D. Zentelis, N. Kotrotsos, D. P. Zagklis, V. Sygouni, F. N. Lamari, **C.A. Paraskeva, Isolation of high-added-value products from grape marc of the plant *Vitis vinifera* L.**, FORTH/ICEHT, PATRAS, GREECE

AKNOWLEDGEMENT

We acknowledge the support of this work by the Project "PPP_Phenolics" (code 03828), which is implemented under the Action "**2nd Call for H.F.R.I. Research Projects** to support Faculty Members and Researchers" funded by **Hellenic Foundation for Research and Innovation.**

Isolation of high-added-value products from grape marc of the plant *Vitis vinifera* L.

A. D. Zentelis[1,2], N. Kotrotsos[3], D. P. Zagklis[3], V. Sygouni[1,3], F. N. Lamari[1,2],

C.A. Paraskeva[1,3,*]

[1]Foundation for Research and Technology, Hellas, Institute of Chemical Engineering Sciences, FORTH/ICE-HT, GR-26504 Patras, Greece

[2] Department of Pharmacy, University of Patras, Greece

[3]Department of Chemical Engineering, University of Patras, GR-26504 Patras, Greece

Keywords: phenolics, isolations, membranes, extraction.

Presenting author email: takisp@chemeng.upatras.gr

‘A complete exploitation of agricultural wastes: Isolation of organic compounds with high added values’

Agro-industrial wastes

- Sugar industry
  - Sugar Beet pulp→ molasses for animal nutrition.
- Fruits and vegetables
  - Pulp after juice extraction (citrus, apples, tomatoes etc.)→ pectin from apple pomace, tomato pomace as animal feed, etc.
  - Coffee beverages
  - Tomato juices
- Vinification
  - Defective wine, grape marc→ alcohol rich solutions production, separation of phenolic content from solids.
- Olive oil production
  - Olive mill wastewater (3-phase extraction)→ separation of phenolic content, fertilizer, biological herbicide, animal feed.

‘A complete exploitation of agricultural wastes: Isolation of organic compounds with high added values’

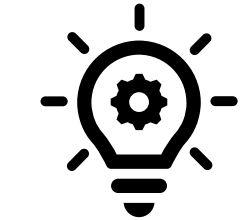
Scope

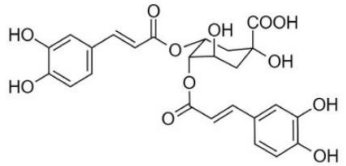
- Large amounts of agricultural by-products are produced every year, some of them rich in phenolic compounds.
- Phenols are antioxidants with high-added value and positive effects to the human health.
- Their separation to produce cosmetic products, food supplements etc., is of great interest.
- For this purpose, a combination of solid-liquid extraction, membrane filtration, liquid-liquid extraction, resin adsorption/desorption following by evaporation and freeze drying is proposed, to produce phenolic concentrates.
- The final products of the proposed process contain a large percentage of the by-products’ phenolic content, in a small fraction of the initial volume.
- This technique, after modification, can be applied to a variety of phenol-rich by-products, allowing the operation of phenol separation plant adjustable to local agricultural activities.

‘A complete exploitation of agricultural wastes: Isolation of organic compounds with high added values’

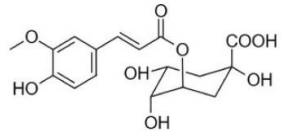
- Idea: To develop a method for the for maximum, cost-effective exploitation of agro-industrial wastewaters, using a combined process of membrane filtration and other physicochemical processes.

- **EFFECTIVE TREATMENT OF AGROINDUSTRIAL LIQUID AND SOLID WASTES (Environment). What we have tested so far…**
  - **OMW (3phase)**
  - **Pomace or Alperujo (2phase)**
  - **Olive leaves**
  - **Winery by-products**
  - Coffee by-products
  - Tomato by-products
- **ISOLATION OF PHENOLS, COMPOUNDS WITH HIGH ADDED VALUE (Profit). What have isolated and purified so far…**
  - **Hydroxytyrosol, Tyrosol (Olive mill wastewaters)**
  - **Oleuropein (olive leaves)**
  - trans-Resveratrol, **catechins (Winery by-products)**
  - Chlorogenic acid, caffeoylquinic acids, dicaffeoylquinic acids and feruloylquinic acids, (coffee by-products)-**COFFECO & COFFEE ISLAND, spin-off company**
  - Lycopene (tomatoes)

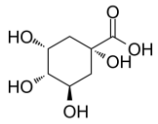




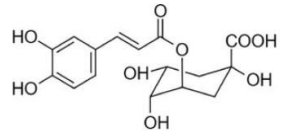
3,4-dicaffeoylquinic acid



5-feruloylquinic acid



Quinic acid



5-caffeoylquinic acid

Derivatives of chlorogenic acid

Part D: Winery by-products

Isolation of high-added-value products from grape marc of the plant *Vitis vinifera* L.

INTRODUCTION

During vinification and the treatment of *Vitis vinifera L.* species fruits, by-products such as grape marc and wine bottom sludges are produced in large quantities.

The high organic load contained in the grapes, combined with the large amounts produced every year, make necessary their treatment before its disposal to the environment.

On the other hand, (the toxicity of grape by-products in the environment) is attributed to their high content in phenolics, known for their high antioxidant activity.

Several treatment techniques, including physicochemical processes, are used to reduce the organic load of grape by-products) with simultaneous isolation of phenolic compounds, which are of high interest and high added value for cosmetic industry, food industry and pharmaceutics.

Isolation of high-added-value products from grape marc of the plant Vitis vinifera L.

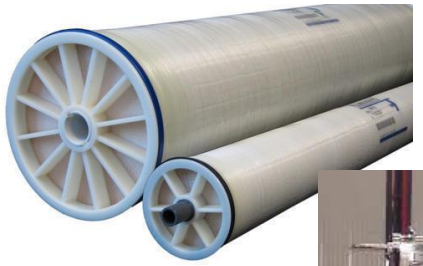
Scope

The present work is focused on the experimental investigation of the parameters during the extraction of phenolic compounds from grape marc Merlot variety), minimizing the amount of extracted carbohydrates.

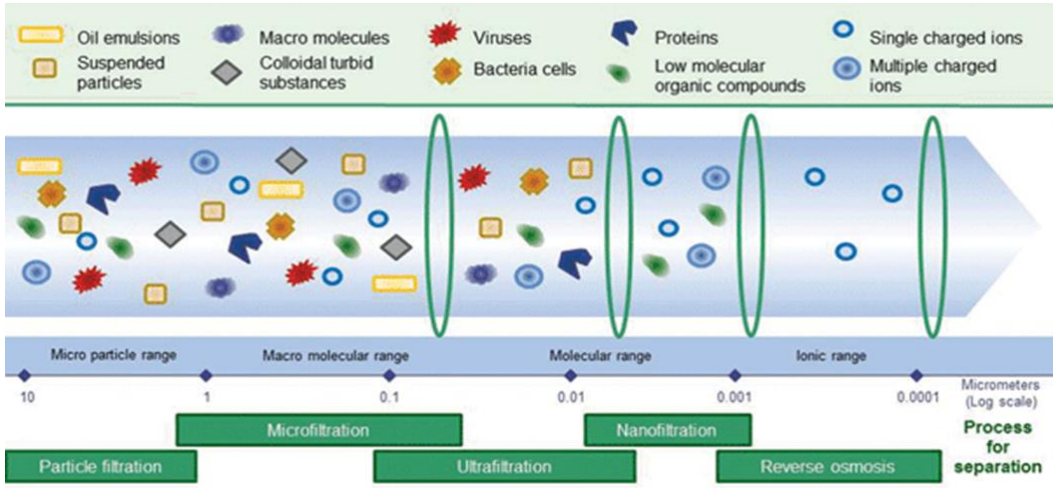
The experimental results obtained from the parametric analysis of the extraction process, were tested in a pilot scale experiments using a membrane system consisting of an Ultrafiltration (UF), two Nanofiltration (NF), and a Reverse Osmosis (RO) membranes.

More specifically, the product obtained from the extraction, was further treated with the pilot-scale membrane system and the final product was characterized considering its Total Phenolic Content (TPC), Total Sugar Content (TSC) and antioxidant capacity with the FRAP method. In addition, a qualitative analysis of the fractions was carried out using LC-MS.

Membrane filtration processes



Separation depends on pore size, Molecular weight cut-off, (MWCO)



Advantages	Disadvantages
Low Energy Consumption	Low selectivity
Readily combined with other physicochemical processes	Short lifetime
Easy to modify and adjust variables	Fouling effects and polarization concentration
Automation	Sensitivity to mechanical resistances
Installation on an industrial scale	Low permeate flowrates
Low Temperature conditions, **no phase changes**	Non-resistance to all chemicals
Application in many fields (food, juice, chemical industry)	? COST
No additional provisions are required	

Preparation of the raw material

- Grape marc- Variety of Merlot
- Harvesting period, August 2019– C. Achaia
- OINIKI- George Karelas, K. Achaia
- I. Kotrotsos, Vasiliko, Achaias

Grape marc
(Merlot)

Natural drying (3 days),
RT

grinding

drying 1-2 days
25^{o}C

Grape marc,
Grinded and dried

Extraction Condition- parameter values

Conditions:

- Solvent type
- *Solid/liquid ratio (w/v)*
- Temperature
- Duration of the extraction

a) **Solvents:** Water, ethanol (EtOH), acetone, Polyethylene glycol (PEG), ethyl acetate,

- aqueous solutions of the above organic solvents (50-50 %)

Parameter Values				
Solvent	Ratio (w/v)	Time	Temperature	Other Parameter
any	1/10	60 min	RT	-

b) Solid/liquid ratio (w/v): 1/5 (w/v), 1/10 (w/v)

Extraction conditions				
Solvent	Ratio (w/v)	Time	Temperature	Other Parameter
Water	Variable	60 min	RT	1/5, 1/10 (w/v)
EtOH 50%	Variable	60 min	RT	1/5, 1/10 (w/v)

Parametric study of extraction conditions

c) Extraction duration

Extraction conditions				
Solvent	Ratio (w/v)	Time	Temperature	Other Parameter
Water	1/5	Variable	RT	10, 30, 60 min
Water	1/10	Variable	RT	10, 30, 60 min

d) Temperature

Extraction conditions				
Solvent	Ratio (w/v)	Time	Temperature	Other Parameter
Water	1/5	60 min	Variable	10, 25, 40, 50 and 60°C

