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Bergamot flavoured olive oil: Comparison between enrichment processes, evaluation of shelf-life and health properties

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ABSTRACT

This study aims to examine the bioactivity of Calabrian extra virgin olive oil enriched with bergamot fruits (*Citrus bergamia* Risso & Poiteau) harvested in Reggio Calabria province (Italy). To extra virgin olive oil (EVOO), *cv* Ottobratica 10 and 20 % of fresh fruit was added during crushing of the olives and 2 % by infusion of freeze-dried bergamot (CFVOOB10, CFVOOB20 and IFVOOB samples, respectively) was added. EVOO, bergamot extract and flavoured samples (FVOOs), were analysed throughout a one-year period. Total phenol content (TPC) as well as total chlorophyll (TChlC) and total carotenoid (TCC) contents were spectrophotometrically determined. In addition, the phenolic profile wasstudied by UHPLC. Free acidity (FA), peroxide values (PV), spectrophotometric indices, α-tocopherol, colour, and antioxidant activity were also assessed. The impact of bergamot addition on lipase, α-amylase, and α-glucosidase was estimated. Expert panellists evaluated the influence on the sensorial attributes, and CFVOOB10 was found to be the most pleasant. CFVOOB10 also showed the lowest PV and the highest FA after the storage. CFVOOB20 showed good protection against lipid peroxidation. Generally, all the FVOOs maintained a better inhibitory activity against the key enzymes related to obesity, compared to the EVOO. Data analyses confirmed that these FVOOs should be considered to be functional with a good sensory profile.

1. Introduction

Extra virgin olive oil (EVOO) is one the key ingredient of the Mediterranean diet (Almanza-Aguilera et al., 2023). It is known for its numerous benefits on human health for the high content of fatty acids, both unsatured, which represent approximately 85 % of its fat composition and mainly constituted by oleic acid, and saturated, which represent approximately the restant 15 % and mainly constitued by palmitic acid. It is known that also its phenolic composition, validated in the last years by the European Food Safety Authority (EFSA) with Directive n. 432/2012 (European Union Commission, 2012) is responsible for the health benefits. Over the last few years, which has seen an increase in pathologies caused by eating disorders such as obesity, developing and testing functional olive oils through the addition of functional molecules, has become a very interesting field (Jimenez-Lopez et al., 2020). The addition of these molecules generates a product that cannot be classed as 'extra virgin olive oil' but is defined as flavoured olive oil (FVOO). Authors have demonstrated how, these new formulations present a different flavour and, depending on the enrichment matrix used, could have greater oxidative stability during storage. Already other authors tested different enrichment processes of an EVOO. Someone drew a comparison between the processes utilized and on the impact of the final quality of the flavoured olive oils (Clodoveo et al., 2016). Results clearly show that the addition in the malaxation step, which does not require the use of solvents, seems to be not only a green technique that is easier and faster to apply than others, such as infusion,

Abbreviations: (AA), Ascorbic acid; (ABTS), 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; (BHT), Butylated hydroxytoluene; (DPPH), 1,1-Diphenyl-2-picryl-hydrazil; (EVOO), Control; (FA), Free Acidity; (FRAP), Ferric Reducing Antioxidant Power; (B), Bergamot extract; (IFVOOB), Bergamot olive oil infusion 2%; CFVOOB10, Bergamot olive oil crushing 10%; CFVOOB20, Bergamot olive oil cruching 20%; (PV), Peroxide Value; (TPC), Total Polyphenols Content; (TChlC), Total Chlorophyll Content; (TCC), Total Carotenoid Content.

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but also shows more effective results in the extraction of phenolic compounds, with a significantly lower level of hydrolysis (Caponio et al., 2016). The region of Calabria is characterized by a Mediterranean climate. This feature makes it suitable for the cultivation of olives. In fact, the region is rich in many varieties very different from one part to another. Ottobratica is one of the most popular varieties, mainly in the west side part of the region. Its promising characteristics are due not only to genetic factors, but also to the milling process, and climate, which never reaches very high levels of temperature or humidity (Rizzitano, 2018; Piscopo et al., 2016). Ottobratica oil shows the highest total phenolic content, compared to other Calabrian varieties, and medium to high tocopherols levels (Sicari et al., 2021). The other parameters, such as sterols, triglycerides and waxes are strongly influenced by the crop season and the harvest year (Giuffrè A.M., 2013). Bergamot (*Citrus bergamia* Risso & Poiteau) is a hybrid of *C. aurantium* x *C. medica* (Nicolosi et al., 2000). Three cultivars have been grown in the Province of Reggio Calabria for centuries (Gioffrè et al., 2020). Due to the economic importance for its geographical area of production, many studies have been conducted to determine the physical and chemical properties of bergamot fruit (Benalia et al., 2023; Maiuolo et al., 2022). The main use of bergamot fruit is for its essential oil which is used in perfumery, even if, recently the juice has also been used for beverages due to its beneficial effects on the human health (Maiuolo et al., 2023). Bergamot fruit by-products were also studied to prepare fortified biscuits (Laganà, Giuffrè, De Bruno, & Poiana, 2022) and vinegar (Di Donna et al., 2020).

Bergamot essential oil, which obtained the PDO (Protected Designation of Origin from the European Union) in 1999, is widely used in the pharmaceutical industries for its antiseptic and antibacterial proprieties, and in the cosmetic industries and in the food industries for its aromatic properties (Giuffrè A.M., 2019). Attempts to cultivate the fruit in other parts of the world have been unable to qualitatively substitute the Italian product, due to its unique combination of climate, pedological characteristics, cultivation techniques, rootstock, the age of the plants and the degree of ripeness at harvest. The phenolic pattern is mainly composed of narirutin, naringin, rutin, hesperidin, and others (Pernice et al., 2009). Bergamot's high content in flavones can exert antioxidant properties (Sicari V. & Pellicanò M.T., 2016a). Moreover, bergamot fruits were able to reduce serum levels of lipids (Lamiquiz-Moneoet al., 2019, Leporini et al., 2021).

In this context, our work aims to evaluate the effect of the addition by infusion or during olive crushing of fresh bergamot fruit on virgin olive oil quality parameters and bioactivity. For this purpose, we have measured the free acidity, peroxide value, spectrophotometric indices, colour, total phenol, carotenoid and chlorophyll contents as well as the α-tocopherol content, single phenolic composition by UHPLC, the antioxidant activity, inhibition of carbohydrate hydrolysing enzymes and pancreatic lipase. The sensory analysis was also assessed. One of the aims of this study was to minimize waste products. For this reason, the decision was taken to use the whole fruit, since both the bergamot and olive oil industries generate many by-products.

2. Materials and methods

2.1. Samples

Olives (Olea europea L.) of Ottobratica cultivar were harvested at Polistena in the province of Reggio Calabria in October 2021. Olive oil extraction was performed by a mini-pressing apparatus (Agrimec Valpesana, Calzaiolo, San Casciano Florence-Italy) consisting of a crushing hammer, a malaxator and a press. The extraction was performed at room temperature and the malaxation step lasted for 40 min. The pressure system does not use water and the pressing phase, once the selected pressure was reached (200 atm), was applied for 20 min. The olive oil was immediately separated from wastewater after extraction by means of a laboratory centrifuge, and it was stored in green glass bottles (100 mL). Bergamot fruits were produced by a local farmer in the province of Reggio Calabria. Following the research aims previously described, three different enrichment processes were carried out, employing two technological approaches: the first, the addition of the fruits directly into the crusher, and the second, their addition into the oil by infusion. For the first approach, the bergamot fruits were sliced and added to the olives in the crusher. The additions were carried out in two different millings, at two different percentages. In the first milling 18 kg of olives and 2 kg of bergamot fruits were used; for the second milling, 16 kg of olives and 4 kg of bergamots were used. 700 mL of 10 % flavoured oil (CFVOOB10) and 700 mL of 20 % flavoured oil (CFVOOB20) were obtained. The extraction was performed at room temperature and the malaxation step lasted for 40 min. The pressure system does not use water and the pressing phase, once the selected pressure was reached (200 atm), was applied for 20 min. For the second approach, bergamots were sliced, frozen at −18 °C for 24 h and then freeze-dried, for as long as necessary so that all the water content was eliminated. After that, the oil was infused at 2 % in the dark and under constant agitation. After 30 days sample IFVOOB was obtained. The obtained flavoured virgin olive oils (FVOOs) were filtered and packaged in green glass bottles with a capacity of 100 mL with a threaded screw cap with drip catcher, and stored in the dark at room temperature, similar to consumer conditions. Analyses were made for EVOO (extra virgin olive oil) and FVOOs (CFVOOB10, CFVOOB20 and IFVOOB) to evaluate their stability during storage at pre-established times: T0 on the day of production; T15 after 15 days from production; T30 after 30 days from production; T60 after 60 days from production; T180 after 180 days from production; T360 after 360 days from production.

