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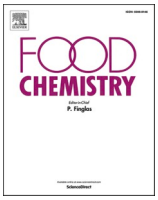

Shelf-life of flavoured olive oil with chili pepper: Comparison between co-milling fresh chili peppers with olives and typical infusion flavouring methods over 18 months of storage

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ABSTRACT

The aim was to study the shelf-life over 18 months of storage (no light and oxygen exposure) of chili peppers flavoured olive oils comparing flavouring methods of co-milling of fresh chili peppers with sound olives at mill scale with temporary and permanent infusion of dried chili peppers in olive oil. Tocopherols, secoiridoids and capsaicinoids by HPLC-DAD, volatile compounds by HS-SPME-GC–MS, and sensory profiles were studied. The decrease in tocopherols and secoiridoids was greater in "infusion" samples, while a significant increase in capsaicinoids was observed in "permanent infusion" samples. The main changes were observed for sensory and volatile profiles: "infusion" samples were defective already after 2 months with significant increase of defects-relating volatile compounds, while "co-milling" samples were defects-free and characterized by nice balance among hotness/heat and pepper fruity/taste during entire storage. "Co-milling" samples showed better shelf-life (18 months) than infusion ones, and even than EVOO control samples (12 months).

1. Introductionf

Flavoured olive oils (FOOs) are olive oil-based condiments, appreciated for their sensory and nutritional properties also by consumers being not familiar with extra virgin olive oils (EVOO). The flavoured olive oils with chili pepper (CPFOOs) are among the most popular ones thanks to their hotness/heat sensory attribute due to capsaicinoids (Caponio et al., 2016; Caporaso et al., 2013; Gambacorta et al., 2007; Loizzo et al., 2021; Trovato et al., 2023; Zellama et al., 2022).

In the European FOOs production, the same operating conditions of virgin olive oil processing extraction are usually carried out, but the application of flavouring operation forbids labelling FOO as "extra virgin olive oil/virgin olive oil" according to the EU law (European Parliament and the Council (EC), 2022a; Cecchi et al., 2023; COI, 2024). The FOOs can be produced following different flavouring methods: (i) The "temporary infusion", consisting of solid-liquid extraction of small-sized dried aromatic plants in olive oil, followed by the solid mechanical separation via filtration or centrifugation; (ii) the "permanent infusion", consisting of continuous solid-liquid extraction of small-sized dried aromatic plants in olive oil over entire conservation and distribution; (iii) the "co-milling", a method in which olive fruits are milled together with fresh aromatic plants; (iv) the "co-extraction", a method in which small-sized fresh/dried aromatic plants are added to olive paste during malaxation; (v) the "direct mixing", consisting of addition of pure compounds, pre-concentrated extracts or essential oils into olive oil. Despite some disadvantages, infusion is to date the most used flavouring method (Baiano et al., 2009; Boulares et al., 2022; Caponio et al., 2016; Caporaso et al., 2013; Cecchi et al., 2023; Clodoveo et al., 2016; Lamas

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et al., 2022; Sousa et al., 2015; Stefanidis et al., 2023; Trovato et al., 2023).

The applied flavouring operations may cause significant effects on the quality of FOOs due to the different extraction approaches. During infusion, the operating conditions (e.g., time, temperature, solid structure and composition, liquid-solid ratio) are the limiting factors, potentially causing microbial growth resulting in the development of molecules responsible for off-flavours but also raising safety issues. Instead, in the case of both the "co-milling" and "co-extraction" methods, the olive paste is a particular biological material at high water activity, where the flavouring process is given by the combination of phenomena such as solid-liquid extraction and enzymatic reactions, which end with the oil filtration step. Recently, Cecchi et al. (2023) showed that the co-milling of sound olives with fresh chili peppers significantly improved the CPFOOs quality in comparison to the typical temporary infusion, producing oils free from sensory defects and with improved sensory, volatile and capsaicinoids profiles. To get these advantages, it has been important to add the fresh chili peppers directly during milling, and not in the dried form during malaxation (as in the case of co-extraction approach). In fact, the use of fresh chili peppers also allowed avoiding the drying step, which causes some off-flavours and the loss of pleasant VOCs typical of chili peppers (Cecchi et al., 2023; Ciafardini et al., 2004; Lamas et al., 2022; Montoya-Ballesteros et al., 2014; Stefanidis et al., 2023).

The oxidative stability plays a key role on commercial diffusion of flavoured oils influencing the product shelf-life (Stefanidis et al., 2023; Zellama et al., 2022), but to date only few studies (Baiano et al., 2009; Boulares et al., 2022; Caponio et al., 2016; Custureri et al., 2023; Peres et al., 2023; Sousa et al., 2015) focused on measuring their storability under real storage conditions (e.g., non-accelerated conditions). In general, the above studies reported an improved oil shelf-life. Peres et al. (2023) studied the storability of FOOs produced at lab-scale by either co-extraction or co-milling of dried thyme and olive fruits of the Cornicabra cultivar. Custureri et al. (2023) studied the storability of FOOs produced at lab-scale by either co-extraction or infusion of ginger and olive fruits of the Ottobratica cultivar. The CPFOO storability was studied by Caponio et al. (2016) and Baiano et al. (2009), comparing different flavouring methods, but without focusing attention on capsaicinoids and the typical VOCs of chili peppers during storage. However, a systematic study concerning the storability of CPFOOs produced by mean of different flavouring techniques and using chili peppers at different ripening degree is missing in the literature.

In this research a wide shelf-life study of CPFOOs produced by different flavouring methods adding fresh or dried chili peppers at different ripening degree was carried out. The aim was to verify the hypothesis that producing CPFOOs by co-milling of fresh chili peppers at two different ripening degrees with sound olives allows improving the oil storability in comparison with those produced by the typical temporary and permanent infusion methods. The evolution of chemical and sensory quality of the CPFOO samples was studied over 18 months of storage, also in comparison with a non-flavoured virgin olive oil sample produced from the same batch of olive fruits. Shelf-life was evaluated keeping into account both the moment in which the samples exceeded the legal limits of the quality parameters for the EVOO category, including the sensory ones, and significant degradation of minor compounds and VOCs typical of virgin olive oil and chili peppers.

2. Materials and methods

2.1. Chemicals

The Milli-Q-system (Millipore SA, Molsheim, France) was used to produce water of ultrapure grade. Acetonitrile with HPLC grade was purchased from Panreac (Barcelona, Spain); ethanol, formic acid, isopropanol and phosphoric acid were purchased from Merck (Darmstadt, Germany). Standards of capsaicin (> 98.5 %) and α-tocopherol (> 97.5 %) were purchased from Merck (Darmstadt, Germany) as well as all the internal and internal standards used for HS-SPME-GC–MS analysis of VOCs (their purity is reported in Cecchi et al. (2019)).

2.2. Raw material: Chili peppers and olive fruits samples

Chili peppers: four batches of organic chili peppers were collected from Azienda Agricola Carmazzi (Viareggio, Lucca, Italy) at two ripening degrees (i.e., the red and the green ripening degree) and in fresh and dried form as already described by Cecchi et al. (2023). Briefly, they were: i) Fresh Red Chili Peppers (**FRCP**), constituted by fresh red fruits of the Jalapeño (40 %), Habanero Rosso (30 %) and Naga Morich (30 %) varieties; ii) Dried Red Chili Peppers (**DRCP**), constituted by the **FRCP** fruits dried in a ventilated oven (40 °C, 60 h, dry weight, 13.2 %); iii) Fresh Green Chili Peppers (**FGCP**), constituted by fresh green fruits of the Jalapeño (40 %), Habanero Rosso (30 %) and Naga Morich (30 %) varieties; iv) Dried Green Chili Peppers (**DGCP**), constituted by the **FGCP** fruits dried in a ventilated oven (40 °C, 60 h, dry weight, 12.9 %). The chili peppers samples were harvested the day before processing.

Olive fruits: a homogenous batch of sound olive fruits of the Frantoio cultivar was processed at maturation index = 3.6 (Uceda & Frias, 1975). They were collected during the 2021 olive oil production year from the Fattoria Altomena (Pelago, Florence, Italy). The olive fruits were harvested on the same day of processing.

2.3. The experimental plan

The olive oil extraction process was carried out at industrial scale as previously described (Cecchi et al., 2023), applying the common operating conditions to obtain filtered EVOO. Seven types of olive oil samples were obtained, as follows (Fig. 1):

- The **EVOO** control sample: it was a non-flavoured extra virgin olive oil sample obtained from 300 kg of olive fruits.
- The **GOO_{green}** flavoured sample: it was a flavoured olive oil obtained by co-milling of 300 kg of olive fruits with 4.5 kg (i.e., 1.5 % *w*/w) of **FGCP**.
- The **GOO_{red}** flavoured sample: it was a flavoured olive oil obtained by co-milling of 300 kg of olive fruits with 4.5 kg (i.e., 1.5 % w/w) of **FRCP**.
- The **FOO_{green}** flavoured sample: it was a flavoured olive oil obtained at lab scale by temporary infusion of **DGCP** into the **EVOO** control samples for 10 days. An infusion ratio of 1.29 % *w*/w was applied for maintaining the same ratio of the above co-milling trial, keeping into account both chili peppers drying yield and olive oil extraction yield.
- The **$FXOO_{green}$** flavoured sample: it was a flavoured olive oil obtained at lab scale by permanent infusion of **DGCP** into the **EVOO** control samples for 18 months. The infusion ratio was equal to the **FOO_{green}** sample.
- The **FOO_{red}** flavoured sample: it was a flavoured olive oil obtained at lab scale by temporary infusion of **DRCP** into the **EVOO** control samples for 10 days. An infusion ratio of 1.32 % w/w was applied for maintaining the same ratio of the above co-milling trial, keeping into account both chili peppers drying yield and olive oil extraction yield.
- The **$FXOO_{red}$** flavoured sample: it was a flavoured olive oil obtained at lab scale by permanent infusion of **DRCP** into the **EVOO** control samples for 18 months. The infusion ratio was equal to the **FOO_{red}** sample.