In all studies the resulting extract was first filtered through a series of sieves and finally processed by centrifugation to remove any suspended particles

Grape marc - Chemical composition and applications

TPC 3.6-4.7% DM

- Phenolic acids
  - gallic acid
  - Syringic acid
  - p-coumaric acid
- Flavan-3-ols
  - (+)-catechin
  - (-)-epicatechin
  - (-)-epicatechin gallate

- Anthocyanidins
  - Malvidin-3-O-glycoside
  - Delphinidin-3-O-glycoside
  - Cyanidin-3-O-glycoside
  - Peonidin-3-Oglycoside

- Stilbenes
  - trans-resveratrol

- Procyanidins (tannins)
  - Procyanidin dimers
  - Procyanidin trimers

- Flavones
  - Quercetin
  - Kaempferol

Chemical composition of Merlot grape marc

Moisture 55-75%	Carbohydrates 17-29%
Fats 7.1-11.4%	Total Phenolic Content, TPC, 3.6 – 4.7%
Proteins 6-10%	Fibers 43-75%
Ashes 4.5-6.1%	

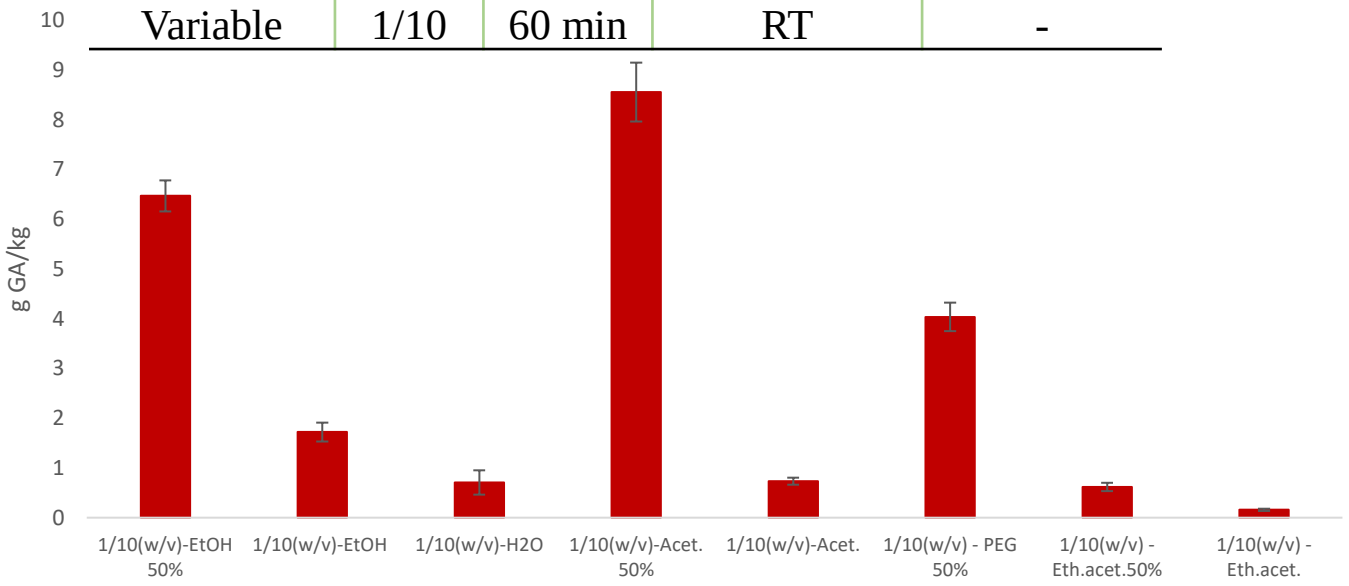
Application sectors	Use
Livestock	**Animal feed**
Agriculture	Fertilizers
Alcohol Distillery	Alcohol and alcoholic beverages
Food Industry	As functional foods, Food supplements Preservatives, Increasing the added value of food
Pharmaceutical industry **Cosmetics**	Supplements Improvement of intestinal flora Cosmetics
Gastronomy	Oils
Coloring	Pigments

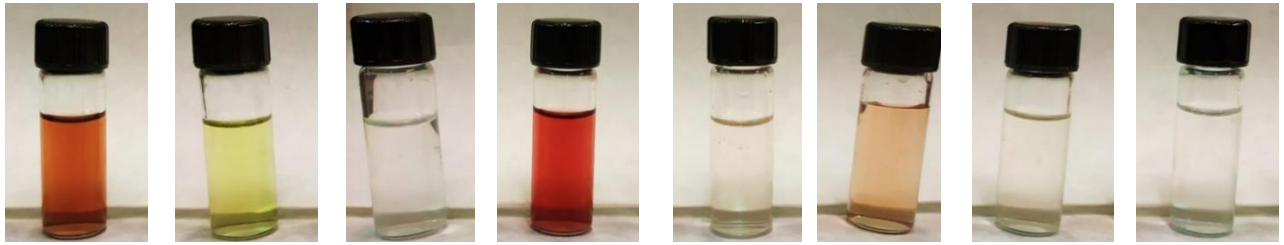
Parametric study of extraction conditions

a) Solvent

Extraction conditions

Solvent	Ratio (w/v)	Time	Temperature	Other Parameter
Variable	1/10	60 min	RT	-





ethanol 50% | ethanol 95% | water | acetone 50% | acetone 100% | PEG 50 % | Eth. acet 50% | Eth. acet

Values of TPC (g GAE/kg)

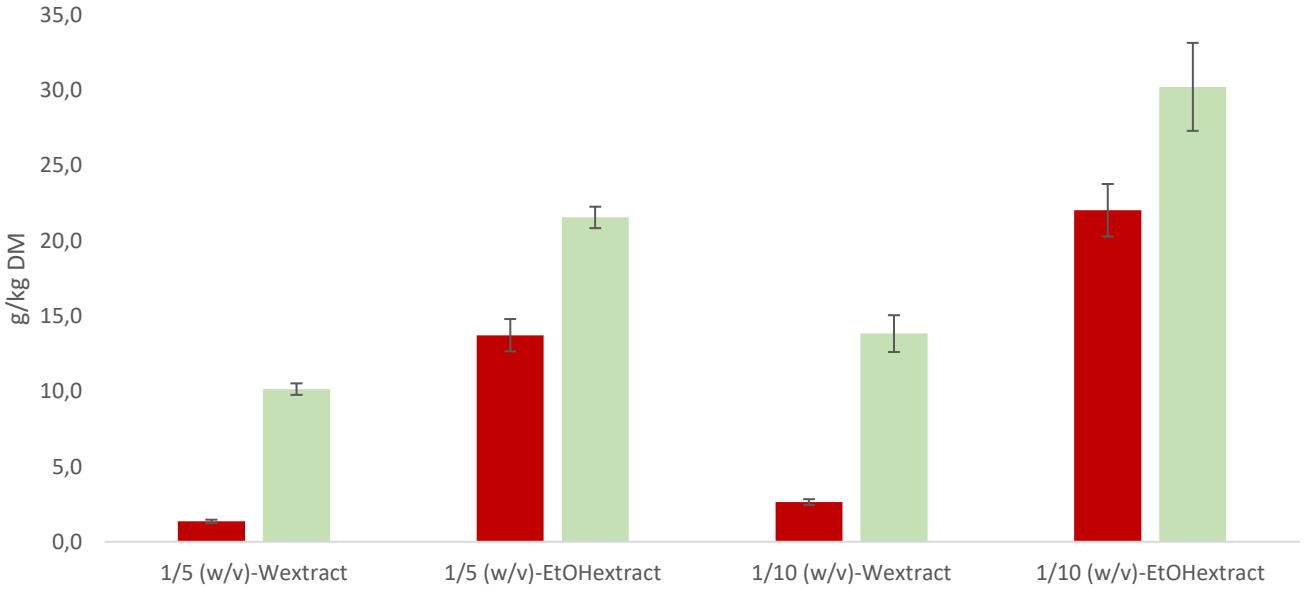
Acetone 50% (8.56 ± 0.59 g GAE/kg)
Ethanol 50% (6.46 ± 0.31 g/kg DM)
PEG 50% (4.03 ± 0.28 g GAE/kg)
Acetone 100% (0.74 ± 0,.7 g/kg)
Ethanol 95% (1.73 g GAE/kg)
water (0.71 ± 0,.4 g GAE/kg)

- Max. acetone 50% & ethanol 50%
- Min. Eth. Acet. & water
- **The presence of water enhances extractability**
- Color → proportional to TPC content
- Green color→ Chlorophyll

Acetone=> not suitable for membranes

Parametric study of extraction conditions

b) Solid/liquid ratio (w/v)



Extraction conditions

Solvent	Ratio (w/v)	Time	Temperature	Other Parameter
Water	Variable	60 min	RT	-
EtOH 50%	Variable	60 min	RT	-

➢ Ethanolic extractions:

High extractability of TPC,
Good ratio of TSC/TPC.
A solid/liquid ratio of 1/10 (w/v) leads to further TPC extraction
A solid/liquid ratio of 1/5 results in a large volume loss

