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DELIVERABLE: E 2.2.1 REPORT FOR THE CHARACTERIZATION OF EACH EXTRACTED ACTIVE COMPONENTS

Work Package: GT2

Responsible partner: Instituto de Tecnologías Químicas Emergentes de la Rioja (INTER)

Contributors partners: AkiNaO (Vanessa Andreu/Annabel Levert)

INTER (Marta Perez)

Delivery date: January 2017

Real delivery date: April 2017 (draft)

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1. Introduction

INTER and AKINAO have received samples from different partners of the project, in order to characterize their properties. The samples received are:

SOURCE	EXTRACTING PARTNER	METHOD OF EXTRACTION	SAMPLES CODES
Pine bark	IPVC	Sohxlet in water	PW1, PW2, PW3
	IPVC	Sohxlet 50% water-50% ethanol	PWE1, PWE2, PWE2
	IPVC	Sohxlet 100% ethanol	PE1, PE2, PE3
	UMINHO	Conventional extraction 100% water	C-H2O-1, C-H2O-2, C-H2O-3
	UMINHO	Conventional extraction 30% water 70% ethanol	C-EtOH-1, C-EtOH-2, C-EtOH-3
	UMINHO	Ohmic extraction 100% water	O-H2O-1, O-H2O-2, O-H2O-3
	UMINHO	Ohmic extraction 30% water 70% ethanol	O-EtOH-1, O-EtOH-2, O-EtOH-3
Peach	UdL	Ultrasound 70% ethanol-30% water	US-Peach-Rep-1, US-Peach-Rep-2
Cucumber	UdL	Ultrasound 70% ethanol-30% water	US-Cucumber-Rep-1 US-Cucumber-Rep-2
Apple	UdL	Ultrasound 70% ethanol-30% water	US-Apple-Rep-1, US-Apple-Rep-2
Red pepper	UdL	Ultrasound 70% ethanol-30% water	US-Redpepper-Rep-1 US-Redpepper-Rep-2
Olive pomace	UMinho	Conventional extraction 100% water	AL-C-H2O-1, AL-C-H2O-2, AL-C-H2O-3
	UMinho	Conventional extraction 50% water 50% ethanol	AL-C-EtOH-1, AL-C- EtOH-2, AL-C- EtOH-3
	UMinho	Ohmic extraction 100% water	AL-O-H2O-1, AL-O-H2O-2, AL-O-H2O-3
	UMinho	Ohmic extraction 50% water 50% ethanol	AL-O-EtOH-1, AL-O- EtOH-2, AL-O- EtOH-3

INTER has developed the physic-chemical characterization of the different samples, pH, density and solubility. In addition, INTER has tested the stability of the different extracts by measuring the evolution of antioxidant capacity along the time.

AKINAO has studied the samples by HPLC-MS, in order to identify the most important components in the extracted products.

2. Results of phys-chemical characterization

INTER has characterized the different samples provided by the other partners. Samples were directly sent in liquid form and as it was received, they were measured. Samples C-EtOH-1 and C-EtOH-2 suffered a problem during the transportation and they were not received.

Samples of olive pomace were received at the end of March and only antioxidant capacity has been measured.

2.1 pH

In order to measure pH of the samples, INTER has used a pH-meter located in our facilities and properly calibrated. The results are shown in next table:

SOURCE	SAMPLES CODES	REPLICA			MEDIA
		1	2	3	
Pine bark	PW	4,33	4,43	4,39	4,38
	PWE	4,15	4,19	4,15	4,16
	PE	4,92	4,73	4,67	4,77
	C-H2O	3,89	3,93	3,93	3,92
	C-EtOH	4,14			4,14
	O-H2O	3,92	3,94	4,01	3,96
	O-EtOH	4,01	4,15	4,08	4,08
Peach	US-Peach	5,07	5,11		5,09
Red pepper	US-Redpepper	5,46	5,45		5,46
Apple	US-Apple	4,86	4,93		4,90
Cucumber	US-Cucumber	5,63	5,65		5,64

The samples of olive pomace were received recently and INTER has not time to perform the characterization and include in this draft of the deliverable.

From the different samples of pine bark, we can observe that:

- Using Sohxlet, we obtained in general less acid species, specially using ethanol. It is also worthy to comment that the of ethanol and water aims to obtain more acid species.
- The results obtained by conventional and ohmic heating are quite similar in terms of pH, offering more acid species for water extraction.

2.2 Apparent density

The density of the sample was measured using a picnometer of Gay-Lussac for liquids of 10 ml. The density values are showing in the next table:

SOURCE	SAMPLES CODES	REPLICA			MEDIA
		1	2	3	
Pine bark	PW	1,00	1,00	1,00	1,00
	PWE	0,975	0,975	0,975	0,975
	PE	0,91	0,91	0,91	0,91
	C-H2O	1,00	1,00	1,00	1,00
	C-EtOH			0,97	0,97
	O-H2O	1,004	1,004	1,004	1,004
	O-EtOH	0,966	0,963	0,964	0,964
Peach	US-Peach	0,951	0,95		0,951
Red pepper	US-Redpepper	0,953	0,953		0,953
Apple	US-Apple	0,952	0,952		0,952
Cucumber	US-Cucumber	0,951	0,951		0,951

The density of the samples is due to the vehicle where the sample is prepared.

2.3 Solubility

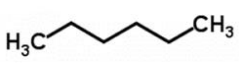
In order to check, the solubility of the obtained samples, INTER has tested visually the solubility in front of different solvents selected by their solvent polarity. See next table:

Solvent	Solvent Polarity Index, *P*
Hexane	0.1
Carbon tetrachloride	1.56
Isopropyl ether	1.83
Toluene	2.4
Methyl-*t*-butyl ether	2.4
Chloroform	2.7
Diethyl ether	2.8
Dichloromethane	3.1
Isopropanol	3.92
Tetrahydrofuran	4.0
Ethyl Acetate	4.4
Methanol	5.1
Acetone	5.1
Dioxane	5.27
Acetonitrile	5.8
Water	10.2

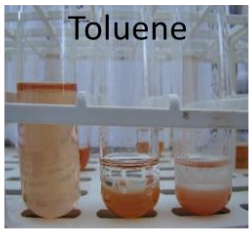
Reference: D. Harris, *Quantitative Chemical Analysis*, 9th ed., 2015.
E. Katz *et al*., Eds., *Handbook of HPLC*, Marcel Dekker, New York, 1998.

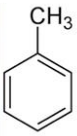
The selected solvents were hexane, toluene, 2-propanol and acetone.

The results obtained for the samples provided by IPVC is shown in next figure:



Sample	Solvent	Solubility
PE1	Hexane	Solid precipitate
PW1		Insoluble
PWE1		Insoluble
PE1	Toluene	Oil extraction
PW1		Insoluble
PWE1		Insoluble
PE1	2-propanol	Soluble
PW1		Soluble
PWE1		Soluble
PE1	Acetone	Soluble
PW1		Soluble
PWE1		Soluble





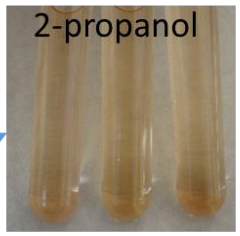
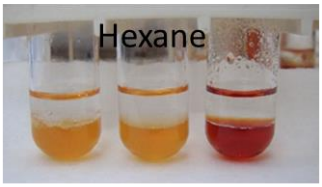
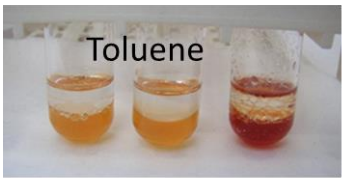
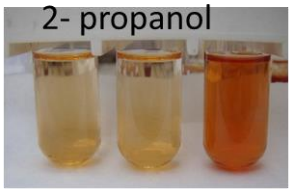


Figure: Solubility studies of IPVC samples

Similar results were obtained in the case of UMinho samples:



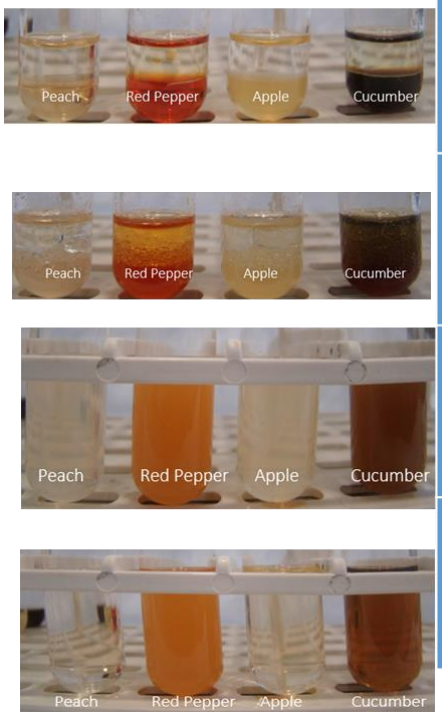




Sample	Solvent	Solubility
C-H2O-1	Hexane	Insoluble
O-H2O-1		Insoluble
C-EtOH-3		Insoluble
C-H2O-1	Toluene	Insoluble
O-H2O-1		Insoluble
C-EtOH-3		Insoluble
C-H2O-1	2-propanol	Soluble
O-H2O-1		Soluble
C-EtOH-3		Soluble
C-H2O-1	Acetone	Soluble
O-H2O-1		Soluble
C-EtOH-3		Soluble

Figure: Solubility studies of UMinho samples

Samples from UdL offered a very similar results:



Sample	Solvent	Solubility
US-Peach	Hexane	Insoluble
US- Red Pepper		Insoluble
US-Apple		Insoluble
US-Cucumber		Insoluble
US-Peach	Toluene	Insoluble
US- Red Pepper		Insoluble
US-Apple		Insoluble
US-Cucumber		Insoluble
US-Peach	2-propanol	Soluble
US- Red Pepper		Soluble
US-Apple		Soluble
US-Cucumber		Soluble
US-Peach	Acetone	Soluble
US- Red Pepper		Soluble
US-Apple		Soluble
US-Cucumber		Soluble

Figure: Solubility studies of UdL samples

As a conclusion of the solubility essays, we can say that the samples are polar, soluble in polar solvents such as acetone, and 2-propanol.

