odisee

**BIT - O**

Biochemical Innovation Team Odisee

# Technical symposium:
# "Phenolic compounds in by-products from apple trees and apples"

# Mapping of the phenolic profile of apple wood

**Hannes Withouck**

**Annick Boeykens**

**Machteld Van den Broucke**

# Introduction

**Waste products from the fruit industry: Fruit growers annually have hundreds of tons of wood waste**

# Introduction

## Waste stream

Spigno G. et al. (2007)[1]: "*There is an increasing processing of agricultural wastes (such as wine-making wastes) as a low-cost source of antioxidants (phenolic compounds)"*

Yilmaz Y. and Toledo T.R. (2006)[2]: "*Skins and seeds of grapes are produced in large quantities by the wine making industry. These byproducts have become valuable raw materials for extraction of polyphenols. "*

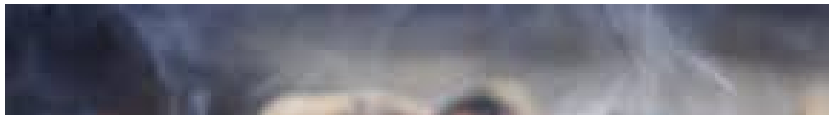
*"Bark pine is a lumber industry sub-product that is produced when wood is transformed. It is a good source of phenolic compounds (Jerez et al., 2007)[3] and its extracts have been reported to have several bioactivities including antioxidants (Ku et al., 2007)[4], cardiovascular benefits (Gohil and Packer, 2002), and anti-diabetic effects (Schafer and Hogger, 2007)."*

*Ghitescu et al. (2015)[5] "Spruce bark (picea abies) is a rich source of phenolic compounds with antioxidant activity."*

# Waste stream – Apple tree wood

Residues from the harvest of apple woods or wood processing are frequently used for applications with low added value such as energy or paper pulp (Dedrie et al., 2015; Ghitescu et al., 2015)[5,6].

Haspenwood creates smoke chips for the barbecue.



**We want to do more: What is the phenolic profile and the antioxidant properties of apple tree wood extracts from the *Malus domestica*, also known as King Jonagold.**

# Introduction

**Waste products from the fruit industry: Fruit growers annually have hundreds of tons of wood waste**

What is happening with the residues:

- Burning
- Smoke chips
- Smoke planks

Literature:

Xü et al. (2010) [7] reported the presence of phloretin in the bark of apple trees. Phloretin has been known mostly as a cosmeceutical ingredient.
Moreira et al. (2016) [8] has shown the potential of apple tree wood residues as a source of phenolic compounds.

The extraction of bioactive compounds from core wood and bark derived from apple wood *(Malus domestica* 'King Jonagold')

# Waste stream – Apple tree wood

Industrial Crops & Products 104 (2017) 210–220

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

## Valorization of apple tree wood residues by polyphenols extraction: Comparison between conventional and microwave-assisted extraction

Manuela M. Moreira[a,*], M. Fátima Barroso[a], Annick Boeykens[b,c], Hannes Withouck[b], Simone Morais[a], Cristina Delerue-Matos[a]

[a] REQUIMTE/LAQV, Instituto Superior de Engenharia do Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal
[b] Odisee University College, School of Technology, Chemistry, Ghent, Belgium
[c] KU Leuven, Faculty of Engineering Technology, Cluster Bioengineering Technology (CBeT), Ghent, Belgium

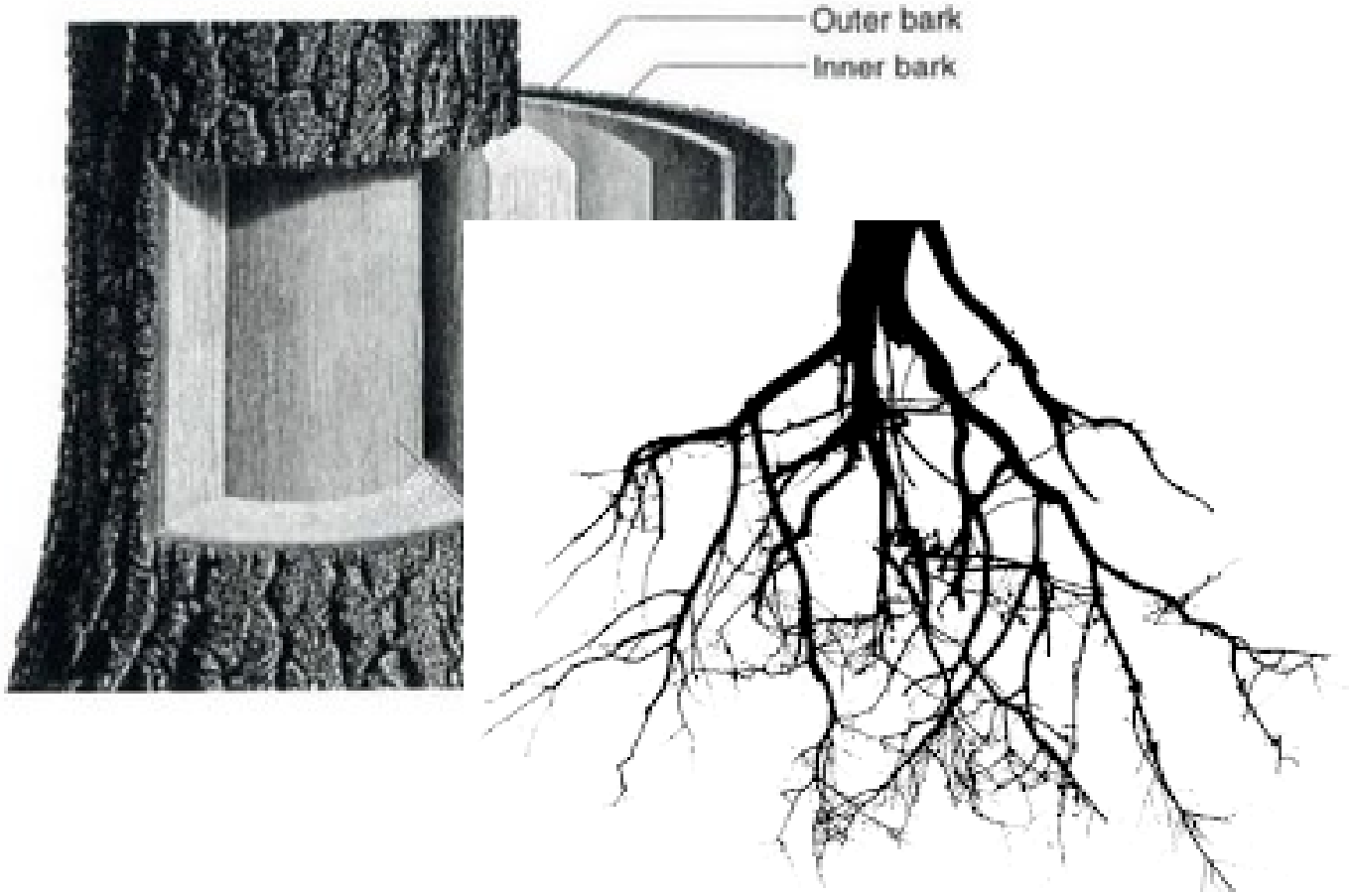
ARTICLE INFO

*Keywords:*
Apple tree wood
Microwave-assisted extraction
Conventional extraction
Antioxidant activity
Phenolic compounds
HPLC analysis

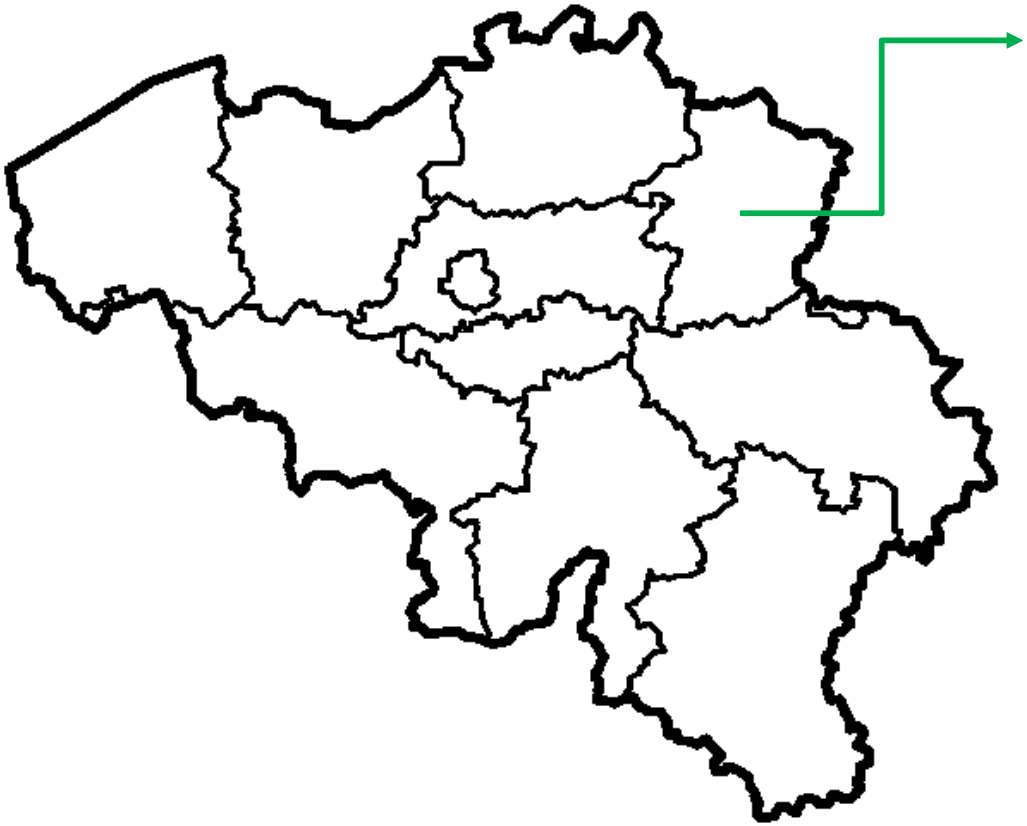
ABSTRACT

For the first time, the characterization of antioxidant activity and phenolic profile of apple tree (*Malus domestica*) bark, core and roots was carried out. Phenolic compounds were extracted from the Belgium apple tree wood residues collected at two seasons, namely summer 2015 and winter 2016, using conventional (CE) and microwave-assisted extraction (MAE) techniques. For each extraction technique, the influence of the most important operational parameters, namely solvent composition, extraction time and temperature, on the total phenolic and flavonoid content, and antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA) and ferric reducing activity power (FRAP) assays were optimized. The phenolic profile from the obtained extracts was also characterized by high-performance liquid chromatography with photodiode array detection (HPLC-PDA). Optimum conditions were: 20 mL ethanol:water 60:40 v/v, 20 min, 100 °C, sample weight 0.1 g for MAE and 20 mL ethanol:water 50:50 v/v, 2 h, 55 °C, sample weight 0.5 g for CE. Root extracts obtained by MAE (the most efficient technique) presented the highest phenolic (47.7 ± 0.9 mg gallic acid equivalents/g dry weight) and flavonoid (17.1 ± 0.8 mg epicatechin equivalents/g dry weight) content, and antioxidant activity (28.4 ± 2.0 mg trolox equivalents/g dry weight and 36.1 ± 2.7 mg ascorbic acid equivalents/g dry weight for DPPH-RSA and FRAP assays, respectively), followed by bark and core wood extracts. HPLC-PDA [illegible] the phenolic composition

# Waste stream – Apple tree wood

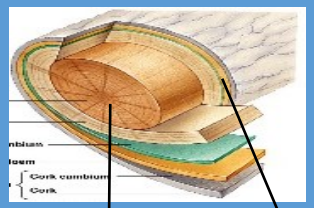


# Apple wood



Haspenwood

# Processing of the apple wood




Core wood

Bark

Root

Shredding

Grinding

Pre-treatment:
- Oven drying at 60 °C
- Freeze Drying
- Fresh storage

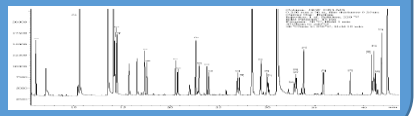
**Extraction**

UAE = Ultrasound Assisted Extraction [9]
WSE = Warm Solvent Extraction [10]
EAE = Enzyme Assisted Extraction [11, 12]

