Journal of Agriculture and Food Research 16 (2024) 101111

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Turmeric-flavoured olive oil: A promising path to natural antioxidant benefits

Irene Maria Grazia Custureri [a], Vincenzo Sicari [a,*], Angelo Maria Giuffrè [a], Rosa Tundis [b], Ana Cristina Soria [c], Monica Rosa Loizzo [b]

[a] Department of Agriculture, University "Mediterranea" of Reggio Calabria, Salita Melissari, Feo di Vito, 89124, Reggio Calabria, Italy
[b] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Edificio Polifunzionale, Via P. Bucci, 87036, Rende, Italy
[c] Institute of General Organic Chemistry (IQOG-CSIC), Juan de la Cierva 3, 28006, Madrid, Spain

ARTICLE INFO

Keywords:
Turmeric
Curcuminoids
Antioxidant activity
Functional
Olive oil

ABSTRACT

The influence of the addition of *Curcuma longa* (turmeric) powder to Ottobratica variety extra virgin olive oil (EVOO) by using malaxation or infusion processes to obtain flavoured virgin olive oil (FVOO) was determined. FVOO was monitored during one year of storage at room temperature in the dark. FVOO obtained malaxation process (CM) showed the lowest free acidity value, irrespective of the time of storage considered. C* values of 7.23 *vs* 6.38 and 6.79 were recorded for the FVOO obtained infusion process (CI) and CM FVOO, respectively. Moreover, CM exhibited the highest radical scavenging activity with IC_{50} values of 9.48 and 3.49 μg mL^{-1} at T0 in DPPH and ABTS tests, respectively. However, the addition of turmeric did not improve the bioactivity of the FVOO against key enzymes involved in metabolic syndrome. Collectively our data have demonstrated, once again, how the enrichment of EVOO with an aromatic and functional matrix such as turmeric does not always lead to an improvement in its intrinsic functional characteristics despite the fact that it may be appreciated by the consumer for its sensorial characteristics.

1. Introduction

Virgin olive oil is extracted from freshly harvested healthy fruits, using mechanical processes (milling, malaxation, centrifugation) that allow the preservation of chemical and sensory characteristics (Commission Regulation (EEC) N° 2568/91) [1]. However, to ensure an effective and positive health impact, olive oil must contain a minimum amount of some bioactive compounds like phenolics, as stipulated by the polyphenols-related health claim (European Commission Regulation (EU) No 432/2012,2012) [2]. Several scientific evidence testifies that the health effects of the Mediterranean diet have been attributed to the consumption of olive oil and to its composition in fatty acids and so-called minor components, such as tocopherols, carotenoids, and polyphenols [3]. Statistics from the World Health Organization (WHO) have shown how the increase in life expectancy has increased in Mediterranean countries compared to that of more developed Western countries, in correlation with the degree of adherence to the Mediterranean diet, where olive oil represents the main condiment with consumption albeit minimal but daily [4]. Although the mechanisms by which olive oil bioactive compounds exert these effects are only just beginning to be addressed, the mounting evidence indicating their antioxidant effects are likely to be the key element since oxidation process is commonly found during the initiation or progression of several pathologies [5].

Furthermore, recent studies have shown that the consumption of virgin olive oil improved some parameters linked to obesity such as plasma lipid profile and insulin resistance [6]. Obesity is increasing worldwide, becoming a public health problem of paramount importance. Every year, 4 million people die from obesity and related diseases [7]. Several authors have shown the presence of oxidative stress in obese patients from metabolic disorders causing a high release of free radicals [8].

To help the body in counteracting oxidative process that acts as disease-promoting process, increasing the dietary intake of natural antioxidants has proven to be useful [9]. Spices and herbs are food matrices naturally rich in antioxidant compounds with great potential to

* Corresponding author.
E-mail addresses: irene.custureri@unirc.it (I.M.G. Custureri), vincenzo.sicari@unirc.it (V. Sicari), amgiuffre@unirc.it (A.M. Giuffrè), rosa.tundis@unical.it (R. Tundis), acsoria@iqog.csic.es (A.C. Soria), monica_rosa.loizzo@unical.it (M.R. Loizzo).

https://doi.org/10.1016/j.jafr.2024.101111
Received 8 February 2024; Received in revised form 11 March 2024; Accepted 14 March 2024
Available online 15 March 2024

possess great potential for human health [10]. Among them *Curuma longa* L (turmeric) has recently attracted consumer interest due its content in bioactive compounds including curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin), compounds characterized by high healthy potential [11–13]. For this purpose, the rhizome was largely used fresh to enrich dishes or to prepare herbal teas, or as a powder in "golden milk" [14,15]. Other functional foods enriched with turmeric are bakery snacks, corn snacks, herbal drinks [16–19].

Over the last decade, emerging consumer trends have highlighted that consumers are looking for new sensory sensations, with greater attention to health and well-being. This gave rise to the need to develop flavoured and fortified olive oils. These new products are the result of the incorporation of traditional aromas and flavours and not through different flavouring processes [20].

Considering that nowadays consumers are particularly attentive to what they eat and to the possibility of introducing fortified foods with health-promoting properties, the objective of this work was to enrich EVOO (extra virgin olive oil) with turmeric powder using two different technological approaches (adding the powder directly to the olive paste and adding the powder directly to the oil via infusion) thanks to the high affinity of that spice to an oily matrix. According to the European Union Commission [21] the addition of some matrices to an extra virgin olive oil generates a product labelled as flavoured virgin olive oil (FVOOs) and no longer as EVOO. By carrying out different *in vitro* tests, the ability of this FVOOs to act as antioxidant or antiradical agent was studied as well as the effect against key enzymes related to obesity, such as α-amylase, α-glucosidase and lipase.

2. Materials and methods

Olives (*Olea europea* L.) from the Ottobratica variety grown in San Giorgio Morgeto (Latitude: 38°23′28"32 N; Longitude: 16°5′10"68 E) (Reggio Calabria, Italy) were collected at random using machinery. The fruits were placed in the usual HDPE (high-density polyethylene) drilled plastic boxes with a capacity of 40 lt. The oil extraction took place immediately using a mini-laboratory apparatus (Agrimec Valpesana, Calzaiolo, San Casciano, Florence, Italy), through the pressing of the olive paste. The mixing of the olive paste was executed for 40 min at ambient temperature. The maximum working pressure was 200 atm, which was reached in approximately 20 min. The extracted substance was then accurately separated from the wastewater.

Turmeric powder was purchased in a local market (Reggio Calabria, Italy). It was added (1% w/w) during olive paste malaxation to obtain tumeric FVOO (CM). At the same time, another enrichment procedure was applied through the infusion of turmeric powder (2% w/w) for 30 days in EVOO to obtain tumeric FVOO (CI). This procedure was conducted in a 500 mL flask, in the absence of light, at ambient temperature, which was carefully closed to prevent the entry of O_2 and under magnetic stirring. All samples (the control oil, EVOO; the one obtained by malaxation, CM; and the one obtained by infusion, CI) were stored for one year at the usual consumer condition (ambient temperature, in the dark, in 100 mL dark glass bottles).

Analyses were conducted at the following times: T0 (day of production); T15 (15 days after production); T30 (after 30 days); T60 (after 60 days); T180 (after 180 days); and T360 (after 360 days).

2.1. Extraction and analysis of the phenolic portion in turmeric powder

The extraction of turmeric powder was performed by an ultrasonic bath extractor (3800-CPXH; Branson, Milan, Italy) as previously described by Zlabur et al. [22]. Approximately 4 g of powder were placed in a tube with 250 mL of distilled water and placed into an ultrasonic bath. The extraction was conducted for 30 min at a pulse mode of 2 s on/4 s off and power of 30%. A temperature of 40 °C was maintained in the bath. The extract was separated by using a centrifigue Nüve NF 1200R (Saracalar Kümeevleri, Ankara, Turkey) at 8000 rpm for 10 min. The mixture was filtered with a Büchner funnel and kept at −4 °C until analysis.

The total phenolic content (TPC) was evaluated as previously described by Choi et al. [23]. The results were expressed as mg gallic acid equivalents (GAE) g^{-1}.

For the quantification of the total flavonoid content (TFC) 0.5 mL of turmeric extract was mixed with 2.5 mL of distilled water and 0.150 mL of $NaNO_2$ 5%. After 5 min 0.300 mL of $AlCl_3$ 10% were added and after a further 5 min 1 mL of NaOH 1 M was added. Finally, 0.550 mL of distilled water were added. After 15 min of incubation at room temperature, the absorbance was measured at 510 nm using an Agilent 8453 UV-VIS spectrophotometer (Agilent Technologies, Milan, Italy). Results are expressed as mg of rutin equivalents (RE) g^{-1}.

