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Bioactive compounds extracted by liquid and supercritical carbon dioxide from citrus peels

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Abstract This work investigated the extraction of bioactive compounds from citrus peels, an agri-food waste. Carbon dioxide (CO_2), an eco-friendly solvent, was used under liquid and supercritical conditions to perform the extractions from orange, tangerine and lemon peels. The possibility of using ethanol as a cosolvent at small percentages up to 20% was also studied. The extraction yield, total polyphenolic content, individual polyphenolic profile, antiradical activity and volatile organic compounds of the extracts were evaluated.

The highest yields were obtained when 20% ethanol was used as a cosolvent in both liquid (at 20 MPa and 20 °C) and supercritical (at 30 MPa and 60 °C) CO_2 extraction. In addition, the extracts obtained with liquid CO_2 + 20% ethanol showed the highest content of naringin (35.26, 44.05 and 19.86 mg g^{-1} in orange, tangerine and lemon peel extracts, respectively) and terpenes, in particular limonene. This type of extract also showed the highest antiradical activity (31.78–59.51 μmolTE g^{-1}) as measured by both ABTS·+ and DPPH·.

These findings show that the extraction with a liquid CO_2 and ethanol mixture could be a valid alternative to traditional solvent extraction using 80% less organic solvent and producing extracts with high antiradical capacity and rich in volatile organic compounds.

Keywords Antiradical activity, green technology, lemon, naringin, orange, polyphenols, tangerine, volatile organic compounds.

Introduction

Due to the increase in waste products resulting from food processing, new strategies and new policies must be developed to manage the increase in waste generated by the agri-food industry. The transformation of citrus fruits produces a high quantity of agro-industrial wastes, especially peels and seeds. Worldwide, citrus fruits (oranges, tangerines, lemons, grapefruits) are cultivated for consumption as fresh or processed fruit. The major citrus producing countries are China, Brazil, the United States, Mexico, India, Spain, Iran, Italy, Nigeria and Turkey. Production and consumption trends are different in these countries; however, 147 million tonnes of citrus are produced annually (Khan, 2021). Approximately 25% of citrus fruits are processed in the food industry to produce citrus juices/citrus-based drinks, jams, marmalades and dehydrated citrus-based products (Panwar *et al.*, 2021). Considering that the disposal of food by-products of plant origin represents both a cost for the industrial operator and a potential negative impact on the environment, the wastes could be used as sources to extract and to isolate potential bioactive compounds of commercial interest, such as proteins, polysaccharides, fibres, aromatic compounds, phenolic compounds, carotenoids and vitamins, which can be used in the food, nutraceutical, pharmaceutical and cosmetic industries (Alexandre *et al.*, 2018).

In citrus fruits, aromatic components provide the characteristic odour and include compounds that have antibacterial activity and bactericidal effects (Lin *et al.*, 2010; Omar *et al.*, 2013). Furthermore, citrus peels have a high content of phenolic compounds such as flavonoids and phenolic acids that have antimicrobial, antiviral, anti-inflammatory and antioxidant activities and their concentrations depend on the citrus species (Ignat *et al.*, 2011; Chocholouš *et al.*, 2013; Palazzolo *et al.*, 2013; Zhang *et al.*, 2019; Singh *et al.*, 2020). Among flavonoids, naringin is the most important compound found in citrus fruits, and its concentration depends on fruit ripeness; in fact, the highest concentration of this compound was found in immature fruit

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(Yusof *et al*., 1990). Several studies have shown that naringin supplementation is beneficial for the treatment of obesity, diabetes, hypertension and metabolic syndrome (Alam *et al*., 2014). Regarding the use of naringin in the food industry, Iturriaga *et al*. (2014) showed that this compound used in chitosan films reduced the lipid oxidation induced by UV light in foodstuffs.

Kang *et al*. (2006) showed that the addition of citrus peel powder inhibited lipid oxidation in salmon meat homogenate. In fact, foods containing fat and oils are subject to oxidative deterioration with the formation of potentially toxic secondary compounds, and the addition of antioxidants in the matrix can be used to prevent deteriorative reactions (Moure *et al*., 2001).

Usually, cold pressing, hydrodistillation and solvent extraction methods are used to extract essential oils. However, new extraction techniques have been developed to improve the quantity of essential oil, to preserve its quality and to consume less energy, such as extraction with supercritical fluids. The most commonly used fluid is carbon dioxide (CO_2) because it is nontoxic, nonflammable and recyclable at room temperature, and it is a gas at atmospheric pressure. CO_2 is easily separable from the solute when the extraction process is complete and available at high purity and very low cost (Donelian *et al*., 2009; Moret *et al*., 2014; Aiello *et al*., 2020). Supercritical CO_2 has high density that allow good solubility and diffusivity 10–100 times higher than those of other liquids, which improve mass transfer and reduce extraction times. The selectivity of this type of extraction can be influenced by adjusting the temperature, pressure (or both) and the density and other properties of the fluid can be varied to adapt to the solubility of different components of specific interest (Donelian *et al*., 2009; Moret *et al*., 2014). Another important aspect of CO_2 use is that the extract can be used without other refinement processes. The refinement processes involve an increase in production costs, use solvents or reagents that pollute the environment, and can reduce the presence of bioactive compounds present in the extracts. The supercritical fluid extraction has high initial set-up costs (Pereira & Meireles, 2007), but process costs can be minimised through optimisation of pressure, temperature, particle size of sample and superficial velocity of CO_2 (Rosa & Meireles, 2005; del Valle-Gonzalo *et al*., 2014). Also liquid CO_2 can be used as solvent but it has lower diffusivity, higher viscosity and higher surface tension than does the supercritical phase. As in the extraction with organic solvents, the efficiency of extraction with liquid and supercritical carbon dioxide is dependent upon its amount and the time it is in contact with the matrices.

In this work, citrus peels of orange (*Citrus sinensis*), tangerine (*Citrus reticulata*) and lemon (*Citrus limon*) were subjected to CO_2 extraction to obtain bioactive compounds. CO_2 was used under liquid and supercritical conditions at different temperature and pressure values and was compared to the solvent (ethanol) extraction used as a control. In CO_2 extractions, the possibility of using ethanol as a cosolvent at small percentages of up to 20% was also studied. The obtained extracts were characterised for extraction yield, total polyphenolic content, individual polyphenolic profile, antioxidant activity and volatile organic compounds.

Materials and methods

Materials

Orange (*Citrus sinensis*), tangerine (*Citrus reticulata*) and lemon (*Citrus limon*) peels were obtained from fruits harvested in the province of Caserta (Campania, Italy).

The peels, composed of flavedo and albedo, were cut into pieces with a diameter of approximately 10 cm, frozen at −18 °C and lyophilised. The lyophilised pieces of peels were subsequently ground with a knife mill (Grindomix M200, Retsch Italia, Verder Scientific Srl, Bergamo, Italy) and sieved to obtain particles with ø ≤ 1 mm. The samples were kept in the dark at −18 °C until extraction.

Chemicals

The carbon dioxide (CO_2) (assay purity 99.9%) used was provided by SOL Spa (Naples, Italy). All solvents and reagents used for experiments were purchased from Sigma–Aldrich Co. (Milano, Italy).

Moisture content

The moisture content of the peels was calculated gravimetrically by weighing approximately 30 g of peels before and after lyophilisation conducted at −50 °C and <0.05 mbar for 48 h. The results were expressed as a weight/weight percentage of water (% w/w).

