Carolea olive oil enriched with an infusion of Capsicuum annuum and C. chinense dried pepper powders to produce an added value flavoured olive oils

CAROLEA OLIVE OIL ENRICHED AN INFUSION OF *CAPSICUUM ANNUUM* AND *C. CHINENSE* DRIED PEPPER POWDERS TO PRODUCE A FLAVOURED OLIVE OIL WITH ENHANCED OXIDATIVE STABILITY

Running title

OLIVE OIL ENRICHED AN INFUSION OF DRIED PEPPER

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Abstract

The effects on the quality and oxidative stability of flavoured virgin olive oils (FVOOs) obtained from Carolea extra virgin olive oli (EVOO) was investigated. The oils were prepared by adding dried pepper powder from *Capsicum annuum* L. "Amando", "Mirasol", "Topepo rosso" and *C. chinense* Jacq. "Aji limo" and "Red mushroom" cultivars. The total phenol, flavonoid, and carotenoid content was monitored in pepper extracts, EVOO, and FVOOs phenolic fractions as well as their oxidative stability. DPPH, ABTS, and FRAP assays were applied to test the antioxidant activity. Interesting results were obtained from FVOO formulated with Aji limo with IC_{50} of 18.8 and 27.6 μg/mL in DPPH and ABTS test, respectively. Moreover, this FVOO showed an induction time of 17.40 h compared to 12.30 h for EVOO.

Pratical applications

Consumers are taking greater responsibility for their own health and they are increasingly turning to their diet to improve it. Virgin olive oil, the main fat of the Mediterranean diet, is per se considered as a functional food—as stated by the European Food Safety Authority (EFSA)—due to its content in healthy compounds. The daily intake of endogenous bioactive phenolics from virgin olive oil is variable due to the influence of multiple agronomic and technological factors. Thus, a good strategy to ensure an optimal intake of polyphenols through habitual diet would be to produce enriched virgin olive oil with well-known bioactive polyphenols.

Keywords *Capsicum;* flavoured olive oil; phenols; carotenoids; capsaicinoids; antioxidant; oxidative stability.

1. INTRODUCTION

The EVOO used was obtained from the Carolea cultivar, widely grown for oil production in the south of Italy, including Calabria, together with other cultivars that characterise the biodiversity of this Region (Giuffrè, 2017; Giuffrè, 2018). It gives a medium fruity oil with hints of bitterness. EVOO is known not only for its shelf-life, but also for its pharmaceutical properties and as an aid against some chronic diseases.

Chilli pepper (genus *Capsicum*) is a widely-consumed spice worldwide. It contains many phytochemicals with antioxidant properties, including carotenoids, flavonoids, phenols, terpenoids, saponins, stilbenes, and nitrogenous compounds (Wahyuni et al., 2013).

The addition of chilli pepper to EVOO has become more popular in recent years, due to consumer demand. The resulting oils, in addition to being flavoured, can also have an extended shelf-life.

The addition of spices or other flavourings means the resulting oil no longer satisfies the European Union Commission definition for extra virgin olive oil, but can be defined as a Flavoured Olive Oils (FVOO).

The main technique to produce FVOO is infusion. In this case powdered chilli pepper was added to EVOO and left to steep in amber bottles, after which it was filtered.

The main objective of this study was to evaluate the effect of 30 days' infusion in Carolea extravirgin olive oil (EVOO) of powdered *C. annuum* L. Mirasol, Amando, and Topepo rosso, *C. chinense* Jacq. Aji limo and Red mushroom cultivars.

For this purpose: *i*) total phenol, flavonoid, and carotenoid contents were assessed in pepper extracts, EVOOs, and FVOOs; *ii*) capsaicin, dihydrocapsaicin, vitamin C, and vitamin E were quantified in all pepper samples; *iii*) the EVOO fatty acid profile was studied; *iv*) the protective effect of pepper extracts on FVOOs' oxidative stability was investigated; *vi*) the antioxidant potential of pepper extracts, EVOO, and FVOOs' phenolic fraction was investigated by different *in vitro* techniques.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Chemicals, reagents and solvents were purchased from Sigma-Aldrich S.p.a. (Milan, Italy).

