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The addition of *Capsicum baccatum* to Calabrian monovarietal extra virgin olive oils leads to flavoured olive oils with enhanced oxidative stability

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Abstract

This study aimed to evaluate the influence of *Capsicum baccatum* L. Aji Angelo and Bishop crown cultivars to the quality parameters of flavoured olive oils (FOOs) obtained by the addition of both fresh and dried pepper powders (1%) to Dolce di Rossano and Roggianella monovarietal extra virgin olive oils (EVOOs). First, pepper extracts were investigated for their total phenolic, flavonoid, carotenoid content as well as phenolic acids, fatty acid profile, and vitamin C and E content. In order to evaluate the impact of both fresh and dried peppers on the oxidative stability of FOOs, the Rancimat test was applied. 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene bleaching (B-CB) and ferric reducing antioxidant power (FRAP) assays were used to investigate the antioxidant potential. Bishop crown dried extracts showed the highest phenolic, carotenoid and vitamin content, whereas Aji Angelo had the highest amount of capsaicinoids. Among EVOOs, Roggianella EVOO showed the highest antioxidant activity as well as the highest induction time (39.6 h). Remarkably, FOO obtained by the addition of Bishop crown dried pepper extract to Roggianella EVOO showed a higher induction time (44.9 h) with respect to the corresponding EVOO.

Keywords: antioxidant activity; *Capsicum*; chemical profile; monovarietal extra virgin olive oil; oxidative stability

Introduction

According to the definition of the European Union Commission (EC, 2003; EEC, 1991), an extra virgin olive oil (EVOO) must be extracted "only from olives with superior quality, cannot undergo any treatment other than washing the fruits, and decanting, centrifuging and filtering the extracted olive oil. It excludes oils obtained from seeds by chemical or mechanical methods or the use of solvent extraction or re-esterification methods, and those mixed with oils from other sources." Thus, the addition of herbs, spices or other fruits to an EVOO generates a product that cannot be called "extra virgin olive oil," but can be defined as flavoured olive oil (FOO) (Baiano *et al.*, 2010). These FOOs can be characterised by improved nutritional values, enriched sensory characteristics and increased shelf-life. Recently, several FOOs have been introduced into the market (Issaoui *et al.*, 2016).

Capsicum genus comprises 30 species, however, only five (*C. baccatum, C. annuum, C. frutescens, C. chinense* and *C. pubescens*) are those mainly cultivated (Tripodi and Kumar, 2019). *Capsicum* phytochemicals include phenolics, carotenoids, capsaicinoids and other metabolites (Wahyuni *et al.*, 2013). Several of these compounds are well-known as antioxidants accounting for the traditional use of peppers as food preserving agents from

ancient times. In the last decades, our research group investigated the chemical composition and bioactivity of many food plants including different *Capsicum* species collected in Calabria (Southern Italy) (Fazio *et al.*, 2018; Loizzo *et al.*, 2015, 2017; Menichini *et al.*, 2009; Tundis *et al.*, 2013).

This study aimed to evaluate the effect of *Capsicum baccatum* Aji Angelo and Bishop crown cultivars on the quality parameters of FOOs obtained by the addition of pepper powder to Dolce di Rossano and Roggianella monovarietal EVOOs. For this purpose: (i) total phenolic, flavonoid and carotenoid contents, as well as vitamins C and E were assessed in both EVOOs, pepper extracts and FOOs; (ii) fatty acids, sterols, phenolics and capsaicinoids were also quantified; (iii) the protective effect of pepper on FOO oxidative stability; and (iv) the antioxidant potential was investigated by different *in vitro* methods.

Materials and Methods

Chemicals and reagents

All reagents were purchased from Sigma-Aldrich S.p.a. (Milano, Italy), whereas analytical-grade solvents were obtained from VWR International s.r.l. (Milan, Italy). The following standards were used: FAME Mixes-Analytical Standards (CRM47885); Cholesterol (PubChem CID: 5997); 24-Methylene-Cholesterol (PubChem CID: 92113); Campesterol (PubChem CID: 173183); Campestanol (PubChem CID: 119394); Stigmasterol (PubChem CID: 5280794); Δ^7-Campesterol (PubChem CID: 5283646); Clerosterol (PubChem CID: 5283638); Clerosterol (PubChem CID: 5283638); β-Sitosterol (PubChem CID: 222284); Sitostanol (PubChem CID: 6743); Δ^5-Avenasterol (PubChem CID: 5281326); $\Delta^{5,24}$-Stigmastadienol (PubChem CID: 286499); Δ^7-Stigmastenol (PubChem CID: 3080632); Δ^7-Avenasterol (PubChem CID: 12795736); Erythrodiol (PubChem CID: 101761); Uvaol (PubChem CID: 92802); 4-Hydroxybenzoic acid (PubChem CID: 135); *p*-Coumaric acid (PubChem CID: 637542); *o*-Coumaric acid (PubChem CID: 637540); Ferulic acid (PubChem CID: 445858); Hydroxytyrosol (PubChem CID: 82755); Tyrosol (PubChem CID: 10393); (+) Pinoresinol (PubChem CID: 73399); 3,4-DHPEA-EDA (PubChem CID: 18684078); *p*-HPEA-EDA (PubChem CID: 11652416); Hydroxytyrosol acetate (PubChem CID: 155240); Apigenin (PubChem CID: 5280443); Luteolin (PubChem CID: 5280445); Chlorogenic acid (PubChem CID: 1794427); Quercetin (PubChem CID: 5280343); Beta-carotene (PubChem CID: 5280489); Vitamin C (PubChem CID: 54670067); Vitamin E (PubChem CID: 14985); Capsaicin (PubChem CID: 1548943); Dihydrocapsaicin (PubChem CID: 107982).

