ARISTOIL

“Reinforcement of Mediterranean olive oil sector competitiveness through development and application of innovative production and quality control methodologies related to olive oil health protecting properties”

PRIORITY AXIS 1: Promoting Mediterranean innovation capacities to develop smart and sustainable growth

OBJECTIVE: 1.1 To increase transnational activity of innovative clusters and networks of key sectors of the MED area

Project website: http://aristoil.interreg-med.eu/

DELIVERABLE Number: 3.2.2
Title of DELIVERABLE: 3.2.2 Agronomic Factors Database
ACTIVITY n. 3.2 Investigation of production parameters influencing the quality of olive oil
WP n. 3: STUDYING

PARTNER IN CHARGE: National and Kapodistrian University of Athens, Department of Pharmacognosy and Natural products Chemistry (UoA)

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Confidential ×	*Public* □	

Date: 31 March 2018

Contents

Agronomic Factors Database for Greek Olive Oils

Investigation of process parameters on the phenolic content of olive oil

Description and aims of the project

The aim of this part of the project was to clarify which are the most crucial parameters that affect the concentration of the phenolic substances in extra virgin olive oil. Our research was not only focused on the natural factors of the olive fruit but also in the mechanical process of the olive oil extraction.

The extra virgin olive oil phenolic fraction has been studied by many researchers the last years, and it is common accepted that the mechanism of its production is a really complex task that has not been totally clarified yet.

Many researchers have come to converge to some basic conclusions, but it's really hard to export some simple instructions for the olive oil producers for an accurate harvest time and extraction method. This is mainly caused by the variety of the climatically factors and the different effect on each olive variety.

In this project we tried to extract some simple conclusions for the factors that affect the EVOO phenolic fraction. The innovation we applied on this work, was the use of the NMR method for the olive oil analysis. This method allows the analysis of the phenolic fraction not only as a total number of phenolic substances but also the analysis of the individual substances. This will help us understand more steps of the production mechanism.

The factors we decided to study was the variety of the olive fruit, the harvest time, the malaxation time and the malaxation temperature.

In order to elaborate this project we tried to simulate the olive oil extraction that take place in a common olive mill with an array of devices in a laboratory scale. This array consists of a crusher that turns the olive fruit in a paste form. The crushing process lasts 5 minutes exactly as happening in an olive mill. The olive paste is transferred in a stainless mixing bowl where the paste is mixed for the rest of the process. Depending on the malaxation temperature we want to apply the mixing bowl is transferred in a water bath where the temperature is checked with a digital thermometer. The olive oil extraction is completed with the collection of specific amount of the olive paste which is centrifuged for five minutes. Finally we collect the olive oil from the top of the tube we proceed to the olive oil analysis of 5 g. The only difference between the original process of extraction is the absence of the water during centrifuge.

We decided to work on two olive varieties that are very common in Greece ''Koroneiki'' and ''Athenolia''. The olive fruit samples for the Koroneiki variety where collected from the 10th of October 2017 till 6th of February 2018 while the Athenolia olive fruit samples were collected from the 20th of November 2017 till the 6th of February 2018.

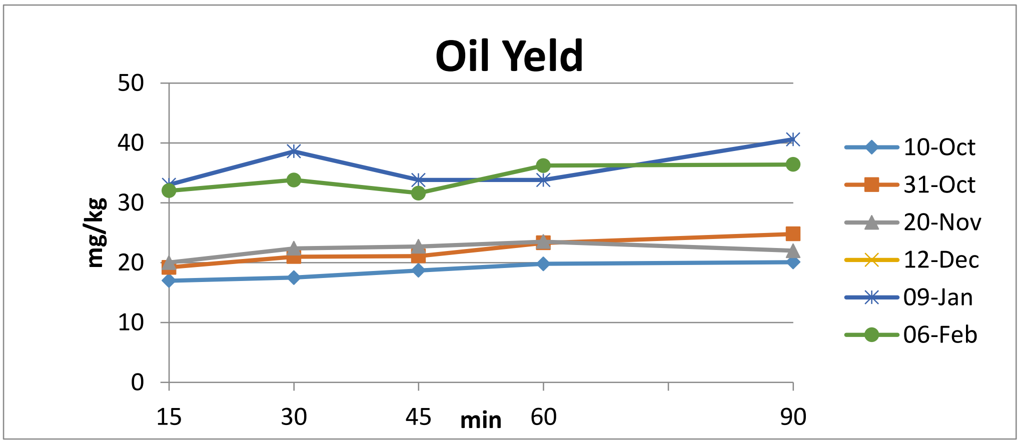
All of the olive fruit samples were collected in different periods from the same trees in the region of Molaoi-Lakonia.

For the Koroneiki variety we collected samples on six different harvest time. On each sample we applied three different malaxation temperatures (23^0C, 28^0C, 32^0C) and we collected olive oil samples in 5 different malaxation time (15, 30, 45, 60 και 90 min). In other words we had 18 cases which include 5 olive oil samples per experiment.

For the Athenolia variety we collected samples in 4 different harvest time and we applied only one malaxation temperature (28^0C). On each experiment we collected olive oil sample in 5 different malaxation time (15, 30, 45, 60 και 90 min).

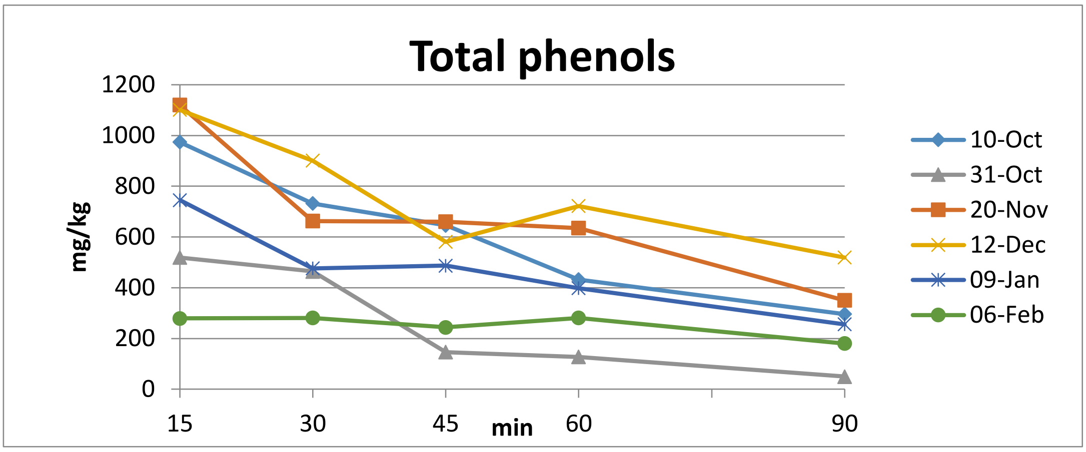
Discussion and Conclusions

After the analysis of the olive oil samples we collected we easily come to the conclusion that independently the variety of the olive fruit, the oil yield increases as the degree of ripening is getting higher. It is commonly accepted that later the olive fruit is collected the higher oil yield is achieved. The Increase of the oil content of the fruit between an unripe and a mature olive can reach up to 100%. We can see in the diagram below an example of the oil yield in koroneiki variety. It is clear that in February the oil content is almost 2 times bigger than the oil content in October.



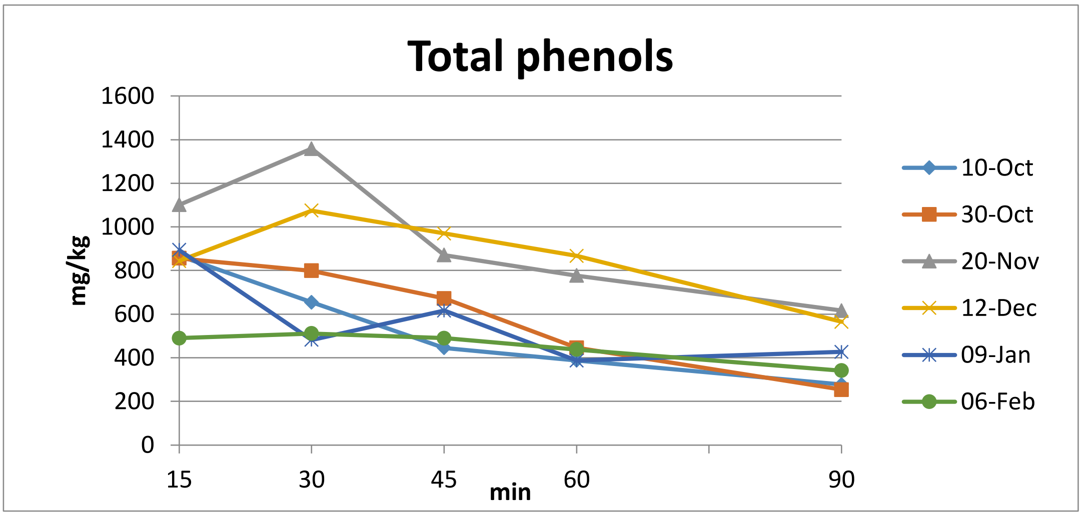
The effect of the malaxation temperature in the oil yield seems to be important too. We could say that high temperature allows the oil yield to achieve the maximum status in a shorter time than in low temperature.

It is well known that the phenolic substances of the EVOO differs from the phenolic substances of the olive fruit. That means that EVOO phenolics like oleocanthal and oleacein cannot be found in the olive fruit. This statement leads to the hypothesis that EVOO phenolics are created after the crushing of the olive fruit. The moment before the crushing the olive oil, even if its in a separated droplets has a zero level phenolic content. After analyzing all the samples we collected we saw that every time we used



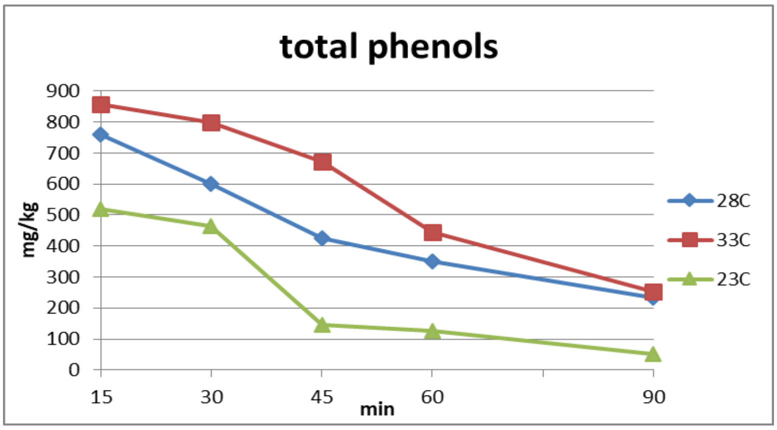
low or medium temperature (till 28C), the total phenolics after 15 minutes of malaxing was reducing.

On the contrary when we applied higher temperature there we two cases out of six



when the total phenols continued to increase till the 30^{th} minute of malaxing follows by rapid reducing.

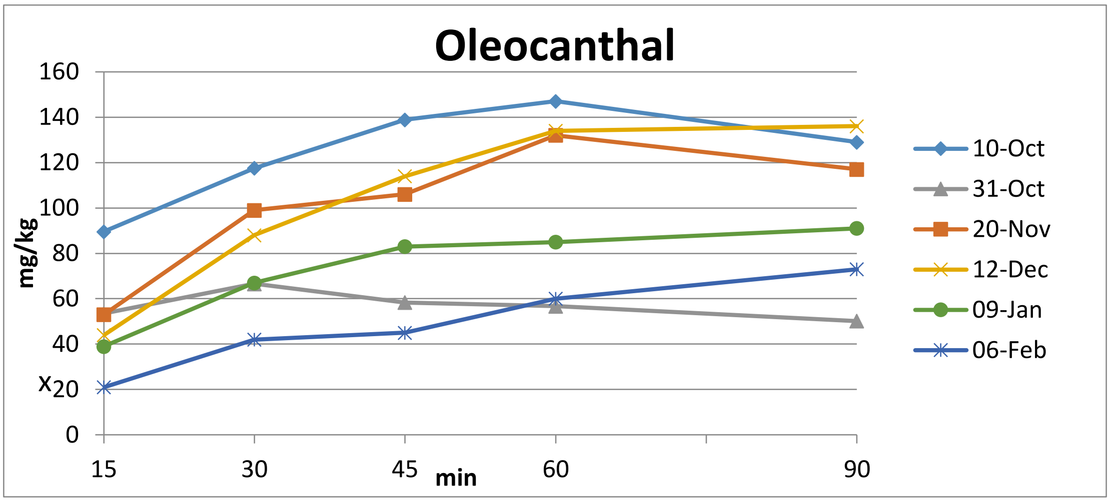
Comparing also the total phenolic substances of EVOO at the same ripening degree for three different temperatures we noted that even a small increase of the temperature leads to higher phenolic content. In 4 out of 6 cases the application of the temperature (28-32C) enriches the olive oil at least for the first 30 minutes of malaxing.

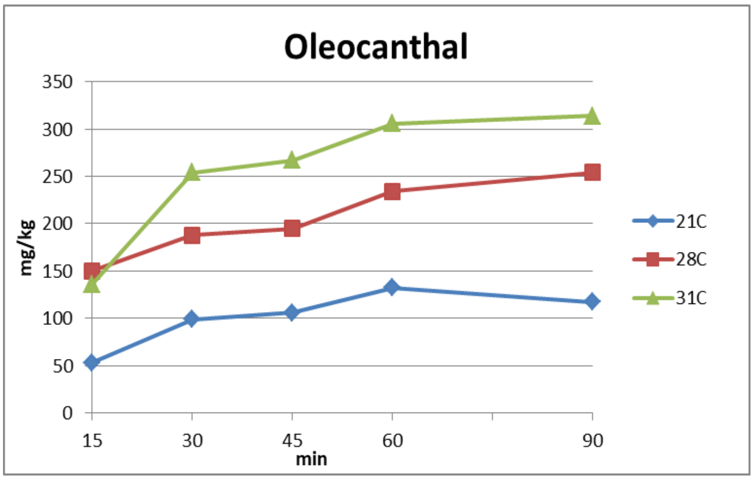


We can safely conclude that the total amount of the EVOO phenolic substances are created at the moment the olive fruit is crushed. Most of the times the creation stops during the first 15 minutes of malaxing. Heating during malaxing is maximizing the performance of the creation step in terms of maximum amount and in some cases elongation of this step.

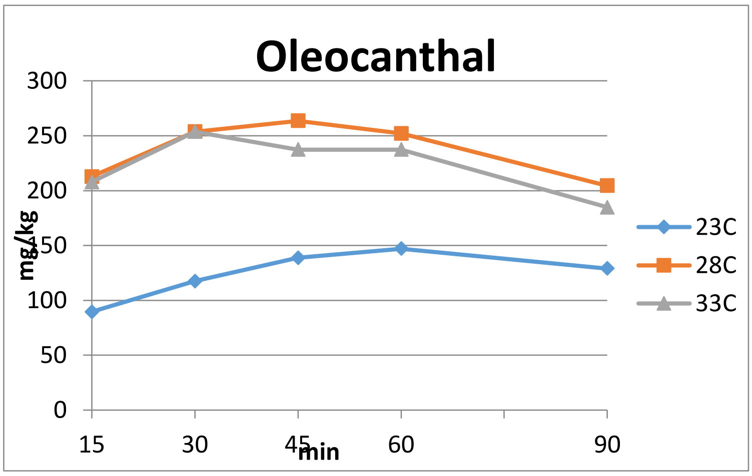
Independently of the total phenolics it turns out that it is also important which specific substances are contained in the phenolic fraction. The individual substances strongly effect on the pharmaceutical characteristic of the olive oil and also in the organoleptic characteristics.

Our study showed that oleocanthal, in all cases is increasing during malaxation process at least for 30 minutes. The increasing of oleocanthal is continuing till the 45th minute of malaxation with the exception of two cases of short ripening degree olive fruits of koroneiki variety. In some cases the increasing of oleocanthal continues till the end of the process especially on middle and high temperature

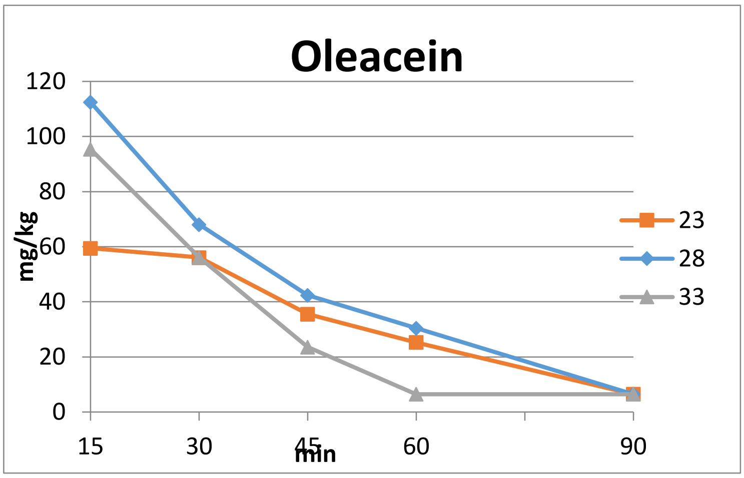


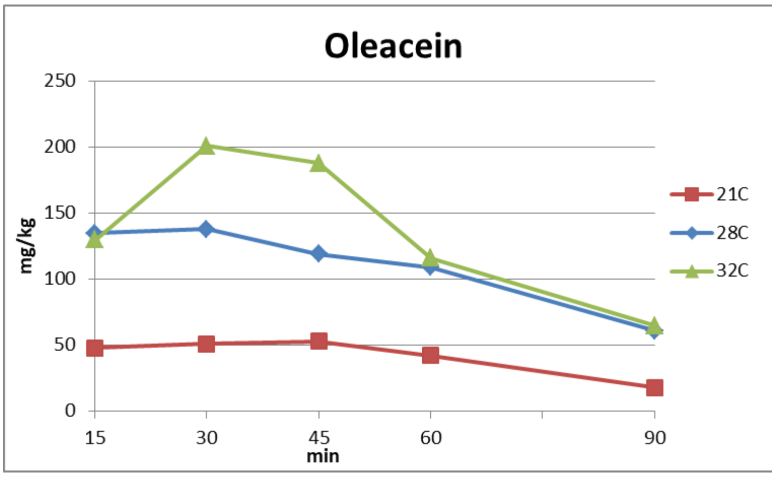


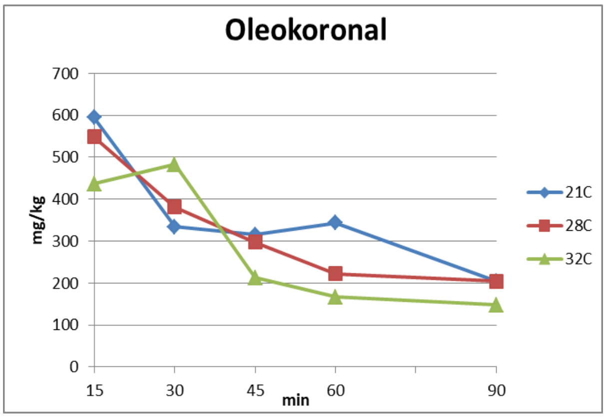
As for the malaxation temperature at the same ripening degree we can easily conclude that heating during malaxation enhance the formation of oleocanthal rapidly. In the six experiments of koroneiki we saw that the application of middle temperature lead to doubling the amount of oleocanthal and in some cases the application of high temperature may lead to tripling the amount of oleocanthal

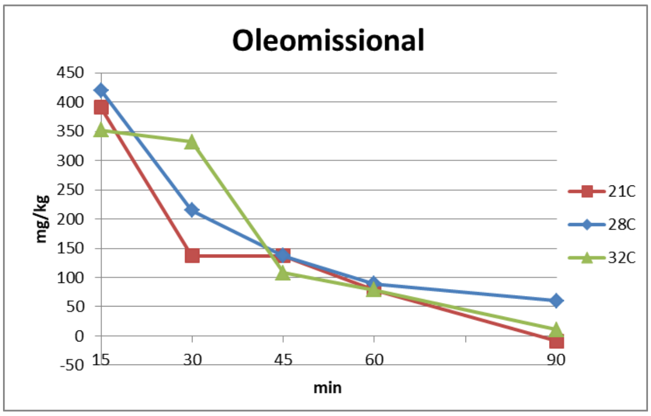


In the case of oleacein we noticed that in some cases we had an increase of oleacein till the 30th minute of malaxing followed by a rapid reducing till the end of the process. We could say that this phenomena takes place in olive fruits with high ripening degree. On the contrary olive fruits with short ripening degree shows an increase from the 15 minutes with the exception of the cases were we applied high temperature. We can also se that the application of heating increases the oleacein level significantly. Finally as we saw in the case of oleocanthal heating can lead to tripling the oleacein levels from the same olive fruit sample.









Finally oleokoronal and oleomissional are two constituents that reduced rapidly during the process of malaxing, There are only two exceptions in this statement where this constituents are increasing till the 30th minute but we have to mention that this two cases coincide with the cases that the total phenolics are increasing. In all cases the level of this two constituents is very high at the beginning of the process and very low in the end. This tendency we noted in oleocanthal oleacein oleokoronal and oleacein enhances the hypothesis that oleocanthal and oleacein are created by the consumption of oleokoronal and oleomissional.

Results

10/10/2017

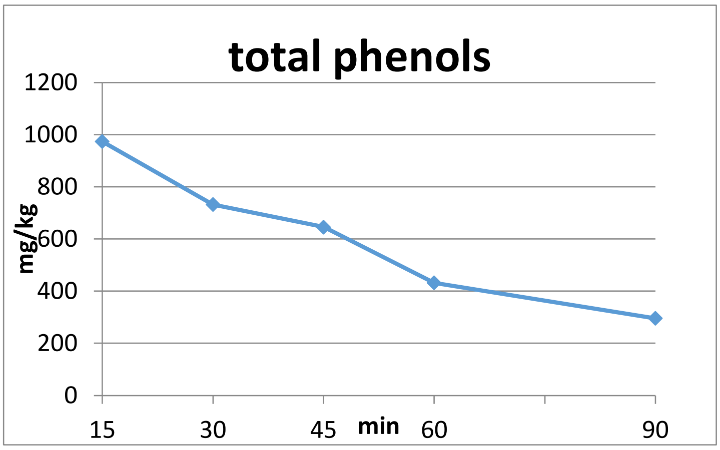
TGI11 Koroneiki Variety

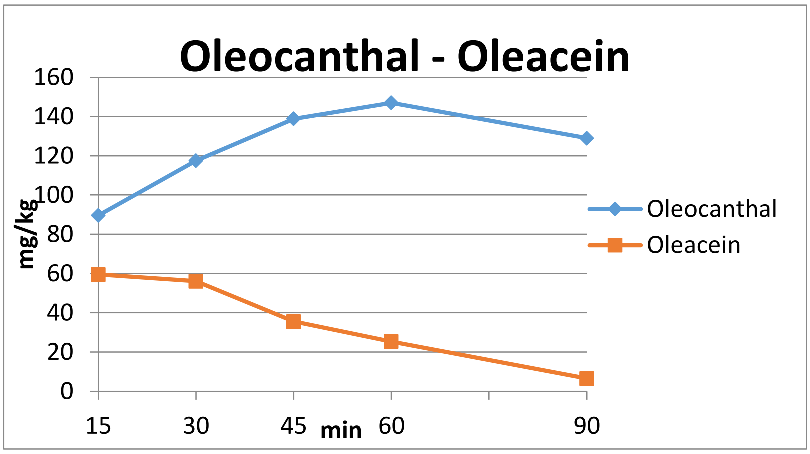
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in

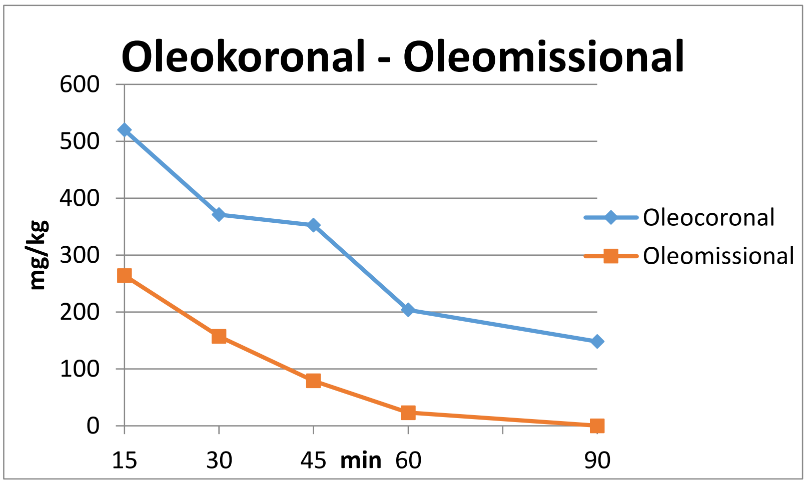
	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI11_15	90	59	13	27	520	264	974	17g
TGI11_30	117	56	6	25	371	157	732	17,75g
TGI11_45	139	36	4	36	353	79	646	19,1g
TGI11_60	147	25	0	36	204	23	432	20,8g
TGI11_90	129	7	0	29	148	0	296	20g

stainless bowl

- Malaxation Temperature / malaxation time:***23,4-23,6^{0}C*** - 15, 30, 45, 60 and 90min



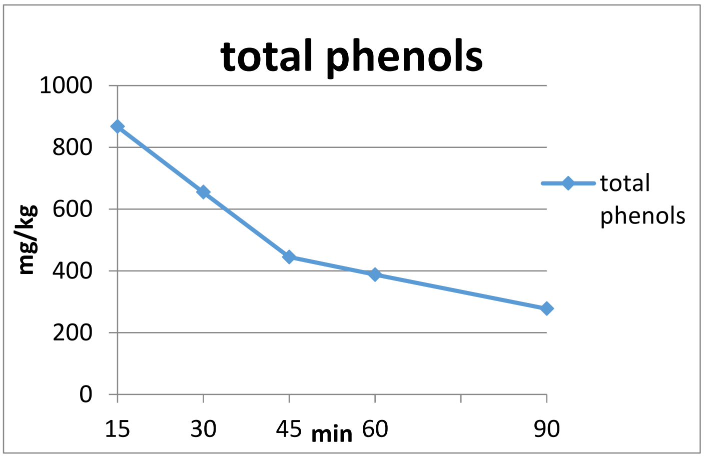


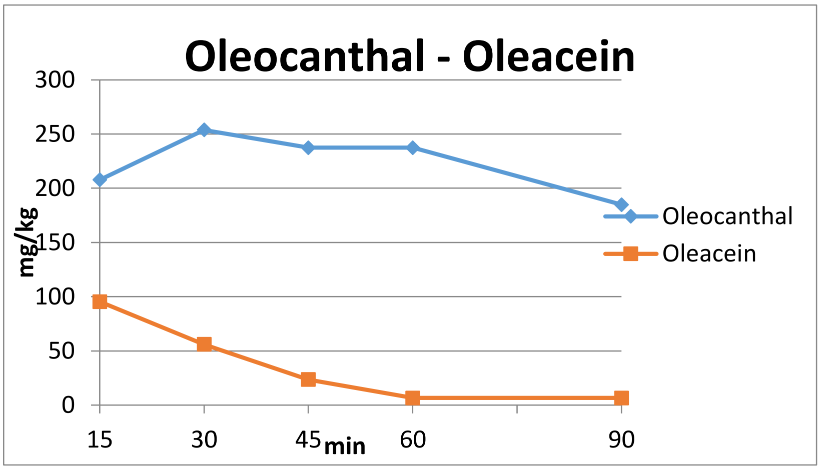


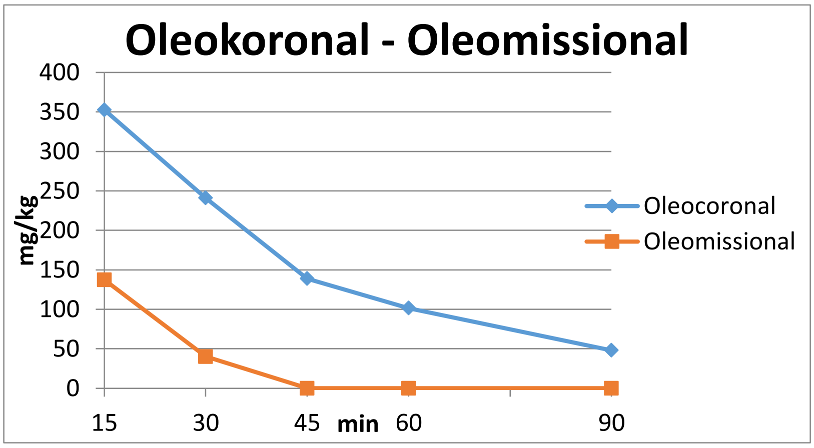
	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI12_15	208	95	28	46	353	137	867	17,9g
TGI12_30	254	56	8	55	241	40	654	19,3g
TGI12_45	237	24	0	57	139	0	445	18g
TGI12_60	237	7	0	60	102	0	388	21,2g
TGI12_90	185	7	0	55	48	0	277	21g

TGI12 Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time: **32,5-33,5^{0}C** - 15, 30, 45, 60 and 90min.







	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI13_15	213	112	25	46	476	288	1161	17g
TGI13_30	254	68	16	50	306	135	829	17,5g
TGI13_45	264	42	8	55	250	35	655	18,7g
TGI13_60	252	30	1	60	260	0	594	19,8g
TGI13_90	204	7	0	50	120	0	364	20,1g

11/10/2017

TGI13 Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time**27,5-29,4^{0}C** 15, 30, 45, 60 and 90min

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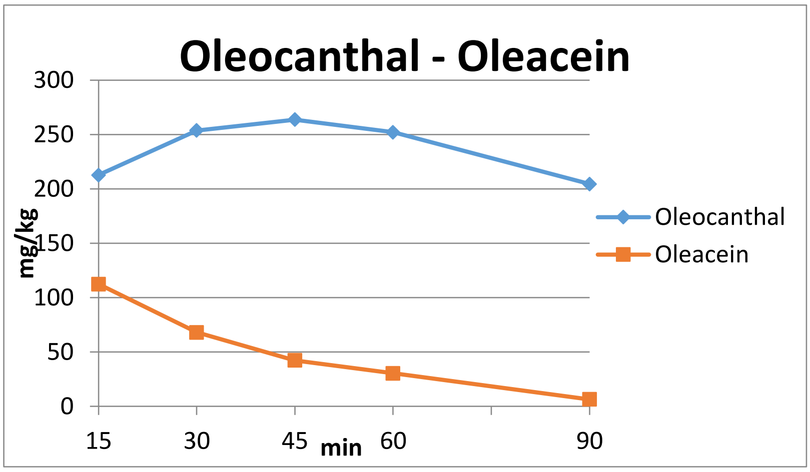
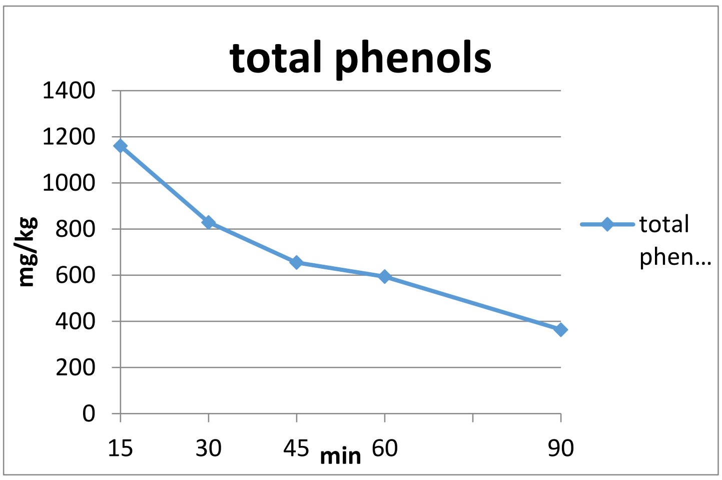
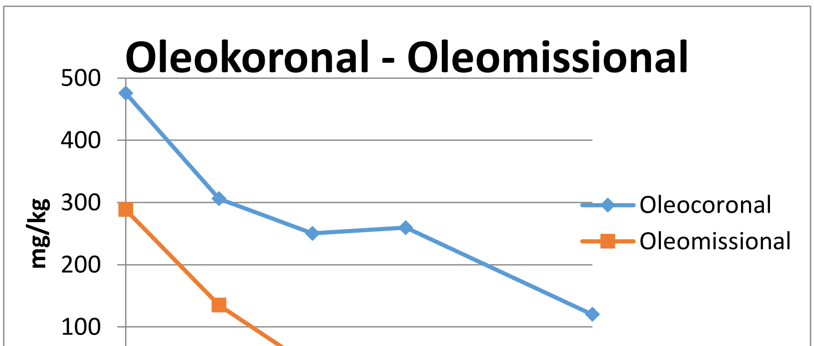
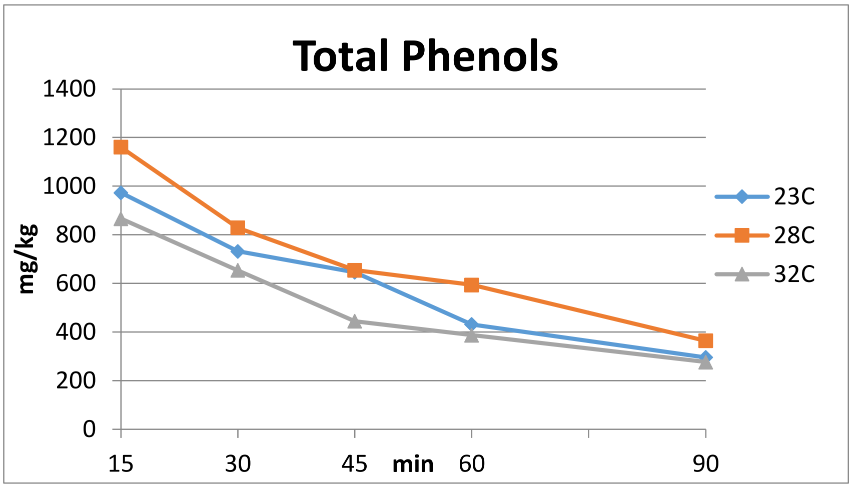






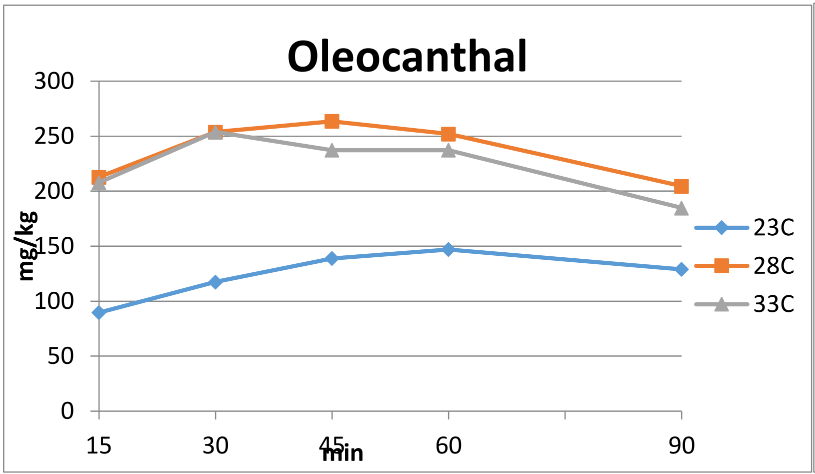


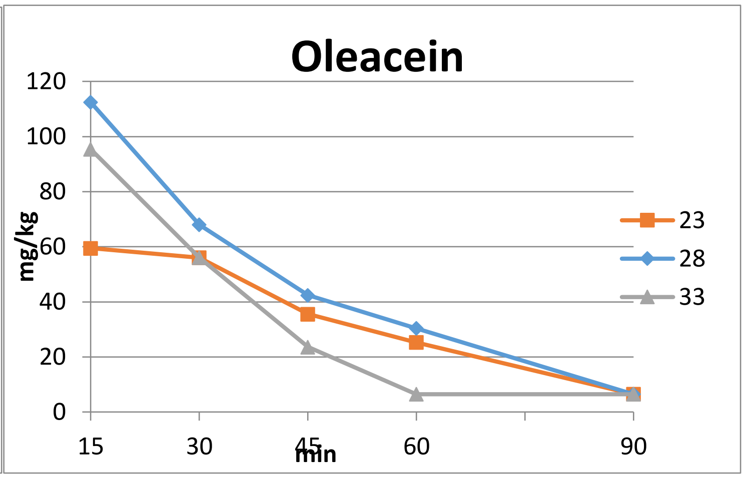
Comparative for koroneiki on 10-10-2017 :

	23	28	33
15	974	1161	867
30	732	829	654
45	646	655	445
60	432	594	388
90	296	364	277

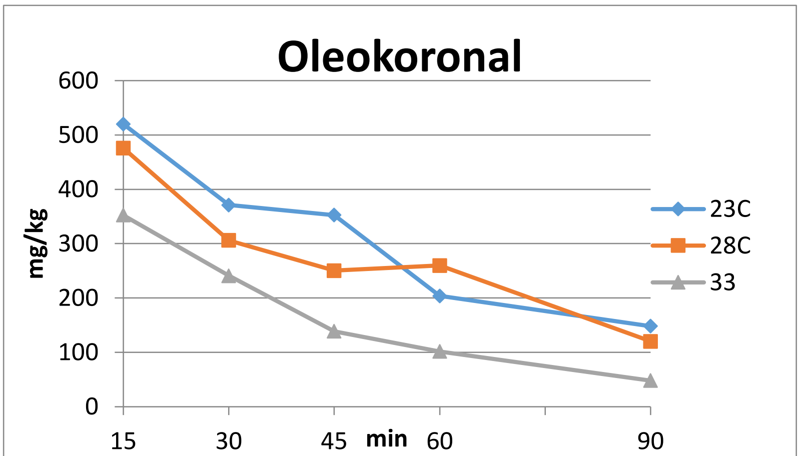
Project co-financed by the European Regional Development Fund

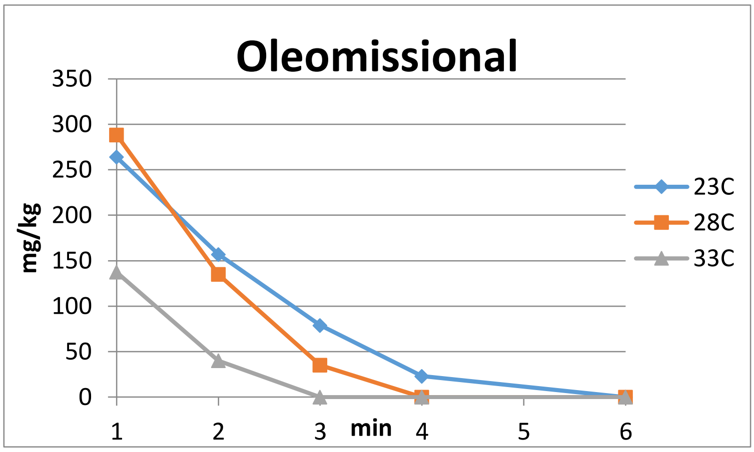
	23C		28C		33C	
	Oleocanthal	Oleacein	Oleocanthal	Oleacein	Oleocanthal	Oleacein
15	90	59	213	112	208	95
30	117	56	254	68	254	56
45	139	36	264	42	237	24
60	147	25	252	30	237	7
90	129	7	204	7	185	7



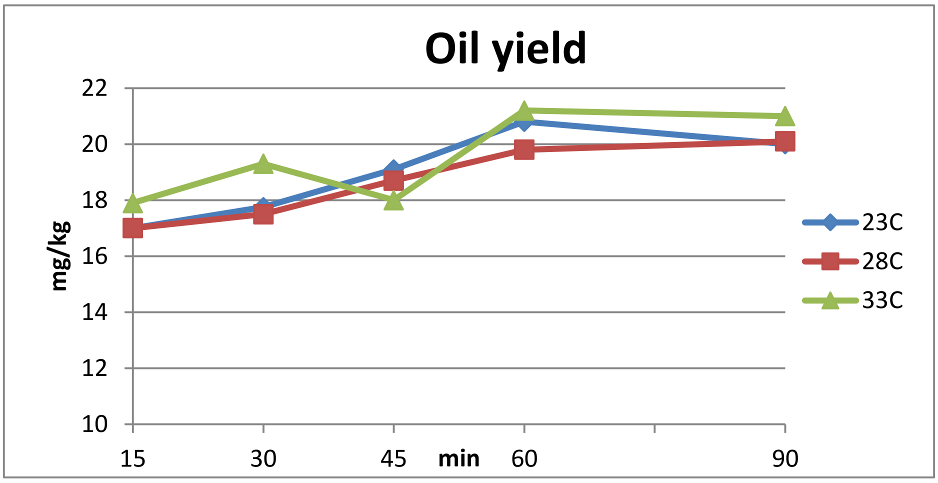


	23C		28C		33C	
	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional
15	520	264	476	288	353	137
30	371	157	306	135	241	40
45	353	79	250	35	139	0
60	204	23	260	0	102	0
90	148	0	120	0	48	0





	23C	28C	33C
G oil\200g paste			
15	**17**	**17**	**17.9**
30	**17.75**	**17.5**	**19.3**
45	**19.1**	**18.7**	**18**
60	**20.8**	**19.8**	**21.2**
90	**20**	**20.1**	**21**



31/10/2017

TGI34: Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI34_15	106	99	45	48	343	215	856	19,6g
TGI34_30	178	112	50	62	269	128	799	21,8g
TGI34_45	232	94	33	67	195	52	672	23,3g
TGI34_60	201	41	8	53	127	16	446	23,3g
TGI34_90	119	7	0	18	111	0	254	24g

- Malaxation Temperature / malaxation time: **32,5-33,5^{0}C** - 15, 30, 45, 60 and 90min.

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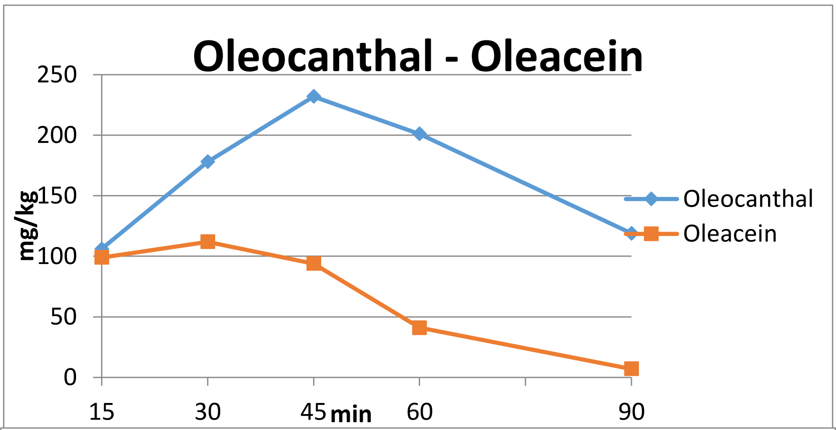
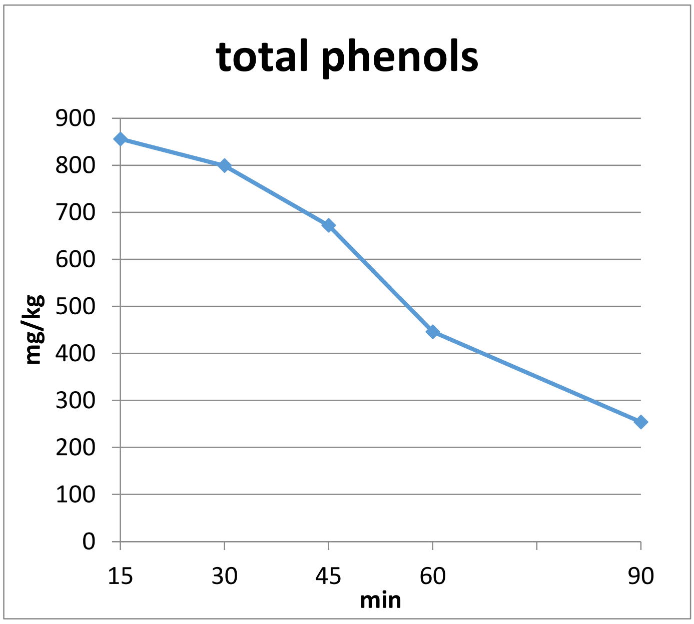
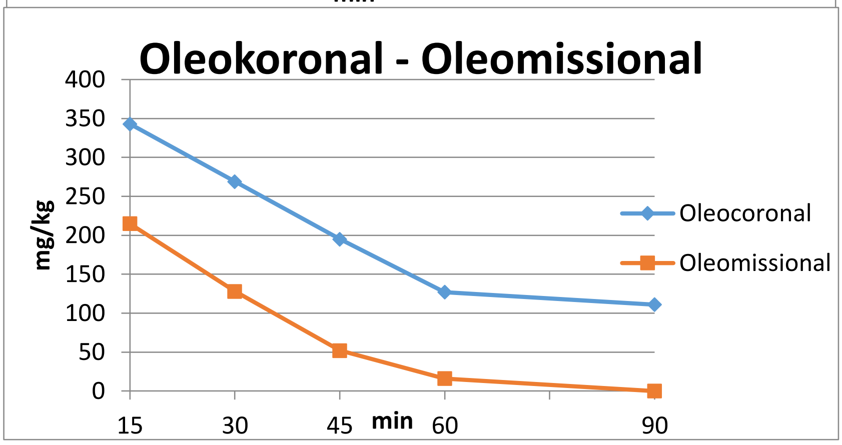


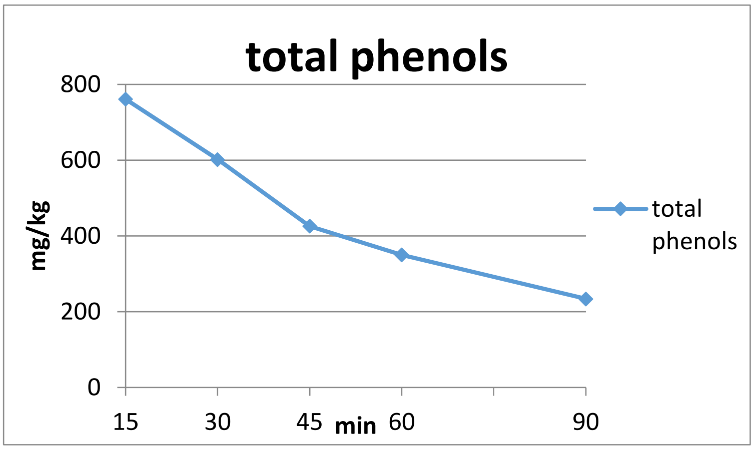


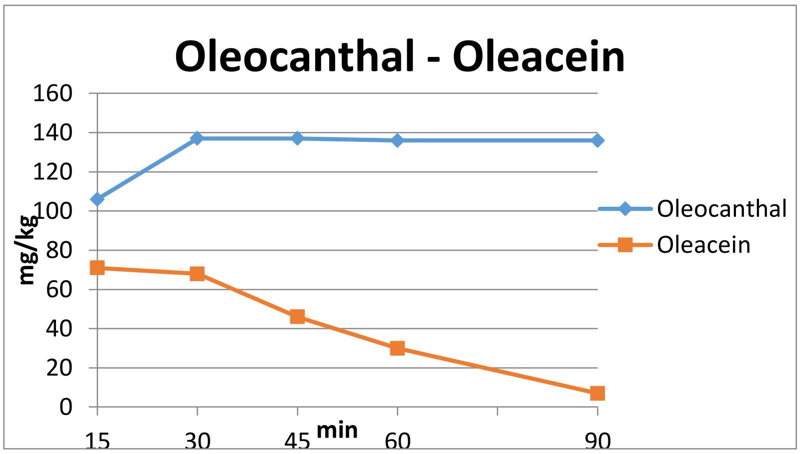


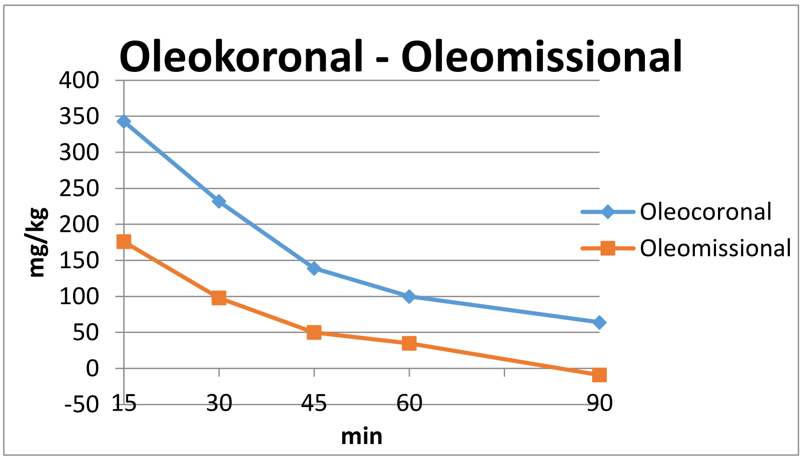
TGI35: Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time: **27,5-29,** - 15, 30, 45, 60 and 90min.

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI35_15	106	71	28	36	343	176	761	19,2g
TGI35_30	137	68	23	43	232	98	602	21g
TGI35_45	137	46	13	41	139	50	426	21,1g
TGI35_60								23,3g
TGI35_90	136	7	0	36	64	0	243	24,8g





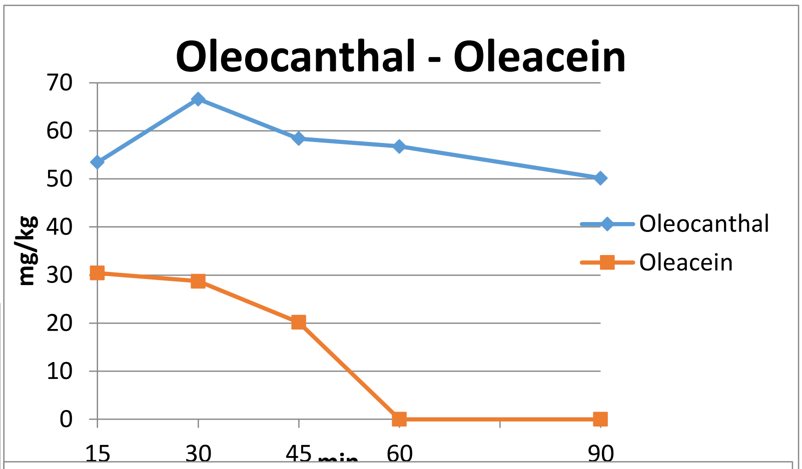


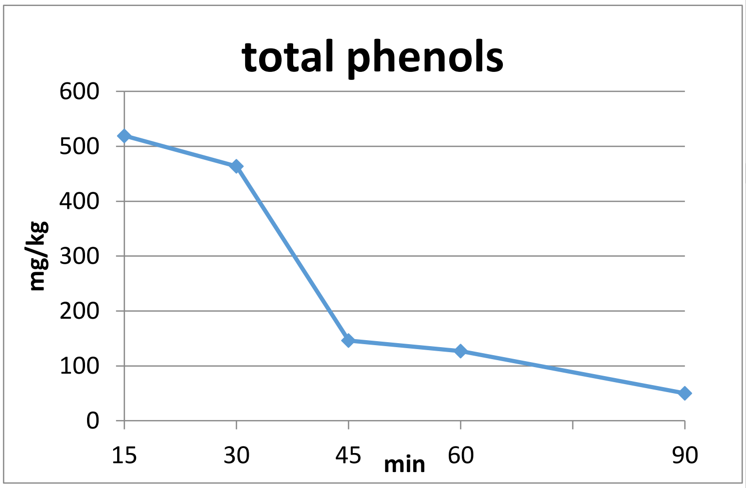
TGI36: Koroneiki Variety

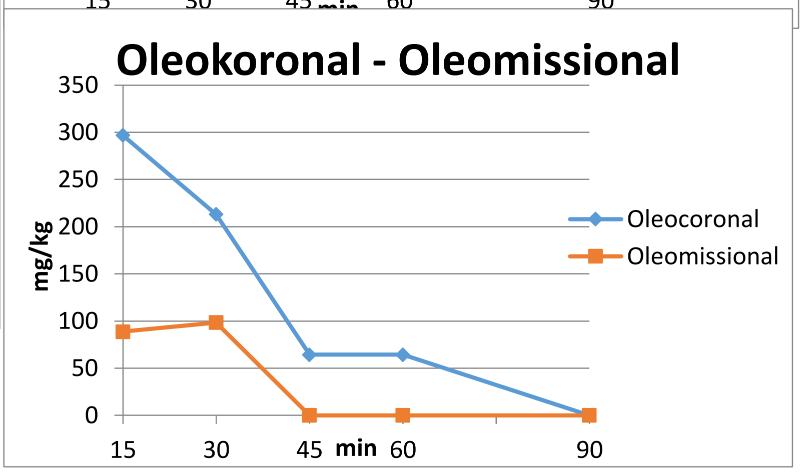
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
15	53	30	16	34	297	89	519	**19.2**
30	67	29	16	41	213	98	464	**20.6**
45	58	20	4	0	64	0	146	**21.6**
60	57	0	6	0	64	0	127	**21.3**
90	50	0	0	0	0	0	50	**23.8**

- Malaxation Temperature / malaxation time:***<u>22-23^{0}C</u>*** - 15, 30, 45, 60 and 90min







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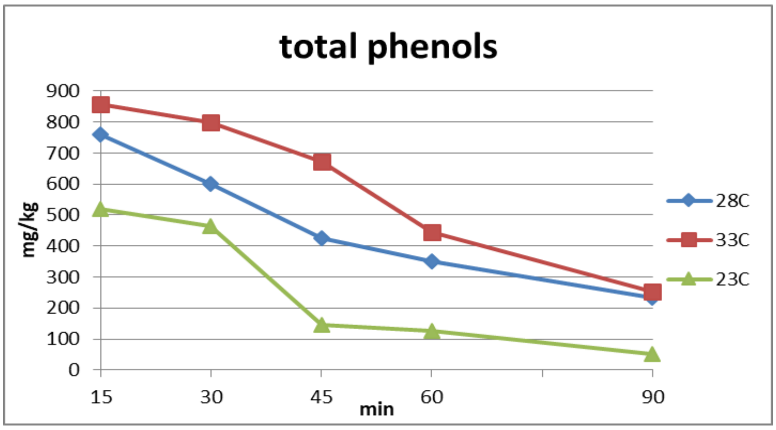
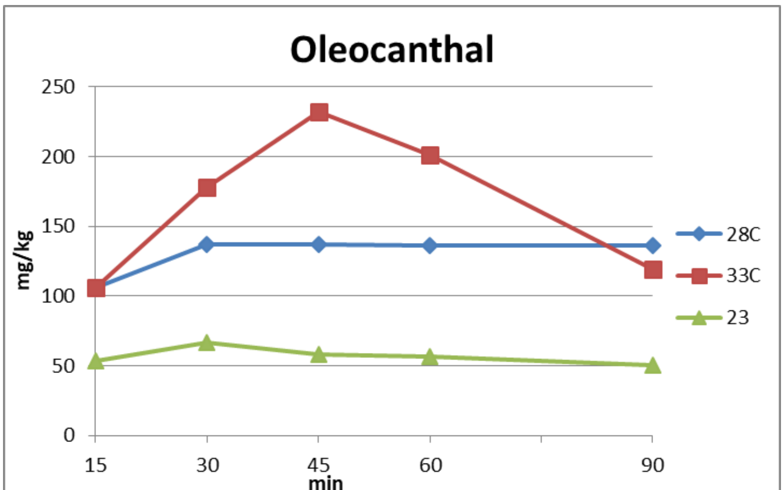
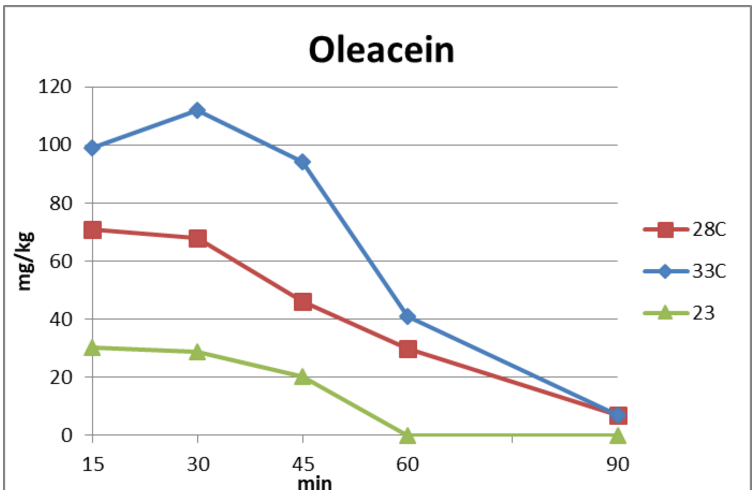
Comparative for koroneiki on 31-10-2017

Time(min)	23	28	33
15	519	761	856
30	464	602	799
45	146	426	672
60	127	350	446
90	50	234	254

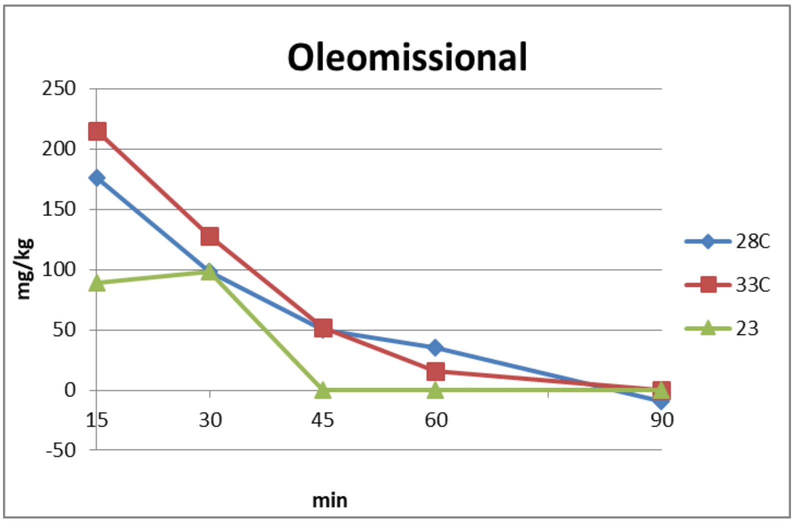


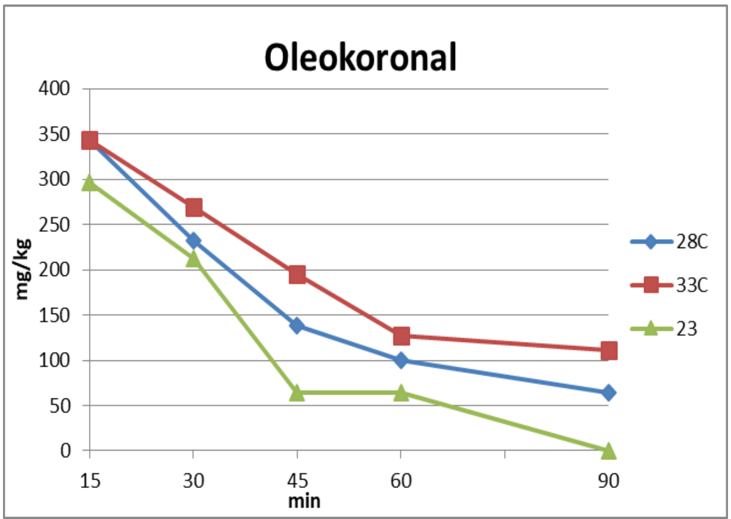
Time (min)	Oleocanthal	Oleacein	Oleocanthal	Oleacein	Oleocanthal	Oleacein
15	53	30	106	71	106	99
30	67	29	137	68	178	112
45	58	20	137	46	232	94
60	57	0	136	30	201	41
90	50	0	136	7	119	7



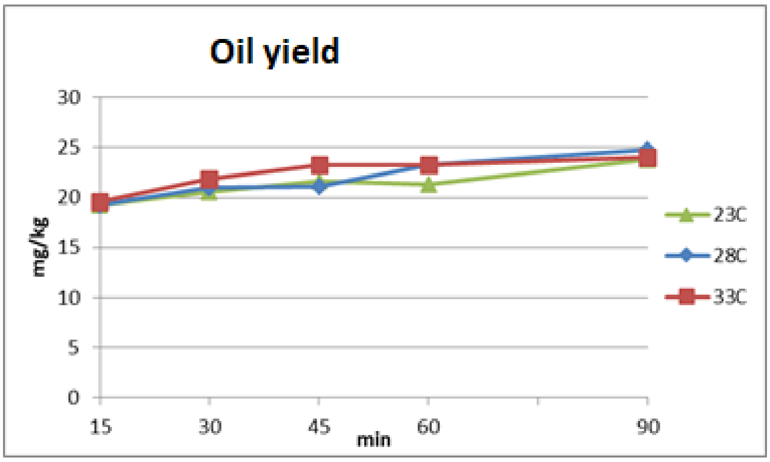


	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional
15	297	89	343	176	343	215
30	213	98	232	98	269	128
45	64	0	139	50	195	52
60	64	0	100	35	127	16
90	0	0	64	-9	111	0





15	**19.2**	**19.2**	**19.6**
30	**20.6**	**21**	**21.8**
45	**21.6**	**21.1**	**23.3**
60	**21.3**	**23.3**	**23.3**
90	**23.8**	**24.8**	**24**



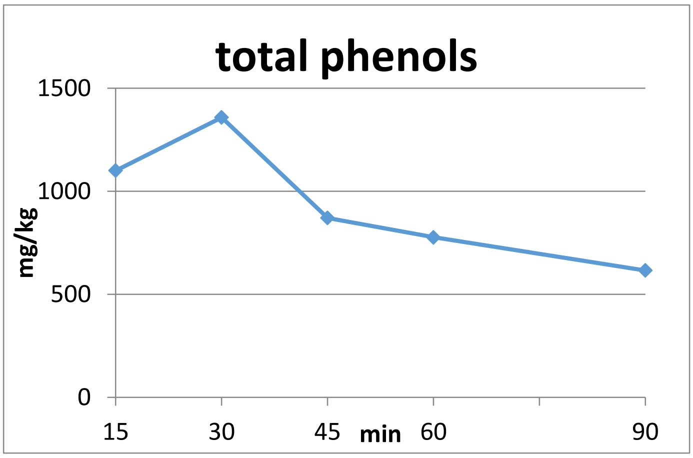
20/11/2017

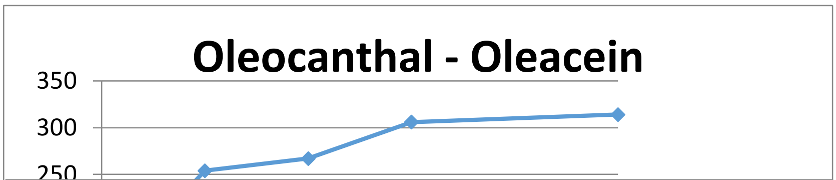
TGI40: Koroneiki Variety

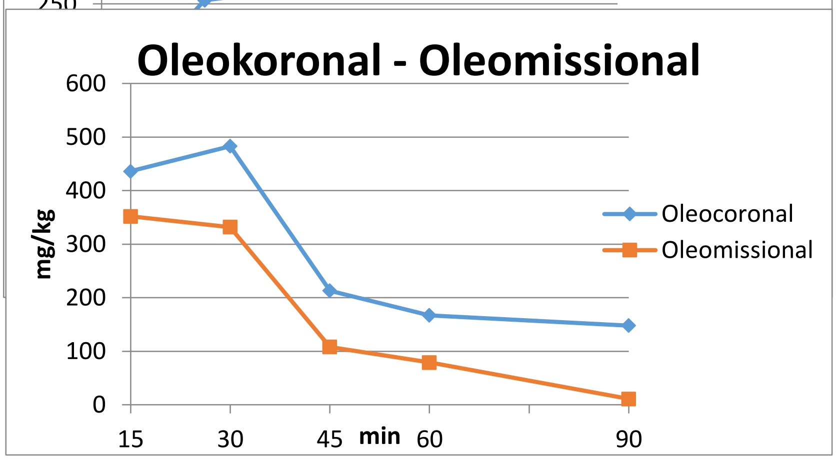
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI40_15	136	130	25	22	436	352	1101	17.8g
TGI40_30	254	201	50	39	483	332	1359	20.8g
TGI40_45	267	188	50	46	213	108	871	22.5g
TGI40_60	306	116	55	55	167	79	777	23.1g
TGI40_90	314	65	18	60	148	11	616	23.1g

- Malaxation Temperature / malaxation time: **32,5-33,5^{0}C** - 15, 30, 45, 60 and 90min.