The infusion trials, time of infusion (only in the case of the FOO samples) and chili peppers form (i.e., sliced), were defined according to preliminary trials (Cecchi et al., 2023). The choice of the level of chili peppers (i.e., 1.5 %) was also selected during preliminary trials where a group of consumers did not accept samples obtained with 3.0 % *w*/w of fresh chili peppers due to a too high level of pungency, while they well accept and appreciated those obtained with 1.5 % w/w of fresh chili

Fig. 1. Scheme of the preparation of non-flavoured (EVOO) and flavoured samples.

peppers (Cecchi et al., 2023).

Samples were obtained in triplicate and were bottled in filled 250-mL dark glass bottles. Samples were stored in the dark at room temperature (i.e., range 18–22 °C) over a storage period of 18 months. The samples were analyzed at time zero (t_0), 2 months (t_1), 6 months (t_2), 12 months (t_3) and 18 months (t_4); t_0 was the day in which the samples by temporary infusion were obtained. The total number of analyzed samples was 99: 7 types of olive oil samples × 3 replicates × 5 storage times, but at t_0 the **FXOO$_{red}$** and **FXOO$_{green}$** samples were the same of the **FOO$_{red}$** and **FOO$_{green}$** samples, respectively. Phenolic compounds, capsaicinoids, tocopherols, volatile profile, sensory profile and the legal quality parameters (European Parliament and the Council (EC), 2022a) such as free acidity, peroxide value and spectrophotometric indices, were measured.

2.4. Legal quality parameters analysis

Free acidity (%), peroxide value (meq$_{O2}$/kg) were evaluated using the CDR OxiTester Analysis System (CDR srl Florence, Italy) as described by Romani et al. (2020), and UV spectrophotometric indices (K_{232}, K_{270} and ΔK) were measured according to the European Commission methods (Romani et al., 2020; European Parliament and the Council (EC), 2022a; European Parliament and the Council (EC), 2022b).

2.5. HPLC-DAD analysis of capsaicinoids and phenolic compounds

According to Guzman and Bosland (2017), the capsaicinoids content was measured by HPLC to be able to convert the capsaicinoids content into the Scoville Heat Units (SHU) via a multiplying factor of 16 in order to measure the pungency attribute intensity. Since preliminary trials were carried out to verify that the peaks relating to capsaicinoids fall in a zone of the chromatogram at 280 nm free from phenolic compounds or other interfering molecules (Cecchi et al., 2023), analysis of capsaicinoids and phenolic compounds were performed together applying a single extraction procedure and running a single HPLC analysis. The chromatographic analysis was carried out using an HP1100 liquid chromatograph system, which was made up of auto-sampler, HP1100 DAD (Agilent Corporation, CA, USA) and a SphereClone ODS (Baiano et al., 2009), 5 µm, 250 × 4.6 mm i.d. column Phenomenex, Bologna, Italy). The conditions of both extraction procedure and HPLC analysis were according to the official IOC method for olive oil phenolic compounds (COI, 2022). Quantitative analysis of phenolic compounds was carried out using the syringic acid as internal standard and the tyrosol as external standard, thus expressing results as mg$_{tyr}$/kg$_{oil}$. Concerning quantitative analysis of capsaicinoids, they were carried out using a calibration curve of capsaicin, as previously described (Cecchi et al., 2023).

2.6. HPLC-FLD analysis of tocopherols

Analysis of tocopherols was carried out through HPLC-FLD analysis. An HP1200L liquid chromatograph system was made up of auto-sampler, FLD (Agilent Corporation, CA, USA) and a C18 Lichrosorb, 5 µm, 250 × 4.6 mm i.d. column (Interchim, Montluçon, France) was used. Eluents were A) acidic water (pH = 3.2, formic acid) and B) CH_3CN, with the following gradient: B were at 95 % at time 0 and stayed in this condition for 5 min, then increased to 100 % in 1 min staying in this condition for 24 min, then coming back to 95 % in 2 min, for a total analysis time of 32 min at a flow rate of 0.8 mL/min. The analysis was performed by injecting 20 µL of a solution of the oil sample prepared by dissolving 80 mg of oil into 1 mL of isopropanol with the aid of a vortex. Chromatograms from FLD were acquired using excitation and emission wavelengths of 295 nm and 325 nm, respectively, according to Gliszczynska-Swigło and Sikorska (2004). Quantitative analysis was carried out using a calibration curve of α-tocopherol (R^2 = 0.9999, range 0–8.76 µg).

2.7. HS-SPME-GC–MS analysis of volatile organic compounds

Analysis of volatile organic compounds (VOCs) was carried out according to a HS-SPME-GC–MS method previously validated (Cecchi et al., 2019). The quantification of 72 target volatile compounds typically present in virgin olive oil was done by means of the Multiple Internal Standard Normalization (MISN) approach. Details of the applied conditions for HS-SPME pre-concentration step, GC separation, MS analysis and quantitative analysis were previously reported (Cecchi et al., 2019; Cecchi et al., 2023).

VOCs typical in the CPFOOs (Cecchi et al., 2023) were also analyzed; tentative identification of the molecules was done through comparison between mass spectra of the analyte and standard NIST08/Wiley98 library database (minimum matching factor = 80 %), and between the retention indexes calculated and those present in the NIST Standard

Reference Database according to van den Dool and Kratz (1963). Quantitation was carried out using calibration lines of the 72 target compounds as described in Cecchi et al. (2023).

2.8. Sensory analysis

Sensory analysis was carried out by a panel of 8 trained panelists (5 males, 3 females, 28–65 age group, all Italian), which were regular consumers of EVOO accustomed to CPFOOs (Cecchi et al., 2023). The profile sheet is reported in the Supplementary file (Fig. S1); it was constituted by the descriptors generated by consensus by panelists in addition to the typical defects and positive attributes of virgin olive oils (COI/T.20/Doc. No15/Rev. 10).

2.9. Ethics & standards requirements for sensory evaluation

In this research study, sensory evaluation was carried out in accordance with the principles set forth in the Declaration of Helsinki for medical research involving humans and was subject to ethical standards that promote and ensure respect for all human participants and protect their health and rights. Since the research study was not of a medical nature, the research protocol was not submitted to an ethics committee for approval, in line with national regulations. Written informed consent was obtained from all participants in accordance with the GDPR (General Data Protection Regulation) 2016/679. Participants could withdraw from the study at any time without giving a reason. The tested products were safe for consumption.

2.10. Data analysis

Mean and standard deviation (SD) of triplicates for all experiments were evaluated using EXCEL in-house routines (Microsoft 365 version). For each measured parameter, a three-factor ANOVA was run for assessing the effect of milling type, ripening stage of chili peppers, storage time, and their interaction. Fisher Least Significant Difference (LSD) post hoc comparison was then carried out in order to find significant differences among samples data. All ANOVA and Fisher LSD analyses were run using OriginPro 2023b (Northampton, Massachusetts, USA).

3. Results

Chemical and sensory data from 6 types of CPFOOs (coming from 2 levels of ripening degree (green and red) of the chili peppers × 3 flavouring methods (temporary infusion, permanent infusion, co-milling)) were studied over storage (5 different storage time for a total of 18 months), and were statistically compared considering ripening degree, flavouring method and storage time as factors. The EVOO samples produced from the same batch of olives and used for infusion experiments were also analyzed as external samples but were not included in the statistical evaluation.

3.1. Evolution of legal quality parameters during storage

Table S1 of the Supplementary file shows the evolution of the legal quality parameters in both flavoured and non-flavoured olive oil samples during storage. All samples were compliant with the EU legal limits for the EVOO category (i.e., free acidity ≤0.80 %, peroxide value ≤20 meq_{O2}/kg_{oil}; $K_{232} \leq 2.50$; $K_{270} \leq 0.22$; $\Delta K \leq 0.01$). The free acidity reached 0.24 % for the GOO_{red} and FOO_{red} samples after 18 months; the peroxide value, the K_{232} and K_{270} never exceeded 9.9 meq_{O2}/kg_{oil}, 2.04 and 0.18, respectively. A significant increase of free acidity occurred after 12 months of storage, while peroxide value and UV spectrophotometric indices did not show significant increases during storage of all samples.

3.2. Evolution of tocopherols during storage

The tocopherol content is reported in Table 1 as total content and content of α, β + γ, and δ forms. α-Tocopherol was the far prevalent form, and it was one and two order of magnitude more abundant than the β + γ- and δ-tocopherol forms, respectively. At t_0, some effects of the flavouring method were highlighted: the tocopherols content in the oil samples flavoured by infusion was greater than in those flavoured by co-milling (GOO), but no significant difference was highlighted among

Table 1
Tocopherols content in non-flavoured (**A**) and flavoured (**B**) olive oil samples measured over 18 months of storage and expressed in mg/kg as mean ± SD of triplicates. For the total tocopherols content in flavoured samples, different letters indicate significant differences among treatments at $p < 0.05$.