Materials, drying process and enrichment procedure

Dolce di Rossano and Roggianella monovarietal EVOOs were supplied in November 2019 by Frantoio Meringolo, Corigliano Calabro (Cosenza, Italy). All samples have accomplished the UNI10939, 2001 certification. EVOOs were stored in green glass bottles without headspace before analysis. Fruits of *C. baccatum* Aji Angelo and Bishop crown cultivars were obtained from Miceli s.r.l. farm (Scalea, Cosenza, Italy) that provides its authentication. Fruits were picked up at the maturity stage, defined by a visual colour change and size measurement. Before analyses, fruits were examined for integrity and absence of insect contamination, devoid of peduncles and seeds and cut into small pieces. To obtain dried peppers, fruits were sun-dried at 35°C for 2 weeks. Both fresh and dried peppers were grounded and the powders (50 mg) were added to 5 g of EVOOs and stirred to obtain FOOs that were left for 30 days in the infusion. After that, FOOs were filtered to remove the powder and analysed. Samples were stored at −20°C until analysis.

EVOOs and FOOs

Quality parameters

Extra virgin olive oil quality parameters (free acidity, peroxide index, UV light absorption sterol and fatty acid profiles) were analysed according to the EC Regulation methods (EU, 2016). To compare oxidative stability of FOOs and EVOOs, Rancimat equipment (Metrohm, Basel, Switzerland) at 98°C and with airflow of 10–12 L/h was used, according to a known protocol (Firestone, 1993).

EVOO phenolic extract and high-performance liquid chromatography (HPLC) analysis

The EVOO phenolic extract was obtained by using a previously described procedure (Montedoro *et al.*, 1992). The obtained extract was dissolved in 1 mL of methanol/water (1:1, *v*/*v*) and after filtration injected into a high-performance liquid chromatography (HPLC) instrument, Knauer instrument (ASI – Advanced Scientific Instruments, Berlin, Germany), coupled with UV-VIS detector of Waters Company (model Waters 486 Tunable), as previously described (Sicari *et al.*, 2010). The mobile phase was constituted by water/acetic acid (98:2, *v*/*v*) (A) and methanol/acetonitrile (1:1, *v*/*v*) (B), with a flow rate of 1 mL/min. Data from three independent experiments were acquired with Clarity software (Chromatography Station for Windows) and expressed as mean ± SD.

Total phenolic, flavonoid, carotenoid and chlorophylls contents

Total phenolic content (TPC) was determined as previously described by Gao *et al.* (2000). The absorbance was

read at 765 nm. For the total flavonoid content (TFC), the method reported by Yoo *et al.* (2008) was applied. Absorbance was read at 510 nm. Total carotenoid content (TCC) and chlorophyll content were determined following a previously reported procedure (Mínguez-Mosquera *et al.*, 1900). According to this method, the index K670 assesses the total chlorophylls and their derivatives, whereas the index K470 assesses TCC.

Capsicum baccatum

Extraction procedure

Both *C. baccatum* Bishop crown and Aji Angelo cultivars (250 g) were subjected to maceration in ethanol (500 mL) for three times. Extracts were stored until analysis at −20°C in amber bottles.

Total phenolic, flavonoid and carotenoid contents

Total phenolic content, TFC and TCC were determined as described for EVOO samples in the previous section. TPC was expressed as mg chlorogenic acid (CA) equivalents/100 g dry weight (DW). TFC was expressed as mg quercetin equivalents (QE)/100 g DW. TCC was expressed as mg β-carotene (βC) equivalents/100 g DW (Menichini *et al.*, 2009).

Capsaicin and dihydrocapsaicin content

Capsaicin and dihydrocapsaicin contents were determined according to a previously reported method (Menichini *et al.*, 2009), by using a GC17A gas chromatograph (GC) (Shimadzu, Milan, Italy) equipped with a Flame Ionization Detector (FID). Analyses were performed in isothermal conditions at 210°C. The capsaicinoids content was done and data are expressed as mean ± SD in μg/g DW.

Vitamin C and E content

The content of vitamin C in pepper samples was determined according to a previously reported method (Klein and Perry, 1982). For determination of vitamin E content gas chromatography-mass spectrometry (GC-MS) analyses were performed (Loizzo *et al.*, 2015). Vitamin content was expressed as mg/100 g DW.

Antioxidant activity of EVOOs and pepper extracts

Radical scavenging activity by ABTS and DPPH tests

Both 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) tests were applied to examine the radical scavenging activity of *Capsicum* extracts and EVOO phenolic fraction using the procedure previously described by Leporini *et al.* (2018). In both cases, ascorbic acid was used as a positive control.

Evaluation of protection of lipid peroxidation

In the β-carotene bleaching (B-CB) test, a mixture of linoleic acid (LA), β-carotene and Tween 20 was prepared and the resulting emulsion was mixed with samples (Loizzo *et al.*, 2019). The absorbance was read after 30 min of incubation at 470 nm. Propyl gallate was used as a positive control.

Ferric reducing activity power (FRAP) assay

Both pepper extract and EVOO phenolic fraction (at a concentration of 2.5 mg/mL) were tested. Also, the ability of samples to protect the iron from redox reaction is evaluated (Loizzo *et al.*, 2019). Butylated hydroxytoluene (BHT) was used as control.

Calculation of relative antioxidant capacity index (RACI)

The relative antioxidant capacity index (RACI) was used to establish antioxidant rank of samples (Sun and Tanumihardjo, 2007).

