

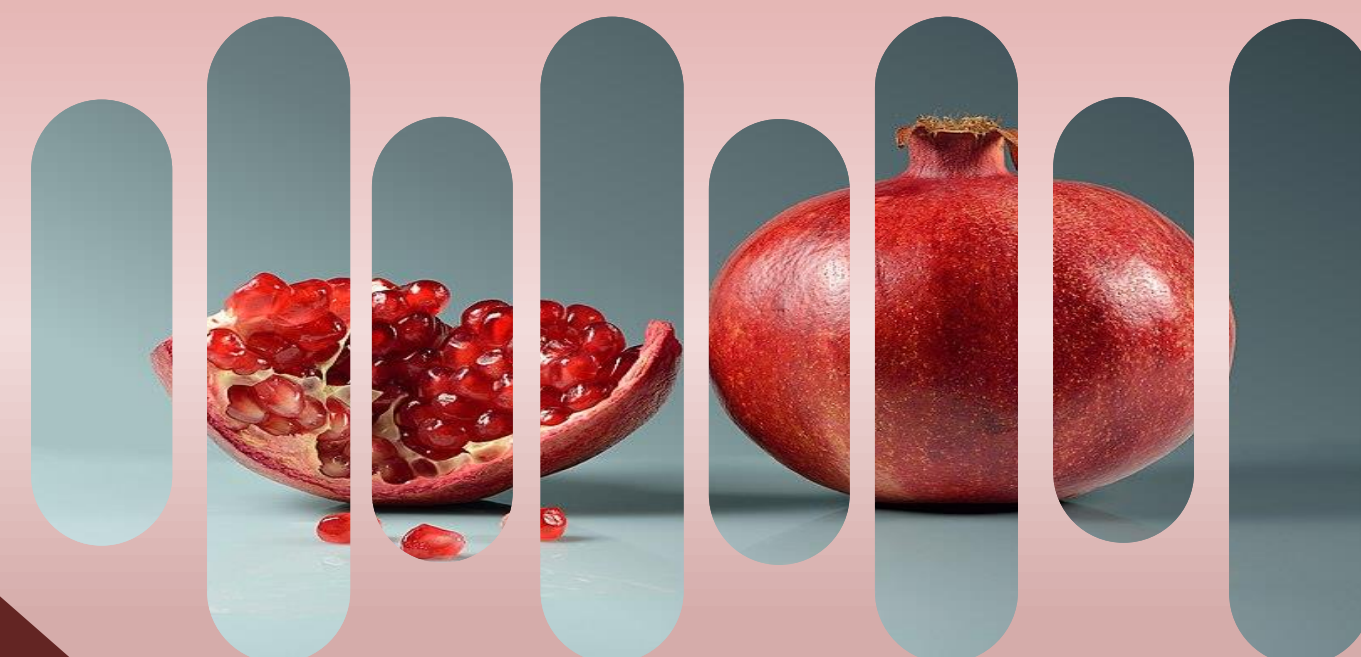


DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY,
SCHOOL OF AGRICULTURE,
ARISTOTLE UNIVERSITY OF THESSALONIKI

*" An holistic approach for the valorization of
pomegranate by-products using green methods "*

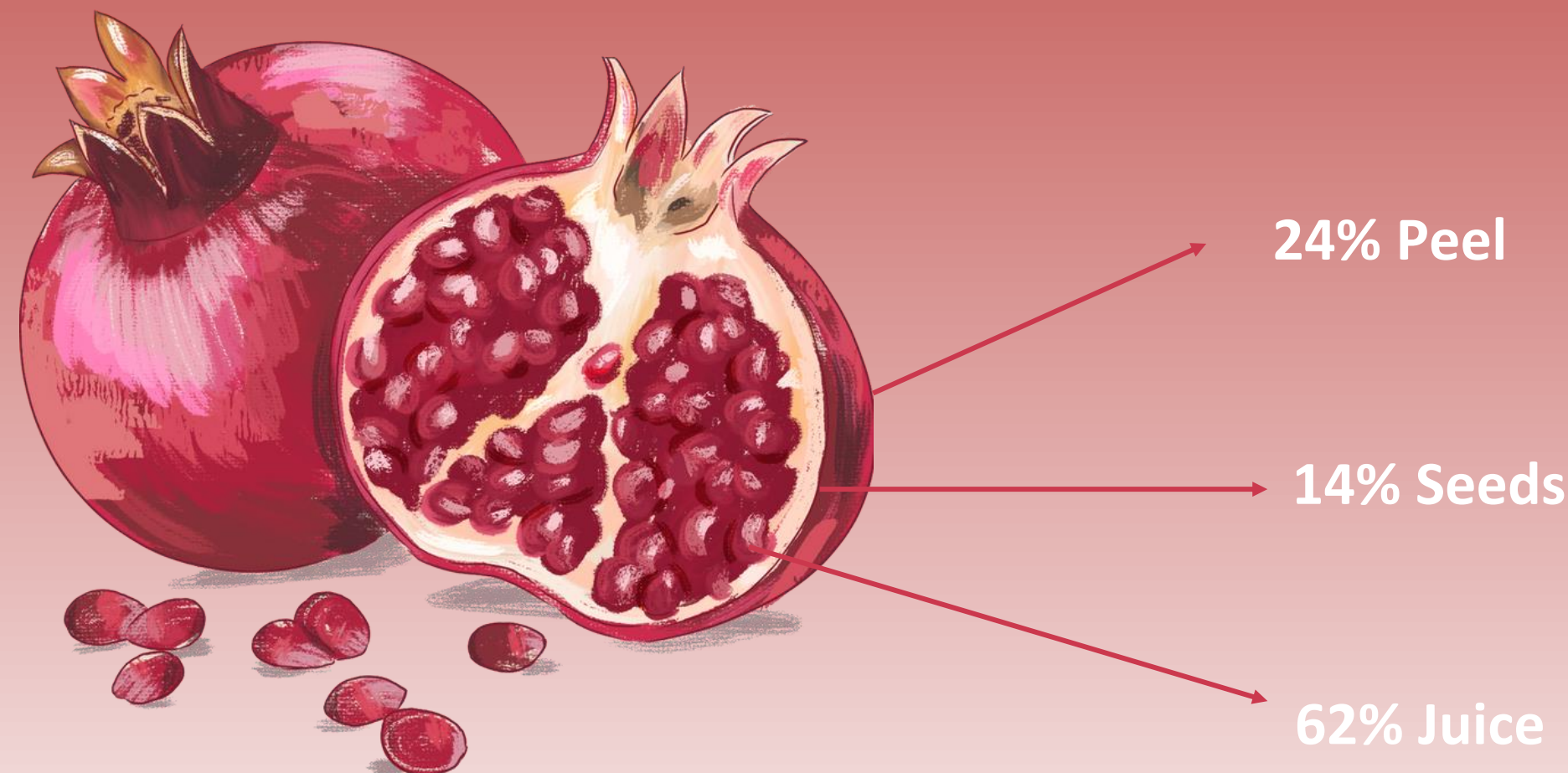
SOLOMAKOU N., KYRIAKOUDI A., KALFA E., KADERIDES K., MOURTZINOS I., GOULA A.M.

CHANIA, JUNE 2023



POMEGRANATE

- Pomegranate is an ancient fruit originating from the Middle East and nowadays the global pomegranate production is around 2 million tons



Scientific classification

Kingdom	<i>Plantae</i>
Division	<i>Tracheophyta</i>
Class	<i>Magnoliopsida</i>
Order	<i>Myrtales</i>
Family	<i>Punicaceae</i>
Genus	<i>Punica</i>

Panth et al., 2017; De Oliveira et al., 2020; Usha et al., 2020).

POMEGRANATE

- Pomegranate can be consumed as fresh fruit or juice and as well as marmalade, jelly, grenadine, sauce and wine
- Pomegranate extracts can be utilized in pharmaceutical and food industries as nutrient supplements

For 1 ton of pomegranate juice



9 tons of by-products



Fresh juice

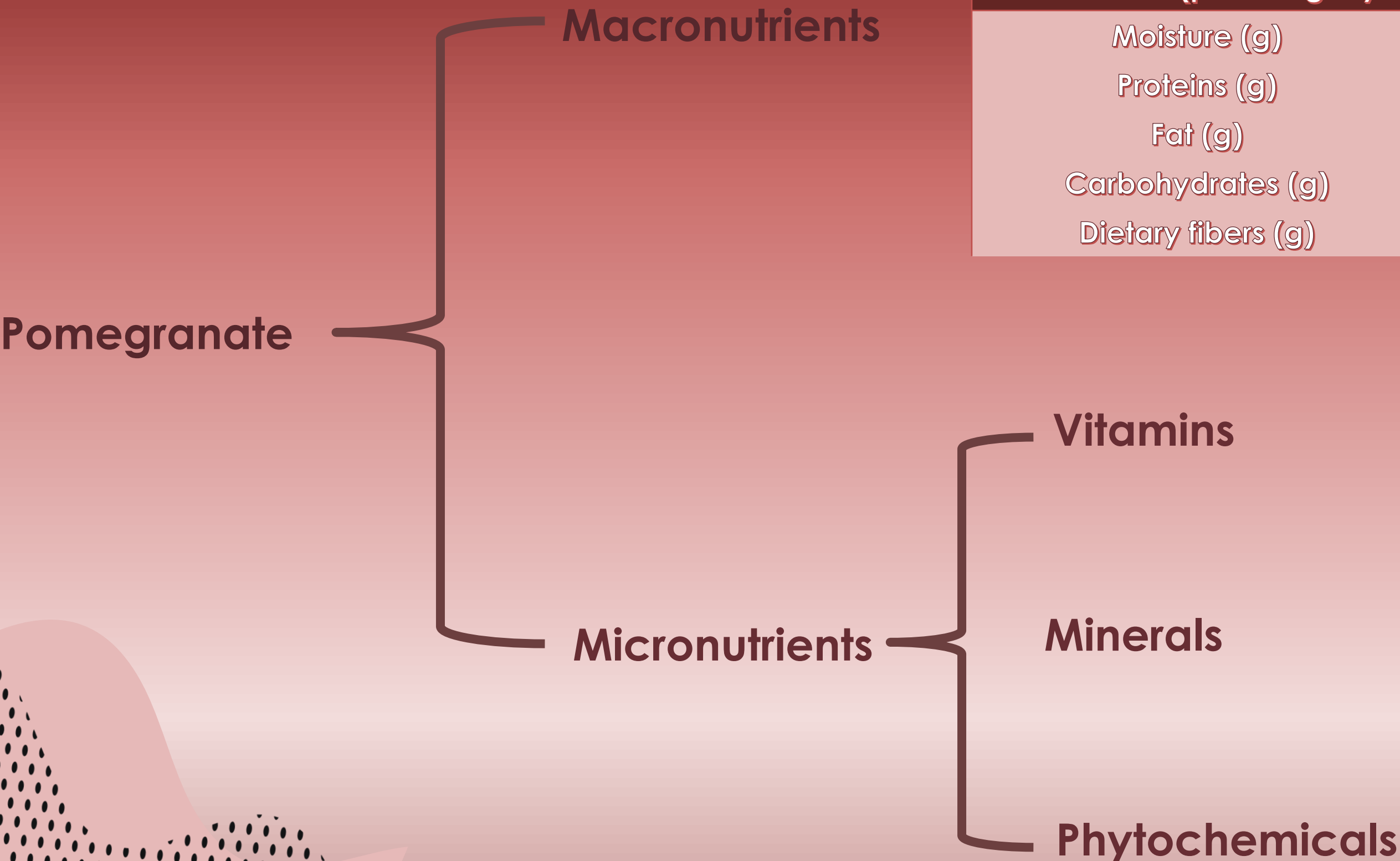


Wine



Marmalade

CHEMICAL COMPOSITION



Chemical composition of pomegranate fruit (per 100 g dry matter)	
Moisture (g)	75-80
Proteins (g)	1-2
Fat (g)	1-2
Carbohydrates (g)	18-20
Dietary fibers (g)	4

