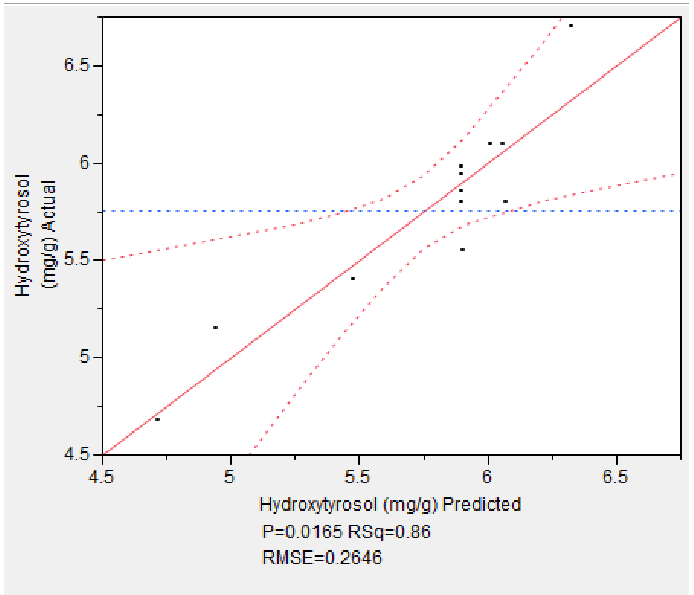
• Hydrolysis time (X3) was decided to be examined separately as a kinetic study on the optimum conditions.

• Both hydrolysis time (X3) and temperature (X4) was set at level +1 of the FD experiment

Level	H2O (mL)	H2SO4 (mL)
1.414	350	3.5
1	335.36	3.35
0	300	3
-1	264.64	2.65
- 1.414	250	2.5

Experimental and modelling results of the CCD

H2O (mL) X1	H2SO4 (mL) X2	TPC (mg/g)	OLEUROPEIN (mg/g)	HYDROXYTYROSOL (mg/g)	TYROSOL (mg/g)
1	1	15.8	1.12	5.55	3.67
1	-1	21.2	1.46	4.68	4.53
-1	1	22.3	1.27	5.8	4.52
-1	-1	17.81	1.04	6.1	2.99
1.4142	0	19	1.25	5.15	3.58
-1.4142	0	21.1	1.37	6.1	3.94
0	1.4142	24.3	1.64	6.7	4.71
0	-1.4142	23.33	1.46	5.4	4.19
0	0	21.22	1.22	5.98	3.51
0	0	20.74	1.24	5.86	3.58



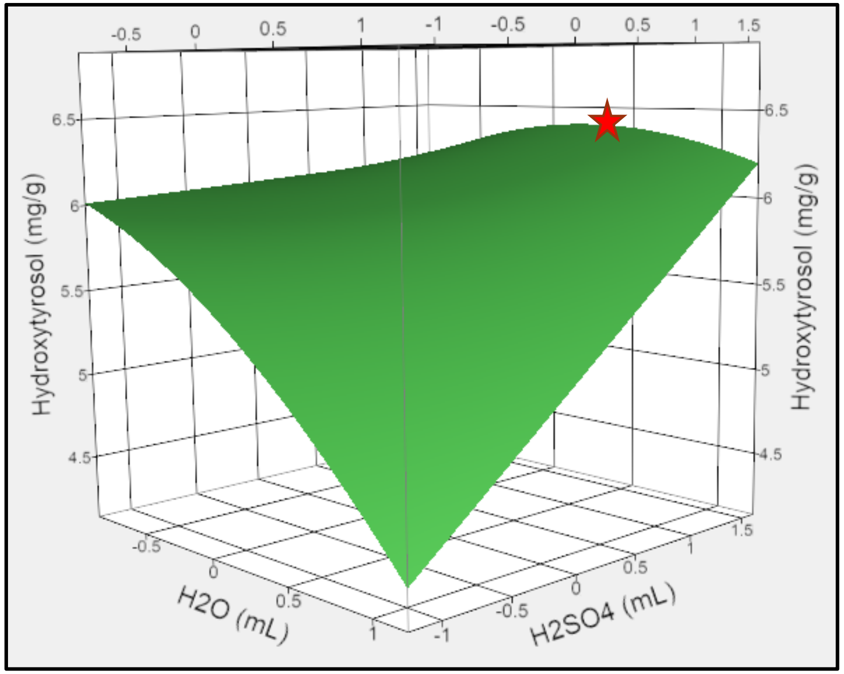
Experimental and Model predictions for the hydroxytyrosol (mg/g)