Statistical analysis

Data were obtained from three different experiments (n = 3) and expressed as means ± standard deviation (SD). Prism GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA) was used to calculate the concentration that yielded a 50% inhibition (IC_{50}) value as a result of the concentration-response curve. *Pearson's* correlation coefficient (*r*) was calculated using Microsoft Excel 2010 software. ANOVA followed by Dunnett's test ($\alpha = 0.05$) was applied to evaluate the differences between data and positive control result in biological assays, however, Tukey's test was used to determine any significant difference among all treatments at different levels *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

Principal component analysis (PCA) is the most common form of factor analysis and is categorised as a multivariate statistical technique. It is used to analyse interrelationships among a large number of variables. For the evaluation of the results of the chemical (TFC, TPC, TCC, DPPH, ABTS, β-carotene-bleaching, FRAP and induction time), analyses of Roggianella and Dolce di Rossano EVOOs and FOOs, PCA was applied.

Results and Discussion

Dolce di Rossano and Roggianella EVOO quality parameters

Based on the results reported in Table 1 and according to EC regulation, both Dolce di Rossano and Roggianella EVOOs could be classified as "extra virgin" (EC, 2003; EEC, 1991).

Table 1. Chemical and qualitative parameters of monovarietal Dolce di Rossano and Roggianella EVOOs produced in Calabria (Italy) during 2018–2019 season.

Quality parameter	EVOO		Significance
	Dolce di Rossano	Roggianella	
Free acidity (%)	0.79 ± 0.02[a]	0.73 ± 0.01[b]	**
Peroxide value ($meqO_2/kg$)	15 ± 1[a]	13 ± 1[b]	**
K232	1.9 ± 0.9[a]	1.8 ± 0.8[b]	**
K270	0.15 ± 0.02[a]	0.15 ± 0.02[a]	n.s.
ΔK	0.001 ± 0.00[a]	0.001 ± 0.00[a]	n.s.
TPC (ppm)	73 ± 2[b]	537 ± 6[a]	**
TFC (ppm)	18 ± 1[a]	15 ± 1[b]	**
TCC (ppm)	3 ± 2[a]	2 ± 3[b]	n.s.
Chlorophyll (ppm)	4 ± 2[a]	4 ± 3[a]	n.s.

TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TCC: Total Carotenoid Content.
** Significance at $P < 0.01$, n.s. not significant.
Results followed by different letters in the same row are significantly different by Tukey's multiple range test.

Our values agreed with those reported by Lavelli and co-workers for EVOO Pendolino, Leccino, Moraiolo and Taggiasca cultivars (Lavelli and Bondesan, 2005).

A significant difference ($P < 0.01$) was observed between the phytochemicals content (TPC, TFC and TCC) of two investigated oils. In particular, Roggianella EVOO showed a TPC value that was seven-time higher than that found for Dolce di Rossano (Table 1). The TPC value is in line with those found by Sicari *et al.* (2017) for Roggianella EVOO from Reggio Calabria whereas lower values were recorded for Calabrian Ottobratica and Carolea EVOO, respectively (Piscopo *et al.*, 2016). A statistically significant difference was also observed in TFC with values of 18 and 15 ppm for Dolce di Rossano and Roggianella EVOOs, respectively.

The pigment content of EVOOs is an important quality parameter since consumers directly evaluate it based on their colour (Gargouri *et al.*, 2013). Moreover, their content is strictly related to EVOO stability. As reported in Table 1, TCC and chlorophyll content were not statistically different for Dolce di Rossano and Roggianella EVOOs. This TCC content is similar to that reported by Borrello and Domenici (2019) for Tuscan EVOOs from Frantoio, Leccino, Moraiolo and Pendolino cultivars. Previously, Tuberoso *et al.* (2016) found chlorophylls values in the range from 6.5 to 10.5 ppm, and from 20.9 to 47.6 ppm with respect to TCC for Sardinian EVOOs Semidana and Tonda di Cagliari, respectively.

EVOO chemical profile

As expected, oleic acid (OA, ω-9) was the main monounsaturated fatty acid (MUFA) whereas palmitic acid (PA) was the major saturated fatty acid (SFA) in both EVOOs (Table 2). A statistical difference was observed in OA/ LA (ω-6) ratio ($P < 0.01$), as Roggianella EVOO showed the highest OA/LA ratio, an indicator of the EVOO stability (Alvarruiz *et al.*, 2003). Our data are in line with data found for Roggianella EVOO from Reggio Calabria province (Sicari *et al.*, 2010). More recently, several Italian monovarietal EVOOs including Leccino, Frantoio, Dolce Agogia and Moraiolo were investigated by Blasi *et al.* (2019). The percentages of OA and PA were quite similar to those found in our samples. Values from 71.84 to 73.20% and from 16.09 to 19.30% were recorded for OA and PA, respectively, in Sari Hasebi and Halhali green EVOOs from Turkey (Yorulmaz and Konuskan, 2017).

In general, the sterolic composition of the two investigated EVOOs was significantly different ($P < 0.01$). As expected, β-sitosterol represented the most abundant phytosterol (Table 3). Two-time higher content of Δ^5-avenasterol was found in Roggianella EVOO with respect to Dolce di Rossano. A statistically significant difference in the campesterol/stigmasterol index was observed between investigated EVOOs. Both EVOOs showed values of campesterol lower than 4%, the maximum limit established by European regulations and by the IOC (EU, 2011; IOC, 2009). Our data were in agreement with those reported for Turkish Sari Hasebi and Halhali green EVOOs, that were found in the range from 80.72 to 87.81% of β-sitosterol, respectively, followed by Δ^5-avenasterol (3.34–5.29% for Halhali and Sari Hasebi, respectively) (Yorulmaz and Konuskan, 2017). The total sterol content was 1698 and 1971 mg/kg for Dolce di Rossano and Roggianella, respectively, i.e. much higher than 1000 mg/kg indicated by the European regulations and by the IOC as a minimum sterol content for an EVOO (EU, 2011; IOC, 2009).