Kalamara, 2014



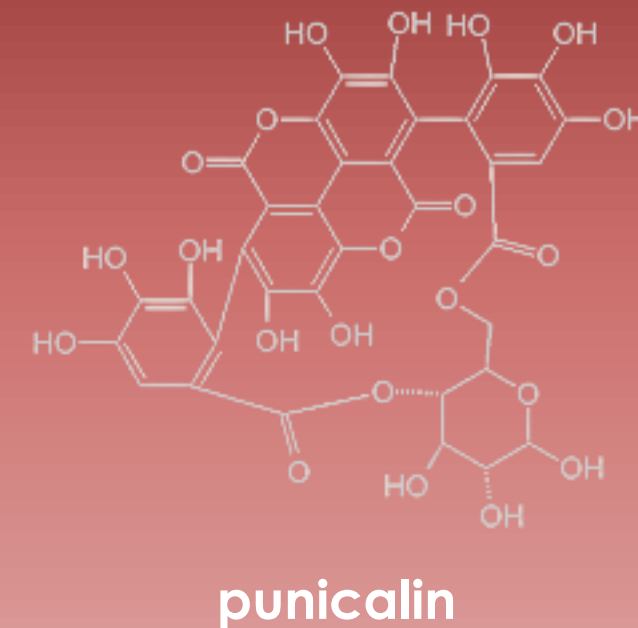
124 different
phytochemicals

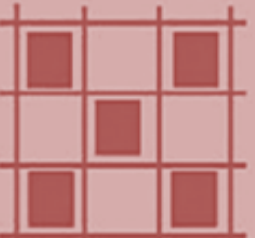
, compared with grape

-

Exclusive source of **punicalin** and **punicalagin**

Pomegranate phenolics { Free/Soluble
Bound/Insoluble



Free Phenolic compounds	Bound Phenolic compounds			
	Chemically bound		Physically entrapped	
	to macromolecules via covalent bonds	to food matrix via ionic bonds	to food matrix (particularly dietary fibers)	within intact cell
				

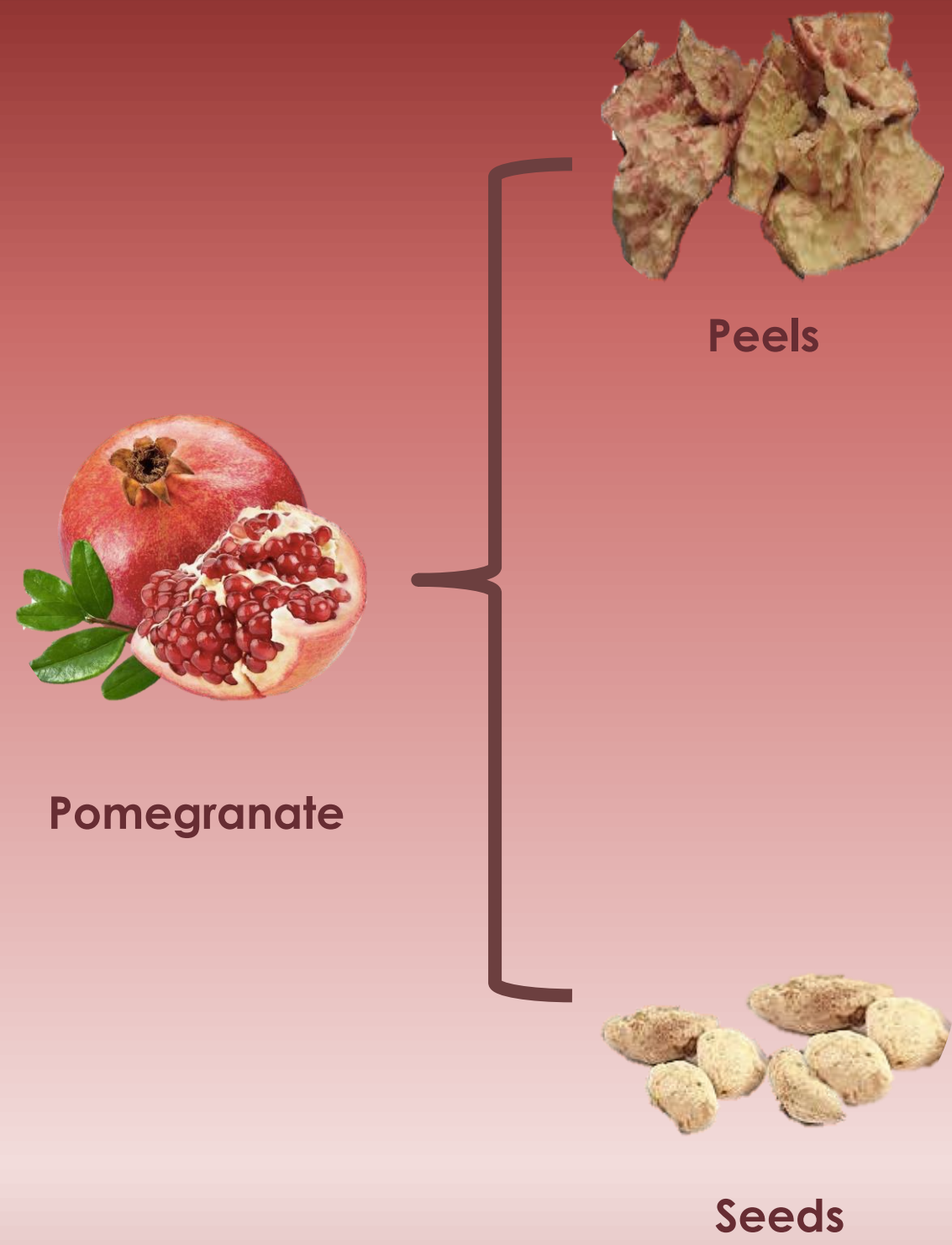
Antioxidant

Anti-Microbial

Anti-inflammatory

A stylized illustration of a pomegranate, showing its characteristic red, seed-filled segments (arils) within a dark red, leathery rind. The pomegranate is positioned in the lower right quadrant of the image, partially overlapping the 'Anti-inflammatory' puzzle piece.

COMPOSITION OF POMEGRANATE BY-PRODUCTS



Pomegranate peel extract phenolics composition (per 100 g dry matter)	
Total phenols (g)	15
Ellagic acid (mg)	44.19
Ellagic acid derivatives (g)	5-6
Punicalagin (mg)	1667.00
Gallic acid (mg)	125.80
Chlorogenic acid (g)	0.02
Coumaric acid (g)	0.05

Spilmont et al., 2015; Gullon et al., 2016

Fatty acid composition of pomegranate seeds	
Punicic acid	79%
Oleic acid	9%
Linoleic acid	6%
Palmitic acid	3%
Stearic acid	2%

Gram et al., 2018

POMEGRANATE PEELS PHENOLICS EXTRACTION

Method	Optimum Conditions	Yield	Reference
UAE	L/S: 32,2/1 mL/g, L: Water, t: 10 min, Amplitude level: 52 %, Pulse: 7/6, T: 35 °C	119.82 mg TAE/g DW	Kaderides et al., 2015
UAE	L/S: 10/1, L: 70 % EtOH, t: 10 min, Intensity level: 105 W/cm ² , Duty cycle: 50%	318.71 mg GAE/g DW	Kazemi et al., 2016
UAE	L/S: 40/1 mL/g, L: Water, t: 10 min, Amplitude level: 40 %, Duty cycle: 50%, T<50 °C	35.60 mg GAE/g DW	Boggia et al., 2016
Indirect UAE	L/S: 44/1 mL/g, L: 59 % EtOH, t: 25 min, T: 80 °C	157.30 mg GAE/g DW	Zivkovic et al., 2018
UAE	L/S: 4/1 mL/g, L: Water, t: 6.2 min, Amplitude level: 60 %	42.20 mg TAE/g DW	Sharayei et al., 2019
MAE	L/S: 40/1 mL/g, L: Water, t: 10 min, Power: 300 W, T<50 °C	23.60mg GAE/g DW	Boggia et al., 2016
MAE	L/S: 60/1 mL/g, L: 50% EtOH, t: 4 min, Power: 600 W	199.4 mg GAE/g DW	Kaderides et al., 2019

***UAE: Ultrasound-assisted extraction, MAE: Microwave-assisted extraction**

POMEGRANATE SEED OIL EXTRACTION

Method	Optimum Conditions	Yield	Reference
Enzymatic	Pre-treatment: Sodium phosphate (50mM), L/S: 8/1 w/v for 10 min, T: 45 °C Extraction: protease 5-100 U/g d.b., T: 45 °C, t: 4-16 h, Stirring: 110 rpm	22,9% oil 13.2% protein	Talekar et al., 2018
UAE	T: 20-65 °C, L/S: 8-20/1 w/v, A: 30-60%, Pulse: 3/4 – 2/1, t: 2-40 min, L: Hexane	18.16 wt %	Kalamara et al., 2015
UAE	T: 20-80 °C, L/S: 4-20/1 w/v, A: 20-60%, Pulse: 5/15 – 2/1, t: 2-40 min, particle size: 0,2-3,4 mm, L: Hexane	446.3 g oil/kg (60%)	Goula, 2013
Supercritical CO ₂ extraction	P: 200-400 bar, T: 60 °C	98.1 g/100g unsaturated fatty acids 1.95 g/100g saturated fatty acids	Silva et al., 2019
Supercritical CO ₂ extraction	P: 320 bar, T: 60 °C	85.4 %	Natolino and Da Porto, 2019
MAE	L: Hexane, P: 220 W, t: 5 min, L/S: 10/1	35.19 %	Cavdar et al., 2017
MAE + Supercritical CO ₂ extraction/Soxhlet	P: 250-600 W, t: 6 min	36.3%	Durdevic et al., 2017
Acid hydrolysis	6 M HCl, T: 100 °C, t: 1h	Increase antioxidant activity	Salazar et al., 2020

