Importance of oleacin and oleocanthal on the oxidative stability of extra virgin olive oil measured by Rancimat

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The influence of oleacin and oleocanthal concentration on thermal oxidative stability of extra virgin olive oil was investigated. A sample of fifty-six oils in their native state (native oil) and cleaned from phenols through a liquid-liquid extraction (dephenolised oil) was oxidised by Rancimat at 130°C. Extension of induction period (E-IP), calculated as the difference between the induction periods of the native and dephenolised oils, was used as key parameter. This was correlated (Pearson's correlation test) with the primary quality indicators (free acidity and peroxide value), fatty acid composition, total phenols, oleacin and oleocanthal concentration, and the sum of these two last compounds (EDAs). Influence of free acidity and fatty acid composition on E-IP was low, while a significant and positive correlation with total phenols, oleacin and EDAs was found. However, no supplementary effect on E-IP was obtained at a concentration of EDAs up to 341 mg kg^{-1} OE (oleuropein equivalent). Finally, a weak correlation was observed between total phenols and oleacin ($r = 0.401$), oleocanthal ($r = 0.417$) and their sum ($r = 0.463$) suggesting that these phenols should be considered as an independent quality indicator for extra virgin olive oil.

Keywords: Olive Oil, Oleacin, Oleocanthal, Oil Oxidative Stability, Rancimat test.

Abbreviations: *DO-IP* (dephenolised oil induction period), *EDAs* (sum of OIN and OAL concentration), *E-IP* (extension of induction period), *EVOO* (extra virgin olive oil), *IP* (induction period), *NO-IP* (native oil induction period), *OAL* (oleocanthal), *OE* (oleuropein equivalent), *OIN* (oleacin), *OP* (other phenols), *TP* (total phenols).

1. INTRODUCTION

Oleacin (OIN) and oleocanthal (OAL) are among the major phenolic compounds determined in virgin olive oil [1]. These are dialdehydic forms of the elenolic acid (Fig. 1) deriving from oleuropein and ligstroside, respectively. OIN and OAL are object of attention for their proven health and sensory properties [2]. Under the health aspect, a positive role was evidenced in the treatment of cardiovascular pathologies, certain kinds of cancers, chronic inflammatory diseases, Alzheimer's disease and *Helicobacter pylori* infection [3-7]. Under the sensory aspect, OIN and OAL are associated with the pungency and bitterness of EVOO [8]. Oxidation is one of the major causes of edible lipid deterioration generating rancid and off-flavours, toxic compounds and nutritional loss. Commonly, the term 'oxidation stability' is used to indicate the period required to make an oil/fat a non-edible product under standardised conditions. This is an important quality factor to estimate the susceptibility to oxidation and, consequently, the shelf life of oils/fats [9]. Numerous accelerated methods have been developed to determine oxidation stability of oils/fats. The Rancimat test is one of the most

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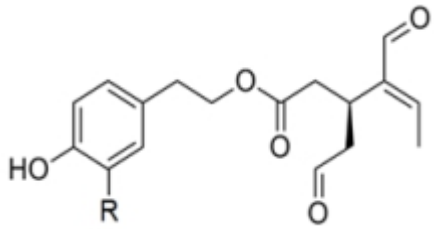

R = OH OLEACIN
3,4-DHPEA-EDA
Dialdehydic form of decarboxymethyl oleuropein aglycone
Dialdehydic form of elenolic acid linked to hydroxytyrosol

R = H OLEOCANTHAL
p-HPEA-EDA
Dialdehydic form of decarboxymethyl ligstroside aglycone
Dialdehydic form of elenolic acid linked to tyrosol

Figure 1 - Formula and nomenclature of oleacin and oleocanthal.

popular methods because it is simple, reproducible, rapid and does not require chemicals or solvents [10, 11]. In the Rancimat apparatus, few grams of oil are treated at variable temperature (50-220°C) while a constant air flow gurgles into the oil. In these conditions, high amounts of volatile compounds are generated and dissolved into a distilled water vessel where the water conductivity is constantly measured. In the final step, the induction period (IP) is determined as the moment in which a rapid increase of water conductivity occurs. The IP (expressed in hours) represents the value of Rancimat oxidative stability.

Rancimat test has been frequently used to study kinetic parameters [12], to compare oxidative stability of different oils/fats [13, 14] and to assay the activity of antioxidant or pro-oxidant substances [15-17].

The good oxidation stability of extra virgin olive oil (EVOO) is attributed to its high monounsaturated fatty acid composition and natural content of antioxidants [18-19]. In this regard, the influence of phenols has been studied largely [20, 21].