2.2. Chemical quality criteria of EVOO, CM and CI

Quality parameters, including free acidity (FA) and peroxide value (PV) were determined according to EEC Regulation [20]. FA was expressed as % of oleic acid whereas PV was expressed as mEq O_2 kg^{-1} [21]. CIELab colour parameters were measured using Konica Minolta CM-700d (Osaka, Japan) Results were reported as chroma (C*).

$$C* = \sqrt{a^2 + b^2}$$

2.3. Extraction of the phenolic portion of EVOO, CM and CI

For the extraction of the phenolic portion of EVOO (control extra virgin olive oil), CM (turmeric flavoured olive oil obtained by malaxation) and CI (turmeric flavoured olive oil obtained by infusion), the procedure of Montedoro et al. was applied [24]. Oils were mixed with a MeOH:H_2O (7:3 v/v) and treated with *n*-hexane. The residue was taken up with hydroalcoholic solution (1:1 v/v) and stored at −20 °C until analysis.

2.3.1. Analysis on the total phenolic content (TPC) and quantification of the individual phenols of EVOO, CM and CI

The total phenolic content (TPC) of EVOO, CM and CI was also determined spectrophotometrically, at 765 nm using the method previously described by Baiano et al. [25]. The quantification of the individual phenols was conducted by a UHPLC-DAD apparatus, coupled with a PDA-1 (photodiode array detector, PLATINblue), provided with a binary pump, a C18A column (1.8 μm, 100 mm × 2 mm), set to 30 °C and with the phenolic portion corresponding to an aliquot of 2 μL. The mobile phases was composed of H_2O suitable for UHPLC systems, acidified until pH 3.1 (by CH_3COOH) and CH_3CN, with a flow rate of 0.4 mL min^{-1}. The detector was set at 254, 280, 330, 350 and 450 nm wavelengths. For the quantification, external standards purchased from Merck (Darmstadt, Germany) were used and the results were expressed as mg kg^{-1} [26].

2.3.2. α-Tocopherol evaluation of EVOO, CM and CI

The oils were mixed with 2-propanol (1:10 v/v), the upper phase was collected and filtered using a nylon syringe filter (0.45 μm pore size). Sample (5 μL) was injected into an UHPLC-DAD apparatus coupled with a fluorescence detector RF-20A/RF-20Axs model (Shimadzu Corporation, Kyoto, Japan) with a flow rate of 0.5 mL min^{-1}. The mobile phase was CH_3OH:CH_3CN (50:50). The detector was set at a 290 nm excitation wavelength and a 330 nm emission wavelength. The identification and quantification were performed by calibration curve, using pure α-tocopherol, and results were expressed as mg kg^{-1} of oil [26].

2.3.3. Antioxidant activity

The ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) test was applied to investigate the radical scavenging ability of the samples using a previously described procedure [27]. The absorbance was measured at 734 nm.

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay was applied using the previously described procedure [27]. Ascorbic acid was used as the positive control in both radical scavenging assays.

The β-carotene bleaching test was done following the previously described procedure [27]. The absorbance was read at λ = 470 nm.

2.3.4. Carbohydrate hydrolysing enzyme and pancreatic lipase inhibitory activity

The α-amylase and α-glucosidase inhibitory activity of EVOO and flavoured oils were determined using the method of Sicari et al. [27]. The absorbances were read at 540 nm.

Pancreatic lipase inhibitory activity was determined as previously described using orlistat as a positive control [27].

2.4. Evaluation of the sensory characteristics of EVOO, CM and CI

The sensory evaluation was conducted by a trained group of seven judges, in accordance with the current legislation and according to the internal regulations of the department. All the panellists were previously informed about the ingredients they tasted. The judging took place using the profile of an extra virgin olive oil with additional attributes from turmeric, according to a 9-point scale where 1 is absent and 9 is extremely perceptible. To describe the sensory characteristics of EVOO, CM and CI, a quantitative descriptive analysis (QDA) was performed. The obtained results were represented with graphical spider plots using Microsoft Office Excel 2014.

The judges evaluated the olfactory and gustatory sensations, moreover they also judged the visual characteristics.

2.5. Statistical analysis

Samples were analysed in triplicate. Analytical data was reported as means ± standard deviation. The analysis of variance (one-way ANOVA) was conducted by applying the post hoc Tukey test (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, not significant at $p > 0.05$.

3. Results and discussion

3.1. Turmeric extract

Turmeric extract (TE) was analysed to evaluate the total phenols and flavonoids content (TPC and TFC, respectively), the antioxidant and the inhibitory activity against key enzymes involved in the sugar and lipid metabolism such as α-amylase, α-glucosidase, and lipase. A TPC value of 29.65 mg GAE g^{-1} was found. This value is strongly affected by the drying method used to make the powder. In fact, Cumroemphat et al. [28] highlighted the differences between the freeze-dried, the hot-air dried and the sun-dried methods, finding the highest values for the freeze-dried sample with 35.7 mg GAE g^{-1}, followed by the hot-air dried sample with 30.5 mg GAE g^{-1}. Concerning the TFC, our data (17.41 mg RE g^{-1}) agrees with those reported by Cumroemphat et al. [28], who found that the TFC values of fresh turmeric correspond to 36 mg RE g^{-1}, while, regardless of the drying process used, the values are around 20 mg RE g^{-1}. A promising radical scavenging activity was observed using TE in both DPPH and ABTS test with half maximal inhibitory concentration (IC_{50}) values of 19.42 and 3.14 μg mL^{-1} respectively, whereas in the β-carotene bleaching test, TE showed a good protective activity against lipid peroxidation with IC_{50} value of 17.06 μg mL^{-1}.

A moderate activity against α-amylase and α-glucosidase was recorded with TE with IC_{50} values of 250.20 and 249.28 μg mL^{-1}, respectively. This data is about 5- and 7- times, higher than the acarbose used as positive control (50.18 and 35.57 μg mL^{-1}, respectively). A similar trend was observed against pancreatic lipase assay in which TE reached a value of IC_{50} correspondent to 228.56 μg mL^{-1}.

Previously, Al-Lahham et al. [29] found IC_{50} values of 69, 50, and 9 μg mL^{-1} in α-amylase, α-glucosidase, and lipase assay, respectively [29].

3.2. Chemical quality criteria of EVOO, CM and CI

The free acidity value (FA) expressed as % of oleic acid, decreased significantly ($p < 0.01$) especially in the CM sample (turmeric flavoured olive oil obtained by 1% malaxation) at T0 (0.68 *vs* 0.53% for the EVOO (control olive oil) and CM samples, respectively). In the CI (turmeric flavoured olive oil obtained by 2% infusion) sample FA recorded values comparable to EVOO for the whole duration of storage (i.e. at T0 0.68 and 0.67% for the EVOO and CI samples, respectively). At the end of storage, CM and CI showed lower percentages in free acidity than the control (0.84 *vs* 0.65 and 0.76% for the EVOO, CM and CI samples, respectively). However, in all of them, at T0 the FA values remained below the 0.80% fixed by European Union Commission to be classified as extra virgin olive [21]. Very controversial data emerged from the analysis of the scientific literature. Caporaso et al. [30] found values of 1.20 and 1.60% after an infusion of 30 days with chilli pepper at 10 and 20% into olive oil, respectively. Instead, Clodoveo et al. [31], underlined how different technological approaches used, generated different % of FA. For the same enrichment matrix (thyme) they found 0.32, 0.29 and 0.34% when the matrix was added by infusion, by malaxation or by sonication of olive paste added with matrix (Supplementary Table S1 a). The known antioxidant potential of turmeric probably expressed its effect decreasing the free acidity value when mixed with olive paste. In another study of an olive oil enriched with *Citrus bergamia* fruits, despite the highest antioxidant properties of this fruit, the acidity of this matrix caused a strong increase in these values [32]. Therefore, in our case, this decrease is also probably due to the strong affinity between turmeric and the oily matrix [19].

The peroxide value (PV) during storage increased significantly in all the samples ($p < 0.01$). As well as the FA, the CM sample possessed the lowest value at T0 (9.45 *vs* 6.14 mEq O_2 kg^{-1} for EVOO and CM, respectively) and at T360 (9.61 mEq O_2 kg^{-1}). As opposed to the CI which after the conservation period, reached values even higher than the control (22.88 *vs* 17.89 mEq O_2 kg^{-1}) (Supplementary Table S1 b).

The addition of turmeric did not significantly ($p > 0.05$) affect the lightness (L*) parameter of the enriched oils. Values responsible for the red-green colour (a*) show a slight decrease in CI 3.00 *vs* 3.42 EVOO. As expected, the parameters that describe the yellow-blue colour (b*) increased in the CI sample 2.49 and decreased in CM 2.01 sample, compared to the control (2.24). Ayadi, Grat-Kamoun and Attia [33] enriched olive oil with seven different Tunisian spices and observed that only the enrichment with thyme caused significant changes in the colorimetric parameters. On the other hand, Ammar et al. [34] enriched an olive oil with prickly pear flowers and observed a slight decrease in L* and b* values and a slight increase in a*. During storage, as expected, the L* value increased and a* and b* decreased. Particularly a* parameters in the CM sample reached negative values starting from 6 months of storage (−0.16) against 0.03 and 0.15 of the CI and EVOO samples, respectively. The Chroma (C*) is the parameter most influenced by the addition. In the unflavoured sample C* values of 7.23 *vs* 6.38 and 6.79 were recorded for the CI and CM samples. Therefore, the EVOO colour is darker and less bright than the CI and CM samples (Supplementary Table S2).