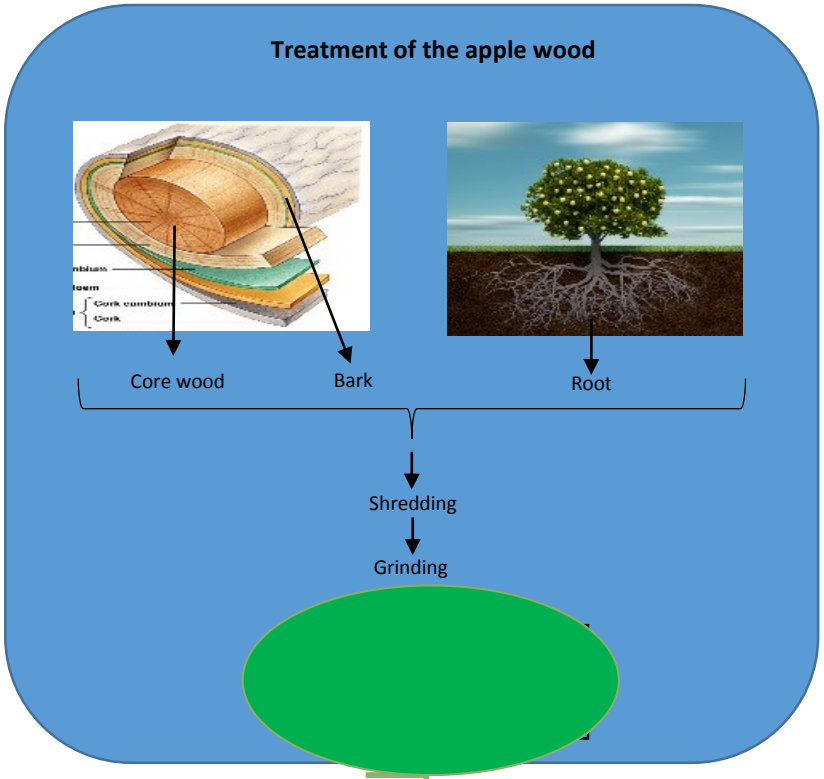
**Data Analysis**

Spectrophotometric analyses → Absorption

RP-HPLC-PDA →



# Processing of the apple wood




PRE - TREATMENT:

- Oven-dried at 60 °C
- Freeze–dried (under vacuum)
- Fresh

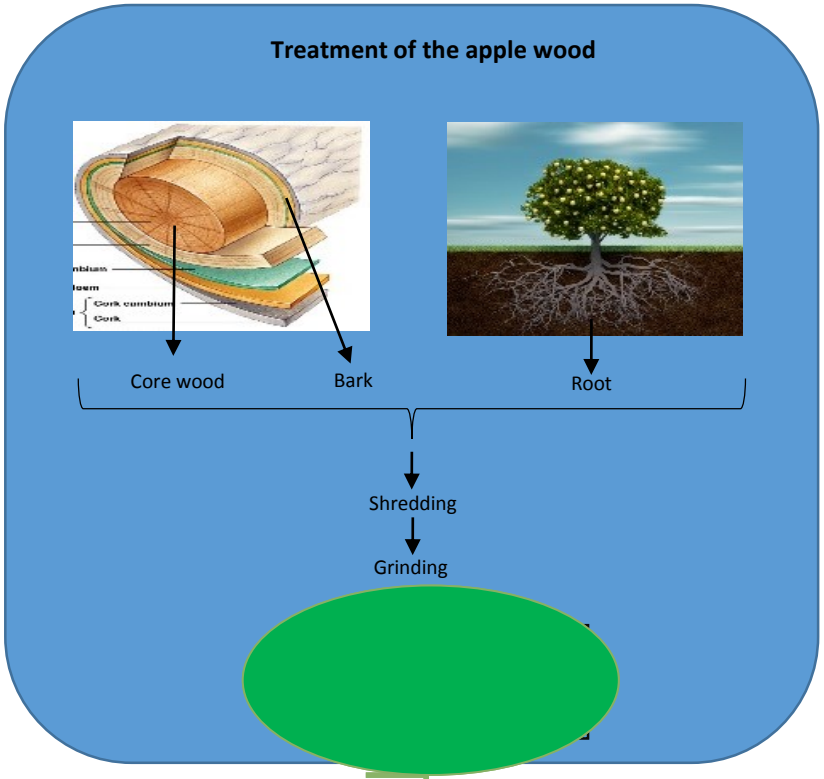
Influence of type of solvent?
Influence of the extraction technique?

**Extraction**

WSE = Warm Solvent Extraction

UAE = Ultrasound Assisted Extraction

# Processing of the apple wood



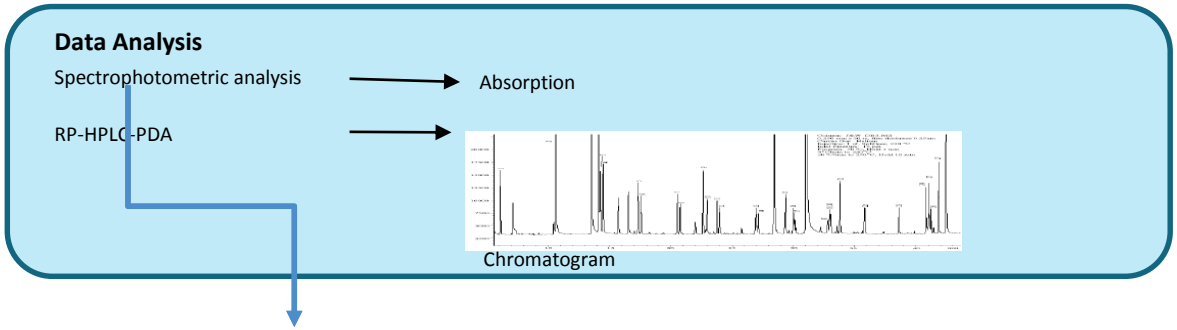

PRE - TREATMENT:

- Oven-dried at 60 °C
- Freeze–dried (under vacuum)
- Fresh

**Extraction**

EAE = Enzyme Assisted Extraction

# Analysis on the apple wood extracts




## Spectrophotometric analysis:

- Total phenolic content (TPC): Folin-Ciocalteu methode [13, 14]
- Total flavonoid content (TFC): Aluminium chloride method [15]
- DPPH-RSA method: 2,2´-diphenyl-1-picrylhydrazyl radical scavenging activity [16]
- FRAP method: Ferric Reducing Antioxidant Power [17]

## RP-HPLC-PDA:

- Calibration with 17 marker compounds
- Identification and quantification of the marker compounds in the extracts

# Analysis on the apple wood extracts

## RP-HPLC-PDA:

- Calibration with 17 marker compounds [18-20]

| **Compound** | **Retention Time (RT)** (minutes) | **Optimum wavelength** (nm) |
|---|---|---|
| (+)-Catechin | 11.888 | 277 |
| (-)-Epicatechin | 17.978 | 277 |
| (-)-Epicatechin gallate | 23.748 | 276 |
| *p*-coumaric acid | 23.983 | 308 |
| Ferulic acid | 26.433 | 321 |
| Caffeic acid | 17.160 | 322 |
| Vanillic acid | 16.507 | 261 |
| Gallic acid | 5.540 | 269 |
| Quercetin | 48.157 | 368 |
| Kaempherol-3-glucoside | 41.214 | 347 |
| Naringenin | 48.157 | 289 |
| Naringin | 34.190 | 282 |
| Rutin | 35.953 | 353 |
| Phloretin | 49.962 | 285 |
| Phloredzin | 36.777 | 284 |
| Procyanidin B1 | 9.117 | 279 |
| Procyanidin B2 | 13.258 | 278 |

# Results

**Core wood**

**Bark**

**Root**

WSE
- Spectrophotometric analysis
- RP-HPLC PDA

UAE
- Spectrophotometric analysis
- RP-HPLC PDA

EAE
- RP-HPLC PDA

# Results

## Core wood

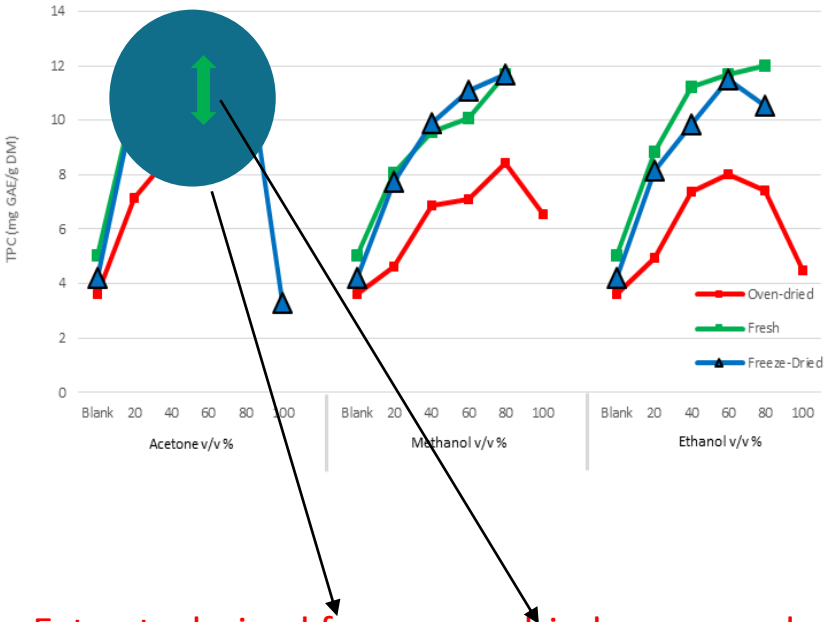
WSE

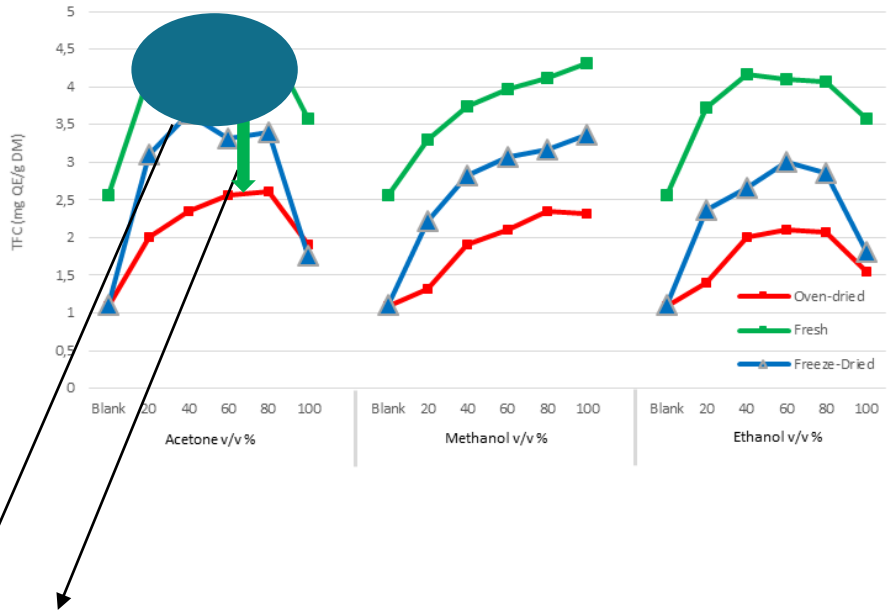
- Spectrophotometric analysis
- RP-HPLC PDA

## Apple tree core wood - WSE

**Spectrophotometric analysis – TPC**

**Spectrophotometric analysis – TFC**



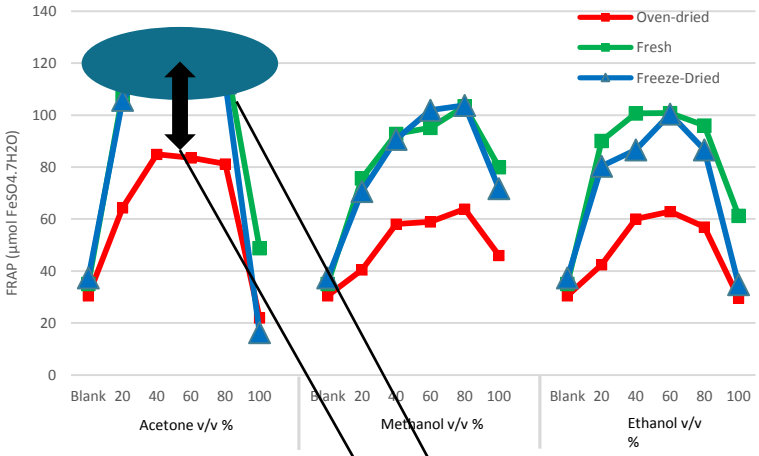

Extracts derived from oven-dried core wood:

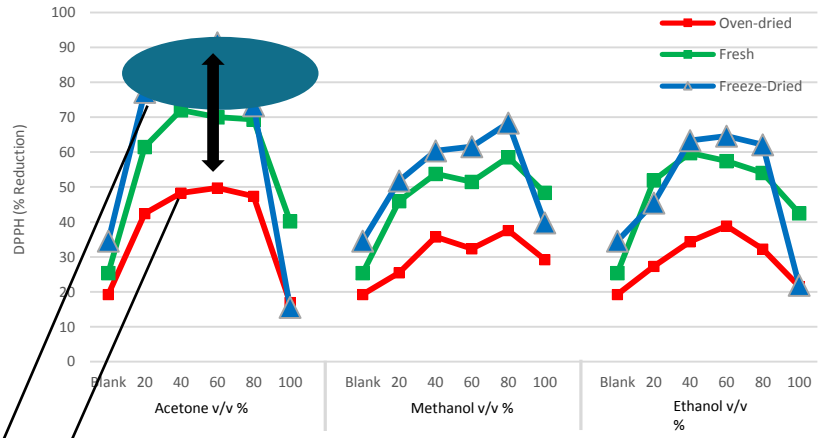
TPC: ⬇

TFC: ⬇

## Apple tree core wood - WSE

### Spectrophotometric analysis – reducing capacity




Extracts obtained with 40% up to 80% v/v solvent mixture show the highest antioxidant activity.