In this study, relationship occurring between OIN and OAL concentration and Rancimat induction time was investigated by using a sample of fifty-six different EVOOs. All oils were characterised for their initial quality indicators (peroxide and free acidity acid value), fatty acid composition and phenolic fraction and oxidised by a Rancimat apparatus at a temperature of 130°C.

2. MATERIALS AND METHODS

CHEMICALS AND OIL SAMPLES

The study sample consisted of 56 commercial Italian extra virgin olive oils, purchased directly from local markets or olive oil mills. Oil samples, listed and described in Table I, varied for the olive cultivar (blend or monocultivar), production year (2015, 2016, 2017) and Italian region (Molise, Apulia, Campania, Tuscany). In each observation year, the oils were collected from October to December and analysed in few days after sampling. All chemicals and solvents were of analytical or HPLC grade and supplied by C.Erba (Rodano, Milan, Italy) and Sigma-Aldrich (St. Louis, MO, USA) companies.

EVOO ANALYTICAL DETERMINATIONS

Compositional variables were determined only on the native oils. Free acidity and peroxide value were determined following the analytical methods described by the EEC Regulation 2568/91 [22]. Fatty acid methyl esters were determined by a MOD-8000 (Thermoquest Instrument, Rodano, MI, Italy) gas-liquid chromatograph equipped with a flame ionization detector and an Alltech EC-1000 FFAP (Alltech, USA) capillary column (30 m × 0.32 mm i.d., film 0.25 µm).

Operative conditions were the following: carrier gas He at 50 kPa; split injection system with a splitting ratio of 1:50; injector and detector temperatures set at 250°C and 270°C, respectively; programmed ramp 150-240°C at 10°C min^{-1}; injected quantity of 1 µL; cold transesterification with 2N methanolic potash.

Phenols were extracted from the oils by liquid-liquid partitioning through the following procedure. Each oil (6 g) was treated four-times with 4 mL of 80% methanol: water (v/v).

The mixture was vortexed for 30 sec and centrifuged at 4,000 *g* for 5 min. The collected supernatant was dried by rotary vacuum evaporator at 40°C by dissolving the dried residue in 2 mL of 80% methanol solvent. Oil phase was recovered as "dephenolised oil"; it was washed twice with small volumes of water to remove any traces of solvent, centrifuged (4,000 *g* for 5 min), filtered on cotton wool and stored at 4°C before the assay.

Phenolic methanol extract was filtered through a 0.45 syringe filter before analysis. Total phenols (TP) was determined by means of Folin-Ciocalteu reagent [23]. An aliquot of each phenolic extract (variable from 0.1 to 0.3 mL) was transferred into a 25-mL volumetric flask containing about 10 mL of water. Therefore, 1.25 mL of Folin-Ciocalteu reagent was mixed thoroughly, by adding, after 3 minutes, 5 mL of 15% (w/v) sodium carbonate. Mixture was brought to volume and left for 2 hours at room temperature in the dark. Finally, the absorbance was measured at 760 nm using a UV–vis spectrophotometer (Varian Cary 100). In the same way, a calibration curve was prepared with increasing amounts of gallic acid and then, the total phenol concentration was expressed as milligrams of gallic acid equivalents (GAE) per kg of oil. Oleacin (OIN) and oleocanthal (OAL) were determined following the

Table I - List, origin and characteristics of the studied extra virgin olive oils. FA: free acidity (%); PV: peroxide value (meqO_2/kg); TP: total phenols (mg GAE/kg); OIN: oleacin (mg OE/kg); OAL: oleocanthal (mg OE/kg); EDAs: OIN+OAL sum (mg OE/kg); SFA: saturated fatty acids (%); MUFA: monounsaturated fatty acids (%); PUFA: polyunsaturated fatty acids (%).Value given are the average of two replicas.

