A comparative study on different extraction techniques to recover polyphenols from winery waste

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Red winemaking process

Destemming

Crushing

Pressing

Completion of fermentation

Sedimentation decanting

Wastes

Wastes

Milogalactic fermentation

Sedimentation decanting

Maturation and natural clarification

Finishing and stabilization

Tournour et al., 2015

Winemaking wastes

- **Winemaking wastes: steams, seeds, peels, marcs**

6 L wine ⟶ 1 kg of grape pomace

-anthocyanins
-flavonols
-flavanols
-phenolic acid
-resveratrol

-**Increase COD, BOD_5**

+ Natural antioxidants
+ Functional food ingredients
+ Healthy benefits: scavening activity agains free radical

Compounds	mg/kg
Oenin	291-445
Catechin	56.2-74.3
Epicatechin	22.6-34.5
Quercetin	2.88-3.6
Rutin	2.2-3.5
Kaempferol	1.8-2.2
Myricetin	0.3-0.5
Total phenols	**791-1127**

Tournour et al., 2015; Rusjan et al., 2007

Composition of winery waste

Fraction / Compound	Percentage in grape (% weight) for fractions / Percentage within the fraction for compounds
Grape Pomace	**13-20%**
Moisture	50-72%
Sugars	Up to 150 g/kg
Fibers	30-40 %
Protein	4%
Tartrate	50-75 g/t
Total flavonol content	29-199 mg/100g dry weight
Grape Seeds	**3-6%**
Essential oil	12-17%
Fibers	40%
Protein	11%
Phenolics	4-7%
Grape Stems	**1.4-7%**
Moisture	55-80%
Phenolics	5.8%
Grape Skins	**65%**

Gonzales-Paramas et al., 2004; Nerantzis et al., 2006

Handling of grape pomace

Current status

Ways to process grape pomace:

- production of alcoholic beverages
- fertilizer
- animal feed
- fuel
- recovery of organic & fatty acids (tartaric acid, malic acid, citric acid)
- production of oil (grape seed oil)
- applications in pharmacological and cosmetic industries

Nerantzis et al., 2006; Lora et al., 2015

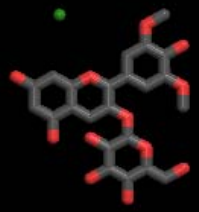
Handling of grape pomace

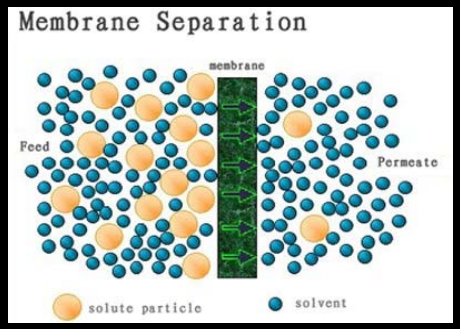
New trend - Valorization of phenolics

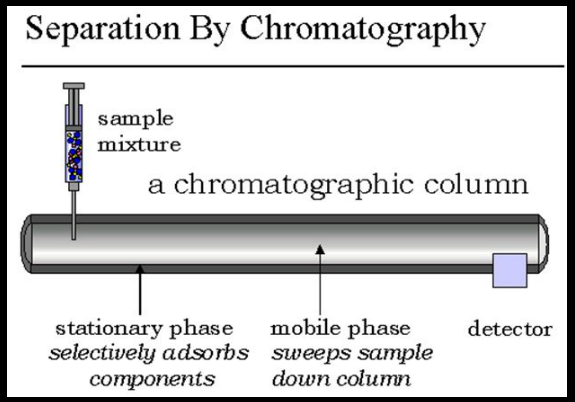
1. • Membrane separation
2. • Chromatographic separation
3. • Adsorption
4. • **Extraction**

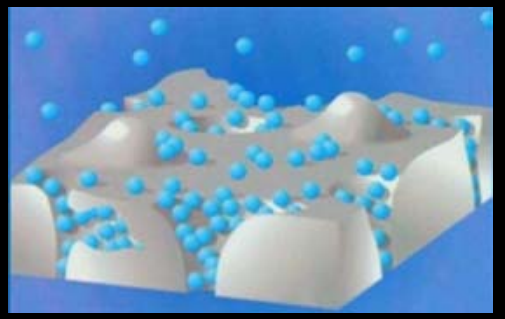
Goula et al., 2015

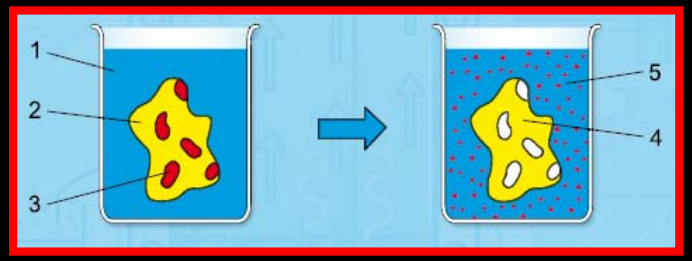
Phenolics







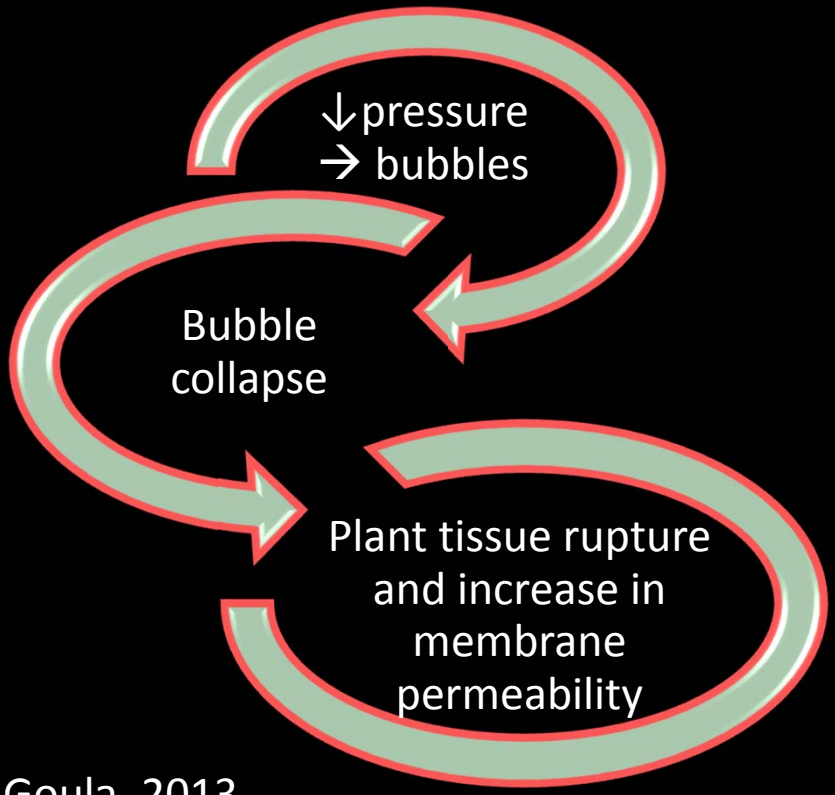




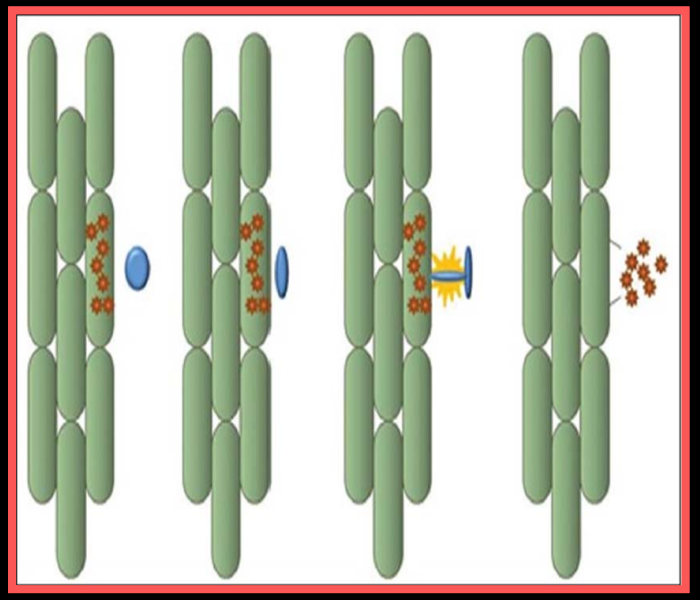
Methods for extraction of phenolics from grape pomace

