

A ‘Mediterranean ice-cream’: Sensory and nutritional aspects of replacing milk cream with extra virgin olive oil

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ABSTRACT

In this study, we explored the sensory and nutritional properties of innovative ice-creams designed totally replacing cow’s milk cream with extra virgin olive oil (EVOO).

Milk and chocolate flavored ice-creams containing 14.1% and 10.2% milk cream or 5.1% and 3.6% of EVOO were produced.

In a triangle test, only the milk-flavored ice-cream with EVOO was distinguished from its traditional counterpart whereas in a quantitative descriptive sensory analysis EVOO ice-creams showed an increased intensity of the descriptor “grassy” that was supported by the greater concentrations of specific volatile organic compounds coming from EVOO, compared to traditional ice-creams. Moreover, EVOO ice-creams contained less saturated fatty acids and more mono- and poly-unsaturated fatty acids along an increased concentration of bioaccessible polyphenols and antioxidant activity measured after an *in vitro* digestion, which was more pronounced in milk-flavored sample. Data showed that EVOO can be a functional fat replacer in ice-cream recipes to produce healthier products.

1. Introduction

Epidemiological evidence shows a negative association between the consumption of extra virgin olive oil (EVOO), the recommended fat in Mediterranean diet, and the risk of cardiovascular diseases (Guasch-Ferré et al., 2014; George et al., 2019), hypertension (Massaro et al., 2020), diabetes (Casas et al., 2018), Alzheimer (Román et al., 2019), colon and breast (Casas et al., 2018).

The chemical composition of EVOO plays a key role in its effects on human health. EVOO bioactive compounds include oleic acid, phenolic compounds, squalene and other minor compounds (Jimenez-Lopez et al., 2020). Among EVOO’s phenolic compounds, liguostroside, oleuropein aglycones and their demethoxy-dialdehydic forms, hydroxytyrosol, tyrosol are the most abundant (Romani et al., 2019). The health claim of EVOO polyphenols to prevent LDL oxidation, thus protecting blood lipids from oxidative damage, has been also authorized by the European Food Safety Authority (EFSA) when the polyphenol content is >200 mg/kg (EFSA Journal, 2011). However, the richer is in polyphenol

the stronger is the bitter and pungent taste of the EVOO and this reduces consumer acceptability (Vitaglione et al., 2013; Cavallo et al., 2019). The interaction between polyphenols and milk proteins in gastronomic preparations has, however, an impact on both the biological activities (Almajano et al., 2007) and sensory properties (Pripp et al., 2005). Binding affinity of polyphenols to proteins is size-dependent and increases with their molecular size (Pérez-Gregorio et al., 2020), therefore the small polyphenols in EVOO may establish weak interaction with milk proteins. Clarifying the characteristics of such interaction and their implications in biological mechanisms underpinning the health benefits upon consumption is crucial to determine the efficacy of EVOO polyphenols as functional ingredients in dairy products (Tosif et al., 2021).

It is a matter of fact that food polyphenols have a low bioavailability as such and several metabolites with many biological properties are formed after consumption in humans (Di Lorenzo et al., 2021). This justifies the plethora of biological effects that food polyphenols can exert *in vivo* that go well beyond the antioxidant activity of parent compounds (Di Lorenzo et al., 2021). The interaction of polyphenols with the other

Abbreviations: EFSA, European Food Safety Authority; EVOO, extra virgin olive oil; LDL, low-density lipoprotein; TAC, total antioxidant capacity; VOCs, volatile organic compounds.

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Table 1

Amount (kg and %) of each ingredient used in the formulations of 4 ice-creams: milk (M), milk with EVOO (MO), chocolate (C), chocolate with EVOO (CO).

	M		MO		C		CO	
	kg	%	kg	%	kg	%	kg	%
Water	2.4	54	3	64	2.4	51	3	60
Sugar	0.7	16	0.7	15	0.8	18	0.9	17
Pregel	0.8	17	0.8	16	0.8	16	0.8	15
EVOO	0	0	0.2	5	0	0	0.2	4
Milk Cream	0.6	14	0	0	0.5	10	0	0
Cocoa powder	0	0	0	0	0.2	5	0.2	5

food components affects their bioaccessibility upon digestive processes and the extent of metabolism they are subjected within the gastrointestinal tract, also through the gut microbiota, or after absorption (Di Lorenzo et al., 2021; Silberberg et al., 2005). Specifically, the complexes polyphenol-protein in foods, may function as polyphenol carriers in the gastro-intestinal tract, improving their stability and solubility in the food matrices and minimizing their biotransformation or delivering them in the lower gut (Tosif et al., 2021).

Whether and how these complexes affect antioxidant activity is still a matter of debate among scientists. Majority of studies showed an increase of *in vitro* antioxidant activity as well as the anti-proliferative effect upon simulated digestion of tea, coffee and cocoa catechins after the interaction with milk proteins (von Staszewski et al 2012; Stojadinovic et al 2013; Tosif et al., 2021). Few studies have been published focusing on EVOO polyphenols and whey proteins.

Recently, an artisanal ice-cream made by adding EVOO (12%) to the conventional ice-cream containing milk cream (14%), thus fortifying the product with EVOO polyphenols, showed a slight bitter taste (Sacchi et al., 2019). To the best of our knowledge, in a previous study ice-creams formulated by fully replacing milk fat with EVOO were developed to evaluate their physico-chemical and sensory properties (Güven et al., 2018).

The aim of this work was to develop innovative ice-creams with milk cream totally replaced by EVOO and explore their sensory and nutritional properties.

2. Materials and methods

2.1. EVOO quality indices, sensory analysis, volatile compounds and total polyphenols

EVOO used in the ice-cream manufacturing was analyzed to assess the acidity, peroxide value (PV), K_{232} , K_{270} , and ΔK , in accordance with the EU official method (EEC Reg. 2568/91). Acidity was expressed as oleic acid percentage (%); PV was expressed as meq O_2 kg^{-1} oil. For the analysis of spectrophotometric indices, an ultraviolet-visible UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) was used.

Sensory analysis of the EVOO was performed by a trained panel according to the EC method (EEC Reg. 2568/91 and subsequent updates).

The analysis of volatile compounds was performed using the solid

Table 2

Nutrition facts of the 4 ice-creams per 100 g. Milk (M), milk with EVOO (MO), chocolate (C), chocolate with EVOO (CO). DRV: Dietary Reference Value.