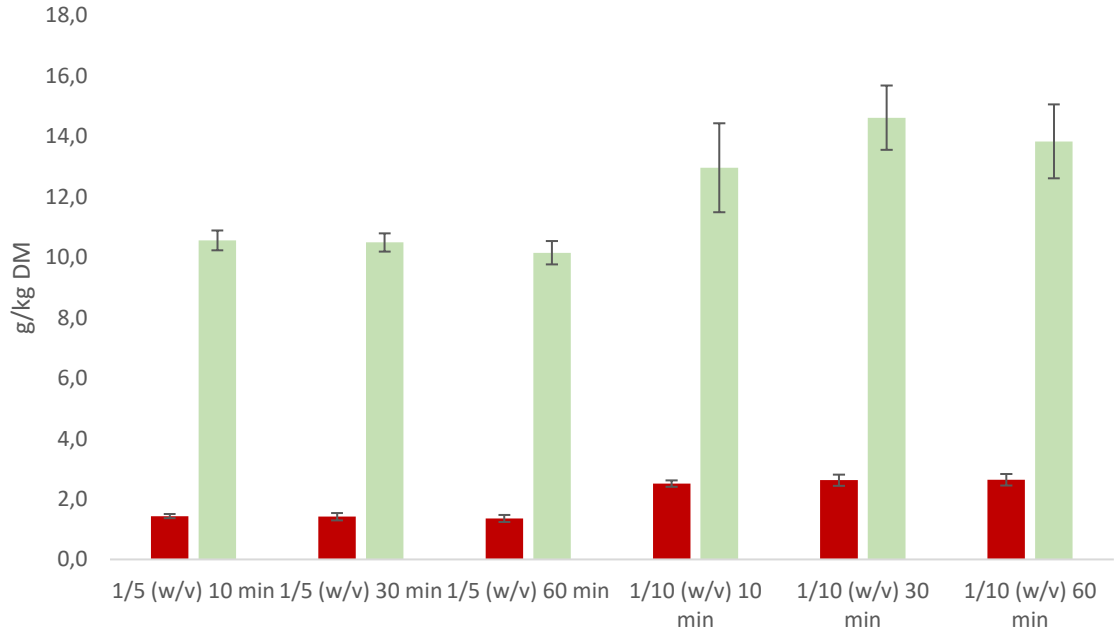
(w/v)	TPC (g GAE/kg DM)	TSC (g GLU/kg DM)	Ratio TSC/TPC	Volume loss (%)
1/5 (w/v) -$W_{extract}$	**1.36 ± 0.12**	**10.15 ± 0,39**	**7.4**	**36.6**
1/10 (w/v) - $W_{extract}$	2,64 ± 0,19	13.83 ± 1.22	5.2	22.0
1/5 (w/v) - $EtOH_{extract}$	13,72 ± 1,08	21.54 ± 0.71	1.5	48.0
1/10 (w/v) -$EtOH_{extract}$	**22.01 ± 1.74**	**30.21 ± 2.92**	**1.3**	**25.3**

➢ Aqueous extractions

Low extractability of TPC, High extractability of TSC
No significant differences are observed between the ext. with S/L ratio of1/5 and 1/10 (w/v).
The S/L ratio 1/5 shows the smallest TPC extractability

Parametric study of extraction conditions

c) Duration of extraction



Extraction conditions

Solvent	Ratio (w/v)	Time	Temperature	Other Parameter
Water	1/5	Variable	RT	-
Water	1/10	Variable	RT	-

- Duration times in each ratio (w/v) appear similar

In terms of TSC and TPC level

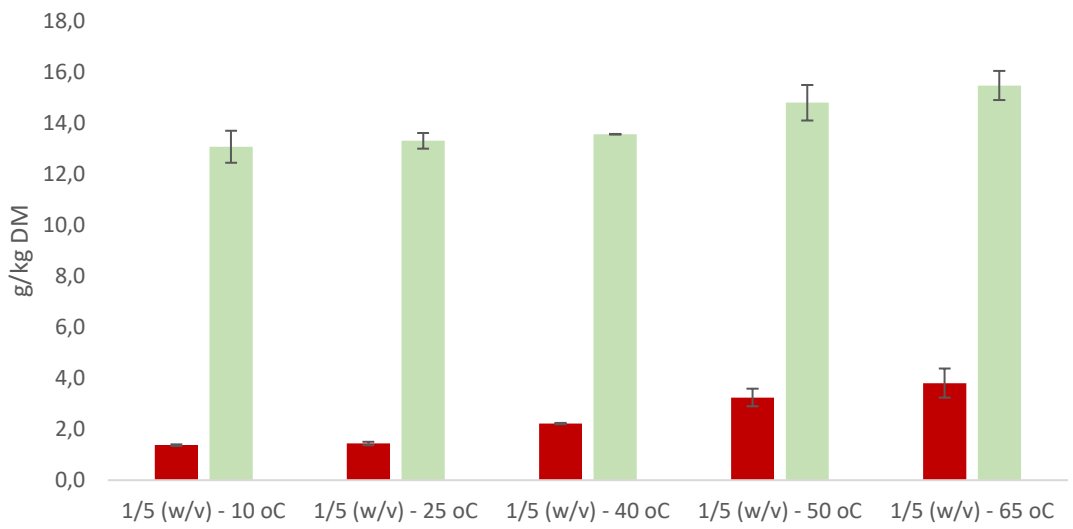
- The TSC/TPC ratio in the extraction 1/5 (w/v) > 1/10 (w/v)
- Extraction of TPC increases from 1/5 (w/v) → 1/10 (w/v) 100%
- Extraction of TSC increases from 1/5 (w/v) → 1/10 (w/v) 35%

(w/v)	TPC (g GAE/kg DM)	TSC (g GLU/kg DM)	Ratio TSC/TPC	Volume loss (%)
1/5 (w/v) – 10 min	**1.44 ± 0.07**	**10.56 ± 0.33**	**7.3**	
1/5 (w/v) – 30 min	1.41 ± 0.13	10.49 ± 0.30	7.4	36.7
1/5 (w/v) – 60 min	1.36 ± 0.11	10.14 ± 0.39	7.4	
1/10 (w/v) – 10 min	2.51 ± 0.11	12.95 ± 1.47	5.1	
1/10 (w/v) – 30 min	2.62 ± 0.18	14.61 ± 1.06	5.5	22.0
1/10 (w/v) – 60 min	2.64 ± 0.19	13.83 ± 1.22	5.2	

Solid/Liquid ratio 1/5 and t=10 min shows the lowest extractability

Parametric study of extraction conditions

d) Temperature



Extraction conditions				
Solvent	Ratio (w/v)	Time	Temperature	Other Parameter
Water	1/5	60 min	Variable	-

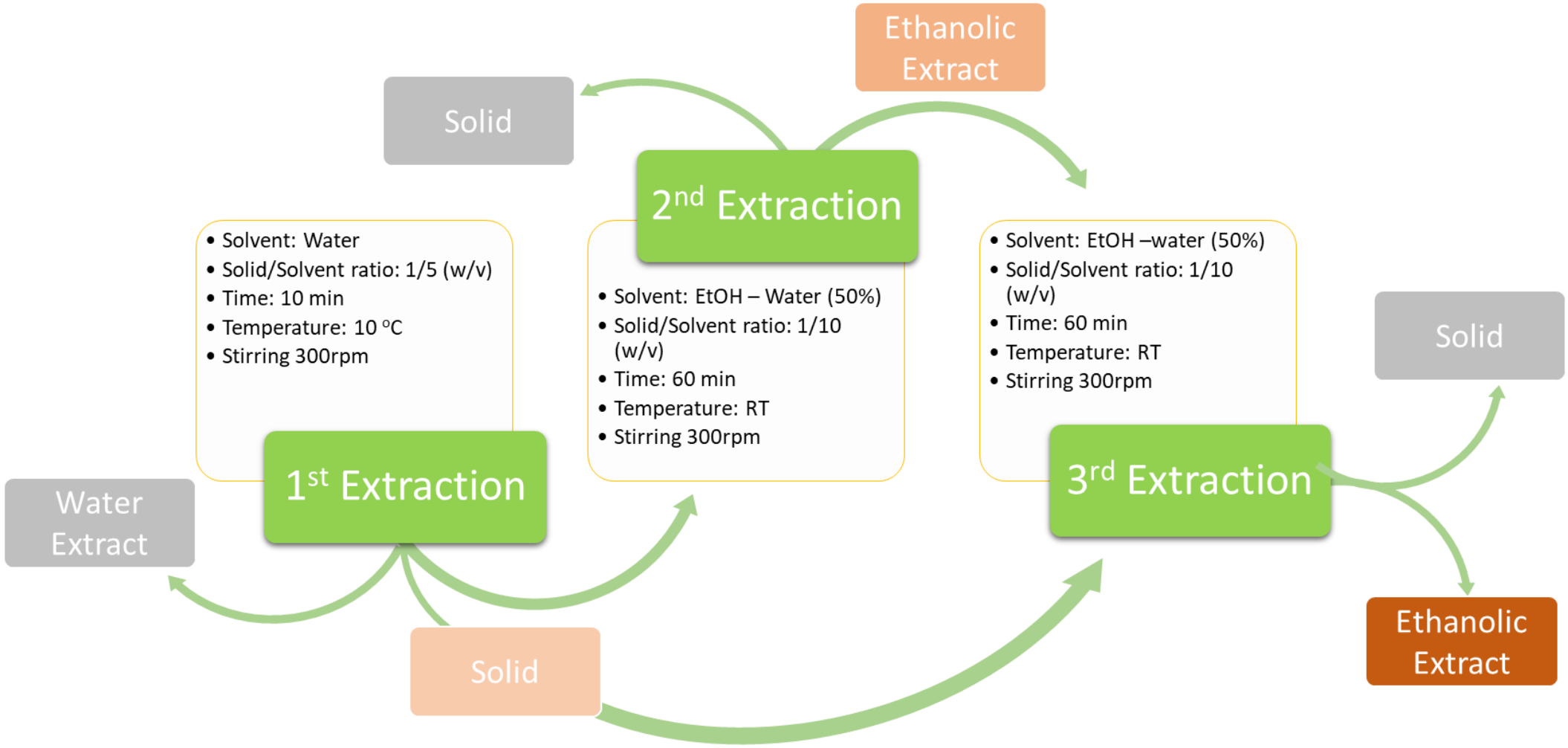
- The extractability of both TSC and TPC increases with increasing temperature
- Increase TPC>TSC → decrease of the ratio TSC/TPC

(w/v)	TPC (g GAE/kg DM)	TSC (g GLU/kg DM)	Ratio TSC/TPC
1/5 (w/v) – 10 ºC	**1.37 ± 0.03**	**13.07 ± 0.63**	**9.5**
1/5 (w/v) – 25 ºC	1.44 ± 0.07	13.30 ± 0.30	9.2
1/5 (w/v) – 40 ºC	2.21 ± 0.02	13.56 ± 0.10	6.1
1/5 (w/v) – 50 ºC	3.23 ± 0.35	14.80 ± 0.69	4.5
1/5 (w/v) – 65 ºC	3.80 ± 0.57	15.47 ± 0.57	**4.0**