Extraction method	Yield (maximum)	Reference
Stirring	170.9±10.7 ppm	Pinelo et al.,2007
Conventional Soxhlet	13.8% w/w GAE	Loui et al., 2004
High pressure extraction	126±9 mg GAE/g	Rodríguez et al., 2007
Supercritical fluid extraction	31.69 mg GAE/g DP	Aliakbarian et al.,2012
Ultrasound-assisted extraction	9.57 mg GAE/g	Thymiatis et al., 2015
Microwave-assisted extraction	3.68 g/100g	Brachim et al., 2013
Enzyme-assisted extraction (protease)	12.8 mg GAE/g	Rodríguez-Morgado et al., 2015

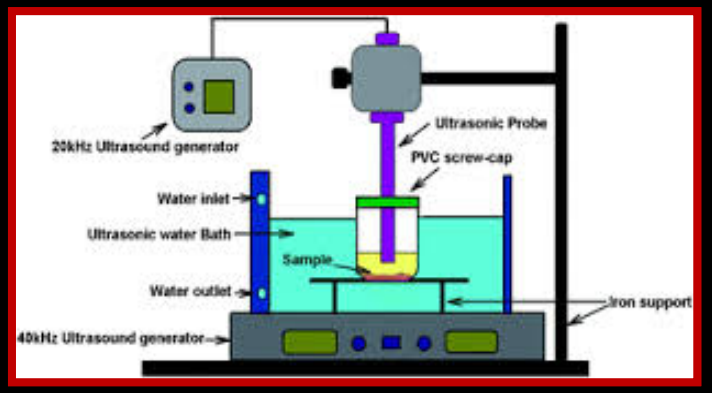
Ultrasound-assisted extraction (UAE)



Goula, 2013



Chemat et al., 2011

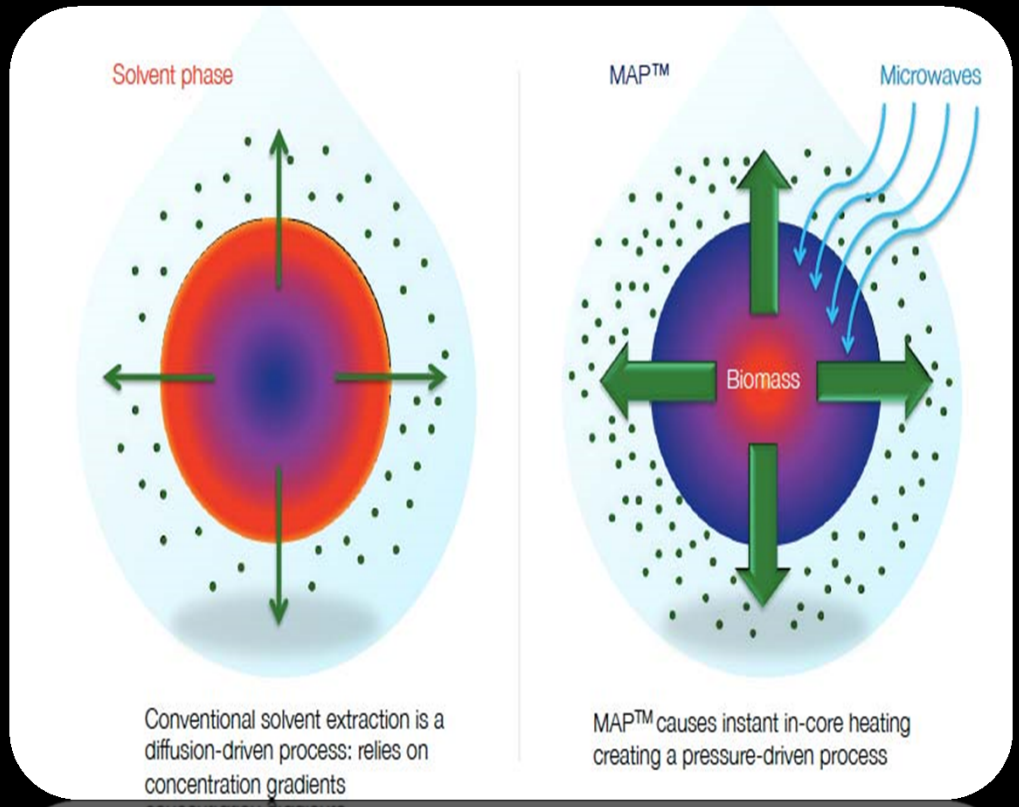


- ✓ Inexpensive-low instrumental requirements
- ✓ Simple and efficient alternative extraction technique

Ghafoor et al., 2009

Microwave-assisted extraction (MAE)

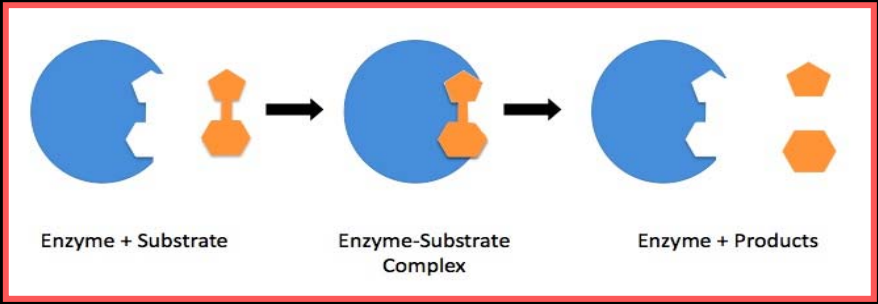
1. Speed
2. Versatility
3. Selectivity
4. Low energy expenditure
5. Minimal solvent consumption
6. Reduced by-product formation

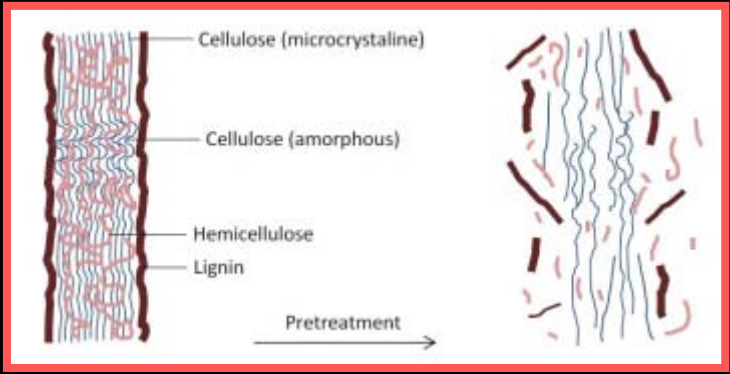


Dai et al., 2001

Enzymatic pre-treatment and extraction

- Ability to *break biological barriers* such as cell walls and membranes; thus, substrates become porous for easy release of bioactive compounds
- Requires *low or moderate temperature* range, which does not damage heat-labile compounds
- The nontoxic nature of enzymes (being protein), which unlike chemical treatment, *does not have residual effects*





Hammed et al., 2013

Trade name of enzyme preparation	Enzyme type
Viscozyme L	β-Glucanase (endo-1,3(4)-)
Porcine liver esterase	Carboxylic esterase
Pectinex 3XL	Polygalacturonase
Ultraflo L	Heat-stable multiactive endo-1,4-β-glucanase endo-1,4-β-xylanase
Flavourzyme 500L	Endo and exo-peptidase
Celluclast 1.5L	Cellulase
Amyloglucosidase (AMG) 300 L	An exo 1,4-α-D-glucosidase
Termamyl 120L	Heat stable α-amylase
Protamex	Hydrolysis of food proteins
Kojizyme 500 MG	To boost soy sauce
Alcalase	Endo peptidase
Neutrase	Protease
Maltogenase	Maltogenic amylase
Pancreatic trypsin	A serine protease
Dextrozyme	Glucoamylase
Promozyme 400L	Pullulanase
Depol 761P	Xylanase
Cellubrix	Mixture of cellulose and β-glucosidase

Published research work on enzyme-aided extraction of bioactive compounds from plants

Sources	Enzymes	Products/biological activities
Black currant pomace	Cellulase 13L (C013L) from *Trichoderma reesei*	Antioxidant phenol
Black currant juice press residues (*Ribes nigrum*)	Grindamyl CA pectinase, pectinases Macer8 FJ and Macer8 R, Novozym 89 acid protease, and pectolytic enzyme Pectinex BE	Antioxidative phenols
Black currants and bilberries	Econase CE, Biopectinase CCM, Pectinex Smash XXL, and Pectinex BE-3L	Flavonols
Blueberry processing waste	9 enzyme with different combinations of pectinase, hemicellulose, cellobiase, and cellulose	Anthocyanins and polyphenolics
Citrus peel	Cellulase MX, Cellulase CL, and Kleerase AFP	Phenolics
Grape pomace	Cellubrix, Neutrase, and Viscozyme	Polyphenols
Apple juice	Pectinex Yield Mash, Pectinex Smash XXL, Pectinex XXL, Pectinex Ultra-SPL, Pectinex AFP L-4, Panzym XXL, and Panzym Yield Mash	Antioxidant phenolic
Apple skin	Pectinex Smash, Novozymes, Celluclast 1.5L, Enzeco, and Sumizyme AP	Phenols
Apple peel	*Thermobifida fusca* cellulase	Antioxidant phenolics