3.3. TPC and α-tocopherol content in EVOO, CM and CI

A great variability in TPC was observed as reported in Fig. 1. Generally, a great variability of TPC is recorded in relation to the olive cultivar, stage of maturity, climatic conditions, area of growth.

Di Lecce et al. [35] studying 11 Italian mono-varietal extra virgin olive oils stated that this diversity is only due to factors related to the genetic background of the olive cultivar under study. CM flavoured oil showed a lower TPC even compared to the control. On the contrary CI

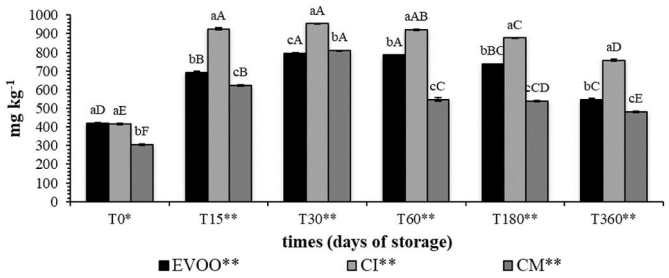

Fig. 1. Total phenolic content (TPC) during one year of storage. Values are expressed as mg kg^{-1}. Data is expressed as means ± S.D. (n = 3)
EVOO: control; CI: turmeric flavoured olive oil obtained by 2% infusion; CM: turmeric flavoured olive oil obtained by 1% malaxation. Differences between samples were evaluated by one-way ANOVA followed by Tukey's post-hoc test. The capital letters indicate the differences in one sample in one year of storage. The lowercase letters i indicate the differences among the samples at the same time of analysis. *$p \leq 0.05$; **$p \leq 0.01$.

exhibited the highest TPC even at the end of the storage (757.88 mg kg^{-1}, about 57% more than CM and 38% more than EVOO. This reduction it could be related to the high temperatures and time of processing of the malaxation of the olive paste mixed with turmeric powder exposing the olive paste to a greater quantity of oxygen and a greater loss of the polyphenols [36]. In the scientific literature there is some contradictory information. Soaeres et al. [36] enriched an extra virgin olive oil with rosemary and basil leaves and found for the same operating conditions a lower level in the basil flavoured olive oil than in the control (135 *vs* 179 mg kg^{-1}) in contrast to the rosemary flavoured olive oil in which higher levels than the control was recorded (188 *vs* 179 mg kg^{-1}). Also, Sousa et al. [37] used many matrices of enrichment and observed how the TPC content is highly variable and influenced by the single matrix, and probably by the phenolic patterns of each one. α-Tocopherol is a molecule rarely present in nature, with high beneficial properties for human health, of which extra virgin olive oil is generally the main natural source. It allows for greater oxidative stability of the oil and is particularly sensitive to sources of light and heat [38]. It is of paramount importance to maintain a good level of a α-tocopherol even in flavoured olive oils. Previously, Sousa et al. [37] found a variable α-tocopherol content depending on the enrichment matrix used varying between a minimum of 174.6 (enrichment with laurel) and a maximum of 191 mg kg^{-1} (enrichment with hot chilli) respect the control EVOO 181 mg kg^{-1}. In our case, the addition influences this content. At T0 the levels of CI and EVOO are very similar, otherwise CM presents a very low level (354.63, 350.01 and 176.56 mg kg^{-1} for EVOO, CI and CM, respectively). During the storage period the CM levels increase is probably due to a greater solubilisation of this molecule, until reaching values at T30 similar to the other samples (234.22, 253.60 and 218.99 mg kg^{-1} for EVOO, CI and CM, respectively). Starting from T180 in CM there is a substantial decrease and at the end of storage only CI maintains the highest levels of α-tocopherol (79.53, 84.55 and 59.2 mg kg^{-1} for EVOO, CI and CM, respectively) (Table 1). Similarly to what was observed in the TPC, treating the olive paste with turmeric powder could also have caused greater degradation of α-tocopherol. Since these molecules are very susceptible to light and heat, the malaxation time to which the olive paste was subjected to promote a greater transfer of bioactive compounds from the matrix to the oil could not have had the desired effect, causing an increase in temperature, as well as greater exposure of the paste to oxygen, thus causing easier degradation of α-tocopherol.

3.4. UHPLC analysis of the phenolic portion of EVOO, CM and CI

Turmeric is rich in curcumin, bisdemethoxycurcumin and demethoxycurcumin. These curcuminoids are hydrophobic molecules responsible for the typical yellowish colour of the rhizome [39]. Moreover, these compounds are known for their high antioxidant potential [22,23,39,40].

TE was characterised by 3590.81, 6385.1 and 10054.7 mg kg^{-1} of bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively (Supplementary Table S3). Cheng-Chao et al. [39] studied 12 samples of Chinese rhizomes and found values ranging between 2.76 and 5.83 mg g^{-1} for bisdemethoxycurcumin, 2.64–7.60 of mg g^{-1} for demethoxycurcumin and 10.16–16.48 mg g^{-1} for curcumin. Differently, Pal et al. [40] evaluated the curcuminoid content in 45 Indian genotypes and estimated that the genotype TCP 2 possessed the highest content with values of 10000, 19800 and 25900 mg kg^{-1} for bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively. This demonstrates the huge variety stemming from the genotypes, the cultivation areas, and the treatments to which the rhizomes are subjected.

CM and CI showed a curcuminoid content which increased in the first 30 days of storage and then decreased after 12 months (Tables 2 and 3). CM at T0 and T360 presented values of 2546.09–1446.45, 3469.76–4660.58 and 4171.60–4540.14 mg kg^{-1} of bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively. In contrast CI at T0 presented values of 1901.42–1994.44, 3624.93–5411.02 and 7285.50–8378.33 mg kg^{-1} of bisdemethoxycurcumin, demethoxycurcumin and curcumin, respectively. Among our samples, the two different technological approaches caused very different and variable trends in the curcuminoids content ($p < 0.01$). In fact, when the matrix was added during malaxation, in which water is still present because the olive drupes are naturally rich in water, due to the curcuminoids' hydrophobic quality, their content is significantly lower in CM. Conversely, when turmeric was added by infusion obviously after the oil had already been filtered and in the absence of water, their content is higher. In addition, the curcumin content in CI was significantly higher in all storage phases than CM. This condition means that infusion gives a higher recovery of these compounds than malaxation. From the analysis of the data, it can be stated that CI, at the end of storage, maintained a greater content of curcuminoids compared to CM. However, regarding antioxidant activity, the situation is the opposite (see paragraphs 3.5). In fact, after 12 months of storage, the DPPH and ABTS values were higher in CM than in CI. This study has allowed us to confirm what was previously stated by Pal et al. [39], namely that the antioxidant activity of turmeric is not due to the presence of curcuminoids alone, but that there are probably other bioactive that contribute to it.

Table 1
α-Tocopherol content of EVOO, CI and CM (values are expressed as mg kg^{-1}) during one year of storage.

	T0	T15	T30	T60	T180	T360	Sign
α-Tocopherol							
EVOO	354.63 ± 5.63^{aA}	261.63 ± 5.96^{aB}	234.22 ± 4.72^{bB}	223.72 ± 5.15^{aB}	246.61 ± 5.72^{aB}	79.53 ± 1.41^{bC}	**
CI	350.01 ± 5.1^{aA}	252.2 ± 5.12^{aB}	253.6 ± 2.20^{aB}	225.13 ± 5.60^{aC}	218.44 ± 4.1^{bC}	84.55 ± 1.44^{aD}	**
CM	176.56 ± 3.18^{bD}	223.93 ± 4.54^{bA}	218.99 ± 4.18^{cA}	203.13 ± 4.91^{bB}	165.81 ± 2.62^{cC}	59.2 ± 2.23^{cE}	**
Sign	*	*	**	**	**	**	

EVOO: control; CI: turmeric flavoured olive oil obtained by 2% infusion; CM: turmeric flavoured olive oil obtained by 1% malaxation. Differences between samples were evaluated by one-way ANOVA followed by Tukey's post-hoc 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. *$p \leq 0.05$; **$p \leq 0.01$.

Table 2
Quantification of phenols in turmeric flavoured olive oil by infusion sample (CI) during one year's storage. Values are expressed as mg kg^{-1}.