2.4 Colour of the samples and evolution

The absorbance pattern of the samples were measured in order to estimate the quantity of components extracted:

IPVC samples: pine bark

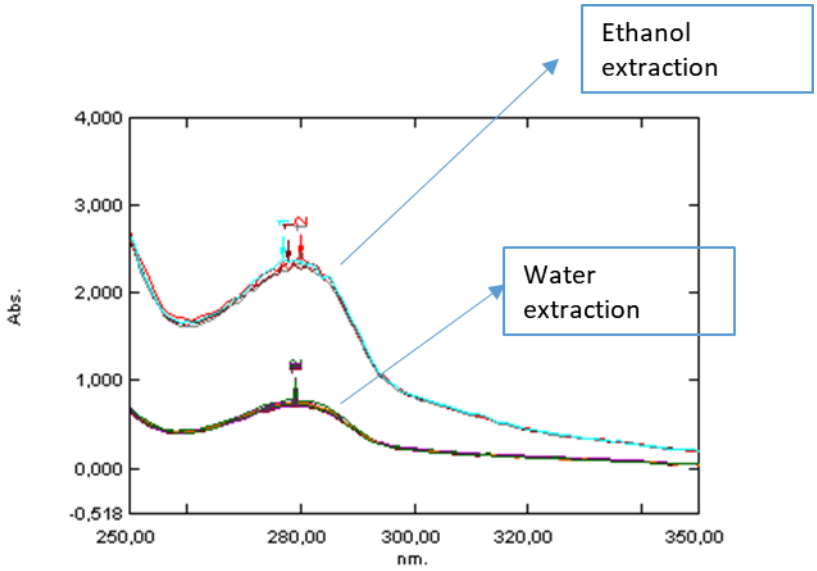


Figure: UV-vis spectra evolution of IPVC samples

The evolution of the samples after 6 months in different conditions is shown in next table:

Sample	Abs. (278nm)	Abs . 6 month refrigerated	Abs . 6 month Tª ambient
PW1	0,794	0,83	1,089
PW2	0,707	0,735	1,102
PW3	0,693	0,666	0,814
PE1	1,375	1,325	1,391
PE2	1,393	1,316	1,397
PE3	1,420	1,343	1,396
PWE1	1,498	1,313	1,445
PWE2	1,559	1,369	1,512
PWE3	1,496	1,376	1,502

Depending on the storage conditions, the samples have shown a reduction or increase in the absorbance. It is worthy to comment that it is complicated to calculate if the increase of colour is positive or negative, due to the generation of more coloured species due to oxidation, or the loss of some species due to degradation.

UMINHO samples: pine bark

Similar studies have been developed with UMINHO samples, and some of them have shown contamination by fungi, even though they are refrigerated. In this case, most of the samples have shown a great reduction of the absorbance, although the samples with ethanol have increased the absorbance in some samples.

The obtained results are shown in next table:

		Samples Refrigerated		Samples Tª ambient	
	18.07.2017	17.11.2017		17.11.2017	
Sample	A_{278}	A_{278} 4month	↓	A_{278} 4month	↓
C-H_2O-1	0,743	Fungi	X	Fungi	X
C-H_2O-2	0,729	Fungi	X	0,445	0,284
C-H_2O-3	0,75	0,648	0,102	0,435	0,315
C-EtOH-3	2,342	2,257	0,085	2,342	0
O-H_2O-1	0,779	0,723	0,056	0,492	0,287
O-H_2O-2	0,709	0,652	0,057	Fungi	X
O-H_2O-3	0,712	0,671	0,041	0,424	0,288
O-EtOH-1	2,245	2,044	0,201	2,129	0,116
O-EtOH-2	2,262	2,17	0,092	2,584	-0,322
O-EtOH-3	2,345	2,389	-0,044	2,644	-0,299

UdL samples: peach, red pepper, apple and cucumber

Some of the samples needed to be diluted in order to not saturate the signal. The results are shown in next graphic:

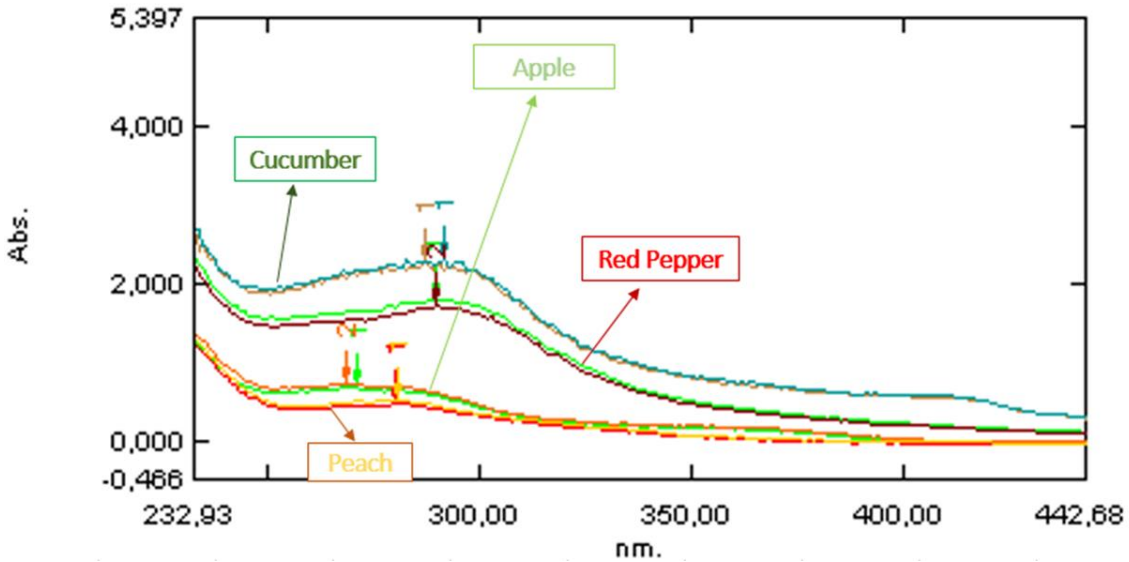


Figure: UV-vis spectra evolution of UMinho samples

The evolution along the months show a reduction of the colour in all the cases except for the samples of apple, where it is observed an slightly increasing in the signals. The results are summarized in next table:

	A_{278nm}	$A_{278\ nm}$ 1month	$A_{278\ nm\ 1month}$	$A_{278\ nm\ 3month}$	$A_{278\ nm}$ 3month
Sample		Tª ambient	Refrigerated	Tª ambient	Refrigerated
Peach Rep 1	0,519	0,524	0,585	0,443	0,496
Peach Rep 2	0,473	0,54	0,543	0,491	0,529
Red Pepper Rep1	1,705	1,624	1,705	1,268	1,739
Red Pepper Rep2	1,608	1,666	1,693	1,209	1,696
Apple Rep 1	0,648	0,814	0,794	0,575	0,733
Apple Rep 2	0,699	0,831	0,846	0,584	0,749
Cucumber Rep 1	2,238	2,168	2,37	1,342	2,268
Cucumber Rep 2	2,208	2,09	2,647	1,377	2,199

2.5 Antioxidant capacity-Stability

In order to understand which are the most interesting samples for encapsulation, and to evaluate their stability, INTER has measured the antioxidant capacity of the samples using ABTS essay. The essay is based on the radical scavenging of the cation radical ABTS:

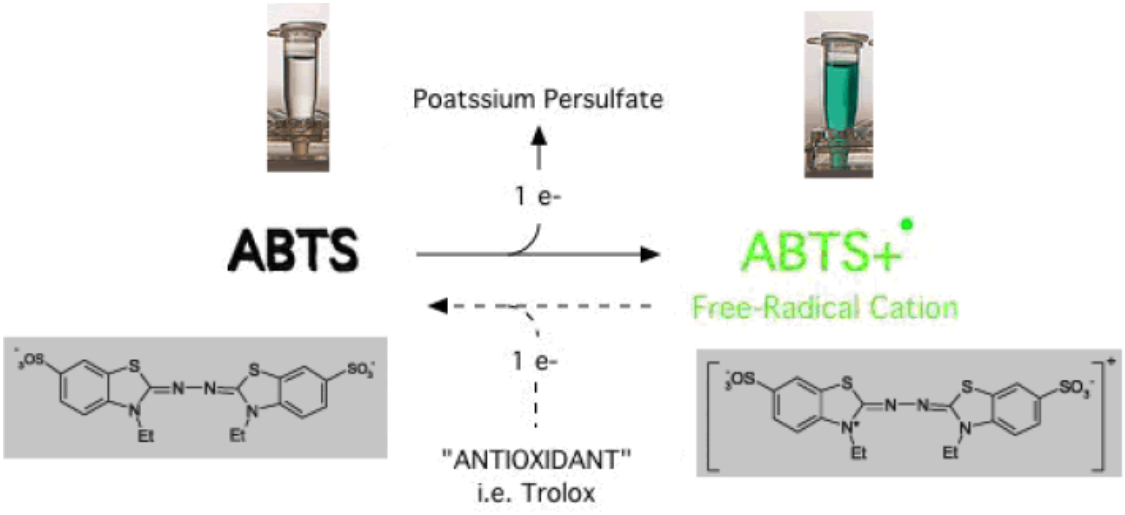


Figure: ABTS chemical reaction

The protocol is the following:

Radical preparation:

$K_2S_2O_8$: 0.066g in 100ml H_2Od

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)): 0.0385g in 10ml H_2Od

10 ml of each solution are mixed and stirred during 14-16 hours in dark conditions, in order to form ABTS radical (after stirring in dark condition, ABTS is stable for 48 h)

Radical stability

Once the radical is obtained, it should be diluted until an absorbance of 0.7±0.02nm at 734 nm is obtained. The stability is measured during 30 minutes.

Measurement conditions

- Sample holder: PMMA

- Blank sample: water

- Range measurement: 700-753nm, maximum 734nm

Measurement of antioxidant capacity of extract

2.5 ml of diluted ABTS radical solution are mixed with 2µL of extract (or diluted extract), the mixture is stirred and kept dark for 30 min, after this time the absorbance is measured and the value at 734 nm is written. All samples are tested by triplicated and the final result is presented as a media. With this data, the inhibition % is calculated:

$$\%\ \text{inhibición ABTS´} = \left[\frac{(ABS_{inicial} - ABS_{final})}{ABS_{inicial}}\right] X100$$

$ABS_{inicial}$ = initial value of ABTS radical

ABS_{final} = final value obtained with the extract after 30 min.