OBJECTIVE OF THE STUDY



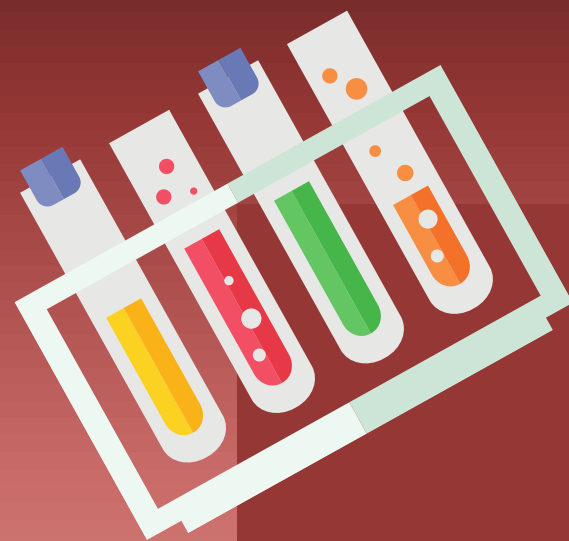
Holistic exploitation of pomegranate by-products as a **source of value-added components**



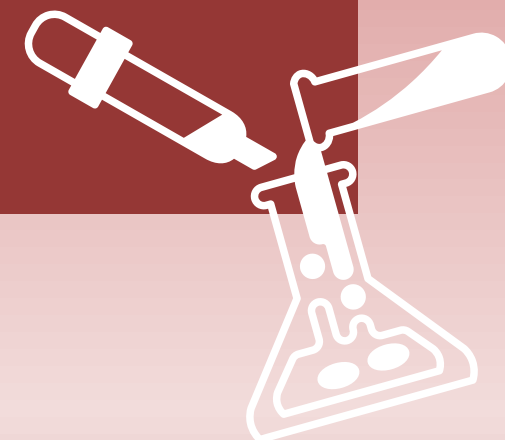
Optimization of recovery of phenolics from pomegranate peels using "green" techniques



Optimization of oil extraction from pomegranate seed using novel methods



MATERIALS AND METHODS



SAMPLES PREPARATION

COLLECTION OF
POMEGRANATE BY-
PRODUCTS

DRYING
(40°C, 24 h)

MILLING

PHENOLICS
EXTRACTION

OIL
EXTRACTION



Variety: Wonderful



POMEGRANATE PEEL EXTRACT

FREE PHENOLICS

ULTRASOUND- ASSISTED
EXTRACTION

OPTIMIZED CONDITIONS



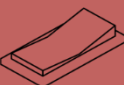
Solvent type: Water



Solvent/Solid ratio: 32/1 mL/g



Amplitude: 40% (50 W)



Pulse duration/Pulse interval ratio: 7/6 s/s



Extraction duration: 10 min



Extraction temperature: 35 °C



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

Kaderides et al., 2015



RP-HPLC-DAD
(punicalagin,
ellagic acid)

- ✓ EtOH
- ✓ MeOH
- ✓ 50% v/v aq. solution of EtOH
- ✓ 50% v/v aq. solution of MeOH

DETERMINATION OF TOTAL
PHENOLICS-
ANTIOXIDANT ACTIVITY

DPPH• assay

ABTS•⁺ assay

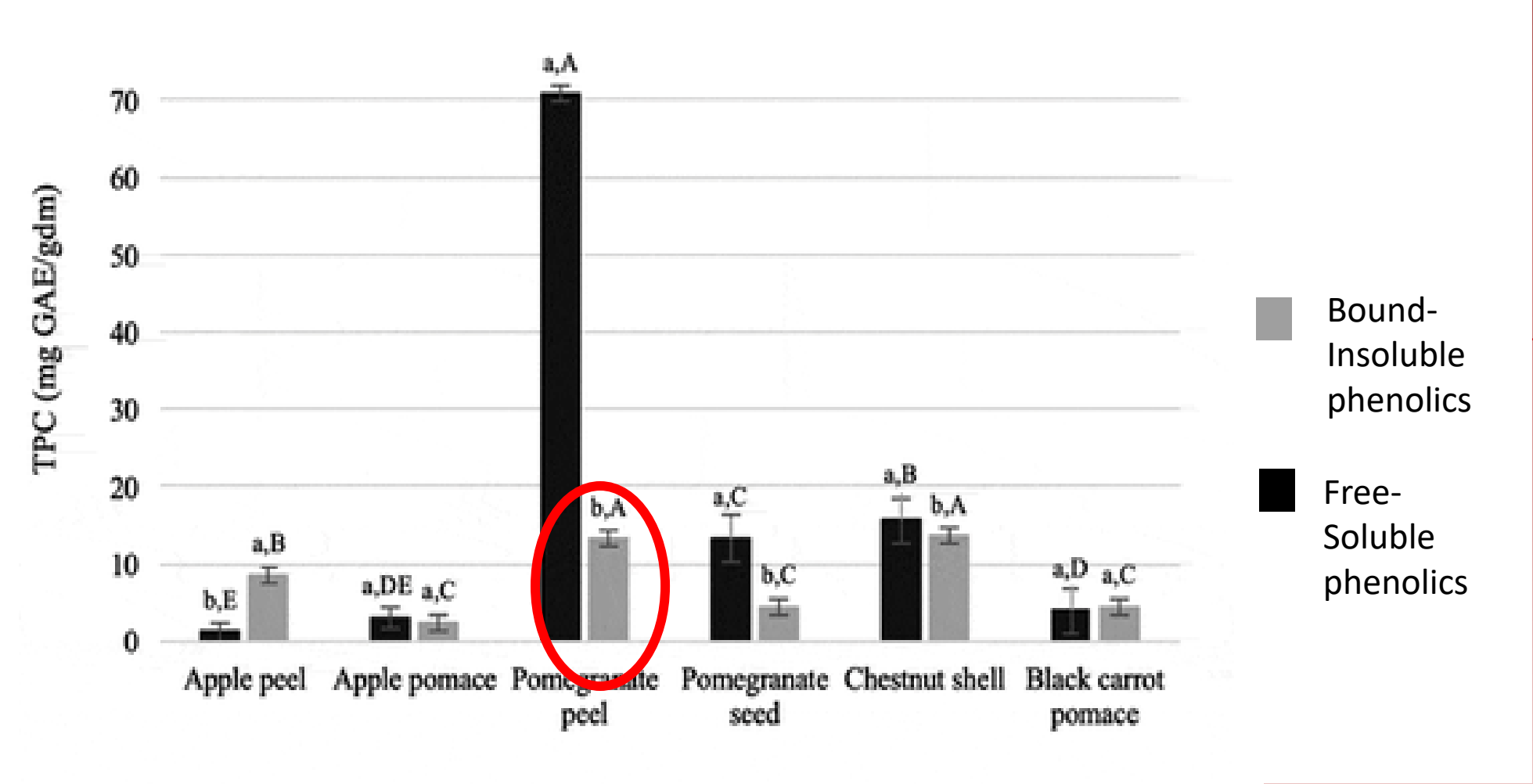
CUPRAC assay

Folin-Ciocalteu
assay



POMEGRANATE PEEL EXTRACT

BOUND PHENOLICS



Source of ellagic acid
(bound form)

Total phenolic content of different plant wastes extracts

Gulsunoglu et al., 2019

RECOVERY OF BOUND PHENOLICS

Extraction of free phenolic compounds

Drying of solid residue

Ultrasound-assisted alkaline hydrolysis of solid residue

Neutralization of solution

Liquid-liquid extraction with ethyl acetate

Evaporation

Redissolution of the dry extract in MeOH



Parameters Levels (RSM Methodology)

L/S	Concentration of NaOH	t
10-49 mL/g	0.5-4 M	3-112 min

DETERMINATION OF TOTAL PHENOLICS-ANTIOXIDANT ACTIVITY

DPPH• assay

ABTS•⁺ assay

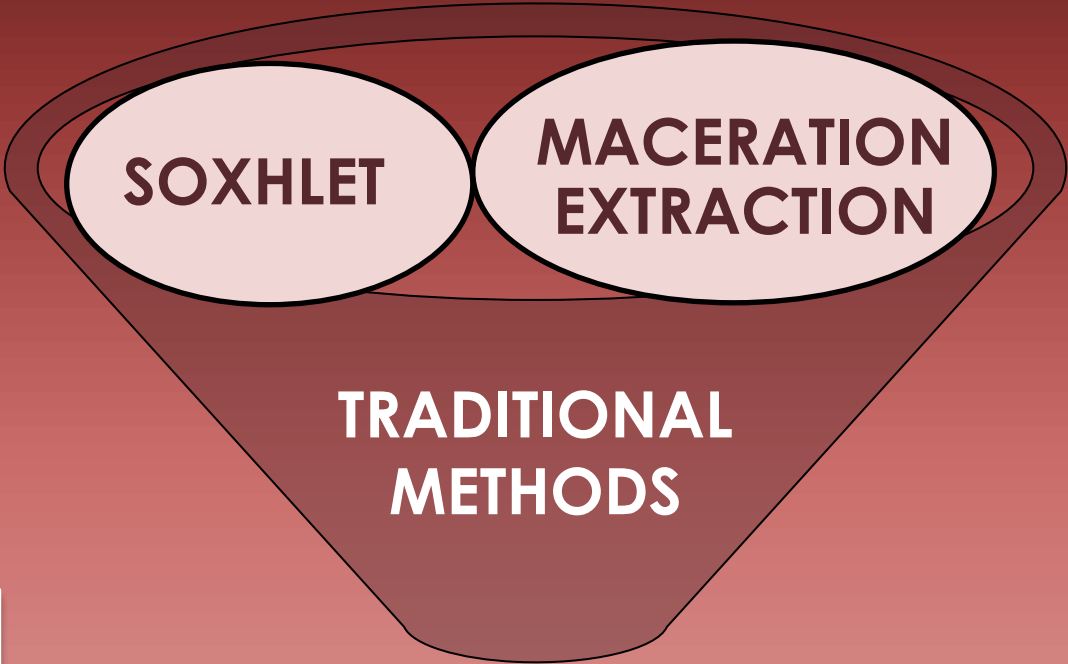
CUPRAC assay

Folin-Ciocalteu assay

RP-HPLC-DAD
(punicalagin,
ellagic acid)



EXPLOITATION OF POMEGRANATE SEEDS



↓

Extraction time

↓

Volume of organic solvent

↑

Extraction yield

Optimum extraction conditions				
L/S (hexane)	Pulse on/off	T°C	Amplitude	t
20 mL/g	$\frac{3}{4}$ s/s	20°C	60%	10 min

Kalamara, 2014

NOVEL “GREEN”
EXTRACTION METHODS

ULTRASOUND- ASSISTED EXTRACTION

ENZYMATIC PRETREATMENT

Parameters Levels (RSM Methodology)			
L/S (water)	Enzyme concentration C.E.	Type of enzyme (T.E.) (% cellulase)	t
2-6 mL/g	2-4%	0-100%	60-240 min