Table 2. Fatty acid composition of monovarietal Dolce di Rossano and Roggianella EVOOs produced in Calabria (Italy) during 2018–2019 season.

Fatty acid	EVOO		Significance
	Dolce di Rossano	Roggianella	
Myristic acid (C14:0)	0.01 ± 0.00[a]	0.01 ± 0.00[a]	n.s.
Palmitic acid (PA, C16:0)	14 ± 2[a]	13 ± 2[b]	**
Palmitoleic acid (C16:1)	1.3 ± 0.1[a]	0.9 ± 0.1[b]	**
Margaric acid (C17:0)	0.02 ± 0.00[a]	0.02 ± 0.00[a]	n.s.
Heptadecenoic acid (C17:1)	0.04 ± 0.00[a]	0.04 ± 0.00[a]	n.s.
Stearic acid (SA, C18:0)	1.4 ± 0.2[b]	1.5 ± 0.1[a]	**
Oleic acid (OA, C18:1)	72 ± 4[b]	79 ± 6[a]	**
Linoleic acid (LA, C18:2)	8.4 ± 0.9[a]	6.6 ± 0.8[b]	**
α-Linolenic acid (ALA, C18:3)	0.40 ± 0.02[a]	0.40 ± 0.02[a]	n.s.
Arachidic acid (C20:0)	0.90 ± 0.03[a]	0.80 ± 0.03[b]	*
Gadoleic acid (C20:1)	0.20 ± 0.01[b]	0.30 ± 0.01[a]	**
Behenic acid (C22:0)	0.10 ± 0.01[a]	0.10 ± 0.01[a]	n.s.
OA/LA	8.6[b]	12.0[a]	**
∑SFA	16.43[a]	15.43[b]	**
∑MUFA	73.54[b]	80.60[a]	**
∑PUFA	8.8[a]	7.0[b]	**
MUFA/PUFA	8.36[b]	11.51[a]	**

SFA: saturated fatty acid; PUFA: polyunsaturated fatty acids; MUFA: monounsaturated fatty acids.
**Significance at $P < 0.01$, * Significance at $P < 0.05$, n.s. not significant.
Results followed by different letters in the same row are significantly different by Tukey's multiple range test.

Table 3. Sterol composition of Dolce di Rossano and Roggianella EVOOs produced in Calabria (Italy) during 2018–2019 season

Sterol	EVOO		Significance
	Dolce di Rossano	Roggianella	
Cholesterol (%)	0.12 ± 0.01[a]	0.07 ± 0.02[b]	**
24-Methylene-Cholesterol (%)	0.07 ± 0.01[b]	0.29 ± 0.01[a]	**
Campesterol (%)	2.23 ± 0.03[b]	2.67 ± 0.05[a]	**
Campestanol (%)	0.16 ± 0.01[a]	0.11 ± 0.01[b]	**
Stigmasterol (%)	0.80 ± 0.02[b]	1.81 ± 0.04[a]	**
Δ^7-Campesterol (%)	0.08 ± 0.01[a]	0.08 ± 0.01[a]	n.s.
Clerosterol (%)	0.87 ± 0.01[b]	0.98 ± 0.02[a]	**
β-Sitosterol (%)	88.21 ± 0.08[a]	81.71 ± 0.06[b]	**
Sitostanol (%)	0.98 ± 0.03[a]	0.63 ± 0.02[b]	**
Δ^5-Avenasterol (%)	5.01 ± 0.05[b]	10.3 ± 0.8[a]	**
$\Delta^{5,24}$-Stigmastadienol (%)	0.62 ± 0.04[a]	0.51 ± 0.01[b]	**
Δ^7-Stigmastenol (%)	0.37 ± 0.01[a]	0.39 ± 0.05[a]	n.s.
Δ^7-Avenasterol (%)	0.51 ± 0.01[a]	0.43 ± 0.01[b]	**
Apparent β-Sitosterol (%)	95.68[a]	94.16[b]	**
Campesterol/Stigmasterol	2.79[a]	1.48[b]	**
β-Sitosterol/Δ^5-Avenasterol	17.61[a]	7.89[b]	**
Total sterols (mg/kg)	1698[b]	1971[a]	
Erythrodiol (%)	1.52 ± 0.02[a]	0.92 ± 0.02[b]	**
Uvaol (%)	0.79 ± 0.01[a]	0.16 ± 0.01[b]	**

All the sterols as well as erythrodiol and uvaol are expressed as percentage of the total sterol content.
** Significance at $P < 0.01$, * Significance at $P < 0.05$, n.s. not significant.
Results followed by different letters in the same row are significantly different by Tukey's multiple range test.

Table 4. Phenolic composition of monovarietal Dolce di Rossano and Roggianella EVOOs produced in Calabria (Italy) during 2018–2019 season.