The first samples received were the samples from IPVC, so we have tested the initial antioxidant capacity ant we have compared the results with the obtained for different samples at environmental temperature and in the refrigerator. The results in the first six months are showing in the next graphic:

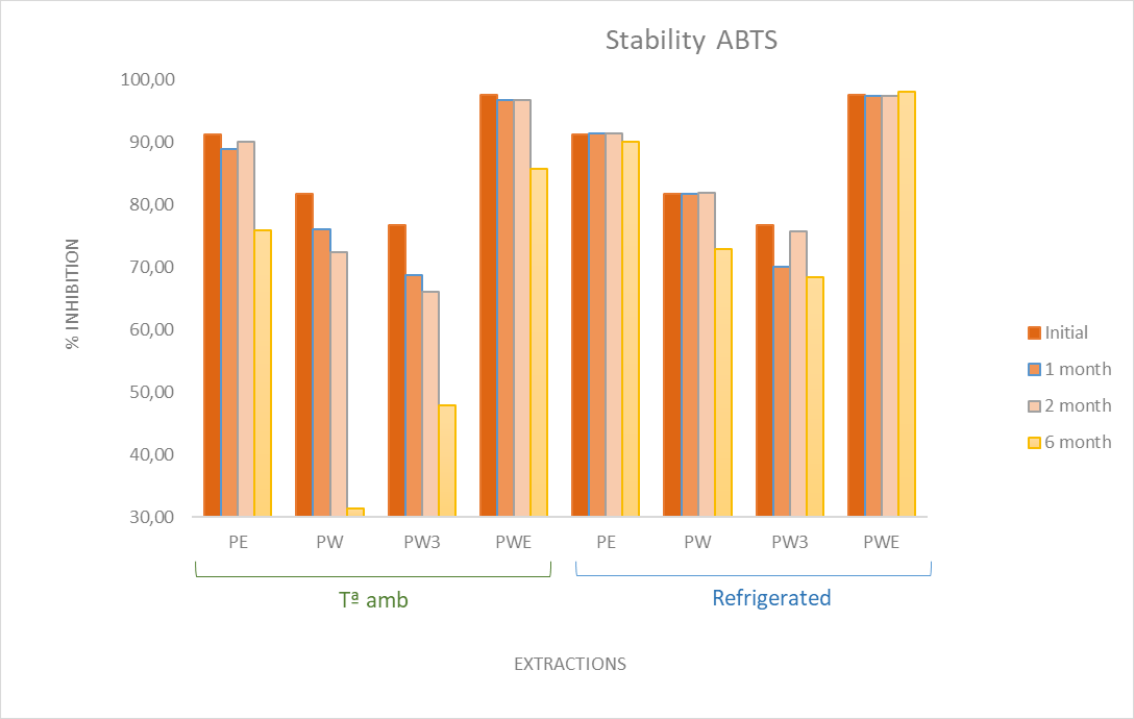


Figure: Comparison of inhibition percentage of IPVC samples after 6 months

In addition, the comparison in the first 9 months of the experiment is shown in the next figure:

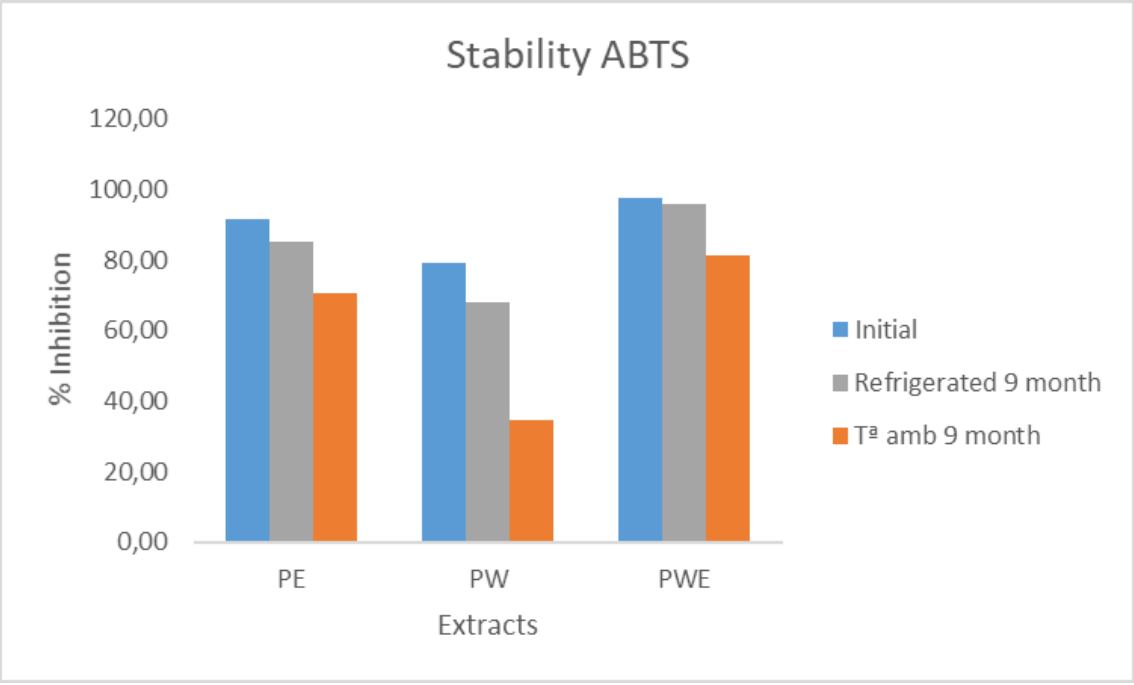


Figure: Comparison of inhibition percentage of IPVC samples after 9 months

From these graphics, we can observe different results:

- Samples obtained with water-ethanol offered the highest antioxidant capacity.
- The extracts are almost stable kept in the refrigerator, although the water extracts start to reduce their activity after 9 months.
- At room temperature, the antioxidant capacity starts to diminish after 3 months.
- The samples extracted with water-ethanol, maybe due to the presence of ethanol are more stable and kept their performance after 9 months.

Similar studies were developed with the samples provided by UMinho. However, the antioxidant capacity obtained for the samples are higher indeed, they are around 100% and we decided to dilute the samples at 16% in order to observe properly the changes between the samples. After three months, we obtained the following results:

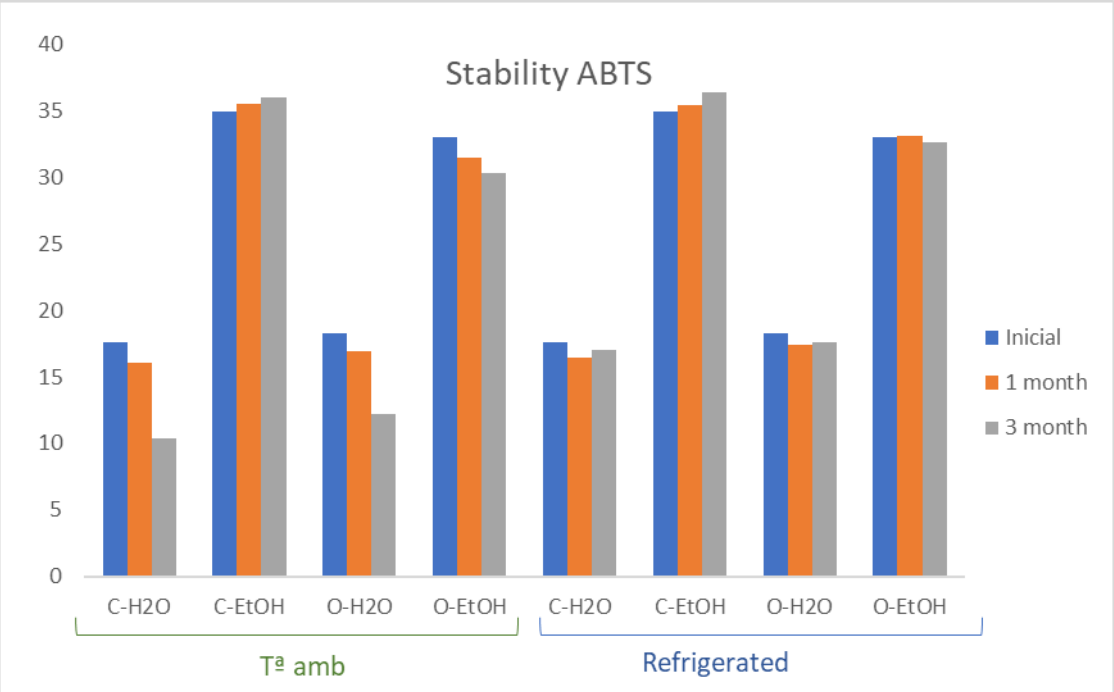


Figure: Comparison of inhibition percentage of UMinho samples after 3 months

In terms of antioxidant capacity, we have obtained the following conclusions with UMinho samples:

- In the refrigerator, all the samples are stable.
- Best results in terms of antioxidant capacity were obtained with conventional extraction methodology in a mixture of water and ethanol.

- After three months, the samples obtained in water solution started to diminish the antioxidant capacity.
- In some of the samples obtained in water, we observed the presence of fungi.

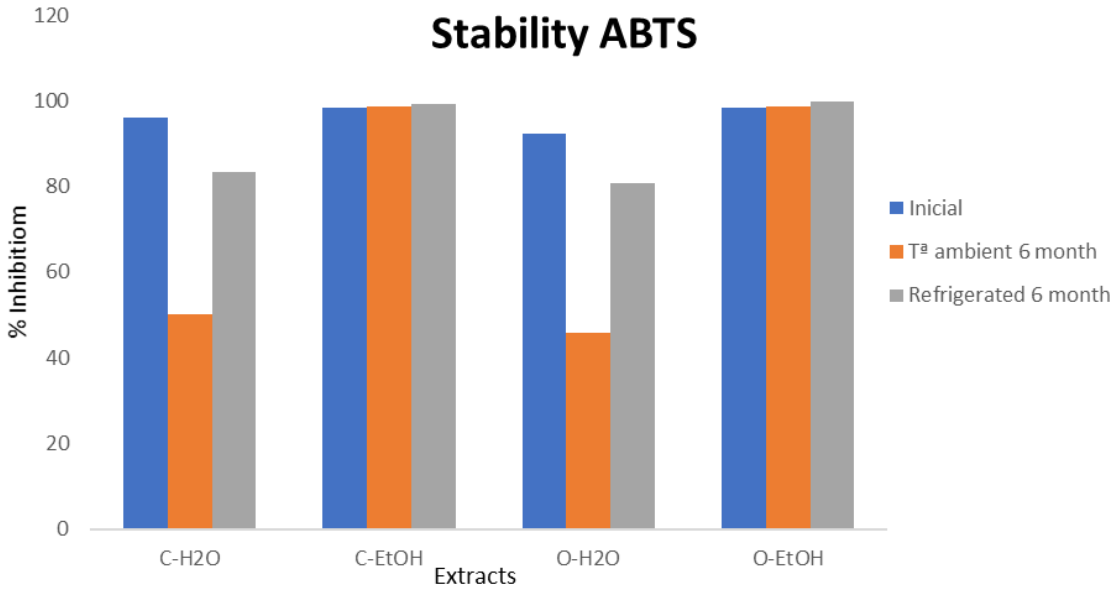


Figure: Comparison of inhibition percentage of UMinho samples after 6 months

After six months, the results offered a similar trend:

- We observed the best performance for the samples obtained with conventional extraction and water-ethanol mixture.
- Water extracts have reduced their capacity at higher rate at environmental temperature than ethanol extract.