2.2. Analytical methods

2.2.1. Bergamot fruit

2.2.1.1. Extraction procedure and phytochemical content. The whole of the bergamot fruit was sliced, frozen at −18 °C for 24 h, freeze-dried for 48 h and ground into a fine powder. The extract was prepared following the method of Gabriele et al. (2017) with some modification. The lyophilized samples were subjected to maceration with ethanol 70 % for 24 h, 1:10 (w:v). The obtained extract (B) was centrifuged for 10 min at 2300 g at 4 °C and the supernatant was collected, filtered with Büchner funnel and stored at 4 °C in the dark until use. Briefly, for total phenolic content (TPC) to one mL of bergamot extract properly diluted was added at five mL of Folin-Ciocalteu 1:10. After five min, four mL of Na_2CO_3 7.5 % was added. It was incubated in the dark at room temperature for two hours. Afterwards, the absorbance was read at 765 using a UV–VIS spectrophotometer. Results are expressed as mg of gallic acid equivalent (GAE)/L of freeze-dried extract (Sepahpour et al., 2018).

For the Total Flavonoid Content (TFC) the aluminum chloride method was used. 0.5 mL of bergamot extract properly diluted was mixed with 2.5 mL of distilled water and 0.150 mL of $NaNO_2$ 5 %. After five min 0.300 mL of $AlCl_3$ 10 % was added and after a further five min, one mL of NaOH 1 M. Finally, 0.550 mL of distilled water was added. After 15 min of incubation at room temperature, the absorbance was measured at 510 nm using a UV–VIS spectrophotometer. Results are expressed as mg of quercetin equivalent QE/L of freeze-dried extract (Sepahpour et al., 2018).

2.2.2. EVOO and FVOOs 2.2.2.1. free acidity, peroxide value, spectrophotometric

Indices EVOO quality parameters were determined according to EEC Regulation (European Union Commission, 2016). Free acidity (FA) was expressed as % oleic acid; peroxide value (PV) was expressed as mEq O_2/kg of oil, indexes of primary and secondary oxidation were measured spectrophotometrically and expressed as K232, K268 and ΔK.

2.2.2.1. Colour. The colour was measured with a colorimeter (Konica

Minolta CM-700 d, Osaka, Japan), according to the international standard CIELab L*, a*, b* and the results were reported as chroma (C*).

2.2.2.2. Chlorophyll and carotenoid content. Pigments were extracted from the oil samples using five mL of oil and five mL of n-hexane. Total contents of chlorophylls (TChlC) and carotenoid (TCC) were determined spectrophotometrically (670 nm and 470 nm, respectively) and expressed as mg/kg of pheophytin and lutein, respectively (Minguez-Mosquera et al., 1991).

2.2.2.3. Sample preparation for the evaluation of α-tocopherol content. α-Tocopherol content was determined using the method described previously by De Bruno et al., 2021. The identification and quantification were performed by calibration curve, using pure α-tocopherol and results were expressed as mg/kg of the oil (De Bruno et al., 2021).

2.2.2.4. Phenolic fraction extraction procedure. Five g of oil samples were mixed with two mL of methanol/water (70:30, v/v), two mL of n-hexane and centrifuged (6000 g, 10 min, 4 °C). The hydro-alcoholic phase containing the phenols was separated from the lipophilic phase, collected, and stored at −20 °C until analysis (Montedoro et al., 1992).

2.2.2.4.1. TPC of evoo and FVOOs. The determination of total phenolic content of EVOO and FVOOs wa determined using the method described previously by Baiano et al., 2009. The total phenol content was determined at 750 nm and expressed as mg GAE/kg of oil (Baiano et al., 2009).

2.2.2.4.2. EVOO and FVOOs phenolic profile. The identification and quantification of phenolic compounds by UHPLC was determined using the method described previously by Romeo et al. The detector was set at 254, 280, 330, 350 and 450 nm. External standards were used for the quantification and results were expressed as mg/kg of oil (Romeo et al., 2019).

2.2.3. Antioxidant activity

The antioxidant power of samples was investigated using multi-target approaches, given the complexity of the oxidative process. Extracts from bergamot (B), EVOO and FVOOs were dried in a rotavapor and re-dissolved in 1 mL of methanol for further analysis.

2.2.3.1. Radical scavenging ability by ABTS and DPPH tests. The 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity radical were performed according to Leporini et al. (2018).

2.2.3.2. β-Carotene bleaching test and FRAP. The protection of lipid peroxidation and the Ferric Reducing Antioxidant Power (FRAP) assay, (β-carotene bleaching test), were performed according to Plastina et al., 2021.

2.2.4. Carbohydrate hydrolyzing enzymes and lipase inhibition test

For the α-amylase inhibitory test, samples were dissolved in ethanol, added to starch solution, and left to react with the enzyme at room temperature for five min. The absorbance was read at 540 nm. Acabarose was used as a positive control (Tundis et al., 2021). In the α-glucosidase assay, a mixture of sample, maltose solution, and enzyme was left to incubate at 37 °C for 30 min. Subsequently, 50 μL of perchloric acid was added, and the mixture was centrifuged. The supernatant was collected and mixed with five μL of DIAN and 300 μL of PGO and left to incubate at 37 °C for 30 min. The absorbance was read at 500 and Acabarose was used as a positive control (Tundis et al., 2021). In the inhibition of pancreatic lipase, extracts were mixed with lipase enzyme, Tris-HCl buffer (pH 8.5), and 4- nitrophenyl octanoate. After 30 min at 37 °C the absorbance was read. Orlistat was used as a positive control (Plastina et al., 2021).

2.2.5. Sensory analysis

EVOO and FVOOs were also assessed by sensory analysis. A tasting panel was formed of seven specialist assessors (age: between 30 and 65). The evaluation was done using 9-point structured scales where 1 is absent and 9 is extremely perceptible. A sensory quantitative descriptive analysis (QDA) was performed to define the sensory profile of each sample. QDA test results were analyzed and reported as a spider graph using Microsoft Office Excel 2014. The sensory analysis was done in accordance with the current legislation and according to the internal regulations of the department. All the panelists were previously informed on the ingredients they tasted.

2.3. Statistical analysis

Samples were analyzed in triplicate. Analytical data were reported as means ± standard deviation. The analysis of variance (one-way ANOVA) was conducted by applying the post hoc Tukey test at $p < 0.01$ (SPSS software, 21.0 version, Armonk, NY, USA). The following symbols were used to indicate the significance: * $p \leq 0.05$; ** $p \leq 0.01$; ns $p > 0.05$ not significant.

3. Results and discussion

3.1. Bergamot extract, phytochemical content and bioactivity

The first step of the study concerned the physicochemical characterization of the bergamot extract and the detection of the antioxidant and enzymatic activity. Bergamot extract (B) was characterized by a high total phenol content (TPC) and total flavonoid content (TFC) (309.12 mg GAE/L of freeze-dried extract and 45.13 mg QE/L of freeze-dried extract).

Phenolic compounds and flavonoids are known to be responsible for antioxidant activity in fruits. The IC_{50} calculated or the extract by DPPH assay reached the value of 35.67 μg/mL. Our data agree with those reported by Trovato et al. (2010) who found a similar IC_{50} corresponding to 25.12 μg/mL for bergamot juice. Previously, Sicari et al. (2016b) evaluated the DPPH radical scavenging activity for a selection of bergamot fruit juice harvested in the main areas of cultivation in Reggio Calabria province, and detected IC_{50} values ranging from 20.5 to 31.4 μg/mL. Moreover, B showed a promising ABTS radical scavenging effect with IC_{50} value of 3.21 μg/mL.