N.	Blend or mono-cultivar	Italian region	Year	FA	PV	TP	OIN	OAL	EDAs	SFA	MUFA	PUFA	MUFA/ PUFA
1	blend	Molise	2015	0.1	9.8	109	23	49	73	17.5	72.9	9.6	7.6
2	blend	Molise	2015	0.2	6.5	277	140	128	268	16.7	75.3	8.0	9.4
3	blend	Molise	2015	0.2	5.3	299	405	145	550	19.7	72.1	8.2	8.7
4	blend	Molise	2015	0.3	4.9	300	74	85	160	19.5	70.6	9.9	7.1
5	blend	Molise	2015	0.2	10.3	310	158	177	335	17.9	73.0	9.1	8.1
6	blend	Molise	2015	0.2	6.8	313	216	174	390	17.7	72.8	9.5	7.7
7	blend	Molise	2015	0.2	9.2	320	164	178	342	16.9	74.1	9.0	8.2
8	blend	Molise	2015	0.2	9.1	346	222	212	434	16.9	73.7	9.4	7.8
9	blend	Molise	2015	0.2	8.4	348	85	149	235	17.8	72.1	10.1	7.1
10	blend	Molise	2015	0.2	5.8	362	357	187	544	16.9	75.9	7.2	10.6
11	blend	Molise	2015	0.2	9.2	391	199	140	339	18.3	72.9	8.9	8.2
12	FS17	Molise	2015	0.2	3.2	403	190	111	302	14.6	76.7	8.7	8.8
13	Coratina	Molise	2015	0.2	5.2	412	203	196	399	12.9	80.8	6.3	12.9
14	Nera Colletorto	Molise	2015	0.3	4.1	416	79	155	234	20.3	67.5	12.2	5.5
15	blend	Molise	2015	0.3	9.4	431	84	171	255	18.9	70.3	10.8	6.5
16	Cazzarella	Molise	2015	0.3	4.0	460	316	334	650	20.4	65.4	14.2	4.6
17	Cazzarella	Molise	2015	0.3	4.2	478	303	358	661	20.7	65.0	14.2	4.6
18	Rumignana	Molise	2015	0.3	4.0	489	345	262	607	16.4	74.2	9.3	7.9
19	blend	Campania	2016	0.2	13.7	121	37	74	110	15.0	79.4	5.6	14.2
20	blend	Campania	2016	0.2	10.5	166	81	89	170	15.2	77.0	7.8	9.9
21	blend	Campania	2016	0.3	12.0	173	99	88	187	15.6	76.5	7.9	9.7
22	blend	Campania	2016	0.1	7.5	178	87	139	226	15.5	78.7	5.8	13.5
23	blend	Campania	2016	0.2	15.1	191	97	108	205	15.3	77.4	7.3	10.6
24	blend	Campania	2016	0.2	11.6	223	210	139	350	15.4	78.3	6.3	12.4
25	blend	Molise	2016	0.5	11.6	187	87	121	208	17.5	72.9	9.7	7.5
26	blend	Molise	2016	0.2	7.9	262	247	156	403	15.3	78.2	6.4	12.1
27	blend	Molise	2016	0.2	11.0	266	180	181	361	15.1	78.1	6.8	11.5
28	blend	Puglia	2016	0.3	10.1	260	73	163	236	13.4	78.9	7.8	10.2
29	blend	Puglia	2016	0.4	8.2	268	77	128	205	14.7	76.7	8.6	8.9
30	blend	Puglia	2016	0.2	8.5	284	114	135	249	16.6	73.5	9.9	7.4
31	Carolea	Calabria	2017	0.2	9.1	431	150	56	206	16.9	77.3	5.8	13.3
32	Coratina	Calabria	2017	0.2	11.1	530	153	167	320	15.5	76.4	8.1	9.5
33	blend	Campania	2017	0.3	7.8	242	194	130	324	15.0	76.0	9.0	8.5
34	Leccio del Corno	Campania	2017	0.2	10.7	427	232	134	366	14.4	78.1	7.5	10.4
35	Leccino	Marche	2017	0.3	10.0	277	257	181	438	16.5	77.1	6.4	12.1
36	blend	Molise	2017	0.3	9.6	177	148	164	312	16.8	74.0	9.2	8.0
37	blend	Molise	2017	0.2	5.5	201	137	108	244	17.2	72.9	9.9	7.3
38	blend	Molise	2017	0.1	6.2	258	314	238	553	17.8	74.0	8.1	9.1
39	blend	Molise	2017	0.3	11.1	278	93	117	210	18.9	70.7	10.4	6.8
40	blend	Molise	2017	0.2	6.4	332	154	221	375	15.4	74.8	9.9	7.6
41	Gentile Mafalda	Molise	2017	0.2	7.1	379	74	67	140	19.3	69.7	11.0	6.3
42	Coratina	Molise	2017	0.3	5.0	384	122	196	318	13.5	79.2	7.3	10.8
43	Coratina	Molise	2017	0.2	4.2	406	223	296	518	13.3	79.6	7.2	11.1
44	blend	Molise	2017	0.2	10.1	416	237	182	418	18.4	73.5	8.1	9.0
45	Gentile Larino	Molise	2017	0.3	8.5	431	313	197	510	18.4	70.3	11.3	6.2
46	Peranzana	Puglia	2017	0.2	13.1	302	270	235	505	17.6	71.3	11.1	6.4
47	blend	Puglia	2017	0.2	9.8	358	161	298	458	17.3	71.5	11.2	6.4
48	blend	Puglia	2017	0.2	8.0	475	283	281	564	19.2	69.8	10.9	6.4
49	Termite Bitetto	Puglia	2017	0.2	5.8	519	331	69	400	18.2	74.4	7.4	10.1
50	Ogliarola Salentina	Puglia	2017	0.2	13.2	625	250	122	372	19.0	70.9	10.0	7.1
51	Coratina	Puglia	2017	0.3	4.2	686	227	211	438	12.9	80.6	6.5	12.3
52	Coratina	Puglia	2017	0.2	5.6	689	193	219	412	13.7	78.9	7.4	10.6
53	Cima di Melfi	Puglia	2017	0.2	4.5	697	161	164	325	15.1	77.6	7.3	10.6
54	Coratina	Puglia	2017	0.3	6.6	705	182	222	404	13.9	78.3	7.8	10.1
55	Nocellara Belice	Sicilia	2017	0.2	12.3	206	87	71	158	18.2	74.5	7.4	10.1
56	Frantoio	Toscana	2017	0.2	13.0	565	185	204	389	18.3	74.6	7.1	10.5
			Minimum	0.1	3.2	109	23	49	73	12.9	65.0	5.6	4.6
			Maximum	0.5	15.1	705	405	358	661	20.7	80.8	14.2	14.2
			Mean	0.2	8.3	358	180	166	346	16.7	74.6	8.7	9.0
			Standard deviation	0.1	3.0	147	90	68	139	2.0	3.6	1.9	2.3