2.2 Plant materials, EVOO and FVOOs formulation

The olive fruits of *Olea europea* Carolea cultivar were collected in Calabria (Italy) during the 2018/2019 season. Carolea EVOO was obtained using continuous mills at the "Meringolo" olive oil mill (Corigliano Calabro, Cosenza, Italy). The EVOO sample received UNI10939, 2001 certification.

Capsicum fruits were obtained from the "Miceli" farm. (39°48'21 N, 15°47'46 E) (Scalea, Calabria, Italy). Table S1 reports their main characteristics. *Capsicum* fruits were collected at complete maturation and dried in the sun for 2 weeks. Subsequently, the dry product was powedered and 50 mg was added to 5 g of Carolea EVOO and stirred to obtain the corresponding FVOO. FVOO were stored for 30 days in amber bottles at -20 °C until analysis.

2.3 Extraction procedure

Capsicum fruits (250 g) were subjected to maceration using ethanol (350 mL) as a solvent (3 × 72 h). The extracts were combined and stored -20 °C until analysis.

2.4 Determination of phytochemical content

The total phenol content (TPC) and the total carotenoid content (TCC) were determined following the procedure previously reported (Gao et al., 2000). Chlorogenic acid equivalents (CAE)/100 g of fresh weight (FW) and mg β-carotene equivalents (βCE)/100 g FW were used to express results on TPC and TCC, respectively. For the total flavonoid content (TFC) the protocol of Yoo et al. (2008) was applied. mg Quercetin equivalents (QE)/g FW were used to express the obtained results.

Gas chromatography (GC) (Shimadzu GC17A, Shimadzu, Milan, Italy) equipped with a flame ionisation detector (FID) was used for capsaicin and dihydrocapsaicin determination in µg/g FW (Menichini et al., 2009).

2.5 Vitamin C and E content

The pepper's vitamin C content was determined according to the method of Klein and Perry, (1982) and expressed as mg/100 g DW. Gas chromatography-mass spectrometry (GC-MS) analysis (Agilent, Milan, Italy) was used for vitamin E quantification in mg/100 g DW.

EVOO quality parameters (acidity, peroxide index, and UV light absorption) were determined according to the procedures described by EC Regulation (EUC, 2013). The oxidative stability index as defined in AOCS Official methods (1993) was investigated by using Rancimat apparatus (Metrohm, Basel, Switzerland). The curve inflection point was defined by induction time and expressed in hours (Karakuş et al., 2017).

2.6 Extraction of phenolic fraction

The EVOO phenolic fraction was obtained following the procedure of Montedoro et al. (1982) using hydroalcoholic solution (7:3 v/v) and then *n*-hexane. After centrifugation, the residue was taken up with hydroalcoholic solution (1:1 v/v) and stored at -20°C until analysis.

2.7 Determination of TPC, TFC, and TCC content in Carolea phenolic fraction

The procedure for spectrophotometric determination of TPC and TFC was the same as that applied to the pepper extract (see paragraph 2.4). In EVOO the TCC was determined as described by Minguez-Mosquera et al. (1991). Concisely, EVOO (5 mL) was mixed with *n*-hexane (1:1, v/v). Results are expressed as ppm.

2.8 Fatty acid analysis

Carolea EVOO fatty acids were determined by GC-MS analyses (Agilent, Milan, Italy) following the procedure previously reported (Leporini et al., 2018).

2.9 Antioxidant activity of pepper extracts and EVOO phenolic fraction

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays were applied to examine the radical scavenging activity of *Capsicum* extracts and EVOO phenolic fraction using the procedure previously described by Loizzo et al.[14] In both cases ascorbic acid was used as a positive control.

Moreover, both pepper extracts and EVOO phenolic fraction (at concentration of 2.5 mg/mL) were tested, also, to evaluate the ability of samples to protect iron from redox reaction (Loizzo et al., 2016). Butylated hydroxytoluene (BHT) was used as control.