The β-carotene bleaching test measures the discoloration of β-carotene due to oxidation caused by the degradation products of linoleic acid because of temperature. The presence of antioxidant compounds inhibits the degradation of β-carotene, the effect visible at a macroscopic level is the persistence of the characteristic orange colour. Sample B showed IC_{50} value of 54.09 μg/mL.

The principle of the FRAP assay, acronym of "Ferric Ion Reducing Antioxidant Power", is based on the ability of the various antioxidants to reduce the Fe (III) at pH 3.6. Bergamot extract exhibited a FRAP value higher than BHT (78.14 *vs* 63.26 μM Fe(II)/g).

Bergamot extract also showed a promising α-amylase and α-glucosidase inhibitory activity with IC_{50} values of 62.21 and 71.46 μg/mL, respectively. A IC_{50} value of 115.27 μg/mL, was found against pancreatic lipase.

The major flavone found in B sample was hesperedin followed by naringin, neoeriocitrin, and neoesperidin (Table S1). This high level of hesperidin was totally in disagreement with that found by Sicari V. and Pellicanò M.T. (2016a) which corresponded to 33.5 mg/L of juice. Differently, the amount in neoesperedin is 47.54 % higher than our results (528.2 mg/L of juice). However, the content in naringin is very similar to our data (554.5 mg/L of juice). These discrepancies could be given by the diversity of the two extracts analysed, since our extract included all the parts of the fruit (juice, peel, seeds, pulp, albedo).

3.2. EVOO and FVOO

The quality parameters (Table 1) obtained from the analyses showed values for EVOO (control) within the limits established by Regulation EEC/2568/91 (European Union Commission, 2016) and the percentage of free acidity (FA) varied from 0.68 at T0 to 0.84 % at T360. The bergamot fruit addition caused a rise in FA in the flavoured olive oils (FVOOs) (Ayadi et al., 2009): the FA values of CFVOOB10 (bergamot olive oil obtained by 10 % enrichment during crushing), CFVOOB20 (bergamot olive oil obtained by 20 % enrichment during crushing) and IFVOOB (bergamot olive oil obtained by 2 % infusion) were higher than the control and above 0.80 %. During storage the co-milled samples had the highest levels. This agreed with findings of other authors who studied olive oil flavoured with lemon (Sacchi et al., 2017). The FA increase is probably due to the more acidic environment during malaxation caused by the acids released from bergamot that promotes the hydrolysis of triglycerides. Regarding the primary compound of oxidation, during the 360 days of storage the unflavoured oil suffered a slight oxidation, but lower than the limits set by the EU Regulation 2568/91 for EVOO (from T0 9.45 to 17.89 mEq O_2/kg at T360). All these data agree with the range of the literature data for Ottobratica cultivar (Almeida et al., 2017; Sicari V., 2017). CFVOOB10 and CFVOOB20 had significantly lower peroxide values compared to the control. The mixture with olive paste improves the oil's stability, in contrast to the infusion that showed similar values to the control throughout storage, possibly because infusion may increase oxygen content and hence oxidation. No significant differences were found for the secondary oxidation coefficient ΔK, which maintained values around of 0.00 throughout storage. Concerning the value of K232 e K268 (Figure S1 (a and b), the results are in accordance with authors (Moustakime et al., 2021) and after one year, both were significantly higher in the FVOOs than the control.

Olive oil colour is one of the first impact parameters for consumers, because it could orient its purchase. The addition of spices or herbs can affect this parameter with a consequent influence on its acceptability to the consumer (Issaoui et al., 2016; Lamas et al., 2022). In EVOO and in the FVOOs there was a decrease in chroma C* (Fig. 1) during storage, with significant differences between the samples ($p < 0.01$). More precisely, in CFVOOB10 and CFVOOB20, C* decreased after than 60 days. Certainly, the colour is intimately linked to the chlorophyll and carotenoid contents, thus the consequence in the reduction of the C* values. The content of this pigment (Table 2) is affected by the method of oil extraction, the level of ripeness of the olives, the cultivar and the storage conditions although pigments in olive oil are directly related to oxidative stability (Emmanouilidou et al., 2021). Summarizing, chlorophyll content was more influenced in the samples produced by co-milling, whereas the carotenoid content was higher in IFVOOB. Carotenoids are not generated naturally in the body, so they must be included in the diet. They are known for controlling metabolic disorders and in the reduction of reactive oxygen species (ROS) (Ascrizzi et al., 2019).

In table 2 are reported the values of the total phenolic content (TPC). EVOO possessed a lower value (418.51 mg GAE/kg of oil) than that found by De Bruno et al. (2021) for the same cultivar (1150 mg GAE/kg), but higher than the quantity detected by other authors (Almeida et al., 2017). These differences are probably due to the fact that TPC related to many variables including period of collection, fruit development, and plant growth (Negro et al., 2019). As expected, olive oil enrichment caused an increase in polyphenols. Despite this, adding the matrix by co-milling increases the volume of the paste and naturally there is a loss of these molecules in the olive mill wastewater, also due to the fact that the acids frees by the bergamot could lead to the scission of the secoiridoid aglycons into simple phenols more likely to be lost with the wastewater (Sacchi et al., 2017). The acidic environment that could have caused the bergamot juice, originated a strong lowering of pH of the olive paste generating an unfavorable condition for the activity of some enzymes, even inhibiting some of it. Moreover, this condition could have influenced the distribution phenomena of the compounds present in the lipid or acqeous phases in the malaxing and filtration processes. This probably caused the strong decrease in polyphenol content, in addition to the increase in free acidity values previously discussed. In contrast to this was IFVOOB, in which the TPC was higher than the other samples, even after one year of storage. In this case the enrichment can be considered an addition because there was no enzymatic process or interaction with theolive paste that affects this kind of compound.

Tocopherols or Vitamin E, are linked with the antioxidant activities and play an important role in the scavenging of the reactive oxygen species (ROS). EVOO is naturally rich in tocopherols. In the literature the highest tocopherol content of Calabrian cultivars (both

Table 1
Quality parameters of EVOO and FVOOs. Free acidity [1]: Values are expressed in%; Peroxide value [2]: are expressed in mEq O_2/kg of oil.

FA[1]	T0	T15	T30	T60	T180	T360	Sign
EVOO	0.68 ± 0.02^{bD}	0.70 ± 0.00^{cCD}	0.41 ± 0.00^{dC}	0.56 ± 0.00^{cB}	0.53 ± 0.05^{cC}	0.84 ± 0.01^{cA}	**
IFVOOB	0.68 ± 0.01^{bC}	0.81 ± 0.02^{bB}	0.80 ± 0.02^{cB}	0.84 ± 0.01^{bB}	0.84 ± 0.00^{bB}	2.14 ± 0.09^{aA}	**
CFVOOB10	0.88 ± 0.03^{aCD}	0.97 ± 0.00^{aC}	0.93 ± 0.02^{bCD}	0.82 ± 0.01^{bD}	1.34 ± 0.00^{bB}	1.77 ± 0.10^{bA}	**
CFVOOB20	0.89 ± 0.02^{aC}	0.94 ± 0.02^{aABC}	1.00 ± 0.04^{aA}	0.95 ± 0.00^{aAB}	0.91 ± 0.00^{aBC}	1.00 ± 0.02^{cA}	**
Sign	**	**	**	**	**	**	
PV[2]							
	T0	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
EVOO	9.45 ± 0.20^{aD}	9.50 ± 0.36^{bD}	10.56 ± 0.25^{aC}	10.95 ± 0.03^{bC}	12.86 ± 0.09^{bB}	17.89 ± 0.09^{bA}	**
IFVOOB	9.43 ± 0.11^{aC}	10.36 ± 0.46^{aC}	10.39 ± 0.77^{aC}	12.85 ± 0.17^{aB}	13.11 ± 0.01^{aB}	16.73 ± 0.06^{cA}	**
CFVOOB10	3.81 ± 0.04^{bC}	3.95 ± 0.03^{dC}	3.77 ± 0.30^{cC}	6.38 ± 0.14^{cAB}	6.13 ± 0.07^{dB}	6.49 ± 0.03^{dA}	**
CFVOOB20	5.73 ± 0.41^{aC}	4.81 ± 0.05^{cD}	5.81 ± 0.11^{bC}	4.81 ± 0.00^{dD}	7.98 ± 0.02^{cB}	19.23 ± 0.19^{aA}	**
Sign	**	**	**	**	**	**	
ΔK							
	T0	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
EVOO	0.00 ± 0.00^{bBC}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{BC}	0.00 ± 0.00^{BC}	0.00 ± 0.00^{bAB}	0.00 ± 0.00^{aA}	**
IFVOOB	0.00 ± 0.00^{bAB}	0.00 ± 0.00^{bAB}	0.00 ± 0.00^{AB}	0.00 ± 0.00^{AB}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bB}	*
CFVOOB10	0.00 ± 0.00^{b}	-0.01 ± 0.00^{b}	0.05 ± 0.08	0.00 ± 0.02	0.01 ± 0.03^{a}	-0.01 ± 0.00^{b}	ns
CFVOOB20	0.02 ± 0.01^{aA}	0.01 ± 0.01^{aAB}	-0.01 ± 0.00^{C}	0.00 ± 0.01^{BC}	-0.01 ± 0.00^{cC}	-0.01 ± 0.00^{bC}	**
Sign	**	**	ns	ns	**	**	