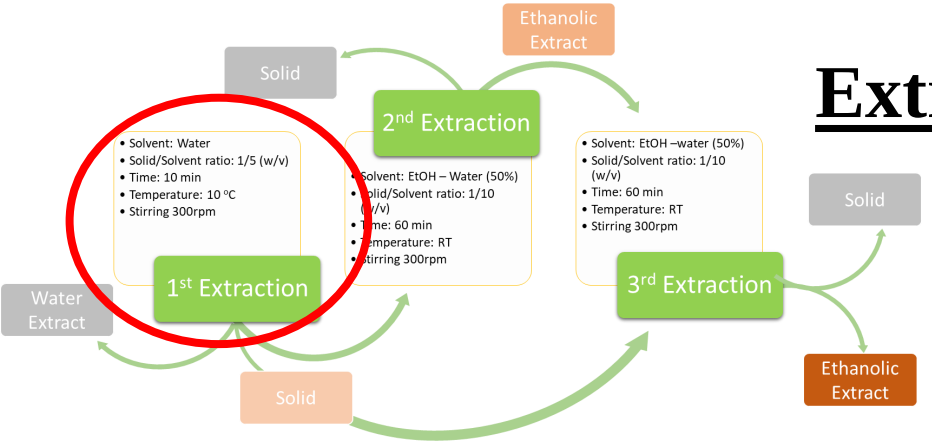
- **Maximum TSC/TPC ratio at 10ºC**
- **Maximum TPC/TSC ration at 65ºC,**

Extraction in pilot plant equipment

Extraction conditions



Extraction in pilot plant equipment



1º stage aqueous extractions

6 kg of grape marc
(Dried and Grinded)

30 L water at 10ºC

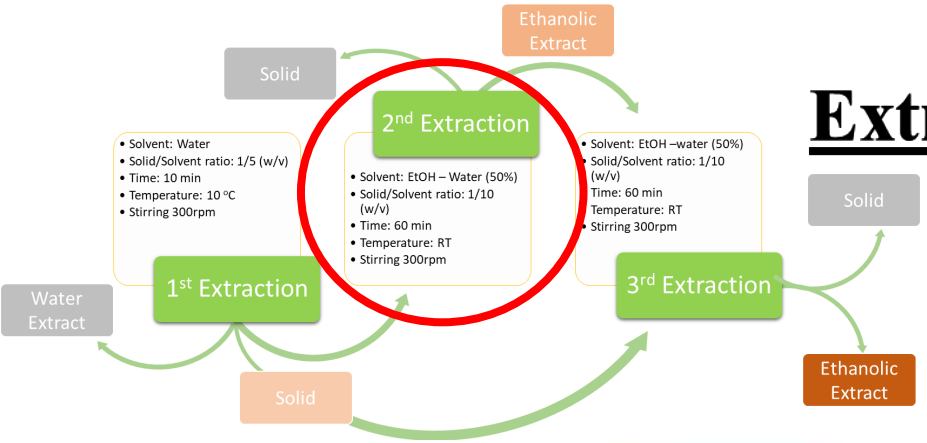
10 min extraction
(stirring)

23 L water extract

Filtration

2.00 mm, 0.600 mm, 0.125 mm

6 kg solid
(+ 6.5 L moisture)



Extraction in pilot plant equipment

2º stage – ethanolic extraction

3 kg of grape marc (with 3 L solution)

27 L EtOH 50% at RT

60 min extraction (stirring)

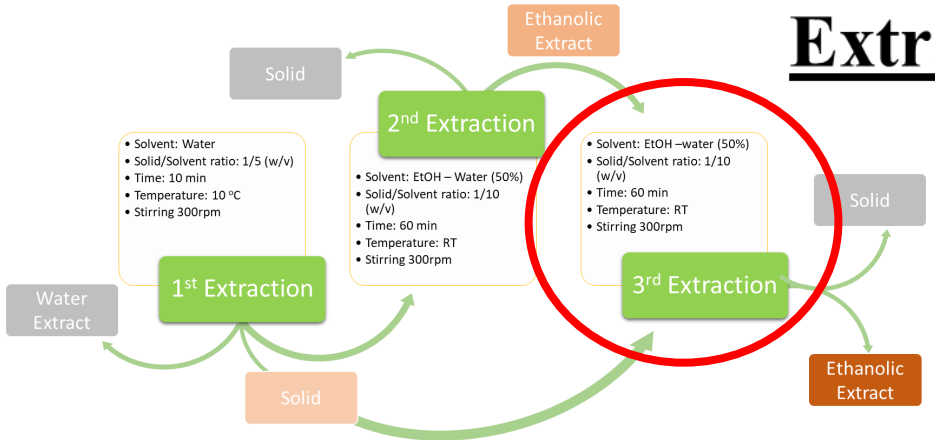
3 kg of grape marc (with 4 L solution)

Filtration

2,00 mm, 0,600 mm, 0,125 mm

24 L ethanolic extract

Extraction in pilot plant equipment



3º stage – ethanolic extraction

24 L ethanolic extract

2.7 kg of grape marc (with 3 L solution)

60 min extraction (stirring)

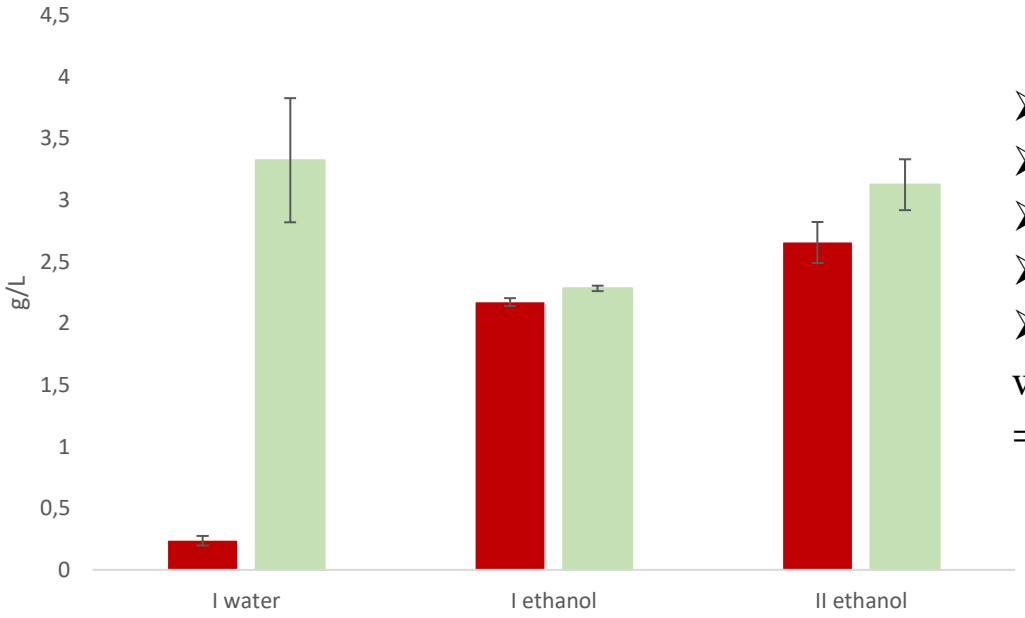
2.5 kg of grape marc (with 5 L solution)

Filtration

2,00 mm, 0,600 mm, 0,125 mm

18 L ethanolic extract

Extraction in pilot plant equipment



- I_{water} : Significant extraction of TSC, small that of TPC
- => ratio TSC/TPC= 14.0
- $I_{ethanol}$: The TSC/TPSC ratio is 1 because a portion of the TSC
- removed during aqueous extraction
- $II_{ethanol}$: Further extraction of TPC as well as TSC,

with the values of the corresponding concentrations
=> 2.65 ± 0.16 (g GAE/L) and 3.12 ± 0.20 (g GLU/L) => ratio TSC/TPC =1.2

Extraction code	TPC (g GAE/L)	TPC (g)	TSC (g GLU/L)	TSC (g)	Ratio TSC/TPC
I_{water}	0.24 ± 0.04	5.64 ± 0,94	3.32 ± 0.50	78.02 ± 11.75	**14.0**
$I_{ethanol}$	2.17 ± 0.03	52.08 ± 0.72	2.28 ± 0.02	54.72 ± 0.48	**1.0**
$II_{ethanol}$	2.65 ± 0.16	47.70 ± 2.88	3.12 ± 0.20	56.16 ± 3.60	**1.2**

- TPC recovery from Iethanol to IIethanol extraction decreases despite increasing of phenolics concentrate (52.14 ± 0.79 → 47.81 ± 2.99 g)
- Solvent loss is an important factor

Antioxidant Capacity- FRAP

	I_{water}	$I_{ethanol}$	$II_{ethanol}$
mmol Fe^{2+}/L	0.13 ± 0.04	21.11 ± 0.39	**30.78 ± 0.42**