Best model predictions are acquired for hydroxytyrosol output (R^2=0.86)

$$Y_{hydroxytyrosol} = \beta 0 + \beta 1\ X1 + \beta 2\ X2 + \beta 3\ X1\ X2 + \beta 4\ X1\ X1 + \beta 5\ X2\ X2$$

Parameter	Estimate	t Ratio
β0	5.895	
β1	-0.3767	-4.03
β2	0.3011	3.22
β3	0.2925	2.21
β4	-0.2113	-2.02
β5	0.00125	0.01

Optimum extraction conditions and validation on the optimum value



<u>Optimum conditions</u>

For X1 was 0.104 (304 mL) and

For X2 was 1.414 (3.5 mL)

$Y_{hydroxytyrosol} = 6.4$ mg/g

HYDROXYTYROSOL mg/g Dry	TYROSOL mg/g Dry	OLEUROPEIN mg/g Dry	TPC (mg/g)
6.51	4.93	1.40	24.82

Recovery of phenolic compounds from treated OMW

Liquid aqueous phase from centrifugation

4-6 g/L of phenolic compounds
3-4% of sugars

Micro & ultrafiltration

Ion-Exchange Resins

~ 80 g/L of phenolic compounds

Evaporation

High Concentrated Extraction rich in Phenolic Compounds

Conclusions and Future Actions

- We illustrated an extraction process of the phenolic compounds from II-phase OMW using acid hydrolysis
- The Factorial Experiment together with the Central composite design gave us an overall extraction of Hydroxytyrosol 6.5 mg/g of dry OMW and a TPC of 24.8 mg/g of dry OMW
- Up to 80% of the initial TPC can be recovered in the aqueous phase
  - Concentration of Tyrosol reached ~5 mg/g of dry OMW
- Next set of experiments will be focused on the recovery of an extract rich in phenolic compounds.