Organic solvent extraction (control)

Approximately 1 g of dried and ground peels (ø ≤ 1 mm) was added to 10 mL of ethanol, stirred for 1 min and allowed to rest for 24 h. Subsequently, the samples were centrifuged at 6000 g for 10 min (PK 131; ALC International Srl, Milano, Italy). The supernatant was collected in a glass tube. The extraction procedure was repeated four times. The solvent was removed under vacuum using a Rotavapor Laborota 4000-Efficient instrument (Heidolph Instrument, Schwabach, Germany). The extract obtained was used as a control, and the extraction method was coded C

(Table 1). The extraction yields were expressed as g extract 100 g dry matter $(DM)^{-1}$. The extracts were stored at −20 °C until analysis.

Supercritical and liquid CO_2 extraction

Approximately 12 g of dried and ground peels (ø ≤ 1 mm) were added to an SFC 4000 extractor (JASCO International Co., Ltd., Tokyo, Japan) equipped with a 50-mL-volume extractor vessel.

Supercritical CO_2 extraction was performed at a flow rate of 10 mL min^{-1} and a temperature of 60 °C at two different pressure values (30 and 20 MPa) and at two different percentages (10% and 20%) of ethanol used as the cosolvent.

Liquid CO_2 extraction was performed at a flow rate of 10 mL min^{-1}, a temperature of 20 °C and at two different percentages (10% and 20%) of ethanol used as the cosolvent.

The extraction method codes are shown in Table 1.

The extraction time was 5 h, with 30 min of the static phase alternating with 30 min of the dynamic phase. The extraction yields were expressed as g extract 100 g DM^{-1}. The extracts were stored at −20 °C until analysis.

Total polyphenol content

The total polyphenol content (TPC) of the extracts was determined by the Folin–Ciocalteu method reported by Benelli *et al.* (2010) with some modifications. Briefly, 50 mg of extract were added to 3 mL of methanol, shaken for 30 s, sonicated for 20 min and filtered with a 0.22 μm PES filter. To 0.1 mL of this solution, 7.9 mL of ultrapure water, 0.5 mL of Folin–Ciocalteu reagent and 1.5 mL of a 20% sodium carbonate solution were added.

The solution was incubated for 120 min in the dark at room temperature, and the absorbance was read at 765 nm using a UV-1601PC UV–Visible scanning spectrophotometer (Shimadzu, Milan, Italy). A calibration curve ($R^2 = 0.99$) was constructed with gallic acid at different concentrations (50, 100, 150, 200, 400, 600 and 800 mg L^{-1}). The results were expressed as mg of gallic acid equivalent/g of extract.

Individual polyphenols by high-performance liquid chromatography analysis

To determine the individual polyphenol concentration, 150 mg of extract were weighed and inserted into a tube with 3 mL of methanol. The mixture was shaken with vortexing for 30 s, sonicated for 20 min and filtered with a 0.22-μm PES filter before injection into the HPLC system.

HPLC analysis was performed following the method of He *et al.* (2011), with some modifications. An HPLC system (Agilent 1100 Series, Santa Clara, USA) equipped with degaser G4225A, DAD detector G1315B and FLD G1221A and a Spherisorb ODS2 (5 μm, 4.6 mm × 250 mm) C18 reversed-phase column was used. The mobile phases were composed of 0.1% formic acid in water (phase A) and acetonitrile (phase B). The elution gradient was as follows: 0–5 min, 10% B; 5–10 min, 15% B; 10–16 min, 15% B; 16–18 min, 18% B; 18–28 min, 30% B; 28–33 min, 40% B; 33–35 min, 50% B; 35 min, returns to initial conditions. The flow was set at 1.0 mL min^{-1}.

Phenolic detection was performed at 260, 280 and 330 nm using a diode array detector (DAD).

To quantify the concentration of compounds, calibration curves of standards (chlorogenic acid, caffeic acid, p-cumaric acid, ferulic acid, vanillic acid, hydroxycinnamic acid cinnamic acid, rutin, naringin, catechin and epicatechin) were constructed. The range of linearity was 1–125 ppm for naringin, 5–50 ppm for rutin and 1–50 ppm for all other standards, while the square of the correlation coefficient (R^2) was 0.9985 for naringin, 0.9985 for rutin, 0.9983 for chlorogenic acid, 0.9984 for caffeic acid, 0.9993 for

Table 1 Yield of extracts obtained by different methods from orange, tangerine and lemon peel

Extraction method					Yield (g 100 g DM^{-1})		
Code	**Solvent**	**Pressure (MPa)**	**Temperature (°C)**		**Orange**	**Tangerine**	**Lemon**
C	Ethanol (control)	amb	amb		35.16 ± 2.07[a]	28.59 ± 4.38[a]	30.69 ± 4.20[ab]
	CO_2			Cosolvent (Ethanol)			
L-10	*Liquid*	20	20	10%	4.48 ± 0.52[d]	4.67 ± 0.47[d]	15.82 ± 0.93[c]
L-20	*Liquid*	20	20	20%	17.49 ± 1.53[b]	17.60 ± 0.61[b]	28.84 ± 5.29[ab]
SC-20-10	*Supercritical*	20	60	10%	5.08 ± 0.84[d]	5.06 ± 0.01[d]	7.51 ± 1.72[d]
SC-20-20	*Supercritical*	20	60	20%	14.56 ± 0.69[c]	13.01 ± 1.19[c]	24.32 ± 0.34[b]
SC-30-10	*Supercritical*	30	60	10%	5.19 ± 0.88[d]	6.40 ± 0.44[d]	15.45 ± 4.79[c]
SC-30-20	*Supercritical*	30	60	20%	17.20 ± 0.89[b]	17.60 ± 1.30[b]	31.24 ± 3.00[a]

[a-d]Different letters in the same column indicate statistically significant differences ($P < 0.05$).

p-cumaric acid, 0.9979 for ferulic acid, 0.9978 for catechin, 0.9933 for vanillic acid, 0.9996 for epicatechin, 0.9923 for hydroxycinnamic acid and 0.9912 for cinnamic acid.

The limit of detection (LOD) and limit of quantification (LOQ) were 2.5 and 5 ppm, respectively, for rutin and 0.5 and 1 ppm, respectively, for the other compounds. The results were expressed as mg of the phenolic compound/g of extract.

Antiradical activity assays

The antiradical activity of the extracts was determined by DPPH· assay and by ABTS·+ assay.

The DPPH· assay is based on the scavenging activity of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·) and was performed as described by Espinosa-Pardo *et al.* (2017), with some modifications. To 20 mg of extract, 3 mL of methanol were added, and the mixture was shaken for 30 s, sonicated for 20 min and filtered with a 0.22-μm PES filter. Subsequently, 1.5 mL of 0.1 mM of DPPH solution was added to 0.5 mL of extract. The solution was kept in the dark for 45 min.

The absorbance was measured at 520 nm using a UV-1601PC UV–Visible scanning spectrophotometer (Shimadzu, Milan, Italy). To evaluate the antiradical activity, a Trolox calibration curve in the range of 10–500 μM was prepared. The results were expressed as Trolox equivalent (TE) μmol g^{-1} of extract.