The samples provided by UdL were studied following the same protocol, in this case it was no needed the dilution of the samples:

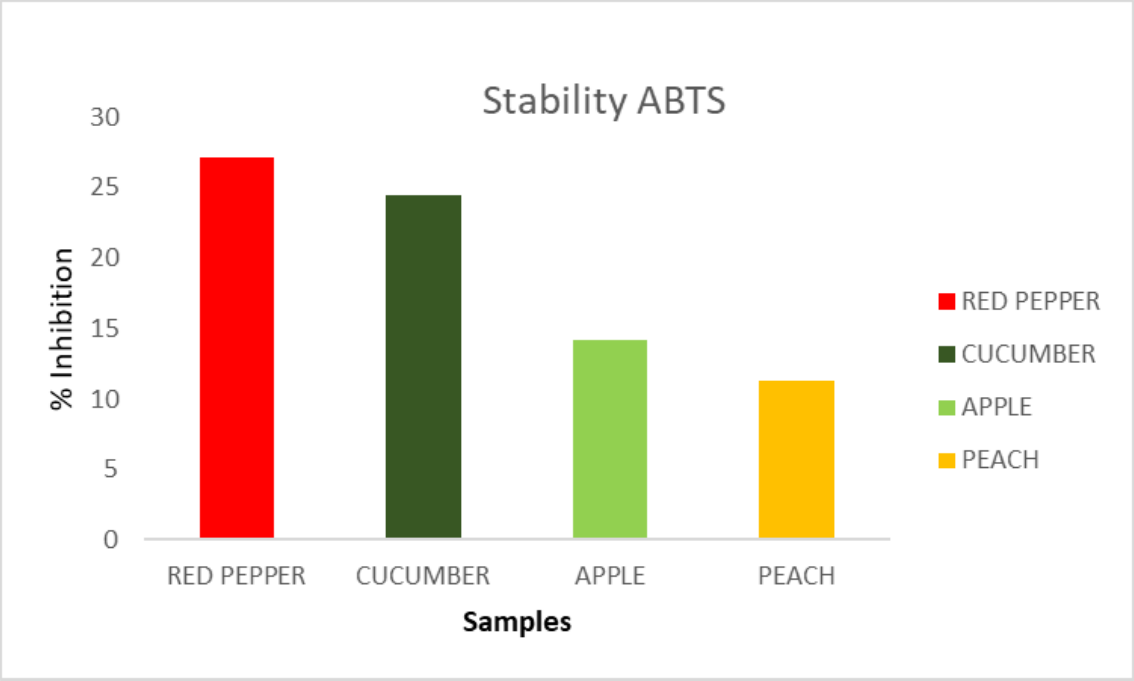


Figure: Comparison of initial inhibition percentage of UdL samples

At this moment, we have not studied the samples after time, because we have received in the last three months.

UMinho samples: olive pomace

Antioxidant capacity were measured for different olive pomace received from UMinho. Sample OP-C-EtOH was lost during transportation. The results are shown in next figures:

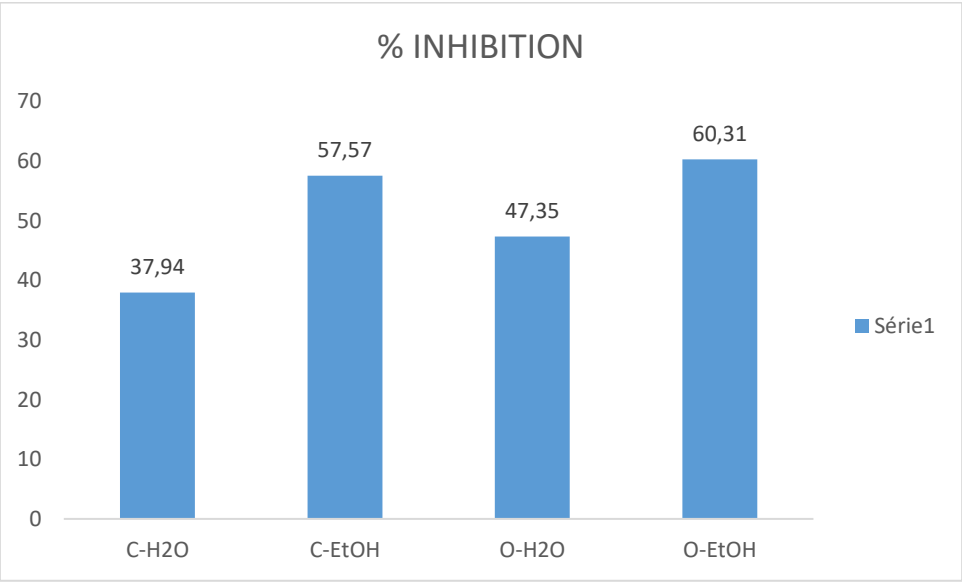


Figure: Comparison of initial inhibition percentage of olive pomace samples

Sample	pH
AL-C-H2O-1	4,71
AL-C-H2O-2	4,68
AL-C-H2O-3	5,06
AL-C-EtOH-1	5,79
AL-C-EtOH-2	5,67
AL-C-EtOH-3	XXXXX
AL-O-H2O-1	4,52
AL-O-H2O-2	4,6
AL-O-H2O-3	4,6
AL-O-EtOH-1	5,3
AL-O-EtOH-2	5,23
AL-O-EtOH-3	5,3

3. Results of HPLC-MS

3.1 Development of the methods

HPLC-DAD-MS analysis was developed for each extract. It was performed on UHPLC-DAD-MS Thermo Scientific (Orbitrap Qexactive) apparatus, with a C18 column. The flow rate was 0.5 ml/minutes. Two gradient were developed according to the nature of the extracts (aqueous, hydroalcoholic or alcoholic). They are shown in the table below.

Aqueous extracts	**Hydroalcoholic and alcoholic extracts**
0-1 min : 95/5 (water/acetonitrile) 15 : 50/50 20-23 : 0/100	0-1 min : 95/5 (water/acetonitrile) 20-23 : 0/100

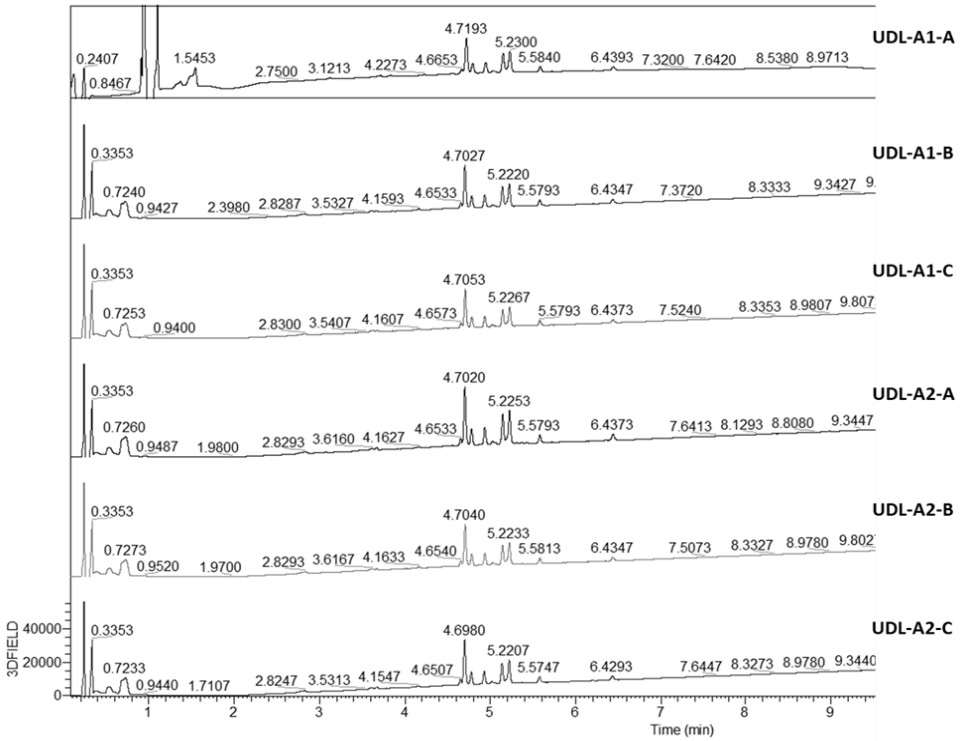
The MS parameters were as follows:

- Ionization mode : electrospray negative (ESI-)
- Collision energy : 35 eV
- Detection : Full scan and MS2
- Mass range : 100-1500

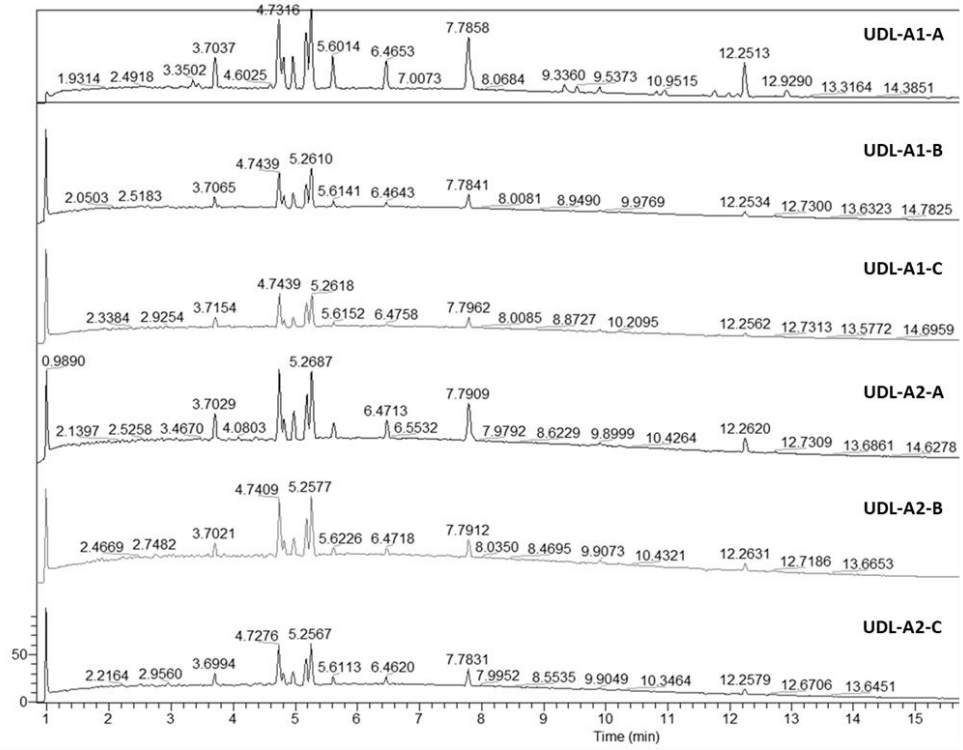
Dried extracts were solubilised in methanol (1mg/ml) and filtrated before analysis. The samples of olive pomace were received recently and AkiNaO has not time to perform the characterization and include in this draft of the deliverable.

3.2 Results

- **Apple :**



PDA total scan chromatograms of hydroalcoholic apple extracts



Base peak chromatograms (ESI-) of hydroalcoholic apple extracts

The different apple hydroalcoholic extracts show very close chemical profile, with major compounds with retention time between 4 and 6 minutes. Two major compounds do not absorb in UV but are visible in mass spectrometry (7.7 and 12.2 minutes).

The table below summarizes the major compounds determined in apple extracts and their tentative identification.