Hammed et al., 2013

Objective

The aim of the present research is to compare the present and traditional extraction techniques to propose an optimum method for isolation of priced compounds from grape pomace

❖ **Comparison of new methods for extraction of phenolic compounds from grape pomace:**

- Microwave-assisted extraction
- Ultrasound-assisted extraction

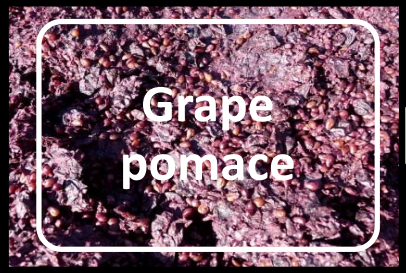
❖ **Study of:**

➡ **effect of grape pomace moisture content on extraction yield**

➡ **enhancement of the optimum extraction treatment by enzymatic pre-treatment**

Materials and methods

Extraction process



Drying

Grinding

Extraction / extraction with enzyme pretreatment

Ultrasound

Microwave

Filtration

Folin Ciocalteu

Determination of phenolic compounds

Factors Affecting the Ultrasound-Assisted Extraction Process

1. Extraction temperature
2. Solvent type
3. Liquid /Solid ratio
4. Amplitude level
5. Pulse duration/Pulse interval ratio
6. Extraction time

130 W, 20 kHz VCX-130 Sonics and Materials (Danbury, CT, USA) with Ti–Al–V probe (13 mm)

Experimental Design for Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds from Grape pomace

❖Response Surface Methodology (31x5 experiments)

Parameters	Levels				
Solvent type (% aqueous ethanol)	0	25	50	75	100
Extraction temperature (T, ^{o}C)	20	30	40	50	60
Amplitude level (A, %)	20	30	40	50	60
Liquid /Solid ratio (mL/g)	8	12	16	20	24

•Every experiment in 5 times: 2, 5, 10, 20, 30 min

Factors Affecting the Microwave-Assisted Extraction Process

1. Microwave radiation power
2. Solvent type
3. Liquid /Solid ratio
4. Extraction time

Microwave system (MultiwaveB30MC030A)
(Anton Paar, Austria)

Experimental Design for Optimization of Microwave-Assisted Extraction of Phenolic Compounds from Grape pomace

❖Response Surface Methodology (31x5 experiments)

Parameters	Levels				
Solvent type (% aqueous ethanol)	0	20	50	80	100
Microwave radiation power (Watt)	100	201	350	499	600
Liquid /Solid ratio (mL/g)	8	11	16	21	24

•Every experiment in 5 times: 1, 2, 3, 4, 5 min

Effect of grape pomace moisture content on extraction yield at the optimum extraction conditions

Agiorgitiko grape pomace

81.7 g water/100 g grape pomace

55° C

Drying
(with and without air)

45° C

Microwave –assisted

Extraction

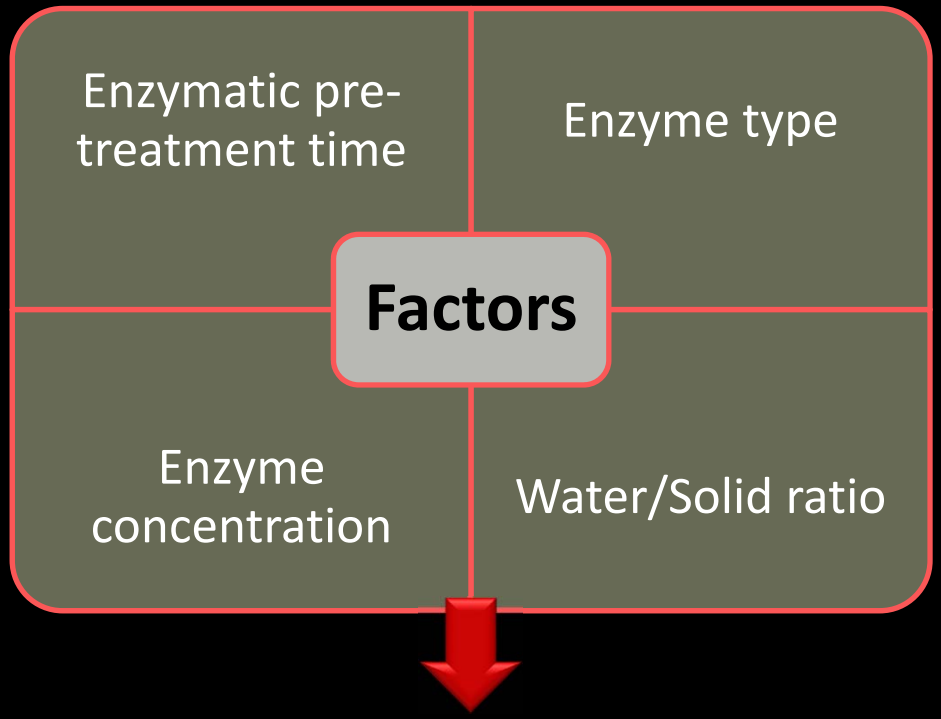
Ultrasound –assisted

Determination of phenolics compound

Folin Ciocalteu

Determination of moisture content of grape pomace with highest extraction yield

Factors Affecting the Extraction Process with Enzymatic Pretreatment



Pectinase

Microwave-assisted and ultrasound-assisted extraction at the optimum conditions

Cellulase

✓**Cellulase**, from *Trichoderma reesei*, with activity 700 EGU/g (EGU, endoglucanase units)

✓**Peclyve V**, pectinase enzyme preparation, concentrated in β-glucosidasic activities

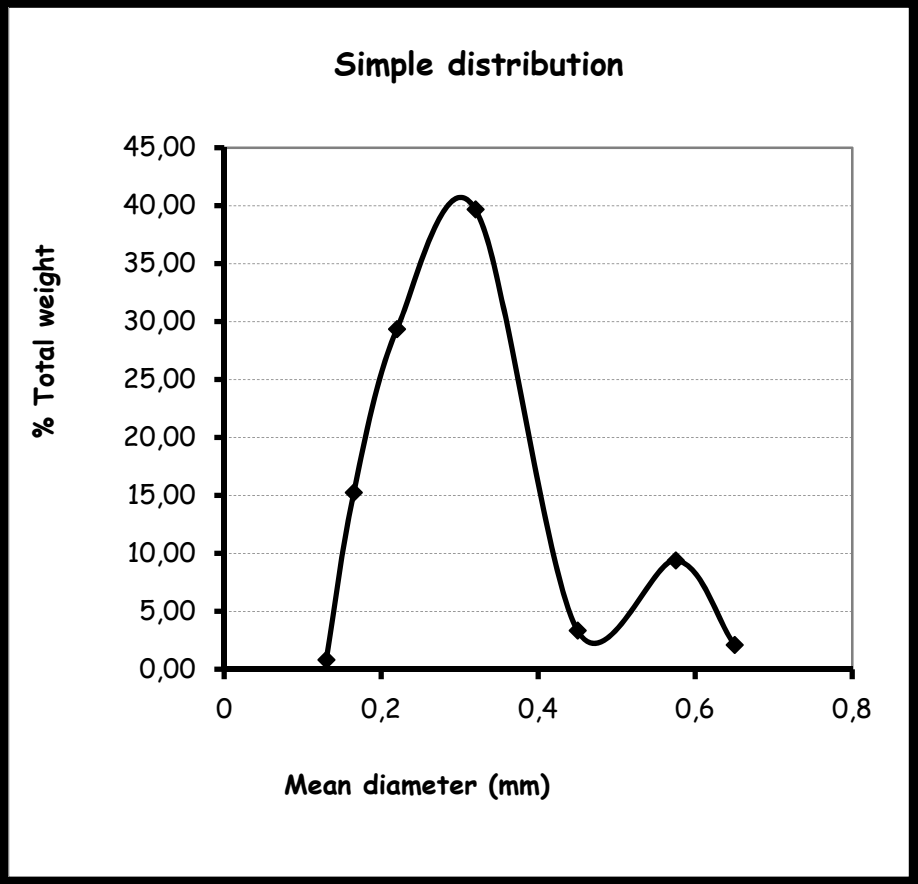
Experimental Design for Optimization of Enzymatic-Assisted Extraction of Phenolic Compounds from Grape pomace