2.10 Statistical analysis

Data are expressed as mean ± standard deviation (S.D.). Prism GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA) was used to calculate IC_{50} value and to perform ANOVA test followed by a multicomparison Dunnett's test (α= 0.05). *Pearson's correlation coefficient* (*r*) and Tukey's multiple range test were also done. Principal component analysis (PCA) was applied by SPSS software for Windows, version 15.0 (Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Phytochemical content of peppers

Extraction yields in the range of 6.2-6.5% were obtained for *C. annuum* Aji limo, Topepo rosso, and Amando. Highest yields were found for Mirasol and Red mushroom (8.2 and 7.1%, respectively). Extracts were analysed in order to evaluate their TPC, TFC, TCC, and capsaicinoid content (Table 1).

C. chinense Red mushroom exhibited the highest TPC value of 623.6 mg CAE/100 g FW, followed by Topepo rosso and Aji limo. Aji limo pepper was characterized by the highest TFC (64.5 mg QE/ 100 g FW), followed by Amando pepper (54.5 mg QE/ 100 g FW).

Carotenoids are responsible for *Capsicum* colour. Except for Aji limo pepper (98.1 mg βCE/100 g FW), the carotenoid content was in the range 227.4-328.1 βCE/100 g FW.

The most well-known phytochemicals of *Capsicum* are capsaicinoids (capsaicin and dihydrocapsaicin) that are responsible for *Capsicum* pungency (Estrada et al., 1998). *C. chinense* Red mushroom showed the highest capsaicin content with value of 2504.4 μg/g FW followed by *C. chinense* Aji limo pepper (2234.5 μg/g FW). The lowest capsaicin content was found in *C. annuum* (410.2-510.2 μg/g FW). The same trend was observed for dihydrocapsaicin. *C. chinense* peppers are characterized by the highest vitamin C and E content with values of 5.6 and 6.0 mg/g FW for Red mushroom, and 5.9 and 6.3 mg/g FW Aji limo, respectively.

Our data on *C. chinense* Red mushroom agree with those reported for *C. chinense* Habanero (Menichini et al., 2009). Several varieties of *C. annuum* have been investigated. Among them, in agreement with our obtained data on *C. annuum* species, are values of *C. annuum* var. *acuminatum* with TPC 970.2 mg CAE/100 g FW, TFC 56.0 mg QE/100 g FW TCC of 324.2 βCE/100 g FW, respectively (Tundis et al., 2016). Significantly lower TFC (5.6 mg QE/100 g FW) and TCC (133.9 βCE/100 g FW) values were found in *C. annuum* var. c*erasiferum*. In another work, Tundis et al. (2016) investigated the evolution of phytochemical content during ripening of *C. annuum* cv Cayenne Golden, acuminatum, Orange Thai and Fiesta. When mature, TPC ranged from 648.6 to 679.6 mg CAE/100 g FW. *C. annuum* cayenne golden showed the highest TPC value. TFC values ranged from 34.9 to 61.5 mg CAE/100 g FW. *C. annuum* acuminatum showed the highest TCC (414.1 βCE/100 g FW). *C. annuum* Orange Thai, acuminatum, and Fiesta are spicier than *C. annuum* Mirasol, Amando, and Topepo rosso with capsaicin content about 3-times higher.

3.2 EVOO quality parameters and chemical profile

The analysis of Carolea EVOO quality parameters showed a free acidity value of 0.37%, a peroxide level of 7.98 meq O_2/kg of oil, and a ΔK value of 0.0024. A free acidity of 0.47%, and a peroxide level of 6.91 meq O_2/kg of oil were found for Carolea EVOO by Piscopo et al. (2016) whereas a mean value of 0.3 g oleic acid/100 g oil was previously, recorded for Frantoio EVOO by Leporini et al. (2018). Our values are in agreement with those reported by Lavelli et al. (2005) for EVOO obtained by Pendolino, Leccino, Moraiolo, and Taggiasca cultivars.