	T0	T15	T30	T60	T180	T360	Sign
Hydroxityrosol	16.15 ± 1.54^{b}	7.37 ± 1.04^{d}	9.27 ± 0.22^{c}	8.26 ± 0.04^{c}	14.67 ± 0.94^{b}	26.17 ± 1.75^{a}	**
Tyrosol	15.61 ± 2.03^{ab}	12.84 ± 0.37^{b}	15.22 ± 2.46^{ab}	14.41 ± 1.01^{ab}	18.44 ± 0.78^{a}	14.21 ± 0.69^{ab}	*
4-Hydroxyphenyl acetate	0.00^{b}	0.00^{b}	2.30 ± 0.75^{a}	0.00^{b}	0.00^{b}	0.00^{b}	*
Chlorogenic acid	1.92 ± 0.19^{c}	8.64 ± 0.19^{a}	6.97 ± 0.01^{c}	7.52 ± 0.08^{b}	0.00^{d}	0.00^{d}	**
Caffeic acid	0.00^{b}	0.00^{b}	4.27 ± 0.01^{a}	0.00^{b}	0.00^{b}	0.00^{b}	*
Vanillic acid	1.47 ± 0.01^{a}	1.30 ± 0.12^{a}	0.19 ± 0.03^{b}	0.00^{b}	0.00^{b}	0.00^{b}	**
Homovanillic acid	1.92 ± 0.01^{b}	7.55 ± 0.73^{a}	1.81 ± 0.13^{b}	1.88 ± 0.06^{b}	1.91 ± 0.10^{b}	0.00^{c}	**
Quercetin 3,4′-diglucoside	0.91 ± 0.07^{c}	2.50 ± 0.22^{a}	0.50 ± 0.04^{d}	0.47 ± 0.01^{d}	0.56 ± 0.11^{d}	1.10 ± 0.04^{b}	**
Ferulic acid	0.00^{b}	0.00^{b}	0.54 ± 0.01^{a}	0.00^{b}	0.00^{b}	0.00^{b}	*
Rutin	0.00^{d}	3.26 ± 0.11^{b}	3.22 ± 0.10^{b}	2.79 ± 0.22^{b}	4.37 ± 0.33^{a}	0.62 ± 0.09^{c}	**
Luteolin-7-*O*-glucoside	3.07 ± 0.91^{d}	15.90 ± 0.28^{a}	3.45 ± 0.15^{b}	3.33 ± 0.11^{b}	3.88 ± 0.50^{b}	3.28 ± 0.25^{b}	**
Oleoropein	0.48 ± 0.08^{a}	0.23 ± 0.00^{b}	0.25 ± 0.01^{b}	0.27 ± 0.05^{b}	0.21 ± 0.05^{bc}	0.12 ± 0.03^{c}	**
Cinnamic acid	0.91 ± 0.36^{bc}	2.49 ± 0.31^{a}	0.66 ± 0.05^{c}	0.60 ± 0.04^{c}	0.72 ± 0.10^{c}	1.16 ± 0.21^{b}	**
Quercetin	12.94 ± 0.55	10.80 ± 0.87	13.18 ± 0.17	12.52 ± 1.78	10.99 ± 1.04	11.93 ± 1.38	ns
Pinoresinol	43.38 ± 0.36^{a}	36.62 ± 3.19^{ab}	44.51 ± 0.12^{a}	43.68 ± 1.96^{ab}	35.22 ± 8.89^{b}	39.19 ± 2.41^{ab}	*
Kaempferol	0.00^{b}	2.43 ± 0.31^{a}	2.56 ± 0.14^{a}	2.47 ± 0.02^{a}	0.00^{b}	0.00^{b}	**
Isoramnetin	0.00^{d}	4.89 ± 0.39^{c}	11.22 ± 0.47^{ab}	11.30 ± 1.48^{a}	9.44±±1.01^{ab}	8.56 ± 2.62^{bc}	**
Apigenin	58.98 ± 11.81^{a}	15.98 ± 1.75^{b}	8.41 ± 0.21^{c}	8.49 ± 0.00^{c}	7.80 ± 0.37^{cd}	1.96 ± 0.55^{d}	**
Apigenin-7-*O*-glucoside	1.80 ± 0.30^{c}	0.00^{b}	0.00^{b}	0.27 ± 0.05^{a}	0.00^{b}	0.00^{b}	**
Bisdemetoxycurcumin	0.00^{e}	1901.42 ± 3.41^{d}	2346.09 ± 11.48^{a}	2186.37 ± 10.98^{b}	2007.78 ± 5.33^{bc}	1994.44 ± 8.76^{c}	*
Demetoxycurcumin	0.00^{f}	3624.93 ± 6.89^{e}	4442.76 ± 9.94^{c}	4001.96 ± 9.43^{d}	4675.80 ± 11.19^{b}	5411.02 ± 8.88^{a}	**
Curcumin	0.00^{d}	7285.50 ± 5.05^{c}	8935.9 ± 12.51^{a}	8112.11 ± 11.04^{b}	7455.52 ± 9.94^{bc}	7285.50 ± 9.62^{c}	*

Data is expressed as means ± S.D. (n = 3). Differences between samples were evaluated by one-way ANOVA followed by Tukey's post-hoc test. The letters indicate the differences in one sample in one year of storage. *$p \leq 0.05$; **$p \leq 0.01$, ns, not significant at $p > 0.05$.

Table 3
Quantification of phenols in turmeric flavoured olive oil by malaxation sample (CM) during one year of storage. Values are expressed as mg kg^{-1}.

	T0	T15	T30	T60	T180	T360	Sign
Hydroxityrosol	29.79 ± 1.32^{c}	35.51 ± 0.63^{ab}	38.19 ± 0.91^{ab}	41.72 ± 1.13^{b}	36.16 ± 4.12^{ab}	70.12 ± 4.51^{a}	**
Tyrosol	40.41 ± 3.04^{d}	49.89 ± 0.63^{c}	41.76 ± 1.79^{d}	59.69 ± 2.72^{b}	27.93 ± 2.60^{e}	217.45 ± 8.41^{a}	**
4-Hydroxyphenyl acetate	0.00^{c}	1.78 ± 0.12^{b}	5.10 ± 1.20^{a}	5.26 ± 0.04^{a}	4.23 ± 1.27^{a}	0.00^{c}	**
Chlorogenic acid	0.00^{d}	4.79 ± 0.05^{c}	10.69 ± 0.50^{b}	12.17 ± 0.38^{a}	9.90 ± 0.33^{b}	0.00^{c}	**
Caffeic acid	0.00^{c}	0.72 ± 0.03^{bc}	0.69 ± 0.03^{bc}	1.47 ± 0.12^{b}	4.41 ± 0.85^{a}	0.00^{c}	**
Epicatechin	0.00^{e}	1.50 ± 0.04^{c}	0.80 ± 0.01^{d}	0.66 ± 0.02^{d}	5.15 ± 0.46^{b}	6.72 ± 0.10^{a}	**
Syringic acid	0.12 ± 0.01^{c}	0.23 ± 0.01^{a}	0.04 ± 0.00^{d}	0.19 ± 0.02^{b}	0.00^{e}	0.00^{e}	**
Vanillin	1.85 ± 0.03^{c}	2.32 ± 0.03^{a}	2.04 ± 0.08^{b}	1.72 ± 0.06^{c}	0.00^{d}	0.00^{d}	**
Homovanillic acid	2.95 ± 0.07^{bc}	3.61 ± 0.14^{a}	3.32 ± 0.25^{ab}	2.59 ± 0.43^{c}	0.00^{d}	0.00^{d}	**
Quercetin 3,4′-diglucoside	6.94 ± 0.06^{b}	8.09 ± 0.29^{a}	5.00 ± 0.09^{c}	5.17 ± 0.34^{c}	5.46 ± 0.51^{c}	6.84 ± 0.29^{ab}	**
Ferulic acid	0.33 ± 0.01^{c}	0.36 ± 0.01^{bc}	0.34 ± 0.01^{bc}	0.37 ± 0.02^{b}	0.53 ± 0.02^{a}	0.00^{d}	**
Rutin	1.74 ± 0.08^{b}	1.94 ± 0.13^{a}	0.78 ± 0.03^{cd}	0.67 ± 0.01^{d}	0.75 ± 0.04^{cd}	0.93 ± 0.10^{cd}	**
Luteolin-7-*O*-glucoside	2.64 ± 0.13^{b}	3.21 ± 0.10^{a}	2.78 ± 0.33^{b}	2.93 ± 0.12^{ab}	0.00^{c}	0.00^{c}	**
Oleoropein	0.10 ± 0.01^{b}	0.11 ± 0.01^{b}	0.09 ± 0.00^{b}	0.11 ± 0.00^{b}	0.25 ± 0.01^{a}	0.25 ± 0.02^{a}	**
Quercetin	5.94 ± 0.16^{b}	6.00 ± 0.50^{b}	8.40 ± 0.75^{b}	9.37 ± 1.41^{b}	7.28 ± 0.90^{b}	14.97 ± 3.89^{a}	**
Luteolin	1.45 ± 0.01^{c}	1.17 ± 0.09^{c}	1.54 ± 0.13^{bc}	2.17 ± 0.12^{ab}	2.22 ± 0.36^{a}	2.44 ± 0.48^{a}	**
Pinoresinol	15.04 ± 0.60^{cd}	17.52 ± 0.96^{ab}	15.18 ± 0.80^{bc}	18.17 ± 0.28^{b}	14.27 ± 1.04^{d}	45.82± 4.87^{a}	**
Kaempferol	3.89 ± 0.04^{cd}	3.21 ± 0.10^{d}	6.27 ± 0.46^{c}	6.71 ± 0.11^{c}	16.78 ± 0.26^{a}	12.65 ± 1.22^{d}	**
Isoramnetin	2.51 ± 0.06^{b}	3.93 ± 0.18^{a}	2.81 ± 0.35^{ab}	3.76 ± 0.22^{ab}	4.08 ± 0.01^{a}	3.43 ± 0.75^{ab}	**
Apigenin	2.97 ± 0.05^{cd}	2.64 ± 0.20^{d}	4.57 ± 0.42^{ab}	5.58 ± 0.03^{b}	9.57 ± 1.57^{a}	0.00^{e}	**
Apigenin-7-*O*-glucoside	0.91 ± 0.05^{b}	1.26 ± 0.05^{a}	1.14 ± 0.03^{a}	1.19 ± 0.05^{a}	0.25 ± 0.01^{c}	0.00^{d}	**
Bisdemetoxycurcumin	2546.09 ± 8.7^{c}	2503.11 ± 10.2^{c}	4630.44 ± 13.9^{a}	4502.54 ± 9.2^{a}	4105.13 ± 13.4^{b}	1446.45 ± 10.1^{d}	**
Demetoxycurcumin	3469.76±7^{e}	3472.9 ± 9.9^{e}	6223.82 ± 14.7^{b}	6082.23 ± 12.3^{c}	6886.25 ± 10.9^{a}	4660.58 ± 5.6^{d}	**
Curcumin	4171.60 ± 10.3^{d}	5008.12 ± 12.3^{c}	8182.91 ± 4.8^{b}	8007.48 ± 9.4^{bc}	8893.52 ± 12.^{a}	4540.14 ± 8.4^{cd}	*