International Olive Oil Council method [24] with the modification described below.
The HPLC instrument (Varian ProStar 330, Mulgrave, AUS) was equipped with a Kinetex 5u C18 100 Å column (150 × 4.6 mm) (Phenomenex, USA); mobile phase was a mixture of H_3PO_4–bidistilled water 0.2% v/v (eluent A), methanol (eluent B), acetonitrile (eluent C); the gradient for the A/B/C eluents was as follows: 0 min 96/2/2%; 24 min 50/25/25%; 27 min 40/30/30%; 36 min 0/50/50%; 49 min 96/2/2%; chromatograms were obtained at 280 nm. The OIN and OAL concentration was quantified by an oleuropein standard calibration curve derived from a plot of area counts versus concentration (mg OE/kg oil). The sum of OIN and OAL concentration was called EDAs.

RANCIMAT TEST

Induction period (IP, hours) was determined by a Rancimat apparatus model 730 (Metrohm AG, Herisau, Switzerland) on 2.5 g of native and dephenolised oil samples heated at 130°C and under a purified air flow rate of 20 L/h. Induction Period Extension (E-IP) was calculated as the difference between the IP of native oil (NO-IP) and that of the colligated dephenolised oil (DO-IP) through the following formula: *E-IP = (NO-IP) - (DO-IP)*. Geometric meaning of the induction period (E-IP) is shown in Figure 2.

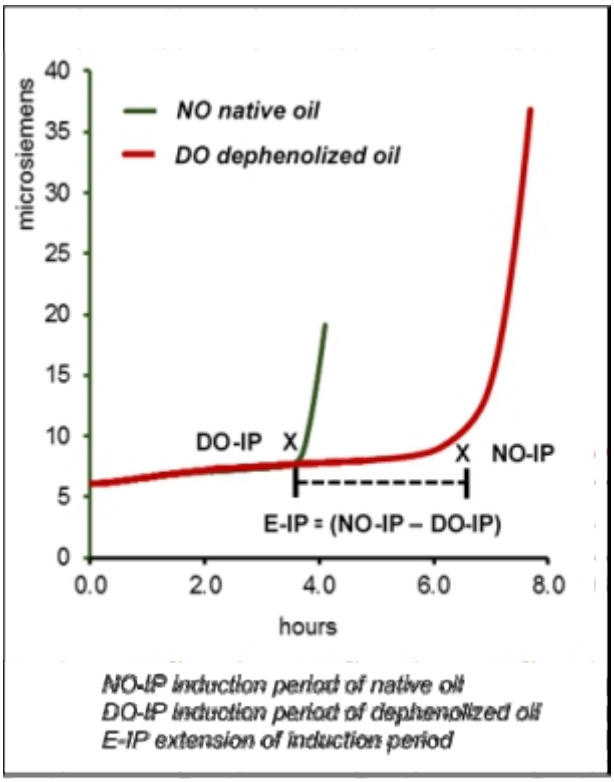

Figure 2 - Geometrical meaning of the extension Rancimat induction period (E-IP).
Legend:
NO native oil
DO dephenolized oil