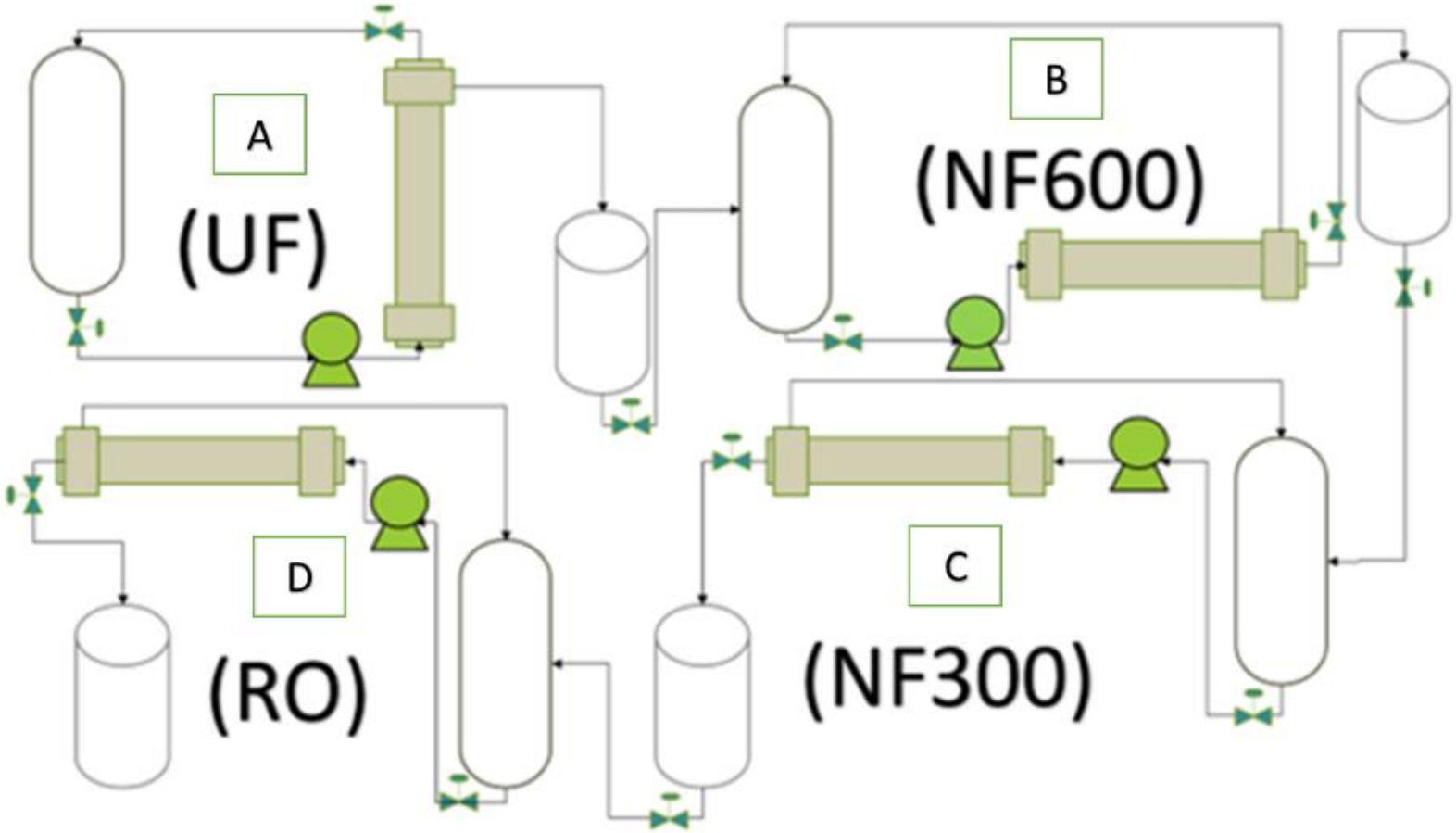
- FRAP => I_{water} : Low Antioxidant Capacity (0.13 mmol Fe^{2+}/L)
  $I_{ethanol}$: High Antioxidant Capacity (21.11 mmol Fe^{2+}/L)
  $II_{ethanol}$: High Antioxidant Capacity (30.78 mmol Fe^{2+}/L)
  The enrichment of the ethanolic extract is confirmed.

Separation by membrane filtration processes

4 types of membranes

1. UF: with MWCO 100 nm
2. NF600: with MWCO 600 Da
3. NF300: with MWCO 150-300 Da
4. RO: (Salt rejection)

NaCl 99%

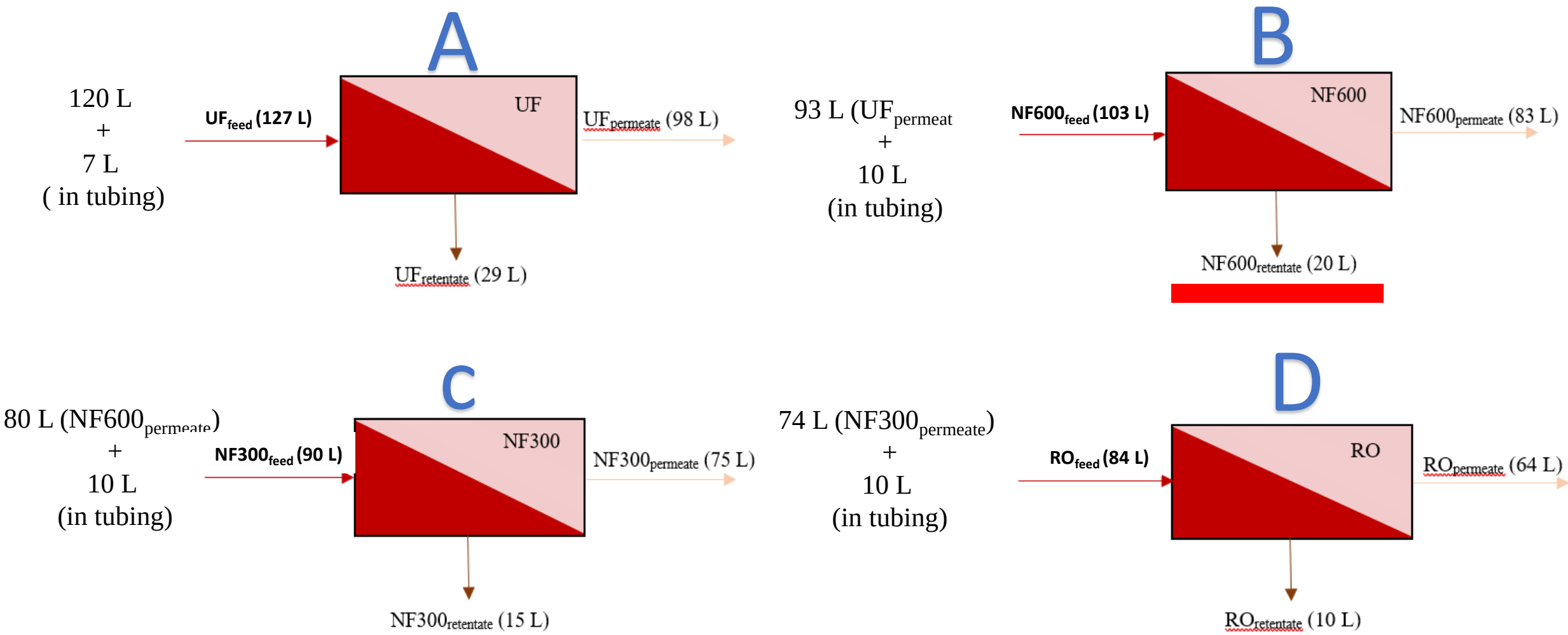


Stream Feed

To the 18 L of ethanol extract was added another 102 L to bring the volume up to 120 L (V with which the whole process started and using a UF membrane)

Upon completion of each process, 2 fractions were produced, Concentrate (Retentate stream) and Permeate (Permeate stream). Each time the filtrate of the previous membrane was used as the feed stream for the next membrane.

Separation by membrane filtration processes



Separation by membrane filtration processes

From Left to the right: $II_{ethanol}$, $UF_{αρχικό}$, $UF_{retentate}$, $UF_{permeate}$, $NF600_{retentate}$, $NF600_{permeate}$, $NF300_{retentate}$, $NF300_{permeate}$, $RO_{retentate}$, $RO_{permeate}$

	$UF_{initial}$	$Uf_{retentate}$	$UF_{permeate}$
TPC (g GAE/L)	0.34 ± 0.02	0.45 ± 0.01	0.31 ± 0.02
TPC (g)	43.52 ± 2.54	13.05 ± 0.29	30.38 ± 1.96
TSC (g GLU/L)	0.37 ± 0.02	0.42 ± 0.02	0,38 ± 0,07
TSC (g)	46.99 ± 2.54	12.18 ± 0.58	37.24 ± 6.86
Ratio TSC/TPC	1.1	1.07	1.2

	$NF600_{initial}$	$NF600_{retentate}$	$NF600_{permeate}$
TPC (g GAE/L)	0.24 ± 0.05	**0.82 ± 0.09**	-
TPC (g)	24.72 ± 5.15	16.41 ± 1.75	-
TSC (g GLU/L)	0.33 ± 0.04	0.70 ± 0.04	-
TSC (g)	33.99 ± 4.12	14.09 ± 0.88	-
Ratio TSC/TPC	1.4	0.85	-

	$NF300_{retentate}$	$RO_{retentate}$
TPC (g GAE/L)	0.09 ± 0,03	0.02 ± 0.01
TPC (g)	1.35 ± 0.45	0.20 ± 0.10
TSC (g GLU/L)	0.27 ± 0.04	0.08 ± 0.01
TSC (g)	**4.05 ± 0.60**	0.80 ± 0.10
Ratio TSC/TPC	3.1	4.0

- UF: Little change in concentration of TPC and TSC, both concentrate and filtrate
- NF600:
- Significant increase in [TPC] in concentrate.
- Much of the phenols were retained at this stage, reasonable proanthocyanidins with a MW of about 600 g/mol
- Due to high [TPC] and reduced solubility results in particles precipitation

Separation by membrane filtration processes

In the concentrate NF600 the max. antioxidant capacity compared to the other fractions

	$II_{ethanol}$	$UF_{initial}$	$NF600_{retentate}$	$NF300_{retentate}$	$RO_{retentate}$
TPC (g GAE/L)	2.65 ± 0.16	0.34 ± 0.02	0.82 ± 0.09	0.09 ± 0.03	0.02 ± 0.01
mmol Fe^{2+}/L	30.78 ± 0.42	2.09 ± 0.04	4.36 ± 0.32	0.47 ± 0.03	0.18 ± 0.03

TOTAL ORGANIC LOAD IN TERMS OF COD

	g O_2/L	g O_2
I_{water}	12.93 ± 7.44	303.9 ± 174.84
UF_{feed}	29.33 ± 0.41	3725.33 ± 52.42
$UF_{retentate}$	32.36 ± 1.55	938.62 ± 44.93
$UF_{permeate}$	28.88 ± 3.02	2830.71 ± 295.88
$NF600_{retentate}$	**47.51 ± 3.05**	950.29 ± 61.08
$NF600_{permeate}$	27.34 ± 3.13	2269.83 ± 259.98
$NF300_{retentate}$	16.45 ± 3.36	246.72 ± 50.42
$NF300_{permeate}$	29.72 ± 1.16	222.15 ± 87,46
$RO_{retentate}$	25.76 ± 2.95	257.65 ± 29.46
$RO_{permeate}$	17.22 ± 4.81	1102.27 ± 307.89