Compound	Amount in EVOO (ppm)		Significance
	Dolce di Rossano	Roggianella	
4-Hydroxybenzoic acid	0.21 ± 0.01[b]	0.6 ± 0.9[a]	**
p-Coumaric acid	0.8 ± 0.2[a]	0.20 ± 0.02[b]	**
o-Coumaric acid	0.15 ± 0.01[b]	0.24 ± 0.02[a]	**
Ferulic acid	0.27 ± 0.02[a]	0.14 ± 0.01[b]	**
Hydroxytyrosol	3.12 ± 0.03[a]	2.9 ± 0.8[b]	**
Tyrosol	4.04 ± 0.04[b]	6.2 ± 0.5[a]	**
(+) Pinoresinol	8 ± 2[b]	22 ± 2[a]	**
(+) 1-Acetoxypinoresinol	14 ± 4[b]	52 ± 7[a]	**
3,4-DHPEA-EDA	17 ± 2[b]	48 ± 5[a]	**
p-HPEA-EDA	5.1 ± 0.9[b]	18 ± 1[a]	**
Hydroxytyrosol acetate	9 ± 1[b]	21 ± 2[a]	**
3,4-DHPEA-EA	3.6 ± 0.3[b]	8.26 ± 0.09[a]	**
p-HPEA-EA	0.87 ± 0.08[b]	1.15 ± 0.01[a]	**
Apigenin	0.00 ± 0.00[b]	0.79 ± 0.01[a]	**
Luteolin	0.48 ± 0.02[b]	1.12 ± 0.08[a]	**
∑ Identified phenolics	66.64[b]	178.52[a]	**

3,4-DHPEA-EDA: Oleacein, *p*-HPEA-EDA: oleocanthal, 3,4-DHPEA-EA: Oleuropein aglycone, *p*-HPEA-EA: Ligstroside aglycone.
** Significance at $P < 0.01$.
Results followed by different letters in the same row are significantly different by Tukey's multiple range test.

The phenolic composition of both Dolce di Rossano and Roggianella EVOOs is reported in Table 4. In general, the total amount of identified phenolic compounds in the two EVOOs was significantly different ($P < 0.01$). As expected, secoiridoids and lignans were the most abundant compounds. Roggianella EVOO resulted to be richer in phenolic compounds than Dolce di Rossano. 1-Acetoxypinoresinol followed by 3,4-dihydroxyphenylethanol-elenolic acid dialdehyde (3,4-DHPEA-EDA) were the two most abundant compounds in both EVOOs. The amounts of these compounds in Roggianella were 3.7- and 2.8-times higher than those in Dolce di Rossano. Roggianella EVOO was also characterised by a significant content of pinoresinol and hydroxytyrosol acetate. Dolce di Rossano EVOO variety showed a slightly higher tyrosol content than Roggianella (Table 4). Roggianella EVOO showed a two-times higher content of luteolin than Dolce di Rossano variety. Among phenolic acids, 4-hydroxybenzoic acid was the main abundant acid in Roggianella oil whereas *p*-coumaric acid was the most abundant in Dolce di Rossano EVOO.

Our data were in line with those previously reported for EVOOs from Roggianella cultivars (Giuffrè and Louadj, 2013). Mean values of 48.54, 38.02 and 16.24 μg/g have been found for 3,4-DHPEA-EDA, 1-acetoxypinoresinol and hydroxytyrosol acetate, respectively. Values in the range 21.11–76.80 μg/g were found for 3,4-DHPEA-EDA in Grignano and Leccino EVOOs (Baiano *et al*., 2009). The lower content of phenolic compounds has been found in Sardinian EVOOs where 3,4-DHPEA-EDA ranged from 1.0 to 11.6 μg/g in Tonda di Cagliari and Semidana, respectively (Tuberoso *et al*., 2016). It is interesting to emphasise that 1-acetoxypinoresinol, one of the main phenolics identified in our Calabria EVOO, was not revealed in Tonda di Cagliari and found in a very low quantity in Tonda di Villacidro, Semidana and Bosana oils. However, Bosana contained a high amount of tyrosol (22.5 ppm). A perusal analysis of literature evidenced a great variability in EVOO phenolic profile due to several factors including geographical origin, genetic factors and environmental conditions. This is particularly true for hydroxytyrosol that was found in a high amount in Colozzese EVOO (16.1 ppm), whereas its amount ranged from 0.2 to 8.8 ppm in Spina and Oliva Grossa samples (Negro *et al.*, 2019). With respect to apigenin and luteolin, a higher amount of both phenols were found by Giuffrè *et al.* (2010) in Roggianella EVOO from Reggio Calabria during the season 2006.

Phytochemical content in C. baccatum cultivars

Fresh and dried *C. baccatum* Bishop crown and Aji Angelo fruits were subjected to extraction by maceration. The highest extraction yields were obtained with dried fruits (Table 5).

Table 5. Investigated *Capsicum baccatum* cultivars.

Capsicum baccatum		Colour	Length (cm)	Width (cm)	Extraction yield (%)
Bishop crown	Fresh	Red	5–7	6–8	6.06
	Dried				29.92
Aji Angelo	Fresh	Red	5–7	6–8	6.06
	Dried				27.90

Table 6. Total phenolic, flavonoid, carotenoid, capsaicin and dihydrocapsaicin, vitamin C and vitamin E content in *Capsicum baccatum* cultivars.

Content	Bishop crown		Aji Angelo		Significance
	Fresh	Dried	Fresh	Dried	
TPC[1]	6.80 ± 0.02^{c}	67 ± 4^{a}	7.4 ± 0.4^{c}	63 ± 2^{b}	**
TFC[2]	1.40 ± 0.03^{c}	10.90 ± 0.02^{b}	1.10 ± 0.01^{c}	11.9 ± 0.2^{a}	**
TCC[3]	2036 ± 8^{d}	4785 ± 9^{a}	2763 ± 7^{c}	3388 ± 8^{b}	**
Vitamin C[4]	3.90 ± 0.22^{b}	5.2 ± 0.3^{a}	3.40 ± 0.09^{c}	4.0 ± 0.1^{b}	**
Vitamin E[4]	5.1 ± 0.5^{b}	5.3 ± 0.2^{b}	5.2 ± 0.4^{b}	5.5 ± 0.9^{a}	**
Capsaicin[5]	44 ± 3^{c}	125 ± 7^{b}	19 ± 5^{d}	175 ± 9^{a}	**
Dihydrocapsaicin[5]	24 ± 4^{c}	58 ± 6^{b}	15 ± 1^{d}	156 ± 7^{a}	**

[1] TPC: Total Phenolic Content, expressed as mg of chlorogenic acid equivalents/100 g dried weight (DW), [2] TFC: Total Flavonoid Content, expressed as mg of quercetin equivalents/100 g DW, [3] TCC: Total Carotenoid Content, expressed as mg of β-carotene equivalents/100 g DW. [4] Expressed as mg/g DW, [5] Expressed as μg/g DW. Results followed by different letters in the same row are significantly different ($P < 0.01$, **) by Tukey's multiple range test.