A. EVOO samples	Tocopherols (mg/kg)			
	α	β + γ	δ	Total
EVOO t0	269.9 ± 8.6	38.1 ± 1.1	1.6 ± 0.1	309.7 ± 9.8
EVOO t1	212.5 ± 7.1	32.6 ± 0.8	1.4 ± 0.2	246.5 ± 7.5
EVOO t3	197.9 ± 5.8	33.8 ± 1.2	1.6 ± 0.3	233.3 ± 6.5
EVOO t4	188.1 ± 2.6	32.8 ± 0.5	1.2 ± 0.1	222.0 ± 3.0

B. Flavoured samples	Tocopherols (mg/kg)			
	α	β + γ	δ	Total
GOOred t0	213.5 ± 9.1	28.3 ± 0.9	1.6 ± 0.1	243.4 ± 10.1 cd
GOOred t1	180.2 ± 7.2	23.2 ± 0.8	1.2 ± 0.1	204.5 ± 7.7 gh
GOOred t3	167.5 ± 4.8	25.4 ± 0.6	1.6 ± 0.6	194.5 ± 5.4 hi
GOOred t4	155.3 ± 0.8	22.7 ± 0.6	1.1 ± 0.1	179.0 ± 1.4 jk
GOOgreen t0	179.9 ± 7.2	23.5 ± 1.6	1.8 ± 0.2	205.2 ± 9.0 gh
GOOgreen t1	166.2 ± 7.7	20.3 ± 0.7	1.1 ± 0.1	187.6 ± 8.3 ij
GOOgreen t3	146.3 ± 3.7	21.5 ± 0.7	1.3 ± 0.1	169.2 ± 3.9 k
GOOgreen t4	133.5 ± 1.0	19.8 ± 0.3	1.5 ± 0.1	154.8 ± 1.4 l
FOOred t0	271.1 ± 7.9	42.0 ± 0.8	2.2 ± 0.1	315.3 ± 8.8 a
FOOred t1	228.4 ± 5.4	32.7 ± 0.4	1.5 ± 0.1	262.6 ± 5.2 b
FOOred t3	201.0 ± 20.4	34.8 ± 3.3	1.4 ± 0.6	237.2 ± 24.3 de
FOOred t4	176.6 ± 1.6	31.0 ± 0.5	1.2 ± 0.1	208.9 ± 2.2 g
FXOOred t0	271.1 ± 7.9	42.0 ± 0.8	2.2 ± 0.1	315.3 ± 8.8 a
FXOOred t1	222.7 ± 7.4	30.4 ± 1.8	1.4 ± 0.4	254.5 ± 9.0 bc
FXOOred t3	202.4 ± 1.3	34.1 ± 1.1	1.4 ± 0.2	237.9 ± 1.8 de
FXOOred t4	190.9 ± 1.8	32.9 ± 0.9	1.7 ± 0.1	225.6 ± 2.8 ef
FOOgreen t0	270.6 ± 6.8	41.9 ± 1.2	2.0 ± 0.2	314.6 ± 8.2 a
FOOgreen t1	220.7 ± 10.1	30.8 ± 2.6	1.2 ± 0.2	252.7 ± 12.6 bc
FOOgreen t3	200.2 ± 8.0	35.9 ± 1.5	1.3 ± 0.5	237.5 ± 9.0 de
FOOgreen t4	181.7 ± 1.2	31.7 ± 0.3	1.5 ± 0.1	214.9 ± 1.6 fg
FXOOgreen t0	270.6 ± 6.8	41.9 ± 1.2	2.0 ± 0.2	314.6 ± 8.2 a
FXOOgreen t1	224.6 ± 12.1	31.0 ± 3.0	1.4 ± 0.1	256.9 ± 14.9 b
FXOOgreen t3	195.1 ± 19.5	34.2 ± 1.2	1.3 ± 0.2	230.5 ± 20.6 de
FXOOgreen t4	179.2 ± 2.9	31.3 ± 0.5	1.3 ± 0.1	211.9 ± 3.5 g

temporary (FOO) or permanent (FXOO) infusion. When compared with the EVOO control sample, the tocopherols content in samples flavoured by infusion was slightly greater, while in those flavoured by co-milling was lower. During storage, significant effects of flavouring method, chili peppers ripening stage and storage time were observed (Table 2). The interaction between flavouring method and ripening stage, and the interaction between flavouring method and storage time were also significant. The tocopherol content significantly decreased in all samples in relation to the flavouring method, according to the following order from higher to lower decrease: FOO samples (approx. 32 % after 18 months), FXOO samples (approx. 30 % after 18 months), GOO samples (approx. 25 % after 18 months). In the case of the control EVOO sample the decrease was approx. 28 % after 18 months. No significant variation during storage occurred in relation to the ripening stage of chili peppers. The α-tocopherol continuously decreased during storage, while the β + γ and δ-tocopherol forms decreased in the first two months and then maintained a similar amount for the remaining 16 months of storage.

3.3. Evolution of phenolic compounds during storage

The evolution of phenolic compounds of virgin olive oils was studied over storage. The content of total phenolic compounds (TPC) and of main phenolic compounds as well as the hydrolysis ratio (i.e., the ratio between the sum of tyrosol+hydroxytyrosol and the total phenolic content (Mulinacci et al., 2013)) are shown in Fig. 2. At time zero, the GOO samples showed the greatest TPC, and the main contribution was given by oleacein and oleocanthal. During storage, the total phenolic content, as well as the oleacein and oleocanthal contents, significantly decreased in all samples in relation to the flavouring method (Table 2), according to the following order for TPC: FOO samples (approx. 32 % after 18 months), FXOO and GOO samples (both approx. 24 % after 18 months). In the case of the control EVOO sample the decrease was approx. 27 % after 18 months. The main decrease was observed in the last 6 months. Degradation of phenolic compounds was also observed by the increase of both hydroxytyrosol+tyrosol content and hydrolysis ratio. No significant variation during storage occurred in relation to the ripening stage of chili peppers and the methods of temporary/permanent infusion.

3.4. Evolution of capsaicinoids during storage

Capsaicinoids are the typical alkaloids of *Capsicum* species, which add the well-known health and sensory properties to the chili pepper (Chopin & Littenberg, 2017; Guzman & Bosland, 2017; Krajewska & Powers, 1988). Capsaicin and dihydrocapsaicin represented almost completely the capsaicinoids fraction in all samples; their content and ratio, and the pungency according to the Scoville Heats Units (SHU) are reported in Table 3. A significant effect of the flavouring method on the capsaicinoids content was pointed out (Table 2) as follows: (i) the greatest total content of capsaicinoids was observed in the flavoured samples by infusion (approx. 35 % higher than in GOO samples), and (ii) the total content of capsaicinoids increased during storage in the flavoured samples by permanent infusion (approx. +57 % after 18 months), while no significant differences were observed for GOO and FOO samples over storage. The pungency values of all samples (SHU) were also consistent with the above behavior (Table 3). As for the effect of chili peppers ripening stage (Table 2), the “green” samples showed a significantly greater content than the “red” samples (approx. 10 % higher); the capsaicin/dihydrocapsaicin ratio was greater in the “red” than in the “green” samples, showing that great amounts of dihydrocapsaicin were typical of the green ripening stage of chili peppers.

3.5. Evolution of volatile organic compounds (VOCs) during storage

The typical VOCs of VOO and CPFOO (Cecchi et al., 2019; Cecchi et al., 2023; Trovato et al., 2022) were studied.

3.5.1. VOCs typical of VOO

The most abundant VOCs in EVOO are the typical compounds from the lipoxygenase (LOX) pathway, which are responsible for positive fruity sensory attributes (Cecchi, Migliorini, & Mulinacci, 2021). For all samples, the evolution over storage of the main single LOX VOCs and

Table 2
Data processing of the main chemicals analyzed. For each molecule/group of molecules, results from three-factor ANOVA are reported, where the factors are the flavouring method (FM), the chili peppers ripening stage (RS) and the storage time (ST). The two-way and three-way interactions are also reported.

	flavouring method (FM)	ripening stage (RS)	storage time (ST)	FM × RS	FM × ST	RS × ST	FM × RS × ST
Total tocopherols	***	***	***	***	***	n.s.	n.s.
Hydroxytyrosol + tyrosol	n.s.	***	***	n.s.	***	*	n.s.
Oleacein	***	n.s.	***	***	*	n.s.	n.s.
Oleocanthal	***	**	***	n.s.	***	*	n.s.
Oleuropein aglycone	n.s.	**	***	***	***	***	*
Total phenolic content	***	n.s.	***	n.s.	*	n.s.	n.s.
Hydrolysis ratio	***	*	***	n.s.	***	n.s.	n.s.
Total capsaicinoids	***	***	***	n.s.	***	*	*
Σ LOX VOCs	***	***	***	***	***	**	***
Σ linear saturated aldehydes	***	***	***	***	***	**	***
Σ linear unsaturated aldehydes	***	***	***	***	***	***	***
Ratio (*E*)-2-hexenal/hexanal	***	***	***	***	***	*	**
Σ VOCs C8	***	***	***	***	***	***	***
Σ branched aldehydes	***	**	***	**	***	**	***
Acetic acid	***	n.s.	***	**	***	***	***
Σ carboxylic acids C3-C6	***	***	***	***	***	***	***
Methyl acetate	***	***	***	***	***	*	**
Methyl propanoate	***	***	***	n.s.	***	***	***
Σ esters of 2-methylbutanoic acid	***	***	***	***	***	n.s.	n.s.
Σ esters of 3-methylbutanoic acid	***	*	***	***	***	n.s.	n.s.
Σ other esters	***	***	***	***	***	n.s.	n.s.
Tetramethyl pyrazine	***	***	***	***	***	**	***
Σ molecules from fermentation	***	***	***	***	***	***	***
Σ branched carboxylic acids	***	***	***	***	***	***	***
Dimethyl sulfone	***	*	***	n.s	***	n.s.	n.s.
2-Methyl propanal	***	***	***	***	***	***	***

For each parameter, (***) indicates a significant effect at $p < 0.001$, (**) indicates a significant effect at $p < 0.01$, (*) indicates a significant effect at $p < 0.05$, (n.s.) indicates a non-significant effect.