Qualitative determination of phenolic compounds by LC-MS

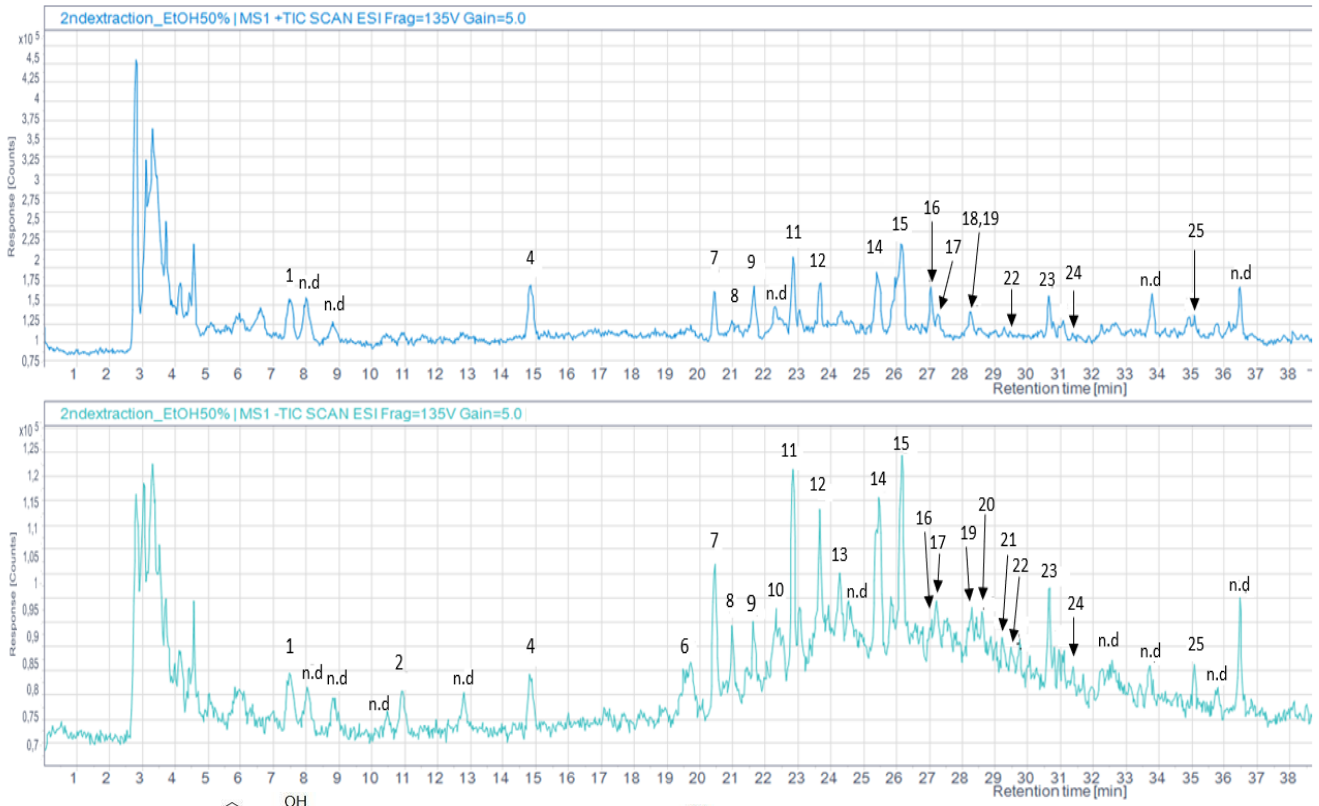
Liquid chromatography system coupled with a single quadrupole spectrometer and an ESI ionization source of the type LC/MSD1260 Infinity II (Agilent Technologies, Inc.)

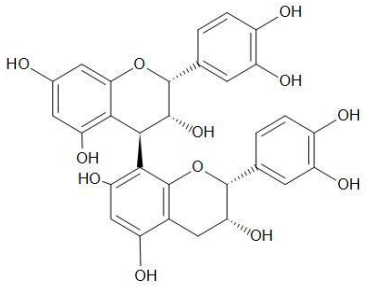
Mobile phase: A) 0,1% Formic acid, B) Methanol, C) ACN
Static phase: Column C18 (Poreshell 120 EC- C18, hydrophobic)
Flow rate 0.3 ml/min
Injection volume (10 µL)
Mass range – MS: 100-1000 m/z

Retention time (Rt) (min)	0,1% Formic acid (%A)	Methanol (%B)	ACN (%C)
0	95	5	0
3	95	5	0
10	85	15	0
12	85	15	0
17	75	15	10
19	75	15	10
29	55	15	30
31	55	15	30
46	0	15	85
47	0	15	85
57	95	5	0
62	95	5	0

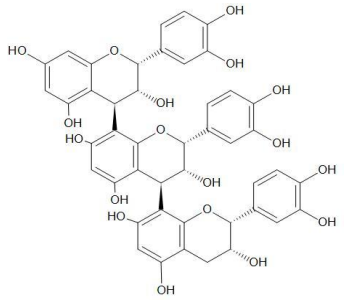
Qualitative determination of phenolic compounds by LC-MS

2nd ethanolic extract





Procyanidin dimer

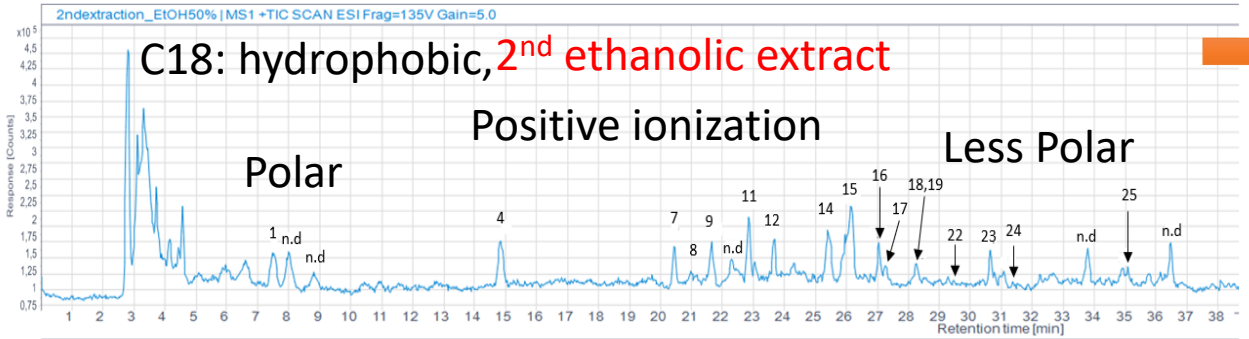


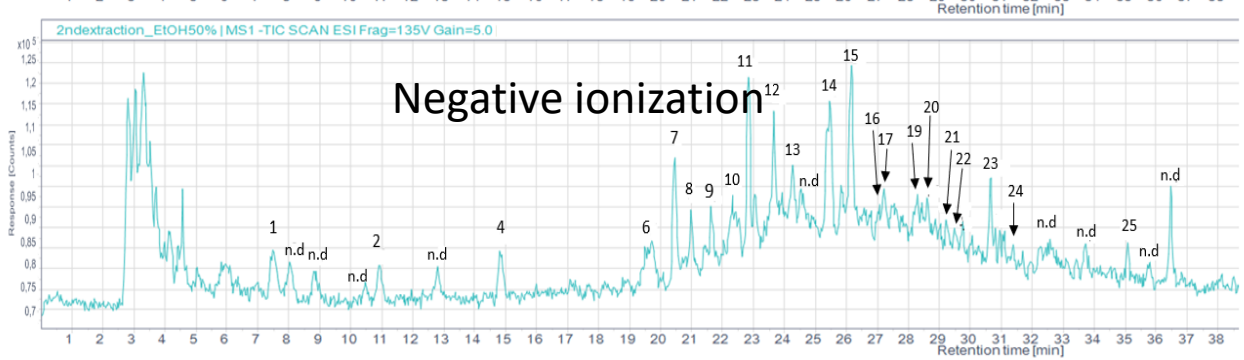
Procyanidin trimer

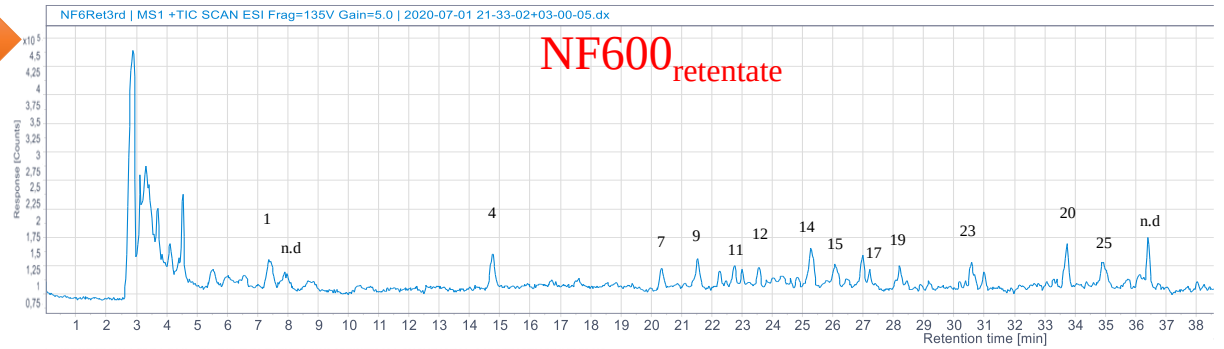
- Benzoic acids
- Amino acids
- Procyanidins dimers, trimers
- Flavan-3-ols
- Anthocyanidins
- Flavanones