Data are expressed as means ± *S*.D. ($n = 3$). EVOO: control; IFVOOB: bergamot olive oil obtained by 2 % infusion; CFVOOB10: bergamot olive oil obtained by 10 % enrichment during crushing; CFVOOB20: bergamot olive oil obtained by 20 % enrichment during crushing. Results followed by letters are significantly different ($p < 0.01$) by post-hoc Tukey's test. The capital letters in the row indicate the differences in one sample in one year of storage. The lowercase letters in the column indicate the differences among the samples at the same time of analysis. Abbreviation: * significance $p \leq 0.05$; ** significance $p \leq 0.01$; ns not significant.

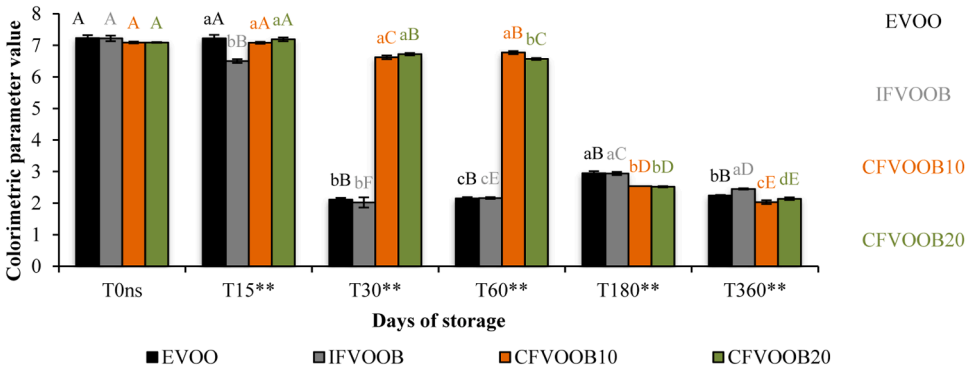

Fig. 1. Chroma* during storage. Data are expressed as means ± *S*.D. ($n = 3$). EVOO: control; IFVOOB: bergamot olive oil obtained by 2 % infusion; CFVOOB10: bergamot olive oil obtained by 10 % enrichment during crushing; CFVOOB20: bergamot olive oil obtained by 20 % enrichment during crushing. Results followed by letters are significantly different ($p < 0.01$) by post-hoc Tukey's test. The capital letters in the row indicate the differences in one sample in one year of storage. The lowercase letters in the column indicate the differences among the samples at the same time of analysis. Abbreviation: ** significance $p \leq 0.01$; ns not significant.

Table 2
Total Phenolic Content (TPC), Total Chlorophyll Content (TChlC), Total Carotenoid Content (TCC) and α-Tocopherol content of EVOO and FVOOs. Values are expressed as mg/kg.

TPC							
T0	T15	T30	T60	T180	T360	Sign	
EVOO	418.51±4.83^{aC}	693.04±54.47^{bD}	796.34±18.44^{aA}	785.20±32.37^{aA}	736.76±19.17^{aB}	546.25±8.95^{bD}	**
IFVOOB	415.09±2.12^{aD}	688.90±40.91^{bA}	802.24±21.96^{bC}	800.11±22.39^{bC}	486.11±0.80^{bC}	457.80±5.33^{aB}	**
CFVOOB10	113.22±13.10^{bB}	160.56±31.74^{aAB}	185.63±3.37^{cA}	166.12±3.68^{cAB}	161.51±6.65^{cAB}	162.90±4.68^{cAB}	*
CFVOOB20	114.4 ± 3.07^{b}	190.98±12.16^{a}	216.43±1.62^{c}	207.88±1.44^{c}	202.72±8.74^{c}	141.88±8.52^{d}	ns
Sign	**	**	**	**	**	**	
TChlC							
	T0	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
EVOO	13.09±0.29^{aB}	13.04±0.37^{cB}	20.06±4.30^{aA}	14.26±1.48^{bB}	13.34±0.09^{bB}	11.03±0.06^{bB}	**
IFVOOB	13.01±0.12^{aC}	19.06±0.98^{aA}	18.37±2.89^{abAB}	16.82±1.04^{aAB}	16.78±0.08^{aAB}	14.97±0.05^{aBC}	**
CFVOOB10	6.38±0.55^{bBC}	12.08±0.39^{cA}	12.41±0.61^{bcA}	10.80±0.17^{cB}	8.17±0.26^{cC}	5.71±0.03^{dC}	**
CFVOOB20	8.86±0.07^{cC}	15.00±0.58^{bA}	9.88±0.38^{cB}	7.99±0.05^{dD}	6.68±0.26^{cF}	4.44±0.17^{cE}	**
Sign	**	**	**	**	**	**	
TCC							
	T0	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
EVOO	6.15±0.1^{aCB}	6.15±0.02^{bCB}	8.41±1.27^{aA}	6.92±0.75^{aAB}	6.51±0.07^{bB}	4.80±0.01^{bC}	**
IFVOOB	6.13±0.4^{aBC}	8.15±0.70^{aA}	7.74±0.92^{aA}	7.48±0.46^{aAB}	6.94±0.10^{aABC}	6.01±0.01^{aC}	**
CFVOOB10	2.01±0.23^{cD}	5.82±0.16^{bA}	4.80±0.19^{bB}	3.66±0.24^{bC}	2.45±0.09^{dE}	1.25±0.09^{dE}	**
CFVOOB20	4.16±0.04^{bB}	5.38±0.25^{bA}	3.98±0.22^{bB}	3.85±0.03^{bB}	2.61±0.00^{cC}	1.69±0.07^{cC}	**
Sign	**	**	**	**	**	**	
α-Tocopherol							
	T0	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
EVOO	354.63±19.36^{aA}	261.63±45.96^{B}	234.22±64.72^{B}	223.72±38.15^{B}	246.61±25.72^{aB}	79.53±1.41^{bC}	**
IFVOOB	353.98±8.08^{aA}	259.00±33.08^{B}	269.13±18.63^{B}	265.72±32.48^{B}	258.60±9.98^{aB}	81.97±2.46^{bC}	**
CFVOOB10	289.81±29.43^{bA}	287.59±1.41^{BC}	289.02±26.60^{B}	251.88±8.62^{B}	205.99±24.90^{abC}	82.13±4.06^{bD}	**
CFVOOB20	278.99±31.20^{bA}	262.34±7.95^{AB}	251.83±10.80^{A}	241.84±8.41^{B}	179.30±16.32^{bC}	88.57±3.31^{aD}	**
Sign	**	ns	ns	ns	*	**	

Data are expressed as means ± *S*.D. ($n = 3$). EVOO: control; IFVOOB: bergamot olive oil obtained by 2 % infusion; CFVOOB10: bergamot olive oil obtained by 10 % enrichment during crushing; CFVOOB20: bergamot olive oil obtained by 20 % enrichment during crushing. Results followed by letters are significantly different ($p < 0.01$) by post-hoc Tukey's test. The capital letters in the row indicate the differences in one sample in one year of storage. The lowercase letters in the column indicate the differences among the samples at the same time of analysis. Abbreviation: * significance $p \leq 0.05$; ** significance $p \leq 0.01$; ns not significant.

autochthonous and allochthonous) was found in October, with a decreasing content during olive ripening (Giuffrè A.M., 2018). Authors evidences how the addition of herbs or spices into an olive oil helps in the protection of the tocopherols degradation, mainly due to the presence of the light or high temperature (Moustakime et al., 2021). Table 2 reports the trend of α-tocopherol content in all samples during storage. The initial level is in accordance with the literature for the control, corresponding to 354.63 mg/kg, and for CFVOOB20 the lowest value was observed (278.99 mg/kg). It confirms that an increase in the olive paste volume can cause a high loss of biochemicals in olive oil mill wastewater. After one year of storage, the α-tocopherol content decreased significantly to values of 79.53, 81.97, 82.13 and 88.57 mg/kg for EVOO, IFVOOB, CFVOOB10, and CFVOOB20, respectively. Despite CFVOOB20 having the lowest level throughout storage, at the end of this period it had the best protective effect against the loss of α-tocopherol, although every FVOO maintained a level slightly higher than the control.