T: 50°C

pH: 5.0

RESULTS AND DISCUSSION



EXTRACTION OF FREE PHENOLICS OF POMEGRANATE PEELS

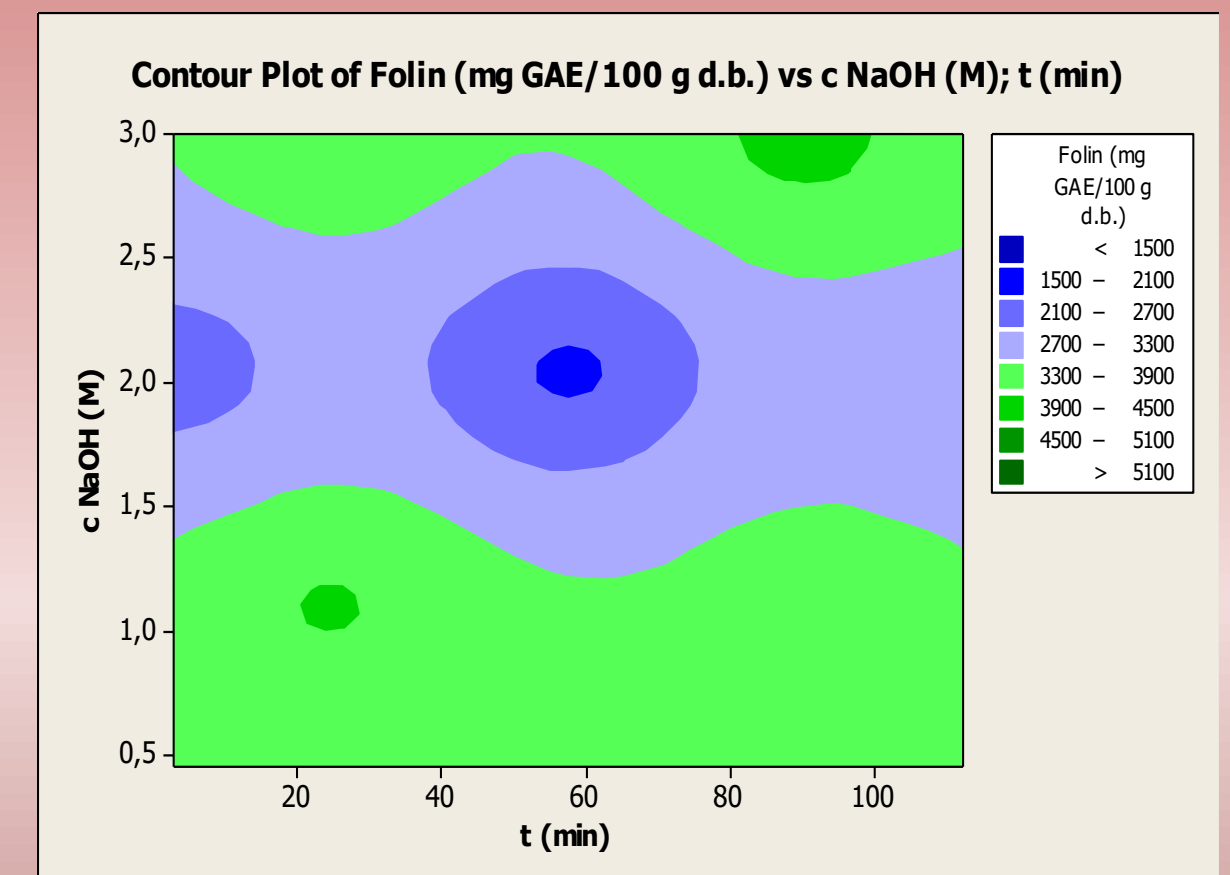
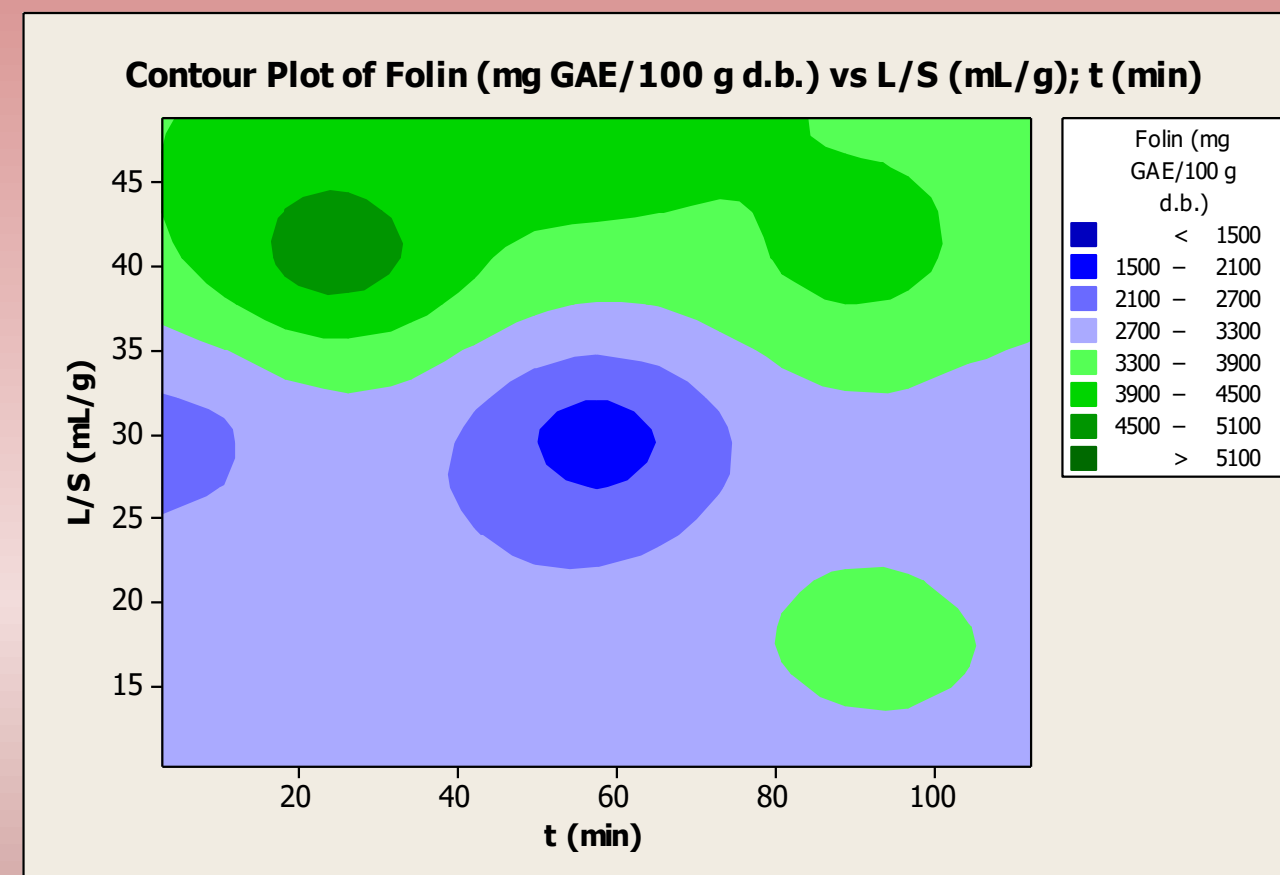
Solvent	Total phenolic content (mg GAE/100 g d.b.)	DPPH (μ mol Trolox /100 g d.b.)	CUPRAC (μ mol Trolox/100 g d.b.)	ABTS (μ mol Trolox/100 g d.b.)	Punicagin content (mg/100 g d.b.)	Ellagic acid (mg/100 g d.b.)
Water	9059 $\pm$ 424	43230 $\pm$ 2069	60496 $\pm$ 2875	1210 $\pm$ 54	30 $\pm$ 4	98 $\pm$ 2
EtOH	2311 $\pm$ 124	10930 $\pm$ 311	10756 $\pm$ 701	565 $\pm$ 26	50 $\pm$ 11	333 $\pm$ 34
MeOH	2926 $\pm$ 124	25411 $\pm$ 578	24510 $\pm$ 1528	762 $\pm$ 26	571 $\pm$ 31	226 $\pm$ 22
50% aq. EtOH	10233 $\pm$ 464	39750 $\pm$ 259	87444 $\pm$ 1215	1686 $\pm$ 145	1274 $\pm$ 83	520 $\pm$ 28
50% aq. MeOH	8419 $\pm$ 510	40231 $\pm$ 2592	67289 $\pm$ 4154	1224 $\pm$ 101	1727 $\pm$ 28	120 $\pm$ 6

ULTRASOUND-ASSISTED ALKALINE HYDROLYSIS

	Mean experimental values	Predicted values
Total phenol content (mg GAE/ 100 g d.b.)	4230 ± 190	4987
A_{ABTS} (μmol Trolox /100 g d.b.)	32397 ± 1817	36230
A_{CUPRAC} (μmol Trolox /100 g d.b.)	29816 ± 1954	31860
Punicalagin (mg/100 g d.b.)	58 ± 3	56
Ellagic acid (mg/100 g d.b.)	1391 ± 94	1477