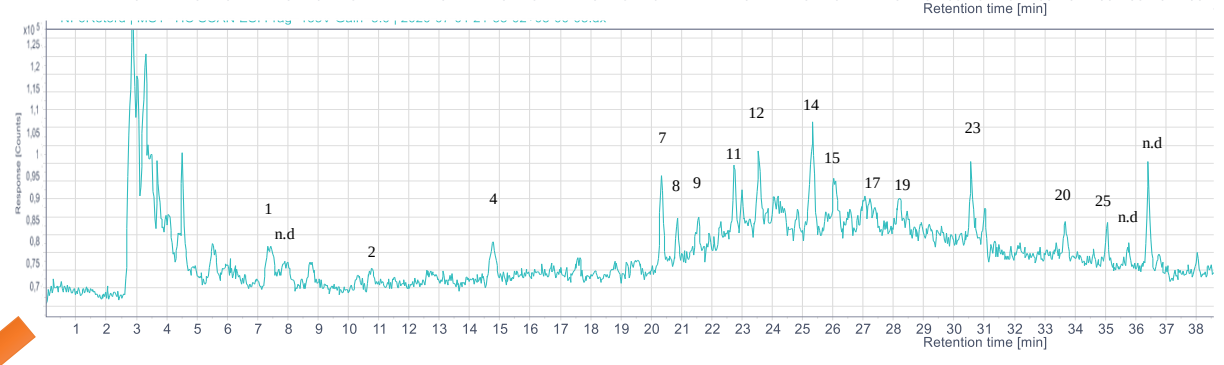
No.	Rt (min)	Tentative	No.	Rt (min)	Tentative
1	7.4	Unknown	15	26.1	(-)-epicatechin
2	10.8	Gallic acid	16	27.0	Unknown
4	14.8	L-phenylalanine	17	27.2	Procyanidin B2 3,3-di-O-gallate
6	19.6	Procyanidin B3	18	28.3	Myricetin-3-O-glucoside
7	20.4	Procyanidin B1	19	28.3	Unknown
8	21.0	Procyanidin trimer B type isomer 2	20	28.6	Procyanidin trimer B type isomer 6
9	21.6	L-tryptophan	21	29.2	(epi)catechin -3-O-gallate
10	22.4	Procyanidin trimer B type isomer 3	22	29.4	Procyanidin B5
11	22.8	(+)-catechin	23	30.6	Delphinidin 3-O hexuronide
12	23.6	Procyanidin B2			Quercetin 3-O galactoside
13	24.2	Procyanidin trimer B type isomer 4	24	31.4	Procyanidin B2 3,3 -di – O-gallate
14	25.4	Procyanidin trimer B type isomer 5	25	35.1	Unknown

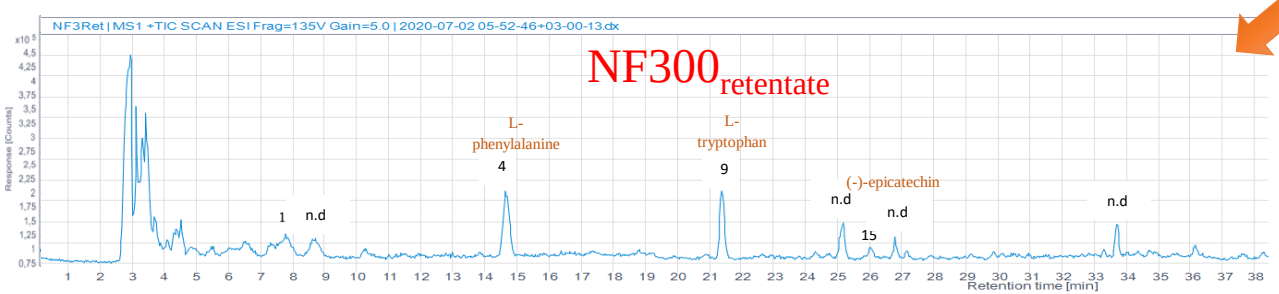
Qualitative determination of phenolic compounds by LC-MS

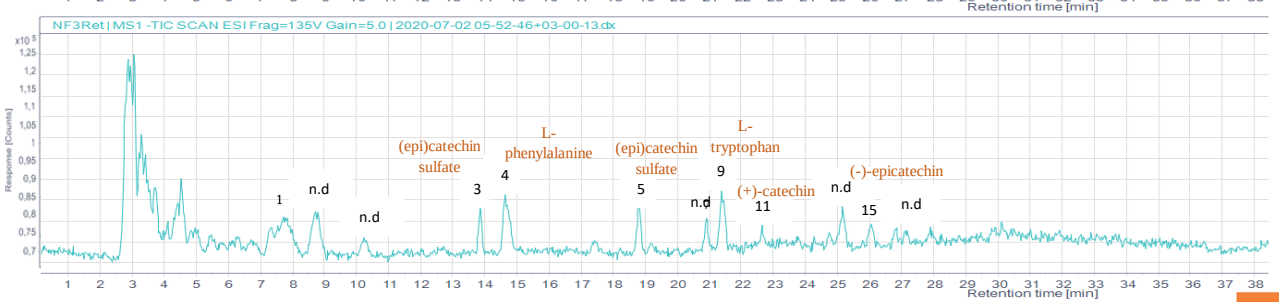


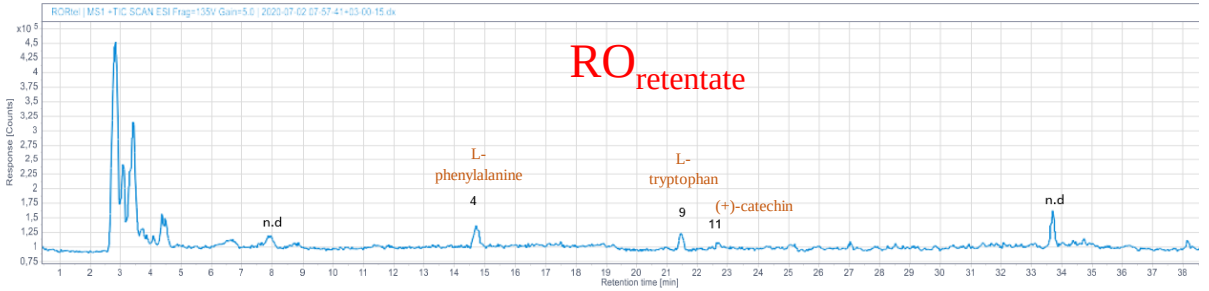


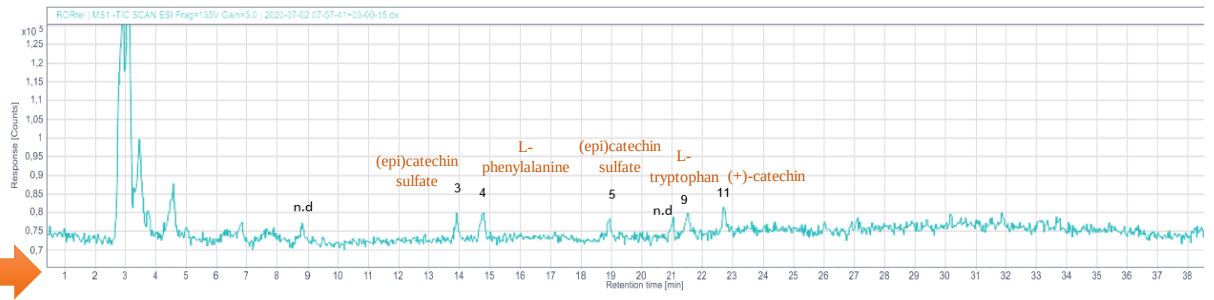












CONCLUSIONS

Separation, Isolation, and Enrichment of phenolic samples was achieved

The $NF_{600retenate}$ fraction showed the highest phenolic content and antioxidant capacity

By using a membrane array, the organic load was reduced to 1/3 of the original which is directly related to the reduction of the phenolic load

Main compounds found: Procyanidins (dimers – trimers), flavan-3-ols, metabolites and amino acids

FUTURE WORK

- Further utilization of fractions rich in phenolic compounds
- The selective isolation of phenolic compounds from condensed fractions using β-cyclodextrins
- Quantification of phenolic compounds occurring in high concentration
- LARGE SCALE PILOT PLANT (300 Kg of grape marc)

THANK YOU FOR YOUR ATTENTION… !!!