EVOO, IFVOO, CFVOOB10 and CFVOOB20 were also analysed to identify and quantify the individual phenolic composition using UHPLC technology. In Table S2 is reported the single phenolic composition of EVOO. It was characterized by a high amount of pinoresinol (43.38 mg/kg), hydroxytirosol (16.15 mg/kg), tyrosol (15.61 mg/kg) and a low quantity of oleoropein (0.86 mg/kg). Sicari et al. (2010) detailed how during one year of storage in an olive oil different enzymatic or hydrolytic processes could induce in substancial changement in the content of phenols. Usually secoiridoid tends to decrease, the phenol alcohols and cinnamic acid increase, as well as the flavonoids. The increase in the latter molecules is probably due to the oxidation of other

phenolic compounds (Sicari et al., 2010). As regards the FVOOs (Table 3), the most common constituents of bergamot were found in different concentrations, it being noticeable that the olive oil reacted differently when the enrichment was carried out by infusion rather than co-milling. Furthermore, the enzymatic process was influenced by the percentage of enrichment. These flavonoids increase during storage due to hydrolytic processes, as demonstrated in the literature (Sicari et al., 2010). The hesperidin content greatly increased during the storage in all the FVOOs. This was inversely proportional to the enzymatic assays: as its content increased, the value of IC_{50} decreased and therefore the activity against the enzyme grew. Also interesting is the naringin content, which followed the same trend as the other flavonoids. It has a high potential against the oxidative process and a strong activity as a scavenger of free radicals. Thus, it was positively correlated with FRAP test in the CFVOOB20 sample and was inversely proportional to β-carotene bleaching test, especially in the CFVOOB10 sample (Ascrizzi et al., 2019). Among identified compounds, it is interesting to note that diosmetin was one of the main abundant compounds. This flavonoid is known for its ability to control glucose metabolism in vivo (Xiaobao et al., 2021). Also of interest was the pinoresinol content, which remained stable in the unflavoured sample, had a slight decrease in IFVOOB during storage, but increased four-fold in CFVOOB10 and CFVOOB20 between T0 and T360. That factor might be due to phenomena of antagonism with other compounds, which decreased their content throughout storage. Another prominent variation among the FVOOs, is the presence of bergamottin in the co-milled samples, 8.44 and 14.78 mg/kg at T0 in CFVOOB10 and CFVOOB20, respectively. This condition denotes that the above-mentioned furocoumarin, which in general has a weak polarity, maximizes its recovery during the pressing of the bergamots with the olives and not with the infusion approach. Bergamottin possesses important pharmacological properties and enhances the bioavailability of drugs thanks to the interaction with cytochrome P450 enzyme (Liu et al., 2017). Regrettably, studies on this molecule are not easy for its rarity and evaluability of this compound.

The radical scavenging activity of EVOO could considered good, thanks to the IC_{50} values for DPPH assay of 12.33 and 29.54 at T0 and T360, respectively and from 3.43 to 15.21 μg/mL at T0 and T360 in ABTS assay (Table 4). However, this activity tended to decrease during all the storage. Among the various Calabrian cultivars, despite belonging to the same area of cultivation, there is an enormous variability in response in these assays, concerning which Leporini et al. (2018) have previously shown IC_{50} values from 45.30 to 256.80 and from 56.30 to 279.60 μg/mL for Calabrian Frantoio EVOO in DPPH and ABTS, respectively. To add bergamots, by infusion or during the crushing, does not seem to produce good results in terms of DPPH. In fact, both enrichment technologies caused a complete loss of the potential scavenger activity of bergamot. On the contrary, in ABTS assay, FVOOs exhibited a higher activity, even at the end of storage, than the control. A total loss in antioxidant power in terms of protection from lipid peroxidation was observed for EVOO at the end of storage (IC_{50} >100 μg/mL) (Table 4). Otherwise, data from the co-milled FVOOs (CFVOOB10 and CFVOOB20) showed a good activity in terms of protection from lipid peroxidation even after one year of storage. This result is probably linked to the high TFC in these extracts.

FRAP assay data shows that, during the year of the storage, the values are lower than the BHT used as positive control (FRAP value 63.26 μM Fe(II)/g). In fact, FRAP values of 25.01 and 4.31 μM Fe(II)/g were recorded at T0 and T360, respectively for EVOO. Promising results were obtained with FVOOs. In fact, CFVOOB20 exhibited a FRAP value of 70.09 μM Fe(II)/g after 360 days' storage (Table 4). This is due to the higher availability of flavonoids and their stability over time in this sample when compared to the others. To sum up, controversial data emerged on the antioxidant activity of a flavored olive oil, probably caused by the matrix or by the techniques used (Loizzo et al.,).

EVOO, CFVOOB10, CFVOOB20 and IFVOOB were also tested to evaluate the potential inhibitory activity against α-amylase and α-glucosidase, two enzymes involved in the hydrolysis of carbohydrates. In EVOO, IC_{50} values from 269.02 to 289.32 and from 137.34 to T360 778.23 μg/mL, at T0 and T360 for α-amylase and α-glucosidase, respectively were found (Table S3). An exponential increase is evident starting from T180 in the α-glucosidase test. On the contrary, in α-amylase test, at T180 the value significantly decreases (240.29 μg/mL), reaching at T360 values very similar to T0, thus maintaining its activity throughout the period considered. All the results are highly significant ($p < 0.01$).

Table S3 shows that the enzymatic activity is higher in the FVOOs (CFVOOB10, CFVOOB20 and IFVOOB) than the control. Promising results in α-amylase test were obtained with CFVOOB20, much more than CFVOOB10 and IFVOOB (52.32 *vs* 63.11 and 77.22 μg/mL at T360, respectively). This property is positively correlated with carotenoid content with a Pearson correlation coefficient of $p = 0.85$. Conversely, in α-glucosidase better results were obtained in CFVOOB10 with IC_{50} values of 60.88 μg/mL at T360, as well as positively correlated with TCC and with $p = 0.63$.

The hypolipidemic activity was evaluated by inhibition of pancreatic lipase, which is involved in the metabolism of fats. A reduction in pancreatic lipase inhibitory activity was observed during storage (IC_{50} values of 143.46 and 312.97 μg/mL at T0 and T360, respectively), with values two-times higher at T360 compared to T0 (Table S4). As previously reported, bergamot possesses inhibitory activity on key enzymes of fat and carbohydrate metabolism (see paragraph 3.1). Also in this case, there is a positive correlation with total carotenoid content in CFVOOB10 and CFVOOB20 ($p = 0.76$ *vs* 0.86, respectively). Notably, IFVOOB preserves this activity up to 6 months of storage, but at 12 months CFVOOB20 showed the best activity with IC_{50} values of 98.16 μg/mL, almost 5-times lower than the EVOO. Therefore, the higher the fruit content in the enrichment process, the better the potential for anti-obesity activity.