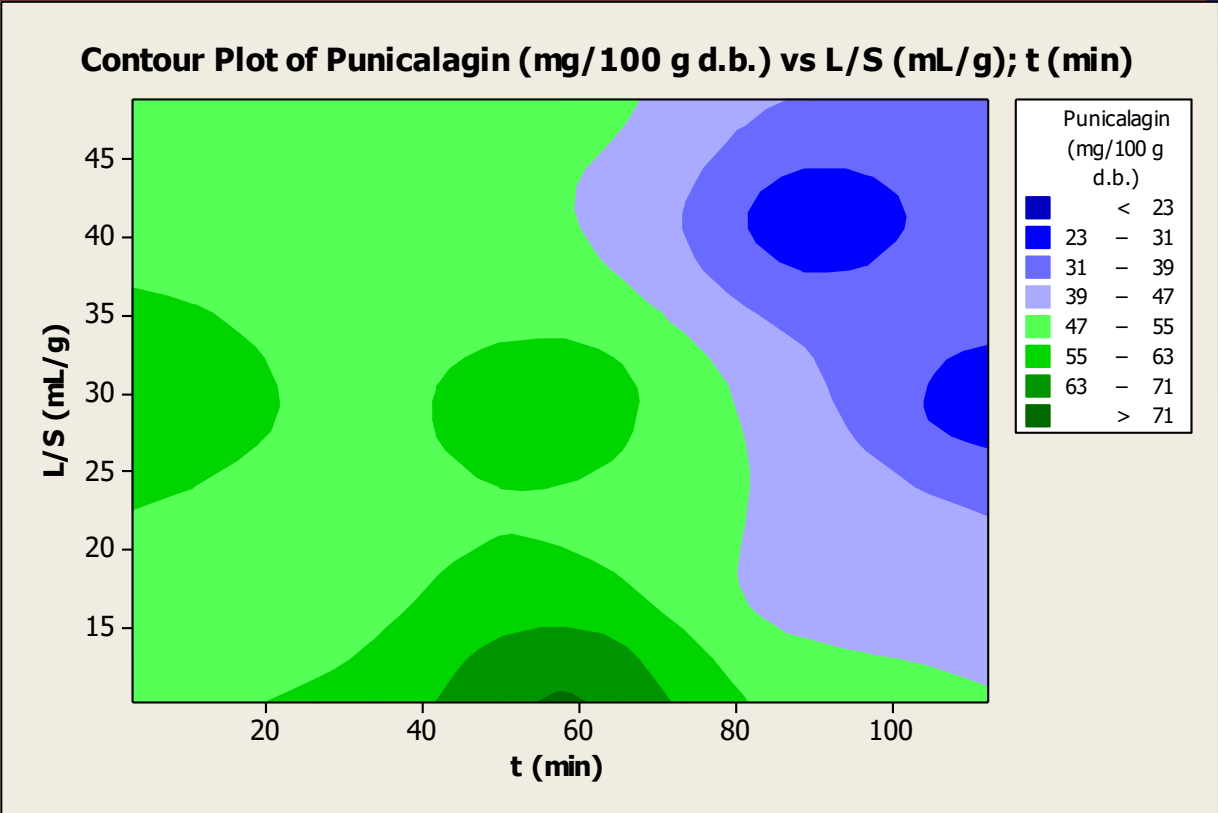
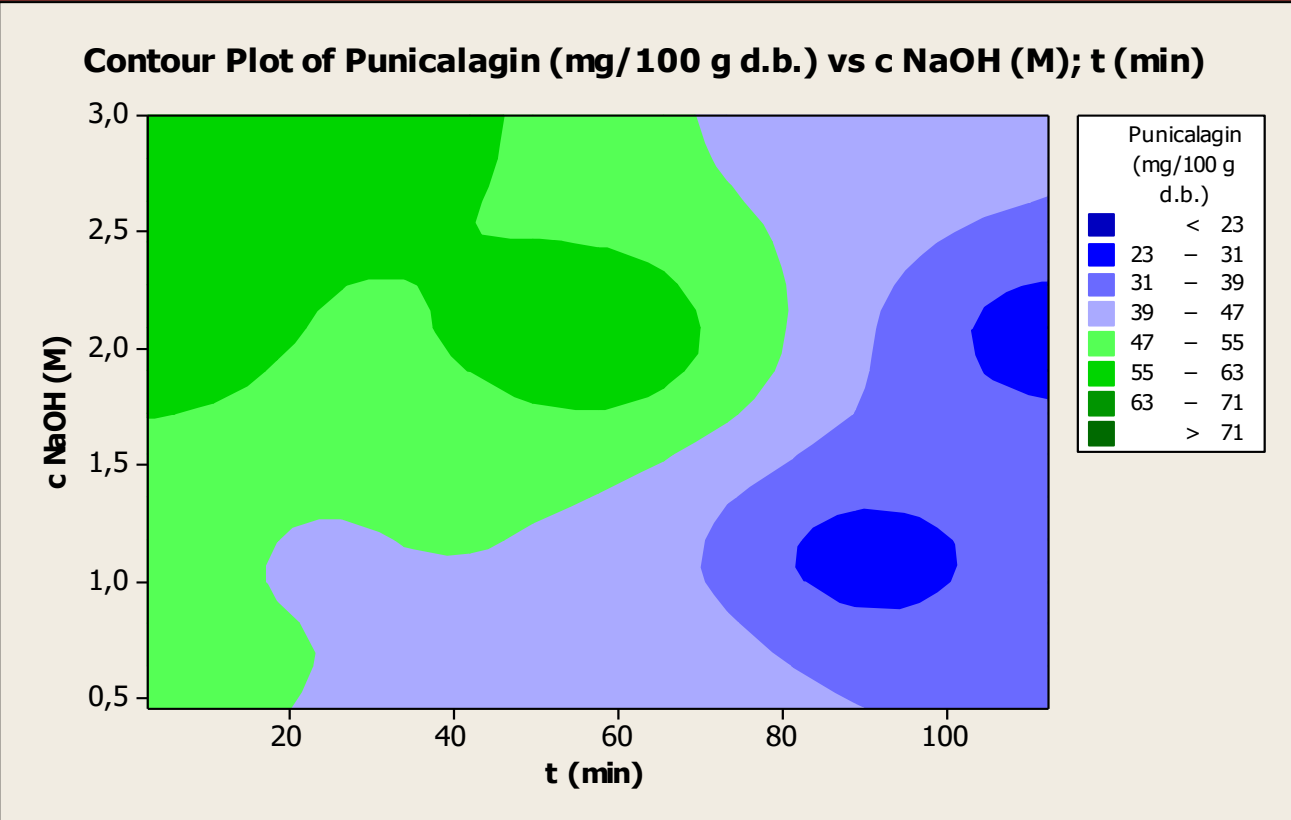
OPTIMUM CONDITIONS
t: 32 min
L/S: 45:1 mL/g
Concentration of NaOH: 1 M

Total phenol content- Folin

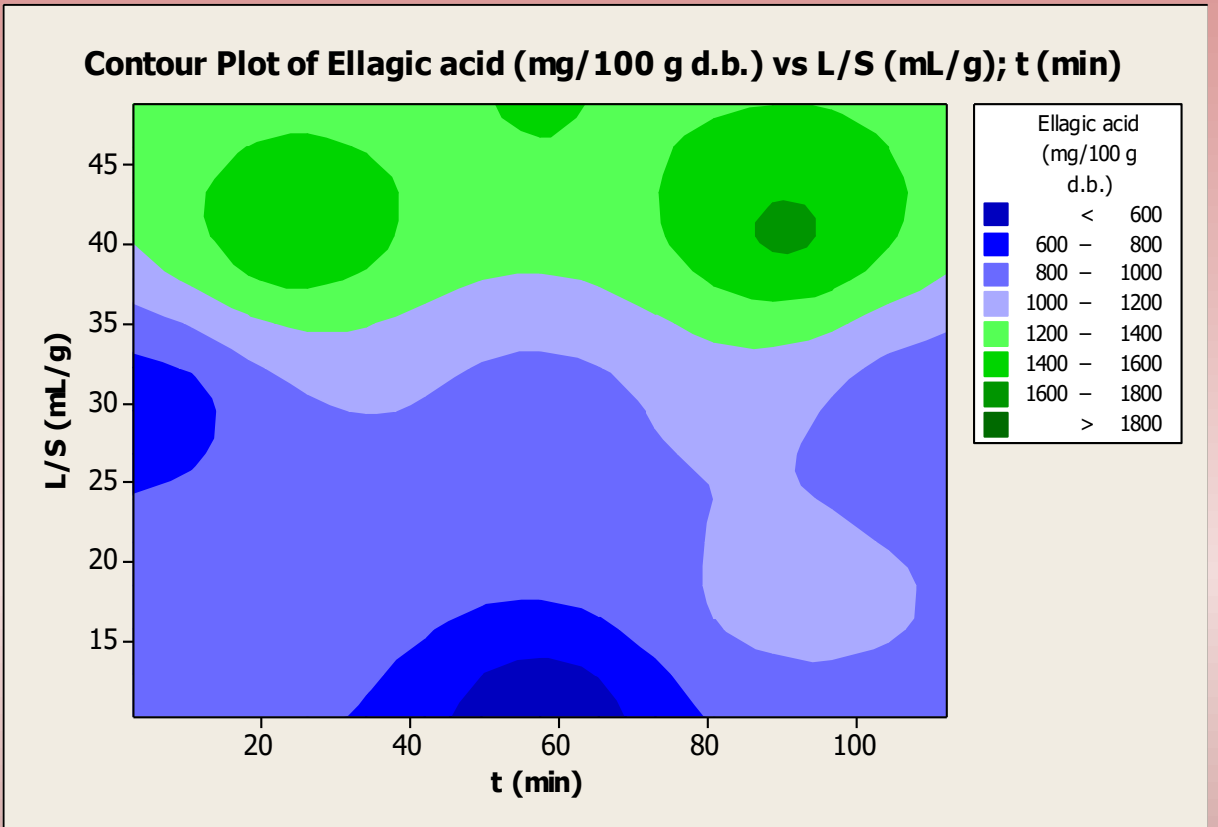
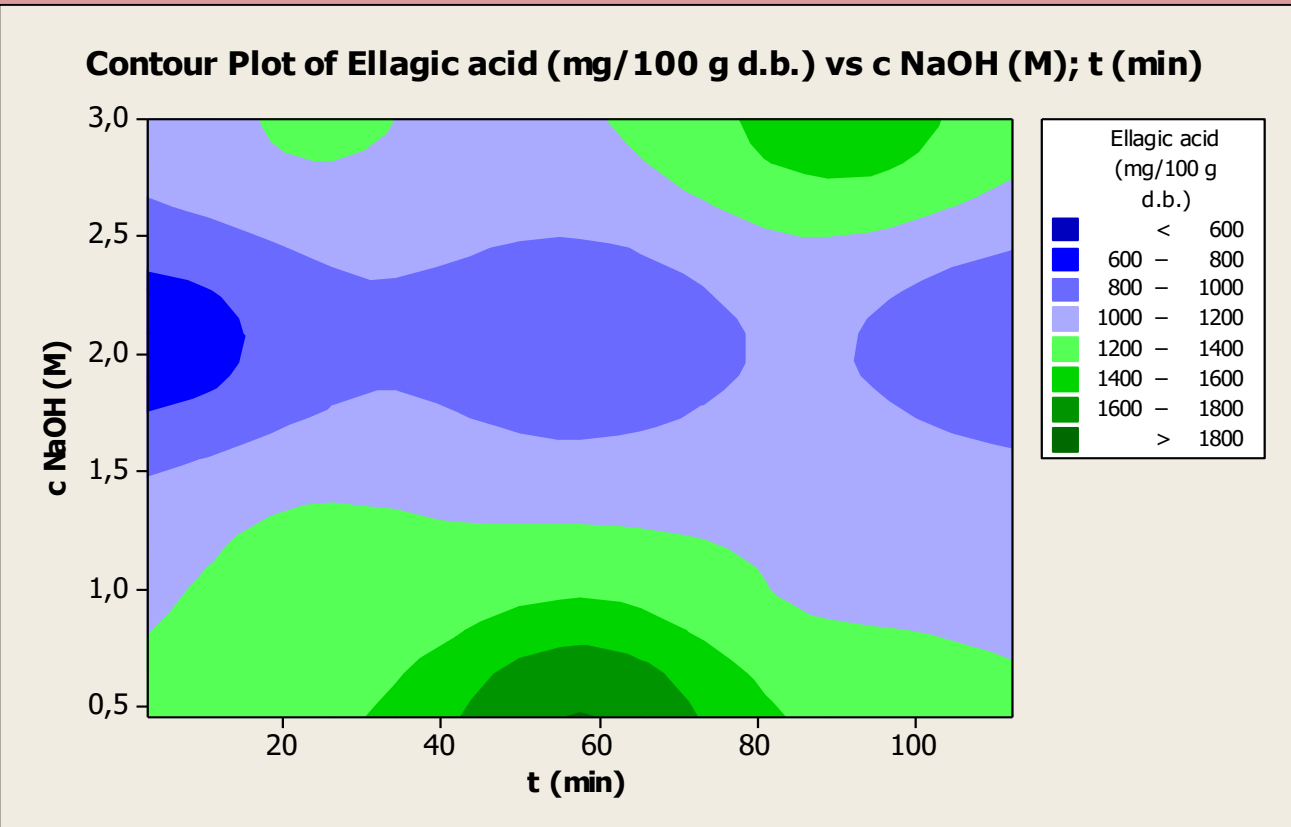