The quantity of phenols in EVOO is not only responsible for the perception of pungency but above all for EVOO resistance to the normal oxidative process. Carolea EVOO showed a TPC of 851.3 ppm, this value is 2-times higher than that found by Piscopo et al. (2016) for the same cultivar (317.44 ppm) (Table 2). Values in the range from 286.73 to 305.65 ppm were found for Ottobratica and Sinopolese EVOO, respectively (Loizzo et al., 2016). Previously, Leporini et al. (2018) showed that TPC varied significantly in EVOO from Frantoio cultivars from different areas of Calabria. However, our data are in agreement with those found for Campania's Frantoio EVOO (Lavelli et al., 2005). A higher TPC value was recorded for Bosana EVOO from Sardinia (Italy) (Del Caro et al., 2006). A TFC value of 28.5 ppm was found for Carolea EVOO phenolic fraction (Table 3). An increase in all phytochemical contents was observed in all FVOOs. In particular, FVOO enriched with Aji limo pepper showed the highest TPC, TFC and TCC with values of 912.1 mg CAE/100 g FW, 42.6 mg QE/100 g FW, 33.1 mg βCE/100 g FW, respectively.

The first qualitative parameter observed by consumers is colour, hence the attention to the EVOO pigment content (Loizzo et al., 2009; Loizzo et al., 2012). Among them carotenoids occupy an important role since they are strong protectors against light induced EVOO oxidation. Previously, Šarolić et al. (2015) investigated Croatian EVOO carotenoid content and found values ranging from 3.86 to 4.75 ppm. Lower values were recorded by Zegane et al. (2015) in Algerian EVOO (0.67-1.70 mg/kg). The TPC, TFC and TCC content was monitored also in FVOOs (Table 3). As it is possible to see, all phytochemical contents are higher in FVOO compared to EVOO. Red mushroom FVOO showed the highest TPC with a value of 912.1 ppm followed by Topepo rosso FVOO (905.6 ppm). The following trend was observed for TFC content in FVOOs Aji limo > Topepo rosso > Red mushroom > Amando > Mirasol. With regard to the TCC, it could be observed that oils flavoured with Red mushroom and Aji limo peppers are characterized by the highest content in carotenoids with values of 28.8 and 25.1 ppm.

Carolea EVOO possessed a high content of oleic acid (Table 3). Among saturated fatty acids (SFA) C16:0 was detected in a significant amount with a value of 13.7% while C18:2 with a percentage of 6.5% was the most abundant polyunsaturated fatty acid. A high oleic/linoleic *ratio* of 11.4 was found for Carolea EVOO, which indicates the high stability of the EVOO (Zegane et al., 2015).

Leporini et al. (2018) previously recorded values ranging from 9.0 to 12.2% for Frantoio EVOOs. Our data are in line with Sicilian EVOO (Biancolilla, Cerasuola, Nocellara Etnea, Nocellara del Belice, and Moresca) (Patumi et al., 2003). More recently, Blasi et al. (2019) reported the fatty acid composition of Frantoio, Dolce Agogia, Leccino, and Moraiolo. Oleic acid was the most abundant with percentages from 76.2 to 78% for Leccino and Dolce Agogia, respectively followed by palmitic acid. Linoleic acid was identified in the range of 6.0 to 7.1% for Dolce Agogia and Frantoio, respectively.

3.3 Antioxidant activity

The antioxidant potential of Carolea EVOO and FVOO phenolic extract as well as pepper extracts was reported in Table 4. Carolea EVOO exhibited a good radical scavenging potential with IC_{50} of 26.6 and 33.5 μg/mL for DPPH and ABTS test, respectively. Previously, Baiano et al. (2009) investigated the evolution of Italian EVOO antioxidant activity during 12 months' storage. The TPC of the investigated Italian EVOO is strictly dependent on the cultivar, area, and time of fruit collection. The analysis of pepper extracts showed that *C. chinense* Aji limo had the highest antioxidant potential with IC_{50} of 11.8 and 18.2 μg/mL, in DPPH and ABTS tests, respectively. A promising FRAP value was also observed (78.8 μM Fe(II)/g). A notable antioxidant potential was observed with Topepo rosso pepper, which showed IC_{50} of 18.9 and 28.4 μg/mL in DPPH and ABTS tests, respectively. This test was positively correlated with TPC (r =0.68) and TCC (r =0.92). A positive correlation was observed also for the TCC and DPPH assay with r value of 0.77. The antioxidant activity of fresh and processed *C. annuum* and *C. chinense* peppers was investigated by Loizzo et al. (2015). Samples characterized by the highest antioxidant activity are also richest in TPC and capsaicinoids. The promising *C. annuum* antioxidant potential was confirmed also by the investigation of Loizzo et al. (2017) who demonstrated how both Pellegrino and Idealino dried pepper samples exhibited ABTS radical scavenging activity with IC_{50} of 45.2 and 45.7 μg/mL, respectively.