Fig. 2. Bar charts representing the evolution of the content of the phenolic compounds detected in both the EVOO and the flavoured olive oil samples as a function of both flavouring method and ripening stage of chili peppers. Data are from triplicates. For each molecule or groups of them, different letters in the graphics indicate significant differences at $p < 0.05$ among the different types of flavoured samples.

their sum (Σ LOX VOCs) are showed in supplementary Fig. S2 and in Fig. 3, respectively. In all samples, the Σ LOX approx. Ranged from 35 to 60 mg/kg, in agreement with previous data on Tuscan EVOOs (Cecchi et al., 2022), and its values were significantly greater in the flavoured samples by infusion than in the GOO samples (Table 2, Fig. 3). A significant increase of the Σ LOX during storage occurred in the $FXOO_{green}$ samples. The behavior of the main single LOX VOCs was consistent with the above results, and an increasing trend was observed for (*E*)-2-pentenal, (*E*)-2-hexenal, (*Z*)-2-pentenol, (*Z*)-3-hexenol, hexyl acetate and (*Z*)-3-hexenyl acetate in the $FXOO_{green}$ samples (Fig. S2). (*E*)-2-Hexenol was the only LOX VOC showing an increasing trend during storage for all the flavoured samples.

The typical VOCs responsible for sensory defects in VOO were studied as VOCs in relationship with oxidative and microbial degradation phenomena, respectively (Angerosa et al., 1996; Cecchi, Migliorini, Giambanelli, et al., 2021; Morales et al., 2005). The first group of VOCs included the sum of linear saturated aldehydes (Σ LSA) and the sum of linear unsaturated aldehydes (Σ LUA) (Fig. 3). The values of Σ LSA and Σ LUA were low in all samples at time zero and significantly increased during storage (Table 2) from approx. 0.5 mg/kg at time 0 to 3.5–5.0 mg/kg after 18 months of storage, and from negligible amounts at time 0 to 0.8–1.1 mg/kg after 18 months of storage, respectively. These increases, in relation to the flavouring method, were greater for the FOO and FXOO than for the GOO samples; the EVOO control samples showed a lower increase. As showed in the supplementary Fig. S3, the main contribution to the Σ LSA was given by hexanal and nonanal: the former compound represented almost completely the Σ LSA content at time 0; then, it showed similar increases for EVOO and GOO samples, and the highest increase was for FOO and FXOO samples. The nonanal was instead absent at time 0, and showed similar increases for EVOO, FOO and FXOO samples with the highest increase for GOO samples. As showed in the supplementary Fig. S4, the main contribution to the Σ LUA was given by the C7 and C10 unsaturated aldehydes with a low contribution from the C9 ones. The (*E*)-2-hexenal/hexanal ratio was also determined according to Gambacorta et al. (2007): the ratio values (supplementary Fig. S5) significantly decreased (Table 2) in all samples, confirming that during storage the level of oil oxidation increased for all samples, but the increase was greater for the flavoured than EVOO control samples. The second group of VOCs included branched aldehydes (i.e., the sum of 2- and 3-methylbutanal), the sum of C8 VOCs, acetic acid, the sum of C3-C6 carboxylic acids and methyl acetate (Fig. 3). A significant increase in the content of all these compounds

Table 3
Capsaicinoid content in flavoured and non-flavoured olive oil samples measured over 18 months of storage and expressed in mg/kg as mean ± SD of triplicates. Pungency according to the scoville heats units (SHU) is also reported. Different letters in each column indicate significant differences at $p < 0.05$.

Sample	capsaicin (mg/kg)	diidrocapsaicin (mg/kg)	Ratio capsa/dihydrocapsa	Total (mg/kg)	Pungency (SHU)
EVOO t0	–	–	–	–	–
EVOO t1	–	–	–	–	–
EVOO t2	–	–	–	–	–
EVOO t3	–	–	–	–	–
GOOred t0	37.0 ± 0.5 j	11.1 ± 0.2 m	3.3	48.0 ± 0.7 o	768.6 ± 11.2
GOOred t1	38.3 ± 0.4 j	12.0 ± 0.2 lm	3.2	50.3 ± 0.6 no	805.2 ± 8.9
GOOred t2	37.5 ± 1.6 j	11.7 ± 0.5 lm	3.2	49.2 ± 2.1 o	786.9 ± 34.1
GOOred t3	37.5 ± 0.2 j	11.0 ± 0.1 m	3.4	48.5 ± 0.3 o	775.9 ± 4.2
GOOred t4	36.9 ± 0.6 j	12.5 ± 0.6 klm	3.0	49.4 ± 1.2 no	790.4 ± 19.2
GOOgreen t0	36.8 ± 0.8 j	15.3 ± 0.3 hij	2.4	52.0 ± 1.1 mno	832.8 ± 17.6
GOOgreen t1	35.5 ± 0.5 j	15.2 ± 0.3 hij	2.3	50.6 ± 0.8 no	809.7 ± 12.5
GOOgreen t2	35.8 ± 0.5 j	15.3 ± 0.3 hij	2.3	51.1 ± 0.7 mno	817.6 ± 11.5
GOOgreen t3	34.9 ± 2.6 j	14.6 ± 1.5 hijk	2.4	49.5 ± 4.1 no	792.2 ± 65.5
GOOgreen t4	36.6 ± 0.4 j	16.6 ± 0.3 gh	2.2	53.2 ± 0.7 mno	851.2 ± 11.2
FOOred t0	46.2 ± 0.8 i	14.1 ± 0.2 ijkl	3.3	60.2 ± 1.0 klm	963.5 ± 16.0
FOOred t1	50.2 ± 0.6 ghi	15.1 ± 0.2 hij	3.3	65.3 ± 0.9 jkl	1044.5 ± 14.0
FOOred t2	51.2 ± 1.2 ghi	15.9 ± 0.4 ghi	3.2	67.0 ± 1.7 ijkl	1072.7 ± 27.0
FOOred t3	45.3 ± 7.2 i	13.1 ± 1.9 jklm	3.4	58.5 ± 9.1 lmn	935.8 ± 145.5
FOOred t4	47.2 ± 0.5 i	15.7 ± 0.5 ghi	3.0	62.9 ± 1.0 jkl	1006.4 ± 16.0
FXOOred t0	46.2 ± 0.8 i	14.1 ± 0.2 ijkl	3.3	60.2 ± 1.0 klm	963.5 ± 16.0
FXOOred t1	55.1 ± 11.9 efg	16.2 ± 4.2 ghi	3.4	71.4 ± 16.1 ghij	1141.7 ± 258.2
FXOOred t2	60.8 ± 2.6 cde	17.9 ± 0.5 fg	3.4	78.8 ± 3.0 defg	1260.2 ± 48.8
FXOOred t3	76.7 ± 7.2 a	21.6 ± 1.5 de	3.6	98.3 ± 8.6 b	1573.1 ± 138.2
FXOOred t4	71.4 ± 12.5 ab	21.6 ± 3.2 de	3.3	93.0 ± 15.7 bc	1488.0 ± 251.2
FOOgreen t0	48.2 ± 1.0 hi	19.8 ± 0.4 ef	2.4	68.0 ± 1.4 hijk	1088.3 ± 22.4
FOOgreen t1	58.1 ± 1.3 def	23.6 ± 0.5 bcd	2.5	81.7 ± 1.8 def	1307.0 ± 28.3
FOOgreen t2	54.4 ± 3.5 efgh	22.6 ± 1.6 cd	2.4	77.0 ± 5.1 efgh	1232.1 ± 81.8
FOOgreen t3	56.9 ± 0.8 defg	21.6 ± 0.3 de	2.6	78.5 ± 1.1 defg	1256.2 ± 17.9
FOOgreen t4	52.0 ± 2.6 fghi	22.6 ± 1.4 cd	2.3	74.6 ± 4.0 fghi	1193.6 ± 64.0
FXOOgreen t0	48.2 ± 1.0 hi	19.8 ± 0.4 ef	2.4	68.0 ± 1.4 hijk	1088.3 ± 22.4
FXOOgreen t1	62.6 ± 7.2 cd	25.0 ± 3.7 bc	2.5	87.6 ± 10.9 cd	1401.2 ± 174.1
FXOOgreen t2	59.7 ± 1.2 de	24.7 ± 1.2 bc	2.4	84.4 ± 2.3 cde	1350.5 ± 37.3
FXOOgreen t3	66.8 ± 11.1 bc	26.0 ± 4.7 b	2.6	92.7 ± 15.9 bc	1483.4 ± 253.9
FXOOgreen t4	76.2 ± 12.2 a	32.6 ± 4.6 a	2.3	108.8 ± 16.8 a	1740.8 ± 268.8

GOOred, olive oil flavoured by co-milling fresh red chili peppers and sound olives; GOOgreen, olive oil flavoured by co-milling fresh green chili peppers and sound olives; FOOred, olive oil flavoured by 10-days infusion of dried red chili peppers in EVOO; FOOgreen, olive oil flavoured by 10-days infusion of dried green chili peppers in EVOO; FXOOred, olive oil flavoured by continuing infusion of dried red chili peppers in EVOO; FXOOgreen, olive oil flavoured by continuing infusion of dried green chili peppers in EVOO; EVOO, extra virgin olive oil control sample.

occurred during storage in relation to the flavouring method (Table 2), with the greatest increases observed for branched aldehydes, acetic acid and methyl acetate. Small increases of both branched aldehydes and acetic acid were observed for GOO and EVOO samples, while greater increases were observed for FOO and, particularly, for FXOO samples: in the FOO samples, the values of branched aldehydes and acetic acid approx. Doubled after 18 months of storage, while in the FXOO samples, the content of branched aldehydes and acetic acid increased approx. From 0.05 to 0.45 mg/kg and approx. From 3 to 25 mg/kg, respectively. The content of methyl acetate showed negligible values and no significant increases during storage in the EVOO, GOO and FOO samples, while a strong increase occurred in the FXOO samples, reaching amounts of 0.4–0.5 mg/kg after 18 months of storage.