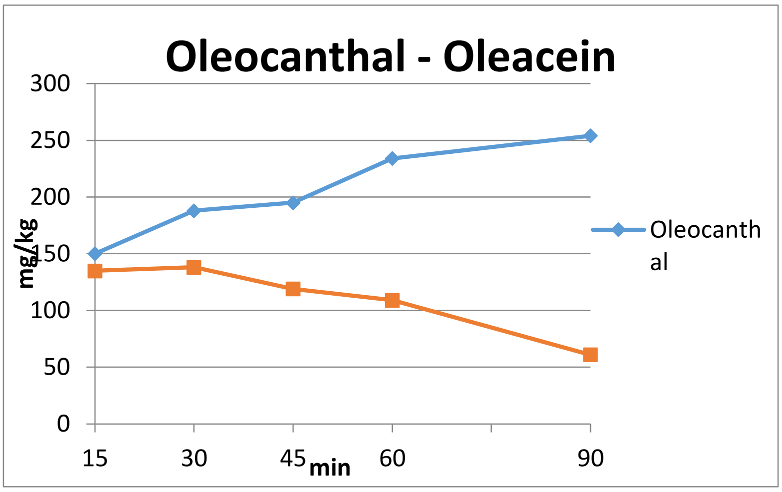


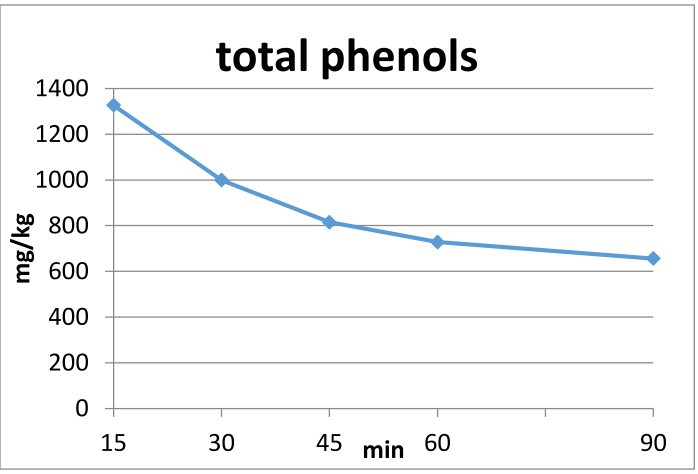
TGI41: Koroneiki Variety

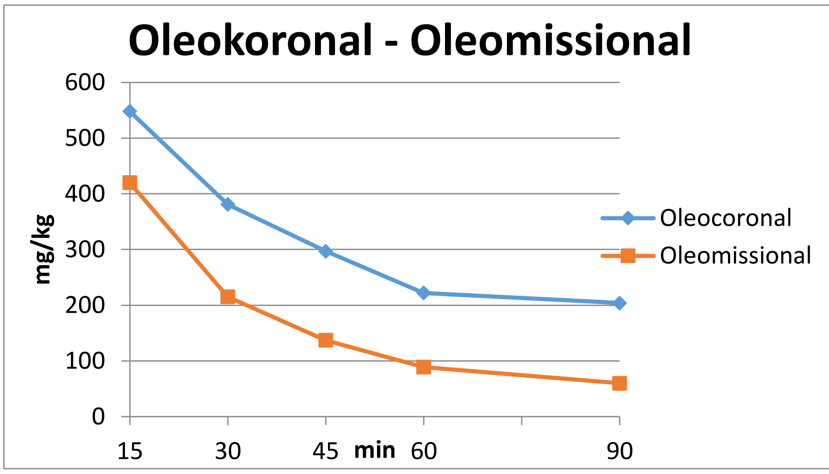
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI41_15	150	135	42	32	548	420	1327	20g
TGI41_30	188	138	40	36	381	215	999	22,4g
TGI41_45	195	119	30	36	297	137	815	22,7g
TGI41_60	234	109	281	46	222	89	981	23,5g
TGI41_90	254	61	18	60	204	60	656	22g

- Malaxation Temperature / malaxation time: **28^{0}C** - 15, 30, 45, 60 and 90min.



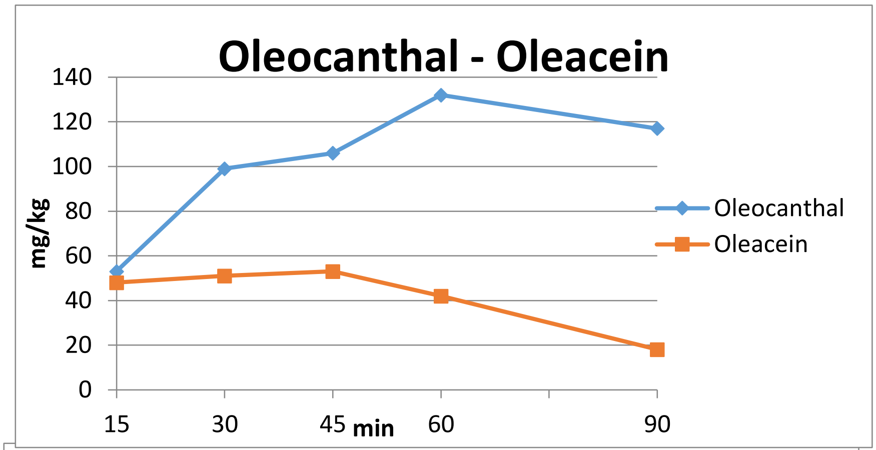


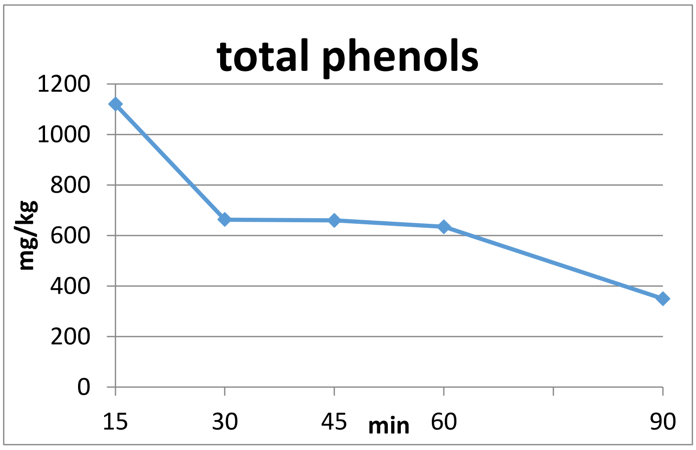


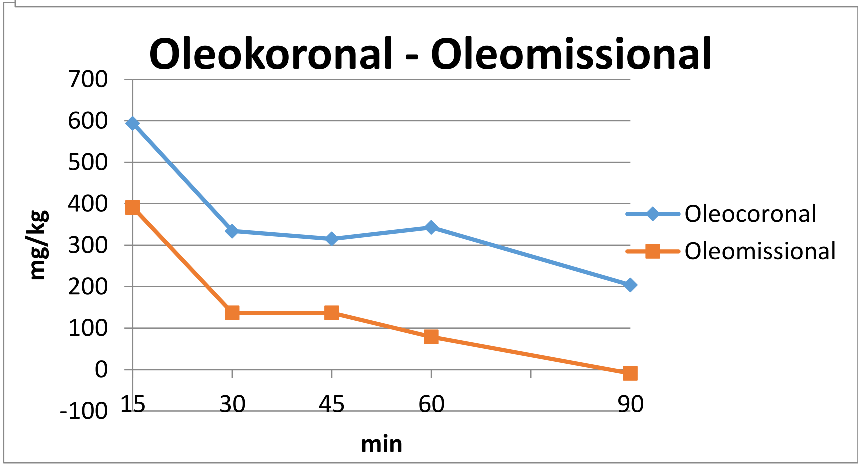
TGI42: Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time: **21^{0}C** - 15, 30, 45, 60 and 90min

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI42_15	53	48	18	15	594	391	1120	18,2g
TGI42_30	99	51	21	20	334	137	663	19,5g
TGI42_45	106	53	33	15	315	137	660	21,6g
TGI42_60	132	42	13	25	343	79	635	19,5g
TGI42_90	117	18	0	22	204	0	362	22,8g

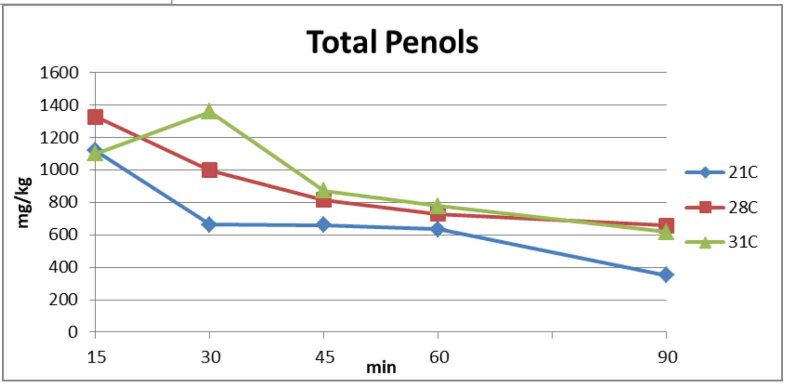




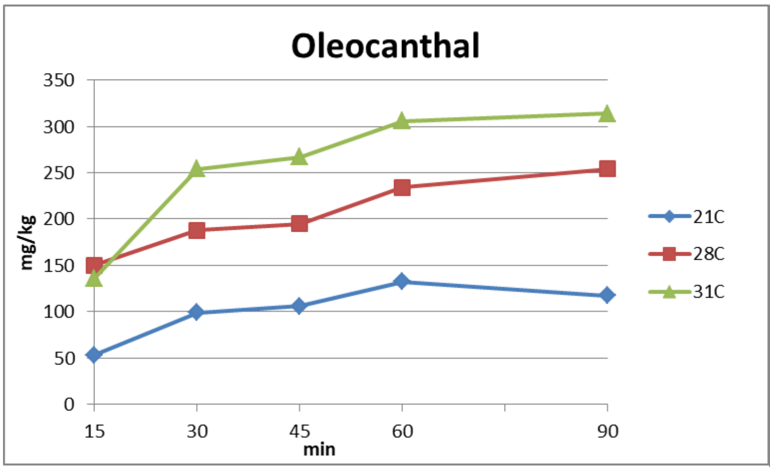


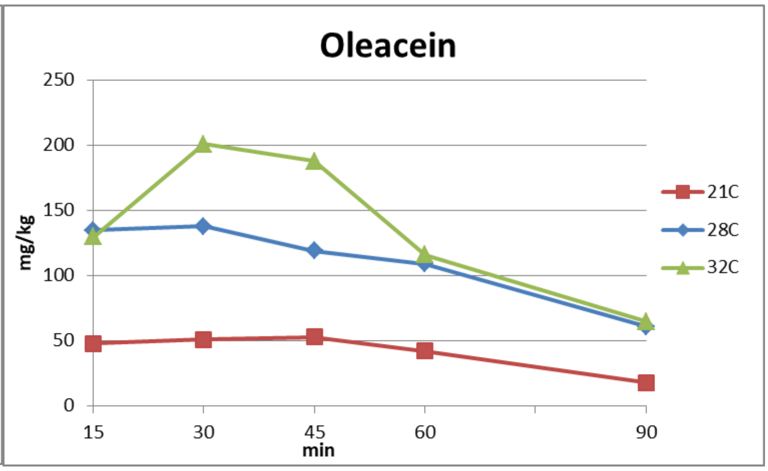
Comparative for koroneiki on 20-11-2017

	total phenols		
	21	**28**	**32**
15	1120	1327	1101
30	663	999	1359
45	660	815	871
60	635	728	777
90	350	656	616

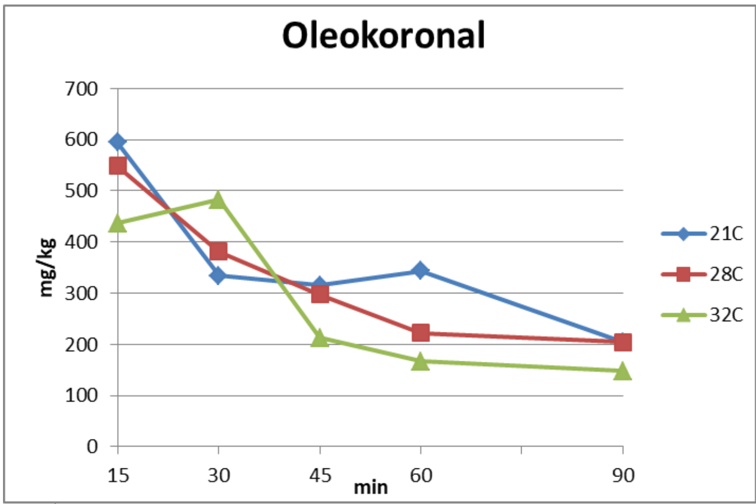


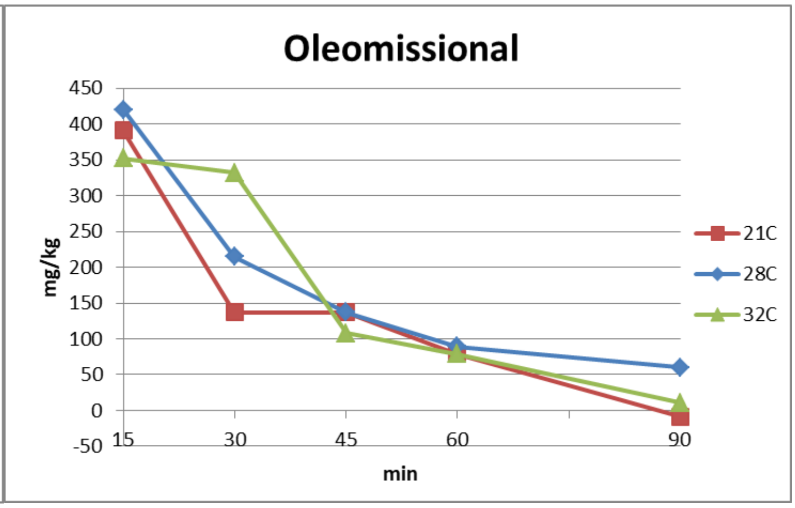
Time(min)	Oleocanthal	Oleacein	Oleocanthal	Oleacein	Oleocanthal	Oleacein
15	53	48	150	135	136	130
30	99	51	188	138	254	201
45	106	53	195	119	267	188
60	132	42	234	109	306	116
90	117	18	254	61	314	65



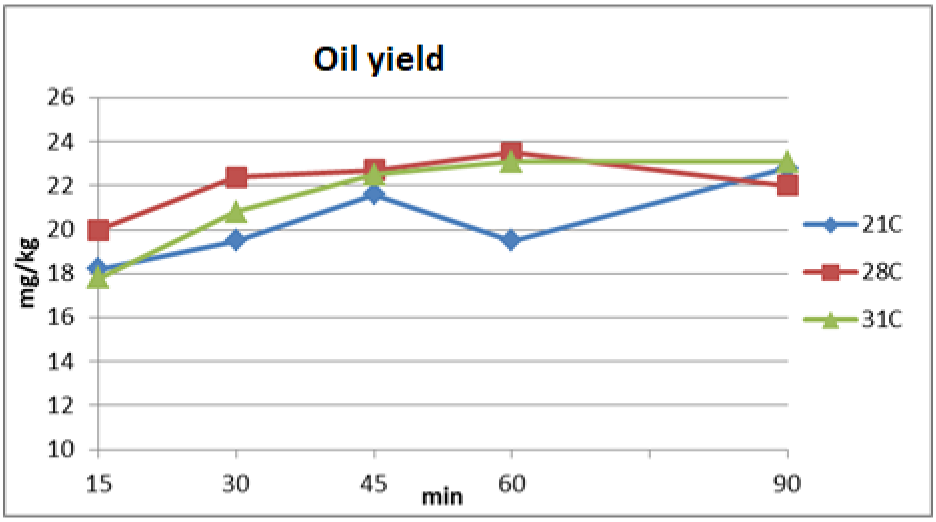


Time(min)	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional
15	594	391	548	420	436	352
30	334	137	381	215	483	332
45	315	137	297	137	213	108
60	343	79	222	89	167	79
90	204	-9	204	60	148	11





15	**18,2**	**20**	**17,8**
30	**19,5**	**22,4**	**20,8**
45	**21,6**	**22,7**	**22,5**
60	**19,5**	**23,5**	**23,1**
90	**22,8**	22	**23,1**

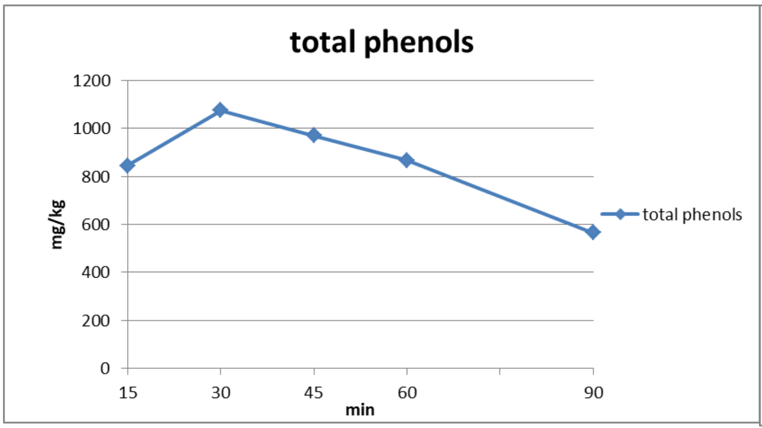


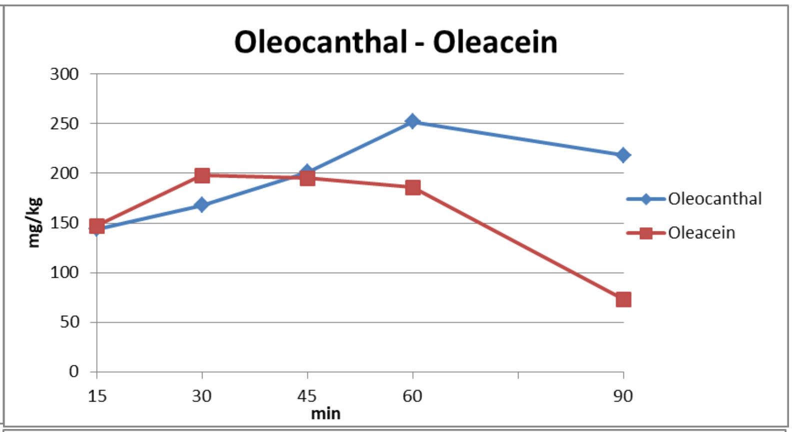
12/12/2017

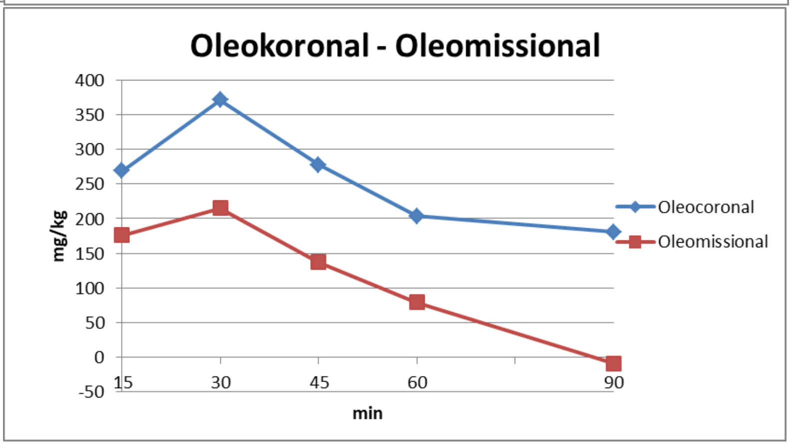
TGI48: Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time: **33^{0}C** - 15, 30, 45, 60 and 90min

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI48_15	144	147	77	32	269	176	844	g
TGI48_30	168	198	84	39	371	215	1075	g
TGI48_45	201	195	98	60	278	137	970	g
TGI48_60	252	186	84	62	204	79	867	g
TGI48_90	218	73	45	57	181	0	574	g





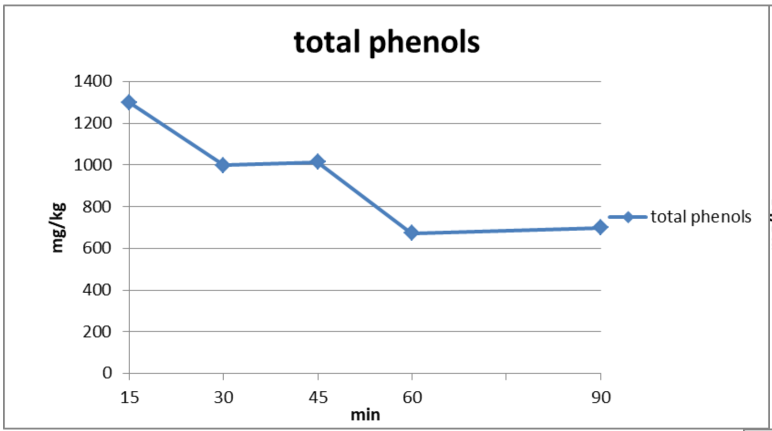


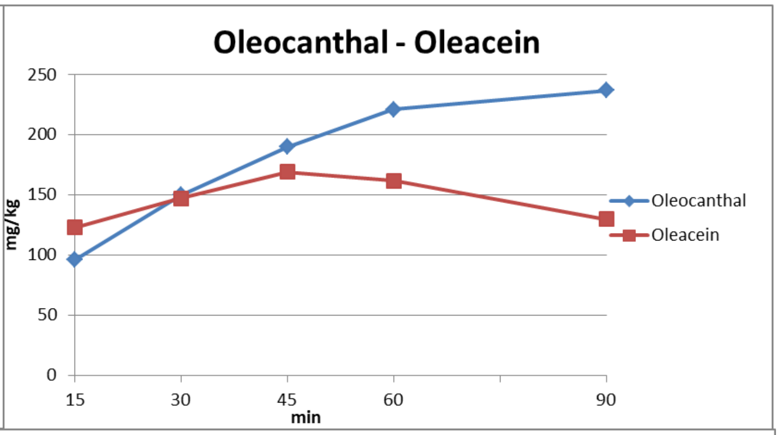
TGI49: Koroneiki Variety

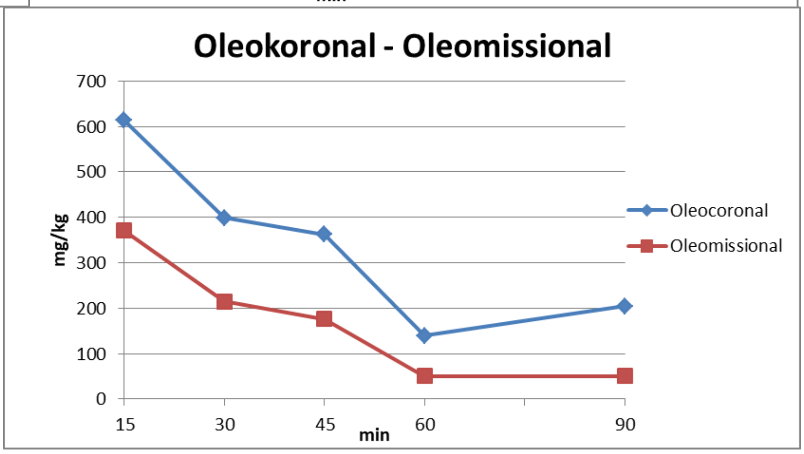
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI49_15	96	123	62	36	613	371	1301	g
TGI49_30	150	147	64	22	399	215	998	g
TGI49_45	190	169	72	46	362	176	1014	g
TGI49_60	221	162	60	41	139	50	672	g
TGI49_90	237	130	40	39	204	50	699	g

- Malaxation Temperature / malaxation time: **<u>28^{0}C</u>** - 15, 30, 45, 60 and 90min





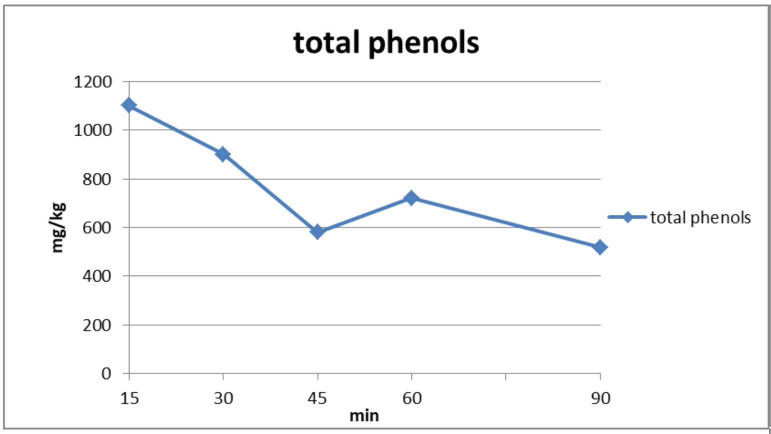


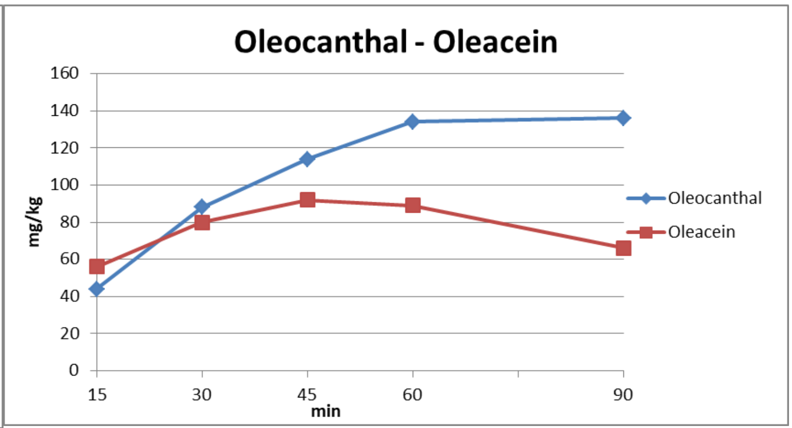
TGI50: Koroneiki Variety

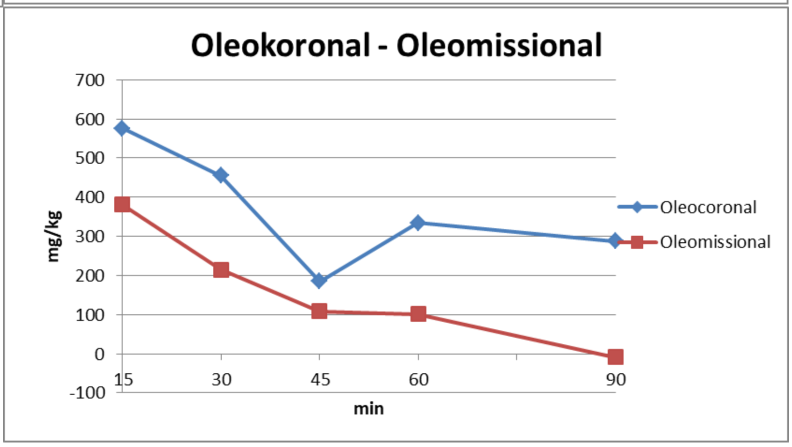
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI50_15	44	56	45	0	576	381	1101	29,3g
TGI50_30	88	80	45	18	455	215	901	30,1g
TGI50_45	114	92	52	29	185	108	581	31,1g
TGI50_60	134	89	35	29	334	101	722	26,9g
TGI50_90	136	66	16	22	288	0	528	32,5g

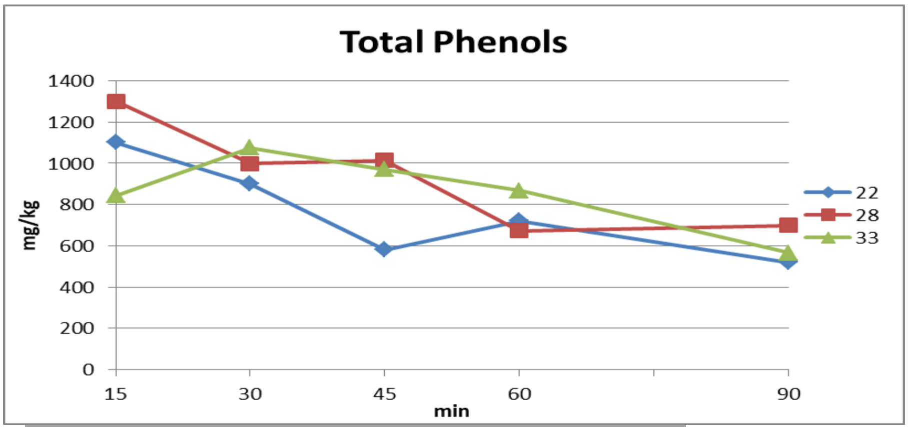
- Malaxation Temperature / malaxation time: **<u>22^{0}C</u>** - 15, 30, 45, 60 and 90min







Comparative for koroneiki on 12-12-2017

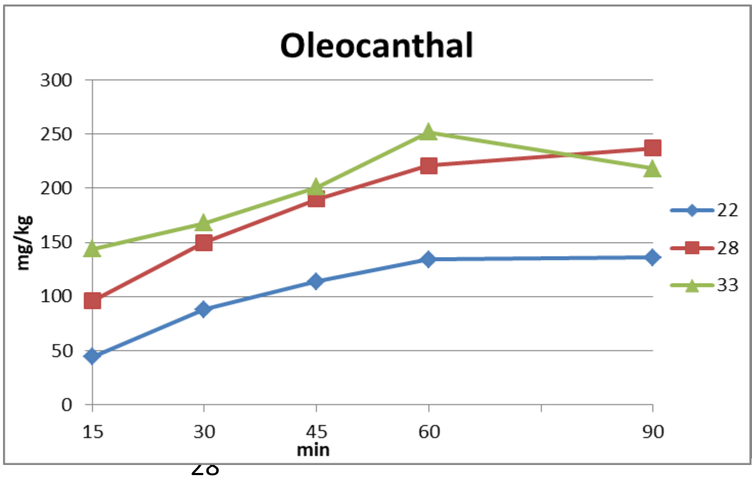


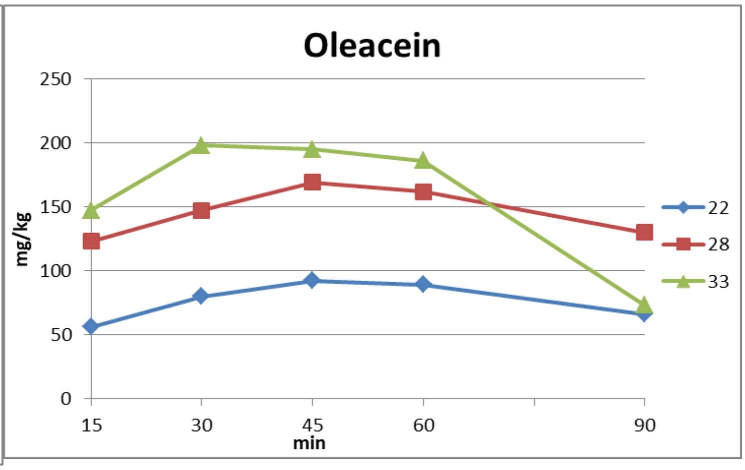
Time	total phenols		
	22	28	33
15	1101	1301	844
30	901	998	1075
45	581	1014	970
60	722	672	867
90	519	699	565

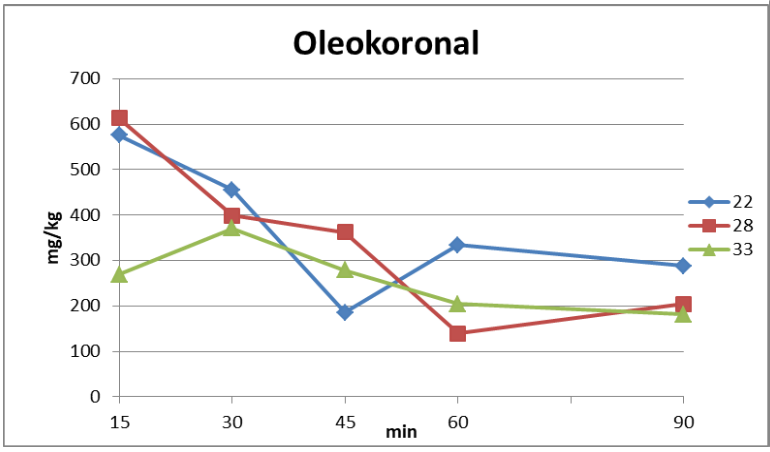
Time(min)	Oleocanthal	Oleacein	Oleocanthal	Oleacein	Oleocanthal	Oleacein
15	44	56	96	123	144	147
30	88	80	150	147	168	198
45	114	92	190	169	201	195
60	134	89	221	162	252	186
90	136	66	237	130	218	73

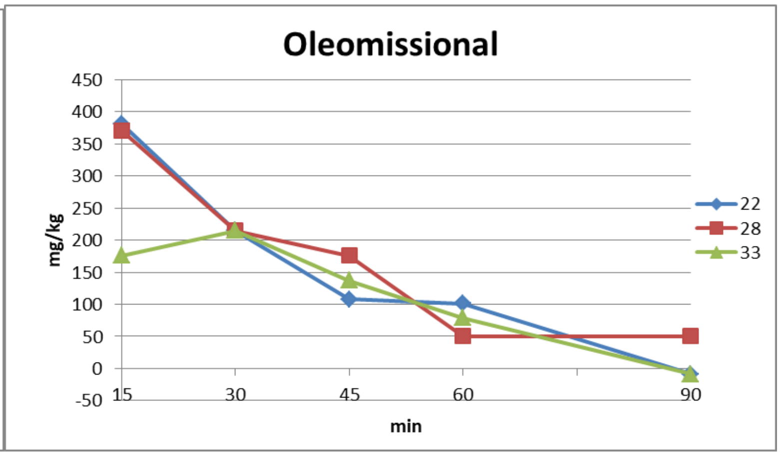
Time(min)	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional
15	576	381	613	371	269	176
30	455	215	399	215	371	215
45	185	108	362	176	278	137
60	334	101	139	50	204	79
90	288	0	204	50	181	0

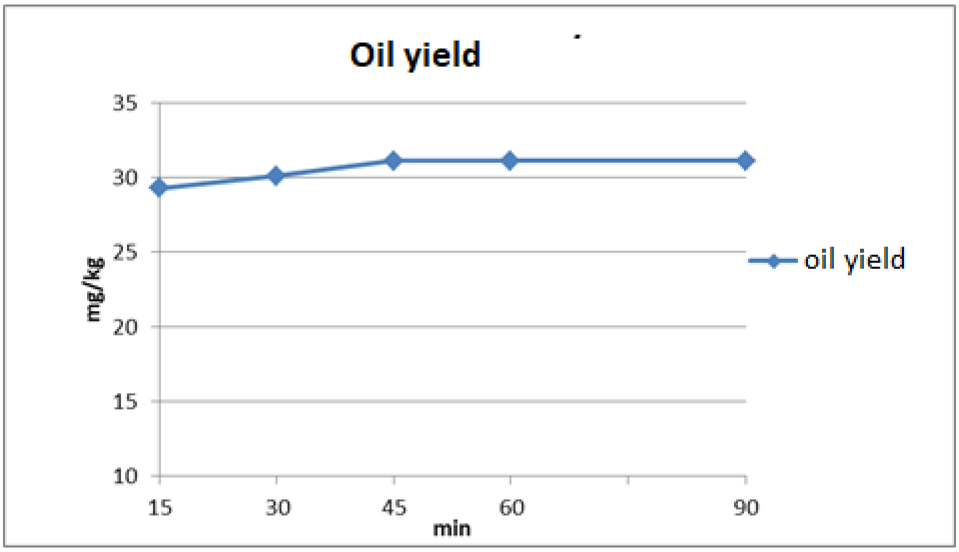
Χρόνος	Oil yield		
	22	28	33
15	**29,3**		
30	**30,1**		
45	**31,1**		
60	**31,1**		
90	**31,1**		






Oleokoronal
mg/kg
min
22
28
33


Oleomissional
mg/kg
min
22
28
33


Oil yield
mg/kg
min
oil yield

09/01/2018

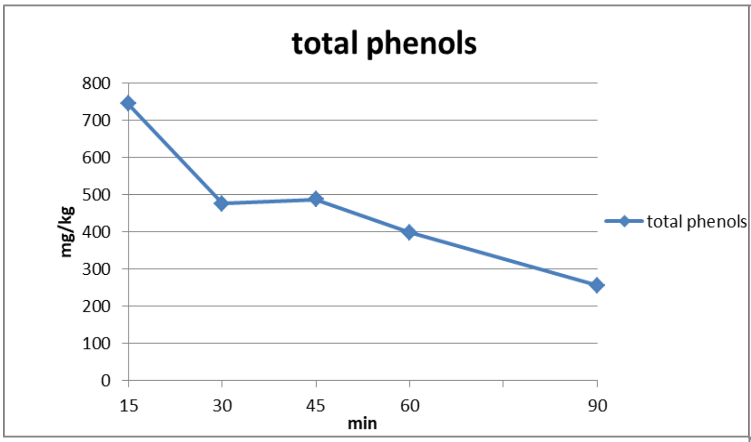
<u>TGI52: Koroneiki Variety</u>

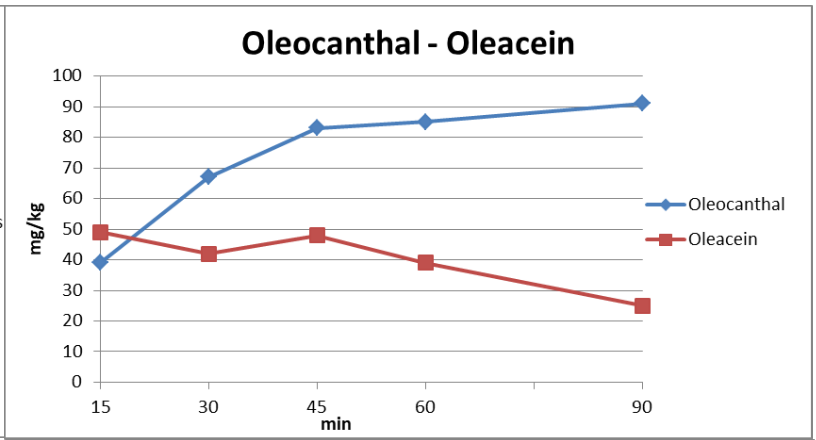
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in

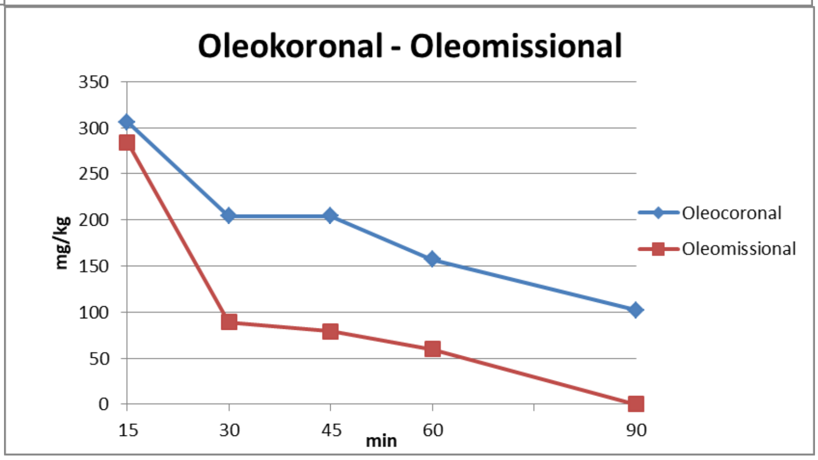
	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI52_15	39	49	45	22	306	284	745	25,9g
TGI52_30	67	42	47	27	204	89	476	27,8g
TGI52_45	83	48	40	34	204	79	487	27,8g
TGI52_60	85	39	30	27	157	60	398	28g
TGI52_90	91	25	6	32	102	0	256	30g

stainless bowl

- Malaxation Temperature / malaxation time: **<u>22^{0}C</u>** - 15, 30, 45, 60 and 90min



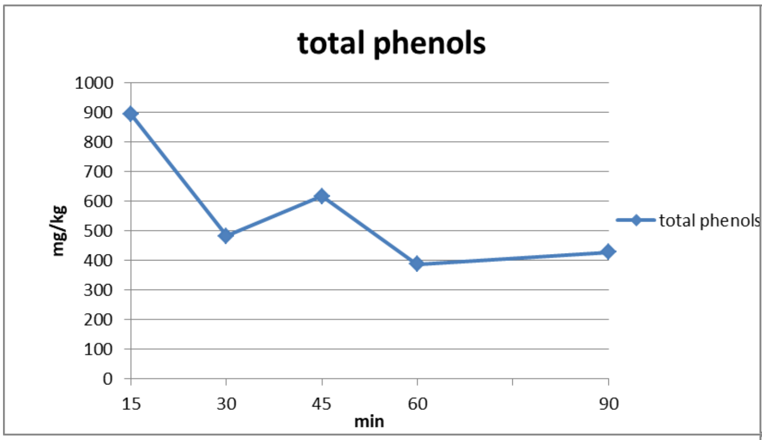


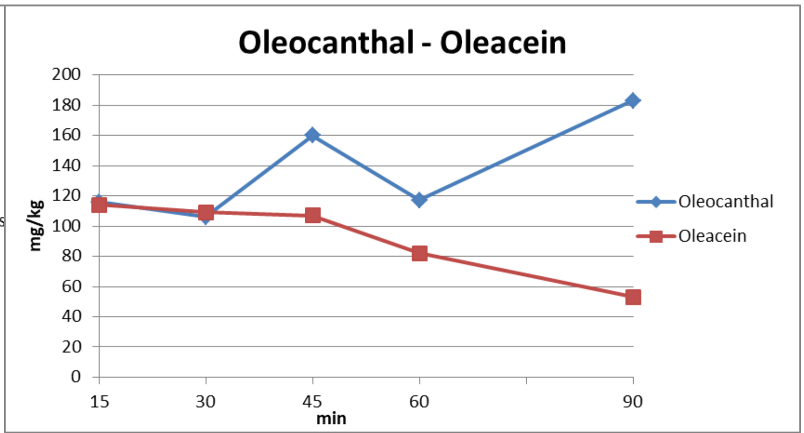


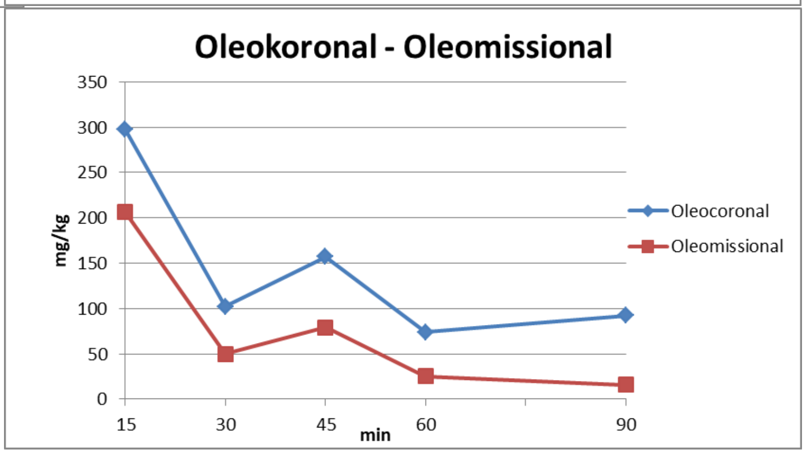
TGI53: Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time: **<u>32^{0}C</u>** - 15, 30, 45, 60 and 90min

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI53_15	116	114	120	41	297	206	894	33g
TGI53_30	106	109	81	34	102	50	482	37,2g
TGI53_45	160	107	69	43	157	79	616	45g
TGI53_60	117	82	52	36	74	25	387	35,6g
TGI53_90	183	53	38	46	92	16	427	37,6g





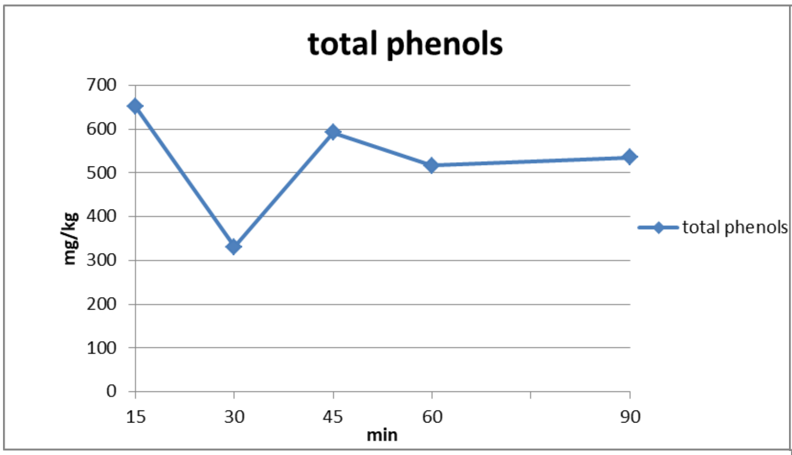


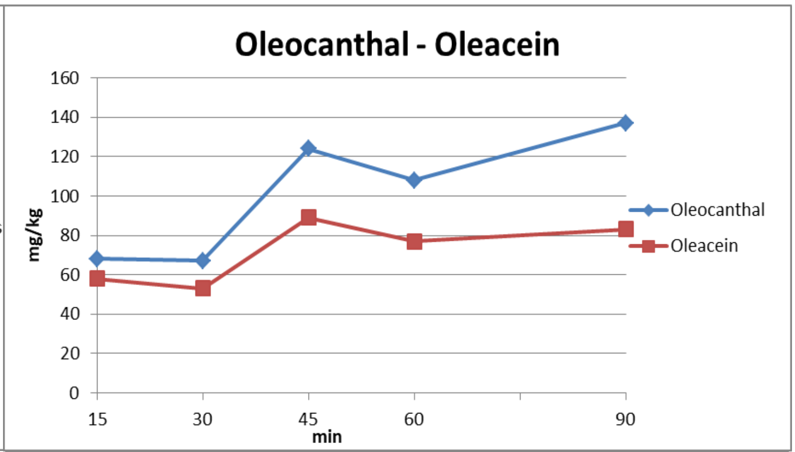
TGI54: Koroneiki Variety

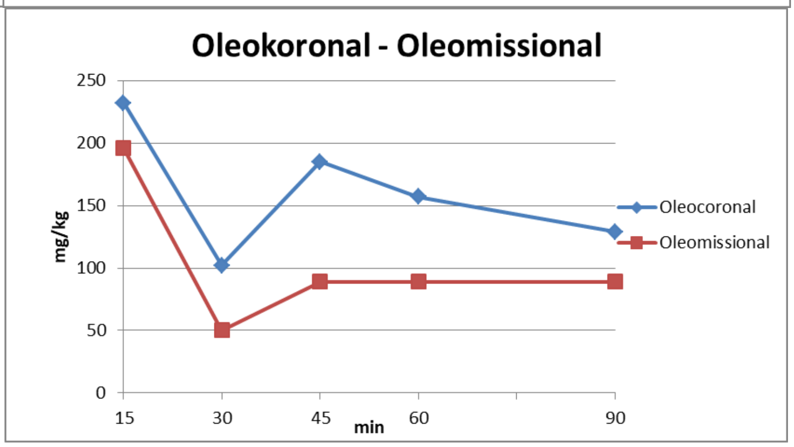
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI54_15	68	58	74	25	232	196	653	33g
TGI54_30	67	53	45	15	102	50	331	38,6g
TGI54_45	124	89	72	34	185	89	592	33,8g
TGI54_60	108	77	55	32	157	89	517	33,8g
TGI54_90	137	83	62	34	129	89	535	40,6g

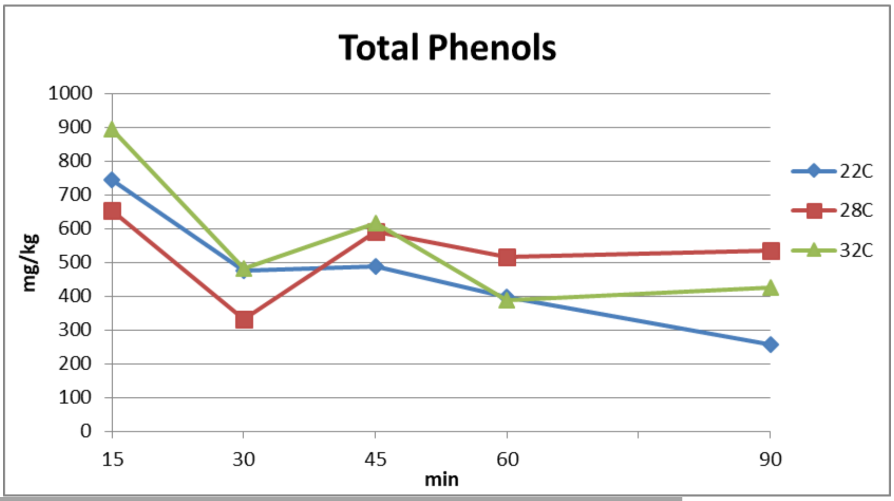
- Malaxation Temperature / malaxation time: **$\underline{28^{0}C}$** - 15, 30, 45, 60 and 90min







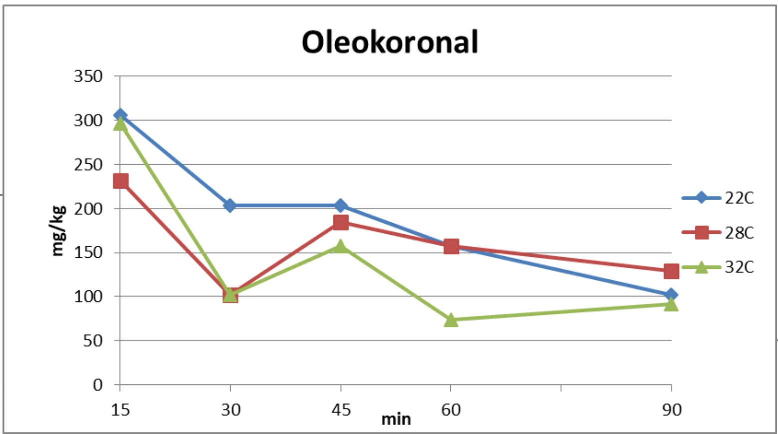
Comparative for koroneiki on 09-01-2018

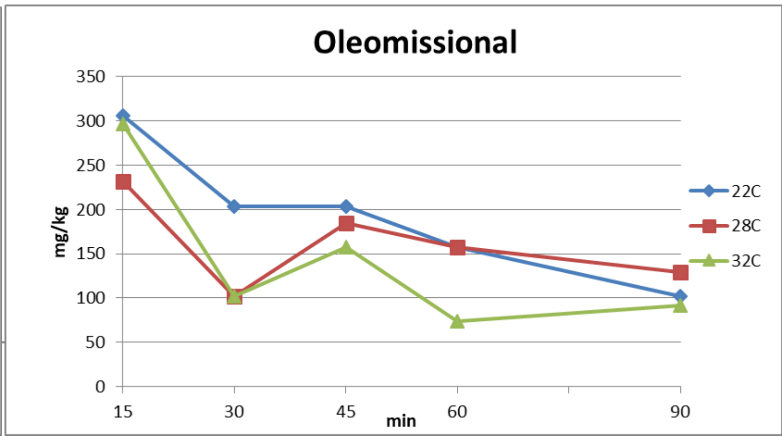


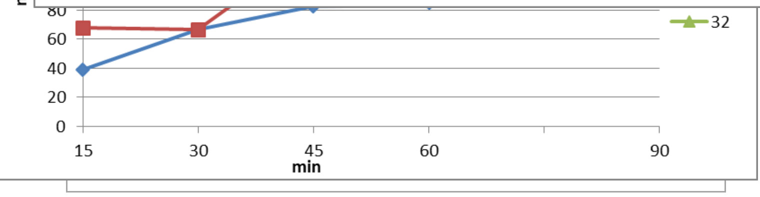
Time(min)	Total phenols		
15	745	653	894
30	476	331	482
45	487	592	616
60	398	517	387
90	256	535	427

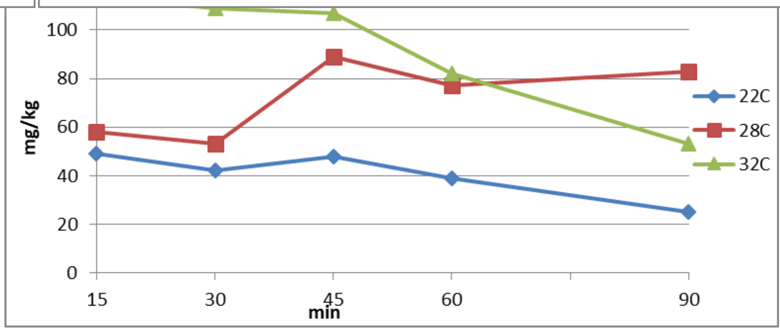
Time(min)	Oleocanthal	Oleacein	Oleocanthal	Oleacein	Oleocanthal	Oleacein
15	39	49	68	58	116	114
30	67	42	67	53	106	109
45	83	48	124	89	160	107
60	85	39	108	77	117	82
90	91	25	137	83	183	53

Time(min)	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional
15	306	284	232	196	297	206
30	204	89	102	50	102	50
45	204	79	185	89	157	79
60	157	60	157	89	74	25
90	102	0	129	89	92	16

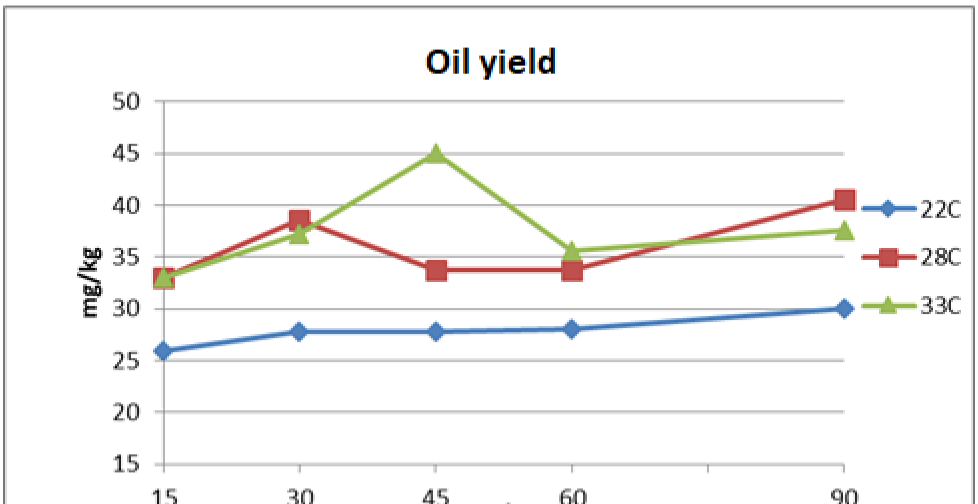








45	27.8	33.8	45
60	28	33.8	35.6
90	30	40.6	37.6

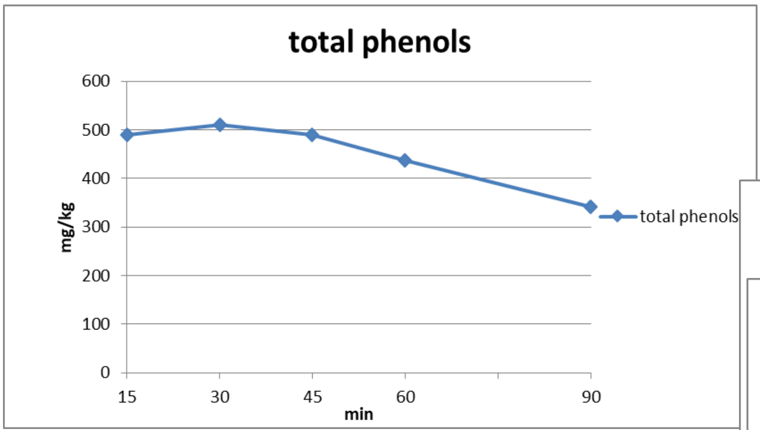


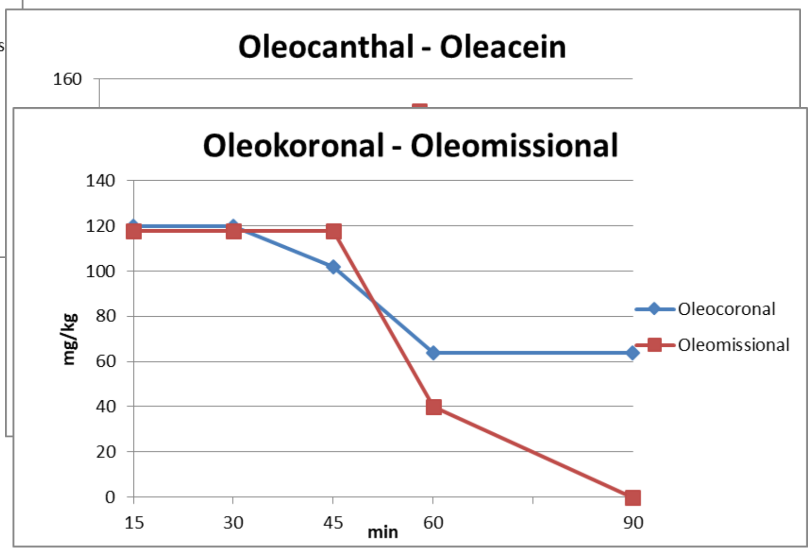
6/02/2018

TGI56: Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time: **32^0C** - 15, 30, 45, 60 and 90min