The highest radical scavenging activity was recorded with FVOO enriched with Aji limo pepper that showed IC_{50} values of 18.8 and 27.6 μg/mL for DPPH and ABTS test respectively, followed by FVOO enriched with Red mushroom (IC_{50} values of 19.3 and 28.9 μg/mL for DPPH and ABTS test, respectively). The same trend was observed, also in reduction of iron with FRAP values in the range 129.8-139.5 for FVOO with Topepo rosso and Red mushroom, respectively. Correlation analysis on EVOO and FVOOs phytochemical content and bioactivity showed that TCC is positively correlated with both ABTS and FRAP test with r value of 0.75. From the analysis of the literature on antioxidant activity of FVOOs a controversial data emerges. This may depend on the matrix used for enrichment (spices, herbs, fruits) and on the different techniques used for obtaining it (infusion or co-processing) (Reboredo-Rodríguez et al., 2017).

According to Caporaso et al. (2013) the radical scavenging potential of a mixture of virgin olive oil and refined olive oil enriched with hot *C. annuum* from Campania (Italy) (20% w/w) showed a greater ABTS radical scavenging activity than the starting olive oil even after only 7 days of infusion. Moreover, the antioxidant activity was correlated to the capsaicinoids and carotenoids released by the pepper during infusion time.

3.4 Effect of the addition of peppers on FVOOs oxidative stability

The effect of the addition of different peppers on FVOOs was investigated by simulating oxidation process using Rancimat apparatus. Carolea EVOO had an induction time of 12.3 h. Generally, all FVOOs are characterized by a higher induction time even if there is a difference depending on the pepper cultivar added to the oil. In particular, the addition of *C. chinense* Aji limo and Red mushroom cultivars peppers to oil resulted in FVOOs with induction times of 17.4 and 15.2 h, respectively. A lesser effect on FVOO's oxidative stability was observed with the addition of *C. annuum* peppers that prolonged the induction time to 14.9, 14.2 and 14.0 h for Mirasol, Amando and Topepo rosso, respectively (Figure 1).

The result of the quotient of the induction time of FVOO and EVOO, namely protection factor (PF), was used as an index of oxidative stability. By using this parameter, the protective activity was demonstrated for all applied pepper extracts with PF values in the range 1.4-1.1 for Aji limo and Topepo rosso or Amando, respectively.

Pearson's correlation coefficient showed that all quantified phytochemicals in FVOOs (TPC, TFC, TCC, vitamins and capsaicinoids) are positively correlated with the oxidative stability measured as induction time. In particular, FVOOs enriched with peppers characterized by high vitamin C and E are more resistant to oxidation (r values of 0.87 and 0.82 for induction time and vitamin C and E content, respectively).

Piscopo et al. (2016) investigated the resistance to oxidation of Calabrian EVOO and found the following order: Carolea > Ottobratica > Sinopolese > Grossa di Gerace. The literature on the effect of the addition of herbs and spices on FVOO oxidative stability is controversial. Previously, Caporaso et al. (2013) showed that the addition of 10-20% w/w of dried *C. annuum* to EVOO determined a reduction of olive oil oxidative stability over a 30-day period. Conversely, Gambacorta et al. (2007) reported an increased oxidative stability in FVOO over 30 days when hot pepper (10-20%) was added by infusion using Dauno EVOO.

3.5 PCA analysis

Principal Component Analysis (D'Agostino et al., 2014). was applied to oils flavoured by an infusion of *Capsicuum annuum* and dried *C. chinense* peppers. By choosing eigenvalues greater than one (>1), the dimensionality was reduced from 11 variables to two principal components (PC). PCA results revealed that the first two principal components explained 92.77 % of total variance. The loadings of first and second principal components (PC1 and PC2) accounted for 59.20 and 33.57 % of the variance, respectively (Fig. 2).