Extracts derived from oven-dried core wood: Antioxidant activity ⬇

## Apple tree core wood - WSE

### RP-HPLC PDA

Resultaten uitgedrukt in mg/g DM

| Phenolic compound | Fresh samples | |
|---|---|---|
| | Ethanol 40 | Water 100 |
| Quercetin | - | - |
| (-)-Epicatechin gallate | 1.494 ± 0.001 | 1.541 ± 0.005 |
| (-)-Epicatechin | 0.373 ± 0.040 | 0.570 ± 0.005 |
| [illegible] | [illegible] | ND |
| [illegible] | [illegible] | 2.512 ± 0.058 |
| Naring[illegible] | [illegible] ± 0.004 | 0.174 ± 0.002 |
| Naringenin | ND | ND |
| Rut[illegible] | [illegible] 0.003 | 0.111 ± 0.002 |
| [illegible] | [illegible] | 5.784 ± 0.003 |
| Phloretin | < LOD | ND |
| **Total Flavonoids** | 17.5 ± 0.4 | 10.7 ± 0.1 |
| Procyanidin B1 | ND | ND |
| Procyanidin B2 | 0.187 ± 0.053 | 0.137 ± 0.004 |
| **Condensed tannins** | 0.187 ± 0.053 | 0.137 ± 0.004 |
| Vanillic acid | 0.135 ± 0.001 | ND |
| Gallic acid | - | 0.401 ± 0.001 |
| Ferulic acid | 0.056 ± 0.008 | ND |
| *p*-Coumaric acid | 0.063 ± 0.008 | 0.066 ± 0.001 |
| Caffeic acid | ND | ND |
| **Total Phenolic Acids** | 0.254 ± 0.017 | 0.467 ± 0.002 |
| **Total amount of polyphenol markers** | **17.9 ± 0.5** | **11.3 ± 0.2** |

# Results

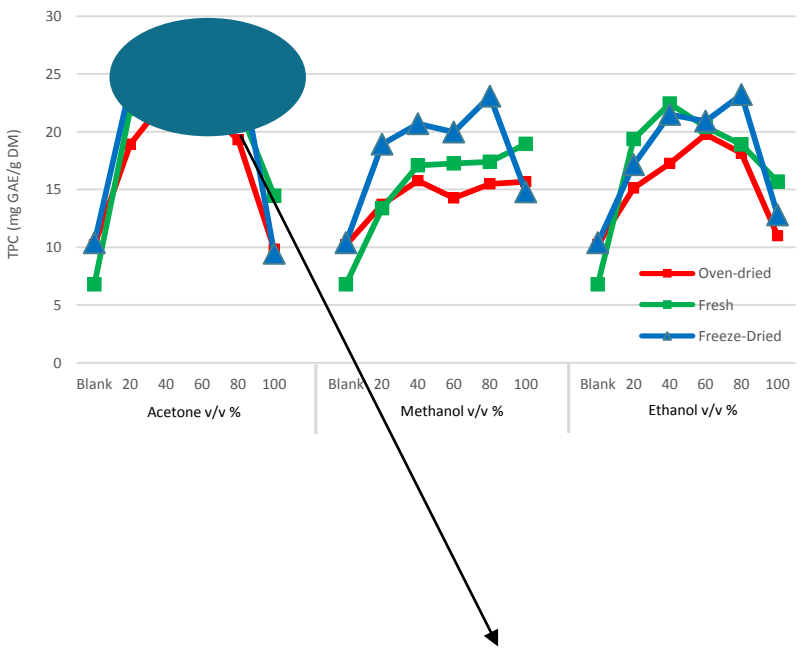
## Bark

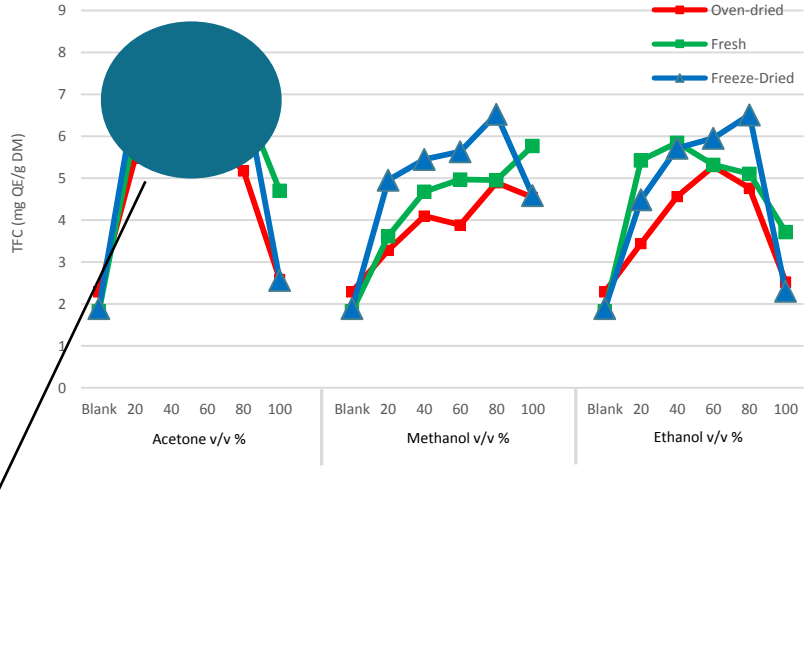
WSE

- Spectrophotometric analysis
- RP-HPLC PDA

## Spectrophotometric analysis – TPC

## Spectrophotometric analysis – TFC

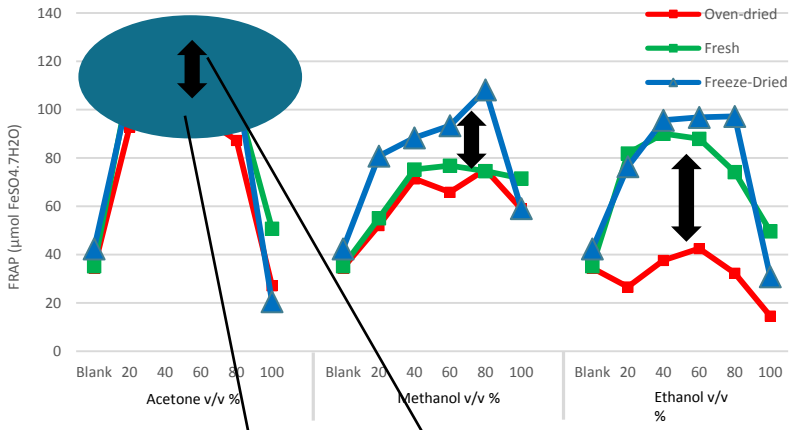


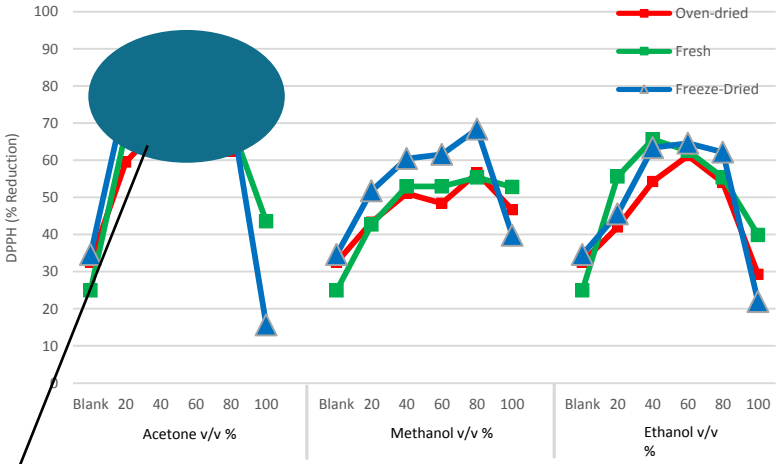




Extractions performed in the presence of acetone/water mixtures:

- Highest TPC
- Highest TFC

# Spectrophotometric analysis – reducing capacity




Extractions performed in the presence of acetone/water mixtures show the highest antioxidant activity.

Extracts derived from oven-dried bark: antioxidant activity

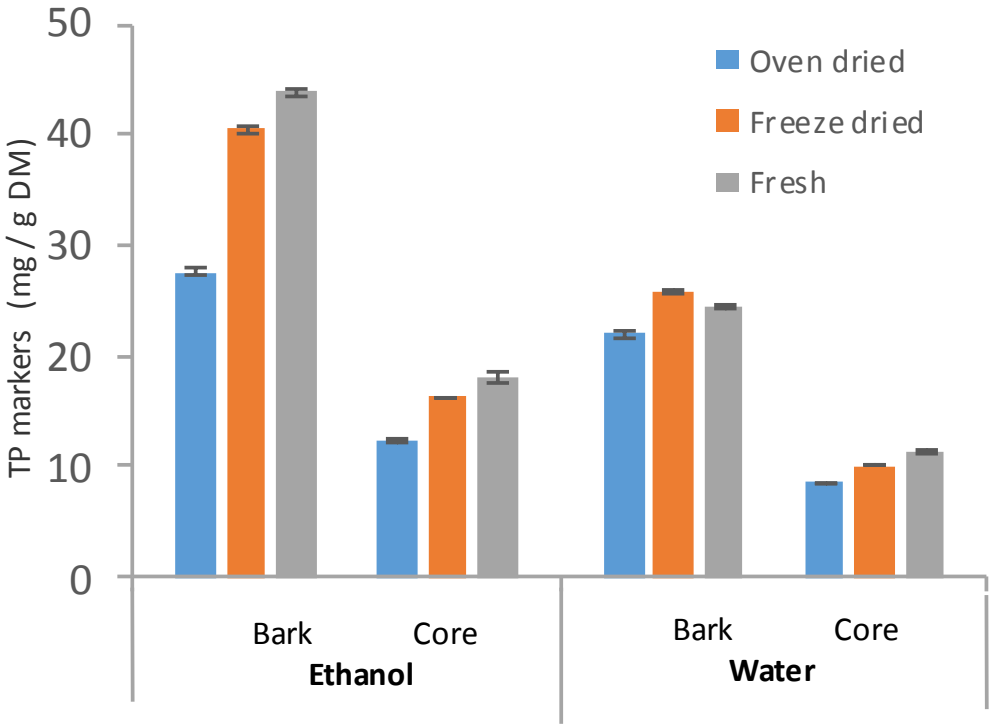
## Apple tree bark- WSE

### RP-HPLC PDA

| Phenolic compound (mg/g DM) | Fresh samples | |
|---|---|---|
| | Ethanol 40 | Water 100 |
| Quercetin | 0.254 ± 0.001 | ND |
| (-)-Epicatechin gallate | 1.410 ± 0.001 | 1.641 ± 0.003 |
| (-)-Epicatechin | 0.678 ± 0.006 | 0.306 ± 0.003 |
| (+)-Catechin | ND | 0.300 ± 0.002 |
| Kaempferol-3-glucoside | 4.789 ± 0.009 | 0.939 ± 0.015 |
| Naringin | 0.169 ± 0.004 | 0.126 ± 0.001 |
| Naringenin | ND | ND |
| Rutin | 0.268 ± 0.002 | 0.055 ± 0.001 |
| | | 20.52 ± 0.02 |
| Phloretin | ND | ND |
| **Total Flavonoids** | 42.3 ± 0.1 | 23.9 ± 0.1 |
| Procyanidin B1 | 0.868 ± 0.008 | ND |
| Procyanidin B2 | 0.140 ± 0.003 | 0.206 ± 0.003 |
| **Condensed tannins** | 1.008 ± 0.011 | 0.205 ± 0.003 |
| Vanillic acid | ND | ND |
| Gallic acid | ND | ND |
| Ferulic acid | 0.144 ± 0.002 | 0.051 ± 0.001 |
| *p*-Coumaric acid | 0.068 ± 0.000 | 0.204 ± 0.001 |
| Caffeic acid | 0.309 ± 0.001 | ND |
| **Total Phenolic Acids** | 0.521 ± 0.003 | 0.254 ± 0.002 |
| **Total amount of polyphenol markers** | **43.8 ± 0.2** | **24.4 ± 0.2** |