Thank you for your attention

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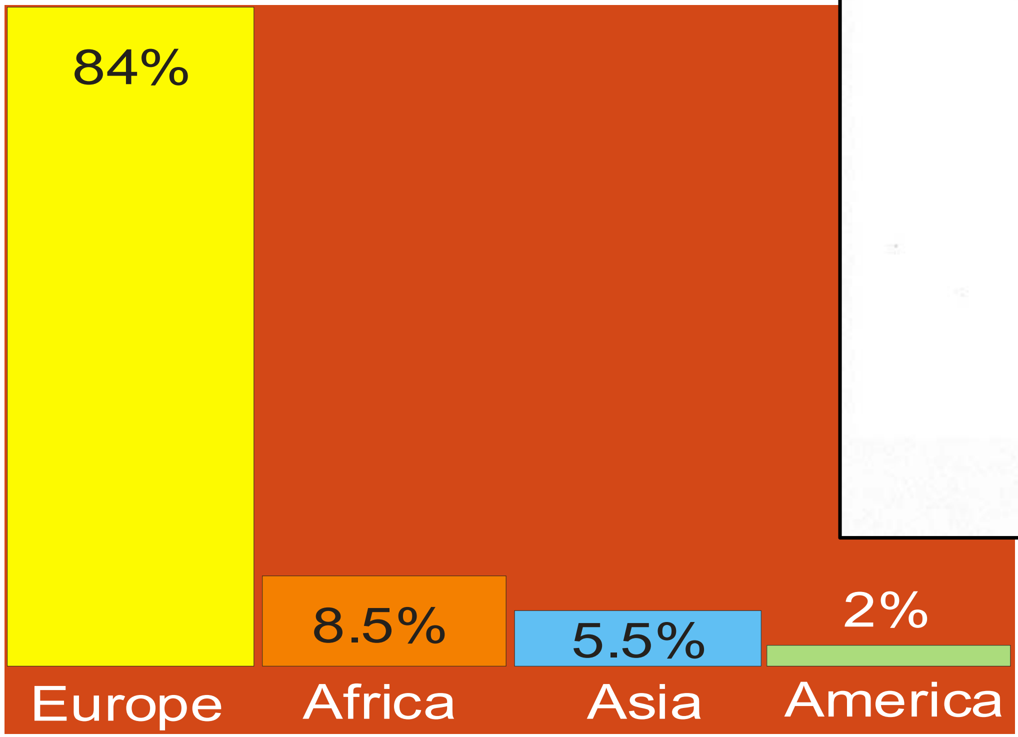
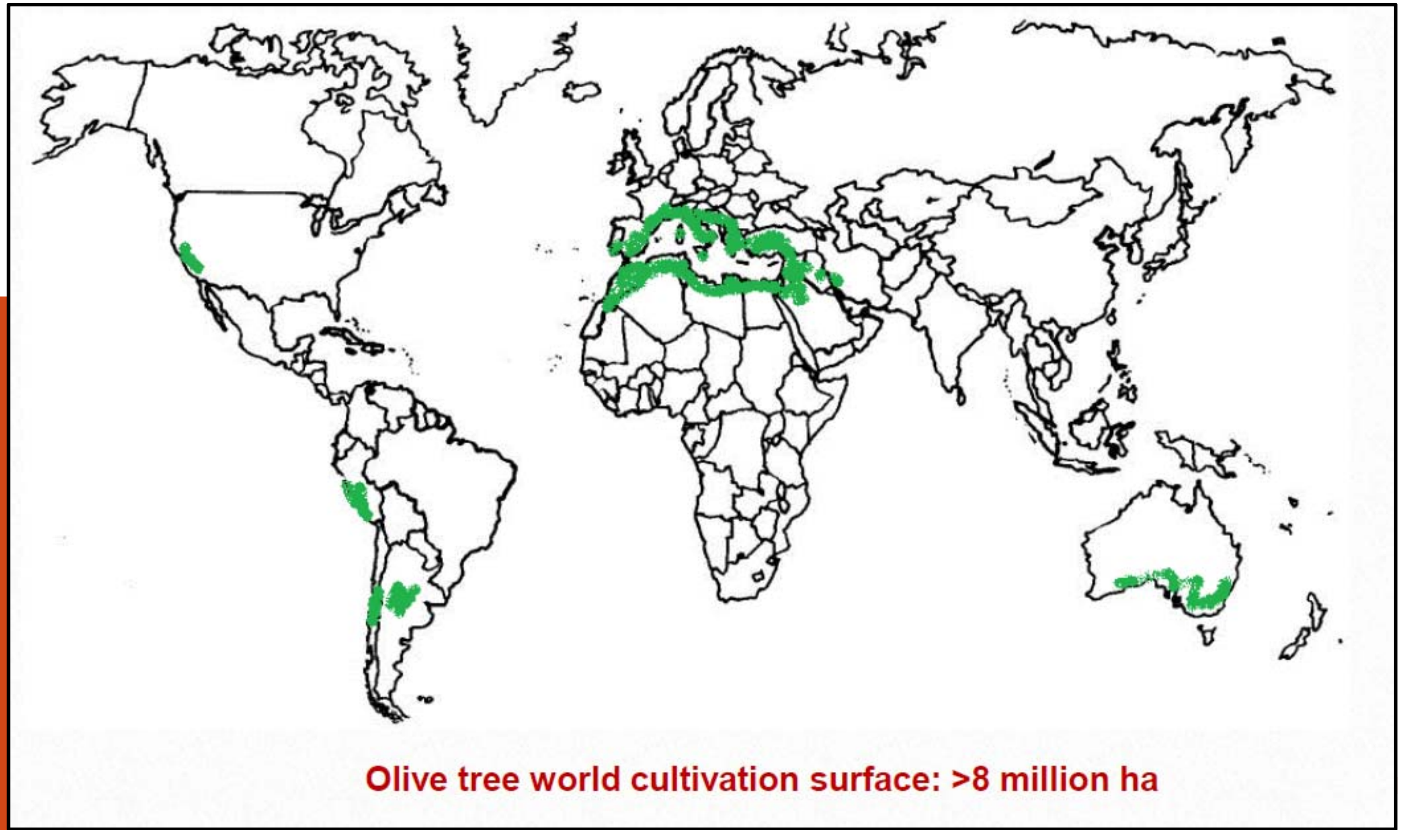
ORGANOHUMIKI THRAKIS