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI56_15	78	112	57	4	120	118	490	32,6g
TGI56_30	72	126	57	18	120	118	511	37,2g
TGI56_45	85	133	40	13	102	118	490	43,6g
TGI56_60	116	143	57	18	64	40	438	31,4g
TGI56_90	127	92	38	20	64	0	341	40,8g

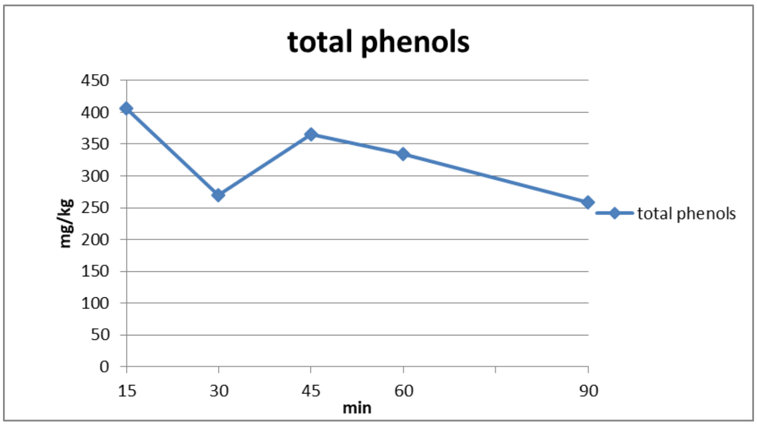


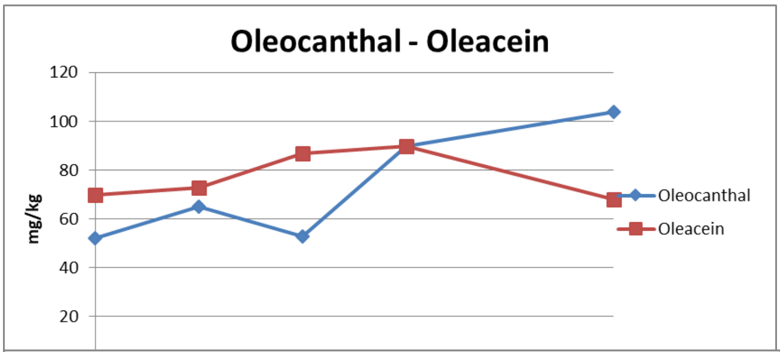


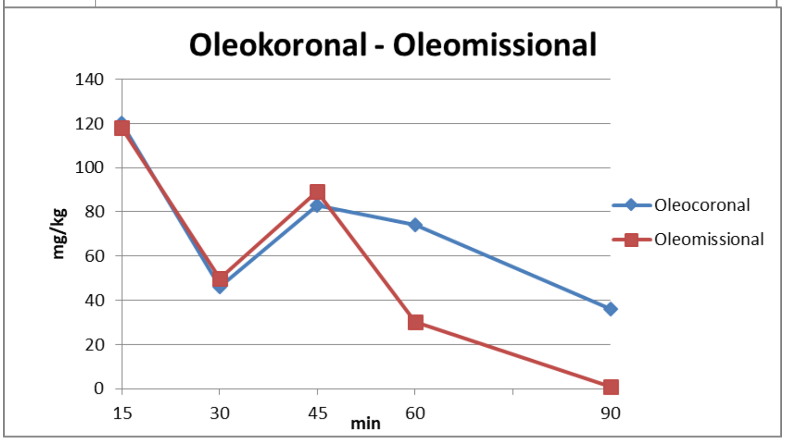
TGI57: Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time: **28^{0}C** - 15, 30, 45, 60 and 90min

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI57_15	52	70	33	13	120	118	406	32g
TGI57_30	65	73	28	9	46	50	270	33,8g
TGI57_45	53	87	42	11	83	89	365	31,6g
TGI57_60	90	90	35	15	74	30	334	36,2g
TGI57_90	104	68	28	20	36	1	258	36,4g



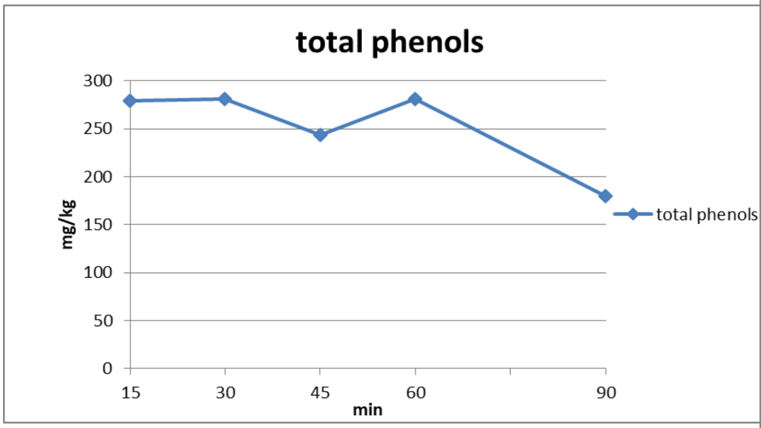


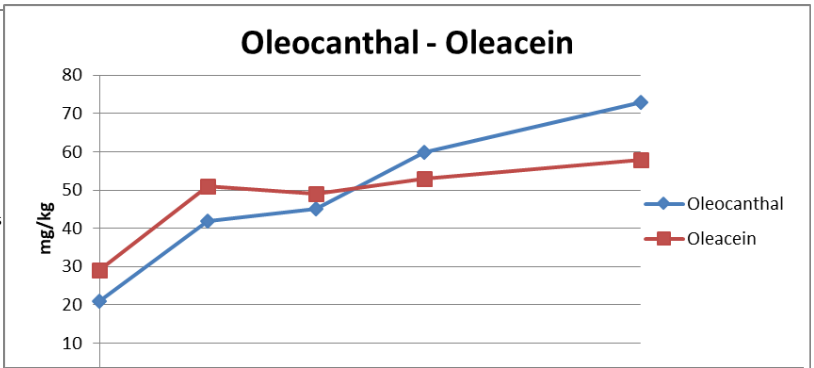


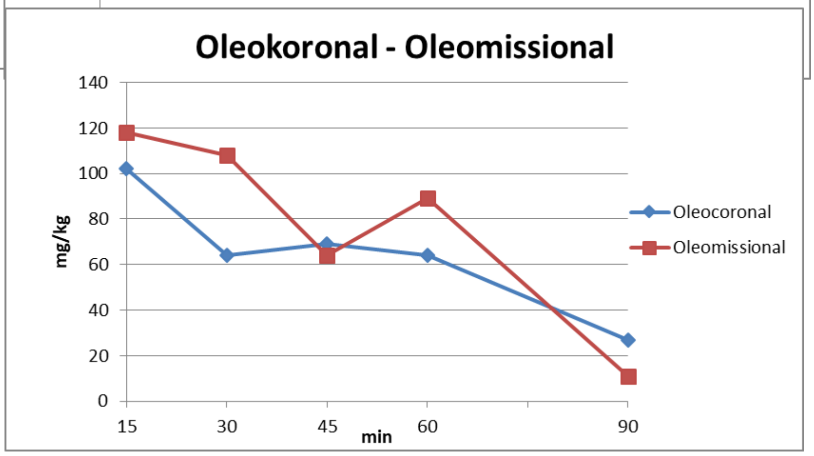
TGI58: Koroneiki Variety

- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl
- Malaxation Temperature / malaxation time: **<u>22^{0}C</u>** - 15, 30, 45, 60 and 90min

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI58_15	21	29	11	0	102	118	280	29,4g
TGI58_30	42	51	16	0	64	108	281	33,2g
TGI58_45	45	49	16	0	69	64	244	37,2g
TGI58_60	60	53	16	0	64	89	281	40,8g
TGI58_90	73	58	11	0	27	11	180	36,6g

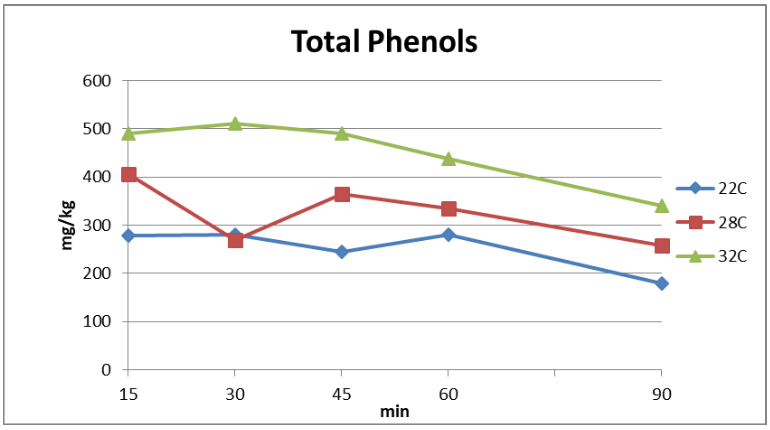




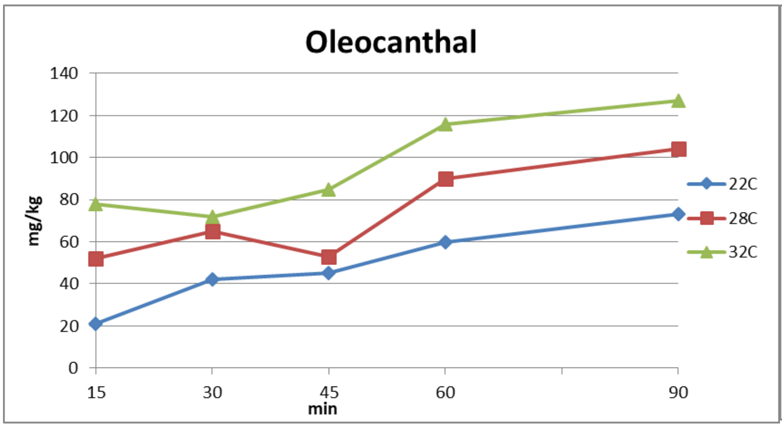


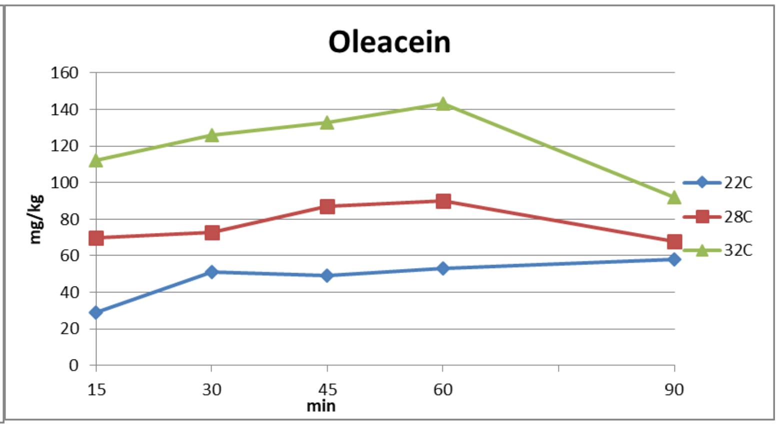
Comparative for koroneiki on 05-02-2018

Time(min)	Total phenols		
15	279	406	490
30	281	270	511
45	244	365	490
60	281	334	438
90	180	258	341

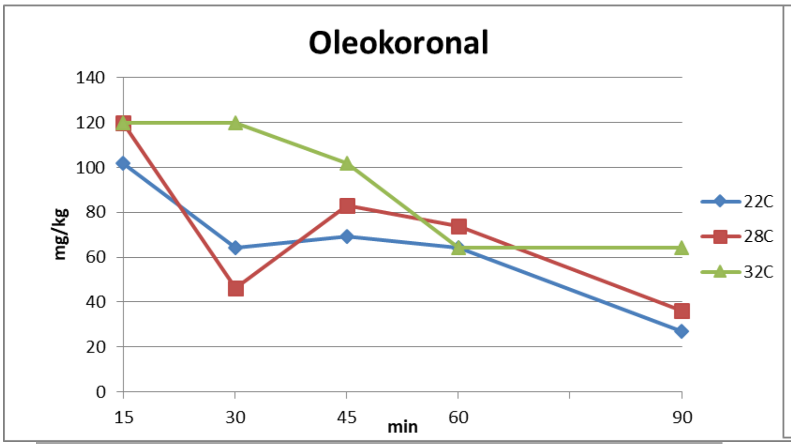


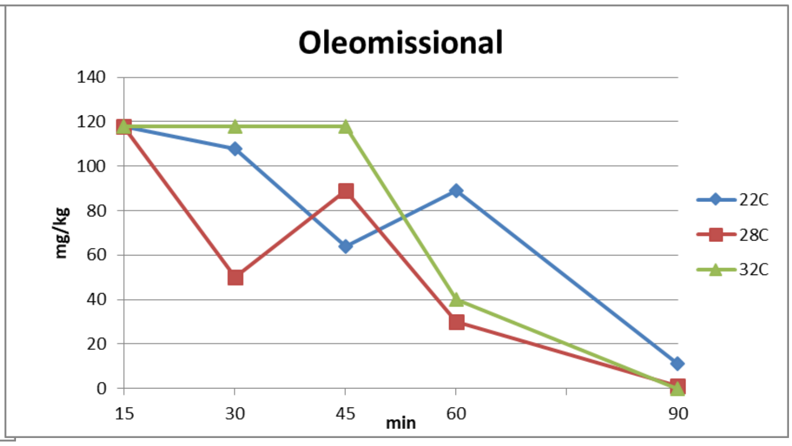
Time(min)	Oleocanthal	Oleacein	Oleocanthal	Oleacein	Oleocanthal	Oleacein
15	21	29	52	70	78	112
30	42	51	65	73	72	126
45	45	49	53	87	85	133
60	60	53	90	90	116	143
90	73	58	104	68	127	92



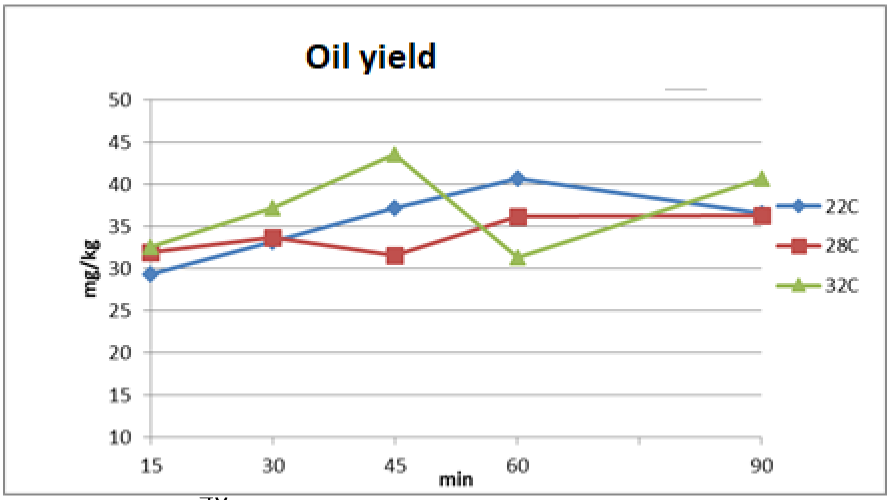


Time(min)	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional	Oleokoronal	Oleomissional
15	102	118	120	118	120	118
30	64	108	46	50	120	118
45	69	64	83	89	102	118
60	64	89	74	30	64	40
90	27	11	36	1	64	0





15	**29.4**	**32**	**32.6**
30	**33.2**	**33.8**	**37.2**
45	**37.2**	**31.6**	**43.6**
60	**40.8**	**36.2**	**31.4**
90	**36.6**	**36.4**	**40.8**

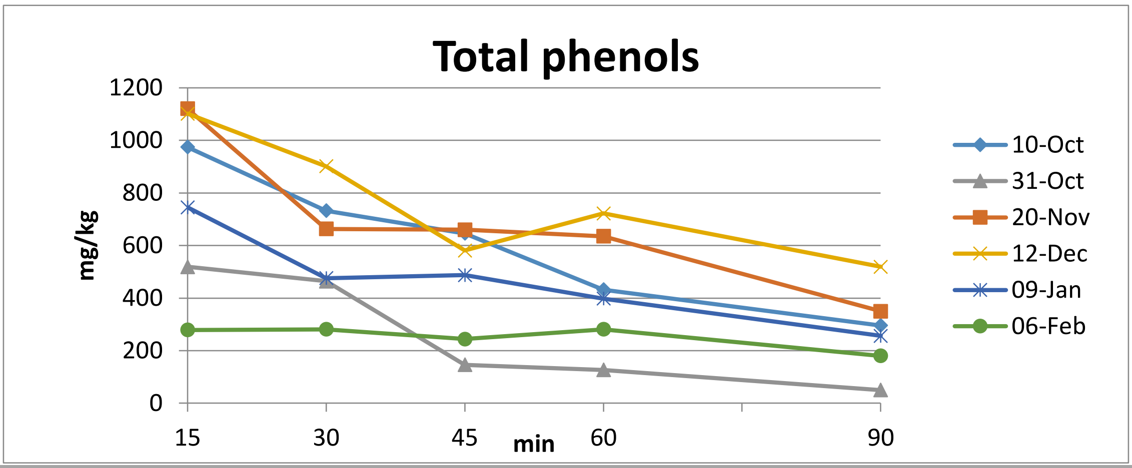


Project co-financed by the European Regional Development Fund

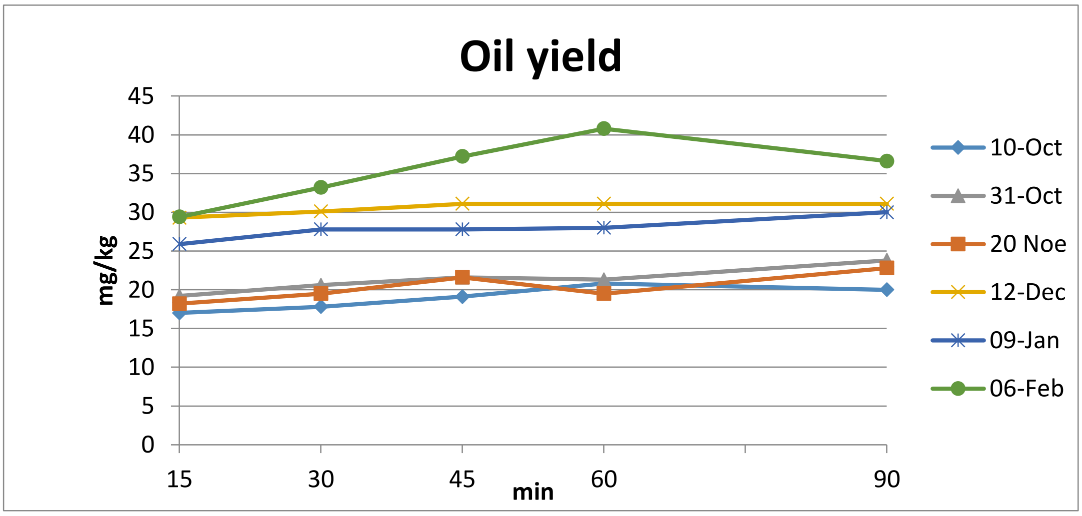
Conclusions for Koroneiki

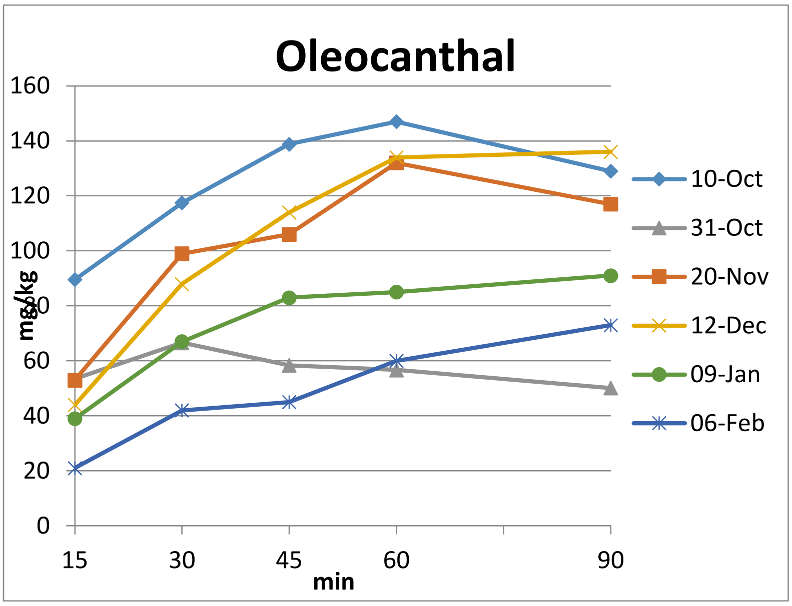
<u>Low temperature</u>

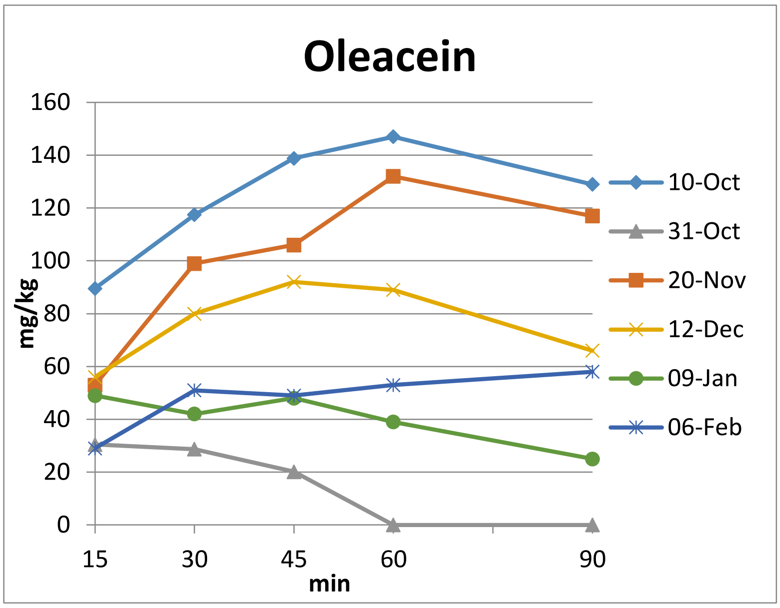
Time	Total phenols					
	10 Oct	31 Oct	20 Nov	12 Dec	09 Jan	06 Feb
15	974	519	1120	1101	745	279
30	732	464	663	901	476	281
45	646	146	660	581	487	244
60	432	127	635	722	398	281
90	296	50	350	519	256	180

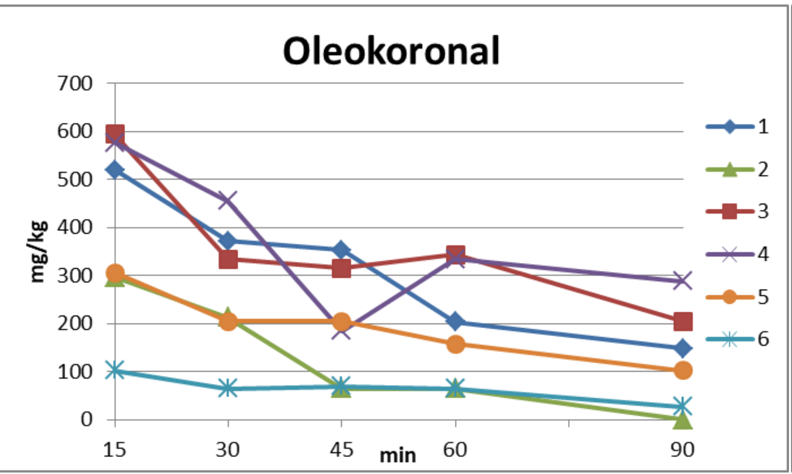


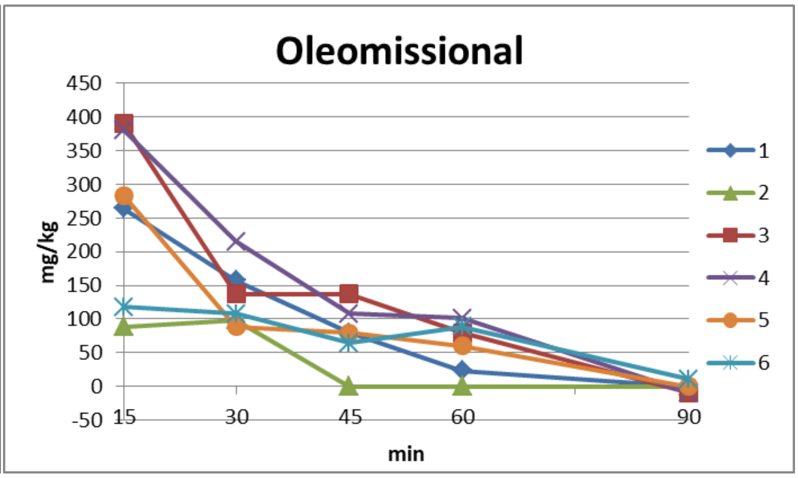
	10 Oct	31 Oct	20 Nov	12 Dec	09 Jan	06 Feb
15	**17**	**19,2**	**18,2**	**29,3**	**25,9**	**29,4**
30	**17,75**	**20,6**	**19,5**	**30,1**	**27,8**	**33,2**
45	**19,1**	**21,6**	**21,6**	**31,1**	**27,8**	**37,2**
60	**20,8**	**21,3**	**19,5**	**31,1**	**28**	**40,8**
90	**20**	**23,8**	**22,8**	**31,1**	**30**	**36,6**




Oleocanthal
mg/kg
min
10-Oct
31-Oct
20-Nov
12-Dec
09-Jan
06-Feb

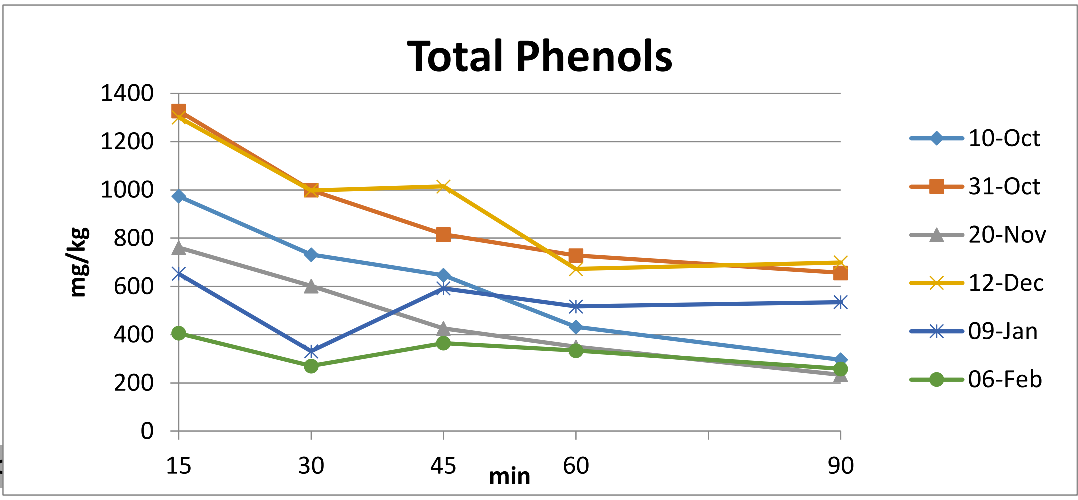

Oleacein
mg/kg
min
10-Oct
31-Oct
20-Nov
12-Dec
09-Jan
06-Feb


Oleokoronal
mg/kg
min

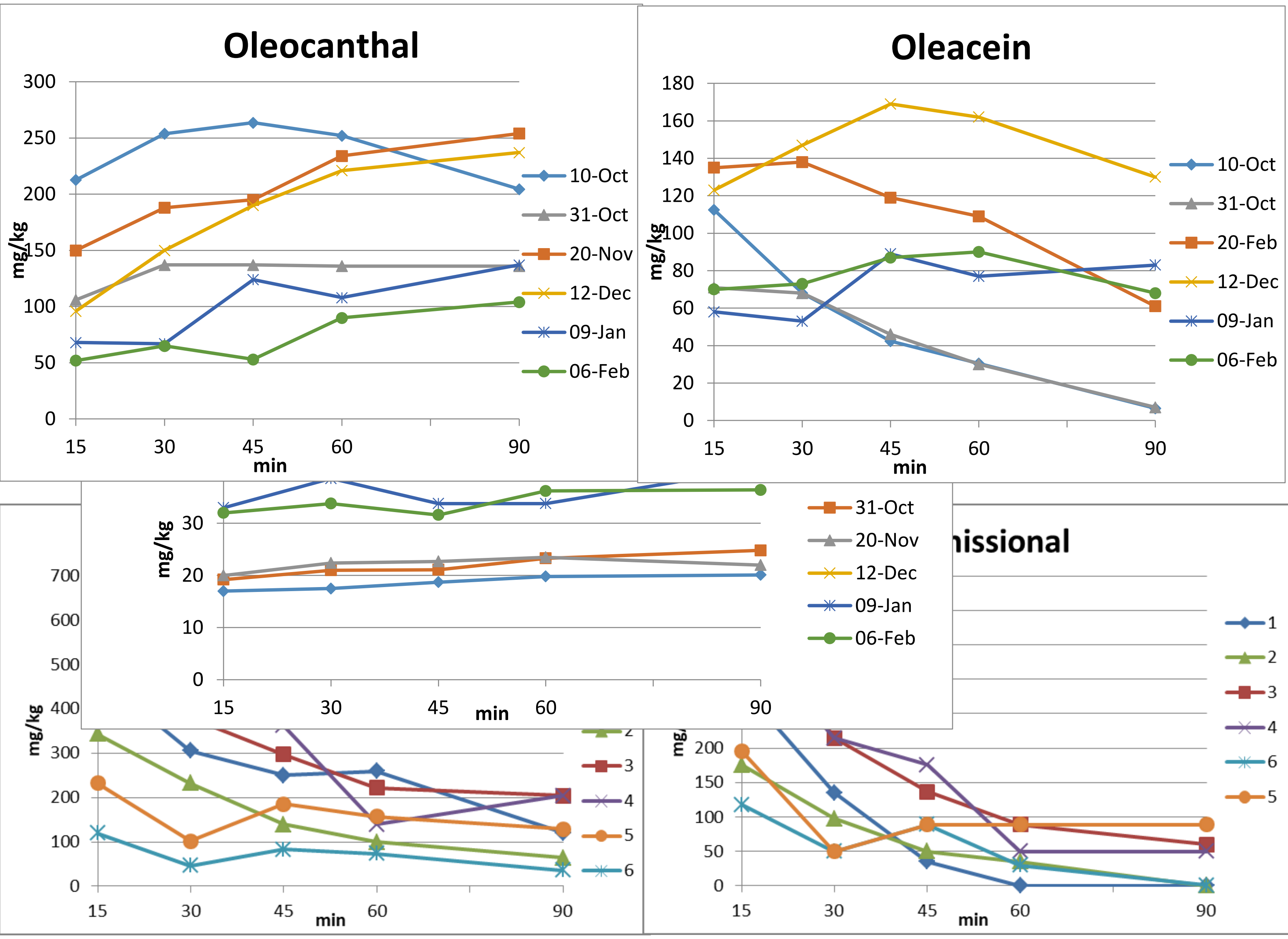

Oleomissional
mg/kg
min

Time	10 Oct	31 Oct	20 Nov	12 Dec	09 Jan	06 Feb
15	974	1327	761	1301	653	406
30	732	999	602	998	331	270
45	646	815	426	1014	592	365
60	432	728	350	672	517	334
90	296	656	234	699	535	258

Medium temperature

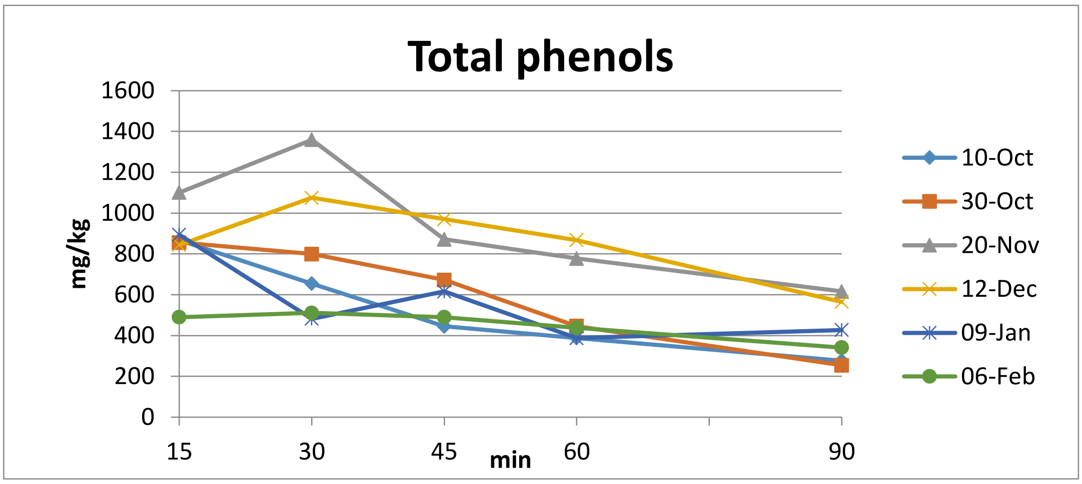


Xp						06 Feb
15	**17**	**19,2**	**20**		**33**	**32**
30	**17,5**	**21**	**22,4**		**38,6**	**33,8**
45	**18,7**	**21,1**	**22,7**		**33,8**	**31,6**
60	**19,8**	**23,3**	**23,5**		**33,8**	**36,2**
90	**20,1**	**24,8**	22		**40,6**	**36,4**

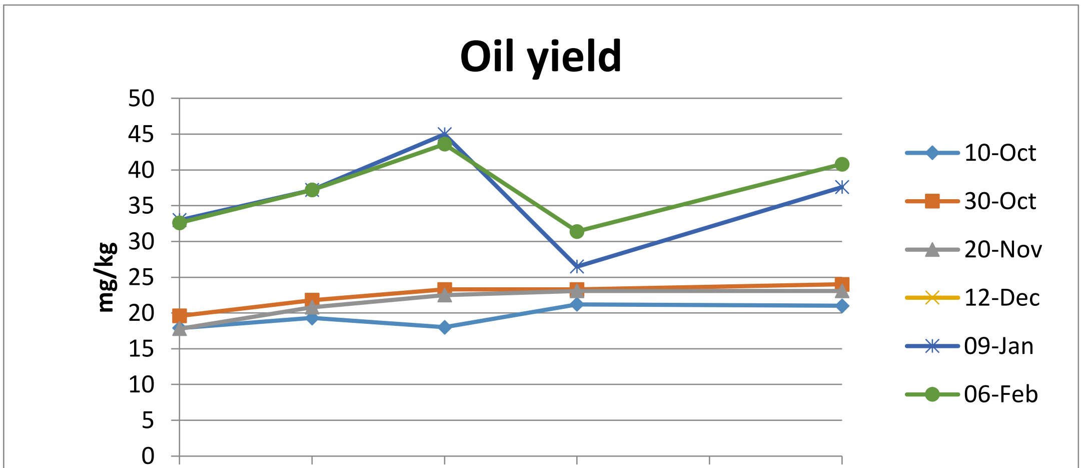

Oleocanthal
mg/kg
min
10-Oct
31-Oct
20-Nov
12-Dec
09-Jan
06-Feb
Oleacein
mg/kg
min
10-Oct
31-Oct
20-Feb
12-Dec
09-Jan
06-Feb
mg/kg
min
31-Oct
20-Nov
12-Dec
09-Jan
06-Feb
mg/kg
min
2
3
4
5
6
nissional
min
1
2
3
4
6
5

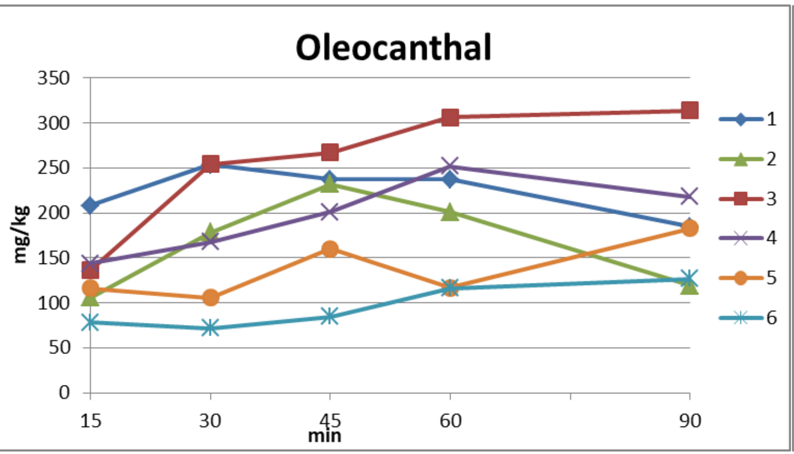
High temperature

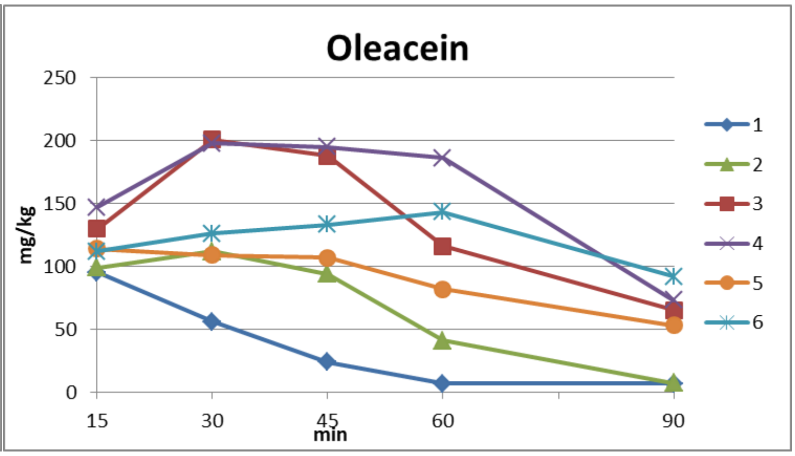
Time			Total phenols			
	10 Oct	31 Oct	20 Nov	12 Dec	09 Jan	06 Feb
15	867	856	1101	844	894	490
30	654	799	1359	1075	482	511
45	445	672	871	970	616	490
60	388	446	777	867	387	438
90	277	254	616	565	427	341

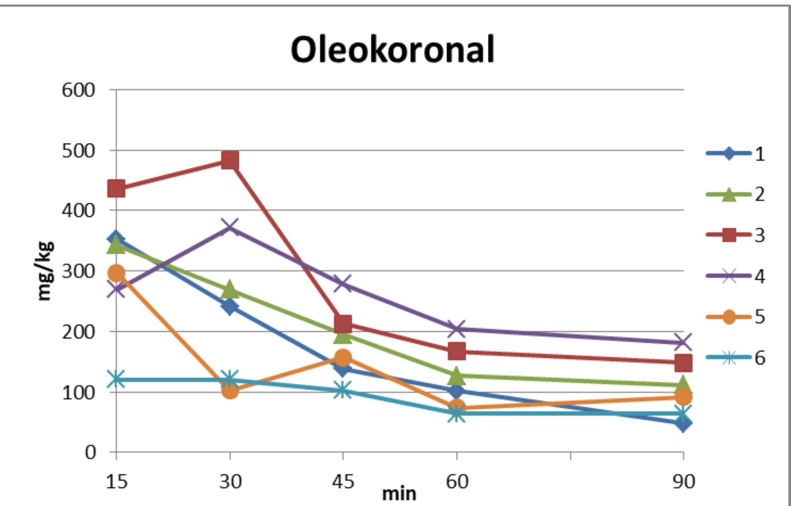


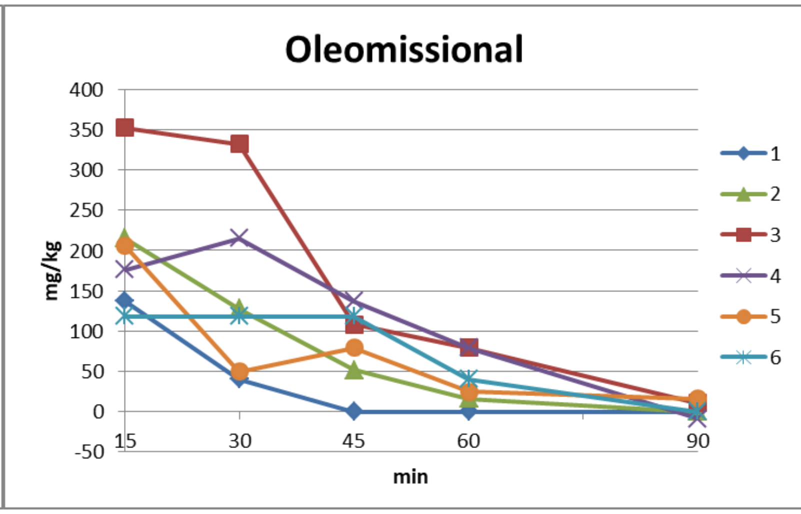
Χρόνος			Oil yield			
	10 Oct	31 Oct	20 Nov	12Dec	09 Jan	06 Feb
15	**17,9**	**19,6**	**17,8**		**33**	**32,6**
30	**19,3**	**21,8**	**20,8**		**37,2**	**37,2**
45	**18**	**23,3**	**22,5**		**45**	**43,6**
60	**21,2**	**23,3**	**23,1**		**35,6**	**31,4**
90	**21**	**24**	**23,1**		**37,6**	**40,8**











1	10 Oct
2	31 Oct
3	20 Nov
4	12 Dec
5	09 Jan
6	06 Feb

Experiments with Athenolia variety

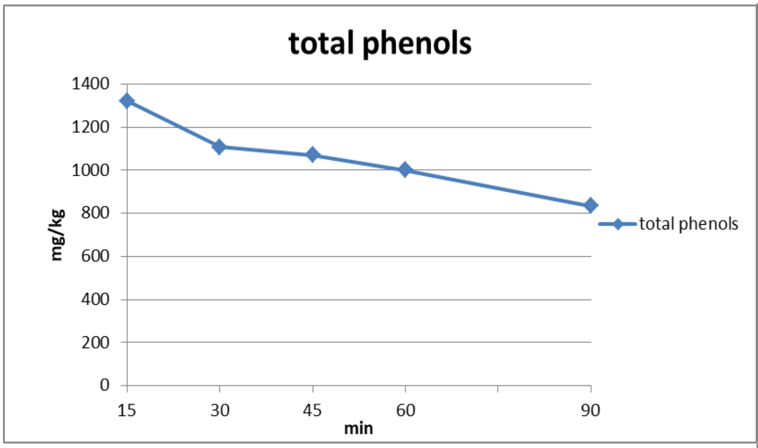
20/11/2017

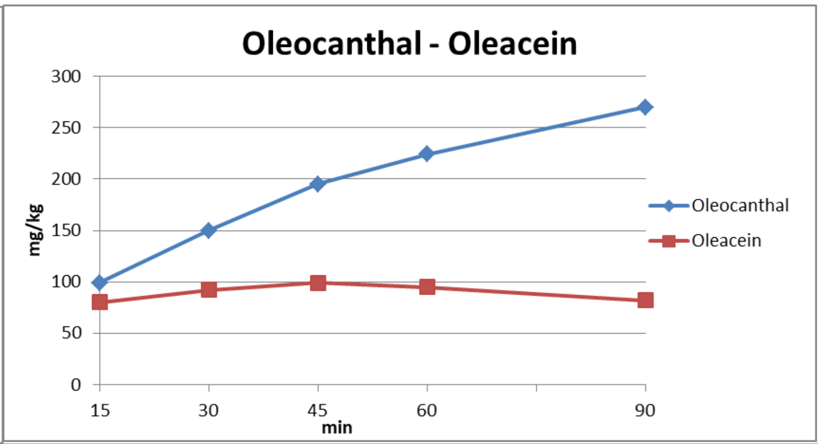
<u>TGI43: Athenolia</u>

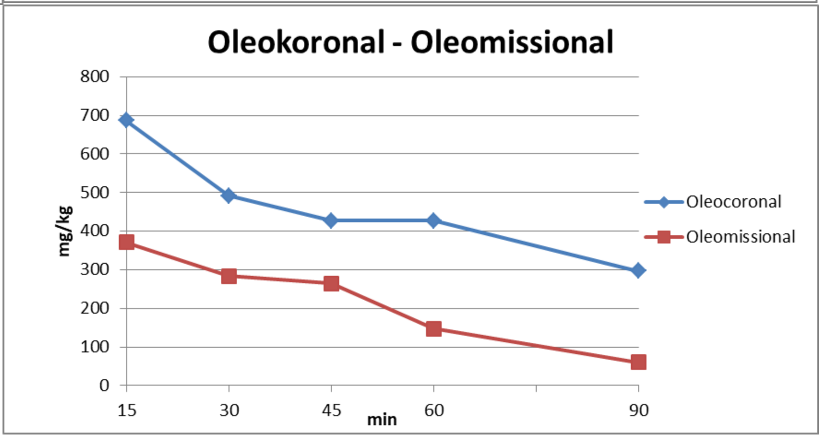
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI43_15	99	80	42	41	687	371	1322	36,7g
TGI43_30	150	92	47	43	492	284	1109	39,6g
TGI43_45	195	99	33	53	427	264	1070	39,5g
TGI43_60	224	95	50	57	427	147	1001	43,2g
TGI43_90	270	82	50	76	297	60	834	43g

- Malaxation Temperature / malaxation time: **<u>28^{0}C</u>** - 15, 30, 45, 60 and 90min







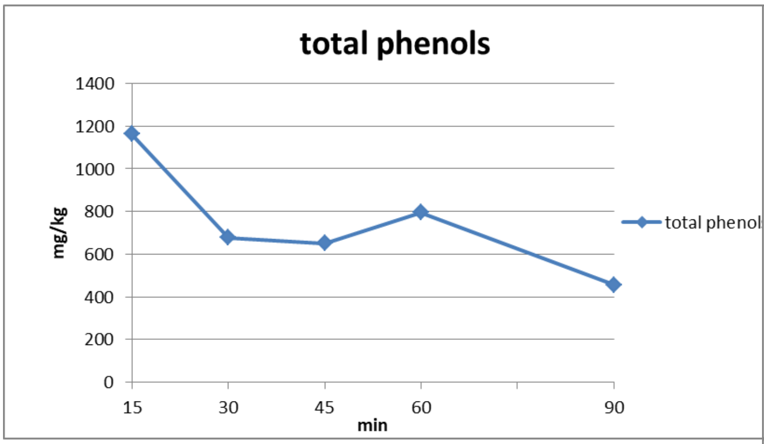
12/12/2017

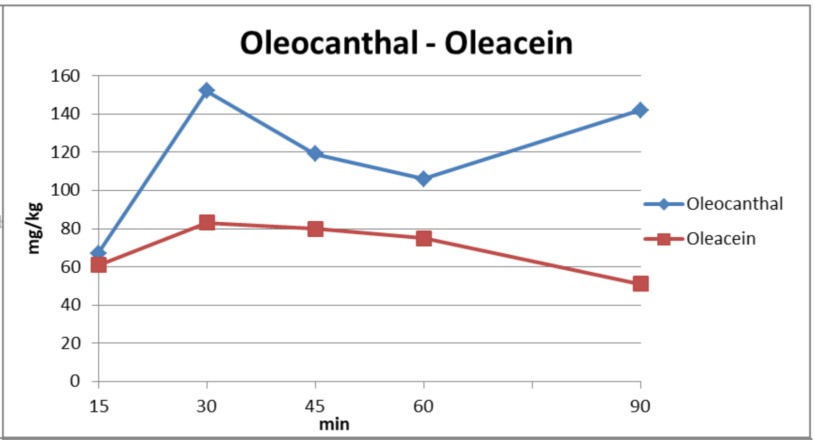
TGI51Athenolia

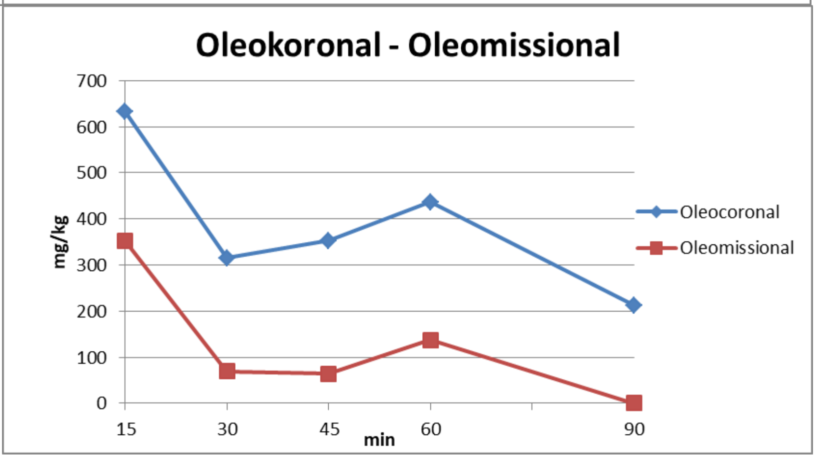
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI51_15	67	61	30	20	632	352	1162	g
TGI51_30	152	83	35	22	315	69	678	g
TGI51_45	119	80	18	18	353	64	652	g
TGI51_60	106	75	18	22	436	137	795	g
TGI51_90	142	51	18	32	213	0	456	g

- Malaxation Temperature / malaxation time: **<u>28^{0}C</u>** - 15, 30, 45, 60 and 90min







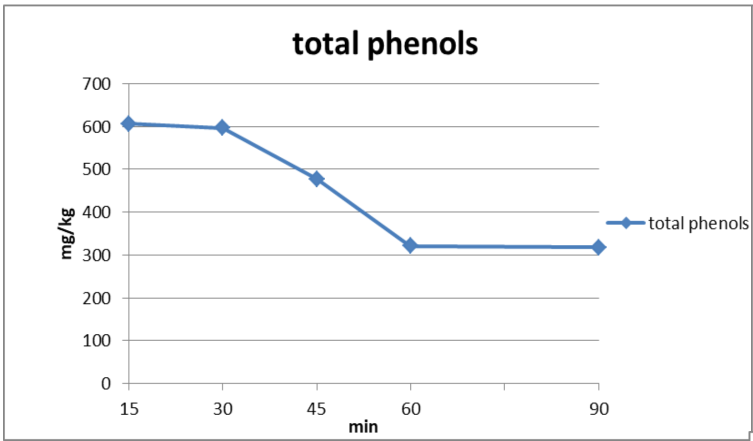
09/01/2018

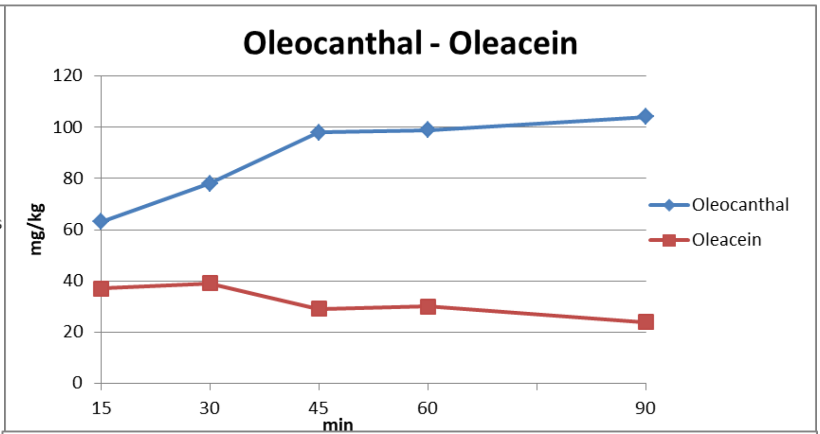
TGI55Athenolia

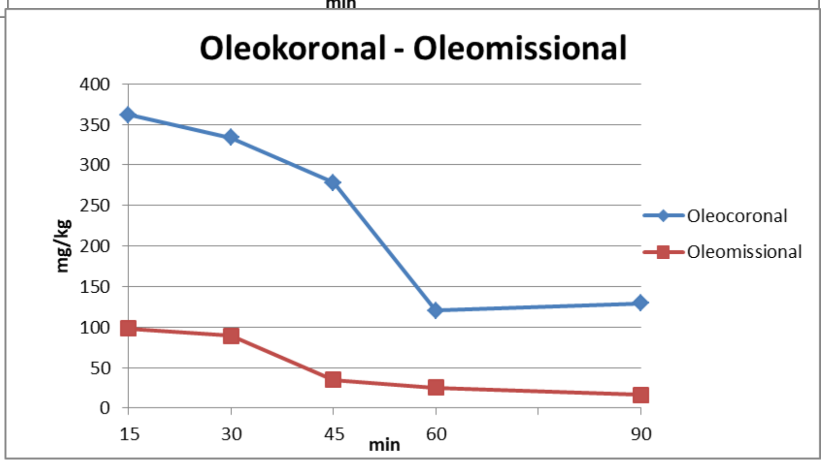
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI55_15	63	37	25	20	362	98	607	45,8g
TGI55_30	78	39	30	27	334	89	597	43,2g
TGI55_45	98	29	16	22	278	35	478	51,2g
TGI55_60	99	30	18	27	120	25	321	48g
TGI55_90	104	24	18	27	129	16	318	49,6g

- Malaxation Temperature / malaxation time: **<u>28^{0}C</u>** - 15, 30, 45, 60 and 90min





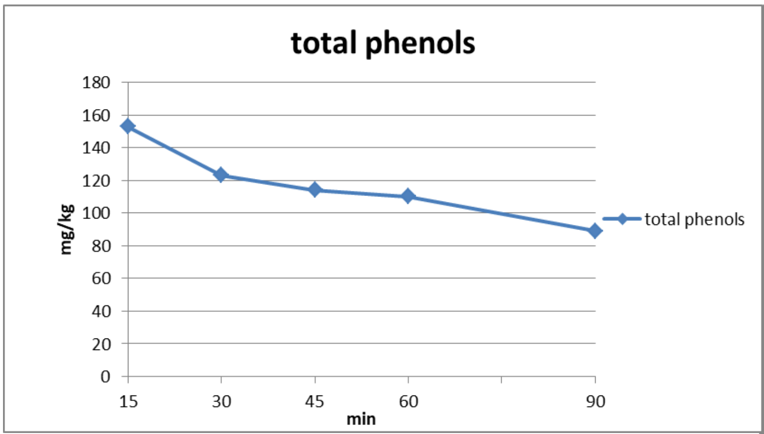


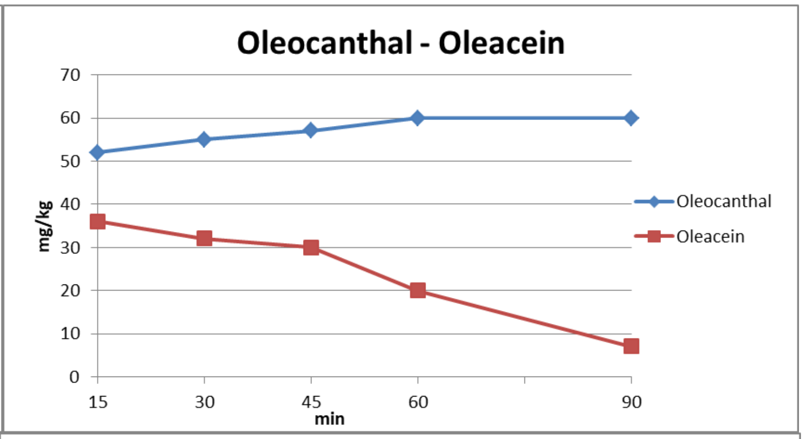
TGI59: Athenolia

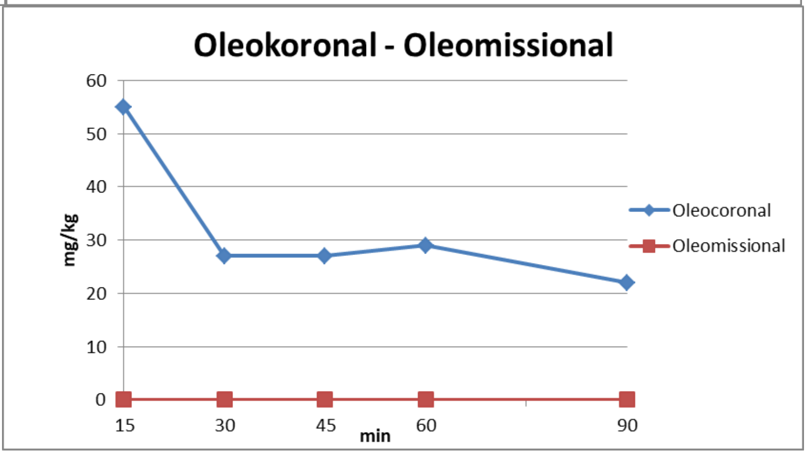
- Olive fruit sample (1,5Kg) was crashed and after 5 min was transferred in stainless bowl

	Oleocanthal	Oleacein	Oleokoronal	Oleomissional	Ligstroside A.	Oleuropein A.	Total Phenols	G oil\200g paste
TGI59_15	52	36	11	0	55	0	153	g
TGI59_30	55	32	0	9	27	0	123	g
TGI59_45	57	30	0	0	27	0	114	g
TGI59_60	60	20	0	0	29	0	110	g
TGI59_90	60	7	0	0	22	0	89	g

- Malaxation Temperature / malaxation time: **<u>28^{0}C</u>** - 15, 30, 45, 60 and 90min

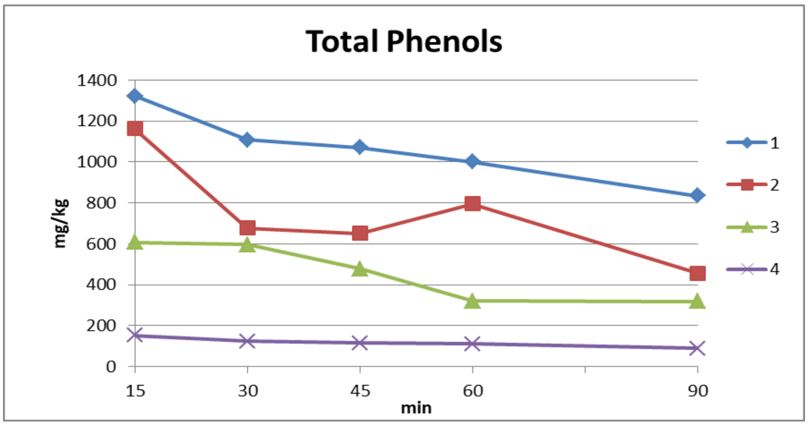


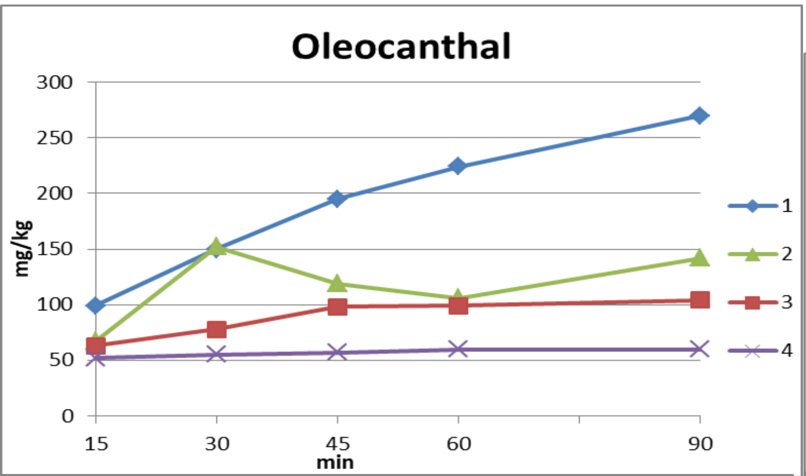


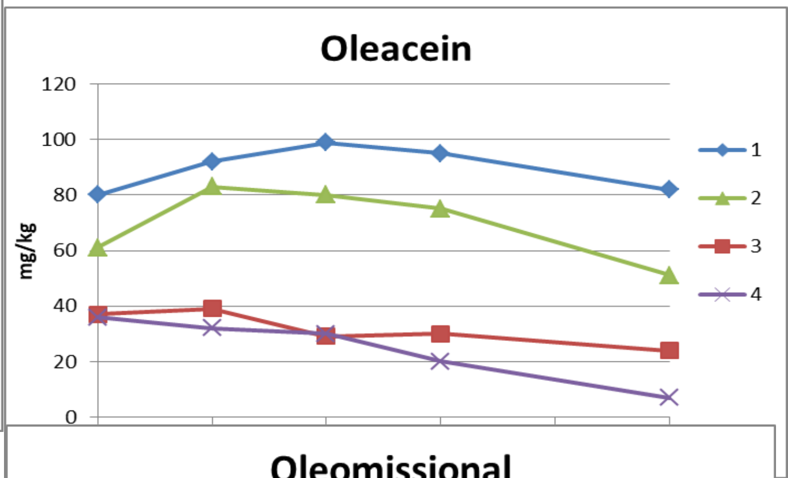


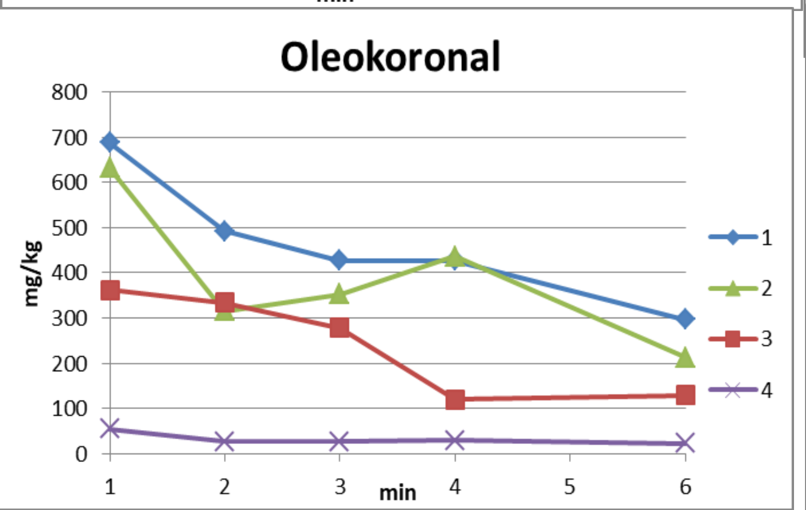
__Conclusions for Athenolia__ –

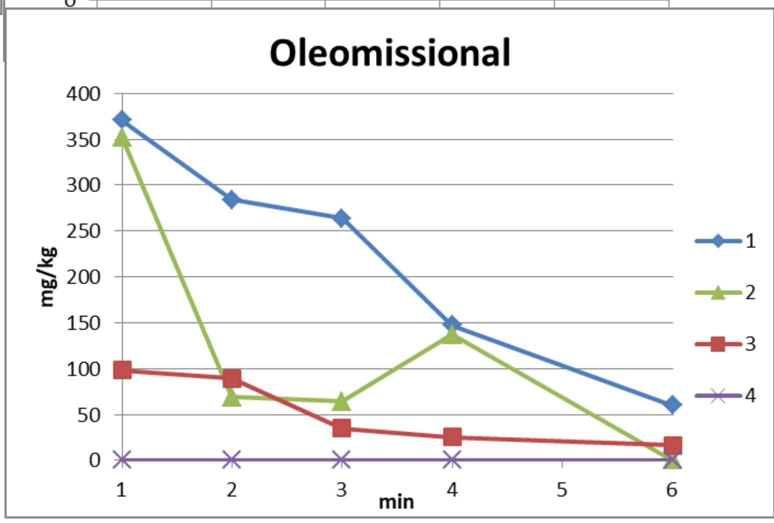
Χρόνος	1	2	3	4
15	1322	1162	607	153
30	1109	678	597	123
45	1070	652	478	114
60	1001	795	321	110
90	834	456	318	89











Agronomic factors database for Spanish olive oils

General results

Total number of analyzed samples: 1222 samples

Distribution of results for the complete cohort:

COMPOUND	MINIMUM	MAXIMUM	AVERAGE	SD
Hydroxytyrosol	0,0	22,2	1,7	1,8
Tyrosol	0,0	0,0	0,0	0,0
Oleacein	0,0	1829,9	303,9	211,4
Oleocanthal	0,0	270,2	55,6	46,8
Oleuropein aglycone	0,0	894,8	218,9	130,9
Oleomissional	0,0	367,1	45,5	46,6
Ligstroside aglycone	0,0	511,2	85,5	72,0
Oleokoronal	0,0	636,7	89,1	83,4
Apigenin	0,0	6,5	0,8	0,6
Luteolin	0,0	6,3	1,3	1,0
Hidroxy derivatives	7,0	2967,6	570,1	312,3
Tyrosol derivatives	2,0	1166,3	230,2	163,9
Total phenolic content	13,0	3734,0	800,4	424,5
Total analyzed compounds	17,2	3737,1	802,5	424,3
Daily intake	0,3	74,7	16,0	8,5

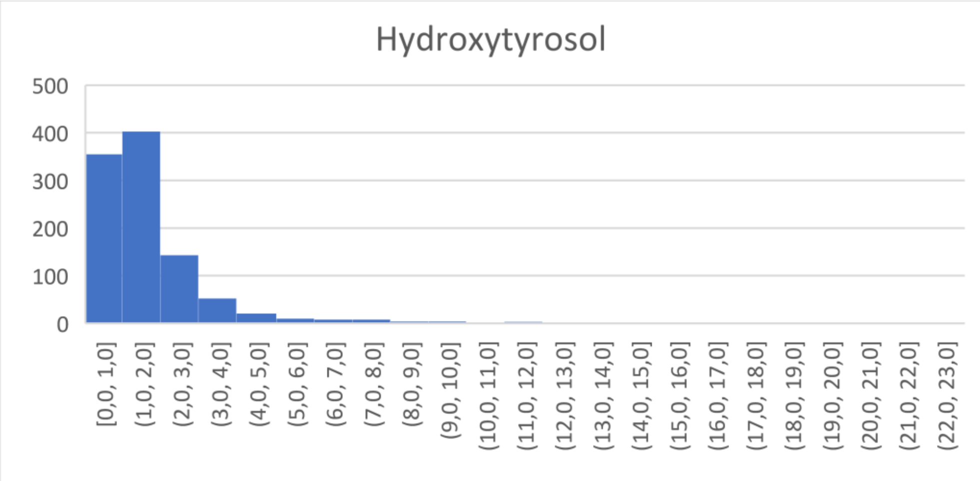
Table 1. Summary of the results on concentrations provided by the analysis of samples collected in Spain in the 2017/2018 season. Concentrations are expressed as mg/kg, except for the “Daily intake” parameter, expressed as mg.