# Core wood VS bark

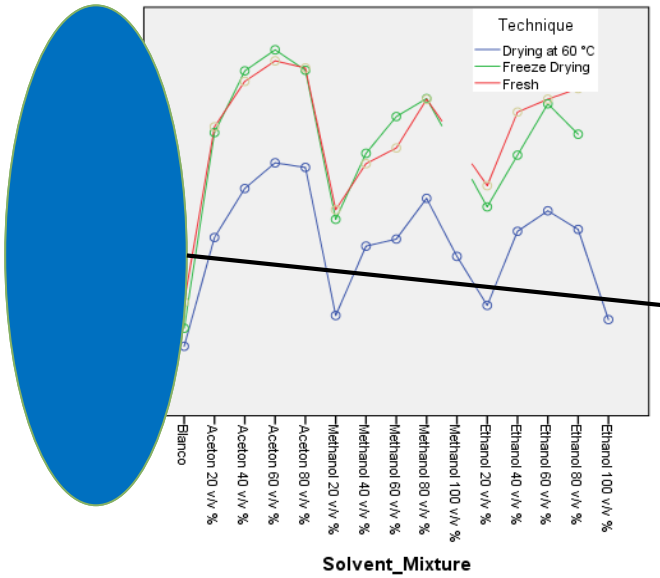
**RP-HPLC PDA**



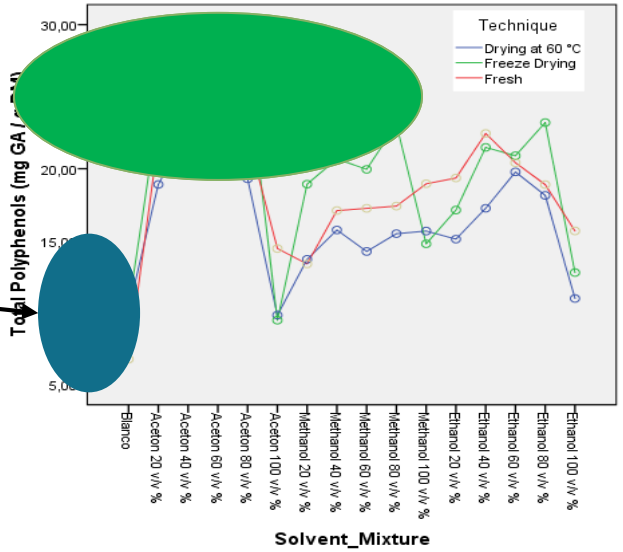
# Core wood VS bark

**Spectrophotometric analysis - TPC**

Core wood




Bark



# Results

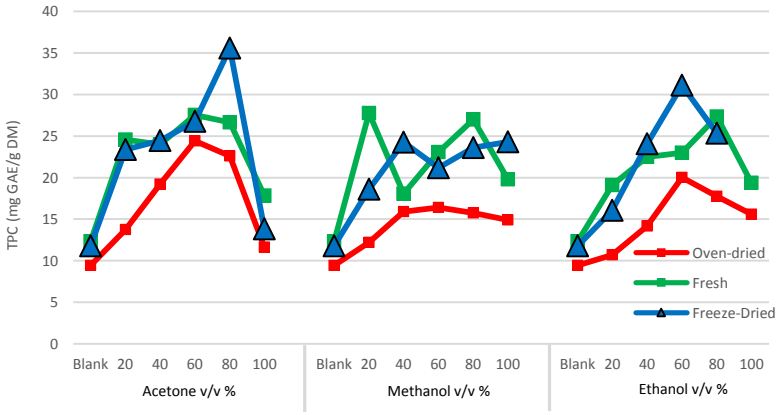
## Root

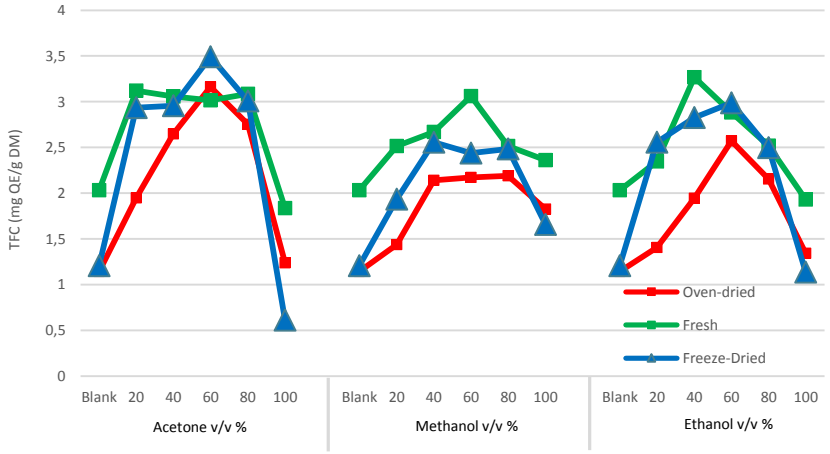
WSE

- Spectrophotometric analysis
- RP-HPLC PDA

## Spectrophotometric analysis – TPC

## Spectrophotometric analysis – TFC

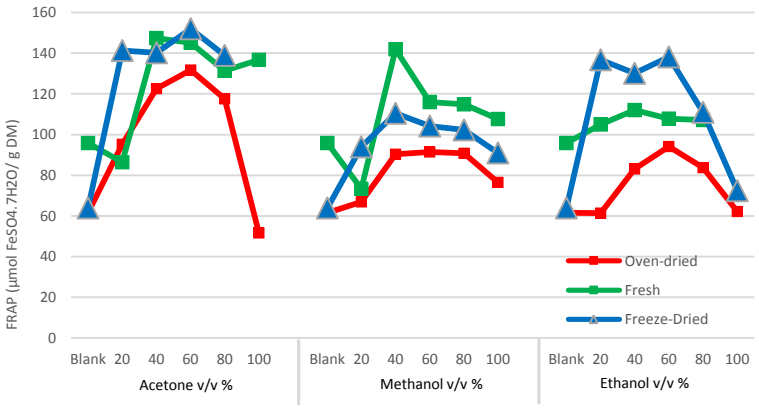


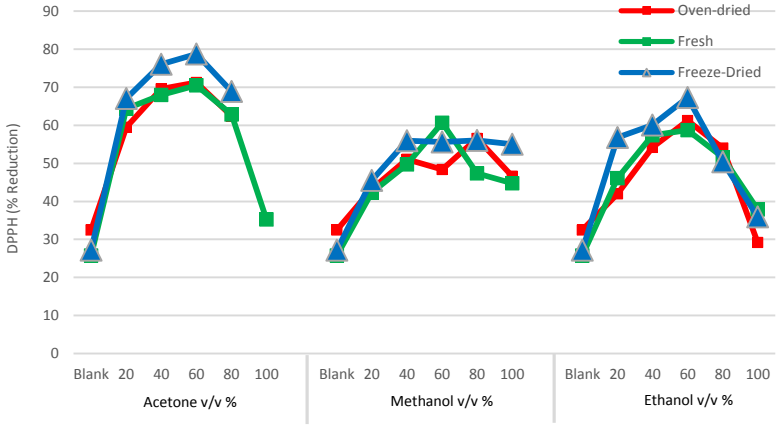




Almost no difference between the type of solvent.

Extracts derived form oven-dried root samples showing lower amount of TPC and TFC.

# Spectrophotometric analysis – reducing capacity



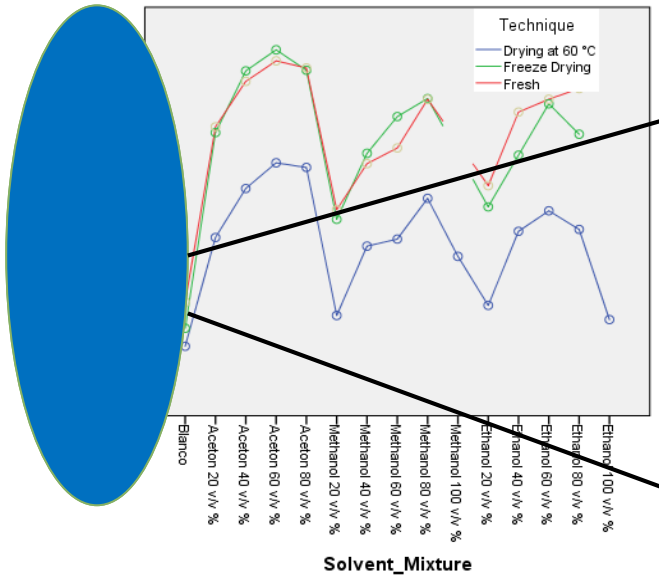

Extractions performed in the presence of acetone/water mixtures show higher antioxidant activity.

Extracts derived from oven-dried root samples (FRAP assay): Antioxidant activity ⬇

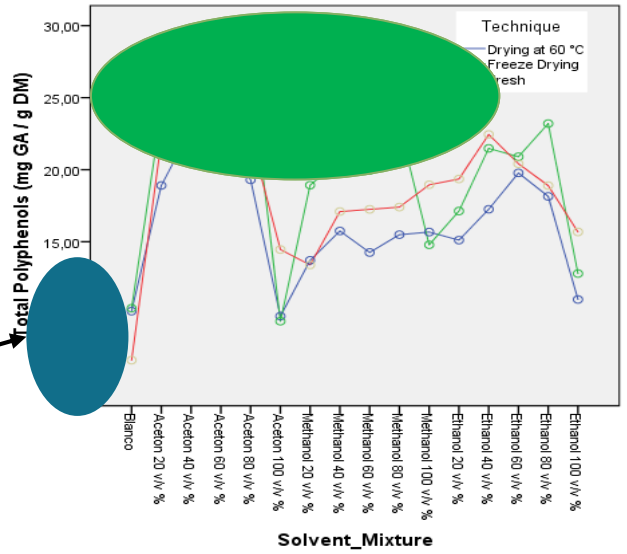
# Core wood VS bark VS Root

**Spectrophotometric analysis - TPC**

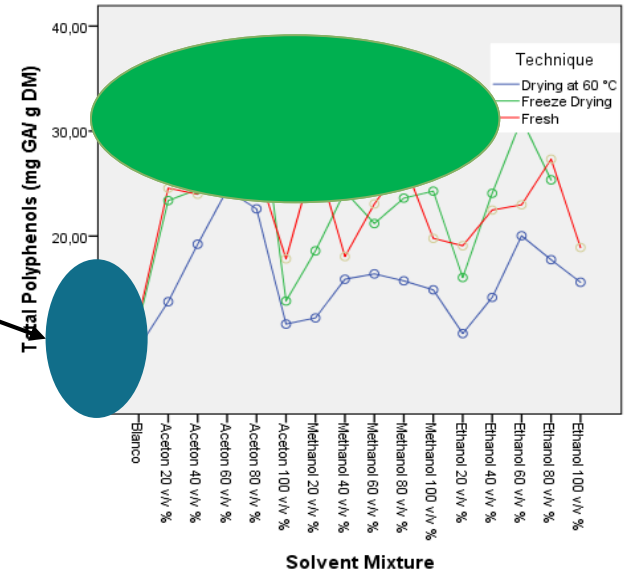
Core wood




Bark




Root



# Results

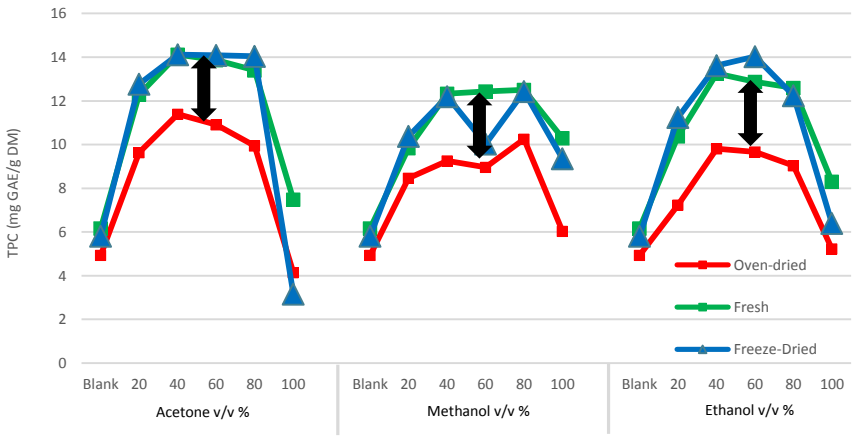
## Core wood

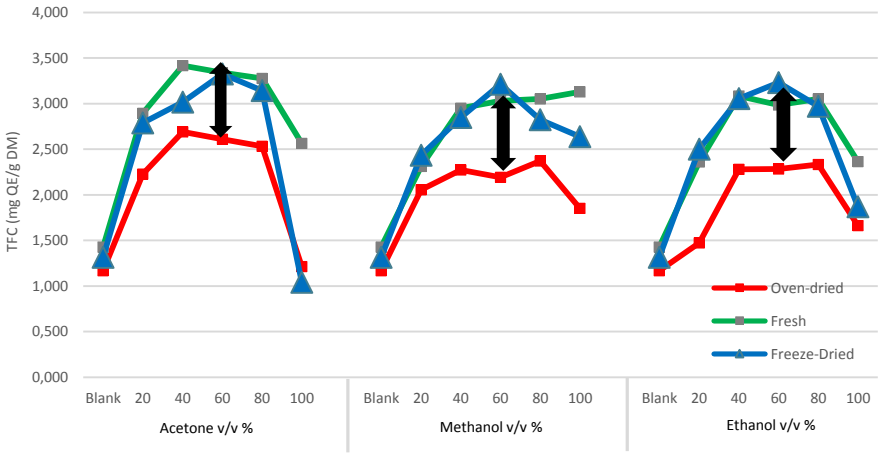
UAE
- Spectrophotometric analysis
- RP-HPLC PDA

## Apple tree core wood - UAE

### Spectrophotometric analysis – TPC

### Spectrophotometric analysis – TFC



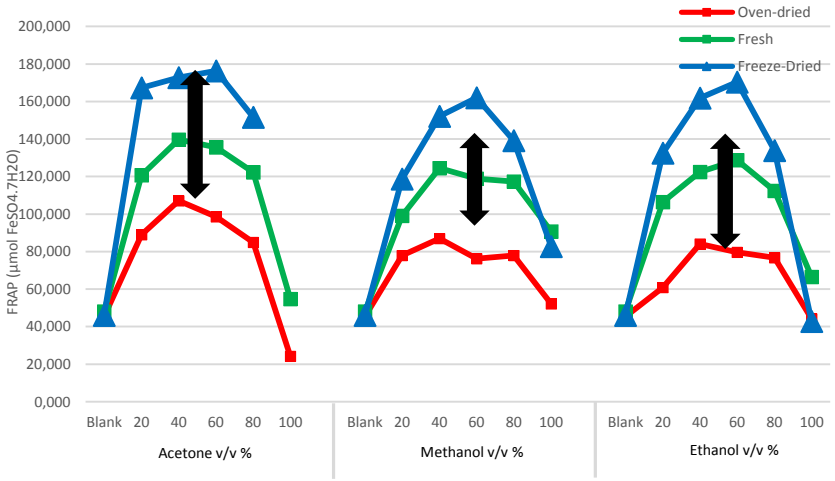

Extractions performed with acetone/water mixtures show similar results as the extractions performed in the presence of ethanol/water mixtures.