	M		MO		C		CO	
	DRV (%)	DRV (%)	DRV (%)	DRV (%)	DRV (%)	DRV (%)	DRV (%)	DRV (%)
Fat (g)	7.6	9.8	7.7	9.9	7.3	9.4	7.0	9.0
Saturated fat (g)	2.9	13.1	0.8	3.6	2.9	13.1	0.7	3.2
Carbohydrates (g)	21.7	7.9	20.7	7.5	24.0	8.7	23.2	8.4
Sugars (g)	17.3	34.6	16.5	33.0	19.2	38.4	18.7	37.4
Proteins (g)	3.9	7.8	3.4	6.8	4.7	9.4	4.2	8.4
Sodium (g)	0.1	5.0	0.1	5.0	0.1	5.0	0.1	5.0
Energy (kcal)	170.8	8.5	165.7	8.3	180.5	9.0	172.6	8.6

phase microextraction technique (SPME) followed by gas chromatography coupled to mass spectrometry (GC/MS) analysis. The SPME device (Supelco Co., Bellefonte, PA, USA) was equipped with a 50/30 μm thick divinyl-benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre coated with 1 cm length stationary phase. The extraction of volatile compounds in EVOO was performed according to Sacchi et al. (2015). Briefly, 3 g of EVOO were added to a 10 mL vial with 10 μL of isobutyl acetate (Sigma-Aldrich, St. Louis, USA; 600 mg kg^{-1} in refined olive oil), that was used as internal standard. The fibre was exposed for 30 min at 40 °C after 10 min at 40 °C for equilibration. The GC/MS conditions are detailed in the par. 2.4.

The extraction and quantification of total phenolic compounds was carried out by using the Folin-Ciocalteu colorimetric assay according to Sacchi et al. (2015). It was performed on EVOO in order to assess the quantity of phenolics that would have been added in ice-cream.

2.2. Ice-cream manufacture

Two differently flavored ice-creams, white milk (M) and chocolate (C) were made by replacing milk cream with EVOO (MO and CO, respectively).

Ice-creams were produced by an artisanal ice-cream laboratory (Bar Siano, Paestum, Salerno, Italy). The four ice-creams were made so that, replacing the milk cream with the EVOO (Table 1), they all had the same amount of fats, carbohydrates, proteins, and other elements (Table 2). Specifically, a commercial semi-finished product for ice-cream (Pregel, PreGel S.p.a., Reggio Emilia, Italy) was added to water, sucrose and milk cream or EVOO ("De Santis", Bitonto, Bari) for M and MO, respectively, plus cocoa powder for C and CO, respectively. All the ingredients were mixed in an ice-cream machine (Frigomat PL Professional, Guardamiglio, Lodi, Italy) and, once ready, the ice-creams were stored at -18 °C and used for the analyses within a week. The semi-finished commercial ice-cream product (Pregel) used in the formulation of ice-creams was made of cow milk powder (65%), dextrose, emulsifiers (E471, E477), dietary fibers, thickeners (E412, E266) and flavors.

2.3. Sensory analysis

A trained panel ($n = 14$) performed sensory analysis of EVOO (EEC Reg. 2568/91 and subsequent modifications) and ice-creams (Sacchi et al., 2019).

A quantitative descriptive sensory analysis (QDA) and a discriminative sensory test (triangle test) were performed on ice-creams.

Several training sessions were carried out to define the sensory attributes to be assessed in ice-creams. Panelists agreed on assessing oily', 'grassy', 'bitterness' and 'pungency' along the 15 attributes usually evaluated in ice-cream (Thompson et al., 2009). All the descriptors are listed in Table S1. A 10-point intensity scale anchored between 0 (not recognized) and 10 (very intense) was employed.

The same panelists also performed triangle tests during which they were presented with three samples, two of which identical, and they were asked to identify the different one. The ice-cream samples were randomly distributed among the panelists.

Table 3
Chemical indices, sensory attributes and volatile compounds of EVOO used in ice-cream formulation.

Chemical index ^a	EVOO	EVOO Law limit ^a
Acidity (% oleic acid)	0.27 ± 0.02	≤0.8
Peroxide value (meq O ₂ kg ⁻¹ oil)	4.93 ± 0.05	<20
K ₂₃₂	2.42 ± 0.01	≤2.50
K ₂₇₀	0.21 ± 0.00	≤0.22
ΔK	-0.001 ± 0.002	≤0.01
Total phenolic compounds (ppm)	339.0 ± 0.2	-
Sensory attribute^b	EVOO	EVOO Law limit^a
Fruity	3.8	>0
Pungency	3.5	-
Bitterness	4.3	-
Volatile compound^c		Odour descriptor^d
Ketones		
1-Penten-3-one	347.16 ± 15.05	pungent
Alcohols		
Ethanol	1548.86 ± 10.29	fruity, sweet
1-Penten-3-ol	198.72 ± 6.56	olive oil, green
cis-2-Penten-1-ol	359.69 ± 24.65	grassy, floral
3-Methyl-1-butanol	50.32 ± 4.64	banana, fruity
1-Hexanol	1538.10 ± 248.37	green, fruity, floral
trans-2-Hexen-1-ol	2208.14 ± 376.52	fruity, herbaceous
cis-3-Hexen-1-ol	849.72 ± 123.39	fruity, green
Aldehydes		
Pentanal	303.11 ± 7.66	herbaceous, grassy, fruity, green
Hexanal	842.43 ± 25.09	freshly cut-grass, green
trans-2-Pentenal	87.87 ± 11.57	grassy, waxy
trans-2-Hexenal	2821.43 ± 3631.444	grassy, green, leafy
cis-2-Heptenal	46.37 ± 12.61	
Esters		
Methyl acetate	59.41 ± 1.06	green, fresh
Ethyl acetate	70.20 ± 5.25	pungent, fruity, pineapple
Hexyl acetate	27.27 ± 4.87	green fruity, fatty
Methyl benzoate	294.97 ± 87.17	fruity
Others		
Toluene	268.31 ± 18.62	nutty, bitter almond, chemical, solvent
o-Xylene	48.73 ± 4.03	geranium
p-Xylene	187.26 ± 30.92	
m-Xylene	79.28 ± 12.56	
β-Ocimene	862.11 ± 282.46	woody, green
Copaene	551.65 ± 177.38	woody, spicy

* EEC Reg. 2568/91 and further modifications.

^a Values are the average of three replicates of analysis (n = 3). Acidity is expressed as oleic acid equivalent (g/100 g). Peroxide value is expressed as meq O₂ kg⁻¹ oil. Phenolic compounds content is expressed as mg kg⁻¹.

^b Sensory attributes are expressed as median on an unstructured 0–10 scale.

^c Volatile compounds level is expressed as µg kg⁻¹.

^d Odour descriptors were taken from literature (Fricke & Schieberle, 2020; Sacchi et al., 2019; Bonvehí, 2005).

2.4. Volatile compounds in ice-creams

The analysis of volatile compounds was performed using the SPME followed by GC/MS analysis.

The volatile compounds of ice-creams were analysed as described by Sacchi et al. (2019). The ice-cream was sampled from the centre of the original container, discarding the top 1 cm of the ice-cream, using a syringe. Five grams of sample were added in a 10 mL dark glass vial, and 10 µL of isobutyl acetate (408 mg kg⁻¹ in water) was used as internal standard. After 10 min at 25 °C the ice-cream was melted, and a magnetic stirring bar and 1.25 g of potassium chloride was added to the vial, which was capped with a polytetrafluoroethylene (PTFE) septum. The content of the vial was stirred for 1 min and then the vial was thermostated at 35 ± 1 °C for 45 min under constant stirring (550 rpm). SPME fibre was inserted through the Teflon septum and exposed to sample headspace for 15 min at 35 °C while stirring. After extraction, the

volatile compounds were desorbed in the injector of CG at 230 °C for 10 min. The SPME device (Supelco Co., Bellefonte, PA, USA) was equipped with a 50/30 µm thick divinyl-benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre coated with 1 cm length stationary phase.