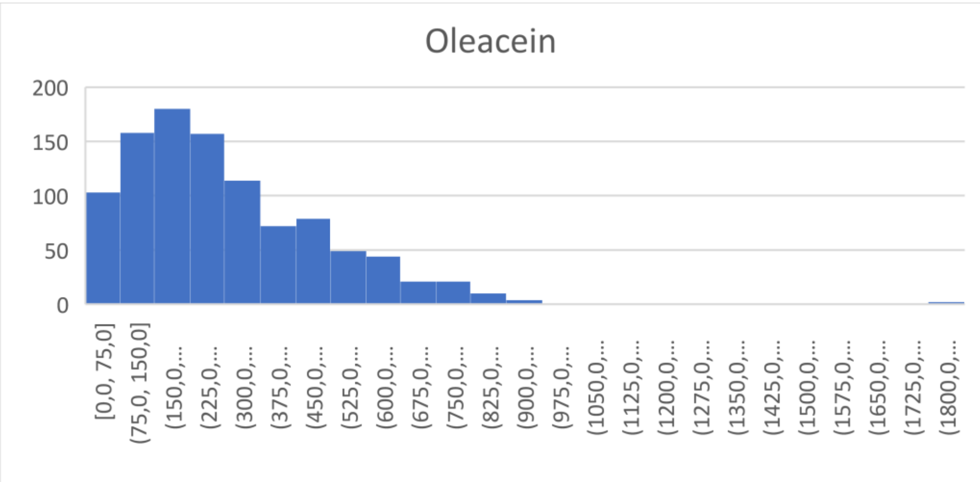
Being:

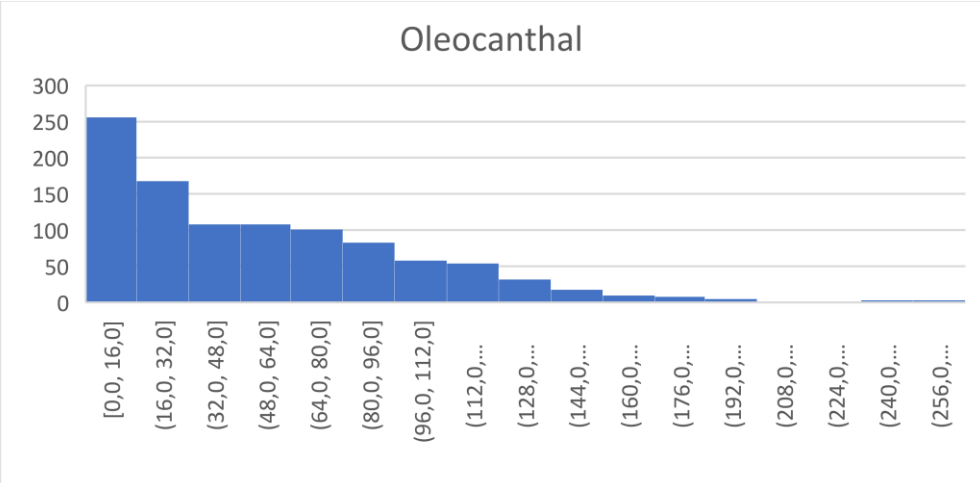
- Total phenolic content: the sum of concentrations of the phenols included in the health claim of the Regulation EU432/2012 and recognized by EFSA.
- Total analyzed compounds: the sum of concentration of all phenols included in Table 1. The main difference as compared to the total phenolic content is the addition of flavonoids, apigenin and luteolin.
- Daily intake: the amount of phenols included in the health claim that are taken by ingestion of 20 g of olive oil.

According to these results it could be concluded that 95% of the samples reported a daily intake amount of 5 mg, which means that they could take benefits from the health claim from a commercial point of view. Additionally, 75% of the samples reported a daily intake amount above 10 mg, which means that three out of four samples were able to provide twice the cut-off amount reported by the EFSA, which is a really significant aspect.

The distribution of concentration for the different compounds is included below:






Oleocanthal
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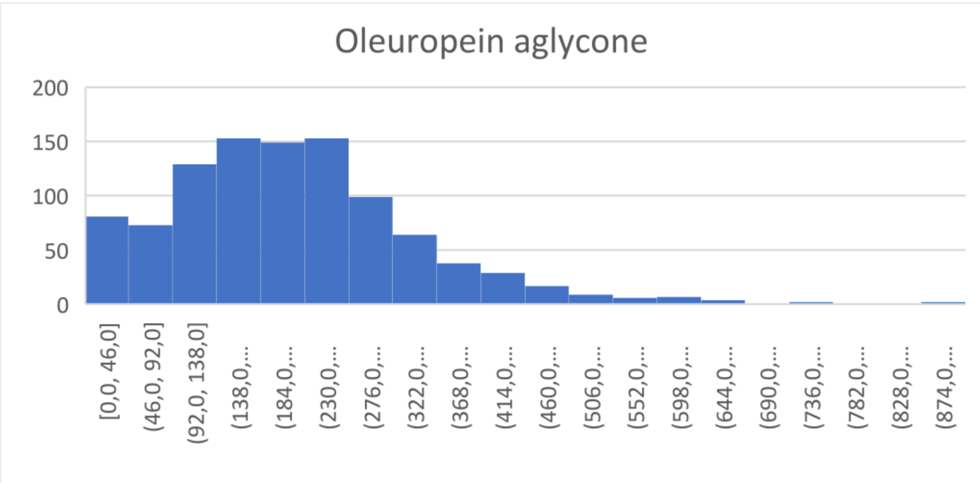
Oleuropein aglycone
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150
100
50
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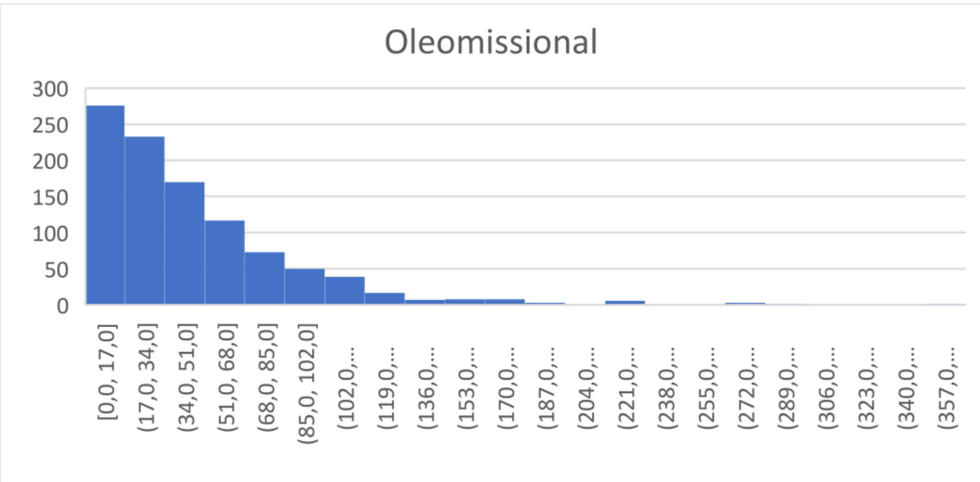
Oleomissional
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200
150
100
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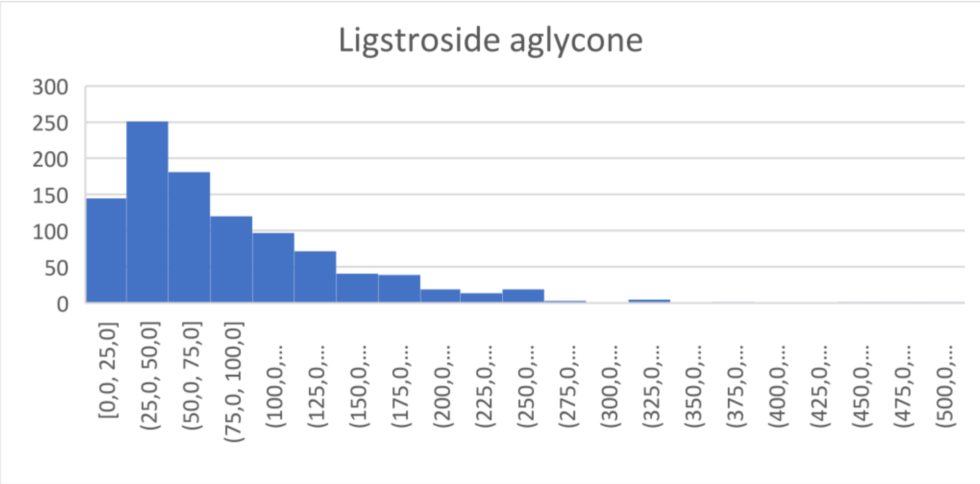
Ligstroside aglycone
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250
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100
50
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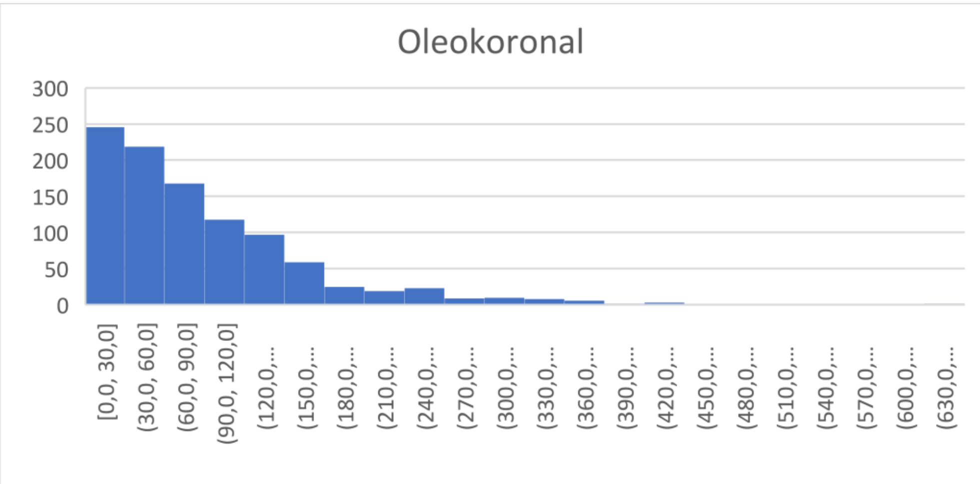
Oleokoronal
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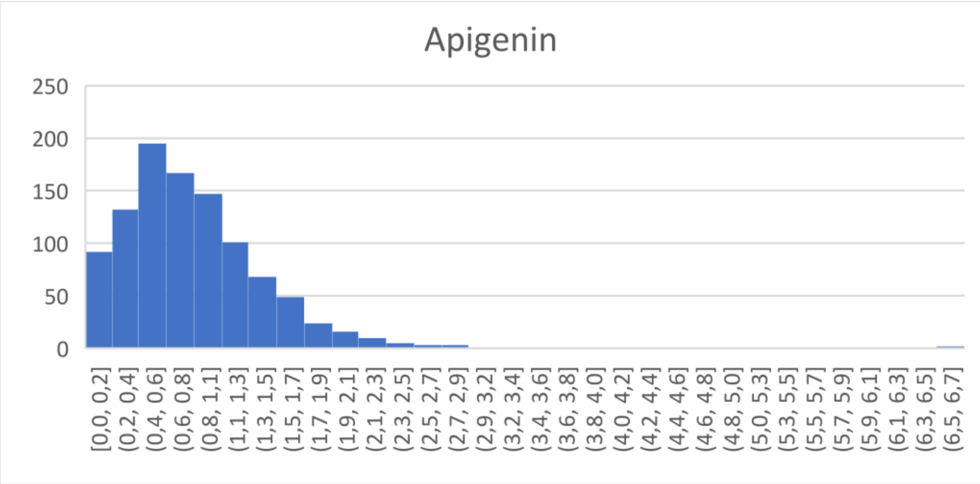
Apigenin
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(2,3, 2,5]
(2,5, 2,7]
(2,7, 2,9]
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(3,2, 3,4]
(3,4, 3,6]
(3,6, 3,8]
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(4,4, 4,6]
(4,6, 4,8]
(4,8, 5,0]
(5,0, 5,3]
(5,3, 5,5]
(5,5, 5,7]
(5,7, 5,9]
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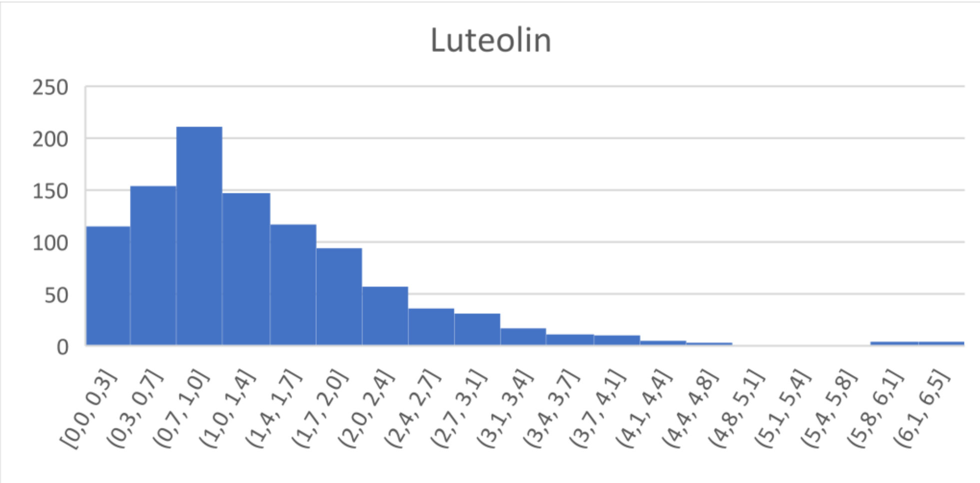
(6,5, 6,7]


Luteolin
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150
100
50
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(3,7, 4,1]
(4,1, 4,4]
(4,4, 4,8]
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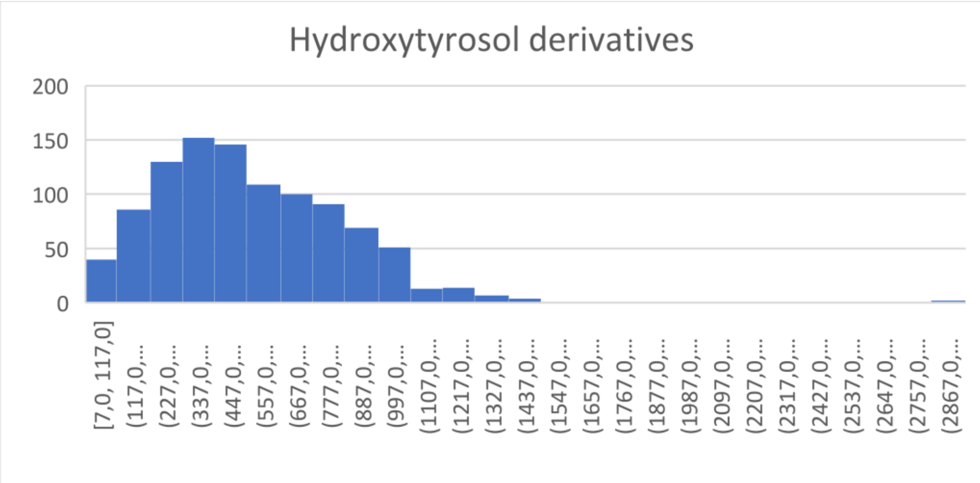
Hydroxytyrosol derivatives
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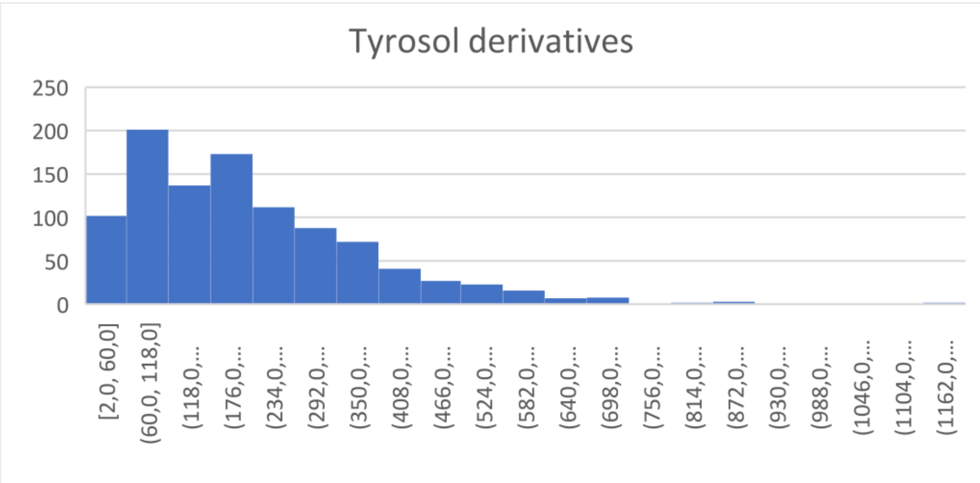
Tyrosol derivatives
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50
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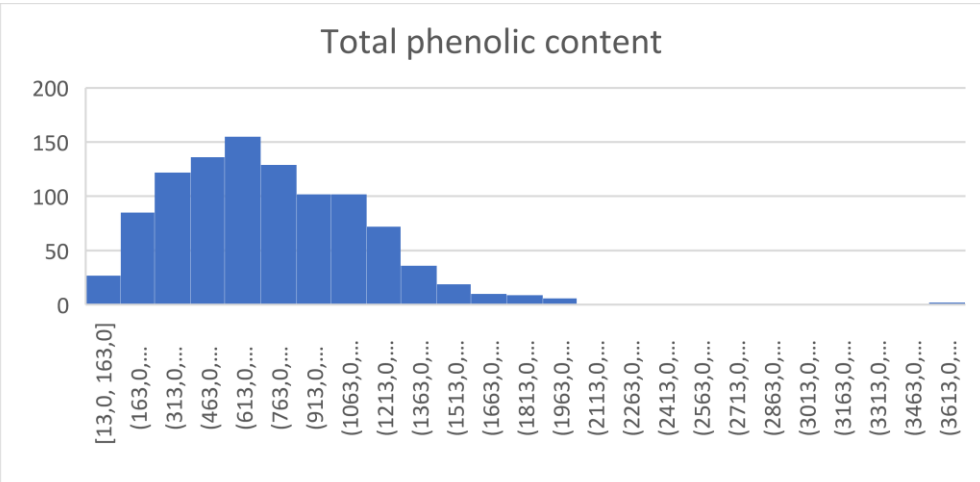
Total phenolic content
200
150
100
50
0
[13,0, 163,0]
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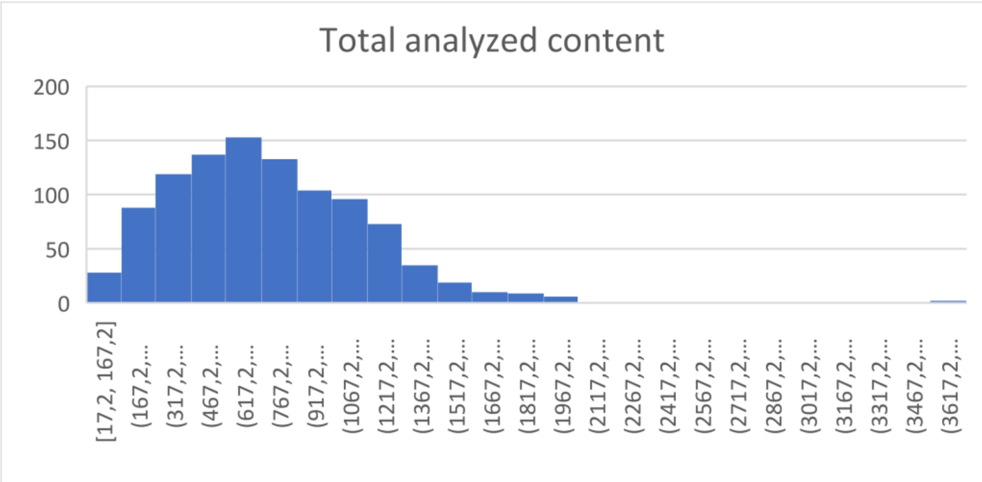


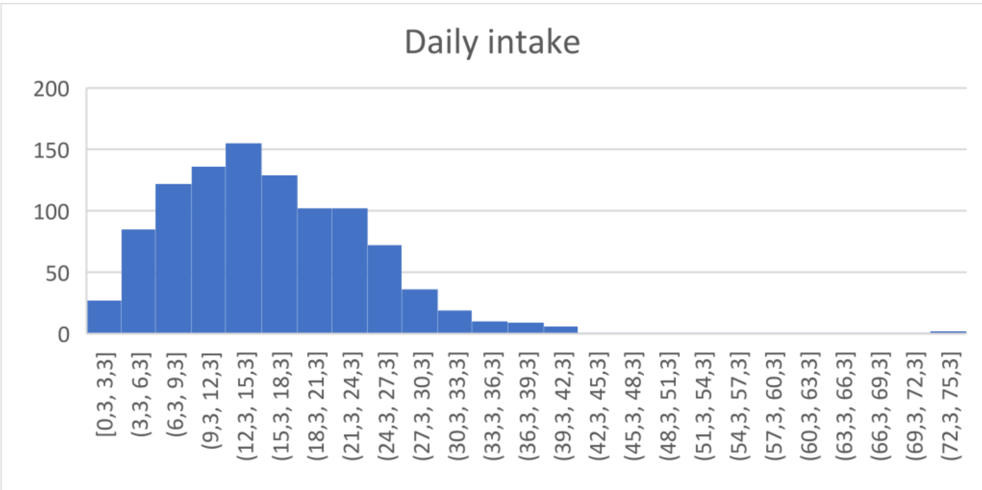


Figure 1. Distribution of the concentrations for all the individual phenols and related parameters found in the cohort of samples analyzed in the 2017/2018 season. The x-axis is expressed as mg/kg for all plots, except for Daily intake parameter, expressed as mg.

As can be seen, a normal distribution was found for the majority of the variables, except for hydroxytyrosol, oleocanthal, oleomissional and oleokoronal. For these compounds, most of the samples were characterized by low concentration of them.

Distribution of samples per harvesting date and cultivar

The samples were collected in a wide period of time that covered seven months, from September to March. This is a really long period, but it is worth noting that the high production in Spain (the highest producer country) obliges to extend the production period as a function of the diversification of cultivars. The next plot provides an overview of the distribution of the samples according to the season:

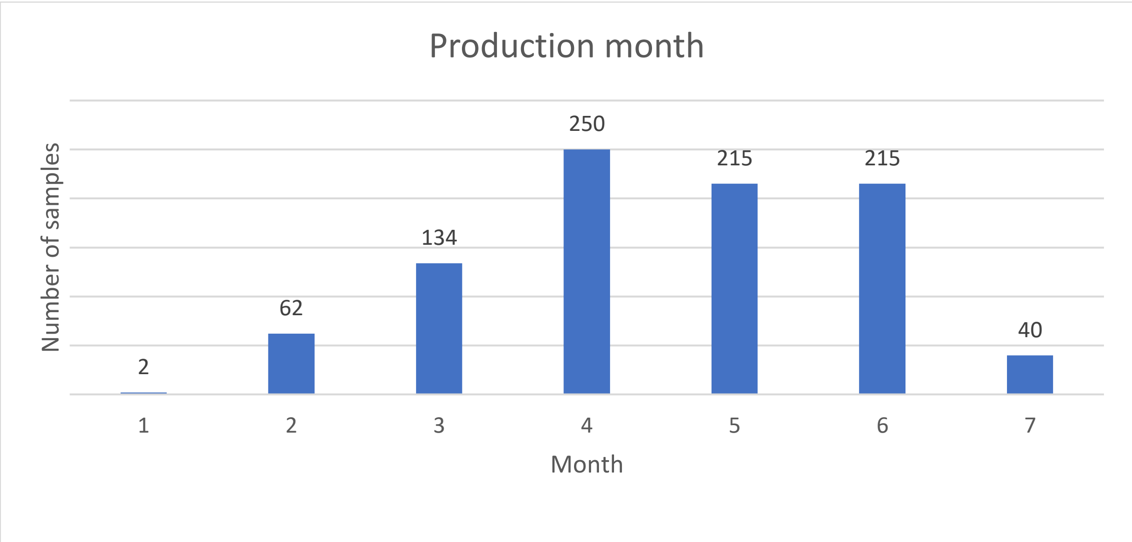


Figure 2. Distribution of samples according to the production month.

Corresponding the numbers to:

1: September

2: October

3: November

4: December

5: January

6: February

7: March

Complementarily, the samples were obtained from a heterogeneous set of cultivars representative of the different areas of Spain:

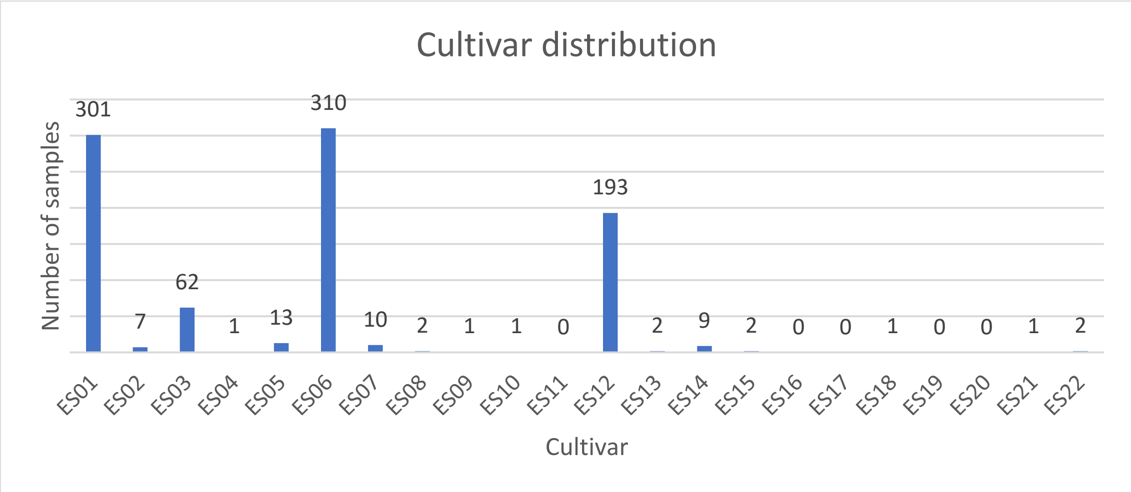


Figure 3. Distribution of samples according to the cultivars used for the production of olive oil.

Being:

ES01: Picual

ES02: Manzanilla Fina

ES03: Arbequina

ES04: Arroniz

ES05: Cornicabra

ES06: Hojiblanca

ES07: Picudo

ES08: Pajarera

ES09: Lechín

ES10: Gordal

ES11: Koroneiki

ES12: Coupage

ES13: Arbosana

ES14: Morisca

ES15: Nevadillo Negro

ES16: Gordalilla

ES17: Frantoio

ES18: Villalonga

ES19: Pico Limón

ES20: Empeltre

ES21: Acebuche

ES22: Verdial

It is worth emphasizing a particular aspect of the Spanish production system, which is typically restricted to traditional cultivation. This is the existence of natural Coupages, samples coming from areas that are characterized by a high cultivar variability, frequently including cultivars that have been preferentially selected along years for several reasons: production criteria, resistance to climatic or disease factors, edafological aspects, etc. It is not a casual aspect that different cultivars dominate each region or that natural Coupages are produced in some of these regions.

The concentration of phenolic compounds as a function of the cultivar is here presented:

PICUAL: 301 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	14,3	1,8	1,8
Oleacein	8,7	826,4	**204,5**	134,8
Oleocanthal	0,0	199,4	**47,6**	37,6
Oleuropein aglycon	20,1	894,8	**283,9**	151,0
Oleomissional	0,0	356,8	**42,9**	39,6
Ligstroside aglycon	9,6	511,2	**129,2**	87,0
Oleokoronal	0,0	636,7	**106,1**	79,7
Apigenin	0,0	3,2	0,6	0,4
Luteolin	0,0	5,9	1,0	0,8
Hidroxytyrosol derivatives	52,7	1512,0	**533,0**	267,8
Tyrosol derivatives	34,3	989,4	**282,9**	165,7
Total EFSA	87,0	2213,9	815,9	401,8
Total Analyzed	87,0	2213,9	817,5	401,5
Intake	1,7	44,3	**16,3**	8,0

MANZANILLA FINA: 7 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	2,3	0,8	0,9
Oleacein	106,8	212,8	**172,4**	42,8
Oleocanthal	45,3	123,1	**90,6**	27,3
Oleuropein aglycon	148,9	192,8	**163,4**	13,9
Oleomissional	21,2	148,6	**78,0**	51,1
Ligstroside aglycon	115,4	196,7	**147,0**	32,0
Oleokoronal	80,6	373,2	**212,5**	108,0
Apigenin	0,1	1,3	0,7	0,5
Luteolin	0,1	1,5	0,7	0,5
Hidroxytyrosol derivatives	340,7	518,0	**414,7**	57,6
Tyrosol derivatives	337,1	597,7	**450,1**	92,4
Total EFSA	711,3	1115,7	864,7	145,9
Total Analyzed	712,3	1117,9	866,1	145,9
Intake	14,2	22,3	17,3	2,9

ARBEQUINA: 62 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	6,0	0,9	0,9
Oleacein	3,0	740,7	**221,7**	156,6
Oleocanthal	2,0	138,3	**48,0**	32,8
Oleuropein aglycon	0,0	134,1	**35,1**	24,3
Oleomissional	0,0	23,7	**1,3**	4,7
Ligstroside aglycon	0,0	31,6	**6,5**	7,9
Oleokoronal	0,0	28,4	**2,8**	6,1
Apigenin	0,0	2,5	0,9	0,5
Luteolin	0,0	6,3	2,5	1,5
Hidroxytyrosol derivatives	7,0	874,8	**258,9**	176,5
Tyrosol derivatives	2,0	165,0	**57,3**	40,3
Total EFSA	13,0	1019,8	316,2	205,4
Total Analyzed	17,2	1020,5	319,7	204,7
Intake	0,3	20,4	**6,3**	4,1

ARRONIZ: 1 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	4,1	4,1	4,1	#¡DIV/0!
Oleacein	533,6	533,6	**533,6**	#¡DIV/0!
Oleocanthal	14,7	14,7	**14,7**	#¡DIV/0!
Oleuropein aglycon	184,5	184,5	**184,5**	#¡DIV/0!
Oleomissional	22,2	22,2	**22,2**	#¡DIV/0!
Ligstroside aglycon	10,0	10,0	**10,0**	#¡DIV/0!
Oleokoronal	2,4	2,4	**2,4**	#¡DIV/0!
Apigenin	0,5	0,5	0,5	#¡DIV/0!
Luteolin	0,4	0,4	0,4	#¡DIV/0!
Hidroxytyrosol derivatives	744,5	744,5	**744,5**	#¡DIV/0!
Tyrosol derivatives	27,2	27,2	**27,2**	#¡DIV/0!
Total EFSA	771,7	771,7	771,7	#¡DIV/0!
Total Analyzed	772,5	772,5	772,5	#¡DIV/0!
Intake	15,4	15,4	**15,4**	#¡DIV/0!

CORNICABRA: 13 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	7,1	1,7	1,9
Oleacein	103,9	641,8	**321,1**	146,2
Oleocanthal	9,4	143,4	**61,6**	51,2
Oleuropein aglycon	94,9	239,7	**152,4**	47,3
Oleomissional	0,0	169,7	**56,9**	65,3
Ligstroside aglycon	45,2	140,8	**82,0**	27,5
Oleokoronal	14,6	314,9	**137,0**	123,3
Apigenin	0,0	1,2	0,4	0,4
Luteolin	0,0	1,4	0,5	0,5
Hidroxytyrosol derivatives	303,2	819,2	**532,0**	135,9
Tyrosol derivatives	100,1	599,1	**280,5**	166,0
Total EFSA	516,9	1314,4	812,6	245,0

Total Analyzed	517,4	1314,6	813,4	244,8
Intake	10,3	26,3	**16,3**	4,9

HOJIBLANCA: 310 samples

COMPPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	14,2	1,9	1,6
Oleacein	22,5	1829,9	**416,4**	226,1
Oleocanthal	1,9	259,4	**51,8**	48,7
Oleuropein aglycon	8,3	762,0	**206,0**	88,0
Oleomissional	0,0	367,1	**39,7**	40,8
Ligstroside aglycon	0,0	274,3	**53,4**	36,8
Oleokoronal	0,0	443,4	**50,8**	46,6
Apigenin	0,0	2,6	0,9	0,5
Luteolin	0,0	4,7	1,4	0,9
Hidroxytyrosol derivatives	37,1	2967,6	**664,1**	326,9
Tyrosol derivatives	11,7	896,3	**156,1**	117,3
Total EFSA	58,2	3734,0	820,1	420,1
Total Analyzed	59,4	3737,1	822,4	420,3
Intake	1,2	74,7	**16,4**	8,4

PICUDO:10 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,1	1,8	0,7	0,5
Oleacein	77,2	618,7	**298,9**	192,5
Oleocanthal	4,4	241,1	**75,2**	67,2
Oleuropein aglycon	60,0	183,0	**120,6**	40,0
Oleomissional	8,1	139,2	**57,4**	40,9
Ligstroside aglycon	36,1	134,2	**59,6**	29,9
Oleokoronal	7,8	331,8	**132,9**	83,9

Apigenin	0,3	2,7	1,6	0,8
Luteolin	0,2	2,9	1,2	0,8
Hidroxytyrosol derivatives	174,6	844,5	**477,6**	220,5
Tyrosol derivatives	151,3	707,0	**267,7**	164,2
Total EFSA	344,4	1551,5	745,3	340,6
Total Analyzed	346,8	1555,2	748,1	340,7
Intake	6,9	31,0	**14,9**	6,8

PAJARERA:2 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	0,8	0,4	0,5
Oleacein	19,9	68,8	**44,4**	34,6
Oleocanthal	39,6	69,6	**54,6**	21,2
Oleuropein aglycon	21,0	25,9	**23,4**	3,4
Oleomissional	2,5	3,0	**2,7**	0,4
Ligstroside aglycon	18,3	25,8	**22,0**	5,3
Oleokoronal	16,6	32,2	**24,4**	11,0
Apigenin	0,0	0,6	0,3	0,5
Luteolin	0,0	0,5	0,2	0,3
Hidroxytyrosol derivatives	48,2	93,6	**70,9**	32,1
Tyrosol derivatives	74,5	127,5	**101,0**	37,5
Total EFSA	168,1	175,8	172,0	5,4
Total Analyzed	168,1	176,9	172,5	6,2
Intake	3,4	3,5	**3,4**	0,1

LECHIN:1 sample

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	2,5	2,5	2,5	#¡DIV/0!
Oleacein	216,4	216,4	**216,4**	#¡DIV/0!
Oleocanthal	104,6	104,6	**104,6**	#¡DIV/0!
Oleuropein aglycon	181,7	181,7	**181,7**	#¡DIV/0!

Oleomissional	36,9	36,9	**36,9**	#¡DIV/0!
Ligstroside aglycon	133,1	133,1	**133,1**	#¡DIV/0!
Oleokoronal	78,6	78,6	**78,6**	#¡DIV/0!
Apigenin	0,7	0,7	0,7	#¡DIV/0!
Luteolin	1,1	1,1	1,1	#¡DIV/0!
Hidroxytyrosol derivatives	437,5	437,5	**437,5**	#¡DIV/0!
Tyrosol derivatives	316,3	316,3	**316,3**	#¡DIV/0!
Total EFSA	753,8	753,8	753,8	#¡DIV/0!
Total Analyzed	755,7	755,7	755,7	#¡DIV/0!
Intake	15,1	15,1	**15,1**	#¡DIV/0!

GORDAL:1 sample

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	0,0	0,0	#¡DIV/0!
Oleacein	51,2	51,2	**51,2**	#¡DIV/0!
Oleocanthal	71,3	71,3	**71,3**	#¡DIV/0!
Oleuropein aglycon	52,8	52,8	**52,8**	#¡DIV/0!
Oleomissional	24,6	24,6	**24,6**	#¡DIV/0!
Ligstroside aglycon	114,8	114,8	**114,8**	#¡DIV/0!
Oleokoronal	157,7	157,7	**157,7**	#¡DIV/0!
Apigenin	0,5	0,5	0,5	#¡DIV/0!
Luteolin	0,7	0,7	0,7	#¡DIV/0!
Hidroxytyrosol derivatives	128,7	128,7	**128,7**	#¡DIV/0!
Tyrosol derivatives	343,9	343,9	**343,9**	#¡DIV/0!
Total EFSA	472,5	472,5	472,5	#¡DIV/0!
Total Analyzed	473,8	473,8	473,8	#¡DIV/0!
Intake	9,5	9,5	**9,5**	#¡DIV/0!

COUPAGE:193 samples

COMPOUND	MIN	MAX	AVERAGE	SD

Hydroxytyrosol	0,0	9,1	1,7	1,2
Oleacein	74,7	1479,5	**369,7**	218,6
Oleocanthal	0,0	270,2	**80,2**	50,6
Oleuropein aglycon	26,4	631,7	**249,5**	118,8
Oleomissional	0,0	283,2	**68,0**	57,7
Ligstroside aglycon	0,0	368,2	**98,4**	61,9
Oleokoronal	0,0	550,6	**121,6**	98,8
Apigenin	0,0	4,5	0,9	0,6
Luteolin	0,0	4,9	1,4	0,9
Hidroxytyrosol derivatives	104,6	2188,8	**688,9**	319,6
Tyrosol derivatives	23,3	1166,3	**300,2**	174,8
Total EFSA	141,4	2849,7	989,1	440,6
Total Analyzed	144,1	2850,4	991,4	440,3
Intake	2,8	57,0	**19,8**	8,8

ARBOSANA:2 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	0,6	0,3	0,4
Oleacein	0,3	127,1	**63,7**	89,6
Oleocanthal	26,5	50,6	**38,5**	17,1
Oleuropein aglycon	9,4	37,6	**23,5**	19,9
Oleomissional	0,0	1,6	**0,8**	1,2
Ligstroside aglycon	0,0	6,3	**3,2**	4,5
Oleokoronal	0,0	0,5	**0,2**	0,4
Apigenin	2,7	6,5	4,6	2,7
Luteolin	3,0	4,2	3,6	0,8
Hidroxytyrosol derivatives	9,8	166,9	**88,3**	111,1
Tyrosol derivatives	33,3	50,6	**41,9**	12,2
Total EFSA	43,0	217,5	130,2	123,3
Total Analyzed	52,6	224,4	138,5	121,5
Intake	0,9	4,3	**2,6**	2,5

MORISCA:9 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	1,4	10,8	6,0	3,5
Oleacein	129,4	601,9	**352,2**	162,9
Oleocanthal	130,7	246,3	**172,2**	41,9
Oleuropein aglycon	84,3	331,5	**205,7**	85,8
Oleomissional	0,0	62,4	**32,2**	24,1
Ligstroside aglycon	71,5	345,1	**157,2**	93,6
Oleokoronal	12,3	240,6	**97,9**	76,4
Apigenin	0,1	0,6	0,4	0,2
Luteolin	0,3	1,0	0,6	0,2
Hidroxytyrosol derivatives	215,1	972,2	**596,1**	248,1
Tyrosol derivatives	222,1	745,4	**427,3**	191,9
Total EFSA	437,2	1656,6	1023,4	407,5
Total Analyzed	437,8	1657,3	1024,4	407,5
Intake	8,7	33,1	**20,5**	8,1

NEVADILLO NEGRO:2 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,1	11,9	6,0	8,4
Oleacein	35,5	112,0	**73,7**	54,1
Oleocanthal	8,9	69,5	**39,2**	42,8
Oleuropein aglycon	136,2	193,6	**164,9**	40,6
Oleomissional	42,4	133,7	**88,0**	64,6
Ligstroside aglycon	100,1	165,0	**132,6**	45,9
Oleokoronal	179,6	322,4	**251,0**	101,0
Apigenin	0,8	1,1	1,0	0,2
Luteolin	0,9	1,5	1,2	0,4
Hidroxytyrosol derivatives	305,5	359,9	**332,7**	38,5
Tyrosol derivatives	414,2	431,5	**422,8**	12,3
Total EFSA	737,0	774,0	755,5	26,2
Total Analyzed	738,7	776,7	757,7	26,8
Intake	14,7	15,5	**15,1**	0,5

VILLALONGA:1 sample

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	1,5	1,5	1,5	#¡DIV/0!
Oleacein	107,9	107,9	**107,9**	#¡DIV/0!
Oleocanthal	0,0	0,0	**0,0**	#¡DIV/0!
Oleuropein aglycon	326,6	326,6	**326,6**	#¡DIV/0!
Oleomissional	291,5	291,5	**291,5**	#¡DIV/0!
Ligstroside aglycon	159,9	159,9	**159,9**	#¡DIV/0!
Oleokoronal	400,6	400,6	**400,6**	#¡DIV/0!
Apigenin	1,3	1,3	1,3	#¡DIV/0!
Luteolin	0,9	0,9	0,9	#¡DIV/0!
Hidroxytyrosol derivatives	727,5	727,5	**727,5**	#¡DIV/0!
Tyrosol derivatives	560,5	560,5	**560,5**	#¡DIV/0!
Total EFSA	1287,9	1287,9	1287,9	#¡DIV/0!
Total Analyzed	1290,1	1290,1	1290,1	#¡DIV/0!
Intake	25,8	25,8	**25,8**	#¡DIV/0!

ACEBUCHE:1 sample

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,8	0,8	0,8	#¡DIV/0!
Oleacein	55,6	55,6	**55,6**	#¡DIV/0!
Oleocanthal	2,8	2,8	**2,8**	#¡DIV/0!
Oleuropein aglycon	40,2	40,2	**40,2**	#¡DIV/0!
Oleomissional	22,0	22,0	**22,0**	#¡DIV/0!
Ligstroside aglycon	20,3	20,3	**20,3**	#¡DIV/0!
Oleokoronal	86,6	86,6	**86,6**	#¡DIV/0!
Apigenin	0,8	0,8	0,8	#¡DIV/0!
Luteolin	0,6	0,6	0,6	#¡DIV/0!
Hidroxytyrosol derivatives	118,5	118,5	**118,5**	#¡DIV/0!
Tyrosol derivatives	109,6	109,6	**109,6**	#¡DIV/0!
Total EFSA	228,2	228,2	228,2	#¡DIV/0!
Total Analyzed	229,6	229,6	229,6	#¡DIV/0!

Intake	4,6	4,6	**4,6**	#¡DIV/0!

VERDIAL:2 samples

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,1	0,9	0,5	0,6
Oleacein	114,0	294,8	**204,4**	127,9
Oleocanthal	12,6	20,4	**16,5**	5,5
Oleuropein aglycon	48,3	148,7	**98,5**	71,0
Oleomissional	8,1	106,1	**57,1**	69,3
Ligstroside aglycon	2,2	25,5	**13,9**	16,4
Oleokoronal	8,7	73,9	**41,3**	46,1
Apigenin	0,2	0,6	0,4	0,3
Luteolin	0,7	1,1	0,9	0,2
Hidroxytyrosol derivatives	170,5	550,6	**360,6**	268,8
Tyrosol derivatives	31,3	111,9	**71,6**	57,0
Total EFSA	201,8	662,5	432,2	325,8
Total Analyzed	202,7	664,2	433,4	326,3
Intake	4,0	13,3	**8,6**	6,5

Table 2. Phenolic profiles provided by the different cultivars included in the 2017/2018 season. The number of samples used for calculations is also pointed out.

Phenolic concentration as a function of the harvesting date

Due to the unbalanced distribution of samples by setting the production month as temporal variable, a new distribution was defined to obtain groups with a balanced composition in terms of number of samples. This new distribution is here presented:

	N	Harvesting date
G1	92	21/09 to 07/11
G2	95	08/11 to 26/11
G3	147	27/11 to 10/12
G4	106	11/12 to 27/12
G5	89	28/12 to 16/01
G6	129	17/01 to 31/01
G7	140	01/02 to 16/02
G8	118	17/02 to 26/03

Table 3. Distribution of samples in eight groups according to the harvesting date.

Eight groups from G1 to G8 were defined with a number of samples ranging from 89 to 147 according to the harvesting date, taking into account in all cases that the collected samples were immediately processed to extract the olive oil. The new distribution is illustrated as follows:

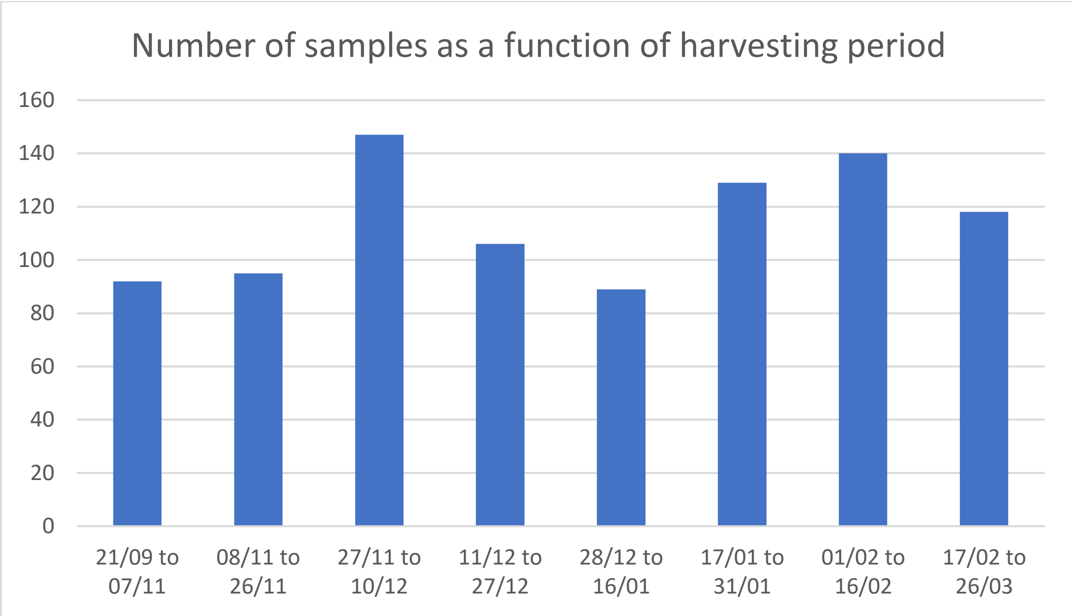
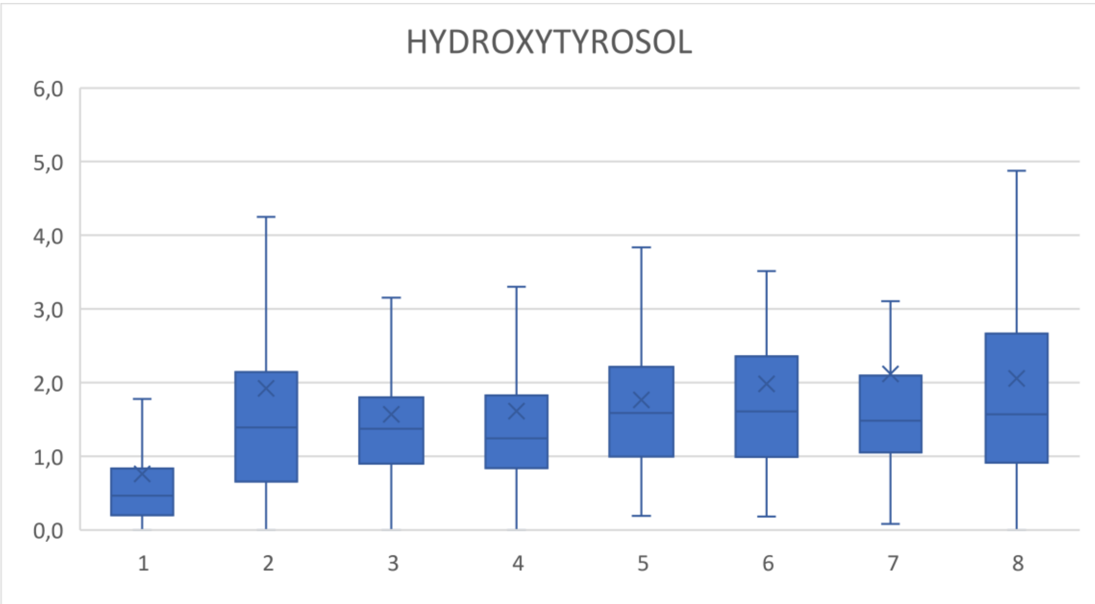
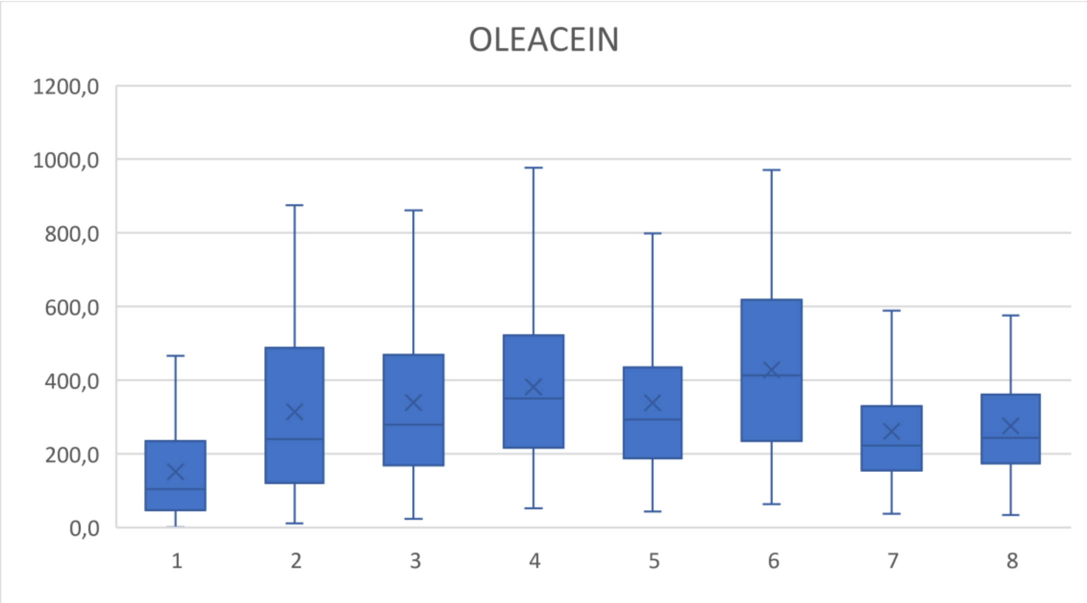
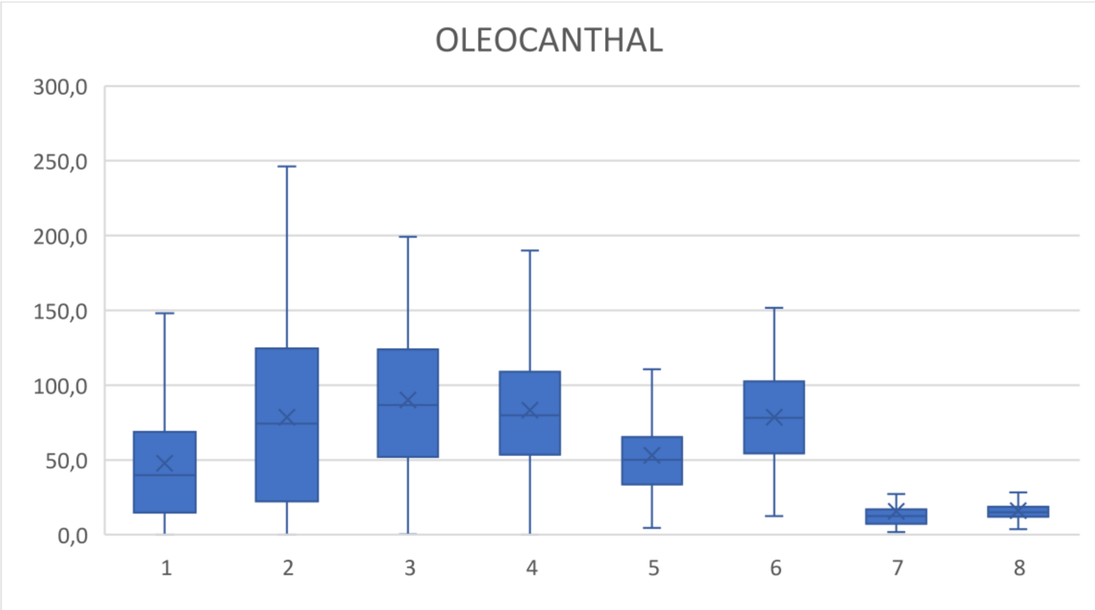


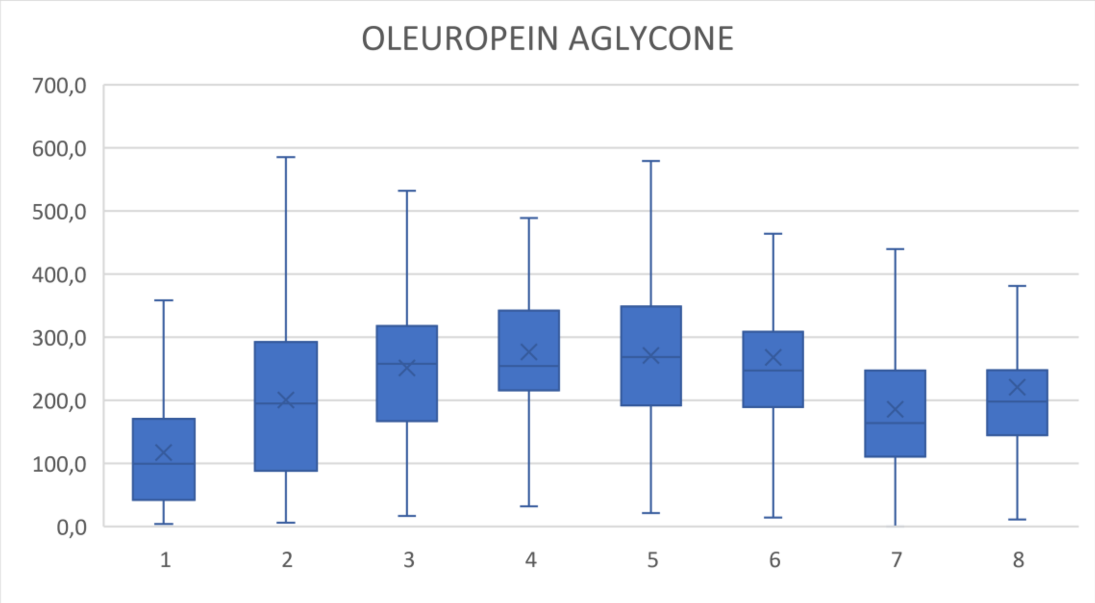
Figure 4. Distribution of samples analyzed in the 2017/2018 season according to the harvesting date.