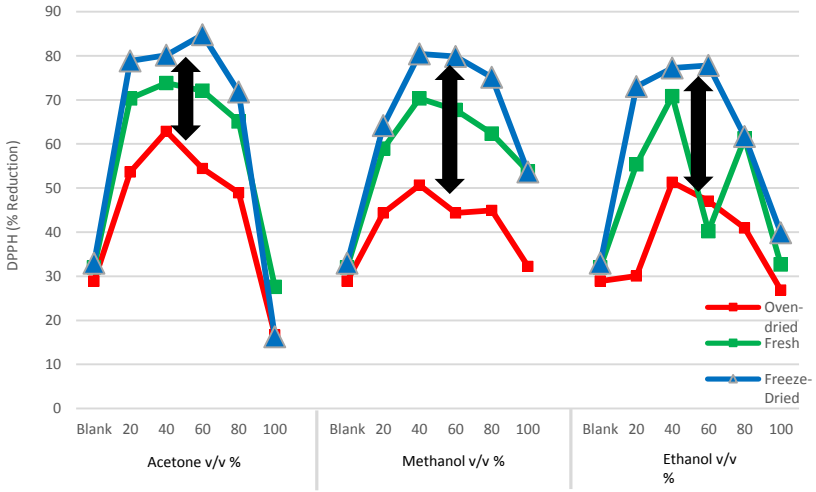
Extracts derived from oven – dried core wood:

TPC: ⬇

TFC: ⬇

# Spectrophotometric analysis – reducing capacity




Extractions performed with acetone/water mixtures show similar results as the extractions performed in the presence of ethanol/water mixtures.

Extracts derived from oven-dried bark: antioxidant activity

## Apple tree core wood - UAE

**RP-HPLC PDA**

| Phenolic compound | Fresh samples | |
|---|---|---|
| (mg/g DM) | Ethanol | Water |
| | 60 | 100 |
| Quercetin | 0.263 ± 0.003 | ND |
| (-)-Epicatechin gallate | 1.4012 ± 0.001 | 1.495 ± 0.011 |
| (-)-Epicatechin | 0.591 ± 0.008 | 0.574 ± 0.003 |
| [illegible] | [illegible] | ND |
| [illegible] | [illegible] | 2.069 ± 0.098 |
| Naringin | 0.177 ± 0.001 | 0.181 ± 0.001 |
| Naringenin | ND | ND |
| Rutin | 0.243 ± 0.004 | 0.115 ± 0.002 |
| [illegible] | [illegible] | 5.126 ± 0.003 |
| Phloretin | ND | ND |
| **Total Flavonoids** | 17.99 ± 0.03 | 9.14 ± 0.78 |
| | | |
| Procyanidin B1 | ND | ND |
| Procyanidin B2 | 0.210 ± 0.127 | 0.129 ± 0.005 |
| **Condensed tannins** | 0.210 ± 0.127 | 0.129 ± 0.005 |
| | | |
| Vanillic acid | 0.173 ± 0.002 | 0.125 ± 0.001 |
| Gallic acid | ND | 0.362 ± 0.001 |
| Ferulic acid | 0.054 ± 0.001 | ND |
| *p*-Coumaric acid | 0.057 ± 0.001 | 0.062 ± 0.001 |
| Caffeic acid | ND | ND |
| **Total Phenolic Acids** | 0.284 ± 0.004 | 0.549 ± 0.003 |
| | | |
| **Total amount of polyphenol markers** | **18.5 ± 0.2** | **9.8 ± 0.8** |

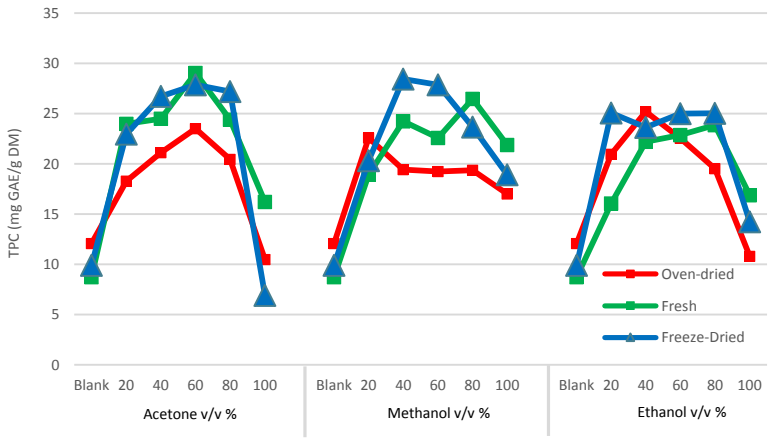
# Results

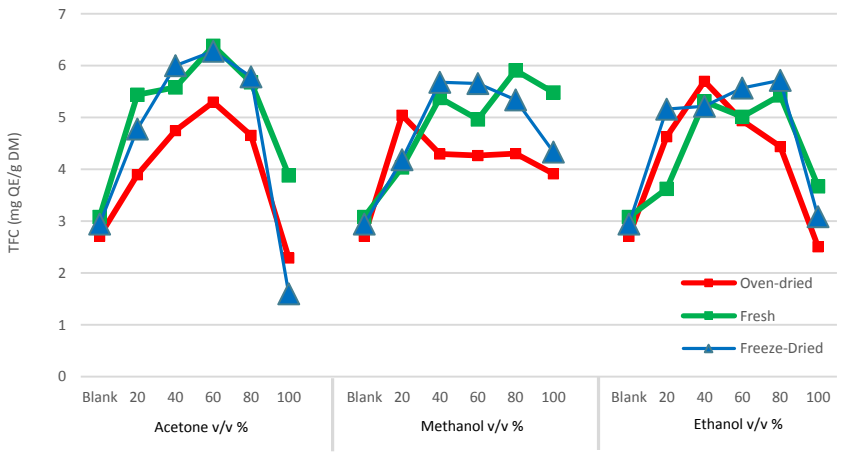
## Bark

UAE
- Spectrophotometric analysis
- RP-HPLC PDA

## Spectrophotometric analysis – TPC

## Spectrophotometric analysis – TFC

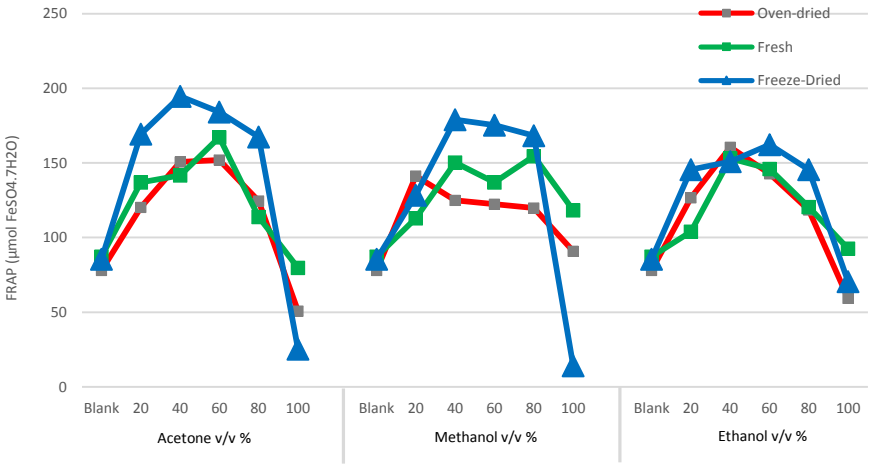


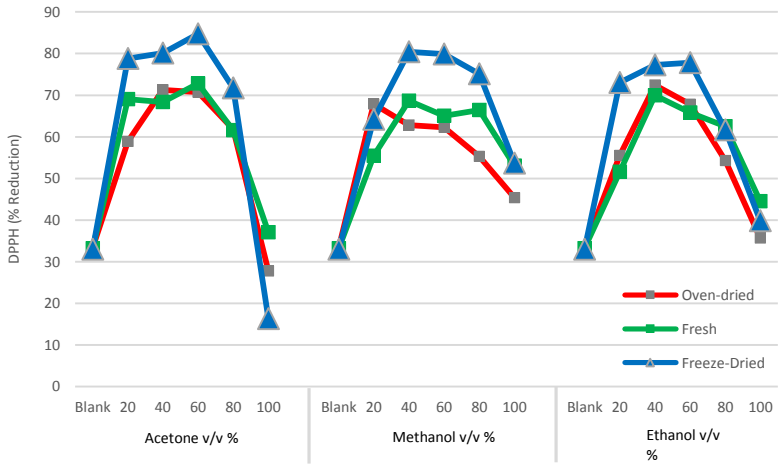




Type of solvent does not influence the result. Pure water and pure solvent show lower results than the combinations water/solvent.

In almost all cases: oven-dried wood samples show decrease in TPC and TFC.

# Spectrophotometric analysis – reducing capacity




Type of solvent does not influence the result. Pure water and pure solvent show lower results than the combinations water/solvent.

In general, the freeze-dried wood samples show the highest reducing capacity.

No difference between fresh and oven-dried wood samples.

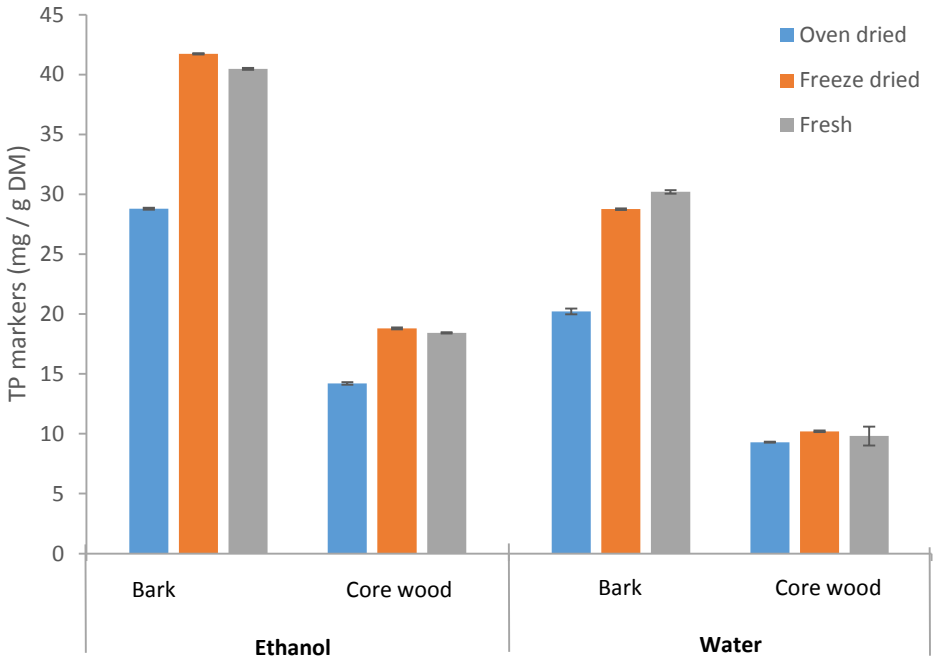
## Apple tree core wood - UAE

### RP-HPLC PDA

| Phenolic compound (mg/ g DM) | Fresh samples | |
|---|---|---|
| | Ethanol | Water |
| Solvent (v/v %) | 60 | 100 |
| Quercetin | 0.255 ± 0.003 | ND |
| (-)-Epicatechin gallate | 1.509 ± 0.001 | 1.470 ± 0.003 |
| (-)-Epicatechin | ND | 0.298 ± 0.002 |
| (+)-Catechin | ND | ND |
| Kaempferol-3-glucoside | 5.836 ± 0.011 | 2.396 ± 0.022 |
| Naringin | 0.243 ± 0.008 | 0.137 ± 0.003 |
| Naringenin | ND | ND |
| Rutin | [illegible] | 0.129 ± 0.002 |
| Phloretin | < LOD | ND |
| **Total Flavonoids** | 40.31 ± 0.08 | 28.43 ± 0.11 |
| Procyanidin B1 | ND | 0.955 ± 0.025 |
| Procyanidin B2 | ND | 0.173 ± 0.004 |
| **Condensed tannins** | ND | 1.128 ± 0.029 |
| Vanillic acid | ND | 0.117 ± 0.001 |
| Gallic acid | ND | 0.312 ± 0.001 |
| Ferulic acid | 0.155 ± 0.001 | 0.066 ± 0.001 |
| *p*-Coumaric acid | ND | 0.152 ± 0.001 |
| Caffeic acid | ND | ND |
| **Total Phenolic Acids** | 0.155 ± 0.001 | 0.647 ± 0.004 |
| **Total amount of polyphenol markers** | **40.5 ± 0.1** | **30.2 ± 0.2** |