The ABTS·+ assay was performed as described by Omar *et al.* (2013), with modifications. To 0.45 mL of extract dissolved in methanol, 2 mL of $ABTS^{\cdot+}$ working solution were added. The solution was kept in the dark for 5 min, and the absorbance was measured at 751 nm using a spectrophotometer. The antiradical activity was calculated using a Trolox calibration curve with different concentrations (10, 50, 100, 150 and 200 μM). The results were expressed as TE μmol g^{-1} of extract.

Volatile organic compounds

The analysis of volatile organic compounds (VOCs) in the extracts was performed using the solid phase microextraction technique (SPME), as reported by Allaf *et al.* (2013), with modifications. Briefly, 100 mg of extract were weighed in a 10-mL vial for headspace analysis. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre was introduced into the vial and kept at 50 °C for 30 min. Subsequently, the fibre was inserted in a gas chromatograph injector, where thermal desorption of the analytes was performed at 250 °C for 10 min in splitless mode. A 6890N GC system equipped with a 5973 mass detector was used.

The VOCs were separated on an HP-5MS capillary column (30 m × 0.25 mm ID × 0.25 μm) of 5% diphenyl 95% dimethylpolysiloxane. The column oven temperature was held at 40 °C for 2 min and increased from 40 °C to 160 °C at 6 °C min^{-1} and from 160 to 210 °C at 10 °C min^{-1}.

Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The ionising electron energy was 70 eV, and mass-to-charge ratios were scanned over the range of 40 to 450 amu in full-scan acquisition mode. The injection and ion source temperatures were 250 and 230 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}.

The compounds were identified using the NIST (National Institute of Standards and Technology) Atomic Spectra Database version 2.0 and verified for retention indices. The relative content of VOCs was calculated on the basis of peak area ratios and expressed in terms of percentage (%).

Statistical analysis

All experiments were performed in triplicate, and the results are expressed as the mean values (±standard deviations) of three replicates. One-way analysis of variance (ANOVA) and Tukey's multiple-range test ($P \leq 0.05$) were performed on the data using XLSTAT software (Addinsoft, New York, NY, USA).

Results and discussion

Extraction yields

Orange peels had 80.75% w/w moisture, in agreement with the findings of de la Torre *et al.* (2019), who reported a range of 80–90%. In tangerine and lemon peels, the moisture contents were 73.52% and 75.04% w/w, respectively, and the results were similar to those of Ghanem *et al.* (2012).

The use of 100% ethanol (control) produced the maximum extract yields of 35.16% for orange, 28.59% for tangerine and 30.69% for lemon peels (Table 1). Only in lemon peels were no significant differences found among the yield values obtained with the control, L20 and SC-30-20 treatments. These data indicate that citrus peels present compounds soluble in solvent with high polarity such as ethanol.

From the experimental data, the extraction time of 5 h, particle size ≤1 mm, the flow rate of CO_2 of 10 mL min^{-1} were the best conditions to obtain the highest yield of extract (data not shown). However, very low extraction yields (<1.5%) were obtained by exclusively using CO_2 in the liquid or supercritical state; thus, this type of extraction was not considered for the subsequent analyses. CO_2, in fact, is an apolar solvent that is selective only for nonpolar components

(Romano *et al.*, 2020) and cannot extract the polar compounds present in citrus peels. Therefore, the use of co-solvent was evaluated to increase the extraction of polar compounds and the yield. In fact, low polarity compounds and small molecules are easily dissolved in CO_2, but large molecules and polar compounds are extracted with the addition of a co-solvent to enhance the extraction yield (Uwineza & Waśkiewicz, 2020). Also different values of temperature and pressure were evaluated because they are factors influencing the extraction efficiency (Ibañez *et al.*, 2012; Azmir *et al.*, 2013).

Among the extractions performed by using CO_2, the highest yields were obtained when 20% ethanol was added in the SC-30-20 (17.20, 17.60 and 31.24% in orange, tangerine and lemon, respectively) and L-20 (17.49, 17.60 and 28.84% in orange, tangerine and lemon, respectively) methods. The addition of ethanol as a cosolvent increased the extraction yield because the intermediate polarity of ethanol influences the creation of a bond between the molecules of the solute and the cosolvent, facilitating the extraction (Benelli *et al.*, 2010).

The SC-20-20 extraction showed a lower yield (14.56, 13.01 and 24.32% in orange, tangerine and lemon peels, respectively) than L-20 even though it was conducted at the same pressure (20 MPa) and with the same percentage of cosolvent (20%). However, in this case, the temperature (60 °C) was higher than L-20. This increase in temperature at 20 MPa causes a decrease in the density of the solvent, thus decreasing the solute solubility and consequently the extraction yields. The effect of temperature on the solute solubility has two opposite effects: on one hand, it increases the vapour pressure of the solutes, enhancing their solubility in the fluid phase and, therefore, the extraction yield; on the other hand, it decreases the solvent density and, therefore, its solvation power. At pressures close to the critical point, the effect of temperature on the solvent density is stronger than on the solute vapour pressure (Espinosa-Pardo *et al.*, 2017). The SC-20-20 yields were also lower than SC-30-20 because in the latter case, the higher pressure of 30 MPa at 60 °C increased the solvent power of CO_2 due to the density increase and improved the penetration of ethanol into the matrix, increasing the extraction yield (Benelli *et al.*, 2010) but too high pressure reduce the extraction capacity (Moret *et al.*, 2014). The same trend was observed in SC-20-10, which produced a yield (7.51%) lower than L-10 and SC-30-10 in lemon peels.

Total polyphenol content

In Table 2, the content of TPC for each matrix is shown.

The TPC of orange peel extract prepared with supercritical CO_2 at 30 MPa ranged from 13.29 to 21.43 mg GAE g extract^{-1} (10% and 20% ethanol, respectively), while in tangerine peel extract, it ranged from 20.04 to 23.84 GAE g extract^{-1} (10% and 20% ethanol, respectively) and in lemon peels it ranged from 11.25 to 23.81 mg GAE g extract^{-1} (10% and 20% ethanol, respectively).

The TPC of the orange peel extract prepared with supercritical CO_2 at 20 MPa ranged from 14.84 to 20.01 mg GAE g extract^{-1} (10% and 20% ethanol, respectively), the TPC of the tangerine peel extract ranged from 15.71 to 38.65 mg GAE g extract^{-1} (20% and 10% ethanol, respectively) and the TPC of the lemon peel extract ranged from 11.29 to 11.90 mg of GAE g extract^{-1} (10% and 20% ethanol, respectively).

The TPC of orange peel extract prepared with liquid CO_2 ranged from 20.98 to 25.25 mg GAE g extract^{-1} (10% and 20% ethanol, respectively), in tangerine peel extract ranged from 31.88 to 33.08 mg of GAE g extract^{-1} (10% and 20% ethanol, respectively) and in lemon peels it ranged from 15.04 to 18.41 mg GAE g extract^{-1} (10% and 20% ethanol, respectively). The TPC of the tangerine extract obtained by the L-20 method was higher than that of the control.