DETERMINATION OF MAIN PHENOLICS

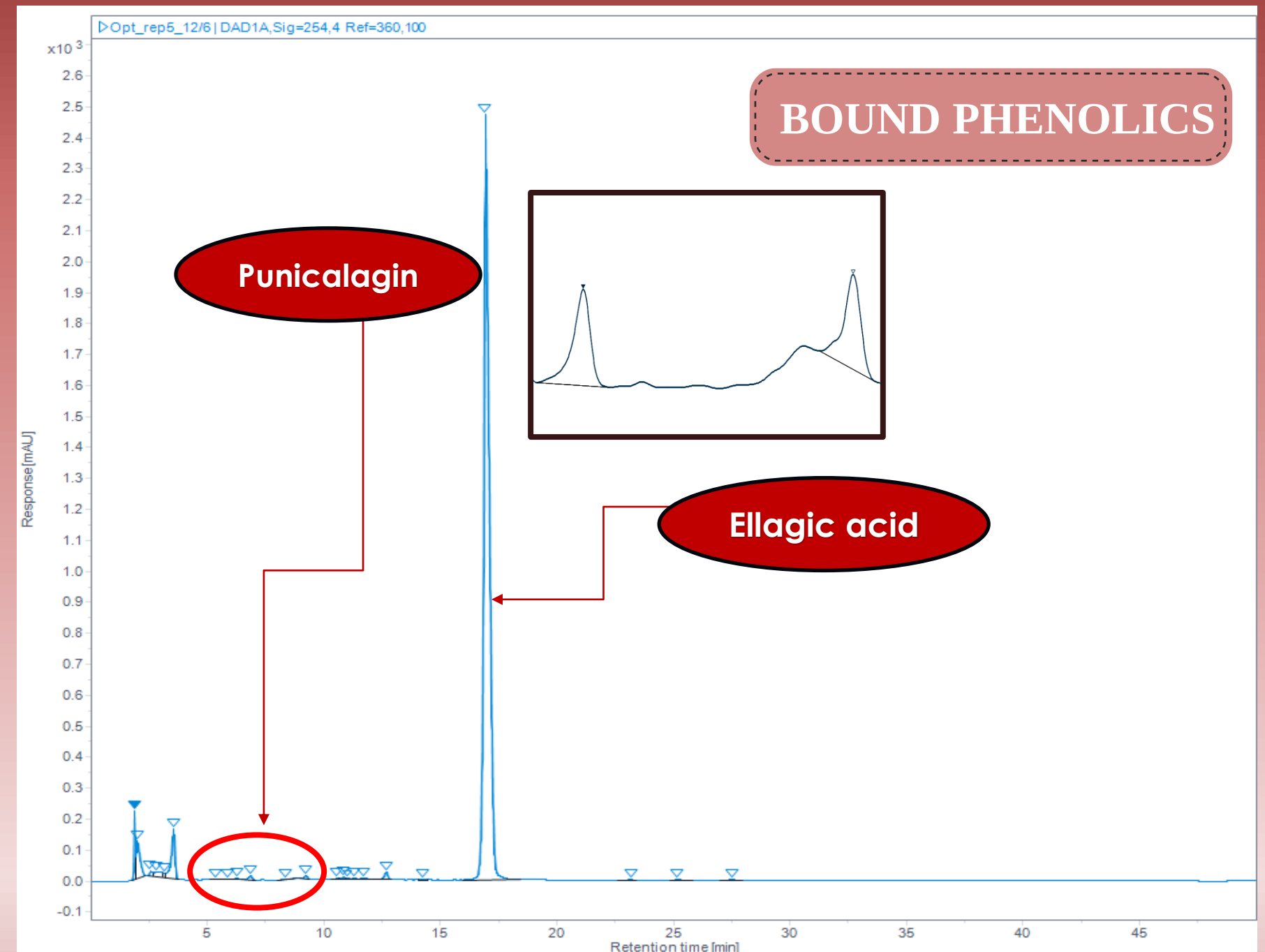
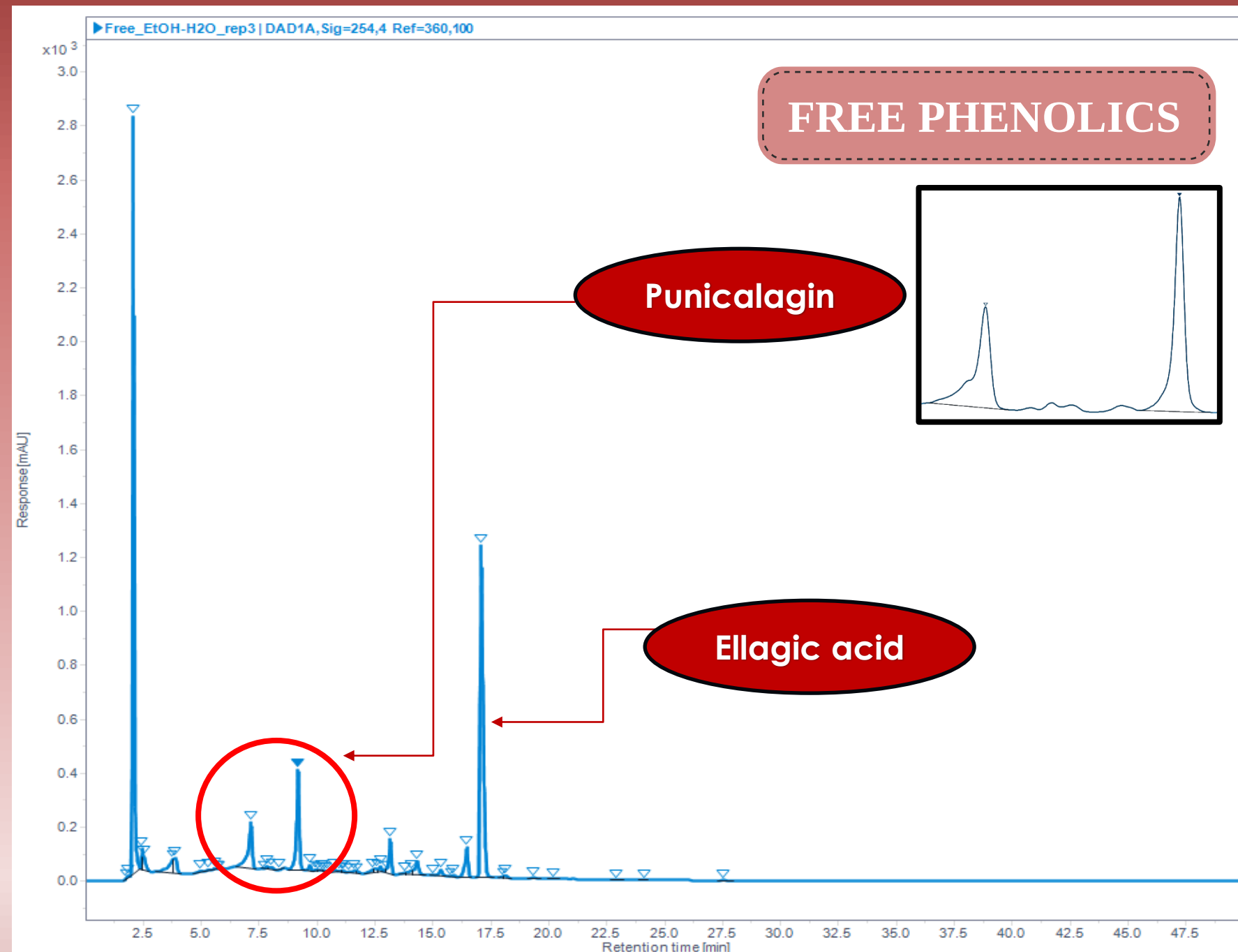
Punicalagin content



Ellagic acid content

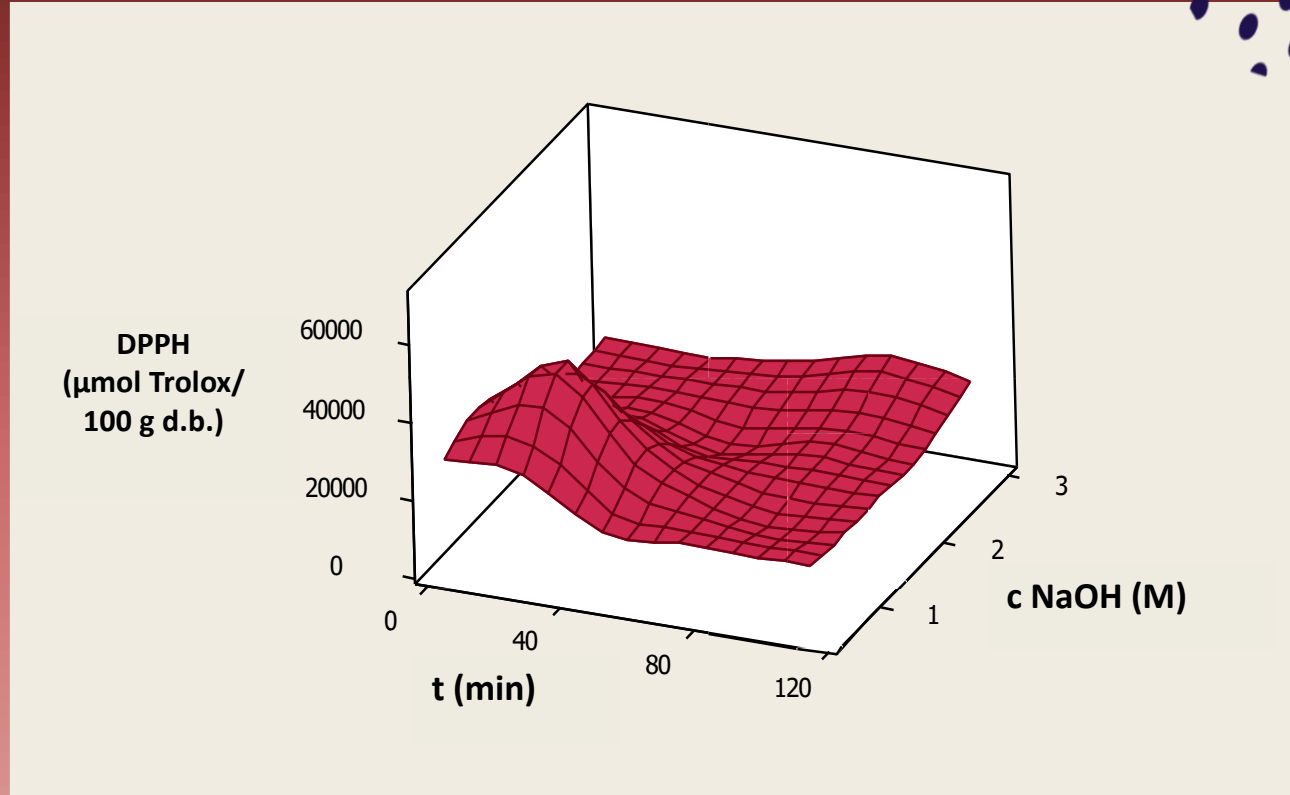
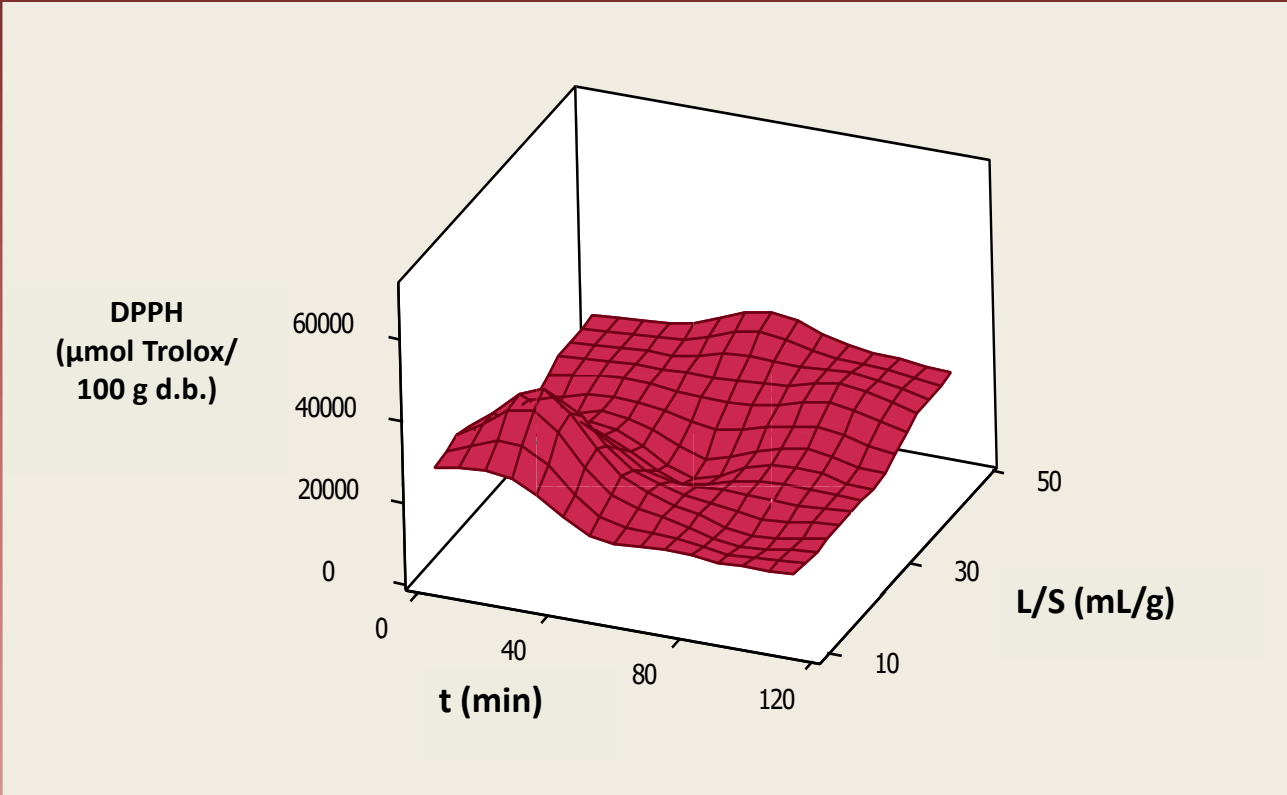