Data is expressed as means ± S.D. (n = 3). Differences between samples were evaluated by one-way ANOVA followed by Tukey's post-hoc test. The letters indicate the differences in one sample in one year of storage. *$p \leq 0.05$; **$p \leq 0.01$.

3.5. Antioxidant activity

Fig. 2 (a and b) and Fig. 3 show the antioxidant potential of EVOO and tumeric flavoured olive oils (CM and CI). The control exhibited interesting activities in both DPPH and ABTS tests with IC_{50} of 12.33 and 3.43 μg mL^{-1}, respectively at T0. At the end of the storage, the control slightly lost its antioxidant potential, reaching values of 29.54 and 15.21 μg mL^{-1}, in DPPH and ABTS tests, respectively. Previously, Baiano et al. [25] evaluated the ABTS radical scavenging activity of different Italian extra virgin olive oils and found the following rank of potency Coratina > Peranzana > Cima di Melfi, Nociara, Leccino > Frantoio, Moraiolo at day of production. In is interesting to note that this rank changed after 6 months storage (Coratina > Peranzana, Cima di Melfi > Fran-toio, Leccino, Moraiolo > Nociara). This evidence is probably the consequence of the phenolic profile changes occurred during storage. Whereas, Sicari [41] found DPPH and ABTS values of 27.37 and 2.52% Ottobratica EVOO harvested in the same cultivation area.

A promising radical scavenging activity was observed with turmeric flavoured oils with IC_{50} values of 9.49 and 9.48 μg mL^{-1} for CI and CM, respectively in DPPH test, and 3.47 and 3.49 μg mL^{-1} for CI and CM, respectively in ABTS test at T0, without significant differences from control sample (EVOO) at the day of production ($p > 0.05$), in both tests. Moreover, CM flavoured oil in DPPH test maintained its potential as a radical scavenger also after storage, with an IC_{50} of 11.36 μg mL^{-1} after 360 days storage. On the contrary CI reached values almost equal to the

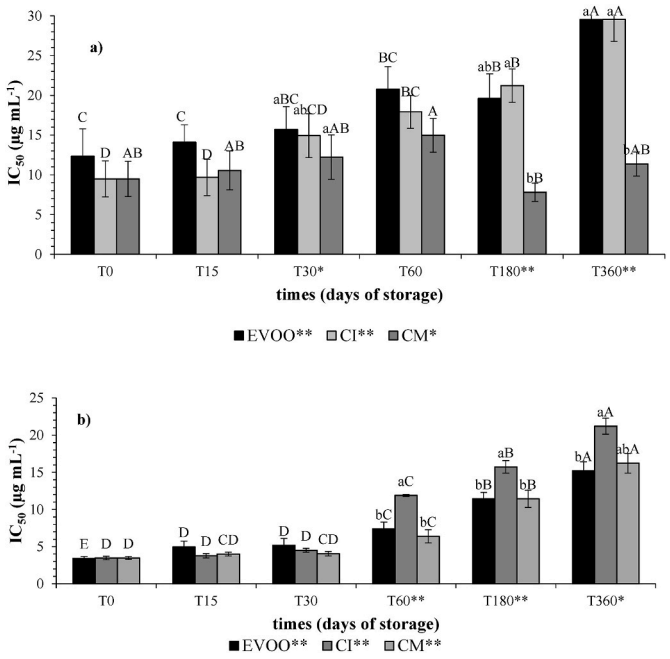

Fig. 2. Antioxidant activity of EVOO, CI and CM against DPPH (Fig. 2a) and ABTS (Fig. 2b) (values are expressed as IC_{50} µg mL^{-1}) during one year of storage

Data is expressed as means ± S.D. (n = 3). EVOO: control; CI: turmeric flavoured olive oil obtained by 2% infusion; CM: turmeric flavoured olive oil obtained by 1% malaxation. Ascorbic acid was used as positive control in both tests (IC_{50} (half maximal inhibitory concentration) values of 5.03 ± 0.82 and 1.78 ± 0.07 µg mL^{-1}, respectively). Differences between samples were evaluated by one-way ANOVA followed by Tukey's post-hoc test. The capital letters indicate the differences in one sample in one year of storage. The lowercase letters indicate the differences among the samples at the same time of analysis. *$p \leq 0.05$; **$p \leq 0.01$; ns, not significant at $p > 0.05$.

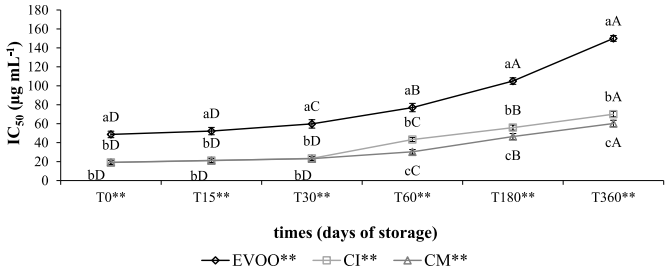

Fig. 3. β-carotene bleaching test, expressed as IC_{50} (µg mL^{-1}) during one year of storage. Data is expressed as means ± S.D. (n = 3).

control (29.55 µg mL^{-1}) at the end of the period of observation. Correlation analysis showed that TPC value was slightly positively correlated with DPPH test with value of 0.32 *vs* 0.19, for CM and CI sample, respectively, conversely to Karacabey et al. [42] who found strong relation between TPC and radical scavenging activity. The addition of matrices does not always improve the antioxidant activity as expected and the positive effect of those additions could be appreciable at the long term [37]. Moreover, in the ABTS test, both CM and CI reached values even higher than the control EVOO (21.21, 16.22 for the CI and CM samples, respectively *vs* 15.21 µg mL^{-1}) at T360. Our results are in agreement with those reported by Sousa et al. [37] who observed very similar values between the control and its related flavoured olive oils and the additions did not improve the activity on the ABTS test, even showing the control olive oil greater activity. Our results are in agreement with those found by Loizzo et al. [43] that evidenced how the infusion process is a valuable approach to obtain flavoured olive oils with and increase radical scavenging potential. In fact, in this works, authors enriched Carolea extra virgin olive oil by the infusion of different *Capsicuum annuum* and *C. chinense*. The flavoured oil infused with Aji limo dry powder resulted the most active in DPPH test with IC_{50} value of 11.8 µg mL^{-1}. This value is 2-times higher than that found for Carolea oil (IC_{50} value of 26.8 µg mL^{-1}). A similar observation was done also in ABTS test. Moreover, Clodoveo et al. [31] evidenced that oils obtained by infusion process with thyme (TI) and oregano (OI) the radical scavenging potential increased significantly by +60% and +33% respect to the control oil, respectively. A similar trend was observed when thyme and oregano spices were added to the olive paste during the extraction process, before the malaxation. In this case the resulting oils (TM and OM) showed an improvement of DPPH radical scavenging potential respect to the infused ones (TI and OI) equal to about 2- and 4-times, respectively.

Positive correlations were also found between this antioxidant potential and the amount of demethoxycurcumin with *Pearson*'s correlation coefficient of 0.72 and 0.65 in DPPH and ABTS, respectively. This demonstrates how this compound, compared to the other two curcuminoids, is the main player in acting as an antioxidant in our flavoured oils.