Figures 2 and Table S2 illustrate the strong correlation that exists between the analyzed variables.

The first component (PC1) is highly positively correlated with FRAP, TPC and OSI; while the second component (PC2) is positively correlated with DPPH and ABTS. Total flavonoids (TFC) show negative correlation for PC1 and PC2. The bi-dimensional PCA analysis clearly classifies the similarities or differences of the enriched extra virgin olive oil. The scores plot analysis clearly classifies the enriched extra virgin olive oils in the upper region of the PCA score plot. The analysis demonstrated that among the enriched oils analyzed they were located in the top right quadrant, which represents the highest FRAP, DPPH, TPC and OSI.

Infusion of *Capsicuum annuum* and *C. chinense* dried peppers in EVOO enriched the oil antioxidant compounds and significantly influenced the chemical composition of these new products (FVOO).

PCA confirmed that all the enriched oils analyzed possess the highest bioactive capacity. Thus, the present results provided the basic data for choosing extra virgin olive oils with higher antioxidant activity for direct consumption.

4. CONCLUSIONS

In recent years, flavoured oil has gained attention to not only flavour meat, fish or salad but also for the potential health benefits of the phytochemicals contained in herbs and spices. In this context, we decided to test the effects of the addition by infusion of different pepper cultivars of *C. annuum* and *C. chinense* to Carolea EVOO. All pepper extracts are rich in bioactive compounds. Several quantified phytochemicals (capsaicin, vitamins C, E TPC and TCC) exert a protective action on the oxidative process, which the oil spontaneously undergoes.

Among peppers tested in the EVOO infusion, *C. chinense* Aji limo and Red mushroom cultivars are the most active. Based on the obtained results FVOOs could be proposed as functional oils characterized by high stability and health properties due their antioxidant potential.

Conflicts of Interest

The authors declare no conflict of interest.

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TABLE 1. GAS PERCENTAGE OF ORANGES PACKED IN FILM

Storage Conditions	Sample Typology	Time (Days)	O_2 (%)	CO_2 (%)
6°C and 80% U.R.	Orange fruits wrapped in Nanoactive film	0	17.27±1.3a	3.54±1.04d
		15	15.16±4.97c	7.33±4.71b
		30	14.8±5.22b	8.86±4.61c
		45	12.7±3.66d	9.10±3.79a

		Sig.	**	**
6°C and 80% U.R.	Orange fruits wrapped in BOPP film	0	19.30±0.42c	1.84±0.45b
		15	20.18±0.28a	0.97±0.49d
		30	19.67±0.32b	1.59±0.59c
		45	18.97±0.25d	2.33±0.38a
		Sig.	**	**

Significance at $P<0.05$. Means in each column with the same letter do not differ significantly.

U.R. = relative humidity

TABLE 2. CHANGES IN THE CONTENT OF PH, TOTAL SOLUBLE SOLIDS AND TOTAL ACIDITY OF THE JUICE FROM ORANGES WRAPPED IN BOPP FILM, NANOACTIVE FILM AND NON-WRAPPED

Analysis	Time (Days)	Control	N.A. Film	BOPP Film	Sig.
Total Soluble Solids (°Brix)	0	13.10±0.10b	13.10±0.10b	13.10±0.10a	
	15	13.30±0.10a	13.40±0.06a	12.70±0.20b	**

	30	13.80±0.10a	13.00±0.06b	13.00±0.10a	*
	45	13.30±0.00a	12.97±0.58b	13.10±0.06a	**
Total Acidity (g/L Citric Acid)	0	11.38±0.14b	11.38±0.14a	11.38±0.14a	
	15	10.09±0.02d	9.31±0.12b	10.91±0.83a	**
	30	11.63±0.02a	9.57±0.19c	9.12±0.19b	**
	45	10.58±0.01c	9.61±0.13d	9.29±0.17b	**
pH	0	3.27±0.04d	3.27±0.04c	3.27±0.04c	
	15	3.39±0.01c	3.54±0.03ab	3.42±0.01b	**
	30	3,45±0,02b	3.48±0.06b	3.49±0.03a	*
	45	3.61±0.02a	3.57±0.01a	3.48±0.01a	**