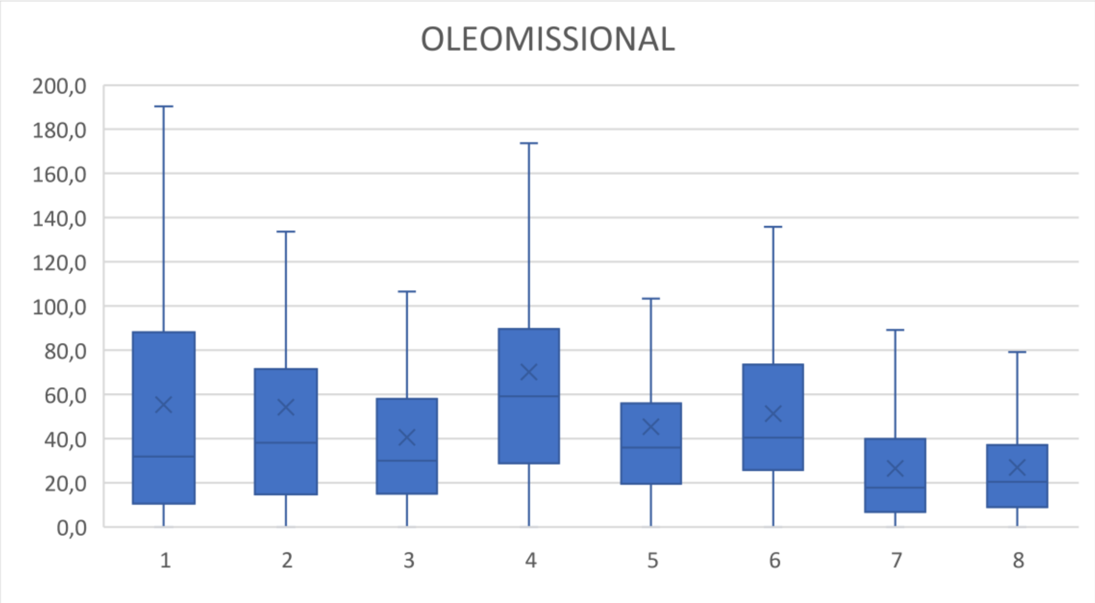
Based on this distribution, the concentration profile for each phenolic compound or associated parameter with the harvesting date is as follows:

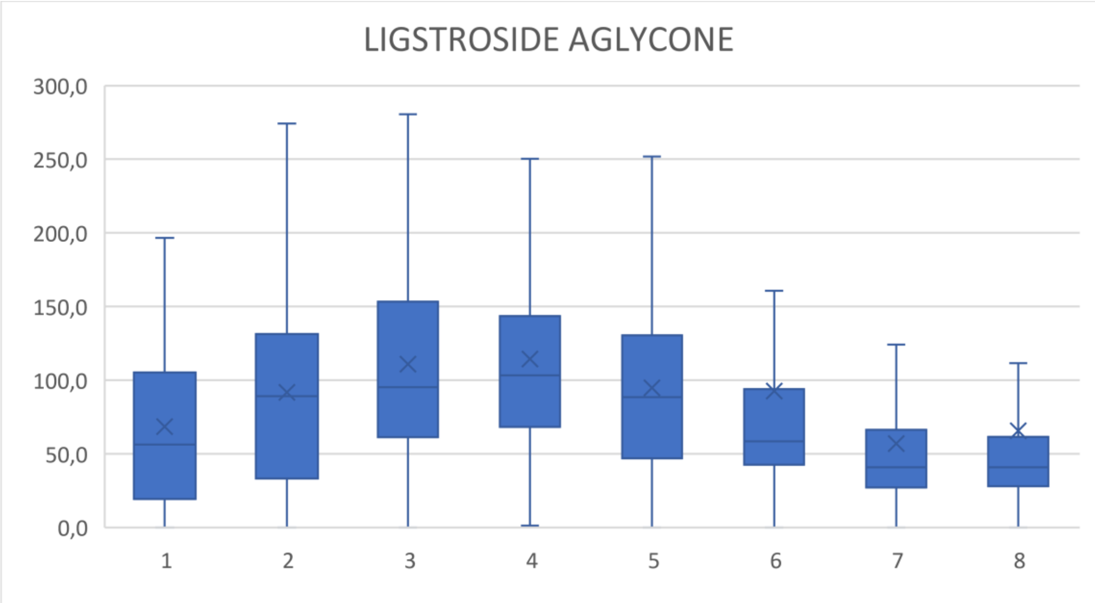


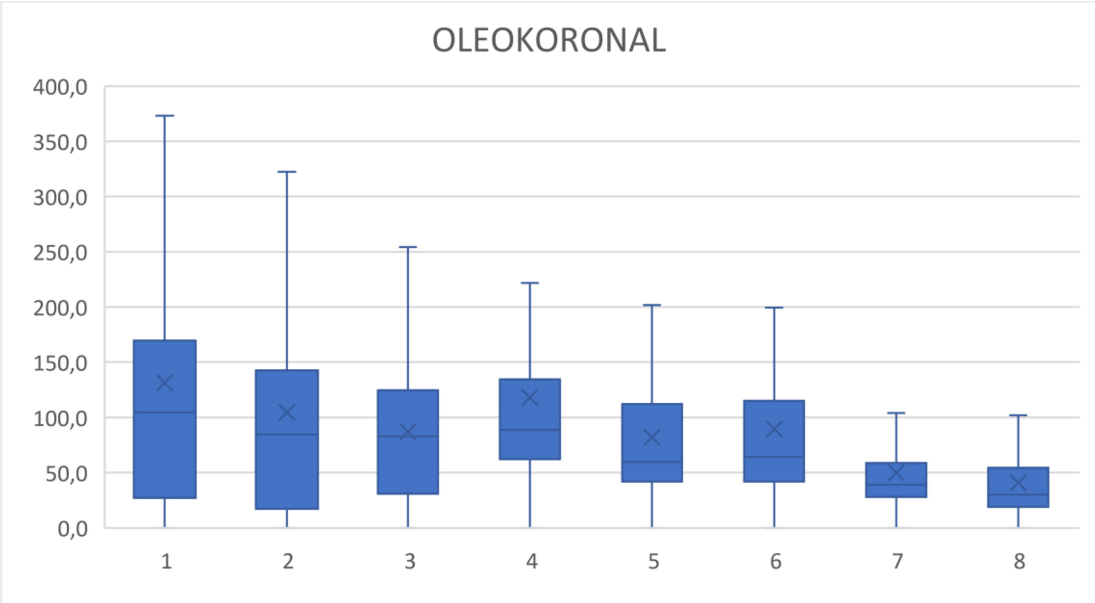

OLEACEIN
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
1
2
3
4
5
6
7
8

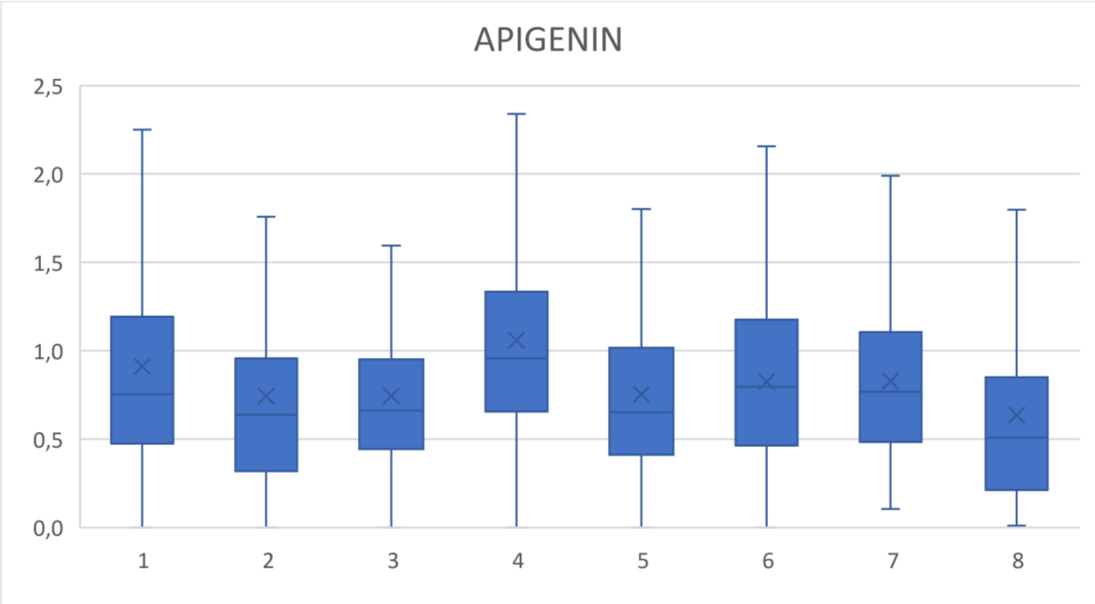

OLEOCANTHAL
300,0
250,0
200,0
150,0
100,0
50,0
0,0
1
2
3
4
5
6
7
8

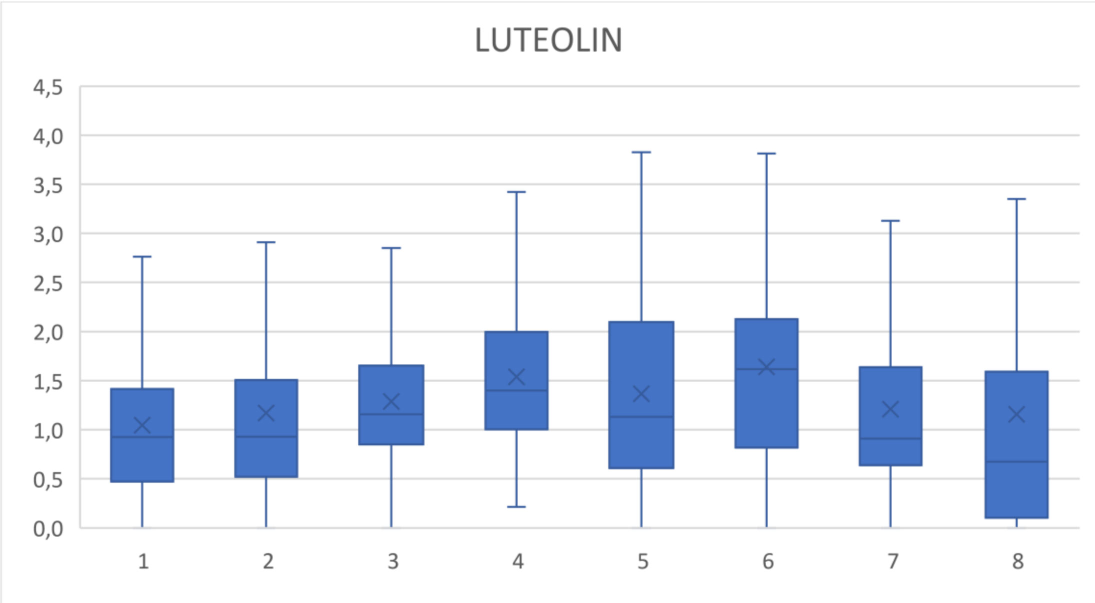

OLEUROPEIN AGLYCONE
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
1
2
3
4
5
6
7
8

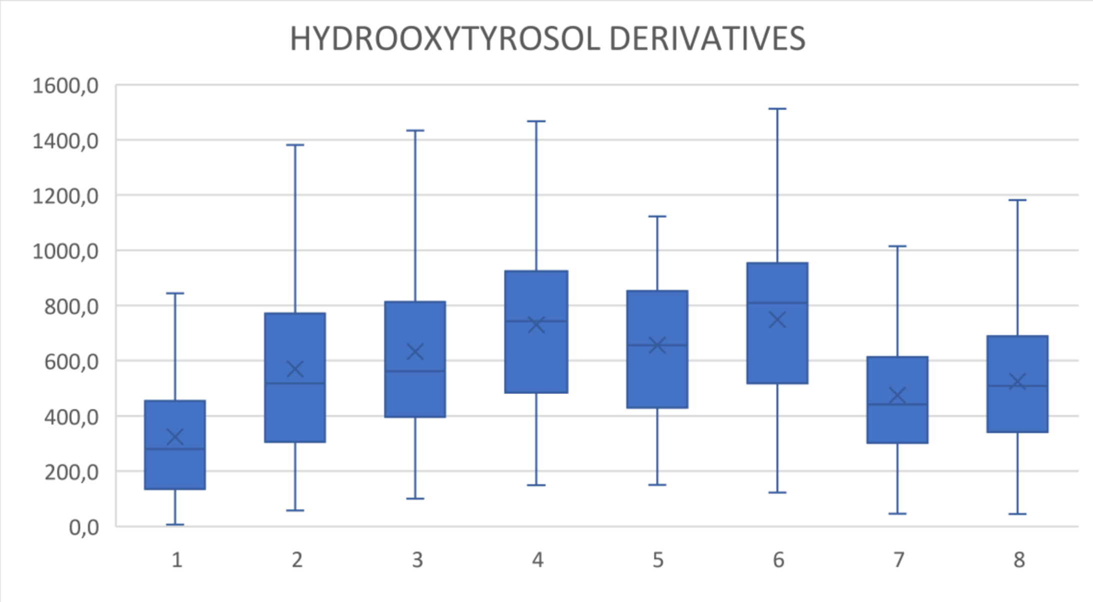

OLEOMISSIONAL
200,0
180,0
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
1
2
3
4
5
6
7
8

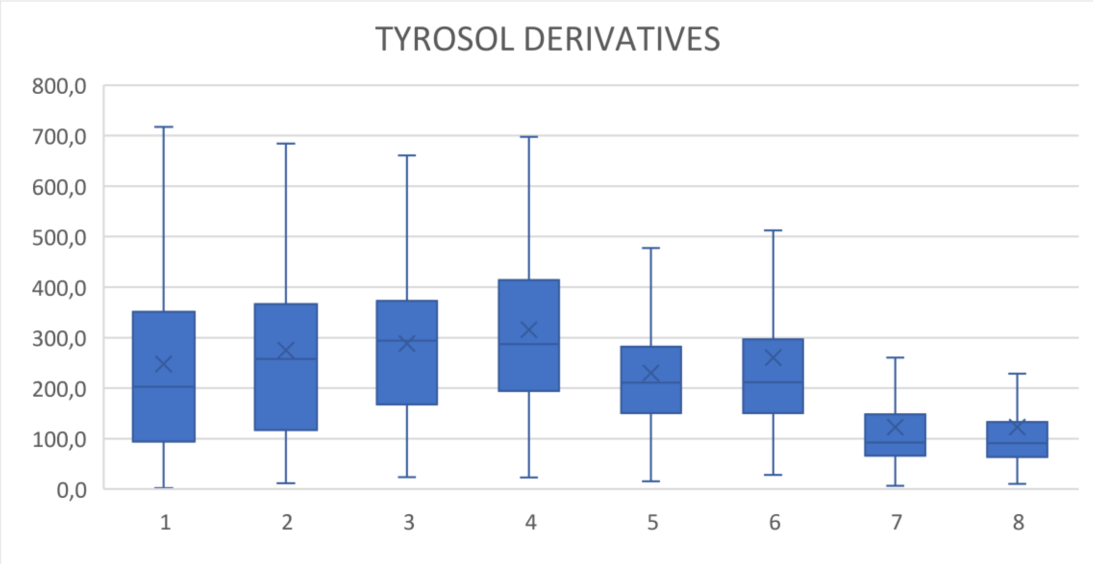

LIGSTROSIDE AGLYCONE
300,0
250,0
200,0
150,0
100,0
50,0
0,0
1
2
3
4
5
6
7
8

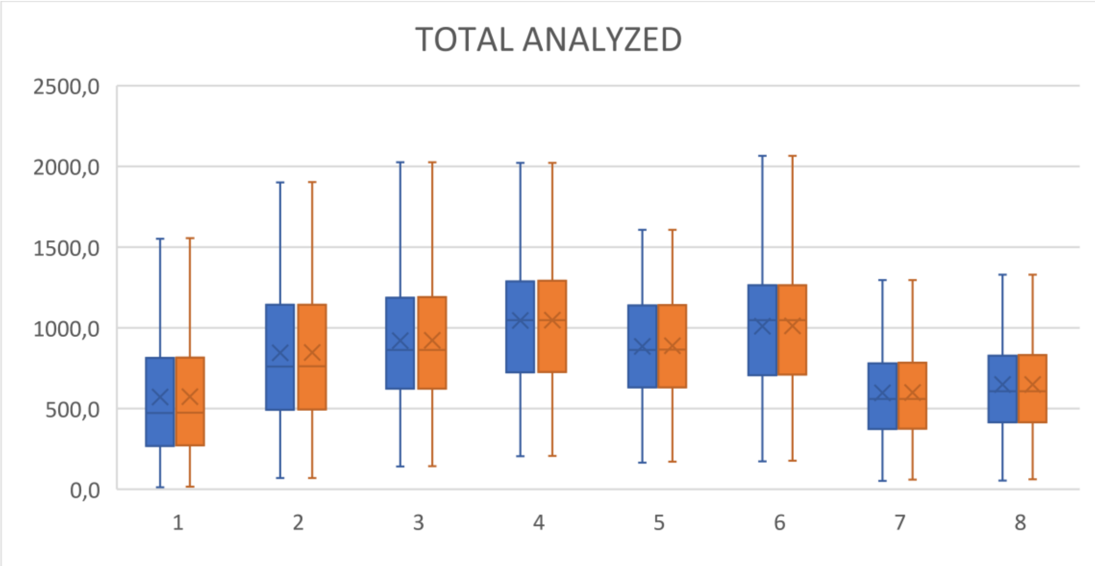

OLEOKORONAL
400,0
350,0
300,0
250,0
200,0
150,0
100,0
50,0
0,0
1
2
3
4
5
6
7
8


APIGENIN
2,5
2,0
1,5
1,0
0,5
0,0
1
2
3
4
5
6
7
8


LUTEOLIN
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
1
2
3
4
5
6
7
8


HYDROOXYTYROSOL DERIVATIVES
1600,0
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
1
2
3
4
5
6
7
8


TYROSOL DERIVATIVES
800,0
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
1
2
3
4
5
6
7
8



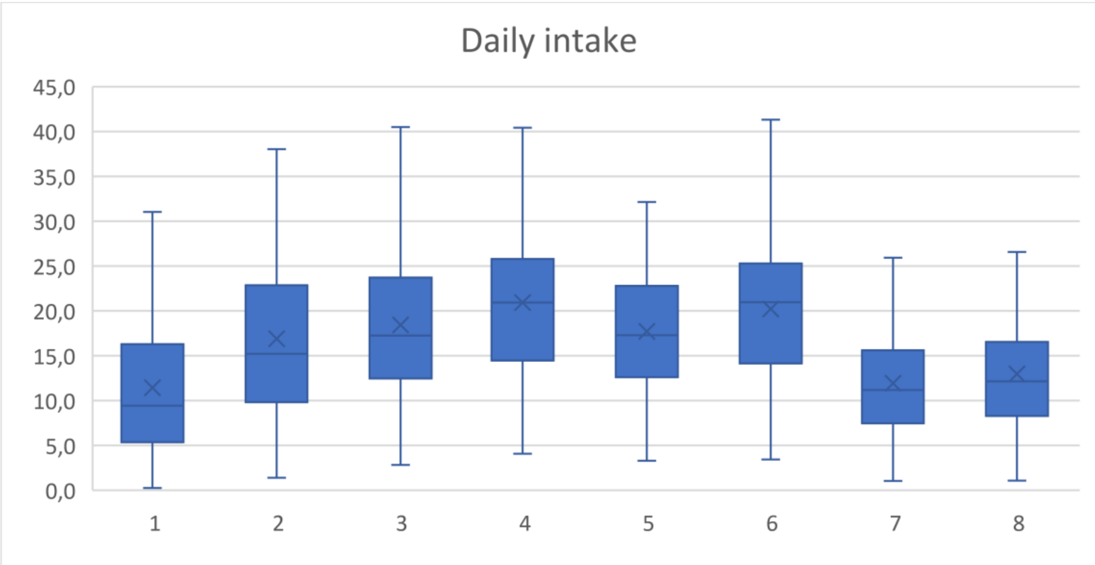


Figure 5. Concentration profiles of the monitored phenolic compounds and associated parameters according to the harvesting date.

These results should be considered in the context of their production. These plots provide, in general terms, a view of the evolution of the concentration of phenolic compounds and associated parameters with the harvesting date. Therefore, no discrimination or filtration of cultivars was carried out. No discrimination attending to geographical origin was applied either. Despite the great number of variability factors affecting these results, interesting conclusions can be deduced in terms of evolution of phenolic concentrations with time. Thus:

- Hydroxytyrosol slightly increased its concentration by increasing the harvesting date, which is predictable. Olive oils produced in February and March are typically characterized by a higher content in hydroxytyrosol than early olive oils produced in October or November.
- Oleacein reached its maximum concentration in the analyzed olive oils in the period encompassing from G3 to G5 or, more precisely, from 27th November to 16th January. At the beginning of the season, it is worth highlighting the high variability in the concentration of oleacein as compared to the last two periodical steps including February and March.
- Oleocanthal reported a quite similar behavior to that observed for oleacein. However, the period encompassing from G2 to G4 led to the maximum levels of this phenol. Therefore, from 28th December the profile of oleocanthal concentration was characterized by a decreasing trend.
- Oleuropein aglycon reached its maximum level in the period between G3 and G6, which means from 27th November to 31st January. Therefore, the behavior of oleuropein aglycon was quite similar to that observed for oleacein, except for the inclusion of G6 in the maximum concentration window for the aglycon derivative.
- Ligstroside aglycon behaved in a similar way to oleuropein aglycone. The performance was quite similar to oleocanthal since the maximum concentration was achieved from 8th November to 16th January. The concentration of ligstroside aglycon starts to show an increasing trend very early, in a way similar to oleocanthal.
- Oleokoronal and oleomissional seemed to offer a decreasing trend by increasing the harvesting date. Nevertheless, the high variability detected at early harvesting dates masked the results partially.

- Luteolin was increased up to G6, being the maximum concentration from G4 to G6, from 11th December to 31st January.
- Apigenin did not show a clear trend as it revealed a sinusoidal profile with maximum concentrations at G4 and G6-G7.
- Hydroxytyrosol derivatives reached their maximum concentration between G3 and G6, from 27th November to 31st January.
- Tyrosol derivatives achieved their maximum content within G3 and G4 groups, which means from 27th November to 27th December.
- As these two families of compounds represent the most concentrated phenolic compounds in olive oil, the profile of the total analyzed compounds is marked by the presence of these two major families.
- The amount of phenols taken per daily ingestion of 20 g of olive oil also was marked by the profile of these two main phenolic families. Thus, the maximum concentration of this parameter was reached from G3 to G6, which is a wide window of time. In fact, these results do not support the fact that early olive oils tend to offer a high phenolic content.

The concentration of phenolic compounds in samples pertaining to the different date groups is here presented:

G1:97

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	5,3	0,8	1,0
Oleacein	0,3	533,6	**148,1**	131,8
Oleocanthal	0,0	241,1	**46,4**	42,0
Oleuropein aglycon	3,9	408,9	**116,7**	87,3
Oleomissional	0,0	356,8	**54,9**	60,9
Ligstroside aglycon	0,0	268,4	**67,6**	56,2
Oleokoronal	0,0	636,7	**130,2**	124,3
Apigenin	0,0	6,5	0,9	0,8
Luteolin	0,0	4,2	1,0	0,8
Hidroxytyrosol derivatives	7,0	961,3	**320,5**	223,9
Tyrosol derivatives	2,0	989,4	**244,2**	191,4

Total EFSA	13,0	1950,7	564,7	387,2
Total Analyzed	17,2	1951,7	566,7	386,9
Intake	0,3	39,0	**11,3**	7,7

G2:76

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	11,9	2,0	2,4
Oleacein	11,2	858,0	**287,6**	209,2
Oleocanthal	0,0	216,6	**73,0**	56,1
Oleuropein aglycon	5,9	605,3	**194,3**	134,1
Oleomissional	0,0	299,3	**53,8**	61,2
Ligstroside aglycon	0,0	345,1	**89,7**	72,1
Oleokoronal	0,0	443,4	**104,5**	106,0
Apigenin	0,0	3,2	0,8	0,6
Luteolin	0,0	5,9	1,2	1,0
Hidroxytyrosol derivatives	57,6	1700,7	**537,7**	330,7
Tyrosol derivatives	11,4	896,3	**267,2**	183,7
Total EFSA	69,3	2596,9	804,9	478,7
Total Analyzed	70,1	2597,7	806,9	478,3
Intake	1,4	51,9	**16,1**	9,6

G3:163

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	8,6	1,6	1,2
Oleacein	22,8	1829,9	**355,9**	255,4

Oleocanthal	0,3	270,2	**93,3**	52,3
Oleuropein aglycon	15,0	762,0	**252,3**	127,2
Oleomissional	0,0	367,1	**42,2**	44,6
Ligstroside aglycon	0,0	334,6	**111,2**	71,4
Oleokoronal	0,0	353,3	**88,6**	69,6
Apigenin	0,0	2,6	0,7	0,4
Luteolin	0,0	3,5	1,2	0,6
Hidroxytyrosol derivatives	99,9	2967,6	**652,0**	364,9
Tyrosol derivatives	23,8	766,4	**293,1**	157,3
Total EFSA	141,4	3734,0	945,1	477,8
Total Analyzed	144,1	3737,1	947,0	477,8
Intake	2,8	74,7	**18,9**	9,6

G4:106

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	14,3	1,6	1,6
Oleacein	52,2	977,1	**381,9**	212,9
Oleocanthal	0,0	247,5	**83,3**	42,8
Oleuropein aglycon	32,0	638,4	**276,6**	115,6
Oleomissional	0,0	291,5	**70,1**	59,6
Ligstroside aglycon	1,1	368,2	**114,3**	68,5
Oleokoronal	0,0	550,6	**118,0**	99,7
Apigenin	0,0	4,5	1,1	0,6
Luteolin	0,2	4,3	1,5	0,8
Hidroxytyrosol derivatives	148,8	1467,8	**730,3**	293,6
Tyrosol derivatives	23,3	1166,3	**315,6**	169,1
Total EFSA	204,1	2550,2	1045,9	400,8
Total Analyzed	207,3	2551,7	1048,4	400,8
Intake	4,1	51,0	**20,9**	8,0

G5:96

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,2	7,1	1,8	1,1
Oleacein	42,8	1085,6	**343,7**	208,7
Oleocanthal	4,6	201,2	**55,7**	31,9
Oleuropein aglycon	20,9	633,0	**275,9**	132,1
Oleomissional	0,0	237,9	**47,5**	44,0
Ligstroside aglycon	0,0	326,1	**98,6**	68,1
Oleokoronal	0,0	378,0	**87,3**	70,5
Apigenin	0,0	2,3	0,7	0,5
Luteolin	0,0	3,8	1,3	0,9
Hidroxytyrosol derivatives	150,4	1537,7	**669,0**	280,8
Tyrosol derivatives	15,2	724,0	**241,6**	142,4
Total EFSA	165,5	2084,0	910,5	379,8
Total Analyzed	170,9	2085,4	912,6	379,4
Intake	3,3	41,7	**18,2**	7,6

G6:130

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,2	14,2	1,9	1,9
Oleacein	63,2	970,4	**422,3**	224,4
Oleocanthal	12,5	151,7	**77,4**	30,0
Oleuropein aglycon	13,8	894,8	**263,3**	140,3
Oleomissional	0,0	182,0	**51,7**	38,8
Ligstroside aglycon	0,0	511,2	**89,2**	93,6
Oleokoronal	0,0	345,4	**86,9**	73,5
Apigenin	0,0	2,2	0,8	0,5
Luteolin	0,0	4,5	1,6	1,0
Hidroxytyrosol derivatives	122,7	1512,0	**739,2**	293,9
Tyrosol derivatives	28,3	961,6	**253,5**	176,0
Total EFSA	172,2	2213,9	992,7	401,8
Total Analyzed	176,4	2213,9	995,1	401,1
Intake	3,4	44,3	**19,9**	8,0

G7:132

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,1	11,9	2,2	2,1
Oleacein	37,1	724,3	**256,5**	140,8
Oleocanthal	1,9	80,1	**12,7**	8,0
Oleuropein aglycon	0,0	645,0	**181,3**	105,0
Oleomissional	0,0	128,4	**23,4**	23,6
Ligstroside aglycon	0,0	270,5	**55,1**	47,5
Oleokoronal	0,0	155,0	**46,8**	31,3
Apigenin	0,1	2,2	0,8	0,4
Luteolin	0,0	6,1	1,2	0,9
Hidroxytyrosol derivatives	45,7	1099,9	**463,4**	217,2
Tyrosol derivatives	6,2	404,8	**114,6**	79,1
Total EFSA	51,9	1504,7	578,0	266,6
Total Analyzed	59,5	1505,5	580,0	266,1
Intake	1,0	30,1	**11,6**	5,3

G8:118

COMPOUND	MIN	MAX	AVERAGE	SD
Hydroxytyrosol	0,0	8,6	2,1	1,7
Oleacein	33,3	716,0	**275,9**	141,4
Oleocanthal	3,8	37,8	**16,1**	5,9
Oleuropein aglycon	11,0	784,1	**220,8**	131,6
Oleomissional	0,0	97,7	**26,9**	23,6
Ligstroside aglycon	0,0	487,2	**65,7**	76,3
Oleokoronal	0,0	202,2	**40,8**	34,9
Apigenin	0,0	2,5	0,6	0,6
Luteolin	0,0	6,3	1,2	1,5
Hidroxytyrosol derivatives	44,4	1182,4	**525,6**	232,4
Tyrosol derivatives	10,4	626,8	**122,5**	105,8

Total EFSA	54,8	1781,6	648,1	309,3
Total Analyzed	62,6	1783,3	649,9	308,7
Intake	1,1	35,6	**13,0**	6,2

Table 4. Phenolic profiles provided by samples produced along the 8 periods of the 2017/2018 season. The number of samples used for the calculations is also pointed out.

Influence of the malaxation step on the phenolic concentration

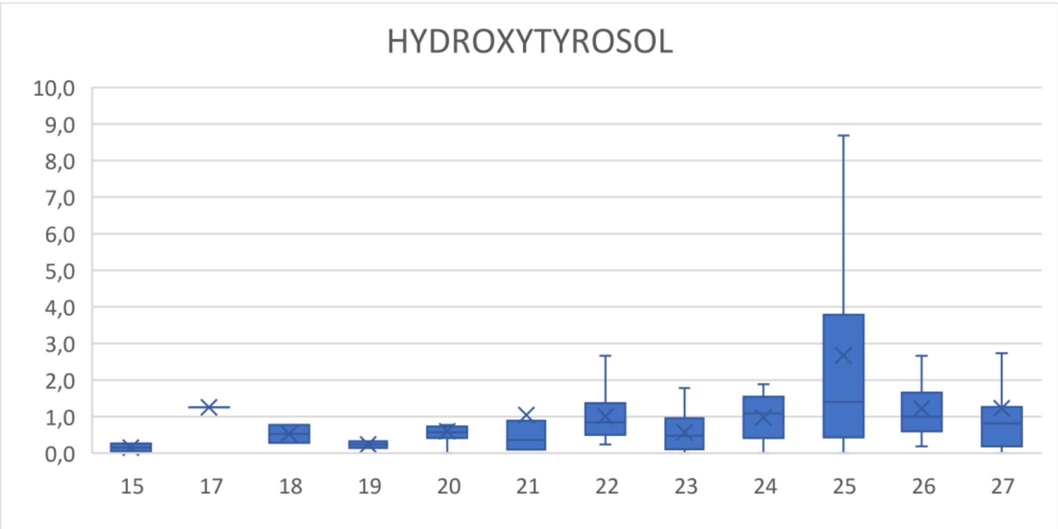
One step of special interest that has proved a relevant impact on the concentration of phenolic compounds is the malaxation time. In the analysis of samples collected during the 2017/2018 season, it was possible to study the effect of malaxation temperature and time on the phenolic concentration of olive oil.

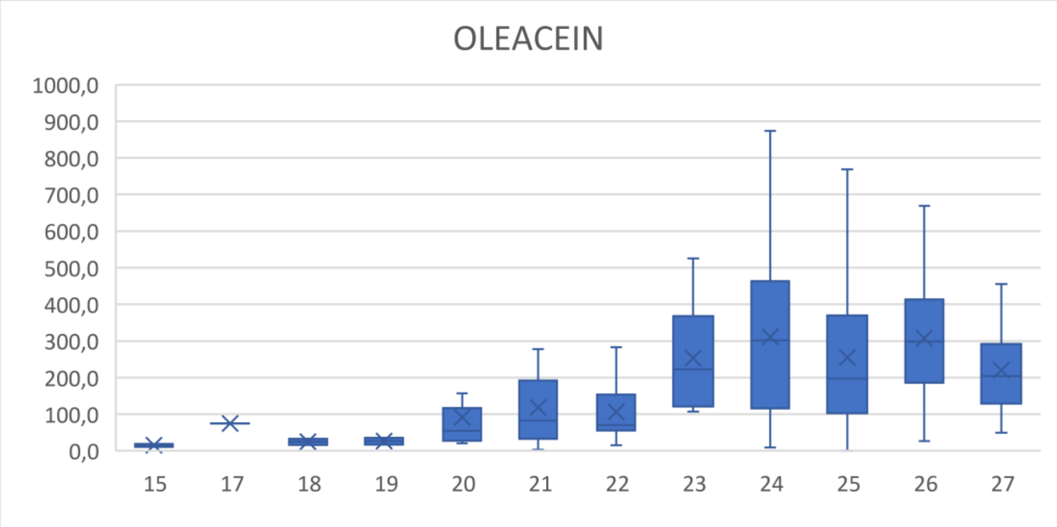
The distribution of analyzed samples with malaxation temperature is as follows:

Samples	Malaxation temperature
2	15
1	17
2	18
2	19
19	20
7	21
16	22
14	23
22	24
58	25
40	26
33	27
3	28
697	30

Table 4. Distribution of samples according to the malaxation temperature.

Maximum malaxation temperature reported was 30ºC, which was used to process most of the samples. Therefore, the distribution of samples was not balanced as can be checked, For this reason, the study was limited to the highlighted area that provides the following results:




OLEACEIN
1000,0
900,0
800,0
700,0
600,0
500,0
400,0
300,0
200,0
100,0
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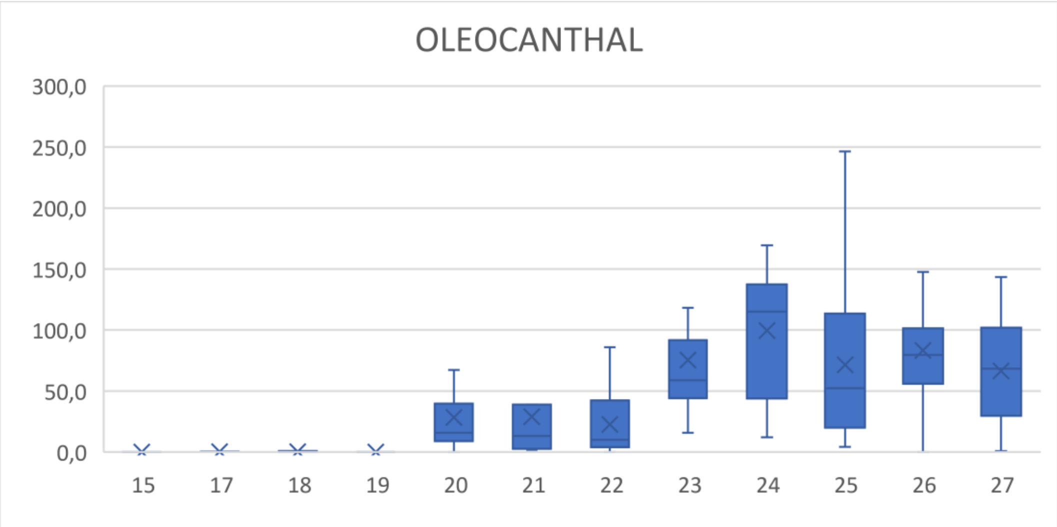
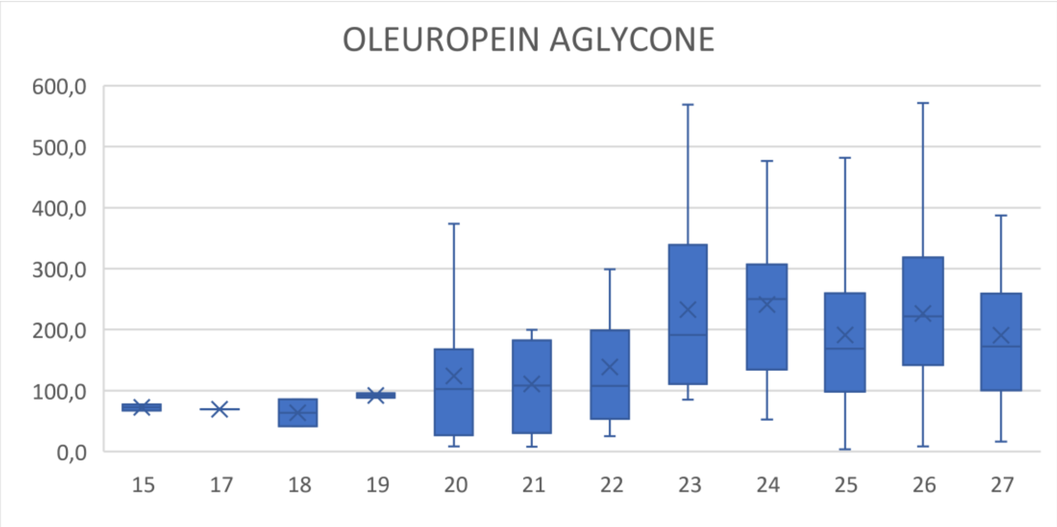
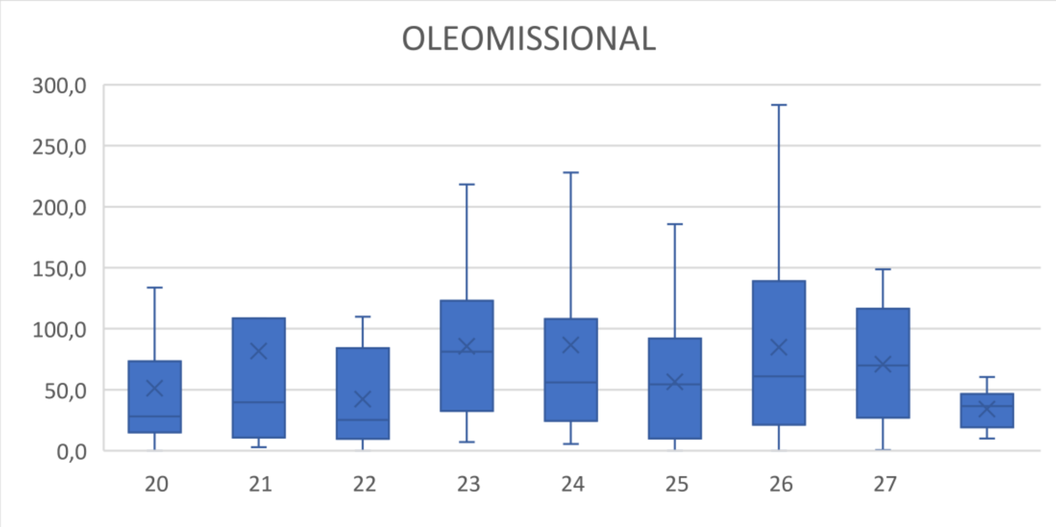
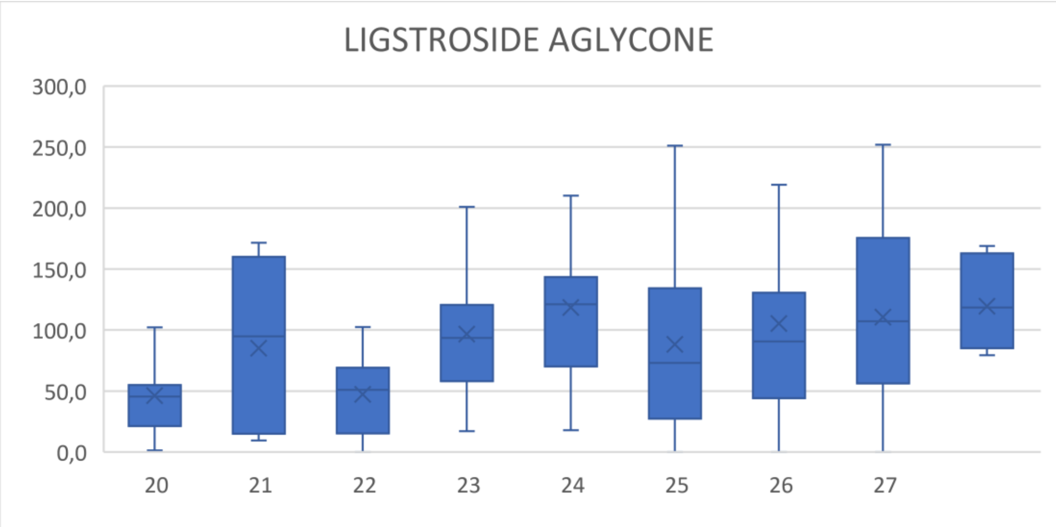
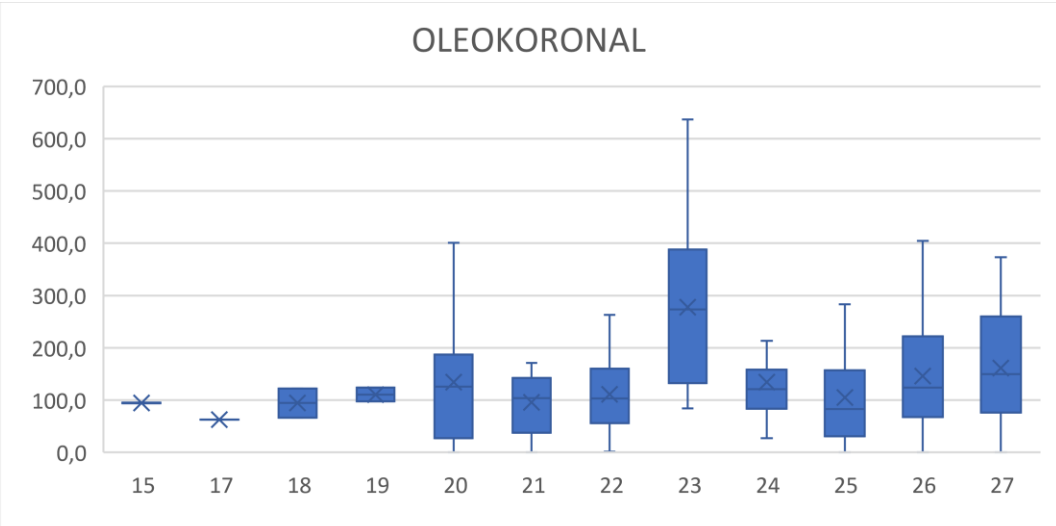
23
24
25
26
27


OLEOCANTHAL
300,0
250,0
200,0
150,0
100,0
50,0
0,0
15
17
18
19
20
21
22
23
24
25
26
27


OLEUROPEIN AGLYCONE
600,0
500,0
400,0
300,0
200,0
100,0
0,0
15
17
18
19
20
21
22
23
24
25
26
27


OLEOMISSIONAL
300,0
250,0
200,0
150,0
100,0
50,0
0,0
20
21
22
23
24
25
26
27


LIGSTROSIDE AGLYCONE
300,0
250,0
200,0
150,0
100,0
50,0
0,0
20
21
22
23
24
25
26
27


OLEOKORONAL
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
15
17
18
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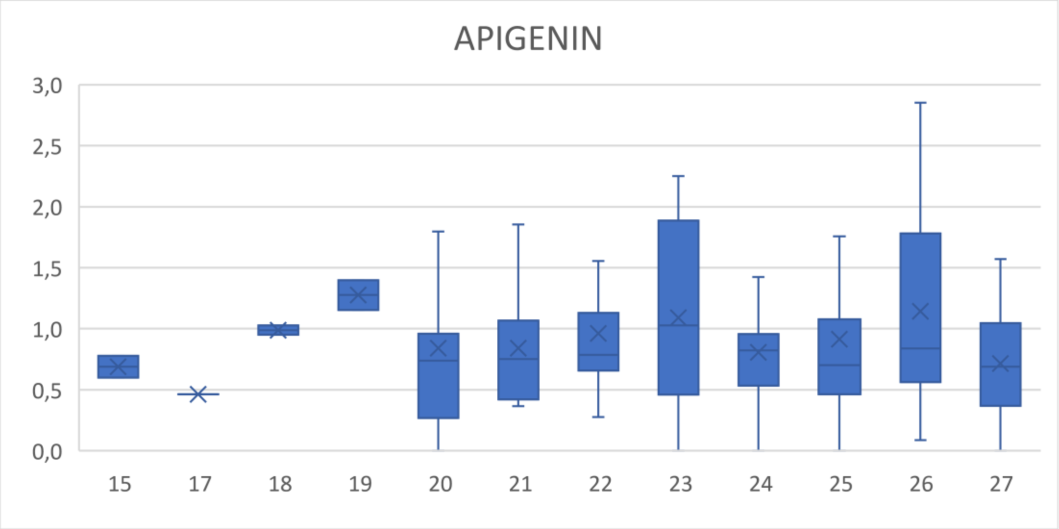
23
24
25
26
27


APIGENIN
3,0
2,5
2,0
1,5
1,0
0,5
0,0
15
17
18
19
20
21
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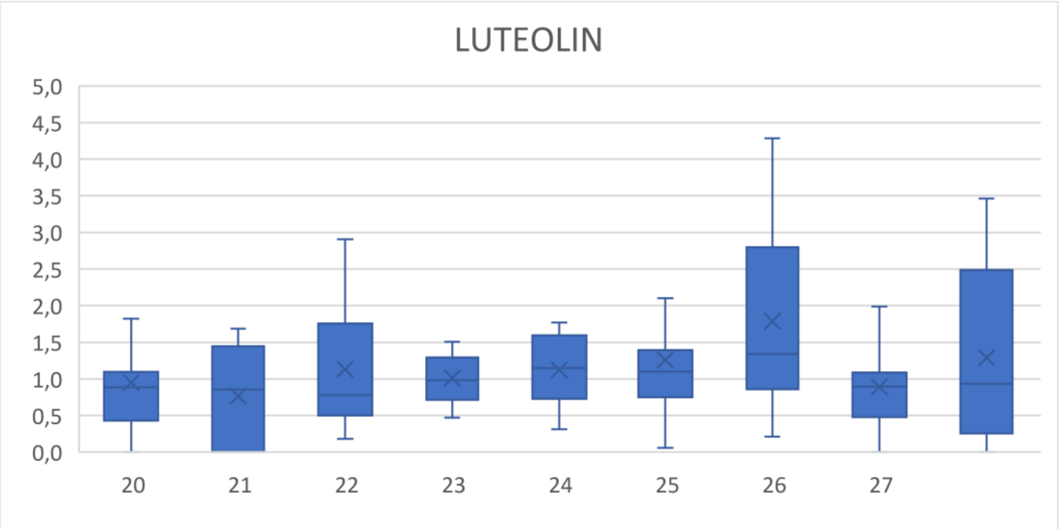
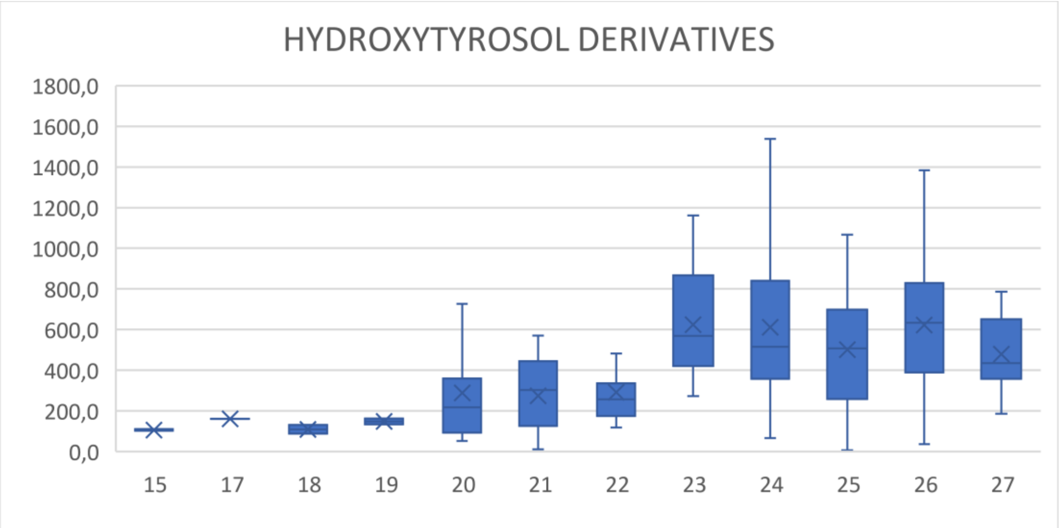
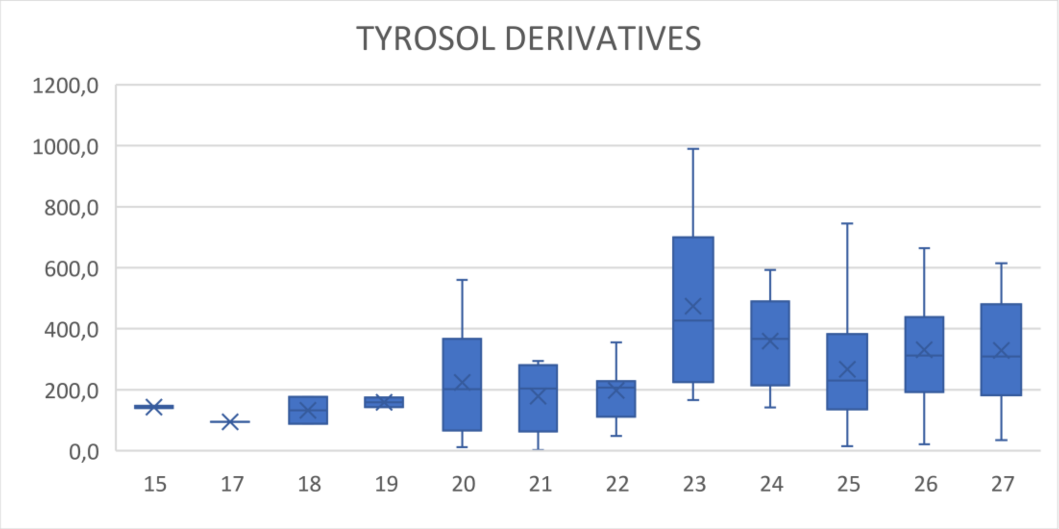
23
24
25
26
27


LUTEOLIN
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
20
21
22
23
24
25
26
27


HYDROXYTYROSOL DERIVATIVES
1800,0
1600,0
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
15
17
18
19
20
21
22
23
24
25
26
27


TYROSOL DERIVATIVES
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
15
17
18
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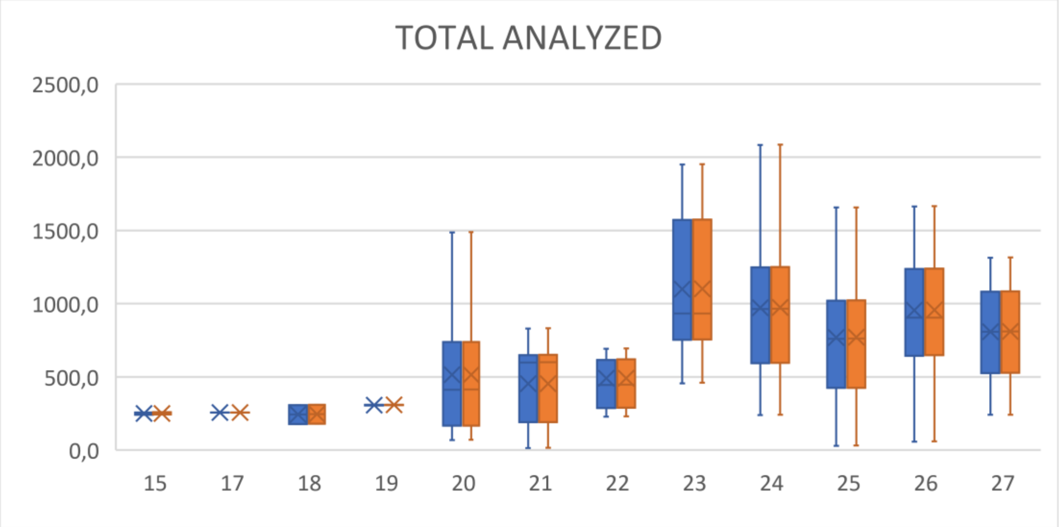
23
24
25
26
27


TOTAL ANALYZED
2500,0
2000,0
1500,0
1000,0
500,0
0,0
15
17
18
19
20
21
22
23
24
25
26
27

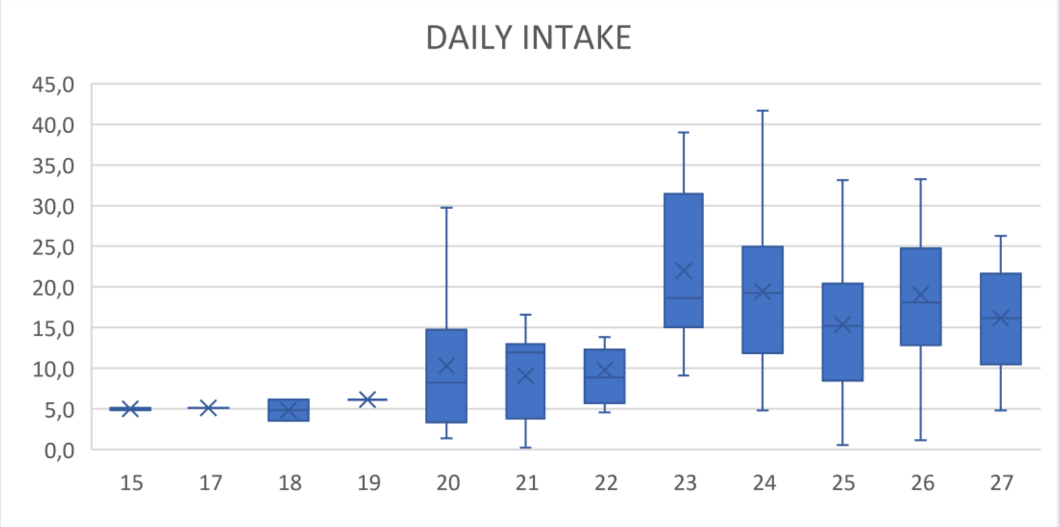

DAILY INTAKE
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
15
17
18
19
20
21
22
23
24
25
26
27

Figure 6. Concentration profiles found for the monitored phenolic compounds and associated parameters according to the malaxation temperature.

According to these results, it can be stated that:

- The temperature range used for studies is quite narrow. This range was from 15º up to 27º. Anyway, it is important to emphasize that these values are not always controlled during all the extraction process, which means that the temperature increases gradually during malaxation unless this step is thermostated, which is not always possible.
- Therefore, it is extended that temperature should not surpass 25–30ºC and, if the purpose is to obtain extra virgin olive oil with high organoleptical properties, the temperature should be always below 28ºC.
- Hydroxytyrosol was detected at higher concentration level in the oils produced with malaxation time at 25ºC. Despite there is a high variability in the data provided at this temperature, it seems clearly that this temperature leads to the highest concentration of this compound.
- Oleacein and oleocanthal reported a similar behavior. Thus, their maximum content was attained when malaxation was carried out at 24ºC.
- Oleuropein aglycon offered a quite similar performance to oleocanthal and oleacein. For oleuropein aglycon it could be set a range from 23 to 26ºC.
- Ligstroside aglycon did not offer an optimum malaxation temperature within the tested range. Anyway, it seems that a temperature increase favor its presence in olive oil.
- Oleomissional and oleokoronal did not give a clear behavior in the tested range. However, there is a slight trend to point out that a temperature around 23–26ºC was the best.
- Apigenin and luteolin did not offer an optimum malaxation temperature in the tested range.

- Hydroxytyrosol and tyrosol derivatives provided a clear message. Enzymatic processes demand for a suited temperature. It can be deduced that these enzymatic reactions, which are directly responsible for the formation of secoiridoid derivatives, are not activated when the temperature is below 23ºC. From this temperature, it seems that the overall concentration of derivatives is constant. In this context, it would be good to obtain samples corresponding to higher malaxation temperatures.
- The same pattern was found for the daily intake, which is evident, considering that the derivatives are the most concentrated contributors to this parameter.

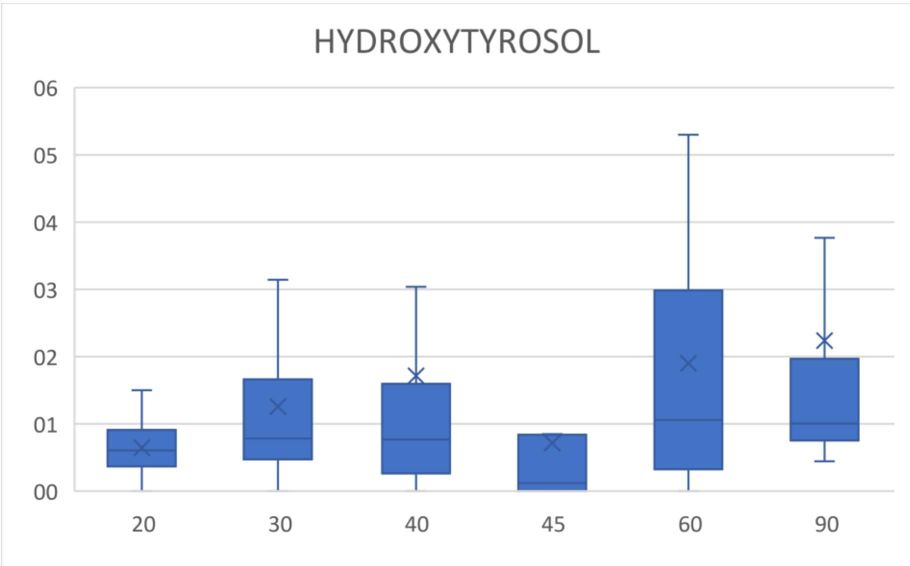
The other parameter affecting the development of the malaxation process is the time, which seems to influence the phenolic composition of virgin olive oils. The distribution of malaxation times used for samples collected within the 2017/2018 season is as follows:

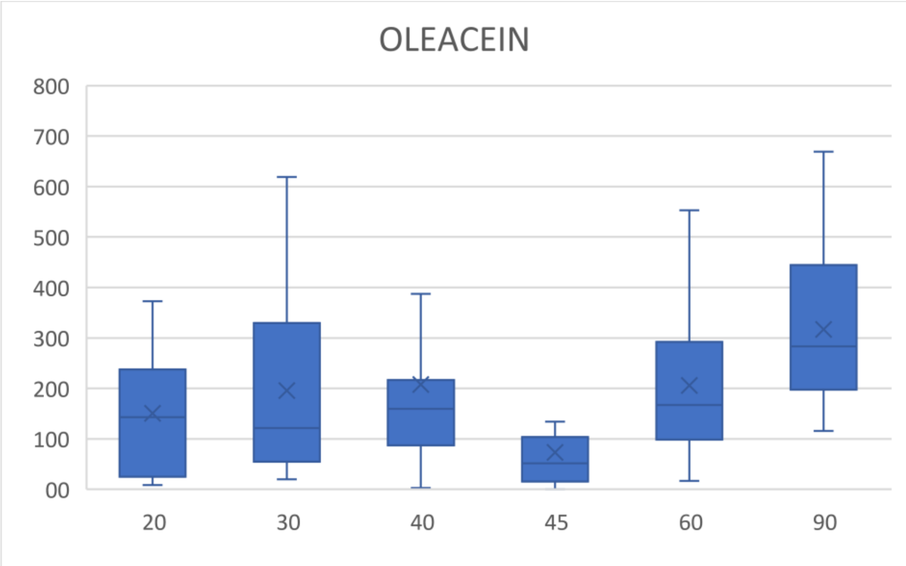
SAMPLES	MALAXATIONTIME
1	7
3	10
4	15
5	17
22	20
2	21
2	23
1	24
3	25
3	27
2	28
1	29
35	30
3	35
27	40
10	45
2	50
3	55
29	60
696	75

38	90
8	120
2	125
13	150
1	160

Table 5. Distribution of samples according to the malaxation time.