# Core wood VS bark

**RP-HPLC PDA**



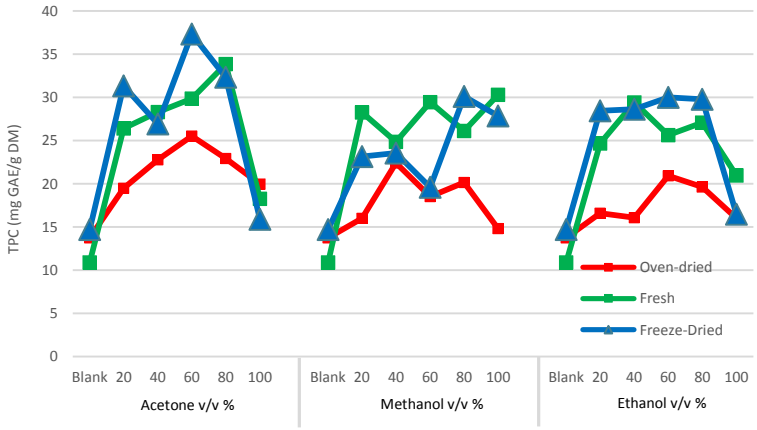
# Results

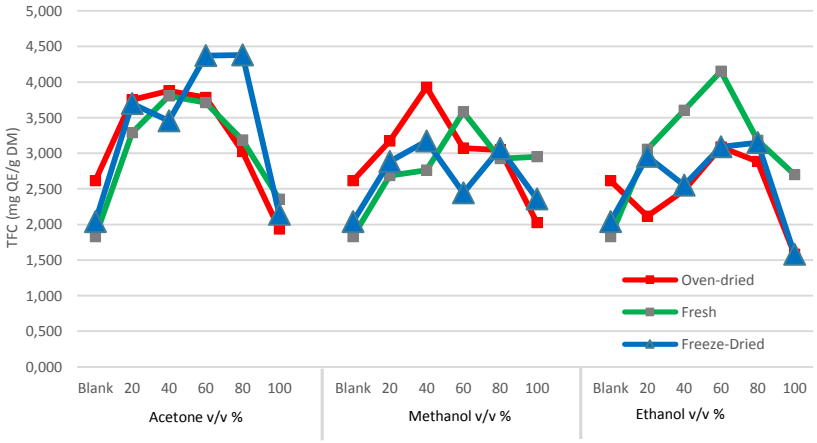
## Root

UAE
- Spectrophotometric analysis
- RP-HPLC PDA

## Spectrophotometric analysis – TPC

## Spectrophotometric analysis – TFC

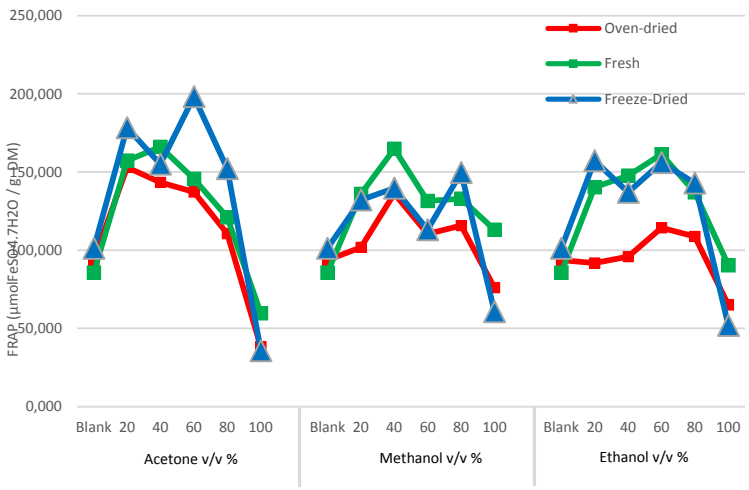


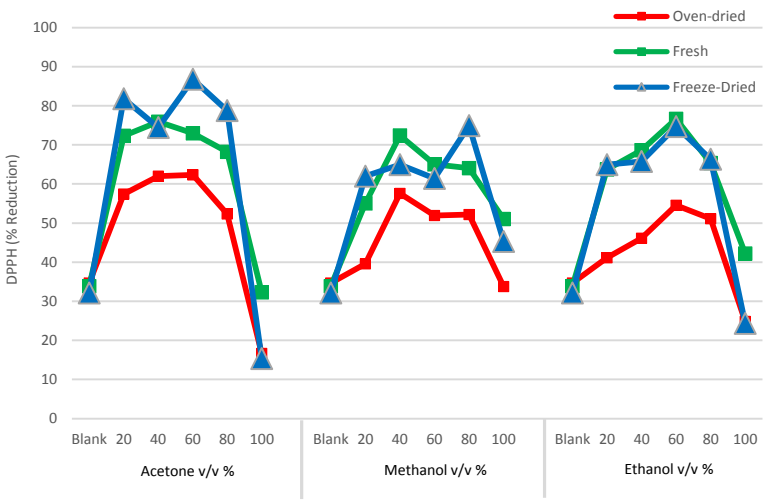




Extractions performed with acetone/water mixtures show the highest results in TPC and TFC. This is the case for all pre-treatment techniques.

Extracts obtained from oven-dried root show lower amount in TPC.

# Spectrophotometric analysis – reducing capacity




Extracts produced in the presence of acetone give the highest results.

Almost no difference between fresh and freeze-dried samples.

In general, extracts obtained by oven-dried samples showed the lowest reducing capacity.

## Apple tree root - UAE

# RP-HPLC-PDA

| Phenolic compound | Lyofilisation | |
|---|---|---|
| | **Ethanol** | |
| Solvent v/v % | 60 | 80 |
| mg Quercetine / g DM | - | 0,279 |
| mg (-) Epicatechin gallate / g DM | - | - |
| mg (-) Epicatechin / g DM | 1,152 | 0,973 |
| mg (+)-Catechine / g DM | - | - |
| [redacted] | 4,901 | 4,800 |
| mg Naringenin / g DM | - | - |
| mg Rutin / g DM | 0,174 | 0,180 |
| mg Phloretin / g DM | 0,039 | 0,032 |
| [redacted] | 51,863 | 52,641 |
| mg Naringin / g DM | 0,140 | 0,137 |
| **Total Flavonoids (mg / g DM)** | **58,27** | **59,04** |
| mg Vanillic acid / g DM | - | - |
| mg Gallic acid / g DM | - | - |
| mg Ferulic acid / g DM | 0,063 | 0,067 |
| mg *p*-coumaric acid / g DM | - | - |
| mg Caffeic acid / g DM | - | - |
| **Total Phenolic Acids (mg / g DM)** | **0,063** | **0,067** |
| Procyanidin B2 / g DM | 0,049 | - |
| Procyanidin B1 / g DM | - | - |
| **TOTAL Polyphenols (mg / g DM)** | **58,38** | **59,11** |