Significance at P<0.05. Means in each column with the same letter do not differ significantly
Sig. - Film, Time, Film*Time
N.A.- nanoactive

TABLE 3. CHANGES IN THE CONTENT OF TOTAL FLAVONOIDS, POLYPHENOLS AND ASCORBIC ACID OF THE JUICE FROM ORANGES WRAPPED IN BOPP FILM, NANOACTIVE FILM AND NON-WRAPPED

Analysis	Time (Days)	Control	N.A. Film	BOPP Film	Sig.
Total Flavonoids (mg/L hesperidin)	0	1209±12.78b	1209±12.78a	1209±12.78c	
	15	987±26,02d	1086±30,05b	1171±23,94c	**
	30	1126±51,11c	1082±57,44b	1285±69,65b	**
	45	1248±59,79a	1179±63.91a	1599±11,84a	**
Total Polyphenols (mg/L gallic acid)	0	5229±151.71a	5229±151.71a	5229±151.71a	
	15	5272±132.12b	5685±152.58b	5396±218.23b	**
	30	5140±110.82c	5284±115.13a	5231±101.89a	**
	45	4501±100.10d	4926±79.54c	4917±114.12b	**
Ascorbic Acid (mg/L)	0	606.65±4.75a	606.65±4.75a	606.65±4.75a	
	15	493.20±4.12c	538.08±1.59b	581.17±1.66b	**
	30	505.83±5.20b	536.19±8.39b	548.99±19.21c	**
	45	498.06±0.00c	550.40±4.14c	501.43±12.43bc	**

Significance at $P<0.05$. Means in each column with the same letter do not differ significantly
Sig. - Film, Time, Film*Time
N.A. - nanoactive

TABLE 4. CHANGES IN THE CONTENT OF HESPERIDIN AND NARIRUTIN OF THE JUICE FROM ORANGES WRAPPED IN BOPP FILM, NANOACTIVE FILM AND NON-WRAPPED

Analysis	Time (Days)	Control	N.A. Film	BOPP Film	Sig.

Hesperidin (mg/Kg hesperidin)	0	265.21±8.36a	265.14±11.03d	265.11±2.45c	
	15	171.02±6.31b	271.12±10.96b	222.28±3.41a	**
	30	99.33±1.54c	229.11±8.46a	221.31±3.58d	**
	45	58.35±2.64d	272.08±4.31c	164.09±4.62b	**
Narirutin (mg/Kg hesperidin)	0	174.45±4.55a	174.45±7.74c	174.45±4.85a	
	15	37.23±1.15d	185.22±5.60a	112.20±8.36b	**
	30	40.07±1.89b	173.14±11.11c	86.23±1.88d	**
	45	34.89±1.06c	206.21±5.29b	89.07±2.48c	**

Significance at P<0.05. Means in each column with the same letter do not differ significantly
Sig. - Film, Time, Film*Time
N.A. - nanoactive

TABLE 5. ANTIOXIDANT ACTIVITY OF THE JUICE FROM ORANGES WRAPPED IN BOPP FILM, NANOACTIVE FILM AND NON-WRAPPED

Analysis	Time (Days)	Control	N.A. Film	BOPP Film	Sig.
Antioxidant Capacity DPPH (-OD^{-3} min^{-1} g dm^{-1})	0	2.54±0.20a	2.54±0.20a	2.54±0.20a	
	15	1.64±0.62b	1.58±0.51d	1.68±0.25d	**
	30	2.22±0.25a	1.76±0.47b	1.72±0.57c	**
	45	1.70±0.26b	2.73±0.56c	2.38±0.17b	**

Significance at P<0.05. Means in each column with the same letter do not differ significantly
Sig. - Film, Time, Film*Time
N.A. - nanoactive