3.5.2. *VOCs typical of CPFOO*

During storage of the flavoured samples, 37 VOCs typical of the flavoured olive oils with chili peppers were evaluated (Cecchi et al., 2023). First, the esters (i.e., the typical constituents of the volatile fraction of fresh chili peppers (Murakami et al., 2019; Trovato et al., 2022)) were evaluated, and their contents were reported as sum of the esters of 2-methylbutanoic acid, sum of the esters of 3-methylbutanoic acid and sum of other esters (Fig. 4). Significant effects of flavouring method, chili peppers ripening stage and storage time were observed (Table 2). The contents of above esters were much greater in the GOO than in the FOO and FXOO samples, and the highest content was in the chili peppers at the green stage of ripening. During storage, a slow decrease was observed in the GOO samples, but no significant decreases were observed in the FOO and FXOO samples. Concerning esters of 2-methylbutanoic and of 3-methylbutanoic acids, as showed in the supplementary Fig. S6, the 4-methylpentyl methylbutanoates were the most abundant, followed by hexyl methylbutanoates, 3-methylbutyl methylbutanoates and (*Z*)-3-hexenyl methylbutanoates. Concerning other esters, supplementary Fig. S7 shows a prevalence of the 4-methylpentyl 2-methylpropanoate and, only for the GOO_{green} sample, of (*E*)-2-hexenyl valerate. The pyrazines, heterocyclic compounds reported in the literature as compounds relating with fresh chili peppers aroma (Trovato et al., 2022), were also measured: only the tetramethyl pyrazine was detected in the flavoured samples by infusion, showing a small increase during storage in the $FXOO_{green}$, while it was not detected in the GOO samples (Fig. 4). Finally, the compounds potentially linked to sensory defects (Cecchi et al., 2023) were measured, and their content was reported as sum of molecules from fermentation, sum of branched carboxylic acids, dimethyl sulfone and 2-methyl propanal (Fig. 4). Significant effects of flavouring method, chili peppers ripening stage and storage time were observed (Table 2). The above compounds were only present in the flavoured samples by infusion, showing an increase with storage time particularly in the FXOO samples. The sum of molecules from fermentation reached very high contents in the FXOO samples (i.e., from approx. 3 to 37 mg/kg in the $FXOO_{red}$ samples and from 7 to 130 mg/kg in the $FXOO_{green}$ samples). As showed in the supplementary Fig. S8, the main contribution to the above values were given by both the *meso* and racemic forms of 2,3-butandiol, followed by acetoin, with low amounts of 2,3-butanedione.

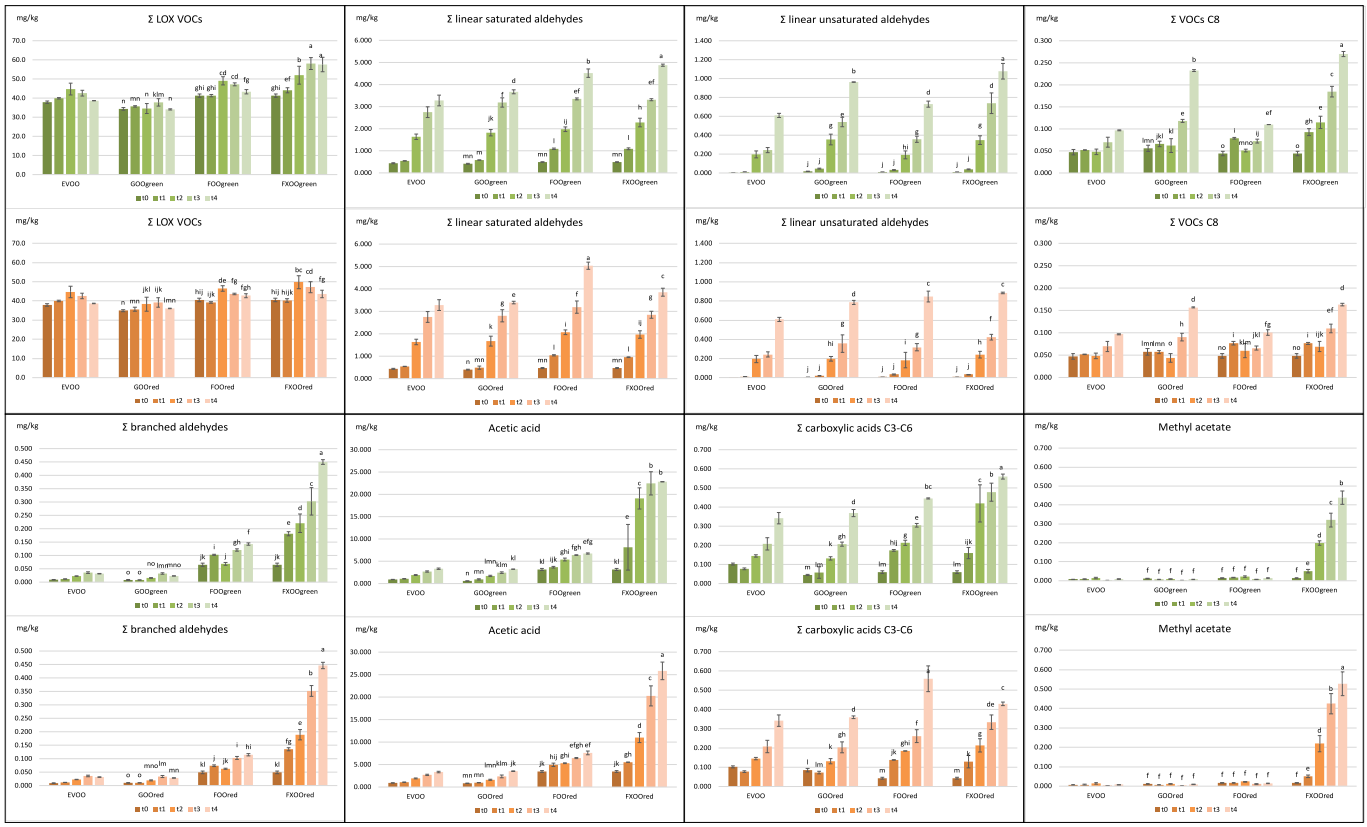

Fig. 3. Bar charts representing the evolution of the content of the volatile compounds detected in both the EVOO and the flavoured olive oil samples as a function of both flavouring method and ripening stage of chili peppers. Data are from triplicates. For each molecule or groups of them, different letters in the graphics indicate significant differences at $p < 0.05$ among the different types of flavoured samples.

3.6. Evolution of sensory profile during storage

The sensory profile of both flavoured and non-flavoured samples was studied during storage in terms of evolution of several sensory attributes as follows: (i) evolution of the negative sensory attributes (i.e., defects) usually detected in defective VOOs; (ii) evolution of the positive sensory attributes usually detected in EVOO and/or CPFOO.

3.6.1. Sensory defects

Table 4 A shows the intensity of the negative sensory attributes during storage. No sensory defect was detected in the GOO samples, while all FOO and FXOO samples were defective as early as the time zero. The winey/vinegary attribute was observed with the highest frequency and intensity in the FOO and FXOO samples; in some cases, the intensity of this attribute was even greater than 3.5, meaning that samples were downgraded to the lampante virgin olive oil category (European Parliament and the Council (EC), 2022a). After 2 months of storage, a general decrease of the winey/vinegary defect was observed as well as the onset of further defects, particularly the rancid in the FOO samples and the fusty/muddy sediment and musty/humid/earthy in the FXOO samples. Noteworthy, in the FXOO samples the onset of a clearly prevailing hay defect was observed. As for the EVOO control samples, they became defective only from 12 months of storage due to onset of the rancid attribute (intensity = 2.3), meaning the sample was downgraded to the virgin olive oil category (European Parliament and the Council (EC), 2022a).