Qualitative determination of phenolic compounds by LC-MS

No.	Rt (min)	Tentative	M.W	Molecular formula	Positive ionization m/z	Negative ionization m/z	Reference	Fraction
1	7.4	Unknown	258	-	259 $[M+H]^+$ 101 $[M+H+2Na]^{3+}$ 141 $[M+H+Na]^{2+}$	257 $[M-H]^-$ 279 $[M+Na-2H]^-$ 295 $[M+K-2H]^-$	-	EEX, NF6, NF3
2	10.8	Gallic acid	170,12	$C_7H_6O_5$	-	169 $[M-H]^-$ 339 $[2M-H]^-$ 125 $[M-CO_2-H]^-$	[38-40]	EEX, NF6
3	13.9	(epi)catechin sulfate	370,3	$C_{15}H_{14}O_9S$	-	369 $[M-H]^-$ 391 $[M+Na-2H]^-$	[40]	NF3, RO
4	14.8	L-phenylalanine	165,19	$C_9H_{11}NO_2$	166 $[M+H]^+$ 120 $[M+H-CO-H_2O]^+$	164 $[M-H]^-$	[41]	EEX, NF6, NF3, RO
5	18.9	(epi)catechin sulfate	370,3	$C_{15}H_{14}O_9S$	-	369 $[M-H]^-$ 391 $[M+Na-2H]^-$	[40]	NF3, RO
6	19.6	Procyanidin B3	578,52	$C_{30}H_{26}O_{12}$	-	577 $[M-H]^-$ 599 $[M+Na-2H]^-$	[38-39], [42]	EEX
7	20,4	Procyanidin B1	578,52	$C_{30}H_{26}O_{12}$	579 $[M+H]^+$ 601 $[M+Na]^+$ 309 $[M+H+K]^{2+}$	577 $[M-H]^-$ 599 $[M+Na-2H]^-$	[38-39], [42]	EEX, NF6
8	21,0	Procyanidin trimer B type isomer 2	866,74	$C_{45}H_{38}O_{18}$	867 $[M+H]^+$ 889 $[M+Na]^+$ 453 $[M+H+K]^{2+}$	865 $[M-H]^-$ 887 $[M+Na-2H]^-$	[38-39], [42]	EEX, NF6
9	21,6	L-tryptophan	204,23	$C_{11}H_{12}N_2O_2$	205 $[M+H]^+$ 227 $[M+Na]^+$ 188 $[M+H-NH_3]^+$	203 $[M-H]^-$ 407 $[2M-H]^-$	[41]	EEX, NF6, NF3, RO
10	22,4	Procyanidin trimer B type isomer 3	866,74	$C_{45}H_{38}O_{18}$	-	865 $[M-H]^-$ 887 $[M+Na-2H]^-$	[38-39], [42]	EEX
11	22,8	(+)-catechin	290,26	$C_{15}H_{14}O_6$	291 $[M+H]^+$ 313 $[M+Na]^+$	289 $[M-H]^-$ 579 $[2M-H]$	[38-39], [42]	EEX, NF6, NF3, RO
12	23,6	Procyanidin B2	578,52	$C_{30}H_{26}O_{12}$	579 $[M+H]^+$ 601 $[M+Na]^+$ 309 $[M+H+K]^{2+}$	577 $[M-H]^-$ 599 $[M+Na-2H]^-$	[38-39], [42]	EEX, NF6
13	24,2	Procyanidin trimer B type isomer 4	866,74	$C_{45}H_{38}O_{18}$	-	865 $[M-H]^-$ 887 $[M+Na-2H]^-$	[38-39], [42]	EEX
14	25,4	Procyanidin trimer B type isomer 5	866,74	$C_{45}H_{38}O_{18}$	867 $[M+H]^+$	865 $[M-H]^-$ 887 $[M+Na-2H]^-$	[38-39], [42]	EEX
15	26,1	(-)-epicatechin	290,26	$C_{15}H_{14}O_6$	291$[M+H]^+$ 313 $[M+Na]^+$ 603 $[2M+Na]^+$	289 $[M-H]^-$ 579 $[2M-H]^-$ 325 $[M-Cl]^-$	[38-39], [42]	EEX, NF6
16	27,0	Unknown	358	-	359 $[M+H]^+$	357 $[M-H]^-$ 315 $[M-CHCOH-H]^-$	-	EEX., NFX
17	27,2	Procyanidin B2 3,3-di-O-gallate	882,73	$C_{44}H_{34}O_{20}$	905 $[M+Na]^+$	881 $[M-H]^-$ 903 $[M+Na-2H]^-$	[38-39]	EEX, NF6
18	28,3	Myricetin-3-O-glucoside	480,4	$C_{21}H_{20}O_{13}$	481$[M+H]^+$ 563 $[M+2ACN+H]^+$	-	[38], [40]	EEX
19	28,3	Unknown	540	-	563 $[M+Na]^+$ 290 $[M+H+K]^{2+}$	539 $[M-H]^-$ 585 $[M+FA-H]^-$	-	EEX. NF6
20	28,6	Procyanidin trimer B type isomer 6	866,74	$C_{45}H_{38}O_{18}$	-	865 $[M-H]^-$ 887 $[M+Na-2H]^-$	[39], [42]	EEX
21	29,2	(epi)catechin -3-O-gallate	442,4	$C_{22}H_{18}O_{10}$	-	441 $[M-H]^-$ 463 $[M+Na-2H]^-$	[38-39], [42]	EEX

Qualitative determination of phenolic compounds by LC-MS

22	29,4	Procyanidin B5	578,5	$C_{30}H_{26}O_{12}$	579 $[M+H]^+$ 309 $[M+H+K]^+$ 331 $[M+2ACN+2H]^+$ 601 $[M+Na]^+$	577 $[M-H]^-$ 599 $[M+Na-2H]^-$	[39]	EEX
23	30,6	Delphinidin 3-O hexuronide	479,08	$C_{21}H_{19}O_{13}^+$	479 $[M]^+$ 501 $[M+Na]^+$ 303 (fragment) 259 $[M+H+K]^{2+}$	477 $[M-H]^-$ 499 $[M+Na-2H]^-$ 955$[2M-H]^{2-}$ 523 $[M+FA-H]^-$	[42]	EEX, NF6
		Quercetin 3-O galactoside	464,38	$C_{21}H_{20}O_{12}$	547 $[M+2ACN+H]^+$	463 $[M-H]^-$ 301 (fragment)	[38-39], [42]	NF6
24	31,4	Procyanidin B2 3,3 -di – O-gallate	882,73	$C_{44}H_{34}O_{20}$	883 $[M+H]^+$ 905 $[M+Na]^+$	881 $[M-H]^-$ 439 $[M-2H]^-$	[39]	EEX
25	35,1	Unknown	566	-	303 $[M+H+K]^{2+}$ 589 $[M+Na]^+$	565 $[M-H]^-$ 611 $[M+FA-H]^-$ 679 $[M+TFA-H]^-$	-	EEX, NF6

Colorimetric assays

Folin-Cioqualteu assay: The F–C assay is a colorimetry method based on SET reactions between the F–C reagent and phenolic compounds. Phenolic compounds are good oxygen radical scavengers, since the electron reduction potential of phenolic radical is lower than that of oxygen radicals and also, phenoxyl radicals are less reactive than oxygen radicals. Thus, scavenging reactive oxygen radicals by phenolic compounds ceased further oxidative reactions. Under this basic condition, dissociation of a phenolic proton leads to the formation of phenolate ion, which is responsible to reduce the F–C reagent. Upon reduction, the intense yellow colour of F–C reagent turns into a blue colour. The absorbance is read at 765 nm..

The single electron transfer (SET) mechanism involves a redox(reduction-oxidation) reaction with an oxidant(radical) as an indicator of reaction endpoint. SET-based assays measure the antioxidant's reducing capacity

Mo(VI) (yellow) + e (from antioxidant) --→ Mo (V) (Blue)

10.3390/antiox9080709

FRAP assay is a typical SET-based method, which measures the reduction of ferric ions (Fe3+)–ligand complex to the intensely blue-colored ferrous ions (Fe2+) complex by antioxidants in acidic media. In another word, this method measure is based on antioxidants to reduce the ferric 2,4,6-tripyridyl-s-triazine complex [Fe3+-(TPTZ)2]3+ to the intensely blue colored ferrous complex [Fe2+-(TPTZ)2]2+ in acidic medium. Measuring the increasing absorption at 593 nm using a spectrophotometer monitors this reduction and results are expressed as micromolar Fe2+ equivalents or relative to an antioxidant standard. However, this assay is non-specific.

$FeSO_4x7H_2O$ is usually used as a reference standard

- SET-based methods detect the ability of an antioxidant to transfer one electron to reduce any compound, including metals, carbonyl groups and radicals. SET reactions are pH dependent. In general, ionization potential values decrease with increasing pH, reflecting increased electron donating capacity with deprotonation.

AH + M3+ → AH+ + M2+.

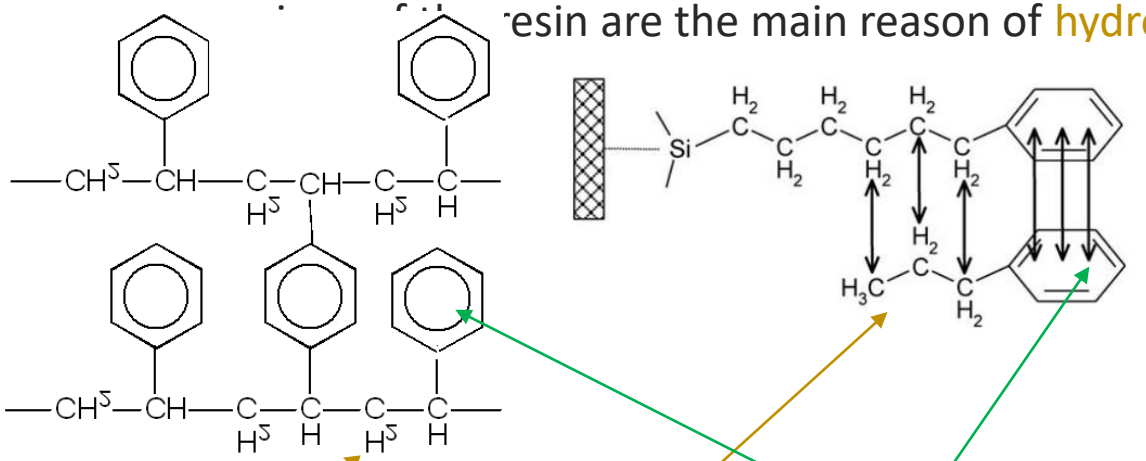
10.1007/s00204-020-02689-3

10.3390/antiox9080709

Interactions between phenolic compounds and resin

Polyphenol compounds can be adsorbed by macroporous resins via physical mechanisms, such as van der Waals forces (electrostatic interaction), hydrogen bonds (when OH- are present), and π-π stacking interactions between phenolics and the benzene rings of resins. Polyphenols contain hydrogen groups and benzene rings and, depending on their structure, exhibit different polarities. Although XAD 16 and XAD 4 have similar polarities, XAD 16 provides a higher surface area and pore volume size and absorbs more polyphenols.

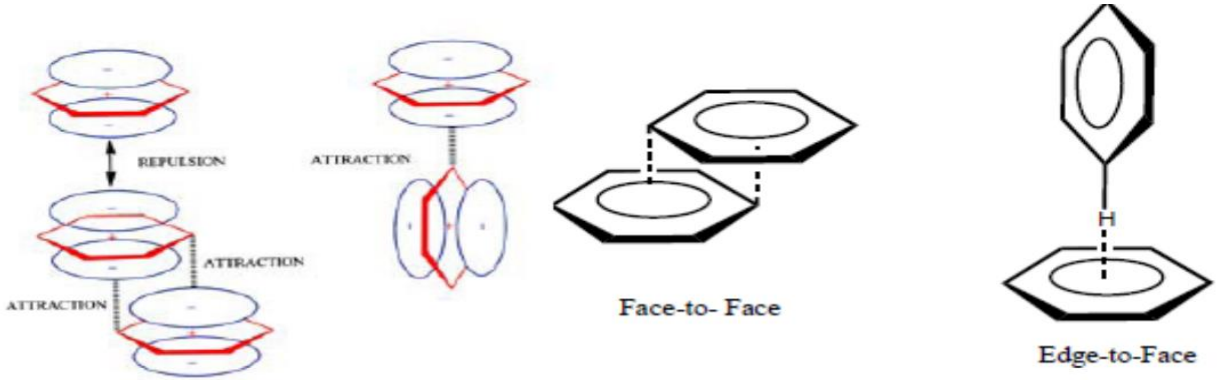
Specifically, associations between compounds non-polar aromatic ring of the phenolic and hydrophobic [illegible]esin are the main reason of hydrophobic interactions.



XAD 16

hydrophobic interactions

electrostatic interactions



π-π stacking interactions