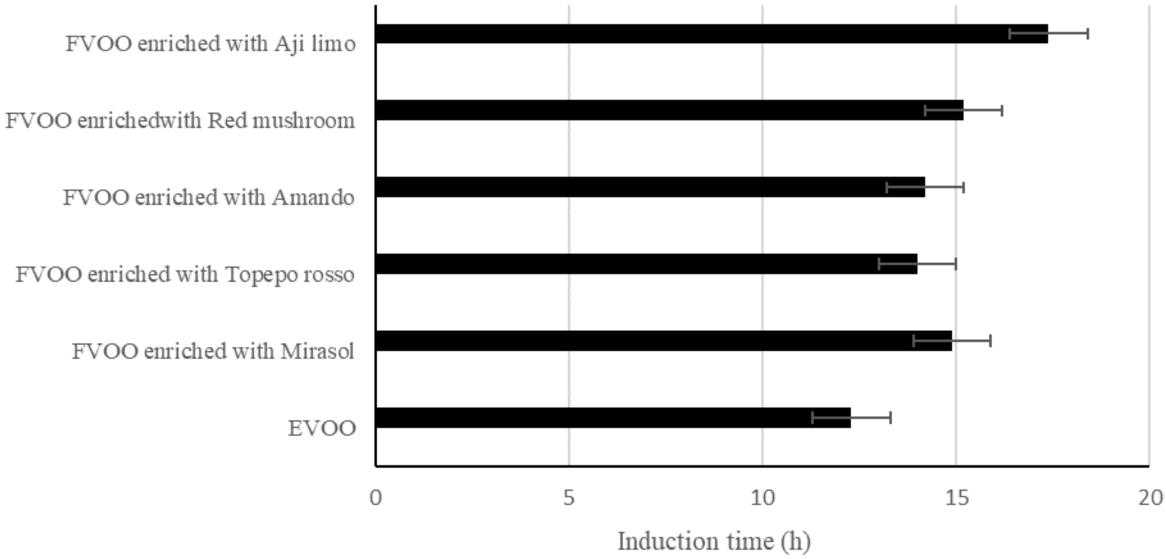

FIGURE 1 Induction time of Carolea EVOO and FVOO

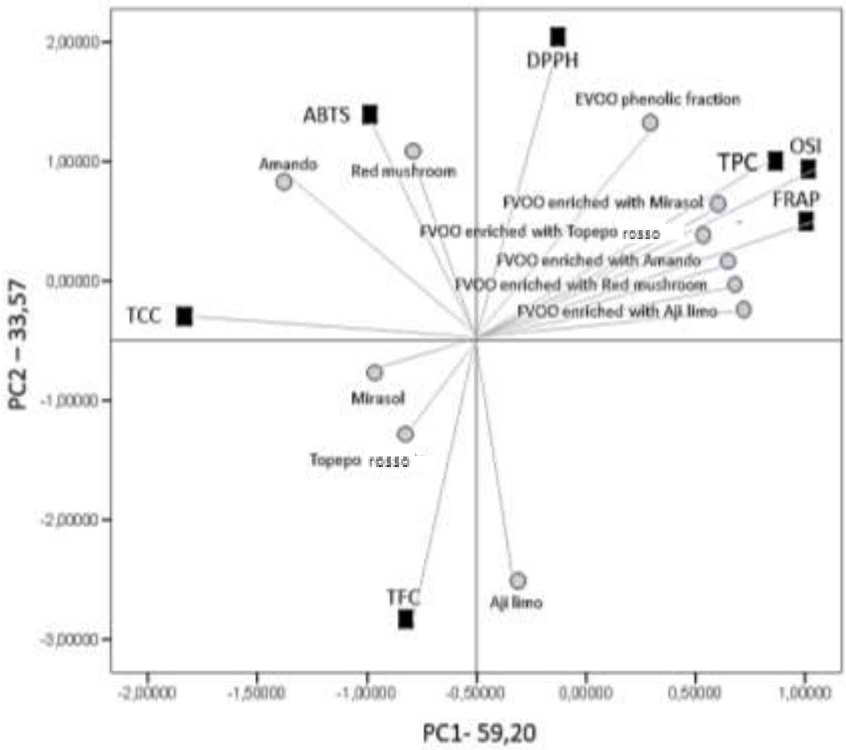

FIGURE 2 **Factor loadings for principal components (PC) PC1 and PC2 and scatter plot of all oil samples**