3.6.2. Positive sensory attributes

Table 4B shows the intensity of positive sensory attributes during storage. Concerning the fruity attributes (i.e., green and ripe olive fruity, fresh and dry pepper fruity), the pepper fruity largely prevailed over olive fruity in GOO samples, and the fresh pepper fruity prevailed over the dry pepper fruit, being greater in GOO_{green} than in GOO_{red} samples. Instead, in FOO and FXOO samples, no fresh pepper fruity as well as no green olive fruity were perceived, and the ripe olive and dry pepper fruity attributes were prevalent. Pepper taste attributes were also perceived in the flavoured samples. The fresh pepper taste characterized the GOO_{green} samples, while similar intensities of fresh and dry pepper taste attributes occurred in the GOO_{red} samples. In the FOO and FXOO samples, dry pepper taste prevailed over fresh pepper taste, and the fresh pepper taste attribute was only perceived at low intensity. In the CPFOOs, the pungency attribute is crucial, since it is the sensory attribute mainly associated with chili peppers due to the presence of capsaicinoids. However, in the CPFOO this attribute is different than the typical pungency of the EVOO, and the panelists described the former as hotness/heat (tongue/mid palate) and the latter as pungency (throat) (Cecchi et al., 2023; Guzman & Bosland, 2017). In the GOO samples, the intensity of the hotness/heat (tongue/mid palate) prevailed over that of pungency (throat). Concerning the other attributes, the bitterness was perceived in the flavoured samples at lower intensities than in the EVOO control samples, likely due to a masking effect given by the pungency and peppery attributes. Other attributes such as green almond, sweet almond and artichoke/thistle were perceived in the EVOO control samples, but at very low intensities in the flavoured samples. Finally, dry tomato was also perceived in the flavoured samples.

4. Discussion

Several processing conditions can influence the quality of CPFOOs. Among them, the quality of olive oil/olives and chili peppers (including their soundness and ripening level), the flavouring method and the percentage amount of chili peppers are critical factors. The literature has

Fig. 4. Bar charts representing the evolution of the content of the volatile organic compounds only detected in the flavoured olive oil samples as a function of both flavouring method and ripening stage of chili peppers. Data are from triplicates. For each molecule or groups of them, different letters in the graphics indicate significant differences at $p < 0.05$.

shown that some of them cause a quality decrease of the CPFOO in relation to the flavouring method, as follows: (i) the low-quality of olive oil using the "infusion" and "direct mixing of extracts" methods; (ii) the low-quality of olives using the "co-milling" and "co-extraction" methods; (iii) the low-quality chili peppers using the "co-milling" method; (iv) the dried chili peppers (characterized by off-flavours and very low amounts of the VOCs typical of chili peppers) using the "infusion" methods (Caporaso et al., 2013; Cecchi et al., 2023; Ciafardini et al., 2004; Lamas et al., 2022; Montoya-Ballesteros et al., 2014; Stefanidis et al., 2023). In this research, the effects of co-milling fresh chili peppers with sound olives and infusion flavouring methods on the quality of CPFOOs during storage were compared for the first time (Baiano et al., 2009; Caponio et al., 2016).

4.1. *Effects of the flavouring methods on the chemical and sensory parameters of the CPFOOs during storage*

The flavouring methods did not affect the variation of legal quality parameters of the oil samples during storage (Table S1). A slight increase of free acidity over storage occurred in all oil samples, suggesting weak hydrolysis of triglycerides during storage. The presence of chili peppers in the FOO and FXOO samples did not caused increases in free acidity greater than in the GOO and EVOO control samples; the above behavior can be explained by both the dried form of chili peppers and the low initial free acidity of the EVOO used for infusion (Custureri et al., 2023; Peres et al., 2023; Zellama et al., 2022). Thanks to the optimal storage conditions applied (i.e., no light exposure, no headspace in the bottles (Cecchi et al., 2019)), the increases in peroxide value and UV indices due to the auto-oxidation of triglycerides were very small, regardless of flavouring methods applied.

The flavouring methods affected the content of minor compounds coming from olive fruits (i.e., tocopherols and phenolic compounds) and chili peppers (i.e., tocopherols and capsaicinoids). The infusion method caused an increase in tocopherols content (Table 1) in FOO and FXOO samples in comparison with GOO and EVOO samples, due to the lipid solubility of chili pepper tocopherols (Custureri et al., 2023; Sousa et al., 2015), but the FOO and FXOO samples had the highest decrease in tocopherols content during storage. Since tocopherols degradation can be ascribed to oxidative phenomena, the flavoured oil samples by co-milling method resulted the most protected to oxidation, meaning that fresh chili peppers had higher antioxidant power than the dried ones. Instead, the lower tocopherols content in the GOO samples (even in comparison with the EVOO control samples), was unexpected and difficult to explain; the co-milling method could have caused not only a lowered tocopherols extractability from chili peppers but also an impediment to extraction of tocopherols from olive paste during malaxation. Concerning phenolic compounds (Fig. 2), the co-milling method led to an increase in total phenolic content in GOO samples and to the lowest phenolic degradation, which occurred mainly in the last 6 months of storage. The GOO samples were enriched in phenolic compounds directly during extraction, also resulting the most protected against oxidation phenomena, as already observed in the case of tocopherols. In all samples, the higher decreasing rate for oleacein and oleuropein aglycone than for oleocanthal was probably caused by the presence in the chemical structure of the first two molecules of the *o*-diphenol moiety, which is more susceptible to oxidation than the monophenol moiety present in the chemical structure of oleocanthal (Napolitano et al., 2010). The capsaicinoids content of oil samples was influenced by the flavouring method, and no significant decrease occurred during storage (Table 3). The infusion method promoted the highest extractability of capsaicinoids from chili peppers, and the permanent infusion even led to increase in capsaicinoids content in FXOO

Table 4
(A) Intensity of the sensory defects in the flavoured and non-flavoured olive oil samples. (B) Intensity of the positive sensory attributes in the flavoured and non-flavoured olive oil samples.

A)

Sample	Fusty / Muddy sediment	Musty/humid/earthy	Winey/vinegary	Rancid	hay
EVOO t0	–	–	–	–	–
EVOO t1	–	–	–	–	–
EVOO t2	–	–	–	–	–
EVOO t3	–	–	–	2.3	–
EVOO t4	–	–	–	3.1	–
GOOgreen t0	–	–	–	–	–
GOOgreen t1	–	–	–	–	–
GOOgreen t2	–	–	–	–	–
GOOgreen t3	–	–	–	–	–
GOOgreen t4	–	–	–	–	–
GOOred t0	–	–	–	–	–
GOOred t1	–	–	–	–	–
GOOred t2	–	–	–	–	–
GOOred t3	–	–	–	–	–
GOOred t4	–	–	–	–	–
FOOgreen t0	–	–	2.0	–	–
FOOgreen t1	0.2	0.3	4.3	0.3	–
FOOgreen t2	–	–	–	–	–
FOOgreen t3	1.1	–	0.6	1.9	–
FOOgreen t4	0.3	–	1.5	1.3	–
FXOOgreen t0	–	–	2.0	–	–
FXOOgreen t1	1.5	1.0	3.2	–	–
FXOOgreen t2	1.4	–	3.6	–	–
FXOOgreen t3	1.5	0.4	1.1	1.0	–
FXOOgreen t4	0.6	2.3	0.9	1.9	5.1
FOOred t0	–	–	1.7	–	–
FOOred t1	–	–	3.0	–	–
FOOred t2	–	–	1.0	–	–
FOOred t3	0.1	–	0.7	2.6	–
FOOred t4	–	–	–	2.3	–
FXOOred t0	–	–	1.7	–	–
FXOOred t1	1.2	0.4	2.4	–	–
FXOOred t2	1.3	1.4	1.0	–	–
FXOOred t3	0.8	0.9	1.3	0.7	–
FXOOred t4	1.0	0.5	–	–	2.2

B)

Sample	Green olive fruity	Ripe olive fruity	Fresh pepper fruity	Dry pepper fruity	Tomato leaf fruity	Bitter	Pungent (throat)	Hotness/Heat (Tongue/ Mid palate)	Astringency
EVOO t0	5.2	–	–	–	–	4.6	5.3	–	3.5
EVOO t1	5.4	4.3	–	–	0.3	5.6	6.1	–	4.0
EVOO t2	–	3.5	–	–	–	5.8	4.7	–	1.2
EVOO t3	–	2.5	–	–	–	2.7	2.7	–	0.9
EVOO t4	–	1.9	–	–	–	3.3	0.5	–	1.6
GOOgreen t0	0.7	1.1	7.0	1.4	0.4	3.4	3.1	7.1	–
GOOgreen t1	–	–	7.2	–	–	1.8	5.4	6.8	0.1
GOOgreen t2	0.3	–	6.2	0.3	–	2.2	3.4	6.6	–
GOOgreen t3	–	0.4	6.3	–	–	1.1	5.4	4.3	1.9
GOOgreen t4	–	1.6	5.5	5.5	–	1.4	2.5	6.8	0.7
GOOred t0	2.1	1.2	5.5	3.8	–	3.3	4.5	6.1	1.3
GOOred t1	–	–	4.9	3.1	–	4.3	4.8	3.8	0.8
GOOred t2	0.2	–	4.0	1.7	–	1.7	2.6	5.4	–
GOOred t3	–	1.3	4.6	2.1	–	2.8	3.0	4.8	0.2
GOOred t4	–	1.7	2.7	3.7	–	3.4	3.5	4.0	1.9
FOOgreen t0	–	3.0	–	2.6	–	2.0	5.6	4.8	0.3
FOOgreen t1	–	2.0	–	0.6	–	0.6	7.8	4.1	0.2
FOOgreen t2	–	1.3	–	1.7	–	2.4	5.1	6.2	–
FOOgreen t3	–	0.6	–	2.0	–	1.8	4.0	6.5	–
FOOgreen t4	–	1.5	–	3.4	–	2.1	2.3	7.6	0.3
FXOOgreen t0	–	3.0	–	2.6	–	2.0	5.6	4.8	0.3
FXOOgreen t1	–	0.7	–	0.4	–	0.2	4.9	5.9	0.1
FXOOgreen t2	–	0.4	–	1.6	–	1.1	6.6	7.2	–
FXOOgreen t3	–	0.6	–	1.5	–	2.9	4.7	6.8	0.4
FXOOgreen t4	–	1.1	–	1.4	–	1.3	5.2	3.3	0.7