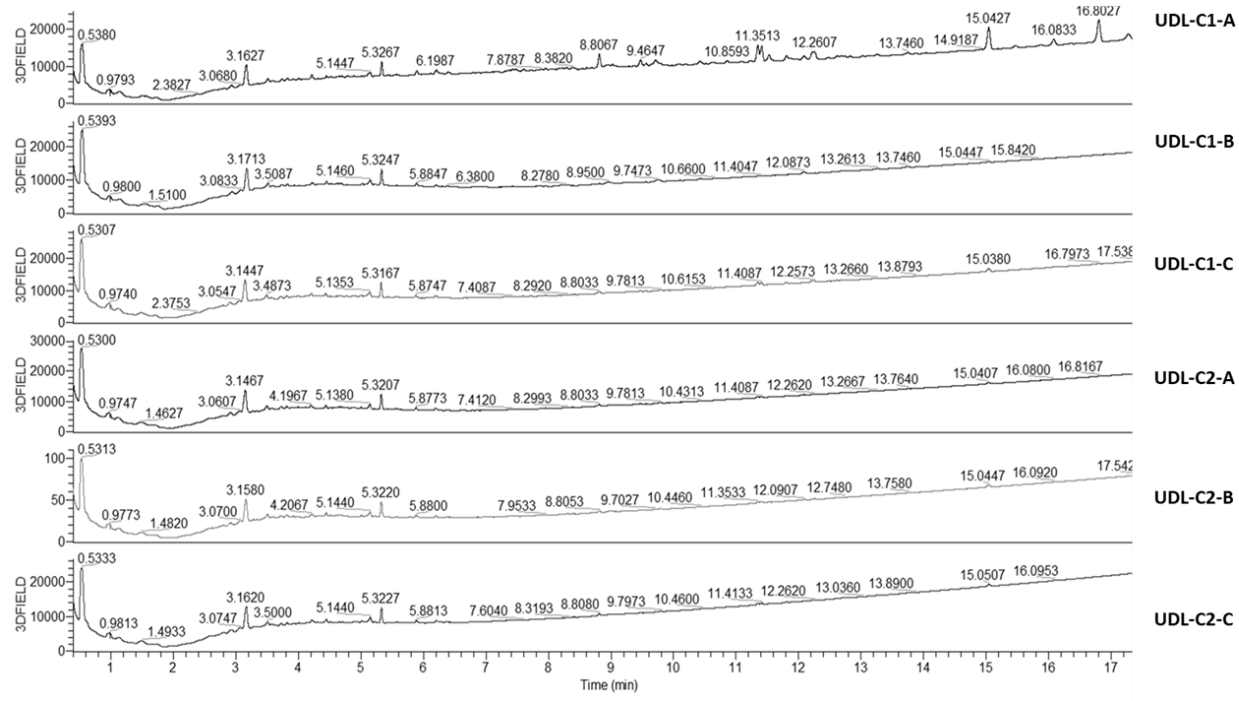
Tentative identification of apple extracts' major compounds

Retention time (minutes)	UV-vis λ max (nm)	MS (base peak / ESI-)	MS2	Tentative identification	Reference
3.7	242	563.2321 (M+FA-H?)	517.2273 (M-H?)	NI	-
4.7	256, 354	463.0862 (M-H)	300.0265	Quercetin 3-galactoside	a
4.8	257, 354	463.0864 (M-H)	300.0263	Quercetin 3-glucoside	a
4.9	256, 355	433.0755 (M-H)	300.0264	Querecetin-3-xyloside	a
5.1	256, 353	433.0754 (M-H)	300.0264	Quercetin-3-arabinopyranoside	a
5.3	256, 349	447.0911 (M-H)	300.0264	Quercitrin	a
5.6	286	481.1330 (M+FA-H) 435.1277 (M-H)	273.0758	Phloridzin	a
6.4	256, 370	301.0341 (M-H)	-	Quercetin	a
7.7	223	329.2319	171.1012	NI	-
12.2	234	295.2266	277.2161	NI	-

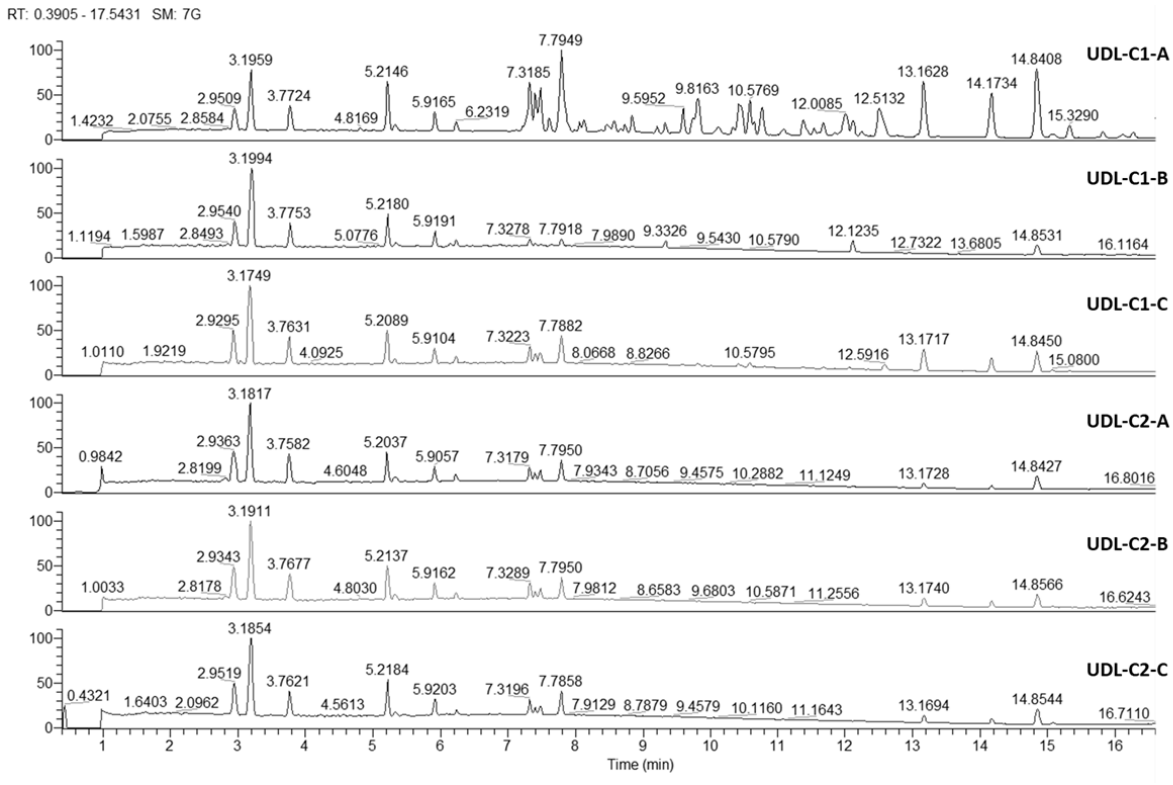
a : M. Ramirez-Ambrosi *et al*, 2013
NI = not identified

The major compounds identified in apple extracts are mainly flavonoids and dihydrochalcones derivatives, in particular glycosylated derivatives.

- **Cucumber**



PDA total scan chromatograms of hydroalcoholic cucumber extracts



Base peak chromatograms (ESI-) of hydroalcoholic cucumber extracts

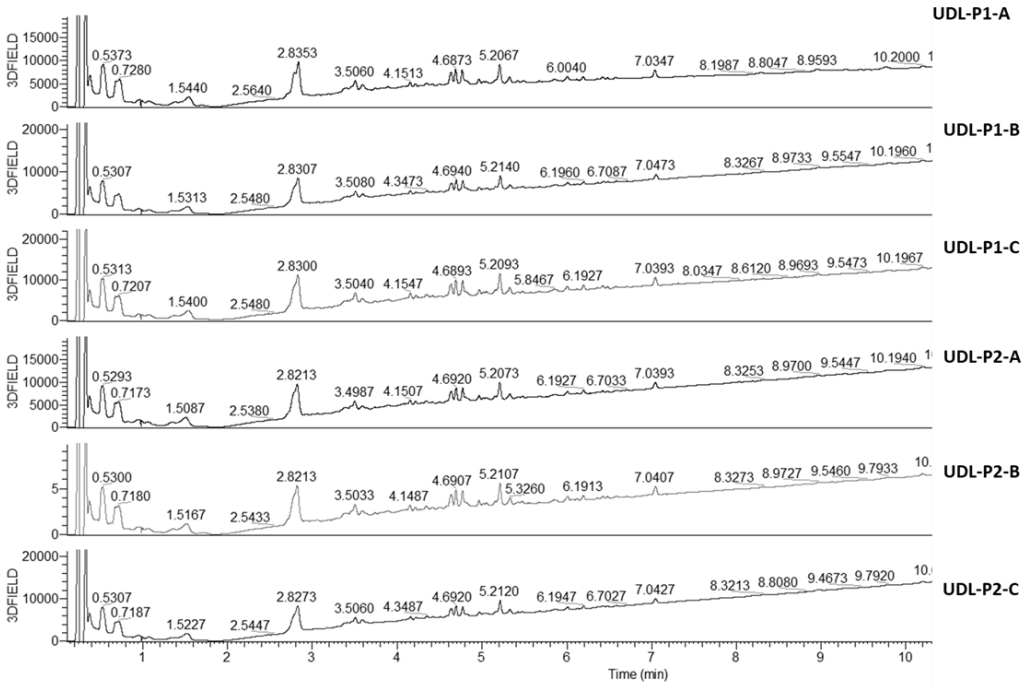
The chemical profile of hydroalcoholic cucumber extracts are quite close, even if the extract UDL-C1-A is quite different. The major compounds have retention time between 2 and 6 minutes. The table below summarizes the major compounds determined in cucumber extracts.

Cucumber extracts' major compounds

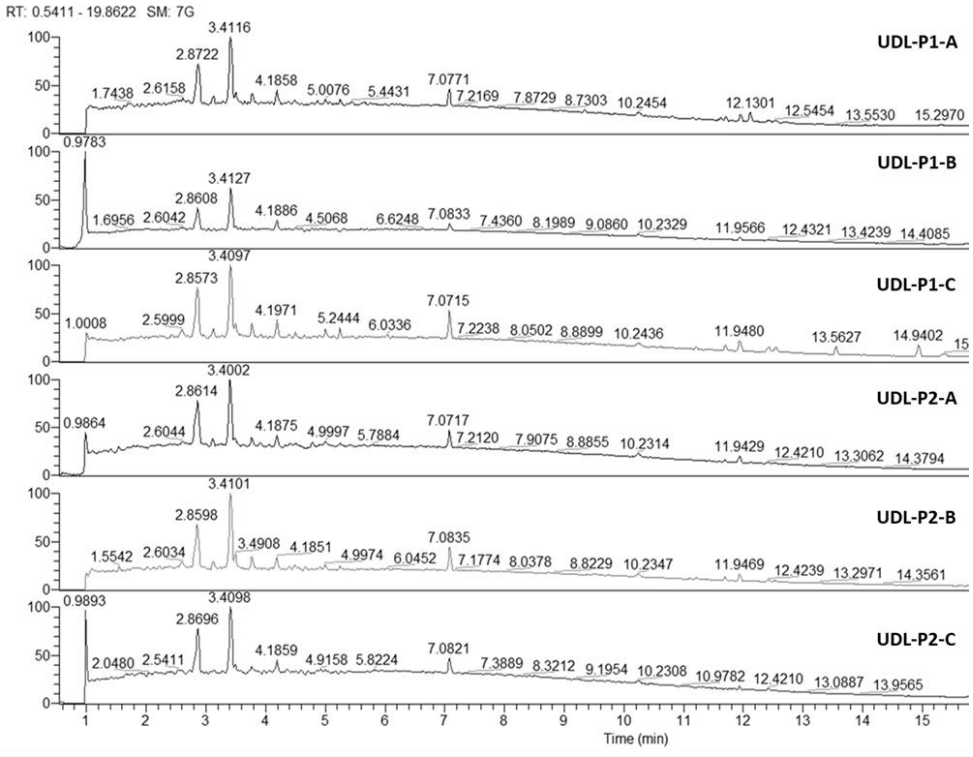
Retention time (minutes)	UV-vis λ max (nm)	MS (base peak / ESI-)	MS2
2.9	301	131.0702	-
3.1	298	210.0766	124.0393
3.7	357	165.0548	124.0393
5.2	219	187.0968	125.0961
5.9	298	238.1082	164.1072
6.2	298	272.0929	124.0393
7.3	221	327.2176	-
7.8	221, 280	329.2334	-
9.3	223, 303	293.1759	236.1053 ; 221.1544
12.1	225, 287	233.1544	217.0616
14.8	225, 287	271.2280	225.2222