Volatile compounds were analysed by a GC/MS Shimadzu model QP5050A (Kyoto, Japan), equipped with a Supelcowax-10 capillary column (60 m, 0.32 mm i.d., 0.5 µm thickness) (Supelco Co., Bellefonte, USA). Temperature was set at 40 °C for 4 min, followed by an increase of 3.5 °C min⁻¹ up to 240 °C and held for 3 min. The helium flow was 1.4 mL min⁻¹. The identification of volatile compounds was performed by comparing retention times and mass spectra obtained by analysing pure reference compounds in the same conditions. The identification of volatile compounds was confirmed by comparing mass spectra with those of the National Institute of Standards and Technology (NIST) database. Mass spectra were recorded at 70 eV. The source temperature was 200 °C and the interface temperature was 230 °C. The quantitative data of volatile compounds was carried out by normalising the peak areas of each compound with respect to the area of the internal standard peak. Peak areas were calculated by using Labsolution acquisition system (GC-MS Solution version 1.20; Shimadzu, Kyoto). Before its use, the fibre was conditioned at 270 °C for 1 h for the analysis. A blank test was performed prior to each analysis. All the analyses were performed in triplicate.

2.5. Fatty acid composition of ice-creams

Lipid extraction from ice-cream was performed using the method by Bligh and Dyer (Bligh and Dyer, 1959; Gonzalez et al., 2003). Briefly, 1 g of each sample was homogenized with 15 mL of CHCl₃/H₂O (1:2 v/v) for 1 min by using a Ultraturrax (Kilka-Werke, Germany). Thereafter, samples centrifuged at 3000 rpm for 5 min and the organic phase was collected in a glass tube. The solid residues was re-extract for 3 times with 15 mL of CHCl₃/CH₃OH/H₂O (1:1:1 v/v) and after being centrifuged, the organic phase was added to the collected phase and filtered through a separator filter paper containing anhydrous sodium sulfate. The extract was dried in a rotatory evaporator (Rotovapor, mod. VV2000, Heidolph) at 35 °C. Finally, the sample was reconstituted in 4 mL of hexane and dried under a gentle stream of nitrogen before stocking at -20 °C.

Fatty acid methyl esters (FAMES) were prepared using potassium hydroxide in a methanol solution (2 N KOH in anhydrous MeOH) according to Romano et al. (2021). Twenty µL of the lipidic extract was dissolved in 2 mL of hexane and added with 1 mL of KOH in a MeOH solution (2 N). The tube was vortexed for 1 min and allowed to react for 4 min at room temperature. One mL aliquot of the upper organic phase was analyzed by high-resolution gas chromatography. samples were injected onto a GC-17A gas chromatograph (Shimadzu Italy, Milan) equipped with a flame ionization detector (FID), and a SUPELCO capillary column SPTM-2560 (75 m length, 0.14 µm i.d. with 0.18 µm coated with poly (bis-cyanopropyl) siloxane (Supelco, New Haven, CT, USA). The oven temperature was held at 150 °C for 13 min, increased at a rate of 5 °C min⁻¹ until 180 °C. This procedure was followed by a second increase in temperature at a rate of 2 °C min⁻¹ to a final temperature of 230 °C, which was held for 20 min. Injector temperature and FID temperature: 240 °C. Carrier gas: Helium. Column flow: 0.3 mL min⁻¹. Split ratio: 1/100. Injected volume: 2 µL. Peaks were assigned matching the retention times of FAMES with those of pure standard compounds (mixture of pure FAMES, Larodan, Malmoe, Sweden) injected under the same conditions.

2.6. Ice-cream in vitro-digestion

The simulated gastrointestinal digestion of the 4 ice-creams was performed using the method described by the guidelines of INFOGEST *in vitro* digestion method (Minekus et al., 2014). Three sequential digestive phases, namely the simulated salivary phase (SSP), gastric phase (SGP)

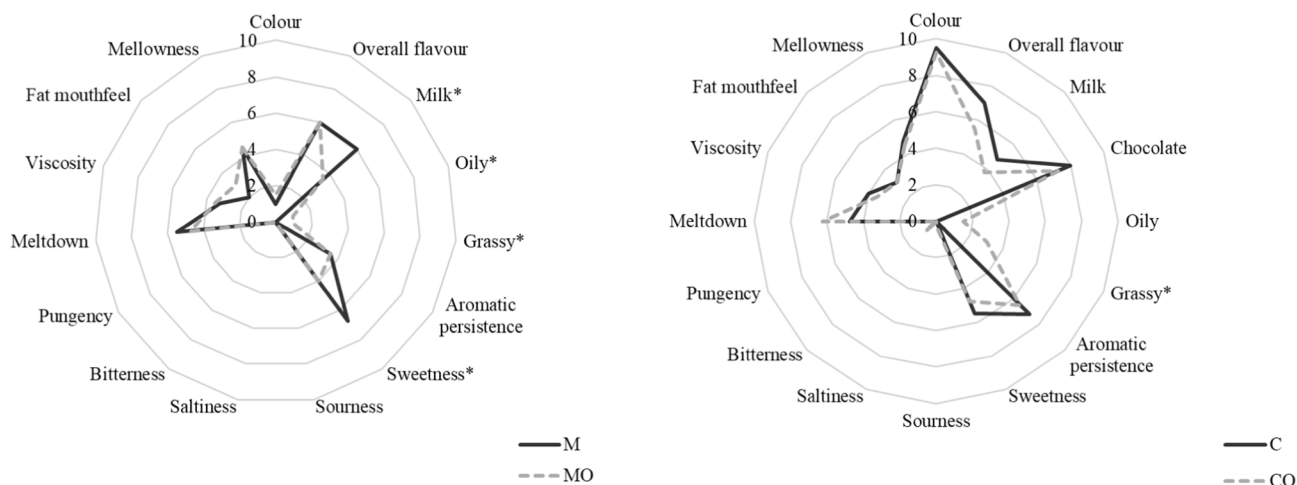


Fig. 1. Radar plot of attributes (mean values) perceived from milk (left) and chocolate (right) ice-creams. M = milk with cream; MO = milk with EVOO; C = chocolate with cream; CO = chocolate with EVOO. *indicates statistically significant differences between the samples ($p \leq 0.05$).

and, intestinal phase (SIP), were carried out.

Oral Phase: 1.75 mL of SSP stock solution (pH 7.0), 0.25 mL of amylase solution 1500 U mL^{-1} (dissolved in SSP), $12.5 \mu\text{L}$ of 0.3 M CaCl_2 and $487.5 \mu\text{L}$ of ultrapure water to a final volume of 5 mL were added to 2.5 g of each sample. The mixture was incubated in thermostatic shaking water bath at 37°C for 2 min (160 rpm).

Gastric Phase: 3.75 mL of SGP stock solution (pH 3.0), 0.8 mL of pepsin solution 25000 U mL^{-1} and $2.5 \mu\text{L}$ 0.3 M CaCl_2 were added the oral bolus. The pH of the mixture was adjusted to 3.0 with 1 M HCl and the volume was made up to 10 mL with ultrapure water. The mixture was incubated at 37°C under shaking for 2 h (130 rpm).