Dried peppers contained the highest amounts of all analysed phytochemicals. In particular, Bishop crown showed the highest TPC and TCC values whereas Aji Angelo had the highest TFC (Table 6). The same trend was also observed in the extracts obtained from fresh samples.

As reported in Table 6, dried pepper extracts were rich in both capsaicin and dihydrocapsaicin capsaicinoids with values ranging from 58 to 125 μg/g DW for Bishop crown and Aji Angelo dried pepper extract, respectively. Bishop crown dried pepper showed the highest vitamin C content while vitamin E was particularly abundant in Aji Angelo dried pepper extract.

Antioxidant activity

Despite it has been previously reported that antioxidant ability determined by DPPH and ABTS *in vitro* assays can significantly differ due to different mechanisms of inactivation of the radicals (Antolovich *et al*., 2002; Plastina *et al*., 2018), we observed similar trends for the investigated samples. Promising radical scavenging activity was found for Roggianella EVOO phenolic extracts in both applied assays (Table 7). These values are better than that reported for different Calabrian monovarietal EVOOs by Sicari (2017) for Sinopolese and Roggianella and Leporini and co-workers (2018) for Frantoio EVOO (average value of 117.2 and 131.9 μg/mL for ABTS and DPPH, respectively). Previously, Negro and co-workers found IC_{50} values in the range 91–160 g/oil for DPPH radical scavenging activity for Oliva Grossa and Spina, respectively (Negro *et al*., 2019). The radical scavenging potential of EVOOs phenolic fraction was positively correlated to TFC and TCC with *r* values of 1.0.

Among investigated peppers, Bishop crown resulted to be the most active with IC_{50} values of 148 and 167 μg/mL for DPPH and ABTS tests, respectively. No significant differences between Bishop crown and Aji Angelo dried pepper extracts were evidenced in the protection from lipid peroxidation. Promising ferric reducing ability was observed for both Dolce di Rossano and Roggianella EVOO, respectively. Previously, Loizzo *et al*. (2017) investigated the antioxidant activity of *C. annuum* and *C. chinense* fresh and processed peppers and found the highest DPPH radical scavenging potential with Effix fresh pepper sample (IC_{50} value of 3.9 μg/mL). A high radical scavenging potential against ABTS has been previously observed for dried *C. annuum* cv Pellegrino and Idealino samples with IC_{50} values of 45.2 and 45.7 μg/mL, respectively (Loizzo *et al*., 2017). Great variability in antioxidant potential was observed with oleoresin obtained from different varieties of fresh peppers including

Table 7. Antioxidant activities of Dolce di Rossano and Roggianella EVOOs and *C. baccatum* extracts.

Sample	ABTS[1]	DPPH[1]	β-carotene bleaching[1]	FRAP (μM Fe(II)/g)	RACI values
EVOO phenolic extracts					
Dolce di Rossano	48 ± 3****	54 ± 2****	205 ± 9****	79 ± 3	71
Roggianella	36 ± 1****	30 ± 1****	127 ± 5****	57 ± 3*	–71
C. baccatum extracts					
Bishop crown					
Fresh	170 ± 2****	162 ± 3****	33 ± 3****	15 ± 1****	0.04
Dried	167 ± 3****	148 ± 3****	21 ± 1***	14 ± 2****	–0.47
Aji Angelo					
Fresh	453 ± 4****	186 ± 2****	77 ± 3****	9.1 ± 0.4****	0.86
Dried	256 ± 4****	157 ± 3****	20 ± 1***	9.2 ± 0.8****	–0.43
Positive controls					
Ascorbic acid	1.71 ± 0.03	5.0 ± 0.8			
Propyl gallate			1.00 ± 0.01		
BHT				63 ± 4	

[1] IC_{50} value (μg/mL). Data are expressed as means ± S.D. (n= 3). DPPH Radical Scavenging Activity Assay, Antioxidant Capacity Determined by Radical Cation ($ABTS^{+}$), β-carotene bleaching test, Ferric Reducing Antioxidant Power (FRAP). Ascorbic acid, BHT and Propyl gallate were used as positive control in antioxidant tests. Differences within and between groups were evaluated by one-way ANOVA followed by a multicomparison Dunnett's test (α= 0.05): ****P < 0.0001, ***P < 0.001, compared with the positive controls.

C. annuum and *C. chinense*. The RACI approach was applied to estimate the rank of antioxidant activity for EVOOs and pepper samples. Roggianella EVOO resulted as the most active one whereas Bishop crown dried pepper extract exhibited the highest antioxidant potential among investigated pepper extracts (Table 7).