Few data concerning cucumber extract characterization are available in the literature. These studies report the presence of organic acid and flavonoid derivatives in cucumber extracts (Abu-Reidah *et al.*, 2012 ; Segarra *et al.*, 2006). Results of LC-MS analysis seems to indicate the presence of small organic acids or amino acid in this extract (low mass and presence of nitrogen compounds suspected due to odd masses).
Bibliographic studies on closely related species are continuing to identify the compounds.

- **Peach :**



PDA total scan chromatograms of hydroalcoholic peach extracts



Base peak chromatograms (ESI-) of hydroalcoholic peach extracts

Chemical profile of peach extracts are very close. Few major compounds have been identified in these extracts. The two mains have retention times of 2.8 and 3.3 minutes. The table below summarizes the major compounds determined in apple extracts and their tentative identification.

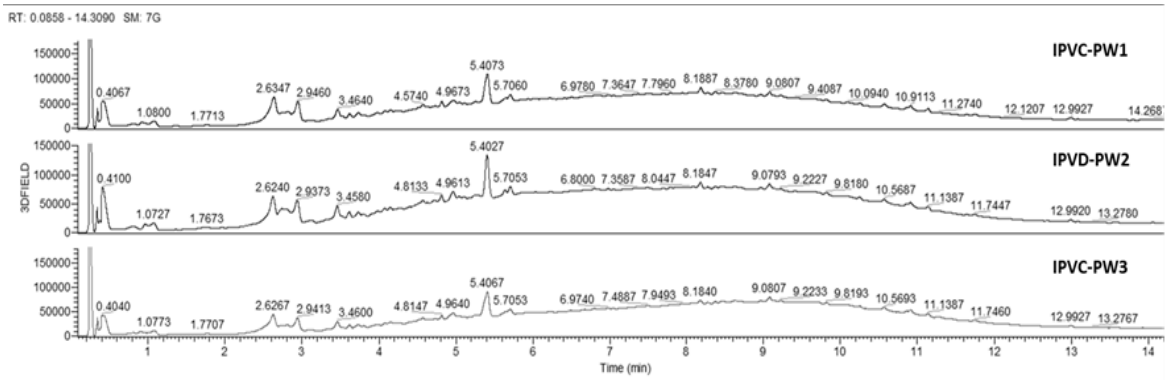
Tentative identification of peach extracts' major compounds

Retention time (minutes)	UV-vis λ max (nm)	MS (base peak / ESI-)	MS2	Tentative identification	Reference
2.8	243, 325	353.0879 (M-H)	191.0555	Chlorogenic acid	a, b, c
3.3	283, 338	340.1037	161.0447	NI	-
4.1	375	377.1818	301.1079	quercetin derivate?	-
7.0	290	271.0613	-	naringenine?	-

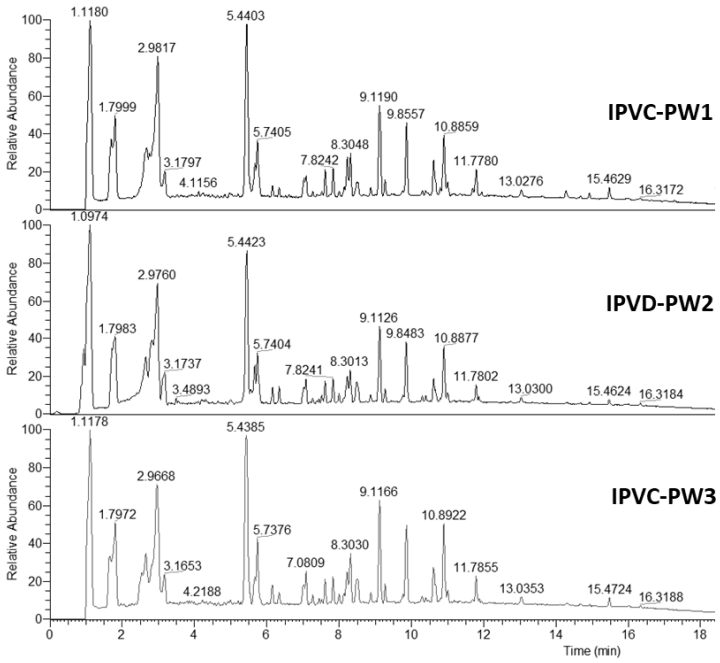
a : A Mokrani *et al.,* 2016 ; b = X Zhao *et al*., 2015 ; c = Tomas-Barberan *et al*., 2001
Not identified

- **Pine bark :**

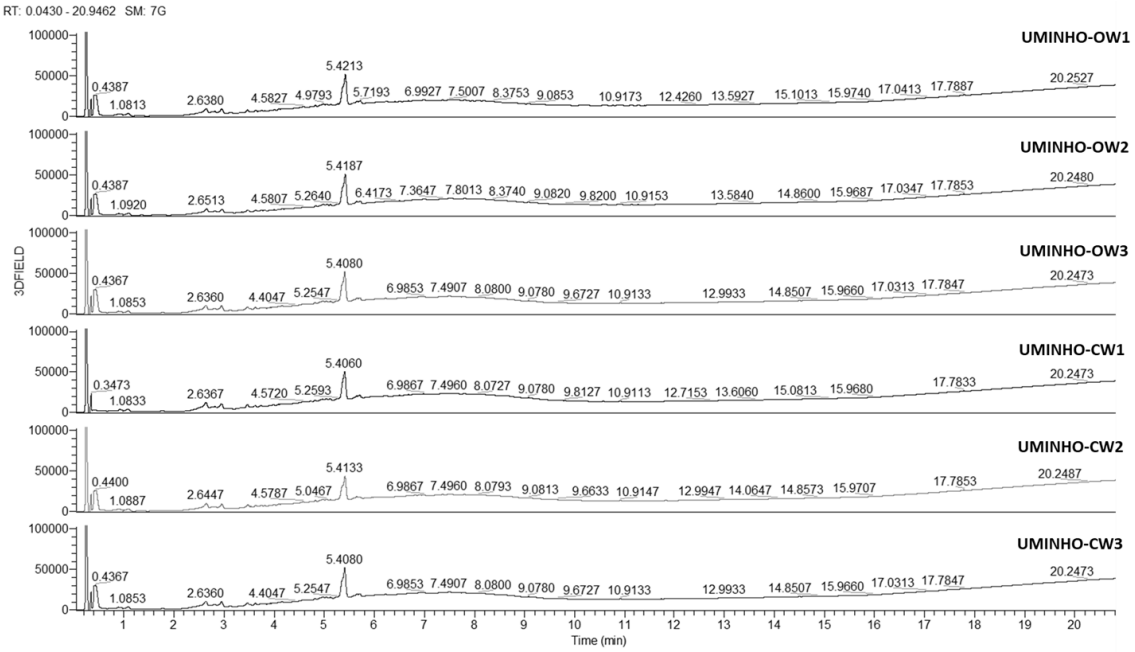
Aqueous extracts :



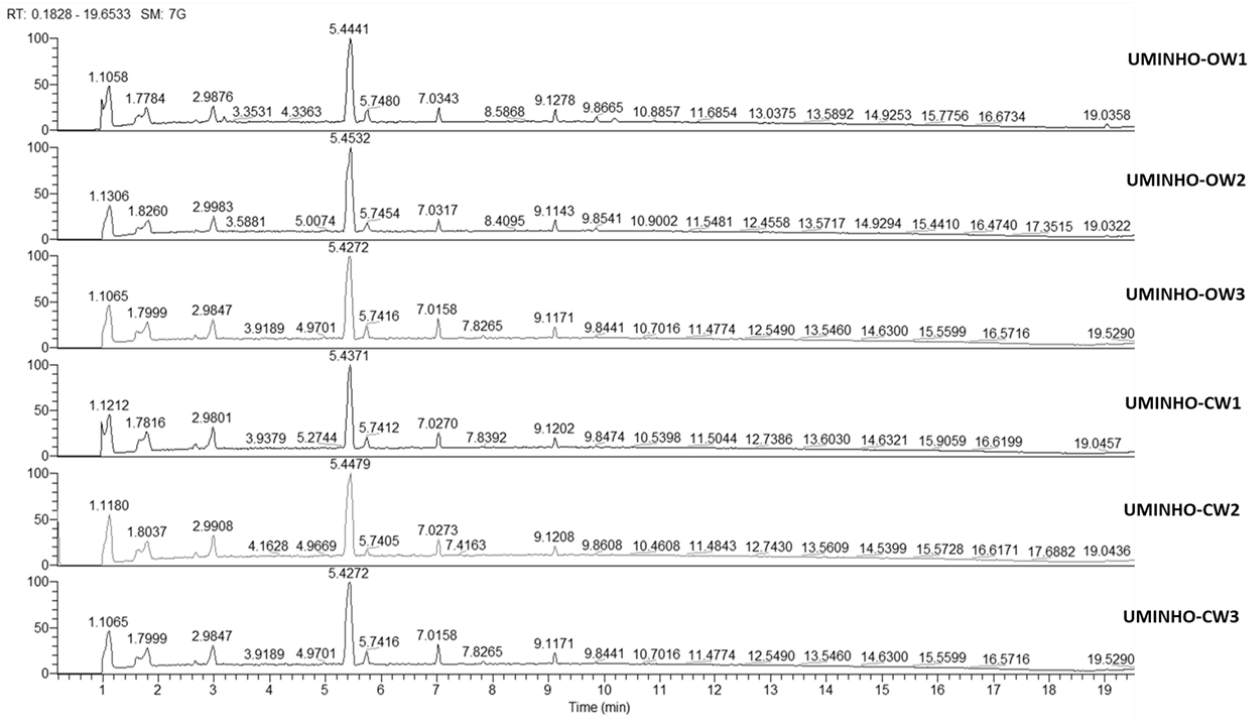
PDA total scan chromatograms of IPVC pine bark aqueous extracts