Intestinal Phase: 10 mL of gastric chyme was mixed with 5.5 mL of SIP stock solution (pH 7), 2.5 mL of pancreatin solution 800 U mL^{-1} , $20 \mu\text{L}$ of 0.3 M CaCl_2 and bile solution 1.25 mL (160 mM) were added. The pH of the mixture was adjusted to 7.0 with 1 M NaOH. Ultrapure water was used to make up the final volume to 20 mL and the mixture was incubated in a thermostatic shaking bath for 2 h at 37°C (160 rpm). At the end of the SIP the samples were centrifuged at 4000 rpm for 10 min to separate the supernatant from the pellet. Supernatants were analyzed for polyphenols and for the total antioxidant capacity (TAC).

2.7. Total polyphenols in ice-creams and digested samples

The extraction of polyphenols from the 4 ice-creams and the digested samples was performed according to Sacchi et al. (2019). One gram of each ice-cream or 1 mL of intestinal digest were mixed with 5 mL pure methanol and maintained for 15 min at room temperature, before being centrifuged at 2500 rpm for 10 min (ALC, Milan, Italy). Then the hydro-alcoholic phase was filtered on 0.22 nm Mimex-GV filters (Millipore, Cork, Ireland). The extracts resulting from ice-creams and the digests were analyzed to determine the total antioxidant activity (TAC) and the concentration of polyphenols.

The concentration of total polyphenols in the methanolic extract was estimated with Folin-Ciocalteu method (Gutfinger, 1981). Briefly, 1 mL of Folin-Ciocalteu reagent (0.2 N) was added to 0.2 mL of each sample. Thereafter 0.8 mL of Na_2CO_3 solution (7.5%) was added and the final mixture was incubated for 30 min at room temperature in the dark. The absorbance values were measured at 765 nm by using a UV-1601 spectrophotometer (Shimadzu, Kyoto). A blank sample was also analyzed. It was made by 0.2 mL distilled water and all the other reagents previously described. Caffeic acid served as a standard for preparing the calibration curve. Results were reported in mg of caffeic acid equivalents per kg of sample.

2.8. Total antioxidant activity of ice-creams and digested samples

The total antioxidant capacity (TAC) was determined in each ice-cream and in the digested samples by the ABTS^{•+} method (Re et al., 1999). Briefly, the reaction was started by adding 1 mL of ABTS^{•+} working solution to 100 μL of the phenolic extract resulting from the undigested and digested sample (as shown in the previous paragraph). After 2.5 min, the absorbance was measured at 734 nm. Analysis was performed in triplicate and expressed as $\mu\text{mol eq. Trolox/g}$ of sample.

2.9. Statistical analysis

Results were expressed as mean \pm SD. Statistical analysis and visualization were carried out in R environment version 4.0.3 (<https://www.r-project.org>). The normal distribution of data was assessed by Shapiro-Wilk test and variables showing a significant positive skewness, were transformed in $\ln(x)$. The differences between the control (conventional ice-creams) and EVOO ice-creams were assessed by a pairwise T-Test. Two-tailed p -values lower than 0.05 were considered significantly different.

3. Results

3.1. EVOO characterization

The chemical and sensory properties of the EVOO used in the formulation of ice-creams, are summarized in Table 3.

Free acidity, peroxide value, K_{232} , K_{270} , and ΔK of EVOO were within the law limits of this market category (EC 2568/91). According to the sensory analysis, medium-intense olive fruitiness (3.8), bitterness (4.3) and pungency (3.5) characterized the EVOO.

The concentration of total phenolic compounds of EVOO was $339.0 \pm 0.2 \text{ mg kg}^{-1}$.

The bitterness and pungency properties are mostly due to the qualitative phenolic profile of the olive oils. Tyrosol, hydroxytyrosol and their secoiridoid derivatives, like oleuropein aglycon, account for approximately 60–90% of the total phenolic compounds of EVOO. The main oleuropein derivatives responsible of EVOO bitterness are the dialdehydic form of decarboxymethyl elenoic acid linked to hydroxytyrosol (3,4-DHPEA-EDA or “oleacein”) and the aldehydic form of elenoic acid linked to hydroxytyrosol (3,4-DHPEA-EA or “oleuropein aglycon”). On the contrary, the dialdehydic form of decarboxymethyl elenoic acid linked to tyrosol (p-HPEA-EDA or “oleocanthal”) and the deacetoxy-ligstroside aglycon (p-HEPA-EA or “ligstroside aglycon”) are reported to be responsible of pungency sensory attribute (Genovese et al.,

Table 4Content of volatile compounds ($\mu\text{g kg}^{-1}$) with indication of their odour descriptors and odour thresholds of 4 ice-creams: milk (M), milk with EVOO (MO), chocolate (C), chocolate with EVOO (CO).