# Results

## Core wood

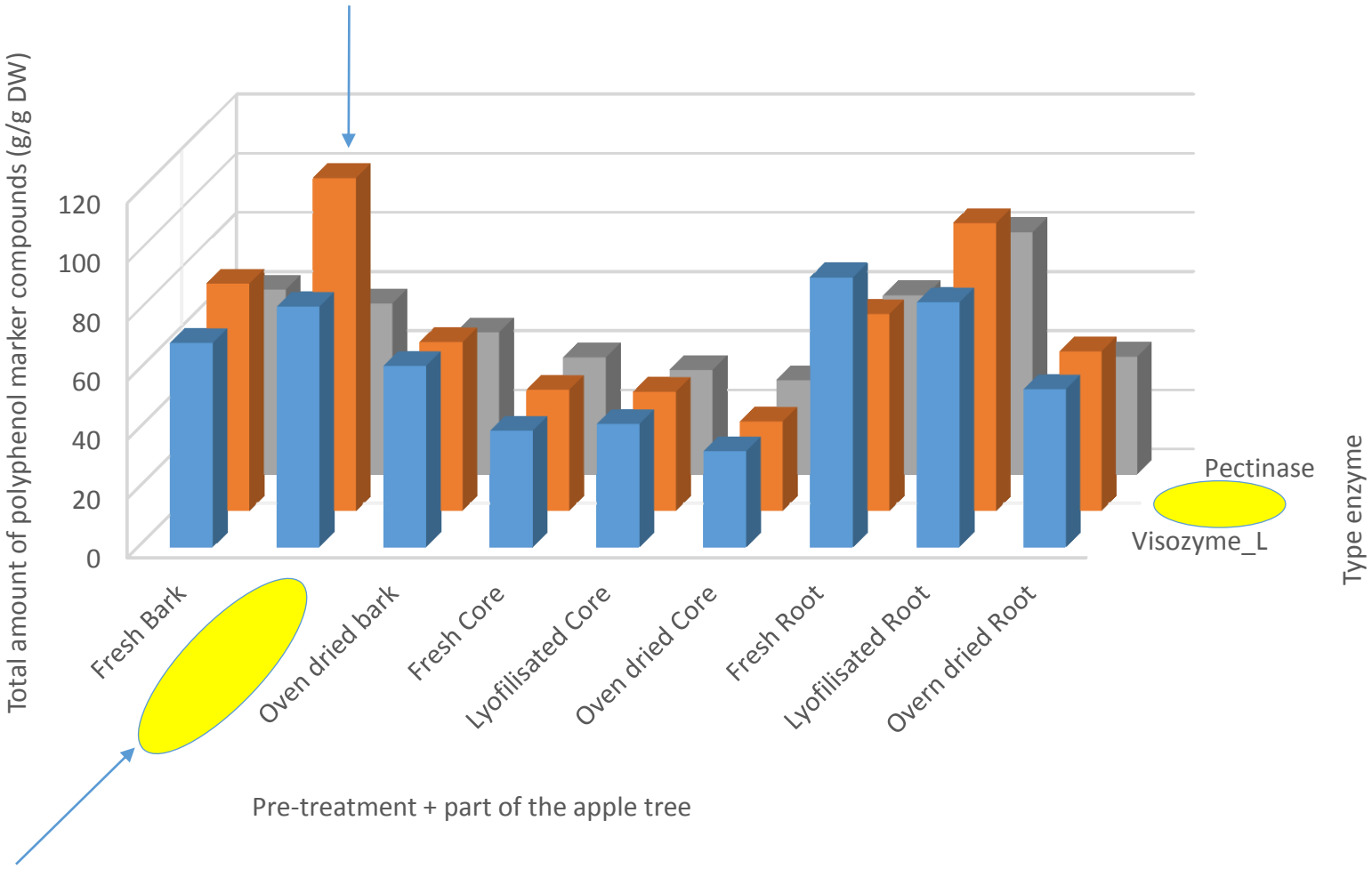
## Bark

## Root

EAE

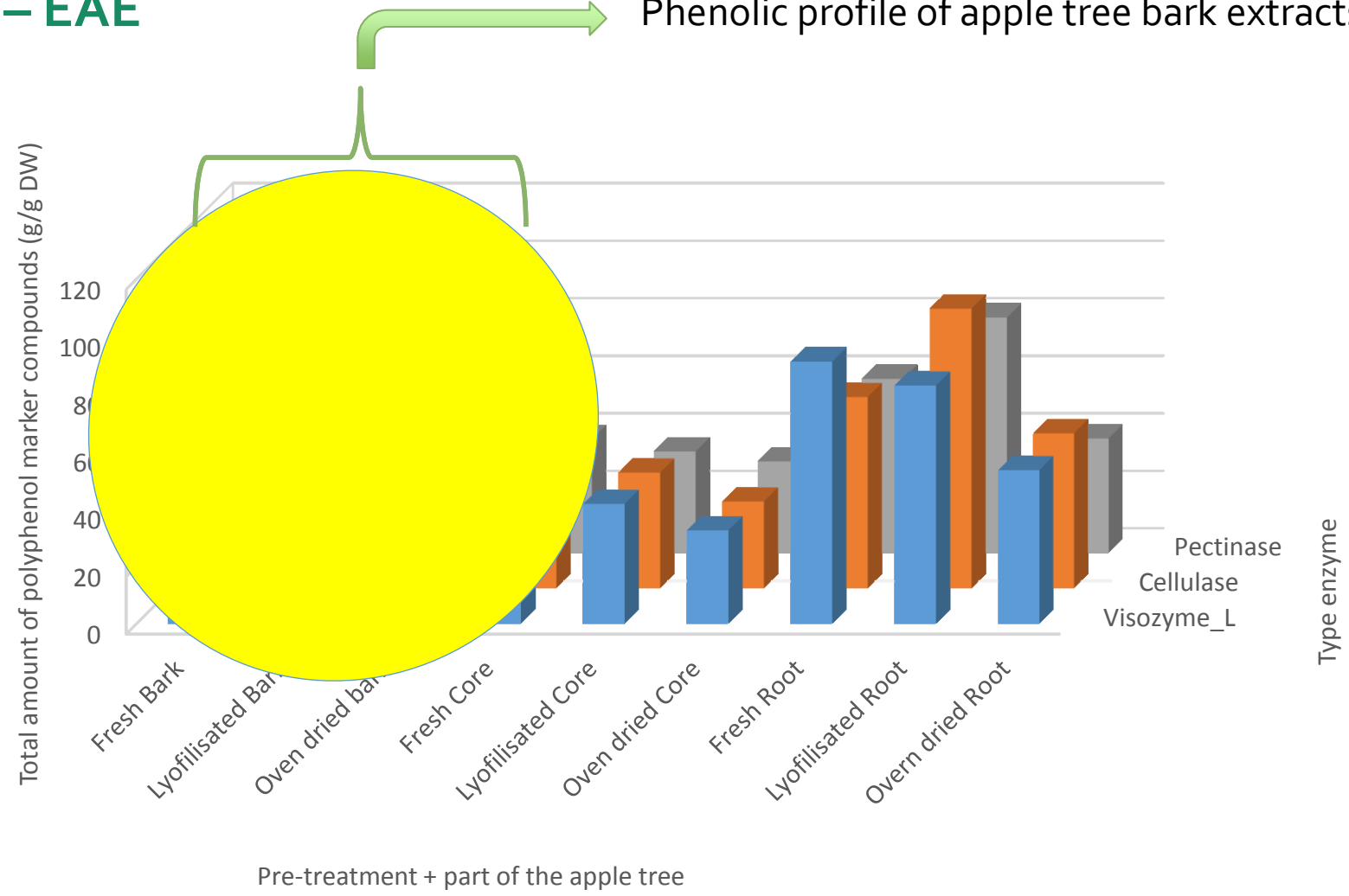
- RP-HPLC PDA

# HPLC – EAE


Total amount of polyphenol marker compounds (g/g DW)
120
100
80
60
40
20
0
Fresh Bark
Oven dried bark
Fresh Core
Lyofilisated Core
Oven dried Core
Fresh Root
Lyofilisated Root
Overn dried Root
Pre-treatment + part of the apple tree
Pectinase
Visozyme_L
Type enzyme

# HPLC – EAE

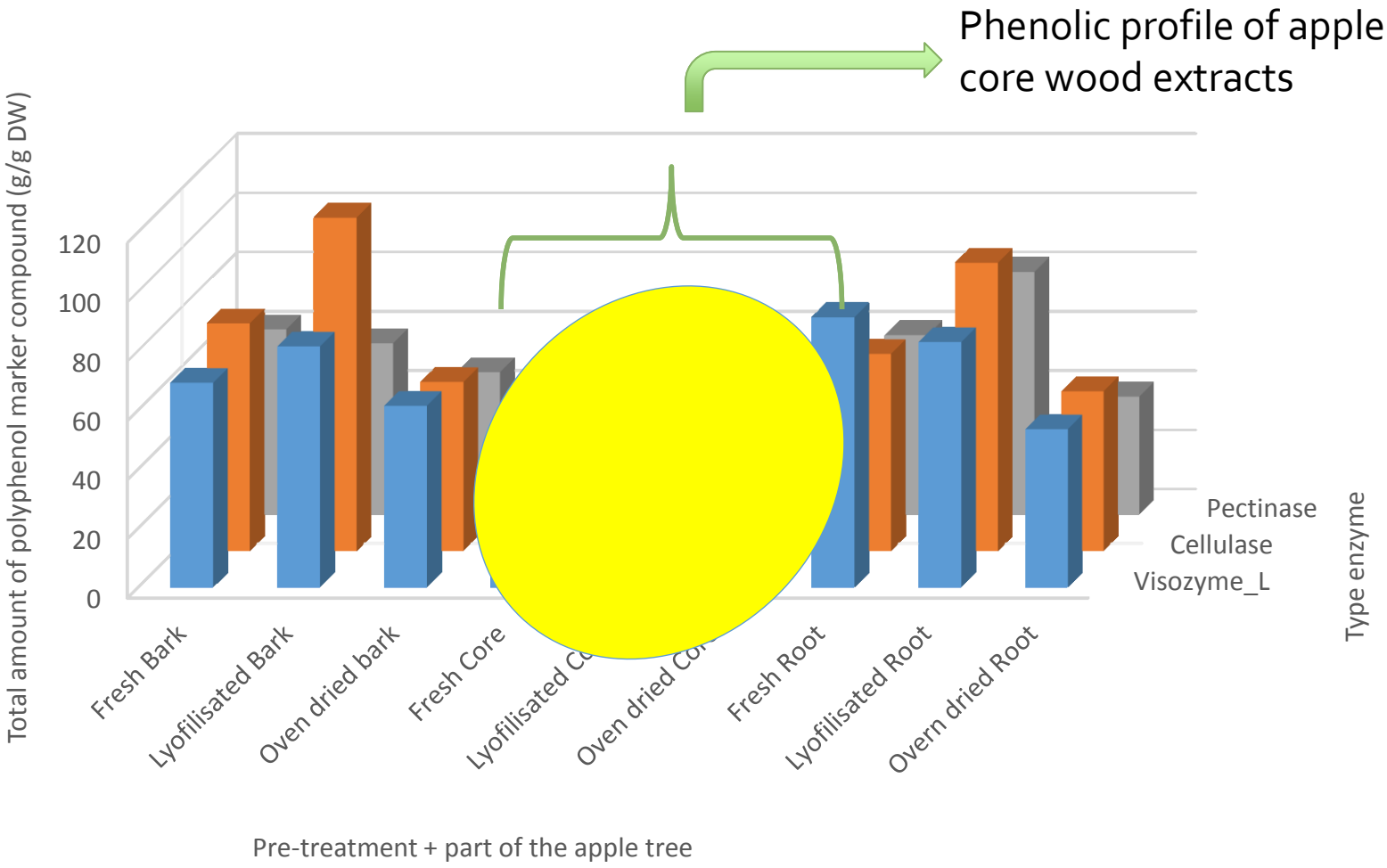
Phenolic profile of apple tree bark extracts



# Phenolic profile of apple tree bark extracts

| Solvent | Bark | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fresh | | | Air dried at 60 °C | | | Lyofilisation | | |
| v/v % | Visozyme_L | Cellulase | Pectinase | Visozyme_L | Cellulase | Pectinase | Visozyme_L | Cellulase | Pectinase |
| **FLAVONOIDS** | | | | | | | | | |
| mg Quercetine / g DM | - | - | - | - | - | - | - | - | - |
| mg (-) Epicatechin gallate / g DM | 2,58 | 2,58 | 3,06 | 3,02 | 3,07 | 3,33 | 3,02 | 3,12 | 3,10 |
| mg (-) Epicatechin / g DM | 0,88 | 0,88 | 0,83 | 0,99 | 1,10 | 0,96 | 1,10 | 1,17 | 0,98 |
| mg (+)-Catechine / g DM | 0,54 | 0,53 | 0,74 | - | - | 0,98 | 0,61 | - | 1,12 |
| [redacted] | 12,07 | 12,75 | 11,49 | 10,49 | 9,95 | 9,51 | 13,72 | 15,95 | 12,86 |
| mg Naringenin / g DM | - | - | - | - | - | - | - | - | - |
| mg Rutin / g DM | 0,52 | 0,50 | 0,40 | 0,67 | 0,50 | 0,45 | 0,67 | 1,00 | 0,39 |
| mg Phloretin / g DM | 0,18 | 0,10 | 0,65 | 0,17 | 0,10 | 0,96 | 0,11 | 0,04 | 0,68 |
| [redacted] | 50,86 | 57,94 | 43,92 | 44,33 | 40,75 | 30,58 | 60,34 | 88,91 | 37,26 |
| mg Naringin / g DM | 0,55 | 0,63 | 0,48 | 0,54 | 0,50 | 0,36 | 0,66 | 0,88 | 0,41 |
| **Total Flavonoids (mg / g DM)** | **68,18** | **75,91** | **61,57** | **60,22** | **55,96** | **47,12** | **80,23** | **111,06** | **56,79** |
| **PHENOLIC ACIDS** | | | | | | | | | |
| mg Vanillic acid / g DM | - | - | - | - | - | - | - | - | - |
| mg Gallic acid / g DM | - | - | - | - | - | - | - | - | - |
| mg Ferulic acid / g DM | 0,16 | 0,21 | 0,10 | 0,27 | 0,23 | 0,12 | 0,26 | 0,49 | 0,10 |
| mg *p*-coumaric acid / g DM | - | - | - | - | - | - | - | - | - |
| mg Caffeic acid / g DM | 0,57 | 0,57 | 0,65 | 0,67 | 0,69 | 0,70 | 0,65 | 0,72 | 0,72 |
| **Total Phenolic Acids (mg / g DM)** | **0,739** | **0,776** | **0,745** | **0,942** | **0,916** | **0,821** | **0,915** | **1,202** | **0,818** |
| **PROCYANIDINS** | | | | | | | | | |
| mg Procyanidin B2 / g DM | 0,21 | 0,16 | 0,21 | 0,20 | 0,22 | 0,12 | 0,26 | 0,24 | 0,24 |
| mg Procyanidin B1 / g DM | - | - | - | - | - | - | - | - | - |
| **Total Procyanidins (mg / g DM)** | **0,207** | **0,164** | **0,209** | **0,196** | **0,222** | **0,124** | **0,257** | **0,240** | **0,240** |
| **TOTAL Polyphenols (mg / g DM)** | **69,13** | **76,85** | **62,52** | **61,35** | **57,10** | **48,07** | **81,40** | **112,51** | **57,85** |

# HPLC – EAE

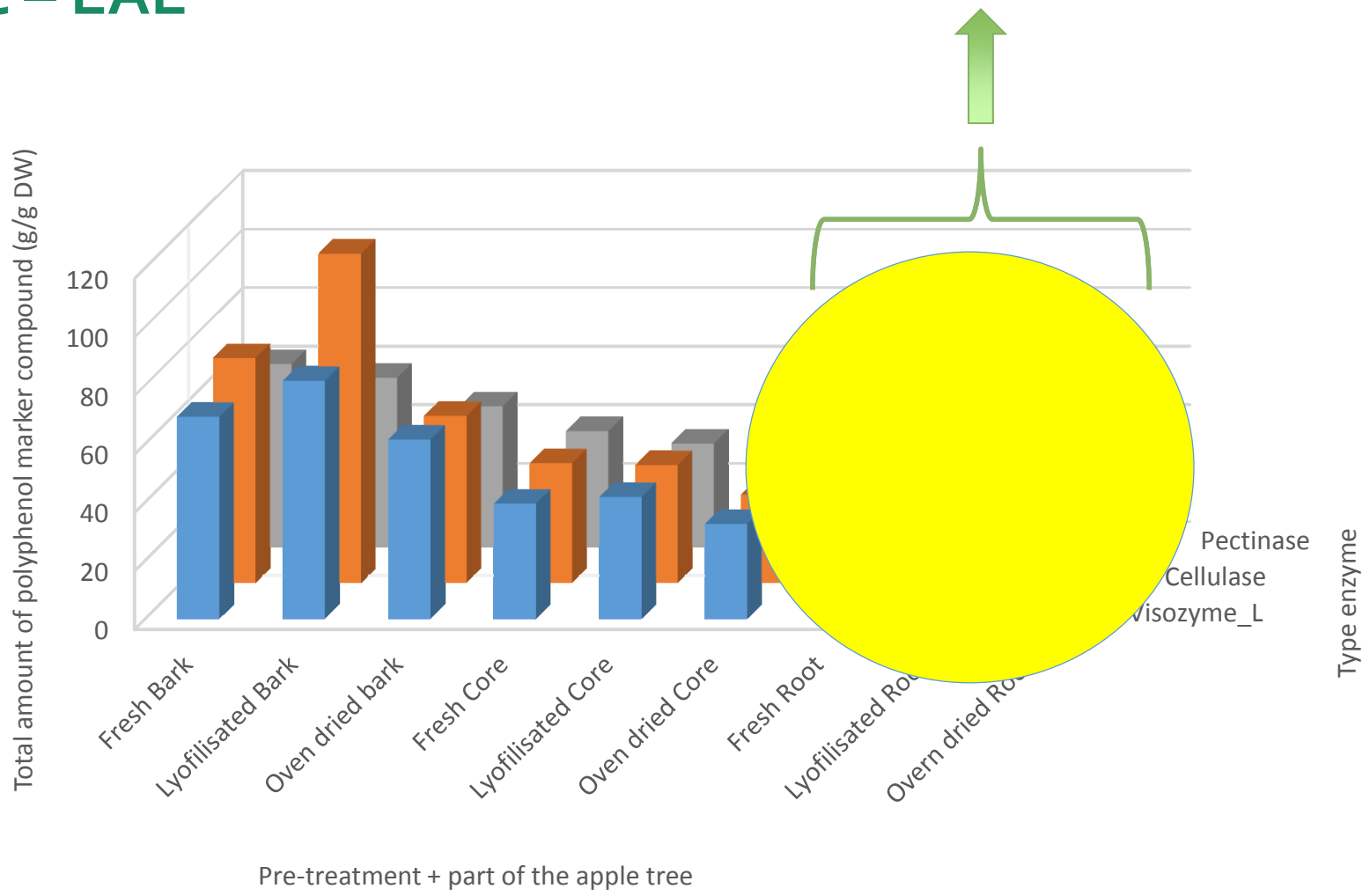

Phenolic profile of apple tree core wood extracts
Total amount of polyphenol marker compound (g/g DW)
120
100
80
60
40
20
0
Fresh Bark
Lyofilisated Bark
Oven dried bark
Fresh Core
Lyofilisated Co
Oven dried Co
Fresh Root
Lyofilisated Root
Overn dried Root
Pre-treatment + part of the apple tree
Pectinase
Cellulase
Visozyme_L
Type enzyme