Base peak chromatograms (ESI-) of IPVC pine bark aqueous extracts



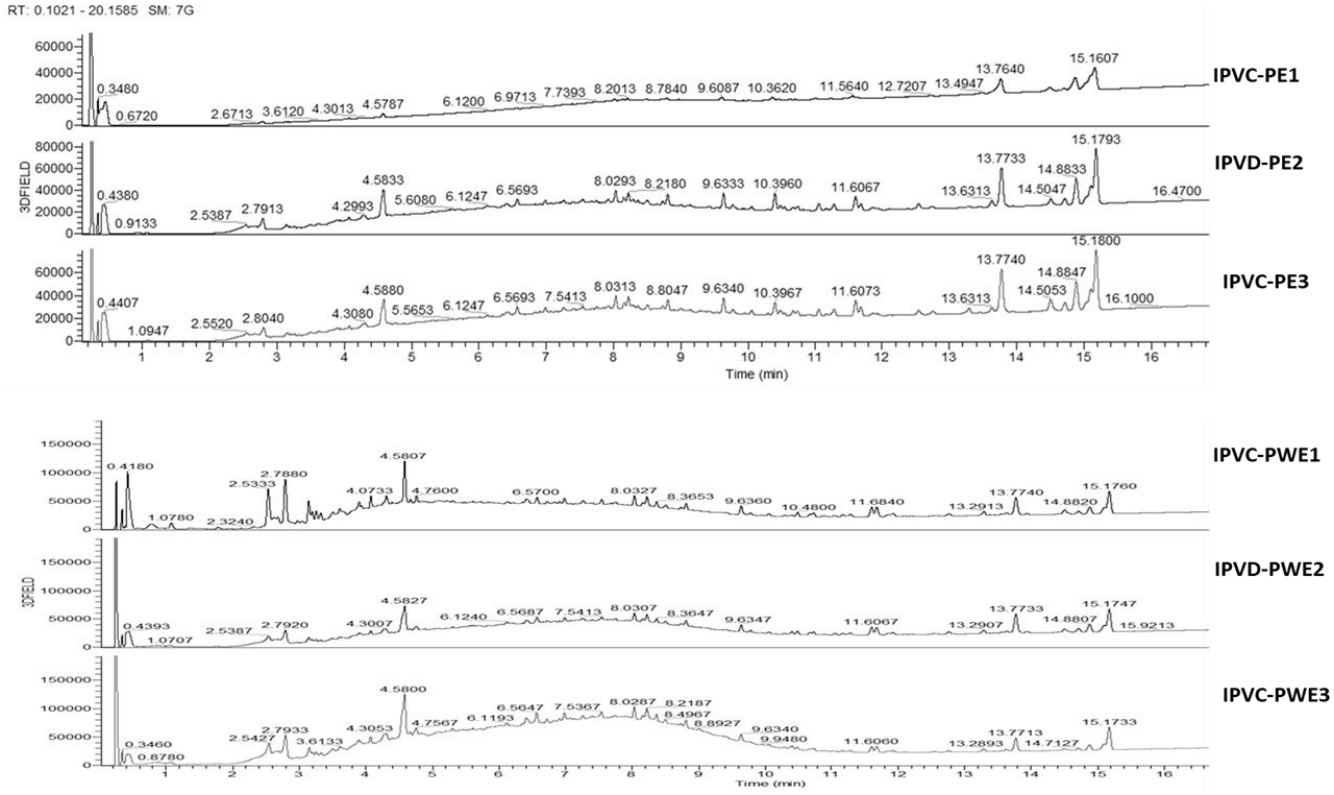
PDA total scan chromatograms of UNMINHO pine bark aqueous extracts



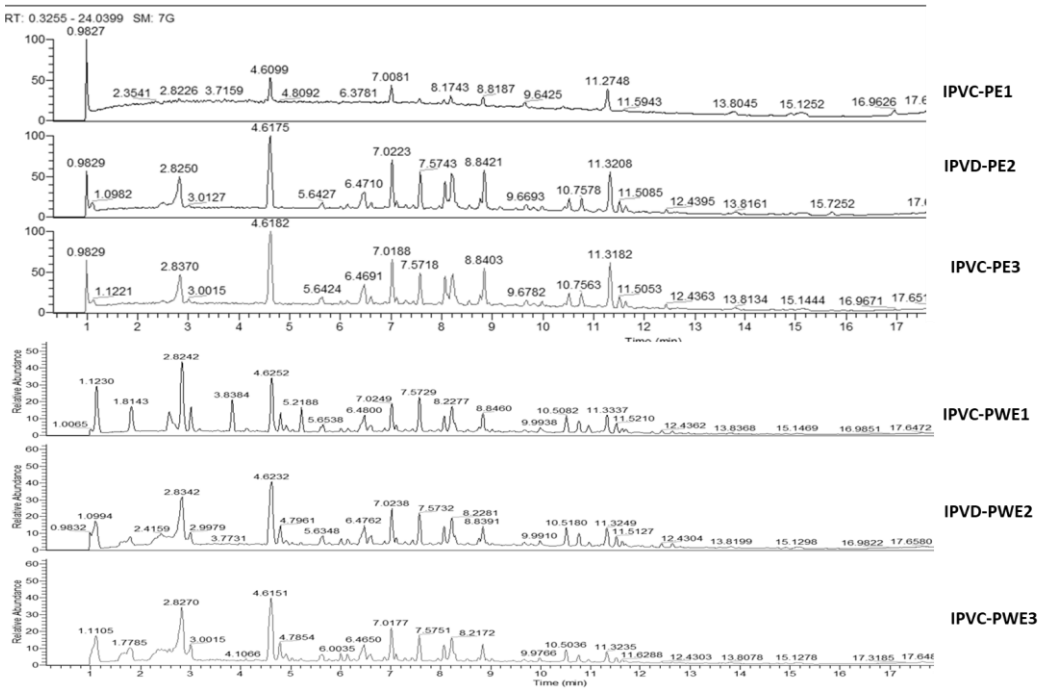
Base peak chromatograms (ESI-) of UNMINHO pine bark aqueous extracts

Pine bark aqueous extracts generated by IPVC and UNMINHO are relatively closed with a major compound with a retention time of 5.4 minutes. The other major compounds have retention time less than 12 minutes, showing they are polar.

Ethanolic and hydroalcoholic extracts :

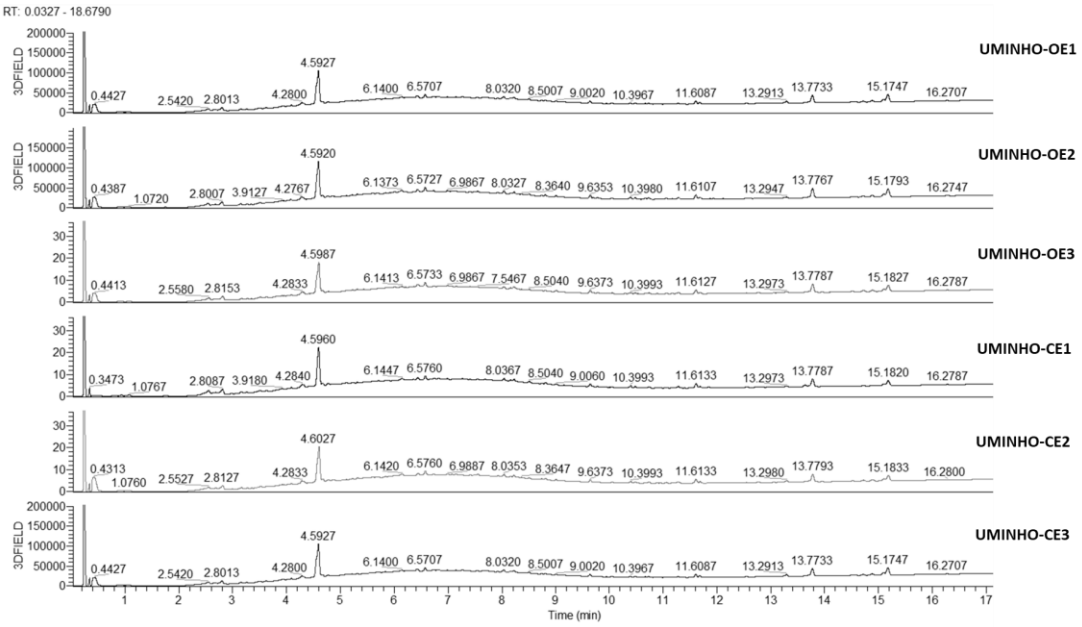


PDA total scan chromatograms of IPVC pine bark ethanolic (up) and hydroalcoholic (down) extracts

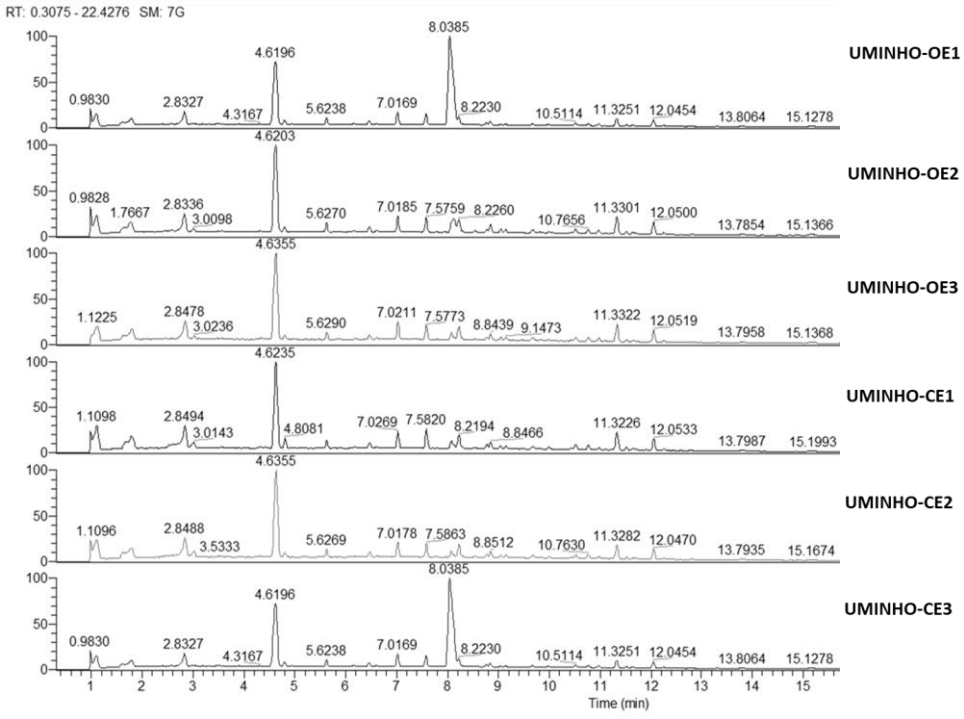


Base peak chromatograms (ESI-) of IPVC pine bark ethanolic (up) and hydroalcoholic (down) extracts

Ethanolic and hydroalcoholic extracts generated by IPVC are relatively close, but non polar major compounds (retention times 10-15 minutes) are more concentrated in ethanolic extracts. A major compound, with a retention time of about 4.5 minutes is detected in all extracts.