The addition of ethanol increased the extraction of TPCs in all matrices, except tangerine when supercritical CO_2 at 20 MPa was used. These findings were similar to those of Espinosa-Pardo *et al.* (2017), who showed an increase in phenolic compounds in the extract of dry orange pomace obtained by supercritical fluid extraction with CO_2 and a small amount (6%) of ethanol as a cosolvent. The TPCs were higher than that of the dry orange pomace extract analysed by Espinosa-Pardo *et al.* (2017), who reported TPCs ranging from 18.0 to 21.8 mg of GAE g extract depending on the pressure and temperature used for the extraction. This result could be due to the longer extraction time (5 h) used in our experiments against 75 min used by Espinosa-Pardo *et al.* (2017). The extraction time, in fact, can influence the extract composition (Uwineza & Waśkiewicz, 2020). Moreover the different matrices analysed, pomace and peels, can have a different initial phenolic content that depends on the growing conditions. However, our results were similar to Benelli *et al.* (2010) that used an extraction time of 300 min to obtain orange pomace extracts with TPC content ranging from 30 a 35 mg GAE g extract^{-1}.

At the same percentage of cosolvent used (10% and 20%), the liquid CO_2 extraction allowed us to obtain TPCs higher than supercritical CO_2 extraction. In fact, in the extract obtained by the L-20 method, the TPC was 25.25, 33.08 and 18.41 mg GAE g^{-1} (in orange, tangerine and lemon, respectively). These values were higher than those found in extracts obtained under supercritical conditions with 20% ethanol (SC-20-20

Table 2 Total phenol content (TPC), phenolic acids and flavonoids in orange, tangerine and lemon peel extracts obtained by different methods

	Extraction method						
	C	**L-10**	**L-20**	**SC-20-10**	**SC-20-20**	**SC-30-10**	**SC-30-20**
Orange							
TPC (mg GAE g extract^{-1})	18.25 ± 0.01^{d}	20.98 ± 0.43^{bc}	25.25 ± 0.19^{a}	14.84 ± 0.32^{e}	20.01 ± 0.01^{c}	13.29 ± 0.01^{e}	21.43 ± 0.05^{b}
Phenolic acids (mg g extract^{-1})	1.67 ± 0.10^{c}	1.96 ± 0.06^{b}	2.57 ± 0.12^{a}	0.80 ± 0.01^{e}	2.17 ± 0.04^{b}	1.03 ± 0.02^{d}	1.66 ± 0.04^{c}
Flavonoids (mg g extract^{-1})	4.87 ± 0.10^{e}	17.28 ± 0.29^{b}	35.94 ± 0.16^{a}	0.68 ± 0.07^{f}	9.50 ± 0.10^{c}	0.72 ± 0.05^{f}	7.88 ± 0.04^{d}
Naringin (mg g extract^{-1})	3.80 ± 0.07^{e}	16.70 ± 0.27^{b}	35.26 ± 0.12^{a}	0.57 ± 0.06^{f}	9.42 ± 0.09^{c}	0.47 ± 0.04^{f}	7.79 ± 0.04^{d}
Tangerine							
TPC (mg GAE g extract^{-1})	31.92 ± 0.03^{b}	31.88 ± 0.08^{b}	33.08 ± 0.06^{a}	28.65 ± 0.19^{c}	15.71 ± 0.01^{f}	20.04 ± 0.15^{e}	23.84 ± 0.09^{d}
Phenolic acids (mg g extract^{-1})	1.00 ± 0.01^{a}	0.65 ± 0.01^{c}	0.90 ± 0.10^{ab}	0.98 ± 0.04^{a}	0.80 ± 0.01^{b}	0.26 ± 0.02^{e}	0.34 ± 0.01^{d}
Flavonoids (mg g extract^{-1})	17.39 ± 0.19^{c}	19.95 ± 1.04^{b}	44.38 ± 0.66^{a}	2.91 ± 0.07^{f}	13.67 ± 0.22^{e}	2.95 ± 0.28^{f}	15.96 ± 1.24^{d}
Naringin (mg g extract^{-1})	16.00 ± 0.18^{c}	19.43 ± 1.02^{b}	44.05 ± 0.65^{a}	2.91 ± 0.07^{e}	13.67 ± 0.22^{d}	2.49 ± 0.22^{e}	15.50 ± 1.21^{c}
Lemon							
TPC (mg GAE g extract^{-1})	30.04 ± 0.01^{a}	15.04 ± 0.22^{d}	18.41 ± 0.10^{c}	11.29 ± 0.20^{e}	11.90 ± 0.28^{e}	11.25 ± 0.02^{e}	23.81 ± 0.08^{b}
Phenolic acids (mg g extract^{-1})	1.07 ± 0.05^{a}	0.48 ± 0.01^{c}	0.32 ± 0.01^{d}	0.25 ± 0.01^{e}	0.67 ± 0.03^{b}	0.14 ± 0.01^{f}	0.48 ± 0.02^{c}
Flavonoids (mg g extract^{-1})	16.67 ± 0.46^{b}	2.66 ± 0.04^{e}	20.29 ± 0.25^{a}	0.39 ± 0.01^{g}	9.86 ± 0.62^{c}	0.94 ± 0.01^{f}	8.37 ± 0.15^{d}
Naringin (mg g extract^{-1})	16.67 ± 0.46^{b}	1.71 ± 0.03^{e}	19.86 ± 0.22^{a}	0.39 ± 0.01^{f}	9.56 ± 0.60^{c}	0.38 ± 0.01^{f}	7.95 ± 0.13^{d}

$^{a\text{-}g}$Different letters in the same row indicate statistically significant differences ($P < 0.05$).

and SC-30-20). The same trend was observed in L-10 extracts compared to those obtained in supercritical conditions with 10% ethanol (SC-20-10 and SC-30-10).

Liquid extraction with CO_2, due to the temperature of 20 °C, did not degrade the phenolic compounds in the orange and tangerine extracts, while the lemon peel extract showed the highest content of TPC (23.81 mg GAE g^{-1}), increasing the extraction temperature (in SC-30-20, where the temperature of extraction was 60 °C at 30 MPa with 20% ethanol), which could be derived from the content of thermally stable polyphenols in the matrix, which were more resistant at high extraction temperatures (Thoo *et al*., 2010). The TPC was influenced by temperature; in fact, it was shown that high temperature could destabilise the phenolic compounds analysed in grape pomace. Pomaces from ripe apple and ripe peach were obtained from fruit juice production (Pinelo *et al*., 2005; Adil *et al*., 2007).

Furthermore, increasing the pressure (30 MPa) and the addition of ethanol (20%) produced an increase in the TPC content in all the types of extract. High pressure changes the distribution and aggregation of phenolic compounds and increases penetration of the solvent into cells by disrupting cell walls and hydrophobic bonds in the cell membrane, which can lead to high permeability and release of antioxidant components (Andrés *et al*., 2016).