3.2.1. *Sensory analysis*

EVOO and FVOOs were also assessed by sensory analysis. In the case of FVOOs, new sensory descriptors were added, ("citrusy", "astringent", "bitter"). The panelists were clearly able to associate the CFVOO10 and CFVOO20 to an enrichment with bergamot fruits, as opposite to IFVOOB in which they were not capable to identify the matrix. Fig. 2 (a and b) report the olfactory and gustatory sensations of EVOO and FVOOs, respectively. First, CFVOOB10 and CFVOOB20 scored a high overall acceptability of 8 and 9 points respectively. In agreement with Sacchi et al. (2017), the fruits belonging to the Citrus, own a positive effect on the olive oil with some defects, covering perfectly all of them when aromatization is performed during the crushing of fresh olives. Other results indicated an increment of citrusy, fruity, bitter, and salty notes. Interesting are also the growth of the attributes "sweet" and "floral", that significantly increased compared to EVOO. The "astringency" attribute underwent a boost in CFVOOB20. However, this result is not always positive. In fact, all panelists agreed that from a sensorial point of view, the 20 % flavouring was too strong, unlike the 10 %, which was overall good, balanced and pleasant to the taste. Regarding IFVOOB, the sensory evaluation was only slightly different from the control (EVOO). In fact, from the olfactory point of view, there was only an increase in the "citrusy" note. However, important increases were in the "sweet", "floral" and "citrusy" notes, to a lesser extent than the previous flavoring technique. Also, in this case the attribute "astringency" is higher. The general acceptability reached an overall grade of 6.

4. Conclusions

The aim of this study was to increase the value of the bergamot fruit by using it in its entirety. Flavoring an olive oil is an ancient practice, but in recent years, the demand for this type of product has increased due to a raised awareness on the part of the consumer, who pays more attention to what they eat and to the beneficial properties of foods. The

Table 3
Single phenolic compounds by UHPLC. Values are expressed as mg/kg.

Phenolic compounds in CFVOOB10 Compounds	T0	T15	T30	T60	T180	T360	Sign
Hydroxytyrosol	13.49±0.22^{c}	7.19±0.08^{d}	20.71±1.74^{bc}	13.74±1.07^{c}	23.16±0.61^{b}	87.57±0.36^{a}	**
Tyrosol	12.76±0.02^{c}	39.69±0.21^{b}	40.59±2.54^{bc}	42.29±4.00^{bc}	45.71±2.03^{b}	53.37±1.09^{a}	**
4-hydroxyphenyl acetate	0.85±0.03^{e}	1.07±0.02^{d}	2.48±0.10^{b}	1.66±0.94^{c}	2.34±0.31^{b}	4.49±0.76^{a}	**
Vanillic acid	0.27±0.00^{c}	1.12±0.03^{b}	1.35±0.07^{ab}	1.69±0.17^{a}	1.40±0.04^{ab}	1.26±0.04^{ab}	**
Homovanillic acid	0.96±0.01^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	**
Vanillin	2.63±0.01^{c}	4.32±0.15^{b}	5.14±0.42^{a}	4.56±0.52^{b}	0.00^{d}	0.00^{d}	**
Chlorogenic acid	11.09±1.06^{c}	25.66±0.43^{a}	27.11±2.96^{a}	26.44±2.93^{a}	18.54±1.02^{b}	9.78±0.51^{d}	**
Quercetin 3,4′-diglucoside	1.74±0.02^{b}	2.43±0.08^{a}	2.25±0.41^{ab}	2.34±0.29^{a}	2.46±0.48^{a}	2.71±0.35^{a}	**
p-Coumaric acid	0.68±0.03^{a}	0.27±0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	**
Ferulic acid	0.33±0.00^{d}	0.93±0.02^{b}	1.40±0.07^{a}	1.39±0.11^{a}	0.98±0.12^{b}	0.65±0.27^{c}	**
Rutin	0.45±0.03^{b}	0.54±0.05^{b}	0.84±0.21^{a}	0.79±0.08^{a}	0.00^{c}	0.00^{c}	**
Luteolin 7-*O*-glucoside	2.69±0.03^{c}	2.69±0.03^{c}	3.83±0.14^{b}	3.98±0.27^{a}	0.00^{d}	0.00^{d}	**
Oleuropein	0.06±0.00^{c}	0.25±0.00^{a}	0.19±0.00^{ab}	0.22±0.03^{a}	0.13±0.01^{bc}	0.12±0.01^{bc}	**
Cinnamic acid	13.39±0.02^{a}	1.61±0.05^{c}	1.32±0.05^{c}	1.68±0.84^{c}	1.89±0.21^{c}	7.44±1.69^{b}	**
Pinoresinol	12.54±0.05^{c}	16.91±0.07^{b}	16.63±1.67^{b}	18.19±2.49^{b}	18.44±0.46^{b}	45.30±1.45^{a}	**
Luteolin	1.32±0.00^{c}	1.71±0.04^{b}	2.73±0.05^{a}	2.58±0.09^{a}	0.00^{d}	0.00^{d}	**
Quercetin	4.64±0.00^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	**
Apigenin	1.38±0.01^{d}	5.27±0.03^{b}	3.15±0.96^{c}	3.61±0.61^{c}	5.74±0.20^{b}	8.78±0.03^{a}	**
Eriocitrin	0.89±0.00^{a}	0.72±0.16^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	**
Neoeriocitrin	0.66±0.00^{b}	0.00^{c}	0.68±0.02^{b}	0.80±0.03^{a}	0.00^{c}	0.00^{c}	**
Narirutin	2.88±0.00^{a}	1.82±0.12^{b}	1.24±0.10^{c}	1.66±0.40^{b}	0.00^{d}	0.00^{d}	**
Naringin	1.03±0.01^{e}	1.97±0.33^{d}	3.32±0.06^{c}	3.79±0.89^{c}	4.74±0.17^{b}	6.73±0.69^{a}	**
Hesperidin	11.30±0.21^{c}	11.91±0.00^{c}	12.31±0.61^{c}	12.97±0.37^{c}	73.37±1.02^{b}	463.12±45.32^{a}	**
Neoesperidin	10.07±0.09^{a}	5.43±0.02^{b}	0.48±0.16^{d}	0.43±0.05^{d}	0.73±0.08^{cd}	0.80±0.20^{c}	**
Didimin	2.15±0.03^{d}	19.67±0.26^{c}	19.26±1.71^{c}	21.21±2.88^{bc}	23.98±0.50^{b}	37.52±0.50^{a}	**
Diosmetin	5.38±0.10^{c}	6.00±0.06^{bc}	6.27±0.21^{bc}	6.55±0.97^{bc}	7.21±0.15^{b}	11.80±3.50^{a}	**
Apigenin 7-*O*-glucoside	0.00^{c}	0.75±0.05^{a}	0.13±0.02^{b}	0.08±0.04^{bc}	0.00^{c}	0.00^{c}	**
Kaempferol	0.00^{e}	4.19±0.04^{c}	3.13±0.26^{d}	3.48±1.19^{d}	4.90±0.05^{b}	6.78±1.43^{a}	**
Isoramnetin	0.00^{c}	0.00^{c}	5.62±0.94^{b}	5.76±1.18^{a}	0.00^{c}	0.00^{c}	**
Bergamottin	8.44±0.66^{b}	9.32±0.45^{a}	8.09±0.76^{bc}	7.56±0.43^{c}	8.08±0.34^{bc}	7.45±0.32^{c}	**
Phenolic compounds in CFVOOB20							
Compounds	**T0**	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
Hydroxytyrosol	2.67±0.01^{e}	9.72±1.31^{c}	8.82±0.96^{d}	9.42±0.63^{c}	15.30±0.77^{b}	20.30±0.16^{a}	**
Tyrosol	13.01±0.02^{d}	19.46±1.84^{c}	29.14±3.33^{a}	21.29±2.48^{b}	18.75±3.84^{c}	9.56±0.69^{e}	**
Vanillic acid	0.22±0.04^{b}	0.18±0.04^{b}	0.22±0.01^{b}	0.00^{c}	0.00^{c}	1.56±0.03^{a}	**
Homovanillic acid	0.28±0.02^{b}	0.46±0.03^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	**
Vanillin	1.40±0.01^{a}	0.99±0.02^{b}	0.95±0.01^{b}	0.89±0.05^{b}	0.77±0.12^{b}	0.28±0.07^{c}	**
Chlorogenic acid	8.56±0.51^{cd}	13.49±2.71^{b}	19.66±3.06^{a}	21.50±1.35^{a}	9.47±0.56^{c}	5.72±0.07^{d}	**
Quercetin 3,4′-diglucoside	2.22±0.02^{c}	3.85±0.98^{a}	3.74±0.61^{ab}	3.22±0.05^{b}	3.15±0.11^{b}	3.07±0.07^{ab}	**
Ferulic acid	0.61±0.04^{c}	0.94±0.15^{c}	1.46±0.10^{ab}	1.57±0.09^{ab}	1.36±0.03^{b}	1.72±0.03^{a}	**
Luteolin 7-*O*-glucoside	1.02±0.01^{b}	1.68±0.50^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	**
Oleuropein	0.07±0.00^{c}	0.17±0.02^{b}	0.16±0.03^{b}	0.16±0.04^{b}	0.11±0.00^{bc}	0.32±0.02^{a}	**
Cinnamic acid	0.75±0.02^{c}	1.83±0.10^{b}	1.64±0.33^{b}	1.86±0.93^{b}	1.84±0.04^{b}	4.32±0.39^{a}	**
Pinoresinol	7.53±0.02^{d}	10.24±1.36^{cd}	13.10±1.39^{c}	13.69±1.16^{c}	15.28±0.47^{b}	31.57±3.08^{a}	**
Luteolin	2.10±0.01^{a}	2.39±0.04^{a}	3.37±0.26^{a}	3.36±0.05^{a}	2.25±0.21^{a}	0.00^{b}	**
Quercetin	0.93±0.02^{b}	1.76±0.30^{a}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	**
Apigenin	13.36±0.02^{a}	1.25±1.04^{e}	2.53±0.44^{d}	2.57±1.14^{d}	4.09±0.24^{c}	5.73±0.4^{b}	**
Isoramnetin 3-*O*-glucoside	0.90±0.01^{b}	1.62±0.18^{a}	0.43±0.16^{cd}	0.55±0.06^{c}	0.00^{d}	0.00^{d}	**
Eriocitrin	0.44±0.02^{c}	0.56±0.03^{c}	1.03±0.01^{b}	1.74±0.03^{a}	0.00^{d}	0.00^{d}	**
Narirutin	0.56±0.00^{c}	0.69±0.18^{bc}	0.96±0.12^{ab}	1.11±0.06^{a}	0.00^{d}	0.00^{d}	**
Naringin	1.57±0.03^{d}	2.10±1.01^{c}	3.20±0.52^{b}	2.86±0.14^{bc}	1.87±0.27^{d}	8.87±1.62^{a}	**
Hesperidin	0.95±0.06^{b}	2.65±0.08^{a}	0.95±0.03^{b}	0.96±0.01^{b}	1.10±0.16^{b}	2.89±0.12^{a}	**
Neoesperidin	0.00	0.36±0.02	0.00	0.00	0.00	0.00	ns
Didimin	7.87±0.31^{c}	12.95±1.52^{b}	12.19±1.62^{b}	13.62±1.02^{b}	14.11±0.47^{b}	28.74±0.75^{a}	**
Diosmetin	2.24±0.00^{c}	12.45±1.02^{b}	12.47±1.68^{b}	12.91±0.98^{b}	15.49±0.39^{b}	31.41±0.08^{a}	**
Apigenin 7-*O*-glucoside	0.68±0.03^{a}	0.66±0.08^{a}	0.47±0.13^{b}	0.08±0.01^{c}	nd^{c}	nd^{c}	**
Kaempferol	2.28±0.00^{bc}	1.29±0.48^{c}	2.95±0.05^{b}	3.16±0.02^{b}	3.09±0.10^{b}	7.67±1.47^{a}	**
Isoramnetin	3.30±0.06^{a}	3.77±0.51^{a}	4.62±0.28^{a}	4.45±0.54^{a}	0.00^{b}	0.00^{b}	**
Bergamottin	14.78±0.77^{c}	16.56±0.87^{a}	15.31±0.67^{b}	14.56±0.62^{c}	13.28±0.34^{d}	15.45±0.87^{b}	**
Phenolic compounds in IFVOOB							
Compounds	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**	
Hydroxytyrosol	10.96±1.95^{e}	25.32±1.51^{d}	34.81±1.74^{c}	46.69±1.32^{a}	39.70±0.73^{b}	**	
Tyrosol	16.66±0.20^{d}	28.98±1.72^{c}	48.20±1.79^{b}	22.99±4.50^{cd}	60.46±3.20^{a}	**	
4-hydroxyphenyl acetate	0.00^{b}	1.86±0.14^{a}	1.85±0.34^{a}	0.00^{b}	0.00^{b}	**	
Chlorogenic acid	2.03±0.04^{a}	1.61±0.10^{a}	1.66±0.07^{a}	0.00^{b}	0.00^{b}	**	
Vanillic acid	0.44±0.01^{c}	1.08±0.11^{b}	2.17±0.15^{a}	0.00^{d}	0.00^{d}	**	
p-Coumaric acid	0.26±0.02^{c}	3.67±0.39^{a}	2.34±0.16^{b}	2.30±0.03^{b}	2.42±0.11^{b}	**	
Quercetin 3–4′-diglucoside	0.00^{c}	3.87±0.65^{b}	3.26±0.06^{b}	0.00^{c}	4.73±0.97^{a}	**	
Ferulic acid	0.58±0.03^{b}	0.72±0.01^{ab}	0.81±0.01^{a}	0.00^{c}	0.00^{c}	**	
Luteolin 7-*O*-glucoside	2.62±0.02^{c}	5.49±0.36^{b}	6.91±1.22^{b}	0.00^{d}	14.93±1.56^{a}	**	
Naringin	1.45±0.14^{e}	6.63±0.54^{d}	15.24±0.45^{c}	23.37±0.36^{b}	77.22±4.22^{a}	**	
Narirutin	0.00^{b}	6.73±0.19^{a}	0.00^{b}	0.00^{b}	0.00^{b}	**	
Oleuropein	0.22±0.03^{c}	1.30±0.08^{b}	2.13±0.20^{a}	1.64±0.13^{b}	0.00^{d}	**	