PDA total scan chromatograms of UNMINHO pine bark ethanolic extracts



Base peak chromatograms (ESI-) of UNMINHO pine bark ethanolic extracts

Ethanolic extracts generated by UNMINHO are quite different of ethanolic extract generated by IPVC. Two major compounds are detected, with retention times of 4.6 and 8.0 minutes. However, there is some difference between the replicates. The table below summarizes the major compounds determined in the different pine bark extracts and their tentative identification.

Tentative identification of aqueous pine bark extracts' major compounds

Retention time (minutes)	UV-vis λ max (nm)	MS (base peak / ESI-)	MS2	Tentative identification	Reference
2.9	280	289.0718 (M-H)	-	catechin	a, b
5.4	290	303.0509 (M-H)	-	taxifolin 1	a, b
5.7	290	303.0510 (M-H)	285.0407	taxifolin 2	a, b
6.9	290	505.1929	-	NI	-
9.1	290	291.1602	-	NI	-
9.8	286	289.1446	245.1546	NI	-
10.8	286	349.2019, 331.1914	-	deihydroxydehydroabietic acid derivate	-
11.7	298	331.1915 (M-H)	-	7,15 – dihydroxydehydroabietic acid	c , d

a = Yesil-Celiktas *et al*., 2009 ; b = P A de Almeida *et al*., 2016 ; c = Lee *et al*., 2018 ; d = Mulholland *et al*., 2017 ; NI = Not identified

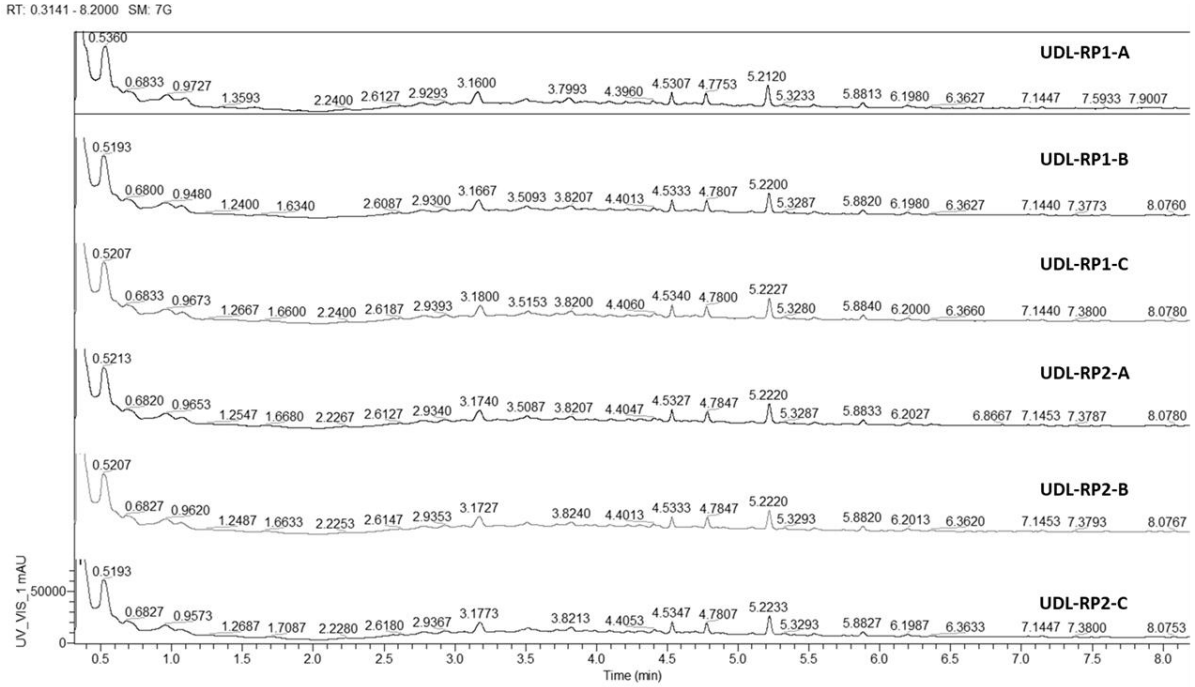
Tentative identification of alcoholic/hydroalcoholic pine bark extracts' major compounds

Retention time (minutes)	UV-vis λ max (nm)	MS (base peak / ESI-)	MS2	Tentative identification	Reference
1.1	259, 295	153.0182 (M-H)	109.0283	dihydroxybenzoic acid	-
1.8	229, 280, 308	137.0233 (M-H)	-	hydroxybenzoic acid	a
2.5	280	577.1349 (M-H)		procyanidine	-
2.8	279	289.0717 (M-H)	245.0818	catechin	b
2.9	244, 323	179.0341 (M-H)	135.0441	caffeic acid	c , d
3.7	263	186.1128	125.0961	NI	-
4.6	290	303.0509 (M-H)	-	taxifolin 1	b
5.2	341	187.0967	-	NI	-
6.4	234, 271, 232, 370	303.1237	-	NI	-
7.0	239	291.1601	246.1581	NI	-
7.5	263	289.1444	-	NI	-
8.0	245, 303	275.1654	233.1184	NI	-
8.7	299	331.1915 (M-H)	-	7,15 – dihydroxydehydroabietic acid	e , f
10.5	284	333.2071	-	Abietic acid derivate?	-
11.2	276	315.1966 (M-H)	-	15 hydroxydehydroabietic acid	e , f

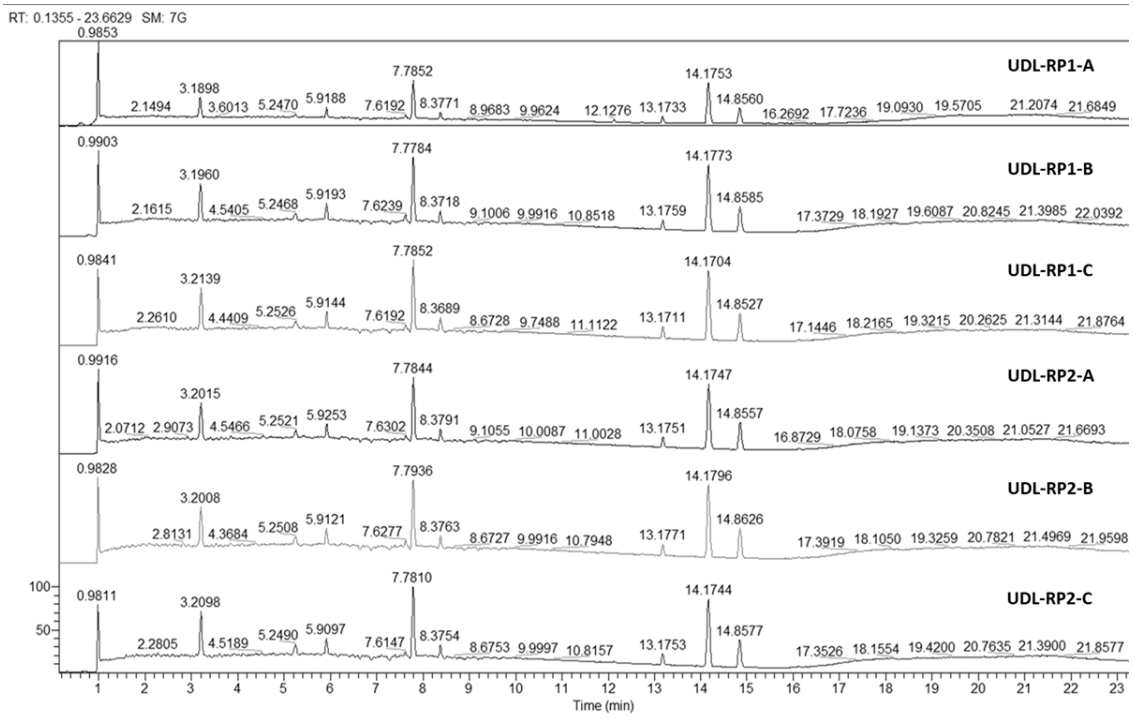
a = Tourino *et al*., 2005 ; b=Yesil-Celiktas *et al*., 2009 ; c = P A de Almeida *et al*., 2016 ; d =Celhay. 2013 ; e = Lee *et al*., 2018 ; f = Mulholland *et al*. 2017
NI = Not identified

The major compound identified in pine bark extract is taxifolin, a flavonoid derivated from quercetine. The presence of dehydroabietic acid derivates is also suspected. These compounds could be involved in antifungal activity against **Plasmopara** ***viticola (Mulholland*** **et al.** ***2017).***

- **<u>Red pepper :</u>**



PDA total scan chromatograms of red pepper extracts



Base peak chromatograms (ESI-) of red pepper extracts

The chemical profile of the different red peper extracts are very closed. These extracts contain several polar (retention times = 3.2 and 7.7 minutes) and less polar compounds (retention times = 13-15 minutes). The table below summarizes the major compounds determined in red pepper extracts and their tentative identification.

Tentative identification of red pepper extracts' major compounds

Retention time (minutes)	UV-vis λ max (nm)	MS (base peak / ESI-)	MS2	Tentative identification	Reference
3.1	298	210.0765	123.0553	NI*	-
5.1	256, 349	447.0932 (M-H)	301.0351 (M-rha-H)	Quercetin rhamnoside	a
5.8	294	238.1081	194.1181	ND	-
7.7	298-337	329.2331	171.1019	**	-
8.3	291	329.2332	171.1018	NI	-
13.1	243	293.2122		NI	-
14.1	225	295.2277 (M+FA-H)?	249.2224 (M-H)?	NI	-
14.8	225	271.2279 (M+FA-H)?	225.2221 (M-H)?	NI	-

a = S Z Mudric *et al.*, 2017
NI = Not identified
*Compound also identified in cucumber extract
** compound also identified in apple extract

UV and mass spectrum obtained for red pepper extract are different from data described in literature. Characterization is still ongoing to try to identify more major compounds.

4. Conclusions

At this moment, the conclusions obtained are preliminary, although we can conclude the following:

- pH of the samples was measured showing different values depending on the extracting media.
- Density is dependent on the media of extraction.
- Solubility studies show that the samples are polar
- The evolution of the colour with time is complicated to correlate with stability, because it is possible to observe increasing or decreasing depending on the type of polyphenols extracted.
- Based on the reduction of antioxidant capacity, we observed the following:
    - Samples prepared with ethanol offered better stability at room temperature, probably due to the ethanol presence.
    - Between all the samples analysed, conventional extraction for pine bark using ethanol-water offered the best antioxidant capacity.
- The results obtained for HPLC-MS showed the presence of different compounds from flavonoids, terpenoids and phenolic acids families. Chemical profile difference between extracts from different process could be detected, in particular for pine bark extracts. These results may be correlated with the biological activity results (expected 10/2018) and the bibliographic data, to compare the activity of the different extracts and try to identify active compounds or families.

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