# Phenolic profile of apple tree core wood extracts

| Solvent | CORE WOOD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fresh | | | Air dried at 60 °C | | | Lyofilisation | | |
| v/v % | Visozyme_L | Cellulase | Pectinase | Visozyme_L | Cellulase | Pectinase | Visozyme_L | Cellulase | Pectinase |
| **FLAVONOIDS** | | | | | | | | | |
| mg Quercetine / g DM | - | - | - | - | - | - | - | - | - |
| mg (-) Epicatechin gallate / g DM | 2,71 | 2,73 | 2,75 | 3,10 | 3,37 | 3,40 | 3,55 | 3,12 | 3,11 |
| mg (-) Epicatechin / g DM | 1,87 | 0,98 | 0,97 | 1,09 | 1,05 | 1,07 | 0,84 | 0,83 | 0,85 |
| mg (+)-Catechine / g DM | 0,83 | 1,00 | 1,30 | 0,86 | 0,88 | 1,25 | 1,08 | 0,86 | 1,52 |
| [redacted] | 14,23 | 15,18 | 14,74 | 11,55 | 10,22 | 10,97 | 14,90 | 14,84 | 13,53 |
| mg Naringenin / g DM | - | - | - | - | - | - | - | - | - |
| mg Rutin / g DM | 0,49 | 0,52 | 0,48 | 0,41 | 0,40 | 0,38 | 0,55 | 0,35 | 0,38 |
| [redacted] | 0,02 | 0,01 | 0,18 | 0,05 | 0,03 | 0,21 | 0,02 | - | 0,49 |
| [redacted] | 17,79 | 18,86 | 17,41 | 14,42 | 13,56 | 12,80 | 18,88 | 18,79 | 13,50 |
| mg Naringin / g DM | 0,37 | 0,40 | 0,22 | 0,33 | 0,33 | 0,19 | 0,40 | 0,17 | 0,14 |
| **Total Flavonoids (mg / g DM)** | **38,32** | **39,69** | **38,04** | **31,81** | **29,85** | **30,27** | **40,22** | **38,96** | **33,52** |
| **PHENOLIC ACIDS** | | | | | | | | | |
| mg Vanillic acid / g DM | 0,24 | 0,26 | 0,27 | 0,25 | - | 0,32 | 0,32 | 0,30 | 0,32 |
| mg Gallic acid / g DM | - | - | - | - | - | - | - | - | - |
| mg Ferulic acid / g DM | 0,10 | 0,11 | - | 0,10 | 0,12 | - | 0,12 | 0,12 | - |
| mg *p*-coumaric acid / g DM | - | - | - | - | - | - | - | - | - |
| mg Caffeic acid / g DM | - | - | 0,65 | - | - | 0,79 | - | - | 0,73 |
| **Total Phenolic Acids (mg / g DM)** | **0,347** | **0,376** | **0,920** | **0,357** | **0,120** | **1,108** | **0,437** | **0,426** | **1,045** |
| **PROCYANIDINS** | | | | | | | | | |
| mg Procyanidin B2 / g DM | 0,77 | 0,90 | 0,64 | 0,33 | 0,27 | 0,48 | 1,07 | 0,92 | 0,77 |
| mg Procyanidin B1 / g DM | - | - | - | - | - | - | - | - | - |
| **Total Procyanidins (mg / g DM)** | **0,772** | **0,898** | **0,637** | **0,327** | **0,273** | **0,481** | **1,071** | **0,918** | **0,767** |
| **TOTAL Polyphenols (mg / g DM)** | **39,44** | **40,96** | **39,60** | **32,49** | **30,24** | **31,86** | **41,73** | **40,31** | **35,33** |

# HPLC – EAE

Phenolic profile of apple tree root extracts



# Phenolic profile of apple tree root extracts

| Solvent | ROOT | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fresh | | | Air dried at 60 °C | | | Lyofilisation | | |
| v/v % | Visozyme_L | Cellulase | Pectinase | Visozyme_L | Cellulase | Pectinase | Visozyme_L | Cellulase | Pectinase |
| **FLAVONOIDS** | | | | | | | | | |
| mg Quercetine / g DM | - | - | - | - | - | - | 0,58 | - | 0,54 |
| mg (-) Epicatechin gallate / g DM | 3,06 | 3,28 | 3,10 | 3,47 | 3,40 | 3,59 | 3,53 | 3,38 | 3,35 |
| mg (-) Epicatechin / g DM | 1,10 | 1,01 | 0,96 | 1,46 | 1,28 | 1,17 | 1,19 | 1,66 | 1,11 |
| mg (+)-Catechine / g DM | 0,63 | 0,64 | 0,89 | - | - | 0,79 | - | 0,78 | 1,01 |
| [redacted] | 11,31 | 10,51 | 12,88 | 5,50 | 6,83 | 6,38 | 13,61 | 16,34 | 11,62 |
| mg Naringenin / g DM | - | - | - | - | - | - | - | - | - |
| mg Rutin / g DM | 0,22 | 0,33 | 0,28 | 0,18 | 0,20 | 0,16 | 0,47 | 0,53 | 0,30 |
| mg Phloretin / g DM | 4,18 | 3,33 | 4,34 | 1,24 | 1,03 | 1,91 | 1,24 | 2,78 | 3,22 |
| [redacted] | 66,87 | 43,87 | 34,58 | 38,40 | 36,66 | 24,37 | 58,09 | 67,57 | 56,93 |
| mg Naringin / g DM | 0,29 | 0,26 | 0,17 | 0,27 | 0,28 | 0,14 | 0,31 | 0,38 | 0,33 |
| **Total Flavonoids (mg / g DM)** | **87,67** | **63,22** | **57,21** | **50,52** | **49,68** | **38,50** | **79,03** | **93,40** | **78,42** |
| **PHENOLIC ACIDS** | | | | | | | | | |
| mg Vanillic acid / g DM | 0,24 | 0,25 | 0,27 | 0,34 | 0,28 | 0,30 | 0,30 | 0,27 | 0,27 |
| mg Gallic acid / g DM | - | - | - | - | - | - | - | - | - |
| mg Ferulic acid / g DM | 0,11 | 0,11 | 0,10 | - | 0,10 | 0,12 | 0,13 | 0,14 | 0,11 |
| mg *p*-coumaric acid / g DM | - | - | - | - | - | - | - | - | - |
| mg Caffeic acid / g DM | 0,65 | 0,61 | 0,62 | - | 0,69 | 0,74 | 0,69 | 0,67 | 0,67 |
| **Total Phenolic Acids (mg / g DM)** | **1,002** | **0,973** | **0,989** | **0,336** | **1,073** | **1,165** | **1,115** | **1,080** | **1,052** |
| **PROCYANIDINS** | | | | | | | | | |
| mg Procyanidin B2 / g DM | 0,17 | 0,19 | 0,16 | 0,13 | 0,15 | 0,15 | 0,27 | 0,38 | 0,19 |
| mg Procyanidin B1 / g DM | 2,38 | 2,18 | 2,21 | 2,48 | 3,03 | - | 2,48 | 2,45 | 2,22 |
| **Total Procyanidins (mg / g DM)** | **2,546** | **2,371** | **2,375** | **2,608** | **3,179** | **0,147** | **2,741** | **2,828** | **2,415** |
| **TOTAL Polyphenols (mg / g DM)** | **91,21** | **66,57** | **60,57** | **53,47** | **53,93** | **39,81** | **82,89** | **97,31** | **81,89** |

# Conclusion

- ✓ Apple wood extracts contain a considerable amount of phenolic compounds with antioxidant activity.

- ✓ Possibility of applying these type of extraction techniques at pilot scale.
  - ➢ *Valorization of the vegetable by-products as natural sources of functional substances for further use*.

- ✓ Phloridzin is the major compound contributing to the phenolic composition, followed by kaempferol-3-glucoside.

- ✓ The highest amount of polyphenols was recovered from fresh samples, not the ideal process due to the large volumes that need to be stored.
  - ➢ *The amount of phenolic compounds and reducing power is considerably higher for freeze-drying process in correlation to oven-drying.*

# Literature


1. Spingo, G.; Tramelli, L.; De Faveri, D.M. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J. Food Eng.* **2007**, 81, 200 – 208.

2. Yilmaz, Y.; Toledo, T.R. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. *J. Food Compos. Anal*. **2006**, *19*, 41-48.

3. Jerez, M.; Selga, A., Sineiro, J.; Torres, J.L;, Nunez, M.J.. A comparison between bark extracts from Pinus pinaster and Pinus radiata: antioxidant activity and procyanidin composition. *Food Chem*. **2007**, 100, 439–444.

4. Ku, C.S.; Jang, J.P.; Mun, S.P.; Exploitation of polyphenol-rich pine barks for potent antioxidant activity. *J. Wood Sci.* **2007**, 53, 524–528.

5. Ghitescu, R.E.; Volf, I.; Carausu, C.; Bühlmann, A.M.; Andrei, G.L.; Valentin, I.P. Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark. *Ultrasonics Sonochemistry*. **2015**, 22, 535-541.

6. Dedrie, M.; Jacquet, N.; Bombeck, P.-L.; Hébert, J.; Richel, A. Oak barks as raw materials for the extraction of polyphenols for the chemical and pharmaceutical sectors. A regional case study. *Ind. Crops Prod.* **2015**, *70*, 316–321.

7. Xü, K.; Lü, H.; Qü, B.; Shan, H.; Song, J. High-speed counter-current chromatography preparative separation and purification of phloretin from apple tree bark. *Sep. Purif. Technol*. **2010**, *72*, 406-409.

8. Moreira, M.; Barroso, F.M.; Boeykens, A.; Withouck, H.; Morais, S.; Delerue-Matos, C. Valorization of apple tree wood residues by polyphenols extraction: Comparison between conventional and microwave-assisted extraction. *Ind. Crops Prod*. **2017**, *104,* 210-220.

9. Meneses, G.T.N.; Martins, S.; Teixeira, J.A.; Mussatto, S.I. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Sep. Purif. Technol.* **2013**, *108*, 152- 158.

# Literature


10. Lazar, L.; Talmaciu, A.I.; Volf, I.; Popa, V.I. Kinetic modeling of the ultrasound-assisted extraction of polyphenols from *Picea abies* bark. *Ultrason. Sonochem.* **2016**, *32*, 191-197.

11. Wang Y. et al., 2011

12. Wang T. et al.2010

13. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144-158.

14. Dvorakova, M.; Guido, L.F.; Dostálek, P.; Skulilová, Z.; Moreira, M.M.; Barros, A.A. Antioxidant properties of free, soluble ester and insoluble-bound phenolic compounds in different barley varieties and corresponding malts. *J. Inst. Brew.* **2008**, *114*, 27-33.

15. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *J. Food Drug Anal.* **2002**, *10*, 178-182.

16. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 1995, 28, 25-30

17. Benzie, F.F.I.; Strain, J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. Anal. Biochem. 1996, 239, 70-76.

# Literature


18. Rubilar, M.; Pinelo, M.; Shene, C.; Sineiro, J.; Nunez, M.J. Separation and HPLC-MS Identification of Phenolic Antioxidants from Agricultural Residues: Almond Hulls and Grape Pomace. *J. Agric. Food Chem.* **2007**, *55*, 10101-10109.

19. Jakobek, L.; Barron, R.A. Ancient apple varieties from Croatia as a source of bioactive polyphenolic compounds. *J. Food Compos. Anal*. **2016**, *45*, 9-15.

20. Tsao, R.; Yang, R.; Young, C.; Zhu, H. Polyphenolic Profiles in Eight Apple Cultivars Using High-Performance Liquid Chromatography. *J. Agric. Food Chem.* **2003**, *51*, 6347-6353.

# BIT - O

Biochemical Innovation Team Odisee

## Thank you for the attention!