Compound	Odour threshold ¹ ($\mu\text{g kg}^{-1}$)	Odour descriptor ²	M	MO	C	CO
Ketones						
Acetone	500000 ^c	solvent, sweet, woody	n.d.	989.35 \pm 249.74	648.29 \pm 36.04 ^a	1109.22 \pm 302.44 ^a
2-Butanone		butterscotch, chocolate	n.d.	352.27 \pm 64.23	1185.05 \pm 51.61 ^a	2607.21 \pm 472.26 ^a
Diacetyl	15 ^b	buttery, creamy	4046.59 \pm 9.59 ^b	5505.83 \pm 218.20 ^a	4563.05 \pm 253.32 ^a	3644.78 \pm 635.94 ^a
2,3-Hexanedione	100 ^c	sweet, creamy, caramellic, buttery	617.14 \pm 88.71 ^a	868.72 \pm 69.02 ^a	n.d.	n.d.
2-Heptanone	140-3000 ^c	cheese, mouldy, fruity	6652.38 \pm 44.73 ^a	3231.62 \pm 235.45 ^b	6311.40 \pm 294.27 ^a	2484.93 \pm 565.23 ^b
2-Nonanone	5-200 ^c	hot milk, fruity	1444.85 \pm 11.48 ^a	552.17 \pm 45.82 ^b	2731.96 \pm 553.82 ^a	1483.82 \pm 66.05 ^a
2-Undecanone	7 ^c	creamy, waxy, fruity	256.60 \pm 62.74	n.d.	425.51 \pm 58.18	n.d.
Alcohols						
Ethanol	100000 ^c	fruity, sweet	n.d.	n.d.	1362.70 \pm 284.10	n.d.
1-Propanol	9000 ^a	alcoholic, fermented, tequila-like	n.d.	n.d.	483.65 \pm 122.98	n.d.
2-Pentanol		sweet, fruity	1039.86 \pm 273.39	n.d.	400.33 \pm 101.16 ^a	548.10 \pm 112.34 ^a
2-Heptanol		green, earthy, oily	n.d.	1245.53 \pm 114.46	325.51 \pm 33.36 ^a	406.41 \pm 80.64 ^a
1-Pentanol	4000 ^c	sweet, fruity, plastic	n.d.	542.05 \pm 73.07	483.28 \pm 24.94 ^a	601.07 \pm 126.98 ^a
1-Penten-3-ol	400 ^c	olive oil, green	n.d.	1017.06 \pm 300.06	n.d.	424.29 \pm 95.97
<i>trans</i> -3-Penten-2-ol		green, grassy, woody	n.d.	590.99 \pm 18.55	n.d.	n.d.
<i>cis</i> -2-Penten-1-ol		grassy, floral	n.d.	n.d.	n.d.	284.07 \pm 45.33
Furfuryl alcohol	2000 ^a	sweet, brown caramellic, breadly, coffee	n.d.	n.d.	381.24 \pm 130.54	n.d.
1-Hexanol	2500 ^c	green, fruity, floral	n.d.	4081.94 \pm 433.05	n.d.	2810.98 \pm 829.47
<i>trans</i> -2-Hexen-1-ol		fruity, herbaceous	n.d.	7056.73 \pm 928.95	n.d.	4595.33 \pm 171.65
<i>trans</i> -3-Hexen-1-ol		green, grassy	n.d.	2013.08 \pm 187.58	n.d.	n.d.
<i>cis</i> -3-Hexen-1-ol	70 ^c	fruity, green	n.d.	n.d.	n.d.	1010.34 \pm 185.05
2-Ethyl-1-hexanol	270000 ^c		223.18 \pm 28.81	n.d.	n.d.	n.d.
Aldehydes						
2-Methylpropanal	0.9 ^b	roasted cocoa	n.d.	n.d.	3839.23 \pm 248.75 ^b	7525.56 \pm 910.89 ^a
2-Methylbutanal	1.9 ^b	musty, chocolate, nutty, furfural	n.d.	263.85 \pm 0.80	10456.97 \pm 1366.02 ^a	14650.85 \pm 3172.26 ^a
3-Methylbutanal	0.4 ^b	green, malty, nutty, chocolate	n.d.	441.12 \pm 20.70	26522.37 \pm 2052.20 ^b	42494.18 \pm 5460.66 ^a
Pentanal	12-42 ^c	herbaceous, grassy, fruity, green	n.d.	3747.62 \pm 227.73	n.d.	n.d.
Hexanal	4.5-5.5 ^b	freshly cut-grass, green	1872.51 \pm 29.97 ^b	8681.96 \pm 200.77 ^a	1690.39 \pm 180.39 ^a	4705.17 \pm 1289.39 ^a
Heptanal	3 ^c	herbaceous, green, oily	n.d.	574.29 \pm 51.74	n.d.	1063.97 \pm 207.02
<i>trans</i> -2-Hexenal	17 ^c	green, fruity, sweet, flowery	n.d.	43678.08 \pm 1769.85	n.d.	13347.64 \pm 3025.51
Octanal	0.7 ^c	green, fatty, oily	n.d.	1361.21 \pm 184.24	478.74 \pm 75.44 ^a	705.57 \pm 111.01 ^a
<i>cis</i> -2-Heptenal		biscuit, fresh milk	n.d.	465.82 \pm 71.49	n.d.	n.d.
Nonanal	1 ^c	green, grassy, fatty, stale	5644.11 \pm 50.47	n.d.	876.77 \pm 229.70	n.d.
Benzaldehyde	350 ^a	almond-like, nutty	n.d.	607.12 \pm 14.94	6947.91 \pm 1470.91 ^a	7434.31 \pm 578.80 ^a
2-Phenylacetaldehyde	4 ^a	honey, nutty, cocoa, berry	n.d.	n.d.	2247.34 \pm 419.79	n.d.
Esters						
Methyl acetate		green, fresh, rum and whiskey-like	n.d.	n.d.	1027.04 \pm 209.27 ^a	2008.70 \pm 280.64 ^a
Ethyl acetate	5-5000 ^c	pungent, fruity, pineapple	n.d.	1662.90 \pm 26.54	n.d.	n.d.
Ethyl propionate	10 ^c	fruity, sweet, apple	2285.77 \pm 363.90 ^b	6639.63 \pm 232.06 ^a	1561.44 \pm 259.06 ^b	3680.85 \pm 162.34 ^a
Ethyl butanoate	1 ^c	sweet, fruity, bubble gum	4139.25 \pm 211.85 ^b	9165.49 \pm 62.75 ^a	3612.76 \pm 877.53 ^b	5363.03 \pm 938.87 ^a
Ethyl benzoate	60 ^c	aromatic, sweet, floral, fruity	n.d.	1066.96 \pm 39.04	n.d.	610.61 \pm 113.52
Pyrazines						

(continued on next page)

Table 4 (continued)

Compound	Odour threshold ¹ ($\mu\text{g kg}^{-1}$)	Odour descriptor ²	M	MO	C	CO
Methylpyrazine	60 ^a	nutty, cocoa, roasted	n.d.	n.d.	2224.96 \pm 501.22 ^a	2615.81 \pm 362.30 ^a
Ethylpyrazine	62 ^b	nutty, musty, fermented, coffee, roasted, cocoa, meaty	n.d.	n.d.	1024.69 \pm 223.63	n.d.
2,3-Dimethylpyrazine	2500 ^a	meaty, caramel, coffee, cocoa	n.d.	n.d.	565.63 \pm 97.86	n.d.
2,5-Dimethylpyrazine	1700 ^a	nutty, peanut, cocoa	n.d.	n.d.	1686.18 \pm 239.24 ^a	2279.44 \pm 285.28 ^a
2,6-Dimethylpyrazine	9000 ^a	coffee, nutty, green	n.d.	n.d.	1052.59 \pm 195.98 ^a	1306.52 \pm 196.19 ^a
2-Ethyl-3-methylpyrazine	130 ^a	nutty, potato	n.d.	n.d.	497.70 \pm 106.22	n.d.
Trimethylpyrazine	1800 ^a	nutty, roasted cocoa, peanut	n.d.	n.d.	1548.18 \pm 387.86 ^a	1472.59 \pm 102.67 ^a
2-Ethyl-3,5-dimethylpyrazine	0.16 ^b		n.d.	n.d.	644.20 \pm 150.87 ^a	810.27 \pm 53.62 ^a
2,3-Diethyl-5-methylpyrazine	0.09 ^b		n.d.	n.d.	279.98 \pm 46.49	n.d.
Tetramethylpyrazine	10000 ^a	nutty, cocoa, musty and vanilla	n.d.	n.d.	3531.66 \pm 862.49 ^a	2715.53 \pm 195.96 ^a
Other compounds						
Toluene		nutty, bitter almond, chemical, solvent	n.d.	n.d.	n.d.	1578.61 \pm 347.35
D-Limonene	10 ^c	citrus, fruity, orange	n.d.	964.61 \pm 232.78	n.d.	456.80 \pm 140.36
o-Xylene		geranium	n.d.	536.63 \pm 152.79	n.d.	519.55 \pm 115.39
Pyrrrole	20000 ^a	sweet, nutty	n.d.	n.d.	767.46 \pm 194.99 ^a	1015.87 \pm 246.65 ^a

Data are shown as mean \pm SD. Different letters indicate statistically significant differences between the control and EVOO ice-cream ($p \leq 0.05$) by a pairwise T-Test.