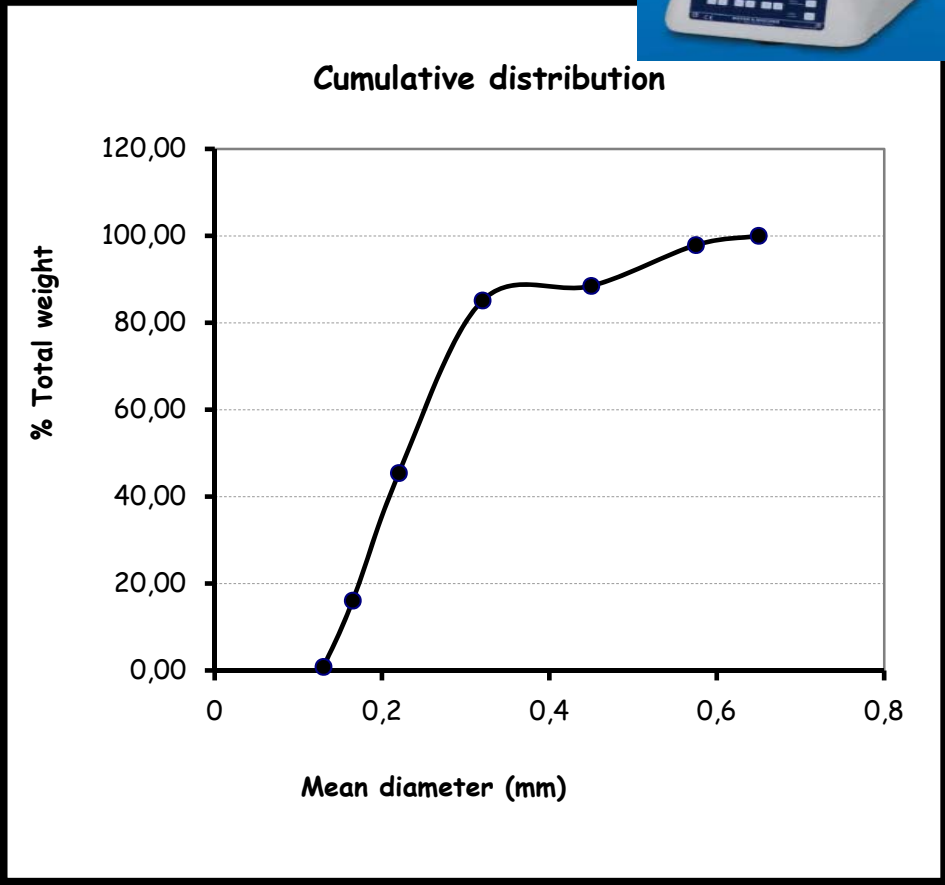
❖Response Surface Methodology (31 experiments)

Parameters	Levels				
Enzymatic pre-treatment time (min)	60	105	150	195	240
Enzyme type (pectinase/cellulase) (% pectinase)	0	25	50	75	100
Enzyme concentration (% dry basis)	2.0	2.5	3.0	3.5	4.0
Water/Solid ratio (mL/g)	20/10	30/10	40/10	50/10	60/10

Results

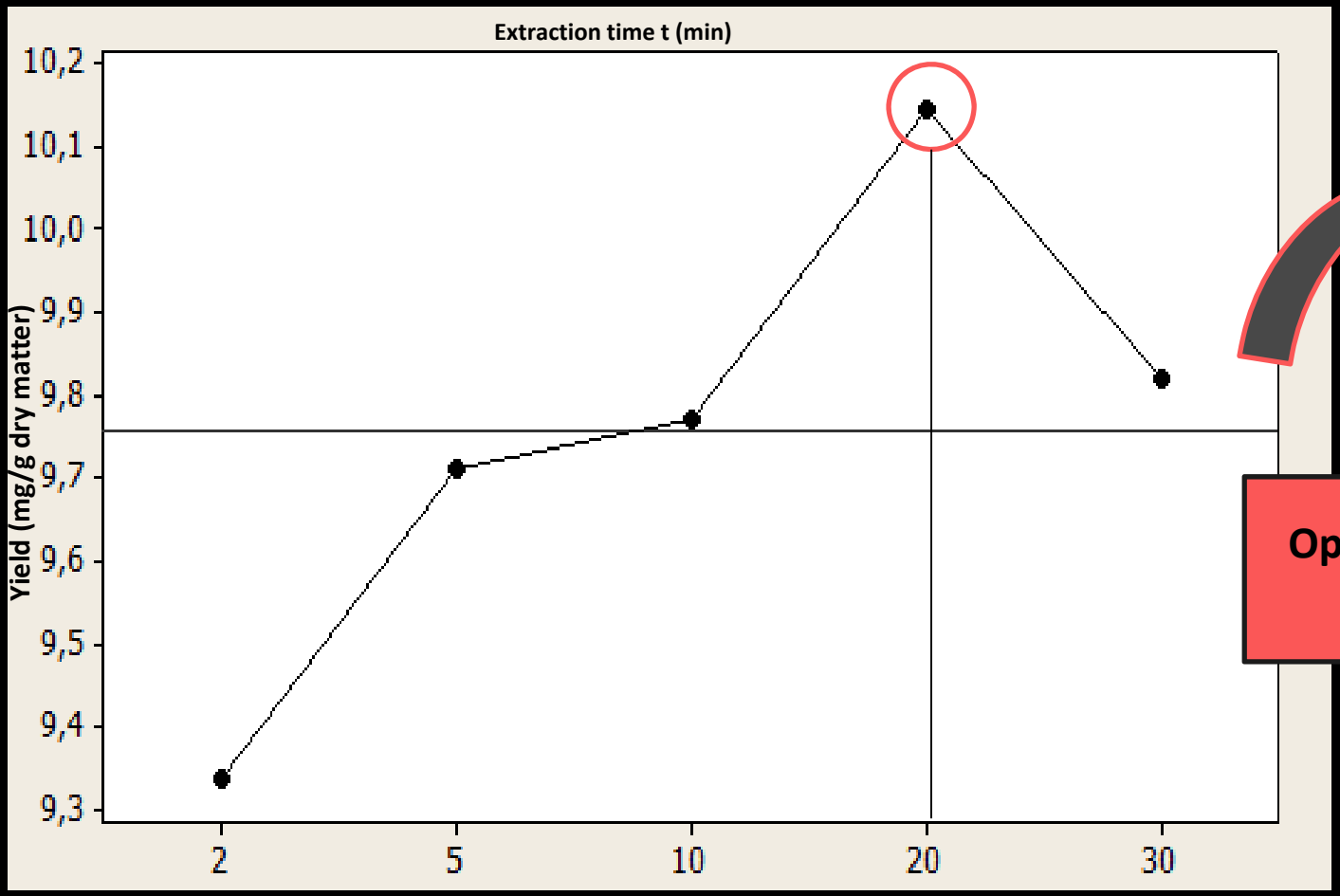
Particle size distribution of milled grape pomace





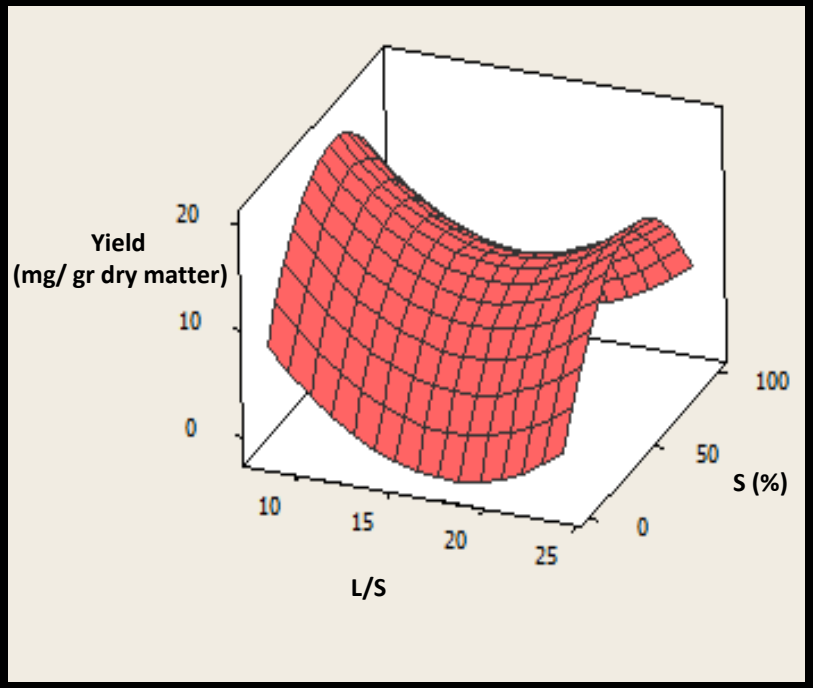
Ultrasound-assisted Extraction
Extraction Yield – Effect of Extraction Time