DETERMINATION OF MAIN PHENOLICS- HPLC ANALYSIS

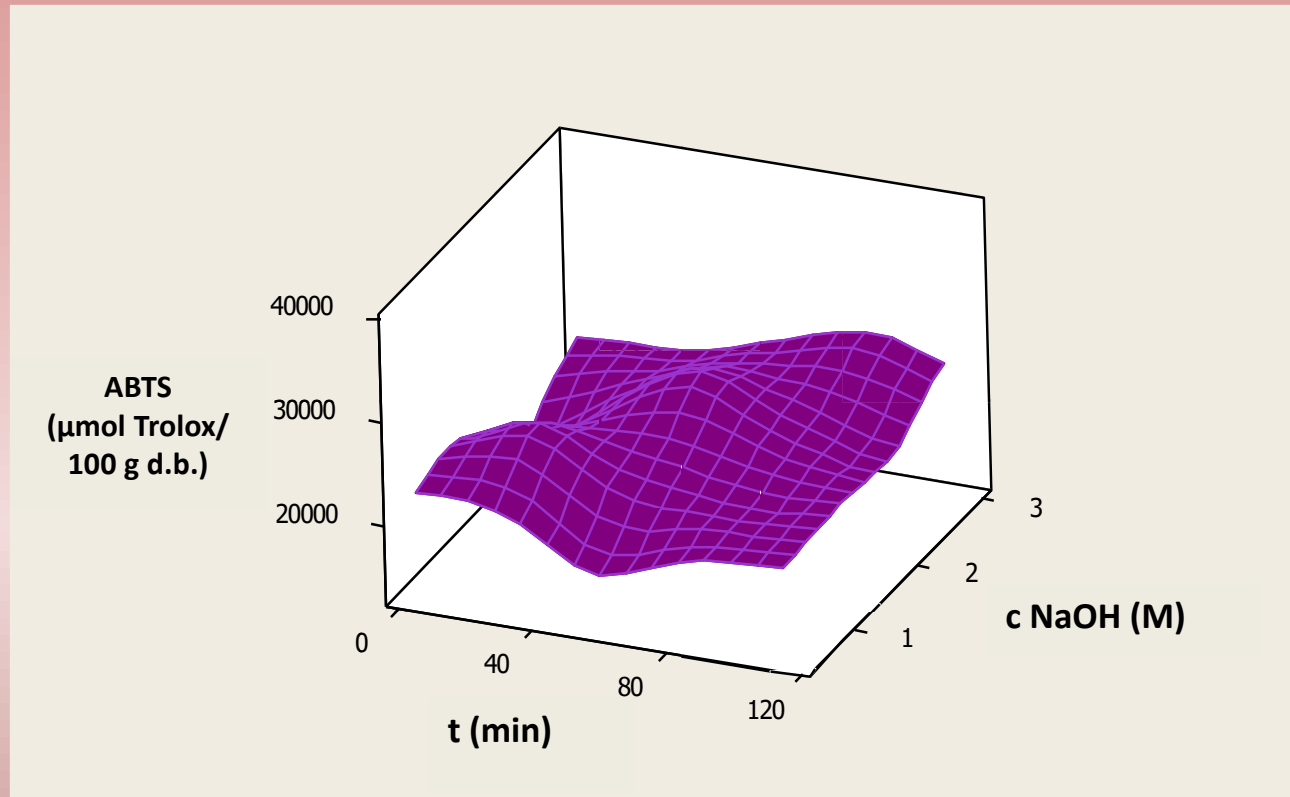
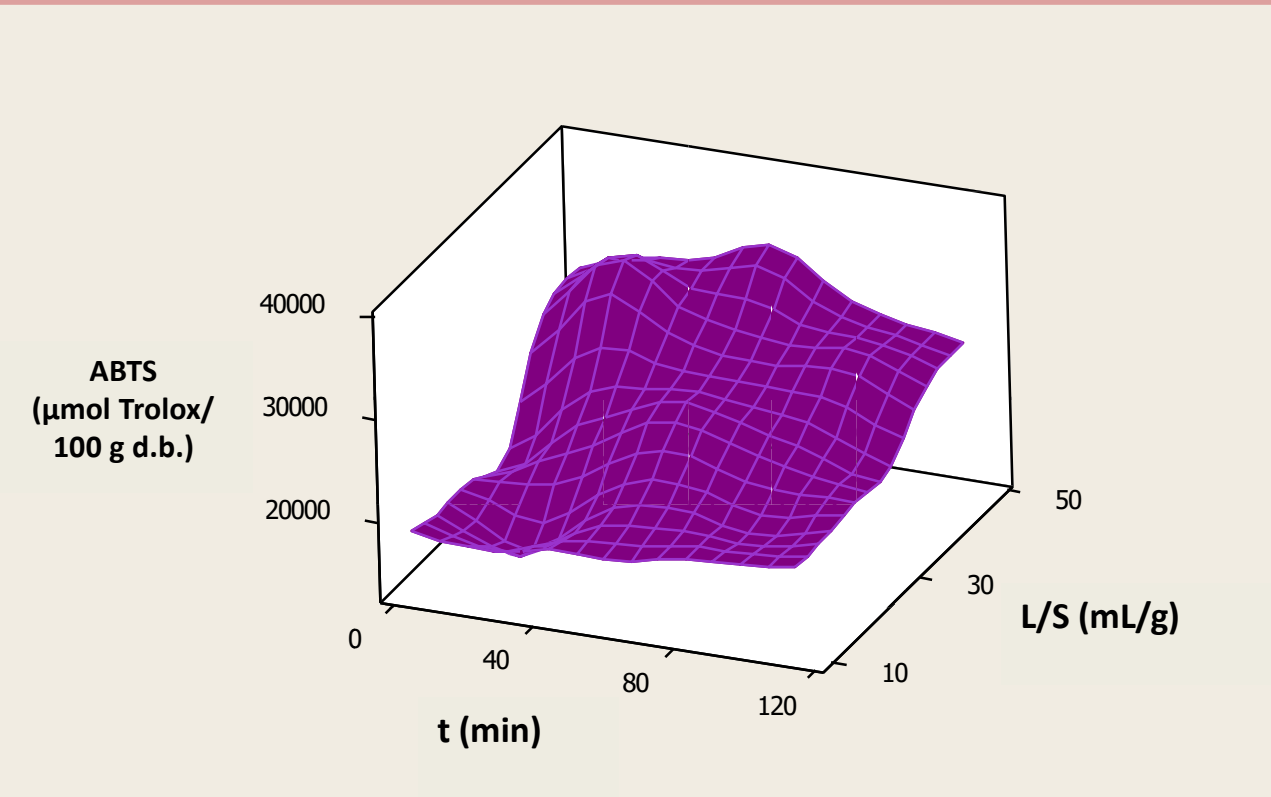