According to these results the highlighted values corresponding to malaxation times from 20 to 90 min were selected for this study. In this interval of malaxation time the following results were obtained for the different phenolic compounds and associated parameters:




OLEACEIN
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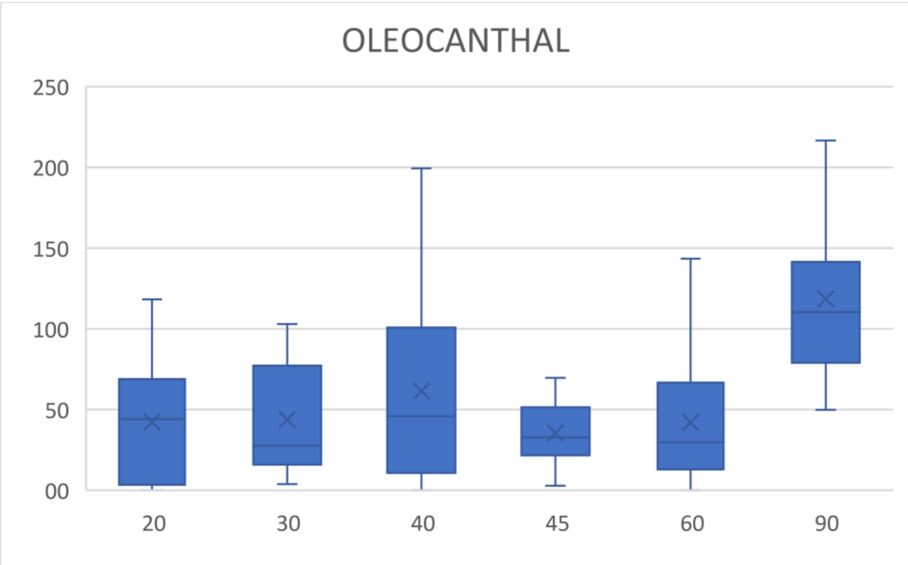
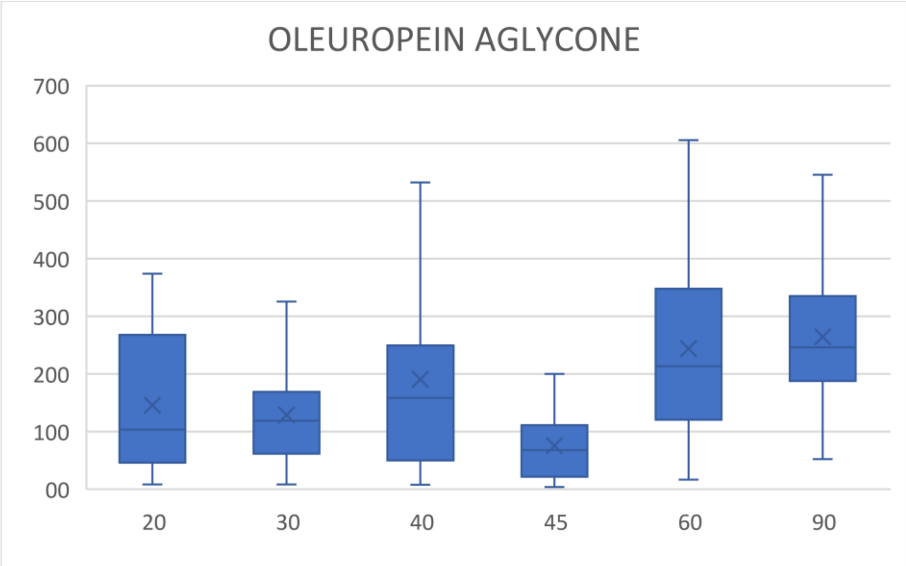
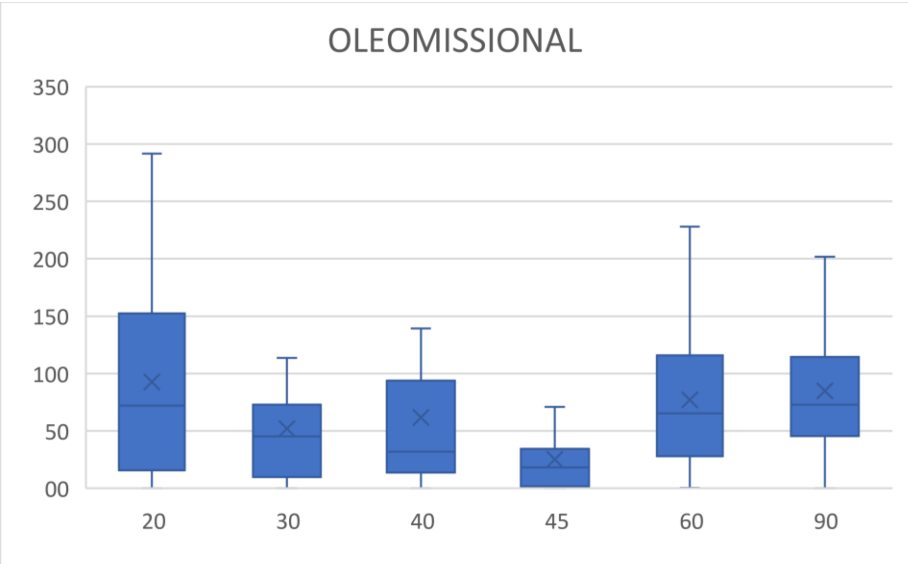
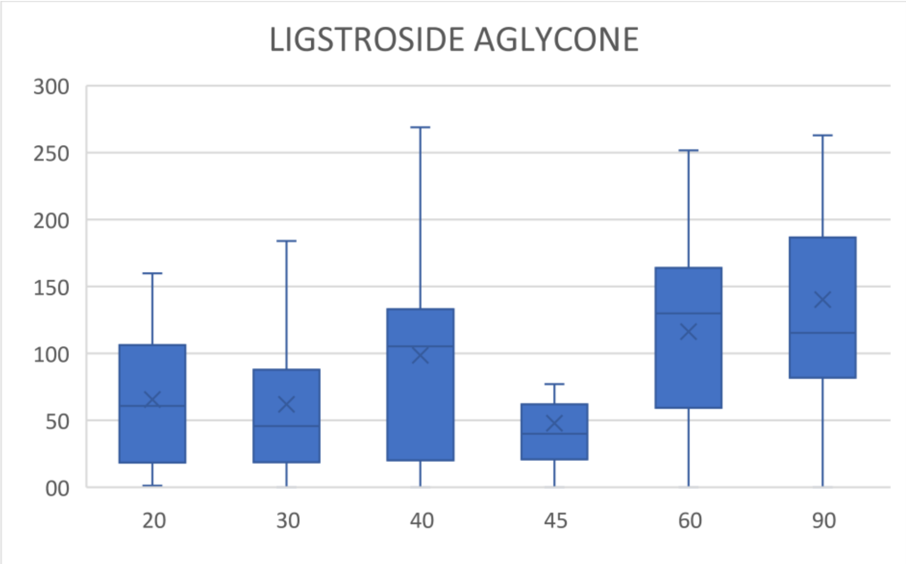
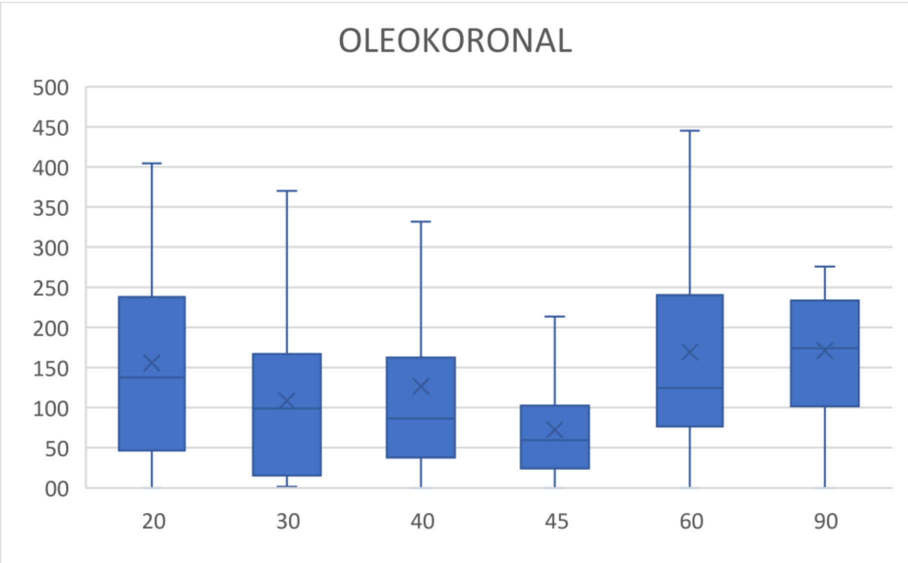
700
600
500
400
300
200
100
00
20
30
40
45
60
90


OLEOCANTHAL
250
200
150
100
50
00
20
30
40
45
60
90


OLEUROPEIN AGLYCONE
700
600
500
400
300
200
100
00
20
30
40
45
60
90


OLEOMISSIONAL
350
300
250
200
150
100
50
00
20
30
40
45
60
90


LIGSTROSIDE AGLYCONE
300
250
200
150
100
50
00
20
30
40
45
60
90


OLEOKORONAL
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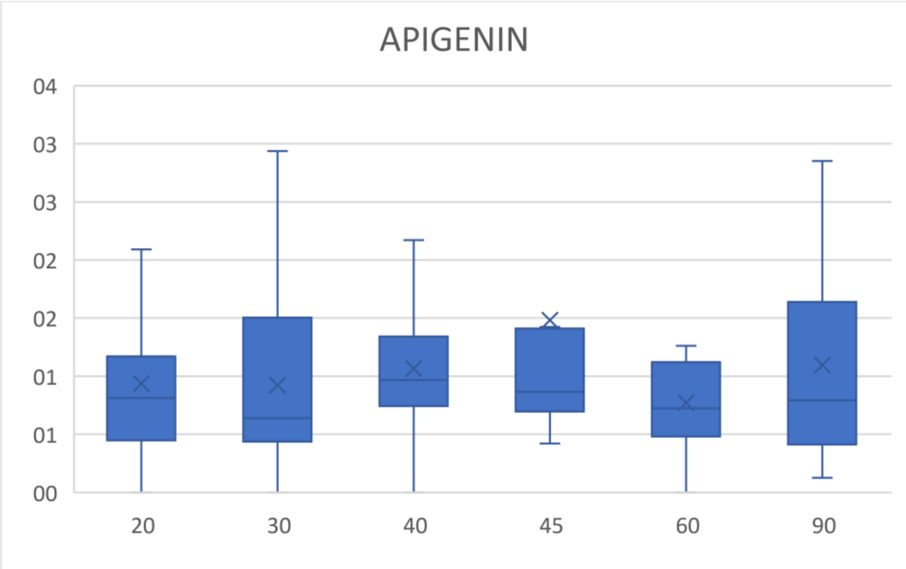
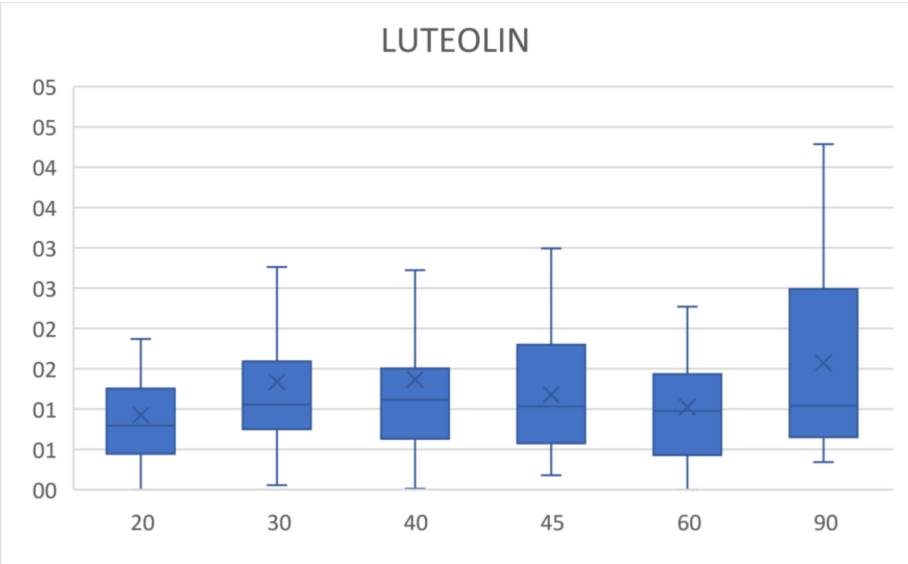
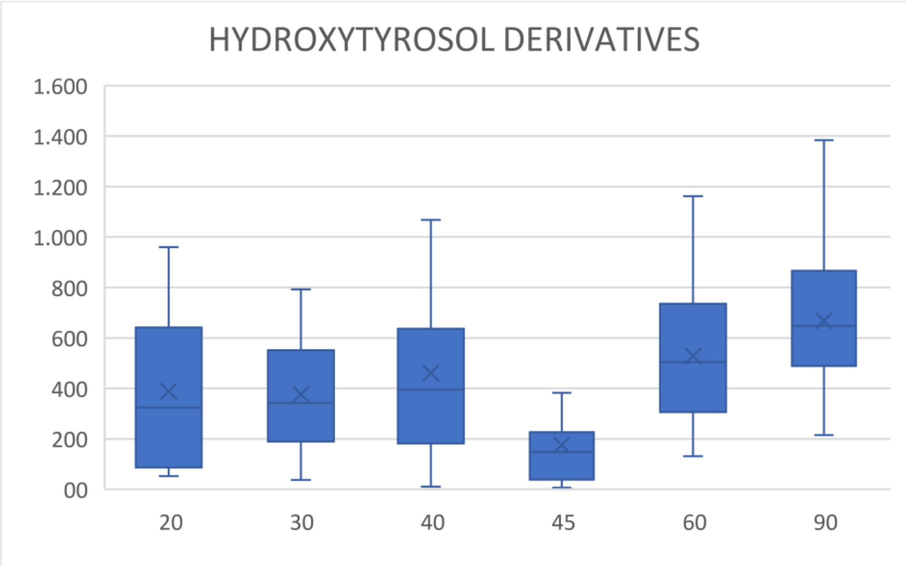
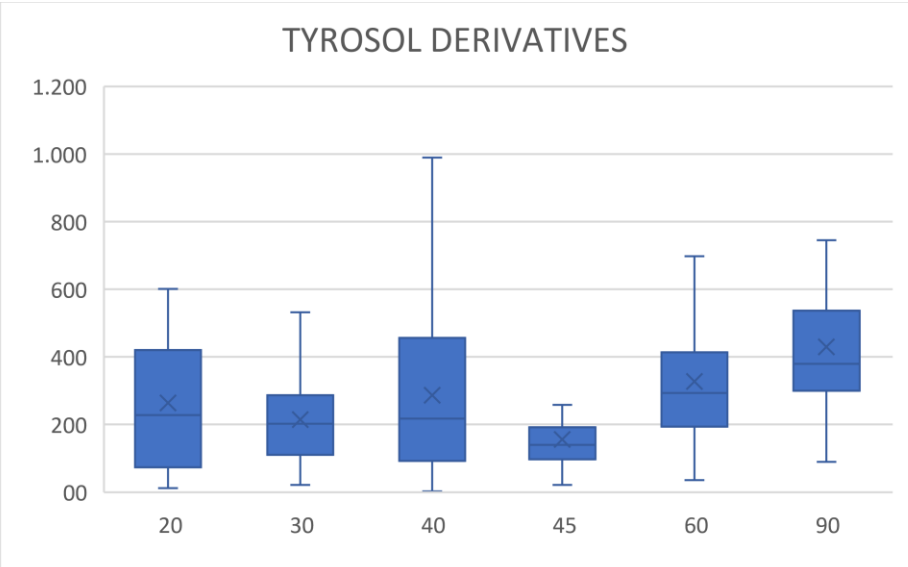
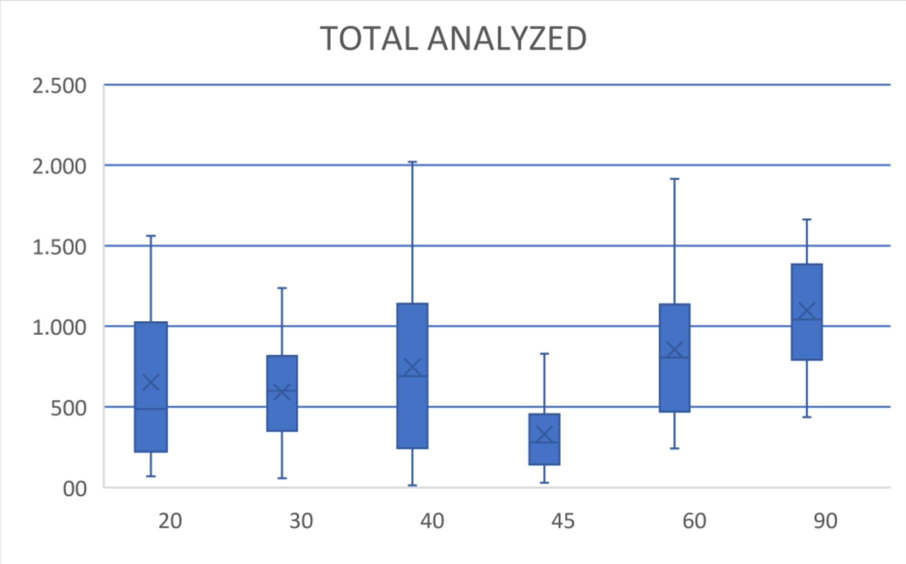
450
400
350
300
250
200
150
100
50
00
20
30
40
45
60
90


APIGENIN
04
03
03
02
02
01
01
00
20
30
40
45
60
90


LUTEOLIN
05
05
04
04
03
03
02
02
01
01
00
20
30
40
45
60
90


HYDROXYTYROSOL DERIVATIVES
1.600
1.400
1.200
1.000
800
600
400
200
00
20
30
40
45
60
90


TYROSOL DERIVATIVES
1.200
1.000
800
600
400
200
00
20
30
40
45
60
90



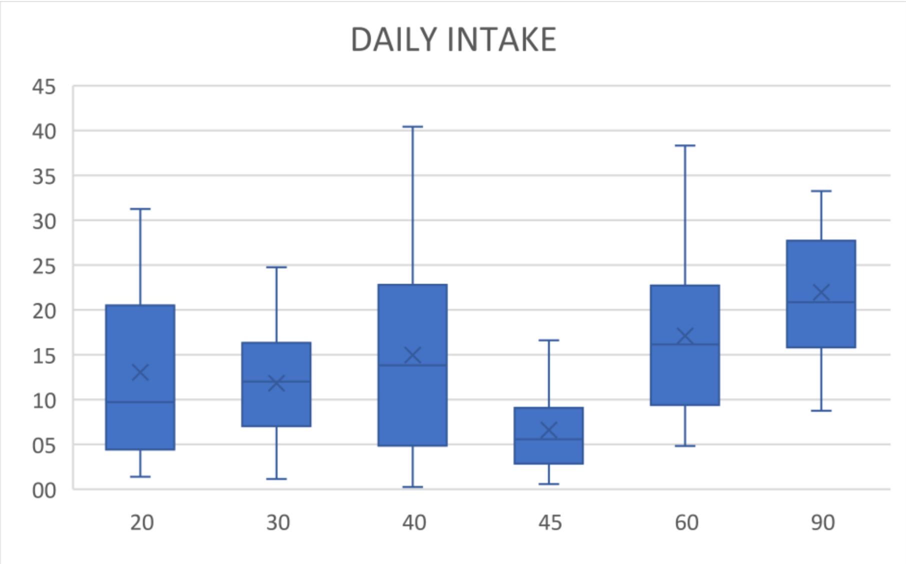


Figure 7. Concentration profiles found for the monitored phenolic compounds and associated parameters according to the malaxation time.

According to these results, it is worth mentioning that in most cases there is a decrease in the concentration of monitored phenols when the malaxation time is 45 min. However, this result could be omitted since the results for this time corresponded to only 10 samples and, particularly, these samples were characterized by a relatively low phenolic concentration. For this reason, the data corresponding to this malaxation time will be omitted in the summary of results, although they are included in the plots.

Considering this study, the next results can be deduced:

-Hydroxytyrosol seems to show an increasing trend up to 60 min, but its content was decreased when the malaxation time was set at 90 min.

-Oleacein and oleocanthal again share a common pattern. They were found at higher levels when malaxation was carried out for longer times. In fact, the maximum content of both phenols was found in samples malaxed for 90 min.

-Oleuropein aglycon and ligstroside aglycon offered the same behavior as oleocanthal and oleacein. Apparently, their concentrations increased when malaxation was prolonged for 90 min.

-Oleomissional and oleokoronal was slightly affected by the malaxation time, although there is an increased level in the oils processed for 60 and 90 min.

-Flavonoids did not report a clear trend along the period of tested times.

-Hydroxytyrosol and tyrosol derivatives reported the same performance as the other secoiridoids, particularly, oleocanthal, oleacin and oleuropein, and ligustroside aglycon.

-The same pattern was found for the total content of phenols and the daily intake parameter, which is again logical attending to the observed results.

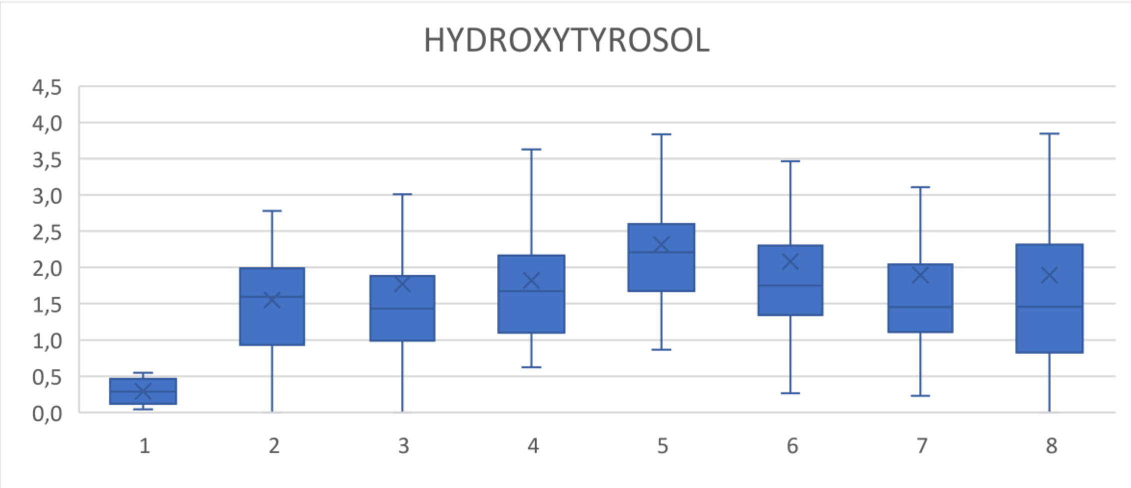
Therefore, in this context, it is quite conclusive that the malaxation time seems to increase considerably the healthy value of virgin olive oils, at least up to 90 min; while the temperature for this process should be in a range from 23 to 28ºC.

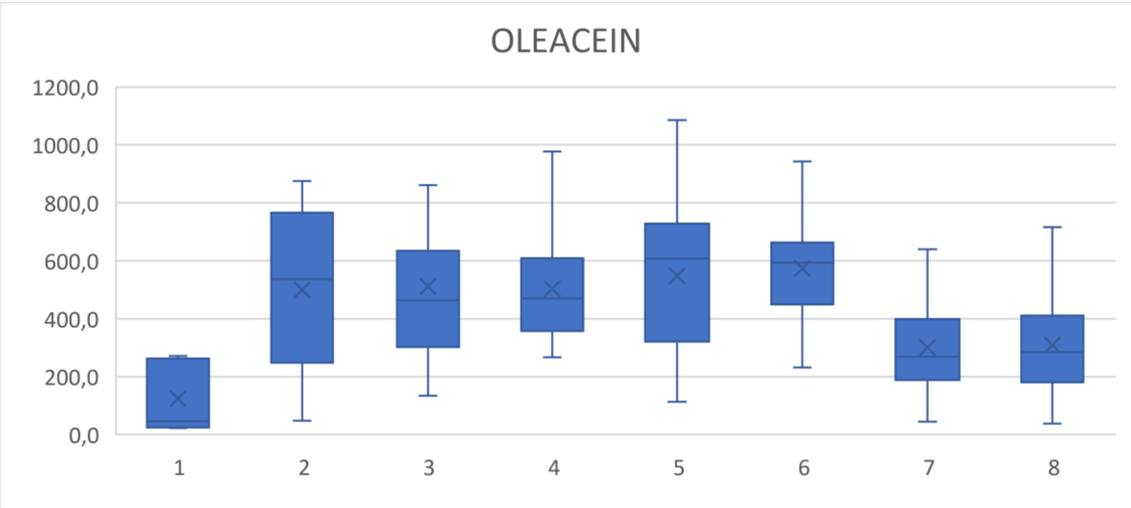
It would be of interest the development of an intervariable test to check the influence of malaxation time and temperature by evaluation of both parameters and their interaction.

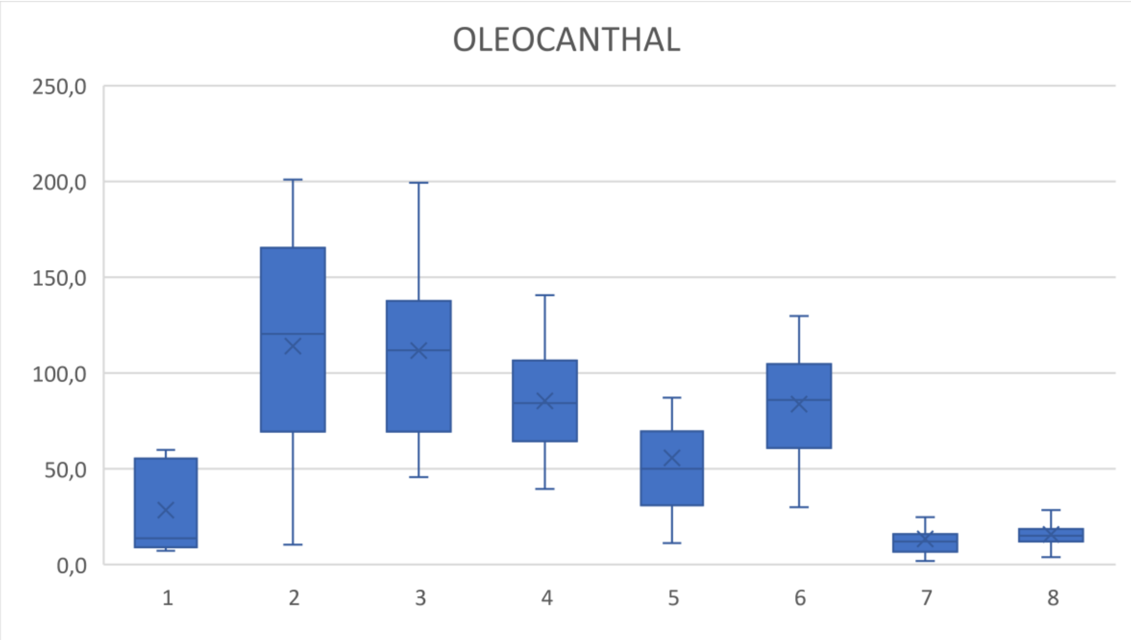
Study of specific cultivars

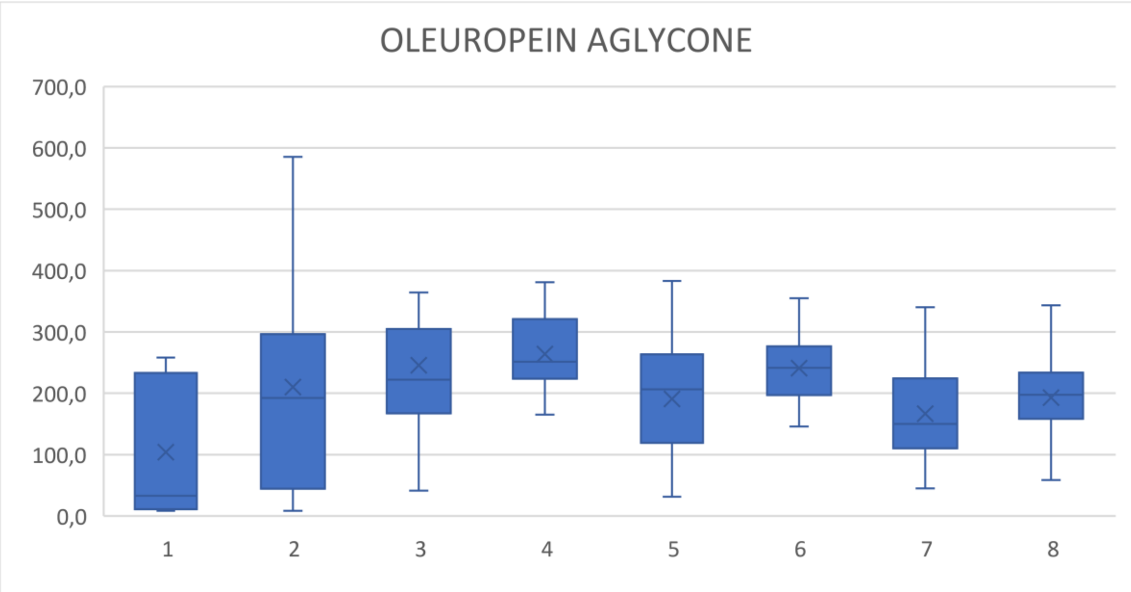
The next step was to evaluate the parameters previously studied for the complete cohort of collected samples in subsets of samples pertaining to the most abundant specific cultivars. These cultivars were Hojiblanca, Picual, Coupage and Arbequina.

Hojiblanca






OLEOCANTHAL
250,0
200,0
150,0
100,0
50,0
0,0
1
2
3
4
5
6
7
8


OLEUROPEIN AGLYCONE
700,0
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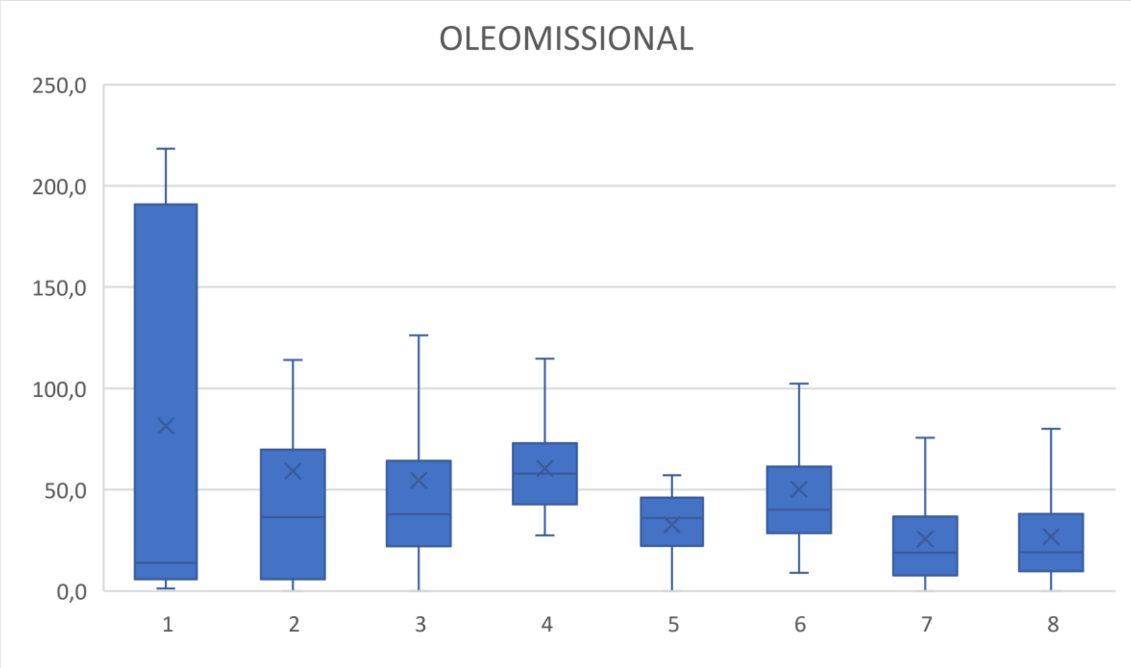
300,0
200,0
100,0
0,0
1
2
3
4
5
6
7
8


OLEOMISSIONAL
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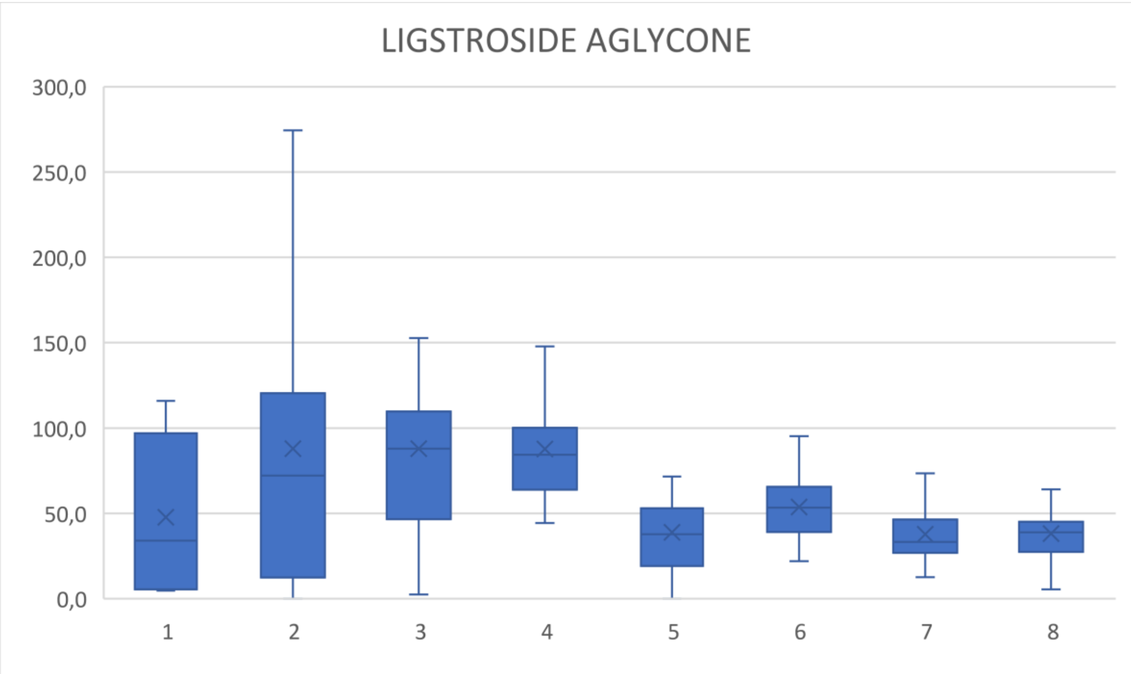
200,0
150,0
100,0
50,0
0,0
1
2
3
4
5
6
7
8


LIGSTROSIDE AGLYCONE
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250,0
200,0
150,0
100,0
50,0
0,0
1
2
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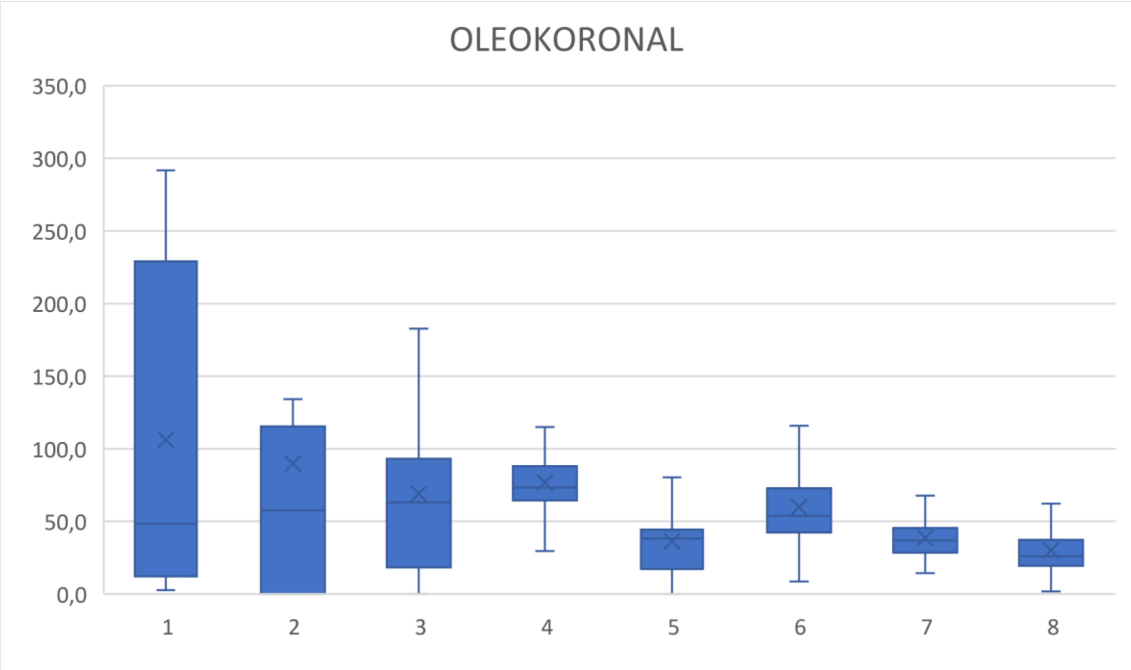
4
5
6
7
8


OLEOKORONAL
350,0
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200,0
150,0
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50,0
0,0
1
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7
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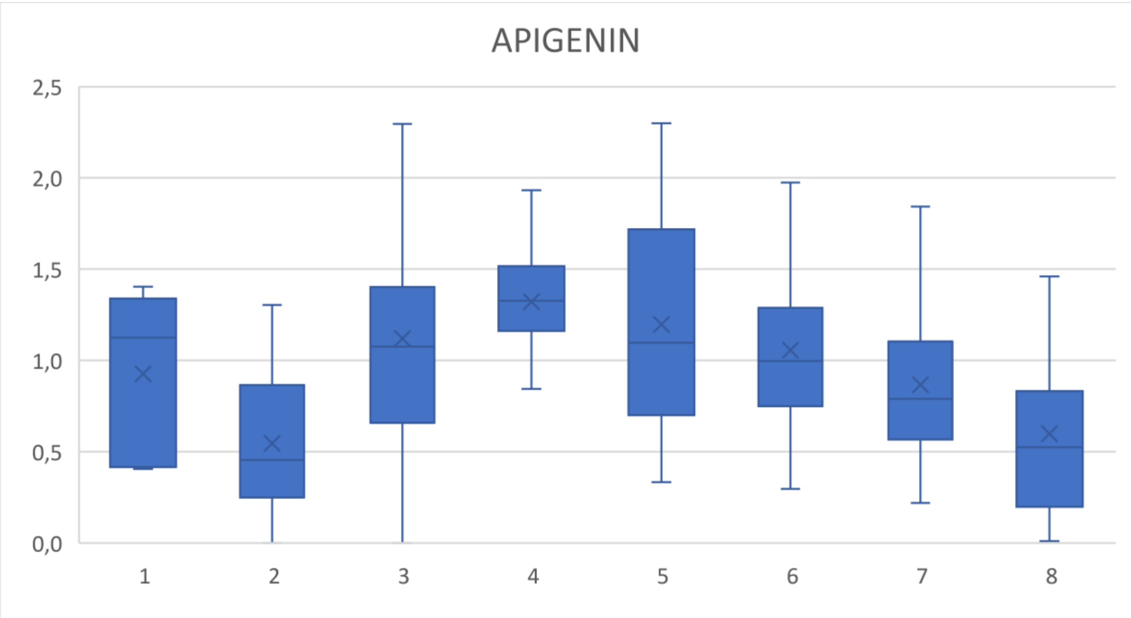
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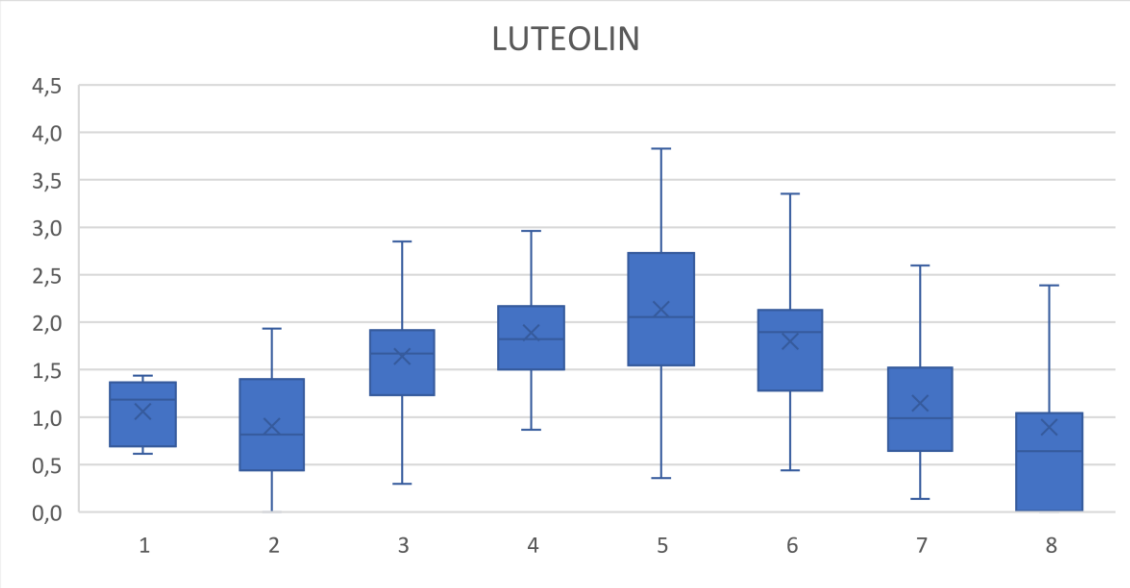
2,0
1,5
1,0
0,5
0,0
1
2
3
4
5
6
7
8


LUTEOLIN
4,5
4,0
3,5
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1,5
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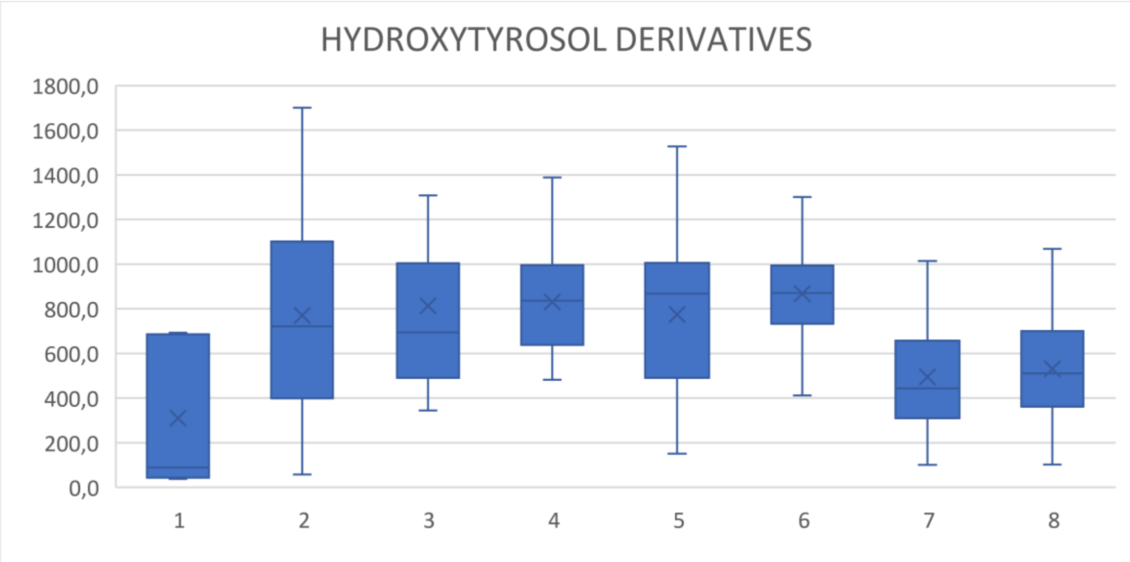
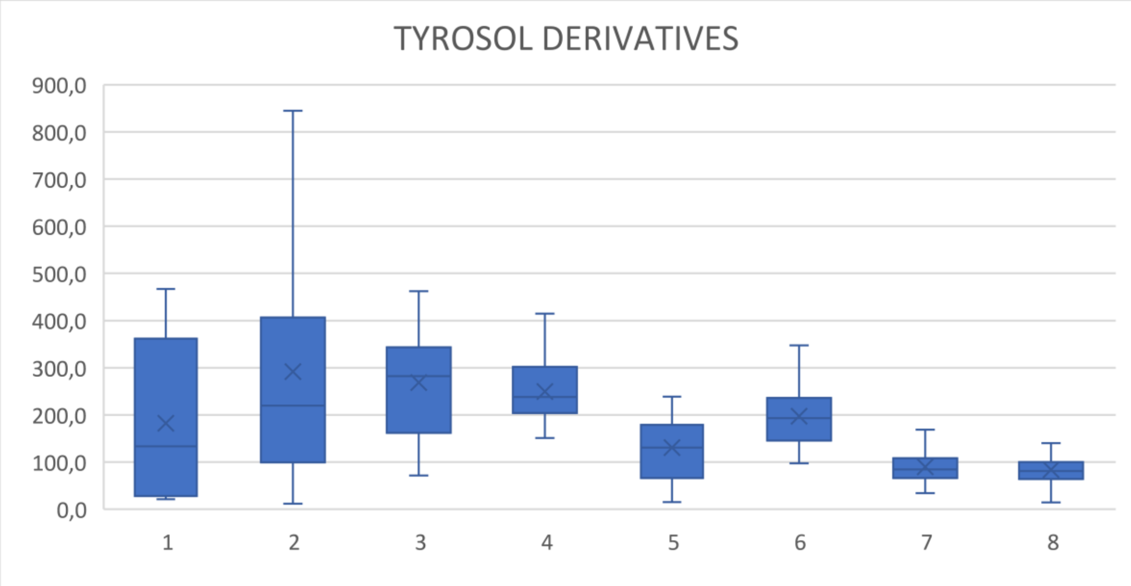
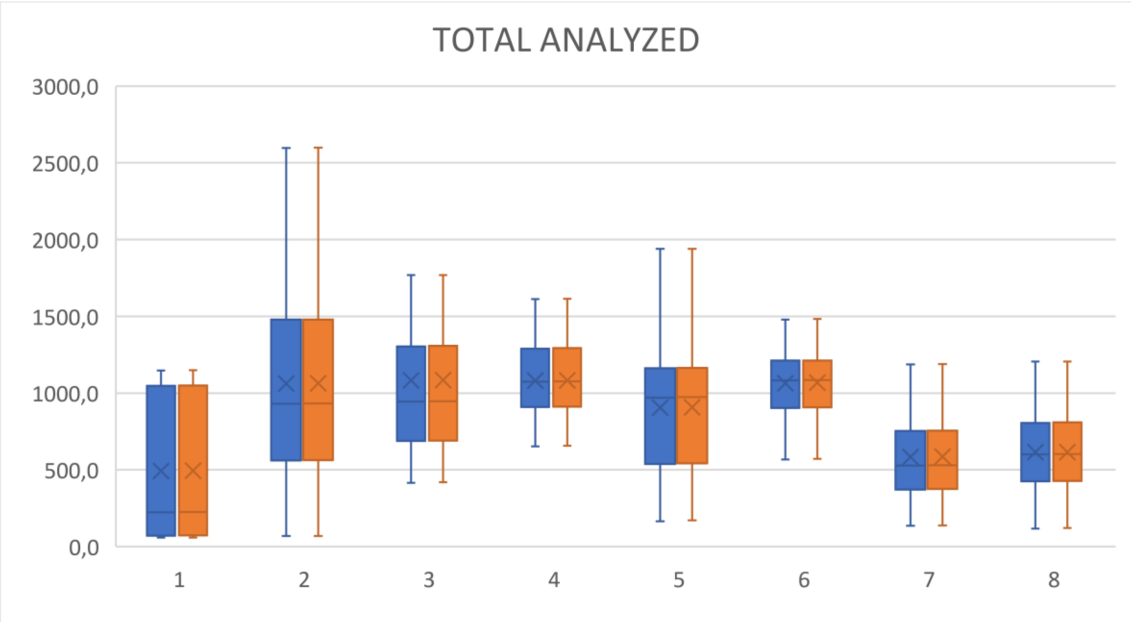
4
5
6
7
8


HYDROXYTYROSOL DERIVATIVES
1800,0
1600,0
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
1
2
3
4
5
6
7
8


TYROSOL DERIVATIVES
900,0
800,0
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
1
2
3
4
5
6
7
8


TOTAL ANALYZED
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2000,0
1500,0
1000,0
500,0
0,0
1
2
3
4
5
6
7
8

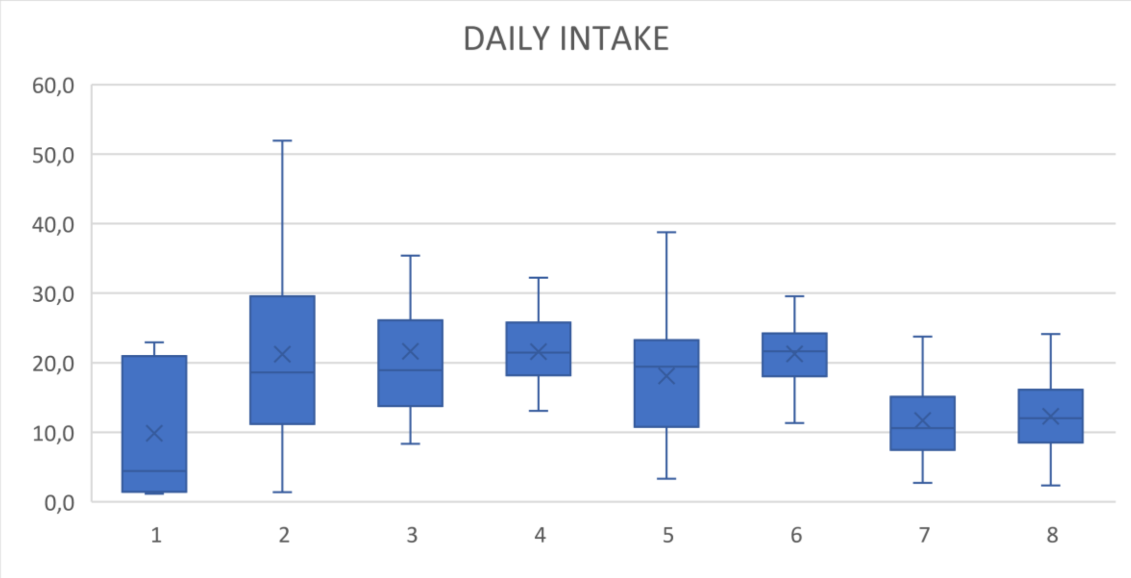


Figure 8. Concentration profiles found for the monitored phenolic compounds and associated parameters in Hojiblanca olive oil samples according to the harvesting date.

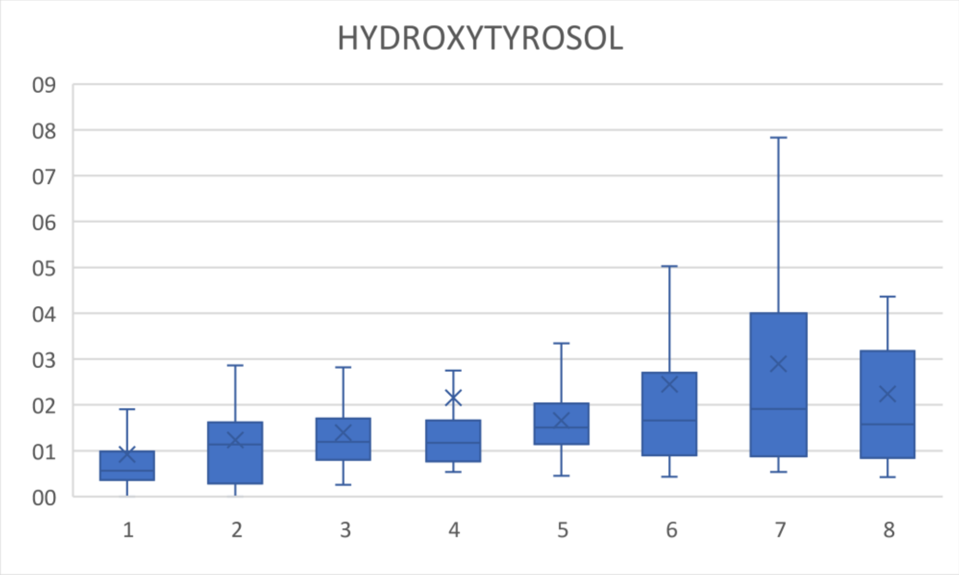
From these results, the following conclusions can be deduced:

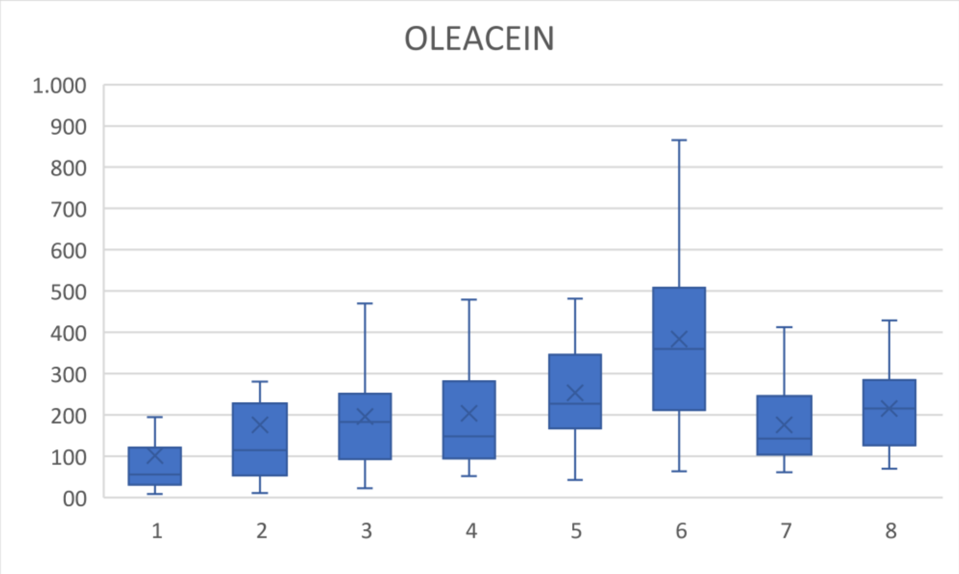
- Hydroxytyrosol decreased its concentration after 16th January.
- Oleacein was characterized by a plateau from 8th November to 31st January, which means that this compound reached a high concentration during a long season period.
- Oleocanthal decreased its concentration from 8th November. In fact, the maximum concentration was reached from 8th November to 10th December.
- Oleuropein aglycon achieved its maximum concentration from 27th November to 31st January. Similar behavior was found for ligstroside aglycon, although the window reaching its maximum concentration was from 8th November to 27th December.
- Oleokoronal and oleomissional decreased their concentrations from the beginning of the season. The first period was characterized in both cases by a high variability. However, the decreasing trend is clearly observed.

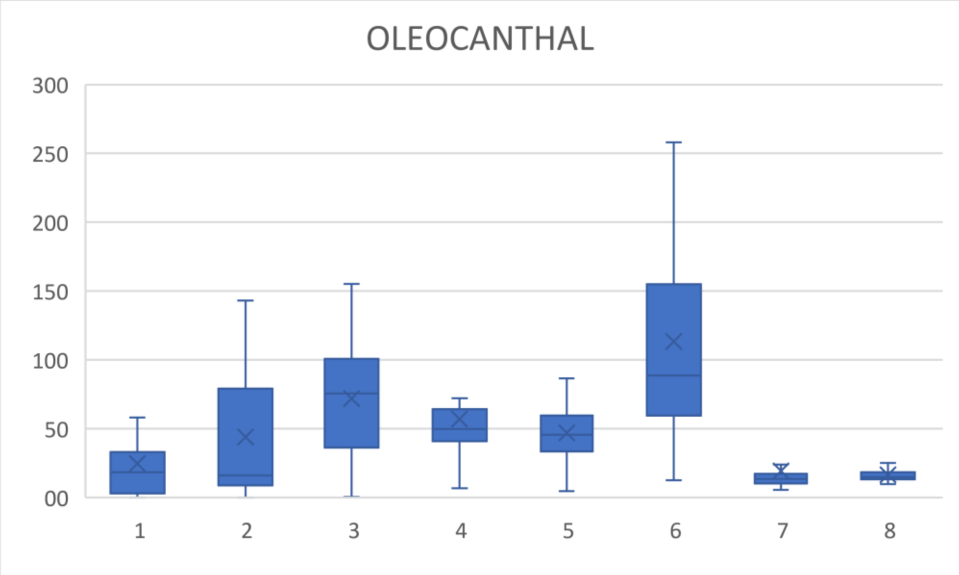
- Luteolin reported its maximum concentration from 28th December to 16th January. On the other hand, apigenin was at its maximum level for a longer period: from 8th November to 31st January.
- Hydroxytyrosol and tyrosol derivatives were marked by the evolution of their corresponding secoiridoids. Thus, hydroxytyrosol derivatives were found at maximum levels in olive oils produced from 8th November to 31st January. Tyrosol derivatives were at maximum level from 8th November to 27th December.
- The daily intake parameter behaved in a similar way to hydroxytyrosol derivatives considering that these compounds, oleacein and oleuropein aglycon, were at higher concentration than tyrosol derivates, oleocanthal and ligstroside aglycon.

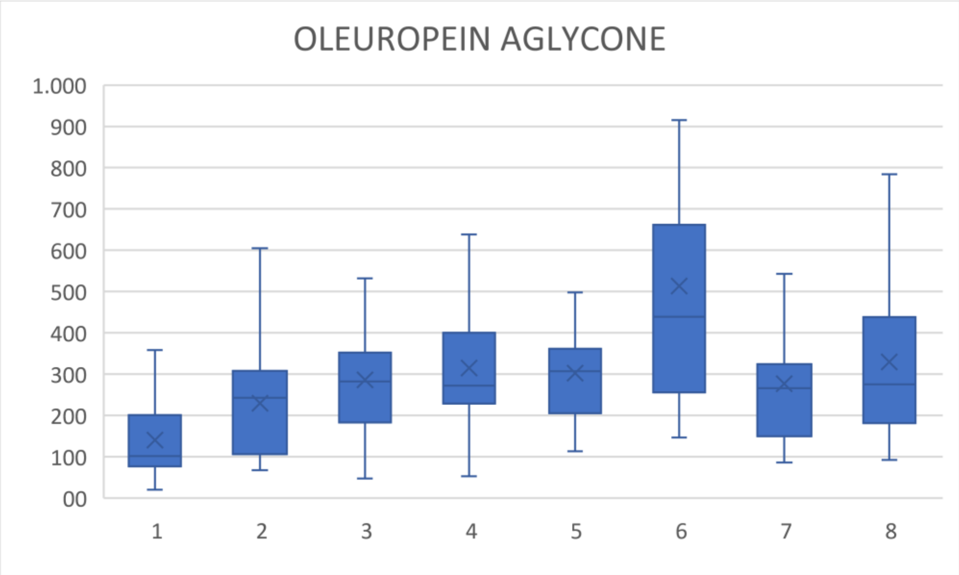
In the case of Hojiblanca there were not samples with enough variability to check the incidence of the malaxation parameters, temperature and time.