❖Yield = 0.61 - 33.88 mg/g dry grape pomace



Optimum extraction time = 18 min

Ultrasound-assisted Extraction Yield
Effects of Various Parameters



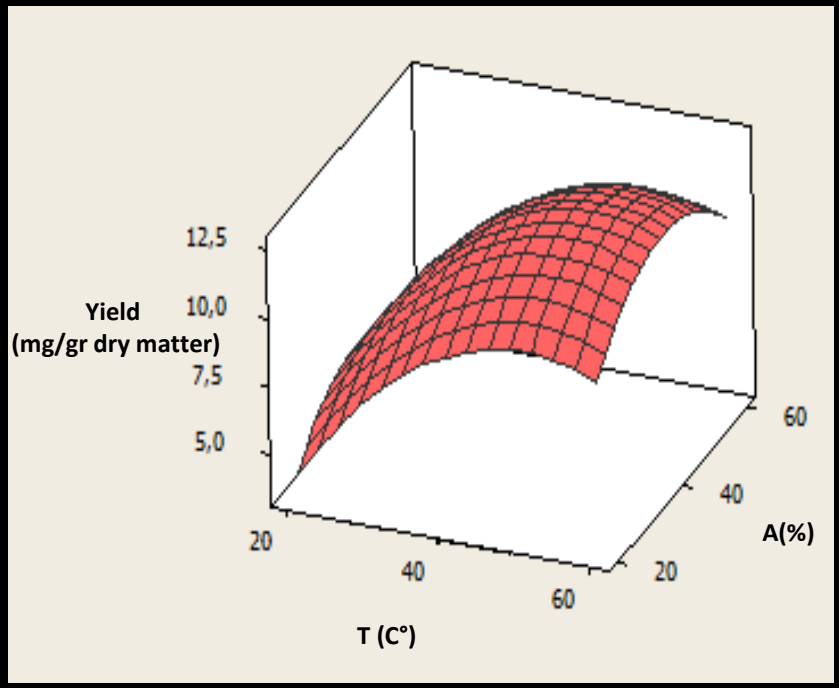
↑ Y ←

T ↑

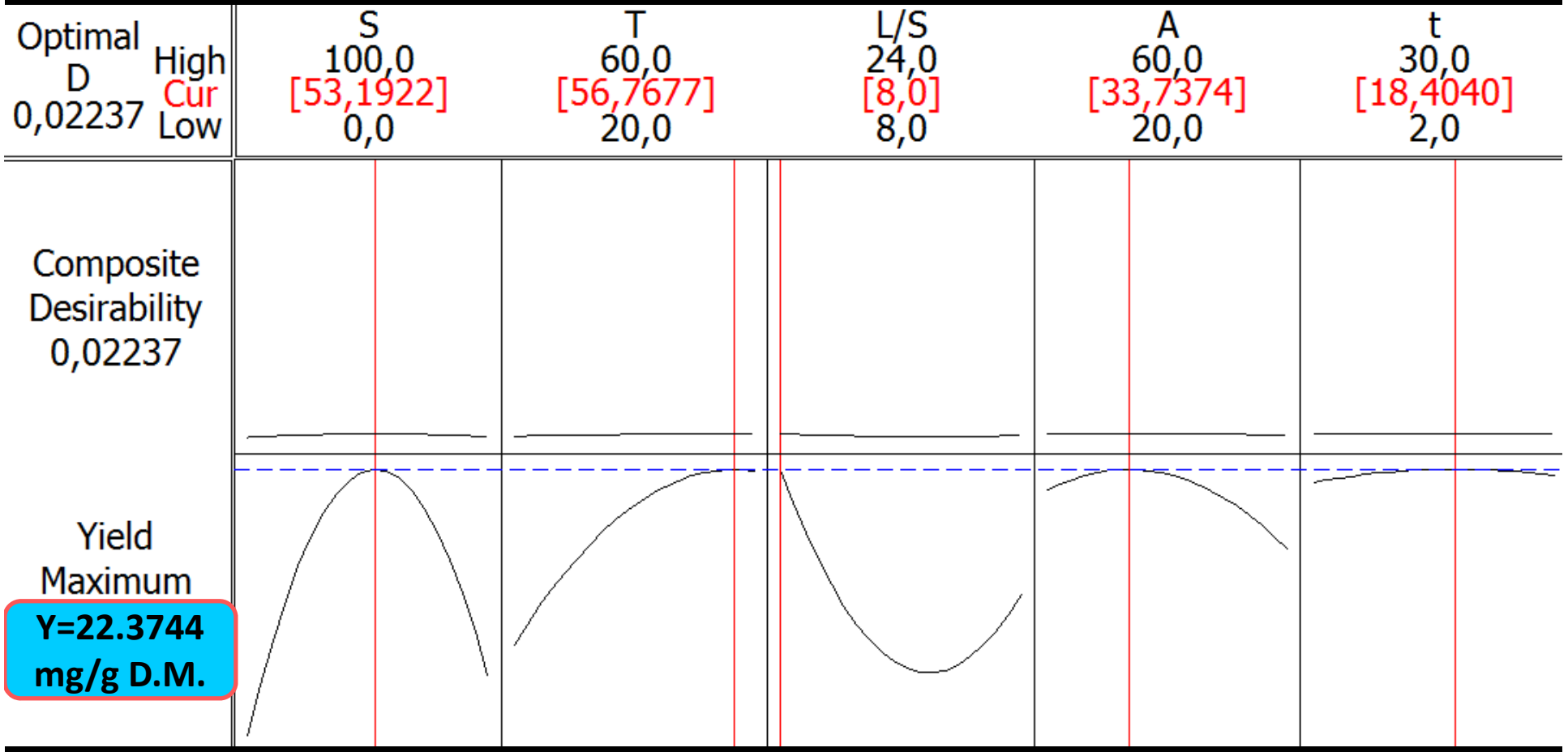
S ↑↓

A ↔

L/S ↔



Optimization of ultrasound-assisted extraction



Empirical model of extraction yield:

$$Y=11.279+0.3798S+0.6837T-3.7561L/S+0.2666A-0.0047S^2-0.0065T^2+0.1043(L/S)^2-0.0057A^2$$

Statistically significant parameters:

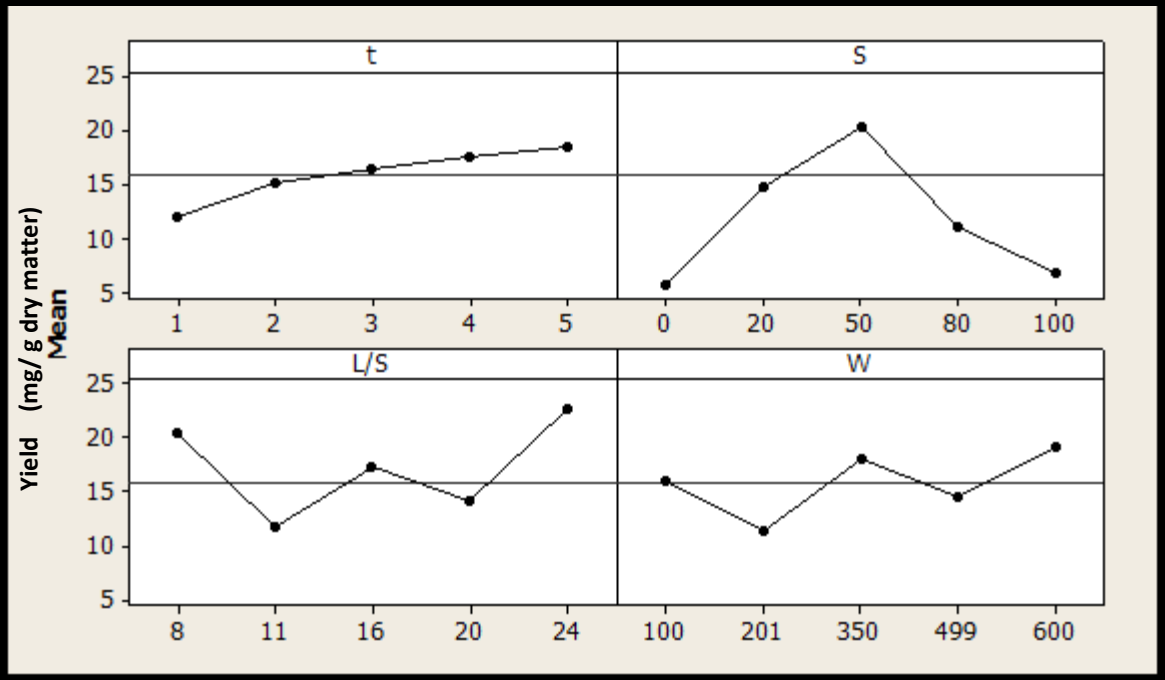
*S (p=0.000), L/S (p=0.000), S*S (p=0.000), L/S*L/S (p=0.000)*

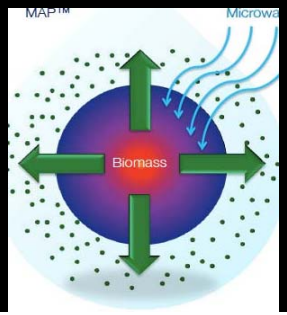
Optimum conditions for maximum yield of phenolics with microwave-assisted extraction