¹ Odour threshold values were taken from literature (calculated in water) (a = Bonvehí, 2005; b = Toci and Boldrin, 2018; c = Leffingwell & Associates).

² Odour descriptors were taken from literature (Fricke & Schieberle, 2020; Sacchi et al., 2019; Bonvehí, 2005).

2021).

EVOO fruity odor was due to the presence of specific volatile compounds. The most important and abundant were *trans*-2-hexenal, 1-hexanol and *trans*-2-hexen-1-ol responsible of grassy and fruity odors (Table 3).

3.2. Sensory analysis

The effect of the replacement of milk cream with extra virgin olive oil (EVOO) in the ice-cream sensory properties was investigated by discriminant test (triangle test) and quantitative descriptive analysis (QDA). In the triangle test, 100% of the panelists correctly recognized MO vs M ($\alpha = 0.001$); conversely, CO was not significantly identified as different from C, as 36% of panelists did not correctly recognize CO vs C ($\alpha > 0.05$).

Fig. 1 shows results of QDA analysis of the ice-creams. In MO compared to M, EVOO increased the intensity of the “grassy” and “oily” descriptors and decreased the intensity of “sweetness” and “milk” descriptors. In chocolate ice-creams only “grassy” descriptor was rated higher in CO than C. Furthermore, in both MO and CO, the in-mouth texture descriptors did not obtain different intensity scores. EVOO, as a lipid matrix, guaranteed a perception of the “overall flavor” descriptor similar to the milk cream. Compared to MO ice-cream, the chocolate in CO masked the perception of “oily” referring to the typical aromatic sensations of vegetal oil.

Overall, the ice-creams containing EVOO were considered “very acceptable” in terms of taste, aroma, and viscosity.

3.3. Volatile organic compounds

The volatile organic compounds (VOCs) identified and quantified in the ice-creams are shown in Table 4, with the indication of their odour descriptors and odour threshold values. In total, 55 VOCs were identified in ice-cream samples, belonging to the chemical class of ketones, alcohols, aldehydes, esters and pyrazines.

2-Heptanone, 2-nonanone and 2-undecanone, responsible for cheesy and milky aroma, were more abundant in M and C. Conversely, esters,

such as ethyl propionate and ethyl butanoate, were quantitatively higher in MO and CO. The replacement of cream with EVOO in the recipe led to the detection, in MO and CO, of some compounds coming from EVOO such as 1-hexanol and *trans*-2-hexenal which were responsible for the greater “grassy” odour perceived in MO and CO vs M and C. On the other hand, chocolate ice-cream was richest in volatile compounds. Pyrazines qualitatively characterized chocolate ice-creams (C and CO) while 2-methylpropanal, 2 and 3-methylbutanal quantitatively. These VOCs, responsible of nutty, roasted, coffee and cocoa odours, have relatively lower odour threshold values than VOCs resulting from EVOO, i.e. 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine (Table 4). This could imply that the volatile compounds of chocolate, compared to the typical volatile compounds of EVOO, caused a masking effect of the EVOO aroma in the ice-cream, corroborating the findings of sensory analysis.

3.4. Fatty acid profile

The lipidic profile of FAMES of M and C was dominated by palmitic (C16:0) accounting for 33.8% and 34.7% of total fat, respectively, with no difference among samples (Table 5). On the other hand, oleic acid (C18:1 *n*-9) was the more abundant fatty acid in MO and CO accounting for 52.0% and 50.5% of total fat. Moreover, in MO and CO, SFA were 1.7-folds lower and MUFA and PUFA were 2- and 2.7-folds higher than the traditional counterparts, respectively.

3.5. Bioaccessibility of polyphenols and antioxidant activity

Fig. 2a and Fig. 2b show the variation of total polyphenol concentration and total antioxidant capacity in MO and CO from the conventional counterparts (M and C, respectively), before and after *in vitro* digestion.

Total polyphenols increased more in MO vs M than in CO vs C, both in the products (before the digestion namely undigested samples) and in the digests (product after the digestion namely digested samples). Specifically, in the products the increase of total polyphenols in MO vs M

Table 5

Fatty acid composition (% weight of total methyl esters) of 4 ice-creams: milk (M), milk with EVOO (MO), chocolate (C), chocolate with EVOO (CO).

		M	MO	C	CO
C4:0	butyric acid	0.80 ± 0.05 ^a	0.43 ± 0.02 ^a	0.77 ± 0.03 ^a	0.26 ± 0.06 ^a
C6:0	caproic acid	1.17 ± 0.07 ^a	0.50 ± 0.03 ^b	1.03 ± 0.08 ^a	0.25 ± 0.03 ^b
C8:0	caprylic acid	0.49 ± 0.06 ^a	0.38 ± 0.02 ^a	0.76 ± 0.02 ^a	0.21 ± 0.01 ^b
C10:0	capric acid	2.43 ± 0.38 ^a	0.91 ± 0.08 ^b	1.85 ± 0.04 ^a	0.54 ± 0.01 ^b
C11:0	undecylic acid	0.27 ± 0.04 ^a	0.09 ± 0.01 ^b	0.17 ± 0.04 ^a	0.04 ± 0.01 ^b
C12:0	lauric acid	2.96 ± 0.27 ^a	1.18 ± 0.12 ^b	2.33 ± 0.06 ^a	0.78 ± 0.05 ^b
C14:0	myristic acid	10.59 ± 0.95 ^a	4.22 ± 0.09 ^b	8.62 ± 0.23 ^a	2.76 ± 0.01 ^b
C15:0	pentadecylic acid	1.09 ± 0.07 ^a	0.43 ± 0.01 ^b	0.83 ± 0.03 ^a	0.28 ± 0.02 ^b
C16:0	palmitic acid	33.83 ± 3.04 ^a	20.72 ± 1.82 ^b	34.71 ± 1.48 ^a	21.06 ± 1.20 ^b
C17:0	margaric acid	0.53 ± 0.03 ^a	0.16 ± 0.01 ^b	0.38 ± 0.02 ^a	0.24 ± 0.01 ^a
C18:0	stearic acid	12.81 ± 1.27 ^a	9.74 ± 0.10 ^a	18.79 ± 0.47 ^a	16.10 ± 0.51 ^b
C20:0	arachidic acid	0.54 ± 0.00 ^a	0.34 ± 0.02 ^a	0.41 ± 0.04 ^a	0.48 ± 0.01 ^a
C22:0	behenic acid	0.17 ± 0.01 ^a	0.15 ± 0.03 ^a	0.15 ± 0.06 ^a	0.22 ± 0.02 ^a
C24:0	lignoceric acid	0.11 ± 0.08 ^a	0.03 ± 0.01 ^a	0.05 ± 0.01 ^b	0.12 ± 0.04 ^a
SFA		67.81 ± 4.28 ^a	39.30 ± 3.80 ^b	70.73 ± 1.80 ^a	43.21 ± 1.3 ^b
C14:1	miristoleic acid	1.06 ± 0.13 ^a	0.35 ± 0.02 ^b	0.65 ± 0.21 ^a	0.23 ± 0.01 ^a
C16:1n-9	palmitoleic acid	1.86 ± 0.24 ^a	0.02 ± 0.01 ^b	1.49 ± 0.05 ^a	0.60 ± 0.07 ^b
C16:1n-7	palmitoleic acid	0.51 ± 0.16 ^a	0.72 ± 0.06 ^a	0.36 ± 0.06 ^a	0.10 ± 0.01 ^b
C18:1n-9	oleic acid	21.21 ± 2.06 ^b	51.96 ± 3.2 ^a	23.37 ± 0.90 ^b	50.52 ± 3.16 ^a
C18:1n-7	vaccenic acid	0.43 ± 0.03 ^a	0.76 ± 0.07 ^a	0.26 ± 0.10 ^b	0.58 ± 0.11 ^a
C20:1	eicosenoic acid	n.d.	0.15 ± 0.01	n.d.	0.18 ± 0.01
MUFA		26.41 ± 2.28 ^b	53.92 ± 2.64 ^a	26.84 ± 0.41 ^b	50.90 ± 0.80 ^a
C18:2n-6	linoleic acid	1.55 ± 0.57 ^b	4.64 ± 0.62 ^a	1.94 ± 0.23 ^b	4.19 ± 0.49 ^a
C18:3n-3	α-linolenic acid	0.31 ± 0.16 ^a	0.44 ± 0.03 ^a	0.39 ± 0.01 ^a	0.36 ± 0.01 ^a
C22:2	docosadienoic acid	n.d.	0.27 ± 0.05	n.d.	0.22 ± 0.03
PUFA		1.90 ± 0.52 ^b	5.10 ± 1.23 ^a	1.86 ± 0.63 ^b	5.01 ± 0.7 ^a