Individual polyphenols

In Table 2, the individual polyphenol profiles of the orange, tangerine and lemon peel extracts are shown. The phenolic compounds determined in the samples were phenolic acids (chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, vanillic acid, hydroxycinnamic acid and cinnamic acid) and flavonoids (rutin, naringin, catechin, epicatechin). A representative chromatogram of the phenolic compounds is presented in Figure 1. The phenolic acids have different dietary health benefits like antioxidant, anti-inflammatory, immunoregulatory, anti-allergernic, anti-atherogenic, anti-microbial, anti-thrombotic, cardioprotective and anti-cancer activities and anti-diabetic properties. Moreover, various phenolic acids are used as functional additives in foods (Teixeira *et al*., 2013; Anlar *et al*., 2018; Rashmi & Negi, 2020). Furthermore, dietary flavonoids have antioxidant effect, anti-cancer, anti-inflammatory and antimicrobial activity (Tripoli *et al*., 2007). The highest concentrations of phenolic acids and flavonoids in orange (2.57 and 35.94 mg g^{-1}, respectively) and tangerine (0.90 and 44.38 mg g^{-1}, respectively) peel extracts were obtained by the liquid CO_2 + 20% ethanol extraction method (Table 2). Additionally, in the lemon peel extracts, the highest concentration of flavonoids (20.29 mg g^{-1}) was obtained by liquid CO_2 + 20% ethanol extraction; in contrast, the highest concentrations of phenolic acids (1.07 mg g^{-1}) were obtained by using the solvent extraction method. Polyphenols are polar compounds and their solubility in CO_2 has been well enhanced by adding polar solvent such as ethanol. Their solubility in exclusively CO_2, instead, is very low because carbon dioxide has nonpolar characteristics. In particular, hydroxycinnamic acids (*p*-coumaric acid, caffeic acid and ferulic acid) are slightly soluble in supercritical

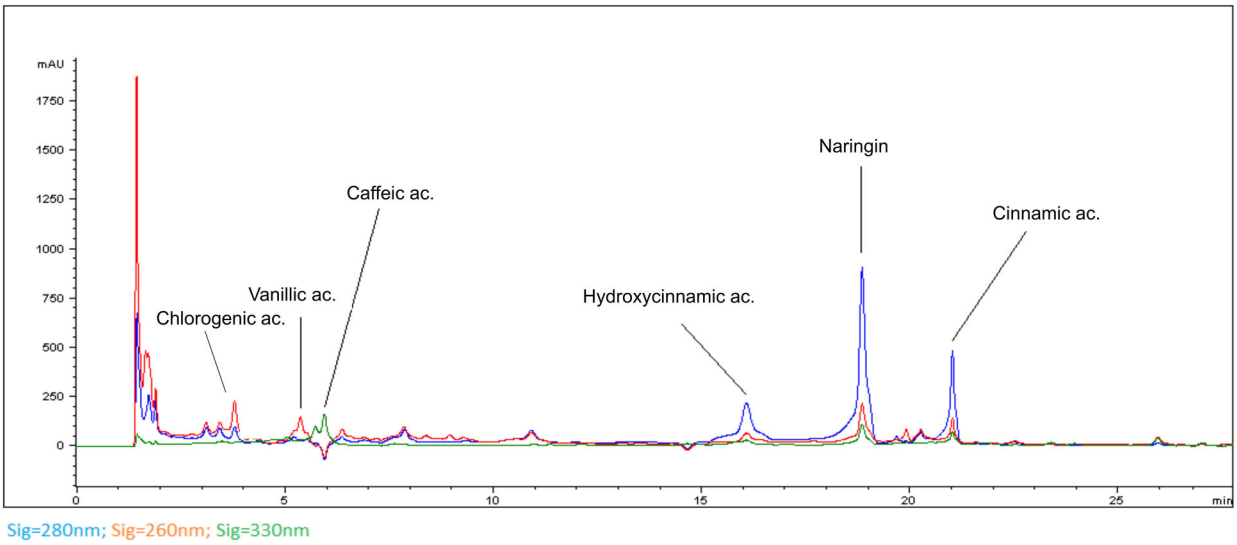

Figure 1 Representative HPLC chromatogram of the phenolic compounds.

CO_2 without addition of a co-solvent (Adil *et al.*, 2007).

Naringin was the most abundant flavonoid, and its concentration ranged from 0.47 to 35.56 mg g^{-1} extract (in SC-30-10 and L-20, respectively) in orange peel extract, from 2.49 to 44.05 mg g^{-1} extract (in SC-30-10 and L-20, respectively) in tangerine peel extract and from 0.38 to 19.86 mg g^{-1} extract (in SC-30-10 and L-20, respectively) in lemon peel extract (Table 2). The naringin has beneficial effects for the treatment of obesity, diabetes, hypertension and metabolic syndrome (Alam *et al.*, 2014). Increasing the ethanol content to 20% (in CO_2 at the same pressure) produced the highest concentration of naringin in all types of extracts. These findings are in agreement with those of Giannuzzo *et al.* (2003), which showed the highest efficiency of naringin extraction when the percentage of ethanol in supercritical CO_2 was increased from 5 to 15%. Furthermore, the temperature also influenced the flavonoid and, in particular, naringin extraction; in fact, when 20% ethanol was added to CO_2 at 20 °C and 20 MPa (conditions to obtain CO_2 in the liquid state), the concentration of naringin was highest (35.56, 44.05, 19.86 mg g^{-1}, in orange, in tangerine and lemon peel extracts, respectively). Conversely, high temperature (60 °C), used to obtain CO_2 in the supercritical state, decreased the naringin concentration in the extracts. Yu *et al.* (2007) showed that the temperature and naringin concentration are inversely correlated in supercritical CO_2 extraction, and they found the highest yield of naringin from grapefruit seeds at 41.4 MPa pressure, 50 °C temperature and 20% ethanol concentration.

Finally, the addition of 20% ethanol to CO_2 also increased the extraction of the other phenolic compounds. while using pure CO_2 in liquid and supercritical extraction, many phenolic compounds were not detected. To increase the extraction of phenolic compounds, it is necessary to modify the polarity of CO_2 by adding ethanol, as reported by Grujic *et al.* (2012). In particular, flavonoids are soluble in pure CO_2, but their solubility can be increased by adding a polar modifier or increasing pressure (Rosa *et al.*, 2008).

Antiradical activity

Two types of antiradical capacity measurements, DPPH· and ABTS·+ assays, were performed to take into account the various modes of action of antiradicals. In fact, the antiradical capacities of extracts depend not only on the extract composition but also on the conditions of the test used. Both methods were based on electron transfer and measured the capacity of an antioxidant to reduce an oxidant, which changes colour when reduced. In Table 3, the antiradical activity of the extracts, as determined by DPPH· and ABTS·+, is shown.

The highest values of antiradical activity as determined by DPPH· were obtained with solvent extraction (C) for all matrices analysed (36.99, 40.38, 69.37 μmolTE g^{-1} for orange, tangerine and lemon peels, respectively). Regarding the extractions with CO_2, the highest values of antiradical activity determined by the DPPH· assay were obtained with the liquid CO_2 + 20% ethanol extraction in all matrices (31.78, 35.94 and 35.75 μmolTE g^{-1} for orange, tangerine and lemon

Table 3 Antiradical activity determined by DPPH· and ABTS·+ assays in orange, tangerine and lemon peel extracts obtained by different methods