Optimum yield: 26.44 mg/g D.M

t (min)	S(%)	W (Watt)	L/S
5	42	408	24

Microwave-assisted extraction – Effect of various parameters





Optimization of Microwave Extraction Yield

Regression analysis

Term	Coef	SE Coef	T	P
Constant	-19.9977	8.14011	-2.457	0.016
t	3.5359	1.61804	2.183	0.032
S	0.7714	0.08316	9.277	0.000
L/S	0.0480	0.63204	0.076	0.940
W	0.0697	0.01811	3.848	0.000
t*t	-0.3330	0.18159	-1.834	0.070
S*S	-0.0063	0.00041	-15.338	0.000
L/S*L/S	-0.0044	0.1645	-0.267	0.790
W*W	-0.0001	0.00002	-4.274	0.000
t*S	-0.0313	0.00865	-3.624	0.000
t*L/S	0.0960	0.05629	1.705	0.092
t*W	0.0001	0.00174	0.046	0.964
S*L/S	0.0004	0.00351	0.128	0.898
S*W	-0.0002	0.00011	-2.112	0.037
L/S*W	-0.0001	0.00071	-0.160	0.874

$R^2 = 0.793$ $S = 3.11367$

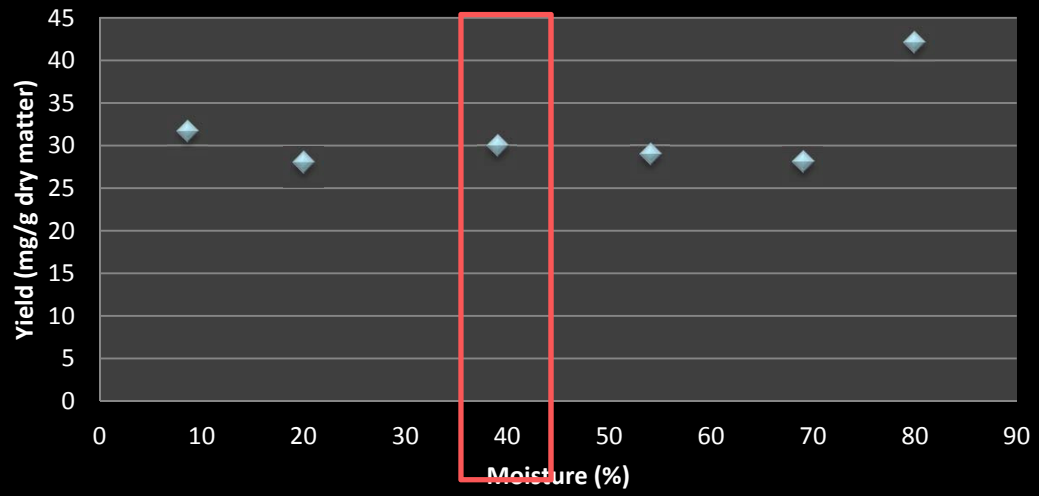
Statistically significant parameters

$P < 0.05$ →

S
W
S*S
W*W
t*S

Effect of grape pomace moisture content on extraction yield

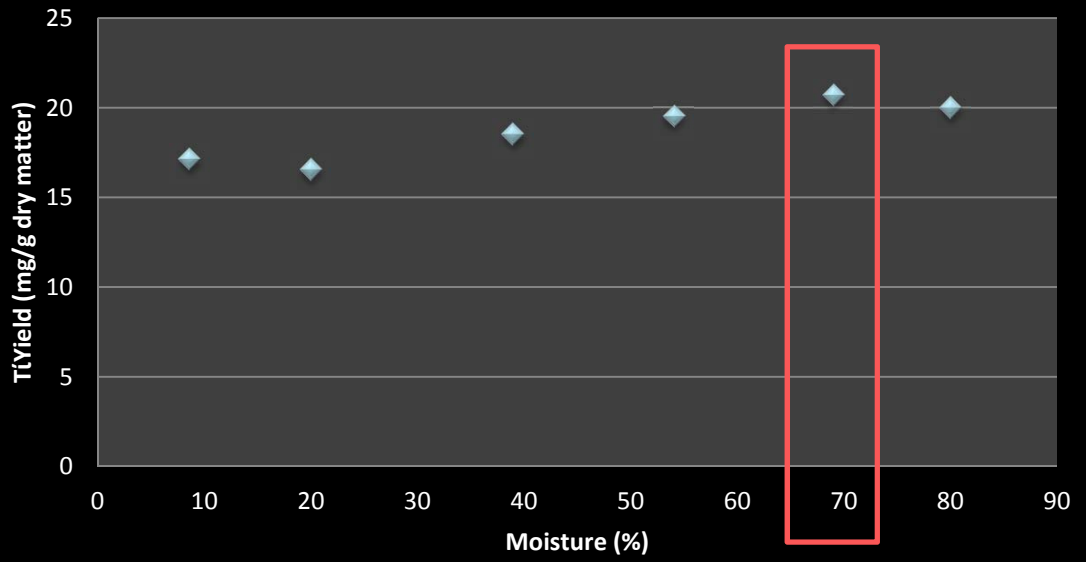
Microwave-assisted extraction & moisture content



Optimum yield in Microwave-assisted Extraction

•Optimum yield: 30.06 mg/g dry matter
•Moisture content: 39% w.b.

Ultrasound-assisted extraction & moisture content

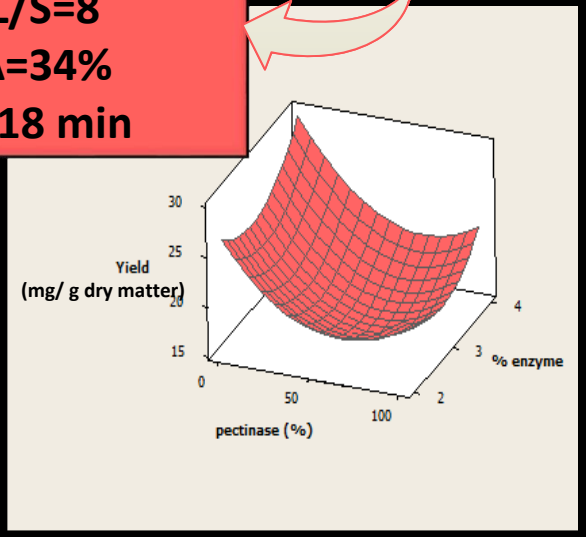


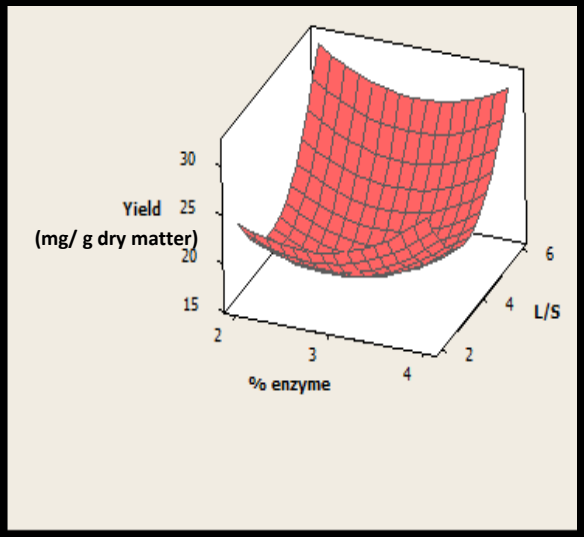
Optimum yield in Ultrasound-assisted Extraction

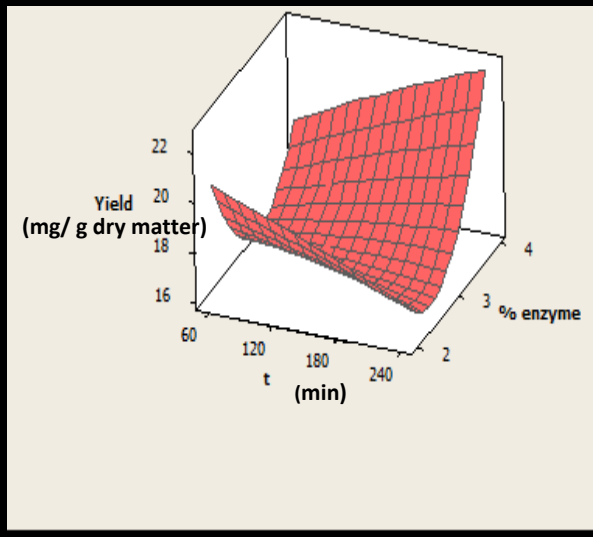
•Optimum yield: 20.72 mg/g dry matter
•Moisture content: 69% w.b.