Protective effect of pepper extracts against oil oxidation

Rancimat apparatus was used to investigate and compare the stability of EVOOs and the corresponding FOOs (Figure 1). In this method, the oxidation is induced by the passage of constant airflow through the oils that is kept under constant temperature. The volatile products of the reaction are collected in deionised water and are measured through the electric conductivity. During the development of the reaction, due to an increase of the conductivity, a curve is drawn from which the induction period is inferred. Stability was expressed as oxidation induction time (expressed in hours). Roggianella EVOO showed a greater induction time than Dolce di Rossano sample (39.6 versus 19.2 h, respectively). This is probably due to the higher amounts of TPC and identified phenolic compounds found in Roggianella EVOO. In line with our data, the following trend for resistance to oxidation has been previously observed for Calabrian EVOOs: Roggianella > Ottobratica > Sinopolese (Sicari, 2017) and Carolea > Ottobratica > Sinopolese > Grossa di Gerace (Piscopo *et al.*, 2016). Remarkably, all FOOs enriched with pepper extracts had a prolonged induction time with respect to the corresponding EVOO. Among them, FOOs obtained by the addition of Bishop crown dried extract to Roggianella EVOO showed the highest induction time of 44.9 h. This possibly depends on the high amount of bioactive phytochemicals, with the only exception of vitamin E and dihydrocapsaicin, found in Bishop crown dried extract. Moreover, by considering the protection factor quotient (PF), Bishop crown fresh pepper-FOO had a PF of 5.7 with respect to Dolce di Rossano EVOO followed by FOO obtained by the addition of the dried extract from the same pepper in Roggianella EVOO (PF = 5.3). Lower values were observed for FOOs enriched with Aji Angelo extracts with PF values ranging from 1.05 to 3.03. It has been previously reported that FOOs obtained by the addition of *C. frutescens* extract did not show any protective effect against the oxidation process (Gouveia *et al.*, 2006). This was probably due to the low content of capsaicinoids. By contrary, a protective effect towards oxidation was observed when dried *C. annuum* pepper was added to EVOO by infusion (10–20% w/w) up to 30 days (Caporaso *et al.*, 2013). The effect of the addition of spices or their extract/essential oils on oil oxidative stability gained attention by researchers working in the food area. The infusion of dried garlic, oregano and rosemary and hot pepper to Dauno monovarietal EVOO led to FOOs in which the formation of primary oxidation products was reduced without any modification on acidity parameter and secondary oxidation compounds (Gambacorta *et al.*, 2007). More recently, Ayadi

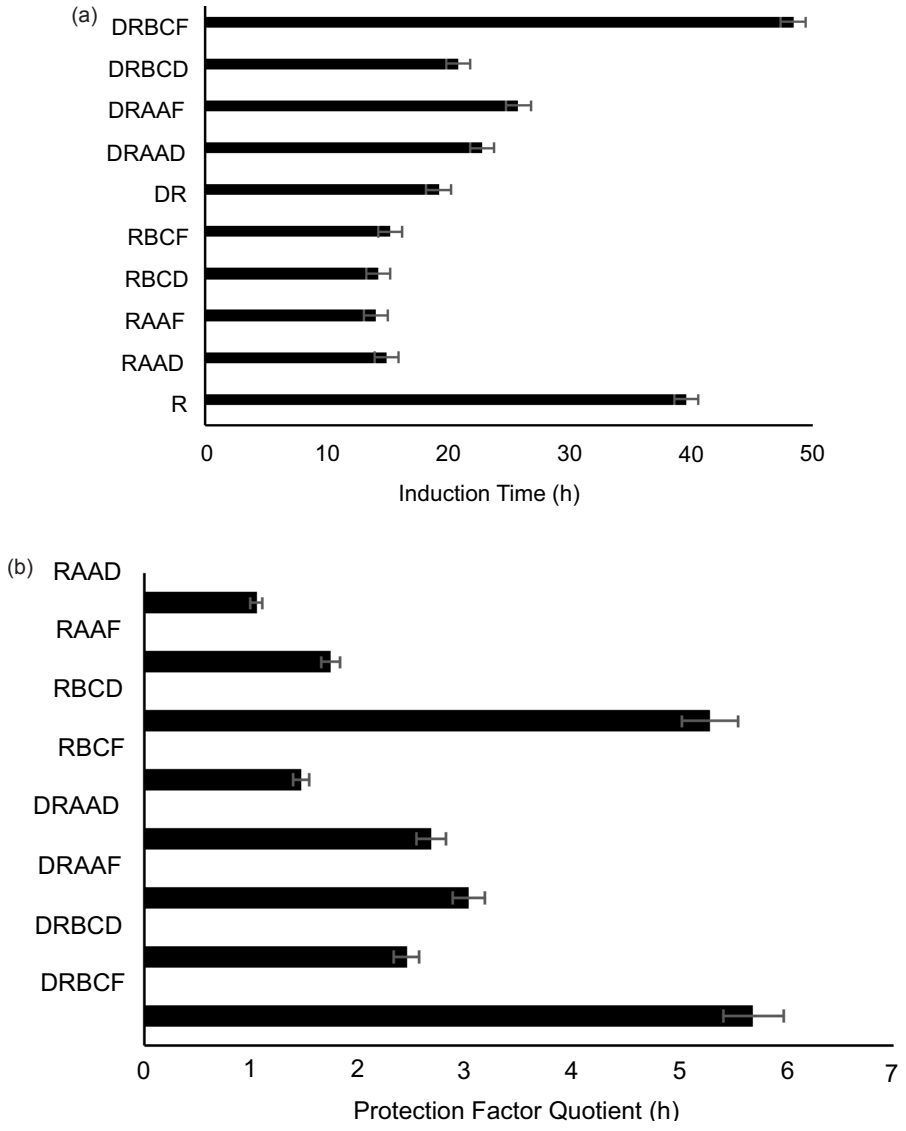

Figure 1. (a) Induction time and (b) Protection Factor quotient of Dolce di Rossano (DR) and Roggianella (R) EVOOs and corresponding FOOs enriched with *C. baccatum* cv Bishop crown (BC) and Aji Angelo (AA) fresh (F) and dried pepper (D) extracts.