	Extraction method						
	C	**L-10**	**L-20**	**SC-20-10**	**SC-20-20**	**SC-30-10**	**SC-30-20**
DPPH· (μmolTE g extract^{-1})							
Orange	36.99 ± 0.09^{a}	28.89 ± 0.02^{bc}	31.78 ± 0.03^{b}	25.67 ± 0.01^{d}	29.62 ± 0.02^{bc}	26.46 ± 0.01 cd	29.79 ± 0.02^{c}
Tangerine	40.38 ± 0.10^{a}	22.16 ± 0.02^{g}	35.94 ± 0.02^{b}	23.72 ± 0.01^{f}	26.97 ± 0.02^{e}	29.93 ± 0.01^{d}	32.3 ± 0.02^{c}
Lemon	69.37 ± 0.09^{a}	22.63 ± 0.08^{e}	35.75 ± 0.67^{b}	20.90 ± 0.10^{f}	27.43 ± 0.38^{d}	27.21 ± 0.02^{d}	31.43 ± 0.02^{c}
ABTS·+ (μmolTE g extract^{-1})							
Orange	38.24 ± 0.08^{c}	38.02 ± 0.15^{c}	56.37 ± 1.01^{a}	28.77 ± 0.02^{d}	42.29 ± 0.22^{b}	28.74 ± 0.08^{d}	54.10 ± 1.66^{a}
Tangerine	39.96 ± 0.26^{d}	38.36 ± 0.05^{d}	59.51 ± 0.37^{a}	52.73 ± 0.33^{b}	27.70 ± 0.02^{f}	35.55 ± 0.53^{e}	48.30 ± 0.09^{c}
Lemon	42.97 ± 0.26^{a}	15.17 ± 0.05^{f}	25.19 ± 0.37^{d}	10.86 ± 0.33^{g}	39.90 ± 0.02^{b}	19.08 ± 0.53^{e}	35.04 ± 0.09^{c}

$^{a\text{-}g}$Different letters in the same row indicate statistically significant differences ($P < 0.05$).

peels, respectively), which could be related to flavonoid and naringin contents that reached their maximum values in the same extracts, as reported in Table 2. The antioxidant activity is also positively correlated with the TPC, as reported by Thaipong *et al.* (2006), who analysed guava extracts obtained with the solvent extraction method, and Barrales *et al.* (2018), who studied orange peel extracts obtained with different extraction methods.

Furthermore, the extracts obtained with liquid CO_2 had a higher antiradical activity than those obtained with supercritical CO_2, and this result could be due to the increase in temperature, set at 60 °C to reach the supercritical state, which causes the removal of a hydroxyl group from the flavonoid structure (Chen *et al.*, 2011), and such structural changes cause the reduction of their antioxidant activity (Sichel *et al.*, 1991).

The ABTS·+ assay values were generally higher than the DPPH· values (Table 3) in orange and tangerine, in agreement with Floegel *et al.* (2011), who found that the antioxidant activity obtained by ABTS·+ assay was significantly higher for fruits and vegetables than that obtained by DPPH· assay. The highly pigmented and hydrophilic antioxidants were better analysed by the ABTS·+ assay than by the DPPH· assay. Therefore, the ABTS·+ assay may be more useful than the DPPH· assay for detecting antiradical capacity in different foods.

Similar to DPPH·, the antiradical activity measured with the ABTS·+ assay showed the highest value (56.37 μmolTE g^{-1} and 59.51 μmolTE g^{-1} in orange and tangerine peels, respectively) with liquid CO_2 + 20% ethanol (L-20), which corresponded to extracts with the highest TPC and naringin (Table 2). The antiradical activity increased when ethanol was added to CO_2 because the addition of ethanol as a cosolvent increased the extraction of antioxidant compounds, as demonstrated by Luengthanaphol *et al.* (2004). For lemon peels, the highest ABTS·+ value was obtained with SC-CO_2-20 MPa (59.02 μmolTE g^{-1}), which corresponded to the extract with the highest phenolic acid content (Table 2). The values obtained were similar to the results obtained by Omar *et al.* (2013), who determined the antiradical capacity (ABTS·+ assay) of peel extracts of various citrus fruits with supercritical CO_2 doped with ethanol, and they showed values of 8.0 μmolTE g^{-1}, 7.8 μmolTE g^{-1} and 10.5 μmolTE g^{-1} in orange, tangerine and lemon peels, respectively.

Volatile organic compounds

Table 4 shows the VOC concentrations in orange, tangerine and lemon peel extracts. VOCs presented in small percentages (<1%) were not reported.

The main VOCs identified were terpenes and sesquiterpenes in all types of extracts.

Among terpenes, limonene was the most abundant compound identified with the highest content in the extract obtained by the Liq. CO_2 + 20% ethanol extraction (52.48% in orange extract, 44.02% in tangerine extract, 43.84% in lemon extract), while a low percentage of this compound was found with the solvent extraction (control) (5.46% in orange extract, 7.52% in tangerine extract, 4.31% in lemon extract). The solubility of terpenes in CO_2 with ethanol is higher than that in CO_2 without cosolvent (Shimoyama *et al.*, 2010). Liquid CO_2 increased the extraction of limonene, as also reported by da Cruz Francisco & Sivik (2002), who showed an increase in the solubility of this compound in liquid CO_2, while the increase in both the temperature and pressure required to reach the supercritical state of CO_2 can degrade the compound (Lopresto *et al.*, 2014). Limonene is largely used in the food industry for its antimicrobial and antioxidant activities (Ibáñez *et al.*, 2020), and it has been shown to have anti-inflammatory, antioxidant, antinociceptive, anticancer, antidiabetic, antihyperalgesic, antiviral and gastroprotective effects (Vieira *et al.*, 2018; Ibáñez *et al.*, 2020). Limonene has

Table 4 Volatile organic compounds (relative percentage) in orange (a), tangerine (b) and lemon (c) peel extracts obtained by different methods