STATISTICAL ANALYSIS

Statistical analysis was performed by SPSS 26,0 statistical software (IBM SPSS, Chicago, IL, USA). Each sample was analysed at least twice repeating the measurement if a standard error was more than 5%. Correlation between the variables were determined by the two-tailed Pearson's test and expressed as coefficient of correlation (r).
Principal components factorial analysis (PCA) was performed on the variables assessed generating a set of two orthogonal axes (PC1 and PC2). The loadings corresponding to the PCs were calculated from the correlation matrix. Finally, the study sample was split by median of EDAs concentration (341 mg OE/kg) into two groups, namely OG1 and OG2, performing the statistical comparisons by the one-way ANOVA with post hoc Tukey's test ($P < 0.05$).

3. RESULTS AND DISCUSSION

OIL COMPONENT VARIABLES

Origin and characteristics of the EVOO samples are given in Table I.
European Union legislation [22] sets for the commercial category of 'extra virgin olive oil' the limits of free acidity and peroxide value at 0.8% and 20 $meqO_2$/kg, respectively. Therefore, considering these variables, all analysed oils belonged to the 'extra virgin olive oil' category. Specifically, range of free acidity was narrow (0.1-0.5%), while the peroxide value (PV) ranged from 3.2 to 15.1 $meqO_2$/kg, with mean value of 8.3 $meqO_2$/kg.
Saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids ranged from 12.9 to 20.7%, 65.0 to 80.8% and 5.6 to 14.2%, respectively. MUFA/PUFA ratios were also calculated being taken as indicative of the oil tendency to undergo autoxidation [25]. This ratio (MUFA/PUFA) showed a wide range (4.6-14.2), with a mean of 9.0.
It is known that the phenols in olive oil may change with olive tree variety and fruit harvesting time [26], oil extraction process [27], oil aging and storage conditions [28]. A significant high variability of the phenolic fraction was found among the samples. TP ranged from 109 to 705 mg/kg GAE, with a mean of 358. The highest TP concentration was obtained in monocultivar oils, specifically those of the *Coratina* (5 oil samples), *Ogliarola Salentina* (1 oil sample) and *Frantoio* (1 oil sample) cultivar. Equally, OIN and OAL concentration showed high variability. Specifically, OIN and OAL ranged from 23 to 405 and 49 to 358 mg/kg OE, respectively.
Pearson correlation matrix of the analysed variables is given in Table II in which the statistically significant

Table II - Correlation matrix (Pearson) between the analysed component variables. Values in bold and underlined are significant at an alpha ≤ 0.01.

Variables	FA	NP	TP	OIN	OAL	EDAs	SFA	MONO	PUFA
FA	**1**								
NP	- 0.057	**1**							
TP	0.053	**-0.383**	**1**						
OIN	-0.100	**-0.338**	**0.401**	**1**					
OAL	0.094	**-0.351**	**0.417**	**0.544**	**1**				
EDAs	-0.019	**-0.390**	**0.463**	**0.911**	**0.841**	**1**			
SFA	0.012	0.027	-0.032	0.186	-0.047	0.143	**1**		
MONO	-0.151	0.088	-0.026	-0.156	-0.231	-0.214	**-0.924**	**1**	
PUFA	0.274	-0.195	0.083	0.088	0.383	0.245	**0.688**	**-0.914**	**1**
MUFA/PUFA	-0.247	0.144	-0.057	-0.078	-0.282	-0.189	**-0.704**	**0.903**	**-0.964**

correlation at an alpha less than 0.01 are underlined. Peroxide value (PV) measures the content of lipid radical and hydroperoxides that are formed during the early stages of oil oxidation [29]. This was significantly and inversely correlated only with the phenolic variables: TP (r = -0.383), OIN (r = -0.338), OAL (r = -0.351) and EDAs (r = -0.390). As regards the phenolic variables, TP was only weakly correlated with OIN (r = 0.401), OAL (r = 0.417) and EDAs (r = 0.463). Actually, while HPLC determination of OIN and OAL is highly selective, the Folin-Ciocalteu method used for TP determination could be affected by potential interferences, including those of the oxidised phenols [17, 30]. This strengthens the statement of *Karkoula et al.* [26] that propose to consider OIN, OAL and EDAs as independent quality indicators, also considering their remarkable biological properties.