Outline

- Scope of the study
  - Valorization of the OMW from II-phase olive mills by recovering high added value phenolic compounds such as hydroxytyrosol, tyrosol and oleuropein
- Introduction
  - The Olive oil production and the current situation in Greece
- Experimental Methodology
  - Implementing an acid hydrolysis process
  - Optimisation of the recovery of the phenolic compounds
    - Factorial Design
    - Response surface methodology
- Conclusions and future recommendations

INTRODUCTION

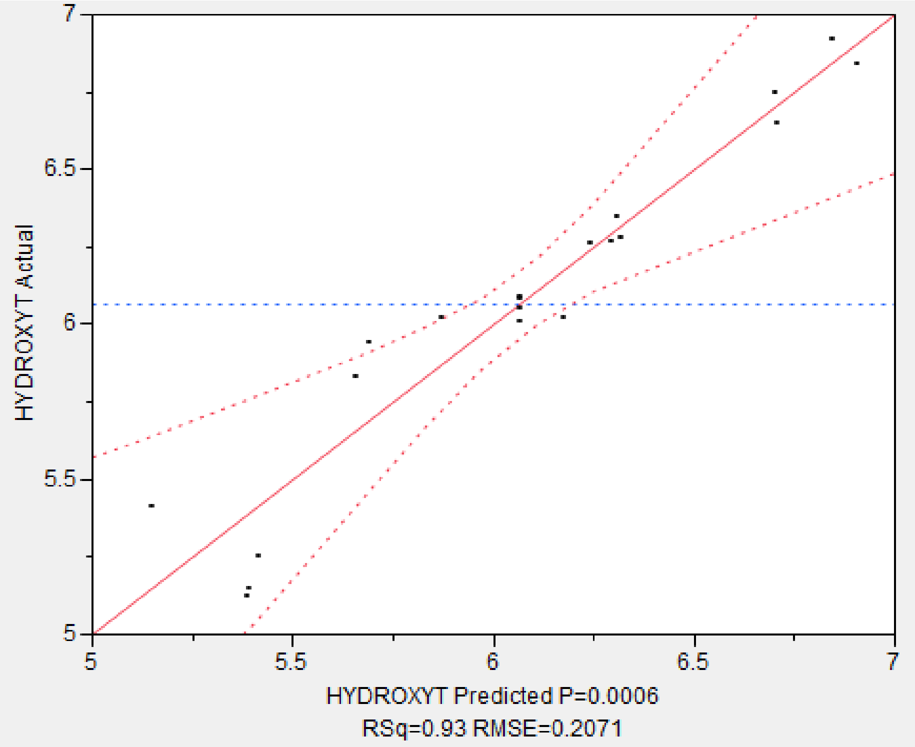




Modelling results (cont')

Ως προς την υδροξυτυροσόλη, το μοντέλο είναι σημαντικό σύμφωνα με το κριτήριο Fisher και το R^2 μεταξύ των πειραματικών και των προβλεπόμενων τιμών είναι 0.93 (βλ. σχήμα 2). Οι τιμές των παραμέτρων παρουσιάζονται στον πίνακα 4 και σημαντικές είναι το θειϊκό οξύ και ο χρόνος.

Parameter	Estimate	t Ratio
β0	6.0645	
β1	-0.07375	-1.42
β2	0.47375	9.15
β3	0.2187	4.22
β4	0.11125	2.15
β5	0.06125	1.18
β6	-0.00125	-0.02
β7	0.03125	0.6
β8	-0.01625	-0.31
β9	-0.07875	-1.52
β10	0.05375	1.04



Σχήμα 2 Πειραματικές και προβλεπόμενες τιμές ως προς την υδροξυτυροσόλη (mg/g)
Πίνακας 4 Οι εκτιμήσεις των παραμέτρων του μοντέλου ως προς την υδροξυτυροσόλη