(*continued on next page*)

Table 3 (*continued*)

Phenolic compounds in CFVOOB10 Compounds	T0	T15	T30	T60	T180	T360	Sign
Hesperidin	24.66±2.22^{c}	14.00±0.8^{d}	9.62±0.87^{e}	34.14±4.19^{b}	71.21±13.75^{a}	**	
Neoesperidin	0.00^{c}	6.99±0.8^{b}	9.78±1.03^{a}	0.00^{c}	0.00^{c}	**	
Cinnamic acid	4.15±1.12^{b}	6.47±0.46^{a}	3.79±0.97^{bc}	2.75±0.07^{c}	0.35±0.02^{d}	**	
Didimin	15.17±0.76^{a}	13.82±0.74^{a}	14.38±0.67^{a}	11.90±0.40^{b}	5.59±0.63^{c}	**	
Quercetin	9.73±0.17^{a}	7.10±0.56^{b}	2.02±0.13^{c}	0.00^{d}	10.78±1.02^{a}	**	
Luteolin	0.00^{b}	3.40±0.20^{a}	3.69±0.11^{a}	0.00^{b}	0.00^{b}	**	
Pinoresinol	41.56±3.05^{ab}	40.56±1.17^{b}	43.23±2.11^{a}	37.65±0.51^{c}	34.44±2.11^{d}	**	
Apigenin	33.64±7.14^{a}	24.94±1.83^{b}	23.56±1.60^{b}	25.12±0.48^{b}	5.83±1.76^{c}	**	
Kaempferol	0.00^{d}	5.84±0.78^{a}	2.06±0.1^{c}	0.00^{d}	3.99±0.45^{b}	**	
Isoramnetin	2.50±0.08^{a}	0.00^{c}	1.30±0.02^{b}	0.00^{c}	0.00^{c}	**	
Isoramnetin 3-*O*-glucoside	0.16±0.01^{a}	0.14±0.06^{a}	0.12±0.02^{a}	0.00^{b}	0.00^{b}	**	
Apigenin 7-*O*-glucoside	1.55±0.19^{a}	1.03±0.01^{a}	1.06±0.15^{a}	0.00^{b}	0.00^{b}	**	

Data are expressed as means ± *S*.D. ($n = 3$). Results followed by letters are significantly different ($p < 0.01$) by post-hoc Tukey's test. Abbreviation: ** significance $p \leq 0.01$.

Table 4
Radical scavenging activity of EVOO and FVOOs against DPPH, ABTS, β-carotene bleaching test (values are expressed as IC_{50} (μg/mL), and FRAP (expressed as IC_{50} (μM Fe(II)/g) assay during the storage.