Enzymatic Pre-treatment & Ultrasound-assisted Extraction at Optimum Conditions - Effects of Various Parameters

S=53 %
T=56 °C
L/S=8
A=34%
t=18 min







↑ **Y** ← **(mg/g D.M.)**

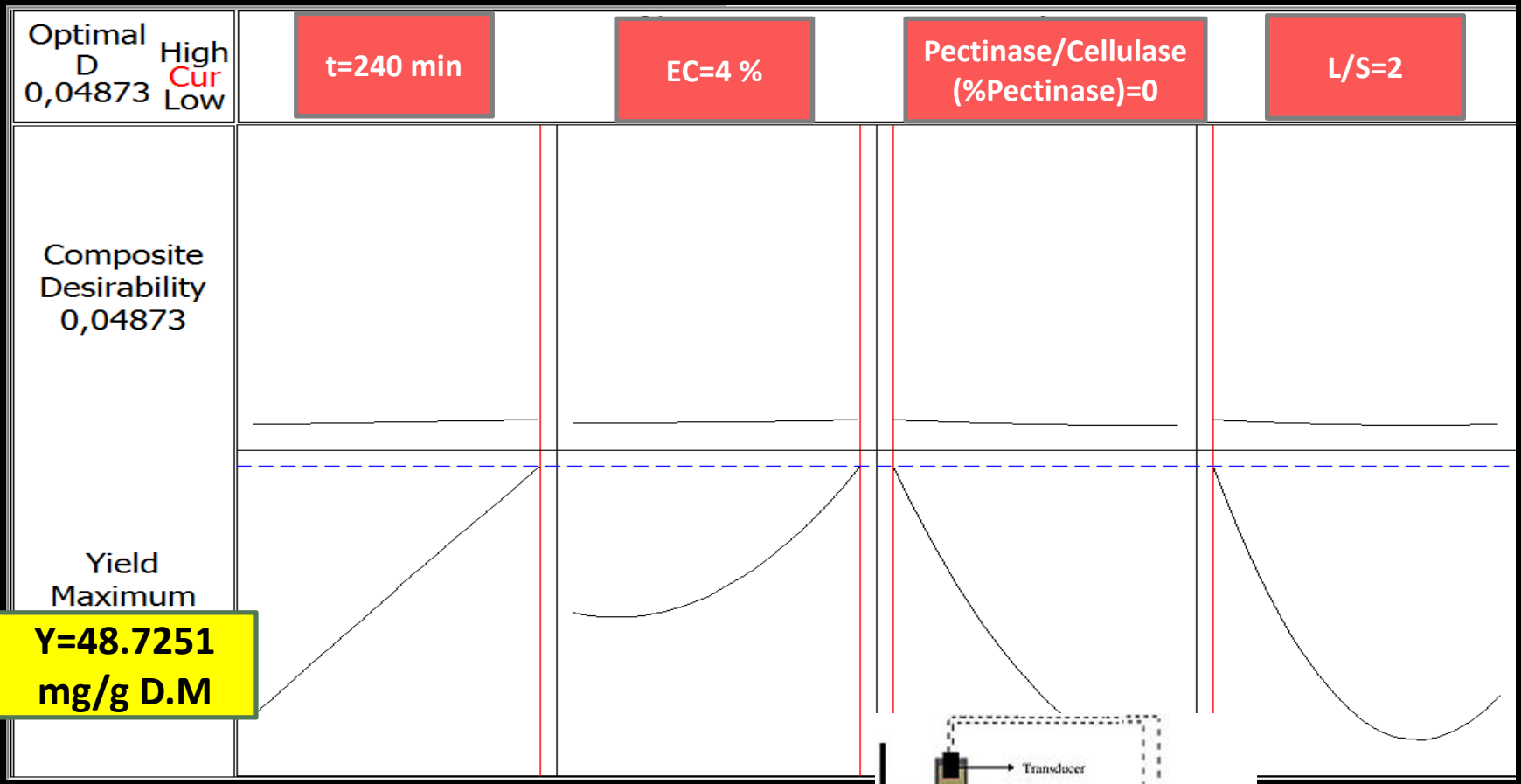
Enzyme (%) ↑
Pectinase (%) ↓
t (min) ↑↓
L/S ↑

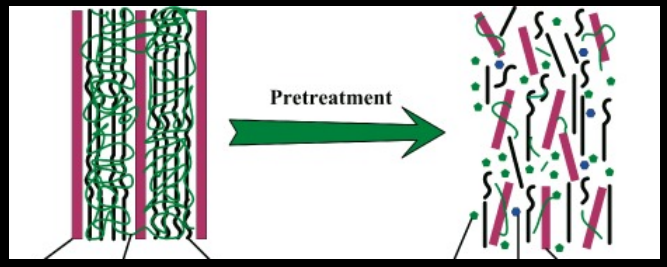
- **R^2 = 0.813**

Statistically significant parameters:

- L/S (p = 0.031)
- Pectinase*Pectinase (p = 0.008)
- L/S*L/S (p = 0.000)
- t*L/S (p = 0.046)

Optimization of enzymatic pre-treatment at ultrasound-assisted extraction





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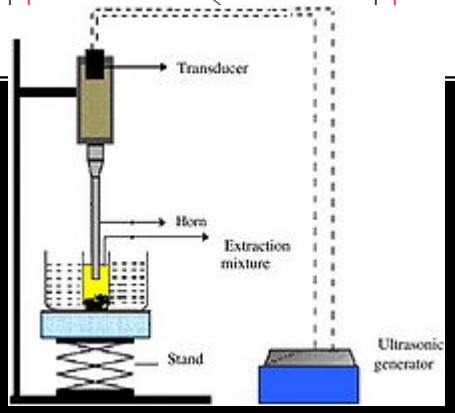
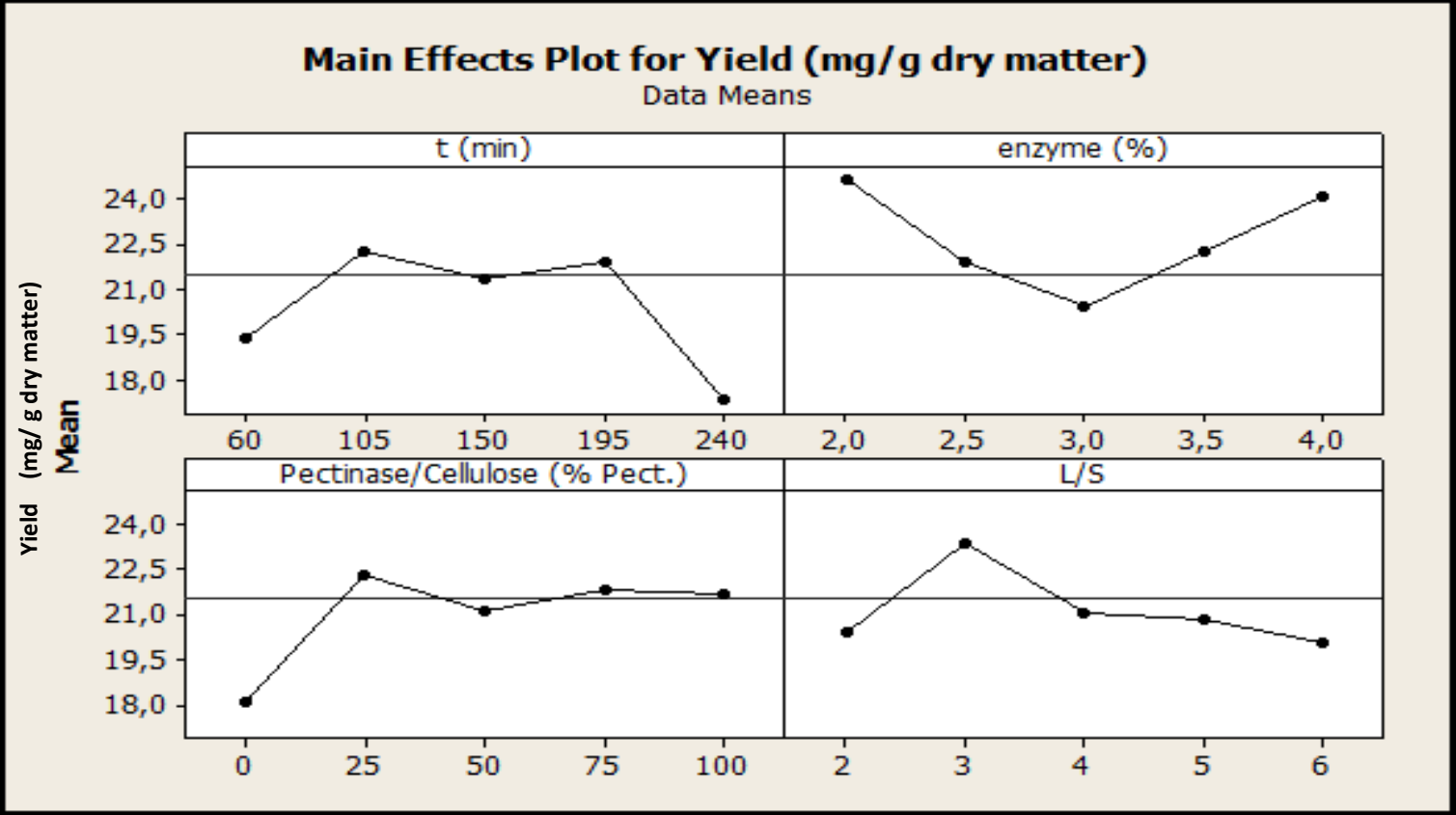