RANCIMAT TEST

The variability of Rancimat results can be seen in the graph of Figure 3. The mean of NO-IP (6.15 h) was roughly twice DO-IP (3.33 h), but the NO-IP range (3.52-9.39 h) was wider than that of DO-IP (2.02-4.43 h). Therefore, DO-IP value was less variable than that of NO-IP and this observation leads us to assume that the average DO Rancimat induction period of 3.3 hours could be a realistic average value for the EVOOs cleaned of their own phenol antioxidant content. However, the Pearson correlation analysis performed between the DO-IP and other determined variables (data not shown) evidenced only a weak significant relationship with the peroxide value (r = - 0.428). In our previous study [31], it emerged that the peroxide species remained exclusively in the dephenolised oil after the recovery of phenolic fraction through 80% methanol solution oil-washing. Therefore, even in this case, although PV of dephenolised oils were not determined, it was reasonable to assume that PV were

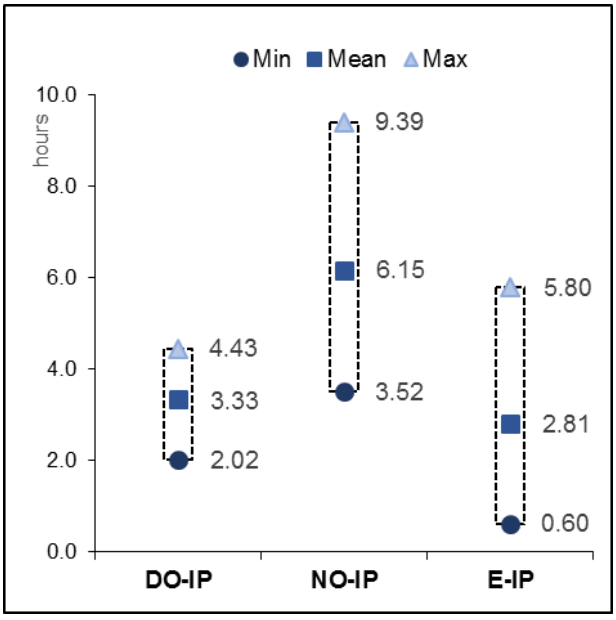

Figure 3 - Minimum, maximum and mean of the induction period of dephenolized (DO-IP) and native oils (NO-IP) and the relative calculated extension (E-IP).

the same found in the parent native oils.

As was to be expected, a significant positive dependence (data not shown) was obtained between E-IP and NO-IP (r = 0.944) supporting our choice to use E - IP as an index to evaluate the influence of each component variables on the extension of oil oxidative stability. By applying factorial analysis, it was found that the first two factors accounted for up to 67.5% of the total variance. The loading plot, shown in Figure 4, revealed four different main sectors. In the second sector on the right side of the graph, E-IP is grouped with all phenolic variables (TP, EDAs, OIN, OAL), confirming the positive role of phenols on the EVOO thermal oxidative stability. However, this finding was largely documented in literature [19-21, 30]. Surprisingly, no relevant association was observed

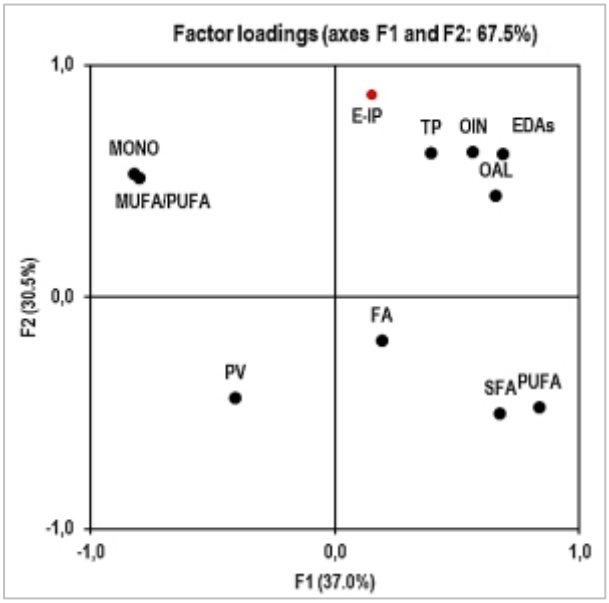

Figure 4 - Loading plot of correlation between factors 1 and 2 for the ail analysed variables.