	Extraction method						
Compound	**C**	**L-10**	**L-20**	**SC-20-10**	**SC-20-20**	**SC-30-10**	**SC-30-20**
(a)							
Limonene	5.46 ± 1.40^{e}	26.93 ± 0.67^{b}	52.48 ± 0.05^{a}	19.74 ± 2.45^{c}	24.59 ± 0.46^{b}	12.71 ± 0.32^{d}	23.08 ± 2.01^{bc}
Terpinene	nd	0.75 ± 0.03^{c}	20.16 ± 1.44^{a}	nd	0.69 ± 0.01^{c}	nd	5.14 ± 1.10^{b}
Linalool	6.93 ± 0.16^{c}	9.53 ± 0.60^{ab}	2.53 ± 0.14^{d}	11.40 ± 1.24^{a}	10.46 ± 0.46^{a}	9.25 ± 0.08^{b}	7.32 ± 0.48^{c}
Terpineol	4.66 ± 1.00^{b}	7.21 ± 0.78^{a}	1.09 ± 0.42^{c}	5.88 ± 0.64^{a}	7.33 ± 0.41^{a}	7.27 ± 0.30^{a}	4.52 ± 0.69^{b}
Σ Terpenes	17.05 ± 1.20^{e}	44.42 ± 0.80^{b}	76.26 ± 1.20^{a}	37.02 ± 1.20^{c}	43.07 ± 0.40^{b}	29.23 ± 0.23^{d}	40.06 ± 1.98^{b}
Decanal	nd	0.76 ± 0.22^{c}	1.56 ± 0.03^{b}	0.48 ± 0.11^{d}	1.80 ± 0.02^{a}	1.70 ± 0.41^{ab}	0.51 ± 0.05 cd
Perillaldehyde	2.36 ± 0.18^{a}	1.69 ± 0.14^{ab}	nd	2.20 ± 0.01^{a}	0.90 ± 0.01^{c}	1.96 ± 0.17^{b}	1.36 ± 0.16^{bc}
Σ Aldehydes	2.36 ± 0.18^{b}	2.45 ± 0.25^{b}	1.56 ± 0.03^{c}	2.68 ± 0.10^{b}	2.70 ± 0.02^{b}	3.66 ± 0.35^{a}	1.87 ± 0.15^{c}
Caryophyllene	2.44 ± 0.04^{ab}	2.97 ± 0.04^{a}	1.05 ± 0.02^{d}	2.45 ± 0.10^{ab}	2.71 ± 0.02^{b}	2.02 ± 0.11^{c}	2.66 ± 0.39^{ab}
Farnesene	1.24 ± 0.17^{a}	1.03 ± 0.04^{a}	0.47 ± 0.06^{d}	0.92 ± 0.13^{ab}	nd	0.87 ± 0.07^{c}	0.61 ± 0.08 cd
Valencene	17.54 ± 0.22^{b}	21.30 ± 1.85^{a}	5.55 ± 1.08^{d}	22.80 ± 0.61^{a}	19.94 ± 1.09^{ab}	12.69 ± 1.86^{c}	20.88 ± 1.19^{a}
Cadinene	3.47 ± 0.03^{a}	3.07 ± 0.34^{a}	1.03 ± 0.02c	3.06 ± 0.08^{a}	3.06 ± 0.02^{a}	1.90 ± 0.22^{b}	2.23 ± 0.33^{b}
Bergamotene	nd	2.33 ± 0.15^{b}	0.43 ± 0.06^{e}	nd	1.32 ± 0.07^{d}	1.80 ± 0.03^{c}	3.22 ± 0.17^{a}
Σ Sesquiterpenes	24.69 ± 0.20^{c}	30.70 ± 1.56^{a}	8.53 ± 0.87^{f}	29.23 ± 0.43^{ab}	27.03 ± 0.21^{d}	19.28 ± 1.05^{e}	29.6 ± 1.05^{b}
1,1-Dimethoxyoctane	1.99 ± 0.25^{b}	2.28 ± 0.03^{b}	nd	4.60 ± 0.26^{a}	nd	4.70 ± 0.48^{a}	2.30 ± 0.04^{b}
Dimethylanthranilate	0.53 ± 0.21^{d}	3.52 ± 0.01^{a}	0.62 ± 0.06^{d}	nd	1.77 ± 0.18^{c}	3.04 ± 0.23^{ab}	2.10 ± 0.51^{b}
Σ Others	2.52 ± 0.18^{d}	5.80 ± 0.02^{b}	0.62 ± 0.06^{f}	4.60 ± 0.26^{c}	1.77 ± 0.18^{e}	7.74 ± 0.35^{a}	4.40 ± 0.41^{c}
(b)							
Limonene	7.52 ± 1.51^{e}	21.80 ± 0.71^{c}	44.02 ± 4.16^{a}	23.93 ± 0.68^{bc}	29.05 ± 1.85^{b}	13.24 ± 1.76^{d}	15.56 ± 0.14^{d}
Terpinene	9.29 ± 1.26^{d}	11.35 ± 0.64^{c}	19.57 ± 0.60^{ab}	18.43 ± 0.75^{b}	21.93 ± 2.39^{a}	11.47 ± 1.22^{c}	11.34 ± 0.26^{c}
Terpinolene	1.11 ± 0.13^{c}	1.60 ± 0.15^{b}	2.32 ± 0.17^{a}	2.37 ± 0.03^{a}	2.38 ± 0.47^{a}	1.51 ± 0.13^{b}	1.40 ± 0.07^{b}
Linalool	3.48 ± 0.36^{b}	3.27 ± 0.5^{ab}	4.18 ± 0.08^{a}	4.26 ± 0.39^{a}	1.98 ± 0.55^{c}	3.00 ± 0.49^{b}	3.01 ± 0.24^{b}
Terpineol	8.33 ± 0.70^{a}	2.01 ± 0.24^{d}	2.03 ± 0.42^{d}	1.99 ± 0.09^{d}	2.01 ± 0.22^{d}	5.23 ± 0.90^{c}	6.30 ± 0.38^{bc}
Carvacrol	2.59 ± 0.04^{a}	1.50 ± 0.09^{c}	nd	nd	0.73 ± 0.31^{d}	1.36 ± 0.11^{c}	2.09 ± 0.03^{b}
Σ Terpenes	32.32 ± 1.04^{g}	41.53 ± 0.18^{d}	72.12 ± 2.37^{a}	50.98 ± 0.55^{b}	58.08 ± 1.34^{c}	35.81 ± 1.09^{e}	39.70 ± 0.16^{f}
Decanal	1.23 ± 0.05^{c}	1.54 ± 0.09^{bc}	2.40 ± 0.82^{a}	2.36 ± 0.07^{a}	1.79 ± 0.29^{b}	0.69 ± 0.19^{d}	0.68 ± 0.01^{d}
Perillaldehyde	2.28 ± 0.09^{a}	2.39 ± 0.08^{a}	0.69 ± 0.13^{c}	nd	0.93 ± 0.13^{c}	2.83 ± 0.38^{a}	1.76 ± 0.02^{b}
Σ Aldehydes	3.51 ± 0.04^{ab}	3.93 ± 0.07^{a}	3.09 ± 0.63^{ab}	2.36 ± 0.07^{d}	2.72 ± 0.14^{c}	3.52 ± 0.21^{ab}	2.44 ± 0.01^{d}
Caryophyllene	1.99 ± 0.10^{c}	4.85 ± 0.07^{a}	1.44 ± 0.28^{d}	0.79 ± 0.07^{e}	2.27 ± 0.22^{c}	3.83 ± 0.15^{b}	3.91 ± 0.08^{b}
Farnesene	3.38 ± 0.22^{c}	7.87 ± 0.95^{a}	2.41 ± 0.16^{d}	0.10 ± 0.01^{e}	3.36 ± 0.92^{c}	6.18 ± 0.36^{b}	6.57 ± 0.22^{b}
Cadinene	0.48 ± 0.01^{e}	0.88 ± 0.05^{b}	1.09 ± 0.02^{a}	0.72 ± 0.01 cd	0.53 ± 0.13^{d}	0.77 ± 0.03 cd	1.00 ± 0.05^{a}
Σ Sesquiterpenes	5.85 ± 0.13^{d}	13.6 ± 0.80^{a}	4.53 ± 0.12^{e}	1.61 ± 0.01^{f}	6.16 ± 0.81^{d}	10.78 ± 0.15^{c}	11.48 ± 0.10^{b}
Dimethylanthranilate	41.81 ± 0.89^{a}	28.13 ± 1.71^{bc}	18.80 ± 2.09^{de}	14.43 ± 0.60^{e}	12.71 ± 1.46^{e}	24.02 ± 0.19^{c}	32.99 ± 0.67^{b}
(c)							
Limonene	4.31 ± 0.09^{e}	22.06 ± 0.95^{c}	43.84 ± 4.71^{a}	13.76 ± 0.43^{d}	30.70 ± 2.76^{b}	11.90 ± 0.18^{d}	12.70 ± 0.67^{d}
Terpinene	2.59 ± 0.07^{d}	1.22 ± 0.15^{e}	16.61 ± 2.12^{a}	6.79 ± 0.10^{c}	11.01 ± 1.37^{b}	6.51 ± 0.06^{c}	2.02 ± 0.46^{de}
Terpinolene	0.60 ± 0.34^{d}	2.16 ± 0.02^{a}	1.68 ± 0.19^{bc}	1.50 ± 0.10^{bc}	1.17 ± 0.14^{c}	0.91 ± 0.03 cd	0.38 ± 0.04^{d}
Linalool	1.16 ± 0.07^{b}	2.06 ± 0.34^{a}	0.77 ± 0.14^{c}	1.14 ± 0.05^{b}	0.49 ± 0.02^{c}	0.69 ± 0.08^{c}	1.92 ± 0.28^{a}
Terpineol	2.69 ± 0.12^{c}	6.01 ± 0.45^{a}	1.18 ± 0.44^{d}	3.59 ± 0.16^{b}	1.03 ± 0.03^{d}	1.89 ± 0.18^{c}	5.26 ± 0.92^{ab}
Neryl acetate	8.15 ± 1.24^{b}	10.22 ± 1.30^{a}	3.76 ± 1.21^{e}	6.65 ± 0.10^{bc}	5.56 ± 0.70^{cde}	4.93 ± 0.29^{d}	10.72 ± 0.68^{a}
Geranyl acetate	5.56 ± 0.58^{ab}	4.88 ± 0.62^{b}	1.78 ± 0.69^{d}	4.77 ± 0.02^{b}	3.24 ± 0.50^{c}	3.24 ± 0.09^{c}	7.39 ± 0.38^{a}
Σ Terpenes	25.06 ± 0.14^{f}	48.61 ± 0.87^{c}	69.62 ± 1.34^{a}	38.20 ± 0.67^{d}	53.20 ± 1.45^{b}	30.07 ± 0.43e	40.39 ± 0.25^{d}
Citrall	1.28 ± 0.44^{d}	3.22 ± 0.34^{b}	1.21 ± 0.50^{d}	3.20 ± 0.03^{b}	2.93 ± 0.35^{c}	0.74 ± 0.13^{d}	6.97 ± 0.55^{a}
Aldehydes	1.28 ± 0.44^{d}	3.22 ± 0.34^{b}	1.21 ± 0.50^{d}	3.20 ± 0.03^{b}	2.93 ± 0.35^{c}	0.74 ± 0.13^{d}	6.97 ± 0.55^{a}
Caryophyllene	6.53 ± 1.73^{a}	5.25 ± 0.56^{a}	5.33 ± 0.32^{a}	5.97 ± 0.32^{a}	6.70 ± 0.28^{a}	5.42 ± 0.03^{a}	3.55 ± 1.13^{b}
Bergamotene	15.10 ± 2.78^{a}	8.51 ± 0.55^{b}	7.27 ± 0.73^{b}	13.42 ± 0.75^{a}	9.27 ± 0.51^{b}	12.52 ± 0.08^{a}	8.83 ± 2.52^{b}
Farnesene	1.40 ± 0.45^{a}	1.12 ± 0.04^{a}	0.71 ± 0.11^{b}	1.38 ± 0.01^{a}	0.64 ± 0.05^{b}	1.22 ± 0.01^{a}	0.84 ± 0.29^{b}
Valencene	1.53 ± 0.14^{b}	1.34 ± 0.01^{b}	nd	nd	2.98 ± 0.38^{a}	2.76 ± 0.14^{a}	1.99 ± 0.52^{a}
Bisabolene	21.06 ± 2.36^{a}	14.34 ± 0.89^{c}	8.23 ± 1.25^{d}	17.38 ± 0.68^{bc}	9.95 ± 1.27^{d}	14.56 ± 0.64^{c}	13.47 ± 1.60^{c}
Σ Sesquiterpene	45.62 ± 1.32^{a}	30.56 ± 0.76^{c}	21.54 ± 0.80^{d}	38.15 ± 0.68^{b}	29.54 ± 0.70^{c}	36.48 ± 0.20^{b}	28.68 ± 1.09^{c}