ANTIOXIDANT ACTIVITY

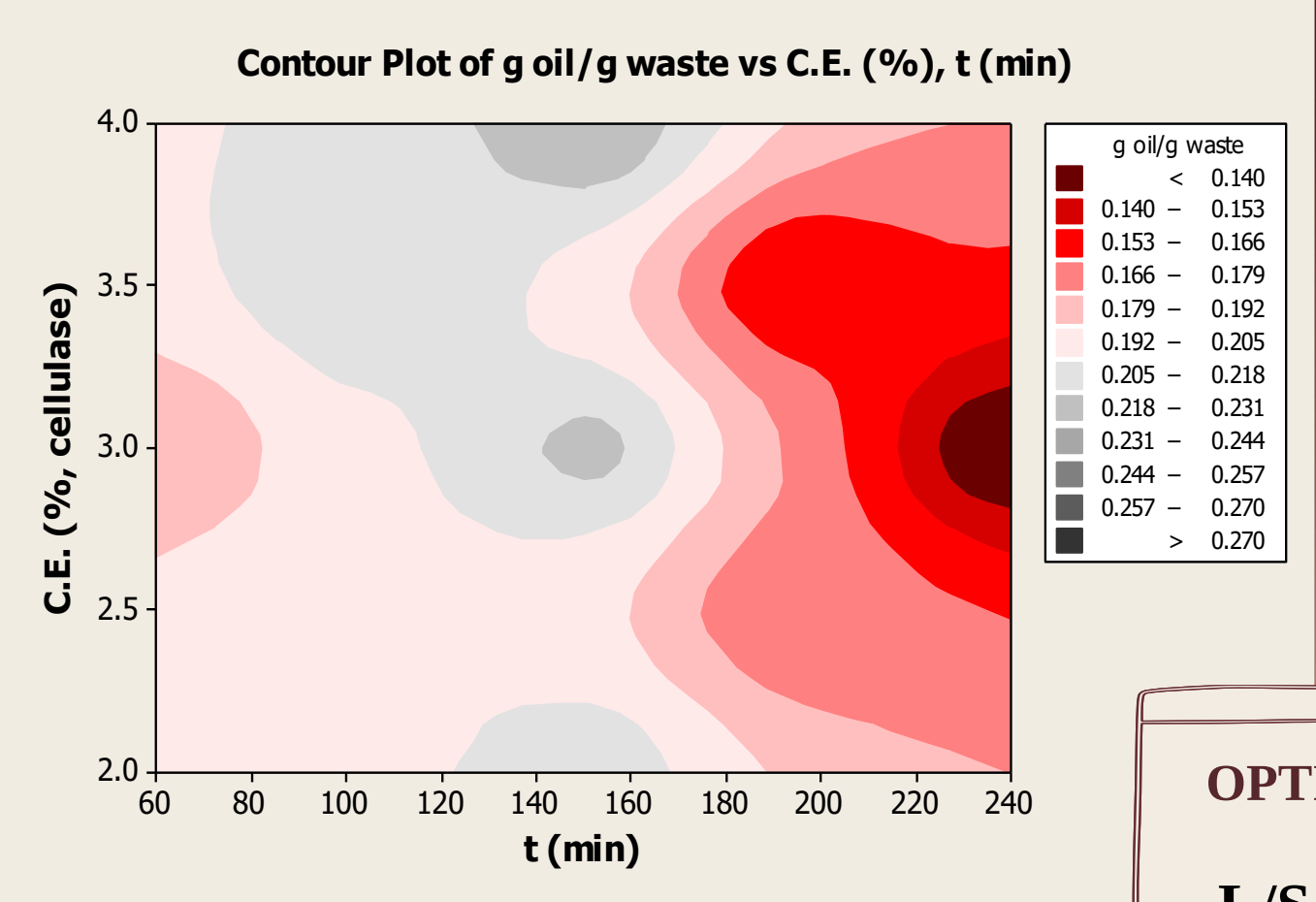
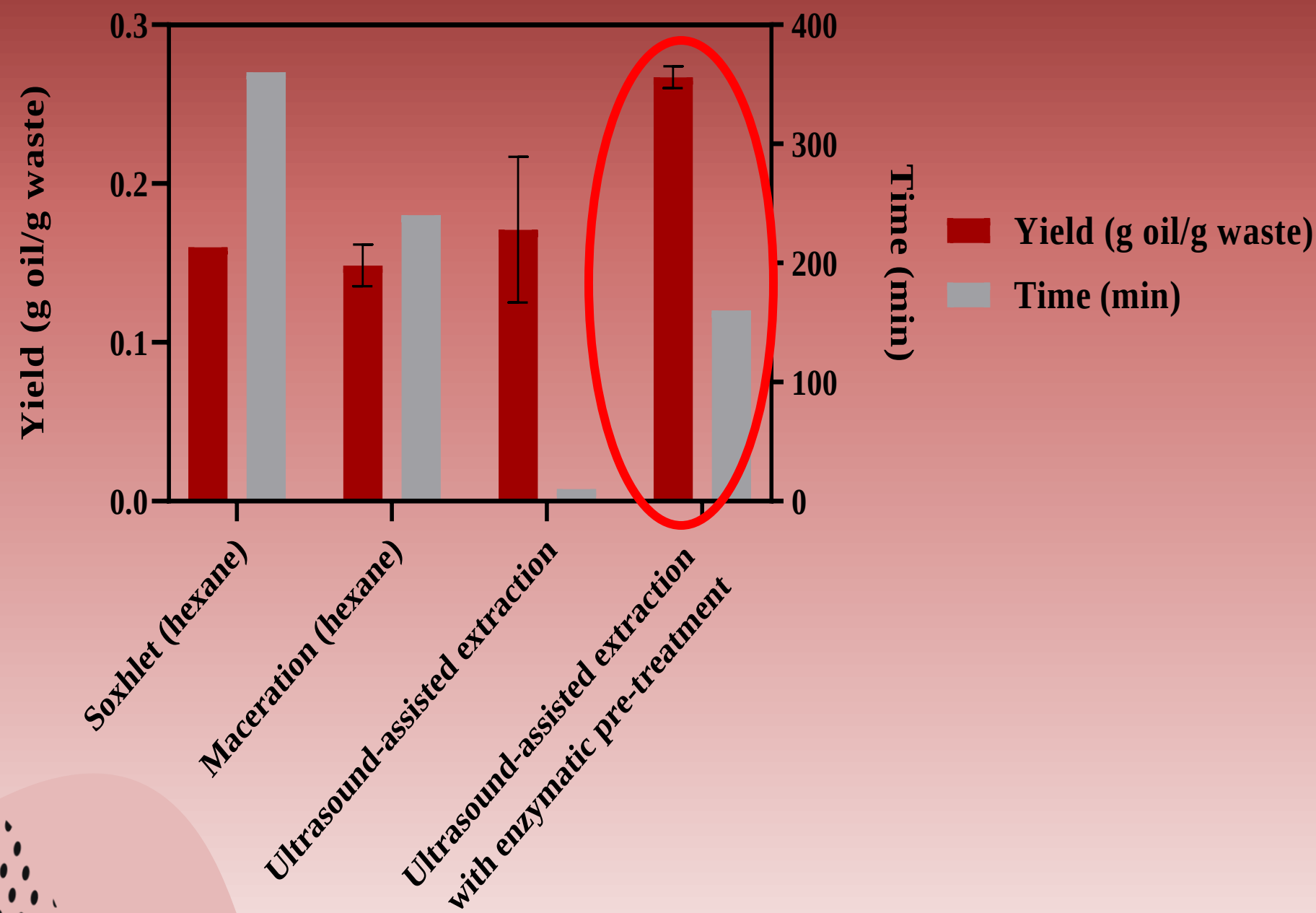
DPPH



ABTS



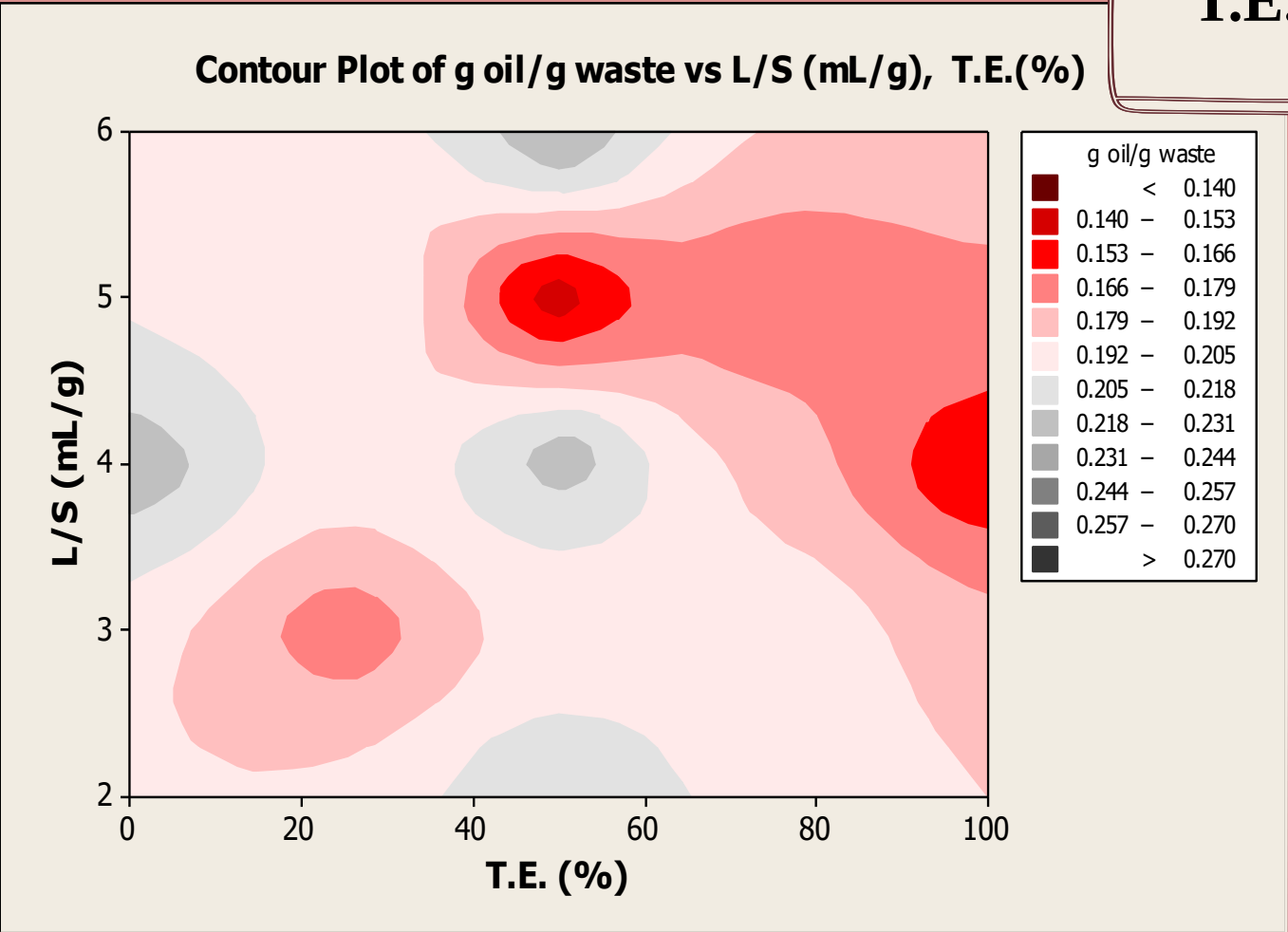
OIL EXTRACTION



0.275 g oil/g waste

OPTIMUM CONDITIONS

**L/S: 4 mL/g, C.E.: 3%,
T.E.: 50%, t:150 min**



CONCLUSIONS



50 % aq. EtOH →

Free phenolics from
pomegranate peels



Ultrasound-assisted
alkaline hydrolysis →

Bound phenolics
(ellagic acid)

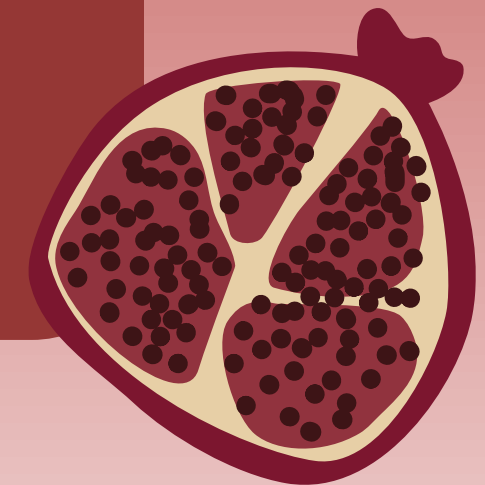


UAE with
enzymatic pre-
treatment →

Oil from
pomegranate seeds



THANK YOU VERY MUCH FOR YOUR ATTENTION!



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