Data are shown as mean ± SD. Different letters indicate differences between the control and EVOO ice-cream ($p < 0.05$) by a pairwise T-Test.

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

almost quintupled that of CO vs C whereas in the digests it doubled.

Total antioxidant capacity similarly increased in both EVOO ice-creams compared to the traditional counterparts. However, digested MO vs M showed a 1.5-folds higher increase in total antioxidant capacity than CO vs C.

4. Discussion

Snacking habits with energy-dense savory and sweet foods have been positively associated with the incidence of obesity and related chronic diseases, in several countries, in both adults and children (Szczepańska et al., 2022; Al-Jawaldehet et al., 2020; Rao et al., 2018). Sweets, including ice-creams, are often demonized for the high sugar and saturated fat

content. Notwithstanding, ice-creams are highly consumed as desserts or snacks by both Americans and Europeans, the average consumption across Europe being estimated as 6.2 L per capita per year (Europe Ice-cream Market Forecast, 2021) and 23 gallons (~87 L) per year in USA (Yuko, 2022).

Therefore, reformulation of ice-creams to optimize nutritional aspects and provide the market with healthier alternative products is a promising strategy to regulate metabolic disease rise worldwide.

In this study we demonstrated that the total replacement of milk cream with EVOO in the traditional recipe of ice-cream led to products valuable for both sensory and nutritional aspects.

Results from sensory analysis highlighted that the presence of EVOO in MO ice-cream increased the intensity of the “grassy” and “oily” descriptors and decreased the intensity of “sweetness” and “milk” descriptors compared to a traditional ice-cream M, whereas other sensory differences in ice-cream were homogeneous. Güven, Kalender and Taşpinar (2018) achieved a similar result, finding significant differences in the “flavour and smell” descriptor in ice-cream developed with olive oil as a total replacement for milk fat, while descriptors “colour and appearance” and “body and texture” were not perceived as different by the panelists. The authors also reported that the differences in flavour were not perceived as significant when fat in ice-cream was partially replaced (1:1 ratio between olive oil and milk cream). Generally, texture defects are more evident in ice-cream if fat is completely replaced by non-fat substitutes such as proteins and/or carbohydrates (Genovese et al., 2022).

The similar scores attributed to “bitterness” and “pungency” descriptors of both flavoured ice-creams developed with EVOO could be explained by the ability of milk proteins to form covalent bonds with phenolic compounds and dampening the intensity of bitterness (Genovese et al., 2015; Ares et al., 2009; Peyrot des Gachons et al., 2021). Furthermore, the replacement of milk fat with EVOO did not lead to differences in the “aromatic persistence” descriptor for both types of ice-cream.

Generally, the fat reduction in ice-cream results in greater odour intensity and less flavour release (Akbari et al., 2019). Conversely, by changing the type of fat in ice-cream, the aroma persistence did not vary significantly as previously reported by Feyzi et al. (2020) in a study on aroma release of saffron ice-cream using *in vitro* and *in vivo* approach. The higher “grassy” odour perceived in MO and CO, is supported by the greater presence of some representative compounds of EVOO such as 1-hexanol, *trans*-2-hexen-1-ol, 1-penten-3-ol, hexanal, heptanal and *trans*-2-hexenal compared to their respective control. These results on volatile compounds agree with those reported by Sacchi et al. (2019), who found 1-penten-3-ol and *trans*-2-hexenal only in the ice-cream developed with the addition of EVOO. These volatile compounds derive from the lipoxigenase pathway and are typical of high-quality EVOO and responsible of its herbaceous odour (Genovese et al., 2021).

Interestingly, panelists could not distinguish CO from C. This may be explained from the high amount of volatile compounds such as pyrazines and aldehydes found in C and CO ice-creams. These compounds derive from roasted cocoa used in the production of chocolate (Fricke & Schieberle, 2020) and are responsible of nutty, roasted, coffee and cocoa odours and are more impactful as odorants than the typical volatile compounds of EVOO, causing a masking effect in ice-cream.

In the respect of nutritional aspects, the replacement of milk cream with EVOO determined a change in fatty acid profile of the ice-cream by reducing SFA while increasing MUFA and PUFA with a concomitant addition of polyphenols in the ice-cream food matrix.

Limiting energy from SFA below the 10% of total energy of the diet is a recommended dietary requirement for Europeans and Americans to reduce the risk of cardiovascular disease (EFSA, 2017; FDA, 2022). Compared to EVOO dairy free ice-creams in US market (Craig & Brothers, 2022), our formulations are similar for fats, SFA and sodium, but contain less carbohydrates and sugars and more proteins. Interestingly, the amount of SFA in MO and CO (3.6 and 3.2% of DRV), is much