(*continued on next page*)

Table 4 (*continued*)

B)

Sample	Green olive fruity	Ripe olive fruity	Fresh pepper fruity	Dry pepper fruity	Tomato leaf fruity	Bitter	Pungent (throat)	Hotness/Heat (Tongue/ Mid palate)	Astringency
FOOred t0	–	1.9	–	3.2	–	1.8	2.7	5.1	0.6
FOOred t1	–	2.3	–	2.1	–	2.1	6.1	3.9	0.6
FOOred t2	–	1.3	1.6	2.3	–	1.6	4.1	5.1	–
FOOred t3	–	0.1	–	1.9	–	1.0	3.9	3.7	0.2
FOOred t4	–	1.6	–	2.1	–	3.9	4.5	4.0	1.2
FXOOred t0	–	1.9	–	3.2	–	1.8	2.7	5.1	0.6
FXOOred t1	–	0.2	–	2.5	–	0.9	4.9	5.6	0.4
FXOOred t2	–	0.2	–	2.6	–	1.5	4.9	6.2	0.1
FXOOred t3	–	0.8	–	2.2	–	1.6	4.6	4.6	0.2
FXOOred t4	–	1.5	–	3.5	–	1.1	4.8	4.5	0.9

Sample	Fresh pepper taste	Dry pepper taste	Green almond	Dry almond	Sweet almond	Artichoke/ Thistle	Dry tomato	Green banana	Yellow apple	Grass	Dry tomato fruity
EVOO t0	–	–	3.4	–	0.4	2.8	–	–	–	2.0	–
EVOO t1	–	–	3.9	–	0.5	3.6	–	–	0.2	2.2	–
EVOO t2	–	–	0.2	–	2.1	1.8	–	–	1.5	–	–
EVOO t3	–	–	0.6	0.2	1.2	1.4	–	0.3	–	–	–
EVOO t4	–	–	–	–	0.2	–	–	–	–	–	–
GOOgreen t0	6.5	–	–	–	0.3	–	–	–	–	–	–
GOOgreen t1	6.0	–	–	–	–	–	0.2	–	–	–	–
GOOgreen t2	6.6	–	–	–	–	–	0.2	–	–	–	–
GOOgreen t3	6.5	–	–	–	–	–	–	–	–	–	–
GOOgreen t4	6.8	2.4	–	–	–	–	–	–	–	–	–
GOOred t0	5.8	3.4	–	–	–	0.7	0.2	–	–	–	–
GOOred t1	4.1	3.3	–	0.3	–	0.2	1.0	0.5	–	–	–
GOOred t2	3.3	2.1	–	–	–	–	1.2	–	–	–	–
GOOred t3	2.9	3.1	–	–	–	0.8	–	–	–	–	–
GOOred t4	1.5	3.7	–	–	–	–	1.0	–	–	–	–
FOOgreen t0	1.1	4.5	–	–	–	–	1.0	–	–	0.2	–
FOOgreen t1	0.6	2.3	–	0.3	–	–	0.4	–	–	0.1	–
FOOgreen t2	–	2.9	–	–	–	–	1.5	–	–	–	1.1
FOOgreen t3	–	2.7	–	–	–	–	–	–	–	–	–
FOOgreen t4	–	3.3	–	–	–	–	–	–	–	–	–
FXOOgreen t0	1.1	4.5	–	–	–	–	1.0	–	–	0.2	–
FXOOgreen t1	–	3.1	–	0.6	–	–	0.5	–	–	–	–
FXOOgreen t2	–	3.5	–	–	–	–	1.6	–	–	–	–
FXOOgreen t3	–	2.8	–	–	–	–	0.1	–	–	–	–
FXOOgreen t4	–	3.1	–	–	–	–	–	–	–	–	–
FOOred t0	–	4.2	–	–	–	0.1	0.2	–	–	–	–
FOOred t1	0.7	3.1	0.1	–	–	0.1	1.1	–	1.0	–	–
FOOred t2	1.7	3.7	–	–	–	–	1.3	–	–	–	1.0
FOOred t3	1.1	2.0	–	–	–	–	–	–	–	–	–
FOOred t4	–	2.1	–	–	–	–	–	–	–	–	–
FXOOred t0	–	4.2	–	–	–	0.1	0.2	–	–	–	–
FXOOredt1	–	2.4	0.7	–	–	–	–	1.4	–	–	–
FXOOredt2	–	4.2	–	–	–	–	1.8	–	–	–	1.2
FXOOredt3	1.1	1.6	–	–	–	–	2.3	–	–	–	–
FXOOred t4	–	3.3	–	–	–	–	–	–	–	–	–

GOOred, olive oil flavoured by co-milling fresh red chili peppers and sound olives; GOOgreen, olive oil flavoured by co-milling fresh green chili peppers and sound olives; FOOred, olive oil flavoured by 10-days infusion of dried red chili peppers in EVOO; FOOgreen, olive oil flavoured by 10-days infusion of dried green chili peppers in EVOO; FXOOred, olive oil flavoured by continuing infusion of dried red chili peppers in EVOO; FXOOgreen, olive oil flavoured by continuing infusion of dried green chili peppers in EVOO; EVOO, extra virgin olive oil control sample.

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samples during storage.

The sensory attributes of all oil samples changed during storage depending on the flavouring method (Table 4). The sensory profile of the flavoured oil samples was different from that of the EVOO samples, but also among each other. The samples flavoured by infusion were overall characterized by hotness/heat (tongue/mid palate), and they showed the presence of sensory defects (i.e., winey/vinegary, fusty/muddy sediment, musty/humid/earthy, rancid) during the entire storage period. Instead, the samples flavoured by co-milling were characterized by the hotness/heat (tongue/mid palate), and pepper fruity and taste, and they were free from sensory defects during the entire storage. The intensity of the hotness/heat attribute in the flavoured samples was directly relating to the presence of capsaicinoids, which were almost entirely represented by capsaicin and dihydrocapsaicin, the

capsaicinoids with the most typical heat sensation (Krajewska & Powers, 1988). The presence of both positive attributes and sensory defects was instead consistent with the VOCs profile. The literature data suggested that the rancid defect is mainly due to the presence of saturated (Σ LSA) and unsaturated aldehydes (Σ LUA) (Angerosa et al., 1996; Cecchi, Migliorini, Giambanelli, et al., 2021; Cecchi, Migliorini, & Mulinacci, 2021; Morales et al., 2005), and experimental data of this research partially agreed, as follows. No rancid defect occurred until 6 months of storage in all oil samples in agreement with the low to medium content of Σ LSA and Σ LUA, while this defect was perceived at 12 and 18 months of storage when higher contents of Σ LSA and Σ LUA were observed (Fig. 3). The GOO samples represented an exception, as the rancid defect was never perceived during storage. The latter behavior can be explained assuming a masking effect given by the combination of hotness/heat sensation and medium to intense pepper fruity and taste, in turn given by the typical volatile compounds of chili peppers (Fig. 4) (Cecchi et al., 2023; Trovato et al., 2022), confirming the importance of interaction and masking effects among different VOCs and other types of molecules on the sensory perception (Belitz et al., 2004; Caporale et al., 2004; Cecchi, Migliorini, & Mulinacci, 2021).

Defects with microbiological origin such as winey/vinegary, fusty/muddy sediment and musty/humid/earthy sensory defects were perceived in both FOO and FXOO samples from the beginning of storage, and they were consistent with the contents of acetic acid, branched aldehydes and other molecules from fermentation (Figs. 3 and 4). In the literature, the increase in branched aldehydes, methyl acetate, acetic acid and molecules from fermentation in VOO and CPFOO has been associated to enzymatic activities of microorganisms on organic substrate (e.g., pectinase activity for methyl acetate, amino acid metabolism for 2- and 3-methylbutanal, fermentation for acetic acid) (Angerosa et al., 1996; Cecchi, Migliorini, Giambanelli, et al., 2021) and to fermentations taking place during mild-conditions drying of chili peppers (Caporaso et al., 2013; Cecchi et al., 2023; Cecchi, Migliorini, Giambanelli, et al., 2021; Toontom et al., 2016).

4.2. Shelf-life of the CPFOOs

In this research, the fresh CPFOO samples were generally proved to be EVOO-based dressings with peculiar sensory attributes (e.g., hotness/heat, pepper fruity and pepper taste) and enriched in molecules with antioxidant activities. The infusion method resulted the most effective to increase the tocopherols and capsaicinoids contents, while the co-milling method resulted the most effective one to increase the content in phenolic compounds. Concerning sensory attributes, the co-milling method led to CPFOO samples (i.e., the GOO ones) free from perceivable defects also giving a nice balance among hotness/heat (tongue/mid palate) and fresh pepper fruity and fresh pepper taste attributes while, unfortunately, the samples flavoured by infusion already showed sensory defects (i.e., winey/vinegary attribute) at t0.