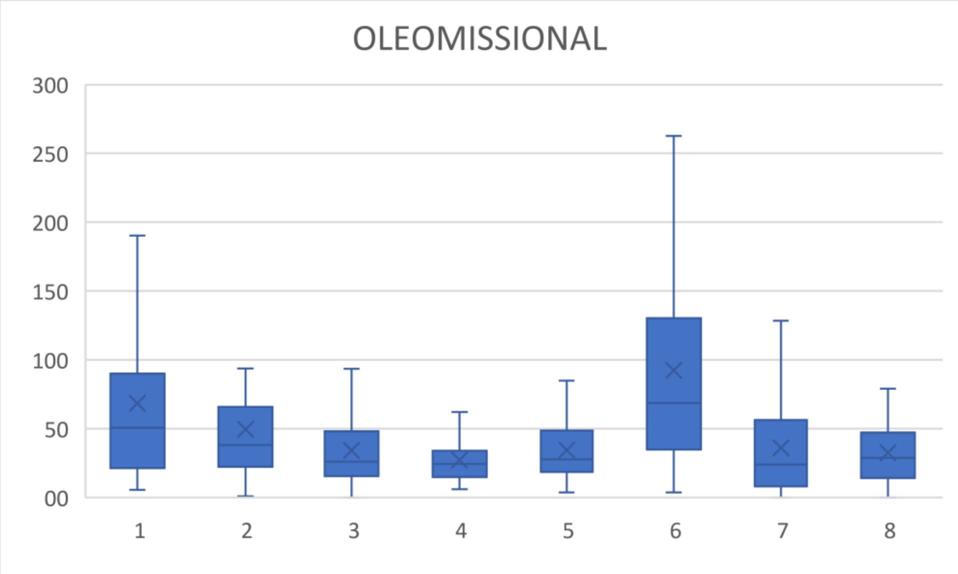
Picual

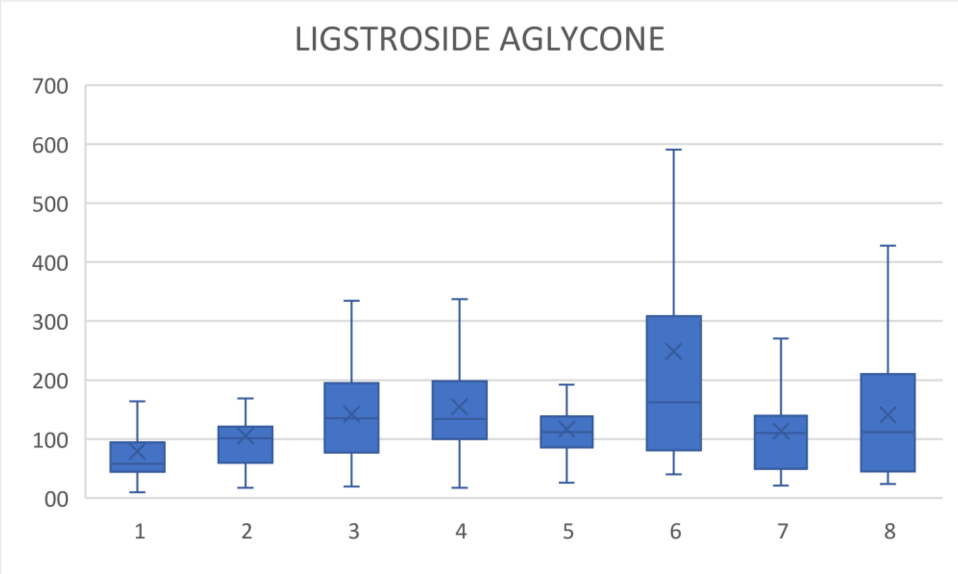

HYDROXYTYROSOL
09
08
07
06
05
04
03
02
01
00
1
2
3
4
5
6
7
8

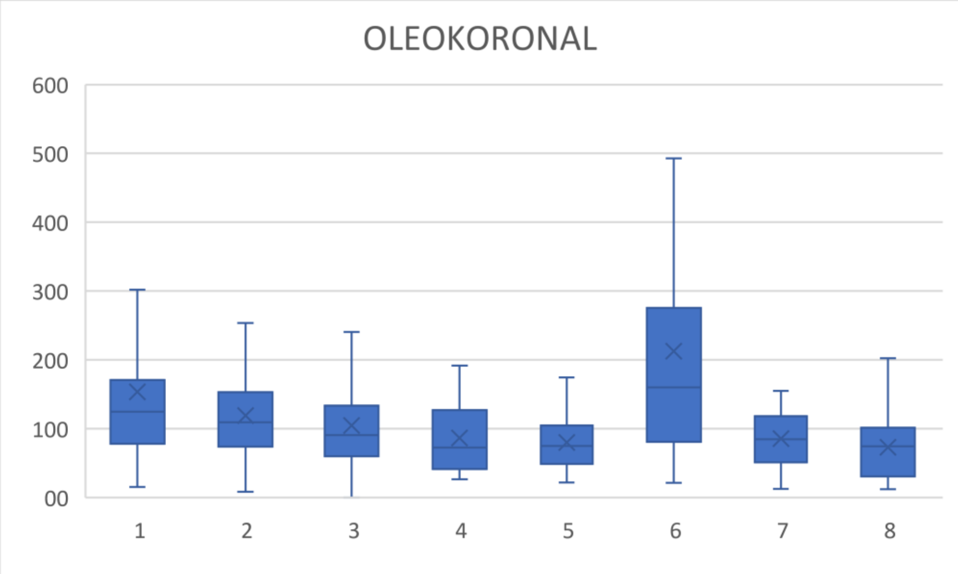

OLEACEIN
1.000
900
800
700
600
500
400
300
200
100
00
1
2
3
4
5
6
7
8

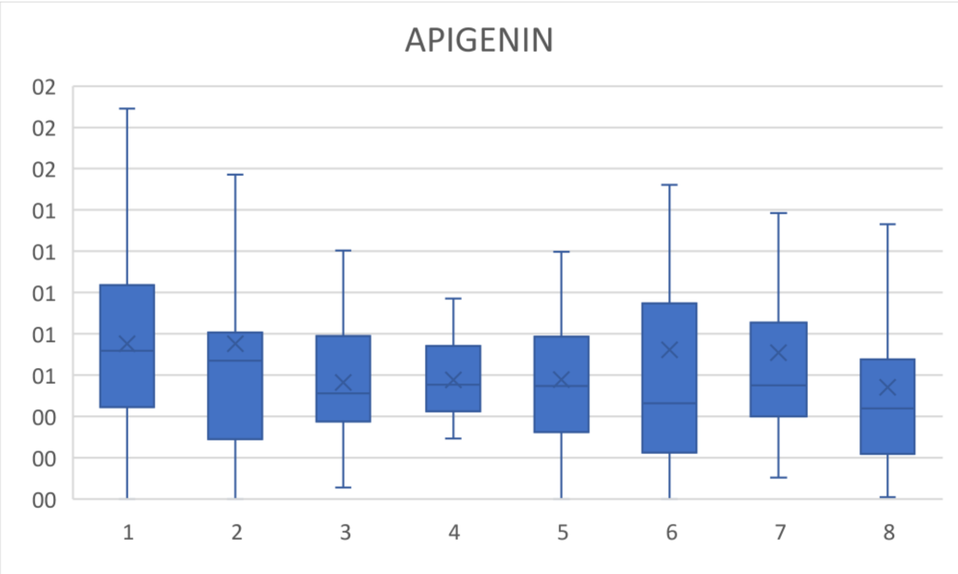

OLEOCANTHAL
300
250
200
150
100
50
00
1
2
3
4
5
6
7
8

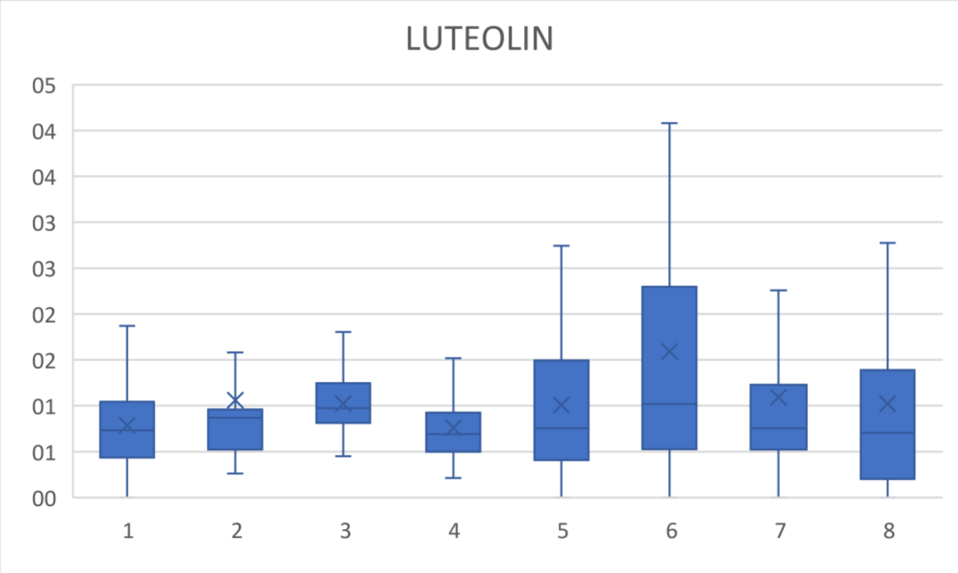

OLEUROPEIN AGLYCONE
1.000
900
800
700
600
500
400
300
200
100
00
1
2
3
4
5
6
7
8

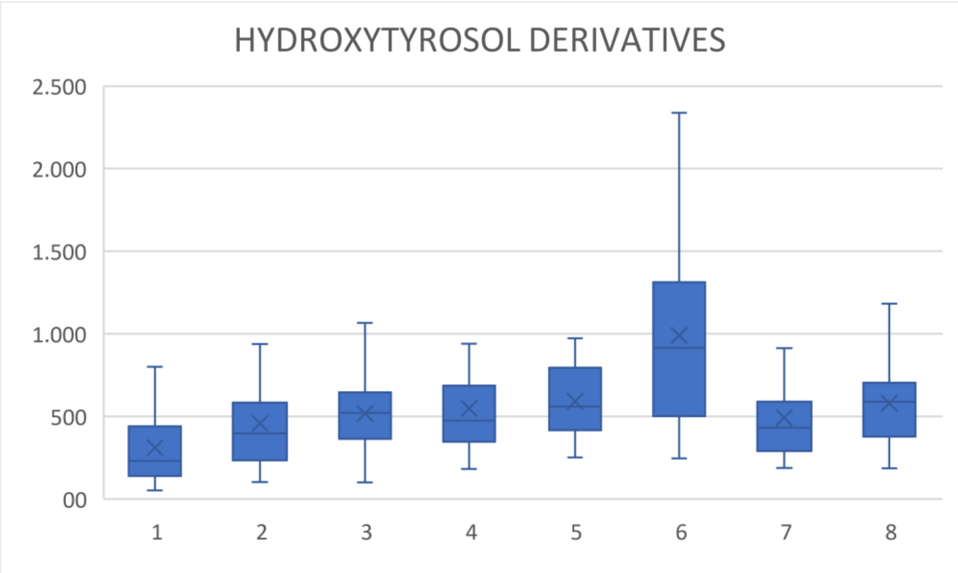

OLEOMISSIONAL
300
250
200
150
100
50
00
1
2
3
4
5
6
7
8

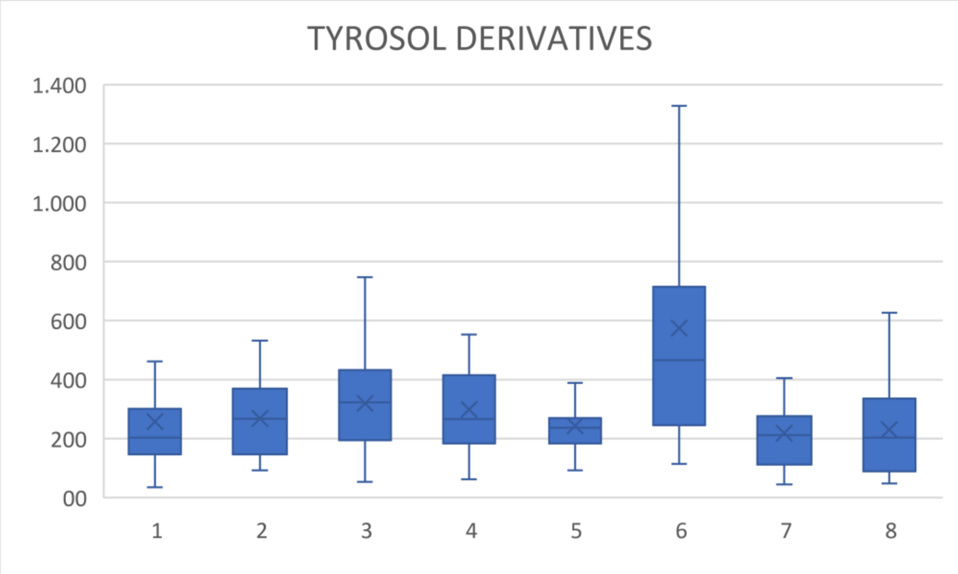

LIGSTROSIDE AGLYCONE
700
600
500
400
300
200
100
00
1
2
3
4
5
6
7
8


OLEOKORONAL
600
500
400
300
200
100
00
1
2
3
4
5
6
7
8


APIGENIN
02
02
02
01
01
01
01
01
00
00
00
1
2
3
4
5
6
7
8


LUTEOLIN
05
04
04
03
03
02
02
01
01
00
1
2
3
4
5
6
7
8


HYDROXYTYROSOL DERIVATIVES
2.500
2.000
1.500
1.000
500
00
1
2
3
4
5
6
7
8


TYROSOL DERIVATIVES
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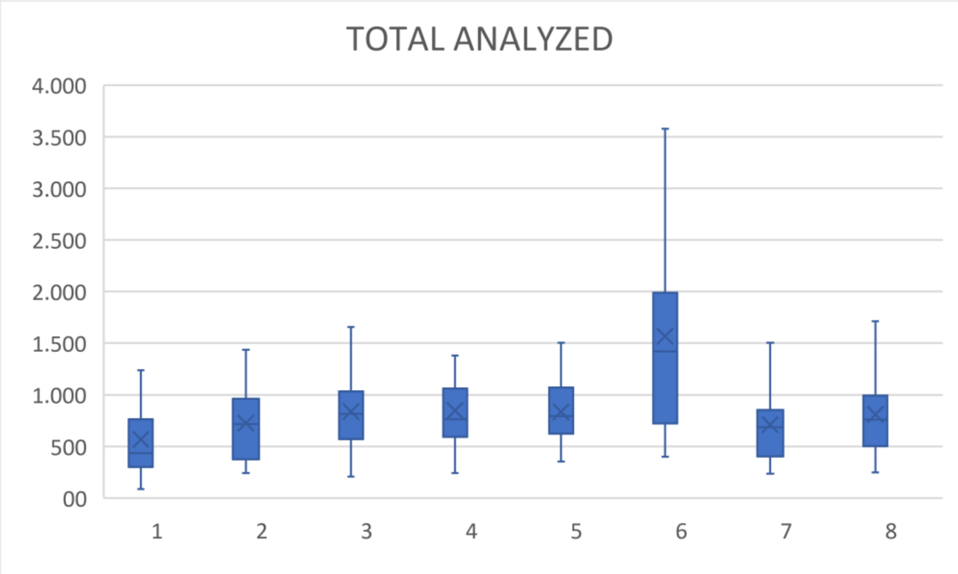
1.200
1.000
800
600
400
200
00
1
2
3
4
5
6
7
8


TOTAL ANALYZED
4.000
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3.000
2.500
2.000
1.500
1.000
500
00
1
2
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4
5
6
7
8

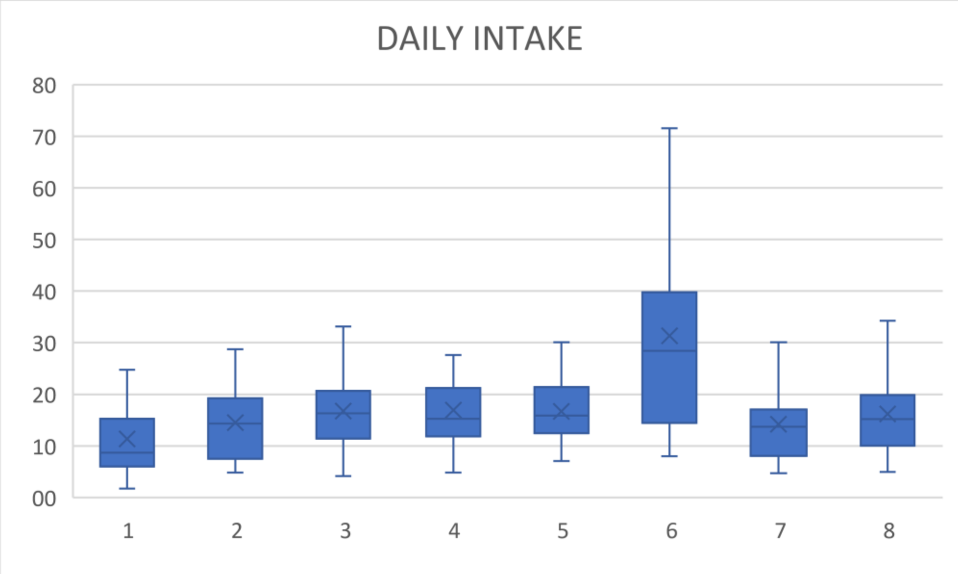


Figure 9. Concentration profiles found for the monitored phenolic compounds and associated parameters in Picual olive oil samples according to the harvesting date.

From these results, the following conclusions can be deduced:

- Hydroxytyrosol reached its maximum concentration from January up to 16th February.
- Oleacein was characterized by an increasing trend up to G6. This last period corresponds from 17th January to 31st January.
- Oleocanthal followed a sinusoidal trend since a first maximum was achieved from 27th November to 10th December, while a second peak was achieved from 17th January to 31st January. After this period, oleocanthal decreased considerably.
- Oleuropein aglycon followed a profile similar to oleocanthal. A first maximum was achieved from 11th December to 27th December, while the second peak was reached from 17th January to 31st January. The same behavior was found for ligstroside aglycon.
- Oleokoronal and oleomissional decreased their concentrations from the beginning of the season, although a different behavior was observed for Picual, since a peak was found within the period 17th January–31st January. According

to this trend, it seems that an external variability source is modifying the observed trend.

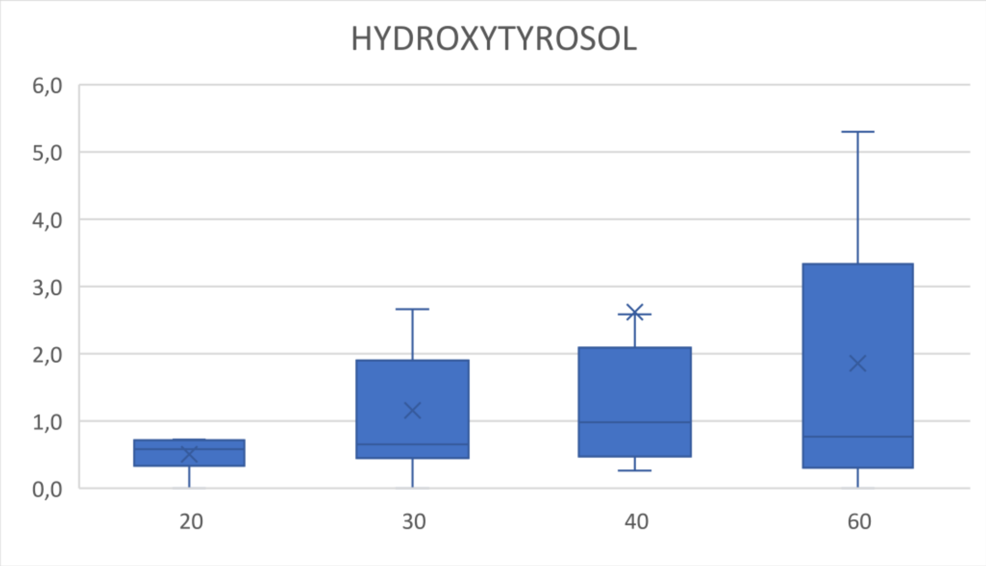
- Luteolin and apigenin did not show a clear trend in the period observed for Picual.
- Hydroxytyrosol and tyrosol derivatives were marked by the evolution of their corresponding secoiridoids. Thus, hydroxytyrosol derivatives were found at maximum levels in olive oils produced from 17th January to 31st January; while tyrosol derivatives were at maximum level from 27th November to 10th December.
- The daily intake parameter behaved in a way similar to hydroxytyrosol derivatives considering that these compounds, oleacein and oleuropein aglycon, were at higher concentration than tyrosol derivatives oleocanthal and ligstroside aglycon.

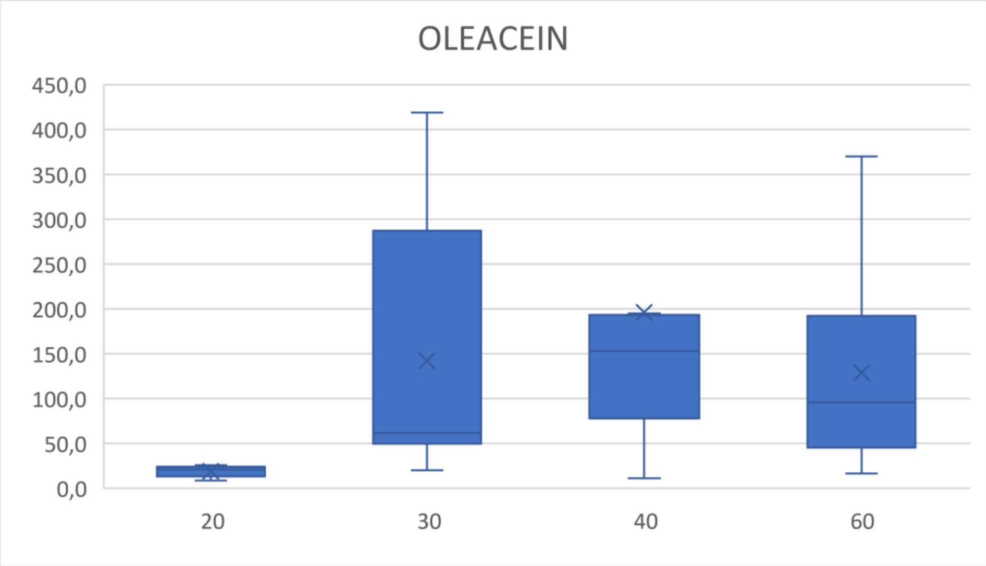
Concerning the malaxation time, this parameter was evaluated within the range from 20 to 60 min. The number of samples considered for each malaxation time is as follows:

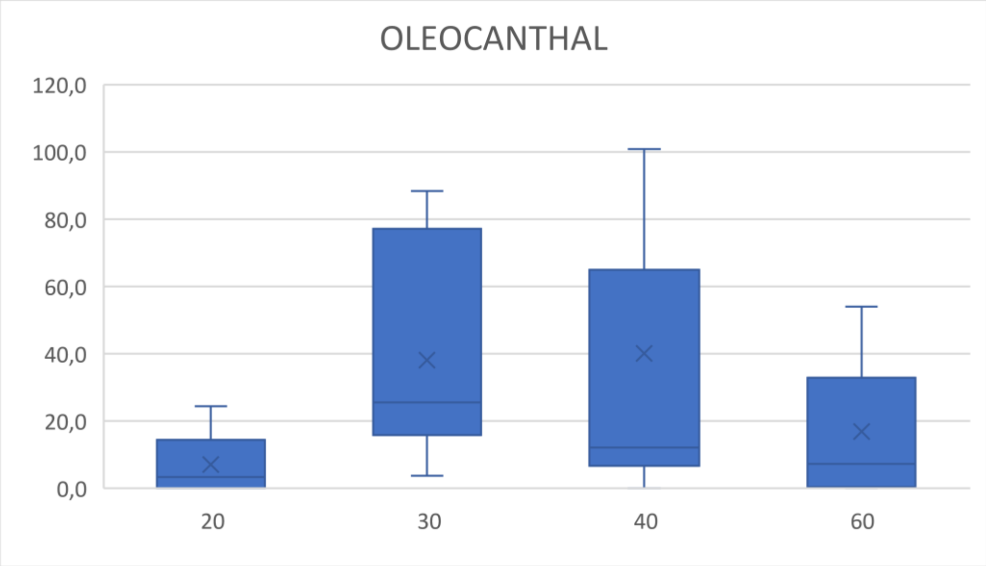
Malaxation time	Picual samples
20	6
30	15
40	13
45	4
60	9
75	253
90	1

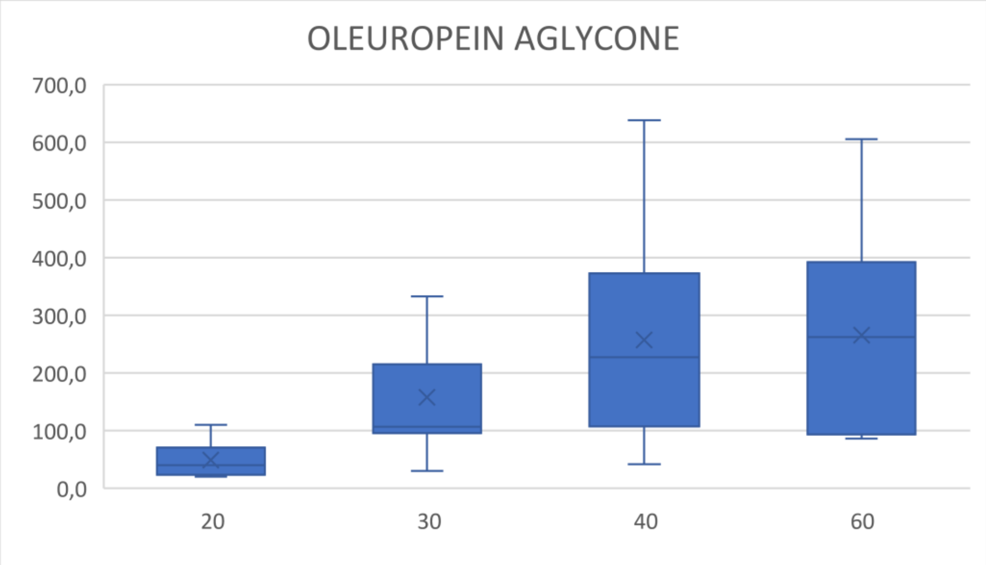
Table 6. Distribution of samples according to the malaxation time.

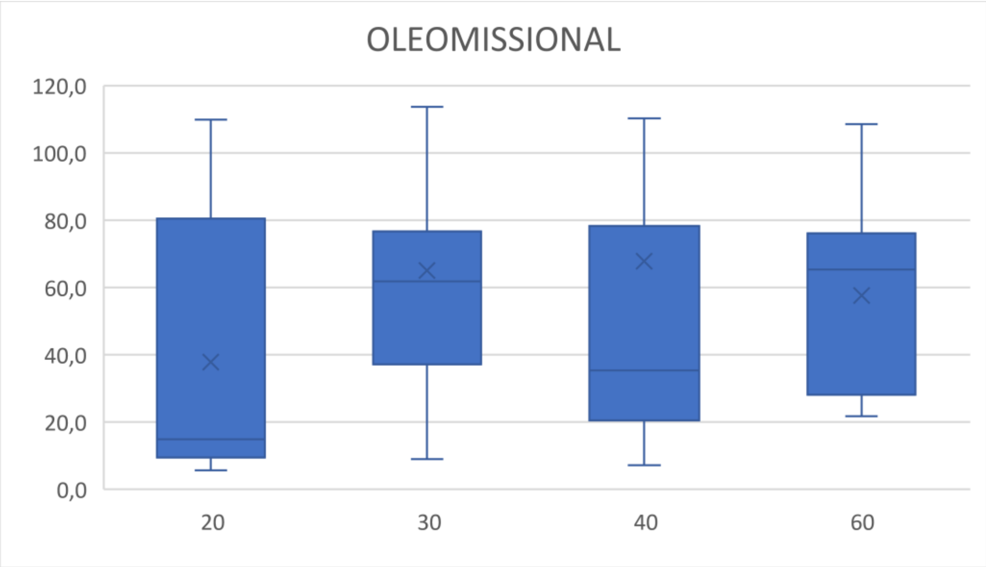
The malaxation times considered for the comparison are highlighted in yellow. The comparison revealed the following results:

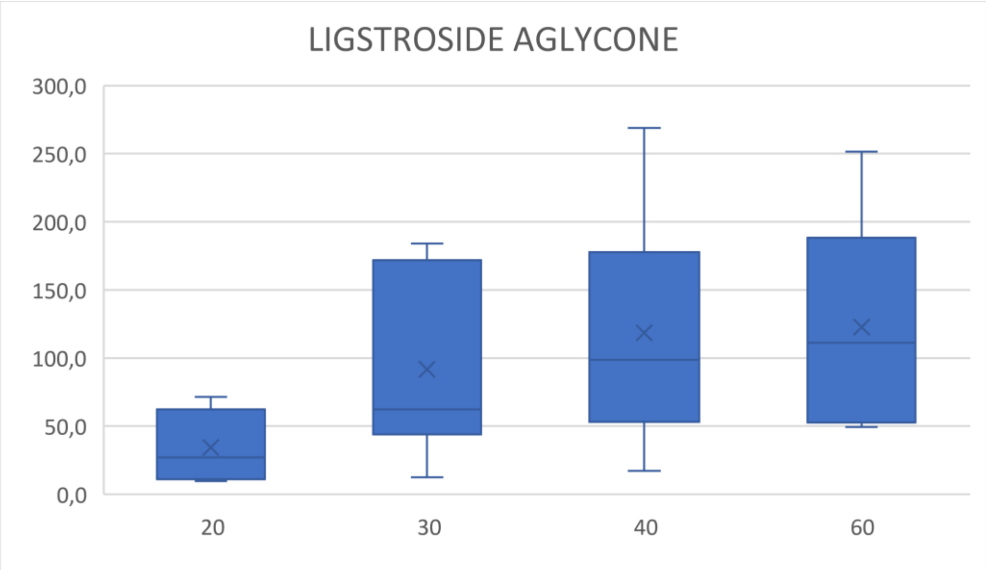

HYDROXYTYROSOL
6,0
5,0
4,0
3,0
2,0
1,0
0,0
20
30
40
60

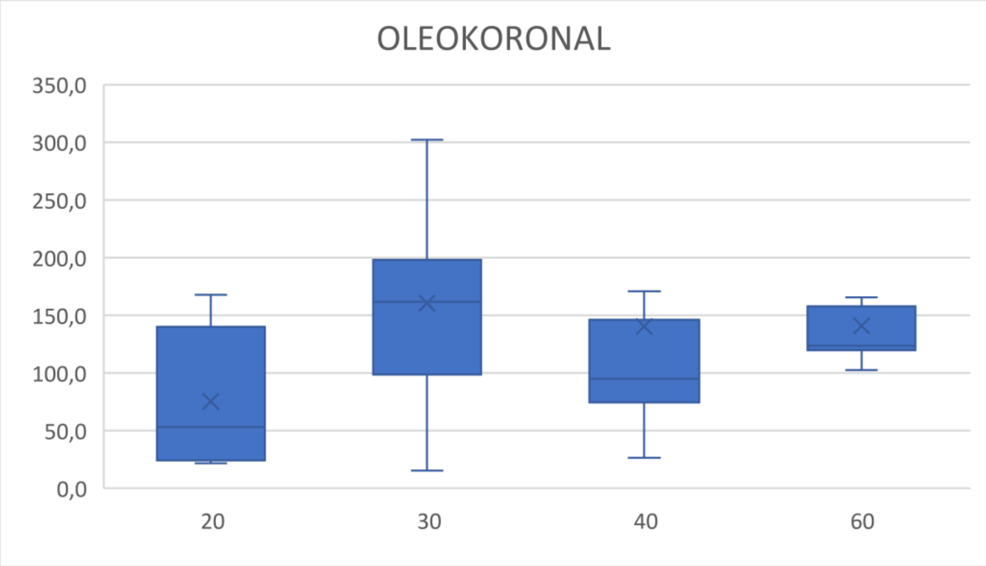

OLEACEIN
450,0
400,0
350,0
300,0
250,0
200,0
150,0
100,0
50,0
0,0
20
30
40
60


OLEOCANTHAL
120,0
100,0
80,0
60,0
40,0
20,0
0,0
20
30
40
60


OLEUROPEIN AGLYCONE
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
20
30
40
60


OLEOMISSIONAL
120,0
100,0
80,0
60,0
40,0
20,0
0,0
20
30
40
60


LIGSTROSIDE AGLYCONE
300,0
250,0
200,0
150,0
100,0
50,0
0,0
20
30
40
60


OLEOKORONAL
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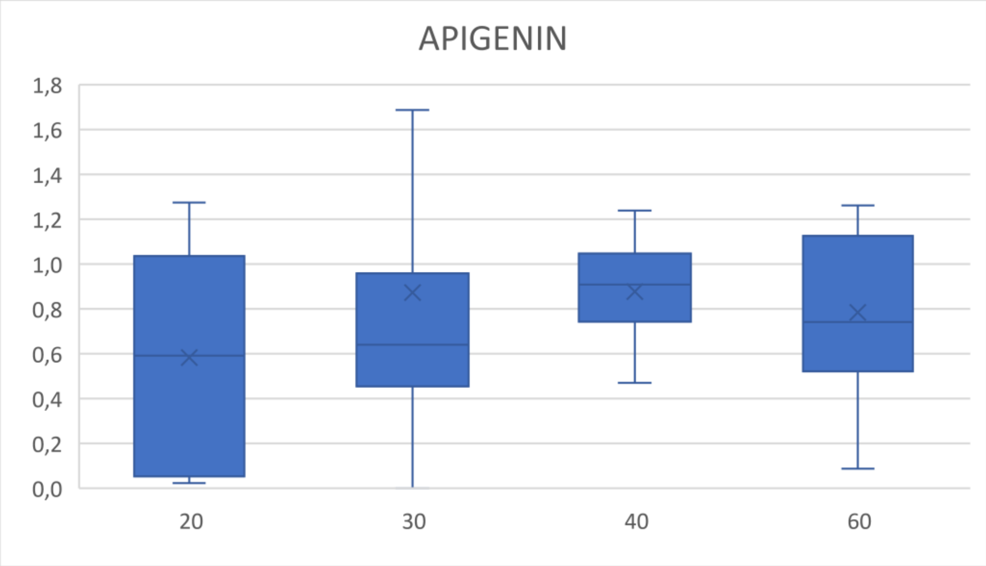
300,0
250,0
200,0
150,0
100,0
50,0
0,0
20
30
40
60


APIGENIN
1,8
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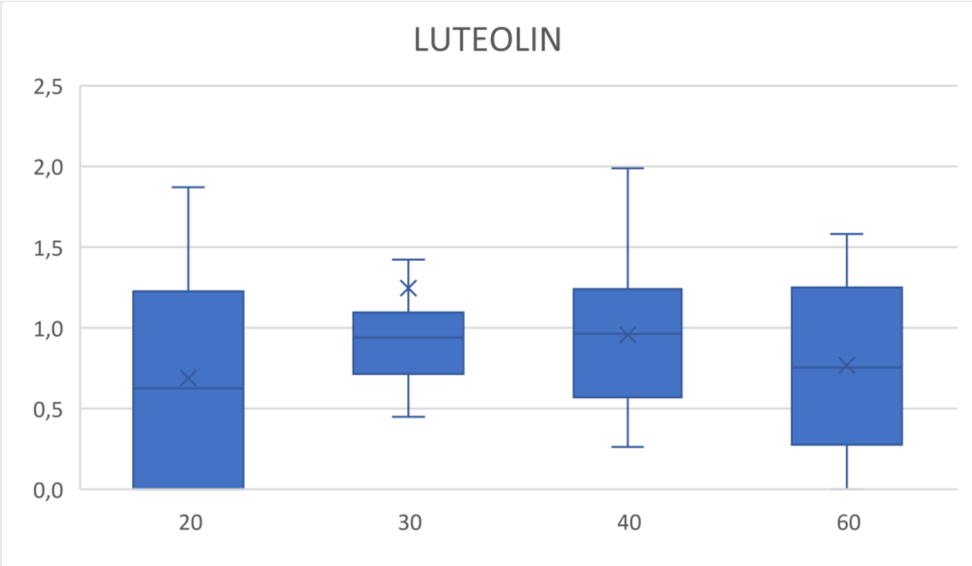
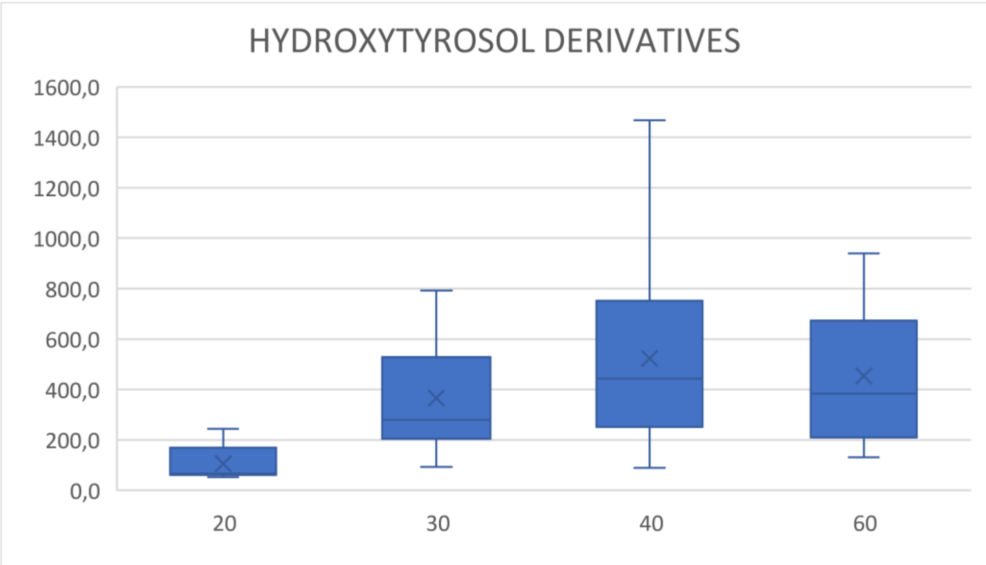
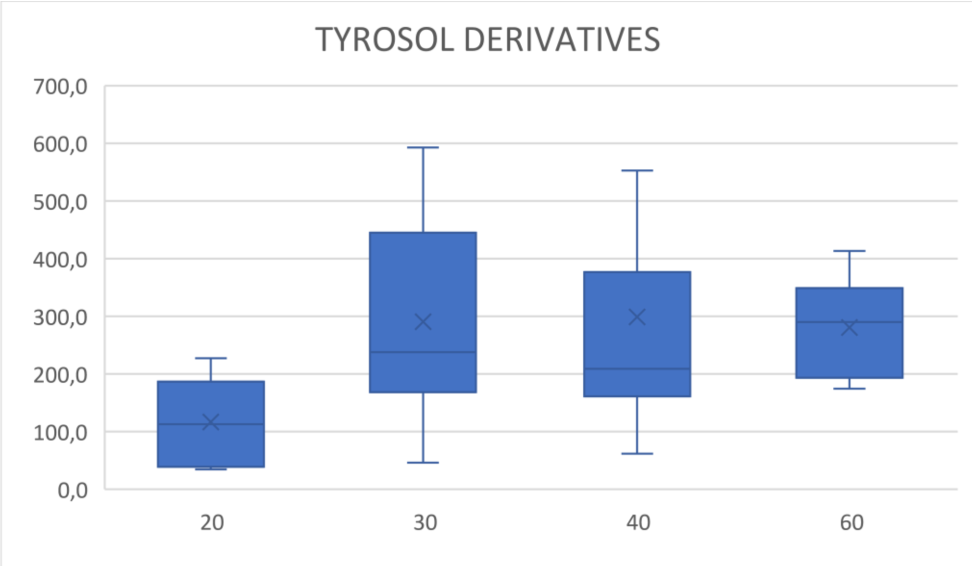
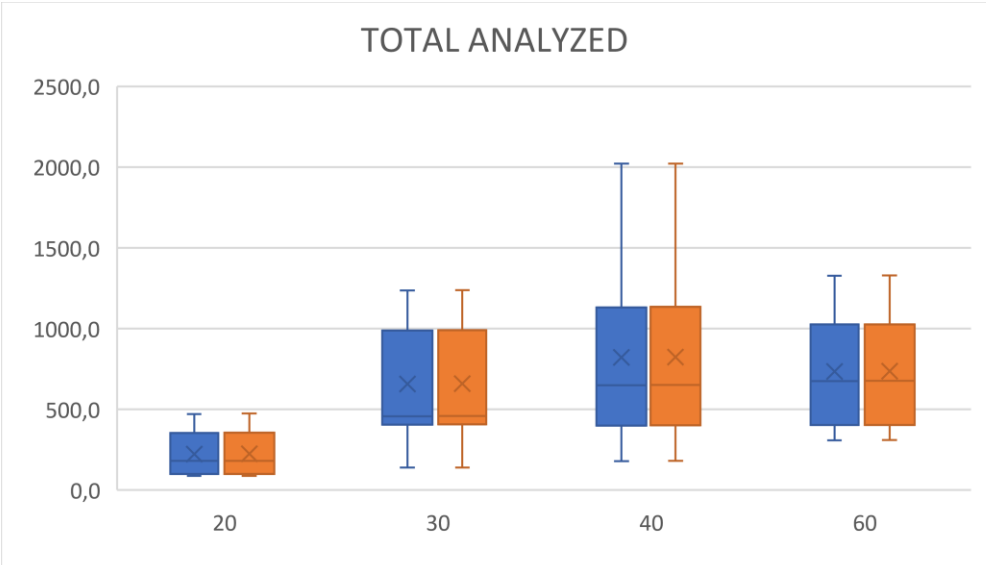
1,2
1,0
0,8
0,6
0,4
0,2
0,0
20
30
40
60


LUTEOLIN
2,5
2,0
1,5
1,0
0,5
0,0
20
30
40
60


HYDROXYTYROSOL DERIVATIVES
1600,0
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
20
30
40
60


TYROSOL DERIVATIVES
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
20
30
40
60


TOTAL ANALYZED
2500,0
2000,0
1500,0
1000,0
500,0
0,0
20
30
40
60

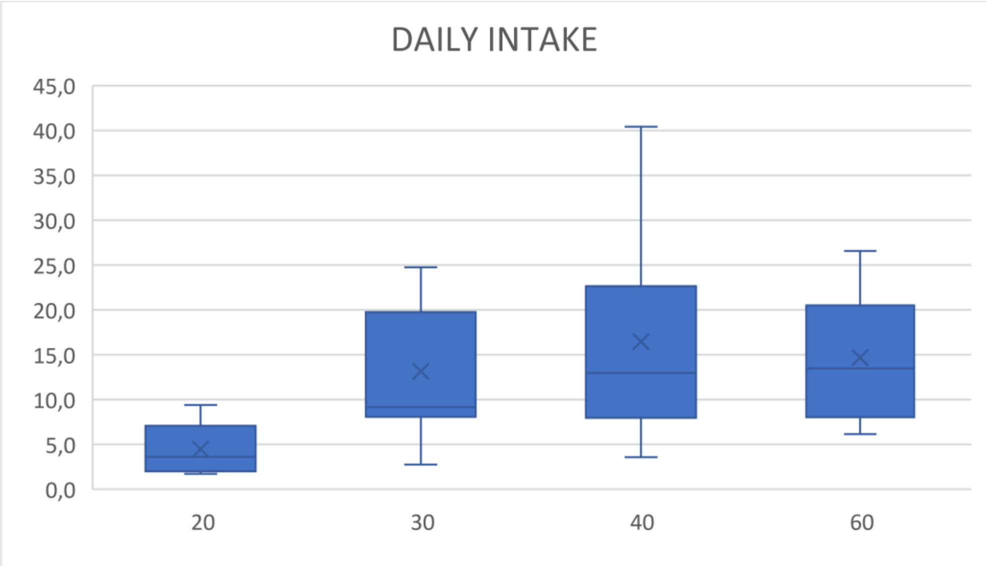


Figure 10. Concentration profiles found for the monitored phenolic compounds and associated parameters in Picual olive oil samples according to the malaxation time.

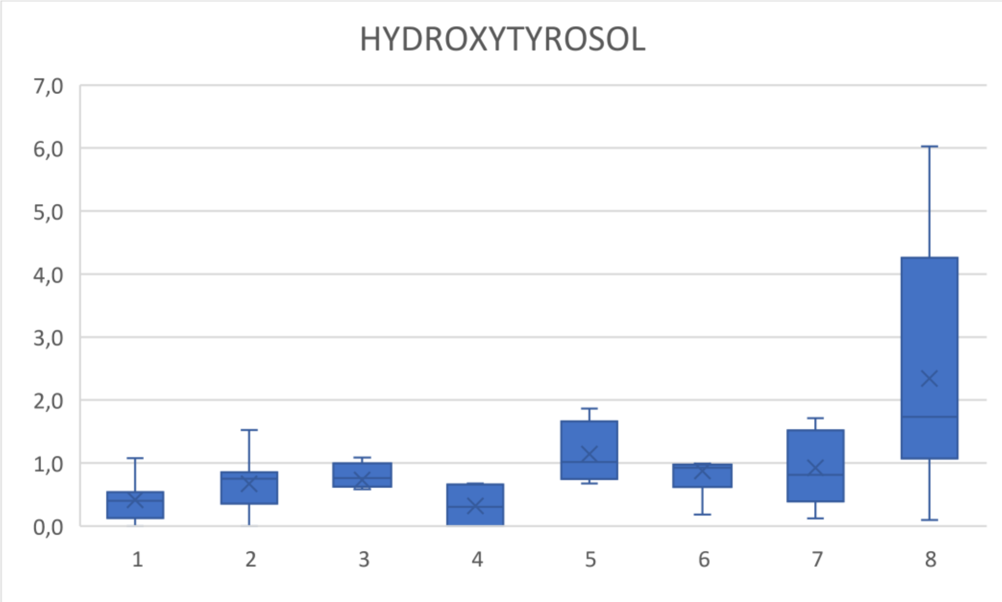
The results that can be deduced from these plots are:

- Hydroxytyrosol increased its concentration when the malaxation time increased.
- Oleacein and oleocanthal had the same behavior. Their maximum levels were attained at 30 min.
- On the other hand, oleuropein aglycon and ligstroside aglycon increased their levels in olive oil when the malaxation time increased. In both cases no differences were observed between 40 and 60 min.
- Oleomissional did not show a clear trend with the malaxation time. Oleokoronal seems to offer an increase of concentration with the increase of the malaxation time.
- Apigenin and luteolin also increased when the malaxation time increased, although 40 min slightly offered higher concentration levels.
- Hydroxytyrosol derivatives reported higher concentrations at 40 min malaxation time, while tyrosol derivatives were higher from 30 to 60 min. Apparently, no differences were observed from 30 min to 60 min.

- As hydroxytyrosol derivatives were more concentrated in Picual olive oil, the daily intake parameter was marked by this behavior. Thus, the optimum malaxation time considering these samples was 40 min.

The malaxation temperature variability of Picual did not allow developing a comparative study due to an unbalanced distribution of samples at different temperature values.

Arbequina


HYDROXYTYROSOL
7,0
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1,0
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1
2
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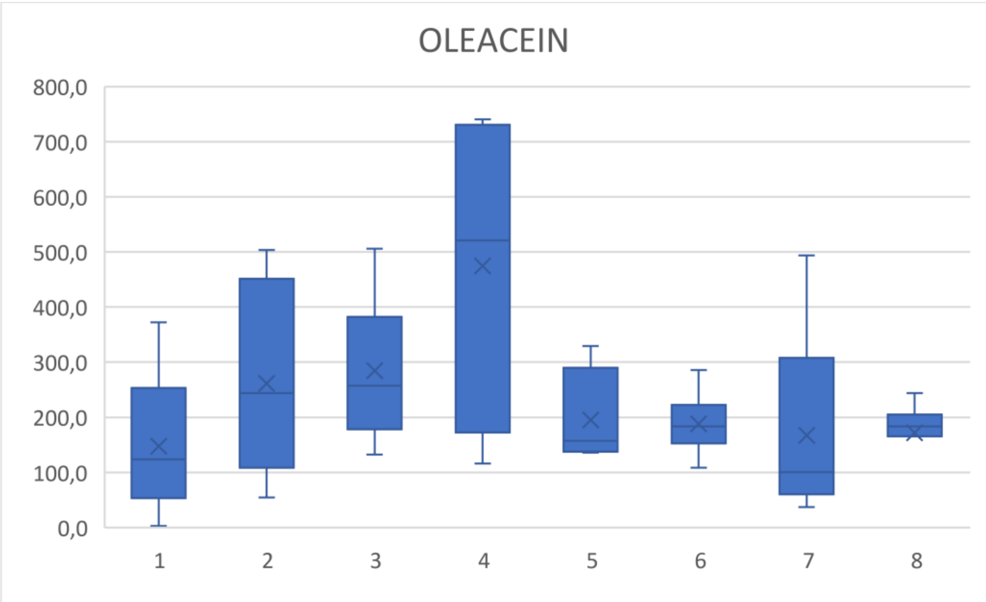
4
5
6
7
8


OLEACEIN
800,0
700,0
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500,0
400,0
300,0
200,0
100,0
0,0
1
2
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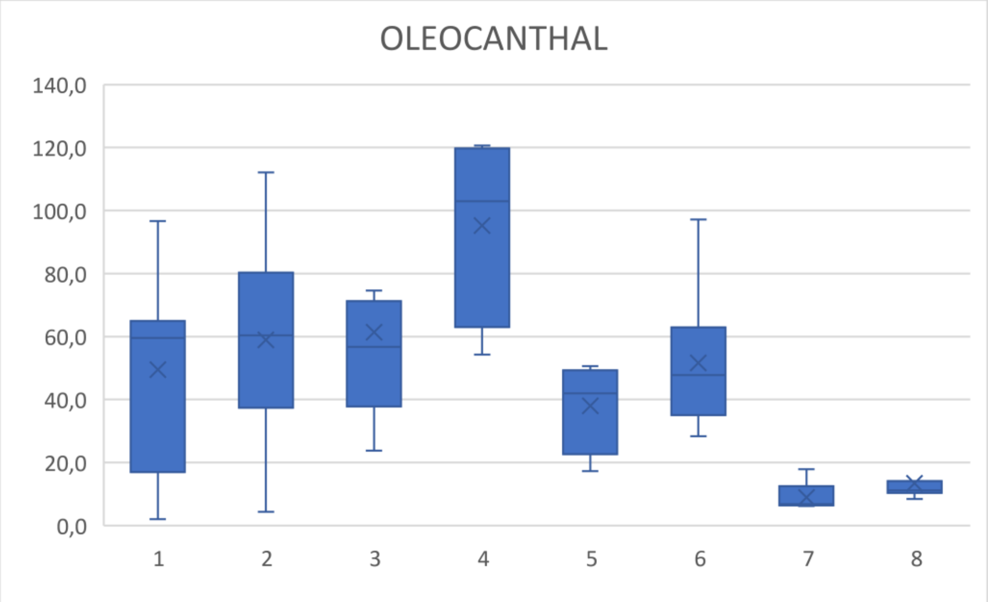
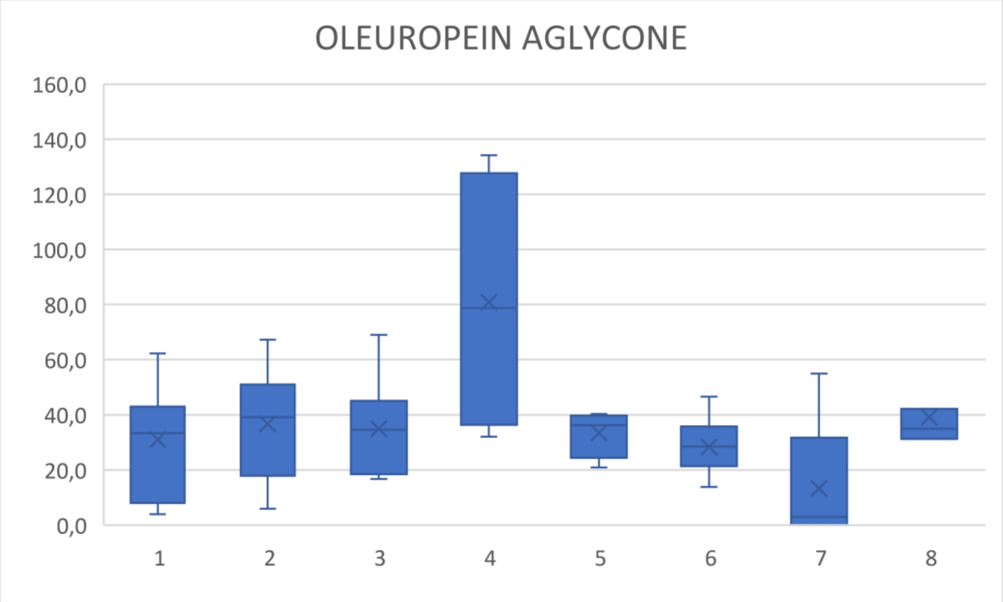
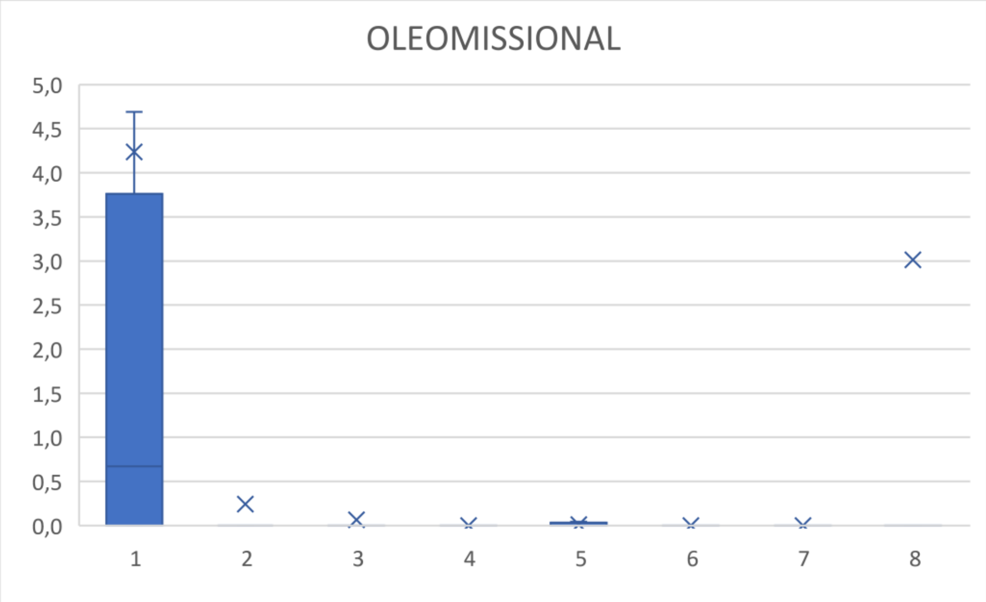
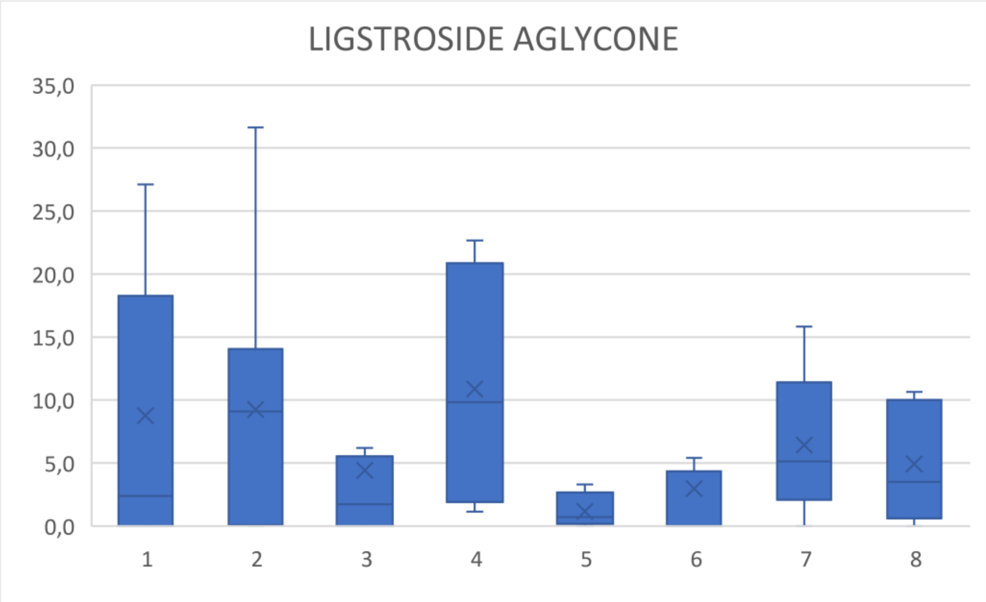
4
5
6
7
8


OLEOCANTHAL
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
1
2
3
4
5
6
7
8


OLEUROPEIN AGLYCONE
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
1
2
3
4
5
6
7
8


OLEOMISSIONAL
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
1
2
3
4
5
6
7
8


LIGSTROSIDE AGLYCONE
35,0
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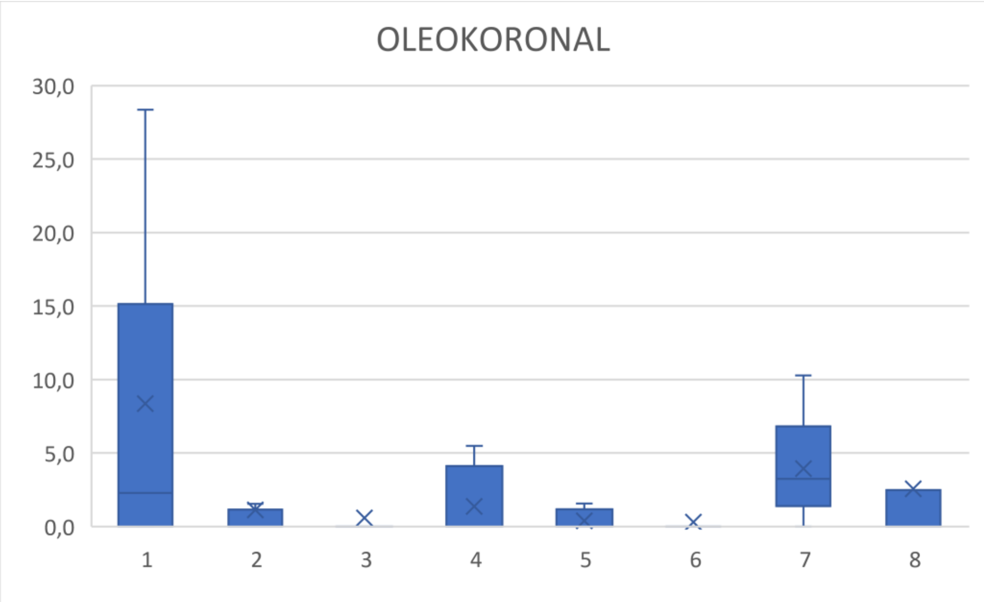
20,0
15,0
10,0
5,0
0,0
1
2
3
4
5
6
7
8


OLEOKORONAL
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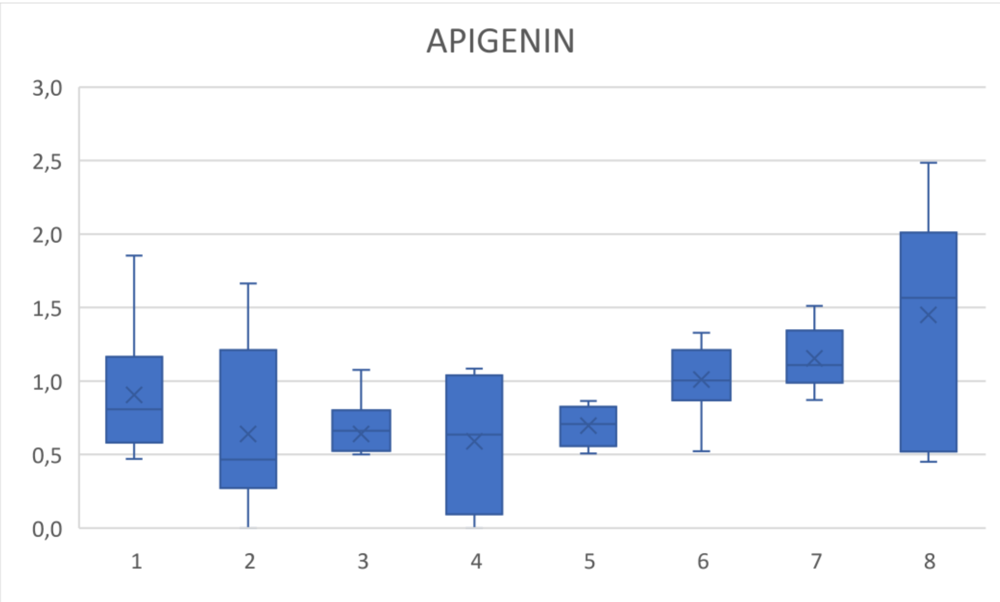
5,0
0,0
1
2
3
4
5
6
7
8


APIGENIN
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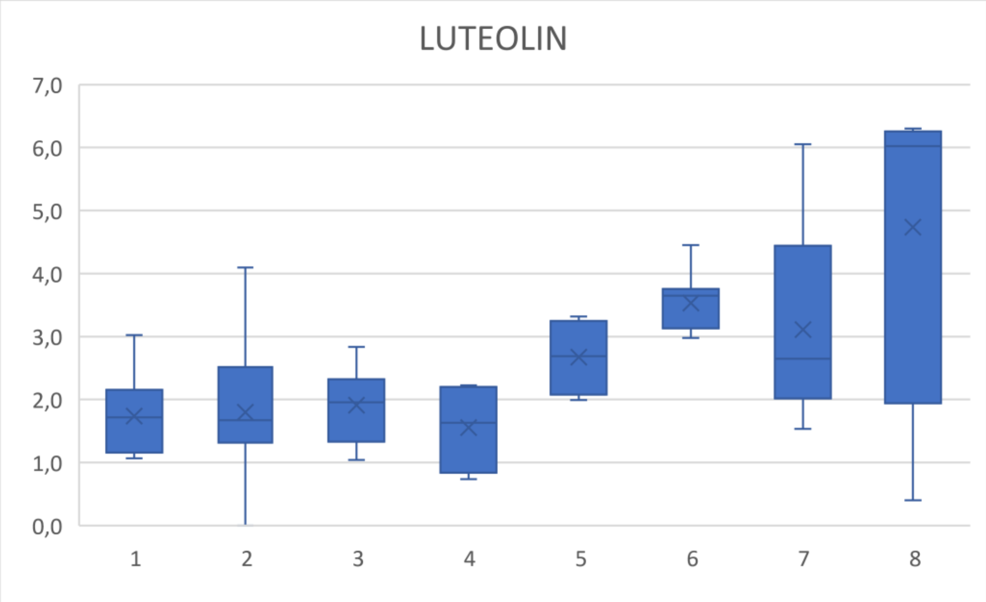
2,0
1,5
1,0
0,5
0,0
1
2
3
4
5
6
7
8


LUTEOLIN
7,0
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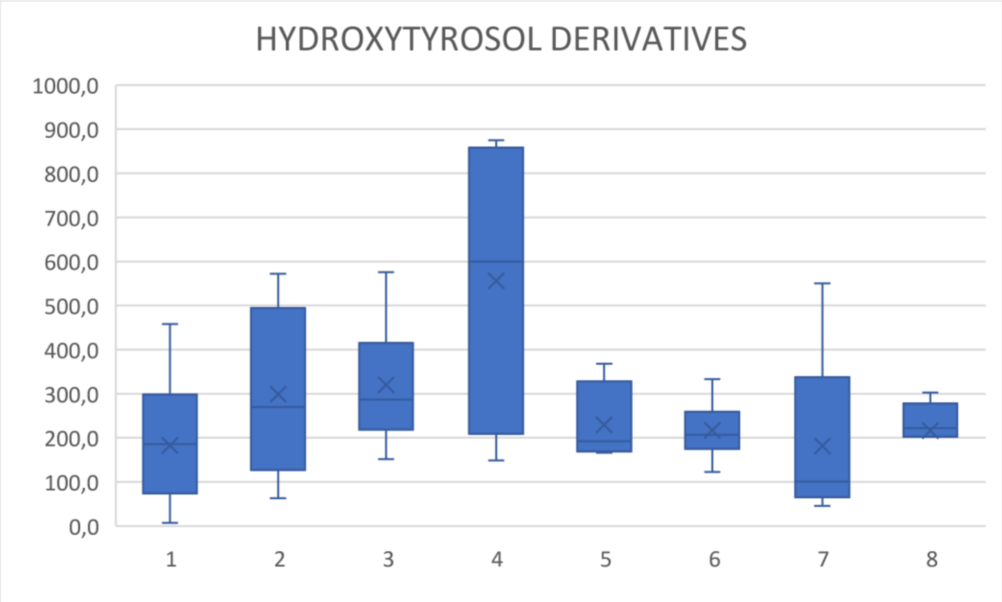
3,0
2,0
1,0
0,0
1
2
3
4
5
6
7
8


HYDROXYTYROSOL DERIVATIVES
1000,0
900,0
800,0
700,0
600,0
500,0
400,0
300,0
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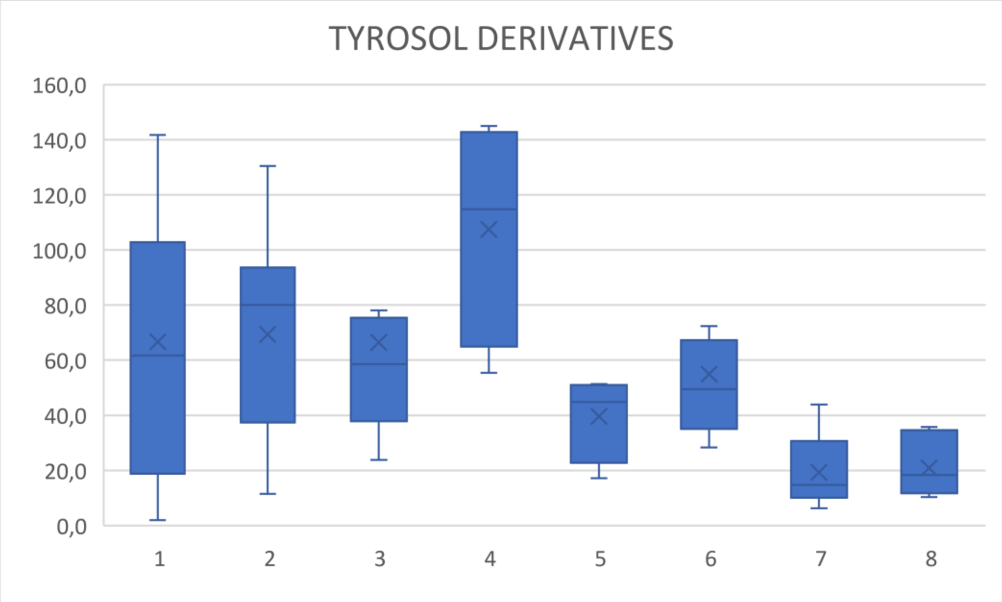
4
5
6
7
8


TYROSOL DERIVATIVES
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
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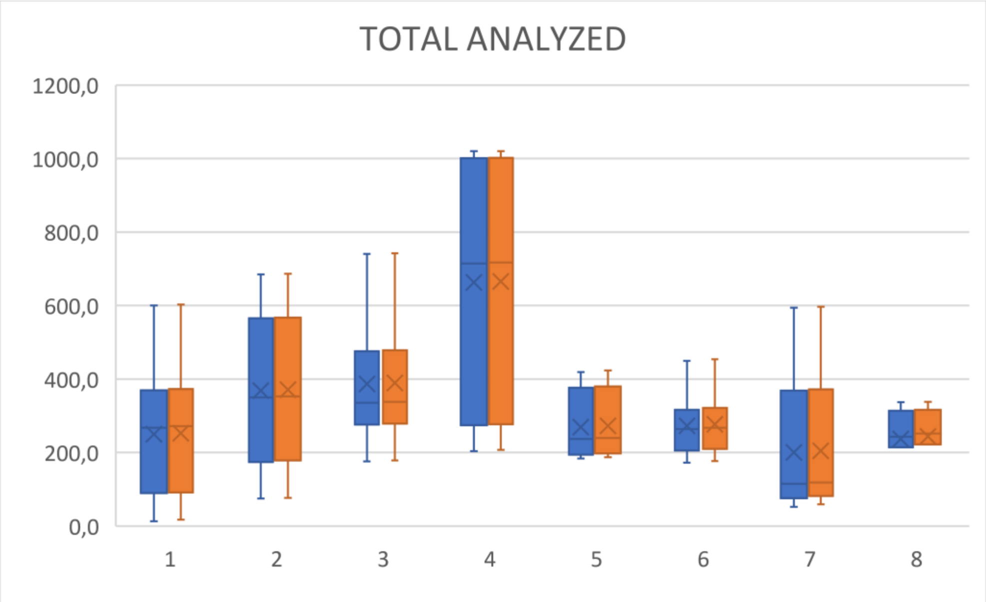
4
5
6
7
8


TOTAL ANALYZED
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800,0
600,0
400,0
200,0
0,0
1
2
3
4
5
6
7
8

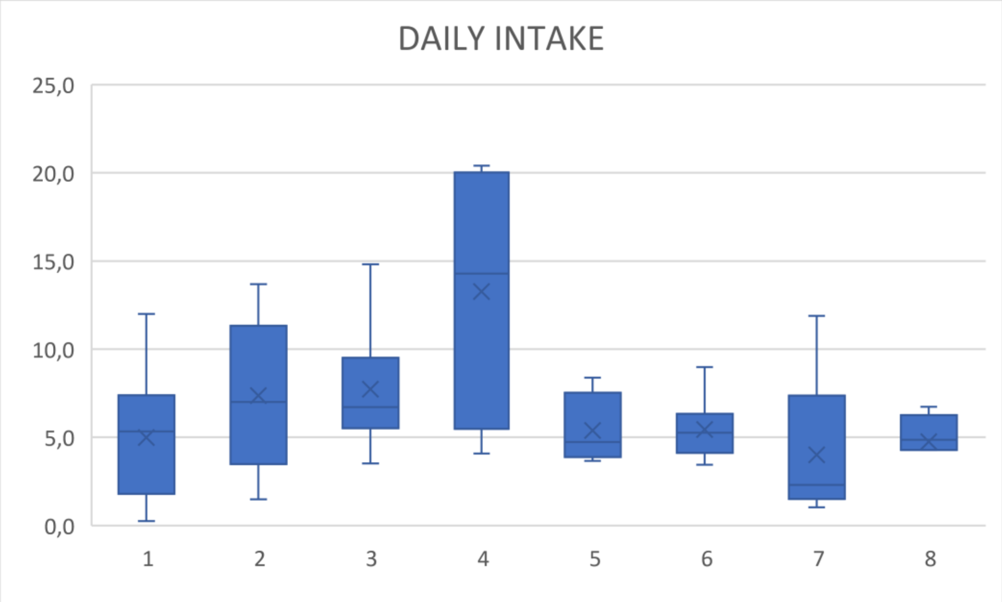


Figure 11. Concentration profiles found for the monitored phenolic compounds and associated parameters in Arbequina olive oil samples according to the harvesting date.