Table III – Mean data comparison of the oils split by median EDAs value (341 mg OE/kg) (OG1<341 and OG2>341 mg OE/kg). Different letter indicates significantly difference at 0.05 level.

	Mean ± Standard Deviation	
	OG1	**OG2**
TP	295 ± 132[a]	414 ± 137[b]
OIN	111 ± 46[a]	249 ± 66[b]
OAL	123 ± 40[a]	208 ± 65[b]
EDAs	234 ± 71[a]	458 ± 92[b]
E-IP	2.14 ± 1.14[a]	3.48 ± 1.09[b]

between E-IP and other remaining variables, especially with the fatty acids. Commonly, the fatty acid composition is considered as a decisive factor on oil oxidation stability and there are large evidences that oil/fat oxidation stability is correlated positively with saturated and negatively with unsaturated fatty acids [32]. Nevertheless, like our findings, a low relationship between the fatty acid profile and Rancimat IP was observed also in other studies [33]. Moreover, the impact of the fatty acid profile on the Rancimat IP was evident especially when oils/fats from different oleaginous raw materials are compared [11, 34].

IMPORTANCE OF OLEACIN AND OLEOCANTHAL

With the aim to understand the effect of OIN and OAL on Rancimat oxidative stability of EVOOs, the samples were split by median of EDAs value (341 mg/kg OE) into two groups (OG1 and OG2). OG1 and OG2 groups had significant differences by ANOVA analysis, shown in Table III, for all phenolic variables. Moreover, the mean of E-IP of OG1 was 60% higher than that of OG2 confirming the positive antioxidant activity of EVOO phenol content. The graphs given in Figure 5 are useful to understand the antioxidant effect produced by the single phenolic variables analysed. A closer correlation between TP and E-IP was obtained compared with the other variables (Fig. 5, Graph 1). However, by comparing the R^2 values, the antioxidant effect of TP appeared to be more relevant in OG1 than OG2.

Regarding EDAs (Fig. 5, Graph 2) significant differences emerged between OG1 and OG2. In OG1, a positive influence of EDAs on EI-P was obtained; conversely, at high doses of EDAS (OG2) any relationships with E-IP was found, while the antioxidant effect tended to decrease. This observation indicates that the higher E-IP found in OG2 was probably due to other phenolic compounds more than to EDAs.

OIN was the more effective component of EDAs as an antioxidant (Fig. 5, Graph 3). Also, in this case, the antioxidant effect of OIN was more relevant in OG1 than OG2 evidencing that high OIN content was not closely correlate with the increase of Rancimat oxidative stability. Finally, OAL showed a secondary importance on oil Rancimat oxidative stability (Fig. 5, Graph 4). However, this was an expected fact, being OAL a mono-phenol (Fig. 1), while OIN was an *o*-diphenol which is recognised to be a more effective radical scavenger compound [36].

Therefore, EDAs concentration up to 341 mg/kg appeared to have no supplementary linear effect on extending the oil oxidative stability. These findings revealed that an excess of OIN and OAL did not correspond necessary to a significant increase in the thermal oxidative stability of EVOO. This could be an important observation, since OIN and OAL are associated with the pungency and bitterness of EVOO [8]. However, it is right to point out the evidences that Rancimat IP cannot be associated with the real shelf life of oils/fats because the formation of volatile products is not as abundant under normal storage condition [31, 32, 37]. Moreover, it was observed that, under the high temperature and oxygen supply condition of Rancimat, the real EVOO's oxidation stability can be minimised by the pro-oxidant effect of the high amount of oxidised phenols [30]. Conversely, the same oxidised phenols are powerful antioxidants at a low temperature and under limited oxygen supply leaving an improvement of the EVOO shelf life [30].

4. CONCLUSIONS

The aim of this study was to investigate on the influence of oleacin and oleocanthal on the EVOO's oxidative stability determined by the Rancimat method. Regarding the other analysed component

variables, it was found that the EVOO rancimat induction period depends especially on the phenolic variables, while a low influence of free acidity and fatty acid composition was provided. A weak correlation was observed between total phenols and oleacin, oleocanthal and their sum (EDAs), suggesting that these last compounds should be considered as independent quality indicators. Oleacin showed antioxidant effect higher than oleocanthal. However, antioxidant effect decreased significantly at concentrations of EDAs higher than 341 mg OE/kg evidencing that an excess of EDAs does not have a supplementary effect on the elongation of oil thermal oxidative stability. Thus, an excessive level of oleacin and oleocanthal could not always be necessary going against the prevailing opinion for which it is crucial to have a very high pungent and bitter taste for an EVOO of the best quality. However, a similar investigation should to be carried out at room temperature to understand also importance of oleacin and oleocanthal on EVOO shelf life.