In the co-processing or infusion with turmeric powder, lead samples were characterised by a high antiradical activity. The protection from lipid peroxidation was evaluated through β-carotene bleaching test. In Fig. 3 it is possible to observe how storage time reduces the protection from lipid peroxidation. EVOO already possessed a poor initial activity and completely lost it starting from 180 days of storage (from 48.72 to > 100 µg mL^{-1} at T0 to T360). Instead, a great potential in protection from lipid peroxidation was observed with both turmeric enrichment processes (malaxation and infusion). The starter values correspond to 19.11 and 19.20 µg mL^{-1} for CI and CM respectively. Until T30, CM and CI maintained very close values. After that, CI started to lose this activity much more than CM, reaching a value at T360 of 70.21 *vs* 60.21 µg mL^{-1} for CI and CM, respectively. Comparing our results with those obtained by Custureri et al. [26] with an enrichment with ginger, by malaxation and infusion approaches, the protection against lipid peroxidation is greater than that of the EVOO, with values approximately 1.3-times higher in the case of the sample obtained by malaxation and 1.5-times lower when comparing the sample obtained by infusion process. This evidence could be explained by the positive correlation existing between curcuminoids and β-carotene bleaching test, especially for desmethoxycurcumin. A similar situation was observed also in β-carotene bleaching test where desmethoxycurcumin resulted positive correlated with CI and CM with *Pearson*'s correlation coefficient of 0.67 *vs* 0.32, respectively.

EVOO: control; CI: turmeric flavoured olive oil obtained by 2% infusion; CM: turmeric flavoured olive oil obtained by 1% malaxation. Propyl gallate was used as positive control (IC_{50} (half maximal inhibitory concentration) values of 1.02 ± 0.01 µg mL^{-1}). Differences between samples were evaluated by one-way ANOVA followed by Tukey's post-hoc test. The capital letters indicate the differences in one sample in one year of storage. The lowercase letters the differences among the samples at the same time of analysis. **$p \leq 0.01$.

Previously, Plastina et al. [44] demonstrated that the addition of different cultivars of dried chilli pepper for infusion resulted in a protective effect against induced oil oxidation. In particular, the flavoured olive oil obtained by the addition of Bishop crown dried pepper to Roggianella EVOO was characterized by the best performance.

3.6. Carbohydrate hydrolysing enzyme and pancreatic lipase inhibitory activity

Table 4 reported data on the inhibition of α-amylase, α-glucosidase, and lipase by EVOO and flavoured olive oil enriched with turmeric by malaxation and infusion processes (CM and CI, respectively).

Table 4
Carbohydrate hydrolysing enzyme and pancreatic lipase inhibitory effect. Values are expressed as IC_{50} (μg mL^{-1}).

	T0	T15	T30	T60	T180	T360	Sign
α-Amylase							
EVOO	269.02 ± 3.77^{bE}	275.21 ± 3.85^{D}	303.38 ± 3.92^{cB}	345.31 ± 4.05^{cA}	240.29 ± 3.87^{cF}	289.32 ± 4.90^{cC}	**
CI	322.85 ± 3.56^{aD}	327.90 ± 3.81^{D}	369.90 ± 3.44^{aC}	501.53 ± 3.35^{aB}	507.11 ± 3.09^{aB}	777.09 ± 6.95^{aA}	**
CM	320.42 ± 3.01^{aCD}	325.29 ± 3.56^{D}	364.44 ± 3.74^{bBC}	417.75 ± 3.85^{bAB}	476.84 ± 3.96^{bAB}	676.21 ± 5.01^{bA}	**
Sign	**	ns	**	**	**	**	
α-Glucosidase							
EVOO	137.34 ± 3.73^{bF}	145.18 ± 3.79^{bE}	198.81 ± 3.82^{D}	337.56 ± 3.90^{aC}	587.49 ± 3.56^{aB}	778.23 ± 4.67^{aA}	**
CI	181.99 ± 3.45^{aB}	184.67 ± 3.21^{aB}	201.50 ± 3.09^{B}	226.74 ± 3.01^{cB}	236.98 ± 1.10^{bA}	489.70 ± 4.07^{bA}	**
CM	181.98 ± 2.09^{aD}	184.09 ± 3.67^{aD}	199.77 ± 3.89^{C}	216.7 ± 4.18^{bB}	233.37 ± 4.23^{cAB}	409.22 ± 4.70^{cA}	**
Sign	**	**	ns	**	**	**	
Pancreatic lipase							
EVOO	143.46 ± 4.85^{bF}	155.52 ± 4.87^{bE}	173.43 ± 4.91^{cD}	206.54 ± 5.01^{C}	253.81 ± 4.81^{cB}	312.97 ± 5.44^{cA}	**
CI	413.94 ± 4.09^{aD}	419.23 ± 4.01^{aD}	474.42 ± 4.22^{aC}	552.05 ± 4.02^{B}	573.11 ± 5.22^{aAB}	823.44 ± 6.55^{aA}	**
CM	410.99 ± 3.10^{aD}	418.80 ± 4.16^{aD}	458.10 ± 4.34^{bC}	504.08 ± 4.11^{B}	546.59 ± 4.01^{bAB}	721.56 ± 4.25^{bA}	**
Sign	**	**	**	ns	**	**	

Data is expressed as means ± S.D. (n = 3). EVOO: control; CI: turmeric flavoured olive oil obtained by 2% infusion; CM: turmeric flavoured olive oil obtained by 1% malaxation. Acarbose was used as positive control in the α-amylase and in the α-glucosidase assays (IC_{50} (half maximal inhibitory concentration) values of 50.18 ± 1.32 and 35.57 ± 0.99 μg mL^{-1}, respectively). Orlistat was used as positive control in the lipase assay (IC_{50} value of 37.44 ± 1.08 μg mL^{-1}). Differences between samples were evaluated by one-way ANOVA followed by Tukey's post-hoc 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. $^{**}p \leq 0.01$; ns, not significant at $p > 0.05$.

Independently from the applied processes, extracts can inhibit enzymes in a concentration-dependent manner. The inhibitory effects of EVOO are highly variable among its varieties and research groups have highlighted how the cultivation area also influences this activity. In fact, Leporini et al. [45] evaluated the ability of EVOOs from Frantoio cultivar harvested in different area of Calabria region and found IC_{50} values ranging from 57.7 to 123.7 μg mL^{-1} in the α-amylase assay, and from 65.6 to 167.7 μg mL^{-1}in α-glucosidase test, assessing as the most active the oils was obtained from drupes harvested in the area of Vaccarrizzo Albanese and as the least active those from the Montalto Uffugo (Calabria, Italy). In addition, Loizzo et al. [46] evaluated, a variety of EVOO from Italy and found IC_{50} ranging from 258 to 2000 μg mL^{-1}, and from 184 to 766 μg mL^{-1} for α-amylase and α-glucosidase test, respectively.

Generally, the addition of turmeric powder to EVOO determined a reduction in enzymes inhibitory activity except in the α-glucosidase inhibition test, in which flavoured samples exhibited a comparable result to the EVOO (IC_{50} values of 137.34, 181.99 and 181.98 μg mL^{-1} for the EVOO, CI, and CM samples, respectively at T0). However, if the data are observed during the storage period of the oils, a significant loss of the inhibitory activity of the enriched oils is found compared to the control at T360, with IC_{50} values of 289.32, 777.09, 676.21 for EVOO, CI and CM, respectively for α-amylase, and 778.23, 489.70, 409.22 for EVOO, CI and CM, respectively for α-glucosidase. A similar trend was observed also in lipase.

These results are disagreed to those found by Custureri et al. [26] after EVOO enrichment with ginger, in which this matrix helped the oil to maintain its functional properties up to one year of storage. These data demonstrate, once again, how the enrichment of an extra virgin olive oil with an aromatic and functional matrix such as turmeric, does not always lead to an improvement in the intrinsic functional characteristics of this food.

3.7. Sensory evaluation

The FVOOs (flavoured olive oils) were tested by a group of expert assessors. They scored different overall acceptability and are listed below in descending order for both approaches: CM > CI (Fig. 4).

The assessors were not able to identify the enrichment matrix. The EVOO was characterised by the presence of slight "muddy" and "sludge" defects. The most characteristic note of CM and CI was obviously the colour, which became a bright yellow. They are also characterised by

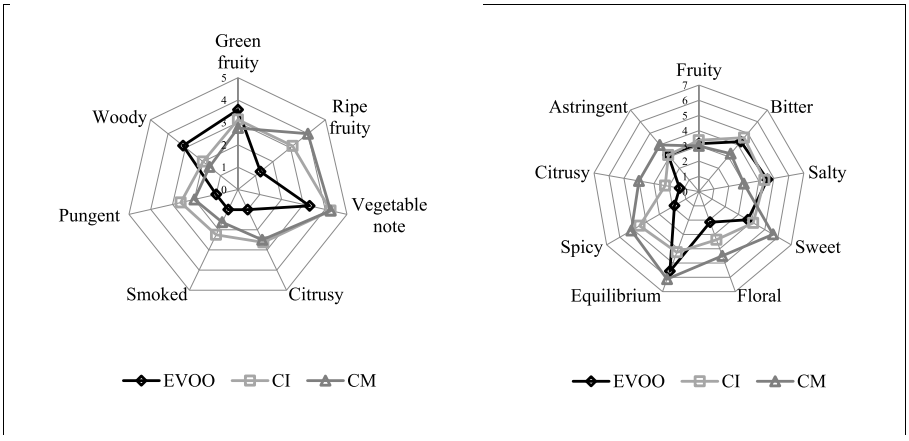

Fig. 4. Sensory evaluation of EVOO: control; CI: turmeric flavoured olive oil obtained by 2% infusion; CM: turmeric flavoured olive oil obtained by 1% malaxation.

high “ripe fruity” and “spicy” attributes. They differed from each other because in CM the defects of the starting oil were covered and its flavour was more balanced than CI, in fact the CM sample resulted the sweetest and most balanced FVOO in the tasting.