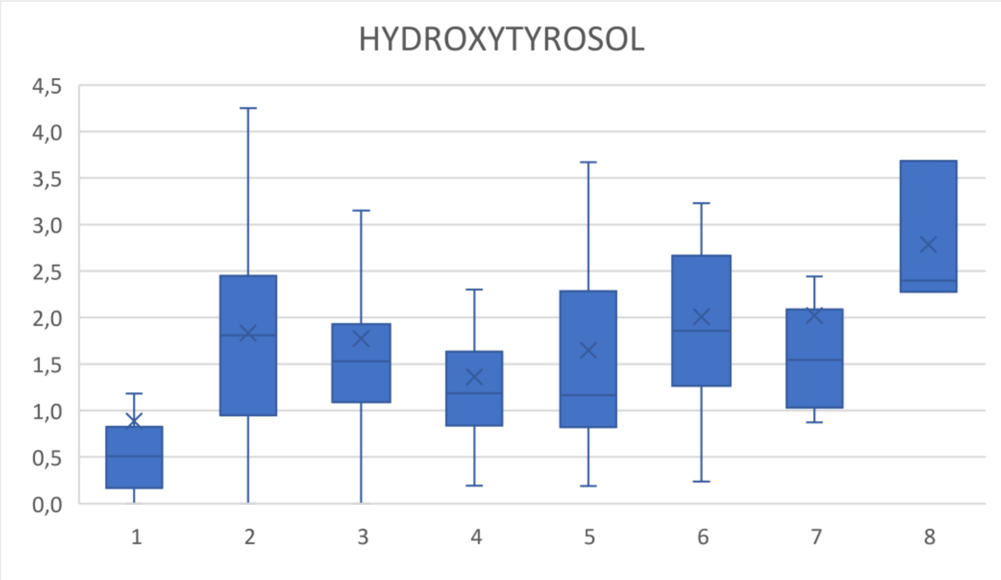
From these results, the following conclusions can be deduced:

- Hydroxytyrosol reached its maximum concentration from January up to the end of the season.
- Oleacein and oleocanthal were characterized by an increasing trend up to G4, which means from 11th December to 27th December. After this period, the concentration of both phenols decreased significantly.
- Oleuropein aglycon and ligstroside aglycon followed a similar profile to oleocanthal. After G4, the concentration of both compounds decreased considerably.
- Oleokoronal and oleomissional were found at very low concentrations during the season. No trends were observed for both phenols.
- Luteolin and apigenin showed a slight increase along the season that led to maximum concentration in February/March.

- Hydroxytyrosol and tyrosol derivatives were also found at maximum levels in December; then, their concentrations decreased significantly. The daily intake parameter also reported the maximum level at the same period.

In the case of Arbequina there were not samples with enough variability to check the incidence of the malaxation parameters, temperature and time.

Coupage


HYDROXYTYROSOL
4,5
4,0
3,5
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2,0
1,5
1,0
0,5
0,0
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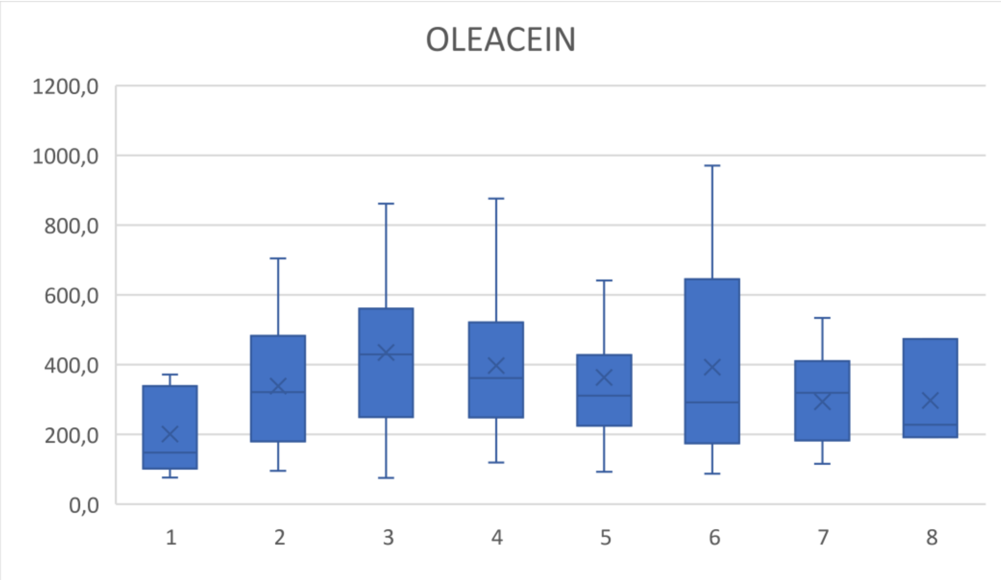
4
5
6
7
8


OLEACEIN
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2
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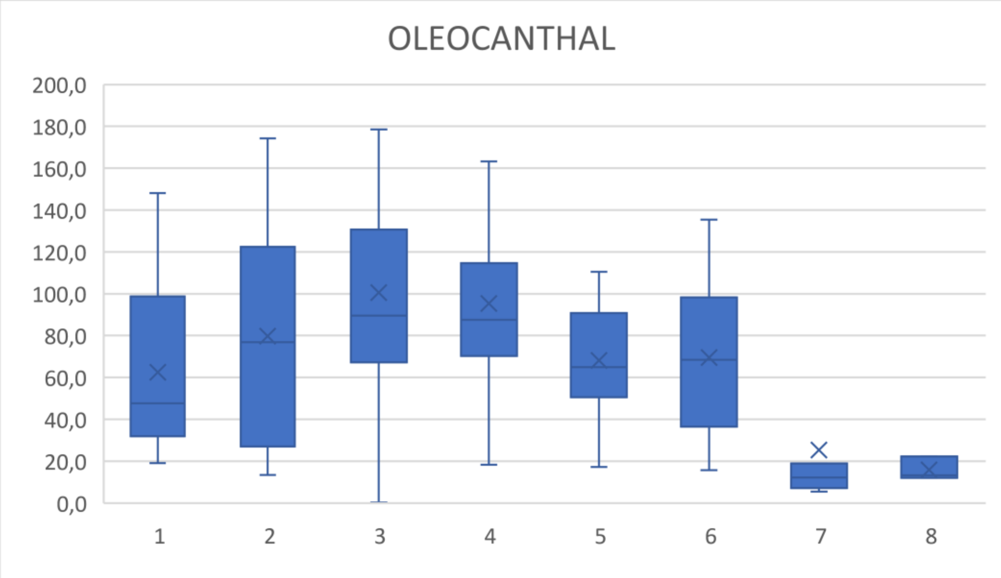
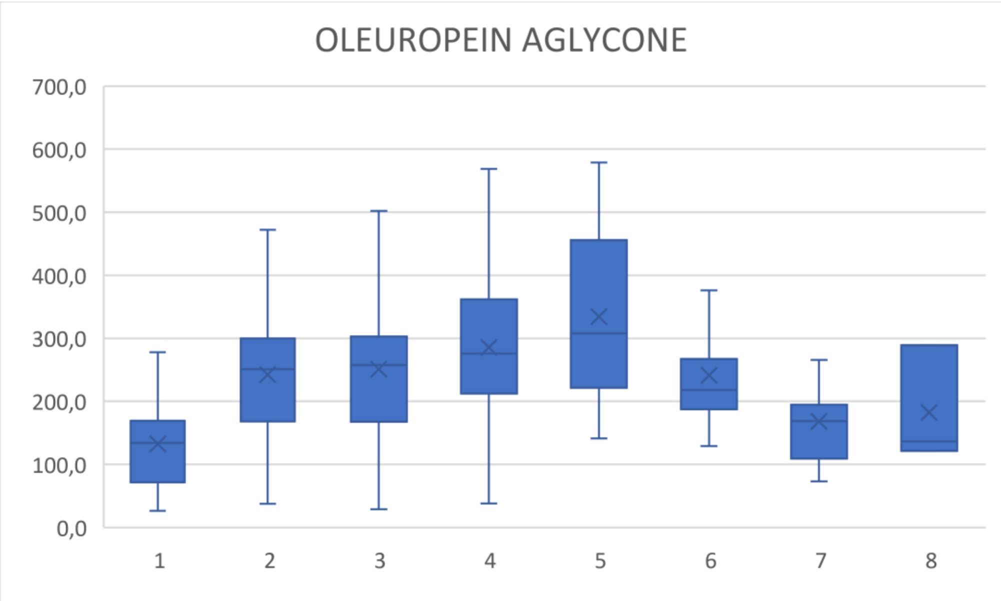
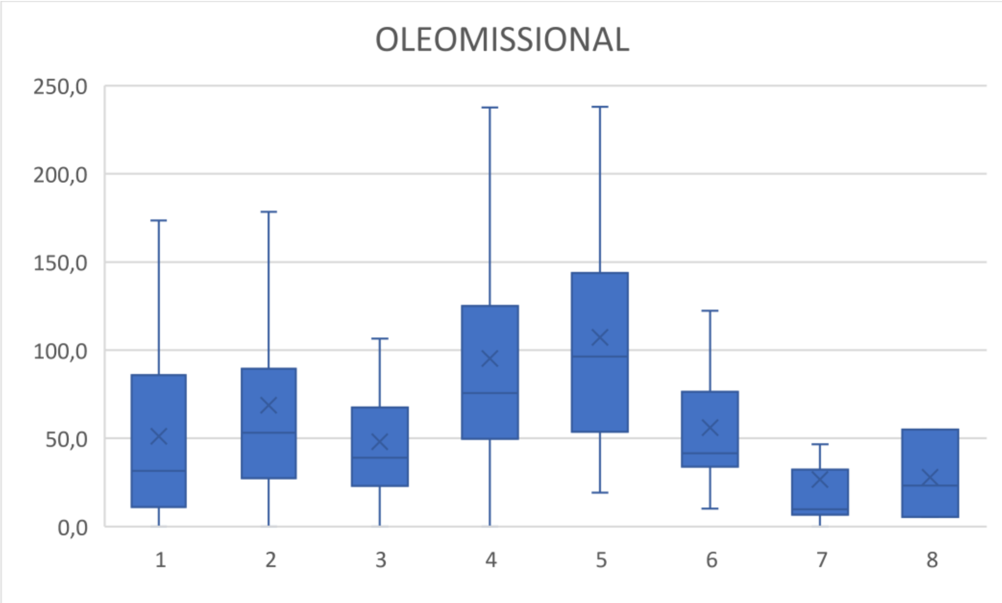
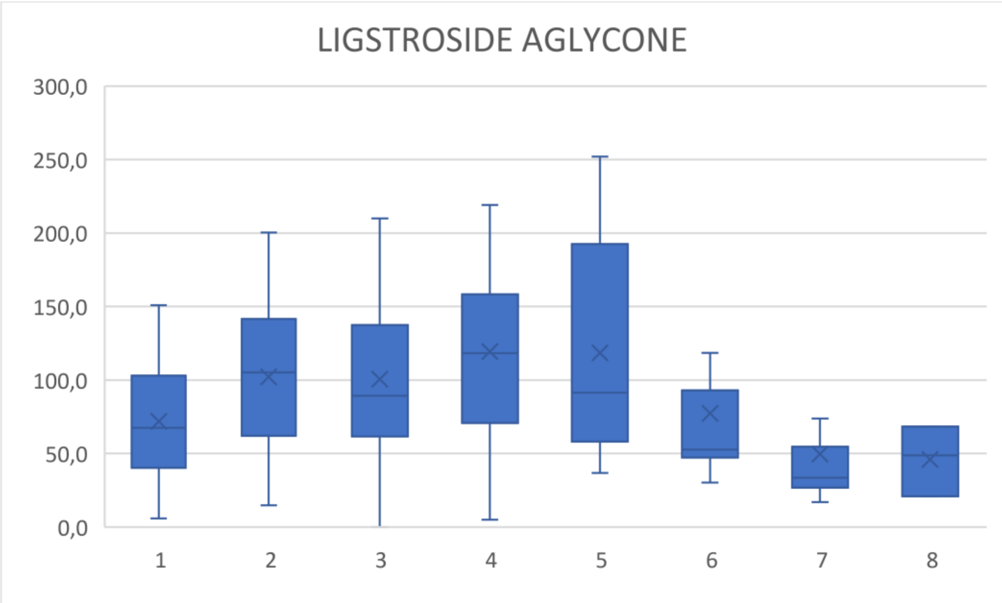
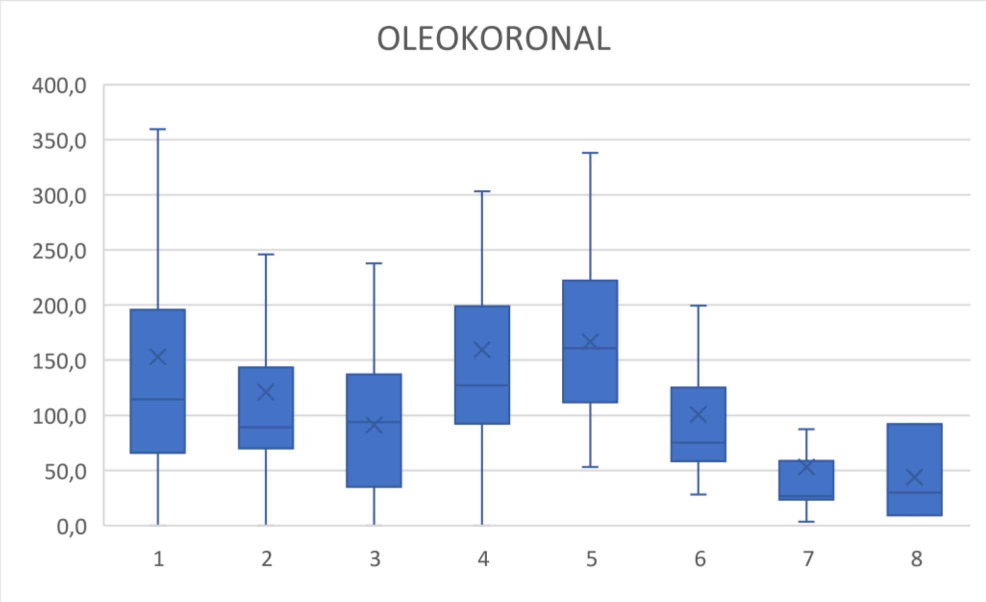
4
5
6
7
8

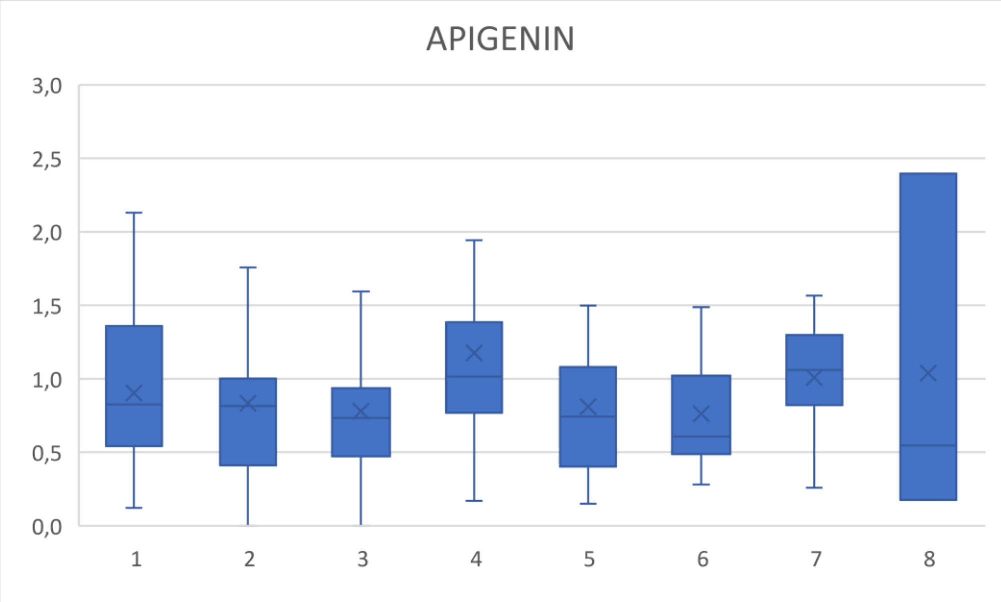

OLEOCANTHAL
200,0
180,0
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
1
2
3
4
5
6
7
8


OLEUROPEIN AGLYCONE
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
1
2
3
4
5
6
7
8


OLEOMISSIONAL
250,0
200,0
150,0
100,0
50,0
0,0
1
2
3
4
5
6
7
8


LIGSTROSIDE AGLYCONE
300,0
250,0
200,0
150,0
100,0
50,0
0,0
1
2
3
4
5
6
7
8


OLEOKORONAL
400,0
350,0
300,0
250,0
200,0
150,0
100,0
50,0
0,0
1
2
3
4
5
6
7
8


APIGENIN
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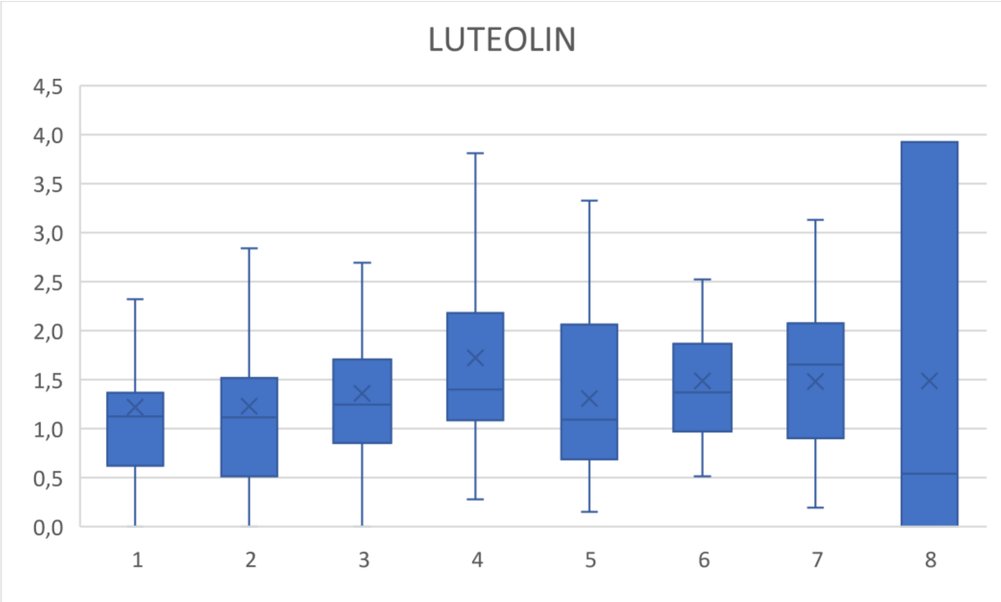
2,5
2,0
1,5
1,0
0,5
0,0
1
2
3
4
5
6
7
8


LUTEOLIN
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
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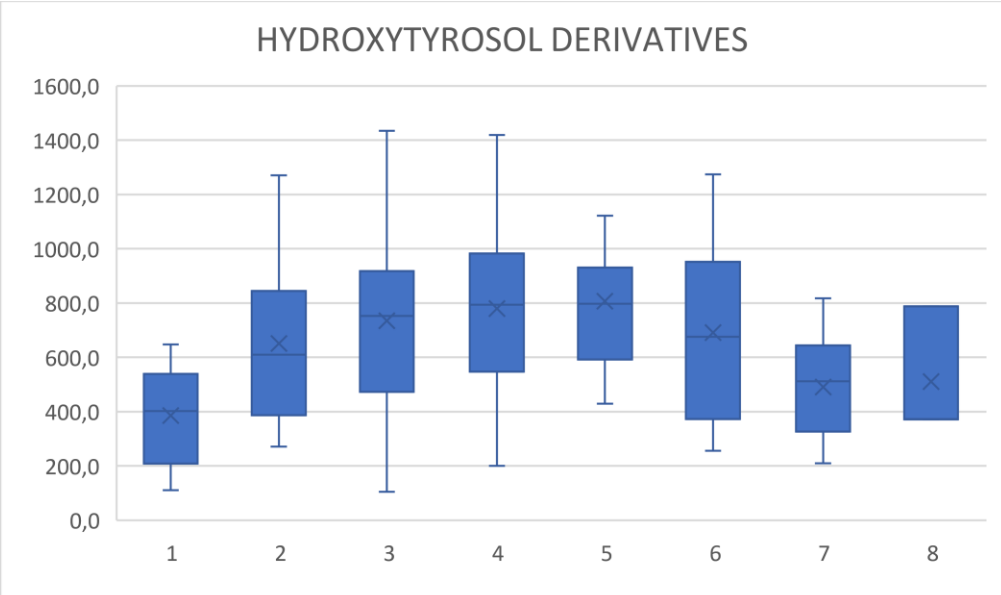
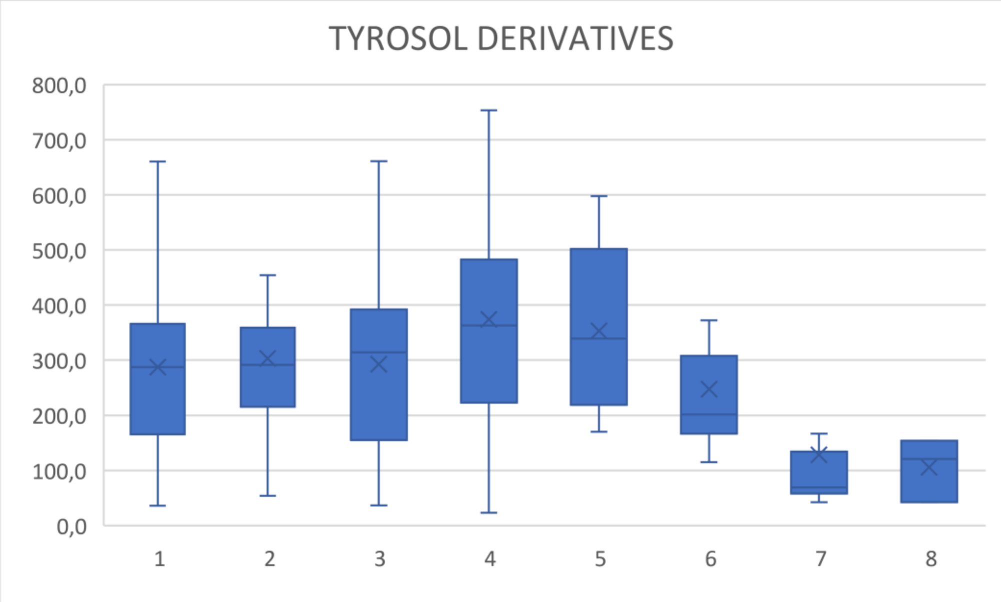
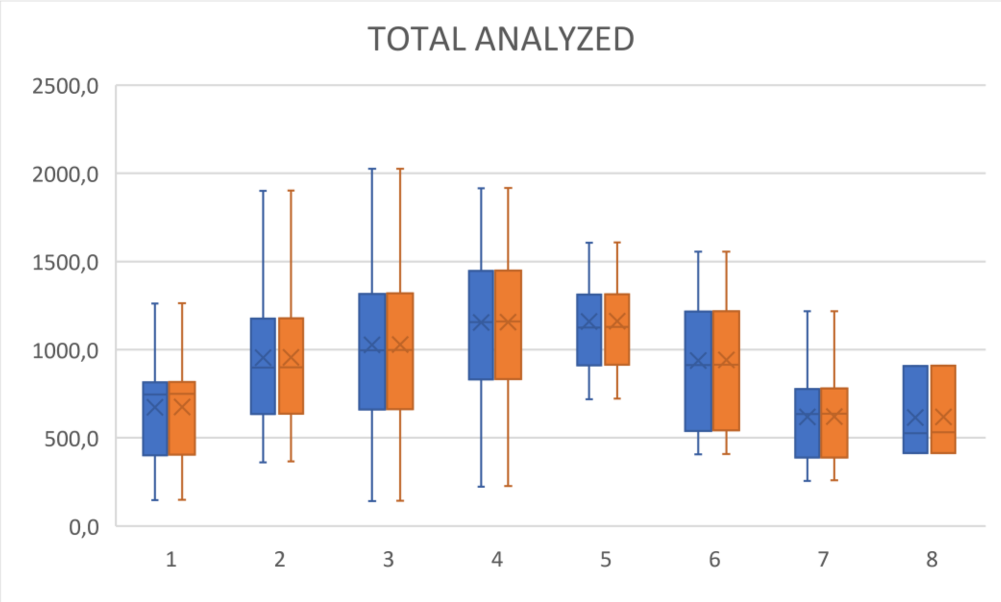
4
5
6
7
8


HYDROXYTYROSOL DERIVATIVES
1600,0
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
1
2
3
4
5
6
7
8


TYROSOL DERIVATIVES
800,0
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
1
2
3
4
5
6
7
8


TOTAL ANALYZED
2500,0
2000,0
1500,0
1000,0
500,0
0,0
1
2
3
4
5
6
7
8

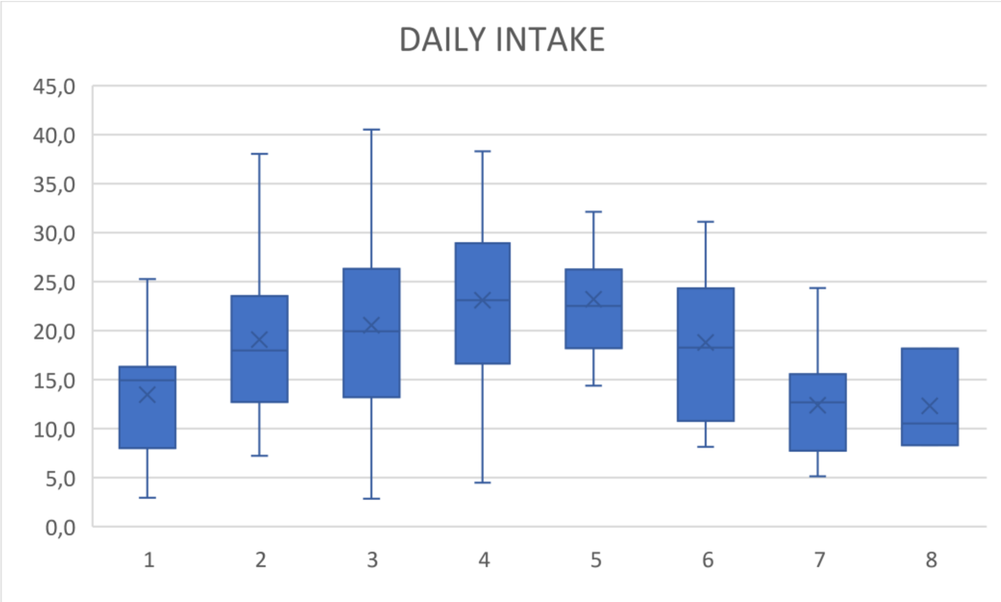


Figure 12. Concentration profiles found for the monitored phenolic compounds and associated parameters in Coupage olive oil samples according to the harvesting date.

According to these results, the following conclusions can be deduced:

- Hydroxytyrosol reached its maximum concentration from January up to end of the season. In fact, the maximum level was found in the last period.
- Oleacein and oleocanthal were characterized by an increasing trend up to G3, which means from 27th November to 10th December. After this period, the concentration of both phenols decreased, particularly that of oleocanthal.
- Oleuropein aglycon and ligstroside aglycon followed a similar profile to oleocanthal but their maximum levels were found from 27th December to 16th January.
- Oleokoronal and oleomissional were characterized by the same pattern as the other aglycons.
- Luteolin and apigenin showed a slight increase along the season that led to maximum concentration in February/March.

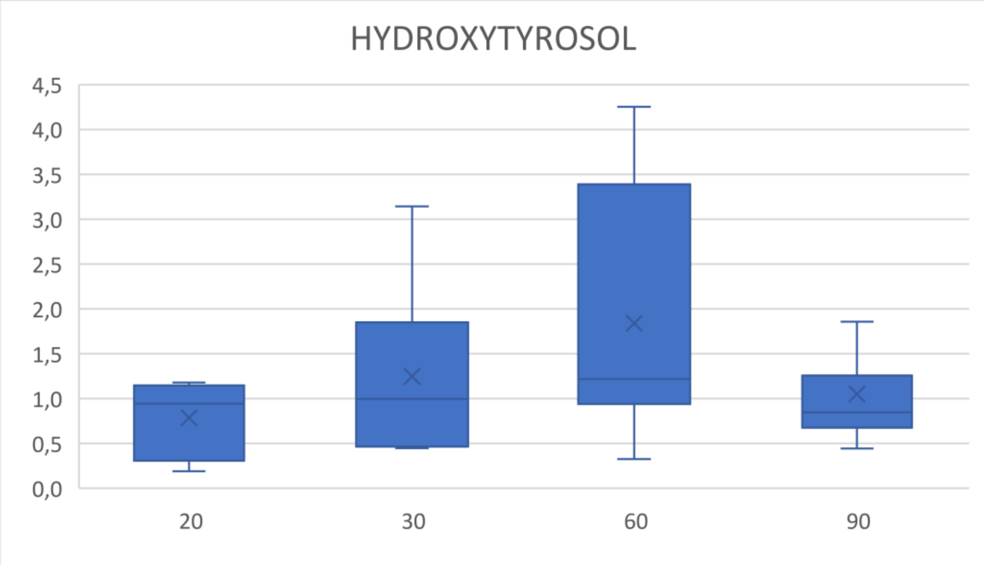
- Hydroxytyrosol and tyrosol derivatives were also found at maximum levels in December/January; then, their concentrations decreased significantly. The daily intake parameter also reported the maximum level within the same period.

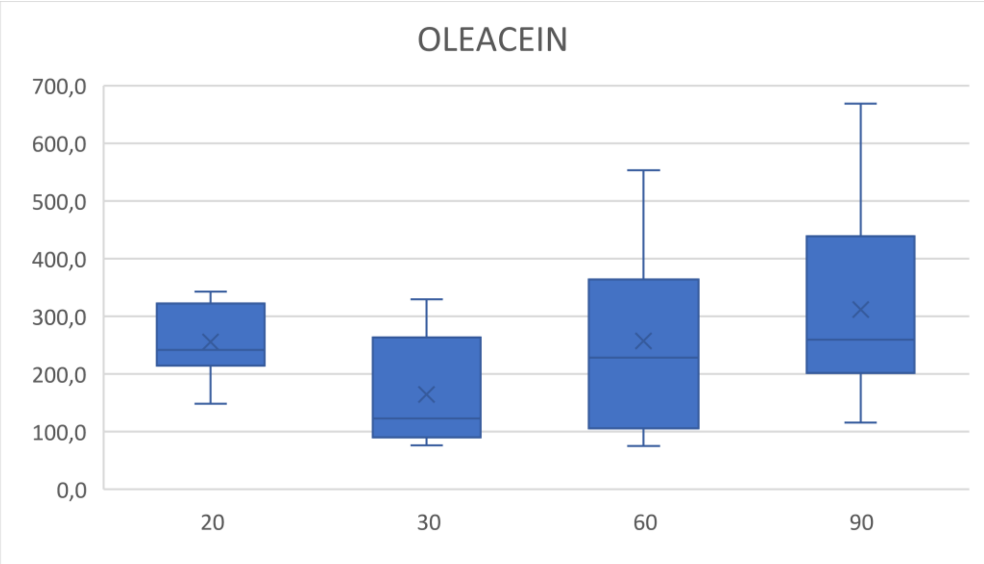
Due to the variability of samples corresponding to Coupage, the study of the malaxation parameters was carried out. Concerning the malaxation time, the distribution of samples was as follows:

Malaxation time	Coupage samples
20	8
30	6
40	1
45	0
60	12
75	28
90	8

Table 7. Distribution of Coupage samples according to the malaxation time.

The samples corresponding to 75ºC were not included in this study since they corresponded to the same origin. These were the results:


HYDROXYTYROSOL
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
20
30
60
90


OLEACEIN
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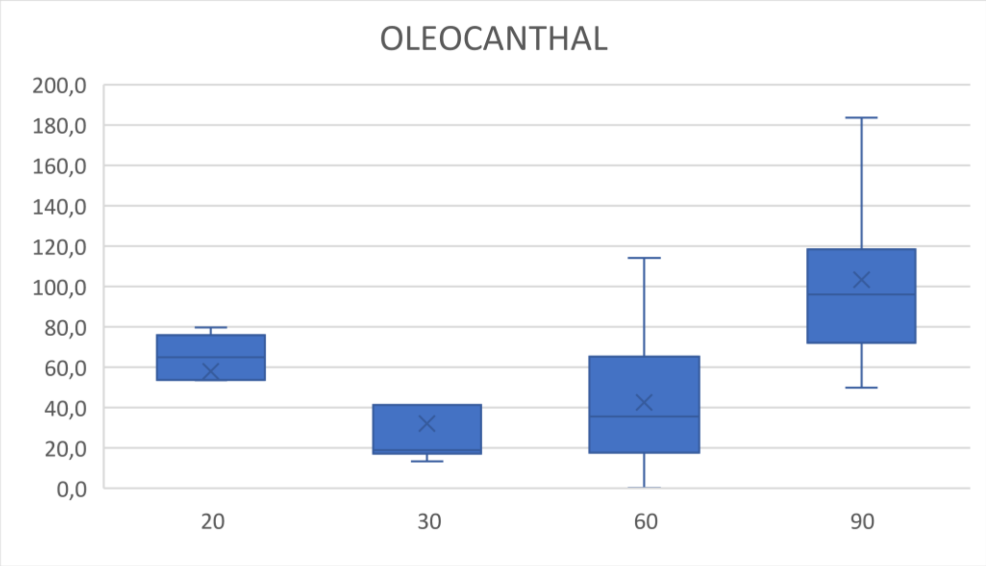
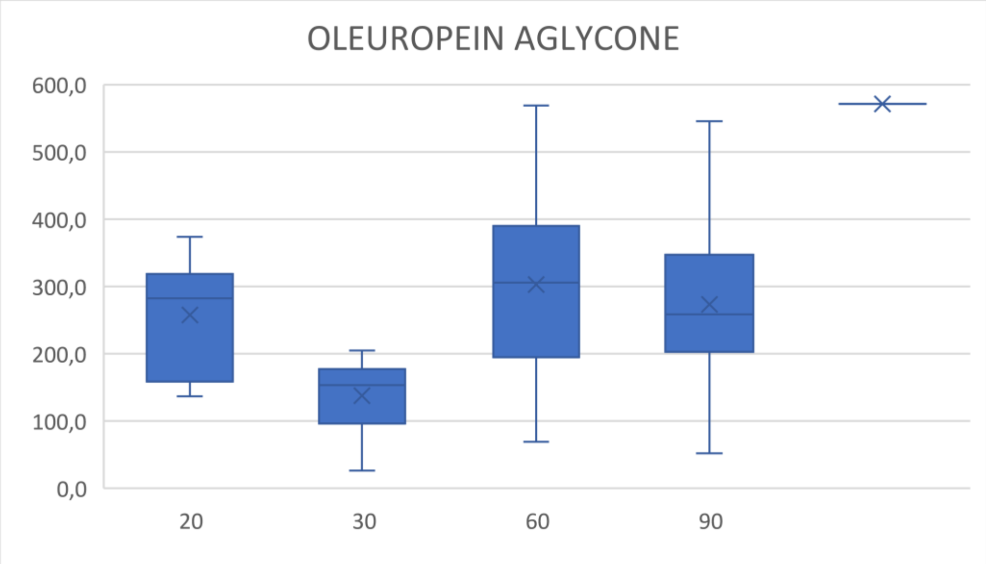
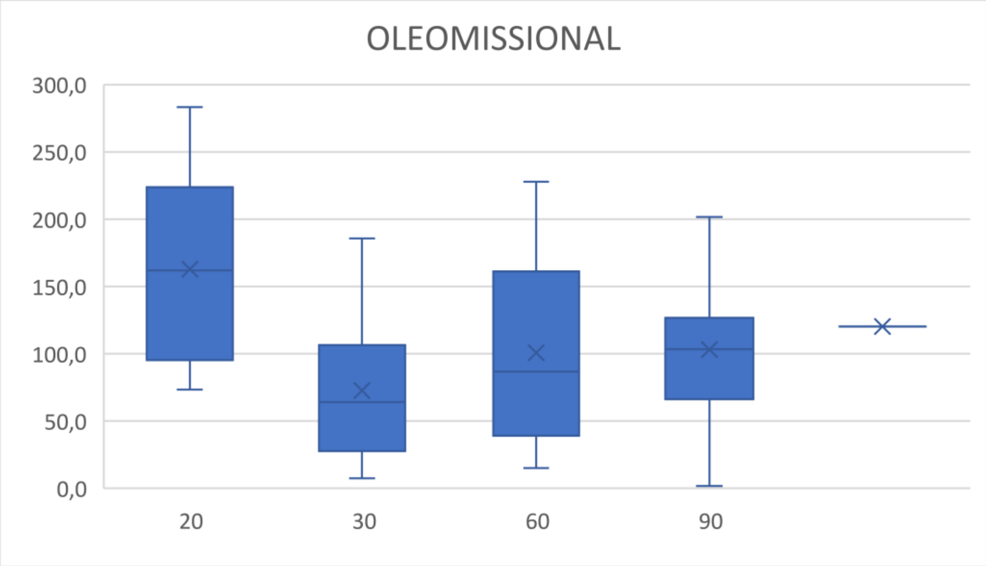
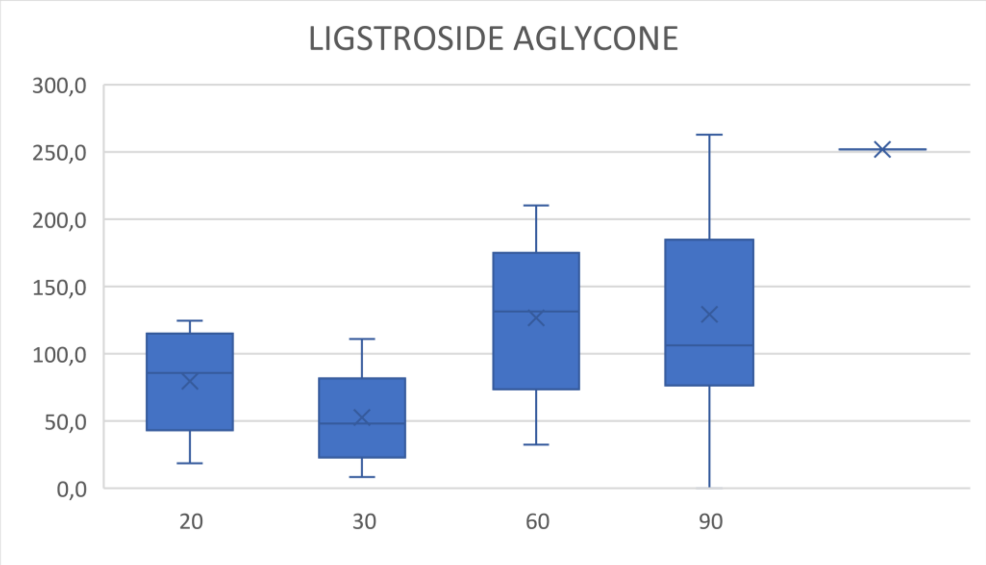
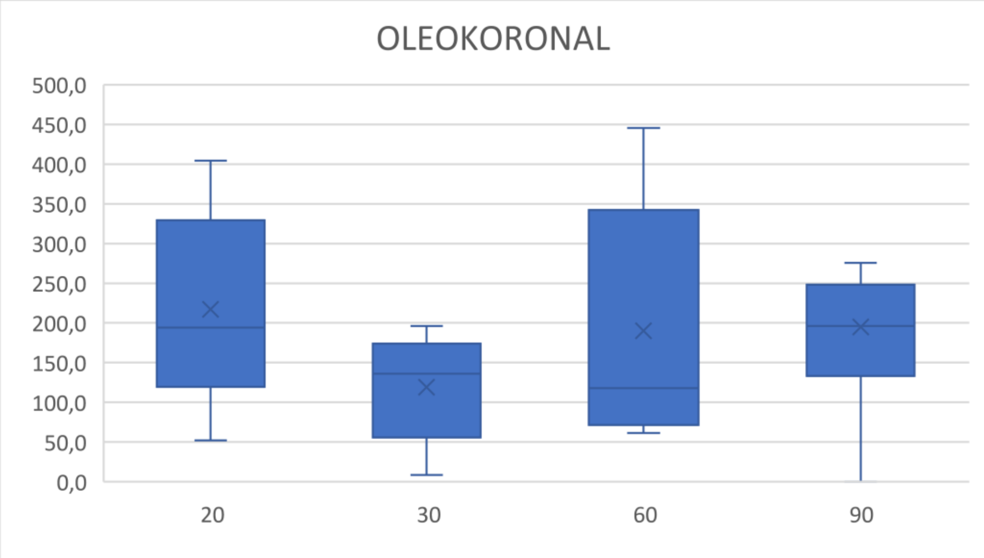
600,0
500,0
400,0
300,0
200,0
100,0
0,0
20
30
60
90


OLEOCANTHAL
200,0
180,0
160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
20
30
60
90


OLEUROPEIN AGLYCONE
600,0
500,0
400,0
300,0
200,0
100,0
0,0
20
30
60
90


OLEOMISSIONAL
300,0
250,0
200,0
150,0
100,0
50,0
0,0
20
30
60
90


LIGSTROSIDE AGLYCONE
300,0
250,0
200,0
150,0
100,0
50,0
0,0
20
30
60
90


OLEOKORONAL
500,0
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400,0
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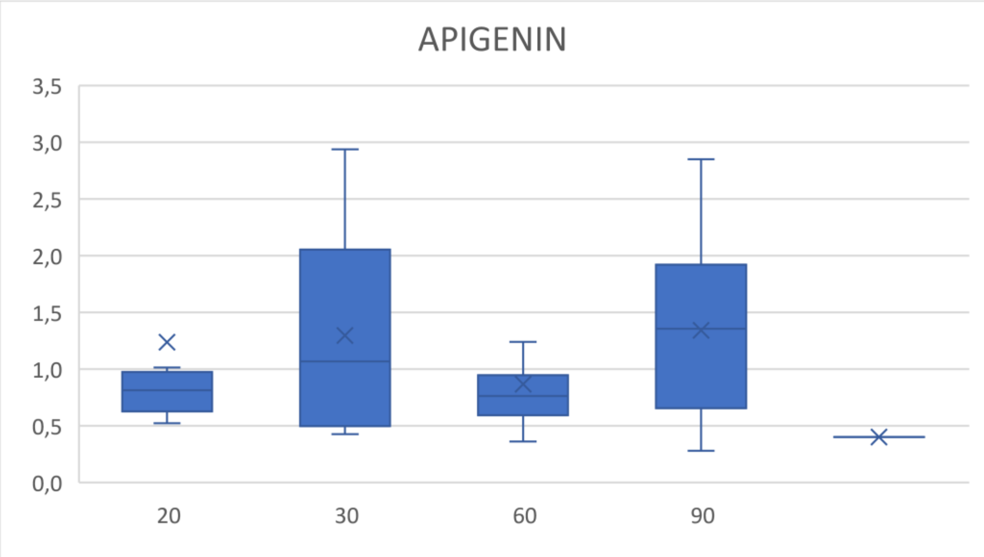
300,0
250,0
200,0
150,0
100,0
50,0
0,0
20
30
60
90


APIGENIN
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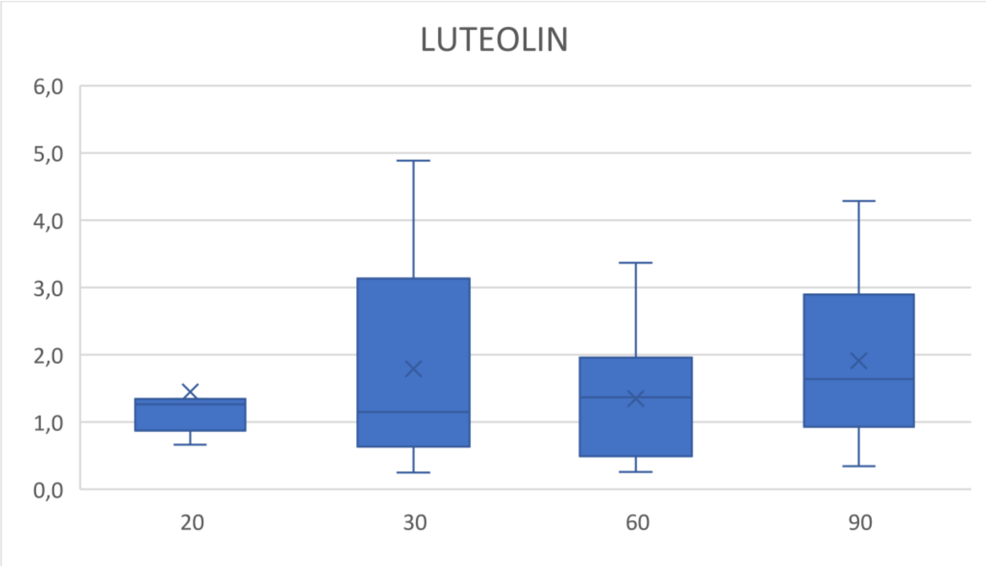
3,0
2,5
2,0
1,5
1,0
0,5
0,0
20
30
60
90


LUTEOLIN
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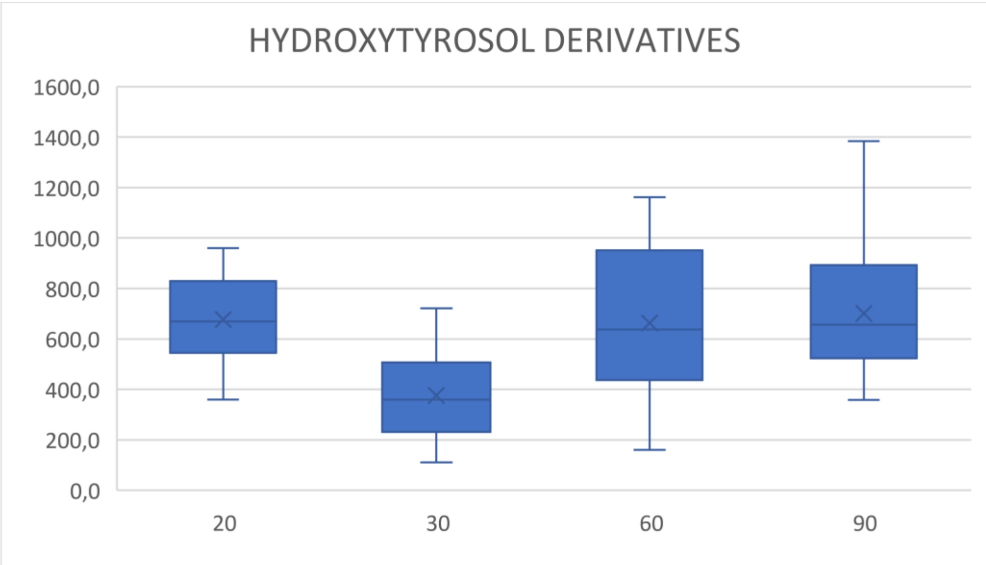
5,0
4,0
3,0
2,0
1,0
0,0
20
30
60
90


HYDROXYTYROSOL DERIVATIVES
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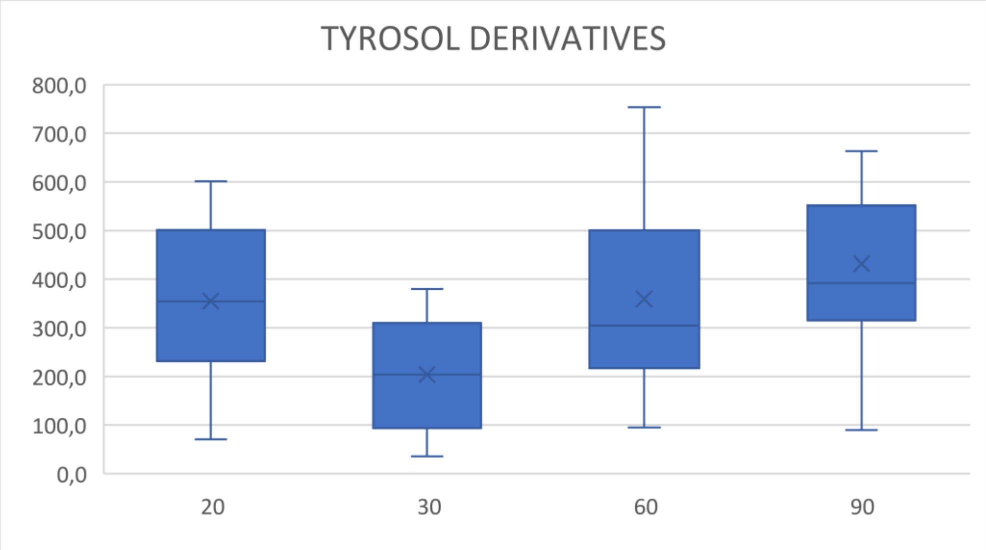
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
0,0
20
30
60
90


TYROSOL DERIVATIVES
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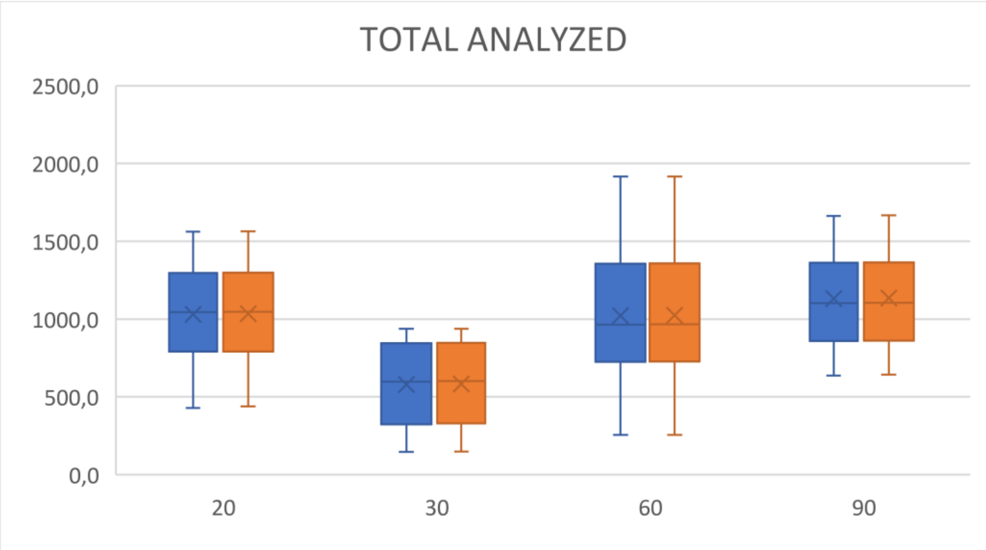
600,0
500,0
400,0
300,0
200,0
100,0
0,0
20
30
60
90


TOTAL ANALYZED
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1500,0
1000,0
500,0
0,0
20
30
60
90

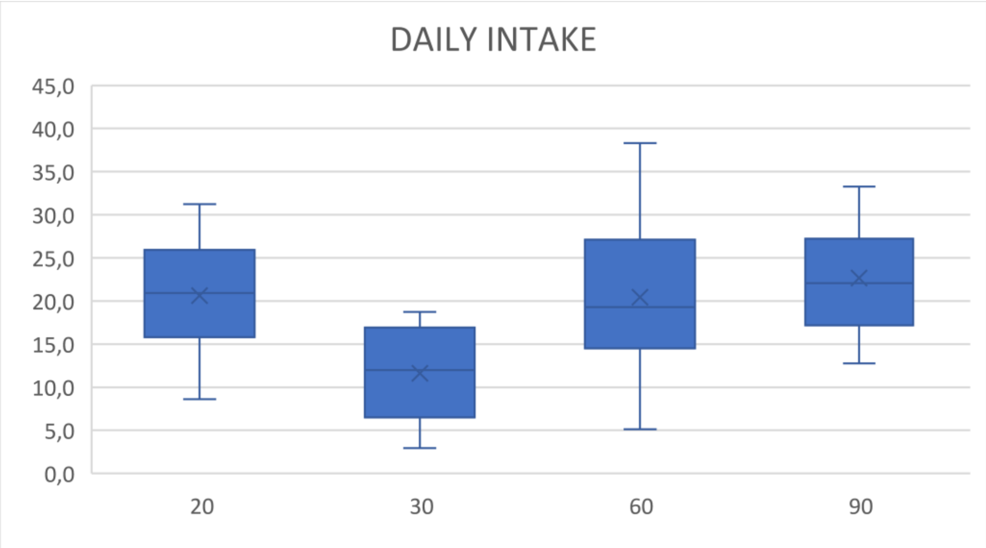
DAILY INTAKE
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
20
30
60
90

Figure 13. Concentration profiles found for the monitored phenolic compounds and associated parameters in Picual olive oil samples according to the malaxation time.

The results that can be deduced from these plots are:

- Hydroxytyrosol increased its concentration when the malaxation time increased up to 60 min. Then, the concentration decreased.
- Oleacein and oleocanthal decreased their concentration up to 60 min and then the concentration increased considerably.
- On the other hand, oleuropein aglycon and ligstroside aglycon increased their levels in olive oil by increasing the malaxation time. In both cases no clear differences were observed between 60 and 90 min.
- Oleomissional and oleokoronal did not show a clear trend with the malaxation time. Nevertheless, it seems that their concentration slightly decreased along the malaxation time.
- Apigenin and luteolin did not show a clear trend with the malaxation time.
- Hydroxytyrosol and tyrosol derivates reported higher concentrations at 60 and 90 min malaxation time.
- The same pattern was observed for the daily intake parameter.

The malaxation temperature was not evaluated in Coupage samples since this parameter was varied in a narrow interval, practically from 24 to 27ºC. Therefore, there is not enough variability considering that this parameter tends to increase during the malaxation process.