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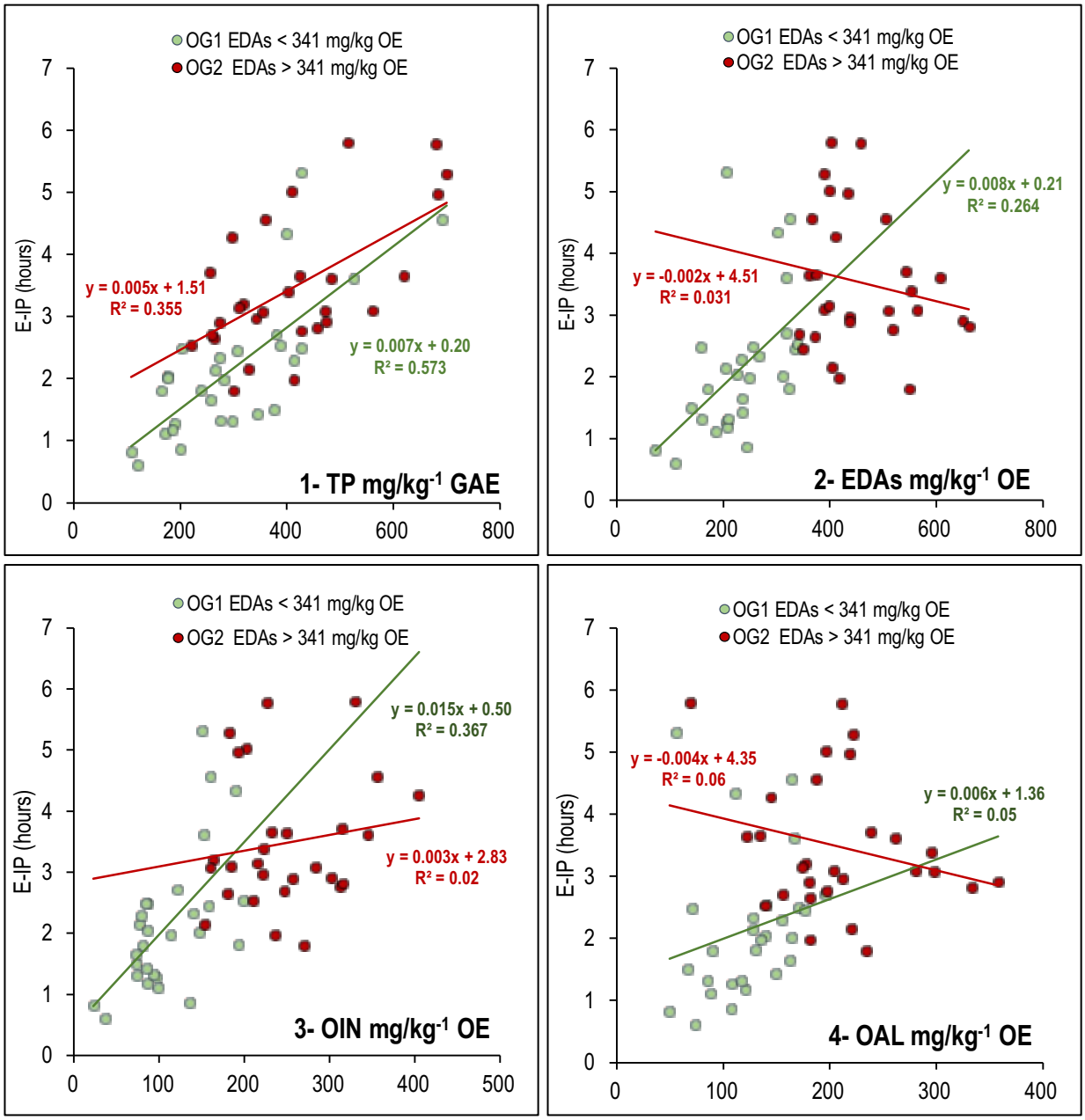

Figure 5 - Relationship between extension Rancimat induction period (E-IP) and analyzed phenolic variables (TP (1), EDAs (2), OIN (3), OAL (4)) relative to the EVOO samplessp lit by median of EDAs value (341 mg/kg OE) in the groups OG1 and OG2.

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