4. Conclusions

Nowadays, the challenge of the food industry sector is to create innovative products to satisfy the demands of consumers who are increasingly attentive to what they consume and to their health. The development of functional or enriched olive oil falls in this category of constant growth. The main challenge lies in creating a novelty, with good organoleptic characteristics, but especially with strong health properties. Concerning our addition of turmeric powder, the processing with olive paste (CM) seems to better protect the oil against oxidation more than the addition by infusion (CI), which reached peroxide values after one year of storage even higher than the control (22.88 *vs* 17.89 mEq O_2 kg^{-1}). The addition of turmeric powder to EVOO generated FVOOs characterized by a higher bioactive phytochemical content compared to the control olive oil. In fact, the main bioactive compounds of turmeric, such as bisdemethoxycurcumin, demethoxycurcumin and curcumin were detected. The addition of turmeric also affected the antiradical potential of the newly obtained flavoured olive oils, especially in malaxation. This demonstrates how curcuminoids do not negatively affect the chemical quality criteria for an olive oil. Regarding the inhibitory activity against the enzymes involved in the digestion of carbohydrates and lipids, FVOOs demonstrated an interesting activity only in α-glucosidase test, even after one year of storage, maintaining an activity approximately 2-times lower, with very similar values between CM and CI, compared to EVOO (778.23 *vs* 489.7 and 409.22 μg mL^{-1} for EVOO, CI and CM, respectively). Moreover, were also enhanced the sensory characteristics. The panellists appreciated the FVOOs, and the new "spicy" note appeared, as well as the colour become bright yellow. Through the application of this study is possible to affirm that among the curcuminoids, demethoxicurcumin is the one that act as main antioxidant and antiradical agent. However, further studies are necessary to optimise some variables in the production, such as malaxation time and temperature, to avoid significant decrease in TPC.

Funding sources

This study was supported and conducted within a PhD research program in “Scienze e tecnologie alimentari” (XXXVI cycle) by Irene M. G. Custureri, who received the grant “Ricerca e Innovazione con caratterizzazione industriale, PON RI 2014–2020”.

The authors are grateful to “*Prim'olio*” for supporting in the sensory evaluation.

CRediT authorship contribution statement

Irene Maria Grazia Custureri: Validation, Resources, Formal analysis, Data curation. **Vincenzo Sicari:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Angelo Maria Giuffrè:** Writing – original draft, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Rosa Tundis:** Resources, Methodology, Formal analysis, Data curation. **Ana Cristina Soria:** Validation, Resources, Methodology, Data curation. **Monica Rosa Loizzo:** Writing – original draft, Validation, Supervision, Resources, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vincenzo Sicari reports was provided by University of Reggio Calabria. Vincenzo Sicari reports a relationship with University of Reggio Calabria that includes:. If there are other authors, they 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

No data was used for the research described in the article.

Abbreviations

AA	Ascorbic acid
ABTS	2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid
C*	Chroma
CH_3CN	Acetonitrile
CH_3COOH	Acetic Acid
CH_3OH	Methanol
CM	Turmeric flavoured olive oil by malaxation
CI	Turmeric flavoured olive oil by infusion
DAD	Diode Array Detection
DPPH	1,1-Diphenyl-2-picryl-hydrazil
EVOO	Control Olive Oil
FA	Free Acidity
GAE	Gallic Acid Equivalent
H_2O	Water
IC_{50}	Half Maximal Inhibitory Concentration
MeOH	Methanol
PV	Peroxide Value
RE	Rutin Equivalent
TE	Turmeric Extract
TFC	Total Flavonoid Content
TPC	Total Polyphenols Content
UHPLC	Ultra-High Performance Liquid Chromatography

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101111.

References

[1] Commission Regulation (EEC) Nº. 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Off. J. Eur. Commun. (L.248, p.1-82)]. .
[2] European Commission, Regulation (EU) No 432/2012 Establishing a list of permitted health claims made on foods other than those referring to the reduction of disease risk and to children's development and health Official Journal of the European Union 136 (2012) 1–40.
[3] J.S. Perona, K.M. Botham, Olive oil as a functional food: nutritional and health benefits, in: R. Aparicio, J. Harwood (Eds.), Handbook of Olive Oil, Springer, Boston, MA, 2013, 10.007/978-1-4614-7777-818.
[4] C. Davis, J. Bryan, J. Hodgson, K. Murphy, Definition of the mediterranean diet; a literature review, Nutrients 7 (11) (2015) 9139–9153, https://doi.org/10.3390/nu7115459.
[5] C. Jimenez-Lopez, M. Carpena, C. Lourenço-Lopes, M. Gallardo-Gomez, J. M. Lorenzo, F.J. Barba, M.A. Prieto, J. Simal-Gandara, Bioactive compounds and quality of extra virgin olive oil, Foods 9 (8) (2020) 1014, https://doi.org/10.3390/foods9081014. PMID: 32731481.
[6] C.C. Tedesco, C. Bonfiglio, M. Notarnicola, M. Rendina, A. Castellaneta, A. Di Leo, G. Giannelli, L. Fontana, High extra virgin olive oil consumption is linked to a lower prevalence of NAFLD with a prominent effect in obese subjects: results from the, MICOL Stud. Nutr. 15 (21) (2023) 4673, https://doi.org/10.3390/nu15214673.
[7] World health organization, https://www.who.int/health-topics/obesity#tab=tab_1 (Last access on November 2023).
[8] M. Barron, H. Hayes, Z. Bice, K. Pritchard, T.L. Kindel, Sleeve gastrectomy provides cardioprotection from oxidative stress in vitro due to reduction of circulating myeloperoxidase, Nutrients 15 (22) (2023) 4776, https://doi.org/10.3390/nu15224776.
[9] A. Serrano, G. Ros, G. Nieto, Bioactive compounds and extracts from traditional herbs and their potential anti-inflammatory health effects, Medicines 5 (3) (2018) 76, https://doi.org/10.3390/medicines5030076.