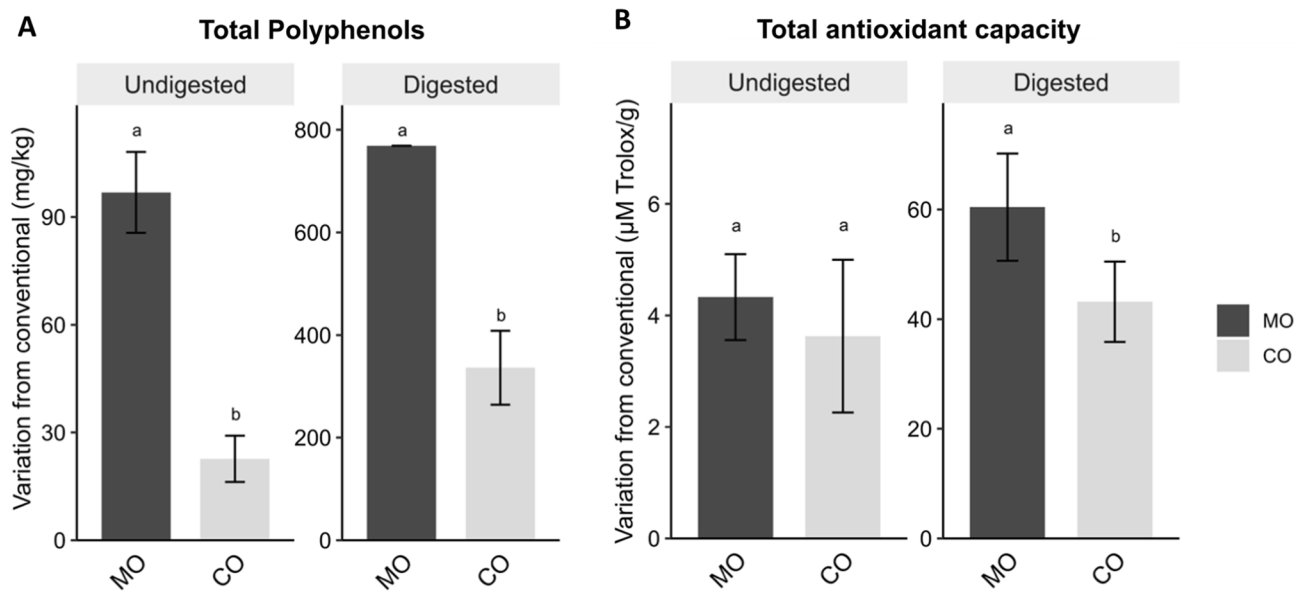


Fig. 2. Variation of total polyphenol concentration (A) and total antioxidant capacity (B) in EVOO-enriched milk (MO) and chocolate (CO) ice-creams from that of conventional ice-creams (M and C), before (undigested) and after (digested) simulated digestion *in vitro*. Different letters indicate significant differences between samples (pairwise T-test, $p < 0.05$).

lower than the 89% of plant-based frozen desserts present in the US market (>5% of DRV) (Craig & Brothers, 2022).

However, some experts have pointed out that only replacing SFA content in the food may not be sufficient to manage cardiovascular risk factors, but the food type, the whole constituents, and their interactions with the food matrix, may be more important (Astrup et al., 2020). Furthermore, despite EFSA and FDA recognized the use of EVOO (about 23 g per day) beneficial and safe for human health, an excessive intake might impair lipid metabolism (Tomé-Carneiro et al., 2020).

Beside the healthy fatty acid profile, using EVOO as ingredient provides the ice-cream with several bioactive compounds such as phenolic compounds, phytosterols, tocopherols, and pigments which may contribute to the protection of cardiovascular, metabolic, immunological and neurological systems through several activities and pathways (Millman et al., 2021). To achieve those benefits, the bioaccessibility of bioactive compounds from the EVOO-enriched food may be fundamental (Rein et al., 2013). Findings of this study demonstrated that polyphenols from ice-creams containing EVOO were bioaccessible *in vitro* thus increasing the antioxidant activity of the digesta compared to the traditional counterparts. Our formulations contain a certain amount of cow milk powder including proteins that could entrap reactive polyphenols, possibly stabilizing them and preventing their oxidation (Cömert & Gökmen, 2022); however, digestive enzymes in the gastrointestinal tract can break down the protein-phenol complexes allowing the release of the phenolic compounds during digestion.

Our data indicated that, although cocoa is a valuable source of polyphenols (Carlsen et al., 2010), the addition of EVOO to chocolate ice-cream (CO) causes a lower increase in bioaccessible polyphenols or antioxidant activity compared to that measured in milk ice-cream (MO). It is likely that the polymeric structure of cocoa procyanidins contributed to physically entrap EVOO polyphenols or protein-phenol complexes in CO, reducing EVOO polyphenol bioaccessibility and antioxidant activity of the digesta compared to MO (Rue et al., 2017; Cömert and Gökmen, 2022).

As a matter of fact, we state here that the Folin-Ciocalteu and ABTS methods used to explore the total polyphenol content and total antioxidant capacity present several pitfalls that limit the direct transfer of our results to *in vivo* conditions (Granato et al., 2018). These methods were used as *in vitro* screening to compare the ice-creams and their potential behavior upon ingestion. However, a chemical characterization of the

ice-creams quantifying the bioactive compounds and an *in vivo* clinical trial are mandatory to prove the biological properties of these innovative ice-creams by evaluating the circulating bioactive compounds and their metabolites, the gut microbial metabolism of nutrients and the inter-individual variations in the biological response (Pellegrini et al., 2020).

After confirmation *in vivo* that milk proteins in the ice-cream matrix allow the targeted delivery of polyphenols in the intestine, future studies may also evaluate whether and at which extent polyphenols extracted from agricultural byproducts coming from the olive oil production may be further incorporated in functional ice-creams (Pérez et al., 2021; Reboredo-Rodríguez et al., 2021). This would allow the re-utilization in the food chain of by-products such as olive mill wastewater or olive leaves that are still rich in polyphenols, in agreement with 2030 Agenda for healthy and sustainable nutrition (United Nations Resolution, 2022).

5. Conclusions

In conclusion, in this study we designed and developed innovative Mediterranean ice-creams by replacing milk cream with EVOO in the traditional recipe of milk and chocolate flavored ice-creams. Sensory analysis demonstrated a high overall acceptability of EVOO-based ice-creams that did not significantly change the original flavour, possibly due to the chemical interactions between EVOO polyphenols and milk proteins that masked bitterness. However, these interactions allowed polyphenols to be released upon digestion *in vitro* thus providing a potential antioxidant protection along the gastro-intestinal tract. The bioaccessibility of polyphenols and antioxidant activity along the reduced ratio between saturated and unsaturated fatty acids made the innovative ice-creams potentially healthy and functional. Future studies should assess whether these properties are maintained *in vivo* in humans.

Ethics statements:

Participants involved in the sensory analysis, were informed on the nature and composition of experimental products and that the products tested were safe for consumption. They gave their consent by signing a document reporting the information related to the products and the indication that they were able to withdraw from the study at any time without giving a reason.

CRedit authorship contribution statement

Silvia Tagliamonte: Formal analysis, Methodology, Data curation, Visualization, Writing – original draft. **Lucia De Luca:** Formal analysis, Methodology. **Antonietta Donato:** Formal analysis, Methodology. **Antonello Paduano:** Formal analysis, Methodology, Supervision. **Andrea Balivo:** Formal analysis, Methodology. **Alessandro Genovese:** Formal analysis, Methodology, Supervision, Writing – review & editing. **Raffaele Romano:** Supervision. **Paola Vitaglione:** Conceptualization, Supervision, Writing – review & editing. **Raffaele Sacchi:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jff.2023.105470>.

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