Enzymatic Pre-treatment & Microwave-assisted Extraction at Optimum Conditions - Effects of Various Parameters

S=42.4 %
L/S=24
W=408 W
t=5 min

❖**Yield = 16.61 - 28.46 mg/g dry grape pomace**



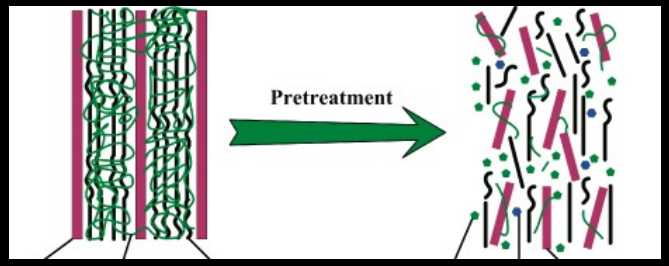
Optimization of enzymatic pre-treatment at microwave-assisted extraction

Optimum Yield:
35.35 mg/g D.M.

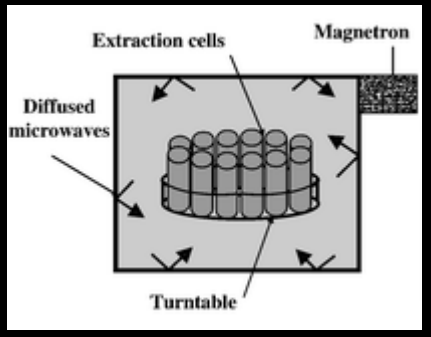
Optimum Conditions			
t (min)	Enzyme (%)	Pectinase/ Cellulase (% Pectinase)	L/S
130	4	0	2

Empirical model of extraction yield:

$$Y=37.92-17.1731EC-0.1681P+6.3016L/S-0.0002t^2+4.047EC^2+0.0164tEC+0.011tP-0.0184tL/S-0.0349ECP-1.9578ECL/S+0.0314PL/S$$

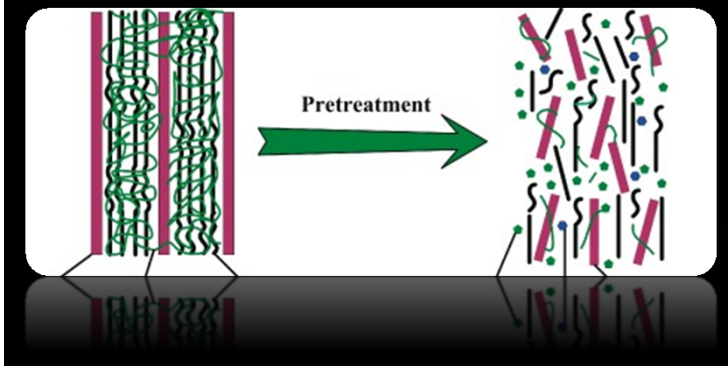


+



To sum up…..

Extraction Method	Optimum Yield (mg/g dry grape pomace)
Ultrasound-assisted	22.37
Microwave-assisted	26.44
Enzymatic pre-treatment and ultrasound-assisted at optimum conditions	48.76
Enzymatic pre-treatment and microwave-assisted at optimum conditions	35.35



Conclusions

- The optimum extraction yield by **ultrasound** extraction was 22.37 mg GAE/g dry grape pomace at 56°C, 53% EtOH as solvent, liquid/solid ratio of 8/1 mL/g, amplitude level up to 34%, pulse duration/pulse interval ratio of 7/6 and extraction time of 18 min.

- The optimum extraction yield by **microwave** extraction was 26.44 mg GAE/g dry grape pomace at 408 W, 42% EtOH as solvent, liquid/solid ratio of 24/1 mL/g and extraction time of 5 min.

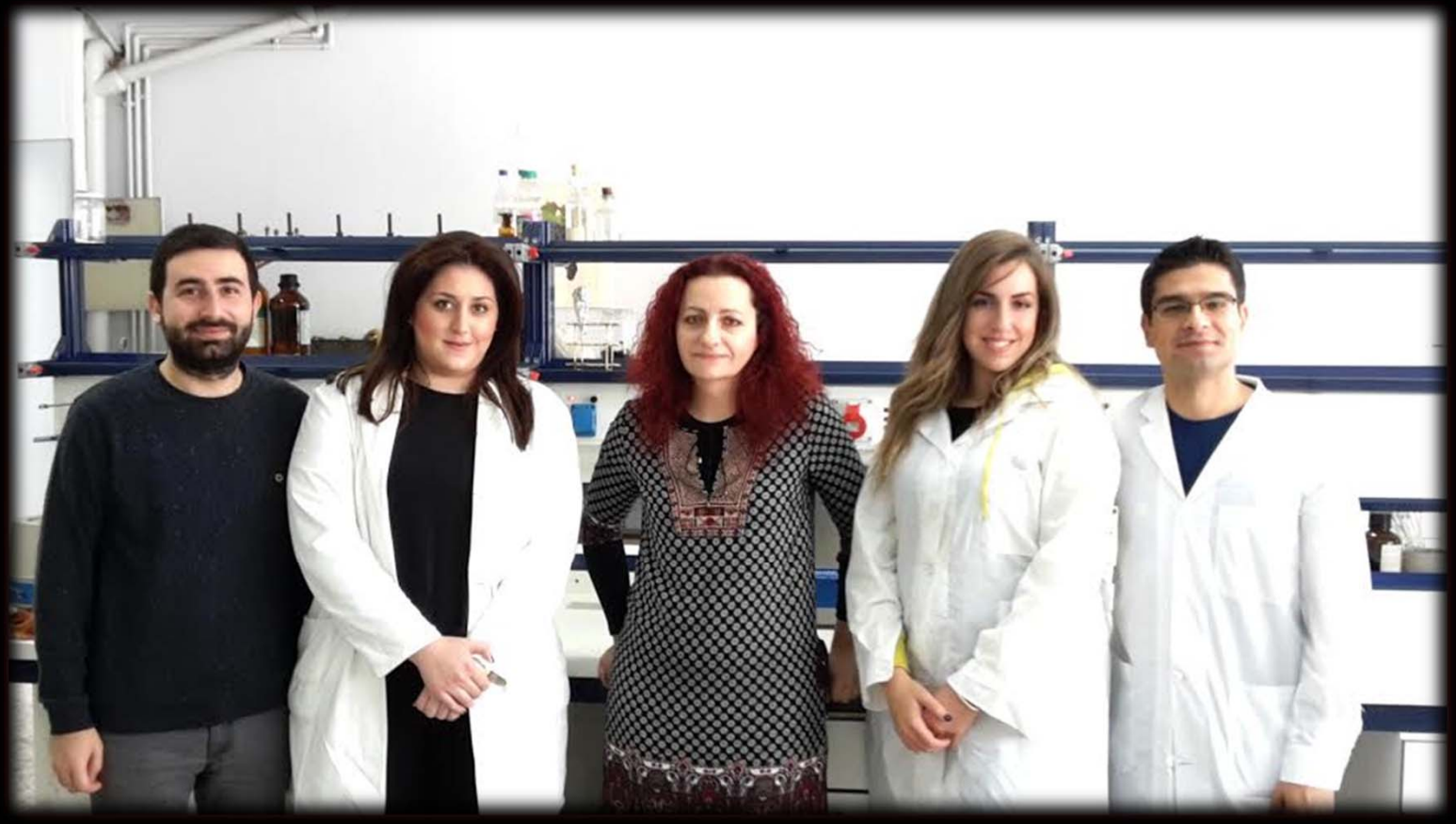
- The optimum **moisture content** in order to maximize the extraction yield was 69% w.b. for the ultrasound extraction and 39% w.b. for the microwave extraction.

- The **enzymatic pre-treatment** using cellulase and pectinase enhance the optimum yield.

Specifically:

➡ Ultrasound extraction with enzymes: optimum yield 48.76 mg/g dry grape pomace, at t=240 min, EC=4%, pectinase=0%, L/S=2

➡ Microwave extraction with enzymes: optimum yield 35.35 mg/g dry grape pomace, at optimum conditions t=130 min, EC=4%, pectinase=0%, L/S=2



Thank you for your attention!