[10] A. Yashin, Y. Yashin, X. Xia, B. Nemzer, Antioxidant activity of spices and their impact on human health: a review, Antioxidant 6 (3) (2017) 70, https://doi.org/10.3390/antiox6030070.
[11] S.J. Hewlings, D.S. Kalman, Curcumin: a review of its effects on human health, Foods 6 (10) (2017 Oct 22) 92, https://doi.org/10.3390/foods6100092.
[12] R.K. Verma, P. Kumari, R.K. Maurya, V. Kumar, R.B. Verma, K.R. Singh, Medicinal properties of turmeric (Curcuma longa L.): a review, Int. J. Chem. Stud. 6 (4) (2018) 1354–1357.
[13] H. Li, A. Sureda, H.P. Devkota, V. Pittalà, D. Barreca, A. Sanchez Silva, D. Tewari, S. Xu, S.M. Nabavi, Curcumin, the golden spices in treating cardiovascular diseases, Biotechnol. Adv. 38 (2020) 107343, https://doi.org/10.1016/j.biotechadv.2019.01010.
[14] F. Idowu-Adebayo, V. Fogliano, A. Linnemann, Turmeric-fortified cow and soya milk: golden milk as a street food to support consumer health, Foods 11 (4) (2022) 558, https://doi.org/10.3390/foods11040558.
[15] K. Singh, S. Sricairatanakool, T. Chewonarin, A. Prommaban, et al., Impact of green extraction on curcuminoid content, antioxidant activities and anti-cancer efficiency (in vitro) from turmeric rhizomes (Curcuma longa L.), Foods 11 (2022) 3633, https://doi.org/10.3390/foods11223633.
[16] A.R. Oliveira, A.E.C. Ribeira, E.R. Oliveira, K.O. Ribeiro, M.C. Garcia, et al., Physicochemical, microbiological and sensory characteristics of snacks developed from broken rice grains and turmeric powder, Int. J. Food Sci. Technol. 55 (7) (2020) 2719–2729, https://doi.org/10.1111/ijfs.14525.
[17] A. Amer, A.E. Rizk, Production and evaluation of novel functional extruded corn snacks fortified with ginger, bay leaves and turmeric powder, Food Prod. Process Nutr. 4 (2022) 4, https://doi.org/10.1186/s43014-022-00083-3.
[18] A.T. Septiana, I. Sitoresmi, P.S. Dewi, Sensory evaluation, antioxidant activity and total of microbial of tamarind- turmeric herbal drink during the storage of refrigerator temperature at various packaging, Food Res. 2 (4) (2018) 391–397, https://doi.org/10.26656/fr.2017.2(4).030.
[19] E. Yilmaz, S. Demirci, E.K. Uslu, Red pepper and turmeric-flavored virgin olive oil oleogels prepared with whale spermaceti wax, J. Oleo Sci. 71 (2) (2022) 187–199, https://doi.org/10.5650/jos.ess21167.
[20] S. Lamas, N. Rodrigues, A.M. Peres, J.A. Pereira, Flavoured and fortified olive oils - pros and cons, Trends Food Sci. Technol. 124 (2022) 108–127, https://doi.org/10.1016/j.tifs.2022.04.013.
[21] European Union Commission, Consolidated Text on the Characteristics of Olive Oil and Olive-Residue Oil and on the Relevant Methods of Analysis, European Union Commission, Brussels, Belgium, 2016. Regulation 01991R2568-IT-04.12.2016-031.005.
[22] J.S. Žlabur, M. Brajer, S. Voc, A. Galic, A. Radman, et al., Ultrasound as a promising tool for the green extraction of specialized metabolites from some culinary spices, Molecules 26 (7) (2021) 1866, https://doi.org/10.3390/molecules26071866.
[23] Y. Choi, W. Kim, J.S. Lee, S.J. Youn, H. Lee, M.Y. Baik, Enhanced antioxidant capacity of puffed turmeric (Curcuma longa L.) by high hydrostatic pressure extraction (HHPE) of bioactive compounds, Foods 9 (11) (2020) 1690, https://doi.org/10.3390/foods9111690, 9(11).
[24] G.F. Montedoro, M. Servili, M. Baldioli, E. Miniati, Simple and hydrolyzable phenolic compounds in virgin olive oil. Their extraction, separation and quantitative compounds and semiquantitative evaluation by HPLC, J. Agric. Food Chem. 40 (1992) 571–1576.
[25] A. Baiano, G. Gambacorta, C. Terracone, M.A. Previtali, C. Lamacchia, E. La Notte, Changes in phenolic content and antioxidant activity of Italian extra-virgin olive oils during storage, J. Food Sci. 74 (2009) 177–183, 10.111/j.1750-3841.2009.010072.x.
[26] I.M.G. Custureri, V. Sicari, M.R. Loizzo, R. Tundis, A.C. Soria, A.M. Giuffrè, Evaluation of quality parameters and functional activity of Ottobratica extra virgin olive oil enriched with *Zingiber officinale* (ginger) by two different enrichment processes during one-year storage, Foods 12 (20) (2023) 3822, https://doi.org/10.3390/foods12203822.
[27] V. Sicari, I.M.G. Custureri, R. Tundis, M.R. Loizzo, Comparison of physicochemical characteristics and bioactivity of olive oil mill wastewaters from traditional and water-saving ARA-controlled three-phase decanter, Sustainability 15 (5) (2023) 3890, https://doi.org/10.3390/su15053890.
[28] T. Chumroenphat, I. Somboonwatthanakul, S. Saensouk, S. Siriamornpun, Changes in curcuminoids and chemical components of turmeric (Curcuma longa L.) under freeze-drying and low-temperature drying methods, Food Chem. 339 (2021) 339, https://doi.org/10.1016/j.foodchem.2020.128121, 1281121.
[29] S. Al-Lahham, N. Jaradat, A. Hamayel, A. Assaassa, F. Hammad, et al., Hexane extract of Curcuma longa L. inhibits the activities of key enzymes and pro-inflammatory adipokines linked to obesity, Eur. J. Integr. Med. 48 (2) (2021) 101400, https://doi.org/10.1016/j.eujim.2021.101400.
[30] N. Caporaso, A. Paduano, G. Nicoletti, R. Sacchi, Capsaicinoids, antioxidant activity, and volatile compounds in olive oil flavored with dried chili pepper (Capsicum annuum), Eur. J. Lipid Sci. Technol. 115 (12) (2013) 1434–1442, https://doi.org/10.1002/ejlt.201300158.
[31] M.L. Clodoveo, T. Dipalmo, P. Crupi, V. Durante, V. Pesce, I. Maiellaro, A. Lovece, A. Mercurio, A. Laghezza, F. Corbo, et al., Comparison between different flavored olive oil production techniques: healthy value and process efficiency, Plant Foods Hum. Nutr. (Dordr.) 71 (2016) 81–87, https://doi.org/10.1007/s11130-016-0528-7.
[32] I.M.G. Custureri, A.M. Giuffrè, M.R. Loizzo, R. Tundis, A.C. Soria, V. Sicari, Bergamot flavoured olive oil: comparison between enrichment processes, evaluation of shel-life and health properties, Appl. Food Res. 4 (2024) 100400, https://doi.org/10.1016/j.afres.2024.100400.
[33] M.A. Ayadi, N. Grati-Kamoun, H. Attia, Physico-chemical change and heat stability of extra virgin olive oils flavoured by selected Tunisian aromatic plants, Food Chem. Toxicol. 47 (10) (2009) 2613–2619, https://doi.org/10.1016/j.fct.2009.07.024.
[34] I. Ammar, A.B. Amira, I. Khemakem, H. Attia, M. Ennouri, Effect of Opuntia ficus-indica flowers maceration on quality and on heat stability of olive oil, J. Food Sci. Technol. 54 (6) (2017) 1502–1510, https://doi.org/10.1007/s13197-017-2581-0.
[35] G. Di Lecce, M. Piochi, D. Pacetti, N.G. Frega, E. Bartolucci, S. Scortichini, D. Fiorini, Eleven monovarietal extra virgin olive oils from olives grown and processed under the same conditions: effect of the cultivar on the chemical composition and sensory traits, Foods 9 (2020) 904, https://doi.org/10.3390/foods9070904.
[36] V.P. Soares, M.B. Fagundesa, D.R. Guerraa, Y.S.B. Leãesa, C.F. Speronia, et al., Ultrasound assisted maceration for improving the aromatization of extravirgin olive oil with rosemary and basil, Food Res. Int. 135 (2020) 109305, https://doi.org/10.1016/j.foodres.2020.109305.
[37] A. Sousa, S. Casal, R. Malheiro, H. Lamas, A. Bento, J.A. Pereira, Aromatized olive oils: influence of flavouring in quality, composition, stability, antioxidants, and antiradical potential, LWT–Food Sci. Technol. 60 (1) (2015) 22–28, https://doi.org/10.1016/j.lwt.2014.08.026.
[38] J. Moustakime, Z. Hazzoumi, K.A. Joutei, Aromatization of virgin olive oil by seeds of Pimpinella anisum using three different methods: physico-chemical change and thermal stability of flavored oils, Grain Oil Sci. Technol. 4 (2021) 108–124, https://doi.org/10.1016/j.gaost.2021.07.001.
[39] I. Cheng-Chao, C.M. Wang, S.P. Li, L.G. Lin, W.C. Ye, Q.W. Zhang, Simultaneous quantification of three curcuminoids and three volatile components of Curcuma longa using pressurized Liquid extraction and high-performance Liquid Chromatography, Molecules 23 (7) (2018) 1568, https://doi.org/10.3390/molecules23071568.
[40] K. Pal, S. Chowdhury, S.K. Dutta, S. Chakraborty, M. Chakraborty, G.K. Pandit, et al., Analysis of rhizome colour content, bioactive compound profiling and ex-situ conservation of turmeric genotypes (Curcuma longa L.) from sub-Himalayan terai region of India, Ind. Crop. Prod. 150 (2020) 112401, https://doi.org/10.1016/j.indcrop.2020.112401.
[41] V. Sicari, Antioxidant potential of extra virgin olive oils extracted from three different varieties cultivated in the Italian province of Reggio Calabria, JABFQ 90 (2017) 76–82, https://doi.org/10.5073/JABFQ.2017.090.011.
[42] E. Karacabey, G. Ozkan, L. Dalgic, S.O. Sermet, Rosemary aromatization of extra virgin olive oil and process optimization including antioxidant potential and yield, TURJAF 4 (8) (2016) 628–635, https://doi.org/10.24925/turjaf.v4i8.628-635.724.
[43] M.R. Loizzo, M. Bonesi, T. Falco, M. Leporini, M. Pagliuso, V. Sicari, R. Tundis, Carolea olive oil enriched with an infusion of *Capsicuum annuum* and *C. chinense* dried pepper powders to produce an added value flavoured olive oils, J. Food Process. Preserv. 45 (2021) 15776, 10.111/jfp.15776.
[44] P. Plastina, R. Tundis, C. La Torre, V. Sicari, A.M. Giuffrè, A. Neri, M. Bonesi, M. Leporini, A. Fazio, T. Falco, et al., The addition of *Capsicum baccatum* to Calabrian monovarietal extra virgin olive oils leads to flavoured olive oils with enhanced oxidative stability, Ital. J. Food Sci. 33 (2021) 61–72, https://doi.org/10.15586/ijfs.v33i1.1937.
[45] M. Leporini, M.R. Loizzo, M.C. Tenuta, T. Falco, V. Sicari, T.M. Pellicanò, R. Tundis, Calabrian extra virgin olive oil from Frantoio cultivar: chemical composition and health properties, Emir. J. Food Agric. 30 (7) (2018) 631–637, https://doi.org/10.9755/ejfa.2018.v30.i7.1743.
[46] M.R. Loizzo, G. Di Lecce, E. Boselli, F. Menichini, N.G. Frega, Inhibitory activities of phenolics compounds from extra virgin olive oils on the enzymes involved in diabetes, obesity and hypertension, J. Food Biochem. 35 (2011) 381–399, https://doi.org/10.1111/j.1745-4514.2010.00390.x.