nd, not detected.

$^{a–f}$Different letters in the same row indicate statistically significant differences ($P < 0.05$).

a typical odour described as lemon-like, orange-like and pleasant (Aguilar-Hernández *et al*., 2020; Ibáñez *et al*., 2020).

In orange extracts, valencene was the sesquiterpene found at the highest concentration, ranging from 12.69% in the extract obtained by SC-30-10 to 22.80% in SC-20-10. This compound is an abundant sesquiterpene, especially in Valencia oranges, and it is used as a marker of fruit maturity. Its concentration is positively correlated with orange flavour quality (Elston *et al*., 2005), and its odour has been described as woody and citrusy (Goldenberg *et al*., 2016). The other compounds identified in orange peel extracts were terpinene, linalool, terpineol, decanal, perillaldehyde, caryophyllene, farnesene and cadinene according to Jokić *et al*. (2020), bergamotene, 1,1-dimethoxyoctane and dimethylanthranilate.

In the tangerine extracts, dimethylanthranilate was the most abundant compound, with percentages ranging from 12.71% in SC-20-20 to 41.81% in the control (C). This compound is responsible for the typical aroma of tangerine (Schieberle *et al*., 2003), and it is used as a fragrance ingredient in cosmetics and detergents (SCCS, 2011); in fact, it provides a mandarin flavour. Potential precursors of this compound are methyl anthranilate and anthranilic acid as intermediates in the aromatic amino acid pathway (Faulhaber *et al*., 1997). Furthermore, terpinene, linalool, terpineol, decanal, perillaldehyde, carvacrol, caryophyllene and cadinene according to Šafranko *et al*. (2021), farnesene according to Xiong & Chen (2020) and 1,1-dimethoxyoctane were also identified.

Finally, in the lemon extracts, the most abundant sesquiterpenes were bergamotene (from 7.27% in L-20 to 15.10% in Control) and bisabolene (from 8.23% in L-20 to 21.06% in Control). Caryophyllene was found in concentrations ranging from 3.55% in SC-30-20 to 6.70% in SC-20-20. These compounds are among the main sesquiterpenes found in lemon essential oil (Njoroge *et al*., 1994), and they provide the odours of wood, spicy and balsamic (Aguilar-Hernández *et al*., 2020). The other compounds identified in lemon peel extracts were terpinene, terpinolene, linalool, terpineol, citrall, neryl acetate, geranyl acetate and farnesene according to Gök *et al*. (2015) and valencene.

Conclusions

Citrus peels are an important economic source due to the presence of bioactive compounds that could be used in the food, cosmetic and pharmaceutical industries. Orange, tangerine and lemon peels derived from waste from the agri-food industry were subjected to liquid and supercritical CO_2 extractions. Among the proposed methods, the highest yields were obtained by adding 20% ethanol as a cosolvent to liquid CO_2 (at 20 MPa and 20 °C) in all types of peels. Ethanol, a solvent allowed in the food industry, was not used in large quantities as in conventional solvent extraction and therefore can be easily eliminated from the extract by evaporation. Carbon dioxide is a nontoxic, nonflammable, recyclable fluid and it is a gas at ambient temperature and pressure that is easily separable from the solute when the extraction process is complete. The extracts produced with this method presented the highest amounts of flavonoids (6285.82 μg g DM^{-1}, 7828.38 μg g DM^{-1}, 5816.9 μg g DM^{-1} in orange, tangerine and lemon extracts, respectively) and limonene (52.48%, 44.02%, 43.84% in orange, tangerine and lemon extracts, respectively). They also showed the highest antiradical activity (31.78–59.51 μmolTE g^{-1}) as measured by both ABTS·+ and DPPH·.

Therefore, extraction with a liquid CO_2 and ethanol mixture could be a valid alternative to traditional solvent extraction using 80% less organic solvent and producing extracts rich in volatile organic compounds and polyphenols with high antiradical capacity.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical approval

Ethics approval was not required for this research.

Author contribution

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