et al. (2009) investigated the quality parameters of FOOs obtained by the infusion of 5% (*w*/*w*) of Mediterranean spices to Chemlali EVOO. Although the addition of spices has led to a slight increase in free acidity, an improvement of their thermal resistance and stability with this hierarchy: rosemary > thyme > lemon > basil ≥ EVOO control was also observed. This could be caused by the transfer of antioxidant compounds from spices to oil during the infusion. More recently, Ammar *et al*. (2017) evaluated the effect on quality parameters including oxidative stability of the addition of *Opuntia ficus-indica* flower extracts to "Chemlali" EVOO that results in FOO1 and FOO2 (5 and 15% (*w*/*w*), respectively). FOO1 was richer in phenolics by 3.4% than FOO2. In disagreement with our data, this addition did not improve oxidative stability. Also, the induction time obtained by the Rancimat method showed values of 2.73 and 2.42 h for FOO1 and FOO2, respectively, in comparison to the control EVOO (2.83 h). The authors speculated that this may be due to a diffusion of undesirable compounds during the flower maceration of olive oil. These compounds can take part in reactions that negatively interfere with oxidative stability (Ammar *et al*., 2017).

The unfavourable effect of EVOO aromatisation on oxidative stability was also observed by Sena-Moreno and co-workers who proposed a new EVOO aromatisation method. In this study, liquid–liquid extractions of saffron aqueous extract in "Arbequina" EVOO was done. The sample obtained from the first liquid–liquid extraction was SO1, whereas SO2, SO3 and SO4 were those obtained from the second, third and fourth

extraction, respectively. SO1 showed the highest safranal concentration. It is worthy of mention that in the first seven months, FOOs were characterised by a reduction of quality parameters including oxidative stability. After that FOOs parameters were still comparable to those of EVOO (Sena-Moreno *et al*., 2018). The impact of enrichment with phenolic compounds from the olive cake and dried thyme on the quality parameters phenolic composition, oxidative stability and antioxidant activity of "Arbequina" EVOO was investigated by Rubió *et al.* (2012). Flavonoids from thyme were characterised by a higher transference ratio (average 89.7%) with respect to secoiridoids from an olive cake (average ratio of 35.3). In each case, all resulted FOOs were more resistant to oxidation with values of induction time of ~8 and ~20 h for control and enriched oil, respectively.

Principal component analysis

The projections of the observations on the first two principal component axes are shown in Figure 2. The accessions are distributed on the factor plane. These two coordinates represent 87.77% of the total variance (PC1 explained 56.17% of total variation, while PC2 explained 31.59% of total variation). The first component (PC1) was positively correlated with TPC, TFC, FRAP, β-carotene bleaching, RACI and ITDR. The second component (PC2) was positively correlated with TPC, ITDR and ITR. Figure 2 shows the space of oils and hot pepper samples (fresh and dried), samples and the bioactive attributes associated with FOOs. The PCA model showed that FOOs were characterised by a higher induction time than the corresponding EVOOs.

Conclusions

Flavoured olive oils gained attention by consumers not only as a new dressing for meat, fish or salad but also for the role of spice phytochemicals on human health. In this context, the protective effect of *C. baccatum* peppers cultivars on the oxidative stability of FOOs obtained by the addition of pepper to two Calabrian EVOOs was evaluated. Roggianella EVOO, characterised by a high phytochemicals content showed a longer induction time. The addition of *C. baccatum* Bishop crown to Roggianella EVOO led to a FOO with an increased induction time with respect to the corresponding EVOO. Promising results were also obtained with FOO resulted by the addition of Bishop crown fresh pepper to Dolce di Rossano. Therefore, the addition of *C. baccatum* Bishop crown peppers was able to enhance the oxidative stability of oils, regardless of the quality of the starting EVOO.

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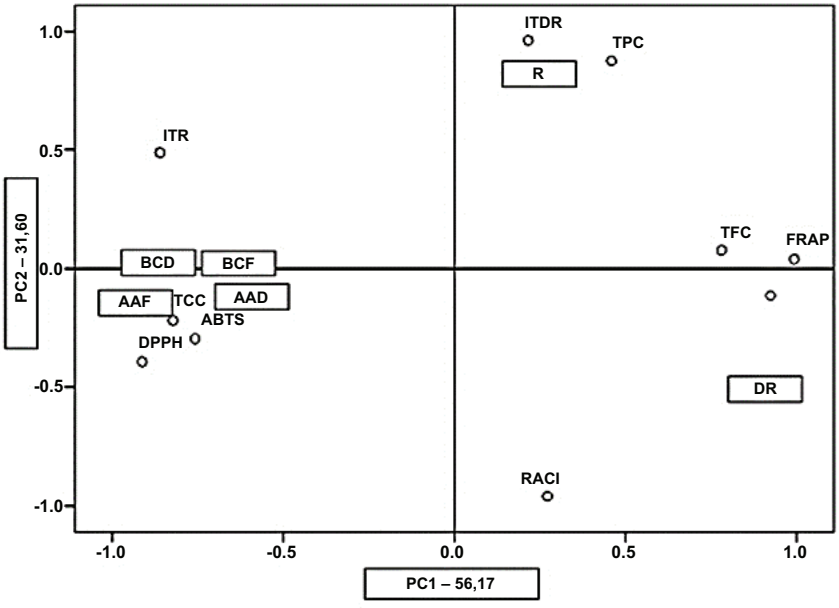

Figure 2. Principal component analysis plot based on bioactivity attributes of extra virgin olive oils (EVOOs) and corresponding flavoured olive oils (FOOs) enriched with C. baccatum pepper extracts. DR: Dolce from Rossano; BCF: Bishop crown Fresh; BCD: Bishop crown dried; AAF: Aji Angelo Fresh; AAD: Aji Angelo dried; R: Roggianella.

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