	T0	T15	T30	T60	T180	T360	Sign
DPPH							
EVOO	12.33±3.45^{cC}	14.09±3.21^{bC}	15.72±2.87^{bBC}	20.77±2.82^{cBC}	19.61±3.09^{cB}	29.54±3.77^{cA}	**
IFVOOB	62.15±2.27^{bAB}	62.27±2.56^{aAB}	59.45±2.76^{aB}	57.09±2.08^{aB}	59.15±2.13^{aB}	68.13±2.44^{aA}	**
CFVOOB10	68.34±2.23^{aA}	63.52±2.18^{aAB}	61.78±2.09^{aB}	49.22±2.47^{bC}	47.45±2.21^{bC}	48.21±2.17^{bC}	**
CFVOOB20	62.13±2.24^{bAB}	60.11±2.32^{aA}	57.36±2.45^{aB}	47.09±265^{bC}	43.11±2.23^{bC}	45.20±2.21^{bC}	**
Sign	**	**	**	**	**	**	
ABTS							
	T0	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
EVOO	3.43±0.25^{B}	4.98±0.77^{aB}	5.16±0.93^{aB}	7.39±0.91^{aB}	11.43±0.86^{aB}	15.21±1.19^{aA}	**
IFVOOB	3.01±0.24^{B}	3.16±0.38^{bB}	2.69±0.27^{bBC}	2.07±0.14^{bC}	3.05±0.19^{bB}	5.11±0.77^{bA}	**
CFVOOB10	3.03±0.56^{A}	2.97±0.34^{bA}	2.44±0.22^{bAB}	2.01±0.13^{bBC}	1.89±0.12^{cC}	1.97±0.22^{bBC}	**
CFVOOB20	3.00±0.26^{A}	2.52±0.18^{bAB}	2.30±0.15^{bABC}	1.98±0.12^{bCD}	1.61±0.10^{cD}	1.85±0.23^{bBCD}	**
Sign	ns	**	**	**	**	**	
β-carotene bleaching test							
	T0	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
EVOO	48.72±3.45^{bD}	52.21±3.89^{bD}	59.83±4.40^{aC}	77.05±4.42^{aB}	>100^{aA}	>100^{aA}	**
IFVOOB	56.16±2.59^{aBC}	59.61±2.88^{aB}	53.28±2.19^{bBC}	50.34±2.08^{bC}	55.19±2.34^{bBC}	88.61±3.46^{bA}	**
CFVOOB10	58.45±2.47^{aA}	57.22±2.52^{aA}	55.26.±2.51^{bAB}	52.16±2.59^{bAB}	50.12±2.01^{bB}	52.31±2.16^{cAB}	**
CFVOOB20	56.12±2.61^{aA}	54.32±2.40^{abAB}	51.98±2.37^{bBC}	50.82±2.35^{bC}	47.89±2.07^{bC}	48.93±2.10^{cC}	**
Sign	**	**	**	**	**	**	
			FRAP				
	T0	**T15**	**T30**	**T60**	**T180**	**T360**	**Sign**
EVOO	25.01±1.20^{cA}	24.71±1.30^{cAB}	23.99±1.52^{cAB}	21.65±1.56^{cBC}	18.21±1.21^{dC}	4.31±0.85^{dD}	**
IFVOOB	64.69±1.97^{aA}	67.09±2.24^{aA}	67.35±2.45^{aA}	69.65±2.81^{aA}	46.37±2.96^{cB}	32.09±2.76^{cC}	**
CFVOOB10	54.12±1.76^{b}	56.31±1.78^{b}	57.45±1.85^{b}	59.13±1.83^{b}	56.49±1.98^{b}	54.13±2.02^{b}	ns
CFVOOB20	63.67±1.92^{aB}	69.83±2.00^{aA}	68.71±2.12^{aAB}	70.31±2.27^{aAB}	68.81±2.29^{aAB}	70.09±2.33^{aAB}	**
Sign	**	**	**	**	**	**	

Data are expressed as means ± *S*.D. ($n = 3$). EVOO: control; IFVOOB: bergamot olive oil obtained by 2 % infusion; CFVOOB10: bergamot olive oil obtained by 10 % enrichment during crushing; CFVOOB20: bergamot olive oil obtained by 20 % enrichment during crushing. Ascorbic acid was used as positive control in both DPPH and ABTS test (IC50 values of 5.03 ± 0.82 and 1.78 ± 0.07 μg/mL, respectively). Results followed by letters are significantly different ($p < 0.01$) by post-hoc Tukey's test. The capital letters in the row indicate the differences in one sample in one year of storage. The lowercase letters in the column indicate the differences among the samples at the same time of analysis. Abbreviation: ** significance $p \leq 0.01$; ns not significant.

Mediterranean diet, of which olive oil is one of the main ingredients, is becoming more popular for its favorable effects on human health. On the basis of this, it is important not only to create an aromatized olive oil with an attractive flavour, but to find a production method that creates an oil with good functional activities, using an optimum percentage of enrichment, which will naturally differ according to the matrix. Our results confirmed that to produce flavoured oil by co-milling is not a simple enrichment but is the result of a complex interaction between the matrix and olives. For the first time, bergamot flavoured olive oils were thoroughly investigated over a one-year period, simulating consumer storage conditions. It was necessary to find the right proportions and technique for enrichment to obtain the best oil in terms of taste and functionalization. Despite the negative effect on the polyphenol content and on some quality indices caused by the lowering of the pH of the olive paste in the co-milled samples (CFVOOB10 and CFVOOB20), the inhibitory activity against the key enzymes linked to obesity menagement remained high, as well as their scavenging activity showed by the FRAP assay. Thus the next challenge could esclude the bergamot juice to limit the acidification of the olive paste in the formulation of new products. Thanks to this study not only the health properties of bergamot been confirmed, but it has been shown that it can also be considered as a 'gourmet oil'.

CRediT authorship contribution statement

Irene Maria Grazia Custureri: Formal analysis, Software. **Angelo Maria Giuffrè:** Conceptualization, Methodology, Resources. **Monica Rosa Loizzo:** Conceptualization, Investigation, Methodology. **Rosa Tundis:** Conceptualization, Formal analysis, Resources. **Ana Cristina Soria:** Conceptualization, Resources, Visualization. **Vincenzo Sicari:**

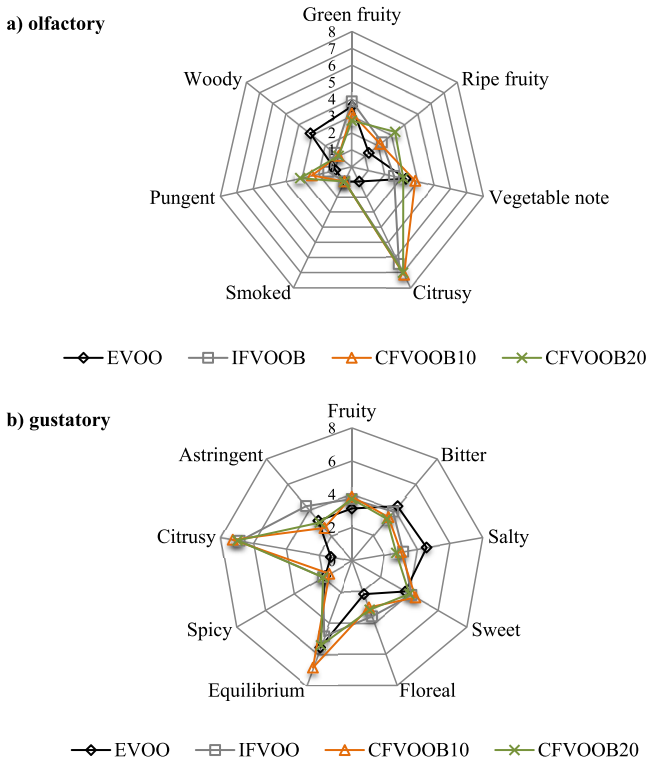

Fig. 2. a) and b) Sensory profile. Abbreviation: EVOO: control; IFVOOB: bergamot olive oil obtained by 2 % infusion; CFVOOB10: bergamot olive oil obtained by 10 % enrichment during crushing; CFVOOB20: bergamot olive oil obtained by 20 % enrichment during crushing.

Conceptualization, Funding acquisition, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Ethical statement

The authors declare that there was no animal or human study involved in this current research paper submitted.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2024.100400.

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