Despite the storage of all samples was carried out under optimal oxidation-protective conditions (i.e., no light exposure, no headspace in the bottle, room temperature), it represented a critical processing step since it was responsible for significant degradation of sample characteristics. All samples were involved in somewhat oxidation phenomena, which caused degradation of tocopherols and phenolic compounds (with consequent decrease in their contents) and, in those samples where the masking effect of sensory perception was not active, the perception of the rancid defect. Samples flavoured by infusion were found to be the most susceptible to oxidation phenomena, due to heat and oxidative damages during air-drying which may have led to high-redox-potentials compounds in dried chili peppers, explaining the behavior above-described. Samples flavoured by infusion were also involved in enzymatic phenomena of spoilage microorganisms, which caused the increase in the content of VOCs responsible for several sensory defects; the residual water in dried chili peppers and solid micro-residues of chili peppers into the oils can explain such microbial activities (Caporaso et al., 2013; Cecchi et al., 2023; Cecchi, Migliorini, Giambanelli, et al., 2021; Montoya-Ballesteros et al., 2014; Toontom et al., 2016).

To evaluate the shelf-life of CPFOOs without a specific EU legislative framework on these oils, reference was made to the UE law of EVOO. All CPFOO samples were compliant with the extra virgin olive oil category, remaining well below the legal limits of the chemical quality parameters over 18 months of storage. Therefore, the assessment of the risk of quality loss of CPFOO samples was evaluated through the sensory profiles. All samples flavoured by infusion resulted characterized by several sensory defects already after 2 months of storage, and therefore were considered at high risk of quality loss. On the contrary, the samples flavoured by co-milling resulted free from sensory defects up to 18 months of storage (i.e., the entire experimental period), and can therefore be considered at very low risk of quality loss. The storability of the samples flavoured by co-milling was even higher than the EVOO control samples, which were downgraded to the virgin olive oil category after 1 year of storage due to the onset of the rancid defect.

It is worth noting that, despite the quality parameters remained well below the legal limits for the EVOO category for all samples over the whole storage period, several samples were perceived defective (Table S1, Table 4), underlying how the sensory quality of the oils is relating much more to the presence/absence of minor compounds such as phenolic compounds, volatile compounds and (in the case of CPFOO) capsaicinoids than to the increase of legal quality parameters.

5. Conclusions

This is the first research article focused on chili peppers flavoured olive oils where vitamin E (i.e., tocopherols), capsaicinoids, typical phenolic compounds of virgin olive oil, typical volatile compounds of virgin olive oil and volatile compounds deriving from co-milling fresh chili peppers and sound olives, in addition to a legal quality parameters and detailed sensory data, were all analyzed for a comprehensive characterization of the flavoured oils. A single analytical method for the simultaneous analysis of typical phenolic compounds of virgin olive oil (i.e., secoiridoid derivatives) and capsaicinoids has been proposed which simplifies the analytical work required to characterize chili pepper flavoured olive oils.

The data of this research confirmed the hypothesis that co-milling of sound olives with fresh chili peppers improves the storability of CPFOOs in comparison with the typical infusion in terms of both sensory quality parameters and degradation of minor compounds and VOCs. In fact, samples flavoured by infusion (FOO/FXOO) were perceived defective during storage, while those flavoured by co-milling (GOO) remained free from sensory defects and characterized by a nice balance among hotness/heat and fresh pepper fruity and taste for the entire storage period (i.e., 18 months). Interestingly, the storability of the latter samples was even improved in comparison to the EVOO control samples. From the chemical viewpoint, GOO samples were by far the poorest in VOCs relating to sensory defects and the richest in positive VOCs from chili peppers such as ester of 2- and 3-methylbutanoic acids over entire storage. In FOO and FXOO samples, fast increases of VOCs relating to defects (i.e., 2- and 3-methylbutanal, 2-methyl propanal, acetic acid, methyl acetate, acetoin, 2,3-butanedione, 2,3-butandiol (both racemic and *meso* forms), branched carboxylic acids) occurred. Over entire storage, samples flavoured by co-milling were the richest in secoiridoid compounds, while those flavoured by infusion were the richest in tocopherols and capsaicinoids.

The results also suggested that the infusion method should be improved, minimizing negative effects of the chili peppers drying. Avoiding the sensory defects of CPFOOs by infusion, the above different flavouring methods can allow for production of different types of chili peppers flavoured oils, suitable for both different segments of consumers and culinary uses. This aspect must be the subject of next research on this topic. Implementation of the co-milling method in olive oil flavouring processing can be considered sustainable in comparison to the

infusion method, since the "co-milling" method has some processing advantages such as: (i) it does not require additional equipment in the oil mill; (ii) it does not require the chili peppers drying step; (iii) it is timesaving and easier to control than the infusion method, requiring keeping under control less operating conditions in relation to the risk of oil off-flavours.

Consent for publication

All authors have reviewed and approved the final version of the manuscript for publication.

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GOOred, olive oil flavoured by co-milling fresh red chili peppers and sound olives; GOOgreen, olive oil flavoured by co-milling fresh green chili peppers and sound olives; FOOred, olive oil flavoured by 10-days infusion of dried red chili peppers in EVOO; FOOgreen, olive oil flavoured by 10-days infusion of dried green chili peppers in EVOO; FXOOred, olive oil flavoured by continuing infusion of dried red chili peppers in EVOO; FXOOgreen, olive oil flavoured by continuing infusion of dried green chili peppers in EVOO; EVOO, extra virgin olive oil control sample.

Hydrolysis ratio: ratio between the sum of tyrosol+hydroxytyrosol and the total phenolic content.

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Σ LOX VOCs: sum of 1-penten-3-ol, 1-penten-3-one, (E)-2-pentenal, (E)-2-pentenol, (Z)-2-pentenol, hexanal, 1-hexanol, hexyl acetate, (Z)-3-hexenal, (Z)-3-hexenol, (Z)-3-hexenyl acetate, (E)-2-hexenal, (E)-2-hexenol, (E)-2-hexenyl acetate, (E)-3-hexenol, (Z)-2-hexenol.

Σ linear saturated aldehydes: sum of pentanal, hexanal, heptanal, octanal, nonanal.

Σ linear unsaturated aldehydes: sum of (E)-2-heptenal, (E)-2-nonenal, (E)-2-decenal, (E,E)-2,4-heptadienal, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal.

Σ VOCs C8: sum of octane, 1-octen-3-one, 1-octen-3-ol, 1-octanol.

Σ branched aldehydes: sum of 2-methylbutanal and 3-methylbutanal.

Σ carboxylic acids C3-C6: sum of propanoic, butanoic, pentanoic and hexanoic acid.

GOOred, olive oil flavoured by co-milling fresh red chili peppers and sound olives; GOOgreen, olive oil flavoured by co-milling fresh green chili peppers and sound olives; FOOred, olive oil flavoured by 10-days infusion of dried red chili peppers in EVOO; FOOgreen, olive oil flavoured by 10-days infusion of dried green chili peppers in EVOO; FXOOred, olive oil flavoured by continuing infusion of dried red chili peppers in EVOO; FXOOgreen, olive oil flavoured by continuing infusion of dried green chili peppers in EVOO; EVOO, extra virgin olive oil control sample.

Σ esters of 2-methyl butanoic acid: sum of 2-methylpropyl-2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, pentyl 2-methylbutanoate, 4-Methylpentyl 2-methylbutanoate, hexyl 2-methylbutanoate, (Z)-3-hexenyl 2-methylbutanoate, (E)-2-hexenyl 2-methylbutanoate, 4-methylhexyl 2-methylbutanoate, 6-Methylhept-4-en-1-yl 2-methylbutanoate.

Σ esters of 3-methyl butanoic acid: sum of 2-methylpropyl-3-methylbutanoate, butyl-3-methyl butanoate, 3-methylbutyl 3-methylbutanoate, 4-Methylpentyl 3-methylbutanoate, hexyl 3-methylbutanoate, (Z)-3-hexenyl 3-methylbutanoate, (E)-2-hexenyl 3-methylbutanoate, 5-methylhexyl 3-methylbutanoate, 6-Methylhept-4-en-1-yl 3-methylbutanoate.

Σ other esters: sum of 4-methylpentyl 2-methylpropanoate, hexyl 2-methylpropanoate, C10 ester (C4 acid, C6 alcohol), trans-2-Hexenyl valerate, 4-Methylpentyl 4-methylpentanoate.

Σ molecules from fermentation: sum of 2,3-Butanedione, acetoin, 2,3-Butanediol racemic, 2,3-Butanediol meso.

Σ branched carboxylic acids: sum of 3-methyl butanoic acid, 4-methyl pentanoic acid, 2-ethyl hexanoic acid.

CRediT authorship contribution statement

Lorenzo Cecchi: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Silvia Urciuoli:** Methodology, Investigation, Formal analysis, Conceptualization. **Francesca Ieri:** Methodology, Formal analysis, Data curation. **Tommaso Ugolini:** Writing – review & editing, Methodology, Formal analysis. **Silvia D'Agostino:** Investigation, Formal analysis. **Carlotta Breschi:** Investigation, Formal analysis. **Diletta Balli:** Methodology, Investigation. **Bruno Zanoni:** Writing – original draft, Supervision. **Nadia Mulinacci:** Writing – original draft, Resources, Project administration, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.141817.

Data availability

Data will be made available on request.

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